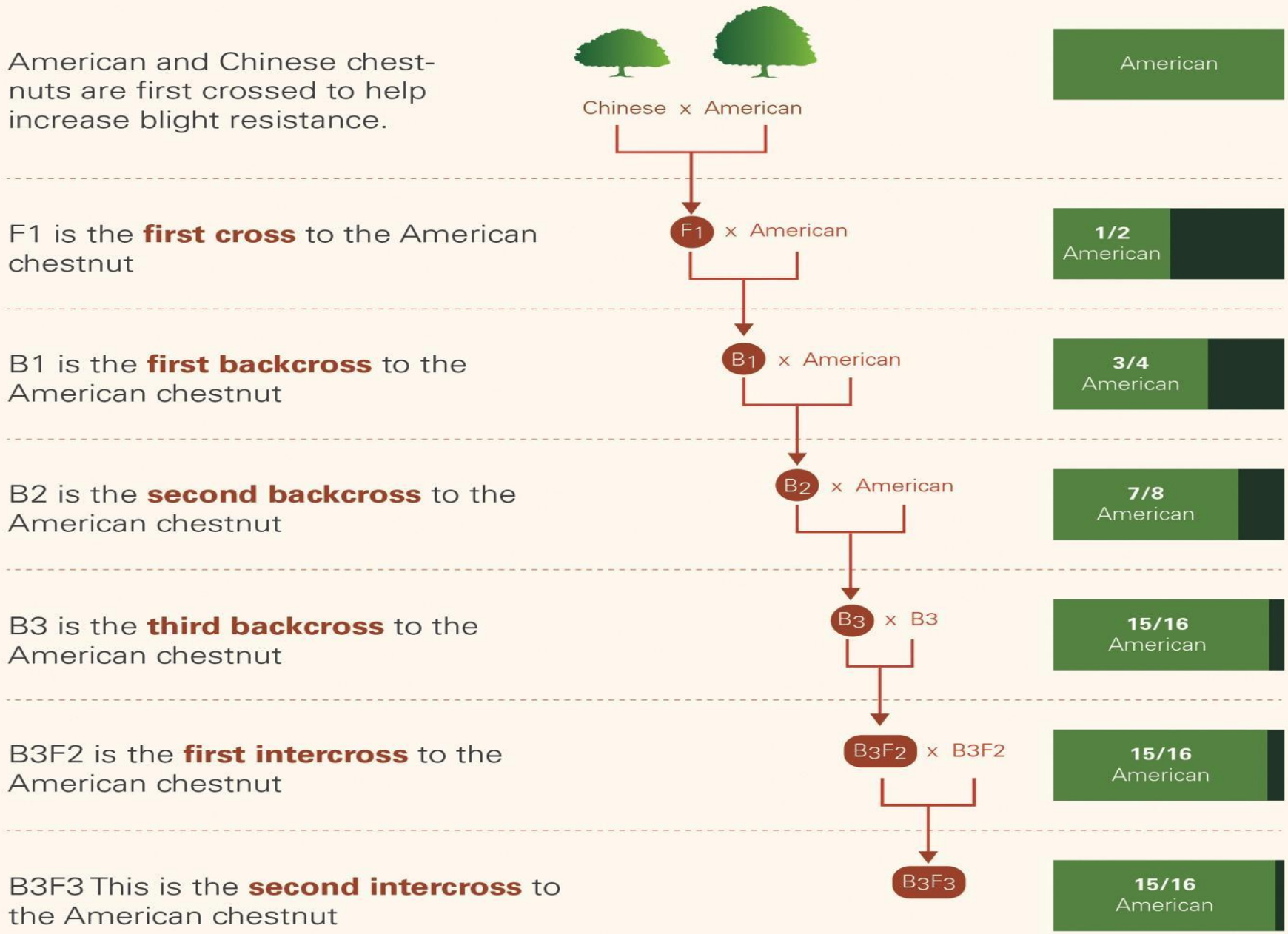
Early screening potentially blight-resistant American chestnut using small stem assays

THOMAS MCNEILL SAIELLI

MID-ATLANTIC REGIONAL SCIENCE COORDINATOR

THE AMERICAN CHESTNUT FOUNDATION



Breeding, testing and evaluation continues. TACF's breeding program will continue to integrate additional sources of blight resistance into the breeding populations.

B3F2 and B4F2 families are planted in seed orchards throughout the state chapters and at Meadowview Research Farms

Cull blight-susceptible trees

Strategies for making final selections in TACF seed orchards

1. Ongoing selections

Hill Craddock and Jared Westbrook assess a tree for blight resistance at the Wagner Farm

Strategies for making final selections in TACF seed orchards

1. **Phenotypic selections**
2. **Progeny tests**

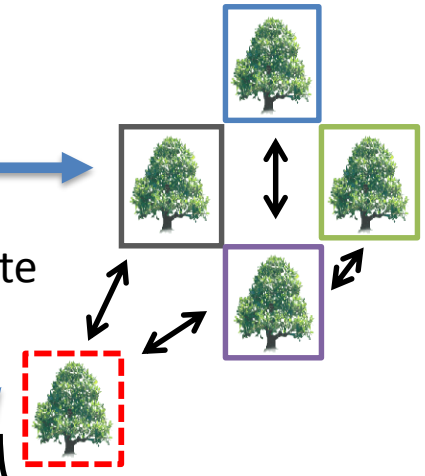
Tom Saielli and Georgia Chapter volunteers putting in a progeny test in the Chattahoochee National Forest

Progeny testing has begun on BC_3F_2 trees that remain after initial culling

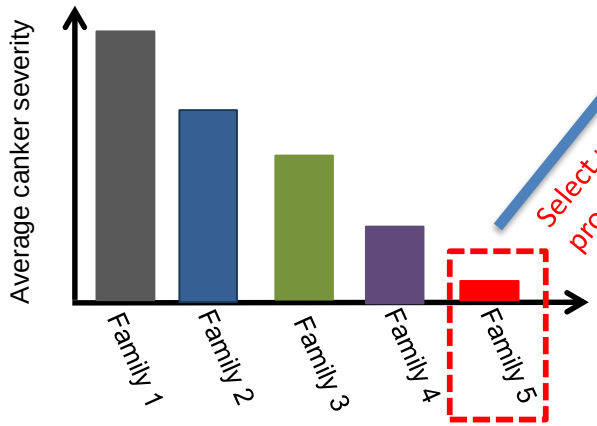
Unselected BC_3F_2 seed orchard

Artificially inoculate & cull susceptible

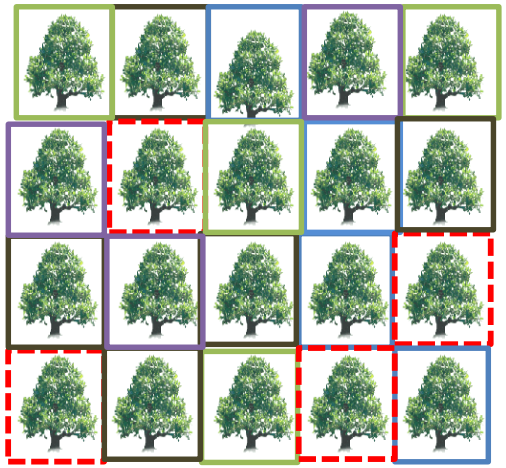
Partially selected seed orchard



Open-pollination among BC_3F_2 survivors



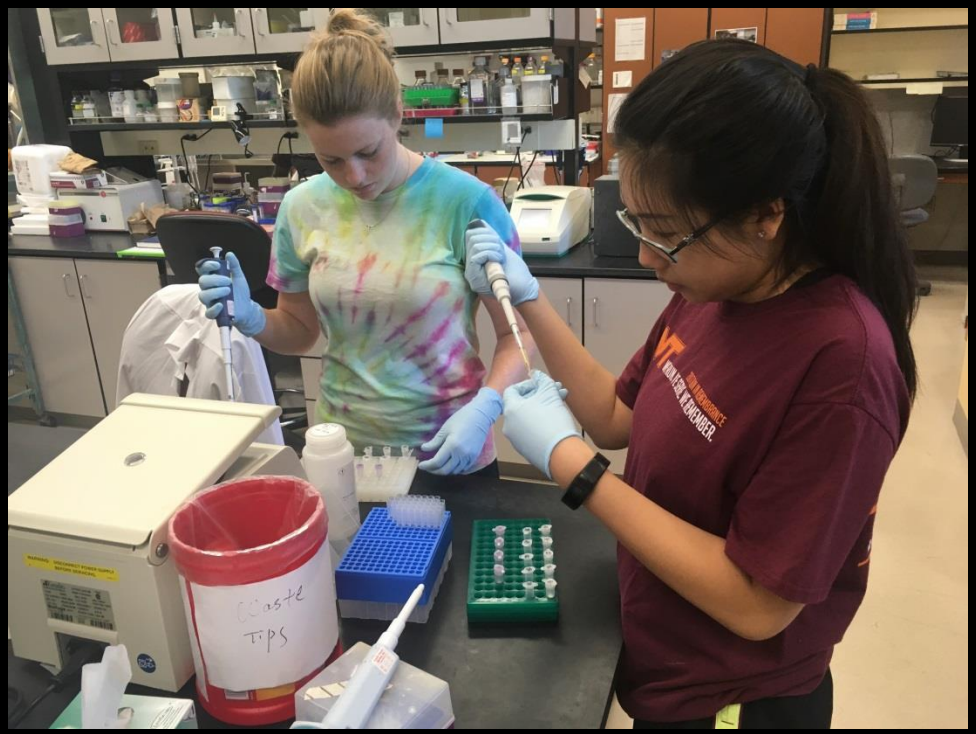
Select parent whose progeny have smallest cankers



Artificially inoculate BC_3F_3 progeny from each selection candidate

Strategies for making final selections in TACF seed orchards

1. Phenotypic selections
2. Progeny tests
3. Genomic selection



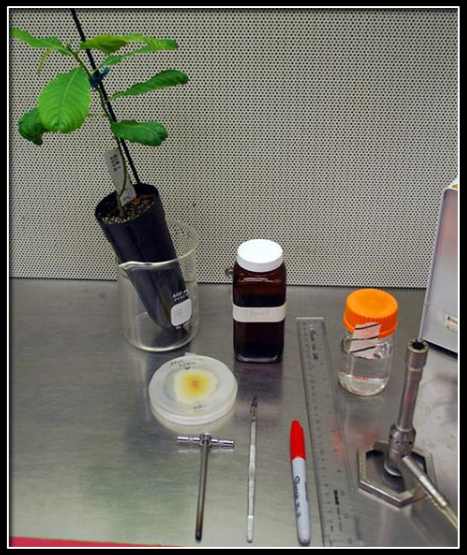
Students at Virginia Tech extract DNA from chestnut leaf samples

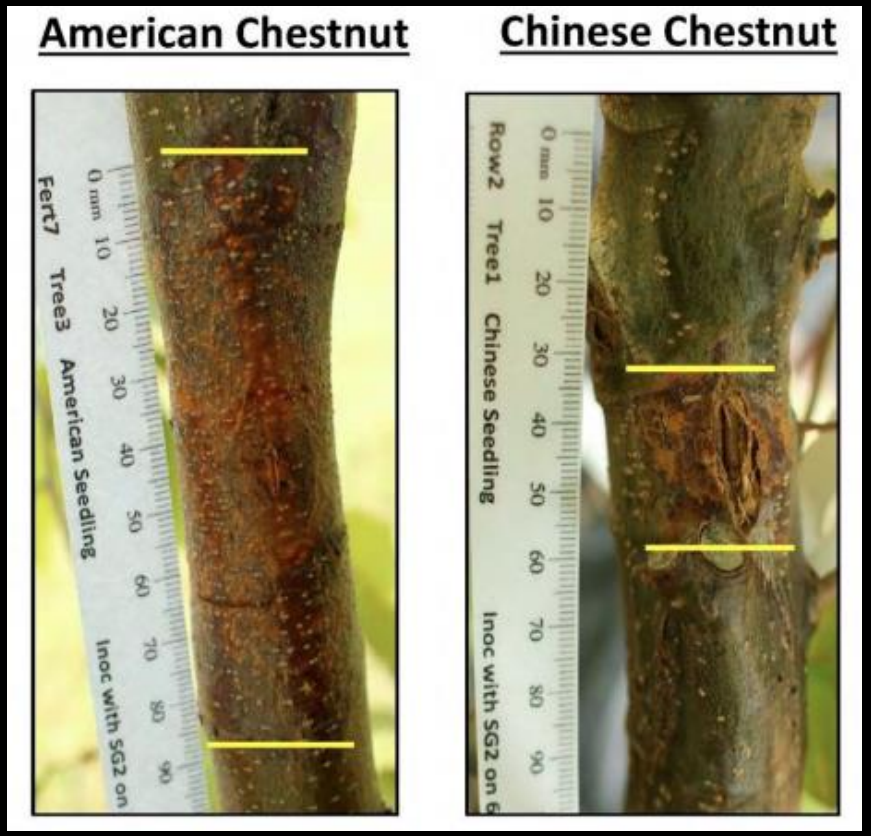
Strategies for making final selections in TACF seed orchards

Accelerating progeny testing with the small stem assay

A seedling at UMD reacts to the small stem assay with significant canker developing, indicating this particular seedling is susceptible.

Small stem assay





Photos: Andy Newhouse, Bill Powell (SUNY-ESF)

SSA progeny testing: Results from the RSC

Why progeny test with small stem assays?

- Obtain results with one year in greenhouse v. three years in field
- Increase sample sizes within families beyond what is feasible in the field

2017 RSC/Meadowview SSA Experimental Design

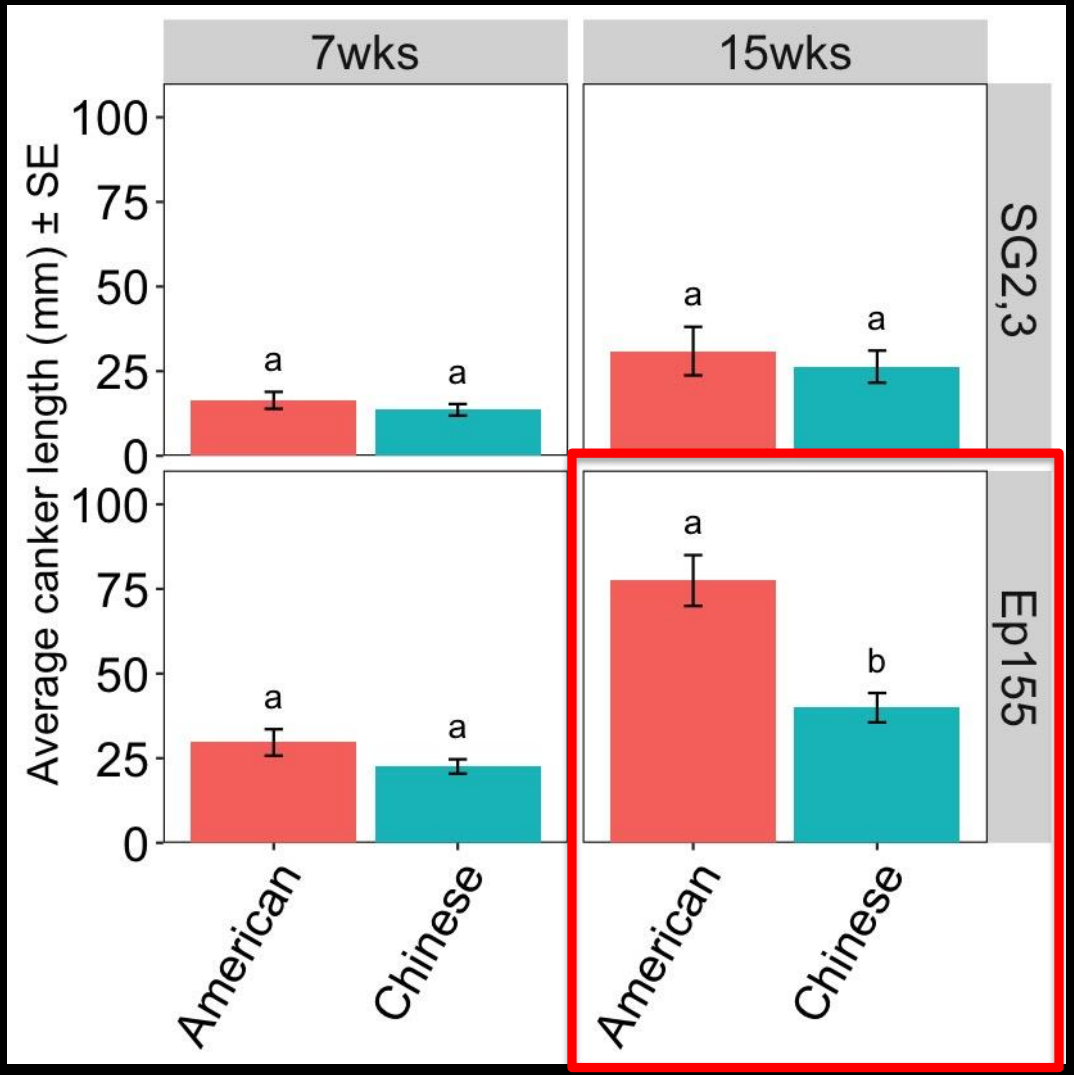
- 69 BC3-F3 families + Chinese and American controls
  - 40 seed sowed per family
- Randomized complete block design
- ½ inoculated with SG2,3, ½ inoculated with Ep155
- Canker length measurements at 7 weeks at 15 weeks post-inoculation

Ep155 canker on a Chinese chestnut 15 weeks post-inoculation

Large Ep155 cankers on BC3-F3s 15 weeks post-inoculation

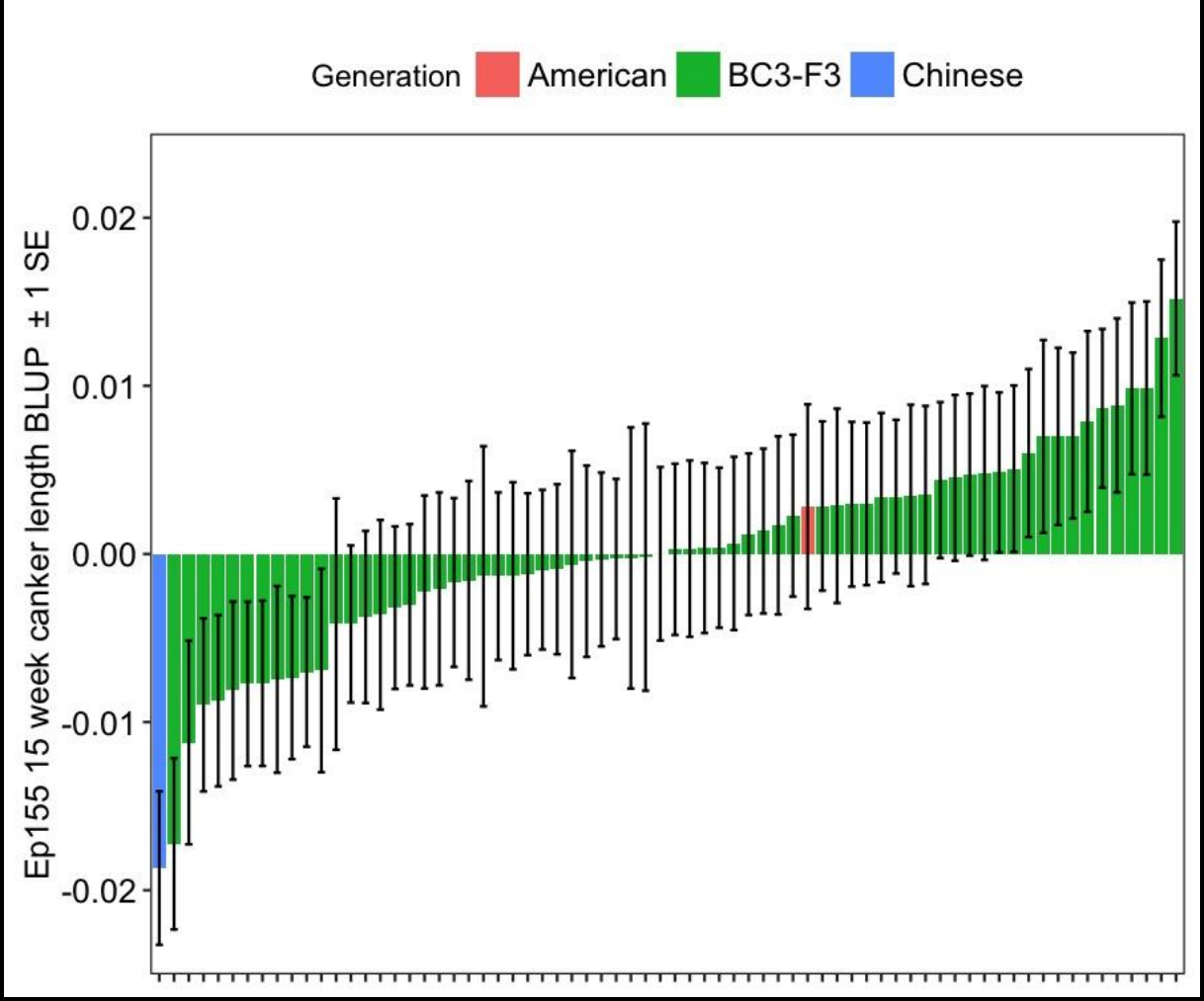
No takes

Can the SSA distinguish the blight resistance of Chinese and American chestnut?

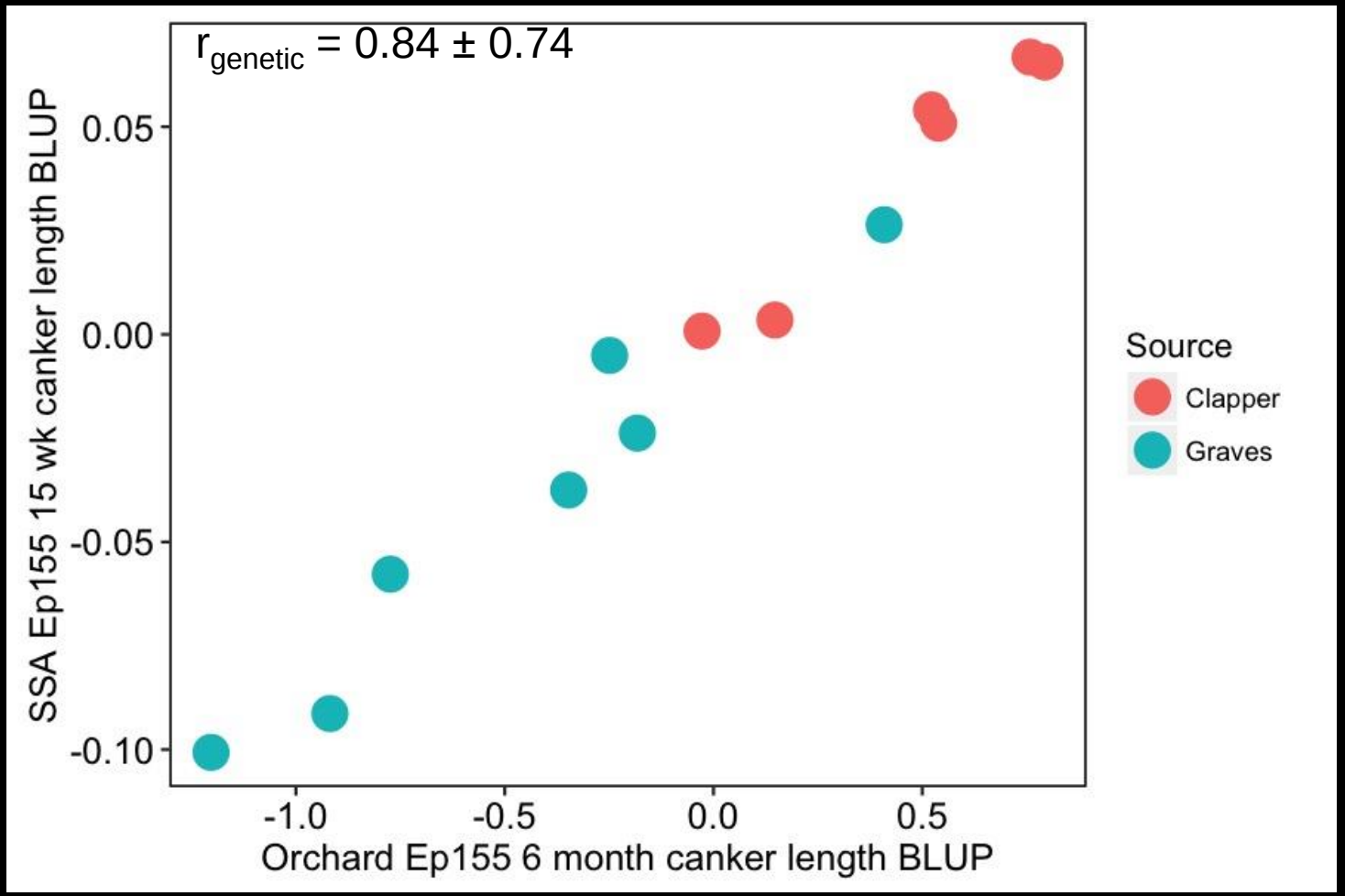


Ep155 cankers significantly different (t-test P<0.001) on Chinese and American chestnut 15 weeks post inoculation

How does genetic variation in canker length among BC3-F3 families compare with Chinese and American chestnut?



Is BC3-F3 family genetic variation in canker length correlated between SSA and orchard progeny tests?



Conclusions

- Greater differentiation in canker lengths between controls and BC3-F3 families with more virulent (Ep155) strain of Cryphonectria parasitica
- Ep155 canker length differentiation more pronounced at 15 week v. 7 week time points
- Preliminary results indicate that family canker length rankings are correlated in the field progeny tests v. SSA

Once we have proof of concept, SSA's may be useful as an early screening tool

Fewer trees planted = less competition

Seedling seed orchards
150 trees per plot
1 foot spacing

Preselected orchards
25 trees per plot
5-10 foot spacing

Chapters Currently Involved in SSA Research

Chapter	Number of seedlings & type of test
Georgia	445 B3F2/B4F2 + controls mostly in D40 containers
Tennessee	1,200 mixed B3F2 and B3F3 + controls mostly in D40 containers, some in 2 gallon containers
Kentucky	820 B3F2 in D40 containers
Maryland	788 B3F2 + controlls in D40 containers
Pennsylvania	1252 B3F3 + Chinese controls in D40 containers
Maine (Roth)	1,260 mixed B3F2 and B3F3 + controls in D40 containers
Maine (Clark)	530 mixed Graves and Clappers + controls in Stuewe's (MT2510) containers
Asheville (RSC)/Meadowview Research Farm	1,450 B3F3 + controls in D40 containers, ½ inoculated with SG2,3, ½ inoculated with Ep155

Preliminary Results

- Many chapters saw very little expression of blight after 8 -10 weeks.
- No wilting and dieback?
- Hold overwinter & attempt again next year?

Hypotheses for no-take?

- Technique
- Inoculum
- Environment
- Timing
- Size of stock

Bruce Levine pilot study: Liquid medium, bigger incision

1. Twelve seedlings growing in 1 gallon containers
2. Ten seedlings inoculated with C. parasitica, two controls

Bruce Levine pilot study: Liquid medium, bigger incision

1. Blight fungus (SG2-3) growing in liquid medium

Bruce Levine pilot study: Liquid medium, bigger incision

1. Blight fungus (SG2-3) growing in liquid medium
2. Larger incision
   - 8.0 x 0.5-1 mm incisions

Bruce Levine pilot study: Liquid medium, bigger incision

1. Blight fungus (SG2-3) growing in liquid medium
2. Larger incision
    - The fungus stuck nicely to the incision. Control trees were inoculated with sterile liquid medium

Bruce Levine pilot study: Liquid medium, bigger incision

1. Blight fungus (SG2-3) growing in liquid medium
2. Larger incision

Results after two weeks

- No cankers on the two controls
- Cankers developed on all ten inoculated seedlings
- Average canker length was 25.0 mm

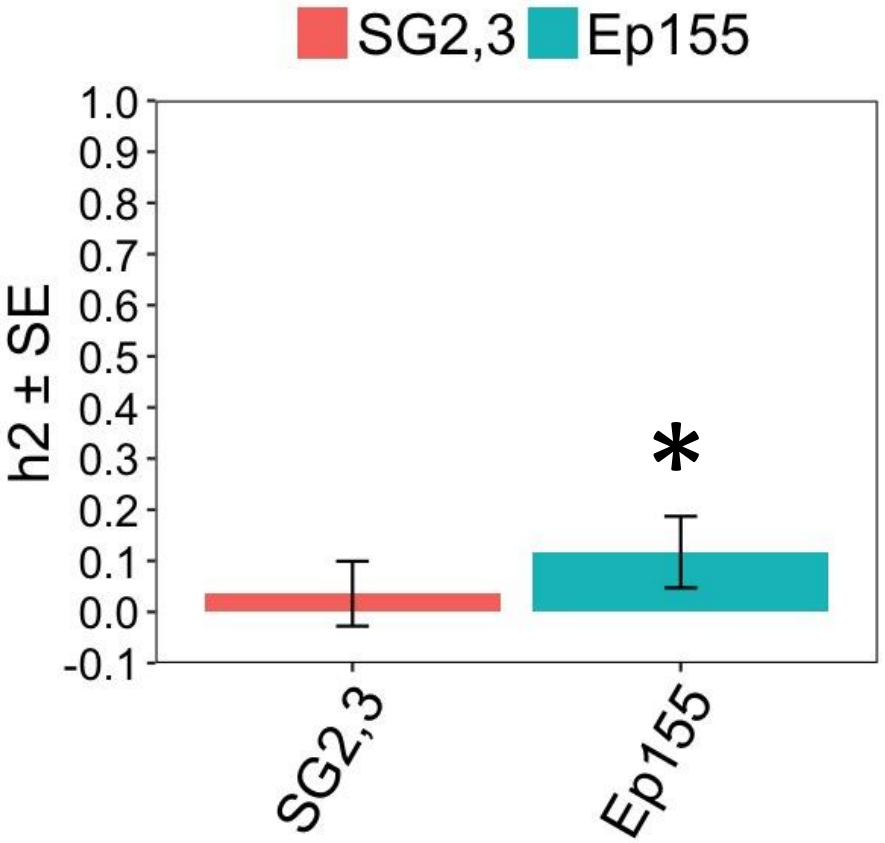
Next Round of Experiments

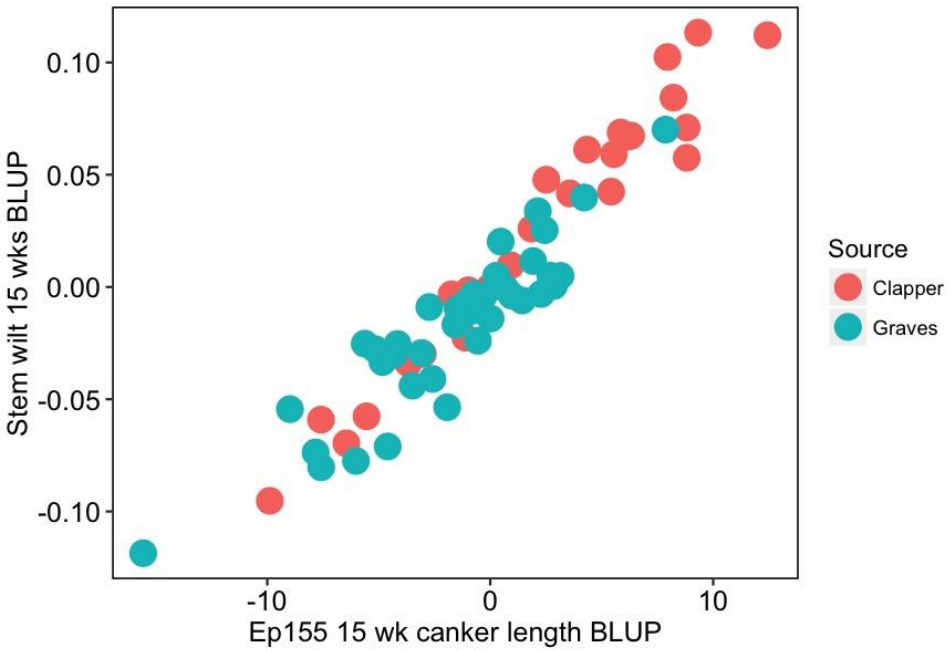
1. Wounding technique
2. Type of media
3. Fungal strain
4. Size of seedling
5. Timing of propagation, inoculation, canker ratings
6. Location of incision

Thank You

THE
AMERICAN
CHESTNUT
FOUNDATION®

Is stem wilt 15 weeks after inoculation heritable and correlated with canker length?



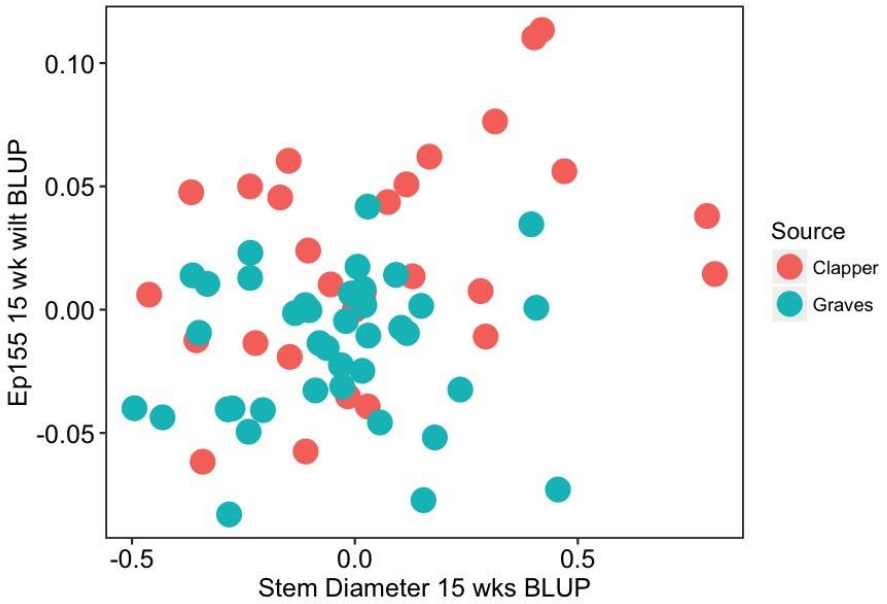


* Denotes BC3-F3 family variance component > 0 (likelihood ratio test P = 0.02)

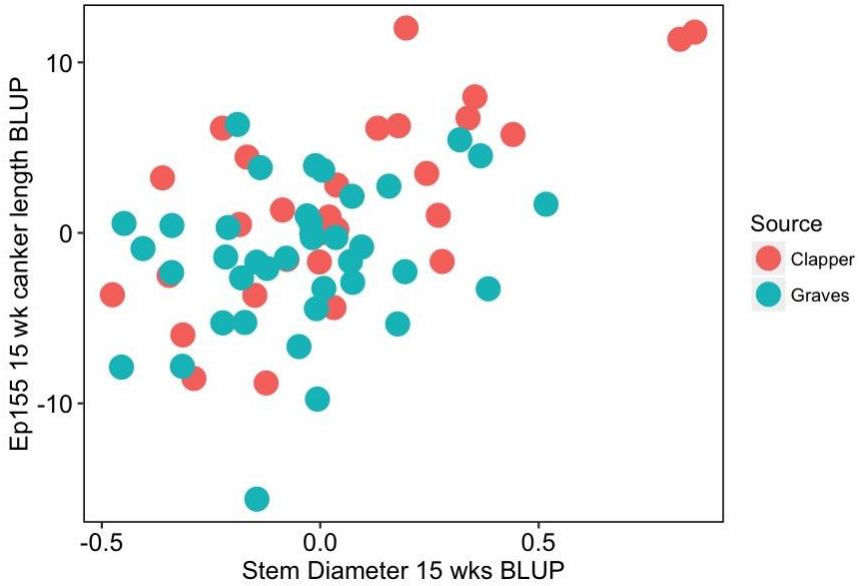
Genetic correlation between stem wilt and canker length 0.84 ± 0.24 (r_g = 0, LRT P = 0.004)

Is stem diameter genetically correlated with canker length or stem wilt?

Heritable variation in stem diameter at seven months age ($h^2 = 0.37 \pm 0.12$)



Wilt v. stem diameter
$r_{genetic} = 0.10 \pm 0.33$
LRT P = 0.73



Canker length v. stem diameter
$r_{genetic} = 0.45 \pm 0.19$
LRT P = 0.03