Control of *Endothia parasitica* Cankers on American Chestnut Sprouts with Hypovirulent Strains

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ABSTRACT.— Inoculation of natural infections of *Endothia parasitica* on 300 American chestnut sprout clumps with a French-derived hypovirulent (H) strain significantly limited canker size. However, control of new infections and untreated cankers in these plots was not observed over a three-year period; probably because the H strain was too restricted in growth and sporulation on American chestnut. In 1977, eight H strains, selected for a range of pathogenicity, compatibility type, and geographic origin, were inoculated as a mixture or individually, or sprayed as a mixture of conidia on 360 native chestnut stems, all previously inoculated with resident virulent strains. All four methods of H treatment significantly limited canker size compared to cankers left untreated. Inoculum containing a mixture of H-strain mycelia was most effective. Whether any of the H treatments will lead to control of secondary virulent infections is still to be determined.

Within 50 years the chestnut blight fungus, *Endothia parasitica* (Murr.) P. J. and H. W. And., reduced the American chestnut *(Castanea dentata* [Marsh.] Borkh.) from a dominant and highly productive member of the forest community to a nearly insignificant understory shrub. In the forest the species has been perpetuated by a persistent succession of sprouts that occur at the base of blight-infected trees. As the original tree or later sprouts become girdled new sprouts arise to maintain the plant.

The phenomenon of hypovirulence associated with certain strains of the blight fungus may provide a means for relieving the tremendous pressure this pathogen has exerted on the American chestnut. Spontaneous curing of European chestnut *(C. sativa* Mill.) in Italian groves apparently resulted from action of these strains (Bonifacio and Turchetti, 1973). Hypovirulent (H) strains are being used to control blight in chestnut groves in France (Anonymous, 1973; Grente and Berthelay-Sauret, 1979) and they have shown promise for control of the disease in this country (Anagnostakis and Jaynes, 1973; Van Alfen *et al.,* 1975; Jaynes *et al.,* 1976).

THE NATURE OF HYPOVIRULENCE

Hypovirulent strains of *E. parasitica* have two important characteristics: 1) low pathogenicity, they are less able to cause disease than normal strains, and 2) curative capacity, they inactivate cankers caused by compatible normal strains, allowing normal healing processes to function. The first clue to the nature of hypovirulence was pro-

vided by French scientists Grente and Sauret (1969a & b), whose experiments suggested that an agent was present in the cytoplasm of affected strains but absent from normal strains. Berthelay-Sauret (1973) and Van Alfen *et al.* (1975) used auxotrophic markers to confirm this suggestion. Day *et al.* (1977) found that all hypovirulent strains tested contain double-stranded ribonucleic acid (dsRNA), a type of genetic material characteristic of many fungal viruses. Normal strains lack this material. Dodds (1979), also at the Connecticut Station, subsequently found that all of the dsRNA in one of the hypovirulent strains obtained from France is contained in unusual membrane-bound, club-shaped, virus-like particles resembling those associated with a severe disease of cultivated mushrooms (Lesemann and Koenig, 1977). Taken together, these observations suggest that hypovirulence in *E. parasitica* is a consequence of viral infection; that is, H strains are diseased. More than one virus-like agent may be involved in hypovirulence.

The curing phenomenon, then, appears to result from transmission of the disease agent from the H strain to the strain causing the canker. The fungus in the canker then becomes diseased (hypovirulent) and unable to continue its attack, and the healing process begins.

The challenge is to establish curative strains in the United States that will control the common virulent (V) strains. Preliminary evidence for a naturally occurring curative strain in Michigan has already been reported (Elliston *et al.,* 1977).

FIELD TESTS OF HYPOVIRULENT STRAINS AS CONTROL AGENTS

Two field tests were established in Connecticut to determine if hypovirulent strains would control cankers on native chestnut sprouts and become naturalized: one test was initiated in the fall of 1974 and the other in the spring of 1977.

Field Test I: 1974.1977

Twelve plots were established in the fall of 1974 and spring of 1975 in Connecticut woodlands. Each plot contained 25 American chestnut sprout clumps within an area of one hectare or less. Each clump used in the test included one or more live stems at least 2.5 cm in diameter, 135 cm from the ground. The sizes and locations of all cankers were recorded. Cankers in six of the plots were left untreated (control plots); cankers in the remaining plots were treated with a hypovirulent strain. Treatment consisted of removing 8.5 mm diameter bark plugs from the two lateral extremities of each canker with a cork borer, filling the holes with disks of potato dextrose agar containing mycelium of the hypovirulent strain, and covering the holes with pieces of masking tape to retard drying. Those plots having the most natural infections were selected to receive the H treatments. We assumed in 1974 that it was only necessary to use an H strain that resulted from conversion of a local V strain (Grente and Sauret, 1969b). Hypovirulent strains derived from combinations of American V with French H (2025) were used.

Stem diameters were recorded annually during the dormant season. Plots were examined monthly during the growing season the first two years and twice the third year. New infections and previously inoculated, but uncontrolled, cankers were re-inoculated through May, 1976.

In the spring of 1976 each set of six plots was split; three of the previously inoculated plots were sprayed with conidia of an H strain (Ep43) as were three of the previously untreated plots. Each plot received 500 ml of H_20 containing 1.7-4.8 x 10^6 spores per ml sprayed on all infections within 250 cm of the ground and in a 45 cm high band around each stem at shoulder height. A handheld, atomizing, mechanical sprayer was used. Applications were made in June, July, and August.

To determine if the H treatments would have an effect on establishment of new cankers, all stems were wounded in April and again in August, 1976, at 90 cm height with a bark sampler that made 5 mm diameter round holes. Of 758 wounds only 13 (1.7 percent) developed infections by October, 1977, and their presence was not significantly correlated with H treatment. The low incidence of infection was unexpected since many of the stems had virulent, sporulating cankers above the wounds to serve as sources of inoculum.

Effects of the agar plug and conidial spray treatments are summarized in Figure 1. Control of H-inoculated cankers during the first season was dramatic: 86 percent (82/95) of the treated compared to 8 percent (3/37) of the untreated cankers showed clear signs of arrest. By the end of the third year the effect had diminished to 13 percent (31/245) controlled compared to no control of 71 untreated cankers. The sharp decrease in control was due in part to regrowth of some cankers but largely to secondary virulent infections that girdled and killed stems having one or more "controlled" cankers on them. The conidial spray treatments had a temporary effect as 15 percent arrested, (11/71) in 1976, the year of application, but this apparent effect was lost by the end of 1977.

There was no evidence that natural spread of H strains and control of secondary infections occurred in any of the plots. A higher incidence of new infections was observed in 1977 in the H-inoculated plots (59) than in the untreated plots (28). This reflects the higher initial incidence of infection (88) in the treated compared to the untreated (32) plots. Considering the higher incidence of disease in H-inoculated plots, 414 infections compared to 172 in the untreated, it is worth noting that the number of girdled stems was about the same in treated (69) and untreated (65) plots. This suggests protection or, at least, delay in stem death in the H-treated plots.

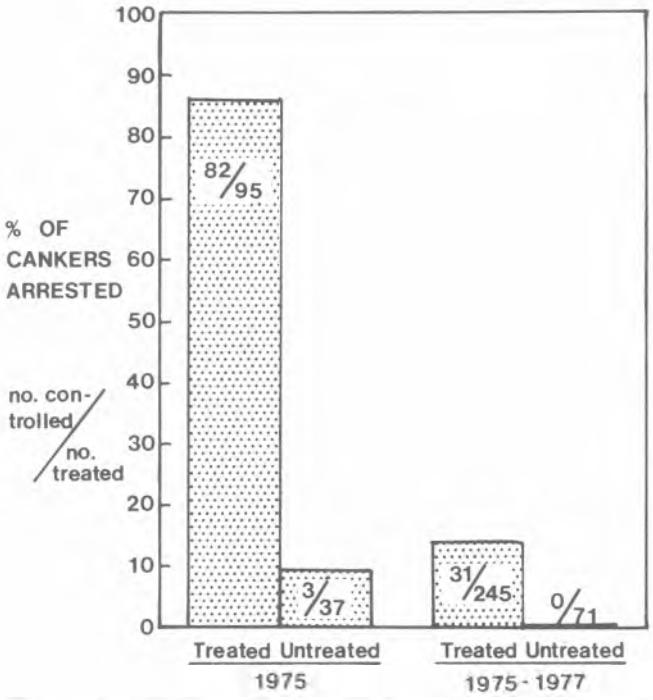

Figure 1. Field test I: Arrest of naturally occurring cankers on American chestnut sprouts inoculated one and three years earlier with French-derived American hypovirulent strains.

These results demonstrated that the H strain could control natural *E. parasitica* infections on American chestnut sprouts. It also suggested, with other research (Elliston, 1979), that the ability of the H strain to colonize host tissue and sporulate could be important to its long-term survival and spread. Other evidence (Elliston and Jaynes, 1977) suggested the presence of vegetative incompatibility among strains of *E. parasitica.* More detailed laboratory studies confirmed this (Anagnostakis, 1977). The effectiveness of H in controlling cankers and spreading in this field test was probably limited by the fact that only one H compatibility type was apparently represented. The 1977 field test was designed with these considerations in mind.

Field Test II: 1977

Fifteen field plots, each containing 24 sprout clumps, were established as described for the 1974-1977 field tests. To eliminate large differences in disease incidence from plot to plot, test trees in each plot were divided into three equal groups and inoculated with infected bark plugs from one of three natural cankers found in or near the plot. The technique consisted of removing 8.5 mm diameter plugs of bark from the periphery of a natural canker, forcing one of these plugs into a 7.5 mm diameter hole made in the American chestnut stem at a height of 1.2 meters and covering it with masking tape. Inoculations were made in May, 1977. Approximately five weeks were allowed between inoculation and treatment to permit canker to develop. The technique was 99 percent effective in establishing cankers.

A group of eight hypovirulent strains (Ep 9, 14, 49, 50, 60, 61, 90, 102) was selected for H treatments. They included representatives from the French-derived American, native American, and Italian collections. The strains represented six compatibility groups, but also varied in pathogenicity, and ability to sporulate.

Four methods of treating cankers were used:

1) Ten liters of a H_20 suspension containing a mixture of spores from the 8 H strains, diluted to 1.0×10^6 - 1.7×10^7 conidia per ml, were sprayed from a knapsack power mist blower throughout the plot but with emphasis on American chestnut stems. Trials, using agar plates as targets at various distances, demonstrated that conidia were well distributed and viable in large numbers at distances up to 8 meters from the nozzle when sprayed through the mist blower. Half of the cankers in each plot were wounded by removing four equally spaced 8.5 mm diameter disks of bark from the margin of each canker with a cork borer immediately before they were sprayed.

2) An agar slurry, prepared by blending together young colonies of all eight strains, was placed in a 50-cc syringe and forced into 4 circular holes cut with a cork borer at 2-5 cm intervals around the canker margin.

3) Eight, approximately equally spaced, 8.5 mm diameter holes were cut around the margin of each canker and one colonized agar disk was inserted into each of the holes. Each hole received inoculum from a different strain. The arrangement of strains around the canker was the same for all cankers.

4) Cankers were treated with agar plugs of one of the eight strains, three cankers, four wounds each, being treated per H strain in each plot. Each of the above treatments was applied to three plots with the other three plots remaining as untreated controls. For treatments three and four, inoculum was taken from the leading edge of actively growing colonies.

H treatments were applied in late June. The height and width of each canker were recorded in late October. The results, reported as average canker areas, are summarized in Figure 2. All treatments with H significantly limited canker size compared to the untreated plots (P=.05). Variation of canker size was great in all the plots. Treatments with the mycelial slurry and the inoculation of all eight strains as separate plugs in each canker were most effective. These treatments provided levels of control that were not significantly different (P=.05). Treatments with one strain per canker and with sprayed spore suspensions were less effective, and not significantly different from each other (P=.05). As expected, wounding prior to spraying conidia enhanced the effectiveness of sprayed H spores.

A total of 93 natural cankers appeared on test trees during the 1977 growing season; their

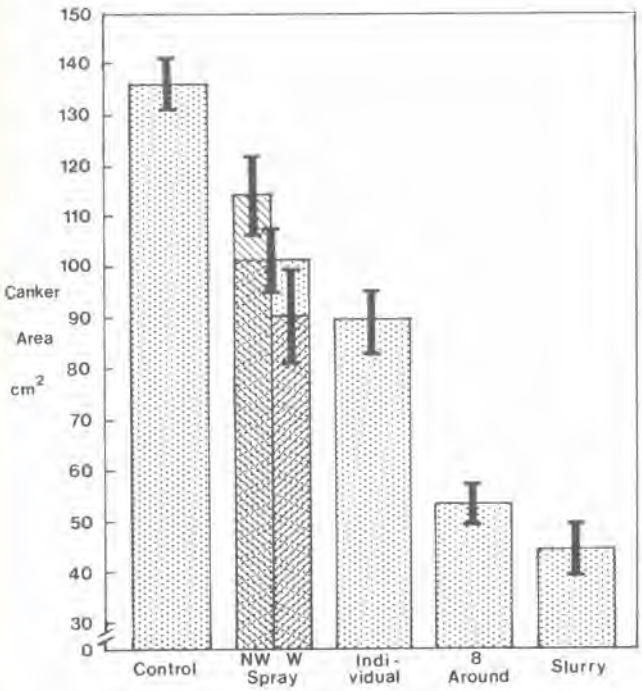

Figure 2. Field test II: Arrest of virulent cankers on American chestnut sprouts treated with eight hypovirulent strains, 1977. Standard error of the mean indicated. Control = cankers received no H treatment; NW = cankers not wounded and sprayed with conidia; W = cankers wounded and sprayed with conidia; Individual = each canker inoculated with a single H; 8 around = all eight H inoculated individually around the canker; Slurry = mix of all eight H inoculated in four places around the canker.

presence was not correlated with treatment.

CONCLUSIONS AND COMMENTS

1. Virulent natural and artificially created cankers can be controlled in the field with hypovirulent strains.

2. Inoculum containing a mixture of H strains appears to act as effectively as the best H in the mix in arresting V canker growth, and its range of effectiveness is enhanced by the presence of several vegetative compatibility groups. Mixtures of H strains of different compatibility groups may offer an effective means of controlling cankers and maintaining specimen trees. To effect natural spread it may be necessary to use strains of H that are capable of growth and long-term survival on chestnut. Thus the selection of H types for inclusion in the mixture would depend on whether immediate control of a canker is desired or more gradual control and natural spread.

3. Spraying conidia of H strains in aqueous suspension may be a useful method for transmitting H to V cankers, especially if the V cankers are wounded before treatment.

4. Artificial wounds made on all trees in Test I were ineffective for detecting natural spread of H. Low incidence of infection of such wounds was observed in all plots.

5. Whether establishment and natural spread of H strains will occur in H-treated areas containing native chestnut, and whether it will result in effective long-term control of virulent strains of *E. parasitica* is still to be determined.

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LITERATURE CITED

Anagnostakis, S. L. and R. A. Jaynes
1973. CHESTNUT BLIGHT CONTROL: USE OF HYPOVIRULENT CULTURES. Plant Dis. Rep. 57:225-226.

Anagnostakis, S. L.
1977. VEGETATIVE INCOMPATIBILITY IN *ENDOTHIA PARASITICA*. Experimental Mycology 1:306-316.

Anonymous (Ministere l'Agriculture et Development Rural).
1973. L'ENDOTHIOSE OU LE CHANCRE DU CHATOIGNIER. Centre Technique du Genie Rural des Eaux et des Forets. Div. Protection Nature. B. P. 114, 38402 St.-Martin-D'Heres.

Berthelay-Sauret, S.
1973. UTILIZATION DE MUTANTS AUXOTROPHES DAN LES RECHERCHES SUR LE DETERMINISME DE "L'HYPOVIRULENCE EXCLUSIVE". (Abst.) Ann. Phytopathol. 5:318.

Bonifacio, A. and T. Turchetti.
1973. DIFFERENZE MORFOLOGICE E FISIOLOGICHE IN ISOLATE DI *ENDOTHIA PARASITICA*. Ann. Accad. Ital. Sci. For. 22:111-131.

Day, P. R., J. A. Dodds, J. E. Elliston, R. A. Jaynes, and S. L. Anganostakis.
1977. DOUBLE-STRANDED RNA IN *ENDOTHIA PARASITICA*. Phytopathology 67:1393-1396.

Dodds, J. A.
1979. DOUBLE-STRANDED RNA AND VIRUS-LIKE-PARTICLES IN *ENDOTHIA PARASITICA*. American Chestnut Symposium Proc., West Virginia University, Morgantown, WV.

Elliston, J. E.
1979. PATHOGENICITY AND SPORULATION OF NORMAL AND DISEASED STRAINS OF *ENDOTHIA PARASITICA* IN AMERICAN CHESTNUT. American Chestnut Symposium Proc., West Virginia University, Morgantown, WV.

Elliston, J. E. and R. A. Jaynes.
1977. BIOLOGICAL CONTROL OF CHESTNUT BLIGHT—A PROGRESS REPORT. Ann. Rept. Northern Nut Growers Assoc. 68:84-90.

Elliston, J. E., R. A. Jaynes, P. R. Day, and S. L. Anagnostakis.
1977. A NATIVE AMERICAN HYPOVIRULENT STRAIN OF *ENDOTHIA PARASITICA*. Proc. Am Phytopath. Soc. 4:83 (Abstr.).

Grente, J. and S. Sauret.
1969a. L'HYPOVIRULENCE EXCLUSIVE, PHENOMENE ORIGINAL EN PATHOLOGIE VEGETALE. C. R. Acad. Sci. Paris 268:2347-2350.

Grente, J. and S. Sauret.
1969b. L'HYPOVIRULENCE EXCLUSIVE EST-ELLE CONTROLLEE PAR DES DETERMINANTS CYTOPLASMIQUES? C. R. Acad. Sci. Paris 268:3173-3176.

Grente, J. and S. Berthelay-Sauret.
1979. BIOLOGICAL CONTROL OF CHESTNUT BLIGHT IN FRANCE. American Chestnut Symposium Proc., West Virginia University, Morgantown, WV.

Jaynes, R. A., S. L. Anagnostakis, and N. K. Van Alfen.
1976. CHESTNUT RESEARCH AND BIOLOGICAL CONTROL OF THE CHESTNUT BLIGHT FUNGUS. Pages 61-70. In J. F. Anderson and H. Kaya, eds. *Perspectives in Forest Entomology.* Academic Press, New York, 428 pp.

Lesemann, D. E. and R. Koenig.
1977. ASSOCIATION OF CLUBSHAPED VIRUS-LIKE PARTICLES WITH A SEVERE DISEASE OF *AGARICUS BISPORUS.* Phytopath. Z. 89:161-169.

Van Alfen, N. K., R. A. Jaynes, S. L. Anagnostakis, and P. R. Day.
1975. CHESTNUT BLIGHT BIOLOGICAL CONTROL BY TRANSMISSIBLE HYPOVIRULENCE IN *ENDOTHIA PARASITICA.* Science 188:890-891.