FHI Project: Transgenics at SUNY-ESF
"Target: A plantable tree"

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Darling 4 transgenic American chestnut planted at the New York Botanical Garden, April 18, 2012

SUNY-ESF Transformation Project Deliverables

William A. Powell (PI) and Charles A. Maynard (coPI)
SUNY-ESF

- Transformations with constructs on hand and characterization of transgenic events.
  - 14 1st generation vector constructs in pipeline (embryo to whole plants)
  - 52 transgenic events
- Second-generation gene constructs and transformations.
  - Cloned 27 cDNA genes from *Castanea mollissima* (21) & *C. seguinii* (6) & sent to Dr. Nairn, UGA, for vector construction
  - Culling protocol established using PCR, qPCR (insert copy number), and RT-qPCR (expression) to reduce unwanted transgenic events
  - 27 pFHI vector constructs in pipeline (embryo to whole plants)
  - 94 transgenic events after culling (>20 trees each = >1,880 trees to test)

  For objectives 1 & 2, many embryo clusters, maintaining over 4624 tissue culture shoots, 218 plants in growth chamber, 244 in field shade tent, 640 transgenic trees in the field (with 534 non-transgenic controls)

- Early blight-resistance assay development.
  - Workshop, leaf assay development, training Allison from UGA, added washing protocol for young field leaves, publication in progress
  - Save years off of plantable tree development
- Early flowering gene tests.
  - Produced FT transgenic events
  - 35S promoter is too strong, but heat-shock promoter works well
  - Other work: can produce pollen in less than a year using highlight treatments (TACF Journal)
  - Other work: started doing crosses with transgenic American chestnut
- Supplemental information at the end

Field plantings: 640 transgenic trees & 534 controls

Transgenic Darling 4 planted in Fall 2008
Transformed in April 2007

Inoculation plots, older in background, newest in front

Shade tents improve production

skip greenhouse
(244 transgenic trees in shade tents)

Transgenic American chestnut in tube

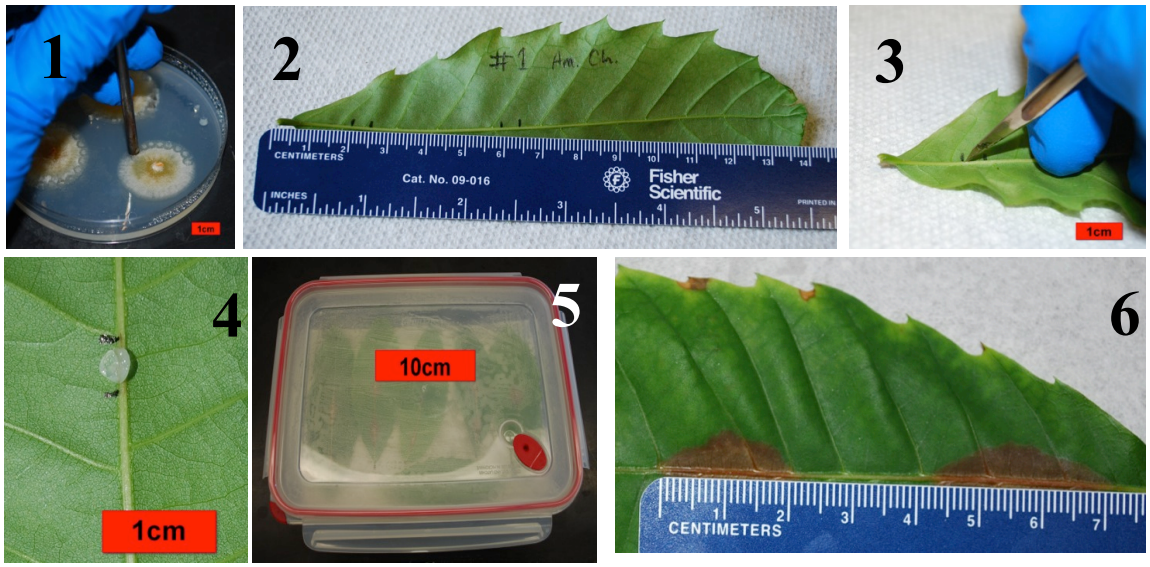
Quicker mycorrhizal colonization in field soil

Traditional stem assay vs. leaf assay

Traditional assays:

4-year-old tree, 1 1/2 inches (3.8cm) in diameter, inoculated with the chestnut blight to evaluate their resistance level after several months.

PA-TACF http://www.patacf.org/patacfactivities.htm



Leaf assays can be done with 6 or more leaves per plant or clonal event, results in 3 to 7 days.

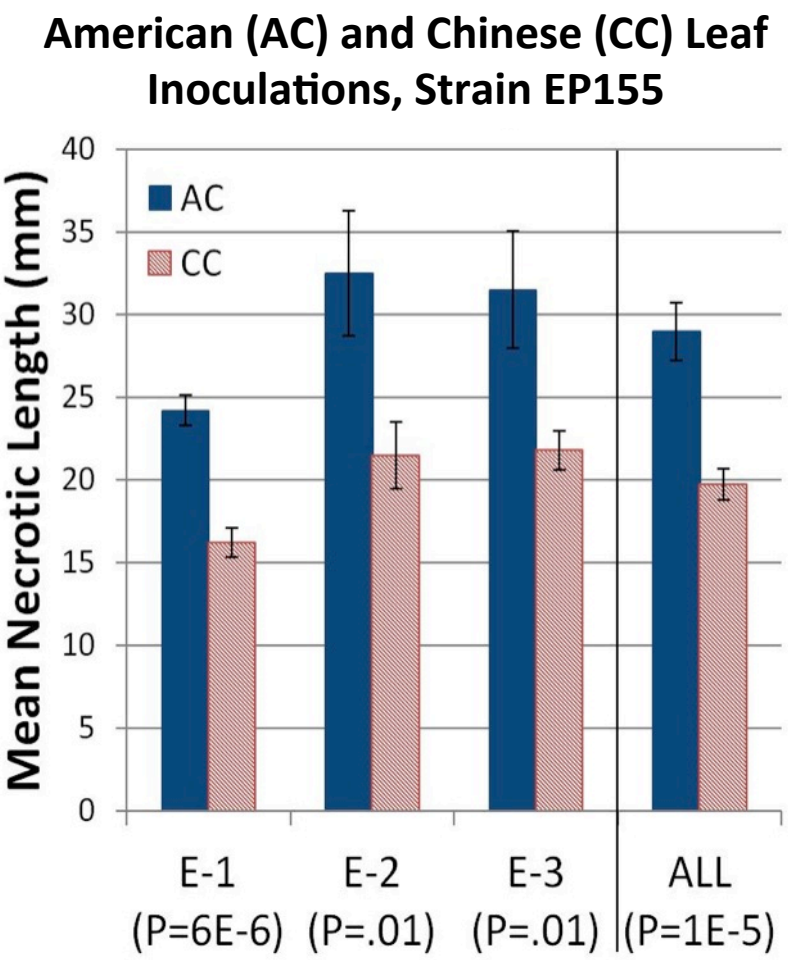
New – washing technique for field trees (May-June)

Savings of ~ 4 years!

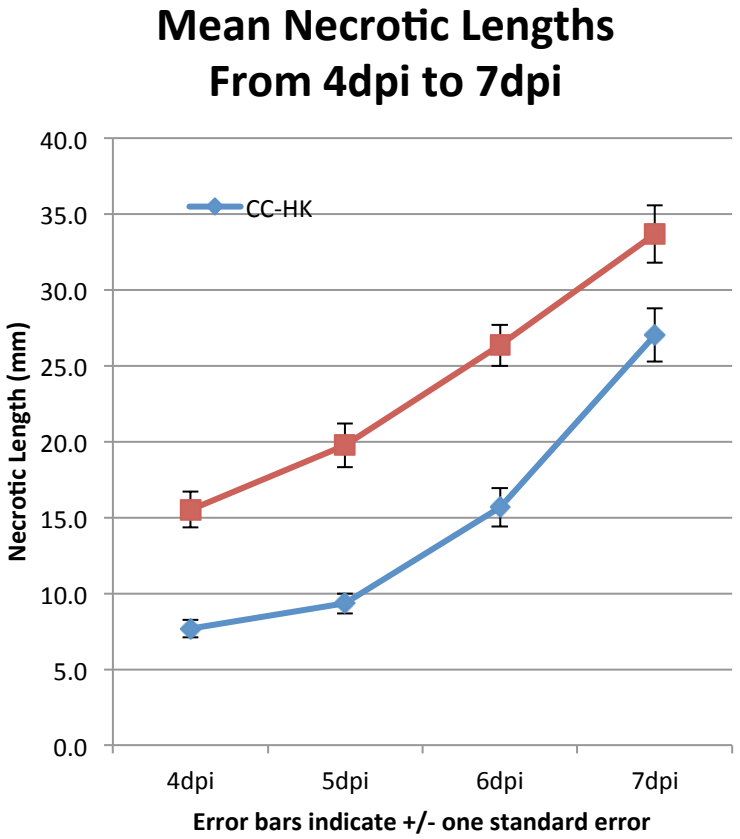
Faster screening for plantable trees

Chinese chestnut consistently shows less necrosis than American chestnut

Comparisons of experiments (7 days)



Comparisons of assay length in days post inoculation (DPI), strain SG2



(Andy Newhouse and Jesse Spitzer)

1st Generation event, Darling 4, shows a level of enhanced blight resistance
EP155 Inoculations 6/14/12
18 DPI, 7/2/12

American chestnut seedlings (susceptible control)

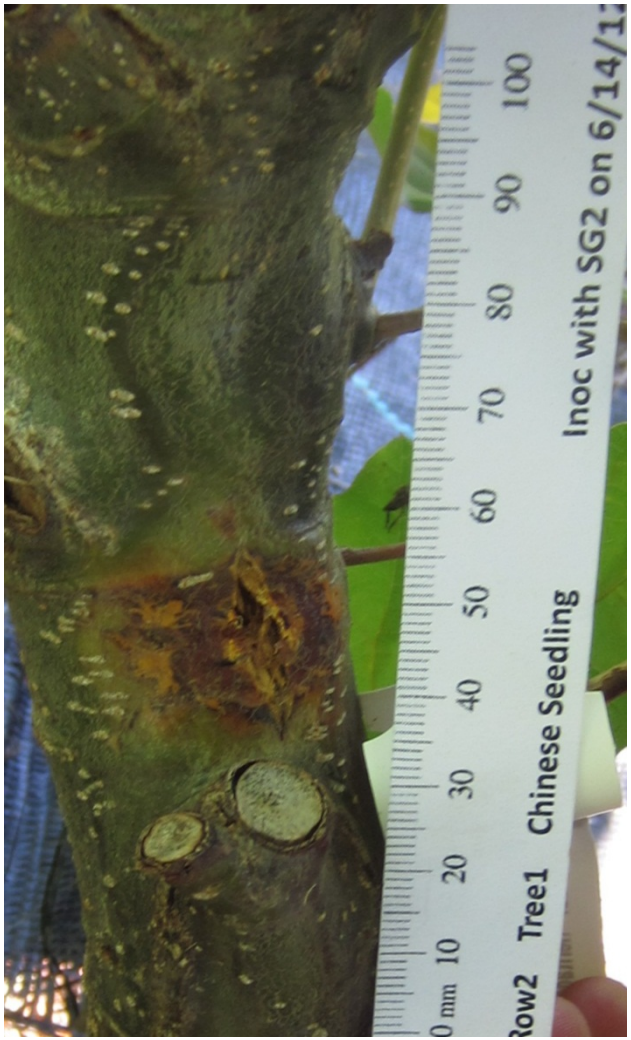
Chinese chestnut, Cropper (resistant control)

(assay ongoing)

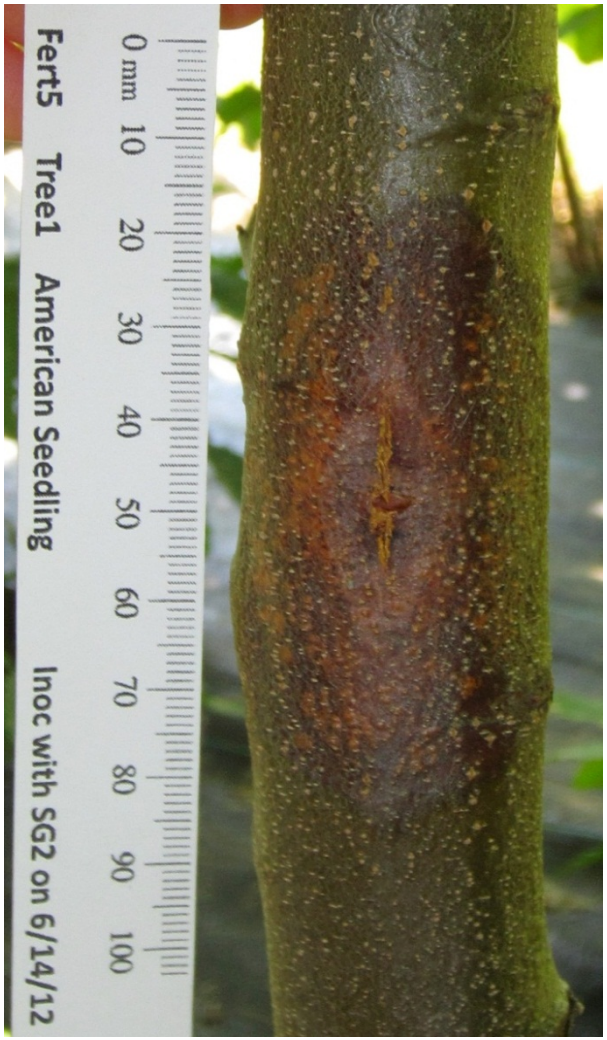
Darling 4, large stems (2.5cm+) (transgenic American chestnut)

Canker size, 7 weeks post-inoculation
SG2-3 inoculations

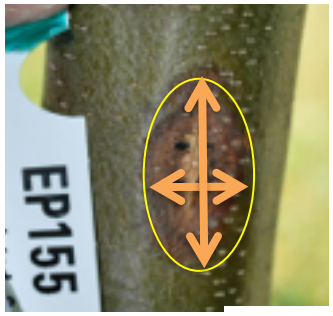
Chinese chestnut, Cropper
(resistant control)

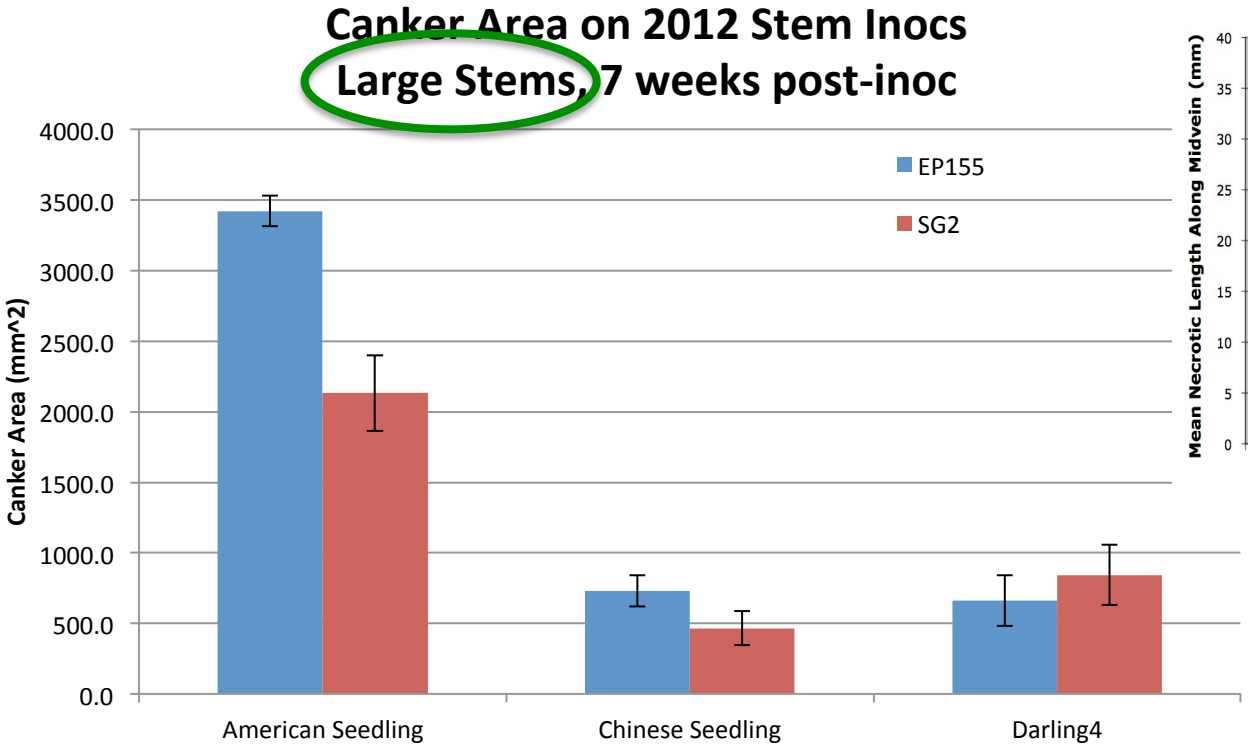


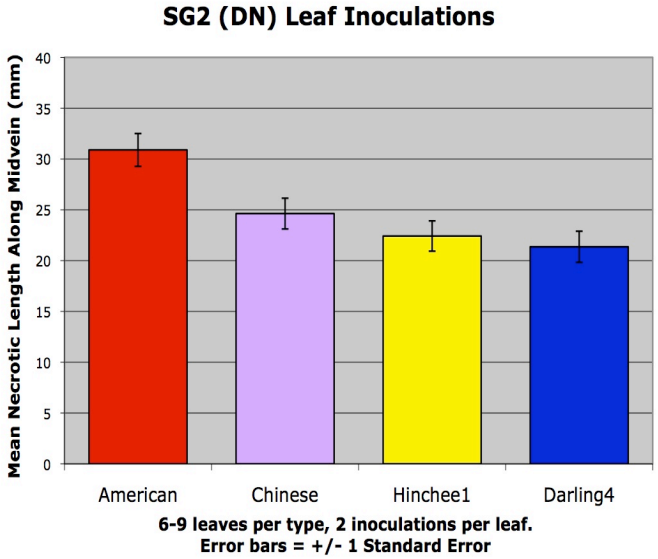
American chestnut seedlings
(susceptible control)



Darling 4, large stems (2.5cm+)
(transgenic American chestnut)







n=4 trees per strain/type, error bars +/-1 SEM, area assumes circular/oval canker

Demonstrates blight resistance can be enhanced in a plantable transgenic American chestnut tree

(how much enhancement in resistance still needs to be tested)

(want only 10 events to move forward, need culling)

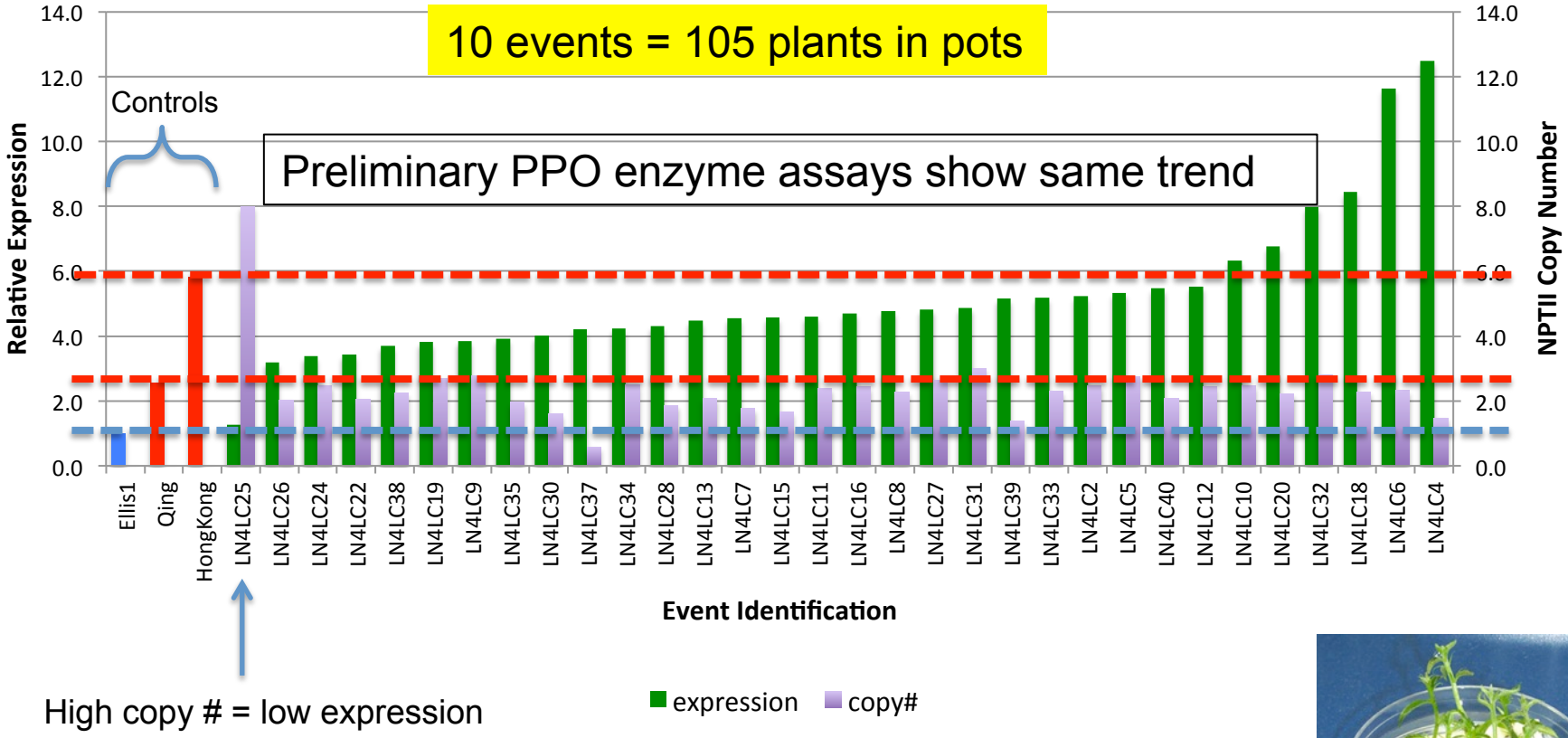
32 pFHI-cmLac events (2nd generation)

Native gene + identical cisgene product

UBQ11 constitutive promoter

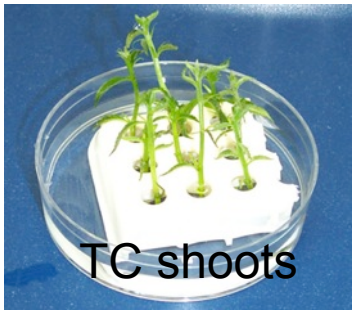
Laccase expression and NPTII Copy Number in LN-4LC Transgenic Events

Tissue culture shoots, quick screen qPCR & RT-qPCR



High copy # = low expression

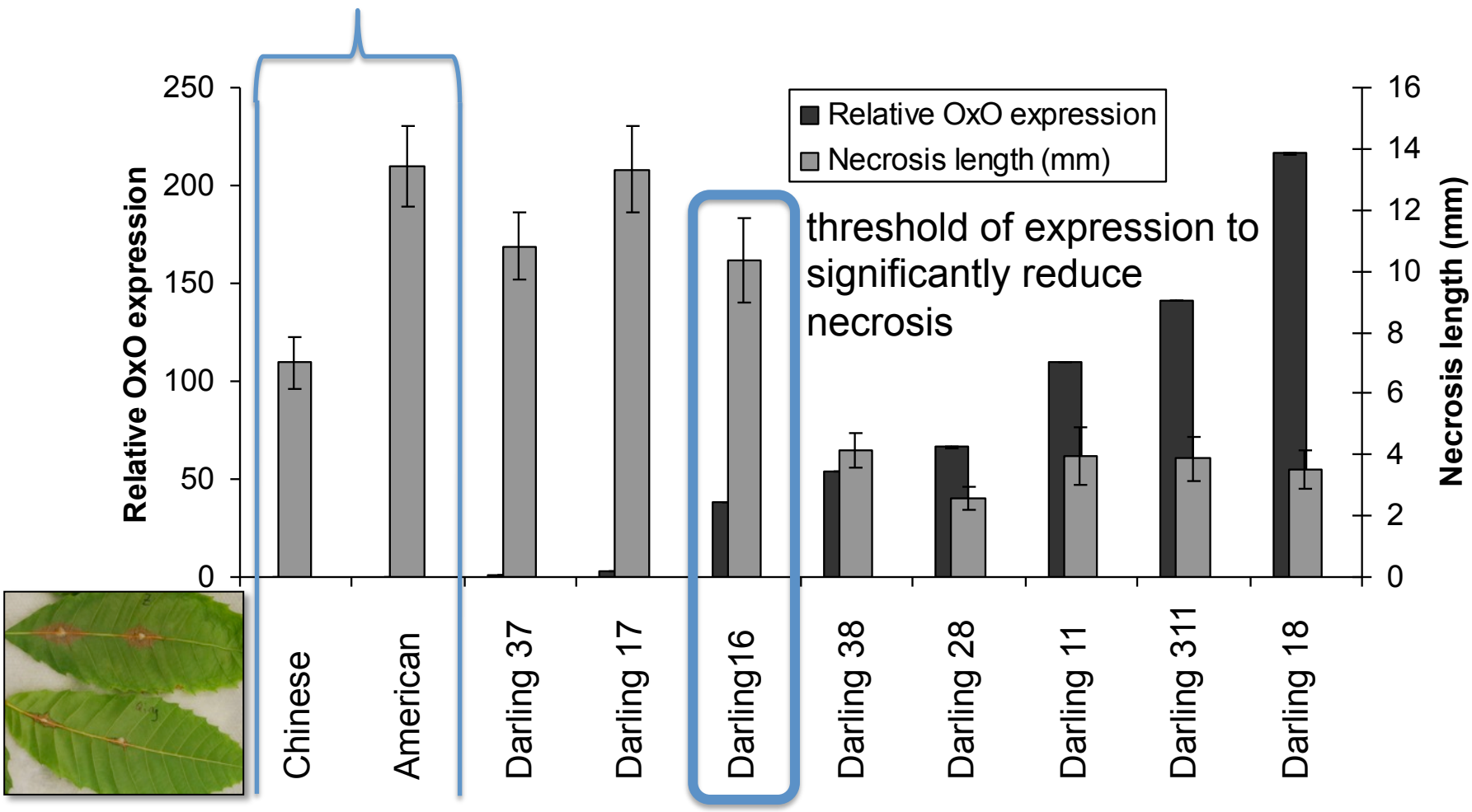
Note: Plantable trees need low insert copy number and the release of few events so as not to multiply copies during breeding and restoration.



(Kathleen Baier)

Combining leaf assays with gene expression assays (RT-qPCR)

Newer Darling trees (p35S-OxO, 1st generation vector)



Gene expression levels are important when choosing a plantable tree

(may need the Goldilocks level of expression)

(Amelia Bo Zhang)

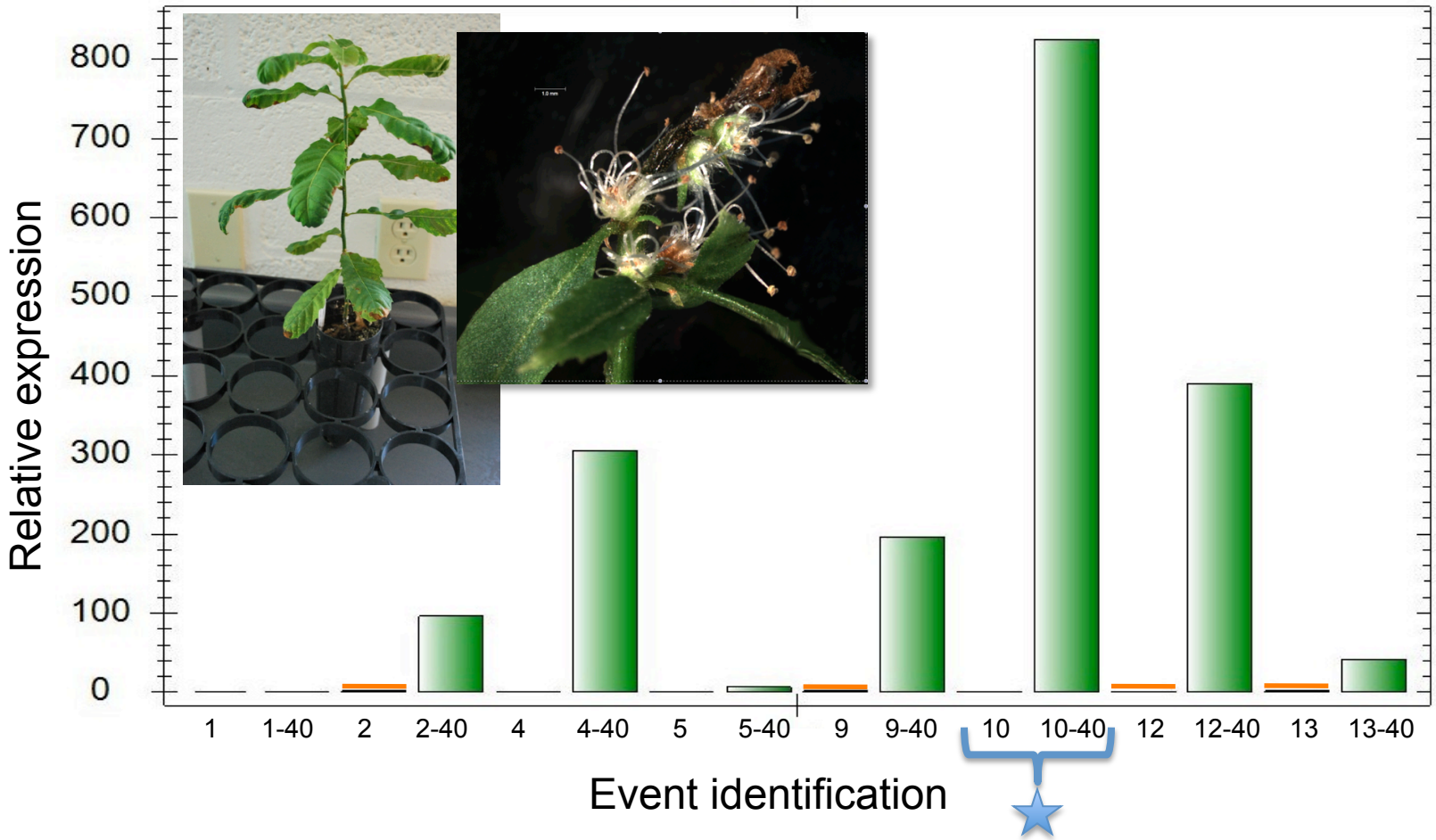
FT1 expression in transgenic American chestnut TC shoots

pOrStFor-HSP:PtFT1 events (vectors from Steve Strauss, OSU)

Orange, ambient temperature (22C)

Green, TC shoots induced at 40C for 4 hrs

(collaborating with High School students to help test these plants)



Accelerate genetic crosses to produce plantable trees

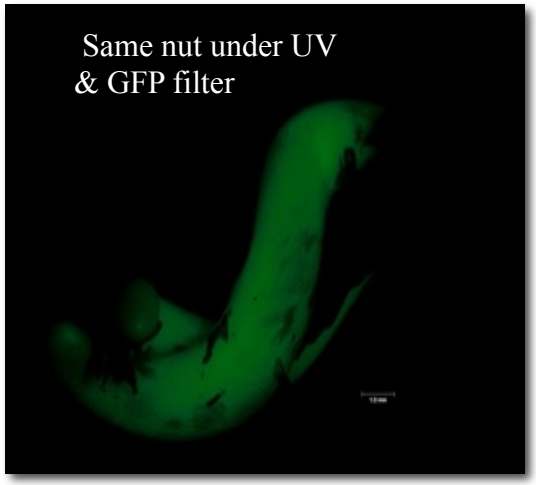
(Kathleen Baier, Lilibeth Northern, & Allison Oakes)

Are the plantable transgenic American chestnut trees fertile and can they pass on the new trait?

Yes!

First cross between transgenic chestnut and wild-type 2011-2012

Seven GFP+ Seedlings planted in field this spring



(Andy Newhouse)

How quickly can new plantable transgenic trees produce pollen for outcrossing to increase genetic diversity?

10-11 months after acclimatization

High-light dose pollen production from transgenic chestnut

Saves 2 years

Kathleen with Chinese chestnut catkins & burs

Hinchee 1 catkins. Pollen collected for 2012 crosses

Outcrossing Darling 4 & Hinchee 1

(to increase genetic diversity of plantable trees)

Questions?

Chuck Maynard measuring 18 inch DBH surviving American chestnut tree

Supplementary information

- Held USDA Compliance Education and Biotechnology Quality Management System (BQMS) Program workshops July 16 & 17, 2012 at SUNY-ESF. Aides the regulatory process for producing a plantable tree.
- Vector construct mentoring from Monsanto researchers, June 21 & 22, 2012 at SUNY-ESF. Gained advice from very experienced researchers how best to construct vectors and items to consider when going for deregulation of a plantable tree.
- Three of the 35S-OxO Darling events are under high-light pollen production in preparation or for 2013 crosses. Increasing genetic diversity of a plantable tree.
- New bioreactor method developed to eliminate need for cotransformations. This is a second bioreactor method using an ebb and flow system for producing plantable trees.
- Cloned the promoter from the Chinese chestnut laccase-like gene and identified a molecular marker to distinguish American from Chinese. This can be used in future vector constructs used to produce a plantable tree.

Supplementary information

- Results from previous USDA BRAG grant demonstrated no significant difference among transgenic Darling 4, Darling 5, Hinchee 1, and Hinchee 2 events and a non-transgenic standard panel of trees including clonal wild-type from tissue culture, American chestnut, hybrids, and Chinese chestnut seedlings with respect to mycorrhizal colonization, insect feeding, and vascular plant colonization. Needed to establish methods to evaluate environmental impacts and of the plantable tree.
- New USDA BRAG continues research on these trees as they progress through flowering and nut production. Insect feeding, leaf litter decomposition, nut composition, and gene expression stability will be tested in the events listed above.
- Established three Chinese chestnut shoot cultures in modified chestnut medium. This provide more accurate control trees when testing the plantable tree.
- From gene expression assays & insert copy number studies, we know that large numbers of inserts suppress transgene and native gene expression. Goal should be to keep insert copy number low (3 or less) in plantable trees.

Supplementary information

- Established three B3F3 trees in shoot culture, developing methods to help clonally propagate the best B3F3 or B3F4 plantable chestnut trees. Small TACF grant for supplies was awarded to help with this work.
- A pilot study has begun to cross a Darling 4 and Hinchee 1 transgenic trees with a B3F3 tree to determine if combining the programs could produce even better plantable trees.
- Additional fenced planting area established at the Lafayette Road Experiment station, SUNY-ESF, to expand field trials.

Field & lab tours are given often, even to small groups. Just let us know if you would like to visit.