Determination of effects of desiccation and frost stresses on the physiological quality of Norway spruce (*Picea abies* [L.] Karst.) seedlings by measurement of electrolyte leakage from the root system

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ABSTRACT: Two-year, bare-rooted seedlings of Norway spruce (*Picea abies* [L.] Karst.) were used in this study. The seedlings were exposed to artificial desiccation and frost stress after spring lifting. After exposure to these factors, electrolyte leakage from the root system was measured. The results showed that artificial desiccation significantly affected the rate of electrolyte leakage. The effect of desiccation was confirmed by the survival of plants and height increment (100% survival and 49% height increment of control seedlings in contrast with plants after 6 hours of desiccation: 9% survival and 2% height increment). On the other hand, frost stress did not affect the rate of electrolyte leakage (the rate of REL did not correlate with the intensity of stress treatment).

Keywords: desiccation and frost stress; electrolyte leakage; Norway spruce; seedlings root system; survival

In Slovakia, afforestation and artificial regeneration (dominant bare-rooted planting stock – 95%) are carried out on an area of about 12,000 ha/year. Repeated reforestation (about 40%) affects significantly the volume of artificial regeneration. These failures are probably caused by a variety of factors, including plantation site conditions, but the poor physiological quality of bare-rooted plants is probably a major contributing factor (ANONYMOUS 1997).

The maximum work with planting stock is concentrated to the spring season. Weather conditions largely influence the work operations in forest nurseries and on planting sites (intensive increase in temperature causes bud flushing). On the other hand, the soil in nurseries is frozen and therefore the plants cannot be lifted. These facts do not contribute to correct handling of planting stock and could lead to the poor physiological quality of plants and seedlings.

Small forest nurseries (without air-conditioned storage) could have problems with short storage of plants and seedlings after lifting. On the other hand, the long transport of planting stock from nursery with air-conditioned storage to a forest site could involve damage to plants (desiccation, frost).

Desiccation can influence water uptake in two ways. Firstly, desiccation can kill roots. A reduction in functional roots will decrease the surface area for water uptake from the existing root system and also the number of root initials for root production. Secondly, by reducing the water potential, desiccation causes stomata closure (MCKAY, WHITE 1997).

Frost damage to roots is not usually a problem as the root temperature is normally buffered by the soil so that roots are rarely exposed to a temperature as low as the surrounding air (MCEVOY, MCKAY 1997a,b). On the other hand, lifting and transplanting of plants could cause damage to the root system if the temperature falls below 0°C.

The roots system is less tolerant than shoots (CALMÉ et al. 1994) and fine roots are damaged at warmer temperatures more easily than woody roots (COLOMBO 1994). The fine roots play a decisive role in water uptake, nutrition and mycorrhiza (LINDSTRÖM 1986). LASSHEIKKI et al. (1991) stated that root freezing damage would result in the loss of some of the fine roots and thereby in a reduction in root growth potential. In contrast with it SARVAŠ (1999) concluded that slight damage to fine roots did not cause plant mortality but damage to mature roots (directly under the root collar) was important for the survival and field performance of planting stock.

Unfortunately, plants subjected to desiccation and frost stresses tend to suffer internal tissue damage that is rarely evident during visual inspection (MCEVOY, MCKAY 1997a) and the determination of such physiological damage to planting stock is very time-consuming (MARTINCOVÁ, NÁROVCOVÁ 2001).

There are many methods to assess physiological damage of plants around the word. The most frequently used method for assessment of physiological damage is measurement of Root Growth Potential (BURDETT et al. 1983; RITCHIE, DUNLAP 1980; LINDQVIST 1998; SIMPSON, RITCHIE 1997, etc.). The disadvantage of RGP method is mainly time requirement (3 weeks), questionable interpretation of results and high cost of the test.

In the last years the method of measurement of electrolyte leakage from the root system was used to find the physiological quality of planting stock in conifers (COLOMBO 1994; FOLK, GROSSNICKLE 1997; GARRIOU et al. 2000; MCKAY 1991,1992,1993,1997; MCKAY, MASON 1991; TINUS 1996, etc.) and broadleaved species (GARRIOU et al. 2000; KERR, HARPER 1992; MCEVOY, MCKAY 1997a,b; SARVAŠ 1998, 1999; SCHÜTE, SARVAŠ 1999, etc.).

The aim of this study was to describe the influence of desiccation and frost on the physiological quality of spruce seedlings after spring lifting and to assess this influence by measurements of electrolyte leakage from the root system.

MATERIAL AND METHODS

Norway spruce (*Picea abies* [L]. Karst.) two-year bare-rooted seedlings that were produced in a nursery of Forest Research Institute in Zvolen were used in this study. Biometrical characteristics and age of tested planting stock are given in Table 1.

On 25. 3. 1999 the plants were lifted from nursery beds and desiccation and frost tests were carried out. In a desiccation test the plants were placed outdoors on concrete floor for 2, 4 and 6 hours. The air temperature was 16°C, cloudy, calm, air humidity 80%. The frost test was carried out under air-climate storage and the seedlings were kept 2, 4 and 6 hours /–2°C. These conditions simulate a stress that could normally impact on seedlings during lifting, handling and transporting.

After artificial stresses the samples (from each stressed variant + control variant, 15 seedlings were used) were prepared for measurement of electrolyte leakage from the root system (REL). The electrolyte leakage was assessed according to the method described by MCKAY (1992). Roots were washed in tap water to remove soil and rinsed in deionised water to remove surface ions. The samples from roots (taken directly under the root collar – 2 cm length) were used. Individual samples were put in 40 ml universal glass bottles containing 30 ml deionised 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 bathing solution was measured using the conductivity meter LF 320 with built-in temperature compensation (25°C). Then the samples were killed in a pressure cooker at 110°C for 10 min. The second conductivity measurement was made 24 h after the sample killing. The total conductivity was as follows:

$$\text{REL} = \frac{\text{conductivity after 24 h}}{\text{conductivity 24 h after autoclaving}} \cdot 100$$

where: REL – root electrolyte leakage.

Next, another 60 seedlings from each variant were planted in the forest nursery instantly after stress treatment (3 replications × 20 seedlings, distance between the rows was 20 cm and distance of seedlings in rows was 15 cm). After the first vegetation period the survival was assessed and height increments of plants were calculated:

$$\text{height increment (\%)} = \frac{\text{autumn height} - \text{spring height}}{\text{spring height}} \cdot 100$$

Variant identification:

1 – unstressed planting stock
2 – planting stock after 2-hour desiccation stress
3 – planting stock after 4-hour desiccation stress
4 – planting stock after 6-hour desiccation stress
I – unstressed planting stock
II – planting stock after 2 hours/–2°C
III – planting stock after 4 hours/–2°C
IV – planting stock after 6 hours/–2°C

The values of electrolyte leakage from roots after artificial frost and desiccation and height increments were evaluated by Tukey's honest significant difference (HSD) test.

RESULTS

ELECTROLYTE LEAKAGE

Desiccation treatment

The results showed that duration of desiccation influenced the rate of electrolyte leakage from roots. The electrolyte leakage from seedlings was 49 and 47% after four and six hours respectively. However, the rate of electrolyte leakage in unstressed seedlings and seedlings after two-hour desiccation stress was 36% and 35%, respectively.

Table 1. Age and morphological traits of spruce seedlings

Characteristic	Spruce
Age	2 + 0
Height (cm) ± s_x	15.9 ± 4.3
Root length (cm) ± s_x	9.9 ± 2.7
Diameter of root collar (mm) ± s_x	3.1 ± 0.7
Shoot diameter (measured 5 cm above root collar) (mm) ± s_x	2.6 ± 0.7

Table 2. The rate of electrolyte leakage (%) and level of significance after artificial desiccation stress

Treatment	REL(%) ± one standard error
I	36 ± 4[a]
II	35 ± 5[a]
III	49 ± 9[b]
IV	47 ± 8[b]

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

Table 3. The rate of electrolyte leakage (%) and level of significance after artificial frost stress

Treatment	REL(%) ± one standard error
I	36 ± 4[a/b]
II	32 ± 4[a]
III	41 ± 12[b]
IV	33 ± 13[a/b]

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

The differences between these two groups were statistically highly significant (Table 2).

Frost treatment

The electrolyte leakage after frost stress reached maximum values from seedlings after 4h/–2°C (41%). In contrast to it, the electrolyte leakage from seedlings treated 2h/–2°C was only 32%. The rate of leakage from unstressed seedlings and after 6h/–2°C was 36% and 33%, respectively (Table 3).

SURVIVAL AND FIELD PERFORMANCE OF PLANTS

Desiccation treatment

The highly significant differences were observed in survival and annual height increment after desiccation stress. The rate of survival in unstressed seedlings and seedlings after 2 hours of desiccation treatment was 100% and 98%. The height increment for both variants was 49% and 42% after the first vegetation period. On the other hand, the plants after 4 and 6 hours of desiccation treatment showed only 49% and 9% rate of survival. The minimum height increment was measured in plants after 6 hours of desiccation (2%) (Table 4 and Fig. 1).

Coefficients of correlation between electrolyte leakage and survival and height increment were calculated. The coefficient of correlation between REL and height increment was –0.93 and between REL and survival –0.88.

Frost treatment

The differences in the rate of survival of plants after frost treatment were not so clear as for plants after desiccation treatment (100% survival of control plants and 80% survival after treatment 6h/–2°C). The frost treatment significantly affected height increment (49% control plants and 25% for plants after 6 h – Table 5 and Fig. 2). The correlation coefficient between height increment and REL was –0.04 and between REL and survival 0.14.

Table 4. Height increment (%) and level of significance after artificial desiccation stress

Treatment	Height increment (%) ± one standard error
I	49 ± 23[a]
II	42 ± 19[a]
III	14 ± 20[b]
IV	2 ± 9[c]

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

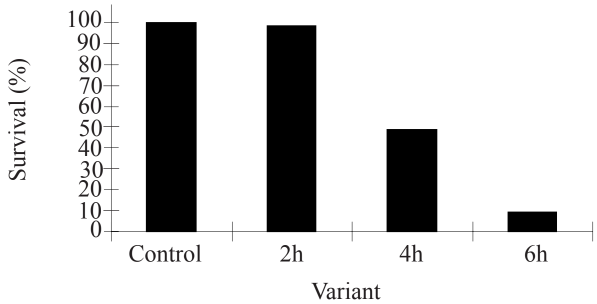

Fig. 1. Survival of spruce seedlings after desiccation stress

Table 5. Height increment (%) and level of significance after artificial frost stress

Treatment	Height increment (%) ± one standard error
I	49 ± 23[a]
II	38 ± 19[a/b]
III	31 ± 20[b/c]
IV	25 ± 9[c]

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

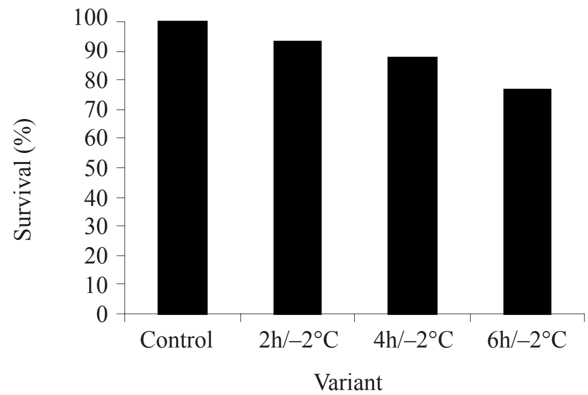

Fig. 2. Survival of spruce seedlings after frost stress

DISCUSSION

In Slovakia, bare-root planting stock is widely used for reforestation. In spring, desiccation and cold (frost) stresses can influence the physiological quality of seedlings and plants. This quality of plants can be endangered during their handling (lifting, grading, short or long storage and transport to planting sites) (GARRIOU et al. 2000). During these operations, seedlings can be exposed to conditions that could cause desiccation (MCKAY 1997) and to reduce the survival of conifers (MCKAY, WHITE 1997; TABBUSH 1987).

In this study the influence of desiccation and frost stress on survival, height increment and electrolyte leakage from roots of spruce seedlings was studied.

Desiccation treatment

After desiccation treatment highly significant differences in the rate of electrolyte leakage were found (two groups). In first group, unstressed seedlings and seedlings after two-hour desiccation treatment had the same significant level of electrolyte leakage (36 and 35%) and the rate of plant survival was 100% and 97%. On the other hand, seedlings after four- and six-hour desiccation treatments showed 49% and 47% electrolyte leakage, respectively, and plants under six-hour desiccation treatment reached only 9% survival and 2% height increment. In general, several studies reported a possibility to find the physiological quality of planting stock by measurements of electrolyte leakage: i) to determine lifting dates for bare-rooted seedlings (high REL values following cold storage usually indicate that root deterioration has occurred, MCKAY 1992, 1993, 1994, 1998; MCKAY, MASON 1991), ii) to assess frost damage to trees and determine the levels of frost hardiness (COLOMBO 1994, 1997; KEATES 1990; MURRAY et al. 1989; NICOLL et al. 1996; SARVAŠ, 1999) and iii) heat damage (BINDER, FIELDER 1995).

Unfortunately, it is problematic to make a comparison of separate results. The methodology of sample preparation and measurement is different and our own tested planting stock was very heterogeneous in these studies (age and geographic origin of plants, different ways of raising in the nursery, etc.). SARVAŠ (1999) found 23% electrolyte leakage from the tap root of unstressed spruce plants and samples from the same planting stock had only 20% electrolyte leakage after desiccation treatment (8 hours –25°C/15% air humidity). The plants were not transplanted and therefore it was not possible to find the influence of stress treatment on survival and growth.

In our study two years old seedlings were used (root collar diameter 3.1 mm) and SARVAŠ (1999) used four-year plants (root collar diameter 12.6 mm). SMIT-SPINKS et al. (1984) described that the hardiness of suberized roots varies with root diameter, generally hardiness increases with increasing diameter. The larger diameter of root collar probably caused the lower rate of electrolyte leakage.

On the other hand, close correlations were found between electrolyte leakage from roots and survival (–0.88) and height increment (–0.93) of plants after the first vegetation period. These results confirm the fact that measurement of electrolyte leakage is a reliable method to determine physiological damage to planting stock after desiccation treatment. But it is necessary to underline that this correlation was calculated from four values only and therefore these results are of orientation character only.

Frost treatment

In contrast with desiccation treatment, no statistically significant influence of frost treatment on the rate of electrolyte leakage from spruce roots was found. The plants under 6 hour/–2°C reached the lowest rate of electrolyte leakage (32%). On the other hand, there was a significant difference in height increment after the first vegetation period after re-planting. The coefficient of correlation between REL/survival was 0.14 and height increment –0.04.

CONCLUSION

The obtained results demonstrate a possibility of decreasing the physiological quality of planting stock by rough handling during lifting and transport of planting stock. This decrease in physiological quality of spruce plants in reflected in poor survival and height increment after the first vegetation period. The results point to the importance of root protection against desiccation and frost during handling. It is very important for successful afforestation program to use undamaged plants only. Therefore the testing of planting stock quality plays a decisive role.

In this study, the deterioration of physiological quality (caused by desiccation) was determined by measurement of electrolyte leakage from the root system. Desiccation treatment for 4 hours and more was found to have a significant impact on survival and height growth of plants after re-planting. This impact was detected by measurement of electrolyte leakage.

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Zisťovanie vplyvu sucha a mrazu na fyziologickú kvalitu semenáčikov smreka (*Picea abies* [L.] Karst.) pomocou merania straty elektrolytu z koreňového systému

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ABSTRAKT*:* V štúdii sa zisťoval pomocou merania straty elektrolytu z koreňového systému vplyv sucha a mrazu na fyziologickú kvalitu dvojročných semenáčikov smreka (*Picea abies* [L.] Karst.). Výsledky ukázali, že umelý stresový faktor sucha významne ovplyvnil úroveň straty elektrolytu z koreňového systému. Vplyvy sucha boli potvrdené aj ujatosťou a výškovým prírastkom sadbového materiálu po prvej vegetačnej perióde – kontrolný variant dosiahol 100% ujatosť a 49% výškový prírastok v kontraste so semenáčikmi po 6 hodinách pôsobenia sucha, ktoré dosiahli 9% ujatosť a 2% výškový prírastok. Na druhej strane mrazový stresový faktor nemal štatisticky významný vplyv na úroveň straty elektrolytu (rozsah REL nekoreluje s intenzitou stresového faktora).

Kľúčové slová: koreňový systém; smrekové semenáčiky; strata elektrolytu; stres suchom a mrazom; ujatosť

V Slovenskej republike sa rozsah umelej obnovy lesa a zalesňovania pohybuje ročne na úrovni 12 000 ha a v drvivej väčšine (v 95 %) sa používa voľnokorenný sadbový materiál. Rozsah opakovaného zalesňovania sa pohybuje okolo 40 %. Pod tento vysoký podiel sa podpisuje okrem objektívnych faktorov (vplyv počasia, nedostatok zrážok v čase výsadby) aj nedostatočná fyziologická kvalita sadbového materiálu.

Práve fyziologické poškodenie sadbového materiálu je veľmi časté v jarnom období, kedy sa uskutočňuje manipulácia s ním. Krátkodobé porušenie technologických postupov pri manipulácii so sadbovým materiálom môže zapríčiniť nízku ujatosť a následný rast lesných kultúr. Ďalším problémom fyziologického poškodenia je jeho diagnostikácia. Toto poškodenie nie je možné určiť vizuálne a prejavuje sa až po výsadbe, kedy už došlo k výrazným finančným stratám. Preto sa hľadajú postupy a metódy, pomocou ktorých by bolo možné určiť tento druh poškodenia sadbového materiálu lesných drevín. Jednou z metód, ktorá je pomerne rýchla (výsledky merania sú k dispozícii do 48 hodín) a finančne nenáročná, je metóda založená na meraní straty elektrolytu z jednotlivých rastlinných častí sadbového materiálu.

Cieľom práce bolo zistiť vplyv umele indukovaného stresového faktora sucha a mrazu na semenáčiky smreka (ujatosť a výškový rast po prvej vegetačnej perióde) a možnosti metódy merania straty elektrolytu určiť tento vplyv.

V práci boli použité dvojročné semenáčiky smreka obyčajného (*Picea abies* [L.] Karst.). Semenáčiky boli vystavené umelému suchu (2,4 a 6 h) a mrazu (2,4 a 6 h/ –2 °C). Po skončení pôsobenia týchto stresových faktorov + nestresované semenáčiky (nulový variant) sa uskutočnilo meranie straty elektrolytu z časti koreňového systému – REL (2cm časť odobratá priamo pod koreňovým krčkom). Následne boli semenáčiky zaškôlkované na záhon lesnej škôlky. Po prvej vegetačnej perióde sa zisťovala ujatosť a výškový prírastok. Výsledky ukázali, že umelý stresový faktor sucha významne ovplyvnil úroveň straty elektrolytu z koreňového systému (strata elektrolytu z nestresovaných semenáčikov dosiahla 36 % a semenáčiky vystavené 6 h stresu suchom dosiahli stratu elektrolytu na úrovni 47 %). Výsledky vplyvu sucha boli potvrdené aj ujatosťou a výškovým prírastkom sadbového materiálu po prvej vegetačnej perióde (kontrolný variant dosiahol 100% ujatosť a 49% výškový prírastok v kontraste so semenáčikmi po šiestich hodinách pôsobenia sucha, ktoré dosiahli 9% ujatosť a 2% výškový prírastok). Koeficient korelácie medzi REL/ujatosťou bol –0,88 a medzi REL/výškovým prírastkom –0,93. Na druhej strane mrazový stresový faktor nemal štatisticky významný vplyv na úroveň straty elektrolytu (rozsah REL nekoreluje s intenzitou stresového faktora). Rozdiely v úrovni ujatosti semenáčikov medzi jednotlivými variantmi neboli také výrazné ako pri semenáčikoch po strese suchom (100% ujatosť pri kontrolnom variante a 80% po mraze –2 °C/6 h). Mráz výrazne ovplyvnil výškový prírastok (49 % pre nestresované semenáčiky a 25 % po mraze –2 °C/6 h). Koeficient korelácie medzi výškovým prírastkom a REL bol –0,04 a medzi REL/ujatosťou 0,14.

Dosiahnuté výsledky potvrdili možnosť zníženia fyziologickej kvality sadbového materiálu počas nesprávnej manipulácie. Toto zníženie sa v práci prejavilo v nízkej ujatosti a výškovom prírastku po prvej vegetačnej perióde. Preto je potrebné ochrane sadbového materiálu proti suchu venovať zvýšenú pozornosť. Na zvýšenie celkovej ujatosti má významnú úlohu testovanie kvality sadbového materiálu.

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