# TESTING SLASH PINE FOR RUST RESISTANCE IN ARTIFICIAL AND NATURAL CONDITIONS

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Field tests to locate southern pines resistant to fusiform rust (*Cronartium fusiforme* Hedgc. & Hunt ex Cumm.) are costly, time-consuming, and often unreliable (Henry and Jewell 1963; Kinloch and Kelman 1965; LaFarge and Kraus 1967). Hence, Jewell (1960) devised an inoculation technique in which telia-bearing oak leaves are suspended over pine seedlings in the cotyledon stage grown under controlled conditions of temperature and moisture. The technique was subsequently modified (Jewell and Mallett 1964, 1967) to increase its capacity and efficiency.

This and related approaches (Arnold and Goddard 1966; Davis and Goggans 1968; Driver et al. 1966) attempt to provide favorable conditions for infection and uniform, abundant supplies of inoculum. The test may be too severe, however, since selections having slight but useful resistance might be eliminated. Since resistance to some diseases varies with age (Patton 1961), tests on juvenile material could be unrealistic. Furthermore, testing during a single life stage with more or less local inoculum could result in selection for specialized rather than generalized resistance (Smith 1968).

This paper describes the relationship between infection rates under artificial conditions and those observed in field plantings at a number of locations and after several years of exposure. Results indicate that response to artificial inoculation is a reliable index of field performance; both types of tests, however, are valuable in breeding for rust resistance.

## MATERIALS AND METHODS

Six slash pines (*Pinus elliottii* Engelm.) were selected in Harrison County, Mississippi, for use in artificial and field inoculation tests (Jewell and Mallett 1967). Three were rust-free (8-7, 11-6, and 18-27), and three were infected (18-40, 18-41, and 18-62). The natural stands involved were about 75 percent infected. Open-pollinated seed was collected over a period of several years from each of the six selections.

Dr. F. F. Jewell[2/] artificially inoculated open-pollinated progenies in identical randomized block designs in 1963 and 1964. Each family was represented by two rows of 18 trees in each of five blocks. Proportions of plants infected per row were determined 9 months after inoculation. Results of one of the individual experiments were reported previously (Jewell and Mallett 1964). For presentation here, the original data were reanalyzed as separate tests and as a single experiment combining both years. The proportion of plants infected was

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transformed to degrees arc sine and subjected to analysis of variance for the randomized block design with more than one observation per experimental unit (Steel and Torrie 1960). Family means were compared by multiple range tests at the 0.01 level.

Field tests of open-pollinated progenies from the same selections were established by Jewell in 1963 at Gulfport, Mississippi, and in 1964 on Crown Zellerbach Corporation land near Bogalusa, Louisiana. The Gulfport planting contains progenies from all six selections in a randomized block design. Each family is represented by two rows of 15 trees in each of seven blocks. The Bogalusa planting consists of five blocks each containing two rows of 15 trees; five families are represented.

Survival, total height, and rust infection were measured annually. Rust infection was observed in terms of three indices: number of stem infections per plant, total number of infections per plant, and proportion of plants infected per row. The proportion infected in the latest year was also analyzed after plants previously infected but currently rust-free had been deducted. Percent of survival and the proportion infected were transformed to degrees arc sine. Row means for the six variables were subjected to analysis of variance for the randomized block design with more than one observation per experimental unit. A combined analysis of both locations was not attempted as the effect would have been confounded with different planting dates. Family means were compared by multiple range tests at the 0.01 level. _In addition, simple correlations were calculated to determine if survival and growth were associated with infection and which index best measured infection.

RESULTS

Artificial inoculation.--Variation among families was significant in both 1963 and 1964. In both years, the proportion of diseased plants from one rust-free and the three infected selections was greater than from the remaining rust-free selections (fig. 1). The combined analysis confirmed the significance of variation among families. However, both the year x family interaction and differences between years were nonsignificant.

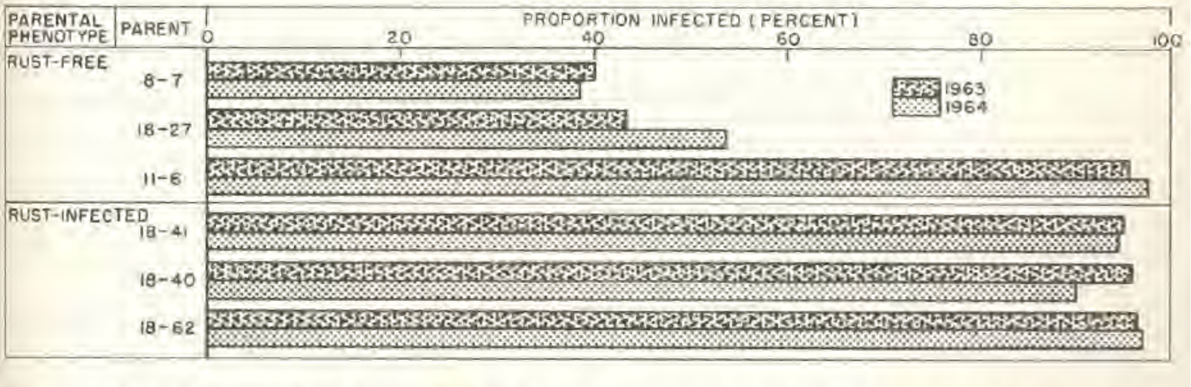


Figure 1.--Artificial inoculation of open-pollinated slash pine seedlings, 1963 and 1964.

Field survival.--Survival at the latest measurement varied significantly among families at Bogalusa, but not at Gulfport (table 1). Mortality was heaviest shortly after planting. Simple correlations between survival and the three indices of infection were nonsignificant.

Table 1.--Mean percentage survival of open-pollinated slash pine progenies

| Parent | Bogalusa, year 4 | Gulfport, year 5 |
|---|---|---|
| 18-27 | 1/86.6 | 67.7 |
| 8- 7 | 86.2 | 88.0 |
| 18-40 | 79.7 | 83.5 |
| 18-41 | ... | 82.5 |
| 11- 6 | 57.3 | 85.1 |
| 18-62 | 54.1 | 84.3 |

1/ Values connected by the same vertical lines do not differ significantly at the 0.01 level according to Duncan's test.

Total height.--Differences among families in total height became apparent after 3 years in the field. After 4 years at Bogalusa, variation among families was significant. A rust-free selection, 11-6, produced the slowest growing progeny. Their mean height was significantly less than that of the tallest family (fig. 2). Differences among families after 5 years at Gulfport were not significant. Regardless of age or location, progenies of both rust-free and infected selections were among the fastest growing lines. Simple correlations between infection rates and height or average annual height increment during the first 3 years were nonsignificant.

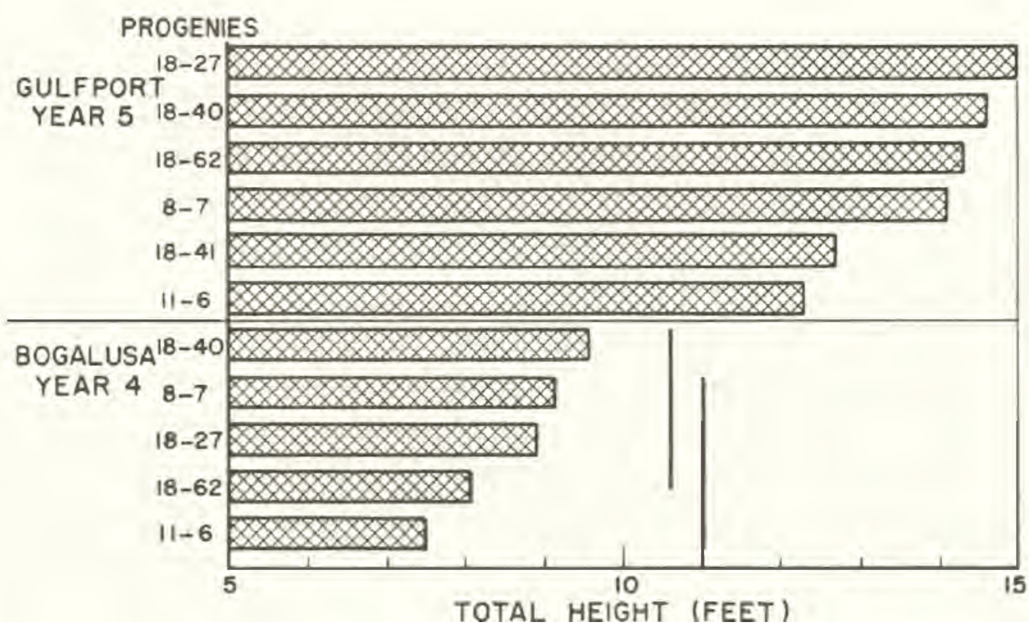


Figure 2.--Mean height of open-pollinated slash pine progenies at two locations. Values connected by the same vertical line do not differ significantly at the 0.01 level according to Duncan's test.

Stem infections.--Stem infections appeared as early as 1 year after planting at both locations. Since then, the number per plant has increased steadily and significant differences among families were noted after 2 years of exposure. Although few changes in family ranking have occurred since, considerable variability was encountered within families. For example, the coefficient of variation at Gulfport ranged from 108 percent during the second year to 65 percent in the fifth year. This high error rate indicates that distinguishing between resistant and susceptible lines required at least 4 to 5 years of exposure. By then, progenies of 18-40 and 18-62 had more stem infections than those of any other selection (table 2). In addition, progeny of 8-7 at Gulfport had fewer stem infections. Simple correlations between numbers of stem infections and "ther infection indices were significant at the 0.01 level.

Table 2.--Mean numbers of infections per plant among open-pollinated slash pine progenies

| Location | Family | Number of infections per plant | |
|---|---|---|---|
| | | Stem | Total |
| Gulfport, year 5 | 18-62 | 1/ 0.65 | 1.71 |
| | 18-40 | .60 | 1.72 |
| | 18-27 | .36 | .87 |
| | 11- 6 | .32 | .62 |
| | 18-41 | .31 | .78 |
| | 8- 7 | .02 | .04 |
| Bogalusa, year 4 | 18-62 | .42 | 1.46 |
| | 18-40 | .40 | 1.90 |
| | 18-27 | .20 | 1.19 |
| | 11- 6 | .14 | .52 |
| | 8- 7 | .04 | .05 |

1/ Values connected by the same vertical lines do not differ significantly at the 0.01 level according to Duncan's test.

Total infections.--Increases in the total number of infections per plant occurred each year and were consistently larger for progenies of rust-infected selections. However, the frequency of new infections fluctuated widely from year to year and considerable variability existed within families. Coefficients of variation decreased with years of exposure, but were never less than 41 percent. However, differences among families have been significant since the second year and family ranking has changed only slightly since the third year. At Gulfport after 5 years of exposure, progenies of 18-40 and 18-62 had more infections than those of all other selections (table 2). Progenies of 18-41, 18-27, and 11-6 had fewer infections than these, but more than those of 8-7. A similar pattern emerged at Bogalusa after 4 years (table 2). Progenies of 8-7 and 11-6 had fewer infections than any others. Simple correlations between total infections and the proportion infected were significant at the 0.01 level.

Proportion infected.--Annual increases in numbers of newly infected plants varied between years and locations (fig. 3). However, family rankings have been similar at both locations since the first infections were noted. Initially, variation within families was large, but coefficients of variation have been less than 30 percent since the third year. Consequently, differences which were evident among families within 2 years of planting have become more definite. At last measurement, variation among families was significant. Regardless of location, fewer progeny of 8-7 were infected than of any other selection (fig. 3). On the other hand, progenies of 18-62 and 18-40 were by far the most frequently infected. Progenies of one rust-infected (18-41) and two rust-free selections (18-27 and 11-6) differed significantly from both the best and worst families and exhibited intermediate levels of infection at both locations. When plants previously infected but rust-free at the last measurement were deducted from the analyses, results were essentially the same (fig. 3).

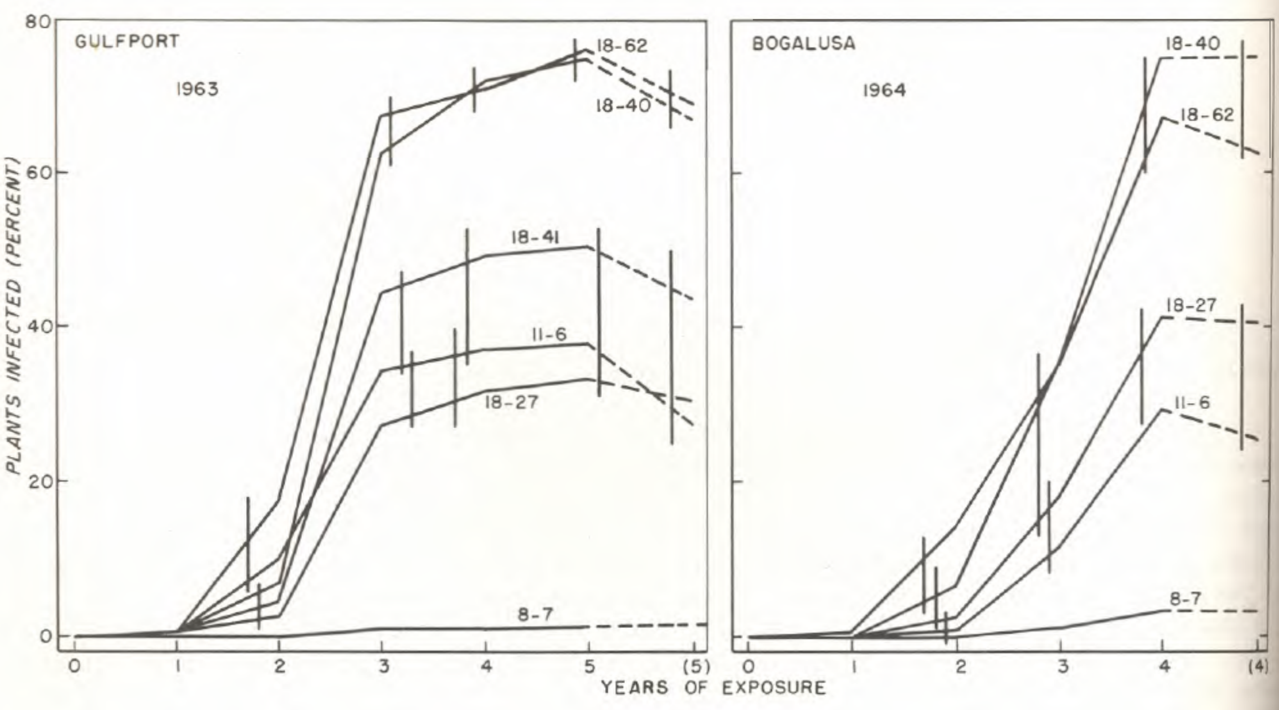


Figure 3.--Mean proportions of plants infected (percent) among open-pollinated slash pine progenies at two locations. Values connected by the same vertical line do not differ significantly at the 0.01 level according to Duncan's test. Years in parentheses denote results after plants previously infected but now healthy were deducted.

## DISCUSSION AND CONCLUSIONS

The three indices of infection gave the six families similar ratings and were significantly correlated. The proportion infected seemed the most reliable index. A curvilinear relation existed between total infections and the proportion of plants infected similar to that found for loblolly pine, (*Pinus taeda* L.), (Kinloch 1968). That is, infections per plant tended to increase faster than the number of infected plants. This relation suggests that infections per plant may be more sensitive in heavily infected areas or when families concerned have only slight resistance. However, this trend did not appear until the fourth and fifth years. In addition, large error terms were encountered in analyses based on stem and total infections. Results from the proportion of plants infected paralleled those obtained after adjustment for plants previously infected but not showing infection at the last measurement. This agreement demonstrates that a single scoring 4 or 5 years after planting can provide a realistic estimate of field performance, at least in controlled, well-replicated plantations.

Combined analysis of the two artificial inoculation tests demonstrated that the technique yields consistent results. Progenies of 8-7 and 18-27 resisted artificial inoculation and performed better than average in the field (fig. 4). While not best in the field, progeny of 18-27 were significantly more resistant than the worst families. Since both these selections demonstrated heritable resistance in both types of test, it appears that they can be used safely in breeding programs as suggested by Jewell and Mallett (1967). This conclusion seems especially sound as these were open-pollinated progeny from a rust-free selection in a stand that was 75 percent infected. Even better field performance can be expected from crossing individuals thus identified. For example, progeny from the cross between 8-7 and 18-27 or its reciprocal averaged only 10 percent infection in a series of artificial inoculations (Jewell and Mallett 1967). Consequently, selection and breeding on the basis of artificial inoculation results should yield rapid improvement. Judgments based on artificial inoculation results err only on the safe side. Of the progenies resistant to artificial inoculation, none were found susceptible in the field (fig. 4) even after several years of exposure at two locations. While some potentially useful selections may be overlooked, susceptible ones will not go undetected.

According to the nursery test (fig. 1), the infected selections, 18-62, 18-40, and 18-41, are undesirable for inclusion in breeding programs. The presence of 18-62 and 18-40 in the upper right quadrant of figure 4 supports this conclusion and further demonstrates the close agreement between artificial and field testing. While 18-41 seems undesirable at first glance, its intermediate field performance (fig. 3) makes outright rejection questionable. Assuming that resistance is controlled by additive genes (Kinloch 1968) or at least not by a single dominant gene (Jewell and Mallett 1967), this intermediacy suggests that 18-41 possesses some genes for resistance. That is, it has a larger complement than 18-62 or 18-40, but smaller than 8-7. Data from previous artificial inoculations (Jewell and Mallett 1967) support this inference. Progeny from the cross between 8-7 and 18-62 were 44 percent infected. Crossing 8-7 with 18-41, however, produced progeny which were only 23 percent infected.

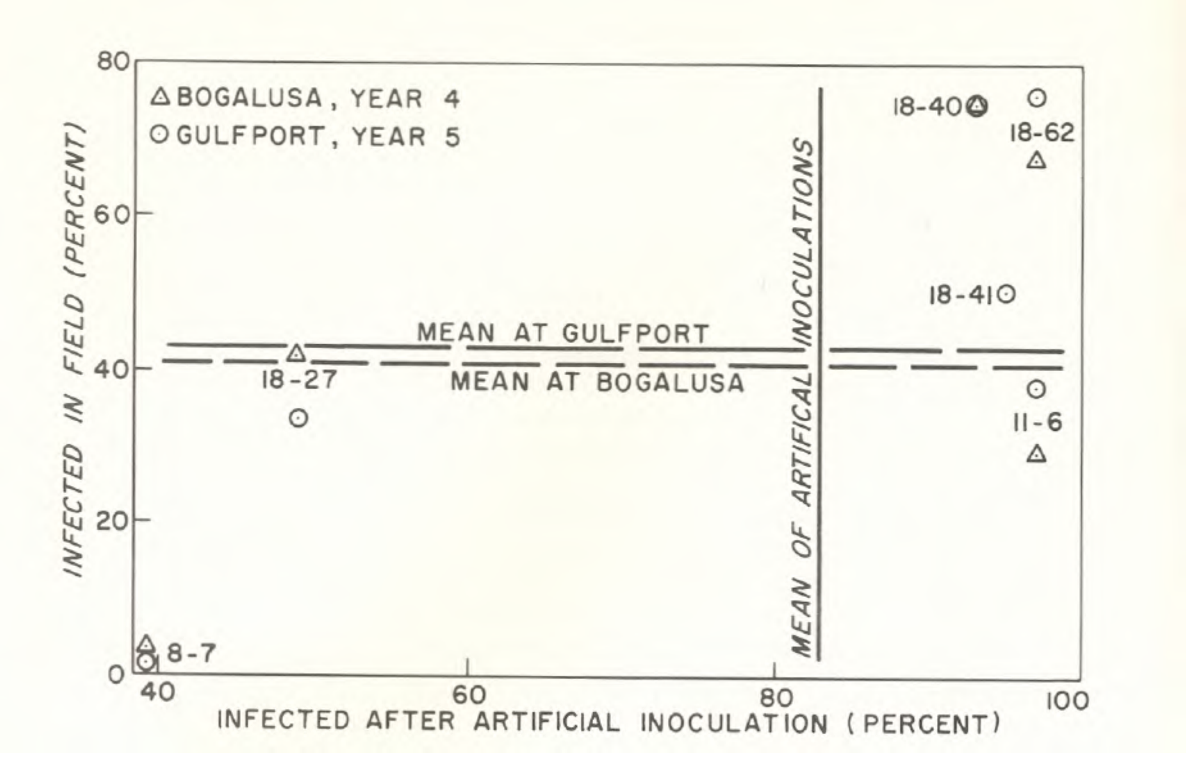


Figure 4.--Relationship between the results of artificial and natural inoculations of open-pollinated slash pine progenies.

Relying on the artificial testing of open-pollinated progenies would have eliminated 18-41. Thus, such tests may be too severe, but they identify the most resistant selections several years before field tests would. The accumulated data suggest a means of circumventing this weakness. For example, partially resistant and potentially useful selections such as 18-41 can be identified by screening control-pollinated progenies or at least progenies of crosses between candidates and specific tester parents like 8-7. Partially resistant selections thus could be located among individuals more desirable from other aspects. Parents with appropriate degrees of resistance could be identified to reforest areas of varying rust hazard.

The rust-free selection, 11-6, performed better in the field than might have been expected on the basis of artificial inoculation alone (fig. 4). Its performance in other Gulfport plantings has also exceeded expectation. This deviation from agreement infers that 11-6, like 18-41, either possesses fewer genes for resistance than 8-7 or has a different form of resistance. Available evidence favors the latter premise. First, 11-6 performed quite differently in two types of test. Second, artificial inoculation of progeny from crosses between 11-6 and 8-7 resulted in 53 percent infection (Jewell and Mallett 1967). Progeny from its cross to 18-62 were 95 percent infected. Thus, 11-6 did not combine as well with 8-7 as 18-41 and was highly susceptible in combination with a susceptible selection.

The field resistance of 11-6 may be dependent upon age or interaction with the environment for its expression. An increase in resistance to blister rust, Cronartium ribicola J. C. Fisch., with age was observed by Patton (1961). The progeny of 11-6 tended to be the slowest growing of those tested (fig. 2).

Hence, its resistance may be an indirect result of mediocre growth. Low vigor and resistance are often related in cases of obligate parasitism (Heimburger 1962; Illy 1966). Whatever the underlying causes, the departure of 11-6 from agreement demonstrates that joint artificial and field tests can identify selections having different forms of resistance. Thus, acceptable genotypes can be combined purposefully, rather than haphazardly, for greater progress.

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