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Original article

Winter variation in physiological status of cold stored and freshly lifted semi-evergreen *Quercus nigra* seedlings

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Abstract

• Water oak (*Quercus nigra* L.) is a tardily deciduous species commonly planted in afforestation projects in the Lower Mississippi River Alluvial Valley, USA. Field performance is often marked by low survival rates and top dieback, which may be associated with poor physiological quality of planting stock.

• We investigated physiological status of cold stored (2–4 °C; CS) and freshly lifted (FL) seedlings during the period between lifting and planting (December – February). In mid-February, seedlings were transplanted into a controlled greenhouse environment for 90 d to evaluate post-transplant growth performance.

• Net photosynthetic rates were positive until late January (generally greater in CS seedlings) and became negative thereafter. FL seedlings generally had lower LT_{50} values from freeze-induced electrolyte leakage (FIEL), reflecting greater cold hardiness. FIEL of foliage provided the best indicator of physiological status, though terminal buds may serve as a suitable substitute. All seedlings experienced top dieback following transplant; CS seedlings had less relative root-collar diameter, height, and root volume increments.

• Cold storing seedlings did not appear to prolong dormancy, increase stress resistance, or hold promise as a means to improve outplanting success. Regardless of storage regime, seedlings appeared to be most cold hardy and perhaps stress resistant until late January.

Mots-clés :
fluorescence de la chlorophylle /
tolérance au froid /
perte d'électrolyte /
LT50 /
photosynthèse nette /
décidu tardif /
stress de transplantation /
Quercus nigra

Résumé – Variation hivernale de l'état physiologique de plants semi décidus de *Quercus nigra* stockés au froid et récemment arrachés.

• *Quercus nigra* L. est une espèce semi décidue, plantée dans les projets de reboisement dans la Basse vallée alluviale du fleuve Mississippi aux Etats-Unis. Les performances en plantation sont souvent marquées par un faible taux de survie et un dépérissement de la flèche du plant, ce qui peut être associé à leur mauvaise qualité physiologique.

• Nous avons étudié l'état physiologique de jeunes plants stockés au froid (2–4 °C ; CS) et récemment arrachés (FL), au cours de la période entre l'arrachage et la plantation (Décembre - Février). À la mi-février, les plants ont été transplantés pour 90 jours dans une serre climatiquement contrôlée, pour évaluer les performances concernant la croissance après transplantation.

• Les taux de photosynthèse nette ont été positifs jusqu'à la fin janvier (généralement plus élevés pour les jeunes plants CS) et sont devenus négatifs par la suite. Les plants FL ont eu généralement des valeurs LT50 inférieures de perte d'électrolyte induit par le gel (FIEL), reflétant une plus grande tolérance au froid. Le FIEL du feuillage fourni le meilleur indicateur de l'état physiologique, même si les bourgeons terminaux peuvent servir comme un substitut approprié. Tous les plants on présenté une perte des feuilles de la flèche à la suite de la transplantation ; les plants CS avaient un rapport relatif racine-diamètre du collet, une hauteur, et des accroissements du volume de racines, moindres.

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• Le stockage au froid des plants ne semble pas prolonger la dormance, ni accroître la résistance au stress de transplantation, ou tenir la promesse d'un moyen d'amélioration de la réussite du reboisement. Indépendamment du régime de stockage, les plants semblent être plus tolérants au froid et peut-être plus résistants au stress jusqu'à la fin janvier.

1. INTRODUCTION

The Lower Mississippi River Alluvial Valley in the southern USA was once covered by over 10 million ha of bottomland hardwood forests (Schoenholtz el al., 2001; Sharitz and Mitsch, 1993). By 1978, this number decreased to 2 million ha (Hefner and Brown, 1985), leaving the region economically marginal for agriculture and susceptible to flooding (Newling, 1990). With the aid of federal conservation programs, afforestation with bottomland hardwoods is increasing. Water oak (*Quercus nigra* L.) is a commonly planted species in these programs but frequently exhibits severe top dieback, resulting in poor seedling performance (Jacobs et al., 2005; Johnson and Krinard, 1985; Lockhart et al., 2003; Michalek et al., 2002). In general, poor seedling quality and/or improper planting dates may lead to reduced survival, shoot dieback, and poor stem form, especially in *Quercus* species (O'Reilly and Keane, 2002). Jacobs et al. (2005) postulated that the top dieback observed in their field trial may have been due to poor physiological conditions of transplanted seedlings. More specifically, it is possible that seedlings were not sufficiently dormant or stress resistant at time of planting.

Operationally, bareroot water oak seedlings are usually lifted in early winter, cold stored (Williams and Stroupe, 2002), and planted from January to mid-April (Michalek et al., 2002; Russell et al., 1998). Several tree nurseries in the southeastern USA (including the nursery used in this study) lift bareroot water oak seedlings from December to March and cold store (2–4 °C) them for 10–14 days prior to outplanting. During the interval between lifting and planting, bareroot stock may be damaged by a range of stress factors (McKay, 1997). Because water oak is a semi-evergreen species, seedlings may incur additional stresses when lifted and outplanted due to transpiring leaf surfaces. It is, therefore, important that water oak seedlings are sufficiently stress resistant when subjected to lifting, storage, and outplanting.

Plants are more likely to be stress resistant when cold hardy and dormant (Faulconer, 1988; O'Reilly et al., 2001; Ritchie, 1986; Sarvaš, 2004). Development of cold hardiness and the ability to withstand stress varies seasonally in relation to bud dormancy status (Burr et al., 1989). Naturally, dormancy is induced by decreasing photoperiods and air temperatures (Larcher, 1995). O'Reilly et al. (2000) hypothesized that lifting seedlings near the end of the growing season and placing them in cold storage might induce dormancy earlier in the season and, correspondingly, increase stress resistance at time of outplanting, resulting in higher survival rates and improved field performance.

Physiological tests can be used to assess dormancy status or detect low vigor seedlings (O'Reilly and Keane, 2002; Prášil and Zámečník, 1998) and the suitability of overwintering in the field or in storage (Puttonen,1997). Chlorophyll fluorescence (CF), net photosynthetic assimilation rates (A), freeze-induced electrolyte leakage (FIEL), and root growth potential (RGP) have been widely used as indicators for assessing dormancy status and ideal periods for lifting and cold storage (O'Reilly and Keane, 2002; Percival, 2004; Puttonen, 1997).

Poor field establishment is not unique to water oak plantings. Bareroot stock often experience high mortality rates following outplanting, with low plant vigor (poor physiological condition) at time of planting as a primary cause of reduced field performance (O'Reilly and Keane, 2002; Percival, 2004). To our knowledge, however, the use of physiological indicators to assess the condition of water oak seedlings has never been published. Therefore, findings of this research should be significant for both improving operational practices and assisting future research on water oak and may have additional applicability to other semi-evergreen oaks and temperate broadleaves.

The primary objectives of the present study were to (i) assess the suitability of cold storage to induce dormancy and improve stress resistance, (ii) examine the utility of A, CF, and FIEL in evaluating seedling dormancy status as they transition through the physiological dormancy cycle during the period when water oak seedlings are commonly lifted and planted, and (iii) evaluate the suitability of plant tissues other than leaves for use in FIEL tests.

2. MATERIALS AND METHODS

2.1. Plant material, sampling, and storage regimes

One-year-old (1+0 bareroot) water oak (*Quercus nigra* L.) seedlings were grown at the Louisiana Department of Agriculture and Forestry Nursery, Monroe, LA, USA (32° 31' – 37"N, 92° 00' – 52.6" W). Acorns were sown on 18 March 2004. Seedlings were top pruned twice. All seedlings had recovered from this treatment and had single, dominant leaders and set terminal buds by the time seedlings were harvested in mid-December. Fertilizer (13N-13P-13K) was applied at 279 kg ha^{-1} prior to planting: and approximately 47.6 kg ha^{-1} of N was applied in solution every 2 weeks throughout the growing season. Average monthly temperatures and total precipitation, as well as deviations from normal, recorded at a nearby weather station are shown in Table I.

Seedlings removed from cold storage (CS) and freshly lifted (FL) from nursery beds were sampled periodically throughout the winter of 2004–2005. On 13 December, immediately prior to the first sampling, a subset (n = 1500) of seedlings was lifted from nursery beds, packaged into bundles of 100 seedlings each in wax lined bags, sealed, and placed in cold storage at 2–4 °C (RH was not controlled) to be the CS seedlings. One day prior to each sampling date (15 December 2004, 12 and 26 January, and 9 and 23 February 2005), 100 seedlings were removed from cold storage and 100 were freshly

Table I. Average (deviation from normal) temperature (°C) and total (deviation from normal) precipitation (cm) from planting of the acorns (March 2004) to last lifting date (February 2005; data from National Oceanic and Atmospheric Administration weather station at Monroe Regional Airport, Monroe, Louisiana).

Year	Month	Average temperature (°C)	Precipitation (cm)
2004	March	16.2 (1.6)	8.92 (–5.13)
	April	18.1 (–0.7)	7.37 (–4.75)
	May	23.1 (–0.4)	24.82 (10.92)
	June	25.6 (–1.7)	28.17 (16.81)
	July	27.2 (–1.4)	19.58 (10.69)
	August	25.6 (–2.6)	9.70 (2.49)
	September	24.7 (–0.2)	0.56 (–8.00)
	October	22.4 (3.3)	18.47 (8.53)
	November	14.7 (1.2)	28.04 (16.74)
	December	8.4 (–0.7)	8.08 (–5.21)
2005	January	10.1 (2.3)	7.70 (–6.17)
	February	11.3 (0.8)	10.06 (–0.97)

lifted from a nursery bed and shipped to Purdue University in West Lafayette, IN, USA. CS and FL seedlings came from the same nursery bed and were lifted by the same nursery personnel using the same operational practice (tractor-mounted mechanical lifter). Roots of all seedlings were sprayed with Viterra Root-Dip® (Amereq, Inc., New York, NY, USA) immediately after lifting in FL seedlings and after removal from storage in CS seedlings to prevent root desiccation during overnight shipping. On each sampling occasion, 37 seedlings were randomly selected from each of the CS and FL seedling bundles for physiological measurements (*A*, CF, and FIEL). The RGP trial was conducted once, from 11 February to 12 May 2005 (beginning two days after the second to last sampling date), for which 15 seedlings were randomly selected from each batch of CS and FL seedlings.

2.2. Net photosynthesis and chlorophyll fluorescence

A and CF were measured using an open gas exchange system (LI-6400; LI-COR, Inc., Lincoln, NE, USA) with an integrated fluorescence chamber head (LI-6400–40 leaf chamber fluorometer). Seedlings were removed from shipping boxes and allowed to acclimate to ambient laboratory conditions (PAR = 10 μmol m^{-2} s^{-1}; temperature = 23.5–24.5 °C) for 1–2 h prior to measurements. Five seedlings were randomly selected from each storage regime. Three leaves from the middle section of the shoot were removed from the twig, wiped clean with a dry paper towel, and placed in the leaf chamber. In instances when one leaf did not cover the leaf chamber area completely, two leaves were pressed together at the margins. *A* and CF were measured concurrently. Measurements were made at photosynthetic photon flux density (PPFD) of 500 μmol m^{-2} s^{-1}, reference CO_2 of 400 ppm, and flow rate at 500 μmol s^{-1} (Apostol et al., 2007), and leaf of temperature of 24 °C. Following enclosure in the leaf cuvette, data were logged when a leaf reached a steady-state value, defined as photosynthesis, conductivity, and sample water with slopes less than 0.5, 0.01, and 1, respectively, for 20 s.

The parameter F'_v/F'_m was used to assess CF. Briefly, leaves were placed across the cuvette and minimum fluorescence of a light adapted leaf that has been momentarily darkened (F'_o) was measured using a weak measurement beam (<1 μmol photons m^{-2} s^{-1}). Maximum fluorescence (F'_m) was determined following a red light saturating pulse (>7000 μmol photons m^{-2} s^{-1}) and centered at wavelength of 630 nm. The F'_v/F'_m ratio ($[F'_m - F'_o]/F'_m$), estimates the efficiency of energy harvesting in PS II in light-adapted leaves (Islam et al., 2008). Recorded values were based on the average of three leaf measurements per seedling.

2.3. Freeze-induced electrolyte leakage

In the first experiment, FIEL was evaluated on CS and FL seedlings using leaf and stem tissues. Two samples (stem and leaf) were extracted from four randomly-selected seedlings for each of the eight test temperatures for a total of 24 CS and 24 FL seedlings. Leaf and stem tissues from a single seedling were assigned to the same test temperature. Leaf samples consisted of five uniform discs (7 mm diameter). One disc was extracted from each of five different leaves. Discs were taken from green (non-necrotic) leaves in the middle portion of the shoot, and the midrib was avoided. Stem tissue samples were 1 cm long and taken from the first to the second cm above the root-collar.

Samples were placed in plastic vials containing 15 ml deionized water and 0.25 g washed quartz sand (Tinus, 2002). The vials were sealed and placed in a programmable freezer set at 2 °C overnight. Control samples were removed before sub-freezing temperature treatments began. The freezing regime (–3, –7, –11, –15, –20, –25, and –30 °C) cooled at a rate of 0.1 °C min^{-1}, and target temperatures were held for 30 min before the corresponding samples were removed. After removal from the freezer, 10 ml deionized water was added to each vial. FIEL was calculated as relative electrical conductivity (EC). The first EC measurements (EC_1) were taken after an overnight leakage period to assess tissue damage caused by the temperature treatments. Samples were autoclaved (121 °C for 30 min) to achieve total cell death. Final conductivity readings were recorded after a second overnight leakage period (EC_2; following Burr et al., 1990). Blank vials were also measured and subtracted from EC_1 and EC_2 of each vial (Ritchie and Landis, 2003). Electrolyte leakage was determined using the equation described in Ingram and Buchanan (1984):

$$\text{Electrolyte leakage}(\%) = z + [(100 - -z)/(100 + e^{-k(temp-B)})]$$

where z was the baseline level of electrolyte leakage, B was the temperature corresponding to the midpoint of the response curve, k was the slope of the line at the midpoint, and *temp* was the treatment temperature. Cold hardiness data was expressed as an index, LT_{50}, the temperature at which 50% of total electrolyte leakage occurred (Jacobs et al., 2008). Non-linear regressions were performed using GraphPad PRISM™ software (GraphPad Software, Inc., San Diego, CA, USA) to fit a logistic sigmoidal function to the data for both CS and FL seedlings on each sampling date.

The tardily deciduous nature of water oak during its dormant phase prompted a second experiment to evaluate use of tissues other than leaves in the FIEL trials. On 25 January 2005, FIEL was evaluated on the following plant tissues of FL seedlings: leaves (5 discs), terminal bud, tap root (1-cm segment excised from tip of the root), and lateral roots (six 1-cm sections ≥2 mm diameter, removed from the central portion of the root mass). Four sample vials were randomly assigned to each of the six test temperatures: 2 (control), –5,

–10, –15, –25, and –35 °C. Samples were processed, and EC_1 and EC_2 were measured similarly to the procedure described in the first experiment. Electrolyte leakage was expressed as percentage of total electrolytes.

2.4. Root growth potential and transplanting performance

The RGP test was designed to determine the viability and performance of the CS and FL seedlings, as a surrogate to field outplanting. The RGP test was arranged as a completely randomized design with 15 seedlings from each storage regime. On 11 February 2005, initial height, root-collar diameter (RCD), and total root volume were measured. Seedlings were planted in individual 5.7 l (1.5 gal) Treepots™ (Stuewe and Sons, Corvallis, OR, USA) filled with Scotts Metro-Mix® 560 media (The Scotts Company, Marysville, OH, USA). Pots were watered to saturation every 2 d. Greenhouse conditions were set to 16 h photoperiod, day/night air temperature and RH at 26/20 °C and 60/50%, respectively, and PPFD of 400 μmol m^{-2} s^{-1} at seedling top height. Because severe top dieback occurred, the trial was maintained for 90 d to allow surviving seedlings to re-sprout.

On 12 May 2005 (90 d after planting), seedlings were harvested to obtain the following measurements: height, RCD, total root volume, and dry masses of taproot, lateral roots, stem, and leaves. All live tissues still attached to the plant were included in biomass measurements. Roots with white tips were counted as new white roots.

2.5. Data analysis

Statistical analysis of the *A*, CF, LT_{50}, and RGP data was performed separately by date using the T-test in SAS 9.1 software package (SAS Institute, Cary, NC, USA) to compare storage regimes. Assumptions regarding error terms, normality, independence, and equal variances, were verified. In the case of unequal variance (CF data on 15 December and 26 January and final dry masses), Satterthwaite approximation of denominator degrees of freedom was used to compute *P*-values. Correlation analyses between leaves and terminal buds, lateral root, and tap root were performed using the correlation procedure of SAS. All statistically significant differences were tested at $\alpha = 0.05$.

3. RESULTS

3.1. Net photosynthesis and chlorophyll fluorescence

Net photosynthetic assimilation rates were significantly higher in CS seedlings ($p = 0.046$, 0.001, 0.023, < 0.001 on 12 December, 26 January, 9 February, and 23 February, respectively), except on 12 January ($p > 0.05$). Mean *A* values for both treatments were positive and showed increasing trends until late January (Fig. 1A). By 9 February, rates had dropped sharply to negative values and showed decreasing trends thereafter. Values for the CF parameter, F'_v/F'_m, did not differ significantly between storage treatments. CF remained fairly steady throughout the measurement period (0.67–0.74, excluding the FL measurement on 26 January; Fig. 1B).

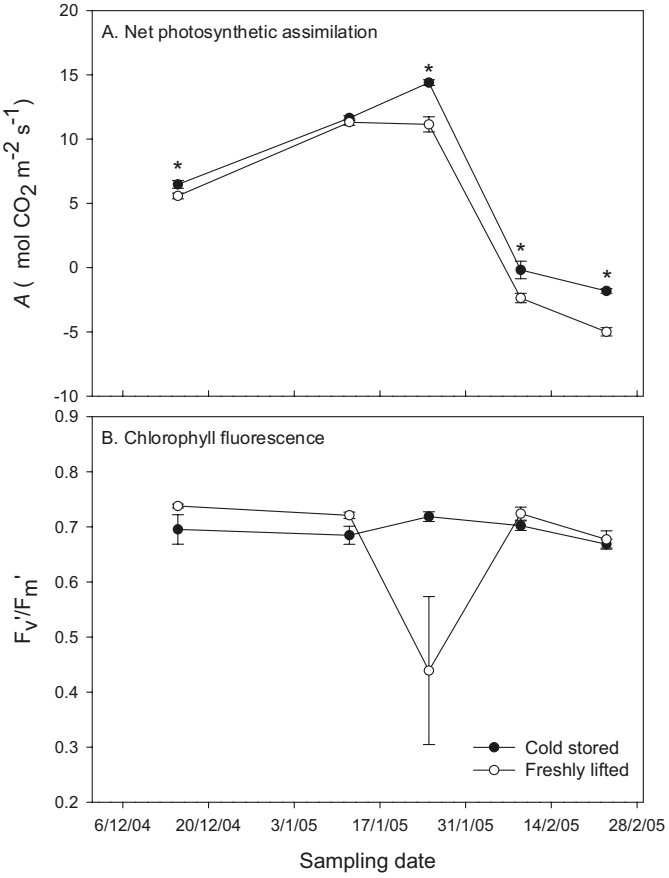

Figure 1. Net photosynthetic assimilation (A) and chlorophyll fluorescence (B) of cold stored and freshly lifted seedlings. Each data point represents mean ($n = 5$) ± SE. Asterisk (∗) at each sampling point indicates significant difference at $P < 0.05$.

3.2. Freeze-induced electrolyte leakage

Mean LT_{50} values from leaf tissues were significantly higher in CS seedlings from 12 January until 9 February ($p = 0.033$, 0.008, and 0.028 on 12 January, 26 January, and 9 February, respectively), indicating that FL seedlings were more cold hardy during that period (Fig. 2A). Mean leaf LT_{50} values in both treatments were steady during sampling dates 2 and 3 (–11 and –15 °C in CS and FL seedlings, respectively) and spiked on the following sampling date (to –8 and –11 °C in CS and FL seedlings, respectively; Fig. 2A). By the next sampling date, 23 February, there was little change in mean LT_{50} values in FL seedlings, but mean LT_{50} values in CS seedlings declined (to –19 °C) and were significantly lower than FL seedlings ($p = 0.001$). There was no difference on 15 December.

Stem LT_{50} values showed a comparable but less consistent response patterns than leaf tissues over the sampling dates. Values were significantly higher in CS seedlings on 12 January ($p = 0.041$) and 26 January ($p = 0.001$) and higher in FL seedlings on 9 February ($p = 0.002$). There were no

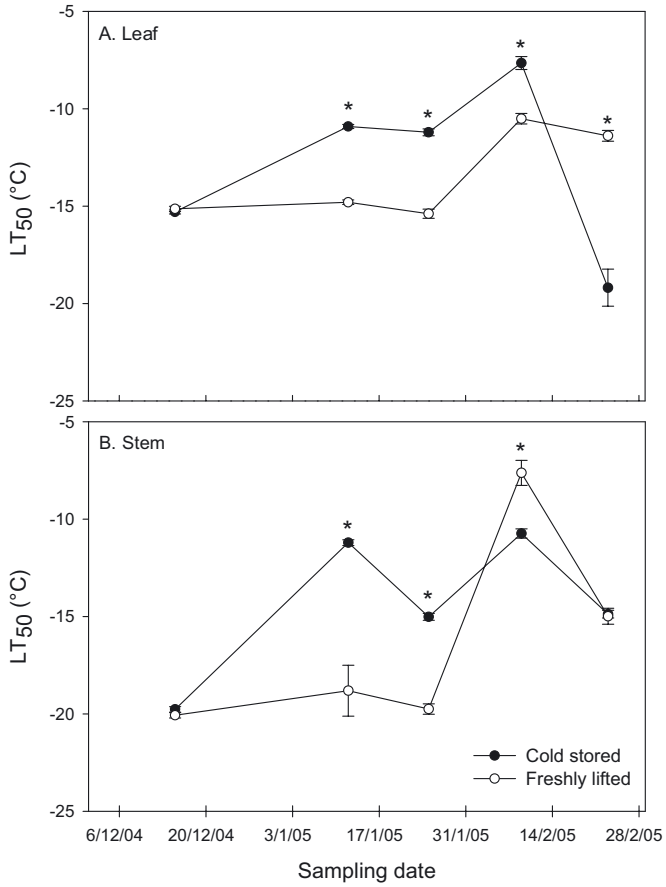

Figure 2. Cold hardiness development of cold stored and freshly lifted water oak seedlings throughout the measurement period. Cold hardiness is based upon the temperature at which 50% of total ion leakage (LT_{50}) occurs for the leaf (A) and stem (B). Each data point represents mean (n = 4) ± SE. Asterisk (∗) at each sampling point indicates significant difference at $P < 0.05$.

differences on the first (15 December) or last (23 February) dates. Stem LT_{50} for FL seedlings was lower (approximately –20 °C) and remained constant from December to January, while values were higher and more variable in CS seedlings (–11 to –20 °C; Fig. 2B). Simillarly to leaves, there was a sharp increase in LT_{50} values on 9 February in both CS and FL seedlings. However, LT_{50} values of CS seedlings were greater than FL seedlings this date only (–11 and –8 °C in CS and FL seedlings, respectively).

In the second study, tissues showed increasing sensitivity (e.g., greater relative FIEL) in the following order: terminal buds, leaves, tap root, and lateral roots. FIEL values increased at –10 °C in tap and lateral root tissues and only rose slightly in response to lower temperatures thereafter (Fig. 3). After –10 °C, FIEL values of terminal bud and leaf tissues rose steadily until maximum damage at the lowest freeze test temperature (–35 °C; Fig. 3). Of the plant tissues examined, there was a significant positive correlation between leaves and

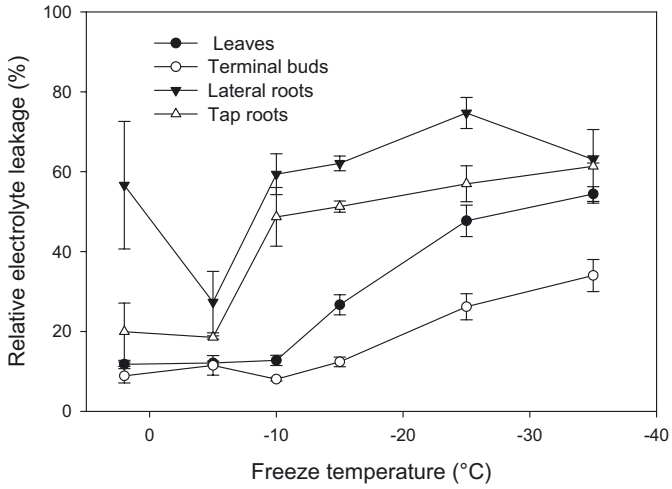

Figure 3. Relative freeze-induced electrolyte leakage of four different tissues of freshly lifted water oak seedlings exposed to a series of freezing temperatures. Seedlings were sampled on 25 January 2005. Each data point represents mean (n = 4)± SE.

terminal buds (r = 0.91554; $p \leq 0.001$), lateral roots (r = 0.45615; p = 0.025), and tap root (r = 0.75891; $p \leq 0.001$).

3.3. Root growth potential and transplanting performance

Initial morphological measurements from 11 February revealed that RCD was significantly greater in CS than in FL seedlings; neither height nor root volume were different at the beginning of the trial (Tab. II). There was no significant difference in survival between the treatments (87% and 93% for CS and FL seedlings, respectively). All seedlings experienced top dieback, resulting in negative height growth (Tab. II). In general, we observed that CS seedlings re-sprouted near the root-collar, but FL seedlings re-sprouted farther above the root-collar on the main stem or from existing branches. Percent change during the 90 d RGP trial revealed significant differences in seedling height, RCD, and root volume with values greater in FL seedlings compared to CS seedlings (Tab. II). No significant differences were detected in final mean dry masses of lateral roots, stem, leaf, or total plant tissues (Tab. II). By the end of the 90 d trial, very few seedlings had new white roots and differences were non-significant.

4. DISCUSSION

4.1. Net photosynthesis and chlorophyll fluorescence

The decline in A rates to net negative values in February indicated that leaves may have been transitioning toward senescence at that time (Herrick and Thomas, 2003; Jurik, 1986). Higher A in CS seedlings was likely due to greater chlorophyll

Table II. Growth of cold stored and freshly lifted water oak seedlings during the 90 d root growth potential trial. Seedlings were planted on 11 February 2005 (initial) and harvested on 12 May 2005 (final). Values represent means (n = 15)± SE.

Growth parameter	Cold stored	Freshly lifted	$P > \|t\|$
Root-collar diameter (mm)			
Initial	8.1 ± 0.6	6.5 ± 0.4	0.030
Final	8.6 ± 0.6	7.9 ± 0.6	0.416
% change	7.3 ± 2.8	22.4 ± 3.7	0.003
Height (cm)			
Initial	66.5 ± 2.5	64.4 ± 1.5	0.477
Final	18.8 ± 3.6	38.8 ± 4.5	0.002
% change	−71.3 ± 5.5	−39.4 ± 7.0	0.001
Root volume (cm^3)			
Initial	12 ± 2	10 ± 1	0.392
Final	17 ± 3	21 ± 3	0.443
% change	44 ± 16	113 ± 19	0.011
New white roots (count)	0.33 ± 0.33	0.20 ± 0.14	0.718
Final dry mass (g)			
Tap root	4.30 ± 0.76	4.29 ± 0.54	0.271
Lateral roots	0.73 ± 0.17	1.34 ± 0.31	0.023
Stem	12.00 ± 2.11	8.46 ± 1.08	0.026
Leaves	5.77 ± 1.12	4.33 ± 0.51	0.008
Total	21.28 ± 3.86	18.42 ± 2.19	0.042

content in leaves of CS seedlings. Cold storage may have impeded dormancy development or delayed chlorophyll breakdown (Itai and Tanahashi, 2008). Chlorophyll content was not measured, but presumed to be the case by the bright green visual appearance of CS seedling leaves compared to the dull, browning foliage of FL seedlings.

CF values over the sampling period did not demonstrate useful information regarding the physiological dormancy cycle of water oak, suggesting this may not be a suitable parameter to assess dormancy status during conditions described in the present study. All measured F'_v/F'_m values were lower than the values found in healthy, nonstressed oak (*Quercus*) species (0.80–0.83; Percival, 2005), indicating that seedlings from both treatments exhibited some degree of stress, possibly from lifting, storage, and/or shipping.

4.2. Freeze-induced electrolyte leakage

The FIEL test uses plant tissues to measure electrolytes that leaked from ruptured cells due to freeze damage (Ritchie and Landis, 2003). Higher FIEL values reflect damaged cell membranes (McKay, 1992) or increased levels of metabolic activity (Harper and O'Reilly, 2000). FIEL has been found to be a good predictor of number of days to budbreak (i.e., depth of dormancy) for northern red oak (*Q. rubra* L.) seedlings (Wilson and Jacobs, 2004). The spike in LT_{50} values of both leaf and stem tissues on 9 February indicates that both CS and FL were experiencing higher physiological activity and may have been emerging from dormancy on this date. The subsequent decline in LT_{50} values (on 23 February) was also reported by Jacobs et al. (2008) in freezer-stored (–2 °C) black walnut (*Juglans nigra* L.) seedlings at the end of the storage period; they suggested that additional freezer storage may have maintained or increased cold hardiness.

Stress resistance (e.g., frost or drought resistance) increases with cold hardiness, though maximum dormancy occurs just before maximum cold hardiness (Fuchigami and Nee, 1987). Based on leaf and stem LT_{50} values in the first study, seedlings should have been most dormant and stress resistant in mid-January and may perform best when outplanted near this time. However, seedlings were still photosynthetically active at this point, so we cannot be certain that this trend is accurate for water oak seedlings. Faulconer (1988) observed that bareroot seedlings lifted early in the season (i.e., during the early stages of cold hardiness development) lost cold hardiness during cold storage; whereas, seedlings lifted later in the season were able to maintain cold hardiness in storage. In our study, CS foliar and stem LT_{50} values on the first measurement date were as low as any values observed during the measurement period (with exception of leaf tissues on 23 February), indicating that seedlings may have been at maximum cold hardiness by the date of lifting and storage initiation. This suggests that cold storage itself may have been the cause of damage to CS seedlings, rather than improper lifting dates.

As FIEL of leaf tissue appeared to be the best indicator of physiological activity in the first study, sampling FIEL of terminal buds may be useful when leaves are absent on water oak and other temperate deciduous broadleaf species (Sarvaš, 2004).

4.3. Root growth potential and transplanting performance

RGP, defined as the ability of a seedling to grow new roots rapidly after outplanting, is essential to survival of most species because existing roots are usually inefficient at water uptake and unable to compensate for transpirational losses (O'Reilly and Keane, 2002). At the beginning of the trial, existing roots (generally consisting of a taproot, several severed first-order lateral roots, and no fibrous roots) of all seedlings in this study were presumably inadequate to extract sufficient water. Moisture stress may have caused shoot dieback and the epicormic shoot formation observed in seedlings in this study and others (Englert et al., 1993; Hibbs and Yoder, 1993). Because water oaks retain their foliage until much later than many other temperate deciduous forest trees species, they may incur additional stresses when lifted and outplanted due to large, transpiring leaf surfaces in relation to root system size, similar to evergreen conifers (O'Reilly and Keane, 2002). Irrigating to augment water supply (Yeiser, 1999) and container production to help maintain fine roots (Williams and Stroupe, 2002) have been found to increase survival and minimize top dieback by reducing moisture stress after outplanting.

Lindqvist and Asp (2002) found that plants lifted late had similar total carbohydrates in shoots but twice as much in roots by the end of 6 months in cold storage, and early-lifted common oak (*Q. robur* L.) seedlings experienced greater top dieback than did late-lifted seedlings, which is similar to CS seedlings in this study (lifted earlier and stored for 8 weeks). This suggests that FL seedlings may have had greater stored carbohydrate reserves that enabled better recovery after planting. However, both stored and current photosynthate have been found to contribute to root growth near the end of a leaf flush in deciduous northern red oak seedlings (Sloan and Jacobs, 2008) and to spring flushes in evergreen cork oak (*Q. suber* L.) seedlings (Cerasoli et al., 2004). This suggests that if soil and other conditions are favorable, photosynthetically active water oak seedlings may be able to produce new roots soon after outplanting with a combination of both stored carbohydrates and current photosynthate.

However, as dormancy status was not monitored prior to lifting in mid-December and the RGP trial was not conducted at every sampling date, it is not entirely evident whether the poorer performance of CS seedlings was a result of inappropriate lifting dates (mid-December), storage duration, and/or planting dates (mid-February for the RGP trial) or whether water oak seedling quality will consistently deteriorate in cold storage. For example, O'Reilly et al. (2002) found that among physiologically active sycamore (*Acer psuedoplatanus* L.) seedlings, those lifted and stored in February performed best after planting.

5. CONCLUSIONS

The physiological test, FIEL of foliar tissue, may be a suitable test to compare batches of seedlings and determine changes in water oak seedling dormancy status. In the absence of leaves, terminal buds may be an appropriate substitute for evaluating physiological dormancy status of water oak and possibly other semi-evergreen deciduous species. *A* may be useful to assess photosynthetic capacity at any point, as well as to compare physiological activity among batches of seedlings. The results of this study did not support the use of CF to compare the physiological quality among batches of seedlings or demonstrate any relationship between CF and dormancy status.

In accordance with water oak survival and growth as commonly observed in field plantings in the Lower Mississippi Alluvial Valley, seedlings performed poorly following transplanting, illustrating the tendency of bareroot water oak seedlings to incur transplanting stresses associated with poor seedling vigor. Results from our greenhouse transplanting trial suggest that storing seedlings at 2–4 °C is not recommended to improve outplanting success. Future studies should examine RGP of water oak seedlings throughout the winter, investigate methods to improve morphology, and explore nursery practices that increase stress resistance and root regeneration capabilities.

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