Early Symptomatology of Fusiform Rust on Pine Seedlings

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ABSTRACT

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The first macroscopic symptom of fusiform rust, caused by *Cronartium quercuum* f. sp. *fusiforme*, on slash pine (*Pinus elliottii* var. *elliottii*) and loblolly pine (*P. taeda*) is red pigmentation that develops in various patterns on stems and needles. Early symptoms on seedlings grown in vermiculite-filled tubes were studied on the basis of proportion of seedlings developing stem pigment and the number of lesions developing on seedling stems and how these changed with time. Seedlings from half-sib families of both slash and loblolly pines showed a rapid rate of increase in both proportion of pigmented seedlings and numbers of stem lesions after an initial lag period. Resistant families had greater rates of increase than susceptible families. Increasing levels of inoculum resulted in larger proportions of pigmented seedlings and larger numbers of stem lesions. High inoculum densities amplified the difference between resistant and susceptible families.

Additional key words: disease resistance, early symptoms.

Fusiform rust of southern pines, which is caused by *Cronartium quercuum* (Berk.) Miy. ex Shirai f. sp. *fusiforme*, is one of the most destructive diseases of forest trees in the United States. Two of the most susceptible hosts, slash pine (*Pinus elliottii* var. *elliottii* Engelm.) and loblolly pine (*Pinus taeda* L.), are also the most widely planted, most intensively managed, and most economically important species in the southeastern USA. Obtaining precise estimates of loss has been difficult (4), but the impact unquestionably amounts to millions of dollars annually (9). Although this disease has been intensively studied for years and many control methods have been proposed, losses continue to increase and the disease continues to spread (2).

Symptoms of fusiform rust include stem and branch galls, which appear relatively late in disease development, and red stem and needle pigment, which appears early. Studies concerned with variation in resistance have focused primarily on galls. Some research on pigment has been done (5,6,8), but much remains unknown about this symptom and its relation to resistance. Better understanding of early symptomatology should help elucidate the nature of resistance, and could lead to a useful method of early screening of pines for resistance. The experiments described here were designed to determine a useful method of describing pigment development on large numbers of seedlings, and to compare and contrast the development of early symptom expression, as affected by inoculum density, on resistant and susceptible pine genotypes.

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MATERIALS AND METHODS

Host material consisted of five slash pine and seven loblolly pine families that differed in resistance to fusiform rust. Rankings of these families in order of decreasing resistance, as determined in previous tests based on proportions of inoculated seedlings that developed galls, were as follows: slash pine 10-226, Jones 18, 2790-3, S-118, and Charlton 2; loblolly pine 10-5, 10-6, 11-20, 15-42, 1536-5, 4625-3, and 4666-4 (H. R. Powers, Jr., *personal communication*). Seeds were sown in flats containing sterile soil,

and transplanted after 2 wk into polypropylene tubes (17 × 100 mm) containing medium grain vermiculite. A modified Hoagland's solution containing 100 μg N/ml, 75 μg P/ml, and 100 μg K/ml (10) was applied at a rate of 25 ml per seedling twice weekly for the duration of the experiment. Basidiospores were collected from oak leaves that had been inoculated with aeciospores collected from trees in Clarke County, GA, in 1974 (isolate 2-74) using the procedure described by Matthews and Rowan (7). Spore densities were determined by a Coulter Counter, and spore suspensions were applied with a chromatography sprayer at a rate of approximately 0.25 ml per seedling. Immediately after inoculation, seedlings were placed into a mist chamber at 20 C for 24 hr. They were then kept in an air-conditioned headhouse at 20–25 C for an additional 24 hr before being transferred to a greenhouse.

To determine the effect of inoculum density (ID), groups of 80 seedlings (43 days old) each of Jones 18 and S-118 were inoculated with spore suspensions having one of the following densities: 5.0×10^4, 2.5×10^5, 0.5×10^6, 1.0×10^6, 2.0×10^6, and 4.0×10^6 spores per milliliter, and examined for symptoms beginning 5 days after inoculation. To determine the influence of host genotype, three (slash) and five (loblolly) replicates of 20 seedlings for each of four slash families and seven loblolly families were inoculated with 2×10^6 spores per milliliter when seedlings were 40 days old, and examined beginning 5 days after inoculation.

RESULTS

Red pigment developed in various patterns on stems and needles. On some seedlings it showed as distinct spots ranging in size from less than 1 to 2 mm. On others, it appeared as large diffuse areas 3–5 mm or more in diameter. When pigmented seedlings were examined, both distinct spots and large areas were scored equally as individual lesions, and seedlings showing either or both of these patterns were scored as pigmented. Data reported below refer only to seedlings with stem pigment, because preliminary studies showed that pigment located on the stem can be seen more easily and measured more rapidly than needle pigment and suggested that stem pigment is more closely correlated with resistance.

Representative curves for the proportion of seedlings developing pigment at different IDs for families S-118 and Jones 18 are shown in Fig. 1. Curves for 2.5×10^5 and 1.0×10^6 were similar to those for 0.5×10^6, and curves for 4.0×10^6 were similar to those for 2.0×10^6 in each family. The time required for 50% of the seedlings to develop pigment is shown in Table 1. Generally, seedlings of Jones 18 reached this point sooner than seedlings of S-118 over the entire range of inoculum levels except at 5.0×10^4 spores per milliliter, where the two families were about the same. Within each family, higher ID shortened the time required to reach 50% pigmentation.

The effect of increasing ID on the percentage of pigmented seedlings at 17 and 50 days after inoculation is shown in Fig. 2A. Trends were similar for both families at other measured intervals. At all inoculum levels, Jones 18 showed larger proportions of pigmented seedlings. At 50 days, seedlings in both families in all treatments, except the lowest ID, reached nearly the same proportion of pigmented seedlings.

The effect of increasing ID on the average number of spots appearing on the stems of seedlings for the same intervals is shown in Fig. 2B. Numbers of spots induced by the lowest inoculum levels remained nearly constant at all measurement times. Trends were similar for both families over the entire measurement period. At all levels, Jones 18 seedlings had larger numbers of spots than S-118. Relatively greater differences in spot numbers appeared between higher inoculum concentrations than between lower concentrations. As a result, differences between families became accentuated at higher inoculum levels with increasing time.

Each of the four slash (Fig. 3A) and seven loblolly (Table 2) families showed a sharp increase in number of pigmented seedlings after an initial lag period. For slash 10-226, the period of increase lasted until 25 days, when it reached a plateau. The period of increase for all other slash pine families was longer and peaked later. Times taken for 50% of the total seedlings to become pigmented differed among the families as follows: 10-226 = 8 days, 2790-3 = 11 days, S-118 = 19 days, and Charlton 2 = 18 days. All loblolly pines tested showed a dramatic increase between 8 and 10 days. Times taken for 50% of the loblolly pine seedlings to become pigmented were: 10-5 = 9 days, 1536-5 = 11.5, 15-42 = 11.5, 11-20 = 12, 10-6 = 12, 4625-3 = 20, and 4666-4 = 22.

The number of stem spots was counted only on the slash pine. Responses were similar to those based on proportion of pigmented seedlings (Fig. 3B). All families showed increasing spot production following an initial lag phase of 5–8 days after inoculation except Charlton 2, which began this increase at 8–10 days. The curve for 2790-3 rose abruptly to a maximum at 25 days. All other families increased more linearly and reached maximums much later than 2790-3.

The average numbers of ungalled seedlings for each family at 65 days after inoculation are shown in Table 3. Both 2790-3 and 10-226

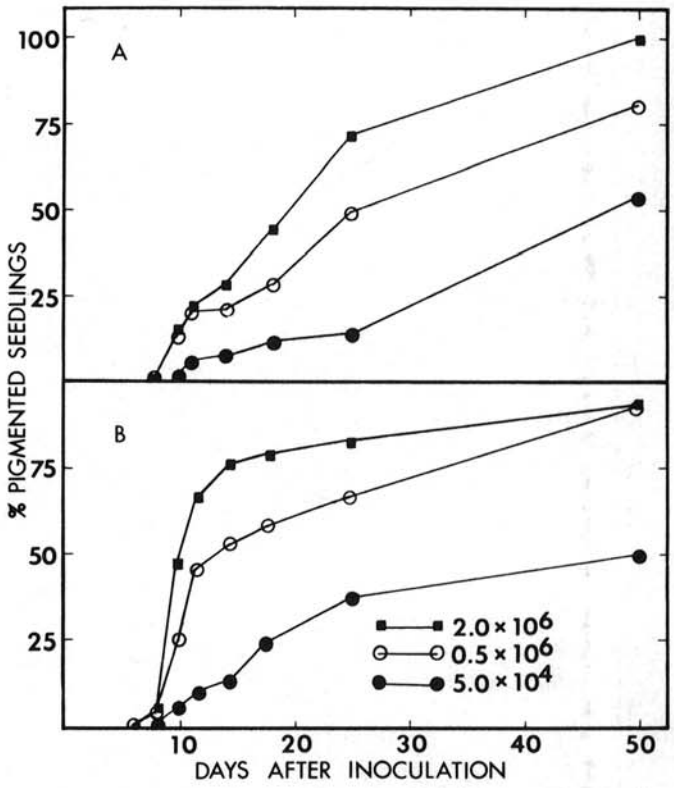

Fig. 1. Percentage of seedling exhibiting stem pigment in slash pine families **A,** S-118 and **B,** Jones 18 at various times after inoculation with 5.0×10^4, 0.5×10^6, and 2.0×10^6 basidiospores per milliliter of *Cronartium quercuum* f. sp. *fusiforme*.

TABLE 1. Days required to reach 50% pigmentation for slash pine families S-118 and Jones 18 inoculated with various concentrations of basidiospores of *Cronartium quercuum* f. sp. *fusiforme*

Family	Resistance rating	Inoculum concentrations (basidiospores/ml)					
		5.0×10^4	2.5×10^5	0.5×10^6	1.0×10^6	2.0×10^6	4.0×10^6
Jones 18	Resistant	52	20	13	13	10	10
S-118	Susceptible	48	34	27	27	22	20

were similar and had much greater proportions of ungalled seedlings than Charlton 2 and S-118. The average numbers of spots occurring at 19 days on seedlings that remained ungalled at 65 days in 2790-3 and 10-226 were significantly larger than numbers of spots on seedlings that became galled (Table 4). Averages for numbers of spots per seedling were similar in both families for seedlings that became galled but differed between families for seedlings that remained ungalled, the average being significantly higher in 2790-3 than in 10-226. Estimates were not calculated for S-118 and Charlton 2 because these families had too few ungalled seedlings.

DISCUSSION

Just as galls are the most conspicuous symptom in later stages of disease development, red pigment is the most conspicuous early symptom. The experimental system developed for this study permits critical examination of early symptoms on large numbers of individual plants. It also permits a convenient method of determining how these symptoms are influenced by inoculum density and host genotype, and how they change with time.

Inoculum density and host resistance are similar in their effects: higher levels of inoculum and higher levels of host resistance both produce greater rates of increase of pigmentation. At the lowest inoculum concentration, stem pigment develops at a much slower rate and involves long term responses. Consequently, differences in pre-gall symptoms between resistant and susceptible families are not evident. Significantly, the lowest concentration used in this test corresponds with spore densities used in current screening techniques in which differences between resistant families are dependably identified on the basis of gall formation. Apparently, no strict correlation exists between the ability to produce pigment under low inoculum loads and gall production. Greater spore concentrations result in amplification of differences in pregall symptoms between the resistant and susceptible families. In order to identify differences in levels of resistance on the basis of pigment development, very high inoculum levels are needed.

All families of both slash and loblolly pines show similar response curves in that there is an increase in pigment after an initial period of no macroscopic response. Although no macroscopic symptoms are evident, some evidence suggests that the lag phase is actually a very active time. Hare (3) found substances in slash pines that inhibited the basidiospores of fusiform rust within 3 days after inoculation. Studies of disease induced alterations of

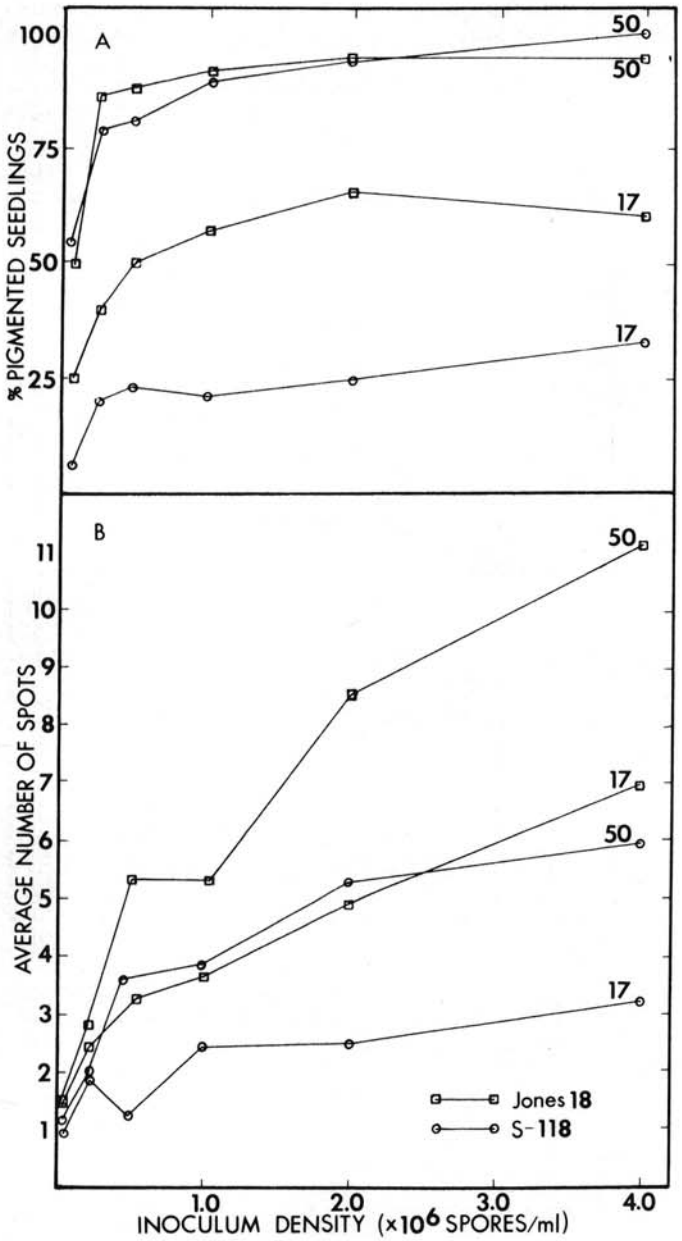

Fig. 2. A, Percentage of slash pine seedlings with stem pigment at 17 and 50 days after inoculation with basidiospores of *Cronartium quercuum* at six different inoculum densities. **B,** Average number of spots per spotted seedling at 17 and 50 days after inoculation.

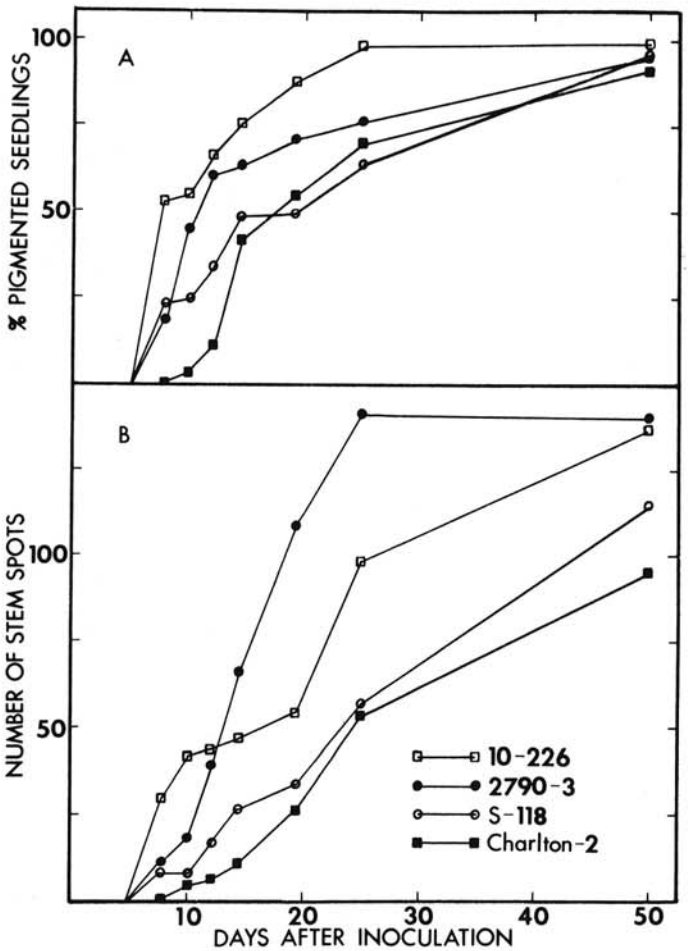

Fig. 3. A, Percentage of seedlings showing stem pigmentation in each of four families of slash pine differing in relative levels of resistance at different times after inoculation with *Cronartium quercuum* f. sp. *fusiforme*. The standard deviations were at 8, 10, 12, 14, 19, 25, and 50 days after inoculation, respectively: 10-226 = 10, 5, 5, 10, 10, 3, 3: 2790-3 = 11, 3, 3, 8, 13, 13, 6: S-118 = 10, 3, 6, 6, 10, 13, 3: and Charlton 2 = 0, 3, 10, 16, 3, 10, 6. **B,** Total number of spots on the stems of 20 seedlings in each family. Standard deviations were: 10-226 = 17, 4, 5, 9, 15, 21, 27: 2790-3 = 8, 6, 4, 22, 58, 71, 22: S-118 = 10, 12, 12, 14, 19, 24, 9: and Charlton 2 = 0, 2, 3, 6, 3, 11, 24.

TABLE 2. Percentage (± SD) of seedlings showing stem pigmentation in each of seven families of loblolly pine, differing in relative levels of resistance, at different times after inoculation with *Cronartium quercuum* f. sp. *fusiforme*

Family	Resistance rating[a]	Days after inoculation				
		8	10	12	14	20
10-5	1	6 ± 2	60 ± 4.4	81 ± 4	83 ± 3	85 ± 9
10-6	2	0	37 ± 1.84	49 ± 14	56 ± 11	77 ± 14
11-20	3	2 ± 3	32 ± 7.44	50 ± 17	60 ± 17	76 ± 15
15-42	4	3 ± 2	29 ± 1.36	56 ± 8	71 ± 4	80 ± 6
1536-5	5	0	33 ± 6.64	58 ± 12	64 ± 11	77 ± 9
4625-3	6	1 ± 2	17 ± 1.04	35 ± 5	46 ± 7	50 ± 15
4666-4	7	1 ± 2	17 ± 3.44	31 ± 8	37 ± 12	40 ± 16

[a] Based on previous studies in which relative resistances of the families were determined by the presence of galls after 9 mo.

respiration rate in slash pine seedlings (J. E. Lundquist, *unpublished*) indicated that large changes occur within 2 days. Because the host responds metabolically to infection long before the first appearance of pigment, the pigment itself may be what Daly (1) refers to as a specious effect or a by-product of the resistance reaction, and not the cause of resistance.

Previous studies have dealt with early host responses coincident with the phase marked, in this research, by an abrupt increase in pigmentation. Walkinshaw (11) identified a reaction in slash pine that produced necrotic host cells between 8 and 10 days after inoculation, but he did not mention whether these cortical responses were correlated with the development of surface pigment. Lewis (6) detected an increase in peroxidase activity 8–10 days after inoculation in loblolly pine seedlings, but he found no significant differences between the resistant and susceptible families tested. He also examined isozyme patterns of peroxidase and polyphenoloxidase at 9 days, but detected no disease induced alterations.

Nearly all seedlings in all families that are followed to the late stages of early symptom development become pigmented. Seedlings that do not have the capacity to develop pigment in the early stages develop this capacity by the later stages. Generally, resistant families reach the maximum level of response sooner than susceptible families. The greater rate of increase for resistant families suggests that resistance to fusiform rust is dependent on the rate at which a host responds to infection. On the basis of previous studies in which relative resistance of the families tested was determined by the presence of galls after 6–9 mo, the proportion of seedlings showing pigment between 10 and 14 days after inoculation for slash pine and at 12 days for loblolly pine appears to be a good early predictor of resistance.

In general, families that had large proportions of pigmented seedlings also developed large numbers of stem spots. A close relationship exists between resistance of individual seedlings within a family and number of spots that develop. Rankings based on pigmented seedlings, however, did not correspond exactly with rankings based on number of spots. For example, the maximum number of spots was reached in 25 days for 2790-3, but the maximum proportion of pigmented seedlings was not attained until at least 50 days. Conversely, 10-226 reached a maximum number of pigmented seedlings by 25 days, but the maximum number of spots did not appear until after 50 days. The relative resistance between families can be estimated better by rankings based on the percentage of pigmented seedlings than on numbers of stem spots.

Pigment production is not a specific response to rust infection alone. Several different stresses can induce the production of stem pigment, including mechanical wounding, dry ice application, applications of toxic organic solvents, UV radiation, and others (J. E. Lundquist, *unpublished*). Studies by Lundquist (*unpublished*) show that the length of time between the application of a stress to pine seedling stems and the pigment response depends on the nature and intensity of the particular stress used. For instance, UV light may induce pigment development in the epidermis of stems of slash pine seedlings in as short a time as 2 days; as opposed to the average of 8 days for the fungus induced pigment. In other words, the host has the capacity of a quick response; yet when infected, the response is delayed. The pathogen avoids inducing pigment development for a longer period of time. But unlike the response induced by the fungus, no difference in response to UV radiation was noted between resistant and susceptible pine selections under the conditions of the experiment.

TABLE 3. Average percentage of seedlings without galls at 65 days after inoculation with *Cronartium quercuum* f. sp. *fusiforme* on four families of slash pine differing in levels of relative resistance

Family	Relative level of resistance	Percent ungalled (± SD)
10-226	high	45 ± 9
2790-3	high	42 ± 3
S-118	low	8 ± 8
Charlton 2	low	2 ± 3

TABLE 4. Average number of spots (± SD) per seedling at 19 days after inoculation with *Cronartium quercuum* f. sp. *fusiforme* on slash pine seedlings that were either ungalled or galled at 65 days after inoculation

Family	Ungalled	Galled
2790-3	10.19 ± 2.22	1.77 ± 0.78
10-226	4.02 ± 1.28	1.43 ± 0.24

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