White Spruce: The Effect of Long-Term Cold Storage Is Partly Dependent on Outplanting Soil Temperatures[1]

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Seedlings of *Picea glauca* were freezer stored for 0 to 30.5 wks. at -5C, and thereafter grown at three different soil temperatures (3,7,11C). Root growth at 11C increased as seedlings received up to 14 wks. storage duration, and decreased thereafter. In contrast, root growth at the lower temperatures simply decreased with storage duration. Root growth performance and stomatal conductance data both suggest that storage duration greater than 22 wks. can be detrimental to seedling development.

INTRODUCTION

Conifer seedlings for freezer storage, lifted at their peak of cold hardiness and stress resistance, are stored from 4 to 8 months in British Columbia. During this period physiological changes occur which may affect subsequent outplanting vigor and survival. Our area of concern is the effect of storage duration on root growth of seedlings, since limited root growth has been implicated as contributing to failure of large plantations of white spruce (*Picea glauca* (Moench.)Voss.) in B.C. (Butt 1986).

The effect of storage on root growth is not straightforward. In general there is a long decline in root growth capacity with storage, although in Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) there is also a transient increase in root growth capacity after two months storage (Ritchie 1987). This temporary increase is also noted in winter-lifted interior spruce and to a lesser extent in lodgepole pine *(Pinus contorta* Dougl.) (Ritchie, Roden and Klein 1985).

The relationship between storage duration and root growth may be even more complex. Husted and Lavender (1988) tested root growth of white spruce seedlings before and after 6 months storage; there was no net change in root growth in 17C soil, which contrasted With a striking loss in root growth in similar seedlings planted in soil at 3C. The idea that the observed effect of storage duration might depend on the temperature at which seedlings were planted, formed the basis of the present experiment.

An additional factor which seemed relevant in this experiment was the role of stored and newly assimilated carbohydrate. One obvious effect of storage is in the depletion of stored carbohydrates. Carbohydrate depletion has been noted in Douglas-fir (Ritchie 1982), in Ponderosa pine (*Pinus ponderosa* Laws.) (Hellmers 1962), and in Engelmann spruce (*Picea engelmannii Parry)* (Ronco 1973), and poor seedling performance has been attributed to reduced carbohydrate levels (Ritchie 1982, Ronco 1973). However, in some cases, new photosynthate rather than stored carbohydrate appears to be critical for root growth. The development of new roots in Sitka spruce (*Picea sitchensis* (Bong.)Carr.) is partly dependent, and in Douglas-fir, entirely dependent upon the carbohydrate source from the shoots (Philipson 1988, van den Dreissche 1987). Whether new root growth is dependent on carbohydrate reserves or on photosynthate or both, is not understood, but it seemed that the seedling's ability to establish a new root system would be enhanced by an active photosynthetic process. We decided to examine in more detail the root growth of stored white spruce seedlings, at soil temperatures that might be encountered in planting sites, in conjunction with measurements of photosynthetic gas exchange during the first month of growth after storage.

MATERIALS AND METHODS

Container grown white spruce (1+0,PSB 313) obtained from the B.C. Forest Service, were lifted and cold stored at -5C for up to 30.5 wks. (7.5 months). All work was done under normal operational conditions. At approximately one month intervals, seedlings were removed, thawed, potted, and grown for 28 days in three soil temperature treatments (3,7,11 C), in a growth chamber at U.B.C. (air temperature 11 C, 480 µmol m^{-2} s^{-1} PPFD, 16 hrs. photoperiod). During this period several gas exchange parameters such as net photosynthesis and stomatal conductance were followed using a Licor 6200 IRGA. Seedlings were measured at 680 µmol m^{-2} s^{-1} PPFD. New root growth was measured at the end of each growth period.

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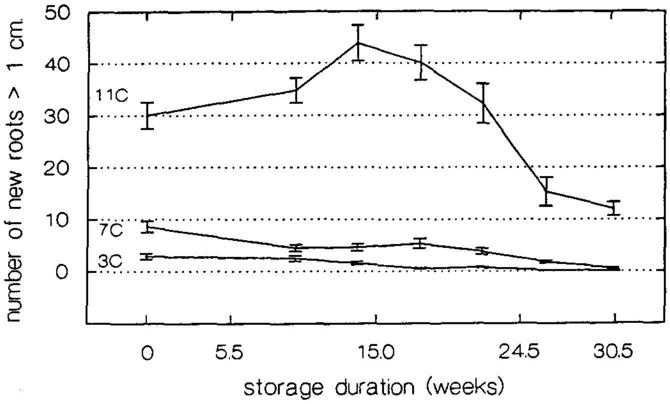

Figure 1 -- New root growth >1 cm. after 28 days at 3,7,11C soil temperatures, and dark freezer storage to 30.5 wks. Error bars are 1 SEM, n=40.

RESULTS

Root growth (as assessed by number of roots longer than 1 cm.) was lower at the colder temperatures (3,7C) than that at 11 C over the entire range of storage durations (Fig. 1). In addition, an interaction between soil temperature and length of storage was evident: unstored seedlings (0 wks. storage) planted at 11C produced 3.5 times as many roots as those planted at 7C, although by 30 wks. storage the ratio had increased to 20 times. At this latter storage duration, root production at both temperatures had decreased. An increase in root growth over storage duration was evident only with the high soil temperature, and peaked at 14 wks. In contrast, the colder soil temperatures (3,7C) showed a negative effect on root growth over the entire storage range.

Stomatal conductance and net photosynthesis measurements were not strongly affected by soil temperature (data not shown), although both variables were affected by storage. The data shown in Figures 2 and 3 show similar patterns to those collected from seedlings growth at the other temperatures.

Figure 2 shows the effect of storage duration on stomata[conductance at various periods up to 28 days after outplanting. The first day after outplanting, stomatal conductance was low, and had the same value after all storage durations. However, by day 4 all seedlings showed an increase in conductance which we interpret as stomatal opening. Following this was a general increase in conductance during the rest of the 28 day period after outplanting (shown in Figure 2 as points vertically above each other). This is attributed to increased water loss by developing new foliage after bud break. Storage duration greater than 22 wks. had some effect on this pattern; seedlings stored for the longer periods showed a , dramatic increase in conductance after day 1.

Just as with stomatal conductance, changes in net photosynthesis were observed in the 28 day growth period after each storage duration, and this pattern of change was affected by storage duration. With increased storage duration (up to 22 wks), the pattern became more complex, although there was a trend to generally higher levels of net photosynthesis. At longer storage durations, (longer than 22 wks.) the pattern changed; rates of photosynthesis started out low and rose to high levels during the observation period.

Figure 4 shows the effect of storage duration on the number of days from plating to terminal bud break (TBB). This interval was not directly affected by soil temperature, although there was a strong effect of storage duration. Over the entire 30.5 wks. storage period the interval to TBB was found to decrease from about 23 to 8 days (average of three soil temperatures).

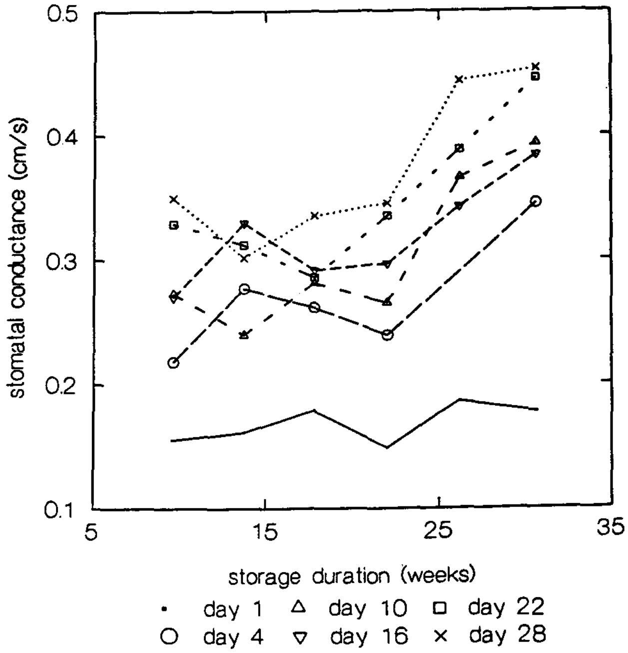

Figure 2 -- Stomata[conductance changes over 28 days growth at 3C soil temperature after varying storage durations, n-40.

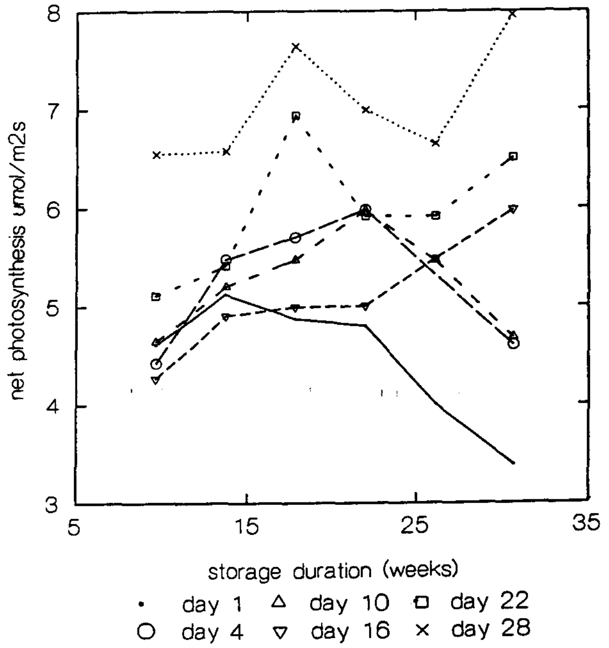

Figure 3 -- Net photosynthesis changes over 28 days growth at 11 C soil temperature after varying storage durations, n-40.

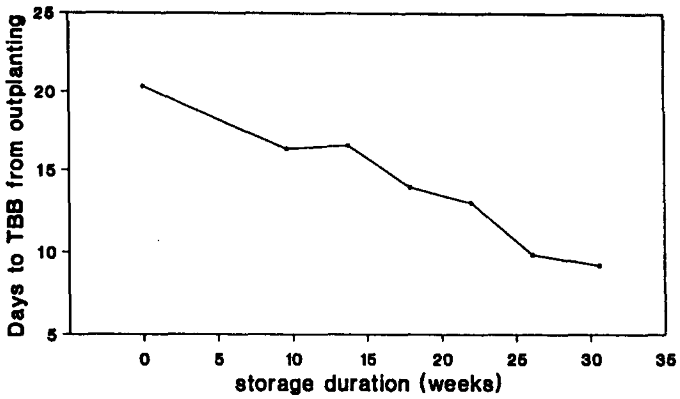

Figure 4 - Days to terminal bud break (TBB) after varying storage durations, n=120.

DISCUSSION

In this study, we observed relatively little effect of soil temperature on the shoot, as judged by the response of photosynthesis, stomatal conductance and the interval to TBB. This is in direct contrast to the findings of DeLucia (1986), in which root temperatures below 8 degrees caused a strong effect on both photosynthesis and conductance in Engelmann spruce.

However, photosynthesis, conductance and interval to TBB were affected by storage duration. The effect on stomatal conductance was fairly straightforward. After all storage durations, the seedlings demonstrated relatively low stomatal conductance for the first 4 days after planting. We interpret this as part of the seedling recovery process after storage, and it may indicate stomatal opening upon relief of water stress. In the case of seedlings stored longer than 22 wks, we observed that the final stomatal conductance increased with extended storage duration. This general trend may be the result of hormonal activity; abscisic acid (ABA), which has been implicated in stomatal control (reviewed in Zeevaart and Creelman 1988) may decrease with long term storage. Abscisic acid has also been implicated in bud dormancy, suggesting that the increased conductance and shortened interval to terminal bud break (Fig. 4) may be both related to declining ABA levels.

After 22 wks. storage the reduction in net photosynthesis was not correlated with stomatal conductance. This suggests stomatal size was not limiting photosynthesis. After 22 wks. storage, a low photosynthetic rate during the initial 4 days of growth indicates impairment of the photosynthetic system.

In contrast to the shoot parameters, root growth was affected by both temperature and storage duration. With regard to temperature, there are several observations. First, there was much more root growth at 11 C than at the lower temperatures. Even at the 11 C lowest root growth (30.5 wks.), the number of new roots was 40% higher than the highest observed at the colder temperatures (0 wks.,7C). Second, root growth at 11 degrees showed a transient increase with storage, similar to that discussed in the Introduction. One suggestion to explain this pattern is that chilling is necessary to produce vigorous new roots (peak at 14 wks.) and, as suggested by Zaerr and Lavender (1974), and van den Driessche (1987), root development may be under hormonal control. The fact that this increase was not noted in roots from 3 and 7C soil suggests that the postulated hormonal effect is soil temperature dependent and that a threshold temperature exists for white spruce which is >7C and <11 C. Below this threshold soil temperature, storage for any duration had a negative impact on root growth after outptanting. We note that this particular temperature threshold may be provenance or elevation specific.

With regard to the effect of duration, the 22 wks. point again seems important. Root growth at 11C Indicates storage durations over 22 wks. severely reduce the seedling's ability to produce new roots. There is a 47% drop in root growth between 22-26 wks.

The decreased interval to TBB is also a factor in reducing root growth, although secondary to the effect of cold soil temperatures. Once bud break occurs, rate of new root development decreases (Mattsson 1981). It is the amount of time photosynthate is available for root growth prior to new foliage development coupled with favorable soil temperature that is important for seedling establishment.

This growth chamber study has shown the complexity of interactions between soil root temperature and storage duration, in relation to carbon fixation, stomatal conductance, and root growth. While this work needs to be followed by field studies, there are some silviculturally relevant implications:

1. Dark freezer storage of white spruce in excess of 22 wks. has a detrimental effect on seedling growth after outplanting. The decline in TBB period and its relationship to root growth has significant implications for nursery practices and planting recommendations.

2. Root growth potential measurements made at relatively warm temperatures may not reflect the actual ability of the seedling to produce roots at lower temperatures.

3. The fact that root growth at low temperatures was adversely affected by all storage durations in this experiment suggests that use of stored seedlings in cold soils may contribute to poor spruce plantation growth.

The key to good establishment depends upon root growth (Mattsson 1981), and as we have seen, root growth depends upon soil temperatures, length of cold storage, and days to TBB. In developing a successful reforestation program careful choice of planting date in consideration with root growth patterns and local planting conditions (soil and light parameters) is necessary to maximize growth potential.

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