INOCULATION OF LOBLOLLY PINES WITH *CRONARTIUM FUSIFORME* AT DIFFERENT LEVELS OF INTENSITY

H. R. Powers, Jr., H. J. Duncan, L. D. Dwinell, and Thomas Miller [1/]

Many research groups in the South are selecting and testing loblolly (*Pinus taeda* L.) and slash (*P. elliottii* var. *elliottii*) pines for possible resistance to fusiform rust caused by *Cronartium fusiforme* Hedgc. & Hunt ex Cumin. Parent trees are usually selected on the basis of several growth and yield traits and freedom from rust infections. To reduce the probability of selecting a tree that has escaped infection, research scientists try to make evaluations in areas where the incidence of disease is very high. We prefer areas where more than 85 percent of the trees are infected.

Once parent trees have been chosen, evaluations of resistance have usually been based on the field performance of either open- or control-pollinated progeny from specific parent trees. Field tests are effective but require at least 3 or 4 years for completion. Furthermore, there is no guarantee that the seedlings will be subjected to infection by the rust in any given area. Heavy rust infections usually occur in nature only once every 4 or 5 years in a given location. During the last 5 years in Greene County, Georgia, only 1966 could have been considered a severe rust year. A progeny test in that year would have given seedlings a vigorous natural screening. Since that time, rust infections have been light to moderate. This means that seedlings planted in the winter of 1966-67, after 4 years in the field, would have been thus far inadequately tested at this location. If rust infections are light during the 1971 and 1972 seasons, these seedlings would miss being tested during the most advantageous period for evaluation.

Because of the difficulties involved in field testing for resistance, plant pathologists have worked for many years to develop a reliable means of artificially inoculating seedlings. Most of the techniques tried have involved suspending oak leaves bearing telial columns above the pine seedlings to be inoculated (Jewell 1960). The high humidity and approximately 70°F. temperature required for heavy infections have usually been maintained in a canvas tent or a combination of tent and shed. Refrigerated moist chambers of various types have also been used to achieve the same results when temperatures are too high. Variations on this basic principal have been developed (Dwinell, in press; Snow and Kais, in press) which give much more accurate control of humidity, temperature, and levels of infection, but facilities are limited in size.

[1/] Senior Plant Pathologist, Forestry Research Technician, and Research Plant Pathologists, USDA Forest Service, Southeastern Forest Experiment Station, Forestry Sciences Laboratory, Athens, Georgia 30601.

Other specialized inoculation techniques, which may vary considerably from normal conditions, have been developed for specific research purposes. In one of these, telial columns are inserted directly into a cut on stems of seedlings (Hedgcock and Siggers 1949). Other techniques use basidiospores that have been cast from telia suspended over water. The spores are then injected into seedlings with a hypodermic needle (Powers 1968), or placed at specific points on the seedling with a fine glass rod (Miller 1970). All of these techniques depart to varying degrees from the normal means of infection, but do permit infection of specific tissues at precise points. These procedures, while achieving definite research objectives, probably would not be satisfactory for evaluating seedling resistance or susceptibility on a large scale.

A key element lacking in all of this work is host material which will give a relatively uniform disease response to specific levels or concentrations of inoculum. Host material of this type would make it possible to simplify the evaluation of the various techniques.

Another serious problem facing researchers, particularly those who make large scale inoculation tests in tents or moist chambers, has been the erratic germination of telia, resulting in uneven patterns of infection. This has been less of a problem when fresh field-collected material was used as inoculum than in cases where year-round inoculations are attempted with inoculum produced under greenhouse conditions. Usually, telia produced in the greenhouse are satisfactory, but occasionally germination is poor. The newer, more carefully controlled inoculation procedures of Dwinell and Snow eliminate some of these difficulties.

It is obvious that several choices of inoculation techniques are possible when evaluating rust resistance of seedlings. During 1969, studies were designed to test open-pollinated progeny from several parent tree selections and also to evaluate the capacity of three inoculation procedures to provide a range of inoculum densities. These tests were designed to provide information on the overall resistance or susceptibility of the selections, to find the range of inoculum levels obtainable with different techniques, and to determine if any possible resistance would hold constant over a wide range of inoculum densities.

METHODS

Three different inoculation procedures were used. The most severe tests were made with the first method, in which seedlings were inoculated in a large temperature-controlled moist chamber at 70°F. Inoculum density was not recorded for each test in this chamber, but a Kramer-Collins spore sampler (Kramer and Pady 1966) used during one run gave a count of over one million basidiospores per cubic foot of air sampled. In this case, northern

red oak leaves bearing telia were suspended 2 to 3 inches above the test seedlings. The second inoculation technique was that described by Miller and used precast basidiospores. This technique involved the deposition of inoculum directly onto the growing tip of the test seedlings by means of a glass rod. The third method involved the use of an inoculation chamber with closely controlled humidity and temperature (Dwinell, in press). This apparatus makes it possible to vary the inoculum density by adjusting the number of telia exposed over each of several streams of moist air entering the chamber. This method provided moderate to light inoculum densities--150,000 to 17,000 spores per cubic foot of air sampled.

The plant material used was open-pollinated progeny from three loblolly pine parent tree selections. Selection 29R was a rust-free tree from a progeny test in Houston County, Georgia. This was an area of high rust incidence. A total of 915 seedlings were available for testing from this parent tree. Selection 30R was an apparently rust-free tree from an area of moderate rust incidence near Athens, Georgia. One rust gall was subsequently found on this selection© Over 700 seedlings from this source were inoculated. The third parent tree, Number 4628-02, was selected from the same area as 29R for use as a highly susceptible check. Only 178 seedlings were available from this selection, and they were only inoculated in the large moist chamber to give an overall basis for comparison. The 29R and 30R seedlings were tested with all the inoculation procedures listed.

Because of the large number of seedlings being tested in our program, we carry out inoculation tests almost constantly in the large moist chamber. The sometimes erratic germination of telia makes it necessary to split seed lots to avoid the possibility of one entire group being tested with a low level of inoculum. The groups of seedlings discussed in this report were tested in the large chamber on 5 different inoculation runs. Seedlings inoculated by Miller's procedure were all done on the same day, and the seedlings inoculated in Dwinell's chamber were done on three different days. In this latter case, the inoculum densities for the three runs were 150,000, 107,000 and 17,000 spores per cubic foot of sampled air, respectively.

RESULTS

Infection of seedlings exposed to the heaviest inoculum densities in the large moist chamber ranged from 100 percent in the case of 4628-02, the most susceptible, to 45 percent with 29R, the most resistant (Table 1). In this test, as well as all others, 30R seedlings were intermediate with 72 percent infection. When the results from all five runs in the large moist chamber were averaged, the incidence of rust was lower, ranging from 81 percent for 4628-02 to 36 percent for 29R. With Miller's less severe technique, the infection levels were 44 percent and 21 percent for 30R and 29R, respectively.

The three different inoculation levels in Dwinell's chamber gave increasingly lower infections with decreasing inoculum densities. There were insufficient seed of 30R for a satisfactory test at the lightest inoculation level, which gave 2 percent infection on 29R.

Table 1.--Results of inoculations of progeny from loblolly Pine selections with basidiotiporeS of Cronartium fusiforme using different Procedures and at different levels of Intensity.

Type of inoculation	Seedlings infected 1/		
	29R	30R	4628-02
	Percent	Percent	Percent
Heaviest inoculation-moist chamber 2/	45	72	100
Average of 5 runs-moist chamber	36	63	81
Point inoculation with glass rod	21	44	
Inoculation with controlled inoculum densities:			
150,000 spores/ft3	11	27	
107,000 1/ 5 17			
17,000	2		

1/ Infection percentages determined by gall development on infected seedlings.

2/ Spore samples from one heavy inoculation run indicated over one million spores per cubic foot of air sampled.

DISCUSSION

The three most important results of this study were the constant resistance of 29R, the correlation between inoculum density and subsequent gall development, and the production of infection rates fairly close to the level

desired. Seedlings from selection 29R were consistently more resistant than 30R throughout the tests. The susceptible check was more susceptible than 30R in the two comparisons that were possible. This means that carefully controlled inoculation tests can produce infection levels ranging from extremely heavy to very light, depending upon the inoculum density. The ability to produce specified infection rates and the development of pine host material that gives a relatively constant infection response means thgt seedlings can be evaluated for rust resistance much more effectively.

In addition, the results of this study indicate that even in the case of selections which eventually prove to be galled in the field, there may be some gain in the level of resistance compared to a susceptible check. Selection 4628-02, which had several rust galls and might represent a poor commercial lot, had from 18 to 28 percent more rust than 30R under relatively heavy infection conditions.

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