Variation in Resistance of Slash Pine to Southern Fusiform Rust 1/

Southern fusiform rust caused by Cronartium fusiforme (Hedge. & Hunt) is widely recognized as the most serious disease of slash pine. The widespread incidence of this disease throughout the species range, and its reduction in timber production, leaves no doubt of the economic importance of fusiform rust.

Although most southern foresters can easily cite cases of plantations with 90% or more of the trees infected, natural selection for resistance to fusiform rust does not appear to be very strong. Some reasons for this condition are: (1) many diseased trees live long enough to pass on susceptible genes, (2) presence of alternate hosts is required and (3) weather conditions must be favorable for disease spread. These afford ample opportunity for highly susceptible trees to escape infection.

Thus; it appears that the most promising procedure for developing strains of slash pine with improved resistance to this disease is to artificially induce disease epidemics to locate resistant types.

This paper concerns early results of procedures used in the University of Florida Cooperative Forest Genetics Research Program to screen slash pine selections for resistance to fusiform rust.

In the Florida program, as in others, selections with observable disease were rejected. However, in a few cases small branch cankers were found in crowns of selected trees after they were established in seed orchards. But even if these trees are thrown out, there is little reason to suspect that the selected trees as a group have greater or less susceptibility than average dominant slash pines, i.e. they are probably representative of the general slash pine population in this respect.

PROCEDURES AND INOCULATION TECHNIQUES

In the spring of 1964 both seed and one-year-old progenies from 40 open-pollinated lines of slash pine selections were chosen for intensive testing. Seedlings of 90 additional lines were retained for limited tests.

For each of the 40 mother tree lines, 20 one-year-old seedlings were potted during January. In March, 30 seeds from each line were planted in 4-inch pots, six seeds per pot. (Germination within lines varied from 20 to 87 per cent, yielding from 6 to 26 cotyledon stage seedlings per line.)

Aeciospores were collected from slash pine cankers in the early spring and stored in a refrigerator. This material produced heavy infection when placed on new leaves of small oaks potted for this purpose. Within two to three weeks, numerous telial columns developed on plants dusted with aeciospores. The pathogen remained in the telial stage for an extended period. Extensive sporidial development was stimulated by keeping infected oak leaves on moist filter paper in Petri dishes at approximately 70° F for 24 hours. This procedure made sporidia production possible at any time desired.

A humidity chamber - a wooden frame covered with canvas - was constructed. A mist

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system inside and an external sprinkler maintained high humidity and provided some cooling by evaporation.

In late April, approximately six weeks after germination, the newly germinated seedlings were placed in the humidity chamber during late afternoon. A section of an oak leaf having abundant sporidia was placed between cotyledons on the apical meristem of each seedling. The mist system was turned on for approximately one hour. The external sprinkler was started and maintained for the entire 48-hour inoculation period.

Second-year-potted seedlings of the same lines were inoculated using the same procedures except that it was necessary to divide the 800 seedlings into four blocks for inoculation in four consecutive periods (five trees of each line per block).

Supplementary trials of inoculation procedures on one-year-old potted seedlings were conducted. The techniques included wrapping moist cotton around infected oak leaves placed on succulent new growth, and inserting leaf sections in incisions made in new growth. Jewell (1960) has used these methods successfully.

Seedlings in the nursery were shaded with a light cotton cloth. Entire infected oak leaves were attached to new growth of each of 10 trees from 31 lines. Of these, five trees of each line were encased in polyethylene bags for two days and the other five were left open. On a number of other lines in the nursery, only the open treatment was attempted.

The nursery bed treatments were applied last and were not completed until May 20. Local inoculum was exhausted by that time, and infected leaves from the vicinity of Albany, Georgia, were used for this phase. The oak leaves were transported in a styrofoam cooler with a thin layer of ice on the bottom. Sporidial development under these cool, moist conditions was excellent.

A tally of infection from cotyledon stage inoculation was made in July and again in late October. The first tally was based primarily on needle symptoms but some galls were forming already. In the second tally, only seedlings with actual galls were classed as diseased.

Infection of seedlings one-year-old at inoculation time was recorded in November and rechecked the following April (disease development progresses more slowly in the older material).

RESULTS

Cotyledon stage inoculations

Seedlings inoculated during the cotyledon stage showed symptoms of infection in July; the different mother tree lines varied from 42.9 to 95.7 per cent infected. Chi-square analysis indicated highly significant heterogeniety of infection rates. Individual lots with infection rates of 50 per cent or less were significantly lower, and those with infection rates above 90 per cent were significantly higher, than the general population tested. Thus, three of the 40 lines of 7.5 percent had a significantly lower incidence of initial infection by fusiform rust (higher resistance) than other lines tested.

The second tally in October showed that some of the seedlings which had definite symptoms including needle lesions and red coloration in July had failed to develop a gall and were apparently healthy. This occurred in 13.7 per cent of initially infected seedlings and was noted in as high as 45.0 per cent of the infections in some mother tree lines. The correlation coefficient for July and October determinations was 0.823. There were significant differences among lines in what might be termed secondary resistance following initial needle infection. Ten per cent of the lines tested had significantly higher resistance of this type.

In July, mean infection rate was 71 per cent. Because of the secondary resistance, only 62 per cent of the seedlings developed galls; rates of galling ranged from 31.6 to 91.3 per cent.

For "all or none" traits such as this where measurement of character expression on an individual plant is not feasible, Robertson and Lerner (1949) and Dempster and Lerner (1950) have proposed these statistical methods for heritability calculations. The formula,

$$\text{heritability} = \frac{\text{genetic improvement}}{\text{phenotypic selection differential}}$$

may be expressed in statistical terms as:

$$\text{heritability} = \frac{\text{heterogeniety Chi square} - (N-1)}{r\ N_o}$$

where N is the number of families, r is the genetic relationship within families, and

$$N_o = \Sigma n - \frac{\Sigma(n^2)}{\Sigma n} - (N-1),$$

n being the total individuals per family. The application of this method to fusiform rust resistance was discussed previously by Arnold (1964) and Goddard and Arnold (1964). Heritabilities calculated were:

Resistance to initial infection--.199 ± .092

Resistance to disease development after infection--.253 ± .121

Combine resistance to gall formation--.237 - .100

Table 1. Comparison of fusiform infection rates on seedlings of two ages and with various inoculation techniques.

Inoculation Method	No. of Plants	Per cent Infected
Cotton-wrap (year old)5/	50 1/	16.0
Oak leaf insertions (year-old)5/	50 1/	24.0
Humidity Chamber (year-old)5/	800 2/	8.75
Humidity Chamber (cotyledon stage)5/	719 2/	62.30
Nursery bed, polyethylene bag6/	156 3/	15.40
Nursery bed, unbagged6/	582 3/	27.30
Humidity Chamber1/	100 1/	7.00
Nursery bed, polyethylene bag	151 4/	15.20
Nursery bed, unbagged	151 4/	27.80

1/ Seedlings of mixed origin.
2/ Seedlings of 40 mother tree lines.
3/ Seedlings of 133 mother tree lines.
4/ Seedlings of same mother tree lines.
5/ All inoculated in April with same inoculum.
6/ All inoculated in May with same inoculum.

Table 2. Infection rates of 22 progeny lines inoculated with fusiform rust at two ages.

	Cotyledon stage in humidity Chamber	Year-old in Nursery bed	Year-old in Humidity Chamber
240-55	43.7	20.0	15.0
270-55	71.4	40.0	00.0
283-55	61.1	00.0	10.0
284-55	81.8	40.0	10.0
289-55	81.8	00.0	10.0
78-56	57.1	80.0	15.0
80-56	42.9	00.0	15.0
106-56	50.0	00.0	00.0
108-56	85.7	00.0	05.0
110-56	55.6	00.0	00.0
116-56	83.3	20.0	10.0
216-56	60.0	33.3	25.0
235-56	70.8	60.0	20.0
269-56	36.4	20.0	20.0
339-56	41.7	40.0	05.0
345-56	90.0	20.0	10.0
18-57	83.3	40.0	10.0
21-57	87.0	60.0	15.0
51-57	64.7	100.0	05.0
65-57	52.6	80.0	05.0
67-57	57.9	20.0	00.0
93-58	61.1	20.0	05.0
Mean	64.9	31.9	9.5

Inoculation of year-old seedlings.

Inoculations of year-old seedlings were not nearly as successful as inoculations during cotyledon stage. Potted seedlings of the same parentage and inoculated with the same inoculum in the canvas chamber in the same manner as the newly germinated ones had an average infection rate of only 8.75 per cent. Because of the spotty infection rates, no differences among lines could be detected.

Both the cotton wrap and oak leaf insertion techniques gave a somewhat higher infection than the humidity chamber. However, resistance to penetration is eliminated by the incision and for that reason the insertion method is inferior.

Nursery bed inoculation is more promising for testing year-old seedlings. The overall infection rate for seedlings not bagged with polyethylene was 27.3 per cent. Nursery bed seedlings bagged had an infection rate of 15.4 per cent. Several comparisons of techniques used on progenies of the same lines and inoculations made during the same period are possible. Although these data do not lend themselves to statistical analysis, comparisons of interest are presented in Table 1.

A detailed listing of infection rates in progenies of 22 lines which were inoculated at two ages and in both the humidity chamber and nursery bed are presented in Table 2. Insufficient trees per line were inoculated in nursery beds for a good definition of line differences although an indication of trends was expected. Correlations between infection rates within lines under these various conditions were extremely low and nonsignificant.

DISCUSSION

It is very obvious that infection rates were far higher when inoculations were made on cotyledon stage seedlings than when made on year-old seedlings. Jewell (1960) reported infection rates on one-vear-old seedlings higher than obtained here, perhaps because he worked with smaller numbers and was more experienced with inoculation techniques. Of the methods tried on older seedlings, the most successful was inoculation of seedlings retained in nursery beds even without means of maintaining high humidity.

Work with cotyledon stage seedlings indicated highly significant differences in rates among progenies of the various selections in both initial infection and actual gall formation, However, a continuous variation of the quantitative type was suggested by these data rather than simple major gene differences as may explain species differences (Jewell, 1964).

There appeared to be a reduction in susceptibility of older seedlings and a lack of correlation between relative infection of lines inoculated at both ages. Possibly improved techniques would increase the infection of older seedlings. Year-old seedlings*potted a tew months prior to inoculation attempts had a less vigorous growth and, consequently, less susceptible tissue than either cotyledon stage seedlings or nursery bed seedlings one year old. Humidity and temperature conditions during the nursery bed inoculations were not ideal for the pathogen.

On the other hand, it is entirely possible that resistance increases with seedling age and that the mechanism of resistance changes. With proper attention to fungicide application, fusiform rust can be almost completely eliminated from a southern pine nursery. However, protection of plantations is not practical. If the mechanism of resistance is different with older seedlings, selection of a strain resistant during the cotyledon stage could be of little value in combating plantation infection.

Future plans in the Florida program include development of methods and testing of year-old progenies. In the spring of 1965 additional nursery bed inoculations were applied. To obtain conditions more favorable for the pathogen, a portable humidity chamber was prepared. It consists of a light metal frame and mist system covered with canvas and an external sprinkler hose to keep the canvas wet. Approximately 25 seedlings per line of 50 mother tree lines were inoculated. In addition, seedlings of 50 crosses between select trees were inoculated to gain more insight into the inheritance of resistance. Definitive data on fusiform resistance should be obtained. If the 1965 inoculations are successful, these tests will be expanded in the future to determine relative susceptibility or resistance of progenies of all selected slash pines used in the program. This would provide a basis for development of a clonal orchard for resistant types and for a breeding program to obtain greater resistance.

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