

Histology and Progression of Fusiform Rust Symptoms on Inoculated Loblolly Pine Seedlings

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ABSTRACT

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Nine months after inoculation of loblolly pine (*Pinus taeda*) seedlings with basidiospores of *Cronartium quercuum* f. sp. *fusiforme*, most symptomatic tissues had necrotic cells in the cortex. When infection caused depressed areas, necrosis included xylem and phloem cells. Whereas tissues in galls longer than 10 mm were heavily and extensively colonized by the rust, tissues from galls shorter than 10 mm had rust mycelium only in small, isolated areas in the cortex, phloem, or xylem. Seedlings with no stem symptoms or with purple stem spots but no swelling when outplanted at 9 mo were symptomless 24 mo after inoculation. Seedlings with galls shorter than 10 mm at 9 mo usually were free from symptoms (63-67%) after 24 mo. Seedlings with galls 10-25 mm long at 9 mo had the highest frequency of galls and aecia at 24 mo, whereas those with galls 10-25 mm long with depressed areas and those with galls longer than 25 mm had the highest mortality rates.

One of the goals in our effort to control fusiform rust disease caused by *Cronartium quercuum* (Berk.) Miyabe ex Shirai f. sp. *fusiforme* is to identify a diversity of genes for resistance in loblolly (*Pinus taeda* L.) and slash (*P. elliottii* Engelm. var. *elliottii*) pines. Considerable heterogeneity in resistance should provide long-term benefits in control by reducing the opportunity for the pathogen to adapt to a limited genetic base. Sources of resistance in both pine species have been shown to vary in response to basidiospore inoculum generated from certain single-gall aeciospore collections (1,7-10,15).

Pines also vary in symptom expression. Variation in symptom expression to the white pine blister rust fungus (*C. ribicola* J. C. Fisch. ex Rabenh.) among species of white pine has been given as evidence for variation in mechanisms of resistance (2). Miller et al (6) described macroscopic and microscopic expressions of four general host responses of slash pine seedlings to infection by *C. q. f. sp. fusiforme*. The relative occurrence of these responses has been determined for some rust-resistant selections of slash and loblolly pines (1,5). Some symptoms on slash pines indicate resistance in the seedling. Nine months after inoculation, Griggs et al (1) outplanted slash pine seedlings with one of three symptom types: stem or needle spots but no stem

swelling, galls <25 mm long, and galls >25 mm long. These seedlings were scored 2 yr later for "growing" galls. Their results suggest that slash pine seedlings with spots and no swelling or with galls <25 mm long resist the rust fungus, whereas those with galls >25 mm long are susceptible. Walkinshaw (14) reported mycelium of the rust fungus was more abundant in the cambium of galls on loblolly pine than in those on slash pine seedlings. In galls 1-10 mm long on slash pine, mycelium was not observed in the cambial area.

The objective of the histological study was to examine the growth patterns of *C. q. f. sp. fusiforme* in loblolly pine seedlings with various symptoms 9 mo after inoculation. The progression of the disease was followed on outplanted seedlings for 2 yr after inoculation to determine the outcome of various symptoms.

MATERIALS AND METHODS

Four-week-old loblolly seedlings were inoculated with basidiospores of the rust as previously described (5). Seeds were collected separately from each of 156 wind-pollinated loblolly pine trees in the USFS-GA Forestry Commission rust-resistant seed orchards in October 1983. Progeny from each tree are hereafter called a family. The maternal parents of the orchard trees are the most likely source of resistance. These maternal parents are the first-generation resistant selections and the seedlings are third-generation, wind-pollinated progeny of these selections. Nine months after inoculation, seedlings representative of nine different symptom types were selected for a histological study; another group of seedlings, with 10 different

symptom types, was outplanted in nursery beds.

Histology. The nine types of symptoms examined histologically at 9 mo were: 1) no stem symptoms, 2) purple needle base, 3) purple stem spot without swelling, 4) purple stem spot without swelling but with a depressed area, 5) <10-mm-long gall, 6) <10-mm-long gall with a purple depressed area, 7) <10-mm-long, one-sided gall encompassing only part of the stem, 8) >10-mm-long gall, and 9) >10-mm-long gall with purple depressed area(s). For symptomless seedlings, the first 3 cm of stem above the cotyledonary node was sectioned. Six seedlings of each symptom type were selected to provide representatives from at least four third-generation families derived from at least three first-generation resistant selections per symptom type. Specimens were processed by standard histological methods (4) and stained with Pianezze III-B stain (13).

Representative sections of each specimen were examined for presence of spermatogonia, for relative amount of rust mycelium, and for host cell hypertrophy, hyperplasia, or necrosis.

Disease progression from 9 to 24 mo.

Ten symptoms were identified at 9 mo for the disease progression study. Seedlings with the first seven symptom types were selected. In addition, seedlings with >10-mm-long galls were separated by two gall lengths, 10-25 mm and >25 mm; those with galls 10-25 mm long were further separated by presence or absence of depressed areas on the galls. Each symptom type was represented by 25-42 seedlings. Seedlings of each symptom type were selected from 10-22 third-generation families derived from 8-14 first-generation resistant selections to maximize the variation in host material (5). Seedlings were planted at 30-cm spacings in raised beds containing soil fumigated by methyl bromide. Data on symptoms and signs of fusiform rust and seedling survival were recorded 24 mo after inoculation.

RESULTS

Histology. Nine months after inoculation, the tissue area affected by the pathogen varied from small purple areas at the needle base and on the stem to large, fusiform galls (Table 1). Large galls with depressed areas were shorter and smaller in diameter than large galls

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without prominent depressed areas. Rust mycelium was infrequently observed in the purple needle base or in the stem spot tissues. Usually, host cells containing rust mycelium were surrounded by necrotic cells in these seedlings. Tissue from small

galls had limited rust mycelium (Fig. 1A), whereas large galls were heavily and extensively permeated by the fungus (Fig. 1B and C). Most symptomatic tissue had necrotic cortical cells present. In purple stem spots, the necrotic area

was underlaid by reaction parenchyma (Fig. 1D). Seedlings with purple stem spots with depressions had necrosis extending into the phloem and xylem tissues (Fig. 1E and F). Spermogonia were common and abundant in large galls without depressed areas but were seen in only one gall with a depressed area. The spermogonia appeared to be mature even when present several host cells below the epidermis.

Table 1. Size of affected area and relative occurrence of fungus and host responses in six loblolly pine seedlings of each of nine symptom types 9 mo after inoculation with *Cronartium quercum* f. sp. *fusiforme*

Symptom	Mean area affected (length × width × depth, mm)	Response ^a		
		Pine cell necrosis	Rust mycelium	Spermo- gonia
1. No stem symptoms	0	0	0	0
2. Purple needle base	0.8 × 0.5 × 0.3	6+	1+	0
3. Purple stem spot	1.5 × 0.8 × 0.4	6+	2+	0
4. Purple stem spot with depressed area	4.6 × 2.1 × 1.0	6+++	1+	0
5. Gall <10 mm long	6 × 3.6	6+	6+	0
6. Gall <10 mm long with depressed area	8 × 3.5	6++	6+	0
7. One-sided gall <10 mm long	3 × 1.6	6+++	4+	0
8. Gall >10 mm long	45 × 6.2	3+	6+++	5
9. Gall >10 mm long with depressed area	22 × 4.0	6+++	6++	1

^aNumber of seedlings with response; relative abundance indicated by plus symbols.

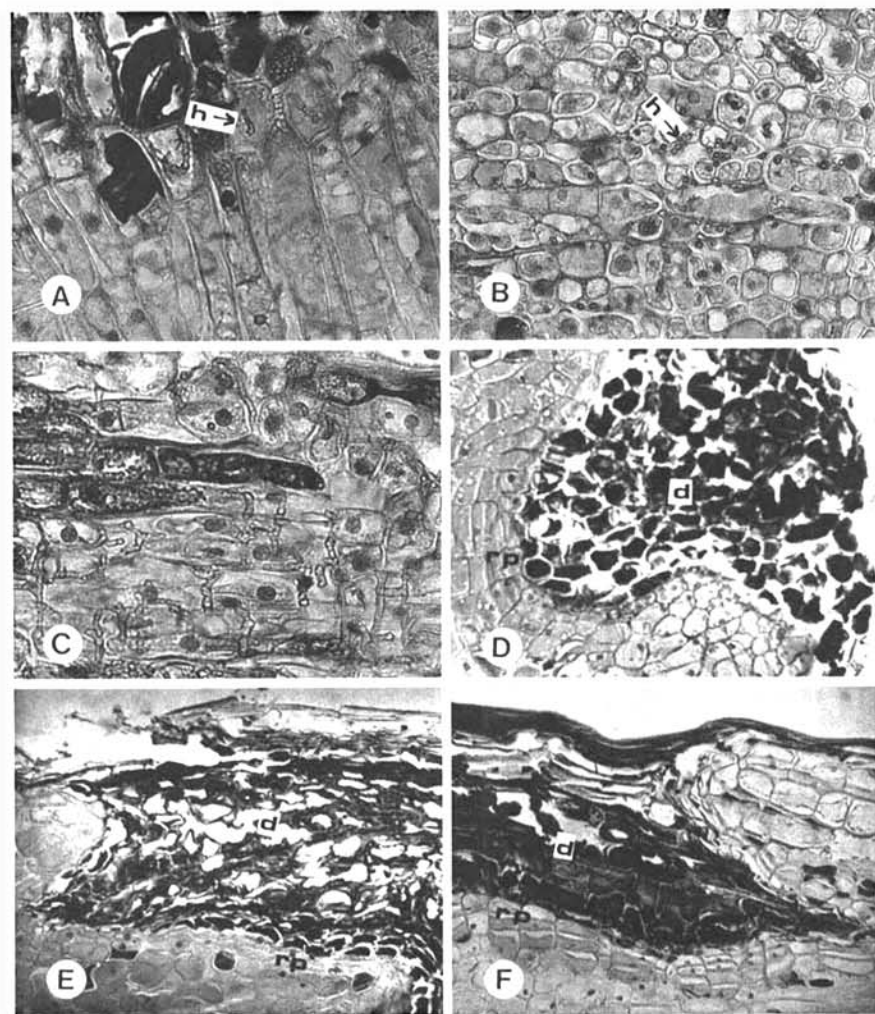


Fig. 1. Tissue from loblolly pine seedlings with various symptoms of fusiform rust disease 9 mo after inoculation: (A) Small gall with limited hyphal (h) development by *Cronartium quercum* f. sp. *fusiforme*, (B) large gall with hyphae (h) common, (C) large gall with extraordinary hyphal colonization, (D) purple stem spot with dead host cells (d) and reaction parenchyma (rp), and (E and F) purple stem spots with depressions showing more extensive tissue involvement.

Disease progression from 9 to 24 mo.

Seedlings with no stem symptoms at 9 mo after inoculation remained symptomless after 24 mo (Table 2). Most seedlings with either type of purple stem spot without swelling at 9 mo were symptomless by 24 mo. Of the seedlings with small, <10-mm-long galls, 63–67% were symptomless after 24 mo. A 2 × 2 contingency table for proportion of symptomless seedlings at 24 mo by symptom types 2–4 (stem symptoms without swellings) compared with types 5–7 (<10-mm-long galls) resulted in chi-square = 20.98, significant at $P < 0.005$. Similarly, a contingency table for proportion of symptomless seedlings at 24 mo for symptom types 5–7 compared with types 8–10 (galls 10–25 and >25 mm long) resulted in chi-square = 74.21, significant at $P < 0.005$. Seedlings with medium-length galls at 9 mo (type 8) had the highest frequency of galls and acacia at 24 mo, whereas seedlings with medium galls with depressed areas or long galls (types 9 and 10) had the highest rates of mortality.

DISCUSSION

The absence of stem symptoms 9 mo after inoculation with *C. q.* f. sp. *fusiforme* was the most common response in loblolly pine seedlings in certain resistant families (5). A sample population of these symptom-free seedlings was examined histologically at 9 mo after inoculation. No mycelium or haustoria were detected in the tissues (Table 1). A second group of these seedlings remained symptomless after 24 mo in the field (Table 2). Other seedlings had needle spots that sometimes occurred at the needle base and contained necrotic tissue extending to the stem. Seedlings with purple needle bases at 9 mo were symptomless at 24 mo. Miller et al (6) suggested symptomless slash pine might have latent infections that would eventually become galls. In the current study, this proved not to be true for loblolly seedlings with no stem symptoms during the first 9 mo after inoculation, just as reported earlier by Griggs et al (1) for slash pine seedlings.

Small galls seem to indicate a resistance response in loblolly pine, as in slash pine (1). In my sample of loblolly pine, 63–67% of the seedlings with any of three types of small galls at 9 mo were symptomless at 24 mo. This study and that of Walkinshaw (14) indicate that

rust mycelium is present to a limited extent in small, 1- to 10-mm-long galls on loblolly pine. By limiting mycelial development, the host resisted the infection in about two-thirds of the outplanted seedlings. Griggs et al (1) reported that most slash pine seedlings with galls < 25 mm long at 9 mo were free from external symptoms after 33 mo. Small galls were significantly more frequent in a resistant slash pine family than in either a moderately resistant or a uniformly susceptible family (1). Similarly, in loblolly pine, most families had the same frequency of small galls as the susceptible control but some resistant loblolly sources had twice as many seedlings with small galls as did other sources (5). Families with significantly greater numbers of seedlings with small galls than other families after greenhouse inoculations may perform better in the field, since small galls indicate resistance.

Purple stem spots without swellings have been considered good indicators of resistance in both slash and loblolly pines (1,5,6,11,16). The mycelium of the rust fungus was confined by necrotic cells in slash pine seedlings with this symptom type (3,6), and such seedlings did not develop galls up to 33 mo after inoculation (1). Loblolly pine seedlings with this symptom were present in most of the 156 families tested (5). In histological sections, the fungus appeared confined by necrotic tissue, and most seedlings with this symptom at 9 mo had no galls 24 mo after inoculation (Table 2).

The histological examination of > 10-mm-long galls with and without depressed areas showed some interesting contrasts in gall size, amount of necrosis, relative amount of rust mycelium, and presence of spermatogonia (Table 1). In galls 10–25 mm long without depressed areas, the obligate parasite thrived and the host remained alive, whereas in galls 10–25 mm long with depressed areas and in galls > 25 mm long, the interaction of pathogen and host created more necrotic tissue because of a high level of susceptibility. The fungus usually overwhelmed the host, and when seedlings with the latter two symptoms were outplanted, many died (Table 2). A few seedlings with medium and large galls were symptomless 24 mo after inoculation. This type of recovery has been reported earlier (12).

Table 2. Progression of disease on loblolly pine seedlings from 9 mo after inoculation with *Cronartium quercuum* f. sp. *fusiforme* to 24 mo by symptom type

Symptom type at 9 mo	Number of seedlings	Seedlings at 24 mo			
		Symptomless ^a (%)	Galled (%)	Dead (%)	Aecia (%)
1. No stem symptoms	40	100	0	0	0
2. Purple needle base	25	100	0	0	0
3. Purple stem spot	42	93	5	2	0
4. Purple stem spot with depressed area	29	83	14	3	0
5. Gall <10 mm long	40	63	37	0	3
6. Gall <10 mm long with depressed area	36	67	27	6	0
7. One-sided gall <10 mm long	32	66	32	3	0
8. Gall 10–25 mm long	40	8	78	13	35
9. Gall 10–25 mm long with depressed area	36	17	44	39	8
10. Gall >25 mm	36	3	50	47	6

^aChi-square = 190 with 9 df, significantly different at $P < 0.005$.

Sporulation by the fungus suggests the most compatible host-parasite interaction, i.e., the fungus is able to survive and propagate in this host-parasite relationship. Loblolly seedlings that support this type of pathogenic relationship would not be useful in resistance breeding programs. The relative amount of rust mycelium present in symptomatic tissue, not the relative amount of necrotic tissue, seemed more closely related to the long-term success of this obligate parasite. Most seedlings that were examined had necrotic tissue present 9 mo after inoculation, and both resistant seedlings and susceptible seedlings of all symptom types had relatively large amounts of necrotic tissue. Only medium and large galls with and without depressed areas had more than minimal amounts of rust mycelium present, and these galls were the type in which the disease continued to develop or resulted in host death within 24 mo of inoculation.

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