Container Longleaf Pine Seedling Morphology in Response to Varying Rates of Nitrogen Fertilization in the Nursery and Subsequent Growth After Outplanting.

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Abstract: A fertilization rate of 2 or 3 mg nitrogen (N) per week for 20 weeks yielded longleaf pine (*Pinus palustris*) seedlings grown inside a greenhouse that survived well and produced good root collar diameter (RCD) growth the first year after outplanting. Of a range of fertilization rates (0.5 to 4 mg N/week), the 2 mg rate yielded seedlings that did not require needle clipping during nursery production, but increased their RCD by 150% the first year after outplanting. The lower rates (<1 mg N) also survived well, but RCD growth was poorer than the 2 mg rate. In the nursery, the 3 mg rate was borderline for requiring clipping to reduce lodging; under customary nursery practices, the 4 mg rate seedlings would have been clipped; root collar diameter growth in the nursery was similar to that of seedlings grown with the 2 mg N rate. Seedlings receiving >3 mg N also survived well and RCD growth after one season in the field was 14% more than that of the 2 mg N rate. We are continuing to monitor seedlings to determine when they exit the grass stage.

Keywords: *Pinus palustris*, container nursery, field performance, fertilization

Longleaf pine (*Pinus palustris* P. Mill.) once dominated the landscape of the southern coastal plain of the United States from eastern Texas to southeastern Virginia, covering nearly 36 million ha (90 million ac) (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 and others 2002). Current estimates are that longleaf pine forest now occupies about 2.2% of its original range (Jose and others 2006). Longleaf pine possesses 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) 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 (Hainds 2002).

For decades, bareroot longleaf pine was used for reforestation. In the last decade, however, container production of longleaf pine has become a popular stocktype for regeneration (Dumroese and others 2005), and the 1999 estimate of longleaf pine seedling production indicated container production would exceed that of bareroot production by a margin of two to one (54 million versus 27 million) (Hainds 2002). Container longleaf pine, with their intact roots systems, survive and grow better than bareroot stock (Boyer 1989; Barnett and McGilvray 1997; South and others 2005), a characteristic noted for container loblolly pine (*P. taeda*) on harsh sites as well (South and Barnett 1986; Barnett and McGilvray 1993). The presence of an intact root system also allows an extension of the planting season (Barnett and McGilvray 1997).

In response to the increase in demand for container longleaf pine seedlings and because of the lack of detailed research dealing with container longleaf pine seedling production, Barnett and others (2002) published interim guidelines for producing quality longleaf seedlings grown in containers. The guidelines suggest suitable needle lengths, needle color, firmness of root plugs, container specifications, bud formation, and other seedling attributes. Of these, root collar diameter (RCD) may be the most important because it is well correlated with seedling performance after outplanting (South and others 1993; South and others 2005). Therefore, the guidelines state that RCD should be at least 4.8 mm (3/16 in), with a target of at least 6.3 mm (1/4 in). Achieving a larger RCD, however, requires application of sufficient nutrients. These additional nutrients 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/m^2 [40/ft^2]), causing needle mortality and allowing foliar disease to develop (Barnett and McGilvray 1997). In many container nurseries, growers clip needles to discourage lodging.

In bareroot nurseries, seedling phenology and the timing, frequency, and severity of clipping can affect survival after outplanting. For most studies, however, a modest clipping of needles prior to outplanting improved survival, apparently through reductions in transpiration (South 1998). It is unclear if these results would be true for container seedlings grown at much higher densities and where seedling root mass is not lost during harvest. Barnett (1984) found that repeated clipping of needles to a short length (5 cm [2 in]) reduced root system size. This led to the interim guideline (Barnett and others 2002) to refrain from clipping needles to a length less than 10 cm (4 in). Identifying a rate of nitrogen fertilization 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.

Our objectives were to: (1) compare morphological and physiological characteristics of container longleaf pine seedlings grown under five liquid fertilizer regimes; and (2) evaluate subsequent survival and growth after outplanting.

Materials and Methods

Nursery Experiment

Our experiment took place in a greenhouse at the USDA Forest Service Southern Research Station in Pineville, Louisiana (31° 22' N, 92° 26' W). Longleaf pine seeds were obtained from Louisiana Forest Seed Company (Woodworth, LA) and sown on 24 April 2004 into Ropak® Multipot #3-96™ containers. Each container was comprised of 96 cavities (441 seedlings/m^2 [41 seedlings/ft^2]) having a volume of 98 cm^3 (6 in^3) and depth of 12 cm (4.8 in). Cavities were filled with a 1:1 (v:v) peat moss to vermiculite medium.

Five weeks after sowing (week 5), we initiated 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). We used Peter's Professional Brand™ 20-19-18 (20N:8P:15K) liquid soluble fertilizer. Two additional applications of fertilizer were made using the same N levels; one made during week 28 and another during 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% of the field capacity weight (Landis and others 1989). Once each week, we calculated the amount of fertilizer to add to a sufficient amount of irrigation water to apply the appropriate mg N/seedling and return the containers to field capacity. Fertigation solutions were carefully applied by hand using a watering can to ensure even distribution of the fertilizer.

Beginning 9 weeks after sowing (21 June) and continuing at 5-week intervals, 30 seedlings were randomly sampled six times from each treatment. This resulted in four samples during the 20-week fertilizer application period; the fifth and sixth samples were collected in November and December, respectively. We measured root collar diameter (mm) and length of the longest needle (cm). 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 17 November 2004, we outplanted 100 randomly selected seedlings from each treatment into an open field site that had been mowed in the Palustris Experimental Forest near McNary, Louisiana (31° 1'N, 92° 37'W). The area is gently sloping (1% to 3%) with Beauregard silt-loam (fine-silty, thermic Plinthaquic Paleudult) 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, we measured the RCD of each seedling. Each seedling was labeled to facilitate remeasurement. Herbicide (Accord™) was applied around each planted seedling on 10 May 2005 in order to reduce competition and promote growth release. About one year later (November 2005), we determined survival and remeasured RCD on surviving seedlings. Survival and RCD increment were analyzed with logistic regression (SAS 2003).

Results

Nursery Experiment

After 20 Weeks of Fertigation—When fertigation commenced, seedlings averaged 5.2% 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 (>1.0 N) resulted in seedlings having RCD >4.6 mm (0.18 in); these rates were not statistically different. The 0.5 N rate, however, yielded seedlings with significantly less RCD (3.7 mm [0.15 in]) than the other rates. For needle length, 3.0 N and 4.0 N produced the longest needles (28 and 30 cm [11 and 11.9 in], respectively). These were significantly longer than 1.0 N and 2.0 N needles (24 and 22 cm [9.4 and 8.7 in]), which were not statistically different. The 0.5 N rate had the shortest needles (20 cm [7.9 in]), which was not statistically different than the 1.0 N. Every rate of N yielded statistically different 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 about 0.6 g of biomass, significantly less than 1.0 N and 2.0 N that averaged 0.7. 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 fertigation made during weeks 28 and 32 caused, in general, total N content to continue to increase, except at the highest rate. For all rates, root N content continued to increase, but shoot N content either remained steady or declined. N concentration in all seedling tissues

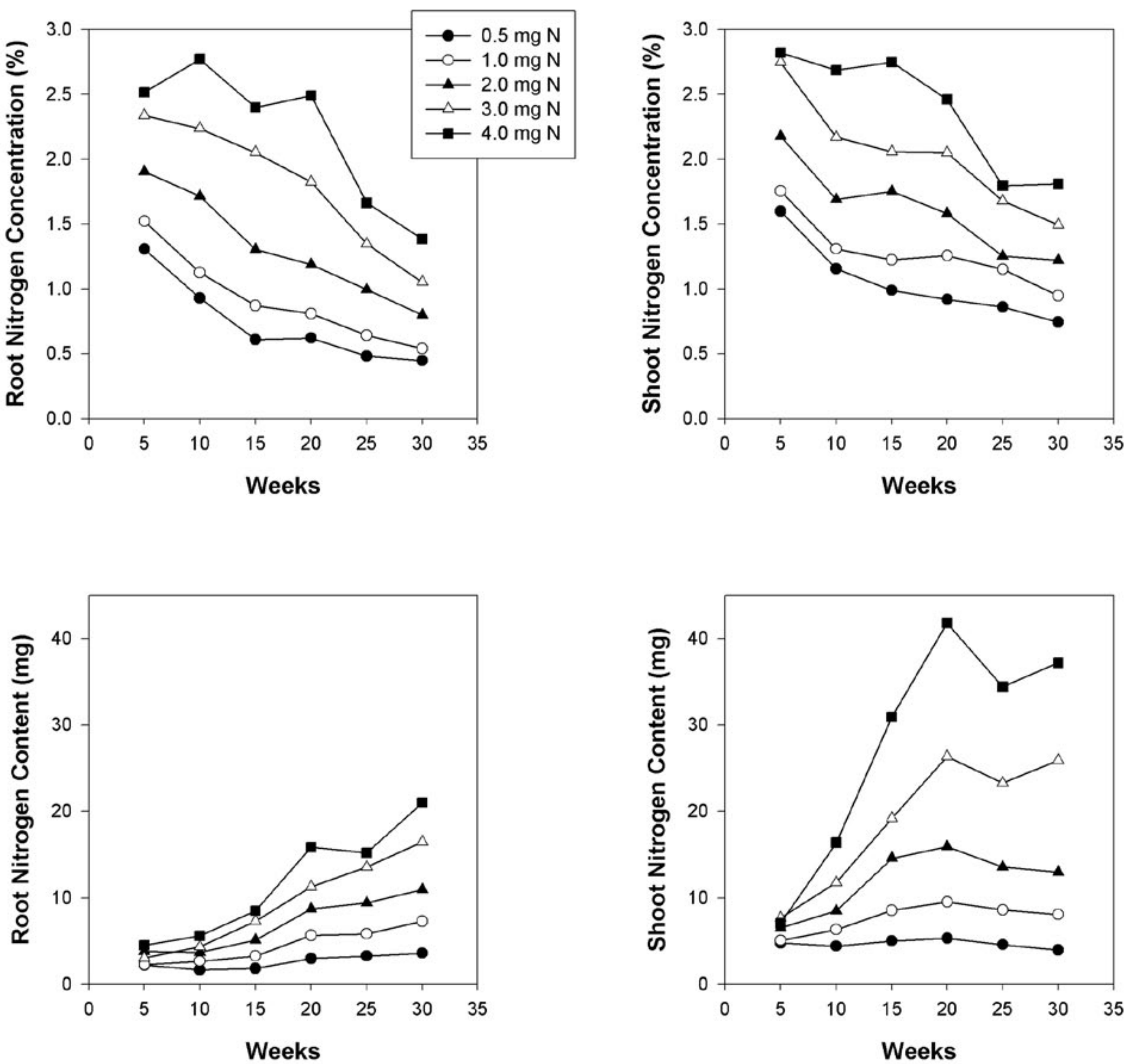

Figure 1—Root and shoot nitrogen concentrations (%) and contents (mg) of longleaf pine seedlings grown in containers in a greenhouse in Louisiana. Seedlings received either 0.5, 1.0, 2.0, 3.0, or 4.0 mg N per seedling per week for 20 weeks, and then twice more during week 28 and 32.

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

Fertilizer rate (mg N/seedling/week)	Root collar diameter	Longest needle length	Root biomass	Shoot biomass
	(mm)	*(cm)*	*(g)*	*(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
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.

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

Fertilizer rate (mg N/seedling/week for 20 weeks + 2 additional applications)	Root collar diameter	Longest needle length	Root biomass	Shoot biomass
	(mm)	*(cm)*	*(g)*	*(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
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.

continued to decline (fig. 1). RCD was > 6.9 mm (0.27 in) for rates > 2.0 N; these were statistically different than the 6.2 mm (0.24 in) for 1.0 N, which was statistically different than the 4.6 mm (0.18 in) yielded by 0.5 N (table 2). Needle length decreased significantly from 35 cm (13.8 in) in 4.0 N to 21 cm (8.3 in) in 0.5 N. Shoot and root biomass followed the same general trend (table 2).

Field Experiment

First-year survival was high in all treatments and not significantly different among treatments ($P = 0.6165$), ranging from 80 to 95%. Interestingly, block 1 had significantly lower survival (78%; $P = 0.0073$) compared among the other blocks with survival of 90 to 92%. Similarly, block 1 had significantly less ($P = 0.0001$) RCD growth (7.6 mm [0.30 in]) when compared among the other blocks (8.4 to 8.9 mm [0.33 to 0.35 in]). The RCD increment was significantly different among treatments ($P < 0.0001$), ranging from 124 to 184% of original RCD, and increased with increasing rate of N applied in the nursery (fig. 2).

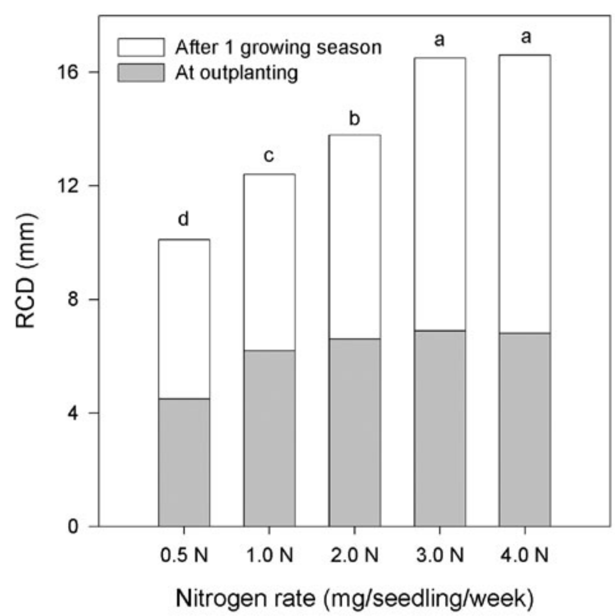

Figure 2—Root collar diameter growth of longleaf pine seedlings grown in containers in a greenhouse in Louisiana. Seedlings received either 0.5, 1.0, 2.0, 3.0, or 4.0 mg N per seedling per week for 20 weeks, and then twice more during week 28 and 32. The gray portion of each bar represent RCD growth in the nursery; the white portion is the RCD increment of growth added after one season in the field. Different letters above bars indicate significantly different means (*P* < 0.05).

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, we chose five rates in order to hone in on N application rates that yielded high values deemed important from a seedling quality standpoint. RCD may be the single most important seedling quality parameter (South and others 1993, 2005) because increasing RCD is associated with enhanced outplanting performance. In this study, increasing the rate of N from 0.5 to 3.0 mg per seedling per week increased RCD during nursery production as well as during the first year after outplanting. Other researchers have also found that increasing fertilizer rates to longleaf pine seedlings increases RCD during nursery production (Jose and others 2003; Dumroese and others 2005), a trait reported in loblolly pine (Sung and others 1997), black spruce (*Picea mariana*) (Quoreshi and Timmer 2000; Timmer and Teng 2004), and Norway spruce (*P. abies*) (Kaakinen and others 2004).

Similar to RCD, shoot biomass increased as N levels increased. Root biomass, however, was different—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 (*P. lambertiana*) and Jeffrey pine (*P. jeffreyi*) (Walker 2001), Douglas-fir (*Pseudotsuga menziesii*) (Jacobs and others 2003), Norway spruce (Kaakinen and others 2004), as well as longleaf pine (Prior and others 1997). This increased root growth response to lower N levels may be an attempt to acquire additional resources. 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 during the entire experiment, while total N content in seedlings increased, similar to previous results with this species (Dumroese and others 2005). Seedlings given the highest N rate (4.0) had a foliar N concentration of 2.5% after the 20-week fertigation period. This is the upper end of the optimum range for conifer seedlings as defined by Landis and others (1989) and borderline for luxury consumption as defined by Dumroese (2003). The next lower rate (3.0 N) yielded a foliar N concentration of 1.8%, a value still within the Landis and others (1989) optimum range; all rates <2.0 N yielded foliar N concentrations below that optimum range. As the seedlings "coasted" from week 25 through week 35, the two additional applications of fertilizer were insufficient to maintain N concentrations; increasing seedling biomass resulted in a dilution effect despite increases in seedling N content. Before outplanting, the N concentration in the highest N application rate dropped from 2.5 to 1.4%, moving the value below the Landis and others (1989) optimum range for conifer seedlings in general. Similar changes were seen in all other N application rates. Despite these drops, however, seedlings that received at least 1.0 mg N/week continued to add RCD, needle length, and biomass.

At outplanting, all seedlings grown with 1.0 N or higher exceeded most, if not all, the minimum guidelines described by Barnett and others (2002). After outplanting, survival was unaffected by the rate of N, but N rate did have an effect on RCD. The growth increment of RCD increased as the rate of N applied in the nursery increased. Recently, South and others (2005) showed 97% of longleaf pine seedlings exit the grass stage when RCD, or ground line diameter (GLD), is >23 mm, a similar value to earlier work (Wahlenberg 1946; Wakeley 1954). They found that on three of four sites, seedlings having an average RCD >16.5 mm (0.65 in) subsequently had an average of 63% exit the grass stage during the next growing season. In our study, seedlings receiving 3.0 N or 4.0 N had RCD >16.5 mm (0.65 in) after the first field season and the increment of growth was greater than that reported by South and others (2005) for seedlings grown in the same container type. It will be interesting to see what happens to our seedlings during the second growing season. Based on South and others (2005), we expect a high proportion of them to exit the grass stage.

Because most longleaf pine seedlings exit the grass stage when RCD >23 mm (0.9 in) (South and others 2005), producing seedlings with large RCDs in the nursery may shorten the time frame after outplanting for seedlings to exit 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) indicates that RCDs in this container type (Ropak® Multipot #3-96™) could approach 11 mm (0.4 in) before field performance might be compromised by "root-binding." For the containers we used and the length of our growing season, however, it may be difficult to achieve much greater RCD growth. We grew longleaf seedlings with RCDs >6.9 mm (0.27 in) with N rates >2.0 N. But once our N rates were either 3.0 N or 4.0 N, seedlings were quite similar in RCD and root biomass; the 4.0 N seedlings were borderline for needing clipping to prevent lodging. Therefore, it appears that the benefit of adding the additional 1.0 mg N per week (3.0 to 4.0 mg) during the growing season duration we used was negligible in terms of seedling growth in the nursery, and survival and growth after outplanting; our 4.0 N seedlings were probably in luxury consumption as defined by Dumroese (2003).

The interim guidelines of Barnett and others (2002) were intended to be transitory, modified as new data were generated on longleaf pine nursery production and subsequent field performance. In most temperate pine species, formation of a solid terminal bud is thought to infer enhanced seedling quality for two reasons. First, large terminal buds generally have numerous needle primordial that will form the new shoot during the subsequent growing season; more primordial provide potential for more shoot growth. Second, and perhaps more importantly, formation of buds in the nursery indicates seedlings are no longer in a rapid growth phase, but are being hardened in preparation for harsher outplanting conditions. In our study, longleaf seedlings were hardened by reducing N applications. Even so, only about 20% of the seedlings in our experiment had what could be called a terminal bud. Given that our survival was high and that the seedlings with the largest increments of RCD after outplanting did not have buds, clearly more research is needed. We have repeated this experiment by growing longleaf seedlings in a greenhouse and in an outdoor growing situation, and outplanting them in the Palustris Experimental Forest. Hopefully additional data on RCD development, bud formation, and growth of seedlings in the field will continue to clarify the guidelines for growing longleaf pine.

Finding an ideal regime, however, will be difficult because of the enormous number of choices in container type and the resulting number of N rates that could be used to grow crops in them. Unfortunately, the optimum rate of N for any particular species in any particular nursery is dependent on many factors, including the idiosyncrasies and management philosophy of the nursery manager (Dumroese and others 1997). From this study, it appears that growers using Ropak®

Multipot #3-96™ can produce seedlings with high survival and growth potential, without the labor for clipping, using an N application rate of 3.0 mg N/seedling/week over a 20-week period. This rate corresponds well with Dumroese and others (2005). We feel this would be a good starting point for developing fertilization regimes specific to local nurseries.

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