Fighting the Spreading Chestnut Blight

Researchers are optimistic that a viruslike disease of the blight-causing fungus may eventually spell salvation for the American chestnut

Will the blight end the chestnut?
The farmers rather guess not.
It keeps smoldering at the roots
And sending up new shoots
Till another parasite
Shall come to end the blight.

Robert Frost penned these lines in the 1930's, and 20 years later the promised parasite turned up in Italy. There viruslike agents have infected the blight-causing fungus, weakening it enough to bring chestnut blight into remission. French plant pathologists imported the strains of sick Italian fungus and used them successfully to control blight in French chestnut orchards.

These encouraging results from Europe have yet to be repeated in the United States. Researchers have brought the weakened strains across the Atlantic and use them to cure individual blight infections, known as cankers, on American chestnut trees. But so far efforts to establish the sick fungus—to have it and its parasites spread naturally to heal untreated cankers and trees—have failed. In trying to understand and reverse the failure, plant pathologists have learned a lot about the American chestnut (*Castanea dentata*), the blight fungus (*Endothia parasitica*), and the viruslike agents infecting the fungus.

American researchers' hopes for a cure for chestnut blight were kindled about 10 years ago, when Jean Grente and Suzanne Berthelay-Sauret of France's National Institute for Agricultural Research in Clermont-Ferrand reported success in treating blighted European chestnut trees (*C. sativa*). Grente and Berthelay-Sauret used weakened, "hypovirulent" fungus strains obtained from the recovering Italian trees to inoculate cankers on a few trees in French orchards. The native fungus caught the disease carried by the imported strains and the treated cankers healed. Researchers have yet to isolate the disease factors, but infer that they may be viruses. They are associated with double-stranded RNA, a common diagnostic of viruses that infect fungi. When a sick fungus fuses with a healthy one, the viruslike agents can enter the healthy fungus, thereby making it hypovirulent.

To Grente's satisfaction, even untreated cankers in the treated orchards started to recover. "Hypovirulent strains . . . spread without any assistance. After 10 years the stand [of chestnuts] can be completely healed," says Grente. He achieved the best results by inoculating about ten cankers per hectare (10,000 square meters) of chestnut orchard.

"If we had a hectare of chestnut trees here and went in and treated ten cankers, in 3 years most trees would be dead," says William MacDonald of West Virginia University. The problem American researchers have come up against is that the weak fungus and its viruslike agents do not spread to untreated cankers and other trees as they do in Europe. The treated canker recovers, but another canker kills the tree. "If hypovirulence had not worked so well in Italy, we would have trouble convincing ourselves that it was possible" for weakened strains to take over the blight, says Sandra Anagnostakis of the Connecticut Agricultural Experiment Station (CAES) in New Haven.

Many factors seem to conspire against a cure for blight in the United States. For one thing, American chestnut is naturally very vulnerable to the fungus. For another, the existing trees are small saplings, which can succumb in a season. And the fungus has become harder to fight, because it has diversified since sneaking into this country late last century as a stowaway on a shipment of oriental chestnut trees.

The diversification of the fungus has produced a block to the spread of the viruslike agents here. Not all strains of fungus can fuse easily with each other, yet individuals must merge for the parasites to be transferred. Anagnostakis has counted more than 80 types of *E. parasitica* that are, to a greater or lesser degree, incompatible with each other. Several types may be present in any small grove of saplings. Moreover, MacDonald has found that different cankers on one tree are more likely than not to be infected by strains that cannot fuse easily with each other. In Europe there are fewer varieties of *E. parasitica*, and those in any given locale tend to be compatible.

To overcome the problem of having to treat a canker with a hypovirulent strain carefully matched to the infecting strain, Richard Jaynes and John Elliston of CAES have tried using a mixture of hypovirulent strains. Such a mixture "appears to overcome the incompatibilities like a synergism," Elliston explains. The viruslike agents are transferred to the infecting strain, "and the canker stops dead in its tracks," he says. Often the tree closes the treated wound and sloughs off the infected bark.

Researchers suspect, however, that simply curing cankers may not be the best approach to reestablishing the American chestnut as an important tree in eastern forests. A cured canker is exactly that: a scarred but healthy patch on the tree. It does not harbor a colony of sick fungus long enough to serve as a reservoir from which hypovirulence can spread to other cankers.

Plant pathologists are coming to the view that active, large, but superficial cankers may be one key to fighting the blight. Such infections are common in Italy, and they are thought to provide a continuous source of the viruslike agents that keep blight in remission there. In superficial cankers, the hypovirulent strains thrive, but do not penetrate the vital inner bark tissues of the tree. In the normal course of blight the fungus kills the cambium. When the canker is large enough to girdle the trunk or a limb, the tree dies above the site of infection.

American researchers hope to find or develop suitable hypovirulent strains and devise a way to introduce them so that they produce superficial and persistent cankers on American chestnut trees. Toward this goal, Elliston has been studying hypovirulent strains of *E. parasitica* and the viruslike agents that infect them. He has identified at least seven or eight different parasitic agents, many of which have unique debilitating effects on the fungus. In Italy, he con-

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cludes, "many agents appear to work cooperatively to weaken the fungus and control blight."

Some chestnut trees in Michigan may help Elliston reach his goal. Lawrence Brewer of Western Michigan University in Kalamazoo spends his leisure time searching for and inspecting chestnut trees in Michigan. Blight hit that state much later than it decimated the eastern forests. According to Brewer, there are still a few large stands of trees in northern Michigan that have never been infected. Moreover, Michigan chestnuts are large, old trees, unlike the saplings that continue to sprout in the East.

A large, surviving chestnut tree in Michigan. [Photo by Lawrence Brewer]

But the big news from Michigan for Elliston and other blight fighters is that "in about 20 places the trees seem to be healing naturally," according to Brewer. Four years ago CAES researchers isolated the fungus from some recovering trees near Rockford, Michigan, and found that some of the strains there are hypovirulent. More recently, hypovirulent strains have been obtained from large, superficial cankers on trees in Tennessee and Virginia. Elliston has found perhaps four or five viruslike agents debilitating these American strains. By analyzing the double-stranded RNA associated with these agents, Allen Dodds of CAES concludes that they are different from their Italian counterparts.

Although in a few cases, superficial cankers harboring hypovirulent strains of *E. parasitica* have developed naturally on American chestnut trees, researchers have thus far been stymied in their efforts to create such cankers. In ongoing field studies plant pathologists at CAES, West Virginia University, and Virginia Polytechnic Institute and State University are testing many hypovirulent strains (and their viruslike agents) and trying many different ways of introducing them in the hope of achieving superficial, persistent cankers to serve as reservoirs of hypovirulence.

A few factors seem to conspire to hamper efforts to establish and spread hypovirulence in eastern forests. One problem is that sick strains do not reproduce as vigorously as healthy strains do. Normally *E. parasitica* procreates both sexually and asexually. The sexual spores spread readily, borne by wind. But sick strains usually do not produce sexual spores. To disperse in the forest, weakened strains must rely on rain, insects, or birds to carry asexual spores or bits of fungus tissue to a new site. Wayne Weidlich of Michigan State University thinks that asexual reproduction may be adequate to spread the hypovirulent strains in Michigan, where in places "chestnut trees are the dominant features in the landscape—visited by lots of insects and birds." But he doubts that "eastern chestnuts [shrubs] in the forest understory will be visited by many vectors to spread the fungus." Another block to the spread of hypovirulence is that not all offspring of a hypovirulent fungus are themselves hypovirulent.

Many researchers believe that hypovirulence would have a better chance of becoming established if American chestnut trees were bred to be less susceptible to blight. *Castanea dentata* is so vulnerable to blight that the small saplings frequently die within a season of catching the disease. There is scant time for a weakened strain to infect and debilitate the deadly one before the tree succumbs.

"If you have a very resistant tree and a pretty sick fungus, you can go with it," says Eyvind Thor of the University of Tennessee, Knoxville. Although, as Weidlich puts it, "there is no such thing as a blight-resistant American chestnut," both Thor and Gary Griffin of Virginia Polytechnic assert that some trees seem to fight the blight better than others. But such trees are too few and far apart to breed in the wild. (Chestnut trees do not pollinate themselves.)

Griffin, cooperating with John Elkins of Concord College in Athens, West Virginia, and Bruce Given of the West Virginia Department of Agriculture, and Thor have independent breeding programs. They are trying to improve the resistance of American chestnut by propagating and crossbreeding trees that seem to be less vulnerable than most. Breeding experiments on trees are slow to bear fruit, however. Thor has been working for 20 years, and he suggested that *Science* call back in 30 to 100 years, "when we might have made a lot of progress."

Following a slightly different tack, Jaynes is hybridizing American chestnut with its oriental cousins, because the latter are much more resistant to blight. Ten years into the effort "some seedlings are displaying desirable features, but it is too early to know what will be their fate as far as blight resistance, shape, and nut quality go," he says.

Unfortunately, *E. parasitica* finds American chestnut bark particularly to its liking. Elkins recently isolated and identified one component of the inner bark that may be a major factor in the species' extreme vulnerability to blight. That substance is a tannin, hamamelitannin, that American and European chestnut bark are rich in, while resistant oriental chestnuts lack it. "*Endothia parasitica* is able to use hamamelitannin rapidly as a source of food. The fungus grows well on a medium that has the material as the only source of carbon," says Elkins. According to Frederick Hebard of Virginia Polytechnic, *E. parasitica* grows so rapidly on American chestnut bark that it appears to act as a driven wedge, cracking through a lignified layer that the tree forms in a futile effort to heal the wound and fight the fungus.

That European chestnuts are slightly better off than *C. dentata* is ascribed by Elkins to a chemical similarity with oriental chestnuts. Elkins hopes that his analyses of chestnut bark will eventually lead to a chemical test for blight resistance. Such a test would be valuable, for instance, to researchers trying to select the most resistant seedlings early in a breeding program.

By encouraging the viruslike agents that afflict the fungus and by strengthening the American chestnut, "we are just helping nature speed up the selection process," says Thor. Contrary to the attitude that prevailed 60 years ago, when foresters gave the American chestnut up for lost, researchers today are full of hope. In time, the chestnut may start to thrive in eastern forests again.

—BEVERLY KARPLUS HARTLINE