Frozen-stored conifer container stock can be outplanted without thawing

Photo by Clare Kooistra

Clare M Kooistra and Jonathan D Bakker |

ABSTRACT

In this study, we outplanted frozen-stored container stock in operational settings and found minimal effects of thawing regime on seedling performance over 2 y. We monitored the performance of 1+0 container stock of 3 coniferous species (lodgepole pine [*Pinus contorta* var. *latifolia* (Pinaceae)], western larch [*Larix occidentalis* (Pinaceae)], and interior spruce [*Picea glauca* and *P. engelmannii* hybrid complex (Pinaceae)]) planted with frozen or thawed root plugs at 2 sites. Differences in chlorophyll fluorescence between frozen and thawed seedlings disappeared within 3 d of outplanting, and differences in budburst phenology disappeared within 6 wk of outplanting. Frozen seedlings had slightly lower diameter and biomass than thawed seedlings but did not differ in condition, height, or sturdiness ratio over 2 growing seasons. Interspecific and environmental differences accounted for much more of the variation in measured attributes than did plug temperature treatment. Eliminating the thaw stage from the seedling production process can yield significant operational advantages.

KEY WORDS
reforestation, storage regime, thawing regime, chlorophyll fluorescence, budburst phenology

NOMENCLATURE
USDA NRCS (2004)

Prompt reforestation is critical in ensuring the sustainability of forestry in harvested areas and is valuable for restoring ecosystem health following crown fire or other natural disturbances. Many plants used in reforestation are produced in nurseries, where seedling quality and performance are optimized through control over tree size, root-to-shoot ratio, nutrient status, and health.

Seedling quality and vigor can be affected by many aspects of the seedling production process, including harvest, storage, thawing regime, transportation, and outplanting technique (McKay 1997). Much attention has focused on determining when to harvest seedlings and how long to store them (Kozlowski and Pallardy 2002). Seedlings are often stored at below-freezing temperatures (–2 °C [28 °F]; the conventional practice in British Columbia) or at above-freezing temperatures (2 to 4 °C [36 to 39 °F]). Doing so provides flexibility in scheduling delivery to outplanting sites (Rose and Haase 1997).

Frozen storage offers several advantages over cool storage, including reduced loss of carbohydrate reserves (Ritchie 1982; Wang and Zwiazek 2001), maintenance of cold hardiness (Schaberg and others 1996), and diminished likelihood of storage molds (Sutherland and others 1989). Seedlings are packaged into bundles before entering frozen storage. Conventional bundling techniques wrap a number of container seedlings together, causing the root plugs to freeze together.

Before outplanting, these seedlings must be thawed so that they can be separated without damaging their root plugs. Technologies are now available to package seedlings individually so that they can be separated while frozen. Elimination of the thaw stage from the seedling production process would streamline the operation and improve the logistics of seedling delivery.

Recent studies have demonstrated that conifer seedlings can tolerate a variety of thawing practices (Rose and Haase 1997; Maki and Colombo 2001). Many studies have shown that seedlings with frozen plugs (frozen seedlings) can be planted directly into the ground with little effect on short-term physiology or first-year growth (Camm and others 1995; Fløistad and Kohmann 2001; Kooistra and Bakker 2002). A few studies have reported some negative effects of rapid thawing (Helenius and others 2004; Helenius 2005), though positive effects have also been demonstrated (Silim and Guy 1998). Overall, these results suggest that seedling health and survival are minimally affected by whether thawing occurs before outplanting.

The study by Kooistra and Bakker (2002) was conducted in a nursery environment, which is substantially different from the environments into which seedlings are outplanted operationally. For example, the seedbed received uniform site preparation, and seedlings were irrigated and fertilized. In addition, soils were warmer (18 to 32 °C [64 to 90 °F]) at the time of outplanting than is usually experienced operationally in northerly latitudes. In this study, we extend the results of Kooistra and Bakker (2002) to operational settings. We used frozen and thawed container stock of 3 coniferous species, replicated the experiment at 2 sites, and monitored seedling performance for 2 growing seasons after outplanting. We were particularly interested in the relative magnitude of the effect due to plug temperature compared with the effects of species identity and large- and small-scale environmental differences among sites and blocks.

MATERIALS AND METHODS

Experimental Design

Lodgepole pine (*Pinus contorta* Dougl. ex. Loud. var. *latifolia* Engelm. ex S. Wats. [Pinaceae]; seedlot 60151), western larch (*Larix occidentalis* Nutt. [Pinaceae]; seedlot 60717), and interior spruce (*Picea glauca* (Moench) Voss and *P. engelmannii* Parry ex Engelm. hybrid complex [Pinaceae]; seedlot 60148) 1+0 seedlings were grown using standard operational methods in 80-ml (5-in^3) containers (Styroblock™ 410 for larch, Copperblock™ 410 for pine and spruce; containers produced by Beaver Plastics, Edmonton, Alberta). In fall 2001, seedlings were harvested, graded, bundled in groups of 15, boxed, and placed in frozen storage at –2 °C (28 °F). During bundling, a layer of nursery grade plastic wrap was placed between the seedlings in each bundle so that they could be separated while frozen.

In spring 2002, root growth capacity (RGC) was measured for 30 seedlings per species using Burdett's (1979) index of root growth. RGC was significantly lower for spruce than for larch and pine (mean values of 3.9, 4.7, and 4.6, respectively), but RGC values >2 are considered viable for operational purposes in British Columbia (Kooistra, unpublished data).

Bundles were randomly assigned to 1 of 2 plug temperature treatments in spring 2002: seedlings were outplanted either with thawed root plugs (thawed seedlings) or with frozen root plugs (frozen seedlings). Seedlings for the thawed treatment were held for 5 d at 10 to 15 °C (50 to 59 °F) before outplanting. Frozen seedlings were kept in the freezer until outplanting. All seedlings were kept in the dark in the freezer and/or cooler.

The field portion of the experiment was conducted at 2 sites (South Fork and Styx Creek) 60 km (37 mi) apart in the interior Douglas-fir (IDF) biogeoclimatic zone, moist warm variant (mw1). Both sites are on crown land in Riverside Forest Products' operating area in the southern interior of British Columbia. South Fork is located on a northeast aspect at 1230 m (4035 ft) elevation and was clear-cut harvested in winter 2002. Styx Creek is located on a southwest aspect at 1090 m (3575 ft) elevation and was clear-cut harvested in autumn 2000. Sites did not receive any site preparation but were fenced to prevent grazing and trampling by livestock. Soils at both sites are brunisols with a restrictive layer at about 35 cm (1.1 ft) and a mor humus form. South Fork has silty loam to loamy sand soils with 50% coarse fragments, while Styx Creek has loamy sand soils with 25% coarse fragments (Riverside Forest Products, unpublished data).

A portable weather station recorded temperature and precipitation at each site during the 2002 growing season. Precipitation from June to September 2002 was 62.4 mm (2.5 in) at Styx Creek and 24.7 mm (1.0 in) at South Fork. Logistical constraints prevented us from maintaining the weather stations during the 2003 growing season, but the region experienced drought conditions both years.

At each site, the experiment had a randomized complete block design with 9 blocks and 6 treatment combinations (3 species × 2 plug temperature treatments). Each treatment combination consisted of a bundle of 15 seedlings outplanted in a row. Rows were 2.5 m (8.2 ft) apart, and seedlings within each row were 0.5 m (1.6 ft) apart. A total of 810 seedlings (9 blocks × 6 treatment combinations × 15 seedlings/bundle) were outplanted at each site. Frozen seedlings were kept in coolers prior to outplanting while thawed seedlings were kept in the cartons used by the BC Ministry of Forests to ship seedlings to outplanting locations around the province. Seedlings were outplanted by 1 person at Styx Creek and 2 people at South Fork.

The Styx Creek site was outplanted on 15 May 2002 between 0900 and 1400 hours. Air temperatures at the time of outplanting were 7 to 12 °C (45 to 54 °F), and the sky was overcast with sunny breaks. The South Fork site was outplanted on 24 May 2002 between 0900 and 1200 hours. Air temperatures at the time of outplanting were 8 to 12 °C (46 to 54 °F), and the sky was partly cloudy. Outplanting occurred near the end of the typical planting season for this biogeoclimatic zone (MoF 1998). In particular, environmental constraints (snow on site) prevented earlier outplanting at South Fork; the recent snowmelt resulted in much wetter soils at the time of outplanting than at Styx Creek.

Warming Trends

We examined the temperatures of seedling root plugs (T_p) and the soil (T_s) using a method modified slightly from that of Kooistra and Bakker (2002). T_p was measured by placing a temperature probe at a 5-cm (2-in) depth between 2 outplanted seedlings held together by elastics. We used 2 probes per treatment combination in each of 3 blocks, for a total of 36 probes per site. Readings were taken every 15 min for at least 7 h after planting. After the temperature measurements were complete, the elastics were removed and seedlings replanted separately. T_p did not differ among species or blocks (data not shown), so the mean T_p was calculated for each plug temperature treatment in each site.

Soil temperature (T_s) was measured at a 5-cm (2-in) depth with 1 probe in each block where T_p was measured. Measurements were made at 15-min intervals. The temperature difference (T_d) between the soil and root plugs was calculated as:

$$T_d = T_p - T_s$$

For frozen seedlings, this temperature difference is negative and rises toward zero as the root plug thaws. For thawed seedlings, the temperature difference is smaller and may be positive or negative.

Seedling Performance

Seedling performance was followed for 2 growing seasons after outplanting. Variables used to assess performance included chlorophyll fluorescence, budburst phenological stage, seedling condition, and growth parameters. Seedlings used to assess warming trends did not differ from other seedlings in performance measures (data not shown) and were included in all analyses.

Chlorophyll fluorescence measurements provide a rapid, nondestructive physiological test of a seedling's photosynthetic capacity (Binder and others 1997; Maki and Colombo 2001; Rose and Haase 2002). Fluorescence measurements compare the basic fluorescence in the dark with the maximum fluorescence when exposed to a saturating light (Fv/Fm) and are reported as quantum yield. We used an EARS-PPM fluorometer (EARS, Delft, The Netherlands) with a modulated (7.2 kHz) 637 nm light to measure fluorescence and a ≈ 1800 W/m^2 saturating light (Anonymous 1993). At each site, fluorescence measurements were taken on the top third of the

TABLE 1

Regressions for aboveground biomass (M, g) of larch, pine, and spruce as a function of seedling height (H, cm), stem diameter (D, mm), and sturdiness ratio (SR). Data are for seedlings at the time of outplanting (initial) and after 1 growing season.

Time / Species	**Equation**	r^2_{adj}
Initial		
Larch[z]	$M = 0.589523 + 0.001344(H)^2 - 0.100179(SR)$	0.916
Pine	$M = \dfrac{1}{-0.083898 + 2.257960(1/D)}$	0.392
Spruce	$M = 0.247476 + 0.001187(H)^2 + 0.096497(D)^2$	0.677
After 1 Growing Season		
Larch[z]	$M = -1.27703 + 0.001612(H)^2 + 0.065233(D)^2$	0.925
Pine	$M = \dfrac{1}{-0.090094 + 1.392132(1/D)}$	0.756
Spruce	$M = 0.996030 + 0.002841(H)^2 + 0.084094(D)^2$	0.484

[z] stem biomass only

shoot of each of 10 seedlings per treatment combination. Seedlings that had not yet been measured were not exposed to the light pulses produced while measuring other seedlings. Measurements were taken before outplanting; frozen seedlings were measured in the freezer and thawed seedlings after thawing had occurred but before they were taken from cool storage. Measurements were also taken for each of 6 nights after outplanting. Fluorescence measurements for larch were taken on the stem (cambial tissue) whereas measurements for pine and spruce were taken on foliage.

The budburst phenological stage of each seedling was assessed visually at the time of outplanting and 4 d, 1 wk, 3 wk, and 6 wk after outplanting. At each date, seedlings were assigned to a phenological category (no activity, terminal bud swollen [larch and spruce] or candling [pine], bud flushing, bud damaged or dead).

Seedling condition was assessed after 1 and 2 growing seasons. At each measurement date, seedlings were subjectively assigned to condition classes (good, fair, poor, dead).

Seedling growth was assessed by measuring height and stem diameter at ground level at the time of outplanting and at the end of each growing season, and by calculating the sturdiness ratio and aboveground biomass of each seedling. Sturdiness ratios (SR) were calculated at each measurement date as height divided by stem diameter. A large SR indicates a tall, slender seedling, while a small SR indicates a short, stocky seedling. Separate biomass regressions were developed for seedlings at the time of outplanting and at the end of the first growing season (logistical constraints prevented us from obtaining similar data at the end of the second growing season). Biomass was related to seedling height and stem diameter. Regressions for initial biomass were based on 30 seedlings per species from the stock types used in this experiment; regressions for biomass at the end of the first growing season were developed by destructively sampling 2 seedlings per treatment combination per block (total of 72 seedlings per species). Stepwise regression was used to identify the combination of variables that best predicted seedling biomass; input variables included height and stem diameter, transformations of height and stem diameter, and dummy variables representing site and plug temperature. For each species, the regression equation with the largest adjusted r^2 value was selected as the best equation. Species varied in how well their biomass was predicted by height and stem diameter (Table 1). Neither site nor plug temperature contributed to the predictive ability of the regressions, indicating that a single regression equation could be applied to each species at each date.

Statistical Analysis

We examined warming trends by modeling T_d as a function of time since outplanting. For each plug temperature treatment at each site, the equation (linear, second order polynomial, or third order polynomial) with the largest adjusted r^2 value was selected as the best-fitting model.

Chlorophyll fluorescence and growth parameters (height, stem diameter, biomass, SR), were analyzed using repeated measures MANOVA to assess the overall effects of site, species, and plug temperature treatment as well as changes in these factors over time. Significant MANOVA results were followed by univariate ANOVA tests to identify the response variables in which significant differences existed. We treated site, species, and plug temperature as fixed factors in the analyses. Blocks were treated as a random factor nested within sites and used as the error term to test the site effect. For factors with > 2 levels, Fisher's LSD was used to identify which levels differed significantly.

Budburst phenological stage and seedling condition are nominal variables and could not be analyzed using ANOVA. Instead, we used contingency analysis to determine whether the proportions of seedlings in each category varied among sites, species, and plug temperature treatments. These analyses used seedlings from all blocks to ensure adequate sample sizes and were repeated for each assessment date.

All analyses were conducted using JMP software (version 5.1.2; SAS Institute Inc, Cary, North Carolina) with $\alpha = 0.05$.

RESULTS

Warming Trends

Soil temperatures ranged from 6 to 11 °C (43 to 52 °F) at South Fork (mean = 8 °C [46 °F]), and from 8 to 13 °C (46 to 55 °F) at Styx Creek (mean = 10 °C [50 °F]). Root plugs of thawed seedlings were within 4 °C (7 °F) of soil temperatures at the time of outplanting (Figure 1). At South Fork, the warming trend of thawed seedlings was best described as a linear function of time since outplanting with a slight positive slope. At Styx Creek, a third order polynomial function fit the data best. The fit lines were always within 2 °C (4 °F) of soil temperature.

Root plugs of frozen seedlings were cooler than the soil at the time of outplanting and exhibited lags before their temperatures began to rise (Figure 1). The warming trends at both sites were best described as third order polynomial functions of time since outplanting. The initial temperature difference (T_d) was greater and root plugs warmed faster at South Fork than Styx Creek. The fit lines were within 2 °C (3.6 °F) of soil temperature after about 5 h at South Fork and 7 h at Styx Creek.

Seedling Performance: Chlorophyll Fluorescence

Chlorophyll fluorescence varied between plug temperature treatments ($F = 8.01$, $P = 0.006$), species ($F = 509.85$, $P < 0.001$), and sites ($F = 34.30$, $P < 0.001$). In addition, interactions of time with plug temperature treatment, species, site, the species x plug temperature interaction, and the site x species interaction were significant ($P \leq 0.01$). Fluorescence values were significantly lower for frozen than thawed seedlings for the first 2 nights after outplanting and tended to be lower for frozen seedlings on the third night ($F = 2.87$, $P = 0.094$) but did not differ between plug temperature treatments on subsequent nights (Figure 2). Fluo-

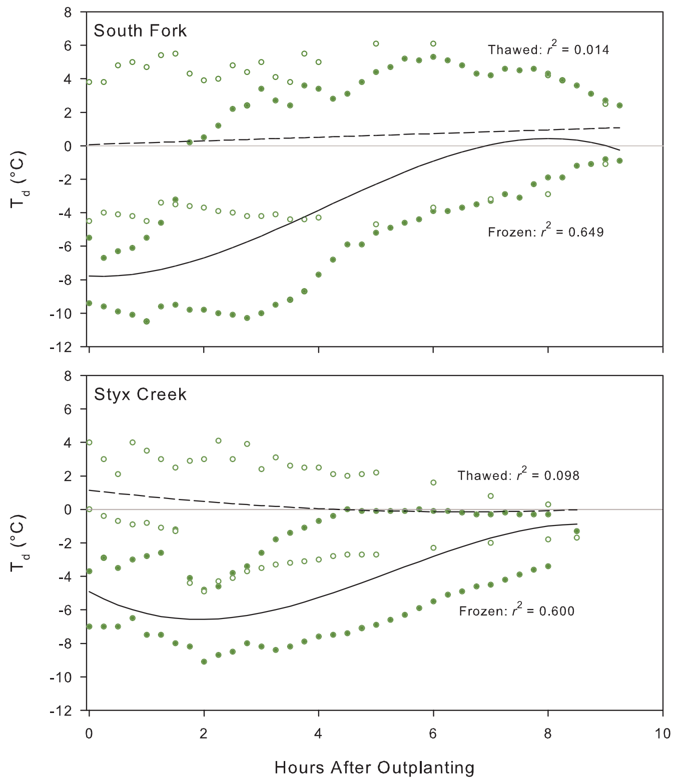

Figure 1. Temperature difference (T_d; °C) between the soil and root plugs planted while frozen (•, solid lines) and thawed (o, dashed lines) as a function of time since outplanting at South Fork (*top*) and Styx Creek (*bottom*). Fit lines are based on $n = 18$ seedlings per plug temperature treatment at each site and are significant at $P < 0.05$. The minimum and maximum values for each plug temperature treatment at each time are also shown. Plug and soil temperatures are equal when $T_d = 0$.

rescence values were lowest for larch and highest for pine, and were lower at Styx Creek than South Fork.

Seedling Performance: Budburst Phenology and Seedling Condition

Plug temperature had a significant effect on budburst for 3 wk after outplanting, as thawed seedlings advanced in phenology faster than frozen seedlings (Figure 3). The magnitude of the plug temperature effect diminished over time (4-d: $\chi^2 = 199.6$, $P < 0.001$; 1-wk: $\chi^2 = 142.6$, $P < 0.001$; 3-wk: $\chi^2 = 19.1$, $P < 0.001$), and was undetectable after 6 wk ($\chi^2 = 2.5$, $P = 0.481$). The effect of plug temperature treatment on phenology was more pronounced for larch than for pine or spruce.

Budburst differed significantly among species and sites. Larch advanced in phenological stage faster than pine or spruce, and more pine seedlings were dead or damaged than larch or spruce seedlings (Figure 3). Seedlings at South Fork advanced faster than those at Styx Creek, though the proportion of dead or damaged seedlings was also higher at South Fork after 6 wk.

After 1 growing season, ≥ 95% of all seedlings were classified as "good" (Table 2) and seedling condition did not vary among

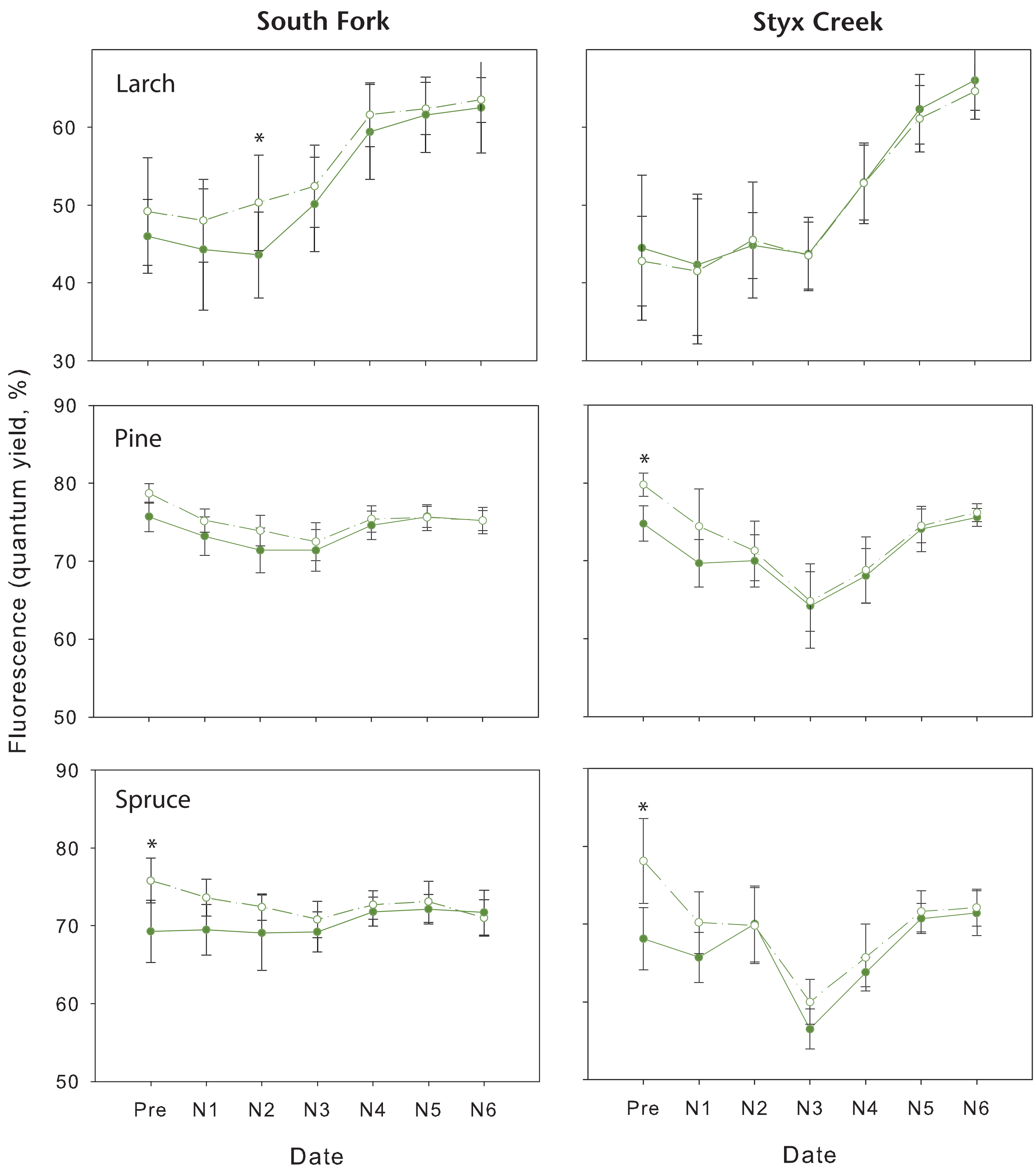

Figure 2. Chlorophyll fluorescence (mean ± SD) of frozen (•, solid lines) and thawed (o, dashed lines) seedlings of larch, pine, and spruce outplanted at South Fork (*left*) and Styx Creek (*right*). Measurements were made on *n* = 10 seedlings per species x plug temperature treatment combination at each site. Pre = preplant measurements; N1 through N6 = measurements made the night after outplanting and for the next 5 nights. An asterisk (*) above a date indicates that fluorescence measurements differed significantly between plug temperature treatments. Note that the y-axis has a different scale for larch than for pine or spruce.

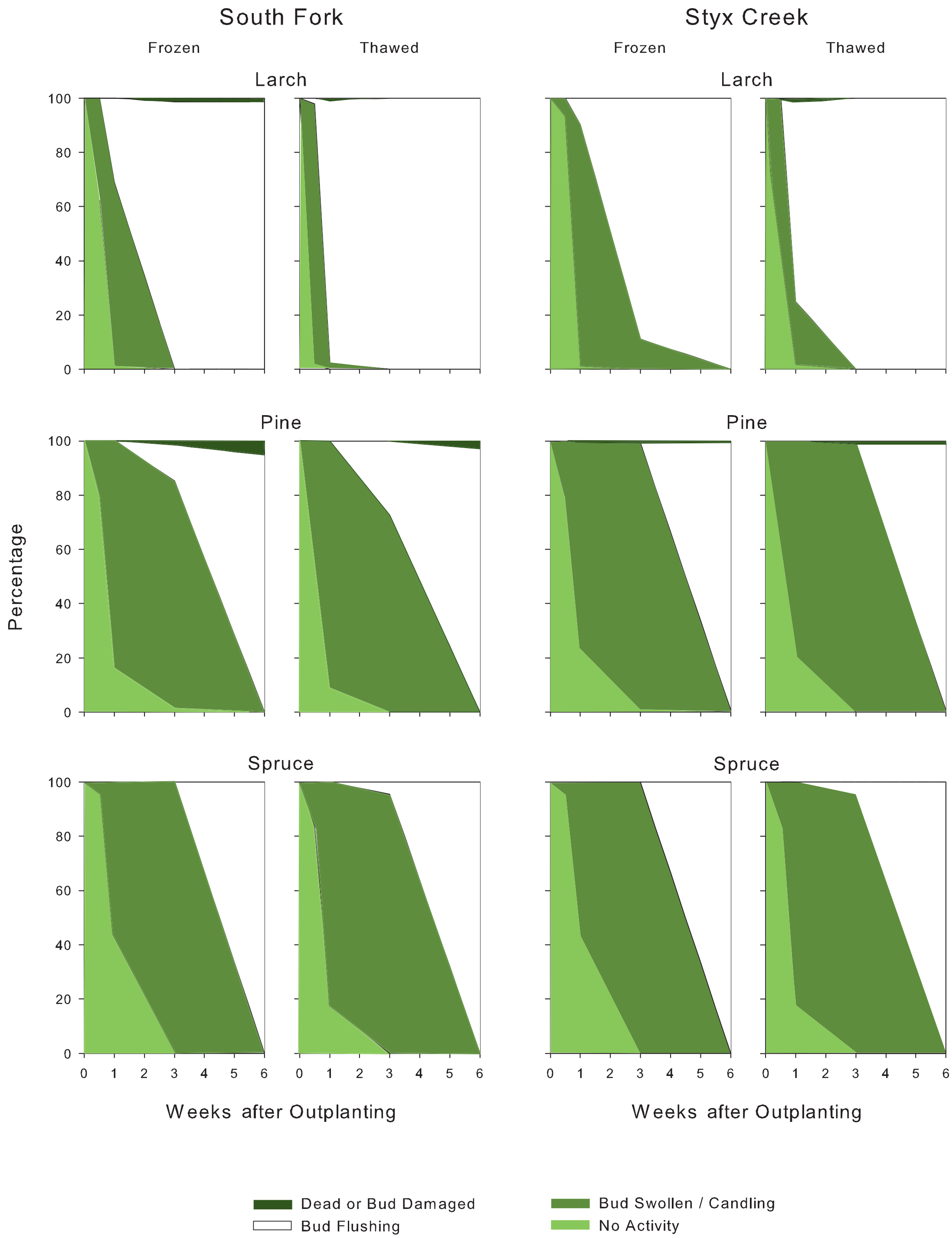

Figure 3. Budburst phenological development of frozen and thawed seedlings of larch, pine, and spruce outplanted at South Fork (*left*) and Styx Creek (*right*). Graphs show the proportion of seedlings in each of 4 phenological stages. Measurements were taken at outplanting and 4 d, 1 wk, 3 wk, and 6 wk after outplanting.

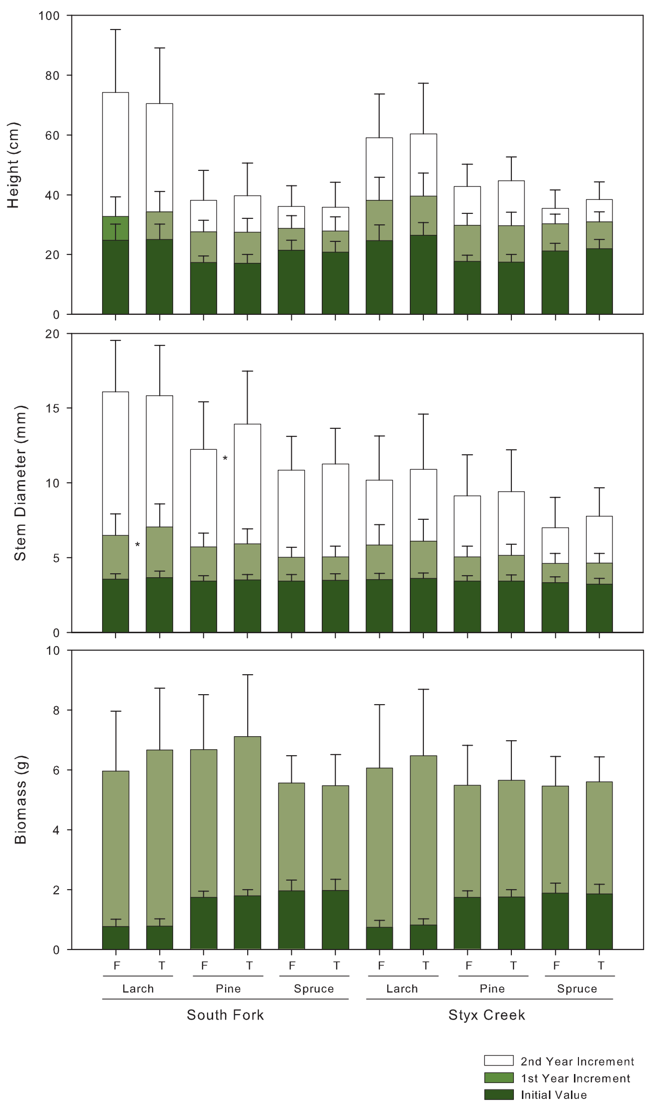

Figure 4. Height, stem diameter, and above ground biomass (mean + SD) of frozen (F) and thawed (T) seedlings of larch, pine, and spruce outplanted at South Fork and Styx Creek. Initial values are shown in dark green, first-year increments in light green, and second-year increments in white. Values that differed between plug temperature treatments for a given species are shown by an asterisk (*) between the plug temperature treatments. Biomass was not calculated in the second year.

plug temperature treatments, species, or sites. After 2 growing seasons, seedling condition still did not vary between plug temperature treatments ($\chi^2 = 2.1$, $P = 0.714$) but varied among species ($\chi^2 = 40.9$, $P < 0.001$) and sites (Table 2). Mortality was highest in larch, and more pine seedlings were assessed as "fair" than for other species. These differences were especially pronounced at South Fork, so seedlings were assessed to be in better condition at Styx Creek ($\chi^2 = 33.4$, $P < 0.001$).

Mean survival was 99% after 1 growing season and 96% after 2 growing seasons. Larch accounted for 57% of the mortality, spruce for 29%, and pine for 14%. Interim visits to assess seedling condition indicated that mortality occurred during the winter (due to rodent herbivory) and during July droughts in both growing seasons.

Seedling Performance: Growth Parameters

Seedling height did not vary between plug temperature treatments ($F = 0.42$, $P = 0.517$) but varied among species ($F = 215.09$, $P < 0.001$) and blocks ($F = 1.78$, $P = 0.049$), and with the site x species interaction ($F = 5.48$, $P = 0.006$). Interactions of time with species and with the site x species interaction were significant ($P < 0.001$) but the interaction with plug temperature treatment was not ($F = 0.05$, $P = 0.955$). Larch grew the most, and spruce the least (Figure 4). Height growth varied more between sites for larch than for pine or spruce, resulting in significant site x species interactions in both growing seasons.

Seedling stem diameter varied between plug temperature treatments ($F = 5.21$, $P = 0.025$) but varied much more among species ($F = 88.36$, $P < 0.001$), sites ($F = 47.43$, $P < 0.001$), and blocks ($F = 4.29$, $P < 0.001$). The site x species interaction was also significant ($F = 3.18$, $P = 0.047$). Interactions of time with species, block, and the site x species interaction were significant ($P < 0.02$) but the interaction with plug temperature treatment was not ($F = 2.28$, $P = 0.109$). At the end of the second growing season, mean stem diameter was 0.6 mm (0.02 in) smaller for frozen than thawed seedlings (Figure 4). In comparison, mean second-year stem diameters varied 4.2 mm (0.17 in) between sites, 4.0 mm (0.16 in) among species, and 7.9 mm (0.31 in) among blocks. Stem diameter growth varied more between sites for larch than for pine or spruce, resulting in a significant site x species interaction in the second growing season.

Seedling biomass differed slightly between plug temperature treatments ($F = 3.20$, $P = 0.078$) but varied much more among species ($F = 10.18$, $P < 0.001$) and blocks ($F = 3.15$, $P < 0.001$), and with the site x species interaction ($F = 6.55$, $P = 0.002$). Interactions of time with block, species, and the site x species interaction were significant ($P < 0.001$). At the end of the first growing season, mean biomass was 0.29 g lower for frozen than thawed seedlings (Figure 4). In comparison, mean biomass varied as much as 0.77 g among species and 2.37 g among blocks.

Seedling sturdiness ratio (SR) did not vary between plug temperature treatments ($F = 0.79$, $P = 0.376$) but varied among species ($F = 73.31$, $P < 0.001$) and sites ($F = 105.38$, $P < 0.001$). Interactions of time with species, site, and the site x species interaction were significant but the interaction with plug temperature was not. SR was significantly lower at South Fork than Styx Creek (Figure 5) and was lowest for pine.

TABLE 2

Condition classes of larch, pine, and spruce seedlings outplanted at South Fork and Styx Creek with frozen or thawed root plugs. Seedling condition was measured after 1 and 2 growing seasons for 1620 seedlings. Data are averaged across blocks and are percentages of seedlings in each class; percentages may not sum to 100% because of rounding error.

Site / Species	After 1 Growing Season				After 2 Growing Seasons			
	Good	*Fair*	*Poor*	*Dead*	*Good*	*Fair*	*Poor*	*Dead*
South Fork								
Larch	96	1	1	1	87	5	1	7
Pine	95	3	1	1	76	20	1	3
Spruce	97	1	1	1	88	8	3	1
Styx Creek								
Larch	95	1	1	3	88	3	3	6
Pine	99	1	0	0	96	3	0	1
Spruce	98	0	0	1	90	4	0	6

DISCUSSION

Warming Trends

Soils at these sites were cooler than in the study by Kooistra and Bakker (2002), and root plugs of frozen seedlings took longer to approach soil temperature (Figure 1). These lags in warming are attributed to the heat absorbed by water within frozen root plugs as it changes state from a solid to a liquid. The rate of thawing in frozen root plugs is inversely related to the soil temperature: less latent heat is available in cooler soils so thawing occurs at a lower rate and more time must elapse before roots can become physiologically active. If frozen seedlings were planted in near-freezing soils, the amount of time required for thawing to occur could be more substantial. The cool soil temperatures at these sites have temporary effects on the plants because soil warms during the summer. In boreal sites, plant growth, particularly belowground, can be reduced by soils that remain cool for the entire growing season (Helenius 2005).

Plug temperatures were measured using paired seedlings, so the warming trends reported here underestimate the rate at which the root plugs of individual seedlings thaw. Single root plugs contain less ice and have higher surface area to volume ratios and therefore warm more rapidly than the paired root plugs used in this study. The 80-ml plug volume used in this experiment is one of the most common sizes used in British Columbia, though root plug volumes used operationally range from 65 to 340 ml (4 to 21 in^3) (MoF 1998). Larger root plugs take longer to warm (Kooistra and Bakker 2002).

Seedling Performance

Frozen and thawed seedlings differed physiologically before and immediately after outplanting, though these differences rapidly disappeared. Fluorescence values were lower for frozen than thawed seedlings (Figure 2), reflecting the more rapid photosynthetic activation of thawed seedlings. Larch exhibited lower fluorescence values in this study than in an earlier study in a nursery setting (Kooistra and Bakker 2002), though values were up to the range reported in the earlier study after 4 nights. Fluorescence values for pine were roughly equivalent with those from the nursery study, while values for spruce began higher in this study than in the nursery study. By the end of the measurement period, fluorescence values were within the range observed for conifer seedlings (Nippert and others 2004; Ritchie and Landis 2005; CM Kooistra, personal observation), though larch values were slightly lower because the foliage had not finished its spring growth.

Fluorescence measurements are a valuable way of rapidly assessing the physiological status of a seedling (Binder and others 1997; Rose and Haase 2002). For example, fluorescence measurements at Styx Creek declined for the first 3 nights following outplanting (Figure 2), likely in response to drying site conditions. The soils at Styx Creek are shallow and were dry at outplanting, and it did not rain until 4 d after planting, after which the fluorescence values of all species rose. The dry conditions at Styx Creek may also explain the more rapid phenological development of seedlings at South Fork (Figure 3).

Seedling phenology was also strongly affected by plug temperature treatment. In particular, thawed larch seedlings advanced in phenology more rapidly than frozen seedlings (Figure 3), as was shown in an earlier nursery study (Kooistra and Bakker 2002) and is frequently observed operationally. The rapid phenological development of thawed larch seedlings predisposes them to injury, particularly to damaged swollen buds during transport and to solarization of foliage that flushes in closed cartons prior to

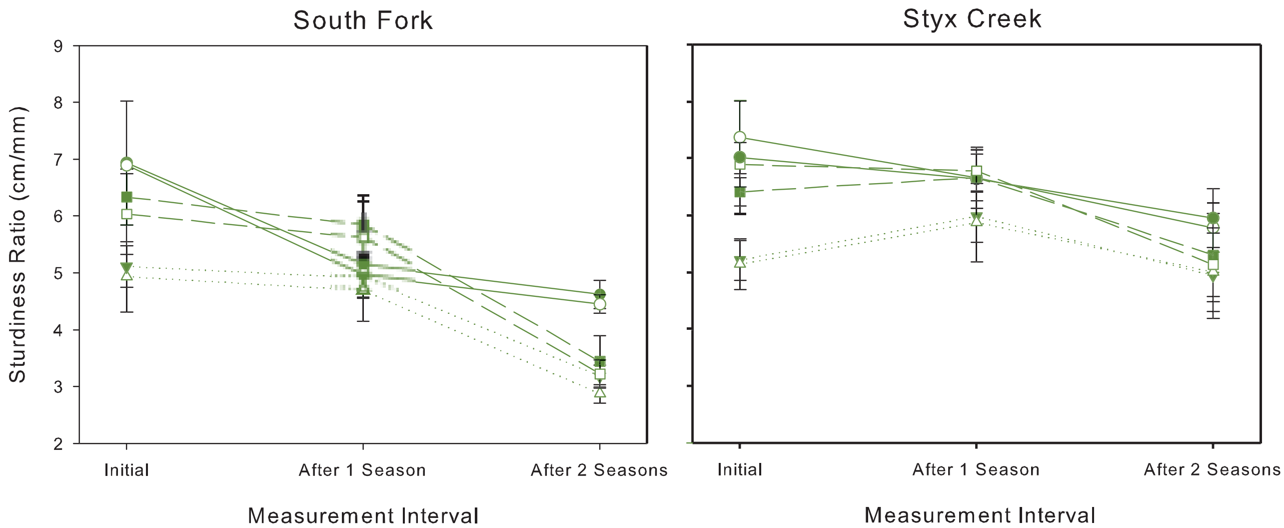

Figure 5. Sturdiness ratio (mean ± SD) of frozen (filled symbols) and thawed (open symbols) seedlings of larch (circles, solid lines), pine (triangles, dotted lines), and spruce (squares, dashed lines) outplanted at South Fork (*left*) and Styx Creek (*right*). Sturdiness ratios were calculated at the time of outplanting and after 1 and 2 growing seasons. Sturdiness ratios did not differ significantly between plug temperature treatments for any species, site, or measurement interval.

outplanting. The slower phenological development of frozen larch seedlings is therefore advantageous from an operational perspective. Similarly, Norway spruce (*Picea abies* [L.] Karst.) seedlings that are rapidly thawed break bud slower (Helenius 2005) and maintain higher freezing tolerance (Fløistad and Kohmann 2001) than seedlings that are slowly thawed. The slower phenological development of rapidly thawed seedlings could be advantageous when outplanting in the early spring or in areas with a risk of late frosts.

The effects of plug temperature treatment on phenology were short-lived: we observed no differences in phenological stage between frozen and thawed seedlings of any species 6 wk after outplanting (Figure 3). Two years after outplanting, seedlings from both plug temperature treatments were thriving, and > 85% of seedlings were rated as "good" (Table 2). As in a previous study (Kooistra and Bakker 2002), larch comprised the majority of seedlings that died. Compared to the other species, pine was in the best condition at Styx Creek but the worst condition at South Fork (Table 2).

Significant site x species interactions indicate that species responded differently to environmental conditions at the 2 sites. In particular, larch exhibited more variation in growth parameters between sites than the other species (Figures 4, 5). Larch also shows a greater response than pine to different watering regimes (Kooistra and Bakker 2002). Pine grew as tall or taller on these sites as it did in a seedling nursery at a lower elevation (Kooistra and Bakker 2002) where conditions were warmer and drier than lodgepole pine prefers.

Plug temperature treatment caused a small but significant reduction in seedling stem diameter and biomass (Figure 4) but no effect on sturdiness ratio (Figure 5). Biomass and sturdiness ratio both integrate height and stem diameter, but biomass is more affected by stem diameter than height (Table 1) whereas SR weights them equally. Plug temperature treatment had no effect on seedling height in this study (Figure 4), whereas Helenius and others (2004) reported that rapid thawing (7 h at 12 °C [54 °F]) negatively affected height growth in the first 2 years at 1 of 2 sites. Overall, much more of the variation in growth parameters was attributable to interspecific differences and to large- and small-scale environmental differences (that is, differences among sites and blocks) than to plug temperature treatment. Similar results are evident in other published studies: Fløistad and Kohmann (2001) report differences between seedlots in frost hardiness and bud break, and Helenius and others (2004) indicate that seedling height after 3 y differed by about 10 cm between sites but by about 5 cm between thawing regimes within sites. Environmental differences will be increasingly expressed in the growth and phenology of seedlings and will further mask initial differences attributable to nursery culture (Grossnickle and Folk 2003). Even differences in stock type have less of an effect on seedlings than differences among outplanting locations (Jones and others 2002; Campbell and others 2003).

CONCLUSIONS AND IMPLICATIONS

Seedlings outplanted with frozen root plugs took several hours to thaw and warm to soil temperature. Fluorescence measurements indicated that seedlings outplanted with thawed root plugs were functioning at a higher level of physiological activity than those outplanted with frozen root plugs, though this difference disappeared within 3 d of outplanting. Frozen seedlings advanced in budburst phenology slower than thawed seedlings. Over 2 growing seasons, frozen seedlings had slightly lower stem diameter and biomass than thawed seedlings, but condition, height, and sturdiness ratio did not differ between plug temperature treatments. Interspecific and environmental differences accounted for much more of the variation in measured attributes than did plug temperature treatment.

This study demonstrated that seedlings of western larch, lodgepole pine, and interior spruce are tolerant of being outplanted with frozen root plugs. Packaging seedlings individually before frozen storage and removing the thawing stage from the seedling production process would provide significant operational advantages with respect to cold storage management, coordination of transportation to planting sites, and the logistics of planting operations.

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AUTHOR INFORMATION

Clare M Kooistra
Conifera Consulting
7305 Ogata Way
Vernon, BC V1H 1C2
cmkooistra@shaw.ca

Jonathan D Bakker
Research Specialist
Ecological Restoration Institute
Northern Arizona University
PO Box 15017, Flagstaff, AZ 86011-5017
Jonathan.Bakker@nau.edu