

**Title:** The establishment of a genetically-diverse population of hybrid American chestnut trees resistant to both chestnut blight incited by *Cryphonectria parasitica* and to Ink Disease incited by *Phytophthora cinnamomi*

**Principal investigator(s):** Joe James, TACF; Steve Jeffers, Clemson University; Paul Sisco, TACF

**Duration:** The third year of a 3-year project.

Joe James's note: "This is an ongoing project, but by 3 years I hope it will have begun to make a significant contribution to the overall breeding program."

**Total amount requested:** \$ 10,500

**Short-term goals of the project:**

1. To evaluate blight-resistant BC<sub>3</sub> selections at Meadowview to see which trees also carry resistance to Ink Disease incited by *Phytophthora cinnamomi* (*Pc*). This will be done by testcrossing each BC<sub>3</sub> selection to pure American chestnut trees in North Carolina, with the assistance of Paul Sisco and the Carolinas Chapter. The testcross families will then be screened for *Pc* resistance at Joe James' farm in South Carolina, with the assistance of Professor Steve Jeffers of Clemson.
2. To save and plant out survivors from the *Pc* screening first in pots, for further evaluation for *Pc* and blight resistance, and then in the field at Joe James' farm in Seneca, SC, to create a population of trees resistant to both diseases.
3. To maintain five orchards of *Pc*-resistant trees from previous years' screening on the farm of Joe James.
4. To maintain an orchard of "Restoration" chestnuts so they can be selected for *Pc* resistance as well as blight resistance.
5. To participate in genetic experiments to determine the number and type of gene action of genes for *Pc* resistance coming from the Asian *Castanea* species.

**Long-term goal of the project:**

To develop a genetically-diverse population of hybrid American chestnut trees resistant to chestnut blight and Ink Disease for restoring American chestnut to regions where *Pc* is present in the soil.

**Narrative:** Ink Disease, incited by *Phytophthora cinnamomi* (*Pc*), will be a major impediment to TACF's goal of restoring the American chestnut tree in areas of the South and Mid-Atlantic States where the disease organism is present in the soil. *Pc*, introduced to southern ports by the early 1800's, is a soil-borne pathogen that destroys the root systems of many plant species<sup>1</sup>. The North American and European *Castanea* species are susceptible to Ink Disease whereas the Asian species are resistant. Joe James, in cooperation with Steve Jeffers, a *Phytophthora* expert at Clemson University, has been screening hybrid chestnut families for resistance to Ink Disease, planting out survivors at Joe's 200-acre farm in Seneca, SC<sup>2</sup>. The plan is to screen these *Phytophthora* survivors for blight resistance when they reach a sufficient size, to develop a population of American-type trees resistant to both diseases.

1. [http://www.cals.ncsu.edu/course/pp728/cinnamomi/p\\_cinnamomi.htm](http://www.cals.ncsu.edu/course/pp728/cinnamomi/p_cinnamomi.htm)
2. <http://www.plpnem.ucdavis.edu/faculty/rizzo/lab/Publications/FichtnerRizzo-SurvivalChalmydosporeProduction-2009.pdf#page=200>

A goal of the project is to screen all BC<sub>3</sub> selections at the Meadowview Research Farm to see which trees contain resistance to Ink Disease as well as to blight. Even though there has been no direct selection for resistance to Ink Disease at the Meadowview Research Farms, some backcross trees selected for blight resistance have also been found to carry resistance to Ink Disease (Table 1). This fact, along with data generated by Professor John Frampton and his student at North Carolina State University, has led Fred Hebard to hypothesize that there is a single, partially-dominant locus for resistance to Ink Disease in the Asian species. More work on the genetics of resistance to Ink Disease is now going on at the Southern Institute of Forest Genetics (Dana Nelson) and at Clemson University (Tatyana Zhebentyayeva), using leaf material from trees at Joe James' farm. To continue screening the Meadowview BC<sub>3</sub> trees, pollen from these trees will be shipped to Paul Sisco in North Carolina and to other chapters, as needed, to create testcross families for screening.

Funds requested will be used to create the testcross families for testing and to provide supplies and labor for both the screening process and the maintenance of the *Pc*-resistant trees at Joe James' farm in Seneca, SC.

Joe James is maintaining two berms and six orchards at his farm (Fig. 1). Seedlings that survive the first screening for resistance to Ink Disease are planted in pots in the first berm, re-inoculated with *Phytophthora cinnamomi*, and allowed to grow for an additional year. Some of the seedlings die during this second year of exposure to the disease organism.

The two-year-old survivors, still in pots, are then transferred to a second berm, where it is hoped that in their third year they will reach sufficient size for inoculation with the milder form of the chestnut blight fungus (Fred Hebard's strain SG1 2-3). The seedlings that are sufficiently resistant to this strain will then be planted out in orchards on Joe James' farm for further evaluation. It is hoped that these will form a population of trees that will produce seed for restoration of American chestnut in regions where Ink Disease is present in the soil.

Here is Joe James' description of the work going on at his farm:

"I, with Fred and Paul's guidance, will be planting the selected trees that come off of the 2<sup>nd</sup> berm at the end of their 3<sup>rd</sup> growing season. At the end of this 3<sup>rd</sup> season, now that the 2<sup>nd</sup> berm is fully operational, I should have some 30 to 60 trees to put in their appropriate orchard site. These trees will have been screened for both diseases. Fred has recently scored 92 trees that were inoculated with blight last summer. Using his criteria 40 trees will be culled and the remaining 52 will be carried forward for further breeding efforts. The exact protocol has yet to be fully designed, but at some juncture these trees, by breeding along generational lines, could be used to inject *Phytophthora* resistance into existing blight resistant populations without diluting their present levels of blight resistance.

I have screened ~ 8500 seedlings from 85+/- families between 2004 and the present. There are 250 *Pc*-resistant trees planted on 5 separate sites on a 248 acre farm. These sites are separated by at least 500 feet. Each site is delineated by generational type; i.e., there is a B<sub>1</sub>, 2 B<sub>2</sub>s, a B<sub>3</sub>, and a B<sub>4</sub> orchard. This farm has been placed on a permanent conservation easement for the main purpose of developing *Pc*-resistant hybrid American chestnut trees. This past winter I instituted a production plan which encompasses three stages over three years. The first year involves planting the nuts in non-infected medium, growing them some 11 to 12 weeks, then inoculating them with a *Pc* strain Steve Jeffers isolated from this farm. By November most have died. All seedlings are then pulled and graded by the number, placement and appearance of lesions on the root system. The best seedlings are selected and planted in 3-gallon pots and buried in the 1<sup>st</sup> berm. Approximately 15 to 25% of these seedlings will then die during the 2<sup>nd</sup>

year. Most will be advanced to a 5 gallon pot and placed in the 2<sup>nd</sup> berm at the end of their 2<sup>nd</sup> growing season. The following May these trees are to be inoculated with the SG 2-3 strain of the blight fungus. Final selections for blight resistance will be made that fall and these selected trees will be placed in the appropriate orchard.

Within the next 2 to 3 years, there will be at least 100 trees that will require controlled pollinations. This past summer I was able to test my first F<sub>2</sub> nuts from *Pc*-resistant parents. The numbers were small, some 70 seedlings, but for B<sub>2</sub>F<sub>1</sub> nuts the survival averages 18.8% for the first growing season and these F<sub>2</sub> nuts survived at a rate of 59%. With B<sub>3</sub>'s it was even more impressive, going from 4.5% in the average F<sub>1</sub> family to 39% in the F<sub>2</sub> seedlings.

To really get things going we need prospective controlled pollinations done. The parents in these pollinations should preferably be from B<sub>2</sub> or B<sub>3</sub> families which have shown from previous *Pc* screenings to at least carry some level of resistance. About 60% of families should fall into that category. The parents should also be from previously blight screened material. The ideal tub planting density is ~ 132 nuts / tub (12 / row). I have 9 tubs presently on line. Therefore, 9 x 132 = 1188 total nuts. If controlled pollinations were done on 30 trees with 25-30 bags/tree, I would have ample material to start with each year. When considering the germination rate, 1188 nuts should produce roughly 1000 seedlings. Then considering the survival rates for B<sub>2</sub>'s and B<sub>3</sub>'s, respectively, of 18.8% and 4.5%, less ongoing mortality, this construct should produce 30 B<sub>3</sub> trees or 120 B<sub>2</sub> trees per year. If after blight inoculation the best ½ were advanced this would yield 15 B<sub>3</sub>'s or 60 B<sub>2</sub>'s for final orchard planting.

### **Timeline for Work:**

Each year, the pattern of work is the same:

January: year-old seedlings from previous year's experiment are rated for resistance to Ink Disease, and best seedlings are transferred to 3-gallon pots and put in first berm; two-year-old survivors are transferred to 5-gallon pots and transferred to second berm.

March: seeds of "Restoration Chestnuts" provided by the Meadowview Research Farms are split into two equal parts, half being planted directly into the field and half into tubs with artificial mix. Seeds from testcross families are planted tubs; begin summer season of maintaining trees in the six orchards at Joe James' farm (weeding, fertilization, deer protection, and labeling).

May: seedlings of sufficient size are inoculated with strains of the blight pathogen, as appropriate

June: controlled pollinations are made in NC and elsewhere to create testcross families and in SC to create F<sub>2</sub>'s, etc, as described in Joe James' narrative.

July: seedlings in tub experiments are inoculated with *Pc*, using strains of the pathogen isolated from Joe James' farm.

Rest of summer: weeding, fertilization, deer protection, and labeling continues

Fall or winter: seedlings inoculated with *Cryphonectria* are evaluated for blight resistance.

### **How Results are to be Measured and Reported:**

Each year Steve Jeffers of Clemson University summarizes the results of the previous year's screening for *Pc* resistance. These results are available by March.

Each year Joe James will be responsible for updating the maps of his orchards and entering the results into the national TACF database.

Each year by October 1 the four Investigators will together write and submit a report to the TACF concerning the previous year's work.

**Breakdown of How Funds will be Spent:**

**Expenses of Joe James at Seneca, SC:**

**Total per year (Seneca, SC) = \$ 8,000**

**Expenses of Paul Sisco to Produce Seed for Screening:**

**Total = \$ 2,500**