Comparing Cold-Stored and Freshly Lifted Water Oak (*Quercus nigra*) Seedlings Based on Physiological Parameters

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Abstract—Water oak is often used in afforestation projects in the Lower Mississippi Alluvial Valley, but its field performance is often poor due to low survival rates and severe top dieback immediately after planting. The poor physiological quality of planting stock may be a contributing factor to this transplanting problem. In this study, cold storage was investigated to increase dormancy status of seedlings. The physiological status of cold-stored and freshly lifted seedlings was assessed from mid-December to late February during one season using chlorophyll fluorescence, net photosynthesis, freeze-induced electrolyte leakage, and root growth potential. Storing seedlings at 2 °C (36 °F) did not appear to induce dormancy or improve stress resistance in water oak seedlings. Regardless of the storage regime, seedlings appeared to be most hardy and dormant until late January.

Keywords: chlorophyll fluorescence, electrolyte leakage, LT_{50}, net photosynthesis, top dieback

Introduction

Water oak (*Quercus nigra* L.) is a common species planted in federal conservation programs in the Lower Mississippi Alluvial Valley in the southern United States. Unfortunately, this species often exhibits poor field survival and performance, including severe top dieback (Johnson and Krinard 1985; Yeiser 1999; Michalek and others 2002; Lockhart and others 2003: Jacobs and others 2005). The top dieback observed in a recent field trial may be due to poor physiological conditions of the transplanted seedlings (Jacobs and others 2005). It is possible that they are neither sufficiently dormant nor stress resistant at the time of planting.

In several tree nurseries in the southeastern United States, bareroot water oak seedlings are lifted from December to March and temporarily placed in cold storage (2 °C [36 °F]) for 10 to 14 days prior to outplanting (Gillett 2005). During the interval between lifting and planting, bareroot stock may be damaged by a range of stress factors (McKay 1997).

The development of cold hardiness and the ability to withstand stress varies seasonally and in relation to the bud dormancy status (Burr and others 1989). Seedlings are more likely to be stress resistant when they are cold hardy and dormant (Ritchie 1986; Faulconer 1988). In an effort to induce dormancy, which prepares the seedlings for the shock of lifting, packing, and storage, seedlings may be cold-stored (O'Reilly and others 2000). Therefore, we hypothesized that lifting seedlings

near the end of the growing season and placing them in cold storage may induce dormancy earlier in the season and, correspondingly, increase stress resistance at the time of outplanting, resulting in higher survival rates and better field performance.

To assess the dormancy status and detect low vigor or damaged seedlings, we used physiological tests, as proposed by Prášil and Zámečník (1998) and O'Reilly and Keane (2002). Chlorophyll fluorescence (CF), freeze-induced electrolyte leakage (FIEL), and root growth potential (RGP) have been widely used as indicators for assessing the development of dormancy status and the ideal period for lifting and cold storage (O'Reilly and Keane 2002; Percival 2004).

The physiological dormancy cycle of water oak has never been studied. Therefore, findings of this research should be significant for both improving operational practices and assisting future research on water oak and other bottomland hardwoods species. The primary aims of the present study are to: (1) assess the suitability of cold storage to induce dormancy; and (2) assess the utility of CF, net photosynthetic rates (*A*), FIEL, and RGP to determine the physiological dormancy status of seedlings as they shift from active growth stage to dormant stage through the period when seedlings are commonly lifted and planted.

This paper will summarize and apply the findings of Goodman and others (forthcoming) to water oak seedling cultivation, afforestation, and general physiological knowledge.

Materials And Methods

Plant Materials and Storage Treatments

Seedlings used in this study were 1-year-old bareroot water oak seedlings from a Louisiana nursery. In mid-December, a subset of seedlings was lifted and placed into cold storage. At 2-week intervals from mid-December to late February, seedlings were taken from cold storage (2 °C [36 °F]) and from nursery beds to be assessed in the laboratory for CF, *A*, and FIEL; only one RGP trial was started in mid-February.

Chlorophyll Fluorescence and Net Photosynthesis

CF and *A* were measured using an open gas exchange system (LI-6400; LI-COR Inc., Lincoln, NE) with an integrated fluorescence chamber head (LI-6400–40 leaf chamber fluorometer; LI-COR Inc [fig. 1]). Seedlings were removed from shipping boxes and allowed to acclimate to ambient laboratory light conditions prior to being measured. Photosynthesis values were based on the average of three leaf readings per seedling. Leaves were selected from the middle section of the shoot, removed from the twig, wiped clean with a dry paper towel, and placed in the leaf chamber. With thin water oak leaves, it was often necessary to overlap two leaves to cover the entire leaf chamber area. CF and *A* were measured concurrently. The parameter Fv/Fm was used to assess CF, specifically, the maximum quantum yield of PSII for light-adapted plants.

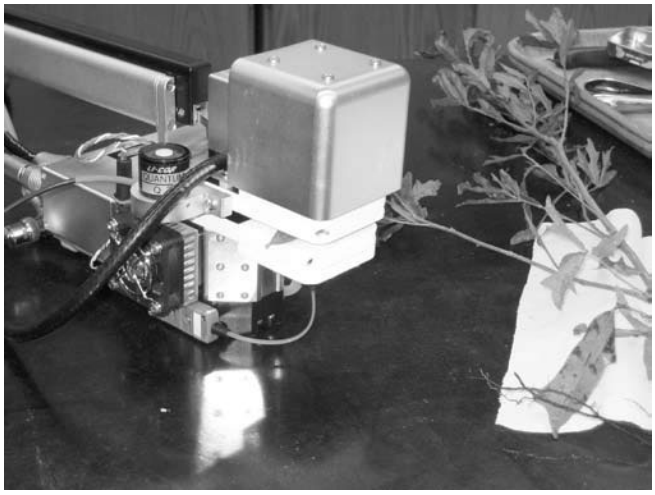

Figure 1—Detached leaf in leaf chamber for chlorophyll fluorescence and net photosynthesis measurements using an open gas exchange system (LI-6400; LI-COR Inc., Lincoln, NE) with an integrated fluorescence chamber head (LI-6400–40 leaf chamber fluorometer; LI-COR Inc.).

Freeze-Induced Electrolyte Leakage (FIEL)

FIEL was evaluated using the stem and leaf tissues on each sampling date. Stem tissue samples were 1 cm (0.4 in) in length, taken 1 cm (0.4 in) above the root collar. Leaf samples consisted of five uniform discs (midrib avoided); one disk was extracted from each of five different leaves, taken from the middle portion of the shoot (fig. 2). Samples were placed in plastic vials containing 1.5 ml (0.05 oz) water and 0.25 g (0.9 oz) sand (fig. 3). Topped vials were placed in a freezer overnight at +2 °C (36 °F). Control samples were removed before sub-freezing temperature treatments began

Figure 2—Foliar samples for freeze-induced electrolyte leakage (FIEL) trial. One uniform disk was taken (midrib avoided) from each of five different leaves, taken from the middle portion of the shoot.

Figure 3—For the freeze-induced electrolyte leakage (FIEL) trial, stem and foliar samples were placed in plastic vials containing 1.5 ml (0.05 oz) water and 0.25 g (0.9 oz) sand.

Figure 4—Cold stored seedling before root growth potential (RGP) trial. Notice the large stem, abundant foliage, and small root system (no fine roots).

(–3, –7, –11, –15, –20, –25, and –30 °C [27, 19, 12, 5, –4, –22 °F]). The freezing regime cooled at a rate of –1 °C/10 minutes (–1.8 °F/10 minutes), and target temperatures were held for 30 minutes before the corresponding sample was removed. After freezing, an additional 10 ml deionized water was added to each vial. FIEL was calculated as relative electrical conductivity (EC). The first EC measurement (EC_1) was taken after an overnight leaking period to assess the tissue damage caused by the temperature treatments. The samples were autoclaved to achieve total cell death, and the final conductivity readings were taken after a second overnight leaking period (EC_2; following Burr and others 2001). Blank vials were also measured and subtracted from EC_1 and EC_2 (Ritchie and Landis 2003). The temperature at which 50 percent of the plant tissues (LT_{50}) died was calculated from the EL values after log transformation using GraphPad PRISM™ software (GraphPad Software, Inc.).

Root Growth Potential and Seedling Growth

The RGP test was designed to test the viability and performance of the cold-stored and nursery bed seedlings as a surrogate to field outplanting. In mid-February, initial height, root collar diameter, and root volume were measured (fig. 4). Seedlings were planted in individual pots filled with a soilless growth medium. Plants were watered every 2 days. Greenhouse conditions were set to 16-hour photoperiod with day/night air temperatures of 26/20 °C (79/68 °F), RH of 60%/50%, and photosynthetic photon flux density (PPFD) of 200 μmol/m^2/s at seedling height. Because of the severe transplant shock and top dieback, the RGP test was run for 90 days. At the end of the test, seedlings were removed from the pots and the roots washed in tap water (figs. 5 and 6). Because of massive new root growth, RGP was expressed as dry mass of lateral roots. In mid-May (90 days after transplanting), seedlings were destructively harvested to obtain the following measurements: final height, root collar diameter, total root volume, and dry masses of foliage, stem, taproot, and lateral roots.

Findings And Application

Net Photosynthesis and Chlorophyll Fluorescence

The CF parameter, Fv/Fm, measurements were lower than the suggested 0.83 for healthy plants (Björkman and Demming 1987), suggesting that seedlings from both treatments were stressed, possibly from lifting, storage, and/or shipping. Nursery bed seedlings consistently had higher

Figure 5—Cold stored seedlings after 90-day root growth potential (RGP) trial. Notice the top dieback, resprouting at or near the root collar, and few new, fine roots.

Figure 6—Nursery bed seedlings after 90-day root growth potential (RGP) trial. Notice the top dieback, resprouting (near the root collar in two trees and higher on the stem in two others), and more abundant new and fine roots. The seedling with the largest root mass experienced almost no top dieback.

Fv/Fm values than cold-stored seedlings, suggesting that nursery bed seedlings were in a slightly better physiological condition. However, CF stayed fairly steady throughout the measurement period, which did not reveal much about the physiological dormancy cycle of this species and is not recommended for diagnostic dormancy status testing for this species.

A rates were higher in cold-stored than nursery bed seedlings, presumably from the higher chlorophyll content in the lush, green foliage of the cold-stored seedlings versus the browning foliage of the nursery bed seedlings. Rates were positive and increasing until late January. By the next measurement in mid-February, they had dropped sharply to negative net values and continued to decline, indicating that the leaves were transitioning towards leaf senescence.

Leaf and Stem Cold Hardiness

Leaf LT_{50} values revealed a more consistent response over the period evaluated than stem tissues and may be a more reliable indicator of the dormancy status of water oak seedlings. In the leaf samples, both cold-stored and nursery bed seedlings followed the same trend of LT_{50} cold hardiness values: they were low until late January and spiked by the next measurement in early February. Generally, the nursery bed seedlings were hardier to the cold than the cold-stored seedlings, also indicating that nursery bed seedlings were in a better physiological condition. Foliar FIEL analysis is very simple procedure and may be done non-destructively, making this cold hardiness assessment technique worth exploring further.

Root Growth Potential and Seedling Growth

In the RGP trial, survival was slightly better among nursery bed than cold-stored seedlings. All seedlings experienced top dieback, even with ample watering. Surviving seedlings resprouted and continued to grow. Net height growth, however, was negative during the trial. Cold-stored seedlings tended to resprout near the root collar, but nursery bed seedlings resprouted farther above the root collar on the stem and/or from branches, resulting in a less negative height increment among nursery bed seedlings (figs. 5 and 6). RGP was expressed as dry mass of the lateral roots, which was higher in nursery bed seedlings.

At the beginning of the trial, the existing roots (consisting of a taproot, a few severed first-order lateral roots, and no fibrous roots) of seedlings received in this study were undoubtedly inadequate to take up necessary quantities of water (fig. 4). RGP, a seedling's ability to grow new roots rapidly after outplanting, is very important to the survival of most species because the existing roots are usually inefficient at water uptake and not able to compensate for transpirational losses (O'Reilly and Keane 2002). Because water oaks retain their foliage until much later than other hardwoods, they may have additional stresses when lifted and outplanted due to the large, transpiring leaf surfaces in relation to the size of the root system, similar to evergreen conifers studied by O'Reilly and Keane (2002). Moisture stress was likely the cause for the shoot dieback and epicormic shoot formation seen in the seedlings in this study and others (Englert and others 1993; Hibbs and Yoder 1993).

Root growth relies on current photosynthate (van den Driessche 1987). Because water oaks retain their leaves and are photosynthetically active until early February (in the season tested), they may have the opportunity to produce new roots soon after outplanting, if timed correctly. Alternatively, most broadleaf trees rely on stored carbohydrates to develop new shoots and buds (Kozlowski and Pallardy 1997). Therefore, nursery seedlings should be cultured to have enough stored carbohydrates to resprout at least once during the establishment phase.

Other nursery culture techniques could attempt to increase lateral root growth and protect the existing root system during lifting, storage, and shipping. Shoot growth could be curbed or shoots pruned to improve shoot:root relations. Alternatively or in addition, leaf fall could be induced to reduce transpirational stress in cold storage and upon outplanting. Other techniques, such as withholding nitrogen, inducing moisture stress, and the timing of budset have been suggested to influence the dormancy cycle (Faulconer 1988).

Given that the RGP trial took 3 months to yield results, it is not applicable to nursery managers for immediate management decisions regarding water oak seedlings. Furthermore, results from an RGP trial should be interpreted cautiously because they are not always correlated with actual field establishment (Rietveld and Williams 1981).

Summary

Our greenhouse transplanting trial showed that cold-stored seedlings performed poorly compared to nursery bed seedlings. Although *A* was higher in cold-stored seedlings, CF was higher (more healthy) in nursery bed seedlings. In addition, LT_{50} values for both stem and leaf tissues were generally lower (more cold hardy) in nursery bed seedlings. Storing seedlings at 2 °C (36 °F) did not seem to deepen dormancy or improve stress resistance compared to seedlings in nursery beds. In fact, cold storage may have been a substantial stress to the seedlings and/or inhibited dormancy and stress resistance development.

In accordance with typical water oak field survival and growth, all seedlings in the study performed poorly. This emphasizes the point that survival and growth are a result of a multitude of morphological, physiological, and environmental conditions. The seedlings received in this study may not have been typical in their morphology or dormancy status prior to lifting and transplanting. In the present study, the seedlings appeared to be most hardy and dormant until late January, regardless of storage regime.

Because seedlings are recommended to be lifted and planted when stress resistant and planted when RGP is high and when environmental conditions are favorable, we recommend that seedlings are lifted and planted promptly in January. Future studies should examine the RGP of water oak seedlings throughout the winter, investigate methods to improve morphology, and explore nursery practices that increase stress resistance in order to discover optimal lifting and planting windows and to alleviate moisture stress. Attention to these themes should assist in improving water oak transplanting success and seedling growth in the outplanting site.

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