Comparison of Time and Method of Mist Chamber Measurement of Root Growth Potential[1]

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Abstract.--Container-grown ponderosa pine, Douglas-fir, and Engelmann spruce seedlings were cold acclimated and deacclimated in growth chambers over 19 weeks. Weekly whole-plant freeze tests and 7- and 14-day root growth potential (RGP) tests indicated 7-day RGP results were misleading during cold acclimation and that the 14-day test period was preferable. During cold deacclimation, both RGP test periods were suitable. Quantification of RGP as total length and total number of new roots per seedling were nearly equally informative from budset to bud break, independent of the length of the RGP test.

INTRODUCTION

Root growth potential (RGP) is the ability of a tree seedling to initiate and elongate new roots when placed into an environment favorable for root growth (Ritchie 1985). It is a measure of seedling physiological quality and vigor. To become established in the field after outplanting, seedlings must be able to utilize new soil reserves of water and nutrients as those reserves in immediate contact with existing roots are depleted. New roots must be produced to accomplish this. Seedlings with a high capacity to produce new roots are likely to become established more rapidly and with less stress than comparable seedlings with a low RGP. For this reason, RGP measurements made prior to outplanting have been found to be positively correlated with the field survival and growth of many species of forest tree seedlings (Burdett 1979, Burdett et al. 1983, Jenkinson 1980, Ritchie and Dunlap 1980, Stone et al. 1961). Measurement of the RGP attribute is currently thought to be the most reliable predictor of field performance of the various seedling quality tests available (Ritchie 1985).

RGP is commonly measured using one of three approaches: the pot test, a hydroponic system, or an aeroponic system. In the pot test, originally developed by Stone (Stone 1955, Stone and Jenkinson 1970, Stone and Schubert 1959), seedlings are potted, several per container, and maintained for 28 days at 20°C under a 16-hour photoperiod and as near field capacity as possible. Seedlings are washed from the medium to assess root growth. While this technique is successful, it has disadvantages (Ritchie 1985). Considerable time is required before results are available, and plant maintenance during that time is expensive. Potting and unpotting of seedlings is not only labor intensive, but requires large quantities of media, can result in root system damage, and does not permit examination of the root system prior to the end of the test period. Burdett (1979) addressed the problem of the lengthy test period by developing a 7-day test in which root growth was accelerated by increasing the day/night temperatures to 30°/25°C. The 7-day and 28-day test results are well correlated in a number of conifers (Ritchie 1985), though not in all species (Ritchie 1984).

The hydroponic system uses temperature-controlled aerated water baths made from aquariums painted black and covered with lids which support the seedlings with the roots submerged. Winjum (1963) used a 28-day test period, while others have successfully shortened the test to between 15 and 21 days (DeWald et al. 1985, Rose and Whiles 1985, Sutton 1980). Hydroponic systems eliminate the disadvantages associated with potting and unpotting of seedlings. Additionally, this technique requires 50% less bench space than the pot test, and the roots are easily measured because they remain clean and unbroken. Ritchie (1984, 1985) found that seedlings tested hydroponically produced about the same length and number of new roots as similar in concurrent pot tests. However, hydroponic culture of tree seedlings can result in steadily decreasing xylem water potential and minimal new root production

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(Rietveld 1986). An additional problem suspected with the hydroponic system is an unsuitability for the testing of container stock because of failure to adequately aerate the root balls.

The aeroponic system includes the use of mist boxes or chambers in which the seedling root systems are suspended (Day 1982, Hileman 1986). A 28-day test period has been used with Pistacia chinensis (Lee and Hackett 1976), but Tinus et al. 1986) have successfully shortened the test to 14 days with conifers by using a warm water mist to accelerate root growth. The aeroponic system has all the desirable characteristics of hydroponics plus some important additional advantages. Seedlings in mist chambers initiate new roots 1 week sooner than potted seedlings (Rietveld 1986) and produce greater numbers of regenerating roots than seedlings in concurrent pot tests (Lee and Hackett 1976). This permits shorter test periods. In addition, the aeroponically-created root environment maintains xylem water potentials similar to those of potted seedlings (Rietveld 1986) and is ideal for the testing of container stock (Tinus et al. 1986). The aeroponic system is rapidly becoming the method of choice for these reasons. USDA Forest Service initiated aeroponic RGP testing at all 11 of its nurseries in 1987.

The most desirable parameter of root growth is total new root surface area, because it is proportional to water and nutrient uptake ability (Newman 1966). However, root surface area is not readily measured. Thus, RGP is usually quantified as total length and/or total number of new roots per seedling (Ritchie 1984). Total new root length is directly proportional to surface area, if, as assumed, the new roots are nearly all the same diameter. If it is further assumed that most new roots are the same length when root growth is measured after a limited period of time, such as 14 or 28 days, then number of new roots will be strongly correlated to new root length, and thus to new root surface area also. Number and length of roots are the consequence of different processes, however. Number of roots per seedling is a measure of the initiation of new roots and the initiation of renewed growth of existing roots (Stone et al. 1963). Total length of new roots produced measures both initiation and elongation (Ritchie and Dunlap 1980). Root initiation and elongation are controlled by different mechanisms (Torrey 1976), and respond differently to factors such as chilling hours (Krugman and Stone 1966), soil temperature (Nambiar et al. 1979), and nutrient status (Nambiar 1980). Thus, it should not be assumed that number and length of roots will always be strongly correlated under all RGP test conditions.

Total length and number of new roots per seedling are thought to be fairly well correlated using the standard pot test (Ritchie 1985). Total number of new roots ($\geq$0.5 cm in length) was correlated (R=0.8667) with total length of those new roots in Pinus taeda using a 28-day got test with an average root temperature of 26.5°C (Larsen and Boyer 1986). When RGP was measured as total number of new roots $\geq$1.25 cm and as total length of new roots $\geq$2.5 cm with a 30-day pot test and 20°C root temperatures, the two approaches gave similar results (Krugman and Stone 1966). This type of data has led to the prevalent procedure of measuring only total number of roots per seedling because of the considerable reduction in the time required to count the roots as opposed to measuring root length (Ritchie 1985). Similar information on the correlation between length and number of roots is unavailable for the aeroponic method and shorter test periods.

A seedling quality test should, ideally, provide the highest quality information, in the shortest possible time, in the most efficient manner, and for the widest range of stock types. Toward this ideal with the RGP test, the objectives of this study were to examine the quality of information provided by 7-day vs 14-day aeroponic tests of container stock from bud set to bud break, with root growth quantified as total length of new roots per seedling vs total number of new roots per seedling. This research was performed within the context of a larger study examining the relationship between root growth potential and two other seedling quality parameters: cold hardiness and bud dormancy.

MATERIALS AND METHODS

Seedlings of ponderosa pine (Pinus ponderosa var. scopulorum Engelm., Chevelon District, Apache-Sitgreaves National Forests, elev. 2,300 m), Douglas-fir (Pseudotsuga menziesii var. glauca (Beissn.) Franco, Cloudcroft District, Lincoln National Forest, elev. 2,700 m), and Engelmann spruce (Picea engelmannii (Parry) Engelm., Springerville District, Apache-Sitgreaves National Forests, elev. 3,000 m) were greenhouse-grown in 400-ml Rootrainer[3] book containers in a peat-vermiculite mix for 9 months (October 1984 June 24, 1985. Greenhouse temperatures ranged from 23 to 28 C daily (average 25°C) and 18 to 21°C at night (average 20°C). Daylength was extended to 22 hours with fluorescent light. Other cultural conditions were as recommended by Tinus and McDonald (1979). During the ninth month, the trees set bud and entered dormancy. The seedlings were then graded and those of uniform size were placed in Percival HL-60 growth chambers for a 4-stage, 19-week cold acclimation and deacclimation regime (table 1). Sodium and multivapor arc lights provided 43,000 lux, and watering was as needed with nutrient solution. At approximately weekly intervals, a sample of 20 seedlings per species was taken for concurrent tests of cold hardiness and root growth potential.

Whole-Plant Freeze Test

Cold hardiness was measured by a whole-plant freeze test. One book of four seedlings of each

[3]Trade names are used for brevity and specificity and do not imply endorsement by USDA or Colorado State University to the exclusion of other equally suitable products.

Table 1.--Cold acclimation and deacclimation conditions.

Stage	Day nos.	Dur-ation (wks)	Day temp. (°C)	Night temp. (°C)	Day length (hrs)	Nutri-ent Solu-tion
1	0-21	3	20	15	10	low N, high PK
2	22-71	7	10	3	10	low N high PK
3	72-105	5	5	-3	10	low N high PK
4	106-133	4	22	22	16	high N

species was placed in each of three styrofoam coolers with the rootballs supported and covered to a depth of 5 cm with dry vermiculite. The coolers, with the lids wired shut and fitted with thermister probes into the crowns of the seedlings, were placed in a 650-liter household chest freezer. Crown temperature was lowered rapidly from ambient to 0°C and at a rate of 3 to 5°C per hour thereafter. A baking pan filled with liquid nitrogen was placed in the freezer to reach temperatures below -25°C. The pan size and degree of foam insulation controlled the rate of temperature fall. Three temperatures, 5°C apart, were selected to bracket the expected LT of the stem tissue. When a cooler reached a selected test temperature, it was removed from the freezer and placed in a refrigerator at 1°C to thaw overnight. The seedlings were then removed from the coolers and placed in a warm greenhouse (day 26°C, night 19°C, 22-hour day).

Extent of injury to each seedling was assessed after 7 days. The percentage of the length of the stem that was killed was estimated by examining the cambium and phloem for browning and loss of tissue integrity. Rates of increasing injury with decreasing temperature were compared across test day and species, and data with similar rates were subjectively placed into six groups. This pooling of data was necessary because 12 trees per species per test day did not provide adequate information for statistical analysis. Injury in the range of 10 to 90% was regressed against temperature for each group, and the 50% injury point (LT_{50}) was estimated by calibration methods (Graybill 1976). The range 10 to 90% was chosen because the relation between injury and temperature was primarily linear, but nonlinear above and below that range.

Root Growth Potential (RGP)

Eight additional seedlings per species were placed in an aeroponic mist box in a greenhouse (day 26°C, night 18°C, long days) to measure RGP. A mist box measuring 1.0 m wide x 2.4 m long x 0.6 m high, was constructed of 5 cm thick rigid urethane foam, and was fitted with a PVC piping, 3-nozzle system 25 cm above the floor of the box. The seedlings were inserted through holes in strips of plywood which formed the top of the box, and were held in place with soft urethane foam plugs. The intact rootballs, suspended within the box, were exposed to 100% relative humidity at 27°C maintained by a warm-water intermittent mist. After 7 and 14 days, the total number of new white roots, $\geq$0.5 cm in length, that had emerged from the rootball were measured to the nearest cm and counted. Tallied roots were marked with tempera paint to prevent duplicate measurement. (The paint was subsequently removed by the mist.) Seedling height and caliper data were also taken. Measurements were made without damage to the seedlings, which were kept in the mist chamber until bud break to assess dormancy status.

RGP was expressed as total number of new roots per seedling and total length of new roots per seedling, at 7 and 14 days. The data sets for total new root length per seedling at 14 days for the three species were selected to assess the significance of possible covariates. There was no trend over time in seedling height or caliper in any of the three species. No consistent covariance existed between RGP and height, caliper, or (height x caliper^2) in Engelmann spruce and ponderosa pine. Seedling height was a significant ($p=.02$) covariate in Douglas-fir, but the contribution of the covariate was so small ($R^2 = .04$) that it did not warrant inclusion in further data analysis. There was no consistent covariace between RGP and caliper or (height x caliper^2) in Douglas-fir.

Box plots were used to flag outliers in the same three data sets (Chambers et al. 1983). Thirteen of the 360 seedlings, with RGP measurements several standard deviations from the weekly mean, were omitted after each seedling was found to be defective in some way, and therefore not properly part of the main population. Weekly means, with 95% confidence intervals, were calculated from the remaining observations for all 12 RGP data sets.

Homogeneity of variances was rejected ($p \leq .005$) for all data sets using Bartlett's test. Welch's test was used for comparing all means within each data set because the data were not suitable for transformation. All hypotheses of equal means were rejected ($p<.0001$). Pairwise comparison of means with an F-protected LSD test, approximated using heterogeneous variance t-tests, resulted in many statistically significant differences ($p=.05$). However, because of the heterogeneous variances, detecting differences between means was not as straight forward as applying a standard least significant difference for all pairs compared. Thus, for ease of interpretation, major differences between means, as determined by the test of non-overlapping 95% confidence intervals (Jones 1984), were established and indicated on Figures 2, 3, and 4. The test of non-overlapping 95% confidence intervals was found to be intermediate between the more conservative Dunnett's T3 test ($p=.05$) (Dunnett 1980) and the more liberal F-protected LSD ($p=.05$). More importantly, the chosen method identified significant changes in RGP which could be readily envisioned as biologically important

differences. Means with 95% confidence intervals for the 12 data sets are presented in Burr (1987).

A correlation analysis between length and number was performed for both 7- and 14-day data, on an individual seedling basis within each species, to determine how well total number of new roots per seedling might indicate total length of new roots per seedling.

RESULTS

Whole-plant Freeze Test

Cold hardiness was gained and lost in response to the four successive temperature stages (fig. 1). Seedlings of the three species did not harden during the first stage with warm temperatures and short days (day 20°C, night 15°C, 10-hour day). Stem cold hardiness, expressed as an LT_{50}, ranged from -11 to -17°C for the three species during these first 21 days. When growth chamber temperatures were lowered to 10°C day and 3°C night in the second stage, there was a lag period of variable length, depending upon the species, before cold hardening of stem tissue proceeded. There was a 1-week lag (test days 21 to 28) in ponderosa pine, a 2-week lag (test days 21 to 35) in Engelmann spruce, and a 2-week lag after the first week of the second stage (test days 28 to 42) in Douglas-fir. Cold hardiness increased after these lag periods until maximum cold hardiness was reached at the end of the third stage (day 5°C, night -3°C) on test day 105.

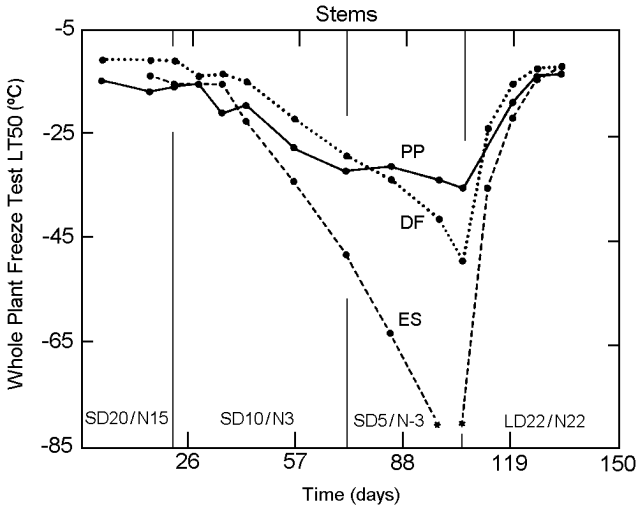

Figure 1.--Stem cold hardiness (LT_{50}) of ponderosa pine (PP), Douglas-fir (DF), and Engelmann spruce (ES) as a function of time, determined by the whole-plant freeze test. Engelmann spruce cold hardiness on test dais 98 and 105 is indicated by asterisks at -80°C. On these two test days there was no injury (LT_0) to stem tissue at -75°C, the lower limit of the freezer. Growth chamber conditions are indicated across the bottom of the graph and are described in table 1.

Maximum stem cold hardiness, expressed as an LT_{50}, reached -35°C in ponderosa pine and -49°C in Douglas-fir. Engelmann spruce cold hardiness on test days 98 and 105 is indicated by asterisks at -80°C (fig. 1). On these two days there was no injury (LT_0) to stem tissue at -75°C, the lower limit of the freezer. Deacclimation began immediately in all three species upon exposure to the fourth stage conditions (day 22°C, night 22°C, 16-hour day). Cold hardiness was rapidly lost and reached minimum levels on test day 133 at the end of the 19 weeks. Stem tissue cold hardiness on test day 133 was -13°C in ponderosa pine and -11.5°C in Douglas-fir and Engelmann spruce.

Bud Dormancy

Dormancy requirements for ponderosa pine were fully met by test day 21, at the end of the first stage, and for both Douglas-fir and Engelmann spruce by test day 71, at the end of the second stage. Bud break occurred during the 18th week of the regime in Engelmann spruce, and during the 19th week in ponderosa pine and Douglas-fir.

Root Growth Potential (RGP)

The RGP patterns were similar, in a general way, for the three species, whether measured as total length or total number of new roots per seedling, after either 7 or 14 days in the mist chamber (figs. 2, 3, 4). RGP was low in the first stage when cold hardiness was at a minimum and dormancy intensity was maximum. RGP remained low for differing portions of the second stage. High, though variable, RGP levels were reached in the second and/or third stages as cold hardiness increased and chilling requirements for bud dormancy were met. Maximum RGP levels were at least 5-fold greater than minimum RGP levels. During the first week of deacclimation in the fourth stage, RGP did not decrease, although approximately 65% of maximum cold hardiness was lost. Following the first week of deacclimation, RGP declined rapidly. Both cold hardiness and RGP had returned to minimum levels at bud 'break.

Correlation analysis within each species indicated that total length and total number of new roots at 7 days were strongly correlated (R=.918 to .933), as were total length and total number of new roots at 14 days (R=.889 to .948) (table 2). The strength of the correlation between length and number at 7 days was similar to that at 14 days in Douglas-fir and Engelmann spruce. In ponderosa pine, the correlation between length and number was stronger at 7 days than after 14 days. The variability in total number of new roots per seedling accounted for 79.0 to 89.9% of the variability in total new root length per seedling, depending upon species and time of measurement. The patterns of the RGP means, expressed as total length and total number of new roots at each of the two measurement times, were thus very similar within each species (figs. 2, 3, 4).

In general, for the three species, changes as large or larger than a 100% increase or decrease

Table 2.--Correlation analysis between total length and total number of new roots per seedling for each species after 7 and 14 days in the mist chamber.

Species	R	R2
Ponderosa pine		
7 days	.93330	.87105
14 days	.88901	.79034
Douglas-fir		
7 days	.92333	.85255
14 days	.94828	.89924
Engelmann spruce		
7 days	.91811	.84293
14 days	.90630	82138

(e.g. doubling) in RGP over time were significantly different, independent of time or method of measurement. Changes in number or length of roots during the 19-week regime were not statistically significant on the same test date when measured at 7 and 14 days. When ponderosa pine RGP was measured as total new root length per seedling (fig. 2A), the first significant increase in RGP during cold acclimation occurred on test day 42 when measured at 14 days, and on test day 56 when measured at 7 days. The decrease in RGP during the third stage was not significantly different from the peak on test day 71 when measured at either time. However, the low RGP levels in the third stage were not significantly different from the earlier low levels, such as between test days 14 and 28. RGP increased on test day 112, after 1 week of deacclimation, when measured at both times, but the increase was significant only at 7 days. RGP then returned to the original low levels. When ponderosa pine RGP was measured as total number of new roots per seedling (fig. 2B), the first significant increase in RGP during cold acclimation also occurred on test day 42 when measured at 14 days, and on test day 56 when measured at 7 days. The decrease in RGP during the third stage was significantly lower than the peak on test day 71 but also significantly greater than the earlier lowest (a) levels, when measured at both 7 and 14 days. The increase in RGP during the first week of deacclimation was significant only when measured at 7 days. RGP then returned to the original low levels.

In Douglas-fir, when RGP was measured as total length or number of new roots per seedling (figs. 3A, 3B), the first significant increase in RGP during cold acclimation occurred on test day 42 when measured at 14 days, and on test day 71 when measured at 7 days. A second significant increase occurred in both the 7- and 14-day measurements by test day 84. This was followed by a significant decrease in RGP on test day 98, when measured at 7 days, which was not significantly different from the earlier lowest (a) levels. The pattern was not the same at 14 days. The changes in RGP during the first week of deacclimation were not significant at either measurement time, and by the end of the fourth stage, RGP had returned to the earlier lowest levels.

When Engelmann spruce RGP was measured as total length or number of new roots per seedling (figs. 4A, 4B), the first significant increase during cold acclimation occurred on test day 42 when measured at 14 days, but did not occur until test day 84 when measured at 7 days. RGP fluctuated from test day 42 to the end of the third stage, on test day 105, when measured at 14 days, though none of the changes were

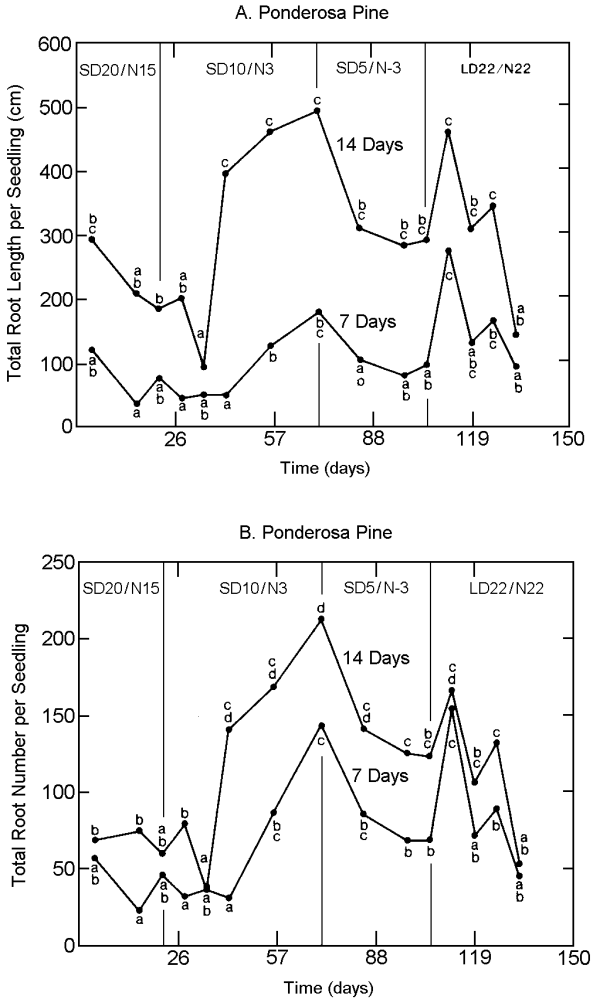

Figure 2.--Ponderosa pine root growth potential expressed as (A) total length of new roots per seedling and (B) total number of new roots per seedling measured after 7 or 14 days in a mist chamber, as a function of time. Within each curve (7 days and 14 days), means with the same letter are not significantly different. Growth chamber conditions are indicated across the top of the graphs and are described in table 1.

statistically significant. There was also no further significant change in RGP during the third stage when measured at 7 days. None of the changes in RGP during the first week of deacclimation were significant when measured after either 7 or 14 days. RGP had returned to fairly low levels at the end of the fourth stage.

Ponderosa pine data were normalized to test day 71, and Douglas-fir and Engelmann spruce data to test day 84, to illustrate the differences and similarities in the patterns of the 7- and 14-day measurements (figs. 5, 6, 7). The normalized ponderosa pine data (fig. 5) made more apparent the 2-week delay in detecting the increase in RGP during cold acclimation when RGP was measured after 7 days. Measurement of total number of new roots per seedling at 14 days best differentiated between the low RGP levels of the third stage and of the first two stages. The increase in RGP during the first week of deacclimation was readily detected when measurements were made after 7 days.

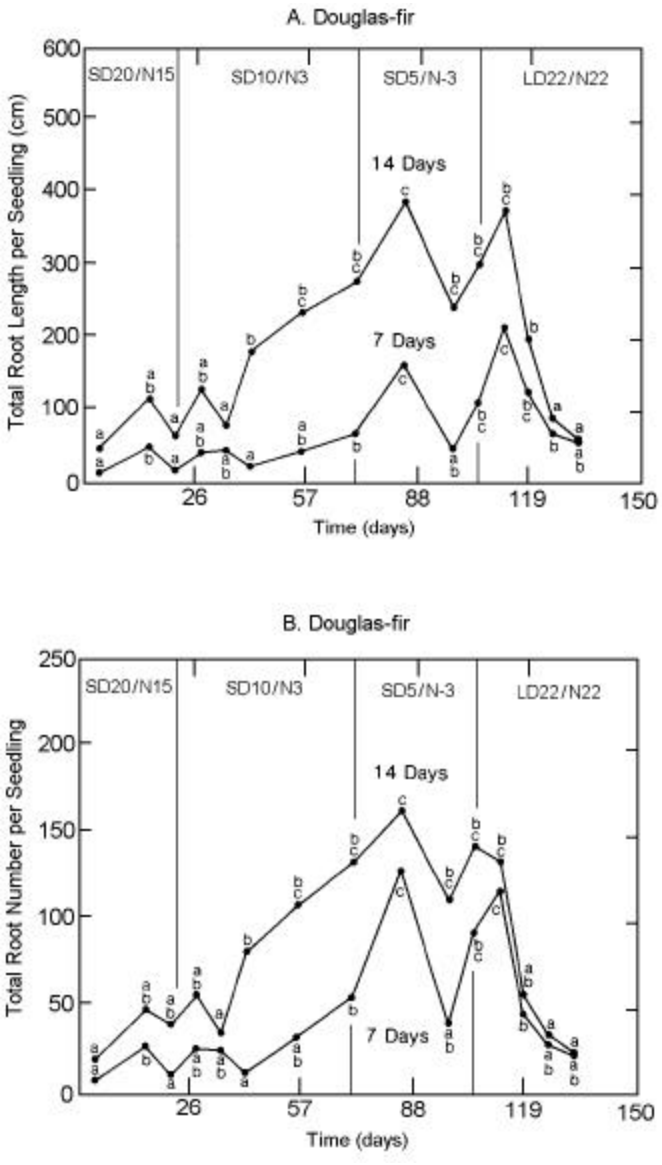

Figure 3.--Douglas-fir root growth potential expressed as (A) total length of new roots per seedling and (B) total number of new roots per seedling measured after 7 or 14 days in a mist chamber, as a function of time. Within each curve (7 days and 14 days), means with the same letter are not significantly different. Growth chamber conditions are indicated across the top of the graphs and are described in table 1.

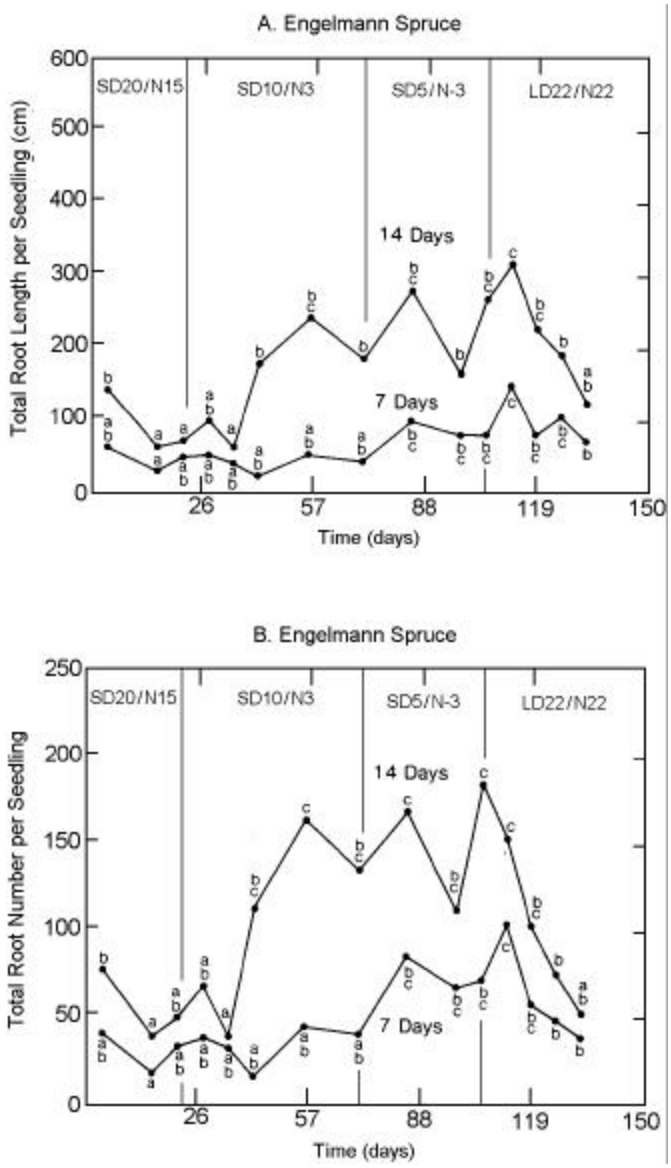

Figure 4.--Engelmann spruce root growth potential expressed as (A) total length of new roots per seedling and (B) total number of new roots per seedling measured after 7 or 14 days in a mist chamber, as a function of time. Within each curve (7 days and 14 days), means with the same letter are not significantly different. Growth chamber conditions are indicated across the top of the graphs and are described in table 1.

The normalized Douglas-fir RGP data (fig. 6) made more apparent the 4-week delay in detecting the increase in RGP during cold acclimation when measured at 7 days. Also apparent was the inability to distinguish the low RGP on test day 98 from the RGP prior to test day 42, when measured at 7 days. When measured at 14 days, the decline on test day 98 indicated a fluctuation during a period of high RGP, rather than a sudden loss of RGP. During the first week of deacclimation, 7-day measurements suggested an increase in RGP more strongly than 14-day measurements.

Normalized Engelmann spruce RGP data (fig.7) indicated that detection of a significant increase in RGP above the low levels prior to cold acclimation in the second stage required an additional 5 to 6 weeks when measured at 7 days. During the first week of deacclimation, 7-day measurements suggested an increase in RGP, while 14-day measurements indicated no change.

DISCUSSIONS AND CONCLUSIONS

The RGP patterns of the three species (figs. 2, 3, 4) were a function of seedling response to simulated seasonal environmental changes created in growth chambers. Nevertheless, these patterns were quite representative of RGP patterns reported in the literature for nursery-grown bareroot seedlings lifted at regular intervals from bud set to bud break (Jenkinson 1980, Ritchie and Dunlap 1980, Stone et al. 1962).

RGP's measured as total number and as total length of new roots per seedling were strongly correlated in all three species, whether measured after 7 or 14 days in the mist chamber (table 2). Number of roots was a good predictor of length, indicating that changes over time in total new root length were mainly the result of changes in the number of roots elongating rather than changes in the elongation rate of the individual roots. Rietveld (1986) found that total number (R^2 =.88) and total length (R^2 =.90) of new roots were strongly correlated to a root area index, using a 17-day aeroponic test. Thus, not only were number and length of new roots well correlated, but both were also good estimators of new root surface area, the parameter of primary interest. Since length and number were nearly equally informative under the test conditions used here, measuring total number of new roots is recommended because it required only 252 of the time necessary to measure total new root length. More information can thus be gained per unit of time spent in data collection by measuring only the number of new roots on a 4-fold larger sample of seedlings than by also measuring total new root length on a 75% smaller sample of seedlings. For example, using the test of non-overlapping 95% confidence intervals, a doubling of the sample size from 8 to 16 seedlings would reduce the size of the confidence interval by 35%. Since a change in RGP of approximately 100% was required to be significantly different with a sample size of 8, a 651 increase or decrease would be significantly different with a sample size of 16. A 4-fold increase in sample size from 8 to 32 would reduce the size of the confidence interval by 56% and a 44% increase or decrease in RGP would be significantly different.

A significant increase in RGP during cold acclimation was detected 2 to 6 weeks earlier in the three species when RGP was measured after 14 days, rather than after 7 days, regardless of whether root number or length was measured. The inability to detect the increase when measured at 7 days was apparently the result of low growth

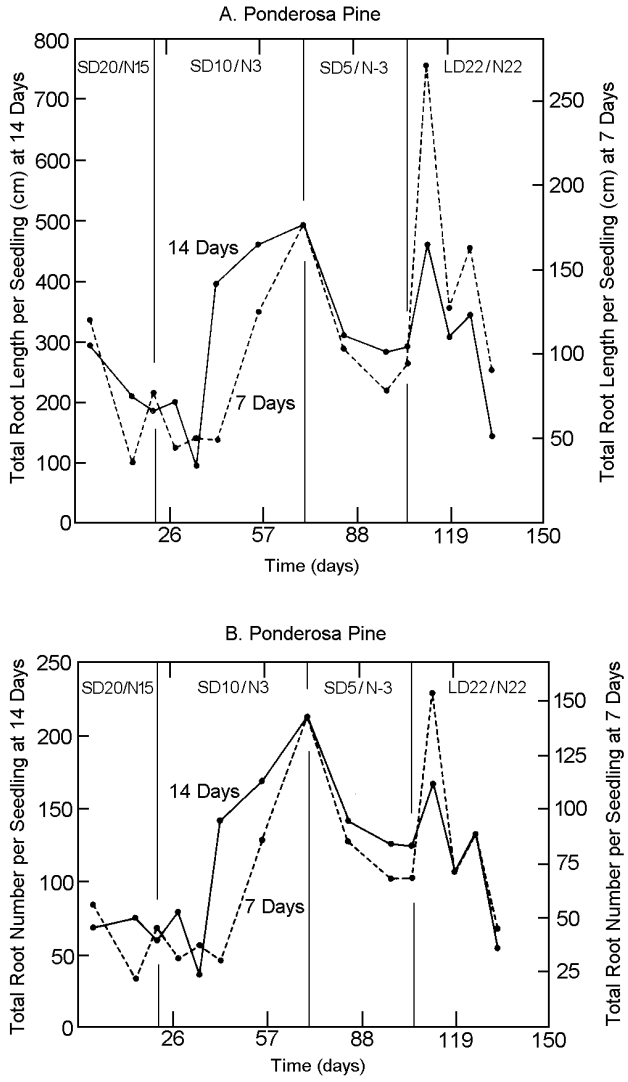

Figure 5.--Ponderosa pine root growth potential expressed as (A) total length of new roots per seedling and (B) total number of new roots per seedling measured after 7 to 14 days in a mist chamber, as a function of time. The 7-day Y-axis scales have been adjusted such that the 7- and 14-day data converge at test day 71. Growth chamber conditions are indicated across the top of the graphs and are described in table 1.

levels during the first 7 days in the mist chamber combined with high levels of growth during the second 7 days (figs. 2, 3, 4). A second disadvantage of 7-day measurement of RGP during the period of cold acclimation was the inability to distinguish between fluctuations in high RGP levels and the low RGP levels prior to the start of cold acclimation. This was particularly true in Douglas-fir (fig. 3) and also in ponderosa pine (fig. 2A). Additionally, all first significant increases in RGP, when measured after 14 days in the mist chamber, occurred on test day 42, whether expressed as total number or total length of new roots. The increase in RGP between test days 35 and 42 corresponded well with the onset of steady, rapid increases in cold hardiness (fig. 1). It marked the end of the plateau period at the beginning of the second stage, during which there was a lag in the development of cold hardiness as well as RGP. No such relationship was apparent between cold hardiness and RGP measured at 7 days. Measurement of RGP after 7 days was not as informative as measurement at 14 days during the period of cold acclimation for these reasons. A 7-day test of RGP prior to cold deacclimation, whether as a routine test of seedling quality or over a period of time to determine lifting windows, could be very misleading.

However, measurement of RGP after 7 days may be a better indicator of the onset of deacclimation than 14-day measurement, especially in ponderosa pine (fig. 5). For example, RGP consistently increased during the first week of

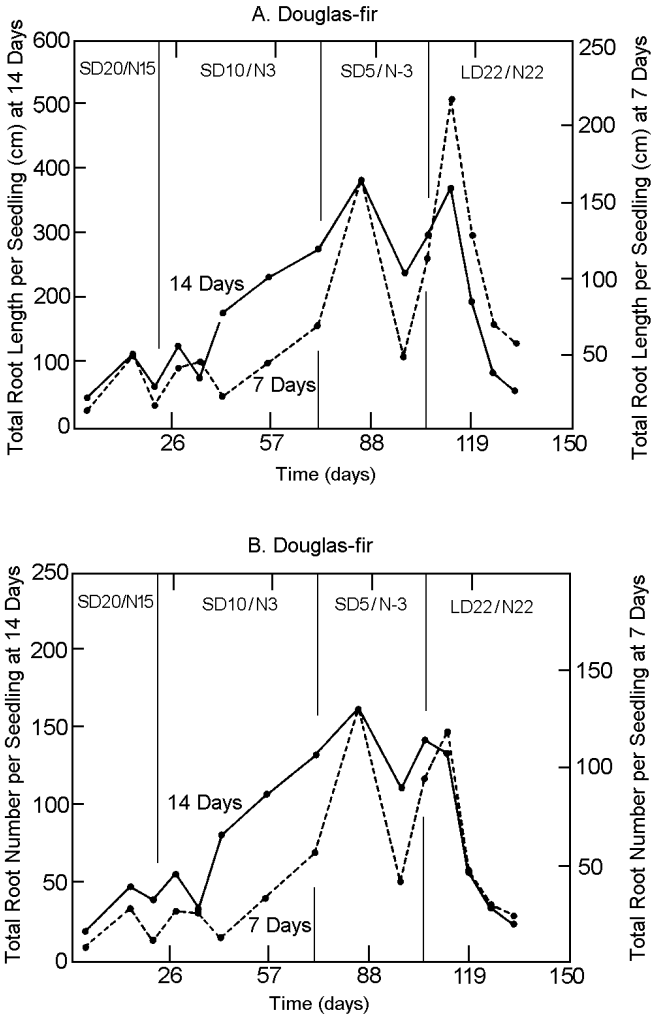

Figure 6.--Douglas-fir root growth potential expressed as (A) total length of new roots per seedling and (B) total amount of new roots per seedling measured after 7 or 14 days in a mist chamber, as a function of time. The 7-day Y-axis scales have been adjusted such that the 7- and 14-day data converge at test day 84. Growth chamber conditions are indicated across the top of the graphs and are described in table 1.

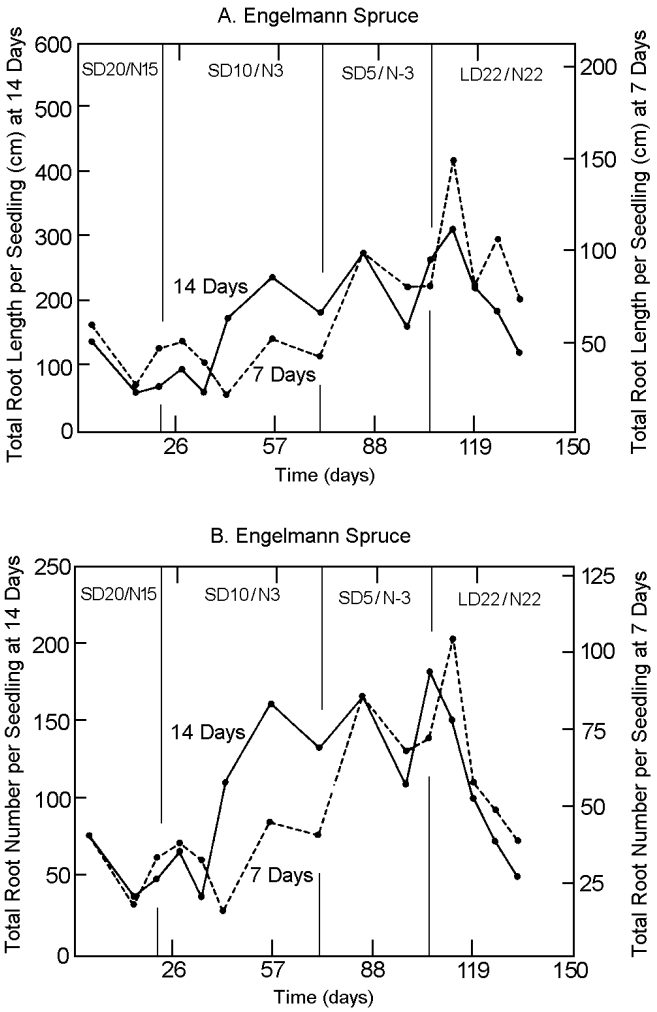

Figure 7.--Engelmann spruce root growth potential expressed as (A) total length of new roots per seedling and (B) total number of new roots per seedling measured after 7 or 14 days in a mist chamber, as a function of time. The 7-day Y-axis scales have been adjusted such that the 7- and 14-day data converge at test day 84. Growth chamber conditions are indicated across the top of the graphs and are described in table 1.

deacclimation when measured after 7 days. Though the increase was significant only in ponderosa pine (fig. 2), the normalized data (figs. 5, 6, 7) indicated that the relative magnitude of the increase was greater at 7 days than at 14 days in all instances. RGP measurement at 14 days during the first week of deacclimation led to the conclusion that no change occurred. The rapid decline in RGP after the first week of deacclimation was as clearly indicated in the 7-day measurements as in the 14-day measurements (figs. 5, 6, 7). This was true largely because the majority of the root growth, especially increases in number of roots, occurred during the first 7 days in the mist chamber. RGP measurements at 7 days are thus recommended if the data are to be used to monitor the rapid loss of stock quality with approaching bud break.

In summary, total length and total number of new roots per seedling were nearly equally informative with container stock under the mist chamber conditions described. Use of number of roots with relatively larger sample sizes is recommended as most efficient and informative. RGP tests of 7 and 14 days in duration yielded different information. On the basis of accuracy and quantity of information provided, the 14-day test is recommended during cold acclimation and the 7-day test is suggested for use during cold deacclimation.

LITERATURE CITED

Burdett, A. N. 1979. New methods for measuring root growth capacity: their value in assessing lodgepole pine stock quality. Can. J. For. Res. 9:63-67.

Burdett, A. N., D. G. Simpson, and C. F. Thompson. 1983. Root development and plantation establishment success. Plant and Soil 71:103-110.

Burr, K. E. 1987. Cold hardiness, root growth capacity, and bud dormancy testing of conifer seedlings. Ph.D. Dissertation, Colorado State University, Fort Collins, Colo., p. 109-115.

Chambers, J. M., W. S. Cleveland, B. Kleiner, and P. A. Tukey. 1983. Graphical methods for qdata analysis. Wadsworth Internat. Group, CA. 395p.

Day, R. J. 1982. Evaluating root regeneration potential of bare-root nursery stock. p. 83-96 In: Huber, R. F., compiler. Proc. 1981 Intermountain Nurserymen's Assoc. meeting, Aug. 11-13, 1981, Edmonton, Alberta. Environ. Can., For. Serv., North. For. Res. Cent., Edmonton, Alberta. Inf. Rep. NOR-X-241.

DeWald, L. E., P. P. Feret, and R. E. Kreh. 1985. A 15-day hydroponic system for measuring root growth potential. U.S.D.A. For. Serv. Gen. Tech. Rep. 5O-54, p. 4-10.

Dunnett, C. W. 1980. Pairwise multiple comparisons in the unequal variance case. J. Amer. Statistical Assoc. 75:756-800.

Graybill, F. A. 1976. Theory and application of the linear model. Section 8.5. Duxbury Press, CA. 704 p.

Hileman, G. R. 1986. Root growth capacity system. U.S.D.A. For. Serv. Gen. Tech. Rep. RM-137, p: 75-76.

Jenkinson, J. L. 1980. Improving plantation establishment by optimizing growth capacity and planting time of western yellow pines. U.S.D.A. For. Serv. Res. Pap. PSW-154. 22p.

Jones, D. 1984. Use, misuse, and role of multiple-comparison procedures in ecological and agricultural entomology. Environ. Entomol. 13:635-649.

Krugman, S. L. and E. C. Stone. 1966. The effect of cold nights on the root-regenerating potential of ponderosa pine seedlings. For. Sci. 12:451-459.

Larsen, H. S. and J. N. Boyer. 1986. Root growth potential of loblolly pine (Pinus taeda L.) seedlings from twenty southern nurseries. Circular 286, Ala. Agric. Exp. Stn., Auburn Univ. 16p.

Lee, C. I. and W. P. Hackett. 1976. Root regeneration of transplanted Pistacia chinensis Bunge seedlings at different growth states. J. Amer. Soc. Hort. Sci. 101:236-240.

Nambiar, E. K. S., G. D. Bowen, and R. Sands. 1979. Root regeneration and plant water status of Pinus radiata D. Don seedlings transplanted to different soil temperatures. J. Exp. Bot. 30:1119-1131.

Nambiar, E. K. S. 1980. Root configuration and root regeneration in Pinus radiata seedlings. N.Z. J. For. Sci. 10(1):249-263.

Newman, E. I. 1966. A method of estimating the total length of root in a sample. J. Appl. Ecol. 3:139-145.

Rietveld, W. J. 1986. A new, more efficient method to evaluate root growth potential of planting stock using a root area index. U.S.D.A. For. Serv. Gen. Tech. Rep. RM-137, p. 96.

Ritchie, G. A. and J. R. Dunlap. 1980. Root growth potential: its development and expression in forest tree seedlings. N.Z. J. For. Sci. 10(1):218-248.

Ritchie, G. A. 1984. Assessing seedling quality. Ch. 23 In: Duryea, M. L. and T. D. Landis, eds. Forest nursery manual: Production of bare-root seedlings. Martinus Nijhoff/Dr. W. Junk Pub., The Hague/Boston/Lancaster. 386p.

Ritchie, G. A. 1985. Root growth potential: principles, procedures, and predictive ability. Ch. 8 In: Duryea, M. L. ed. Evaluating seedling quality: principles, procedures, and predictive abilities of major tests. Workshop held October 16-18, 1984. Forest Research Laboratory, Oregon State University, Corvallis. 143p.

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. U.S.D.A. For. Serv. Gen. Tech. Rep. 5O-54, p. 25-33.

Stone, E. C. 1955. Poor survival and the physiological condition of planting stock. For. Sci. 1:90-94.

Stone E. C., E. E. Gilden, D. W. Cooper, and R. J. Malain. 1961. Planting dates for Douglas-fir seedlings in California forest lands. Calif. Agric. 15(8):15-16.

Stone, E. C. and J. L. Jenkinson. 1970. Influence of soil water on root growth capacity of ponderosa pine transplants. For. 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. For. Sci. 8:288-297.

Stone, E. C. and G. H. Schubert. 1959. Root regeneration by ponderosa pine seedlings lifted at different times of the year. For. Sci. 5:322=332.

Stone, E. C., G. H. Schubert, R. W. Benseler, F. J. Baron, and S. L. Krugman. 1963. Variation in the root regenerating potentials of ponderosa pine from four California nurseries. For. Sci. 9:217-225.

Sutton, R. F. 1980. Planting stock quality, root growth capacity, and field performance of three boreal conifers. N.Z. J. For. Sci. 10(1):54-71.

Tinus, R. W., K. E. Burr, S. J. Wallner, and R. M. King. 1986. Relation between cold hardiness, root growth capacity, and bud dormancy in three western conifers. U.S.D.A. For. Serv. Gen. Tech. Rep. RM-137, p. 80-86.

Tinus, R. W. and S. E. McDonald. 1979. How to grow tree seedlings in containers in greenhouses. U.S.D.A. For. Serv. Gen. Tech. Rep. RM-60. 256p.

Torrey, J. G. 1976. Root hormones and plant growth. Ann. Rev. Plant Physiol. 27:435-459.

Winjum, J. K. 1963. Effects of lifting date and storage on 2+0 Douglas-fir and noble fir. J. For. 61:648-654.