Resistance Responses in Half-Sib Loblolly Pine Progenies after Inoculation with *Cronartium quercuum* f. sp. *fusiforme*

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ABSTRACT

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Open-pollinated progeny from 156 loblolly pine trees in the USFS-Georgia Forestry Commission orchards were inoculated with *Cronartium quercuum* f. sp. *fusiforme*. Nine months after inoculation, the incidence of galls among the 156 families ranged from 2 to 97%. The susceptible control family had significantly more galls than 116 of the 156 other families tested according to the 0.70 disease ratio computation (percent galls test family/percent galls susceptible control = ≤0.70). The 0.70 disease ratio was more conservative than a chi-square test of independence in separating resistant and susceptible families. Responses to infection were compared between resistant and susceptible families to determine the relative frequency of resistant responses. The absence of stem symptoms was the most common resistant response in many resistant families. The third-generation progeny derived from resistant selections 2318, 10-5, and 42R had 50% or more seedlings with this response. Needle spots were early symptoms of infection, but the frequency of seedlings with needle spots at 3 and 6 mo was not correlated with gall frequency at 9 mo. Some third-generation progeny had high frequencies of stem spots without swellings (symnos) at 3, 6, and 9 mo after inoculation as a resistant response. Stem spots present at 6 mo but no longer visible at 9 mo were present in other resistant selections. The ratio of short, medium, and long galls among 90 families was 10, 29, and 62%; however, gall length varied significantly among families. Galls on seedlings in certain resistant families were more frequently long than were galls on seedlings in susceptible families. Progeny from some resistant families had twice as many short galls as the average.

Additional key words: fusiform rust, *Pinus taeda*.

Resistance appears to be the best means of limiting damage by *Cronartium quercuum* (Berk.) Miyabe ex Shirai f. sp. *fusiforme* in young pine plantations. Loblolly (*Pinus taeda* L.) and slash (*P. elliottii* Engelm. var. *elliottii*) pines have been planted in seed orchards for the production of progeny with high levels of resistance to fusiform rust disease (12). The rust-resistant loblolly pine seed orchards being developed cooperatively by the USDA Forest Service and the Georgia Forestry Commission include both clonal and seedling stock (12). Scions for grafted trees in the clonal orchard came from rust-free individuals from families with outstanding characteristics for growth and yield as well as low indices of rust in field progeny tests. The seedling seed orchards were established with rust-free survivors of the concentrated basidiospore spray (CBS) inoculation system (6,12). Some trees in both types of orchard have common maternal parents. Because most orchard trees were the product of wind pollination, the maternal parents of the orchard trees are the most likely source of resistance. These maternal parents are designated resistant selections in this paper. The seedlings tested in this study are third-generation wind-pollinated progeny of these selections.

The relative resistance of each orchard tree is evaluated by exposing 4-wk-old seedlings to the disease in the CBS inoculation system (6). Seedlings with galls 9 mo after inoculation are rated susceptible, whereas those without galls are resistant. Our standard measure of familial resistance is the disease ratio (DR), computed by dividing the percentage of seedlings with galls in the test family by that of the standard susceptible control (16). Test families with a DR >0.70 are considered susceptible, those with DR ≤0.70 are resistant; however, no information on the statistical validity of the 0.70 DR has been presented. The percentage of seedlings with galls after CBS inoculation generally has correlated well with gall development on seedlings of the same families planted in the field (9,12,15,16).

Pine seedlings vary in their response to infection by *C. q.* f. sp. *fusiforme*. Jewell et al (3,4) observed red spots on primary needles and cotyledons 4–7 wk after inoculation of most (69–99%) slash pine seedlings. These early symptoms were used to measure the success of inoculations. However, Miller and Cowling (7) reported a low frequency of stem galls from inoculation of primary needles. Purple spots have been observed on the stem as soon as 2 wk after inoculation (8) and may or may not develop into galls (20). Purple stem spots without swelling are called symnos. Purple stem spots vary in their persistence; some are transient, being present soon after inoculation but absent by 9 mo, whereas others remain visible for at least 9 mo (8). Symptomless seedlings have less frequently been reported. Miller et al (8) reported symptomless seedlings were caused by the failure of basidiospores to penetrate the host or by subliminal infections with no macroscopic symptoms. Walkinshaw et al (20) reported 12% of slash pine seedlings from 30 families were symptomless, but this characteristic was not highly correlated with field performance. Griggs et al (1) found only 32 of 1,296 slash pine seedlings from three families free of needle and stem symptoms and classified these as escapes. Most susceptible loblolly and slash pine seedlings develop a fusoid gall by 9 mo after inoculation. However, small galls on slash pine seedlings tend to stop expanding because of limited mycelial colonization, and the seedling recovers from the inactive infection (5,8,17). Galls have been classified as small if they are <10 mm (19) or <25 mm (1) long 9 mo after inoculation. Hoff et al (2) related six symptoms of white pine blister rust, caused by *Cronartium ribicola* A. Fisch., to six mechanisms of resistance. More than 50% of seedlings of six of eight white pine species were symptom free 9–36 mo after exposure to the white pine blister rust fungus.

The various symptom types described for rust diseases of pines may be due to different genetic components, and a diversity of resistance genes is the goal of most efforts to control diseases through breeding. This study determined the statistical validity of the 0.70 DR and the relative occurrence of different symptom types in a sample of phenotypically resistant loblolly pine from clonal and seedling seed orchards. Patterns of symptom expression by families at 3 and 6 mo after inoculation were compared with patterns at 9 mo to characterize early symptoms of resistance and susceptibility.

MATERIALS AND METHODS

Seed 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 156 families were divided into seven groups of 19–25 families each for inoculation. After stratification, seed were sown in a peat moss-vermiculite-perlite (4:4:1, v/v/v) mixture. Germinated seedlings were transplanted individually into 5- × 15-cm-diameter plastic tubes. Six replications of 20 seedlings each per family were inoculated 4 wk after transplanting. Replications within each group of families and a susceptible control family (4666-4) were randomly inoculated. Inoculation dates were 7, 8, and 9 May and 20, 21, 28, and 29 August 1984. Basidiospores were obtained from oaks inoculated with a 1974 mass collection of aeciospores from Clarke County, GA, and were used at a rate of 5×10^4/ml in the CBS system (6). Basidiospore germination was 90% or more on 1.5% water agar after 24 hr at 20 C.

Symptoms of infection were recorded 3, 6, and 9 mo after inoculation. At 3 and 6 mo, presence of no symptoms, needle spots, symnos, and galls were recorded for each seedling. At 9 mo, no symptoms, symnos, and galls were recorded. Multiple symptoms occurred on many seedlings; however, the symptom indicating the greatest susceptibility in the order galls, symnos, needle spots, and no symptoms were used in most analyses. For the seedlings inoculated on the four August dates, galls were placed in three size classes (<10 mm, 10–25 mm, and >25 mm long) at 9 mo.

Most data were subjected to chi-square analyses. A regression analysis of needle spots at 3 and 6 mo with galls at 9 mo was made for the May data.

RESULTS

Galls as indicators of relative susceptibility. Nine months after inoculation, 2–97% of the seedlings in different families had galls (Table 1). The average percentage of galls per family was 70% for susceptible families and 41% for resistant families. Even though the susceptible control family varied in incidence from 67 to 92% galls, resistant and susceptible families could be identified by the 0.70 DR. The 156 orchard trees are second-generation selections, and 116 were rated as resistant, based on a DR for the family of less than 0.70. Within inoculation dates, chi-square tests of independence indicated that gall frequency was significantly more common in susceptible than in resistant families. The <0.70 DR was more conservative than a chi-square analysis in discriminating between resistant and susceptible families, since the latter analysis indicated the differences were significant by 0.001 level of probability. Furthermore, chi-square tests of percentage galls between the susceptible control and individual families revealed significant differences were present even between families with DR ⩽0.80 for all seven inoculation dates.

Frequency of symptoms. Needle spots occurred early and frequently with 79, 70, and 69% of the seedlings inoculated on 7, 8, and 9 May, respectively, having needle spots. However, the regression analysis of the frequency of needle spots on seedlings within families at 3 and 6 mo with the frequency of galls on seedlings within families at 9 mo indicated there was no correlation.

Three and six months after inoculation, resistant families always had larger percentages of seedlings with no symptoms and needle spots only than did susceptible families (Table 2). Conversely, more seedlings in susceptible families had symptoms on the stem either as galls or as symnos than did those in resistant families. The percentages of symnos in the two groups were not much different at any time, whereas seedlings with galls were most frequent in susceptible families even at 3 mo after inoculation. Data from two inoculation dates presented in Table 2 are representative of those from the seven inoculations. The data from all seven inoculations were similar in that the chi-square comparisons indicated the proportion of seedlings with the different symptoms in susceptible and resistant families were significantly different at each observation time for each inoculation date. Some seedlings in resistant families with the symnos symptom at 6 mo had no stem symptom at 9 mo. These transient stem spots occurred on approximately 10% of the resistant seedlings. There was a decline in the frequency of seedlings with needle spots as only symptoms and symnos between 3 and 9 mo after inoculation. In resistant families, most of these seedlings became symptomless, whereas in susceptible families galls formed.

The resistant sources of the third-generation progeny had a significant effect on the occurrence of symptoms. Third-generation progeny of resistant sources 10-5, 2318, and 42R (group I) had more seedlings with no symptoms and fewer with symnos than did progeny from other resistant sources (Table 3). Chi-square tests indicated the two groups were significantly different. Group I families were first noted because of the frequent occurrence of seedlings with no stem symptoms (i.e., no symptoms or needle spots only). This group did have large numbers of seedlings with no stem symptoms, but group II also had the largest number of resistant seedlings in the no stem symptom categories. Symnos were more common in group II than in group I. Symnos were more common in some resistant sources like 15-42, T605, and T601, but

TABLE 1. Frequency of galls on seedlings in resistant and susceptible families of loblolly pine 9 mo after inoculation with *Cronartium quercuum* f. sp. *fusiforme*

Inoculation date	Control[a] (%)	Disease class	Families (no.)	Seedlings with galls (%)	
				average	range
5-7-84	85	R[b]	19	40	8–57
		S	4	69	60–79
5-8-84	78	R	15	41	12–52
		S	9	64	56–77
5-9-84	76	R	16	35	9–53
		S	3	73	65–83
8-20-84	91	R	19	48	26–63
		S	3	75	66–84
8-21-84	92	R	12	48	34–60
		S	12	81	67–97
8-28-84	67	R	16	35	2–45
		S	8	59	47–81
8-29-84	88	R	19	39	25–51
		S	1	62	···
Summary		R	116	41	
		S	40	70	

[a] Seedlings of susceptible control, 4666-4, inoculated each date.
[b] Resistant (R) and susceptible (S) classes based on a disease ratio > or ⩽ 0.70 of the galls in the susceptible control for each family. Classes within dates are significantly different according to chi-square tests of independence $P > 0.001$.

TABLE 2. Frequency of four responses on loblolly pine seedlings in fusiform rust resistant and susceptible families 3, 6, and 9 mo after two inoculation dates

Inoculation date	Mo after inoculation	Disease class	Seedlings with response (%)			
			No[a]	NSO[b]	Symnos[c]	Galls
5-8-84	3	R[d]	17	28	25	31
		S	10	23	23	45
	6	R	35	7	17	41
		S	21	5	13	61
	9	R	53	···	6	41
		S	34	···	5	61
8-21-84	3	R	19	35	24	23
		S	8	32	23	37
	6	R	16	21	16	47
		S	8	7	9	75
	9	R	47	···	6	48
		S	14	···	4	83

[a] No = Symptomless.
[b] NSO = Needle spots as only symptom.
[c] Symnos = Stem spot with no swelling.
[d] R = Resistant. S = Susceptible.

TABLE 3. Frequency of various responses to fusiform rust infection in two groups of third-generation resistant families 6 and 9 mo after inoculation

Inoculation date	Resistant group[a]	Families (no.)	Seedlings with responses (%) 6 mo No[b]	NSO[c]	Symnos[d]	Galls	9 mo No	Symnos	Galls
8-20-84	I	3	30	28	4	38***[e]	58	1	41**[f]
	II	16	18	16	23	44	39	12	49
8-21-84	I	6	16	30	10	44***	54	1	45*
	II	6	16	14	22	48	39	10	51
8-28-84	I	8	45	18	5	33***	64	2	35 NS
	II	8	27	16	23	34	51	14	35
8-29-84	I	7	46	13	5	37***	58	2	40 NS
	II	12	34	13	15	38	52	9	39

[a]Group I = Families of 42R, 2318, and 10-5; II = families of all other resistant sources.
[b]No = Symptomless.
[c]NSO = Needle spots as only symptom.
[d]Symnos = Stem spots with no swelling.
[e]Probable ratio of symptoms at 6 mo equal $P>0.001$*** according to χ^2.
[f]Probable galls at 9 mo equal $P>0.01$**, >0.05*, NS according to χ^2.

TABLE 4. Effect of family susceptibility on the percentage of fusiform rust galls in three size categories

Inoculation date	Disease class	Seedlings with galls (%) gall length (mm) <10	10–25	>25
8-20-84	Resistant	16	29	54*[a]
	Susceptible	10	34	55
8-21-84	Resistant	6	12	82***
	Susceptible	7	20	73
8-28-84	Resistant	5	38	57***
	Susceptible	13	45	43
8-29-84	Resistant	8	25	66 NS
	Susceptible	7	21	72
Average		10	29	62

[a]Proportion of seedlings with galls in the three size classes tested by χ^2 within inoculation date. NS = Not significantly different, or significant * $P>0.05$, *** $P>0.005$.

TABLE 5. Effect of resistant source of third-generation loblolly pine families on the percentage of fusiform rust galls in three size categories

Inoculation date	Resistant source	Families (no.)	Seedlings with galls (%)	Galls (%) length (mm) <10	10–25	>25
8-29-84	10-5	5	38	3	18	79***[a]
	29R × Ark	5	43	10	28	61
8-28-84	10-5	5	31	5	24	71***
	10-6	5	55	20	39	41

[a]Proportion of seedlings with galls in the three size classes significantly different for the two resistant sources at $P>0.005$.

even in these sources more resistant seedlings had no stem symptoms than symnos.

Gall length at 9 mo. Resistant families did not limit gall length as a response (Table 4). More galls on seedlings in resistant families were in the longest (>25 mm) category than were galls on seedlings in susceptible families.

Families from many resistant sources had seedlings with long galls. Resistant source 10-5 had five families in both the 8/28 and 8/29 inoculations, although one of these families had only 2% of the seedlings with galls. More than 70% of the galls formed on seedlings in these families were in the longest category (Table 5). Resistant source 29R × Ark also had a low percentage of seedlings with galls, but the distribution of gall length was different from that of galls on seedlings of 10-5. Seedlings of resistant source 10-6 were more susceptible; however, more galls were in the two shortest categories than were galls on seedlings from 10-5.

DISCUSSION

Presence or absence of galls at 9 mo has been the standard means of separating resistant and susceptible seedlings and families. In this study, 116 of the 156 families tested had significantly fewer galls than the susceptible control family based on the <0.70 DR as the cutoff point for resistant families. Chi-square tests of independence were somewhat less conservative than the 0.70 DR in discriminating between resistant and susceptible families. The 0.70 DR has been a useful standard for roguing the most susceptible trees in the USFS-GA Forestry Commission orchards and for assessing the relative resistance of trees with <0.70 DR.

The superior performance of the progeny of some resistant selections in field plantings has been documented. In 1971, Zobel et al (21) reported 10-5 as a maternal parent had progeny with an infection level in field progeny tests of 4% compared with 72% infection in a susceptible family. Family 10-5 had the fewest galls of seven families tested in CBS inoculations and in field exposure for 5 yr (9). Progeny of 10-6 had 12% infection compared with 57–67% infection in three susceptible sources (21). Powers and Kraus (12) reported a good correlation between percentage of seedlings galled after CBS inoculations and subsequent natural infection on nongalled survivors. In CBS inoculations, second-generation seedlings from the resistant selections used here have consistently had fewer galls than susceptible controls (9–14).

The absence of stem symptoms was the most common response in these resistant loblolly pine families. The relative frequency of this symptom type in resistant slash pine families has not been indicated (8). The resistance screening center near Asheville, NC, has not used symptomless seedlings in its resistance index because this symptom type was not well correlated with the field resistance of its sample population of slash pine families (20). Because 156 third-generation families from many resistant selections of loblolly pine were tested in this study, we were able to confirm the frequent occurrence of symptomless seedlings with certain selections. Most notable selections for symptomless seedlings were 10-5, 2318, and 42R, with 50% of the third-generation progeny in this category. Further studies are needed to determine how this response relates to mechanisms of resistance.

Needle spots caused by infection by *C. q.* f. sp. *fusiforme* were recorded to determine the frequency of all symptoms of infection, and the role of needle infections in gall formation. Needle spots occurred commonly at 3 mo but were not indicative of percentage of galls at 9 mo. However, the high percentage of seedlings with needle spots did indicate that most seedlings were infected. Stem symptoms usually appeared distinct from needles or needle spots and were often visible as early as needle spots. Direct stem infection appears to be the typical means of gall initiation for young loblolly pine seedlings in CBS inoculations.

Seedlings with symnos appear to have the highest probability of resistance. Whereas symptomless seedlings could have been disease escapes, those with stem spots resisted the fungus in an obvious manner. Walkinshaw et al (20) found the second highest positive correlation for this symptom with field results in 30 slash pine families. Miller et al (8) gave no indication of the frequency of occurrence of purple spots in their slash pine populations. In this study, third-generation wind-pollinated progeny of resistant selections 15-42, T601, and T605 had the highest frequency of stem spots at all three observation times, suggesting the mechanism of resistance from these sources often produces this type of symptom in response to the fungus.

The classic phenotype of a susceptible seedling is one with a fusoid stem gall that often results in host death in the field. Deviations from the fusoid shape suggest that the host is limiting spread of the pathogen. Walkinshaw et al (20) have shown that when the frequency of galls is high, gall characteristics become useful indicators of resistance. Thirty slash families averaged 86% galls after CBS inoculations, and gall shape and texture were the most useful characteristics for ranking susceptibility (20). Forty-three loblolly families from Livingston Parish, LA, averaged 70% galls, and the ratio of gall length to width was used to indicate variation in susceptibility, with ratios nearest 1.0 indicating resistance (18). In our study of 156 families, the average incidence of galls was 49% with a range of 2–97%. These 156 families were readily separated into resistant and susceptible groups by their disease ratios. In these families, gall length and texture appear to be less useful. Although we characterized gall length and texture, the latter character was not reported. In contrast to the results of Snow et al (18), longer galls tended to be more common in resistant than in susceptible families (Table 4). Considerable variation in gall length occurred among families. Because resistant source 10-5 had the highest frequencies of long galls, it is apparent that restriction of gall development is not a standard indicator of resistance. Resistance sources with a high proportion of short galls (e.g., 10-6) may be of even greater value than the DR data indicate if short galls in loblolly pine are similar to the short stabilized galls described as a resistance response in slash pine (1,5,8,14). Walkinshaw (19) reported that short galls in loblolly pine contained rust mycelium, whereas those on slash did not. However, if a high proportion of short galls stop expanding, even seedlings with short galls could be considered resistant rather than susceptible.

The results of this study demonstrate that most rust-free survivors of CBS inoculations and of field exposure in orchards produce progeny that are much more resistant to fusiform rust than are a susceptible control family. For these loblolly families, the presence or absence of galls when used to establish a disease ratio provides rapid separation of resistant and susceptible parents. In addition, certain resistant parents produce a high proportion of seedlings that never develop stem symptoms. Other resistant parents produce seedlings that express resistance with stem spots but no swellings. Short galls may be a third useful mechanism of resistance. Studies of these four symptom types and the basis for the variation in response are under way.

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