



# Progress Report

## Moving Closer to 10,000 Trees

### Activities undertaken between Oct. 16, 2015 and October 15, 2016

This has been a very productive year and many strides have been made towards achieving our goal. Last year's report left off with our tissue culture plants being rooted and acclimatized in our new lab space and growth chambers. This year, we:

- began a three-phase establishment of blight resistant American chestnut seed production orchards.
- established a diversity orchard with trees from throughout the American chestnut's natural range.
- communicated regularly with all three U.S. regulatory agencies (EPA, USDA, and FDA) and the Canadian regulatory agencies to determine what is needed for a successful regulatory review.

### Planting new blight resistant American chestnut seed production orchards

Seed orchards of the blight resistant American chestnut trees and wild-type mother trees are being established in three phases (Fig. 1, left), with this year being the first phase. Both transgenic trees and wild-type mother trees will be multiplied, rooted, acclimatized, greenhouse grown, and planted in orchards located at Tully and Zoar Valley, New York. The orchards will be fenced to protect from deer, and irrigated to optimize growth. The plantings will be designed to optimize intercrossing between a lead transgenic event (Darling 54 or Darling 58) and an American chestnut mother tree line (Ashale or Zoar). Due to expected inheritance patterns, about half the nuts produced from these orchards will produce fully blight resistant trees (Fig. 1, right). The orchards will be expanded over the next three growing seasons, pending continued funding. Once we complete the regulatory review and receive approval for distribution, these orchards will provide the nuts to grow seedlings for the public and for restoration of this important tree.

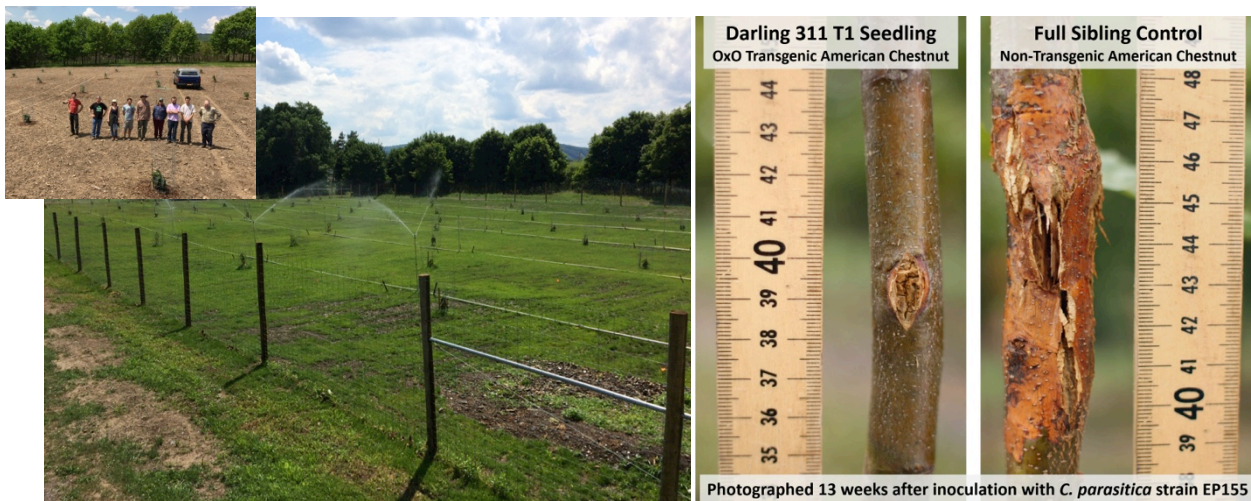


Figure 1. Left + inset: New seed production orchard being established, Tully, NY. Right: inoculated stems showing blight resistance we expect to be inherited by seedlings in these orchards. These are sibling offspring of older 'Darling' events, both of which were infected with the blight fungus. Trees expressing the OxO gene have high levels of blight resistance, as demonstrated by the seedling on the left, compared to the one at right, which did not inherit the OxO gene.

## Establishing an American chestnut diversity orchard

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We have received wild-type American chestnuts from different parts of its native range and have planted them in a “diversity orchard”. When these trees are mature, they will be hand pollinated with pollen from our blight resistant ‘Darling’ American chestnut trees. Seedling offspring will be tested for the oxalate detoxifying enzyme, and pending regulatory approval, resulting blight resistant transgenic trees can be distributed to the regions where the mother tree originated. This process will enhance genetic diversity and local adaptation of the trees being distributed to areas outside of New York and surrounding states.

## Laying the groundwork for the regulatory review

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The regulatory review for genetically engineered plants is very rigorous and thorough. It is especially complicated for our project because it was established based on older laws and regulations for crop plants, some of which are not directly applicable to forest-type trees. Therefore, it is very important for our not-for-profit project to learn as much as possible about the process prior to submission of our application, so that we don’t waste time or money. Thus we have several experienced *pro bono* advisors helping the project, and we have been in frequent communication with the U.S. and Canadian federal regulators through face-to-face meetings and phone conferences.

This has led us to acquire help from the USDA’s IR-4 project, who will formally submit our application to the EPA, saving us hundreds of thousands of dollars in fees. It is still expensive to complete all the tests needed for the review, but with this and other help we are striving to make the best use our supporters’ funds. We hope to submit our applications in 2017, and barring any unforeseen difficulties, we expect the process to take about three to four years to complete. During that time, our seed production orchards will be maturing, so seedlings should be ready for distribution as soon as approval is granted.



**Figure 2. 2015/2016 American chestnut research team at SUNY-ESF**

Left to right: Bill Powell, Drew Teller, Allison Oakes, Linda McGuigan, Andy Newhouse, Kristen Stewart, Dakota Matthews, Yoks Bathula, Tyler Desmarais, Vern Coffey, and Chuck Maynard

New as of fall 2016, not pictured: Alex Levine, Erik Carlson, & Xueqing (Shellie) Xiong