Are we ready to move forward with a regulatory test case?


Blight resistant American chestnut tree will establish a new paradigm
No transgenic plants have been deregulated for use in a restoration program

American chestnut regulatory model for other forest species

Ash tree marked for removal on Tejah Ave, Syracuse, NY

Emerald ash borer is spreading despite best efforts to stop it.

Dr. Paula Pijut (USFS)
Purdue Univ.
developing

Bt Ash

Loss of Hemlock to woolly adelgid in NC

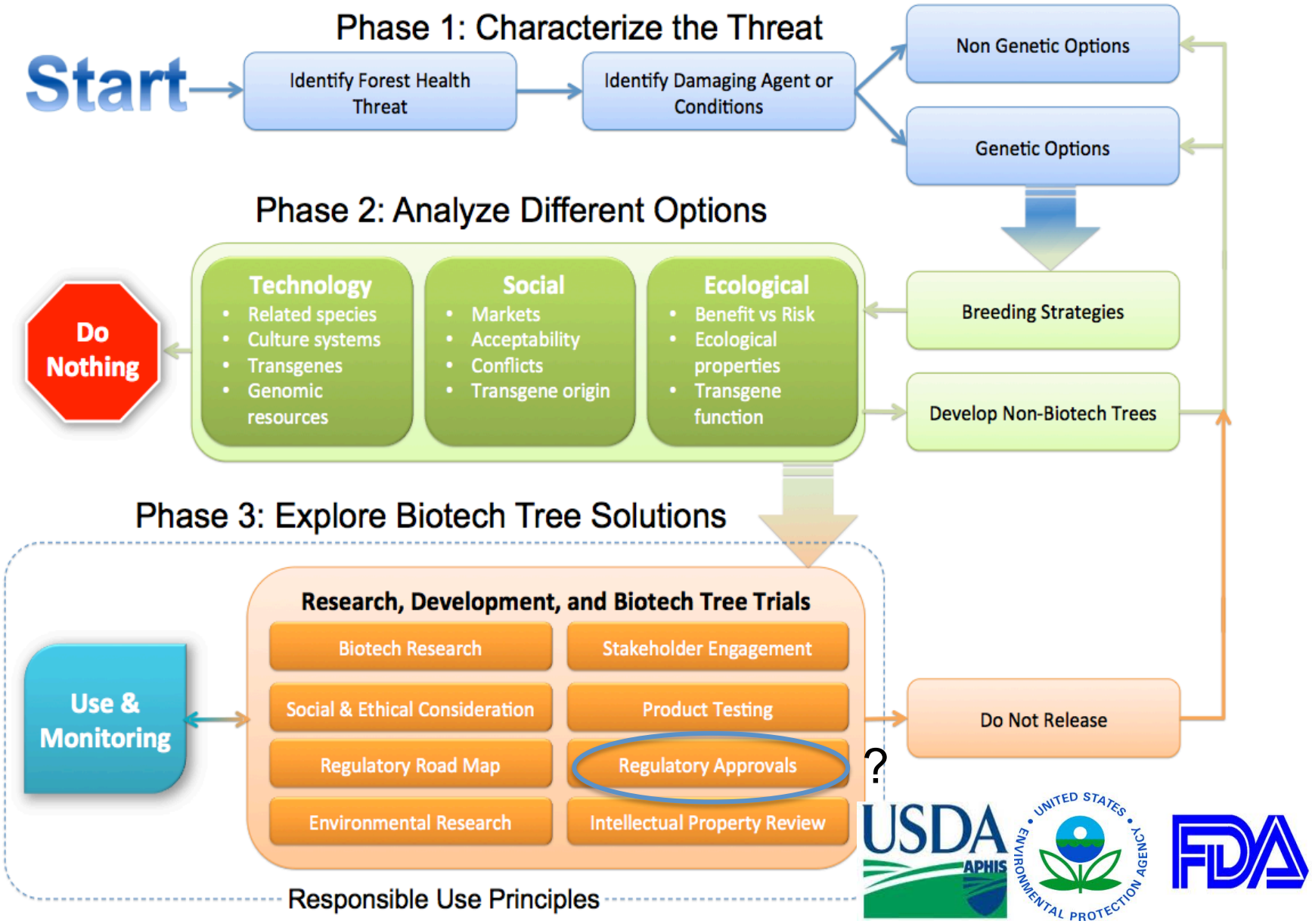
American elm
(DED and **elm yellows**)

Black walnut
(thousand canker disease)

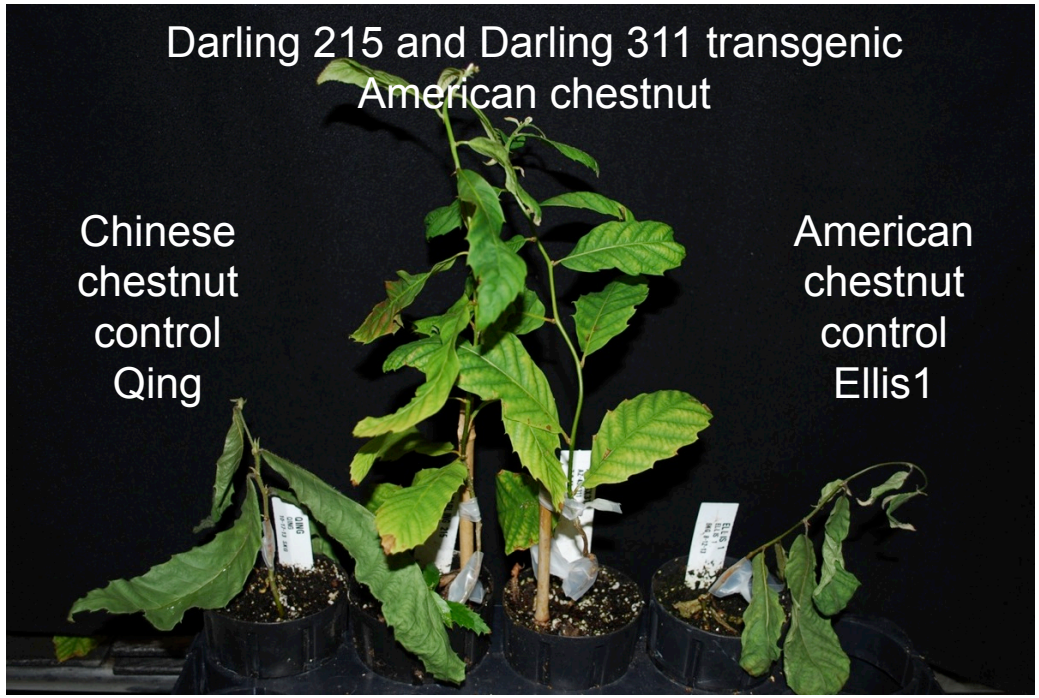
And more…

Need a roadmap through deregulation of restoration trees

The Forest Health Roadmap



- We have “version 1.0” blight resistant American chestnut trees using the oxalate oxidase (OxO) gene
  - Darling 4 was the “beta” version – proof-of-concept for enhancing blight resistance
  - Version 1.1 might be OxO + Lac
  - Version 2.0 trees to follow with stacked genes and *Phytophthora* resistance



The safety of OxO can be easily understood by the public because they eat it all the time (gluten free)

The regulators may view it as GRAS:

"If there is no demonstrated hazard from the PIP, exposure to a PIP expressing plant itself is not a risk endpoint."

Kough & Edelstein, Chap 10, C.A. Wozniak and A. McHughen (eds.), *Regulation of Agricultural Biotechnology:* 163 *The United States and Canada*, DOI 10.1007/978-94-007-2156-2_10,

EPA asked if there was a easy identifier…

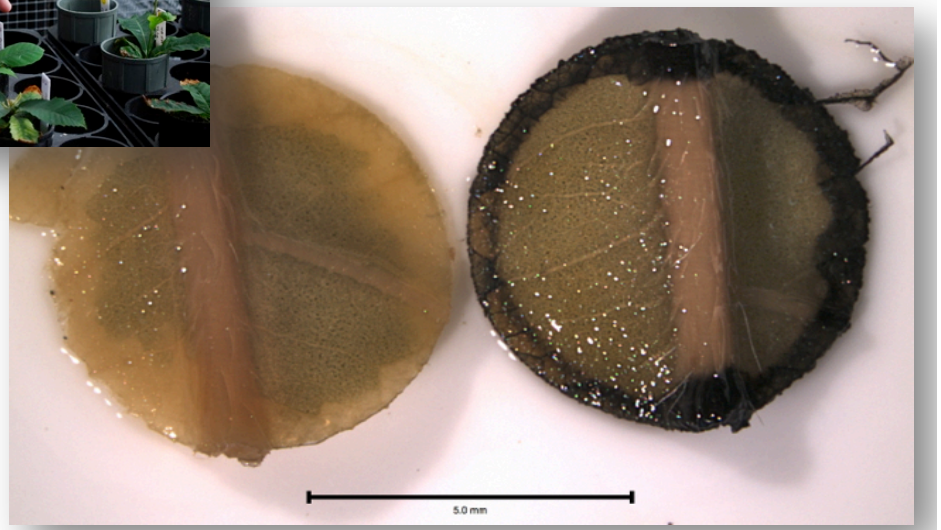
Yes!

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.

A single, dominant resistance gene can rescue the genetic diversity of surviving chestnut population, and maybe aid the breeding program

ESF

AC
Surviving tree

X

AC

R_{OxO}

T1 ~50% R_{OxO}

~ 50% 0

CC

$R_1R_1\ R_2R_2\ R_3R_3$

X

AC

$r_1r_1\ r_2r_2\ r_3r_3$

F1 hybrid $R_1r_1\ R_2r_2\ R_3r_3$ X $r_1r_1\ r_2r_2\ r_3r_3$

$r_1r_1\ r_2r_2\ r_3r_3\ R_{OxO}$

Kim Steiner suggested capturing these genes by crossing

3X

BC1-3

$R_1r_1\ R_2r_2\ R_3r_3$

$R_1r_1\ R_2r_2\ r_3r_3$

$r_1r_1\ R_2r_2\ R_3r_3$

$R_1r_1\ r_2r_2\ R_3r_3$

X $r_1r_1\ r_2r_2\ r_3r_3$

Blight resistance assays select a mix of genotypes.
Must screen many, many trees.

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 (10 acre max)
    - 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

Small scale 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 transgenics 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.

Metabolomics studies support similar or lower risks than hybrid breeding.

Metabolites are the intermediates and products of metabolism. The term metabolite is usually restricted to small molecules.

Question: Does genetic engineering cause more or less change than traditional hybrid breeding?

Transgenic: most modified available & intermediate resistance
– 4 or 5 transgenes, 2 vectors, multiple inserts

Hybrids: first generation backcross
- less complex than many on the market and in nut orchards

This project is supported in part by **Biotechnology Risk Assessment Grant Program** competitive grant no. 2012-33522-19863 from the USDA National Institute of Food and Agriculture and the Agricultural Research Service.

Mass Spectrometry (MS)-based Metabolomics

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Nancy Engle
Madhavi Martin
Stacy Evans
Cassie Bruno
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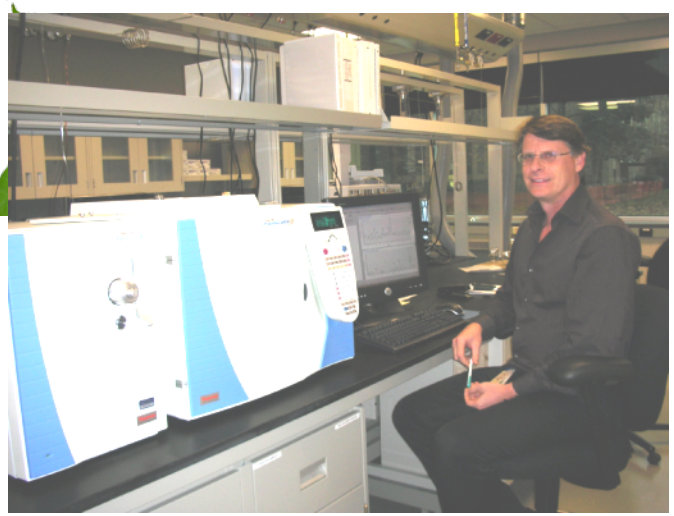
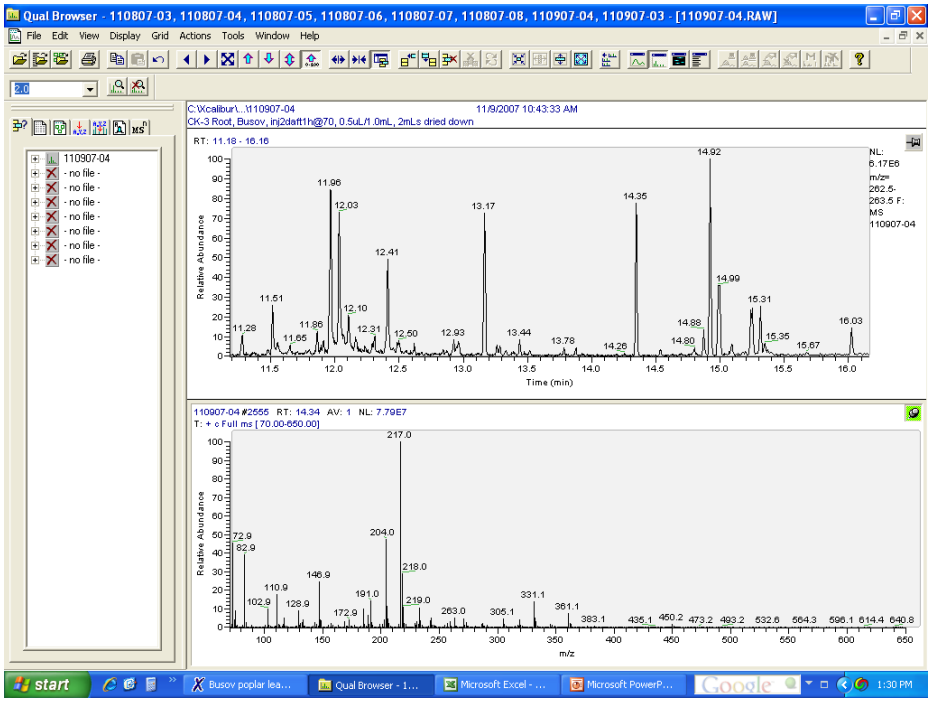


ThermoElectron Polaris GCQ
-Ion trap (GC-MS/MS)
-Structural information
-Narrow candidate unknown metabolites

Waters GCT Premier TOF-MS
-Accurate mass
-Elemental composition
-High speed, sensitivity, dynamic range

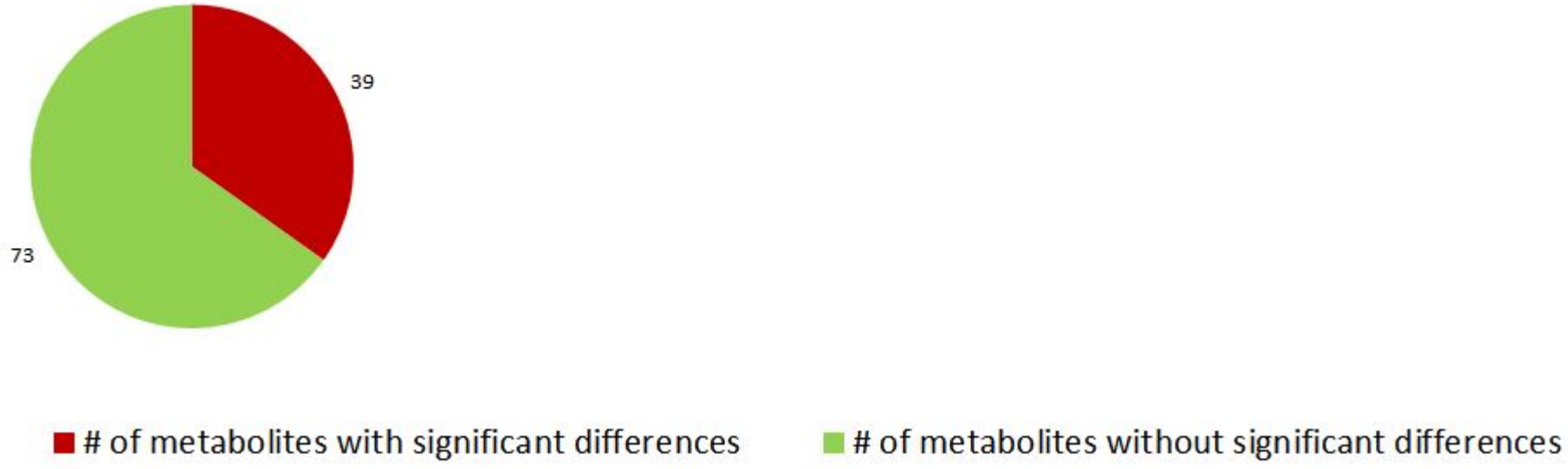


GC-MS
GCQ
GC-ToF-MS

Genetic engineering produces fewer changes than traditional breeding:

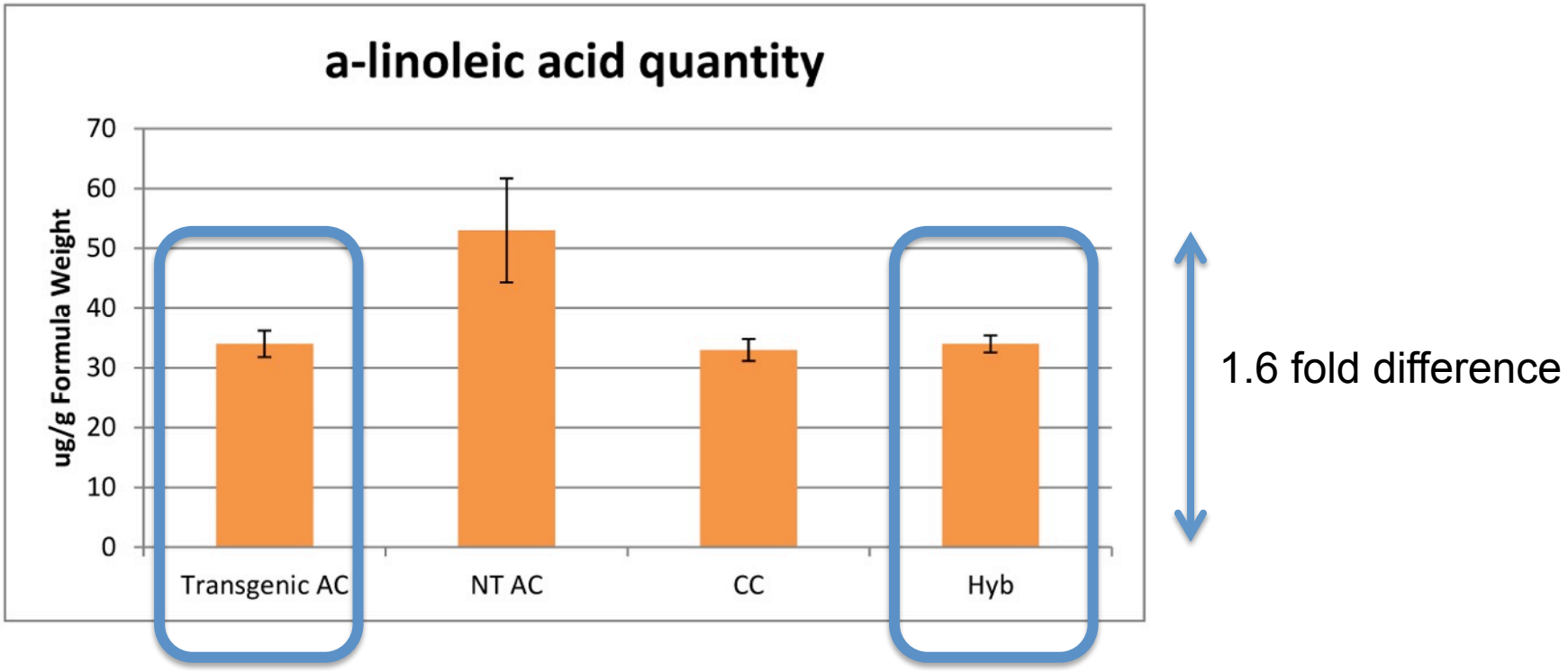
Number of significant changes (red) in 112 metabolites

American chestnut
vs
Chinese chestnut



*BC1 = backcross hybrid: (American X Chinese) X American

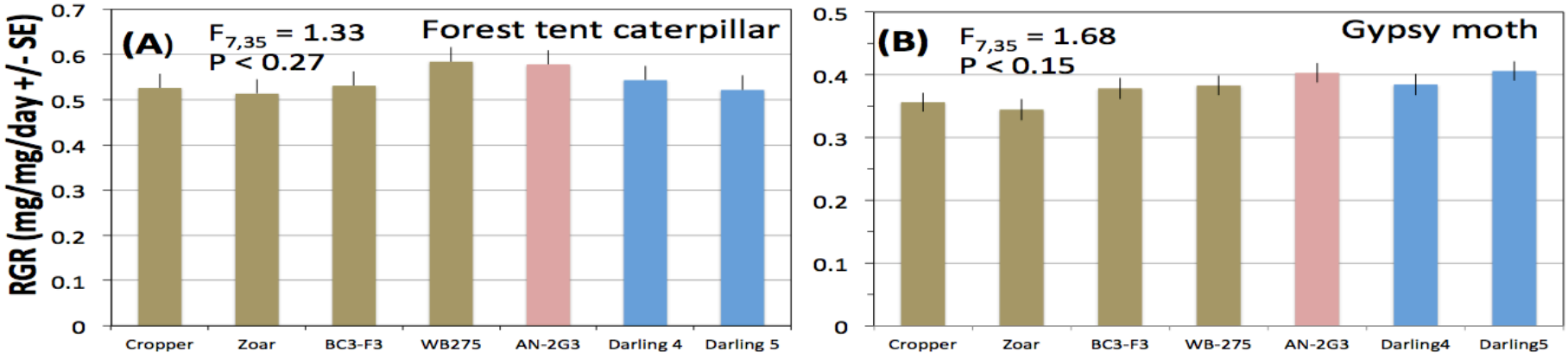
The single significantly different metabolite in leaves of transgenic American chestnut is small and the same as in the BC1 Hybrid & Chinese



Same change in transgenic & backcross hybrid

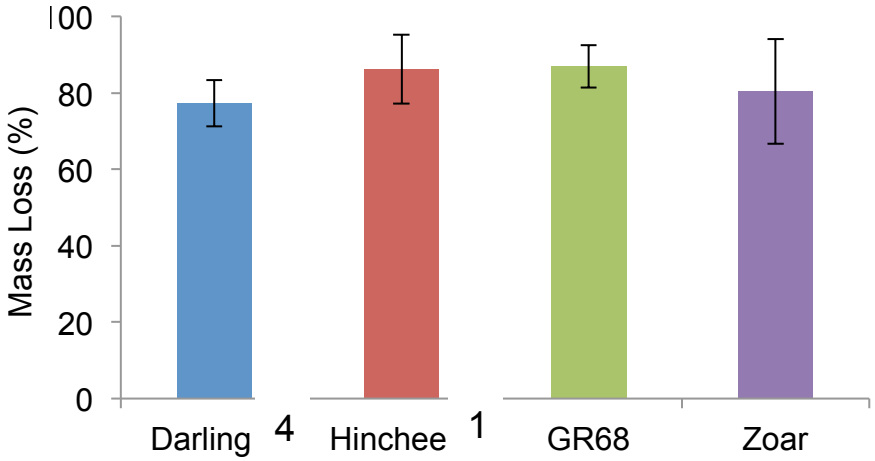
Insect herbivory on leaves backs up metabolomics
No significant difference

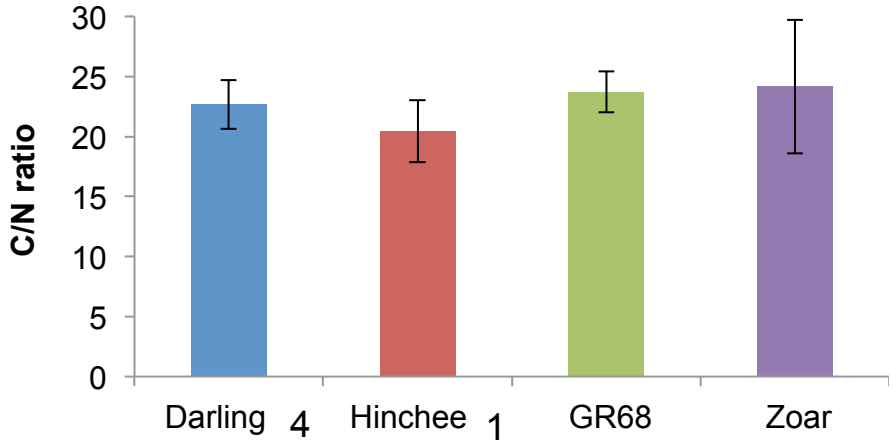
Dr. Dylan Perry (Professor)



Decomposition of chestnut foliage backs up metabolomics
No significant difference

Amanda Gray (M.S. Student) & Dr. Russ Briggs (Professor)





Conclusion

Even when adding five transgenes, genetic engineering made fewer and smaller changes in metabolites than conventional breeding.

The few changes had no biological significance with respect to insect feeding or leaf litter decomposition.

1000 feet effective pollination isolation distance

Example 1: “trees only 100 feet apart will experience reduced pollination success, and trees
1000 feet apart are essentially reproductively isolated.”

P. A. Rutter. 1990. Chestnut Pollinators Guide. Badgersett Research Corporation, Bulletin 1. (http://www.badgersett.com)

Example 2:

Midwest Nut Producers Council Journal - Late Spring 2012 - Volume 1, Issue 1

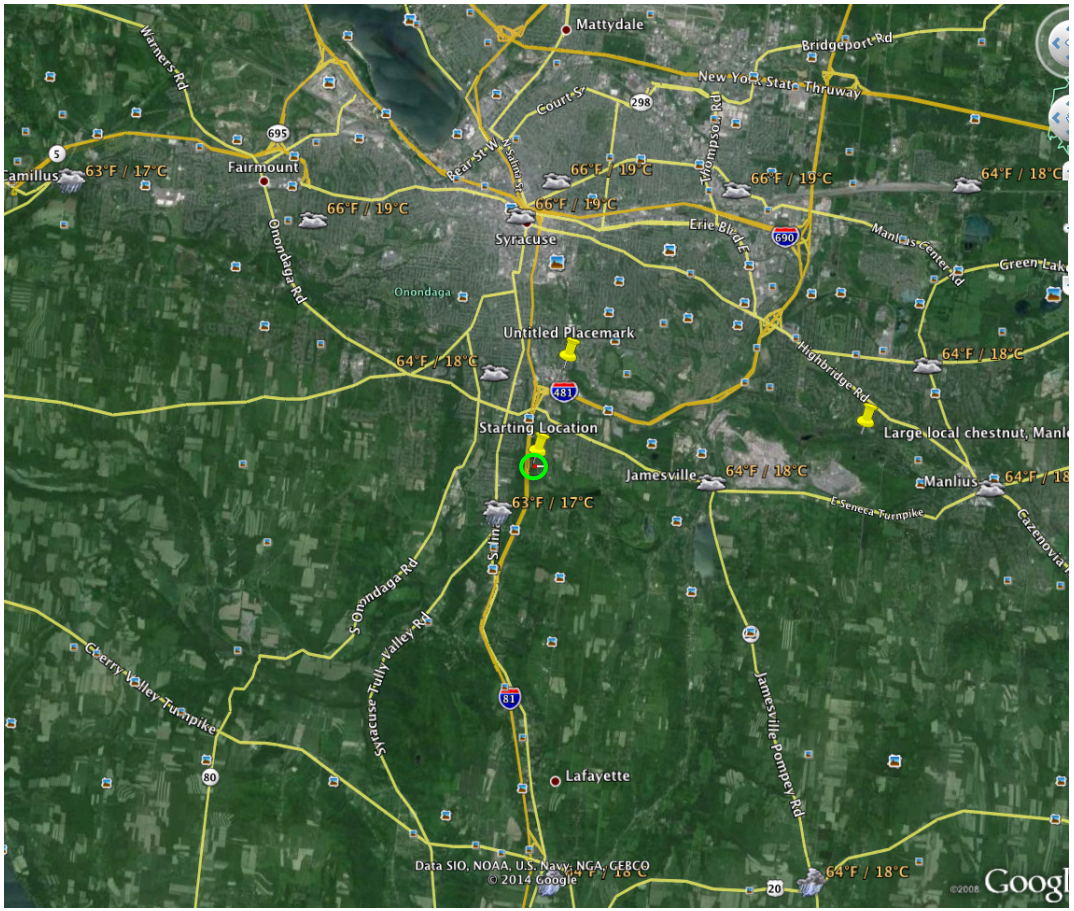
What can growers do to minimize damage from IKB?

Don’t allow Chinese chestnuts to pollinate the ‘Colossal’ trees.

Keep Chinese trees at least 1,000 feet away from the ‘Colossal’ trees in your orchards so that their pollen doesn’t pollinize the trees.

* ‘Colossal’ - European/Japanese hybrid

FHI might want to double to 2000 ft (~609 meters)



Education & Outreach works

Example 1: SUNY-ESF Library

Transgenic American elm, 6 yrs

Example 2: New York Botanical Garden

Transgenic American chestnut, 3rd yr

How long will it take?
Transgenic Plum (HoneySweet) example: 7 years

American chestnut
5 years?

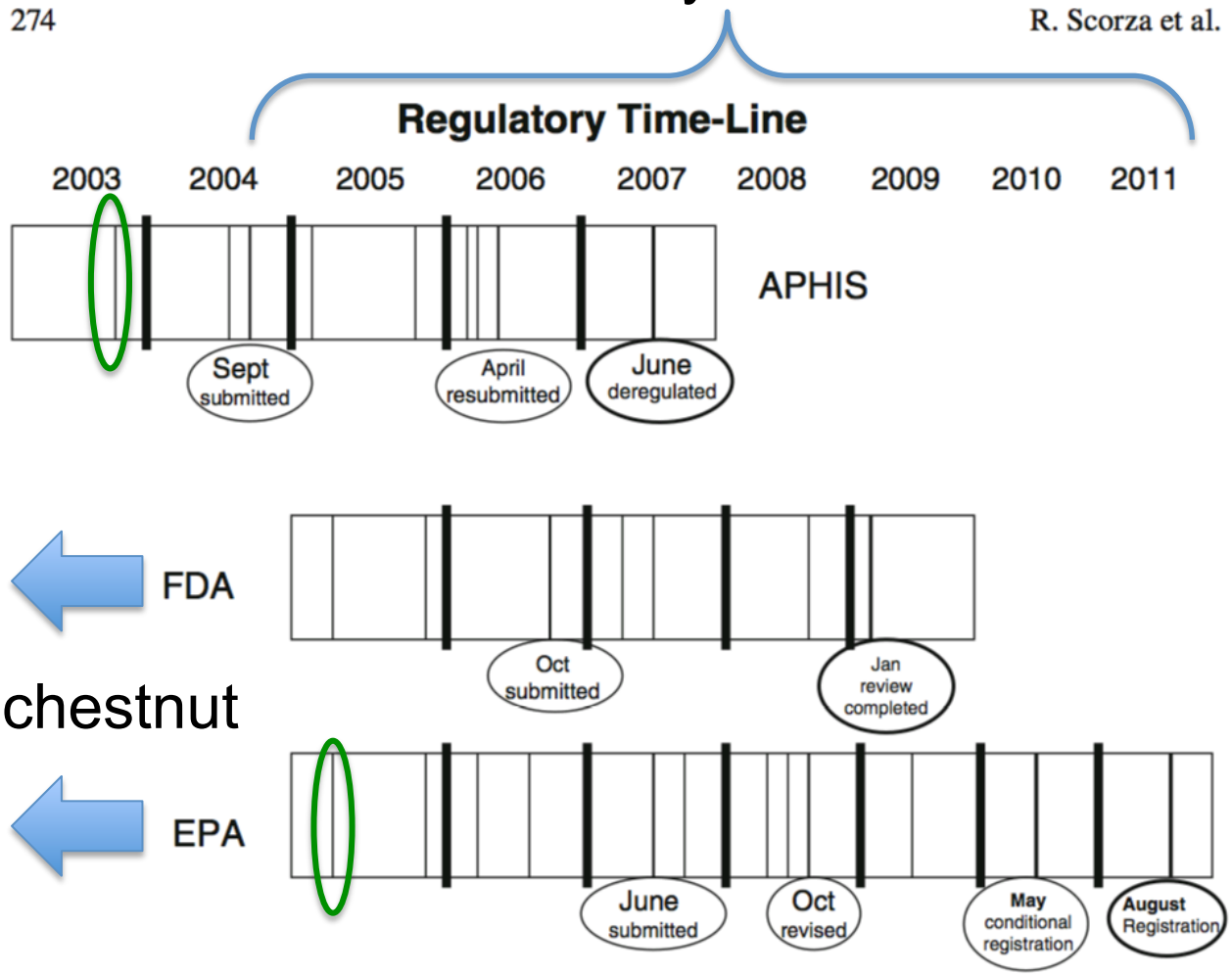


Fig. 12.2 Schedule of regulatory consultations (*thin lines*), submissions and approvals for 'HoneySweet' plum. Thin vertical lines indicate dates of meetings between regulators and applicant

What deregulation may look like. (starting the discussion)

- Produce 10,000 blight resistant American chestnut trees during the regulatory review
  - Tight spacing, < 10 acres
  - Continue small scale environmental research.
  - Education & outreach programs should parallel review process.

- Once deregulated, give out "**monitored trees"**.
  - Trees will be easily identifiable (OxO assay kit).
  - All first recipients agree to make annual reports (5 years?)
    - Similar to TACF's agreement with backcross trees but simpler
    - TACFNY members first (invested) & then others who agree
  - Simple web-based/app data collection
    - **TreeTaggr** or something similar
    - Record growth, flowering, nut production, health, any unusual observations, etc. (questions developed by ecologists)

- Newer, improved versions may follow.
  - Phytophthora resistant, stacked genes

100's of lay researches helping primary researchers evaluate "monitored" trees

- Comparisons to mother trees
  - TACFNY already has a wild-type "mother" tree planting program started (see Allen Nichols).
  - Used to enhance genetic diversity.
- Advantage of having many locations on private lands
  - Would provide a broader range of environments that just a few test sites & would supplement larger designed experiments.
- Other plantings
  - 3 large scale research plots
  - Mine land reclamation
  - Botanical gardens
  - Some historic sites

- Wait on national forests and some park lands

Questions?

"Optimism is the faith that leads to achievement; nothing can be done without hope."
Helen Keller

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

Exploring deregulation/registration to promote research & outreach

- We need a test case to identify a path that has minimal paperwork requirements given the public good & ecological goals, but is responsible and rigorous in its science and outreach.
  - This will not be easy
  - Will need a lot of help from various stakeholders
  - Need to hire dedicated personnel

from *Pandora's Picnic Basket*
by Dr. A. McHughen