Forest Health Initiative – Phase 2

Year 3 Report: January 2015 – December 2015

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. 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:

- 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. Carlos Rodriguez Franco, Acting 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 3 Accomplishments Overview:

<u>Biological Science:</u>

- Nine gene constructs that have been screened and culled for candidate gene expression over background expression were moved forward through shoot multiplication, rooting, acclimation, growth in the greenhouse, and leaf assays.
- Seven cisgene and one transgene events showed promising results from the leaf assays. The one transgene was stilbene synthase from grape.

- Transfer of the 32 remaining CP B3F3 somatic seedlings from cultures initiated in 2012 to TACF collaborators for planting.
- Producing somatic embryos for germination that are engineered with 9 blight-resistance and *Phytophthora*-resistance candidate genes.
- New B3F3 and transgenic somatic seedlings were produced during summer and fall 2015.
- Transgenic somatic seedlings (48) representing 13 CGs and 1 reporter construct (transgenic control) and 9 wildtype somatic seedlings were planted in the nursery at UGA's Whitehall Forest on October 16, 2015.
- Transgenic somatic seedlings (58) representing 25 events with 7 blight resistance and Phytophthora-resistance candidate genes and 3 reporter gene constructs and 23 wildtype trees from 6 clones, including 3 TACF B3F3 clones, were transferred to Virginia Tech cooperators on May 16, 2015.
- Transgenic somatic seedlings (132) with 18 blight resistance genes, Phytophthora-resistance candidate genes and reporter genes (transgenic controls) are currently growing in greenhouse and should be ready to transfer to Virginia Tech cooperators in for spring 2016 planting or to use in Phytophthora resistance screens.
- New approach to in vitro screening of chestnut clones for *Phytophthora* resistance using shoot cultures is being tested at Clemson University by Dr. Steve Jeffers.

- A total of 286 trees have been planted at Powell River and 360 at Kentland Farms.
- Due to permitting issues, planting scheduled for Kentland in spring 2015 was moved to the Powell River Project mine site. Planted mix of transgenics and pure American chestnuts for a total of 95 trees. Majority of trees planted on May 27, 2015, trees with 23RN gene planted Sep 14, 2015 after the permit was amended to include them.
- Results from Powell River include:
    - Nut-derived trees had more height growth (8.8 ± 5.8 cm) in the third growing season than SE-derived trees which, on average, lost height (-31.7 ± 10.9 cm) ($p=0.008$). There were no significant differences in total or year 3 height growth among genotypes, likely because of the large difference between the SE-derived and nut-derived trees within genotypes.
    - On average, ground line diameter (GLD) increased over the entire experiment after all three years (0.97 ± 0.20 cm) and over the third growing season (0.29 ± 0.16 cm).
    - Several trees were observed to exhibit blight symptoms, but thus far there are no significant effects of genotype or method of production (nut-derived or SE-derived) on blight incidence. While there are no significant differences among constructs yet, it is notable that none of the surviving Cyst1, ETF, or TAGL exhibited any symptoms of blight, particularly since the TAGL trees were among the best survivors of the cohort.
    - The difference between the occurrence of blight symptoms in non-transgenic and transgenic trees was significant. Overall, 86.4 % of non-transgenic trees in this cohort have some symptoms of blight, compared to 51.5% in the transgenic trees.
    - Trees and associated soil samples from the Powell River Project tested positive for *Phytopthera cinnamomi*.

- Results from Kentland Farms include:
    - SE-derived trees are surviving slightly better after two full years (51.8% vs. 42.8%).
    - Tissue-derived Graves trees grew the most, followed by nut-derived trees, which on average died back in the previous growing season.
    - One Graves nut-derived and one SE-derived tree also showed symptoms of blight.
    - None of the plant or soil samples collected in spring 2015 from Kentland Farm tested positive for Phytopthera.

    - In spite of poor soil, the Powell River Project site is amenable to restoration with transgenic chestnut; with excellent survival/growth and very little evidence of cankers on transgenic plants.
- One particular transgene (CBS) resulted in prodigious flowering. Continued monitoring is necessary to determine if this is a direct effect of the transgene or perhaps a side-effect of plant health/vigor coming out of the greenhouse.

<u>Social Science / Outreach</u>

- A large random and representative nationwide survey of the U.S. public, as well as additional stakeholders and interest groups consisting of government agency representatives, university scientists, members of nongovernmental organizations, and representatives of private forest-based companies (i.e., experts) has been completed.
- Some highlights of major results include:

- In total, 89% of the public and 72% of experts perceive of chestnut blight as a moderate or extreme threat to the health of forests, and 72% of the public and 66% of experts consider climate change a moderate or extreme threat.
- A large majority of the public (82%) and experts (77%) believe it is important to restore American chestnut trees to their native range.
- More than 60% of the public and 75% of experts believe that humans should find ways to change trees so they are no longer affected by chestnut blight. Likewise, only 10% of the public and 3% of experts believe that humans should take no action and let chestnut blight take its natural course.
- The majority of the public (58%) and experts (81%) would vote in favor of using genetic modification to address chestnut blight. There was more support for using genetic modification to address chestnut blight than for addressing climate change (51% public, 55% experts) and increasing forest growth for commercial purposes (49% public, 62% experts).
- The majority of the public (58%) and 84% of experts would vote in support of changing genes in American chestnut trees to help them resist chestnut blight. In addition, 55% of the public and 70% of experts would vote in support of adding a gene from wheat (i.e., OxO gene) to help trees resist chestnut blight.
- The majority of the public was not, however, supportive of breeding American chestnut trees with chestnut trees from Asia (45% vote in support) or adding genes from other distantly related organisms (42%) to help trees resist blight, even though these approaches were supported by the majority of experts (82% and 67%, respectively).

- Continued 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.
- Required datasets that match tree species with geographic area have been developed.
- The first of two database alterations have begun by developers.
- GIS technician is parsing final data set for the lookup.
- Developers are working on the API requirements and coding for database searches.

Regulatory and Management

- Complete ongoing reports and paperwork for field trial permits, material movement, planting reports and permit amendments.
- Developed an approved regulatory and communications plan for 2015.
- Webinar given at APHIS-BRS, March 26, 2015 on US and International regulatory policies regarding GE trees and how those policies might evolve. This was an internal meeting with 20 attendees in person and 20 remotely.
- Prepared a Study Statement of Task for a National Academy of Science study to Examine the Potential for Biotechnology to Address Forest Health .
- Presented the study to the Board on Agriculture and Natural Resources at their meeting on Dec 9, 2015. The study was positively received and further coordination with NAS is underway to move this idea forward in the process.
- Complete overhaul of FHI website – 70% complete.