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The Persistence of Container Nursery Treatments on the Field Performance and Root System Morphology of Longleaf Pine Seedlings

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Abstract: In recent decades, container stock has become the preferred plant material to regenerate longleaf pine (*Pinus palustris* Mill.) forests in the southeastern United States. We evaluated the effects of container nursery treatments on early and long-term field performance in central Louisiana. Seedlings were grown in four cavity volumes (60–336 mL) with or without copper oxychloride root pruning (Cu or no-Cu) and fertilized at three nitrogen (N) rates. Across treatments, 91% of the seedlings emerged from the grass stage by the second field season, and 88% of the seedlings survived eight years after outplanting (Year 8). Seedlings grown in the largest cavities had greater total heights and stem diameters than those cultured in the 60- and 95-mL cavities through Year 8. Seedlings receiving the least amount of N in the nursery were consistently smaller in stature through Year 8 than seedlings receiving more N. Field growth was unaffected by copper root pruning through Year 8. Foliar mineral nutrient concentrations and seedling nutrient contents of Year 2 seedlings did not respond to nursery treatments. Independent of nursery treatments, seedlings excavated in Year 2 had at least 60% of their first-order lateral roots (FOLRs) originating from the top 4.0 cm of the taproots. The Cu-root-pruned seedlings had twofold the percentage of FOLRs egressed from the top 8.0 cm of the root plug when compared with the no-Cu seedlings. Moreover, the Cu root pruning treatment decreased the percentage of root plug biomass allocated to FOLRs, total within root plug FOLR lengths, and FOLR deformity index. The effects of increasing cavity volume or N rate on the root plug FOLR variables were opposite those of the Cu root pruning treatment. Our results suggest that a tradeoff may exist between seedling stature and a more natural FOLR morphology in outplanted container longleaf pine seedlings.

Keywords: cavity volume; copper oxychloride; root deformity index; fertilization; field performance; first-order lateral root; *Pinus palustris*; root pruning; root system morphology

1. Introduction

Longleaf pine (*Pinus palustris* Mill.) ecosystems covered 37 million ha in the southeastern United States before European settlement. Many factors, including unsustainable harvest for naval stores and timber products between the late 1800s and early 1900s, conversion to agriculture uses, replacement with faster-growing loblolly pine (*Pinus taeda* L.) and slash pine (*Pinus elliottii* Engelm.) plantations, urbanization, and exclusion of natural fire regimes, have reduced this ecosystem by about 97% [1–5]. The remaining 1.4 million ha contain some of the most biologically diverse habitats outside of the tropics [6]. Over the past three decades, a consortium of land managers has been actively restoring longleaf pine forests and the species they support [2,7]. In 2009, the America's Longleaf Restoration Initiative proposed a range-wide conservation plan with a 15-year goal of increasing this ecosystem from 1.37 to 3.24 million ha via planting, improving, and maintaining existing forests with relic longleaf pines by silvicultural treatments including prescribed fire [8]. Half of this increase is targeted to occur in 16 significant geographic areas across the nine southern states where most of the original longleaf pine grew; 13 of these areas are within 200 km of the Gulf or Atlantic coasts [8]. A concern in these coastal areas is the frequency of strong tropical storms, including hurricanes [2]. Longleaf pine suffered less wind damage than loblolly pine after Hurricanes Hugo in South Carolina [9] and Katrina in Mississippi [10].

Compared with bareroot seedlings, container longleaf pine seedlings generally have a wider planting window and greater first-year field survival [11]. In 2008, about 90% of longleaf pine seedlings planted were container-grown [12]. Because much of this stock type is intended for outplanting in hurricane-prone areas, prudent managers should consider seedling quality variables, especially those concerned with root system morphology. At the onset of large-scale use of container seedlings in the late 1970s, a limited understanding of seedling quality led to the production of stock in unsuitable containers (i.e., those with smooth interior walls [13]) with or without poor crop management (i.e., growing seedlings too large or too long in their containers [14]), which contributed to juvenile stem instability after outplanting. For example, toppling in container lodgepole pine (*Pinus contorta* Dougl.) plantations in British Columbia, which was widespread but generally affected less than 15% of the outplanted seedlings, was attributed to the development of spiraling lateral roots in the root plug [14,15]; poor root system configurations in container lodgepole pine and Douglas fir (*Pseudotsuga menziesii* var. *glauca* Franco) persisted for 11 years after outplanting [16].

To address root spiraling, an early container modification was the addition of vertical ridges to the interior wall to reduce lateral root spiraling by directing primary lateral roots to grow downward along the interface between the cavity wall and the growing medium [17]. Even so, the resulting caged appearance of root systems was suspected to cause root system anchorage failure and thus sapling stem instability [15,18–20]. To reduce this cage effect, interior container walls were coated with copper (Cu) compounds to stop primary lateral roots from elongating once they reached the cavity wall [19,21]. This treatment reduced the incidence of leaning in lodgepole pine three years after outplanting [22]. Longleaf pine seedlings cultured in Cu-coated cavities grew more higher-order roots than those in non-Cu cavities [11,20,23] and favored biomass allocation to the taproot and secondary lateral roots [20,23,24]. One year after outplanting, longleaf pine seedlings cultured in Cu-coated cavities had a larger fraction of primary lateral roots emerging from the top 5 cm of the root plug than seedlings cultured without Cu [20]. A third cavity modification involves openings along the cavity wall that cause lateral roots to air-prune, similar to the way taproots air prune at the bottom of containers. Air-pruned interior lodgepole pine (*P. contorta* var. *latifolia*) seedlings had new root growth evenly distributed along their root plug in a root growth potential test [25], and outplanted air-pruned Scots pine (*Pinus sylvestris* L.) seedlings had improved root system structure and stem straightness compared with seedlings not air-pruned [26]. Similarly, air-pruned longleaf pine seedlings had fewer deformed primary lateral roots and a larger taproot compared with seedlings grown without air-pruning at the end of nursery production and 14 months after outplanting [27].

One to two years after outplanting, routine assessments of longleaf pine seedlings include survival, ground-line diameter, and the percentage of seedlings emerged from the grass stage. Various silvicultural treatments, such as prescribed fire; chemical and mechanical vegetation control; mulching; and site preparation, have improved early field performance [28–34]. Most of these studies, however, did not specify seedling quality attributes before outplanting. Target seedling attributes for container longleaf pine have been proposed and updated, with root collar diameter (RCD) (>4.75 mm) and fascicle length (>10 cm) being the non-destructive, objective, and minimum standards of quality suggested [35,36]. Work to improve target seedling attributes and the early field performance of planted container longleaf pine seedlings continues. For example, in a recent field trial, seedlings grown in combinations of Cu root pruning and container size, seedling size at outplanting, and subsequent first-year field growth were compared [20]. Larger containers yielded seedlings with greater RCD one year after outplanting [20], and seedlings produced with Cu root pruning, despite having similar RCD to control seedlings at outplanting, had larger stature five years after outplanting [37]. One study has shown that higher rates of supplied nitrogen during nursery production can improve longleaf seedling RCD and height three years after outplanting compared with lower rates [38].

To our knowledge, the current study is the first to link attributes of container longleaf pine seedlings delivered from the nursery (e.g., RCD and nutrient concentrations) with early field performance (e.g., survival, emergence from the grass stage, root system morphology, seedling biomass allocation, and nutrient concentrations and contents) and long-term field performance (e.g., survival, total height, and stem diameter) of outplanted longleaf pine seedlings. We hypothesized that the seedling quality attributes, as influenced by nursery treatments, would have persistent relationships with early and long-term seedling field performance. This study is a long-term field evaluation of the crop of longleaf pine seedlings cultured in four cavity volumes with or without Cu root pruning and with one of three N fertilization rates during production [24].

2. Materials and Methods

The container longleaf pine seedlings we studied were cultured in a greenhouse at the United States (U.S.) Department of Agriculture Forest Service, Rocky Mountain Research Station in Moscow, ID, USA (46.7° N, 117.0° W) [24]. The treatments were 24 factorial combinations of four container cavity volumes, three N fertilization rates, and two copper root pruning treatments. Seedling responses to treatment effects at the end of nursery production are reported [24].

To summarize seedling culture, seeds from a Louisiana seed source were sown on 15 May 2007 in Styroblock™ containers (no copper, no-Cu) and equivalent-sized Copperblock™ containers (Cu) having cavity volumes of 60, 95, 125, and 336 mL with depth (cm)/top diameter (cm) of 13/2.8, 12/3.6, 12/4.2, and 15/5.9, respectively [24]. Container cavities were filled with a 1:1 (v:v) sphagnum peat moss/vermiculite growing medium. Beginning four weeks after sowing, seedlings were fertigated weekly for 19 weeks (a total of 20 applications) in a manner such that N concentration of the fertigation solution applied to each cavity volume was proportional. We followed the protocol of relative N rates of 0.5 (low N, LN), 2 (medium N, MN), and 4 mg N week^{-1} (high N, HN) [38]. When the mass of the water of the medium reached 75% of the container capacity, fertigation or irrigation solution volumes of 6, 10, 13, and 34 mL were applied to each seedling in the 60-, 95-, 125-, and 336-mL cavities, respectively, to return the water mass of the medium back to container field capacity [39]. The fertigation solution contained fixed concentrations of macro- and micro-nutrients (Peters Professional® S.T.E.M.™, the Scotts Company, Marysville, OH, USA) and chelated Fe (Sprint 330, Becker Underwood, Inc., Ames, IA, USA). Ammonium nitrate was added to the fertigation solution to achieve the desired N amount per seedling per cavity volume for each N rate. For example, a total of 6, 24, or 49 mg N was applied to the seedlings grown in the 60-mL cavities at LN, MN, or HN rates, respectively, whereas, those grown in the 336-mL cavities received a total of 34, 137, and 274 mg N for the LN, MN, and HN rates, respectively [24]. Foliar N concentrations were about 0.8%, 1.4%, and 1.8% for the LN, MN, and HN treatments, respectively, at the end of nursery production [24]. Seedlings were extracted from containers on 4 December 2007 (29 weeks after sowing), boxed, shipped, and stored in a cold room at the U.S. Department of Agriculture Forest Service, Southern Research Station in Pineville, Louisiana until outplanting.

2.1. Seedling Quality

Before outplanting, three seedlings were randomly sampled from each of the 24 nursery treatments and assessed for RCD, number of fascicles, number ofFOLRs, and length of the longest FOLR (LFOLR). We classified FOLRs as those emanating from the taproot and having a rigid structure and diameter >0.5 mm when measured 5 mm from the taproot [40].

2.2. Outplanting

The study site is on the Palustris Experimental Forest (31.0° N, 92.6° W) in Rapides Parish, Louisiana, and has a gently sloping (1%–3%), moderately well-drained, and slowly permeable Beauregard silt loam soil (fine-silty, siliceous, superactive, thermic Plinthaquic Paleudults) [41]. Grasses and forbs, the dominant vegetation before study establishment, were mowed and burned in July 2007. The study was established in a randomized complete block experimental design with three blocks. A total of 15 29-week-old seedlings from each of the 24 nursery treatments were outplanted in each plot with custom planting punches on 11 December 2007. The plots were 15 consecutive planting locations every 0.6 m in planting rows that were 1 m apart. The site was prescribed burned in late May 2013.

2.3. Field and Laboratory Measurements

2.3.1. Growth and Survival

We measured seedling RCD at the time of outplanting. Ground-line diameter (GLD) was measured after the first and third years, and diameter at breast height (DBH) was measured at the end of the fifth and eighth years. Seedling total height (hereafter, height) was measured after 1, 2, 3, 5, and 8 years in the field. Seedlings were deemed emerged from the grass stage when their height exceeded 12 cm [30].

2.3.2. Root System Analysis

For root system analyses, we selected either two seedlings (March 2009; 15 months after outplanting) or one seedling (October 2009; 22 months after outplanting) from each of the 24 nursery treatments in each block. A total of six and three seedlings from each treatment were excavated 15 and 22 months after outplanting, respectively. We hand excavated seedling root systems within a 25-cm radius of the stem and to the depth of the deepest root. Seedlings were bagged and transported to the laboratory. Root systems were severed from their shoots at the root collar, and soil and residual growing medium were rinsed from the roots under running tap water. Care was given to avoid untangling lateral roots within the root plug. Seedling root systems were placed on a root plug template corresponding to their original cavity volume. Numbers of sinker roots (vertically oriented adventitious roots initiated near the callus tissue proximal to the air-pruned tip of the taproot) [42,43] and non-sinker adventitious roots (non-vertically oriented roots initiated near the taproot tip callus tissue) were counted. Taproot depth or depth of the deepest sinker root at the point where it terminated or assumed an angle that was greater than 45 degrees from the vertical position was used as the surrogate value for maximum vertical anchorage depth.

The diameter (measured 5 mm from the origin on the taproot) criterion for FOLRs in seedlings excavated in Year 2 was increased to ≥3.0 mm. Those primary lateral roots within the root plug and with <3-mm diameter were classified as fine roots. Within the root plug, each FOLR was assessed for its origin on the taproot (depth from root collar), diameter, egress point on the root plug template, and total length within the root plug. Three 4-cm zones, 0–4.0, 4.1–8.0, and 8.1–12.0 cm, were delineated from the root collar downward on the taproot or the root plug template for each of the four cavity volumes. Seedlings grown in the 60- and 336-mL cavities had a fourth zone: 12.1–13.0 cm and 12.1–15.0 cm, respectively. Each FOLR was assigned to the appropriate zone based on its origin and egress points. For each seedling, FOLR origin and egress in each zone was expressed as the percentage of the total seedling's FOLR number.

To calculate the deformity score for a FOLR, a value of 1 was assigned for each of the following morphological abnormalities associated with only the portion of a FOLR within the root plug: (1) >4 cm between the point of origination from the taproot and the point of egress from the root plug; (2) ascending; (3) >5 cm of the FOLR spiraling around the root plug; and (4) kinked or zigzagged. These morphologically unnatural traits are usually not present in naturally grown longleaf seedling root systems. The FOLR deformity index (DI) for a seedling was the sum of deformity scores for all FOLRs.

2.3.3. Biomass Allocation and Nutrients

The biomass of foliage, stem, and different root components internal and external to the root plug among the excavated seedlings was determined after drying each component to equilibrium (70 °C). The percentage of biomass allocation to various components, e.g., fascicles, stems, taproot+ (= taproot plus sinker roots plus non-sinker adventitious roots), FOLRs, and fine roots, was determined. The percentages of root biomass within the root plug (hereafter, plug root biomass) allocated to the taproot, FOLRs, and fine roots were also determined. On each excavation date, one seedling from each of the 24 nursery treatments in each block was analyzed for mineral nutrients. Oven-dried seedling foliage, stem, root plug taproot, and root plug FOLR were ground in a Wiley mill, and sieved through a 2-mm screen. Tissue nutrient concentrations were determined by A & L Plains Agricultural Laboratories, Inc. (Lubbock, TX, USA).

2.4. Statistical Analysis

Analysis of variance (ANOVA) was used to examine the effects of cavity volume, N rate, and Cu root pruning on stock quality variables at outplanting: RCD (mm), FOLR number, longest FOLR length (cm), and fascicle number. Similar ANOVA were used to evaluate seedling and sapling field performance variables—RCD (mm), GLD (mm), DBH (mm), height (cm), survival (%), and seedling emergence from the grass stage (%)—and Year 2 excavated seedling parameters: biomass (g) and biomass allocations (%), plug root biomass (g) and allocations (%), FOLR number, proportion of FOLR originating from each taproot zone (%), proportion of FOLR egressing from each root plug zone (%), mean and total FOLR length within the root plug (cm), and seedling FOLR DI. The ANOVA were conducted by SAS® PROC GLM ($\alpha = 0.05$) [44]. For variables deemed significant, mean separation was done using the Bonferroni t-test for each comparison at α level of 0.05. The correlation between the vertical anchorage depth and root anchorage biomass was obtained from a linear regression of vertical anchorage depth on root anchorage biomass.

3. Results

3.1. Stock Quality at Outplanting

Seedlings cultured in 366-mL cavities consistently had greater values with respect to all variables than those in 60-mL cavities (Table 1). Seedling RCD increased with increasing cavity volume. The HN and MN seedlings had greater RCD than the LN seedlings (Table 1). The rates of N did not affect the number of FOLR or the length of the longest FOLR. Number of fascicles, however, increased with the increasing N rate. Copper root pruning increased seedling RCD and reduced the length of the longest FOLR without affecting the numbers of FOLR or fascicles (Table 1). Among all two- and three-way treatment interactions, only the cavity volume × N rate interaction significantly affected the fascicle number ($p = 0.0377$). The fascicle numbers among seedlings cultured in 60- or 95-mL cavities did not respond to N rates. The fascicle numbers of the LN seedlings did not respond to cavity volume. The 336-mL seedlings fertilized at MN had more fascicles than the MN seedlings of smaller cavities, whereas the 336-mL HN seedlings had comparable fascicle numbers to that of the 125- and 95-mL HN seedlings.

3.2. Early and Long-Term Field Performance

Across treatments during the first 5 years after outplanting, survival was 97% and mortality was not associated with specific nursery treatments. Between Years 6 and 8, survival decreased an additional 9%, with twice as much mortality occurring among seedlings grown in 60-mL cavities as that of seedlings grown in larger cavity volumes. Similarly, twice as much mortality was observed in the LN seedlings as in seedlings grown at higher N rates during the same time period. Copper root pruning did not affect mortality between Years 6 and 8. Regardless of treatments, seedlings that died between Years 6 and 8 were smaller at the end of Year 5 than those that survived (110 vs. 235 cm height; 6.3 vs. 28.5 mm DBH) and also had smaller RCD at outplanting than those that survived (4.7 vs. 5.5 mm).

Table 1. Means (1 standard error of the mean, SE) and *p*-values for seedling root collar diameter (RCD), number (#) of first-order lateral roots (FOLRs), length of longest FOLR (LFOLR), and fascicle number in response to nursery treatments at the end of nursery production. Within each treatment, means for each variable followed by the same letter are not significantly different at the Bonferroni-adjusted 0.05 level.

Treatment	RCD (mm)	FOLR (#)	LFOLR (cm)	Fascicle (#)
		Cavity volume (mL)		
60	3.65 (0.18) a	2.8 (0.4) a	10.1 (1.0) a	6.5 (0.4) a
95	5.01 (0.17) b	3.1 (0.3) a,b	12.3 (0.6) a,b	9.9 (1.1) a
125	5.84 (0.33) c	4.8 (0.4) b,c	10.3 (0.5) a	10.3 (1.9) a
336	7.13 (0.24) d	5.7 (0.7) c	13.8 (1.2) b	16.9 (2.1) b
p-value	<0.0001	<0.0001	0.0018	<0.0001
		Nitrogen rate		
Low	4.86 (0.28) a	3.9 (0.3)	10.8 (0.7)	6.9 (0.4) a
Medium	5.55 (0.34) b	4.3 (0.5)	11.8 (0.8)	10.9 (1.4) b
High	5.82 (0.35) b	4.1 (0.5)	12.2 (0.9)	15.0 (1.9) c
p-value	0.0013	0.7258	0.2101	<0.0001
		Root pruning		
No-Cu	5.14 (0.27) a	3.9 (0.4)	13.4 (0.5) b	11.3 (1.5)
Cu	5.67 (0.26) b	4.3 (0.4)	10.0 (0.7) a	10.6 (0.9)
p-value	0.0127	0.4086	<0.0001	0.5891

Nursery treatments of cavity volume and N rate significantly affected all the seedling morphological variables up to eight years after outplanting (Table 2). From Years 1 to 5, seedling height increased with the increasing cavity volume (Figure 1a). Eight years after outplanting, seedlings from 366-mL cavities were taller than those from 60- and 95-mL cavities, and those from 125-mL cavities were taller than those from 60-mL cavities (Figure 1a). Seedlings fertilized with HN or MN in the nursery were always taller than the LN seedlings (Figure 1b). Except for Year 1, the MN and HN seedlings were comparable in height up to eight years in the field. Seedling heights did not respond to Cu root pruning for eight years after outplanting (Table 2, Figure 1c).

Table 2. *p*-values for the effects of nursery treatments (cavity volume, N rate, and Cu root pruning) and their two- and three-way interactions on seedling morphological variables assessed at outplanting (Year 0) and up to eight years after outplanting (Year 8) using analysis of variance. The measured morphological variables are as follows: RCD, root collar diameter; GLD, ground-line diameter; HT, height; EMGS, percent emergence from the grass stage; and DBH, diameter at breast height.

Variable	Cavity Volume (C)	Nitrogen Rate (N)	Copper Root Pruning (Cu)	C × N	C × Cu	N × Cu	C × N × Cu
RCD-Year 0	<0.0001	<0.0001	0.0003	0.0020	0.8631	0.7274	0.0412
GLD-Year 1	<0.0001	<0.0001	0.5702	0.5174	0.3485	0.7078	0.0835
HT-Year 1	<0.0001	<0.0001	0.0843	0.3809	0.2679	0.2534	0.5140
EMGS-Year 1	<0.0001	<0.0001	0.1034	0.3494	0.5656	0.4868	0.6788
HT-Year 2	<0.0001	<0.0001	0.1484	0.0458	0.1653	0.7920	0.2804
EMGS-Year 2	0.0003	0.0002	0.6159	0.1688	0.6792	0.5754	0.3431
GLD-Year 3	<0.0001	<0.0001	0.3764	0.8142	0.1071	0.4136	0.5348
HT-Year 3	<0.0001	<0.0001	0.2205	0.1711	0.2517	0.9654	0.3698
DBH-Year 5	<0.0001	<0.0001	0.6495	0.3578	0.0871	0.5675	0.1485
HT-Year 5	<0.0001	<0.0001	0.4894	0.3255	0.1149	0.5319	0.1114
DBH-Year 8	<0.0001	0.0003	0.2853	0.1955	0.2073	0.3605	0.4752
HT-Year 8	<0.0001	<0.0001	0.6922	0.0639	0.0613	0.4151	0.1327

A two-way cavity volume x N rate interaction significantly affected Year 2 seedling height (Table 2). Among seedlings grown in 60- and 95-mL cavities, Year 2 height did not respond to N rates. The LN seedlings cultured in 125-mL cavities were shorter in Year 2 than seedlings receiving higher N rates.

The LN seedlings in 336-mL cavities were shorter in Year 2 than the 336-mL HN seedlings but were similar to the 336-mL MN seedlings in Year 2 height. At each N rate, seedlings cultured in 95-mL cavities were as tall in Year 2 as those in 60- and 125-mL cavities but were shorter than those cultured in 336-mL cavities.

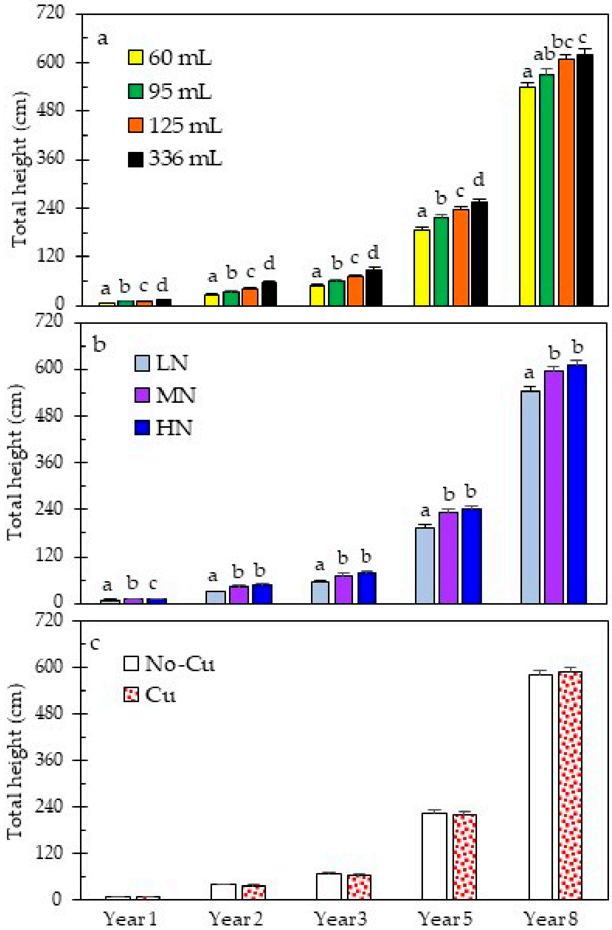

Figure 1. Longleaf pine seedling total height in response to nursery treatments from one to eight years after outplanting. (**a**) Cavity volume; (**b**) nitrogen rate: low (LN), medium (MN), and high (HN); and (**c**) copper (Cu) root pruning. Within a treatment and year, the same letter indicates no significant differences at the Bonferroni-adjusted 0.05 level. Error bars represent 1 SE.

By the end of Years 1 and 2 after outplanting, more seedlings grown in 125- and 366-mL cavities emerged from the grass stage than the 60-mL seedlings (Figure 2a). In Year 1, the 366-mL seedlings had the highest percent emergence from the grass stage among seedlings grown in the four cavity volumes. The LN seedlings had less emergence than seedlings fertilized with higher N rates in the first two years of outplanting (Figure 2b). Copper treatment did not affect seedling emergence from the grass stage (Table 2, Figure 2c). By the end of Year 2 after outplanting, more than 80% of seedlings had emerged from the grass stage among all 24 nursery treatment combinations.

Independent of the nursery treatments, seedlings that emerged from the grass stage in each of the first three years after outplanting were grouped and analyzed. Through eight years in the field, seedlings that emerged in the first year were taller than those that emerged in the second and third years, and those that emerged in the second year were taller than seedlings that emerged one year later (Figure 3a). Seedlings that emerged from the grass stage in the first, second, and third years after outplanting had, respectively, 6.2, 5.1, and 4.5 mm RCD at outplanting (Figure 3b). Similar to heights, the stem diameters of seedlings that emerged in the first year were greater than those that emerged in the second and third years, and those that emerged in the second year had greater stem diameters than seedlings that emerged in the third year.

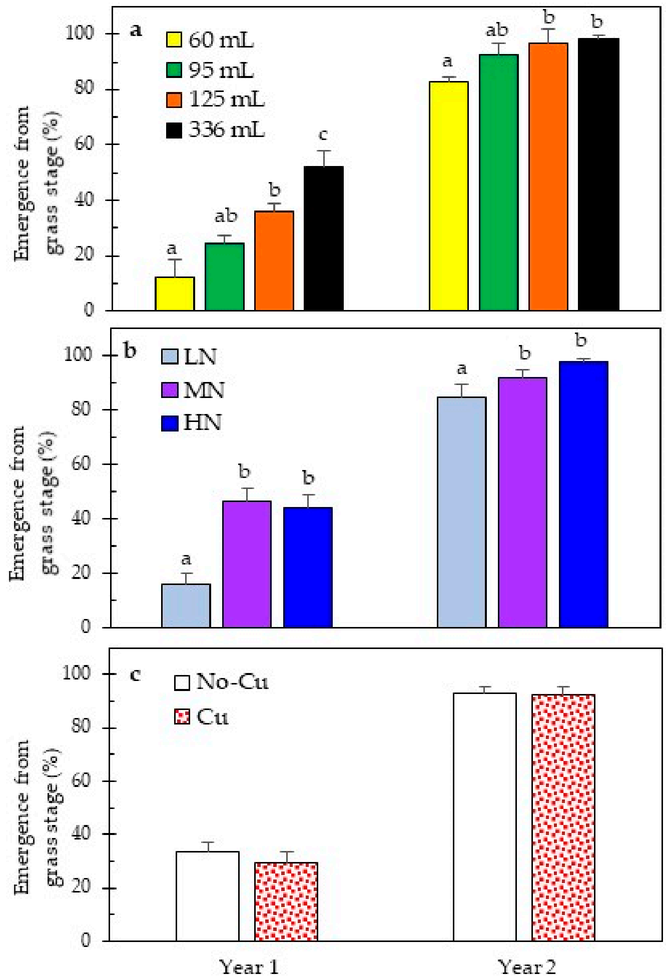

Figure 2. Percent emergence from the grass stage by longleaf pine seedlings in response to nursery treatments during the first two years after outplanting. (**a**) Cavity volume; (**b**) nitrogen rate: low (LN), medium (MN), and high (HN); and (**c**) copper (Cu) root pruning. Within a treatment and year, the same letter indicates no significant differences at the Bonferroni-adjusted 0.05 level. Error bars represent 1 SE.

Between the time of and eight years after outplanting, seedling RCD, GLD, and DBH were significantly affected by cavity volume and N rate (Table 2). Seedlings grown in 336-mL cavities always had larger stem diameters than those grown in 60- or 95-mL cavities; those grown in 125-mL cavities always had larger stem diameters than those grown in 60-mL cavities (Figure 4a). Seedlings fertilized with HN or MN had comparable stem diameters that were larger than those of the LN seedlings from Year 1 to 8 (Figure 4b). While copper root pruning increased RCD at outplanting, it did not affect stem diameters in subsequent years after outplanting (Figure 4c).

A two-way cavity volume × N rate interaction significantly affected RCD-Year 0 among all outplanted seedlings at outplanting (Table 2). Root collar diameters of the LN seedlings were smaller than those of the MN and HN seedlings grown in 95- and 125-mL cavities, but this effect was not observed among seedlings grown in 60- or 336-mL cavities. The HN seedlings grown in 125-mL cavities had greater RCD than the HN seedlings grown in 95-mL cavities, whereas, the MN or LN seedlings grown in 125-mL cavities had comparable RCD to seedlings in 95-mL cavities and fertilized at the same N rate. A three-way cavity volume × N rate × Cu root pruning interaction significantly affected seedling RCD at outplanting (Table 2). For seedlings cultured in 60- or 336-mL cavities, RCD was not affected by N rates or Cu root pruning. For seedlings in each of the 12 cavity volume and N rate treatment combinations, Cu root pruning did not affect RCD. Regardless of Cu treatment, RCD of the 95-mL LN seedlings was not different from that of the 60-mL seedlings fertilized at any of the three N rates.

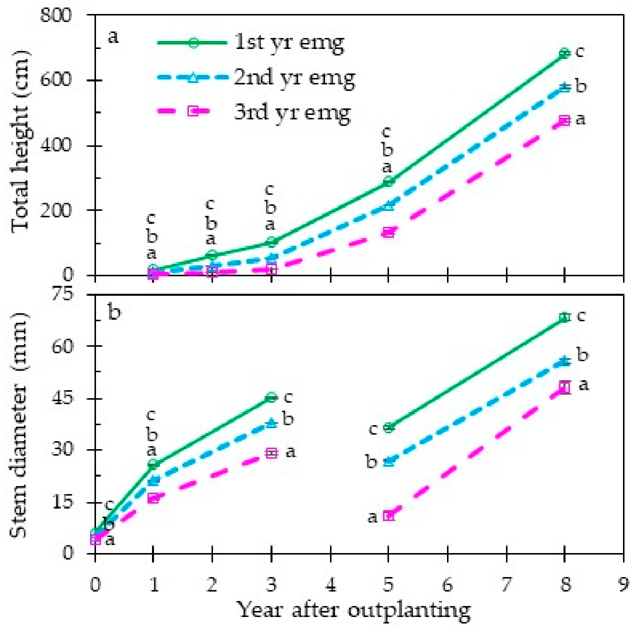

Figure 3. Seedling height (**a**) and stem diameter (breast height diameter for Years 5 and 8) (**b**) of longleaf pine seedlings that emerged from the grass stage during the first, second, or third year in the field. For each year, the same letter indicates no significant differences at the Bonferroni-adjusted 0.05 level. Error bars represent 1 SE.

3.3. Seedling Physiology

3.3.1. Seedling Biomass and Allocation

We did not find any significant two- or three-way interactions for the seedling biomass or its allocation to seedling components among seedlings excavated 15 and 22 months after outplanting (March and October 2009, respectively). The main effects of cavity volume and N rate significantly affected the seedling biomass on both excavation dates (Table 3). Seedlings grown in the larger cavities (336 and 125 mL) had significantly greater seedling biomass than those grown in the smallest (60 mL) cavities for both excavation dates (Table 3). The biomass of the HN and MN seedlings was significantly greater than that of the LN seedlings on both dates.

The fractions of seedling biomass allocated to seedling components responded inconsistently to the three treatment types between the two excavation dates. Among the 15-month seedlings, the fractions of seedling biomass allocated to all the seedling components did not respond to cavity volume or Cu treatment. Compared with the LN seedlings, the HN seedlings allocated larger and smaller fractions of biomass to foliage 15 and 22 months after outplanting, respectively. Moreover, for the 22-month seedlings of all the treatments, the Cu treatment affected the fraction of biomass allocation in FOLRs (Table 3). Among the 22-month seedlings, the 336-mL seedlings had lower and greater fractions of biomass allocation in foliage and stems, respectively, compared with the 60-mL seedlings. Across treatments, between March and October 2009, the biomass of seedlings, foliage, and stems increased two- (71.2 vs. 216.7 g), 4.4- (22.6 vs. 122.4 g), and fourfold (8.5 vs. 42.7 g), respectively, whereas, the biomass of taproot+ (21.6 vs. 29.9 g) and FOLRs (16.7 vs. 19.7 g) increased less than 0.4-fold during the same time.

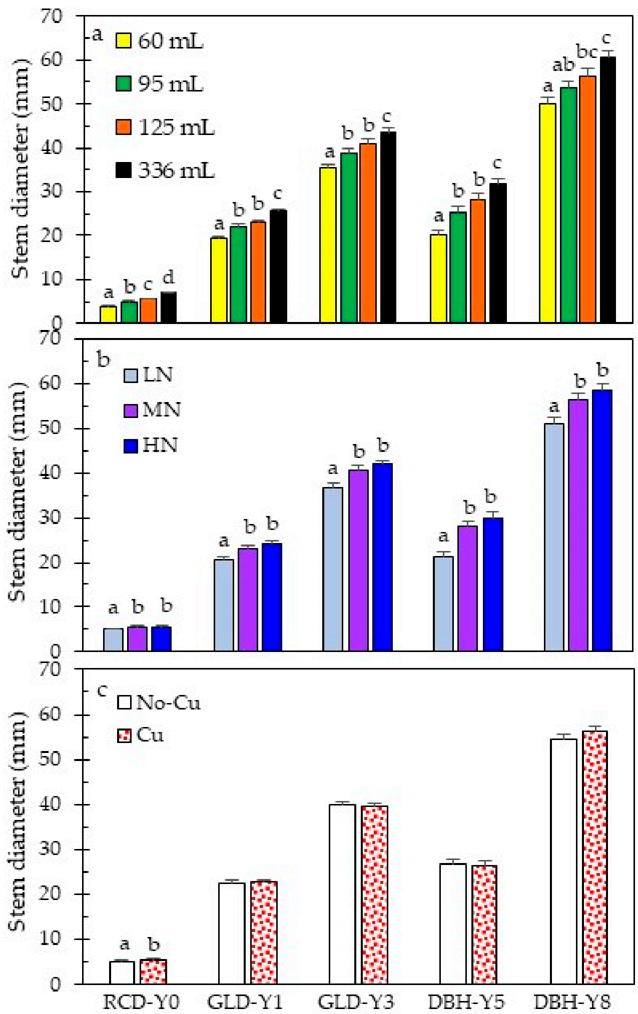

Figure 4. Longleaf pine seedling stem diameter, expressed as root collar diameter (RCD) at outplanting (Y0), ground-line diameter (GLD) in Year 1 (Y1) and Year 3 (Y3), and diameter at breast height (DBH) in Year 5 (Y5) and Year 8 (Y8), in response to nursery treatments. (**a**) Cavity volume; (**b**) nitrogen rate: low (LN), medium (MN), and high (HN); and (**c**) copper (Cu) root pruning. Within a treatment and year, the same letter indicates no significant differences at the Bonferroni-adjusted 0.05 level. Error bars represent 1 SE.

Table 3. Means (1 SE) and *p*-values for the seedling biomass and its allocation to seedling components in response to nursery treatments. Seedlings were excavated 15 and 22 months after outplanting. The seedling components are as follows: taproot+, taproot plus all sinker roots and all adventitious roots; FOLR, first-order lateral root; and fine root, non-FOLR from taproot within the root plug. Within each treatment and excavation, means followed by the same letter are not significantly different at the Bonferroni-adjusted 0.05 level.

Treatment	Seedling Biomass (g)	Foliage (%)	Stem (%)	Taproot+ (%)	FOLR (%)	Fine Root (%)
15-Month Seedlings (March 2009)						
Cavity volume (mL)						
60	45 (4) a	31.1 (0.7)	12.8 (0.8)	31.9 (1.4)	21.2 (1.4)	3.0 (0.2)
95	64 (5) a,b	32.4 (0.8)	11.3 (0.6)	32.6 (2.1)	21.4 (1.5)	2.3 (0.2)
125	68 (6) b	30.7 (1.0)	11.7 (0.9)	29.7 (1.7)	25.3 (1.8)	2.5 (0.2)
336	108 (9) c	32.8 (0.8)	12.2 (0.5)	26.8 (1.5)	25.9 (1.0)	2.3 (0.6)
p-value	<0.0001	0.1632	0.4325	0.0483	0.0545	0.5555

Table 3. *Cont.*

Treatment	Seedling Biomass (g)	Foliage (%)	Stem (%)	Taproot+ (%)	FOLR (%)	Fine Root (%)
			Nitrogen rate			
Low	53 (7) a	30.1 (0.7) a	11.5 (0.4)	33.4 (1.8) b	21.7 (1.6)	2.7 (0.2)
Medium	72 (6) b	31.9 (0.6) a,b	11.7 (0.7)	30.5 (1.4) a,b	23.1 (1.2)	2.8 (0.4)
High	88 (7) b	33.2 (0.7) b	12.8 (0.6)	26.4 (0.9) a	25.5 (0.9)	2.1 (0.2)
p-value	<0.0001	0.0071	0.2905	0.0013	0.1326	0.2356
			Root pruning			
No-Cu	74 (7)	31.2 (0.6)	11.6 (0.3)	31.3 (1.2)	23.3 (1.0)	2.7 (0.3)
Cu	69 (5)	32.3 (0.6)	12.4 (0.6)	29.3 (1.2)	23.7 (1.1)	2.4 (0.2)
p-value	0.3918	0.1579	0.2830	0.2165	0.7918	0.4260
			22-Month Seedlings (October 2009)			
			Cavity volume (mL)			
60	133 (22) a	60.0 (1.9) b	16.4 (1.8) a	14.7 (0.9)	7.5 (0.8)	1.5 (0.3) b
95	210 (20) a,b	57.5 (1.2) b	18.6 (1.3) a,b	13.5 (0.8)	9.6 (0.8)	0.9 (0.2) a
125	228 (26) b	56.5 (1.4) a,b	20.3 (1.4) a,b	13.2 (0.8)	9.4 (0.7)	0.7 (0.1) a
336	296 (33) b	52.0 (1.5) a	23.6 (1.7) b	13.7 (1.0)	10.1 (0.8)	0.5 (0.1) a
p-value	0.0001	0.0015	0.0040	0.5560	0.1334	0.0003
			Nitrogen rate			
Low	149 (16) a	59.4 (1.0) b	15.4 (0.9) a	15.5 (0.6) b	8.6 (0.7)	1.2 (0.2)
Medium	242 (28) b	57.1 (1.4) a,b	20.5 (1.5) b	12.5 (0.9) a	9.1 (0.7)	0.7 (0.2)
High	259 (22) b	53.0 (1.6) a	23.3 (1.4) b	13.3 (0.7) a,b	9.6 (0.7)	0.8 (0.1)
p-value	0.0005	0.0014	<0.0001	0.0101	0.5928	0.0260
			Root pruning			
No-Cu	234 (22)	55.4 (1.1)	20.1 (1.2)	13.5 (0.7)	10.3 (0.6) b	0.8 (0.1)
Cu	200 (18)	57.6 (1.2)	19.4 (1.2)	14.1 (0.6)	8.0 (0.5) a	1.0 (0.1)
p-value	0.1416	0.1099	0.6246	0.4740	0.0071	0.2345

3.3.2. Mineral Nutrient Concentrations and Contents

With a few exceptions, seedlings excavated 15 or 22 months after outplanting generally did not exhibit significant treatment differences among mineral nutrient concentrations of foliage, stems, and within the root plug taproot and FOLR tissues. Three exceptions were noted in the 15-month samples, but it is unclear if these differences were biologically significant. Compared with the HN seedlings, the LN seedlings had a higher foliar N concentration (1.25% vs. 1.30%) and a higher stem K concentration (0.40% vs. 0.46%). The 60-mL seedlings had a higher stem K concentration than the 366-mL seedlings (0.47% vs. 0.38%). Among the 22-month seedlings, most of the significant treatment effects took place in the stems. The LN seedlings had significantly higher stem N and Mg concentrations than the HN seedlings (1.21% vs. 0.95% N and 0.15% vs. 0.12% Mg). The 60-mL seedlings had significantly higher stem N, P, K, Mg, and Zn concentrations than the 336-mL seedlings (1.22% vs. 0.93% N, 0.12% vs. 0.09% P, 0.41% vs. 0.30% K, 0.14% vs. 0.11% Mg, and 44 vs. 33 ppm Zn). Across treatments, most tissue nutrient concentrations were significantly different between March and October except for concentrations of foliar P and Mg (Table 4), stem P and Cu, taproot N, and FOLR P. The total seedling contents for each nutrient significantly increased from March to October in Year 2 (Table 4): the total seedling contents of P, K, Mg, and Cu increased by greater than twofold; seedling N, Zn, Ca, and Fe contents increased between 1.4- and 1.8-fold; and seedling B and Mn contents increased onefold or less.

Table 4. Across nursery treatments means (1 SE) of foliar nutrient concentrations and total seedling nutrient contents among individuals excavated 15 and 22 months after outplanting. For the 22-month seedlings, means followed by = are not significantly different at α = 0.05 from the means of the 15-month seedlings.

Nutrient	15-Month Foliage Concentration	15-Month Seedling Content (mg)	22-Month Foliage Concentration	22-Month Seedling Content (mg)
	(%)	(mg)	(%)	(mg)
N	1.27 (0.01)	680 (46)	0.89 (0.01)	1884 (121)
P	0.08 (0.002)	54 (4)	0.08 (0.002) =	163 (10)
K	0.52 (0.01)	269 (18)	0.56 (0.02)	962 (61)
Mg	0.11 (0.002)	73 (5)	0.11 (0.002) =	219 (12)
Ca	0.44 (0.01)	135 (9)	0.20 (0.01)	340 (22)
	(ppm)	(mg)	(ppm)	(mg)
Cu	5.6 (0.1)	0.3 (0.0)	4.5 (0.1)	1.0 (0.1)
B	15 (1)	2 (0.1)	20 (1)	4 (0.3)
Zn	63 (1)	3 (0.2)	40 (1)	8 (0.4)
Fe	52 (2)	8 (1)	114 (9)	19 (1.7)
Mn	665 (16)	19 (1)	241(8)	35 (2.5)

3.4. Seedling Root System

3.4.1. Vertical Anchorage

The outplanted seedlings grew from zero to six adventitious roots near the taproot tip callus tissues, with most seedlings forming two or three adventitious roots. For some seedlings, however, their taproots continued to extend from the root plug bottom after outplanting. The presence or numbers of egressed taproots, sinker roots, or non-sinker adventitious roots in outplanted seedlings did not respond to nursery treatments. Across all treatments, 43% and 49% of the 15- and 22-month seedlings, respectively, had egressed taproots, whereas, 47% and 38% of the 15- and 22-month seedlings formed sinker roots, respectively. Non-sinker adventitious roots were found in 8% and 18% of the 15- and 22-month seedlings, respectively. Fewer than 4% of all the seedlings grew both sinker and adventitious roots. Fewer than 3% of all the seedlings had no vertically egressed roots.

Among the 15-month seedlings, the HN seedlings had greater vertical anchorage depth than seedlings that received less N, and the 125-mL seedlings had greater vertical anchorage depth than the 60-mL seedlings (Table 5). Copper root pruning did not affect the vertical anchorage depth of seedlings from either date (Table 5). The vertical anchorage depth of the 22-month seedlings did not respond to the nursery treatments (Table 5). However, a significant two-way cavity volume × Cu root pruning interaction ($p = 0.0215$) showed that for the 22-month seedlings, Cu root pruning increased the vertical anchorage depth for the 95-mL seedlings (28.5 vs. 14.5 cm) but decreased the vertical anchorage depth among seedlings grown in 60- (28.9 vs. 20.8 cm), 125- (27.2 vs. 23.3 cm), and 336-mL cavities (25.1 vs. 13.4 cm). For both dates, root anchorage biomass in the HN seedlings was greater than that of the LN seedlings and the 336-mL seedlings had greater root anchorage biomass than the 60-mL seedlings (Table 5). The correlation between vertical anchorage depth and root anchorage biomass was significant with r^2 values of 0.1349 and 0.1065 for the 15- and 22-month seedlings, respectively.

3.4.2. Plug Root Biomass and Allocation

For the 15- and 22-month seedlings, the plug root biomass responded significantly to cavity volume, N rate, and Cu root pruning without significant two- or three-way interactions (Table 6). For both excavations, the 60-mL seedlings and the LN seedlings had the least plug root biomass among cavity size and N rate treatments, respectively (Table 6). The Cu seedlings also had less plug root biomass than the no-Cu seedlings on both dates (Table 6). Percent plug root biomass allocations to the taproot and FOLR responded in opposite ways. For example, 15 months after outplanting, seedlings grown in 336-mL cavities had a lower percent allocation of plug root biomass to the taproot and higher percent allocation to FOLR compared with seedlings grown in 60- and 95-mL cavities (Table 6). Similarly, plug root biomass allocation to the taproot and FOLR in the HN seedlings was lower and higher, respectively, than that in the LN seedlings. At 22 months, plug root biomass allocation to the taproots did not respond to cavity volume or N rate treatments, but plug root biomass allocation to FOLR was greater in the 336-mL seedlings and the HN seedlings compared with the 60-mL seedlings and the LN seedlings, respectively (Table 6). Contrary to the effects of increasing cavity volume and N rate, Cu root pruning decreased plug root biomass allocation to FOLR but increased allocation to the taproot on both dates (Table 6). The percent allocation of plug root biomass to fine roots was unaffected by any nursery treatments on both dates except that it was significantly greater among the 60-mL seedlings compared with the seedlings grown in larger cavities 22 months after outplanting (Table 6).

Table 5. Means (1 SE) and *p*-values for vertical anchorage depth and root anchorage biomass in response to nursery treatments. Seedlings were excavated 15 and 22 months after outplanting. Vertical anchorage depth was taproot depth or depth of the deepest sinker root at the point where it terminated or assumed an angle that was greater than 45 degrees from the vertical position. Root anchorage biomass was the biomass of the taproot and all sinker roots in full lengths. Within each treatment and excavation, means followed by the same letter are not significantly different at the Bonferroni-adjusted 0.05 level.

Treatment	15-Month Vertical Anchorage Depth (cm)	15-Month Root Anchorage Biomass (g)	22-Month Vertical Anchorage Depth (cm)	22-Month Root Anchorage Biomass (g)
		Cavity volume (mL)		
60	18.7 (1.8) a	13.7 (1.3) a	24.9 (3.3)	18.3 (3.0) a
95	19.3 (2.0) a,b	19.1 (1.8) a	21.5 (3.1)	26.6 (2.9) a,b
125	26.5 (2.1) b	18.0 (1.2) a	25.3 (3.7)	26.2 (2.5) a,b
336	20.2 (2.7) a,b	27.9 (3.0) b	19.3 (3.2)	35.7 (3.7) b
p-value	0.0229	<0.0001	0.4557	0.0008
		Nitrogen rate		
Low	18.2 (1.7) a	15.7 (2.0) a	19.8 (2.1)	21.0 (2.6) a
Medium	19.0 (1.8) a	20.6 (1.7) a,b	19.9 (2.6)	27.5 (3.0) a,b
High	26.3 (2.0) b	22.7 (2.0) b	28.5 (3.4)	31.6 (2.7) b
p-value	0.0019	0.0151	0.0345	0.0110
		Root pruning		
No-Cu	22.9 (1.7)	21.2 (1.9)	23.9 (2.5)	29.5 (2.8)
Cu	19.4 (1.4)	18.2 (1.3)	21.5 (2.2)	23.9 (1.8)
p-value	0.0731	0.1288	0.4248	0.0510

Table 6. Means (1 SE) and *p*-values for plug root biomass and its allocation to components (FOLR, first-order lateral roots; fine root, non-FOLR from taproot) within the root plug in response to nursery treatments among seedlings excavated 15 and 22 months after outplanting. Within each treatment and excavation, means followed by the same letter are not significantly different at the Bonferroni-adjusted 0.05 level.

Treatment	Plug Root Biomass (g)	Taproot (%)	FOLR (%)	Fine Root (%)
		15-Month Seedlings (March 2009)		
		Cavity volume (mL)		
60	14.1 (1.1) a	66.5 (1.9) a	24.3 (2.3) a	9.2 (0.7)
95	20.4 (1.4) b	64.1 (2.3) a	29.2 (2.3) a	6.7 (0.5)
125	20.6 (1.5) b	59.6 (2.3) a,b	32.8 (2.4) a,b	7.6 (0.6)
336	33.8 (2.7) c	53.2 (2.1) b	40.0 (2.1) b	6.8 (1.4)
p-value	<0.0001	0.0004	<0.0001	0.2207

Table 6. *Cont.*

Treatment	Plug Root Biomass (g)	Taproot (%)	FOLR (%)	Fine Root (%)
		Nitrogen rate		
Low	17.9 (2.2) a	63.9 (2.5) a	28.1 (2.5) a	8.0 (0.7)
Medium	22.9 (1.8) b	61.2 (1.9) a,b	31.0 (1.9) a,b	7.7 (0.9)
High	25.9 (2.1) b	57.3 (1.7) b	35.7 (2.1) b	6.9 (0.7)
p-value	0.0005	0.0517	0.0220	0.5985
		Root pruning		
No-Cu	24.2 (1.9) a	58.1 (1.6) a	34.5 (1.7) a	7.4 (0.7)
Cu	20.5 (1.5) b	63.2 (1.7) b	29.0 (1.8) b	7.8 (0.6)
p-value	0.0188	0.0139	0.0110	0.7219
		22-Month Seedlings (October 2009)		
		Cavity volume (mL)		
60	17.3 (2.3) a	66.8 (2.5)	23.1 (2.6) a	10.1 (1.7) a
95	28.3 (2.9) b	64.8 (2.5)	29.2 (2.9) a,b	6.0 (1.0) b
125	29.2 (2.7) b	62.3 (2.7)	32.6 (2.8) b	5.2 (0.6) b
336	41.7 (3.8) c	59.1 (2.6)	37.3 (2.8) b	3.5 (0.5) b
p-value	<0.0001	0.1089	0.0005	<0.0001
		Nitrogen rate		
Low	22.4 (2.3) a	67.4 (2.1)	25.1 (2.5) a	7.4 (1.1)
Medium	29.9 (3.4) b	61.6 (2.3)	33.0 (2.3) b	5.4 (1.0)
High	35.1 (231) b	60.7 (2.2)	33.6 (2.7) b	5.8 (0.9)
p-value	0.0006	0.0382	0.0057	0.1919
		Root pruning		
No-Cu	33.6 (2.8) a	58.5 (1.8) a	36.2 (2.0) a	5.3 (0.9)
Cu	24.6 (1.9) b	68.0 (1.5) b	24.9 (1.8) b	7.1 (0.7)
p-value	0.0004	0.0001	<0.0001	0.0591

3.4.3. Morphology of FOLRs

FOLRs within the Root Plug

None of the nursery treatments significantly affected the origination pattern of FOLRs from the taproot within the root plug. Across all treatments and excavation dates, at least 60% and 28% of all root plug FOLRs originated in the 0–4.0-cm and 4.1–8.0-cm zones of the taproot, respectively. Fewer than 9% of all root plug FOLRs originated in the 8.1–12.0-cm and >12.0-cm zones of the taproot.

At 15 months, a two-way cavity volume × Cu root pruning interaction ($p = 0.0300$) significantly affected mean FOLR length within the root plug. Copper treatment decreased mean FOLR length more among the 336- (12.8 vs. 7.9 cm) and 60-mL seedlings (8.6 vs. 4.0 cm) than among the 125-mL seedlings (8.6 vs. 6.7 cm). On both excavation dates, all root plug FOLR variables responded to cavity volume, N rate, and Cu root pruning with two exceptions (Table 7). In the first exception, the FOLR number did not respond to Cu treatment, and in the second exception, mean FOLR length was not affected by N rate. Seedlings grown in 336-mL cavities had greater values for all within plug FOLR variables than seedlings grown in smaller cavities on both dates except they had a similar FOLR number compared with the 22-month, 95-mL seedlings (Table 7). On both dates, HN seedlings had more FOLR, greater total FOLR lengths, and a greater FOLR DI than the LN seedlings; and Cu root pruning decreased mean and total FOLR lengths and FOLR DI (Table 7).

FOLR Outside the Root Plug

Copper root pruning as a main or interaction effect consistently altered the egress pattern of FOLR from 0 to 12.0 cm of the root plug on both dates (Table 8). Compared with the 15-month no-Cu seedlings, the 15-month Cu seedlings had higher percentages of FOLR egress from the 0–4.0-cm (3.5% vs. 18.3%)

and 4.1–8.0-cm (22.7% vs. 36.9%) zones of the root plug, and smaller percentages of egress from the 8.1–12.0-cm (57.9% vs. 38.8%) and >12.0-cm (15.9% vs. 5.0%) zones. Similar Cu root pruning effects on FOLR egress pattern were also observed among the 22-month seedlings as among the 15-month seedlings except Cu did not affect FOLR egress percent from the >12.0-cm zone. A significant two-way cavity volume × Cu root pruning interaction (p = 0.0056) among the 15-month seedlings showed that Cu treatment decreased FOLR egress from the 8.1–12.0-cm zone of all the seedlings except for seedlings grown in the 336-mL cavities. Among the 22-month seedlings, a significant two-way cavity volume × Cu interaction (p = 0.0077) showed that Cu treatment increased percent FOLR egress from the top 4.0 cm of the root plug in all the seedlings except for the 336-mL seedlings.

Table 7. Means (1 SE) and p-values for morphological variables of the first-order lateral roots (FOLR) within the root plug in response to nursery treatments for seedlings excavated 15 and 22 months after outplanting. The morphological variables are as follows: FOLR number; mean FOLR length, average length of FOLR within the root plug; total FOLR length, sum of lengths of FOLRs within the root plug; and DI, deformity index, the sum of deformity scores for FOLR within the root plug. Within each treatment and excavation, means followed by the same letter are not significantly different at the Bonferroni-adjusted 0.05 level.

Treatment	FOLR Number	Mean FOLR Length (cm)	Total FOLR Length (cm)	DI
		15-Month Seedlings (March 2009)		
		Cavity volume (mL)		
60	5.7 (0.4) a	6.3 (0.7) a	36.2 (5.0) a	3.0 (0.6) a
95	6.6 (0.5) a	8.0 (0.6) b	54.5 (5.9) a	4.8 (0.7) a
125	7.5 (0.6) a	7.6 (0.4) a,b	59.0 (5.6) a	5.3 (0.6) a
336	9.6 (0.6) b	10.4 (0.7) c	101.7 (10.3) b	9.2 (1.1) b
p-value	<0.0001	<0.0001	<0.0001	<0.0001
		Nitrogen rate		
Low	5.8 (0.5) a	7.9 (0.6)	49.4 (7.3) a	4.4 (0.7) a
Medium	7.6 (0.5) b	8.2 (0.5)	64.9 (7.0) a,b	5.8 (0.7) a,b
High	8.7 (0.5) b	8.2 (0.6)	74.2 (8.3) b	6.6 (0.9) b
p-value	<0.0001	0.6912	0.0047	0.0165
		Root pruning		
No-Cu	7.3 (0.5)	9.9 (0.4)b	76.9 (7.2) b	7.4 (0.7) b
Cu	7.4 (0.4)	6.2 (0.3) a	48.1 (4.2) a	3.8 (0.4) a
p-value	0.7158	<0.0001	<0.0001	<0.0001
		22-Month Seedlings (October 2009)		
		Cavity volume (mL)		
60	6.3 (0.6) a	7.0 (0.7) a	46.2 (6.9) a	4.4 (0.8) a
95	8.2 (0.8) a,b	7.3 (0.7) a	64.1 (10.9) a	6.6 (1.5) a
125	7.9 (0.7) a	7.8 (0.6) a	64.7 (8.3) a	7.1 (1.1) a
336	10.9 (0.9) b	10.4 (0.9) b	117.7 (15.9) b	12.7 (2.0) b
p-value	0.0006	<0.0001	<0.0001	0.0002
		Nitrogen rate		
Low	6.8 (0.6) a	7.3 (0.6)	51.8 (7.2) a	5.4 (1.1) a
Medium	8.5 (0.8) a,b	8.5 (0.6)	76.6 (10.9) a,b	8.4 (1.5) a,b
High	9.8 (0.7) b	8.6 (0.7)	91.2 (12.6) b	9.3 (1.4) b
p-value	0.0076	0.0665	0.0033	0.0378
		Root pruning		
No-Cu	8.8 (0.6)	10.2 (0.5) b	94.9 (9.8) b	10.6 (1.2) b
Cu	7.8 (0.6)	6.1 (0.3) a	51.5 (6.1) a	4.8 (0.8) a
p-value	0.1806	<0.0001	<0.0001	<0.0001

Percent FOLR egress from the root plug was not affected by N rate at 15 months (Table 8). However, at 22 months, N rate significantly affected percent FOLR egress from the 4.1–8.0-cm zone, with the

HN seedlings having lower percent FOLR egress compared with the MN and LN seedlings (26.0% vs. 42.4% MN and 36.3% LN). In contrast, the HN seedlings had greater percent FOLR egress from the >12.0-cm zone than that of the lower N seedlings (19.0% vs. 8.6% MN and 3.2% LN). Percent FOLR egress responded inconsistently to cavity volume between excavation dates except that cavity volume affected FOLR egress from the >12.0-cm zone on both dates (Table 8). Among the 15-month seedlings, percent FOLR egress from the 8.1–12.0-cm zone of the 125-mL seedlings were comparable to that of the 95-mL seedlings (61.4% and 55.9%) and greater than that of the 60- and 336-mL seedlings (41.7% and 34.5%). Percent FOLR egress from the 8.1–12.0 cm root plug of the 95-mL seedlings was greater than that of the 336-mL seedlings. Among the 22-month seedlings, percent FOLR egress from the top 4.0 cm of the root plug of the 95-mL seedlings (22.6%) was comparable to that of the 60- and 125-mL seedlings but greater than that of the 336-mL seedlings (8.2%).

Table 8. *p*-values for the effects of nursery treatments (cavity volume, N rate, and Cu root pruning) and their two- and three-way interactions on percent egress of the first-order lateral roots from each of four root plug zones in seedlings excavated 15 and 22 months after outplanting using analysis of variance.

Egress from Root Plug (cm)	Cavity Volume (C)	Nitrogen Rate (N)	Copper Root Pruning (Cu)	C × N	C × Cu	N × Cu	C × N × Cu
15-Month Seedlings (March 2009)							
0–4.0	0.5016	0.9933	<0.0001	0.5024	0.1036	0.8204	0.4237
4.1–8.0	0.0627	0.1681	0.0219	0.7383	0.8408	0.7116	0.5560
8.1–12.0	0.0005	0.3796	0.0002	0.8830	0.0056	0.3341	0.7559
>12.0	<0.0001	0.2303	<0.0001	0.4101	<0.0001	0.8293	0.9987
22–Month Seedlings (October 2009)							
0–4.0	0.0311	0.7943	<0.0001	0.8622	0.0077	0.1007	0.8669
4.1–8.0	0.8383	0.0230	<0.0001	0.7750	0.3028	0.1679	0.7650
8.1–12.0	0.1274	0.2828	<0.0001	0.7692	0.3711	0.6554	0.3101
>12.0	<0.0001	<0.0001	0.1314	0.0001	0.3872	0.1218	0.6118

The pattern of the FOLR egress from the root plug for both excavation dates, as affected by Cu root pruning treatment, was further analyzed by stratifying FOLRs based on their taproot origination zones (Figure 5). Copper root pruning significantly altered percent FOLR egress from various root plug zones (Figure 5). The 15-month Cu seedlings had a greater percent of FOLR that originated from the top 4.0 cm of the taproot and egressed from the top 4.0 cm of the root plug than the 15-month no-Cu seedlings (Figure 5a). In contrast, the 15-month no-Cu seedlings had a greater percent of FOLRs that originated from the top 4.0 cm of the taproot and egressed from the 8.1–12.0-cm and >12.0-cm root plug zones than the Cu seedlings (Figure 5a). The 15-month Cu seedlings also had more of the 4.1–8.0-cm-originating FOLRs egressed from the 4.1–8.0-cm zone of root plug than the no-Cu seedlings. Similar effects of Cu treatment on increasing egress from the 0–4.0 and 4.1–8.0 cm root plug and decreasing egress from the bottom two root plug depths were also observed among the 22-month seedlings (Figure 5b). It is noteworthy that a few FOLRs egressed from the root plug zone shallower than their taproot origination zone. For example, 1.1% and 0.2% of the FOLRs originating in the 4.1–8.0-cm taproot zone egressed from the 0–4.0-cm root plug zone in the 15-month Cu and no-Cu seedlings, respectively (Figure 5a). In the 22-month seedlings, these values were 2.4% and 1.5%, respectively. Some seedlings also had upward egress by FOLRs originating in the 8.1–12.0-cm zone (Figure 5a,b).

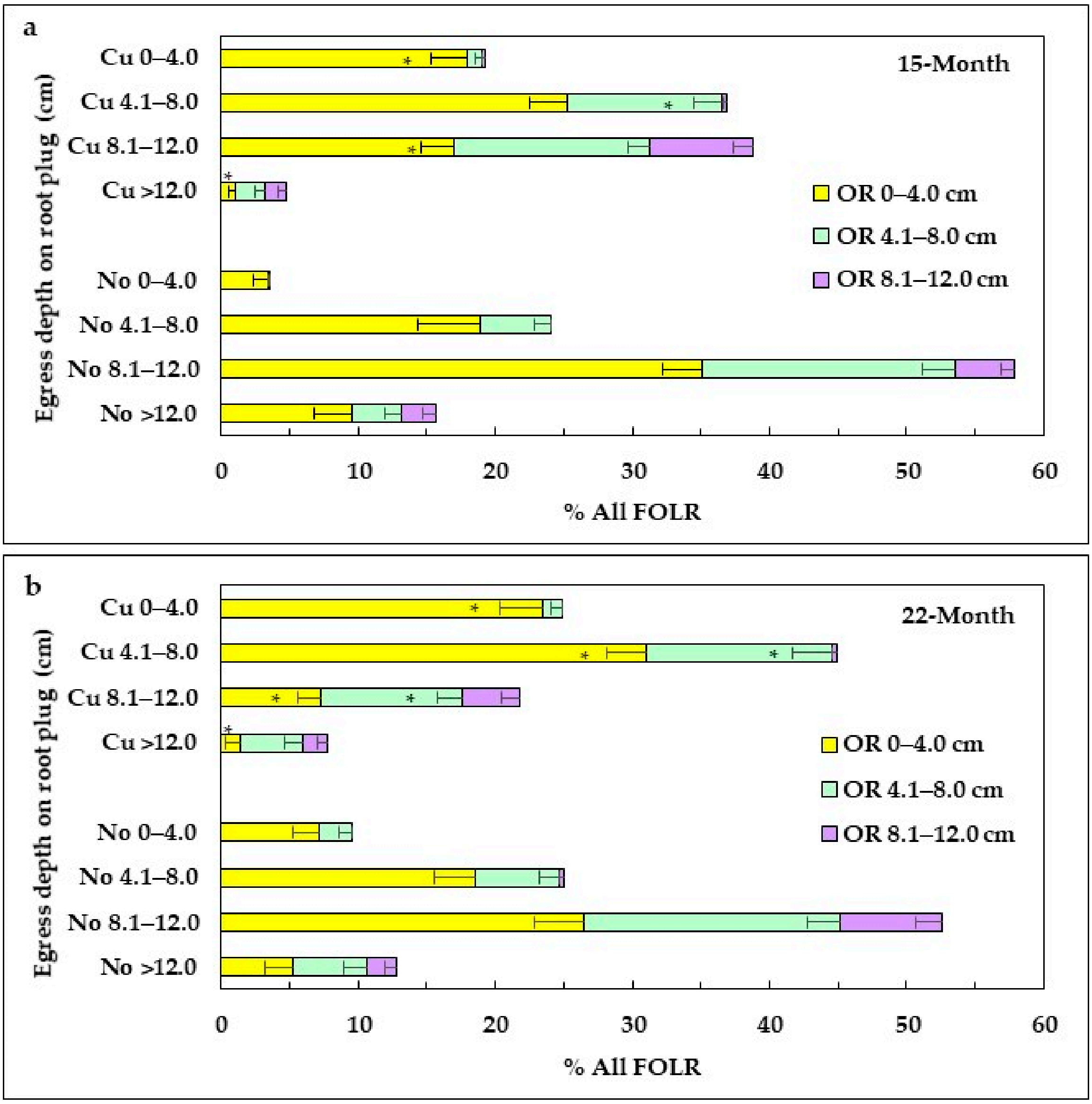

Figure 5. Percent first-order lateral roots (FOLRs) originating from each zone of the taproot (OR 0–4.0, 4.1–8.0, and 8.1–12.0 cm) and egressed from each of the four root plug zones (0–4.0, 4.1–8.0, 8.1–12.0, and >12.0 cm) in response to Cu root pruning among longleaf pine seedlings. (**a**) 15 months after outplanting; (**b**) 22 months after outplanting. Within a root plug zone and excavation, asterisks associated with Cu seedlings indicate that Cu root pruning significantly changed percent FOLR egress at the Bonferroni-adjusted 0.05 level. Error bars represent 1 SE.

4. Discussion

4.1. Seedling Quality Attributes

Outplanting high-quality seedlings, as defined by the target plant concept [45] and evaluated with standard methodologies [46], is essential to the success of forest plantation establishment. Assessments of seedling quality, however, should include the root system [47,48]. A few studies have reported positive container-grown longleaf pine seedling growth responses (e.g., RCD, fascicle number, nutrient concentrations, FOLR number, shoot and root system biomass, and cold hardiness) to treatments such as container cavity volume, N rate, and Cu root pruning at the end of nursery production [20,23,24,38,49]. Similar to the findings in the study by Dumroese et al. [24], the seedlings in our study responded positively to increasing cavity volume and N rate for various growth variables (Tables 1 and 2; Figure 4).

The literature is inconclusive with respect to the effect of Cu root pruning on seedling parameters, such as RCD and root biomass. In our study, Cu root pruning increased seedling RCD at outplanting (Table 1, Figure 4c), and this is in agreement with the findings of Dumroese et al. [24] but is contrary to the findings of Sayer et al. [23]. While the literature shows that Cu root pruning has increased longleaf pine seedling root system biomass and altered root system allocation pattern by increasing biomass allocation to the taproot and secondary lateral roots and decreasing biomass allocation to primary lateral roots at the end of nursery culture [20,23], it also shows that Cu has increased taproot biomass but not total root biomass [24]. Nevertheless, several studies have shown the inhibitory effect of Cu root pruning on the elongation of primary lateral roots in conifers [19–21,23,50,51]. We observed that Cu root pruning significantly reduced lengths of the longest FOLRs in the seedlings without affecting the number of FOLRs prior to outplanting (Table 1). During manufacturing, the ribs inside the Copperblock^{TM} containers are not coated with Cu and this is apparently to allow a few lateral roots that are not in contact with the Cu coating to extend vertically downward and improve root plug firmness. It is possible that the longest FOLR of some of the Cu seedlings in our study were a result of this process.

In loblolly pine seedlings, the number of FOLRs may be affected by different nursery protocols (such as seedbed densities) [40,52]. In our study and prior to outplanting, we used a diameter criterion of > 0.5 mm to qualify primary lateral roots. This may partially explain why seedlings cultured in 336-mL cavities had more FOLR than the 60- and 95-mL seedlings (Table 1). Regardless, loblolly pine seedlings with more FOLRs consistently grow to a larger size in nursery beds [40] and for at least seven years after outplanting [52]. This seedling quality attribute is highly heritable among half-sib families of loblolly pine [40]. Although the seedlings of the current study were from a mixed seed source, the FOLR number may still be a useful parameter in assessing longleaf pine seedling quality.

4.2. Field Performance Attributes

Across all treatments, 97% of outplanted seedlings survived to Year 5; of the seedlings that died by this time, 56% of them had RCD < 4.75 mm at outplanting. In addition, about 61% of seedlings that died between Years 6 and 8 had RCD < 4.75 mm at outplanting. Together, these results give credence to the interim guidelines that suggest a minimum RCD of 4.75 mm for container longleaf pine seedlings [35,36]. However, 35% of all seedlings that survived eight years in the field were below this RCD threshold at outplanting, and this suggests that overall nursery culture (e.g., cavity volume and N rate), rather than a discrete RCD threshold, had a high impact on long-term seedling survival. For example, on the one hand, 97% of the 60-mL seedlings and 58% of the LN seedlings that died by Year 8 had RCD < 4.75 mm at outplanting. On the other hand, 82% and 50% of the surviving seedlings in Year 8 that were cultured in 60-mL cavities and fertilized at LN, respectively, had RCD < 4.75 mm at outplanting. Only 21% and 29% of the surviving seedlings in Year 8 that were cultured in cavities larger than 60-mL and fertilized at higher N rate than LN, respectively, had RCD < 4.75 mm at outplanting. Our data are in agreement with the guidelines suggested by Barnett et al. [35] and Dumroese et al. [36], that in addition to a discrete 4.75-mm RCD threshold, other variables (e.g., fascicle number and root systems) should be considered in determining seedling quality at outplanting.

One possible cause for the twofold mortality rate observed for the 60-mL and LN seedlings between Years 6 and 8 compared with the seedlings of all the other treatments may have been the prescribed fire applied in Year 6. In the year before the prescribed fire, the 60-mL and LN seedlings that died between Years 6 and 8 had a three- to fourfold lower DBH and were half the heights of their cohorts of the same nursery treatments that survived. Similarly, Haywood et al. [37] also showed that a prescribed fire applied 14 months after outplanting killed more longleaf pine seedlings cultured in 60-mL cavities than those in larger cavities [37]. Seedlings of lower quality, having less growth, may not develop sufficiently to survive a prescribed burn, a regular management practice in longleaf pine forest management.

Together with survival, traditional field performance indicators (i.e., seedling heights and stem diameters) in our study reflected the quality of planting stock and demonstrated that positive growth effects of nursery treatments, such as cavity volume and N rate, can persist for at least eight years after outplanting (Table 2, Figures 1 and 4). Our results extend the current understanding of how culturing longleaf seedlings at 2 or 4 mg N per seedling weekly affects growth beyond three years after outplanting as reported in [38]. In contrast to research that showed that Cu-root-pruned seedlings cultured in 108- or 164-mL container cavities yielded a growth benefit through five years [37], our seedlings showed no DBH or height response to Cu root pruning (Table 2, Figures 1 and 4). In the current study, Year 5 seedlings were about 50 cm taller than the Year 5 seedlings established in central Louisiana in 2004 [37]. Again, the overall early and long-term field performance of longleaf pine in the current study (Figures 1 and 4) indicates the benefits of growing seedlings in 336-mL cavities or fertilizing seedlings at MN and HN levels.

4.3. Grass Stage Duration

The residence time of seedlings in the grass stage serves as an essential indicator of planting stock quality for longleaf pine [38] and possibly other *Pinus* species with a grass stage [53,54]. Among the 95-mL seedlings fertilized with MN or HN in the current study, about 27% and 97% emerged by the end of the first and second years in the field, respectively (Figure 2b). In a study established in a grass-dominated area in central Louisiana, longleaf pine seedlings cultured in 108-mL cavities reached >90% of emergence from the grass stage between three and four years after outplanting [37]. Moreover, in our study, 69% of the HN seedlings cultured in 336-mL cavities emerged from the grass stage during the first field season (Figure 2a). Our data show that without any post-establishment silvicultural treatments (such as herbicide application or prescribed fire), some nursery treatments can enhance seedling emergence from the grass stage of development.

Seedlings that emerged from the grass stage in Year 1 were larger in stature compared with those that emerged in Years 2 or 3 through the eight years (Figure 3). According to a financial study on establishing longleaf pine forests, the bare land value of a longleaf pine stand decreases by 6% with each additional year of grass-stage residence [55]. Although comparatively greater costs are incurred by growing and planting longleaf pine cultured in 336-mL cavities and fertilized with 4 mg N seedling^{-1} weekly for 19 weeks, this nursery protocol may have its merit if these extra-large and nutrients-loaded seedlings are planted on harsh sites with heavy plant competition [56]. These results have applications for other pine species that have a grass stage [53,54].

4.4. Mineral Nutrition

Foliar N concentrations increased from about 0.8% in the LN seedlings to 1.4% and 1.8%, respectively, in the MN and HN seedlings used in our study [24], similar to the results of Jackson et al. [38] (0.6%, 0.9%, and 1.5%) with similar N rates. The HN and MN rates used in this study [24] and by Jackson et al. [38] produced seedlings with higher foliar N concentrations than seedlings given an extra fall fertilization of ammonium nitrate in a study by Rodríguez-Trejo et al. [57] (0.9%). Our October concentrations of foliar N (Table 4) were between those observed in greenhouse-grown (2.6%) and field-grown (0.75%) longleaf pine [58]. During the second field season, our seedling fascicles had concentrations of N, Mg, and Ca similar to, and P and K higher than, those of longleaf pine fascicles six years after outplanting in central Louisiana [32]. The foliar concentrations of major nutrients in our study met the thresholds for longleaf pine except that N was lower (0.89% vs. 0.95%) [59]. All the physiological parameters assessed in the current study, such as the short residence time of the grass stage, normal levels of mineral nutrients, and good height and stem diameter growth, support our hypothesis that the field performance of high-quality planting stock persists for several years.

4.5. Biomass Allocation

Our study provides detailed analyses of seasonal biomass development during the second field season. Seedling biomass responded positively to cavity volume and N rate but not Cu treatment (Table 3). Across all treatments, the seedling biomass increased twofold from 15 months (March) to 22 months (October) (Table 3). The pattern of seedling biomass allocation to seedling components changed between 15 and 22 months, with increasing allocation to fascicles and stems and decreasing allocation to roots (Table 3). These shifts in allocations were associated with seedlings emerging from the grass stage, which increased from 57% in March to 90% in October. A slightly different biomass allocation pattern among grass-stage seedlings was observed in another longleaf pine study in central Louisiana [20]. Specifically, one year after outplanting, grass-stage seedlings allocated 60%, 10%, and 30% of their seedling biomass to foliage, stems, and root systems, respectively [20]. Both studies show that the emerging seedlings increase their biomass to the stems and decrease their biomass allocation to root systems within the excavated area.

4.6. Root System Morphology

Conifer seedlings cultured in hard-walled cavities exhibited an unnatural root form with most of their primary lateral roots extending vertically along the circumference of the root plug [14,17]. Once outplanted, these seedlings have root egress from the bottom of the root plug [14,20,60]. In longleaf pine, roots that egressed from the plug bottom may be either a taproot, adventitious roots from the air-pruned taproot, or FOLRs [27,42,61], but these roots do not necessarily extend vertically into the soil profile [18,27,61]. In our study, we did not include the vertically extending FOLRs in potential vertical anchorage depth for two reasons. First, these non-geotropic FOLRs are likely to turn horizontally [43,61]. Second, for the Year 2-excavated seedlings, most of FOLRs did not extend into the soil profile as deep as the egressed taproot or the deepest sinker root. Lack of consistent vertical anchorage depth responses to nursery treatments over two excavation dates (Table 5) indicated that vertical anchorage depth maybe impacted by other factors (e.g., poor planting technique that jams the root plug bottom into the bottom of the planting hole or the presence of a hardpan) in addition to nursery treatments. Three years after outplanting, Cu root pruning reduces the number of leaning lodgepole pines [22]. We did not observe any leaning individuals up to eight years in our study. Thus, benefits of greater vertical anchorage depth and root system biomass are yet to be verified among young longleaf pines.

Seedlings cultured in 336-mL cavities or fertilized at MN or HN had greater plug root biomass than the rest of the seedlings, whereas, Cu root pruning decreased plug root biomass (Table 6). These opposite treatment effects between increasing cavity volume or N rate and Cu root pruning also existed in plug root biomass allocation (Table 6). Specifically, an increase in cavity volume or N rate increased percent plug root biomass allocation to FOLR and decreased percent allocation to the taproot. The opposite occurred by Cu root pruning. Some studies report that Cu treatment increased root biomass allocation to the taproots and secondary lateral roots of longleaf pine seedlings at the end of nursery production [20,23,24]. Our findings extend observations of nursery treatment effects on seedling root systems up to two years after outplanting.

We observed that after container-grown seedlings were outplanted, the effects of Cu treatment on FOLR within the root plug were still evident. Specifically, we observed that between 15 and 22 months after outplanting, the no-Cu and the Cu seedlings increased by 3.8 and 0.3 g within the root plug FOLR biomass, respectively. Furthermore, the physical constraints of hard-walled cavities are of a configuration and architectural nature. For example, total FOLR lengths and DI were reduced by Cu root pruning, whereas, increasing cavity volume or N rate yielded greater total FOLR lengths and higher DI (Table 7). As the FOLR of the outplanted seedlings elongated to intercept soil water and nutrients, the within root plug mean FOLR lengths basically remained constant between 15 and 22 months after outplanting (Table 7). However, DI of the within root plug FOLR increased during this time (Table 7). It should be noted that the Cu root pruning treatment reduced, but did not eliminate DI on both dates. One explanation for this is that the non-Cu-coated ribs inside the Cu-coated cavities did not inhibit FOLR elongation. Similarly, air lateral root pruning, via discontinuous cavity side vents, decreased but did not eliminate FOLR spiraling in longleaf seedlings at the end of nursery production and 8 and 14 months after outplanting [27]. Continued research is needed to gauge the long-term FOLR morphology of planted longleaf pine in response to nursery treatments. By understanding the long-term consequences of FOLR deformities that develop during seedling production, current cultural tools can be improved to produce seedlings of better quality.

Nearly 60% of the FOLR originated from the top 4 cm of the taproots regardless of nursery treatments. However, Cu root pruning consistently affected the egress pattern of FOLR from the root plug (Table 8). Compared with the no-Cu treatment, Cu lateral root pruning generally increased the percentage of FOLR egress from the upper 8 cm of the root plug and decreased FOLR egress from deeper than 8 cm of the root plug (Figure 5). These FOLR egress patterns were similar to those of longleaf pine seedlings excavated one or three years after outplanting [18,20]. The Cu root pruning treatment contributed to maintaining the natural root system of container-grown seedlings with more lateral roots egressing into the top 8–10 cm of the soil profile (Figure 5) [18,20]. Our observed FOLR egress patterns differed from new root egress patterns in 30-day root growth potential tests where vertical distributions of new roots in number [11,23] and biomass [20] were noted by depth for the Cu seedlings compared with the no-Cu seedlings. Roots quantified in the root growth potential test are newly emerged from the root plug and are higher-order roots branching from FOLRs. Dissimilar patterns of root egress between FOLRs and new roots of planted Cu seedlings demonstrate that root growth potential tests serve as an indicator of seedling quality [47,48] but are not a substitute for root system excavation and assessments of root system morphology.

In our study, the Cu root pruning treatment resulted in more FOLR egress into a shallower portion of the soil profile (Figure 5), but it did not increase seedling growth during the eight years of observation (Figures 1 and 4). Therefore, in our study, there was no benefit of Cu root pruning on lateral root interception of water or nutrients in a zone near the soil surface as suggested [20,60]. Instead, Cu root pruning may have benefited sapling physical stability. Young container lodgepole pine trees sometimes lean or topple by pivoting about a point below the ground due to primary lateral roots extending vertically from the tip of the root plug [14]. With Cu lateral root pruning, more lateral roots egressed horizontally into the upper portion of the soil profile, and the risk of toppling was decreased [14,19,22]. In addition to enhancing a more natural FOLR egress pattern, Cu root pruning in our study improved longleaf pine root system architecture by reducing within root plug FOLR biomass, lengths, and DI. There exists a tradeoff for cultural treatments that maximize seedling stature (Figures 1 and 4) but also increase lateral root system deformity. Further research is needed to evaluate the impact of this tradeoff, especially where the threat of high sustained wind exists.

5. Conclusions

Quality attributes of container-grown longleaf pine seedlings, as affected by nursery treatments, were linked to seedling field performance up to eight years after outplanting. Increasing cavity volume or N rate decreased residence time in the grass stage and enhanced the stature of outplanted longleaf pines. Although the copper oxychloride root pruning treatment did not affect seedling field growth, it promoted an egress of first-order lateral roots from the root plug similar to the origination pattern of these roots from the taproot. Moreover, Cu root pruning decreased mean and total lengths and deformity index of first-order lateral roots within the root plug of seedlings excavated in the second year, whereas, increasing cavity volume or N rate exhibited the opposite effects on these morphological parameters. There seemed to be a tradeoff between seedling stature and root system morphology after outplanting that may have important implications for tree stability.

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