

Small stem assays

MAY BE A RELIABLE SCREENING TOOL FOR TESTING AMERICAN CHESTNUT RESISTANCE TO *CRYPHONECTRIA PARASITICA*

By Tom Saielli, Mid-Atlantic Regional Science Coordinator and Bruce Levine, MD Chapter

The American Chestnut Foundation (TACF) is screening American chestnut backcross families for blight tolerance using small stem assays (SSAs). In the small stem assay, the stems of containerized chestnut seedlings are inoculated with the fungal pathogen *Cryphonectria parasitica* and assessed for differences in blight tolerance between families. For sixteen weeks post-inoculation, volunteers and TACF staff assessed blight canker severity and survival to detect differences in blight tolerance across families. We also wanted to determine whether a strong correlation exists between the SSA and standard field inoculations.

Since 2017 TACF staff and cooperators have been experimenting with SSAs. After some initial hurdles we now see promising results. In SSAs conducted at Meadowview Research Farms and Penn State, differences in canker severity and survival were detected among BC₃F₃ families screened at both locations. There was also a correlation between survival in containerized SSAs and canker severity in field test seedlings.

2018 Progeny test results

In 2018 TACF, Penn State University, and several cooperators assisted in SSA progeny tests of Meadowview BC₃F₃s. The SSAs were performed on 30-40 seedlings from 107 BC₃F₃ families, Chinese, F₁, and American controls. The technique involved making a 1 x 5 mm incision with a cork borer and inoculated with the highly virulent Ep155 strain of *C. parasitica*.

Tolerance was measured by monitoring time to wilt over 16 weeks. Time to wilt measures the number of days before seedlings wilt from the inoculation. The blight tolerance of the BC₃F₃ hybrid families and controls all segregated as expected. The Chinese controls had the highest blight tolerance, the American controls had among the lowest tolerance, and the F₁ controls had intermediate tolerance. The blight tolerance of BC₃F₃ chestnuts ranged from susceptible to intermediate. Among the BC₃F₃ families, 10 were more blight tolerant than F₁ hybrids (**Figure 1**), whereas the remaining BC₃F₃ families were less tolerant, on average. The heritability of blight tolerance, which is the proportion of variation in survival days that is genetically



Volunteers with the Kentucky and Tennessee Chapters, as well as Eastern Kentucky University and the Forest service, help plant the BC₃F₂ seedlings from the New Kent SSA program. These seedlings will be monitored for years and eventually re-screened for blight resistance. Photo by Tom Saielli.



Seedlings in the SSA trials produced a range of canker severity from A) small healing cankers, B) Intermediate cankers, to C) severe cankers. The observed variability indicates good genetic segregation among the chestnut families tested and help us determine the best, and the worst, trees and families being tested in the breeding program. Photos by Tom Saielli.

determined was 0.37 ± 0.07 . These results help us determine which Meadowview BC_3F_2 parents are sufficiently blight-tolerant to keep and which ones are inferior, and need to be culled from the breeding program.

2018 Correlation between SSAs and field tests

Among the BC_3F_3 families screened using SSAs, 37 were also evaluated for blight tolerance in field progeny tests conducted between 2011 and 2016. A goal of the SSAs this year was determining if there is a correlation between the blight tolerance rankings in the 2018 SSAs and field progeny tests.

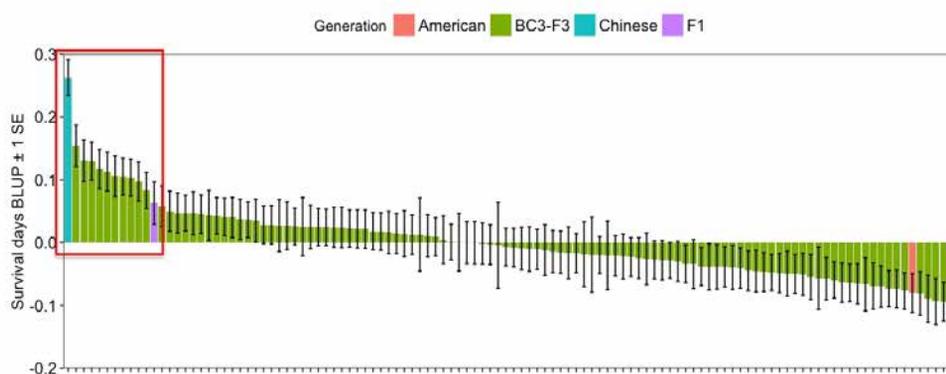
The advantage of using SSAs for progeny testing is results on family average blight tolerance can be obtained in one year versus 4 years in the field. We can also screen higher numbers of each family per year with the SSA method.

In the SSAs, the survival days measurement for blight tolerance is negatively correlated with the average canker severity of the same families inoculated in the field ($r = -0.75 \pm 0.30$). (Figure 2). This confirms that blight tolerance is correlated in SSAs and field progeny tests.

Early screening BC_3F_2 s

SSAs are also being tested as a method to screen large numbers of BC_3F_2 seedlings prior to planting in seed orchards. BC_3F_2 seedlings rated roughly equivalent to F_1 controls would be planted in seed orchards. This method would allow more seedlings to be screened and require fewer trees planted in each plot. This would allow for wider spacing than the standard seed orchard protocol.

Figure 1: SSA survival spectrum in BC_3F_3 families

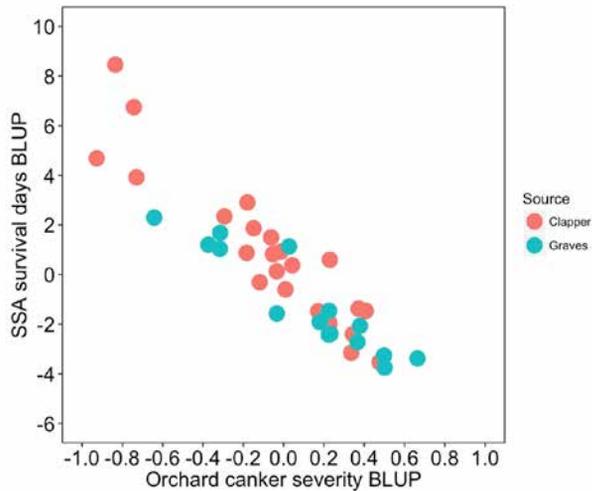


“Time to wilt” was the metric by which resistance was measured for 107 BC_3F_3 families and controls. Ten families survived as long or longer than F_1 s. No backcross families survived as long as Chinese chestnut controls $h^2 = 0.37 \pm 0.07$

In 2018 TACF, in cooperation with the Virginia Department of Forestry, Maryland and Virginia TACF, and Virginia Master Naturalists, conducted experiments at the New Kent Forestry Center, in New Kent VA. 450 seedlings from three BC_3F_2 families (Fleming, WSSC and Hart), Chinese, American, and F_1 controls were propagated in one-gallon containers and inoculated following standard SSA protocol. Cankers were assessed for twelve weeks.

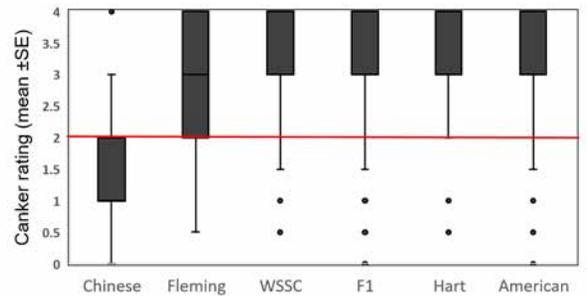
Tolerance was measured by rating cankers on a 0-4 scale (0 = completely healed, 1 = a small, callused canker, 2 = a swollen and callused cankers, 3 = a large, stroma-covered cankers, and 4 = dead or dying seedlings). Chinese chestnuts were the most tolerant, American chestnuts were the least, and the F_1 s and 3 BC_3F_2 families ranged from susceptible to intermediate tolerance. Of the 120-150 seedlings screened per family in this study, approximately 20-45 seedlings would be selected for transplanting to the seed orchard based on these results.

Figure 2: Survival days in SSA negatively genetically correlated with canker severity in orchard tests



Thirty-seven BC_3F_2 families screened in 2018 using small stem assays were previously inoculated in orchard progeny tests. Small stem assay time to wilt date correlated well with progeny test canker severity data ($r = -0.75 \pm 0.30$).

Figure 3: SSA canker ratings for BC_3F_2 families and controls



Canker ratings among BC_3F_2 families and controls meet predicted values, with Chinese being most resistant, Americans being most susceptible, and F_1 s and hybrids ranging from poor to intermediate resistance. Only BC_3F_2 families with scores less than or equal to 2.0 would be transplanted into seed orchards, allowing for the removal of the most susceptible (>2.0) seedlings prior to planting.

Proof of concept?

The BC_3F_2 families screened at New Kent have been planted at the seed orchard at Eastern Kentucky University, Richmond KY, where they will be re-inoculated under standard field testing protocols in 2-3 years. Results will be compared to SSA results in order to determine if enough correlation exists between the two methods to rely on SSAs for early screening in seed orchard establishment. Additionally, in a 2017 experiment, two Maryland sources of BC_3F_2 families were screened with SSAs and planted in seed orchards for long-term evaluations.

These results provide reasonable assurance that SSAs can be useful for progeny tests, and potentially as an early screening tool. The use of SSAs by TACF chapters can save valuable resources, time and labor planting and managing seed orchards, as well as improve the quality of the trees established in our orchards.



Volunteers at the New Kent Forestry Center tagging the seedlings with barcodes and prepping to ship to EKU to be planted in the seed orchard. Photo by Tom Saielli.



Volunteer collects the final canker measurements at the New Kent Forestry Center. In addition to collecting data, the volunteers are tagging the seedlings with barcodes and prepping to ship to EKU to be planted in the seed orchard. Photo by Tom Saielli.