Chestnut Breeding in the United States for Disease and Insect Resistance

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Chestnut: A Prized Tree with a Long History

The genus *Castanea* (family Fagaceae) is found in north temperate climates around the world, and is highly prized in many different cultures for its nutritious nuts and valuable timber. Selection for larger, better-tasting nuts has been ongoing in Asia and Europe for centuries. Jaynes (48) quotes Eynard et al. as saying that selections of *C. crenata* Siebold & Zuccarini were known in Japan more than 1,000 years ago. In China, there are at least four species of *Castanea*. Liu Liu wrote in her treatise that chestnuts were found in the ruins of an ancient city in China, demonstrating that they were in use there 6,000 years ago (17). Early trade routes moved European chestnut trees (*C. sativa* Miller) west of their native range (in the Caucasus mountains), and the Romans then moved them across their empire to provide support posts for grapevines, as well as for the nuts (25,37). Cultivar selection in Turkey, Italy, Spain, and Portugal has been extensive, and regional favorites developed.

The many uses of the wood of American chestnut (*C. dentata* (Marshall) Borkhausen) made this "all purpose" tree extremely valuable in its native range in North America (Figs. 1 and 2). Nut production was important as a food source for rural families and many species of birds and animals. The other American species in the genus *Castanea* are classed as chinquapins, and may be divided into several or lumped as a single species (11,55). The small nuts from these trees and bushes serve primarily as mast for wildlife (69).

Botany

Most chestnuts produce two kinds of flowers, usually at the ends of the branches where they are exposed to full sun, although the American chinquapins produce their flowers on spurs at the sides of the branches. Long, cylindrical male catkins have dense stamens that bear the anthers. These produce abundant pollen that is both windborne and carried by insects. The female flowers are prickly involucres with 6 to 14 ovules. The stigmas bristle out of the end of the involucre and are receptive after most of the catkins have bloomed, and remain so for 1 to 2 weeks (Fig. 3). Catkins at the base of the female flowers delay blooming until the female flowers are receptive (duodichogamy). Trees that are interspecific hybrids are often "male sterile" and produce catkins that never bloom to produce pollen (Fig. 4). Phenology was elaborated by Bounous et al. (20). The nuts form only if the trees are cross-pollinated, but rare self-fertile trees have been reported. All of the species (6 or 13 depending on whether you believe the lumpers or the splitters) are cross-fertile in both directions, but there appear to be some weak genetic barriers between certain species (48,49). Nuts are generally three per bur, and the involucre splits into four sections that fold back to release the ripe nuts (Fig. 5). The *Castanea* called chinquapins have single nuts in each bur, and the involucre splits into two sections. Branches of most *Castanea* rarely continue to grow beyond the flowers, but since American chinquapin female flowers are formed on short spurs, the main branches continue to elongate. Chestnuts are not hard-shelled like other tree nuts, so they are

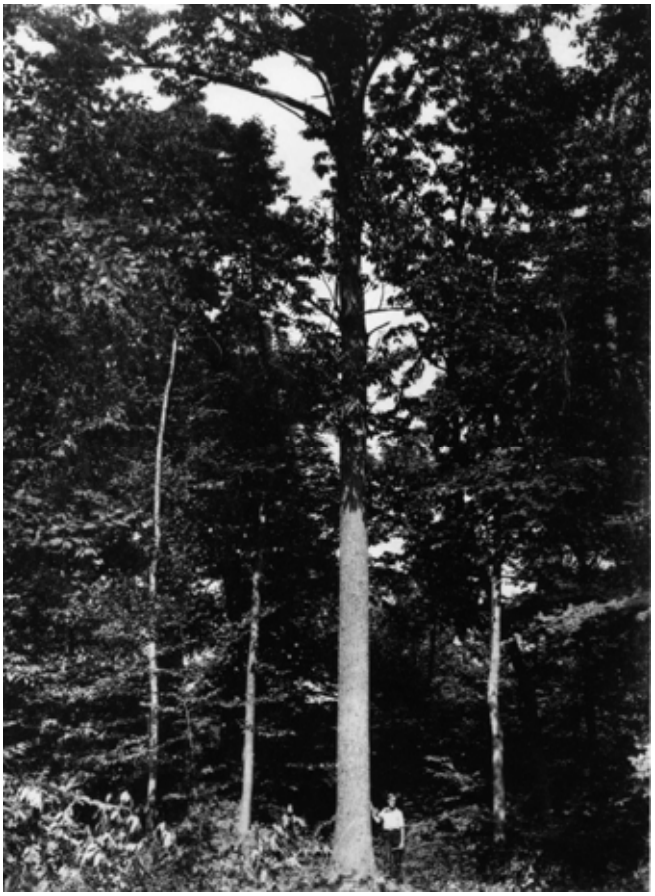

Fig. 1. An American chestnut tree photographed in Scotland, CT in 1905. The tree was 83 feet tall, 27 inches in diameter, and 103 years old, and it occupied 900 square feet (44).

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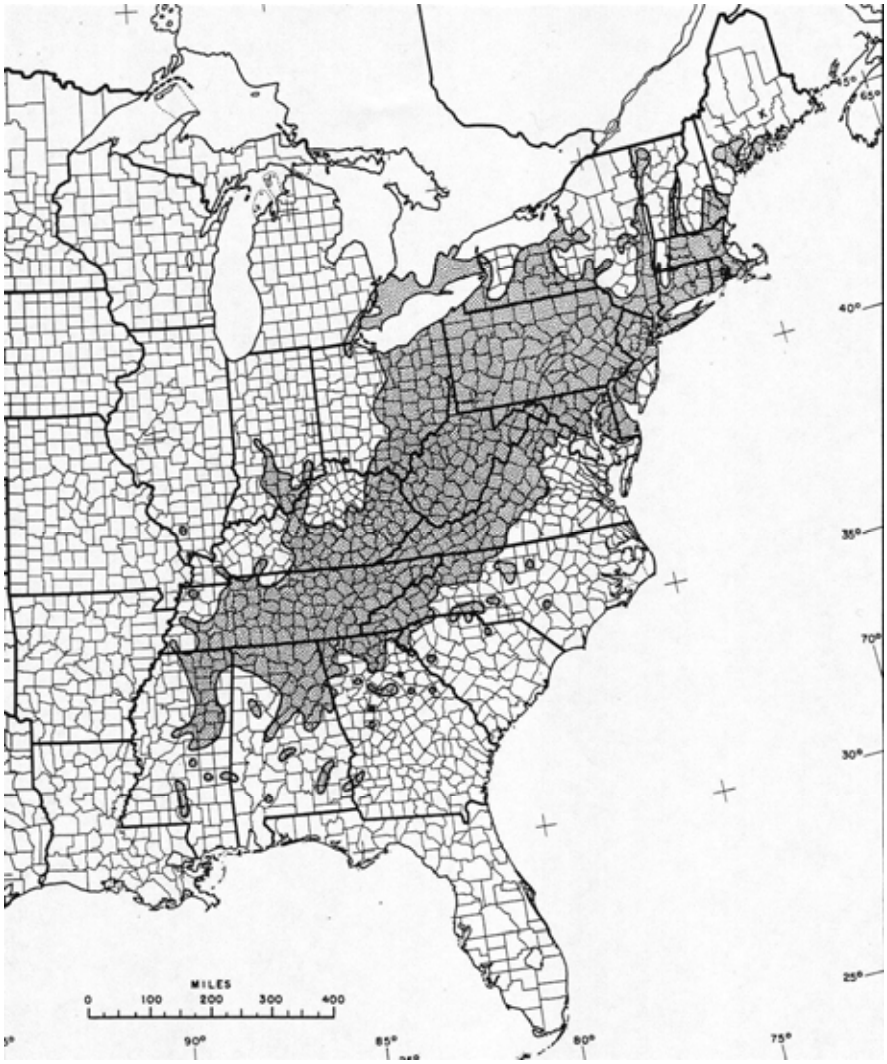

Fig. 2. Native range of American chestnut trees, *Castanea dentata*, in the United States, from J. R. Saucier, 1973, USDA Forest Service Fact Sheet 230.

Fig. 3. Chestnut flowers: male catkins with dense stamens and female prickly involucres with stigmas.

highly perishable and cannot be allowed to dry out. Over the winter in the ground, or in stratification, the carbohydrate in the nut tissue (the cotyledons) gradually changes to sugar, providing "antifreeze" for survival of the embryo (P. N. Gordon, *personal communication*). After 3 months of cold, the radicals start developing, and the tap and fibrous root systems are formed.

Early Importations

The appeal of chestnut, both for timber and for nut production, has prompted importation and experimentation in the United States for many years. In 1773, Thomas Jefferson grafted European chestnut cuttings onto American chestnuts at his home, Monticello, near Charlottesville, VA (25). When E. I. Du Pont de Nemours moved from France to Bergen Point, NJ (in 1799) and then to Brandywine, DE (in 1802), he planted European chestnuts for himself and gave them to friends and acquaintances (37).

G. Harold Powell's 1900 report from the Delaware Agricultural Experiment Station discusses the early history of Japanese chestnut

Fig. 4. Flowers on an interspecific cross of *Castanea* have catkins that form but do not bloom ("male sterile"), but the involucres are usually normally receptive.

trees in the United States. They were first imported by S. B. Parsons of Flushing, NY, who obtained seed from plant collector Thomas Hogg in 1876 (71). These "Parsons' Japan" were sold as original seedlings and their offspring, and three planted in Connecticut in 1876 are still alive and well (Fig. 6). William Parry of New Jersey imported 1,000 grafted trees from Japan in 1882, and selected many of the early named cultivars. In the West, Luther Burbank imported 10,000 nuts from Japan in 1886 and sold selected seedlings by mail-order and to other nurseries.

Early Breeding Work

Our first records of crosses between *Castanea* species typify the whole history of chestnut breeding in the United States: the work was done by both a professional botanist and an interested amateur. Walter Van Fleet was an associate editor of Rural New Yorker magazine when, in 1894, he used pollen of an American chestnut on flowers of the European (or European-American) cultivar 'Paragon' and planted the progeny in Little Silver, NJ (81).

Amateur nut grower George W. Endicott of Villa Ridge, IL had a Japanese chestnut tree that he called 'Japan Giant', but which Detlefsen and Ruth say was probably cultivar 'Coe' selected by Luther Burbank and sold by J. H. Hale of Connecticut (32). In 1899, Mr. Endicott used pollen from an American chestnut tree to produce Japanese × American hybrids (the female parent is always listed first). From five resulting seeds, he raised three trees and named them 'Daniel Boone', 'Blair', and 'Riehl'. 'Boone' produced six burs when it was 17 months old (Fig. 7) (32). 'Blair' and 'Riehl' produced nuts when they were 4 and 5 years old, respectively. These three trees were planted near each other in an isolated field, and Mr. Endicott collected seed from 'Boone', planting 25 seedlings in 1906 and 150 seedlings in 1909. The male parents of these seedlings were, presumably, 'Blair' and 'Riehl'. Mr. Endicott died in 1914 and never saw the results of this second generation cross. When Detlefsen and Ruth examined them in 1920, they described the segregation for tree size and shape, and nut size among this progeny (32).

Fig. 5. An American chestnut bur showing the three nuts still held in the involucre which has split into four sections.

Fig. 6. Japanese chestnut tree, *Castanea crenata*, thought to have been planted in 1876. This tree is in front of the Bee and Thistle Inn in Old Lyme, CT.

Ink Disease and Chestnut Blight: Diseases that Threaten Chestnut Survival

Two serious diseases of chestnut trees changed the direction of chestnut research in the United States. Ink disease, caused by the root pathogen *Phytophthora cinnamomi* Rands., was discovered to be the cause of widespread death of chestnuts and chinquapins in the southern United States, which had been observed since about 1850 (60). This imported pathogen probably came into the southern United States before 1824. Ink disease was reported on chestnuts, walnuts, and cork oaks in Portugal in 1853, and in 1904 Prunet said that "The Black Foot Disease of Chestnut...[is]…of all the diseases the most to be feared" (72).

In 1912, Hopkins reported on his surveys in the southern United States and quoted Mr. Jones of Riceboro, GA: "…in 1823 a great fall of rain occurred and late 1824 was also very rainy, in 1825 many chinquapin trees died and continue to do so up to 1845, if the disease is not stopped, trees will be exterminated" (47).

The ink disease pathogen was widely distributed by 1945 (27) (Fig. 8). The organism enters the roots or root collar of trees through wounds, killing the tissue and turning it black (Fig. 9). It is easily spread in wet soils, and was originally called a "water mold" because of its motile spores (zoospores), but is no longer classified

Fig. 7. Drawing of a bur with nuts from chestnut cultivar 'Boone'.

with the true fungi. Trees killed by this organism do not sprout, and never recover. In 1950, Crandall noted that cork oak trees from Portugal had been extensively introduced into the United States to exactly the areas where *P. cinnamomi* became established: the southern United States and California (26). Ink disease is still a major problem in Europe, and the breeding program begun in 1986 at INRA in France used resistant Japanese chestnuts in crosses with European chestnuts to produce cultivars resistant to the pathogen (73). Since ink disease is still a threat to chestnuts in the southern United States, breeding programs must select for resistance if plantings are to succeed. When The Connecticut Agricultural Experiment Station (CAES) seed was sent to Cecile Robin in France and Mollie Bowles and John Frampton at North Carolina State University for testing, seedlings that were *C. dentata* × *C. crenata* were completely resistant, and the backcross (*C. dentata* × *C. crenata*) × *C. dentata* progeny segregated 1:1 for resistance, suggesting that a single dominant gene in Japanese chestnut trees confers resistance (*unpublished*). Since production of resistant chestnut trees has lagged behind demand, efforts to find a chemical control have increased (38).

The second chestnut disaster was the introduction of chestnut blight disease, which was first found in the United States in 1904. The pathogen causing the lethal cankers is an Ascomycete now known as *Cryphonectria parasitica* (Murr.) Barr (formerly *Endothia parasitica* (Murr.) And. & And.) (18). The discovery of chestnut blight in the Bronx Zoo was described by Merkel (58) as follows: "…a few scattered cases which occurred [on American chestnut trees] during the summer of 1904. Early last June [1905] this disease was noticed on so many widely scattered trees of all sizes that specimen branches and an appeal for information were sent to the USDA."

Finally, when Haven Metcalf and J. Franklin Collins wrote their 1909 Bulletin, they stated: "The theory…that the Japanese chestnuts were the original source of infection, has been strengthened by many facts.…While the disease has spread principally from the vicinity of New York City there is much to indicate that it occurred at other points at an early date. Chester's Cytospora on a Japanese chestnut noted at Newark, Del., in 1902, may have been the bark disease. Observations by the junior writer indicate that this disease may have been present in an orchard in Bedford County, Va. as

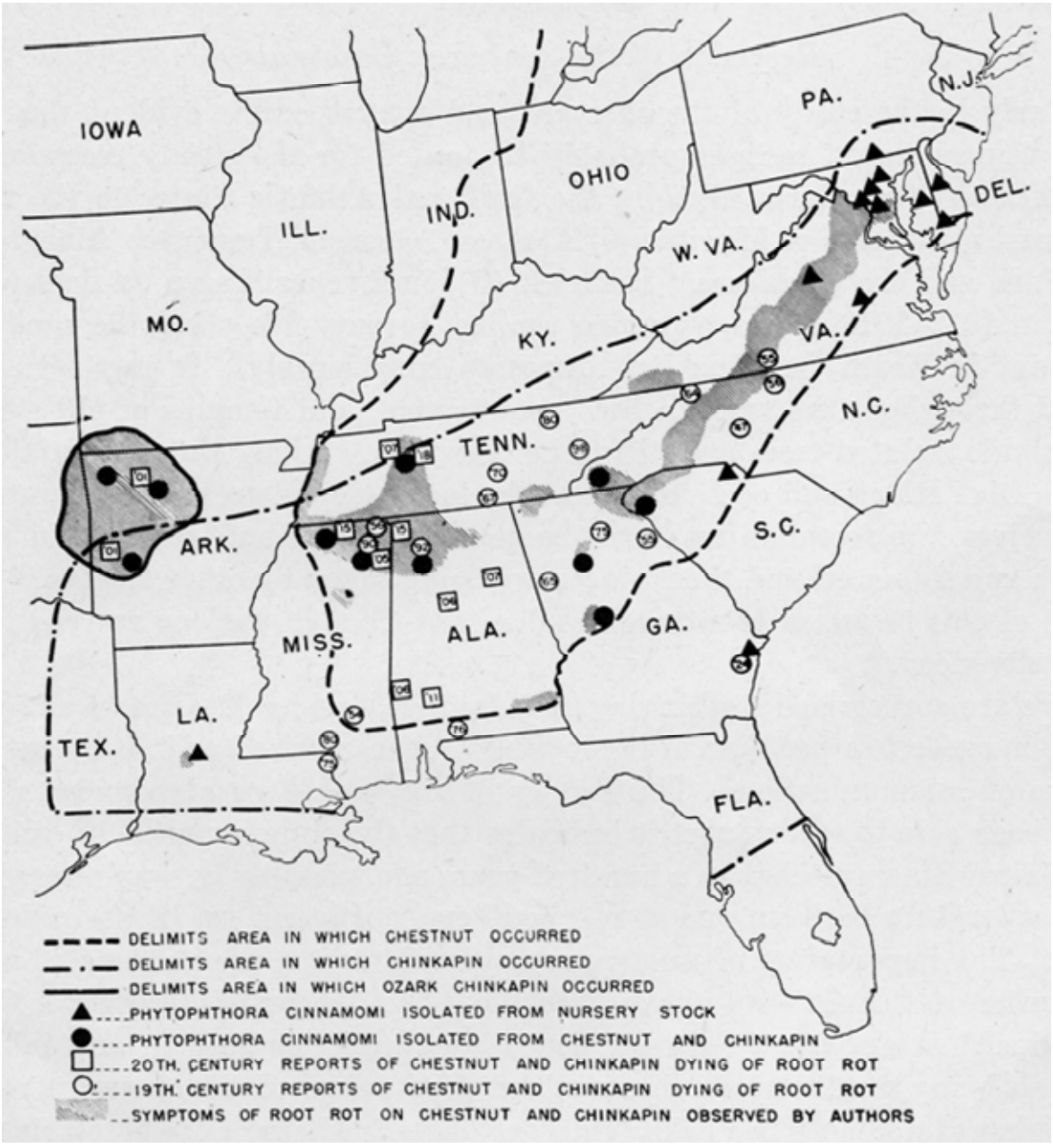

Fig. 8. Map of the distribution of *Phytophthora cinnamomi* in 1945, from G. S. Crandall, G. F. Gravatt, and M. M. Ryan (27).

early as 1903, and…It becomes more and more evident as this disease is studied that diseased nursery stock is the most important factor in its spread to distant points" (59).

Any or all of the early Japanese imports could have carried the disease. Certainly, the Bronx Zoo was not responsible for bringing it in, even though their sharp-eyed grounds people first recognized the problem. People anxious to plant something new and different do not always notice problems on old and common plants.

The Search for Resistance

From the earliest discovery of chestnut blight disease, many kinds of treatments were tried to control it. Nothing worked, so a major forest tree was reduced to a multiple-stemmed shrub (9,54). The fungus enters wounds, grows in and under the bark, and eventually kills the cambium all the way around the twig, branch, or trunk, and everything distal to this canker then dies (Fig. 10). Pycnidia form on the bark surface producing abundant conidia, and perithecia then develop and produce ascospores (16). The sticky masses of conidia are easily spread by rain-splash and by birds and insects that frequent the branches and trunks (74), and ascospores are shot out into the air during wet conditions (16). Nuts can become infected with the pathogen, which grows under the nut shell and breaks through the shell to form pycnidia (31). The fungus rarely infects the "root collar" at the base of the tree, which is full of dormant embryos that sprout in response to the death of the main trunk, and the process starts all over again. Sprout clumps survive today that are the remnants of the original trees. Plant explorer Frank Meyer found chestnut blight disease in both China and Japan, and reported that Asian trees were often very resistant to the disease and developed few symptoms when infected (29,77,78). This was taken as circumstantial proof that Asian trees imported into the United States had brought the blight with them. In 1912, the Plant Quarantine Act was passed to reduce the chances of such a catastrophe happening again (84).

The U.S. Department of Agriculture (USDA), Bureau of Plant Industry responded to the crisis by importing chestnuts from Japan and China, searching for replacement chestnut trees with resistance to both chestnut diseases (Fig. 11) (29,79). The records of these USDA chestnut importations, and their distribution, are now housed at CAES.

Breeding Chestnuts for Resistance to Chestnut Blight

When chestnut blight disease started killing Van Fleet's trees in New Jersey in 1907, he began using *C. crenata* trees with obvious resistance, and crossed them with an Allegheny chinquapin (*C. pumila* Miller) grown from seed collected in Virginia in 1889 (82). Van Fleet moved to Maryland in 1910 to work for the USDA, and expanded his chestnut crosses to include some of the newly imported *C. mollissima*. In 1914, he detailed his crossing methods: he removed male catkins as they appeared, covered the female flowers with paper bags, applied the desired pollen with a brush, a fingertip, or by flicking or lightly drawing the catkins over the stigmas (81). One of his best hybrids was called "S-8," its row and tree location in the nursery (Fig. 12). The female parent was his *C. pumila* from Virginia and the male parent was one of his listed as "several named varieties of Japan chestnut including 'Parry's Giant,' 'Killen,' and 'Hale'" (82). Seedlings of this tree were widely

Fig. 9. Symptoms of infection by *Phytophthora cinnamomi* on a Chinese chestnut tree (*Castanea mollissima*). Infected tissue of roots and lower stems are "inky" black, and become liquified.

Fig. 10. Chestnut blight canker caused by *Cryphonectria parasitica* on an American chestnut tree (*Castanea dentata*). The fungus enters through wounds such as the broken branch on the left, and grows in and under the bark to kill the cambium. Orange stromata with pycnidia break through the bark and conidia are extruded when the bark is wet. Photo by R. A. Jaynes.

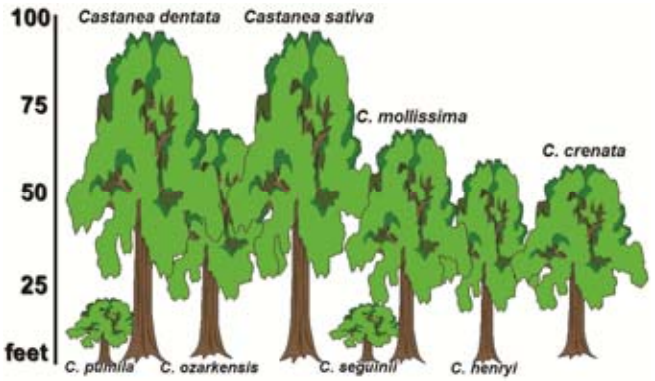

Fig. 11. Relative sizes of trees in the genus *Castanea*.

distributed by the USDA, and survivors can still be found (Fig. 13). He worked on chestnut (and rose) breeding until his death in 1922.

The USDA Yearbook of Agriculture for 1937 carried an article by H. L. Crane, C. A. Reed, and M. N. Wood, in which they discuss nut breeding in the United States (28). Their work at Beltsville, MD and the work of R. B. Clapper, F. H. Berry, and G. F. Gravatt at Glenn Dale (Bell), MD, and A. S. Colby at the experiment station in Urbana, IL involved making and testing hybrids for their resistance to chestnut blight disease (28). Pure cultures of the blight fungus were used to inoculate trees, or the trees were planted out and allowed to live or die from natural infections of chestnut blight disease. The field notes and breeding records from the USDA chestnut breeders from 1931 to 1958 are now at CAES.

Fig. 12. Fruiting branch of Van Fleet's chestnut hybrid S8 at Bell, MD. Photographed in 1943 by R. B. Clapper.

Fig. 13. A seedling of Van Fleet's hybrid chestnut S8 planted in 1939 by E. S. Wright in Knoxville, GA. R. Fair is shown under the tree in 1996.

Clapper worked from 1925 to 1949 breeding chestnut trees for forest plantings (Fig. 14) (33). The hybrid that he was most excited about was the result of the cross (*C. mollissima* M16 × *C. dentata* FP 555) × *C. dentata* FP 555. The first cross was made in 1935 and the backcross in 1946 (Fig. 15). M16 was a seedling from Plant Introduction seed #34517 from Tientsin, China (imported 1912), and FP555 was a sprout clump from a chestnut near the road on the front fence line of C. Campbell, across the road from the Plant Introduction Garden (34). The ortet of Clapper's best tree was one of these backcrossed hybrids (#B26:3146) planted in a USDA chestnut test plot near Carterville, IL in 1949 (Fig. 16). Little and Diller named the tree 'Clapper' (56). This tree survived until 1978, when it died of chestnut blight disease (19), but ramets are still growing at the CAES farm in Hamden, CT. The USDA chestnut breeding work was continued by Jesse D. Diller and Fredrick H. Berry until the project was terminated about 1960. They planted test plots of Asian chestnut trees in 19 locations and mixed plantings of hybrid and species chestnut trees in 15 locations in the Eastern United States. The latter plantings had 1,746 trees, including 500 hybrid seedlings and 541 Chinese chestnuts from the USDA nursery at Bell, MD (Fig. 17), and 705 hybrid seedlings from CAES (19,75).

The contribution of many interested nut growers has been very important, both in spurring on the scientists and in educating the public. In Connecticut, physician R. T. Morris planted many kinds of chestnuts and experimented with crosses and culture (61,62). His property, Merribrooke, in Stamford and Greenwich, CT is now

Fig. 14. R. B. Clapper examining a group of seedlings from 1931 and 1932 crosses of Japanese and American chestnuts. Photo in Bell, MD in 1938.

Fig. 15. A group of seedlings from the cross of *Castanea mollissima* M16 × *C. dentata* F.P. 555 made in 1935. Trees photographed in Bell, MD in 1946.

a park, and many interesting chestnut trees can still be found there. Alfred Szego of Long Island, NY tried many combinations of species, searching for the best nut tree (80).

E. M. Meader at the University of New Hampshire purchased seed of Asian chestnut trees in the market in Seoul, Korea in 1947 and used them in a breeding program with American chestnut trees to produce improved orchard trees that are suitable for New England climates. He was well known as a fruit breeder, and felt that the Korean chestnuts were more suitable than the Chinese for the shorter seasons in the north (R. A. Jaynes, *personal communication*).

Ongoing Breeding Programs

The longest continuing chestnut breeding program in the United States is that in Connecticut. Woodlot owners and nut growers encouraged CAES to study chestnut management before, and disease resistance after chestnut blight disease engulfed the state (44,70). Arthur H. Graves started his career at Yale University in New Haven, CT, and in 1921 took a job at the Brooklyn Botanical Garden in New York. In 1914, he wrote: "The most hopeful indications for chestnut in North America in the future lie along the line of breeding experiments.…Work of this kind is extremely valuable and, although slow in yielding results, may eventually prove to be the only means of continuing the existence in our land of a greatly esteemed tree" (39).

He collected and planted chestnut trees on land that he owned in Hamden, CT, and started making crosses in 1930. He soon began a long association with geneticist Donald F. Jones at CAES, who had planted Chinese chestnut trees from the USDA at Lockwood Farm, a CAES facility in Hamden, CT. Initial crosses of Japanese chestnut trees on Long Island with American pollen from the USDA yielded hybrids with low resistance (Fig. 18), and Graves began crossing these with Chinese chestnut trees to "increase resistance."

Fig. 16. The 'Clapper' chestnut planted in Carterville, IL in 1949, is examined by R. A. Jaynes in 1962.

Fig. 17. Richard Day pollinizing Chinese × American hybrids in the Bell, MD orchard in 1956.

He made crosses every year, planting hybrids and trees from the USDA on the land in Hamden, and when he retired in 1947, he moved back to Connecticut to work full-time with CAES on chestnut breeding. He used inoculations of pure cultures of the blight fungus into stems, and of spores into wounds, to induce chestnut blight disease, and published his ratings of species resistance from tests made over 30 years (40). In 1949, Graves sold 8.3 acres of his land to the Sleeping Giant Park Association, who then gave it to the State of Connecticut, stipulating that the property was to be reserved for CAES tree-breeding experiments. This is probably the finest collection of species and hybrids of chestnut in the world. In 1962, he reported that he and his associates at CAES had made more than 250 combinations of all the species of chestnuts (41). Two of his students, Hans Nienstaedt and Richard A. Jaynes, maintained these trees, made crosses, and contributed greatly to our general knowledge of chestnut. For his doctoral thesis, Jaynes made crosses between all the species, most in both directions, and confirmed the diploid chromosome number of *Castanea* as 24 (48,49).

The early Connecticut breeding work focused on making hybrids that were combinations of species, inoculating the trees with the blight fungus to test their resistance, and looking for ideal trees with good nut quality or with good timber form that could be propagated clonally. Jaynes joined the Experiment Station staff in 1962, and with Graves (who died in December of that year) published a bulletin on Connecticut hybrid chestnuts and their culture (53). The demand for chestnut trees with good quality, large nuts has continued steadily, and many of the Connecticut cultivars are still available from nurseries (50). In addition, Jaynes cooperated with the Virginia Division of Forestry to plant over 10,000 hybrid chestnut seedlings in the Lesesne State Forest in Virginia. These are still monitored in order to make selections for timber trees.

Many Asian trees in Connecticut have been evaluated for survival in the New England climate (some for 135 years, by 2012), and selected trees have been used in controlled crosses to make new hybrids (2,770 crosses in 80 years). The hybrids have been tested for resistance to chestnut blight (by inoculating 5-year-old trees, or just letting them grow in a planting where blight is rampant), and tested for resistance to ink disease by several cooperators. The trees also have been evaluated for timber form and for nut quality. There are no tests that can be made on seedlings to determine their degree of resistance to chestnut blight. Trees must be grown for at least 5 years before inoculating with blight cultures or allowing natural blight infections to eliminate the most susceptible individuals. Early indications that determination of tannin types and levels might be useful for selection were found to not be valid (6).

Fig. 18. A. H. Graves with one of his Japanese (or Japanese × European) × American hybrids, the Smith tree. The tree has been "inarched" to keep it alive in spite of a chestnut blight canker at the base. Sprouts from the root collar were sharpened to a point and inserted under the bark above the canker. A graft union formed, and the resulting bridge allowed xylem and phloem flow.

The Discovery of a Biological Control for Chestnut Blight

An extra boost to breeding chestnuts came with the discovery in Italy that something was allowing blight-infected chestnut trees (*C. sativa*) to survive. French scientist Jean Grente collected samples from the heavily callused cankers, isolated the fungus, and found that the *C. parasitica* in those cankers was greatly changed in appearance and virulence (42). He called them "hypovirulent" strains, and demonstrated that the genetic determinant was carried in the cytoplasm of the fungus and passed from strain to strain by anastomosis. He used hypovirulent (H) strains for biological control in orchards in France, growing them in a laboratory set up for that purpose. When we wrote to him in 1972 and asked for cultures of his hypovirulent strains, he sent them under our import permit. We found that the cytoplasmic agent was a double-stranded RNA (dsRNA) virus encapsulated in pleomorphic vesicles (Fig. 19) (30,35). Using these strains, we were able to keep a virulent strain from killing American chestnut trees in the greenhouse by coinoculating with a hypovirulent strain (15). Interest in these viruses brought many other scientists into the project, and a federal re-

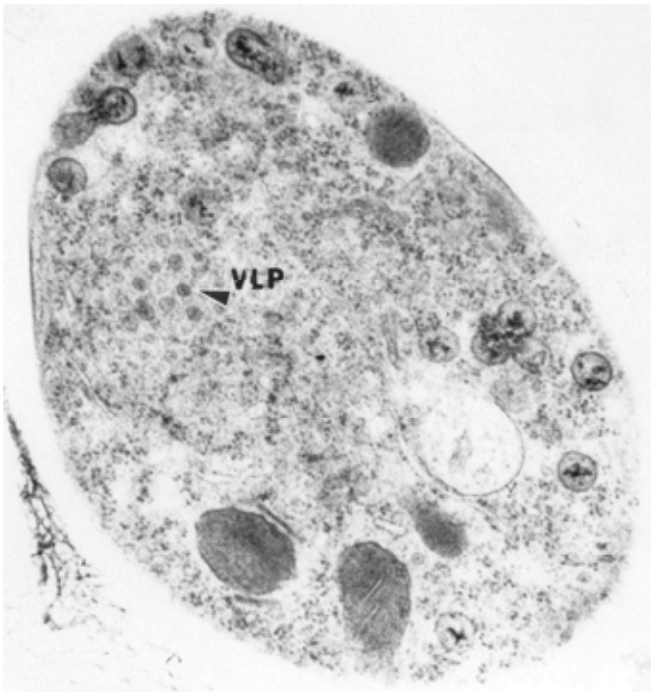

Fig. 19. Virus-like particles (VLP) in a transmission electron microscope section of a *Cryphonectria parasitica* hypha with European double-stranded RNA viruses.

gional project was formed (now called NE-1033, http://nimss.umd.edu/homepages/home.cfm?trackID=10058).

There is a vegetative incompatibility (vic) system in the pathogen that restricts virus transfer, since the virus can only be transferred from strain to strain by hyphal anastomosis (Fig. 20) (1,2,4). As sequence data for this vic system become available, the nature of this restriction is being elucidated (23). When we were given permission to try controlling blight with hypovirulent strains at the CAES farm in Hamden, we were concerned that vic gene differences might reduce our chances of determining the usefulness of the biocontrol. We decided to use mixtures of hypovirulent strains to circumvent the problem of vegetative incompatibility (51). Jaynes planted 70 American seedlings sent by Ivan Thor at the University of Tennessee, and when blight became obvious on them, we treated every canker we could reach by inoculating a mixture of hypovirulent strains around the canker margin. Now the trees are 36 years old, and half of them keep dying back from chestnut blight, sprouting, and dying again. A third of them died back once and the sprouts then continued to grow, with heavily callused cankers from the base to near the top. The rest of the trees never died back, and have heavily callused cankers from the base to near the top. Branches die, new cankers form, but trees survive and nut production is heavy (Fig. 21). There is no pattern to the location of the "good" trees in this planting, nor any obvious difference in the cankers. It is clear that all American chestnut trees are not equal in resistance to chestnut blight disease, and that the presence of hypovirulent strains in the fungal population can allow some trees to survive.

Our hopes for a cure for chestnut blight in the forest were first encouraged (3,5), but as other hardwood trees in forest plots became larger, competition for light, water, and nutrients resulted in the chestnut trees dying back (57). We can use this biocontrol to keep American trees alive in an orchard setting, or in a forest clearcut for several years, and having an easily obtained source of *C. dentata* for breeding purposes is a distinct advantage.

With Jaynes' retirement from CAES in 1983, responsibility for the chestnut breeding program fell to me. At the urging of Charles R. Burnham, a retired geneticist, Connecticut records were searched for hybrids that were products of susceptible × resistant trees, and any that were backcrossed again to the susceptible parent species. Burnham felt that a few generations of backcrossing and selecting, followed by crosses of those products, would allow selection of trees that had the form and nut quality needed, combined with resistance to chestnut blight (22). These trees would produce "true to type" offspring and allow reforestation with chestnut. Besides convincing me to adopt this breeding system, he wanted to get volunteers throughout the range of American chestnut to pollinate local trees with pollen from hybrid trees with some resistance. As these people were found and organized, two people took up the job of forming a nonprofit foundation to oversee the work. Philip Rutter met Burnham in 1982 and with the help of lawyer Donald Willeke, incorporated The American Chestnut Foundation (TACF). This allowed them to raise funds to acquire land and hire help to supervise a breeding program of "chapters" of the foundation in many states. They hired F. V. Hebard, a plant pathologist, to start the breeding and to plant the seedlings on a farm in Meadowview, VA. Hebard drove to Connecticut to use trees at CAES, bagging female flowers, coming back in 2 weeks to pollinate, and then returning in the fall to collect the nuts. After several years of this, a breeding population was established, and crosses were being made with American chestnuts found flowering in Virginia and many other places where chapters of TACF were established (www.acf.org). Their advanced backcross populations are now planted in seed orchards for large-scale propagation of the trees.

The third breeding program in the United States is the American Chestnut Cooperators' Foundation (ACCF). In the 1970s, they searched for American chestnut trees that had survived even though the rest of the population around them had died of chestnut blight disease. When samples of blight cankers from these trees were sent to CAES, we found dsRNA viruses in the *C. parasitica* strains that were causing cankers but not killing the trees (36,52). The estimated sizes of these dsRNA viruses differed from that of the European hypovirulence viruses in the *C. parasitica* that we had imported, and their level of virulence reduction in the fungus was variable. *C. parasitica* from surviving trees in Michigan also contained different virus types, leading to naming of the various virus species (45). Since then, sequencing has been done to further identify the viruses (65), and our knowledge of the nature of hypovirulence greatly advanced. Gary Griffin's tests found that some of these old, surviving trees in the southern United States had significant resistance to chestnut blight compared to other members of their local population (43). Longer survival may have given time for viruses from other fungi to infect the *C. parasitica*. It is also possible that these trees have some genetic factors that allow them to survive better when viruses do get into the mycelium of the

Fig. 20. Vegetative incompatibility (vic) genes in *Cryphonectria parasitica* result in barrage lines of dead cells and pycnidial production when strains with different alleles are paired on potato dextrose agar. The pair in the center on the left have no barrage line and are presumed to be vegetatively compatible.

Fig. 21. American chestnut trees (*Castanea dentata*) kept alive with hypovirulence viruses which cause a biological control of chestnut blight disease. Trees are now 35 years old, and there have been no treatments with hypovirulent strains of the fungus since 1981. All the trees are heavily cankered, half keep dying back and sprouting as they do in normal forest conditions, but the rest continue to grow.

fungi. ACCF scientists have collected scions from selected old survivors and grafted them onto native American chestnut rootstocks. They now have several breeding orchards of these grafted trees, along with first- and second-generation selections from the ACCF breeding program, some of which are protected with hypovirulence treatments to help with survival. Seed from cross-pollinations are being distributed to cooperating growers for testing throughout the natural range of American chestnut (www.ppws.vt.edu/griffin/accf.html).

Chestnut Trees for Nut Production

Interest in growing chestnut trees in U.S. orchards for nut production has waxed and waned for centuries, encouraged by the Northern Nut Growers Association (www.NorthernNutGrowers.org). In 1983, only 162 commercial Chinese chestnut orchards were reported in the eastern United States, even though yearly imports of chestnuts were at 4.5 million kg at that time (68). Nutrients in the nuts have been examined in both open-pollinated and hand-pollinated nuts. Pure American chestnuts are always found to have large amounts of oleic acid, which may contribute to the perceived sweetness of the nuts of this species (76). Using nuts from controlled crosses, it was clear that the male parent in the cross has no influence on the size of the nuts, but does have an effect on the nutrient contents (7,8,13,14). Thus, orchard design should consider selection of cultivars that produce large nuts in the selected location (climate, soil), placement of optimal pollinizer trees, and selection of pollinizers that will result in nuts with ideal nutrient content.

There have been few directed breeding programs in North America to produce superior orchard chestnuts. On the West Coast, the work of Luther Burbank, Albert Etter, and Felix Gillet in California and Jack Gellatly in British Columbia produced trees suitable for those climates and soils (63). Growers with seedling populations of trees tend to find one that produces bigger, tastier nuts, and if it can be propagated, they give clones to their friends. Several of the French chestnut cultivars produced by the INRA to survive ink disease were imported by Hill Craddock at the University of Tennessee at Chattanooga, kept in quarantine for 3 years, and then shared with U.S. nurseries. They survive well on the West Coast, but have not been widely successful in the eastern United States. The most commonly planted cultivar in the United States is 'Colossal', a Japanese-European hybrid of unknown origin, which was propagated in large numbers by a nursery in California (64). They also sold cultivar 'Nevada' as a pollinizer for 'Colossal'. If someone wanted to plant an orchard of 200 trees that were at least 4 ft. tall, they were the sole source.

A chestnut cultivar is, by definition, a clone (ramet) of the original (ortet) tree, and cultivar names are enclosed in single quotes. The International Society for Horticultural Science has a Cultivar Registration Authority organization, and I am the registrar for cultivars of *Castanea*. A list of the names that have been used for cultivars and some of their characteristics can be found on the CAES web page at: www.ct.gov/caes/cwp/view.asp?a=2815&q=376864. Since chestnut trees cannot be propagated by rooting cuttings, the scions of the cultivars must be grafted onto suitable chestnut root stocks. Confusion in naming trees ("But I got that scion wood from my friend Joe and he said it was cultivar 'Enormous'!") has resulted in misunderstandings about the characteristics of the cultivars. Using DNA markers, Jeanne Romero-Severson at Notre Dame has begun to sort out the named cultivars of butternut (46), and when she moved on to cultivars of chestnut, she found 6 distinct types of chestnut trees with the name 'Colossal' in U.S. orchards (J. Romero-Severson, *personal communication*). The location of the ortet is known (M. Nave, *personal communication*), and samples from that tree will help to define the cultivar.

A chestnut growers' association was formed in 1992 as the Western Chestnut Growers, and then expanded as the Chestnut Growers of America (www.chestnutgrowers.com). Meetings are held yearly to discuss problems and solutions for growers trying to make money selling the nuts. Tests of minerals in leaves and soil were initiated by growers in this group in hopes of finding optimal soil nutrients for good nut production. No clear patterns have yet emerged, but tests are still being made on orchard and forest plantings monitored by CAES. Progress in improved nut production and marketing has been rapid in both the Missouri Center for Agroforestry and the Michigan Chestnut Cooperative (www.centerforagroforestry.org and www.chestnutgrowersinc.com).

Insect Problems

Since many of the commercial nut-producing chestnut cultivars are pollen sterile, an orchard of chestnut trees must have well-spaced compatible trees to serve as a pollen source to assure good nut set. The trees need adequate water, protection from deer and from bear. In addition, the grower must be prepared to deal with several insect pests. Ambrosia beetles have become a serious problem in the southern United States (66), and two different weevils (*Curculio sayi* and *C. caryatypes*) lay their eggs through the ripening burs (21,83). The eggs hatch when the nuts are released from the burs and the weevil larvae eat their way out of the nuts. The presence of "little white worms" among the nuts will keep customers from buying them again. Chemical control is possible, guinea fowl allowed to forage under the trees can significantly reduce the weevil population, or the freshly harvested nuts can be given a hot water dip to kill the eggs. This pest must be controlled or the nuts will be unmarketable. None of the species of chestnut have been shown to be completely resistant to weevil infestation, so no directed breeding for resistance has been possible.

The Asian chestnut gall wasp *Dryocosmus kuriphilus* was brought into the United States in 1974 by a grower in central Georgia who brought twigs of Japanese chestnut from Japan to graft in his orchard (67). Its spread has been slow, but its distribution now includes many eastern states. This insect lays its eggs in developing leaf and flower buds, and when the eggs hatch, gall formation is initiated, resulting in death or deformation of the leaves or flowers. Drastic reduction in the nut crop can result. *D. kuriphilus* is parthenocarpic and reproduction is rapid. No pesticides have been found to be effective for control, but several parasitoids are being studied at the University of Kentucky (24). Breeding for resistance to this insect may be possible using American and Chinese chinquapins, *C. pumila*, *C. ozarkensis*, and *C. henryi*. Their resistance has not been thoroughly studied, but individuals have been observed to be free of infestation in plantings with other chestnut species that were heavily infested (8). A group of 93 CAES trees from 1993 crosses of *C. dentata* × (*C. ozarkensis* × *C. mollissima*) were planted in North Carolina where gall wasp was established. After 14 years, the 36 survivors clearly had some resistance to gall wasp infestation, and renewed effort was put into breeding nut cultivars with chinquapins for resistance to gall wasp (12).

Future Possibilities

A giant step forward in understanding the genetics of chestnuts was taken in 2006 with a grant from the NSF. This multi-institutional project was dedicated to the development of genomic resources for the *Fagaceae*, including chestnut. All of genomics resources developed by the now-completed project are available at the Fagaceae Genomics Project (FGP) website (www.fagaceae.org). These include ESTs (expressed sequences tags, the DNA sequence from functional genes) and EST-SSR (EST sequences with simple sequence repeats) and annotation for the ESTs. The website also has a listing of available resources and data mining tools. Crosses between two F1 (CAES trees in Hamden, CT) that were American × Chinese with the same Chinese parent and different American parents produced seedlings that were grown by TACF in Virginia. Crosses onto the same Chinese parent with pollen from another highly blight-resistant Chinese tree produced seedlings planted in Connecticut. Additional Chinese seedlings were made with a tree at the TACF farm in Virginia and planted there. With the genomic resources from the FGP, and DNA from the trees planted in CT and at TACF, collaborators at Clemson and at the United States Forest Service constructed a genetic map and a physical map for Chinese

chestnut (*C. mollissima*). The physical map was developed from two BAC libraries constructed using partial digestions with *Eco*RI (10× coverage) and *Hin*dIII (11× coverage). Information from this project and from TACF inoculations of segregating chestnut progeny suggest that three genes, on different chromosomes, are responsible for the resistance of Chinese chestnut trees to chestnut blight.

Since American chestnut trees can now be kept alive using biological control by hypovirulence in orchards where they have no serious competition, it is much easier to carry out a breeding program than when chance-blooming American chestnut trees in the woods must be sought. My plan for restoration of timber chestnuts in Connecticut involves using multiple hypovirulent strains of *C. parasitica* to treat American chestnut sprouts in the forest after a timber harvest, and then planting advanced backcrossed ("nearly American") hybrids among them. The next generation of seedlings in the plot will have resistance genes from the planted trees, and all the diversity that evolved in that small population of American chestnuts. Succeeding generations will allow natural selection of the chestnut trees most fit for that site.

Selections continue to be made in Connecticut for better orchard as well as timber trees (10). Advanced seed orchards of backcrossed timber trees with resistance from Chinese and from Japanese chestnut will allow us to make restoration plantings in eastern forests. Information gleaned from the Fagaceae project will help us to focus future breeding to more effectively develop the kinds of trees that we need. The work will continue, and the renewed interest in chestnuts should allow cooperation with many people and institutions to speed our progress toward usable chestnut timber stands and a new nut market in the United States.

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The author bagging female flowers on an American chestnut tree. Photo by Pamela Sletten.

Sandra Lee Anagnostakis

Dr. Anagnostakis was born in Coffeyville, KS, and attended college at the University of California at Riverside, where she received a Bachelor's degree in the spring of 1961. In graduate study at the University of Texas at Austin, she worked with C. J. Alexopoulos in mycology studying the genetics of slime molds. After receiving a Master's degree in botany, she joined the staff of The Connecticut Agricultural Experiment Station in the Department of Genetics (1966). She completed her Doctor of Agronomy degree at Justus-Liebig University in Giessen, West Germany in 1985, working with Professor J. Kranz. Sandra has worked on the genetics of various fungi, including those that cause corn smut disease and Dutch elm disease. She has been working on chestnut blight disease (caused by *Cryphonectria parasitica*) since 1968. After completing basic studies with the fungus, she imported Hypovirulent (virus containing) strains from France (1972) and demonstrated that they could be used in the United States for biological control of the disease. She has worked on the ecology of the blight fungus and its control by hypovirulence, and on studies of virulence in the fungus and resistance in the trees. She continues the Experiment Station project on chestnut tree breeding experiments to produce better timber and orchard trees. She is an Agricultural Scientist in the Department of Plant Pathology and Ecology.