Seedling Inoculations with Fomes annosus Show Variation in Virulence and in Host Susceptibility

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ABSTRACT

Variation in the virulence of 23 isolates of *Fomes annosus* to nursery-run *Pinus taeda* was demonstrated by inoculating seedlings with infested beech branch segments. Most of the isolates caused 85-100% mortality of *P. taeda* seedlings; however, four of the isolates caused only 50-60% mortality. The isolates reacted similarly in several trials on *P. taeda*. Variation in host susceptibility was shown on conifer and hardwood species with 10 isolates of the fungus. On coniferous hosts the average mortality was: *P. elliottii* var. *elliottii*, 82%; *P. echinata*, 80%; *P. taeda*, 74%; *P. virginiana*, 72%; *Abies fraseri*, 67%; *P. palustris*, 65%; *P. strobus*, 64%; and *Juniperus virginiana*, 28%. Conifers other than *J. virginiana* appeared to be highly susceptible to *F. annosus*. In a second trial with *P. elliottii* var. *elliottii*, *P. palustris*, and *P. taeda* seedlings, the average mortality was 72, 64, and 63%, respectively. When seedling mortality due to *F. annosus* is a factor in regeneration of pine plantations, these results indicate there would be little advantage in favoring one pine species over another. Essentially no mortality of the hardwood species, *Liriodendron tulipifera* or *Plantanus occidentalis*, occurred due to *F. annosus*. Phytopathology 60:1743-1746.

Variation among isolates of *Fomes annosus* (Fr.) Karst. has been shown in a number of studies of growth rate, pH opt, final pH of substrate, pigment production, capacity to utilize various carbon and nitrogen sources, ability to decay wood, toxin production, and temp of inactivation (1, 2, 3, 5, 7, 14, 16, 19). None of these studies linked variation in cultural characters to pathogenicity.

Although there are over 140 reported hosts of *F. annosus* (4, 20, 21), few have been confirmed through Koch's postulates. Some attempts have been made to rate the relative susceptibility of the host species. From field observations, Low & Gladman (13) rated *Abies alba* as highly resistant, *A. grandis* as less resistant, and all other conifers grown in the British Isles as susceptible, with *Tsuga heterophylla* being exceptionally susceptible. Also from England, Wallis (22) reported that *F. annosus* damage was relatively low in stands of *Pinus nigra* var. *calabrica* as compared with stands of *P. sylvestris*. In a survey of the southern USA, Powers & Verrall (17) found that losses to *F. annosus* were similar in stands of slash pine (*P. elliottii* var. *elliottii*) and loblolly pine (*P. taeda*). Froelich & Dell (8) reported greater losses in slash pine than in loblolly.

Field studies to compare susceptibility are generally limited because of lack of uniform infestations. Kuhlman & Ross (12) found no differences in susceptibility of seedlings of seven species planted on infested sites because of the low incidence of infection. In inoculation studies, however, roots of 13-year-old longleaf pine, *P. palustris*, were less susceptible than those of slash or loblolly pines (9). Greenhouse studies provide uniform conditions for testing large numbers of seedlings and fungus isolates.

The purposes of this study were to determine the variation in virulence of isolates of *F. annosus* from various hosts and geographic locations on nursery-grown loblolly pine seedlings and to measure the susceptibility of 10 conifer and hardwood species to infection by 10 isolates of *F. annosus*. A portion of this study was previously described (10).

MATERIALS AND METHODS.—Seedlings were 1 year old, except the Fraser fir seedlings, which were 4 years old and had been grown in North Carolina Division of Forestry nurseries. Seedlings were wounded at the root collar and inoculated with a 20- to 25-g infested beech branch segment (11). The geographic source and host plant of all isolates are given in Table 1.

The repotted seedlings were randomized on greenhouse benches. Isolations for *F. annosus* were made from roots and stems of dead seedlings each day, and from living seedlings at the conclusion of the experiments (11).

Variation in virulence.—Each of 23 isolates of the fungus were used to inoculate 20 loblolly pine seedlings. The isolates were chosen to encompass broad geographic and host ranges. Twelve of the isolates were tested 2 additional times. Controls were inoculated with noninfested beech segments.

A virulence index was calculated from the daily mortality data. The index for each seedling equalled 60 days minus the days until death of the seedling plus an additional 4 points if *F. annosus* was recovered. The index for each treatment was the sum of the indices for all seedlings divided by the total number of seedlings per treatment. The index data were subjected to an analysis of variance, and multiple comparisons among means were made with Duncan's multiple range test.

Variation in susceptibility.—The 10 hosts were: shortleaf (*P. echinata* Mill.); loblolly (*P. taeda* L.); longleaf (*P. palustris* Mill.); slash (*P. elliottii* Engelm. var. *elliottii*); white (*P. strobus* L.) and Virginia pine

TABLE 1. Variation among isolates of *Fomes annosus* as indicated by a virulence index and mortality of loblolly pine seedlings

Isolate source	Isolate host	Isolate no.	Virulence index[a,b]	% Mortality[a,c]
South Carolina	*Pinus elliottii* Engelm. var. *elliotti*	195	47 a	100 (85)
South Carolina	*P. palustris* Mill.	293	47 a	100
South Carolina	*P. taeda* L.	292	46 ab	100 (78)
Georgia	*P. elliottii* var. *elliottii*	133	45 ab	100
South Carolina	*P. palustris*	125	45 ab	95
South Carolina	*P. taeda*	167	45 ab	95
India	*P. griffithii* McClel.	118	45 ab	95 (85)
North Carolina	*P. taeda*	128	44 ab	95 (85)
Pennsylvania	*P. rigida* Mill.	120	43 ab	95
North Carolina	*Chamaecyperis thyoides* (L.) B.S.P.	113	43 ab	95
North Carolina	*P. taeda*	291	43 ab	95
California	*P. muricata* D. Don	121	42 ab	95
North Carolina	*P. strobus* L.	137	42 ab	90
North Carolina	*Juniperus virginiana* L.	126	41 ab	90 (80)
California	*P. jeffreyi* Grev. & Belf.	114	39 abc	90
South Carolina	*P. palustris*	290	39 abc	85
England	*P. nigra* Arn.	169	38 abc	85 (48)
England	*P. sylvestris* L.	168	32 bcd	75 (73)
North Carolina	*P. echinata* Mill.	289	32 bcd	75 (85)
Washington	*Pseudotsuga menziesii* (Mirb.) Franco	115	26 cde	60 (73)
Australia	*Araucaria cunninghamii* Ait.	216	25 cde	60 (70)
Norway	*Picea abies* (L.) Karst.	117	23 de	60 (63)
New Zealand	*Agathis australis* Salisb.	145	17 e	50 (55)
New Zealand	*A. australis*	63		(40)
		Control		5 (0)

[a] Each treatment consisted of 20 seedlings.
[b] Virulence index = (60 days until death + 4 if *F. annosus* was recovered) for each 20 seedlings/20. Values followed by the same letter do not differ significantly at the .01 level.
[c] Data in brackets are the average mortality of two additional experiments of each of 20 seedlings.

(*P. virginiana* Mill.); eastern redcedar (*Juniperus virginiana* L.); Fraser fir (*Abies fraseri* [Pursh] Poir.); yellow-poplar (*Liriodendron tulipifera* L.); and American sycamore (*Platanus occidentalis* L.). Sixteen seedlings of each species were inoculated with each of 10 isolates of the fungus, so that 160 seedlings of each species were inoculated. Yellow-poplar and sycamore have not been reported as hosts and therefore were included as controls.

Loblolly, slash, and longleaf pine seedlings were inoculated in a second test with nine isolates of the fungus. Controls were similarly treated, but with noninfested beech segments.

RESULTS.—*Variation in virulence.*—All isolates of *F. annosus* caused some mortality of loblolly pine seedlings (Table 1). Both the percentage mortality and the virulence index demonstrated that most of the isolates of *F. annosus* tested were highly virulent to loblolly pine seedlings. Isolate 145 was the least virulent. The average mortality data in brackets for the two additional tests indicate that all the isolates except 169 performed consistently.

Variation in susceptibility.—Yellow-poplar and sycamore seedlings reacted as nonhosts (controls) for the isolates of *F. annosus* (Table 2). Water-soaked areas appeared on the leaves of some seedlings of both species, but only a few individuals died. Mortality of redcedar averaged only 28%. An analysis of variance of the mortality data for the conifers showed that variations due to hosts, isolate, and interaction of host

TABLE 2. Percentage mortality[a] for 10 host species inoculated with each of 10 isolates of *Fomes annosus*

Host	Isolate 63	117	120	145	168	114	289	195	126	118	Avg. for host
Slash Pine	50	69	94	69	75	88	88	94	100	94	82
Shortleaf Pine	75	56	56	75	88	69	88	94	94	100	80
Loblolly Pine	32	63	88	44	69	69	88	100	100	88	74
Virginia Pine	32	32	81	56	81	88	88	94	81	88	72
Fraser Fir	75	44	13	69	88	75	63	88	69	88	67
Longleaf Pine	44	50	69	63	50	63	88	56	69	94	65
White Pine	50	56	19	56	69	69	81	88	81	75	64
Redcedar	38	32	0	0	38	63	19	19	50	25	28
Yellow-Poplar	0	0	0	0	0	0	0	6	6	6	2
Sycamore	0	0	0	6	0	6	0	0	0	6	2
Avg. for isolate	40	40	42	44	56	59	60	64	65	66	54

[a] Each percentage is based on 16 seedlings.

and isolate were significant at the 1% level. An individual degree of freedom comparison showed that slash and shortleaf pine seedlings were more susceptible than were longleaf and white pine. When loblolly, slash, and longleaf seedlings were inoculated with nine isolates in a second test, however, there was no significant difference in mortality among the three species. Mortality due to *F. annosus* averaged 72% for slash, 64% for longleaf, and 63% for loblolly.

Recovery of *F. annosus* from the seedlings varied considerably among the 10 isolates. Percentage recovery from the seedlings for the various isolates was as follows: 145, 8; 63, 18; 120, 20; 117, 21; 114, 35; 289, 37; 168, 41; 118, 46; 126, 46; and 195, 48.

DISCUSSION.—*Variation in virulence.*—The virulence of 23 isolates of *F. annosus* varied only slightly when compared under greenhouse conditions on nursery-run loblolly pine seedlings. The isolates came from 16 different host species and 10 different states or countries. Isolates 289, 291, and 293 were chosen because they were present in stumps, but no damage was noted in adjacent trees. This heterogeneous collection of isolates performed similarly in their pathogenicity to loblolly pine seedlings. Isolates 145 and 216 from *Agathis* (New Zealand) and *Araucaria* (Australia) and 115 and 117 from *Pseudotsuga* (Washington, USA) and *Picea* (Norway) were less virulent than other isolates tested. *Fomes annosus* is not yet a problem in pine stands in Australia and New Zealand, although it has been reported on *Araucaria* and *Agathis* (23). The present study suggests that isolates from those countries are not as virulent on pine as are other isolates of the fungus. *Fomes annosus* usually causes heart rot in *Pseudotsuga* and *Picea* rather than mortality, as it does in pines. The two isolates from *Pseudotsuga* and *Picea* appeared somewhat less virulent on pine than the isolates from pine.

Recovery of *F. annosus* from the seedlings was generally easily accomplished; however, the less virulent isolates were often replaced by *Trichoderma* spp., and were less readily recovered from both the dead seedlings and the inoculum blocks. These isolates usually killed a few seedlings rapidly (8-18 days) but caused no further mortality. Failure to compete with the soil-borne fungi led to replacement of *F. annosus* in inoculum blocks and, consequently, low mortality of seedlings.

Spores of *F. annosus* are air-borne and have been collected up to 200 miles at sea (18). Because of this widespread dispersal, there is little chance for geographic or host specific segregation by the fungus. The small variation in virulence among the isolates indicates that variation in the pathogen would be unimportant in screening for resistance in *Pinus* spp.

Variation in susceptibility.—One of the major uses of the seedling inoculation method is screening for variation in susceptibility. Two nonhost species survived the inoculation, indicating that the method is accurate to that degree. The relative field susceptibility of the other eight species was unknown, although mortality of all of them occurs in the field.

Redcedar appears to be highly susceptible to infection by *F. annosus* in the Piedmont region of the Carolinas (6, 15). Dwyer (6) reported that 0-39% of redcedar in 15 stands in South Carolina had conks. In many stands redcedar are dead and have *F. annosus* conks but the pine overstory is not affected. Nevertheless, in this study redcedar was the least susceptible coniferous species. The soil may have influenced the results since the potting soil was very sandy whereas Piedmont soils are generally heavy clays.

The small variation in susceptibility among the six pine species indicates that little reduction in seedling losses to *F. annosus* could be achieved by favoring the planting of one species over another. Although the statistical analysis indicated some variation in the initial experiment, it is doubtful that the differences would be useful. The second experiment with loblolly, slash, and longleaf pines showed no significant difference in susceptibility among the three species. Hodges (9) reported infection of 11, 86, and 70% of longleaf, loblolly, and slash pine roots, respectively, of 13-year-old trees following inoculation. The low rate of infection he reported for longleaf may reflect a physiological difference between roots of seedlings and older trees, especially since longleaf seedling roots have a large, succulent cortex. Because longleaf seedlings have a large cortex, results obtained by this inoculation method may not be indicative of the resistance of large trees of this species. Kuhlman & Ross (12) have reported mortality of longleaf seedlings planted on infested sites.

Previously, I (11) noted that some loblolly pine seedlings showed symptoms similar to those described as a reaction to fomannosin, a toxin produced in vitro by *F. annosus* (1). In this study, many of the hardwood seedlings had water-soaked areas on their leaves. Some loblolly and longleaf seedlings had water-soaked fascicles, and these pine seedlings died sooner than the other seedlings. Although seedlings inoculated with several isolates exhibited this symptom, in no case did all the seedlings of one species show this symptom in response to a single isolate. This intraspecific variation in reaction suggests that various degrees of susceptibility to the fungus may exist within these species.

Establishing high infection rates under uniform conditions in the field is difficult and impractical with *F. annosus*, and therefore greenhouse screening appears to be the most satisfactory approach for evaluating variation in susceptibility. The consistent reaction of the isolates in three trials and the generally consistent reaction of the host species affirm the general reliability of the technique. Because a program for genetic improvement of planting stock in the Southeast is well developed, progeny for testing intraspecific variation are available and are being used.

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