

Blight Control and Restoration

OF THE AMERICAN CHESTNUT

Efforts to restore the American chestnut focus on natural blight resistance found in a few surviving trees of the species in the natural range, strains of the blight fungus with reduced virulence (hypovirulence), and introduction of Asian chestnut blight resistance into American chestnut. These approaches may be used alone or in combination. Also critical are site selection and other forest management practices.

By Gary J. Griffin



The chestnut blight was first observed on American chestnut (*Castanea dentata*) in 1904 at the Bronx Zoological Park in New York City (Roane et al. 1986). A girdling sunken canker on the stem of the tree kills the vascular cambium and results in foliar blight symptoms. The fungus causing the disease was introduced from Asia and is known as *Cryphonectria parasitica*. Within 40 years, the disease was found in all areas of the natural range of American chestnut, which extended from Maine south to Alabama and Mississippi.

Within the Appalachians, almost all stems of canopy American chestnut trees in the former Oak-Chestnut Forest and Mixed Mesophytic Forest regions (Braun 1950) were killed by the disease. American chestnut, northern red oak (*Quercus rubra*), and white oak (*Quercus alba*) were the only species that were principal dominants in both forest regions (Braun 1950), and about 25 percent of the canopy trees were American chestnut. Many understory American chestnut trees have survived blight, however, even to the present time, except on the most mesic sites. Blight incidence on these understory trees typically is low (less than 30 percent), and new stump sprouts usually maintain the rootstocks of blight-killed understory stems.

Chinese chestnut (*Castanea mollissima*) and Japanese chestnut (*Castanea crenata*) are blight resistant, and European chestnut (*Castanea sativa*) is less susceptible than American chestnut (Roane et al. 1986). A few large Amer-

ican chestnut trees (greater than 10 inches dbh) in the natural range have survived blight, and the largest one reported is a low-altitude tree (660 feet) located near the base of the northern Blue Ridge in Virginia. Research on the large, surviving American chestnut trees has identified three factors associated with survival: (1) low levels of blight resistance in the trees, (2) presence in cankers of low to high percentages of hypovirulent strains of the blight fungus (strains with reduced virulence) that are infected with fungal viruses, and (3) favorable sites (Griffin et al. 1983). Survival of individual trees most often has been associated with two or all three of these factors. In Virginia, most large, surviving American chestnut trees grow at low altitude (400 to 1,500 feet).

Current restoration efforts for American chestnut have centered on four approaches: (1) hypovirulent strains of the blight fungus, (2) Asian blight resistance, (3) natural blight resistance in American chestnut, and (4) forest management activities.

Hypovirulent Strains

Natural chestnut blight control in Europe is widespread (Heineger and Rigling 1994) and has been associated with hypovirulent strains of the chestnut blight fungus that are infected with one type of fungal virus (*Cryphonectria hypovirus 1*) (Hillman et al. 1995). These hypovirulent strains of the blight fungus also have been used successfully to artificially inoculate cankers on European chestnut trees for the

purpose of blight control. In contrast, natural hypovirulent strains of the chestnut blight fungus have not been found to be widespread on American chestnut trees. On large, surviving American chestnuts, hypovirulent strains with viruses are associated with nonkilling, or superficial, cankers. In these cankers, only the outermost bark tissues are infected and killed by the blight fungus, and the fungus does not colonize the vascular cambium.

Numerous attempts have been made to control blight on clearcut and other American chestnut trees by artificial inoculation of natural or artificially established blight cankers with individual hypovirulent strains or mixtures of hypovirulent strains of European and American origin (Jaynes and Elliston 1980). In general, these attempts have not been successful (Hobbins et al. 1994), although some blight control (presence of abnormal cankers and more live stems per chestnut sprout clump) has been obtained in one instance nine years after inoculation with hypovirulent strains (Anagnostakis 1990). Subsequent studies have shown that the virus, associated with the hypovirulent strains of European origin, did not persist at sites where they were introduced (Peever et al. 1997). However, integrated management of chestnut blight, incorporating hypovirulence, has resulted in a high level of chestnut blight control (Dierauf et al. 1997).

Several factors may be responsible for the failure of hypovirulent strains to control blight in American chestnut



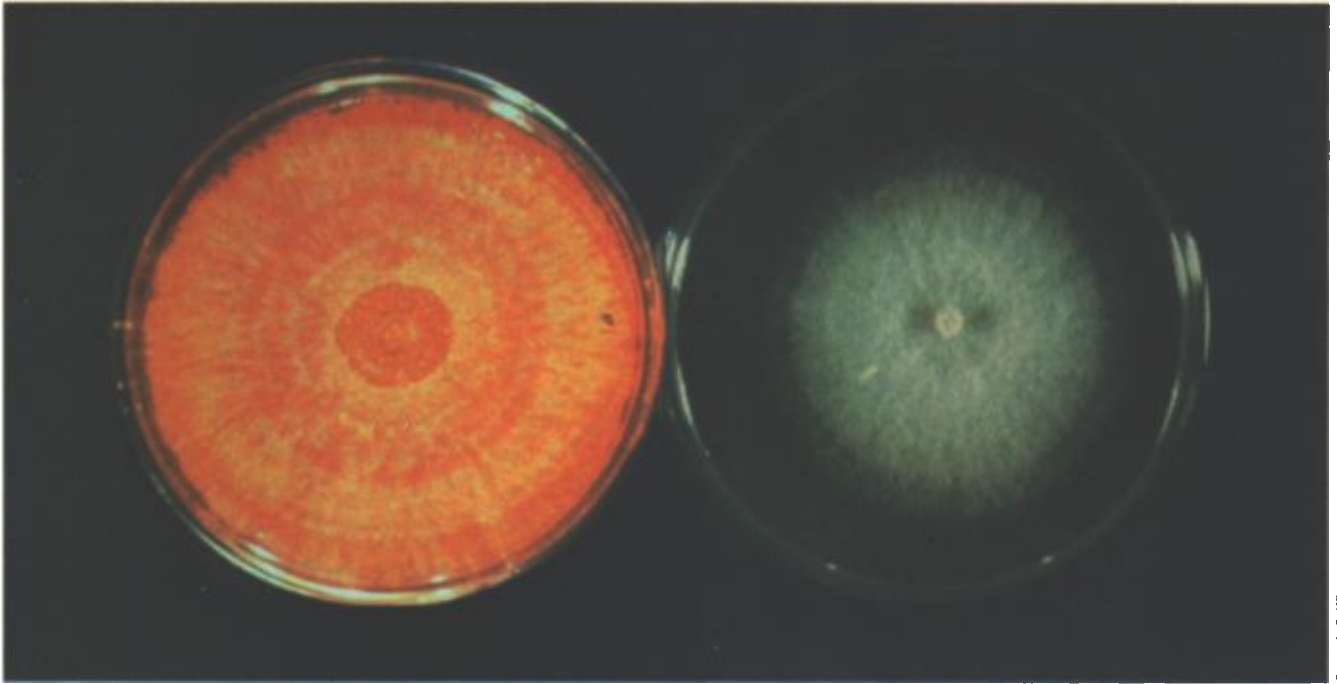
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The largest American chestnut (40 inches dbh) to survive blight in the natural range of the species is located at the base of the northern Blue Ridge in Virginia. This tree has a low level of blight resistance and is infected with hypovirulent strains of the chestnut blight fungus.

trees: (1) high blight susceptibility of American chestnut, (2) abundance of virulent inoculum of the blight fungus at forest sites, (3) restricted movement of the hypovirulence viruses among the many strains of the chestnut blight fungus in cankers, (4) environmental stress at many forest sites, and (5) superficial canker instability.

The first three factors are closely linked to each other. The high susceptibility of American chestnut leads to a high biomass of blight fungus in bark tissues and the formation of abundant sexual and asexual spore-producing bodies on the bark surface. The sexual fruiting bodies, through airborne spore (ascospore) formation, lead to abundant and diverse types of viru-

lent, virus-free blight fungus strains that are readily disseminated to nearby and distant American chestnut trees. The diverse blight fungus strains differ from each other in compatibility, which can prevent the transfer between strains of the viruses associated with hypovirulence (Anagnostakis 1983; Hobbins et al. 1994). The transfer of viruses between fungal strains is dependent on the fusion of fungal filaments (hyphae) that are compatible or only weakly incompatible with each other. In contrast to American chestnut, the more blight-resistant European chestnut supports less sexual reproduction of the blight fungus and fewer strains differing in compatibility (Heineger and Rigling 1994).



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Laboratory cultures of the chestnut blight fungus are normally yellow-orange in color (left). When infected with hypovirulence virus of European origin, they are white in appearance (right). These white hypovirulent strains, in combination with low levels of blight resistance in American chestnut, have been used to produce a high level of blight control.

To circumvent the factors limiting spread of hypovirulence viruses on American chestnut trees, blight fungus strains have been genetically modified so that the sexual spores will give rise to strains containing hypovirulence virus of European origin (Anagnostakis et al. 1998). The genetically modified strains have the ability to transmit virus under laboratory conditions to all compatibility types of the blight fungus resulting from sexual reproduction. Field release studies on American chestnut have shown that the genetically modified strains survived for a short period of time, two years, in the research plots. The low or nonproduction of spore-producing structures in the field on American chestnuts by hypovirulent strain EP 713 (Griffin and Griffin 1995), containing the same virus as the genetically modified strains, suggests this is a limiting factor in the long-term efficacy needed for practical levels of blight control and replacement of virulent strains. The rapid killing of American chestnut by the abundant wild-type virulent strains in forest plots is an additional limiting factor to be overcome by this novel approach to augment hypovirulence virus spread.

Asian Blight Resistance

Early efforts at breeding a blight-resistant chestnut by crossing Asian chestnut species with American chestnut generally were not successful. Trees with a high level of resistance often had poor forest-tree form and had less than 50 percent American chestnut parentage. Berry (1980) found that 27 percent of 705 hybrid chestnut trees from the Connecticut Agricultural Experiment Station and 12 percent of 500 hybrids from the USDA program in Maryland were surviving in 15 plots established from 1947 to 1955 in the eastern United States.

Burnham (1981) proposed using the backcross method of breeding, in which blight-resistant genes from Chinese chestnut are transferred to American chestnut by backcrossing Chinese \times American hybrids ($C \times A F_1$) with the most resistance to American chestnut three or more times and then intercrossing the most blight-resistant progeny to obtain the level of blight resistance of Chinese chestnut and the forest-tree form of American chestnut. The American Chestnut Foundation has an intensive breeding program to implement the backcross method. Artificial inoculation with virulent strains

has been used in progeny tests to identify blight resistance.

So far, three sources of Chinese chestnut blight resistance and 15 to 20 American chestnut lines from one area have been used in crosses. Molecular mapping has been used to identify genetic regions associated with blight resistance in a $C \times A F_2$ family, and can be used in the future to ensure the efficiency of the backcross method (Kubisiak et al. 1997). Good progress has been made in producing the F_1 generation of the third backcross (BC_3-F_1) and the best selections have vigorous, upright growth. In some of this breeding work, large, surviving American chestnuts have been used (P. Sisco and F. Hebard 1999, pers. commun.).

The Virginia Department of Forestry, in cooperation with the Connecticut Agricultural Experiment Station, planted approximately 12,000 hybrid chestnut seedlings, with Chinese and Japanese sources of blight resistance, at the Lesesne State Forest, Virginia, between 1969 and 1976 (Jaynes and Dierauf 1982). Some seed sources had Chinese, Japanese, and American parentage. In 1981, one of the most promising seed parents for production of seedlings with good

form, vigor, and field blight resistance (natural infection) was C13, a Chinese cross of unknown parentage. More recently, the better hybrids have been used in a backcross program in which large, surviving American chestnuts in Virginia have been used as the female parent. To date, approximately 10 hybrids, with good form and field blight resistance, and 10 American chestnuts have been used to produce the first backcross (BC_1-F_1), and these BC_1-F_1 trees and six American chestnuts have been used to produce the second backcross (BC_2-F_1) (J. Scrivani 1999, pers. commun.).

Resistance in American Chestnut

Thor at the University of Tennessee and Given at the West Virginia Department of Agriculture established breeding programs with large, surviving American chestnut trees. Scions of these trees were grafted into chestnut rootstocks to establish seed orchards in both states, and field resistance was used to screen out less-resistant trees. The Thor orchard has been destroyed, but the American Chestnut Cooperators' Foundation (ACCF) works with the West Virginia Department of Agriculture to distribute seedlings from the Given orchard and the Elkins seed orchard at Concord College, West Virginia, to cooperating members of ACCF. So far, 60,453 seedlings from open pollinations have been distributed. ACCF has also distributed 20,000 seed nuts from Virginia Tech seed orchards containing first-generation American chestnut progeny from parents that have been identified to have low levels of blight resistance by artificial inoculation with a standard virulent strain (Elkins and Griffin, unpublished data). In controlled pollination tests, generally a low frequency of blight resistance has been found in progeny of crosses between surviving American chestnut trees from different geographic areas, but crosses between trees from the same geographic area or site have yielded high percentages of progeny with low levels of blight resistance, based on artificial inoculations with the standard virulent strain. Combining blight resistance from different geographic areas may lead to a higher

level of resistance in future generations.

Radiation breeding was used by Dietz (Roane et al. 1986) to produce a blight-resistant American chestnut, and several large plantations were established. So far, no highly blight-resistant American chestnut has been reported for the 18,000 trees planted between 1962 and 1977, but some of the trees planted at the Lesesne State Forest have demonstrated low levels of blight resistance following artificial inoculation trials with a standard virulent strain (Dierauf, Elkins, and Griffin, unpublished data).

Forest Management Activities

Site factors may be critical to maintaining blight control for the long periods required for chestnut timber production. Change of a superficial canker over winter into a killing canker has been a problem in maintaining chestnut blight control for long periods in clearcuts with natural or artificially introduced hypovirulence, especially at higher altitudes (above 2,500 feet) (Griffin et al. 1993).

High-elevation sites are important because American chestnut understory trees are found in Virginia at highest density at about 2,500 to 4,000 feet elevation in the Ridge and Valley section of the former Oak-Chestnut Forest region. The rootstocks of these trees may be very important to blight control efforts in the future, especially following clearcutting. To examine whether the loss of blight control might be partly a high-altitude problem, bark plugs from superficial cankers on American chestnut trees at low altitude (780 feet) in Virginia, and pure cultures of hypovirulent isolates of the blight fungus from the same cankers, were inoculated on stems of blight-free American chestnut sprouts in a clearcut at high altitude (3,500 feet).

For both bark plugs and hypovirulent isolates, the cankers that were produced following inoculation were highly superficial, based on small bark-core assays, after the first growing season. However, the superficiality rating of these cankers decreased greatly (disease developed at the vascular cambium) following one to several winters at the high-altitude site (Griffin et al.

1993; Griffin and Griffin 1995). Cankers on the low-altitude trees, which tested as blight-susceptible, remained superficial over the test period and beyond. Other tests have indicated that high-altitude American chestnut trees may be under physiological stress from low temperatures in mid- to late winter. For example, electrolyte leakage from American chestnut bark tissue disks, an indicator of physiological stress on cell membranes, was much greater during the first week of March for high-altitude (3,900 feet) trees than for low-altitude (530 feet) trees (fig. 1).

This greater physiological stress at high altitude during late winter and early spring may decrease host defense mechanisms in American chestnut toward weak pathogens, such as hypovirulent strains. In addition, colonization of the superficial cankers by virulent strains can occur at high altitude (Griffin and Griffin 1995). Similarly, severe blight cankers caused by virulent strains, and sometimes stem death, were found on the normally highly blight-resistant Chinese chestnut growing only at high altitude or in frost pockets (Jones et al. 1980). Drought stress (Gao and Shain

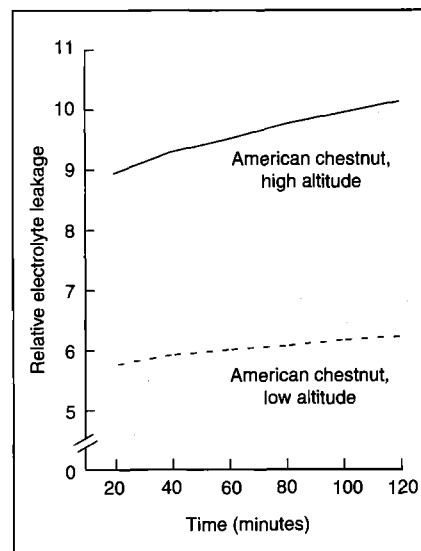


Figure 1. Low-temperature stress on American chestnut at high altitude during late winter is indicated by the greatly increased electrolyte leakage from bark tissues collected at high altitude (3,900 feet) versus low altitude (530 feet). This stress may adversely affect blight control at high altitude.

1995) or xeric sites also may contribute to increased blight susceptibility. The best American chestnut blight control in managed forest clearcuts has been observed on mesic sites (Griffin et al. 1991).

Hardwood competition has been identified as a critical factor not only to blight control but to survival of American chestnut rootstocks following chestnut stem death. Following clearcutting, American chestnut grows as rapidly as any hardwood. This rapid growth is followed by an epidemic of chestnut blight over a 10-year period (Griffin et al. 1991). On xeric and intermediate sites, stump sprouts grow vigorously after the stem has been killed by blight, and this cycle may be repeated several times. On mesic sites, however, the combination of high levels of hardwood competition, which reduces light to young stump sprouts, and browse damage results in death of the sprouts and rootstocks. American chestnut rootstock survival over all sites was inversely proportional to competing hardwood basal area (Griffin et al. 1991). Thus, American chestnut is being lost for any future blight control on mesic sites following clearcutting. These sites have the greatest potential for producing large American chestnut trees.

Controlling hardwood competition by periodic cutting resulted in high American chestnut survival and the natural development of superficial cankers (blight control) that were associated with hypovirulent strains (Griffin et al. 1991, 1993). On mesic and intermediate sites, this forest management practice increased stem size, promoted mast production, and maintained the survival of individual stems for several years beyond that in check plots having no removal of competing hardwoods. Blight control was greatest on mesic sites.

Restoration of American Chestnut

A high level of blight control has been obtained on American chestnut trees that were grafted in 1980 with



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In 1999, an observer points to a 15.7-inch dbh stem on a two-stem American chestnut tree grafted in 1980 from a large survivor and later inoculated with a mixture of hypovirulent strains of the blight fungus. Arrow points to a highly superficial (nonkilling) blight canker on a companion stem. This high level of blight control is typical throughout the sawtimber-size tree.

scions from large survivors (Dierauf et al. 1997). Cankers on the trees were inoculated with a mixture of hypovirulent strains in 1982 and 1983. The trees grow on a low-altitude (1,350 feet), mesic site in the northern Blue Ridge at the Lesesne State Forest, Virginia. The largest tree in 1999 was 61 feet tall, had a 95 percent live crown ratio, a dbh of 15.7 inches, and almost all cankers were superficial. In this integrated management system, hardwood competition is kept low, and tests indicated that host resistance factors in the trees and spread of hypovirulent strains, containing hypovirulence virus of European origin, were associated with blight control (Robbins and Griffin 1999).

This management system may be suitable for restoring American chestnut in intermediate- to low-altitude

wildlife clearing margins or group selection openings with American chestnut rootstocks; the site should be mesic, such as northerly slopes, cove slopes, benches, or lower slope positions. Large quantities of mast could be produced, and the trees should also reach sawtimber size.

For chestnut timber production on a larger scale, a high level of blight resistance likely will be needed, especially at high-altitude forest sites. The backcross approaches, described above, offer great promise for restoration if the high level of blight resistance of the Chinese (or Japanese) chestnut can be retained, if this resistance is stable at high altitude, and if few or no undesirable characteristics are carried over from the Chinese chestnut resistance sources. Also, use of the best selections from crosses between American chestnuts with low levels of blight resistance, including sources from high altitude, may lead to progeny with levels of blight resistance suitable for outplanting. Diversity, vigor, true breeding for blight resistance, and good forest-tree form will be needed in the chestnut sources used in seed orchards for restoration by all breeding programs.

chards for restoration by all breeding programs.

Different regions or sites may require American chestnut sources adapted to these areas. Forest clearcuts and old fields on intermediate to mesic sites where American chestnut is indigenous should be favorable for outplanting the American chestnut seedlings, which can be vigorous and six to seven feet tall at one year when grown under optimal nursery conditions (P. Kormanik 1998, pers. commun.). Forest management involving control of competing hardwoods developing from stumps and seeds should enhance blight control and tree establishment in clearcuts. We have used short tree shelters with some success to protect American chestnut seedlings in forest plots from browse damage, which is very important in the Appalachians.

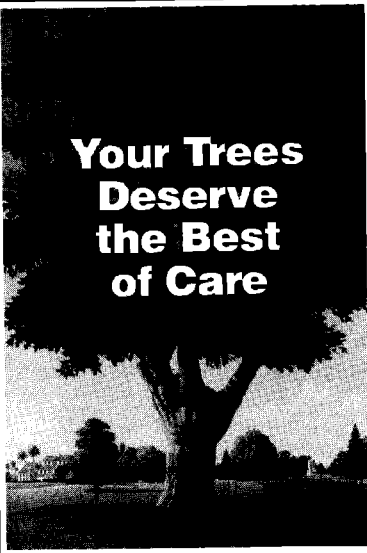
Hope for the Future

The prognosis for American chestnut restoration is good. At this point, chestnut breeding for blight resistance probably has the greatest potential for restoration. The blight control strategies presently under development by university scientists, state forestry researchers, and chestnut research groups are generally sound, but they should not be carried out in isolation from the problems encountered in diverse forest environments, including the varied behavior of American chestnut-blight fungus interactions in these diverse environments.

If American chestnut is to be reestablished as an important component in eastern hardwood forests, forest-based research on chestnut blight control should be given the same priority as laboratory-based research. Grassroots interest among the public for restoring the American chestnut is enormous. Underpinning and interacting with the chestnut research groups and state and federal foresters is a very large and enthusiastic base of support from individual citizens, native plant societies, schools, and youth groups.


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