Applying biotechnology to conserve and restore trees for Georgia's urban forest

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North American Forest Species Under Threat

- American chestnut (*Castanea dentata*) – chestnut blight, Phytophthora root rot
- American elm (*Ulmus americana*) – Dutch elm disease
- Eastern hemlock/Carolina hemlock (*Tsuga canadensis/Tsuga caroliniana*) – Hemlock woolly adelgid
- All North American ash (*Fraxinus)* species– Emerald ash borer
- Sugar maple (*Acer saccharrum*) – Asian longhorn beetle
- Black walnut (*Juglans nigra*) – Thousand cankers disease
- Oaks (*Quercus* spp.) – Sudden oak death
- Redbay/swamp bay/sassafras (*Persea* and *Sassafras* spp.) – Laurel wilt
- Fraser fir (*Abies fraseri*) – Balsam woolly adelgid
- Port Orford Cedar (*Chamaecyparis lawsoniana*) – POC root disease
- Atlantic white cedar (*Chamaecyparis thyoides*) – overcutting, loss of habitat

Some approaches to combatting established, invasive forest tree pests and pathogens

- Eradication
- Containment (quarantine)
- Biological control (predators, parasitoids)
- Chemical control
- Host resistance
  - Employ natural resistance within species using selection and breeding
  - Hybrid breeding—introgress genes from resistant relatives
  - Mutation breeding
  - Genetic engineering

Genetic resistance success story:

Multiple Dutch elm disease-resistant American elm varieties have been propagated via rooted cuttings

‘Princeton’ American elms on the UGA campus

‘Valley Forge’ and ‘New Harmony’ varieties were developed by A.M. Townsend and L.R. Schreiber at the US National Arboretum

The impact of host genetic resistance programs can be greatly leveraged by *in vitro* mass clonal propagation

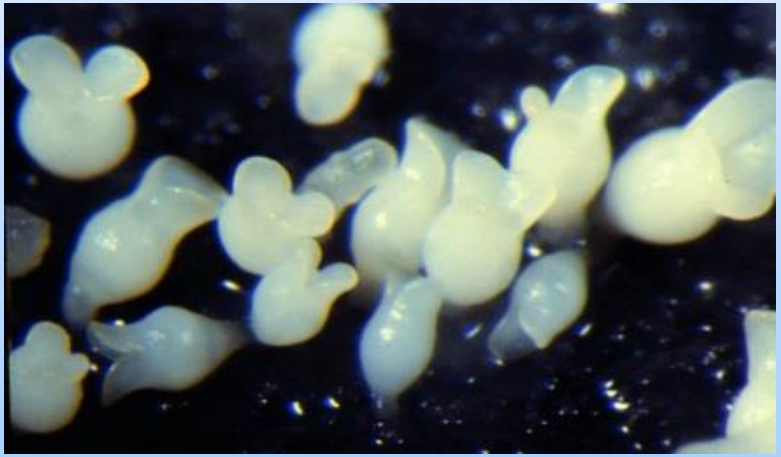
Types of *in vitro* propagation

- Micropropagation (Axillary shoot culture)
- Organogenesis (Adventitious shoots)
- Somatic embryogenesis

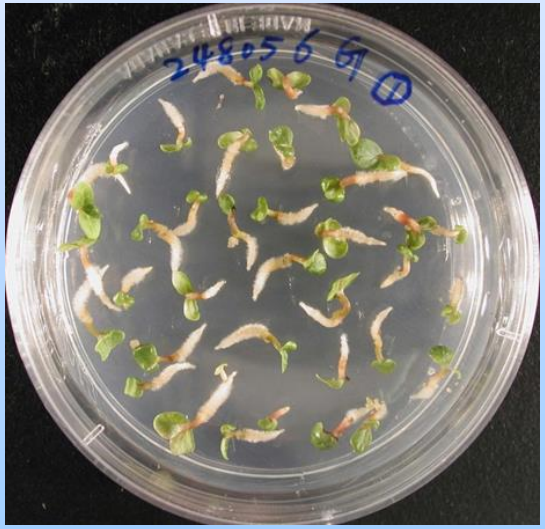
In vitro propagation (tissue culture) is performed in a sterile bench to prevent contamination by fungi and bacteria

Somatic Embryogenesis (SE):

A process by which structures (“somatic embryos”) resembling seed embryos are produced asexually. These somatic embryos can be germinated like seeds to produce clonal seedling-like plantlets (“somatic seedlings”)



Yellow-poplar somatic embryos



Germinating somatic embryos

Somatic seedlings

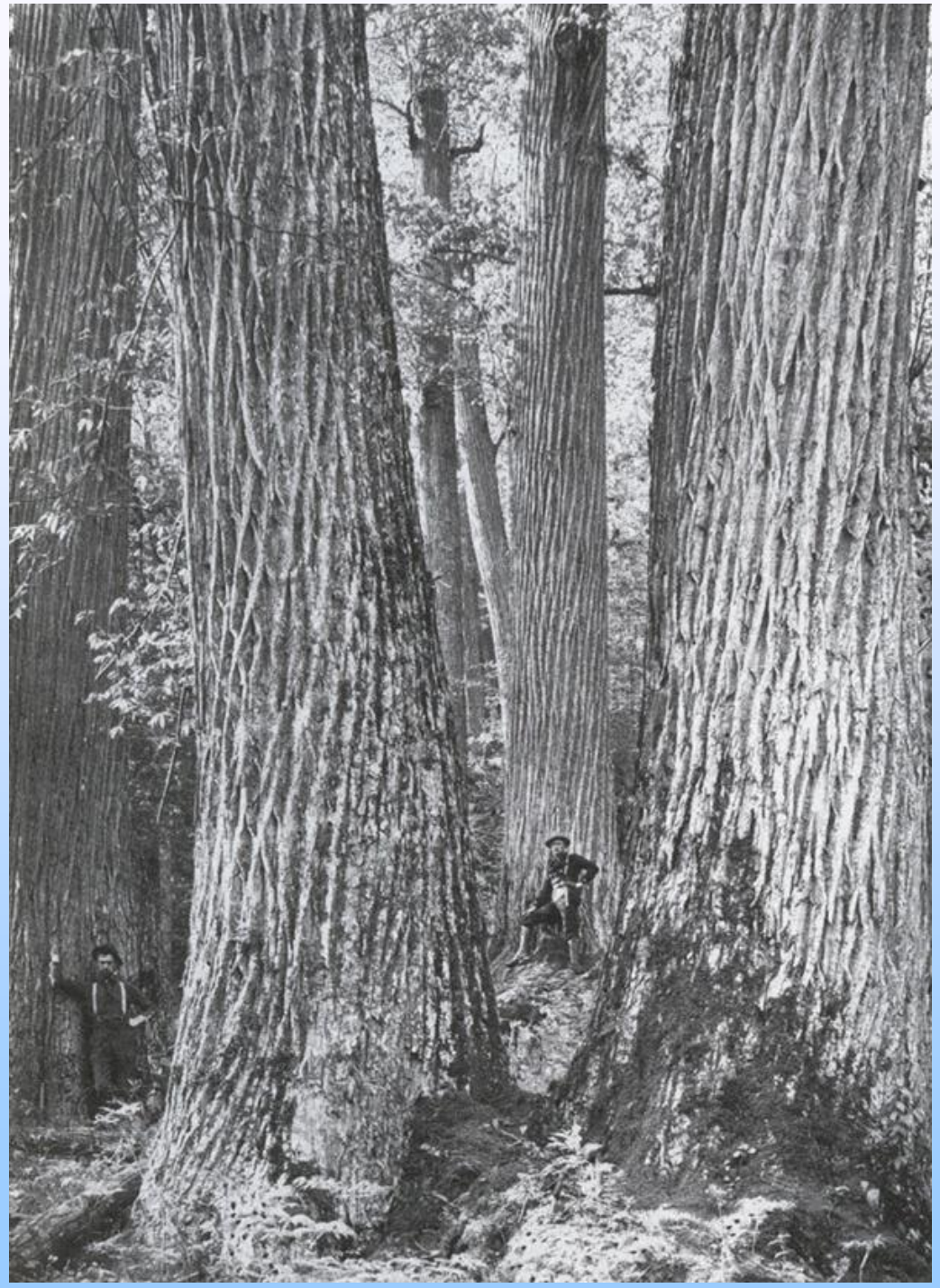
Potential applications of SE systems for threatened forest species

- For mass clonal propagation of promising pest- or pathogen-resistant/tolerant genotypes generated by selection and breeding programs
- To help conserve genetic diversity of threatened species populations
  - SE cultures are easy to cryostore and recover
- To provide a means of regenerating transgenic trees engineered for insect- or disease-resistance/tolerance
  - For candidate resistance gene testing
  - For deployment – could happen for one forest tree soon

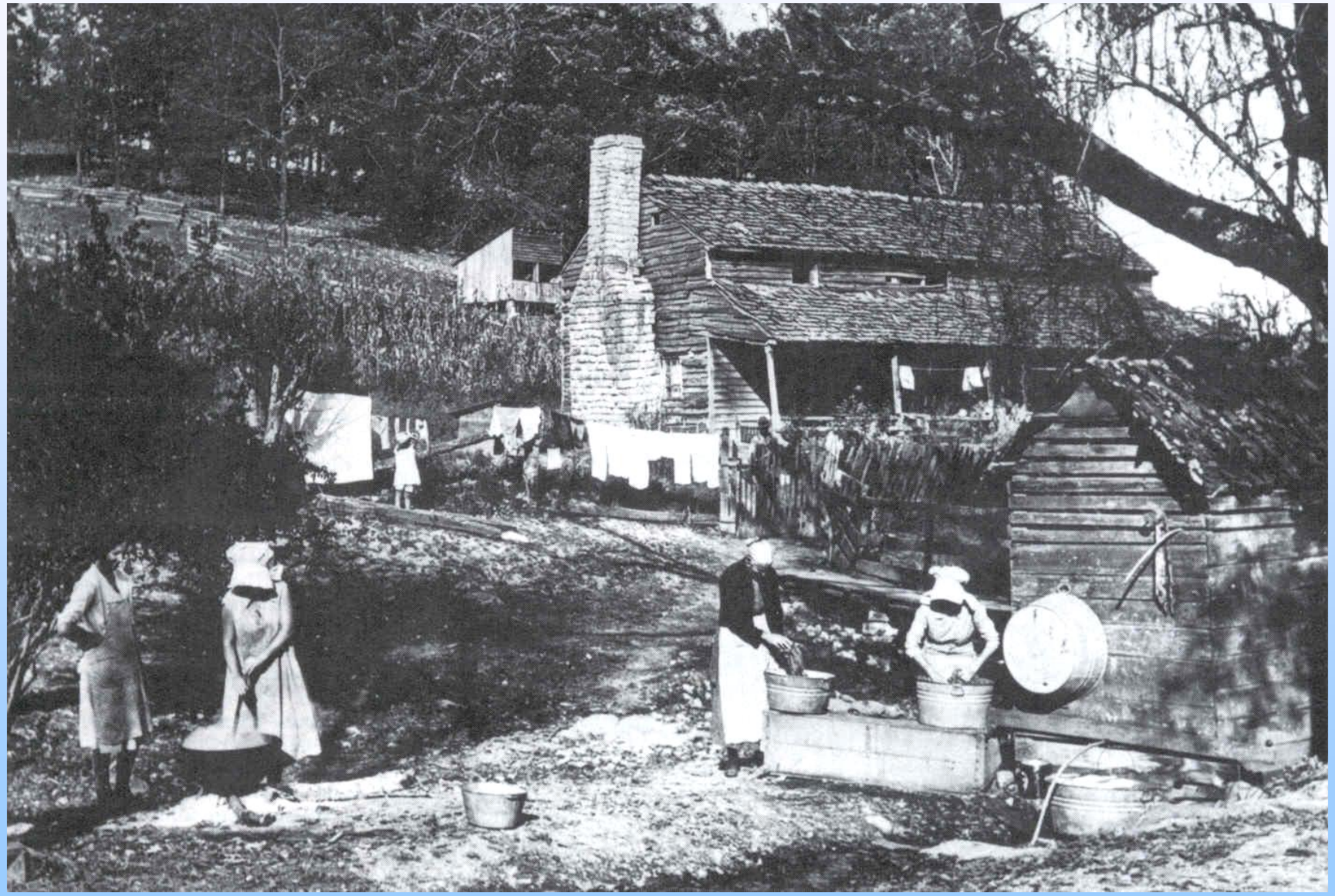
Examples of development and application of in vitro propagation for threatened tree species

- American chestnut (*Castanea dentata*)
- Eastern and Carolina hemlocks (*Tsuga canadensis* and *Tsuga caroliniana*)
- Ash species (*Fraxinus pennsylvanica, Fraxinus americana, Fraxinus latifolia*)
- Franklin tree (*Franklinia alatamaha*)

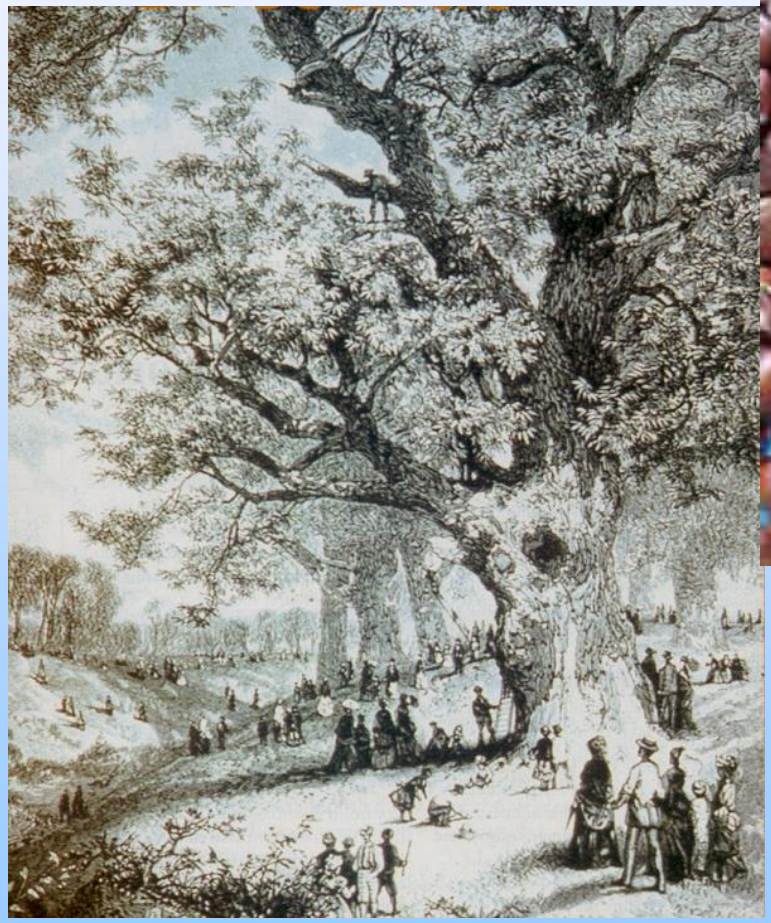
American chestnut in the Great Smoky Mountains of North Carolina, c. 1900

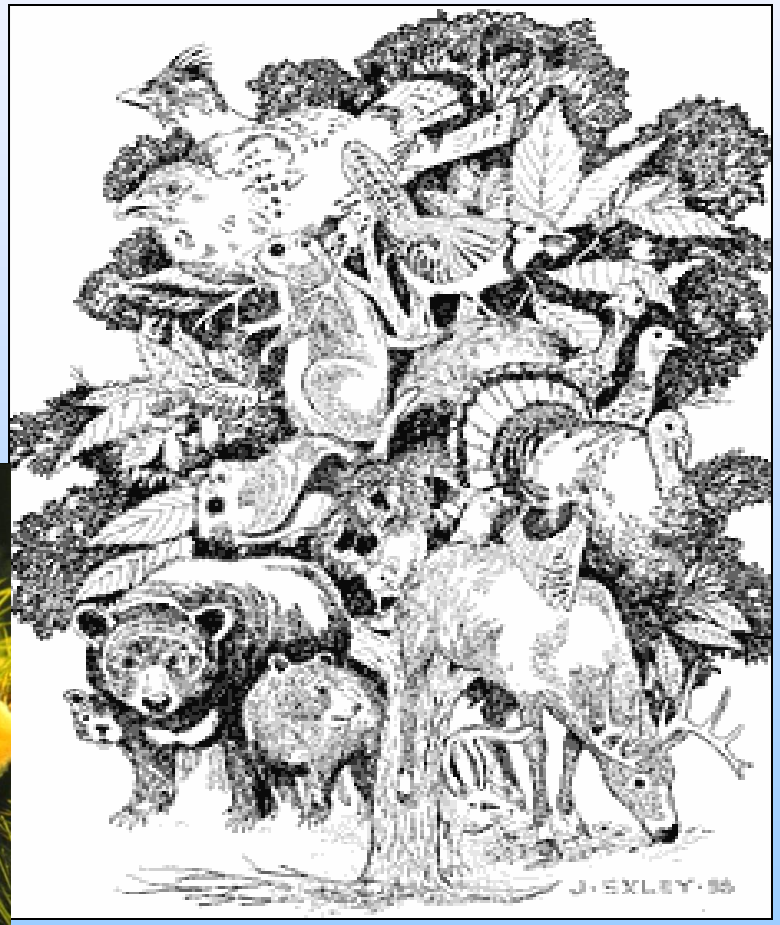


American Chestnut Was Central to Appalachian Life



Nuts from American chestnut were a staple for people and wildlife



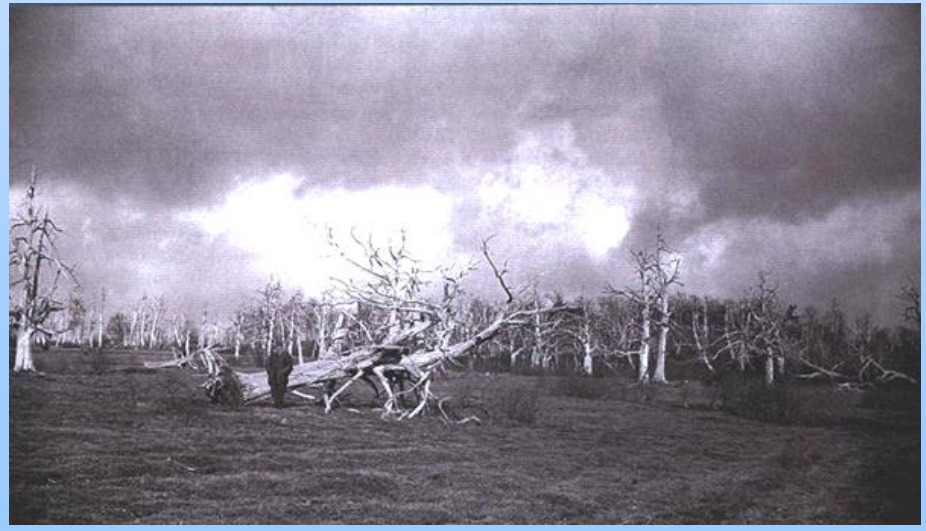


The chestnut blight fungus (*Cryphonectria parasitica)* was accidentally introduced to the U.S. on Asian chestnut trees

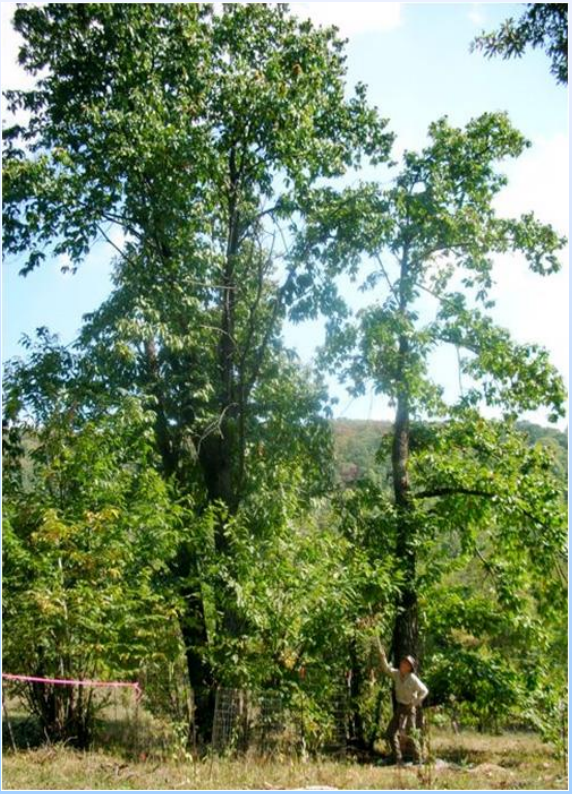
The fungus is necrotrophic, killing tissue with oxalic acid. The infection forms a sunken canker, eventually girdling the tree

Chestnut blight wiped out ~4 billion American chestnut trees between 1904 and 1950

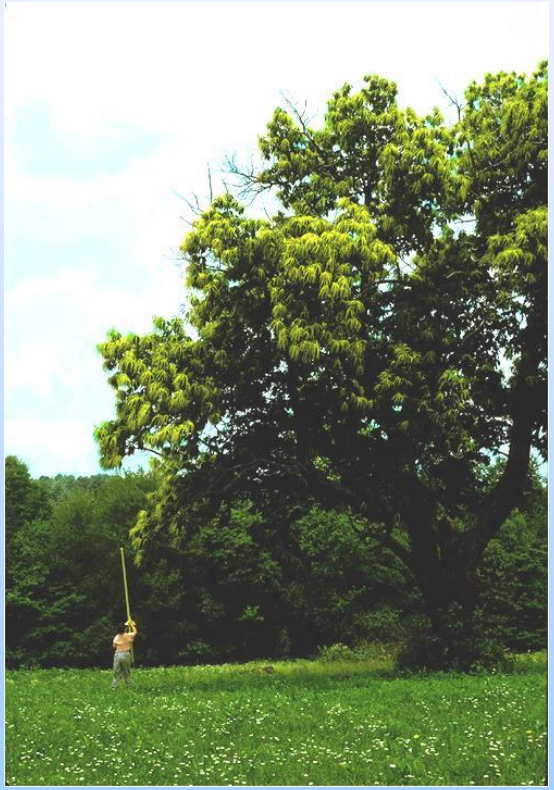
Photo courtesy Great Smoky Mountains National Park Library and The American Chestnut Foundation



The American Chestnut Cooperators Foundation (ACCF) breeds among Large Surviving American (LSA) chestnuts to produce progeny with blight resistance



Thompson and Ragged Mountain Trees

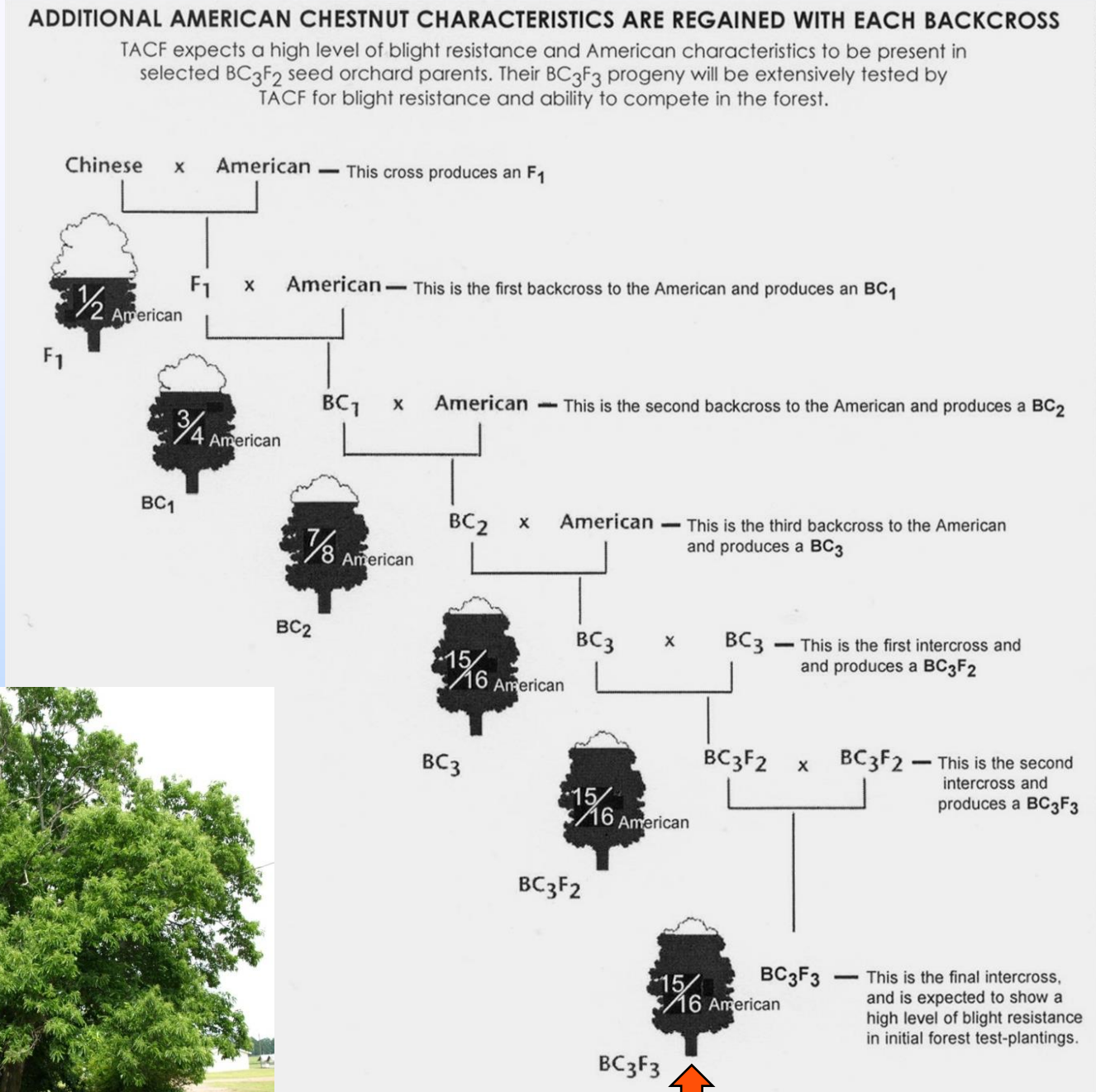


Amherst, VA Tree

Adair County, KY Tree

The American Chestnut Foundation's (TACF) backcross breeding program with Chinese chestnut

Chinese chestnut is blight resistant, but is not a canopy tree



Open-pollinated seed production

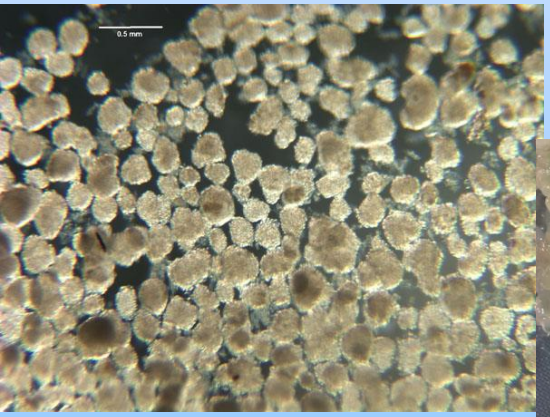
Development of SE technology for American chestnut and hybrid chestnuts at UGA

- 1989 – first embryogenic cultures established
- 1997 – first somatic seedlings germinated
- 2000 – cryopreservation applied to cultures
- 2001 – first somatic seedlings planted
- 2005 – Suspension culture-based system established

American Chestnut Medium Recipe

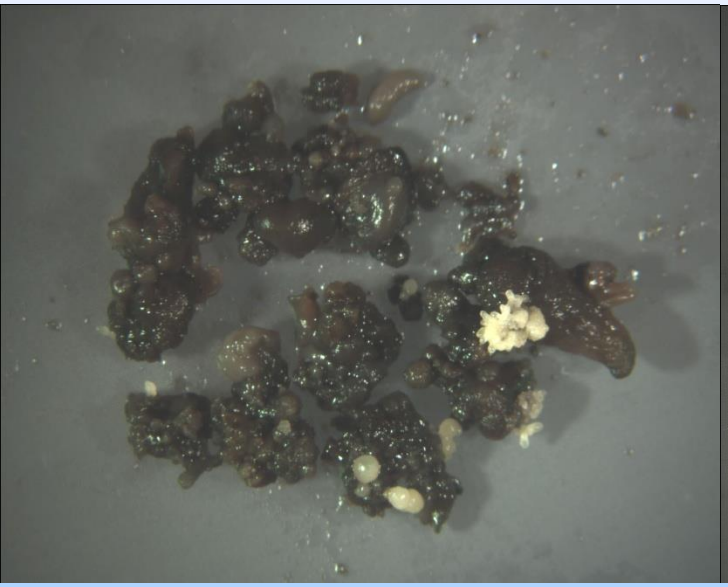
- Lloyd and McCown's Woody Plant Medium major salts (N, P, K, Ca, Mg)
- Lloyd and McCown's Wood Plant Medium minor salts (Mn, Zn, Cu, B, Mo)
- Murashige and Skoog's Iron (Fe)
- Schenk and Hildebrandt's vitamins (thiamine, nicotinic acid, pyridoxine)
- 30 g/l sucrose
- 0.5 g/l L-glutamine
- 2 or 4 mg/l 2,4-D (auxin)
- Gelled with Phytagel gellan gum

Scaled-up production using suspension cultures

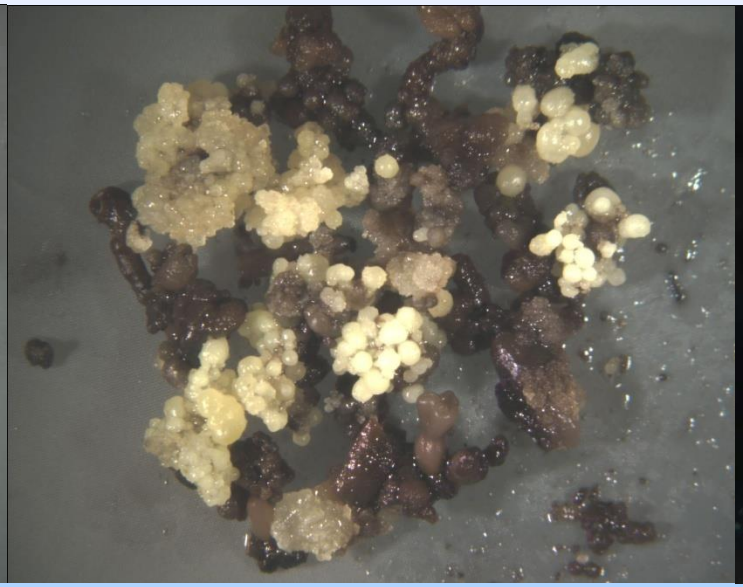


Cryostorage of Embryogenic Cultures in Liquid Nitrogen

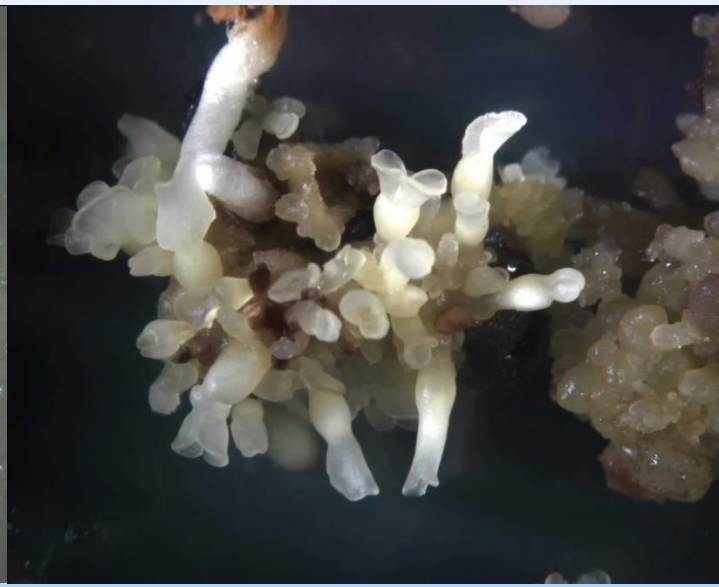
Regrowth of American chestnut embryogenic material following removal from liquid nitrogen



1 week



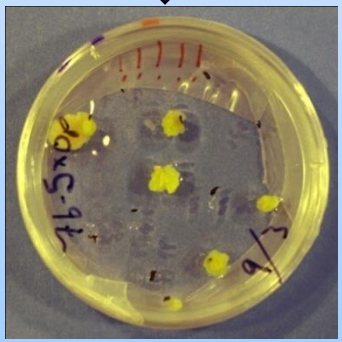
2 weeks



SE production

Selection/breeding programs, SE and cryostorage are a powerful combination for chestnut restoration

Selected trees crossed to get seeds



Seeds from crosses used to start SE cultures

"Captured" SE cultures

Somatic seedling production

Copies of all cultures cryostored

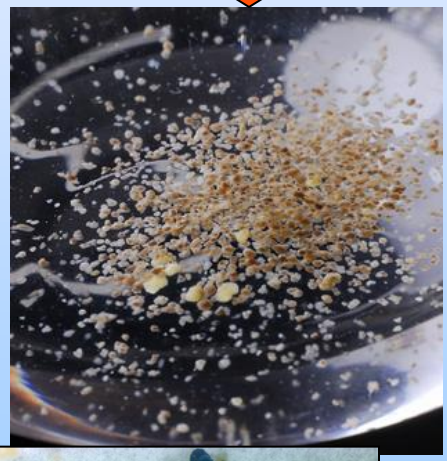
Screening Results

Somatic seedlings screened/field tested

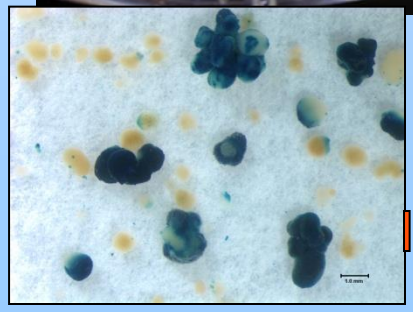
Best clones recovered from cryo and mass propagated for planting

Embryogenic cultures are used as target material for gene transfer

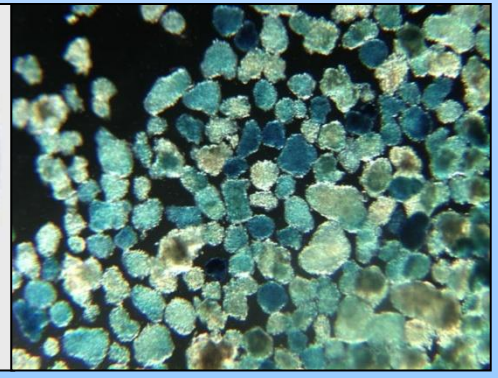
1. Agrobacterium infection of chestnut embryogenic cells



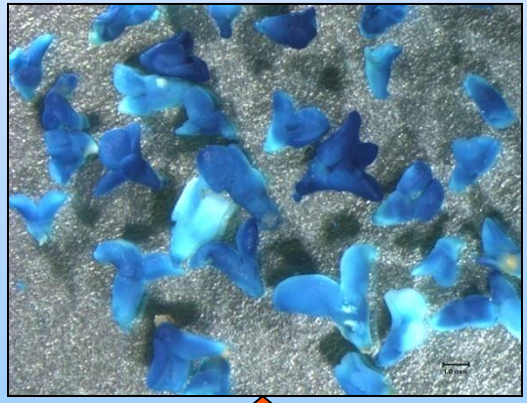
2. Selection of transgenic events



3. Proliferation of individual events



4. Somatic embryo production



5. Somatic seedling production

Dr. Bill Powell's (SUNY-ESF) results of inoculations with chestnut blight fungus with transgenic oxalate oxidase (*OxO*) chestnut

American chestnut

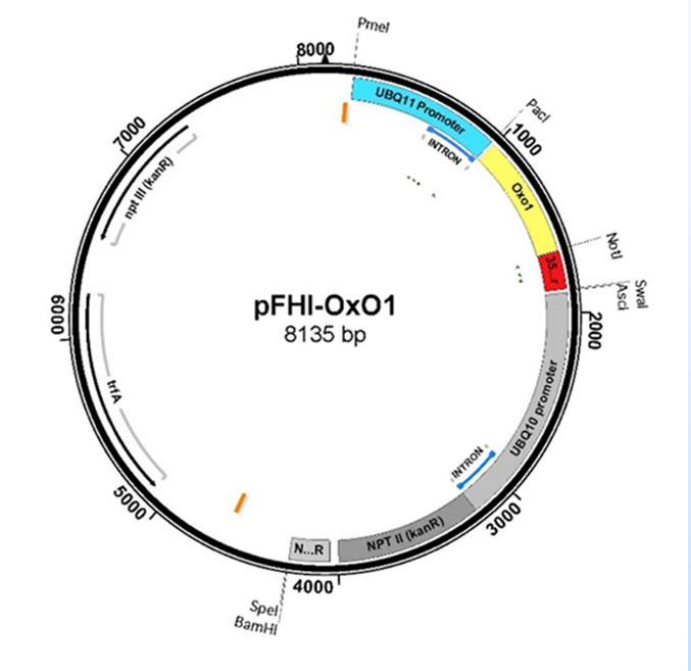
Chinese chestnut

Darling 58 (transgenic *OxO*)

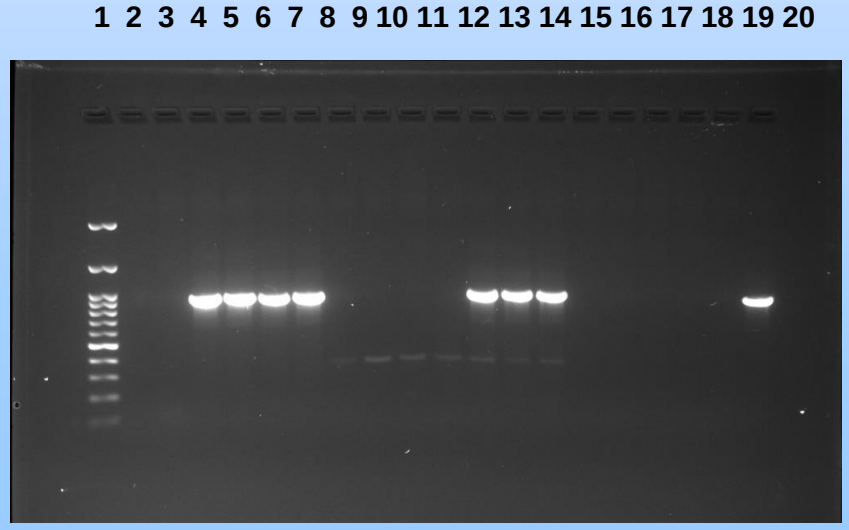
SUNY-ESF is currently pursuing "nonregulated" status from USDA, EPA and FDA for American chestnut trees engineered with the *OxO* gene

What my lab is working on with TACF now?

New *OxO* transformations with new lines and DNA vectors



- Over 80 PCR-positive *OxO* transgenic events in 9 new American chestnut culture lines, representing all regions of the range, (including GA!) have been generated
- Somatic embryos produced from multiple PCR-positive lines, including Georgia lines
- First PCR-positive shoots produced from somatic embryos should be large enough to root soon.

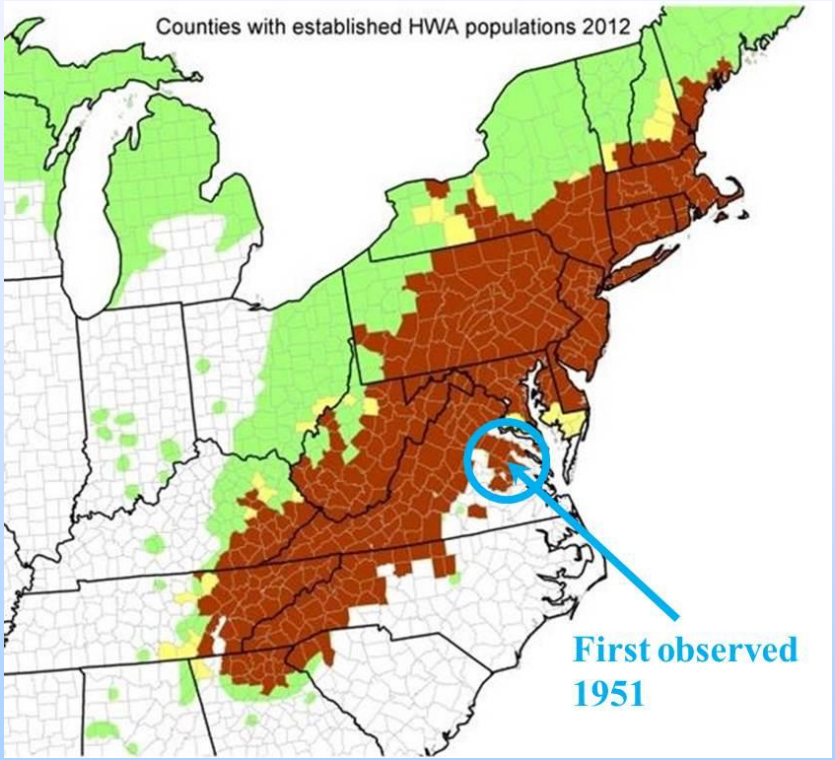


But I want to plant some chestnuts now!

Some sources of blight-resistant chestnut trees

- Hybrid backcross nuts from TACF—join your Georgia Chapter of TACF (https://tacf.org/ga/about-us/)
- Route 9 Cooperative (https://route9cooperative.com/) sells hybrid nuts for planting to produce trees for nut production or timber production
- Chestnut Hill Nursery (https://chestnuthilltreefarm.com/) sells Dunstan hybrid chestnuts

Eastern and Carolina hemlocks and Hemlock Woolly Adelgid (HWA)

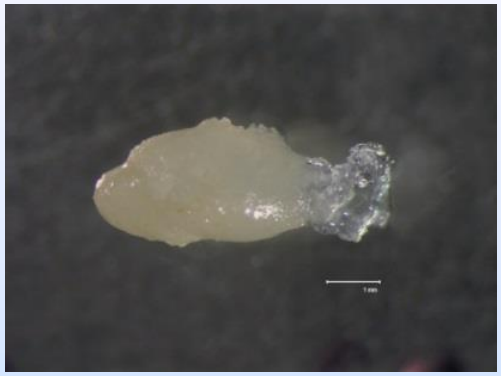
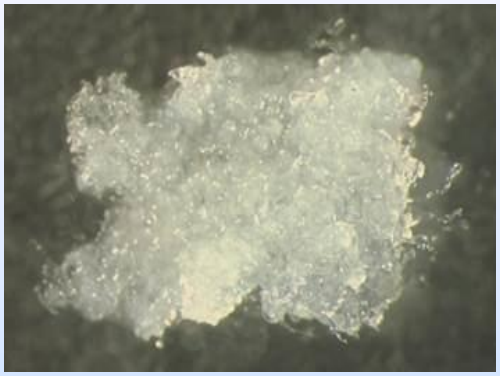


Great Smoky Mountains National Park

Uninfested Counties
Infested Counties
Newly Infested in 2012
Native Range of Hemlock

HWA was accidentally imported from Japan and first seen in the eastern U.S. in the 1950s. Both hemlock species in the Eastern US may be lost

SE propagation of hybrid hemlocks (bred with HWA-resistant Asian species) for clonal testing and restoration

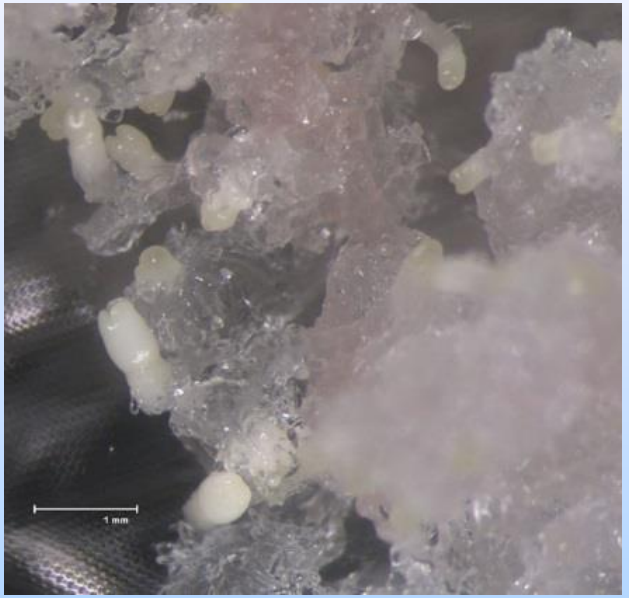





Breeding between North American and Asian hemlocks (*T. caroliniana* x *T. chinensis* and *T. caroliniana* x *T. sieboldii*) was performed by NC State University collaborator Dr. Ben Smith, and seeds were cultures to start hybrid hemlock cultures. Somatic seedlings now being tested by Dr. Smith for resistance/tolerance to HWA

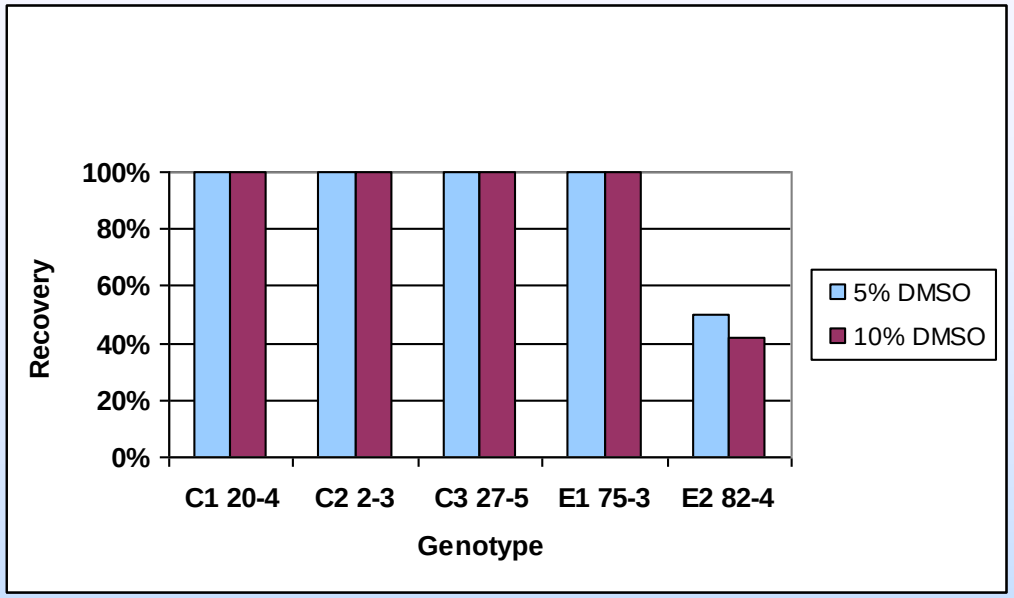
Propagating clones derived from seeds of surviving, putatively HWA-tolerant hemlocks

Cultures were initiated from seeds collected from eastern hemlocks in the “Bulletproof” stand in NJ and the Lee stand in NC

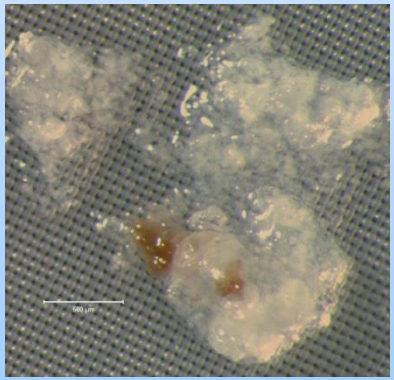


Somatic embryos from NJ Tree No. 3.5

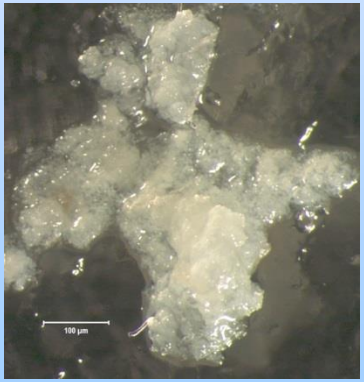
Cryopreservation of hemlock germplasm



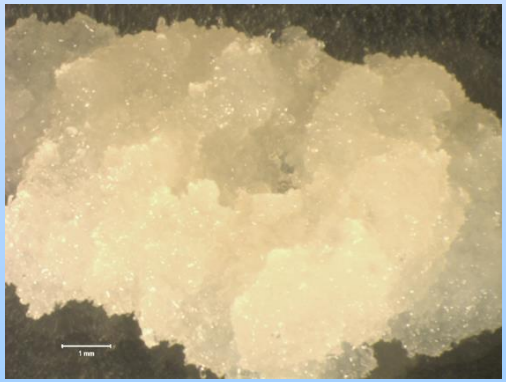
Regrowth of hemlock callus following recovery from cryostorage



2 weeks



4 weeks



7 weeks

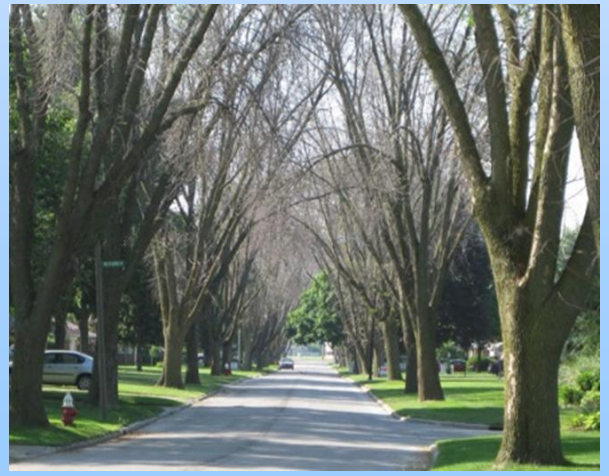
One of the Carolina hemlock cultures successfully cryostored was from one of the few surviving Tallulah Gorge, GA trees

White ash (*Fraxinus americana*) and green ash (*Fraxinus pennsylvanica*) are valuable landscape trees and forest trees for wood products

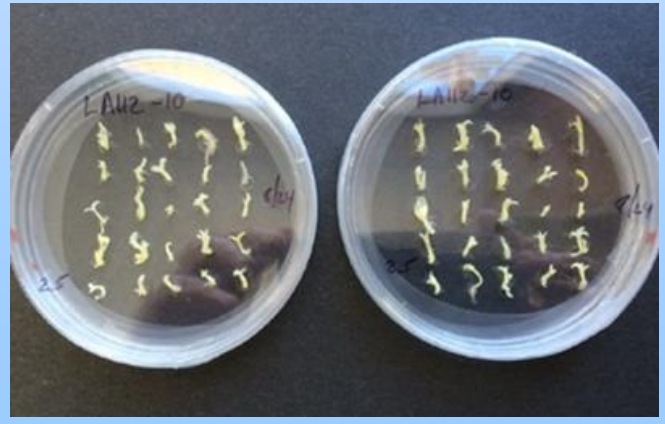
White ash is the best wood for baseball bats

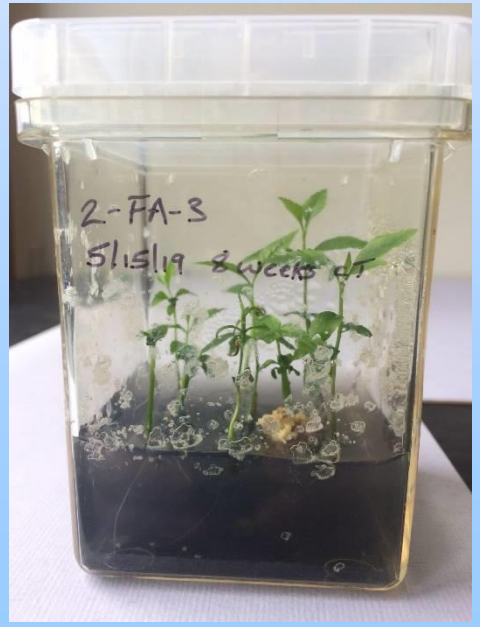
All North American ash species, including white Ash and Green Ash are attacked by the Emerald Ash Borer (EAB)

- EAB accidentally introduced from Asia
- EAB first discovered in Michigan in 2002
- EAB has already killed millions of ash trees in 35 US states and Canada
- First sightings in Georgia summer 2013

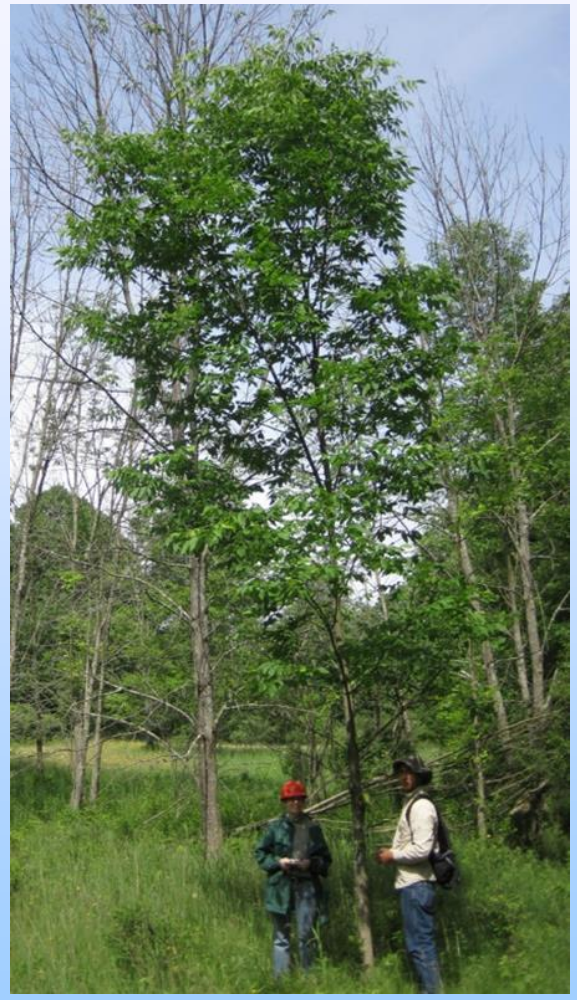


In vitro culture for clonal propagation of ash trees

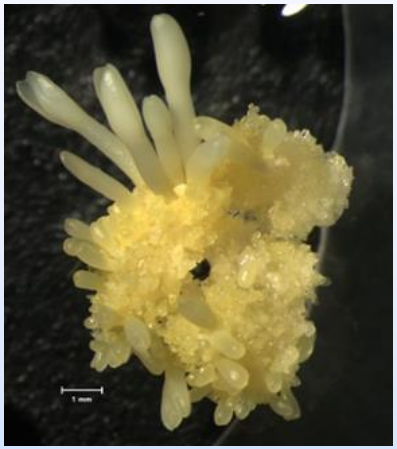




Lingering ash somatic embryogenesis for clonal testing for EAB resistance and to create new ash varieties for restoration



Lingering white ash tree in Michigan (photo courtesy Dan Herms)

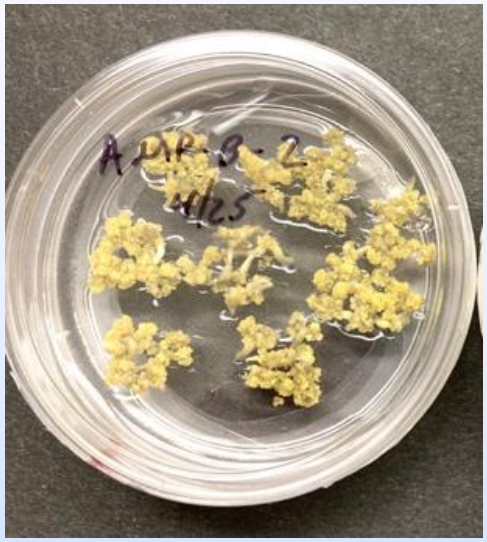


White ash SEs from OP seed collected from lingering white ash parent

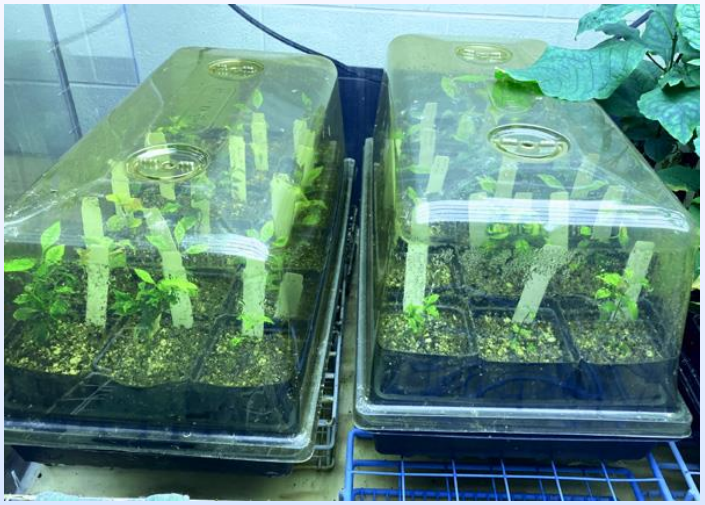
SEs from cross between lingering green ash parents

First field test of lingering ash clones at UGA's Whitehall Forest in 2022

Georgia lingering ash cultures



Embryogenic cultures were started from lingering green ash collected in Atlanta Memorial Park (AMP) by Trees Atlanta cooperators and in Murray Co., GA, by GFC cooperators.



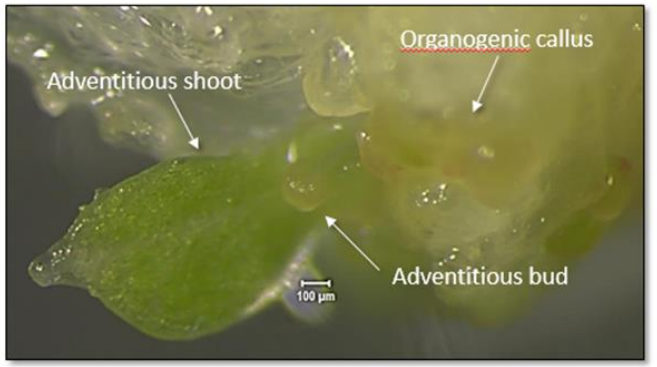
AMP lingering ash plantlets undergoing acclimatization February 2024 (above) and the same plantlets in May 2024 (below)

Plantlets from Georgia lingering ash clones

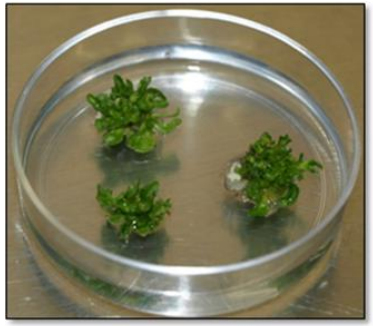
Franklinia alatamaha (Franklin tree), a Georgia native, has not been seen in the wild since 1803— We can propagate it in vitro via shoot cultures

In vitro Plantlet Regeneration of *Franklinia alatamaha*

Explant- Immature fruit



Shoot Organogenesis



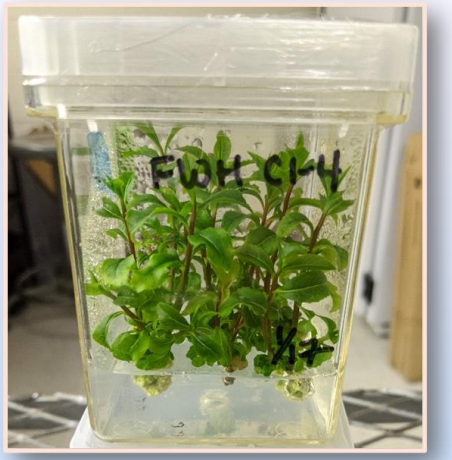
Shoot multiplication

Shoot elongation

Rooting & Acclimation

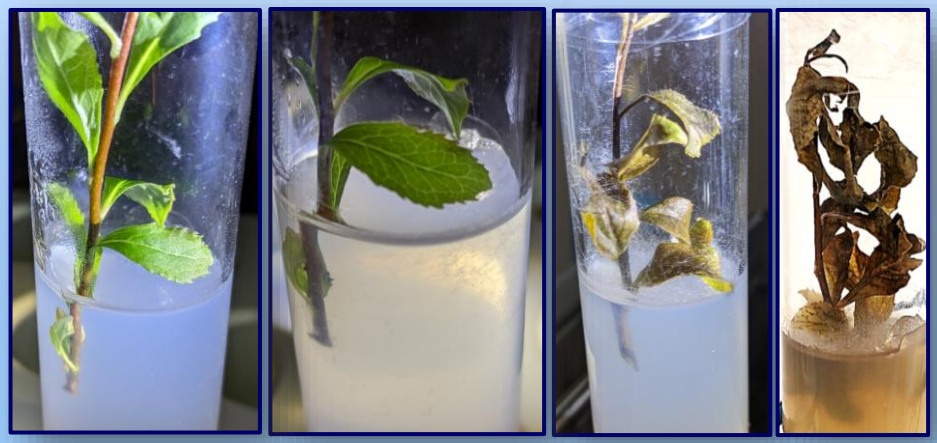
Producing root rot resistant Franklinia using mutagenized shoot cultures

Franklinia tree killed by Phytophthora root rot



Franklinia shoot culture

Franklinia nodal segments were gamma-irradiated for mutagenesis



Disease progression with shoots infected in vitro

These mutagenized shoots have survived for 10 months without any disease symptoms

Lab Personnel

Ryan Tull
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Professional II

Heather Gladfelter
Postdoc

Paul Montello
Research
Professional III

Ryan McNeill
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Nicole Locke
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Acknowledgements

Forest Health Initiative

The American Chestnut Foundation

The American Chestnut Cooperators Foundation

Frank and Evelyn Schmidt Foundation

Horticultural Research Institute

USDA Forest Service

Camcore

Trees Atlanta

Jared Westbrook (TACF)

Bill Powell (SUNY-ESF)

Chuck Maynard (SUNY-ESF)

Rusty Rhea (USFS)

Robert Jetton (Camcore)

Ben Smith (NCSU)

Mark Mayer (NJ Dept. of Agriculture)

Dan Herms (Davey Tree Expert Co.)

Diane Hartzler (Ohio State University)

Brandon Merz (Georgia Forestry Commission)

Brian Williams (Trees Atlanta)

Emily Roth (Trees Atlanta)