Change of cold hardiness in bare-rooted Norway spruce planting stock during autumn and its effect on survival

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ABSTRACT: The objective of this study was to test a method of measurements of electrolyte leakage for determining an optimal autumn lifting date. The second objective was to obtain information about the effects of different autumn lifting dates on survival of Norway spruce (*Picea abies* [L.] Karst.) plants. A significant difference was found between lifting dates for the values of root electrolyte leakage (REL) from unstressed plants, but without clear tendency. The values of electrolyte leakage from shoots (SEL) were very stable (11–13%). On the other hand, the electrolyte leakage from roots (REL) decreased in dependence on different lifting date after artificial frost stress. On the first lifting date (end of September) the REL values were 77%. On the last lifting date (8 November) the REL values were 56%. The same tendency was found for SEL values (decrease from 63% at the end of September to 17% on 8 November). Differences were also found in the survival of plants. The plants lifted on earlier dates had nearly 100% mortality, which decreased with later date of lifting. The results of this study showed that cold hardiness of planting stock increased during autumn and was higher for shoots than for roots. The first results showed that it is possible to optimize the autumn lifting date of spruce planting stock by measurements of electrolyte leakage from shoots after artificial frost test.

Keywords: cold hardiness; electrolyte leakage; lifting date; spruce planting stock

The annual rate of reforestation in the Slovak Republic is about 12,000 ha (ANONYMOUS 2000) and about 95% of the total area is planted with bare-rooted stock. Generally, the reforestation program is carried out in spring and it is affected by weather conditions (temperature and rainfall).

In the last years, weather conditions have been characterized by extreme temperature amplitudes. After low temperatures in February a warm period came in March and lead to the budbreak of plants in nursery beds. On the other hand, the bare-rooted stock for reforestation can not be lifted due to frozen soil. These facts affect the physiological quality of planting stock very negatively. The poor physiological quality of plants causes low survival and field performance.

Approximately 39% of the sites have to be replanted due to plantation failures. These failures are probably caused by a variety of factors, including plantation site conditions, but plant quality of bare-rooted plants is probably a major contributing factor (ANONYMOUS 2000).

One solution is plant lifting from nursery beds in autumn and their cold storage during winter. This process has its own advantages. First of all, it is independent of weather conditions in spring. It is possible to suspend the time of reforestation works. On the other hand, the disadvantage of using cold storage during winter is additional cost and complicated work.

Many conditions must be satisfied for successful cold storage. There are *technical conditions* in climatic storage (air temperature and humidity). The *health condition of plants* is another very important agent. After lifting it is necessary to carry out strict health selection of plants and to bring only healthy and mechanically undamaged stock to climatic storage. The third factor that affects successful winter indoor storage is *dormancy of planting stock* during autumn lifting. According to FOLK and GROSSNICKLE (1997) 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 cycle of dormancy development and release and changes in cold hardiness levels in nursery plants were cued to seasonal changes in photoperiod, temperature, precipitation and other environmental factors.

In Central Europe weather conditions have very unstable development and therefore it is very difficult to fix the term of autumn lifting according to date. Many parameters have been studied to find an optimal date of autumn lifting of planting stock. In Northern Europe it is dry weight of terminal buds (ROSVALL-ÅHNEBRINK 1985), visual damage and subsequent growth, electrical impedance GLERUM (1973, 1985), O'REILLY et al. (2000) used shoot and root mitotic index.

It is generally accepted that primary effects of freezing are due to the membrane disruption (STEPONKUS 1984) and cold hardiness of all tissues increases over autumn sample dates reaching maximum hardiness at midwinter

(SIMPSON 1994). This injury of cell membranes could be determined by: browning technique (TIMMMIS 1977), electrolytic conductivity method (COLOMBO et al. 1984), electrical impedance method (GLERUM 1973), electrical impedance ratio method (GLERUM 1985).

The objective of this study was to test a method of measurements of electrolyte leakage after artificial frost stress for determining an optimal autumn lifting date for bare-rooted Norway spruce planting stock.

The second objective was to obtain information about the effects of different autumn lifting dates on survival of Norway spruce after cold storage.

MATERIALS AND METHODS

Norway spruce transplants (2 + 3) were used in this study. (Fatransko-podtatranská) seed zone 2, approximate altitude of mature stand 1,200 m above sea level. The plants were grown at Jochy Nursery (830 m a.s.l., annual temperature 6°C). The data on air temperatures were obtained for a weather station in Liptovský Hrádok (7 km from Jochy Nursery).

The mean heights of transplants were 40 ± 10.5 cm and their mean root collar diameter 7.1 ± 3.0 mm. Plants were lifted at one week interval from 20. 9. 1999 to 8. 11. 1999 (8 sample dates). On each occasion 140 plants were lifted. Directly after lifting the plants were carefully controlled and only plants without mechanical damage were used. On each sample date 100 plants were placed in polyurethane bags (in each bag 25 plants were spliced with root system – shoots were out of the bag). These bags were placed to a refrigerated storage house (air temperature 1–2°C and air humidity 92–95%). Directly in the nursery, samples of shoots and roots were taken from 20 plants for an electrolyte leakage test. The remaining 20 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 refrigerator (temperature 2°C). After two hours, the plants were put to a climatic room for 20 hours (freezing test, temperature –16°C). After this artificial frost treatment and one hour at room temperature the samples of roots and shoots were taken for electrolyte leakage measurements.

On 25. 4. 2000, the cold storage of plants finished and the plants were transported to Beňuš forest enterprise where the plants were set out on the next day. Electrolyte leakage from roots (REL) and shoots (SEL) ($n = 20$) was measured directly before planting. The plants were set out in a randomized block design on clear-cut area Jasenienok (1,400 m a.s.l., soil pH 3.1, spacing of plants 1.5 m). During the vegetation period the plants were maintained free using weed cutting. In this randomized block plants ($n = 25$) were arranged according to the date of lifting and two replications were used (Table 1).

The modified method by 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 of roots (taken directly under root collar – REL) and shoots (taken 5 cm under shoot bud – SEL) was 2 cm. Individual samples were put to 40ml universal glass bottles containing 30 ml deionized water of conductivity < 3 μS/cm. The bottles were capped and left at room temperature for 24 h. The bottles were shaken (5×) and the conductivity of bath solution was measured using the conductivity meter LF 320 with built-in temperature compensation 25°C. Then the samples were killed through autoclaving at 110 °C for 10 minutes. The second conductivity measurement was made in 24 hours after autoclaving. The total conductivity was as follows:

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

Electrolyte leakage was calculated for all measurements. The index of injury (I_t) according to FLINT et al. (1967) was calculated for plants after artificial frost test.

$$I_t = \frac{\text{EL frozen} - \text{EL unstressed}}{1 - \dfrac{\text{EL unstressed}}{100}}$$

According to GLERUM (1985) the main environmental factors that trigger the frost hardiness process are temperature and photoperiod and therefore average temperature (M_t) was calculated for each lifting date:

$$M_t = \frac{\text{daily temperature 5 days before lifting date}}{5}$$

Statistical analysis

The rate of electrolyte leakage was treated with analysis of variance, and Tukey's HSD test was used for mean separation of treatments at the 5% level. The survival dates were transformed to arcsine square roots before analysis.

Table 1. Arrangement of plants according to lifting date in randomized block

2	6	2	6
8	7	5	1
4	2	3	7
5	1	4	8

1 – lifting on 20. 9. 1999 5 – lifting on 18. 10. 1999
2 – lifting on 27. 9. 1999 6 – lifting on 25. 10. 1999
3 – lifting on 4. 10. 1999 7 – lifting on 1. 11. 1999
4 – lifting on 11. 10. 1999 8 – lifting on 8. 11. 1999

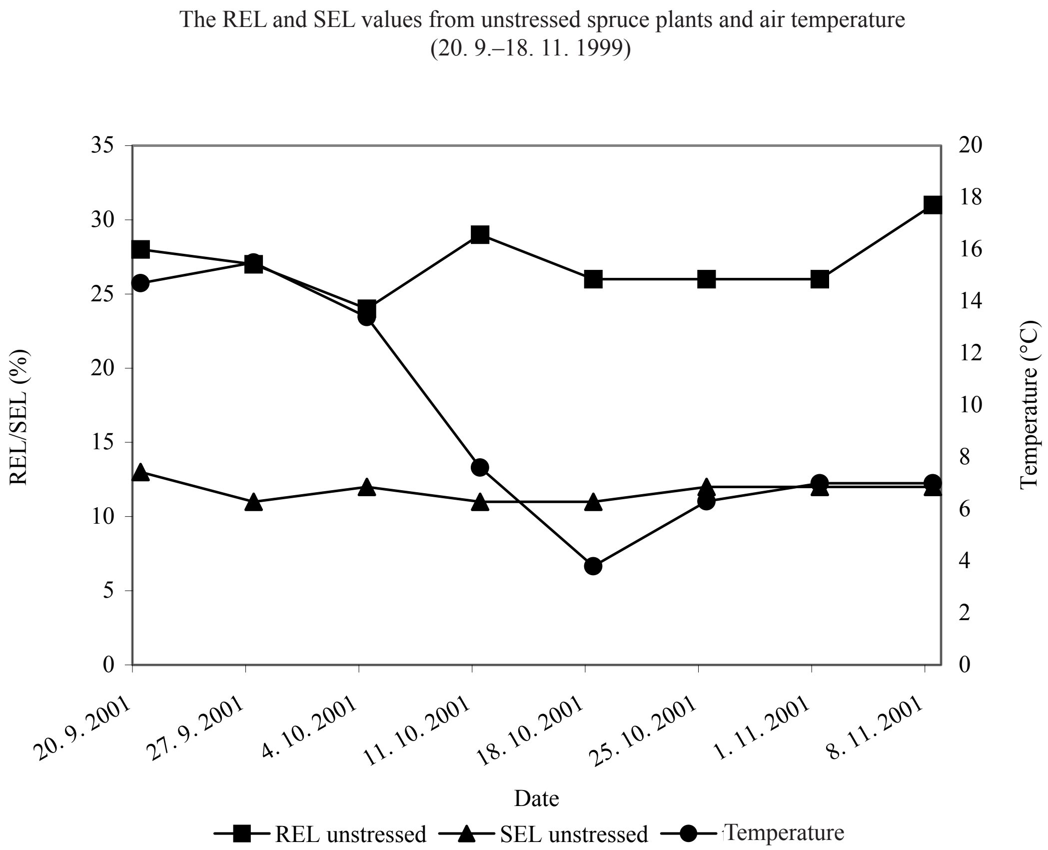

Fig. 1. The rate of electrolyte leakage from shoots and roots and daily temperatures on different lifting dates

RESULTS

Electrolyte leakage from unstressed plants

Differences were found in electrolyte leakage rates between the shoots and roots of unstressed plants (Table 2). The REL values were 24–31% without any clear tendency during autumn. The SEL values showed lesser amplitudes (11–13%) than roots. The changes of temperature did not affect electrolyte leakage from unstressed plants (Fig. 1).

Table 2. The rate of electrolyte leakage ± one standard error from roots (REL) and shoots (SEL) directly after lifting in autumn 1999 (unstressed plants)

Variant	REL%	SEL%
1	$28 \pm 3.9^{a/b}$	13 ± 5.5^{a}
2	$27 \pm 3.5^{a/b}$	11 ± 1.6^{a}
3	24 ± 5.6^{b}	12 ± 3.0^{a}
4	$29 \pm 5.0^{a/b}$	11 ± 1.4^{a}
5	$26 \pm 4.8^{a/b}$	11 ± 2.6^{a}
6	$26 \pm 5.3^{a/b}$	12 ± 4.4^{a}
7	$26 \pm 5.6^{a/b}$	12 ± 3.0^{a}
8	31 ± 6.5^{a}	11 ± 2.2^{a}

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

Electrolyte leakage after artificial frost test

The rate of electrolyte leakage decreased during autumn. REL decreased from 77% (on 20 September) to 56% (on 8 November). The SEL values decreased from 63% to 17% during autumn (Table 3). The same trend was shown by the index of injury of shoots (I_t).

Field performance

The plants were set out at the end of April and their survival was observed after the first vegetation period. The rate of electrolyte leakage was measured before planting (Table 4). The electrolyte leakage from roots amounted to 50% (lifting date on 20. 9.) and it was statistically significantly different from the other lifting dates.

In this study the plants were placed in cold storage for 217 days (lifting date 20. 9. 1999). The electrolyte leakage in these plants was 50% for REL and 35% for SEL and the plants had 100% mortality. On the other hand, the plants lifted as the last ones (8. 11. 1999) reached 28% REL and 31% SEL and 96% survival (169 days in cold storage, Fig. 2).

DISCUSSION

Spring planting of bare-rooted stock is very often used in afforestation and reforestation programs in Central

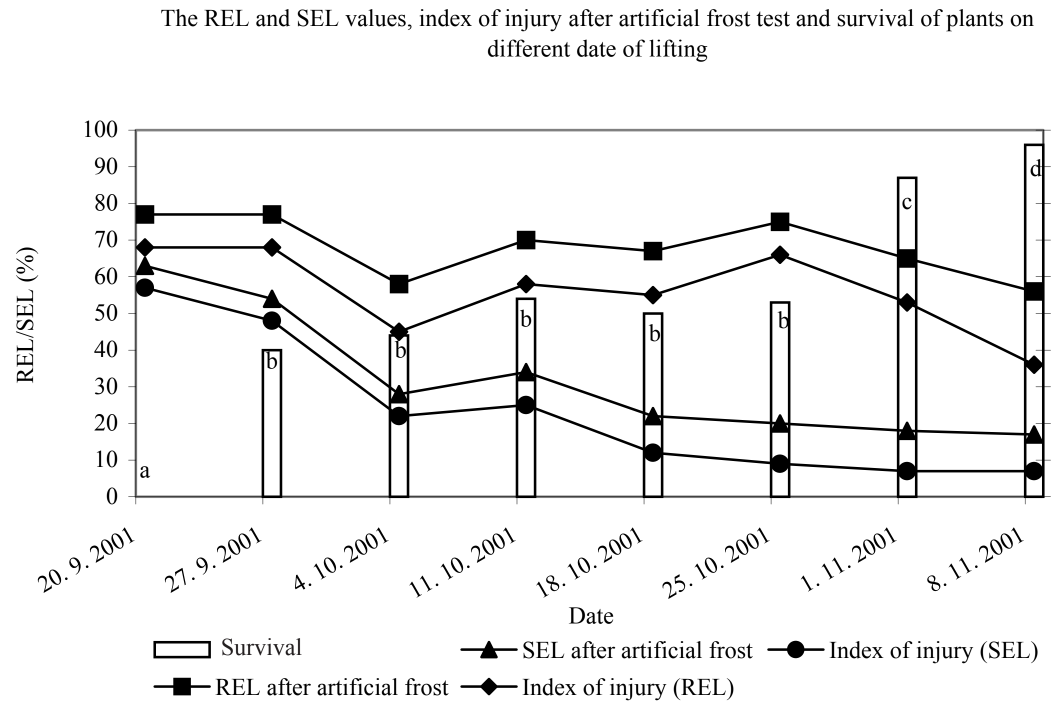

Fig. 2. Survival of plants and electrolyte leakage from plants lifted on different dates. (The survival dates were transformed to arcsine square roots before analysis and Tukey's HSD test was used for means separation of treatments at the 5% level)

Table 3. The rate of electrolyte leakage ± one standard error with index of injury (calculated similarly to FLINT et al. 1967) from roots (REL%) and shoots (SEL%) directly after frost treatment in autumn 1999

Variant	REL%	$I_{t(REL\%)}$	SEL%	$I_{t(SEL\%)}$
1	77 ± 6.8[a]	68	63 ± 6.4[a]	57
2	77 ± 6.6[a]	68	54 ± 5.3[b]	48
3	58 ± 10.6[d]	45	28 ± 6.8[d]	22
4	70 ± 6.2[b/c]	58	34 ± 8.1[c]	38
5	67 ± 10.4[c]	55	22 ± 4.2[e]	12
6	75 ± 11.8[a/b]	66	20 ± 4.4[e/f]	9
7	65 ± 10.9[c]	53	18 ± 5.0[f]	7
8	56 ± 9.7[d]	36	17 ± 4.3[g/f]	7

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

Europe. But these programs can be affected negatively by weather conditions. High temperatures come after low temperatures, influencing the budbreak of plants in nursery beds. One solution to this problem is autumn lifting of planting stock and cold storage during winter. A crucial factor for successful overwintering of planting stock is its dormancy during autumn lifting. There are many papers dealing with this issue (BURR et al. 1990; COLOMBO 1994; FLINT et al. 1967; LINDSTRÖM, MATTSSON 1989; MARTINCOVÁ, HRABÍ 1985; O'REILLY et al. 2000; SAMPSON et al. 1997; TINUS, BURR 1997; TINUS 1996; etc.). On the other hand, there is relatively little information about determination of optimal lifting date by help of cold hardiness assessment in Central Europe.

Bare-rooted 5-years old plants of Norway spruce were used in this study. These plants had a relatively small mean

Table 4. The rate of electrolyte leakage ± one standard error from roots (REL) and shoots (SEL) for plant lifting in autumn 1999 (measurement on 25. 4. 2000)

Variant	REL%	SEL%
1	50 ± 15[a]	35 ± 12[a]
2	29 ± 4[b]	28 ± 7[a]
3	30 ± 7[b]	34 ± 7[a]
4	29 ± 5[b]	27 ± 11[a]
5	31 ± 8[b]	29 ± 5[a]
6	34 ± 7[b]	29 ± 9[a]
7	35 ± 7[b]	36 ± 7[a]
8	28 + 4[b]	31 ± 4[a]

Tukey's HSD test was used for means separation ± standard error of treatment at the 5% level. Different letters show significant differences height of shoots and height standard error. This was probably affected by seed provenance. The plants from mountain areas grew more slowly and their height variation was caused by pollination of some trees with pollen from trees situated at lower locations (MRÁČEK, LOKVENEC 1974).

The method of measurement of electrolyte leakage from roots and shoots was used for the first testing. This method is based on this fact: when the tissue is injured, the site of injury is the cell membrane that loses is selective permeability (GLERUM 1985). COLOMBO et al. (1984) found that measurement of electrolyte leakage expressed in percent is an excellent technique for determining the frost hardiness of coniferous seedlings.

In this study a different procedure for induction of artificial frost stress was used. In other studies climatic rooms were used in which the temperature gradually decreases to a target temperature. This moderate decrease in temperature (i.e. 1–6°C degrees per hour) causes formation of intercellular ice that may or need not be lethal, depending on the hardiness of the plant (GLERUM 1985).

In this study the temperature –16°C was used (without moderate decrease in temperature). This temperature caused intracellular freezing. There were two reasons for this procedure:

a) the climatic room for a moderate decrease in temperature was not at disposal yet,
b) primary testing of the method of electrolyte leakage measurement for determination of cold hardiness.

Electrolyte leakage from unstressed plants

A difference was found between lifting dates in the values of root electrolyte leakage (REL), but without clear significance. The values of electrolyte leakage from shoots (SEL) were constant. Probably, the changes in REL values could be caused by rainfall and different development of dormancy in shoots and roots. According to HOFFMAN (1974), shoot dormancy precedes the cessation of root growth in most temperate tree species and NICOLL et al. (1996) found for Sitka spruce that root growth stopped for more than 3 months after shoot growth ceased.

Electrolyte leakage after artificial frost test

Electrolyte leakage from roots (REL) decreased in dependence on different lifting dates. On the first lifting date (end of September) the REL values were 77%. On the last lifting date (8 November) the REL values were 56%. These differences were statistically significant. The same tendency was found in SEL values (decrease from 63% at the end of September to 17% on 8 November). Index of injury showed the same tendency as electrolyte leakage for both plant parts. These results confirm the fact that shoots are mostly hardier than roots (SMIT-SPINKS et al. 1985; LINSTRÖM, MATTSON 1989; LYR et al. 1992). COLOMBO (1995) wrote that shoots were generally frost hardier than roots, differences in hardiness along the stem and root axes were gradual, rather than abruptly differing at the shoot-root interface.

These results showed that planting stock in dormancy is hardier than during the vegetation period. O'REILLY et al. (2000) stated that the temperature that caused 50% mortality of needles (LT_{50}) decreased from –5°C in September to –35°C in early December. The same results were reported by SIMPSON (1994), who found for white spruce that cold hardiness of buds, foliage and stems increased over autumn sample dates reaching maximum hardiness at midwinter.

The results of this study cannot be interpreted with lethal temperature but the index of injury showed that frost damage of shoots significantly decreased with date of lifting and on the last three dates it was under 10%.

It is necessary to underline that these first results and development of frost hardiness are affected by different combinations of temperature, day length and precipitation. Consequently, the physiological status of seedlings is not necessarily the same on each calendar date, each year, but instead it is a complex function of many interacting factors that can vary with year, location, and genotype (BURR 1990).

Field performance

A clear difference in the survival of plants lifted on different dates was found. The plants lifted on 20 September were dead. In contrast, the plants lifted on 8 November had 96% survival rate.

Except different lifting dates the planting stock quality is affected by duration of cold storage. MCKAY (1992) wrote that fine roots of *Picea sitchensis* could tolerate 90 days storage commencing at any time from mid-December to early April. Unfortunately, it was impossible to carry out the same storage term for all variants.

CONCLUSIONS

The results of this study show that different lifting dates affect cold hardiness of spruce planting stock, and this hardiness is higher for shoots than for roots. The first results show that it is possible to optimize the autumn lifting date for spruce plants by measurements of electrolyte leakage from shoots after an artificial frost test. On the other hand, except the lifting date the survival of plants was also affected by different length of storage. These results could be used for further research on cold hardiness of bare-rooted and containerized planting stock in forestry nursery practice (effect of different fertilization, genetic provenance of seeds, time of sowing, etc.).

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Zmena v odolnosti na mráz pri voľnokorennom sadbovom materiáli smreka a jej vplyv na ujatosť

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ABSTRAKT: Cieľom štúdie bolo testovanie metódy merania straty elektrolytu na určenie vhodného termínu jesenného vyzdvihovania. Druhým cieľom bolo získať údaje o vplyve rôzneho termínu vyzdvihovania na ujatosť sadbového materiálu smreka

(*Picea abies* [L.] Karst.). Zistili sa významné rozdiely v hodnotách straty elektrolytu z koreňov (REL) v závislosti od termínu vyzdvihovania pri nestresovanom sadbovom materiáli, ale bez jasnej tendencie. Hodnoty straty elektrolytu zo stonky (SEL) boli stabilné (11–13 %). Na druhej strane hodnoty straty elektrolytu z koreňov klesali v závislosti od dátumu vyzdvihovania po umelom mrazovom strese. V prvý termín vyzdvihovania (koniec septembra) hodnoty straty elektrolytu (REL) boli 77 %. V posledný termín vyzdvihovania (8. novembra) tieto hodnoty dosiahli 56% úroveň. Rovnaká tendencia bola zistená aj pri hodnotách straty elektrolytu zo stonky (pokles zo 63% úrovne koncom septembra na 17% 8. novembra). Rovnako boli zistené rozdiely v ujatosti sadeníc. Sadenice vyzdvihnuté v prvých termínoch mali až 100% mortalitu, ktorá klesala s neskoršími termínmi vyzdvihovania. Dosiahnuté výsledky poukazujú na fakt, že odolnosť na mráz pri sadbovom materiáli sa zvyšuje počas jesene; táto odolnosť je vyššia pri stonke ako pri koreňovom systéme. Prvotné výsledky ukázali, že pomocou merania straty elektrolytu zo stonky po umelom mrazovom strese je možné optimalizovať termín jesenného vyzdvihovania sadbového materiálu smreka.

Kľúčové slová: odolnosť na mráz; strata elektrolytu; dátum vyzdvihovania; sadbový materiál smreka

Rozsah umelej obnovy lesa sa na Slovensku pohybuje okolo 12 000 ha. Pri tejto obnove sa na 95 % používa voľnokorenný sadbový materiál. Smrek patrí k veľmi dôležitým drevinám používaným pri tejto umelej obnove. Drvivá väčšina prác spojených s umelou obnovou lesa a zalesňovaním sa uskutočňuje v jarnom období, kedy sú tieto práce veľmi ovplyvnené priebehom počasia (teplota a zrážky). Tieto faktory môžu negatívne ovplyvniť fyziologickú kvalitu sadbového materiálu (manipulácia s napučaným sadbovým materiálom, nedodržanie technologických termínov atď.). Následne sa znížená fyziologická kvalita sadbového materiálu môže negatívne prejaviť na ujatosti a nedostatočnom raste po výsadbe.

Cieľom štúdie bolo získať prvotné informácie o vplyve rozdielneho termínu jesenného vyzdvihovania na ujatosť sadbového materiálu smreka. Ďalším cieľom bolo testovanie metódy merania straty elektrolytu po mrazovom teste na určenie optimálneho termínu vyzdvihovania.

V štúdii sa použil voľnokorenný sadbový materiál smreka obyčajného (2 + 3) (*Picea abies* [L.] Karst.). Sadenice boli od 20. septembra do 8. novembra v týždenných intervaloch vyzdvihované zo záhonu lesnej škôlky. Z každého termínu vyzdvihovania bolo umiestnených 100 sadeníc do polyetylénových vriec (v každom vreci boli umiestnené 4 zväzky – nadzemná časť bola mimo vreca). Tento sadbový materiál bol následne umiestnený do klimatizovaného skladu (teplota vzduchu 1–2 °C, vzdušná vlhkosť 92 až 95 %). Priamo v lesnej škôlke boli odobraté vzorky zo stonky a z koreňového systému (20 kusov) pre meranie straty elektrolytu. Zvyšných 20 kusov vyzdvihnutých sadeníc bolo po prenesení do laboratória Lesníckeho výskumného ústavu použité na mrazový test. Počas tohto testu boli celé sadenice v igelitovom vreci a umiestnené pri teplote 2 °C. Po dvoch hodinách boli sadenice prenesené do klimatizovanej komory (–16 °C), kde boli ponechané 20 hodín. Po skončení testu sadenice boli ponechané hodinu pri izbovej teplote a následne sa odobrali vzorky z koreňa a stonky na zistenie straty elektrolytu.

Dňa 25. apríla sa ukončilo skladovanie sadeníc v klimatizovanom sklade a sadenice boli nasledujúci deň vysadené. Pred výsadbou sa zistila úroveň straty elektrolytu z koreňového systému a stonky (n = 20) a sadenice boli vysadené.

Na základe dosiahnutých výsledkov je možné konštatovať, že úroveň straty elektrolytu zo stoniek nestresovaného sadbového materiálu bola stabilná (11–13 %) počas celého obdobia vyzdvihovania sadbového materiálu. Hodnoty straty elektrolytu z koreňa kolísali v rozmedzí 24–31 % bez výraznej tendencie. Na druhej strane hodnoty straty elektrolytu výrazne klesali v závislosti od termínu vyzdvihovania pri sadeniciach po umelom mrazovom teste (pokles z 63% úrovne 20. septembra na 17% 8. novembra pri stonke). Rovnako bol pokles a štatisticky významné rozdiely zaznamenané aj v hodnotách straty elektrolytu z koreňového systému (z 77% na 56% úroveň).

Rovnako boli zistené rozdiely v hodnotách straty elektrolytu po ukončení skladovania sadbového materiálu v klimatizovanom sklade. Sadbový materiál, ktorý bol vyzdvihnutý 20. septembra, dosiahol hodnotu straty elektrolytu na úrovni 50 %. Boli zistené aj rozdiely v ujatosti sadbového materiálu v závislosti od termínu jeho vyzdvihovania. Sadenice vyzdvihnuté 20. septembra mali 100% mortalitu. Na druhej strane sadenice vyzdvihnuté 8. novembra dosiahli 96% ujatosť. Je zrejmé, že rozdielny termín jesenného vyzdvihovania výrazne ovplyvňuje mrazuvzdornosť sadbového materiálu. Na základe získaných výsledkov merania straty elektrolytu je zrejmé, že tieto zmeny je možné určiť pomocou merania straty elektrolytu zo stonky. S vysokou pravdepodobnosťou je možné tvrdiť, že rozdielny termín vyzdvihnutia sadbového materiálu ovplyvnil aj jeho ujatosť, ak keď nie je možné kvôli rozdielne dlhému termínu skladovania v klimatizovanom sklade tento vplyv presne stanoviť.

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