VEGETATIVE COMPATIBILITY AND HYPOVIRULENCE CONVERSION IN *ENDOTHIA PARASITICA*: STATE OF THE ART

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ABSTRACT.-- This *paper reviews the recent work on vegetative compatibility (v-c) in Endothia parasitica and relates v-c to hypovirulence conversion. Endothia parasitica has at least 128 v-c groups but hypovirulence conversion is not as limited as vegetative compatibility. For example, seven isolates with hypovirulence from four v-c groups converted isolates from 38 of 48 v-c groups tested. Recent evidence indicates that at least some alleles for v-c have a quantitative rather than a qualitative effect.*

Vegetative compatibility (v-c) refers to the characteristic enabling two hyphae to make contact, fuse, and exchange cytoplasm or nuclear material. Often the negative response, vegetative incompatibility, is used because incompatibility occurs more commonly than compatibility. In *Endothia parasitica,* hypovirulence (H) due to a cytoplasmic factor is spread by hyphal fusion and cytoplasmic exchange. In many ascomycetes, heterokaryon compatibility rather than vegetative compatibility is used to describe the portion of the parasexual cycle in which hyphal fusion results in two or more genetically different nuclei in a vegetative hypha. Heterokaryons of *E. parasitica* can form when compatible isolates of normal virulence are paired but never form from pairing hypovirulent isolates (Anagnostakis 1981; Puhalla and Anagnostakis 1971; Van Alfen et al. 1975). Puhalla and Anagnostakis (1971) found heterokaryons formed in pairings between virulent, auxotrophic mutants on minimal media but conidia from the heterokaryons infrequently were prototrophic. Thus, the mycelium maintained the heterokaryotic condition but the conidia were generally uninucleate and ended the condition. Van Alfen et al. (1975) used auxotrophic mutants to demonstrate that H was transferred from a hypovirulent isolate to a virulent isolate. Paired inoculations of a H, lysine, auxotroph and a virulent, methionine auxotroph yielded only H, methionine auxotrophs from two restricted cankers. Besides indicating that the H factor was extrachromosomal, this sample suggested heterokaryosis had not occurred. Subsequently, Anagnostakis (1981a) reported heterokaryons never resulted from pairings of virulent and H isolates in culture or on the host even when auxotrophs produced from the same original isolate were used. Apparently, the addition of the H factor in the cytoplasm has changed the capacity of the hyphae to maintain the heterokaryon even in culture. Anagnostakis (1981b) has also reported the infrequent occurrence (1:36 isolates) of a heterokaryon in nature. Since heterokaryons do not form in pairings of H and virulent isolates, there is

less chance for the parasexual cycle to initiate new genetic combinations to possibly offset the effect of H. The normal sexual cycle offers greater probability of new genetic combinations than does the parasexual cycle. However, Elliston (1978) indicated H strains produced perithecia infrequently compared to virulent strains. Genetic integrity seems to be clearly maintained in *E. parasitica* as will be indicated again later.

A technique developed by Anagnostakis (1977) has proven useful in determining v-c groups among virulent isolates of *E. parasitica.* This technique took advantage of observations made by Andes (1961) and Rizet (1952). Vegetative incompatibility in *Podospora anserina* was indicated by a clear zone between colonies because hyphae died back to septa after anastomosis. Perithecia formed on each side of the clear zone in what was called a barrage, provided that the isolates were of opposite mating type (Rizet 1952). Three types of response between single ascospore isolates of *E. parasitica* were a clear zone of inhibition, a line of pycnidia, and a smooth merging of mycelia (Andes 1961). Anagnostakis (1977) made pairings of *E. parasitica* isolates on Difco potato dextrose agar with methionine and biotin (dPDAmb) and placed the dishes in the dark at 25 C for 5 to 10 days. Pairs either had a complete merging of mycelia or formed a row or rows of pycnidia (a barrage). Merging pairs were classified as being from the same v-c group whereas presence of barrage indicated different v-c groups. Initially, 28 v-c groups were identified among isolates from Italy, France, and the United States (Anagnostakis 1977). Later, when 77 different v-c groups were identified, Anagnostakis (1980) indicated there would need to be alleles at least at seven loci to produce that number of groups and 2^7 could code for 128 possible v-c groups.

The larger the number of v-c groups present in a canker or in a forested area, the more difficult it would seem to be to control the disease by maintaining a population of compatible H isolates. We have used the Anagnostakis method to determine the number of v-c groups present in individual cankers and among cankers on the same tree and in small groves. Individual cankers often have two or more v-c groups present. The isolated tree at Bonair, Tennessee, which yielded 35 percent H isolates of the total *E. parasitica* isolates present (Kuhlman this proceedings), had four v-c groups present in the six cankers. One canker had all four groups, whereas four cankers had three groups, and one had two. The 41 cankers from near Buchanan, Virginia, (Kuhlman this proceedings), yielded an average of 2.3 v-c groups per canker. We are still determining the number of distinct v-c groups among the 93 groups identified in individual cankers, but estimate 12 to 15 groups present in the area of approximately an acre.

Both the incompatible (barrage) response and the compatible (merge) response vary in discreteness among isolates. Most isolates merge only with a few isolates in a typical response; others merge with isolates from several v-c groups, whereas a few isolates are incompatible even with themselves. This suggested that some isolates contained either hyphae or nuclei from several v-c groups. Ten hyphal tip isolates were made from each of five isolates with typical, broad, or incompatible responses. The ten hyphal tip isolates within each of the 15 parent isolates were paired with each other and all were compatible with all other propagules within the family. Anagnostakis (1977) also reported no segregation for v-c groups from single conidial isolates of either virulent or H isolates.

The merge response is typical when isolates are selfed, whereas the barrage is understood to be a response to incompatibility and failure to establish cytoplasmic exchange. Anagnostakis (1977) recognized the relationship of v-c grouping and conversion of virulent isolates by H isolates was not directly related since virulent isolates from 13 v-c groups were controlled by H isolates from 3 v-c groups in field experiments. A laboratory method for determining conversion capacity of H isolates utilizes growth of H and virulent isolates on a cellophane film over dPDAmb (Anagnostakis and Day 1979). At 28 C on a 16-hour day, conversion may occur within 2 days and up to 8 days. The cellophane film increases the probability of hyphal contact between the two isolates; however, in our lab, conversion is frequent on dPDAmb at room temperature (19 to 24 C) without cellophane. Under these conditions, conversion is apparent after 4 to 10 days of growth.

Anagnostakis and Day (1979) paired seven H isolates from four v-c groups with virulent isolates from 48 v-c groups. Their data are presented as the response of the virulent isolate to the seven H isolates (Table 1). Twenty-three of the 38 v-c groups that were converted were converted by H isolates

Table 1. Data from Anagnostakis and Day (1979) showing conversion (C) of virulent isolates from 48 v-c groups by seven H isolates from four v-c groups

Virulent isolates: v-c group number(s)	H isolate: v-c group:	113 10	4b 10	A 17	B 38	5A 40	5B 40	5C 40
40		C	C	C	C	C	C	C
17		C	C	C		C	C	C
38		C	C		C	C	C	C
10		C	C			C	C	C
26		C	C				C	C
21			C		C	C		C
36				C		C	C	C
3, 32		C	C		C		C	
48			C		C		C	C
8, 18		C	C		C			
12		C	C	C				
43					C	C	C	
19, 37			C				C	C
28			C		C	C		
34			C	C	C			
31							C	C
7		C	C					
50					C		C	
41, 45, 46			C				C	
4			C		C			
25, 27		C						
1, 6, 9, 22			C					
23					C			
15						C		
39, 42, 47, 49							C	
20								C
2, 5, 11, 14, 16, 29, 30, 33, 35, 44								

from two or more v-c groups. Anagnostakis and Day (1979) said that "hypo virulence conversion capability" was less restrictive than v-c of normal isolates. On the other hand, conversion by different isolates from v-c groups 10 and 40 varied considerably even though isolates within each group were from the same parent isolates. Although 27 v-c groups were converted by H isolates from v-c 10, only 11 were converted by both isolates of group 10. Similarly, only 5 of 25 v-c groups that were converted by v-c 40 isolates were affected by all three isolates.

Virulent isolates within a v-c group also vary in response to H isolates. Data from Bonair, Tennessee, are presented in Table 2. Three to five isolates from the four v-c groups were paired with 13 H isolates. In each v-c group, some isolates were not converted by H isolates that converted other

Table 2. Conversion (C) of virulent isolates from four v-c groups by 13 H isolates from the same American chestnut source near Bonair, Tennessee

Virulent isolate	v-c group	H isolate[a/] 1-8	1-15	2-11	2-13	3-8	3-20	4-7	4-12	5-2	5-11	6-1	6-5	6-19
1-13	A			C			C							
2-14	A			C										
6-2	A			C			C							
3-9	D						C							
4-5	D						C						C	
5-7	D						C							
6-18	D												C	
1-12	E	C								C				C
2-3	E	C		C			C			C				C
4-31	E	C		C			C			C				C
5-4	E	C								C				C
6-4	E	C					C			C				C
1-2	B		C	C	C	C			C		C	C		
2-27	B		C		C	C			C		C	C		
3-17	B				C	C			C		C	C		
4-16	B		C		C	C			C		C	C		
5-13	B			C	C	C			C					
6-14	B		C		C	C			C		C	C		

[a/] Isolate number = canker number - chip number.

isolates within the group. Hypovirulent isolates 2-11 and 3-20 converted virulent isolates in three v-c groups. These data indicate that grouping isolates by v-c groups is helpful in terms of conversion potential but exceptions to conversion are not unusual either positively or negatively.

The discrepancy between v-c groups among virulent isolates and conversion of virulent isolates within and among v-c groups by H isolates may be explained in several ways. One likely explanation is that conversion requires only minute infection of H cytoplasm into the virulent cytoplasm. Field data indicate conidia carry enough of the H factor to provide control of treated

cankers (Kuhlman this proceedings). In laboratory tests, hyphal contact needs only permit exchange of a single dsRNA particle to establish conversion so that disruption of the cytoplasmic exchange after hyphal walls are dissolved seemingly would not prevent conversion.

Anagnostakis (1980) explained the discrepancy from a genetic point of view. Matings of isolates from some v-c groups yielded progeny of only the parent v-c groups, suggesting that only one allele segregates. Some matings yielded progeny of four v-c groups indicating alleles at two loci were different. Some parent isolates have different alleles for compatibility at four loci and the progeny should represent 16 (2^4) v-c groups. If we presume the seven alleles that Anagnostakis (1980) has identified function in various portions of plasmogamy, i.e. hyphal contact, cell-wall breakdown, cytoplasmic exchange, etc., each allele may function in one step of this process. If several alleles are the same in two isolates, plasmogamy may proceed far enough to permit exchange of the H factor even though it is subsequently disrupted by an incompatibility in gene function.

Conversion of virulent isolates by H isolates is affected quantitatively by the number of genes that differ for compatibility (Anagnostakis and Waggoner 1981). Anagnostakis and Waggoner (1981) paired virulent and H isolates which differed by 0, 1, 2, and 5 v-c genes. The fewer genes different, the smaller the canker development on American chestnut. Pairs that differed by five v-c genes formed cankers which were not significantly different in size from those formed by two virulent isolates from the same v-c groups. Although Anagnostakis and Waggoner (1981) suggest their results are similar to Caten's (1972; 1973) results with *Aspergillus amstelodami,* they apparently differ in regard to the frequency of occurrence of quantitative alleles. Caten (1972) initially stated that transfer of vegetative death, a cytoplasmically inherited trait, between strains of *A. amstelodami* was completely prevented if strains differed by more than one gene. Later, Caten (1973) reported the discovery of a gene locus that had a quantitative rather than qualitative effect. This locus restricted but did not abolish heterokaryon formation. Four other gene locations acted qualitatively with any one individually determining incompatibility. Further genetic work will be needed to determine if Anagnostakis and Waggoner's (1981) conclusions regarding the additive nature of the v-c genes in *E. parasitica* are true for all gene pairs or only for a few of the seven involved. The long-term effects on canker development and tree survival of quantitative versus qualitative traits also need to be evaluated to be certain disease control is achieved.

Vegetative compatibility grouping is helpful in establishing possible relationships among virulent isolates but conversion of virulent isolates by H isolates is necessary to confirm the relationships. A definitive test for conversion and thus for vegetative compatibility should include: pairing of virulent and H isolates, noting changes in virulent colony morphology that suggests conversion, transferring the converted isolate, retention of H morphology by the transfer, and conversion of the normal isolate by the converted transfer. These steps are necessary because isolates often seem to change their growth habit when confronted by another isolate. By subculturing the apparent convert, the permanence of the change can be confirmed. At least, occasional pairing of converted isolates with "parent" virulent isolates seems advisable.

Field work with v-c has been less intensive because our knowledge in this area is just developing. Early work at Connecticut (Elliston and Jaynes 1977; Jaynes and Elliston 1978; 1980) utilized a single H strain to arrest canker development during a growing season. After three growing seasons and repeated treatments, 13 percent of the cankers (31:245) were arrested whereas none of the untreated control cankers was arrested. No attempt to match v-c groups of virulent and H isolates was made. Similarly, a second experiment in 1977 utilized eight H isolates from six v-c groups that were unselected for compatibility with virulent isolates. After two growing seasons, canker size and tree mortality were less in H isolate-wounded treatments than in nontreated, nonwounded treatments.

In the southern Appalachians, inoculated American chestnut trees have survived better following treatment with compatible H isolates or with a random selection of 28 H isolates than did check trees that were wounded and treated with agar (Kuhlman this proceedings). In the experiment started in 1978, one virulent-H combination was an erroneous choice that was not compatible and all the trees died. This emphasizes the need for ensuring compatibility for successful treatment. Since trees in check treatments died in 15 months, the need for rapid deployment of the H isolates to control virulent isolates is apparent. After several growing seasons, some live treated trees have signs of *E. parasitica* and symptoms of renewed infections. This renewed activity appears to be due to secondary infections by virulent isolates from other v-c groups. Whether inoculations with H isolates from several v-c groups can provide long-term protection against secondary infections remains to be determined.

Vegetative incompatibility is one impediment to control of chestnut blight with H in the eastern United States. Previous work has indicated that conversion of virulent isolates occurs 20 to 50 percent of the time between any two virulent and H isolates (Anagnostakis 1979; Grente and Berthelay-Sauret 1978; Kuhlman this proceedings; Puhalla and Anagnostakis 1971). Using the conservative 20 percent conversion, we calculated 14 randomly selected H isolates were needed to ensure 95 percent probability of conversion of any virulent isolate. In field studies, 28 H isolates have reduced tree death and girdling by virulent isolates. Long-term protection requires the survival of 14 H isolates according to this estimate. However, this estimate assumed each H isolate was independent of other isolates (i.e., each had a 20 percent possibility of converting any virulent isolate). Anagnostakis and Waggoner (1981) have indicated conversion is dependent on the number of similar v-c alleles in the two isolates. Thus two H isolates from v-c groups with only a single allele different are more likely to respond in the same manner to a virulent isolate than are two H isolates from v-c groups differing by five to seven alleles. Selection of H isolates from a broad spectrum of the 128 v-c groups might provide a 40 percent conversion rate and reduce the number of H isolates needed for control to six. Increasing our knowledge of the role of v-c may enhance the prospects for biological control of chestnut blight with cytoplasmic hypovirulence.

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