Forest Health Initiative

Year 3 Cumulative Report:
July 1, 2009 – September 30, 2012

Executive Summary

The Forest Health Initiative (FHI) is a three-year project exploring whether there is an appropriate and valuable role for biotechnology in protecting and restoring North America's forest ecosystems. These ecosystems are under increasing threats. FHI's focus on biotechnology is driven by the need for new tools to fight a growing number of pests, diseases and pollutants—and a host of new stresses induced by climate change—that each year destroy millions of acres of native forests and native trees. America's once-dominant Chestnut and Elm forests have been virtually wiped out by invasive disease. The fight is on to save the Eastern Hemlock of the Southern Appalachians, the ash forests of the Midwest, and the suddenly vulnerable conifer stands of the American west from both non-native and native pests and diseases.

FHI believes the best way to fully explore the many scientific, environmental, social and regulatory challenges surrounding the use of biotechnology to protect natural forests is to develop a test tree that responds to an existing threat to forest health. FHI is currently supporting an effort to revive the American chestnut by developing and testing in confined field trials a transgenic variety that could provide a safe and effective way to rapidly achieve blight resistance.

The specific goals of this program include developing:

1. A holistic approach for biotechnology to address emerging forest health threats by assessing not just the science but the societal and regulatory issues concurrently.

2. Biotechnology tools (i.e., the science and technology) derived from genomics, marker-assisted selection, and transformation of American chestnut to be used to save other forest tree species that are threatened by catastrophic loss.

3. Concurrent dialogue with all stakeholders to better understand concerns, inform our science and regulatory efforts, and create a more informed citizenry about forest threats and opportunities to overcome these threats.

4. Acceptable protocols for testing transgenic forest trees, integrating biotechnology with traditional breeding and silvicultural practices, and using these appropriately for forest health purposes.

The project provided funds to accomplish objectives in three areas: 1) Scientific development of genetically engineered (GE) trees for forest health and, in particular, expansion of GE American chestnut as a model for testing forest health issues; 2) Regulatory and Intellectual Property needs/issues for GE forest trees, utilizing GE American chestnut as a test case; and 3) Addressing societal concerns about GE trees to answer forest health problems.

The project is organized around three fully coordinated groups in pursuing the initiative's objectives through a "braided" approach. These include a Science Group, a Social/Environmental Group and a Policy/Regulatory Committee. The entire effort is overseen by a Steering Committee.

FHI is funded by the US Forest Service, the US Endowment for Forestry and Communities, and Duke Energy and is guided by a Steering Committee that includes experts from the Environmental Defense Fund and The Nature Conservancy.

Steering Committee Members:

- Carlton Owen, *Chair*, President & CEO, US Endowment for Forestry & Communities; sponsor
- Dr. Jim Reaves, Deputy Chief for Research and Development, USDA Forest Service; sponsor
- Dr. Steven Hamburg, Chief Scientist, Environmental Defense Fund
- Mariann Quinn, Director, EHS Policy and Strategy, Duke Energy; sponsor
- Dr. Peter Roussopoulos, Retired – USDA Forest Service
- Dr. Faith Campbell, Senior Policy Representative, The Nature Conservancy

Year Three Annual Meeting Summary: January 2009 –August, 2012
The Science, Social/Environmental and Regulatory/Policy groups actively organized and pursued the objectives of the FHI over the past three years. The third Annual Meeting was held in Washington, DC, August 9 – 10, 2012. Thirty five participants attended the meeting from the Steering Committee, Science Advisory Committee, Science Team, Social and Environmental Group, Regulatory and Policy Committee, as well as representatives from the regulatory agencies and invited stakeholders. The meeting highlighted research results, complimentary policy work, and showcased the FHI Roadmap, a product of the Social/Environmental group, which is designed to support civil discourse and inform decisions about how to respond to forest health threats. This meeting also included an invited talk by Mr. R. Neil Sampson, President, The Sampson Group, who spoke on *Forest Health and the World Around Us*.

The Society of American Foresters partnered with the FHI to host a day of briefings and meetings on Capitol Hill on August 8th. The FHI group's goal was to educate and update Congress and Congressional staff members on the progress of the Forest Health Initiative programs in the last three years, discuss accomplishments in the last year, and outline plans for the future of the American chestnut project and the FHI roadmap. John Heissenbuttel, Susan McCord, Adam Costanza, and Dr. John Carlson presented a power point presentation in two separate briefings held in the House of Representatives and the Senate Agriculture Committee Hearing Rooms. The briefings were followed by meetings with staff members from the office of briefing cosponsor Representative Kurt Schrader (D-OR) and Representatives Thompson (R-PA), the office of Representative David Price (D-NC), and members of the House and Senate Agriculture Committee professional staff.

Key Results. The three-year effort was designed to produce various types of plantable American chestnut trees with improved blight resistance using contemporary, accelerated methods of genetic improvement. The FHI has resulted in:

- Powerful genomic resources: Complete genome of the Chinese chestnut and a list of genes and markers that accelerate genetic improvement of blight resistance, through conventional breeding as well as transgenic trees.
- Accelerated breeding: Genomic and genetic resources to produce >95% American chestnut with Chinese chestnut blight resistance with 1 to 2 fewer and shorter cycles of breeding. This has the potential to condense 50 years of breeding into 15 years.
- Transgenic testing: Potential of >99.999% pure American chestnut with Chinese chestnut blight resistance candidates within 15 months.

- Clonal testing: Blight resistant trees can be produced by the thousands as demanded by restoration efforts -- applies to conventionally bred as well as transgenic trees.

- Unexpected, significant products: Early screen for blight resistance condenses 5 years of field screening to 1 year. Markers for *Phytophthora* resistance enable selection of ink disease resistant trees. Transgenic and conventional acceleration of early flowering enables greater diversity of planting stock. Memorandum of Understanding with TACF formalizes a first-ever partnership on clonal development and testing.
- New collaborators and approaches: Proves that synergies emerge when transparency, mutual respect and accountability are high priorities.

Policy. The FHI is working with regulatory processes to determine the steps required to use advanced biotechnology tools in developing blight resistant American chestnuts that could someday flower and propagate naturally in the wild. This work is paving the way for using the tools on other tree species with health challenges in U.S. forests. The FHI has resulted in:

- Dialogue with agencies and ongoing Q&A.
- Intellectual property review.
- Biological dossier of American chestnut for use by the regulatory agencies.
- Review of the regulatory landscape.
- Roadmap development.
- Field trial and demonstration plot coordination.

Social and Environmental. The FHI is founded on the understanding that science cannot be addressed in a vacuum but rather must be developed in concert with societal understanding and needs. Extensive dialogues have been undertaken with stakeholders and the results incorporated into the attached "Forest Health Roadmap." This will be a valuable tool supporting civil discourse and informing decisions when considering biotechnology to address forest health.

Experience is showing that the science-driven, braided approach pioneered by the program, can be used as a template for other forest health challenges such as the Emerald Ash Borer, the Hemlock Woolly Adelgid, Thousand canker disease, and many others.

Next Steps. Continue supporting the pipeline of candidate resistant trees, evaluate material as effectively as possible, and address range issues. Set up some demonstration sites to engage the public and field trials to determine the efficacy of resistance and possible unintended expressions. Expand the social norm research component to possibly include forest landowner and public surveys. Develop some communication plans to include publications in journals and other media to include the roadmap and a policy essay.

I. Science Highlights

The Science Group is comprised of researchers from US Forest Service, Penn State University, University of Georgia, SUNY-ESF, and Clemson University. These institutions cover much of the natural range of the American chestnut and each has a long-standing and successful research program in this area. Summary research reports for each organization (UGA, SUNY-ESF, PSU, USFS) are attached.

Genomics – Genome sequencing

- New high quality gDNA was prepared from tissue harvested directly from a Vanuzem ramet in the TACF orchard.
- Two new gDNA libraries were constructed and sequencing initiated.
- Final genome assembly expected by December, 2012.Sequencing of the BAC clone contigs tiling the three major resistance QTLs was conducted. The assembly of the QTL sequences revealed 994 genes in QTL cbr1, 548 genes in QTL cbr2, and 410 genes in QTL cbr3.
- Identification of genes involved in blight resistance:
    - Over 500 candidate disease resistance genes identified in previous genome assembly and transcriptome (RNA sequence) analyses.
    - In total the three QTL contain 194 genes related to disease resistance, among which most likely candidate genes for blight resistance are being selected. Several gene sequences have been provided to the transformation team.
- More complete genes with promoters are being developed for cis-gene approach.

Germplasm–Breeding and Testing

- Provided immature seeds to the Clonal testing team from a variety of germplasm backgrounds.
- Mapping data is providing a mechanism to choose the most resistant trees with the least amount of Chinese chestnut genome from TACF material.
- QTL analysis completed and information passed to the Genomics and Transformation teams.
- 96 BACs were selected from the integrated map for cytogenetics work. This will look at large-scale genomic changes (ie, translocations) between American chestnut and Chinese chestnut.
- Phytophthora resistance mapping is underway.

ClonalTesting & Gene Transfer

- Over 450 new embryogenic cultures from American chestnut, Chinese chestnut, B3F3 and other hybrid chestnuts captured and cryostored.
- First every TACF B3F3 somatic seedlings generated.
- 27 chestnut candidate genes and 4 heterologus candidate genes transformed into multiple chestnut genotypes.
- Over 800 transgenic somatic seedlings in pots, with thousands more in the pipeline at UGA.

Transgenic Testing

- Transformation with constructs on hand and characterization of transgenic events: 12 1st generation vector constructs in the pipeline (52 transgenic events).
- Second-generation gene constructs and transformations: 27 pFHI vector constructs in the pipeline.
- Over 600 transgenic American chestnut trees in the field, 1,900 in pots, and thousands more in the pipeline at SUNY representing 1st and 2nd generation events.
- Established a screening process using PCR and RT-qPCR to cull less promising events, thereby streamlining the regeneration process and reducing labor costs.
- Early blight-resistance assay developed, saving 3 to 4 years off of plantable tree development.
- Early flowering gene tests. Can now produce pollen in less than a year using high light treatments.

- Began doing crosses with transgenic American chestnut and shown that transgenes are heritable and expressed in offspring.

II. Social/Environmental Highlights

The Social/Environmental Group (SEG) is comprised of a diverse mix of more than 20 stakeholders including environmental groups, state and federal agencies, landowners, social interest and professional organizations. It was organized to ensure that all stakeholder groups had the opportunity to provide guidance on whether and how biotechnology should be deployed to address forest health challenges.

FHI Roadmap

In response to a need for a series of science-based questions to evaluate the best available options for addressing forest health challenges, including using GE, a roadmap was developed that includes all of the information generated by all three groups of the FHI. The roadmap is available on the FHI website at www.foresthealthinitiative.org.

The goal of the roadmap is to support civil discourse and inform decisions about how to respond to current and potential forest health threats. The emphasis is on deciding if and how to intervene, with a focus on when genetic approaches to improving tree resistance might be appropriate to pursue. The roadmap might, for example, guide an analysis of research options for a public sector agency such as the US Forest Service, by a company considering the development and marketing of a transgenic resistant tree, or a certification or stewardship system deciding whether to endorse research or commercial deployment. Genetic options include conventional breeding and diverse biotechnologies, including genetic engineering. The threats to forest health of concern include biotic agents such as diseases and insects, and abiotic stresses such as climate shifts. Both exotic and native biotic agents, and the effects of variation in climate, are germane. A summary flow chart presents the general logical approach to decision making, but because of the complexity of forest-stressor interactions, general incompleteness of scientific understanding, and variable social perspectives on when intervention is appropriate, it is presented as a "guide or roadmap." Accompanying material provides added explanations and examples to help inform the use of the roadmap in practice. Information supporting this process will come from a variety of sources including agencies, researchers, and other informed stakeholders.

It is organized to first consider the state of knowledge and capacity about the forest health threat and tree genetic tools, second to evaluate the desirability of various kinds of breeding research, and finally to evaluate, if the research is successful, the desirability of release in wild or planted forests. This material scrutinizes transgenic tree research and release in depth.

FHI Outreach

Since the SEG was established it has developed draft principles and a draft outreach and communications plan. In October 2010 the full FHI group discussed these materials and suggested to the FHI Steering Committee that a professional communications consulting firm be contracted to refine messages and develop a presentation that could be used by all FHI participants in outreach efforts.

In December 2011 the Steering Committee agreed to engage Burness Communications to develop outreach messages and materials. Since then Burness has reviewed all of the SEG material, developed some key messages and a power point presentation for use by FHI participants. To date the power point presentation has been used for webinars with USDA Forest Service representatives, House and Senate staffers, and the Forest Guild. The video is available for viewing on the FHI website, www.foresthealthinitiative.org.

III. Regulatory/Policy Highlights

The Regulatory/Policy Committee is overseen by the Institute of Forest Biotechnology with input from the three US regulatory agencies with oversight of GE trees: USDA APHIS, EPA, and FDA. Other members include experts in intellectual property law and NEPA law.

General Policy Response Plan

The following 8 components are integral to a policy plan that quickly responds to forest health threats with biotechnology tools. The left column shows the relative order of components grouped into phases, and how early to start working on them. Relative timing of phases is important, but there is overlap and many components can happen concurrently.

Phase 1

Start 5 years prior to possible deregulation and use

1. **Open lines of communication with policy stakeholders**
   Objective: Foster open discussions to guide biotechnology efforts
   Communicating with key policy stakeholders is a critical step because it will help guide all of the following steps that have regulatory implications. In general, the earlier an initial communication is made the better. However, planning for a product that is more than five years away will make the discussion less concrete to regulating agencies.

2. **Engage a wide spectrum of additional stakeholders**
   Objective: Increase understanding between FHI participants and stakeholders
   Creating a positive, collaborative dialogue with many types of stakeholders is critical because healthy forests are a social good. Topics should include the environmental and economic impact of the forest health threat and the corrective options available.

Phase 2

Start 3-5 years prior to possible deregulation and use

3. **Review intellectual property**
   Objective: Understand which biotechnology options are available
   Intellectual Property (IP) holders are stakeholders with legal recourse based on patents they own. IP reviews, also referred to as patent landscapes, help researchers determine what direction research is moving in the field, can help identify competitors, and identify the geographic areas where research is occurring. More specifically, a detailed review of patents can be used as an examination of potential patent problems that may interfere with future research in an area and commercialization of discoveries. However, a patent landscape is not a legal opinion on freedom to operate.

4. **Assemble a biological dossier**
   Objective: Provide stakeholders a single biological information resource
   A detailed document covering both the forest health threat and the tree species in danger helps regulating agencies, stakeholders, and research scientists communicate more effectively by referring to common information. This information is very useful in making regulatory decisions and environmental assessments.

5. **Review the regulatory landscape**
   Objective: Understand the framework that regulatory agencies work within
   A review of the current state of regulations and related lawsuits that control or influence the use of biotech trees for forest health is necessary before a regulatory course can be planned. This step also helps guide scientific efforts and public interactions. In the U.S., there are three government agencies that can have jurisdiction over the development and deployment of biotech trees:
   - U.S. Department of Agriculture's (USDA) Animal Plant Health Inspection Service (APHIS)
   - U.S. Environmental Protection Agency (EPA)
   - U.S. Department of Health and Human Services' Food and Drug Administration (FDA)

Phase 3

Start 3-1 years prior to possible deregulation and use

6. **Query agencies to define a regulatory course**
   Objective: Gather specific information about the regulatory process
   In order to develop a concrete plan to use a biotech tree for forest health, routine interactions with regulatory agencies are necessary. Since regulators are mandated to respond to discrete requests, this step will involve a number of iterative questions and answers to and from the agency.

7. **Prepare an environmental report**
   Objective: Assess the risks and benefits of using the biotech tree
   The U.S. National Environmental Policy Act (NEPA) applies to all three agencies in situations that could potentially have a large effect on the environment. It is likely that NEPA will play an increasingly large role in the regulation of biotech trees in the future. Preparing an environmental report is a major step in addressing NEPA issues. In addition, this document is a useful tool to further engage regulatory and public stakeholders on project details.

8. **Interact with agencies on future regulations**
   Objective: Increase the effectiveness of regulations that deal with biotech trees
   Regulations change slowly over time while forest health threats move quickly. By and large, environmentally focused stakeholders believe that helping agencies evolve their regulations is critically important to ensure that the risks of using advanced biotechnologies are reduced, while the benefits from these technologies are increased.

This generalized policy rapid response plan was synthesized during the FHI's policy work, which is ongoing, and will likely evolve as the FHI's efforts progress. To date the process has been very iterative and is a result of many collaborators from academia, industry, government, and non-profit organizations.