EFFECTS OF SELECTED NORTH AMERICAN AND ITALIAN CYTOPLASMIC HYPOVIRULENCE AGENTS ON NORTH AMERICAN AND ITALIAN STRAINS OF ENDOTHIA PARASITICA.

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ABSTRACT.--*Each of four North American and two Italian cytoplasmic hypovirulence (CH) agents were transmitted on agar into each of 22 isolates of Endothia parasitica. Half of the isolates were from North America and half from Italy. Eleven vegetative compatibility groups were represented. Three of the isolates from North America and five from Italy had previously been naturally infected with CH agents and freed of these by single conidial isolation. The others had no known history of natural infection with CH agents. Each agent caused a characteristic disease in each isolate, regardless of geographic origin or previous history.*

Introduction

Cytoplasmic hypovirulent (CH) strains of the chestnut blight fungus, *Endothia parasitica,* studied to date contain double-stranded RNA (dsRNA), have abnormal cultural characteristics, and reduced abilities to cause chestnut blight (Day et al. 1977; Elliston 1978; Dodds 1980). Because dsRNA is characteristic of fungal viruses (Lemke and Nash 1974), virus-like agents are assumed to be involved.

The abnormalities found among CH strains are diverse. Previous work suggests that a variety of virus-like CH agents may contribute to this diversity (Dodds 1980; Elliston unpublished data). Differences in responses of strains to individual CH agents may also contribute to the diversity. This study was conducted to explore this latter aspect of the diversity question. In this work, the effects were determined by introducing four American and two Italian CH agents individually into representative American and Italian strains.

Materials and Methods

Cytoplasmic hypovirulence agents. Table 1 lists the CH agents used, their sources, and the effects they had on cultural characterisitcs and pathogenicity of the source strains. All but HI_1 behaved as single agents; HI_1 appears to be a mixture that includes HI2.

Table 1. Sources and effects of six CH agents on cultural characteristics and pathogenicity of source strains.

CH agent	Source strain	Geographical origin	Cultural characteristics: Radial symmetry[a]	Radial striations[b]	Concentric rings[c]	Growth rate[d]	Pathogenicity[e]
H_{M_1}	EP-60	Michigan	-	-	-	+	-
H_{M_2}	EP-60	Michigan	+	+	+	++	++
H_{T_2}	EP-234	Tennessee	-	-	-	+	+
H_{V_1}	EP-700	Virginia	-	-	-	+	-
H_{I_1}	EP-419	Italy	+	-	±	++	++
H_{I_2}	EP-419	Italy	+	+	+	++++	+++
None			+	+	+	+++	+++

[a] + = radially symmetric; - = radially asymmetric.

[b] + = radially striated; - = not radially striated.

[c] + = concentric rings present; - = concentric rings absent.

[d] + = slow; ++ = moderate; +++ = fast; ++++ = very fast.

[e] - = nonpathogenic; + = very weakly pathogenic; ++ = moderately pathogenic; +++ = highly pathogenic.

Strains of *Endothia parasitica* and cultural conditions.. Table 2 lists the 22 strains of *E. parasitica* used as recipients of CH agents, their geographical sources, vegetative compatibility (v-c) group, and other pertinent information. Eight of the strains were normal single conidial isolates of strains naturally infected with CH agents.

Stock cultures were maintained on slants of Difco potato dextrose agar amended with 0.1 g L-methionine and 0.1 mg biotin per liter (PDAmb) at 4 C. Colonies used for inoculum were grown on 20 ml PDAmb in 100 x 15 mm petri dishes for 7 to 9 days at 20 C under fluorescent lights with a 16 hr photoperiod (standard conditions). Plates were sealed with a single layer of parafilm and inverted under the lights.

Determination of cultural characteristics. Cultural characteristics were determined with 7-day-old colonies grown under the conditions described. Inoculum for these colonies consisted of 8 mm diameter plugs cut with a sterile cork borer from the advancing margins of inoculum colonies. Four cultures of each strain were grown and examined.

Transmission experiments. The CH agents were transmitted from donor strains (those containing CH agents) to recipient strains (those lacking CH agents) on PDAmb. Donor and recipient strains in the same v-c group were paired on 100 x 15 mm plates containing 20 ml agar. Those in different groups were paired on 150 x 15 mm plates containing 40 ml agar to permit more extensive interactions. A vertical line was drawn with a marking pen across the bottom of each plate through the center. In most experiments, three donor inoculum blocks were placed near the center ca. 5 mm apart and 2 mm to the left of the line, with mycelium facing down and leading mycelium directed toward the line. Recipient inoculum blocks were placed similarly opposite the donor blocks ca. 2 mm to the right of the line. Colonies of donor and recipient strains used as controls were grown with only donor or recipient inoculum arranged as described. Three or four replicates of each pair and control

Table 2. Strains of *Endothia parasitica* used as recipients of CH agents.

Strain	Designation	Isolate/type source	Geographical origin	Vegetative compatibility group
EP-42	BAR V	MI[a]	Connecticut	5
EP-144	7-8-1	MI	West Virginia	30
EP-155	p17+3	MI	Connecticut	40
EP-305	14A	MI	Connecticut	40
EP-366	p12-5	SAI[b]	Connecticut	39
EP-393	338x290,p7t2-3	SAI, cre,[c] met-[d]	Connecticut	5
EP-502	None	MI	Connecticut	10
EP-516	EP-6-b-4	SCI,[e] EP-6,met-	Connecticut	8
EP-523	60N	SCI,EP-60-b-9	Michigan	9
EP-589	234N	SCI,EP-234	Tennessee	24
EP-709	700N	SCI,EP-700	Virginia	49
EP-46	1012	MI	Tuscany, Italy	11
EP-62	None	SCI,EP-49	Tuscany, Italy	12
EP-65	None	SCI,EP-51	Tuscany, Italy	11
EP-67	None	SCI,EP-50	Tuscany, Italy	10
EP-408	2-4	MI	Tuscany, Italy	12
EP-409	3-8	MI	Tuscany, Italy	40
EP-421	22-8-b-6	SCI,22-8	Tuscany, Italy	11
EP-427	28-1	MI	Piedmont, Italy	20
EP-432	38-1	MI	Campania, Italy	30
EP-462	58-2	MI	Campania, Italy	30
EP-505	27-9-c-76	SCI,27-9	Piedmont, Italy	40

[a] Mass isolate.
[b] Single ascospore isolate.
[c] Cream mutant.
[d] Methionine-requiring mutant.
[e] Single conidial isolate.

were used in each experiment. Plates were sealed with parafilm and incubated under standard conditions.

Donor strains used in early transmission experiments included single conidial derivatives of source strains and a few products of earlier transmission experiments. New infection products were added to the list of donors as they were obtained. Recipient strains not successfully infected in early experiments were paired with the new donors as they became available. To check that transmission had occurred in cases of difficult transmission, products were paired with the uninfected form of the recipient strain. Rapid transmission between a product and its corresponding uninfected form was taken to indicate that the CH agent had been transmitted successfully.

Field pathogenicity test. Eight of the 22 sets of strains were tested for short term pathogenicity in eight 11 to 14 cm d.b.h. (diameter at 1.4 m) American chestnut sprouts in the forest. Each set included a recipient strain in its uninfected form and the same strain in each of five infection states. On each tree, an approximately 3 m length of trunk with smooth bark was divided into eight nearly equal regions. Sets of strains were assigned to positions (regions) on the eight trees according to an 8 x 8 Latin square design. Infection states within a strain set were arranged randomly within the region assigned to the set. Inoculations were made as described (Elliston 1978). Canker length and width were measured to the limits of infection or injury two months after inoculation. Canker areas were calculated using the formula

for an ellipse.

Results

Transmission experiments and cultural characteristics of products. Each of the six CH agents was successfully transmitted into each of the 22 recipient strains of the fungus. The culture collection numbers for the products are listed in Table 3. Each agent similarly affected cultural characteristics of all 22 recipient strains. Two representative sets of strains are shown in Figure 1. The most variable effects were observed with $H1_1$ infections. Subcultures of strains infected with this mixture of agents differed in pigmentation, amount of aerial mycelium, arrangement of pycnidia, and density and organization of leading mycelium.

Table 3. Culture collection numbers for products of transmission experiments

CH agent	Recipient strain										
	42	144	155	305	366	393	502	516	523	589	709
A. American Recipient Strains											
H_{M_1}	1025	843	844	845	833	832	836	552	544	715	839
H_{M_2}	1056	852	868	869	855	851	864	570	524	850	863
H_{T_2}	1060	894	905	906	878	902	892	600	629	653	910
H_{V_1}	1057	913	915	916	718	719	911	806	716	804	708
H_{I_1}	1058	776	779	781	848	818	803	923	395	820	823
H_{I_2}	1059	821	780	805	822	819	775	924	721	722	824
B. Italian Recipient Strains											
	46	62	65	67	408	409	421	427	432	462	505
H_{M_1}	837	791	838	788	793	846	786	847	795	796	799
H_{M_2}	857	859	858	865	860	870	811	808	853	854	871
H_{T_2}	886	893	887	885	901	907	810	809	895	877	908
H_{V_1}	790	792	912	789	794	798	717	801	914	797	800
H_{I_1}	761	762	763	765	767	812	757	771	773	849	769
H_{I_2}	759	760	764	766	768	816	758	772	922	813	770

Pathogenicity tests. The results of the pathogenicity test are summarized in Table 4. No statistically significant differences were found among areas of cankers caused by the eight strains in the uninfected state or when infected with agents Hm_1 or H12. With strains infected with agents HM2, HT2, and $HI_?$, a few statistically significant differences were found but no

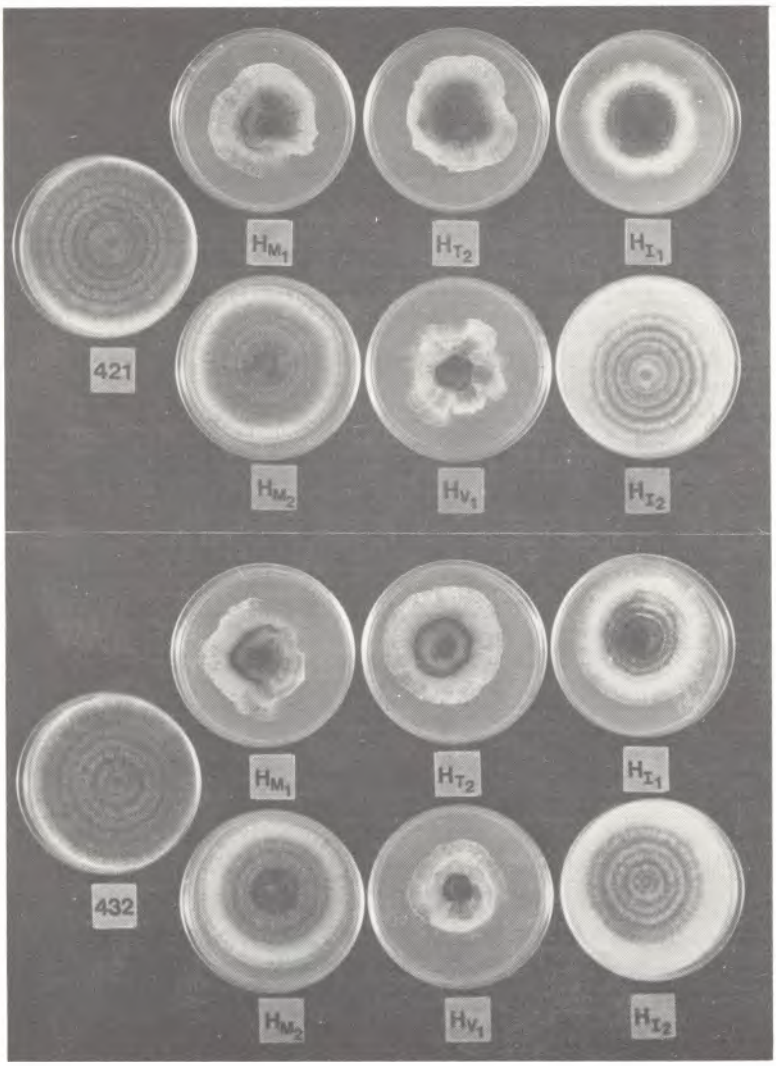

Figure 1. Representative recipient strains in their uninfected forms (left) and infected with six CH agents; A, recipient strain EP-421; B, recipient strain EP-432.

consistent patterns were observed. The statistical analysis of the data indicated highly significant differences occurred among trees ($F=4,994$), but not among inoculated regions within trees ($F=0.21$).

Discussion

In the study reported here, the five individual CH agents each had consistent effects on cultural characteristics of recipient strains whether the recipients were from North America or Italy or previously infected or not previously infected. Even EP-393, the cream mutant, showed consistent changes in colony morphology when infected with the agents; only colony color was distinct. Cultural characteristics of strains infected with H11, the only mixture of agents used, were least consistent within subcultures of a given strain. This inconsistency may reflect the involvement of a mixture of agents. The inconsistency Anagnostakis and Day (1979) and Anagnostakis (1981) reported among derivatives of European strains may also have been caused by mixtures of agents.

Table 4. Mean canker area for eight recipient strains and their infection products

Recipient strain	Mean canker area, cm^2, for strain infected with CH agent: y/					
	None	H_{M_1}	H_{M_2}	H_{T_2}	H_{I_1}	H_{I_2}
EP-144	54.0 z/	0.5	7.0 bc	0.8 b	16.8 ab	37.2
EP-155	58.6	0.8	11.4 a	1.6 a	18.1 a	42.6
EP-523	57.6	0.6	5.8 c	0.8 b	13.3 abcd	44.7
EP-589	56.8	1.0	8.4 abc	0.7 b	16.0 abc	52.0
EP-408	53.7	0.4	8.8 abc	0.8 b	12.4 bcd	51.3
EP-432	54.6	0.5	9.2 ab	1.5 a	9.9 d	42.2
EP-421	51.8	0.4	10.5 a	0.8 b	11.4 cd	45.7
EP-505	62.7	0.8	10.3 ab	2.0 a	13.7 abcd	42.4

y/ Means within columns not followed by the same letter are significantly different at $P \leq 0.05$, based on Duncan's "new multiple range test".

z/ Mean for eight cankers two months after inoculation.

The pathogenicities of the eight recipient strains in the uninfected form were not significantly different. This is in contrast with results reported previously by Jaynes and Elliston (1980) and may be due to having inoculated all strains into each tree in this experiment thus eliminating differences due to trees. Highly significant differences were found among trees in this study, but not among inoculated regions within trees. These results support the conclusion (Elliston 1978) that all strains in a pathogenicity determination should be inoculated into each tree, if that is feasible.

For the most part, a given CH agent affected the pathogenicities of the eight recipient strains similarly. The few significant differences found by statistical analysis may have been due more to the method of measuring cankers than to actual differences in amounts of colonized tissue. Measurements were made to the maximum limits of disrupted tissue. With weakly and moderately pathogenic strains, callus tissue often developed at the margin of the colonized tissue and sometimes caused uncolonized bark tissue at the top and bottom of the site to split and die back, enlarging the total amount of disrupted tissue and the calculated canker areas. The amount of callus was not uniform from site to site. The extent to which this affected the results is not clear.

Tha availability of many sets of recipient strains infected with a variety of CH agents will permit detailed comparisons of dsRNA patterns to determine if each agent has a consistently different pattern of dsRNA components. These strains will also be useful for establishing novel mixed infections and for preparing slurries (Jaynes and Elliston 1980) containing specific CH agents or combinations of agents for better defined studies of canker control.

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