Influence of physiological status at the time of lifting on cold storage tolerance and field performance of Douglas fir and Sitka spruce

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Abstract

The physiological status of Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) and Washington origin Sitka spruce (*Picea sitchensis* (Bong.) Carr.) seedlings was assessed at the time of lifting. Seedlings of both species were sampled periodically from October to May and after cold storage in 1995/96 and 1996/97. Douglas fir only was sampled also in 1997/98. The field performance of seedlings that were planted in a field trial concurrently with the physiology work was evaluated. Shoot cold hardiness showed a clear seasonal pattern and was a good indicator of readiness of seedlings for lifting for planting or cold storage. Seedlings can be safely lifted for field planting when their shoots have hardened to -10 °C in Douglas fir (November to March) and -20 °C in Sitka spruce (late November/early December to March). Equivalent values for judging long-term (more than three months) cold storage tolerance are -20 and -30 °C, respectively. Root electrolyte leakage (REL) values should be considered to augment this information and to assess post-storage vitality. REL values should be <25% for cold storing Douglas fir, but the test is not reliable for judging its readiness-to-lift for field planting. In Sitka spruce, REL values of <20% and <15% indicate readiness for lifting for field planting and cold storage, respectively. Although more resistant to handling stresses later in the season, Douglas fir performs best in the field when freshly planted early in the lifting season (November to December), but Sitka spruce performs best when planted during the period of highest stress resistance (late November/early December to early March).

Keywords: cold hardiness, root electrolyte leakage, dormancy, stress resistance.

Introduction

The role of planting stock quality in ensuring good establishment success has come into clearer focus in Ireland in recent years. While the morphological (or visual) characteristics of plant quality (for example root collar diameter, height and shoot:root ratio) are of key importance (Thompson 1985), it is often the physiological (or non-visual) attributes of the stock that have the greatest impact upon field performance (Ritchie 1984). Physiological quality is, in turn, affected by many factors, including cultural practices used in the nursery. However, plant handling and storage practices probably have the greatest effect on quality (McKay 1997).

In Ireland, most planting stock is lifted in the nursery from about November to March, packed in co-extruded polyurethane bags and dispatched for field planting. In addition, the

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plants may be stored under ambient conditions for some weeks before planting, either at the nursery, in transit, at sorting depots, or at the planting sites. The seedlings[1] may be subjected to a variety of stresses during this chain of events, including desiccation, rough handling, and lack of light (see Tabbush 1988, McKay 1997). Plant condition also may deteriorate because of the depletion of stored food reserves during storage under ambient conditions, prior to planting (Puttonen 1986). In addition, a significant proportion of seedlings is currently placed in cold or freezer storage for a period before planting. The use of such storage allows flexibility in carrying out lifting operations; the stock is ready for dispatch for field planting when field conditions are favourable. However, the quality of the seedlings may deteriorate while in cold storage. Some plantation establishment failures have been attributed to this factor. While steps should be taken to minimise or avoid the potential stresses outlined above, in practice some stresses will always occur. The ability of seedlings to withstand these stresses varies with dormancy status and associated cold hardiness levels (Ritchie 1986).

The results of previous studies provided useful information on the annual cycle of physiological development in Douglas fir (O'Reilly *et al.* 1999) and Sitka spruce (O'Reilly *et al.* 2000) seedlings in Ireland. However, the effect of lift date on the ability to cold store and on subsequent field performance also required investigation, since field growth responses were not entirely consistent from year to year. The physiological status of seedlings at lifting and/or following cold storage was determined using shoot cold hardiness and root electrolyte leakage (REL). The survival and first-year height increment of the seedlings were evaluated in field trials established in tandem with the physiology work. The results are used to give guidelines on the best time to lift for planting or cold storage. In addition, recommendations are made on the best methods for assessing plant physiological quality.

Materials and methods

Plant material, sampling and cold storage

The Douglas fir were 1u1 (non-transplanted, undercut) seedlings {seed source: Darrington, Washington, US; seed description/seed zone 91(797) 403; 48° 15' N, 121° 36' W; <300 m elevation} from the None-so-Hardy Limited nursery at Ballymurn, Co Wexford (52° 27', 6° 29'; 70 m elevation) in 1995/96 and 1996/97, and 2+1 transplants {seed source: Chehalis/Centralia Washington, US; seed description/seed zone: (797) 241; 46° 43' N, 122° 58 W; <150 m elevation} from the Coillte nursery at Ballintemple, Co Carlow (52° 44' N, 6° 42' W, 100 m elevation). They were lifted at periodic intervals in 1997/98 from October to May each year and dispatched to University College Dublin (UCD) for analysis. Similarly, 2+1 Sitka spruce {seed source: seed description/seed zone (797) 030; 46° 58' N, 123° 53' W; <150 m elevation} from the Coillte Camolin nursery, Co Wexford (52° 38', 6° 26'; 65 m elevation) was lifted for analysis in 1995/96 and 1996/97, but not in 1997/98. Descriptions of the nursery soils are given in Table 1.

[1] Seedling is used in a generic sense to include all types of planting stock, including transplants.

Table 1. *Soil characteristics at study site nurseries.*

Nursery	*Ballymurn*	*Ballintemple*	*Camolin*
Soil type	*Sandy clay loam*	*Sandy loam*	*Clay loam*
pH	na[1]	5.7	5.5
Organic matter %	na	6-8	9
Sand %	na	66	35
Silt %	na	19	45
Clay %	na	15	20

[1] Information not available

The mean heights and diameters of the seedlings are shown in Table 2. Detailed descriptions of the cultural practices used at each nursery are not available, but they were similar to those described by Mason (1994a, 1994b).

Table 2. *Morphological characteristics of the Douglas fir and Sitka spruce seedlings used in the study.*

Species	*Douglas fir*			*Sitka spruce*	
Year	1995/96	1996/97	1997/98	1995/96	1996/97
Nursery	BN[1]	BN	BE	CN	CN
Height[3] cm	47.8 (0.48)[2]	44.8 (0.51)	47.4 (0.29)	41.2 (0.53)	48.9 (0.44)
Diameter[3] mm	6.7 (0.13)	6.7 (0.11)	8.6 (0.08)	6.7 (0.13)	6.7 (0.08))

[1]Nursery source abbreviations: Ballymurn (BN), Ballintemple (BE) and Camolin (CN).
[2]Values in parentheses are standard errors.
[3]Based on 20 plants from each of four lift dates (replicates) for each species in each year.

At each sampling date, 150 plants of each species were loosened by machine and lifted by hand, placed in polyurethane co-extruded bags (50 seedlings per bag) and dispatched the same day to UCD. In an identical manner, three bags containing 50 seedlings each (total 150) were dispatched for cold storage (1 - 2 °C) on each of four occasions in 1996/97, and on five occasions for Douglas fir in 1997/98. Upon removal from the cold store in May (after different duration of storage, depending on the lift date), 100 seedlings (two bags) were dispatched to the field trial, while 50 (one bag) were retained for the physiological assessments. Seedlings were also lifted and dispatched for field planting on most lifting occasions, as described below.

Observations and measurements of physiological parameters

All physiological tests/ assessments were carried out on plants just after lifting, but the REL test alone was performed on cold stored plants.

<u>Shoot cold hardiness</u>

Cold hardiness tests were carried out over a period of two to three days, commencing on the date of lifting. On each test occasion, one first-order excised lateral shoot (10 to 15 cm long, 2 to 4 mm base diameter) from the current year's growth, from each of 15 seedlings was subjected to one of a series of three to five target freezing temperatures in the range of -3 to -35 °C in a programmable freezer. The first test was carried out overnight starting on the day of lifting, while the remaining tests were carried out over the following two to three days. Air temperature was cooled from 5 °C at 5 °C h^{-1} until the desired target temperature was reached; it was held at this for three hours and then warmed at 10 °C h^{-1} to 5 °C. After treatment, the shoots were placed in beakers containing tap water and held in a heated (18 - 23 °C day/15 - 18 °C night; 16 h photoperiod) greenhouse for two to three weeks. Cold hardiness was determined by the extent needle tissue damage. Needle damage was scored (after Cannell *et al.* 1990) as: 0, no damage; 1, < 50% of needles killed; 2, > 50% killed but less than 100% killed; 3, all needles dead. The temperature at which 50% of the needles (LT_{50}) died was interpolated from these data, assuming that these scores represented 0, 33, 66, and 100 % damage, respectively.

<u>Root electrolyte leakage</u>

REL is used as an indicator of root vitality and potential field performance (McKay and Mason 1991). The principle of this test is that the movement of ions into and out of cells is largely controlled by the cell membrane. When root tissue is placed in distilled water (that has almost no ions) some ions will move through the membrane and surrounding tissue into the water through osmotic pressure. The amount of leakage depends on the level of damage to the membrane (McKay 1992). It is usually measured using a conductivity meter. However, active roots will leak more ions than inactive roots, making REL an indicator of root activity also. To determine if root damage has occurred however, REL values must be interpreted relative to the expected seasonal (baseline) values.

The REL tests were carried out at the time of lifting and following cold storage, using a method similar to that described by McKay and Mason (1991). (REL data are not available for Douglas fir removed from cold storage in February 1997/98 due to a processing error.) After washing the roots of the 15 seedlings to remove most of the soil, approximately 300 to 500 mg (fresh weight) of fine (< 2 mm diameter) roots were removed from the central portion of the root of each plant and placed in beakers containing tap water. The excised roots were washed thoroughly three times in tap water. The roots were then rinsed three further times in distilled water and placed in 28 ml universal vials with 17 ml distilled water. The vials were capped, agitated, and allowed to incubate at room temperature (18 - 20 °C) for 18 hours. After incubation, the conductivity of the bathing solution was measured using a conductivity meter with inbuilt temperature compensation (Delta Ohm, HD8706, Padova, Italy). All root samples were then killed by placing the vials in an oven at 90 °C for two hours. The samples were allowed to cool to room temperature (for four hours) before taking the second conductivity reading. The initial 18 hours conductivity reading was expressed as a percentage of the second reading.

<u>Field performance</u>

A separate trial was established each year in tandem with the physiology work. At approximately four to five week intervals each year, seedlings were dispatched for planting at a field site at the Coillte Tree Improvement Centre, Kilmacurra, Co Wicklow (52° 56' N, 6° 09' W, 120 m elevation). The soil at Kilmacurra had a pH of 5.7, 7% organic matter, and sand, silt and clay fractions of 40, 32 and 27%, respectively. Each year the field site was cleared of weeds prior to planting using Roundup at 2 l/ha (720 g glyphosate). Thereafter weeds were removed by hand at regular intervals.

The field trial was set down as spilt-plot with four blocks, comprising two storage treatments (freshly lifted and three to seven months cold storage) as the main treatments and six to eight lifting dates as the sub-treatments. Each of the four blocks contained one replicate of most of the storage treatment x lifting date combinations, as a row plot of 20 seedlings. Because plants were not placed in the cold store on all lifting dates, some plots were incomplete. Spacing was approximately 50 cm between rows and 30 cm within rows.

Survival (per subplot) and height increment were recorded at the end of the first growing season of each year. Because there was some variation in initial planting stock size, the height increment data were analysed as percent of initial height, measured before growth began in the spring. Subplot means were used in all of the data analyses.

Data analysis and presentation

Because the exact time of sampling varied from year to year, comparison of calendar date effects on response data were difficult to carry out. Therefore, the means and standard errors on each sample date for each year and 95% confidence intervals are presented. Unless otherwise stated, each seedling observation/value was treated as a single replicate.

Separate analyses were carried out on the root electrolyte leakage values (after arc sine square root transformation) for each year using an analysis of variance in SAS (1989) to test for the effects of lift date, storage treatment (no storage, or storage until May). In addition, the REL values recorded for seedlings from each lift date before and after cold storage until May were compared using a t test.

The field survival (after arc sine square root transformation) and percent height increment (plot mean) data for each year were subjected to an analysis of variance (SAS 1989) to test for block and lift date effects, separately for each storage (no storage, cold stored until May/June) treatment. The factorial split-plot model for the two factors was not tested because the cold storage treatments were not carried out at all lift dates. Means for each date were also compared using LSD tests. The performance of cold stored stock was compared with that of the freshly planted stock of same lift date using a t-test.

Meteorological data

Dormancy and cold hardiness development and growth are strongly influenced by weather (Lavender 1984). Because measurements were not taken at the nurseries, temperature data were obtained from weather stations closest to or most representative of the weather at the nurseries. Degree-days was calculated by accumulating temperature sums above each daily mean value (degree days = mean-5 °C; mean=maximum-minimum/2) for the April to October period of each year. Similarly, the cumulative chilling from September to March each year was calculated using temperature sums ≤5°C.

Air temperature data were obtained for Johnstown Castle, Co Wexford, approximately 17 and 35 km from Ballymurn and Camolin nurseries, respectively, for the 1995 to 1997 period. Temperature data from Kilkenny (data for Oakpark, Carlow, not available in late 1999) were used for 1997/98 (about 40 km from Ballintemple Nursery). Weather data were not available for the planting site. However, for months in which comparisons could be made between the each of the two stations and Kilmacurra, degree-day sums differed little between stations (Figure 1).

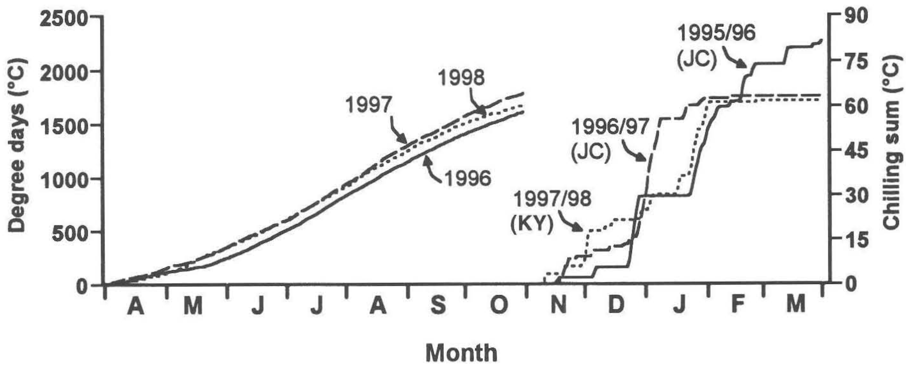

Figure 1. *Accumulated degree-days (>5 °C) from April to October and daily chilling sums (<5 °C) from November to March for Johnstown Castle (JC) and Kilkenny (KY).*

Results

Cold hardiness

Since cold hardiness is expressed as an LT_{50} value (temperature that kills 50% of the shoots), many shoots will be actually damaged by temperatures about 2 to 4 °C warmer than those shown (Figure 2).

The shoots were least hardy in early October (about -5 °C) in both species and became most hardy by December/February in each year (Figure 2). Interestingly, Douglas fir did not harden greatly after late November (ca. -15 °C) in 1997/98, whereas the shoots hardened greatly (ca. -25 °C) after this time in the other years (Sitka spruce was not sampled in 1997/98). The shoots hardened to about -25 °C in Douglas fir and -35 °C (lower limit of freezer) or lower in Sitka spruce by early January in 1995/96 and 1996/97. Thereafter, shoots maintained high hardiness levels until mid to late February, then dehardened rapidly during February and March, achieving similar hardiness levels to those attained in October. The seasonal pattern of change in cold hardiness was remarkably similar each year in Sitka spruce, but the shoots dehardened sooner in 1996/97 than in 1995/96 in Douglas fir. Douglas fir shoots were significantly less hardy from December to February in 1997/98 than in the other years ($p \leq 0.01$). Shoots were significantly ($p \leq 0.05$) hardier during deacclimation from January to April in 1995/96 than in 1996/97 (too few data were available for 1997/98 to assess significance), but differences were not significant between the latter two years.

Root electrolyte leakage

REL was significantly (all $p \leq 0.01$) influenced by lift date in both lifting seasons and date of cold storage in 1996/97 (both species) and 1997/98 (Douglas fir only, Figure 3). Root electrolyte leakage was much higher in 1997/98 than in other years in Douglas fir. REL also was generally higher in Douglas fir than in Sitka spruce.

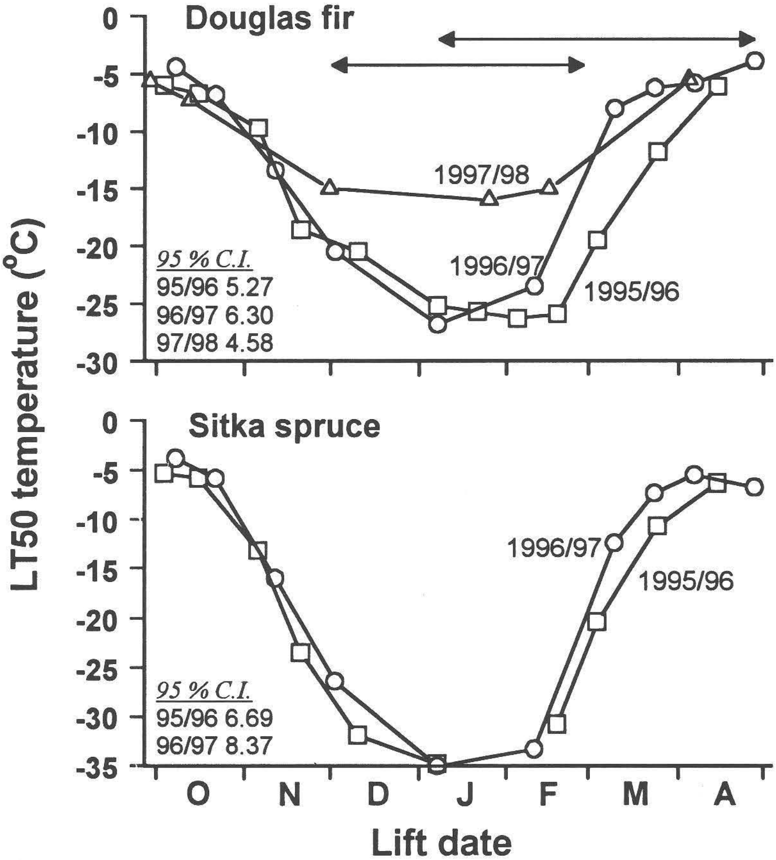

Figure 2. *Seasonal change in cold hardiness of Douglas fir and Sitka spruce.* Periods where cold hardiness differed significantly between lift dates are indicated by arrows. (C.I. is confidence interval).

In Douglas fir in 1995/96 and 1996/97, REL values were relatively high in October and early November (27-37%), then declined to low values (ca. 20%) by January to early February (Figure 3). Thereafter, REL remained relatively similar in 1995/96 (20-25%), but increased to high values (ca. 40%) by March in 1996/97. In 1997/98, REL decreased from 53% in September to about 38% in late November. Values ranged from 36-39% until early March. Thereafter, REL increased again, reaching 45% by early May.

In Sitka spruce, REL was generally low (12 - 21%) and showed no clear seasonal trend in 1995/96 (Figure 3). In 1996/97, REL decreased rapidly from a high (29%) in October to a low (ca.15%) from late November to early February. Thereafter, REL increased gradually to a high (20%) in May.

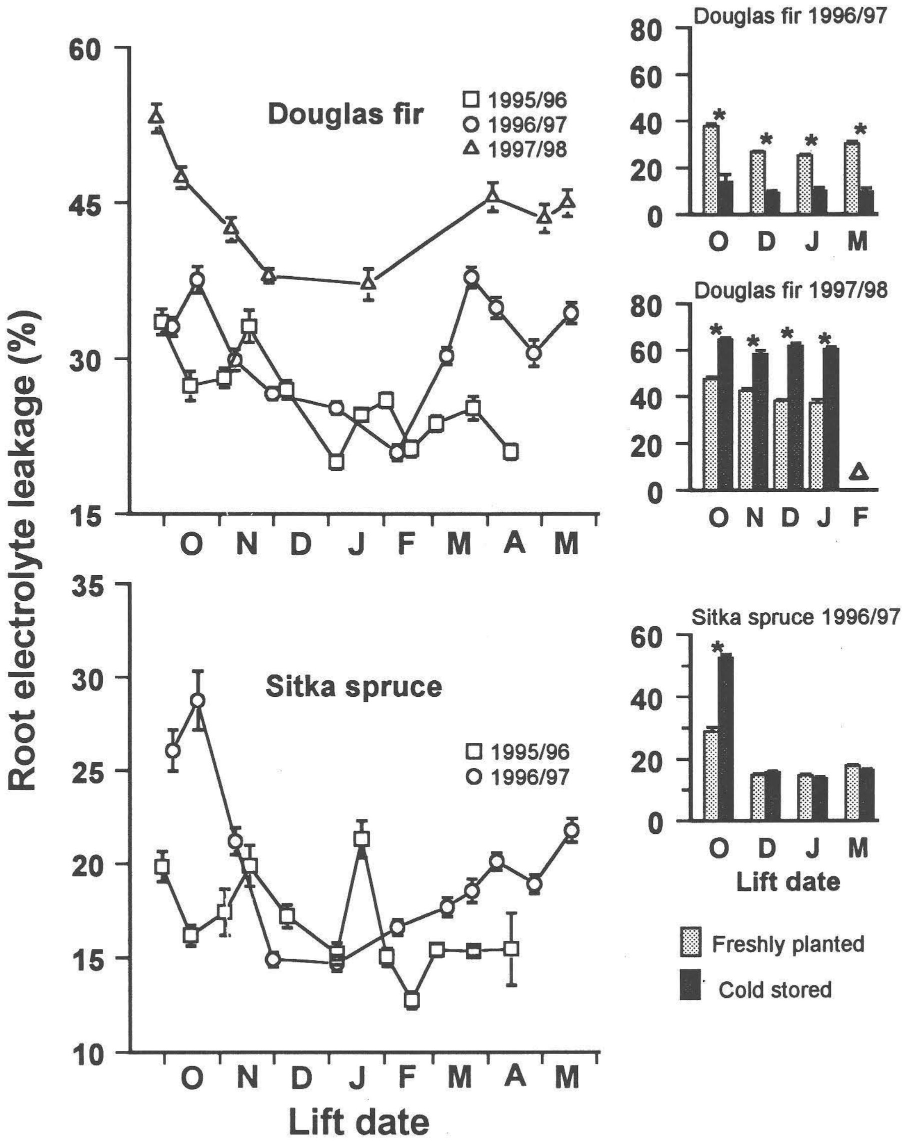

Figure 3. *Root electrolyte leakage of Douglas fir and Sitka spruce seedlings at time of lifting.* Insets show values for stock freshly lifted and following cold storage until May. Vertical bars on symbols indicate standard errors. Significant differences between cold stored and freshly lifted stock are indicted (*).

In Douglas fir, REL was lower ($p \leq 0.01$) following cold storage than at the time of lifting in 1996/97, whereas the reverse was the case in 1997/98 (Figure 3). Survival in the field was much higher in 1996/97 than in 1997/98, in agreement with this trend (Table 3a). In Sitka spruce in 1996/97, REL values after storage were similar to those at the time of lifting in December, January and March, but REL was higher ($p \leq 0.01$) after storage for stock lifted in October. Field mortality also was highest for Sitka spruce lifted to the store in October (Table 3b).

Table 3a. *Survival of Douglas fir seedlings freshly lifted or cold stored until May and planted three to four days later.*

1995/96		*1996/97*			*1997/98*		
Month	*Freshly lifted*	*Month*	*Freshly lifted*	*Cold stored*[2]	*Month*	*Freshly lifted*	*Cold stored*
Oct	99a[1]	Oct	98a	25b*	Oct	88bc	0b*
Nov	98a	Dec	96a	98a	Nov	98ab	0b*
Dec	99a	Jan	99a	98a	Dec	100a	14b*
Jan	100a	Mar	91a	97a	Jan	81c	11b*
Mar	99a	Apr	91a		Feb	96ab	79a
Apr	100a	May	55a		Mar	100a	
					Apr	93ab	
					May	89bc	
p≤	ns[3]		0.0001	0.0001		0.0005	0.0001

[1] Means followed by the same letter are not significantly different.

[2] Cold storage lift dates indicated (*) are significantly different from the freshly lifted stock of that date.

[3] Not significant.

Table 3b. *Survival of Sitka spruce seedlings freshly lifted or cold stored until May and planted three to four days later.*

1995/96		*1996/97*		
Month	*Freshly lifted*	*Month*	*Freshly lifted*	*Cold stored*[2]
Oct	100a[1]	Oct	100a	41.0b*
Nov	100a	Dec	100a	97.5a
Dec	100a	Jan	98.8a	100a
Jan	100a	Mar	100a	100a
Mar	100a	Apr	96.3a	
Apr	98.9a	May	92.5a	
p≤	ns[3]		0.0500	0.0001

[1] Means followed by the same letter are not significantly different.

[2] Cold storage lift dates indicated (*) are significantly different from the freshly lifted stock of that date.

[3] Not significant.

Field performance

The survival of freshly lifted seedlings was generally very high (>90%), except for those planted early or late in the season (Table 3). In both species, differences in survival among lift dates were significant ($p \leq 0.05$) in 1996/97 and 1997/98, but not in 1995/96. Height increment (as percent of initial height) of seedlings showed larger differences among planting dates (Figure 4). Height increment in Douglas fir was best for stock that was freshly planted early in the season, gradually declining to low values for those planted in April and May. Although similar to Douglas fir in the 1995/96 season, the effect of planting date on height increment of Sitka spruce was less clear in 1996/97.

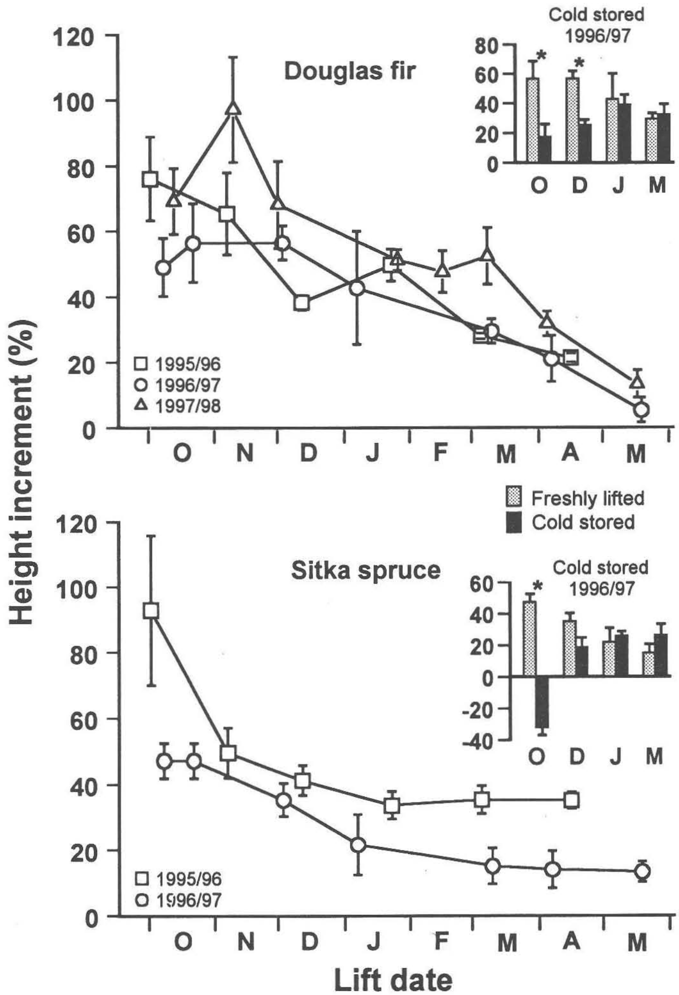

Figure 4. *End-of-season height increment as percent of initial height of seedlings freshly lifted or cold stored until May and planted three or four days later in 1995/96, 1996/97 and 1997/98 spruce (Douglas fir only).* Vertical bars on symbols indicate standard errors.

The survival (Table 3) and height increment (Figure 4) of Douglas fir and Sitka spruce seedlings that had been cold stored from January or March until the time of planting in May were similar to values for the freshly planted stock. However, there were significant differences ($p \leq 0.05$) between the storage treatments for other lift dates. Most of the Douglas fir seedlings that were cold stored in 1997/98 died. Sitka spruce cold stored from October had poor survival and many leading shoots died back, resulting in negative height increment overall

Discussion

The results reported in this paper are a continuation of earlier work (O'Reilly *et al.* 1999, 2000). For this reason, the recommendations on optimum lift/storage dates given here differ slightly than if they were based solely on the results shown in this paper.

Physiological status at the time of lifting

Cold hardiness displayed a clear seasonal trend each year in both species, as found in earlier studies carried out in Ireland (O'Reilly *et al.* 1999, 2000). Cold hardiness varies seasonally mainly in response to seasonal changes in photoperiod and chilling temperatures (normally considered those <5 °C) (Larcher 1995), but species may differ in their relative response to these factors. Cold hardiness acclimation and deacclimation was remarkably similar in 1995/96 and 1996/97 in Sitka spruce (Figure 2). However, shoots of Douglas fir deacclimated significantly earlier in 1996/97 than in the slightly cooler 1995/96. Hybrid larch (*Larix x eurolepsis* Henry) shoots sampled from seedlings lifted from the same nursery (Ballymurn) also dehardened sooner in 1996/97 than in 1995/96 (O'Reilly *et al.* 2001). While differences in cold hardiness acclimation were not significant for the two species in this study, such differences were evident in seedlings of both species sampled from Ballintemple Nursery in earlier studies (O'Reilly *et al.* 1999, 2000). The effect was pronounced in Douglas fir only, similar to the trend detected in this study for the deacclimation phase. Sitka spruce is a coastal species in its native habitat and therefore cold hardiness acclimation and deacclimation may be more heavily influenced by photoperiod than in Douglas fir, which covers a wider longitudinal range (Colombo *et al.* 2001).

Perhaps the most surprising outcome in this study was the observation that Douglas fir did not harden to temperatures colder than -17 °C in 1997/98 (Sitka spruce was not sampled that year). The reasons for this result are not fully clear, but some hypotheses may be advanced. First, a different provenance of Douglas fir was used in 1997/98 than in the two previous years, although all were from a similar part of the species range in Washington. Provenance can have a large impact on the cold hardiness of conifer seedlings (Cannell and Sheppard 1982, Nicoll *et al.* 1996, Colombo *et al.* 2001). Second, three-year-old transplanted seedlings were sampled in 1997/98 from Ballintemple Nursery, whereas two-year-old undercut stock from Ballymurn were used in the two previous years, which may have further confounded treatment effects. Cold hardiness of conifer seedlings is influenced greatly by nursery cultural practices and climatic differences between nurseries (Colombo *et al.* 2001). However, it is unlikely that these factors fully explain the cold hardiness differences. Chilling sum accumulations were similar in Ballymurn in 1996/97 as in Ballintemple in 1997/98 (Figure 1), yet cold hardiness levels differed between these years. Chilling temperatures have a major impact on cold hardiness development in

Douglas fir (Burr *et al.* 1989, O'Reilly *et al.* 1999). Furthermore, in previous research carried out on Douglas fir sampled from Ballintemple Nursery, seedlings became hardy to temperatures colder than -25 °C in three consecutive years, including the very mild 1994/95 (O'Reilly *et al.* 1999), whereas this did not happen in the relatively cool 1997/98. Another possible explanation is that nutrient levels were sub-optimal for cold hardiness development. Nutrient availability is known to have a large effect on the development of cold hardiness in conifer seedlings (Colombo *et al.* 2001). However, there is no evidence to support this claim since shoot tissues were not analysed to determine nutrient levels.

The seasonal course of root electrolyte leakage was less clear in both species, perhaps reflecting fluctuations in root activity. Providing soil temperatures are adequate, root growth may occur throughout much of the winter in Ireland (O'Reilly *et al.* 1999, 2000). Nevertheless, REL was generally lowest in the December/February period each year (except for high peak in January for Sitka spruce in 1995/96), and values increased later in the cooler spring of 1995/96 than in 1996/97. High REL values indicate root damage or high root activity levels (Harper and O'Reilly 2000). REL was exceptionally high in Douglas fir in 1997/98, probably because the roots were more active that year than in other years. The shoots of the seedlings were also less cold hardy during the winter of 1997/98, suggesting that the plants were generally more active then than in other years. The higher than expected REL for Sitka spruce stock lifted in January 1995/96 may have been the result of damage caused during lifting under wet conditions. Although O'Reilly *et al.* (2000) suggested that REL values <16% might indicate readiness for lifting in Sitka spruce, the high variation in REL values among lift dates in both species in this study indicates that the test is of limited value for this purpose. The results reinforce the view that no one test can be relied upon to determine plant quality (Puttonen 1997).

Lifting for field planting

Seedlings lifted for field planting should be sufficiently resistant to the stresses of normal lifting and handling operations; otherwise they will perform poorly after planting. However, post-planting field conditions may also be important. Seedlings of some species, for example, may benefit from planting at certain times of the year because warm soil temperatures allow new root growth. New roots are more efficient in supplying water for the plant's needs than nursery roots (Larcher 1995). Good quality plants may die if planted into a cold soil, probably due to the gradual loss of water during transpiration, water that the plant is unable to absorb through the old roots.

The results from this study showed that the date of planting was important for Douglas fir, but less so for Sitka spruce, which is largely in agreement with earlier findings (O'Reilly *et al.* 1999, 2000). Douglas fir performed best when planted between October and early December. The main reasons for the superior performance of Douglas fir planted at these times are (i) the higher root growth potential of the seedlings at this time (data on file), and (ii) soil temperatures are warm enough to permit root growth. Soil temperatures are unfavourable for root growth from about late December to February, although this is the period when the plants are most resistant to handling stresses. Although soil temperatures also may be favourable for root growth in March/April, root growth potential is very low (shoot activity appears to take precedence) and resistance to handling stresses is declining; poor performance can be expected from late planting. Therefore, it is recommended that Douglas fir should be planted in autumn and early winter, provided soil temperatures are >5 °C. For this reason, the planting season may vary with soil type and location of site in Ireland. In addition to their effects on competition for nutrients and water, the presence of competing vegetation may lower soil temperatures (Low and Greig 1973), thus reducing seedling field performance. Soil preparation and vegetation control seems to be more important in ensuring the successful establishment of Douglas fir than for most other conifers (Tabbush 1988).

The biggest risk in planting Douglas fir during the recommended period, especially in October, is that the plants are not highly stress resistant and may suffer from damage during handling, or while in temporary storage prior to planting. The seedlings will be most resistant to these handling stresses when the shoots have hardened to ≤-10 °C. Nevertheless, recent research results indicate that it should be possible to store Douglas fir stock (in the shade) for a period equivalent to 1,500 degree hours (base >5 °C) (Harper and O'Reilly 2000). This is equivalent to about 12-19 days during the October to December period, based upon 1997 temperatures at Ballintemple nursery. The period of safe storage may be much shorter in some years than in others (especially in October), and in milder coastal and southern locations than at this nursery, but may be longer during colder periods. For this reason, the use of both ambient air and soil temperature data in judging lifting windows is advocated, rather than relying solely on calendar date, especially for Douglas fir.

Planting date had little effect on the survival and height increment of Sitka spruce, but height increment was lowest for those planted late in lifting season. Although field performance was good for stock planted in October and November (especially since planting took place within two to four days of lifting), it is recommended that under operational conditions stock should not be lifted unless they are hardy to -20 °C, commencing about mid-November (Figure 2). Sitka spruce will withstand the stresses of lifting and handling when hardy to this or lower temperatures (O'Reilly *et al.* 2000). There is some evidence that the lifting of QCI Sitka spruce could commence about two to three weeks earlier than this, commencing in early November (O'Reilly and Keane 1996). Lifting should cease when the shoots have dehardened to temperatures warmer than -20 °C, in about early March (Figure 2). Sitka spruce of Washington provenance is less resistant to the stresses of lifting and handling during October and early November than Douglas fir, so planting should be avoided during this period. Unlike Douglas fir, the main criterion in deciding when to lift Sitka spruce is when stress resistance levels are sufficient to permit safe handling. The results of this study and earlier work (O'Reilly *et al.* 2000) indicate that the window of opportunity for lifting Sitka spruce may not change greatly from year to year, since hardiness levels appear to respond strongly to photoperiod. Nevertheless, lifting might be delayed until December in an exceptionally mild year, such as occurred in 1994/95 (O'Reilly *et al.* 2000).

Lifting for cold storage

A major focus of this research was to determine the effect of cold storage on plant quality, especially for Sitka spruce. Cold hardiness is a very useful indicator of readiness to lift stock for planting or cold storage (Tinus and Burr 1997). Field performance after planting was good following long-term (>three months) cold storage until May for Douglas fir and Sitka spruce seedlings; these had achieved cold hardiness levels of -20 and -30 °C, respectively. This confirms results from earlier studies (O'Reilly *et al.* 1999, 2000). The levels of cold hardiness were achieved by the period January to February in Douglas fir and December to early March in Sitka spruce. Shoots of Douglas fir did not harden below -17 °C in 1997/98, and stock was not highly storable that year, reinforcing the validity of the cold hardiness test. The reason why Douglas fir did not harden off and become storable in 1997/98 is unclear, as discussed earlier.

The results of this study confirm the view that long-term cold storage of Douglas fir is viable, despite the problems encountered in 1997/98. However, it is essential that hardiness levels be determined to confirm that the plants are 'storable', whereas this may not be necessary for lifting Sitka spruce for cold storage from January to March. In addition, the quality of the stored Douglas fir should be monitored more often while in cold storage than Sitka spruce, and all seedlings should be removed for planting by April or early May at the latest. The REL test is useful for monitoring quality in storage (McKay and Mason 1991). Although the post-storage performance of Douglas fir stock was poor in 1997/98, survival in the field was better for seedlings placed in storage in February than on other lift dates. Seedlings stored in December, January or February had similar cold hardiness levels, but the duration of cold storage was shortest for those lifted in February. The period of cold storage of Douglas fir should be kept as short as possible (preferably from February), perhaps until April or early May, but not until June (see also O'Reilly *et al.* 1999).

Although field survival was excellent, height increment was usually slightly less than that of the stock lifted freshly lifted on same date. The shorter growing season available to seedlings planted after cold storage is probably the main reason for this outcome. The buds of seedlings planted late (May/June) usually do not flush until four to five weeks later, thus limiting the period available for growth. In another study of hybrid larch carried out in Ireland, the growth of seedlings following cold storage was also reduced (O'Reilly *et al.* 2001). Foresters should be aware that additional vegetation management might be necessary for sites planted late with cold-stored stock. Furthermore, there may be a higher risk of drought stress shortly after planting (even leading to mortality) for cold stored stock, since new roots (which absorb most of the water required by the plant) may not have been initiated prior to the onset of the drought.

In general, REL was a good indicator of post-storage root vitality for Sitka spruce, as

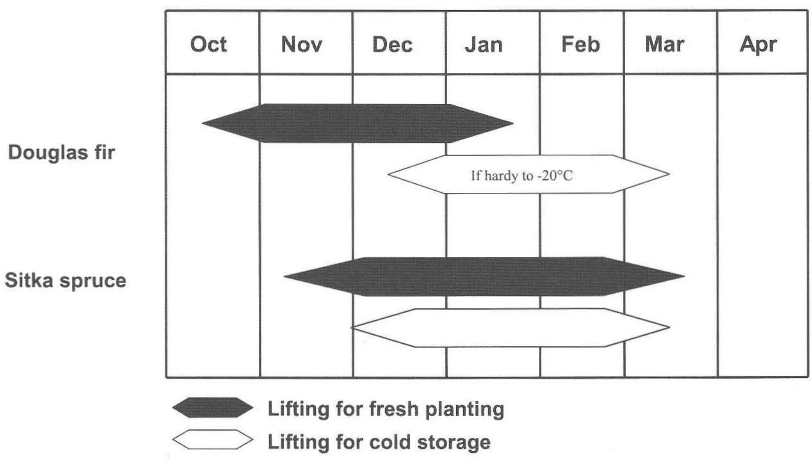

Figure 6. *Recommended lifting dates for Douglas fir and Sitka spruce.* Recommendations are less reliable where bars narrow - seedlings should be planted soon after lifting, or may not be ready for cold storage during these periods.

found previously in this and other species (McKay and Mason 1991, McKay 1992, 1993, 1998, O'Reilly *et al.* 2000). However, the relationship between REL and field performance was less clear for Douglas fir. For example, in 1996/97, post-storage REL values were generally low (indicating good quality), but performance was poor for stock lifted for cold storage in October. Nevertheless, the REL data corroborated the findings for the cold hardiness data in 1997/98; REL values were high at the time of lifting and were >60% after cold storage. Very high REL values were associated with high mortality in Douglas fir planted after cold storage that planting season.

Conclusions and implications for forestry practice

Seedlings can be safely lifted and handled when their shoots have hardened to -10 °C in Douglas fir (November to March) and -20 °C in Sitka spruce (late November/early December to March) (Figure 5). Equivalent values for judging cold storage tolerance are -20 and -30 °C, respectively. However, the height increment of freshly planted Douglas fir declines almost linearly with date of planting from November to May; early planting is recommended (Figure 5). Root electrolyte leakage values could be used to support this information and in assessing post-storage vitality, although the test was less useful for this purpose in Douglas fir. In Sitka spruce, REL values of <20% and <15% indicate readiness for lifting for field planting and cold storage, respectively. REL values should be <25% for cold storing Douglas fir, but the test is not reliable for judging readiness to lift for field planting. Sitka spruce appears to respond more strongly to seasonal changes in photoperiod than Douglas fir; the safe lifting windows may vary little from year to year in Sitka spruce.

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