# Comparison of Two Pine Seedling Container Systems Used at the Resistance Screening Center

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*Single-tree Ray Leech tubes were compared with 20-tree tray containers, which are currently used in fusiform rust resistance screening. The tubes appear to be suitable for resistance screening and solve many of the problems encountered with the 20-tree trays.*

Fusiform rust is the most damaging forest disease in the Southeast. Out of about 13.8 million acres of loblolly and slash pine, at least 10 percent of the trees are infected (*1*).

Many of these pine trees possess some genetic resistance to fusiformrust. Indeed, loblolly and slash pine trees have been found to produce a higher proportion of progeny resistant to the rust than is seen among pines in general (*2*). A Forest Service Resistance Screening Center was established at Asheville, N.C., in 1973 to screen seedlots for resistance to fusiform rust. These screening tests enable clients to evaluate the rust resistance of their seed-producing pines more quickly and with less expense than is possible through field progeny tests. The screening process requires the rearing of pine seedlings from seed, so they can be inoculated with rust spores and their resistance measured. This article reports on tests of a variety of tree seedling containers that appeared to be suitable for rearing test seedlings.

Since 1973; seedlings to be rust resistance screened have been grown in a plastic tray filled with a 3:2:1 (by volume) mixture of sterilized soil, sand, and peat. Twenty trees are grown in each tray. Although reliable results can be obtained using the current container, there are several problems: (1) Seedlings produced cannot easily be separated for outplanting; (2) root diseases can easily spread from tree to tree; (3) soil fertility varies; (4) nutrients cannot be controlled as well as in a soilless growing medium; (5) seedlings are usually not uniform; (6) outplanting success is poor; and (7) labor costs are high. These problems led to a search for a different container system that would better meet the needs of the Resistance Screening Center.

## Methods

At the outset, 15 container types were considered. Trial seedlings were grown in the three container types considered most applicable to center procedures. The Ray Leech super cell container (10 $in^3$, 1-½ in top diameter, 2° taper, and 8-½ in long) best met the requirements for 1-year seedling maintenance at the center and an experimental design of 20 trees per replication (fig. 1).

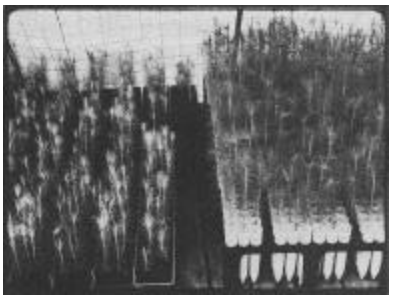

**Figure 1.—***The currently used trays (left) and the new containers (right) under evaluation.*

To properly compare the currently used trays with the test containers, it was necessary to make side-by-side comparisons. Slash and loblolly pine—one susceptible seed source, one resistant, and four of unknown resistance of each species—were grown and inoculated with rust spores in each of the two container systems. Seed sources were all half-sib families, except for two bulk collections. There were 10 containers of 20 trees for each of the 12 seedlots. At 6 weeks, one-half of the seedlings were inoculated at day one (run 1) and the remainder 2 days later (run 2). This comparison was replicated three times at 1-month intervals.

The standard containers already in use were treated as usual. This involved germinating seeds in a tray filled with vermiculite and transplanting the seedlings before the seedcoat was shed into 13- by 5- by 4.5-inch plastic trays filled with a 3:2:1 (by volume) mixture of sterilized soil, sand, and peat. These were fertilized with Miracle-Gro at the label rate 2, 4, and 6 months after transplanting.

The Ray Leech containers were filled with a 5:4:1 (by volume) mixture of peat, vermiculite, and perlite to within 1-½ inches of the top. One seed was placed in each of the 300 containers for each seedlot. One hundred seeds from each seedlot were placed in a vermiculite-filled tray for "fill-in" transplants as needed. Most seeds germinated in the containers. Two hundred containers, each containing one seedling, were selected from each seedlot. These seedlings received a one-half concentration of Miracle-Gro 1 week after germination and full concentrations at 1-month intervals thereafter. Each tube received 40 milliliters of the fertilizer solution at each application.

The percentage of seedlings with fusiform rust galls in each set of 20 seedlings was measured at 6 months for slash pine and 9 months for loblolly pine. The statistical design appropriate for this evaluation was a split plot, with the treatments (i.e., tray or container) being the whole plots, and the 20-tree units of seedlots being the split plots.

Treatments and seedlots were considered fixed effects. That is, any inference concerning these two factors was valid only for the two treatments and specific sets of six seedlots. On the other hand, the treatments and the 20-tree units were considered random effects.

The analysis of variance (ANOVA) and other statistical procedures answered several questions of interest. In particular, the ANOVA determined whether the overall level of infection was the same for both tray and container systems and whether as many or more seedlings were brought through the test with the container system. In the analysis, the dependent variable for the former question was the percentage of seedlings galled, and for the latter, percentage surviving. (Since both dependent variables are percentages, the square root transformation was used for the purpose of stabilizing variances.) Table 1 gives the mean percentage galled and surviving for each system.

The same analysis was used to determine if the difference between treatments in the container systems was the same as or less than the difference between treatments with the trays. In addition, separate ANOVA's were calculated analyzing the percentage galled data from each system. These were used to see if the variability between groups of 20 seedlings was the same or lower with the Leech cell system. The *F*-tests from these two analyses are given in table 2, which contains the mean percentage galled for each system, by run.

Finally, two questions concerning rankings of the seed sources were considered. First, the trays-by-container correlation of the six seed source means determined whether rankings of seedlots from both systems were consistent. Second, the correlation of run 1 by run 2 seed source means indicated that seed source rankings

**Table 1.**—*Mean percentages galled and surviving for each system*

| | Percent galled | | Percent surviving | |
|---|---|---|---|---|
| Test | Trays | Containers | Trays | Containers |
| 1 | 66 | 70* | 88 | 96** |
| 2 | 88 | 94 | 95 | 98** |
| 3 | 72 | 74 | 97 | 93* |
| 4 | 89 | 90 | 81 | 100** |
| 5 | 73 | 76 | 92 | 83 |
| 6 | 93 | 91 | 79 | 95** |
| x | 80 | 83 | 89 | 94* |

*Difference of mean percentages of trays and containers significant (.05 level).

**Difference of mean percentages of trays and containers highly significant (.01 level).

**Table 2.**—*Mean percentage of seedlings galled and F, t, and z test values for each container system by inoculation run*

| | Trays | | Containers | | | | | |
|---|---|---|---|---|---|---|---|---|
| Test | Run 1 | Run 2 | Run 1 | Run 2 | $F^1$ | $F^2$ | $t^3$ | $z^4$ |
| 1 | 61 | 71 | 67 | 72 | .54 | 1.04 | 4.23* | 1.65 |
| 2 | 84 | 91 | 93 | 95 | .86 | 1.87 | 2.16 | .76 |
| 3 | 72 | 71 | 71 | 77 | 11.56** | 1.21 | 11.42** | .43 |
| 4 | 90 | 88 | 91 | 90 | .28 | 1.98 | 2.96* | -.58 |
| 5 | 74 | 72 | 75 | 77 | 1.07 | 1.23 | 9.16** | .69 |
| 6 | 91 | 95 | 89 | 93 | .58 | 1.79 | 1.23 | 1.40 |

[1]F-statistic for comparing run 1-run 2 differences among trays and containers.

[2]F-statistic for comparing differences in variation among 20-tree units between trays and containers.

[3]Value of t used to compare rankings of seedlots in trays to ranking of seedlots in containers. Significant positive value indicates similarity in rankings of seedlots between trays and containers, nonsignificant positivevalue indicates lack of similarity.

[4]Value of z used to compare differences in rankings of seedlots from the two runs for both trays and containers. Positive value indicates increase in similarity in rankings of seedlots from the two runs with the containers; negative value indicates decrease.

*Significant (.05 level)

**Highly significant (.01 level)

from the two runs were more consistent with one system than with the other.

The correlations upon which the t-statistics were based were those of the seedlot means, not the rankings of the means. Correlations could have been done on the rankings, but it was preferable to use the actual data for such a correlation. The t- and z-statistics, shown in table 2 for each test, indicate the outcome of these statistical procedures.

## Results

In four of the six tests, the mean percentage of seedlings surviving in the containers significantly exceeded that of the trays (table 1). Five of the six tests indicated a slightly higher, but not significant, difference in percentage of seedlings galled.

Table 2 indicates that, in five of the six tests, the difference between inoculation runs in mean percentage galled for trays did not vary significantly from the corresponding difference for containers. In one case, there was a significantly greater difference between the runs with containers than those with trays.

The second column of F-statistics in table 2, comparing tray versus container variation attributable to 20-tree units, indicates no significant differences in any of the tests. Hence, there was no significant difference in the sampling variation between the two systems.

The four significant values of the t-statistic indicate that the ranking of seedlots with the Leech tube system was similar to the ranking in the 20-tree tray system. In the remaining tests, the positive value of *t* indicates that the correlation of rankings of seedlot means within treatments was positive, but not significantly so. In both of these tests, however, the three seed sources with the lowest percentage galled were the same with each system.

None of the z-statistics were significant, indicating no statistical difference between the systems in rankings of seedlot means from the different runs. However, five of the six values of z were positive, possibly indicating a slightly greater consistency of seed source rankings between runs for the Leech cell containers.

## Discussion

It appears that Ray Leech tube containers are suitable for resistance screening, since the tubes did not markedly change the amount of infection or the difference in the amount of infection between runs. The sampling variation was the same with both systems. The seed source rankings of the tubes in the two runs in a test were equal or better in agreement than those for the trays.

Without regard to runs, there was no evidence that seed source rankings from the two

systems were not essentially in agreement. However, only six seed sources were compared at any one time, and firm conclusions about differences in seed source rankings must come from examination of many more families. Also, any differences in rankings might be eliminated by changing the fertilization or other growing conditions in the tubes.

In addition, it-appears that Ray Leech tubes solve many of the problems encountered with the current 20-tree tray system. More seedlings completed the tests in the tubes than in the trays. This was largely because of replacement of dead trees before inoculation and reduction of damping off after inoculation.

Further, although agreement of seed source rankings of the two runs with tubes was not statistically better than the corresponding agreement for trays, the data suggest additional testing may establish such a result. This would imply that conditions in the tubes from run to run are more uniform than with the trays; a more desirable situation for resistance screening purposes.

**Literature Cited**

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