DISEASE INCIDENCE, SYMPTOMATOLOGY, AND VEGETATIVE COMPATIBILITY TYPE DISTRIBUTION OF *ENDOTHIA PARASITICA* ON OAK AND CHESTNUT HOSTS IN NORTH CAROLINA

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ABSTRACT.--American *chestnut, scarlet oak, white oak, and post oak were identified as hosts of Endothia parasitica in the North Carolina mountains and Piedmont. Symptomatology of the oak hosts, and disease incidence by region are given. A diversity of vegetative compatibility (v-c) types were collected from each host. Similar specific v-c types were found on the different hosts, however the relative frequencies varied between hosts. Scarlet oak, because of its range, has the greatest potential for use in hypovirulence research.*

In the United States, *Endothia parasitica* has been reported on several species of oaks including: scarlet *Quercus coccinea*, post *Q. stellata*, and white *Q. alba* oaks. Unfortunately, little has been published concerning *E. parasitica* on these hosts other than confirmation of etiology and descriptions of symptom expression (Anderson and Babcock 1913; Rankin 1914; Clapper et al. 1946; Ham 1967). A more complete knowledge of these oak hosts is needed to determine their importance as virulent inoculum sources and as potential reservoirs of hypovirulent (H) strains of *E. parasitica.* This study was designed to determine the symptomatology, regional disease incidence, and patterns of vegetative compatibility (v-c) types on the hosts of *E. parasitica* in the North Carolina Piedmont and mountains.

Materials and Methods

Mountain and Piedmont Surveys. These two surveys included all 21 mountain counties of North Carolina, and 12 Piedmont counties selected for their large oak populations. Gridded county highway maps were used to locate 6 randomly chosen plots in each county. In the field, each plot was described and systematically subdivided into 5 subplots, employing the methodology of the U. S. Forest Service Renewable Resources Evaluation Project (1977). Subplot boundaries were established with a 10-factor basal area panama tube. Within each subplot, all American chestnuts, *Castanea dentata,* and all oaks with a minimum 2.5 cm d.b.h. were identified and examined for the presence of *E. parasitica.* Each infection was characterized and appropriate symptom or sign material collected for laboratory isolation. Suscepts between subplots and additional stands of suscepts identified by local U. S. Forest

Service personnel were also investigated, as time permitted. Several additional collections of *E. parasitica* were made to supplement the isolate base for v-c testing. The first of these involved periodic sampling of American chestnuts along the entire length of the Blue Ridge Parkway (BRP) in North Carolina. A second set of isolates, obtained from scarlet, post, and white oaks, was amassed in a limited survey at Stone Mountain State Park, North Carolina. The last group of isolates (to be called Mixed survey) represents cultures obtained on various trips throughout the state during 1979 to 1980.

Vegetative compatibility testing. *Endothia parasitica* was best isolated from infected host tissue collected from either the canker margin (American chestnut), loosened bark (post oak), or involuted inner bark tissues (scarlet and white oaks). Samples were flame sterilized, plated onto potato dextrose agar (PDA) and incubated on a laboratory bench at room temperature. *Endothia parasitica* usually appeared 2 to 3 days later. Isolates were then transferred to PDA slant culture for long-term refrigerated storage.

Vegetative compatibility types were determined using the method of Anagnostakis (1978). Survey isolates were first grown on PDA amended with 100 mg/1 methionine and 1 mg/1 biotin. Mycelial plugs were removed from culture margins and paired in new plates of the same media with known v-c testers supplied by the Connecticut Agricultural Experiment Station (v-c types 1 to 63). These plates were incubated at 25 C in the dark and later examined for vegetatively compatible (merge) or incompatible (barrage formation) reactions. Each survey isolate was initially paired with ten v-c testers. Isolates that were not compatible with any of these testers were then paired with an additional 10 v-c testers. After the third series in this manner, all remaining survey isolates were tested against all remaining v-c testers. At least three pairings were made between each unknown and v-c tester.

Results

Mountain and Piedmont surveys. Eleven species of oak were examined during the course of this study, eight of which were not infected by *E. parasitica.* These species, and the total number of stems examined were: northern red oak *Q. rubra,* 424; chestnut oak *Q. prinus,* 353; southern red oak *Q. falcata,* 162; black oak *Q. veZutina,* 117; water oak *Q. nigra,* 22; willow oak *Q. phelios,* 13; blackjack oak *Q. marilandica,* 12; and chinkapin oak *Q. muehlenbergii, 1.* American chestnut, scarlet oak, post oak, and white oak were identified as hosts of *E. parasitica* in this survey (Figures 1, 2 and 3). Incidence of infection varied by species and region (Table 1). Disease incidence for all hosts was slightly higher in the mountains (11.4 percent) than in the Piedmont (10.5 percent) or in both regions combined (11.1 percent).

American chestnut was found primarily as small stump sprouts in the mountain counties. The low incidence of infection (17.0 percent) probably reflects the large number of these sprouts and their associated juvenile resistance. There was no evidence, based on visible symptomatology or subsequent cultural characteristics, that any cankers on American chestnut contained H strains of *E. parasitica.*

Figure 1. Symptomatology of *Endothia parasitica* on oak. Swollen butt of scarlet oak showing multiple cankers and rough involuted bark.

Figure 2. *Endothia* canker of post oak. Note cracked bark and various degrees of callus formation.

Figure 3. *Endothia parasitica* on white oak.

Table 1. Percent incidence of *Endothia parasitica* on various hosts in a 33-county survey of North Carolina

Host	Piedmont[a/]	Mountains	Regions combined
American chestnut	0.0 (1)	17.0 (866)	17.0 (867)
Scarlet oak	25.9 (174)	8.6 (406)	13.8 (580)
Post oak	14.5 (69)	50.0 (2)	15.5 (71)
White oak	1.0 (310)	1.0 (364)	1.0 (674)
Total	10.5 (554)	11.4 (1638)	11.1 (2192)

[a/] Number in parenthesis is total trees.

Infected scarlet oaks were most often characterized by the swollen butt condition, as first described by Ham (1967). Basal cankering by *E. parasitica* resulted in various degrees of basal swelling, callus formation, involution of bark, and distortion of wood tissues beneath cankered areas. Infected tissues were sometimes localized, but more typically the entire basal circumference was involved. Pycnidia, (and rarely perithecial stromata), were usually present in bark crevices. Orange mycelial fans were always present. *Endothia parasitica* was also isolated from small bole cankers. These cankers, which never exceeded a few centimeters in diameter, were sometimes found on trees that also exhibited swollen butt (Table 2). Infected scarlet oaks were found in the Piedmont and mountains, although infected trees were almost three times more prevalent in the Piedmont. In both regions, diseased trees were usually found in groups, rather than as randomly scattered trees. The d.b.h. of infected trees ranged from 11.9 to 78.7 cm inches with a mean of 31.2 cm.

Table 2. Symptomatology of *Endothia* canker on Scarlet oak in North Carolina from the 1979-80 33-county survey

	Number of Trees		
Symptomatology	Piedmont	Mountains	Regions combined
Swollen Butt	31	31	62
Bole cankers	5	3	8
Both symptoms	9	1	10
Total	45	35	80

Post oak was found primarily in the Piedmont, where 14.5 percent of the total 69 trees exhibited one to numerous irregular bole cankers. These cankers were typified by cracked or missing bark, and an abundance of fungal tissue. This tissue was sometimes so extensive that the limits of individual cankers could not be delineated. Examination of the canker face, which was often surrounded by callus, and the interior portions of loosened bark revealed numerous pycnidia and rarely perithecial stromata. Completely callused cankers were sometimes observed. Infected post oaks, like scarlet oaks, were usually found in groups. The d.b.h. of the infected trees ranged from 16 to 53.1 cm with a mean of 30 cm.

The disease incidence of white oak was 1 percent in both the mountains and the Piedmont. *Endothia parasitica* on this host species was associated with a range of symptomatology including: basal swelling and cracking, gall formation, and wounds within stem crotches. Orange fan material was always present.

Vegetative compatibility testing. In this study, 278 isolates of *E. parasitica* were obtained from 247 trees. Some of these isolates, even upon repeated testing, showed compatible reactions with more than one of the v-c testers. It was decided for the purposes of this study, to list each compatible reaction as a separate v-c type. Thus, 1 survey isolate could be placed with 2 or more different v-c types. Following this procedure, our 278 isolates yielded 332 compatible reactions with 48 different v-c testers. This data, summarized by host species in Table 3, shows that a diversity of v-c types were collected from each host species. An additional 55 isolates did not show compatible reactions with any of the v-c tests.

Table 3. Distribution of vegetative compatibility (v-c) types among hosts of *Endothia parasitica* in North Carolina, 1979 to 1981

Hosts	Trees	v-c typed isolates	Number of different v-c groups[a/]
American chestnut	117	163	34
Scarlet oak	103	134	36
Post oak	12	16	11
White oak	10	11	8
Chestnut species	3	6	6
Oak species	2	2	2
Total	247	332	

[a/] v-c groups as delineated by the Connecticut Agricultural Experiment Station.

The v-c types found most often on American chestnut were similar to those found most often on the other hosts of *E. parasitica* (Table 4). However, the relative frequency among these v-c types did vary between hosts. The number

Table 4. Frequency of the most common vegetative compatibility (v-c) types on hosts of *Endothia parasitica* in North Carolina, 1979 to 1981

Host				
American chestnut (163 isolates)[a/]	Scarlet oak (134 isolates)	Post oak (16 isolates)	White oak (11 isolates)	All hosts (332 isolates)
43 (28)[b/]	9 (33)	9 (4)	9 (4)	9 (54)
24 (18)	19 (11)	18 (2)		43 (37)
9 (13)	24 (7)	37 (2)		24 (25)
15 (10)	43 (7)			19 (24)
19 (10)	42 (6)			15 (17)
44 (10)	44 (5)			44 (16)
				8 (13)

[a/] Listed by vegetative compatibility (v-c) type and number of trees yielding that type.

[b/] This v-c number corresponds to those assigned by the Connecticut Agricultural Experiment Station.

of different v-c types by survey was: Mountain (26 v-c types), Piedmont (26), Blue Ridge Parkway (32), Stone Mountain State Park (16), and Mixed collection (22). If all of the collections made in the mountain region are considered as a unit (Mountain, Blue Ridge Parkway, and Stone Mountain surveys), then 42 different v-c types are represented. The most common v-c types found in these collections are listed in Table 5.

Scarlet oak was the only host species with large numbers of infected individuals in both the mountains and Piedmont. The v-c data for this species were examined for regional differences in v-c type distribution. In the Mountain survey, 53 isolates were obtained from 40 trees. Vegetative compatibility testing identified 21 different v-c types. These data were similar to that obtained from the Piedmont, which yielded 55 isolates from 46 trees representing 25 v-c types. The ratio of number of trees to v-c types was 1.0:1 and 1.84:1 for the mountains and Piedmont, respectively. In both regions, the two most common v-c types were v-c 9 (mountains: 9 isolates, Piedmont: 5 isolates) and v-c 19 (15,5). Thus, there did not appear to be large regional differences in v-c type distribution. Multiple isolations made on 15 scarlet oaks showed that 9 of these trees contained more than 1 v-c type per tree. Vegetative compatibility types differed between bole cankers and swollen butt on the same tree and within a single swollen butt. Examination of v-c type distribution within a small geographic area (Stone Mountain State Park survey) showed that many v-c types were present (12 v-c types on 10 trees). This trend was also noticed with American chestnut on the BRP survey.

Table 5. Frequency of the most common vegetative compatibility (v-c) types of *Endothia parasitica* by survey in North Carolina, 1979 to 1981

Mountains[a] 97 isolates	Piedmont 66 isolates	Blue Ridge 100 isolates	Stone Mountain 23 isolates	Mixed[b] 46 isolates	Mountain Region[c] 220 isolates
9 (17)[d]	9 (20)	43 (15)	9 (6)	43 (7)	43 (29)
43 (12)	19 (6)	24 (10)	37 (2)	9 (6)	9 (28)
4 (9)	42 (5)	15 (7)	43 (2)	24 (5)	24 (18)
15 (7)	56 (5)	44 (7)		19 (4)	15 (15)
19 (7)	41 (3)	19 (6)		34 (3)	19 (14)
24 (7)		8 (5)		44 (3)	44 (12)
8 (5)		9 (5)			8 (10)
					4 (10)

[a] Listed by vegetative compatibility (v-c) type and number of trees yielding that type.

[b] Isolates obtained on several trips to different sections of North Carolina.

[c] Total of Mountain, Blue Ridge Parkway, and Stone Mountain State Park surveys.

[d] The v-c numbers correspond to those assigned by the Connecticut Agricultural Experiment Station.

Discussion

While white oak was only rarely (1 percent) infected by *E. parasitica*, diseased scarlet and post oaks were found in significant numbers in North Carolina. The disease incidence on post oak, found only in the Piedmont, was 14.5 percent. This figure compares very closely with the results of Bryan (1960) who, in a survey of the North Carolina, South Carolina, and Georgia Piedmont, found 14.3 percent of the post oaks infected. The observation of pycnidia and the perithecial stromata on infected trees, and the collection of viable spores of *E. parasitica* in preliminary stem flow experiments, indicates that post oak should be considered as a source of virulent inoculum of *E. parasitica*. Post oak appeared to be the most susceptible of the oaks in this survey, because cankers can coalesce and cause mortality. The ability of H strains of *E. parasitica* to heal virulent cankers on this host needs to be determined. However, as post oak is not often found intermixed with American chestnut, its role in the restoration of the chestnut may be limited.

While *E. parasitica* did not appear to be an important mortality factor on scarlet oak in this study, it must be considered as an important source of

virulent inoculum. Infected trees, usually with asexual or sexual fruiting structures, were found in significant numbers in both the Piedmont (25.9 percent) and the mountains (8.6 percent). Cross inoculation studies designed to test the relative pathogenicity of *E. parasitica* obtained from scarlet oak and the other hosts of the pathogen, are in progress at Duke University. The widespread distribution of scarlet oak may offer opportunities for disease control using hypovirulent strains of *E. parasitica.* If hypovirulent strains of the pathogen could be established on scarlet oak, they would be well situated to serve as hypovirulent inoculum reservoirs. Whether these inoculum sources can be established or maintained on scarlet oak is unknown at present. The rough bark that typifies swollen butt, and the involutions and irregular nature of the infection will make establishment and evaluation of H strains difficult.

A diversity of v-c types were collected from each host of *E. parasitica.* Similar results have been reported by other researchers working with American chestnut in the United States (Anagnostakis and Waggoner 1981; MacDonald and Double 1978). The v-c types isolated most frequently from American chestnut in this study were similar to those isolated most frequently from other hosts of *E. parasitica.* The relative frequencies of these v-c types however, did vary between hosts. Studies with scarlet oak, found in both the mountains and Piedmont, showed similar degrees of v-c type diversity and similar specific v-c types in these two regions. More than one v-c type was sometimes present within a single scarlet oak with multiple infections or from several trees within a limited geographical area. These results suggest that any treatment with H strains of *E. parasitica* on infected oaks should utilize batch mixtures of several v-c types.

It is not known why some of our isolates gave compatible reactions with more than one v-c tester. Presumably, these results identify v-c types that are genetically related. This theory is supported by the fact that certain patterns of v-c types were repeatedly involved in these multiple reactions.

We feel that research on the oak hosts of *E. parasitica* should continue. While it is true that scarlet and post oaks are not commercially important species, they do play an important role in the maintenance of inoculum levels of *E. parasitica* in North Carolina. Furthermore, the potential for conversion of their cankers to hypovirulent forms of the pathogen needs to be evaluated. Scarlet and post oak may be important in the control of *E. parasitica* in North Carolina.

Literature Cited

Anagnostakis, S. L. Testing *Endothia parasitica* strains for vegetative incompatibility. MacDonald, William L.; Cech, Franklin C.; Luchok, John; Smith, Clay, eds. Proceedings of the American chestnut symposium; 1978 January 4-5; Morgantown, WV. Morgantown: West Virginia University Books; 1978:101-102.

Anagnostakis, S. L.; Waggoner, P. E. Hypovirulence, vegetative incompatibility, and the growth of cankers of chestnut blight. Phytopathology 71:1198-1202; 1981.

Anderson, P. J.; Babcock, D. C. Field studies on the dissemination and growth of the chestnut blight fungus. 1913; Penn. Chestnut Tree Blight Comm. Bull. 3. 46 p.

Bryan, W. C. Losses from defect in Piedmont hardwoods. 1960; USDA For. Serv. Southeast. For. Exp. Stn.; Stn. Pap. 109. 31 p.

Clapper, R. B.; Gravatt, G. F.; Stout, D. C. *Endothia* canker on post oak. Plant Dis. Rep. 30:381; 1946.

Ham, D. L. The etiology of *Endothia parasitica* on scarlet oak. Durham, NC: Duke University; 1967. 25 p. MS Thesis.

MacDonald, W. L.; Double, M. L. Frequency of vegetative compatibility types of *Endothia parasitica* in two areas of West Virginia. MacDonald, William L.; Cech, Franklin C.; Luchok, John; Smith, Clay, eds. Proceedings of the American chestnut symposium; 1978 January 4-5; Morgantown, WV. Morgantown: West Virginia University Books; 1978:103-105.

Rankin, W. H. Field studies on the *Endothia* canker of chestnut in New York State. Phytopathology 4:233-260; 1914.

Renewable Resources Evaluation Project. Forest survey field instructions for South Carolina. 1977; USDA For. Serv., Southeast. For. Exp. Stn. 36 p.

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