Using Frost Hardiness as an Indicator of Seedling Condition[1]

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Abstract.--Knowing the frost hardiness of conifer seedlings is of benefit to nursery managers and seedling users even if the potential for actual frost damage is not of major concern. Examples are presented illustrating the ability of comparative hardiness testing to reveal variation in seedling phenology brought about by genetic, cultural, and environmental factors. Implications for the timing of cultural practices and lifting windows are discussed.

Introduction

The physiological condition of conifer seedlings during the lifting and planting season is of critical importance to the success of reforestation efforts. This subject has received much emphasis in recent years, reflected by ongoing efforts to estimate seedling quality using a variety of physiologically based tests, such as root growth potential (RGP), stress tests, dormancy release index (DRI), frost hardiness (FH), and others (Duryea, 1985). Although these tests are founded upon sound physiological theory, their success in accurately predicting stock performance in operational settings has been mixed. One reason for this is that quality tests can only assess potential stock performance. Even with high quality seedlings, poor handling or severe environmental stresses may still result in performance problems.

Another source of uncertainty has been the fact that seedling physiological condition may change between the time of testing and the time the seedlings are lifted or planted. Seedling condition, of course, changes continually throughout the year; this is reflected in the seasonal development of RGP, FH, and other seedling attributes. When a seedling lot is tested one time during the planting season, the results give a "snapshot" indication of the general condition of the seedlings on the date tested. This approach has proven to be satisfactory for the routine screening of large numbers of seedling lots, and for identifying lots with severe quality problems. However, because of the continual changes that seedlings undergo, a detailed understanding of seedling physiology can be obtained only through a "motion-picture" approach, that is, tracking seedling conditioning through tests conducted at intervals during the lifting/planting season. This can be done with several tests, either alone or in combination. Ritchie (1980) showed how RGP changes seasonally, rising from low levels in the fall to a midwinter peak, and then falling again in spring. Frost hardiness follows a similar pattern, and both appear to be related to the dormancy cycle. For the past several years, International Paper has used the physiological tracking approach for assessing proper lifting dates for seedling lots grown at its Kellogg Nursery. Each of the major tests has been utilized in this context; this paper will focus on the usefulness of frost hardiness testing as an

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indicator of seedling condition at various times throughout the planting season. The interplay of seedling genetics, nursery cultural practices, and environmental factors, specifically chilling hour accumulation, will be discussed with regard to their influence on hardiness development, and by implication, on proper lifting window.

Background

Reforestation is most successful when seedlings are handled at the time of maximum stress resistance. Stress resistance is an abstract term which is difficult to quantify. It includes such attributes as drought tolerance and frost hardiness, and is generally considered to be linked to the seedling dormancy cycle. While dormancy and stress resistance are difficult or time consuming attributes to quantify, it is relatively easy to measure frost hardiness. Although frost hardiness testing has received much attention in the past, interest has usually been limited to assessing the potential for frost damage to seedlings. As part of International Paper's seedling monitoring program, we have adopted as a working hypothesis that, as frost hardiness increases, overall resistance to stresses of all kinds also increases (Faulconer and Thompson, 1985). The basis for this assumption is the fact that frost hardiness develops as a result of metabolic changes such as cessation of active growth and physiological dehydration of various seedling tissues, indicative of a lowered state of metabolic activity for the entire seedling. Additionally, years of observations have indicated that maximum reforestation success is achieved in midwinter, when frost hardiness is at its peak, regardless of whether any frost damage has occurred. Tracking the seasonal development of frost hardiness thus becomes of interest even if the potential for actual frost damage to seedlings is not of major concern.

The rate at which seedlings enter dormancy and begin to develop resistance to stress is controlled by three categories of factors: the genetic background of the seedling lot, nursery cultural practices, and other environmental influences such as photoperiod and cool temperatures. If one or more of these factors differs between seedling lots, the timing and rate of their hardiness development may also differ, resulting ultimately in varying optimum lift dates for the seedlinqs. If the development of frost-hardiness is followed beginning early in the fall, divergent trends in hardiness development can be identified early enough to be used as a guide for lifting schedules and for assessing the storability of seedlings.

Methods

Frost hardiness testing is begun in the fall, as soon as hardening commences. Samples are lifted at biweekly intervals usually beginning on or about October 1. Each sample lot is divided into three or four sub samples, which are subjected to a gradient of increasingly severe simulated whole plant frosts in a programmable freezing chamber. Temperatures are chosen at which 20%, 50%, and 80% mortality is expected. After freezing, seedlings are placed in a greenhouse for five days to allow damage symptoms to develop. Damage to cambium, buds, and needles is then evaluated visually using the "browning" method. For each temperature run, percent mortality is estimated based on the severity of damage to the various tissues. Mortality is then plotted against temperature, and the LT-50, or lethal temperature for 50% of the seedling sample, is interpolated from the resulting line. The LT-50 is the term from which the hardiness development curves are derived. For a more detailed description of this and other methods of evaluating frost hardiness, see Burr et al (1986) and Schuch (1987).

As the season progresses, the frost hardiness development curve for each lot is plotted on a chart. This enables direct comparison of the hardening trends between seedling lots. Hypothetical example curves showing typical divergence of hardening trends are illustrated in figure 1. In this example, on any given sample date there is a spread of several degrees C in the LT-50s between these lots. If a target hardiness of, for example, -15 C is desired before lifting, then a comparison such as provided by figure 1 indicates a difference of several weeks for the opening of the lifting window between lots.

The remainder of this paper provides actual examples of divergent hardening trends and discussions of the causes of divergence. All examples are for coastal Douglas-fir grown at International Paper Kellogg Nursery. This data has been collected as part of our routine seedling monitoring program conducted each fall and winter. Frost hardiness monitoring ends as the seedlings are lifted and sent to the field; for that reason the following hardiness development curves end during midwinter.

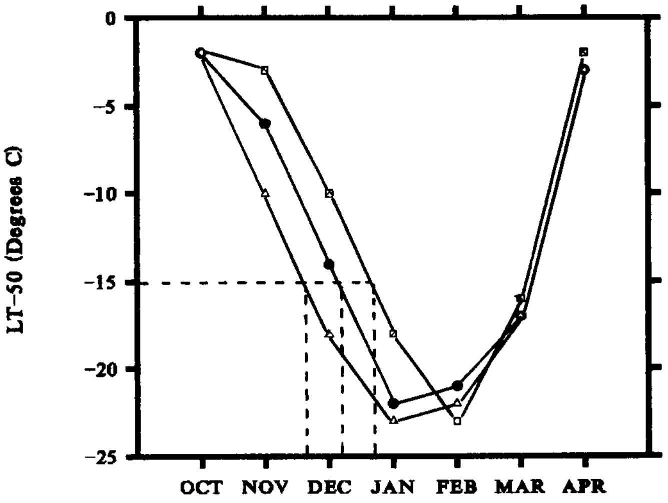


Figure1. Typical divergence of frost hardiness development trends between three seedling lots.

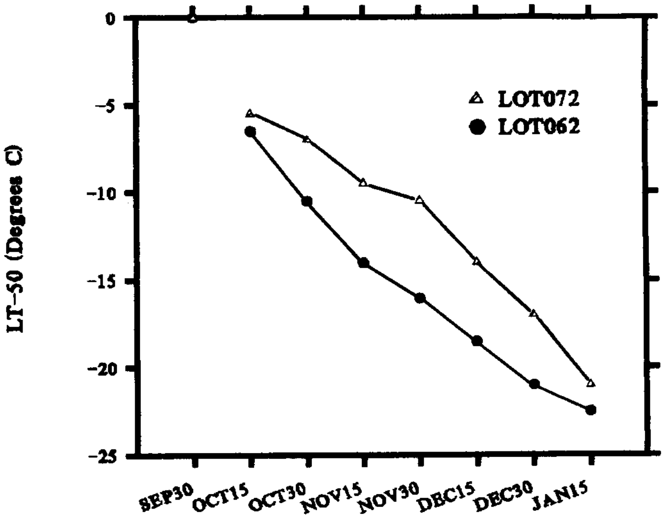


Figure 2. Comparison of frost hardiness development for two 2+0 lots from different seed sources (Oregon zones 072 and 062).

Genetic Variation

Jenkinson (1984) discussed at length the phenomenon of seed source lifting window. By plotting several years of plantation survival data versus lift date, for numerous seedling lots from the USFS Humboldt Nursery, he established that different seed sources have varying safe lifting windows. Because all seedlings were from the same nursery, receiving essentially the same cultural practices and exposed to the same climatic conditions, the factor responsible for lifting window variation was evidently seed source genetic variation. If the mechanism by which the genetic component influences lifting window is by determining the rate and timing of hardiness development during the fall, then variation in seed source lifting windows should be predictable by comparative frost hardiness testing of the various seed sources.

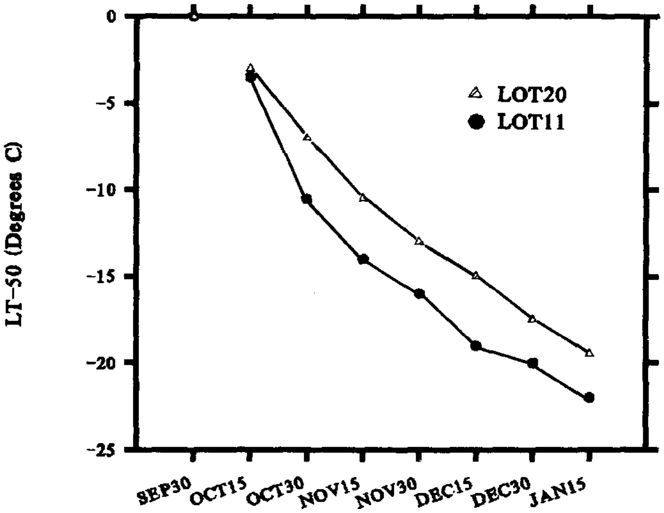
Figure 2 illustrates the frost hardiness development curves for two seedling lots at Kellogg Nursery in 1987-88. Both lots were 2+Os and were subjected to identical cultural practices and climatic conditions during both years in the nursery (in fact, the sample areas for the two lots were in adjacent beds). Seedlings from zone 072 0.5 (southern Oregon coast) lagged dramatically in hardiness development as compared to those from 062 1.0 (mid-Oregon coast). On any given sample date, the hardiness of the 072 lot, in terms of LT-50, was from 3 to 6 degrees C behind the 062 lot. In terms of lifting schedules, a more useful way to interpret this data is to say that the 072 seedlings were two to three weeks behind in hardiness development.

The tendency of seedling lots from the southern Oregon coast to lag behind more northerly or inland lots in hardiness development has been observed repeatedly for each year frost hardiness tests have been conducted. Jenkinson (1984) also found that the lifting windows for provenances from this general region consistently open later than for other sources evaluated. For two seed sources similar in origin to those illustrated in Figure 3 (072 Powers and 061 Alsea), he discovered a spread in the opening of the lifting window nearly identical to the spread between the frost hardiness development curves of the corresponding Kellogg lots. This suggests that fall hardiness development trends and seed source lifting windows are directly related. If so, then frost hardiness testing would offer nursery managers a substantial shortcut for establishing lifting windows for various seed sources.

Nursery Cultural Practices

Nursery cultural practices can have a great impact on the induction of dormancy in seedlings, and on the subsequent development of hardiness. Practices such as the withholding of nitrogen or induction of moisture stress are designed to cause the cessation of active growth in preparation for the fall and winter. These practices interact with, and to an extent sometimes override, the genetic component controlling dormancy development, potentially resulting in an additional source of variability in hardening trends between seedling lots.

The most important phenological effect of cultural manipulation of nursery seedlings is probably the timing of final budset, which in nurseries can occur anytime from midsummer to autumn. Frost hardiness tests indicate that hardiness development can be strongly affected by the timing of budset. Figure 3 illustrates the FH development curves for two seedlots from Kellogg Nursery. In this example, the two lots were sown with the same seedlot (zone 252 1.0) in the spring of 1986. Lot 1 was sown for 2+0 seedlings, whereas lot 2 was lifted after the first year and transplanted for 1+1 production. The genetic background of the lots was identical, as was nursery environment and climate. The divergent hardening trends between the lots must therefore be due to the variation in cultural regimes for the two stock-types. The 1+1 lot reached target height early in the second year, and the seedlings were "shut down" by mid-July through moisture stress treatments. For the 2+0 lot, in contrast, height control was achieved partially through top-mowing, which though effective, can delay final bud set. As a result, the timing of budset differed significantly for the two lots.



Figure'3. Comparison of frost hardiness development trends for 2+0 and 1+1 seedlings sown with the same seedlot (Oregon zone 252 1.0).

Lavender and Stafford (1984) demonstrated the importance of early budset in order for seedlings to properly respond to the cool temperatures which condition seedlings in the fall and early winter. They showed that a period of mild, short days occurring after budset was necessary for subsequent cool weather to be fully effective in satisfying chilling requirement. The frost hardiness curves for these two lots indicates that early budset will also hasten the subsequent development of hardiness. This suggests that cold hardiness and fulfillment of chilling requirement are physiologically linked, which was hypothesized by Ritchie (1986). It would appear then that the timing of the lifting window is determined by the efficiency with which seedlings respond to fall and winter chilling, which can be measured by rate and degree of frost hardiness attainment.

Environmental Conditions

Besides genetics and nursery cultural practices, the third major variable affecting seedling hardiness development is the nursery climate, especially exposure to cool temperatures. As discussed above, genetics and cultural practices interact to produce seedlings that are either more or less predisposed to efficiently respond to chilling. From then on, the amount of chilling actually received is the most important determinant of hardiness 'development.

Nursery climate varies geographically between nurseries, and annually within a single nursery. One commonly used method to deal with this variability is to quantify the duration of cool temperatures experienced by seedlings. Hours during which the temperature is less than a specified minimum are defined as chilling hours, and the accumulated number of such hours experienced by seedlings is used as a guide for predicting seedling condition.

Although use of chilling hour accumulation is easy, inexpensive, and provides an instantaneous assessment of seedling condition (one can always know the number of hours accumulated on any given day), sole reliance on chilling has several disadvantages. First, as discussed earlier, seedling lots which have been exposed to the same amount of chilling may be in very different stages of hardiness development. Secondly, there is apparent disagreement regarding the effective temperature range of a chilling hour. Jenkinson (1984) defines it as being less than 10 C, whereas Ritchie (1986) uses temperatures below 6 C.

Other researchers have used only temperatures between 0 and 5 C in the belief that very cold temperatures retard the physiological processes driven by chilling. Finally, there is uncertainty as to the effect of interruptions of chilling accumulation by unseasonably warm temperatures.

The type of uncertainty which can result from sole reliance on chilling hours as a guide is illustrated in figures 4 and 5. Figure 4 represents graphically the accumulation of chilling hours (defined here as hours cooler than 6 C) at Kellogg Nursery for two consecutive years, 1985-86 and 1986-87. Due to mild weather in the fall of the second year, chilling accumulation lagged far behind that of the first year. The oft-cited 300 hour minimum requirement before safe lifting may commence was not reached until mid-January, about six weeks later than the previous year. Figure 5 compares frost hardiness development for the two years for zone 252 2+0 Douglas-fir. Although development in 1986-87 did lag behind that of the previous year, the delay was not nearly so dramatic as might have been expected from the chilling hour data. One possible explanation is that cultural practices differed somewhat between the two years and offset the difference in chilling. More likely is that in 1986, temperatures slightly outside the arbitrary range, which did not count toward the cumulative total, were still effective in stimulating hardiness development and in satisfying chilling requirement.

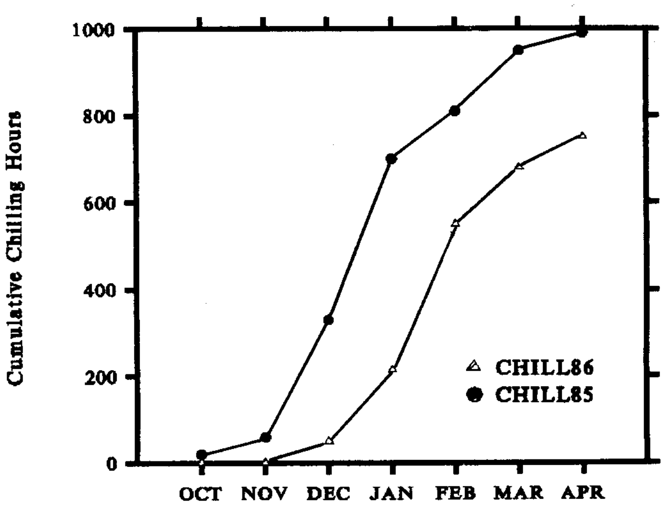


Figure 4. Chilling hour accumulation at Kellogg Nursery for 1985-86 and 1986-87.

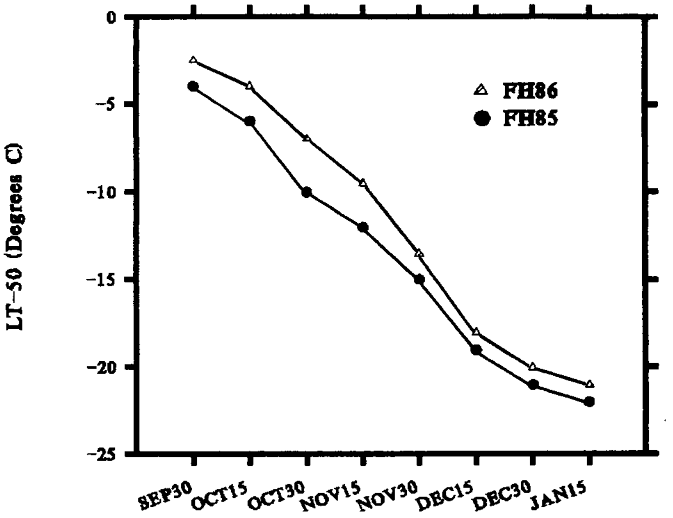


Figure 5. Comparison of frost hardiness development trends for zone 252 1.0 2+0 seedlings in 1986-86 and 1986-87.

Because of the variability between the large numbers of seedling lots produced at most nurseries, and because chilling hours are apparently poorly defined, reliance on chilling hour accumulation alone as an indicator of seedling condition will likely result in an overly generalized and potentially inaccurate assessment of the status of nursery seedlings. Different species and seed sources may have different chilling requirements in terms of number of needed hours, and they may be responsive to different temperature ranges. Attempting to establish guidelines which would account for the multitude of seed sources, and for the variability introduced by cultural practices, would be a monumental task. Much easier is to simply measure the seedlings' integrated response to the genetic, cultural, and climatic factors responsible for their hardiness development.

Frost Hardiness and Storage

The preceding sections have illustrated how frost hardiness testing can detect differing rates of hardiness development between seedling lots. At this point it is still uncertain what hardiness level should be attained before lifting, storage, and planting may proceed safely. However, some preliminary work measuring the effects of cold storage on frost hardiness has provided some clues. Figure 6 illustrates a portion of a typical hardiness development curve for Douglas-fir 2+0 seedlings tested during the fall and early winter of 1987. On each lift date, one sample was tested immediately; another was placed in cold storage to be retested on the next lift date. The objective was to determine whether hardiness continued to develop in storage, and to compare the hardiness of stored seedlings with those which remained in the nursery. For the first lift dates, when seedlings were still in the early stages of hardiness development, an apparent loss of hardiness occurred during storage. Later, as the hardiness of seedlings in the nursery beds deepened, it appears that an ability to maintain hardiness in storage developed. Viewing frost hardiness as an indicator of overall seedling physiological status, this suggests that the physiological stability of seedlings in storage increases as hardiness deepens. In this example, it appears that lifting and storage before attainment of an LT-50 of approximately -15 C will result in a loss of seedling vigor.

Other observations have indicated that storage of seedlings lifted after significant dehardening has begun also results in further loss of hardiness (Ritchie 1986). It is generally recognized that the quality of seedlings lifted either too early or too late will decline in storage. By measuring the amount of hardiness lost in storage, it should be possible to quantify "too early" and "too late" in terms of LT-50 on the lift date.

In contrast to these results, Burr (1989) found that interior Douglas-fir continued to harden or even reversed dehardening when placed in cold storage, regardless of the hardiness level at the time storage commenced. However, this work was conducted with containerized seedlings which remained upright and undisturbed in the containers during the storage treatments. The storage treatments discussed in the previous paragraph involve bare-root seedlings which have been lifted from the beds and stored horizontally in tightly packed paper bags, similar to operational storage practices at a bare root nursery. The contrast in effect on frost hardiness development between the two differing storage treatments suggests that the shock associated with bare root lifting and storage prevents or retards further physiological changes during storage which would result in continued hardiness development. The fact that undisturbed seedlings which are placed in storage are capable of further physiological development serves to emphasize the importance of minimizing the stresses associated with bare root lifting, and to conduct the lifting when resistance to stress is at its peak.

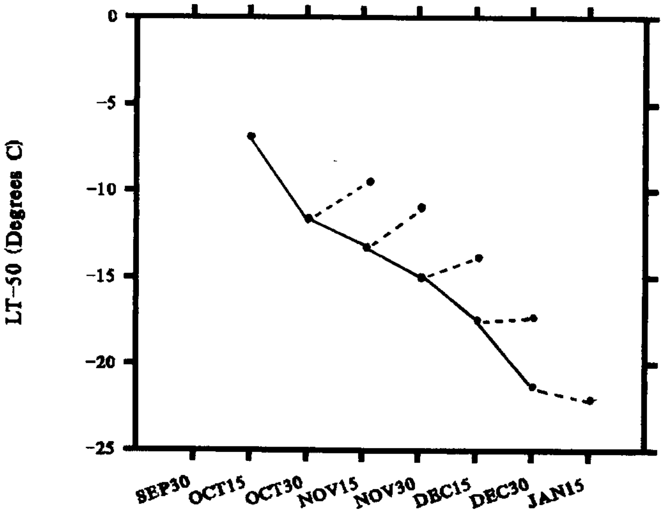


Figure 6. Effect of cold storage on frost hardiness development of coastal Douglas fir. Dotted lines connect LT50 points from fresh samples with stored samples from the same lift date.

Conclusion

The foregoing observations regarding the value of frost hardiness testing as an indicator of seedling condition have resulted from several years of International Paper's operational seedling monitoring program. More formal research is needed to confirm the hypotheses presented in this paper and to further investigate the relationship of frost hardiness to other physiological attributes of nursery seedlings. Specifically, the correlation between frost hardiness and overall stress resistance should be more firmly established, and more information is needed regarding the effects of storage on frost hardiness. In the meantime, however, there is little doubt that comparative frost hardiness testing can reveal significant differences between the phenological cycles of different seedling lots, with important implications for the timing of cultural practices and lifting operations.

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