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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

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Highlights

- Elevated temperature resulted in increased height growth, delayed onset and shortened duration of autumn frost hardiness development in Norway spruce seedlings.
- Elevated temperature increased variation between genotypes in height growth and frost hardiness development.
- Elevated atmospheric CO_2 concentration had no effect on the development of height or autumn frost hardiness in Norway spruce seedlings.

Abstract

The mean temperature during the potential growing season (April–September) may increase by 1 °C by 2030, and by 4 °C, or even more, by 2100, accompanied by an increase in atmospheric CO_2 concentrations of 536–807 ppm, compared to the current climate of 1981–2010, in which atmospheric CO_2 is at about 350 ppm. This may affect both the growth and frost hardiness of boreal trees. In this work, we studied the responses of height and autumn frost hardiness development in 22 half-sib genotypes of one-year-old Norway spruce (*Picea abies* (L.) Karst.) seedlings to elevated temperatures and atmospheric CO_2 concentration under greenhouse conditions. The three climate treatments used were: T+1 °C above ambient and ambient CO_2; T+4 °C above ambient and ambient CO_2; and T+4 °C above ambient and elevated CO_2 (700 ppm). The height growth rate and final height were both higher under T+4 °C compared to T+1 °C. Temperature increase also delayed the onset, and shortened the duration, of autumn frost hardiness development. Elevated CO_2 did not affect the development of height or frost hardiness, when compared to the results without CO_2 elevation under the same temperature treatment. Higher temperatures resulted in greater variation in height and frost hardiness development among genotypes. Three genotypes with different genetic backgrounds showed superior height growth, regardless of climate treatment; however, none showed a superior development of autumn frost hardiness. In future studies, clonal or full-sib genetic material should be used to study the details of autumn frost hardiness development among different genotypes.

Keywords climate change; cold acclimation; tree growth; needles; seedlings; *Picea abies*
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1 Introduction

Projected atmospheric warming during the growing season may affect the development of height growth and autumn frost hardiness in young seedlings of boreal conifers. On one hand, warming may increase the growth of boreal conifer seedlings (Brix 1972). On the other hand, warming may negatively affect the development of autumn cold hardiness (Chang et al. 2016). This is because the development of autumn cold hardiness is triggered by decreasing temperature and photoperiod length (Beck et al. 2004; Chang et al. 2016), and warming may cause asynchrony in these environmental triggers (Chang et al. 2016). In addition, genotype transfers, used in practical forestry in the boreal zone, may negatively affect autumn cold hardening, as they are commonly transferred northward and northwestward, and have longer growing periods compared to the local genotypes (Skrøppa and Magnussen 1993). Failure to develop sufficient cold hardiness in autumn may result in frost damage, leading to a decreased vitality in the seedlings, an increased risk of wood-related defects, and mortality (Langvall 2000). This may also result in long-term economic losses for forestry. Therefore, a better understanding is needed concerning how genotype affects the development of autumn cold hardiness under climate change conditions.

In Finland, Norway spruce (*Picea abies* (L.) Karst.) is important to the forestry industry and accounts for 30% of the current volume of growing stock (Peltola 2014). Based on many previous studies, growth and the development of autumn cold hardiness in Norway spruce seedlings are affected by photoperiod length and the prevailing temperature during the growing season (Aronsson 1975; Christersson 1978; Skrøppa 1991; Dalen et al. 2001; Rostad et al. 2006). They are also affected by geographical origin and genotype (Johnsen and Apeland 1988; Skrøppa 1991; Hannerz and Westin 2000, 2005). In addition, temperature conditions during flowering (Johnsen et al. 1996), seed maturation of parent trees (Skrøppa et al. 2007), and the availability of growing resources, such as nutrients (Luoranen et al. 2008), all affect the development of autumn cold hardiness in Norway spruce seedlings.

Relatively little is still known about the onset and dynamics of development of autumn cold hardiness, in different genotypes of young Norway spruce seedlings under elevated temperature and atmospheric CO_2 concentration. According to Ruosteenoja et al. (2016), the mean temperature during the potential growing season (April–September) may increase by 1 °C by 2030, and by 4 °C, or even more, by 2100, accompanied by an increase in atmospheric CO_2 concentrations of 536–807 ppm, compared to the current climate of 1981–2010, in which atmospheric CO_2 is at about 350 ppm.

In this work, we studied the responses of height growth and autumn frost hardiness development in 22 half-sib genotypes of one-year-old Norway spruce seedlings to elevated temperature and atmospheric CO_2 concentration under greenhouse conditions. The following climate treatments were used: T+1.0–1.5 °C above ambient and ambient CO_2-baseline climate treatment; T+4.0–4.5 °C above ambient and ambient CO_2; and T+4.0–4.5 °C above ambient and elevated CO_2 (700 ppm). We hypothesized that the climatic treatment and genotype would together affect the height growth and autumn frost hardiness development in young Norway spruce seedlings. We also hypothesized that some genotypes due to their half-sib origin and hybrid background of mother parent-trees would show both superior height growth and earlier autumn frost hardiness compared to others.

2 Materials and methods

2.1 Experimental design and seed material

Seed material was collected from a Norway spruce clonal trial, from trees that were harvested in winter 2015. The trial was established in 1974 in Imatra, southeastern Finland (61°08´N, 28°48´E, 60 m a.s.l.; 1300 growing degree days). Seeds of half-sib origin were collected from openly pollinated mother trees, representing 22 different genotypes, including five southern Finnish (F) and two southwestern Russian (R) clones, two Finnish–Swiss (F × S), eight Finnish–German (F × G), two Finnish–Latvian (F × L), and three Finnish–Estonian (F × E) provenance hybrid clones (Table 1).

Table 1. Origins of clones and provenance hybrid clones (Gtype) used as seed donors.

Gtype	Geographical origin	Geographical location and elevation: parent 1			Geographical location and elevation: parent 2		
		Latitude (N)	Longitude (E)	Elevation (m a.s.l.)	Latitude (N)	Longitude (E)	Elevation (m a.s.l.)
Finland × Switzerland (F × S)							
V455	3/E1845 Muonio × E1771 Wintschgau	67°58´	23°40´	150–250	46°30´	10°25´	< 800
V456	4/E1845 Muonio × E1771 Wintschgau	67°58´	23°40´	150–250	46°30´	10°25´	< 800
Finland × Germany 1 (F × G1)							
V447	6/E1832 Rovaniemi × E1770 Spiegelau	66°29´	25°40´	250	48°57´	13°25´	700–800
V448	8/E1832 Rovaniemi × E1770 Spiegelau	66°29´	25°40´	250	48°57´	13°25´	700–800
V449	10/E1832 Rovaniemi × E1770 Spiegelau	66°29´	25°40´	250	48°57´	13°25´	700–800
Finland × Germany 2 (F × G2)							
V49	E5520 Loppi × E1893 Carlsfeld	60°37´	24°26´	120	50°24´	12°35´	900
V302	H3270 Loppi × E1766 Carlsfeld	60°37´	24°26´	120	50°24´	12°35´	900
Finland × Germany 3 (F × G3)							
V381	K1413 Pieksänmaa × E1890/2 Schielbach	62°23´	27°04´	160	50°24´	12°18´	400–600
V382	K1413 Pieksänmaa × E1890/6 Schielbach	62°23´	27°04´	160	50°24´	12°18´	400–600
V383	K1411 Pieksänmaa × E1890/8 Schielbach	62°23´	27°04´	160	50°24´	12°18´	400–600
Finland × Latvia (F × L)							
V469	E2672 Loppi × E943/2 Goldingen	60°44´	24°30´	120	57°38´	22°00´	< 100
V470	E2672 Loppi × E943/3 Goldingen	60°44´	24°30´	120	57°38´	22°00´	< 100
Russia (R)							
V325	513/2 Pskov Oblast	–	–	–	57°48´	28°26´	100–200
V327	513/8 Pskov Oblast	–	–	–	57°48´	28°26´	100–200
Finland × Estonia (F × E)							
V386	K1420 Pieksänmaa × E949/1 Perawald	62°22´	27°04´	160	58°00´	27°30´	< 100
V388	K1420 Pieksänmaa × E949/4 Perawald	62°22´	27°04´	160	58°00´	27°30´	< 100
V389	K1420 Pieksänmaa × E949/5 Perawald	62°22´	27°04´	160	58°00´	27°30´	< 100
Finland (F)							
V47	Tree157, +8 Pornainen	60°29´	25°30´	30	–	–	–
V332	E2937/4 Pöytyä	60°43´	22°51´	90	–	–	–
V43	Tree300, +44 Miehikkälä	60°47´	27°30´	60	–	–	–
V48	Tree92, +44 Miehikkälä	60°47´	27°30´	60	–	–	–
V465	E3821/1 Ruokolahti	61°19´	28°55´	100	–	–	–

The greenhouse experiment was conducted at the Haapastensyrjä research station of the Natural Resources Institute Finland (Luke) in southwest Finland (60°37´N, 24°25´E) in 2015. To investigate the effects of potential future climatic conditions on height growth and frost hardiness development in the 22 half-sib genotypes of one-year-old Norway spruce seedlings, we subjected them to three different climate treatments in separate greenhouse rooms (Fig. 1), similar to the methodology of Zeps et al. (2017). In each greenhouse room (11.5 m × 7.3 m), temperature and CO_2 concentration could be adjusted independently from the other greenhouse rooms. During the study period, the mean monthly temperature (i.e., the ambient outdoor temperature) ranged from +16.0 °C in July, to 4.0 °C in October. The mean temperature during the study period was +10.5 °C. The frost-free period in 2015 lasted from May 16 (calendar day – CD – 136) to October 5 (CD 278).

For the 2030 temperature scenario (Ambient T+1 °C), the temperature was maintained in real-time in the first greenhouse room at 1.0–1.5 °C above the ambient outdoor temperature. For the 2100 temperature scenario (Ambient T+4 °C), the temperature was maintained in real-time in the second greenhouse room at 4.0–4.5 °C above the ambient outdoor temperature. Elevation of the atmospheric carbon dioxide was considered only for the 2100 temperature scenario (Ambient T+4 °C + CO_2), where, in the third greenhouse room, in addition to the temperature elevation, the CO_2 concentration was elevated to 700 ppm, starting from May 18 (CD 138). The elevated CO_2 level was kept higher in the daytime, but was allowed to decrease at night to a threshold of 650 ppm. 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 were based on CMIP3 – Coupled Model Intercomparison Project Phase 3 (Special Report on Emissions Scenarios, SRESA1B; Jylhä et al. 2009), which correspond quite well with the most recent climate change projections of CMIP5 (e.g., Representative Concentration Pathway, RCP4.5; Ruosteenoja et al. 2016).

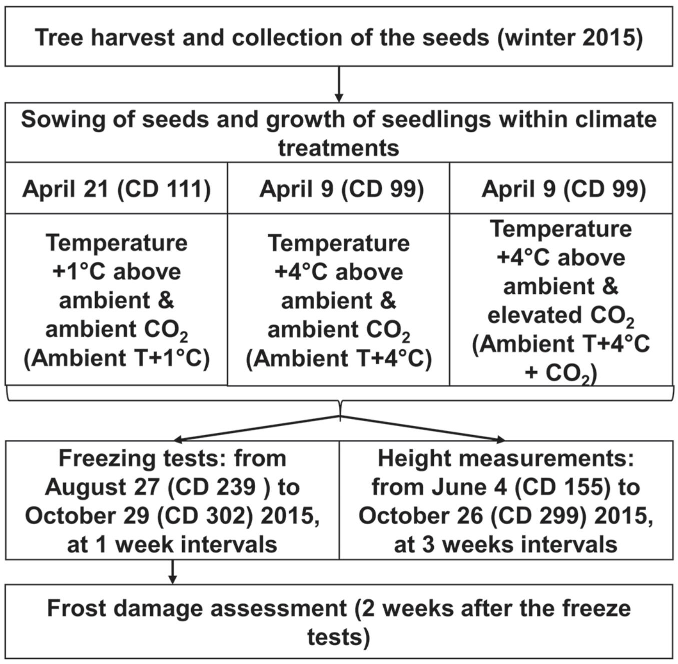

Fig. 1. Outline of experimental layout. CD – calendar day.

To simulate the prolonged growing season expected under elevated temperature, according to Ruosteenoja et al. (2011), 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). The seeds were sown in Lännen B15 boxes with 96 containers (12 containers per row × 8 containers per column), filled with pre-fertilized peat (NPK 16–4–17). Seeds representing a certain genotype were planted in one column in the box, with a random assignment of the box column to the genotype. The boxes were placed on rolling tables in random order, 30 boxes per rolling table. The general level of seed germination was 69%. Therefore, two seeds per container were sown. One randomly chosen seedling was removed in cases where both seeds germinated in the container. Because of limited seed availability, replications of the climate treatments could not be performed.

In order to minimise any effects of heterogeneity in the growing conditions on the results of the experiment, the rolling tables were rotated about themselves by 180 degrees, four times per day. In addition, the positions of the rolling tables in each growing room were swapped once a week. Irrigation was applied to the seedlings around twice a week, regardless of climate treatment. The seedlings were evenly irrigated in each box. The minimal weight of each box with seedlings was 8 kg before irrigation, while the maximal weight was 10 kg after irrigation.

2.2 Height and frost hardiness assessment

Height (mm) was measured in 20–24 seedlings per genotype, for each climate treatment, in a period from June 4 (CD 155) to October 26 (CD 299) in 2015, once every three weeks. Artificial freeze tests were used to study the development of autumn frost hardiness. In total, 10 freeze tests were performed. They started on August 27 (CD 239) and continued until October 29 (CD 302), at seven-day intervals. Initially, it was planned that 5280 seedlings would be subjected to freeze testing (8 seedlings × 10 freeze test days × 22 genotypes × 3 climate treatments = 5280 seedlings); however, due to mortality, only 5034 seedlings (95%) were exposed to freeze testing.

For the freeze tests, the boxes with seedlings were moved from the growing rooms to a 19 m × 21 m freezing room. The freezing room was equipped with four heat exchangers, located in the corners. 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 $h^{-1,}$ 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^{-1}) to the ambient temperature. The boxes with seedlings were placed on a thermo-isolated surface. Peat-filled boxes with no seedings were placed around the experimental material to limit cold air overflow around the edges, and for root system protection.

After the freezing treatment, the boxes with seedlings were returned to their respective greenhouse rooms. One person visually scored injuries to the needles, two weeks after the freeze test (Andersson 1992). Injury-scoring was based on the rate of chlorophyll breakdown, which resulted in browning of the needles. We applied an 11-point classification of the damage, which included classes from 0% (all needles intact) to 100% (all needles injured) damage, with 10% intervals.

2.3 Data analyses

The effects of the climate treatments on the mean height growth of the seedlings for each genotype was determined using a nonlinear mixed-effect model, based on the Hossfeld IV function (Zeide 1993; Mehtätalo et al. 2015):

$$H_{rit} = asym_{rit} + \frac{hmin_{rit} - asym_{rit}}{1 + \left(\frac{day_{rit}}{xmid_{rit}}\right)^{scal_{rit}}} + \varepsilon_{rit}$$

$$asym_{rit} = x'\beta_1 + a_{rit}$$

$$hmin_{rit} = x'\beta_2 + b_{rit} \qquad (1)$$

$$xmid_{rit} = x'\beta_3$$

$$scal_{rit} = x'\beta_4$$

, where H_{rit} is the mean height of seedlings during the day_{rit} for a certain genotype i under climate treatment r; $asym_{rit}$ is the final height; $hmin_{rit}$ is the height at the start of height measurement; $xmid_{rit}$ is the day that half the height was reached ($asym_{rit} – hmin_{rit}$); $scal_{rit}$ is the scaling variable that controls the width of the curve; fixed part $x'\beta$ includes the climatic treatment effect; a_{rit} and b_{rit} are random effects of $asym_{rit}$ and $hmin_{rit}$, respectively, that include the genotype effect; and ε_{rit} is the error term, with a normally distributed residual variance and zero mean. Random effects are assumed to be independent, with common bivariate normal distributions. Hypothesis tests of the fixed effects were based on the approximate conditional t-test (Pinheiro and Bates 2000).

The effects of within-genotype variations on height growth were considered in further analyses, using a nonlinear mixed-effect model. This was fitted separately for each climate treatment data subset because fitting to the complete dataset was not successful.

$$H_{ijt} = asym_{ijt} + \frac{hmin_{ijt} - asym_{ijt}}{1 + \left(\frac{day_{ijt}}{xmid_{ijt}}\right)^{scal_{ijt}}} + \varepsilon_{ijt}$$

$$asym_{ijt} = x'\beta_1 + c_{ijt} \qquad (2)$$

$$hmin_{ijt} = x'\beta_2 + d_{ijt}$$

$$xmid_{ijt} = x'\beta_3 + f_{ijt}$$

$$scal_{ijt} = x'\beta_4$$

, where j stands for seedling within genotype i; fixed part $x'\beta$ includes the genotype effect; c_{ijt}, d_{ijt}, and f_{ijt} are random effects of $asym_{ijt}$, $hmin_{ijt}$, and $xmid_{ijt}$, respectively, that include the seedling level within the level of the box. The nonlinear models were fitted using the nlme package (Pinheiro et al. 2017) for R (R Core Team 2017).

A binary logistic mixed-effect model (GLMM) was used to study the effects of climate treatment and genotype on the development of autumn frost hardiness:

$$y_{ijt} \sim Bernoulli\ (p_{ijt})$$

$$logit(p_{ijt}) = \beta_0 T0_{ijtk} + \beta_1 T1_{ijtk} + \beta_2 T2_{ijtk} + \beta_4 D_{ijtk} + \beta_5 D^2_{ijtk} + a_i^{(1)} T0_{ijtk} + a_i^{(2)} T1_{ijtk} \qquad (3)$$

$$+ a_i^{(3)} T2_{ijtk} + b_t + d_{jt}$$

, where y_{ijtk} is the number of needles damaged by frost, p_{ijt} is the probability of needle damage; $\beta_0 T0_{ijtk}$, $\beta_1 T1_{ijtk}$, and $\beta_2 T2_{ijtk}$ are fixed effects for the climate treatment, $\beta_3 D_{ijtk}$ and $\beta_4 D^2{}_{ijtk}$ are fixed effects of the freeze test day. The second order of the freeze test day term was needed to provide a satisfactory fit to the data. The random part includes the by-climate-treatment effects for genotype

a_i, and the effect of the box b_t nested by the freeze day effect d_{jt}. For analysis, we converted percent damaged needles to number of damaged needles.

For the conversion, we assumed that each seedling had the same number of needles (estimated based on eight measured seedlings, representing different heights and genotypes). For example, 10% needle damage for a seedling was converted to number of damaged needles by 0.1 × mean number of needles per seedling. We also removed calendar days 295 and 302 from the analysis because of an absence of frost damage variation. We applied the lme4 package (Bates et al. 2015) for fitting GLMM in R. Hypothesis tests of the fixed effects were based on the Wald t-test, with the Satterthwaite approximation for decrease of freedom, as implemented in the lmerTest package (Kuznetsova et al. 2016).

Based on Eq. 3, we estimated the days with 99%, 50%, and 1% of damaged needles in seedlings for each genotype and climate treatment. The duration of frost hardiness development was calculated for each climate treatment as being the difference between the days with 99% and 1% damaged needles, as averaged over all genotypes within each the climate treatment. We applied the ggplot2 package for R (Wickham 2009) for data visualization.

3 Results

3.1 Effects of climate treatment and genotype on height development

The values for the final mean height (*asym*) for Ambient T+1 °C (119 mm) differ from those of Ambient T+4 °C (146 mm) and Ambient T+4 °C + CO_2 (144 mm) (Fig. 2, Table 3). The day (*xmid*) half the mean final height was reached (*asym–hmin*) is earliest under Ambient T+4 °C + CO_2 (CD

Table 2. Parameter estimates and standard errors (SE) for final mean height (*asym*), mean height at height measurement start (*hmin*), day (*xmid*) half mean height was reached (*asym–hmin*), and scaling variable (*scal*) and random effects for final mean height (*asym*) and mean height at height measurement start (*hmin*) of height development model (Eq. 1).

Parameter	Treatment	Fixed part		Random part						
				Variance	Correlations					
		Estimate	SE		*asym* Ambient T+1 °C	*asym* Ambient T+4 °C	*asym* Ambient T+4 °C + CO_2	*hmin* Ambient T+1 °C	*hmin* Ambient T+4 °C	*hmin* Ambient T+4 °C + CO_2
asym	Ambient T+1 °C	118^a	2.22	10.202	1	0.671	0.808	0.722	0.488	0.719
	Ambient T+4 °C	147^b	3.01	17.602		1	0.841	0.396	0.898	0.822
	Ambient T+4 °C + CO_2	144^b	3.87	19.902			1	0.455	0.708	0.921
hmin	Ambient T+1 °C	29^a	1.65	1.402				1	0.5	0.639
	Ambient T+4 °C	35^b	1.96	2.402					1	0.854
	Ambient T+4 °C + CO_2	37^b	1.91	2.902						1
xmid	Ambient T+1 °C	204^a	1.1	–						
	Ambient T+4 °C	201^b	1.1	–						
	Ambient T+4 °C + CO_2	201^b	1.1	–						
scal	Ambient T+1 °C	17.60^a	1.443	–						
	Ambient T+4 °C	14.66^b	1.292	–						
	Ambient T+4 °C + CO_2	15.55^b	1.314	–						
ε_{rjt}	–	–	–	10.302						

Different letters following estimated values indicate differences among climate treatments ($p < 0.05$). Ambient T+1 °C – 1 °C above ambient and ambient CO_2, Ambient T+4 °C – 4 °C above ambient and ambient CO_2, Ambient T+4 °C + CO_2 – 4 °C above ambient and elevated CO_2.

Table 3. Parameter estimates for final mean height (*asym*), mean height at height measurement start (*hmin*), day (*xmid*) half mean height was reached (*asym–hmin*), scaling variable (*scal*), and genotypes (Gtype) of by-climate-treatment height development model (Eq. 2).

Gtype	Origin	Ambient T+1 °C				Ambient T+4 °C				Ambient T+4 °C + CO_2			
		asym	*hmin*	*xmid*	*scal*	*asym*	*hmin*	*xmid*	*scal*	*asym*	*hmin*	*xmid*	*scal*
V455	F × S	111	31	202	18.38	159	38	199	14.98	142	35	195	14.20
V456	F × S	107	31	205	18.86	147	32	197	14.94	145	33	197	14.28
V447	F × G1	134	28	202	16.39	170	37	199	14.86	178	35	197	13.46
V448	F × G1	119	29	203	16.61	141	37	198	15.82	130	35	197	15.00
V449	F × G1	117	27	201	17.43	146	36	199	15.32	139	34	194	14.03
V49	F × G2	128	27	205	16.97	178	37	200	14.50	159	33	196	13.34
V302	F × G2	136	30	203	17.92	175	35	197	14.17	184	37	197	13.63
V381	F × G3	123	30	203	19.27	131	35	196	15.98	127	32	199	14.18
V382	F × G3	122	33	203	17.98	154	39	197	14.70	130	38	196	14.00
V383	F × G3	132	32	204	18.54	124	34	203	17.47	128	34	200	14.66
V469	F × L	121	27	205	16.40	136	33	202	14.39	132	32	197	14.31
V470	F × L	102	25	206	18.02	127	28	201	15.78	105	27	199	16.00
V325	R	102	28	204	17.18	127	35	202	15.99	117	34	195	15.86
V327	R	110	24	201	18.55	134	31	198	15.02	134	32	194	16.16
V386	F × E	133	31	203	18.42	161	36	199	14.71	174	38	197	13.85
V388	F × E	112	28	201	18.38	150	36	198	14.39	151	35	196	14.63
V389	F × E	119	29	205	17.91	142	34	200	14.66	155	37	200	14.85
V47	F	124	27	204	18.17	155	33	202	15.37	170	31	196	13.32
V332	F	101	30	204	18.87	111	34	196	16.59	120	34	194	14.71
V43	F	128	29	203	16.02	140	37	205	16.46	138	34	198	14.22
V48	F	112	30	201	18.97	148	39	198	16.04	144	39	195	14.94
V465	F	124	28	205	17.07	159	37	200	14.54	162	36	195	14.12
All genotypes		119	29	203	17.83	146	35	199	15.30	144	34	196	14.44

Mother tree origins are Finland × Switzerland (F × S), Finland × Germany (F × G), Finland × Latvia (F × L), Finland × Estonia (F × E), Russia (R), and Finland (F). Numbers 1, 2, and 3 for F × G mark different crosses for mother trees. Ambient T+1 °C – 1 °C above ambient and ambient CO_2, Ambient T+4 °C – 4 °C above ambient and ambient CO_2, Ambient T+4 °C + CO_2 – 4 °C above ambient and elevated CO_2.

196), followed by Ambient +4 °C (CD 199), and Ambient +1 °C (CD 203) (Table 3). For *asym* and *xmid*, the climate treatments, except for Ambient T+4 °C and Ambient T+4 °C + CO_2, differed from each other significantly ($p < 0.05$) (Table 2).

Variations in final height among the genotypes (Table 2, Fig. 3) was the highest under Ambient T+4 °C + CO_2, followed by Ambient T+4 °C, then Ambient T+1 °C. Relatively high correlations between final height and climate treatment (Table 2) 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 (Fig. 3).

3.2 Effects of climate treatment and genotype on frost hardiness development

Climate treatment significantly ($p < 0.05$) affected the probability of frost damage (Table 4). Seedlings grown under Ambient T+1 °C developed frost hardiness the earliest, followed by Ambient T+4 °C, and then Ambient T+4 °C + CO_2 (Fig. 2, Table 4). This was opposite to the duration of frost hardiness development, 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 (Supplementary file S2, available at https://doi.org/10.14214/sf.9980). Frost damage probability differed significantly ($p < 0.05$) between climate treatments, except for Ambient T+4 °C and Ambient T+4 °C + CO_2 (Table 4).

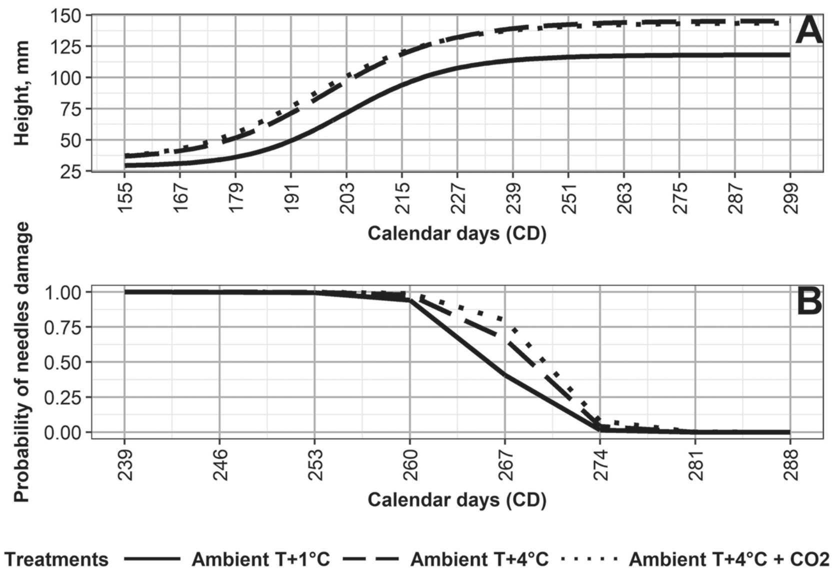

Fig. 2. **(A)** Height development of Norway spruce seedlings, and **(B)** probability of autumn frost damage in needles of Norway spruce seedlings, as averaged by the following climate treatments: 1 °C above ambient and ambient CO_2 (Ambient T+1 °C), 4° C above ambient and ambient CO_2 (Ambient T+4 °C), and 4 °C above ambient and elevated CO_2 (Ambient T+4 °C + CO_2).

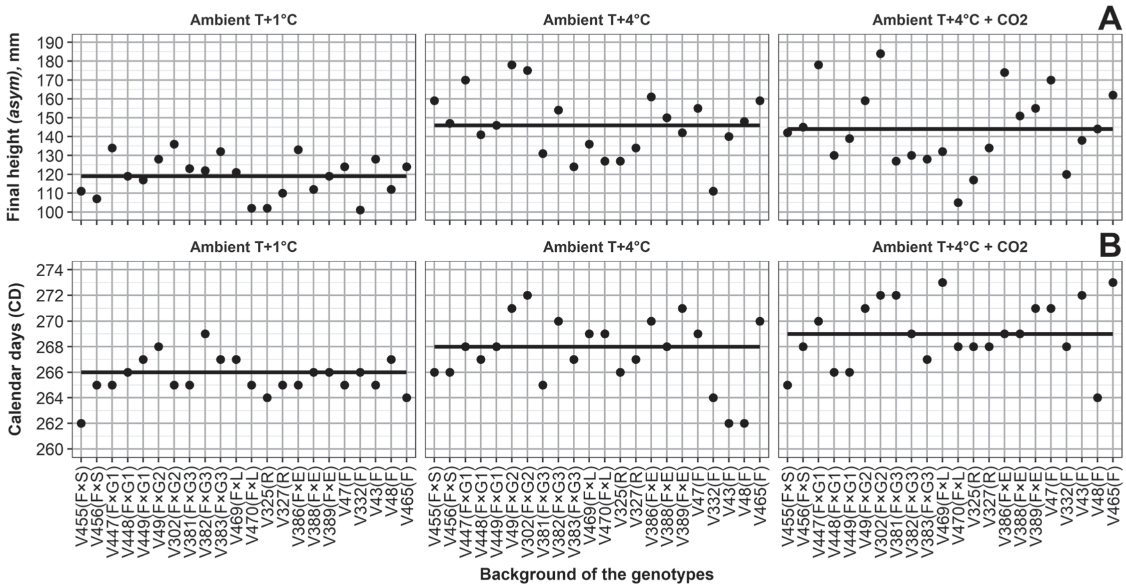

Fig. 3. **(A)** Final heights for Norway spruce genotypes, and by-climate-treatment mean final heights (solid line). **(B)** Calendar days of 50% frost damage probability in seedling needles for Norway spruce genotypes, and by-climate-treatment mean calendar days of 50% frost damage probability (solid line). Mother tree origins are Finland × Switzerland (F × S), Finland × Germany (F × G), Finland × Latvia (F × L), Finland × Estonia (F × E), Russia (R), and Finland (F). Numbers 1, 2 and 3 for F × G mark different crosses for mother trees. Climate treatments are Ambient T+1 °C – 1 °C above ambient and ambient CO_2, Ambient T+4 °C – 4 °C above ambient and ambient CO_2, Ambient T+4 °C + CO_2 – 4 °C above ambient and elevated CO_2.

Table 4. Parameter estimates, standard errors (SE), and random effects of the frost hardiness development model (Eq. 3).

Parameters	Fixed part			Random part				
				Parameters	Estimates	Correlations		
	Estimate	SE	p-values			Ambient T+1 °C	Ambient T+4 °C	Ambient T+4 °C + CO_2
Ambient T+1 °C; β_0	8.2463184[a]	0.4665176	< 0.001	variance ($a_i^{(1)}$)	0.72219^2	1	0.15	–0.02
Ambient T+4 °C; $\beta_0 + \beta_1$	9.3113819[b]	0.4869418	< 0.001	variance ($a_i^{(2)}$)	1.29778^2		1	0.38
Ambient T+4 °C + CO_2; $\beta_0 + \beta_2$	10.006337[b]	0.5008949	< 0.001	variance ($a_i^{(3)}$)	1.26624^2			1
Day; β_3	–0.1186385	0.0366558	–	variance (d_{jt})	0.01072^2			
Day^2; β_4	–0.0067618	0.0008185	< 0.001	–	–			
–	–	–		Box; variance (b_t)	1.67323^2			

Different letters following estimated values indicate differences among climate treatments ($p < 0.05$). Ambient T+1 °C – 1 °C above ambient and ambient CO_2, Ambient T+4 °C – 4 °C above ambient and ambient CO_2, Ambient T+4 °C + CO_2 – 4 °C above ambient and elevated CO_2.

Variation in frost hardiness development between genotypes (Table 4, Fig. 3) was the highest under Ambient T+4 °C and Ambient T+4 °C + CO_2, followed by Ambient T+1 °C; however, variation in the probability of frost damage between the boxes with seedlings was higher than the variation caused by any climate treatment. Correlations between the climate treatments ranged from –0.02 between Ambient T+1 °C and Ambient T+4 °C + CO_2 to 0.38 between Ambient T+4 °C and Ambient T+4 °C + CO_2 (Table 4). This shows that, in terms of frost hardiness development, the genotypes perform quite differently among the climate treatments. 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 (Fig. 3).

4 Discussion

4.1 Effects of climate treatment on height development

We hypothesized that changing environmental conditions, such as elevated temperature and atmospheric CO_2 concentration, together with genotype, affected the development of height growth and autumn frost hardiness in one-year-old seedlings of Norway spruce. We also hypothesized that some genotypes had both superior height growth and earlier autumn frost hardiness compared to others. In line with our first hypothesis, a higher growth rate (*xmid*) and mean final height (*asym*) was observed for seedlings of different genotypes, under Ambient T+4 °C and Ambient T+4 °C + CO_2, compared to Ambient T+1 °C (Table 3). A similar kind of response in height growth to elevated temperature was observed in one-year-old Sitka spruce (*Picea sitchensis* (Bong.) Carrière) and white spruce (*Picea glauca* (Moench) Voss) by Brix (1972), for example. On the other hand, in our study, differences in the sowing time between climate treatments might have affected the length of growing period under different climate treatments, and so the final height of the seedlings. The free height growth pattern of the one-year-old seedlings may have also contributed to differences observed in the height growth. Contrary to our first hypothesis, we found no significant effect ($p > 0.05$) of elevated atmospheric CO_2 concentration either on height growth rate or final height of the seedlings, regardless of genotype (Table 2).

4.2 Effects of climate treatment on frost hardiness development

Our results show that the development of autumn frost hardiness was delayed by 5–7 days in seedlings of different genotypes under Ambient T+4 °C and Ambient T+4 °C + CO_2, compared to Ambient T+1 °C (Suppl. file S2). This is in line with earlier findings, e.g., by Chang et al. (2016), who found impairment in autumn frost hardening in the three-year-old seedlings of the eastern white pine (*Pinus strobus* L.) that were grown under an elevated temperature. In our 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. Thus, elevated temperature shortened the period of frost hardiness development, regardless of the applied CO_2 concentration. On the other hand, the temperature may have simultaneously prolonged free shoot growth in the seedlings and, thus, indirectly affected the development of autumn frost hardness. In previous studies, a strong relationship has been observed between autumn frost hardiness development and bud-set (i.e. growth cessation) in Norway spruce seedlings from an identified seed source (provenance genetic level) (Johnsen and Apeland 1988). On the other hand, a weaker or nonexistent relationship has been observed in Norway spruce seedlings of half-sib and full-sib families (Johnsen and Apeland 1988; Skrøppa 1991).

We found no significant ($p > 0.05$) effect of elevated atmospheric CO_2 concentration on frost hardiness development of Norway spruce seedlings of different genotypes when comparing the performance of seedlings under Ambient T+4 °C and Ambient T+4 °C + CO_2. In a previous study by Dalen et al. (2001), elevated CO_2 concentration was found not to affect frost hardiness development in one-year-old seedlings of Norway spruce, and nor was it found to affect the onset of bud dormancy in young seedlings of Sitka spruce in another study (Murray et al. 1994). On the other hand, Bigras and Bertrand (2006) 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. Contradictorily, Chang et al. (2016) found that an elevated atmospheric CO_2 concentration delayed the development of autumn frost hardiness in eastern white pine seedlings. In such previous studies, however, different experimental setups and methodologies of frost hardiness assessment were applied, which makes it difficult to compare the results between the different studies.

4.3 Effects of genotype on height and frost hardiness development

The elevation of temperature increased variability in the development of height growth and frost hardiness among and within 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 in our study, regardless of climate treatment; however, the greater height growth of these genotypes could not be explained by geographical origin of the mother trees (data not presented).

In this study, a rather low consistency in the development of autumn frost hardiness was found across the climate treatments among the half-sib genotypes. This could imply that relatively high genetic variation exists in the genotypes. In addition, relatively high variability between the boxes with seedlings was observed (Table 4, Suppl. file S1); however, it was not possible to separate the effects of genetic factors and environmental conditions on frost hardiness development within the genotypes because of their half-sib origins. Based on our study, the selection of genotypes with early frost hardiness for projected future climate requires cloned or full-sib seedling material to reduce uncontrolled genetic variation within the genotypes, and to separate the genetic and environmental components of the variation.

Similarly to height growth, the development of autumn frost hardiness of the tested half-sib genotypes could not be explained by geographical origin of the mother trees (data not presented). In a previous study by Hannerz and Westin (2000), the development of autumn frost hardiness in Norway spruce seedlings from an identified seed source (provenance genetic level) was affected by genotype origin. Thus, the genetic level (provenance, half-sib, and full-sib) of the tested seedling material may undermine expected geographical trends in the development of height growth and autumn frost hardiness.

5 Conclusions

The climate treatments applied in this study affected the height growth rate and final height of young Norway spruce seedlings, regardless of genotype. Elevated temperature delayed the onset, and shortened the duration, of autumn frost hardiness development. We found no effects of elevated atmospheric CO_2 concentration on the development of height or autumn frost hardiness. Higher temperature also increased variation in height and frost hardiness development among genotypes. Some genotypes showed superior height growth, regardless of climate treatment, but they did not show earlier autumn frost hardiness development. We could not separate the effects of genetic factors and environmental conditions on height and frost hardiness development within the genotypes because of their half-sib origin. In future studies, the clonal or full-sib genetic material should be used to study detailed differences between genotypes in the simultaneous development of height and autumn frost hardiness. Factorial experiments, with a higher number of replicates for each genotype, are also needed for such studies.

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Total of 33 references.

Supplementary files

S1: Random effects for final mean height (*asym*), mean height at start of height measurement (*hmin*), day (*xmid*) half mean height was reached (*asym–hmin*) in by-climate-treatment height development model (Eq. 2),
S2: Calendar days (CD) of 99, 50, and 1% seedling needle frost damage (NFD) for different gen-

otypes (Gtype) and climate treatments. Duration of frost hardiness development averaged for climatic treatments,

available at https://doi.org/10.14214/sf.9980.