

# Using Electrolyte Leakage for Evaluating Hardwood Seedling Cold Hardiness

Barrett C. Wilson  
Douglass F. Jacobs

Barrett C. Wilson is a Graduate Research Assistant with the Hardwood Tree Improvement and Regeneration Center, Department of Forestry and Natural Resources, Purdue University, 195 Marsteller St, West Lafayette, IN 47907-2033; telephone: 765.496.6686; e-mail: barrett@fnr.purdue.edu. Douglass F. Jacobs is an Assistant Professor with the Hardwood Tree Improvement and Regeneration Center, Department of Forestry and Natural Resources, Purdue University, 195 Marsteller St, West Lafayette, IN 47907-2033; telephone: 765.494.3608; e-mail: djacobs@fnr.purdue.edu.

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**Abstract:** This paper describes the use of electrolyte leakage (EL) from stem tissue as a potential method for assessing cold hardiness of hardwood seedlings. The EL method has seen little use with North American hardwoods, but has successfully predicted conifer hardiness in both controlled and operational settings and has been used experimentally on hardwoods in Europe. Three species of hardwoods—northern red oak (*Quercus rubra* L.), black walnut (*Juglans nigra* L.), and black cherry (*Prunus serotina* Ehrh.)—were evaluated for hardiness at 4 temperatures (3, -10, -20, -40 °C [37, 14, -4, -40 °F]) after being subjected to 3 storage regimes (freezer, cooler, ambient) of varying duration. Higher EL values at lower temperatures and longer durations represent an increase in cell damage and loss of hardiness. For all species, an increase in EL over time (storage duration) corresponded to a decrease in the number of days required for budbreak under greenhouse conditions. EL levels did not appear to be related to height growth after 3 months. The EL trends were similar for all species and storage temperatures, while the 3 species exhibited differing responses to storage temperatures when placed in a greenhouse. The data presented here is preliminary. Further research is needed to gauge the effectiveness of EL as a predictor of seedling hardiness and quality for commonly produced hardwoods.

**Keywords:** seedling quality, electrolytes, budbreak, freezing, *Quercus rubra*, *Juglans nigra*, *Prunus serotina*

## Introduction

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Testing cold hardiness is an important component of hardwood seedling quality assessment programs. Cold hardiness provides a measure of dormancy status (Ritchie 1984), predicts the ability to tolerate stresses associated with lifting, storing, and planting (O'Reilly and others 1999), and provides an indication of field performance potential (Pardos and others 2003). Physiological methods of testing cold hardiness can be rapid (McKay 1992), allowing for timely decisions in nursery management systems. All phases of hardwood seedling production and establishment will benefit from further cold hardiness and quality assessment research.

Many methods have been employed for evaluating hardiness of plants. Visual methods, such as whole plant freeze tests and shoot tissue browning, have been effective (Timmis 1976; Liu and others 1998), but are more time consuming. Water relations (Ameglio and others 2001), bud mitotic activity (Calme and others 1994), abscisic acid concentration (Li and others 2003), soluble sugar concentration (Tinus and others 2000), and chlorophyll fluorescence (Rose and Haase 2002) are among the many physiological indicators that have been used on conifers. Measurement of these indicators, while more rapid, has seen limited application to hardwood forestry. The electrolyte leakage (EL) method was chosen for this study. It has been used extensively in conifer research and applications, where it was shown to be a useful and effective approach to determining hardiness (Colombo and others 1995; Bigras 1997).

Electrolytes are ions which are located within the cell membrane. Unstressed, undamaged plant cells will maintain these electrolytes within the membrane. As the cells are subjected to physical or environmental stresses, their membranes lose

integrity. Damaged membranes allow electrolytes from injured cells to flow into the xylem. An estimation of damage can be made by comparing the conductivity of the leaked contents from injured and uninjured tissues in solution (Mattsson 1996).

For conifers, needles are the most commonly sampled tissues for EL (Burr 1990). Sampling of leaves is particularly applicable to evergreens, which can be sampled and tested throughout the dormant period. Roots (McKay 1992) and stem tissues (Colombo and others 1995) have also been used. The efficacy of EL in predicting cold hardiness has resulted in its use in operational practice at some nurseries, particularly for determining lifting windows and storability (Tinus 1996). For hardwoods, there is relatively little information available. Most EL research in the past was performed using roots of European species (Edwards 1998; McKay and others 1999; O'Reilly and others 2001). Important hardwood species of eastern North America have not been well represented. Therefore, the objectives of this study were: 1) to evaluate EL from stem tissues as a method for estimating hardiness and growth potential in eastern hardwoods; and 2) to observe changes in dormancy status that occur in response to different methods and duration of post-lifting storage.

## Materials and Methods

One-year-old (1+0) bareroot seedlings of northern red oak (*Quercus rubra* L.), black walnut (*Juglans nigra* L.), and black cherry (*Prunus serotina* Ehrh.) of bulk seed origin were lifted from Vallonia Nursery, Vallonia, IN in November 2002 and divided among 2 storage treatments: cold storage (3 °C [37 °F]) and freezer storage (-2 °C [28 °F]). A third treatment consisted of seedlings remaining in the nursery to receive ambient environmental conditions during the storage period. Cold and freezer stored seedlings were bundled in kraft paper rolls with moistened peat moss, similar to those in Figure 1. Cold storage was in a thermostatically controlled cooler and freezer storage was in a Conviron PGR-15 (Controlled Environments Ltd, Winnipeg, MB) growth chamber. At monthly intervals from January until April, 21 trees of each species were removed from each of the storage regimes.

With the exception of ambient trees in January (frozen soil), 5 trees of each species/storage combination were potted into 4-gal (15-l) Treepots™ containers (Stuewe and Sons, Inc, Corvallis, OR) using Scotts Metro-Mix 366-P (Scotts Co, Marysville, OH) and placed in a greenhouse after each removal from storage. Greenhouse environmental conditions were maintained at 23.9 °C (75 °F) day and 17.8 °C (64 °F) night, with a photoperiod determined by natural daylength. Water containing a complete fertilizer solution was applied as needed. The number of days to first budbreak (DBB) was recorded for each seedling as an indicator of the dormancy level. Measurements of height were recorded at potting and at 30, 60, and 90 days after potting (DAP). Height was measured from media level to the base of the terminal bud (Figure 2). Readings from 30, 60, and 90 DAP were expressed as percent increase from initial height measurements. These observed values provided an estimation of performance potential when the seedlings were subjected to the given treatment conditions.



Figure 1—Seedling packaging system using kraft paper bundles.

For the EL procedure, a 1-cm (0.4-in) long section of stem, cut at both ends, was removed from the top third of 16 seedlings from each species/storage combination. The 16 stem samples were individually placed into 20 ml (0.7 oz) copolymer polypropylene vials (RPI Corp, Mt Prospect, IL) containing 15 ml (0.5 oz) of deionized water. Four samples



Figure 2—Measuring the height of black cherry.

were randomly assigned to 1 of 4 freeze test temperatures—3 (control), -10, -20, -40 °C (37, 14, -4, -40 °F). Sample vials were capped and the control treatment placed into a refrigerator where it was not exposed to freezing temperatures. The remaining treatments were placed into a Cryomed 1010 freezing unit (Thermo Forma, Marietta, OH) cooled by liquid nitrogen (Figure 3). Upon reaching each successive treatment, the respective vials were removed and placed in a refrigerator to thaw overnight. Sample vials of all treatments were then removed from refrigeration to complete thawing at room temperature. After thawing, stem EL values (total dissolved solids, ppm) were measured with a HI 9813 portable conductivity meter (Hanna Instruments, Inc, Woonsocket, RI). After recording initial values, vials were placed in a Getinge/Castle autoclave (Getinge USA, Inc, Rochester, NY) for steam sterilization at 110 °C (230 °F) for 20 minutes to attain a level of complete EL. Conductivity for each sample was calculated as Percent EL = Initial EL/Complete EL x 100. For the purpose of this paper, preliminary results from the -20 °C freeze test will be discussed.

## Results and Discussion

### Electrolyte Leakage

For samples frozen to -20 °C, there was a similar trend for all species when exposed to progressively longer storage durations. For northern red oak, EL from stem tissues was 20 to 25% after 2 months storage and rose steadily to the 40% range after 5 months. There were no clear differences between ambient, cooler, or freezer stored stock. EL from black cherry samples increased sharply from 20 to 30% after 2 months to around 75% at 5 months. Freezer stored black cherry seedlings exhibited the highest levels of leakage, particularly after 4 and 5 months of storage. Black walnut samples began at 15 to 30% and ended at 50 to 60%. Black walnut seedlings stored in the cooler had the lowest levels and those left in the nursery during winter and early spring had the highest levels.

The increase in EL with prolonged storage of seedlings seems to indicate that the chilling requirement was supplied



Figure 3—Cryomed freezing chamber.

in storage, resulting in a loss of dormancy and cold hardiness with continued exposure. Individual species showed different responses to the different storage regimes. The response of northern red oak did not differ among storage regimes. This might be attributed to a greater chilling requirement or higher level of dormancy, as evidenced by the lower EL levels compared to the other species. The high EL of freezer stored black cherry seedlings may be a result of a low chilling requirement and an increased sensitivity to freezing temperatures in a storage environment. High EL levels in black walnut seedlings receiving ambient conditions could indicate that cold and freezer storage regimes are effective in maintaining a lower degree of physiological activity for this species.

### Dormancy Status

Days to first budbreak was used as an indicator of seedling dormancy status. The number of DBB followed a trend that was opposite to that of EL. While EL increased with longer storage duration, DBB decreased over the same period. Northern red oak required more DBB than the other species. DBB decreased from around 45 days after 2 months storage to between 0 and 20 days after 5 months. Seedlings that were in the nursery had already leafed out after the 5-month period, while freezer-stored trees required about 20 days. Black cherry seedlings needed the fewest days for budbreak. After 2 months, DBB was 15 to 20 days. This decreased to 0 to 10 days after 5 months. Black walnut seedlings followed the same trend, with DBB decreasing from 25 to 30 days to 0 to 20 days. As was the case with the oaks, ambient cherry and walnut had broken bud before the end of the 5-month storage duration. Freezer stored seedlings also required the most DBB.

DBB decreased with longer storage durations, while stem EL levels increased. Fewer DBB and high EL levels both point to an increase in physiological activity and loss of hardiness. While a relationship between EL and DBB might be indicated by the given results, there were some unexpected outcomes. For example, freezer-stored seedlings of all species tended to have the highest EL values, but required the most DBB as well. Reasons for this are unclear. This may be associated with high levels of shoot desiccation in the freezer. Packaging materials did not completely enclose the seedlings, thereby exposing much of the stem tissue to freezing conditions. Consequently, there was a higher occurrence of terminal bud mortality compared to cold stored and ambient seedlings, particularly in black cherry and black walnut. This mortality may have altered seedling budbreak patterns.

### Seedling Height

Height measurements did not appear to be consistently associated with EL, but rather depended on storage conditions. Cooler stored northern red oak showed a height increase of 50 to 100% in the greenhouse following a 2- to 4-month storage period, while freezer and ambient seedlings had increases of 20 to 30%. For black cherry, cooler stored seedlings increased in height from 100 to 200%. Freezer and ambient seedlings decreased (225 to 175% and 150 to 75%,

respectively) during the period. Ambient and cooler stored black walnut seedlings increased in height (25 to 55%) while those stored in the freezer decreased (15 to 5%).

Declines in height growth for freezer stored black cherry and black walnut seedlings might be attributed to high levels of terminal bud mortality. Cooler stored seedlings of all species exhibited the highest increases in height as storage duration was extended. Otherwise, it is difficult to discern trends in height growth that may be related to EL and hardiness level.

## Future Directions

Future research into EL can be extended to include a number of different plant tissues, such as roots and buds. Other characteristics, such as lateral versus terminal buds or fine versus coarse roots, could also be useful. The method of packaging seedlings for storage should also be considered, particularly when dealing with subfreezing conditions. In addition, because the seedlings for this project were germinated from seeds collected over a relatively large geographic range in Indiana, it is likely that genetic differences are responsible for some variation. Therefore, it may be helpful to reduce this variation by using half-sib plant material. Rapid methods for measuring cold hardiness and dormancy status will benefit nursery managers by allowing prompt assessment of seedling storability and performance potential. This study will provide useful information for planning and implementing related projects in the future.

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