EFFECTS OF CONTAINER CAVITY SIZE AND COPPER COATING ON FIELD PERFORMANCE OF CONTAINER-GROWN LONGLEAF PINE SEEDLINGS

Shi-Jean Susana Sung, James D. Haywood, Mary A. Sword-Sayer, Kristina F. Connor, and D. Andrew Scott[1]

Abstract—Longleaf pine (*Pinus palustris* Mill.) seedlings were grown for 27 weeks in 3 container cavity sizes [small (S), medium (M), and large (L)], and half the containers were coated with copper (Cu). In November 2004, we planted 144 seedlings from each of 6 container treatments in each of 4 replications in central LA. All plots were burned in February 2006. Cavity size or Cu had no effect on seedling survival after one growing season in field. Small seedlings had a lower survival rate than either M or L seedlings from May through November of the second growing season; and Cu did not affect seedling survival the second year. Seedlings of all treatments had 88 to 98 percent survival after 2 years. More than 40 percent of the Cu-L and L seedlings had heights exceeding 12 cm and were considered coming out of the grass stage, whereas fewer than 5 percent of the Cu-S and S seedlings were coming out of the grass stage. Season, but not container treatments, affected photosynthetic rates and chlorophyll contents.

INTRODUCTION

Extensive harvest of longleaf pine (*Pinus palustris* Mill.; LLP) for timber and naval store products between the late 1800s and early 1900s, conversion of lands that had supported LLP to agricultural uses or to plantations of other fast-growing pine species, and exclusion of fire from the landscape, all contributed to the disappearance of about 96 percent of the pre-European settlement LLP ecosystems in the South (Brockway and Outcalt 1998, Landers and others 1995, Outcalt 2000). For the last two decades, many public, industrial, and private land managers and owners have been actively restoring LLP ecosystems in the Southern United States (Barnett 2002, Boyer 1989, Lander and others 1995). In most of the artificial LLP regeneration efforts, the planting of container-grown LLP seedlings usually has had a higher survival rate than bare-root stock plantings (South and others 2005 and references cited therein). However, one noted drawback of using container-grown stock for planting is that the established plantings have some sapling toppling in strong wind (South and others 2001). Other seedling qualities of container-grown LLP may also contribute to planting failure. For example, the root-bound index, the ratio of seedling ground-line diameter to container cavity top diameter, has been used to predict field survival and performance of container-grown seedlings (South and others 2005). Root-bound indices of greater than 28 percent have been associated with poor survival of LLP two years after planting (South and others 2005).

Improvements in the morphological quality of container stock root systems have been attempted by adding ridges to the cavity or coating it with copper (Cu). Cavity ridges help reduce root spiraling by training primary lateral roots to grow vertically (Barnett and Brissette 1986). Slow release of low-concentration Cu stops seedling lateral roots from elongating when they reach the cavity wall (Ruehle 1985). In a root growth potential test, LLP seedlings grown in Cu containers produced more new roots than those grown in non-Cu containers or bareroot seedlings (South and others 2005). Lodgepole pine (*P. contorta* Dougl.) grown in Cu-coated containers had fewer leaning seedlings three years after planting than those from non-Cu containers (Krasowski 2003).

In 2004, a study comparing the effects of different container cavity sizes and Cu coating on LLP seedling growth, field performance, and tree stability was begun in central LA. This report concentrates on the field performance of planted LLP seedlings for two growing seasons. Specifically, we assessed seedling survival, height growth, photosynthetic rate, and chlorophyll (Chl) content.

MATERIALS AND METHODS

Seedling Culture

Longleaf pine seeds from a Florida seed orchard mix were sown in containers in April 2004. There were six container treatments: three cavity sizes and two (with and without Cu) cavity coating treatments. Cavity top diameter (cm)/depth (cm)/volume (ml) for the small (S), medium (M) and large (L) cavity sizes were 2.8/13.3/60, 3.5/14.9/93, and 4.2/15.2/170, respectively. Cavity numbers per m^2 for S, M, and L containers were 756, 530, and 364, respectively. Styroblock ® and Copperblock® containers (Beaver Plastics Ltd, Edmonton, Alberta, Canada) of these cavity dimensions were used for no Cu and Cu-coating treatments. Copper oxychloride is the coating's active ingredient. Approximately 700 LLP seedlings were grown for each of the 6 container treatments.

[1]Research Plant Physiologist, Supervisory Research Forester, and Research Plant Physiologist, USDA Forest Service, Southern Research Station, Pineville, LA, Research Plant Physiologist, USDA Forest Service, Southern Research Station, Auburn, AL, Research Soil Scientist, Forest Service, Southern Research Station, Pineville, LA, respectively.

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We adapted protocols for growing LLP that were established by Barnett and McGilvray (1997, 2000) for this study. Specifically, the growth medium was a 1:2 mixture of commercial peat moss and vermiculite. The growth medium contained Osmocote® 19-6-12 slow release fertilizer (The Scotts Miracle Grow Company, Marysville, OH) at a rate of 3.6 kg/m^3. Between mid-May and the end of September 2004, we applied a 0.05- to 0.06-percent solution of water soluble fertilizer (Peters Professional® 20-19-18, J. R. Peters Inc., Allentown, PA) weekly until root plugs were saturated. Between July and mid-October 2004, Benlate® fungicide (Dupont, Wilmington, DE) was applied twice monthly. We watered the seedlings as needed. Seedlings were grown for 27 weeks under ambient light in a greenhouse where air temperature was maintained at 20 to 25 °C. Six weeks before planting, fertilization was stopped and watering was reduced to encourage bud set. All needles were trimmed to 15 to 20 cm two weeks before lifting.

Field Experiment

Study site—Our study is located on the Palustris Experimental Forest within the Kisatchie National Forest in Rapides Parish of central LA (31°11'N, 92°41'W). The soil is a moderately well-drained, gently sloping Beauregard silt loam (fine silty, siliceous, thermic, Plinthaquic Paludults). Mima mounds of Malbis fine sandy loam (fine loamy, siliceous, thermic, Plinthic Paleudults) are scattered across the study area. The dominant vegetation before we established the study was composed of grasses and forbs. In May 2004 we prepared the site for planting by broadcast application of a tank mixture of glyphosate and triclopyr herbicides. Two weeks later, the area was rotary mowed.

Experimental design—We established the field study using a randomized complete block design with four replications. In summer 2004, 24 treatment plots of 0.0576 ha (24 by 24 m) each, were established in the 3.5-ha site. Treatment plots were blocked based on apparent soil drainage using depth-to-mottles and plot concavity or convexity as indicators of drainage. Seedlings grown in the six cavity treatments (S, Cu-S, M, Cu-M, L, and Cu-L) were randomly assigned to a plot in each block.

Plantation establishment—In early November 2004, 27 week old container-grown LLP seedlings were lifted and planted on the same day. We planted seedlings at 2 by 2 m spacing using a planting punch customized for each of the three cavity dimensions. Treatment plots are 12 rows of 12 trees. All plots were burned in February 2006 as part of the routine management of the site. By August 2006, all LLP seedlings grew new needles, and ground-cover vegetation returned to the site.

Field and Laboratory Measurements

In November 2005, we surveyed all seedlings for survival in each plot, as well as in May, July, September, and November 2006. Seedling height growth was measured on the interior 64 (8 rows of 8 seedlings) seedlings in December 2005 and 2006. Seedlings taller than 12 cm were considered as growing out of the grass stage (Haywood 2000).

In July 2006, three seedlings from each of the 24 plots were randomly selected and tagged for long-term measurements of photosynthetic rate and Chl content. We measured photosynthesis rate using a LiCor 6400 portable, open-system infrared gas analyzer (LiCor, Lincoln, NE). Photosynthetic active radiation was set between 1400 and 1600 µE/m^2/sec with a red-blue light source inside the measuring chamber (3 by 2 cm); and the CO_2 level for the reference chamber was set at 400 ppm. The middle sections of two three-needle fascicles were enclosed in the photosynthesis chamber during measurements. After measurements, we harvested those fascicles, stored them on ice, and transported them to the laboratory for needle surface area measurement and Chl analysis.

Needle sections of 6 to 8 cm in length, including the parts (3 cm length) that were measured for photosynthesis, were measured for surface area using the displacement method devised by Johnson (1984). Next, these needle sections were cut into segments of 0.2 to 0.3 cm. Needle segments were extracted for Chl a and Chl b using N, N'-dimethylformamide (DMF) (Moran and Porath 1980). No tissue grinding was involved with this protocol. Culture tubes containing DMF and needle segments were placed in the refrigerator until the segments lost all color. Absorbance of the DMF extract was read at 664 nm and 647 nm by a DU-70 spectrophotometer (Beckman Coulter Inc., Fullerton, CA). Contents of Chl a and Chl b were calculated using the simultaneous equations of Porra and others (1989).

Statistical Analysis

Percentages for survival and seedlings out of the grass stage were arcsine transformed [arcsin (percent square root)] before analysis (Steel and Torrie 1980). The transformed survival percentages were compared for the October 2005, May, July, September, and November 2006 measurement dates using a repeated measures randomized complete block design model (α=0.05) (SAS Institute 1991). In the analysis, Cu (yes and no) and container size (large, medium, and small) were the two treatment levels, and we tested for copper x container-size interactions. Survival was also compared by date using a randomized complete block design model, and if significant treatment effects were found, plot mean comparisons were made with Duncan Multiple Range Tests (α=0.05). Likewise, we compared seedling heights after the first (October 2005) and second (November 2006) growing seasons and the percentage of seedlings out of the grass stage (greater than 12 cm tall) after the second growing season. Photosynthesis rate and Chl a + b content of longleaf pine seedlings were compared for the July, October, November, and December 2006 measurements, in addition to comparisons of container size and Cu coating across all months.

RESULTS AND DISCUSSION

Seedling Survival

There were no cavity size and Cu coating interactions for seedling survival of either year. Cavity sizes and Cu coating had no effect on LLP seedling field survival after one growing season (table 1). The trend was repeated for Cu coating during the second growing season. Seedlings grown in S

Table 1—Effects of container cavity size and copper coating on the first and the second year field survival of longleaf pine seedlings. There was not size and copper coating interaction for either year. Seedlings were grown in containers for 27 weeks and then planted in central LA in November 2004

Treatment	Oct-05	May-06	Jul-06	Sep-06	Nov-06
			Seedling survival, percent		
Container size					
Large	98.3a[a]	97.1a	96.9a	96.9a	96.9a
Medium	98.5a	96.4a	96.4a	96.4a	96.4a
Small	97.1a	91.4b	90.6b	90.6b	90.5b
			Seedling survival, percent		
Copper coating					
Yes	98.9a	96.3a	96.0a	96.0a	95.9a
No	97.0a	93.6a	93.3a	93.3a	93.3a

[a]Least square means within the same column followed by the same letter were not significantly different at the 0.05 level.

containers had lower survival than those grown in M or L containers for all measurement months during the second growing season (table 1). Between November 2005 and May 2006, S and Cu-S treatments lost quite a few seedlings (fig. 1). This mortality might be associated with the prescribed fire conducted on the study in February 2006. After May 2006, there was hardly any mortality in any treatment (table 1, fig. 1). Overall survival for LLP seedlings of all treatments ranged between 88 and 98 percent 2 years after planting. This rate of survival was as good as in the study by Haywood (2000) and better than values reported by others (Boyer 1989, Ramsey and others 2003, Rodriguez-Trejo and others 2003, South and others 2005).

Seedling Growth

The Cu-L seedlings were shorter than the L seedlings one year after planting (fig. 2). This trend was reversed two years after planting. In either year, Cu coating did not affect height growth of cavity size M or S. Seedlings grown in S and Cu-S containers were the shortest in both growing seasons (fig. 2). One of the reasons for LLP regeneration failure has been that some LLP seedlings remain in the grass stage for several years (Haywood 2000, South and others 2005). Typically, such seedlings were shaded by herbaceous and woody vegetation, and therefore more prone to infection by brown-spot needle blight (*Mycosphaerella dearnessii* M.E. Barr.). In our study, the percentage of seedlings growing out of the grass stage after two growing seasons was positively associated with container cavity size (fig. 3). However, Cu coating did not affect the percentage of seedlings growing out of the grass stage (fig. 3). Weed control may shorten the time

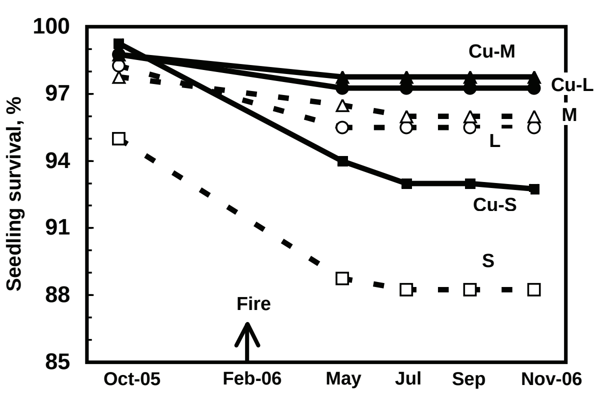

Figure 1—Effects of container cavity size and copper (Cu) coating on longleaf pine seedling field survival for two growing seasons after planting. Seedlings were grown in containers for 27 weeks in a greenhouse and planted in central LA in November 2004. Treatments were: Cu-L: large size cavity with Cu coating; L: large size cavity; Cu-M: medium size cavity with Cu coating; M: medium size cavity; Cu-S: small size cavity with Cu coating; and S: small size cavity. Arrow indicates when the study area was burned.

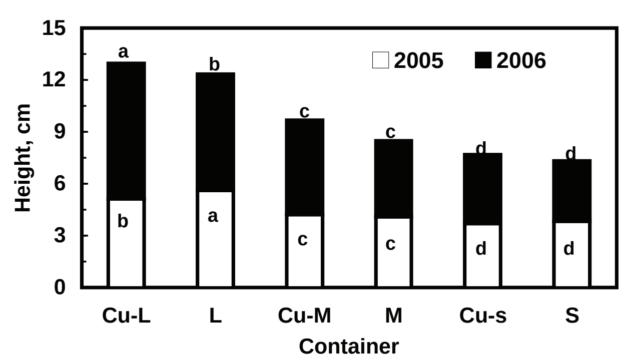

Figure 2—Effects of container cavity size and copper coating on longleaf pine seedling height growth in the field at the end of the first (2005) and second (2006) growing seasons. Seedlings were grown in containers for 27 weeks in a greenhouse and planted in central LA in November 2004. Treatments were: Cu-L: large size cavity with Cu coating; L: large size cavity; Cu-M: medium size cavity with Cu coating; M: medium size cavity; Cu-S: small size cavity with Cu coating; and S: small size cavity. Least square means with the same letter for each year were not significantly different at the 0.05 level.

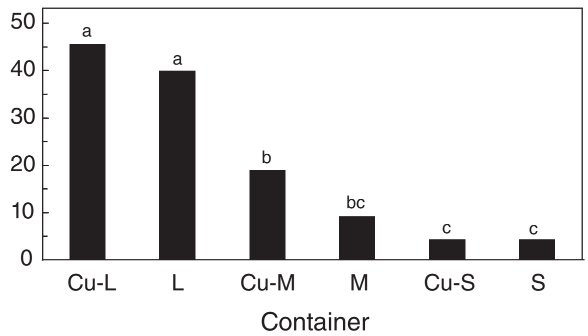

Figure 3—Effects of container cavity size and copper coating on percentage of longleaf pine seedlings growing out of the grass stage at the end of the second growing season. Seedlings were grown in containers for 27 weeks in a greenhouse and planted in central LA in November 2004. Treatments were: Cu-L: large size cavity with Cu coating; L: large size cavity; Cu-M: medium size cavity with Cu coating; M: medium size cavity; Cu-S: small size cavity with Cu coating; and S: small size cavity. Least square means with the same letter were not significantly different at the 0.05 level.

the LLP seedlings remaining in the grass stage (Haywood 2000, Ramsey and others 2003). Planting LLP grown in large containers should be considered an option for shortening the time seedlings remain in the grass stage.

Physiological Attributes

Photosynthetic rates we measured (table 2) were within ranges similar to rates measured in a greenhouse study of LLP by Jose and others (2003). The S seedlings in our study had a slightly lower photosynthetic rate than seedlings of the other five treatments (data not shown). Container treatment did not affect seedling Chl a+b contents (data not shown). Season, however, had significant but different effects on photosynthetic rate and Chl a+b content (table 2).

For the month of July, with excessively high temperatures and dry conditions, the photosynthesis rate was lower than in October (table 2). In a loblolly pine (*P. taeda* L.) study located near our study, photosynthetic rates for July and August were much lower than those measured in October and November (Tang and others 1999). Further, the needles sampled in July had lower Chl a+b than those measured in October and November (table 2). Because all plots were burned in February 2006, needles sampled in July, although similar in length to those sampled in the other months, might not have been physiologically mature. The lower Chl a+b content will also affect negatively the photosynthetic rate as shown here between July and October measurements.

Mean ambient temperatures during photosynthesis measurements in November and December were 14 °C and 19 °C, respectively. Such temperatures in central LA were lower and higher than mean day temperatures for November and December, respectively. Although the December needles had only 56 percent of the November Chl a+b level, their photosynthetic rate was 92 percent of that in November needles (table 2). Temperatures apparently are very critical during photosynthesis measurements in the winter season.

Table 2—Photosynthetic rate and chlorophyll a + b content (Chl) of longleaf pine seedlings in 2006. Seedlings were grown in containers for 27 weeks and then planted in central LA in November 2004

Month	Photosynthetic rate	Chlorophyll a+b content
	μmol/m²/sec¹	nmol/cm²
Jul	5.80b[a]	15.50c
Oct	7.17a	20.63b
Nov	5.06c	25.55a
Dec	4.66d	14.48d

[a]Least square means within the column followed by the same letter were not significantly different at the 0.05 level.

Decreased levels of Chl a+b in December needles were typical of most coniferous needles in winter (Kostner and others 1990). Nevertheless, with such active photosynthesis in December when day time temperatures are in the high teens, LLP in this area can continue its growth in these winter months when rainfall is ample and competing vegetation is dormant.

PRACTICAL APPLICATIONS

For artificial LLP regeneration using container stock, it is recommended that seedlings be grown in container cavity of at least 98 ml (Barnett and McGilvray 1997), a little larger than the M containers used in this study. The extra cost of growing seedlings in M containers rather than the S containers can be justified by the greater survival and accelerated height growth of M stock. Of course, it is up to the land managers whether they want to invest in LLP seedling stock grown in L containers. On sites where vegetation competition is severe, taller seedlings growing out of the grass stage will be less shaded by competition and become more tolerant of fire. Greater than 40 percent (versus 9 to 19 percent) of seedlings growing out of the grass stage for L and M seedlings, respectively, might justify the extra cost.

Although the advantage of Cu coating on early seedling field performance was not definitive in our study results, we believe that more evenly distributed root systems along the length and circumference of the root plug of longleaf pine seedlings grown in Cu cavities (Mary Anne Sword Sayer, personal observation) will enable them to perform better when there is a severe drought or when strong wind threatens to topple LLP saplings.

CONCLUSIONS

When care was taken for growing and planting LLP seedlings, even those grown in small cavity size containers had 88 percent survival two years after planting. Mean height growth and number of seedlings growing out of the grass stage increased with container cavity size. Container size and Cu coating did not affect photosynthesis rate or chlorophyll content. Our study showed that photosynthesis was still active in winter months. We will be monitoring this study continuously for some physiological attributes and, later, for morphological attributes such as sapling root system architecture and stability.

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