Small Stem Assay for Chestnut Blight Resistance in Segregating Full-Sib Families of F2 Chestnut Trees

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THE AMERICAN CHESTNUT FOUNDATION®

BACKGROUND

The efforts to restore the American chestnut began in the 1920s with attempts to introduce blight resistance into the American chestnut species by hybridizing it with the Asian species. In 1981 Charles Burnham hypothesized that three generations of backcrossing and selection for blight resistance would be sufficient to recover trees with American chestnut morphology and Asian chestnut levels of disease resistance. The American Chestnut Foundation was founded in 1983 to test Burnham's proposal (Burnham, Rutter and French 1986).

The TACF state chapters currently participating in the breeding program have been challenged by the difficulty and long time periods required to screen hybrid seedling progeny for blight resistance – a process that can take from 5 to 7 years and requires large commitments of land and other resources. Small stem assays offer an attractive alternative in that they can be completed in one or two years, using container-grown plants in a greenhouse or nursery setting (Powell et al. 2007). This application has been used by The American Chestnut Foundation with plans to continue to use this method in future progeny testing (Westbrook 2018).

Objectives

Figure 1. Demonstration of the size of the F2 seedlings that were inoculated.
Photo by: Dr. Hill Craddock

This study focuses on the results of the inoculation of 535 chestnut seedlings during their first growing season. In 2018, 402 seedlings of F2 hybrid families derived from 'Nanking' Chinese, and open pollinated seedlings of *Castanea dentata* and *C. mollissima* used as controls, were screened in a randomized complete block design.

Hypothesis

The hypothesis we are testing is whether the F2 population segregates as expected.

We predict that the American and Chinese will exhibit significant differences in resistance to *Cryphonectria parasitica* inoculation. The F1 population will exhibit an intermediate blight resistance between the American and Chinese chestnut trees.

Furthermore, we predict that the F2s will display a full range of resistance, from highly resistant to low resistance to the effects of chestnut blight with most individuals having an intermediate resistance.

If these hypotheses are supported by the experiment, then the small stem assay will be a valuable source for progeny testing for The American Chestnut Foundation.

METHODS

- Seeds were planted in two gallon containers in early February to early March. Seeds planted were from two families of F2s (NK5 and NK6), one group of F1s, and American and Chinese controls.
- In June seedlings were moved outside to a drip irrigation line. The seedlings were organized in a randomized block design.
- Plants were arranged into 3 blocks (roughly 200 trees per block) with a guard row between each block to prevent a pseudo replication.
- Seedlings were inoculated at the end of July, approximately four months after planting, with *Cryphonectria parasitica* isolate EP-155.
- Although about 666 seeds were planted only 535 seedlings grew to the desired stem diameter of 4 mm.
- Using a grafting knife, a rectangular incision was made 10mm long and 2mm wide (figure 2).

Figure 2. Size of incision that was made for the inoculum. Incision size was 10mm long and 2 mm wide.

- The mycelial margin was cut into 10x2mm pieces.
- After the inoculum was applied to the incision a piece of parafilm was wrapped around the stem at the point of inoculation.
- Seedlings were screened at eight weeks and again at sixteen weeks. Cankers were measured and seedlings that did not survive were removed from the trial.

Figure 3. One of the fifteen F2 hybrids exhibiting Chinese levels of resistance to *Cryphonectria parasitica.*
Photo by: Dr. Hill Craddock

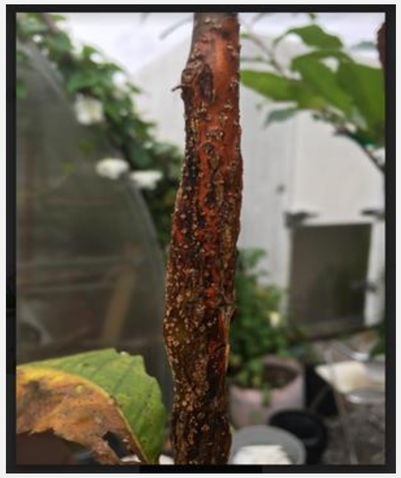

Figure 4. Swollen canker on a surviving F2 hybrid.
Photo by: Dr. Hill Craddock

Preliminary Results

The Chinese controls had the largest survival percentage of 62.79%. The NK6 F2s had second largest survival percentage of 3.87% followed by the NK5 F2s with 2.94% of seedlings that survived. Only 1.18% of the F1 population survived the inoculation and 0.0% of the American control seedlings survived.

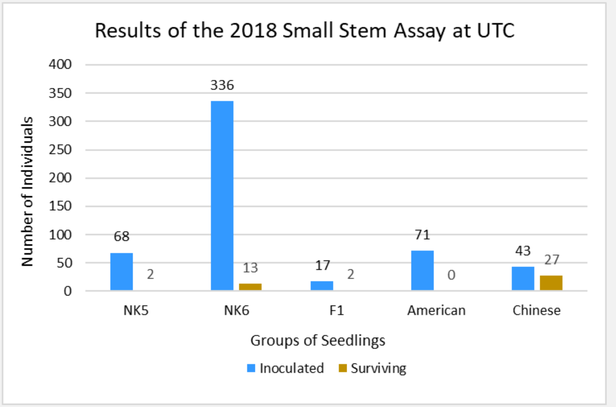

Figure 5. Number of inoculated and surviving chestnut hybrids, F1s and F2s, and control groups, American and Chinese seedlings during the 2018 small stem assay.

CONCLUSIONS

The results of our 2018 small stem assay appear to support our hypotheses. The American and Chinese controls exhibited differences in resistance; 63% of Chinese seedlings survived and 0.0% of American seedlings survived. The F2 seedlings displayed a full range of resistance to *Cryphonectria parasitica*. We judged 96% of the F2 seedlings to be susceptible and removed them from the trial. The few very best F2s had swollen cankers similar to the cankers on the Chinese controls. We selected 4% of the surviving F2s for an orchard planting. Further statistical analysis will be completed with the 8-week canker measurements data to determine the resistance displayed by the F1s. If further statistical analysis continues to support the hypotheses, then the small stem assay may have real value for progeny testing of F2s, including the thousands of B3F2s and B4F2s that we are currently generating at the Chapter level. An effective small stem assay in the nursery will help to drastically reduce the number of trees planted in our Chapter seed orchards.

ACKNOWLEDGMENTS

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