EARLY IDENTIFICATION OF FUSIFORM RUST RESISTANT SLASH PINE FAMILIES THROUGH CONTROLLED INOCULATION

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The impact of fusiform rust on many slash pine plantations needs no further elaboration. A viable alternative to susceptible planting stock is urgently needed. As a temporary expediency, planting a resistant species such as shortleaf pine in certain areas where high infection rates can be anticipated could be advantageous. In the long run, however, for sites on which slash pine is the most productive species, resistant varieties of slash pine should be developed.

As discussed in the previous paper by Schmidt and Goddard (1971), some fairly resistant lines of slash pine are being identified in regular progeny tests. However, there are frequent inconsistencies in comparing results from different tests. Although some field tests provide useful information, in many other tests the disease incidence is so low that little or no reliance can be placed on family rust ratings. Also, at best three, or preferably, five years are required for disease evaluation.

To provide rust data for all select families used in the University of Florida Cooperative Forest Genetics Program, and to obtain this information at a more rapid pace than possible through the standard progeny testing program, a special rust screening project is underway. Because they presumably already have other desirable traits, progenies of seed orchard clones receive first consideration. Other potential sources of rust resistance are also being tested.

PROCEDURES

The current screening program *was* started in 1969 on a modest scale to test procedures and has been continued annually with slight modifications. Seed resulting from open pollination in orchards (half-sibs) were pregerminated in petri dishes and planted in small peat pots. The pots were arranged in flats, each flat containing 48 seedlings. Randomly placed in each flat were single seedlings from 46 families plus seedlings of one line which had repeatedly shown very high susceptibility in field tests, included as a susceptible check. Fifty such flats of seedlings were moved in mid-May, approximately two months after germination, to shelves in a screening shed constructed for maintenance of high humidity.

Telia-bearing oak leaves were distributed on wire frames directly over the seedlings. To assure an ample supply of infected oak leaves, locally collected aeciospores were used to inoculate expanding laurel and water oak leaves during the spring a few weeks prior to pine inoculation. Moist muslin was draped over the wire frames and shelves, and canvas over the entire shed

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was kept wet, maintaining a relative humidity within the shed of 100%. Glass slides were placed in each flat to monitor the number and distribution of Cronartium fusiforme sporidia. Seedlings were kept in the shed for 72 hours and then placed in nursery beds.

The same seedlings were again inoculated at approximately one year of age in the nursery beds by similar procedures. Metal frames were placed over the nursery beds to support the frames of oak leaves, and the entire outer frame was covered with canvas. The covering tent was kept wet to maintain high humidity around the seedlings. Slides for spore counts were again distributed through the bed.

Gall counts were made the winter after shed inoculation and again in December after the nursery bed inoculations. Separate tally by gall location identified infection from shed or nursery inoculation. Data are presented as percent of trees galled in each family.

For 1970 shed inoculations, seedlings were grown on Jiffy 7 peat pellets. The flats hold 7 rows of 10 pellets. Half-sib families were placed in 10-tree rows. There are six shelves in the shed and each shelf constituted a block. Row-plots were randomized on each shelf.

Included were half-sib families of 163 select slash pines and 138 half-sib families from a special rust free seed production area. As 10-tree plots of over 300 families repeated six times exceeded the capacity of the inoculation shed, three groups of progenies were inoculated in sequential inoculations during the first two weeks of April. In Group I were 112 select tree families plus 25 half-sib families fram the special seed production area. Group II consisted of 113 families from the seed production area. In Group III were 51 families of Buckeye Cellulose Corporation selections grown by them and brought to the shed for inoculation. The susceptible check was included in each group.

The special rust free seed production area referred to above was established by Brunswick Pulp and Paper Company in a young slash pine plantation severely infected with fusiform rust (over 90% of trees with galls). All infected trees were removed. Residual trees are now producing cones and open-pollinated progenies of individual trees in this stand were inoculated as described above.

A third phase of the rust screening project is establishment of seedlings from the same families (not the identical seedlings used in shed and nursery phases) in four high risk locations--one each in east Georgia, west Georgia, north Florida and south Alabama. Initial plantings of these tests were made in 1971. No data are yet available and evaluation will not be completed until at least three years after establishment.

Testing for rust resistance of progenies of the several hundred clones used in the Florida program will not be completed before 1975, and ultimate success of the project must be based on reliable identification **of resistant** lines. However, useful preliminary data from the controlled inoculation phases are already being generated.

RESULTS

Comparison of Shed and Nursery Inoculations

Results of both shed and nursery bed inoculations are available for the 46 select families initially inoculated in 1969. Mean infection resulting from shed inoculation was 48.1% with individual family means ranging from 22.5 to 69.2%. Infection resulting from inoculation at age 1 year averaged 59.3%, ranging from 15.4 to 88.5%. In both cases, there were significant differences among individual families. Family rust percents from the two inoculations had a correlation coefficient (r) of 0.52 (Figure 1). This

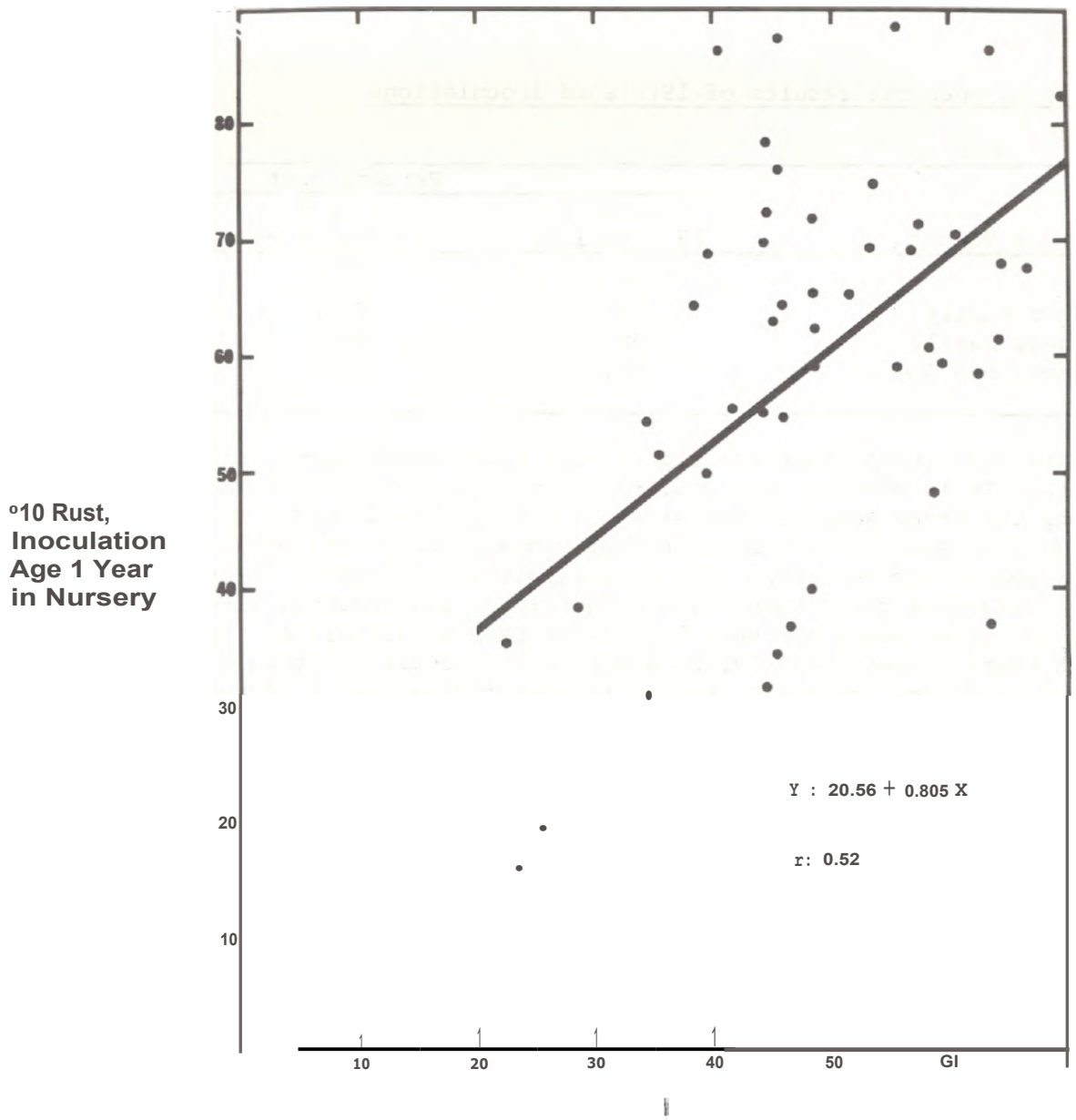

Figure 1.--Percent rust infection of individual slash pine select tree families inoculated at 6-8 weeks following germination plotted against percent rust infection of the same individuals inoculated at age I year.

value with 45 degrees of freedom is highly significant but does not indicate that resistance to infection during the primary needle stage is highly predictive of resistance at the later developmental stage. However, at least two of the families had consistently low infection following both inoculations. Additionally, five of the ten families with lowest infection rates as young seedlings were among the ten lowest when inoculated at one year of age in the nursery bed. Similarly, most of the families with high susceptibility in the shed had high rust percent from nursery inoculations.

1970 Shed Inoculations

General results of inoculations in the shed in 1970 are summarized in Table 1.

Table 1.--General results of 1970 shed inoculations

	Percent Rust		
Mean Values	Group I 137 families	Group II 113 families	Group III 51 families
Test	40.7	53.4	77.5
Lowest Family	7.7	22.2	44.8
Highest Family	80.7	85.7	91.7
Susceptible Check	51.7	73.1	69.2

Within each group there are significant differences among infection percents. It is obvious that uniformity in infection percent was not achieved among the three separate inoculations. Yet, in each case, sufficient mo-culuni with satisfactory distribution was applied to differentiate among the families in any one test. The susceptible check, chosen on the basis of high infection rates under field conditions, was somewhat disappointing as it was seldom among the very highest in rate of infection. However, in each block of each inoculation group, a substantial portion of susceptible check seedlings had galls, giving further indication of adequate quantity and distribution of sporidia.

In the first inoculation group, 25 families from the Brunswick Pulp and Paper Company seed production area were included along with 112 select families. The average infection rate of the progenies of special rust free selections was 41.6% in contrast to 39.8% for the plus tree families. It appears that neither the general resistance level nor frequency of highly resistant families was greater for rust selections than for the general plus tree selections, at least in the early primary needle stage.

Relationship to Field Test Results

Data on rust from natural inoculation in regular progeny tests were available for approximately 70 of the families included in 1970 shed inoculations. Although only a few of these families were in any one test or series of tests, comparisons of rust infection percentages under the two

conditions do indicate trends. Correlation coefficients were calculated using family rust infection percents for lines inoculated in contrast to results in a single field test or the mean rust percents in a series of field tests (Table 2). The r values were rather variable, but the values

Table 2.--Correlation coefficients comparing rust infection percents of families common to field progeny tests and 1970 shed inoculations

Field Progeny Tests	No. of families in common	Mean Infection		
		Shed	Field Tests-1/	
Test 6-4	7	42.5	10.1	.67
Test 6-6	7	42.5	8.7	.78
Test 6-7	7	42.5	9.9	-.27
Means, Tests 6-4, 6-6, 6-7	7	42.5	9.6	.83*
Means, Tests 6-10, 6-15	5	34.4	20.0	.71
Means, Tests 1-6, 1-7	6	39.6	21.7	.32
Means, Tests 1-8, 1-9	12	35.3	15.6	.61*
Means, Tests 1-8, 1-9 1-12, 1-13	6	31.1	20.7	.56
Means, Test 4-3, 4-4, 4-5	5	44.5	62.1	.44
Means, Test 7-6, 7-7, 7-8, 7-9	6	38.2	31.2	.66

* Significant at .05 level

1/ Means are for the common families, not for the entire test.

for series of tests were quite high in most cases. If the mean infection in several tests is the best estimate of relative resistance under field conditions, it appears that controlled inoculation gave a fairly good indication of relative resistance. For families not adequately field tested, shed inoculation results can be accepted with some confidence.

DISCUSSION

On the basis of two years results from shed inoculations and one year results from nursery inoculations, it is evident that there is substantial genetic variation in slash pine in respect to susceptibility to fusiform rust and that relative susceptibility can be differentiated by the procedures used. There is no evidence of complete resistance among the slash pine families tested, but some lines are consistently low in percentage of trees infected.

The significant correlation coefficient for infection percents from shed and nursery inoculations indicates fair consistency of performance at the two stages of development. The repeatability was not as high as would be desired. However, utilizing data from 1969 shed inoculations and from inoculations of the same seedlings in nursery beds one year later, resistant families were identified.

Inoculation during the primary needle stage also gives a fairly good indication of families more likely to have reduced infection rates under field conditions. Correlation coefficients for rust following shed inoculation versus natural inoculation, although with limited numbers of families, indicated a rather strong positive agreement, especially when mean values for several field tests were used. Results obtained are in general agreement with those reported by Dinus (1968) for six slash pine families compared for inoculation under artificial and natural conditions.

Rather intensive selection against fusiform rust susceptible trees in an area of high infection was disappointing. The proportion of trees with high levels of resistance among progenies was no greater than with general plus tree selections. These results are in direct contrast to a similar study reported by Dinus (in press) in this conference. The conflicting findings may in part be related to test locations. In Dinus' study, phenotypic rust resistant selections were made in Mississippi and progenies inoculated with rust from Mississippi. In our case, selections were made in a stand near Jessup, Georgia, and the progenies were exposed to rust from Gainesville, Florida. Thus, there is possibility of variation in the rust fungus. Also, data to date are related only to susceptibility of progenies during the primary growth stage and perhaps increased resistance during later developmental stages will become apparent.

There are some suggestions of changes in relative resistance with increased age. Some lots appear to be much more susceptible at the primary needle stage than at later stages of development. The reverse may also be true with some lots seemingly increasing in susceptibility with age. There is enough shifting of relative resistance level at later stages to question selection for resistance solely on the basis of early inoculation. Variation in relative rust infection between tests, either under natural or controlled conditions, is enough to doubt the reliability of any single test. However, in conjunction with data from natural inoculations at later stages of development, early inoculations provide substantial help in identification of resistant seed sources.

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