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Biophysical changes in the roots of Scots pine seedlings during cold acclimation and after frost damage

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ABSTRACT

Root system health is a key factor for seedling quality and it is a prerequisite for the proper growth of seedlings after out-planting. If the seedlings are moved to freezer storage too early in the season, roots can be damaged and thereby the quality of seedlings declines. We aimed to develop a novel method to assess an appropriate time for moving the seedlings to freezer storage in the autumn and to detect possible root damage during overwintering. One-year-old containerized Scots pine seedlings were measured before, during and after freezer storage, and after a series of frost exposure tests. The electrical impedance spectra (from 4 kHz to 200 kHz) of roots changed during cold acclimatization. The impedance loss factor (δ) of roots at 50 kHz frequency decreased when the threshold of frost tolerance was exceeded in the freezing tests. Root hydraulic conductance (K_r) increased before and during the freezer storage. In the initial phase of cold acclimatization, K_r increased considerably after exposure to frost temperatures, indicating damage, but that effect disappeared with frost hardening. In regrowth tests, the largest number of new root tips was observed after exposure to −12 °C in the freezing tests just before and after freezer storage, and after exposure to −30 °C during the freezer storage. During the freezer storage, shoot growth declined at higher exposure temperatures than root tip formation, suggesting that roots and specifically root tips would not be the primary reason for declined shoot growth. We conclude that the biophysical measurements of roots are useful for assessing the condition of the root system for overwintering applications in tree seedling nurseries, and that roots tolerated lower temperatures than previously thought.

1. Introduction

Planting with container seedlings is the most common forest regeneration method in the Nordic countries. The good condition of the root system determines the quality of the seedlings used in regeneration, being a prerequisite for their successful field performance too (Grossnickle and MacDonald, 2018). Outdoors, roots are regarded to be sensitive to damage during overwintering, especially if there is no protective snow cover (Sakai and Larcher, 1987; Colombo et al., 1995), thus declining the quality of the seedlings. In order to improve the quality, freezer storage has become a common overwintering method, both in the Nordic countries and in North America (Landis et al., 2010). In Finland, approximately one half of a total of 155 million seedlings produced annually are taken to freezer storage and the other half overwinters outdoors.

The right time to move the seedlings from the nursery field to the freezer storage is a key phase in the cultivation of seedlings in the boreal zone. For successful storage, the seedlings should be kept in the field conditions long enough to reach a sufficient degree of frost hardiness. This is important for the resilience of the seedlings in the freezer storage too, even though the temperatures are not as extreme as in the field conditions (Landis et al., 2010). In practice, the timing for moving the seedlings to freezer storage in the nurseries is often based on assessment of the water content of the apical shoot or the shoot electrolyte leakage (SEL) test but they do not take into account the frost hardiness of roots (Rosvall-Åhnebrink, 1982; Lindström et al., 2014).

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Cold acclimatization includes a multitude of physiological and biochemical changes in cells that are driven by the increasing night length and decreasing temperature. These changes prepare cells and organs to tolerate apoplastic freezing and its dehydration consequences (Weiser, 1970; Uemura and Steponkus, 1994; Grossnickle and South, 2014). Cold acclimatization develops at a different pace in shoots and roots. Therefore, shoots may be sufficiently frost tolerant for storage while roots are not (Stattin et al., 2000) and too early transfer to cold storage may damage the roots. Delayed transfer increases the risks of being exposed to night frosts, causing direct damage, and also to snowfall and the formation of an ice layer, whereupon the packing of seedlings for freezer storage is impossible. In freezer storage, the risks of being damaged during overwintering and harsh spring conditions with a high variation of temperature and light irradiance are also avoided.

The functional integrity and vitality of the root systems has often been assessed by relative electrolyte leakage (REL) and root growth potential (RGP) (Burdett, 1979; McKay, 1998; Grossnickle and MacDonald, 2018). In REL, the results are typically limited to a small part of the root system and they may be confounded by washing the roots (Radoglou et al., 2007; Repo and Ryyppö, 2008; Korhonen et al., 2015, 2018). RGP is a slow test method but it is useful for assessing the condition of root systems and overall seedling vitality and growth in controlled growing conditions in tree seedling nurseries (Burdett, 1979; Grossnickle and MacDonald, 2018). Root injuries may be detected by dyeing with triphenyl tetrazolium chloride (TTC) but the reactive substances of root tissues may bias the results, especially in conifers (Sutinen et al., 1996; Richter et al., 2007). X-ray and magnetic resonance imaging are non-destructive methods but they are limited by soil properties, scanning time and resolution for fine roots (Asseng et al., 2000; Metzner et al., 2014; Brackin et al., 2017). Electrical impedance spectroscopy (EIS) gives information of the electrolyte balance in cells as affected by various factors, for example, by freezing stress (Zhang and Willison, 1992; Repo et al., 1994). If there are changes in the balance, the proportion of the current along different routes changes. In EIS, the alternating electric current of different frequencies is driven through the sample. Complex impedance is obtained from the relation between voltage and current (regarding both amplitude and phase) and this is formed of a real part (Z_{Re}) and an imaginary part (Z_{Im}). A low-frequency current will pass along the routes with the lowest impedance, like routes along the apoplastic space in plant tissues, whereas a high-frequency current may pass barriers like cell membranes. EIS has been used to study root growth and morphology (Rajkai et al., 2005; Cao et al., 2011), as well as mycorrhiza formation and freezing injuries in roots (Repo et al., 2014, 2016), but it has not yet been used for assessing root vitality in nursery applications.

Another potential method to study root vitality is based on hydraulic conductance (K_r), which is a measure of roots' capacity to transport water (Tyree et al., 1995). Low soil temperatures and water/freezing stress in roots typically decreases K_r and thus inhibits water uptake (Nardini et al., 1998). However, K_r increased when the water was injected to the roots by a high pressure flow meter (HPFM) from the opposite direction compared to a normal situation (i.e. to get reverse-flow K_r) (Leinonen et al., 2011). This is explained by the facilitated passage of water from roots to soil in injured root tips. Therefore, this method may potentially indicate impaired root structure and function but it has not yet been tested on overwintering applications in tree seedling nurseries.

In this study we aimed to develop a fast and non-destructive method for assessing the frost hardiness of roots in order to assess both the freezer storability of nursery seedlings in autumn and frost damage in roots after overwintering (either in freezer storage or outdoors). The biophysical properties of the roots of Scots pine seedlings were measured by EIS and using an HPFM before, during and after freezer storage and after exposing the roots to different freezing temperatures. The results were compared with commonly used methods for monitoring changes in frost hardiness, in other words through monitoring root and shoot growth in the regrowth tests, the water content of shoots, and the frost hardiness and chlorophyll fluorescence of needles.

2. Material and methods

2.1. Seedling cultivation and samplings

One-year-old Scots pine seedlings were cultivated in PL-81F containers (cell volume 85 cm^3, 81 cells per container, 546 cells/m^2; Lännen Oyj, Iso-Vimma, Finland) in the Natural Resources Institute Finland (Luke), in the Suonenjoki nursery (Eastern Finland, 62°39′N, 27°03′E, 142 m a.s.l.). The seedlings were grown from a seed lot EY/FIN T03-13-0205 collected from a 1.5-generation seed orchard (sv 434) located in Rantasalmi, Finland (62°05′N, 28°14′E, 80 m a.s.l.) consisting of 36 clones with a utilization area for temperature sum of 1060–1260°days (daily mean temperature > 5 °C). The seeds were sown on May 20, 2015, and grown first in an unheated greenhouse until July 29. Then the containers were moved to an outdoor growing area and back to an unheated greenhouse on September 9 to avoid damage by early night frosts. On November 17, approximately 2000 good quality seedlings (without border seedlings) were selected from circa 50 containers, packed into cardboard boxes (70 seedlings/box), and moved to a freezer storage (−3 °C) at RH 90–100%. The temperature in three of the boxes was recorded during the freezer storage with iButton sensors (Ds1921G-F5#, Maxim Integrated, USA). The rest of the seedlings in the greenhouse were moved outdoors to overwinter under the snow cover. The outside air temperature was recorded at the weather station at the height of 2 m, close to the greenhouse.

The air temperature was 3 °C at the time of moving the seedlings from the unheated greenhouse to the freezer storage (on November 17) (Fig. 1). In ten days, the temperature inside the cardboard boxes decreased to −2 °C and in one month to −3 °C (±0.5 °C). Outside air temperature was 1 °C in the middle of March when two containers of seedlings were dug from under the snow cover (ca. 30 cm) for the reference measurements. The freezer storage was switched off on April 8, and thereafter the storage temperature increased slowly. At the last sampling time, at the end of April, both outside and storage temperature were 4 °C.

The seedlings were sampled for the measurements three times before being moved to the freezer storage (September 21, October 18 and November 17, 2015), twice during the freezer storage (January 16 and March 15, 2016) and once after completion of the freezer storage but while still in the storage room (April 25). Before freezer storage, 540 seedlings were sampled in September (test temperatures 3 °C, −5 °C, −8 °C, −12 °C, −18 °C and −30 °C) and 630 seedlings in October and November (test temperatures 3 °C, −3 °C, −6 °C, −12 °C, −18 °C, −30 °C and −45 °C) in nine containers and transported to the Luke/

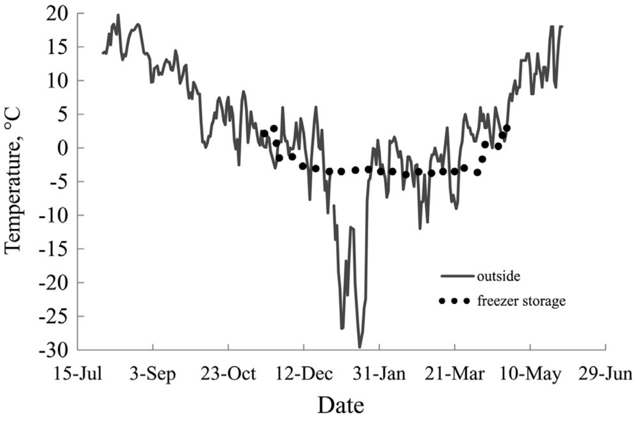

Fig. 1. Daily mean outside air temperature before, during and after freezer storage and the temperature in the seedling boxes during the freezer storage.