

## ROOT GROWTH POTENTIAL OF SOUTHERN PINE SEEDLINGS GROWN AT THE W. W. ASHE NURSERY

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Abstract. Root growth potential (RGP) of major species and seed sources grown at the W. W. Ashe Nursery was measured over the course of the 1987-88 lifting season. The effects of accumulated chilling in the nursery bed and days in cold storage on RGP varied with species, and in some cases for seed sources within species. For the sources of loblolly and shortleaf pines evaluated, accumulated chilling largely determined RGP. The relative importance of chilling versus duration of storage varied by seed source for slash and longleaf pines.

**The reforestation improvement program (RIP) is a joint venture within the USDA Forest Service between the National Forest system and research. Initiated in 1985, RIP is a nation-wide comprehensive system for monitoring every phase of the reforestation process. The long-term goal of the program is to make reforestation on National Forest lands a highly predictable process leading to reliable establishment of fast growing plantations. An additional objective is to learn more about basic tree seedling ecophysiology. One aspect of RIP is to measure root growth potential (RGP) of the major species and seed sources grown at each participating Forest Service nursery.**

Root growth potential is recognized as the best comprehensive measure of seedling quality now available (Ritchie 1984). It is a measure of the ability of a seedling to initiate and elongate new roots when held in an environment favorable for root growth.

For many species, RGP has been shown to be a good predictor of field survival (Ritchie and Dunlap 1980, Feret and Kreh 1985, Larsen and others 1986). Relatively high RGP is also considered to be correlated with seedling vigor and tolerance to desiccation and physical damage that often accompany lifting, storing, and outplanting of nursery stock. Seedling RGP is affected by many factors, including genotype, nursery practices such as fertilization and root culture, and the timing of lifting and duration of cold storage. There is a seasonal periodicity in RGP that results in peak RGP coinciding with fulfillment of the chilling requirement for dormancy release (Ritchie and Dunlap 1980). This periodicity makes RGP testing a valuable tool for scheduling lifting and planting operations to coincide with the period when seedlings are least susceptible to damage.

**Techniques for measuring RGP and the value of the results as a measure of seedling quality were developed by Stone and his coworkers (Stone 1955, Stone and Schubert 1959, Stone and others 1962, Stone and Jenkinson 1970). This initial work was done with ponderosa pine (*Pinus ponderosa* Laws.) and Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco). It has since been used as a measure of quality for numerous species, both conifers and hardwoods, around the world (Ritchie 1985).**

There is no standard procedure for conducting RGP tests (Ritchie 1985). Rooting medium is usually either sand or a peat-vermiculite mix, although hydroponic and airoponic systems are coming into increasing use. Temperature and photoperiod regimes and the length of the period under these conditions vary with the objectives of the test. The measure of RGP also varies. The number of new roots over some specified length or the number and total length of new roots are most often reported. Other measures include volume or surface area before and after the test. An abbreviated method with only 7 growing days that was used by Burdett (1979) gave good correlations between a simple 0 to 5 score and field survival.

Results from RGP tests have been used in several ways. A primary use has been for relating RGP to calendar date or accumulated chilling to determine optimum lifting dates, or "lifting windows," for use in subsequent years at a particular nursery (Jenkinson 1984). Another use is for determining safe storage length for seedlings lifted on a particular date or with a certain chilling period (Carlson 1985). The abbreviated RGP tests have also been used to cull batches of lodgepole pine (*P. contorta* Dougl. ex Loud.) nursery stock (Burdett 1979). For the southern pines, RGP tests have been used to compare nurseries, seed sources or families, time of lifting, and the effects of cultural and handling practices (Brissette and Roberts 1984, Brissette and Carlson 1987, Feret and others 1985, Larsen and Boyer 1986, Rose and Whiles 1985).

The objective of this paper is to present the trends in RGP for pine seedlings lifted from Ashe Nursery during the 1987-88 season. Because only 1 year of data is available, no attempt is made to define optimum lifting windows or storage lengths. Rather, by presenting statistically significant results graphically, we hope the reader will realize just how dramatically chilling and storing can affect new root growth.

These evaluations will continue at Ashe Nursery. As additional results are obtained over the next few years, appropriate lifting windows and cold storage durations will be refined for the species and seed sources grown there.

## MATERIALS AND METHODS

During the 1987-88 lifting season, four pine species were evaluated: loblolly (*P. taeda* L.), shortleaf (*P. echinata* Mill.), slash (*P. elliottii* Engelm.), and longleaf (*P. palustris* Mill.). Loblolly pine was represented by seed orchard sources from Alabama, Louisiana, north and south Mississippi, and Texas. A Georgia seed orchard lot was the only source of shortleaf pine evaluated. Slash pine was represented by seed orchard sources from Alabama, Florida, and Mississippi. Both seed orchard and forest-collected lots of longleaf pine from several geographic sources were evaluated: seed orchard lots from south Alabama and Louisiana, and seed orchard and forest-collected lots from north Alabama, Florida, and Mississippi.

**Seedlings for RGP testing were drawn at random from seedling lots in the packing shed on five dates. The dates and corresponding hours of accumulated chilling (0 to 8°C) at 20 cm above the ground are shown in table 1. From each tested lot, 40 seedlings were separated into groups of 10. One group was put**

into RGP testing within 24 hours of lifting. All other seedlings were put into cold storage (approximately 3°C) and then into RGP tests after 7, 14, and 21 days.

**Table 1.--Dates and accumulated chilling (0 to 8°C) at 20 cm above the soil surface for seedlings lifted for RGP testing at Ashe Nursery in 1987-88**

<u>Life</u>	<u>Date</u>	<u>Accumulated chilling</u> <u>hr</u>
1	Dec. 15	340
2	Jan. 13	580
3	Jan. 29	680
4	Feb. 19	820
5	Feb. 29	880

The RGP tests were conducted in large mist chambers under airoponic conditions. The root zone temperature was maintained at 27°C, and the photoperiod was 24 hours under fluorescent lights. The test duration was 21 days. RGP was measured as both the number of new roots greater than 1 cm long and as the total length of those new roots. Before RGP testing, each seedling was measured for root collar diameter, number of first-order lateral roots that were greater than 1 mm in diameter adjacent to the taproot, and whether a terminal bud had formed. At the end of each RGP test, the number of seedlings that broke bud during the test was recorded.

The data were summarized as means of the 10-seedling test groups. The data were first analyzed using analysis of variance (ANOVA) to determine whether the interactions involving seed source or the main effects of seed source were significant within each species. A significance level of p=0.05 was used. If there were significant differences, then sources were grouped using Duncan's Multiple Range Test.

Data from the appropriate groups (species or seed source) were fitted to a quadratic response-surface regression model. RGP (number of new roots greater than 1 cm long) was the dependent variable, and accumulated chilling in hours and duration of cold storage in days were the independent variables. Because only 1 year of data was available, optimum values for chilling hours and storage duration were not estimated from the models.

The response surfaces estimated by the models were smoothed with bivariate interpolation and then plotted on a three dimensional graph. The plots are presented to show the trends in RGP as it was affected by chilling and cold storage.

## RESULTS AND DISCUSSION

For loblolly pine, there were no significant interactions or main effects due to seed source. In a total of 170 10-tree RGP tests there was an overall mean of 14 new roots greater than 1 cm in length and a total mean length of new roots of 30 cm per seedling (table 2).

Table 2.--Overall average RGP, measured as both number of new roots greater than 1 cm long and total length (cm) of new roots, for species and seed sources evaluated at Ashe Nursery in 1987-88

Species/source	n <sup>a/</sup>	Mean RGP		r <sup>b/</sup>
		Number	Total length	
Loblolly	170	14	30	.96
GA shortleaf	19	18	42	.96
Slash				
AL + FL	57	13	21	.97
MS	15	19	34	.91
Longleaf				
FL	36	6	14	.93
MS	35	6	12	.94
LA	17	13	31	.97
N.AL	106	10	18	.91
S.AL	20	8	14	.77

<sup>a/</sup> n is the number of 10-tree tests represented.

<sup>b/</sup> r is the correlation coefficient between the number and length of new roots.

For Georgia shortleaf pine, nineteen 10-tree tests were evaluated. The mean number of new roots was 18, and the average length of new roots was 42 cm (table 2).

Among the slash pine sources, there was an interaction between seed source and days of cold storage ( $p=0.0126$ ) for the number of new roots. For the length of new roots, the interaction between seed source and days of cold storage, and main effects of seed source were significant ( $p=0.0001$  and  $0.0457$ , respectively). The Mississippi source differed from the Alabama and Florida sources in number and length of new roots (table 2).

For longleaf pine, there was a source by chilling hour interaction for both number ( $p=0.0058$ ) and length ( $p=0.0001$ ) of new roots. The main effect of seed source was also significant for both measures of RGP ( $p=0.0011$  for number and  $p=0.0001$  for length). The overall mean RGP measured as both number and length of new roots for each source is shown in table 2. Because of the differences among the sources, Louisiana and north Alabama sources were selected for further discussion.

The correlation between number and length of new roots was very high, ranging from 0.91 to 0.97, except for south Alabama longleaf pine, for which it was only 0.77 (table 2). Because of these high correlations, the results of the response-surface regressions are presented only in terms of the number of new roots.

Figure 1 shows the response surface estimated for all the tested sources of loblolly pine combined. The regression of RGP on chilling hours and days stored was highly significant ( $MSE=82.66$  and  $F=16.0$ ) and explained 33 percent of the variation in RGP. Although ANOVA showed seed source to be nonsignificant, much of the unexplained variation is probably attributable to physiological and/or morphological source differences. Evidently, RGP peaked at around 600 hours of accumulated chilling and then gradually declined. Duration of cold storage had little effect on RGP until late in the lifting season, then RGP dropped steadily to zero by 21 days.

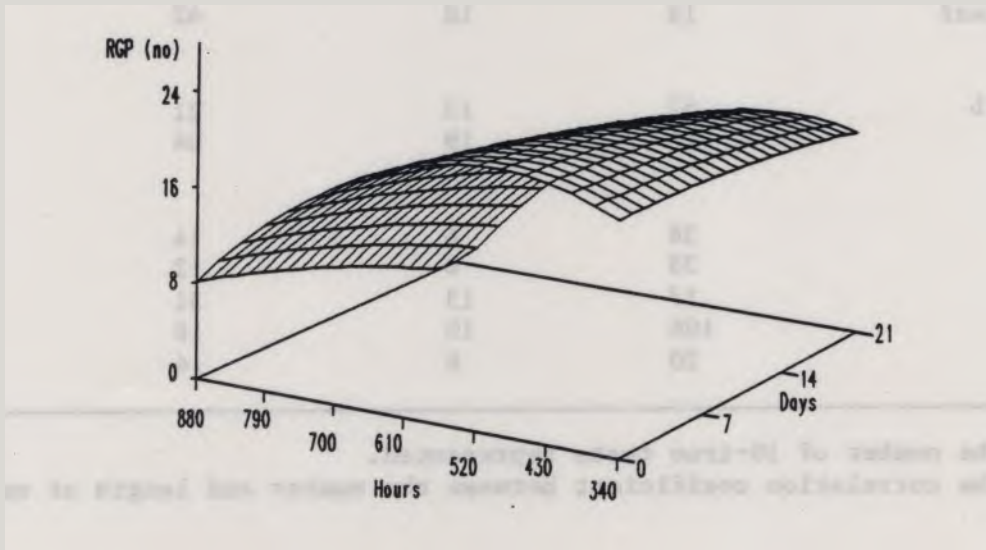


Figure 1.--Response surface for RGP of loblolly pine estimated by quadratic regression on hours of accumulated chilling ( $0-8^{\circ}\text{C}$ ) in the nursery bed and days in cold storage.

The effects of chilling and cold storage on the RGP of Georgia shortleaf pine are estimated by the response surface shown in figure 2. The regression was very highly significant ( $MSE=30.89$  and  $F=17.1$ ), and it explained 87 percent of the variation in RGP. In this case, RGP was very dependent on accumulated chilling, with a very sharp peak at about 610 hours. Storage of up to 21 days had little effect on RGP throughout the season.

Figure 3 shows the estimated response surface for RGP of the Alabama and Florida sources of slash pine as it was affected by chilling and storing. Again, the relationship was very highly significant ( $MSE=80.48$  and  $F=11.9$ ) and explained 54 percent of the variation in RGP. The most striking feature of the response surface is that it predicts RGP dropping to zero late in the lifting season, regardless of storage duration. The peak in RGP was about the same as that for the loblolly pine sources that were tested.

Mississippi slash pine showed a very different response pattern (figure 4). The regression was highly significant ( $MSE=117.29$  and  $F=6.2$ ) and explained 77 percent of the variation in RGP. Unlike the Alabama and Florida sources, the RGP of the Mississippi source steadily increased with accumulated chilling. Also, this source was extremely sensitive to storage, with even 1 week's storage being detrimental to RGP throughout the season. The recovery

of RGP at 21 days, after declining during shorter periods of storage, is difficult to explain. Feret and others (1985) observed a similar response in Virginia loblolly pine and speculated on the causes. They suggested that such a response may be due to: 1) a cyclic pattern in RGP, 2) stress causing a physiological burst of new root growth before seedling death, or 3) a combination of the two phenomena.

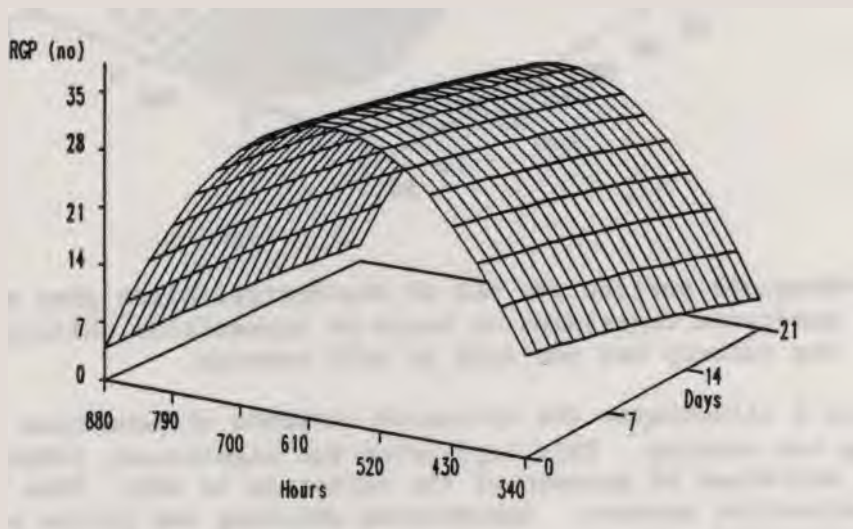


Figure 2.--Response surface for RGP of Georgia shortleaf pine estimated by quadratic regression on hours of accumulated chilling (0-8°C) in the nursery bed and days in cold storage.

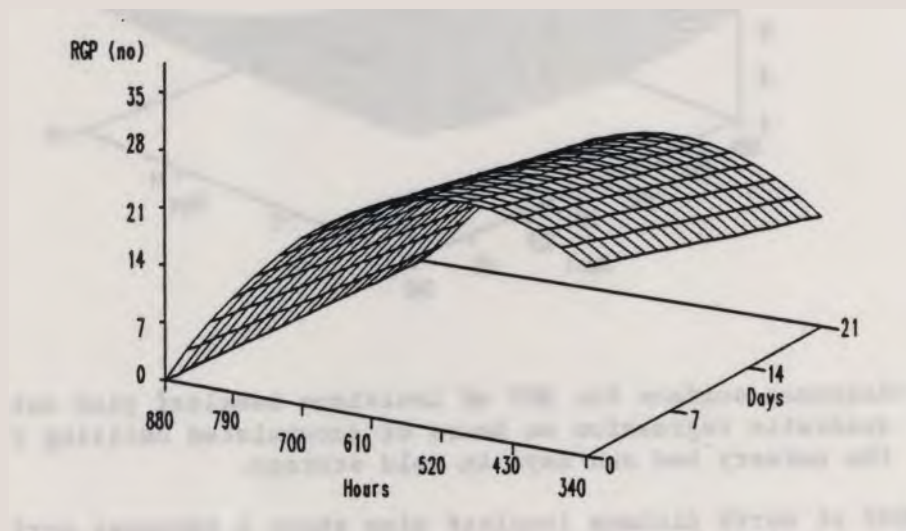


Figure 3.--Response surface for RGP of Alabama and Florida slash pine estimated by quadratic regression on hours of accumulated chilling (0-8°C) in the nursery bed and days in cold storage.

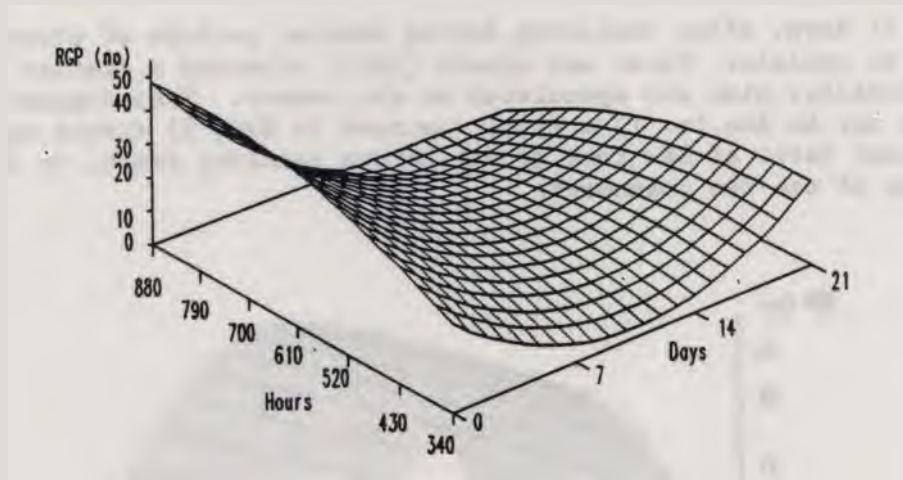


Figure 4.--Response surface for RGP of Mississippi slash pine estimated by quadratic regression on hours of accumulated chilling (0-8°C) in the nursery bed and days in cold storage.

Figure 5 illustrates the estimated response of Louisiana longleaf pine to chilling and storing. This regression was significant (MSE=35.15 and F=4.5) and explained 67 percent of the variation in RGP. This source also shows a distinctive pattern. Accumulated chilling had little effect on RGP of the fresh seedlings. However, after about 370 chilling hours, RGP showed a progressive decline with increased storage.

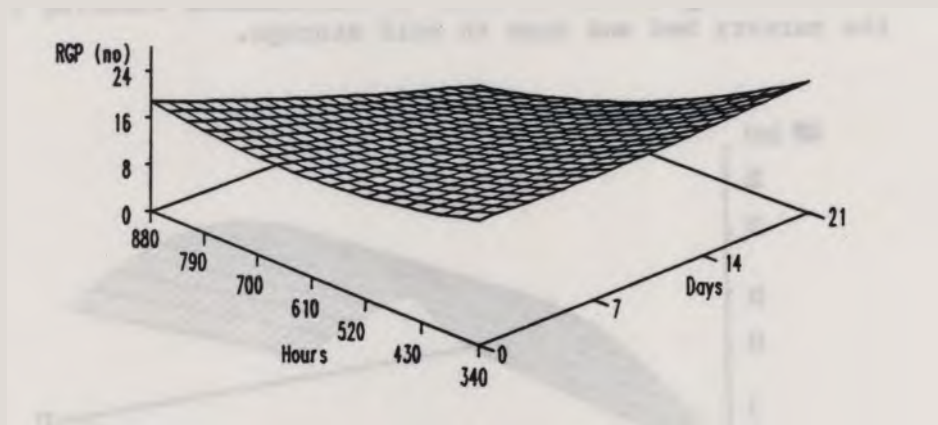


Figure 5.--Response surface for RGP of Louisiana longleaf pine estimated by quadratic regression on hours of accumulated chilling (0-8°C) in the nursery bed and days in cold storage.

The RGP of north Alabama longleaf pine shows a response surface similar to that of Georgia shortleaf pine (figure 6). The regression is very highly significant (MSE=44.82 and F=6.8), but it explains only 25 percent of the variation in RGP. As with the Georgia shortleaf pine, RGP peaked at about 610 hours. Although RGP declined in storage early in the lifting season, storage had little effect after about 450 chilling hours.

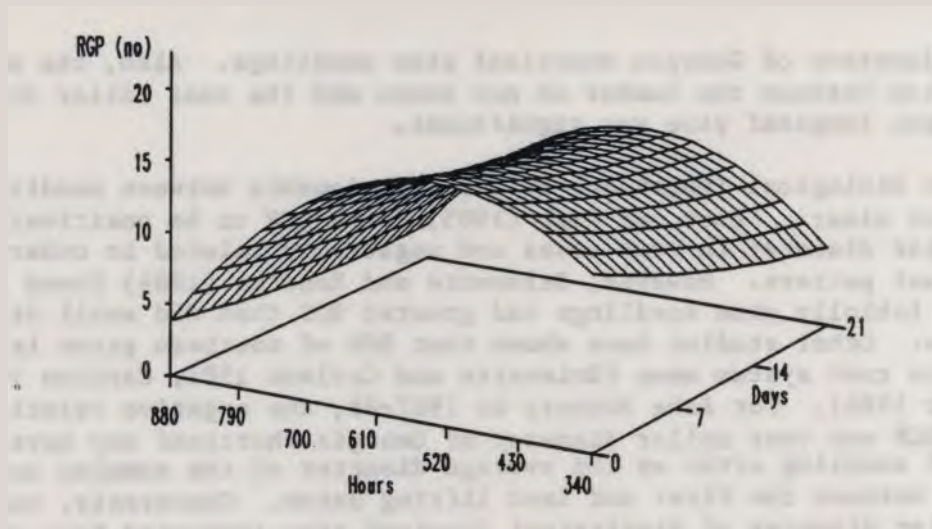


Figure 6.--Response surface for RGP of north Alabama longleaf pine estimated by quadratic regression on hours of accumulated chilling (0-8°C) in the nursery bed and in cold storage.

With loblolly pine, Carlson (1985) showed that bud dormancy is as responsive to chilling accumulated in cold storage as it is to natural chilling. However, he also showed that the RGP of seedlings lifted before the fulfillment of their chilling requirement does not increase in storage as might be expected. Instead, the RGP of early lifted seedlings (207 chilling hours) declined in storage, and storage of seedlings lifted at 500 chilling hours had no effect or improved RGP (Carlson 1985). He concluded that cold storage probably reduced RGP through dehydration of root tissue of the less suberized, early lifted seedlings.

In our evaluations, lifting began at 340 chilling hours, and only Mississippi slash pine (figure 4) had a marked decline of RGP with storage at that time. The RGP of the other species and sources was either unaffected or showed only a slight decline in storage after the earliest lift. The differences between our results and Carlson's (1985) suggest that, although RGP had not yet peaked by our earliest lifting date, the roots were probably more suberized than those of the early—lifted seedlings he studied, and thus less susceptible to damage in storage.

As the lifting season progressed, the tendency for reduced RGP after storage was probably the result of changing dormancy status associated with the initiation of bud break (Garber and Mexal 1980), rather than changes in the degree of root system suberization. Therefore, before peaking RGP may depend on both dormancy status and the susceptibility of the roots to dehydration; after peaking RGP probably depends primarily on dormancy status.

There were inconsistent relationships between RGP and the other seedling attributes measured. Positive correlations between both numbers and length of new roots and the number of first—order lateral roots were significant for loblolly pine, but not for any other species or sources within species. Both measures of RGP were significantly, but negatively, correlated with root



**collar diameters of Georgia shortleaf pine seedlings. Also, the negative correlation between the number of new roots and the root collar diameter of Mississippi longleaf pine was significant.**

The biological importance of the relationship between seedling size and RGP is not clear. Feret and Kreh (1985) found RGP to be positively related to root collar diameter in some cases and negatively related in others, with no significant pattern. However, Brissette and Roberts (1984) found that large diameter loblolly pine seedlings had greater RGP than did small diameter seedlings. Other studies have shown that RGP of southern pines is positively related to root system mass (Brissette and Carlson 1987, Carlson 1986, Larsen and Boyer 1986). For Ashe Nursery in 1987-88, the negative relationships between RGP and root collar diameter of Georgia shortleaf may have been the **result of sampling error as the average diameter of the sampled seedlings declined between the first and last lifting dates. Conversely, the average root collar diameter of Mississippi longleaf pine increased from 10.6 to 12.6 mm between the first and last lifting dates, probably because of continued diameter growth in the nursery. In this case, the negative correlation between RGP and root collar diameter was most likely the result of the late-lifted seedlings having lower RGP due to their dormancy status, even though they were larger.**

In some cases RGP was related to the proportion of seedlings that broke bud during the 21-day test period. In loblolly pine and Alabama and Florida slash pine, there was a significant negative correlation between both measures of RGP and the proportion of seedlings that broke bud during the test. For south Alabama longleaf pine, the correlation was also negative and significant for number of new roots, but not length of new roots. All these relationships agree with the general pattern of RGP decline just before bud break (Ritchie and Dunlap 1980). However, the relationship did not hold for all the RGP tests conducted at Ashe Nursery in 1987-88. A better relationship may have been apparent if the number of days to bud break, a measure of dormancy intensity, had been used rather than just the proportion of seedlings that broke bud during the test.

#### SUMMARY AND CONCLUSIONS

Lift and store recommendations cannot be made based on just 1 year of testing. Also, this study did not include field testing to validate the importance of RGP to field performance of the sources evaluated. However, the data do clearly show several important relationships that a nursery manager should be aware of, including:

1. **Species and sources within species may differ dramatically in optimum lifting dates.**
2. **In general, the ability of southern pines to produce new roots is very responsive to the amount of chilling that accumulates before they are lifted. However, after sufficient chilling, seedlings are capable of breaking bud, with an associated decline in RGP.**

3. **The ability of seedlings to maintain physiological vigor while in cold storage is source dependent and changes during the lifting season.**

The measurement of RGP will continue at Ashe Nursery as part of the RIP. The goal of these evaluations is to define the target lifting dates, based on chilling hours, and safe storage lengths through the lifting season for the major sources grown at the nursery. If consistent results can be obtained over a 3-year period, then tentative recommendations will be made. Those recommendations will then be refined as additional data are collected in subsequent years. Also, beginning with the 1988-89 crop, field planting of samples from the same batches of stock used in the RGP tests is planned. The results of those outplantings will strengthen the recommendations based on RGP testing.

#### LITERATURE CITED

- Brissette, J.C. and W.C. Carlson. 1987. Effects of nursery bed density and fertilization on the morphology, nutrient status, and root growth potential of shortleaf pine seedlings. p. 198-205. In. Phillips, D. (ed.). Proc. Fourth Biennial Southern Silvicultural Research Conf. USDA Forest Serv. Gen. Tech. Rpt. SE-42.
- Brissette, J.C. and T.C. Roberts. 1984. Seedling size and lifting date effects on root growth potential of loblolly pine from two Arkansas nurseries. *Tree Planters' Notes* 35(1):34-38.**
- Burdett, A.N. 1979. New methods for measuring root growth capacity: their value in assessing lodgepole pine stock quality. *Canadian J. Forest Res.* 9:63-67.
- Carlson, W.C. 1985. Effects of natural chilling and cold storage on budbreak and root growth potential of loblolly pine (*Pinus taeda* L.). *Canadian J. Forest Res.* 15:651-656.
- Carlson, W.C. 1986. Root system considerations in the quality of loblolly pine seedlings. *South. J. Appl. Forestry* 10:87-92.
- Feret, P.P. and R.E. Kreh. 1985. Seedling root growth potential as an indicator of loblolly pine field performance. *Forest Sci.* 31:1005-1011.
- Feret, P.P., R.E. Kreh and L.E. Dewald. 1985. Root growth potential of stored loblolly pine seedlings. p. 18-24. In. Shoulders, E. (ed.). Proc. Third Biennial Southern Silvicultural Research Conf. USDA Forest Serv. Gen. Tech. Rpt. S0-54.
- Garber, M.P. and J. G. Mexal. 1980. Lift and store practices: their impact on successful establishment of southern pine plantations. *New Zealand J. Forestry Sci.* 10:72-82.
- Jenkinson, J.L. 1984. Seed source lifting windows improve plantation establishment of Pacific slope Douglas-fir. p. 115-141. In: Duryea, M.L. and G.N. Brown (eds.). Proc. Seedling Physiology and Reforestation Success. Martinus Nijhoff/Dr W. Junk Publishers, Dordrecht/Boston/London.**

- Larsen, H.S. and J.N. Boyer. 1986. Root growth potential of loblolly pine (*Pinus taeda* L.) seedlings from twenty southern nurseries. Circular 286, Alabama Agric. Exp. Stn., Auburn Univ., Auburn, AL.
- Larsen, H.S., D.B. South and J.M. Boyer. 1986. Root growth potential, seedling morphology and bud dormancy correlate with survival of loblolly pine seedlings planted in December in Alabama. *Tree Physiology* 1:253-263.
- Ritchie, G.A. 1984. Assessing seedling quality. p. 243-259. In: Duryea, M.L. and T.D. Landis (eds.). *Forest Nursery Manual: Production of Bareroot Seedlings*. Martinus Nijhoff/Dr W. Junk Publishers, The Hague/Boston/Lancaster, for Forest Res. Lab., Oreg. St. Univ., Corvallis.
- Ritchie, G.A. 1985. Root growth potential: principals, procedures, and predictive ability. p. 93-104. In: Duryea, M.L. (ed.). *Proc. Evaluating Seedling Quality: Principals, Procedures and Predictive Abilities of Major Tests*. Forest Res. Lab., Oreg. St. Univ., Corvallis.
- Ritchie, G.A. and J.R. Dunlap. 1980. Root growth potential: its development and expression in forest tree seedlings. *New Zealand J. Forestry Sci.* 10:218-248.
- Rose, R.W. and R.P. Whiles. 1985. Root growth potential and carbohydrate shifts in previously cold stored loblolly pine seedlings grown in hydroponic culture. p. 25-33. In: Shoulders, E. (ed.). *Proc. Third Biennial Southern Silvicultural Research Conf.* USDA Forest Serv. Gen. Tech. Rpt. S0-54.
- Stone, E.C. 1955. Poor survival and the physiological condition of planting stock. *Forest Sci.* 1:90-94.
- Stone, E.C. and J.L. Jenkinson. 1970. Influence of soil water on root growth capacity of ponderosa pine transplants. *Forest Sci.* 16: 230-239.
- Stone, E.C., J.L. Jenkinson and S.L. Krugman. 1962. Root-regenerating potential of Douglas-fir seedlings lifted at different times of the year. *Forest Sci.* 8:288-297.
- Stone, E.C. and G.H. Schubert. 1959. Seasonal periodicity in root regeneration of ponderosa pine transplants--a physiological condition. p. 154-155. In: Proc. Society of American Foresters 1958 National Conv.**