Vegetative Compatibility and Hypovirulence Conversion Among Naturally Occurring Isolates of *Cryphonectria parasitica*

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Accepted for publication 13 January 1984 (submitted for electronic processing).

ABSTRACT

Kuhlman, E. G., and Bhattacharyya, H. 1984. Vegetative compatibility and hypovirulence conversion among naturally occurring isolates of *Cryphonectria parasitica*. Phytopathology 74:659-664.

Virulent (V) isolates of *Cryphonectria parasitica* were readily recovered from both sunken cankers and swollen superficial cankers on American chestnut trees in the southern Appalachians. Hypovirulent (H) isolates of *C. parasitica* made up 38% of the population in six swollen superficial cankers on one isolated tree in Tennessee but were infrequently recovered from 9 sunken and 52 swollen superficial cankers from North Carolina, Virginia, and Italy. A cluster analysis was used to group V isolates by vegetative compatibility (v-c) and by conversion susceptibility. Isolates from the Tennessee tree had four distinct v-c groups identified by the merge-barrage response and each canker averaged 2.7 v-c groups. Forty-one cankers on 19 trees near Buchanan, VA, yielded 2.3 v-c groups per canker. Pairings among 93 randomly selected isolates from these within canker groups indicated 17 v-c groups were present in the 303-m-long study area, with an average range of 134 m per v-c group. Susceptibility to conversion by six H isolates from the area was present in 80% of the 93 isolates. Although susceptibility to conversion was widespread (average 217 m per H isolate), the H isolates occurred in only four of 41 cankers over a distance of 56.7 m. V isolates were identified with broad susceptibility to conversion by H isolates from several v-c groups and H isolates were identified with broad capacity for conversion of V isolates from several v-c groups. When American chestnut saplings were inoculated with H isolates, 52% were live and healed over, 28% were live and infected, and 20% had dieback to the inoculation point in 27–29 mo. Inoculation of saplings with V isolates resulted in 3% live and 97% with dieback.

Additional key words: *Castanea dentata*, chestnut blight, *Endothia parasitica*, Koch's postulates.

The hypothesis that hypovirulent (H) isolates of *Cryphonectria parasitica* (Murr.) Barr (=*Endothia parasitica* (Murr.) P.J. & H.W. Anderson) were the cause of superficial swollen cankers on European chestnut (*Castanea sativa* Mill.) in Italy has been supported by several authors (14–16,26). Sunken cankers resulted in shoot dieback, whereas superficial swollen cankers did not cause dieback. According to this hypothesis, H isolates of *C. parasitica* were most common in superficial swollen cankers, whereas only normal, virulent (V) isolates were present in sunken cankers. The ratio of H to V isolates was directly related to the ratio of sunken to superficial swollen canker symptoms. Conflicting evidence about this hypothesis has been presented. In rebuttal, Palenzona (24) reported that the occurrence of H and V isolates was unrelated to the frequency of sunken cankers near Torino, Italy. In support of the hypothesis, two reports indicated hypovirulent isolates may be responsible for survival of large American chestnuts (*C. dentata* (Marsh.) Borkh.) in the eastern United States (17,18).

Grente and Sauret (16) suggested hypovirulence was cytoplasmically transmitted because conidia from H isolates yielded H and V cultures, whereas those from V isolates yielded only V cultures. Van Alfen et al (27) used auxotrophic isolates of *C. parasitica* to confirm that hypovirulence was a cytoplasmically transmitted determinant. Anagnostakis and Day (2) developed a laboratory method for transmitting the H factor to V isolates. This method can be used to demonstrate the infectious or invasive nature of hypovirulence and therefore confirms that hypovirulence is cytoplasmically transmitted (11). Jaynes and Elliston (18) broadened the definition of hypovirulence to include departures in cultural morphology from "normal" isolates, whereas cytoplasmic hypovirulence for them indicated a cytoplasmic factor causing reduced virulence. We have used the original definition of hypovirulence as a cytoplasmically transmitted determinant (16,27).

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Conversion of V isolates to H isolates requires the exchange of cytoplasm that contains the H factor from H to V isolates. Anagnostakis (1) developed a technique for separating V isolates into vegetative compatibility (v-c) groups. Although only isolates within v-c groups presumably readily exchange cytoplasm, several reports (1,2,19) indicated conversion of V to H isolates was not limited to within v-c groups.

The presence of H isolates of *C. parasitica* has been reported from American chestnuts in several areas of the eastern United States (6,9,18,19); however, no attempts have been reported to quantify the occurrence of H isolates or to determine if conversion capacity was a barrier to spread of the H factor. The present study determined the relative occurrence of H and V isolates in sunken and superficial swollen cankers, grouped isolates from within two areas by v-c and by susceptibility to conversion by native H isolates, and determined the relative pathogenicity of H and V isolates. Portions of this study were presented in a preliminary report (19).

MATERIALS AND METHODS

Study areas. The presence of nongirdling superficial swollen cankers on larger American chestnut trees was the criterion for selecting study areas near Buchanan, VA, Franklin, NC, and Robbinsville, NC. H isolates have been reported previously from cankers at Buchanan (Natural Bridge), VA, and Bonair (Cumberland), TN (9,18,19). Two cankers from European chestnuts in Italy were received in response to a request for superficial swollen cankers, but those cankers had more sunken canker tissue than swollen canker tissue and we classified them as sunken cankers.

Isolations. Twenty bark and wood chips were removed from each of 41 cankers on 19 trees at Buchanan and 6 cankers on 1 tree at Bonair. At these two locations, half of the chips were taken from around the canker margin and half were systematically taken across the canker center. Twelve to 30 chips were removed from each canker from Franklin, Robbinsville, and Italy with no attempt to sample the canker margin separately from the canker center.

Chips were surface-sterilized in 65% ethyl alcohol and plated on Difco potato-dextrose agar (PDA). Isolates of *C. parasitica* were subcultured on PDA and incubated under white fluorescent lights

(16-hr photoperiod) to subjectively separate V and probable H isolates (21).

Conversion and v-c groups. Each *C. parasitica* isolate from each canker at Buchanan and Bonair was paired with all other isolates from that canker to test for merge groups within cankers in a modification of Anagnostakis' technique (1) as described previously (21). Compatible isolates within cankers based on the merge response were designated as a group. One randomly selected isolate from each group within a canker was paired with randomly selected isolates from all other groups within that study area.

Probable H isolates were paired with compatible V isolates (same v-c group within a canker) on PDA with 100 mg of methionine and 1 mg of biotin per liter (PDAmb) (1) to confirm the presence of a cytoplasmic factor that was infectious and affected the cultural growth of V isolates. Anagnostakis and Day's (2) conversion method was modified by eliminating the cellophane and by placing 5-mm agar disks with mycelium of the V and H isolates 10 mm apart at the center of the petri dish. Plates were incubated at 25 C with a 16-hr photoperiod (white fluorescent light). Conversion was determined 4–11 days after pairing by changes in growth habit of the V isolate. Subcultures from converted sectors were grown on PDAmb and compared with subcultures of the V isolate to confirm conversion. Conversion response of the randomly selected isolates was determined in pairings with six H isolates from Buchanan and with 13 H isolates from Bonair.

A cluster analysis (10) was used to group isolates within the Bonair and Buchanan areas by v-c response and by conversion response to H isolates from the study area (22). In performing the cluster analysis to determine v-c groups, the distance between two isolates was defined as a function of the number of times both isolates merged with each other and with any of the other 16 or 91 isolates. Similarly, in performing the cluster analysis to determine conversion groups, the distance between two isolates was defined as a function of the number of times both isolates were converted by the same H isolate(s).

Pathogenicity. Pathogenicity of H and V isolates was evaluated by inoculating small American chestnut saplings growing near Franklin, NC. Saplings were 1.3–2.5 cm in diameter at the single inoculation point and free of blight symptoms. In 1980, 14 isolates were used, including each of four V and H isolates randomly selected from our collection, one V and one H isolate from Buchanan, and four H isolates of intermediate pathogenicity from Italy (7; J. E. Elliston, *personal communication*). Two inoculation times, April and June, and six replicates per isolate at each inoculation time were used. In May 1981, nine V and nine H isolates from the Bonair, Buchanan, and Italian collections were randomly selected. The sequence for the 18 isolate treatments was randomly determined for each of eight replicates. All isolates were grown on PDA.

A 7-mm bark disk was removed from the wood with a cork borer and a 7-mm agar disk with mycelium of the V or H isolate was placed in the wound. The inoculation area was covered with masking tape for 6 wk. Sign and symptom development was observed in both experiments for 27–29 mo after the inoculations.

RESULTS

Isolation. Isolates of *C. parasitica* were recovered from 1,097 of 1,460 chips from 67 blight cankers (Table 1). Thirty-seven of the 1,097 isolates were designated H because they had an infectious property that affected their growth in culture and could convert related V isolates to the abnormal growth habit. H isolates were present in both sunken and swollen superficial cankers. The six cankers on the Bonair tree yielded 28 H isolates out of 73 *C. parasitica* isolates recovered (38%). Four cankers at Buchanan yielded single H isolates and one canker from Italy yielded five H isolates.

The six swollen superficial cankers on the Bonair tree occurred on the main stem from its base to a height of 9.75 m. Neither height of the canker above the ground nor canker size influenced the frequency of H isolate recovery (Table 2). Recovery of *C. parasitica* was poorest from the large basal canker. Bark chips taken from the canker margin had a lower ratio of H isolates per total *C. parasitica* isolates (9/32) than did chips from the canker center (19/41).

Grouping by v-c and conversion. *Bonair area.* All isolates within each canker were tested for the merge response with other isolates from that canker. Each canker had two to four merge groups so that the six cankers yielded 18 groups. One isolate from each of the 18 groups was randomly selected for pairing with the other 17 isolates. The 18 isolates had a merge pattern that indicated four v-c groups were present in the six cankers. The cluster analysis also identified four groups (labeled 1–4) (Table 3). The eight isolates in group 1 merged with four to eight of the isolates in the group, whereas all isolates in other groups merged with all other isolates within each respective group. Each canker had two or three v-c groups (Table 2).

Conversion of the 18 randomly selected V isolates by 13 H isolates from Bonair was generally similar for members of the four v-c groups (Table 3); however, V isolates 618 and 405 in v-c group 1 were converted only by H isolates 320 and 605, which converted no other isolates in group 1. H isolates 211 and 320 converted isolates from three and four v-c groups, respectively, whereas the other H isolates converted isolates within only single v-c groups.

Buchanan area. When all isolates of *C. parasitica* from each canker from Buchanan were paired with each other, there appeared to be one to five merge groups per canker so that the 41 cankers had 93 within-canker groups. Randomly selected isolates of each of the 93 groups were paired in all combinations to determine v-c grouping within the Buchanan study area. The merge response occurred an average of seven times per isolate, with variations from 1 to 23. Use of cluster analysis resulted in 17 v-c groups with 2–15 isolates per group (Table 4). Four of the isolates did not cluster with other isolates. Pairing among the 93 randomly selected isolates confirmed the within-canker grouping in 37 of 41 cankers. That is, the groups within cankers remained distinct according to the

TABLE 1. Occurrence of hypovirulent (H) isolates of *Cryphonectria parasitica* in sunken and swollen superifical cankers on American and European chestnuts

Location	Cankers[a] (no.) ss	sk	Range DBH[b] of trees (cm)	Sample chips (no.)	Isolates (no.)	(no.)
Buchanan, VA	34		2–28	680	499	4
Bonair, TN	6		43	120	73	28
Franklin, NC	10		5–30	258	176	0
Robbinsville, NC	8		3–8	200	187	0
Buchanan, VA		7	2–8	140	103	0
Italy		2	4–6	62	59	5
Total	58	9		1,460	1,097	37

[a] ss = Infections with abundant callusing and no dieback distal to canker. sk = Infections produced sunken tissue across canker face or death of tree distal to canker.
[b] dbh = Diameter breast height.

TABLE 2. Canker and isolate data for six chestnut blight cankers on an American chestnut tree near Bonair, TN

Canker No.	Height (m)	Area (cm^2)	*Cryphonectria parasitica* Total isolates (no.)	H isolates (%)	v-c groups[a] (no.)	Conversion groups[b] (no.)
2	9.75	3,098	11	45	3	3
3	8.23	1,445	11	36	2	2
1	6.71	5,266	14	43	3	3
5	6.10	13,651	14	43	3	3
6	5.49	7,966	17	29	3	4
4	0–3.66	39,945	6	33	2	2

[a] v-c = Vegetative compatibility groups based on merge or barrage responses among isolates.
[b] Conversion groups based on conversion to the hypovirulent condition by contact with one or more H isolates.

cluster analysis in 37 of 41 cankers. Twelve cankers had only one v-c group present, whereas 29 cankers had two to five groups present. The average area of the 12 cankers with single v-c groups was 1,368 cm^2 compared with 4,342 cm^2 for the 29 cankers with two or more v-c groups.

The 93 within-canker isolates were paired with six H isolates recovered from four of the cankers in 1979, 1980, and 1981. Conversion of 74 isolates (80%) occurred in pairings with one or more of the H isolates (Table 4). Cluster analysis of this response formed five conversion groups (Table 4). Isolates in v-c group 1 had the most similar conversion response because 13 of 15 isolates were in conversion group 1. Isolates in other v-c groups tended to be present in several conversion groups or contained so few isolates that no trends were indicated.

The six H isolates were assigned to v-c groups on the basis of the conversion of a V isolate from within their canker of origin. The H isolates converted isolates in three to 11 v-c groups (Table 4). H isolates 545 and 543 converted 34 and 48 randomly selected isolates from nine and 11 v-c groups; however, the isolates in v-c group 1, to which those H isolates belong, were converted most readily only by those two H isolates. H isolates 546 and 547 and v-c group 2 responded in an opposite manner, with only four and three v-c groups being converted by the H isolates, but some V isolates in v-c group 2 were susceptible to conversion by each of the six H isolates.

TABLE 3. Eighteen virulent (V) isolates of *Cryphonectria parasitica* from Bonair, TN, representing within-canker v-c groups clustered by merge response and by conversion (C) response with 13 hypovirulent (H) isolates

V isolate	v-c group[a]	H isolate													C group[b]
		115	211	213	308	412	511	601	108	502	619	320	605	407	
102	1	C	C	C	C	C	C	C							I
227	1	C		C	C	C	C	C							I
416	1	C		C	C	C	C	C							I
614	1	C		C	C	C	C	C							I
513	1		C	C	C	C									IV
317	1			C	C	C	C	C							I
618	1												C		IV
405	1											C	C		IV
431	2	C							C	C	C	C			II
203	2	C							C	C	C	C			II
604	2								C	C	C	C			II
112	2								C	C	C				II
504	2								C	C	C				II
113	3	C										C			III
602	3	C										C			III
214	3	C													V
309	4											C			III
507	4											C			III

[a]Grouping based on cluster analysis of merge response of pairs of V isolates.
[b]Grouping based on cluster analysis of conversion response of pairs of V and H isolates.

TABLE 4. Clustering of within-canker virulent (V) isolates from Buchanan, VA, by vegetative compatibility (v-c) and conversion response. Conversion of virulent isolates by six hypovirulent (H) isolates recovered from the study area. v-c groups are subjectively arranged by relative occurrence in conversion groups

v-c group	Total isolates	V isolates (no.)											
		Conversion group					Not converted	H isolates (by culture number) converting					
		I	II	III	IV	V		543	545	544	514	546	547
1	15	13	1	1	0	0	0	14[a]	14[a]	2	1	0	0
3	9	4	4	0	0	0	1	8	4	0	0	0	0
7	4	3	0	1	0	0	0	4	3	1	0	0	0
9	4	2	1	0	0	0	1	3	2	0	0	0	0
15	3	1	1	0	0	0	1	2	2	0	0	0	0
16	3	0	1	0	0	0	2	1	0	0	0	0	0
NG[b]	2	2	0	0	0	0	0	2	2	0	0	0	0
4	8	0	0	5	2	0	1	0	0	5	4	0	0
6	4	0	0	3	0	1	0	1	1	4	3	2	1
8	4	0	1	1	1	0	1	0	0	2	3	0	0
10	3	0	0	1	1	0	1	1	0	1	1	0	0
11	3	0	0	2	0	0	1	0	0	2	2	0	0
14	3	0	0	1	0	0	2	0	0	1	1	0	0
NG[b]	1	0	0	1	0	0	0	0	0	1[a]	1[a]	0	0
2	14	1	6	4	0	2	1	10	4	8	5	10[a]	9[a]
5	4	1	0	1	0	0	2	1	1	1	1	0	0
12	3	1	0	0	0	2	0	1	1	0	0	2	0
13	3	0	0	0	0	1	2	0	0	0	0	1	1
17	2	0	0	0	0	0	2	0	0	0	0	0	0
NG[b]	1	0	0	0	0	0	1	0	0	0	0	0	0
V isolates	93	28	15	21	4	6	19	48	34	28	22	15	11
v-c groups	17	8	7	10	3	4	13	11	9	10	9	4	3

[a]One V isolate was from v-c source of H isolate.
[b]NG = isolates not grouped.

The 41 cankers at Buchanan occurred on 19 trees growing in a 303-m-long study area with only minor departures from a straight line. Cankers with four of the six H isolates were used as the plot center. Isolates in v-c group 1 were recovered from some cankers on trees throughout the 303-m-long area (Fig. 1). Other v-c groups occurred on cankers for 1–279 m within the study area, with an average of 134 m for the 17 v-c groups. Because 80% of the representative isolates were converted in culture, it was possible to determine the range of susceptibility to conversion in the virulent population for each of the six H isolates. Susceptibility to conversion was present over an average of 217 m of the study area (Fig. 2). Susceptibility to conversion by three of these H isolates was present throughout the study area; however, the three H isolates occurred only on three cankers on two sprouts from one stump.

Pathogenicity. V isolates of *C. parasitica* from American and Italian sources caused dieback of most American chestnut saplings (Table 5). In contrast, American chestnut saplings most often formed healing callus at the inoculation site of H isolates. H isolates from Italy more often produced symptoms of infection than did H isolates from America. One of four Italian H isolates used in 1980 and one of three used in 1981 produced swollen superficial cankers on half of the saplings and normal cankers on the other half.

DISCUSSION

H isolates of *C. parasitica* were not commonly associated with swollen superficial cankers on American chestnut trees in Virginia and North Carolina (Table 1). An isolated American chestnut near Bonair, TN, had a population of *C. parasitica* composed of 38% H

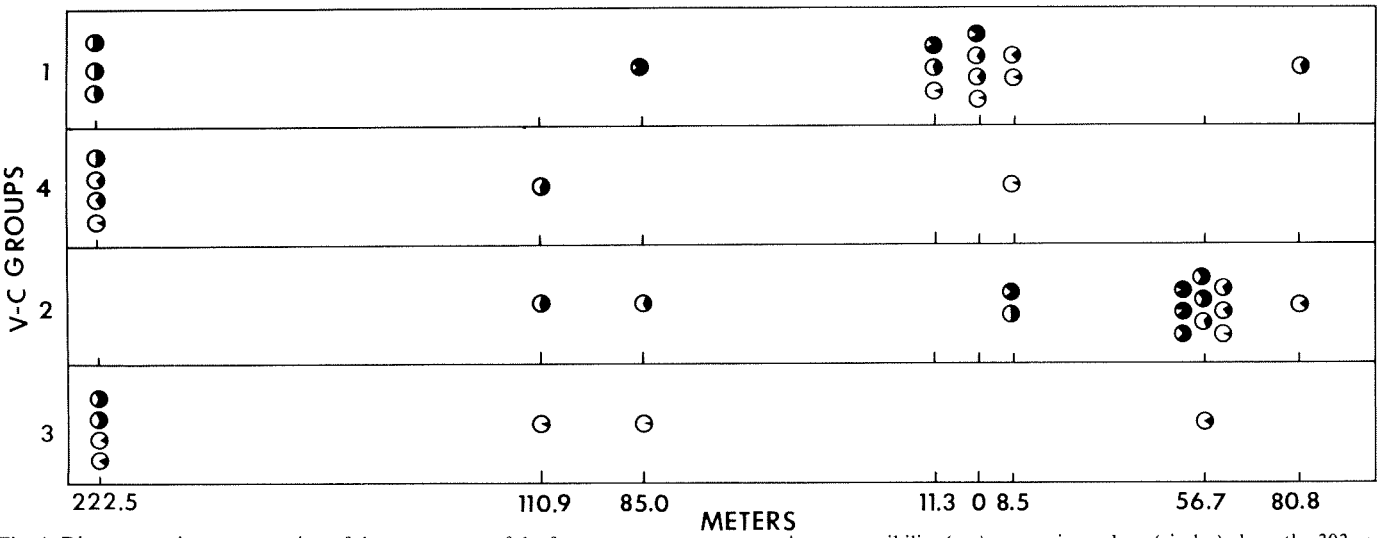

Fig. 1. Diagrammatic representation of the occurrence of the four most common vegetative compatibility (v-c) groups in cankers (circles) along the 303-m study area near Buchanan, VA. Occurrence is indicated by the number of cankers and the relative canker area (dark areas) colonized by virulent isolates of *Cryphonectria parasitica* of each v-c group. Four of six hypovirulent (H) isolates occurred in cankers at the 0-m mark.

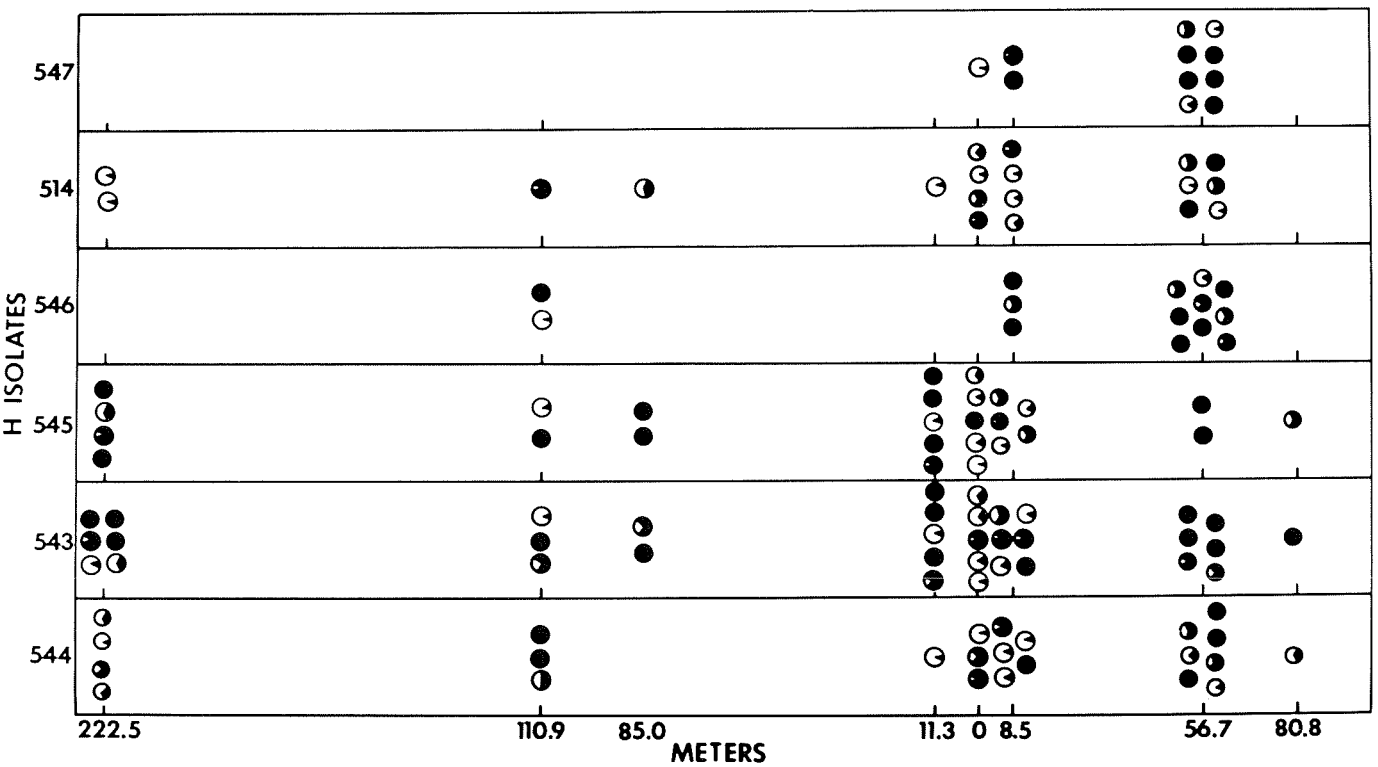

Fig. 2. Occurrence of susceptibility to invasiveness of the hypovirulent (H) factors from each of six H isolates of *Cryphonectria parasitica* among virulent (V) isolates in cankers (circles) at various distances from the 0-m mark where four of six H isolates were recovered, whereas isolates 546 and 547 occurred in a canker at 56.7 m. Occurrence is indicated by the number of cankers and the relative canker area (dark areas) colonized by V isolates susceptible to conversion.

isolates. The blight infections on this tree were unusually superficial and the only obvious symptom of infection was roughened bark. This tree is a codominant with its crown in the overstory with other dominant trees of this mixed hardwood stand. No other American chestnuts were seen along the 3.2-km trail through the hardwood stand to the tree. The tree is on the site of an abandoned farm and may have been planted, although American chestnut was native to that region of the Cumberland Plateau. This combination of isolated existence and common occurrence of H isolates appears similar to conditions in Michigan (5). The cankers at Buchanan and Franklin were sampled because of the presence of several large individual trees and the swollen appearance of many cankers. The largest tree at Buchanan had a basal canker extending 2.7 m up the trunk. Although this canker was swollen and yielded one H isolate, the tree died within a year.

Chestnut blight infections with swollen cankers have often intrigued pathologists. Shear et al (25) illustrated both sunken and swollen cankers. In 1950, Graves (13) noted that a swollen canker on a "blight resistant" American chestnut occurred on a thin soil. Graves (13) suggested the unfavorable site showed the tree was resistant. Our study areas were either ridge tops or north-facing slopes where adverse weather might be expected. Fast-growing American chestnuts in clear-cuts or release cuttings commonly develop sunken cankers. Adverse growth conditions may slow tree growth and canker development and thereby promote swollen cankers. Thus environment rather than host or pathogen may often be the cause of swollen cankers.

Isolates of *C. parasitica* designated H on the basis of an infectious cytoplasmic factor differed dramatically from V isolates in pathogenicity on American chestnut saplings (Table 5). Randomly selected V and H isolates from American sources used in 1980 are almost on opposite ends of the pathogenicity spectrum. Because the isolates were selected randomly, they can be considered representative of more than 100 isolates of each type in our collection. Even isolates of intermediate pathogenicity from Italy had limited pathogenicity under our conditions. Recently, Jaynes and Elliston (18) suggested that isolates with reduced pathogenicity commonly were associated with large surviving American chestnuts. They used Elliston's (7,8) definition of hypovirulent isolates, which includes changes in cultural morphology, presence of dsRNA, and/or number of single-conidial colony types. Only the seven isolates in their study with two or more single-conidial colony types can be considered to have an infectious cytoplasmic factor according to the criteria of Fincham et al (11) for demonstrating extranuclear inheritance. These isolates produced canker areas 18% as large as the single virulent standard did, whereas six isolates with one single-conidial colony type produced cankers 70% as large as the standard (18). Their other 11 isolates cannot be designated H or non-H on the basis of an infectious cytoplasmic factor. Unless evidence is presented linking changes in cultural morphology with genetic control of virulence, these isolates seemingly should be considered expressions of the range of virulence and cultural morphology for V isolates.

H isolates from swollen superficial cankers in the southern Appalachians failed to satisfy two of Koch's postulates. First, H isolates were not regularly associated with swollen superficial cankers. Second, when H isolates were recovered from blight cankers they did not produce swollen superficial symptoms. The H isolates from the Bonair tree with its very superficial cankers were nearly avirulent, as was a random selection of H isolates from our culture collection. Two of six H isolates from Italy produced the swollen superficial symptom, but at least one of these was from a sunken canker. H isolates apparently are common in Italy and Michigan (5,6,15,24,28); however, a rigorous survey in Italy found H isolates were not limited to superficial cankers (24).

The presence of one to five v-c groups in individual cankers at Bonair and Buchanan indicated multiple infections occurred. Previously, Kuhlman (21) reported that secondary infections by V isolates of cankers caused stem dieback in trees that had shown signs of healing. The Bonair tree also had H isolates from two to four conversion groups in each canker and therefore was a unique example supporting the Grente-Sauret hypothesis (14–16).

Vegetative and conversion incompatibility have been viewed as possible barriers to spread of hypovirulence in *C. parasitica* (1–3,11,20). In the Bonair tree, we found similar v-c groups and conversion capacity in each of the six cankers spread vertically over 9.75 m. Although 17 v-c groups were present in the Buchanan cankers, the four major groups had spread horizontally along much of the 303-m study area. Susceptibility to conversion was present in 80% of the 93 canker groups confronted with six H isolates from the area. Conversion capacity was widespread for each of the H isolates and should not have limited spread. Because the H condition occurred infrequently in our sample, other factors must limit its spread. For example, some H isolates sporulate less than V isolates (7) and this could limit spread.

A correlation analysis of the frequency of merge response among the 93 V isolates from Buchanan versus the conversion susceptibility to the six H isolates indicated $r = 0.044$, whereas the merge response among 18 V isolates from Bonair versus conversion susceptibility to 13 H isolates revealed $r = 0.79$. The isolated occurrence of the Bonair tree resulted in a relatively homogenous mix of V and H isolates. The Buchanan cankers were more widely distributed and had many nearby American chestnut stands to produce a heterogeneous mix of V isolates, whereas the H isolate population was low.

With 50 mass isolates, Anagnostakis (1) reported all V strains within a v-c group merged with each other, whereas pairs of strains from different groups formed barrage zones. From the Buchanan area, 21 of 93 randomly selected isolates formed a barrage response when selfed. Single-hyphal-tip isolates from these isolates had demonstrated the isolates were not multiple strains (20). MacDonald and Double (23) paired all isolates within four plots to delineate nine v-c groups, then used two isolates per v-c group as testers for other plots. Isolates not compatible with the testers were paired with each other to identify five other v-c groups and 23 isolates that were incompatible with any isolates. We designated within-canker v-c groups by pairing all isolates in a canker. Randomly selected isolates from all within-canker groups were then paired with each other. In the Bonair tree, v-c groups were easy to identify because pairs of isolates within groups almost always merged with each other, whereas pairing among isolates between groups never produced the merge response. For the Buchanan study area, v-c grouping was more complex because the 93 within-canker groups did not merge in distinct, exclusive groups. We believe this lack of exclusiveness indicates that v-c groups overlap in complex patterns perhaps controlled by the number of similar genes, as Anagnostakis and Waggoner (3) have suggested for conversion capacity. Pairings among the 93 isolates subjectively suggested several major v-c groups but the cluster analysis provided an objective method for categorizing groups. Initially, we used an

TABLE 5. Pathogenicity of hypovirulent (H) and virulent (V) isolates of *Cryphonectria parasitica* 27–29 mo after inoculation of American chestnut saplings

Isolate type and source	Inoculation date(s)	Isolates (no.)	Reps. (no.)	Symptoms (%)		
				Dieback	Live/ infected	Live/ healed
V isolate						
American	Apr., June 1980	5	12	93	3	3
American	May 1981	6	8	100	0	0
Italian	May 1981	3	8	100	0	0
H isolate						
American	Apr., June 1980	5	12	8	12	80
Italian	Apr., June 1980	4	12	19	48	33
American	May 1981	6	8	29	27	44
Italian	May 1981	3	8	36	32	32
Weighted averages						
V isolates				97	1.5	1.5
H isolates				20	28.0	52.0

SAS cluster analysis program that was readily accessible (12). In comparing the response of any two isolates to another isolate, the SAS program used a distance function that gave equal measure of similarity to merge-merge (1-1) and barrage-barrage (0-0) responses. Using this definition of distance, clusters included isolates that did not merge with other members of the group. To avoid this discrepancy, we decided to define distance between two isolates only in terms of the merge-merge (1-1) responses with respect to other isolates. Results of cluster analyses reported in this paper are based on an International Mathematical and Statistical Libraries (4) cluster analysis program that allows user flexibility in the initial distance matrix.

Grouping *C. parasitica* isolates by v-c is an attempt to indicate the relative ease of anastomosis formation and subsequent cytoplasmic exchange. Anagnostakis (1) developed the technique to identify compatible V isolates. However, H isolates did not behave in a predictable manner with regard to v-c in field studies because three H isolates from three v-c groups limited canker development by V isolates in five to nine v-c groups. Recently, Anagnostakis and Waggoner (3) suggested the limits of conversion of a V isolate to an H isolate were determined by the number of different v-c alleles between the H and V isolates. Canker size 90 days after inoculation was directly related to the 0, 1, 2, or 5 v-c allele differences among the H and V isolate pairs. Our data suggest v-c groups and conversion groups vary in discreteness. In the small sample from Bonair, three v-c groups were distinct, with complete compatibility among the two to five isolates in each group. The fourth group had barrage responses among some isolates within the group. Two of the H isolates converted V isolates in three or four v-c groups, whereas 10 H isolates converted only isolates in single v-c groups. Cluster analysis was used to objectively separate isolates in the Buchanan study into v-c groups because many isolates merged with isolates from more than one v-c group. V isolates within v-c groups also varied in their response to the six H isolates (Table 4). This variation is shown in the conversion group clusters and in conversion by individual H isolates. The V isolates within v-c groups usually occurred in two or more conversion groups. In Table 4, the v-c groups are subjectively arranged by relative occurrence in conversion groups. This arrangement indicates most conversions of V isolates in the first six v-c groups resulted from isolates 543 and 545, and in the next six v-c groups, by isolates 544 and 514 (Table 4). Some isolates in v-c group 2 were converted by each of the H isolates. These data indicate some V isolates have a broad susceptibility to invasiveness of hypovirulence from divergent isolates and, conversely, some H isolates can convert V isolates from many v-c groups. The H isolates with broad conversion capacity are of special interest because a few individuals of broad conversion capacity could be used in an inoculum slurry for canker treatment. Isolates with this broad conversion capacity have been identified (22).

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