Forest Health Initiative – Phase 2
Year 2 Report: January 2014 – December 2014

EXECUTIVE SUMMARY

Background:
The Forest Health Initiative (FHI) is a collaborative effort supported by the USDA Forest Service, the U.S. Endowment for Forestry and Communities, and Duke Energy, to support groundbreaking public research into the potential of using biotechnology to address forest health issues.

Originally envisioned with a challenge grant by the U.S. Endowment for Forestry and Communities, FHI is guided by a multi-stakeholder Steering Committee and supports work in a braided approach where scientific study operates openly in a collaborative effort with social/environmental and regulatory networks.

The primary purpose is to support core activities needed to promote the health and restoration of threatened forest tree species. These activities include biological science research that is creating technology platforms for genome-guided breeding and genetic engineering[1]. The activities of this biological research are reinforced by parallel activities focused on social, environmental and policy aspects of biotechnology, in order to engage broad communities of users seeking options for restoration of threatened forest tree species. The American chestnut (*Castanea dentata*) is the case study.

The FHI is a directed effort at developing and demonstrating a Rapid Response Plan for forest health issues. The RRP includes a careful analysis of the situation followed by a directed research effort that integrates work across the realm of biotechnologies, including genome sequencing and bioinformatics, population genotyping for breeding, early screening for disease resistance, micropropagation of the best genetic materials, and transformation of native genotypes with resistance genes from related and other plant species. All information generated is in the public domain and will be made readily available for reference and use.

In short, the FHI seeks to involve scientists, policymakers, and social, environmental and economic stakeholders in determining how biotechnology may be used in addressing forest health challenges.

Goals:
While the phase I work of the FHI continues to develop a plantable tree, and determining if it is appropriate from scientific, social, and regulatory standpoints, the second 3-year phase of the FHI envisions what would be necessary to develop a tree ready for planting in a forest landscape. Areas to be investigated for these landscape-ready trees include:

[1] Nelson, CD, WA Powell, CA Maynard, KM Baier, AE Newhouse, SA Merkle, CJ Nairn, L Kong, JE Carlson, C Addo-Quaye, ME Staton, FV Hebard, LL Georgi, AG Abbott, BA Olukolu. 2013. The Forest Health Initiative, American chestnut (*Castanea dentata*) as a Model for Forest Tree Restoration: Biological Research Program. Acta Hort 1019:179-189.

- Determine which disease resistance gene(s) work, leading to their optimization and improved selection.
- Determine how these gene(s) can best be used to step up to larger scale plantings while ensuring accommodation of genetic diversity.
- Work with the three regulatory agencies (APHIS, EPA, FDA) that are necessary for landscape use of the biotech trees.
- Work with restoration organizations on a plan to test and use GE American chestnut trees, creating at least 3 field tests over 3 years.
- Conduct social science research to determine the extent and conditions under which the public might support a role for biotechnology in battling threats to forest health.

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. John Davis, Professor, University of Florida

- Bill Toomey, Program Director, Forest Health Protection, The Nature Conservancy

Year 2 Accomplishments Overview:

Biological Science:

- 24 gene constructs are in line to be regenerated to whole plants from shoot cultures and/or waiting for leaf assays to be done.
- Testing methods to screen for enhanced blight resistance using tissue culture plants. This would eliminate the need for regeneration and acclimatization for less promising plants, saving 6 months or work and expense.
- 4 peer reviewed articles in scientific journals.

- Somatic seedlings representing 9 B3F3 (hybrid backcross) clones should be ready for transfer to VA Tech May 2015.
- Transgenic somatic seedlings and wildtype controls representing 21 events with 16 blight resistance candidate genes should be ready for transfer to VA Tech May 2015.
- Somatic seedlings from crosses between pure large, surviving (putatively blight resistant) American chestnuts were planted out on sites in the Jefferson and George Washington National Forests in the fall 2014.
- Chestnuts engineered with 2 different constructs with candidate Phytophthora resistance genes and transgenic control trees with 2 different reporter gene constructs were planted in Phytophthora resistance screening tubs at Joe James' Chestnut Return Farm on 7/3/14.
- New crop of transgenic chestnuts representing 9 events with 4 Phytophthora resistance candidate genes and combinations are growing in the greenhouse now.
- New approach to screening chestnut callus for Phytophthora resistance in well-plates is being tested now with Dr. Steve Jeffers (Clemson Univ.).
- Planted mix of transgenics and cloned crosses for a total of 90 trees on May 12, 2014 at the Powell River Project mine site. Recorded initial health, height, and ground line diameter.

- Weed control applied around trees at Kentland (mechanical and chemical) and the Powell River Project (mechanical).
- Recorded bud break stage approximately twice per week at Kentland farm planting until the majority of trees had leafed out.
- Collected monthly health/vigor data for all three cohorts from May to November.
- Measured end-of-growing season height and ground line diameter (GLD) for all live trees in November 2014.
- Beginning data analysis for year 2 data of cohort 1, and preliminary data analysis for year 1 of cohorts 2 and 3.
- Beginning plans for manuscript preparation.
- Presented poster on first planting cohort at the TACF annual meeting on October 18 in Front Royal, VA.

Social Science / Outreach

- Phase I (stakeholder focus groups) is complete and results were presented at the last annual meeting in Syracuse.
- Phase II (nationwide public sample) survey instruments were designed and revisions were made in response to comments from stakeholders who participated in Phase I, FHI stakeholders, and SAC members (including at the last annual meeting in Syracuse).
- An extensive pre-test (i.e., pilot test) of the Phase II instruments was conducted in Fall 2014.
- Final revisions to the Phase II survey instruments were made and the sampling design was finalized in Winter 2014.
- Data collection (i.e., survey administration) for Phase II has now begun and will continue through the first few months of 2015 (multiple mailings of survey instruments are necessary to increase response rates). Preliminary results of these Phase II surveys will be presented at the next annual meeting and in the next interim report.

- Began work on "Public Outreach Tool: Zip Code Lookup for Nearby Trees Under Health Threats" by defining and implementing software to allow priority tree species to be displayed based on a postal code input on a website or mobile device.
- Database requirements and datasets have been identified.
- The first of two database alterations have begun by developers.
- GIS technician is parsing final data set for the lookup.

Regulatory and Management

- Developed a standard movement variance for the FHI. Got approval for this variance from APHIS. This variance meets the stringent biosecurity standards APHIS requires to move living, genetically modified trees, while saving FHI members time, effort, and money whenever trees need to be moved over state lines.
- All regulatory information was put into a shared folder for all FHI Permit holders to access. This creates a common set of documents that streamlines permit applications.
- Created a process with APHIS to house master 'superset' permits with the FHI for each researcher. This reduces the time burden on individual scientists by putting the upfront agency communications primarily in the hands of the IFB to get the permit approved.
- Completed a report to be In Accordance with Responsible Use Principles that was presented to the Steering Committee at the July meeting in Syracuse.