

EVALUATION OF MINE SPOIL SUITABILITY FOR THE INTRODUCTION OF AMERICAN CHESTNUT HYBRIDS IN THE CUMBERLAND PLATEAU¹

Michael E. French², Christopher D. Barton, Donald Graves, Patrick N. Angel, and Frederick V. Hebard

Abstract: American chestnut (*Castanea dentata* (Marsh.) Borkh.) was formerly the most important hardwood species throughout the forests of eastern North America. The introduction of an exotic fungal blight (*Cryphonectria parasitica* (Murr.) Barr) in the early 20th century decimated *C. dentata* populations. Blight-resistant chestnut hybrids may soon be available for widespread distribution through The American Chestnut Foundation's breeding program, although the development of blight-resistant hybrids is only the first step of the restoration process. For successful introduction, more information must be attained about site requirements necessary for successful establishment and growth of American chestnut. Surface mine spoils in the Appalachian coal region and elsewhere may prove suitable for the establishment of founder populations of blight-resistant chestnut hybrids which may then act as reservoirs for chestnut dispersal into surrounding forests. Six research plots composed of three different loose-graded spoil types have been constructed in the Cumberland Plateau of eastern Kentucky to evaluate their effects on tree performance. The three spoil types are: (1) predominately brown weathered sandstone; (2) predominately gray un-weathered sandstone; and (3) equally mixed brown and gray sandstones and shale. *C. dentata* sub-plots were planted within each of the six plots to serve as proxies for the blight-resistant chestnut hybrids. Preliminary data suggest that initial survival rates are significantly higher (100%) on the mixed spoil material over that of the weathered brown sandstone (79.5%). Survival on the un-weathered grey sandstone (93.2%) was not significantly different than that observed on the other two spoil types.

Additional Key Words: reforestation, restoration, hardwood.

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Introduction

American chestnut (*Castanea dentata* (Marsh.) Borkh.) was formerly a dominant hardwood species throughout much of the eastern deciduous forests (Braun, 1950). The accidental introduction of *Cryphonectria parasitica* (Murr.) Barr, a fungal pathogen of Asiatic origin, was first noticed in New York City in 1904 and, within 50 years, the fungal blight had spread throughout the majority of the chestnut's natural range causing the loss of American chestnut as a canopy tree (Merkle, 1906; Heald, 1926; Keever, 1953; Roane et al., 1986). Throughout the native range, fewer than 30 trees of the estimated pre-blight population of 4 billion have proven themselves capable of surviving long periods of infection (Hebard, 2004). American chestnut was formerly an economically important hardwood throughout its range, supplying timber of high quality, providing an abundant annual nut crop that was an important food source for both humans and animals, and producing tannic acid which was extracted from the bark and was widely used in leather tanning processes (Frothingham, 1912; Steer, 1948; Lord, 2004).

Cryphonectria parasitica is an ascomycete fungus that infects the American chestnut through wounds of the bark. The pathogen then grows in the bark, forming mycelial fans which infect healthy tissues (Hebard et al., 1984). The fungus then attacks the phloem, vascular cambium, and xylem, effectively girdling the tree, but leaving the root system alive. Chestnuts have the capacity to produce stool sprouts (stump sprouts) which was noted in early literature because of the potential for management of chestnut forests through coppicing (Zon, 1904; Frothingham, 1912; Buttrick and Holmes, 1913). This ability to sprout has retained American chestnut's presence in eastern forests, but what was once a dominant overstory tree has been reduced to occasional understory shrub.

Since 1983, The American Chestnut Foundation (TACF) has been selectively breeding the few surviving American chestnuts that produce flowers with Chinese chestnuts (*Castanea mollissima*), with the hope of one day restoring this venerable tree throughout its native range (Burnham, 1981; Burnham et al., 1987). By crossing the surviving American chestnuts with Chinese chestnuts and back-crossing the offspring with different American chestnuts three times, TACF has produced hybrid chestnuts that are essentially 15/16 American chestnut in character and 1/16 Chinese chestnut in character (Fig. 1). At each step in the back-cross procedure, the hybrids were tested for blight resistance. Only trees with a high blight resistance were used in successive breeding stages. Hybrid trees with strong blight resistance that are 15/16 American chestnut in character will then be intercrossed with other 15/16 American trees twice (again, resistance is tested at each step), to ensure a high level of homozygosity for blight resistance alleles in the final product (i.e. the B₃F₃ generation). The ultimate goal of TACF's breeding strategy is to breed most of the Chinese characteristics out of the advanced backcrosses while retaining the blight resistance possessed by Chinese chestnuts, in effect, producing trees that are true-breeding for blight resistance and essentially American chestnut in all other characteristics. TACF is currently producing their first B₃F₃ trees, and expect them to be available for release around 2010 (P. Sisco, personal communication, 2005).

THE AMERICAN CHESTNUT FOUNDATION'S BACKCROSS BREEDING PROGRAM

*With each cross, additional American chestnut characteristics are regained.
Only at the final cross, however, is blight resistance equal to that of the
Chinese parent again reintroduced.*

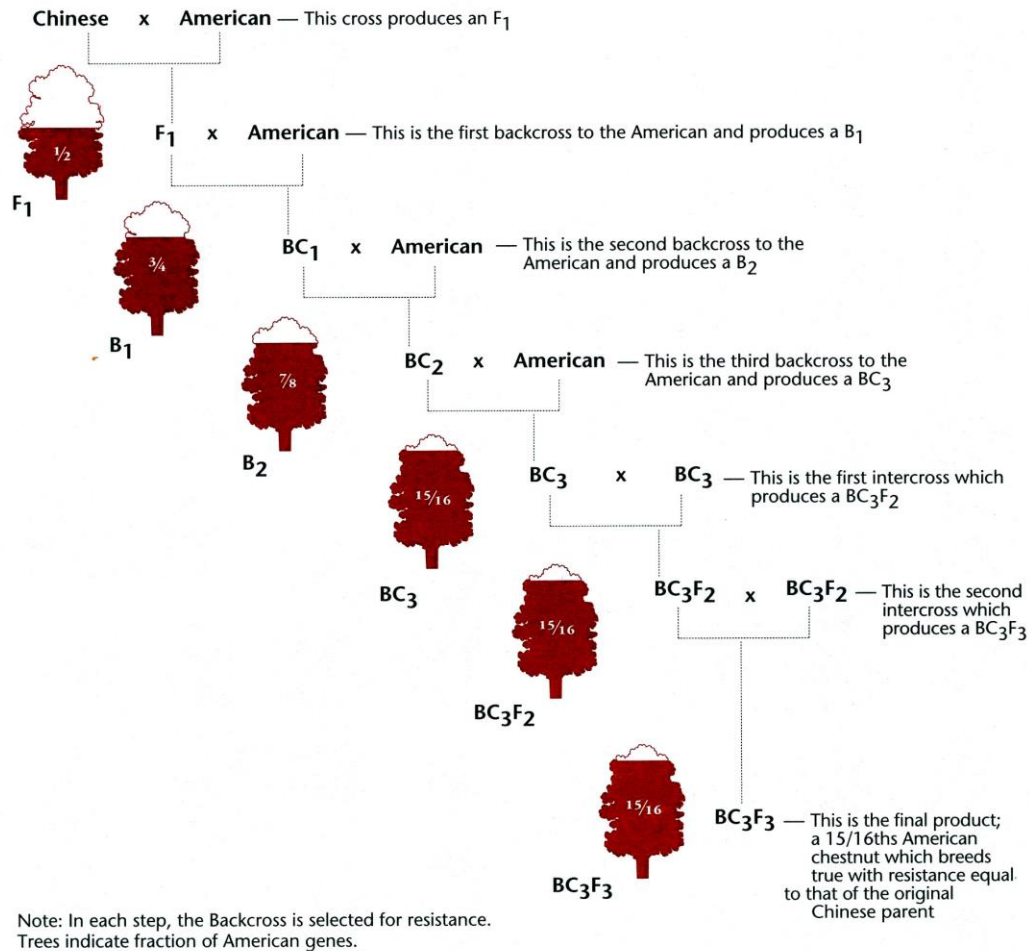


Figure 1. The American Chestnut Foundation's breeding strategy.

Historical accounts report that American chestnut repeatedly showed fast growth in both height and diameter, and the potential to compete with other native hardwoods in a range of settings (Zon 1904; Graves 1905). Modern afforestation efforts throughout the eastern United States use numerous species, but American chestnut is not commonly used since they will soon die from infection caused by blight. As such, little modern data exist that pertain to plantation growth of American chestnut in relation to other species. Less data exist describing how TACF hybrids fare in growth and survival in a mixed-species plantation. One recent study (Jacobs and Severeid, 2004) demonstrated that American chestnut experienced phenomenal plantation growth, out competing black walnut (*Juglans nigra*) and red oak (*Quercus rubra*) and showed high initial survival (>95%) five years after sowing.

For future chestnut backcross reintroduction efforts, much information is needed concerning the site requirements and optimal growing conditions of TACF's backcross trees. High seedling mortality due to the presence of *Phytophthora cinnamomi*, a filamentous protist that is the causative agent of a root rot disease has been observed in experimental plantings in Kentucky and elsewhere (Brosi, 2001). The presence of *Phytophthora* root rot has long been associated with high soil moisture, so future restoration efforts will likely focus on well-drained sites. Little work has been done to explore the possibility of American chestnut reforestation on reclaimed mines, although it is now a priority of TACF (C. H. Keiffer, personal communication 2005; TACF, 2004).

As TACF advanced backcrosses become available, surface mined lands appear to hold great potential to serve as a springboard for chestnut re-introduction into eastern forests. Reforestation experiments on loose mine spoils in the Appalachian region have shown high survival and good growth rates (Angel et al., 2006). Un-compacted spoils are initially devoid of pathogenic microbial communities which may aid chestnut establishment. These sites are also well-drained which may aid in hindering the establishment and colonization by *Phytophthora cinnamomi*. The Appalachian coal region falls almost entirely within the natural distribution of American chestnut (Fig. 2 & 3).

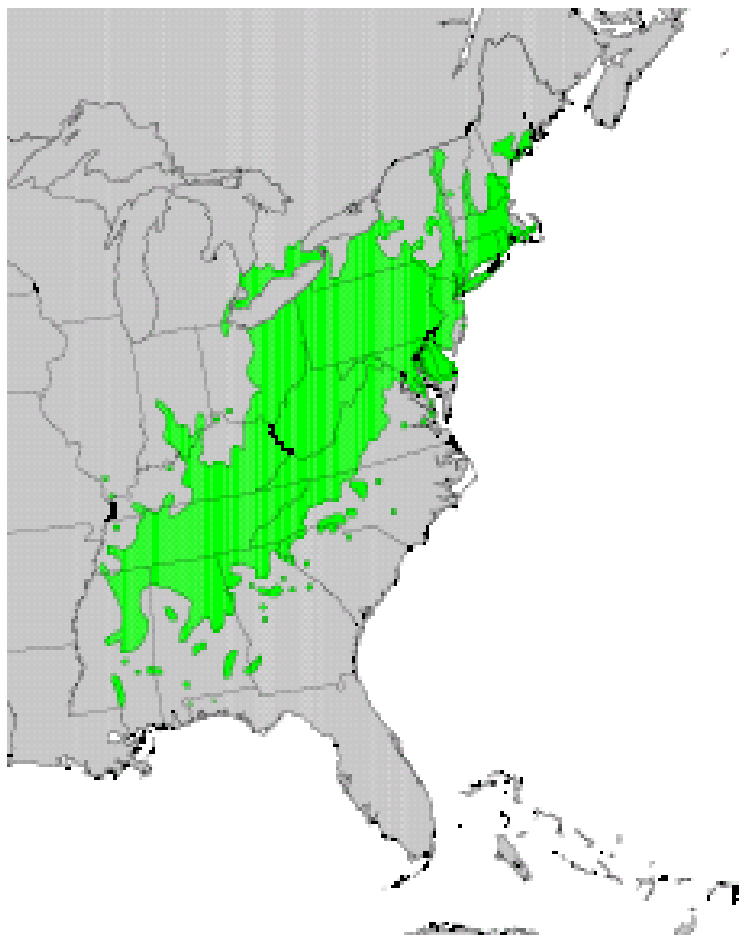


Figure 2. Native range of American chestnut. From Little, E.L., Jr., 1977, Atlas of United States trees, volume 4, Minor Eastern Hardwoods: U.S. Department of Agriculture Miscellaneous Publication 1342, 17 p., 230 maps.

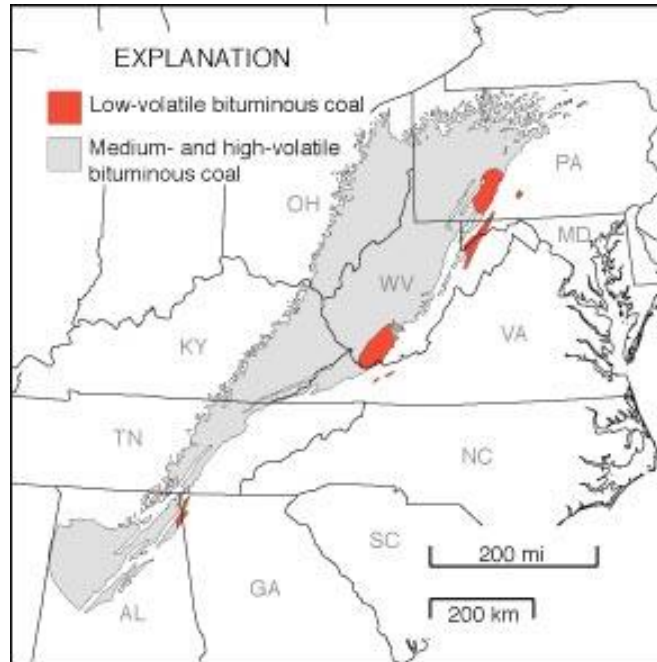


Figure 3. The Appalachian Coal Region. From: www.pubs.usgs.gov/fs/fs115-99

Should mine spoils prove conducive to chestnut growth, establishing founder populations that could naturally disperse into the surrounding forests throughout the range of the Appalachian coal region would aid TACF's goal of restoring the chestnut. Furthermore, newly reclaimed sites provide a "blank slate" where competition problems from established vegetation that are associated with forest plantings can be avoided. The purpose of this research is to investigate some specific aspects of American chestnut growth and survival on different spoil materials and to determine whether *Phytophthora* root rot will impact American chestnut reforestation efforts on mined lands.

Materials and Methods

Research plots were established in March 2005 on an active mountaintop removal operation for the purpose of evaluating tree performance, conducting water characterization studies and determining the mineralogical, chemical and physical characteristics of three different types of loose-graded spoils (see Fig. 4 – Aerial photograph of research plots). The mine site is located on Bent Mountain on Brushy Fork near the community of Meta in Pike County Kentucky (latitude N 37° 35' 50", longitude W 82° 23' 38"). Three spoil types are involved: (1) predominately brown weathered sandstone; (2) predominately gray un-weathered sandstone; and (3) equally mixed brown weathered and gray un-weathered sandstones. The three spoil types are the three treatments in this experiment and they are applied as a one-way treatment structure in a completely randomized design structure. Each of the three treatments was installed in a square plot that measures approximately 63 meters on each side. The total area of each plot is 4050 square meters. The three treatments were replicated once, creating a total of six plots or experimental units (see Fig. 4). The three treatments were randomly assigned to the six experimental units. Each of the six plots was physically separated and isolated from each other

by a 2.5-meter buffer zone where no loose spoil was dumped. The buffer zones will also serve as an access to drain pipes and instrumentation installed in the six plots.



Figure 4. Research plots consisting of differing loose dump spoil material

A rectangular foundation for the six plots was prepared with spoil material from the mountaintop removal operation. The foundation, which is about 3.5 hectares in size, was orientated along the long axis in a northwest-southeast direction on a 2% slope with the highest elevation occurring on the southeast edge. The foundation was then divided in half along the short axis and graded on a 2% slope so that the center line of each plot is at a lower elevation than the northeast and southwest edges (forming a “bathtub” configuration to facilitate the flow of water to the center and then to the exit point of each plot). The spoil in the foundation was then highly compacted by repeated passes of both rubber tired and tracked heavy equipment so that it is impervious to water.

At the exit point of each plot and in the area of the buffer zones, a pit was dug into the compacted foundation that measured 2 meters by 2 meters and 1 meter deep. The floor of the pit slopes to a 20-centimeter diameter hole drilled through about 5 meters of the compacted foundation and about 12 meters of rock overburden to an abandoned underground mine in the Peach Orchard coal seam below the plots. The drainage system to the underground mine below the plots was installed to completely isolate each plot so that no drainage from one plot can mix with drainage from any other plot. Spoil was dumped out of the end of large dump trucks onto the compacted foundation into tightly placed piles that are about 3.7 meters deep. The spoil piles were placed in parallel rows in such a way that they closely abut one another across each of the six plots. The spoil piles were then “struck-off” with one pass of a small bulldozer (D-5) down the length of each parallel ridge of spoil, pushing it into the parallel valleys on both sides as specified in Reclamation Advisory Memorandum Number 124 issued by the Kentucky

Department of Natural Resources (KDSMRE, 1997). Soil samples were collected from the sites and are currently being analyzed.

Four species of tree seedlings of 1:0 nursery stock were planted into the loosely graded spoil of the six plots on April 2, 2005. The four species were white oak (*Quercus alba*), red oak (*Quercus rubra*), yellow-poplar (*Liriodendron tulipifera*), and green ash (*Fraxinus pennsylvanica*). One sub-plot measuring 7 meters by 7 meters was established in the spring of 2006 on each of the six plots to determine the survivability of American chestnuts (*Castanea dentata*) planted in the different spoil types. Each American chestnut sub-plot consists of approximately 23 container-grown seedlings planted on approximately 1.5 meter by 1.5 meter spacing.

The chestnut seed used in this study were pure American chestnuts that were harvested from an orchard grown, open-pollinated tree to minimize pedigree effects. All seedlings were germinated in an artificial soil medium and raised for approximately 1 month in planting tubes and cared for in a greenhouse prior to out-planting. When out-planted, seedlings were assigned numbers and were protected from herbivory by 18-inch, plastic tree shelters. The seedlings will be evaluated for height growth, diameter growth and survival on an annual basis. The study will be analyzed by ANOVA as a completely randomized design with two replications. Treatment effects will be considered statistically significant if the level of probability for random occurrence is less than 0.05.

Results and Discussion

In this study, the preliminary data indicate that American chestnut survived and grew well in all spoil media, and that no statistically significant differences were observed on height or root collar growth between any treatments, although statistically significant differences in survival were observed (Table 1). Average height growth for seedlings planted in the weathered brown sandstone was highest (27.1 cm), followed by seedlings planted in the mixed spoil material (25.5 cm). Seedlings planted in the un-weathered gray sandstone showed the least growth (24.0 cm) after one growing season. Average root collar diameter was the same for all plots (4.5 mm).

Table 1. Growth and Survival of American chestnut seedlings on differing mine spoils

	<u>Avg. Ht. growth (cm)</u>	<u>Avg. RCD (mm)</u>	<u>Survival %</u>
Mixed Sandstone and Shale	25.5 a†	4.5 a	100 a
Un-weathered Gray Sandstone	24.0 a	4.5 a	93.2 ab
Weathered Brown Sandstone	27.1 a	4.5 a	79.5 b

† Values followed by the same letter within a column are not statistically significant at p=0.05 level.

Seedlings had the best survival rate (100%) in the spoil consisting of mixed sandstone and shale for the first growing season. Survival rates were also high on the un-weathered gray sandstone plots (93.2%) and weathered brown sandstone plots (79.5%). The difference in survival rate on the mixed spoil plots was statistically significant from that of the weathered brown sandstone plots at the $p = 0.05$ level of significance. Incidence of blight was highest on seedlings planted in the weathered brown sandstone plots (40.9%), followed by seedlings in the mixed sandstone and shale plots (28.3%). Incidence of blight was lowest on the seedlings planted in the un-weathered gray sandstone material (25.0%). Indicators of stress (i.e. basal sprouting and the formation of two leaders) were also measured for all seedlings, and were highest on seedlings planted in the mixed sandstone and shale plots (34.8%), followed by seedlings in the weathered brown sandstone (31.8%). Seedlings in the gray un-weathered sandstone had both the lowest incidence of blight, and the lowest number of trees indicating stress (20.5%).

The performance of these seedlings is not necessarily indicative of how American chestnut would fare in an operationally planted mine reclamation, since these seedlings were planted as containerized transplants. This was done due to the low availability of bareroot stock, and to ensure that nut depredation problems associated with direct-seeding would be avoided. Chestnuts grow optimally when direct-seeded, although sufficient measures must be taken to ensure that the nuts are not injured by the elements and wildlife. The seedlings used in this study were planted while they were still actively growing, whereas most commercial operations field plant 1-0 or 2-0 bareroot stock during a period of dormancy. There may be a time lag between the performance of these seedlings compared to what might be expected from bareroot stock or from direct-seeded chestnuts.

The presence of *Phytophthora cinnamomi* has not yet been detected in any of the research plots, which is potentially a positive sign for future reclamation efforts involving chestnuts. *Phytophthora*, when present, is often immediately detected. In this study, the root systems of dead trees appeared healthy, with no visible signs of rot induced necrosis. All dead seedlings could be attributed to infection by chestnut blight which can be expected when using blight-susceptible pure American chestnuts (Fig. 5).



Figure 5. A swollen canker caused by chestnut blight.

No conclusions have yet been determined, as this is only the first year of a multi-year study, although it appears that all three mine spoils tested have the capacity to support chestnut growth. It appears that the brown sandstone plots may foster the best tree growth, but may also demonstrate the highest mortality. The trees will continue to be monitored for growth and survival, as well as above and below-ground biomass after the final growth measurements have been taken and soils on the research plots will be tested for the presence of *Phytophthora*.

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