Influence of Needle Age and Inoculum Spore Density on Susceptibility of Longleaf Pine to Scirrhia acicola

A. G. Kais

Principal Plant Pathologist, Southern Forest Experiment Station, Forest Service, U. S. Department of Agriculture, Gulfport, Mississippi 39501.

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ABSTRACT

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Susceptibility of fascicled needles to infection by *Scirrhia acicola* decreased as needles elongated and matured. Almost all needles of young seedlings inoculated at age 8 wk were severely infected within 4 wk of inoculation. For more mature seedlings (12 wk of age or older when inoculated), moderate infection occurred within 8-12 wk. Young plants with newly expanding needles were so susceptible that even light inoculations produced rapid, massive tissue mortality. In contrast, needles of seedlings inoculated at 14 wk of age were moderately resistant to both light and heavy inoculations. For mature seedlings, the needle tissue proximal to the stem was more susceptible to infection than either medial or distal tissue of the same needles.

Additional key words: brown-spot needle blight, *Lecanosticta acicola*, spore density, disease resistance, *Pinus palustris*.

Brown-spot needle blight, caused by *Scirrhia acicola* (Dearn.) Siggers, limits longleaf pine (*Pinus palustris* Mill.) regeneration in the South by reducing plant vigor and delaying the onset of rapid height growth (12). Until recently, research on the disease was hampered because reliable testing was possible only in the field (3). Now, a newly developed inoculation technique (8) allows testing to be completed in 8-10 mo by inoculating seedlings under optimum conditions of light, humidity, and temperature. This testing process could be accelerated by inoculating plants at the age when they are most susceptible to infection. The experiments described here employ the new inoculation technique in a greenhouse to study the relationship of needle age and inoculum density to disease development. Susceptibility of specific tissue zones of fascicled needles were examined.

MATERIALS AND METHODS

Preparation of seedlings and inocula.—Three experiments were conducted during winter and spring 1975. Longleaf pine seeds randomly selected from a bulk collection (Alabama source) were germinated in 5-cm-diameter peat pots and then transplanted into 10-cm-diameter plastic pots. Potting medium was a 4:1 (v/v) ratio of sandy loam to vermiculite. All plants were grown on greenhouse benches under a 16-hr photoperiod maintained by natural and supplemental fluorescent lighting. Individual seedlings were measured for fascicled needle lengths and then covered with polyethylene bags 3 days before inoculation. These measurements were made so that the inoculated needle tissue could be differentiated from the tissue that developed after inoculation. Fascicled needle growth results from cell division in the intercalary meristem at the base of the needles (9). In these tests, only inoculated portions of needles were evaluated for infection.

Conidial isolates of *Lecanosticta acicola* (Thum.) Syd., the imperfect state of *Scirrhia acicola*, were obtained from longleaf pine seedlings on the Harrison Experimental Forest, Saucier, Mississippi. Subsequent single-spore isolates were maintained on malt agar slants at 25 C. Spore suspensions were prepared by rinsing 3-wk-old cultures with sterile distilled water and then screening the resulting suspensions through four thicknesses of cheesecloth. Approximately 3 ml of suspension were atomized on each seedling to the point of run-off; the plants were then rebagged for 10 days. The number of spores deposited per unit of needle surface was uniform throughout the study. All plants were examined for infection 4, 8, and 12 weeks after inoculation. At such times, a visual estimate was made of the total surface area of lesions and necrotic tissue present on inoculated portions of the fascicled needles of each seedling. Infection then was expressed as a percentage of the total needle tissue originally inoculated.

Experiments.—In the first experiment, 8-, 10-, 12-, 13-, and 15-wk-old seedlings were evaluated for susceptibility 4, 8, and 12 wk after their common inoculation date. At that time, fascicled needle lengths of the inoculated seedlings ranged from 2.5 - 4.0 cm for the 8-wk-old seedlings to 21.0 - 24.0 cm for the 15-wk-old seedlings. Five plants of each age group were inoculated with a mixture of five isolates; spore density was 5×10^5 spores/ml. Thus, the design consisted of five randomized blocks analyzed as split plots over time.

The second experiment tested the relationship of symptom development on plants of different ages to inoculum dosage; a single isolate was applied at either 5×10^4 or 5×10^5 spores/ml. Forty plants were divided into four completely randomized blocks, each consisting of two plants at 8, 10, 12, 14, and 16 wk of age; one plant of each age received the high-density inoculum, and the

other the low. All plants were inoculated at the same time. Infection levels were recorded 12 wk after inoculation.

The third experiment compared susceptibility to infection of different tissue zones on fascicled needles of mature (14-wk-old) seedlings. In this experiment, fascicled needles were considered to have three segments of equal length—one proximal, one medial, and one distal to the stem. Twelve plants with needle lengths ranging from 19.2 to 27.0 cm were divided into four completely randomized blocks of three seedlings each. Each seedling possessed approximately 10-15 fascicled needles. On one seedling of each block, only the apical segments of the needles were inoculated; on another, only the medial segments were inoculated; and on the third, only the distal segments of the needles were inoculated. In each case, inoculum was excluded from the remaining two segments of the needles by covering them with paper towels. The inoculum was a mixture of spores from five isolates and spore density was 5×10^5 spores/ml.

RESULTS

Young, newly expanding, fascicled needles of longleaf pine (2.5 - 6.0 cm) were extremely susceptible to brown-spot needle blight; whereas the older, expanded needles (21.0 - 24.0 cm) were relatively resistant (Table 1). Maximum infection appeared 4 wk after inoculation on seedlings inoculated at age 8 or 10 wk, whereas maximum infection appeared between 8 and 12 wk after inoculation on seedlings inoculated at 12 wk of age or older. Eight-wk-old seedlings with young needles were most susceptible to infection; more than 95% of their needles were severely infected. Regardless of seedling age at inoculation, initial symptoms appeared on all plants within 2 wk of inoculation. The relative amount of infection on inoculated portions of fascicled needles appeared to decrease as needles elongated and matured.

Except for the youngest and the oldest seedlings, there was significantly more infection on plants inoculated with inoculum suspension having a heavy spore density (5×10^5 spores/ml) than on those that received inoculum with a light spore density (5×10^4 spores/ml) (Table 2). Young plants with expanding needles were so susceptible that inoculation resulted in rapid infection and massive tissue mortality. In contrast, mature fascicled needles of older plants were moderately resistant, even to heavy inoculation. For both spore densities, the newly expanding fascicled needles of the youngest plants were most susceptible, whereas the mature expanded fascicled needles of the oldest plants were the most resistant.

Results obtained by inoculating three segments of mature fascicled needles also showed the youngest needle tissue to be most susceptible to infection (Table 3). Although initial symptoms appeared on all inoculated tissue within 2 wk, the youngest tissue of the mature fascicled needles—that proximal to the stem—had much higher percentages of infected needle tissue than the medial or distal portions of the same needles.

TABLE 1. Susceptibility of longleaf pine needles of various ages to *Scirrhia acicola*

Plant age (wk) at inoculation	Infection after different periods of incubation (%)[y]		
	4 wk	8 wk	12 wk
8(2.5 - 4.0)[z]	96 a	97 a	97 a
10(5.5 - 6.0)	76 b	74 b	75 b
12(10.5 - 12.0)	31 e	47 d	53 c
13(15.5 - 18.0)	17 f	32 e	34 e
15(21.0 - 24.0)	7 g	17 f	22 f

[y]Mean visual estimate of infected tissue as a percentage of needle tissue inoculated. Values followed by the same letter do not differ significantly ($P = 0.05$).

[z]Numbers in parentheses indicate needle length (cm) at the time of inoculation.

DISCUSSION

These experiments indicated that susceptibility of longleaf pine to brown-spot needle blight was associated with age of fascicled needle tissue. Young expanding needles were susceptible, whereas mature needles were relatively resistant. In all cases, the primary and cotyledonous needles of inoculated plants were moderately resistant to infection. Although in another study infection occurred only on immature needles 7.5 - 15.0 cm long (14), the present results indicate that infection occurs as soon as needles emerge and continues to the time when needle elongation ceases (30 - 40 cm). Consequently, effective fungicidal control of the disease could probably be achieved by spraying seedlings as new needle flushes occur. Hence, disease control should require fewer than the four to seven fungicide applications now being recommended (7).

TABLE 2. Percentage of infection on longleaf pine seedlings of various ages after inoculation with two spore densities of *Scirrhia acicola*

Plant age at inoculation (wk)	Infection (%) following inoculation with a spore density (no./ml) of:	
	5×10^4 (light)	5×10^5 (heavy)
8(5.5 - 7.0)[y]	71 a[z]	71 a
10(9.5 - 13.0)	18 cd	64 a
12(14.5 - 17.0)	10 de	34 b
14(18.5 - 21.7)	5 de	29 bc
16(26.0 - 33.0)	4 e	12 de

[y]Numbers in parentheses indicate needle length (cm) at the time of inoculation.

[z]Mean visual estimate of infected tissue as a percentage of needle tissue inoculated. Values followed by the same letter do not differ significantly ($P = 0.05$).

TABLE 3. Susceptibility of various needle zones of 14-wk-old longleaf pine seedlings inoculated with *Scirrhia acicola*

Needle zone	Infection (%) at different periods of incubation[z]		
	4 wk	8 wk	12 wk
Proximal	28 b	30 b	44 a
Medial	5 e	13 cd	16 c
Distal	2 e	10 d	12 cd

[z]Mean visual estimate of infected tissue as a percent of needle tissue inoculated. Values followed by the same letter do not differ significantly ($P = 0.05$).

Susceptibility to infection is strongly influenced by the interaction of needle tissue age and spore density. For best results in greenhouse tests, spore density for 10- to 12-wk-old seedlings should be between 1×10^5 and 3×10^5 spores/ml. This inoculation of moderately susceptible fascicled needles with a relatively low spore density should prevent severe infections which might mask treatment responses or minor infections that make it difficult to differentiate treatments. Therefore, total duration of inoculation experiments can be reduced from the 8-10 mo previously required (8) to approximately 6 mo. This technique is useful for screening longleaf pines for disease resistance and for detecting pathogenic variability.

The brown-spot symptoms produced on seedlings in the present study differed from those usually observed in the field. After greenhouse inoculations, plants were blighted only on the inoculated portions of fascicled needles, and no infections appeared on needle tissue that developed after the inoculation date. Also, under greenhouse conditions, the proximal or basal zones of fascicled needles were susceptible to infection. In contrast, field-infected seedlings commonly have three distinct zones: (i) a dead apical portion, (ii) a mottled middle portion, and (iii) a green basal zone (15). Apparently, young susceptible needle tissue is inoculated repeatedly, which produces massive tissue mortality. Subsequent infection of developing tissue results in the mottled appearance of the middle zone. In contrast to greenhouse tests, the proximal portions of needles in the field appear resistant to infection even when inocula are plentiful (4). However, as tips of longleaf pine needles are killed in the field, they bend outward and downward to resemble a tussock of dead grass drooping around the stem of the plant. It is possible that the drooping needles trap and transport spores contained in water droplets to the needle ends. Drooping needles may also form a barrier to protect basal needle tissue from spore inoculation by water splash. In either case, the basal tissue of seedlings in the field would experience lighter infection and would remain comparatively green or disease-free.

For other pine species, *S. acicola* has been reported to attack either young or old fascicled needles. In the field, it infects young fascicled needles of Scots (*Pinus sylvestris* L.) (13) and white pine (*P. strobus* L.) (2) and the older, mature needles of loblolly (*P. taeda* L.) (1, 11) and red pine (*P. resinosa* L.) (10). However, recent greenhouse tests indicated that young fascicled needles of Scots, longleaf, sand (*P. clausa* Chapm. Vasey), jack pine (*P. banksiana* Lamb.), loblolly, and red pine were susceptible to the pathogen (5, 6, 8). For loblolly and red pine, these differing results may be due to: (i) extremely virulent strains of the fungus, (ii) the latent period required by the host for expression of disease symptoms, and (iii) increased susceptibility of test plants by exposure to greenhouse conditions. Consequently, spraying of all fascicled needles as they emerge might give satisfactory control of the disease on any of the pine hosts.

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