Occurrence of Darluca filum on Cronartium strobilinum and C. fusiforme Infecting Oak

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ABSTRACT

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The mycoparasite *Darluca filum* commonly was found in uredial sori of *Cronartium strobilinum* on *Quercus minima* and *Q. pumila* in Florida. Isolates of *D. filum* were inoculated and established on uredia and telia of *C. fusiforme* present on six oak species in North Carolina. Pycnidia of *D. filum* often formed within the base of the telia of *C. fusiforme*.

Additional key words: Quercus spp., biological control, fusiform rust, cone rust, hyperparasite.

Darluca filum (Biv. Bern. ex Fr.) Cast. has been reported to be a cosmopolitan mycoparasite on numerous genera and species of rust fungi (1, 2). Most often it has been found on the uredial and telial stages, although occasionally it has been reported on the aecial stage (1, 2). It has not been reported previously on *Cronartium* spp. One aspect of our research is a survey for mycoparasites of *Cronartium fusiforme* Hedgc. & Hunt ex Cumm. and an evaluation of the potential of these organisms as biological control agents. This paper reports the occurrence of *D. filum* on *C. strobilinum* (Arthur) Hedgc. & Hahn on naturally-infected oaks in north Florida and the successful inoculation of *C. fusiforme* with *D. filum* in North Carolina.

MATERIALS AND METHODS

Darluca filum was collected in September 1969 from *C. strobilinum* uredial sori on leaves of dwarf live oak [*Quercus minima* Sarg.) Small] and running oak (*Q. pumila* Walt.) (5) from a mixed pine-hardwood stand in Leon County, Florida. Isolations of single germinated conidia were made on potato-dextrose (PDA) and lima bean agars (3). Subcultures were maintained on malt extract-yeast extract agar (MYE) (2% malt extract, 2% dextrose, 0.1% peptone, 2.5% agar, 0.25% yeast extract) and glucose yeast extract agar (1% glucose, 0.2% yeast extract, 2.5% agar). Spore suspensions were prepared by flooding culture plates with sterile deionized water, scraping the culture surface, and filtering through cheesecloth.

The susceptibility of uredia and telia of *C. fusiforme* on oak leaves to infection by *D. filum* was determined under field conditions in eastern North Carolina. The inoculum was sprayed onto the lower leaf surface with a Sprayon Jet-pack sprayer (Sprayon Products, Inc., Cleveland, Ohio). Check plants were sprayed with sterile deionized water. After inoculation, branchlets were covered with wet plastic bags that were left partially opened for 1-12 day periods of moist incubation.

RESULTS AND DISCUSSION

Natural occurrence on Cronartium strobilinum.—Numbers of uredia with and without pycnidia of *D. filum* were determined on natural infections of *C. strobilinum* on dwarf live oak leaves collected from north Florida in September 1974. Pycnidia of *D. filum* were found on 25 of 39 leaves and 91% of the more than 800 uredia were parasitized.

Susceptibility of C. fusiforme.—In early May 1975 immature leaves on sapling oaks were inoculated with a water suspension of *C. fusiforme* aeciospores. Immediately afterward, we applied *D. filum* inoculum or check treatments. Infection by *D. filum* was determined 14 days after inoculation. Sixteen percent of 1,308 uredial and/or telial sori of *C. fusiforme* on water oak (*Q. nigra* L.) contained pycnidia of *D. filum* (after 1 day of incubation in wet plastic). After 7 days in moist incubation, 6% of 304 sori on black oak (*Q. velutina* Lam.) and 54% of 452 sori on willow oak (*Q. phellos* L.) were infected. No sori on check leaves were parasitized.

Effect of incubation time.—To determine the effect of incubation period on rate of infection, a study was established in Halifax County, N. C. on 22 May 1975. *Cronartium fusiforme*-infected leaves on three branchlets of five water oaks, two laurel oaks (*Q. laurifolia* Michx.), two willow oaks, two blackjack oaks (*Q. marilandica* Muench.), and one white oak (*Q. alba* L.) were treated and branchlets were moist-incubated for 6 or 12 days in the inoculated treatments and for 6 days in the check treatments. Sori with and without pycnidia of *D. filum* were counted on two or three leaves per branchlet, 6, 12, and 42 days after inoculation. The 42-day sample was taken because observations indicated a considerable increase in infection of sori had occurred. Since these

were correlated samples, differences between means were compared with Tukey's conservative honestly significant difference test (7).

After inoculation, pycnidia were found in rust sori on all five oak hosts. Frequency of both rust and mycoparasite varied considerably, and the low incidence of rust on blackjack and white oaks precluded any statistical analysis on oak species effect. Moist incubation for 12 days did not significantly increase the infection by *D. filum* over that at 6 days (Table 1). The 73% infection by the mycoparasite after 42 days in the first treatment was significantly greater than that observed after 6 and 12 days in either of the inoculated treatments. Simultaneous inoculation of wheat with *Puccinia recondita* and *D. filum* produced heavier *D. filum* infection when moist-incubated for 8, 15, or 16 days rather than for 1 or 3 days (6, 8).

The age of the uredia and telia inoculated in these studies was not known. All leaves had signs of *C. fusiforme* when they were inoculated with *D. filum*. Since uredia first were observed in the area on 6 May, most leaves were probably no longer susceptible to rust infection by 22 May. For this reason our inoculations probably were on existing rust infections. Keener (4) also successfully inoculated existing rust infections on 17 hosts with 11 isolates of *D. filum*.

Although check treatments were established before the inoculated treatments, some spores in the latter treatments must have settled into the partially opened bags and produced the low infection levels after 6 and 12 days. An unexpected and interesting result was the 20% infection level in the water-check treatment after 42 days (Table 1). Since numbers of pycnidia increased in all treatments between days 12 and 42, either environmental conditions during this period were conducive for local spread, or many pycnidia were formed only after longer incubation.

Leaves from untreated branchlets on inoculated water and laurel oaks and from adjacent (<20 feet) and distant (>100 feet) trees of these species also were examined after 42 days. Eighteen percent of the rust sori on leaves from untreated branchlets on treated trees were infected with *D. filum*. Because we did not sample these branchlets at the two earlier times, we do not know if these infections resulted from our treatments or from secondary spread. Leaves from adjacent and distant trees of each of these two species had 0.2 and 0% infection for water oak and 0 and 3% infection for laurel oak. The low infection levels on adjacent water oaks and distant laurel oaks suggest that occasional natural infections occur.

Infected telia of *C. fusiforme* often had pycnidia growing at the base of the telial column (Fig. 1). The pycnidia weakened the telia and resulted in the telia breaking off prematurely. Small infected uredia with pycnidia and the bases of broken infected telial columns appeared similar in some cases. Telia in various stages of maturity that occurred under these conditions further

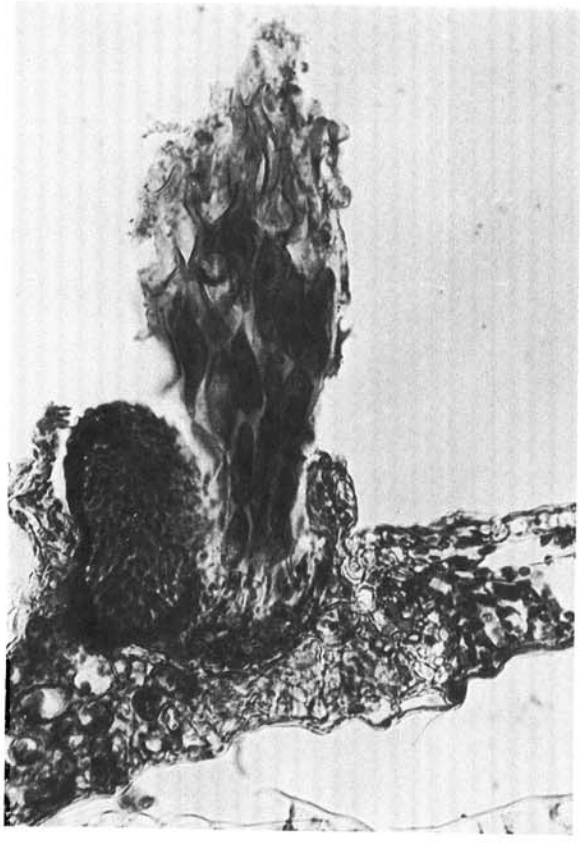

Fig. 1. Telial column of *Cronartium fusiforme* with a pycnidium of *Darluca filum* at the base (× 390).

TABLE 1. Occurrence of pycnidia of *Darluca filum* in uredia and/or telia of *Cronartium fusiforme* on five oak species[y] 6, 12, and 42 days after inoculation with *D. filum* spores or water

Inoculation treatment	Moist incubation (days)	Uredia and/or telia at days after inoculation: 6 Total (no.)	6 With *D. filum*[z] (%)	12 Total (no.)	12 With *D. filum*[z] (%)	42 Total (no.)	42 With *D. filum*[z] (%)
D. filum	6	2814	37 bc	4131	43 bc	2673	73 d
D. filum	12	2456	37 bc	3231	42 bc	2637	61 cd
Water	6	2191	3 a	3950	1 a	4069	20 ab

[y]Water, laurel, willow, blackjack, and white oaks.

[z]Numbers with a common letter are not statistically different ($P = 0.01$) according to Tukey's test for honestly significant differences (hsd = 28%).

confounded any assessment of the effect of *D. filum* on telial development.

Potential for biological control.—The efficacy for biological control of *C. fusiforme* by *D. filum* needs to be assessed. Rapid spread and colonization by *D. filum* would be necessary to affect telial formation which normally occurs within 2 weeks after uredia appear.

In contrast to *C. fusiforme, C. strobilinum* has a life cycle that seems more suited for natural biological control by *D. filum*. The uredial stage appears soon after aeciospore infection in late spring. As new oak leaves mature, the urediospores continue to infect susceptible tissue until late fall, when telia develop. Pycnidia, having overwintered on evergreen leaves of several oak hosts, would be avilable for spread throughout the spring, summer, and fall. In our collection, made in September, 91% of the uredia had been parasitized. Studies are now underway to determine the effect of parasitism by *D. filum* on uredial and telial development and basidiospore release in both *C. fusiforme* and *C. strobilinum*.

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