

# Effects of light exposure in freezing temperatures on winter damage to foliage of Norway spruce container seedlings in mid and late winter: Pilot experiments in open field

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**ABSTRACT:** Norway spruce (*Picea abies* [L.] Karst.) is widely planted for reforestation in the boreal zone. It is sensitive to frost and high irradiance during the growing season, and also to winter damage to foliage, which cause growth losses in reforestation. This study made a pilot attempt to examine the needle damage and seedling vigour on hardened Norway spruce seedlings under freezing temperatures (0 to –20°C) using natural and artificial light exposure from a day to weeks in an open field in mid and late winter in central Finland. The treatments induced needle browning and decreased seedling vigour, which reduced shoot and root growth during the following growing season. Visibly damaged, mottled needles of one-year terminal shoots had practically no healthy-looking cells. The new buds, however, were healthy and were able to grow during the following season. Our results suggest that, above the snow cover, other environmental factors, e.g. freezing temperatures and wind, rather than radiation intensity induced the observed needle damage found immediately after the treatments, and the subsequent growth reduction in the following growing season. The preliminary methods used outdoors in winter could not distinguish all the different environmental conditions and their mechanisms of effects on seedlings, which demonstrates the need for further method development in controlling experimental conditions of air temperature, radiation intensity, and air current in future research of seedling winter damage outdoors.

**Keywords:** cell damage; frost; irradiance; needle colour; *Picea abies*

In the boreal zone, Norway spruce (*Picea abies* [L.] Karst.) is widely planted for reforestation (GROSSNICKLE 2000). In Finland, for example, Norway spruce is planted on more than 70% of the annual Finnish outplanting area (YLITALO 2013). Norway spruce is a shade-tolerant, climax species, which is sensitive to frost during the growing season. In winter, frost and high irradiance may lead to damage and desiccation of foliage (LEIKOLA, RIKALA 1983; ÖRLANDER 1993; GROSSNICKLE 2000; LANGVALL et al. 2001).

Low-temperature photoinhibition in conifer needles, by exposure to high irradiance, usually leads to reversible needle yellowing (CHRISTERSSON, VON

FIRCKS 1988; BALL 1994; GERMINO, SMITH 1999; GROSSNICKLE 2000; ROBAKOWSKI 2005). Photoinhibition caused by high irradiance and frozen soil seems to be associated with a decreased number of thylakoids in the grana stacks, decreased concentration of chlorophylls, and lower chlorophyll/carotenoid ratio, but usually these phenomena recover in the following growing season (ÖQUIST 1986; SUTINEN et al. 1990; SUTINEN et al. 2000).

In winter, freezing temperatures, especially with dry, windy air, can lead to direct frost desiccation and needle browning above the snow surface at the time when the roots cannot provide enough water from the frozen soil to replace the water lost by the foliage

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(e.g. RUMMUKAINEN, VOIPIO 1981; TRANQUILLINI 1982; GROSSNICKLE 2000). Rapid changes in air temperature may also cause freezing injuries in tissues, thus increasing water loss further (CHRISTERSSON, VON FIRCKS 1988; BALL 1994; GERMINO, SMITH 1999; GROSSNICKLE 2000; ROBAKOWSKI 2005). Several combinations of winter stresses may result in varying types of seedling winter damage, which may be irreversible, like in the case of needle browning, and lead even to death in early spring (HADLEY et al. 1991; KRASOWSKI et al. 1996; GROSSNICKLE 2000).

Outplanted seedlings, whose foliage has been damaged by winter desiccation, can grow from new buds that usually remain unharmed (RUMMUKAINEN, VOIPIO 1981). However, if more than 50% of the needles of seedlings are damaged (browned) at the tree nursery, the planting success of seedlings has been observed to weaken markedly (TERVO, KAUTTO 1999). Winter damage of foliage can reduce the growth of outplanted seedlings at least during the first post-injury growing season (KRASOWSKI et al. 1996; LANGVALL et al. 2001).

Winter damage in Norway spruce seedlings is a common problem in reforestation, as it results in growth losses. Occurrence and symptoms of winter foliage damage are easily seen in forest tree seedlings, but the combined mechanisms of the damage and necessary silvicultural control measures are not fully known. It is not entirely understood how the timing, length and intensity of light together with freezing temperatures affect the viability and winter desiccation damage of outplanted conifer seedlings. The aim of this work was to study the effects of the intensity and duration of light radiation exposure on winter damage in hardened Norway spruce seedlings under freezing temperatures in an open field in mid and late winter.

## MATERIAL AND METHODS

**Study site and seedling material.** All the experiments were conducted on a site at an operational forest nursery in Suonenjoki, central Finland (62°64'N, 27°05'E). The site was a flat, north-facing plot at 140 m a.s.l. where a nearby building wall on the southern side provided some weather shore.

For all experiments, short-day treated, second-year Norway spruce seedlings were grown in PL64F containers using local seed from a seed orchard (Lannen Plant Systems, BCC Oy, Säkylä, Finland) (HEISKANEN 2013). All the seedlings were placed in plastic bags in the autumn and stored in a freezer (−3, ..., −4°C) until the next spring.

**Preliminary experiment.** For a preliminary study, a total of 48 uniform seedlings were randomly selected in September 2011 (mean height = 30.5 cm, SD = 8.2). The seedlings were transplanted into 2-litre pots filled with a mixture of 25 vol.% of light Sphagnum peat and 75 vol.% of coarse sand (containing 24.7 mass-% of particle size < 0.6 mm). The mixture had a mean bulk density of 1.195 g·cm<sup>−3</sup> and total porosity of 54.3% (for determinations in detail, DUMROESE et al. 2011; HEISKANEN 2013).

One half of the seedling pots were irrigated fully before the winter storage and the other half so that 45% of the total porosity became filled with water (i.e. to air-filled porosity of 24.4%). At the end of February 2012, two thirds of the seedling pots were moved from winter storage and installed by groups to an open field at the nursery, where the pots were assigned to a randomized block design to reduce possible variability in field conditions. The treatments used for the pots were: (i) irrigation before winter storage (full or half), (ii) winter storage time (until March or May), and (iii) height position in the snowy field (root collar at a height of 12 or 24 cm from the soil surface) (Table 1). Seedlings that were stored until May 2012 had a height position of 12 cm only (i.e. pots were on the soil surface). There were 6 treatments in all. In each of the 8 blocks used, there was one seedling from each of the 6 treatments, making a total of 48 seedlings. This experiment attempted to monitor a situation in mid and late winter when seedlings have suddenly emerged from the snow cover and are then exposed to lingering (for several weeks) freezing temperatures.

Minimum and maximum air temperatures in the field were −16 and 5°C, respectively, from 28<sup>th</sup> February to 9<sup>th</sup> April, when the freezing temperatures ended (data not shown). The seedlings in the field were kept manually covered with snow, 3 cm above the root collar, until the bare soil started to be revealed in spring. In mid-April, the pots that were still in the freezer storage, were allowed to warm

Table 1. Treatments and number of seedlings in the preliminary experiment

Treatment	<i>n</i>	Autumn watering (W)	Winter storage time (S)	Field position (K)
S+W+K−	8	yes +	until May +	lower
S+W−K−	8	no −	until May +	lower
S−W+K+	8	yes +	until March −	higher
S−W−K+	8	no −	until March −	higher
S−W+K−	8	yes +	until March −	lower
S−W−K−	8	no −	until March −	lower

up to the ambient temperature of about 0°C on 25<sup>th</sup> April. Then they were moved to a temperature of 5°C before being moved to the field on 2<sup>nd</sup> May, after which the diurnal air temperatures were above 0°C (data not shown). Seedlings in the field were irrigated once or twice a week, and monitored for growth and vigour until visible bud set.

**Experiment 1.** A total of 204 uniform seedlings were randomly selected for the experiment, like in the preliminary experiment (mean seedling height = 25.3 cm, SD = 3.8). In September 2012, the selected seedlings were loaded into small 3 by 4 row containers (12 cells) that had been cut out of PL81F-containers, and were stored in a freezer like in the preliminary experiment.

The first set of 4 small containers (each with 12 seedlings) was placed in an open field at the forest nursery in the afternoon on 19<sup>th</sup> February 2013, preceding the light exposure experiment the next day. The containers were covered with snow up to the root collar. This experiment attempted to monitor a situation within a day or two in mid and late winter, when seedlings have emerged from the snow cover and are then exposed to freezing temperatures.

The next morning, each set of container seedlings that had been brought onto the field the preceding afternoon, was placed on a slowly rotating plate (0.4–0.5 rpm). This was done in order to minimize light treatment differences owing to the spatial position of the seedlings. Ambient natural light was supplemented either with a higher artificial light (light source Bp 1.2-HMV, Ludvig Pani Ltd, Austria, see SMOLANDER et al. 1987; PELTONIEMI et al. 2005) at a 30 degree angle, or with a lower light source (two Philips metal halide lamps, HPI-T 400W, Amsterdam, The Netherlands) from above at a 90 degree angle onto the plate. The shaft of the higher light intensity was restricted and this limited the number of seedlings that could be used in the experiment. Light transmission measurements were taken at the seedling shoot level using a quantum sensor (LI-185B, LI-COR, Lincoln, NE, USA). An air fan with low resultant pressure was used to remove possible extra heat from the seedling shoots.

On 20<sup>th</sup> February, the first seedling set was exposed to light at 07:30 and kept on for 7 h until 14:30. Full light yielded the mean photosynthetically active radiation of either about 800 or 1,400  $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  at the seedling shoot level. Because of the date and the north-facing experiment site, little direct sun light was received (Fig. 1). Air temperature was below 0°C at the screen height (Fig. 1) as well as at the seedling shoot level (data not shown) during the light exposure experiments.

Two subsequent light exposure runs (each with 48 seedlings) were made on consecutive days with both light intensity levels. Therefore, 192 seedlings in all were exposed to the light treatments (8 time occasions from 0 to 7 h  $\times$  6 seedlings  $\times$  2 light intensities  $\times$  2 runs). Six random seedlings were sampled hourly from the containers, starting at 07:30 in each run. Of these removed seedlings, some (128 in all) were first thawed at 5°C in the dark for a couple of days, and then for a few days in shaded natural light, before being moved into a greenhouse, where they were grown until visible bud set. Extra seedlings from 5 small containers (60 seedlings) were also thawed directly from the winter storage without any treatments outside (0-treatment), and were

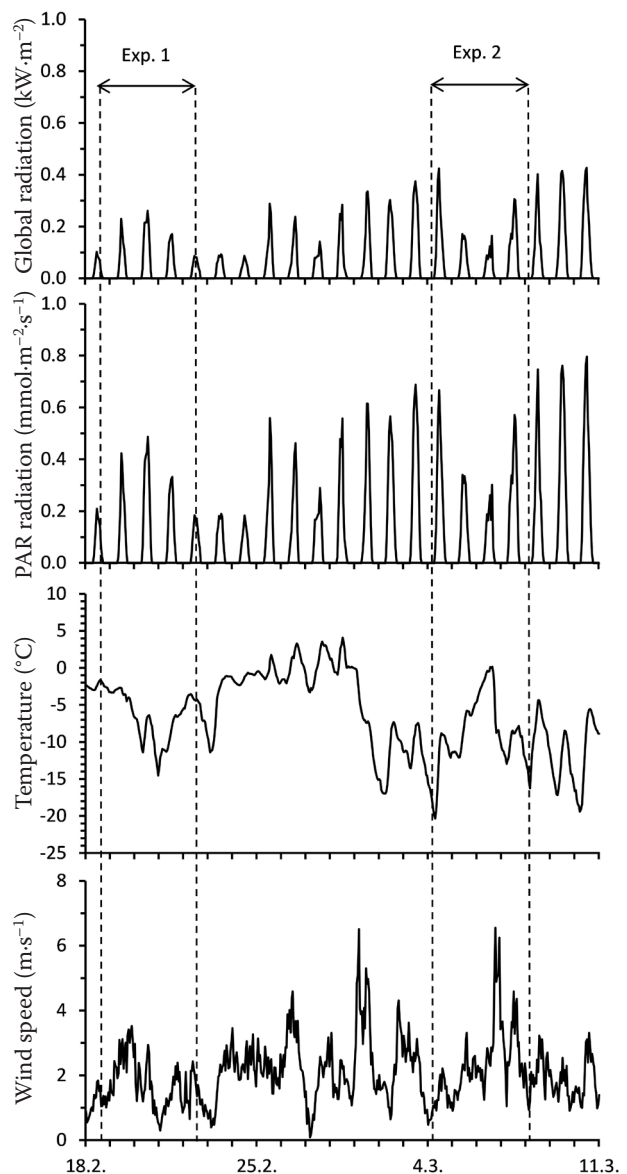


Fig. 1. Ambient weather conditions during winter desiccation experiments 1 and 2 from the adjacent weather station at a height of 2 m (date on the x-axis given as day.month. in the year 2013)

Table 2. Light level (PAR) and duration (in days) treatments and number of seedlings (*n*) in experiment 2

Treatment	Duration	<i>n</i>	Treatment	Duration	<i>n</i>
1,400 PAR	1 d	4	800 PAR	1 d	30
1,400 PAR	2 d	4	800 PAR	2 d	30
1,400 PAR	3 d	4	800 PAR	3 d	30
1,400 PAR	4 d	4	800 PAR	4 d	30
–	–	–	800 PAR	7 d	30
1,400 Ctrl	1 d	4	800 Ctrl	1 d	30
1,400 Ctrl	2 d	4	800 Ctrl	2 d	30
1,400 Ctrl	3 d	4	800 Ctrl	3 d	30
1,400 Ctrl	4 d	4	800 Ctrl	4 d	30
–	–	–	800 Ctrl	7 d	30

800, 1,400 PAR – supplied light intensity level in  $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ; Ctrl – parallel treatment with no exposure to artificial light; additional seedlings were also treated for tissue samples to microscopy (in all 64 for 1,400 PAR and 1400 Ctrl and in all 60 for 800 PAR and 800 Ctrl)

grown simultaneously with the treated seedlings in the greenhouse for comparison. During the growing time in the greenhouse, the mean temperature was between 15 and 25°C (night-day), relative humidity 45–80% (day-night) and light  $260 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  for 12 hours a day, about 50 cm above the seedling shoot level.

In addition, 2 random seedlings from the 6 removed seedlings (at each hour of each run) were taken for tissue samples. From these 2 seedlings, the terminal shoot of the first seedling was immediately sampled for microscopic analysis. The terminal shoot of the second seedling was sampled after 5 days in the greenhouse. Thus, 16 seedlings were taken in all for tissue samples to microscopy at each run (8 h  $\times$  2 seedlings). In addition, 12 extra seedlings were thawed from the freezer storage without any treatment (0-treatment) and used for control tissue samples (76 tissue samples in all).

**Experiment 2.** A total of 456 uniform seedlings were randomly selected and stored over winter, like in the previous experiments (mean seedling height = 22.6 cm, SD = 1.4). On 4<sup>th</sup> March 2013, 8 random small containers were moved from the freezer to the open field and covered with snow. The next morning, 4 containers were placed onto the rotating plate and under the higher light intensity (1,400  $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ), similarly to Experiment 1 (Table 2). Light was turned on at 07:30 and blacked out at 14:30. The other 4 containers were left nearby as a control treatment without artificial light. From each of the 8 containers, 3 random seedlings were removed on 4 consecutive afternoons at 14:30. Of these, 2 seedlings were taken for tissue sampling,

one immediately and the other one after growing for 5 days in the greenhouse, like in Experiment 1 (64 samples in all). So, 4 seedlings from each container were left growing in the greenhouse until bud set (32 in all).

In parallel, a total of 360 seedlings in 30 small containers were also moved to the open field and covered with snow up to the root collar (mean seedling height = 26.4 cm, SD = 1.8). Half of these (15 containers) were left as a control treatment without artificial light. The other half was exposed to the lower light intensity from 07:30 to 14:30, using 4 lamps from above (800  $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  at seedling shoot level). After the 4th day, no artificial light was supplied (Table 2). The position of the containers was changed daily to minimize spatial dependence. This experiment attempted to monitor a situation within a week in mid and late winter when seedlings have emerged from the snow cover and are then exposed to freezing temperatures.

After days 1, 2, 3, 4, and 7, at 14:30, 3 small containers (36 seedlings) from both the light exposure and control treatments were removed for thawing at 5°C, and put under shaded natural light (room with uncovered side windows) for a week. From each removed container, one seedling was taken for microscopic analysis immediately before thawing and the other after growing for 5 days in the greenhouse, like in Experiment 1 (60 seedling samples in all). The other 300 seedlings were left in the greenhouse for growing and measurements, like in Experiment 1. In addition, extra seedlings (the same batch as that in Exp. 2) were taken from 5 small containers (60 seedlings) and were thawed directly from the winter storage without any treatment outside and were grown simultaneously with the treated seedlings in the greenhouse for comparison (0-treatment).

**Seedling measurements.** After the exposure experiments, the seedlings were grown until visible bud set in the terminal leader shoot and were then measured for growth, vigour, and needle colour. Needle colour for green and brown hues was assessed separately for the lower, middle, and top parts of the current-year shoot (HEISKANEN 2005). Seedling vigour was rated in categories according to growth and visually determined needle colour as follows; 1 high vigour, 2 slightly weakened (some chlorosis), 3 weakened (chlorotic needles), 4 very weak (very chlorotic or brown needles) and 5 dead. Any seedling in vigour categories 1 or 2 was counted as vigorous. After bud set, seedlings were harvested and measured for dry masses of different shoot compartments (old and new stems and nee-



dles). In the preliminary experiment, root egress into the sand-peat mixture outside the peat plug was also measured.

For microscopic analysis, 2 cm of the top of the sample seedlings were taken after the treatments in Experiments 1 and 2. Immediately after cutting, the seedling tops were put to test tubes containing fixative solutions (2% glutaraldehyde in cacodylate buffer, 0.07 M, pH 7.0). Cross-sectional samples (size 0.5–1 mm) of five different needles per seedling were cut within two days at 3 mm from the needle tip, in a drop of the fixative solution, and subsequently prepared as described previously (SOIKKELI 1978; SUTINEN et al. 1990). The cross-sections (1.5  $\mu\text{m}$  thick) of each sample were cut using LKB 2128 Ultratome (Bromma, Sweden), double-stained with 1% toluidine blue and 1% p-phenylene diamine (KIVIMÄENPÄÄ et al. 2004), and digitally photographed (Leica CD Camera, Heerbrugg, Switzerland) under a light microscope (Leica DM2500, Germany). The cross-sectional area of needle mesophyll, the intercellular area, and the areas of healthy and differently damaged cells were measured using Adobe Photoshop software (version CS6).

The terminal buds were fresh-cut longitudinally to two halves. In order to analyse the condition of the buds, the outer surface and the inner parts of the longitudinally cut buds were investigated under a stereomicroscope (Wild, Heerbrugg, Switzerland).

**Data analysis.** In the preliminary experiment, the nonparametric Friedman's test was used to analyse overall and pairwise differences between the treatment levels in the randomised block design. The tested seedling variables were growth of terminal shoot, dry mass of new shoot, dry mass of outgrown roots, needle colour and seedling vigour (the last two are on an ordinal scale). A nonparametric test was applied because of the simplicity of the experimental layout, and the low number of replicates and deviations from normal distribution of the tested variables.

In Experiment 1, the Mann-Whitney U test and analysis of covariance were used at a time level of 0 hours to assess whether conditions and handling were similar for both light treatments (800/1,400  $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) in all the test runs. In Experiments 1 and 2, the nonparametric Kruskal-Wallis test, analysis of covariance or two-way analysis of variance were used to analyse overall and pairwise differences between the light treatment levels (0-treatment, 800 and 1,400  $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ), and between the light exposure time levels (0-treatment as a control). The Kruskal-Wallis test was used for

the proportion of browned needles, due to deviations from normal distribution. Analysis of covariance was used for the growth of terminal shoots and growth of diameter, where the initial height (and/or initial diameter) was used as a covariate. Different seedlings were used at each time level for light treatments 800 and 1,400  $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , so all seedling observations were independent of each other. Because there was no time level for 0-treatment, the effect of the light treatment factor and the light exposure time factor were analysed separately. Because there was no 0-treatment for the areas of mesophyll, extracellular space and healthy cells in needles, two-way analysis of variance was used. The data analyses were carried out using IBM SPSS Statistics software, version 20.

## RESULTS

In the preliminary experiment, all the seedlings that were in the winter storage until May before moving to the field (treatments S+W+K- and S+W-K-) were in high vigour at seedling harvest in June, while the seedlings that were moved from the winter storage to the field at the end of February were all weakened or very weak at harvest. However, vigour for treatment S-W+K+ did not differ from these two treatments (Friedman  $P > 0.05$ ). In addition, the dominant foliar colour of old terminal shoots was green in the seedlings that were moved to the field in May, while the colour was brown (dried out) or needles were fallen in the other seedlings although treatments S-W+K+ and S-W-K- did not differ from these two treatments (Friedman  $P > 0.05$ ). Correspondingly, new shoot growth and root egress from the peat plug into the surrounding soil were greater in the seedlings moved to the field in May (Fig. 2), although Friedman's pairwise comparisons showed no difference between the S+W+K- treatment and other treatments (the test could not be performed for outgrown roots because of too many zero or missing values).

In Experiment 1, the proportion of browned needles in the top part of the shoot (Mann-Whitney U test  $P = 1.00$ ), as well as shoot and diameter growth (covariance analysis  $P > 0.27$ ) of the seedlings grown in the greenhouse, did not differ between light intensity levels at time 0 hours, which suggests similar conditions and handlings for both light intensity levels in all the test runs. Terminal shoot growth in the greenhouse did not differ along with the light exposure hours per day in the field (covariance analysis  $P = 0.09$ ). Needle browning tended to decrease along with the light

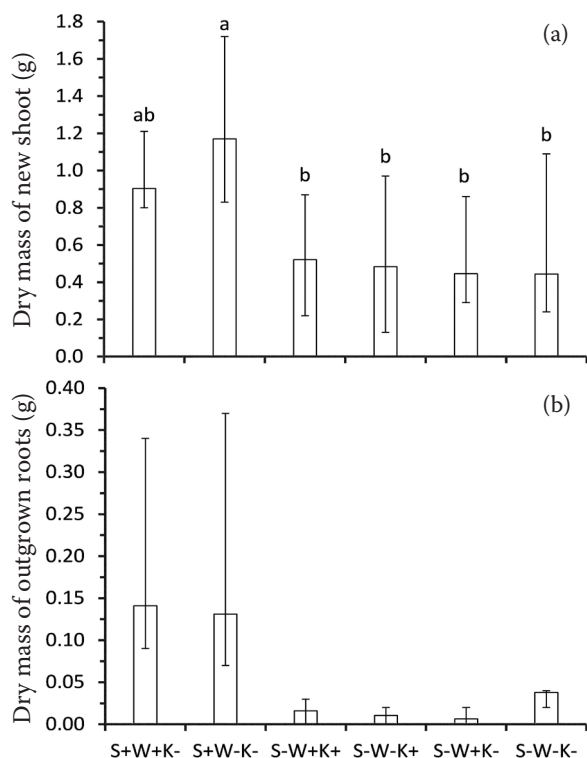


Fig. 2. Dry masses of new shoots and outgrown roots in the different treatments at harvest in the preliminary experiment (median and range,  $n = 8$  seedlings per treatment); different letters above the bars show significant pairwise differences ( $P < 0.05$ ) in Friedman's test (the test could not be performed for roots due to too many zero or missing values); S – winter storage time, W – watering before storage, K – height position in the field, - and + indicate lower or higher treatment level, respectively

exposure hours in the field but there were no significant differences (Kruskal-Wallis  $P = 0.82$ ). However, 0-treated seedlings grew slightly better in the greenhouse than seedlings treated with  $800 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  light in the field but as well as seedlings treated with  $1,400 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  light (covariance analysis  $P = 0.01$ ) (Fig. 3). The proportion of browned needles in the top part of the shoot at the end of the greenhouse grow-

ing was also significantly lower in the 0-treatment (i.e. not exposed to field conditions outside) compared to the light-treated seedlings (hours combined, Kruskal-Wallis  $P < 0.001$ ).

In Experiment 2, terminal shoot growth or diameter growth in the greenhouse did not differ between 0-treated and light treated seedlings in the field (covariance analysis  $P > 0.05$ ) (Fig. 4). However, average terminal shoot growth in the greenhouse was significantly lower at day 7 (covariance analysis  $P < 0.001$ ). Instead, the proportion of browned needles in the top part of the shoot at the end of greenhouse growing was lower in 0-treated seedlings (Kruskal-Wallis  $P < 0.001$ ). 0-treated seedlings also had the least browned needles, and seedlings in the field at day 7 had the most (Kruskal-Wallis  $P < 0.001$ ) (Fig. 4).

No healthy cells were seen in the damaged, browned (mottled) needles of one-year-old terminal shoots at the end of the greenhouse growing that followed the light exposure experiments. Furthermore, no difference in needle anatomy was found between the treatments. For example, before greenhouse growing in Experiment 2, areas of mesophyll, extracellular space and healthy cells for needles in terminal shoots showed no significant differences by covariance analysis through four days with or without  $1,400 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  light supplement (Figs 5 and 6). The studied buds were always healthy looking.

## DISCUSSION

The results showed that exposing shoots of Norway spruce seedlings to freezing temperatures without snow cover in mid and late winter for about a week can induce seedling damage, first in old needles (needle browning) later in spring, and subsequently as decreased seedling vigour and

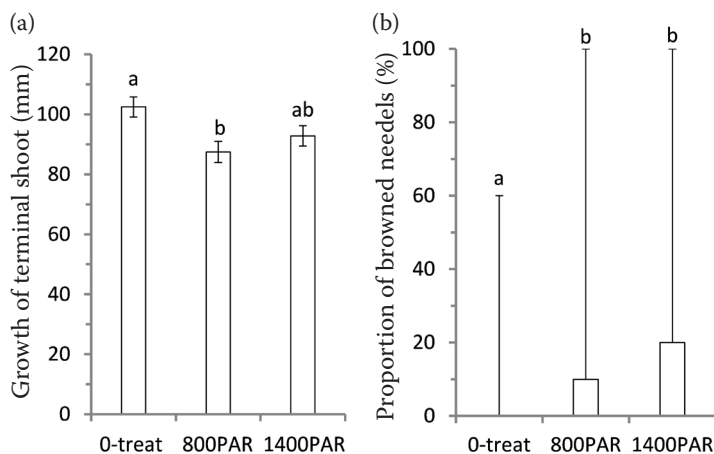


Fig. 3. Length of terminal shoot (model adjusted mean  $\pm$  SE) and proportion of browned needles (median and range) in the top part of the shoot after greenhouse growing in experiment 1 ( $n = 55-60$  seedlings per treatment);

different letters above the bars show significant pairwise differences ( $P < 0.05$ ) by covariance analysis (growth) or Kruskal-Wallis test (needles); 800 and 1,400 PAR – supplied light intensity level in  $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  with treatment hours combined; 0-treatment – seedlings with no exposure to field conditions

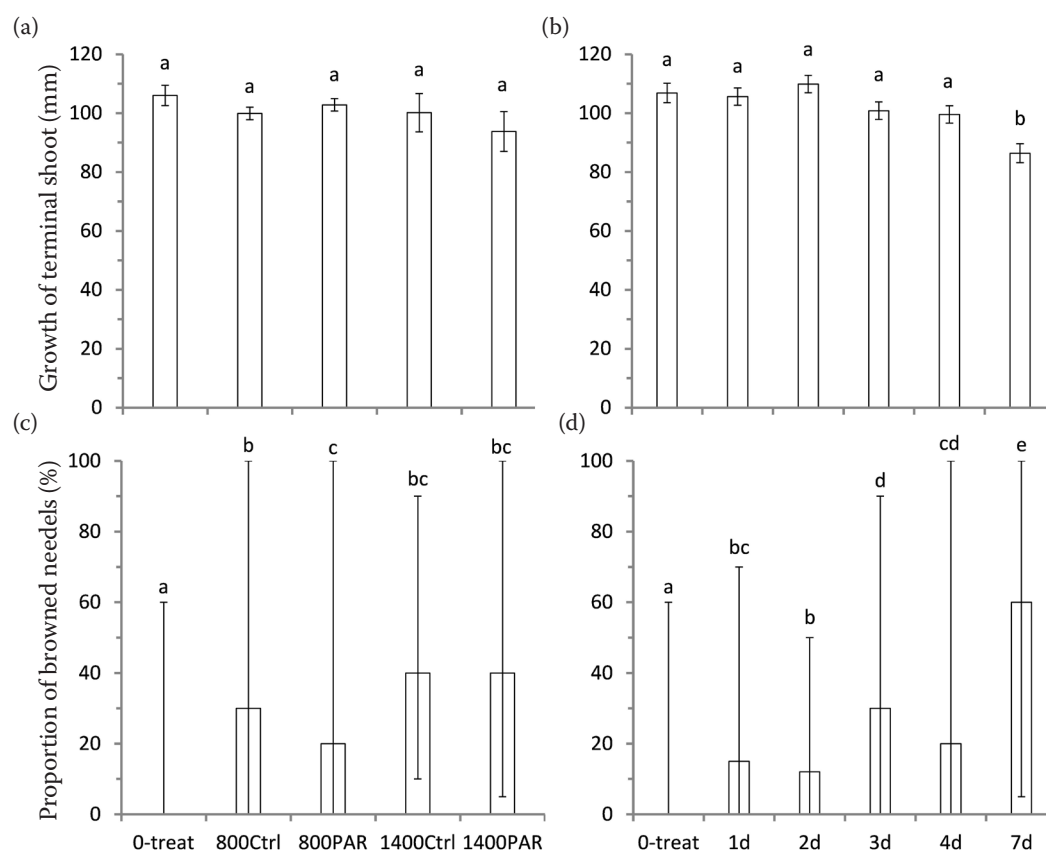


Fig. 4. Length of terminal shoot (model adjusted mean  $\pm$  SE) and proportion of browned needles (median and range) in the top part of the shoot after greenhouse growing in experiment 2 ( $n = 60$  seedlings per 0-treatment,  $n = 150$  per each 800-treatment,  $n = 16$  per each 1,400-treatment and  $n = 60$ –68 per each day 1d–7d);

different letters above the bars show significant differences ( $P < 0.05$ ) by covariance analysis (growth) or Kruskal-Wallis test (needles), (a–c): 800 and 1,400 PAR – supplied light intensity levels in  $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  with days combined; Ctrl – control seedlings with no exposure to artificial light; (b–d): 0-treatment is compared to the combined light treatments by number of days, after the 4th day, no artificial light was supplied, 0-treat – seedlings with no exposure to field conditions.

shoot growth in the following growing season. The studied artificial light radiation intensities yielded no clear difference in seedling winter damage compared to natural light intensities. Practically no healthy cell existed in the damaged browned needles of one-year-old terminal shoots. Shoot exposure to freezing temperatures in February for several weeks (preliminary experiment) in natural light induced almost total needle browning, which consequently reduced new shoot growth to about a half and root growth to one or two tenths in the following growing season in comparison with seedlings with no browned needles (5% or less). In all the studied experiments, the foliar damage was observed but the buds were intact and were able to grow in spring, which supports observations in practical plantations (RUMMUKAINEN, VOPIO 1981).

Seedling growth showed no coherent change in the post-damage growing season when the seedlings were under varying light exposure hours per

day in the preceding winter (Exp. 1). Instead, the proportion of browned needles from the old top shoot decreased along with the light exposure hours. Furthermore, the proportion of browned needles tended to be higher in the 1,400 than in the 800  $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  light treatment. This probably indicates that after transfer to the treatment the acclimation to the new light and temperature conditions improved during the day.

New shoot growth showed no coherent change either along with the light exposure time within a week in the preceding winter (Exp. 2), but the proportion of browned needles of the old shoot tended to increase, while the light intensity level had no clear effect. This suggests that the freezing temperatures rather than the radiation conditions induced the observed seedling damage. However, it is possible that radiation may have a greater impact on the winter foliage damage at non-freezing temperatures in late winter or early spring (TRANQUILLINI 1982; HADLEY et al. 1991; NEUNER et al. 1999).

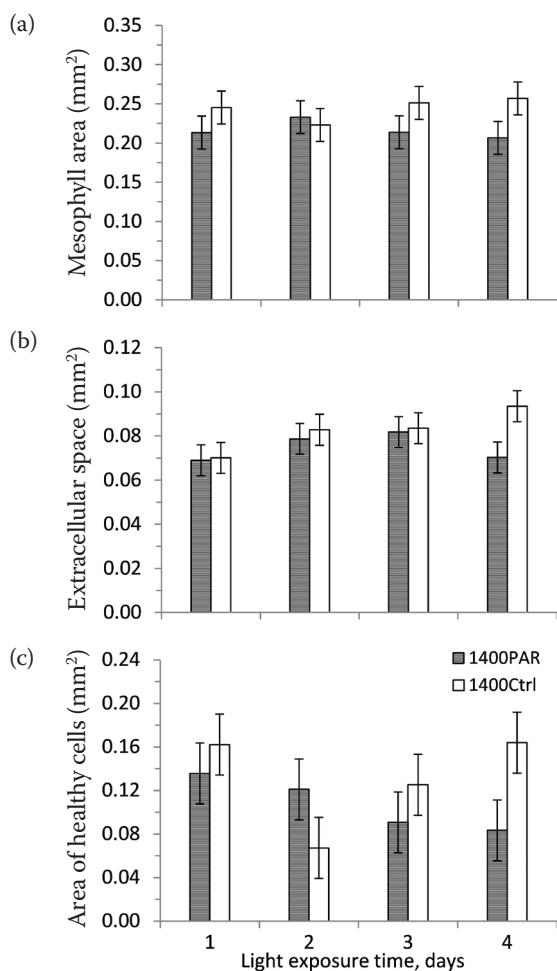


Fig. 5. Areas of mesophyll, extracellular space, and healthy cells for needles in terminal shoots before greenhouse growing in Experiment 2 (model adjusted mean  $\pm$  SE,  $n = 4$  seedlings per light treatment  $\times$  day combination); 1,400 PAR – supplied light intensity level in  $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  and Ctrl – control seedlings not exposed to artificial light

In previous studies, winter foliage damage was reported to reduce the growth of planted seedlings at least during the post-injury growing season (KRASOWSKI et al. 1996; LANGVALL et al. 2001). Planting success of a seedling has also been observed to weaken markedly when more than 50% of the needles of the seedling are damaged (browned) at the tree nursery (TERVO, KAUTTO 1999). Older and sturdier seedlings usually tolerate winter damage better than younger and taller seedlings (KRASOWSKI et al. 1996). Genetic tolerance to winter desiccation in Norway spruce seedlings may be attributable to a high degree of hardiness possessed by early flushing and short seedlings (DANUSEVICIUS et al. 1999). Seedlings with low or no post-planting root growth may be more subject to winter desiccation. Therefore, site preparation treatments that improve rooting and seedling establishment,

such as mounding, may reduce overwinter injuries (KRASOWSKI et al. 1996). On the other hand, seedlings in high mounds may be susceptible to winter foliage damage because they emerge first from the snow cover in springs (HEISKANEN et al. 2013).

In general, decreasing temperatures below  $-3^{\circ}\text{C}$  can damage actively growing shoots and needles (CHRISTERSSON, von FRICKS 1988; REPO 1992). In Norway spruce seedlings, new root tips grow after the soil temperature has increased to  $5^{\circ}\text{C}$  but root growth is still negligible below  $8^{\circ}\text{C}$  (VAPAAVUORI et al. 1992). On the other hand, first-year Norway spruce seedling roots (tested as root growth capacity) and shoots have been shown to survive and grow in spring, when exposed to temperatures not lower than  $-16^{\circ}\text{C}$  in January (LINDSTRÖM 1986). In winter, the soil temperature is usually near  $0^{\circ}\text{C}$  under snow cover (GROSSNICKLE 2000). Minimum temperatures of about  $-2^{\circ}\text{C}$  on the surface of a ploughing tilt under snow cover have been mea-

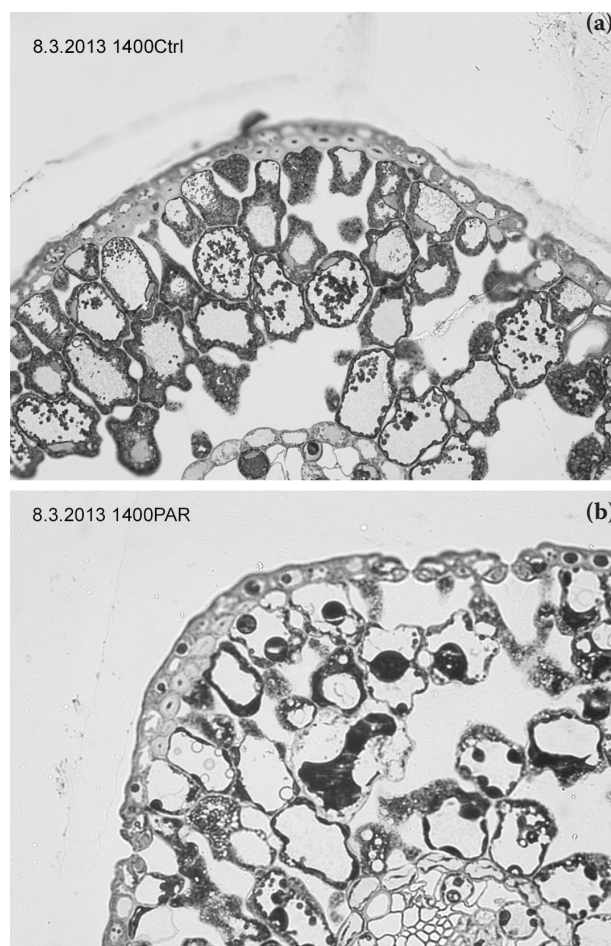


Fig. 6. Example of needle cross section image showing mesophyll, extracellular space, and cells in terminal shoots before greenhouse growing in Experiment 2;

1,400 PAR – supplied light intensity level in  $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  and the Ctrl – control seedlings not exposed to artificial light



sured in mid-Sweden (LINDSTRÖM, TROENG 1995), while in cold winters, temperatures as low as  $-10^{\circ}\text{C}$  have been observed on a ploughing tilt under snow cover and  $-6^{\circ}\text{C}$  under the humus layer in Finland (KUBIN 1990).

In conclusion, Norway spruce seedlings exposed to freezing temperatures, with natural or artificial light, outdoors in mid and late winter showed damage to needles and decreased seedling vigour, which consequently reduced shoot and root growth in the first growing season following the damage. The buds were not damaged in any treatment, and they were able to grow in the next spring. The results suggest that above the snow cover, other environmental factors, e.g. the duration and level of freezing temperatures and wind, rather than the radiation conditions induce the observed winter damage to foliage and subsequent reduced growth in Norway spruce seedlings. However, radiation may have a greater impact on seedling winter damage at non-freezing temperatures in late winter or early spring, which this idea could merit further research. Furthermore, research may be needed into how artificial light radiation corresponds to natural light conditions. The preliminary methods used here outdoors did not distinguish all the different environmental conditions and their mechanisms of effect on seedlings, which may also demonstrate the need for further method development in controlling experimental conditions at air temperature, radiation intensity and air current for future research in seedling winter damage outdoors.

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