Determining Optimal Lifting Time of Nursery Stock for Cold Storage

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As one of several physiological parameters for determining the optimal lifting time of barerooted nursery stock for cold storage, shoot and root frost hardiness were studied as possible indicators of storability. During autumn (September to December) 1997, seedlings of pedunculate oak (*Quercus robur*) and Scots pine (*Pinus sylvestris*) were lifted at 7 and 6 occasions, respectively. At each lifting date seedlings for field performance trials were stored at -1C, while samples of shoot tips and fine lateral roots were frozen to - 5, -10, -15, and -20C. A control sample was kept at +2C. Frost damage was assessed using the electrolyte leakage method. In April 1998 the cold stored seedlings were planted for field performance. The results indicate that shoot frost hardiness at -20C can be used as an indicator of storability, and that the relatively simple, fast, and inexpensive method described here has potential for operational use in the future.

INTRODUCTION

For several decades, autumn lifting and subsequent cold storage of nursery stock have been common practice in Scandinavia in order to overcome peaks of demand for spring time planting and to prolong planting time until early summer (in this text the term "cold storage" refers to storage in the range of a few degrees above or below 0C). It has for some time been common knowledge that lifting and subsequent cold storage of seedlings before a certain state of physiological maturity is reached has detrimental effects on survival and growth after planting the following year (Omi et al., 1994; Sønderhousen and Bøvre, 1980). For many deciduous species leaf abscission is a fairly good indicator of physiological maturity and is often used in practice. However, juvenile forms of some species, e.g., *Quercus robur* and *Fagus sylvatica*, do not drop all their leaves in autumn and the state of physiological maturity can therefore be difficult to determine. The same difficulty is obvious for conifer species.

Storage of barerooted seedlings is a drastic environmental change. An important prerequisite for storability, therefore, must be that seedlings have reached a high level of stress resistance towards cold and dehydration. Maximum stress resistance has been found to coincide with maximum bud dormancy (Hermann, 1967; Lavender and Wareing, 1972), and measurement of dormancy status is, therefore, a possible parameter for determining optimal lifting time. Measuring bud dormancy, however, may take weeks (Ritchie, 1984) and is not suitable for operational nursery purposes where results often are required within a few days.

Shoot frost hardiness correlates quite well with development of stress resistance (Lavender, 1991), and could be an alternative parameter to dormancy. Several methods for assessing frost hardiness are available:

Visual Evaluation. A rather simple and widely used technique is visual evaluation of frozen tissue (tissue browning). Major disadvantages with this method are the long incubation period of 1 or 2 weeks (Anisko and Lindstrom, 1996; Stergios and Howell, 1973) and the element of subjectivity in the evaluation.

Shoot Tip Dry Matter Content. Rosvall-Åhnebrink (1985) found that the dry matter content of shoot tips (SDM) was correlated with shoot frost hardiness and it has for some years been used rather commonly in Swedish nurseries as a parameter for storability. It is relatively simple and inexpensive to measure, but practical experience, as well as later experimental results, have revealed some uncertainty about the reliability of this parameter (Mattsson,1998 pers. comm.; Lindstrøm and Håkansson, 1996).

Chlorophyll Fluorescence. This parameter is a measure of the functionality of the photosynthetic apparatus and provides information about both dormancy status and frost hardiness in conifers (Strand and Öquist, 1988). The method is fast and has also proven to be useful in determining seedling storability (Vidaver et al., 1989), but the equipment is expensive and the use is restricted to conifers and other evergreen species.

Electrolyte Leakage. Physiological injuries of cell membrane integrity caused by freezing or desiccation can be detected with the electrolyte leakage (EL) method (Dlugokecka and Kacperska-Palacz, 1978; McKay and White, 1997; Wilner, 1960). Cells within plant tissue (e.g., shoot or root sections) exposed to frost, will be more or less injured depending on the severity of the freezing process or the degree of frost tolerance of the tissue. Freezing of extracellular water causes an efflux of water from the cytoplasm through the cell membrane out into the extracellular lumen. This process often leads to alterations in the cell membrane, apparently affecting the ion transport properties, which results in increased leakage of electrolytes (Palta and Li, 1980). The frost damage can be quantified by placing the frozen tissue in deionized water for a specific period of time (e.g., 24 h) which allows electrolytes to leak out of the injured cells. The resulting increase in electrical conductivity of the water is a measure of the freezing damage. The EL method is relatively fast, simple, and inexpensive to perform in comparison with other physiological tests, and could be done by larger nurseries or offered as a service by commercial laboratories.

The objective of this investigation was to study shoot and root frost hardiness as possible parameters for detecting the optimal lifting time of barerooted seedlings for cold storage. For frost hardiness assessment, the EL method was chosen because it is relatively fast, simple, and inexpensive and because of its potential use as a more universal method for detecting plant tissue injuries under operational nursery conditions. Two model species, pedunculate oak (*Q. robur* L.), and Scots pine (*P. sylvestris* L.), were selected because of their wide distribution and economic importance in Scandinavian and European forestry.

MATERIALS AND METHODS

Sampling. From September until December 1997, seedlings of *Q. robur* (1+0) and *P. sylvestris* (2+0) were lifted at 7 and 6 occasions, respectively, in a commercial nursery and immediately transported to the Department of Ornamentals in Årslev. Lifting dates for *Q. robur* seedlings were 15 Sept., 6 Oct., 27 Oct., 10 Nov., 24 Nov., and 8 Dec. and for *P. sylvestris*. 15 Sept., 6 Oct., 27 Oct. 17 Nov., and 8 Dec. At each lifting date samples were prepared for freezing tests, pre-planting measurement of root growth potential (data not presented here), and field performance. The freezing test included a frost treatment and subsequent evaluation of frost injury with the EL method. Seedlings for the root growth potential test and field performance were stored at -1C until April 1998.

Freezing Test. At each occasion shoot tip and fine root samples of 75 seedlings of both species were prepared for freezing tests. From each seedling a 3-cm-long stem segment was cut out right below the apical bud, and root samples were prepared from fine lateral roots (400 to 900 mg per sample, diameter 2 mm, and approximately 3 cm long). Shoot tips and roots were washed in tap water and afterwards carefully rinsed in deionized water to avoid contamination with surface ions. Each sample was put into a 25-ml plastic bottle and capped. The 75 samples were divided into four sets of 15 replicate samples per target freezing temperature and a set of 15 samples was kept at +2C as control. The 60 samples were frozen in a programmable freezer at a rate of 2C h^{-1} to the target temperatures -5, -10, -15, and -20C. Each set of samples was kept at the respective target temperature for 60 min, before they were removed from the freezer and placed at -1C to thaw overnight.

Evaluation of Frost Injury. Twenty ml of deionized water, with a known low electrical conductivity (C0, mS cm^{-1}), were added to each bottle and the samples were left to leak in darkness at room temperature for 24 h ±15 min. After shaking the samples briefly, the resulting conductivity of the water (C1, mS cm^{-1}) was measured with a conductivity meter. The samples were then autoclaved at 110C for 60 min to obtain maximum possible electrolyte leakage. After cooling samples to room temperature, a second reading of the conductivity (C2, mS cm^{-1}) was made. The root electrolyte leakage (REL) and shoot electrolyte leakage (SEL) were calculated as relative conductivity (RC):

$$RC = \frac{C1 - C0}{C2 - C0} \times 100\ \%$$

Relative conductivity (RC) expresses the injury caused by freezing as a percentage of maximum injury and thus eliminates the effect of sample size. To eliminate the effect of membrane damage caused by environmental factors in the nursery and during the lifting process, shoot and fine root frost hardiness of both species were expressed in terms of $SEL_{diff-20}$ and REL_{diff-5}, respectively. The $SEL_{diff-20}$ and REL_{diff-5} are defined as the difference in SEL or REL of samples frozen to -20 or -5C and the respective control samples, kept at +2C, according to Lindström and Håkansson (1996). Low values indicate that the tissue is frost hardy in the range of the specified temperatures.

Field Performance. In April 1998, cold stored seedlings of both species were planted in an experimental field at the Research Center in Årslev for assessment of field performance. Trials were laid out in a randomized block design with five replicate blocks of 19 seedlings per lifting date. Strips of lawn grass were sown between seedling rows to simulate weed competition in a controlled reproducible way. Measurements of first-year field performance (survival and growth) have not been completed yet. At this point only survival in August 1998 has been recorded, while growth parameters (height and stem diameter increment) will be measured during the winter 1998-99.

RESULTS AND DISCUSSION

Field survival in August 1998 of *P. sylvestris* was clearly affected by lifting time (Table 1). Lifting in September and early October and subsequent cold storage had a detrimental effect on field survival, while lifting by the end of October or later resulted in satisfactory field performance. Survival of *Q. robur* seedlings was high (100%), irrespective of lifting date. Growth data (height and stem diameter increment) has not been recorded yet, but there are clear visual differences between lifting dates, showing poor performance of early lifted seedlings.

The freezing test results showed a considerable increase in shoot frost hardiness in terms of $SEL_{diff\text{-}20}$, and for both species the values stabilized around 0% to 8% from 27 Oct. (Table 1). The increase in root frost hardiness of *Q. robur* during the period

Table 1. Shoot tip and fine root frost hardiness and first-year field survival (August 1998) of *Quercus robur* and *Pinus sylvestris* seedlings lifted on different dates during autumn 1997. Shoot and root frost hardiness is expressed as $SEL_{diff\text{-}20}$ and $REL_{diff\text{-}5}$ respectively, i.e., the difference in SEL or REL of samples frozen to -20 or -5C and the respective control samples.

Quercus robur

	Lifting date					
	15 Sept.	6 Oct.	27 Oct.	10 Nov.	24. Nov	8 Dec.
$SEL_{diff\text{-}20}$ (%)	58.0	35.5	3.2	7.7	5.5	3.1
$REL_{diff\text{-}5}$ (%)	28.9	18.2	19.1	20.3	21.5	17.8
Field survival (%)	100	100	100	100	100	100

Pinus sylvestris

	Lifting date				
	15 Sept.	6 Oct.	27 Oct.	17 Nov.	8 Dec.
$SEL_{diff\text{-}20}$ (%)	35.2	22.6	4.9	5.4	0.5
$REL_{diff\text{-}5}$ (%)	43.5	26.8	31.8	9.2	17.7
Field survival (%)	0	27	97	100	100

was small in comparison with the development in shoots. For *P. sylvestris* there was a general but rather unstable increase in root frost hardiness. A rather good correlation ($r^2 = 0.97$) between shoot frost hardiness and storability, in terms of post-storage field survival, was obtained for *P. sylvestris*. The stabilization of $SEL_{diff\text{-}20}$ coincided with 97% to 100% field survival of *P. sylvestris* seedlings lifted 27 Oct. and later, which indicates satisfactory storability from that date. The described method of combined freezing test and electrolyte leakage measurement was able to detect the onset of maximum seedling storability for at least *P. sylvestris*, with threshold values of $SEL_{diff\text{-}20}$ for storability in the range of 0% to 5%. Similar results have been obtained by Lindström and Håkansson (1996) for *P. sylvestris* and Norway spruce, *Picea abies* (L.) Karst. With these promising results in mind, we hope to develop the method further and to introduce it on a more operational scale as a future service for nurseries.

LITERATURE CITED

Anisko, T. and **O.M. Lindstrom** 1996. Survival of water-stressed *Rhododendron* subjected to freezing at fast and slow cooling rates. HortScience 31:357-360.

Dlugokecka, E. and **A. Kacperska-Palacz.** 1978. Re-examination of electrical conductivity method for estimation of drought injuries. Biol. Plant. 20:262-267.

Hermann, R.K. 1967. Seasonal variation in sensitivity of Douglas-fir seedlings to exposure of roots. For. Sci. 13:140-149.

Lavender, D.P. 1991. Measuring phenology and dormancy. pp. 403-422. In: Lassoie, J.P. and T.M. Hinckley (eds.) Techniques and Approaches in Forest Tree Ecophysiology. CRC Press, Boca Raton.

Lavender, D.P. and **P.F. Wareing** 1972. The effects of daylength and chilling on the responses of Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) seedlings to root damage and storage. New Phytol. 71:1055-1067.

Lindström, A. and **L. Håkansson.** 1996. EC-metoden - et sätt at bestämma skogsplantors lagringsbarhet. Inst. för Skogsproduktion, Sveriges Lantbruksuniversitet. Stencil no. 95.

McKay, H.M. and **I.M.S. White** 1997. Fine root electrolyte leakage and moisture content: indices of Sitka spruce and Douglas-fir seedling performance after desiccation. New. For. 13:139-162.

Omi, S.K., R. Rose and **T.E. Sabin.** 1994. Fall lifting and long-term freezer storage of ponderosa pine seedlings: Effects on starch, root growth, and field performance. Can. J. For. Res. 24:624-637.

Mattsson, A. 1998. Dalarna University, Herrgårdsvägen 122, s-776 98 Garpenberg, Sweden

Palta, J.P. and **P.H. Li.** 1980. Alterations in membrane transport properties by freezing injury in herbaceous plants. Evidence against the rupture theory. Physiol. Plant. 50:169-175.

Ritchie, G.A. 1984. Assessing seedling quality. pp. 243-259. In: Duryea, M.L. and T.D. Landis (eds.) Forest nursery manual: Production of bareroot seedlings. Martinus Nijhoff and Dr. W. Junk Publishers for Forest Research Laboratory, Oregon State University.

Rosvall-Åhnebrinck, G. 1985. Innvintring af plantor för höstplantering eller vinterlagring. Skogsfakta Konferens 7:33-37.

Stergios, B.G. and **G.S. Howell, Jr.** 1973. Evaluation of viability tests for cold stressed plants. J. Amer. Soc. Hort. Sci. 98:325-330.

Strand, M. and **G. Öquist** 1988. Effect of frost hardening, dehardening and freezing stress on in vivo chlorophyll fluorescence of seedlings of Scots pine (*Pinus sylvestris* L.). Plant Cell. Environ. 11:231-238.

Sønderhousen, E. and **O. Bøvre.** 1980. Vinteropbevaring af bøg (*Fagus sylvatica* L.). Statens Planteavlsforsøg, Medd. nr. 1552.

Vidaver, W., , W.D. Binder, G.R. Lister, R.C. Brooke and **P.M.A. Toivonen.** 1989. Assessment of photosynthetic activity of nursery grown *Picea glauca* seedlings using an integrating fluorometer to monitor variable chlorophyll fluorescence. Can. J. For. Res. 19:1478-1482.

Wilner, J. 1960. Relative and absolute electrolyte conductance for frost hardiness of apple varieties. Can. J. Plant Sci. 40:630-637.