Relation Between Cold Hardiness, Root Growth Capacity, and Bud Dormancy in Three Western Conifers[1]

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Abstract.--Ponderosa pine, Douglas-fir, and Engelmann spruce seedlings were greenhouse container grown, then cold acclimated and deacclimated in growth chambers over 19 weeks. Stem cold hardiness, new root length at 14 days, and days to budbreak were measured weekly. During acclimation, root growth capacity had doubled when stem cold hardiness reached -22 °C. During deacclimation, root growth capacity was not lost when two-thirds of maximum cold hardiness was lost. At budbreak, both cold hardiness and root growth capacity were minimum.

INTRODUCTION

The tree nursery industry has long recognized the need for accurate measures of seedling quality (Duryea 1985, Rook 1980). To date, morphological tests predominate, because they are quick and easy to perform, and there is a long history of correlation with survival and growth in the field. Physiological testing at an operational level is still in its infancy, because the tests require more expensive instrumentation and are frequently time-consuming, and in many cases we don't know how to interpret the results. Nevertheless, physiological testing has the prospect of eventually being a far better predictor of field performance than morphological characteristic.

To become established in the field, seedlings must first make root contact with the surrounding soil (Tinus 1974), and it is the new white root tips that are the low resistance pathway for water uptake (Carlson 1986). This is why root growth capacity (RGC) has become an important test and has been found well correlated with field survival and growth (Jenkinson 1980, 1984).

Next, the seedling must grow in height. Meeting chilling requirements for budbreak is rarely a problem with bare-root stock, but can be with container-grown seedlings. More important, budbreak has long been the criterion for judging plantability of stock, yet spring budbreak is the last in a series of physiological changes from winter dormancy to summer growth.

To be a useful management tool, a test has to yield results early enough to change the course of events. Measuring bud dormancy by counting days to budbreak takes much too long. Assessing root growth capacity by the pot test takes 28 days; this can be cut to 7-14 days in the aeroponic mist box (Ritchie 1985, DeWald et al. 1985, Burdett et al. 1983), but probably not less. However, there are tests for cold hardiness that can be done in a matter of minutes to 2 days (Burr et al. 1986, Greer 1983a, 1983b, Pelkonen and Glerum 1985, Colombo et al. 1984, Andrews et al. 1983). Knowing the cold hardiness and the rate of acclimation or deacclimation is valuable for protection of the seedlings, such information could also be used as a quick estimator of bud dormancy and root growth capacity if a good, consistent relation between the three parameters could be found.

The purpose of this study was to find whether such a relation exists. Our research showed that RCG and cold hardiness tests can indicate loss of quality weeks before visible budbreak.

MATERIALS AND METHODS

Ponderosa pine (*Pinus ponderosa* Laws., Chevelon District, Apache-Sitgreaves National Forest, elev. 2,300 m), Douglas-fir (*Pseudotsuga menziesii* var. *glauca* (Beissn.) Franco, Cloudcroft District, Lincoln National Forest, elev. 2,700 m), and Engelmann spruce (*Picea engelmannii* (Parry) Engelm., Springerville District, Apache-Sitgreaves National Forest, elev. 3,000 m) were seeded in

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400-m1 Rootrainers[2] in peat-vermiculite in October 1985. They were grown in greenhouses at Flagstaff, Ariz., with night temperatures averaging 18-21 °C and day temperatures 23-25 °C until April 1986, when day temperatures began to rise, reaching about 28 °C by June. Daylength was extended to 22 hours with fluorescent light. Other cultural conditions were as recommended by Tinus and McDonald (1979). On June 24, the seedlings were sorted; those of uniform size were placed in four Percival HL-60 growth chambers under 43,000 lux from sodium and multivapor arc lights and kept watered as needed with nutrient solution under a cold acclimation and deacclimation regime (indicated in table 1). All seedlings were of a single population that went through the same succession of stages. At weekly intervals, samples of seedlings were taken for concurrent tests of cold hardiness, root growth capacity, and bud dormancy.

Table 1.--Conditions of cold acclimation and deacclimation

Stage	Duration (weeks)	Day temp. (°C)	Night temp. (°C)	Day length (hours)	Nutrient solution
1	3	20	15	10	Low N, high PK
2	7	10	3	10	Low N, high PK
3	5	5	-3	10	Low N, high PK
4	4	22	22	16	High N

Cold hardiness was measured by a whole-plant freeze test. One book of four seedlings of each species was placed in each of three styrofoam coolers, and the rootballs supported and covered to a depth of 5 cm with dry vermiculite. The coolers were instrumented with thermistor probes in the crowns of the seedlings, the lid wired shut, and the coolers placed in a 650-L household chest freezer. The temperature was lowered rapidly from ambient to 0 °C and at a rate of 3-5 °C per hour thereafter. To reach temperatures below -25 °C, a baking pan was placed in the freezer and filled with liquid nitrogen. The size of pan and degree of insulation controlled the rate of temperature fall. For each species, three temperatures were selected 5 °C apart, which were expected to encompass the LT_{50} of the stem. When a designated styrofoam cooler reached one of these benchmark temperatures, it was removed from the freezer and placed in a refrigerator at +1 °C where it thawed overnight. The seedlings were then removed from the coolers and placed in a warm greenhouse (day 26 °C, night 19 °C, 22-hour day).

After 7 days, the stems were sliced open and the cambium and phloem examined for browning and cell turgor. For each seedling, the proportion of stem that had been killed was estimated. Rates of increasing injury with decreasing temperature were compared across days and species, and data with similar rates was subjectively placed into 5 groups. This pooling of data was necessary because 12 trees for a particular day did not provide adequate information for statistical analysis. For each group, injury in the range 10-90% was regressed against temperatures, and the 50% injury point was estimated by calibration methods (Graybill 1976). The range 10-90% was chosen because the relation between injury and temperature was primarily linear, but nonlinear above and below this range.

At the same time the cold hardiness test was run, eight additional seedlings per species were placed in an aeroponic mist box in a greenhouse (day 26 °C, night 18 °C, ambient day length) to measure root growth capacity. The seedling stems were inserted through holes in plywood strips and held in place with urethane foam plugs. The intact rootballs were exposed to 100% relative humidity at 27 °C maintained by a warm-water intermittent mist. After 14 days, the new white roots that emerged from the rootball were measured (cm ± 1) and counted, and RGC expressed as total new root length per seedling. RGC was measured without damaging the seedlings, which were then returned to the mist box.

The seedlings were left in the mist box until they broke bud. A seedling was considered to have broken bud when 50% of its buds had broken. Days to 50% budbreak were recorded when four of the eight seedlings had broken bud. When this was not observed directly, days to 50% budbreak were calculated by linear interpolation. The observations were plotted against day number during acclimation and deacclimation, with intervals plotted for the interpolated points. The endpoints of the intervals indicate actual measurements.

RESULTS AND DISCUSSION

Budbreak proceeded approximately as expected from previous work (Lavender 1985), but with some differences between species with respect to timing (fig. 1). Engelmann spruce did not break bud at all until day 42, or after 1,176 chilling degree hours below 7 °C. Chilling requirements were fully met by day 71, after 2,800 chilling degree hours. Days to 50% budbreak remained stable at about 21 days until the deacclimation period, during which days to 50% budbreak declined to zero in 21 days.

Douglas-fir did not break bud until day 56, after 1,960 chilling degree hours. Chilling requirements were fully met by day 71 (2,800 hours). Thereafter, days to 50% budbreak remained stable at about 26 days until the deacclimation period, during which days to 50% budbreak declined to zero in about 28 days.

Ponderosa pine did not require any chilling to break bud, and broke bud even at the earliest test dates except at day zero. After 21 days at day

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[2] Trade names are used for brevity and specificity and do not imply endorsement by USDA or Colorado State University to the exclusion of other equally suitable products.

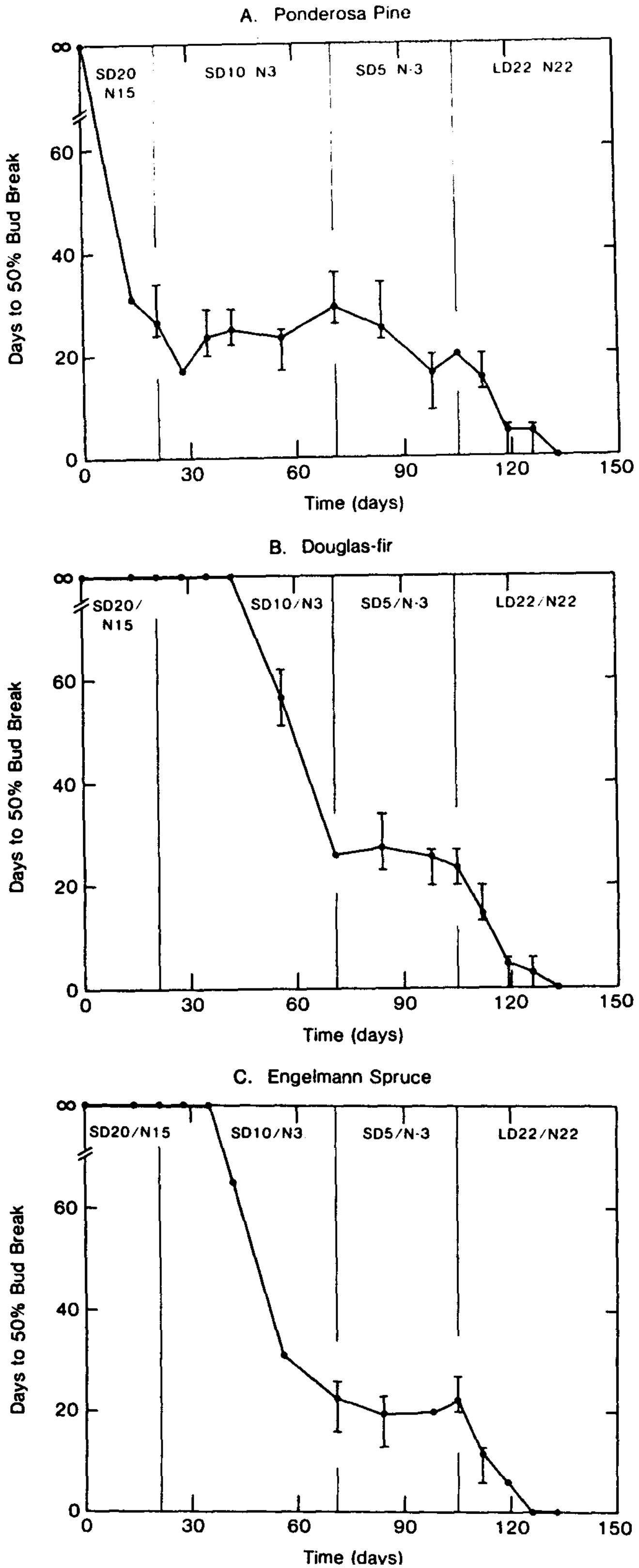

Figure 1.--Days to 50% budbreak of (A) ponderosa pine, (B) Douglas-fir, and (C) Engelmann spruce as a function of time. Temperature stages are described in table 1. Plotted intervals indicate interpolated values, the endpoints of the intervals being the actual measurements.

20 °C, night 15 °C, days to 50% budbreak declined to a plateau between 18 and 30 days, but with more variability than Engelmann spruce and Douglas-fir. Days to 50% budbreak declined to zero in about 28 days.

Root growth capacity (fig. 2) of Douglas-fir (fig. 2B) was low (100 cm per seedling) at the beginning of the acclimation period. Two weeks into the second stage of hardening (day 10 °C, night 3 °C) RGC began a rapid rise to a 400 cm peak at day 84 during the third stage of hardening (day 5 °C, night -3 'C). RGC dropped to about 250 cm and then rose to a second peak 1 week into deacclimation. Because of variability, the two peaks separated by a valley may not be distinguishable statistically, but the second peak especially makes sense biologically. Many workers have found an inverse correlation between root and shoot growth, with a particularly strong burst of root growth shortly before budbreak (Riedacker and Arbez 1983, Jenkinson 1980, El Nour and Riedacker 1984). After more than 1 week under deacclimation, RGC declined to a low level (50 cm) at budbreak.

RGC of Engelmann spruce (fig. 2C) was initially low (70 cm); but 2 weeks into the second stage of hardening, RGC rose abruptly to about 230 cm per seedling with a lot of variability. Maximum RGC (320 cm) occurred 1 week into deacclimation; but again, because of variability, the peak may not be statistically distinguishable from the preceding plateau. After the peak, RGC dropped rapidly with the approach of budbreak.

RGC of ponderosa pine (fig. 2A) began at midrange (290 cm) and declined to a low of 100 cm. Two weeks into the second stage of hardening, RGC rose abruptly to over 400 cm, where it remained until the third stage, when RGC again dropped to 300 cm. As with Engelmann spruce and Douglas-fir, a second peak appeared 1 week into deacclimation, followed by a rapid decline as budbreak approached. Again, because of variability, these relations are not clearcut. However, the pattern (fig. 2) is remarkably similar to the one reported by Jenkinson (1980) for bare-root seedlings of the Arizona ecotype (Read 1980, 1983) grown in California nurseries.

Although there are distinct differences between species, the pattern of RGC during cold acclimation and deacclimation showed some very interesting similarities. First, the rise in RGC early in the second stage of hardening occurred at the same time for all three species, although the rise was abrupt in ponderosa pine and Engelmann spruce and more gradual in Douglas-fir. Second, maximum RGC was five to seven times minimum RGC. Third, a second peak (albeit not distinct statistically) occurred 1 week into deacclimation, or 2-3 weeks before budbreak, depending on species. Fourth, RGC declined rapidly as budbreak approached. Although RGC appears to have multiple peaks for each species (fig. 4 below), the variability in the data (fig. 2) is too great to know if the peaks are real.

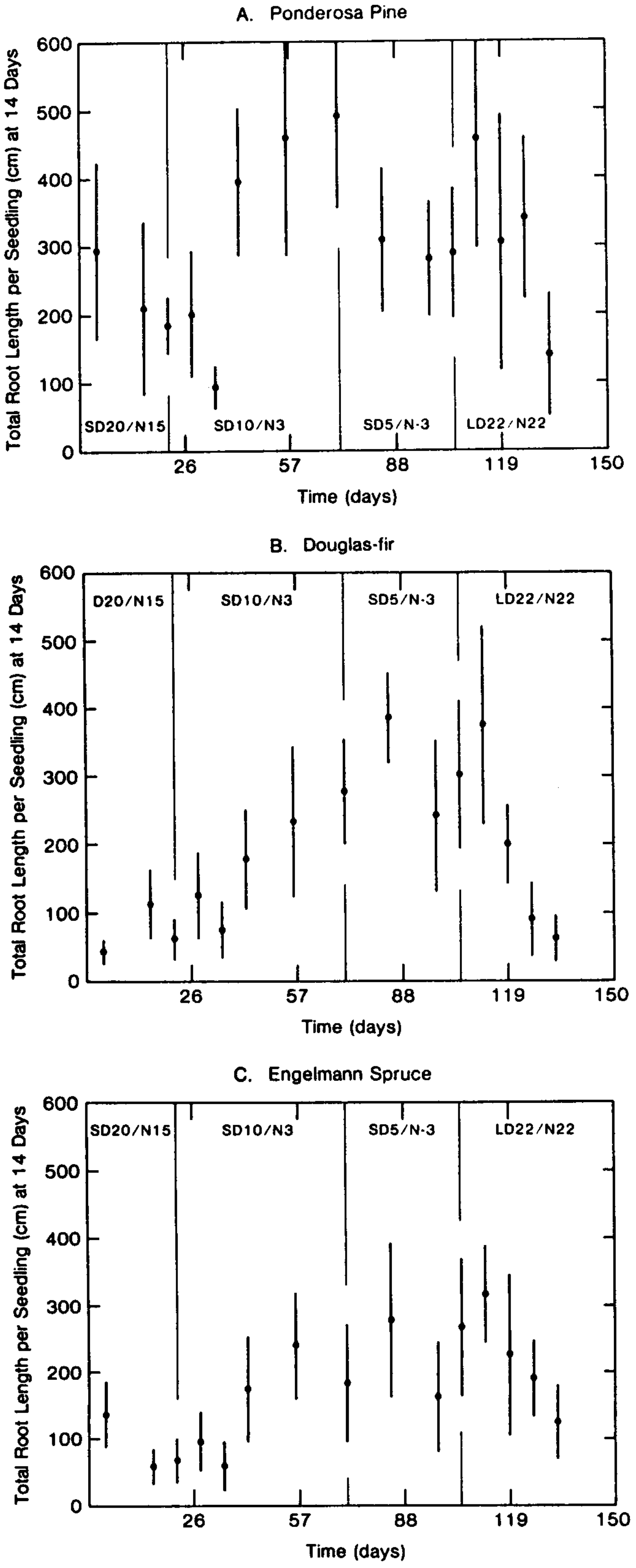

Figure 2.--Root growth capacity as total new root length per seedling at 14 days of (A) ponderosa pine, (B) Douglas-fir, and (C) Engelmann spruce as a function of time. Vertical bars are 95% confidence levels. Temperature stages are described in table 1.

Cold hardiness of each species (fig. 3) was gained and lost as a function of the four successive temperature stages. In the first stage (day 20 °C, night 15 °C, 10-hour day), ponderosa pine (fig. 3A) did not harden, but stem cold hardiness was about -16 °C (LT$_{50}$). During the second stage, there was no hardening for the first week. Thereafter, hardening proceeded at about 0.4 °C per day until maximum hardiness was reached at about -32 °C on day 71. Upon entering stage 4 (day 22 °C, night 22 °C), deacclimation began immediately and proceeded at about 1 °C per day to a minimum hardiness at about -14 °C.

Douglas-fir stems (fig. 3B) started at minimum hardiness of -11 °C and did not harden during the first temperature stage. During the second stage, there was no significant hardening for 3 weeks. Thereafter, hardening proceeded at about 0.5 °C per day, reaching an LT(50) of -47 °C by the end of stage 3 (day 105). Gain in hardiness was continuous with no sign of leveling out, as there was in ponderosa pine. Therefore, -47 °C may not represent maximum possible cold hardiness of Douglas-fir stems. Upon entering stage 4, deacclimation began immediately and proceeded at about 2.3 °C per day to -11 °C.

Like ponderosa pine and Douglas-fir, Engelmann spruce (fig. 3C) did not harden during the first stage, nor did it harden during the first 2 weeks of the second stage. Thereafter, Engelmann spruce stems hardened at about 1 °C per day from about -15 °C to -63 °C on day 84. By day 93, 27 days into the third stage, Engelmann spruce was unkillable at -77 °C, which was the low limit of the freezer. Upon entering stage 4, Engelmann spruce deacclimated to -35 °C in 1 week, a rate of at least 6 °C per day. The rate of dehardening declined rapidly thereafter, finally reaching a minimum hardiness of -13 °C.

The principal differences between species seem to be in their rate of hardening and the maximum attainable hardiness, which is much in keeping with the altitude of their native habitat. In Arizona, ponderosa pine grows from 1,800 to 2,700 m, Douglas-fir from 2,400 to 3,000 m, and Engelmann spruce from 2,700 to 3,300 m.

In addition, there are some striking similarities between species. No hardening occurred at warm temperature even with a short day. Based on work on deciduous species, partial hardening might have been expected (Ketchie 1985, George and Burke 1977), but does not seem to occur in conifers (Aronsson 1975) except in high latitude seed origins (Cannell and Sheppard 1982). When the temperature was lowered to day 10 °C, night 3 °C, there was a 1-3 week lag before hardening began. On the other hand, there was no lag in the loss of cold hardiness when deacclimation was initiated, and the rate of deacclimation was two and one-half to **six times** faster than acclimation. Aronsson (1975) has made similar observations on Scots pine and Norway spruce.

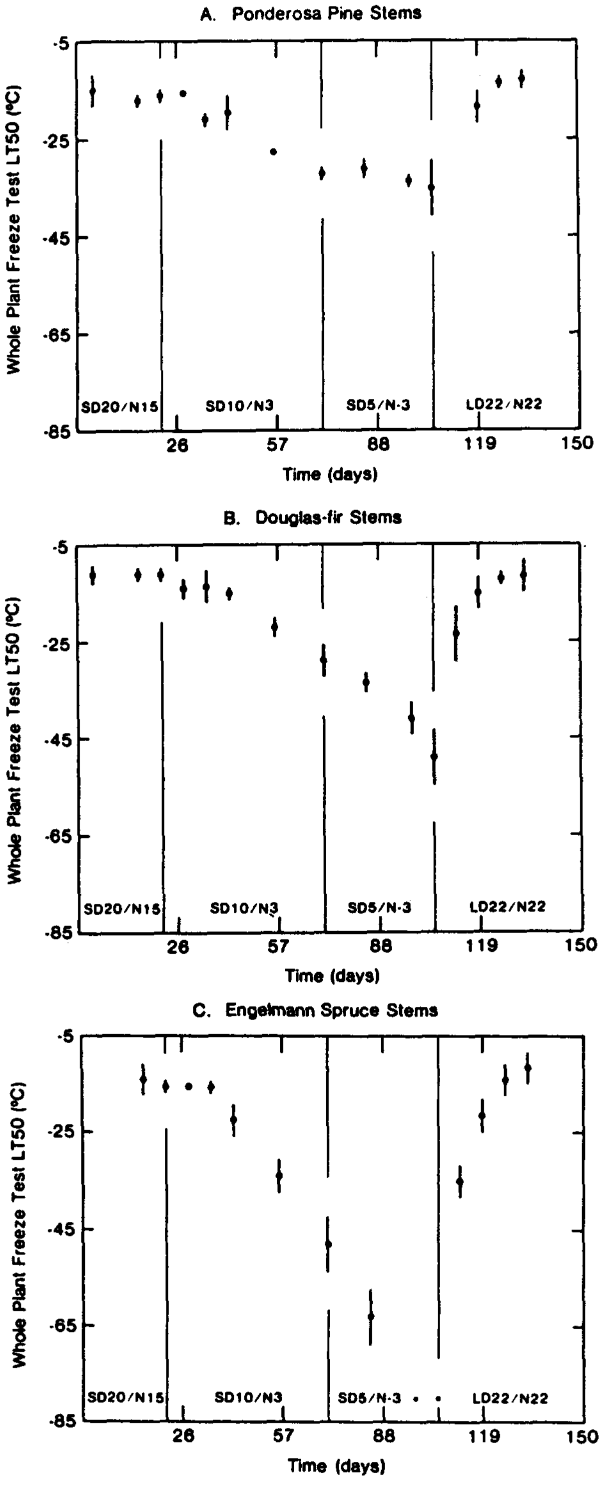

Figure 3.--Stem cold hardiness of (A) ponderosa pine, (B) Douglas-fir, and (C) Engelmann spruce as a function of time. Each LT_{50} and its 95% confidence interval were calculated by calibration methods. The confidence interval is for the mean of 12 observations. Temperature regimes are described in table 1. For day number 112, ponderosa pine was less hardy than the warmest temperature used. On day numbers 98 and 105, Engelmann spruce is indicated at -77 °C, but the stems were not visibly injured.

When cold hardiness, RGC, and bud dormancy are compared, some interesting and possibly useful relations emerge (fig. 4). During acclimation, when stem LT_5 reached -22 °C, root growth capacity had just doub9ed. RGC was then on a high plateau in the case of ponderosa pine and Engelmann spruce, and on a continuing rise in the case of Douglas-fir. If this relation were to hold up under further testing, measuring cold hardiness could become a quick way to determine when to begin fall lifting in bare-root nurseries.

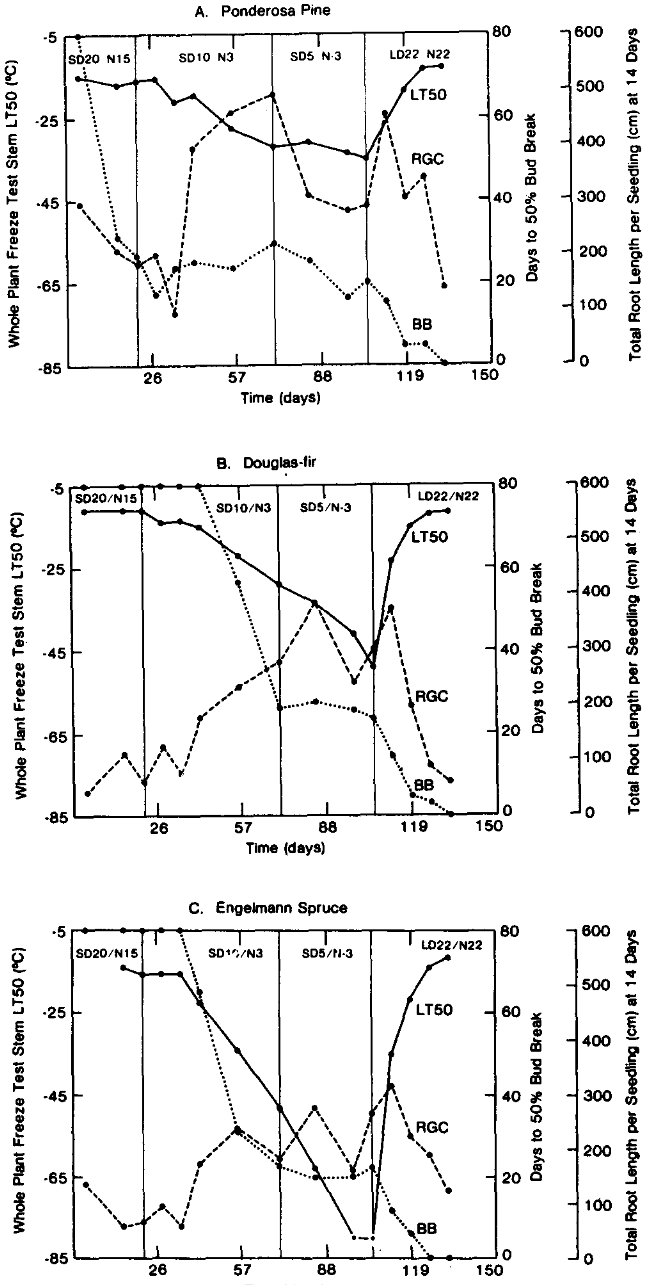

Figure 4.--Composite showing relation between stem cold hardiness ($_{\text{LT59}}$), root growth capacity (RGC), and days to 0% budbreak (BB) as a function of time for (A) ponderosa pine, (B) Douglas-fir, and (C) Engelmann spruce.

Satisfaction of bud chilling requirements occurred at a level of cold hardiness that varied with species: in ponderosa pine -15 °C (no hardening), Douglas-fir -29 °C, and Engelmann spruce -48 °C. This relation may be species- and ecotype-specific, further testing will be required to substantiate this. However, some relation between cold hardiness and bud dormancy is likely as evidenced by the successful use of chilling degree hours to predict bud dormancy in many species (Cannell and Smith 1983, Owens et al. 1977).

During deacclimation, the putative second peak in RGC coincided with a two-thirds loss of stem cold hardiness and one-third of the time toward budbreak from the plateau representing days to budbreak after the chilling requirements were met. This relation could be very useful for determining planting stock quality in the early spring by measuring cold hardiness. By the time 50% budbreak occurred, both RGC and cold hardiness were at their minimum. It is therefore crucial to know the physiological condition of the seedling weeks and even months before 50% budbreak (Lavender 1985).

For ponderosa pine, requirements for budbreak were met about 2 weeks before the precipitous rise in RGC, but in Douglas-fir and Engelmann spruce meeting of bud chilling requirements occurred close to the first peak in RGC. For all three species days to 50% budbreak declined steadily from the beginning of deacclimation, whereas RGC appears to peak and then decline. A pulse of root growth prior to budbreak has been observed in many species (Ritchie and Dunlap 1980).

In this experiment, temperatures were selected for rapid deacclimation. Under less favorable conditions deacclimation would be slower, but the same sequence of events would probably occur. Whether the observed relations between cold hardiness, RGC, and budbreak will be the same under different conditions remains to be tested.

CONCLUSION

Relationships between cold hardiness, RGC, and bud dormancy have been found that support a hypothesis by Ritchie (1985) that such relations exist. At present, it is uncertain how far these results can be generalized. Other ecotypes of ponderosa pine (Jenkinson 1980, Read 1983) and Douglas-fir (Jenkinson 1984) are known to behave differently in many ways. Less is known about Engelmann spruce. Furthermore, these relations were observed under a single set of temperature and photoperiod conditions. Therefore, it would be a mistake to apply them immediately without further testing.

These results suggest cold hardiness testing could be a promising avenue for a quick estimate of RGC and bud dormancy. The whole-plant freeze test is not particularly fast, as it takes 7 days and sometimes longer, but faster tests are becoming available (Burr et al. 1986). It also appears that meaningful information from an RGC test may not be obtainable in less than 14 days (Burr, Tinus, and Wallner, unpublished data), and certainly not in less than 7 days (Burdett et al. 1983).

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