Effect of Thawing Duration and Temperature on Field Performance of Frozen-Stored Norway Spruce Container Seedlings

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Increasing use of frozen storage in nurseries at northern latitudes calls for thawing methods that are safe, economical and easy to apply on a large scale. The easiest and most economical method would be to thaw seedlings in the same boxes they were stored in. However, doing this safely requires more knowledge about how long and at what temperatures seedlings should or can be kept in the boxes without reducing field performance. In this study, 1-yr-old frozen-stored Norway spruce (*Picea abies* (L.) Karst.) container seedlings were thawed for 4, 8 or 16 days at 4 or 12 °C in cardboard boxes before planting on a reforestation site and on experimental field in mid-June. Some seedlings were also planted on these locations after thawing for only 7 hours at 12 °C in order to separate frozen root plugs. We found some evidence that planting seedlings after short thawing periods (7 hours at 12 °C and 4 days at 4 °C), under which conditions the root plugs remain completely or partly frozen, has a negative effect on field performance of Norway spruce seedlings. Thawing over a 4–8 day period in cardboard boxes at ca. 12 °C appears to ensure complete thawing of the root plugs and unaffected field performance, but is short enough to prevent the growth of mould.

Keywords frozen-planting**,** height growth, *Picea abies*, storage, survival
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1 Introduction

Increasing numbers of nurseries at northern latitudes overwinter container seedlings in frozen storage (–1 to –5 °C) in cardboard boxes (see Camm et al. 1994, McKay 1997). For example, Finnish nurseries deliver annually over 80 million Norway spruce (*Picea abies* (L.) Karst.) container seedlings (Västilä 2002), of which it is estimated that more than 30% are currently frozen-stored. Frozen seedlings are thawed according to a variety of methods before outplanting (Mattson 1978, Niiranen 2001, Paterson et al. 2001).

Previous research on frozen-stored conifer seedlings has mainly focused on the short-term physiological effects of different thawing methods (Camm et al. 1995, Fløistad and Kohmann 2001), or on the effects of planting frozen seedlings (Camm et al. 1995, Kooistra and Bakker 2002). Fløistad and Kohmann (2001) showed that there are physiological advantages in thawing seedling bundles rapidly in the air (15 °C) or by immersion in water (8 °C) compared with slow (8 weeks) thaw. However, due to practical constraints, such methods are difficult to apply on a large scale in boxes. Likewise, planting frozen seedlings, even if relatively safe in warm soil (Camm et al. 1995, Kooistra and Bakker 2002), cannot be done on a large scale unless packaging is modified to facilitate separation of frozen root plugs.

The easiest and most economical way to thaw seedlings would be to keep them in the boxes they were stored in. However, studies are needed to determine how long and especially at what temperatures initially frozen seedlings should or can be kept in the boxes without affecting their physiological quality and subsequent field performance. While high air temperatures (> 15 °C) would thaw frozen root plugs rapidly, they might also result in depletion of carbohydrates (Puttonen 1986) and moulding (Hocking 1971), which suggests that thawing period should be as short as possible. On the other hand, the time needed to thaw the root plugs may be too short to permit the frozen-stored seedlings to resume normal physiological processes before planting (Grossnickle and Blake 1985, Mattson and Troeng 1986).

The objective of this study was to compare different thawing duration and thawing temperature combinations with respect to the long-term field performance of frozen-stored Norway spruce container seedlings.

2 Material and Methods

2.1 Seedling Material

Norway spruce seedlings were raised in hard plastic containers (Blockplant 12 × 12 -container, cell size 66 cm^3, Panth-Produkter Ab, Sweden) in a greenhouse at a local commercial nursery (Fin Taimi Oy, Tuusniemi nursery, 62°55´N, 28°19´E) according to standard culturing procedures. Seeds originated from a registered orchard producing seeds adapted to conditions in central Finland were sown on 11 May 2000. On 24 October 2000, seedlings were extracted from the containers and placed upright in plastic trays (110 seedlings per tray) that were inserted into cardboard boxes (2 trays i.e. 220 seedlings per box). Altogether 14 boxes selected for the experiment were kept in frozen storage (–3 °C) until 25 May 2001, when they were transported to the Suonenjoki Research Station (62°39´N, 27°03´E, altitude 142 m a.s.l.) and stored at –4 °C until used. A random sample of 60 seedlings had the following characteristics (mean ± SD): height 12.6 ± 1.8 cm, shoot dry weight 0.40 ± 0.08 g and root dry weight 0.15 ± 0.04 g.

2.2 Experimental Sites

The effect of different thawing duration and thawing temperature combinations on seedling field performance was studied on an experimental field and reforestation site at Suonenjoki in central Finland between June 2001 and September 2003. The field was harrowed with a farm harrow in May 2001. The soil (0–15 cm) was coarse sand (67% by mass of fractions 0.2–0.6 mm and 26.5% of fractions < 0.2 mm, organic material 1.2%). The reforestation site (altitude 126 m a.s.l.) had been logged in spring 2000 and patch-scarified in autumn 2000. The site was a gentle slope with variation in soil moisture from intermediate on the upper slope to mesic on the lower slope, and classified as *Myrtillys* type according to the Finn-

Table 1. Monthly mean air temperature and precipitation during the growing season (May–September) in 2001, 2002 and 2003, and the long term (air temperature: 1972–2003 and precipitation: 1975–2003) averages (mean ± SD) at the Suonenjoki Research Station.

Month / Year	Temperature (°C)				Precipitation (mm)			
	2001	2002	2003	1972–2003	2001	2002	2003	1975–2003
May	7.6	10.8	10.1	9.0 ± 1.8	55	32	56	39 ± 18
June	14.2	15.7	12.0	14.4 ± 2.0	61	105	72	69 ± 34
July	18.7	18.3	19.9	16.6 ± 1.7	81	73	68	82 ± 29
August	14.6	17.1	14.3	14.2 ± 1.4	80	48	81	76 ± 35
September	10.4	8.7	10.0	9.0 ± 2.4	74	36	28	57 ± 31

ish classification of forest types (Cajander 1925). The soil was fine sand (37 to 59% of fractions < 0.2 mm and 29–34% of fractions 0.2–0.6 mm, organic material 3.5–6.1%). In the experimental field, weeds were removed in August every year during the study period. Due to scarification before planting, no weed control was applied on the reforestation site.

2.3 Treatments

Seedlings were taken out of the freezer 4, 8 or 16 days before planting and thawed in boxes at either 4 or 12 °C. Other seedlings were taken out of the freezer on a planting day and thawed for 7 h at 12 °C to separate frozen root plugs. This procedure resulted in 7 different thawing duration and temperature combinations. Two seedling boxes (440 seedlings) were randomly assigned to each combination. Two holes (3 cm × 9 cm) were made in each box to ensure ventilation during thawing. No sign of softening was detected in the root plugs after 7 h thawing at 12 °C (frozen-planting). The root plugs of the seedlings thawed for 4 or 8 days at 4 °C were also still partly frozen (especially in the middle of the seedling tray). It was possible to separate the root plugs without causing any visible damage to roots. Seedlings thawed for 16 days at 12 °C had some greyish fungal mycelia on their needles, but this disappeared soon after planting. Seedlings received no irrigation before planting.

2.4 Experimental Design and Measurements

Differently thawed seedlings were planted on the reforestation site (14 June) and on the experimental field (15 June) in a randomized block design consisting of 5 blocks divided into seven plots (one plot for each thawing duration and temperature combination). In each plot there were 18 seedlings (a total of 1260 seedlings: 2 sites × 5 blocks × 7 plots × 18 seedlings). Because of the lack of proper patches in a plot on the reforestation site, two seedlings were occasionally planted in the same patch (60 cm × 120 cm), but with at least 50 cm spacing between seedlings. On the experimental field, spacing was 40 cm between seedlings and 80 cm between rows (plots). On both planting days weather was sunny or partly cloudy, and the air temperature varied between 17–19 °C. Soil temperatures were 14 °C (reforestation site) and 18 °C (experimental field) measured at a depth between 0–10 cm with a digital thermometer (Sensotherm 100, GWB, West Germany). Soil water content was relatively high on both sites due to rain showers on 12 June (14 mm) and 13 June (9 mm). Weather conditions during the growing season (May to September) 2001, 2002 and 2003 did not differ considerably from the long-term average (Table 1).

The height of each seedling was measured to the nearest 0.5 cm from ground level immediately after planting and again on mid-September 2001, 2002 and 2003. In addition, seedlings were visually rated as vigorous (no visible damage), weakened (part of the needles brown or shed) or dead (all needles brown or shed) after every growing season.

2.5 Statistical Analysis

Since the differences in seedling height at planting between treatments were statistically significant ($p<0.001$, ANOVA) on both sites, height growth data obtained from the randomized block design were subjected to an analysis of covariance (seedling height at planting as a covariate). However, as the effect of the covariate was nonsignificant, it was omitted, and the data were subjected to analysis of variance (ANOVA) in which thawing duration and thawing temperature combination was treated as a fixed factor and block as a random factor (mixed effects model). Tukey's test was used to locate the differences between treatment means. Proportions of dead seedlings in different thawing treatments were arc sine-transformed before subjecting to ANOVA. Data were analyzed using SPSS 11.0 for Windows.

3 Results

Seedling mortality was low on the reforestation site (Fig. 1A). Thawing treatments had no significant effect on either first, second or third year mortality. The amount of weakened seedlings was also low (<1% after the first year and approximately 2% after the second and third year). On the experimental field, frozen-planting (7 h thawing at 12 °C) increased mortality significantly during the first year ($P=0.001$). However, there was no significant increase in mortality during the second and third year (Fig. 1B). After the first and second year, weakened seedlings were found only among seedlings thawed for 4 days at 4 °C (8% and 2%, respectively). A few weakened seedlings (1 to 3%) were found in all treatments after the third year.

Thawing treatments had no effect on either first-, second- or third-year height growth in the reforestation site (Fig. 1C). In the experimental field, thawing treatment had a significant effect on first- and second-year height growth ($P<0.001$ and $P=0.027$), but not on third-year height growth. Seedlings planted frozen or thawed for 4 days at 4 °C had the poorest height growth during the first, and especially during the second year ($p<0.05$, Fig. 1D). Higher thawing temperature (12 °C) seemed to improve height growth slightly both on the reforestation site (excluding the 8-day thawing period) and in the experimental field. However, the differences between treatments were minor (Fig. 1C and 1D).

4 Discussion

On the homogenous but rather coarse-textured experimental field, mortality increased and height growth was poor when the root plugs were completely frozen (7 hours thawing at 12 °C) at the time of the planting. In addition, height growth was decreased when the root plugs were still partly frozen (4 days thawing at 4 °C) at the time of the planting. One explanation for the increased mortality and poor height growth may have been water stress caused by restricted uptake of water from the frozen root plug. However, since the soil temperature was relatively high (≥ 14 °C) on both sites, root plugs probably thawed rapidly after planting. According to Kooistra and Bakker (2002), and also to our own unpublished results from a previous study, small frozen root plugs (66–80 cm^3) thaw within about 2 h in warm soil (15–23 °C). Even though water stored in the root plug became rapidly available to the roots after thawing, the seedlings may not have been able to take up water sufficiently due to lack of water permeability of the existing root system (Grossnickle and Blake 1985). This, together with high air temperature and solar radiation at the planting site, may have resulted in seedling water deficit and subsequent increase in mortality and decrease in height growth. It is also possible that the roots of frozen planted seedlings were slightly damaged while the root plugs were separated, which would have further depressed the ability of root system to take up water. On the other hand, however, the results from the reforestation site agree with those of earlier studies (Camm et al. 1995, Kooistra and Bakker 2002): the effect of frozen-planting on field performance was minor (especially in warm and moist soil) compared to the effect of differing planting environments.

Even a relatively long thawing period (16 days) at 12 °C had no negative effect on the field performance of frozen-stored seedlings. Seedling

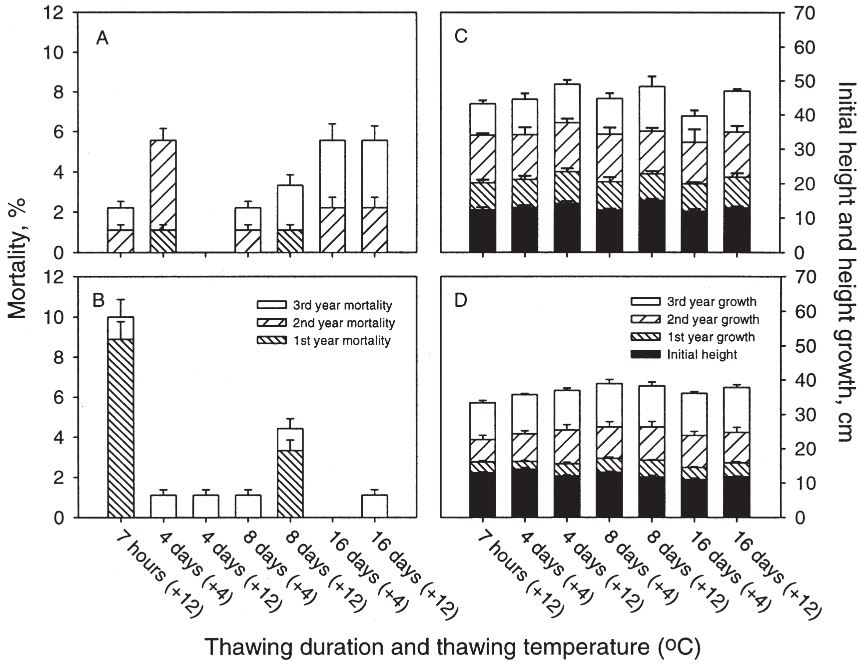

Fig. 1. Mortality, initial height and first, second and third year height growth (block means ± SE) of 1-yr-old frozen-stored Norway spruce container seedlings exposed to different thawing duration and thawing temperature combinations before planting on a reforestation site (A, C) and on an experimental field (B, D).

respiration at this temperature was apparently so slow that it had no major effect on carbohydrate reserves and subsequent seedling field performance (the temperature in the boxes was probably < 12 °C at the beginning of the thawing period because of the heat absorbed by the melting ice). According to Puttonen (1986), depletion of carbohydrate reserves of Scots pine (*Pinus sylvestris* (L.) seedlings during storage resulted in substantially reduced shoot length and high mortality only when the carbohydrate reserves fell below ca. 2%. However, this threshold value was only reached at a storage temperature of 20 °C after a three-week period. Thus, with respect to carbohydrate reserves, the temperatures used in the present study can be considered safe even during thawing periods exceeding 16 days. On the other hand, the mould observed in seedlings thawed for 16 days at 12 °C may be of greater concern during long thawing periods. Low light intensity, moderate temperature and high air relative humidity prevailing in seedling boxes during the thaw are all factors that have been reported to favour the growth of mould (Peterson et al. 1988, Dugan and Blake 1989, Peterson and Sutherland 1990). This emphasizes the importance of adequate ventilation in seedling boxes during extended thawing periods.

None of the thawing methods used in this study (excluding frozen-planting) was clearly deleterious to long-term seedling field performance. However, the thawing temperature of 4 °C was clearly too low for seedlings to be thawed and ready for planting, even after 8 days. On the other hand, the 16-day thawing period at 12 ºC resulted in moulding, which may, when excessive, negatively affect field performance. Consequently, thawing for 4–8 days at 12 °C, with which incomplete thawing of the root plugs and moulding can be avoided, appears to be the best alternative.

To further ease the thawing procedure and the delivery of the seedlings from the nursery, future research should focus on thawing frozen-stored seedlings outdoors under varying (day/night) temperature and radiation conditions.

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