RESPONSES OF THREE TEXAS LOBLOLLY PINE FAMILIES TO AECIOSPORE ISOLATES OF CRONARTIUM QUERCUUM F.SP. FUSIFORME

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Abstract.--Identifying aeciospore isolates of the fusiform rust fungus with virulence toward resistance in specific pine sources is the first step in our effort to ensure that a variety of resistance types are present in the resistant population. Open-pollinated seed from three Texas trees in the USFS/Georgia Forestry Commission orchard that had demonstrated good resistance to composite isolates of the rust were challenged by 13 specific isolates of the rust from Texas, Arkansas, Louisiana, and Georgia. Nine months after inoculation frequency of galls differed significantly for family, isolate, and family x isolate interaction. Gall frequency varied from 31-80, 7-55, and 26-55% for the three families. Isolates from TX and AR were more virulent on two of these families than were isolates from GA and LA.

Keywords: Fusiform rust, Cronartium quercuum f.sp. fusiforme, loblolly pine, resistance, virulence

INTRODUCTION

Resistance in loblolly (Pinus taeda L.) and slash pines (P. elliottii Engelm. var. elliottii) to fusiform rust disease needs to have a broad genetic base to ensure its stability against the very heterogenous causal fungus, Cronartium quercuum (Berk.) Miyabe ex Shirai f. sp. fusiforme. Rust resistance in agronomic crops is often overcome by the development of virulent isolates of a fungal pathogen. In these crops, resistance is often controlled by single genes, and some isolates of the pathogen have compatible genes for virulence that enable them to attack an otherwise resistant host plant. A broad genetic base of resistance in southern pines should reduce the risk of a virulent strain of the pathogen that previously occurred at a low frequency becoming dominant and causing severe losses.

Virulent isolates of the pathogen are useful for identifying different mechanisms or genes for resistance in the host population. Isolates virulent toward resistant loblolly and slash pines have been identified (Griggs and Walkinshaw 1982; Kinloch and Walkinshaw 1991; Snow and Griggs 1980; Kuhlman 1989, 1990, 1992; Powers 1985; Powers and Matthews 1979, 1980; Powers et al. 1977, 1978; Snow et al. 1975, 1976; Walkinshaw and Bey 1981). Virulent isolates produce gall frequencies in specific resistant families 1.5-2.0 times

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those caused by most isolates (Kuhlman 1990). Five of 21 loblolly pine families inoculated with six virulent, single-gall aeciospore isolates were uniformly, highly resistant (averaged <30% of seedlings galled). The other 16 families varied widely in response to the isolates and usually were susceptible (>65% seedlings with galls) to at least one isolate (Kuhlman 1992). One of the highly resistant families, 152-60, was from a Texas Forest Service collection. Powers and Matthews (1979, 1980) reported that isolates from the geographic source of some resistant material were often more virulent on that source than were isolates from other geographic sources. Single-gall isolate TX-3 was more aggressive on progeny of two Texas trees than were single-gall isolates from Georgia or South Carolina (Powers and Matthews 1979). Therefore, isolates from Texas appeared to be a possible source of virulence towards resistance in trees from Texas.

The objectives of this study were: (1) to identify rust isolates that are virulent on progeny from one or more resistant loblolly pines from Texas; (2) to determine if isolates from Texas are more virulent on Texas sources than isolates from other regions; and (3) to compare the responses of progeny from three Texas trees to 13 rust isolates.

METHODS

Seed Sources

Half-sib progeny from three trees in the seedling seed orchard (SSO) of rust resistant loblolly pines developed by the Georgia Forestry Commission and the USDA Forest Service were selected for this study. These trees came from a mass collection from a Texas Forest Service seed orchard. Progeny of tree 152-60 have a disease ratio (DR) (=frequency of galls in test family/frequency of galls in a susceptible control) of 0.30 in several concentrated basidiospore spray (CBS) system inoculations with composite isolates of the rust fungus. When progeny of this tree were inoculated with six single-gall isolates of the rust, 21% developed galls (Kuhlman 1992). Progeny of tree 151-79 have a DR of 0.33, whereas those of 152-266 have a DR of 0.54 after inoculation with composite isolates of the fungus.

Rust Isolates

Thirteen aeciospore isolates were studied. Eleven were from single galls and two were composite collections from several galls in an area. Two single-gall isolates, 152-201 and 3327-13, were chosen as avirulent controls since each produced galls on only 17% of the seedlings of 152-60 (Kuhlman 1992). Those two isolates and another single-gall isolate (G741) were from Georgia. Five isolates from Texas included three from single galls (TX-3, SG-A, and SG-1), and two from composite collections (SAT and LAT). Of the other five isolates, three were from single galls in Arkansas (77A-1, 77A-6, and 77A-10) and two were from single galls in Louisiana (LLL-3 and LLL-7).

Study Design

Seeds were moist, cold stratified for 6 weeks before planting in vermiculite in germ trays. Seedlings were transplanted 20 to a flat in a mixture of gravel and Fafard II Mix. When the seedlings were 6 weeks old, eight flats of each source were inoculated in the CBS system with one of the isolates. Four flats of each source were inoculated with one of the isolates on one day and the other four flats of each source were inoculated on a second day. Flats were randomly assigned to isolate treatment, and the order of isolate treatment was random. After inoculation the flats were placed in a moist chamber at 20 C for 24 hours, then moved to a greenhouse for 9 months.

Each seedling was examined for the presence or absence of galls 3, 6, and 9 months after inoculation. At 9 months, lengths of galls on live seedlings and rust associated mortality (RAM) also were observed. Seedlings that had galls at 3 or 6 months and were dead at 6 or 9 months were designated RAM.

The data at 9 months were subjected to a factorial analysis of variance with the SAS GLM procedure. Treatments were compared with Tukey's Studentized Range (Tukey's) at the 0.05 level. To elucidate regional trends in virulence, the incidence of galls by isolates from each state for individual families were compared.

RESULTS AND DISCUSSION

Frequency of galls, RAM, and gall length were each significantly affected by pine family, isolate, and the interaction of family x isolate (Table 1).

Table 1. Sources of variation, degrees of freedom, mean squares and significance for gall frequency, RAM, and gall length.

		Mean square		
Source	DF	Gall frequency	RAM	Gall length
Pine families	2	0.79286 ***[1]	0.15944 ***	1325.461 2 ***
Rust isolates	12	0.27215 ***	0.13250 ***	279.6769 ***
Fam x isolate	23	0.13014 ***	0.03686 ***	118.9067 *
Error	246	0.01158	0.01186	65.0282

1 ***=P<.001 *=P<0.05

Families varied significantly in the frequency of galls after inoculation with the 13 isolates (Table 2). Family 151-79 had the largest range in susceptibility from 31% with LLL-7 to 80% with 77A-1. Family 152-60 had the most resistant responses; six isolates produced infections on less than 25% of the seedlings. The highest virulence toward 152-60 was 55% by isolate TX-3. Although family 152-266 had the least variation in response to the 13 isolates, the amount of infection with LLL-3 was more than twice that with SG-A and TX-3. Thus, on each of the three families, some isolates caused infections at least twice as frequently as other isolates.

Unique reactions of individual families to specific isolates led to a statistically significant family times isolate interaction. Note, for example, the gall frequency in families 151-79 and 152-60 inoculated with LAT (60 and 46%, respectively) and 77A-10 (38 and 23%, respectively). Incidence decreases by over 20%, whereas family 152-266 had 31% galls with LAT and 51% with 77A-10. Isolates 77A-6 and SGA had similar gall frequencies on families 151-79 and 152-60, but family 152-266 had 49% galls with 77A-6 and only 26% with SG-A.

Table 2. Frequency of seedlings with galls in three loblolly pine families nine months after inoculation with each of 13 single-gall isolates of *Cronartium quercuum* f.sp. *fusiforme*.

Isolate	State of Origin	Composite (C) or Single gall (S)	Family 151-79	Family 152-60	Family 152-266
77A-1	AR	S	80 a[1]	52 a	
SAT	TX	C	76 ab	48 a	39 abcd
SG-A	TX	S	65 ab	53 a	26 d
TX-3	TX	S	62 ab	55 a	27 d
SG-1	TX	S	62 ab	51 a	31 bcd
77A-6	AR	S	60 b	41 ab	49 abc
LAT	TX	C	60 b	46 a	31 cd
77A-10	AR	S	38 c	23 bc	51 ab
LLL-3	LA	S	37 c	11 c	55 a
3327-13	GA	S	36 c	7 c	37 abcd
G741	GA	S	36 c	16 c	45 abcd
152-102	GA	S	33 c	22 bc	29 d
LLL-7	LA	S	31 c	13 c	35 bcd

[1] Within columns, numbers followed by the same letter do not differ siginficantly (Tukey's = 0.05).

Isolates from Texas and Arkansas were more virulent on families 151-79 and 152-60 than were isolates from Louisiana and Georgia, producing 1.5-4.0 times as many galls (Table 3). Family 152-266 had more variation in response to the individual isolates and did not have significant differences in average infection among isolates from the four States. The results with the former two families support the observations of Powers and Matthews (1979) that isolates from Texas are more virulent on Texas sources, but also provide evidence of variations among rust isolates from Texas.

Table 3. Association of family susceptibility with state of origin of the rust isolates.

State of Origin of Isolates	Isolates (No.)	Family 151-79	Family 152-60	Family 152-266
Texas	5	65 a[1]	51 a	31 a
Arkansas	3	54 a	38 a	50 a
Louisiana	2	33 b	12 b	40 a
Georgia	3	35 b	15 b	37 a

[1]Within columns, numbers followed by the same letter do not differ significantly (Tukey's=0.05).

Rust associated mortality of seedlings was affected by family, isolate, and the interaction of family by isolate. Family 151-79 had the most RAM just as it had the highest frequency of seedlings with galls. However, in all three families the isolate causing the highest frequency of galls did not cause the highest frequency of RAM (Table 4). Isolate TX-3 produced the highest frequency of galls on family 152-60 but the 14% of seedlings with RAM is near the mean for this family. Similarly isolate LLL-3 produced the highest frequency of galls (55%) on family 152-266, but the lowest frequency of RAM (6%) on the same family. Also on family 152-266, isolate 77A-10 produced 51% galls and the highest RAM of 29%.

Family 151-79 had the greatest average gall length in addition to having the greatest frequency of galls and RAM. However, family gall length response to isolates was independent of the gall-frequency and RAM responses (Table 5). Family 151-79 inoculated with isolate 3327-13 had a low frequency of galls, but the galls that formed were the longest in this family. Also, on family 151-79, isolate SG-1 produced many galls, but the galls were short. Similar variations occurred with the other two families. Isolates LLL-3 and LLL-7 were avirulent to family 152-60 but they had the longest and shortest galls, respectively, for this family.

CONCLUSIONS

Inoculation of three Texas families with isolates of the fusiform rust fungus from four States resulted in considerable variation in infection suggestive of three different types of resistance. Isolates 77A-1 and SAT from Arkansas and Texas, respectively, were highly virulent on family 151-79. Texas isolates were most virulent on families 151-79 and 152-60, but a Louisiana isolate produced the most infection on family 152-266. RAM and gall length appeared to be independent of incidence of galls.

Table 4. Frequency (%) of rust associated mortality among seedlings in three loblolly pine families after inoculation with each of 13 single-gall isolates of <u>Cronartium quercuum</u> f.sp. <u>fusiforme.</u>

	Family		
Isolate	151-79	152-60	152-266
SG-A	37 a[1]	30 a	15 ab
LAT	33 ab	13 bc	8 b
SG-1	31 abc	25 ab	13 ab
77A-1	28 abcd	22 ab	
SAT	26 abcd	15 abc	11 ab
77A-6	22 abcde	10 bc	21 ab
TX-3	17 abcde	14 bc	9 ab
LLL-7	12 bcde	0 c	12 ab
77A-10	10 cde	4 c	29 a
152-102	9 cde	5 c	7 b
G741	8 cde	2 c	7 b
3327-13	6 de	1 c	6 b
LLL-3	3 e	0 c	6 b

[1]Within columns, numbers followed by the same letter do not differ significantly (Tukey's=0.05).

Table 5. Average length of galls among seedlings in three loblolly pine families after inoculation with each of 13 single-gall isolates of <u>Cronartium quercuum</u> f.sp. <u>fusiforme.</u>

	Family		
Isolate	151-79	152-60	152-266
3327-13	38.0 a[1]	19.9 ab	26.7 ab
LLL-3	36.1 ab	32.0 a	31.5 a
SAT	33.5 ab	25.3 ab	21.4 ab
77A-1	32.5 ab	23.6 ab	
TX-3	31.4 ab	27.7 ab	18.0 b
77A-6	31.3 ab	30.2 a	31.2 a
G741	30.7 ab	17.7 ab	29.5 ab
152-102	30.3 ab	24.9 ab	24.1 ab
LAT	30.1 ab	22.8 ab	17.9 b
77A-10	28.7 ab	30.8 a	25.4 ab
SG-A	28.6 ab	24.4 ab	17.4 b
LLL-7	23.5 ab	13.1 b	21.5 ab
SG-1	22.1 b	18.8 ab	20.3 ab

Within columns, numbers followed by the same letter do not differ significantly (Tukey's=0.05).

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