Frequency of Vegetative Compatibility Types of *Endothia parasitica* in Two Areas of West Virginia

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ABSTRACT.—The vegetative compatibility types of 202 virulent isolates of *Endothia parasitica* were determined. Isolates were obtained from cankers on American chestnut stems found in two clear-cut areas within West Virginia. Eighty-nine percent (179) of the isolates were classified in 14 vegetative compatibility groups. Twenty-three isolates (11 percent) were vegetatively incompatible with all others or did not give a consistent reaction when paired on agar. Study areas with the highest incidence of infection contained the greatest number of vegetative compatibility types while those with the least infection contained the fewest. Only 25 percent of the trees with multiple cankers were infected by isolates of the same vegetative compatibility type.

The phenomenon of vegetative incompatibility has been described for several ascomycetous fungi (Caten, 1972) and recently for *Endothia parasitica* (Murr.) P. J. and H. W. And. (Anagnostakis, 1977). Anagnostakis (1977) has demonstrated that when virulent strains of *E. parasitica* are paired on an amended potato dextrose agar medium one of two reactions may occur. The mycelia of the strains either merge (vegetatively compatible) or interact by forming a barrage (vegetatively incompatible). When the vegetatively incompatible response occurs a line of pycnidia are formed at the interface of the two strains.

The transfer of the cytoplasmic determinants of hypovirulence requires that hyphal anastomosis occur between virulent and hypovirulent strains (Van Alfen *et al.,* 1975). If vegetative incompatibility limits or precludes successful transfer then hypovirulent strains may fail to restrict canker causing virulent strains, thus limiting the effectiveness of hypovirulence in the forest. This study was undertaken in conjunction with other hypovirulence field trials to determine if different vegetative compatibility types occur in West Virginia and if so, with what frequency.

MATERIALS AND METHODS

Sixteen study plots with abundant American chestnut regeneration were established in 10-15 year-old clear-cut areas in West Virginia. Plots 1-8 are located in the George Washington National Forest and are approximately 80 km distant from plots 9-16, in the Monongahela National Forest. Each plot is approximately 20 m square and contains from 20-50 living chestnut stems ranging in size from 1-20 cm, 1.3 m above the ground. Only living but cankered stems greater than 2.5 cm were used in this study.

Mass isolations of mycelium from cankers were made by removing 1 cm bark plugs from the advancing margins of the canker. Four or more bark plugs/canker were cultured on a potato dextrose agar (Difco) medium amended with biotin (5 ug/1), methionine (100 mg/1) and streptomycin (3 mg/1). Transfers were made from the advancing edge of the resulting cultures to obtain pure cultures of *E. parasitica*.

Isolates obtained from plots 5-8 were first paired with each other in all combinations using the procedures of Anagnostakis (1977). When a pattern of vegetative compatibility (v-c) emerged, so that isolates could be grouped, two isolates/group were selected to serve as testers. These test isolates were than paired with isolates from plots 1-4 and 9-16. All isolates which were vegetatively incompatible with the test isolates were then paired with each other to determine if additional v-c groups could be established.

RESULTS

One-hundred and seventy-nine (89%) of the 202 *E. parasitica* isolates obtained from plots 1-16 were classified into 14 vegetative compatibility groups (Table 1). Groups A -G were established as the result of the original pairings of isolates from plots 5-8. Almost 80 percent of all isolates could be accounted for by one of these seven groups, with type A being by far the most common type encountered. Twelve percent (23 isolates) of the isolates could not be classified because they were vegetatively incompatible with all other isolates or did not give a consistent reaction when paired.

Trees in plots 1-4 had the highest incidence of infection and also contained the greatest number of v-c groups (Table 2). Only 12 percent of the trees in plots 9-12 were cankered and only 5 v-c groups were found. Group B, which occurred commonly in plots 1-8 (George Washington Forest), was not recovered from the Monongahela Forest plots.

An example of the distribution of v-c types is given for plot 1 (Fig. 1). Even though cankers of the same v-c type frequently occurred near each other, the distribution of types, when all plots were

Table 1
Vegetative compatibility types of 202 *Endothia parasitica* cankers in plots 1-16.

Group	Number of Isolates	(Percent)
A	47	24
B	26	13
C	18	9
D	26	13
E	10	5
F	21	11
G	10	5
H	4	2
I	2	1
J	4	2
K	5	3
L	2	1
M	2	1
N	2	1
Incompatible Isolates	23	11

Table 2
Occurrence of vegetative compatibility types in plots 1-16.

Plots 1-4	Plots 5-8	Plots 9-12	Plots 13-16
A	A	A	A
B	B		
C	C	C	C
D	D	D	D
E	E		E
F	F	F	F
G	G	G	
H			H
I			
J	J		
K			K
L	L		
M			
			N
Total Types			
13	9	5	8
% Trees Cankered			
61%	47%	12%	22%

considered, was highly variable. Similar results were observed when trees with multiple cankers were examined. When two or more cankers occurred on the same tree they were more commonly caused by isolates of different rather than similar v-c type (Table 3).

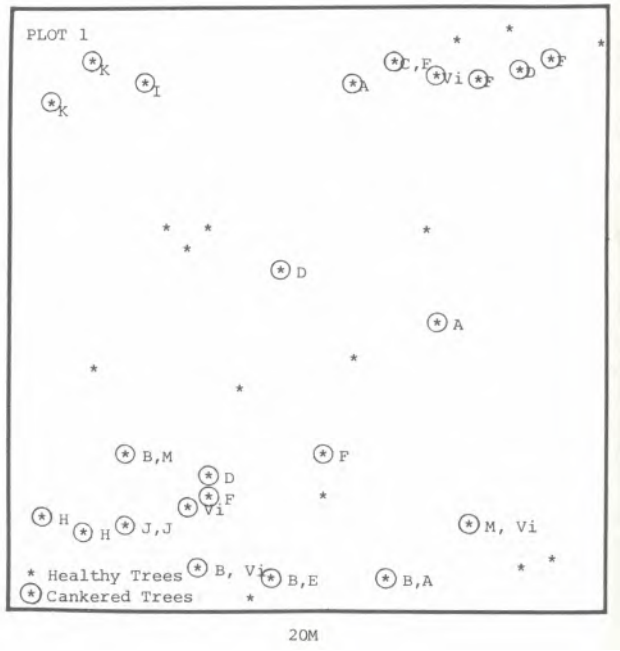

Figure 1. Distribution of vegetatively compatible (A-N) and incompatible (Vi) types of *Endothia parasitica* isolated from American chestnut.

DISCUSSION

The occurrence of more than one vegetative compatibility type was not surprising. Anagnostakis (1977) encountered as many as 28 types among isolates obtained from the United States and Europe. We could not, however, anticipate the frequency or distribution of v-c types among infections that occur in clear-cut areas where regeneration was predominately chestnut.

The occurrence of cankers caused by different v-c types on the same tree or on trees near one another was unexpected. This observation lends additional circumstantial evidence to previous findings that ascospores are the primary inoculum (Heald *et al.*, 1915). Anagnostakis (1977) has demonstrated that strains obtained from ascospores of a single perithecium are commonly of different v-c type. In other tests we found (unpublished data) that single conidial isolates obtained from different pycnidia produced by the same strain are identical to the parent in v-c type. Therefore, if conidia served as the primary source of inoculum then cankers would more commonly be of identical v-c type. This was not the case, even on trees with multiple cankers where rain-washed conidia from existing cankers would seem to function ideally as primary inoculum for new cankers on the same stem. We must also consider whether the frequency of the v-c types we found is a valid representation of their natural occurrence. Provided this is the case, then some explanation of incidence is necessary. While several explanations are possible, consideration of the natural v-c segregation ratios of ascospores from numerous cankers may provide an answer.

Table 3
Number of American chestnut trees with single and multiple *Endothia parasitica* cankers in Plots 1-16.

	Cankers/Tree					Total
	One	Two	Three	Four	Five	Cankered Trees
Plots 1-4	46	20(3;17)*	5(1;4)	2(1;1)	1(0;1)	74
Plots 5-8	31	5(2;3)	5(2;3)	—	—	41
Plots 9-12	10	2(1;1)	—	—	—	12
Plots 13-16	16	1(0;1)	—	—	—	17
Total	103	28(6;22)	10(3;7)	2(1;1)	1(0;1)	144

*Same compatibility type; different compatibility type.

If the phenomenon of vegetative incompatibility proves to be a barrier to the transmission of the determinants of hypovirulence in the field, then the 14 v-c groups and the other vegetatively incompatible strains we found may represent a formidable obstacle to the successful establishment of hypovirulent strains. In the event that strains are developed or evolve that control cankers caused by virulent strains of the seven major v-c types, less common v-c strains could persist to maintain the disease. In contrast, if ascospore segregates of common v-c strains give rise to less common types, control of common v-c strains might in turn result in the elimination of minor strains. This would appear to be a possible outcome if strain frequency is attributed to genetic segregation.

At present it is essential that the relationship between vegetative compatibility and hypovirulence be unraveled. Field trials are currently underway to meet this objective. Hopefully, vegetative incompatibility in *E. parasitica* will not be an obstacle to the success of hypovirulence in North America.

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LITERATURE CITED

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

Caten, C. E.
1972. VEGETATIVE INCOMPATIBILITY AND CYTOPLASMIC INFECTION IN FUNGI. J. Gen. Microbiol. 72: 221-229.

Heald, F. D., M. W. Gardner, and R. D. Studhalter.
1915. AIR AND WIND DISSEMINATION OF ASCOSPORES OF THE CHESTNUT-BLIGHT FUNGUS. J. Agr. Res. 3:493-531.

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