Can Foliage Water Content Measurements Replace Freezer Tests in Determining a Safe Lifting Time For Frozen Storage of Conifer Seedlings?[1]

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Several researchers have reported a strong relationship between the frost hardiness of planting stock and its shoot water content (Pellet and White 1969, Rosvall-Åhnebrink 1977, Jonsson et. al. 1981, Colombo 1990, Calmé et. al. 1992). The last cited work was done in Quebec. Calmé et. al (1992) examined the relationship between shoot water content and injury sustained in a -10°C freezer test by containerized seedlings of three species (Jack pine, white, and black spruce). They reported that frost hardy seedlings had shoot dry to fresh weight ratio of less than 0.3. Consequently, they suggested that measuring shoot water may rapidly predict seedling frost tolerance. In fact, the dry weight to fresh weight ratio of excised shoot tips (terminal 2 cm) has been used operationally in Sweden to determine safe lifting time for storage of conifer seedlings. Seedlings are lifted when the ratio is 0.31 or less (Hultén and Lindell 1980).

The possibility of a simple and rapid test to determine safe lifting time for seedling storage is an exciting one. Unfortunately, there are problems with the reliability of the water content test. Calmé et. al. (1992) reported that photoperiod shortening alone or coupled with moisture stress, and different levels of NPK fertilization had no effect on the relationship between frost tolerance and shoot water content. Non-hardy seedlings had high water content while it was low in the hardy ones. Lindström [4] (pers. comm. 1994), however, thought cultural treatments may influence the relationship.

In Sweden, seedlots from locations north of the nursery must reach lower water content to be lifted than the local seedlots. The reverse is the case for seedlots south of the nursery (Lindström - pers. comm. 1994). Based on the Swedish perspective, a great deal of experience is required from nursery managers to interpret the water relation based measurement correctly.

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In British Columbia, safe time to lift for freezer storage is determined by the -18°C test recommended by Simpson (1985)[5]. The test is simple and reliable but results of visual injury assessment are available only after one week. So far, faster methods of injury evaluation have not been operationally adopted in British Columbia.

This report takes advantage of data gathered for other studies in 1991 and in 1994. Foliage water content was monitored in these studies and related to results of the -18°C test. Therefore, our results are not directly comparable to reports based on measurements of shoot water content. We treat this report as a preliminary assessment of the method for more thorough examination, should it be warranted.

MATERIALS AND METHODS

Plant material and sampling:
A number of seed sources and treatments were tested. Seedlots and treatments used for testing should be viewed merely as a variety of material available at the nursery. Seed source locations and special cultural treatments are given in the results section.

In 1991, all tests were made on transplants growing outdoors in a nursery bed at Red Rock Research Station. They were either, planted in the spring of 1991, following freezer storage, or sown in the spring, grown in a greenhouse culture, and transplanted into the nursery bed in August. These were not truly cultured seedlings; however, we were interested in the evaluation of end-of-season trends. The 1994 testing was made on current-year sown seedlings growing in a greenhouse culture.

Nine randomly collected seedlings were sampled per seedlot or treatment in 1991, and 15 in 1994. Means of water content measurements and foliage injury in freezer tests are presented here. In most cases, foliage samples for water content determination were taken from seedlings used in the freezer tests. With few exceptions, both procedures were carried out on the same dates.

Freezer tests:
The standard -18°C freezer test was used as it is done routinely in British Columbia nurseries. Two-thirds of the collected seedlings were frozen while one third served as controls. Evaluation of visible injury was done after one week in a hydroponic culture at forcing conditions.

Water content measurements:
Four needles were sampled at mid-day from the terminal 2 cm of each seedling. The needles were placed into numbered glass vials, then weighed (fresh weight) on the laboratory precision balance. In 1991, the needles were processed in pairs - two per sampled tree. In 1994, a sample consisted of four needles. After weighing, the leaves were placed on a microwave-safe plastic tray with numbered compartments and microwaved at low power for 10 min. They were weighed, then returned to the microwave for one more minute at low power and weighed again to determine if their dry weight stabilized (no further decline). This was usually sufficient to obtain a reliable dry weight measurement. Dry weight to fresh weight ratios were then calculated.

RESULTS AND DISCUSSION

Seedlings remaining outdoors in a nursery bed in 1991:

Seedlot 8791 (latitude 54°36' N, elevation 1219 m)
Seedlot 8791 was sown in PSB 313B[6] containers in March 1991 at two different nurseries: Red Rock Research Station (RRRS) and Cowichan Lake

[5] Simpson, D.G. Measuring cold hardiness. Presentation at the Forest Nursery Association of British Columbia Annual Meeting, Sept. 23-26, Duncan, B.C.

[6] All container types mentioned in this report are styroblock containers produced by Beaver Plastic Limited of Edmonton, Alberta. Providing trade names does not constitute endorsement by BCFS.

Research Station (CLRS). Following growth at an extended, 23 h photoperiod, different short day treatments (SDT) were applied at each nursery to induce dormancy. A 14 h photoperiod SDT was applied at RRRS in early July for two weeks. A 10 h photoperiod SDT for three weeks was applied in mid-July at CLRS. The CLRS stock was air shipped to Red Rock and both batches were planted into the nursery bed at RRRS on August 6.

There was a rapid increase in dry weight to fresh weight ratio in RRRS and CLRS seedlings between August 7 and September 10 (Fig. 1 A-B). The first freezer test was made on September 10. On that date, both the RRRS and CLRS seedlings had mean foliage injury greater than 25%. Foliage dry to fresh weight ratio was about 0.42 on September 6. It obviously stabilized and remained at this value in all measurements after September 6. Mean foliage injuries in all freezer tests after September 10 were very low (Fig 1A-B).

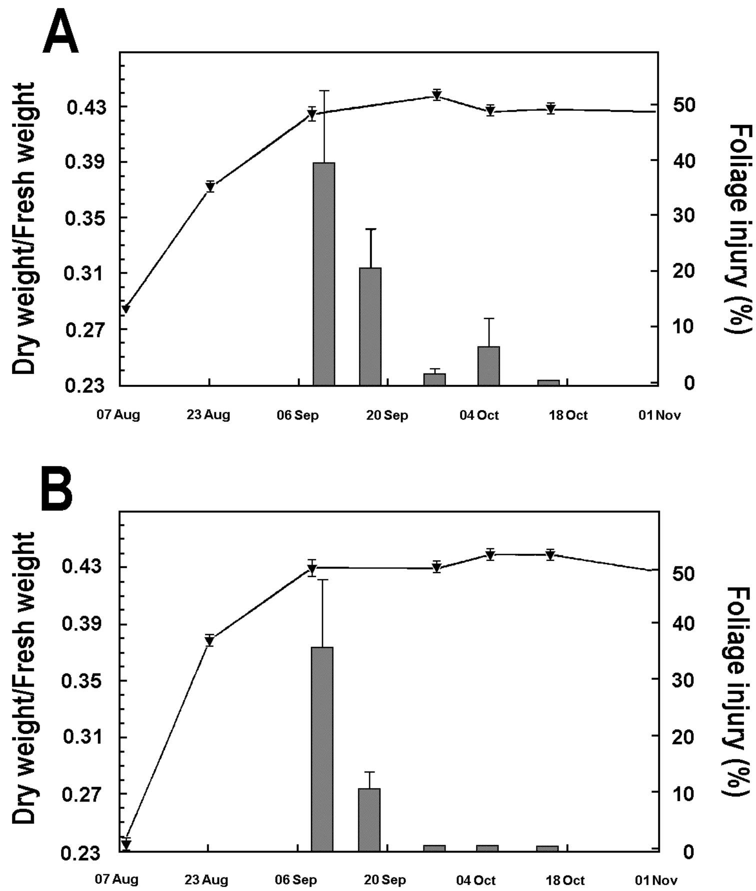

Figure 1 A-B. Changes in mean foliage water content expressed as a dry to fresh weight ratio (line graph) and mean foliage injuries resulting from -18°C freezer tests for seedlings of seedlot 8791 transplanted in August from greenhouse culture into a nursery bed at Red Rock Research Station. Capped vertical lines show standard errors of the means.
Figure 1A: **Stock grown in Red Rock greenhouses prior to transplanting.**
Figure 1B: **Stock grown in Cowichan Lake Research Station greenhouses prior to transplanting.**

Seedlot 8779 (latitude 55°44'N, elevation 1067 m)

Two stock types were available in seedlot 8779. One was a 2+0 PSB 415B container type with seedlings sown on June 1, 1990 and grown in Industrial Forestry Services (IFS) greenhouses near Prince George. The other stock was 1+0 PSB 415B sown in February and grown in the Hybrid nursery greenhouses, near Vancouver. IFS nursery used reduced N fertilization to induce budset. No particular dormancy induction treatment was applied at Hybrid nursery. Rather, budset was promoted by naturally occurring shorter days at the nursery's latitude, relative to that of the seed source. Both batches were planted at Red Rock on August 6.

The Hybrid stock passed the first freezer test made on September 9, and was considered ready for lifting. Dry to fresh weight ratio was about 0.42 on that date (Figure 2). It continued to increase until November reaching about 0.45 - 0.46. Injury in all freezer tests after that date was very low (Figure 2). The IFS stock had 27% injury and dry/fresh weight ratio of about 0.41 on September 9. It passed the freezer test on September 20. The weight ratio increased, then declined (but not below 0.42) between September 10 and November 1 (Figure 2). The IFS stock was only 2% above the acceptable injury limit on September 10. It is possible that it was ready for lift at that date (visual assessments of injury are not that precise).

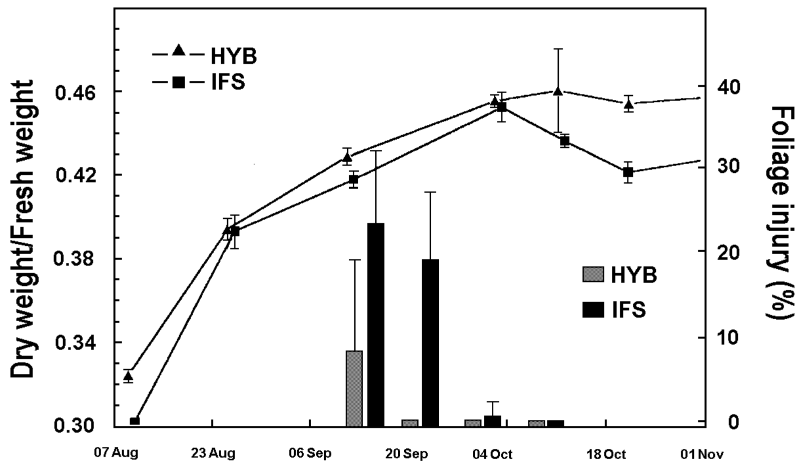

Figure 2. Changes in foliage water content (dry to fresh weight ratio) shown as a line graph, and mean freezer test foliage injury (vertical bars) in seedlings of seedlot 8779. Seedlings were transplanted into a nursery bed at Red Rock in August 1991 as 2+0 PSB 415B stock grown at Industrial Forestry Services (IFS) nursery and as 1+0 PSB 415B grown at the Hybrid nursery. Capped vertical lines indicate standard errors of the means.

Seedlot 1822 (latitude 54°45' elevation 853 m)

PSB 415B 2+0 stock was planted in late May 1991 following freezer storage. It sustained mean foliage injury higher than 30% in the September 9 test even though foliage dry to fresh weight ratio already reached about 0.43 (not shown). However, the ratio was very stable after that date (0.43-0.44) and foliage injury was minimal or none.

All the above seedlots were from sources north of, and higher in elevation than RRRS. The seed sources were also north of the nurseries where they were grown prior to transplanting into beds at RRRS. It is difficult to judge to what extent the effects of previous growing conditions were nullified by the common post-transplanting conditions at RRRS (latitude 54°45'N, elevation 620 m). For seedlot 8791, there appears to be very little if any significant effect of previous treatments and nursery of origin. In seedlot 8779, the difference between stock types (different age and nursery of origin) was both in frost hardening and foliage water content patterns. The latter difference occurred only after late September. It is not certain whether the observed differences were due to the nursery locale (latitude, growing regime), age of stock, or both.

We remind the reader that these are outdoor-grown seedlings and they cannot be compared to greenhouse-grown ones. Nevertheless, the above examples do show close a relationship between water (or dry matter) content and frost resistance of spruce seedlings. For these outdoor-located seedlings the threshold value of dry to fresh weight ratio of about 0.42-0.43 appears to be well related to the stage when the seedlings sustain less than 25% foliage injury in the -18°C test.

1994 testing of seedlings from greenhouses:

This time, container seedlings grown in a greenhouse culture rather than outdoors were studied. Figure 3 A-B shows changes in dry to fresh weight ratio in seedlings of four seedlots ranging widely in latitudes and altitudes of seed sources and either untreated (Figure 3A) or subjected to SDT of 14 h for two weeks in early June (Figure 3B). All seedlings were grown in PSB 415B styroblocks and were sown at the same date in February. Seed source locations are as follows:

Seedlot 30664 latitude 51°36’N, altitude 480 m;

Seedlot 6863 latitude 54°56’N, altitude 960 m;

Seedlot 8779 latitude 55°44’N, altitude 1067 m;

Seedlot 35075 latitude 58°25’N, altitude 450 m;

Figure 3 shows no obvious stabilization of foliage water content in seedlings of any seedlot whether SDT treated or not, until possibly very late in the season. Therefore, it is necessary to look for threshold values in water content corresponding to the dates when seedlings sustained lower than 25% foliage damage in freezer tests and were considered ready for lift. Outcomes of freezer tests resulting in injury higher than 25% are not shown on Figures 3A-B to prevent their overcrowding. Instead, Table 1 presents lift dates determined by freezer tests, mean foliage injury, and mean foliage dry to fresh weight ratios on these dates.

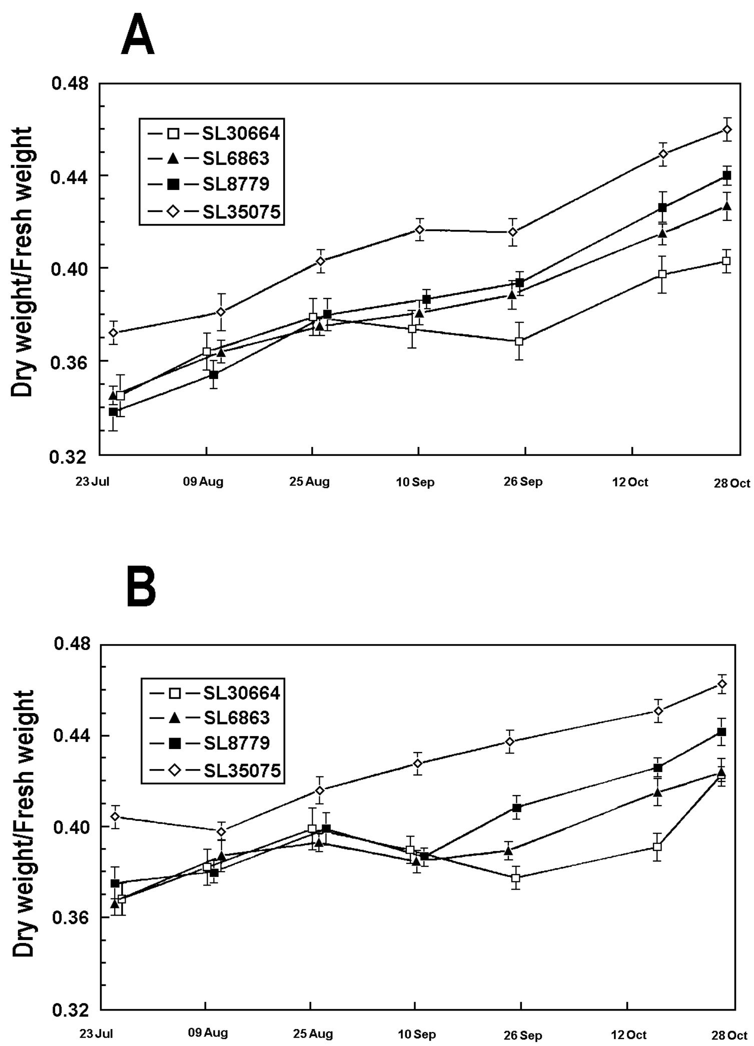

Figure 3A-B. Changes in foliage water content (dry to fresh weight ratio) in seedlings of four different seedlots (SL) of interior spruce toward the end of their first growing season. All seedlings were sown on the same date and grown under a greenhouse culture in PSB 415B containers.
Figure 3A: Seedlings untreated with short day treatment.
Figure 3B: Seedlings from a 14 h short day treatment applied for 2 weeks starting June 9, 1994.

The northernmost seedlot 35075 was ready for lift early. It had consistently lower foliage water content than did other seedlots throughout the observation period, whether short-day treated or not (Figures 3A-B). This is consistent with the earlier discussed effect of seedlot location. The trend is for lower water content (higher dry to fresh weight ratio) in seedlots north of, and for higher water content in those south of the nursery when at similar levels of frost hardiness (Lindstöm, pers. comm. 1994). It is particularly obvious if seedlot 35075 is contrasted with seedlot 30664, which came from a similar elevation but almost seven degrees of latitude south. Seedlot 8779 is an exception to the theory. This seedlot is from north of the nursery and from a higher elevation than all the other seedlots. However, it had a low dry to fresh weight ratio, compared to the other seedlots, when ready to lift as determined by the -18°C test (Table 1). Seedlot 6863 can be considered local by latitude but about 300 m higher in elevation, relative to nursery location. It was ready to lift at about 0.42 dry to fresh weight ratio. In most cases the ratio was higher at ready to lift date in short-day treated than in the untreated seedlings (Table 1) indicating there may be some influence of these treatments on the discussed relationship. The dry to fresh weight ratio separated he seedlots in accordance to the latitude of seed origin in untreated and SDT treated seedlings in late September (Figures 3A-B). By that time, however, some seedlots and treatments would have already been lifted based on the -18°C.

Table 1.Mean foliage water content and mean foliage injury (plus and minus standard error) on the date considered safe to lift for seedlings of four seedlots and untreated (UNTR) or treated with a short day (SDT) of 14h for 2 weeks in early summer.

			Means on that date	
Seedlot	Treatment	Date when ready to lift	Foliage injury (%)	Foliage dry: fresh weight ratio
30664	UNTR	Oct 20	12±3	0.404±0.005
30664	SDT	Oct 20	17±3	0.424±0.003
6863	UNTR	Oct 11	9±2	0.416±0.005
6863	SDT	Oct 11	12±4	0.416±0.006
8779	UNTR	Sept 19	4±2	0.394±0.005
8779	SDT	Sept 19	22±6	0.409±0.005
35075	UNTR	Sept 19	4±2	0.416±0.006
35075	SDT	Sept 19	14±8	0.438±0.005

Note:Seedlings were considered ready to lift once mean foliage damage in a -18°C freezer test was below 25%.

SUGGESTIONS AND CONCLUSIONS

At this time, it is not possible to replace freezer tests with water content measurements to determine safe lifting time for conifer seedlings. The data shows, though, that it would be worthwhile to undertake more work in this area as it may be possible to reduce the number of freezer tests. Many issues not addressed by this study must be realized and answered to gain a better understanding of the water content measurement technique.

These are:

1) Are whole shoot or shoot tip based measurements of water content more consistent and better related to seedling frost resistance than foliage based measurements? In this report, the dry to fresh weight ratio of foliage at lifting dates was always higher than the shoot-tip based ratio threshold of 0.31 used for lifting time determination in Sweden.

2) What about the great range of seed source elevation (from sea level to > 1000m) and latitude combinations characteristic of British Columbia seed sources ? Does it make the water content measurement test too complicated to interpret for reliable practical use?

3) What is the influence of seedling age and size on the dry to fresh weight ratio of foliage and of shoots?

4) Should further studies relate directly between weight based measurements and storability rather than be compared to one temperature based freezer test?

5) What technical details could be improved to make the technique more reliable?

The -18°C test works well in determining safe lift time in British Columbia nurseries. The water content measurement must prove to be equally reliable to be considered useful in practice. If it does so, its low cost, simplicity, and speed will offer real benefits.

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