Hypovirulence Conversion in *Endothia parasitica*

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ABSTRACT

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A laboratory method developed by J. Grente was used to test the abilities of seven related hypovirulent strains of *E. parasitica* to convert 49 virulent vegetative compatibility (v-c) tester strains to hypovirulence. The success of conversion, its rate, and the stability of the converts are all influenced by vegetative compatibility. Rapid conversions (2 days or less) always occurred between strains of the same v-c group and were common in certain incompatible pairings. In other incompatible pairings, conversion was slow and erratic, and 10 of the testers were not converted. Examination of dsRNA components in two series of replicated incompatible rapid conversions showed that variation and stability of the dsRNA patterns also were influenced by the v-c group of the recipient strains. Mechanisms for maintaining stable dsRNA genomes and for generating variants of these genomes contribute to the polymorphism and adaptability of this biological control agent.

Additional key words: biological control, mycovirus, vegetative compatibility.

Infectious hypovirulent strains (H) of the chestnut blight pathogen *Endothia parasitica* (Murrill) Anderson are either nonpathogenic or have reduced pathogenicity and can transmit this character to virulent wild-type strains (V) (see the review by Anagnostakis [2]). A V that acquires hypovirulence is said to be converted. If conversion takes place in a canker on the chestnut (*Castanea* spp.) host, the canker often is contained and healing tissue growth occurs. If pathogenicity is sufficiently reduced the converted strain cannot destroy the vascular cambium, the tree may remain alive, and the canker can serve as a source of further hypovirulent inoculum. Thus, infectious hypovirulence provides a biological control for chestnut blight. All infectious hypovirulent strains examined so far contain dsRNA (3); however, those that contain dsRNA vary widely in pathogenicity and some are nearly as pathogenic as the V isolates (4).

Transmission of hypovirulence determinants and the resulting conversion occurs following hyphal anastomosis between hypovirulent and virulent strains (8,9). The frequency and stability of anastomoses between different strains of *E. parasitica* are determined by several genes that govern vegetative compatibility (v-c) (1,2). As in other Ascomycetes, vegetative incompatibility occurs between paired homokaryotic mycelia that have different alleles at one or more of the controlling loci (heterogenic incompatibility) and it results in the death of fused cells. Field observations suggest that vegetative incompatibility can restrict conversion. Results of laboratory tests by Grente and Sauret (5-7) and also those of Anagnostakis (*unpublished*) appear to confirm this. They show that a given H strain will readily convert only certain V strains and that it converts other V strains either with difficulty or not at all. In this paper we report the results of laboratory conversion tests with European H strains and analyses of the dsRNA components in converted strains.

MATERIALS AND METHODS

Laboratory conversion tests were conducted by a method similar to that of Grente and Sauret (5,6 and *personal communication*). Inocula of H and V strains were paired on one side of a cellophane disk placed on PDAmb (Difco potato dextrose agar, supplemented with methionine [100 mg/L] and biotin [1 mg/L]). Pairings were incubated at 28 C in a 16-hr photoperiod (white fluorescent light) for 8 days. Conversions of V strains by European white or pigmented H strains resulted in sectors of mycelial growth having the white appearance characteristic of European white H strains. These sectors started at the interface between the two strains and enlarged daily (Fig. 1). At 28 C, wild-type *E. parasitica* require ~8 days to grow from one side of an 8.5-cm petri dish to the other. Rings of pycnidia that form on the colonies in response to photoperiod serve as convenient "day markers." White sectors that appeared within the first 2 days were called rapid conversions and those that appeared within 3–8 days were called slow.

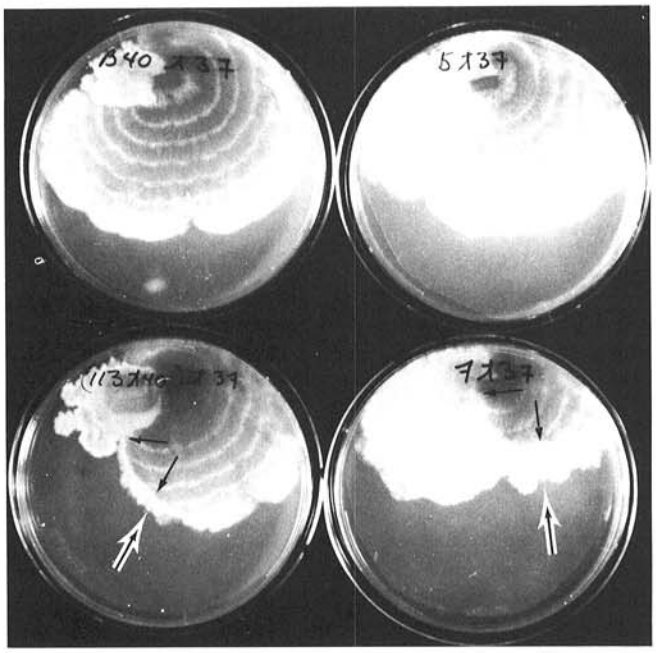

Fig. 1. Four different hypovirulent strains of *Endothia parasitica*, all v-c 40, (left) paired with a normal strain which is v-c 37. In the top two plates there has been no visible conversion. The bottom left plate has a sector (indicated by arrows) of white converted mycelium beginning in day-ring 4. The sector in the plate on the right starts in the first day-ring.

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The cultures that were used are listed in Table 1.

Mycelia for extraction of dsRNA were harvested as 7- to 10-day-old colonies grown on the surface of autoclaved wettable cellophane film (DuPont 193-PUDO) cut into 8 cm diameter disks and placed on PDAmb. The method of extraction and analysis of dsRNA was that described by Day et al (3). Samples corresponding to approximately 0.1 g (wet weight) of mycelium were electrophoresed on 5% polyacrylamide gels for 10 hr at 4 mA/gel. Gels were stained with propidium iodide (~5 μg/ml) and photographed in UV light through a yellow filter.

Tests for vegetative compatibility were described by Anagnostakis (1).

RESULTS AND DISCUSSION

Hypovirulence conversion. Several different hypovirulent strains were tested against a set of 49 tester strains, each of which represented a different vegetative compatibility group. All of these testers are virulent. The results show that vegetative incompatibility does not necessarily prevent conversion to hypovirulence (Table 2). The seven hypovirulent strains of Table 2 represent four v-c groups and converted all except 10 of the 49 v-c testers used. All replicates of tests of vegetatively compatible pairs, for example 113 + v-c 10, showed rapid conversion. In many cases involving paired strains of different v-c groups, replicated tests did not agree. If at least one test was scored rapid, this result was entered even though results of the other tests were either slow or negative. Similarly, if at least one test was scored slow this result was entered even though the other tests were negative. Clearly an element of chance governs incompatible conversions, and when their frequency is low only a large number of replicated tests will reveal them. For this reason we cannot conclude that any given pairing always will be negative.

All the conversion events for 4b7 shown in Table 2 resulted in formation of white sectors even though the donor strain was pigmented. The difference between this strain and its parent, strain 113, may reflect the differences in their dsRNA components (3) but also are confounded by the variation shown in replicate tests.

Five of the hypovirulent strains used in the tests were products of previous conversions and show patterns of conversion that differ from that of the original hypovirulent source strain. Their origins are described in Table 1. Strain A, derived from 78, was chosen because its conversion was very rapid; all of its mycelial growth after pairing was white. Strain B, derived from strain 153, was the result of a very slow conversion in which the white sector appeared 6 days after pairing. The pairing that produced strain A was repeated six times (see below), and rapid conversion always occurred. The pairing which produced strain B was repeated 12 times and none resulted in conversion. When strains A, B, and 113 were paired with strain 155 (v-c 40), conversion by 113 was rapid, and that by A and B was slow (each taking 4 days). We expected these new converts (A40, B40, and 113-40) to interact identically with the 49 virulent testers since their nuclear genotypes for v-c group were presumed identical. All three, as expected, gave rapid conversions with v-c 40, but also gave rapid conversions with v-c 10 and v-c 17 (all replicates were rapid). Their differences with respect to other incompatible conversions are of doubtful significance in view of the variation among replicate tests discussed earlier.

TABLE 1. *Endothia parasitica* strains utilized in hypovirulence conversion experiments

Strain number	Description and source
Original hypovirulent strains:	
113	subculture of B2025, white; from a treated canker, Gonfaron, France, 1970, (J. Grente, 1972)
4b7	subculture of JR2043, pigmented; from a single conidium of B2025, (J. Grente, 1972)
Virulent strains used in conversion studies:	
78	v-c 17, single ascospore clone from canker, (Hamden, CT, 1976)
153	v-c 38, mass isolate from canker, (Cockaponset State Forest, CT, 1977)
155	v-c 40, mass isolate from canker, (Bethany, CT, 1977)
302	v-c 17, mass isolate from canker } three separate test areas,
303	v-c 17, mass isolate from canker } (Cockaponset State Forest, CT, 1977)
304	v-c 17, mass isolate from canker }
305	v-c 40, mass isolate from canker, (Mt. Tom State Park, CT, 1978)
Hypovirulent strains resulting from conversion of virulent strains:	
A	113 (v-c 10) + 78 (v-c 17) → white (v-c 17) = "A" (conversion on cellophane)
A40	A (v-c 17) + 155 (v-c 40) → white (v-c 40) = "A40" (conversion on cellophane)
B	113 (v-c 10) + 153 (v-c 38) → white (v-c 38) = "B" (conversion on cellophane)
B40	B (v-c 38) + 155 (v-c 40) → white (v-c 40) = "B40" (conversion on cellophane)
113-40	113 (v-c 10) + 155 (v-c 40) → white (v-c 40) = "113-40" (conversion on cellophane)
v-c tester strains:	
Three v-c testers are listed above (strains 78, 153, and 155). The origins of the others are similar. Twenty-eight of them are listed in (1). Of 50 v-c testers now in use, 49 are virulent.	

TABLE 2. Conversion tests on cellophane over PDAmb using seven hypovirulent (H) strains of *Endothia parasitica* and 49 virulent vegetative compatibility (v-c) testers.

	H strains						
	113 (v-c 10)	4b7 (v-c 10)[a]	A (v-c 17)	B (v-c 38)	113-40 (v-c 40)	A40 (v-c 40)[a]	B40 (v-c 40)[a]
Rapid conversions v-c #	10,12,17,40	10,17,19,28,32,34,40	17	3,21,23,28,38,48	10,17,40	10,17,18,24,26,32, 37,39,40,41,42,43,45, 46,47,48,49,50	10,17,19,36,37, 38,40
Slow conversions v-c #	3,7,8,18,25,26, 27,32,38	1,3,4,6,7,8,9,12,21, 22,26,37,38,41,45, 46,48	12,34,36,40	4,8,(32),34,40, 43,50	15,21,28,36,38,43	3,19,31,36,38	20,21,26,31,48
Total	13	25	5	13	9	23	12

[a] All 49 tests were replicated at least twice.

[b] Testers 2, 5, 11, 14, 16, 29, 30, 33, 35, and 44 were not converted by any of these H strains, tester 13 contains dsRNA and is not included here.

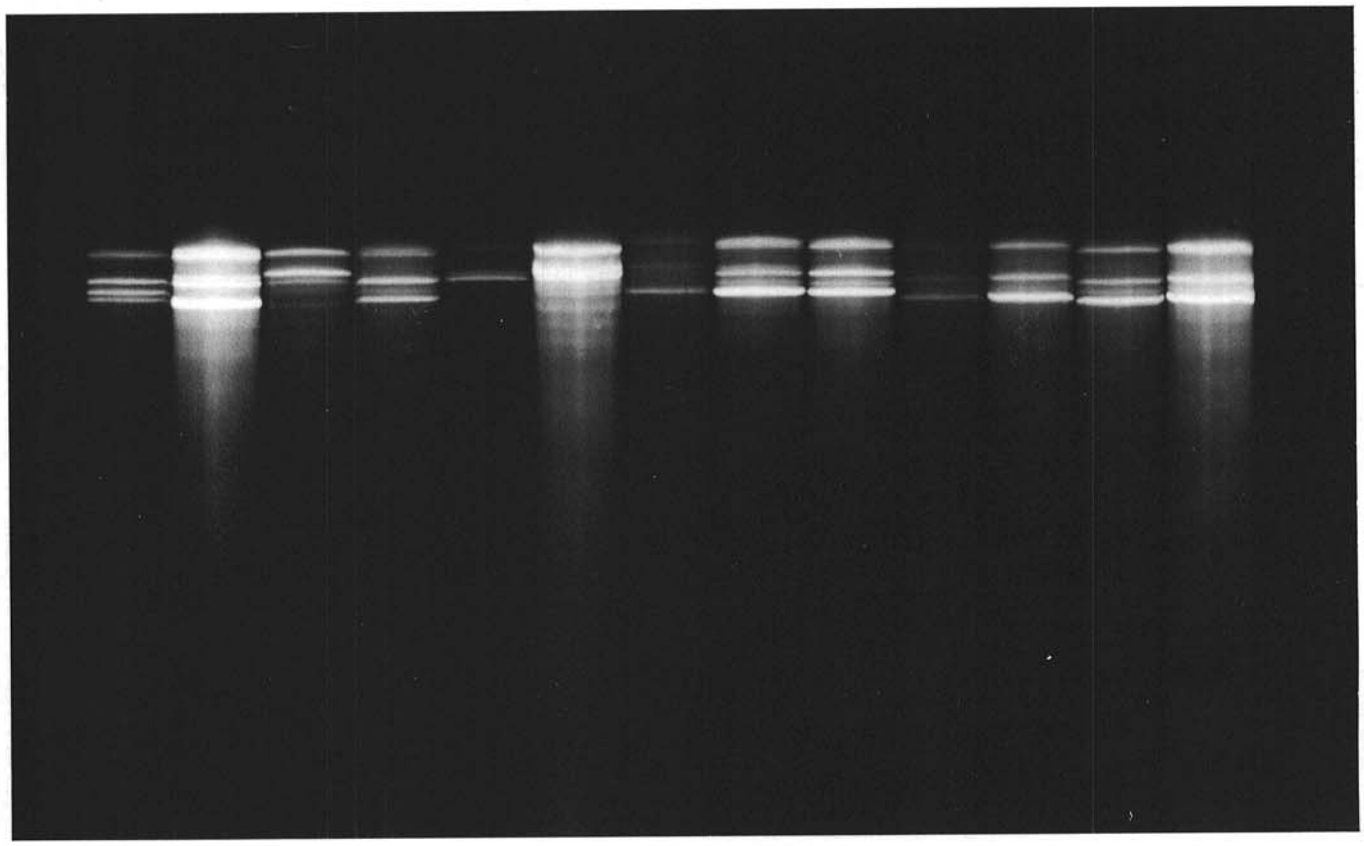

Fig. 2. Polyacrylamide electrophoresis gels stained with propidium iodide showing dsRNA banding patterns of (left to right): 113, six replicates of 113 + 78, and six replicates of 113 + 155. Each gel received extract equivalent to 0.1 g wet weight of mycelium.

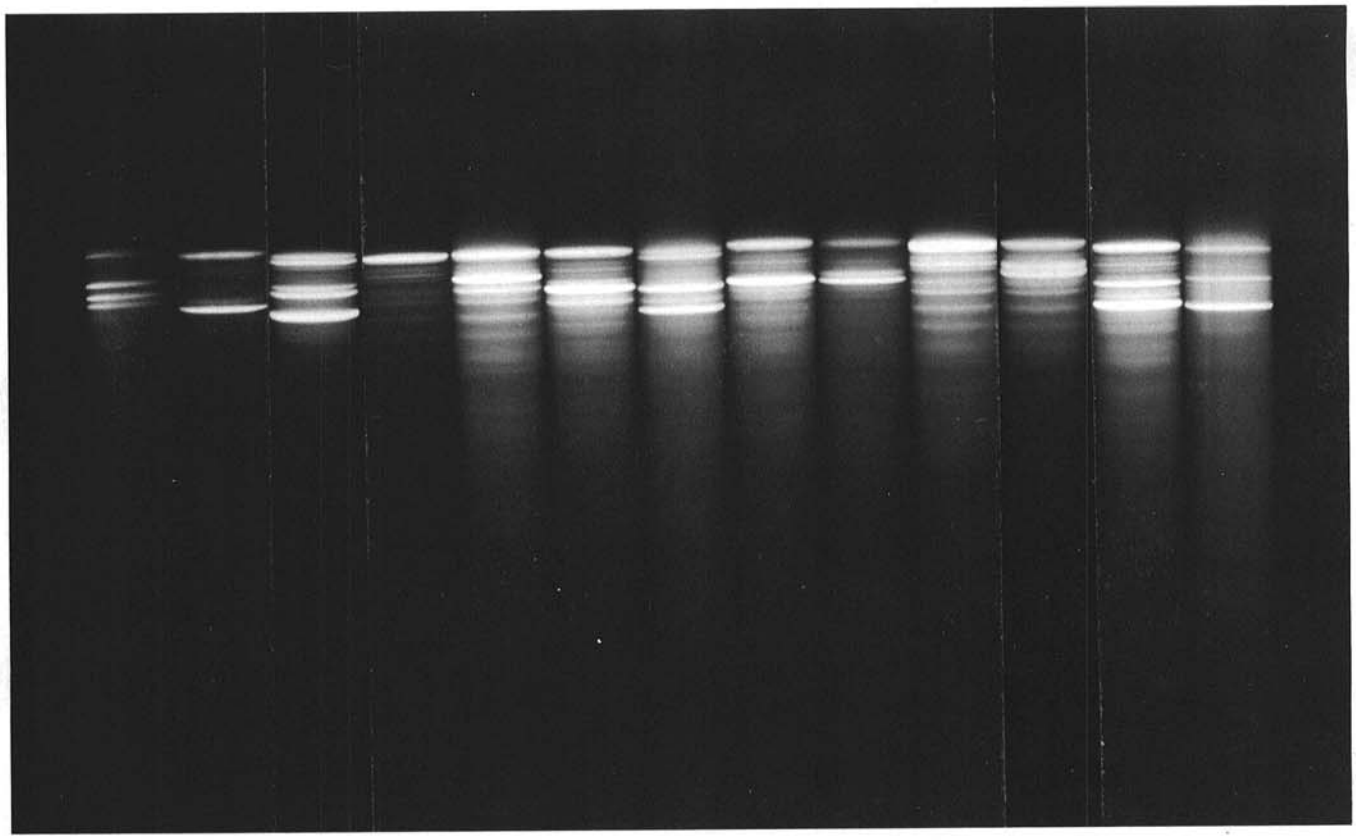

Fig. 3. dsRNA patterns extracted from six replicates of 113 + 78 after subculturing twice and five times. The gel on the extreme left is from 113. The remaining gels are arranged as six pairs; the gel from the second transfer of a replicate is on the left of each pair, the gel from the fifth transfer is on the right.

TABLE 3. dsRNA patterns of hypovirulent strains of *Endothia parasitica* which are the products of different v-c 17 and v-c 40 virulent wild types converted to hypovirulence by pairing with 113 (v-c 10)

Strains paired	Pairings made (no.)	Patterns (no.)	No. like 113(v-c 10)[a]
113 + 78 (v-c 17)[a]	6	6	1?
113 + 302 (v-c 17)	7	5	1
113 + 302 (v-c 17)	7	4	0
113 + 304 (v-c 17)	7	7	0
113 + 155 (v-c 40)	6	1	6
113 + 305 (v-c 40)	7	1	7

[a] Vegetative compatibility group.

dsRNA components. Hypovirulence is associated with the presence of dsRNA. Our working hypothesis is that dsRNA is transmitted via hyphal anastomoses at the time of conversion. The dsRNA patterns for the three original converts of strain 155 (A40, B40, and 113-40) differ quite widely. This, or a difference in some other transferred cytoplasmic element, may explain the differences in the abilities of these three hypovirulent strains to convert the 49 virulent testers. The several dsRNA components may influence the ease with which incompatible interactions take place, or it may reflect differences in requirements for the maintenance or replication of dsRNA.

The recognition of a convert depends on the ability of dsRNA to replicate and produce a phenotypic change in the recipient strain. Although the v-c groups of interacting strains will determine the frequency and stability of hyphal anastomoses, and hence the likelihood of conversion occurring, other host genes may govern dsRNA replication and expression. To explore this possibility, the product of a slow conversion (strain B) and those of two rapid conversion (strains A and 113-40) were extracted for dsRNA analysis. These strains were then subcultured three successive times and extracted again. The patterns of dsRNA obtained from strains B and 113-40 did not change after subculturing, but that for strain A did. More pairings were made, and six replicates each of 113 + 78 (v-c 17) and 113 + 155 (v-c 40) were tested further. Twelve replicates of 113 + 153 (v-c 38) failed to produce converts. Strains 78, 153, and 155 did not contain detectable dsRNA by the method described above.

The gels (Fig. 2) show six distinct patterns for the converts of 78 (v-c 17) but only one pattern, very similar to that of 113, for the converts of 155 (v-c 40). Each of the twelve strains and 113 were reexamined after three further subcultures. The dsRNA patterns shown by 113 and the set of six conversions of 155 (v-c 40) were unchanged after subculturing. However, the six converts of 78 (v-c 17) showed further variation. Fig. 3 illustrates dsRNA preparations obtained after two and after five subcultures of each of the six converts of 78 (v-c 17). Although some dsRNA patterns are similar before and after subculturing (pairs 4 and 6) the others differ greatly. Evidently the dsRNA patterns which were derived from 113 in the genetic background of 78 are both varied and unstable.

In view of the differences in dsRNA patterns between converts of 78 (v-c 17) and 155 (v-c 40) a further test was carried out by obtaining seven replicate conversion products of each of three other virulent isolates of v-c 17 (strains 302, 303, and 304) and one other v-c 40 (strain 305). All of those conversions were rapid.

Extraction of all four virulent isolates showed them to be free of dsRNA. The dsRNA patterns of the replicate converted strains that were obtained are summarized in Table 3.

The genetic backgrounds of converted strains appear to have profound effects on the stability and nature of the dsRNA they acquire through conversion by 113. Since all conversions in Table 3 were rapid it seems unlikely that infrequent anastomoses were responsible for transfer of some but not all components of 113 to strains of v-c 17. Dodds has found that 113 characteristically has four or five dsRNA components with approximate molecular weights of 6.2, 5.9, 5.0, 4.6, and 4.4 + 10^6 (Dodds, *personal communication*). As can be seen in Fig. 2, the derived patterns are often more complex than might be expected from simple reassortment of the dsRNA components if 113 carries a multicomponent virus or several distinct viruses. These patterns include bands in other positions. The differences between patterns found in extractions carried out after two or five transfers (shown in Fig. 3) also are more complex than can be explained by random reassortment of dsRNA components. One possible explanation is that configurational instability results from the action of dsRNA nucleases present in v-c 17 strains. If they are absent or ineffective in our two v-c 40 strains, the relative constancy of patterns seen in this background might be expected.

In *E. parasitica*, some vegetatively incompatible pairings allow the transmission of hypovirulence determinants. Therefore, what we may call "hypovirulence conversion compatibility" is less stringent than the vegetative compatibility defined by an earlier test (1). The presence of the dsRNA may override vegetative incompatibility, or some genetic differences leading to vegetative incompatibility may allow more cytoplasmic exchange than others. Even in an incompatible interaction there may be sufficient cytoplasmic contact for sufficient time to permit transfer of dsRNA before cell death occurs. This might be tested with other cytoplasmic markers. As a rough approximation, a given H strain is able to convert about 25% (5–50%) of the 49 virulent v-c tester strains. Thus far, only two of the 49 have not yet been converted by any of the H strains tested (Table 3 and Anagnostakis, *unpublished*). Thus, the existence of more than 70 v-c groups in *E. parasitica* (Anagnostakis, *unpublished*) is not as great a barrier to transmission of hypovirulence among strains as it first appeared to be.

When V strains 155 and 305 (both v-c 40) are converted to H strains by pairing with 113 (v-c 10), the dsRNA genome of 113 appears to be completely transferred and is stable in those strains after subculturing. However, there is great variation in dsRNA genomes among H strains resulting from conversion of strain 78 (v-c 17) by 113. Three other V strains (v-c 17 from different sources) also have variable dsRNA genomes after conversion with 113. This may be due to some interaction of the nuclear genes which determine v-c 10, v-c 40, and v-c 17, or it may be characteristic of all conversions of v-c 17 and v-c 40 strains.

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