BREEDING FOR RESISTANCE TO CHESTNUT BLIGHT AT THE UNIVERSITY OF TENNESSEE

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At one time the Appalachian forests were commonly of the oak-chestnut type. This forest type was dominated by the American chestnut (*Castanea dentata*) which reached 120 feet in height and a diameter of 8 feet. Based on economic factors, C. *dentata* consistently ranked first in importance on many different sites in this region. However, since the introduction of E. *parasitica* around the turn of the century the American chestnut decreased in importance and is presently uncommon in community composition. The initial successional patterns have subsequently been identified (Thor and Summers, 1971; Woods and Shanks, 1959).

The role played by E. *parasitica* in initiating the decline of C. *dentata* and subsequent successional patterns is that of a pathogen. There is still uncertainty, however, as to whether E. *parasitica* is establishing a host-parasite association through physical action of the mycelium or through the use of a marasmin.

BREEDING PROGRAMS

Since the introduction of E. *parasitica* research programs were initiated in hope of getting offspring resistant to the fungus. Interspecific hybridization between C. *dentata* and the resistant oriental species C. *mollissima* and C. *crenata* has not yet given successful results as far as producing resistant forest trees. Therefore, an American chestnut intraspecific breeding program was initiated in 1960 by the Forestry Department at The University of Tennessee. This research was based on the hypothesis that crossing surviving American chestnut trees possessing some resistance to the blight fungus may result in occasional progenies with more resistance than either parent. A number of trees that appeared to have some degree of resistance were selected from Tennessee, north Georgia, and western North Carolina (MacDonald and Thor, 1967). Scion wood was collected from these trees and grafted on Asiatic chestnuts. This program is still underway and to date a total of 40 American chestnut trees have been selected, For the past two years controlled crosses in the grafted orchard have yielded a good harvest of nuts which are now being grown in progeny tests (Thor, 1973).

Another phase of the chestnut breeding program at The University of Tennessee is the irradiation of American chestnut seeds. In the hope of causing a mutation which produces blight resistance, several thousand seeds have been submitted to ^{60}Co irradiation (Thor and Barnett, 1969). Thus far this approach has been fruitless. Even though some trees grow large enough to produce nuts, they seldom get taller than 10 feet before being killed back by the blight.

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BIOASSAY

A major difficulty confronting breeding programs is the lack of a test to determine blight resistance of young progenies. Natural infection occurs in 5 to 25 years; obviously there is a need for a more satisfactory assay procedure, Barnett (1967) recognized the need for early determination of progeny resistance and undertook an investigation of the chemistry of inner bark of chestnut. This study was based on the observation that species of Castanea contain high concentrations of phenolic-like compounds and that cankers also contain several of these compounds, He extracted the inner bark of individual chestnut trees with four different solvents, chloroform, diethyl ether, acetone, and methanol. Chromatographic comparison of extracts from sound and infected bark from the same tree, and from both American and Chinese bark revealed dramatic qualitative and quantitative variation among individual trees. Furthermore, the effect of crude extracts upon the growth of the blight fungus was tested, and the chloroform extract of infected inner bark inhibited in vitro growth of the fungus more than any other extract (Barnett, 1972).

The chloroform extract was further investigated and components of the crude extract were separated by column chromatography into 67 fractions. In the development of the column the polarity of the solvent system was changed by going from pure benzene through diethyl ether, mixtures of diethyl ether-methanol and benzene-methanol (Samman, 1973). These fractions were bioassayed with E. parasitica by a method similar to that of Barnett (1973).

Results of the bioassay indicated the existence of some fungus-inhibiting chemicals that could be extracted (Samman and Barnett, 1973). Also, the test indicated that some fractions collected with a mixture of benzene-methanol from the column are more inhibitory to fungal growth than all the controls (Table 1). The fact that these compounds were eluted with a benzene-methanol mixture indicates that they are highly polar (Samman, 1973).

Table 1.--Effect of five chloroform fractions which significantly reduce in vitro growth of E. parasitica.

Fraction #	Mean diameter	% reduction of growth
67	8.4	44
66	9.2	39
64	10.9	28
65	11.8	22
60	11.9	21

Mean of 7 controls 15.11

CHEMICAL ANALYSIS

Analysis of the fractions on thin layer chromatography (TLC) revealed that these samples were relatively pure. Also, none of the components were positively identified by TLC although many standards, ranging from simple phenols to phenolic acids and flavonid glycosides, were tested on the same chromatogram. Chromophoric reagents, such as ferric chloride and vanillin, both specific tests for phenolics, gave negative results with all the fractions; therefore, the assignment of these compounds to phenolics was not possible.

The most important fraction (number 66, Table 1) is a two milligram sample of yellow oily material. This sample was further investigated and structural analysis was attempted. The infrared spectrum has revealed an absence of any aromatic compound; no absorption bands were observed at 1600 cm^{-1} or at 1500 cm^{-1} where phenyl ring-stretching bands usually are found. Also, C----H stretching is only apparent on the lower frequency side of 3000 cm^{-1} which indicates that only aliphatic stretching is present and no aromatic stretching occurs. An absorption band observed at 3500 cm indicates that a hydroxyl group is present, another band at 1730 cm^{-1} indicates the presence of a carbonyl group (-C=0), probably a carboxyl acid. The possibility of another carbonyl group is also indicated (Figure 1). These observations explain the high polarity of the fraction as indicated by chromatographic data. Proton nuclear magnetic resonance (NMR spectroscopy) confirms these findings and further indicates a lack of aromatic and olefinic hydrogens. High resolution mass spectral analyses of fungistatic oil and silylated derivatives have been less rewarding; unfortunately, there was no identifiable parent ion or high mass signal of sufficient strength for peak matching. However, the mass spectra indicate a long chain hydrocarbon portion which was obscured in the infrared spectra by chloroform contamination.

WORK IN PROGRESS

Determination of a complete structure of fungistatic compounds was hindered by the unavailability of sufficient amounts of bark extracts. Only small trees, a couple of inches in diameter, had been available. Fortunately, an American chestnut tree thirty years old, 45 feet in height, and 9 inches in diameter was located and permission obtained for its harvest (Thor, 1975). The stem had a dozen cankers giving evidence of repeated infections and ability of this tree to restrict the growth of the fungus. This tree provided 1000 grams of infected bark and 600 grams of sound inner bark. Four grams of crude extract were obtained from each sample and they are now being separated into different components by liquid column chromatography. We hope a definite statement regarding the exact chemical nature of fungistatic compounds will be possible following analyses of these components.

Another phase of the chestnut research program at The University of Tennessee is the demonstration and characterization of any toxic substances excreted by E. <u>parasitica.</u> The advance of mycelium into the sound inner bark tissue is usually accompanied by a necrosis of tissue in advance of the mycelium. This observation suggests that the fungus excretes a substance toxic to inner bark tissue. Tissue of C. <u>dentata</u> will be

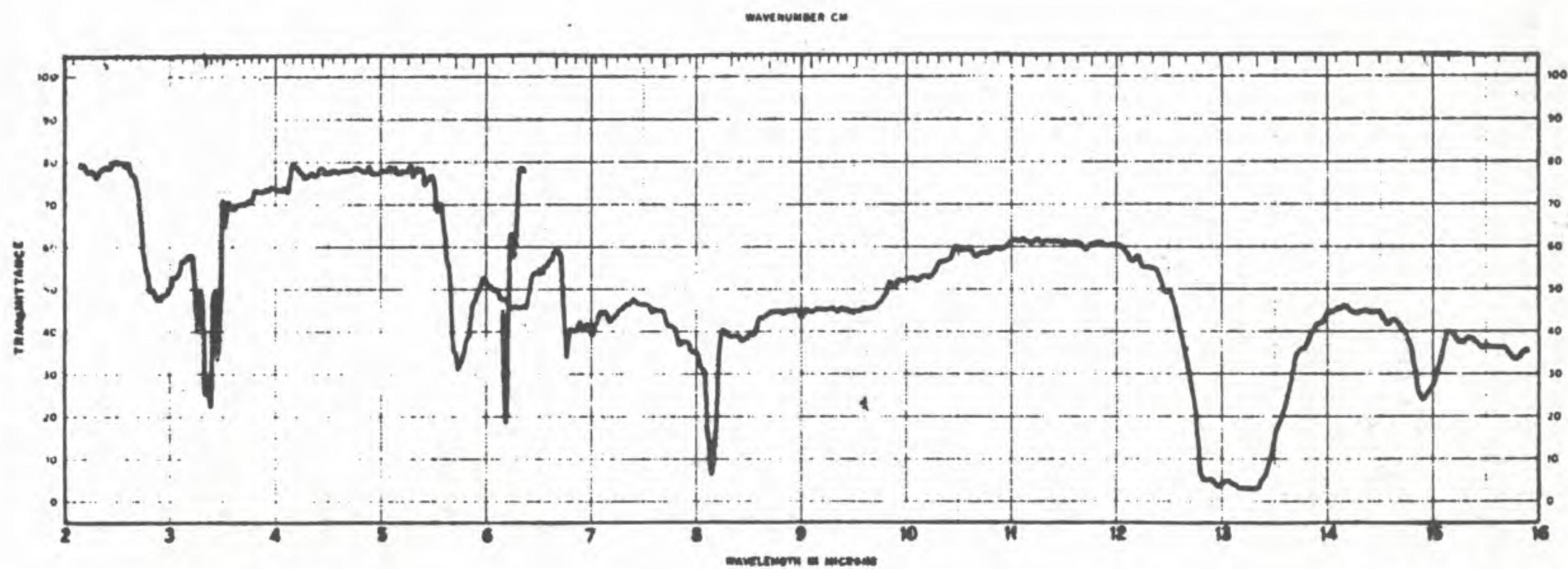

FIGURE 1. The infrared spectrum of fraction number 66, chloroform solvent, taken as thin film.

subjected to excretions of E. parasitica followed by an attempt to detect any subsequent cellular damage. Detection of a toxic effect may allow isolation and characterization of the toxic substance. Culture material of E, parasitica of known origins has been collected and is now included in our tests since several strains of the chestnut blight organism may exist (MacDonald et al„ 1962). We will study variation among geographic strains of the fungus, especially the effect of their excretions on the callus tissue of American chestnut.

Callus tissue of C, dentata, induced and cultured in vitro, will permit a high degree of control over experimental conditions, The best culture media for growing American chestnut callus tissue are being surveyed. Cultures of inner bark sections were started on a Whitmore and Rier medium and on a Marashigi and Skoog plus sucrose, L-cysteine, casiene hydrolisate and ferric citrate medium. Cultures on Whitmore and Rier showed definite growth of callus after two weeks but the callus died back when transferred to a fresh medium; this death of callus on handling may complicate use of tissue culture in bioassay of the fungus. Both liquid and solid phases of each medium are being investigated; liquid cultures are used to obtain friable tissue that could be used for propagation of more callus tissue, while solid cultures are more appropriate for use in bioassays with the fungus.

LITERATURE CITED

Barnett, P. E. 1967, Some problems involved in development of early selection criteria for Castanea dentata trees resistant to Endothia parasitica. M.S, thesis, the University of Tenn.

Barnett, P. E, 1972. A comparative study of phenolics in chestnut (Castanea), and their relationships with resistance to Endothia parasitica. Ph.D. Diss„ The University of Tenn.

Barnett, P. E. 1973. Micro-bioassay method applied to the chestnut blight fungus, Plant Dis, Repts. 57:672-675.

MacDonald, R. D. and E. Thor. 1967. American chestnut in the Southern Appalachians. Jr. of Forestry 65:121-122.

MacDonald, R. D., E. Thor, and J. O. Andes. 1962. American chestnut breeding program at the University of Tennessee. Proc. 53rd Annual Report Northern Nut Growers Assn. pp. 19-21.

Samman, S. N. 1973. Identification of some compounds in the inner bark of American chestnut (Castanea dentata). M.S. thesis, The University of Tenn.

Samman, S. N. and P. E. Barnett 1973. Effects of compounds from chestnut inner bark on the growth of Endothia parasitica. Proc. 12th Southern For. Tree Improv. Conf. pp. 343-346.

Thor, E, 1973, The American chestnut 1973. National Parks and Conserv. Magazine 47(9).9-12.

Thor, E. 1975. Hope for the American chestnut. Tenn. Conserv. 41(7):2-4.

Thor, E. and P. E. Barnett, 1969, Breeding for resistance to chestnut blight, Proc. 2nd World Consult. Forest Tree Breeding 15/3.

Thor, E. and D. D. Summers. 1971. Changes in forest composition on Big Ridge Natural Study Area, Union County, Tennessee. Castanea 36:114-122,

Woods E. W. and R. E. Shanks 1959. Natural replacement of chestnut by other species in the Great Smoky Mountains National Park. Ecology 40:349-361.