# The American Chestnut: Breeding and Restoration

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**TACF NEW ENGLAND REGIONAL BREEDING PROGRAM**
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THE AMERICAN CHESTNUT FOUNDATION®

# American Chestnut 101

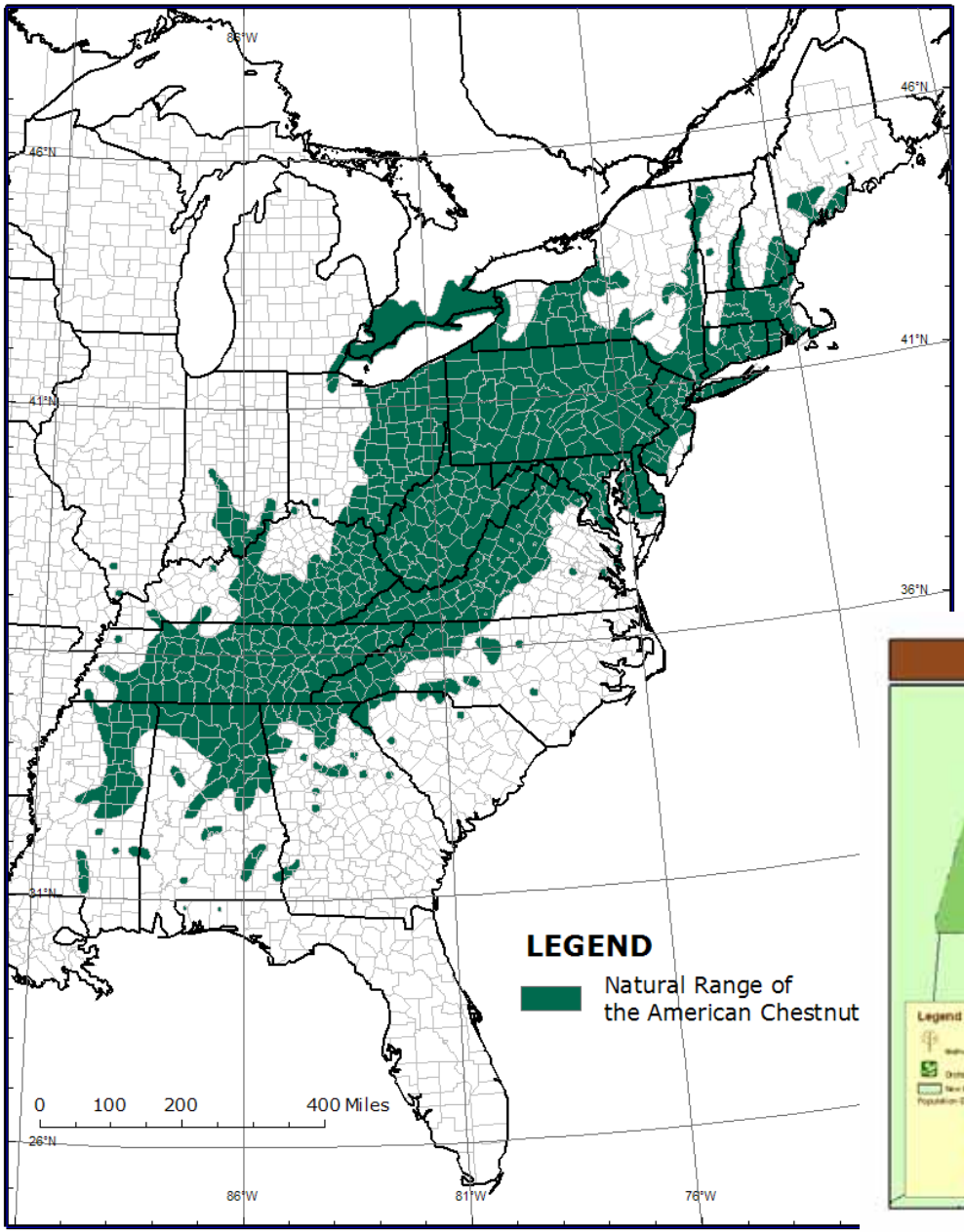

## THE BASICS

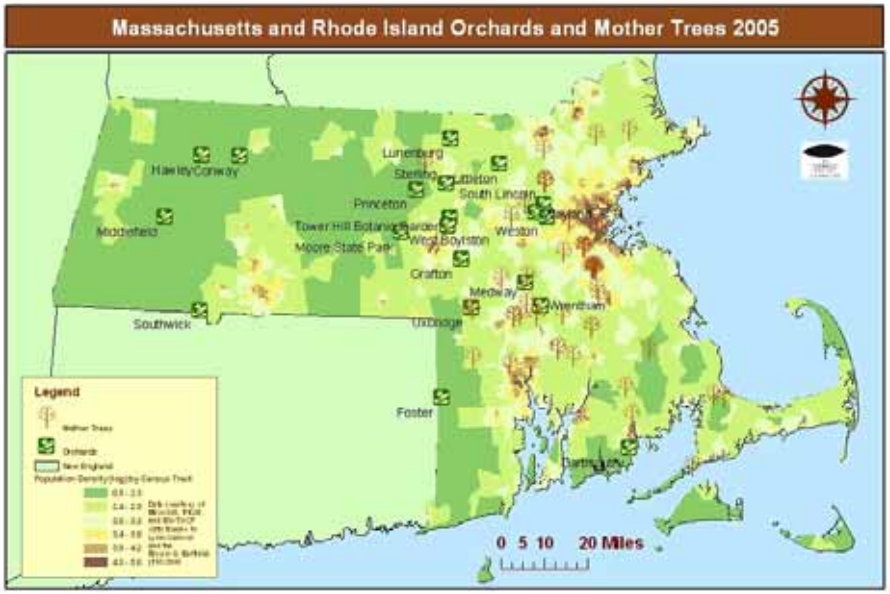
# American chestnut: *Castanea dentata*

- Member of the *Fagaceae* family
  - Beech *(Fagus*), chestnut (*Castanea*) and oak (*Quercus*)
- Species of *Castanea* native to north America
  - ***Castanea dentata* – American chestnut**
  - *Castanea pumila* – Chinquapin or Allegheny Chinquapin
  - *Castanea ozarkensis* (*Castanea pumila* var. *ozarkensis*)– Ozark Chinquapin
- Non-native *Castanea* species
  - ***Castanea mollissima* – Chinese chestnut**
  - *Castanea crenata* – Japanese chestnut
  - *Castanea sativa* – European chestnut
  - *Castanea henryi* – Henry's chinquapin (China)
  - *Castanea seguinii* – Seguin chestnut (China)

# Historic Range

- Most of MA
- All of RI
- Limited by cold 0r poor chestnut site conditions



Simple, Alternate

Deeply toothed, teeth hooked or curved in

Narrow taper at base

Small, pointed buds

# American Chestnut ID: Leaves

# American Chestnut ID: Flowers

Male and female flowers

Self-infertile

Flower in late June, after risk of frost

Wind and insect pollinated

Densely spiny bur, more so than Horsechestnut

Nuts three to a bur, large, brown and shiny

Un-pollinated nuts are flat and rectangular

# American Chestnut ID: Burs and Nuts

Timber-form

Bark has wide, flat ridges

# American Chestnut ID: Mature Tree

Root-collar sprouts are most common today

# American Chestnut ID: Root-Collar Sprouts

Girdling, sunken stem cankers

Orange fruiting bodies

Water sprouts, drought symptoms

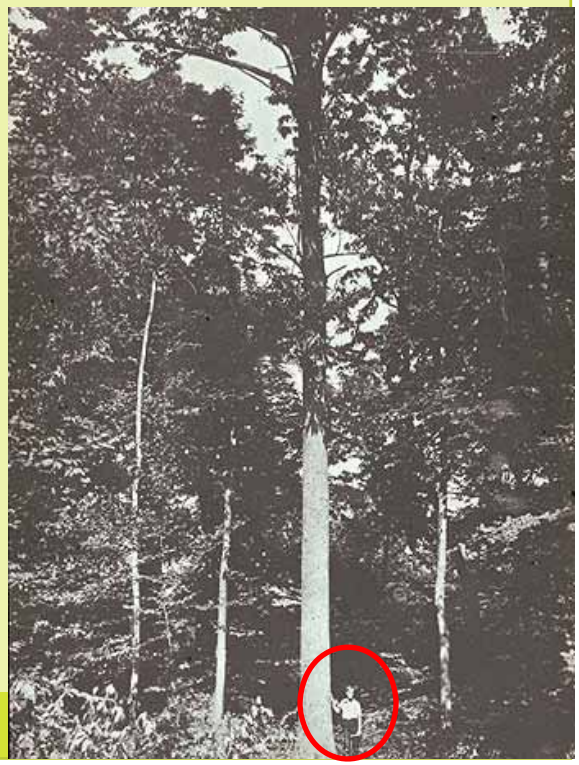
# American Chestnut ID: Blight (*Cryphonectria parasitica*)

# American Chestnut: The Past 100 Years

**PRE-BLIGHT USES, BLIGHT INTRODUCTION AND SPREAD, SPECIES RESTORATION WORK, AND LESSONS LEARNED**

# American Chestnut: The Tree

- Major component of eastern forests
- Fast growth, large, extremely rot resistant
- High-value timber species
- Nuts valuable to wildlife
- Tannins used in tanning leather
- Nuts valuable to people and livestock
- Culturally significant



# Chestnut Blight

- Blight first identified in New York in 1904
- Fungus (*Cryphonectria parasitica*) girdling canker
- Spread quickly, functionally wiped out chestnut as over-story tree by 1950's
- Two types of spores – airborne ascospores and sticky, spiral conidia (asexual spores)

# Early Restoration Attempts

- Cultural methods
  - Tree surgery, fungicide, forest gap barriers, eventually removal
- Identifying natural resistance among American chestnuts
  - 90% of existing trees determined to have escaped blight
  - Small number existing with a low level of natural blight resistance
- Replacement tree to fill niche
  - Expeditions to Asia, high hopes for Chinese chestnut
  - Ultimately failed, due to poor understanding of Chinese chestnut habitat requirements
  - Chinese chestnut since proven too small to compete in a mature forest setting

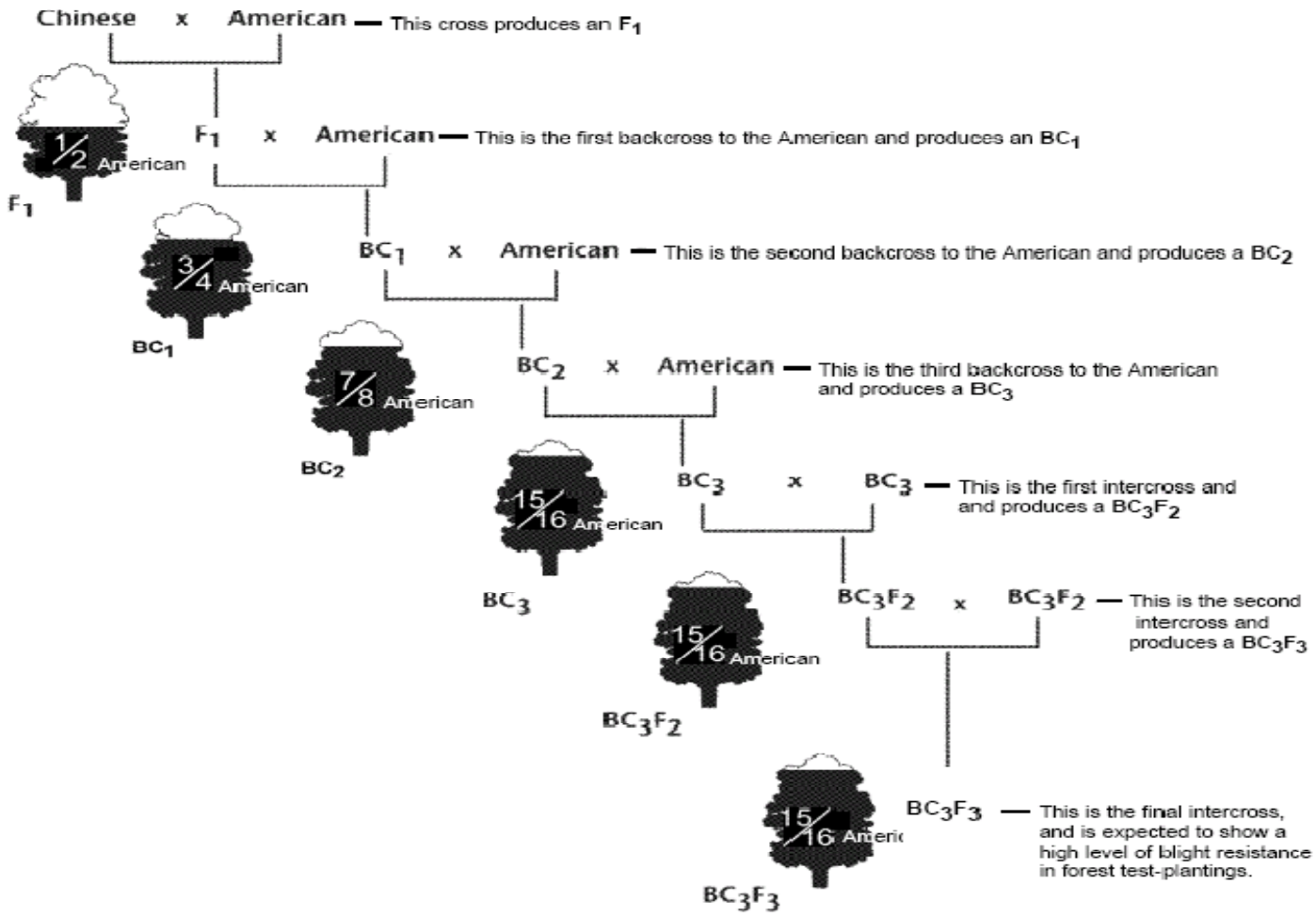
# Early Breeding

- USDA Breeding Program
  - American chestnut x Chinese chestnut, Japanese chestnut or other
  - Only 50% American chestnut, or less if backcrossed to another Asian chestnut parent
  - Poor American characteristics and blight resistance – project abandoned in 1960's
- CAES Breeding Program
  - Started by Arthur Graves in 1920's and continued today
  - Wide variety of hybrids and backcrosses
  - Looking for blight-resistance and timber form
  - Working with the fungus as well

# THE AMERICAN CHESTNUT FOUNDATION'S BACKCROSS BREEDING PROGRAM

## ADDITIONAL AMERICAN CHESTNUT CHARACTERISTICS ARE REGAINED WITH EACH BACKCROSS.

TACF expects a high level of blight resistance and American characteristics to be present in selected $BC_3F_2$ seed orchard parents. Their $BC_3F_3$ progeny will be extensively tested by TACF for blight resistance and ability to compete in the forest.




**Note:** In each step, the backcross is selected for resistance. Trees indicate average fraction of American genes with no selection.

# TACF Chestnut Restoration

- Breed a genetically diverse and regionally-adapted population of blight-resistant American chestnuts
  - Utilize state chapter system to breed 20 lines/resistance source in each participating state (16 chapters in 19 eastern states)
    - Include greater diversity and local adaptation to breeding stock
    - Breed enough resistant lines to ensure genetic diversity and avoid inbreeding (surpass 50/500 rule)
- Test "final" crosses in trial plantings – USFS and other cooperators
- Conduct restoration plantings w/in the native range
  - Employ a grid system for range (USGS 7.5 min quads)
  - Establish at least ½-acre plantings of 50+ trees within each grid cell
  - Should allow for natural spread and cross-pollination over time

# Breeding A Blight-Resistant Chestnut

## TACF'S BREEDING PROGRAM

# Genetics Refresher

- **Allele** – one possible state or form of a gene, distinguished from other alleles by phenotypic effects (Alleles for flower color: R = red, r = white)
- **Heterozygote** – An individual with different alleles for a particular trait (Rr)
- **Homozygote** – An individual with identical alleles for a particular trait (RR or rr)
- **Genotype** – the specific allelic or genetic constitution of an organism (genetic make-up)
- **Phenotype** – the observable properties of an organism that are genetically controlled (**expression** of genetic make-up)

# Genetics Refresher

- **Dominant trait** – one that is expressed in a heterozygote
  - RR or Rr are both red because “R” is dominant
- **Recessive trait** – one that is not expressed in a heterozygote
  - Only rr is white because “r” is recessive
- **Incomplete dominance** – Expression of heterozygous phenotype that is distinct and often intermediate to that of either parent
  - RR is red, rr is white and Rr is pink

# Genetics Refresher

## Dominant/Recessive Inheritance

| | R | r |
|---|---|---|
| **R** | RR | Rr |
| **r** | rR | rr |

Rr x Rr

R = red
r = white

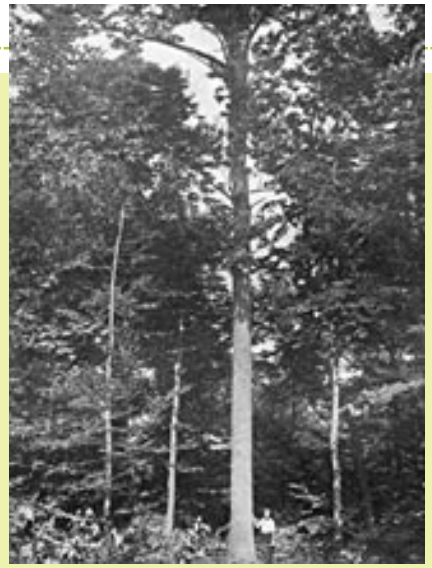
## Incomplete Dominance

| | R | r |
|---|---|---|
| **R** | RR | Rr |
| **r** | rR | rr |

Rr x Rr

# Backcross Breeding

- American chestnuts are fully susceptible to blight
- Some Chinese chestnuts are fully resistant to the blight, all have moderate resistance
- Blight-resistance is **incompletely dominant**
  - Evidence from field tests
  - Segregation of resistant vs. non-resistant offspring
  - Ratios observed have led to:
    - 2 or 3 genes for blight resistance
    - Trees homozygous for blight resistance more resistant than heterozygous trees (based on observations and ratios)

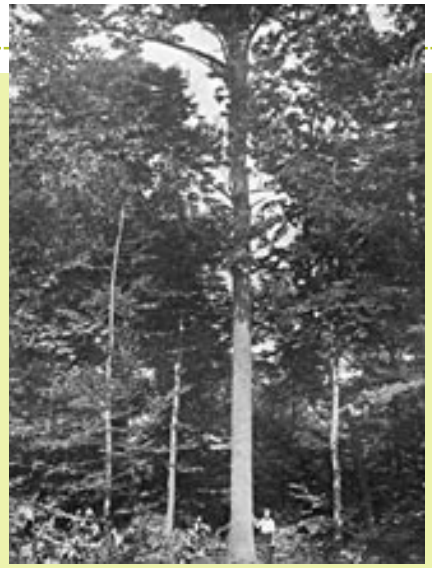


American chestnut

Chinese chestnut

# Backcross Breeding

- Combination of hybrid and backcross breeding
- Hybrid cross captures blight-resistance
  - American x Chinese
- Backcrosses breed out Chinese chestnut character and include more American chestnut character
  - Repeat enough times to re-capture desired amount of American character (3 or 4 times)
- Intercrosses (hybrid of two backcrosses) increase blight-resistance further
  - Each intercross increases chance of breeding trees homozygous for resistance (2 times)



American chestnut

Chinese chestnut

# TACF Backcross Breeding Program

- First generation hybrid to capture blight-resistance
  - American x Chinese = F1: ½ American, ½ Chinese. All are moderately resistant
- Offspring backcrossed with American chestnut over several generations to capture more American character
  - F1 x American = Backcross 1 (BC1): ¾ American, ¼ Chinese. 1 out of 8 are moderately resistant.
  - BC1 x American = B2: 7/8 American, 1/8 Chinese. 1 out of 8 are moderately resistant.
  - BC2 x American = B3: 15/16 American, 1/16 Chinese 1 out of 8 are moderately resistant.
- Intercross resistant offspring to increase blight-resistance
  - BC3F1 x BC3F1 = B3F2: 15/16 American. 1 out of 64 are highly resistant.
  - BC3F2 x BC3F2 = B3F3: 15/16 American. **All are highly resistant.**

# TACF Backcross Breeding Program

| | rrr | |
|---|---|---|
| RRR | RrRrRr | 3R |
| RRr | RrRrrr | 2R |
| rRR | rrRrRr | 2R |
| RrR | RrrrRr | 2R |
| Rrr | Rrrrrr | 1R |
| rrR | rrrrRr | 1R |
| rRr | rrRrrr | 1R |
| rrr | rrrrrr | 0R |

Resistance in backcross generations. Note: no offspring can be homozygous for resistance in these generations.

# TACF Backcross Breeding Program

| | RRR | RRr | rRR | RrR | Rrr | rrR | rRr | rrr |
|---|---|---|---|---|---|---|---|---|
| RRR | RRRRRR | RRRRRr | RrRRRR | RRRrRR | RRRrRr | RrRrRR | RrRRRr | RrRrRr |
| RRr | RRRRrR | RRRRrr | RrRRrR | RRRrrR | RRRrrr | RrRrrR | RrRRrr | RrRrrr |
| rRR | rRRRRR | rRRRRr | rrRRRR | rRRrRR | rRRrRr | rrRrRR | rrRRRr | rrRrRr |
| RrR | RRrRRR | RRrRRr | RrrRRR | RRrrRR | RRrrRr | RrrrRR | RrrRRr | RrrrRr |
| Rrr | RRrRrR | RRrRrr | RrrRrR | RRrrrR | RRrrrr | RrrrrR | RrrRrr | Rrrrrr |
| rrR | rRrRRR | rRrRRr | rrrRRR | rRrrRR | rRrrRr | rrrrRR | rrrRRr | rrrrRr |
| rRr | rRRRrR | rRRRrr | rrRRrR | rRRrrR | rRRrrr | rrRrrR | rrRRrr | rrRrrr |
| rrr | rRrRrR | rRrRrr | rrrRrR | rRrrrR | rRrrrr | rrrrrR | rrrRrr | rrrrrr |

Resistance in intercross generations. Note: one cross should be homozygous for all resistance genes, and many homozygous for one or two resistance genes.

# TACF Backcross Breeding Program

- F1 - all are moderately resistant
- All backcrosses: 1 out of 8 theoretically should be moderately resistant. The rest will vary in resistance from none to less than moderate
- First intercross: 1 out of 64 theoretically should be highly resistant
- Second intercross: all theoretically should be highly resistant

# TACF Backcross Breeding Program

- 6 generations of crossing
- Typically takes 5 years  (at least) to grow chestnuts to size appropriate for blight-resistance trials
- Typically takes 5-7 years (at least) to get chestnuts to flower
- This means at a minimum, our program should take 30-40 years to complete
- TACF is in its 26$^{th}$ year and producing 6$^{th}$ generation material for the 2$^{nd}$ or 3$^{rd}$ year– how is that possible?!!

# Sources of Resistance: A Head Start

## 'Graves'

- First backcross tree (BC1) developed by Arthur Graves at the CAES in 1953
- Chinese chestnut grandparent, ‘Mahogany’
- Tree showed good blight-resistance
- Material still available in 1980’s

## ‘Clapper’

- First backcross tree (BC1) developed in Indiana
- Named for R.B. Clapper with the original USDA breeding program
- Tree showed good timber for and blight-resistance
- Material still available in 1980’s

# THE AMERICAN CHESTNUT FOUNDATION'S BACKCROSS BREEDING PROGRAM

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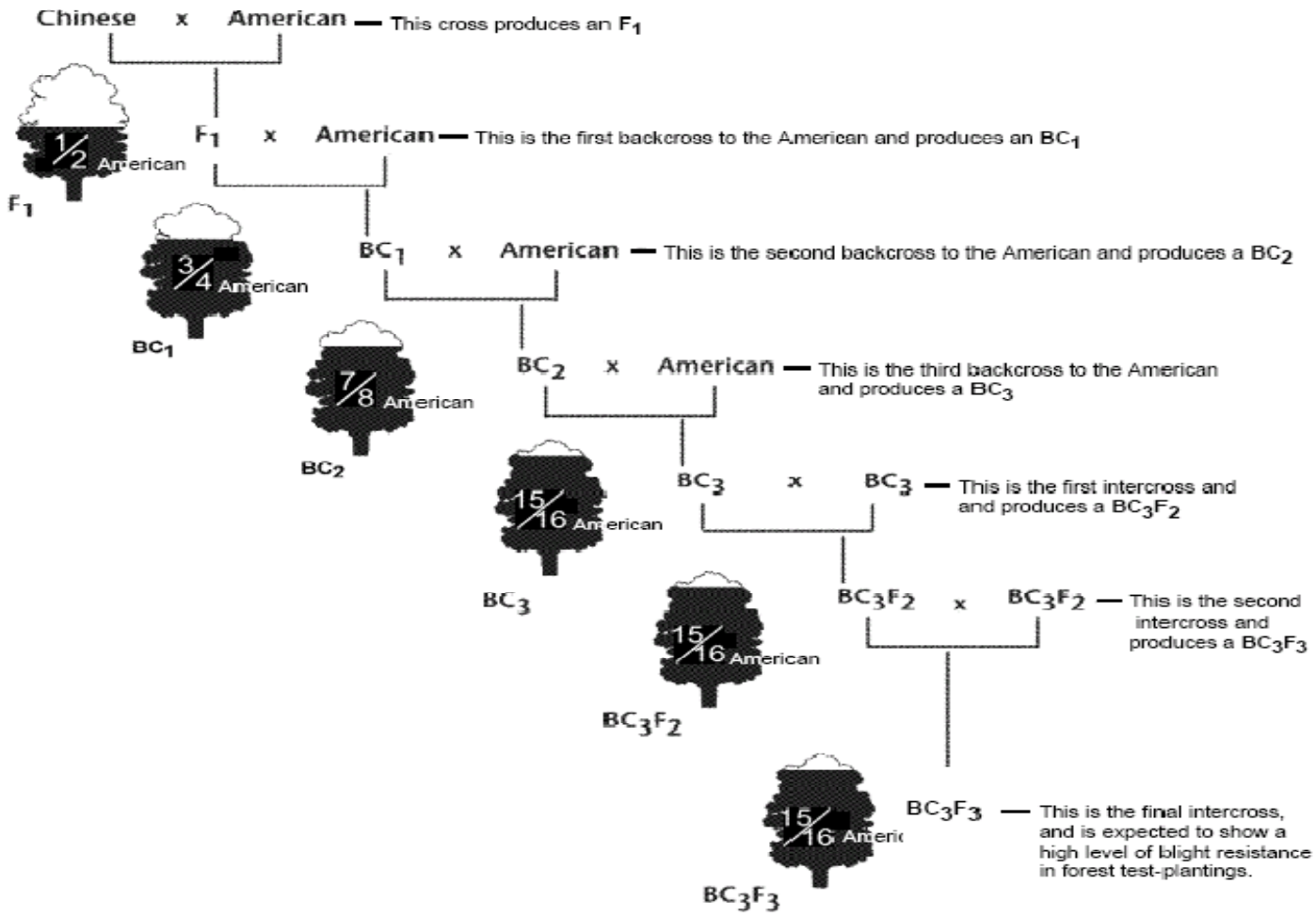


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# TACF Backcross Breeding Program

## Meadowview

- Started with ‘Graves’ and ‘Clapper’ sources of resistance
- Completed 4 generations of breeding since 1986
- Provides backcross pollen to state chapters
- Working to develop additional sources of resistance
  - More time-consuming, need to start at F1 cross

## State Chapters

- Identify local mother trees
- Use BC2 (or even BC3) pollen from Meadowview to complete final backcross generation locally
- Breed final two intercross generations locally
- Allows state chapters to complete breeding work more quickly, while still incorporating local genetics and adaptations

# Breeding Locally: The South Kingstown, RI Orchard

**A LOOK AT THE MA/RI CHAPTER BREEDING PROGRAM AND THE FUTURE OF THE SKLT ORCHARD**

# Pollinating a Mother Tree

- Native, flowering and accessible
- MA/RI Chapter actively pursues chestnut reports
  - Several trees pollinated every year
- Coordinate access, monitor flower development and request pollen from Meadowview Research Farms

# Controlled Pollination!!

# 2009 RI Mother Trees

- 2 Mother Trees pollinated
  - E. Greenwich, RI (~500 nuts)
  - Exeter, RI (~200 nuts)
- Seed stored over the winter
- Will be planted in spring of 2010

# Breeding Orchards

- At least 1-1.5 acres
- Plant at least three backcross lines
  - A “line” is the offspring from a native mother trees crossed with a blight-resistant pollen from our Meadowview Research Farms
- Suitable conditions: full sun, well-drained acidic soil, protection from wildlife, appropriate management and care available for at least 15 years

# Breeding Orchards

- Plant lines from controlled pollinations, as well as controls:
  - Pure American chestnut
  - Pure Chinese chestnut
  - F1 hybrids (American chestnut x Chinese chestnut)
- Grow seedlings to 1.5-3” diameter
- Inoculate with blight fungus, rate infection and select based on resistance and American character

# South Kingstown Breeding Orchard

- Partnership with MA/RI TACF, URI Master Gardeners and South Kingstown Land Trust
- Appropriate site located in 2008
- Orchard controls established in 2009
- Full orchard planting planned for 2010

# South Kingstown, RI Breeding Orchard

- Approximately 1 acre
- Currently planted: controls - American, Chinese and F1
  - American and Chinese planted as seed
  - F1 planted as seedlings
- Plant breeding lines in spring of 2010
  - Material determined this winter, likely from RI mother tree(s)
- Full sun, well-drained soil
- Deer fence, weed and vegetation control, tube shelters (rodent protection)
- Orchard manager – Rudi Hempe – as well as support from SKLT, URI Master Gardeners and MA/RI TACF

# South Kingstown Orchard: Future

- Plant breeding lines
- Grow to ~1.5-3” diameter
- Challenge trees with blight fungus
- Select the 2-4 most resistant trees from each breeding line
- Remove all but selected trees and allow them to cross-pollinate
- Produce BC3F2 seed for a seed orchard

# Seed Orchards

- Plant with nuts produced by crossing selected BC3 trees
  - These are the nuts produced by selected trees left in the breeding orchard
  - The nuts planted are from the BC3F2 generation
- Each resistance source has it's own seed orchard
  - No mixing of resistance sources at this generation
- Plant in 9, 1-acre blocks
  - Each block contains representatives from every line created with a particular source of resistance
  - Each block contains 150 trees from a particular line, or ~3,000 trees

# Seed Orchards

- Much higher density planting –
  - Rows 5 ft apart, trees 1 ft apart
- Inoculation at much younger age
- Selected trees produce BC3F3 seeds
  - Should all be highly blight-resistant and appropriate for restoration plantings

# A Note on Pollen…

- Pollens sent from Meadowview Research Farms are collected from selected trees at various stages of breeding (usually BC2 or BC3)
- 2 major sources of resistance (‘Clapper’ and ‘Graves’) but others are being developed
- Breeding goal for state chapters using ‘Graves’ and ‘Clapper’ is to produce 20 distinct lines per source
  - Should be enough to prevent inbreeding depression in future breeding generations
  - Capture local genetics and increase diversity of entire program

# Taking Breeding Further

- MA/RI Chapter has completed 20 lines of both ‘Graves’ and ‘Clapper’
- Now moved on to a *new* source of resistance: ‘Nanking’
- Proved to be the most blight-resistant grafted Chinese chestnut importation
  - Brought to US in 1935, collected near Shanghai
  - Released by USDA in 1949
  - Identified as highly blight-resistant in 1980’s
  - Currently about 20 pollen lines of BC2 ‘Nanking’ available for breeding

# Future Plans for ‘Nanking’

- Need a minimum of 15 lines from ‘Nanking’ resistance to prevent future inbreeding depression and population collapse
- MA/RI Chapter will need to pollinate additional chestnuts and plant breeding orchards to reach this goal
- A lot of work still ahead – contact MA/RI Chapter if you would like to help!

# Upcoming TACF Events

- MA Chapter Annual Meeting – Tomorrow!
  - National Grid Facility in Worcester, MA
  - Updates on Chapter work and future plans
  - Chestnut Cold Tolerance – A Restoration Concern
    - Dr. Paul Schaberg, USFS, South Burlington, VT
- CT Chapter Annual Meeting
  - March 6, 2010 – Hartford, CT
  - Genomics, insects and chestnut ID
- TACF 27th Annual Meeting
  - Shepherdstown, WV – October, 2010

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