**Author(s):** Tapani Repo, Julien Ballandras, Jouni Kilpeläinen, Dongxia Wu & Timo Domisch

**Title:** Early-stage detection of root freezing injuries of Scots pine (*Pinus sylvestris* L.) seedlings by impedance loss factor and hydraulic conductance

**Year:** 2023

**Version:** Published version



**Please cite the original version:**

Repo, T., Ballandras, J., Kilpeläinen, J., Wu, D. & Domisch, T. (2023) Early-stage detection of root freezing injuries of Scots pine (*Pinus sylvestris* L.) seedlings by impedance loss factor and hydraulic conductance. Physiologia Plantarum, 175( 3), e13919. Available from: https://doi.org/10.1111/ppl.13919

Received: 5 January 2023 | Revised: 16 March 2023 | Accepted: 21 April 2023
DOI: 10.1111/ppl.13919

SHORT COMMUNICATION

Physiologia Plantarum

# Early-stage detection of root freezing injuries of Scots pine (*Pinus sylvestris* L.) seedlings by impedance loss factor and hydraulic conductance

Tapani Repo[1] | Julien Ballandras[1,2] | Jouni Kilpeläinen[1] | Dongxia Wu[1,3] | Timo Domisch[1]

[1]Natural Resources Institute Finland (Luke), Joensuu, Finland

[2]Association Groupe ESA, Angers, France

[3]Hebei Agricultural University, Faculty of Horticulture, Baoding, People's Republic of China

**Correspondence**
Tapani Repo, Natural Resources Institute Finland (Luke), Yliopistokatu 6B, FI-80100 Joensuu, Finland.
Email: tapani.v.repo@gmail.com; ext.tapani.repo@luke.fi

**Funding information**
Academy of Finland, Grant/Award Number: decision No. 311455; Natural Resources Institute Finland, Grant/Award Numbers: 41007-00004000, 41007-00112100

**Edited by** Y. Utsumi

## Abstract

The condition of the root system affects the quality of seedlings in forestry and horticulture. Previously, the electrical impedance loss factor ($\delta$) and the reverse-flow hydraulic conductance ($K_r$) of the roots of Scots pine seedlings were found to increase when assessed a few days after frost damage. How these variables change with time after the root damage is unknown. We arranged an experiment with 1.5-year-old Scots pine seedlings exposed to $-5°C$ or $-30°C$, with the control seedlings kept at 3°C. Then, $\delta$ and $K_r$ of roots were monitored for 5 weeks in favorable growing conditions. The properties of the roots were observed to be in a dynamic state after the damage. A significant difference in $\delta$ was found between the test temperatures $-30°C$ versus $-5°C$ and 3°C ($p = 0.004$ and $p < 0.001$, respectively). The clearest effect of freezing injuries on $\delta$ of roots was observed in the first measurement 1 week after the freezing test. The temperature significantly affected $K_r$, too, with a significant difference between the low-temperature treated plants $-30°C$ versus $-5°C$ and control ($p < 0.001$, respectively). The difference in $K_r$ between $-30°C$ and the other two temperatures increased with time and was the largest in the last samples, taken after 5 weeks. We conclude that the impedance loss factor may detect root damage if the measurements occur early enough after the damage, but a longer time difference (3–5 weeks) is needed according to the reverse-flow hydraulic conductance.

## 1 | INTRODUCTION

The condition of the root system is an important trait that affects the survival and growth of seedlings in forestry and horticulture, as well as defining the quality of nursery seedlings (Grossnickle & MacDonald, 2018; Ritchie et al., 2010). The condition may be affected by different abiotic stress factors, such as frost, drought, and excess of water, in different phases of seedling production, as well as after planting in field conditions. It is important to know the state of roots before the seedlings are dispatched from the nurseries for planting in the forests and gardens in the spring, and also in the fall before the seedlings are moved to frost storage for overwintering. If the roots are damaged, the effects will appear in the growth of the shoot, with a delay typically after the growing season has started, thus, affecting regeneration success (Grossnickle & MacDonald, 2018; Nilsson et al., 2010; Riikonen & Luoranen, 2018). Failures in forest regeneration may require replanting of the stand, which causes economic losses for both the seedling producers and forest owners.

There are limited direct methods for assessing the condition of root systems, as well as for the early detection of potential damage, and therefore, new methods to study the belowground processes of trees are needed. If the roots are damaged, their physiological condition is unstable and probably changes with time depending on the growth conditions. In bare root seedlings, relative electrolyte leakage (REL) has been successfully used to detect frost injuries in roots; however, in some cases, it has failed (Ritchie et al., 2010). In pot seedlings of Scots pine (*Pinus sylvestris* L.), the leakage of electrolytes from frost-damaged roots took place into the soil and/or by washing the roots with water (Repo & Ryyppö, 2008). Therefore, the REL method failed to detect root damage unless the root samples were cleaned from the soil and placed in the test tubes before the freezing tests (Radoglou et al., 2007; Repo & Ryyppö, 2008). Root growth potential (RGP) is based on detecting the number of new root tips on the surface of the root plugs of seedlings after growth under favorable conditions for 10–14 days (Grossnickle, 2000; Grossnickle & South, 2014; McKay, 1998; Ritchie et al., 2010). RGP varies during the annual cycle, which may limit the applicability to assess the physiological condition of the root system (Grossnickle & Ivetic, 2022). In the triphenyltetrazolium chloride (TTC) test, changes in root color may indicate whether the root is damaged (Richter et al., 2007; Sutinen et al., 1996). The method is laborious and undamaged roots may have chemicals that give a similar color change as damaged ones, which may bias the results.

The hydraulic and electrical properties of the roots of forest tree seedlings have been shown to change through changes in frost hardiness and freezing injuries (Colombo & Asselstine, 1989; Di et al., 2019; Korhonen et al., 2019; Repo et al., 2016). Hydraulic properties of roots can be measured by pressurizing roots from outside of the roots in a chamber or through the cut surface of the stem. In the former case, the weight of the roots changes according to the water protruding through the cut surface of the stem, with the amount of change depending on the condition of the root system (Ritchie, 1990). In the latter case, pressurized water is driven into the root system through the cut surface of the stem, and hydraulic conductance is obtained by pressure-volume analysis (therefore termed as the reverse-flow hydraulic conductance, $K_r$) (Tyree et al., 1995). Because damaged roots, especially root tips, leak water more easily than intact roots, their hydraulic conductance is higher (Korhonen et al., 2019; Leinonen et al., 2011; Ritchie, 1990). Hydraulic properties of roots of black spruce (*Picea mariana* Mill.) and Douglas fir (*Pseudotsuga menziesii* Mirb.) seedlings were found to change with root growth after soil thawing in spring or after freezing damage (Colombo & Asselstine, 1989; Ritchie, 1990). In freeze-damaged seedlings, those changes were correlated with seedling vigor and mortality (Ritchie, 1990). In controlled freezing tests of Scots pine seedlings during hardening and dehardening, reverse-flow hydraulic conductance of roots increased if damaged (Di et al., 2019; Korhonen et al., 2019; Leinonen et al., 2011; Repo et al., 2016). In the regrowth tests, those changes were connected with new root tip formation and root biomass (Di et al., 2019; Korhonen et al., 2019).

Electrical impedance spectroscopy (EIS) has been used for aboveground tree organs (Glerum, 1985; Repo et al., 1994; Repo, Zhang, Ryyppö, & Rikala, 2000; van den Driessche & Cheung, 1979; Zhang & Willison, 1992) and roots (Cao et al., 2011; Di et al., 2019; Repo et al., 2012, 2016) to detect their responses to environmental stresses. In EIS, an electric current of different frequencies is driven through the sample. The current carrying capacity of different cell compartments depends on the electrolyte balance between the apoplast and symplast, which is further affected by the condition of cell membranes. Due to the cell membrane damage and consequent leakage of the symplastic ions to the apoplastic space, the apoplastic electrical resistance decreases. Changes in the current carrying properties are used for assessing the frost hardiness of plants after controlled freezing tests (Repo et al., 1994, 2000; Zhang & Willison, 1992). The equivalent model for the root/soil system is more complex than for aerial parts, which poses a challenge for the formulation of a proper model and the estimation of the model parameters (Cao et al., 2011; Dalton, 1995; Repo et al., 2012). Therefore, a simpler form of EIS, that is, a single frequency (50 kHz) measurement of the impedance loss factor (δ), was used for the detection of root damage (Di et al., 2019). The loss factor increased with freezing injuries. Recently, a classification analysis of the impedance spectra of roots of Scots pine seedlings, using the approach of machine learning, could separate damaged and undamaged root systems and detect the mycorrhizal colonization of the roots (Repo et al., 2014, 2016).

It is unknown how the biophysical properties of roots, that is, the impedance loss factor and reverse-flow hydraulic conductance, change with time after freezing injuries. If they change, it will affect the diagnostic value of these variables for detecting injuries in roots. We, therefore, aimed to monitor $\delta$ and $K_r$ of roots of Scots pine (*P. sylvestris* L.) for 5 weeks after the controlled freezing tests at temperatures that caused either serious, slight, or no root damage. We hypothesized that the $\delta$ and $K_r$ of roots change after cellular injuries, and the time after the occurrence of damage should therefore be considered in the application of those methods.

## 2 | MATERIALS AND METHODS

The experiment was run with 1.5-year-old Scots pine (*P. sylvestris* L.) seedlings (pot type Pine 81, pot volume 100 $cm^3$, Pohjan Taimi Ltd.) that were cultivated and freezer stored (−3°C) at a tree seedling nursery in Eastern Finland (Pohjan Taimi Ltd., Juuka, Finland, 63°14′ N, 29°15′ E). Seeds were sown in the pots on May 5, 2018, raised in the greenhouse until mid-July, when they were moved to a nursery field to harden for winter, and moved to freezer storage (−3°C) on December 20, 2018. In the nursery cultivation, fertilization took place after germination with Kekkilä Forest-Superex (NPK 22-5-16, Kekkilä-BVB), and in the nursery field first with Kekkilä Peat-Superex

(NPK 11-5-26) and at the end of August with Kekkilä Autumn-Superex (NPK 0-20-24).

For the experiment, four cardboard boxes with 80 seedlings in each were taken from the freezer storage in the early summer of 2019 and transported to the Biosphere Laboratory in Joensuu (Natural Resources Institute Finland and University of Eastern Finland). Because it was aimed to monitor the changes in the impedance loss factor and hydraulic conductance with time after root injuries, not to assess the threshold temperature of freezer-stored seedlings, the seedlings were allowed for some degrees of dehardening before the start of the freezing tests at $-5°C$ and $-30°C$ with the control 3°C. Dehardening took place in a growth chamber (PGW36, Conviron Ltd.) at 18°C with the boxes closed for 1 day to slow down thawing and then with the boxes open for 4 days in long-day conditions (16/8 h for day/night, photon flux density 200 μmol $m^{-2}$ $s^{-1}$).

Ninety seedlings were taken from the cardboard boxes and placed in plastic containers for each temperature in the whole-plant freezing tests. In the freezing test chambers (ARC 300/ −55/+20, Arctest), the cooling rate from 5°C to −3°C was 2°C $h^{-1}$. The temperature of −3°C was maintained for 5 h to freeze the soil. Then the cooling continued at 2°C $h^{-1}$ to the target temperatures (−5°C and − 30°C), which were maintained for 4 h. The rate of thawing to 5°C was 5°C $h^{-1}$. The control seedlings were maintained at 3°C. After the treatments, the seedlings were raised in a growth chamber in long-day conditions (16/8 h for day/night, 18°C temperature, RH 75%, photon flux density 200 μmol $m^{-2}$ $s^{-1}$). Watering occurred by immersing the pots in tap water to a depth of 3 cm for 30 min, 2–3 times per week.

At each sampling time (5 times at 1-week intervals), nine randomly selected seedlings per treatment were used to measure the electrical impedance spectra (EIS), that is, real ($Z_{Re}$) and imaginary ($Z_{Im}$) part of impedance, and hydraulic conductance of roots. The different seedlings were measured by both methods at each sampling time. In EIS, a non-destructive set-up with two electrodes was used. A stainless-steel needle electrode (diameter 0.3 mm, Kangsheng Europe GmbH) was pushed into the stem 2 mm above the point of the first lateral root. Another electrode was a stainless-steel plate electrode with peaks protruding into the soil substrate at the bottom of the pots. The measurements were carried out at 42 frequencies between 90 and 200 kHz (EIS-101, Simitec Ltd.). The measurement of each seedling was repeated twice, with the stem electrode set perpendicularly between the measurements. The input voltage level was 200 mV ($V_{pp}$). The impedance loss factor (Equation (1)) was calculated at 50 kHz (β-dispersion range; for dispersion ranges, see Schwan, 1988) that was previously found to give the best resolution for root damage in Scots pine (Di et al., 2019).

$$\delta = \tan^{-1}\left(\frac{Z_{Im}}{Z_{Re}}\right) \tag{1}$$

At each sampling time, the reverse-flow root hydraulic conductance ($K_r$, in grams per megapascal per second) was measured for nine seedlings per treatment using a high-pressure flow meter (HPFM) (Dynamax). The stem was cut 15 mm above the root collar while the root system remained intact. The bark was peeled off below the cut point of the stem, and the capillary tube of the HPFM was connected to the cut surface with a coupling set. The measurement is based on monitoring water flow by gradually pressurizing the root from 0 to 0.55 MPa (Tyree et al., 1995). The reverse-flow $K_r$ was obtained from the linear relationship between water flux and applied pressure (Tyree et al., 1995; Voicu et al., 2008). The mean value of three repeated seedling measurements was used in further analyses. At the last sampling time, the length of the new shoot and the length of five new needles of each seedling were measured with a ruler with 1-mm accuracy.

The impedance loss factor and hydraulic conductance data were analyzed through liner regression ("lsmeans" package in R).

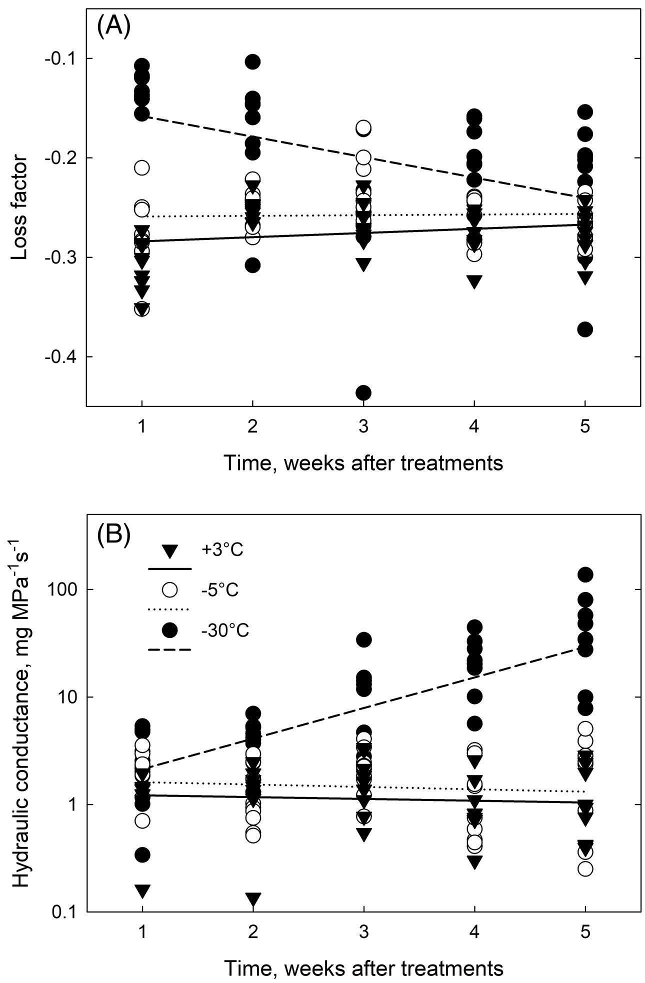


**FIGURE 1** Impedance loss factor of roots (A) and hydraulic conductance of roots (B) of Scots pine seedlings, which were exposed to 3°C (control), and to −5°C and − 30°C in one phase of dehardening in the whole-plant freezing tests. Different seedlings were measured at 1-week intervals after the temperature treatments ($n = 9$ for each treatment at each sampling time). The regression line of −30°C differs significantly from −5°C and 3°C ($p = 0.004$ and $p < 0.001$ for the loss factor and $p < 0.001$ for the hydraulic conductance, respectively).

**TABLE 1** ANOVA table for the effects of time since temperature exposure (time), exposure temperature (temp) and their interaction on impedance loss factor and reverse-flow hydraulic conductance of roots of 1-year-old Scots pine seedlings that were exposed to two freezing temperatures (−30°C and − 5°C, and control (3°C) in one phase of dehardening in controlled conditions.

| | Loss factor | | | Hydraulic conductance | | |
|---|---|---|---|---|---|---|
| | Df | *F*-value | *p*-Value | Df | *F*-value | *p*-Value |
| Time | 1 | 3.9 | 0.0504 | 1 | 22.4 | 5.813E-6 |
| Temp | 2 | 37.6 | 1.41E-13 | 2 | 25.6 | 4.311E-10 |
| Time × temp | 2 | 8.5 | 0.0003 | 2 | 22.3 | 4.771E-9 |

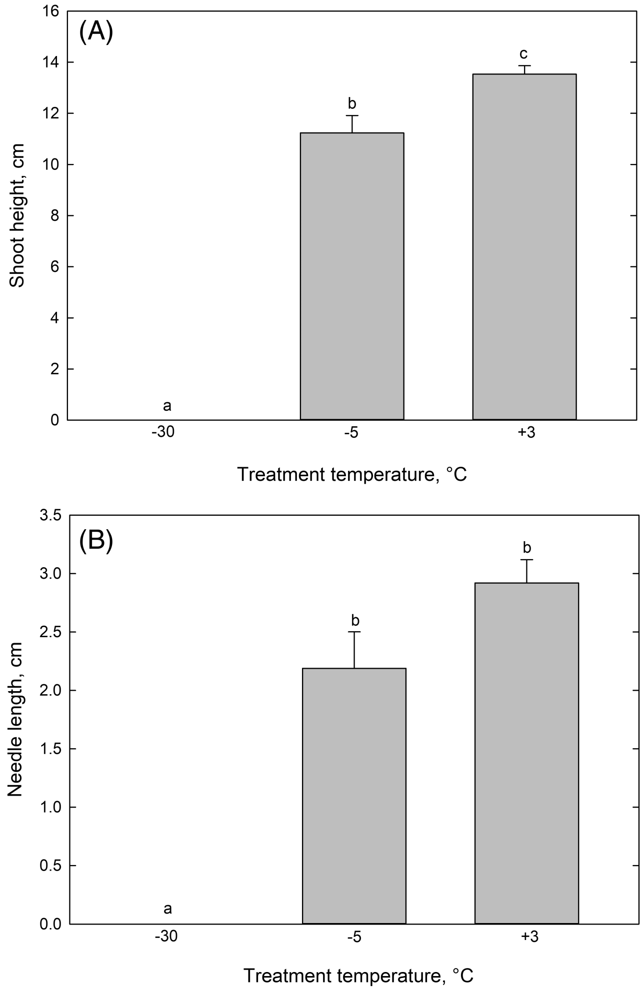


**FIGURE 2** The length of the new shoot (A) and needles (B) of Scots pine seedlings exposed to different temperatures in whole-plant tests and raised in favorable growth conditions for 5 weeks. The different small-case letters indicate significant differences ($p \leq 0.05$) between the treatments. The bars indicate standard errors ($n = 9$).

We compared whether the regression slopes differed between temperature treatments. Time (weeks 1–5) and temperature treatments (−30°C, −5°C, and +3°C) were treated as categorical factors. Specifically, we wanted to know whether the loss factor or the hydraulic conductance differed between the temperature treatments and whether interactions existed between time and temperature treatments. First, we examined the ANOVA *p*-value from the interaction of loss factor and temperature treatments and then compared the slopes with pairwise comparisons with Tukey's correction for *p*-values. Needle and shoot length data were analyzed using the Kruskal–Wallis rank sum test and, subsequently, the Wilcoxon rank sum test with pairwise comparisons.

## 3 | RESULTS

The impedance loss factor significantly increased as a result of root injuries (Figure 1A, Table 1). A significant difference in $\delta$ was found between the test temperatures −30°C vs. −5°C and 3°C ($p = 0.004$ and $p < 0.001$, respectively), and the clearest effect was observed in the first measurement after 1 week from the freezing test. The differences disappeared during the observation period of 5 weeks, however. Temperature had a significant effect on $K_r$, with significant differences between the test temperatures −30°C vs. −5°C and 3°C ($p < 0.001$ respectively). There was no difference in $K_r$ among the treatments after 1 week from the freezing treatment (Figure 1B, Table 1), but the difference between −30°C and the other two temperatures developed with time. The difference was the largest in the last sampling after 5 weeks in the growth chamber.

There were no new shoots or needle growth in the seedlings that were exposed to −30°C and all the seedlings exposed to this temperature died (Figure 2). Shoot and needle elongation slightly decreased after exposure to −5°C compared to 3°C, but the decrease was significant for shoot growth only (Figure 2A). No root growth was observed in the seedlings exposed to −30°C whereas new root tip formation was observed on the surface of the peat plug in the other two temperature treatments.

## 4 | DISCUSSION

The results support our hypothesis that the root systems are physiologically in continuous change after the damage occurred. This affects their electrical and hydraulic properties and should therefore be considered in the applications of these methods. In laboratory studies, the occasion of damage is usually known quite accurately, especially if the treatments are projected to the roots. It is, therefore, possible to schedule the measurements properly for the detection of root damage. However, in the field conditions, that is, in the tree seedling nurseries, the damage may have occurred at different times during the annual cycle depending on the stress factor, and therefore the occasion of damage will most probably remain unknown. Meanwhile, the physiological condition of the root system may have changed, which affects the results, depending on the assessment method. In addition, the seasonal changes in the physiological properties of the root system may add an amount of variation/change to the loss factor and hydraulic conductance, too (Di et al., 2019).

The impedance loss factor of the seriously damaged roots increased, as assessed shortly after the freezing test and thawing of the soil in the pots. This accords with the previous study, in which the $\delta$ increased, especially in the seedlings that were not considerably frost-hardened (Di et al., 2019). However, the difference in $\delta$ between damaged and undamaged roots decreased over time after the damage. This may be connected to the leakage of the electrolytes from damaged roots to the soil, as observed previously by REL (Repo & Ryyppö, 2008), whereupon a new balance of the electrolyte concentrations between root cells and soil was formed. The results suggest that it is possible to assess root damage by $\delta$ if the measurement takes place within 2 weeks from the occurrence of the damage. If the measurements were conducted later, the assessment might fail. In that case, the values of the damaged roots returned near the level of the undamaged roots of non-frost-exposed seedlings. This may not be explained by the recovery of root cells from damage in favorable growing conditions but by processes other than those directly connected with the cell membrane damage. To identify the differences between damaged and undamaged roots by the EIS method after three or more weeks, an approach other than the measurement of $\delta$ at a single frequency, for example, classification analysis of the electrical impedance spectra (Repo et al., 2016), is needed.

The reverse-flow hydraulic conductance of roots increased as a result of serious root damage (exposed to $-30^\circ$C), but there was no difference between control and mild-frost exposed seedlings. Furthermore, the difference in $K_r$ between undamaged and seriously damaged seedlings increased with time. In the previous study with Douglas-fir seedlings, freezing injuries of roots cleaned of soil and fully hydrated were detected by pressure-volume analysis too, based on the weight loss by pressurization of roots in a chamber (Ritchie, 1990). In the reverse-flow measurement of $K_r$, no cleaning of soil from roots is needed, and therefore root system stays intact, which makes the measurements easier. The results of the current study accord with the previous study, in which $K_r$ of roots of Scots pine seedlings, as assessed after 3 weeks from freezing treatment, increased exponentially with respect to the decrease of the exposure temperature (Korhonen et al., 2019). In another experiment with unhardened Norway spruce (*Picea abies* L. Karst) seedlings exposed to a series of low temperatures between 5°C and $-12^\circ$C, $K_r$ of roots also increased in relation to the exposure temperature if measured immediately after the freezing test (Leinonen et al., 2011). However, the difference in $K_r$ between the undamaged and damaged roots was almost one order of magnitude less than here or in the previous study (Korhonen et al., 2019), which is probably due to the change of $K_r$ with time after damage, as observed in the present study. Both the previous and present studies indicate that it is possible to detect root damage by $K_r$. The new finding here was that the difference between undamaged and damaged roots increased with time, being the largest in the last measurement 5 weeks after the occurrence of the damage. This indicates that the hydraulic resistance of the water conduits in the xylem cells of the stem and roots gradually decreased during the 5 weeks. It is probable that with a prolonged time, that is, longer than 5 weeks, drying blocks the stem vessels, leading to a decrease in hydraulic conductance, even though the decomposition of the damaged roots may have the opposite effect. This would deserve further studies, however.

In conclusion, to our knowledge, this was the first time to show that the electrical impedance loss factor and reverse-flow hydraulic conductance of roots change with time after freezing injuries but in a different manner, depending on the time scale since the damage occurrence. Both methods are nondestructive for roots, although the measurement of hydraulic conductance is destructive for the shoot. The measurement of the impedance loss factor is fast, whereas the measurement of the reverse-flow hydraulic conductance takes some more time to be completed. Since the difference in the hydraulic conductance between nondamaged and seriously damaged roots increased with time after the occurrence of damage, it would have a higher diagnostic value than the loss factor in assessing the condition of the root system in cases where the occasion of damage is unknown. The measurements were carried out in one phase of dehardening for the seedlings with no, mild or serious injuries in their root systems. Further studies would be deserved for roots with different frost hardiness levels and freezing test temperatures covering the range of different degrees of injuries, the effects of other abiotic stressors than frost, as well as the effect of soil in the root/soil-measurements of impedance loss factor.

## AUTHOR CONTRIBUTIONS

*Conceptualization*: Tapani Repo, Jouni Kilpeläinen, Dongxia Wu, and Timo Domisch. *Methodology*: Tapani Repo, Julien Ballandras, Jouni Kilpeläinen, Dongxia Wu, and Timo Domisch. *Measurements*: Julien Ballandras. *Data curation*: Julien Ballandras and Tapani Repo. *Original draft preparation*: Tapani Repo and Julien Ballandras. *Writing*: Tapani Repo, Julien Ballandras, Jouni Kilpeläinen, Dongxia Wu, and Timo Domisch. All authors have read and agreed to the published version of the manuscript.

## ACKNOWLEDGMENTS

This study was funded by the Natural Resources Institute Finland (Luke) (projects 41007-00004000 and 41007-00112100) and the Academy of Finland (decision No. 311455).

## DATA AVAILABILITY STATEMENT

The data is available from the authors on request.

## ORCID

*Tapani Repo* https://orcid.org/0000-0002-7443-6275
*Jouni Kilpeläinen* https://orcid.org/0000-0003-0966-7648
*Timo Domisch* https://orcid.org/0000-0001-7026-1087

## REFERENCES

Cao, Y., Repo, T., Silvennoinen, R., Lehto, T. & Pelkonen, P. (2011) Analysis of willow root system by electrical impedance spectroscopy. *Journal of Experimental Botany*, 62, 351–358. Available from: https://doi.org/10.1093/jxb/erq276

Colombo, S.J. & Asselstine, M.F. (1989) Root hydraulic conductivity and root growth capacity of black spruce (*Picea mariana*) seedlings. *Tree Physiology*, 5, 73–81. Available from: https://doi.org/10.1093/treephys/5.1.73

Dalton, F.N. (1995) In-situ root extent measurement by electrical capacitance methods. *Plant and Soil*, 173, 157–165. Available from: https://doi.org/10.1007/BF00155527

Di, B., Luoranen, J., Lehto, T., Himanen, K., Silvennoinen, M., Silvennoinen, R. et al. (2019) Biophysical changes in the roots of scots pine seedlings during cold acclimation and after frost damage. *Forest Ecology and Management*, 431, 63–72. Available from: https://doi.org/10.1016/j.foreco.2018.04.008

van den Driessche, R. & Cheung, K.-W. (1979) Relationship of stem electrical impedance and water potential of Douglas-fir seedlings to survival after cold storage. *Forest Science*, 25, 507–517.

Glerum, C. (1985) Frost hardiness of coniferous seedlings: principles and applications. In: Duryea, M.L. (Ed.) *Proceedings: evaluating seedling quality: principles, procedures, and predictive abilities of major tests*. Workshop held October 16-18, 1984. Corvallis: Forest Research Laboratory, Oregon State University, pp. 107–123.

Grossnickle, S.C. (2000) *Ecophysiology of northern spruce species. The performance of planted seedlings*. Ottawa, Ontario, Canada: NRC Research Press, p. 409.

Grossnickle, S.C. & Ivetic, V. (2022) Root system development and field establishment: effect of seedling quality. *New Forests*, 53, 1021–1067. Available from: https://doi.org/10.1007/s11056-022-09916-y

Grossnickle, S.C. & MacDonald, J.E. (2018) Why seedlings grow: influence of plant attributes. *New Forests*, 49, 1–34. Available from: https://doi.org/10.1007/s11056-017-9606-4

Grossnickle, S.C. & South, D.B. (2014) Fall acclimation and the lift/store pathway: effect on reforestation. *The Open Forest Science Journal*, 7, 1–20. Available from: https://doi.org/10.2174/1874398601407010001

Korhonen, A., Lehto, T., Heinonen, J. & Repo, T. (2019) Whole-plant frost hardiness of mycorrhizal (*Hebeloma* sp. or *Suillus luteus*) and non-mycorrhizal scots pine seedlings. *Tree Physiology*, 39, 526–535. Available from: https://doi.org/10.1093/treephys/tpy105

Leinonen, L., Roitto, M., Lehto, T., Calvo-Polanco, M., Zwiazek, J.J. & Repo, T. (2011) Voiko juurten vedenjohtokykyä käyttää taimien pakkasvaurioiden arviointiin? *Taimiuutiset*, 1, 22. (in Finnish) https://issuu.com/metla/docs/taimi-1-11

McKay, H.M. (1998) Root electrolyte leakage and root growth potential as indicators of spruce and larch establishment. *Silva Fennica*, 32, 241–252. Available from: https://doi.org/10.14214/sf.684

Nilsson, U., Luoranen, J., Kolström, T., Örlander, G. & Puttonen, P. (2010) Reforestation with planting in northern Europe. *Scandinavian Journal of Forest Research*, 25, 283–294. Available from: https://doi.org/10.1080/02827581.2010.498384

Radoglou, K., Cabral, R., Repo, T., Hasanagas, N., Sutinen, M.-L. & Waisel, Y. (2007) Appraisal of root leakage as a method for estimation of root viability. *Plant Biosystems*, 141, 443–459. Available from: https://doi.org/10.1080/11263500701626143

Repo, T., Cao, Y., Silvennoinen, R. & Ozier-Lafontain, H. (2012) Electrical impedance spectroscopy and roots. In: Mancuso, S. (Ed.) *Measuring roots – an updated approach*. Berlin Heidelberg: Springer-Verlag, pp. 25–49. Available from: https://doi.org/10.1007/978-3-642-22067-8_2

Repo, T., Korhonen, A., Laukkanen, M., Lehto, T. & Silvennoinen, R. (2014) Detecting mycorrhizal colonisation in scots pine roots using electrical impedance spectra. *Biosystems Engineering*, 121, 139–149. Available from: https://doi.org/10.1016/j.biosystemseng.2014.02.014

Repo, T., Korhonen, A., Lehto, T. & Silvennoinen, R. (2016) Assessment of frost damage in mycorrhizal and non-mycorrhizal roots of scots pine seedlings using classification analysis of their electrical impedance spectra. *Trees*, 30, 483–495. Available from: https://doi.org/10.1007/s00468-015-1171-x

Repo, T. & Ryyppö, A. (2008) Electrolyte leakage method can give misleading results concerning the frost hardiness of roots. *Plant Biosystems*, 142, 298–301. Available from: https://doi.org/10.1080/11263500802150548

Repo, T., Zhang, G., Ryyppö, A. & Rikala, R. (2000) The electrical impedance spectroscopy of scots pine (*Pinus sylvestris* L.) shoots in relation to cold acclimation. *Journal of Experimental Botany*, 51, 2095–2107. Available from: https://doi.org/10.1093/jexbot/51.353.2095

Repo, T., Zhang, G., Ryyppö, A., Rikala, R. & Vuorinen, M. (2000) The relation between growth cessation and cessation and frost hardening in scots pine of different origins. *Trees*, 14, 456–464. Available from: https://doi.org/10.1007/s004680000059

Repo, T., Zhang, M., Ryyppö, A., Vapaavuori, E. & Sutinen, S. (1994) Effects of freeze-thaw injury on parameters of distributed electrical circuits of stems and needles of scots pine seedlings at different stages of acclimation. *Journal of Experimental Botany*, 45, 823–833. Available from: https://doi.org/10.1093/jxb/45.6.823

Richter, A.K., Frossard, E. & Brunner, I. (2007) Polyphenols in the woody roots of Norway spruce and European beech reduce TTC. *Tree Physiology*, 27, 155–160. Available from: https://doi.org/10.1093/treephys/27.1.155

Riikonen, J. & Luoranen, J. (2018) Seedling production and the field performance of seedlings. *Forests*, 9(740), 1–4. Available from: https://doi.org/10.3390/f9120740

Ritchie, G.A. (1990) A rapid method for detecting cold injury in conifer seedling root systems. *Canadian Journal of Forest Research*, 20, 26–30. Available from: https://doi.org/10.1139/x90-004

Ritchie, G.A., Landis, T.D., Dumroese, R.K. & Haase, D.L. (2010) Assessing plant quality. In: Landis, T.D., Dumroese, R.K. & Haase, D.L. (Eds.) *The container tree nursery manual*, Vol. 7, Ch 2. Washington, DC, USA: U.S. Department of Agriculture, Forest Service, pp. 17–82.

Schwan, H.P. (1988) Dielectric spectroscopy and electro-rotation of biological cells. *Ferroelectrics*, 86, 205–223. Available from: https://doi.org/10.1080/00150198808227015

Sutinen, M.-L., Mäkitalo, K. & Sutinen, R. (1996) Freezing dehydration damages roots of containerized scots pine (*Pinus sylvestris*) seedlings overwintering under subarctic conditions. *Canadian Journal of Forest Research*, 26, 1602–1609. Available from: https://doi.org/10.1139/x26-180

Tyree, M.T., Patiño, S., Bennink, J. & Alexander, J. (1995) Dynamic measurements of roots hydraulic conductance using a high-pressure flowmeter in the laboratory and field. *Journal of Experimental Botany*, 46, 83–94. Available from: https://doi.org/10.1093/jxb/46.1.83

Voicu, M.C., Zwiazek, J.J. & Tyree, M.T. (2008) Light response of hydraulic conductance in bur oak (*Quercus macrocarpa*) leaves. *Tree Physiology*, 28, 1007–1015. Available from: https://doi.org/10.1093/treephys/28.7.1007

Zhang, M.I.N. & Willison, J.H.M. (1992) Electrical impedance analysis in plant tissues: the effect of freeze-thaw injury on the electrical properties of potato tuber and carrot root tissues. *Canadian Journal of Plant Science*, 72, 545–553. Available from: https://doi.org/10.4141/cjps92-068

**How to cite this article:** Repo, T., Ballandras, J., Kilpeläinen, J., Wu, D. & Domisch, T. (2023) Early-stage detection of root freezing injuries of Scots pine (*Pinus sylvestris* L.) seedlings by impedance loss factor and hydraulic conductance. *Physiologia Plantarum*, 175(3), e13919. Available from: https://doi.org/10.1111/ppl.13919