Some Effects of Cold Storage on Seedling Physiology[1]

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Abstract.--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 which provide energy for growth and information for phenological development. This affects many important physiological variables which influence seedling quality. This paper summarizes several years of storage physiology research on Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) conducted by Weyerhaeuser Company.

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.

INTRODUCTION

Cold or frozen storage of planting stock enables nurserymen and foresters to bridge the gap between fall or winter lifting and 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 millenia tree species have adapted to use these factors as sources of both energy for growth and information for driving phenological development (Campbell 1978).

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

This paper will consider some important physiological processes and variables and outline the manner in which they respond to the cold storage environment. It is 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

Enumerable physiological processes and variables affect seedling physiological quality. Among those which are 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.

1Paper presented at the Western Forest Nursery Council Meeting, [Tumwater, Washington, August 12-15, 1986).

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[3]The author thanks Dr. Nicholas Wheeler for reviewing and Ms. Joan Ruthford for preparing this manuscript.

Cold storage impacts photosynthesis and respiration in two ways. First, absence of light stops photosynthesis; 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 non-structural carbohydrate (TNC) concentrations in January were highest in foliage (Ritchie 1982). During the first two months of storage foliar TNC was respired more rapidly than stem or root TNC (fig. 1.). During the following 10 months a near-linear decrease in TNC occurred in all tissues - the result being that during one year the seedlings had consumed roughly half their food reserves. Storage temperature also affects the rate of loss of food reserves. Douglas-fir seedlings stored at -2°C contained about 2.5 mg/g 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 mg/g 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 (Lavender 1985). 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, spring-like 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 (Ritchie 1984). 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.

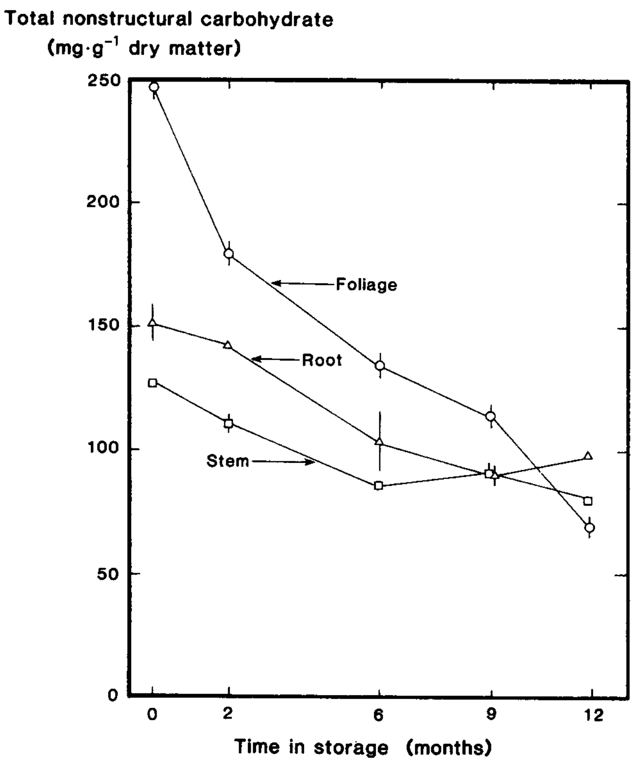

Figure 1.--Changes in total nonstructural carbohydrate concentrations in foliage, stems, and roots of 2+0 Douglas-fir seedlings lifted January 27, 1978 and stored at -1°C. Vertical bars = + 1 standard error. Reproduced with permission from Canadian Journal of Forest Research 12(4): 908,1992.

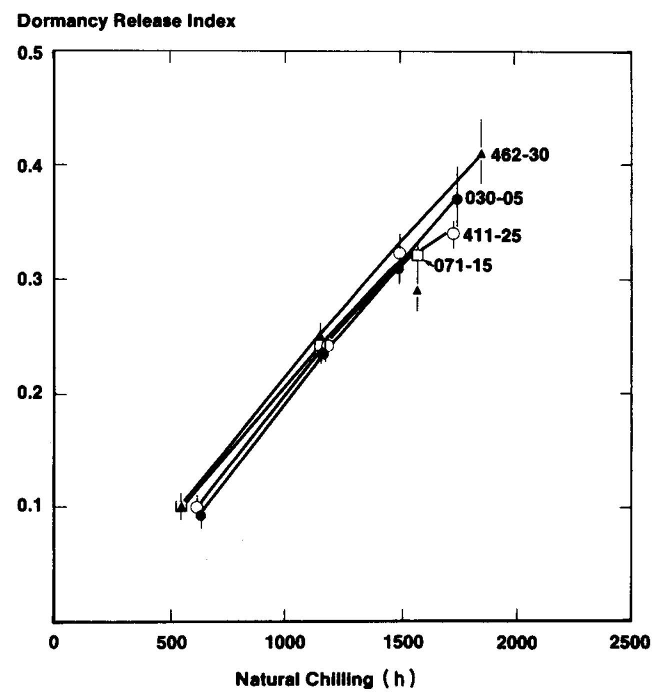

Figure 2.--Dormancy release index in 2+0 Douglas-fir seedlings as a function of natural (nursery) chilling. Data are for the winter of 1979-1980. Each point is a mean (± standard error) of 15 seedlings held in a forcing environment. A chilling hour is defined as one during which the air temperature is 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 which affect this relationship. First, seedlings are exposed no longer to daily fluctuating light and temperature; and second, they are held at a temperature which 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 value. These are plotted as circles on the figure. We then held back samples of these seedlings for storage at -1°C for two [**[]] and six [^]** months, then removed them and again determined the DRI values. In each case, seedlings were far more dormant following 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.) and interior spruce (*Picea glauca-engelmannii* complex) with similar results (Ritchie et al. 1985) 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.

RGP is strongly affected by cold storage. In 2+0 Douglas-fir a very clear pattern has emerged over several seasons of testing. RGP 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: two-month storage is nearly always beneficial - the greatest benefit being gained with fall and early-winter lifted stock, while six-month 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 (Ritchie et al. 1985) quite different patterns were observed (fig. 4). The reasons for these differences are not

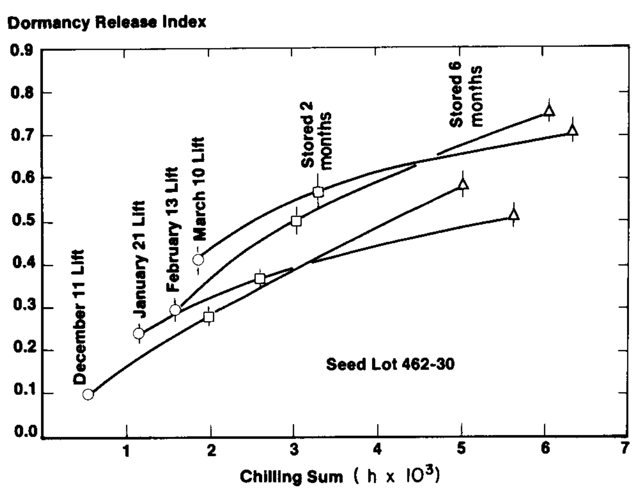

Figure 3.--Dormancy release index in 2+0 Douglas-fir seedlings from a western Oregon Cascade seed zone during winter of 1979-1980. Seedlings were lifted at four times during winter and stored at -1°C for 2 or 6 months. Chilling sum is the sum of nursery chilling plus hours held in storage. Reproduced with permission from *Canadian Journal of Forest Research* 14(2): 188, 1984.

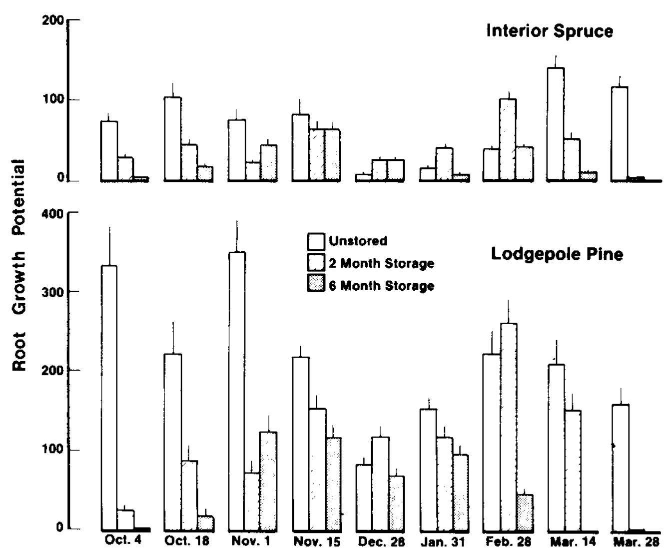

Figure 4.--Root growth potential (number of new roots per seedling) of 2+0 lodgepole pine and interior spruce seedlings lifted throughout winter from a central British Columbia nursery. Seedlings were tested immediately after lifting and after 2 and 6 months in -1°C storage. Each value is a mean (standard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

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. With hardier northern species such as white spruce (_Picea lauca_ (Moench.) Voss) and lodgepole pine, midwinter hardiness can approach -80°C.

Hardiness develops in fall in response first to shortening photoperiod then to increasing exposure to cold nights (Glerum 1985). 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, since carbohydrate reserves undergo a net loss during storage and since 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 which do exist (table 1) tend to confirm the above predictions. 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 de-harden in storage.

More research is needed on this question for it has important implications. Suppose, for example, that seedlings are fall-lifted for mid-winter planting. If they have not developed adequate hardiness before lifting and are planted on a very cold site they might suffer considerable winter damage. One

Table 1.-- Estimated values of LT5o (deg. C)[1] for lodgepole pine and interior spruce seedlings at time of lifting and following 2 or 6 months in storage at -1°C. Reproduced with permission from _Canadian Journal of Forest Research_ 15(4): 640, 1985.

Date Lifted	Storage Period (months)	Date Tested	Lodgepole Pine	Interior Spruce
October 4, 1982	0	October 4, 1982	(-20)[2]	(-26)
	2	December 4, 1982	(-14)	-27
	6	April 4, 1983	----	-14
November 1, 1982	0	November 1, 1982	-29	-30
	2	January 1, 1983	-26	-26
	6	May 1, 1983	-20	-25
March 28, 1983	0	March 28, 1983	-18	<-18
	2	May 28, 1983	(-11)	<-18
	6	September 28, 1983	-7	<-18

[1] LT5o = lethal temperature for 50% of the test population.
[2] Values in parentheses are extrapolations, others are interpolations of percent injury over temperature curves from whole-plant freeze tests.

wonders how much overwinter damage can be attributed to this cause. On the other hand, seedlings which are lifted in winter can be held well into spring for high elevation planting where exposure to low temperatures is expected. In the table 1 data, for instance, seedlings lifted in November were still hardy to -200 or -250C 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 -- i.e. they are able to tolerate substantial desiccation of both tops and root systems without incurring appreciable damage (Ritchie and Shula 1985, Ritchie 1986). By spring (March) when growth begins, water relations properties shift abruptly to a far less favorable status and seedlings become very sensitive to waterrelated stresses. The success of the midwinter 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 researched. One might speculate, however, that since carbohydrates are depleted in storage, and since water relations reflect osmotic relations which depend to a degree on dissolved carbohydrates in the cells, that we would see a gradual deterioration of seedling water status with time in cold storage. This might partially explain why storage beyond six to nine months almost invariably results in poor performance of planting stock. This is an interesting and important question and deserves to be investigated.

SUMMARY AND CONCLUSIONS

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.

LITERATURE CITED

Campbell, R. K. 1978. Regulation of bud-burst timing by temperature and photoregime during dormancy. p. 19-34. In: Proc. N. Amer. For. Biol. Workshop, 5th, 1977.

Glerum, C. 1985. Frost hardiness of coniferous seedlings: principles and applications. p. 107-123. In: Proceedings: Evaluating seedling quality: principles, procedures, and predictive abilities of major tests. Workshop held October 16-18, 1984. Forest Research Laboratory, Oregon State University, Corvallis.

Lavender, D. P. 1985. Bud dormancy. p. 7-15. In: Proceedings: Evaluating seedling quality: principles, procedures, and predictive abilities of major tests. Workshop held October 16-18, 1984. Forest Research Laboratory, Oregon State University, Corvallis.

Ritchie, G. A. 1982. Carbohydrate reserves and root growth potential in Douglas-fir seedlings before and after cold storage. Can. J. For. Res. 12:905-912.

Ritchie, G. A. 1984. Effect of freezer storage on bud dormancy release in Douglas-fir seedlings. Can. J. For. Res. 14:186-190.

Ritchie, G. A. 1986. Relationships among bud dormancy status, cold hardiness, and stress resistance in 2+0 Douglas-fir. New Forests 1:29-42.

Ritchie, G. A., J. R. Roden, and N. Kleyn. 1985. Physiological quality of lodgepole pine and interior spruce: effects of lift date and duration of freezer storage. Can. J. For. Res. 15:636-645.

Ritchie, G. A., and R. G. Shula. 1984. Seasonal changes of tissue-water relations in shoots and root systems of Douglas-fir seedlings. For. Sci. 2:538-548.