THREE AMERICAN TRAGEDIES: CHESTNUT BLIGHT, BUTTERNUT CANKER, AND DUTCH ELM DISEASE

Scott E. Schlarbaum (1), Frederick Hebard (2), Pauline C. Spaine (3), and Joseph C. Kamalay (4)

Abstract. Three North American tree species, American chestnut (Castanea dentata), butternut (Juglans cinerea), and American elm (Ulmus americana), have been devastated by exotic fungal diseases over the last century. American chestnut was eliminated from eastern forests as a dominant species by chestnut blight (Cryphonectria parasitica). Butternut is presently being extirpated, as butternut canker disease (Sirococcus clavigigenti-juglandacearum) spreads into northern populations. Urban and forest American elm populations have been decimated by Dutch elm disease (Ophiostoma ulmi and O. nova-ulmi). A combination of basic and applied research has been directed toward developing resistant trees of each species. Resistant American elms are now available for planting in urban settings. The prospects for reintroduction of resistant American chestnut, butternut, and American elm into eastern forests appear to be promising.

Forest ecosystems are subjected to many biotic and abiotic stresses. Native insects and diseases, droughts, windstorms and wildfire periodically impact forests or specific tree species, leaving dead or weakened trees. The effects of these stresses may be manifested locally or over a large area, yet they do not cause species extinction. In contrast, exotic pests can threaten the continued existence of a species (cf. United States Congress, 1993). Often host species have not evolved genetic resistance to exotic pests, as coevolutionary processes have not occurred.

Three prominent North American tree species, American chestnut [Castanea dentata (Marsh.) Borkh.], butternut (Juglans cinerea L.), and American elm (Ulmus americana L) have been severely impacted by three exotic fungal diseases, chestnut blight [Cryphonectria parasitica (Murr.) Barr], butternut canker (Sirococcus clavigigenti-juglandacearum Nair, Kostichka & Kuntz), and Dutch elm disease [Ophiostoma ulmi (Buis.)Narruf. and O. nova-ulmi]. Below is a brief account of the impacts of these diseases on their host species, examples of research approaches for disease control, and a prognosis for the future of each species.

EXOTIC PESTS AND THE AMERICAN CHESTNUT

The American chestnut was once the dominant hardwood species in the eastern United States. The tree was important to native Americans because it produced large crops of nuts eaten by wildlife and humans, in contrast to the oaks, hickories, and other trees that have replaced the chestnut (Schlarbaum 1989). The species was used in many different ways by early European settlers, providing food and timber, food for domesticated animals, and tannin. Prior to the European colonization of North America, American chestnut was found in vast stands from Maine to Florida, with the largest trees occurring in the southern Appalachians. During the 19th century, however, introduced fungal diseases would change the species composition of eastern North American forests. An exotic fungal disease, Physophtthora cinnamomi Rands, infested southern populations of American chestnut and the related Allegheny chinkapin as early as 1824 (Crandall et al. 1945). This root rot disease, thought to have caused mortality of chestnuts and chinkapins in low, moist areas, constricted the natural range. This fungal disease was followed by the more commonly known chestnut blight, which spread throughout eastern hardwood forests at a rate of 24 miles per year. By the 1950s, virtually all mature American chestnuts had succumbed to the disease. American chestnut is now a minor understory component, existing as sprouts from old stumps and root systems (Anagnostakis 1995).

There have been two primary research approaches to restore chestnuts to the American forest: the use of hypovirulent strains and breeding.

Hypovirulence research: In 1953, European chestnut (C. sativa) trees infected with blight were observed to be healing (Biraghi, 1953). Further investigation of this phenomenon revealed that unusual strains of C. parasitica were associated with healing cankers (Grente and Berthelay-Sauret 1978). The factors responsible for the healing from the unusual or "hypovirulent" (sensu Grente) strains were found to be transmissible to normal strains through hyphal anastomosis, and would convert the normal strains to hypovirulent, thereby demonstrating potential for biocontrol. Subsequently, the presence of unencapsidated double stranded RNA (dsRNA) molecules were discovered in cytoplasm of hypovirulent strains, and the dsRNA was confirmed to be a virus (Day et al. 1977). Using molecular biology, Choi and Nuss (1992a,b) demonstrated that the genes of the virus were the cause of hypovirulence.

A problem with using hypovirulent strains as biocontrol has been the lack of vegetative compatibility with certain virulent strains. Without vegetative compatibility, transformation does not occur, and the virulent strain will eventually cause mortality. Another
problem with hypovirulent strains is the relatively limited mode of dispersal. The virus exists in the cytoplasm and therefore, does not become involved in the sexual process, i.e., is not contained in the ascospores. Ascospores are disseminated by wind, while the virus containing conidia are not airborne, and have to rely upon animal or water (rain) vectors for dispersal. Despite these limitations, hypovirulent strains have been used to effect recovery from chestnut blight in certain situations (Scibilia and Shain 1989, Anagnostakis 1990, MacDonald and Fulbright 1991, Brewer 1995).

Molecular biology has been used to address the limitations of hypovirulent strains (Choi and Nuss 1992b). The molecular structure of the virus revealed that there were only two genes that were responsible for causing debilitation of the fungus. These genes were transferred to the fungal nucleus using genetic engineering techniques, thereby allowing for subsequent integration into virulent strains through sexual recombination. For every cross, approximately 50 percent of the progeny will have the debilitating genes. Sexual recombination will also broaden the vegetative compatibility range of hypovirulent strains. The effectiveness and spread of the transgenic fungus are currently being evaluated in field conditions. The fungus has been found to survive for two years, produce hypovirulent spores, and was effective in controlling chestnut blight (Anagnostakis, personal communication).

**Breeding research:** Two strategies were pursued to breeding a blight resistant American chestnut: breeding within the American chestnut gene pool and hybridization with Asian chestnut species.

1. Breeding with American chestnut populations: Although chestnut blight had essentially removed mature chestnuts from eastern forests, there were occasional surviving trees that were thought to possess some resistance. Enzymatic studies of inner bark tissue revealed resistance differences, albeit low, among trees (Samman and Barnett 1973, McCarroll and Thor 1985). Cross pollinations were made among putative resistant trees, but resistance could not be increased to an acceptable level and so the approach was abandoned (Thor 1978, Schlarbaum, personal observation).

2. Hybridization with Asian chestnuts: Resistance in Asian chestnut species, particularly *C. mollisima* Bl. (Chinese chestnut) and *C. crenata* Sieb. & Zucc. (Japanese chestnut) was evident to scientists in the early 1900's. Breeding and testing programs were initiated by state and federal agencies.

**Early (pre-1960) breeding programs:** The U.S. Department of Agriculture and the Connecticut Agricultural Experiment Station vigorously tried to breed blight-resistant chestnut trees between the 1930s and the 1960s. The initial hybrids generated by these programs were not as blight resistant as the oriental chestnut parent. To increase resistance, a breeding strategy was adopted that crossed the first hybrids back to a resistant parent, either a Chinese or Japanese chestnut. Unfortunately, this strategy produced trees more similar to oriental chestnut phenotypes, e.g., short and branching, which were not competitive in eastern forests (Schlarbaum *et al.* 1994).

Despite the failure to produce a blight resistant American chestnut, the early breeding programs left an extremely valuable legacy of knowledge and germplasm. Methods were developed for testing trees for blight resistance. Hybrids generated in the later phase of these programs gave the first indication that blight resistance is partially dominant and controlled by only two genes. Additionally, the genetic material accumulated and developed by the old breeding programs has proved to be valuable to current breeding efforts. These materials include: two partially blight-resistant first backcrosses (BC1), the "Graves" tree, and the "Clapper" tree, first generation hybrids, and pure Chinese chestnut.

**Backcross Breeding Strategy:** A number of breeding programs are breeding blight-resistant American chestnut trees using the backcross method (Burnham *et al.* 1986, Burnham 1990). This breeding strategy will transfer blight resistance from Chinese chestnut to American chestnut, while retaining the desirable growth, form, and adaptability of the American chestnut. Highly blight-resistant progeny were recovered after intercrossing first hybrids between Chinese and American chestnut or intercrossing first backcrosses.

There is now evidence that only a few genes control blight resistance in Chinese chestnut, specifically, two or three incompletely dominant genes. The evidence was provided by a combination of crossing and molecular biology. In addition, the use of molecular techniques to accelerate the breeding process is now considered to be feasible. A genetic map of chestnut with regions associated with blight resistance identified, could be used to screen newly germinated nuts for blight resistance. This may enable several generations of backcrossing to be bypassed, yet still produce trees that have proportions of American parentage similar to those of trees bred using conventional backcrossing.

Blight resistant American chestnut may soon be available for general reforestation. The American Chestnut Foundation estimates that by 2012, nuts will be produced from the most advanced breeding lines that can be used in reforestation.

**Chestnut gall wasp another exotic pest of chestnut:** Although blight resistant chestnuts may be available in the near future, Phytophthora cinnamomi will still effectively restrict planting to upland sites. On these sites, chestnuts will then be challenged by
yet another exotic pest, the chestnut gall wasp (*Dryocosmus kuriphilus* Yasumatsu). Infestations by this insect were first reported in 1974 (Payne et al. 1975) and now have spread north into Tennessee and North Carolina. Chestnut gall wasp larvae feed upon bud and flower tissue forming a characteristic gall and producing a toxin that can kill the infested branch. Severe infestations can cause tree mortality.

**BUTTERNUT CANKER DISEASE AND BUTTERNUT**

Butternut (syn. white walnut) is a highly valued hardwood species native to eastern North American forests. The tree is closely related to black walnut (*Juglans nigra* L.) and can occur on cove hardwood, dry, and riparian sites. The wood of butternut is highly valued for carving and for furniture, e.g., cabinets. Butternuts were often planted on farmsteads, close to the house. Nut kernels were used in baking, and cultivars have been selected for orchard production (Millikan and Stefan 1989). The husk surrounding the nut was often used to dye fabrics. In the American Civil War, the color of Confederate uniforms was created using butternut husks as a source of dye.

Currently, many butternut populations are being devastated by an exotic fungal disease that causes multiple branch and stem cankers. The causal agent of butternut canker is *Sirococcus clavigignenti-juglandacearum*, a mitosporic fungus belonging to the large group of Fungi Imperfecti. This large group encompasses those fungi where only the asexual stage of reproduction has been found and the sexual stage remains unknown. Currently, this *Sirococcus* species is thought to be an introduced pathogen, due to its sudden appearance on butternut. The disease was first observed in Iowa in 1967 (Renlund 1971), but is believed to have spread from the southeastern coastal region. The age of the cankers suggests that the fungus first appeared in North America approximately 40-50 years ago (Anderson and LaMadeleine 1978).

In 1995, the Forest Service estimated that 77 percent of the butternuts in the Southeast were dead (USDA Forest Service 1995). Surviving butternuts are now usually found in riparian zones, and the majority of trees are heavily infected and not reproducing. In contrast to American chestnut, butternuts usually will not sprout after stem death. Young trees are subject to mortality, and fungal spores can be carried on the fruit husks (Prey and Kuntz 1982). Therefore, when a population becomes infected, that particular gene pool has the potential to be permanently lost. The rapid decimation of butternut populations has been considered so severe that the U. S. Fish and Wildlife Service has listed the species as a "species of Federal concern."

In response to the devastating effects of the butternut canker, two research and development efforts have been formed to address this problem. The USDA Forest Service, North Central Experiment Station, initiated a cooperative effort with northern states and northern National Forests to locate surviving butternuts and graft putative resistant trees into clone banks to preserve the germplasm. Cooperators are instructed on identification of butternut canker and conservation of germplasm (Nicholls et al. 1978, Ostry et al. 1994). Research is being conducted to develop laboratory and field protocols to screen trees for resistance, host range studies, *in vitro* clonal propagation (Pijut 1993), and the role of insects in dissemination of the fungus. A continuing series of progress reports document the research activities of this group.

A coalition has also been formed in the southeastern United States, by the University of Tennessee, USDA Forest Service, Southern Region and Southern Forest Research Experiment Station, Great Smoky Mountains National Park, Tennessee Division of Forestry, and USGS Biological Research Division. This coalition is working to locate surviving trees or populations, characterize sites, identify trees with putative resistance, develop screening methodology for disease resistance, study fungal physiology, and preserve germplasm.

Progeny/gene conservation tests were established at five locations in 1994 and three additional locations in 1995. One planting was established under infected butternut trees for increased disease pressure. This planting will be closely monitored for disease spread and resistant genotypes or resistant families. Seeds collected in 1996 are presently being grown at the East Tennessee State Nursery to provide experimental material for additional plantings and research activities.

Pathology studies have centered around developing screening methods to identify butternut resistance. These studies include wounding and mycelial inoculation of seedlings under different fertilization regimes, wounding and spore inoculation of seedlings, and log inoculations to study pathogenicity. When possible, different genetic families (open-pollinated) are used for inoculation. Additionally, research has been conducted on physiology and transmission of the fungus.

Currently, the lack of knowledge about the physiology and genetics of *Sirococcus clavigignenti-juglandacearum* hinders the formation of a comprehensive strategy for protecting the butternut species. The survival of large butternut trees in localities where the majority of butternut trees have been destroyed suggest that genetic resistance may be present. Resistance is present in nut selections from another *Juglans* species. Heartnut (*Juglans sieboldii* var. *cordiformis* (Maxim.) Rehd.), a Japanese walnut nut selection, has shown resistance to butternut canker and could be used in a breeding program. Using either natural resistance or resistance in heartnut, a backcross breeding approach coupled with the development of a methodology for disease resistance screening has the potential
to restore this important tree species to eastern forests.

DUTCH ELM DISEASE AND AMERICAN ELM

American elm usually occurs in a mixture of other hardwood species, commonly on bottomland sites with rich, well-drained loam soils. The species' distribution is throughout eastern North American forests, extending well into the Great Plains. The streets of North American cities were once lined with American elms, a fast growing, stress tolerant tree, with a vase-shaped crown. Wood from the species was used for furniture, flooring, construction, hardwood dimension, and veneer.

Forest and urban populations of American elm have been devastated by two strains of Dutch elm disease (DED), a non-aggressive strain (Ophiostoma ulmi) and an aggressive strain (O. nova-ulmi). The disease entered the country on shipments of unpeeled veneer logs from Europe. Dying American elms were first observed in Cleveland, Ohio in 1930 (May 1930). The disease spread through eastern forests from three infection centers (cf. Stipes and Campana 1981) and had spread through most of the country by 1977. Dutch elm disease has proven to be the most devastating shade tree disease in the United States (Karnosky 1977).

Some forest populations, however, still contain large American elms, ca. 29"+ dbh. Other native elm species, such as red elm (Ulmus rubra Muhl.), can be infected with DED, but appear to have greater resistance.

Attempts to breed resistance into American elm using other Ulmus species generally failed. American elm is a tetraploid, while other elm species have diploid chromosome complements (Santamour 1969), and a reproductive barrier exists between the two ploidy levels. Fortunately, American elms exist that are susceptible to infection, but are tolerant to the disease. Tolerant trees are clonally propagated by rooted cuttings. Dr. A. M. Townsend, The U. S. National Arboretum, estimates that only 1 in 100,000 American elm trees are tolerant to Dutch elm disease (Becker 1996). Two new cultivars, "Valley Forge" and "New Harmony," were released by the U. S. National Arboretum in 1996 (U. S. National Arboretum, 1996). A small number of American elm trees which have survived the two DED epidemics are identified each year over the wide range of this species. Seeds or cuttings from each tree subjected to an established screening protocol are selected for tolerance to this deadly wilt disease. Ideally, different resistances can be brought together by hybridizing widely separated elms. To this end, pollen from the trees which survived DED epidemics is being used in controlled crosses with DED tolerant selections.

A cooperative project between the USDA Forest Service and the U. S. National Arboretum has been initiated to study the genetics of host resistance in the field and at the molecular level. Four tolerant selections have been crossed. The resulting progenies will be DNA fingerprinted and evaluated for disease tolerance to construct a genetic map. The genetic map could be used to guide further tree selection in breeding programs and to understand quantitative inheritance of disease tolerance. It is estimated that at least 10 percent of the progeny trees will have DED tolerance greater than the parent trees.

Although trees with good tolerance to DED have been found, very little is known about the mechanisms of tolerance. Research has been conducted to identify American elm defense reactions at the biochemical level using cell suspension cultures (Gringas et al. 1997). It will be important to recognize similarities and differences in the mechanisms of DED tolerance in the varied selections to enable the synthesis of unique genetic combinations. In addition, any breeding programs directed toward improving disease resistance would benefit from a reliable tissue culture screening method. Such a technique could be used to eliminate years of effort in the evaluation of germplasm. The cultures will also be used to isolate defensive chemicals and identify genes responsible for tolerance. Differences among cell cultures in toxin tolerance and changes in gene expression shown by the amount and type of newly synthesized proteins have been detected. Studies by USDA Forest Service scientists are planned to investigate the impact of elm cell secretions on the fungus and associated toxins.

REINTRODUCTION OF AMERICAN CHESTNUT, BUTTERNUT, AND AMERICAN ELM

A critical question that arises in relation to reintroduction of these species to eastern forests is whether they can reoccupy the niche they formerly held and successfully compete and reproduce. For butternut and American elm, there are enough existing naturally reproducing populations that detailed studies can be made on the silvicultural requirement for successful establishment. No such studies can be made on American chestnut on sites within the former natural range. However, there is indirect evidence on the growth characteristics of the species that suggest a strategy.

Blight-resistant American chestnut trees will probably have no difficulty in reclaiming certain sites from the relatively slower growing oaks and hickories. Species such as yellow-poplar (Liriodendron tulipifera) and red maple (Acer rubrum) will be vigorous competitors, but the growth rate of chestnut seedlings suggests that chestnut will be able to compete with these seedlings (Schlarbaum, personal observation). In blight-free regions in the midwest, chestnut seedlings have been able to usurp niches formerly filled by oak and other northern hardwoods. Chestnut sprouts in clear cuts provide indirect evidence of the species'
growth rate potential. American chestnut sprouts dominate the site until infected by the blight fungus.

Another significant problem is in the mechanics of generating enough seed for widespread reforestation of these species. Seed production from the endpoints of breeding programs usually occurs in a seed orchard, under the auspices of a university, state, or federal tree improvement program. Unfortunately, government-based tree improvement programs are rapidly disappearing due to the relatively high cost and long time periods required to generate tangible products associated with this type of research and development program (Schlarbaum 1995). Until this trend is reversed, general reforestation with resistant genotypes of these species will be hampered.

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1. Department of Forestry, Wildlife & Fisheries, The University of Tennessee, Knoxville, TN 37901-1071

2. The American Chestnut Foundation, Wagner Research Farm, Meadowview, VA 24361

3. USDA Forest Service, Southern Research Station, 320 Green Street, Athens, GA 30602

4. USDA Forest Service, Northeastern Forest Experiment Station, 359 Main Rd., Delaware, OH 43015-8650.