Genetic Improvement and Root Pruning Effects on Cherrybark Oak (*Quercus Pagoda* L.) Seedling Growth and Survival in Southern Arkansas

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Abstract

Cherrybark oak is a highly desirable hardwood species across the Southeastern United States. Silvicultural techniques for establishment have been carefully studied, but advances in tree improvement have yet to be realized. Cherrybark oak seedlings of genetically improved and unimproved stock were tested in field plantings in southern Arkansas and in a controlled pot study for root pruning effects. After 2 years, initial growth advantages of improved stock were no longer present; however, improved stock averaged 19 percent higher survival compared with unimproved seedlings. The improved stock also had greater resprouting after top dieback, indicating more resiliency. In the root pruning study, seedlings with pruned roots were easier to plant, had better survival, and exhibited less transpiration and stomatal conductance. Also, larger roots of the improved stock were more apt to be uncovered by erosion, potentially killing the tree. Larger roots systems are considered more desirable, but caution must be taken when planting. The larger root systems of genetically improved cherrybark oak seedlings make proper planting more challenging. However, pruning may offer a remedy making the seedlings easier to plant and more drought hardy initially.

Introduction

Bottomland hardwood forests are important contributors of ecological richness, mast for wildlife, and wood products in the Southern United States (Wharton et al. 1982). Among hardwoods, red oaks (*Quercus* subgroup *Erythrobalanus*) are ecologically and economically valuable. Despite the high desirability of red oaks, natural regeneration failures in stands historically dominated by these oaks has been well documented (Clatterbuck and Meadows 1992, Hodges and Janzen 1987, Lorimer 1989, Oliver et al. 2005). The lack of natural oak regeneration on many sites has resulted in some landowners planting oaks to ensure this taxa remains viable for future generations, provides wildlife habitat, conserves the natural environment, and produces high-value products (Michler et al. 2005). For example, oak afforestation by planting is an increasingly common, if sometimes risky, practice to restore mid-successional forests. In recent years, numerous silvicultural techniques have been developed to improve the survivorship and growth rates of planted red oaks, including the use of tree shelters, competition control, and a range of site preparation techniques (Burgess et al. 1990, Hansen and Tolsted 1981). In spite of these efforts, oak seedling production in the Southern United States is only a fraction of overall seedling production but has increased over the years from 17.8 million oak bareroot seedlings and 154,000 containerized seedlings in the 2008 and 2009 seasons (Enebak 2011) to more than 23 million seedlings overall in 2016 (Hernández et al. 2017). As oak seedling planting will likely continue at a high level into the foreseeable future, managers should adopt field planting practices using the best quality nursery stock available while balancing costs and risks with gain potential.

Although most of the research effort related to artificial regeneration of oaks has been on mechanical site practices or competition treatments (Collins and Battaglia 2008, Holladay et al. 2006, Leonardsson et al. 2015), more attention recently has been directed to the biological component of planting, including nursery practices (e.g., lifting depth and seedling sizes), which can improve the growth and survival performance of most hardwoods (Collins and Battaglia 2008, Farmer and Pezeshki 2004). Perhaps more importantly, using genetically improved hardwood seedlings has the potential to be as important as for stock quality as silvicultural practices such as irrigation, fertilization, weed control, and root culturing practices in plantation and nursery settings (Jacobs 2003). The potential for gains in survival and growth through hardwood tree improvement has yet to be greatly explored. Although desirable, these gains are elusive because of many challenges, including long generation and reproductive cycles, intermittent seed crops, difficulty in controlling pollination, overall higher production costs, and greater monetary risk in the case of planting failure (Dickmann et al. 1980, Lantz 2008). Limitations to using improved hardwood seedlings are gradually changing. Starting in 2012, the Arkansas Forestry Commission began offering improved (second generation) cherrybark oak (*Quercus pagoda* Raf.) seedlings to the public. Cherrybark oak is one of the most widely distributed and prized of the red oaks in the Eastern United States, desired for its fast-growing, high-quality wood and abundant hard mast for wildlife (Ezell and Hodges 1994, Putnam 1951). In addition, research has shown that cherrybark oak may be particularly amenable to tree improvement programs. Adams et al. (2007) found cherrybark oak had high family heritability for height (0.5 to 0.7) and diameter (0.55 to 0.7), which opens the door for improvement. These results are in line with previous studies on heritability for other oak species such as Nuttall oak (*Quercus texana* Buckley) and are higher than the 0.36 heritability estimated for white oak (*Quercus alba* L.) height growth (Gwaze et al. 2003, Rink 1984).

Although improved cherrybark oak seedlings may offer significantly better volume growth over unimproved seedlings, this improvement comes at premium—improved seedlings sell for $400 per 1,000 seedlings, or twice the cost of unimproved seedlings (Adams et al. 2015). More study is needed to determine if the added expense of improved cherrybark oak seedlings can be realized by increased returns. Some questions regarding nursery practices and genetic improvement (and their interactions) can be addressed even at an early stage. The objective of our research was to evaluate the growth and survival characteristics of a genetically improved variety of cherrybark oak compared with unimproved seedlings 2 years after planting at two field sites. Because the large root size in the improved stock was a hindrance during planting, three distinct root pruning treatments were examined for both improved and unimproved cherrybark oaks in a parallel study. These two studies were intended to provide one of the first field assessments of genetic improvement in cherrybark oak.

Methods

Field Planting Study

During the winter of 2011–2012, two sites were prepared for this study in South Arkansas. Sites were on the University of Arkansas at Monticello's Teaching and Research School Forest in Drew County (Monticello site) and at the University of Arkansas's Southeast Research and Extension Center in Hempstead County (Hope site). The Monticello plantings were installed on two formerly pine-dominated stands slightly east of the city of Monticello (N 33° 37' 12.31", W 91° 44' 0.38"). The previously pine-dominated stands had been salvaged and cleared following a tornado in 2010. The Hope location (N 33° 43' 9.76", W 93° 31' 49.92") was formerly an abandoned pasture that was cleared and brush-hogged prior to planting. The Monticello site was on Grenada and Henry silt loams (cherrybark oak SI_{50} = 24 - 26 m), and the Hope site was on a Una silty clay loam (SI_{50} = 27 m) (USDA NRCS 2017).

In March 2012, 1-year-old, bareroot, open-pollinated (half-sib) second-generation improved cherrybark oak seedlings and 1-year-old unimproved woods-run cherrybark oak seedlings grown at Arkansas Forestry Commission's Baucum Nursery (North Little Rock, AR) were planted by hand with a hardwood dibble on a 2.43 by 3.04 m spacing at both sites (figure 1a). The overall study design was a randomized complete block at two sites: Monticello and Hope. Each site had two blocks within which improved or unimproved seedlings were randomly assigned to plots. Following planting, a pre-emergent sulfometuron methyl herbicide (Oust XP, DuPont, Wilmington, DE) was applied over the top of seedlings at a rate of 146 ml ha^{-1}. Manual vegetation control was conducted during the first 2 years to reduce woody competition (mainly from "volunteer" loblolly pine [*Pinus taeda* L.]) (figure 1b).

Ground line diameter (GLD; measured to the nearest 0.1 cm) and seedling height (measured to the nearest cm) were recorded for a subset of seedlings that were systematically selected from each plot (i.e., every third tree), resulting in 342 seedlings across the entire study being measured. These seedlings were measured prior to planting, at the beginning of the first growing season (May 2012), at the end of the first growing season (October 2012), at the beginning of the second growing season (May 2013), and at the end of the second growing season (October 2013) (figure 1c). Seedling survival was measured in October 2012 and June 2013. Some seedlings flagged as dead in the October 2012 assessment were actually only top killed and resprouted the following spring—these seedlings were recorded as resprouts during the analysis (figure1d).

Figure 1. (a) Chemical site preparation was conducted using backpack sprayers followed by (b) seedlings planted in January. Each year, the trees were assessed as being (c) alive or (d) dead. (Photos by J. Adams, January–March 2012)

Root Pruning Study

While installing the field study, the large root width and length for the improved stock challenged the planters, even though seedlings had been undercut. Often, the root mass was larger than the standard hardwood dibbles used to plant these seedlings (figure 2a), although the unimproved stock generally had smaller rooted seedlings (figure 2b). Thus, three root pruning treatments at different intensities were evaluated in a separate study to evaluate potential tradeoffs between initial seedling size and ease of planting. For this study, 80 cherrybark oak seedlings (40 improved and 40 unimproved) were randomly selected from the Baucum Nursery in November 2013. A large volume of soil was extracted around each seedling to maintain an intact root system. All seedlings were measured for GLD, initial height, and number of first-order lateral roots (FOLR; a lateral root with > 1 mm diameter at the point of attachment on the taproot). Ten trees from each of the genetically improved and unimproved seedling stocks were randomly assigned to one of four categories: (1) no pruning (NoP); (2) pruning of the taproot to 21 cm long (P21); (3) pruning of the taproot to 21 cm and all FOLRs to 2 cm in length (P21-2); and (4) pruning of the taproot to 10 cm long (P-10).

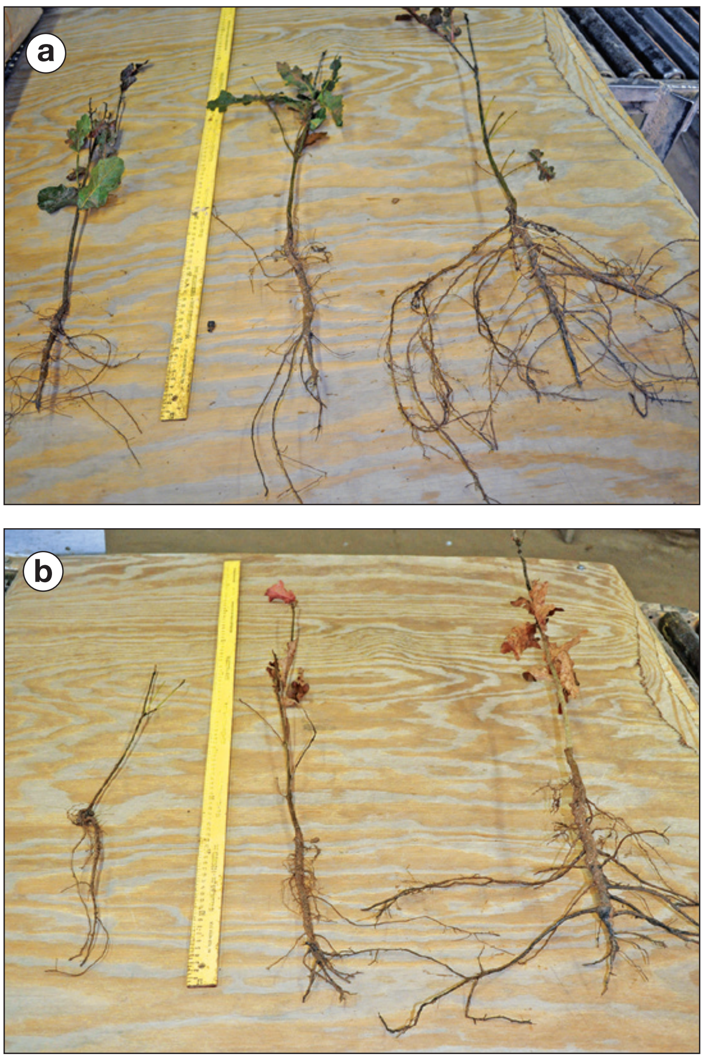

Figure 2. Small, medium, and large cherrybark oak seedlings of (a) improved stock and (b) unimproved stock from the Arkansas Forestry Commission nursery. (Photos by J. Adams, January 2011)

In November 2013, immediately after initial measurements and root pruning treatment, the seedlings were planted in 11.4-L plastic growth bags filled with Earthgro® topsoil (Hyponex Corporation, Marysville, OH) and randomly assigned to one of four blocks in a pasture on the University of Arkansas at Monticello campus and protected from deer browsing with an electric fence (figure 3a). Seedlings were watered and manual weed removal was conducted every 3 days. Every 2 days, trees were monitored for bud break and survival (figure 3b, 3c, and 3d). Height was recorded weekly, and GLD was measured at the conclusion of the study in May through June 2014, at which time all plants had either experienced bud break or were dead.

At the conclusion of the study, all surviving seedlings were assayed for photosynthetic activity, conductance, and transpiration using a LI-6400XT Portable Photosynthesis System with the 6400-40 Leaf Chamber Fluorometer (LI-COR; Lincoln, NE). Relative humidity in the leaf chamber was kept between 60 and 70 percent, carbon dioxide (CO_2) of the reference was set to ambient CO_2 concentration (400 µmol CO_2 mol^{-1}), flow rate was set to 500 µmol s^{-1}, and the internal photosynthetic active radiation was set to 700 µmol m^{-2} s^{-1}. This photosynthetic rate was selected to match the average ambient radiation across the season of measurement for southern Arkansas and was determined by empirical data previously collected in the area in previous years. The first mature leaf at the top of the dominant shoot was selected for the assay and inserted into the 2 cm^2 chamber so that the chamber was completely covered by the leaf. Each leaf was left in the chamber until readings stabilized, then a multiphase single flash was emitted, and photosynthetic related variables were recorded.

Figure 3. (a) Seedlings in the pruning study were placed in soil bags with treatments randomized spatially. Optimally, the seedlings (b) grew from an apically dominant stem; however, (c) many resprouted near the base with the seedling expressing top dieback. Much of the mortality or dieback was related to (d) erosion of soil near the seedling base exposing roots. (Photos by J. Adams, May 2013)

Data Analyses

Field plantings were analyzed for treatment effects on GLD, height, and survival at the end of the second growing season. Survival was also assessed in the third growing season. A mixed-model was used for analysis of variance (ANOVA) of GLD and height in which site and block within site were random factors, treatment was a fixed factor, and all interactions were random factors. Survival was analyzed using the same general linear mixed model form with a specified binomial distribution and a logit link function. Because so many trees were found to resprout at the beginning of the third year, Fisher's exact test of independence was conducted to determine if resprouting was associated with stock type. Also, the resprout data were linked with data previously reported by Adams et al. (2015) and Mustoe and Adams (2013) and analyzed using a general linear mixed model form with a specified binomial distribution and a logit link function. Means separations were conducted using an F-protected Fisher's Least Significant Difference at an alpha level of 0.05. These analyses were conducted using SAS software (SAS Institute 1999). Finally, Pearson correlation coefficients were calculated between height and GLD across measurement times.

For the root pruning study, ANOVA was conducted using a mixed model in which stock treatment, pruning treatment, and their interaction were fixed effects, and block was a random effect. When appropriate, differences among treatments were determined using F-protected Fisher's Least Significant Difference test at the alpha level of 0.05. Effects on survival were also analyzed using a mixed model of the same form but with a specified binomial distribution and a logit link function. After the primary analysis, an unanticipated issue seemed to affect survivorship patterns—extreme rain events had washed soil out of some of the pots during the study and exposed lateral roots immediately below the root collar, resulting in 35 of the 80 plants with some root exposure (figure 3d). To determine how this exposure affected mortality rates, a Chi-square test was conducted in which root exposure occurrence or nonoccurrence was partitioned with seedling survival or mortality. To further delineate the major factors affecting survival in this rooting study, a tree building method was used to determine which major factors (i.e., tree attributes) and their respective thresholds contributed to seedling survival. Tree building was conducted using R software and the "rpart" package with a method = "class" option (Breinman et al. 1984, R Core Team 2008).

Results

Field-Grown Cherrybark Oak Development

At the end of the second growing season, genetically improved cherrybark oak seedlings had greater survival than the unimproved seedlings ($p = 0.02$) and continued to have greater survival the following spring ($p < 0.01$; figure 4). Site did not have a significant effect on survival ($p = 0.77$) by the end of the study. Likewise, stock type did not have an effect on height ($p = 0.87$) or GLD ($p = 0.77$) at the end of the two growing seasons.

In the spring of 2013, an increase in survival was observed during the preceding year. This increase was because approximately 25 percent of the seedlings identified as dying during the second year apparently were only top killed and resprouted the following spring. Improved stock had significantly more ($p = 0.01$) resprouting, resulting in a 4.3-percent increase in surviving trees during the previous year compared with unimproved stock, which had only a 1.7-percent increase in surviving trees during the previous year.

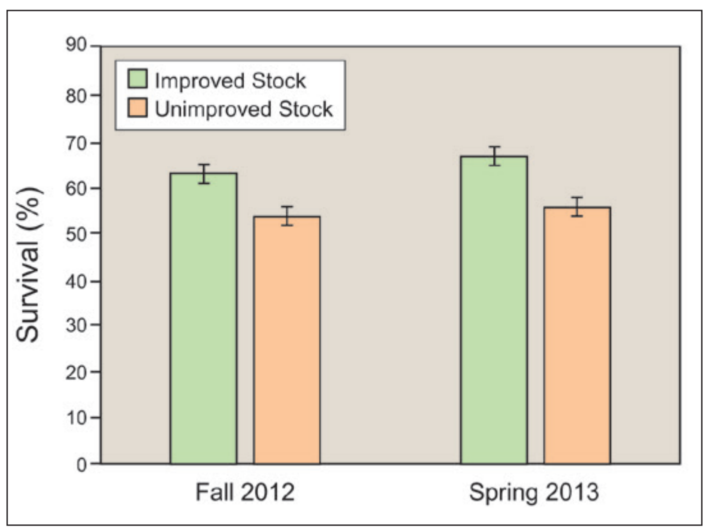

Figure 4. Survival of genetically improved and unimproved cherrybark oak seedlings at the end of year 2 and the beginning of year 3. Increases over time were due to seedlings resprouting that had been previously considered dead.

The correlation between height and GLD across all seedlings strengthened over time regardless of stock type. Pearson correlation coefficient (r) between the two traits at each measurement period were r = 0.50 at planting, increasing to r = 0.71 after the first growing season, and r = 0.91 at the end of the second growing season. Age-age correlations were weak for either planting height or planting GLD, with traits at year 1 or 2, with R-values ranging from 0.09 to 0.47. Growth at the end of year 1, however, correlated with growth at the end of year 2 much better with a height-to-height correlation of 0.67 and GLD-to-GLD correlation of 0.79.

Root Pruning Study

At the time of planting, unimproved seedlings were 21.5 percent taller than the improved stock, but the improved stock had 16.4 percent larger GLD (both $p \leq 0.01$) and 40 percent more FOLR than the unimproved stock ($p = 0.02$). In May 2014, shoot growth did not vary significantly by pruning treatment, stock type, or their interaction ($p = 0.99$). Similarly, no stock or pruning treatment differences occurred in leaf-level net photosynthesis (i.e., net CO2 assimilation rate; both $p > 0.34$). Transpiration and conductance, however, differed by pruning treatment ($p = 0.04$ and 0.02, respectively), with the unpruned seedlings having significantly higher levels of conductance and transpiration (figure 5a and b). Both stock type and pruning treatment significantly affected survival ($p = 0.04$ and $p < 0.01$, respectively) but not the interaction ($p = 0.79$). The improved seedlings had 25 percent higher mortality than the unimproved stock (figure 5c). The unpruned seedlings had the highest mortality, and those in the most intensive pruning treatment (reducing taproot length to 10cm) had the highest survival (figure 5c). Using this analysis, a decision tree was created (figure 6).

The additional analysis to determine effects of root exposure because rain washed soil out of the pots showed that seedlings with unexposed roots had 76.7 percent survival, whereas seedlings with root exposure had only 28.6 percent survival. Further analysis showed that unimproved cherrybark oak seedlings had 57.5 percent root exposure compared with only 30 percent of improved seedlings ($p = 0.01$). Among treatments, the no-prune treatment had 80 percent seedling root exposure, the two treatments pruned to 21 cm had approximately 50 percent of seedlings with root exposure, and the 10 cm pruning had no exposure.

Discussion

Cherrybark oak seedlings were established on suitable sites with good planting stock using appropriate techniques, but survival was poor after two growing seasons in the field planting study,

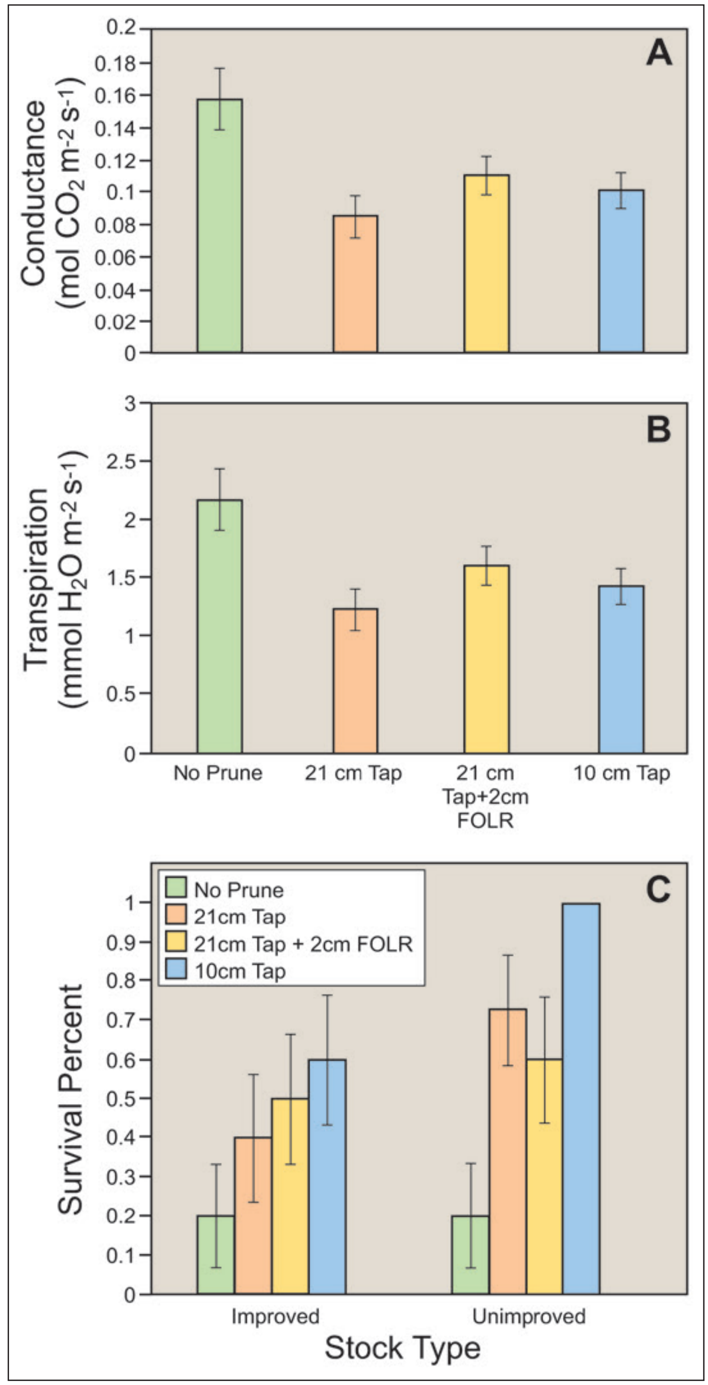

Figure 5. Average (a) conductance (mol CO_2 m^{-2} s^{-1}), (b) transpiration (mmol H_2O m^{-2} s^{-1}), and (c) survival of improved and unimproved cherrybark oak seedlings subjected to varying root pruning treatments.

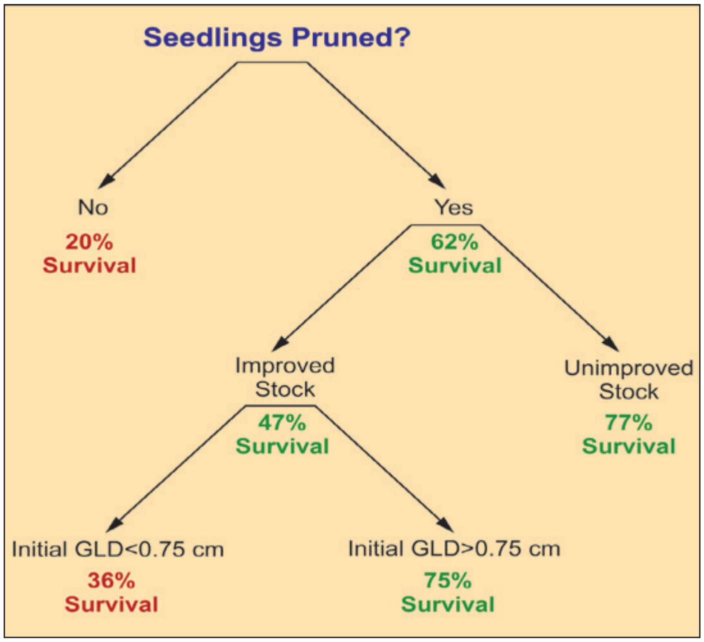

Figure 6. A decision tree for estimating cherrybark oak seedling survival developed from data in the root pruning study in which three levels of factors affected data.

with an average survival of 60 percent across the two sites. Low survival rates of planted hardwoods are not unusual even in research settings (Holladay et al. 2006, Jacobs et al. 2004), and operationally could be a deterrent to some landowners concerned about losing their investments in the reforestation effort. Many factors affect early survival of oak seedlings and can be hard to identify. In this study, we attribute the relatively high mortality rate to severe drought during the first growing season. The 2012 growing season was one of the driest years on record across much of Arkansas, a condition further exacerbated by near-record growing season high temperatures (Runkle et al. 2017). Under these challenging circumstances, it is important to note that the improved cherrybark oak stock still had significantly better survival than the unimproved stock and were more apt to resprout from dieback. This increase in survival may prove to be one of the biggest benefits of the improved seedlings by helping to ensure sufficient minimum stocking is achieved more cost effectively.

Another way to potentially overcome high seedling mortality has been to plant larger, better developed seedlings. Studies with northern red oak (*Quercus rubra* L.) have shown that seedlings with more FOLR have greater survival and growth (Kormanik et al. 1997). Seedlings with greater root development also tend to be initially taller, which helps under some circumstances. Grossnickle (2005) recommended taller seedlings for sites with high plant competition but low environmental stress. When taller seedlings are planted on sites where soil water and nutrients are more limiting than light, however, taller seedlings can actually exhibit lower survival than shorter seedlings (Boyer and South 1987). Although the present study had both mechanical and chemical competition control, drought conditions may have negated initial size advantages of the improved seedlings (Adams et al. 2015) for growth in the following years. The initial size differences between the two stock types may have affected the ability to resprout after dieback during the summer drought. Such size effects on successful sprouting have been documented for decades in coppice species such as *Salix* spp. and *Populus* spp. (Burgess et al. 1990, Hansen and Tolsted 1981).

Size effects on survival may be a manifestation of root:shoot variations that are often used to assess seedling quality. Across 14 oak species, hydric oak species had more shoot weight per unit root weight and greater height allocation in the first 1 to 2 years compared with xeric adapted species (Conner 1997). Furthermore, Gazal and Kubiske (2004) studied Shumard oak (*Quercus shumardii* Buckley) and cherrybark oak and found that larger ratios of root volume to shoot volume sustained higher evapotranspiration rates across both moist and drought conditions. Thus, hydric-associated species seem to have adapted to the low occurrence of water deficits these species could face. Artificial regeneration and management of seedlings potentially changes this dynamic. Undercutting or field pruning of bareroot seedlings, commonly done as a nursery practice, alters the root:shoot and improves the ease of planting, but this process may have other side effects. Barden and Bowersox (1989) found that pruning initial radicles prior to acorn planting combined with a later lateral root pruning to a depth of 25 cm increased the number of new roots on 1-0 red oak seedlings. Beckjord and Cech (1980) found that root pruning had no negative effect on northern red oak 1-0 seedlings as long as two-thirds of the taproot was left intact. These studies suggest that early pruning may lead to a later proliferation of roots, but that more developed seedlings may suffer greater impacts from root pruning during lifting.

Other studies have shown that root pruning can negatively impact seedlings and result in decreased height growth. For example, light root pruning of 25 percent of individual root length was found to have a negative effect on initial height growth in Nuttall oak (Farmer and Pezeshki 2004). Harrington and Howell (1998) determined that even lightly pruning taproots (i.e., pruning the portion of the taproot with a diameter < 1 mm) was enough to reduce height growth in loblolly pine.

Potentially, the decrease in height growth and subsequent decrease in net photosynthesis feeds back to root production, as the photosynthates are not present to support further root growth (Grossnickle 2005). Although a net reduction in photosynthesis has been shown to occur in Monterey pine (*Pinus radiata* D. Don) for at least the first 30 days following initial planting of root-pruned seedlings (Stupendick and Shepherd 1980), the current study did not detect differences among leaf-level photosynthesis rates across pruning treatments, although stomatal conductance and transpiration rates decreased after pruning. This phenomenon inversely mirrored the survival rates, which were better in the pruned seedlings and supports the supposition put forth that floodplain oaks with large root systems may have poor morphology to adapt to a dry growing season as they experience excess water loss (Gazal and Kubiske 2004). Thus, pruning may alter the physiology of the seedlings, causing seedlings with larger shoots to decrease their stomatal transpiration. Although we did not prune the roots for the field planting component, small root systems may also have an advantage as they are simply easier to plant and pose less risk for eventual root exposure and subsequent mortality.

Conclusions

Our examination of genetically improved cherrybark oak seedlings showed that having a larger seedling and root system may increase survival and resprout in the field, although mechanically limiting the size of the roots may aid in proper planting. Thus, seedlings with a larger GLD but with a trimmed root mass may be the optimum for a successful seedling. Still, further study is needed to assess the long-term effects of pruning the seedlings for field use over multiple summer droughts.

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