Lack of Host Specificity in North Carolina Isolates of *Endothia parasitica*

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ABSTRACT

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Two isolates from each of five hosts of *Endothia parasitica* (American and oriental chestnut, post, scarlet, and white oaks) and agar controls were cross-inoculated onto 10 (1980) and 11 (1981) trees of each host. Results were evaluated after 4 mo for the chestnuts and 2 yr for the oaks. The oak isolates were generally as pathogenic as the isolates collected from chestnut hosts. Relative host susceptibility, as determined by rate of canker growth and degree of callus formation, was, in order of decreasing susceptibility: American and oriental chestnut, and post, scarlet, and white oaks. Little external evidence of infection was visible on the oaks after 2 yr; in addition, neither basal nor bole inoculations produced typical "swollen butt" symptoms on scarlet oak as seen in nature.

Additional key words: *Cryphonectria parasitica*, *Quercus*.

The introduction of *Endothia parasitica* (Murr.) P. J. & H. W. Anderson (=*Cryphonectria parasitica* (Murr.) Barr) into the United States at the turn of the century and the ensuing devastation of the American chestnut (*Castanea dentata* (Marsh.) Borkh.) raised concern about the vulnerability of other eastern hardwoods, especially the closely related oaks. Early reports and inoculation studies (6,17,19) indicated that the fungus was saprophytic to mildly parasitic on several host species, including white (*Quercus alba* L.), black (*Q. velutina* Lam.), scarlet (*Q. coccinea* Muenchh.), and chestnut (*Q. prinus* L.) oaks. In 1917, Shear et al (19) inoculated 32 species of eastern hardwoods, including chestnut, white, and red oaks, with *E. parasitica*. Only four of 70 inoculations on oak were rated as parasitic, all of which were on a single white oak, which was eventually girdled by the fungus. These same authors reported no subsequent loss of pathogenicity on American chestnut after the fungus passed through red maple or scarlet oak (19). In 1946 Clapper et al (5) reported cankers, branch dieback, and mortality of post oak (*Q. stellata* Wang.) that was naturally infected by *E. parasitica*. Isolates of the fungus caused typical cankers when inoculated onto *Castanea* spp.; however, experimental inoculations on post oak did not produce cankers after 8 mo. Live oak (*Q. virginiana* Lam.) was first reported as a host of *E. parasitica* in 1960 when dieback was investigated by May and Davidson (14). Isolates of *E. parasitica* from live oak produced cankers on American chestnut (14) and young live oaks (3). While investigating "swollen butt" of scarlet oak, Ham (9) inoculated *E. parasitica* onto eight species of oak including scarlet, white, southern red (*Q. falcata* Michx.), northern red (*Q. rubra* L.), post, blackjack (*Q. marilandica* Muenchh.), willow (*Q. phellos* L.), and black oaks. All four inoculations on post oak and one on white oak were successful, as were inoculations on seven of eight scarlet oaks. Inoculations of all other hosts were unsuccessful, although *E. parasitica* was recovered from one control inoculation on red oak. More recently, Jaynes (10) inoculated 40 species of native and introduced woody plants including white, chestnut, black, and turkey (*Q. laevis* L.) oaks with American virulent and hypovirulent strains of *E. parasitica*. At the end of 3 mo, only the inoculations on *Castanea* species were classified as positive.

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We have done a survey of the mountains and Piedmont of North Carolina and found that *E. parasitica* was present on post, scarlet, and white oaks throughout these regions (16). The present study was designed to determine the relative pathogenicity of *E. parasitica* isolates collected from chestnut and oak species, furnish data on relative host susceptibility, and describe the symptomatology of early canker development on the oak hosts.

MATERIALS AND METHODS

Host selection. All host trees (American and oriental chestnut, post, scarlet, and white oaks) selected for cross-inoculation were judged healthy and free of any *Endothia* infections. The American chestnut study plots were located within a 10-yr-old clearcut at the Coweeta Hydrologic Laboratory in Franklin, NC. All other inoculation plots were located in the Duke Forest. Oriental chestnut will be used to designate this host species because the exact parentage is unknown, but the trees appeared to be primarily Japanese chestnut (*C. crenata* Sieb. & Zucc.).

Isolate selection. All 421 isolates of *E. parasitica* in the Duke culture collection, collected primarily from the 1979–1980 North Carolina survey (16), were stratified by host species and regional origin (mountain or Piedmont). Two isolates per host (one from each region) were then randomly selected, yielding a total of 10 isolates. All Piedmont isolates from the oaks and oriental chestnut were derived from the Duke Forest. Mountain-region isolates from post and scarlet oaks and that from oriental chestnut were obtained near Stone Mountain and Bent Creek, respectively. Because our culture collection did not contain a Piedmont isolate of *E. parasitica* from American chestnut, both American chestnut isolates were of mountain origin. All isolates were maintained on Difco potato-dextrose agar (PDA).

First inoculation series, 1980. Ten American chestnuts were inoculated on 25 June 1980 and 10 trees each of the other hosts were inoculated between 23 and 25 May 1980. In all cases, bole inoculations started at 0.5 m aboveground (position 1) and progressed upward at 15-cm intervals (Fig. 1). A random-number table was used to assign each of the 11 treatments (10 isolates of *E. parasitica* plus a sterile PDA control) to one of the 11 inoculation positions on the bole. Inoculations were performed as described by Jaynes and Elliston (11). The sequence of treatment positions for tree number 1 was repeated for trees 3, 5, 7, and 9, and reversed for trees 2, 4, 6, 8, and 10. A different sequence of treatments was randomly determined for each host. Bole inoculations were

performed on all five host species.

Earlier field observations indicated that bole cankers on scarlet oak were confined to a small branch-stub area, whereas basal cankers often resulted in large, encircling multiple cankers or swollen butt. A second set of inoculations was installed along two basal circumferential rings on the same scarlet oaks used for the bole inoculations to determine if the difference was due to a positional effect. As with the bole inoculations, the treatment sequence was randomly predetermined, then reversed for alternate trees (Fig. 1).

Because of rapid canker enlargement, the studies on American and oriental chestnut were evaluated approximately 4 mo after inoculation. Canker length and width measurements were recorded to determine canker area. The presence of fungal fan material, fruiting bodies, and callus tissue was also noted. Cankers on the oak hosts expanded very slowly, so these trees were left intact for 2 yr and finally evaluated between 3 June and 14 July 1982.

Second inoculation series, 1981. To confirm the slow canker expansion observed on the oaks in the first inoculation series, a second series was installed in 1981. In this second study, we collected data for 11 trees of each host, except American chestnut. The same 10 isolates of *E. parasitica* were used; however, rather than reversing the sequence of treatments between alternate trees, the treatment sequence was randomized for each tree of each host. The rest of the procedure was identical to that described for the 1980 study.

In both inoculation series, mean canker areas were determined for each isolate on each host and subjected to analysis of variance and Duncan's multiple range test.

RESULTS

Canker development. A summary of canker characteristics, including mean canker size and percentage of infection, incidence of callus formation, and amount of sporulation are listed in Tables 1 and 2. All cankers on American and oriental chestnuts in the first inoculation series were classified as normal for their respective hosts. The second series of inoculations on oriental chestnut did

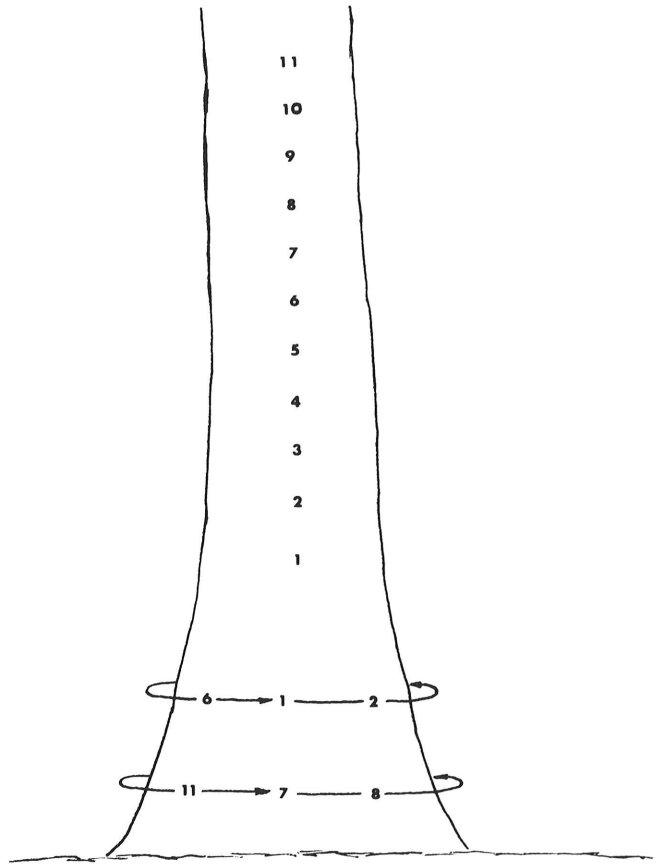

Fig. 1. Diagrammatic representation of inoculation positions used in the cross-inoculation study. The 11 inoculation points on the main stem were used for all five host species (American and oriental chestnut, post, scarlet, and white oaks). The circumferential basal positions pertain only to scarlet oak.

TABLE 1. Summary of canker characteristics from the 1980–1982 *Endothia parasitica* cross-inoculation experiment[a]

Host	Inoculations[b] (no.)		Infection[c] (%)		Frequency of callus (%)		Frequency of *E. parasitica* sporulation		Mean canker size[d] (mm^2)
	V	C	V	C	V	C	V	C	
American chestnut	100	10	98	50	0	12	98	40	8,206
Oriental chestnut	100	10	94	10	0	50	87	0	3,555
Post oak	100	10	84	20	16	21	13	0	5,906
Scarlet oak bole	90	9	92	89	87	44	1	0	301
Scarlet oak base	90	9	93	100	88	78	1	0	255
White oak	100	10	47	60	57	40	1	0	153

[a] Two isolates from each host species and a sterile control cross-inoculated onto 10 trees of each host.
[b] V = Virulent inoculum, C = control inoculum (sterile potato-dextrose agar).
[c] Percentage of infection by *E. parasitica* based on presence of mycelial fan or pycnidia.
[d] Approximate incubation time: American and oriental chestnut = 4 mo, oak species = 24 mo.

TABLE 2. Summary of canker characteristics from the 1981–1982 *Endothia parasitica* cross-inoculation experiment[a]

Host	Inoculations[b] (no.)		Infection[c] (%)		Frequency of callus (%)		Frequency of *E. parasitica* sporulation		Mean canker size[d] (mm^2)
	V	C	V	C	V	C	V	C	
Oriental chestnut	110	11	91	27	0	9	24	0	196
Post oak	110	11	84	55	8	9	0	0	275
Scarlet oak bole	110	11	89	73	2	9	4	0	128
Scarlet oak base	110	11	90	64	3	18	5	0	126
White oak	110	11	62	55	38	27	0	0	84

[a] Two isolates from each host species and a sterile control cross-inoculated onto 11 trees of each host.
[b] V = Virulent inoculum, C = control inoculum (sterile potato-dextrose agar).
[c] Percentage of infection by *E. parasitica* based on presence of mycelial fan or pycnidia.
[d] Approximate incubation time = 12 mo.

not perform as expected. After 13 mo, fungal fruiting was sparse, mycelial fan development was poor, and the mean canker areas averaged only 10% of the areas recorded after 4 mo in the first inoculation series.

Canker development on post oak was very slow. At the end of 12 mo (both 1980 and 1981 inoculations), there was little external evidence of the pathogen. Destructive sampling of several 1-yr-old cankers showed that the fungus was active and primarily located in the outer bark. Some necrosis was present in the inner bark, but these areas were small, and closely centered on the inoculation site. Even after 2 yr, there was still relatively little external evidence of fungal activity, although pycnidia were present within 13% of the old inoculation wounds and cracking of the bark was visible on close inspection. However, as the outer bark of these 2-yr-old cankers was removed in successive layers, it became evident that the fungus had developed extensively in both the outer and inner bark, with mycelial fans often extending to one-half the circumference of the tree.

Most inoculations on scarlet oak also showed little external evidence of infection at the end of 1 yr. Four bole and six basal inoculations (1981 series) did produce pycnidia and mycelial fans within the inoculation wound. At the end of the 1980 study (2 yr) callus formation was observed on 87% of the main stem and 88% of the basal inoculations (Table 1). Most of the inoculations had a central callus plug, in which the old inoculation wound was filled with callus tissue. This tissue apparently did not confine the fungus, however, as evidenced by the ring of necrotic inner bark tissue that surrounded the plug. This zone of necrosis varied in width and shape. The remainder of the callused inoculations (2–8%) were either completely callused or callused at the canker margin.

Canker development was poorest in white oak. When both studies were evaluated in June 1982, between 38 and 53% of both years' inoculations had completely callused. The anomaly was a single tree from the 1980 study, on which the fungus produced several large, lethal-type cankers that almost girdled the tree.

Relative host susceptibility. The five host species examined in this study showed different degrees of host susceptibility to *E. parasitica*. Based on the rate of canker development and degree of callus formation the most susceptible host species was American chestnut, followed in order of decreasing susceptibility by oriental chestnut and post, scarlet, and white oaks (Tables 1 and 2). Analysis of variance showed that there were no significant differences between main stem and basal inoculations on scarlet oak ($P = 0.05$) after 2 yr.

Relative virulence of isolates. Data from both inoculation series were not combined for statistical analysis because incubation times and inoculation techniques varied. Tree-to-tree variation within each host was significant ($P = 0.01$) for all species except scarlet oak main stem inoculations (1980 series) and post oak (1981 series). In the first inoculation series, isolate effects were strongly significant ($P = 0.01$) on American and oriental chestnut and basal scarlet oak inoculations, but not significant on the other hosts ($P = 0.05$). Interactions between isolate, host, and tree were not significant. Variation due to isolate was only weakly significant ($P = 0.06$) on oriental chestnut and post oak in the 1981 series and not significant on the other hosts. For this reason, only the data from the 1980 series will be presented here. The data from the 1981 series is available elsewhere (15).

The relative virulence of the different isolates was best delineated on the chestnut hosts (Table 3). On American chestnut, the three most virulent isolates were derived from scarlet, post, and white oaks. On oriental chestnut, the most virulent isolate was obtained from scarlet oak. The mean canker areas produced by the other isolates on this host could not be separated statistically, indicating that the oak isolates were as virulent as the chestnut isolates. On the more resistant hosts, variation in canker size within isolates was so large that canker means could not be separated by a Duncan's multiple range test, even though mean canker areas differed by as much as a factor of 32 between treatments.

DISCUSSION

Although only 10 isolates of *E. parasitica* were used, the results of this study showed that the virulence of some oak isolates was equal to or greater than the two isolates collected from American chestnut. In fact, some of the most virulent isolates in this study were obtained from post and scarlet oak. Nash and Stambaugh (16) found significant numbers of oaks infected by *E. parasitica* throughout the mountain and Piedmont regions of North Carolina. The present study gives added significance to that survey work by confirming that the oak hosts represent sources of inoculum capable of killing American chestnut trees. The amount of inoculum produced on the oak species is unknown. Gravatt (7) reported that fruiting of *E. parasitica* was rare on post oak; however, on the Duke Forest, pycnidia were found on exfoliating bark and callus folds of post oak cankers and on the stumps of infected trees felled 1 yr previously. Viable conidia were routinely collected from all of these sources. Perithecial stromata were rarely found. Further research is needed to quantify the amount of inoculum that is actually disseminated from infected oaks to American chestnut and thus assess its epidemiological importance.

The ranking of hosts with respect to susceptibility to the pathogen was in agreement with the studies and observations of others (4,8,9,17,19). As expected, American chestnut was the most susceptible host, followed in order of decreasing susceptibility by oriental chestnut and post, scarlet, and white oaks. Susceptibility was subjectively based on rate of canker development and degree of host callus production over a 2-yr period. These parameters may be influenced by several factors including host vigor, site quality, host age, and age of infection. Long-term survival and growth impact studies would clarify the role of *E. parasitica* on the oaks.

TABLE 3. Mean canker area produced by isolates of *Endothia parasitica* on five chestnut and oak hosts in North Carolina, 1980–1982[a]

	Host					
Isolate[b]	American chestnut	Oriental chestnut	Post oak	Scarlet oak bole	Scarlet oak base	White oak
Scarlet oak-Mtns	12,351[c] a[d]	8,066 a	8,430 a	811 a	173 bc	111 a
Post oak-Mtns	11,309 ab	3,318 b	7,581 a	464 ab	392 a	40 a
Scarlet oak-Pied	8,761 abc	4,326 b	7,742 a	418 ab	304 ab	64 a
Oriental chestnut-Mtns	8,806 abc	2,878 b	7,677 a	262 b	310 ab	428 a
Post oak-Pied	7,624 bc	3,884 b	8,188 a	201 b	233 bc	170 a
American chestnut 2-Mtns	8,523 abc	3,790 b	6,230 a	206 b	190 bc	175 a
American chestnut 1-Mtns	9,034 abc	4,074 b	5,260 a	182 b	182 bc	178 a
White oak-Mtns	10,265 ab	2,268 b	4,825 a	131 b	112 c	61 a
Oriental chestnut-Pied	6,134 c	3,616 b	5,181 a	213 b	200 bc	274 a
White oak-Pied	5,795 c	2,797 b	3,590 a	256 b	220 bc	51 a
Control	1,617 d	86 c	264 a	171 b	188 bc	127 a

[a] Incubation times: American and oriental chestnut = 4 mo; post and white oak = 24 mo; scarlet oak = 25 mo.
[b] Mtns = Isolate from North Carolina mountains; Pied = isolate from North Carolina Piedmont.
[c] Values represent mean canker size (mm^2) of 10 cankers per isolate on each host.
[d] Within columns, values followed by the same letter do not differ significantly according to Duncan's multiple range test ($P = 0.05$).

High levels of infection were recorded in our control treatments, especially on the more resistant hosts (Tables 1 and 2). Furthermore, mean canker size of the control treatment could not be statistically separated from other treatments on several of the oaks (Table 3). Obviously, the integrity of the sealed check wounds was not maintained throughout the long incubation period. We believe these infection rates were due, in part, to high levels of local inoculum that originated from a 50-yr-old infected oriental chestnut plantation or from naturally infected oaks found throughout Duke Forest. The degree of natural infection and variability encountered on the oak hosts suggests that pathogenicity trials are best conducted on the chestnut species.

The least susceptible of the hosts in this study was white oak. Many of the inoculations on this species were completely callused after 2 yr, and in only a few instances did the fungus appear truly parasitic. Although *E. parasitica* could probably kill weakened white oaks, the fungus appears to function primarily as a secondary wound invader on this host. These conclusions are supported by the fact that only 1% of 674 white oaks examined in the North Carolina survey were infected with *E. parasitica* (16).

The slow development of *E. parasitica* on post oak, and the subtle external symptomatology may explain why Clapper et al (5) reported no canker development on post oak 8 mo after inoculation. Our results indicate that 2 yr would be necessary for distinctive external symptoms or signs to appear. *E. parasitica* grew inconspicuously in the outer bark of post oak during the first year, and only later proliferated within the inner bark tissues. Similar observations were made on many scarlet and white oak inoculations during this study and by Clapper et al (5).

Although *E. parasitica* can be isolated from very small basal cankers on scarlet oak, the symptomatology most often associated with this host is the swollen butt condition. Such trees contain numerous basal cankers, involuted and buried bark, and large amounts of callus tissue (9). Smaller, discrete bole cankers have also been reported on this host in North Carolina (16). Neither of these two canker types were reproduced in this study, nor were any significant differences observed in canker size between basal and main stem inoculations. All inoculations on scarlet oak produced very small cankers (Tables 1–3). Thus, Koch's postulates have yet to be proven for *E. parasitica* and the swollen butt condition. If this symptomatology is caused by *E. parasitica*, then it must be the result of a long-term host-pathogen interaction. This interaction probably includes changes in host susceptibility, the presence of vectors or wounding agents, or combinations of these factors. Carpenter ants (*Camponotus*) are often associated with swollen butt of scarlet oak and do carry spores of *E. parasitica* (2,19; Nash, *unpublished*); however, their role as a vector or wounding agent has not been fully determined (1,2,18–21). Most of the cankers on scarlet oak in this study were characterized by some degree of callus formation, primarily by a central callus plug surrounded by limited necrosis. An interaction of this kind could conceivably produce the characteristic swollen butt condition of scarlet oak; only long-term studies will verify this relationship. Although it is known that *E. parasitica* can kill post oak (5), there is no direct evidence that the fungus can kill mature scarlet oak.

The slow growth of *E. parasitica* on scarlet oak might be used to advantage in biocontrol efforts involving hypovirulent (H) strains of the fungus. Efforts to establish H strains of *E. parasitica* have often been unsuccessful in this country because American chestnuts inoculated with H strains are highly susceptible to subsequent lethal infections arising from natural virulent inoculum (12,13). Direct inoculations of H strains onto scarlet oak would probably fail because of natural host resistance and the inherent low virulence of H strains. However, the application of H strains to existing cankers may result in conversion of the virulent strains and the establishment of the H type on a host resistant to later natural infection.

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