Interaction of Cronartium fusiforme and Cronartium quercuum with Quercus velutina

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ABSTRACT

Cronartium fusiforme interacted differentially with black oak (*Quercus velutina*), whereas *C. quercuum* developed normal uredia and telia. The types of infection observed were similar to four of the *Puccinia graminis* types (0, 0;, 1, and 4), and were not influenced by the age of host leaves. Half-leaf inoculation tests of lots of aeciospores and urediospores of *C. fusiforme* and *C. quercuum* showed no variability between rust collections within a species. Therefore, black oak seedlings of known resistance to *C. fusiforme* infection (types 0; and 1) can be used to differentiate between *C. fusiforme* and *C. quercuum*. Phytopathology 61:1055-1058.

Additional key words: rust susceptibility, host-parasite interaction, disease differentiation, epiphytology of rusts.

The important pine stem rusts in southeastern USA are fusiform rust, caused by *Cronartium fusiforme* Hedgc. & Hunt ex Cumm., and eastern gall rust, caused by *Cronartium quercuum* (Berk.) Miyabe. These heteroecious pathogens have a similar alternate host (oak) range, and cannot be distinguished morphologically. On the aecial or pine host, they have been differentiated on the basis of gall morphology (9). Because intermediate forms exist (2), gall morphology is not an accurate means of identifying these fungi (2, 5).

Preliminary work on the relative susceptibility of southern oaks to *C. fusiforme* and *C. quercuum* revealed that *C. fusiforme* interacted differentially with black oak (*Quercus velutina* Lam.). In contrast, the interaction of *C. quercuum* with black oak uniformly resulted in the normal development of uredia and telia. Because of the importance of distinguishing between these fungal species in breeding trees for rust resistance, this study was undertaken to characterize and further define the interactions of *C. fusiforme* and *C. quercuum* with black oak. A preliminary report has been made (1).

Materials and Methods.—Black oak seedlings from 10 open-pollinated trees in Georgia and North Carolina were grown in a 1:1:1 soil mix (sand:loam: pine bark) in 4-inch plastic pots in the greenhouse. New leaf growth was induced by a 16-hr photoperiod.

Both mass and single-gall aeciospore collections of *C. fusiforme* and *C. quercuum* were used as inoculum. Spore lots of *C. fusiforme* were taken from loblolly (*Pinus taeda* L.), slash (*P. elliottii* Engelm. var. *elliottii*), longleaf (*P. palustris* Mill.), pond (*P. rigida* var. *serotina* [Michx.] Loud.), and pitch (*P. rigida* Mill.) pines. Aeciospores of *C. quercuum* were taken from shortleaf (*P. echinata* Mill.), Virginia (*P. virginiana* Mill.), jack (*P. banksiana* Lamb.), and sand (*P. clausa* [Chapm.] Vasey) pines. Aeciospore collections were preserved and stored according to the technique of Roncadori & Matthews (6). When urediospores were needed, seedlings of northern red oak (*Quercus rubra* L. Michx. f.) were inoculated with aeciospores of selected spore lots. Before use, the aeciospores or urediospores were rehydrated for 24 hr at 20 C in an atmosphere saturated with water vapor.

Two general inoculation procedures were employed. In the first method, black oak seedlings were inoculated by dusting 6- or 7-day-old leaves with a mixture of spores and talc (1:10), using a bell jar under a vacuum equal to 10 inches of mercury (7). This technique produced a uniform coverage of spores on the undersurfaces of the leaves.

The second procedure was a half-leaf inoculation method similar to that used by Miah & Sackston (3). Aeciospores or urediospores were suspended in distilled water containing 0.01% (v/v) Tween 20 (polyoxyethylene sorbitan monolaurate) or talc (1:100). The suspension was daubed onto half the underside of the leaf with a sterile cotton swab so that seven half-leaves of each black oak seedling were inoculated with seven different spore collections. One noninoculated half-leaf of each plant served as control. After inoculation, the seedlings were placed in an incubation chamber with an atmosphere saturated with water vapor at 20 C for 24 hr, then moved to a greenhouse or controlled-environment chamber.

The types of infection observed were similar to four of the *Puccinia graminis* types originally described by Stakman & Levine (8). The infection types produced by *C. fusiforme* on black oak (Fig. 1) are as follows: no macroscopically recognizable symptoms (type 0); hypersensitive flecks (type 0;); necrotic tissue developing around either the uredia or telia (type 1); and uredia and telia developing normally (type 4).

Symptomatology.—Initial observations on symptom development and infection types were made on 50 randomly selected black oak seedlings inoculated with aeciospores of either *C. fusiforme* (Lot 5-66) or *C. quercuum* (Lot 6-66) using the bell jar technique. In this and subsequent experiments, the seedlings were examined every other day for 3 weeks to follow the progression of symptoms.

Leaf age.—The influence of leaf age on the interaction of *C. fusiforme* and *C. quercuum* was determined by inoculating groups of 10 seedlings of known sensitivity to *C. fusiforme* (types 0;, 1, and 4) with aeciospores of *C. fusiforme* (Lot 5-66) and *C. quercuum* (Lot 6-66) when the leaves were 4, 6, and 8 days old.

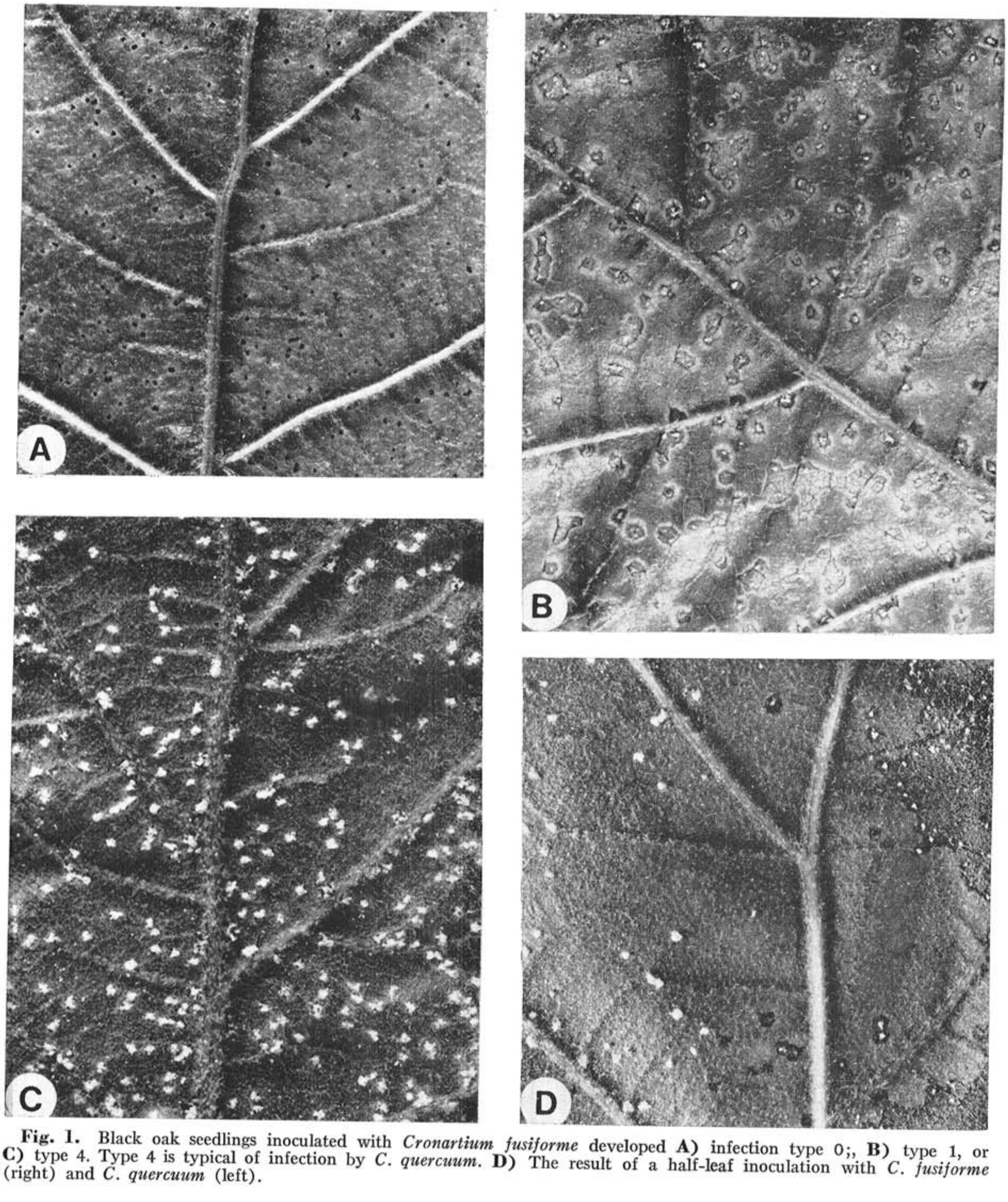

Fig. 1. Black oak seedlings inoculated with *Cronartium fusiforme* developed **A)** infection type 0;, **B)** type 1, or **C)** type 4. Type 4 is typical of infection by *C. quercuum*. **D)** The result of a half-leaf inoculation with *C. fusiforme* (right) and *C. quercuum* (left).

The bell jar technique was used to inoculate these seedlings.

Aeciospore and urediospore inoculations.—The half-leaf inoculation procedure was used to determine if the infection types were different for collections of spores from different aecial hosts. Aeciospore lots of *C. fusiforme* and *C. quercuum* from different pine hosts were assayed on single seedlings selected at random from each of the 10 open-pollinated families. The half-leaves were coded, and the inoculation scheme was such that a spore lot did not occur at the same leaf position more than twice. This was done to eliminate errors due to the effect of leaf position. Ten such experiments were conducted using 19 spore collections of *C. fusiforme* and 8 of *C. quercuum*. The half-leaf procedure was used to inoculate black oak seedlings with urediospore collections of *C. fusiforme* and *C. quercuum*.

Distribution within black oak families.—During the course of the aeciospore and urediospore inoculations, it became apparent that all members of a black oak family did not react in the same manner to *C. fusiforme*. To determine the distribution of infection types within black oak families, 50 seedlings from each family were inoculated with aeciospores of *C. fusiforme* (Lot 5-66) using the bell jar technique.

RESULTS.—*Symptomatology.*—*Cronartium fusiforme* interacted differentially with the black oak seedlings and produced all four types of infection. The hypersensitive flecks (type 0;) were apparent 3-4 days after inoculation. Black oak seedlings inoculated with *C. quercuum* developed only normal uredia and telia (type 4). Infection types 2, 3, and X described by Stakman & Levine (8) have not yet been observed on black oaks inoculated with these pine-oak rusts.

Leaf age.—Infection types resulting from the interaction of *C. fusiforme* with black oak were the same at each age, and were the same as had been determined previously. That is, if the seedling's infection had been 0;, the infection following these inoculations was 0;, regardless of leaf age. There is some possibility of confusing type-0; and type-1 reactions on seedlings whose leaves are older than 8 days. Microscopic examination, however, usually reveals the tip of an aborted telial column in the center of the necrotic area.

Aeciospore and urediospore inoculations.—Since the data from each experiment followed the same basic pattern, the results of only one are presented (Table 1). The spore collections produced uniform infection types on the oak seedlings, and the response to *C. fusiforme* by a hypersensitive reaction or a type-4 reaction was not related to the source of the aeciospores. For example, if the interaction of the seedlings with *C. fusiforme* Lot 9-96 from slash pine resulted in the development of hypersensitive flecks (type 0;), the seedling interacted in the same manner with all other collections of *C. fusiforme*. Regardless of a seedling's reaction to *C. fusiforme*, a susceptible response (type 4) was produced by all lots of *C. quercuum*.

The relationships observed in urediospore inoculations were similar to those obtained with the aeciospore collections. This was true for albino urediospores of *C. fusiforme* and for urediospores of *C. quercuum* originating from jack pine in Wisconsin (Table 1).

Distribution within black oak families.—It is apparent from Table 2 that the within-family distribution of infection types was highly variable. The mean distribution was 3% for infection type 0, 30% for 0;, 45% for 1, and 22% for type 4 for all families. Infection type 0 probably resulted from the physiological or chronological age of the seedlings at the time of inoculation, rather than from true immunity. (This study is being expanded, and the distribution of infection types is to be compared with the infection type of the maternal parent.)

DISCUSSION.—Over a period of years, Hedgcock & Siggers (2) inoculated 27 species of oaks with aeciospore or urediospore of *C. fusiforme* and *C. quercuum*. They did not report that any tested species interacted differentially with these pine-oak rusts. With black oak, the failure to note a differential response was probably due to the small sample size (11 seedlings were inoculated with aeciospores of *C. fusiforme;* two with urediospores). Furthermore, the seedlings could have been from a single maternal parent, such as family 6 (Table 2), in which case these within-family differences would have been missed. This result emphasizes the need for adequately extensive sampling for host-range studies.

As oak leaves mature, they become increasingly resistant to infection by aeciospores of *C. fusiforme* (7) or *C. quercuum* (4), and uredial development is restricted sooner than telial development. This phenomenon, fortunately, did not influence the reaction of the

TABLE 1. Infection types recorded on black oak seedlings inoculated with aeciospores or urediospores of *Cronartium fusiforme* and *C. quercuum*

			Black oak seedling[a]									
Rust	Pine host	Lot no.	1	2	3	4	5	6	7	8	9	10
C. fusiforme[d]												
	Slash	9-65	0;[b]	1	1	1	0;	4	0;	1	4	1
	Loblolly	1-67	0;	1	1	1	0;	4	0;	1	4	1
	Longleaf	2-68	0;	1	1	1	0;	4	0;	1	4	1
	Pond	7-65	0;	1	1	1	0;	4	0;	1	4	1
C. quercuum[d]												
	Shortleaf	5-67	4	4	4	4	4	4	4	4	4	4
	Sand	17-68	4	4	4	4	4	4	4	4	4	4
	Virginia	6-66	4	4	4	4	4	4	4	4	4	4
C. fusiforme[e]												
	Slash	1-66U	0;	1	0;	1	1	4	0;	4	0;	1
	Slash	WSP-1[c]	0;	1	0;	1	1	4	0;	4	0;	1
	Loblolly	5-66U	0;	1	0;	1	1	4	0;	4	0;	1
C. quercuum[e]												
	Virginia	6-66U	4	4	4	4	4	4	4	4	4	4
	Jack	Wis-IU	4	4	4	4	4	4	4	4	4	4

[a] Using a half-leaf inoculation method, the aeciospore or urediospore collections were assayed on one seedling from each family of black oak.

[b] Infection types: 0 = no macroscopically recognizable symptoms; 0; = hypersensitive flecks; 1 = necrotic tissue developing around either the uredia or telia; and 4 = uredia and telia develop normally.

[c] Albino urediospores.

[d] Aeciosporic inoculation.

[e] Urediosporic inoculation.

TABLE 2. Distribution of infection types of *Cronartium fusiforme* within black oak families[a]

Families		% Infection by types[b]			
No.	Location	0	0;	1	4
1	Rock Eagle, Ga.		55	45	
2	Athens, Ga.	2	22	74	2
3	Washington, Ga.	2	65	24	10
4	Madison, Ga.	2	9	76	13
5	High Shoals, Ga.	6	40	53	
6	Toccoa, Ga.		4	4	92
7	Carnesville, Ga.	6	60	34	
8	Carnesville, Ga.	2	14	52	32
9	Asheville, N.C.	4	9	39	48
10	Asheville, N.C.	4	18	49	26
	Mean	3	30	45	22

[a] Fifty seedlings from each open-pollinated family were inoculated with *C. fusiforme* (Lot 5-66).
[b] Infection types: 0 = no macroscopically recognizable symptoms; 0; = hypersensitive flecks; 1 = necrotic tissue developing around either the uredia or telia; and 4 = uredia and telia develop normally.

black oak seedlings to infection by *C. fusiforme* or *C. quercuum* reported here.

Peterson (5) considers *C. fusiforme* and *C. quercuum* to be at least racially distinct on the basis of host ranges, distribution, symptoms, and serological comparison. Although the differential responses reported here are not sufficient to determine whether these rust fungi are separate species or races, it does provide circumstantial evidence to support Peterson's contention.

The majority of black oaks are hypersensitive to infection by *C. fusiforme* (Table 2); cursory examination in the field over 2 years confirmed this. The fact that high percentages of black oak seedlings are resistant to infection by *C. fusiforme* suggests its limited importance in the epiphytology of fusiform rust of southern pines. Black oak is, however, an excellent host for *C. quercuum*, and plays an important role in the epiphytology of eastern gall rust.

The use of black oak seedlings for differentiating between *C. fusiforme* and *C. quercuum* provides a rapid means of confirming the identity of field-collected aeciospores. This technique should, therefore, prove valuable for identifying inoculum to be used in screening pine seedlings for resistance to these pine-oak rusts. It is essential to use black oak seedlings whose resistance to infection (types 0; and 1) by *C. fusiforme* has been predetermined.

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