Fertilizer-Induced Changes in Susceptibility to Fusiform Rust Vary Among Families of Slash and Loblolly Pine

S. J. Rowan

Principal Research Plant Pathologist, U.S. Department of Agriculture, Southeastern Forest Experiment Station, Forestry Sciences Laboratory, Carlton Street, Athens, GA 30602.

Accepted for publication 23 March 1977.

ABSTRACT

ROWAN, S. J. 1977. Fertilizer-induced changes in susceptibility to fusiform rust vary among families of slash and loblolly pine. Phytopathology 67:1280-1284.

Effects of fertilization on susceptibility (percent of seedlings infected) to fusiform rust (caused by *Cronartium fusiforme*) were observed in 11 artificially inoculated families of slash pine, and seven families and one geographic race of loblolly pine. Fertilization (N + P + K + Fe) increased the susceptibility of all families, but significant family × fertilization interactions were found in both loblolly and slash pine. This finding implies that seedling susceptibility tests in tree improvement programs should include seedlings both fertilized and not fertilized prior to inoculation. The length of the shoot (the assumed infection court), when seedlings were inoculated 8 wk after emergence, was affected significantly by fertilization, family, and their interaction. Shoot length, however, was not correlated with the percentage of seedlings that became infected. Thus, susceptibility in young slash and loblolly pine seedlings is not strongly controlled by the size of the infection court.

Additional key words: tree disease, tree improvement.

Fusiform rust (caused by *Cronartium fusiforme* Hedgc. & Hunt ex Cumm.) is a serious threat to the feasible management of slash (*Pinus elliottii* Engelm. var. *elliottii*) and loblolly (*P. taeda* L.) pines. Phenotypic selection has demonstrated resistance in both pine species (1, 4, 5, 9). Fertilization with nitrogen and phosphorus causes an increase in the susceptibility (i.e., increased percentage of galled seedlings) of pine seedlings to fusiform rust in field and greenhouse culture (2, 8). Because certain families are more efficient than others in their utilization of nutrients (5), fertilization may increase the susceptibility of some families more than others. Kinloch and Stonecypher (4) found only a small genotype × site interaction among 103 loblolly families. Their data, however, are from field experiments in which infection percentages were the result of multiple natural inoculations, and site fertility was not an imposed treatment. Although results of fusiform rust susceptibility tests conducted in field and greenhouse conditions appear correlated (11), a lack of uniform fertility in greenhouse tests can cause marked differences between duplicate inoculations (8) or result in poor correlation of field and greenhouse test data.

The primary objective of the present study was to determine if significant fertility and family interactions exist in greenhouse tests, and if seedlings should be grown in both uniformly fertile and infertile conditions for rust screening tests.

MATERIALS AND METHODS

Seeds from three control-pollinated and eight wind-pollinated slash pine parent trees (seeds from a single parent tree constitute a family) were soaked 24 hr in water and planted in flats of builder's sand (99.6% sand, 0.4% silt, trace clay). Seeds from one control-pollinated and six wind-pollinated parents, and from one geographic race of loblolly pine were stratified for 30 days and planted in flats of builder's sand. The geographic race of loblolly pine is a collection of trees native to Livingston Parish, Louisiana, whose progeny are inherently resistant to fusiform rust. Seedlings of this race will hereafter be referred to as a family. For each family, 20 seedlings that emerged during a 4-day period were transplanted into each of 10 plastic flats (33×13×11 cm). The flats contained a mixture of sandy loam, sand, and pine bark (2:1:1, v/v). The mixture contained 60.8% sand, 11.6% silt, 27.6% clay, less than 4.5 μg/g of total N, 6.5 μg/g P, 40 μg/g K, 87.7 μg/g Ca, 24.5 μg/g Mg, 67 μg/g Mn, 0.15 μg/g B, 4 μg/g Zn, and 1.8% organic matter. The pH was 5.2.

For 12 wk, beginning 2 wk after transplanting, five replicates of each family were each fertilized weekly with 400 ml of a nutrient solution. The other five flats per family were not fertilized. The nutrient solution, made the day of application, contained 552 μg/ml N, 199 μg/ml P, 338 μg/ml K, and 5.5 μg/ml Fe at pH 5.5. Sources of mineral nutrients were urea, ammonium phosphate, potassium phosphate, potassium nitrate, and chelated sodium ferric diethylenetriamine pentaacetate. Monthly applications were made during the 6 mo following the above 12-wk period, and two bimonthly applications were made during the last 5 mo of the study.

Aeciospores (source 2-74) collected from galls on loblolly pines in Clarke County, Georgia, in 1974 were used to inoculate northern red oak (*Quercus rubra* L.) seedlings. Basidiospores were collected from the oak leaves, and suspensions containing 75,000 spores per ml were used to inoculate 8-wk-old pine seedlings (6, 7, 8).

Basidiospore germination was 85% on 1.5% water agar at the time of inoculation.

At the time of inoculation the hypocotyl length, number of cotyledons, and height from soil line to needle tip were recorded for each seedling. Height to the base and top of each fusiform rust gall and height to the terminal bud were recorded for each seedling 3, 6, 9, and 12 mo after inoculation. Seedlings then were cut at ground line, and the fresh weights of tops were recorded.

The vertical growth of each stem gall was determined by comparing the heights of each gall's base and top when first observed to their heights 12 mo after inoculation. Shoot length (all was succulent new growth) at time of inoculation was taken as seedling height less hypocotyl length. The effects of the fertilization and family variables on seedling growth and susceptibility to fusiform rust were tested in a randomized complete block design with five replications (2×8×5 for loblolly and 2×11×5 for slash pines). Statistical evaluations of data included analysis of variance, rank correlation, discriminant function, and correlation analysis (3, 10). Means were ranked by use of Duncan's new multiple range test. The relationship between shoot length and susceptibility to fusiform rust was assessed by correlation analysis, analysis of variance, and discriminant function analysis to determine if tall seedlings in each flat were more susceptible than short ones (3). These latter two analyses were made because of the possibility that familial resistance and growth rate may interact and mask possible correlations between shoot length and susceptibility to the disease.

RESULTS

Susceptibility.—Fertilization significantly increased the susceptibility of seedlings of all families of loblolly and slash pine to *C. fusiforme* (Tables 1, 2). Significant family × fertilization interactions were found in both pine species (Fig. 1). Three types of interactions were noted: (i) one family was as susceptible as another at low fertility, but significantly more susceptible at high fertility (Fig. 1-G, H, I, K, L, M, N, and P); (ii) one family was significantly more susceptible than another at low fertility, but of equal susceptibility at high fertility (Fig. 1-B, C, D, E, F, J, and O); and (iii) one family was more susceptible than another at low fertility, but less susceptible at high fertility (Fig. 1-A).

TABLE 1. Effect of fertilization on, and relationship of shoot length at time of inoculation to, incidence of fusiform rust in artificially inoculated families of loblolly pine

Family	Seedlings infected		Shoot length at inoculation	
	Nonfertilized (%)	Fertilized[a] (%)	Nonfertilized (mm)	Fertilized (mm)
LP[b]	12.1 A[c]	64.6 D	26.3 A	64.8 B
L-40R	20.0 B	88.0 G	32.2 A	84.5 F
11-23	22.0 B	78.0 E	32.5 A	84.3 F
1531-5	25.0 BC	78.8 EF	31.0 A	76.1 DE
11-20	25.3 BC	57.0 D	28.8 A	80.8 EF
7-56	26.0 BC	77.0 E	30.1 A	68.6 BC
2516	27.4 BC	89.0 G	31.0 A	73.3 CD
L-36X	30.4 C	86.0 FG	32.3 A	79.3 DEF

[a]Fertilization applied (N+P+K+Fe) weekly during first 12 wk, monthly during next 6 mo, and bimonthly during final study period.
[b]The abbreviation LP refers to the Livingston Parish geographic race of loblolly pine.
[c]Means followed by a common letter in each pair of columns do not differ ($P = 0.05$) according to Duncan's multiple range test.

TABLE 2. Effect of fertilization on, and relationship of shoot length at time of inoculation to, incidence of fusiform rust in artificially inoculated families of slash pine

Family	Seedlings infected		Shoot length at inoculation	
	Nonfertilized (%)	Fertilized[a] (%)	Nonfertilized (mm)	Fertilized (mm)
Jones-18	39.0 A[b]	68.0 F	45.9 ABC	71.2 GH
Jones-2	43.0 AB	86.0 GH	48.9 ABC	68.9 FGH
S-37R	50.0 ABC	84.9 GH	46.6 ABC	62.4 EF
Jones 11-2	52.0 ABC	80.9 FG	44.7 AB	56.7 DE
1794	53.0 BC	92.0 GH	45.8 ABC	61.9 EF
1787	55.0 BC	88.9 GH	48.2 ABC	62.7 EF
G-188	56.0 BCD	94.9 H	52.7 CD	74.1 H
Jones 11-1	58.0 CDE	87.8 GH	47.4 ABC	63.5 EF
3010-4	61.0 CDE	85.0 GH	43.7 A	63.9 EFG
Dooly-19	67.0 DE	79.8 FG	52.1 BCD	69.1 FGH
1744	71.0 E	95.0 H	58.8 DE	76.2 H

[a]Fertilization applied (N+P+K+Fe) weekly during first 12 wk, monthly during next 6 mo, and bimonthly during final study period.
[b]Means followed by a common letter in each pair of columns do not differ ($P = 0.05$) according to Duncan's multiple range test.

To assess the reliability of calculating the percentage of seedlings infected earlier than 12 mo after inoculation, infection percentages 3, 6, or 9 mo after inoculation were compared with those at 12 mo (Table 3). Readings were judged reliable when all significant family × fertilization interactions were identified. These interactions became evident when about 80% of the infected seedlings were identifiable. The family × fertilization interaction was not significant at 3 mo, barely significant at 6 mo, and very significant 12 mo after inoculation. Three mo after inoculation, estimates were unacceptable. The greatest error was for nonfertilized seedlings of loblolly family 11-23 (32%), and the most reliable reading was for fertilized seedlings of slash family Jones 11-1 (98%). Six mo after inoculation the reliability of readings was fairly precise for all except nonfertilized loblolly; 9 mo after inoculation all readings were acceptable, but reliability was increased by taking readings 12 mo after inoculation.

Gall length.—The length of galls 12 mo after inoculation was significantly (P = 0.01) affected by fertilization, family, and their interaction (Table 4). Galls were significantly longer on fertilized than nonfertilized

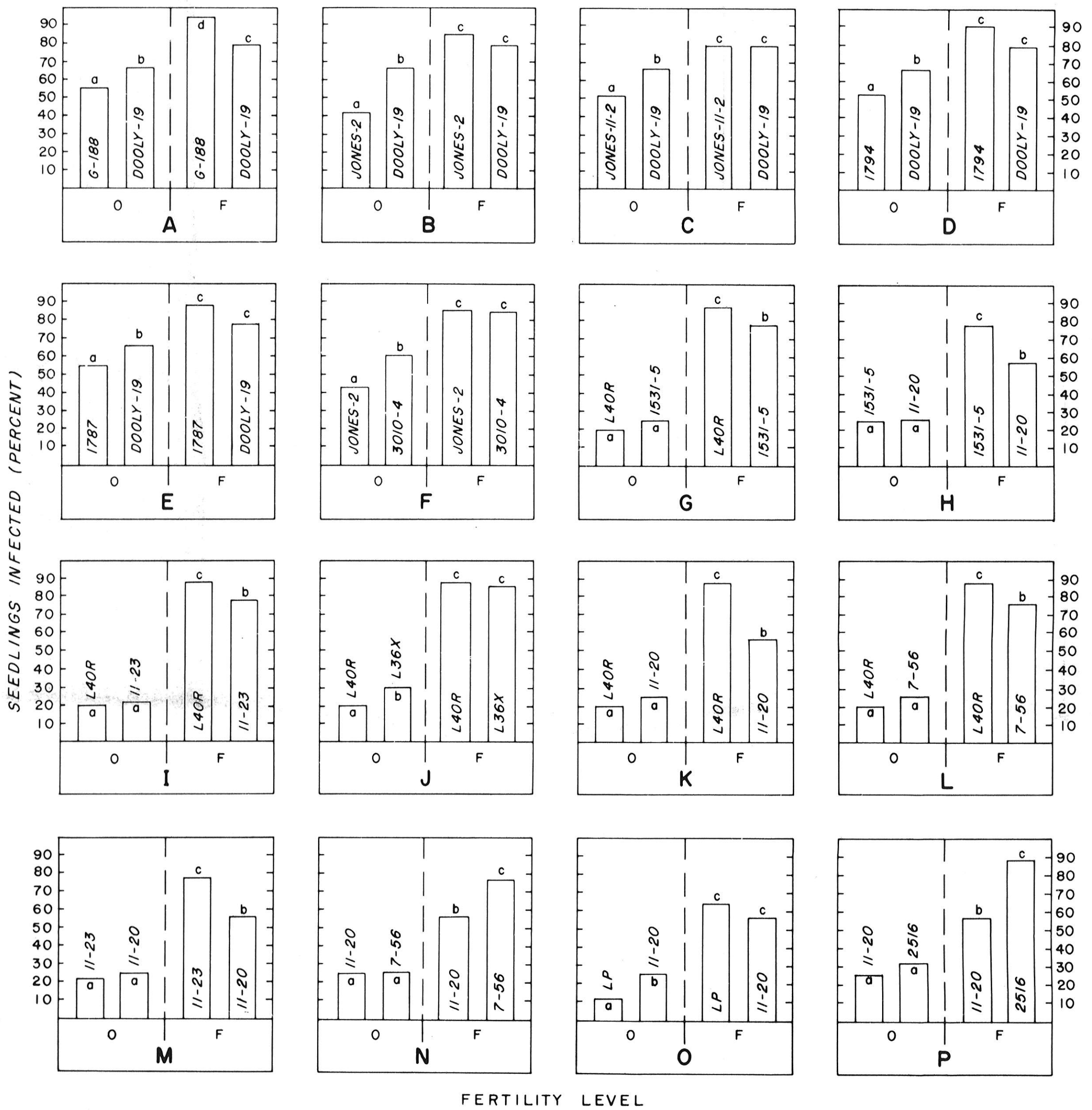

Fig. 1. Statistically significant interactions ($P = 0.05$) between fertilization and family affecting the relative susceptibility of slash (A to F) and loblolly (G to P) pine seedlings to fusiform rust. All points on graphs are means of five replicates, and points labeled by a common letter do not differ significantly (P = 0.05; Duncan's multiple range test). "0" = nonfertilized and "F" = fertilized. Fertilization (N+P+K+Fe) applied weekly during first 12 wk, monthly during next 6 mo, and bimonthly during final study period.

seedlings of all families except Jones-18.

Gall length was not correlated with percentage of seedlings infected (r = .004). The average gall was significantly shorter on nonfertilized loblolly seedlings (41.2 mm) than on nonfertilized slash seedlings (55.6 mm). The average gall was significantly longer on fertilized loblolly seedlings (122.0 mm) than on fertilized slash pine seedlings (110.0 mm).

Shoot length at time of inoculation.—The length of shoots at time of inoculation was significantly affected by fertilization, family, and their interaction in both slash and loblolly pines (Table 1, 2). Shoots, or a portion thereof, are the infection court for *C. fusiforme*, but among treatments shoot length was not correlated with the percentage of seedlings infected (r = .003). A discriminant function analysis and a conventional analysis of variance both indicated, however, that tall seedlings in each flat were significantly ($P = 0.01$) more susceptible than short seedlings.

Cotyledon number and hypocotyl length.—The number of cotyledons and the length of hypocotyls were significantly affected by family, but not by fertilization or by the family × fertilization interaction. Neither the number of cotyledons nor the length of hypocotyls was correlated with the percentage of seedlings infected (r = .0002 for both). Both the number of cotyledons and hypocotyl length were significantly greater on slash than on loblolly pine seedlings. These data suggest that neither cotyledons nor hypocotyls were a part of the infection court at time of inoculation.

TABLE 3. Detection of fusiform rust gall formation 3, 6, and 9 mo after artificial inoculation, as percentage[a] of seedlings observed galled 12 mo after inoculation of two *Pinus* species

Pinus spp. and fertility level	Months after inoculation 3 (%)	6 (%)	9 (%)
Slash			
Nonfertilized	74.6	92.8	97.7
Fertilized[b]	88.3	94.8	97.8
Loblolly			
Nonfertilized	61.2	73.3	94.5
Fertilized	86.3	96.1	97.8

[a]Percent reliability = [(no. seedlings infected at observation interval)/(no. seedlings infected 12 mo after inoculation)] × 100.

[b]Fertilization applied (N+P+K+Fe) weekly during first 12 wk, monthly during next 6 mo, and bimonthly during final study period.

DISCUSSION

Susceptibility of slash and loblolly pine seedlings to fusiform rust was increased more by fertilization in certain families than in others. Significant family × fertilization interactions involved seven of the 11 slash pine families and all eight families (including the one geographic race) of loblolly pine. Although the length of the assumed infection court (shoot length above cotyledons) at time of inoculation was not correlated with susceptibility among experimental treatments, tall slash and loblolly pine seedlings within treatments were significantly more susceptible than short ones. I previously reported that, in a single seed lot of loblolly pine, the size of the infection court was correlated with susceptibility to fusiform rust (8). These observations suggest that infection court size strongly influences the likelihood that a seedling will become infected, but that familial resistance and other factors may mask the importance of the infection court size. Dinus and Schmidtling (2) also found no correlation between seedling height and rust incidence in a field fertilization test. Familial resistance is a very important factor controlling incidence of fusiform rust, but the rank-order of families may be influenced by fertilization × family interactions.

Slash pine appears to develop visible galls faster than loblolly, and fertilized seedlings of both species develop galls faster than nonfertilized ones. Although gall length and percentage of seedlings infected were not correlated, gall length is probably a reasonably good index of the compatibility of fungus and host genotypes. Infection data apparently can be taken 6 mo after inoculation if only main treatment effects are needed, but when possible interactions between fertilization and families are to be measured it is best to wait 12 or more mo after inoculation

TABLE 4. Effect of fertilization on length of galls on seedlings of slash and loblolly pine 12 mo after artificial inoculation

Slash pine family	Gall length Nonfertilized (mm)	Gall length Fertilized[a] (mm)	Loblolly pine family	Gall length Nonfertilized (mm)	Gall length Fertilized (mm)
Jones-18	40 A[b]	50 ABCD	LP[c]	31 A	113 C
Jones-2	63 CDE	117 FGH	L-40 R	57 B	115 CD
S-37R	57 ABCDE	116 FGH	11-23	47 AB	139 F
Jones 11-2	43 AB	106 FG	1531-5	30 A	119 CDE
1794	53 ABCD	113 FGH	11-20	43 AB	104 C
1787	48 ABC	130 H	7-56	44 AB	117 CD
G-188	70 DE	130 H	2516	35 A	136 EF
Jones 11-1	51 ABCD	99 F	L-36X	44 AB	134 DEF
3010-4	51 ABCD	100 F			
Dooly-19	61 BCDE	130 H			
1744	75 E	120 GH			

[a]Fertilization applied (N+P+K+Fe) weekly during first 12 wk, monthly during next 6 mo, and bimonthly during final study period.

[b]Means followed by a common letter in each pair of columns do not differ ($P = 0.05$) according to Duncan's multiple range test.

[c]The abbreviation LP refers to the Livingston Parish geographic race of loblolly pine.

when the family × fertilization interaction is most significant.

The significant effects of family and fertilization interactions upon susceptibility of seedlings to fusiform rust confirm previous speculation (8) that both fertilized and nonfertilized seedlings should be inoculated for reliable test results.

LITERATURE CITED

1. BARBER, J. C., K. W. DORMAN, and E. BAUER. 1957. Slash pine progeny tests indicate genetic variation in resistance to rust. U.S. Dep. Agric., For. Serv., Southeast. For. Exp. Stn. Res. Notes 104. 2 p.
2. DINUS, R. J., and R. C. SCHMIDTLING. 1971. Fusiform rust in loblolly and slash pines after cultivation and fertilization. U.S. Dep. Agric., For. Serv., South. For. Exp. Stn. Res. Pap. SO-68. 10 p.
3. FREESE, F. 1964. Linear regression methods for forest research. U.S. Dep. Agric., For. Serv., For. Products Lab. Res. Pap. FPL 17. 136 p.
4. KINLOCH, B. B., JR., and R. W. STONECYPHER. 1969. Genetic variation in susceptibility to fusiform rust in seedlings from a wild population of loblolly pine. Phytopathology 59:1246-1255.
5. LA FARGE, T., and J. F. KRAUS. 1967. Fifth-year results of a slash pine polycross progeny test in Georgia. Proc. Ninth South. Conf. For. Tree Improv. 1967:86-91. Eastern Tree Seed Lab., Macon, Georgia.
6. LAIRD, P. P., and W. R. PHELPS. 1975. A rapid method for mass screening of loblolly and slash pine seedlings for resistance to fusiform rust. Plant Dis. Rep. 59:238-242.
7. MATTHEWS, F. R., and S. J. ROWAN. 1972. An improved method for large-scale inoculations of pine and oak with Cronartium fusiforme. Plant Dis. Rep. 56:931-934.
8. ROWAN, S. J., and K. STEINBECK. 1977. Seedling age and fertilization affect susceptibility of loblolly pine to fusiform rust. Phytopathology 67:242-246.
9. SCHMIDT, R. A., and R. E. GODDARD. 1971. Preliminary results of fusiform rust resistance from field progeny tests of selected slash pines. Proc. Eleventh South. Conf. For. Tree Improv. 1971:37-44. Eastern Tree Seed Lab., Macon, Georgia.
10. STEELE, R. G. D., and J. H. TORRIE. 1960. Principles and procedures of statistics. McGraw-Hill, New York. 481 p.
11. WELLS, O. O., and R. J. DINUS. 1974. Correlation between artificial and natural inoculation of loblolly pine with southern fusiform rust. Phytopathology 64:760-761.