

Diversity of Vegetative Compatibility Groups of *Cryphonectria parasitica* in Connecticut and Europe

S. L. ANAGNOSTAKIS, Plant Pathology and Ecology, Connecticut Agricultural Experiment Station, New Haven 06504, and B. HAU and J. KRANZ, Tropeninstitut, Justus-Liebig-Universität, Schottstrasse 2-4, 6300 Giessen, West Germany

ABSTRACT

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Strains of the chestnut blight fungus (*Cryphonectria parasitica*) were isolated from natural cankers in Connecticut and compared with strains isolated in France and Italy for vegetative compatibility (v-c) type. When American and European populations were compared using diversity indices, less v-c diversity was found among European than among Connecticut *C. parasitica*.

Potentially lethal cankers of chestnut blight, caused by *Cryphonectria parasitica* (Murr.) Barr (formerly *Endothia parasitica* (Murr.) And.), can be controlled by inoculating the cankers with strains of the fungus that contain cytoplasmic genes for hypovirulence (2,10). Hypovirulence appears to be spreading naturally in Italy (21), where it was first discovered, and in France (11,12), where it was introduced. After several years of experimental introduction of strains of *C. parasitica* with European hypovirulence into American chestnut (*Castanea dentata* (Marsh.) Borkh.) stands, there is no evidence that this hypovirulence is spreading measurably in the United States.

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The cytoplasmic determinants for hypovirulence (2,13) cannot always be transmitted between strains of the fungus when the strains are of different vegetative compatibility (v-c) types (6,7). For a number of years, we have been collecting data on the diversity of *C. parasitica* populations in Connecticut and in Europe. The v-c types of strains were tested (1,5) and recorded, and the total numbers of types found have been reported (5). Without censusing the populations, we cannot know exactly how many v-c types are present, and in what proportions, on the two continents. However, diversity of v-c in a sampling throughout Connecticut, Italy, and France would reflect the genetic diversity present in the populations and the potential numbers of v-c types possible. This v-c diversity could influence the ease with which hypovirulence spreads through the *C. parasitica* population.

MATERIALS AND METHODS

A survey of *C. parasitica* in 48 counties in Connecticut was made with the help of the gypsy moth survey team from the Department of Entomology at the Connecticut Agricultural Experiment Station. Whenever they found a stand of

American chestnut or a single living tree, they took one to five bark samples if any chestnut blight cankers were present. R. A. Jaynes, J. E. Elliston, and S. L. Anagnostakis collected additional samples.

Bark samples were placed on 2% water agar in petri dishes and incubated at 25–27 C with 16 hr per day of white fluorescent light. Mycelium grew into the agar around the bark sample within 1–2 days. This initial growth on water agar effectively eliminated bacterial contamination. Small pieces of agar with mycelium were transferred to sterile petri dishes (100 × 15 mm) each containing 30 ml of Difco potato-dextrose agar (PDA) and incubated as described previously. When other fungi were abundant on the samples, isolations were made under a dissecting microscope from erumpent stromata of *C. parasitica*. Cultures were maintained by weekly transfers to PDA and incubated at 25–27 C or stored on PDA slants in screw-capped tubes at 4 C.

J. Grente sent strains of *C. parasitica* from his collection of strains isolated from orchards in southern France, where the farmers reported the arrival of blight, and from Corsica and Italy, where he traveled to examine chestnut orchards (12). Italian strains and bark samples were sent by L. Mittempergher or T. Turchetti or collected by P. R. Day or J. E. Elliston. These represented a few samples taken in each of many areas where hypovirulence was known to be present.

Tests for v-c were made by transferring small cubes (less than 3 mm on a side) of mycelium in PDA from the edges of

rapidly growing cultures less than 7 days old. Pieces for testing were placed on PDA not more than 5 mm apart and incubated at 25–27 C in the dark for 4 days. At the end of this time, mycelia that were vegetatively compatible had grown together, forming confluent mycelium. Incompatible mycelia had grown to a meeting point in the agar but remained separated by a “barrage” line composed of dead cells with no covering aerial mycelium. If these test plates were then incubated for 2 days at 25–27 C with 16 hr of white fluorescent light per day, pycnidia formed along both sides of the barrage, making it more obvious. Occasionally the barrage was faint and pycnidia did not form along its edge (4).

Lebeda (17) has suggested that the genetic diversity expressed as diversity of virulence phenotypes among individuals in populations of plant-pathogenic fungi can be examined by calculating diversity indices of the kind used in population ecology to express species diversity (14). These indices are based on the probabilities of recovering samples with certain phenotypes from populations with certain diversities.

The simplest measure of diversity in a population is the number of species or phenotypes (S) in a sample from a population divided by the number (N) of individuals in the sample. The division of S by N is an attempt to make this measure of diversity independent of sample size.

The most commonly used index that takes into account more details of population structure is called H' (14,19,20).

$$H' = -\sum_i p_i \log_e p_i,$$

where p_i is the fraction of the whole sample represented by each phenotype ($s_1/N, s_2/N, s_3/N$, etc.) from a population with S phenotypes.

When all individuals have the same phenotype, $H' = 0$, and when all have different phenotypes, H' has its highest value.

RESULTS AND DISCUSSION

A total of 165 *C. parasitica* isolates were obtained in Connecticut. These were divided into 67 v-c types, many of which (38) were found only once (Fig. 1); only three were found more than 10 times. We might predict from this that for every three isolates obtained in Connecticut, a new v-c type will be found. We still do not know what the maximum total may be, because this depends on the number of v-c gene loci in *C. parasitica* and the number of alleles at each locus (3). For example, laboratory crosses of v-c 5 × v-c 10 strains (found near each other twice in the survey) have produced a maximum of 106 v-c types (3). Gene-pool mixing may occur over long distances, because the initial epidemic of *C. parasitica* in North America was estimated to have spread at about 20 mi./yr (8).

Grete (12) paired 148 of his *C. parasitica* strains in a v-c test and found that they fell into 22 v-c types. Most of these had been isolated from 18 provinces in France; three came from Italy. He sent strains representing the 22 types for comparison with our type testers and for comparison with 49 strains in our collection that were Italian in origin. Thirty-three v-c types were found among the 197 total isolates. These data are also presented in Figure 1 for comparison with the data from Connecticut. There were 15 v-c types found only once but seven found more than 10 times. The diversity of European strains, with one v-c type found for every six isolates, appears to be less than found in Connecticut.

When the data for the Connecticut and European surveys were used to calculate diversity indices S/N and H' , the highest values of diversity were found in data from Connecticut. Connecticut S/N was 0.41 and H' was 3.83, whereas European S/N was 0.17 and H' was 2.74.

Even though the total number of samples of the pathogen is rather small, it appears by these measures of diversity that the Connecticut and European populations of *C. parasitica* differ from each other in v-c diversity.

Nash (18) similarly sampled the population of *C. parasitica* in North Carolina and compared isolates with 48 Connecticut v-c tester strains. He found 41 distinct v-c types among 327 survey isolates, 40 isolates that did not give clear reactions, which he divided into 17 clusters, and 55 isolates that were not like any of the 48 Connecticut v-c tester strains (18, some of his data are also reported in 15). From these data, we get $S/N = 0.21$ (58/272) and an $H' = 3.35$. Because Nash's isolates were collected over a large area as were the Connecticut isolates, his data can be compared with the Connecticut and European data. We have no way of knowing how many additional v-c types were present among the 55 “different” strains. At the extremes, they all could have been of different v-c types, making his $S/N = 0.35$ and his $H' = 3.9$, or all the same, so that $S/N = 0.18$ and $H' = 3.24$.

Bazzigher et al (9) reported data on v-c types of *C. parasitica* in Switzerland, where they found 12 v-c types among 124 strains isolated from 25 locations. This represents one v-c type for every 10 strains isolated ($S/N = 0.097$ and $H' = 1.47$).

Censusing (examining every member of a subpopulation) may give different data from sampling (examining representatives of subpopulations). This is especially true when many types are represented only once, because H' assumes that N is a very large number and weights the single recoveries heavily. Hutchinson (14) has discussed this problem. MacDonald and Double (data

in 16) have censused v-c types of *C. parasitica* in 16 chestnut plots (each 400 m²) in two national forests in West Virginia. They found 33 clear v-c types, 4 clusters, and 88 types found only once (or lost). Calculating this as 125 types, their $S/N = 0.14$ and $H' = 3.57$. Kuhlman and Bhattacharyya (15) censused *C. parasitica* on 19 trees along a 303-m transect in Virginia and used cluster analysis to establish 17 v-c clusters and 4 singles among 93 isolates. This yields an S/N of 0.23 and an H' of 2.76.

Because hypovirulence can sometimes be transmitted between incompatible strains (4,7,15,16), Kuhlman has proposed that hypovirulence conversion incompatibility is a more realistic system to consider when trying to control chestnut blight. Previous conversion success using seven randomly selected hypovirulent strains and 49 virulent strains (6) was only 30% (100 conversions per 343 pairings). In a much larger test, Kuhlman (16) paired 27 hypovirulent strains with 118 virulent strains and found that 14% (448/3,186) of the pairs resulted in conversion. Selective tests for conversion between v-c types that barraged weakly resulted in rapid conversions in 86% (62/72) of the pairings (4), but more important is Kuhlman's finding that mixtures of four to 11 hypovirulent strains resulted in conversion of 82–95% of his 118 virulent strains (16).

In practical terms, this means we can increase the chance of controlling a canker by using a mixture of hypovirulent strains. However, spread within a subpopulation or population may require that our mixtures of hypovirulent strains reflect the v-c diversity of the virulent strains in the population. Perhaps a lower diversity of v-c types in the European *C. parasitica* population is part of the reason hypovirulence appears to spread more easily in Europe than in the United States.

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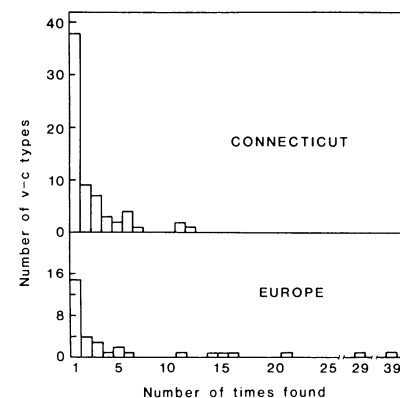


Fig. 1. Vegetative compatibility (v-c) diversity of *Cryphonectria parasitica* in Europe and Connecticut.

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