

Outlook for Blight-Resistant American Chestnut Trees

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Abstract: Culminating 20 years of breeding efforts, in spring 2008, The American Chestnut Foundation (TACF) delivered its first 500 chestnuts to the USDA Forest Service for testing on National Forest lands. The expectation is that these seedlings will be more resistant to chestnut blight (*Cryphonectria parasitica*) than are pure American chestnut trees (*Castanea dentata*). Greater numbers of seeds will be distributed in coming years, as more trees start producing nuts and as these trees become larger. Meanwhile, the breeding program at TACF continues, incorporating different sources of resistance and broadening the genetic base by breeding to surviving American chestnut trees from Maine to Alabama. An additional challenge in the southern United States is the fact that American chestnut is highly susceptible to the root rot organism *Phytophthora cinnamomi*, which is found in warm, poorly-drained soils. Both Chinese (*Castanea mollissima*) and Japanese (*Castanea crenata*) chestnut trees are resistant to this soil pathogen. A volunteer member of TACF, Joseph B James of Seneca, SC, is cooperating with Clemson University scientist Steve Jeffers to breed American chestnut trees resistant to both blight and root rot. In addition, Scott Enebak of Auburn University is determining ways of keeping the root rot pathogen out of forest nurseries where chestnut seedlings are grown.

Keywords: American chestnut, *Castanea dentata*, *Cryphonectria parasitica*, *Phytophthora cinnamomi*, disease resistance, genetic diversity, ecosystem restoration

Introduction

One hundred years ago, the American chestnut (*Castanea dentata*) was a dominant canopy tree in the Appalachian Mountains, making up one quarter of the forest. Its tannin-filled, naturally rot-resistant wood was used for fences, shingles, utility poles, and industrial purposes, as well as for making furniture and decorative woodwork. The yearly nut crop, which was as abundant as it was consistent, provided a bounteous harvest for wildlife, free-range hogs and turkeys, and for humans themselves, who sold the nuts for cash income. Then, in the late 19th century, a fungal pathogen, *Cryphonectria parasitica*, was introduced on Japanese chestnut trees (*Castanea crenata*) imported to the United States in the days before there were any plant quarantine regulations. American chestnut had almost no resistance to this pathogen, which spread quickly by both airborne and sticky spores. Within 50 years of its discovery in the Bronx Zoo of New York City, chestnut blight had effectively eliminated chestnut as a freely reproducing, dominant tree species. Both the Federal government and private individuals, like Arthur Graves of Connecticut, tried to breed blight-resistant forest-type chestnut trees by crossing American chestnut with both Japanese and Chinese (*Castanea mollissima*) chestnut trees. But the hybrid F1 trees that were the product of these early breeding programs were not sufficiently resistant to the blight, and not tall enough to compete in a forest environment. The Federal program was abandoned in 1960.

Fortunately, chestnut breeding did not end with the demise of the Federal program. Charles Burnham of the University of Minnesota, a professor of corn genetics, felt that the early efforts had made a major mistake. Instead of stopping with 50/50 American/Chinese hybrids, he proposed a series of additional backcrosses to American chestnut to get more of an American-type tree, followed by at least one generation of intercrossing to bring blight resistance up to a high level. In 1983, Burnham and some fellow scientists and laymen organized The American Chestnut Foundation (TACF) as a private, non-profit corporation to pursue the backcross breeding strategy. In 1989, a farm was leased in southwest Virginia, and a scientist, Fred

NOTE: All Sisco figures are on pages 64 to 68.

Hebard, was hired to direct the program. Starting with the best BC₁ (backcross one) progeny of the previous Federal and private programs, and taking care to promote fast growth and precocious flowering, Hebard was able to produce three more generations and complete the first stage of Burnham's program by 2005. In the spring of 2008, the first 500 BC₃F₃ seeds from selected, blight-resistant American-type trees were provided to the USDA Forest Service for testing on National Forest lands in northeast Tennessee and southwest Virginia.

TACF's breeding program has grown more ambitious and complex than Burnham originally envisioned. Additional sources of resistance from various Chinese and Japanese chestnut cultivars are being investigated, and genetic diversity is being increased by volunteer efforts in 15 state chapters from Maine to Alabama.

Unfortunately chestnut blight is not the only disease that is a threat to the survival of American chestnut. Another pathogen introduced from Asia, *Phytophthora cinnamomi*, destroyed most chestnut trees in the Piedmont South before chestnut blight arrived in North America. *Phytophthora cinnamomi* is a pathogen of many plant species, including Fraser fir (*Abies fraseri*), but it is particularly virulent on American chestnut. The disease spreads by water-borne spores in the soil. The spores are susceptible to freezing soil temperatures, so it moves slowly and doesn't survive well at high elevations. Chinese and Japanese chestnut species have resistance to the disease, so it is possible to breed for resistance to both chestnut blight and *Phytophthora* root rot. Joe James, a retired surgeon from Seneca, SC, has teamed up with Steve Jeffers, an expert in *Phytophthora* spp. at nearby Clemson University (Clemson, SC), to do just that.

With the dedicated efforts of professionals and volunteers, the first tests of TACF's elite hybrid trees will be planted in spring 2009. This is the beginning of a major forest restoration effort that will continue through most of the 21st century.

Materials and Methods

The outlines of the breeding program at TACF (fig. 1) were first presented in detail by Burnham and others (1986). A recent summary of results to date can be found in Hebard (2006). Two superior BC₁ hybrids were selected to begin TACF's breeding efforts: the 'Clapper' selection from the former USDA program (Clapper 1963), and a BC₁ tree from Arthur Graves' program in Connecticut (Anagnostakis 2007). A third source of resistance was added to the program by making a cross between the Chinese cultivar 'Nanking' and several different American chestnut trees in southwest Virginia. 'Nanking' is a selection from the old USDA program, is precocious, bears large nut crops, and, most importantly, is highly resistant to the blight (Jaynes 1979; Hebard and others 1984).

Because clearcutting was more common in the early 1980s, large numbers of flowering American chestnut trees were present in the Mount Rogers National Recreation Area of Virginia when TACF's breeding program began in 1989. The 15 state chapters of TACF have also found many flowering trees in their own areas, allowing breeding of blight-resistant chestnuts for local adaptation (fig. 2).

Selection for both blight resistance and American chestnut characteristics is practiced in each backcross generation, with only the most resistant and most American trees being advanced. Each backcross is to a different American chestnut tree to prevent inbreeding. Resistance is only partially dominant, so the selections in the backcross generations are only moderately resistant to the blight, as measured by the diameter of canker growth 5 months after inoculation (fig. 3). Backcross progeny are also selected for American characteristics, including canoe-shaped leaves, lack of leaf hairs, small stipule size, cylindrical, pointed, and hairless buds, and red stem color (fig. 4). Trees with a strong central leader are also preferred over very branchy trees, although even American chestnut will spread out when grown in the open (fig. 5).

The efforts of Joe James and Steve Jeffers to select hybrids with resistance to *Phytophthora cinnamomi* and *Cryphonectria parasitica* were outlined by Jeffers and others (2007) at an IUFRO conference at Asilomar, CA. Seeds from hybrid families, as well as pure Chinese and pure American chestnut controls, are planted in sterile soil mix in deep tubs (fig. 6). When the seedlings are 2 to 3 months old, they are inoculated with *P. cinnamomi* grown on vermiculite (fig. 7). The following December or January, the root systems of surviving seedlings are inspected and rated on a 0 to 3 scale, with "0" having no apparent lesions on the roots and "3" being dead. Surviving seedlings rated "1" or "0" are then planted on the farm of Dr. James in Seneca, SC, where *P. cinnamomi* is already present in the soil. The inoculum used on the seedlings is prepared from *P. cinnamomi* isolates from dying chestnut trees from the James farm (fig. 8). The plan is to then inoculate the trees that survived infection with *P. cinnamomi* with the chestnut blight pathogen when they reach sufficient size (> 2.5 cm [1 in] dbh).

Results and Discussion

In 2006, TACF's Meadowview Research Farms (Meadowview, VA) produced the first BC₃F₃ seeds from the 'Clapper' source of resistance. The first 500 seeds for testing were given to the USDA Forest Service in fall 2007. The expectation is that all of the BC₃F₃ seedlings will have higher levels of resistance than pure American chestnut, but this needs to be proven. TACF's official Testing Protocol was published in the Journal of TACF (Ad Hoc 2004). The USDA Forest Service Southern Research Station has recently devoted an entire issue of its magazine *Compass* to the American chestnut. Payne (2008) provided one of the best articles in this issue, describing the testing of chestnut seedlings by the USDA Forest Service.

Because TACF's breeding program has been ongoing for 20 years, a pent-up demand exists among TACF's members and many other landowners for the best blight-resistant seeds. Almost daily, someone in TACF gets a call from a person saying, "I have 20 acres of land I just cleared and I'd love to have some of those blight resistant chestnut seeds."

To give a reasonable and consistent answer to these callers, TACF's Science Advisory Cabinet composed the following:

The American Chestnut Foundation (TACF) is breeding chestnut trees for ability to survive the blight disease, which killed almost all our native American chestnut. TACF's goal

is to confer on American chestnut the ability to thrive in our nation's woodlands, as it once did. Last fall, TACF began harvesting nuts that it expects will be suitable for planting back into the forest. However, these breeding lines are still in the testing phase and their value needs to be proven on many forest sites until 2015 to 2020.

A gradual increase in seed production is expected over the next few years. These will be distributed to cooperators who are assisting in a formal, rigorous testing program. In addition, seeds that are not needed for this purpose will be distributed principally to members of TACF for informal testing. At the same time, TACF is continuing its breeding program to make further gains in disease resistance and forest competitiveness.

Even with seeds of highly blight-resistant chestnut available, reforestation efforts will not be easy. Many forest creatures relish the taste of the nuts, and deer will defoliate small seedlings quickly. Seedlings need to be grown above deer browse height, transplanted, and cared for until they can survive on their own. In the past few years, drought has been a major problem. In poorly-drained sites, *P. cinnamomi* can destroy an entire planting. Other pests, such as gypsy moths (*Lymantria dispar*), Japanese beetles (*Popillia japonica*), Oriental chestnut gall wasps (*Dryocosmus kuriphilus*), and Asian ambrosia beetles (*Xylosandrus crassiusculus*) can also cause extensive damage, especially to young trees.

Nevertheless, chestnut has advantages compared to many other tree species. It can grow quickly, up to 2 m (6 ft) per year from large root systems, and, once established, can tolerate dry conditions.

Summary

The "Environmental Success Story of the 21st Century" could well be the reestablishment of American chestnut as a dominant tree species in the Appalachian forests of eastern North America. The first 500 seeds of putatively

blight-resistant American chestnut trees were given to the USDA Forest Service in spring 2008, and seedlings grown from these seeds at Flint River Nursery (Montezuma, GA) will be outplanted for tests on USDA Forest Service land in the spring of 2009.

More information about the progress in breeding blight-resistant chestnuts can be found at the website of The American Chestnut Foundation (URL: <http://www.acf.org>).

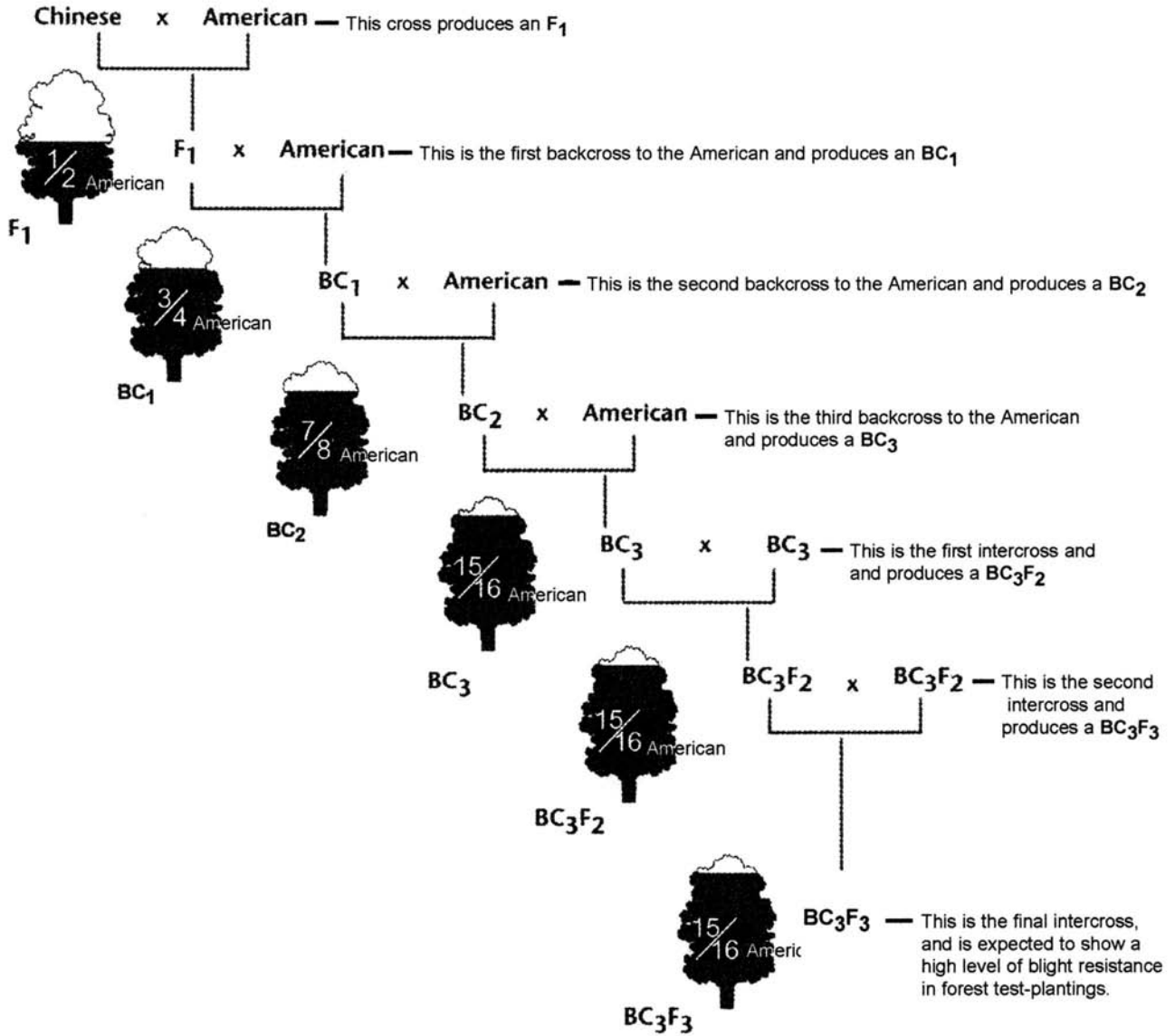
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THE AMERICAN CHESTNUT FOUNDATION'S BACKCROSS BREEDING PROGRAM

ADDITIONAL AMERICAN CHESTNUT CHARACTERISTICS ARE REGAINED WITH EACH BACKCROSS.

TACF expects a high level of blight resistance and American characteristics to be present in selected BC₃F₂ seed orchard parents. Their BC₃F₃ progeny will be extensively tested by TACF for blight resistance and ability to compete in the forest.



Note: In each step, the backcross is selected for resistance. Trees indicate average fraction of American genes with no selection.

Figure 1. The stages in the backcross breeding program of The American Chestnut Foundation. Each generation takes about 6 years.

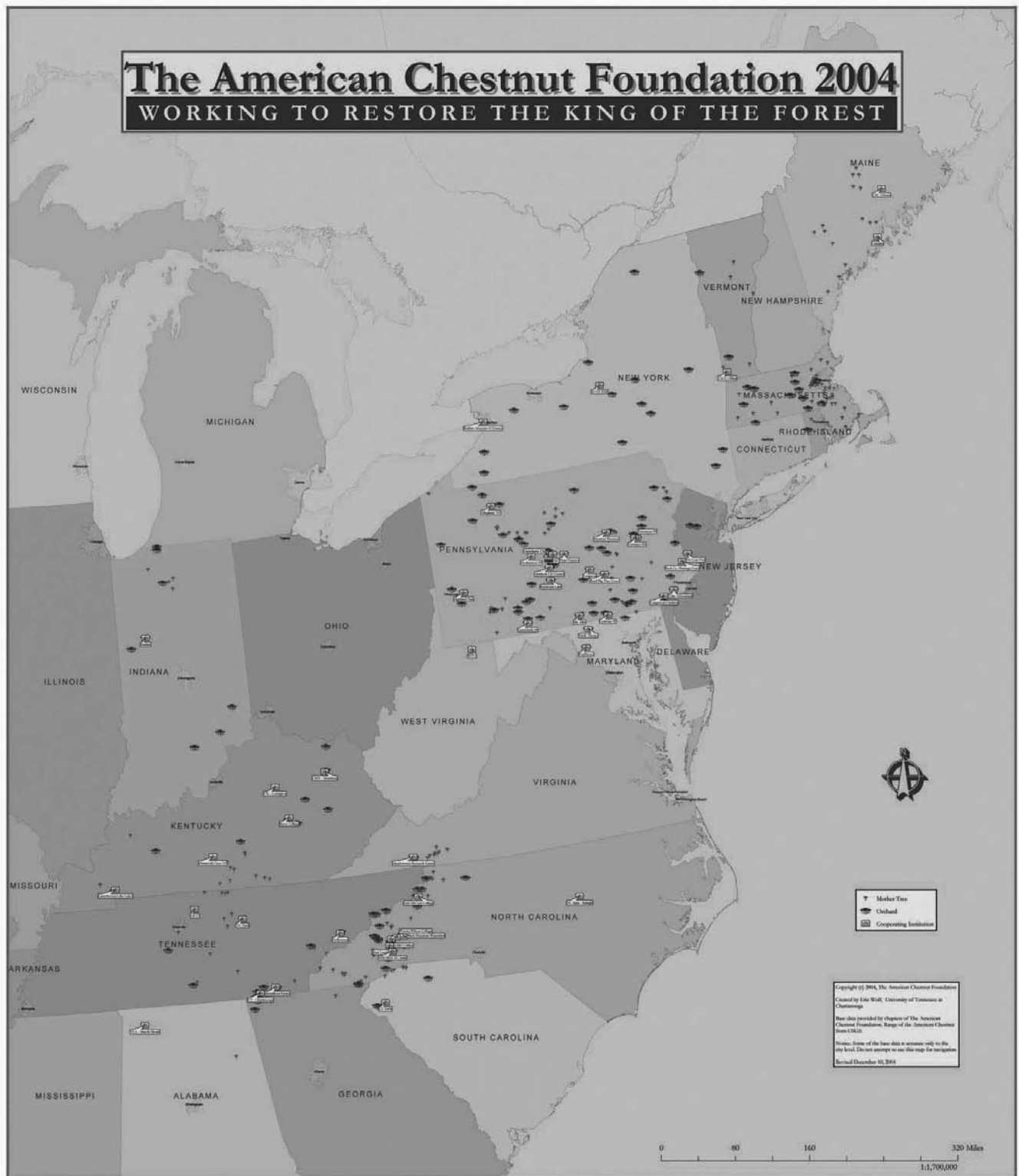


Figure 2. TACF's national effort as of 2004. Volunteers in 15 state chapters are using trees in their areas to obtain local adaptation. Each small green dot represents a surviving American chestnut tree that has been used in the breeding program. The multiple trees indicate orchards where trees are being grown.



Figure 3a. Reaction of a moderately-resistant hybrid tree after inoculation with the blight fungus. A canker walls off the growth of the fungus.

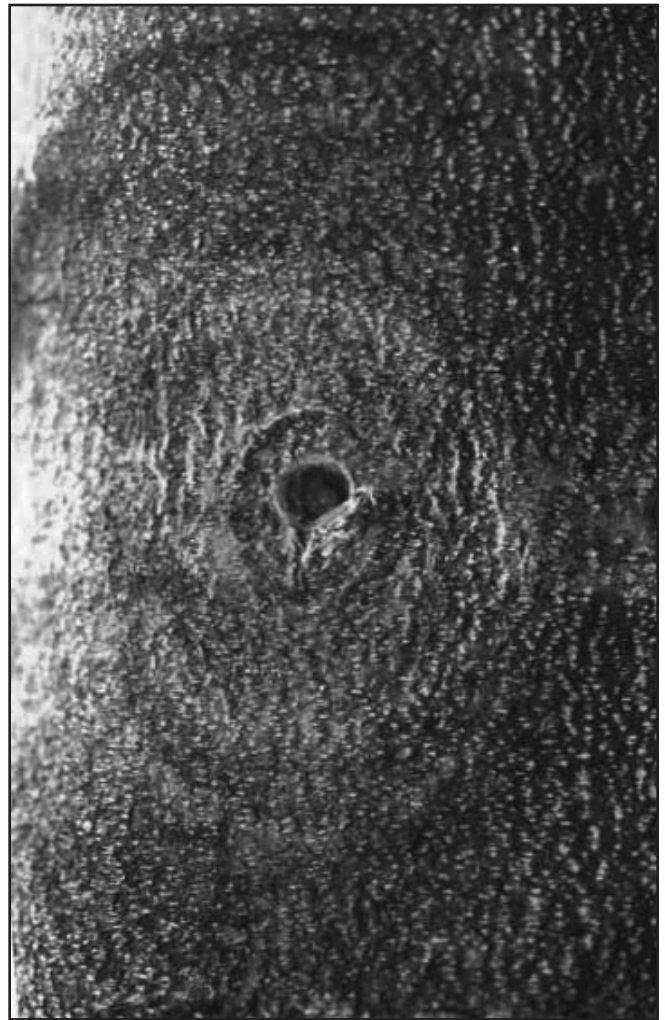


Figure 3b. Reaction of a susceptible hybrid tree after inoculation. The fungus grows in concentric circles from the inoculation point, producing a sunken canker that will soon girdle the tree.

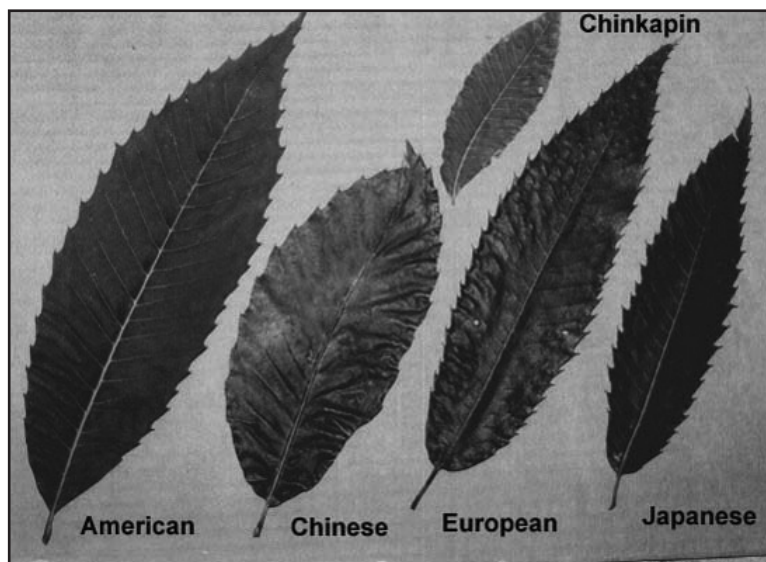


Figure 4. Leaves of the four major chestnut species, plus the leaf of the Allegheny chinkapin (*Castanea pumila*). American chestnut leaves are long in relation to their width, tapered at both ends, and their teeth are prominent and curve inward like the waves of an ocean.



Figure 5a. Surviving American chestnut tree in Dexter, ME. When grown in the open, American chestnut is a spreading, branchy tree. (Photo courtesy of Maine Chapter—TACF.)



Figure 5b. Surviving American chestnut tree in Atkinson, ME. When grown in forest competition, American chestnut easily loses its lower limbs and has a long, limbless bole. (Photo courtesy of Maine Chapter—TACF.)



Figure 6. Joe James of Seneca, SC, confers with Clemson graduate student Inga McLaughlin, while Steve Jeffers and postdoctoral research associate Jae-soon Hwang work in the background. Hybrid chestnuts from many families are planted in sterile media to test for resistance to *Phytophthora cinnamomi*. Seeds are planted close together and families are separated by colored stakes in the planting tubs.



Figure 7. Rice grains impregnated with *Phytophthora cinnamomi* are used to inoculate hybrid chestnut seedlings after about 2 months of growth in the sterile medium. More recently, vermiculite has been used in place of the rice grains to prevent contamination. (Photo courtesy of Steve Jeffers.)



Figure 8. Steve Jeffers, Professor of Plant Pathology at Clemson University, holds a dying hybrid chestnut seedling dug from the farm of Joe James in Seneca, SC. *Phytophthora cinnamomi* was isolated from such seedlings to be used in screening families of hybrid chestnut for resistance to the disease.

The content of this paper reflects the views of the authors, who are responsible for the facts and accuracy of the information presented herein.