

#### When Will Restoration Begin – 4.1

Restoration of the American chestnut may begin when there are seedlings available with sufficient average blight resistance to provide the foundation for a self-perpetuating stand of chestnut. The American Chestnut Foundation (TACF) is pursuing two avenues for capturing blight resistance in American chestnut: conventional breeding and genetic modification through transformation (transgenics). Integration between these methodologies is being studied and considered. Ultimately, high levels of disease resistance and ecological adaptation will be required for large-scale restoration, but out planting referred to as restoration trials are planned for earlier less-than-perfect material.

Selection in the Meadowview B3F2 seed orchards is expected to be completed in 2021, and from state breeding programs in following years. Nuts from these orchards will have variable levels of blight resistance and on average less resistance than Chinese chestnut. However, the B3F3 seedlings will be acceptable for restoration-like activities because we expect a percentage to live to become long-term survivors in the forest canopy. Surviving trees will have the chance to improve in future generations, and unacceptable trees should lose out as the result of human and natural selection.

An estimated 50,000 nuts could be available as early as 2022 from the Meadowview B3F2 seed orchards after final phenotypic selections. As they are completed, B3F2 orchards in state programs will incrementally add production capacity as well as regional genetic diversity. These partially-resistant populations will comprise the first trees available for restoration trials by TACF. How these are distributed and whether they will be sold are matters yet to be decided.

As the first restoration trials are underway with B3F3 seedlings, the Meadowview and state breeding programs will continue by combining Clapper, Graves, and other sources of resistance in two additional generations of intercrossing and selection. These steps will incrementally improve average resistance and increase the percentage of trees with high resistance in restoration populations.

When transgenic American chestnut trees are registered for general use, their pollen will be provided for crossing with previously unused wild American chestnut sources. This step is needed to introduce regional adaptability, minimize inbreeding in restored populations, and ensure the capture of a high level of existing American genetic diversity in the future restored species. Offspring from the first plantations of outcrossed trees can be used for the earliest restoration trials with transgenic trees. These seedlings could be available by 2030. Beyond this step, the plan is to continue breeding for two more generations to incorporate additional diversification and *Phytophthora* resistance into transgenic populations.

We will continue to improve the quality of our trees, and at any point in time the best trees available will be used for restoration trials, or large-scale restoration.