SUNY College of Environmental Science & Forestry (ESF)
The American Chestnut Foundation (TACF)

A not-for-profit, university and public collaboration to

Restore the American chestnut tree

Restoring a keystone species has never been done before!

Mountainsides white with chestnut blooms near Skyland Cabins, Shenandoah National Park, 1912. Courtesy of Shenandoah National Park, National Park Service, Skyland Glass Slide Photograph Collection, Item #205

Wood products

Medicinal uses

Science News *from research organizations*

Chestnut leaves yield extract that disarms deadly staph bacteria

Extract shuts down staph without boosting its drug resistance

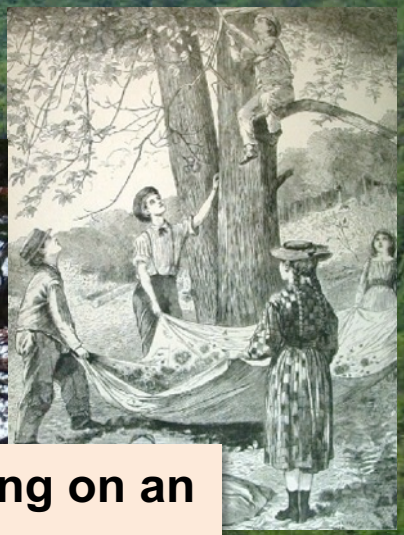
Date: August 21, 2015

Source: Emory Health Sciences

Summary: The study of a chestnut leaf extract, rich in ursene and that it that blocks Staphlococcus aureus virulence and tectable resistance.

Agricultural

Social/historical

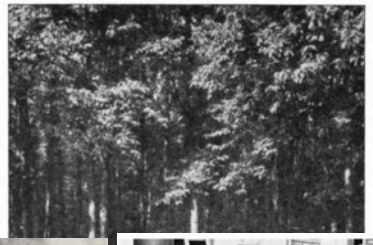


Chestnuts roasting on an open fire,
The Christmas Song
(by Torme and Wells in 1946)

1915 William Bray (first Dean of ESF college) and American chestnut

(5) *Sand Beds of the Syracuse, Phoenix and Rochester Districts.*

On the whole, these sands appear to offer better drained and aerated soil than types hitherto considered. Perhaps this fact, together with more intensive cultivation, will account for the general absence of heath-shrub vegetation. Also their "warmth" (growing out of better drainage and aeration) together with the fact that they lie in a region of ameliorated climate (Zone B of low elevation and under lake influence) will no doubt account for the feature which it is desired to emphasize in this connection; namely, the occurrence of heavy growth of chestnut, oaks, hickory and tulip-tree. The finest bit of chestnut forest that I have seen in New York grows upon these sand beds near Phoenix. Fig. 34. It is claimed that in certain districts the farmers realize more income from the sale of chestnuts than from all other farm products.

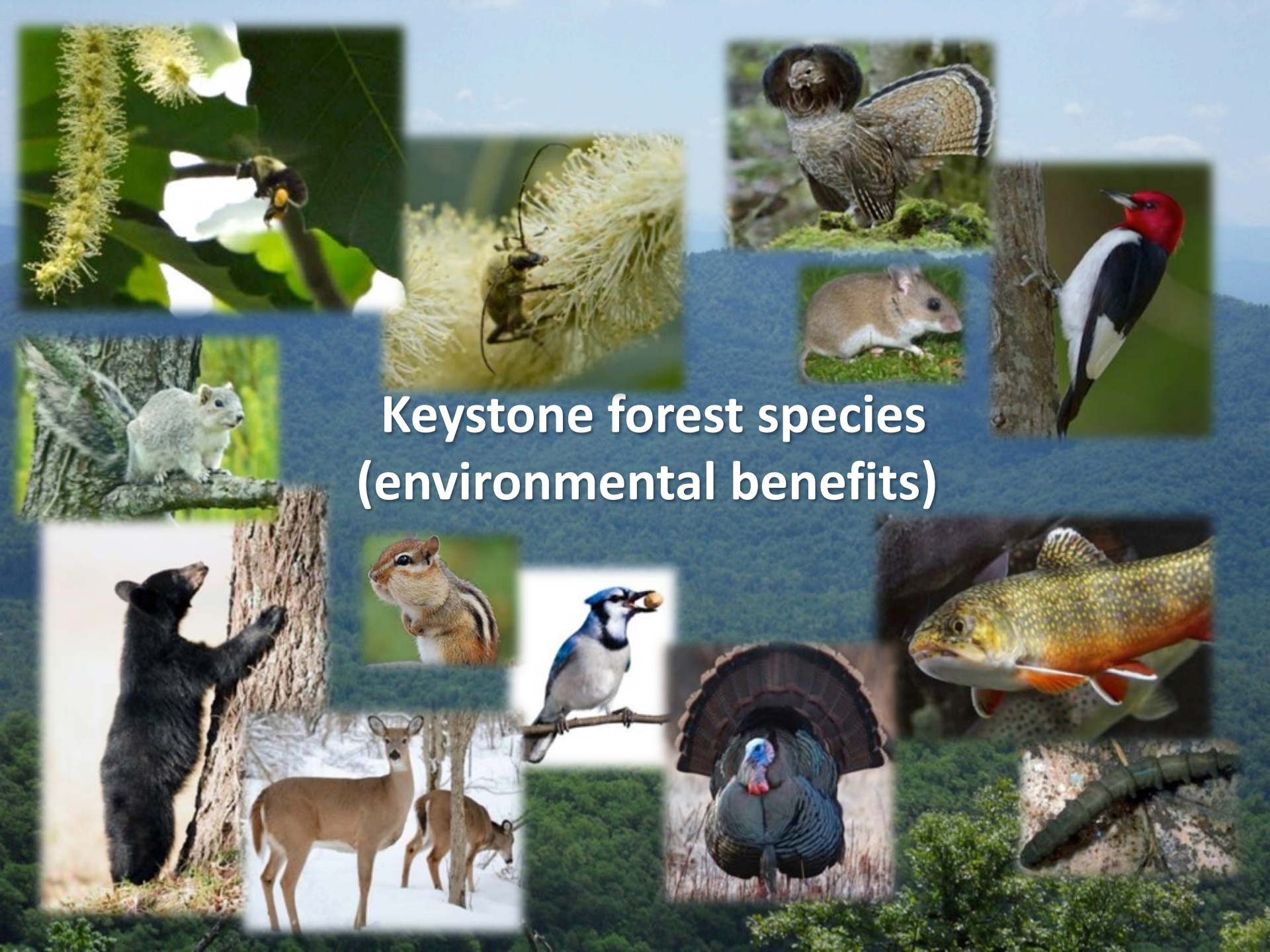


"It is claimed that in certain districts the farmers realized more income from the sale of chestnuts than all other farm products."

A chestnut vendor in Baltimore, Md. [Photo: Library of Congress/Detroit Publishing Co.]

Not orchards, but wild trees

Bray, W. L. (1915). *The development of the vegetation of New York State* (Vol. 16, No. 2). The University.


Keystone forest species
(environmental benefits)

Giants of the Eastern Forests

Under the spreading "American" chestnut tree

photo in MI, 1980s by Alan D. Hart

What happened? Chestnut Blight

Invasive fungal specie:
Cryphonectria parasitca

ALL CHESTNUT TREES HERE ARE DOOMED

Blight Extending All Over This Country and Not One May Be Left Standing.

LAST TWO IN BRONX DYING

There Were 1,500 In Beautiful Grove There—Death of the Chestnuts Has Improved Hemlock Forest.

The chestnut blight or canker is still a great piece of wickedness in the tree world. Dr. W. A. Murrill, Assistant Director of the New York Botanical Garden, has just returned from a trip to Virginia, where he saw the spread of the dreaded thing. People outside New York.

CHESTNUT TREES FACE DESTRUCTION

Trees Worth Millions Dying in This State from a Canker for Which There Is No Remedy.

EATS BENEATH THE BARK

Sprays and Other Attempts to Check Spread of the Parasite Unsuccessful —Trees in Botanical Park Doomed.

The wail of the chestnut tree lover is heard from all parts of New York. Long Island, and adjacent country. Thousands trees, amounting to millions of dollars value, are dying, the victims of the at deadly plant parasite known, the stnut canker. (Diaporthe parasitica

Little or No Danger to Sober Paragon Chestnut Trees from Chestnut Blight

THERE is a widespread interest just now relative to the Chestnut Blight (*Draporthe parasitica*) and we feel that the purpose of this booklet will not have been entirely fulfilled without a frank discussion of this matter as applied to the Sober Paragon Chestnut. Much of the agitation over the chestnut blight has been greatly exaggerated —little more than a "scare" induced by certain sensational newspapers and magazines.

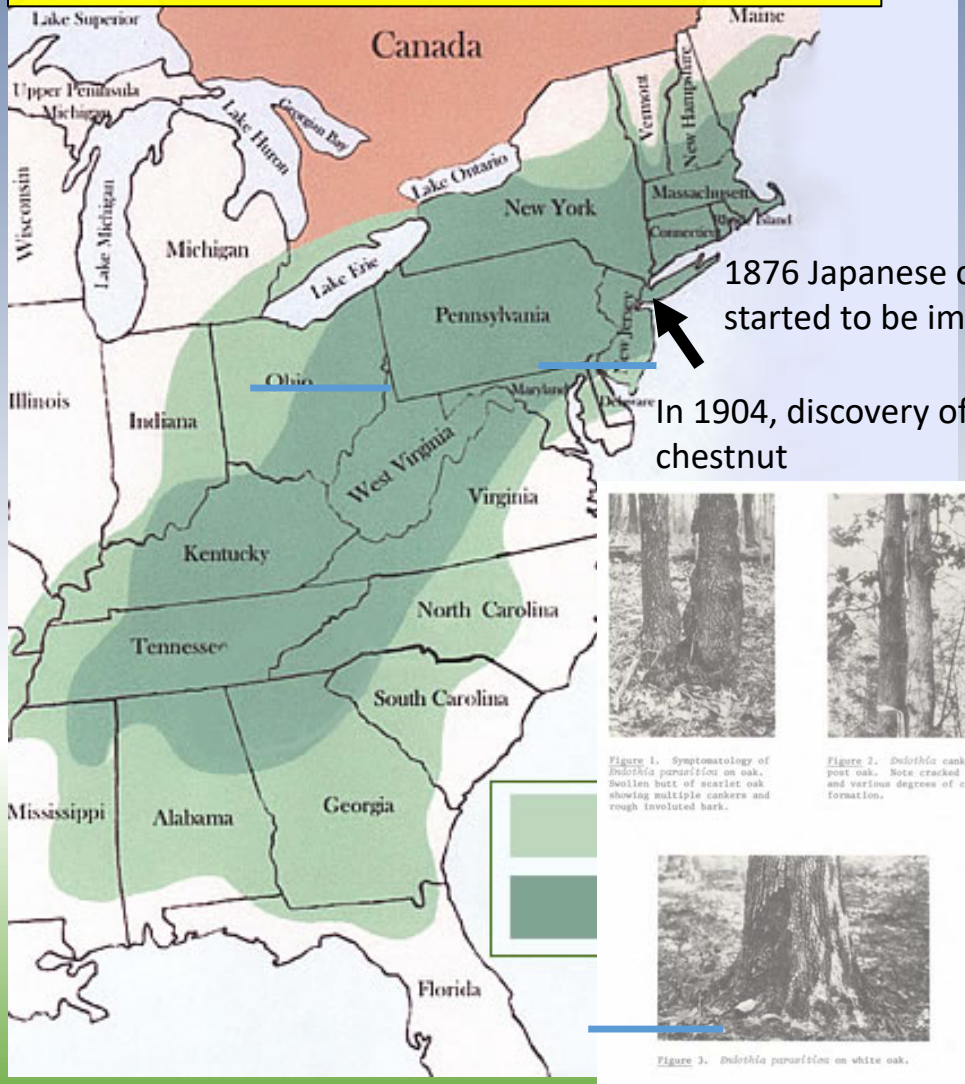
It is true that some native chestnut trees in the Eastern United States have been destroyed by this disease—but it was discovered as far back[illegible]d is, therefore, not spreading so rapidly as many suppose, since most sections of the country have never b[illegible]by it at all.

The Chestnut Blight can[illegible]

Distant view of part of 300 acre plot of Paragon chestnut grove, near Paximos, Pennsylvania.

Chestnut blight in the U.S.

~50 years spread through natural range killing ~4 billion American chestnut trees

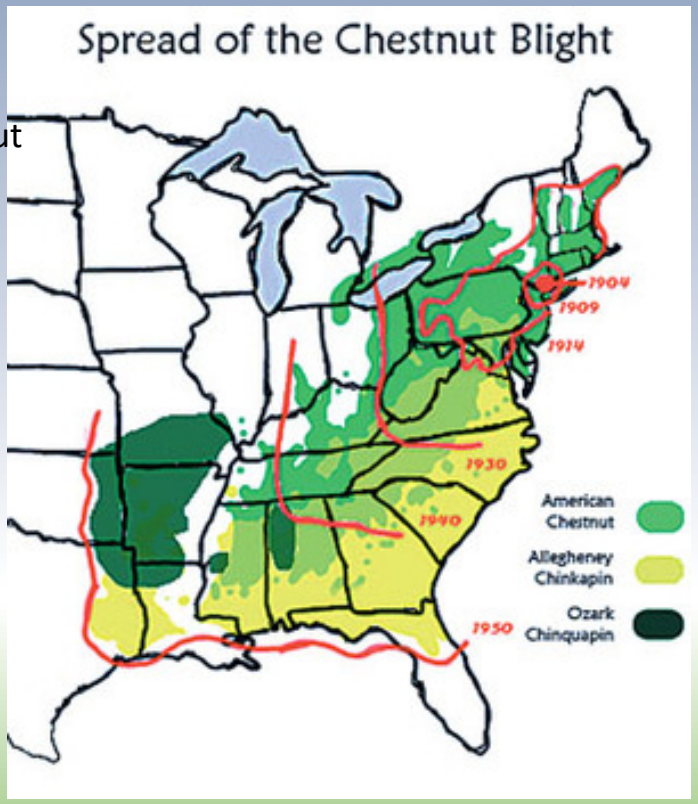


1876 Japanese chestnut started to be imported

In 1904, discovery of chestnut

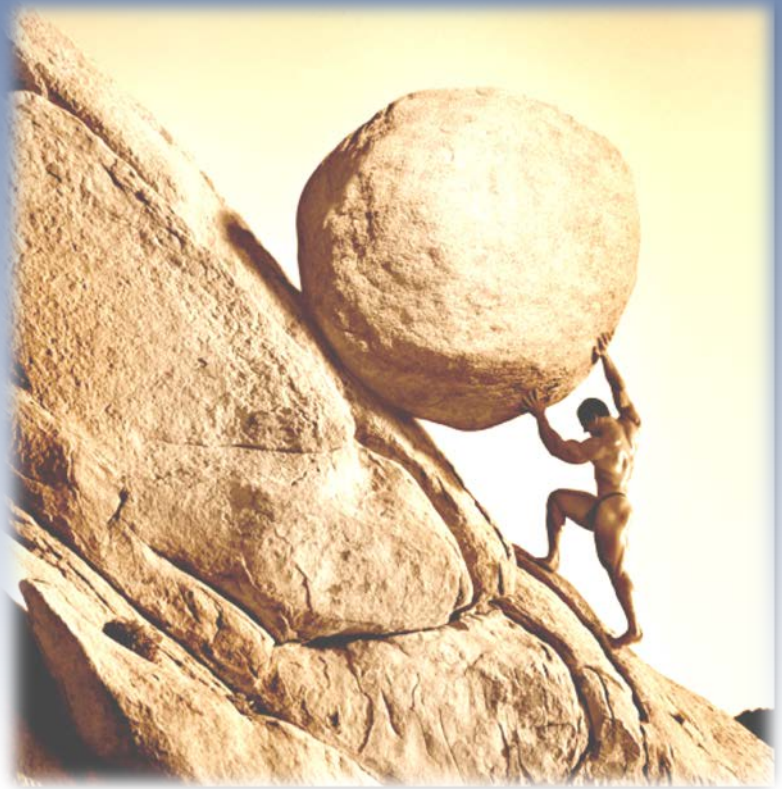
Chestnut blight on related species:

Allegheny Chinkapin, *C. pumila* var. pumila
Ozark Chinquapin, *C. pumila* var. ozarkensis



Chestnut blight also survives on oaks

Today American chestnuts survive in their roots

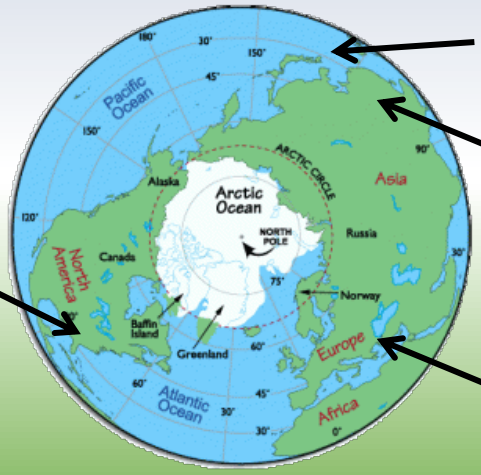


Stuck in a
Sisyphus-like cycle

After over a century of unsuccessful attempts (fungicides, mutational breeding, silvicultural practices, hypovirulence, etc.) at combating the blight, what are the **choices** for restoration today?

Chestnut hybrids are OK for ornamentals or crops,

Not for restoration



Japanese chestnut
C. crenata

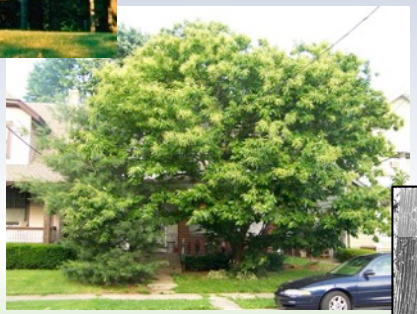
Chinese chestnut
C. mollissima

American chestnut
C. dentata

European chestnut
C. sativa

Are hybrids suitable for restoration?

American chestnut
Castanea dentata



Chinese chestnut
Castanea mollissima

Lion: *Panthera leo*

Tiger: *Panthera tigris*

Liger: *Panthera* hybrid

Better ways:

TACF Meadowview Farm, VA
Dr. Fred Hebard (started 1983)

Dr. Jared Westbrook (current)

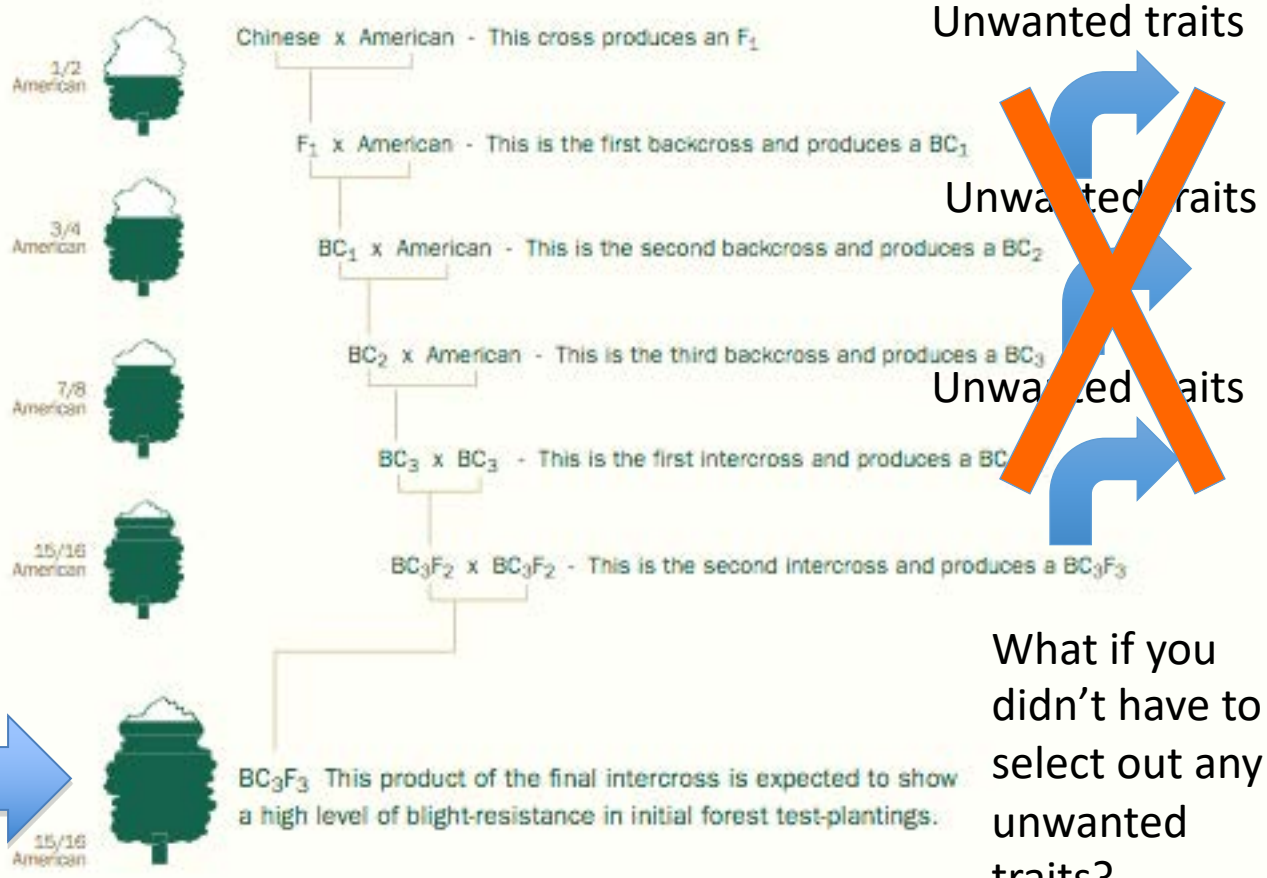
Goal is for 1/16 Chinese chestnut genome to contain the required **9 blight resistance loci on different chromosomes! (#genes?)**

Figure 1.

THE AMERICAN CHESTNUT FOUNDATION BACKCROSS BREEDING PROGRAM

With each cross, additional American chestnut characteristics are regained. Only at the final cross, however, does blight resistance approach that of the Chinese parent

>30,000 CC genes + >30,000 AC genes



Unwanted traits

Unwanted traits

Unwanted traits

What if you didn't have to select out any unwanted traits?

Note: In each step, the Backcross is selected for resistance through the process of inoculation and for American characteristics by visual observation.

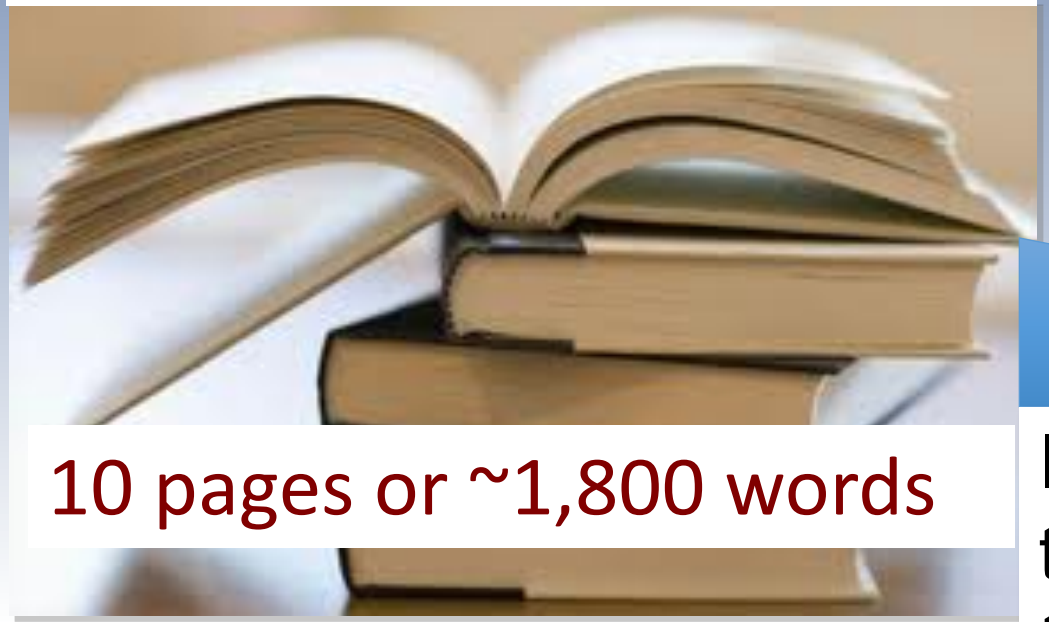
Hybrid Breeding vs. Genetic Engineering:

Why GE is useful for restoration

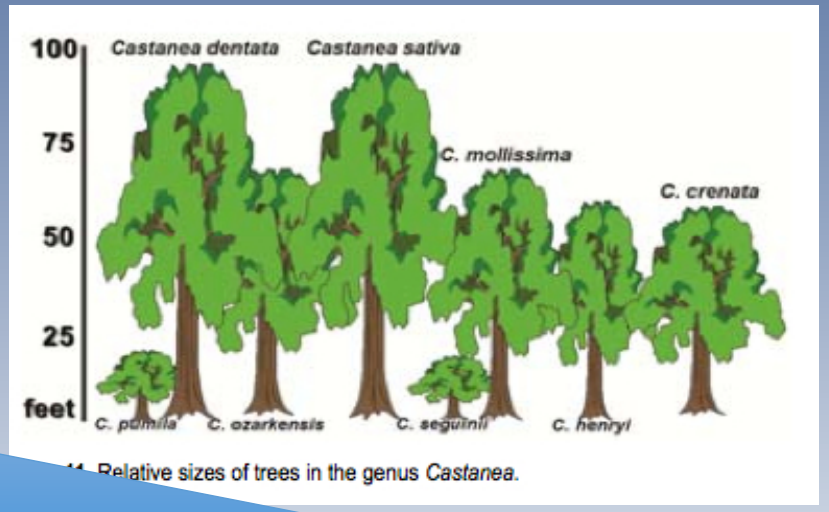
Chestnut has > 30,000 gene pairs

Hybrids = ~½ Chinese chestnut genes

Backcross = ~1/16 Chinese chestnut genes:



10 pages or ~1,800 words



Relative sizes of trees in the genus *Castanea*.

Making very small changes, adding only 2 words

It was very exciting at that season to roam the then boundless chestnut woods of Lincoln, …

blight resistant

Henry David Thoreau, "Walden: or Life in the Woods," 1899

100% American chestnut + blight resistance

Oxalate oxidase (OxO) from wheat

ubiquitous enzyme in many plants & fungi

(non-gluten enzyme, non-allergen)

Table 5.2a. *Cultivated food plants with identified oxalate oxidase gene and/or enzyme*

CULTIVATED FOOD PLANTS		
Common Name	***Scientific Name***	**Reference or NCBI Gene ID**
Peanut	*Arachis hypogaea*	(Wang et al. 2010)
Oat	*Avena sativa*	(Lane et al. 1991)
Beet	*Beta vulgaris*	(Obzansky and Richardson 1983)
African oil palm	*Elaeis guineensis*	(Rusli, Idris, and Cooper 2015)
Strawberry	*Fragaria ananassa*	(Dahiya et al. 2010)
Barley	*Hordeum vulgare*	(Sugiura et al. 1979)
Banana	*Musa paradisica*	(Anjum, Sundaram, and Rai 2014)
Rice	*Oryza sativa*	(Carrillo et al. 2009)
Date palm	*Phoenix dactylifera*	LOC103698783
Peach & Apricot	*Prunus spp.*	(Liang et al. 2010)
Sorghum	*Sorghum bicolor*	(Satyapal and Pundir 1993)
Spinach	*Spinacia oleracea*	(Laties 1950)
Cacao	*Theobroma cacao*	(Gesteira et al. 2007)
Wheat	*Triticum aestivum*	(B. G. Lane et al. 1993)
Corn	*Zea maize*	(Vuletić and Šukalović 2000)

Oxalate oxidase (OxO) from wheat

ubiquitous enzyme in many plants & fungi

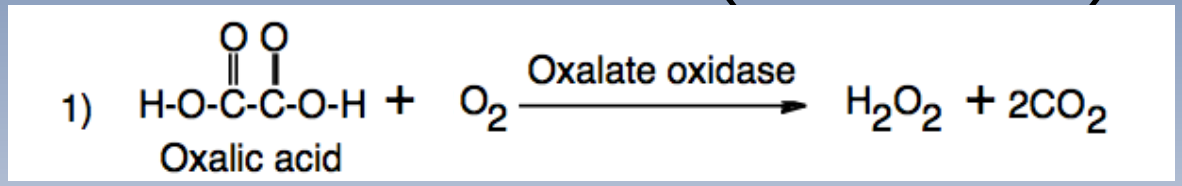
(non-gluten enzyme, non-allergen)

WILD PLANTS AND FUNGI		
Common Name	***Scientific Name***	**Reference or NCBI Gene/Protein ID**
Insulin plant	*Costus pictus*	(Sathishraj and Augustin 2012)
Perennial ryegrass	*Lolium perenne*	(Davoine et al. 2001)
Azalea	*Rhododendron mucronatum*	(Sakamoto et al. 2015)
Castor bean	*Ricinus communis*	LOC107261123
Wild einkorn (wheat progenitor)	*Triticum urartu*	EMS64919.1
Narrowleaf cattail	*Typha angustifolia*	ASM56683.1
Mosses	*6 spp.*	(Laker, Hofmann, and Meeuse 1980)
Split-gill mushroom	*Schizophyllum commune*	SCHCODRAFT_15706
Dermatophytic fungus	*Trichophyton rubrum*	TERG_03492

Oxalate oxidase (OxO) from wheat

ubiquitous enzyme in many plants & fungi
(non-gluten enzyme, non-allergen)

Detoxifies oxalate (oxalic acid)



Not a pesticide (more like an antitoxin)

Does not kill the fungus, no 'cidal' activity.

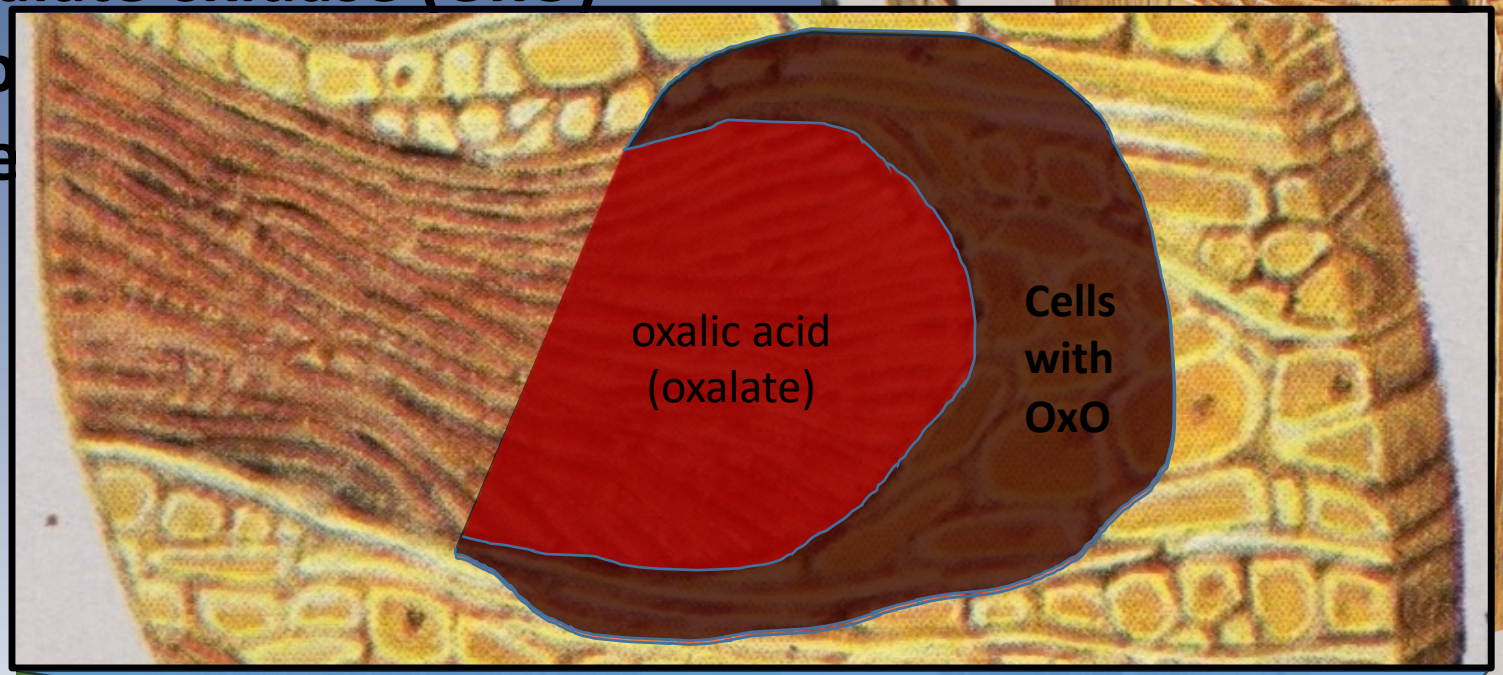
Since the fungus survives, less selective pressure to overcome the oxalate oxidase.

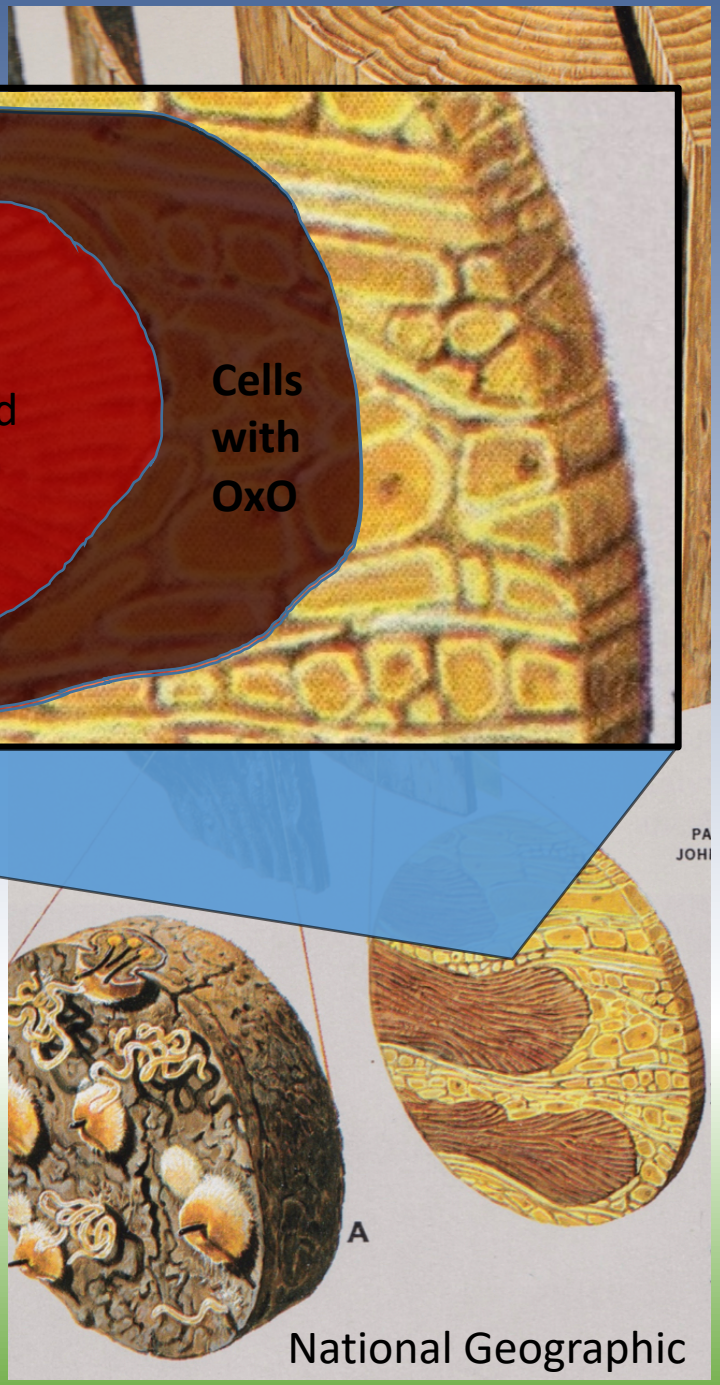
Tolerance has also been suggested to be a more evolutionarily stable form of defense than other forms of resistance since it increases host fitness without directly affecting the pathogen community (Tiffin, 2000), avoiding the "arms race" of specific resistance genes or mechanisms (Rosenthal and Kotanen, 1994; Strauss and Agrawal, 1999; Roy and Kirchner, 2000).

Oxalate oxidase (OxO)

pro

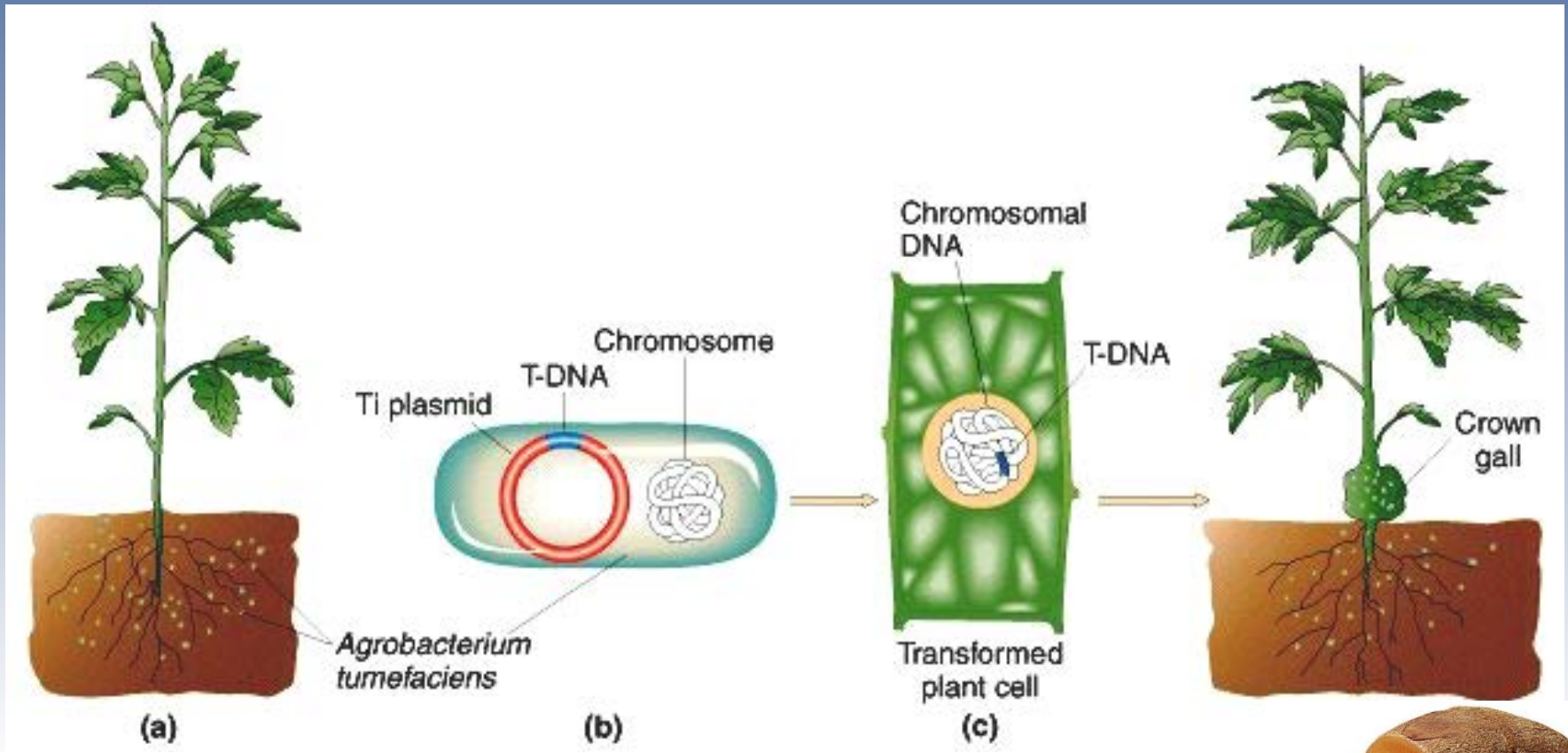
che





How do you get the gene in?

Agrobacterium: a natural plant genetic engineer



The genome of cultivated sweet potato contains *Agrobacterium* T-DNAs with expressed genes: An example of a naturally transgenic food crop

Tina Kyndt[a,1], Dora Quispe[a,b,1], Hong Zhai[c], Robert Jarret[d], Marc Ghislain[b], Qingchang Liu[c], Godelieve Gheysen[a], and Jan F. Kreuze[b,2]

[a]Department of Molecular Biotechnology, Ghent University, 9000 Ghent, Belgium; [b]International Potato Center, Lima 12, Peru; [c]Beijing Key Laboratory of Crop Genetic Improvement/Laboratory of Crop Heterosis and Utilization, Ministry of Education, China Agricultural University, Beijing, China, 100193; and [d]Plant Genetic Resources Unit, US Department of Agriculture, Agricultural Research Service, Griffin, GA 30223

Chestnut tissue culture

How to make a tree from a seed, the hard way.

Dr. Xing and Dr. Maynard based on S.A. Merkle
Modified by Sharon LaPierre, Linda McGuigan, Allison Oakes, and others.

Isolated immature embryos

~2 week window

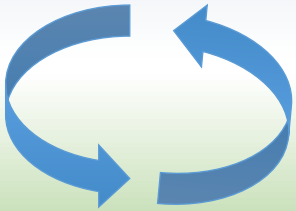
Embryo Extraction

~ 1 in 1000 embryos produces a somatic embryo culture

Somatic embryo culture

Somatic embryogenesis

Seed



Maintained for over 9 years

Bioreactor

First 24 months, now <12 months

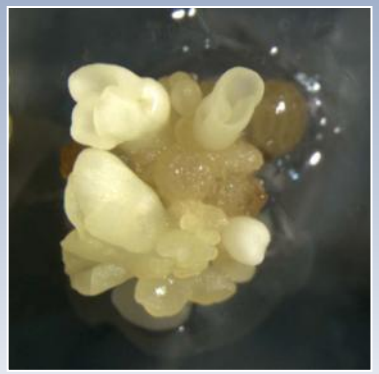
Agrobacterium-mediated transformation and selection

Germination of embryos

Seed

Somatic embryogensis

Heart, Torpedo, and early cotyledon stages

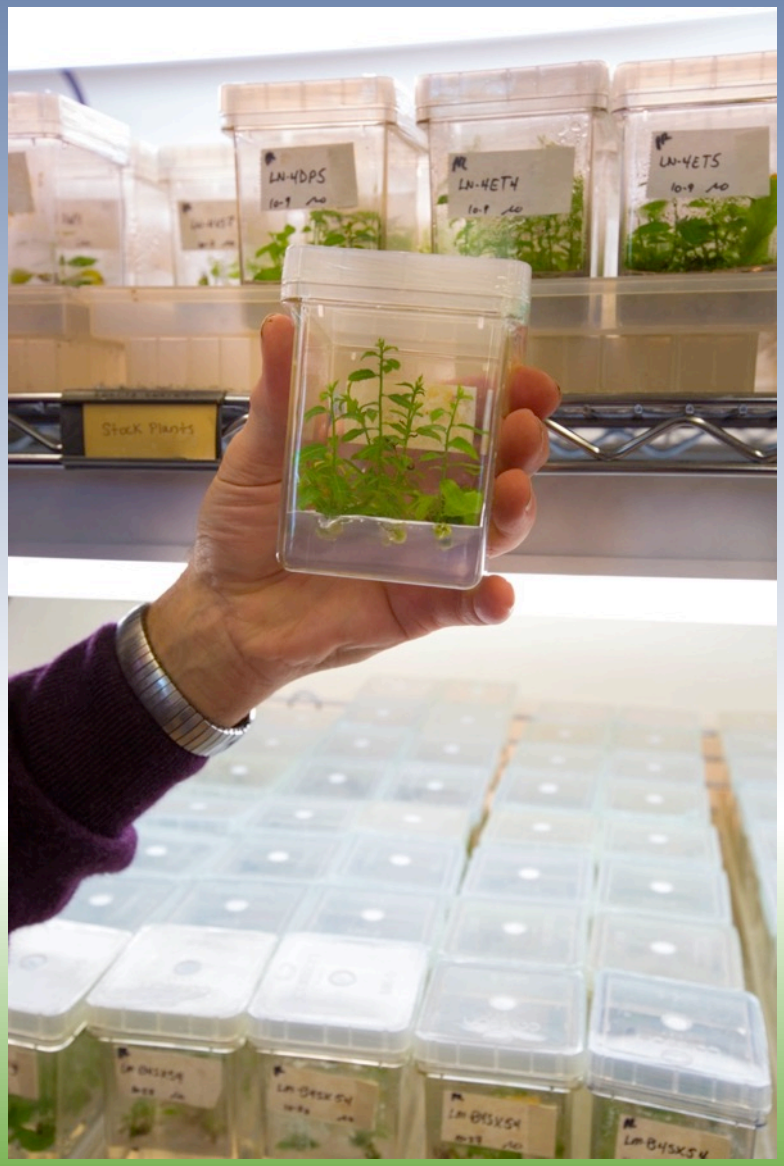


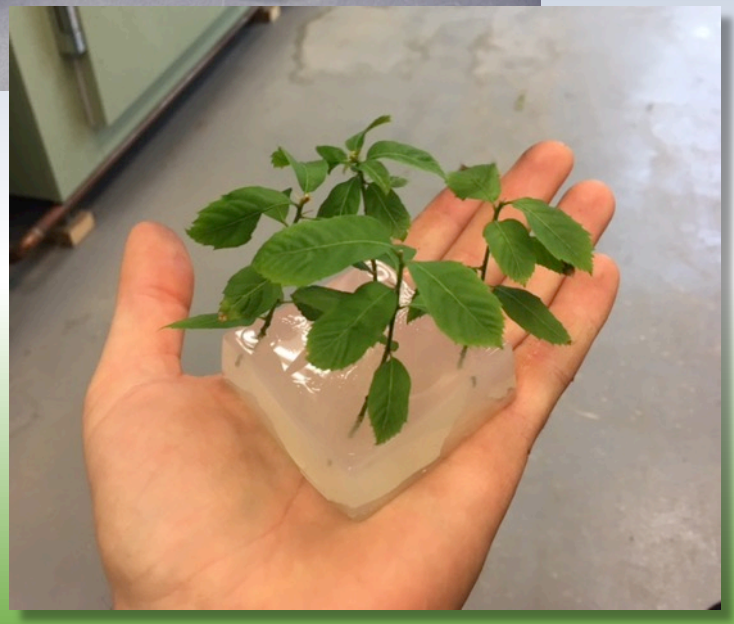
Cotyledon stage & shoot formation

Shoots

Multiplication

Preparing for rooting





Rooting

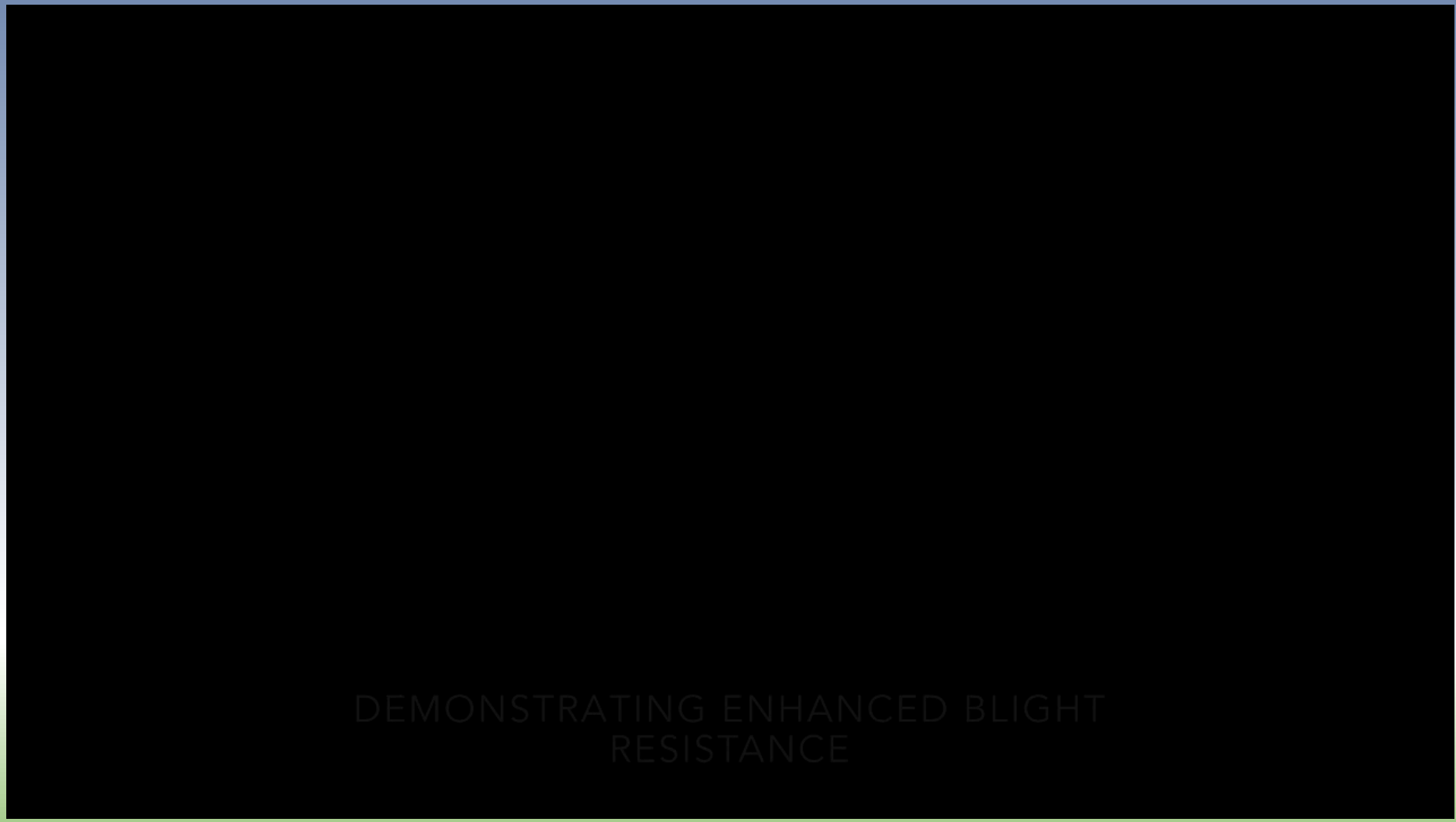
Hormone dip, peat pots, & begin acclimatization

Acclimatization

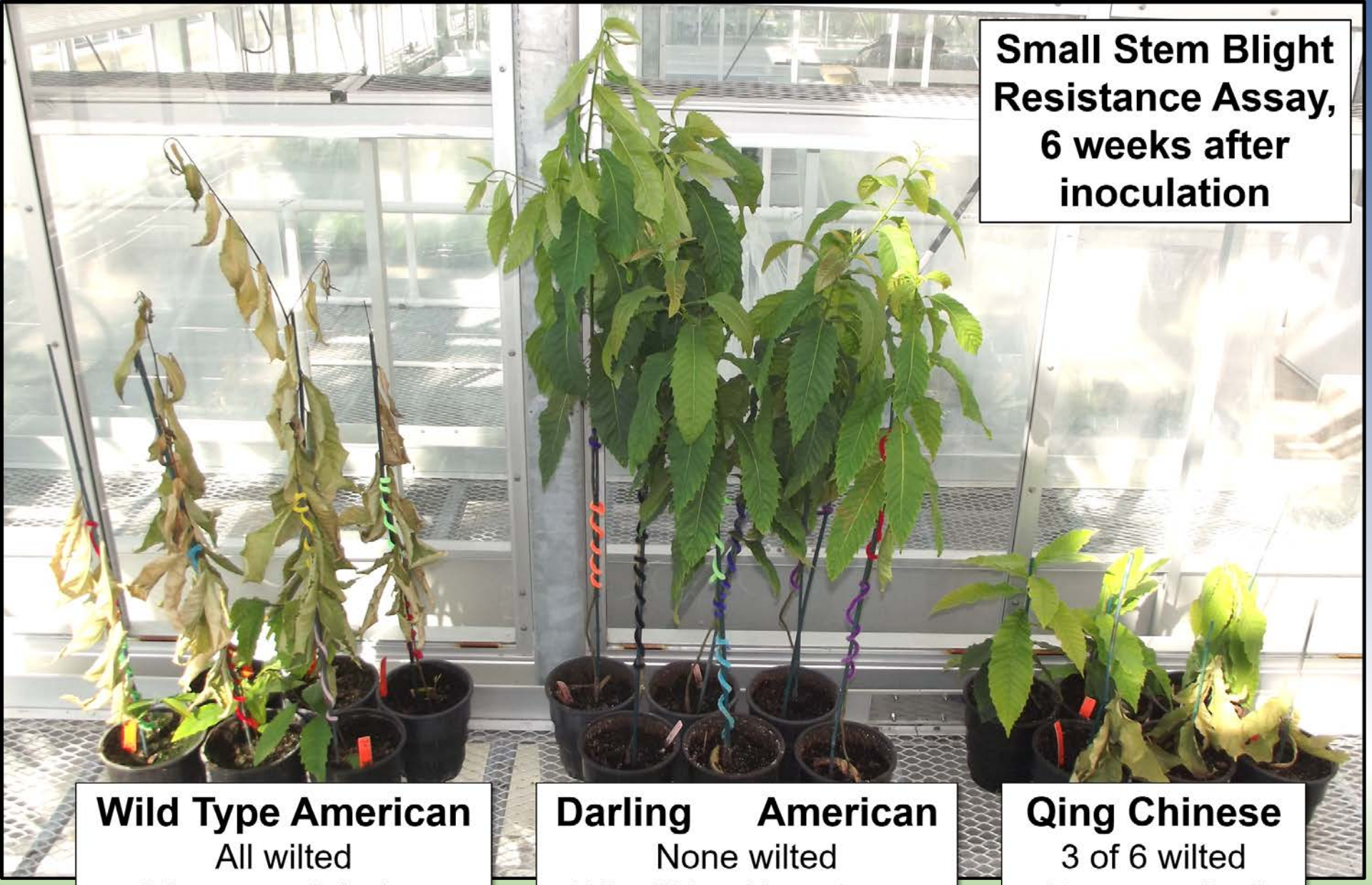
In field 2006 to today…

How do we test for blight resistance?

Goal, blight resistance ≥ Chinese
Small stem assay (2.5-3mm dia.)

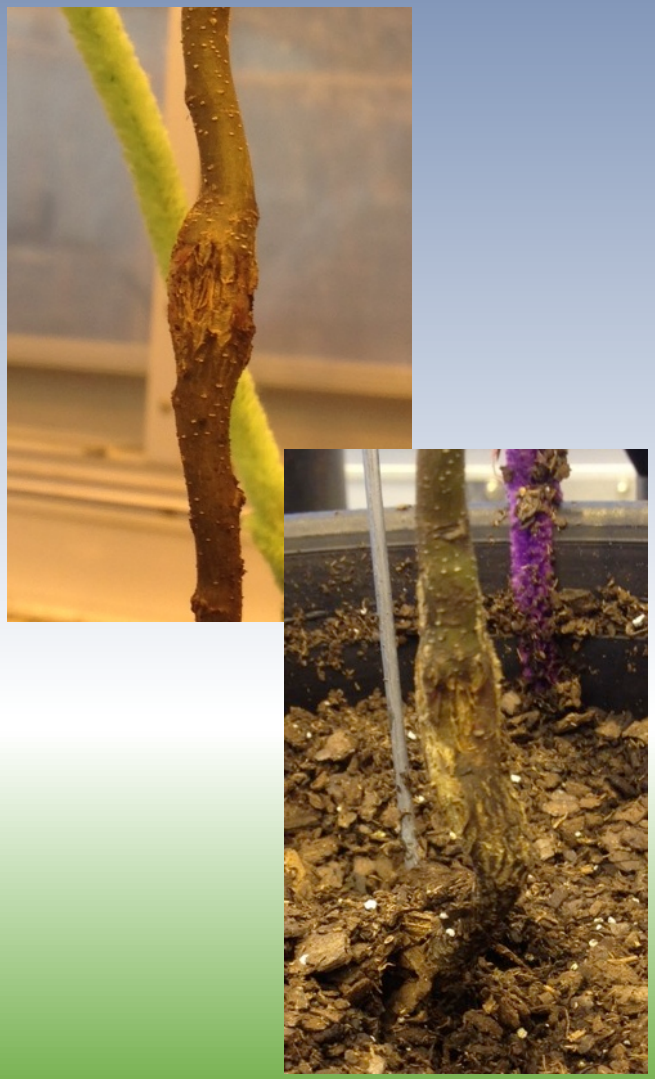


http://www.esf.edu/chestnut/resistance.htm

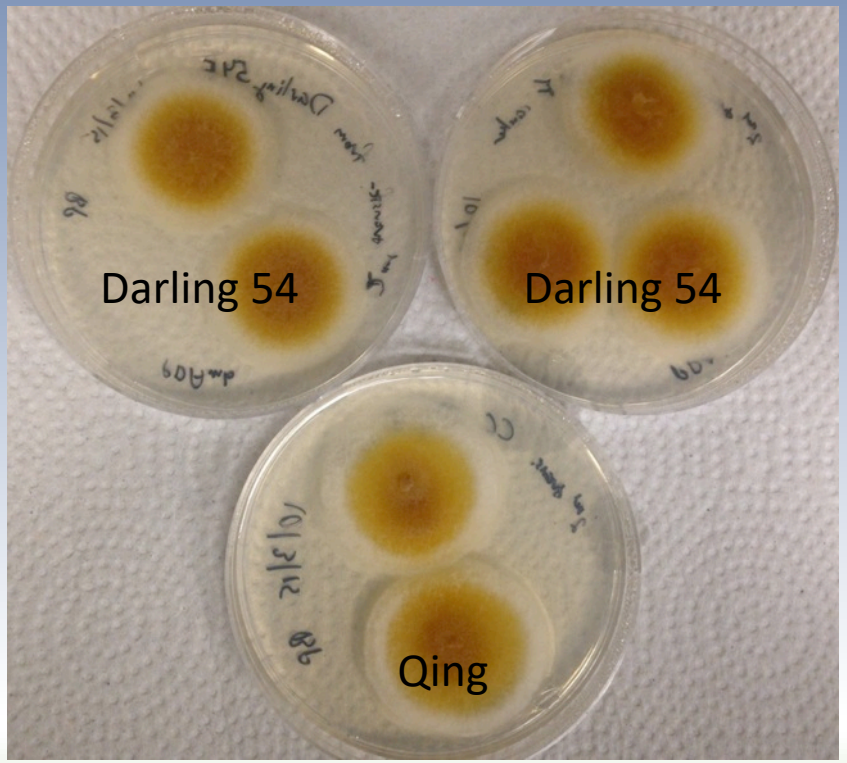

Small Stem Blight Resistance Assay, 6 weeks after inoculation
Wild Type American
All wilted
(New growth below inoculation site)
Darling American
None wilted
(All still healthy ~1 year after photo)
Qing Chinese
3 of 6 wilted
(2 more wilted since photo)

Isolation of *C. parasitica* from small stem assay cankers

Example of Darling 54 cankers



Isolation of *C. parasitica* 52 DPI

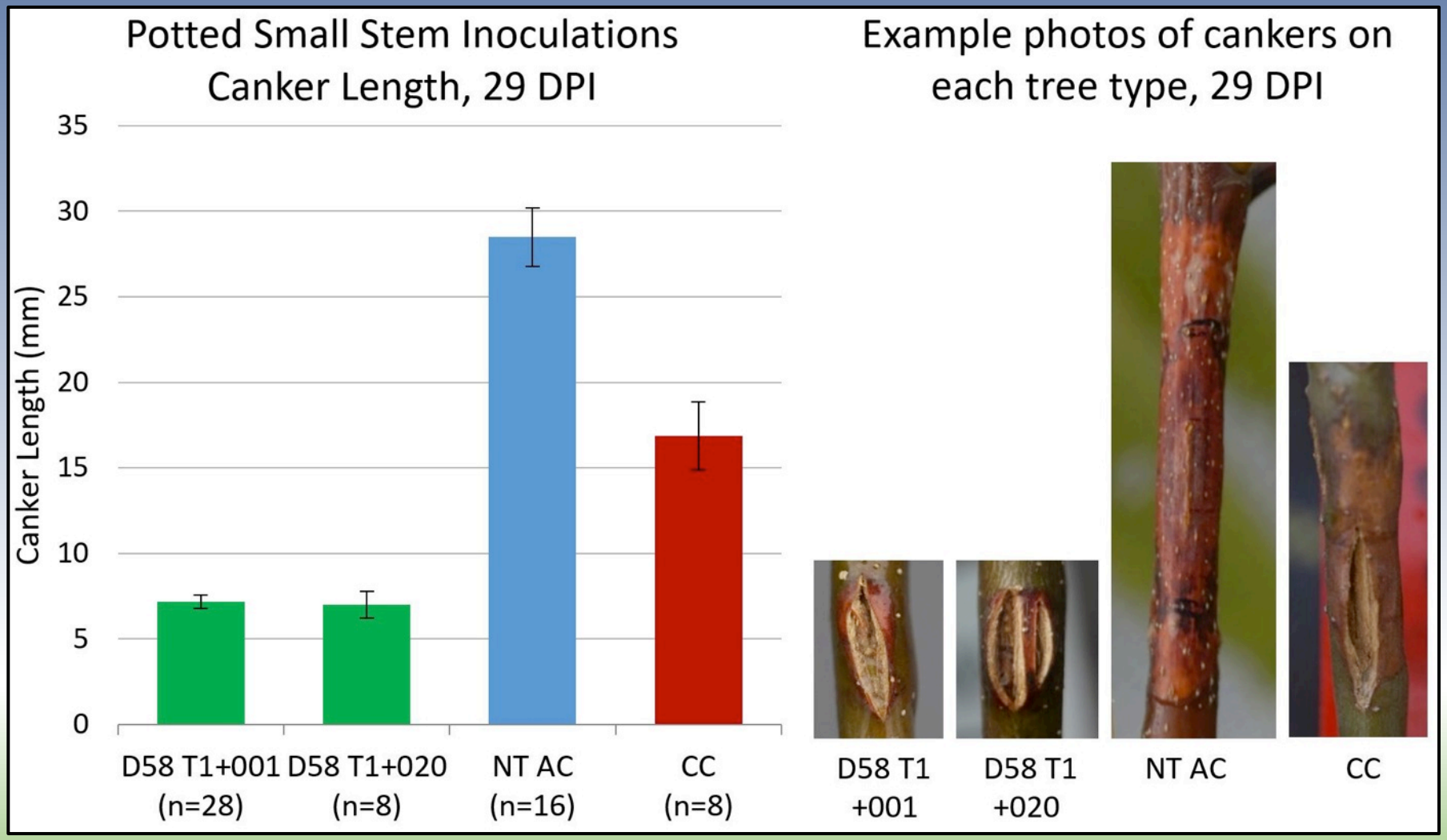


Tree & fungus co-exist.

Inheritance of blight tolerance

Pollination with transgenic pollen

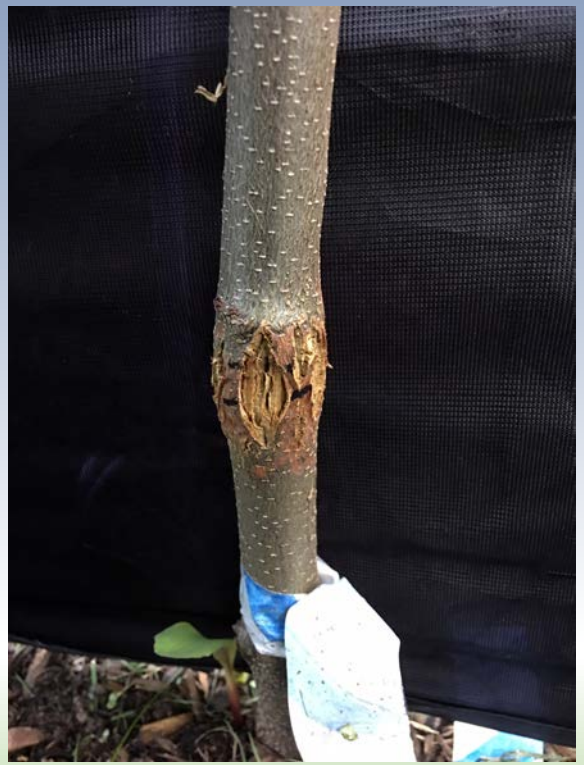
Small stem inoculations 29 DPI
D58 T1s from tissue culture



(Andy Newhouse)

Chinese, OxO American, and wt American chestnut Field inoculations with *Cryphonectria parasitica*

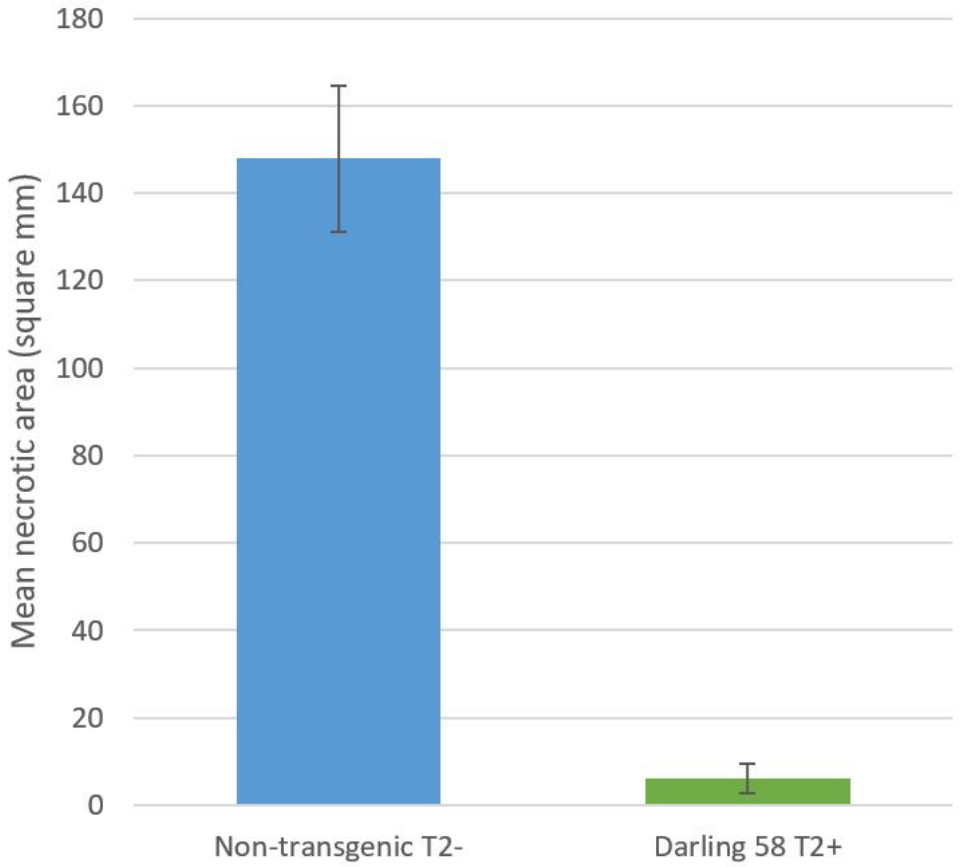
Chinese chestnut

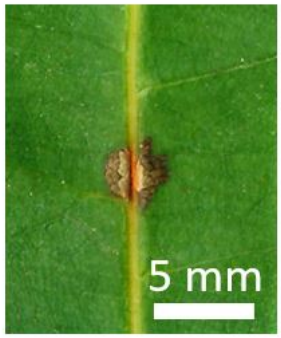


OxO American chestnut

wt American chestnut

Third generation (T2) leaf assays showing protection from the blight fungus





n=10 leaves/type. Error bars indicate +/- one standard error of the mean.

Federal regulatory review:

Non-regulated status

USDA APHIS

Voluntary -
No further questions

FDA

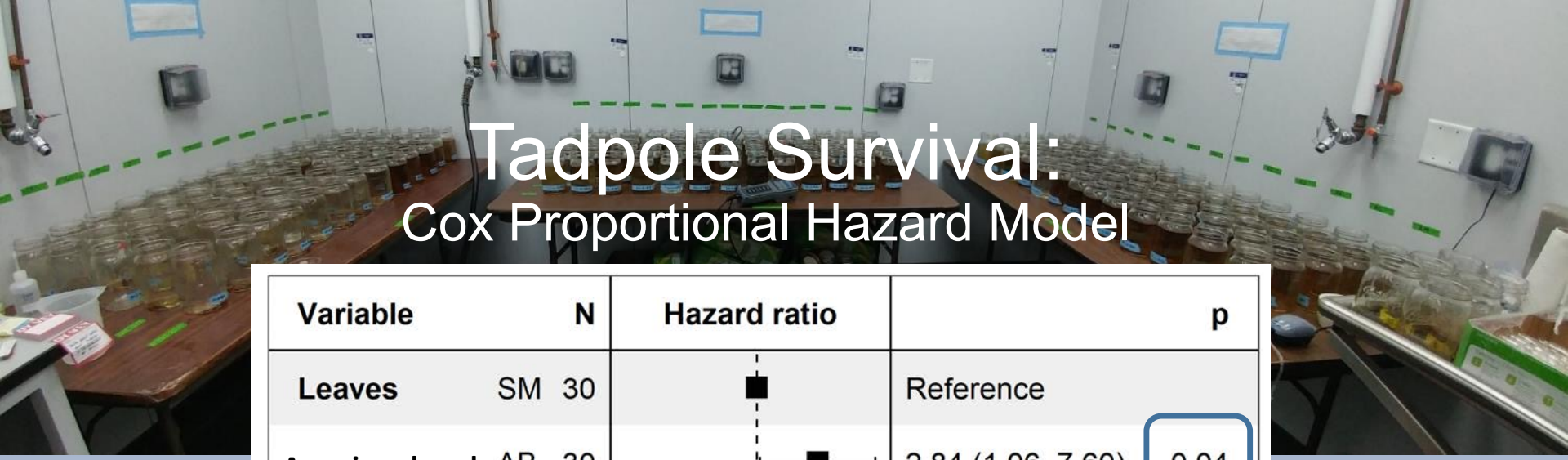
Registration?

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

New paradigm for regulators

Many typical comparative studies,
plus additional experiments
for restoration trees

(slide by Andy Newhouse)



Tadpole Survival:
Cox Proportional Hazard Model

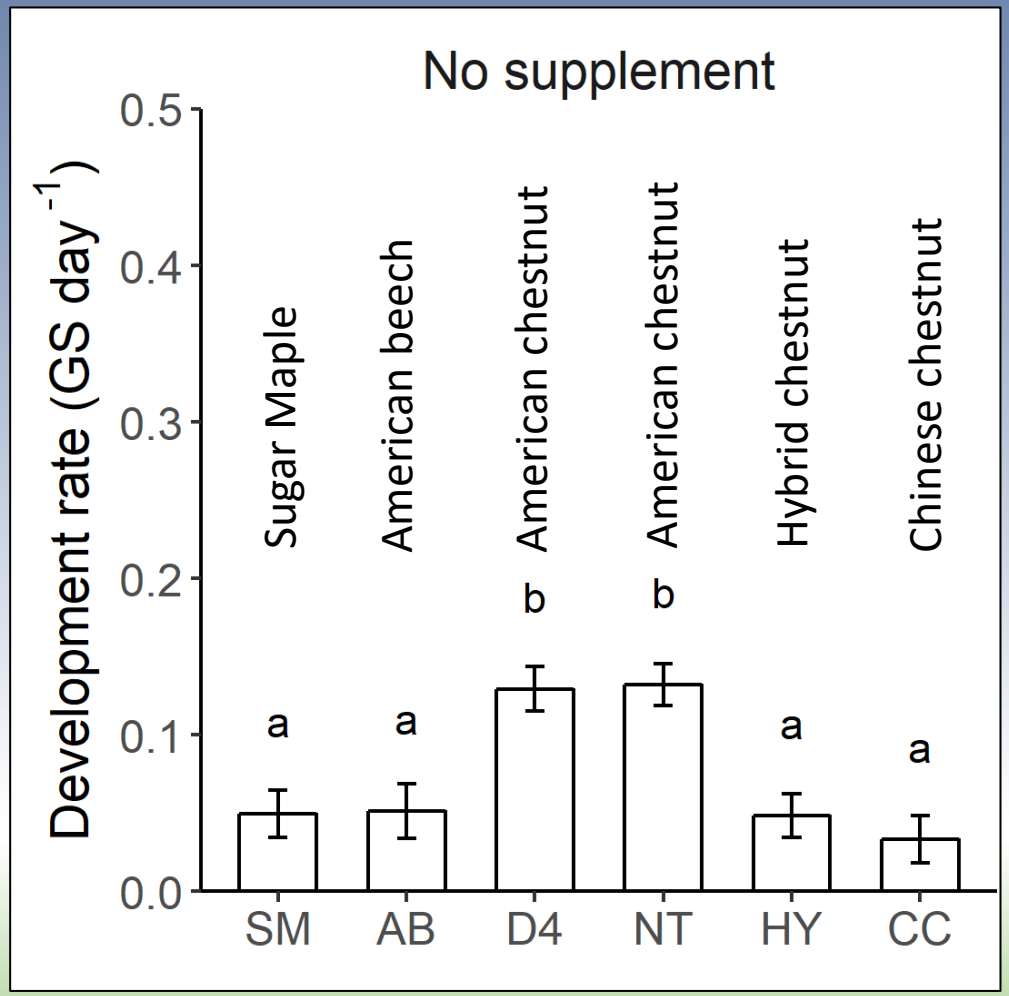
Variable		N	Hazard ratio	p
Leaves	SM	30	Reference	
American beech	AB	30	2.84 (1.06, 7.60)	0.04
	D4	30	0.99 (0.32, 3.06)	0.98
	NT	30	0.43 (0.11, 1.71)	0.23
	HY	30	0.44 (0.11, 1.76)	0.25
	CC	29	0.96 (0.31, 2.97)	0.94
Supplement	No	89	Reference	
	Yes	90	0.48 (0.23, 0.98)	0.05

0.2 0.5 1 2 5

Increasing Survival Hazard

SM – Sugar Maple
AB – American beech
D4 – Transgenic
NT – WT American chestnut
HY – Hybrid chestnut
CC – Chinese chestnut

Tadpole Development



Goldspiel, HB, Newhouse, AE, Gibbs, JP, and Powell, WA. 2018. Effects of Transgenic American Chestnut Leaf Litter on Growth and Survival of Wood Frog Larvae. Restoration *Ecology*, 27:371-378

A unique opportunity with the Darling lines of blight resistant American chestnut:

Rescuing the surviving genetic diversity.

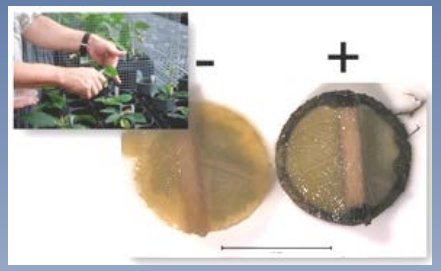
18 in. DBH American chestnut
Manlius, NY

Unique feature of the 'Darling' American chestnut trees:

Rescuing genotypes surviving trees

ESF

Supplier of pollen



Regionally adapted

Genotypes
½ mother & ½ father

pollen

seed

Parental allelic composition

Transgenic American chestnut

Continue to maximize out-crossing

Allows:
Allelic rescue, local adaptation, and increases genetic diversity

TACFNY LSC "Mother" Trees
Or surviving wild population
Or backcross trees

Offspring
50% OxO & fully blight resistance

Restoration foci: mine reclamation, historic sites, private land owners, etc.

Century long restoration
Supporters of all ages

Testing restoration planting

CFCA Youth Conservation Day

(currently under permits)

Leaving a **legacy**:
Will tell their grandchildren:
"I planted that tree!"

Do you think the American chestnut is important?

Very important!

We need you to spread the word
(friends, family, social media, political representatives)
Respond during regulatory open comment period

Join TACF: www.acf.org

Support ESF chestnut research: www.esf.edu/chestnut/

Allen Nicholes, Pres. New York Chapter of The American Chestnut Foundation

American chestnut planting demonstration

https://www.youtube.com/watch?v=vVc1oLXelmg


"We humans are more than consumers, we have gifts of our own to give to the earth."
Dr. Kimmerer at the U.N.
Thank you!
www.esf.edu/chestnut
Questions?