DETECTION AND EVALUATION OF HYPOVIRULENCE IN AND RESISTANCE TO ENDOTHIA PARASITICA IN SURVIVING AMERICAN CHESTNUTS AND ASSOCIATED OAKS IN NORTH CAROLINA

W. J. Stambaugh and B. L. Nash

School of Forestry and Environmental Studies
Duke University
Durham, North Carolina 27706

ABSTRACT.--Cultural hypovirulence in Endothia parasitica from four American chestnuts (d.b.h. 15.6 to 33.0 cm), two each in Iredell and Alexander Counties is reported. Plans for further evaluation of hypovirulence and/or resistance in these and additional candidate trees, as found, are described.

The search for resistance in large American chestnut, *Castanea dentata*, survivors of the epidemic caused by *Endothia parasitica* now has an added dimension with recognition that some strains of the pathogen are in themselves diseased or, by Elliston's (1981) new terminology, cytoplasmically hypovirulent (CH), as probably caused by virus-like agents (Day et al. 1977). Theoretically, the agent(s) in CH strains can be transmitted via hyphal fusion with virulent (V) strains in normally lethal cankers and reduce pathogenicity to the extent that bio-control and tree survival are attained. Transmission between CH and V strains is governed, however, by their vegetative compatibility (v-c) of which there are 77 known types in North America (Anagnostakis and Waggoner 1981). Despite this low probability for compatible CH and V strains to pair in nature, some chestnuts have survived either by means of CH in the pathogen, resistance of the host, possibly both, or disease escape. At least 20 large surviving American chestnuts in seven states have been shown to yield abnormal (potentially CH) isolates of *E. parasitica* (Jaynes 1981). Such trees need to be assessed for the possible interaction of CH and resistance to their survival.

Candidate trees for this type of study are not likely to be found by conventional survey methods. For example, our systematic survey to determine the incidence of *E. parasitica* on oaks and chestnut in the mountain and Piedmont counties of North Carolina (Nash and Stambaugh this proceedings) did not detect a single chestnut larger than 6.8 cm d.b.h. nor did the isolates from 247 trees of all species sampled show any cultural abnormality or potential CH. Quite by chance, however, in November 1980, we were apprised of a 28 cm American chestnut near Union Grove in Iredell County found by a local lumber mill buyer. Since then, the owner of the property has reserved the tree for our use and has led us to three more study trees within a 4.8 km radius of the first tree (Table 1).

Table 1. Data summary from surviving American chestnuts in Iredell (IR) and Alexander (AX) Counties, North Carolina

Tree number	D.B.H.	Number stems	Cankers height	Tissue samples	Number of isolates[a/] T	SUBC	AB
	cm		m				
IR/1	28.4	18	0 - 11.0	260	127	20	2
IR/2	33.0	14	0 - 7.9	168	140	118	12
AX/1	15.6	7	0 - 7.3	73	61	55	8
AX/2	24.6	5	0.9 - 7.0	49	46	35	13

[a/] T = total isolates obtained; SUBC = growth observed through two to four subcultures; and AB = culture abnormal, i.e. morphologically hypovirulent.

This paper reports our preliminary findings from these trees and outlines our plans to enlist the aid of some 148 hardwood lumber mill operators and buyers (NC Forest Service 1979), and through them, the logging contractors, by asking all individuals thus contacted to report the locations of surviving chestnuts, as found in the 26 counties where chestnut is known to occur (Buttrick 1925; Saucier 1973). From this solicitation and response, we anticipate detection of a sufficient number of additional trees to meet our study needs.

Materials and Methods

Candidate trees will be grouped by localities, as reported, to facilitate ground-scouting. During the first visit, a candidate tree will become a study tree if it meets a diameter minimum of 21.6 cm and is either canker-free or displays sublethal infection. Determination of ownership and permission to sample will precede the second visit and will be scheduled during tree dormancy so that at least 20 scionwood cuttings can be taken simultaneously with procurement of 10 to 20 bark core samples per canker perimeter. Isolates from this material will be screened for within-tree v-c type frequency and distribution by the methods of Anagnostakis (1978) and for the appearance of cultural CH by observation of colony growth through four 9 cm plate subcultures; those isolates expressing cultural CH will be tested against within-tree normal V isolates for conversion capacity, using the methodology of Anagnostakis and Day (1979). Their pathogenicity will be compared with V isolates using paired inoculations of living sprout stems of American chestnut. If CH is indicated by preliminary laboratory findings, the source tree will be revisited to determine whether CH strains have spread from this locus by sampling all trees symptomatic of *Endothia* infection within cardinal-direction transect radii of 152 m. The isolate yields from this population sample will be screened in the laboratory for cultural CH detection and intra-tree v-c type distribution as compared with those present in the source tree.

All scionwood material will be stored at 5 C until March when side grafts will be made to Chinese chestnut, C. *mollissima*, rootstock already planted on two sites in the Duke Forest. Once established, the scions will be inoculated by standardized procedure to determine whether the source tree actually possesses some degree of resistance.

Results

Symptomatology. Tree data given in Table 1 actually represent only three locations since AX/1 and 2 were only 6 m apart. All trees displayed full, healthy crowns with cankering confined to main stems. Canker distribution ranged from ground level to the first few live branches at 7 to 11 m (except for AX/2). Basal cankers were deep seated with pronounced marginal callus. Tree IR/1, the first to be discovered, had the most distinctive and uniform symptoms expressed as roughened, slightly fusiform-shaped swellings (Figure 1). *Endothia parasitica*, in relation to these symptoms, was superficial in the outer bark and necrosis, if any, was shallow. The lowermost canker in tree AX/2 was also unusual in that it extended continuously from 0.9 to 3.6 m and appeared as exposed, roughened inner bark with a definite orange cast. All other cankers were atypical of chestnut infection in that callusing was quite evident.

Figure 1. Lower stem of a surviving, 28.5 cm d.b.h. American chestnut in Iredell County, North Carolina showing fusiform swellings associated with *E. parasitica* infection.

Isolate Yield and Characterization. Cankers on all trees were sampled to a height of about 6 m, i.e. within the safe-use limits of our Swedish climbing ladder. Isolation yield of *E. parasitica* from all canker-perimeter, bark-core samples taken per tree is shown in Table 1. Yields ranged in frequency from 48 to 94 percent, as represented by IR/1 and AX/2, respectively.

Pairing of all within-tree isolates among themselves to determine the number of v-c types is nearing completion with v-c type maxima relative to total isolates (Table 1) reduced to 14 (IR/1), 80 (IR/2), 41 (AX/1), and 38 (AX/2). Observations on colony growth of 20 or more isolates per tree through two to four subcultures, to date (Table 1), has revealed at least 2 to 13 abnormal strains from each, as based on reduced growth, lack of pigment, and irregular colony margin, singly or in combination. An additional 60 isolates were obtained from eight scarlet oaks, *Quercus coccinea*, one white oak, *Q. alba*, and an additional 12.7 cm d.b.h. chestnut, all within 365 m of AX/1-2; none of these isolates have shown cultural abnormalities as yet.

Scionwood Grafts. Thirty-one branch tip cuttings from IR/1 were side grafted to Chinese chestnut rootstock on April 2 and 3, 1981; by June, 58 percent of these attempts were judged successful but this dropped to 13 percent by fall. Scionwood from other sources, namely an 82 cm American chestnut on Carter Mountain in Wilkes County and a susceptible American chestnut sprout in Watauga County, gave slightly better grafting results. Survival this fall, was 3/14 (22 percent) and 5/15 (33 percent) respectively.

Discussion

Our results at this stage are primarily descriptive of symptoms in four large surviving American chestnut trees and suggestive that cytoplasmic hypovirulence in the pathogen may be contributing to their survival.

Certainly, symptomatology as described and particularly as illustrated in Figure 1 is atypical of cankering caused by virulent strains of *E. parasitica*. The superficial position of the fungus in stem swellings and the pronounced callus formation in most other cankers is taken as evidence that either resistance, cytoplasmic hypovirulence, or both are functioning.

Our isolation results from each of the study trees (Table 1) indicated detection of some culturally abnormal isolates of the pathogen. These were usually expressed as drastically reduced growth rates on potato dextrose agar in plate culture and deeply lobed colony margins. We recognize that demonstration of cytoplasmic hypovirulence in these isolates will require substantiation, including: 1) conversion ability in dual culture with V-strain isolates (Anagnostakis and Day 1979) with due attention to cultural stability problems (Van Alfen et al. 1978); and 2) pathogenicity compared with that of V-strain isolates by inoculation and measurement of necrosis in living American chestnut. Our plans for the former will match potential CH-strain isolates with V-strain isolates on the basis of within-tree/canker v-c type patterns once that data is complete. For the latter, all culturally abnormal isolates accumulated by next spring and representative V-strain isolates will be inoculated on American chestnuts within a sprout stand located in Watauga County. Since isolates from the AX and IR trees have shown considerable latent expression of growth abnormality which is not easily detectable in tube culture, we are routinely following colony growth in Petri plates through four subcultures.

If CH-strain presence can be adequately demonstrated in any or all of the AX and IR trees, their accessibility at the extreme northern juncture of the two counties and their proximity to one another within an 8.1 km^2 area, should prove a valuable asset to our studies. In the search for additional study trees, four other American chestnuts in Buncombe, Caldwell, and Wilkes counties, with d.b.h.'s ranging from 29 to 82 cm, meet our specifications and will be examined pending permission from the respective owners.

Literature Cited

Anagnostakis, Sandra L. Testing *Endothia parasitica* strains for vegetative incompatibility. MacDonald William L.; Cech, Franklin C.; Luchok, John; Smith, Clay, eds. Proceedings of the American chestnut symposium; 1978 January 4-5; Morgantown, WV. Morgantown: West Virginia University Books; 1978: 101-102.

Anagnostakis, Sandra L.; Day, Peter R. Hypovirulence conversion in *Endothia parasitica.* Phytopathology 69:1226-1229; 1979.

Anagnostakis, Sandra L.; Waggoner, Paul E. Hypovirulence, vegetative incompatibility, and the growth of cankers of chestnut blight. Phytopathology 71:1198-1202; 1981.

Buttrick, P. L. Chestnut in North Carolina. N.C. Geol. and Econ. Survey; 1925; Econ. Paper 56. 4 p.

Day, P. R.; Dodds, J. A.; Elliston, J. E., Jaynes, R. A.; Anagnostakis, S. L. Double-stranded RNA in *Endothia parasitica.* Phytopathology 67:1393-1396; 1977.

Elliston, John E. Hypovirulence and chestnut blight research: fighting disease with disease. J. Forestry 79:657-660; 1981.

Jaynes, Richard A. Abnormal strains of *Endothia parasitica* associated with large surviving American chestnut trees. (Abstr.). H. Clay Smith. U.S. Forest Service American chestnut cooperators' meeting; 1980 January 8-9; Pipestem, WV; 1981; USDA For. Serv. Gen. Tech. Rep. NE-64. p. 11.

North Carolina Forest Service. Buyers of forest products in North Carolina. 1978; N.C. Dept. Nat. Res.; 169 p.

Saucier, J. R. American chestnut...an American wood *(Castanea dentata)* (Marsh) Borkh.). USDA FS-230; 1973; 6 p.

Van Alf en, Neal K.; Jaynes, Richard A.; Bowman, James T. Stability of *Endothia parasitica* hypovirulence in culture. Phytopathology 68: 1075-1079; 1978.

Acknowledgment

Authors thank Don Rogers and Harvey Barron of the North Carolina Forest Service for field assistance.