

# American Chestnut (*Castanea dentata*) (O-hã-yah'tah)

## Rescuing a Keystone Tree Species from an Invasive Pathogen

SUNY College of Environmental Science & Forestry, Syracuse, NY  
*In partnership with The American Chestnut Foundation*

### Current ESF research team:

Bill Powell (Director)

Chuck Maynard (Emeritus)

Linda McGuigan (TC lab Manager)

Allison Oakes (Post doctoral fellow)

Kaitlin Breda (Admin assistant)

Jeff Zarnowski (Greenhouse/field Man.)

Andy Newhouse (Reg. Man., BRAG tech, PhD student)

Dakota Matthews (Mol. Biol. Lab Man./MS grad student)

Hannah Pilkey (Breeding & Production Manager/MS grad student)

Erik Carlson (MS grad student)

Josh Mott (MS grad student)

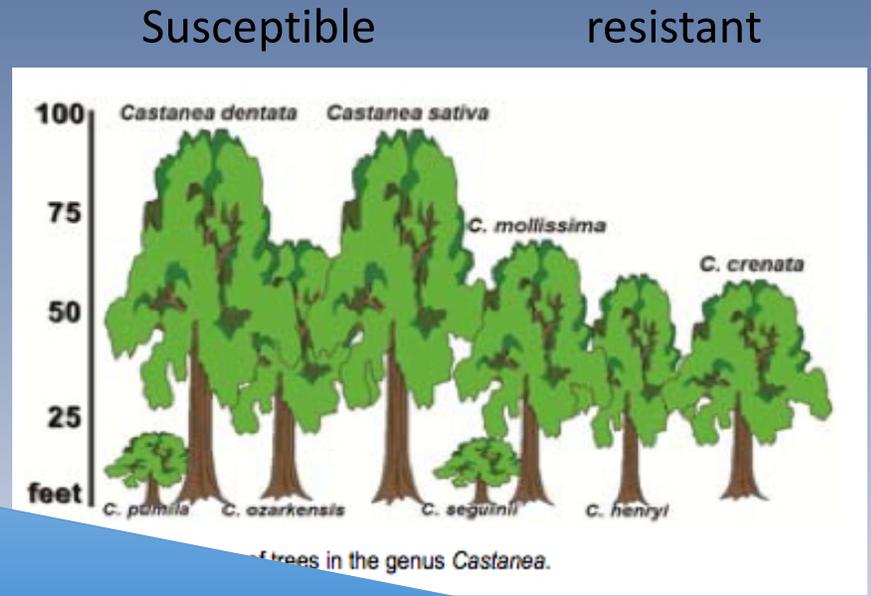
Many undergrads, high school students, collaborators, and volunteers...

**The work of well over 100 people over 30 years**

# Why GE is useful for restoration

Chestnut has ~ 30,000 gene pairs

Hybrids (example Dunstan) = ~½ or more Asian chestnut genes, one allele for every gene pair (short vs. tall canopy tree)



Making very small changes, adding only 2 words



It was very exciting at that season to roam the then boundless <sup>blight tolerant</sup> chestnut woods of Lincoln, ...

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

**100% American chestnut + blight tolerance**

**Oxalate oxidase (OxO) from wheat**  
(non-gluten enzyme, non-allergen)  
ubiquitous enzyme in many plants, fungi, & bacteria

**2 of the 3 sisters contain OxO**



### CULTIVATED FOOD PLANTS

Common Name	Reference
Peanut	(Wang <i>et al.</i> , 2010)
Oat	(Lane <i>et al.</i> , 1991)
Sugar beet	(Arnon and Whatley 1954)
Tea	(Fu <i>et al.</i> , 2018)
African oil palm	(Rusli <i>et al.</i> , 2015)
Finger millet	(Akbar <i>et al.</i> , 2018)
Strawberry	(Dahiya <i>et al.</i> , 2010)
Barley	(Sugiura <i>et al.</i> , 1979)
Tomato	(Sun <i>et al.</i> , 2019)
Banana	(Anjum <i>et al.</i> , 2014)
Rice	(Carrillo <i>et al.</i> , 2009)
Scarlet runner bean	(Chipps <i>et al.</i> , 2005)
Date palm	(NCBI, 2018b)
Peach & Apricot	(Liang <i>et al.</i> , 2010)
Rye	(Lane, 2000)
Sorghum	(Satyapal and Pundir, 1993)
Spinach	(Laties, 1950)
Cacao	(Gesteira <i>et al.</i> , 2007)
Wheat	(Lane <i>et al.</i> , 1993)
Corn	(Vuletić and Šukalović, 2000)

### WILD AND ORNAMENTAL PLANTS

Common Name	Reference
Goatgrass	(NCBI, 2017)
Spiny amaranth	(Goyal <i>et al.</i> , 1999)
Ramie	(Xuxia <i>et al.</i> , 2012)
Bougainvillea	(Srivastava and Krishnan, 1962)
Stiff brome	(NCBI, 2018a)
Rubber bush	(Freitas <i>et al.</i> , 2017)
Mexican tea (epazote)	(Nagahisa and Hattori, 1964)
Insulin plant	(Sathishraj and Augustin, 2012)
Common sunflower	(Maksoud, 1996)
Three-cornered hypnum moss	(Houget <i>et al.</i> , 1927)
Various mosses (12 species)	(Datta and Meeuse, 1955)
Perennial ryegrass	(Davoine <i>et al.</i> , 2001)
White lupin	(Wojtaszek <i>et al.</i> , 1997)
Common ice plant	(Michalowski and Bohnert, 1992)
Switchgrass	(Matthews & Powell)
Castor bean	(NCBI, 2018c)
Azalea	(Sakamoto <i>et al.</i> , 2015)
Wild einkorn (wheat ancestor)	(NCBI, 2013)
Narrowleaf cattail	(Du <i>et al.</i> , 2018)

### FUNGI AND BACTERIA

Abortiporus mushroom	(Grąz <i>et al.</i> , 2016)
White rot fungus	(Aguilar <i>et al.</i> , 1999)
Mycorrhizal fungus	(Mäkelä <i>et al.</i> , 2010)
Endophytic bacterium	(Kumar and Belur, 2016)
Split-gill mushroom	(NCBI, 2016a)
Dwarf bunt fungus	(Vaisey <i>et al.</i> , 1961)
Dermatophytic fungus	(NCBI, 2016b)
Bacterium	(Koyama, 1988)

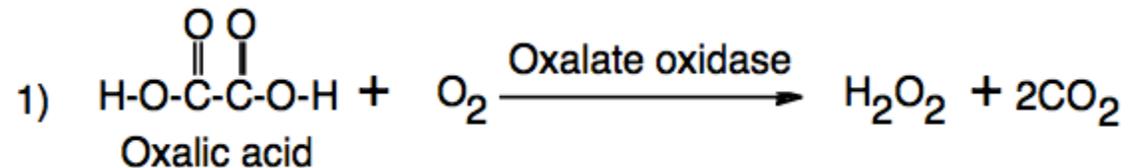
acteria



# 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.

**So the fungus and tree can now coexist.**

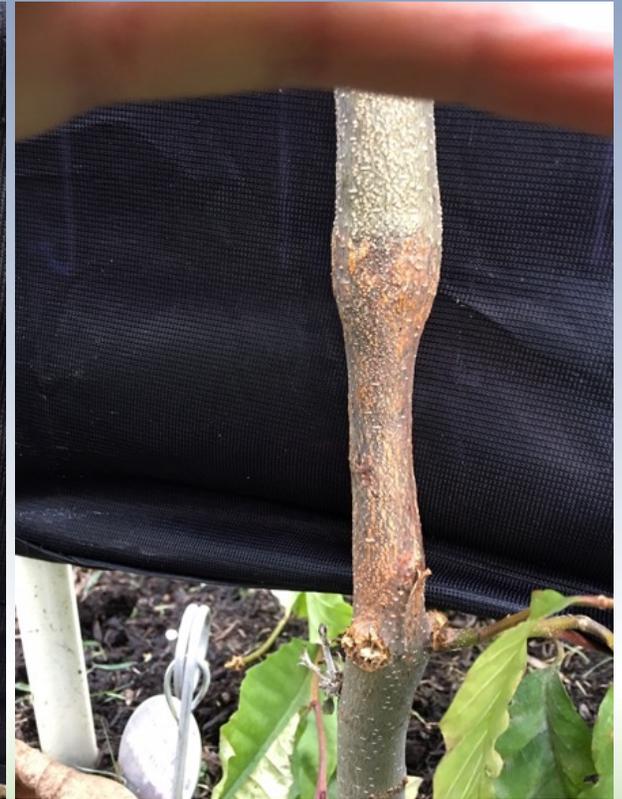
# Chinese, OxO American, and wt American chestnut Field inoculations with *Cryphonectria parasitica*



Chinese chestnut



OxO American chestnut  
Sibling OxO+

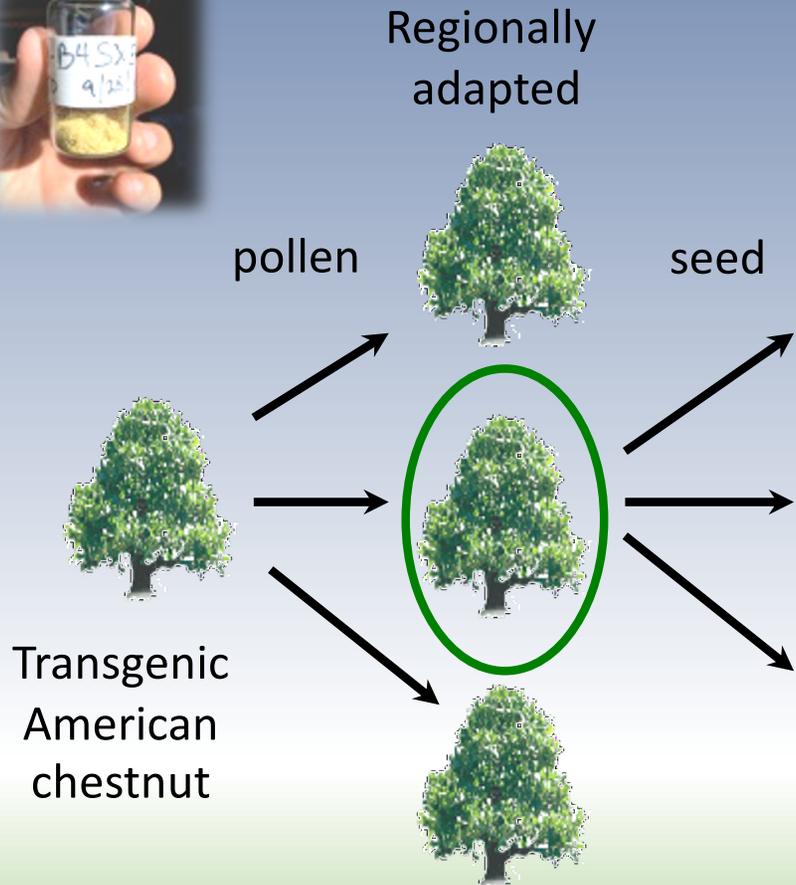


wt American chestnut  
Sibling OxO-

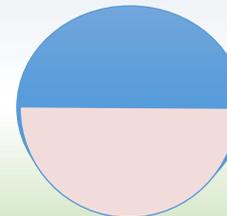
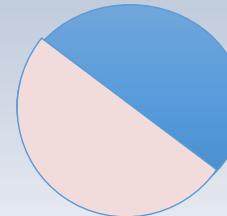
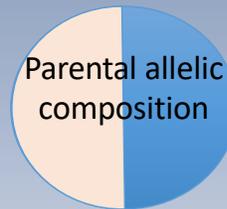
# Rescuing genotypes surviving trees

## Unique feature of dominant tolerance trait

### Supplier of pollen



Genotypes  
 $\frac{1}{2}$  mother &  $\frac{1}{2}$  father



Collaboration with TACF:  
1-3 generations of out-crossing  
for horticultural distribution  
3-5 generations for forest restoration



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 tolerant



**Retains 100% of its original traits.**

## **Conservation:**

Even after 100 years, the wild-type chestnut offspring will be produced from the transgenic trees.

Not true for other breeding methods.

"We humans are more than consumers,  
we have gifts of our own to give to the  
earth."

Dr. Kimmerer at the U.N.

