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Differences in growth, wood density and wood anatomy in Norway spruce genotypes, and development of height and autumn frost hardiness in their seed offspring

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Academic dissertation

To be presented, with the permission of the Faculty of Science and Forestry of the University of Eastern Finland, for public criticism in the auditorium BOR100 in the Borealis Building of the University of Eastern Finland, Joensuu,
on 13th of December 2019, at 12 noon.

Title of dissertation: Differences in growth, wood density and wood anatomy in Norway spruce genotypes, and development of height and autumn frost hardiness in their seed offspring

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Dissertationes Forestales 284

https://doi.org/10.14214/df.284

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ISSN 1795-7389 (online)
ISBN 978-951-651-658-8 (pdf)

ISSN 2323-9220 (print)
ISBN 978-951-651-659-5 (paperback)

Publishers:
Finnish Society of Forest Science
Faculty of Agriculture and Forestry at the University of Helsinki
School of Forest Sciences at the University of Eastern Finland

Editorial Office:
Finnish Society of Forest Science
Viikinkaari 6, FI-00790 Helsinki, Finland
http://www.dissertationesforestales.fi

Levkoev E. (2019). Differences in growth, wood density and wood anatomy in Norway spruce genotypes, and development of height and autumn frost hardiness in their seed offspring. Dissertationes forestales 284. 34 p. https://doi.org/10.14214/df.284

ABSTRACT

The main aim of this work was to study the magnitude of phenotypic variation in different growth, wood density (WD) and wood anatomy properties, and correlations between these, in 41-year-old clones and provenance hybrid clones of Norway spruce (*Picea abies* (L.) Karst.). Additionally, the development of height and autumn frost hardiness in their seed offspring, under different temperature and/or atmospheric CO_2 concentration treatments, were studied under greenhouse conditions over one growing season.

Local Finnish clone V43, and two Finnish–German V449 and V381 provenance hybrid clones had higher stem volume than the average of the 25 genotypes included in the study (Paper I). They had also relatively high overall WD compared to the average of all the genotypes. The geographical transfer of father parent trees affected the overall WD. The averages for tracheid length, double cell-wall thickness (2CWT) in both earlywood (EW) and latewood (LW), and lumen diameter in LW, differed in five genotypes selected for detailed analyses of wood anatomy (Paper II). These parameters and the number of rays correlated with the widths and wood densities of EW and LW. The 2CWT of the tracheids closest to resin canals differed from that in the normal tracheids (Paper II). These differences may be partly affected by the origins of the genotypes.

Under greenhouse conditions, elevated temperatures increased the height growth in seedlings. It delayed the onset of autumn frost hardiness development and shortened its duration. Elevated CO_2 did not affect the development of height and frost hardiness. None of the genotypes showed both superior growth and frost hardiness (Paper III).

A need for further studies on wood anatomy – for example, to consider genotype-specific variations in structural compounds – emerged based on this work. The obtained understanding of phenotypic variation in different genotypes may provide support for tree breeding in the future.

Keywords: *Picea abies*, growth, wood density, wood anatomy, seedlings, frost hardiness

ACKNOWLEDGEMENTS

Through these words I want to express my gratitude to the people who helped me greatly on my path as a young researcher. Their care, which came to me in different forms and ways, was very important. I'm grateful to my main supervisor, Prof. Heli Peltola, who showed great kindness, generosity and support to the stranger knocking at her door and asking for an opportunity to learn under her supervision. Your constant support on every stage of this journey has been very valuable to me. I'm grateful to my supervisor, Doc. Pertti Pulkkinen, for his help with a greenhouse experiment and interesting philosophical discussions. I'm grateful to my supervisor, Dr. Katri Luostarinen, for introducing me to the research field of wood anatomy. I'm also grateful to my supervisor, Prof. Lauri Mehtätalo, for helpful and interesting advise on statistics.

I acknowledge my co-authors – Dr. Veli-Pekka Ikonen, Dr. Antti Kilpeläinen, Mr. Raimo Jaatinen, Prof. Jyrki Kangas and Prof. Anatoly Zhigunov – for their contributions to improving this research. Mr. Asko Harju, Mr. Aulis Leppänen, Mr. Veli Suihkonen and Ms. Minna Mäkinen from the Natural Resources Institute Finland (LUKE) are acknowledged for their help with the fieldwork and greenhouse experiment. Mr. Jarmo Pennala from the School of Forest Sciences, University of Eastern Finland (UEF), is acknowledged for helping with the X-ray analyses and tracheid length measurements.

I also acknowledge the pre-examiners of my thesis, Professor Emeritus Barbara Lachenbruch from the Oregon State University, USA and Professor Hardi Tullus from the Estonian University of Life Sciences, Estonia, for their invaluable reviews and comments.

The scholarships for the early stage researcher, Eino Levkoev, from the Heikki Väänänen Fund, UEF, in 2015, and from the Faculty of Science and Forestry, UEF, in 2016-2019, are acknowledged. The Tornator company and Imatra city are acknowledged for providing the sample trees for this study, and the LUKE is acknowledged for the maintenance of a greenhouse experiment. The support provided by the Strategic Research Council of the Academy of Finland for this work under the FORBIO project (decision numbers 293380 and 314224 for years 2015–2017 and 2018–2021), led by Prof. Heli Peltola at the School of Forest Sciences, UEF, is also acknowledged.

In Joensuu, October 2019,

Eino Levkoev

LIST OF ORIGINAL ARTICLES

This thesis is based on the following papers, which are referred to in the summary by their Roman numerals. Articles are reproduced with the kind permission of publishers.

I	Levkoev E., Kilpeläinen A., Luostarinen K., Pulkkinen P., Mehtätalo L., Ikonen V.-P., Jaatinen R., Zhigunov A., Kangas J., Peltola H. (2017). Differences in growth and wood density in clones and provenance hybrid clones of Norway spruce. Canadian Journal of Forest Research 47: 389–399. https://doi.org/10.1139/cjfr-2016-0285
II	Levkoev E., Luostarinen K., Pulkkinen P., Mehtätalo L., Ikonen V.-P., Peltola H. Relationship of wood anatomy to growth and wood density in clones and provenance hybrid clones of Norway spruce. Manuscript.
III	Levkoev E., Mehtätalo L., Luostarinen K., Pulkkinen P., Zhigunov A., Peltola H. (2018). Development of height growth and frost hardiness for one-year-old Norway spruce seedlings in greenhouse conditions in response to elevated temperature and atmospheric CO_2 concentration. Silva Fennica 52(3), 9980, 15 pp. https://doi.org/10.14214/sf.9980

Eino Levkoev (Levkoev E.) was the primary author of all these papers. The primary author was responsible for the writing of all the papers and all the data analysis. Experiments in all studies (Papers I-III) were planned together with Prof. Heli Peltola, Doc. Pertti Pulkkinen and Dr. Katri Luostarinen. Statistical data analysis was supported by Prof. Lauri Mehtätalo. The co-authors improved the papers by revising and commenting on them.

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1 INTRODUCTION

1.1 Background of the study

Norway spruce (*Picea abies* (L.) Karst.) accounts for about 30% of the total volume of growing stock in Finland and it is an important raw material source in the forestry industry (Peltola 2014). An increase in forest growth per unit of land area on the forestland assigned for timber production, could help to fulfill the increasing demand for wood in the forestry industry, while ensuring sustainable provisioning of other ecosystem services and the multiple use of forests. Resource-efficient wood production and utilization require both an increase in the average amount of harvested wood per unit of land area and an appropriate quality of wood. Wood production per unit land area may be enhanced through the use of various silvicultural measures, such as appropriate thinning regimes, forest fertilization and rotation length (see, e.g., Nilsen 2001; Routa et al. 2013, 2019; Bergh et al., 2014). On the other hand, the use of improved seed and seedling material in forest regeneration could simultaneously provide both a higher yield and quality of wood material through breeding, compared to the use of unimproved regeneration material (Zubizarreta Gerendiain et al. 2007; Ruotsalainen 2014).

Based on previous findings from provenance trials in Finland and Sweden, the first round of seed orchards is able to provide around a 10% genetic gain for growth in Norway spruce (Ruotsalainen and Nikkanen 1998; Rosvall 2001). In the third round of seed orchards, the theoretical genetic gain for growth is estimated to be 20–25% for this species (Rosvall 2001; Haapanen et al. 2015). However, most existing Norway spruce seed orchards in Finland are either situated in relatively old or relatively young stands, which do not have high seed production capacity. This may lead to rapid fluctuations and a lack of sufficient seed supply over time (Ruotsalainen 2014; Haapanen et al. 2017). The problem of low availability of improved regeneration material in Norway spruce may be tackled in the future, at least partially, by the use of vegetative propagation (e.g., cloning and somatic embryogenesis). Based on this, higher genetic gain could also be achieved because of the absence of pollen contamination (Haapanen et al. 2015).

In forest tree breeding, volume growth has typically been used as the measure of productivity, and as a selection trait of primary importance, whereas wood density (WD) has traditionally been used for wood quality assessment due to its informativeness and simplicity of measurement. Also, WD correlates well with various utility properties of wood, such as strength, stiffness and wear-resistance (Saranpää 2003; Horáček et al. 2017). However, the observed genetic-phenotypic relationship between volume growth and WD is generally negative (Zobel and Jett 1995). This makes the selection of genotypes considering both these traits complicated. However, the existence of nonsignificant or weak positive relationships between volume growth and WD has also been observed, for example, in some Norway spruce clones (Zobel and Jett 1995; Bujold et al. 1996; Zubizarreta Gerendiain et al. 2007).

The use of WD alone in the quality assessment of wood could be misleading, however, as WD itself is a complex parameter, largely determined by various factors, among which wood anatomy (WA) is of prime importance. For example, WD in softwood species is affected by cell size, which comprises tracheid lumen diameter (LD) and wall thickness, by the proportion of earlywood (EW) and latewood (LW) and the amount of ray cells (Zobel and van Buijtenen 1989). In addition, it can be affected by resin, located in resin canals

(Barger and Ffolliott 1971; Rissanen and Sipi 2002), and by the presence of compression wood (Tyrvänen 1995; Gryc and Horáček 2007). However, it may be possible to simultaneously obtain both higher growth (e.g., stem volume) and relatively high WD, together with the desired anatomical characteristics of the wood, through, for example, the selection of already existing suitable genotypes or the hybridization of local and foreign parent trees (Gerhold and Park 1986; Magnussen and Yeatman 1988; Ruotsalainen 2014; Luostarinen et al. 2017).

Additionally, the adaptation capacity to changing environmental (climate and site) conditions should be considered in the selection of regeneration material for practical forestry, especially when genotype transfers are involved (Olsen 2010; Ruotsalainen 2010). Genotypes originating from milder climatic conditions (e.g., lower latitude and closer to sea level), for example, tend to have longer growing periods and higher total growth (e.g., stem volume), but lower WD (White et al. 2007), compared to genotypes from harsh environments (Persson and Persson 1997; Skrøppa et al. 1999).

Contemporary breeding programs should also consider the risks related to climate change. In Finland, based on multi-model mean climate change projections (i.e., representative concentration pathways RCP4.5 and RCP8.5), during the potential growing season (April–September), the mean temperature may increase by 1°C by 2030 and by 3–5°C by 2070–2099, compared to the reference period of 1981–2010 for the current climate (Ruosteenoja et al. 2016), whereas the amount of precipitation may not increase greatly (7–11% by 2070–2099). At the same time, an increase in the atmospheric CO_2 concentration is expected, from 350 ppm (1981–2010) to 536–807 ppm by 2070–2099. On one hand, climate warming may reduce the growth of Norway spruce, especially in southern Finland, on soils with low soil-water availability (Kellomäki et al. 2018), due to the creation of sub-optimal temperature conditions and increased drought (Ruosteenoja et al. 2016, 2018). It may also impair the development of autumn cold hardiness through causing asynchrony in environmental triggers (Chang et al. 2016), such as a decrease in temperature and photoperiod length (Beck et al. 2004; Chang et al. 2016). This is because the climate warming may facilitate a longer growing period, while the photoperiod length will remain the same. As a consequence, a decrease in the vitality of seedlings and an increase in mortality and wood-related defects are expected (Langvall 2000), which may lead to negative consequences for the forests and forestry.

In Norway spruce seedlings, height growth and the development of autumn cold hardiness are affected by photoperiod length and the prevailing temperature conditions during the growing season (Aronsson 1975; Christersson 1978; Skrøppa 1991; Dalen et al. 2001; Rostad et al. 2006). Also, they are affected by geographical origin and genotype (Johnsen and Apeland 1988; Skrøppa 1991; Hannerz and Westin 2000, 2005). In the boreal zone, genotypes transferred northward and northwestward may weaken the autumn cold hardening while enhancing the growth as a result of longer growing periods, when compared to the local genotypes (Skrøppa and Magnussen 1993). Based on previous studies, autumn cold hardiness is also affected by temperature conditions during the flowering (Johnsen et al. 1996) and seed maturation of parent trees (Skrøppa et al. 2007), as well as by the availability of nutrients (Luoranen et al. 2008).

Compared to the effects of prevailing temperature conditions on the development of autumn cold hardiness for different genotypes of conifer species, the effects of elevated CO_2 have been contradictory. This may partially be due to the different experimental setups and methodologies used for frost hardiness assessment. For example, no effect of elevated CO_2 concentration on frost hardiness development was found in one-year-old seedlings of Norway spruce (Dalen et al. 2001); whereas, in one-year-old seedlings of black spruce (*Picea mariana* (Mill.) Britton, Sterns & Poggenb.), it enhanced frost hardiness (Bigras and Bertrand 2006), in comparison to three-year-old eastern white pine (*Pinus strobus* L.) seedlings (Chang et al. 2016).

Overall, a better understanding is still required of the magnitude of phenotypic variation in relation to different growth and wood property traits (e.g., WD and WA characteristics), and their correlations, in different Norway spruce genotypes (e.g., Finnish clones and provenance hybrids) growing in experimental field trials. As a result, some WA characteristics may need to be considered in the future in the selection of genotypes for forest breeding, and further, for regeneration material in practical forestry. This may be needed to increase both the resource-efficiency of wood production and its utilization in forestry. At the same time, the adaptation capacity of different genotypes to changing environmental (climate and site) conditions should be considered.

1.2 Aims and hypotheses of the study

The main aim of this work was to study the magnitude of phenotypic variation in relation to different growth, WD and WA properties, and the correlations between these, in 41-year-old clones and provenance hybrid clones of Norway spruce, grown in a clonal field trial established in 1974 in southeastern Finland (Papers I and II). Additionally, the development of height and autumn frost hardiness was studied in their seed offspring under different temperature and/or atmospheric CO_2 concentration treatments, under greenhouse conditions over one growing season (Paper III). The following hypotheses were tested in different studies:

i) Some provenance hybrid clones may have higher growth and overall WD than the Finnish clones (Paper I);
ii) Genotype affects growth, WD and WA properties, and their relationships (Papers I and II); and
iii) The climate treatment and genotype will together affect the height growth and autumn frost hardiness in the seed offspring of clones and hybrid clones. Also, hybrid clones will show superior height growth and earlier autumn frost hardiness compared to the offspring of Finish clones, regardless of climate treatment (Paper III).

2 MATERIALS AND METHODS

2.1 Experimental data

The material for this study was collected from a Norway spruce clonal trial, which was established in 1974 on agricultural soil in Imatra (28°48′E, 61°08′N, 60 m a.s.l.; 1300 growing degree days). The trees represented 119 genotypes (clones and hybrid clones) and were produced through grafting. The genotypes were replicated in 20 blocks (four repetition trees per block, 80 trees per genotype). In spring 2015, 9 to 10 trees with good cone crops, covering 25 genotypes, mostly of hybrid origin (Table 1), were randomly harvested.

Good cone crops were required for the harvested sample trees because their seed material of half-sib origin (hereafter, called genotypes) was used in the study presented in Paper III. Stem diameters at 1.3 m and 6 m from the stem base and the stem height of the harvested trees were measured for further calculation of the stem volume. Sample discs approximately 30 cm thick were taken from each tree at 1.3 m height for further laboratory analyses on the growth, WD and WA characteristics (Papers I, II). For this purpose, radial wood segments (5 mm × 5 mm, 5 mm × 20 mm and 5 mm × 10 mm in cross-section) were cut in a south–north direction from the southern side of the sample discs with a twin-blade circular-saw as shown in Fig. 1.

The selection of the genotypes for detailed WA measurements (Paper II) was based on differences in growth and WD values (assessed in Paper I). Consequently, five genotypes were selected. Six trees per genotype were used for WA measurements (Paper II), except for Finnish–Swiss hybrid V455 (Table 1), from which one damaged tree was excluded. In Paper III, some genotypes were excluded from the experiment due to their low seed yield and low level of seed germination.

2.2 Laboratory measurements (Papers I, II)

Wood density measurements (Paper I)

Wood segments were stored under fixed climate conditions (temperature 20 ± 2°C, relative humidity 65 ± 2%), until they reached a 12% moisture content on a dry-weight basis. Then, the wood specimens were scanned in batches, using a direct-scanning ITRAX X-ray microdensitometer (Cox Analytical Systems, Göteborg, Sweden). The X-ray radiographic images were analyzed using a density profile analysis package to determine intraring density profiles for each sample, from pith to bark. The density profiles were processed using Excel macros to determine the following parameters for individual rings: ring width (RW, mm); EW and LW width (EWW and LWW, mm) and their percentages; mean WD (g/cm^3); minimum and maximum WD (g/cm^3); and EW and LW densities (EWD and LWD, g/cm^3). The mean of the maximum and minimum intraring densities were used as a threshold for EW (< mean) and LW (> mean) in each ring, following a previous study by Zubizarreta Gerendiain et al. (2007). WD measurements were also used in the analysis presented in Paper II.

Tracheid length measurements (Paper II)

Tracheid length measurements were made for groups of 2–3 rings in radial wood segments (5 mm × 20 mm). Samples of 2–3 rings were macerated in a 1:1 (v/v) solution of acetic acid and hydrogen peroxide for 24 h at 60°C. Thereafter, the fibers were separated with a glass rod in a small volume of water and washed to remove all the macerating solution. The L&W Fiber Tester (AB Lorentzen Wettre, Kista, Sweden) was then used to produce two-dimensional images of these fibers and, based on these, the lengths of tracheids were measured (10-12 measurements per sample tree).

Wood anatomy measurements (Paper II)

The radial wood segments were sectioned into shorter pieces, in order to be cut with a microtome and mounted on a slide. Before sectioning, the wood was softened in warm water (60°C) for 5–7 days before sectioning. Cross-sections, 15–20 μm thick, were cut using a rotary microtome (Microm). The sections were stained with safranin-alcian blue (Fagerstedt et al. 1996), after which they were mounted on slides using DePex. The WA characteristics were measured for the first and then every fifth annual ring, and on every fifth millimeter of the radial wood segment. The anatomical measurements were carried out using a Leica stereomicroscope and a Leitz Laborlux 12 light-microscope with a Micropublisher 5.0 camera and using Image Pro 7.0 software. Using the Leica microscope, the number of rays crossing a tangential transect of 1 mm in length was counted in the middle of the measured ring (Carlqvist 2010). In addition, the number of resin canals was counted for each measured ring, separately for areas (mm^2) of EW and LW. A border between the EW and LW was defined visually, where the double cell wall thickness (2CWT) was clearly thickened. The resin canals were classified as normal or traumatic (see, e.g., Wimmer and Grabner 1997).

The 2CWT of the tracheid, and the tracheid LD in the radial direction, of four cells in both the EW and LW, were measured using a Leitz microscope. These measurements were carried out for each sampled annual ring, from the pith to the bark. In addition, from both tangential sides of the resin canals (if both sides were available), the radial thicknesses of two 2CWTs of the nearest (first order) and second nearest (second order) tracheids were measured.

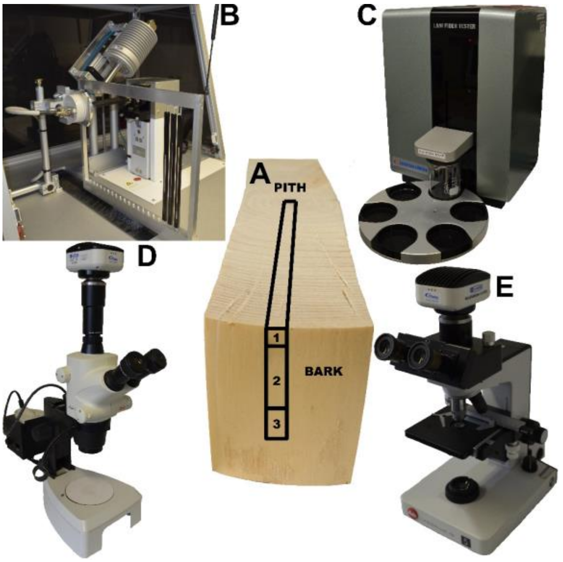

Figure 1. Scheme of cuts of wood segments (A) for WD measurements (1), tracheid length measurements (2) and cross-sectional WA measurements (3) from the south side of the discs. Layout of the ITRAX X-ray microdensitometer (B), the L&W Fiber Tester (C), the Leica stereomicroscope (D) and the Leitz Laborlux 12 transmitting-light microscope (E).

Table 1. Genotypes (Gtype) studied in Papers I–III, and the geographical origins of their parent trees.

Gtype	Geographical origin	Studied in paper	Geographical location and elevation: mother parent tree			Geographical location and elevation: father parent tree		
			Latitude (N)	Longitude (E)	Elevation (m a.s.l.)	Latitude (N)	Longitude (E)	Elevation (m a.s.l.)
Finland × Switzerland (F × S)								
V454	1/E1845 Muonio × E1771 Wintschgau	I	67°58′	23°40′	150–250 (200)	46°30′	10°25′	< 800 (799)
V455	3/E1845 Muonio × E1771 Wintschgau	I–III	67°58′	23°40′	150–250 (200)	46°30′	10°25′	< 800 (799)
V456	4/E1845 Muonio × E1771 Wintschgau	I–III	67°58′	23°40′	150–250 (200)	46°30′	10°25′	< 800 (799)
Finland × Germany 1 (F × G1)								
V447	6/E1832 Rovaniemi × E1770 Spiegelau	I, III	66°29′	25°40′	250	48°57′	13°25′	700–800 (750)
V448	8/E1832 Rovaniemi × E1770 Spiegelau	I, III	66°29′	25°40′	250	48°57′	13°25′	700–800 (750)
V449	10/E1832 Rovaniemi × E1770 Spiegelau	I, III	66°29′	25°40′	250	48°57′	13°25′	700–800 (750)
Finland × Germany 2 (F × G2)								
V49	E5520 Loppi × E1893 Carlsfeld	I, III	60°37′	24°26′	120	50°24′	12°35′	900
V302	H3270 Loppi × E1766 Carlsfeld	I–III	60°37′	24°26′	120	50°24′	12°35′	900
Finland × Germany 3 (F × G3)								
V381	K1413 Pieksänmaa × E1890/2 Schielbach	I, III	62°23′	27°04′	160	50°24′	12°18′	400–600 (500)
V382	K1413 Pieksänmaa × E1890/6 Schielbach	I, III	62°23′	27°04′	160	50°24′	12°18′	400–600 (500)
V383	K1411 Pieksänmaa × E1890/8 Schielbach	I, III	62°23′	27°04′	160	50°24′	12°18′	400–600 (500)

Table 1 continued.

Gtype	Geographical origin	Studied in paper	Geographical location and elevation: mother parent tree			Geographical location and elevation: father parent tree		
			Latitude (N)	Longitude (E)	Elevation (m a.s.l.)	Latitude (N)	Longitude (E)	Elevation (m a.s.l.)
Finland × Latvia (F × L)								
V469	E2672 Loppi × E943/2 Goldingen	I, III	60°44′	24°30′	120	57°38′	22°00′	< 100 (99)
V470	E2672 Loppi × E943/3 Goldingen	I, III	60°44′	24°30′	120	57°38′	22°00′	< 100 (99)
V471	E2672 Loppi × E943/6 Goldingen	I	60°44′	24°30′	120	57°38′	22°00′	< 100 (99)
Russia (R)								
V325	513/2 Pskov Oblast	I, III	—	—	—	57°48′	28°26′	100–200 (150)
V327	513/8 Pskov Oblast	I, III	—	—	—	57°48′	28°26′	100–200 (150)
Finland × Estonia (F × E)								
V386	K1420 Pieksänmaa × E949/1 Perawald	I, III	62°22′	27°04′	160	58°00′	27°30′	< 100 (99)
V388	K1420 Pieksänmaa × E949/4 Perawald	I, III	62°22′	27°04′	160	58°00′	27°30′	< 100 (99)
V389	K1420 Pieksänmaa × E949/5 Perawald	I, III	62°22′	27°04′	160	58°00′	27°30′	< 100 (99)
Finland (F)								
V47	Tree157, +8 Pornainen	I, III	60°29′	25°30′	30	—	—	—
V328	E2969/5 Pornainen	I	60°29′	25°30′	30	—	—	—
V332	E2937/4 Pöytyä	I–III	60°43′	22°51′	90	—	—	—
V43	Tree300, + 44 Miehikkälä	I–III	60°47′	27°30′	60	—	—	—
V48	Tree92, + 44 Miehikkälä	I, III	60°47′	27°30′	60	—	—	—
V465	E3821/1 Ruokolahti	I, III	61°19′	28°55′	100	—	—	—

2.3 Greenhouse experiment (Paper III)

Experimental design

The greenhouse experiment was conducted at the Haapastensyrjä research station of the Natural Resources Institute Finland (Luke) in southwestern Finland (24°25´E, 60°37´N) in 2015. The effects of different temperatures and CO_2 treatments on height growth and frost hardiness development were investigated during one growing season in seedlings produced from seeds that were harvested from sample trees in spring 2015. In this study, three different climate treatments (scenarios) were used in separate greenhouse rooms (Fig. 2). Elevation of the atmospheric CO_2 was considered only for the 2100 temperature scenario (Ambient T+4°C + CO_2), while in the third greenhouse room, in addition to the temperature elevation, the CO_2 concentration was elevated to 700 ppm, starting from May 18 (calendar day [CD] 138). The temperature was not allowed to fall below 0°C in any of the greenhouse rooms during the experiment. Seedlings under all climate treatments were grown in ambient light. The temperature and CO_2 concentration projections for future climate in this study correspond quite well with the most recent climate change projections of CMIP5 (e.g., RCP4.5; Ruosteenoja et al. 2016).

Prolonged growing seasons are expected under elevated temperatures (Ruosteenoja et al. 2016). Therefore, sowing for Ambient T+1°C was done on April 21 (CD 111), and for Ambient T+4°C and Ambient T+4°C + CO_2 on April 9 (CD 99). Two seeds per container were sown because germination was at 69%. Further, one randomly-chosen seedling was removed in cases where both seeds germinated in the container. Heterogeneity in the growing conditions was minimised by shifting the position of the tables on which the boxes containing the seedlings were placed. Irrigation was applied to the seedlings twice a week, regardless of climate treatment. Due to low seed yield, several genotypes were excluded from the experiment (Table 1).

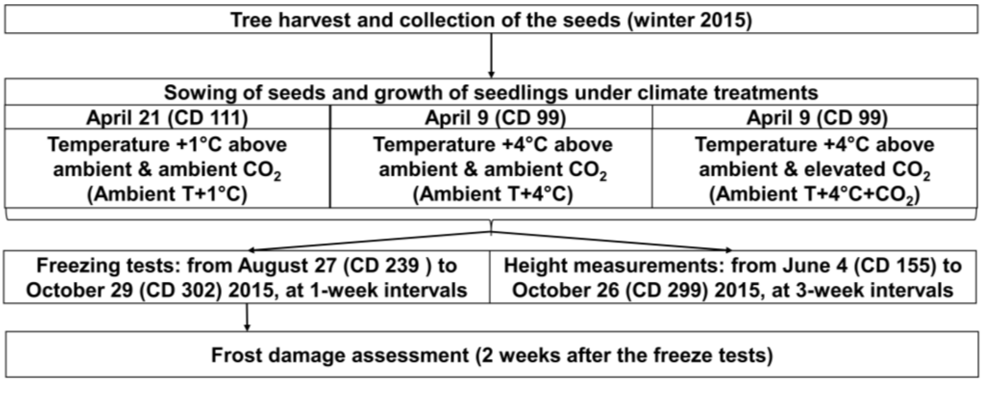

Figure 2. Outline of experimental layout. CD – calendar day.

Height and frost hardiness assessment

The height (mm) was measured for each genotype in 20–24 seedlings per genotype. Height measurements started from June 4 and ended on October 26. Height measurements were taken at 3-week intervals (see Fig. 2).

Artificial freeze tests were used to study the development of autumn frost hardiness. They started on August 27 (CD 239) and continued until October 29 (CD 302) (Fig. 2). Initially, 7040 seedlings were planned to be subjected to freeze tests (8 seedlings × 10 freeze test days × 22 genotypes × 4 climate treatments = 7040 seedlings). However, because of survival issues, 6598 seedlings (≈ 94%) were ultimately freeze tested. The freeze tests were conducted in a 19 m × 21 m freezing room. The treatments were arranged so that eight seedlings per genotype per climate treatment were tested on the same day. The freezing treatment consisted of a gradual decrease in temperature, by 3°C/h, until –10°C was reached. The seedlings were kept at –10°C for 2 h before the temperature was gradually raised (by 1°C/h) to the ambient temperature. After the freezing treatment, the seedlings were returned to their respective greenhouse rooms. One person visually scored injuries to the needles, two weeks after the freeze test, following the methodology of Andersson (1992). Injury-scoring was based on the rate of chlorophyll breakdown, which resulted in browning of the needles. An 11-point classification of the damage was applied, which included classes from 0% (all needles intact) to 100% (all needles injured) damage, with 10% intervals.

2.4 Data analysis

Differences in traits between genotypes

For each sample tree, the stem volume (m^3) was calculated based on the values of tree height and diameter at 1.3 m and 6 m (Paper I), according to the function of Laasasenaho (1982). Depending on the case, arithmetic or weighted cross-sectional means were calculated for the measured parameters (Table 2). The stem mass was calculated for each tree by multiplying stem volume by WD (Paper I). The phenotypic coefficient of variation (CV, %) was calculated by normalizing the standard deviation (σ) by the mean (μ) of the variable (e.g., LWW, LWD and 2CWT) for each genotype, and by multiplication of the quotient by one hundred (Papers I, II).

R software (R Core Team 2018) was used for the statistical analyses. Differences in various growth traits and WD traits among genotypes were tested using one-way analysis of variance (ANOVA). Pairwise analysis using Tukey's test ($p < 0.05$) and Dunnett's test was applied to the studied variables based on Levene's test for equality of error variances (Paper I).

In paper II, differences in WA characteristics among genotypes were tested by setting contrast in the ANOVA function, and by applying a Bonferroni correction. A paired-sample t-test was applied to test for differences among the 2CWTs in normal tracheids and those located next to resin canals. Also, differences between the first- and second-order 2CWTs located next to resin canals in both EW and LW were tested by paired-sample t-test ($p < 0.05$). Phenotypic correlations among the studied properties were calculated using the Pearson correlation procedure ($p < 0.05$) (Papers I, II). A linear mixed-effect model (see Eq.1

in Paper I) was used to study how the geographical origin of the parent trees is associated with the diameter, height, stem volume and overall WD of the genotypes.

Height and frost hardiness assessment

The effects of the climate treatments on the mean height growth of the seedlings for each genotype were determined using a nonlinear mixed-effect model based on the Hossfeld IV function (Zeide 1993; Mehtätalo et al. 2015) (see Eq. 1 in Paper III). Hypothesis tests of the fixed effects were based on the approximate conditional t-test (Pinheiro and Bates 2000). Further, to consider the effects of within-genotype variations on height growth, a nonlinear mixed-effect model (see Eq. 2 in Paper III) was fitted separately for each climate-treatment data subset. The nonlinear models were fitted using the nlme package (Pinheiro et al. 2017) for R.

A binary logistic mixed-effect model was used to study the effects of climate treatment and genotype on the development of autumn frost hardiness (see Eq. 3 in Paper III). The lme4 package was applied (Bates et al. 2015) for fitting the binary logistic mixed-effect model in R. Hypothesis tests of the fixed effects were based on the Wald t-test, using the Satterthwaite approximation for decrease of freedom, as implemented in the lmerTest package (Kuznetsova et al. 2016). Based on Eq. 3 in Paper III, days with 99%, 50% and 1% of damaged needles were estimated for each genotype and climate treatment. The duration of frost hardiness development was calculated for each climate treatment as the difference between the days with 99% and 1% damaged needles, as averaged over all genotypes for each climate treatment.

Table 2. Parameters studied in Papers I–III. SD – standard deviation, CV – phenotypic coefficient of variation.

Paper	Parameters
Paper I	**Arithmetical mean, SD, CV, percentages of genotype means relative to the mean of all studied genotypes:** Diameter at 1.3 m (cm), stem height (m), stem volume (m^3), stem mass (kg), EWW (mm), LWW (mm), LWW (%), RW (mm).
	Weighted cross-sectional mean, SD, CV, percentages of genotype means relative to the mean of all studied genotypes: EWD ($g*cm^{-3}$), LWD ($g*cm^{-3}$), WD ($g*cm^{-3}$).
	Model-based estimates: Effect of geographical origin of the genotype on growth and WD traits
Paper II	**Weighted cross-sectional mean, SD, CV, percentages of genotype means relative to the mean of all studied genotypes:** Tracheid length (mm)
	Arithmetical mean, SD, CV, percentage of the genotype means relative to the mean of all studied genotypes: 2CWT in EW (µm), 2CWT in LW (µm), LD in EW (µm), LD in LW (µm), wall/lumen ratio in EW, wall/lumen ratio in LW, density of normal resin canals in EW (no./mm^2), density of normal resin canals in LW (no./mm^2), density of traumatic resin canals in EW (no./mm^2), density of traumatic resin canals in LW (no./mm^2), first-order 2CWT next to resin canals in EW (µm), second-order 2CWT next to resin canals in EW (µm), first-order 2CWT next to resin canals in LW (µm), second-order 2CWT next to resin canals in LW (µm), ray frequency (no./mm)
Paper III	**Model-based estimates:** Height development and frost damage assessment

3 RESULTS

3.1 Phenotypic variation and correlations among different growth and wood-density traits and the effect of geographical origin on these (Paper I)

Among the growth and WD traits studied, the lowest phenotypic variation was observed in cross-sectional LWD and EWD, followed by WD (range 3.7–4.3%) and tree height (5.5%). The highest variation was observed for stem volume (23.4%). The average variation was observed for diameter at breast height, RW, LWW and EWW (10.00–13.0%).

The genotypes differed among each other in studied traits (see Tables A1, A2 and A3 in Paper I). The Finnish clone V43 showed superior volume and diameter growth (40.7% and 19.8% higher than the respective means over all genotypes), and had higher than the average tree height (103.4% higher than the respective means over all genotypes) and WD the same as the mean over all genotypes. Also, Finnish clone V43 had the largest RW (20.0% higher than the mean). The Finnish–Swiss hybrid V455 had the highest WD (10.9% higher than the mean). Finnish–Latvian hybrid V469 was the tallest (7.0% higher than the mean).

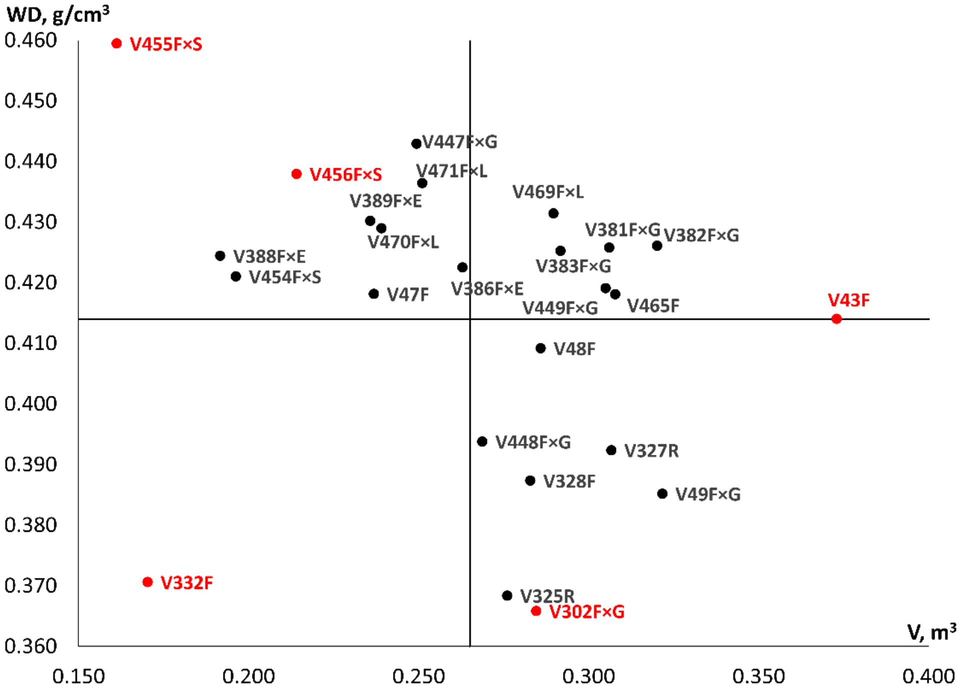

Figure 3. Relationship between mean stem volume (V) and WD. The vertical and horizontal lines are the overall mean stem volume and WD, respectively, for the 25 genotypes. The genotypes for which the wood anatomy was studied in Paper II are shown in red. Parent tree origins are Finland × Switzerland (F × S), Finland × Germany (F × G), Finland × Latvia (F × L), Finland × Estonia (F × E), Russia (R) and Finland (F).

The phenotypic correlation among height, diameter at breast height and stem volume was strong and positive (range 0.65–0.94). The phenotypic correlation among the cross-sectional mean growth parameters varied from weak to strong (range 0.28–0.96). Among the cross-sectional mean WD traits, the phenotypic correlation ranged from 0.70 between EWD and LWD and 0.92 between EWD and WD. Phenotypic correlations between diameter and WD and between stem volume and WD were, in general, negative and moderate (see Table 3 in Paper I). Negligible insignificant phenotypic correlations between these traits were also observed for the Finnish–German hybrids V49 and V448, Finnish–Latvian hybrids V471 and V469 and Finnish clones V47, V48 and V43 (see Table A4 in Paper I).

The increase in latitudinal transfer distance of the parent trees compared with the latitude (elevation effect considered) of the trial tended to decrease the height, diameter at breast height and stem volume, but the effect was not significant ($p > 0.05$). The overall WD was significantly affected only by latitude (elevation effect considered) of the father parent tree ($p < 0.05$), a 1° increase in which decreased the WD by 0.006 g/cm^3 (see Table 2 in Paper I).

3.2 Phenotypic variation among different wood anatomy characteristics and their correlation with growth and wood density traits (Paper II)

Among the WA characteristics studied, tracheid length and ray frequency showed the lowest phenotypic variation, and densities of traumatic and normal resin canals showed the highest phenotypic variation in most of the genotypes (see Table 2 in Paper II). Tracheid length and 2CWT in EW and LW were affected both by the genotype (see Table 2 in Paper II) and cambial age (Fig. 4).

Finnish–Swiss hybrid V456 and Finnish clone V43 had the longest tracheids, while Finnish–German hybrid V302 had the shortest. Finnish clone V43 had the highest mean 2CWT among the genotypes, in both EW and LW (see Table 2 in Paper II). Finnish–German hybrid V302 had the highest mean LD in LW among all genotypes.

The first- and second-order 2CWTs next to the resin canals did not differ among the genotypes in either EW or LW. In the Finnish–Swiss V455 and Finnish–German V302 hybrids, the mean 2CWTs in the first- and second-order tracheids in EW were smaller ($p < 0.05$) than the mean 2CWT in the normal EW tracheids (see Table 2 in Paper II). In Finnish clones V332 and V43, only the 2CWT in the second-order tracheids in EW was smaller ($p < 0.05$) than in the normal tracheids. In LW, the mean 2CWTs in the first- and second-order tracheids were smaller ($p < 0.05$) than the mean 2CWT in the normal tracheids in all genotypes. Also, in the LW, in Finnish clone V43 and Finnish–Swiss hybrids V455 and V456, the first-order mean 2CWTs were smaller ($p < 0.05$) than the second-order ones (see Table 2 in Paper II).

The density of traumatic resin canals had greater variation both in the EW and LW, when compared to the variation in the normal resin canals. Also, the LW had a higher density of both traumatic and normal resin canals than the EW. No statistical difference was found among the genotypes in either normal or traumatic resin canal densities in the EW and LW. The mean number of rays per tangential millimeter was similar in all genotypes (see Table 2 in Paper II).

Both genotype and wood type (EW or LW) affected the strengths and directions of the correlations (Table 4) among the WA characteristics and the cross-sectional growth traits (EWW and LWW) in most cases. Regardless of the genotype, the number of rays per tangential millimeter and tracheid length had a consistent direction of correlation both in the

EW and LW. Correlations among other WA characteristics and EWW and LWW had genotype-specific directions and strengths (Table 4).

Also, genotype and wood type (EW or LW) affected the strengths and directions of correlations between the WA characteristics and the cross-sectional WD parameters (EWD and LWD) in all cases (Table 4). Finnish–Swiss hybrid V455 differed from the other genotypes in the direction of correlations between EWD and anatomical parameters such as tracheid length, LD, the 2CWT/LD ratio, and number of rays per tangential millimeter. In the LW, Finnish–German hybrid V332 differed from the other genotypes in the directions of correlations between LWD and anatomical parameters such as tracheid length, 2CWT, and 2CWT/LD ratio (Table 4). Correlations among other WA characteristics and EWD and LWD had genotype-specific directions and strengths (Table 4).

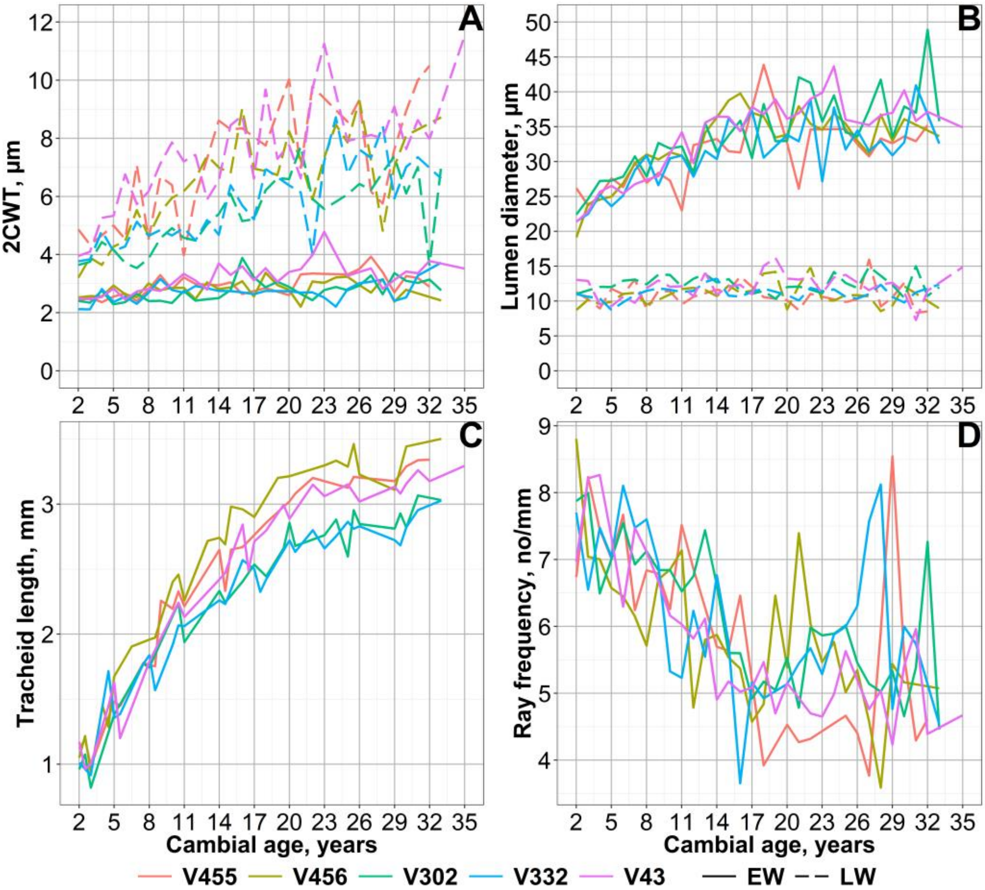

Figure 4. Tracheid double sell-wall thickiness (2CWT) (A) and tracheid lumen diamater (B) in EW and LW and tracheid length (C) for groups of 2–3 annual rings, and density of rays per tangential millimeter in annual rings (D), in Norway spruce genotypes by cambial age. The genotypes are Finnish–Swiss hybrids V455 and V456, Finnish–German hybrid V302 and Finnish clones V332 and V43.

Table 3. Magnitude of different variables for genotypes studied in Papers I and II in relation to the overall mean of all genotypes. '+' – value higher than the mean, '-' – value lower than the mean for all genotypes.

Anatomical characteristic	V455 (F × S)	V456 (F × S)	V302 (F × G)	V332 (F)	V43 (F)
	%	%	%	%	%
Paper I					
Diameter (1.3), cm	-	-	+	-	+
Stem height, m	-	+	-	-	+
Stem volume, m^3	-	-	+	-	+
WD, g/cm^3	+	+	-	-	+
Paper II					
Tracheid length, mm	+	+	-	-	+
Tracheid 2CWT in EW, µm	+	-	-	-	+
Tracheid 2CWT in LW, µm	+	+	-	-	+
Tracheid lumen diameter in EW, µm	-	+	+	-	+
Tracheid lumen diameter in LW, µm	-	-	+	-	+
Wall/lumen ratio, EW	+	+	-	-	+
Wall/lumen ratio, LW	+	+	-	-	+
Density of normal resin canals in EW, no./mm^2	+	-	-	+	-
Density of traumatic resin canals in EW, no./mm^2	-	+	-	+	+
Density of normal resin canals in LW, no./mm^2	+	-	-	+	-
Density of traumatic resin canals in LW, no./mm^2	+	-	+	-	-
First-order 2CWT next to resin canals in EW, µm	+	+	-	-	+
Second-order 2CWT next to resin canals in EW, µm	+	+	-	-	+
First-order 2CWT next to resin canals in LW, µm	+	+	-	-	+
Second-order 2CWT next to resin canals in LW, µm	+	+	-	-	+
Ray frequency, no./mm	+	-	+	+	-

3.3 Effects of climate treatment and genotype on height and frost hardiness development of seedlings (Paper III)

The highest mean final height of the seedlings was observed for Ambient T+4°C (146 mm) and Ambient T+4°C + CO_2 (144 mm), which differed ($p < 0.05$) from Ambient T+1°C (119 mm) (Fig. 5; see Tables 2 and 3 in Paper III). Similarly, the day (xmid) half the mean final height was reached (asym-hmin) was the earliest under Ambient T+4°C + CO_2 (CD 196) and Ambient T+4°C (CD 199), which differed ($p < 0.05$) from Ambient T+1°C (CD 203) (see Tables 2 and 3 in Paper III).

Variations in final height among the genotypes (see Fig. 3 and Table 2 in Paper III) was the highest under Ambient T+4°C + CO_2, followed by Ambient T+4°C and Ambient T+1°C. Relatively high correlations between final height and climate treatment (see Table 2 in Paper III) also indicate that, in general, the performance of the genotypes is quite similar, regardless of climate treatment. For example, V302, V386 and V447 were consistently among the tallest genotypes, regardless of climate treatment (see Fig. 3 in Paper III).

Development of frost hardiness started earliest under Ambient T+1°C, which differed ($p < 0.05$) from Ambient T+4°C and Ambient T+4°C + CO_2 (Fig. 5, see Table 4 in Paper III). Duration of frost hardiness development, however, was opposite, being 32 days under Ambient T+1°C, 28 days under Ambient T+4°C, and 27 days under Ambient T+4°C + CO_2 (see Supplementary file S2 in Paper III, available at https://doi. org/10.14214/sf.9980).

The highest variation in frost hardiness development was observed for Ambient T+4°C and Ambient T+4°C + CO_2, followed by Ambient T+1°C (see Fig. 3 and Table 4 in Paper III). However, the variation in the probability of frost damage between the boxes where the seedlings were grown was higher than the variation caused by any climate treatment. The genotypes performed quite differently among the climate treatments, according to the correlations between the climate treatments (see Table 4 in Paper III). For example, V43 showed the highest frost tolerance among the genotypes under Ambient T+4°C (based on days of 50% frost damage probability estimates), average frost tolerance under Ambient T+1°C, and the lowest frost tolerance under Ambient T+4°C + CO_2 (see Fig. 3 in Paper III).

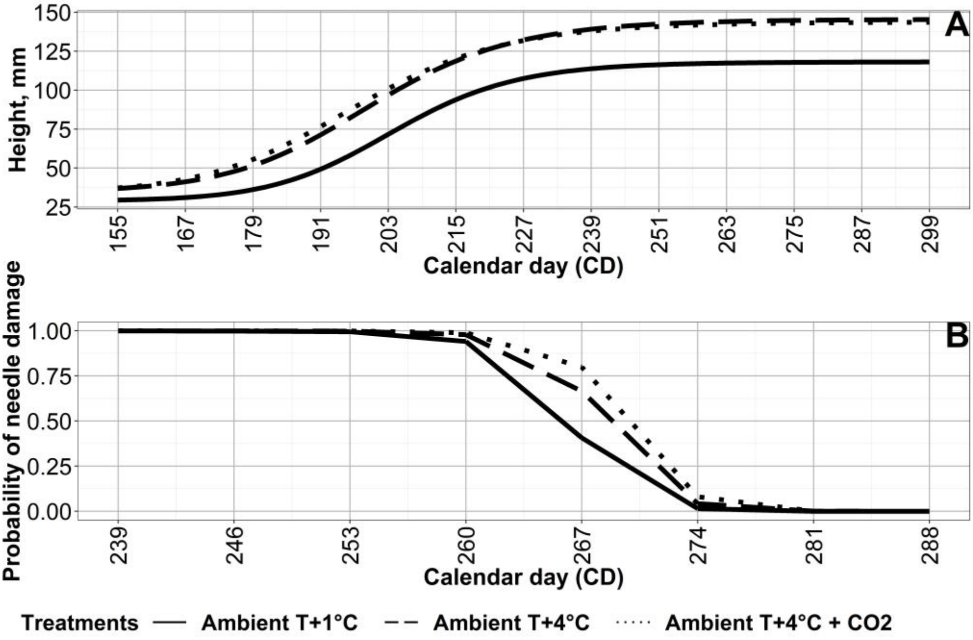

Figure 5. Development of height (A) and dynamics of the probability of autumn frost damage (B) in needles of different genotypes in Norway spruce seedlings, averaged for different climate treatments. Legend: 1°C above ambient and ambient CO_2 (Ambient T+1°C), 4°C above ambient and ambient CO_2 (Ambient T+4°C) and 4°C above ambient and elevated CO_2 (Ambient T+4°C + CO_2).

Table 4. Phenotypic correlations between WA characteristics (studied in Paper II) and EWW, LWW, EWD and LWD for different genotypes.

Anatomical characteristic	EWW					LWW					EWD					LWD				
	(F × S)		(F × G)	(F)		(F × S)		(F × G)	(F)		(F × S)		(F × G)	(F)		(F × S)		(F × G)	(F)	
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Tracheid length, mm	↓↓↓	↓↓	↓↓	↓↓	↓↓	↓↓	↓↓	↓↓	↓↓↓	↓↓	—	↓↓	↓↓	↓↓↓	↓↓↓	↑↑	↑	—	↓↓	↑↑
Tracheid double cell-wall thickness, EW	↓	↓	↓	—	↓↓	—	—	↓	—	↓	—	—	↓	—	↓↓	—	—	—	—	↑
Tracheid double cell-wall thickness, LW	↓↓↓	↓↓	↓↓	↓↓	↓	↓↓↓	↓↓	↓↓	↓	↓	—	↓↓	↓↓	↓↓	↓↓	↑↑	↑	↑↑	—	↑↑
Tracheid lumen diameter, EW	↓↓	↓	↓↓	↓	↓↓	↓↓	↓↓	↓↓	↓↓	↓	—	↓↓	↓↓	↓↓	↓↓	—	—	—	↓↓	↑↑
Tracheid lumen diameter, LW	—	↓	—	↓	—	—	—	—	—	—	—	↓	—	—	—	—	—	—	—	—
Wall/lumen ratio, EW	—	—	—	—	—	—	↓↓	↑	↓↓	—	—	↑	↑	↑↑	↑	—	—	—	↑	—
Wall/lumen ratio, LW	↓↓	↓↓	↓↓	↓↓	↓↓	↓↓	↓↓	↓↓	↓	↓	—	—	↓	↓	↓	↑	—	↑↑	—	↑
First-order 2CWT next to resin canals, EW	—	—	—	—	—	↓↓	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Second-order 2CWT next to resin canals, EW	—	↓	↓	—	↓	—	—	↓↓	—	—	↓↓	—	—	—	—	—	—	—	—	↑
First-order 2CWT next to resin canals, LW	—	↓↓	—	—	↓↓	—	↓↓	—	—	—	—	—	—	—	↓↓	—	↑↑	—	—	—
Second-order 2CWT next to resin canals, LW	—	↓	↓↓	—	↓↓	—	↓	↓	—	—	—	↓↓	—	—	↓↓	—	↑	—	—	—
Normal resin canals no./mm^2, EW	—	—	↑	—	↑	—	—	—	↑	—	—	—	↑	↑↑	↑	—	—	—	↑	—
Normal resin canals no./mm^2, LW	—	—	—	—	—	—	↑	—	—	—	—	—	—	—	—	—	—	↑	—	—
Traumatic resin canals no./mm^2, EW	—	—	↑	—	—	—	—	—	↑	—	—	—	—	↑↑	↑	—	—	—	—	—
Traumatic resin canals no./mm^2, LW	↑	—	—	—	—	—	—	—	—	—	—	—	↑	—	↑↑	↓	—	—	—	—
Normal + traumatic resin canals no./mm^2, EW	—	—	↑	—	—	—	—	—	↑↑	—	—	—	—	↑↑	↑↑	—	—	—	↑	—
Normal + traumatic resin canals no./mm^2, LW	↑	—	—	—	—	↑	—	—	—	—	—	—	↑	—	—	↓	↓	—	—	—
Ray frequency, no./mm	↑↑	↑↑	↑↑	↑	↑↑	↑	↑↑	↑↑	↑	↑	—	↑	↑↑	↑↑	↑↑	↓	—	—	↑	↓

Note: Upward arrows indicate positive correlation, downward arrows negative correlation. Single arrows indicate weak correlation ($0 \le |r_p| \le 0.33$), two medium ($0.33 < |r_p| \le 0.66$) and three strong ($0.66 < |r_p| \le 1$). Parent tree origins are Finland × Switzerland (F × S), Finland × Germany (F × G) and Finland (F). 1 – V455, 2 – V456, 3 – V302, 4 – V332, 5 – V43.

4 DISCUSSION

4.1 Evaluation of experimental approaches and the methodology applied

In this work, the first aim was to study the magnitude of phenotypic variation in different growth and WD traits (Paper I) and WA characteristics (Paper II), and the correlations among these, in 41-year-old clone and provenance hybrid clone trees of Norway spruce (Papers I, II). The second aim was to study the effects of elevated temperature and/or atmospheric CO_2 concentration treatments on the development of height and autumn frost hardiness in half-sib Norway spruce seedlings grown over one growing season under greenhouse conditions (Paper III). These seedlings were produced from seeds collected from mother parent trees of Finnish clones and the provenance hybrid clones used in Papers I and II.

The criterion for harvesting the sample trees for the studies presented in Papers I and II was that they should have a good cone yield, which might have decreased the observed phenotypic variation, especially in tree height, as such trees represented mainly dominant and co-dominant trees in the field trial. Nonetheless, since the sampling criterion was the same for all genotypes, the results obtained would be expected to be comparable among genotypes. Suppressed (and partly also intermediate) trees are also typically harvested in Finland in the first commercial thinning, regardless of tree species (Äijälä et al. 2014).

The number of harvested sample trees per genotype was also quite limited in these studies. However, because they represented clones and provenance hybrid clones, the sample size was expected to be quite sufficient to reveal possible differences among genotypes, the magnitude of phenotypic variation, and correlations among different growth, WD and WA properties, respectively.

The WA measurements were time-consuming and laborious, which affected the number of genotypes, sample trees and measurement protocols that were used under laboratory conditions. All growth, WD and WA properties were measured under laboratory conditions, using the methodologies applied in many previous impact studies (e.g., Zubizarreta Gerendiain et al. 2007, 2008; Luostarinen et al. 2017). Unfortunately, due to the relatively small number of genotypes included in the different studies, genetic correlations could not be calculated among the different properties in Papers I and II.

In the greenhouse study (Paper III), it was not possible to replicate the climate treatments, or use ambient conditions as a control for elevated temperature and/or atmospheric CO_2 concentration treatments. Therefore, the results of the Ambient T+4°C and Ambient T+4°C + CO_2 treatments were compared to those of Ambient T+1°C. The greenhouse experiment also involved fewer genotypes than were used in Paper I due to the lack of sufficient seed yield and a low level of germination of the seeds for some genotypes. The half-sib origin of the seedlings also hindered separation of the effects of genetic factors and greenhouse environmental conditions. Differences in the sowing time between climate treatments might also have affected the length of growing period under different climate treatments, and as well as the final height of the seedlings. Despite this, general effects of the genotypes and the different climate treatments on the development of height and frost hardiness could be observed.

4.2 Growth and wood density traits in Norway spruce genotypes (Paper I)

The studied genotypes differed among each other in growth and WD traits. This could be a result of hybridization (Table 1) and the geographical transfer of the genotypes (see, e.g., Andalo et al. 2005; Barzdajn et al. 2016; Kapeller et al. 2012). However, the change in latitudinal transfer distance of the parent trees compared with the latitude of the clonal trial did not explain the differences in growth traits in the studied genotypes (see Table 2 in Paper I). At the same time, the transfer of father parent-tree genotypes from south of Imatra negatively affect overall WD (see Table 2 in Paper I). Zubizarreta Gerendiain et al. (2009) reported similar results in Norway spruce in another Finnish trial. The differences among genotypes in growth and yield traits could also be explained by their adaptation capacity to environmental conditions at the trial site, such as photoperiod (Ekberg et al. 1979) and prevailing temperature conditions (Ståhl 1998; Skrøppa et al. 1999; Savva et al. 2006; Kapeller et al. 2013). For example, the Finnish–German hybrids tended to show higher than average mean stem volume over all genotypes, while all Finnish–Swiss and Finnish–Estonian hybrids showed the opposite. As was hypothesized, some provenance hybrid clones simultaneously had both higher stem volume and overall WD than the local Finnish clones. However, the stem volume was largest for local Finnish clone V43 (Fig. 3).

Phenotypic correlations between stem volume and WD traits were also affected by genotype. The correlations were moderately or strongly negative for most of the genotypes. However, in local Finnish clone V43, the correlation was negligible, suggesting that an increase in volume did not substantially decrease its overall WD. The Finnish–German V449, V381, V382 and V383 and Finnish–Latvian V469 hybrids showed both relatively high stem volume and overall WD (Fig. 3), despite negative relationships existing between these traits. This suggests that hybridization could offer the means to affect different properties (e.g., Gerhold and Park 1986; Magnussen and Yeatman 1988; Ruotsalainen 2014).

4.3 Wood anatomy characteristics in Norway spruce genotypes (Paper II)

The studied genotypes differed among each other in WA characteristics and in their relations to growth and WD. The development of tracheid length, the 2CWT and LD in both EW and LW were affected by cambial age (Fig. 4), as has also been reported in previous studies (e.g., Saren et al. 2001; Sirviö and Kärenlampi 2001; Jaakkola et al. 2007; Buksnowitz et al. 2010). Tracheid length correlated negatively with EWW and LWW in all genotypes (see Table 4 in Paper II), which is in line with some earlier findings (see, e.g., Stairs et al. 1966; Yang and Hazenberg 1994; Herman et al. 1998; Mäkinen et al. 2007). However, the longest tracheids were observed in both the slow-growing Finnish–Swiss hybrids V455 and V456, and in the fast-growing Finnish clone V43 (see Tables 2 and 3 in Paper II). Therefore, this does not necessarily mean that only slow-growing genotypes have long tracheids.

Based on the phenotypic correlation values, LD affected EWD in most of the genotypes. A similar observation has previously been reported by Lindström (1997). Despite a positive correlation between the 2CWT and LD in EW, 2CWT correlated with EWD only in two fast-growing (high stem volume) Finnish–German hybrid V302 and Finnish clone V43. However, the correlation was negative. In the slow-growing (low stem volume) Finnish–Swiss hybrid V455, neither the 2CWT nor the LD correlated with EWD. Therefore, in addition to environmental factors (e.g., Campelo et al. 2006), WA characteristics might be affected by growth rate, as predetermined by genotype (Irbe et al. 2015) and genotype origin, as was observed in Paper I.

In LW, 2CWT positively correlated with LWD in most of the genotypes, while LD was found not to affect LWD in any of the genotypes. Previously, Luostarinen et al. (2017) suggested that weak correlations between LWD and 2CWT in LW in Finnish clone V332 was due to atypically thin tracheid walls in the LW, with a high homogeneity of tracheid properties. However, in the present study, Finnish–Swiss hybrid V456 showed an equal 2CWT and wall/lumen ratio in the LW as Finnish clone V332, while having higher homogeneity for that property. Therefore, different proportions of structural compounds, which could be predetermined by the genotype (Sandak et al. 2015), might have affected the LWD in V332.

Resin canals and rays tended to increase EWD. The density of resin canals in the EW correlated positively with EWD in Finnish–German hybrid V302 and Finnish clones V332 and V43. The frequency of rays also correlated positively with EWD in Finnish–Swiss hybrid V456. In Finnish–Swiss hybrid V455, no correlations between EWD and density of resin canals and ray frequency were found. In the LW, on the contrary, resin canals and rays tended to decrease LWD, especially in Finnish–Swiss hybrid V455. However, in Finnish clone V332, with the lowest LWD, a positive correlation between LWD and ray density was similar to that in the EW. Previously, contradictory observations on the effect of resin canal density on WD have been reported. Hannrup et al. (2004), for example, found that resin canal density does not affect WD in Norway spruce. Luostarinen et al. (2017), on the contrary, found significant correlations ($p < 0.05$) between these traits. Our results suggest that these contradictions could be explained by a genotype effect.

4.4 Height growth and the development of autumn frost hardiness in seedlings in response to elevated temperature and/or atmospheric CO_2 concentration (Paper III)

A higher growth rate and mean final height was observed for seedlings of different genotypes under Ambient T+4°C and Ambient T+4°C + CO_2 treatments, compared to Ambient T+1°C (See Table 3 in Paper III). A similar effect of elevated temperature on height growth was observed in one-year-old Sitka spruce (*Picea sitchensis* (Bong.) Carrière) and white spruce (*Picea glauca* (Moench) Voss) by Brix (1972). The free-height growth pattern of the one-year-old seedlings may have also contributed to differences observed in the height growth. No significant effect of elevated atmospheric CO_2 concentration, either on height growth rate or final height of the seedlings, was found, regardless of genotype (See Table 2 in Paper III). Also, temperature elevation increased the variability in the development of height growth and frost hardiness among and within the genotypes. This result is in line with the previous findings of Andalo et al. (2005) in white spruce genotypes. The half-sib genotypes V302, V386, and V447 were consistently among the tallest, regardless of climate treatment.

The onset of the development of autumn frost hardiness was delayed by 5–7 days under Ambient T+4°C and Ambient T+4°C + CO_2, compared to Ambient T+1°C (See Suppl. file S2 in Paper III). In earlier studies, such as that of Chang et al. (2016), an impairment in autumn frost hardening in three-year-old seedlings of eastern white pine (*Pinus strobus* L.), grown under elevated temperature, has also been observed. However, in the present study, the cessation of autumn frost hardiness development was observed by October 7 (CD 280), regardless of climate treatment. As a result, the duration of frost hardiness development was shorter under Ambient T+4°C and Ambient T+4°C + CO_2, compared to Ambient T+1°C. On the other hand, the temperature may have simultaneously prolonged free-shoot growth in the seedlings, thus indirectly affecting the development of autumn frost hardiness.

No significant effect of elevated atmospheric CO_2 concentration on frost hardiness development in Norway spruce seedlings of different genotypes was found when comparing the performance of seedlings under Ambient T+4°C and Ambient T+4°C + CO_2. Possibly high genetic variation also led to low consistency in the development of autumn frost hardiness across the climate treatments among the half-sib genotypes. In previous studies on conifer species, contradictory results have been shown for the CO_2 concentration effect on frost hardiness development. For example, no effect of CO_2 concentration on frost hardiness development in one-year-old seedlings of Norway spruce was found by Dalen et al. (2001). Bigras and Bertrand (2006), however, reported an enhancement of frost hardiness in one-year-old seedlings of black spruce (*Picea mariana* (Mill.) Britton, Sterns & Poggenb.) under an elevated CO_2 concentration, while Chang et al. (2016) found that an elevated atmospheric CO_2 concentration delayed the development of autumn frost hardiness in eastern white pine seedlings.

5 CONCLUSIONS AND FUTURE PROSPECTS

In this work, differences were found among genotypes in growth and WD traits and their relationships. Both high stem volume and high WD were simultaneously observed for some of the hybrids (e.g., the Finnish–German V382). In some genotypes, a negligible relationship was also observed among these traits (e.g., the Finnish clone V43). On the other hand, both high stem volume and WD were observed in Finnish clone V465, although these properties were negatively related to each other. Also, as opposed to what was hypothesized, none of the studied hybrids were superior to Finnish clone V43, which had the highest stem volume and relatively high WD. A superior stem volume and relatively high WD had also been observed in Finnish clone V43 in an earlier study, by Zubizarreta Gerendiain et al. (2007), at a younger age of the same field trial. The five genotypes, for which WA characteristics were studied in detail, differed also both in growth and WD values, and in WA characteristics, and in their relations to growth and WD. The present study demonstrated the possibility of finding genotypes with different WA characteristics, which may be desired in the future for different technological processes and wood products. Moreover, the differences in WA observed in the genotypes suggest that they differ in proportion of structural compounds.

Under elevated temperature treatments, seed offspring of the studied genotypes grew taller and faster under greenhouse conditions. However, no significant effect of elevated atmospheric CO_2 concentration was found, either on the height growth rate or final height of the seedlings, regardless of genotype. These results may be at least partially explained by sufficient water availability for the seedlings under greenhouse conditions. On the other hand, an elevation in temperature delayed the development of autumn frost hardiness and shortened its duration, while an elevated atmospheric CO_2 concentration had no significant effect on these. The half-sib genotypes exhibited rather low consistency in the development of autumn frost hardiness across the climate treatments, which might imply relatively high genetic variation. Therefore, no genotypes with both superior height growth and autumn frost hardiness were observed in this study.

Based on this research, a need emerged for further studies on WA; for example, considering genotype-specific variations in structural compounds. In addition, this study provides support for future studies on the identification of loci related to phenotypic properties, differences in which were observed among the studied genotypes. A better understanding of the magnitude of phenotypic variation on different growth, WD and WA properties, and the correlations between and among these in different genotypes, may also provide support for the future work of tree breeding. It might also be found to be beneficial, considering the need to adapt to the changing climate and the need for raw material with better known properties in the wood-based bioeconomy.

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