“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

Company Developer	# Products
Monsanto	34
AgrEvo	10
Calgene	9
Dow AgroSciences	9
Pioneer	7
Syngenta	7
Bayer	4
Aventis	3
DeKalb	2
Du Pont	2
Agritope	1
Asgrow	1
BASF Plant Science	1
Bayer CropScience / M.S. Technologies	1
Bejo	1
Ciba-Geigy	1
DNA Plant Tech	1
Florigene	1
Genective	1
JR Simplot	1
Monsanto / Forage Genetics	1
Northrup King	1
Novartis Seeds	1
Okanagan Specialty Fruits	1
Plant Genetic Systems	1
Stine Seed Farm, Inc.	1
Upjohn	1
Vector Tobacco	1
Zeneca & Petoseed	1
Total	**106**

University/Gov Developer	# Products
Cornell University	1
U of Saskatchewan	1
University of Florida	1
USDA/ARS	1
Total	**4**

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

Prepared form data available at APHIS BRS on-line database: http://www.aphis.usda.gov/brs/status/BRS_public_data_file.xlsx
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."

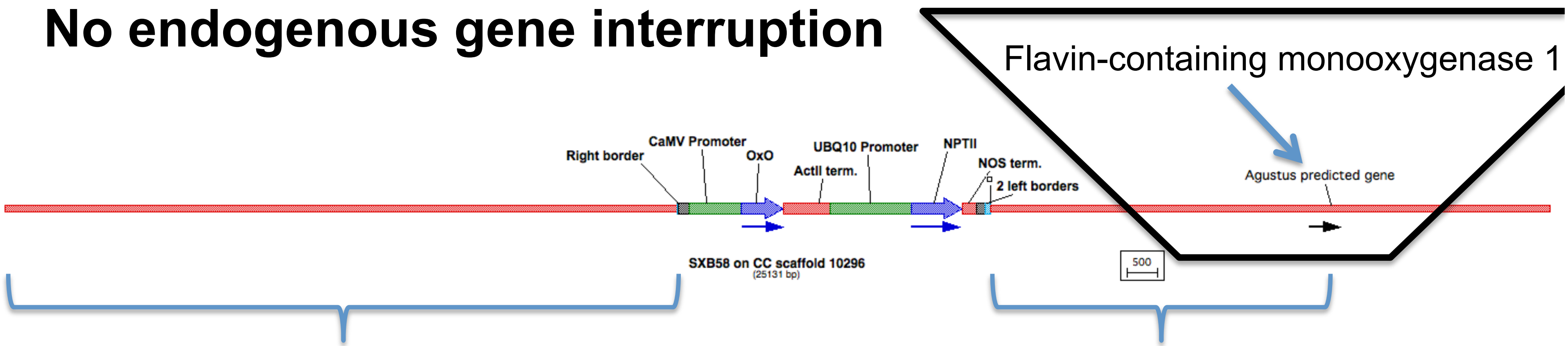
Schnell, J., Steele, M., Bean, J., Neuspiel, M., Girard, C., Dormann, N., … Macdonald, P. (2015). A comparative analysis of insertional effects in genetically engineered plants: considerations for pre-market assessments. *Transgenic Research*, *24*(1), 1–17. http://doi.org/10.1007/s11248-014-9843-7

‘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

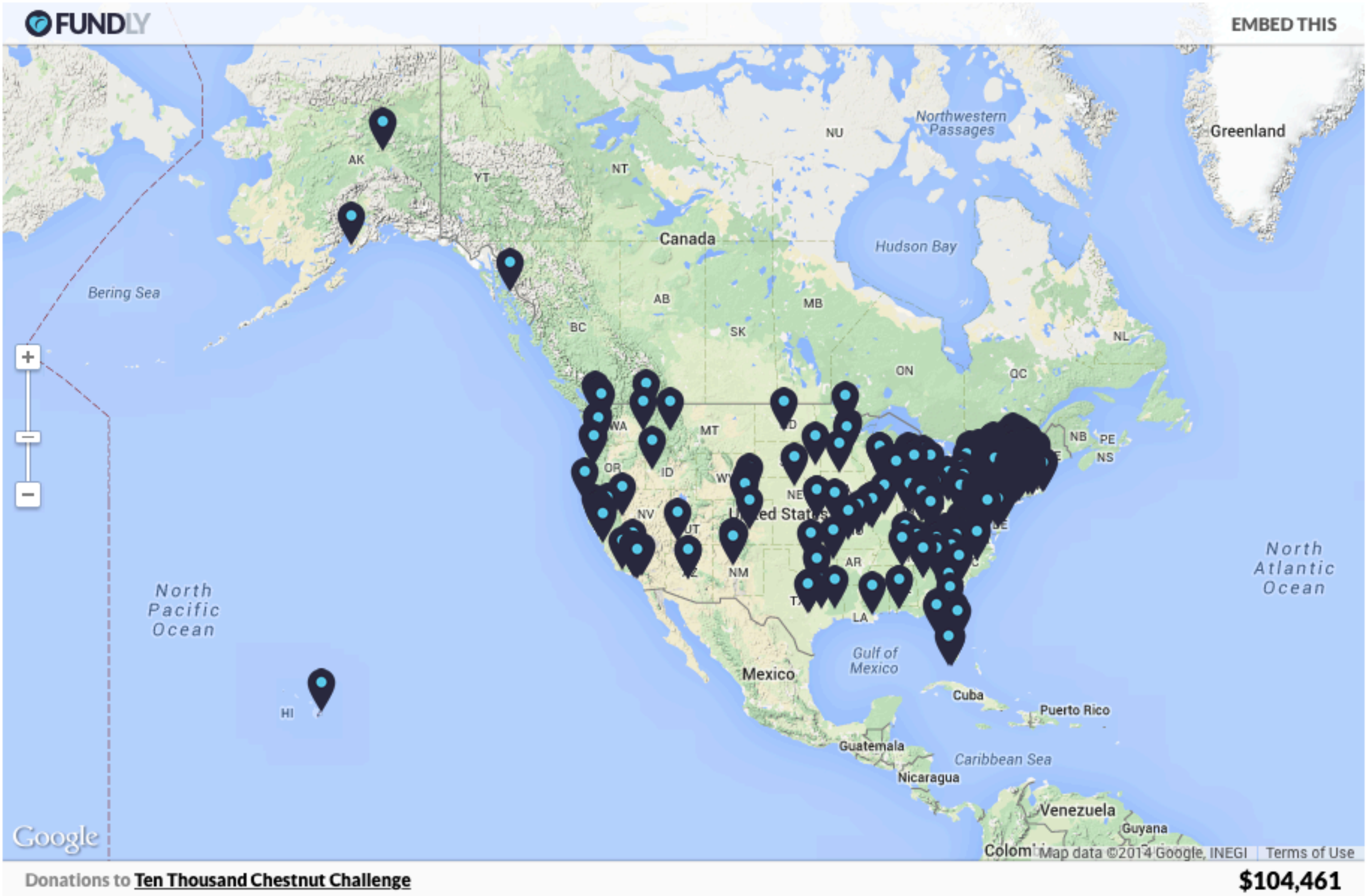
Public Outreach & Education 2014-2015

- 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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EPA

- 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

http://www.aphis.usda.gov/biotechnology/petitions_table_pending.shtml
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 H_2O_2 and 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?

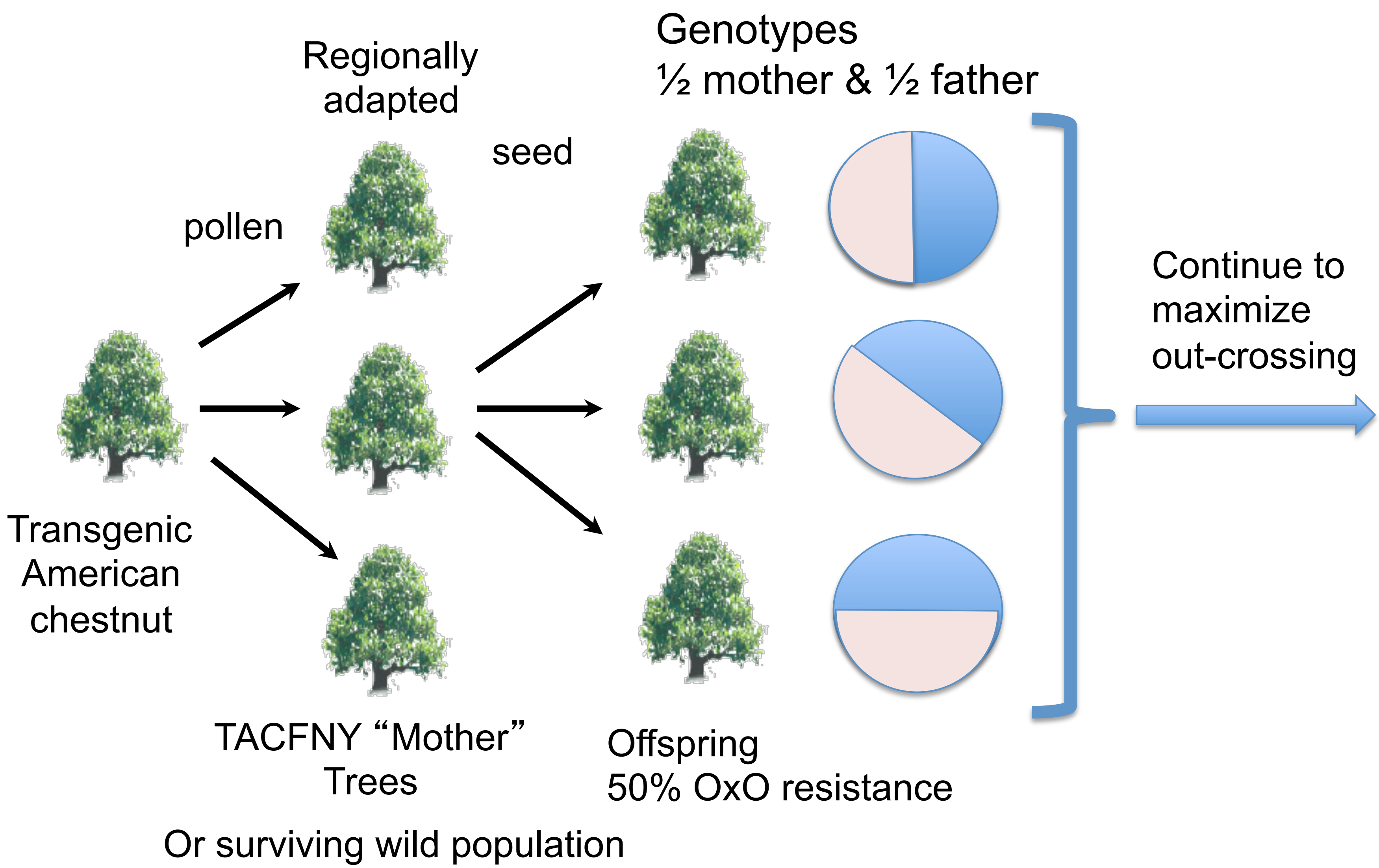
Large spreading American chestnut tree
in MI, 1980's by Alan D. Hart

"For myself I am an optimist - it does not seem to be much use being anything else"
Winston Churchill

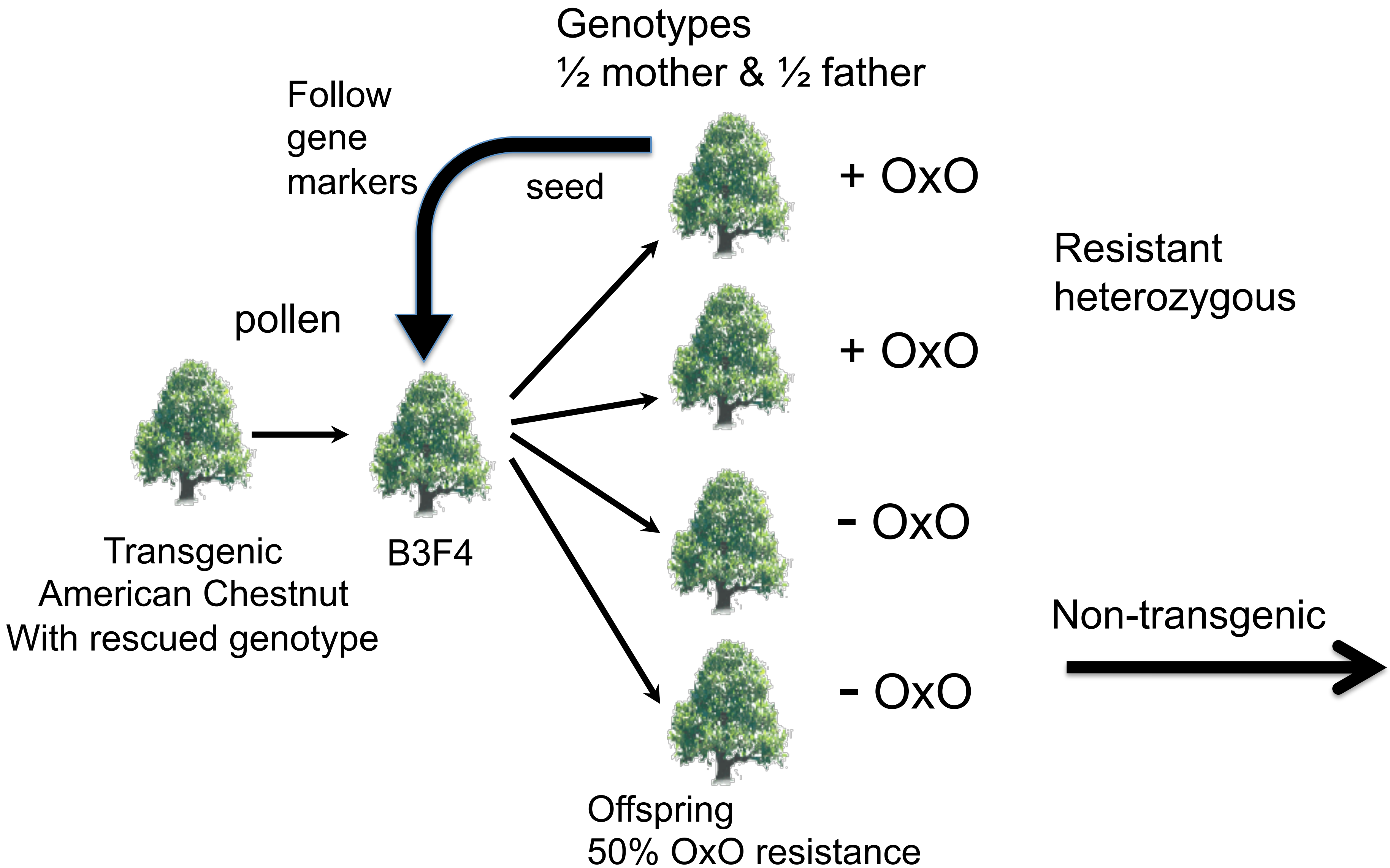
In addition to direct restoration, what else can be done with a dominant blight resistance gene such as OxO?

Rescue of the surviving genetic diversity & aid in breeding

Mother tree project Allen Nichols (TACFNY) **& outcrossing to surviving trees to rescue genotypes**

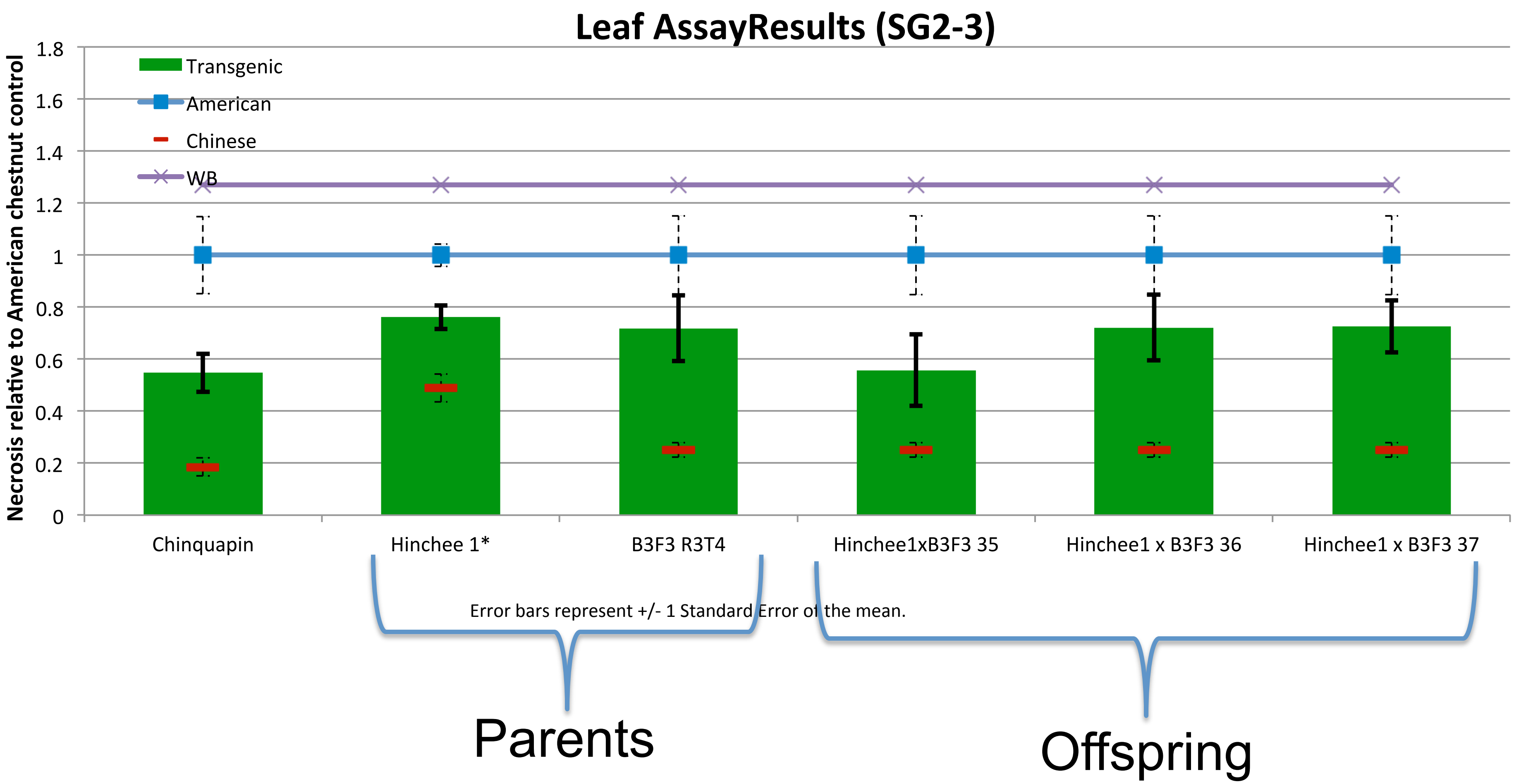


Using hemizygous resistance to add surviving genotypes to the backcross program (Kim Steiner idea)



Example of maintaining resistance when crossing to stack genes

B3F3 x intermediate resistant transgenic



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 a easy identifier…

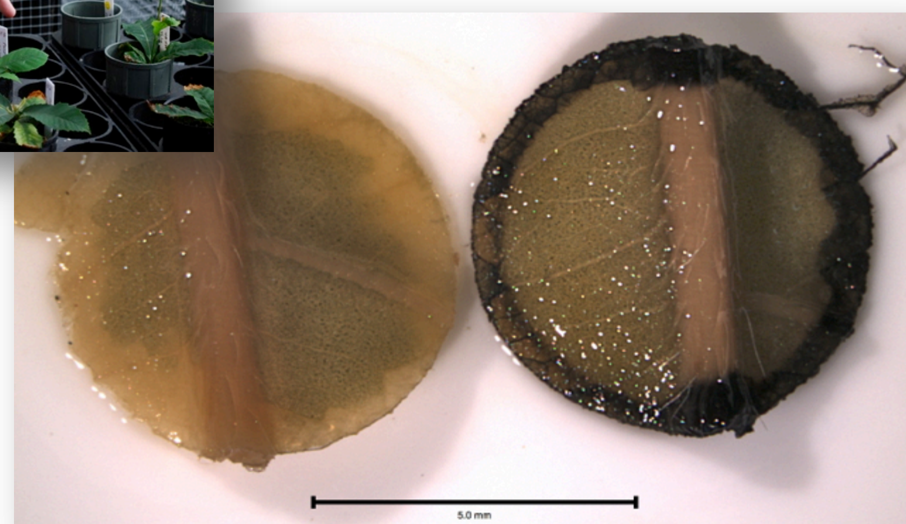
Yes!

Quick screen for OxO gene

OxO assay

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

\- +



Note: Can't be done with a cisgene.

Why pursue deregulation now?

- 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