# VIRULENT ISOLATES OF CRONARTIUM QUERCUUM F. SP. FUSIFORME MAY IDENTIFY DIFFERENT RESISTANCE GENES

E. G. Kuhlman[1]

Abstract.--Aeciospore isolates from six single fusiform rust galls varied in virulence towards 20 resistant loblolly pine families. Susceptibility of the pine families expressed as the percentage of seedlings with galls 6 months after inoculation, varied from immune (0% galls) to susceptible (77% galls). Some isolates were virulent on progeny of normally resistant pine selections, producing as many galls as on progeny of a susceptible selection. These isolates are relatively host specific and further host specificity should be possible by creating single spore lines.

The major benefit of identifying different resistance genes is to develop broadly based resistance in the pine population to reduce the possibility of catastrophic epidemics. An immediate benefit of identifying different resistance mechanisms is the gain we can expect in a breeding program that combines different resistance genes. Some controlled crosses between resistant trees have produced significantly more resistant progeny than do other crosses. The improvement appears to be due to combining resistance genes controlling different resistance mechanisms.

## INTRODUCTION

In basic plant pathology, virulence is defined as a quantitative measure of pathogenicity, i.e., the ability to cause disease. In plant breeding, virulence is a quality of a pathogen to overcome defense mechanisms (resistance) in a host plant. H.H. Flor's (1956) pioneering research on flax rust demonstrated that a single virulent gene enabled a pathogen to infect an otherwise resistant host. Hence, the gene-for-gene hypothesis: for every gene for resistance in the host plant there is a corresponding and specific gene for virulence in the pathogen. Two characteristics of plant parasite systems with known gene-for-gene relations are: (1) Resistance is usually dominant and controlled by single genes. (2) Virulence is usually recessive and controlled by single genes (Christ et al 1987). This situation is demonstrated in the following table.

[1] Principal Plant Pathologist, Southeastern Forest Experiment Station, USDA Forest Service, Athens, Georgia. This research was supported in part by a cooperative agreement with the Georgia Forestry Commission.

Table 1. Resistant (R) or susceptible (S) responses of four varieties of a plant to four isolates of a pathogen with various combinations of virulence (V) and avirulence (v) genes towards the resistance genes in the varieties.

| Pathogen | Plant | | | |
|---|---|---|---|---|
| | $R_1R_1$ | $R_1r_1$ | | $R_2R_2$ |
| | S | S | S | R |
| $V_1v_1$ | R | R | S | R |
| $V_1V_1$ | R | R | S | R |
| v2v2 | R | R | S | S |

Fusiform rust and various cereal rust fungi are macrocyclic, heteroecious rusts. They have five different spore stages and their life cycle is completed on two different host species. On flax and cereals, the urediniospores occur on the economically important host species. Urediniospores are repeating spores since they can infect other plants of the same host species (wheat to wheat). The repetition enables virulent strains of the rust to spread rapidly within a susceptible host population. Urediniospores are dikaryons with an N+N nuclear condition. These nonfused nuclei function as though they were fused. Thus in Table 1 the pathogen could have v1v1, Vivi, or V1V1. Plants with the R1R1 or R1r1 genomes would be resistant to the latter two genotypes but susceptible to the former, since virulence is a recessive trait. Cereal pathologists and geneticists monitor the rust population and then utilize varieties of wheat that are resistant to strains of rust present in wheat growing areas.

Loblolly and slash pines are infected by basidiospores of Cronartium quercuum (Berk.) Miyabe ex Shirai f. sp. fusiforme, the fusiform rust fungus. Spread from pine to pine does not occur. Basidiospores are products of meiosis and are haploid. If the gene-for-gene relationship is present in this host-pathogen system, then a basidiospore can be either only V or v towards resistance genes in the pine host. Avirulence towards resistance genes is usually more commonly encountered than is virulence. Only two of 56 single-gall isolates had virulence towards family 11-20 (Powers et al 1977). However, several authors (Powers et al 1977, Powers 1985, Snow et al 1970, 1975) have shown variations in virulence towards resistant pine families among isolates of fusiform rust. One of the reasons we more commonly find avirulence is that we often use mass inoculum composited from multiple galls. In the concentrated basidiospore spray (CBS) system we routinely use aeciospores collected from 8 to 30 galls. If virulence genes are present in only one of eight galls, the composite inoculum does not express the virulence (Matthews et al 1979, Powers et al 1977, Kuhlman in press). This failure to express virulence is analogous to expecting a mass pollination to show the superior pine growth from one of 8-30 pollen sources. In field progeny tests, trees are exposed to inoculum originating from many galls which could also mask the presence of virulence genes. However no field test nor any CBS test can make a regionwide evaluation of rust susceptibility of any set of pine families. There

simply is too much variation in the virulence of the pathogen population to make any such evaluation possible. What we can do is to select for rust resistance in field tests and CBS inoculation tests. Then virulent isolates can be used in CBS tests to identify selections with different genes or mechanisms for resistance.

The objective of the present study was to group rust resistant loblolly pine families on the basis of their variations in susceptibility to basidiospores from single-gall aeciospore isolates.

## METHODS

### Rust Isolates

Six single-gall aeciospore isolates with virulence towards resistant pine families were selected (Table 2). Virulence towards resistant loblolly (Pinus taeda L.) or slash pine (P.elliottii Engelm. var. elliottii) families had been demonstrated when the isolate caused a significant increase in the number of seedlings with galls over that caused by a control isolate.

Table 2. Response of resistant pine families to single-gall isolates as indicated by percentage of seedlings galled by the virulent isolate compared to those galled by isolates avirulent towards that resistance source (control).

| Pine family | Virulent isolate | Virulent/Control Galled(%) | Virulent/Control Ratio |
|---|---|---|---|
| Loblolly | | | |
| 10-5 | LHNC-2 | 83/42 | 1.98 |
| 11-20 | | 73/34 | 2.15 |
| 29R | 0-1 | 87/39 | 2.23 |
| 2318 | 152-102 | 46/35 | 1.31 |
| Slash | | | |
| 3327-13 | 3327-13 | 57/33 | 1.73 |
| 10-226 | 10-226 | 85/45 | 1.89 |

### Pine Families

Seeds from each of 21 loblolly pine families were stratified, germinated, and planted 20 seedlings per flat.

Experimental Design

21 Loblolly pine families
6 Rust isolates
126 Treatments

5 Replications/treatment
630 Flats

20 Seedlings/flat
12,600 Seedlings

Inoculation

Stored aeciospores of the six rust isolates were rehydrated prior to inoculating northern red oak (Quercus rubra L.) leaves. Basidiospores produced by these infections were concentrated at 50,000 per ml. for the CBS inoculation of the 6-week old pine seedlings. All 126 flats in a replication were inoculated on one day.

Data on symptom expression were taken 3 and 6 mo. after inoculation. A final reading will be made at 9 mo. The percentage of seedlings with galls after 6 mo. in each flat was transformed to an arc sin value prior to analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) comparisons.

## RESULTS

Six months after inoculation, seedlings with galls within the 126 family x isolate treatments varied from 0-77% (Table 3). Susceptible family 4666-4 had the highest frequency of seedlings with galls when Dnoculated with each of the six isolates (Table 4).

Table 3. Ranges and means by isolates of percentages of seedlings with galls 6 mo. after 21 loblolly pine families were inoculated with each of six single-gall aeciospore isolates.

| | Isolate | | | | | |
|---|---|---|---|---|---|---|
| Value | 0-1 | LHNC-2 | SC-35 | 152-102 | 3327-13 | 10-226 |
| Range | 0-70 | 19-77 | 5-71 | 3-77 | 12-73 | 0-74 |
| Mean | 28 | 41 | 33 | 37 | 34 | 29 |

The ANOVA indicated all sources of variation (replication, isolate, family and isolate x family) were highly significant (P>0.0001). Therefore DMRT were used to compare isolate within family and family within isolate differences.

Table 4. Effects of family (F) and fungal isolate (I) on the percentages of seedlings with galls in *21* loblolly pine families 6 months after inoculation with basidiospores derived from six single-gall aeciospore isolates of Cronartium quercuum f. sp. fusiforme.

| | | Fungus isolate | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Resistance | 0-1 | | | LHNC-2 | | | SC-35 | | | 152-102 | | | 3327-13 | | | 10-226 | | |
| Family | Source | % | F[a] | I[b] | % | F | I | % | F | I | % | F | I | % | F | I | % | F | I |
| 42R | 42R | 26 | B | b | 69 | A | ab | 26 | B | d-g | 50 | AB | bcd | 35 | B | b-g | 31 | B | b-f |
| 11-41 | 11-41 | 32 | AB | b | 39 | A | c-g | 6 | C | hi | 3 | C | h | 26 | AB | e-h | 20 | B | d-g |
| 11-20 | 11-20 | 32 | B | b | 42 | B | c-f | 63 | A | ab | 32 | B | c-g | 36 | B | b-g | 29 | B | b-f |
| 4666-4 | 4666-4 | 70 | A | a | 77 | A | a | 71 | A | a | 77 | A | a | 73 | A | a | 74 | A | a |
| 152-60 | TFS | 18 | A | b | 20 | A | g | 15 | A | ghi | 19 | A | fg | 17 | A | h | 23 | A | c-g |
| 151-791 | HH | 17 | A | b | 19 | A | g | 29 | A | d-g | 25 | A | efg | 20 | A | gh | 32 | A | b-f |
| 10-6 | 10-6 | 24 | A | b | 32 | A | efg | 34 | A | c-f | 40 | A | b-f | 28 | A | d-h | 38 | A | b-e |
| 153-190 | 10R | 19 | AB | b | 30 | A | efg | 33 | A | c-g | 28 | A | efg | 19 | AB | gh | 10 | B | gh |
| 153-517 | 7-56 | 26 | BC | b | 46 | A | c-f | 16 | C | fgh | 36 | AB | c-g | 28 | ABC | e-h | 13 | C | fgh |
| 10-5 | 10-5 | 30 | C | b | 60 | A | abc | 39 | ABC | cde | 51 | AB | bc | 52 | AB | bc | 35 | BC | b-e |
| 152-387 | 10-5 | 0 | B | c | 36 | A | d-g | 5 | B | i | 24 | A | efg | 31 | A | c-h | 0 | B | i |
| 153-424 | 29RX10-5 | 27 | A | b | 29 | A | efg | 19 | A | efg | 25 | A | efg | 26 | A | e-h | 7 | B | h |
| 29R | 29R | 55 | A | a | 39 | A | c-g | 54 | A | bc | 41 | A | b-e | 40 | A | b-g | 41 | A | bc |
| 151-576 | T605 | 29 | AB | b | 41 | A | c-f | 36 | AB | c-f | 18 | BC | g | 12 | C | h | 19 | BC | efg |
| 151-307 | 1495-35 | 29 | A | b | 25 | A | fg | 26 | A | d-g | 28 | A | d-g | 23 | A | fgh | 41 | A | bcd |
| 151-334 | T601 | 25 | AB | b | 37 | AB | d-g | 46 | A | bcd | 30 | AB | c-g | 19 | B | gh | 27 | AB | b-f |
| 151-627 | 2318 | 21 | B | b | 42 | A | c-f | 30 | AB | d-g | 43 | A | b-e | 38 | A | b-g | 23 | AB | c-g |
| 151-445 | 10-31 | 24 | B | b | 42 | AB | c-f | 26 | AB | d-g | 43 | AB | b-e | 46 | A | b-f | 35 | AB | b-e |
| 11-10 | 11-10 | 26 | A | b | 36 | A | d-g | 44 | A | bcd | 52 | A | bc | 51 | A | bcd | 49 | A | b |
| 1995-5 | 1995-5 | 30 | D | b | 51 | ABC | b-e | 37 | BCD | c-f | 59 | A | ab | 55 | AB | b | 32 | CD | b-f |
| 151-438 | 11-9 | 34 | B | b | 56 | A | bcd | 37 | AB | c-f | 53 | AB | bc | 47 | AB | b-e | 38 | AB | b-e |

[a]Within family treatments (horizontal comparisons) isolate percentages with the same letter (A-D) do not differ significantly according to Duncan's Multiple Range Test (P=0.05).

[b] Within isolate treatments (vertical comparisons) family percentages with the same letter (a-i) do not differ significantly according to Duncan's Multiple Range Test (P=0.05).

Some families had distinctly different responses to the six isolates. Family 42R (a selection by Harry Powers and John Kraus) was highly susceptible to isolates LHNC-2 and 152-102 but it was resistant to the other four isolates (Table 4). Note that family 42R's response to LHNC-2 was not different from susceptible family 4666-4's response to that isolate. Westvaco's family 11-41 was resistant to all isolates and highly resistant to isolates SC-35 and 152-102. Family 11-20 was as susceptible as 4666-4 to isolate SC-35 but it was resistant to the other five isolates. These were four distinct responses by the first four families. This is the type of identification of different resistance responses hoped for with virulent isolates.

Whereas family 4666-4 was uniformly susceptible to the six isolates, three families (152-60, 151-791 and 10-6) were uniformly quite resistant (Table 4). Families 153-190 and 153-517 were also quite resistant to all isolates but 153-517 was highly resistant to isolates 10-226 and SC-35, and 153-190 was highly resistant to 10-226.

Families 10-5 and 29R were most suceptible to isolates LHNC-2 and 0-1,respectively (Table 4). Family 10-5 was resistant to isolates 0-1, SC-35 and 10-226 whereas family 29R did not vary significantly in susceptibility to the six isolates. Tree 152-387 is an open-pollinated progeny of 10-5 that has produced highly resistant seedlings against our standard inoculum (3% with rust galls). In this study progeny of 152-387 were immune to isolates 0-1 and 10-226, highly resistant to SC-35, and resistant to the other three isolates. Tree 153-424 is a full sib of 29R and 10-5, its progeny have averaged less than 20% with galls with our standard inoculum. In this study family 153-424 was most resistant towards isolate 10-226, but was also very resistant towards all sources.

Both of these progenies of 10-5 in our seedling seed orchard have greater resistance to some of the six isolates than does 10-5. That is, the progeny of 152-387 were more resistant than those of 10-5 to all isolates except 3327-13. Similarly, progeny of 153-424 were more resistant than those of 10-5 to isolates LHNC-2, 152-102, 3327-13 and 10-226, and this family (153-424) was more resistant than 29R to 0-1, SC-35 and 10-226. In our routine screening with a composite isolate, we have tested 24 full-sib families of 29Rx10-5. Half of these, including 153-424, averaged galls on only 13% of the seedlings whereas the other 12 families averaged 41% with galls. These results suggest that half the families have resistance genes from both parents whereas the other half have resistance from only one parent.

Single-gall isolates were used in this study because they had shown enhanced virulence towards some resistant pine families. Single-gall isolates are probably quite heterogeneous, since a gall may have been initiated by more than one basidiospore and spermatization occurs annually to add new genomes. Studies are now underway to isolate single aeciospore lines of the pathogen each with a genome of two nuclei. Single-aeciospore lines will help us to determine if the gene-for-gene concept applies to the fusiform rust disease. Differences in virulence among these lines should be due to a few genes.

## CONCLUSION

Six virulent isolates of C. q fusiforme indicated many different sources of resistance among 21 loblolly pine families. This approach to examining sources of resistance should help to insure that diverse sources of resistance are present in rust resistance orchards. The fungus has a tremendous potential for variation so many sources of resistance may be necessary to prevent epidemic outbreaks of rust in putatively resistant plantings.

## LITERATURE CITED


Christ, B.J., C.O. Person and D.D. Pope. 1987. The genetic determination of variation in pathogenicity. Pp. 7-19. In: Wolfe, M.S., and Caten, C.E., Populations of Plant Pathogens: Their Dynamics and Genetics. Blackwell Scientific Publications. Oxford.

Flor, H.H. 1956. The complementary genic system in flax and flax rust. Adv. Genet. 8:29-54.

Kuhlman, E.G. In Press. Frequency of single gall aeciospore isolates of Cronartium quercuum f. sp. fusiforme highly virulent towards three resistant loblolly pine families. Phytopathology.

Matthews, F.R., Powers, H.R., Jr., and Dwinell, L.D. 1979. Composite vs. single-gall inocula for testing resistance of loblolly pine to fusiform rust. Plant Dis. Rep. 63:454-456.

Powers, H.R., Jr. 1985. Response of sixteen loblolly pine families to four isolates of Cronartium quercuum f. sp. fusiforme. Pp. 88-96. In: Proc. Rusts of Hard Pines Working Party Conference S2.06-10. IUFRO. University of Georgia, Athens.

Powers, Jr., H.R., F.R. Matthews, and L.D. Dwinell. 1977. Evaluation of pathogenic variability of Cronartium fusiforme on loblolly pine in the Southern USA. Phytopathology 67:1403-1407.

Snow, G.A., Dinus, R.J., and Kais, A.G., 1975. Variation in pathogenicity of diverse sources of Cronartium fusiforme on selected slash pine families. Phytopathology 65: 170-175.

Snow, G.A., and Kais, A.J., 1970. Pathogenic variability in isolates of Cronartium fusiforme from five Southern States. Phytopathology 60: 1730-1731.