Interactions of Virulent and Hypovirulent Strains of *Endothia parasitica* on American Chestnuts in North Carolina

E. G. Kuhlman

USDA Forest Service, Southeastern Forest Experiment Station, Research Triangle Park, NC 27709

ABSTRACT—Rate of canker development by virulent (V) and hypovirulent (H) strains of *Endothia parasitica* in paired combinations on stems of American chestnut in a 0.2-hectare area were determined 12 weeks after inoculation. The analyses of variance showed highly significant treatment and replication (tree) differences in canker length, width and area, callus formation, and type of isolate recovered. In spite of large tree-to-tree variation, treatment differences within each of the three major groups, HH, HV, and VV, usually were distinct. The tree-to-tree variation is apparently due to variation in host susceptibility. Apparent conversion of virulent to hypovirulent strains occurred in 34 percent of the HV treatments. Callus formation was inversely related to canker size. Sectoring in cankers by virulent isolates resulted in the appearance of diverse colony types in cultures.

During the summer of 1976, preliminary studies were undertaken to adapt French and Connecticut hypovirulent isolates of *Endothia parasitica* (Murr.) P. J. & H. W. And., to North Carolina because geographic differences in compatibility were reported to affect the transfer of the hypovirulence factor (Grente and Sauret, 1969). The results of these studies were hard to interpret due to weak experimental design and difficulty in proving conversion of virulent North Carolina strains to hypovirulent strains. Therefore in 1977, a study similar to the initial study reported from Connecticut (Van Alfen *et al.*, 1975) was undertaken. The objectives of this study were to determine rate of canker development by virulent and hypovirulent strains in various combinations on stems of American chestnut *(Castanea dentata* [Marsh.] Borkh.) and to recover adapted hypovirulent strains of *E. parasitica*.

METHODS

The interaction of virulent (V) and hypovirulent (H) isolates of *E. parasitica* on stems of American chestnut were evaluated by paired inoculations of VV, HV, and HH. Each of three experiments utilized three virulent and three hypovirulent strains. The three experiments had one virulent and one hypovirulent strain in common so that a total of seven virulent and seven hypovirulent strains were tested. In each experiment there were 19 treatment pairs consisting of three virulent selfed, three hypovirulent selfed, two crossings of virulents (V1+V2, V2+V3) and two crossings of hypovirulents (H1 +H2, H2+H3) and all nine virulent and hypovirulent (HV) combinations. The 19 treatments consisted of five VV, five HH, and nine HV combinations. The 19 treatments were randomized on each of six tree replications in each experiment.

Isolates of *E. parasitica* that were used included: virulent strains from the study area (C1, C5, C6, C8, C12), from Linville Falls, North Carolina (L2), and from Mt. Kisco, New York (NY); and hypovirulent strains from France (3), from Connecticut (43, 52, 53, 54), and from North Carolina (62, 66). The hypovirulent strains from Connecticut and North Carolina were derived from French isolates.

The study area included 0.2 ha of mixed hardwoods on the Coweeta Experimental Forest near Franklin, North Carolina. Inoculations were made on July 6, 1977, by pairing isolates side by side on the trunks of 8-13 cm diameter breast height (dbh) chestnut sprouts. A 7-mm diameter cork borer was used to remove a pair of bark disks down through the cambium. The disks were <3 mm apart. The

same cork borer was used to cut disks of Difco potato dextrose agar (PDA) with the various isolates of the fungus. The PDA disks were put in the bark and covered with masking tape to reduce evaporation. Nineteen isolate treatments were made on each tree starting 15 cm from the ground and proceeding up the trunk at 13-cm intervals on alternate sides of the tree for approximately 2.5 m. Trees were at least 3 cm in diameter at the highest inoculation point and were judged to be free of the blight.

Twelve weeks later callus formation was noted as being absent (0), or present in one (1) or both (2) of the inoculations. Canker length and width were measured from the outer edges of the inoculations. Isolations onto PDA were made from the edge of the canker at the extreme lateral margins and directly above and below each of the inoculations in each treatment pair. Developing colonies were rated either virulent, intermediate, hypovirulent, or not *E. parasitica* on the basis of colony color and growth rate.

All data were subjected to an analysis of variance and treatment differences were compared using Duncan's Multiple Range Test.

RESULTS

The analyses of variance showed highly significant (P=0.01) treatment and replication (tree) differences in canker length, width, and area in callus frequency and in type of isolate recovered for each of the three experiments. The error term involving treatment-tree interactions was not significant in 14 of 15 analyses. Therefore the tree-to-tree variation did not change the treatment effects.

The averages for each of the three inoculation treatment groups, HH, HV, and VV, were usually statistically distinct (P=0.05) (Table 1). Treatment HH caused cankers with the smallest width and length and the highest frequency of callus formation. Treatment VV produced cankers with the largest width and length and the lowest frequency of callus formation. Averages for the HV treatment were intermediate in all cases and significantly different from either VV or HH in seven of the nine averages shown in Table 1.

Some of the variation in canker development is shown by comparing canker width by the V and H isolates which were used in all three experiments (Table 2). The VV treatment, C8+C8, used in all three experiments, produced cankers with average widths of 28.0, 28.5, and 48.7 mm. In contrast the HH treatment, 43+43, had average widths of 5.8, 8.5, and 8.8 mm. In the HV treatments wide variation in canker width occurred; for example, in experiment 1 canker width in the HV combinations ranged from 8.8-33.5 mm.

Thirty-four percent of the HV treatments yielded hypovirulent isolates from the canker margin lateral to the V inoculum, thereby implying conversion of virulent to hypovirulent. Virulent isolates recovered from VV cankers were diverse in appearance. Apparently sectoring occurred in the canker so that 2-4 colony types were recovered from some inoculations. *Trichoderma viride* Pers. ex S. F. Gravy and *T. harzianum* Rifai. were the secondary fungi isolated most often.

DISCUSSION

Van Alfen *et al.* (1975) did not indicate the amount of variation in canker size in their study, nor did they pair all H and V combinations. It was thought chance selection could have produced their results. This study indicates that although considerable variation in canker size may occur, canker growth was reduced by HV treatments (Table 1). Griffin *et al.* (1977) have shown that some single conidial isolates from virulent colonies form cankers similar to those formed by H isolates. However, all of the V isolates in this study formed large cankers.

Chestnut cankers grow faster in length than in width, which suggested measurements of canker lengths might more readily demonstrate treatment effects. However, the analysis of combined treatments for canker length (Table 1) indicates some loss relative to canker width in discriminating among the treatments. Thus, canker width may be a better indication of treatment effect than is canker

Table 1
Length and width of cankers and callus formation by American chestnut sprouts 12 weeks after paired inoculations with virulent (V) and/or hypovirulent (H) isolates of *Endothia parasitica*.

Experiment	Inoculation Treatment VV	HV	HH
	Canker width (mm)		
1	40a[Y]	23b	8c
2	27a	19b	7c
3	22a	11b	7c
	Canker length (mm)		
1	66a	54b	22c
2	54a	47a	21b
3	54a	32b	18c
	Callus frequency[z]		
1	.036c	.389b	1.143a
2	.166b	.296b	1.334a
3	.414c	.868b	1.379a

[Y] Numbers followed by different letters within an experiment are significantly different according to Duncan's Multiple Range Test (P=0.05).

[z] 0 = no callus, 1 = callus on one side of inoculation, 2 = callus on both sides.

length. Survival of the tree is also more affected by lateral canker growth.

Callus formation occurred more frequently in the paired H treatments than in the HV or VV treatments (Table 1). American chestnut has a tremendous capacity for callus formation. The reduced virulence of the H strains provides a longer time for callus formation. Thus, there was an inverse relationship between callus formation and canker size.

The difference between the three experiments in average canker size in the VV and HV treatments (Table 1) might have been explained by the relative virulence of the isolates. However, isolate C8 was used in all three and had similar differences between experiments (Table 2). Since the experiments were conducted simultaneously within a 0.2-ha area with an apparently uniform environment, the differences in canker size are probably due to variation in host susceptibility.

Determining conversion of virulent strains to hypovirulent strains *in vivo* remains a difficult problem. Van Alfen *et al. (1975)* showed conversion with virulent auxotrophic isolates; however, wild strains have no such markers. Isolates recovered from VV inoculations showed variation in colony appearance analogous to sectoring in culture. This variation will not only confuse the identification of converted virulent strains but it may indicate variation in compatibility groups within a mass isolate.

LITERATURE CITED

Grente, J., and S. Sauret.
1969. L'HYPOVIRULENCE EXCLUSIVE, PHENOMENE ORIGINAL EN PATHOLOGIE VEGETALE. Compte Rend. Acad. Sci. Paris (Series D) 268:2347-2350.

Griffin, G. J., J. R. Elkins, G. Tomimatsu, and F. V. Hebard.
1977. VARIATION IN PATHOGENICITY OF AMERICAN ISOLATES OF ENDOTHIA PARASITICA ON AMERICAN CHESTNUT. Proc. Am. Phytopath. Soc. 4:108.

Van Alfen, N. K., R. A. Jaynes, S. L. Anagnostakis, and P. R. Day.
1975. CHESTNUT BLIGHT: BIOLOGICAL CONTROL BY TRANSMISSABLE HYPOVIRULENCE IN ENDOTHIA PARASITICA. Science 189:890-891.

Table 2

Width of *Endothia parasitica* cankers 12 weeks after paired inoculations of the virulent (C8) and hypovirulent (43) isolates with other isolates in each of the three experiments. Twelve other treatment pairs were used in each experiment.

Common Isolate[y]	Experiment 1		Experiment 2		Experiment 3	
	Isolate	Width (mm)	Isolate	Width (mm)	Isolate	Width (mm)
*C8+	*C8	48.7a[z]	*C8	28.5ab	*C8	28.0a
*C8+	43	20.0cd	43	12.8cde	43	7.8cde
*C8+	3	27.7cd	53	25.2abc	54	9.2cde
*C8+	52	8.8d	62	6.2e	66	9.0cde
43+	*C6	33.5bc	*C5	24.0abc	*C12	19.3abcd
43+	*L2	22.0cd	*C1	28.5ab	*NY	7.8cde
43+	43	8.8d	43	8.5e	43	5.8e

[y] Asterisk indicates virulent isolate.

[z] Within an experiment numbers followed by the same letter are not significantly different (P=0.05).