# Efficacy of Darluca filum for Biological Control of Cronartium fusiforme and C. strobilinum

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Accepted for publication 24 August 1977.

ABSTRACT

KUHLMAN, E. G., F. R. MATTHEWS, and H. P. TILLERSON. 1978. Efficacy of Darluca filum for biological control of Cronartium fusiforme and C. strobilinum. Phytopathology 68: 507-511.

In Florida, in a pure stand of *Quercus minima, Darluca filum* naturally infected 93% of the *Cronartium strobilinum* sori; only 0.8% of the sori contained telia. In an adjacent mixed stand more sparsely populated with *Q. minima,* only 32% of rust sori were infected and 26% had telia. Spread of *D. filum* effectively controlled the rust within the pure oak area, but not in the more sparsely populated area. In greenhouse studies, simultaneous inoculations with *C. fusiforme* and *D. filum* on water oak and northern red oak produced only rust sori. When rust inoculations preceded *D. filum* inoculations by 4-21 days, sori of *C. fusiforme* were infected by the mycoparasite, but this infection did not reduce the number of telia formed in sori. After inoculation of sori of *C. fusiforme* with *D. filum,* moist incubation for only 4 hr resulted in some infection. However, maximum infection occurred after 16-24 hr of moist incubation. Pycnidia of *D. filum* began forming in rust sori 4 days after inoculation. Half-leaf inoculations of 8- or 14-day-old rust infections with *D. filum* reduced basidiospore casts 7-21 days later in comparison with casts from control half-leaves. Because of the short irregular cycle of *C. fusiforme* on oak, biological control of *C. fusiforme* by *D. filum* does not appear to be practical.

*Additional key words: Quercus virginiana, Q. nigra,* and *Q. rubra.*

*Cronartium fusiforme* Hedgc. & Hunt ex Cumm. causes southern fusiform rust, the most important tree disease in the southern United States. The pathogen causes fusiform galls on stems and branches of loblolly and slash pines. Aeciospores from galls on pine infect leaves of members of the red oak group in early spring. Sori of the rust appear on the leaf surface 6-10 days after infection. Sori may have uredia briefly but telia soon develop in the same sori or more commonly in sori without formation of uredia. Telia function for 4-6 wk, producing basidiospores which are capable of infecting new pine shoots (1).

*Cronartium strobilinum* (Arthur) Hedgc. & Hahn infects and destroys young female strobili of slash and longleaf pines. Aeciospores from affected strobili infect leaves of evergreen oaks in late spring or early summer. Uredial sori appear in the spring, and urediospores infect new flushes of growth throughout the growing season. Uredial sori of *C. strobilinum* persist from early summer until telia formation in December or January (2).

The growth habits of the oak hosts affect the microclimatic conditions for rust and mycoparasite infections. Water (*Quercus nigra* L.), northern red (*Q. rubra* L.), and live (*Q. virginiana* Mill.) oaks range in size from seedlings to mature 12-m-high trees in most stands, whereas dwarf live oak [*Q. minima* (Sarg.) Small] is a low-growing (<1 m high) understory shrub that spreads by underground runners. The first two oak species are hosts for *C. fusiforme,* and the latter two are hosts for *C. strobilinum.*

Previously, we (5) showed that *Darluca filum* (Biv.-Bern. ex Fr.) Cast. is a mycoparasite of *C. fusiforme* and *C. strobilinum.* Although *D. filum* has been known as a parasite of many rust fungi, no studies have been made of its effect on the development of spore stages of the *Cronartium* rusts. To evaluate the potential of *D. filum* for biological control of these two pine rusts, a series of experiments was carried out to determine: (i) the conditions necessary for infection of *C. fusiforme,* (ii) the effect of infection of *C. fusiforme* telia on basidiospore production, and (iii) the effect of natural infections of *D. filum* on development of *C. strobilinum.* Some of this work has been reported in a preliminary note (4).

## MATERIALS AND METHODS

**Natural infections of Cronartium strobilinum.**—On 14 October 1975, in Leon County, Florida, a 0.2-ha pure stand of *Q. minima* was examined for the presence of uredia of *C. strobilinum* and pycnidia of *D. filum.* Oaks with both uredia and pycnidia on the leaves were marked for further observation. Some leaves from current and older growth flushes were collected, and the uredia with and without pycnidia were counted. On 28 January 1976, leaves from the pure *Q. minima* stand (stand I), a mixed stand sparsely populated with *Q. minima* (stand II) within 37 m of the pure stand, and three separate groups of *Q. virginiana* (stands III and IV had large trees, and stand V had saplings) all within a 3-km radius were examined for the presence of noninfected telia and uredia of *C.*

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*strobilinum* and also telia and uredia infected by *D. filum*. On 26 February 1976, another count of sori on leaves from four of the groups was made. One group of *Q. virginiana* (stand V) had been destroyed in a fire.

**Inoculation studies.**—In each experiment, susceptible leaves of greenhouse-grown seedlings of water oak and northern red oak were sprayed with aeciospores of *C. fusiforme* (6). Water suspensions of *D. filum* conidia were sprayed (5) on these leaves at various time intervals following the rust inoculation. Moist incubation was provided for 24 hr in a 20-C chamber equipped with a humidifier for inoculations with *C. fusiforme* and in wet plastic bags at room temperature (18-24 C) for incubations with *D. filum*. Infection of *C. fusiforme* was determined 14 days after inoculation with *D. filum* by examining rust sori for pycnidia at ×50. Rust age was the number of days following inoculation with *C. fusiforme*.

To determine the effect of rust age on *D. filum* infection, six rust-infected seedlings of each oak species were sprayed either with conidia of *D. filum* or with water 0, 4, 7, 14, and 21 days after aeciospores were applied. Flecking caused by infection with *C. fusiforme* was visible on oak leaves 4-6 days after inoculation of host plants growing in the greenhouse. Uredia and telia appeared 6-10 days after inoculation, and telia continued to appear up to 16 days after inoculation.

Five to ten seedlings of each oak species with 10- to 14-day-old rust infections were inoculated with *D. filum* conidia and incubated in wet plastic bags for 4, 8, 16, or 24 hr to determine the minimum and optimum periods of moist incubation necessary for infection of rust sori.

The effect of inoculum density on sorus infection was studied with six concentrations of *D. filum* conidia ranging from $3.1 \times 10^4$ to $1 \times 10^6$ spores/ml. Conidia at each concentration were sprayed on 14-day-old rust infections on each of 10 water oak seedlings.

On 28 April 1976, approximately 50 leaves on each of two branchlets on each of 12 water oak trees in Wake County, North Carolina, were sprayed with aeciospores of *C. fusiforme* and incubated for 24 hr in wet plastic bags. Two or 3 wk later when signs of the rust were visible, 10-15 leaves on one branchlet on each tree were brushed with a water suspension of *D. filum* conidia and incubated in wet plastic bags. The other branchlet served as a rust-only check. At weekly intervals after the *D. filum* inoculation, an inoculated leaf, an adjacent noninoculated leaf, and a leaf from the rust-only check branchlet were examined for *D. filum* pycnidia.

Six northern red oak seedlings with 14-day-old rust infections were sprayed with *D. filum* conidia. One leaf from each seedling was examined 2, 4, 6, and 8 days after this inoculation to determine how rapidly pycnidia were formed.

**Effect of Darluca filum parasitism on basidiospore cast.**—Seedlings of water oak with 8- or 14-day-old rust infection and of northern red oak with 8-day-old rust infection were inoculated with *D. filum* conidia or sprayed with water. In each case, whole leaves were inoculated with *C. fusiforme;* then, one half of each of these leaves was brushed with a suspension of *D. filum* conidia and the other half with water only. After an additional 7, 14, or 21 days, one leaf from each seedling was divided into approximately equal proximal and distal portions. Rectangular strips, 4 × 20-50 mm, were cut from the inoculated and water-check quarters of each portion, and sori were counted until approximately 200 telia per quarter leaf had been sampled. On leaves with few sori, the entire inoculated and check halves were used. Basidiospores were cast from telia-bearing leaf sections by suspending the sections in a chamber over acidified water (6). Basidiospores were counted with a model B Coulter Counter (Coulter Electronics, Inc., Hialeah, FL 33010) and converted to basidiospores per telium. Differences in numbers of basidiospores per telium between paired observations were subjected to a Student's *t*-test for statistical significance.

## RESULTS

**Natural infections of Cronartium strobilinum.**—In October 1975, *D. filum* had infected 64% of all uredial sori on leaves of *Q. minima* in the pure stand (stand I) (Table 1). In January 1976, noninfected telia were common in the three collections (stands III, IV, and V)

TABLE 1. Estimates of *Darluca filum* infection of *Cronartium strobilinum* sori at three sample times during 1975-1976 in Leon County, Florida, oak stands

| Criteria | October 1975 | January 1976 | | | | | February 1976 | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Stand[a] | Stand[a] | | | | | Stand[a] | | | |
| | I | I | II | III | IV | V | I | II | III | IV |
| Leaves examined per stand (no.) | 52 | 28 | 10 | 20 | 20 | 20 | 33 | 33 | 33 | 33 |
| Sori per stand (no.) | 6,399 | 3,126 | 1,072 | 3,040 | 3,698 | 2,786 | 4,393 | 3,072 | 5,603 | 5,594 |
| Sorus development category: | | | | | | | | | | |
| Telia only (%) | 0 | 0.2 | 13 | 43 | 59 | 31 | 0.8 | 25 | 74 | 61 |
| Telia with *D. filum* (%) | 0 | 0 | 2 | 0.2 | 2 | 3 | 0 | 1 | 3 | 3 |
| Uredia only (%) | 36 | 15 | 51 | 53 | 27 | 11 | 6 | 43 | 12 | 26 |
| Uredia with *D. filum* (%) | 64 | 85 | 33 | 7 | 12 | 55 | 93 | 31 | 12 | 10 |
| Total sori with *D. filum* (%) | 64 | 85 | 35 | 7 | 14 | 57 | 93 | 32 | 14 | 13 |

[a]Stand I = pure *Quercus minima* stand; stand II = sparsely populated *Q. minima* stand; stands III and IV = *Q. virginiana* large trees; and stand V = *Q. virginiana* saplings. Stand V was destroyed in a fire before February 1976.

from *Q. virginiana,* less frequent in the more sparsely populated *Q. minima* stand (stand II), and virtually absent in the pure stand (stand I) of *Q. minima*. The final collection in February 1976 showed increased production of noninfected telia on *Q. virginiana* and in the more sparsely populated *Q. minima* stand, but no significant change in such telia on *Q. minima* or in *D. filum* infection in any area.

**Inoculation studies.**—Simultaneous inoculations of oak leaves with *C. fusiforme* and *D. filum* produced only rust infections (Table 2). When the rust inoculation preceded that of *D. filum* by at least 4 days so that some signs of the rust were present, infection by *D. filum* did occur. On northern red oak, susceptibility of the rust to *D. filum* increased significantly with time after inoculation, whereas on water oak a uniformly high level of susceptibility occurred after 14 days. The proportion of sori with telia varied from 73-100%, but the variation was not related to *D. filum* infection.

Moist incubation for only 4 hr was sufficient for infection of rust sori by *D. filum;* however, maximum infection occurred with 16 or 24 hr of moist incubation (Table 3).

Inoculum concentrations of 31, 62, 125, 250, 500, and 1,000 $\times$ 10$^3$ conidia/ml of *D. filum* resulted in 6, 13, 18, 34, 58, and 74% infections, respectively, of *C. fusiforme* sori on water oak leaves. The amounts of infection of *C. fusiforme* by *D. filum* resulting from the three lowest inoculum concentrations were not significantly different from each other, whereas the three highest concentrations resulted in significant increases.

On water oak in North Carolina, field inoculations of *C. fusiforme* with *D. filum* resulted in 22, 66, 70, 90, and 89% infection, respectively, 1 to 5 wk later. Sori on leaves that were 10-13 cm from the inoculated leaves had 0, 1.7, 0.2, 16, and 55% infection during the same 5-wk period, whereas sori on check leaves >120 cm from the inoculated leaves had no *D. filum.*

No pycnidia were seen in sori 2 days after inoculation of rust-infected northern red oak leaves with conidia of *D. filum;* however, the percentages of sori with pycnidia after 4, 6, and 8 days were 2.6, 27.5, and 35.9, respectively.

TABLE 2. Infection of *Cronartium fusiforme* (Cf) by *Darluca filum* (Df) and telial production on northern red oak and water oak leaves at different intervals between inoculations

| Days between Cf and Df inoculations | *D. filum* (Df) or check (Ck) | Sori with *D. filum* (%)[a] red oak[b] | Sori with *D. filum* (%)[a] water oak[c] | Sori with telia (%)[a] red oak[d] | Sori with telia (%)[a] water oak[d] |
|---|---|---|---|---|---|
| 0 | Df | 0 | 0 | 96 | 92 |
| | Ck | 0 | 0 | 96 | 73 |
| 4 | Df | 5.3 | 2.9 | 94 | 87 |
| | Ck | 0 | 0 | 99 | 92 |
| 7 | Df | 22.4 | 49.9 | 87 | 93 |
| | Ck | 0 | 0 | 97 | 89 |
| 14 | Df | 70.7 | 78.6 | 86 | 93 |
| | Ck | 0.1 | 0 | 94 | 95 |
| 21 | Df | 88.6 | 85.2 | 91 | 91 |
| | Ck | 0 | 0 | 98 | 100 |

[a]Determined 14 days after inoculation with *D. filum;* six seedlings per treatment, 3,000-5,000 sori per treatment.
[b]$LSD_{.01}$ = 17.3.
[c]$LSD_{.01}$ = 15.4.
[d]Differences not significant.

TABLE 3. Effect of duration of moist incubation on infection of *Cronartium fusiforme* sori by *Darluca filum*

| Moist incubation (hr) | Northern red oak: Seedlings (no.) | Northern red oak: Sori with *D. filum* (%)[a] | Water oak: Seedlings (no.) | Water oak: Sori with *D. filum* (%)[a] |
|---|---|---|---|---|
| 4 | 10 | 12 | 5 | 5 |
| 8 | 6 | 26 | 5 | 24 |
| 16 | 6 | 57 | 5 | 64 |
| 24 | 9 | 59 | 5 | 67 |
| | $LSD_{.01}$ = | 15 | | 17 |

[a]Oak leaves were inoculated with aeciospores 10-14 days prior to inoculation with *D. filum* conidia. Sori with *D. filum* were determined 14 days after inoculation with *D. filum.*

TABLE 4. Effect of infection by *Darluca filum* on number of *Cronartium fusiforme* basidiospores cast from water oak leaves

| Rust age (days) at: *D. filum* inoc. | Rust age (days) at: spore cast | Proximal (P) or distal (D) leaf section | Seedlings[a] (no.) | Telia with *D. filum* (%) check | Telia with *D. filum* (%) inoc. | Basidiospores/telium[b] (no.) check | Basidiospores/telium[b] (no.) inoc. |
|---|---|---|---|---|---|---|---|
| 8 | 15 | P | 11 | 0 | 68 | 669 | 211* |
| | | D | 4 | 0 | 71 | 11 | 36 |
| 8 | 22 | P | 14 | 0 | 64 | 1,116 | 242** |
| | | D | 10 | 0 | 74 | 595 | 27** |
| 14 | 21 | P | 14 | 0 | 76 | 984 | 293** |
| | | D | 10 | 0 | 78 | 400 | 117** |
| 14 | 28 | P | 12 | 0 | 81 | 1,358 | 381** |
| | | D | 10 | 0 | 85 | 607 | 208* |
| 14 | 35 | P | 9 | 0 | 70 | 1,736 | 472** |
| | | D | 4 | 0 | 81 | 944 | 405** |

[a]One leaf/seedling.
[b]Double asterisk (**) or single asterisk (*) indicates figure is significantly different from the control at $P = 0.01$ or $P = 0.05$, respectively.

**Basidiospore cast following Darluca filum parasitism.**—Infection of 8-day-old rust sori by *D. filum* reduced the basidiospore casts of 22-day-old rust sori in comparison with casts of the noninoculated checks (Table 4). At age 15 days, the casts were light, but on the proximal half of water oak leaves there was a significant reduction in basidiospores due to infection by *D. filum*. The variation in casts both within and between leaves caused some differences to be nonsignificant.

Infection of 14-day-old rust sori by *D. filum* reduced the basidiospore casts on the inoculated portion compared to those for the check portion, 7, 14, and 21 days later (Table 4). Basidiospore casts were heavier on all proximal and distal check quarters than on their respective inoculated quarters. More spores per telium were cast from all proximal check quarters than from the distal check quarters from the same leaves.

Measurements of 50 pairs of telia from water oak leaves 28-35 days after rust inoculation indicated that those infected with *D. filum* averaged 550 μm in diameter compared to 1,270 μm for check telia on the same leaves.

On northern red oak, infection of 8-day-old rust sori by *D. filum* resulted in casts at 15 days that were not significantly different from those of the check quarters. Basidiospore casts ranged from 70 to 265 spores per telium. At 22 days, the inoculated proximal quater averaged 535 spores per telium, significantly less than the 1,525 spores per telium from the proximal check. Similarly, at 22 days the distal quarters also showed significant differences: an average of 556 spores per telium for the check compared with 264 spores per telium for the inoculated.

## DISCUSSION

Within the 0.2-ha pure stand of *Q. minima* in Florida, *D. filum* appears to have exerted biological control of *C. strobilinum* by significantly reducing the number of telia. Uredia of *C. strobilinum* are formed from June to December, whereas telia form only in late December through February. In the pure stand, infection by *D. filum* averaged 64% in October and 93% in February. More importantly, telia were rarely found, whereas 26% of all sori on *Q. minima* in an adjacent, sparsely populated stand had telia. Since basidiospore cast and subsequent infection of the pine strobili normally occur during January and February, the inoculum potential in the pure stand would be considerably reduced. The infection by *D. filum* in the stand only 37 m away was 32-35%, indicating that spread of the mycoparasite was very limited. In these two areas, *Q. minima* grows less than 0.5 m tall. Since the conidia of *D. filum* ooze from the pycnidia in long sticky columns, we assume the spores are spread by water splashing to nearby hosts and cannot be dispersed over long distances. The pure stand is thickly covered with *Q. minima* sprouts that provide optimum conditions for both the rust and the mycoparasite, whereas in the adjacent stand these oak sprouts are more scattered. Two of the collection areas of *Q. virginiana* (stands III and IV) had large open-grown trees and low infection levels by *D. filum*. Unfortunately, the third area (stand V), which contained saplings and an intermediate *D. filum* infection, was destroyed by fire.

Keener (3) successfully inoculated existing sori of a number of rust species with *D. filum*. Recently, simultaneous inoculations with *Puccinia recondita* and *D. filum* were successful if followed by 3-15 days of moist incubation (7). With 1 day of moist incubation, *C. fusiforme* was susceptible to infection only after it had become well established on its host. Four days after inoculation with *C. fusiforme*, some leaf flecking was present, but signs of infection were not evident macroscopically. The low infection rate by *D. filum* at 4 days indicates the small amount of rust tissue available for parasitism. Compared with water oak, northern red oak exhibited more infection by *D. filum* up to the maximum age of 21 days, presumably because more rust tissue was available.

When sufficient moisture was present, germ tubes from conidia of *D. filum* rapidly penetrated *C. fusiforme* on leaf surfaces after the relatively short periods of 4-8 hr. Maximum infection occurred after 16-24 hr. In vitro, germination of *D. filum* conidia usually exceeds 85%; thus, it was surprising that a high spore concentration was necessary to produce 74% infection. However, no attempt was made to determine the density of rust sori per unit of leaf area, and the rust density effect is probably as important as inoculum concentration.

For biological control of *C. fusiforme*, rapid formation of pycnidia and conidia by *D. filum* following infection is essential for secondary spread of the mycoparasite. Under greenhouse conditions, some pycnidia formed in 4 days, and significant numbers were present after 6 days. This capacity of *D. filum* for rapid multiplication is an asset in biological control.

Telia of *C. fusiforme* that were infected by *D. filum* produced fewer basidiospores than noninfected telia. The only exceptions were from 15-day-old rust infections on northern red oak. Since telia begin to appear 6-10 days after inoculation, these telia probably were only 5-9 days old and somewhat immature. When 14-day-old rust infections on water oak were inoculated with *D. filum*, telial size was reduced and there was a significant reduction in basidiospore casts 7, 14, and 21 days later. The heavy *D. filum* infection reduced basidiospore production two- to threefold.

Basidiospore casts were made 7, 14, and 21 days after inoculation to determine if the *D. filum* infection affected only a portion of the telial column. Because telia mature basipetally, new tissues may have been added after infection. Although the average number of basidiospores per telium in the inoculated treatments increased with time, an analysis of variance showed no significant difference among the means. The increase in basidiospore cast with time in the proximal check treatment also was nonsignificant.

The proximal portion of the check half of the leaf produced significantly more spores than did the distal portion at four of the five sample times. Initially, we thought this within-leaf variation was due to leaf maturity since the leaf matures acropetally. However, the 28- and 35-day-old infections did not reverse this trend even though telia should have been mature. These differences must have been due to physiological differences in the proximal and distal leaf segments.

The potential of *D. filum* as a practical means for widespread biological control of *C. fusiforme* or *C. strobilinum* seems limited at this time. In north-central Florida, *D. filum* will persist from year to year on the evergreen oak hosts of *C. strobilinum*. In localized areas, *D. filum* infection is heavy and control is being exerted, but the same level of control may not occur in adjacent stands.

Although sori of *C. fusiforme* are readily parasitized by *D. filum* following inoculation, this rust provides a less likely candidate for biological control than does *C. strobilinum*. *Cronartium fusiforme* is active on its oak hosts for less than 2 mo, and therefore a simultaneous and rapid buildup of the mycoparasite and rust would be necessary each year. Although inoculations of oaks with the rust and mycoparasite in North Carolina produced high infection levels by the mycoparasite in 2 wk, infection of sori on adjacent noninoculated leaves required an additional 3 wk to reach 55% in spite of abundant rainfall from mid-May through June. Maintenance of the mycoparasite in nature would be doubtful even if it should be capable of surviving for 10 mo. Since oak hosts of the rust are deciduous, *D. filum* spores would have to originate from dead leaves on the forest floor. Furthermore, the amount of rust on oak in an area varies greatly from year to year because of the short period of oak susceptibility and aeciospore dissemination and the variations in environmental conditions. When rust incidence is low, *D. filum* would be severely restricted. Finally, uredia of *C. fusiforme* are very short-lived; thus, the mycoparasite could not build up on uredial sori as it does on *C. strobilinum* where the uredia persist for 6-7 mo.

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