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Impact of soil pressure and compaction on tracheids in Norway spruce seedlings

Roman Gebauer · Daniel Volařík · Milena Martinková

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Abstract We tested the effect of soil compaction on Norway spruce seedlings in terms of the size and theoretical volume flow rate of the tracheids. The results show that soil pressure limits growth in the diameter of the lumens of tracheids in all parts of seedlings studied. The tracheids of the roots with primary xylem had larger lumens than those of the roots and shoots with secondary xylem in both unloaded and loaded seedlings. This corresponds to the higher cumulative theoretical volume flow rate of the tracheids from roots with primary xylem than those from roots and shoots with secondary xylem. Although the volume flow rate of tracheids, according to the Hagen-Poiseuille law, was directly proportional to the quadratic power of the capillary diameter (tracheid lumen), the cumulative curve of the theoretical hydraulic volume flow rate was higher or relatively comparable in loaded seedlings. An explanation for these findings is that there were higher gradients of water potential values in roots and leaves in loaded seedlings because the lengths of the conductive pathways were 27% shorter than in unloaded seedlings. We hypothesise that trees have adapted to different stresses by shortening their conductive pathways to maintain a transpiration rate similar to that of non-stressed trees. These results concerning the impact of soil compaction on tracheid diameter and volume flow rate improve our understanding of the growth and functioning of different conifer organs and the mechanisms underlying the efficiency of water transport through the root xylem to the shoot.

Keywords Adaptation · Anatomy · Volume flow rate · Stress

Introduction

Soil compaction, i.e., decreasing soil porosity and water-holding capacity, limits the ability of roots to grow. The limited root growth results in the slower growth of the aboveground parts of trees (Halverson and Zisa 1982; Cochran and Brock 1985; Tuttle et al. 1988). The operation

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R. Gebauer (✉) · D. Volařík · M. Martinková
Institute of Forest Botany, Dendrology and Geobiocenology, Mendel University of Agriculture and Forestry, Zemědělská 3, 61300 Brno, Czech Republic
e-mail: gebo@email.cz

of heavy machines on the surface compacts the soil to a depth of at least 15 cm (Nadyezhdina et al. 2006), where a majority of the roots of most trees are located (Sands and Bowen 1978; Kozlowski 1999). Also animals and humans, which exert around ten times lower force on soil as the heavy machines, could seriously compact soil (Gómez-Limón and de Lucio 1995; Kozlowski 1999; Serengil and Özhan 2006). Therefore, it is necessary to better understand the process of compaction and its effects on the soil and the growth of vegetation.

If we consider that roots grow deeply into soils, we can assume that they are affected by the mechanical pressure of the soil above them. When a root pushes its way through the soil, it must generate a force greater than the mechanical resistance of soil aggregates by either relocating or deforming them. So, roots must overcome the axial and radial pressures of the soil as well as its frictional resistance (Richards and Greacen 1986). The relationship between these forces varies according to soil texture, cohesive and adhesive soil characteristics and, to a certain extent, the root shape and radius; however, roots are predominantly affected by the axial pressure of the soil (Abdalla et al. 1969; Bengough and Mullins 1990).

The exposure of roots to mechanical pressure induces a number of physiological changes, which have already been well described on a macroscopic level. For example, root elongation decreases upon exposure to mechanical pressures (Sarquis et al. 1991; Bengough and MacKenzie 1994; Croser et al. 1999; Gebauer and Martinková 2005; Konopka et al. 2009). The root tip, which is generally round, becomes concave, the root thickness behind the meristem increases, and the root meristem, as well as the elongation zone, become shorter (Eavis 1967; Atwell 1993; Croser et al. 2000; Clark et al. 2003; Konopka et al. 2008).

There are changes on the microscopic level in response to compaction; for example, root cortical cells start to enlarge radially, rather than axially (Veen 1982), and the xylem structure is modified. The external pressure on the *Fagus sylvatica* trunk resulted in inhibition of vessel development (Bauer and Eschrich 1997). Christensen-Dalsgaard et al. (2008) studied impact of mechanical loading on three tropical species and found a decrease in vessel size, vessel area fraction and specific conductivity in accordance with the increase in mechanical loading. The structure of the xylem is maximally adapted to its main function, i.e., transport of a large amount of water. A reduction in the diameters of conductive xylem pathways, caused by insufficient mineral nutrition (Krasowski and Owens 1999), lack of water (Sperry and Saliendra 1994; Alder et al. 1996) or soil compaction, may have a great impact on the function of different parts of the conductive system. In spite of this, the vast majority of hydraulic studies have focused on the water transport from base of the trunk to the top of the tree (Christensen-Dalsgaard et al. 2007), and very few on the water transport characteristics of the whole plant (including root system).

The aim of this study was to learn how seedlings of Norway spruce react to low long-term soil compaction in terms of the size and theoretical volume flow rate of the tracheids. For this purpose we created a specific pressure of 5.1 kPa on the soil surface, which is only 5% of the short-term pressure exerted by heavy machines (Nadyezhdina et al. 2006). This work is a follow-up to a study on the influence of long-term soil pressure on root growth (Gebauer and Martinková 2005).

Materials and methods

Experimental design

Root containers were used to measure the impact of the long-term soil pressure on root growth during the 2003 growing season (Gebauer and Martinková 2005). Two root

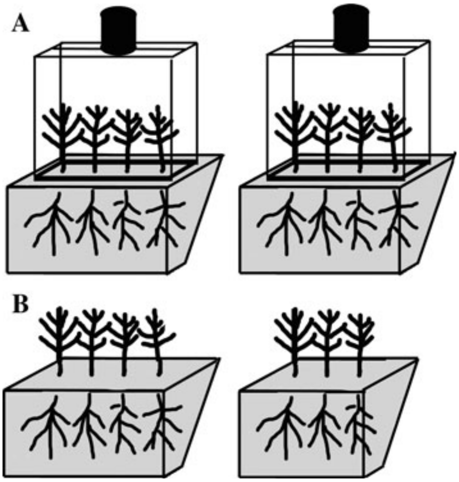

Fig. 1 A schematic picture of the experimental set-up. Two root containers were loaded with iron cylinders to cause a specific pressure of 5.1 kPa on the soil surface (**a**) and two identical containers were not loaded (**b**). The containers were planted with 2-year-old unbudded seedlings of Norway spruce (*Picea abies*/L./Karsten). Eight seedlings were used in the loaded containers (loaded seedlings) and seven in the unloaded containers (unloaded seedlings)

containers were loaded with iron cylinders to cause a specific pressure of 5.1 kPa on the soil surface; as a control, two identical containers were not loaded (Fig. 1). The iron cylinders were placed on construction which was built from iron sticks 8 mm in diameter and 3 cm wide iron straps which exert the pressure on the soil. The length of iron sticks was 1 m to avoid microclimate differences between seedlings. The containers were packed in polythene ferruginous compounds to prevent leakage. Containers were planted in late April with 2-year-old unbudded seedlings of Norway spruce (*Picea abies*/L./Karsten) that were placed in a regularly irrigated hotbed in the Botanical Garden and Arboretum of Mendel University of Agriculture and Forestry in Brno. Seven seedlings were used in the unloaded containers (these will henceforth be referred to as the unloaded seedlings), and eight were used in the loaded containers (henceforth the loaded seedlings). The 2-year-old unbudded seedlings were taken from a tree nursery where they were grown under the same soil and seedling competition conditions. For the experiment, seedlings with the same tree height (42 ± 4 cm including the root system) and the same root-collar diameter (5 ± 0.5 mm) were chosen.

Seedling harvesting

At the end of the experiment, both the unloaded and loaded seedlings were taken out of the root containers, and their root systems were separated from their aboveground systems. The root systems were cleaned thoroughly, and ten fine roots from the distal part (around 10 cm long) of the root system were sampled from each seedling. Altogether, there were 70 and 80 samples from unloaded and loaded seedlings, respectively. Regarding the aboveground systems, three lateral shoots from the current year of growth were sampled from each seedling (i.e., 21 and 24 samples were taken from unloaded and loaded seedlings, respectively). The root and shoot samples were fixed in FAA solution (90 ml 70% ethanol, 5 ml frozen acetic acid and 5 ml 40% formaldehyde; Němec et al. 1962).

Preparation for photomicrography

In the case of the root systems, histological sections were taken from behind the root tip (metaxylem) and then from proximally older places along the root axis where diameter growth had already occurred (secondary xylem—root). Sections from the aboveground systems were sampled at the base of the current-year shoots (secondary xylem—shoot). To highlight contrasts, the preparations were dyed using phloroglucinol + HCl to stain the lignified cell walls in red (see Němec et al. 1962; Prasad 1986). Stained sections were placed in glycerol, and evaluated by scanning with a microscope connected to a digital camera that was connected to a computer in the Biometric Laboratory of Mendel University of Agriculture and Forestry.

Processing of micrographs

For the purpose of examining the root metaxylem (henceforth in the text only the term metaxylem will be used), proximally older roots with secondary structure and current-year secondary xylem of shoots, 20, 30 and 16 micrographs were used, respectively, for unloaded and loaded seedlings. Selected micrographs were manipulated in Adobe Photoshop so that, in the samples taken behind the root tips, the lumens of all of the metaxylem tracheids were manually dyed with contrast colour. In samples from proximally older places (secondary roots) and in current-year old shoots, the lumens of several rows of tracheids with diameter growth from the pith to the cambial zone were also manually dyed with contrast colour (Fig. 2). Through further editing in Adobe Photoshop (using the function Replace Colour) the other colours in the image were changed to white. Finally, the colour image was changed to black-and-white images recording only selected lumens (Fig. 1). Subsequently, the parenchymatic cells, resin ducts and places with hardly visible lumens were depicted inside the area delimited by the dyed lumens. These images were then processed as described above.

Micrograph analyses

The processed micrographs were analysed using the ImageTool3.00 program (The University of Texas Health Science Centre in San Antonio). The following parameters were determined: total analysed area (A_m), lumen area of tracheids (A_{lum}), lumen diameters in two directions, i.e., the major and minor axis diameters (d_{max} and d_{min}, respectively) and the total areas of the parenchymatous phloem/xylem rays, resin ducts and places where the lumens were hardly visible (A_o). The size-class distribution of major and minor axis diameters (d_{max} and d_{min}) of tracheids was calculated in 2-µm increments.

The number of tracheids examined in the individual segments of the metaxylem, secondary xylem of root and secondary xylem of current-year shoots varied in the range of 13–51, 78–2,238 and 125–1,321, respectively. The total number of tracheids measured was 17,400.

Cell wall area

The cell wall area ($C_{\%}$) of the cross-sections of shoots and roots was calculated according to Eq. 1:

$$C_{\%} = 100 - 100 \cdot \left(\frac{\sum_{i=1}^{i=n} A_{lum}}{A_m - A_o} \right). \tag{1}$$

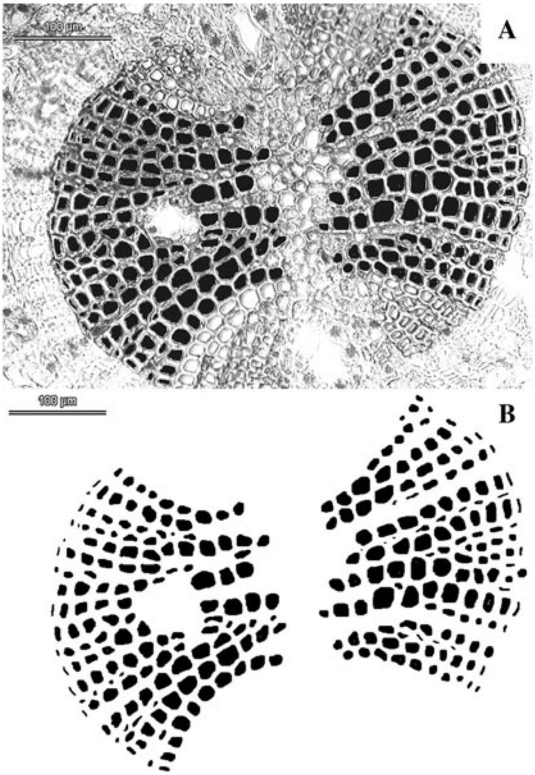

Fig. 2 A photograph of the cross-section of a secondary xylem of fine roots of an loaded seedling that was manipulated in Adobe Photoshop to show how several rows of cells of secondary xylem involved in diameter growth from the pith to the cambial zone were dyed (**a**). Through further editing in Adobe Photoshop, a black-and-white image, recording only selected lumens, was obtained (**b**)

Theoretical potential volume flow rate

The theoretical potential volume flow rate (Q_{th_lum}) was calculated using the Hagen-Poiseuille equation (Zimmermann 1983; Davis et al. 1999; Zwieniecki et al. 2001) for 100 tracheids ($n = 100$) distributed according to the proportion of the major axis length falling within particular 5 μm intervals and for the mean of one single tracheid (Q_{th_mean}). Since the shape of the tracheid lumen was not circular, we applied a minor modification to the formula for one tracheid, as recommended by Nobel (2005) (Eqs. 2 and 3):

$$Q_{th\_lum} = \sum_{i=1}^{i=n} \left(\pi r_{lum}^4 \Delta\Psi\right) / (8\eta\Delta x); \quad \left[g\,h^{-1}\right] \tag{2}$$

and

$$r_{lum}^4 = d_{max}^3 d_{min}^3 / \left(8d_{max}^2 + 8d_{min}^2\right); \tag{3}$$

where η is water viscosity at 20°C (2.78 e^{-13} MPa h), $\Delta\Psi$ is the difference in water potential between the root and leaf and Δx is the length of the water pathway between the root and leaf, which reached 0.75 m for unloaded seedlings and 0.55 m for loaded seedlings. The maximum difference in water potential ($\Delta\Psi$) was not measured, but comparable values were taken for leaves from an adult Norway spruce stand, where it reached about $\psi = -2$ MPa (Prof. J. Cermak, personal communication). Values for root (xylem) water potentials were taken similarly to those for the surrounding soil water potential, which reached −0.05 MPa in typical soil moisture conditions (Taiz and Zeiger 2002). Subsequently, cumulative theoretical potential volume flow rate curves (Q_{th_cum}) were created.

Statistics

We analysed the differences in the major (d_{max}) and minor axis diameters (d_{min}), the lumen areas of tracheids (A_{lum}) and the theoretical potential volume flow rate of tracheids (Q_{th_lum}) between loaded and unloaded samples. Histograms were used to assess the statistical distribution of studied variables; the Shapiro–Wilk test was used to assess data normality. Most of our variables did not conform to the assumption of normality; therefore, we used the Wilcoxon rank sum test for differences in medians as a nonparametric analogue of the *t*-test. Statistical analyses were carried out using the R statistical program (R Development Core Team 2007).

Results

Distributions of tracheid major and minor axes in unloaded seedlings

The sizes of the tracheid major axis diameters (d_{max}) for both the metaxylem and secondary xylem of fine roots of unloaded seedlings were similar and were a little lower for the secondary xylem of current-year shoots, ranging from 5.1 to 28.2 μm, 2.2 to 31.4 μm and 2.1 to 22.7 μm, respectively (Fig. 3). Differences were found between the mean major axis diameters of tracheids. The mean major axis diameter was 51% greater in the tracheids of the metaxylem than those of the secondary xylem of current-year shoots (Table 1). The distribution of tracheids was shifted to the left side of the graph in the secondary xylem compared to the metaxylem of current-year shoots (Fig. 3).

The sizes of the tracheid minor axis diameters (d_{min}) were similar for the metaxylem and secondary xylem of fine roots in unloaded seedlings and were a little lower for the secondary xylem of current-year shoots, ranging from 2.2 to 23.4 μm, 1.5 to 24.5 μm and 0.7 to 16.9 μm, respectively (Fig. 3). Larger differences were found in the mean minor axis diameter than in the mean major axis diameter. The mean minor axis diameter was 55% greater in the tracheids of the metaxylem than in the secondary xylem of current-year shoots (Table 1). The tracheid distribution was shifted to the left side of the graph in the secondary xylem compared to the metaxylem of current-year shoots (Fig. 3).

Distributions of tracheid major and minor axes in loaded seedlings

The sizes of the tracheid major axis diameters (d_{max}) of metaxylem and secondary xylem in loaded seedlings were similar and were a little lower for secondary xylem of current-year shoots, ranging from 4.3 to 27.5 μm, 2.1 to 31.4 μm and 2.1 to 21.3 μm, respectively. The mean major axis diameter of experimental seedlings was 53% greater in the tracheids of the metaxylem than in the secondary xylem of current-year shoots (Table 1). The tracheid distribution was shifted to the left side of the graph for the secondary xylem of current-year shoots compared to that of the metaxylem (Fig. 3).

The sizes of the tracheid minor axis diameters (d_{min}) for the metaxylem and secondary xylem of fine roots in loaded seedlings were similar and were a little lower for the secondary xylem of current-year shoots, ranging from 2.3 to 23.6 μm, 0.7 to 23.9 μm and

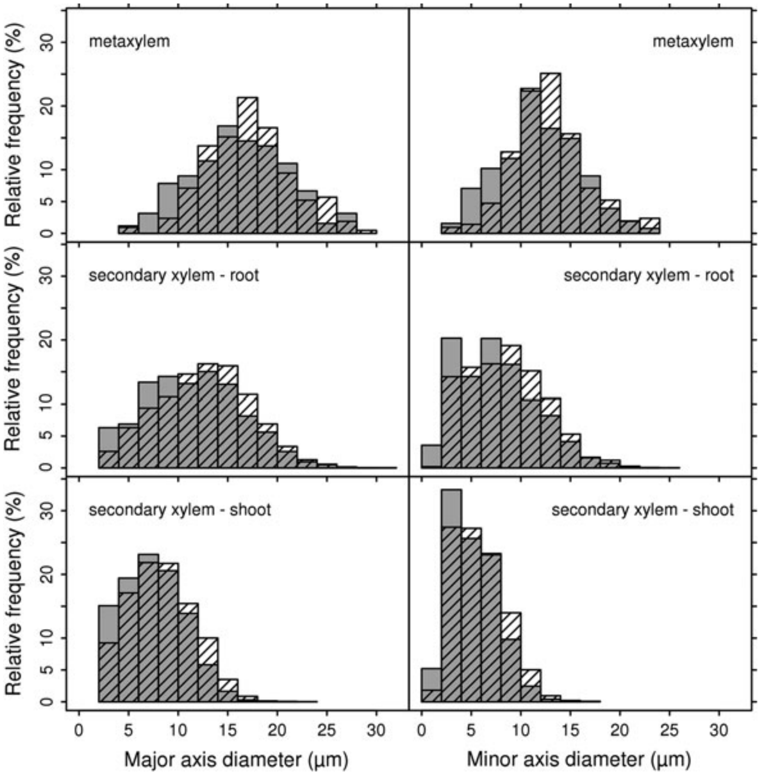

Fig. 3 A histogram of the size distribution of the major and minor axis diameters tracheids in the metaxylem, secondary xylem of roots and secondary xylem of shoots in loaded seedlings (*grey columns*) and unloaded seedlings (*hatched columns*)

Table 1 Mean major axis diameter and mean minor axis diameter of the tracheid lumens of unloaded and loaded seedlings for metaxylem, secondary xylem of roots and secondary xylem of shoots

	Unloaded seedlings		Loaded seedlings	
	Major axis diameter (μm) Mean ± SD	Minor axis diameter (μm) Mean ± SD	Major axis diameter (μm) Mean ± SD	Minor axis diameter (μm) Mean ± SD
Metaxylem	17.1 ± 0.6*	12.8 ± 0.5*	16.1 ± 0.6*	11.9 ± 0.5*
Secondary xylem—root	12.6 ± 0.2**	8.5 ± 0.2**	11.5 ± 0.1**	7.6 ± 0.1**
Secondary xylem—shoot	8.3 ± 0.1**	5.8 ± 0.1**	7.5 ± 0.1**	5.2 ± 0.1**

* Statistically significant differences in means between unloaded and loaded seedlings at $\alpha < 0.05$ (Wilcoxon test); ** statistically significant differences in means between unloaded and loaded seedlings at $\alpha < 0.01$ (Wilcoxon test)

0.7 to 15.7 μm, respectively (Fig. 3). Differences were found when comparing the mean minor axis diameters. The mean minor axis diameter of the tracheids of the metaxylem was 60% greater than that of the secondary xylem of current-year shoots (Table 1). The tracheid distribution was shifted to the left side of the graph for the secondary xylem compared to the metaxylem of current-year shoots (Fig. 3).

Comparison of the tracheid distribution and cell wall area between seedlings

The mean major axis diameters of the metaxylem, secondary xylem of fine roots and secondary xylem of current-year shoots were higher by 6, 10 and 11%, respectively, in unloaded seedlings compared to loaded seedlings (Table 1). This pattern corresponds to a shift in the size of the tracheids of loaded seedlings to the left side of the size distribution (narrower diameter) compared to unloaded seedlings (Fig. 3).

The mean minor axis diameters of the metaxylem, secondary xylem of fine roots and secondary xylem of current-year shoots were 7, 13 and 13%, respectively, higher in unloaded seedlings than in loaded seedlings (Table 1). This pattern corresponds to a shift in the size of the tracheids of experimental seedlings to the left side of the size distribution (narrower diameter) compared to unloaded seedlings (Fig. 3).

The cell wall areas of the total cross-sectional area of the metaxylem, secondary xylem of fine roots and secondary xylem of current-year shoots were 9, 1 and 1% lower, respectively, in unloaded seedlings than in loaded seedlings (Table 2).

Theoretical potential volume flow rate

The mean theoretical potential volume flow rate of a single tracheid was similar in unloaded and loaded seedlings and was greater in roots than in shoots for both treatments (Fig. 3). The differences in the mean theoretical potential volume flow rate of a single tracheid in the metaxylem versus the secondary xylem of current-year shoots were 95 and 96% in unloaded and loaded seedlings, respectively (Fig. 4).

The percentages of tracheids with a major axis diameter wider than 15 μm in the metaxylem, secondary xylem of fine roots and secondary xylem of current-year shoots were 67, 32 and 2%, respectively, out of the total sum of cells in unloaded seedlings. However, the volume flow rates of these cells constituted 92, 76 and 15%, respectively, of the total theoretical potential volume flow rate (Fig. 5). In the case of loaded seedlings, the percentages of tracheids with a major axis diameter wider than 15 μm in the metaxylem, secondary xylem of fine roots and secondary xylem of current-year shoots were 61, 25 and 1%, respectively. However, of the total number of cells, the calculated volume flow rates of these cells, of the total theoretical potential volume flow rate measured, were 92, 75 and 11%, respectively (Fig. 5). From these results, the importance of larger tracheids to the overall volume flow rate of trees is obvious.

Table 2 Comparison of cell wall distribution, i.e. the percentage of the cross sections occupied by cell walls area versus lumens area, of metaxylem, secondary xylem of roots and secondary xylem of current-year shoots of unloaded and loaded seedlings of Norway spruce (*Picea abies*/L./Karsten)

	Unloaded seedlings Cell wall distribution (%) Mean ± SD	Loaded seedlings Cell wall distribution (%) Mean ± SD
Metaxylem	50.6 ± 2.7*	56.4 ± 4.24*
Secondary xylem—root	66 ± 5.9	67 ± 6.4
Secondary xylem—shoot	70.3 ± 5.3	70.8 ± 4.1

* Statistically significant differences in means at $\alpha < 0.05$ (Wilcoxon test)

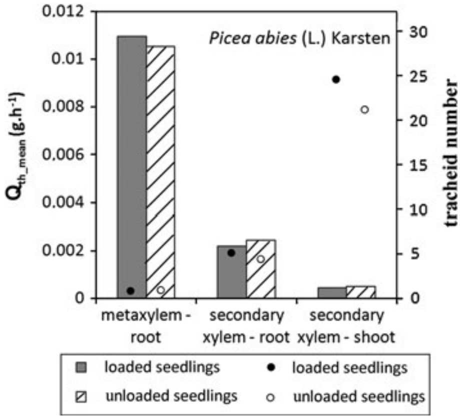

Fig. 4 Mean theoretical potential volume flow rate of single tracheids (Q_{th_mean}) of lumens of the metaxylem, secondary xylem of fine roots and secondary xylem of current-year shoots in loaded (*grey columns*) and unloaded seedlings (*hatched columns*). The *dots* in the graph (*full dots* loaded seedlings, *open dots* unloaded seedlings) show the number of tracheids whose sum of water flow rates will be the same as in the single metaxylem tracheid

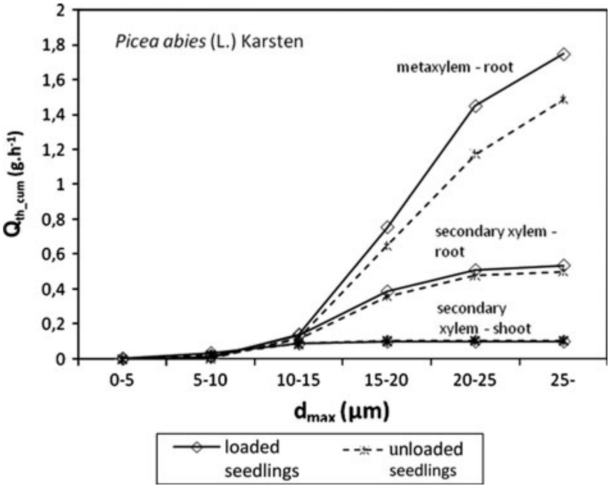

Fig. 5 Cumulative curves of the potential theoretical volume flow rate (Q_{th_cum}) using the Hagen-Poiseuille equation adapted according to Nobel (2005). The *curves*, generated in intervals of 5 μm, were determined from values of the major axis diameters of tracheid lumens of metaxylem, secondary xylem of fine roots and secondary xylem of current-year shoots in unloaded and loaded seedlings

The curve for the cumulative theoretical potential volume flow rate of loaded seedlings gradually exceeds that of unloaded seedlings up to a maximum of 15 and 7% for the metaxylem and secondary xylem of fine roots, respectively (Fig. 4). The curve of the cumulative potential volume flow rate for the secondary xylem of current-year shoots was 7% higher at the maximum point for unloaded seedlings compared to that for loaded seedlings (Fig. 5)

Discussion

Our research shows that soil pressure (in our case, created by a long-term mechanical load) limits the growth of lumen tracheids in diameter in all of the parts of seedlings studied

(Table 1, Fig. 3). The tracheids of the primary xylem (metaxylem) had larger lumens than those of the secondary xylem, as previously reported by Krasowski and Owens (1999) who studied the response of tracheids in long lateral roots of *Picea glauca* seedlings to nitrogen availability. In that study, the mean tracheid diameter was between 15 and 20 µm. In our study, the mean tracheid diameter was smaller than those reported by Sarén et al. (2001) for *Picea abies* and by Qiu-yu et al. (2005) for *Picea koraiensis*, but they studied older trees. Because we studied tracheid diameter in the primary xylem, the finding that the tracheid diameter is lower close to the pith (Sarén et al. 2001; Domec et al. 2009) could explain the lower tracheid diameter in comparison to adult trees.

The fact that the tracheid lumens of loaded seedlings were smaller (Table 1, Fig. 3) may be explained as a defence against the formation of air bubbles within the conductive system (McElrone et al. 2004) that can be caused by poor water availability, since increasing soil density decreases the soil porosity and water capacity (Sands and Bowen 1978; Gomez et al. 2002). The mechanisms of molecule cohesiveness and tension create a negative hydrostatic pressure in xylem, making the whole system more or less susceptible to water column damage and interrupting the conductive pathways by the penetration of air. This results in a conflict between the structural requirements for the minimisation of porosity on the one hand and the requirements for the maximum efficiency of the structure of the conductive system on the other (Hacke and Sperry 2001). The trade-off between conduit diameter and the susceptibility to freezing-induced cavitation was found also by Hacke and Sperry (2001). However, this relationship is weak with regards to drought-induced cavitation (Hacke and Sperry 2001). In that case they found a strong relationship between the thicker of tracheid double wall relative to its maximum diameter and the loss of hydraulic conductivity.

Secondary xylem tracheids in current-year shoots were smaller than those in roots of both unloaded and loaded seedlings. This finding corresponds to the fact that there was a lower cumulative potential theoretical volume flow rate of tracheids in shoots than in tracheids of secondary xylem fine roots and metaxylem (Fig. 5). These results support the conclusion that the volume flow rate of roots is higher than the volume flow rate of shoots (Alder et al. 1996; Martínez-Vilalta and Pockman 2002; McElrone et al. 2004). Roots, especially fine roots, are more predisposed than branches to the creation of air cavities in conductive systems, which are caused by drought (Sperry and Saliendra 1994; Alder et al. 1996; Martínez-Vilalta and Pockman 2002; Domec et al. 2009). Cermák et al. (2002) also found a higher volume flow rate in the trunk than in the petioles of *Laurus azorica* trees, and determined that 100 mm^2 of foliage area was served by almost 11 petiole vessels and/or by almost one single stem vessel. In our case, one single tracheid of metaxylem is served by 4–5 tracheids of secondary xylem of fine roots and by 21–25 tracheids of secondary xylem of current-year shoots (Fig. 4). The fact that tracheid diameters increase downwards from leaves to roots may be explained by the gradient of auxin, which is produced by the young growing leaves and is the major signal involved in the control of all aspects of xylem differentiation (Aloni and Zimmermann 1983; Aloni 1987). Hejnowicz (1997) and Domec et al. (2009) argue that in branches, smaller tracheids with thicker walls may result from branch cells being subjected to lower xylem pressure and therefore lower turgor during their expansion phase. The effect of soil compaction on seedlings was that it increases tapering (narrower conduits distally) of tracheids between secondary xylem of root and shoot by 26% compare to unloaded seedlings (Table 1). The tapering of conductive elements upwards from roots to stem tips has been studied in work that tested theoretical models (West et al. 1999: the West, Brown and Enquist, WBE model). It has been hypothesised that plants have evolved a network of xylem conduits with a tapered structure, which minimises the cost of water transport from roots to leaves (McCulloh and Sperry 2005; Anfodillo et al. 2006).

The cell wall area was statistically different between unloaded and loaded seedlings only for metaxylem tracheids (Table 2). For these reasons, we speculate that a lower rate of root elongation (Sarquis et al. 1991; Bengough and MacKenzie 1994; Croser et al. 1999; Gebauer and Martinková 2005) as well as thickening behind the root tip (Abdalla et al. 1969) under conditions of mechanical pressure on the soil volume is caused by a higher energy investment in the formation of a primary root structure (metaxylem).

Although the volume flow rate of tracheids, according to Hagen-Poiseuille law, was directly proportional to the quadratic power of the capillary diameter (tracheid lumen) (Zimmermann 1983; Tyree and Ewers 1991), the cumulative curve of the theoretical volume flow rate was higher or relatively comparable in loaded seedlings, whose tracheid lumen diameters were smaller than those in unloaded seedlings (Fig. 5). However, the lengths of the conductive pathways, i.e. the distance from the root tips to leaves, in loaded seedlings were 27% shorter than in unloaded seedlings. This finding explains why at comparably higher gradients of water potential values in roots and leaves, i.e. in loaded seedlings, the final speed of flow is higher in tracheid lumens of smaller diameters. Shortening of conductive pathways is a well-known reaction of trees to stress (e.g. drought, hypoxia and salt stress) (Kozlowski 1971; Nicholas 1998; Marron et al. 2002; Schulze et al. 2005). Our results show that this reaction enables seedlings to retain the same speed of water flow and, therefore, for example, carry out photosynthesis. We hypothesise that in this way trees have adapted to different stresses by shortening their conductive pathways in order to maintain a transpiration rate similar to that of non-stressed trees; thus, they will have smaller tracheids or vessels. This hypothesis will be tested in future experiments.

Differences in the diameter of the cell, the thickness of the cell wall and the shape of the lumen reflect changes in the cambium and the environmental factors affecting it (Sarén et al. 2001). The trunk, branches and roots of a tree fulfil different functional roles (Duncham et al. 2007), so it is not surprising that we found anatomical and hydraulic differences between them and also between seedlings growing in different environments. These results on the impact of soil compaction on tracheid diameter and volume flow rate contribute to the improvement of our understanding of the growth and functioning of different conifer organs, and the mechanisms underlying the efficiency of water transport through the root xylem to the shoot.

Conclusion

The tracheid lumens of loaded seedlings were smaller in all studied plant parts. This could be an important defence against the formation of air bubbles within the conductive system that can be caused by poor water availability in compacted soil.

Roots of stressed seedlings are more sensitive to mechanical pressure in younger stages of their ontogeny, when they had narrower tracheids and higher energy requirements for the formation of xylem (a higher number of conductive elements with thicker cell walls) compare to unstressed seedlings. On the other hand, xylem in plants exposed to stress is structurally more resistant to embolism.

Trees that have adapted to stress by shortening their conductive pathways and decreasing the diameters of tracheids or vessels can have the same volume flow rate as unstressed trees by increasing the water potential gradient between roots and leaves.

It is important to note that it is not possible to evaluate root functions on the sole basis of the morphological parameters of roots, such as root diameter, root surface area or root length, but a histological analysis including tracheid diameter, cell wall area or tracheid distribution should also be taken into account.

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