INOCULATION TECHNIQUES IN STUDIES OF RUST RESISTANCE

by

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As the title listed in the program denotes, this subject is a rather broad one. To cover it thoroughly would take up far too much time here. Also, due to my rather limited experience in this field I don't feel qualified to discuss all the various aspects associated with it. Rather, the discussion will be limited to our work along this line which was started very recently.

In any research program in which disease resistance of plants is concerned, some means of testing for such resistance is required., There wee, of course, depending on circumstances, several different methods which can be employed in resistance testing. However, in general, these can be divided into three broad groups. One group involves resistance testing under field conditions where dependance is placed on nature to provide proper inoculum and conditions suitable for infection of the experimental plants. In contrast to this is testing under artificial conditions where plants are subjected to concentrated amounts of inoculum under controlled conditions thought most suitable for infection. Usually, such testing is more severe than would occur under natural conditions. The third group would include methods combining in proper balance, both field and artificial resistance testing. There are, of course, disadvantages and advantages for all three groups. However, we believe that combining field and artificial testing best serves most purposes for the most accurate judging of results. This is the assumption under which the disease resistance program at the Southern Institute of Forest Genetics has been started. Here research has begun to develop resistance among species of Southern pine susceptible to Cronartium fusiforme, commonly known as fusiform rust. This is a heteroecious rust with its aecial and pycnial stages on pine and the unredial and telial stages on various species of oak.

Our initial efforts were aimed toward developing a method of inoculation by which large numbers of selected progenies could be tested simultaneously under controlled conditions. In April 1956 inoculation experiments were carried out on slash pine plants of two ages; 30 day old nursery seedlings and 1-year-old potted plants.

For the nursery seedlings, bulk slash seed was sown in six 4' x 4' nursery bed sections at 60 seed per square foot, Four of these sections were used for inoculation and two were set aside as checks, receiving no inoculum other than from natural sources. At 24-30 days of age the seedlings were inoculated under canvas tents of two types; a single frame tent

and a double frame tent. High humidity was maintained with a water mist.

Inoculum was in the form of small oak branches with telial-bearing leaves attached, inserted into the bed soil so the leaves formed a partial canopy over the seedlings.

Slides of Inoculation Tents

The seedlings were covered by each tent for about 72 hours:. When the tents were removed after this period small water droplets were present on the needles of the seedlings and the bed soil was quite moist. This indicated that a high humidity had been maintained inside the tents as no water had been added to this space for 64 hours. At the end of 6 weeks small red spots and bands were noted on the cotyledons of a large number of the inoculated plants. Hand suctions through these spots and microscopic examination showed typical Cronartium mycelium present in all sections. This was followed by a more thorough sampling of the spotted needles and rust mycelium was found in all cases. It was then concluded that these spots - were probably the early symptoms of Cronartium infection.

Slides on Needle Spots

At this time the bed density was reduced to 30 seedlings per square foot. All seedlings were examined and the percentage with these spots were 69 and 98 in the beds inoculated under

Slide - Infected Seedlings at 5 Months of Age

This indicates to us that these spots are rather good indicators of infection and possibly can be used in future work to judge early suscepti- bility or resistance. At least we know now what to look for.

Inoculation of potted 1-year-old slash pines was successful when the new flush of growth was wrapped in telial-

Slide Inoculation methods used on Potted Plants

Another technique yielding limited success but troublesome to apply was the insertion of telial columns into the new growth stem tissue. Very few infections resulted from this method.

Slide - Single Stem Infection from Cotton and Leaf Wrap Slide Multiple Infection from Wrapping Method Slide - Infection from Insertion and Failure

No needle spots were observed on the infected potted plants, but large purple blotches were noted on the stems in locations now occupied by the swellings. Whether these are indications or symptoms of stem infection is not certain at this time.

The overall results of this exploratory work are encouraging. With what we have learned in this past years' work and with what we hope to learn in the future, we believe we can develop a very satisfactory technique for testing for rust resistance in the southern pines. There are however, many factors that will require further study. What has been mentioned here are the results of one years' work only. We believe these can be repeated, but in doing so, several improvements in the methods will need to be worked out to refine the operation. For example, studies on the placement of inoculum in the seedbed are needed. We need to standardize the inoculum supplied, as to age, locality of collection, and most important, to use the right rust as inoculum. We have two rusts attacking the stems and branches of pine which have primarily the same oak hosts. These rusts must be separated for use in resistance testing. Another fact that required clarification is the time and the conditions of temperature and humidity required by the rust and the experimental plants for successful infection to occur. Also, the most suitable spacing of the plants to be tested is not known.

These are just a few of the problems ahead of us in this work. To work them out effectively will require time, but it is hoped this can be done in the very near future.