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Root Growth Potential and Field Survival of Container Loblolly Pine Seedlings Fall Fertilized with Nitrogen

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ROOT GROWTH POTENTIAL AND FIELD SURVIVAL OF CONTAINER LOBLOLLY PINE SEEDLINGS FALL FERTILIZED WITH NITROGEN[1]

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Abstract—Two studies investigated the effects of fall nitrogen fertilizer applications on the root growth potential (RGP) and field performance of container loblolly pine seedlings (*Pinus taeda* L.). The seedlings were sampled at 4 chilling levels ranging from 100 to 550 hours (0 to 8° C). Seedlings propagated for the first study may have had a hidden nutrient deficiency and therefore the fall diammonium phosphate (DAP) application at rates of 202 kg N/ha and 67 kg N/ha increased RGP 43% and 32%, respectively. The growing season mineral fertilizer application rate was increased in the second study which may explain why fall nitrogen applications at 202 kg N/ha did not increase RGP. In general, RGP increased as exposure to chilling hours increased. Fall fertilization increased total seedling weight. Analysis of covariance indicated that RGP may be a function of total seedling weight and not a direct response to fertilizer treatment or chilling level. For the first study, survival was not significantly affected by the fall DAP treatments.

INTRODUCTION

The majority of studies concerning the effects of fall nitrogen applications have concentrated on the northern forest tree species (Anderson and Gessel 1966, Benzian et al. 1974, Thompson 1982, van den Driessche 1985, 1988, Margolis and Waring 1986a, 1986b). The influences of fall nitrogen applications have not been extensively studied for loblolly pine (*Pinus taeda* L.). Earlier observations for loblolly pine suggest that fall nitrogen applications had a detrimental effect on outplanting performance (Ursic 1956, Gilmore et al. 1959, Shoulders 1959). Poor performance might be the result of altering the bud dormancy cycle. For loblolly pine, several studies have investigated the relationship between bud dormancy, storability, root growth potential (RGP) and outplanting performance (Garber 1983, Boyer and South 1985, Larsen et al. 1986, Dewald and Feret 1988).

RGP is considered to be an important measure of seedling quality (Ritchie 1985). As bud dormancy is released by chilling, increases in RGP have been observed for loblolly pine (Rhea 1977, Johnson and Barnett 1985, Carlson 1985, Larsen et al. 1986). Few studies have reported the influence of nursery cultural practices on the interrelationship between the chilling requirement and other seedling processes. Therefore, the effects of fall nitrogen fertilization on RGP as it relates to chilling level were examined on container-grown loblolly pine seedlings.

MATERIALS AND METHODS

1984-85 Study

Seed from two half-sibling seed lots of loblolly pine (*Pinus taeda* L.) were sown in 144 cm^3 plastic cone containers filled with a coarse sand. Seed sources were from geographically different locations. The ortet of the northern family (No. 0208) was from Virginia (approx. 36.90°N lat. 76.95°W long.) while a southern ortet (No. 0056) was from Georgia (approx. 32.07°N lat. 81.54°W long.). Containers (98) were held in plastic trays which covered a 0.186 square meter area. Seedlings were grown outside at Auburn, Alabama.

Seedlings were sprayed with a Captan spray preceding germination for damping-off control. During the growing season, the seedlings were fertilized every two weeks beginning on May 21, 1984 alternating applications of a 20-20-20 (N-P_2O_5-K_2O) and a 15-30-15 fertilizer. Six applications were made supplying 170 kg/ha/year of nitrogen. The seedlings were watered approximately every other day until the end of August

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when watering was reduced. Both water and fertilizer were withheld promoting bud-set and hardening-off.

The study was conducted as a randomized complete block split plot design with four replications. Whole plots consisted of three levels of a fall application of diammonium phosphate (DAP) fertilizer treatment, while family represented the subplots. DAP was applied with a water can at levels equivalent to 0 (control), 67, and 202 kg N/ha over two application dates of September 28, 1984 and October 5, 1984.

Chilling hours, defined as the number of hours seedlings experienced temperatures between 0 and 8°C, were monitored using a digital temperature recorder. The temperature recorder was placed in a weather box located at seedling height. When chilling levels reached approximately 100, 250, 400, and 550 hours (Table 1), seedlings were sampled from each replication for each treatment and family (subplot). The variables measured were root growth potential and field survival and height growth.

Table 1. Sampling dates and associated chilling hours (0-8° C) for the 1984-85 and 1985-86 root growth potential and outplanting experiments.

Sampling Date	Proposed Chilling Hours	Actual Chilling Hours
	— 1984-85 Fall N Study —	
November 13, 1984	100	97
November 29, 1984	250	238
January 6, 1985	400	406
January 19, 1985	550	559
	— 1985-86 Fall N Study —	
December 10, 1985	100	99
December 29, 1985	250	254
January 11, 1986	400	397
January 30, 1986	550	569

Root Growth Potential

Root growth potential (RGP) for unstored and stored seedlings was measured using a hydroponic method described by Ritchie (1984). RGP testing was conducted in a heated, glass greenhouse. Photoperiod was extended to 16 hr using 300 W incandescent light bulbs. Ten seedlings from each subplot were randomly sampled at each chilling level. Five seedlings were placed in 49 liter plastic bags and the bags placed in cold storage at 4°C for a 10-week period. The other five seedlings were tested immediately. Following a 30-day test period, seedlings were removed from the RGP test and placed in cold storage until root counts could be made. RGP was determined as the number of new roots (white roots) greater than or equal to 1 cm in length. Following the 10-week storage period, RGP was determined in a similar manner for the stored seedlings.

Field Performance

Twenty seedlings from each subplot were randomly sampled at each chilling level for outplanting. Ten of the seedlings were inserted in 49 liter plastic bags and the bags placed in cold storage at 4°C for 10 weeks. The remaining 10 seedlings were outplanted immediately. The outplanting site was located approximately 16 km north of Auburn, Alabama. Soil at the site was classified as a Gwinnett sandy loam (United States Department of Agriculture 1981). Seedlings were removed from their containers and planted in rows on a spacing of 61 cm by 91 cm using a dibble bar. Following planting, initial height and ground-line diameter were recorded. Stored seedlings were outplanted in a similar manner 10 weeks later. Subsequent measurements included first and second year height and survival.

1985-86 Study

A second study continued the examination of RGP response to late-season nitrogen application. The same two loblolly pine families reported previously were used in this study. Seed were sown in the plastic cone containers filled with a peat-perlite-vermiculite-pine bark mixture. For control of fusiform rust (*Cronartium quercuum* f. sp. *fusiforme*), seeds and seedlings were treated with triadimefon. Also, seedlings were sprayed periodically with malathion to control aphids. During the growing season, the seedlings were fertilized once a week beginning on May 11, 1985 applying similar fertilizer types used in the 1984-85 study. By August 31, about 155.3 mg N had been applied to each seedling. Seedlings were watered every other day until August. Fertilization was stopped and watering was reduced in August to promote bud-set and hardening-off.

The study design was a randomized complete block split-plot with four replications. Whole plots consisted of the fertilizer treatments and the subplots were the families. Fertilizer treatments consisted of comparisons between DAP, ammonium nitrate (AN), and a control.

DAP and AN were applied on November 3, 1985 at a rate equivalent to 202 kg N/ha. Seedlings for RGP were sampled at the same chilling levels stated for the 1984-85 study (Table 1).

Data Analysis

For each study, analysis of variance for a randomized complete block split-plot design was conducted using Statistical Analysis System procedures (Statistical Analysis System 1987). The main effects of fertilizer treatment, family, and storage were considered fixed. Analysis for the main effect of chilling level was handled as a split-plot in space and time (Steel and Torrie 1980). Plot means were used as the observations for all studies. Analysis of covariance was done to aid in RGP data interpretation. Where appropriate, simple correlation coefficients were calculated to identify relationships between certain variables. Differences within main effects and their interactions will be discussed as significant at the ≤ 5% level of probability.

RESULTS

1984-85 Study

Application of the DAP fertilizer did not significantly alter the nitrogen (N) concentration of foliage (avg.=0.70%) and roots (avg.=0.43%) for seedlings sampled in January. Stems of fall fertilized seedlings had higher N concentrations (0.41%) than the control seedlings (0.33%).

The main effects of fall fertilizer treatment, family, chilling level, and storage each had a significant influence on RGP (Table 2). RGP was enhanced 43 and 32 percent over the control when treated with 202 and 67 kg N/ha, respectively. Georgia seedlings produced an average of 12 more new roots per seedling than Virginia seedlings. An increase of about four new roots per seedling occurred with each addition of 150 chilling hours. Ten weeks of storage reduced RGP by about 9 percent.

Any significant interactions between main effects were small, usually involving fewer than 5 new roots. An exception is the chilling by storage interaction (P>F=0.0003). Storage reduced RGP by 54 percent at the 100 chilling hour level (Table 3). At 250 hours and greater, RGP was improved or remained unchanged with the 10 weeks of storage.

Table 2. Seedling biomass and root growth potential (RGP) of seedlings tested for RGP, 1984-85 Fall N Study.

Treatment	Original Weight (cm)	Root Collar Diameter (mm)	Total Oven-Dry Weight (g)	Root Growth Potential (#)
Nitrogen Level[1]				
Control	17.8 ns	3.5 ns	2.05 ***	29 ***[2]
67 kg N/ha	17.8	3.7	2.45	43
202 kg N/ha	17.6	3.8	2.53	51
Family				
Virginia	15.6 ***	3.6 ns	2.16 ***	35 ***
Georgia	19.8	3.7	2.52	47
Chilling Level[3]				
100	17.7 ns	3.6 *	2.11 ***	35 *
250	17.2	3.7	2.29	39
400	18.1	3.7	2.40	43
550	18.0	3.8	2.51	48
Storage (10 wk)				
No	-	3.6 ns	2.27 ***	43 ***
Yes	17.7	3.7	2.40	39

[1] Nitrogen levels were applied over two application dates of September 9 and October 5, 1984 using Diammonium Phosphate.

[2] ns, *, **, *** = not significant, and significant at the 5%, 1% and 0.1% probability level, respectively.

[3] Chilling Level = the number of hours the seedlings experienced temperatures between 0 to 8° C before sampling.

The fertilizer treatments increased seedling oven-dry weight (Table 2). The Georgia seedlings were heavier than the Virginia seedlings. At each higher level of chilling, seedlings sampled for RGP were heavier. When compared with other oven-dry weight measures, total seedling oven-dry weight had the highest simple correlation coefficient with RGP (r=0.7685; P>t=0.0001; n=180). An analysis of covariance, with total seedling oven-dry weight as the covariate, was conducted to determine if the main effect results were a function of seedling size or a physiological response altering RGP. While their significance levels were greatly reduced, adjusting RGP for total weight showed that only the main effects of fertilizer treatment (P>F=0.0433) and storage (P>F=0.0079) remained significant. The analysis of covariance indicated that the chilling by storage interaction was not significant (P>F=0.3034).

Table 3. Root growth potential of seedlings sampled at four chilling hour levels in relation to no storage or 10 weeks of storage.

Chilling Hour Level[2] (0 - 8° C)	Root Growth Potential[1] No Storage	Storage
	— 1984-85 Study —	
100	48	22
250	38	39
400	39	46
550	48	48
	— 1985-86 Study —	
100	60	59
250	53	35
400	76	36
550	92	42

[1] Root Growth Potential = The number of new, unsuberized roots greater than 1 cm in length.
[2] The chilling hour by storage treatment interaction for both studies is significant at the 0.1% probability level.

First-year field growth and survival was not significantly affected by the fertilizer treatments (Table 4). The Georgia seedlings had greater growth and survival than the Virginia seedlings. Chilling level did not significantly influence first-year growth, but, as chilling level increased, survival increased. Storage reduced survival by 8 percent. Significant family and storage effects were still evident the second year after outplanting. Also, a small increase in second-year height growth was observed for the fertilized seedlings.

Any significant interactions between main effects represented small differences in height growth or survival. The exception was the first-year chilling by storage interaction. First-year survival was reduced 24 percent and 9 percent by storage when seedlings received 100 and 250 chilling hours, respectively (Table 5). Survival was slightly improved or remained unchanged with storage for seedlings receiving 400 and 550 chilling hours.

1985-86 Study

Fall N applications did not significantly increase N concentrations in foliage (avg.=1.03%), stems (avg.=0.47%), and roots (avg.=0.53%) of seedlings

Table 4. First-year and second-year field survival and height growth for the 1984-85 Fall N study.

Treatment	First Year			Second Year		
	Total Ht. (cm)	Height Growth (cm)	Survival (pct)	Total Ht (cm)	Height Growth (cm)	Survival (pct)
Nitrogen Level[1]						
Control	30 ns	12 ns	75 ns	61 *	30 *	72 ns[2]
67 kg N/ha	31	13	76	63	32	74
202 kg N/ha	32	14	79	67	35	77
Family						
Virginia	28 ***	12 **	72 ***	58 ***	29 **	70 **
Georgia	34	14	82	70	36	78
Chilling Level[3]						
100	30 *	12 ns	64 **	60 ns	31 ns	61 ns
250	31	13	78	63	33	76
400	32	13	82	65	33	80
550	32	14	83	66	34	80
Storage (10 wks)						
No	31 ns	13 ns	81 ***	66 **	34 **	77 **
Yes	31	12	73	62	31	72

[1] Nitrogen Levels were applied to seedlings over two application dates of September 28 and October 5, 1984 using Diammonium Phosphate.
[2] ns, *, **, *** = not significant, and significant at the 5%, 1% and 0.1% probability level, respectively.

Table 5. First year outplanting survival of seedlings sampled at four chilling hour levels in relation to no storage or 10 weeks of storage for the 1984-85 Fall N study.

Chilling Hour Level[1] (0 - 8° C)	Percent Survival	
	No Storage	Storage
100	76	52
250	83	74
400	80	84
550	83	83

[1] The chilling hour level by storage interaction is significant at the 5% probability level.

Table 6. Seedling biomass and root growth potential (RGP) of seedlings tested for RGP, 1985-86 Fall N Study.

Treatment	Original Height (cm)	Root Collar Diameter (mm)	Total Oven-Dry Weight (g)	Root Growth Potential (#)
N Fertilizer[1]				
Control	41 ns[2]	5.8 ns	6.48 ns	54 ns
AN	41	5.8	6.63	60
DAP	41	5.8	6.65	58
Family				
Virginia	37 ***	5.8 **	6.16 **	50 **
Georgia	45	5.9	7.01	65
Chilling Level[3]				
100	41 ***	5.8 ***	6.22 ***	60 ***
250	39	5.6	5.87	43
400	41	5.9	6.74	58
550	43	6.1	7.50	67
Storage (10 wks)				
No	41 ns	5.8 ***	6.62 ns	71 ***
Yes	41	5.9	6.55	43

[1] AN = Ammonium Nitrate, DAP = Diammonium Phosphate; Application rate was 202 kg N/ha applied on November 3, 1985.
[2] ns, *, **, *** = not significant, and significant at the 5%, 1% and 0.1% probability level, respectively.
[3] Chilling Level = the number of hours the seedlings experienced temperatures between 0 to 8° C before sampling.

sampled in January. Fertilizer treatments did not significantly affect seedling weight or RGP (Table 6). The Georgia seedlings were heavier and had higher RGP than the Virginia seedlings. RGP decreased by 28 percent from 100 to 250 chilling hours. All chilling levels above 250 hours showed increases in RGP. Storage reduced RGP by 39 percent. The signficant chilling by storage interaction (P>F=0.0001) showed that storage did not reduce RGP when seedlings were sampled at 100 chilling hours, but storage did reduce RGP when sampling occurred after longer periods of chilling (Table 3).

High correlation coefficients between RGP and total seedling oven-dry weight (r=0.6122; P>t=0.0001; N=192) again necessitated the use of analysis of covariance. The main effects of chilling and storage remained significant. Also, the chilling by storage interaction remained significant (P>F=0.0001). The Georgia seedlings appear to have a higher RGP than the Virginia seedlings because of their larger size.

DISCUSSION

The enhanced RGP of seedlings fall fertilized in the first study may be explained by increases in seedling size and the poor seedling nutrient status. Loblolly pine continues adding shoot and root weight after height growth has stopped in the fall (May 1984). The Georgia seedlings were heavier than the Virginia seedlings. Seedlings sampled at each higher chilling level had greater total oven-dry weights. Analysis of covariance indicated that the family and chilling effect on RGP was confounded by total seedling oven-dry weight. Covariate analysis did not remove the significant chilling effect for the second study. However, the apparent relationship between the lower weight for seedlings sampled at the 250-hour level and their lower RGP casts doubt on the significant chilling effect.

Fall fertilization of Douglas-fir (*Psuedotsuga menziesii* (Mirb.) Franco) has improved RGP (van den Driessche 1985). Improving the nutrient content of the seedlings in the first study would be expected to improve RGP. The improved seedling N concentration in the second study may have resulted in the lack of a fall fertilizer effect on RGP.

It is hypothesized that the ability of seedlings to withstand storage is improved after fulfillment of the chilling requirement for winter bud rest (Garber and

Mexal 1980). The chilling experienced by the seedlings appeared effective in reducing the days-to-budbreak (Williams and South 1992). For the first study, RGP and field survival was not affected by long-term storage after 250 or more chilling hours. However, the analysis of covariance, with total oven-dry weight as the covariate, removed the significant chilling by storage interaction for RGP. Perhaps, the larger seedlings sampled at the higher chilling levels withstand long-term storage better than the smaller seedlings. The opposite result was observed for the second study. RGP was reduced by long-term storage for seedlings experiencing 250 or more chilling hours. Storage may reduce RGP regardless of the terminal bud rest status as influenced by chilling (Carlson 1985). In addition, chilling requirements for storage may differ from the amounts necessary for winter rest release (Boyer and South 1985).

Fall N fertilization improved the size and weight of the container seedlings without significantly altering the bud dormancy cycle (Williams and South 1992). Fall N fertilization may prove more beneficial for seedlings with known or hidden nutrient deficiencies. Any gains in RGP as a result of increasing seedling size may be negated by cold storage regardless of when the seedlings are lifted. Future research should concentrate on the type, timing and amounts of fall mineral fertilization. In addition, future investigations should study the relationship between growing season bareroot cultural practices and fall mineral fertilization.

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