Towards a forest health paradigm based on host genetics and participatory breeding

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Invasive pests and pathogens cause extensive damage to native forest trees and the ecosystems that depend on them and, given the continuing increase in globalization and mean global temperature, introductions and spread of destructive invasive species are predicted to increase. To address these issues, the newly formed Forest Health Research and Education Center (FHC) at the University of Kentucky is focused on developing host resistance in forest trees and understanding the broader impacts of forest health issues on society. As is well known, the longstanding issues related to working on the genetics of forest trees presents a large challenge to breeding host resistance. However, they are no excuse to opt out and depend on shorter-term, less reliable or less environmentally friendly options. Instead, we argue that the long-term, proactive development of genetic, genomic and biotech resources in foundational forest tree species is critical to the implementation of host resistance when and if pest and pathogen problems arise. In addition, to develop these resources and implement these breeding programs over range-wide spatial- and multi-generational time-scales, participatory research networks will be required. We will discuss these concepts in relation to the ongoing work of the FHC.

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Outline

- Tree Genetics as a tool in Forest Health
  - how we can use genetics more efficiently and effectively
    - a lot of species needed
      - tough budget environment
    - invasive pests, climate change
- Forest Health Initiative– chestnut project
    - www.ForestHealthInitiative.org
  - approach, results, status
- Forest Health Research and Education Center
    - www.ForestHealthCenter.org
  - approach, status

Need for rapid response with tree genetics

- Typically tried last with forest trees, even though this can be seen as the best alternative
  - Why?
    - Potential faster fixes are tried first
    - Genetics seen as very long term, which is basically true
    - Why?
      - Trees have long generation times, can be difficult for genetics and breeding
      - Trees are usually difficult to vegetatively propagate, making clonal testing difficult
      - Genetics programs are not well organized and connected--usually due to lapses in funding

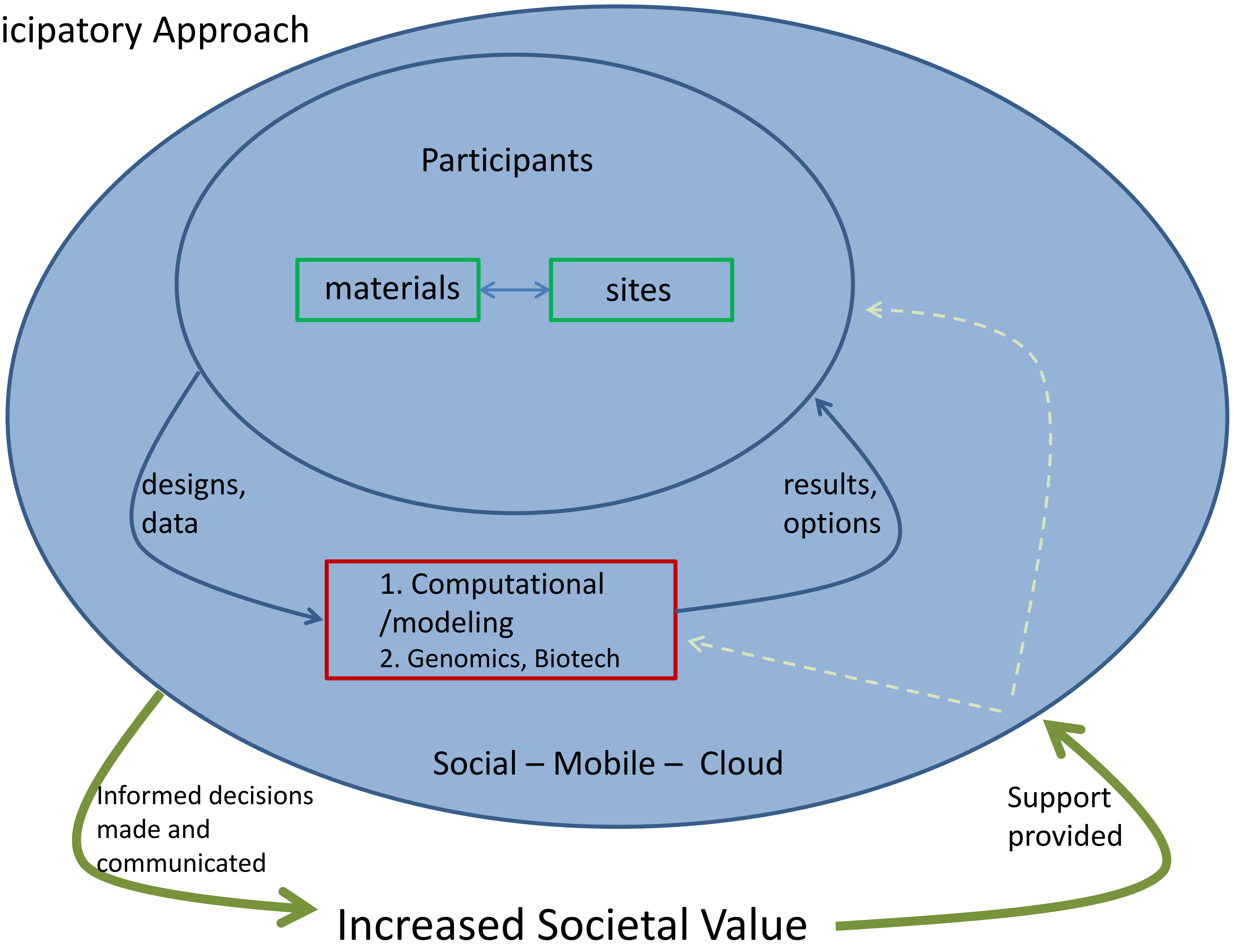
Doesn't need to be the case

- Tree resistance is often the best, sustainable option
- Need to maintain capacity to respond
  - Readily available germplasm
  - Trained personnel and interested citizens
  - Ongoing tools and approaches developments
- Ramp-up effort early, starting with some capacity
- Distribute work over a network of participants

Time for new approach

- Maintain genetics network for important forest tree species
- Each node participates with species in their local area
  - Nodes are made up of one or more professionals and students, and interested citizens (participatory tree improvement)
  - Nodes collect, evaluate and maintain germplasm
    - Local source, plus some more distant sources
      - an ‘incomplete block’, with composite provenancing
    - Several nodes (networked) provide a complete-enough block

A Participatory Approach



No-analog Physical and Fiscal Environments require

- Diverse germplasm (species, genotypes)
- Testing and Selecting in many, diverse physical environments over time
- Inexpensive or highly efficient methods to buffer against fiscal variability

Additional Alternative Breeding Approaches

- Participatory Tree Improvement (PTI)
- No FS-crosses Selection (e.g., BwB)
  - Milan Lstiburek’s excellent talk earlier today
- Rapid-cycle Breeding (e.g., FasTrack)

Breeding Approaches

- Accelerated or Rapid-cycle breeding
  - Induce early flowering to shorten generation time
    - Cultural treatments– GA4/7, glasshouse, top-grafting
    - Transgenic– premature activation of flowering genes (i.e., FasTrack Breeding)

FasTrack (early, continuous flowering) Plum

Breeding Approaches

- FasTrack breeding
  - Early and continuous flowerings possible
    - Need to optimize plant size and flower induction
  - Requires transformation and propagation of the transformants
    - Native plant virus may be used as transgene vector
  - Transgene is removed by selection
  - Early genetic evaluation needed
    - QTL for simple traits
    - Genomic Selection (GS) for complex traits

Part 1-- Summary and Conclusions

- Biodiversity is key to forest and human health
  - Assisting biodiversity through tree genetic resource management (conservation and improvement) is a critical human endeavor
- Innovative approaches are needed in Tree Breeding to accomplish this endeavor
  - Low cost genome technologies along with social and mobile technologies offer great opportunities for innovation
- Our further challenge is to commit/re-commit to making a stronger case for managing/developing tree genetic resources

Forest Health Initiative (FHI)

www.foresthealthinitiative.org

Forest Health Initiative
Advancing Forest Health through Biotechnology

- FHI was/is a new approach– focused on the premise that genomics and biotechnology can rapidly identify and develop resistance, but this must be done in the current social context
  - 1. can biotech (especially GMOs, when they're the 'best' possible or only option) be accepted (and de-regulated) in forest health situations?
  - 2. if not, why?, if so, under what conditions?

"One of the anomalies of modern ecology is the creation of two groups, each of which seems barely aware of the existence of the other. One studies the human community, almost as if it were a separate entity, and calls its findings sociology, economics and history. The other studies the plant and animal community and comfortably relegates the hodge-podge of politics to the liberal arts. The inevitable fusion of these two lines of thought will constitute the outstanding advance of the present century." ***Aldo Leopold, 1935.***

FHI-- chestnut

- In second 3-yr phase, utilizing American chestnut as a model for the FHI approach
- In first phase
  - Genetic engineering approach pursued (SUNY, UGA)
  - Genome mapping and sequencing pursued (Penn State, USFS, Clemson, TACF)
  - Both efforts integrated over the web through bioinformatics (Clemson, Penn State)
- In second phase
  - Field testing of best lines (GM Am chestnut, cloned backcross chestnuts)

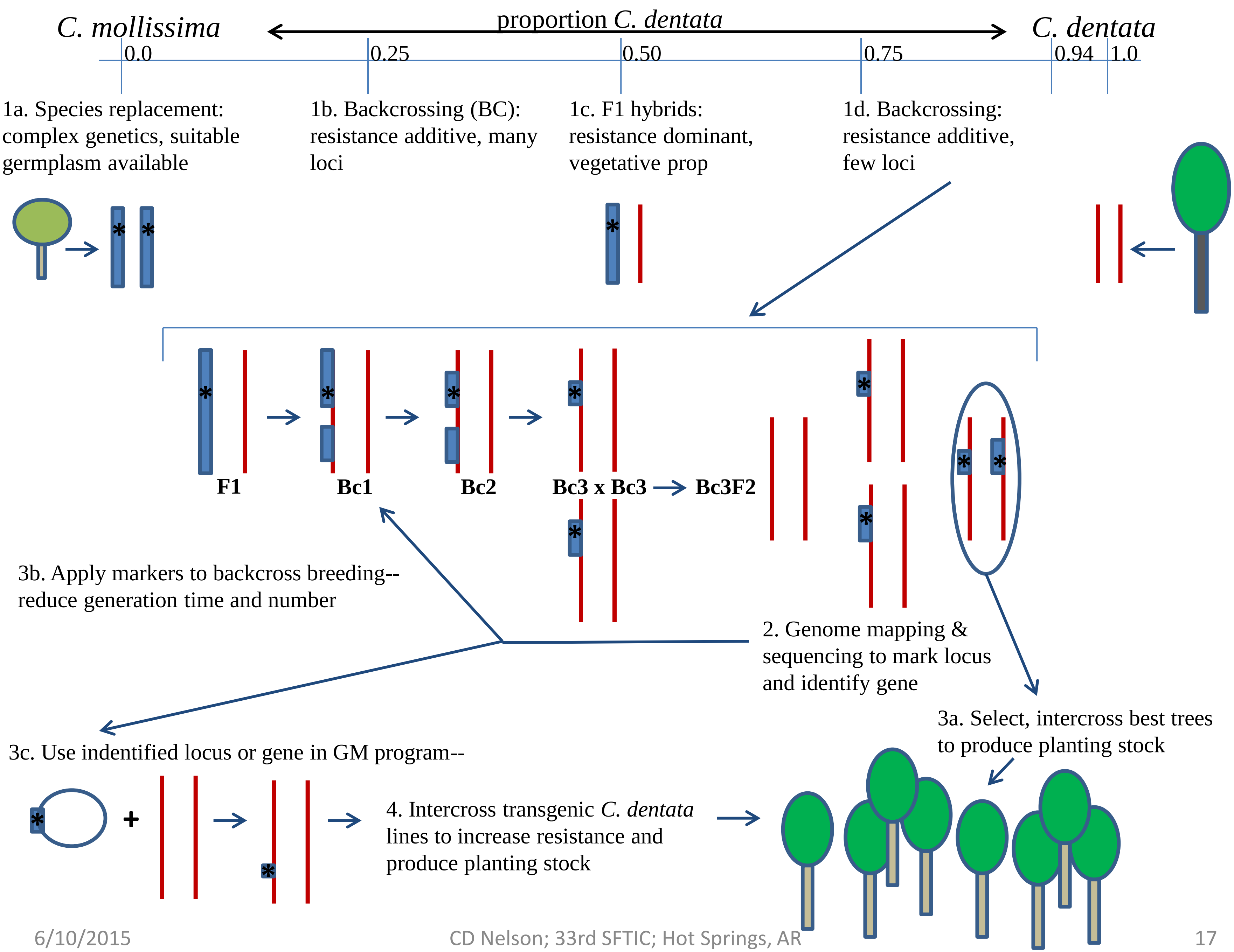


Fig. 5 in Jacobs, Dalgleish, Nelson. 2013. New Phytologist.

FHI-- chestnut

- Social sciences
  - Road map– a decision-tree for prioritizing approaches (from do-nothing to GM)
    - Advisory board activity
    - Included wide-array of interests
  - Survey of opinions on GM in forestry and restoration
    - Oregon State University and others

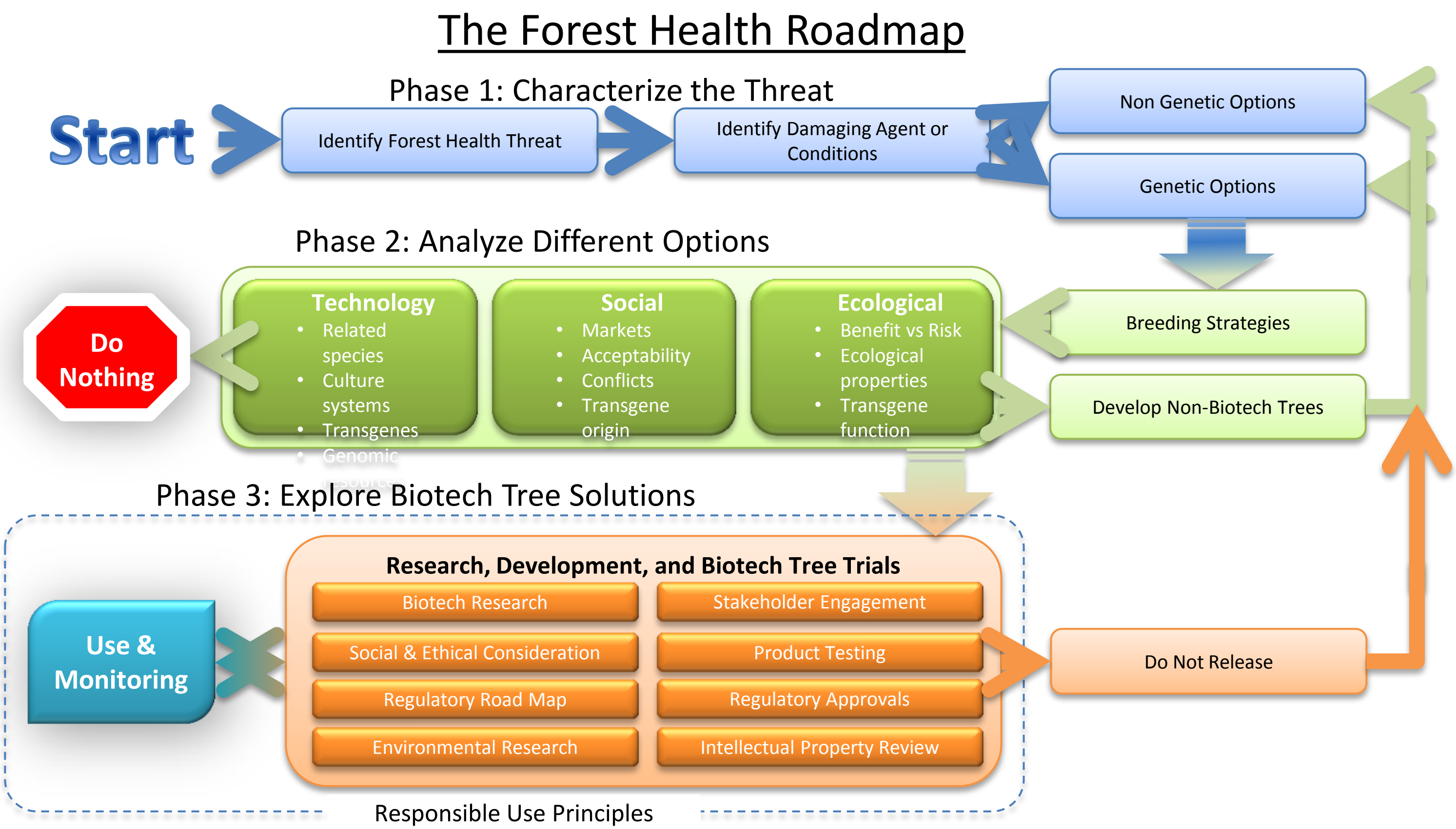
Exploring Biotechnology to Protect Forest Health

Susan McCord, Executive Director (susan.mccord@forestbiotech.org) & Adam Costanza, President (adam.costanza@forestbiotech.org) – Institute of Forest Biotechnology, North Carolina, U.S.A.

About the Forest Health Initiative – ForestHealthInitiative.org

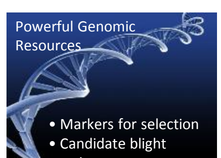
The Forest Health Initiative (FHI) is a collaborative effort to fully explore the many scientific, environmental, social, and regulatory challenges surrounding the use of biotechnology to protect natural forests by developing a test tree that responds to an existing forest threat. The initiative is supporting work to revive the American chestnut with genome-informed bred and engineered varieties modified with blight resistance enhancing genes from a closely related species, the Chinese chestnut. This tree was chosen because of the available data on the genetics of blight resistance and active transformation and breeding programs.

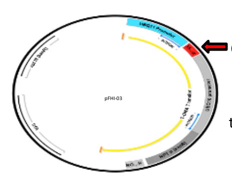
The Forest Health Roadmap



Exploring Biotechnology Tools

One of the most important criteria in effectively preserving forest health is speed. FHI's focus on biotechnology is driven by the need for new tools to fight a growing number of pests, diseases, and pollutants that each year degrade or destroy millions of acres of native forests. FHI's work products are all in the public domain and are intended for social benefit.





Candidate Gene
31 Candidate Genes have been cloned and transformed using this vector.

FHI Working Groups

Science group: Implementing genomic and biotech research strategies to produce and test blight resistant American chestnuts.

Social/Environmental group: Creating a stakeholder driven process that identifies risks, benefits, and options for biotech and non-biotech approaches.

Policy group: Maintaining strict regulatory compliance while interacting with agencies to help guide initiative projects.

Initiative Sponsors: U.S. Endowment for Forestry and Communities; Duke Energy
Project Secretariat: Institute of Forest Biotechnology — Science • Dialogue • Stewardship

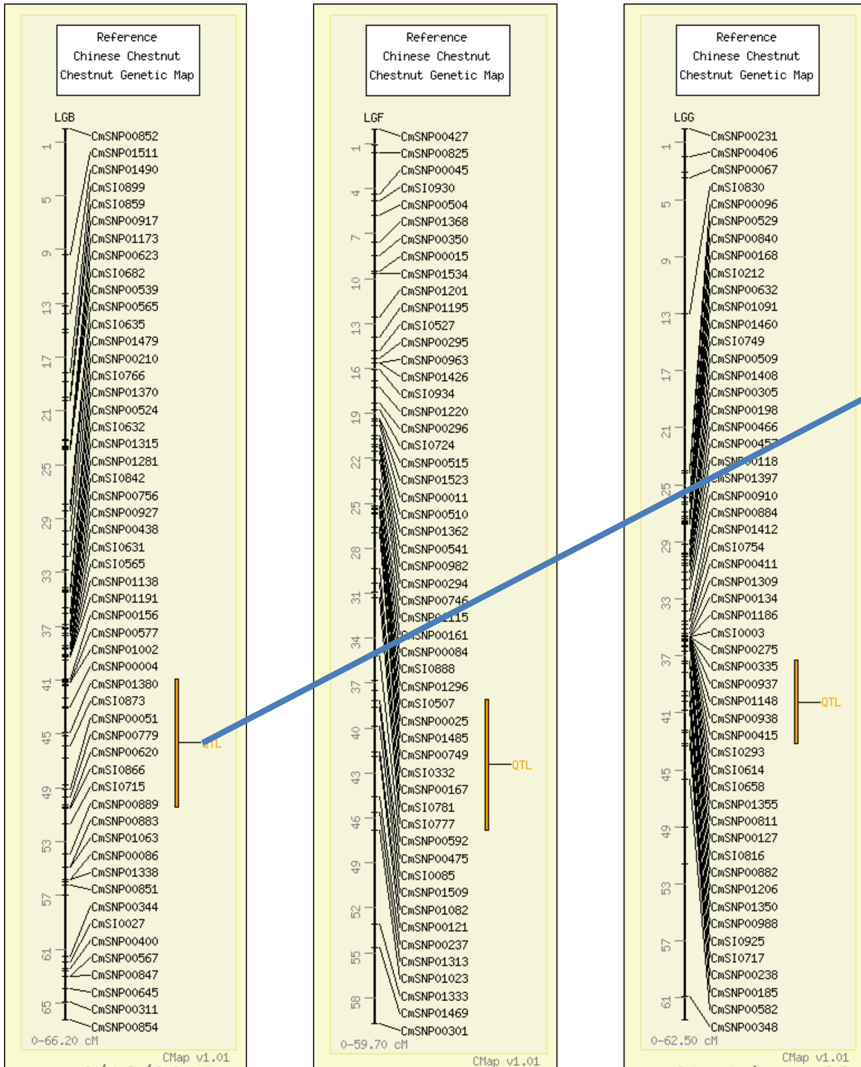
FHI-- chestnut, biological sciences

- Much progress in GM line development at SUNY
  - Some lines using genes discovered in mapping/sequencing Ch chestnut genome
  - **Most promising lines to date use oxalate oxidase gene from wheat** (having better disease defense properties than related genes in chestnut)
- Much progress in clonal development of backcross (BC) lines by UGA and TACF
- Candidate genes for blight and root rot resistances identified
  - DNA markers for marker-assisted breeding in development by TACF, VaTech, USFS, Clemson
  - Genes being tested (lab, GH, field) in GM trees by SUNY, UGA, VaTech

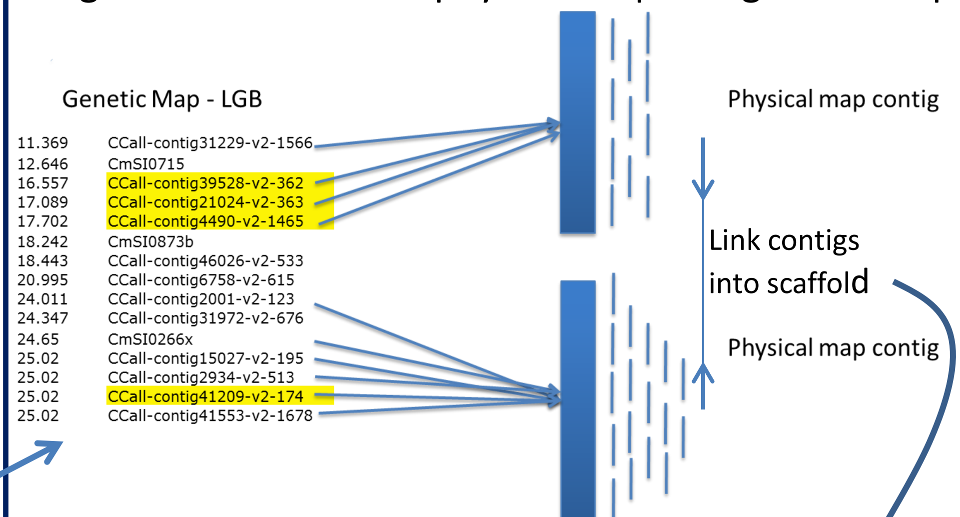
Chinese chestnut: integrated mapping; blight resistance QTL sequencing

3 Linkage groups with blight-resistance QTL:

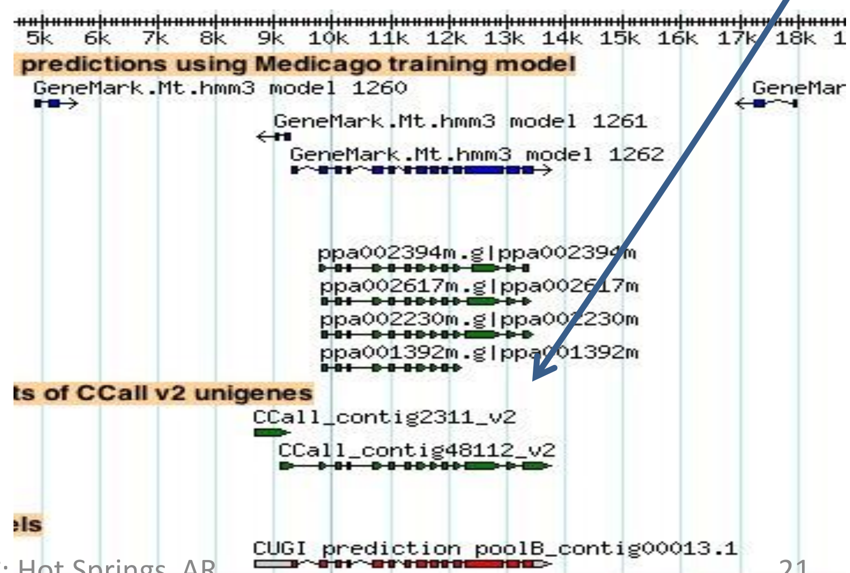
QTL cbr1 on LG_B | QTL cbr2 on LG_F | QTL cbr3 on LG_G



Alignment of QTL *Cbr*1 physical map and genetic map



Genome browser for assembled QTL:



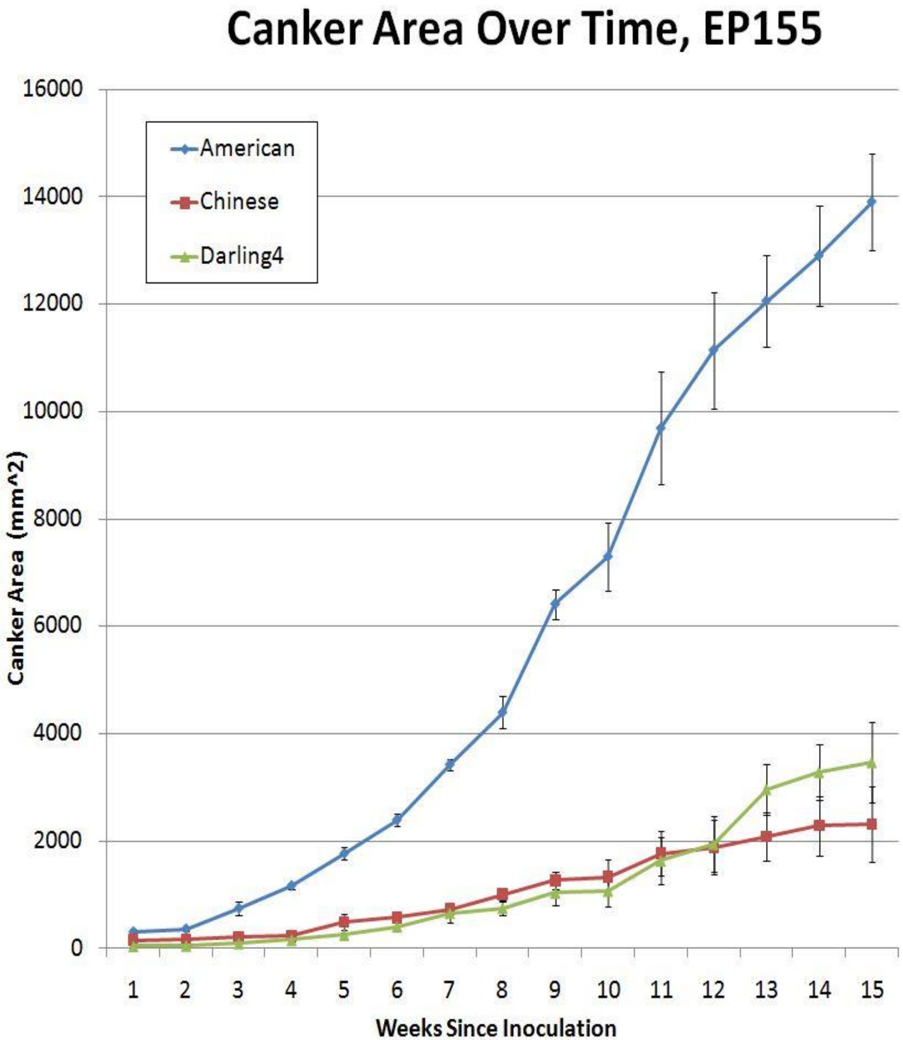
(Nelson, Carlson, Staton-- FHI)

FHI: Candidate Gene List (1/3)

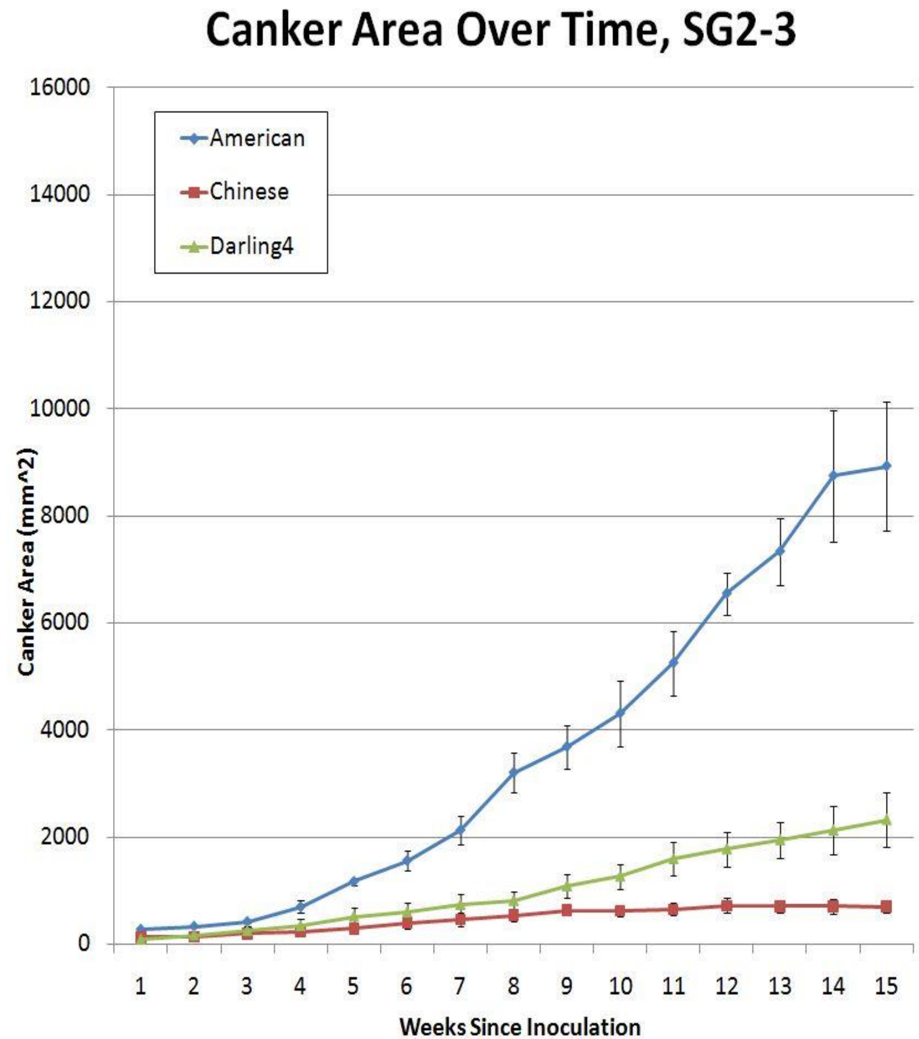
order	CCcontig	Uniprot_BestHit	Linkage_Group	cDNA_status	BinaryVector	BV_status	TransPipe
1	CCall-contig8901_v2	**beta-1 3 glucanase**	?	Cloned&sent	pFHI-B13Gluc	received	SUNY-ESF
2	CCall-contig2586_v2	**CBS domain protein**	?	Cloned&sent	pFHI-CBS1	received	SUNY-ESF
3	CCall-contig11269_v2	**UDP glucosyltransferase**	B, G	Cloned&sent	pFHI-UDP	received	UGA
4	CCall-contig8443_v2	**Thaumatin-like protein**	G	Cloned&sent	pFHI-Thau	received	UGA
5	CCall-contig9278_v2	**DAHP synthase (DHS1)**	G	Cloned&sent	pFHI-DAHP	received	SUNY-ESF
6	CCall-contig8996_v2	**Acid phosphatase**	G	Cloned&sent	pFHI-AcPhos	received	UGA & SUNY-ESF
7	CC454-contig42836_v2	**Laccase/diphenol oxidase**	B	Cloned&sent	pFHI-Lac1	received	SUNY-ESF
8	CCall-contig18406_v2	**Proline-rich protein**	G	Cloned&sent	pFHI-PRP1	received	SUNY-ESF
9	CCall-contig19527_v2	**Ethylene-response transcription factor**	F	Cloned&sent	working	waiting	for UGA
10	CC454_contig2466_v2	**Unknown function**	E	working	working	waiting	TBD
11	CCall_contig39658_v2	**Lipid transfer protein (LTP)/proteinase inhibitor**	G	working	working	waiting	TBD
12	CCall_contig_2055_v2	**Lipid transfer protein SSH**	?	Cloned&sent	working	waiting	TBD
13	CCall_contig4992_v2	**Cysteine proteinase inhibitor**	E	Cloned&sent	working	waiting	TBD
14	CC454_contig41915_v2	**Allene oxide cyclase (AOC)**	?	working	working	waiting	TBD

Field assays of Darling 4 & controls demonstrating enhanced blight resistance

(data from W. Powell, SUNY)



n=4-5 trees per type, error bars = +/- 1 standard error of the mean



n=4 trees per type, error bars = +/- 1 standard error of the mean

(Powell Lab, SUNY)

Involving the Public in Planting Transgenic American Chestnuts (SUNY-ESF)

Issues-- chestnut

- Candidate gene testing– generally slow and expensive
  - Many screens along the way to GM field tests; regulated by APHIS (and EPA and FDA)
  - Early Resistance Assays (leaf, small stem) improve this situation
- Successful genes need to be bred into adapted, diverse germplasm before release
  - Could go relatively faster than BC breeding, because it can be completed in fewer generations
    - but then you have resistant tree increase, planting stock production, and chestnut-based silviculture
- BC breeding with DNA marker information is a good approach, but still long-term
  - Test current B3F2 parents for presence of Ch resistance genes
  - Could add additional Ch resistant sources in relatively less time

Part 2-- FHI Conclusions

- FHI hypothesis is tentatively accepted
  - 'plantable trees' have been achieved in 3 years
  - much knowledge and infrastructure developed
- Continuing work will
  - evaluate the new materials under field conditions
  - complete and fully integrate sequencing & mapping
  - apply mapping resources to breeding programs
- **Adapt FHI process and apply to new species**

New York Botanical Garden Planting

(18 April 2012, W. Powell and C. Maynard, SUNY-ESF)

Part 3-- Road Map for Future

- Learn from past
  - Make tree genetics an ongoing priority
    - Decentralize the activity to provide sustainability
    - Network the activity to provide stability & community
- Proposal for a new center dedicated to such
  - Forest Health Research & Education Center (FHC)
    - USDA Forest Service– Southern Research Station, University of Kentucky (Forestry, CAFE), Kentucky Division of Forestry

FHC Vision

- Virtual center w/many Research and Education partners focused on solving the most pressing forest health problems
  - located at the University of Kentucky-- integrated Biological & Social Sciences Research program with Outreach & Education program
- Research
  - Biology
    - Genetics/physiology of resistance to stress (biotic and abiotic)
    - Management options, including breeding and biotechnology
  - Sociology
    - Human dimensions of new technology in forest management
      - Genomics and biotechnology to conserve and restore
      - Participatory research & development
- Education
  - Teach/impact forest land owners, managers and policy makers
    - through Development and Outreach of participatory programs

Forest Health Research and Education Center (FHC)

- Organizing/Managing Partners
  - USDA Forest Service, Southern Research Station (SRS)
    - Dana Nelson (SRS)
  - University of Kentucky, College of Agriculture (Forestry, Plant Sciences, Entomology, Plant Pathology, Horticulture, Ag Economics)
    - Red Baker (Forestry); Bert Abbott (Biological Science Team Lead); Andrew Stainback (Social Science Team Lead); Jeff Stringer (Outreach Team Lead)
  - Kentucky Division of Forestry (KDF)
    - Leah MacSwords, Chair of Advisory Board
      - comprised of constituent partners (open to all interested parties)
- Three post-docs funded and starting now
  - Anna Conrad (Biological Sciences); Xiaoshu Li (Social Sciences); Ellen Crocker (Education/Outreach)

Forest Health & Restoration Research & Education Center

Mission: Develop and transfer science and technology to enhance tree health and forest restoration

Research

Understand social dimensions of applications

Education

Resistance and tolerance to abiotic and biotic stress; Adapted populations for forest restoration

Teach landowners/managers, policy makers; Improved forest management, policy

Development & Outreach

Extend research & education to the field

Improved tree performance under stress
Improved tree populations for forestry and horticulture
Improved adoption of new technologies and best management practices

www.ForestHealthCenter.org