Changes in cold hardiness of silver fir and larch bare-rooted seedlings during autumn and spring

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ABSTRACT: The objective of this study was to obtain information about changes in cold hardiness of larch and silver fir seedlings during autumn and spring by help of measurements of electrolyte leakage from shoots (SEL) and root system (REL). The values of electrolyte leakage from the untreated (control) root system of silver fir decreased during autumn (from 28% on September 25 to 24% on November 27). Minimum values were reached on March 26. A decrease in electrolyte leakage was found for silver fir shoots (SEL) (the maximum was detected on October 2 – 12% and minimum on November 27 – 7%). Contrary to REL, SEL increased in March. The rate of electrolyte leakage from treated (after artificial frost) roots and shoots decreased during autumn (REL and SEL minimum on November 27). The change in the rate of electrolyte leakage from untreated larch roots was similar to that from silver fir roots during autumn. The values continually decreased from 26% (on September 25) to 12% (on November 27). The course of electrolyte leakage from the treated root system was similar for both species. The differences between electrolyte leakage from larch shoots (treated und untreated ones) were statistically significant, but without any clear tendency during autumn.

Keywords: cold hardiness; electrolyte leakage; larch and silver fir bare-rooted seedlings

The low temperature is a limiting agent for natural distribution of plants (BURKE et al. 1976). Generally, there are no problems with frost damage to dormant seedlings and plants during winter in Central Europe. The trees in dormancy are able to tolerate low temperatures in winter.

On the other hand, there exists a great risk of frost damage to planting stock in spring or autumn. In this period, the plant parts are physiologically active and therefore their cold hardiness is lower. This damage was shown in a decrease in the physiological quality of planting stock (SARVAŠ 2001; O'REILLY et al. 2000).

According to FOLK and GROSSNICKLE (1997) the timing of autumn lifting was considered to be crucial for subsequent field performance, and LAVENDER (1984) stated that dormancy was strongly influenced by weather. O'REILLY et al. (2000) wrote that the cycle of dormancy development and release and changes in cold hardiness levels in nursery plants were related to seasonal changes in photoperiod, temperature, precipitation and other environmental factors. In addition, successful overwintering is affected to a large extent by two factors: seedlings stress resistance and environment (COLOMBO 1997). Therefore, in the first step it is needful to determine the stress resistance (cold hardiness) of different plant tissues and in the second step to suggest available methods how to increase cold hardiness in forest nurseries.

To determine frost damage to shoots, at first the planting stock is subjected to artificial frost and then the degree of damage is measured. It is generally accepted that primary effects of freezing are due to the membrane disruption (STEPONKUS 1984). This injury of cell membranes can be determined by various techniques: browning technique (TIMMIS 1977), electrolytic conductivity method (COLOMBO 1997), electrical impedance method (GLERUM 1973), electrical impedance ratio method (GLERUM 1985).

Manipulations of the irrigation and fertilization regime (LANDIS et al. 1989) and regulation of photoperiod (LANDIS et al. 1992) were used to retard height growth and to initiate hardening of container seedlings. The most promising method is the use of regulated photoperiod. In Canada and in Sweden, photoperiod control, also called short-day (SD) treatment, is used routinely for growing of conifer seedlings (LUORANEN, RIKALA 1997).

It is necessary to underline that for practical determination and regulation of planting stock cold hardiness under nursery practice, the basic research of physiological changes in plants was carried out. On the results of this basic research it was possible to assess objective criteria for determination of cold hardiness. After determination of cold hardiness of planting stock it was possible to modify suggested new cultural practices for increasing the cold hardiness (modification of temperature, photoperiod, fertilization, etc). The basic research of these new technological processes of plant raising was carried out because the technology of increasing the cold hardiness could negatively affect the morphological parameters

Table 1. Age and morphological traits of silver fir and larch seedlings (2000–2001)

Characteristic	Silver fir	Larch
Age	4 + 0	2 + 0
Height (cm)	14.8	35.0
Diameter of root collar (mm)	3.9	3.9

of planting stock (HAWKINS, DRAPER 1991; BIGRAS, D'AOUST 1993).

The objective of this study was to acquire the first information about changes in cold hardiness of larch and silver fir seedlings during autumn and spring by help of measurements of electrolyte leakage from shoots and root system.

MATERIALS AND METHODS

Silver fir (*Abies alba* Mill.) and larch (*Larix decidua* Mill.) seedlings cultivated in a nursery of Forest Research Institute in Zvolen were used in this study. The biometrical characteristics and age of tested planting stock are given in Table 1. Unfortunately, no complex information about plant raising in the nursery is available.

Seedlings were lifted at one-week intervals in autumn (from September 25 to November 27) and in spring (two sample dates, March 12 and 26). On each occasion 30 seedlings were lifted. Directly after lifting the seedlings were checked carefully and only plants without mechanical damage were used (untreated seedlings). Samples of shoots and roots were taken from 15 plants to prepare the procedure of electrolyte leakage measurement. The remaining 15 plants were transported to a lab of Forest Research Institute in Zvolen and used for cold hardiness tests. These whole plants were placed in polyurethane bags and to a refrigerator (temperature 2°C). After two hours, the plants were put to a climatic room for 20 hours (temperature –16°C). After this artificial frost treatment and one hour in the room temperature samples from roots and shoots were taken for electrolyte leakage measurement (treated seedlings).

Table 2. Weather conditions from September 2000 to March 2001

Month	Precipitation (mm)	Air temperature (°C)				Note*
		mean	temperature deviation	max.	min.	
September	24.2	13.4	0.2	27.8	1.9	Maximum temperature between September 10–14 (21.3 to 28.8°C) Minimum temperature on September 7 and 26 (3.6 and –1.1°C)
October	41.1	11.1	2.9	25.4	–3.3	Maximum temperature on October 4 and between 13–15 (24.0 to 27.5°C) Minimum temperature between October 23–24 (0.4 to –5.5°C)
November	98.8	6.8	3.3	14.0	–3.8	Maximum temperature on November 14 and 17 (13.3 and 18.6°C) Minimum temperature on November 11–12, 28, and 30 (–1.4 to –3.8°C)
December	50.6	0.4	1.8	10.5	–13.0	Maximum temperature between December 11–13 (9.0 to 14°C)
January	74.1	–1.2	2.8	5.5	–13.2	Maximum temperature 8 January (5.5 to 9.5°C) Minimum temperature between 15–16 January (–8.4 to –18.2°C)
February	27.3	–0.7	0.8	10.7	–13.2	Maximum temperature 8 and 9 February (9.5 and 15.6°C) Minimum temperature 3 and between 25– 27 February (–8.5 to –22.7°C)
March	66.5	4.3	1.5	14.9	–4.1	Maximum temperature 12 and 25 March (10.5 and 19.1°C) Minimum temperature between 2, 3, 7 and 28 March (–3.0 to –9.3°C)

*Max. and min. temperatures were not available for Sliač weather station. The range of these temperatures was obtained from 10 weather stations in Central Slovakia

Table 3. The rate of electrolyte leakage ± one standard error from silver fir roots (REL) and shoots (SEL) (2000–2001)

Data	Untreated		Treated	
	REL (%)	SEL (%)	REL (%)	SEL (%)
25. 9.	28 ± 4[a]	11 ± 4[a/b]	73 ± 5[a/b]	28 ± 8[b]
2. 10.	28 ± 2[a]	12 ± 2[a]	67 ± 4[a/b/c]	29 ± 9[b]
9. 10.	28 ± 3[a]	13 ± 3[a]	75 ± 5[a]	39 ± 8[a]
16. 10.	25 ± 1[a/b]	10 ± 2[a/b]	65 ± 5[c/d]	21 ± 6[b/c]
23. 10.	–	–	68 ± 4[a/b/c]	19 ± 7[c/d]
6. 11.	25 ± 2[a/b]	10 ± 2[a/b]	63 ± 5[c/d/e]	17 ± 4[c/d]
13. 11.	–	10 ± 2[a/b]	57 ± 3[e]	14 ± 3[c/d]
20. 11.	25 ± 1[a/b]	9 ± 2[a/b]	60 ± 5[d/e]	15 ± 4[c/d]
27. 11.	24 ± 2[a/b]	7 ± 1[b]	50 ± 9[f]	12 ± 3[d]
12. 3.	–	9 ± 2[a/b]	–	13 ± 3[c/d]
26. 3.	19 ± 7[b]	13 ± 7[a]	62 ± 9[c/d/e]	18 ± 13[c/d]

Tukey's HSD test was used for means separation ± standard error of treatment at the 5% level. Different letters show significant differences

The modified method according to McKAY (1992) was used for all electrolyte leakage measurements. The root system was washed in cold tap water to remove soil and rinsed in deionized water to remove surface ions. The sample length from main root (taken directly under the root collar) and shoot (taken directly under the shoot bud) was 2 cm. Individual samples were put to 40 ml universal glass bottles containing 30 ml deionized water of conductivity < 3 μS/cm. The bottles were capped and left at a room temperature for 24 h. The bottles were shaken (5×) and the conductivity of bathing solution was measured using a conductivity meter LF 320 with built-in temperature compensation 25°C. After that the samples were killed through autoclaving at 110°C for 10 minutes. The second conductivity measurement was made 24 hours after the autoclaving. The total conductivity was:

$$\text{REL/SEL}(\%) = \frac{\textit{conductivity after } 24\ h}{\textit{conductivity } 24\ h \textit{ after autoclaving}} \cdot 100$$

Air temperatures, and rainfall dates were obtained from Sliač weather station (Table 2), 4 km from the nursery.

Statistical analysis

Data on the rate of electrolyte leakage was processed by analysis of variance, and Tukey's HSD test was used for mean separation of treatments at the 5% level.

RESULTS

Silver fir

Table 3 shows the results of electrolyte leakage from silver fir roots and shoots during autumn 2000 and March 2001. The values of electrolyte leakage from the untreated root system decreased during autumn (from 28% on September 25 to 24% on November 27). Minimum values were reached on March 26. The same tendency was measured for electrolyte leakage from silver fir shoots (the maximum was detected on October 2 – 12% and minimum November 27 – 7%). Contrary to REL, SEL increased in March.

The rate of electrolyte leakage from treated roots and shoots decreased during autumn (REL and SEL minimum on November 27).

Generally, the electrolyte leakage from treated and untreated seedlings decreased during autumn. The exception was October 9, when the values of electrolyte leakage increased in comparison with those on October 2. The increase was statistically significant for treated shoots (from 29% to 39%).

Larch

The rate of electrolyte leakage from untreated larch roots was similar to that in silver fir roots during autumn. The values continually decreased from 26% (on September 25) to 12% (on November 27). The course of electrolyte leakage from treated roots was similar like in untreated roots except on November 9, when the rate of electrolyte leakage reached maximum values (89%). The differences in electrolyte leakage from shoots (treated und untreated ones) were statistically significant, but without any clear tendency during autumn (Table 4).

DISCUSSION

The planting stock is likely to be resistant to stresses when cold hardy and dormant (BURR 1990; MARTINCOVÁ 1990; RITCHIE 1989; TINUS, BURR 1997, etc.). Cold hardiness is defined as the ability of plants to survive without damage at temperatures below 0°C (LARCHER 1985).

Table 4. The rate of electrolyte leakage ± one standard error from larch roots (REL) and shoots (SEL) (2000–2001)

Data	Untreated		Treated	
	REL (%)	SEL (%)	REL (%)	SEL (%)
25. 9.	26 ± 4[a]	8 ± 1[b]	79 ± 4[b]	49 ± 19[a/b]
2. 10.	–	–	75 ± 4[b]	59 ± 11[a]
9. 10.	26 ± 4[a]	10 ± 5[b]	89 ± 5[a]	62 ± 18[a]
16. 10.	20 ± 2[b]	8 ± 2[b]	73 ± 5[b]	59 ± 13[a]
23. 10.	–	–	77 ± 6[b]	45 ± 18[a/b]
6. 11.	17 ± 3[b/c]	17 ± 9[b]	62 ± 5[c]	54 ± 14[a]
13. 11.	14 ± 2[c/d]	40 ± 15[a]	55 ± 8[c]	38 ± 12[b/c]
20. 11.	13 ± 2[c/d]	35 ± 17[a]	55 ± 11[c]	54 ± 21[a]
27. 11.	12 ± 2[d]	15 ± 3[b]	56 ± 12[c]	22 ± 7[b/c]
12. 3.	15 ± 2[b/c/d]	14 ± 2[b]	–	16 ± 2[d]

Tukey's HSD test was used for means separation ± standard error of treatment at the 5% level. Different letters show significant differences

Dormancy status, cold hardiness levels, root growth potential and other physiological attributes are key determinates of plant physiological condition (O'REILLY et al. 2001) and physiological condition of planting stock at the time of planting is an essential factor of successful field establishment (COLOMBO, NOLAND 1997; SARVAŠ 2002, 2003, etc.).

The cold hardiness of planting stock is a very important factor for raising of bare-rooted and containerized planting stock. The lifting of bare-rooted planting stock can influence the field performance potential for a long time. It is needful that the planting stock is under dormant status and cold-hardy during lifting and handling because it is most likely to be resistant to stresses (LAVENDER 1984; RITCHIE 1989; SARVAŠ 2001, 2003, etc.). Detection of frost damage or poor root growth potential saved million dollars over the last ten fifteen years in North America alone (DUNSWORTH 1997). On the other hand, there is little information about cold hardiness of planting stock in Central Europe.

Silver fir

The electrolyte leakage from untreated seedlings slightly decreased during autumn. On the other hand, a rapid decrease was found in electrolyte leakage from treated seedlings during autumn (REL – from 73% on September 25 to 50% on November 27 and SEL – from 28% on September 25 to 13% on December 12). Apart from October 9, when electrolyte leakage reached maximum values. According to LAVENDER (1984) dormancy and cold hardiness development and growth are considerably influenced by weather and O'REILLY et al. (2000) found that the annual cycle of physiological development and release followed a seasonal pattern. On October 4 the temperature reached 25°C and it probably caused the increase in electrolyte leakage on November 10.

In spring 2001, the electrolyte leakage increased as a response to the beginning of physiological activity of seedlings. According to REPO and PELKONEN (1986), JUNTILLA (1989) dehardening occurs primarily in response to rising temperatures in the spring.

Larch

The REL values from untreated and treated seedlings markedly decreased during autumn. O'REILLY et al. (2000, 2001) suggested that REL could be used as an indicator of root activity for hybrid larch and Sitka spruce seedlings. Low values may be associated with the processes of winter hardening (ZHAO et al. 1995). Our results go with this detection. According to O'REILLY et al. (2001) REL values (from fine roots) under 20% in larch may indicate that root activity levels are low enough to allow the lifting process. In this study, the REL (from mainly root) values under 20% from untreated seedlings were reached after October 6.

The differences in SEL values were statistically significant, but without any clear tendency. Larch is a deciduous conifer, losing its needles primarily in response to decline of photoperiod and cool temperature (MATTSSON, LASHEIKKI 1998). In the study of O'REILLY et al. (2001) cold hardiness (LT_{50}) increased from 0 to –4°C in mid-October to nearly –30°C in mid-December. This cold hardiness (LT_{50}) was assessed by measurements of electrolyte leakage after different freezing temperatures in the range of –3 to –35°C. All needles were removed from the shoots after freezing. In our study the temperature –16°C was used (without moderate decrease in the temperature) and the needles were not removed. The differences in SEL values were highly probably caused by physiological changes in needles during autumn.

CONCLUSION

The results of this study show the changes in cold hardiness of silver fir and larch seedlings at the autumn and spring beginning. The measurement of electrolyte leakage could be used as an indicator of cold hardiness of planting stock. In addition, it is needful to carry out further research on the cold hardiness of planting stock. On the basis of these results it could be possible to optimize cultural practices (sowing, fertilization, lifting, handling, cold storing, etc.) for planting stock in forest nurseries.

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Received for publication November 5, 2003
Accepted after corrections January 19, 2004

Zmeny mrazuvzdornosti voľnokorenných semenáčikov smrekovca a jedle počas jesene a jari

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ABSTRAKT: Cieľom štúdie bolo získať prvotné informácie o priebehu zmien v odolnosti na chlad pri semenáčikoch smrekovca a jedle. Toto zisťovanie sa uskutočnilo počas jesene 2000 a jari 2001 pomocou merania straty elektrolytu. Strata elektrolytu z koreňového systému jedľových kontrolných semenáčikov klesala počas jesene (z 28% úrovne 25. septembra na 24% úroveň 27. novembra). Minimálne hodnoty straty elektrolytu boli zaznamenané 26. marca. Bol zaznamenaný pokles hodnôt straty elektrolytu z koreňového systému jedle (kontrolný variant) – maximálna hodnota bola zaznamenaná 2. októbra –12 % a minimálna

27. novembra – 7 %. Naproti hodnotám straty z koreňového systému hodnoty straty elektrolytu zo stonky stúpali v marci. Hodnoty straty elektrolytu z koreňového systému smrekovca klesali počas jesene. Tieto hodnoty kontinuálne klesali z 26 % (25. septembra) na 12 % (27. novembra). Priebeh hodnoty straty elektrolytu z koreňov po strese mrazom bol rovnaký pri oboch drevinách. Rozdiely v hodnotách straty elektrolytu zo stoniek smrekovca (kontrolných aj po mrazovom teste) boli štatisticky významné, ale bez jasnej tendencie.

Kľúčové slová: odolnosť na mráz; strata elektrolytu; voľnokorenné semenáčiky smrekovca a jedle

Nízka teplota je limitujúcim faktorom pre prirodzené rozšírenie rastlín. Vo všeobecnosti nie sú v strednej Európe problémy s odolnosťou na mráz pri sadbovom materiále počas zimy. Sadbový materiál v dormantnom stave je schopný znášať nízke teploty.

Na druhej strane existuje veľké nebezpečenstvo mrazového poškodenia sadbového materiálu počas jarného a jesenného obdobia. Sadbový materiál je vplyvom nástupu (resp. ukončovania) fyziologickej aktivity schopný znášať prípadné poškodenia nízkymi teplotami na veľmi rozdielnej úrovnil Úroveň dormancie a tým mrazuvzdornosť v tomto období nie je ovplyvňovaná len environmentálnymi faktormi (teplota, dĺžka a intenzita fotoperiódy), ale významnými faktormi sú aj samotné postupy pestovania sadbového materiálu v lesnej škôlke (intenzita a termín hnojenia, zavlažovanie, úprava fotoperiódy atď.). V škôlkárskej praxi (Kanada, Švédsko) je bežným technologickým postupom úprava dĺžky fotoperiódy ako faktora na zvýšenie mrazuvzdornosti pri dopestovávaní krytokorenného ihličnatého sadbového materiálu. Je ale potrebné zdôrazniť, že pred praktickým stanovením a následnou úpravou mrazuvzdornosti bolo potrebné uskutočniť výskum fyziologických zmien sadbového materiálu a na základe týchto výsledkov navrhnúť jednotlivé vhodné postupy na zvyšovanie odolnosti na mráz.

Cieľom štúdie bolo získať informácie o zmenách mrazuvzdornosti pri voľnokorenných semenáčikoch jedle a smrekovca počas jesene a jari pomocou merania straty elektrolytu zo stonky a koreňového systému.

V štúdii sa použili štvorročné, resp. dvojročné semenáčiky jedle a smrekovca. Semenáčiky boli vyzdvihované v týždňových intervaloch (od 25. septembra do 27. novembra) zo záhona lesnej škôlky. Semenáčiky boli vyzdvihnuté rovnako v dvoch termínoch v nasledujúcu jar. V každom termíne bolo vyzdvihnutých 30 semenáčikov. Priamo po vyzdvihnutí boli z koreňového systému a stonky odobraté vzorky (15 semenáčikov) na meranie straty elektrolytu (kontrolný variant). Zvyšných 15 semenáčikov bolo použitých na umelý mrazový test. Semenáčiky boli zabalené do polyuretánového vreca a umiestnené do chladničky na dve hodiny pri teplote 2 °C. Následne boli premiestnené do klimatizovanej komory na dobu 20 hodín pri teplote –16 °C. Po tomto umelom mraze boli semenáčiky ponechané hodinu pri izbovej teplote a následne boli odobraté vzorky z koreňa a zo stonky.

Na základe dosiahnutých výsledkov je možné konštatovať, že hodnoty straty elektrolytu mierne klesali pri kontrolných semenáčikoch jedle počas jesene. Na druhej strane bol zistený prudký pokles hodnôt straty elektrolytu pri semenáčikoch po umelom mraze (REL z úrovne 73 % 25. septembra na 50% úroveň 27. novembra), rovnaký prudký priebeh zmien straty elektrolytu bol zaznamenaný aj pri stonke (z 28% úrovne 25. septembra na 12% úroveň 27. novembra).

Hodnoty straty elektrolytu z koreňového systému smrekovcových semenáčikov výrazne klesali počas jesene po mrazovom teste. Rozdiely v hodnotách strát elektrolytu zo stonky boli štatisticky významné, ale bez jednoznačnej tendencie. Rozdiely v hodnotách straty elektrolytu boli pravdepodobne výrazne ovplyvnené fyziologickými zmenami v ihliciach počas jesene.

Získané výsledky preukázali možnosti zisťovania mrazuvzdornosti pomocou merania straty elektrolytu. Je potrebné uskutočniť ďalšie výskumy zmien v odolnosti na mráz a na základe týchto výsledkov optimalizovať celkový postup dopestovávania sadbového materiálu.

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