Seedling Quality Tests: Root ElectrolyteLeakage

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Introduction

Roots are among the most fragile parts of plants and, hence, are sensitive to many environmental and operational stresses. These include high and low temperatures (Lindström and Mattson 1989, Stattin and others 2000), desiccation (McKay and Milner 2000), rough handling (McKay and White 1997), improper storage (McKay and Mason 1991, McKay 1992, Harper and O'Reilly 2000) and even water logging and disease. It is sometimes possible to detect root damage using the time-honored thumbnail scraping and browning examination, but often damage is invisible or impossible to quantify. A more rigorous test and useful test is called root electrolyte leakage (REL). It measures the health and function of root cell membranes, so REL can be used as an indication of root injury and therefore seedling quality.

The REL technique can be traced back to the early work of Wilner (1955, 1960), but Helen McKay and her co-workers in the United Kingdom were among the first to use REL to evaluate bareroot nursery stock. REL has also been used in Canada (Folk 1999), and is currently one of a battery of seedling quality tests developed by the Ontario Ministry of Natural Resources (Colombo and others 2001). In the United States, however, electrolyte leakage has only been used to test the cold hardiness of foliage but, to our knowledge, REL is not being used.

REL has many desirable features: the procedure is relatively simple, uses readily available equipment, and produces results quickly. However, interpretation of these results can be problematic due to species, seedlot and seasonal interactions.

Theory

Water in roots is contained within two different systems – the symplast and the apoplast (see Ritchie and Landis 2003). The symplast includes all tissues that are enclosed within cell membranes (that is, the cell contents), while the apoplast includes everything else (that is, xylem elements, cell walls and voids). Apoplastic water is nearly pure, while symplast water contains a variety of ions. The semi-permeable membranes surrounding the symplast allow water to pass freely, but not the ions. As cell membranes become degraded through damage, disease, or age, they loose the ability to contain ions. So, if you were to measure the quantity of ions that leaked across damaged root membranes, this would provide an estimate of the relative viability of the root system (Palta *and others* 1977). If the damaged tissues are placed in distilled water, the amount of membrane leakage can be easily and quickly measured with a standard nursery device—an electrical conductivity (EC) meter. This is the basis of the REL test.

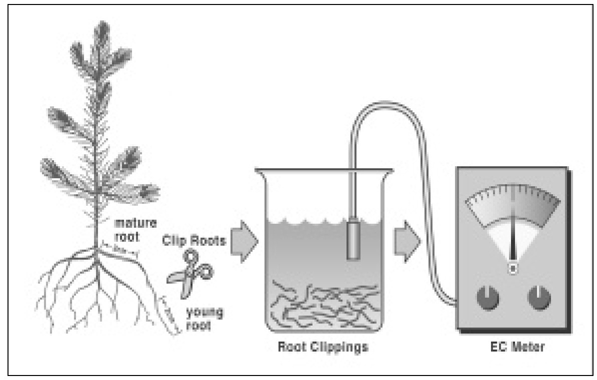

Figure 1. In a root electrolyte leakage (REL) test, root viability is rated by measuring the relative permeability of root cell membranes.

Measurement Procedure

The technique that is most often used (McKay 1992, 1998) has changed little from the initial protocol described by Wilner (1955). The steps are as follows (Figure 1):

1. Roots are first washed in water to remove soil, then in deionized water to remove any surface ions that may be present.
2. A central mass of roots is removed from the plant. With tree seedlings, this is often a band about 1 in. (2.5 cm) wide running across the mid-section of the root system.
3. Roots with diameter greater than 0.08 in. (2 mm) are removed from the sample leaving only "fine" roots.
4. Fine roots are placed into a 1.7 in^3 (28 ml) glass vessel containing 1 in^3 (16 ml) of deionized water.
5. The vessel is then capped, shaken, and left at room temperature for about 24 hours.
6. The conductivity of the solution ("C_{live}") is measured with a temperature-compensated electrical conductivity meter.
7. The root samples are removed and killed by autoclaving at 100 °C (212 °F) for 10 minutes.
8. The conductivity of the solution surrounding the dead root samples ("C_{dead}") is measured.
9. The REL is calculated as the ratio of the EC of the live roots divided by the EC of the dead roots: $REL=(C_{live}/C_{dead}) \times 100$.

The Biological Significance of REL

McKay (1998) offers the following explanation for why the REL test has application as a seedling quality test. After outplanting, the main cause of seedling mortality is transplant shock induced by water stress. A newly planted seedling must be able to extract water from the surrounding soil using its existing roots, and REL measures the viability of the root system. A low REL reading indicates high root viability, allowing water uptake to mitigate transplant shock.

Applications of REL in Nurseries

The REL test is most often used to assess effects of cold damage to roots, poor storage conditions, root exposure causing desiccation, or rough handling of tree seedlings. Nearly all the published work has been with commercial conifer seedlings, primarily Douglas-fir *(Pseudotsuga menziesii*), spruces, pines, and larch. Use of REL to detect freezing damage to roots is applied in one of two contexts: evaluation of cold hardiness test results, and detection of root injury following unseasonably cold weather or sun exposure.

Cold hardiness testing. Classic cold hardiness testing involves two steps: (1) exposing test seedlings to a predetermined sub-freezing temperature (or range of temperatures), and (2) after an incubation period, determining the amount of damage sustained by the frozen tissues (Ritchie 1991, Burr and others 2001). REL is a quick and quantitative way of measuring root damage in Step 2. For example, root samples from bareroot Norway spruce (*Picea abies*) seedlings were exposed to either -5 °C (23 °F) or -10 °C (14 °F) bi-weekly from September 22 through December 15, 1997 in Sweden (Stattin and others 2000). As winter progressed, the difference in REL cold - treated and untreated seedlings became smaller, indicating that the seedlings were becoming increasingly more cold hardy (Figure 2).

Detecting cold or heat injury. Because they are exposed, the roots of container seedlings are easily injured by extreme temperatures. This is especially true when container seedlings are over-wintered outdoors under snow, as is done in eastern Canada and Scandinavia (Lindstrom and Mattson 1989). If snow fails to accumulate, or there is a sudden warm period, container crops are exposed and their roots can be severely damaged. The REL test is ideally suited for making rapid assessment of potentially damaged nursery stock (for example, Coursolle and others 2000).

Determining lifting windows. REL has been used as a direct indicator of the best time for harvesting Sitka spruce (*Picea sitchensis*) and Douglas-fir in the United Kingdom (McKay and Mason 1991).

Monitoring quality of stored seedlings. REL can also be used to monitor seedling quality during overwinter storage (McKay 1992, 1998, McKay and Morgan 2001). In one test (McKay 1998), spruce and larch seedlings were lifted throughout winter, beginning October 1, and

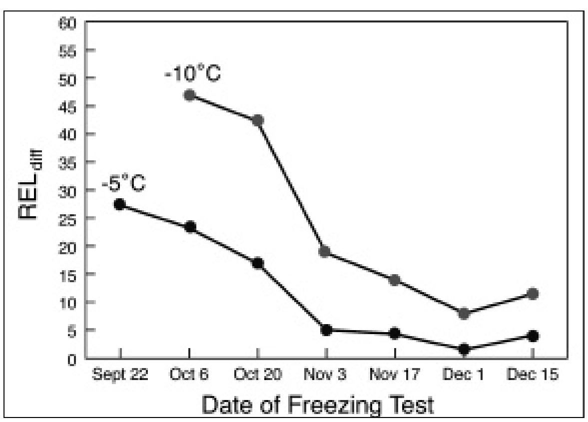

Figure 2. Changes in root electrolyte leakage (REL_{diff}) of outdoor grown Norway spruce seedlings measured biweekly from September 22 through December 15. REL_{diff} is the increased electrolyte leakage from roots following exposure to -5 °C or -10°C compared with leakage from unfrozen seedlings (Modified from Stattin and others 2000).

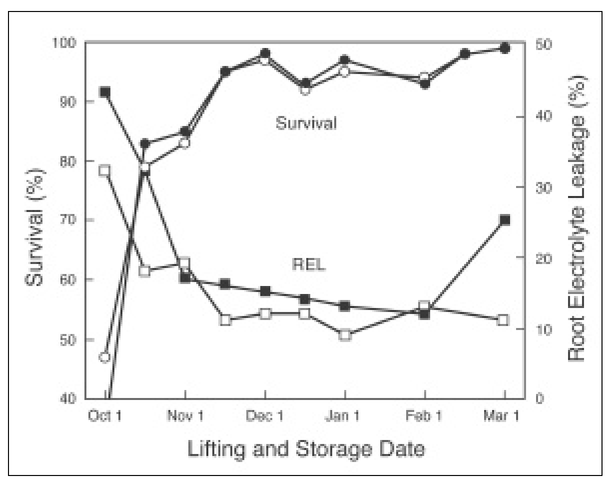

Figure 3 - Mean survival after two growing seasons and root electrolyte leakage (REL) measured after cold storage of Sitka spruce (dark symbols) and Japanese larch (open symbols) planted in April 1990 after storage at +1 °C on different dates in 1989-1990 (modified from McKay 1998).

then placed in storage at +1 °C (33 °F). All seedlings were removed from storage in April, tested for REL, and then outplanted. With both species, REL decreased and survival increased as lifting was delayed (Figure 3).
In another experiment, Douglas-fir seedlings were lifted in October, November, December, and January in Ireland (Harper and O'Reilly 2000). They were "warm stored" at 15 °C (59 °F) for 7 and 21 days, and then tested for REL. REL readings taken at the time of lifting decreased with later harvest dates indicating that the seedlings were becoming more hardy. For each lift date, however, the readings increased sharply with storage duration suggesting that warm storage contributed to fine root degradation (Figure 4).

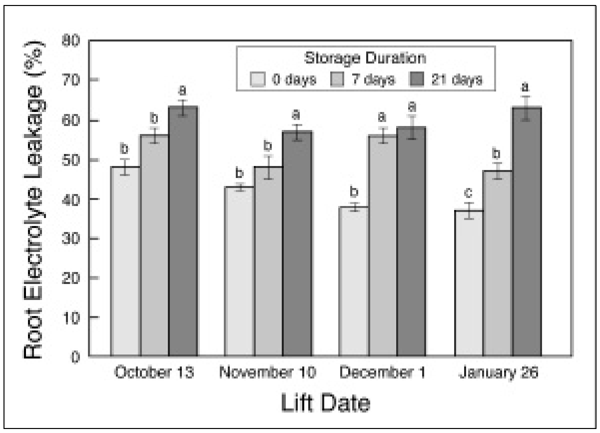

Figure 4 - Effects of zero, one and three weeks warm storage duration on root electrolyte leakage in Douglas-fir seedlings. Within the same lift date, bars with different letters are statistically significant. (Modified from Harper and O'Reilly 2000).

Desiccation and rough handling effects. REL has also been used to evaluate the effect of root desiccation in several studies. Bareroot Sitka spruce and Douglas-fir seedlings were held in controlled environment chambers with their roots exposed to drying conditions for up to three hours (McKay and White 1997). They were then measured for REL and outplanted on several sites in Britain. The REL readings increased with the intensity of the desiccation treatment indicating root injury. This was confirmed when the desiccation treatments had poor outplanting performance on sites with low spring rainfall.

Rough handling in combination with root desiccation was assessed in Douglas-fir, Sitka spruce, Japanese larch and Scots pine (*Pinus sylvestris*) using REL (McKay and Milner 2000). Rough handling consisted of dropping bags of seedlings from a height of 3 meters (9.8 ft). Desiccation was achieved by exposing roots to warm dry air for five hours. Although effects varied with lift date and species, REL was significantly higher in stressed seedlings than in un-stressed seedlings across species and treatments.

REL as a Predictor of Outplanting Performance

The ultimate objective of any seedling quality test is to predict how well nursery stock will survive and grow after outplanting, and many studies have used REL for this purpose. Unfortunately, results have been mixed. With Sitka spruce and Japanese larch seedlings, for example, REL was closely related to both survival and height growth (Figure 5). In Sitka spruce and Douglas-fir seedlings, REL was correlated with survival on some sites but not others (McKay and White 1997). REL predicted establishment of Japanese larch (*Larix leptolepis*) seedlings to some extent, but Root Growth Potential (RGP) was a better predictor (McKay and Morgan 2001). Similar results were found with black pine

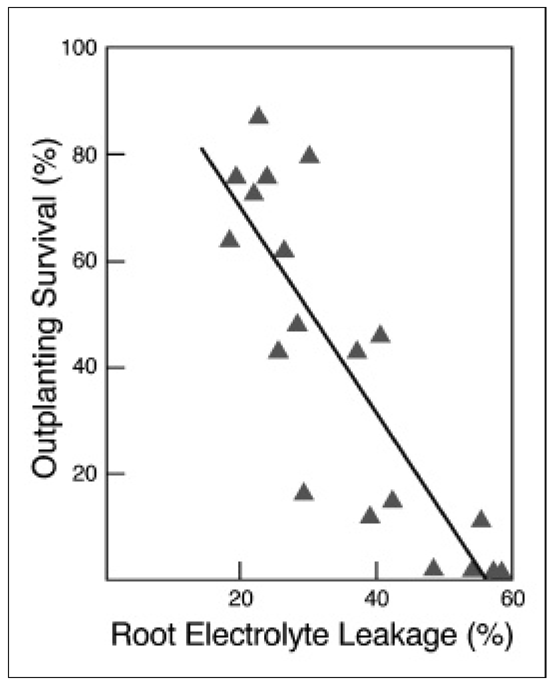

Figure 5—Root electrolyte leakage has been closely correlated with outplanting performance (modified from McKay and Wason 1991).

(*Pinus nigra)* (Chiatante and others 2002), while Harper and O'Reilly (2000) reported that REL was a poor predictor of survival potential in warm-stored Douglas-fir seedlings.

Limitations of REL

So, why does REL predict survival in some cases but not others? As with many things "the devil is in the details."

Genetics. REL has been shown to vary with species and even seed sources within species. For example, jack pine and black spruce, following exposure to a range of damaging root temperatures, had REL values in the range of 27% to 31%, while white spruce exposed to the same temperatures had REL between 36% and 38% (Coursolle and others 2000). Sitka spruce seedlings from Alaska, the Queen Charlotte Islands (QCI) and Oregon provenances, were evaluated for their ability to withstand root drying and rough handling (McKay and Milner 2000). Oregon and QCI seedlings exposed to root drying had lower REL values than Alaska seedlings, while Alaska and QCI seedlings, when exposed to rough handling had lower values than Oregon seedlings. Douglas-fir had higher REL values than Sitka spruce, Scots pine, and Japanese larch, regardless of the type of stress encountered. Two coastal seedlots of Douglas-fir (British Columbia) gave different relationships between REL and survival (Folk and others 1999).

Dormancy status. McKay and Milner (2000) found that the resistance to stresses mentioned above varied seasonally and was correlated with the intensity of bud dormancy, as measured with a Dormancy Release Index (for definition, see Ritchie and Landis 2004). A similar result was reported by Folk and others (1999) for Douglas-fir seedlots. They argued that REL must first be calibrated to bud dormancy status before it can be effectively used to assess root damage in Douglas-fir.

Seedling age. REL gave good correlations with survival in two-year-old black pine seedlings, but the correlations were weak for one-year-old seedlings (Chiatante and others 2002). The authors speculate that the efficiency of REL as a seedling assessment tool could be closely related to the developmental state of the root system.

Summary and Conclusions

Electrolyte leakage from fine roots is a robust and easily measured parameter that has a rapid turn-around time and can be used to evaluate the viability of seedling root systems.

REL measures the ability of membranes within the root system to contain ions. Damaged membranes tend to leak ions so, if ion leakage is quantified, it can provide an indicator of root viability.

REL has been used successfully to evaluate the effects of cold damage, rough handling, desiccation, cold and warm storage, and other stresses on root viability and seedling vigor.

REL is sometimes closely correlated with seedling survival, but in other cases these correlations are weak. This is because factors other than root damage can affect REL. Some of these factors are species, seedlot, seedling age, season, and bud dormancy intensity. When REL is calibrated for these effects it can offer a simple, easy test of seedling root system viability.

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