Breeding for Chestnut Blight Resistance at UTC: Small Stem Assays are essential for the Recurrent Genomic Selection program of The American Chestnut Foundation

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Abstract

The Fortwood Street Greenhouse and Nursery is participating in chestnut breeding under the rubric of the TACF Recurrent Genomic Selection (RGS) program, making controlled crosses (hand pollinations) and screening for resistance to Cryphonectria parasitica using small-stem assays (SSAs). Continuing a 30-year long tradition of backcross breeding at the TACF Chapter level, volunteers of the Tennessee Chapter performed RGS-directed crosses in 2023, 2024, and 2025, using the best, selected parents from the backcross breeding programs of the southern chapters of TACF (Carolinas, Georgia, Alabama, and Tennessee). We screened the resulting progeny during their first years of growth, in containers, in the nursery, using small stem assays that involved inoculations with virulent strains of the blight pathogen. Tissue samples were collected from leaves for DNA sequencing and genomic analyses. Cankers were measured and ranked 90 days post inoculation. We also genotyped thousands of non-inoculated seedlings. Selections were made in the SSAs based on the incited canker phenotype. Selections in the non-inoculated trees were based on the genomic prediction models developed by TACF. Selected trees, including those with the best blight-resistance phenotypes from the SSAs and the genotyped non-inoculated seedlings were planted into experimental orchards of two types: the very best seedlings were planted in a seed orchard design, and the other selections were allocated to progeny tests for long-term phenotyping.

Small Stem Assay

The Small Stem Assay (SSA) is a method of screening first-year seedlings for chestnut blight resistance by inoculating with Cryphonectria parasitica. The incited cankers are then measured after 60 or 90 days, depending on the type of inoculation (Figure 1) and ranked using a subjective scale. There may not be enough resolution in the assay to confidently make selections within families, but we can use family averages to predict heritability of the resistant phenotypes when these data are added to the RGS model. Covariates include the size of the plant and so we also collect data on seedling height and crown diameter (Figure 2). Figure 3 shows an example of the unusual corky bark phenotype associated with the OXO+ seedlings in the 2024 UTC SSA.

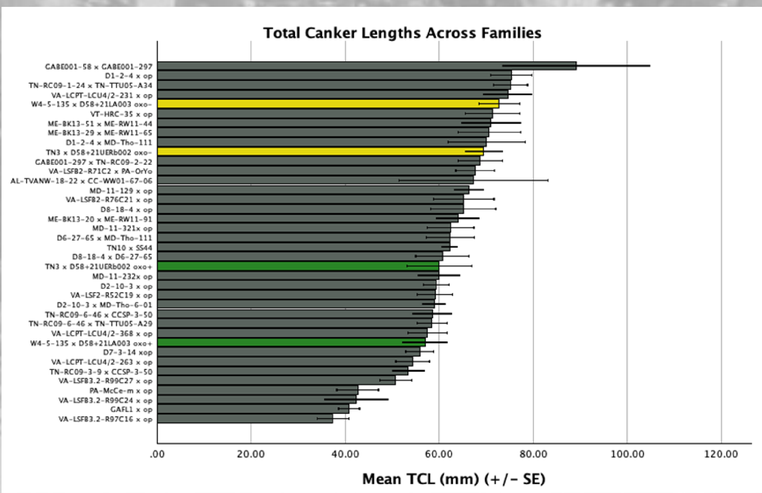

Figure 1. Total canker lengths from the 2024 Small Stem Assay show that canker sizes vary between families. Highlighted data represent two full-sibling OXO families. OXO- progeny tended to have smaller and less severe cankers than their OXO+ siblings but exhibited unusual corky bark symptoms.

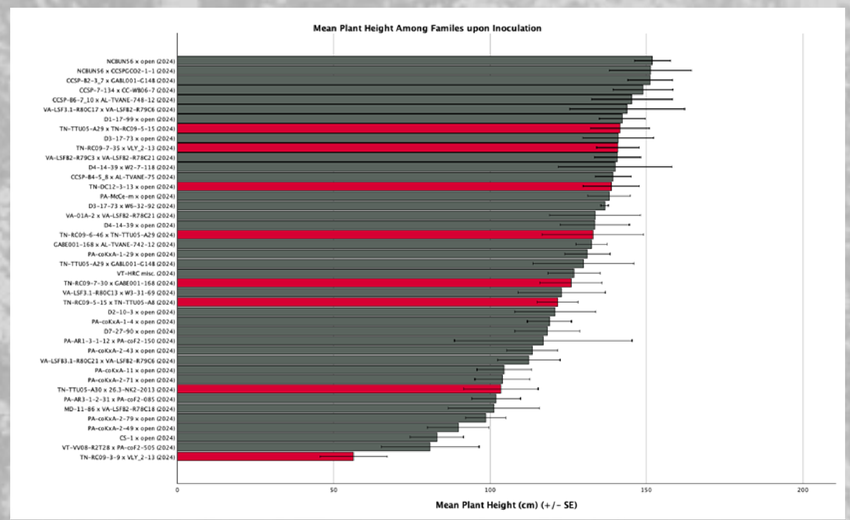

Figure 2. Mean plant height (cm) of 43 full-sib and half-sib families in the 2025 UTC Small Stem Assay. Plant heights were measured on the day of inoculation in July. Highlighted data represent families of Tennessee Chapter selections.

Figure 3. All OXO+ seedlings in the 2024 UTC SSA developed unusual corky growths on the bark. The corky phenotype was not associated with the point of inoculation, sometimes occurring along the entire length of the stem, and was never observed in the OXO- seedlings.

Hand Pollinations

We continue to collect pollen and make crosses in TN-TACF backcross orchards and using naturally occurring wild trees – work that began in 1996. In 2024 we made 27 RGS directed crosses in two TN-TACF orchards (Figure 4 and 5). In 2025 we made 19 crosses in one orchard. Some of the collected pollen was shipped to other Southern Chapters breeders to make their priority RGS crosses, or dried and frozen to preserve the germplasm.

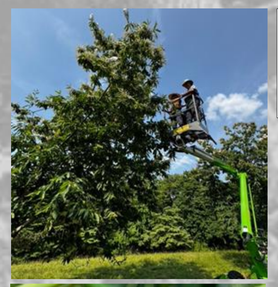

Figure 4. Dr. Craddock and Dr. Zannini use pollen collected from TN-TTU05- A29 (F1) to cross with TN-RC09-6-46 (B3).

Figure 5. Chestnut pistil ready for pollination. On emasculation day, the staminate catkins are removed, and the pistil is bagged. On pollination day, the bag is removed, the flower is pollinated, and the bag is resecured with a color-coded zip tie corresponding to the cross.

Harvest

Open-pollinated and hand-pollinated seeds are harvested using ladders and mechanical lifts before bur dehiscence (Figure 7). In 2024, we harvested 2553 seeds from resulting from hand pollinations (and another open pollinated 62,464 seeds in 76 families). For 2025, we predict an even larger harvest. Seeds are stratified for four months at 35F in a walk-in cooler. Seeds from UTC have been distributed to other TACF locations, including New England, Penn State, Meadowview, Berry College, the Linville River Nursery, and Asheville for SSAs (in a common garden experimental design), and for phytophthora root rot screening.

Figure 7. Chestnuts ready for harvest (left). Harvesting chestnuts from the orchard ladder (middle). Labeled bags of burs are stored in the walk-in cooler until they are shucked and processed (right).

Long-Term Blight Phenotype Ratings

Tennessee currently has 22 backcross and/or germplasm conservation orchards distributed widely across the state. In 2025, we made a concerted effort to visit backcross orchards in the region to assess blight resistance phenotypes. The rubric for long-term phenotypes includes 11 scoring criteria. Several good candidate trees were identified in 2025 that we recommend be added to the RGS program (Figure 8).

Figure 8. Selections are based, in part, on long-term blight phenotype observations in backcross orchards. Shown here, Jeremy Gooch is marking the best-ranked tree in his Yuma, TN orchard with a green marking paint. These highest performing individuals will be considered for inclusion in the RGS breeding program.

Recurrent Genomic Selection

According to TACF, Recurrent genomic selection (RGS) involves using a computer model to associate the DNA profile of a tree (genotype) with field-measured responses to disease like canker size (phenotype). This allows us to make accurate predictions of a tree's resistance from DNA alone, if the tree is related to trees we have already evaluated in the field. This requires that each mother tree in the TN-TACF program and its progeny be sampled for genotyping. In 2024 we sampled tissues from 94 seedlings in the SSA. In 2025, we collected leaf tissue samples from the 600 seedlings in the 2025 UTC SSA and more than 1200 of their container-grown siblings in the nursery.