



# American Chestnut Research & Restoration Project SUNY-ESF

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Species Protection Through  
Disease Resistance:  
Moving from the Lab  
to the Field





# Genes & Vectors



## 1. Oxalate oxidase (OxO) gene from wheat

- A. Detoxifies oxalic acid produced by the blight fungus & protects the lignin produced by the tree

p $\Delta$ VspB-OxO, pTACF3, pTACF7, p35S-CNO, p35S-OxO

## 2. ESF39 or ESF12 antimicrobial peptide

- A. Kills the blight fungus, *Cryphonectria parasitica*
- B. Might also be useful against *Phytophthora* cankers

pTACF6, pTACF7, pCWEA1

## 3. Chitinase from *Trichoderma*

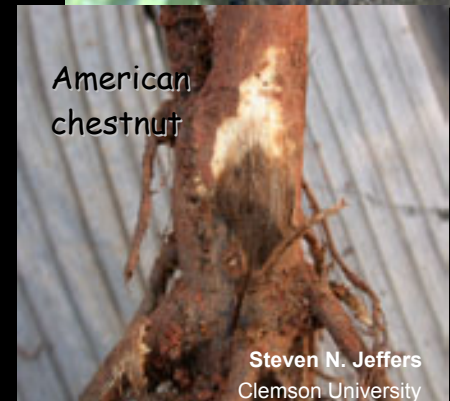
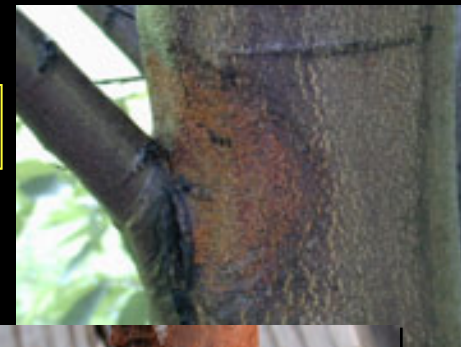
- A. Degrades the cell wall of *C. parasitica*

p35S-CNO

## 4. Ac-AMP1.2 antimicrobial peptide from Amaranth

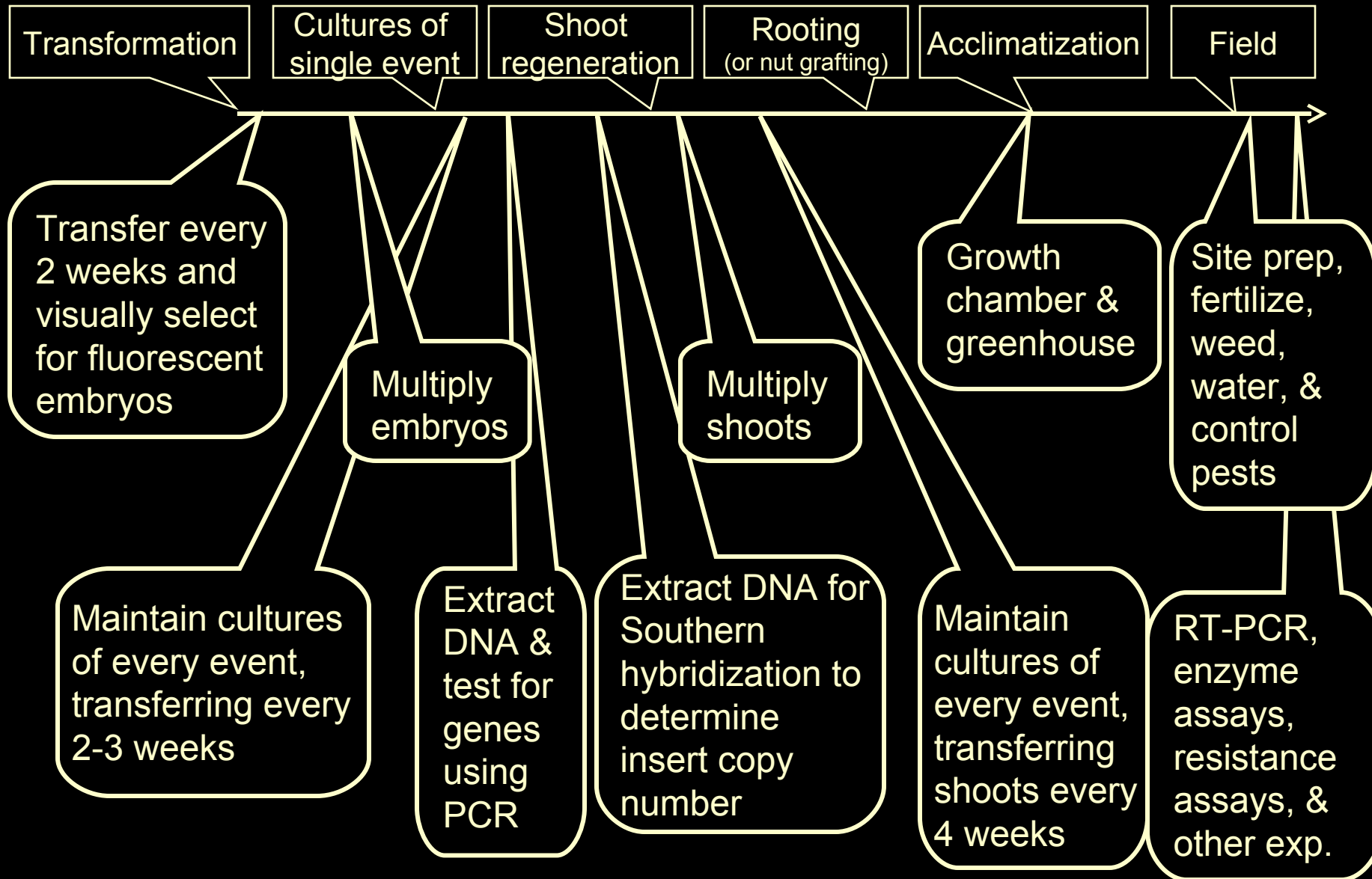
pCWEA1, pCA1

Control vectors: pGFP & pWVK147

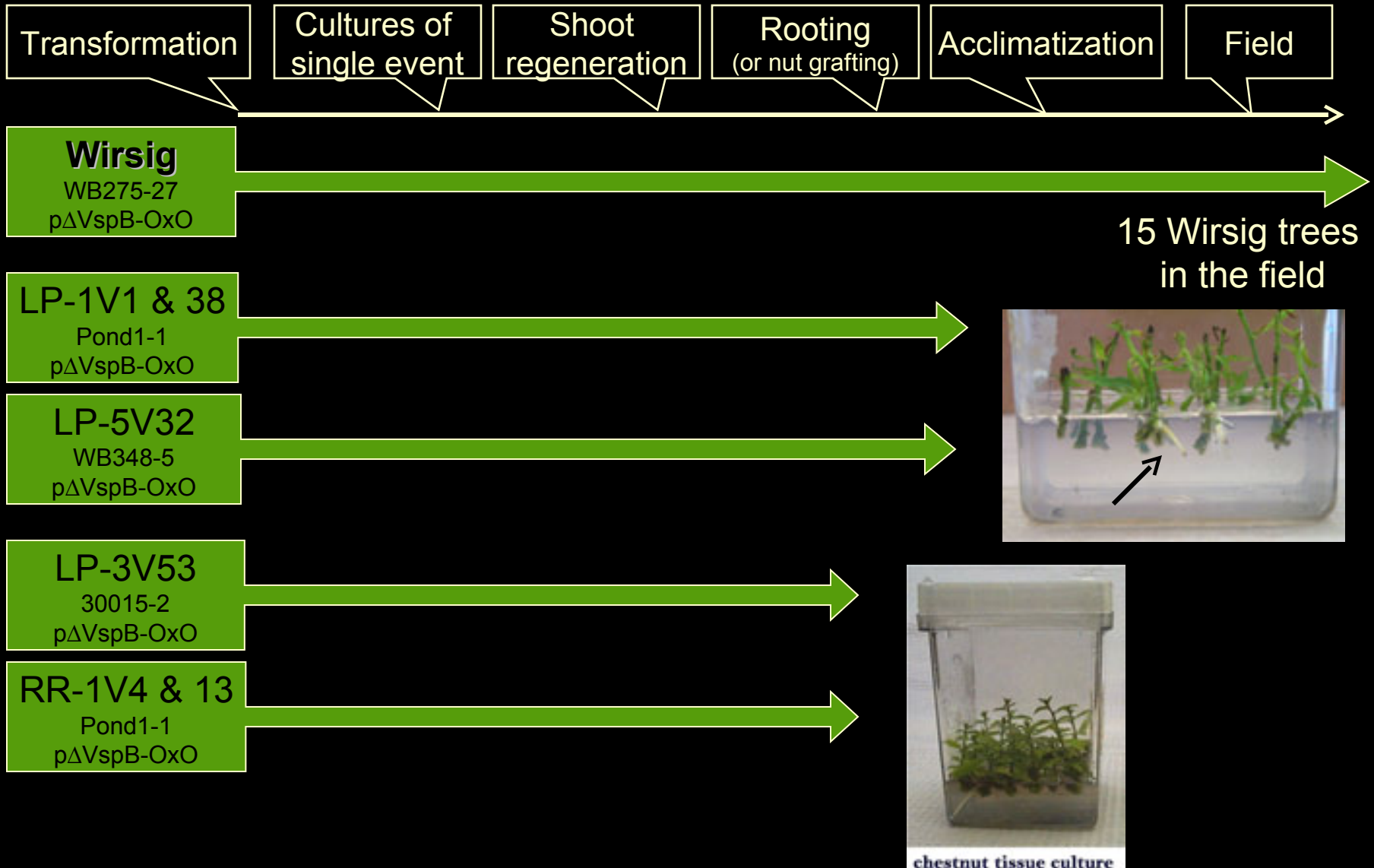


Steven N. Jeffers  
Clemson University

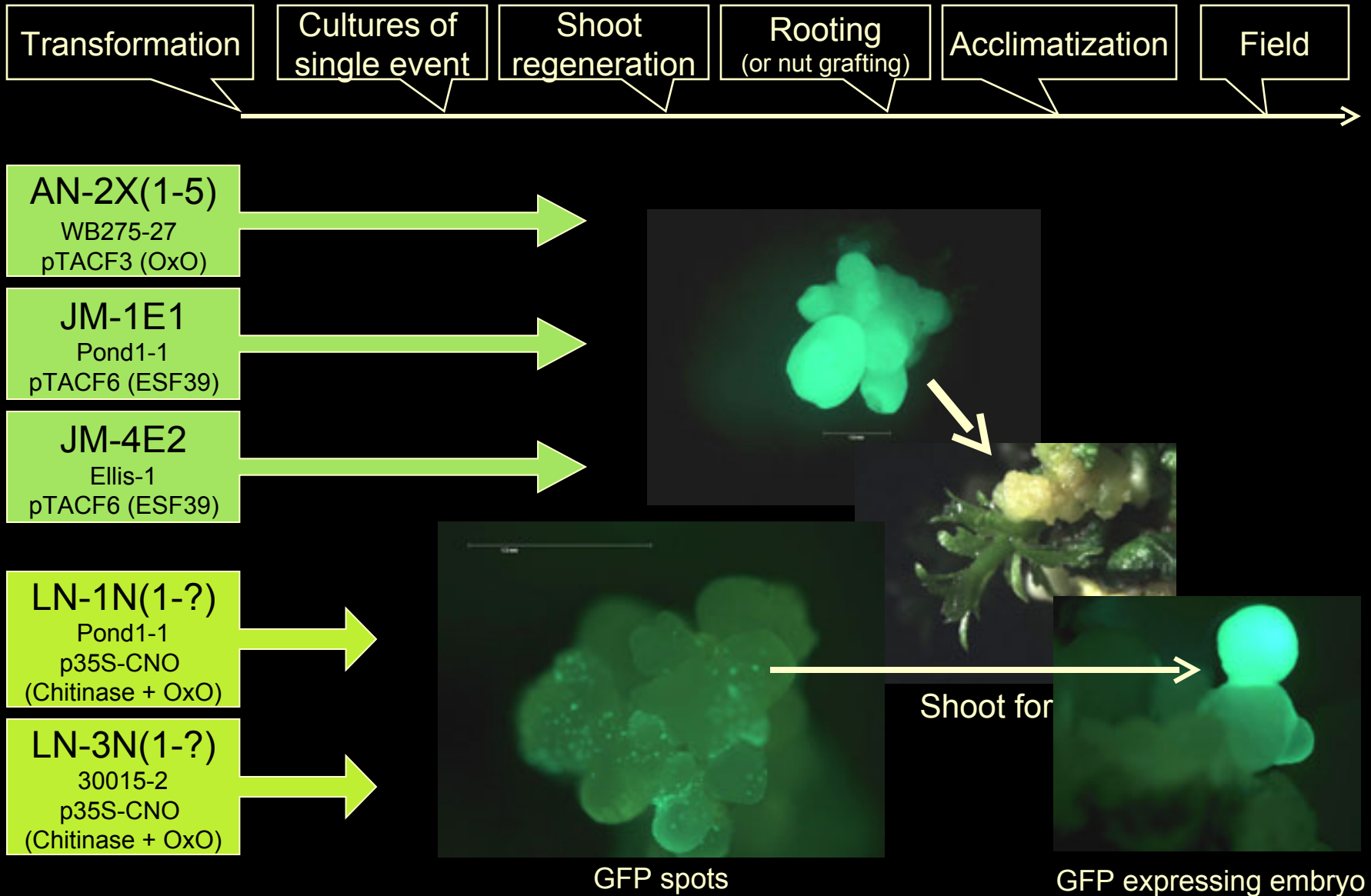
# American Chestnut Transformation & Regeneration Pipeline (~18 months)



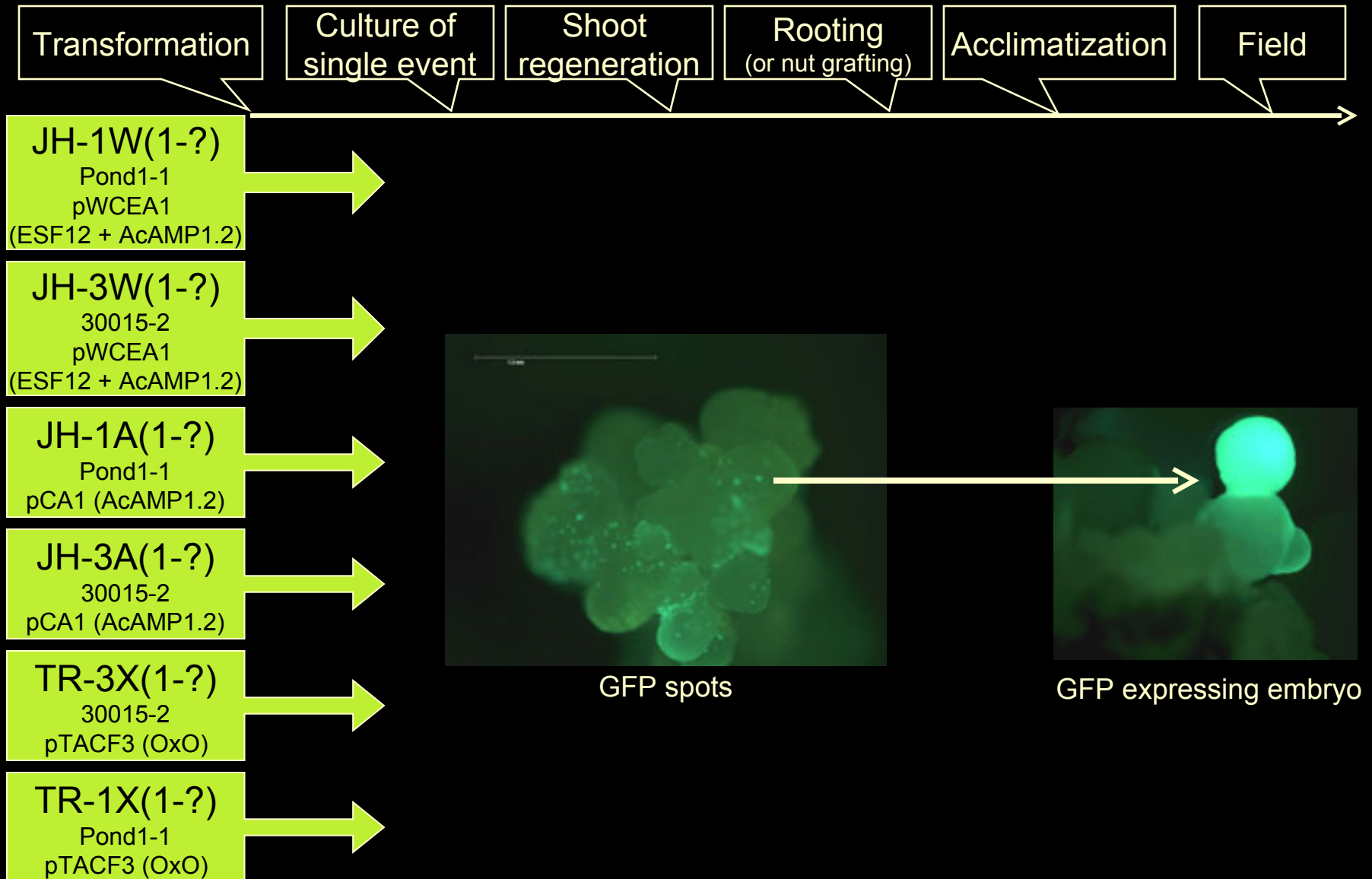
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Transformation

Culture of  
single event

Shoot  
regeneration

Rooting  
(or nut grafting)

Acclimatization

Field

LN-1P(1-?)

Pond1-1  
pTACF7  
(OxO + ESF39)

AZ-1C(1-?)

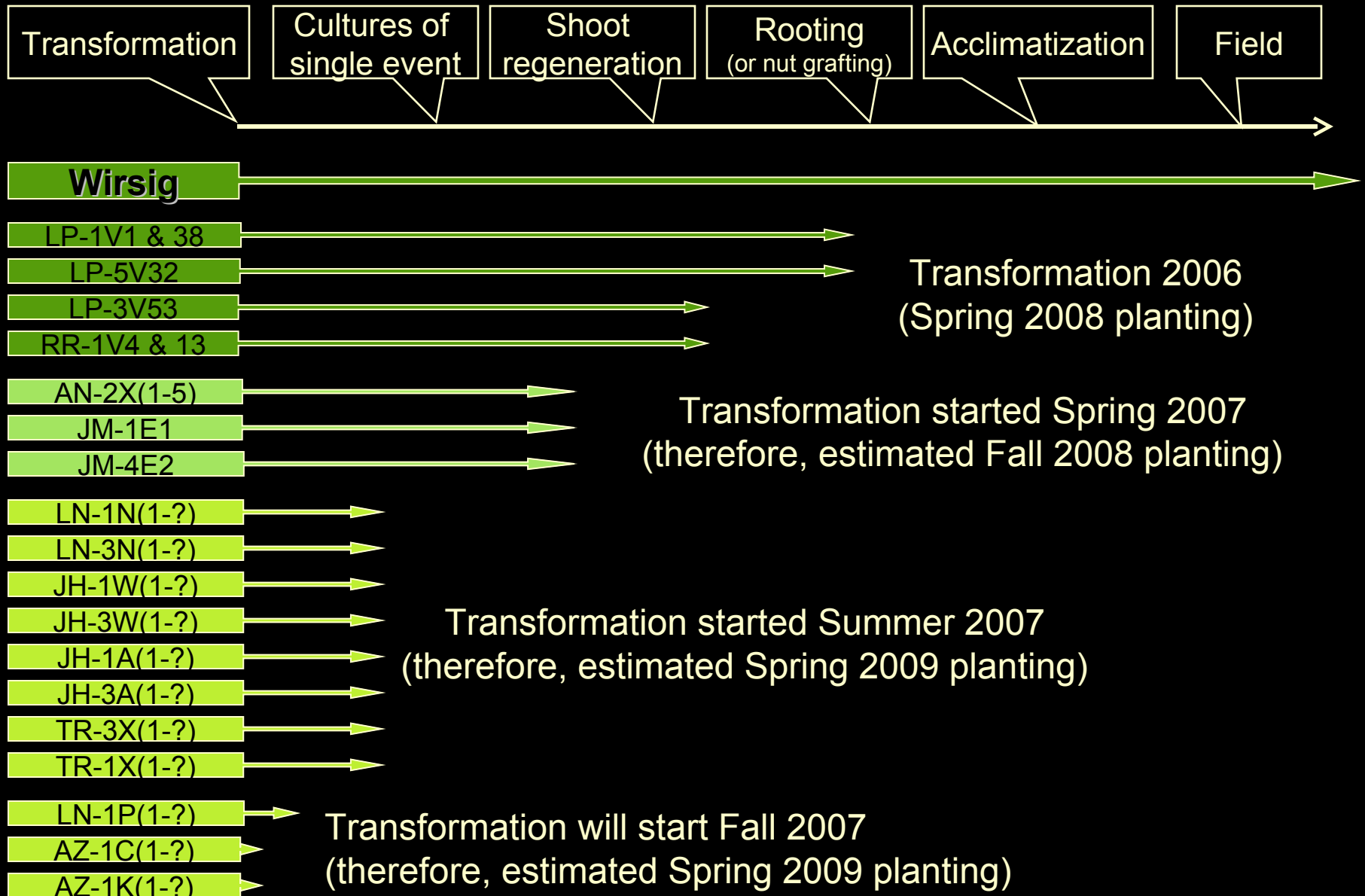
Pond1-1  
p35S-OxO  
(constitutive OxO)

AZ-1K(1-?)

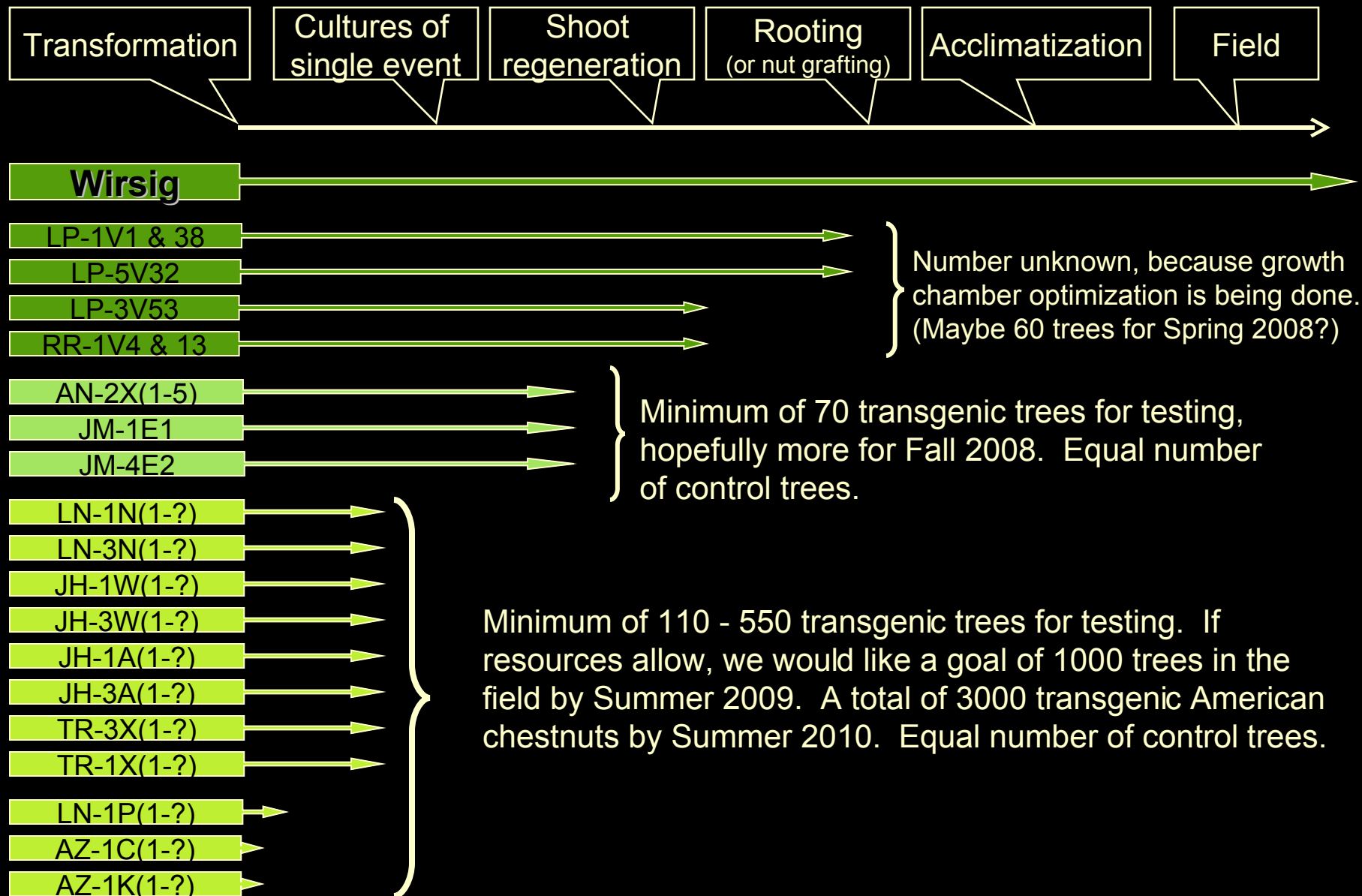
Pond1-1  
pWVK147  
(empty vector control)



# American Chestnut Transformation & Regeneration Pipeline (~18 months)



# Number of trees (try for minimum of 10 trees per event)



# Report on the 2006 & 2007 Syracuse Field Trials

- How is the Wirsig tree doing?
- The 2007 planting

**Transgenic American chestnut 'Wirsig'  
planted on 6/7/06**



# Transgenic American chestnut 'Wirsig' a week later



# Transgenic American chestnut 'Wirsig' four months later



# Transgenic American chestnut 'Wirsig' early Spring 2007



# Transgenic American chestnut 'Wirsig' early August 2007

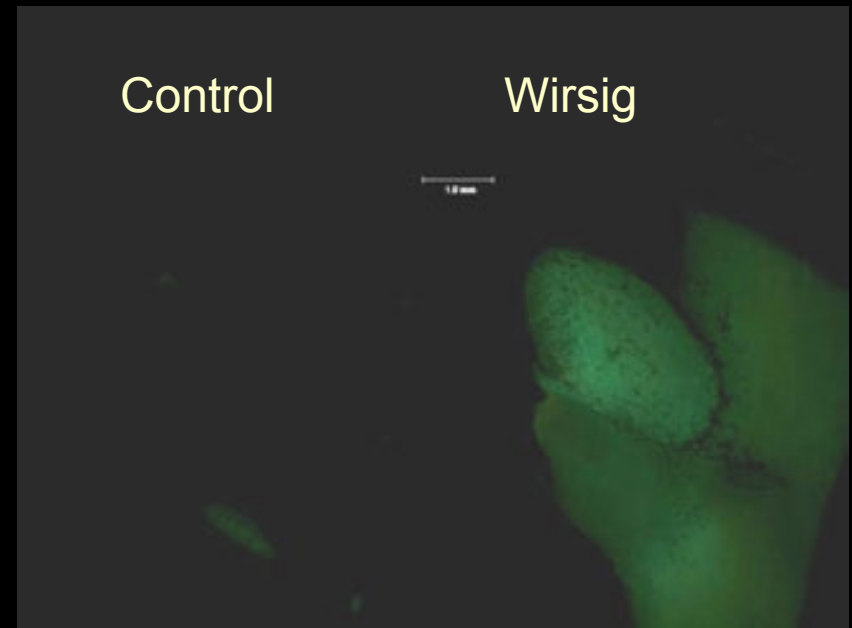




# Wirsig Variety (LP-2V28 event) 2 growing seasons later (~15 months)

White light

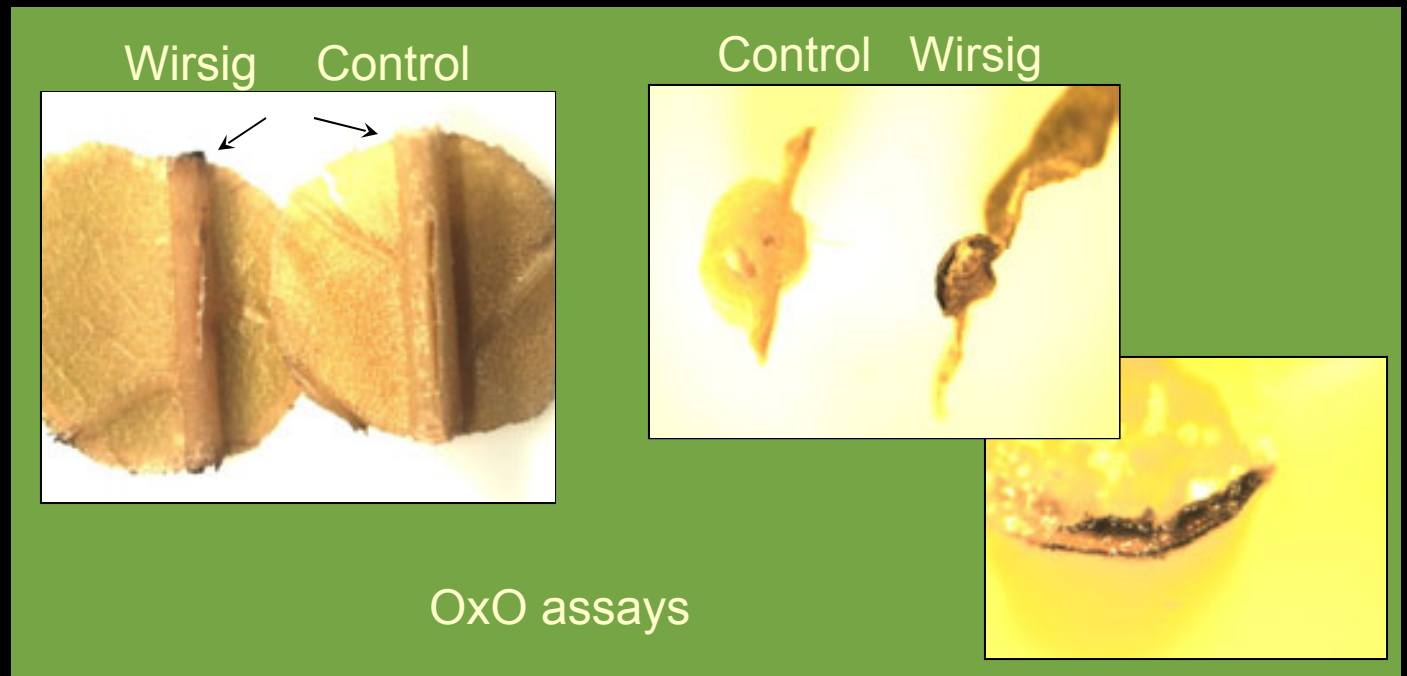
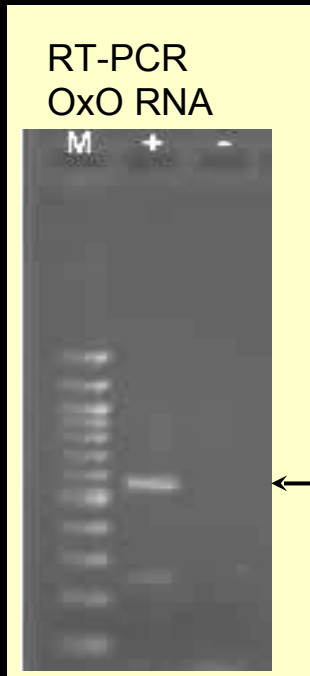
UV+GFP filter



Stable transformation



# Wirsig Variety (LP-2V28 event) 2 growing seasons later (~15 months)



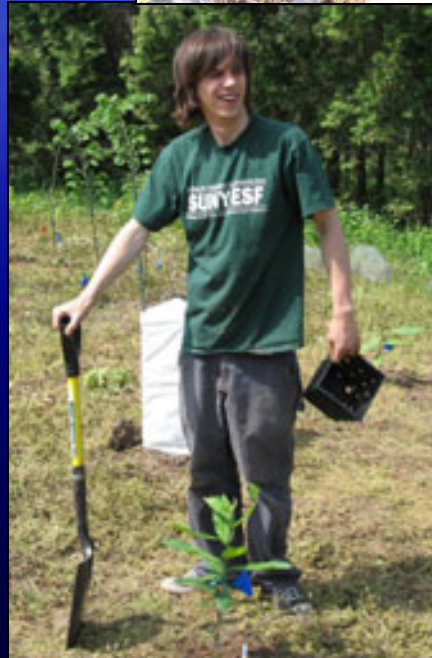
Stable transformation

# The 2007 Planting

First, we checked the roots.



# The Planting Event



# Chestnut Planting 5-15-07

Row	Tree 1	Tree 2	Tree 3	Tree 4	Tree 5	Tree 6
6	LP28, GB4	Dalton Chinese	Dalton Chinese	LP28, AD11	Amer PM3, Rep2, SS5	Amer PM1, SS3
5	LP28, GB2	Amer PM6, Rep2, SS3	Dalton Chinese	Dalton Chinese	Amer PM10, Rep2, SS2	LP28, GB3
4	LP28, AK7	Amer PM10, Rep2, SS3	LP28, AN4	Dalton Chinese	Dalton Chinese	Amer PM2, Rep2, SS4
3	LP28, AC7	Dalton Chinese	Dalton Chinese	American seedling	LP28, AD12	Amer PM11, Rep2, SS2
2	LP28, AM18	LP28, GB1	Amer PM3, Rep2, SS4	Amer PM10, Rep 2, SS5	Dalton Chinese	Dalton Chinese
1	Dalton Chinese	LP28, AN3	Amer PM3, Rep2, SS3	Amer PM3, Rep2, SS1	Dalton Chinese	LP28, AO14
Extra Row	LP1, P1-1, AO4	Nut Graft: NGP2. Rand nut, LP28 scion, grafted 1/18/07	Nut graft: NGP1. Wilson8 nut, LP44 scion, grafted 1/3/07	LP28, AD8	WIRSIG (planted 2006)	empty

## KEY:

Shaded cells indicate transgenic American chestnut

LP28 event = clone WB275-27 with p/spB-OxO

LP1 event = clone Pond1-1 with p/spB-OxO

LP44 event = clone WB275-27 with p/spB-OxO

AN3, AO14, etc. refer to the individual rooted shoot as per Linda's records

GB\_\_ = transgenic LP28 from Greg Boyd's experiment (numbers were added at date of planting)

American chestnuts from Alison Oakes' Potting Mix Study, spring 2007

# Chestnut Planting 5-15-07

Row	Tree 1	Tree 2	Tree 3	Tree 4	Tree 5	Tree 6
6	LP28, GB4	Dalton Chinese	Dalton Chinese	LP28, AD11	Amer PM3, Rep2, SS5	Amer PM1, SS3
5	LP28, GB2	Amer PM6, Rep2, SS3	Dalton Chinese	Dalton Chinese	Amer PM10, Rep2, SS2	LP28, GB3
4	LP28, AK7	Amer PM10, Rep2, SS3	LP28, AN4	Dalton Chinese	Dalton Chinese	Amer PM2, Rep2, SS4
3	LP28, AC7	Dalton Chinese	Dalton Chinese	American seedling	LP28, AD12	Amer PM11, Rep2, SS2
2	LP28, AM18	LP28, GB1	Amer PM3, Rep2, SS4	Amer PM10, Rep 2, SS5	Dalton Chinese	Dalton Chinese
1	Dalton Chinese	LP28, AN3	Amer PM3, Rep2, SS3	Amer PM3, Rep2, SS1	Dalton Chinese	LP28, AO14

The experiment contains:  
 12 transgenic plantlets  
 12 Chinese seedlings  
 12 American seedlings

The trees will be inoculated when they are large enough to provide good blight-resistance evaluations (2009 or 2010?).

# Transgenic American Chestnut

The original shoot died, but the new shoot shows dramatic growth.



# Other Ongoing or Recently Completed Projects

- Selecting locations for future field trials
- Small-stem resistance assay
- Potting mix study
- Increasing plantlet production capacity
- Nutgrafting

# 2008 Field Season Goals

- Outplant at least 10 trees from each of at least 5 transformation events (along with seedling controls)
- Working with the NY Chapter of TACF, identify and prepare at least three suitable sites for 2009 ... field tests

# Seedling resistance assay



# Differences in small stems

Chinese



American



# Small-stem resistance assay

American

Chinese



# Chestnut Potting Mix Study

A photograph of chestnut leaves. The leaves are generally green, but several prominent ones show significant yellowing and chlorosis, particularly along the veins and in the center of the leaf. The background shows more green foliage and a black plastic nursery tray.

Experimental Design

**11 Mixes**

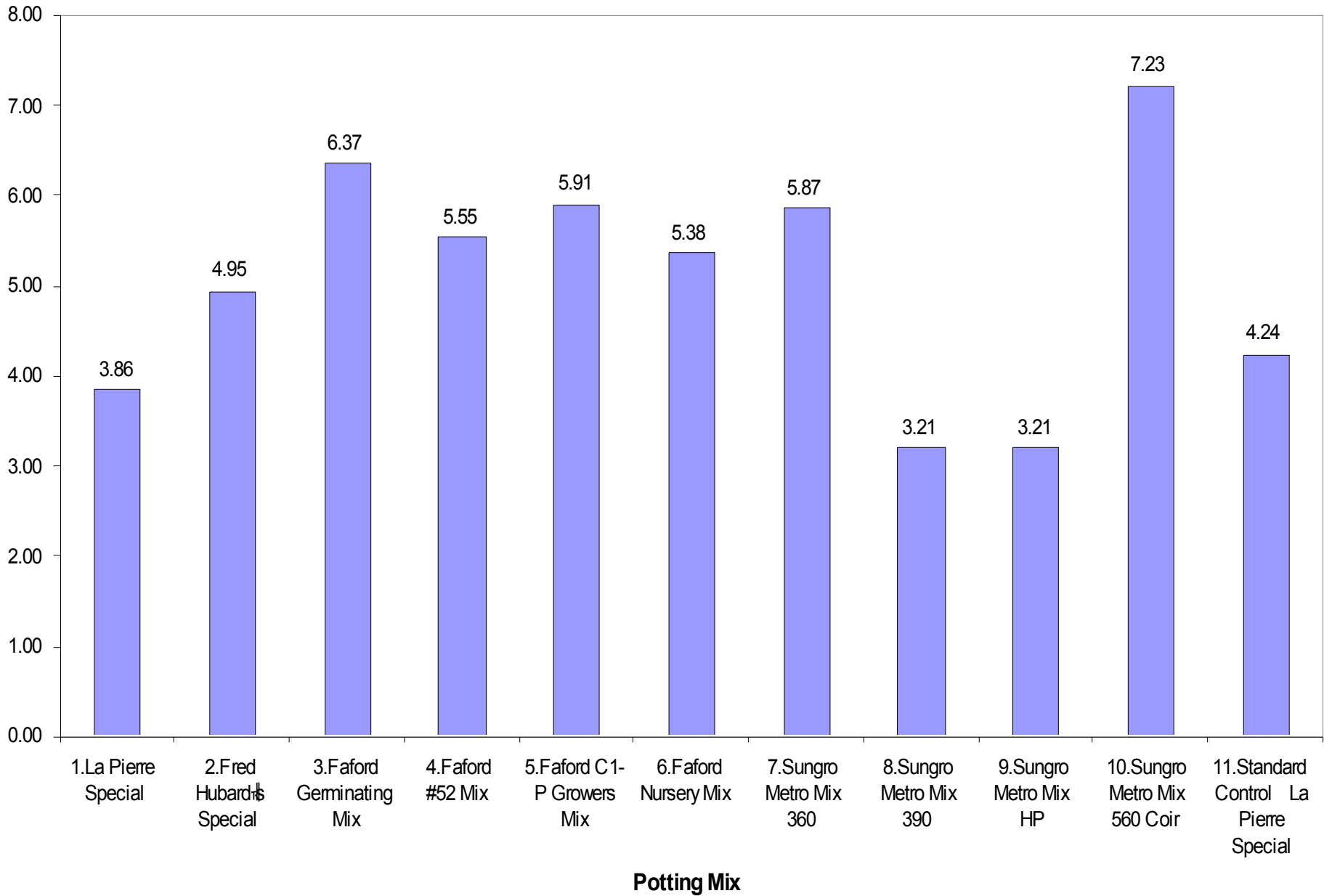
**5 Seedlots**

**3 Replications**

# Potting Mixes Tested

1. La Pierre Special (2 peat: 1 vermiculite: 1 perlite)
2. Fred Hebard's Special (1 peat: 1 vermiculite: 1 perlite)
3. Faford Germinating Mix
4. Faford #52 Mix
5. Faford C1-P Growers Mix
6. Faford Nursery Mix
7. Sungro Metro Mix 360
8. Sungro Metro Mix 390
9. Sungro Metro Mix HP
10. Sungro Metro Mix 560 Coir
11. Standard Control – La Pierre Special

# Ave. Total Dry Weight (g) by Potting Mix



# And the Winners Are:



# Results

## Avoid:

1. La Pierre Special (2 peat: 1 vermiculite: 1 perlite)
2. Fred Hebard's Special (1 peat: 1 vermiculite: 1 perlite)
3. Faford Germinating Mix
4. Faford #52 Mix
5. Faford C1-P Growers Mix
6. Faford Nursery Mix
7. Sungro Metro Mix 360
8. **Sungro Metro Mix 390**
9. **Sungro Metro Mix HP**
10. Sungro Metro Mix 560 Coir
11. Standard Control – La Pierre Special

**#8 - Sungro Metro  
Mix 390**



**#9 - Sungro Metro  
Mix HP**



# Building Capacity

## Greenhouse renovations

Pouring a new floor - **completed**

Installing new high-intensity lighting - **ongoing**

Installing humidity control - **design phase**

Replacing the growth room - **ongoing**

# Our old growth room (the “dungeon”) has seen better days.



- At least 30 years old
- Limited shelf space
- Very unreliable



**The new growth chambers are assembled and on their way.**



# Ongoing Activities

- Regenerating transgenic cell lines
- Building up stock plants
- Optimizing acclimatization parameters

# Nutgrafting Using American Chestnut Tissue Culture Shoots



**Linda McGuigan**





# Questions?

