The Potential for Increased Virulence of Cronartium fusiforme on Resistant Loblolly Pine

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ABSTRACT

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Aeciospores of *C. fusiforme* were collected from individual rust galls on progeny of a resistant loblolly pine parent tree and from galls representing the general rust population on nearby trees. Seedlings of three half-sib families then were inoculated with basidiospores derived from each of these individual galls. These families included seedlings of the resistant source from which half of the aeciospores were collected, a second resistant family, and a susceptible check. The percentages of infection on both resistant families were significantly less than on the susceptible check family. There were no statistically significant differences in virulence between the inocula collected on resistant seedlings and the wild-type inocula. However, there was a slightly higher level of virulence among the inocula originating from the resistant family.

Additional key words: disease resistance, fusiform rust, epidemiology, *Pinus taeda*.

Fusiform rust, which is caused by *Cronartium fusiforme* Hedgc. & Hunt ex Cumm., is responsible for increasingly severe damage on southern pines (5, 8, 10). Because of economic and environmental considerations, selecting and breeding for resistance is the most promising method of disease control. Effective resistance to fusiform rust already has been demonstrated in both slash (*Pinus elliottii* Engelm. var. *elliottii*) (2) and loblolly (*Pinus taeda* L.) pines (14), and resistance is markedly increased by crossing resistant individuals of a single species (6). In view of these facts, special seed orchards have been established to produce resistant seed.

Resistance in bulk collections of loblolly pine seed from certain geographic areas also has been demonstrated (13). During the past 3 yr, seed from one such area, Livingston Parish, Louisiana, has been collected, sold in large quantities to numerous agencies in several states, and widely planted in the Atlantic and Gulf Coastal Plains. The possibility that the use of these seedlings, plus other resistant material from seed orchards, might result in an increase in the virulence of the pathogen is of concern to pathologists and tree breeders. Artificial inoculations have shown that the virulence of *C. fusiforme* is highly variable (7, 11), and striking increases in virulence in the fungus population have occurred where resistant slash pines have been planted (12). Since loblolly is the most widely planted species of pine in the South, it is vital to determine whether the planting of millions of resistant loblolly pines will affect the virulence of the fungus population.

This study was designed to determine whether inocula collected from infected trees of a resistant loblolly pine family differed in virulence from inocula derived from the general rust population in the same area.

MATERIALS AND METHODS

Loblolly pine seedlings were grown from seed of three half-sib (offspring having only one parent in common) families. Families 29R and 11-20 are resistant to fusiform rust, and family 3838-3 is highly susceptible. The parent trees of 29R and 3838-3 originated in the Georgia Piedmont, and the 11-20 parent was from coastal South Carolina. The relative resistance of these families was determined previously by artificial inoculations and observations of field plantings.

Separate collections of aeciospores were made from nine individual galls on half-sib progeny of resistant family 29R in a 4-yr-old experimental planting in Greene County, Georgia. For comparison, aeciospores also were collected from nine galls in a commercial planting of loblolly pine growing within 1.5 km of the 29R planting. Hereafter, inocula from the latter collections will be referred to as the wild-type. Spore collections were handled separately and processed and stored according to the procedures outlined by Roncadori and Matthews (9).

To produce basidiospore inocula, seedlings of northern red oak (*Quercus rubra* L.) were inoculated separately with aeciospores from each of the 18 collections. Basidiospores harvested from the oak leaves were used in a concentrated basidiospore spray for pine inoculation (3, 4). About 2 to 3 days after the pine seeds had germinated, the seedlings were transplanted into flats containing 20

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seedlings each. At 4 wk of age, the seedlings were carried on a conveyor belt under an aqueous spray containing basidiospores. Inocula were adjusted to 50,000 spores per ml, and 8 ml was sprayed on each flat of seedlings. In vitro germination of basidiospores immediately prior to inoculation was 85% or greater for each of the rust inocula. Each of the 18 rust inocula was tested on 80 seedlings (four flats) for each of the three pine families; thus, 4,320 seedlings were inoculated. Immediately after inoculation, the seedlings were placed in a mist chamber and held at 21 C for 24 hr. Then they were grown in the greenhouse for 9 mo.

Infection data were based on the percentage of 9-mo-old seedlings having active galls. The statistical design was a nested factorial analysis of variance with individual gall collections nested within sources (inoculum from resistant pines vs. wild-type). Means were compared according to Duncan's multiple range test (1).

RESULTS AND DISCUSSION

There were highly significant differences among percentages of infection of the three host families (Table 1). There were also highly significant differences among inocula collected from each of the two sources–for example, among inocula collected from resistant pines. The interaction between inocula within sources × families also was highly significant. There was, however, no significant difference in infection levels produced by inocula originating on resistant pines and the wild-type inocula.

The differences in levels of infection among the three host families were expected since these differences in levels of resistance were part of the experimental design. The overall average percentages of infection on each of the three host families were close to those observed in previous tests, family 11-20 being more resistant than 29R and 38-38-3 being highly susceptible (Table 2).

There also were significant differences among the nine inocula within each of the two sources (resistant and wild-type). Additional analyses showed that most of this variation was attributable to differences within inocula from the resistant source. However, this effect was confounded because of the significance of the highest-order interaction, that of inocula within sources × families. This interaction indicated that the inocula within the source did not react the same on the three pine families (Table 2). An example of this variation is shown by V-8, which was of average virulence on 11-20, higher than average on 29R, and below average on 3838-3. The differences among inocula within sources, and the significant inocula within sources × family interation, again emphasize the highly variable nature of *C. fusiforme* (7).

Of major importance in this study was the lack of a significant difference between the two rust sources. The overall averages of infection for the two source groups were remarkably similar—52% for the wild-type inocula and 55% for the inocula from the resistant pines. However, an arithmetically higher level of infection was produced by the inocula derived from resistant family 29R (Table 2); this higher level may be biologically, if not statistically, meaningful.

The fact that differences between the two sources were not detected in the analysis is particularly important to those interested in the development and deployment of rust-resistant loblolly pines in the southeastern USA. There was no overwhelming increase in virulence of the pathogen which could overcome the resistance of a relatively resistant pine family. This finding is in marked contrast to the results in slash pine; the inocula from

TABLE 1. Analysis of variance for nested factorial study to test pathogenic variation between populations of *Cronartium fusiforme* on three loblolly pine families

Source of variation	d.f.	SS	MS	F[a]
Pine families	2	70,956	35,478	90.3 **
Rust sources	1	350	350	0.8 n.s.
Families × sources	2	1,269	634	1.6 n.s.
Inocula within sources	16	7,183	449	3.0 **
Inocula within sources × families	32	12,583	393	2.67**
Error	162	23,844	147	
Total	215	116,185		

[a]The abbreviation n.s. = not statistically significant, $P = 0.05$, and the double asterisks ** = statistically significant, $P = 0.01$.

TABLE 2. Incidence of infection on seedlings of three half-sib families of loblolly pine 9 mo after inoculation with *Cronartium fusiforme* from resistant trees or from wild-type sources

Collection type and No.	Infection on host family[y] 11-20 (%)	29R (%)	3838-3 (%)	Mean
Wild-type				
C-4	42 de	40 de	88 a	57
C-5	36 de	53 cd	68 bc	52
C-6	36 de	41 de	75 ab	51
C-7	39 de	33 e	90 a	54
C-8	36 de	34 de	90 a	53
C-10	33 e	67 bc	81 ab	60
C-11	34 de	34 de	69 bc	46
C-14	26 e	41 de	76 ab	48
C-15	33 e	26 e	79 ab	46
Mean	35	41	80	52
Resistant[z]				
V-3	41 cd	33 cd	81 a	52
V-6	41 cd	35 cd	64 ab	47
V-8	39 cd	63 ab	69 a	57
V-12	27 d	43 cd	80 a	50
V-16	39 cd	64 ab	80 a	61
V-17	42 cd	47 bc	81 a	57
V-18	24 d	39 cd	80 a	48
V-19	27 d	41 cd	80 a	49
V-21	49 bc	83 a	74 a	69
Mean	37	50	77	55
Family Mean	36	46	79	

[y]Infection percentages within collection types followed by the same letter do not differ significantly at $P = 0.05$ as determined by Duncan's multiple range test.

[z]Spores from galls on family 29R.

resistant slash pines were four times more virulent than the general rust population (12). The presence of virulent inocula such as V-21, however, may indicate a trend toward more virulent strains of rust developing on resistant pines. If so, plantings of resistant loblolly pines should be monitored to check on future shifts in pathogenicity within the population of *C. fusiforme*.

CONCLUSIONS

From the standpoint of breeding and selecting for rust resistance, it is encouraging that the loblolly pine families tested in this study did not sustain the same increase in virulence that was recorded on slash pine. This lack of increase indicates that forest land managers can utilize the new, resistant strains of loblolly pine without being concerned about the pathogen showing a fourfold increase in virulence within a single planting cycle. It is also encouraging that 11-20, a resistant source different from that on which the inocula were collected, maintained its level of resistance regardless of the source of the inoculum. This finding may indicate that these two families represent fundamentally different sources of resistance and that the threat of increased virulence can be offset by shifts in host genotypes.

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