

Population Dynamics of *Cryphonectria parasitica* in a Mixed-Hardwood Forest in Connecticut

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Most of this work is from a thesis submitted by the first author to Justus-Liebig Universität, Giessen, in partial fulfillment of the requirements for a Ph.D. degree.

We would like to thank Marie C. Maier and Pamela Sletten for excellent technical assistance.

Accepted for publication 2 December 1986 (submitted for electronic processing).

ABSTRACT

Anagnostakis, S. L., and Kranz, J. 1987. Population dynamics of *Cryphonectria parasitica* in a mixed-hardwood forest in Connecticut. *Phytopathology* 77: 751-754.

A natural population of the chestnut blight fungus, *Cryphonectria parasitica*, was found early in a cycle of the blight epidemic, and the vegetative compatibility profile of the population was censused from summer 1982 through fall 1985. All of the American chestnut sprouts in a 60- × 60-m area in this mixed-hardwood forest were examined regularly, and new cankers on living trees at least 2.5 cm diameter at breast height (dbh) were sampled. *C. parasitica* was isolated, and the vegetative compatibility (v-c) types of the isolates were determined. The typed isolates were then referred to as strains. The strains were paired in the laboratory

with strains of *C. parasitica* containing European hypovirulence (H) determinants to convert them to H strains by cytoplasmic transfer across anastomosis bridges. Each new H strain was inoculated around the margin of the canker from which its progenitor had come to halt canker expansion. In 1982 we found six v-c types in 12 cankers on four (living) trees. By November 1985 we had found 48 v-c types in 272 cankers on 42 trees. By the end of 1985, 25 cankers that had not been treated had yielded isolates with the phenotype of European hypovirulent strains.

Additional key words: biological control, chestnut blight, *Endothia parasitica*, hypovirulence, vegetative compatibility.

The dynamics of populations of pathogens that infect perennial plants might be expected to be rather different from epidemics on annual crops. The rust and blast diseases on Pangola grass in the Caribbean show interlocking cycles of epidemic (17). Genotypes of homothallic species, or species with no sexual reproduction, may vary little from one cycle to the next, but offspring of sexually reproducing species will face selection pressures for fitness as well as the vagaries of the founder effect. The latter is based on the chance survival of a few genotypes at the end of an epidemic cycle (16). Chestnut blight disease is caused by the ascomycete *Cryphonectria parasitica* (Murr.) Barr (formerly *Endothia parasitica* (Murr.) And.). The fungus was introduced into the United States around 1900 and has reduced the population of native chestnut trees (*Castanea dentata* (Marsh.) Borkh.) to groups of understory shrubs that are infected, killed back to the ground, and resprout from the root-collar (9). Because the trees are usually killed before they are big enough to reproduce sexually, and two sexually mature trees are needed for cross pollination, there has been little opportunity for evolution of resistance. Few

publications have dealt with the ecology of this introduced pathogen and whether a stable relationship has been established between the tree and its pathogen. May (22) and others (16) have discussed various kinds of plant-herbivore interactions hoping to find ways of modeling the apparently cyclic nature of population numbers. Hebard (15) has suggested that one cycle of the blight epidemic in a clear cut takes about 15 yr.

The phenotype vegetative compatibility (v-c) is controlled by several nuclear genes in *C. parasitica* (1,3,8), as it is in many other fungi (18). Individuals can be identified as having a certain v-c type, and thus belonging in a given v-c group. MacDonald and Double (21) censused 16 subpopulations of *C. parasitica* in two clear-cut areas in West Virginia for v-c. They found different common types in the two areas. Between the time of their preliminary report in 1978 (21) and their final report on these plots (in 20), the number of v-c types found in the areas increased.

In this paper, we report data for a census conducted of the phenotype v-c of *C. parasitica* strains isolated from living American chestnut trees in a mixed-hardwood plot in Connecticut through four early years of a cycle of the blight epidemic.

MATERIALS AND METHODS

American chestnut trees were found in a stand of mixed

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hardwoods (chestnut, oak, beech) in Rocky Hill, CT. There were no trees older than about 30 yr in the area, and no standing dead trees. We were told by the owner that dead trees and occasional mature trees had been harvested since about 1900. The forested area occupied approximately 0.8 ha and about half of this contained numerous sprouts of American chestnut. Chestnut blight has been present in this area since about 1917, and old stromata of the blight fungus were present on stumps of cut chestnut trees.

The area with the highest density of chestnut (about 60 × 60 m) was mapped (Fig. 1). Blight incidence was low in the summer of 1982, and eight chestnut trees averaged about 12 m in height and 17 cm diameter at breast height (dbh). There were 42 smaller trees or sprout clumps (78 total stems) that averaged about 6 m in height and 4 cm dbh. There were three chestnut stumps with very small sprouts. The living trees that were at least 2.5 cm dbh were numbered with white latex paint, and blight cankers numbered and sampled. As small trees grew to this size, they were numbered.

All trees were examined weekly in the fall of 1982 and spring to fall 1983, 1984, and 1985. New cankers were sampled as soon as they were seen. Bark samples were taken from the canker margins, and *C. parasitica* isolated as previously described (11). A total of 272 isolates (one per canker) was taken from new cankers on 42 trees or sprout clumps from the fall of 1982 through the fall of 1985.

Cultures were maintained by weekly transfers to Difco brand potato-dextrose agar (PDA) and incubated at 25–28 C or stored on PDA slants in screw-capped tubes at 4 C.

Isolates from the Rocky Hill plot were paired with each other in vegetative compatibility tests (1,8,11), tester type strains designated, and new types designated if strains were not compatible with the tester strains. Strains that did not form barrage lines when paired with a tester strain on PDA were considered to have the same v-c phenotype as the tester strain, and therefore, to be in the same v-c group. Pairings were also made with Connecticut v-c type tester strains (11). Typed isolates were then referred to as strains. Our use of the word "strain" does not imply physiologic race.

The strains were paired in the laboratory with *C. parasitica* strains containing Italian hypovirulence (H) determinants to convert them to H strains by cytoplasmic transfer across anastomosis bridges (2,10). Italian H strains contain Type 2 dsRNA (13). This genome determines lack of pigment, i.e., "white" phenotype, and lack of virulence (4,7,14,25). The phenotype is easily recognized in vitro, a useful "marker" for field studies. New strains were initially paired with three H strains that represented the two most common v-c groups in the plot and a third that was found in the first season (Rocky Hill v-c 1, RH 2, and RH 5). If no conversion occurred, they were paired with eight more strains with different v-c types. If conversion failed with these, other H strains

were selected from our H strain library for pairing. Converted mycelium was isolated to PDA for further growth and to confirm the morphology. Each new H strain was inoculated around the margin of the canker from which its progenitor had come, and all treatments successfully halted canker expansion (method reviewed in 4,7,14,25).

New isolates were always carefully examined after several days of growth on PDA for evidence of the phenotype associated with European hypovirulent strains (described above and in 4,7,14,25). Any isolate that appeared to lack pigment was transferred again to PDA for further examination.

In 1985, all cankers that had already been sampled were also sprayed weekly with a water suspension of conidia from 2-wk-old PDA cultures of the three H strains used in initial conversion tests.

Progeny of *C. parasitica* formed in the Rocky Hill plot were also examined. *C. parasitica* is homothallic but preferentially outcrosses (one mating type locus with two known alleles) (7,23). Hypovirulent mycelia usually do not produce perithecia, and H determinants have never been found among ascospore progeny. Although canker expansion was halted by treatment with hypovirulent inoculum, protoperithecia may have formed before H determinants reached the centers of the cankers. Bark samples with perithecia were excised from tree 9/canker 1, 10/2, 24/2, 24/8, 28/1, and 28/2. These were examined under a dissecting microscope, mature perithecia were removed singly, and single ascospore clones derived as described previously (23). These progeny were tested for compatibility with the mycelium in their progenitor cankers and with other Rocky Hill v-c types.

The diversity of vegetative compatibility phenotypes found in populations of *C. parasitica* has been recently discussed (11). Index S/N is the number of phenotypes found divided by the total number of samples. The index H' , frequently used by ecologists (11,16), is:

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

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.

RESULTS

The incidence of new blight cankers at Rocky Hill over the four sampling years is graphed in Figure 2. The number of trees infected went from 4 to 19 to 36 to 42, whereas the number of cankers increased from 12 to 57 to 159 to 272. Infections developed throughout the 60 × 60-m area monitored, but there were seven trees that had no visible cankers in the fall of 1985. These were trees 17, 19, 42, 43, 45, 48, and 49, with dbh's of 10, 12, 3, 7, 7, 2.5, and 2.5 cm in 1985.

The diversity of v-c types of *C. parasitica* from Rocky Hill is



Fig. 1. Map showing the location at Rocky Hill, CT, of 50 American chestnut trees (*Castanea dentata*) that are single stems or multiple sprouts at least 2.5 cm diameter at breast height. Not shown are the many oaks, beeches, and woody shrubs in the plot.

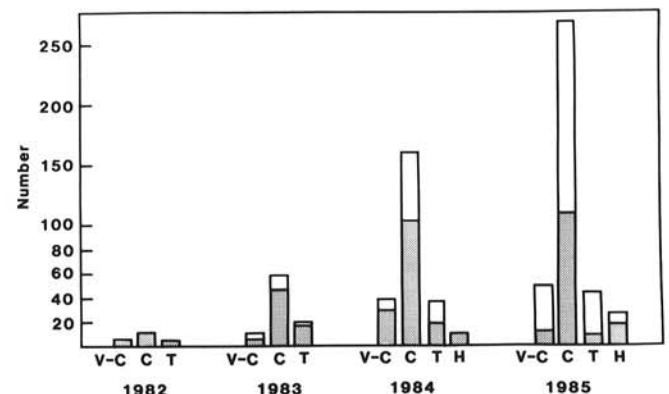


Fig. 2. Number of *Cryphonectria parasitica* v-c types, cankers (C), infected trees (T), and cankers that were hypovirulent (H) when first sampled (i.e., without treatment) on the American chestnut trees at Rocky Hill, CT. The total bar heights indicate cumulative number and the shaded parts indicate the number new for each year.

shown in Figure 3. The types recovered most frequently (RH 1, RH 2, and RH 4) had never been found in Connecticut before. We previously reported v-c type diversity for a sampling of cankers of *C. parasitica* throughout Connecticut (11) as $S/N = 0.41$ and $H' = 2.74$. If we calculate the diversity of v-c types in this censused plot (excluding five H isolates that could not be typed) we get $S/N = 0.18$, and $H' = 2.8$. The type recovered most often from the Rocky Hill plot was RH 1, which was found with similar frequency among ascospore progeny from RH 1 cankers and among isolates from new cankers (Table 1).

Some trees were repeatedly infected by strains of *C. parasitica* in the same v-c group; others developed cankers that were caused by strains of several new v-c types. For example, in 1984, two trees developed four new cankers, all with mycelium in the same v-c group as resident cankers. In the same season, 11 trees developed 17 new cankers with mycelium in the same v-c group as resident cankers, and 29 new cankers that were caused by strains with v-c types not previously found on those trees (29/50 new v-c types = 58%).

Progeny from the cankers sampled in 1983 (trees 9 and 24) and in 1984 (trees 10, 24, and 28) are described in Table 2 as being of the same or different v-c type as the mycelia in their progenitor cankers.

The number of isolates that were hypovirulent went from zero (in 1982 and 1983) to nine (in 1984) to 25 (in 1985) (Fig. 2). These were usually recovered from trees with other cankers that had been sampled and treated previously.

DISCUSSION

Hebard (15) followed blight progress in clear-cut areas in Virginia and concluded that spread of virulent (V) strains of *C. parasitica* is relatively inefficient at low population levels. This would explain why American chestnut trees sometimes become fairly large (10 cm dbh) before increase in canker number become logarithmic.

A large mass of inoculum of *C. parasitica* must be present in any stand of chestnut trees with several blight cankers. Conidia may be dispersed by rain-splash, on the fur or feathers of animals and birds, or by insects, whereas ascospores may be dispersed by these methods, and may also be airborne (9). The largest amount of inoculum at Rocky Hill probably came from the cankers present on living trees, since decay organisms quickly invade the bark when

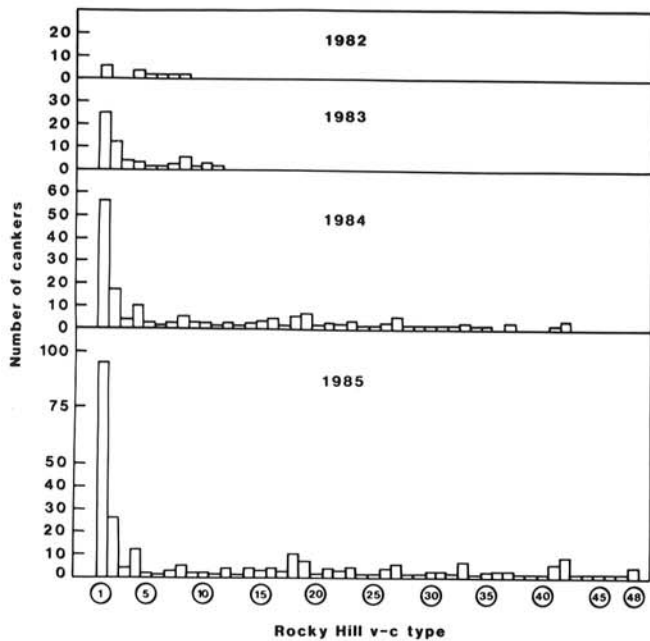


Fig. 3. Number of *Cryphonectria parasitica* strains of each v-c type present on American chestnut trees at Rocky Hill, CT. Cankers were sampled in the fall of 1982 and spring to fall 1983, 1984, and 1985. The v-c type numbers are Rocky Hill numbers and not Connecticut v-c type numbers.

the trees die (12). The trees could also have been infected by inoculum from the leaf litter, the base of cut, dead trees, or from cankers on trees outside the 60- × 60-m area studied.

Rankin's 1914 paper (24) contains some of the best early field ecology done on this disease. He believed, based on all of his observations, that the majority of primary infections of healthy chestnut trees were caused by windborne ascospores, although both ascospores and conidia could infect wounds.

MacDonald and Double (21) concluded that the new infections in 16 chestnut plots in two 10- to 15-yr-old clear-cut areas in West Virginia were frequently the result of ascospore inoculum because two or more cankers on the same tree were more commonly caused by strains of *C. parasitica* of different rather than similar v-c types. In Rocky Hill, 58% of strains from cankers that developed in 1984 on trees with resident cankers were not the same v-c type as the strains from those resident cankers.

The ascospore progeny isolated from perithecia in Rocky Hill cankers showed surprisingly little variation in v-c type (Table 2). Either both mating types (genotypes *A* and *a*) were present in the same v-c phenotype (i.e., strains v-c 1, *A*, and v-c 1, *a*), or a great deal of selfing occurred (which is possible in *C. parasitica*; 7,23). The lack of variation might also be due to strong linkages between the v-c genes preventing recombination. The combined effective inoculum of ascospores and conidia present on a given tree was, therefore, very conservative of v-c type. That is, if new cankers on a tree were to be caused only by conidia or ascospores formed on cankers on that tree, most of them would have v-c types similar or identical to those of the cankers already present.

In spite of this, the new cankers that developed were caused by strains in ever-changing v-c groups as time progressed (Fig. 3). Not only were many strains not like strains in resident cankers on their own trees, but v-c types were recovered that were not present in any of the cankers on the 50 trees of the plot. From this we can conclude that many of the new cankers that arose from one season to the

TABLE 1. Strains from new cankers and progeny of *Cryphonectria parasitica* from vegetative compatibility (v-c) RH 1 cankers on *Castanea dentata* at Rocky Hill, CT, listed as total numbers and percentages recovered in v-c group RH 1

| Strains | Progeny | Strains | Progeny | Strains |
|----------|---------|---------|---------|---------|
| 1983 | 1983 | 1984 | 1984 | 1985 |
| Total 45 | 123 | 102 | 299 | 113 |
| RH 1 20 | 56 | 31 | 97 | 41 |
| 44% | 46% | 30% | 32% | 36% |

TABLE 2. Progeny of *Cryphonectria parasitica* from perithecia in cankers on *Castanea dentata* at Rocky Hill, CT, distinguished by Rocky Hill vegetative compatibility (v-c) type

| Year | Tree/canker (v-c) | Perithecium | Number of progeny in each group | |
|--------------|-------------------|-------------|---------------------------------|-----------|
| | | | v-c-like canker mycelium | other v-c |
| 1983 | 9-1 (RH 1) | 1 | 56 | 67 |
| | 24-2 (RH 2) | 1 | 93 | 7 |
| 1984 | 10-2 (RH-1) | 1 | 12 | 37 |
| | 28-2 (RH 1) | 1 | 30 | 20 |
| | | 2 | 20 | 41 |
| | | 3 | 10 | 29 |
| | 24-8 (RH 1) | 1 | 11 | 39 |
| | | 2 | 14 | 36 |
| 28-1 (RH 25) | 1 | 37 | 8 | |
| | 2 | 13 | 35 | |
| | 3 | 25 | 24 | |

next were certainly not initiated by conidia from resident cankers. Those conidia would have had the same v-c type as the progenitor mycelium.

It could be argued that increased diversity in v-c phenotype of *C. parasitica* might be directed by effective dispersal of H conidia from resident cankers. These might cause conversion of compatible virulent inoculum on the surface of the bark, preventing infection, or conversion of small incipient virulent cankers, preventing them from reaching noticeable size. Some evidence of H spread through experimental plots has been seen in most of our previous experiments. See, for example, data in (6) where isolates taken from three of 15 virulent control cankers at the end of the experiment were clearly hypovirulent. If hypovirulence is forcing diversification at Rocky Hill, a sharp increase in the number of new isolates that have the European hypovirulence phenotype should be observed in the next few years.

The change in the v-c profile of the population could also be due to the normal genetic drift, due to sexual reproduction and recombination of alleles. Several gene loci determine v-c type in *C. parasitica* (1,3,8). A total of 48 v-c types was recovered from cankers at Rocky Hill. Recombination of pairs of alleles at five loci would yield 32 phenotypes, and at six loci, 64 phenotypes. Recommendation of pairs of alleles at four loci and three alleles at one more locus would produce 48 phenotypes.

The phenotype v-c has been examined in members of several fungal species collected over wide areas, but few studies have concentrated on the members of small populations. MacDonald and Double (21, and in 20) have reported v-c diversity in 16 small subpopulations of *C. parasitica* in West Virginia. In 1978 (22) they had a total of 37 v-c types among 202 isolates. One type predominated (47/202), six other types were each found 10 times or more, and 23 types were found only once. This yields diversity indexes of $S/N = 0.18$ and $H' = 2.65$, almost exactly what we found at Rocky Hill. As more cankers were sampled in subsequent years, strains that formed very weak barrages (5) were classified as clusters (19). When these populations were reported in 1984 (in 20) there were 125 v-c types and clusters among more than 800 isolates, yielding a decrease in S/N to 0.14 and an increase of H' to 3.57. The index H' is especially sensitive to the presence of singles in data, and the singles in MacDonald and Double's 1984 data (in 20) had risen to 123. Their most common type still predominated, with 88 isolates. It is not possible to examine the changes in individual subpopulations because the data are reported compiled, but in their 1978 paper (21), MacDonald and Double noted that one type that was frequently recovered in one area (26 times) was not recovered at all in the other area.

Kuhlman and Bhattacharyya (19) censused *C. parasitica* on 19 trees along a 303-m transect in Virginia and used cluster analysis to establish 17 v-c clusters and four singles among 93 isolates. This yields an S/N of 0.23 and an H' of 2.76.

There are now hypovirulent strains in 48 v-c groups deployed in our Rocky Hill plot. In spite of this, only 25 spontaneous H cankers have been identified. These represent at least eight v-c types (five could not be typed due to their H morphology).

We must now examine our data on conversion of these Rocky Hill strains by our standard H strains in light of Kuhlman's suggestion (19,20) that conversion clusters can be identified that can facilitate choice of strains for treatment. Sampling and spraying of conidia from hypovirulent strains will continue and more information will be compiled about the genetic diversity of this subpopulation of the Connecticut population of *C. parasitica*.

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