

EFFECTS OF LIQUID FERTILIZER APPLICATION ON THE MORPHOLOGY AND OUTPLANTING SUCCESS OF CONTAINER LONGLEAF PINE SEEDLINGS

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Abstract—Of a range of fertilization rates (0.5, 1.0, 2.0, 3.0, and 4.0 mg nitrogen (N) per seedling per week) applied for 20 weeks, the 2.0-N and 3.0-N seedlings produced good root collar diameter (RCD) growth (6.9 and 7.1 mm, respectively) and needle length ≤ 30 cm. Root collar development did not differ significantly in seedlings receiving the 4.0-mg-N treatment from those receiving 2.0-mg or 3.0-mg, but needles grew to 35 cm in 4.0-N, surpassing the 30-cm limit to avoid clipping. Seedling survival (95 percent) was higher in 3.0-N seedlings one year after outplanting. RCD growth in 3.0-N was 14 percent greater than 2.0-N seedlings, but not different between the 3.0-N and 4.0-N seedlings. Height and RCD growth remained statistically similar between 3.0-N and 4.0-N seedlings after 2 years. Emergence from the grass stage increased as the amount of fertilizer increased, but given similarities in field performance between the two highest N rates, the extra 1.0-mg N per seedling per week was not economically justified.

INTRODUCTION

Longleaf pine (*Pinus palustris* P. Mill.) once dominated the landscape of the Southern Coastal Plain of the United States, from eastern TX to southeastern VA, covering nearly 36 million ha (90 million acres) (Noss and others 1995). Development of the railroad system in the late 1800s and early 1900s across the Southern United States allowed intense harvesting of longleaf pine, drastically reducing its presence in the landscape (Outcalt 2000, Barnett 2002). Current estimates are that longleaf pine forests now occupy about 2.2 percent of the species' original range (Shibu and others 2006). Longleaf pine trees yield high quality logs, straight boles, and a high resistance to fire, insects, and disease when compared to other southern pine species (Gjerstad and Johnson 2002); and longleaf pine forests are critical to the survival of many unique, threatened, and endangered species (Outcalt 2000). For these reasons, recent Federal incentive programs have encouraged longleaf pine reforestation (Hains 2002).

For decades, bareroot longleaf pine seedlings were used for reforestation. In the last decade, however, container production of longleaf pine seedlings has become a popular method used in regeneration (Dumroese and others 2005). In 2005, the number of container longleaf pine seedlings produced exceeded that of bareroot production by a margin of more than four to one (52 million versus 12 million) (Personal communication. 2006. Mark Hains, Research Coordinator, The Longleaf Alliance, 12130 Dixon Center Rd, Andalusia, AL 36420). With their intact roots systems, container longleaf pine survive and grow better than bareroot stock on ideal or adverse planting sites (Boyer 1989, Barnett and McGilvray 1997, South and others 2005), a characteristic also noted for container loblolly pine (*P. taeda*) (South and Barnett 1986, Barnett and McGilvray 1993). The presence of an intact root system also decreases moisture stress and reduces transplant shock, allowing an extension of the traditional planting season (Barnett and McGilvray 1997).

Due to the increasing demand for container longleaf pine seedlings and the lack of detailed research dealing with container longleaf pine seedling production, Barnett and others (2002) published interim guidelines for producing quality container-grown longleaf pine. The guidelines describe preferred and unacceptable seedling specifications for needles, root collar diameter (RCD), the root system and root plug, and also container cavity size. Of these seedling attributes, RCD is the most important because it is well correlated with seedling performance after outplanting (South and others 1993, South and others 2005). The guidelines recommend that RCD should be at least 4.8 mm (3/16 inch), but preferably 6.3 mm (1/4 in) or greater. Achieving larger RCDs, however, requires application of additional nutrients, which can cause needles to grow long and potentially lodge with nearby seedlings (Dumroese and others 2005). Lodging can prevent efficient application of irrigation and fertigation solutions of container seedlings grown at very high densities (for example, $>400/\text{m}^2$ [40 ft²]) and can cause needle mortality and allow foliar disease to develop (Barnett and McGilvray 1997). In many container nurseries, growers clip needles to discourage lodging.

In bareroot nurseries, the timing, frequency, and severity of clipping and seedling phenology can affect survival after outplanting, but in most studies a modest clipping of needles prior to outplanting improved survival, apparently through reductions in transpiration (South 1998). It is unclear if the results found for bareroot production would be similar for container seedlings grown at much higher densities and where seedling root mass is not lost during lifting. Barnett (1984) found that repeated clipping of needles to a short length (5 cm [2 inches]) reduced root system size. This finding led to the interim guideline to refrain from clipping needles to a length less than 10 cm (4 inches) (Barnett and others 2002). Identification of a nitrogen fertilization rate that promoted RCD development without excessive needle growth could allow nursery managers to avoid this labor-intensive nursery practice, especially if optimum outplanting performance could be maintained. Therefore, our objectives were to: 1) compare morphological and physiological characteristics of container

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longleaf pine seedlings grown under five liquid fertilizer regimes; and 2) evaluate subsequent survival and growth after outplanting.

MATERIALS AND METHODS

Greenhouse Experiment

The experiment took place in a greenhouse at the U.S. Forest Service, Southern Research Station in Pineville, LA (Lat 31.359 N, Long 92.436 W). Longleaf pine seed were obtained from Louisiana Forest Seed Company (Woodworth, LA) and sown on April 24, 2004, into Ropak® Multipot #3-96™ containers. Each container consisted of 96 cavities (441 seedlings/m² [41/ft²]) with a volume of 98 cm³ (6 cubic inches) and depth of 12 cm (4.8 inches). Cavities were filled with a 1:1 (v:v) peat moss to vermiculite medium.

Five weeks after sowing, five fertilizer treatments: 0.5, 1.0, 2.0, 3.0, and 4.0 mg nitrogen (N) per seedling per week for 20 weeks (weeks 6 through 25) were applied using Peter's Professional Brand™ 20-19-18 (20N:8P:15K) liquid soluble fertilizer. Two additional applications of fertilizer occurred, using the same N levels, during week 28 and week 32 (first and last week of November, respectively). For each treatment we used 12 containers (60 containers total).

Seedlings were irrigated whenever the container weight dropped to 75 percent of the field capacity weight (see Landis and others 1989). Once a week, the amount of fertilizer to add to a sufficient amount of irrigation water was calculated in order to apply the appropriate mg-N/seedling and return the containers to field capacity. Fertilizer solutions were carefully applied by hand using a watering bucket to ensure even distribution of the fertilizer.

Beginning nine weeks after sowing (June 21) and continuing at 5 week intervals, 30 seedlings were randomly sampled 6 times from each treatment. This resulted in 4 samples during the 20 week fertilizer application period, whereas the fifth and sixth samples were collected outside the application period, in November and December, respectively. Seedling RCD (mm) and length of the longest needle (cm) were measured. After carefully washing the roots, shoots and roots were separated and dried at 70 °C (160 °F) for 48 hours to determine dry root and shoot biomass (g). Once dried, samples were finely ground and analyzed for N concentration using a LECO-2000 CNS Elemental Analyzer (LECO Corp., St. Joseph, MI). For each dependant variable, data were analyzed using analysis of variance and when appropriate, means were compared using Duncan's multiple range test (SAS 2003).

Field Experiment

On November 17, 2004, 100 randomly selected seedlings from each treatment were outplanted on an open, mowed field site in the Palustris Experimental Forest near McNary, LA (Lat 31.017 N Long 92.617 W). The area is gently sloping (1 to 3 percent) with Beauregard silt-loam (fine-silty, thermic Plinthaquic Paleudults) soils that are moderately drained and slowly permeable (Kerr and others 1980). The site develops a perched water table during prolonged wet periods in winter and can be droughty in summer (Kerr and others 1980). The site was divided into four blocks, with 25

seedlings per treatment per block. Before outplanting, the RCD of each seedling was measured and labeled to facilitate re-measurement. In order to reduce competition and promote growth release, herbicide (Accord™) was applied around each planted seedling on May 10, 2005. One year after outplanting, we re-measured seedling RCD and calculated survival. On December 11, 2006, we re-measured RCD and measured the height (cm) of each seedling. We considered seedlings out of the grass stage when they reached ≥ 10 cm in height and ≥ 25 mm RCD (Wahlenberg 1946). We analyzed all dependent variables using logistic regression (SAS 2003).

RESULTS

Greenhouse Experiment

After 20 weeks of fertigation—The day prior to the initial fertilizer application (during week 5), seedlings were randomly selected and analyzed for N concentration, averaging 5.2 percent N. From that point, root and shoot N concentrations decreased in every rate but total N content increased, more so as N application rate increased (fig. 1). Increasing the N fertilization rate increased seedling size (table 1). All rates ≥ 1.0-mg-N/seedling/week resulted in seedlings having RCDs > 4.6 mm; these rates were not different. The 0.5-N rate, however, yielded seedlings with smaller RCD (3.7 mm) than the other N rates. For needle length, 3.0-N and 4.0-N produced the longest needles (28 and 30 cm, respectively), which were longer than 1.0-N and 2.0-N needles (24 and 22 cm, respectively). The 0.5-N rate had the shortest needles (20 cm), not significantly different than the 1.0-N seedlings. Every increased rate of N application resulted in increasing amounts of shoot biomass, ranging from 0.6 g at 0.5-N to 1.7 g at 4.0-N. Root biomass was an exception, the highest rates of N (3.0 and 4.0) had 0.6 g biomass, significantly less than 1.0-N and 2.0-N which averaged 0.7 g. Like the other variables, 0.5-N was significantly different and lowest at 0.5 g.

After 30 weeks of fertigation—The two additional applications of fertilizer made during weeks 28 and 32 caused total N content to increase, except at 4.0-N. For all rates, root N content continued to increase, but shoot N content either remained steady or declined (fig. 1). RCD was ≥ 6.9 mm for rates ≥ 2.0-N; these were statistically different than the 6.2 mm for 1.0-N which was statistically different than the 4.6 mm yielded by 0.5-N (table 2). Needle length decreased significantly from 35 cm in 4.0-N to 21 cm in 0.5-N. Shoot and root biomass followed the same general trend (table 2).

Field Experiment

First-year survival was high (80-95 percent) in all treatments and did not differ significantly among treatments ($P = 0.6165$). Interestingly, Block 1 had significantly lower survival (78 percent; $P = 0.0073$) compared to the other Blocks, which had survival rates of 90-92 percent. Similarly, Block 1 had significantly less ($P = 0.0001$) RCD growth (7.6 mm) when compared to the other Blocks (8.4 to 8.9 mm). The RCD increment was significantly different among treatments ($P < 0.0001$), ranging from 124 percent to 184 percent of original RCD, and increased with increasing rate of N applied in the greenhouse (fig. 2).

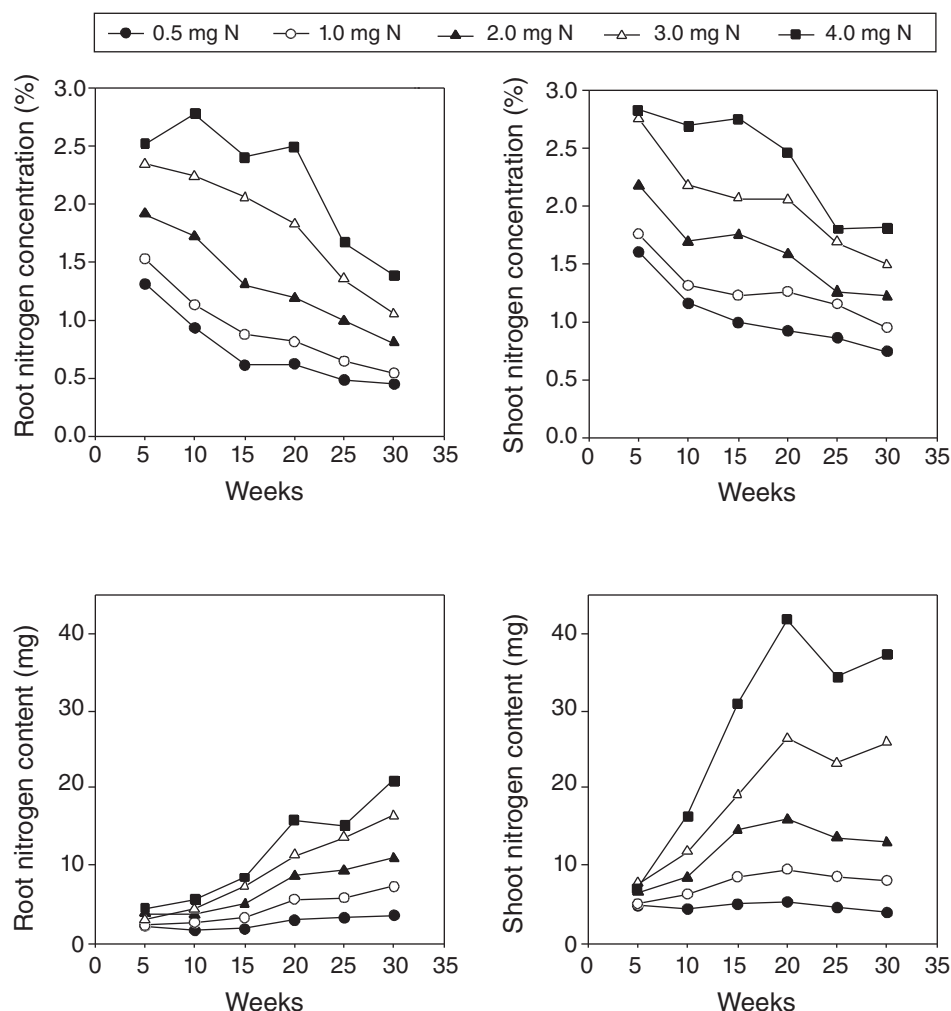


Figure 1—Root and shoot nitrogen concentrations (%) and contents (mg) of longleaf pine seedlings grown in containers in a greenhouse in Louisiana. Seedlings received five treatments: 0.5, 1.0, 2.0, 3.0, or 4.0-mg-N per seedling per week for 20 weeks, and then twice more during weeks 28 and 32.

Seedling survival after 2 years remained high (79-84 percent) and was significantly different among N treatments ($P = 0.0248$). Survival of the 0.5-N (81 percent) and the 4.0-N seedlings (79 percent) was significantly less than the 3.0-N seedlings (94 percent). No significant differences in survival occurred among the 1.0-N, 2.0-N, and 3.0-N treatments, which averaged 89 percent. The RCD increment increased with increasing N treatment after 2 years ($P = 0.0001$). The 3.0-N and 4.0-N treatments were not different, but their RCD increased significantly more than the lower N rates (table 3). The 0.5-N and 1.0-N seedlings did not differ in RCD growth after 2 years (table 3).

Grass stage emergence was different among treatments as N rates increased ($P = 0.0001$). The 0.5-N treatment had less RCD than 1.0-N, but both were similar in seedling height, while neither grew to the height (10 cm) or RCD (25 mm) necessary for seedlings to exit the grass stage (table 3). Treatments ≥ 2.0 -N met the criteria for grass stage emergence with 2.0-N having less height and RCD than

3.0-N and 4.0-N, while the latter two rates did not differ in either growth category (table 3).

DISCUSSION

For container longleaf pine, reported rates of N application varied from 2 to 5 mg-N/week for 20 weeks (see Dumroese and others 2005). In this study, five rates were chosen in order to determine N application rates that yielded growth parameters deemed important from a seedling quality standpoint. RCD may be the single most important seedling quality parameter because increasing RCD is associated with enhanced outplanting performance (South and others 1993, South and others 2005). We found that increasing the rate of N from 0.5 to 3.0 mg per seedling per week increased RCD in the greenhouse, as well as in the first year after outplanting. Other researchers have also reported that increasing fertilizer rates to longleaf pine seedlings increases RCD during nursery production (Jose and others 2003, Dumroese and others 2005), a trait also reported in loblolly pine (Sung and others 1997), black spruce (*Picea mariana*)

Table 1—Mean (standard error) for seedling morphological characteristics after 20 weeks of fertilization

Nitrogen fertilizer rate (mg/seedling/week)	Root collar diameter (mm)	Longest needle length (cm)	Root biomass (g)	Shoot biomass (g)
0.5	3.7 (0.1) b	20.0 (0.9) c	0.48 (0.03) c	0.58 (0.02) e
1.0	4.6 (0.1) a	22.0 (0.9) cb	0.70 (0.03) a	0.76 (0.04) d
2.0	4.9 (0.2) a	24.2 (1.0) b	0.73 (0.03) a	1.01 (0.05) c
3.0	4.7 (0.2) a	27.6 (1.2) a	0.62 (0.03) b	1.29 (0.07) b
4.0	5.0 (0.2) a	30.4 (1.3) a	0.64 (0.02) b	1.70 (0.06) a
<i>P</i> value	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Different letters within columns indicate significantly different means using Duncan's multiple range test at alpha = 0.05.

(Quoreshi and Timmer 2000, Timmer and Teng 2004), and Norway spruce (*P. abies*) (Kaakinen and others 2004).

Shoot biomass also increased as N levels increased. Root biomass, however, reacted differently. The lower rates (1.0 and 2.0) produced more root biomass during the 20 week fertigation period than the higher rates (3.0 and 4.0). Similar findings have been reported for sugar pine (*P. lambertiana*), Jeffrey pine (*P. jeffreyi*) (Walker 2001), Douglas-fir (*Pseudotsuga menziesii*) (Jacobs and others 2003), Norway spruce (Kaakinen and others 2004), and longleaf pine (Prior and others 1997). This response to lower N levels—more root growth—may be the seedling's attempt to acquire additional nutrients. The 0.5-N rate, however, yielded the lowest root biomass, indicating insufficient N availability and probably a severe N deficiency.

Nitrogen concentrations in the shoots and roots decreased in each treatment over the course of the experiment, while total N content in seedlings increased. These trends are similar to previous study results with longleaf seedlings (Dumroese and others 2005). Seedlings given the highest N rate (4.0) had a foliar N concentration of 2.5 percent after the 20 week fertigation period. This is at the upper end of the optimum range for conifer seedlings (Landis and others 1989) and

perhaps borderline for luxury consumption (Dumroese 2003). The next lower rate (3.0-N) yielded a foliar N concentration of 1.8 percent, a value still within the optimum range, but all rates $\leq$ 2.0-N yielded foliar N concentrations below that optimum range (Landis and others 1989). As the seedlings "coasted" from week 25 through week 35, the two additional applications of fertilizer were insufficient to maintain N concentrations; increased seedling biomass resulted in a dilution effect despite increases in seedling N content. Before outplanting, N concentration in the highest N application rate dropped from 2.5 to 1.4 percent, thereby moving below the optimum range for conifer seedlings (Landis and others 1989). Similar changes were seen in all other N application rates, but despite these drops, seedlings having received at least 1.0 mg-N/week continued to add RCD, needle length, and biomass.

At outplanting, all seedlings grown using rates $\geq$ 1.0-N exceeded many of the minimum guidelines described by Barnett and others (2002). Survival was not affected by the rate of N, but the rate of application did have an effect on RCD. The increment of new RCD increased as the rate of N applied in the greenhouse increased. This trend continued for 2 years with both RCD and height growth. Wahlenberg (1946) used a height of 10 cm and RCD of 25 mm as a guideline to determine when seedlings emerge from the grass stage.

Table 2—Mean (standard error) for seedling morphological characteristics after 30 weeks of fertilization

N fertilizer rate (mg/seedling/week for 20 weeks + 2 additional applications)	Root collar diameter (mm)	Longest needle length (cm)	Root biomass (g)	Shoot biomass (g)
0.5	4.6 (0.2) c	20.6 (0.7) d	0.81 (0.04) c	0.53 (0.03) e
1.0	6.2 (0.2) b	22.7 (1.1) cd	1.35 (0.05) b	0.86 (0.05) d
2.0	6.9 (0.2) a	24.9 (0.9) c	1.37 (0.04) b	1.07 (0.04) c
3.0	7.1 (0.2) a	29.6 (1.3) b	1.57 (0.08) a	1.74 (0.08) b
4.0	7.2 (0.2) a	35.0 (1.2) a	1.52 (0.09) a	2.06 (0.09) a
<i>P</i> value	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Different letters within columns indicate significantly different means using Duncan's multiple range test at alpha = 0.05.

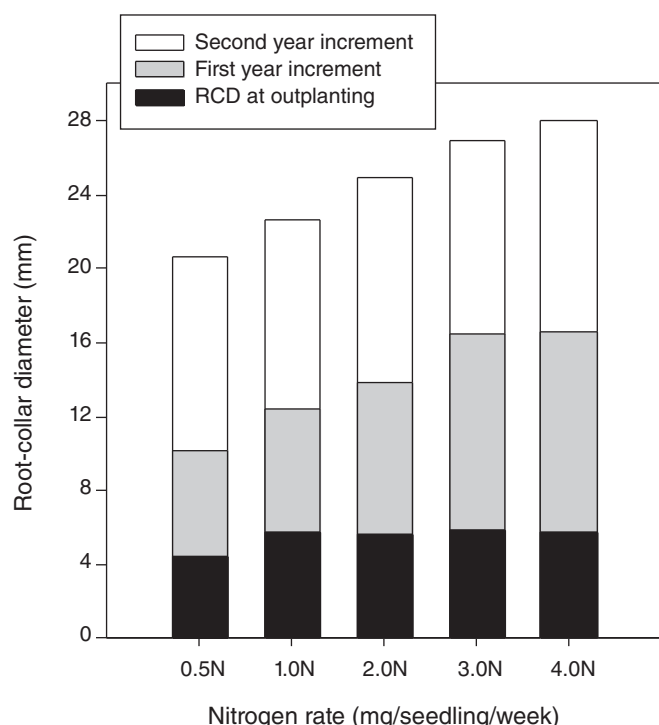


Figure 2—Root collar diameter growth of longleaf pine seedlings grown in containers in a greenhouse in Louisiana. Seedlings received five treatments: 0.5, 1.0, 2.0, 3.0, or 4.0 mg N per seedling per week for 20 weeks, and then twice more during weeks 28 and 32. The black portion of each bar represent RCD growth in the nursery; the gray portion is the RCD increment of growth added after one season in the field; the white portion is the RCD increment of growth added after two seasons in the field.

Based on these criteria, 62 percent of 3.0-N and 67 percent of 4.0-N seedlings had begun height growth 2 years after outplanting. The 2.0-N seedlings had 44 percent out of the grass stage, while height and RCD at this N level averaged 10 cm and 25 mm, respectively. Block 1 in the outplanting study had more grasses and woody stems, leading to

significantly lower RCD and survival compared to the other three blocks. The percentage of seedlings exiting the grass stage would have been greater if Block 1 contained less competition. Deficiencies in nutrients, moisture, or sunlight can extend the time seedlings spend in the grass stage, which can differ from seedling to seedling. With a higher percent out of the grass stage, it appears 3.0-N and 4.0-N seedlings are able to more effectively compete for resources.

Because most longleaf pine seedlings emerge from the grass stage when RCD $\geq$ 25 mm (Wahlenberg 1946), producing seedlings with large RCDs in the nursery may shorten the time seedlings remain in the grass stage. Increased applications of N during hardening are known to foster RCD growth (Montville and others 1996) and recent work (South and others 2005, South and Mitchell 2006) indicate that RCDs in this container type (Ropak #3-96) could approach 11 mm before field performance might be compromised. For the containers used and the length of the growing season, however, it may be difficult to achieve much greater RCD growth. We grew longleaf seedlings with RCDs $\geq$ 6.9 mm using N rates $\geq$ 2.0-N. In particular, the 3.0-N or 4.0-N seedlings were similar in RCD and root biomass, but the 4.0-N seedlings were borderline for needing clipping to prevent lodging. Therefore, it appears that the benefit of adding an additional 1.0-mg N per week (3.0 to 4.0 mg) during the growing season was negligible in terms of seedling growth in the nursery, as well as survival and growth after outplanting. Seedlings at the 4.0 N-level were probably in or near to luxury consumption.

Finding an ideal N regime, however, will be difficult because of the enormous number of container types and N rates that could be used to grow longleaf seedling crops. Unfortunately, the optimum rate of N for any particular species in any particular nursery depends on many factors, including the idiosyncrasies and management philosophy of the nursery manager (Dumroese and Wenny 1997). From the studies we have considered, including our own, it appears that growers using Ropak Multipot #3-96 can produce seedlings with high survival and growth potential using a N application rate of

Table 3—The least squared means for initial (standard error) root collar diameter of seedlings outplanted in November 2004, diameter increment growth from 2004 until November 2006, final diameter measured in 2006, and final height measured in 2006

Treatment	Diameter			Height & (standard error))
	Initial (2004)	Increment (2004 to 2006)	Final (2006)	
0.5	4.53 (0.09) a	16.16 (0.62) a	20.64 (0.62) a	7.56 (0.73) a
1.0	5.72 (0.09) bc	17.00 (0.60) a	22.70 (0.60) b	8.45 (0.70) a
2.0	5.59 (0.09) bc	19.35 (0.60) b	24.90 (0.60) c	10.70 (0.71) b
3.0	5.95 (0.09) c	21.05 (0.58) c	26.93 (0.58) d	13.48 (0.68) c
4.0	5.74 (0.09) bc	22.40 (0.63) c	28.04 (0.63) d	14.46 (0.74) c
P value	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Different letters within columns indicate significantly different means using Duncan's multiple range test at alpha = 0.05.

3.0 mg N/seedling/week over a 20-week period, without the labor of clipping. This rate corresponds well with Dumroese and others (2005) and would be a good starting point for developing fertilization regimes specific to local nurseries.

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