Thawing Regimes for Freezer-Stored Container Stock

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Three thawing regimes were applied over a 6-week period to frozen Douglas-fir (Pseudotsuga menziesii *(Mirb.) Franco.), western larch* (Larix occidentalis *Nutt.), and ponderosa pine* (Pinus ponderosa *Dougl. ex Laws.) container stock: (1) rapid thaw followed by cold storage, (2) slow thaw, and (3) freezer storage followed by rapid thaw. Seedlings were outplanted to 3 sites in north-central Washington. A subsample of seedlings were evaluated for root growth potential (RGP) at the time of outplanting. Seedling performance was assessed after the first and second growing seasons. Although there were significant differences among species, thawing regime did not affect seedling growth or survival after 2 growing seasons nor did it affect RGP. The results indicate that seedlings can tolerate variations in thawing practices that may occur due to weather or other circumstances beyond control. However, it is noted that it may be best to keep seedlings in freezer storage for as long as possible in order to prevent storage molds.* Tree Planters' Notes 48 (1/2): 12-17; 1997).

Freezer storage of container seedlings, although an accepted practice in the nursery industry, is still a relatively misunderstood technique in some forest nurseries and reforestation organizations. Research and experience have shown that freezer storage can be a valuable management tool to a successful reforestation program. Freezer storage gives the nursery greater flexibility by allowing for lifting during late autumn and shipping the following spring. This results in a more balanced work load at the nursery and an effective "surge buffer" between nursery and field production (Hee 1987). Colombo and Cameron (1986) found that freezer storage of container black spruce—Picea *mariana* (Mill) B.S.P.- allows managers to safely delay budset of a late-sown crop, thereby reaching minimum acceptable height, without the risk of winter damage associated with outdoor storage. Furthermore, freezer storage is more suitable for periods in excess of 2 months, because carbohydrate depletion and storage molds can be a problem with long-term cold (2 °F) storage (Ritchie 1982, 1984).

Freezer storage is often necessary to maintain crop dormancy when late-season planting is required in snowed-in units, especially for stock to be planted to high-elevation sites. Odlum (1992) noted that black spruce seedlings kept in frozen storage had greater subsequent root and shoot growth than those wintered outdoors, especially for those outplanted at a later date. Ritchie (1984, 1989) found that the rate of dormancy release in bareroot Douglas-fir—Pseudotsuga *menziesii* (Mirb.) Franco—seedlings was substantially retarded by freezer storage compared to those left in the nursery bed resulting in an expansion of the planting window and a higher, more uniform, physiological quality. Likewise, Lindström and Stattin (1994) found that freezer-stored seedlings of Norway spruce *(Picea abies* (L.) Karst.) and Scots pine *(Pin us sylvestris* L.) had a greater tolerance to freezing in the spring than those that were stored outdoors.

A concern with freezer storage is the thawing process. One thawing method commonly used is to allow the stock to thaw very slowly at temperatures just above freezing over a period of several weeks. Another method is to place seedlings in an area with ambient temperatures for several days prior to outplanting. The standard thawing practice for Weyerhaeuser nurseries is to spread seedling pallets out and allow them to thaw at ambient temperature (10 to 15 °F) for 3 to 5 days (bareroot seedlings) and for 10 to 15 days (container seedlings) (Hee 1987). Whether thawed rapidly or slowly, field foresters prefer to have the stock thawed just prior to outplanting. However, changing weather conditions or other circumstances beyond control can result in thawed stock being held for several weeks in cold storage prior to outplant. Hee (1987) noted that it is best to plant seedlings as soon as they have thawed, but also noted that they can be held in cooler storage after thawing for up to 4 weeks without detriment.

The objective of this study was to examine the effects of 3 thawing regimes on the subsequent quality of 3 species of container-grown conifer seedlings outplanted to 3 sites. The thawing regimes were designed to simulate circumstances typically encountered with frozen stock. The null hypothesis was that there would be no differences in seedling field performance for any of the species due to thawing treatment.

Materials and Methods

Douglas-fir, western larch *(Larix occidentalis* Nutt.), and ponderosa pine *(Pinus ponderosa* Dougl.) container stock (1-year-old Styro-8) were used in this study. For each species on each outplanting site, seedlings were from the same seedlot. Seedlings were grown and freezer stored under standard nursery practices.

Seedlings were shipped frozen to the Leavenworth District of the Wenatchee National Forest in late March to early April 1995, depending on the expected date of planting for each site. Three thaw schedule treatments were applied over a 6-week period as follows:

1. Seedlings were placed under a rapid thaw (5 days at 7 °C = 44.6 °F) 6 weeks before expected outplanting, then held in cold storage (1 °C = 33.8 °F) until outplanting.
2. Seedlings were placed in cold storage for a slow thaw (6 weeks) before outplanting.
3. Seedlings were kept in freezer storage (-2 °F = 28.4 °F) until 1 week before outplanting, when they were placed under a rapid thaw.

Telog temperature recorders (Model 2103, Telog Instruments Inc., Victor, NY) were placed with seedlings in each thawing treatment. Because there were a limited number of Telogs available and because Telog data cannot be examined until it is downloaded to a computer, additional digital temperature probes were placed with the seedlings and monitored weekly.

Seedlings were outplanted to 3 sites on the Wenatchee and Okanogan National Forests in north-central Washington as follows:

- Twisp District, Okanogan National Forest; high-elevation (1,372 m = 4,500 ft) dry site. The slope is 10 to 40% with a northeastern aspect, with light slash and vegetation. All 3 species were planted on June 1, 1995.
- Leavenworth District, Wenatchee National Forest; low-elevation (610 m = 2,000 ft) dry site in area burned by 1994 wildfire. Annual precipitation is 53 to 76 cm (20 to 30 in). Soil is sandy to clay loam. The slope is 60% and the burned trees (avg. dbh = 10 cm = 4 in) were left standing. Douglas-fir and ponderosa pine were planted on April 20, 1995.
- Naches District, Wenatchee National Forest; high-elevation (1,219 m = 4,000 ft) temperate site. The slope is 15% with a western aspect. Douglas-fir and western larch were planted on May 31, 1995.

Seedlings were outplanted at about the same time that the site was scheduled to be operationally planted. Because of late-winter conditions, the 6-week thawing period was extended by 7 to 10 days for seedlings planted on the Twisp and Naches Districts. For each site, all seedlings were planted on the same day. Seedlings were planted at a spacing of 1.5 x 1.5 m (= 4.9 x 4.9 ft).

Initial height and survival were measured and recorded 2 weeks after outplanting and again at the end of the first and second growing seasons (September 1995 and August 1996). In addition, a damage/vigor assessment (incidence of browse, chlorosis, etc.) was recorded for each seedling.

In addition to the outplanted seedlings, a subsample of 15 seedlings of each species/treatment from the Leavenworth and Twisp sites were sent to International Paper's Lebanon facility shortly after seedlings were outplanted (that is, after treatment) and evaluated for root growth potential. These seedlings were potted and allowed to grow in a greenhouse for 3 weeks, then evaluated for the number of seedlings with new roots.

The experimental design consisted of a split-plot design with 5 blocks, 2 or 3 species per site (whole plots), 3 thaw treatments (subplots), and 10 seedlings in each block/species/treatment for a total of 450 seedlings on the Twisp site and 300 seedlings on the Leavenworth and Naches sites. All seedlings were labeled and randomly planted within a block.

An analysis of variance (ANOVA) was performed on all data to determine if thaw treatment has a significant effect on subsequent seedling performance. Differences among mean values for species and treatment were determined using Fisher's protected least significant difference procedure. Statistical Analysis Software (SAS Institute 1989) was used for all data analyses.

Results

It took about 5 days to accomplish the rapid thaw (treatments 1 and 3) and about 3 weeks for the slow thaw (treatment 2) (figure 1).

As would be expected, there were significant differences in field performance among species on each site (figures 2 and 3). However, there did not appear to be any meaningful differences among thawing treatments. During the first season, there were significant treatment by species interactions for both height and growth on the Leavenworth and Naches sites (figure 2). However, despite the statistical significance between treatments, the differences in first-year average height and growth may not be significant from a reforestation perspective, as the differences are small (1 to 3 cm = .4 to 1.2 in) and the ranking does not follow any pattern with regard to the treatments. For example, treatment 1 Douglas-fir had the greatest height on the Leavenworth site, whereas treatment 3 Douglas-fir had the greatest height on the Naches site. Similarly, treatment 3 ponderosa pine had the most growth on the Leavenworth site whereas treatment 1 western larch had the most growth on the Naches site. During the second growing season, there were no significant differences among thawing treat-

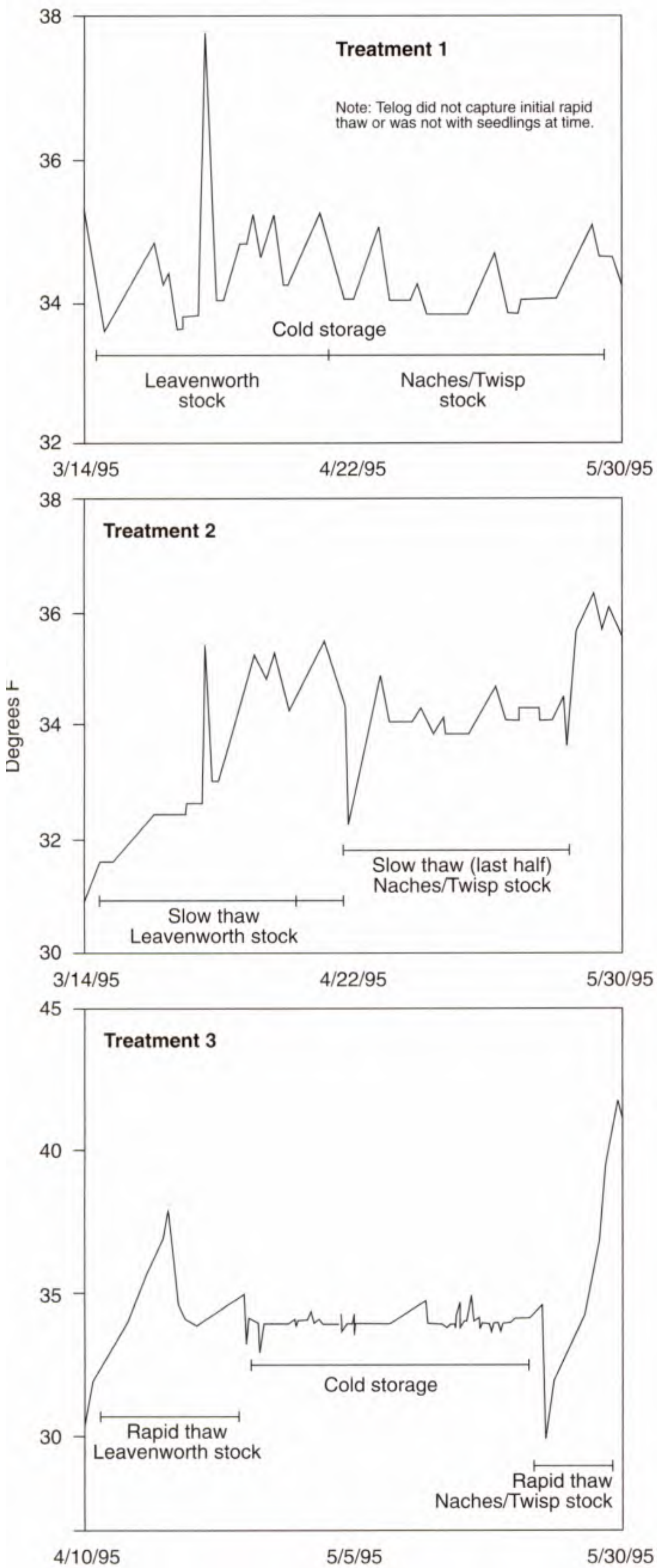

Figure 1—*Output from Telog temperature recorders showing the thawing process of each treatment.*

ments for total height, seasonal height growth, or total height growth on any of the 3 sites (figure 3).

Survival averaged 77% on the Twisp site and 96% on the Leavenworth site regardless of species or treatment. On the Naches site, survival was not influenced by treatment but was very poor for Douglas-fir (23%) compared to western larch (83%). Thaw treatment had no effect on root growth potential.

Discussion

We found that thawing regime did not affect subsequent seedling field performance. In a similar study, Camm and others (1995) reported that there were very few differences between white spruce *(Picea glauca* (Moench) Voss) and Engelmann spruce *(Picea engelmannii* Parry) seedlings planted either directly from the freezer or after 9 days of thawing. The latter broke bud 3.3 days earlier than those planted directly from the freezer but had a less uniform budbreak. Height, shoot and root mass did not differ after 3 months of growth. Camm and others (1995) suggest that a suitable on-site operational protocol for rapid thawing might be to lay frozen bundles on the ground at ambient temperature overnight. Additional possible benefits to this approach that they mention include reductions in handling costs, secondary storage facilities, and losses caused by refrigerator failure (Camm and others 1995).

The idea of a long, slow thaw has been to allow normal physiological processes to fully resume prior to planting. However, this may not be necessary because recovery of water potential after thawing spruce seedlings took hours, not days, once ice crystals left the roots (Camm and others 1995). As a result, these authors recommend against the practice of slowly thawing seedlings for up to several weeks before shipping to the plantation site because fungi *(Botrytis* spp.) often proliferate on seedlings held above freezing in the dark for extended periods. Another study showed that steady-state respiration rates increase significantly during thawing and hence have the potential to greatly deplete carbohydrate reserves, especially over time (Levesque and Guy 1994).

On the other hand, Odlum (1992) stated that rapid thawing of stock can result in damage or mortality attributable to shoots rapidly rising to ambient thaw temperature, while seedling plugs remain frozen, due to their higher water content,. Thus, foliar transpiration without water availability from the roots results in desiccation. Odlum recommended that stock be thawed slowly as described by Koistra and others (1989); seedlings are first exposed to 5 °C until completely thawed. Our findings do not suggest the need for this.

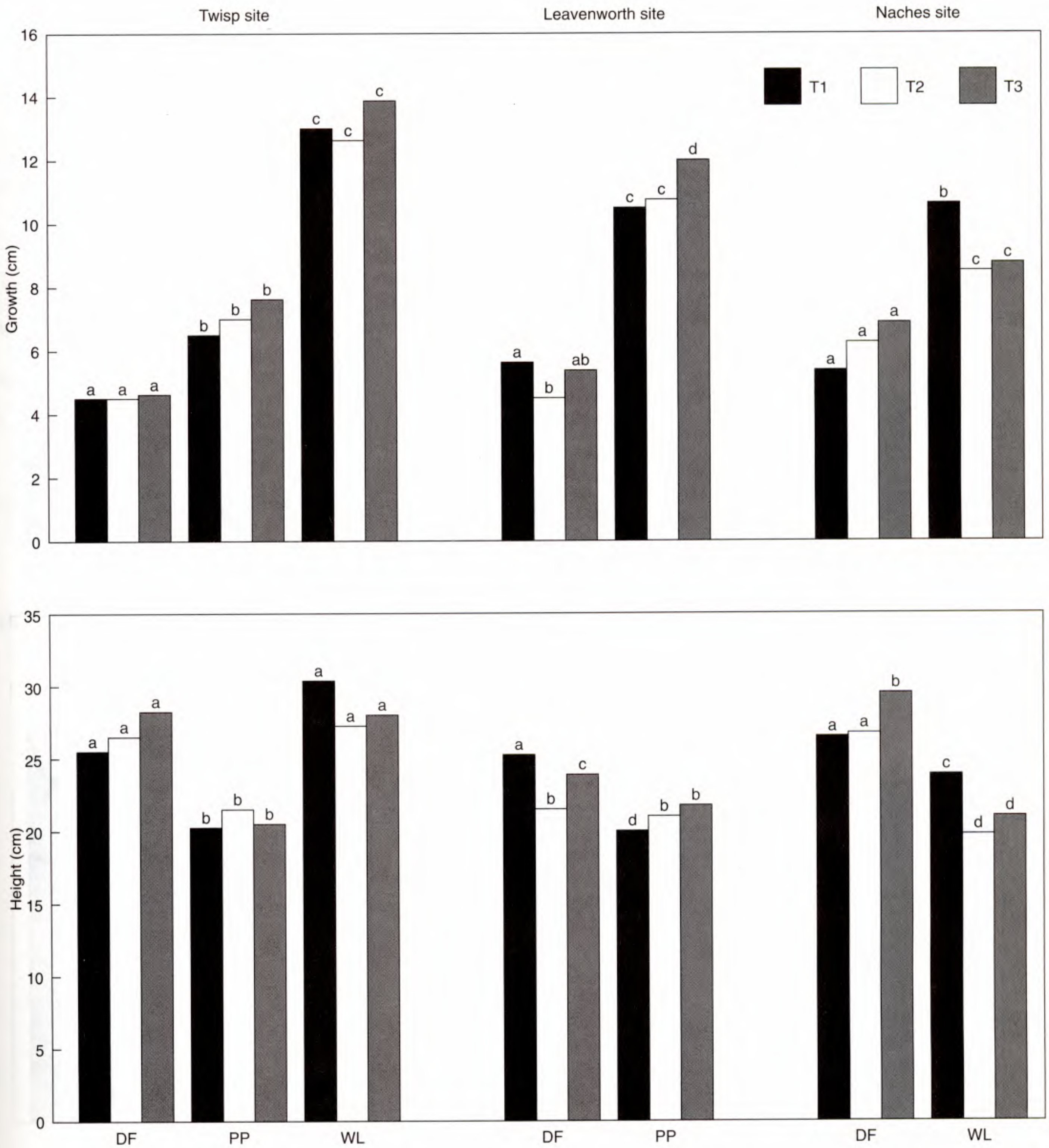

Figure 2—*Total seedling height and growth after the first growing season in the field (1995). On each site, bars with different letters are significantly different at the* $\propto \leq 0.05$ *level.*

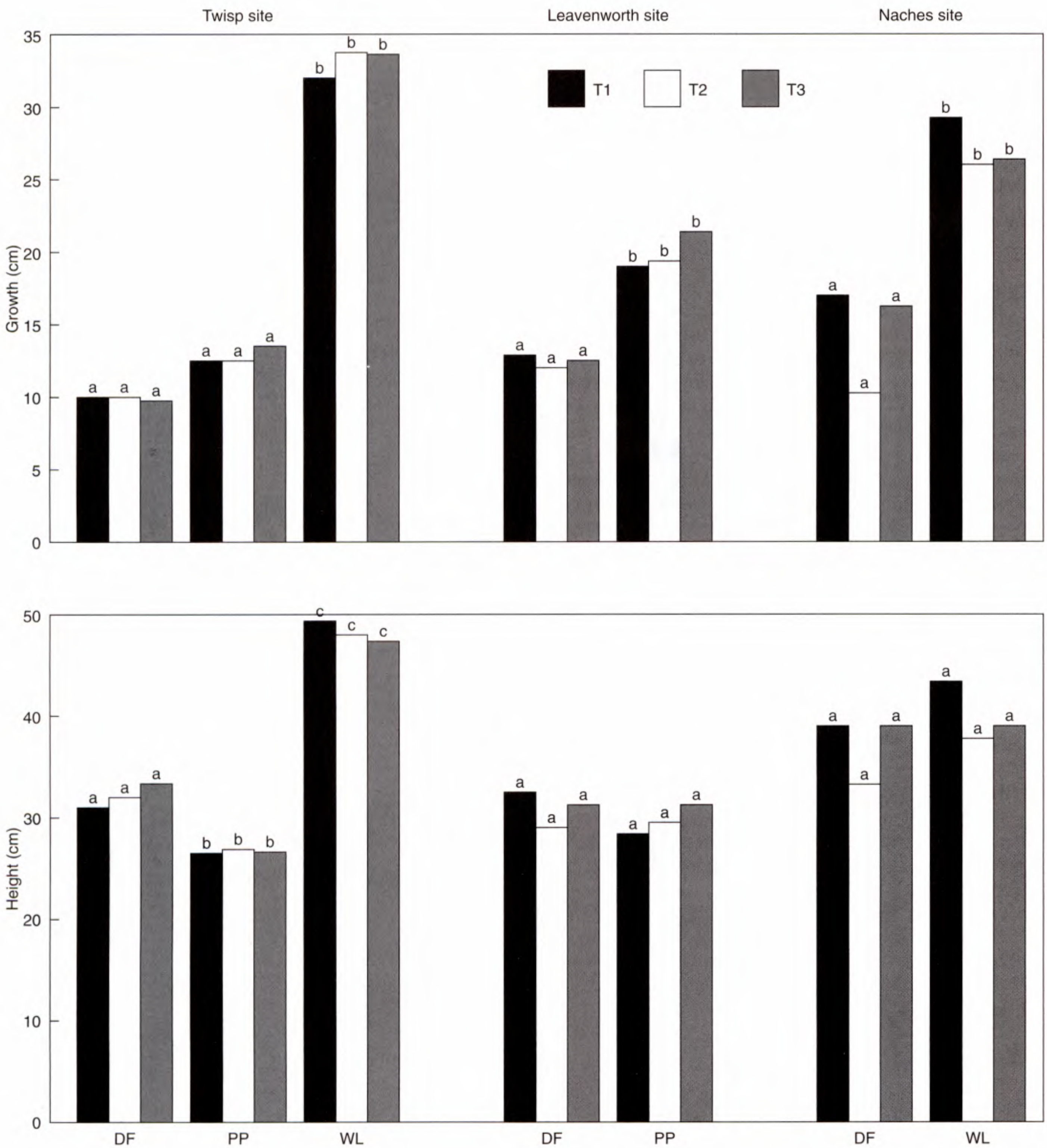

Figure 3—*Total height and growth after 2 growing seasons (1996). Although species differed significantly, there were no significant differences between thawing treatments. On each site, bars with different letters are significantly different at the ≮ 5. 0.05 level.*

Conclusions

Despite assertions in the literature of damage to seedlings caused by either rapid or slow thawing, the results of our study indicate that container seedlings can withstand variations in thawing regimes, as we described, without any detrimental effect to their subsequent field performance. However, managers concerned with post-storage fungal infection should consider using short thawing intervals.

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