ATTEMPTS TO CONTROL CHESTNUT BLIGHT WITH SLURRY AND CONIDIAL SPRAYS OF HYPOVIRULENT STRAINS

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ABSTRACT.--Four field plots were established in 1978 in forested areas with high densities of native chestnut sprouts. Treatments were repeated each year for four years and consisted of mist-blowing solutions of conidia of cytoplasmic hypovirulent (CH) strains and inoculating natural cankers with slurries of CH strains. Treated areas consisted of all the sprouts within a 25 m radius. Competing hardwoods were cut or killed. Stems in the 25 to 50 m radius have been monitored for control of the blight and spread of CH strains from the treated center. Data on stem survival, stem size, canker persistence, new cankers, and canker location are being obtained. Preliminary results will be presented. Inoculation with CH slurries is keeping stems alive longer. There is some evidence of the establishment of persistent cankers within the treated area as well as in the outer perimeter. However, new infections of normal strains are prevalent in both the inner treated area and the outer untreated area.

Control of virulent (normal, V) infections of *Endothia parasitica* on American chestnut by inoculation with agar slurries and even conidial sprays of hypovirulent (H) strains has been previously demonstrated (Jaynes and Elliston 1978; 1980). However, natural spread of the control agent(s), giving protection from secondary infections on the same or neighboring stems, has not been clearly shown. This failure of hypovirulent spread in the eastern United States, in contrast with the situation in Italy and France, has been attributed to: 1) a difference in the host species, *Castanea dentata* vs. *C. Sativa;* 2) a smaller density and stem diameter of host trees; 3) a lack of appropriate vectoring mechanisms; 4) use of the wrong combination of hypovirulent strains; 5) other differences in the parasite, including more vegetative compatibility groups and an increased ability to produce perithecia in the United States (Elliston 1981; 1982); 6) an overabundance of natural V inoculum compared to the small quantities of H inoculum introduced into test plots to date. Some of these problems might be overcome by establishing long-term test plots in areas with high densities of American chestnut, where mixtures of hypovirulent strains could be introduced repeatedly and any competing hardwoods could be removed. Any spread of H strains could be monitored from the central treatment area.

Materials and Methods

In 1978 four field plots were established in northeastern Connecticut in forested areas with high densities of native chestnut sprouts. Partial cutting of the overstory hardwoods had previously occurred in all plots. Hypovirulent treatments were repeated each year for 4 years and consisted of mist-blowing solutions of conidia into the plot and/or hand inoculating natural cankers with slurries of H strains. All treatments were during the growing season (May to October). The first year the stems in all four plots were examined three times (June, July, and September) and all cankers detected within 21/4 m of the ground were inoculated with a slurry of H strains. The combinations of sprays and slurry inoculations were different for each plot (Table 1). One plot, TF, received no spray treatments.

Table 1. Hypovirulent treatments of four plots for 4 years.

Plot	Treatments[a/]	Year and number of treatments				
		1978	1979	1980	1981	Total
GS	Slurry 5	3	1	1	1	6
	Spray 5	1				1
GN	Slurry 10	3		1	1	5
	Spray 5	1	2	1	1	5
TF	Slurry 5	3	1	1	1	6
TN	Slurry 10	3		1	1	5
	Spray 10	2	2	1	1	6

[a/] Cultures of 5 stocks included: EP-4 French, 43 French-American, 47 Italian, 60 American, 172 American. Cultures of 10 stocks included, in addition: EP-49 Italian, 51 Italian, 88 American, 92 American, 171 American.

Prior to the first treatment, five normal cankers per plot were sampled and typed for vegetative compatibility (v-c). The 20 cankers represented 16 v-c Types (4, 8, 9*, 10*, 11, 18, 19*, 20, 24*, 26, 30, 41, 43, 44, 67, 70-those with an asterisk were isolated from two cankers). The H strains were of French, Italian, and American origin and included a wide range of pathogenicities. Seven of the 10 H strains have been typed and six v-c groups are represented (5, 9, 10, 11, 12, 13-two in group 12).

Treated areas consisted of an inner circle of 25 m radius (0.2 ha). At the start of the experiment, competing hardwoods were killed and American chestnut stems girdled with chestnut blight were cut and removed. All live American chestnut stems 2.5 cm in diameter or larger at 135 cm above ground within the 25 m radius were included in the treated area. American chestnut stems in the 25 to 50 m radius (0.6 ha) were also monitored for control of the blight and spread of H strains from the treated center. Data on stem survival, stem size, canker persistence, new cankers, and canker location were obtained in each of the 4 years from the inner treated and outer untreated circles.

The culturing and inoculating techniques of the H strains were as previously reported (Jaynes and Elliston 1978; 1980). Slurries were inoculated around the periphery of each canker. Conidia were sprayed from a back-pack mist-blower, 9 liters/plot. The total number of conidia sprayed in each treatment ranged from 1.2 to 12.1 x 10^{11}. With few exceptions concentrations of conidia of the component strains were within 4 to 5 fold of each other, e.g. 3 to 15 x 10^{7} per ml.

Endothia parasitica was isolated from a few persistent and apparently healing cankers present in the outer circle. These isolates were characterized for morphology on potato dextrose agar and two were tested for dsRNA as previously described (Dodds 1980; Scharf and DePalma 1981).

Results

There are many parameters that need to be analyzed including survival of chestnut stems, changes in basal area, number and location of cankers, persistence of cankers, persistence of infected stems, and isolation and characterization of H strains beyond the treated area. The following results are preliminary and incomplete.

The percentage of stems surviving at the end of 4 years is presented in Figure 1. As expected, a significantly higher percentage of stems have survived in the inner treated circle compared to the outer untreated circle. Average survival in the inner circle was 81 percent compared to 58 percent for the outer. Average stem diameter over 4 years has increased more for stems in the inner circle, 46 percent compared to those in the outer, 12 percent (Figure 2).

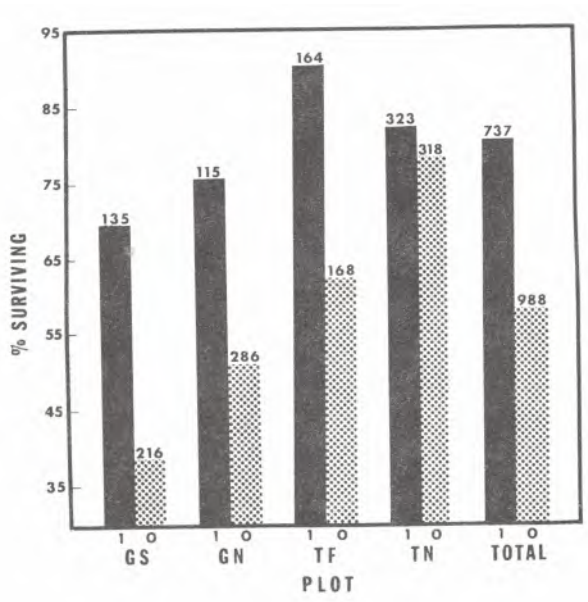

Figure 1. Percentage of stems surviving in 1981 for the inner treated (I) and outer untreated (0) areas of each plot based on the total number of stems that attained at least 2.5 cm d.b.h. in the 4-year period. Total stems indicated at the top of the bars.

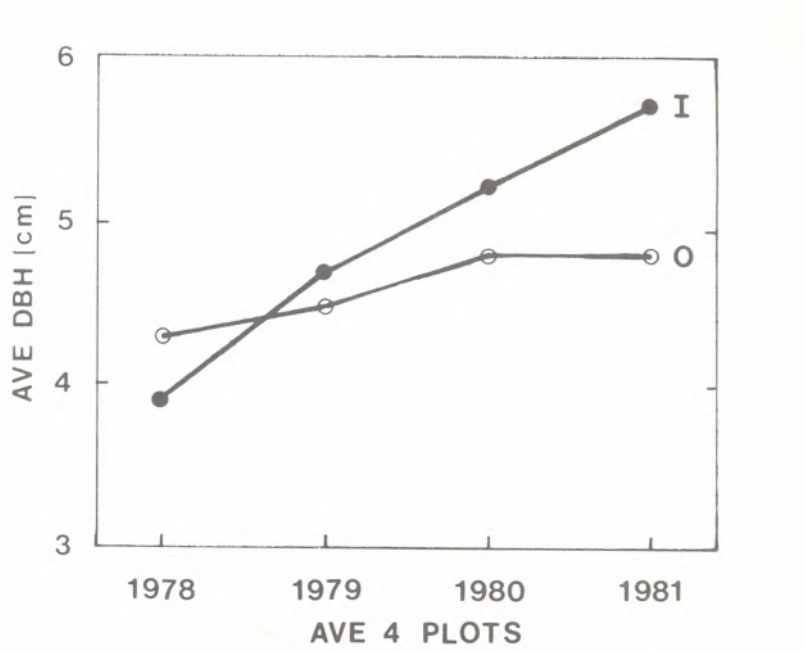

Figure 2. Average stem diameter of American chestnuts in the four inner (I) treated and four outer (0) untreated plots.

The percentage of stems surviving in 1981 in each successive 5 m annulus of the inner and outer circles is shown in Figure 3 for 1 and 3 years. The lines represent the best fit for inner and outer circles. Except for plot GN survival in the inner treated circles was unrelated to distance from the plot center. Survival in the untreated outer circle generally decreased with increasing distance from the treated area. However, survival in the innermost annulus (26 to 30 m) of the untreated outer circle tended to be the same as survival within the treated circle.

Isolations taken from two persistent cankers outside the 25 m radius of plot TN (at 33 m) were tested for dsRNA. One isolate contained dsRNA, the other did not. This tree has been girdled with a superficial canker since 1980 but was still growing vigorously in 1981. Isolates have been obtained from other "healing" cankers in the untreated outer areas but have not been tested for pathogenicity or dsRNA.

Discussion and Conclusions

Inoculation of natural cankers on American chestnut with mixtures of H strains significantly prolongs the survival of the chestnut. The H strains are having an apparent positive effect beyond the treated area.

The tendency for higher survival in the portion of the untreated outer circle nearest the treated circle suggests that hypovirulent strains are moving slowly outward from the treated inner circle. The isolation of one strain containing dsRNA from the outer circle supports this suggestion, although it can not be unequivocally stated that no dsRNA strains were present prior to treatment in 1978. The better survival could also result, not from spread

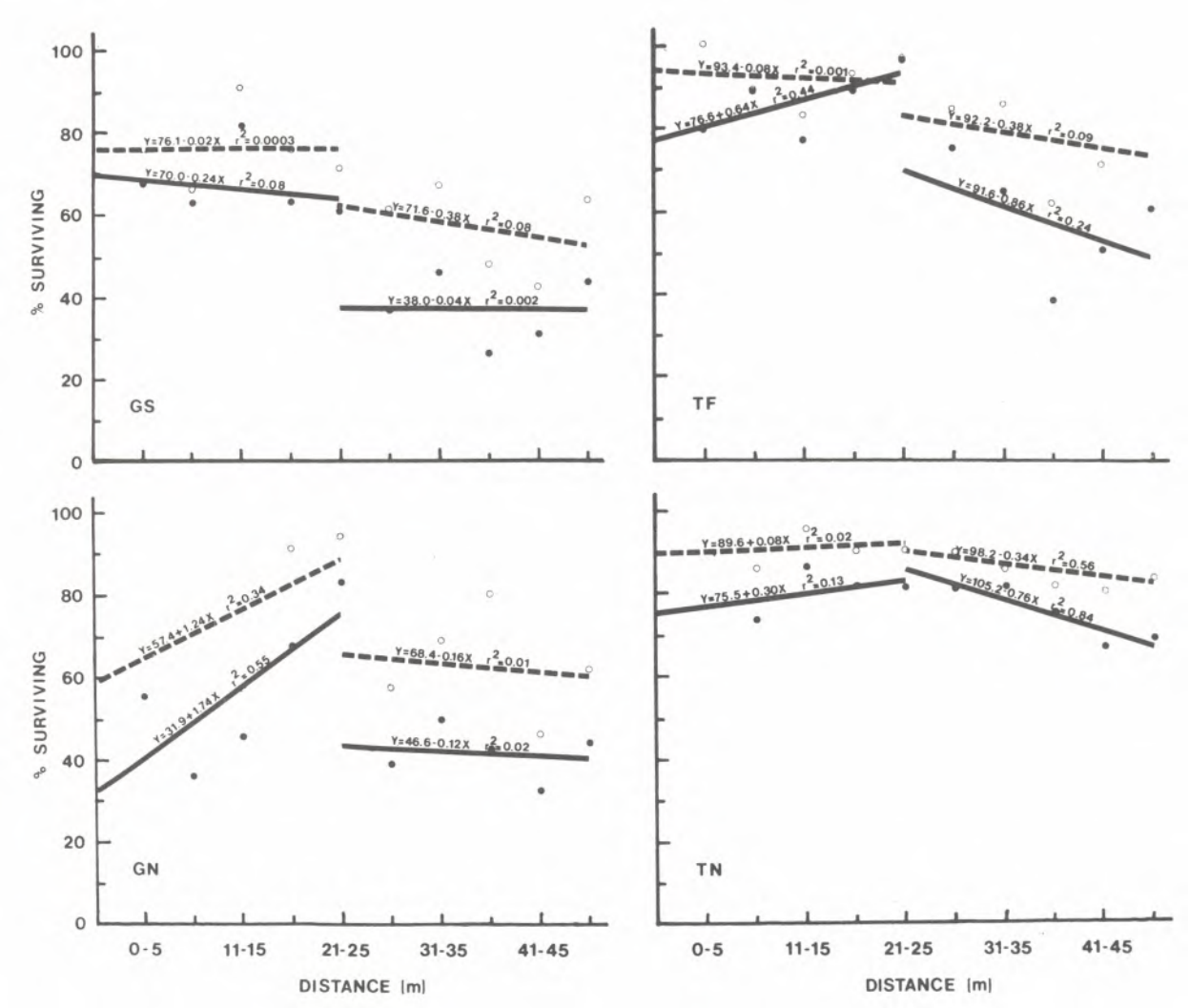

Figure 3. Percentage of stems surviving in 1981 in each section of 5 m radius from 0 to 50 m for each of the four plots based on the total number of stems that attained at least 2.5 cm d.b.h. Three-year (1978 to 1981) data indicated by closed circles and solid lines and 1-year (1980 to 1981) data by open circles and dashed lines.

of H strains, but from reduced virulent inoculum from the treated inner circle. Competition from hardwoods may also be a significant factor in the outer areas of the plots.

On plot GN the generally increased survival with increasing distance from center of the treated circle may simply be due to the small number of stems in the center of this plot. (The only stems lost to vandalism were in the center of plot GN.) Because the treatments for each plot differed (Table 1), it is not possible to determine whether a particular slurry or conidial spray was more effective.

This experiment was designed to determine natural spread from a central treated area. The results have not been dramatic but suggest that spread may be occurring. Even in France, spread of H and control of untreated

cankers is only first observed about 5 years after treatment (Grente and Berthelay-Sauret 1978). No further H treatments are planned for at least three of these four plots, but we expect to continue to observe the trees for survival and monitor spread of H strains.

Better combinations of H strains and application techniques are required for practical control of cankers on treated stems and subsequent spread of the controlling agents. The control observed in these plots can be attributed to the high density of stems, elimination of competition, and repeated inoculation with a mixture of strains.

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