NEW DEVELOPMENTS IN CHESTNUT RESEARCH

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The American chestnut (*Castanea dentata* Borkh.) was lost as a dominant tree in our Eastern forests so long ago that most of us can hardly imagine a forest composed largely of chestnut, Furthermore, it it difficult to comprehend the full economic and ecological effects that resulted from the demise of this species as a major component of our forests.

The history of research on chestnut is long but not notable for its successes:

A. Quarantine of the introduced fungal parasite (*Endothia parasitica* Murr, Anderson) was initially given major emphasis, but clear cutting and burning of host plants proved impractical in limiting spread by the wind borne spores.

B) Fungicides and other chemicals were ineffective in controlling the disease.

C) Introduced species of chestnut were either not resistant or not good forest trees.

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D) Other native or introduced species failed to fill the ecological niche with products as valuable to man.

E) Highly resistant American chestnut trees with desirable growth and form were not found,

F) Breeding disease resistance from species such as the Japanese (C. crenata Sieb. & Zucc.) and Chinese (C. mollissima Bl.) chestnut into a tree that is otherwise like the American has not yet succeeded because of linkage and polygenic inheritance of the important characteristics.

With this discouraging introduction let me now summarize three areas of chestnut research which, singly or combined, may significantly affect our future use of chestnut trees.

ASEXUAL PROPAGATION BY ROOTING CUTTINGS

Successful rooting of chestnut cuttings was first described by Shreve and Miles (1972) and the method found effective with several hybrids by Jaynes (1974). Success depends on the use of cuttings of sprout origin either from the base of the tree or from large limbs that have been cut back. Greenwood cuttings 12-20 cm long are taken with leaves nearly or just recently fully expanded. They are lightly wounded near the basal end and dipped for 1 to 2 sec. in 5,000 to 8,000 ppm indolebutyric acid dissolved in 95% ethyl alcohol and placed in an intermittent mist propagation bed. The medium is 2 parts peat to 1 part coarse perlite, Roots generally appear in 3 to 8 weeks with 75% rooting common for several clones.

Considerable refinement may be needed for commercial application of the rooting technique to obtain uniform rooting, high survival, and subsequent flushing. For instance, there are clear differences among clones for rooting and later survival. Thus choosing selections to propagate will be important. However, the significant point is that for the first time a method has been demonstrated to give excellent rooting and survival of several chestnut clones Asexual propagation of selected American or hybrid clones from cuttings can now be given serious consideration.

CHESTNUT BLIGHT CONTROL WITH A SYSTEMIC FUNGICIDE

Since the discovery of the chestnut blight fungus in New York City in 1904 there has been no means to prevent infection of specimen American chestnuts growing within the natural range. (Isolated trees in the Midwest and West have survived as a results of no inoculum; they are escapes rather than resistant trees,)

Recently Jaynes and Van Alfen (1974) reported that the growth of Endothia parasitica is reduced in trees pressure-injected with a solution of Lignasan (Methyl 2-benzimidazolecarbamate hydrochloride or MBC). This is the same material that is being injected into elm trees to successfully control the Dutch Elm disease (Ceratocystis ulmi Buism. C. Mor.). Lignasan is most effective with American chestnut trees if injected before infection occurs. Experiments in progress should determine if infected trees can be cured and then kept free of disease with annual injections. There is significant fungistatic activity of the material in bark and branches 9 months after injection, Thus for the first time we have a chemical means of protecting and possibly curing chestnut trees susceptible to Endothia _parasitica_.

BIOLOGICAL CONTROL OF CHESTNUT BLIGHT

Biological control of a plant disease may sound like science fiction but we have evidence that an altered form of _Endothia parasitica_ can inactivate the virulent form (Van Alfen et al., 1975). Our original strain was obtained from Grente in France who isolated it from stands of the blight-susceptible European chestnut (C. _sativa_ Mill). He called it hypovirulent because of its near avirulent growth on trees usually susceptible to _Endothia parasitica._

Van Alfen et al. (1975) determined that hypovirulence was caused by a cytoplasmic determinant that is transferred to hyphal anastomosis in host tissue and in culture Transmission of this determinant limits host invasion by virulent isolates and cures established cankers in host trees.

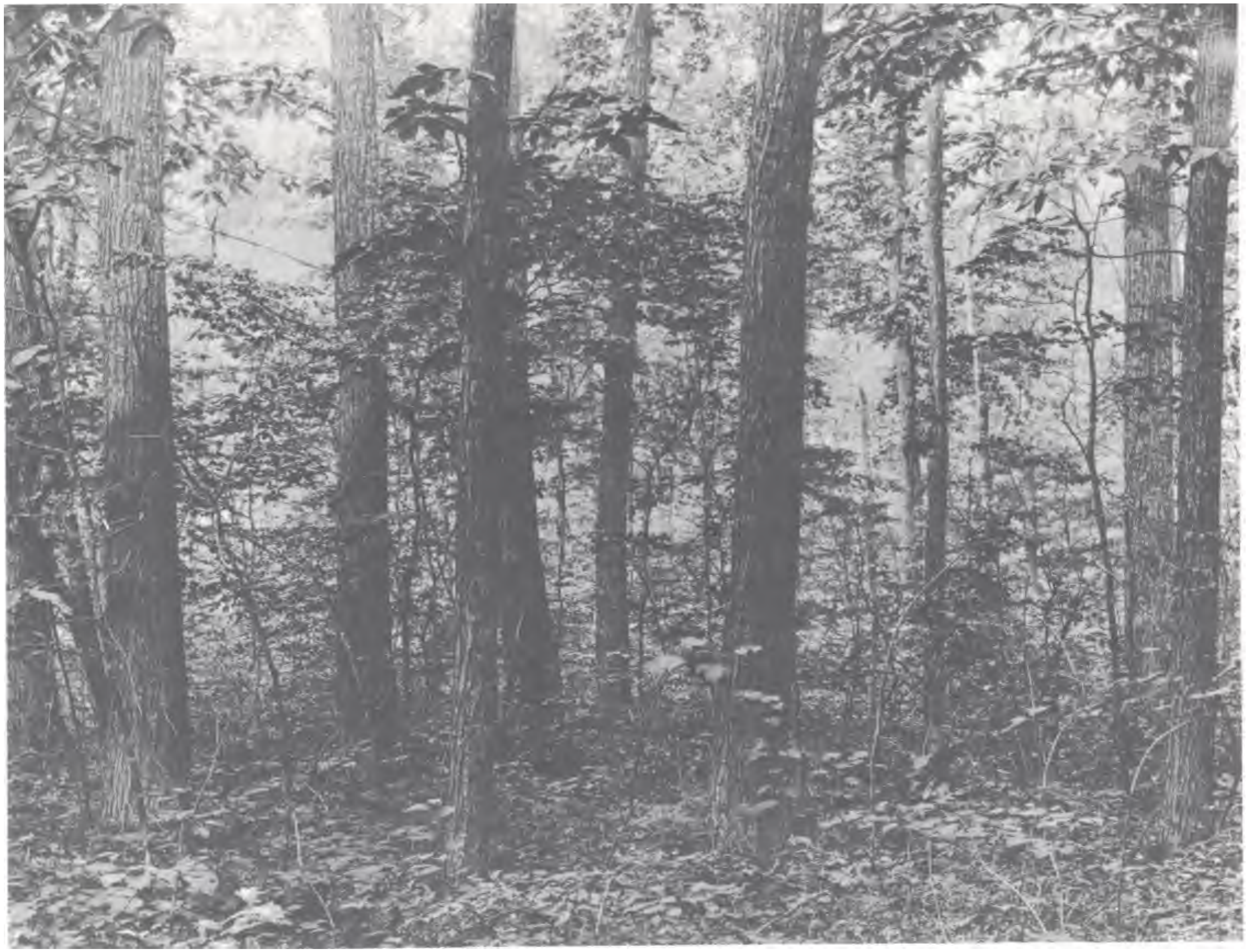
Our initial experiments used American chestnut seedlings under controlled conditions in the greenhouse and field. During the dormant season of 1974-1975 twelve plots were established in native Connecticut woodlands. Each plot contained 25 native sprout clumps each with one or more stems 1" dbh or larger. In half the plots naturally occuring cankers were inoculated with the hypovirulent strain, Results are preliminary but impressive. Average diameter growth of cankers in the control plots from June 18 to July 23, 1975, (5 weeks) was 3.0 cm whereas cankers inoculated earlier in the year with hypovirulent showed a _decrease_ in diameter over the same 5 weeks of 1.2 cm; that is, an actual healing from the edges of the canker, Such remission is startling when one realizes our inability to exert any significant control over such cankers for 70 years and because control is brought about by another strain of the same fungus.

Whether hypovirulence will establish itself and spread to developing cankers on other American chestnut trees in our native forests is yet to be determined. We are hopeful. In the meantime cankers can be controlled by inoculating them with the hypovirulent strain. Regardless of ultimate success in widescale control of the chestnut blight fungus in our native forests these results have opened up a whole new area of inquiry: can we generate hypovirulent forms of other fungi to control other serious plant diseases?

SUMMARY

Recent results suggest that re-establishment of chestnut in the fields and forests of eastern United States may, indeed, be in the forseeable future. Chestnut selections can be propagated asexually by rooting cuttings. Susceptible trees can be protected with a systemic fungicide, Virulent cankers can be controlled by inoculating them with a hypovirulent strain of _Endothia parasitica._

Second growth American chestnut prior to introduction of the Chestnut blight fungus.



Pure stand of American chestnut at Scotland, Connecticut, 1905, prior to infection by the chestnut blight fungus.

LITERATURE CITED

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DISCUSSION

Cameron - A question for Dr. Holmes. Those elms that survived your treatments, do you ever expose any of those to beetle attack?

Holmes - It was natural in the state of our nursery. We have difficulty with beetles coming in and giving the disease to trees that we don't want them to give the disease to. Both where the trees grew first in the nursery and where we put them later, there are large elms nearby and Dutch Elm Disease cannot be removed from the environment. We didn't force beetle attack upon our surviving elms by taking logs containing beetles and leaving them here and there in the nursery, These elms will continue to be exposed to beetle attack where they are standing now. They are rather small trees; and although you can give the disease to one-year-old trees if you inoculate properly (and we have done this in the greenhouse), they tend not to get Dutch Elm Disease from beetles, I don't know why; entomologists might say something about all the beetles flying above the small elms, I don't know. But we sometimes have trees die from natural infection in this nursery, that are no more than 2½ to 3 meters tall.

Schreiner - I'd like to ask if isn't it a fact that age of the seedlings is reflected in resistance?

Holmes - There is a controversy in literature about this.

Schreiner - I was under the impression that there is a change in resistance after about eight years.

Holmes - Well, there may well be. Of course, it would have been nice to have left these trees until we were past their age of controversy, but we needed the space. We get away from that by giving the survivors additional inoculations later. There is a report in literature that you cannot give Dutch Elm Disease to young trees unless you keep them first in the dark for a while, which would appeal to Phil Wargo who is studying stress in trees, and so I tried doing at one time. We got huge corrugated cartons that are used for office furniture and put them over small trees, but later I found it didn't make any difference. In some of the work where I was trying to study the progeny crosses between different cultures of Dutch Elm Disease fungus, I had to use greenhouse facilities in the winter. So I had trees that were only one-year-old and I used a scalpel. I stuck the tip of the scalpel against the stem and held the drop of inoculum there by surface tension on the scalpel and against the stem and then pressed, and the inoculum was drawn in, it was pushed in by the atmospheric pressure, And these trees came down very nicely with Dutch Elm Disease, at the age of one year. This proved to be so with all three sorts of things - rooted cuttings that came from roots, rooted cuttings from shoots, and seedlings. In fact I'm going to be doing this again very shortly. We have a visitor to the Shade Tree Laboratories, Dr. Dilbagh Singh from Blackburn College in Carlinvilles, Illinois, who is studying the effect of drought upon Dutch Elm Disease by growing elms in hydroponic solutions and creating artificial drought by chemical changes in the hydroponic solutions. He just left, to go off to a botanical meeting, leaving me the task of inoculating all these trees as soon as I get back to Amherst from my little vacation. The main thing in inoculating tiny trees in pots is not to go right through the stem with the knife and get it in your finger!

Schreiner - Could I ask one more question? Has climatic control been eliminated as a possibility in rust resistance in loblolly pine?

Hicks - Well, no it hasn't, Dr. Schreiner, and some of the east Texas sources being resistant in east Texas may well be due to climatic factors. That is, we don't have very much rust in east Texas, and it isn't obvious whether east Texas loblolly pine is resistant or whether the disease is not climatically well adapted to our conditions. But I believe the best evidence for genetic resistance is in the fact that these east Texas sources when planted along with sources from Georgia, North Carolina, etc., in provenance studies have shown up consistently resistant as compared with the other sources. But the fact that we have low incidence in east Texas is probably a dual genetic-environment phenomenon. Slash pine, by the way, gets rusted pretty badly in some parts of east Texas, so the fungus is alive and well.

Little - I would like to verify the statement that genetic resistance has shown up in the provenances study: for example, in loblolly pine from the Eastern Shore of Maryland. Incidentally, loblolly pine from Eastern Shore of Maryland may well be a natural gradation to what we call pond pine that is also a source of greater resistance for fusiform rust. In the Eastern Shore provenances planting the local source was much less susceptible than North Carolina or some other sources. In connection with your study, you might mention that Peter Smouse did some work on natural hybridization among pone, pitch, loblolly, and shortleaf pines, published with L. C. Saylor in the Annals of the Missouri Botanical Gardens. and Pete claims that what we call pond pine on the Eastern Shore of Maryland is a gradation between pitch and pond.

Holmes - I wonder if I may make a comment relevant to predisposition to applied stress. We studied this summer the effect of salt-with-*Verticillium,* and salt alone, and *Verticillium* alone, and neither of the two, on sugar maples. And I found my *Verticillium* to be not very potent by itself, because I could not see more symptoms on the *Verticillium*-tested trees than on the control trees. The salt alone showed some symptoms, but the trees with salt AND ALSO with Verticillium added to the soil (later) were KILLED! So it looked like the *Verticillium* was much more severe if there was salt present. There wasn't very much difference in times, a couple of weeks between adding salt and adding the *Verticillium* Wherever we added the *Verticillium* we wounded the roots by cutting down vertically into the soil with a knife in four places but that didn't help cause symptoms where there was no salt.

Ledig - Is there any evidence that trees more resistant to decline have higher levels of glucanase or chitinase activity or suffer less decline after stress?

Wargo - The last slide showed that trees that survive appear to be as high or close to the same levels as the controlled trees, in some cases. We have not gone in and prelooked and then stressed. That is our next step. We are just in the stage of identifying that stress does influence these enzyme systems. And now we would like to go in and see if we can predetermine in terms of enzyme activity. It might not be initial enzyme activity - it might be something like changes in phenolic compounds. We have so many things changing, and one of the things we are finding is a tremendous increase in the amount of phenolics in defoliated trees. This increase alone could be what is causing our differences of activity by inhibiting enzyme activities. We are going to look at pre-stress and then see what happens, The same thing you would be doing with tree vigor.

Schreiner - May I make one suggestion? I think you are not using the best "guinea pig"; why not use poplar clones?

Wargo - I like maple syrups better. It's a reason to go into Vermont and New Hampshire.

Schreiner - With poplar as a "guinea pig" clonal tests have shown that Valsa sordida will be thrown off under good growing conditions. Valsa nivea which grows at lower temperatures and seldom bothers us on a good site in a warmer climate. And this can happen in one or two growing seasons.

Wargo - That is a possibility.

Holmes - Might I say a word - not as much as a defense of maples but for the people who work the maple. Just as elm, maple has been very much planted in cities and towns, partly through the mistaken feeling whereby people feel there must be one particular tree to replace is the elm, when they should have gone to diversity. The maples are suffering very much in the cities and towns. You can find thousands of them with dead tops. So, just as there is a great public demand to save the American elm, there is also a great public demand to find out what is wrong with the maple in our cities and towns. About 1/3 of all our inquiries at the Shade Tree Laboratories are concerned with maple (excluding thousands of routine samples that by law must be collected from wilting elms and must be sent to us).

Schreiner - May I answer that? I agree with you, but for basic research the "public" seldom gives a damn about what you are working with. The use of "guinea pigs" has long been an expected and respected procedure in research.

Wargo - Your point is well taken. We have identified at least a potential system, now we are working with tomato plants, by the same token are even a little easier to grow than poplar.

Holmes - Now that we have gotten to tomatoes, I remember that at the Connecticut Agricultural Experiment Station in the 1950's they were studying chemotherapy and Dutch Elm Disease by the use of tomatoes. All we need to do is to discover a long-lived, shade-giving tomato, suitable for planting along city and town streets.

Schreiner - You may even end up with an elm that bears tomatoes.

Ledig - Did I understand that you had taken these surviving elms, made cuttings, and then planted them out and reinoculated them?

Holmes - I did not mean to suggest that we have gone so far. We have marked the trees from which to take cuttings, and that was done only about a month ago. Up until this point we were submerged in handling the seed, the seedlings, the transplanting, the inoculations, the screening, etc. Now that that is cut off, our attention can more freely be turned to making the cuttings. But this will not be our first effort at making elm cuttings. Recently we have had very poor results with efforts to propagate famous elms for the Elm Research Institute. And yet I have made cuttings years and years ago from Dr. Welch's elm trees (in the early 1950's) successfully. Evidently, I've lost my touch and so I would appreciate the best information from anyone here on what is the true path to success in rooting elm cuttings.

Schreiner - Have you tried layering branches. The method has been used in Europe.

Holmes - That is a good idea, I thought you were going to say is there any chance of using poplars. Yes, that is a very good idea - I hadn't tried that.

Santamour - Sort of a comment, I guess. We have been very successful at rooting elm cuttings in every manner imaginable - at least once, and as Frances said, the next year you try a particular system that worked once, it may not work again. The most consistent success with rooting has been to bring the tree indoors - get yourself some space, and dig the beast up, move into a cool greenhouse in mid-winter and let it start growing much earlier than it would outside. That gives you some very nice succulent shoots and you have 80 to 90 percent success by rooting these, starting very early in the season. Don Lester has used leaf-bud cuttings, whatever they are - I hope he tells us how. Apparently, he is multiplying them like fruitflies - fantastic. There are a number of ways to do it, but you have to get the things consistent.

Holmes - That makes me feel much better.