“You can’t learn to swim unless you are willing to jump into the water.”

Preparing for first of many regulatory reviews of blight resistant American Chestnut Trees

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Who will be the partners?
Forest Health Initiative,
The American Chestnut Foundation,
NYS American Chestnut Research & Restoration Project at SUNY-ESF
And likely others.
Overview of Presentation

• What is the regulatory context with respect to chestnut restoration
• Why use the oxalate detoxifying enzyme for chestnut blight resistance?
  – Pros and cons
• Engage Public & Stakeholders
• Who do we work with & steps toward non-regulated status
• Additional uses of a deregulated tree
Genetically Modified Chestnut trees and exotic chestnut trees are being planted

- Exotic chestnut trees
  - *C. mollissima* (China), *C. crenata* (Japan), & *C. sativa* (Europe)
    - Not adapted to our forests

- Hybrid chestnut trees
  - Various crosses of 5 species from around the world
  - *C. dentata, C. mollissima, C. crenata, C. sativa, and C. seguinii*

- X-ray and Gamma ray radiation bred chestnuts starting back in 1955

- Transgenics can’t be considered in a vacuum
  - The scientific consensus is that genetic engineering is as safe (and sometimes safer) as traditional breeding

- **People should have choices on which to plant**
Why use the oxalate detoxifying enzyme as the first regulatory test case?

• Most effective resistance enhancing gene in American chestnut to date
  – Dominant resistance
  – Can be used to rescue the American chestnut population’s genetic diversity still surviving today

• The OxO gene has value in itself to promote world food security
  – Can enhance fungal resistance in many crops
  – Regulatory cost preventing development
Products approved – Companies vs. Universities

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<th>Company Developer</th>
<th># Products</th>
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<td>USDA/ARS</td>
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Total 106

Cost & effort stop universities and not-for-profit organizations

Slide prepared by ArborGen LLC
Environmental studies to date show transgenic American chestnuts are promising and **support that deregulation is a “safe” path forward**

**USDA NIFA Biotechnology Risk Assessment Grants ($880K)**

**Comparing “worse case scenario available” transgenic events to traditional breeding**

- **Collaborators at SUNY College of Environmental Science & Forestry:**
  - **Dr. Parry** – Entomologist
  - **Dr. Briggs** – Forest soils, Silviculture
  - **Dr. Nowak** - Vegetation Management, Silviculture and Forest Ecology, Production Ecology and Plant Ecophysiology, Invasive Exotic Plant Control, Biogeography and Cultural Landscapes, Sustainable Management and Certification Systems
  - **Dr. Horton** – Environmental Mycologist, Mycorrhizal Ecologist
  - **Dr. Leopold** – Plant Ecologist, Dendrologist
  - **Dr. Maynard** – Woody plant tissue culture, genetic engineering a blight-resistant American chestnut, conventional forest genetics & tree improvement, forest ecology, forest health, restoration ecology
  - **Dr. Powell** – Molecular Biology, Plant Pathology, Forest Biotechnology
- **Collaborators outside SUNY ESF**
  - **Dr. Tschaplinski** (Oak Ridge National Labs) – metabolomics.
  - **Dr. Sweeney** (Stroud Water Research Center) - the role of streamside forests in the structure and function of stream and river ecosystems.
Possible drawbacks know from breeding?

• We know from classical hybrid breeding examples in chestnut that problems can arise.
  – Sterility (usually male)
  – Internal Kernel Breakdown
  – Dwarf growth
  – Mixed traits from different species
  – New unexpected traits (example tissue culture requirements change)

• None of these have been seen in the transgenic events
  – Less likely because making smaller changes
  – Solved the same way as with breeding, just pick a different offspring (event) to move forward
Possible drawbacks specific to the oxalate detoxifying enzyme?

- In transgenic sunflower (Hu et al., 2003)
  - One of three events hypersensitive response-like lesion mimicry
    - Browning on leaves as if fighting of a disease
  - Only in the highest OxO expressing event
  - Other two were normal
- Not reported in other transgenic plants expressing OxO
- Have not been observed in our American chestnut events
- But likely there is a “Goldie Locks” optimum level of expression we should obtain
  - High resistance but no hypersensitive response-like lesion mimicry
- Could be controlled by regulated promoters
  - Producing wound-inducible promoter events for testing
What about possible insertion effects?

Also occur spontaneously and in traditional breeding:

“Genetic changes similar to insertional effects occur in plants, namely as a result of the movement of transposable elements, the repair of double-strand breaks by non-homologous end-joining, and the intracellular transfer of organelle DNA.”

'Darling B58' event insertion site
(cloned & sequenced flanking DNA and searched genomic database)

Location: single insert, CC scaffold 10296 (20Kb)  
– John Carlson’s lab

No endogenous gene interruption

>10.9Kb to next gene  
5.5Kb to nearest gene

No significant change in flanking gene expression.

Note: Chinese chestnut allele would cause significant changes.
Who do we work with?

• **US Environmental Protection Agency**
• **US Department of Agriculture APHIS/Biotechnology Regulatory Services (BRS)**
• **US Food & Drug Administration**
• **US Forest Service (USFS, under USDA – advisory role)**
• **US Fish and Wildlife Service (FWS, under Department of the Interior – advisory role)**

- Bipartisan political help at state and federal levels
- Encouraging public & stakeholder support and participation
- Encourage support from select environmental organizations
Public Outreach & Education 2014-2015

• 34 public presentations & continuing (my team and me)
  – Project Learning Tree
    • Environment & conservation curriculum K-12
  – ReLeaf Conference
    • NY Dept of Environmental Conservation
  – Ozark Chinquapin Foundation
  – NYDEC Indian Nations Conference
    • Haudenosaunee (Iroquois)

• 39 popular press articles & continuing
  – Including Scientific American, Poplar Science, etc.

• Help from biotech outreach organizations
  – Biotechnology Learning Project
  – Alliance for Science
  – Possible documentaries

• Graduate student teaching in STEM program
• SUNY-ESF President, Quentin Wheeler, and Dept Chair, Don Leopold, actively presenting the chestnut projects in their talks
30 day crowd funding campaign
Goal: $50K, but raised over $104K: 553 donors, 719 supporters
Donations from 48 states & 6 countries (Brazil, Canada, Germany, The Netherlands, New Zealand, and Portugal)
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• Fees
  – Registration & tolerance exemption (PRIA B820): estimated $303,878
  – If SAP review is triggered: estimated $364,653
  – But, if the USDA IR-4 Project submits the registration & petitions for us, there will be no charges

• Continuing fees ~$3,200/yr, maybe forever
  – Exemption or rule change?
  – U.S. Forest Service holds license?
    • Or U.S. Fish & Wildlife Service
  – TACF holds license?
  – Establish new 501-3C to hold license?
  – SUNY ESF holds license?
Step 1: regulatory review

- Choose lead event(s)
- ‘Darling B58’
  - High resistance
  - Single insertion
  - Only two genes: Oxo & selectable marker, NPT2

- Choose a “target” date for submission
  - Optimally, submit to EPA, USDA, and FDA at same time

- Nov. or Dec. 2015 submission target
  - Depending on data accumulation
  - Back-up date: Nov. or Dec. 2016

- Keep the public engaged through all steps
  - Maintain transparency (website, twitter, facebook, TACF newsletters, public presentations, news releases.)
Estimate the review to take 5 years
Deregulation by BRS – public access

• 1996 Virus resistant Papaya (Hawaii)
  – Decision in 198 days (~6.5 months)

• 2007 Virus resistant Plum
  – Decision in 1010 days (~2.8 years)

• 2009 Virus resistant Papaya (Florida)
  – Decision in 1734 days (~4.75 years)

• 2015 Non-browning Apple
  – Decision in 1714 days (~4.7 years)

Citrus greening resistant orange – predicted 4 years

Slide prepared by ArborGen LLC
Step 2

- Write draft application
  - Core draft, and then...
  - 3 formats – EPA, USDA, and FDA

- Write petition for EPA tolerance exemption
  - Model after Citrus greening orange exemption

- In house review to find where data is missing
  - Do additional experiments to fill in deficiencies

- Submit only after all data is in place
Key points supporting exemption
EPA tolerance exemption

• OxO is **not a pesticide**
  – Detoxifies the pathogen’s oxalate into \( \text{H}_2\text{O}_2 \) and \( \text{CO}_2 \) which can be used by the plant
  – Disarming the pathogen, changing lifestyle to a saprophyte as seen on oak trees
  – Would be considered a **Plant Incorporated Protectant**

• OxO is **not a known allergen**
  – Allergen Online database searches
  – Negative with 80 mer search (Standard precautionary)
  – Negative with 8 mer search (Most precautionary)
Key points supporting exemption
EPA tolerance exemption (2)

• OxO is not a gluten protein
  – Often asked because it comes from wheat
  – Negative results from Celiac Disease Novel Protein Risk Assessment Tool

• OxO is not a toxin
  – in fact it *detoxifies* a known toxin, oxalate
  – Negative results on Toxin & Toxin Target Database
Key points supporting exemption
EPA tolerance exemption (3)

• OxO is safely eaten by billions of people and pets worldwide in wheat
  – exactly same enzyme

• OxO is a common enzyme found in many edible plants
  – All cereal crops (wheat, rice, corn, barley, sorghum, etc.)
  – Many other plants (strawberry, banana, peanut, azalea, tomato, cacao, potato, apricot, pea, dates, oil palm, beet, arabidopsis, Costus pictus (Insulin plant)
  – Therefore people eat orthologs of this gene and enzyme all the time
Key points supporting exemption
EPA tolerance exemption (4)

• OxO will be consumed in lower quantities from chestnut than from other plant sources
  – Wheat consumption in the U.S. has fluctuated over the past century between **110 and 225 pounds** per capita per year
  – chestnut consumption in U.S. is **0.1 pounds** per capita
  – Korea has the highest consumption per capita at 4.0 pounds

• OxO does not persist in the environment
  – In leaf litter activity is lost when leaf dies
Step 3

• Work with the regulators as the review progresses
• Keep the public engaged
• Work on next generation events
  – Changing promoters, stacking resistance, add Phytophthora resistance
    • Small changes can be made as amendments to registration
    • Large changes will require more review, but still easier the second time
Questions?

“For myself I am an optimist - it does not seem to be much use being anything else”

Winston Churchill

Large spreading American chestnut tree in MI, 1980’s by Alan D. Hart
In addition to direct restoration, what else can be done with a dominant blight resistance gene such as OxO?

Rescue of the surviving genetic diversity & aid in breeding
Mother tree project Allen Nichols (TACFNY) & outcrossing to surviving trees to rescue genotypes

Transgenic American chestnut

Regionally adapted

Seed

Genotypes ½ mother & ½ father

Continue to maximize out-crossing

Or surviving wild population

Offspring 50% OxO resistance

TACFNY “Mother” Trees

Pollen

50%
Using hemizygous resistance to add surviving genotypes to the backcross program (Kim Steiner idea)

Genotypes
½ mother & ½ father

Follow gene markers

Transgenic American Chestnut With rescued genotype

B3F4

seed

+ OxO

Resistant heterozygous

Non-transgenic

- OxO

- OxO

Offspring 50% OxO resistance
Example of maintaining resistance when crossing to stack genes
B3F3 x intermediate resistant transgenic

Leaf Assay Results (SG2-3)

- **Transgenic**
- **American**
- **Chinese**
- **WB**

Error bars represent +/- 1 Standard Error of the mean.

Parents

Offspring
Pro Bono Advice to date
(bold most frequent)

- John Dougherty (TACFNY Science Advisor)
- Val Giddings (Senior Fellow at Information Technology & Innovation Foundation)
- John French (retired EPA, ESF Alum)
- Michael Braverman (USDA IR-4 project)
- Ralph Scorza – USDA Honey Sweet plum
- Rick Tinsworth (Reg. Consultant)
- Phil Hutton (Reg. Consultant)
- Vicky Foster (Reg. Consultant – Orange, Citrus Greening Resistance)
- Robin King (IR-4)
- Ian Nadar (retired Plant Pathologist – Canada connections)
- Dave Lee (Bio – Biotech Industry Org)
- And others
Talking with regulators to gather information & prepare for eventual regulatory review

- 6/14/13: Poster at Biotechnology Risk Assessment Grant (BRAG) Project director’s meeting (with USDA, EPA, and FDA regulators)
- In the fall 2013, we were told by the FHI that they preferred the cisgenes and we would have to go on our own with the OxO gene
- 1/10/14: Washington DC meeting: USDA APHIS BRS representatives
- 6/5/14: Presentation at BRAG Project director’s meeting (with USDA, EPA, and FDA regulators)
- 6/6/14: Presentation and meeting with EPA representatives in Washington, DC
- 3/11/14: Conference call with EPA representatives
- 9/10/14: **USDA IR4 project**, Biopesticide workshop, **American chestnut selected as priority**
- 5/9/15: Teleconference with representatives from USDA, EPA, and FDA
EPA asked if there was an easy identifier...
Yes!
Quick screen for OxO gene

Make into a simple screening kit. Use for testing OxO persistence. Testing outcross offspring.

OxO assay

Note: Can’t be done with a cisgene.
To do top rate environmental studies, you need to plant thousands of trees and we need open pollination

- Current studies are limited by:
  - plot size
  - flower inspection, removal, or bagging
  - limiting growth to control flowering
  - cost of regulatory compliance
  - risk of escape
    - Not due to safety, but because regulated

- Small scale environmental studies are ongoing