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CHESTNUT RESTORATION FOR CONNECTICUT

Chestnuts in Connecticut's Early Forests
Connecticut was heavily forested in 1600, but by the early 1800's, forest covered only about 20% of the state. Now trees have grown up on land no longer farmed, and we are back to about 60% tree cover. In 1910, when chestnut blight disease started killing our chestnut trees, half of the standing timber was chestnut and there were about 130 million mature American chestnut trees in the state. Chestnut was the only wood used for telephone poles and most of the railroad ties were chestnut. The trees were tall and straight, and after clear-cutting, they easily out-competed the other hardwoods to dominate the forests, making pure stands.

American chestnut, *Castanea dentata,* is native from southern Maine to northern Georgia, all along the Appalachian mountain range. In the early 1800's, trees in the southern coastal part of this range were killed by ink disease. This disease is caused by *Phytophthora cinnamomi*, and is still present in that region (6). Fortunately, this organism cannot overwinter in Connecticut, and so is rarely a problem here. However, chestnuts in Connecticut and other areas continue to be challenged by another disease, chestnut blight disease. The chestnut blight fungus, now called *Cryphonectria parasitica* (Murr.) Barr, entered the U.S. on infected Japanese chestnuts that were first imported in 1876. Japanese chestnut trees were also available from catalogs, so mail-order movement of infected trees helped to spread the fungus. Insects and small animals that walk over the cankers also helped to spread the disease (5, 6). Chestnut blight disease reduced the American chestnuts to understory shrubs, which die, sprout from the base, die, and sprout again. The fungus is now present throughout the original range of *C. dentata*, and has spread to many areas of the Midwest where chestnuts were planted.

The Experiment Station and Chestnuts
The first plant pathologist at The Connecticut Agricultural Experiment Station (CAES), George Clinton, studied the progress of chestnut blight disease through our native chestnuts. The pathogen was described, and the species was eventually named *Endothia parasitica* by another CAES scientist, Paul J. Anderson.

Chestnut Breeding in Connecticut
Chestnut breeding work began early in the U.S., but the only program that has continued without interruption is the program at CAES. In 1930, Arthur Graves made his first crosses of American and Japanese chestnut, and began a long collaboration with CAES geneticist Donald Jones. Graves gave CAES about 9 acres of

his land in Hamden, CT with plantings of species and hybrids, to insure the continuation of Connecticut's chestnut breeding program. Graves' students Hans Nienstaedt and Richard Jaynes of Yale University, made many of the hybrids that are still part of the current breeding program.

My early work at CAES included studies of the basic genetics of the blight fungus (and the system of vegetative compatibility that restricts hyphal fusion and the transfer of biocontrol viruses from one strain to another) and tests of extracellular enzymes produced by the fungus (1, 2, 14).

Our breeding plan was first based simply on making hybrids of blight-resistant Asian trees with susceptible American trees and testing the hybrids for resistance to chestnut blight disease (4). When it became clear that at least two genes were responsible for this resistance, we began a back-cross breeding program based on the plan of Charles Burnham (8). Asian trees are crossed with American trees, and the hybrids (partially blight-resistant) are crossed to American trees again. If there are two resistance genes, one out of four of the progeny from these back-crosses have one copy of both resistance genes, giving them partial resistance. If there are three genes for resistance, one out of eight of the progeny will have one copy of all three resistance genes. Trees with partial blight resistance are crossed again to American trees. Repeated back-crossing increases the percentage of American genes in the hybrids, and selecting for partial resistance insures passage of the resistance genes. A final cross of two trees with partial resistance should result in one of sixteen trees having two copies of two resistance genes (or one of sixty-four trees having two copies of three resistance genes), which will make them fully resistant to the chestnut blight fungus (8). In order to control pollination, female flowers are bagged in late June to protect them from pollen; selected pollen is subsequently put on the flowers in July and the bags are closed. Many hybrids are male sterile—catkins form, but the flowers never bloom to produce pollen. This is only seen in interspecific hybrid trees, but is a feature valued by nut growers who want to plant orchards of male sterile trees with a few pollen-producing trees for yields of nuts that are uniform.

CAES has what is probably the finest collection of species and hybrids of chestnut in the world for use in this breeding program; seven *Castanea* species are represented. The breeding program will be greatly helped by studies using molecular genetics, which are currently underway (12). Trees of two kinds are being chosen: for timber (tall and straight, with little energy put into forming nuts) and for orchard or nut production (short and spreading with maximum energy put into forming large, good-tasting nuts). Both kinds of trees must have resistance to chestnut blight disease and be well-adapted to our climate. We are also starting to select our trees for resistance to ink disease, caused by the root pathogen *P. cinnamomi*.

Biological Control of Chestnut Blight Disease

In the late 1950's, a chestnut recovery phenomenon was discovered and studied by Jean Grente in France. He called the system "hypovirulence," because the chestnut blight fungus that he isolated had less than normal ability to kill chestnut trees, and the "fungal sickness" could spread. We found that this is due to a viral infection of *C. parasitica* (6, 7, 9, 10) that is transferred from strain to strain when the hyphae fuse. The genes of

three kinds of these (dsRNA) viruses have now been sequenced, and the viruses placed in the genus *Hypovirus* by Bradley Hillman and his collaborators (11). In Connecticut, hypovirulence can keep trees alive in the forest and orchards, and we have studied the many kinds of organisms (e.g., birds, squirrels, ants, and beetles) that move both killing and curing strains of the fungus from tree to tree (3, 6). There has not been a general recovery of forest chestnuts in Connecticut, but 10 to 15% of American trees are kept alive and flowering.

Synthesis of Breeding and Biological Control

American chestnuts

Chestnut seeds of four kinds of hybrids were planted in Griswold at the Connecticut State Nursery in the spring of 1998. The resulting 500 chestnut trees were lifted in the spring of 2000 and 100 of them were planted in a clear-cut in Prospect on land owned by the Town of Prospect and managed by the Connecticut Water Company, and 25 were planted in a clear-cut at Sessions Woods Wildlife Area in Burlington. These trees are being evaluated for survival under forest competition conditions. Of the remaining seedlings, the 200 best were planted in an orchard at the CAES laboratory and farm in Windsor. The Windsor trees with the best timber form, hardiness, and blight resistance have been selected over the years since planting, and the rest have been removed. There were 50 trees left in 2006 and their offspring were planted in 2007 in the Goodwin State Forest, in the Farmington Town Forest, at Windsor, and on private land in Connecticut and New York. In the fall of 2007, we sent 2,500 nuts from the best of the Windsor trees to Georgia to be raised for us in a commercial nursery. The resulting trees were returned to us in the spring of 2009 and represent the best of our selections. We planted 780 of these in Griswold at the CAES Griswold Research Center, formerly the Connecticut State Nursery. These will serve as our "seed orchard" for producing timber chestnut trees for the forests of Connecticut. The rest will be planted in state and private forests throughout the Northeast.

Ozark chinquapin

Ozark chinquapins (*Castanea ozarkensis*) are timber trees found on the Ozark Plateau in Oklahoma and Arkansas. They have a single nut in each bur, as do the Allegheny chinquapins (*C. pumila*) and Chinese chinquapins (*C. henryi*). Chestnut blight disease has recently reached their native range on the Ozark Plateau, and this disease, in combination with forest fire damage and land disturbance, seriously threaten their survival. We have started testing Ozark chinquapins for resistance to chestnut blight. We have 65 of these from Oklahoma and Arkansas ranging in age from 6 years old to 73 years old. In addition, we have many hybrids of *C. ozarkensis* crossed with various Asian chestnuts and ranging in age from 5 years old to 50 years old. Since these hybrids have already been evaluated for form, similarity to *C. ozarkensis*, and resistance to chestnut blight, they will be useful in a back-cross breeding program to improve the fitness of the chinquapins. We have been crossing our Ozark chinquapins with each other and with Chinese chinquapins, Japanese chestnuts, and hybrids, and these will be back-crossed to *C. ozarkensis*. Using all of these methods we expect to produce trees that will have a better chance of surviving in their native habitat.

The Next Problem

Of course, no project is ever quite "finished." The Asian chestnut gall wasp, *Dryocosmus kuriphilus*, was introduced into the U.S. in 1974 by a grower who evaded

plant quarantine (13). The insect lays its eggs in leaf and flower buds, resulting in defoliated trees with no flowers. Entomologist Jerry Payne chronicled the devastation of orchards of Chinese chestnut trees planted in the state of Georgia. We have reports of infestations throughout Alabama, North Carolina, and into Tennessee, and now in Ohio and Pennsylvania. Unfortunately, now that the insect has reached the part of Tennessee where most of the mail-order companies get their chestnut trees for retail sale, it is possible that gall wasp will be inadvertently shipped all over the United States, just as the blight fungus was. Our breeding work must now include selection for resistance to Asian chestnut gall wasp. Jerry Payne has observed that American and Chinese chinquapins (*Castanea pumila, C. ozarkensis,* and *C. henryi*) resist infestation, and some cultivars of *C. crenata* have some resistance. Once again, our collection of species and hybrids is being used to make new progeny for testing in North Carolina where the insect is now endemic. Preliminary results are encouraging. Of 93 trees planted in 1995, 36 have survived the droughts, chestnut blight, deer, rabbits, and weed competition for 14 years. Among the survivors, 30 had no wasp galls and 6 had few galls. We hope to understand how resistance is inherited and will incorporate this resistance into our trees as quickly as possible.

Project Logic

The crosses that have produced blight-resistant trees for timber have, by necessity, used a rather narrow genetic base, even though different trees were used as parents in each generation. Since the native populations of American chestnuts in Connecticut continue to sprout, by using our biological control, we will be able to keep many of them alive and flowering. The same is true of the Ozark chinquapins in Oklahoma and Arkansas. Now, if we plant resistant trees in the forests where native trees survive, natural crossing will incorporate blight resistance, ink disease resistance, gall wasp resistance, and all of the native genetic diversity into the future generations. The first generation offspring will be intermediate in resistance, but in subsequent generations, trees with full resistance to these problems can be produced.

Since we now live in a world where travel and transport of pests and pathogens is all too easy, global communication and cooperation is our hope for the future.

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