Greater Diversity of Vegetative Compatibility Groups of *Cryphonectria parasitica* on Scarlet Oak than on Post Oak in North Carolina

B. L. NASH, Former Graduate Student, and W. J. STAMBAUGH, Professor, School of Forestry and Environmental Studies, Duke University, Durham, NC 27706

ABSTRACT

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The distribution of vegetative compatibility (v-c) groups of *Cryphonectria parasitica* on cankered post oak (*Quercus stellata*) and scarlet oak (*Q. coccinea*) was investigated in North Carolina. Of 37 separate cankers on 12 post oaks, 35 had one v-c group and two had two v-c groups. The groups were the same or differed among cankers on single trees. On scarlet oak, several different v-c groups were present within small portions of infected butt sections. The number of different v-c groups within single scarlet oaks varied from one to five. Distribution patterns of v-c groups were not predictable, even among trees located close to each other.

Recent attempts at biological control of chestnut blight have centered on the use of hypovirulent (H) strains of the fungus *Cryphonectria parasitica* (Murr.) Barr (=*Endothia parasitica* (Murr.) Anderson & Anderson) (3,16). Control is "presumably achieved" when debilitating viruslike particles are transferred from H strains of *C. parasitica* to healthy, virulent (V) strains via hyphal anastomosis, causing them to become hypovirulent (3,7,8,16). The host is then able to resist the debilitated pathogen. Hyphal unions are most readily formed between strains of the same or closely related vegetative compatibility (v-c) groups (4–6,10). The impact of v-c diversity on transmission of H agents in the field is unknown. Studies on American chestnut (*Castanea dentata* (Marsh.) Borkh.) found many v-c groups within individual cankers, trees, and stands (2,9,11,12) and suggested that vegetative incompatibility could be a major obstacle to the spread of H strains. Other laboratory studies have demonstrated the existence of H strains with "broad conversion capacity," i.e., the ability to convert V strains in several different v-c groups (5,6,10).

C. parasitica infects several species of oak, including post (*Quercus stellata* Wang.), scarlet (*Q. coccinea* Muenchh.), and white (*Q. alba* L.) (13,15). Because isolates of *C. parasitica* from oak are pathogenic on American chestnut (15), and because oaks are found throughout the range of American chestnut, the v-c groups on oak may contribute to v-c diversity on chestnut. In a systematic survey of the North Carolina mountains and Piedmont (14), we identified 8, 11, and 36 different v-c groups from white, post, and scarlet oaks, respectively. Specific v-c groups most common on American chestnut were also the most common groups on the oaks on a regional basis (14). The purpose of the present study was to investigate the distribution of v-c groups within cankers on individual trees and among cankered trees within a stand.

MATERIALS AND METHODS

Initially, 12 post oaks and 10 scarlet oaks infected with *C. parasitica* and situated on the Duke Forest (Durham and Orange counties, North Carolina) were chosen for v-c testing. All cankers were well developed and judged typical in morphology for each host species.

Loose, exfoliating bark from 37 discrete cankers on 12 post oaks was removed with a hand ax and discarded. Six to 10 samples, distributed across the canker face, were then obtained with an increment hammer. Individual samples were approximately 4 mm in diameter and 10 mm long. In the laboratory, the samples were flame-sterilized, planted on Difco potato-dextrose agar (PDA), and incubated on a laboratory bench at room temperature. Pure cultures of *C. parasitica* were transferred to PDA slants for long-term storage.

Methods similar to those of Anagnostakis (1,2) were used to determine v-c groups. Isolates to be tested were first grown on PDAmb (Difco PDA amended with 100 mg/L of methionine and 1 mg/L of biotin). By means of a 3-mm cork borer, mycelial plugs were removed from the colony margin and transferred to fresh plates of PDAmb. These plugs were arranged so that each plate contained either all or 10 isolates from an individual canker paired against each other. All pairings were replicated three times. The plates were incubated in the dark at 25 C and examined periodically for isolate interactions. Isolates that merged were classified as compatible and those that formed barrages, as incompatible (1,10). Pairings that gave intermediate reactions or both reactions were repeated. One or two isolates were chosen to represent each group of compatible isolates from each plate, and these representatives were then successively paired with each other to determine the number of v-c groups per canker. After characterization of isolates within cankers, representative isolates from each canker were paired against each other to determine the v-c groups within each canker on each of the post oaks with multiple cankers. Isolates were not compared among trees for post oak.

Unlike post oak cankers, basal cankers of scarlet oak are not discrete and the outer bark is generally firmly attached. A hand ax was used to expose a portion (619–1,254 cm^2) of infected inner bark from 10 scarlet oaks (SO-1 through SO-10, Table 1). Tissue samples were collected and treated as described for post oak.

An additional five scarlet oaks (SO-11 through SO-15) were selected to examine v-c distribution patterns within and among trees in a local infection center. Each of the five trees had well-developed multiple cankers, with pronounced swelling that encircled the entire basal circumference to a height of approximately 1 m. The cankered portions of these trees were more extensive than the infected areas on the first 10 scarlet oaks. Each so-called swollen butt was arbitrarily divided into 20–28 sections, and each section was sampled intensively. Isolate collections from each section of each scarlet oak were kept separate so that distribution of v-c groups could be determined both within and among trees. All v-c representatives from each canker section were paired against each other. In addition, all representatives from SO-12 through SO-15 were paired against each other to determine the distribution of v-c groups between trees. Distances between these trees were measured and the resulting v-c patterns mapped.

RESULTS

Testing showed that only two of the 37

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cankers on post oak had more than one v-c group within a single canker (Table 2). Both cankers were on the same tree (PO-15) and had the same two v-c groups. Seven of the 12 post oaks had more than one canker. Five of the seven (four with two cankers and one with eight) had the same v-c group in all of the cankers; the other two (PO-1 and PO-15) had more complex distributions. Each of the three cankers on PO-1 had only one v-c group per canker; two cankers contained the same v-c group and the third, a different group. Of the 13 cankers on PO-15, two each had the same two v-c groups and 11 had only one v-c group. Every v-c group identified in these 11 cankers was one of the two v-c groups found in the other two cankers on PO-15.

Initially, only small portions of 10 scarlet oaks (SO-1 through SO-10) were examined for v-c groups. Seven of these trees yielded only one v-c group within the sampled area, two yielded two groups, and one yielded three groups (Table 1). Because it appeared that the distribution of v-c groups might be more complex on scarlet oak than on post oak, five additional trees were selected and intensively sampled throughout their symptomatic portions. Four of these trees (SO-12 through SO-15) were located close to each other, affording an opportunity to check the distribution of v-c groups both within and among trees. These four trees, with their v-c groups, are mapped in Figure 1. The maximum distance between these trees was 67.6 m. SO-12 and SO-13 were large trees whose bases had expanded to make contact. Six different v-c groups were isolated from these two trees, with only one group common to both trees. SO-14 had five different v-c groups (one in common with SO-13), and SO-15 had three groups. SO-15 also had a v-c group in common with SO-13, but it was a different group than that shared by SO-13 and SO-14. Another of the three v-c groups isolated from SO-15 was designated group I_a. This group merged with group I from SO-14, but I and I_a reacted differently when paired separately against other v-c groups. If I and I_a are considered as one v-c group, then 10 different v-c groups were identified from these four trees. SO-11 was located in another stand on the Duke Forest and yielded only one v-c group in 97 isolates representing the total basal area.

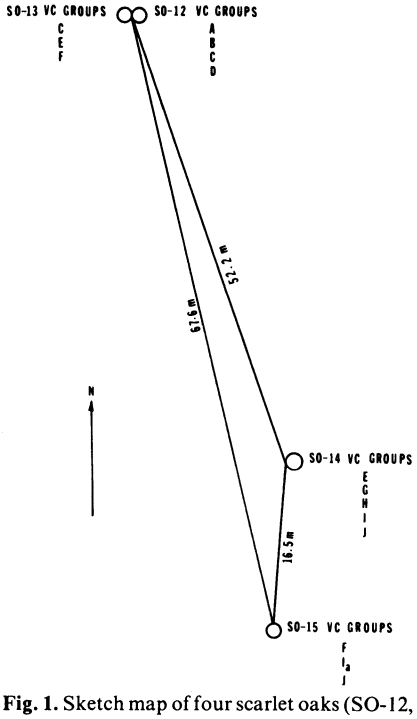

Fig. 1. Sketch map of four scarlet oaks (SO-12, SO-13, SO-14, and SO-15) infected with *Cryphonectria parasitica* on the Duke Forest, showing location of the trees and the vegetative compatibility (v-c) groups associated with each tree. The letters assigned to each v-c group are for delineation only and have no relationship to any other studies.

DISCUSSION

Cryphonectria cankers on scarlet oak appear to have a greater diversity of v-c groups than do those on post oak. Most cankers on post oak contained a single v-c group, as did most cankers on a single tree. Asexual fruiting structures were often observed on post oak in North Carolina, and stemflow samples contained large numbers of conidia. This may be a mechanism by which multiple cankers of the same v-c group could be initiated on individual trees.

Our intensive study of infected scarlet oaks indicated that a single swollen butt could contain a single v-c group or up to five different groups (Table 1, Fig. 1). Distribution among trees showed no

Table 1. Vegetative compatibility (v-c) groups isolated from scarlet oaks infected with *Cryphonectria parasitica* on the Duke Forest, North Carolina

Tree	dbh (cm)	Portion of basal canker sampled (cm^2)	Isolates screened (no.)	Maximum v-c groups per sample area[a] (no.)
SO-1	68	824	6	1
SO-2	52	632	11	1
SO-3	48	720	10	1
SO-4	45	695	9	1
SO-5	50	1,006	2	1
SO-6	30	619	16	2
SO-7	34	695	17	2
SO-8	73	1,254	10	3
SO-9	48	1,099	17	1
SO-10	66	813	12	1
SO-11	41	Entire base	97	1
SO-12	46	Entire base	155	4
SO-13	39	Entire base	148	3
SO-14	34	Entire base	174	5
SO-15	26	Entire base	220	3
			Total 904	

[a] Methods similar to those of Anagnostakis (1,2) were used to determine v-c groups. All isolates collected from an individual sample area were paired in culture to determine the number of v-c groups in that sample area.

Table 2. Vegetative compatibility (v-c) groups isolated from post oaks infected with *Cryphonectria parasitica* on the Duke Forest, North Carolina

Tree	dbh (cm)	Cankers sampled (no.)	Isolates screened (no.)	Maximum v-c groups per canker[a] (no.)	Maximum v-c groups per tree[b] (no.)
PO-1	47	3	31	1	2
PO-2	21	2	24	1	1
PO-3	15	2	12	1	1
PO-4	20	1	21	1	1
PO-5	15	1	6	1	1
PO-6	12	1	13	1	1
PO-7	20	1	19	1	1
PO-8	29	2	14	1	1
PO-9	18	1	3	1	1
PO-11	35	2	32	1	1
PO-12	41	8	43	1	1
PO-15	27	13	101	2	2
		Total 37	319		

[a] Methods similar to those of Anagnostakis (1,2) were used to determine v-c groups. All isolates collected from an individual canker were paired in culture to determine the number of v-c groups in that canker.

[b] Representative isolates from each canker were paired in culture to determine the number of v-c groups in each tree.

apparent pattern. SO-12 and SO-13, which touched each other at their bases, shared only one of six v-c groups. This was the same number of v-c groups SO-13 shared with SO-14 and SO-15, located 52.2 and 67.6 m away, respectively. SO-12 was the same distance as SO-13 from SO-14 and SO-15 but shared no v-c groups with those two trees.

Data on v-c groups at the canker, tree, or stand level have not been published for the oak hosts, but several studies have been done on American chestnut. Anagnostakis (1) reported that 10 v-c groups were present among the single-ascospore progeny from 13 perithecia obtained from one canker. A single perithecium from this canker contained six v-c groups. MacDonald and Double (11) studied the frequency of v-c groups in 16 plots of chestnut regeneration in West Virginia and reported that 31 of 41 trees with multiple cankers had different v-c groups in different cankers. Four years later, McDonald et al (12) found that although 47% of the 880 isolates from these plots could be classified into six v-c groups, there were 37 well-defined v-c groups plus 85 isolates that were incompatible with each other and the 37 defined groups. Kuhlman and Bhattacharyya (9) reported that four v-c groups were represented in six cankers on a single, isolated American chestnut at Bonair, Tennessee; four cankers had three groups and two cankers had two groups. At Buchanan, Virginia, 41 cankers on 19 American chestnuts studied by Kuhlman and Bhattacharyya (9) had one to five (av. 2.3) v-c groups per canker (9). Direct comparisons are not possible, but these various studies suggest that v-c diversity in American chestnut is probably greater than that observed in scarlet oak and possibly much greater than that in post oak.

The greater degree of uniformity in v-c groups on post oak was somewhat surprising. Although occasionally found on post oak cankers, perithecia of *C. parasitica* were not found on infected scarlet oaks. We had thought that the presence of the sexual stage would increase v-c diversity.

Under the conditions of our v-c tests, groups I and I_a (from SO-14 and SO-15) merged with each other but were not consistent when tested against other v-c group representatives. This type of reaction has been observed before (4,10,13,14) and supports the theory that v-c groups may not be discrete entities but instead may be members of different merge groups or networks (4,10,13). Members within a merge group would have different degrees of relatedness with other members of the group. There is a second hypothesis that would explain these results. Because Cryphonectria cankers on scarlet oak are long-lived and not discrete, a "swollen butt" could result from multiple *Cryphonectria* infections. We used mass isolates in this study, and more than one v-c group may have been present within each mass isolate.

It is not known whether inoculum of *C. parasitica* is disseminated from infected oaks to chestnut. The potential impact of this inoculum should be investigated. Scarlet oak is of particular interest because it is found throughout much of the present range of American chestnut and, unlike American chestnut, can tolerate repeated infection by *C. parasitica*. This study indicates that greater v-c diversity exists on scarlet oak than on post oak in the North Carolina Piedmont.

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