Response of black walnut seedlings to storage duration and temperature

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Abstract

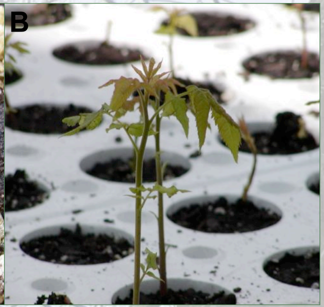
Cold hardiness testing can be useful for estimating the stress resistance and performance potential of forest tree seedlings. There is little information of quick and reliable testing procedures for eastern hardwood species. The objectives of this study were to 1) apply the electrolyte leakage (EL) procedure to cold hardiness testing (at 3, -10, -20, and -40 °C) of container-grown black walnut seedlings using tissue from terminal and basal portions of individual stems, 2) evaluate growth characteristics in an optimal growing environment, and 3) examine how hardiness and growth potential change in relationship to storage duration (0, 2, 4, or 6 months) and temperature (-2 or 3 °C). Preliminary data show that storage temperature has little effect on dormancy status, height, or root-collar diameter of seedlings after two months in a growth chamber. Root growth, as measured by the change in root volume, tended to be greater for cold-stored seedlings. Height and root growth increased with storage duration, while the number of days required for terminal budbreak decreased. Freezer-stored seedlings generally had higher EL values and were considered less stress tolerant. EL values at 3 and -10 °C remained low with increasing storage duration, while values at -40 °C stayed high across all dates. EL measured at -20 °C was the most indicative of physiological change occurring over time, staying below 40% for the first two months, before becoming greater than 60% after 4 and 6 months. These changes corresponded to changes in growth potential and dormancy. While further testing of relationships between EL and other physiological and morphological parameters is needed, data presented here give evidence that cold hardiness testing is useful for determining stress resistance and growth potential of black walnut seedlings.

Background

Cold hardiness assessment provides a measure of dormancy status (Ritchie 1984); predicts the ability of seedlings to withstand stresses associated with lifting, storing, and planting (O'Reilly et al. 1999); and provides an indication of field performance potential (Pardos et al. 2003). The electrolyte leakage (EL) method was chosen for this study. It has been used reliably with conifers (Colombo et al. 1995, Bigras 1997, Tinus and Burr 1997) and similar results are expected with hardwoods.

Electrolytes are contained within cell membranes which are sensitive to environmental stresses. Cold temperatures reduce enzymatic activity, alter metabolism, and decrease the photosynthetic capacity of plant tissues (Dubey 1997). These changes are often associated with increases in membrane permeability and loss of integrity (Campos et al. 2003). As the cells are subjected to stress, electrolytes leak into surrounding tissues. An estimation of cell damage can be made by comparing the conductivity of the leaked contents from injured and uninjured tissues (McNabb and Takahashi 2000).

A

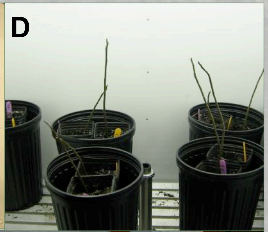

B


Methods and Findings

Walnut seeds (**A**) were sown in 500 ml styroblocks (**B**) in March 2003. The seedlings were grown in a greenhouse until October 2003, when they were placed in an lathe house to induce hardening. In December 2003, two groups of seedlings were removed and placed into either cooler (3 °C) or freezer storage (-2 °C). A sample of seedlings was immediately removed from each storage regime for initial assessment of morphology and cold hardiness. Subsequent assessments occurred after 2, 4, and 6 months of storage. Root volume, height (**C**), and root-collar diameter were measured before the seedlings were potted into 1-gallon containers and placed in a growth chamber (**D**) for two months. Final measurements were made at the end of the two-month period. Terminal budbreak was also monitored (**E**).

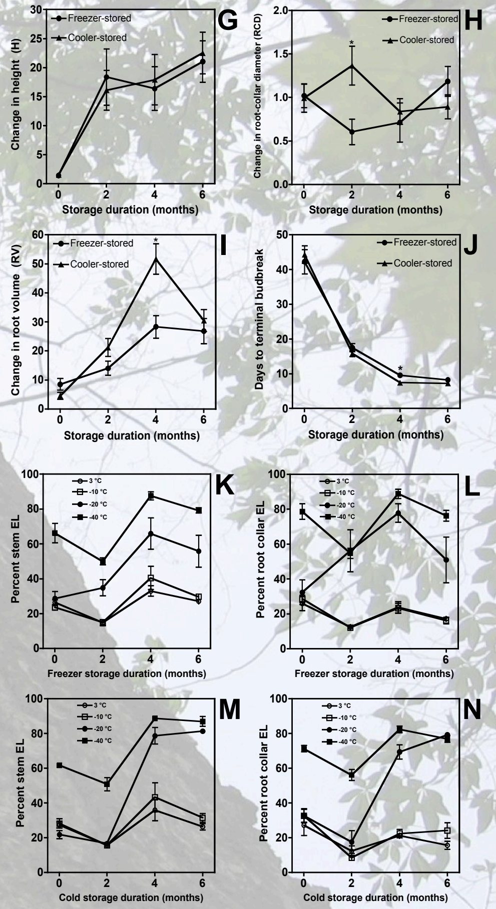
For EL, seedlings were randomly assigned to each of four temperatures: control (3), -10, -20, and -40 °C. Terminal and basal sections of stems were used for testing. Individual samples were placed into 20-ml vials with deionized water. The control treatment was placed into a refrigerator and remaining treatments were placed into a programmable freezing unit. The rate of decreasing temperature was -0.25 °C/minute and each test temperature was held for 30 minutes before decreasing again. After each hold time, treatment vials were removed and placed in refrigeration to thaw. After thawing, conductivity was measured in each vial (**F**). Afterwards, the vials were autoclaved at 110 ˚C for 20 minutes to kill all tissue for maximum conductivity. EL was expressed as a percentage of this maximum.

Budbreak, height, and root-collar diameter (**G, H, J**) were similar for both storage temperatures. The change in root volume was somewhat greater for cooler-stored seedlings (**I**). Height and root volume increased with storage duration, while the number of days required for terminal budbreak decreased. Freezer-stored seedlings generally had higher EL values and were less stress tolerant, particularly at shorter storage durations (**K-N**). EL measured at -20 °C was the most indicative of physiological change occurring over time, staying below 40% for the first two months, before rising over 60% after 4 and 6 months (**K-N**). These changes corresponded to changes in growth potential and dormancy.

D


E


F

Conclusion

Our findings provide evidence that cold hardiness testing is useful for estimating stress resistance and growth potential of black walnut seedlings. In addition to EL, future research may include other parameters useful for determining cold hardiness, such as chlorophyll fluorescence.

References

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