# Mycoparasitic Effects of *Scytalidium uredinicola* on Aeciospore Production and Germination of *Cronartium quercuum* f. sp. *fusiforme*

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## ABSTRACT

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Maximum aeciospore production by *Cronartium quercuum* f. sp. *fusiforme* on 10 loblolly pines averaged $111 \times 10^3$ spores per square millimeter of gall tissue. In areas of the same galls parasitized by *Scytalidium uredinicola*, aeciospore production was reduced 72% to 31,000 spores per square millimeter. Rapid dispersal decreased the aeciospore population on nonparasitized and parasitized gall tissues to 13 and 23%, respectively, of the maxima after 1 wk. Germination of aeciospores from heavily parasitized gall areas was significantly reduced compared with that from nonparasitized or lightly parasitized areas. Although significant differences in aeciospore germination occurred among sampling dates during the sporulation seasons, no linear relationship was detected between germination and time.

*Additional key words:* biological control, mycoparasite, fusiform rust.

Aeciospore production, dispersion, and germination are critical links between the pine and oak hosts for *Cronartium quercuum* (Berk.) Miyabe ex Shirai f. sp. *fusiforme*, the cause of fusiform rust of southern pines. Since fusiform rust is a heteroecious rust, spread to new pine hosts is dependent on infection of the oak hosts. Oak leaves are susceptible to infection only during a 4–6 wk period during oak leaf emergence and maturation (4). Aeciospore production usually starts prior to oak leaf emergence, but it continues through the period that oak leaves are susceptible (5). Aeciospore production starts with a tremendous volume of spores that are readily collected for storage (3), but rapid dispersion leaves only a light powder of spores on the surface of the gall. This production and dispersion has not been quantified.

The mycoparasite, *Scytalidium uredinicola* Kuhlman, Carmichael, and Miller, appeared to decrease production, slow dispersion, and reduce germination of aeciospores (2). If aeciospore dispersion is slowed and viable aeciospores are present for a longer period during the emergence of new oak leaves, the mycoparasite could be beneficial to its host *C. quercuum* f. sp. *fusiforme*. However, reduced aeciospore germination could offset this benefit. Aecial sporulation occurs both with and without the mycoparasite on the same gall, but some gall tissue does not sporulate. The objectives of this study were to measure the production and dispersion of aeciospores by parasitized and nonparasitized gall tissues and to determine the influence of aecial age, *S. uredinicola*, and other microorganisms on aeciospore germination.

## MATERIALS AND METHODS

**Aeciospore production and dispersion.** Ten basal stem galls on 10 8-yr-old loblolly pine (*Pinus taeda* L.) trees in the Sandhills State Forest, Patrick, SC, were selected for this study. All had immature aecia at the beginning (4 April 1978). Total gall area and areas sporulating were mapped by wrapping clear acetate sheets around the gall and outlining these areas on the sheet. Aecial sporulation with and without *S. uredinicola* (2) were mapped each time spores were collected. The area of gall surface sporulating each date was determined by measuring the outlined areas on the acetate sheets with a polar planimeter.

The populations of aeciospores of *C. quercuum* f. sp. *fusiforme* and conidia of *S. uredinicola* present at each sampling date were estimated by collecting three to 10 spore samples evenly distributed over the sporulating areas of each gall. For each sample a 15-mm-long section of cork borer with an inside diameter of 6 mm (28.27 $mm^2$) was pressed into the gall tissue. Spores within the cork borer

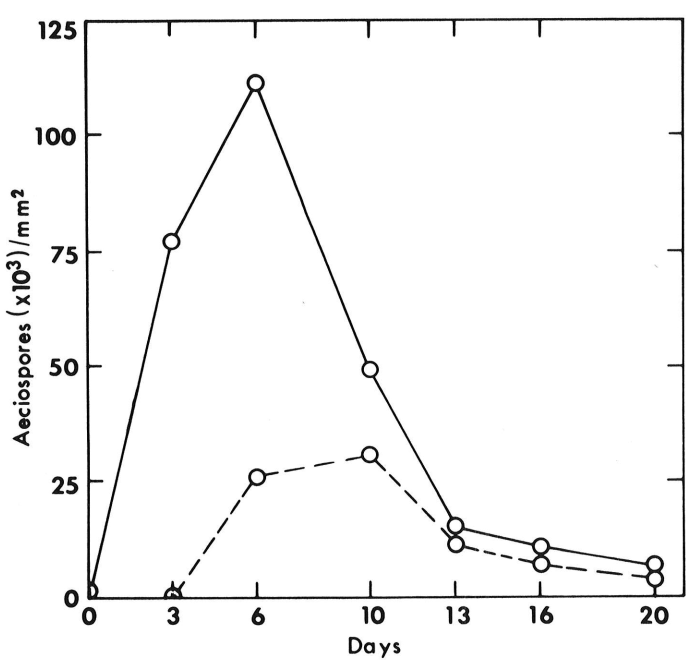


**Fig. 1.** Average aeciospore production per square millimeter of gall surface in unparasitized areas (solid line) and in areas parasitized by *Scytalidium uredinicola* (dashed line) following the appearance of unruptured aecia on 4 April 1978.

TABLE 1. Comparison of estimated actual and potential aeciospore production by galls caused on loblolly pine by *Cronartium quercuum* f. *fusiforme* as reduced by *Scytalidium uredinicola* and potential production if no mycoparasitism had occurred

| Gall no. | Gall area ($cm^2$) | Sporulating area (%) | Sporulating area parasitized by *S. uredinicola* (%) | Aeciospore production: Estimated ($\times 10^9$) | Aeciospore production: Potential ($\times 10^9$) |
|---|---|---|---|---|---|
| 1 | 639 | 100 | 54 | 2.9 | 5.0 |
| 2 | 1,135 | 95 | 55 | 8.8 | 12.4 |
| 3 | 406 | 66 | 28 | 4.2 | 5.6 |
| 4 | 1,196 | 81 | 4 | 10.2 | 10.5 |
| 5 | 737 | 69 | 24 | 4.8 | 5.7 |
| 6 | 520 | 92 | 0 | 7.1 | 7.1 |
| 7 | 991 | 84 | 9 | 6.7 | 7.2 |
| 8 | 1,227 | 59 | 5 | 3.8 | 3.9 |
| 9 | 1,158 | 97 | 48 | 12.5 | 16.1 |
| 10 | 1,123 | 88 | 56 | 5.4 | 7.9 |
| | Average % or total spores | 83 | 28 | 66.5 | 81.4 |

segment were collected with a cyclone spore collector. Samples were collected six times at 3- or 4-day intervals for 20 days.

Spores were mixed with 3–7 ml of distilled water containing two drops of Triton X-100 per liter. One milliliter of the spore suspension was pipetted into 19 ml of acidified water (adjusted to pH 2 with HCl). A Model B Coulter Counter was used to determine the number of spores in the suspension. Separate counts of aeciospores and conidia were made by adjusting the threshold limits. Aeciospore counts were averaged for areas with and without *S. uredinicola*.

**Aeciospore germination.** Aeciospores were collected from galls on loblolly pines near Apex and Tillery, NC, and on slash pines (*P. elliottii* Engelm. var. *elliottii*) in the Sandhills State Forest each week during sporulation from 1974–1976. Thus, observations at the three plantations for 3 yr yielded nine data sets. Galls were selected on the basis of abundant, fresh-appearing aeciospores. However, once a gall was sampled, weekly collections were made as long as spores were present on the gall. Additional galls were added during a season to maintain a sample size of 10–15 galls per location whenever possible. During the 3-yr period, 674 spore samples were processed. Eleven comparisons were made of aeciospore germination from gall areas on loblolly pines either nonparasitized or relatively free of *S. uredinicola* with aeciospore germination from areas heavily colonized by *S. uredinicola*.

Aeciospores were collected dry in a test tube and held in an ice chest until they were processed. Aeciospores were suspended in sterile distilled water and stirred vigorously prior to spreading 1-ml aliquots on 2% water agar (WA) and on Czapek's agar (CA). After 48 hr of incubation at room temperature, the percentage germination on WA was determined for 200 aeciospores per sample. CA plates were held at room temperature for 7 days before bacterial and fungal colonies were counted. Population estimates of aeciospores and slower-growing microorganisms (eg, *S. uredinicola*) were made by counting these on 61 $mm^2$ of agar surface (1% sample) at $\times 100$ magnification.

## RESULTS

**Aeciospore production and dispersion.** In sporulating, nonparasitized areas of the galls, maximum aeciospore production occurred after 6 days (Fig. 1). On that date, the average number of aecisopores per square millimeter of gall surface was $111 \times 10^3$ (range $53–210 \times 10^3/mm^2$). Rapid aeciospore dispersion reduced the average number of aecisopores to $49 \times 10^3$ or 44% of the maximum production 4 days later (day 10) and to $15 \times 10^3$ (13% of the maximum) after 1 wk (day 13). Two wk after the maximum (day 20), the galls had an average of $6 \times 10^3$ aeciospores per square millimeter or 5% of the maximum still on the gall.

Maximum aeciospore production in areas of the galls parasitized by *S. uredinicola* was significantly less than in the nonparasitized areas and occurred 4 days later (day 10). The average maximum

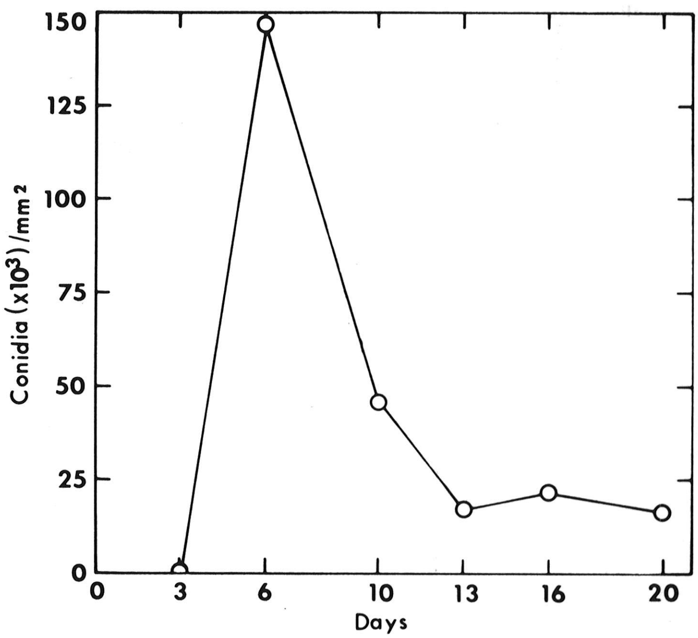


**Fig. 2.** Average conidial production by *Scytalidium uredinicola* per square millimeter of parasitized gall surface after 4 April 1978.

production in the parasitized areas of nine galls was only $31 \times 10^3$ spores per square millimeter which was 72% less than the maximum production in the nonparasitized areas (Fig. 1). Aeciospore production was significantly less in the parasitized areas in comparison to the nonparasitized areas at 5 of the 6 sample days according to *t*-tests. On day 16, only $7 \times 10^3$ aeciospores per square millimeter were present on the parasitized areas.

The shape of the curve for average number of conidia of *S. uredinicola* present was similar to that for aeciospores in nonparasitized areas (Fig. 2). On days 13–20, the number of conidia remained high.

The percentages of the sporulating areas that were parasitized by *S. uredinicola* varied from 0 to 56% (Table 1). The estimated aeciospore production per gall based on samples from parasitized and nonparasitized areas varied from $2.9–12.5 \times 10^9$. The potential production was calculated from the nonparasitized samples to be $5.0–16.1 \times 10^9$ aeciospores per gall. Thus, the parasite reduced the total production for these 10 galls by $14.9 \times 10^9$ aeciospores.

**Aeciospore germination.** An analysis of variance of the transformed percentage germination data by year and location indicated highly significant differences in germination by collection date in 6 of the 9 yr by location data sets. One of these data sets for the Sandhills State Forest in 1975, ranks the percentage of germination and also shows that germination remained high during

TABLE 2. *Cronartium quercuum* f. sp. *fusiforme* aeciospore germination ranked according to percentage of germination from 148 collections made during a 13-wk period at the Sandhills State Forest, SC, in 1975 and from 83 collections during a 7-wk period in 1974 at Tillery, NC

| Sandhills, 1975 | | Tillery, 1974 | |
|---|---|---|---|
| Week | Germination (%) | Week | Germination (%) |
| 1 | 75 a[z] | 4 | 59 a[z] |
| 5 | 72 a | 2 | 52 a |
| 4 | 68 a | 3 | 51 ab |
| 6 | 59 ab | 5 | 49 ab |
| 3 | 56 ab | 6 | 38 ab |
| 8 | 54 ab | 1 | 36 bc |
| 7 | 41 bc | 7 | 15 c |
| 2 | 40 bc | | |
| 9 | 40 bc | | |
| 10 | 31 c | | |
| 11 | 26 c | | |
| 12 | 24 c | | |
| 13 | 1 d | | |

[z]Numbers followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

the first 8 wk of this long 13-wk set (Table 2). More typically, eg, Tillery 1974, aeciospore germination for a given 6-to 8-wk period had variations at both the beginning and the end of the season that were significantly different (Table 2).

The most common microorganisms associated with the aeciospores were *S. uredinicola, Penicillium* sp., *Cladosporium* sp., and bacteria. The latter three occurred so infrequently and in such low numbers that no effect of their presence could be determined. Two statistical comparisons were made with *S. uredinicola.*

In a *t*-test comparison, germination was significantly better ($P = 0.01$) if aeciospores were collected from gall areas that were either nonparasitized or relatively free of *S. uredinicola* in contrast to aeciospores from areas heavily colonized by *S. uredinicola.* The average reduction in germination was 28% for 11 paired samples.

Linear correlations of percentage *S. uredinicola* conidia in the total spore population to percentage aeciospore germination were made separately by year for each of the two North Carolina collections. Fisher's Z transformation was used to test the hypothesis that $\rho = 0$ (Table 3). The inverse effect of *S. uredinicola* populations on aeciospore germination is evident in the table. However, the r values indicate the relatively small effect of the *S. uredinicola* population on aeciospore germination.

## DISCUSSION

**Aeciospore production and dispersion.** The average maximum number of aeciospores produced in nonparasitized areas of the galls was $111 \times 10^3/\text{mm}^2$. Once the peridium breaks and the spores are released, more aeciospores may be produced by the aecial mother cells. Since only spores from the three-to-10 sample points chosen at each date were removed, this sampling did not measure the total number produced by each gall but rather the number present on the gall at each date. Therefore the $111 \times 10^3$ spores per square millimeter is a conservative estimate of the capacity of the rust.

Kais and Walkinshaw (1) have indicated the rate of discharge for aeciospores by *C. quercuum* f. sp. *fusiforme* at peak sporulation varied from 0.5 to 6 mg of spores per square centimeter in 24 hr. The reason for the 10-fold variation in production vs the fourfold variation (Table 1) reported here may be due to differences in sampling procedures.

Previously, Kuhlman et al (2) illustrated several levels of destruction of the aecium by *S. uredinicola.* This study has quantified the reduction in aeciospore production as being 72% of the average maximum in nonparasitized tissue. In this 10-gall sample only 28% of the sporulating tissue was parasitized. Therefore the reduction in spore production for these galls was 20% ($72 \times 28$). For this mycoparasite to be useful in biological control, factors enabling it to parasitize most of the sporulating surface will need to be identified.

For the production and dispersion study, galls were selected on the basis of presence of unruptured aecia. The mycoparasite was not noted until sporulation was occurring. In this sample, nine of 10 galls were parasitized with an average 28% of the sporulating surface affected. The reduction in aeciospore production by $14.9 \times 10^9$ aeciospores by this infection is significant. However, had all the sporulating surfaces been parasitized, production would have been reduced by $58.6 \times 10^9$ aeciospores.

The presence of the mycoparasites delayed maximum production by 4 days in these areas of the galls. This delay probably is the result of the reduced pressure on the outer bark. In nonparasitized areas the maturing aecium rapidly pushes its way through the bark; however, in parasitized areas the smaller aecium takes more time to break through. The delay in spore release enables the mycoparasite to continue its destruction of aeciospores. This delay did not prolong the production and release of aeciospores.

**Aecispore germination.** Besides destroying aeciospores, *S. uredinicola* also renders other aeciospores nongerminative. In paired tests of relatively uncontaminated aeciospores vs aeciospores heavily parasitized by *S. uredinicola*, the reduction in germination of 28% was significant. The inverse relationship that increasing *S. uredinicola* populations have on aeciospore germination is shown by the data from Apex and Tillery.

Previously, in Mississippi, a rapid decrease in germination was reported in a 17-day period following rupture of the aecial peridium (1). In 1975 in the Sandhills State Forest, high aeciospore germination occurred through the eighth wk of sporulation. Thus, aeciospores on galls can remain viable and free of other organisms for several weeks. These collections were from aecial pustules with the most fresh, abundant aeciospores. This selection may have somewhat diminished the age effect, but it does indicate a long-term production of viable spores.

TABLE 3. Correlation coefficents of aeciospore germination and production of *Scytalidium uredinicola* conidia

| | Location | |
|---|---|---|
| Year | Tillery | Apex |
| 1974 | −.24* | −.10 |
| 1975 | −.47** | −.26** |
| 1976 | −.58** | −.51** |

[a]Asterisks, * and **, indicate that the correlation coefficients differ significantly from the null hypothesis, $\rho = 0$, at $P = 0.05$ and $P = 0.01$, respectively.

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