Parasite Interaction with Sporulation by *Cronartium quercuum* f. sp. *fusiforme* on Loblolly and Slash Pine

E. G. Kuhlman

Plant pathologist, USDA Forest Service, Southeastern Forest Experiment Station, Forestry Sciences Laboratory, Research Triangle Park, NC 27709.

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ABSTRACT

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Scytalidium uredinicola was the most common parasite of the aecial stage of *Cronartium quercuum* f. sp. *fusiforme* in three pine plantations during four seasons. This mycoparasite was more frequently associated with aeciospores of the rust on loblolly pine in two North Carolina plantations than on slash pine in a South Carolina plantation. *Tuberculina maxima* was present on galls on loblolly pine during both aecial and spermatial sporulation, but it was not present on galls on slash pine. In the spring, *T. maxima* occurred several weeks before aecia appeared and remained independent of the rust aecia. However, galls parasitized by *T. maxima* one spring less commonly had aecia the following spring than did galls without *T. maxima*. Parasitism by *T. maxima* was less frequent in the fall and had no effect on aecial sporulation the following spring. Galls with aecia alone, aecia with *S. uredinicola*, or neither of these were most likely to have the same sporulation type the following spring. Adult *Epuraea lengi*, a nitidulidid beetle, commonly fed on both aecial and spermatial tissue. Larvae of *E. lengi* fed on aeciospores and larvae of an unidentified diptera fed on spermatia.

Additional key words: Pinus taeda, P. elliottii var. *elliottii,* biological control, ecology, aphids, mites, *Dioryctria,* fusiform rust.

The major control strategy for fusiform rust of loblolly pine (*Pinus taeda* L.) and slash pine (*P. elliottii* Engelm. var. *elliottii*) has been the selection and breeding of trees for rust resistance. Biological control of the disease organism, *Cronartium quercuum* (Berk.) Miyabe ex Shirai f. sp. *fusiforme,* is an alternative that has received less attention. Recently the association of two hyperparasites, *Scytalidium uredinicola* Kuhlman et al and *Tuberculina maxima* Rostrup, with rust galls on pine was described (4,5), but the effect of these organisms on rust epidemiology has not been well quantified.

Parasitism by *T. maxima* was thought to reduce aeciospore production by *C. ribicola* Fisher. Hubert (1) suggested that invasion of the spermatia by *T. maxima* reduced subsequent aeciospore production. Later, *T. maxima* was implicated as a major cause of reduced *C. ribicola* canker activity in both antibiotic-treated and untreated plots (6). Galls of *C. comandrae* Pk. were usually inactive the season after infection by *T. maxima* (7). Aecial and spermatial sporulations of *C. quercuum* f. sp. *fusiforme* are known to fluctuate from year to year within plantations (2); how much hyperparasites and insects influence this fluctuation is the subject of this paper.

MATERIALS AND METHODS

Fusiform rust galls in two loblolly pine plantations in North Carolina and one slash pine plantation in South Carolina were observed weekly during aecial and spermatial seasons from 1974–1977 (2). Sporulation of fungi other than the rust and the presence of insects on each of the approximately 200 tagged galls per plantation were noted during aecial and spermatial sporulation. All data are based on live galls present each season. Chi-square analyses were used to compare the effects of hyperparasites on aecial sporulation.

RESULTS

Occurrence of *S. uredinicola* and *T. maxima*. Conidia of *S. uredinicola* were produced in the spring in aecial sori of *C. quercuum* f. sp. *fusiforme* but were never associated with spermatia in the fall (Table 1). *S. uredinicola* was most common on galls on loblolly pine at Apex, NC, and least common on galls on slash pine at Patrick, SC. *Tuberculina maxima* was not recognized in 1974. In subsequent years, *T. maxima* was observed in both seasons on galls on loblolly pine but it was never seen on galls on slash pine. The occurrence of sporodochia of *T. maxima* was independent of aecial sporulation by *C. quercuum* f. sp. *fusiforme* that same season. Some sporodochia appeared several weeks before the appearance of aecia. Furthermore, sporodochia usually occupied areas of the gall that did not support aecia. Finally, only 49% (97/199) of the galls infected with *T. maxima* in the spring had aecia present.

To determine if galls on slash pine had less infection by mycoparasites than galls on loblolly pine, fungi sporulating on 100 additional slash pine galls were compared with fungi on 100 galls in a loblolly pine plantation at Patrick in April 1976. Aecia occurred on 76 and 85% of the galls on slash and loblolly pine, respectively; *S. uredinicola* occurred on 21 and 19%, respectively; and *T. maxima* occurred on none. On that date, 56% of the galls in the main study at Patrick had aecia and 10% had *S. uredinicola.* This limited sample suggests that geographic location rather than host species of the rust limits the occurrence of *T. maxima.* The longer duration of spermatial sporulation that occurs in North Carolina may favor infection by *T. maxima* (2).

Mycoparasite effect on aecial sporulation. Galls parasitized by *T. maxima* were generally as likely to produce aecia as were nonparasitized galls in the same season, but were significantly less likely to have aecia the following spring than were nonparasitized galls (Table 2). For example, at Tillery in 1976, 41% of the galls with *T. maxima* present that spring had aecia, whereas 33% of galls without *T. maxima* had aecia. In contrast, only 17% of galls parasitized the previous spring had aecia, whereas 44% of nonparasitized galls had aecia.

Parasitism of galls by *S. uredinicola* one spring did not suppress

subsequent aecial sporulation as had *T. maxima* (Table 3). However, galls with aecia alone, aecia with *S. uredinicola,* or neither were most likely to have the same type of sporulation the 2nd yr. For example, 177 galls had aecia alone in 1974 and 67% of these had aecia alone in 1975, whereas the total population had only 40% with aecia only. The results in Table 3 are the combined totals for the three plantations for one of three year-to-year sequences; chi-square analyses indicated highly significant differences within individual plantations for each of the three year-to-year combinations.

Occurrence of insects and other organisms. Adult *Epuraea lengi* Parsons, a nitidulidid beetle, were observed on galls in both the spring and fall (Table 4). Although the small size and brown color of adults made them difficult to see, their presence early in a season indicated aecia or spermatia were developing under the bark. Larvae of *E. lengi* were most often seen on aecia that persisted for several weeks, whereas larvae of an unidentified dipterous species were present only in the fall in spermatial fluid.

Larvae of *Dioryctria* sp. were common inhabitants of galls on slash pine. Their tunnels usually went into the gall wood, and their activity was evident from frass and resin drops. In May 1975, 80 of 177 live galls at Patrick had symptoms of *Dioryctria* larvae; however, their presence had little effect on sporulation except that the resin impeded spore release.

Aphids (*Cinara watsoni* and *C. pinivora* [?]) frequently congregated on galls at Tillery and Apex, but they were never seen in areas of the gall that had sporulation by the rust fungus. Mites (*Coraboides* sp. and *Paracheyletia pyriformis* Banks) were recovered from aeciospore samples from Apex in 1975. Yellow jackets (*Vespula maculiforms* [Buysson]) occasionally visited galls and appeared to feed on drops of spermatial fluid; during any fall season, less than five were observed in each plantation. *Cladosporium, Trichoderma,* and *Penicillium* spp. infrequently sporulated in areas of the gall where aecial sori had persisted for a long time.

DISCUSSION

Scytalidium uredinicola was the most common mycoparasite in all three pine plantations. It occurred in 27, 51, and 9% of the live galls at Tillery, Apex, and Patrick, respectively. Since 44, 65, and 76%, respectively, of the live galls at these locations produced aecia (2), and since *S. uredinicola* sporulated only in aecial sori, the percentages of galls with aecia that were infected with *S. uredinicola* at Tillery, Apex, and Patrick were 61, 78, and 12%, respectively. Infection by *S. uredinicola* has been shown to drastically reduce aeciospore production (3). Therefore, the high incidence of hyperparasitism at Tillery and Apex must significantly reduce inoculum available for oak infection in comparison with that available at Patrick.

Sporulation by *T. maxima* was more commonly observed in the spring than in the fall (Table 1). New sporulation by *T. maxima* commonly preceded aecial sporulation by the rust. Infections by *T. maxima* probably occur in spermatial areas, but attempts to inoculate 300 galls of *C. quercuum* f. sp. *fusiforme* in any stage of activity with *T. maxima* spores and mycelium have been unsuccessful (*unpublished).* In contrast, both spermatial and aecial areas of *C. ribicola* are readily infected by *T. maxima* (8–10). The inoculum density of *T. maxima* used for inoculting fusiform rust galls may have been inadequate.

Fusiform rust galls parasitized by *T. maxima* one spring were less likely to produce aecia the following spring than were galls without *T. maxima.* This association of reduced sporulation by *C. quercuum* f. sp. *fusiforme* and prior infection with *T. maxima* confirms observations made for *C. ribicola* (6) and *C. comandrae* (7).

Many small sporodochia of *T. maxima* occurred at the margin of fusiform rust galls where spermatia were common, but aeciospores were less frequently observed. Since aeciospores and sporodochia usually occurred in separate areas, it is not surprising that the percentage of galls that had aeciospores was about the same for galls infected with *T. maxima* as for galls without *T. maxima.* Previously Kuhlman and Miller (5) indicated that sporodochia of *T. maxima* and aecial mother cells arose in the same cell layers of the gall. The present data suggest that *T. maxima* parasitizes these cell layers for 1 yr before aecia are produced.

The two insects most commonly found on galls, *E. lengi* and the unidentified diptera, may have been even more frequently associated with sporulation than these observations indicated. Adult *E. lengi* were small and inconspicuous and were more apparent before than during sporulation. Both types of larvae were most evident on galls that sporulated for several weeks. Although the movement of dipterous larvae called attention to spermatia,

TABLE 1. Percentage of live galls of *Cronartium quercuum* f. sp. *fusiforme* with *Scytalidium uredinicola*[a] (*S.u.*) and *Tuberculina maxima*[b] (*T.m.*) during aecial and spermatial seasons in three plantations[c]

Host and plantation	Aecial		Spermatial	
	S.u. (%)	*T.m.* (%)	*S.u.* (%)	*T.m.* (%)
Loblolly pine				
Tillery, NC	27	20	0	5
Apex, NC	51	21	0	6
Slash pine				
Patrick, SC	9	0	0	0

[a] *S. uredinicola* was present during four aecial seasons.
[b] *T. maxima* was recognized during three aecial and two spermatial seasons.
[c] Approximately 200 live galls were examined in each plantation.

TABLE 2. The effect of parasitism of fusiform rust galls by *Tuberculina maxima* in the same spring and in the previous spring on production of aecia by *Cronartium quercuum* f. sp. *fusiforme*

	Galls with aecia (%)					
	Tillery			Apex		
T. maxima	1975	1976	1977	1975	1976	1977
Present same spring	56	41	23	79	56	26
Absent same spring	62	33	29	88	73	37
Present previous spring		17[a]	13[b]		40[a]	18[b]
Absent previous spring		44	32		75	39

[a,b] Number significantly different from number below it. [a] $P = 0.01$, [b] $P = 0.05$.

TABLE 3. Number of live fusiform rust galls with combinations of aecia, aecia with *Scytalidium uredinicola*, and neither spore form in 1974 in relation to sporulation type in 1975

1974		Percentage of sporulation type in 1975		
Sporulation type	No. of galls[a]	Aecia alone	Aecia and *S. uredinicola*	Neither
Aecia alone	177	67	24	9
Aecia and *S. uredinicola*	141	8	84	9
Neither	205	39	18	44
Total or average	523	40	38	23

[a] Galls that remained alive through spring 1975.

TABLE 4. Percentage of sporulating galls of *Cronartium quercuum* f. sp. *fusiforme* with *Epuraea lengi* adults and larvae and dipterous larvae in three plantations

Host and plantation	Spring			Fall		
	E. lengi		Dipterous larvae	*E. lengi*		Dipterous larvae
	adults	larvae		adults	larvae	
Loblolly pine						
Tillery, NC	13	18	0	8	0	9
Apex, NC	29	33	0	10	0	24
Slash pine						
Patrick, SC	24	46	0	2	0	25

destructive sampling would have been necessary to ensure recording the presence of either larval species on every gall. Spore masses of *S. uredinicola* and *T. maxima* were eaten by the larvae which were yellow, orange, grey green, or purple depending upon the food source. Aeciospore dispersion is so rapid once the spores are mature (3) that it is doubtful the larvae reduce the inoculum by even 1% under mild climatic conditions and in the absence of *S. uredinicola*. Under these conditions, the primary substrate for the larvae may be the aecial mother cell area whose function is completed when parasitism occurs. The dipterous larvae may aid spermatization.

Adult *E. lengi* often tunneled in the gall tissue that contained aecial initials, and they may be a vector of *S. uredinicola*. If so, *E. lengi* and *S. uredinicola* may have a mutually beneficial relationship because the fungus delays the dispersion of aeciospores and this delay appears to secure a food supply for *E. lengi* larvae. Attempts to infect wounded or unwounded galls with *S. uredinicola* were unsuccessful when inoculum was applied to erupted aecial or spermatial areas or to inactive galls (*unpublished*).

Among the organisms studied in these plantations, *S. uredinicola* and *T. maxima* provide the most effective biological control of aecial production. Infection by *T. maxima* directly reduces the frequency of aecial sporulation. However, the more common infection by *S. uredinicola* reduces aeciospore production and germination (3). To better utilize these mycoparasites for biological control, we need more information about their etiology, epidemiology, and genetic variation.

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