EFFECTS OF COMPOUNDS FROM CHESTNUT INNER BARK ON THE GROWTH OF *ENDOTHIA PARASITICA*

Safiya N. Samman and Paul E. Barnett [1/]

Abstract. --Fractions of the chloroform extract from inner bark of American chestnut were separated. Seven of the 25 fractions used in an assay inhibited growth of *Endothia parasitica* in *vitro.* These results provide a basis for further work toward developing a screening technique for blight resistant American chestnut selections and progenies.

American chestnut *(Castanea dentata)* was nearly eliminated after introduction of the fungus *Endothia parasitica* into this country in the late 1800's. Many attempts were made to control the blight. Interspecific hybridization with Asiatic species at one time appeared to provide a solution, but hybrids have not been successful forest trees. Radiation breeding until now has not given promising results. A selection breeding program has been under way at the University of Tennessee for about twelve years, and a total of 40 American chestnut trees have been selected to date. Last year controlled crosses in the grafted orchard yielded a good harvest of nuts and the seedlings are now being grown for a progeny test.

One hindrance in such breeding programs is the lack of an efficient test for screening progenies for blight resistance. A period of ten to fifteen years is required for each breeding cycle. Thus a method for early determination of progeny resistance is needed to accelerate the breeding program providing savings in time, labor, space, and funds.

Barnett (1967) initiated a study of chestnut inner bark, based on the observation that species of *Castanea* contain high concentrations of phenolics (tannins) and that infected cankers also contain a great variety of these compounds.

Barnett (1972) collected and air dried sound and infected inner bark from American chestnut trees. This material was ground in a Wiley mill and extracted in a Soxhlet apparatus. In order to partially separate some of the more polar compounds the material was extracted in sequence with petroleum ether, chloroform, diethyl ether, acetone, and methanol. Thin-layer chromatography disclosed considerable qualitative and quantitative variation in the phenolics of individual American chestnut trees. A dramatic difference was also noted between chromatographic patterns of sound and infected bark samples from the same tree. These differences were further investigated by determining the effect of the crude extracts upon the growth of the blight fungus in a potato-dextrose-agar culture medium.

[1/] Laboratory assistant, Forestry Department, The University of Tennessee, Knoxville, and Botanist, TVA Division of Forestry, Fisheries and Wildlife Development, Norris, Tenn; respectively

Chloroform-soluble extracts from infected American chestnut trees inhibited *in vitro* growth of the fungus more than any other extract. This paper presents preliminary work on the composition and activity of the chloroform-soluble extract from infected chestnut inner bark.

METHODS

Twenty grams of dry ground bark were defatted with petroleum ether and then extracted with 250 ml of chloroform in a Soxhlet apparatus overnight. The extract was reduced to dryness and taken up in 20 ml of chloroform. Five ml of the extract was fractionated with 1000 ml of 9:1 benzene:methanol followed by 1000 ml of 8:2 benzene:methanol, on a silica gel column of activity III by the "dry column" method of Loev and Goodman (1967). However, rather than cutting the column according to their method, 95 fractions, 15 ml each, were collected with an automatic fraction collector. Each fraction was evaporated to dryness under nitrogen and taken up in 4 ml chloroform. Fractions which absorbed light at 290 millimicron were assumed to contain phenolic compounds (Silvestein and Bassler, 1967).

Figure 1 is the elution chromatogram obtained from the column. Twenty-five peak fractions and eight controls of chloroform solvent were spotted within equally spaced 1.9 cm circles on Whatman #3 MM chromatographic paper. The experiment consisted of three replications distributed completely at random. After spotting, the paper was exposed to a germicidal lamp for 30 minutes, then transferred to 15 cm, sterile, petri dishes. Twenty-five ml of PDA prepared according to Barnett (1972) was poured over the paper and dishes were swirled to yield a layer approximately 1 mm deep. A 1.5 mm PDA plug containing a single germinated pycnidiospore was then transferred to each circular area.

Two assays for fungal growth were made. In the first assay a volume of each sample containing a total of 0.1 absorbance unit at 290 millimicron was used. The second assay included 50 microliters of each fraction and controls regardless of absorbance value. Colonies were cultured at room temperature and diameters measured after seventy-two hours.

The first assay was made to determine the effect of each fraction on fungal growth when the concentration of each component was 0.1 absorbance unit; thus, roughly similar concentrations of each fraction was used. Results expressed as absorbance units can be converted to concentration once absorbing materials are identified. The second assay allowed observation of retarding effects of fractions on fungal growth at the relative concentrations found in the inner bark.

Analysis of variance and a Duncan Multiple Range Test were used to test data obtained from diameter measurements of the colonies.

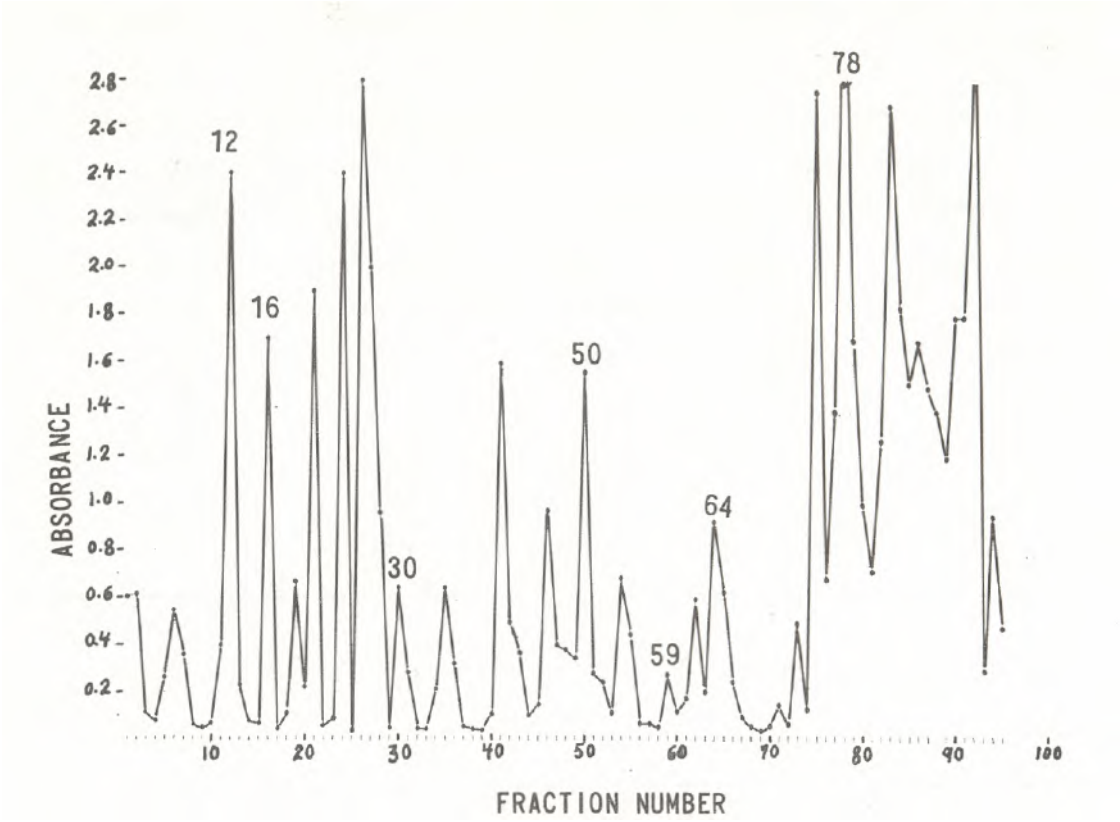

Figure 1.--Elution chromatogram of the 95 fractions collected from the silica gel column.

RESULTS AND CONCLUSION

The analysis of variance showed that some fractions significantly inhibited colony growth. Duncan's Multiple Range Tests indicated that seven of the twenty-five fractions were significantly more inhibitory to fungal growth than all the controls. The other 18 fractions had no statistically significant effect on growth and in no case did they promote growth beyond that of the controls. Mean diameter growth of colonies which were inhibited after 72 hours is presented in Table 1.

Table 1 includes the 7 fractions which inhibited fungus growth significantly in both assays; two additional fractions were more inhibitory than controls in the first assay only. This observation indicates that active fractions inhibit in <u>vitro</u> growth of the blight fungus similarly over a fairly wide range of concentrations; however the inhibition of fraction number 59 is greatly enhanced by a seven-fold increase in concentration. If this observation is confirmed in vivo then selection of resistant individuals would be greatly simplified.

Table 1.--Effect of some chloroform fractions on in *vitro* growth of *Endothia parasitica.*

Fract. No.	First Assay (equal absorbance) Mean Diam. growth (mm)	% of control	Volume (μl)	Second Assay (all vol. 50μl) Mean Diam. growth (mm)	% of control
12	5.0	43.9	42	6.6	55.5
16	4.5	39.5	58	3.4	28.6
30	6.2	54.4	154	7.9	66.4
50	5.3	46.5	64	5.6	47.1
59	4.4	38.6	351	7.6	63.8
64	6.1	53.5	108	6.5	54.6
78	6.2	54.4	24	6.2	52.1
Mean of 7 fractions	5.4	47.4		6.3	53.4
Mean of 8 controls	11.4	100.0		11.9	100.0

The above preliminary results indicate existence of some extractible, fungus inhibiting chemicals that might be used as a screening factor in an American chestnut selection breeding program. Identification of these compounds is the next step. More research is needed to determine the fungicidal effect of components in pure form and to correlate the results with resistance in nature. Such knowledge would provide a valuable tool for selection of blight resistant American chestnut trees.

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