Some Effects of Cold Storage on Seedling Physiology

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*When tree seedlings are lifted from the nursery in winter and placed into cold storage, they are no longer exposed to the natural environmental factors that provide energy for growth and information for phenological development. This affects many important physiological variables that influence seedling quality. This paper summarizes several years of storage physiology research on Douglas-fir (*Pseudotsuga menziesii *(Mirb.) Franco). Stored carbohydrates are depleted, dormancy release is slowed, and cold hardiness is gradually lost in cold storage. Root growth potential may increase, decrease, or remain constant, depending on lift date, storage duration, and species. Effects of cold storage on seedling water relations have not been adequately investigated.* Tree Planters' Notes 38(2):1115; 1987.

Cold or frozen storage enables nursery managers and foresters to hold fall- or winter-lifted nursery stock until spring planting. Because of this, it has become an invaluable tool in forest regeneration operations in the Pacific Northwest.

In the natural outdoor environment, tree seedlings are exposed to strong diurnal fluctuations in air and soil temperature, light intensity and duration, soil and atmospheric water status, and other factors. Over the millennia, tree species have adapted to use these factors as sources of both energy for growth and information for driving phenological development (1).

When seedlings are lifted from the nursery or greenhouse and stored in the cold and dark, they no longer experience these environmental changes. Rather, temperature remains low and constant, light is absent, and humidity is very high.

In this paper, I will discuss some important physiological processes and variables and outline the manner in which they respond to the cold storage environment, based almost entirely on our research and experience with coastal Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) seedlings at Weyerhaeuser. When available and pertinent, data from other conifers are also cited. The focus of the review is on seedling quality.

Physiological Variables Affected

Innumerable physiological processes and variables affect seedling physiological quality. Among the variables strongly affected by cold storage are: (1) carbohydrate reserves, (2) bud dormancy status, (3) root growth potential, (4) cold hardiness, and (5) water relations.

Carbohydrate reserves. Nearly all plant food reserves are stored in the form of starch and sugars. These are produced ultimately by photosynthesis and are consumed by respiration to sustain plant growth and metabolism. Both photosynthesis and respiration are strongly temperature-dependent, and photosynthesis requires light.

Cold storage affects photosynthesis and respiration in two ways. First, the absence of light stops photosynthesis and second, low temperature decreases the rate of respiration. The net effect is that seedlings burn up their supply of reserve carbohydrates in storage, but they do so very slowly.

In an experiment with 2+0 Douglas-fir, total nonstructural carbohydrate (TNC) concentrations in January were highest in foliage (4). During the first 2 months of storage, foliar TNC was respired more rapidly than stem or root TNC (fig. 1). During the following 20 months, a near-linear decrease in TNC occurred in all tissues, the result being that during 1 year the seedlings had consumed roughly half their food reserves. Storage temperature also affects the rate of loss of food reserves. Douglas-fir

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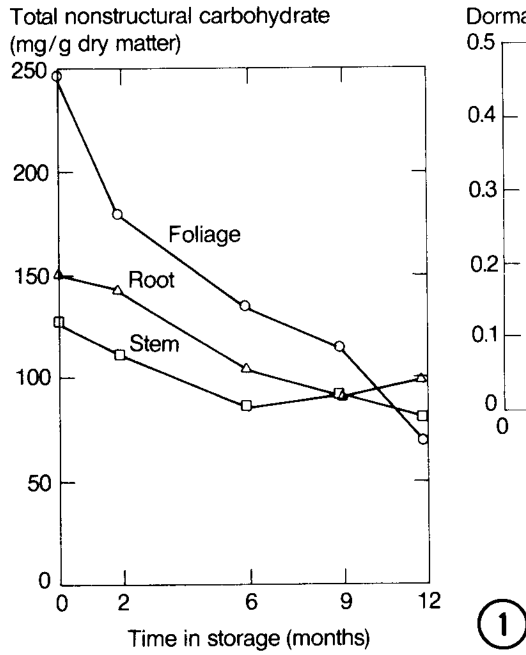
seedlings stored at -2 °C contained about 2.5 milligrams per gram more TNC after 6 months than did those stored at +2 °C (Ritchie, unpublished data).

It would be valuable to know how much food reserve is necessary to ensure survival and adequate early growth, but this information is not yet available. As a first approximation, 10 to 12 milligrams per gram might be a reasonable estimate.

Bud dormancy status. By late fall (October) in the coastal Pacific Northwest, conifer seedlings normally will have reached the peak of dormancy (3). As winter progresses, continual exposure to temperatures below about 6 °C (chilling) acts to release dormancy. By March, dormancy release is complete and seedlings will break bud and begin growing upon exposure to warm, springlike conditions.

This progress through dormancy to dormancy release can be visualized by plotting a dormancy release index (DRI) curve over the accumulation of hours of chilling temperatures. DRI for Douglas -fir is calculated as the number of days to terminal budbreak of seedlings held in a warm, forcing environment (DBB) divided into 10 (5). As dormancy release progresses through winter the DRI value approaches unity. This relationship is shown for 2+0 Douglas -fir seedlings of four seed zones in figure 2.



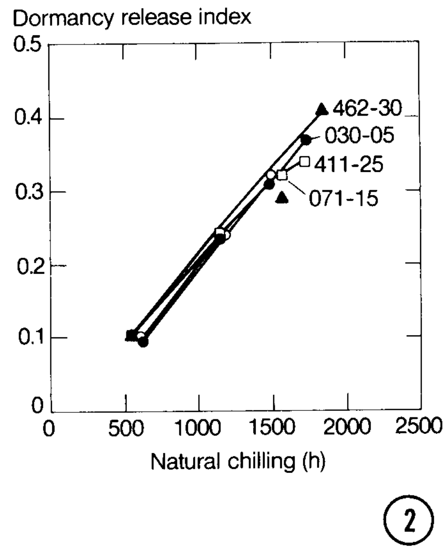
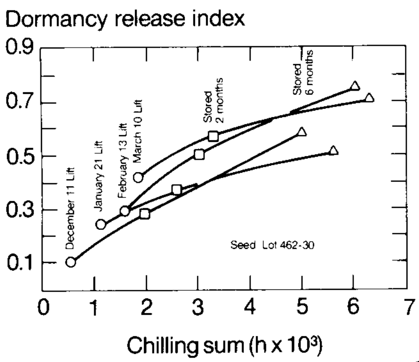


Figure 1—*Changes in total nonstructural carbohydrate concentrations in foliage, stems, and roots of 2 + 0 Douglas-fir seedlings lifted January 27, 1978, and stored at − 1 °C. Vertical bars ± 1 standard error. Reproduced with permission from Canadian Journal of Forest Research 12(4):908; 1982.*

Figure 2—*Dormancy release index in 2 + 0 Douglas-fir seedlings as a function of natural (nursery) chilling. Data are for the winter of 1979–80. Each point is a mean (± standard error) of 15 seedlings held in a forcing environment. A chilling hour is defined as one during which the air temperature is below 6 °C. Reproduced with permission from Canadian Journal of Forest Research 14(2):188; 1984.*

When seedlings are lifted from the nursery and placed into cold storage, several things occur that affect this relationship. First, seedlings are no longer exposed to daily fluctuating light and temperature; and second, they are held at a temperature that is apparently not very efficient at releasing dormancy. The net effect is that dormancy release does occur-but at a much reduced rate.

In the experiment illustrated in figure 3, we lifted Douglas -fir seedlings on four dates during winter and determined their DRI values. (These are plotted as circles on the figure.) We then held back samples of these seedlings



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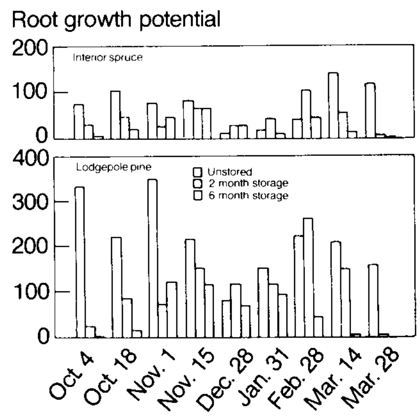

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Figure 3—*Dormancy release index in 2 + 0 Douglas-fir seedlings from a seed zone in the western Oregon Cascades during winter of 1979–80. Seedlings were lifted at one of four times during winter and stored at −1 °C for 2 or 6 months. Chilling sum is the sum of nursery chilling and hours held in storage. Reproduced with permission from Canadian Journal of Forest Research 14(2):188; 1984.*

Figure 4—*Root growth potential (number of new roots per seedling) of 2 + 0 lodgepole pine and interior spruce seedlings lifted throughout winter from a central British Columbia nursery. Seedlings were tested immediately after lifting and after 2 and 6 months in −1 °C storage. Each value is a mean (± standard error) of 20 seedlings. Reproduced with permission from Canadian Journal of Forest Research 15(4):639, 1985.*

for storage at -1 °C for 2 (squares) and 6 (triangles) months, then removed them and again determined the DRI values. In each case, seedlings were far more dormant after storage than they would have been had they been allowed to remain in the nursery beds. Similar experiments were performed with lodgepole pine (*Pinus contorta* Dougl. ex Loud.), and interior spruce (*Pinus glauca-englemannii* complex) with similar results (7), suggesting that this may be a relatively common response in many conifers.

The practical implication of this phenomenon is that one can lift stock in fall or winter when it is dormant and hold it in a dormant condition well into spring for spring planting. It is primarily because of this relationship that cold storage works as well as it does.

Root growth potential. Root growth potential (RGP) is not a physiological process per se. However, it integrates many important physiological processes in the seedling and, for this reason, has become a popular and useful indicator of seedling vigor. The rationale is that if there is any problem with the seedling physiologically, it should show up as a decrease in the seedling's ability to produce roots.

Root growth potential is strongly affected by cold storage. In 2+0 Douglas-fir a very clear pattern has emerged over several seasons of testing. Root growth potential is low in fall and early winter, increases and peaks in December and January, then decreases in February to a low in March. With respect to cold storage: 2 months of storage is nearly always beneficial, the greatest benefit being gained with stock lifted in fall and early winter; while 6 months of storage is rarely beneficial, especially when stock is lifted in late winter or spring.

This relationship is apparently not universal, however. In a study with lodgepole pine and interior spruce (7) quite different patterns were observed (fig. 4). The reasons for these differences are not known and until the underlying mechanisms driving RGP are understood it will probably be necessary to develop this information for each species and, perhaps, each nursery as well.

Cold hardiness. A seedling's ability to endure subfreezing temperatures varies dramatically over the course of the year. In summer, exposures to -5 °C are sufficient to kill Douglas-fir seedlings. But in mid-winter these same seedlings can easily withstand temperatures below -20 °C. Hardier northern species such as white spruce (*Picea glauca* (Moench.) Voss) and lodgepole pine can withstand midwinter temperatures approaching -80 °C.

Hardiness develops in fall in response first to shortening photoperiod then to increasing exposure to cold nights (2). As nights become increasingly colder, seedlings become more and more hardy. Increasing photoperiod and higher temperatures in spring cause seedlings to lose hardiness rapidly. This is why late frosts can be so damaging.

One would suspect that removal of a seedling from these environmental signals by placing it in cold storage would interfere with the development of natural hardiness. Further, because carbohydrate reserves undergo a net loss during storage and hardiness development requires an expenditure of metabolic energy, one would expect a loss of hardiness with time in storage.

Unfortunately, very little information exists on the effect of cold storage on hardiness in tree seedlings. However, the limited data that do exist (table 1) tend to confirm the above predictions. Pine and spruce seedlings lifted early in winter while hardiness was developing did not continue to harden in storage, rather they slowly lost hardiness. Seedlings lifted in spring continued to deharden in storage.

Table 1—*Estimated values of lethal temperatures for 50 percent of the test population (LT_{50}, in °C) for lodgepole pine and interior spruce seedlings at time of lifting and after 2 or 6 months in storage at −1° C*

Lifting date storage period	Treatment date	Lodgepole pine	Interior spruce
October 4, 1982			
0	October 4, 1982	(−20)	(−26)
2 mon	December 4, 1982	(−14)	−27
6 mon	April 4, 1983	—	−14
November 1, 1982			
0	November 1, 1982	−29	−30
2 mon	January 1, 1983	−26	−26
6 mon	May 1, 1983	−20	−25
March 28, 1983			
0	March 28, 1983	−18	−18
2 mon	May 28, 1983	(−11)	−18
6 mon	September 28, 1983	−7	−18

Values in parentheses are extrapolations, the remaining values are interpolations of percent injury over temperature curves from whole-plant freeze tests.

Taken from Canadian Journal of Forest Research 15(4):640, 1985.

More research is needed on this question, for it has important implications. Suppose, for example, that seedlings are lifted in the fall for planting in midwinter. If they have not developed adequate hardiness before lifting and are planted on a very cold site, they might suffer considerable winter damage. One wonders how much overwinter damage can be attributed to this cause. On the other hand, seedlings that are lifted in winter can be held well into spring for planting at high elevations where exposure to low temperatures is expected. In the table 1 data, for instance, seedlings lifted in November were still hardy to -20 or -25 °C when tested the following May.

Water relations. Seedling water relations are very complex, and a complete discussion is far beyond the scope of this paper. Suffice it to say that during mid-winter conifers exhibit very "favorable" water relations properties, that is, they are able to tolerate substantial desiccation of both tops and root systems without incurring appreciable damage (6,8).

By spring (March) when growth begins, water relations properties shift abruptly to a far less favorable status and seedlings become very sensitive to water-related stresses. The success of the mid-winter lifting window may be due in part to these highly favorable seedling water properties.

The question is: what effect does storage have on seedling water status? Do seedlings maintain favorable water status in storage or does water status deteriorate? Unfortunately this question has not been studied. One might speculate, however, that because carbohydrates are depleted in storage, and because water relations reflect osmotic relations that depend to a degree on dissolved carbohydrates in the cells, we would see a gradual deterioration of seedling water status with time in cold storage. This might partially explain why storage beyond 6 to 9 months almost invariably results in poor performance of planting stock. This is an interesting and important question and deserves to be investigated.

Summary and Conclusion

When tree seedlings are held in cold, dark storage for prolonged periods of time, they are separated from the sources of environmental energy and information they need to develop in synchrony with the changing seasons. This affects many important physiological processes and variables. Effects of cold storage on dormancy release, carbohydrate depletion, and root growth potential have been studied and are reasonably well understood at least in an empirical sense. Effects on some other important variables such as cold hardiness and water relations are less well known.. On balance, positive effects of cold storage heavily outweigh negative effects, hence it has become a widespread and very useful practice throughout most of the Pacific Northwest.

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