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Root frost tolerance, seasonal variation in root growth, and field performance of one-year-old Russian larch seedlings with simulated root freezing damages

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ABSTRACT

Planting healthy seedlings with optimal growth potential is essential for proper growth and survival in forest regeneration. Assessing the seedling quality prior to planting is therefore important. In this Icelandic study, effects of root damage induced with artificial freezing in young Russian larch seedlings were examined using the root growth capacity method (RGC). Frost tolerance of roots varied during the winter, and root growth in undamaged seedlings fluctuated, indicating seasonal variations in growth rhythm. The LT_{50} value for root frost tolerance was −13.9°C in late January, but already at −10.6°C (LT_{10}) root damages were severe. After one growing season, shoot elongation was significantly lower in seedlings frozen to −9°C, −13.5°C, and −15.5°C by 23%, 54%, and 72%, respectively, compared with undamaged seedlings. Control seedlings and seedlings frozen to −9°C achieved 100% survival after the first growing season. Survival in seedlings frozen to −13.5°C and −15.5°C was 85% and 27%, respectively. After the second growing season, survival decreased in all frost-damaged seedlings. The ongoing mortality demonstrates the long-lasting effects of planting seedlings with damaged root systems, and the fluctuation in root frost tolerance of young Russian larch seedlings during winter emphasises the need for care when seedlings are moved to outdoor storage.

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Russian larch; root growth; root frost tolerance; root damage; root growth capacity; field performance

Introduction

To avoid freezing and winter damage in forest seedlings in nurseries, hardening must be synchronised with the seasonal climatic cycle (Colombo et al. 2001). The ability of trees to cold harden is determined genetically but controlled by environmental cues (Levitt 1980; Aitken and Hannerz 2001; Bigras et al. 2001). The main factor inducing cold acclimation in conifer shoots is increasing night length in late summer, followed by near-zero and freezing temperatures in autumn, resulting in an increased level of hardiness (Grossnickle 2000; Bigras et al. 2001; Colombo et al. 2001). Roots are less cold tolerant than shoots (Smit-Spinks et al. 1985) and the environmental signals for their cold acclimation are different from shoots. The cessation of root growth and root hardening in conifer seedlings is induced by lowering the temperatures of the growing substrate. Root growth can continue even if shoots are dormant if the substrate temperatures are sufficiently high (Lyr and Hoffmann 1967). Consequently, root growth terminates later in autumn than shoot growth and starts earlier in spring (Bigras and Dumais 2005). Therefore, in nurseries, unprotected roots are particularly vulnerable to frost during autumn (Colombo et al. 1995; Dumais et al. 2002).

Roots require exposure to low temperatures to reach adequate root hardiness to withstand overwintering outdoors (Colombo et al. 2001; Bigras and Dumais 2005). Temperatures below 5°C are effective to harden roots and alternating warm/cold root temperatures results in less cold hardy roots compared to continuous cold root temperatures (Colombo 1994; Colombo et al. 2001). Stattin et al. (2000) found that the root freezing tolerance of bare root seedlings of Norway spruce (*Picea abies* (L.) Karst.) could be improved if the seedlings were exposed to 5.5°C for six weeks prior to frozen storage. As root frost tolerance is species specific (Lindström and Nyström 1987; McKay 1994; Bigras et al. 2001) and each species has a unique fall acclimation pattern, it is difficult to define universal culture practices across all regional forest regeneration programmes (Grossnickle and South 2014).

Root frost tolerance is also age related. The roots of late sown seedlings are less frost hardy than seedlings sown earlier, and root frost tolerance also differs among provenances. Lindström and Nyström (1987) found that one-year-old Norway spruce and Scots pine (*Pinus sylvestris* L.) seedlings from northern provenances gained root frost tolerance earlier in autumn than those from southern provenances, and younger seedlings (sown in July) were more sensitive to root frost than older seedlings (sown in May) in the fall. Furthermore, younger parts of the roots are more sensitive to low temperatures than mature parts (Smit-Spinks et al. 1985). The roots closest to the stem are usually the most tolerant, while the root tips are the least hardy (Colombo et al. 1995).

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Although the majority of seedlings in conventional forest nurseries in the Nordic countries are stored in freezers (Nilsson et al. 2010) there is some proportion of the stock stored outdoors during winter. Approximately 50% of the 155 million conifer seedlings produced annually in Finland are overwintered outdoors (Di et al. 2019), in Iceland the corresponding number was 92% of 3.5 million seedlings during the winter of 2019–2020 (Personal communication, Þórveig Jóhannsdóttir, 2020). In Denmark, approximately 50% of all produced seedlings are overwintered outdoors (Personal communication, Torben Leisgaard, 2020), and in Sweden, approximately one-third of the 381 million forest seedlings delivered in 2019 were overwintered outdoors (Personal communication, Ellinor Edwardson and Claes Uggla, 2020). In Norway, 90–100% of seedlings sold at the age of two-year-old are stored outdoors during the first year of production, but only 20–25% of saleable seedlings are stored outdoors (authors' observations). Storing seedlings outdoors is generally thought of as more hazardous than using freezers to store seedlings in forest nurseries, especially for seedling roots (Lindström 1986; Sakai and Larcher 1987; Colombo et al. 2001). Damage is often related to a lack of isolating snow cover and unstable weather conditions during winter (Lindström 1986; Dumais et al. 2002). The predicted climate changes are likely to increase these risk factors in the future (IPCC 2013); therefore, nurseries could face increased risks in outdoor storage. Damage to the root system is often hard to visualise as seedlings with damaged roots can appear quite normal (Ritchie 1990; Bigras 1998). In forest regeneration, planting high quality seedlings with optimal growth potential improves seedlings growth and the probability to survive after planting (Grossnickle 2012; Dumroese et al. 2016; Grossnickle and MacDonald 2018b). If root growth does not occur just after planting, seedlings can be exposed to water stress, which can lead to increased mortality (Burdett 1990; Grossnickle 2000, 2012).

Russian larch (*Lariz sukaczewii* (Dyl.)) has been an important forest species in Iceland for decades, and its proportion of the total forest seedling has been, on average, 22% since 2000 (Gunnarsson et al. 2000-2020). Frost tolerance in the roots of *Larix* species has been examined mainly in bare-rooted seedlings. McKay (1994) used electrolyte leakage to determine frost hardiness in excised fine roots of 2-year-old undercut and wrenched Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), Japanese larch (*Larix kaempferi* (Carr.)), Scots pine, and three provenances of Sitka spruce bareroot seedlings (*Picea sitchensis* (Bong.) Carr.) (Alaskan, Queen Charlotte Islands, and Oregon). Japanese larch and Sitka spruce developed much greater root frost hardiness of −12°C and −13°C, respectively compared to Douglas-fir (−4°C) and pine (−7°C). The root frost hardiness of Sitka spruce also increased with the provenance's latitude. Hansen and Eriksen (1993) found the maximum root frost tolerance of four-month-old Japanese larch seedlings to be −10°C and short-day treatment during autumn did not affect root frost tolerance. To our knowledge, the root frost tolerance of young containerised Russian larch seedlings has not previously been investigated. Determining the low-temperature tolerance of the seedlings at this critical time in production can be of great importance for nurseries to be able to arrange adequate protection or avoid the weather conditions most likely to damage seedlings after being transported to outdoors storage.

In Iceland, larch seedlings are sold as one-year-old seedlings. The species has commonly been sown twice during the year for the maximum utilisation of greenhouses in early April and June. The seedlings from April sowing are moved outdoors in June and kept outdoors during the summer until short-day treatment in August to induce dormancy (Personal communication, Katrín Ásgrímsdóttir, nursery manager, 2021). The Icelandic summer is short and has a relatively low average temperature; therefore, the June sowing needs to be grown in the greenhouse as long as the natural photoperiod allows height growth to meet the desired height. Seedlings are moved from the greenhouse to winter storage outdoors in late January after being kept at 4°C ± 2°C in the greenhouse during autumn and early winter. In January, weather conditions can be very harsh, with freezing temperatures and limited snow cover (Icelandic Meteorological Office 2022). The greater the difference between greenhouse conditions and outdoor temperatures, the greater the risk of intracellular freezing damage (Colombo et al. 2001). Warm soil that favours root growth may delay root hardening, making the seedlings susceptible to frost damage when moved outdoors (Ryyppö et al. 1998). Therefore, temperature control in greenhouses at this stage of production is very important. Successful storage of container seedlings is one of the most challenging and important aspects of nursery management (Landis et al. 2010) and it is important to know the root frost tolerance of the species.

The root growth capacity (RGC) of seedlings has been used since 2007 in Iceland to evaluate functional integrity and vitality before delivery (Personal communication, Hrefna Jóhannesdóttir, 2021). Seasonal variation in root growth has been reported in Scots pine, Norway spruce, lodgepole pine (*Pinus contorta* Dougl. Ex. Loud.) (Mattsson 1986; Lindström and Nyström 1987), and Siberian larch (*Larix sibirica* Ledeb.) (Mattsson and Lasheikki 1998). RGC is used routinely to estimate the seedling quality, and it is necessary to realise when root growth is suppressed because of natural conditions to be able to interpret the outcome of the RGC test correctly.

The aims of this study were to (1) monitor seasonal variation in root growth of Russian larch seedlings during winter and early spring, (2) investigate the ability of Russian larch roots to frost harden at non-freezing temperatures, (3) examine at what level root frost tolerance is at the time seedlings are moved outdoors for winter storage, and (4) examine the growth and survival of seedlings with damaged and undamaged root systems during two growing season after planting.

Materials and methods

Plant materials

The provenance of the Russian larch used in the present study was "Lassinmaa" (62,04°N, 25,09°E). The seedlings were produced in Sólskógar, a commercial forest nursery in Akureyri (65,66°N, 18,10°W) in northern Iceland. Russian larch was sown on 10 June, with one seed per cavity in plastic conical multipots (BCC, HIKO-93, Gislaved, Sweden). Each pot had a volume of 93cm^3, with 526 cells per m^2 (40 cavities per tray). The growing medium was medium course sphagnum peat, fertilised (0.8 kg m^{-3} N-P-K in the proportions of 16-8-16 with micronutrients) and limed (2 kg m^{-3}) (M6, Kekkilä Oy, Tuusula, Finland). Fertilisation (Kekkilä Stock Superex, NPK 19-4-20, Kekkilä, Co., Tuusula, Finland) began on 22 June with irrigation water at an electrical conductivity (EC) rate of 0.8 mS cm^{-1}. Ferilisation ended in late September. Seedlings were cultivated in a heated greenhouse. Seedlings were air pruned in raised beds during cultivation to ensure no root development outside the containers. In autumn and winter, when temperatures outdoors of the greenhouse fell towards 0°C, the temperature in the greenhouse was maintained as close to 4°C as possible, until seedlings were transferred outdoors in January to be overwintered in a white plastic tunnel.

Freezing procedures

To induce experimental damage to roots, artificial freezing took place at the Icelandic Agricultural University's freeze-testing laboratory at Möðruvellir, Akureyri, on 11 December and 27 January (Table 1).

On 11 December 2011, a preliminary test was conducted to explore the level of frost tolerance in roots before plants for the field trial were frozen. Twenty seedlings were frozen for each treatment to −3°C, −10°C, −15°C, or −20°C, and then root growth was evaluated for each treatment, as described below (about RGC test).

On 27 January, seedlings designated for planting in field trials and for root growth measurements were frozen to −9°C, −13.5°C, −15.5°C, −17°C, and −20°C. We used information from the preliminary test to determine the freezing temperatures. During both freezing procedures, 20 control seedlings were kept at 4°C in dark.

Table 1. Timeline of work components during the study in the years 2011, 2012, and 2013.

Work components	Year 2011	Year 2012	Year 2013
Sowing in sphagnum peat	10th June		
Fertilisation began	22nd June		
Fertilisation ended	September 25th		
Preliminary freezing test	11th December		
RGC* 1	14th December		
Freezing test for field trial seedlings		27th January	
Seedlings moved to outdoor storage		29th January	
RGC 2		1st February	
RGC 3, only for control seedlings		17th April	
All treatments planted in field trial		2nd June	
RGC 4		4th June	
Measurement of field trial		6th September	5th September

*Root growth capacity.

Before freezing, the trays with the seedlings were watered to ensure that the root substrate would freeze evenly. At first, the temperature was lowered from 4°C to 0°C over a period of two hours. The freezing rate was then set to −0.5°C per hour. Target temperatures were maintained for four hours. Samples were slowly thawed again by raising the temperature by 0.5°C per hour until 0°C was reached. The temperature was then set to 4°C to ensure slow thawing. The root substrate temperature was logged during the freezing procedures using data loggers (One Wire, Embedded data systems).

Seedlings designated for planting were placed in conventional white plastic tunnels in the nursery on 29 January to be overwintered before being transported to the planting site on 2 June. To avoid potential non-intentional freezing damage, the trays in the trial were surrounded by other trays. Data loggers were placed in the root substrate and inside the plastic tunnel (HOBO H8 Pro Temp/Temp External Data Logger, Onset Computer Corporation, Pocasset, MA, USA) to monitor the temperature during winter in the plastic tunnels. Weather data were obtained from a weather station nearby in Akureyri.

Measurement of root growth

The root growth capacity test (RGC) was used to measure root growth, as described in Mattsson (1986) using a BCC root growth capacity table (BCC, AB, Landskrona, Sweden). Twenty seedlings from each treatment were randomly chosen and divided into four replicates, with five seedlings in each replicate. RGC tests were performed on 14 December 2011 and 1 February, 17 April, and 4 June 2012 (Table 1). The growing medium used for RGC tests was 50% peat (F6, Kekkilä Oy, Tuusula, Finland) and 50% perlite. Seedlings were grown in artificial light (Philips 58W/840 New Generation) with a 16-hour daylength and 8-hour night, at 20°C air and soil temperature. Measurements of root growth were performed after three weeks. The growing substrate and seedlings were removed from the growing table, and the growing substrate was removed carefully from the root system. All new white roots that had grown outside the original root system were cut off. Then root samples were pooled together, with five seedlings in each sample, dried for 48 h at 70°C, and then weighed.

To evaluate seasonal variation in root growth, control seedlings from freezing tests on 11 December 2011 and 27 January 2012 and additional root growth tests on untreated (control) seedlings from 17 April and 4 June were used (Table 1).

Field trial

All seedlings were planted 15 km south of Akureyri (65,32°N, 18,89°W) on 2 June 2012 in six blocks; there were 10 seedlings for each of the five treatments in each block, for a total of 300 seedlings. The site was dry, with homogeneous vegetation, indicating dry and poor soils, with *Kobresia myosuroides* as the main vegetation species. Because of the Icelandic soils' high phosphor-retention and low availability of nitrogen, fertilisation at planting is a standard work component of afforestation in Iceland (Óskarsson and Sigurgeirsson 2001). All seedlings were fertilised with Sprettur (Carrs Fertilizers, Scotland), a blend of NP (23-5.2). Ten grams of fertiliser was scattered by hand in a 10 cm circumference around each seedling.

The annual height increment, stem diameter, and seedling mortality were evaluated in September before needle fall in 2012 and 2013. Seedlings were classified as dead if the seedlings had no needles. During winter 2012–2013, frost damage in shoots was common. The shoot tips, buds and stems in the upper part of seedlings had visual damage. The damaged part of the seedlings was measured in cm and proportion of damage to the total height was calculated for each plant in all treatments. Since there was not a significant difference of frost damage between treatments (p-value = 0.1042, data not shown) stem diameter was used as a parameter of growth in autumn 2013.

Meteorological data

In December 2011, when seedlings were still stored in the greenhouse, the average temperature was unusually low, −4.1°C, with only 40 min of sunshine hours. In January 2012, the average temperature was 0.3°C, with 4.1 sunshine hours (Icelandic Meteorological Office 2022). From February to June 2012, the lowest temperature registered in the growing medium in outdoor storage was −2°C.

The summer of 2012 was unusually dry (Table 2). However, in September, precipitation was three times higher than the average, at around 140 mm; a large part of that precipitation fell as snow at the beginning of the month. In May 2013, precipitation was 49 mm, which was more than average. However, the rest of the summer months were dry or had average precipitation (Icelandic Meteorological Office 2022).

Statistical analyses

The statistical programme R (R Core team Version 4.0.4) was used to analyse the data. Prior to analysis of the dry weight of new roots, the normality of each treatment was checked by inspecting histograms and boxplots. When there were clear violations of the normality assumption, the Kruskal – Wallis test was used to test all treatments. If significant, further analysis was performed with the post-hoc Kruskal conover test described in the PMCMR package (Pohlert 2014).

Frost hardiness is often expressed as the lethal temperature (LT_{50}) that indicates the temperature at which 50% of exposed plants or plant parts die (Lappi and Luoranen 2018). When calculating LT_{50} and LT_{10} for the freezing test conducted on 27 January, logistic regression was used. A generalised linear model using a binomial distribution was used to fit a curve to temperature and alive/dead data. The MASS library in R was used to calculate the median lethal temperature for LT_{50} (50% survival) and LT_{10} (90% survival) with the function dose.p. The Saphiro–Wilk test and boxplot were used to check the normality of stem diameter and shoot elongation and proportion of frost damages. A one-way analysis of variance (ANOVA) was used to analyse stem diameter, followed by a Tukey post-hoc test to run pairwise comparisons among each of the treatments. Shoot elongation was not normally distributed, and transformation by logarithmic function was not successful in correcting skewness. Therefore, its overall treatment difference was compared by the Kruskal–Wallis test. If the Kruskal–Wallis test was found to be significant further analysis was done with the post-hoc Kruskal conover test described in the PMCMR package (Pohlert 2014). Statistical analysis of proportional survival and frost damages was performed in the same way.

Table 2. Total precipitation (mm) and proportion of average precipitation (%) in the growing season of 2012 and 2013 (Icelandic Meteorological Office 2022).

Year	Month	Total precipitation (mm)	Proportion of average precipitation (%)
2012	June	9.9	35
–	July	25.4	77
–	August	8.4	25
–	September	140	258
2013	May	49.0	153
–	June	8.1	29
–	July	31.3	95
–	August	18.8	53

Results

Root growth in undamaged seedlings

Seasonal variation in root growth of undamaged seedlings was studied through RGC measurements on four occasions during winter 2011–2012 (Table 1). Root growth decreased significantly ($p = 0.046$) from December to April, with 70% less dry weight of the roots in April (Figure 1). In June, dry weight was about 55% more than in April; however, the difference was not statistically significant.

Preliminary root damage test

The frost treatments in the preliminary test on 11 December (Table 1) resulted in a gradient of root damage. There was no significant difference in root growth between the control and treatment groups frozen to −3 and −10°C (Figure 2). When seedlings had been frozen at −15°C and −20°C root growth was significantly lower ($p = 0.0037$).

Pre-planting root damage

The results from the preliminary test in December indicated that the root system of the seedlings would tolerate −10°C (Figure 2), since there was a significant difference in the dry weight of roots between the treatment exposed to −10°C and the treatment exposed to −15°C ($p = 0.0012$). Therefore, when the time came to induce pre-planting root damage in January, the chosen temperatures ranged from −9°C to −20°C. RGC cultivation in January showed 84%, 97%, and 99% less root growth in seedlings frozen to −9°C, −13.5°C and −15,5°C compared to control seedlings ($p = 0.0039$) (Figure 3). After freezing to −17.5°C and −20°C, no seedlings formed new roots; therefore, these temperatures were omitted from Figures 3, 5, and 6.

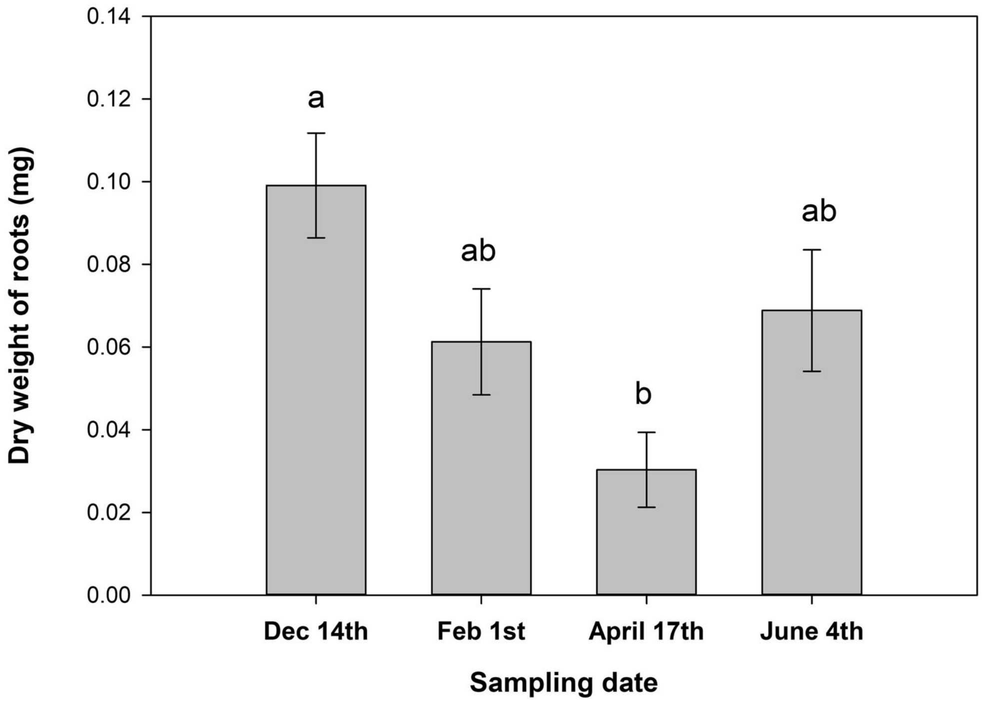

Figure 1. Average dry weight (mg ± SE) of new roots of Russian larch (Larix sukaczewii Dyl.) seedlings grown in an RGC (Root Growth Capacity) table sampled on four occasions: 14 December 2011 and 1 February, 17 April, and 4 June 2012. Different letters above the bars indicate significant differences between dates found by Kruskal–Wallis and the posthoc Kruskal conover test ($p = 0.046$), $n = 20$.

The result from the logistic regression analysis indicated that temperature was significantly associated with the probability of seedlings survival ($p < 0.0001$) (Figure 4). In January, the LT_{50} was −13.9°C (SE ± 0.46), and LT_{10} (10% of exposed seedlings dead) was −10.6°C (SE ± 0.96).

Annual increment and stem diameter

In the first growing season, the average shoot elongation was statistically significantly different between treatments ($p <$ 0.0001). Control seedlings added on average, 7.1 cm to their height (Figure 5). The shoot elongation of seedlings artificially

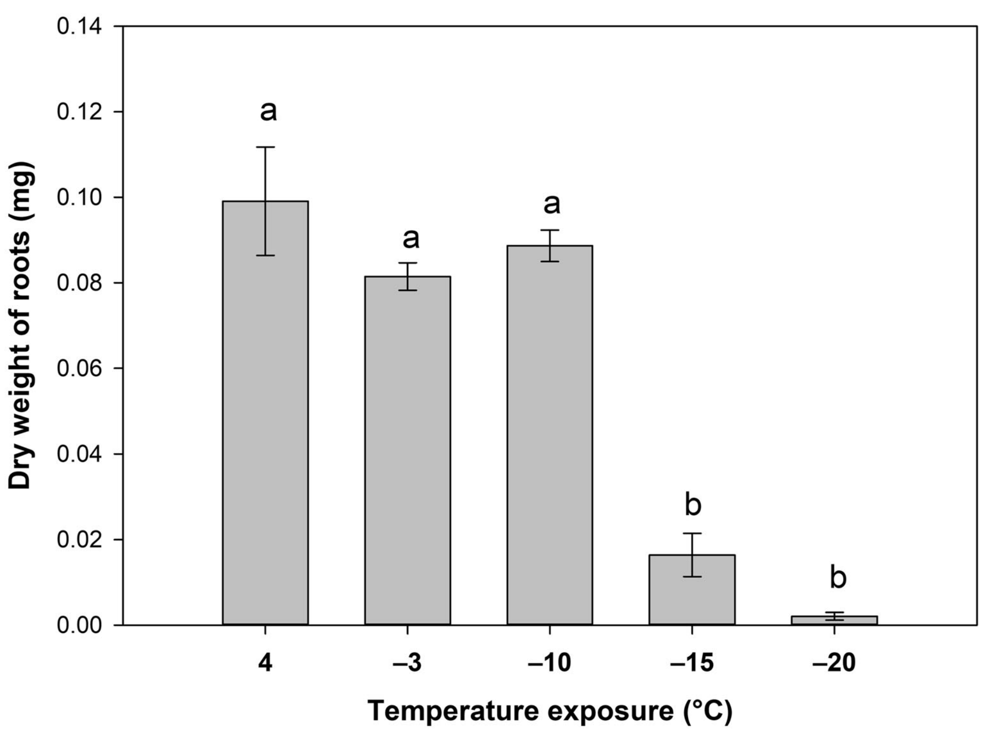

Figure 2. Average dry weight (mg ± SE) of new roots of Russian larch (Larix sukaczewii Dyl.) seedlings grown in an RGC (Root Growth Capacity) table after exposure to different temperatures and sampled on 11 December 2011. Different letters above the bars indicate significant differences between treatments found by Kruskal – Wallis and the posthoc Kruskal conover test ($p = 0.0037$), $n = 20$.

frozen to −9°C, −13.5°C, and −15.5°C was reduced compared to the control treatment by 23%, 54%, and 72%, respectively.

During winter 2012 and 2013, seedlings in the experimental trial were severely damaged by frost. Therefore, the root collar diameter was used as a parameter for growth after the growing season in 2013. The diameter of seedlings exposed to pre-planting freezing to −13.5°C and −15.5°C was significantly lower than control seedlings ($p < 0.0001$), with a 47% and 82% lower average stem diameter, respectively (Figure 6).

Survival in autumn 2012 and 2013

After the first growing season, survival was 100% in the control seedlings and seedlings exposed to −9°C before planting (Table 3). Survival for seedlings exposed to −13.5°C and −15.5°C before planting was 85% and 26.7%, respectively. After the second growing season, survival decreased in all treatments except for the control seedlings, which still had 100% survival. The seedlings exposed to a pre-planting temperature of −9°C had 91.6%, on average, survival and had significantly lower survival than control seedlings ($p = 0.04$). Survival had decreased significantly in seedlings with pre-planting temperatures of −13.5°C and −15.5°C to 63.3% and 20%, respectively.

Discussion

Seasonal variation in root growth

Healthy and vital seedlings with optimal growth potential are essential for forest regeneration (Grossnickle and MacDonald 2018b). Therefore, it is important to assess seedling quality prior to planting (Templeton and Colombo 1997; Bigras and Dumais 2005; Dumroese et al. 2016; Grossnickle and MacDonald 2018a). Root growth capacity is a widely used method to indicate root system integrity (Grossnickle and MacDonald 2018a). Nevertheless, this seedling quality test comes with pros and cons. Simpson and Ritchie (1997) debated about the root growth potential method. One of Simpson and Ritchie's concerns is that root growth potential varies seasonally both in the nursery and in overwinter cold storage. With that in mind, it is important to make the measurements close to the time of planting and acquire knowledge about seasonal variation in root growth to be able to interpret the correct outcome of the RGC test. Mattsson and Lasheikki (1998) reported variations in root growth of Siberian larch seedlings during two growing seasons, from the sowing date in April 1992 until early September 1993. They found that root growth peaked in the middle of September but declined rapidly to a very low level in October due to needle loss during fall, with subsequent low photosynthetic capacity. Root growth remained low during winter, but in May, rapid root growth began again and remained high during summer. Seasonal variations in root growth were also found in our study. There was a clear variation in root growth in undamaged seedlings during the seven-month period from December to June. The dry weight of roots was lowest in April, right at the time when RGC tests are usually conducted in Iceland (author's notes). The low root growth capacity at that time did not result in low growth or survival in undamaged seedlings compared to the seedlings exposed to freezing damage in the field trial, indicating that root growth suppression at this time of year is not related to

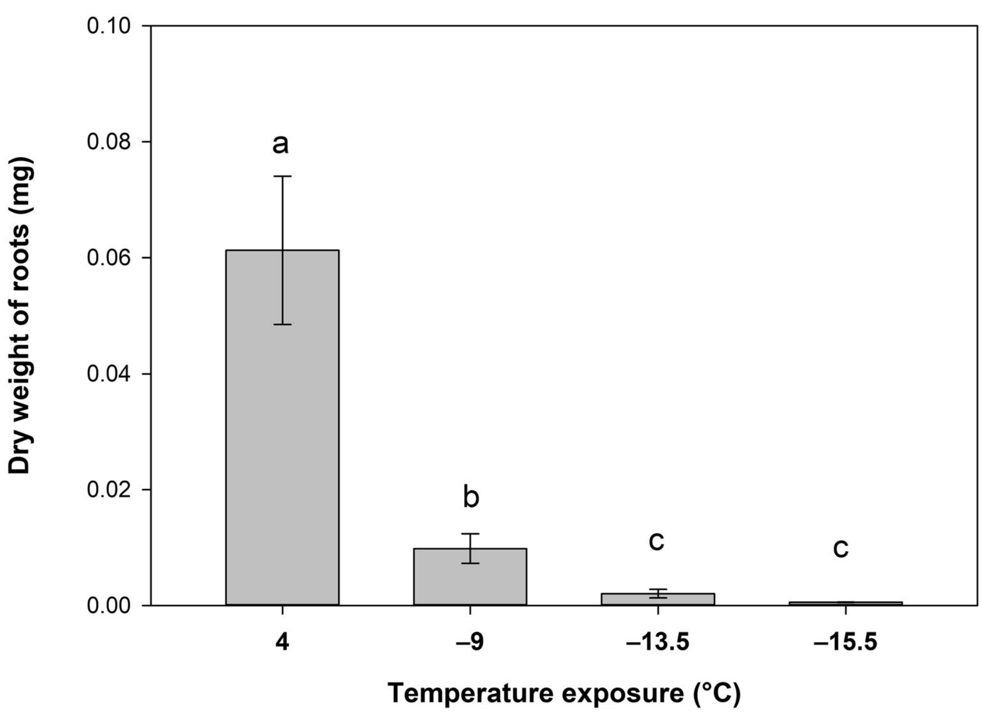

Figure 3. Average dry weight (mg ± SE) of new roots of Russian larch (Larix sukaczewii Dyl.) seedlings grown in an RGC (Root Growth Capacity) table after exposure to different temperatures and sampled on 27 January 2012. Different letters above the bars indicate significant differences between treatments found by Kruskal – Wallis and the posthoc Kruskal conover test ($p = 0.0039$), $n = 20$.

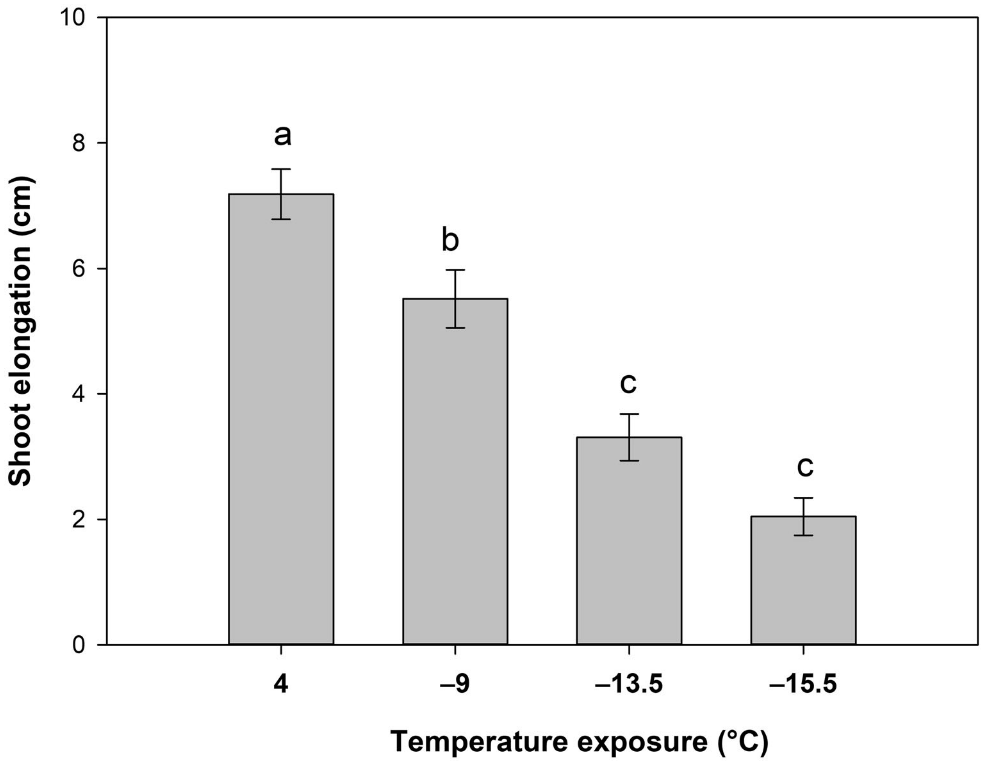

Figure 5. Average (±SE) shoot elongation (cm) of Russian larch (Larix sukaczewii Dyl.) seedlings with pre-planting frost exposure to roots after one growing season. Different letters above the bars indicate significant differences between treatments found by Kruskal–Wallis and the posthoc Kruskal conover test ($p < 0.0001$), $n = 60$.

low seedling quality but might be associated with effects interacting with stress resistance or the dormancy status of the seedlings (Cannell et al. 1990; McKay 1992). It is a widely held belief that root growth is highly dependent on current photosynthate, since conifers are apparently unable to mobilise reserve carbohydrates for root growth (Ritchie and Dunlap 1980; Simpson and Ritchie 1997). The sharp increase in root growth in May in the study by Mattsson and Lasheikki (1998) was interpreted as a response to more favourable growing conditions in terms of light intensity and temperature. This is also the most likely explanation for the rapid, but not significant, increase in root growth in June compared to root growth in April in our study.

Pre-planting root damage and frost tolerance

Lowering freezing temperatures caused decreased root growth and survival following both freezing occasions. The reason for this is probably that increasingly larger proportions of root systems are being damaged, causing a lower ability of the seedlings to grow roots. Since fine roots are the least frost tolerant, freezing episodes kill them, leaving the root system without the necessary fine roots for nutrient and water uptake mechanisms (Kramer and Kozlowski 1979).

When the time came to induce pre-planting root damage in January, the chosen temperatures ranged from −9°C to −20°C since the preliminary test of 11 December had shown significant difference in the dry weight of roots between treatments exposed to −10°C and −15°C. Surprisingly, all frozen seedlings had significantly more damage in January than in December, indicating less frost tolerance in the roots. The reason for this change in root frost tolerance remains unclear. Root dehardening occurs with temperature increase (Bigras and D'Aoust 1992; Bigras and Dumais 2005). During early winter, periods of clear, sunny weather can heat greenhouses and cause plants to lose dormancy (Landis et al. 2010). December 2011 was unusually cold (average temperature −4.1°C), while January was mild in Akureyri (average temperature 0.3°C) (Icelandic Meteorological Office 2022). This may have caused higher temperatures in the greenhouse in January. In our study temperatures during the storage time in the greenhouse were not logged. McKay (1994) found that the root frost hardiness of Japanese larch and Sitka spruce was closely related to soil temperature. Two-year-old seedlings reached a level of −12°C to −13°C for root frost hardiness in February, but it decreased sharply from March to −6°C by the beginning of April. The dehardening of root frost hardiness was apparently related to temperature. This was also reported in Stattin (1999), for one-year-old containerised Scots pine, where root freezing tolerance was reduced when soil temperature was increased.

In our study, Russian larch roots were able to frost harden at non-freezing temperatures to some extent, but freezing tolerance fluctuated during the winter. It can be expected that root frost tolerance in Russian larch can develop differently based on environmental conditions. McKay (1994) found that different species and provenances developed root frost hardiness in response to different aspects of the winter environment and concluded that they may not have a similar process of root frost tolerance formation every winter because of different

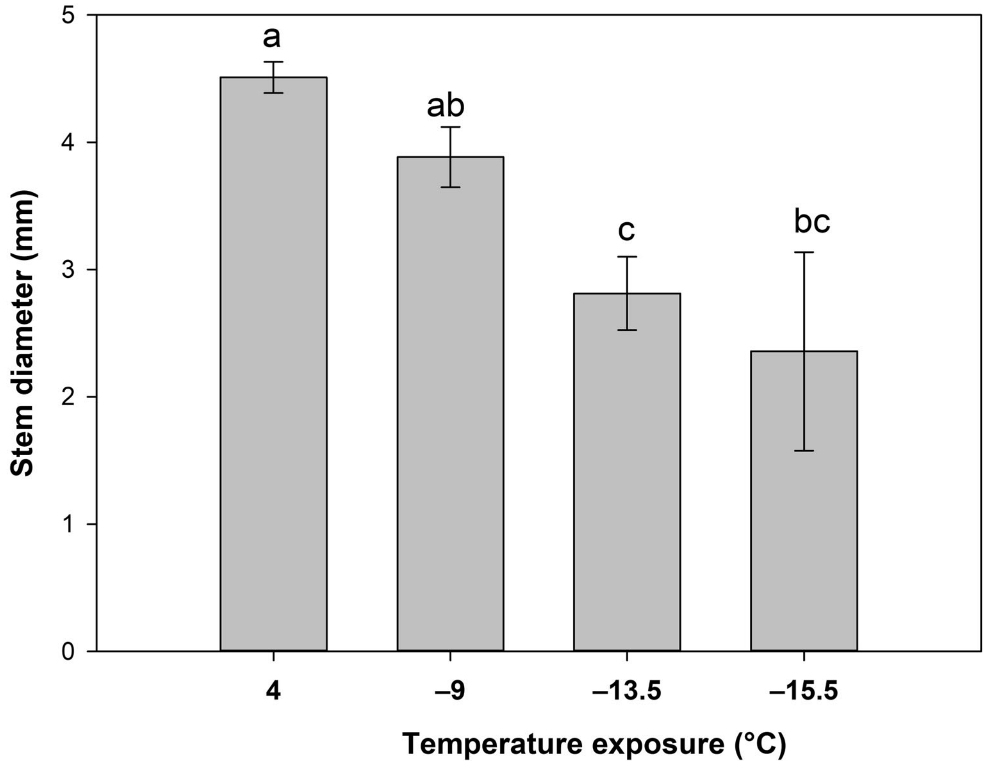

Figure 6. Average (±SE) diameter of Russian larch (Larix sukaczewii Dyl.) seedlings with pre-planting frost exposure to roots after two growing seasons. Different letters above the bars indicate significant differences between treatments found by one-way ANOVA, followed by a Tukey post-hoc test ($p < 0.0001$), $n = 60$.

combinations of temperature, day length, and precipitation. In our experiment, the LT_{10} value showed that at −10.6°C (±SE), severe damage to the root can be expected in Russian larch seedlings. After exposure to −13.9°C (±SE), 50% mortality can be expected based on RGC tests.

Shoot elongation, stem diameter, and survival after planting

Growth and survival decreased with increasing pre-planting frost exposure the first and the second year after planting in the field. These results are in line with the results of

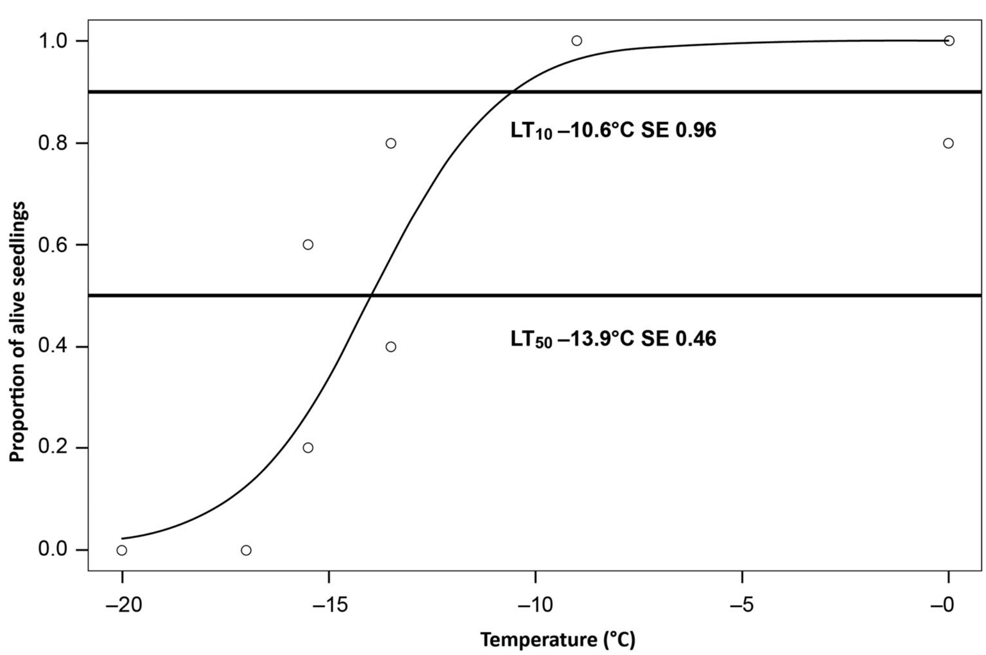

Figure 4. Proportion of alive Russian larch (Larix sukaczewii Dyl.) seedlings (circular symbols) after exposure to five different temperatures on 27 January 2012, as determined by logistic regression and a generalised linear model, using a binomial distribution to fit a curve. The upper black line shows the temperature (±SE) at which 10% of the seedlings were dead. The lower black line shows the temperature (±SE) at which 50% of the seedlings were dead, $n = 20$.

Table 3. Average survival (±SE) of Russian larch (Larix sukaczewii Dyl.) seedlings with different pre-planting temperature exposure to roots after the first (2012) and second (2013) growing seasons in the field.

Exposure temperature (°C)	Average survival (%)			
	2012		2013	
4	100.0 ± 0	a	100 ± 0	a
−9	100.0 ± 0	a	91.7 ± 4.0	b
−13.5	85.0 ± 3.4	b	63.3 ± 8.0	c
−15.5	26.7 ± 6.1	c	20.0 ± 7.7	d

Note: Column values in the same year followed by the same letter are not statistically different at $P < 0.05$ significant level found by Kruskal Wallis and the posthoc Kruskal conover test ($n = 60$).

Dumais et. al (2002) who examined the effects of simulated root freezing during fall on the growth and physiology of white spruce (*Picea glauca* (Moench) Voss), black spruce (*Picea mariana* (Mill. BSP)), and jack pine (*Pinus banksiana* Lamb.). They found that net photosynthesis, stomatal conductance, and nitrogen concentration of current-year foliage decreased with increasing root damage, causing less growth in seedlings with 40–50% of the root system damaged. In their study, seedlings with less frost damage were only slightly affected, which was due to abundant summer precipitation. They also found that seedlings grew better at the wet site and attributed it to the higher soil water content than at the dry site. In our study, the land type was considered rather dry and poor. Precipitation was unusually low during the first growing season in the field, with a record high number of sunshine hours. The year after, June also had only a fraction of the usual precipitation (Icelandic Meteorological Office 2022).

Although survival in the seedlings frozen to −9°C was 100% in the first year, significantly lower shoot elongation than in the control treatment indicated that the root system was also damaged in that treatment. After a second growth period, the survival of this treatment declined significantly to 91.7%, which indicates that the effect of root damage on survival did not necessarily occur in the first year. This was also shown by Bigras (1998) were two-year-old black spruce plants were damaged to varying degrees with freezing temperatures or root pruning. Mortality in damaged seedlings was low during the first growing season, but increased after the second growth period. In that study, the effects of damage to the root system were still evident after three growing seasons in shoot height and stem diameter (Bigras 1998).

In the study of Bigras (1998) the effects of damage were more severe in frozen treatments compared with pruned treatments. This was explained by fine roots being more frost sensitive than large roots in conifer seedlings. Therefore, frozen seedlings suffered from the complete destruction of fine roots, while pruned treatments still had some fine roots available. This is an important factor in understanding the severity of frost damage to the root systems of forest seedlings. If all or a large majority of the fine roots are damaged at planting, the seedlings' opportunities to become coupled to the hydraulic cycle of the planting site are significantly reduced (Burdett 1990; Grossnickle 2000). However, favourable conditions at the planting site, such as soil moisture content or abundant summer precipitation, can help newly planted seedlings with damaged root systems (Bigras 1998; Coursolle et al. 2002).

Conclusion

In this study, seasonal variation in the root growth of Russian larch seedlings was detected. Suppression in root growth is high right at the time when RGC is usually measured during the spring in Iceland. This should be kept in mind when interpreting the results from regular RGC measurements. Our study showed Russian larch roots' ability to frost harden at non-freezing temperatures. The results provide insight into root frost tolerance during winter in young Russian larch seedlings, but more information is needed about how rapidly the root frost tolerance of larch is lost in relation to an increase in root zone temperature. Mortality, due to a damaged root system, occurred not only in the first year but was still ongoing after two growing seasons. These results emphasise the importance of ensuring the quality of forest seedlings before planting.

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