Target Seedling Symposium

Chapter 7
The Target Seedling Concepts: Bud Dormancy and Cold-Hardiness

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ABSTRACT

Bud dormancy and cold-hardiness vary markedly throughout the annual growth cycle of trees in the tem - perate zone and have a profound impact on the ability of tree seedlings to withstand lifting, storage, and outplanting stresses. The Degree Growth Stage model is a useful tool for visualizing the changes in bud dormancy and cold-hardiness and their relationship to changes in other physi-ological attributes, such as root growth potential and stress resistance. Relationships among these attributes pro-vide an opportunity to infer the status of one from another. The level of cold-hardiness can be used to infer bud dormancy status, as well as general stress resistance, at the time of lifting because all are correlated with perfor-mance, and cold-hardiness is easiest to measure. Practical approaches for measuring bud dormancy and cold-hardi-ness, and for routine monitoring for associated physiologi-cal targets, are discussed.

7.1 Introduction

Cyclic changes in bud dormancy and cold-hardiness have evolved in temperate zone trees in response to the stresses imposed by the annual climatic cycle. The rates of development and loss of bud dormancy and cold-hardiness of nursery-grown tree seedlings are cued in any particular year by naturally occurring changes in climatic factors, such as temperature, photoperiod, and precipitation, as well as by nursery cultural practices, such as irrigation, fertilization, and pruning. Consequently, changes in bud dormancy and cold-hardiness do not occur linearly through time, such as by a specific calendar date, but rather as a complex function of many interacting factors which can vary by year, location, and genotype. Even so, nurseries have typically established lifting, storage, and outplanting schedules for the species and ecotypes they grow based on historically successful calendar dates.

Today, measurement of cold-hardiness and bud dormancy, as well as other physiological attributes, morphological parameters, and climatic data, can establish the reasons why these historical schedules are usually successful. In addition to improving our understanding of the physiological processes behind stock performance and our ability to set physiological targets, this information can be extremely useful when atypical situations arise. Challenges may occur, for example, when unusual climatic conditions alter seedling physiology too far from the historical norm; when cultural practices alter seedling physiology so that it is no longer synchronized with the natural environment; and when lifting, storage, or outplanting must be rescheduled for operational reasons, further disrupting physiological development. In many such situations, a thorough knowledge of whole-plant physiological condition will greatly improve our ability to make decisions that will best enhance stock quality and performance (Duryea 1984, 1985, Lavender 1984).

Since bud dormancy and cold-hardiness status cannot be determined simply as a function of time, or by virtue of association with visible changes in morphology, the best approach for their accurate assessment is periodic testing in a wide variety of genotypes during the dormant period, when these two attributes best reflect stock quality. This chapter discusses the annual growth cycle of temperate zone trees and the associated changes in bud dormancy, cold-hardiness, and related physiological attributes, to provide a foundation for establishing appropriate bud dormancy and cold-hardiness targets. In addition, practical approaches for measuring bud dormancy and cold-hardiness, and for their routine monitoring, are discussed.

7.2 Annual Growth Cycle

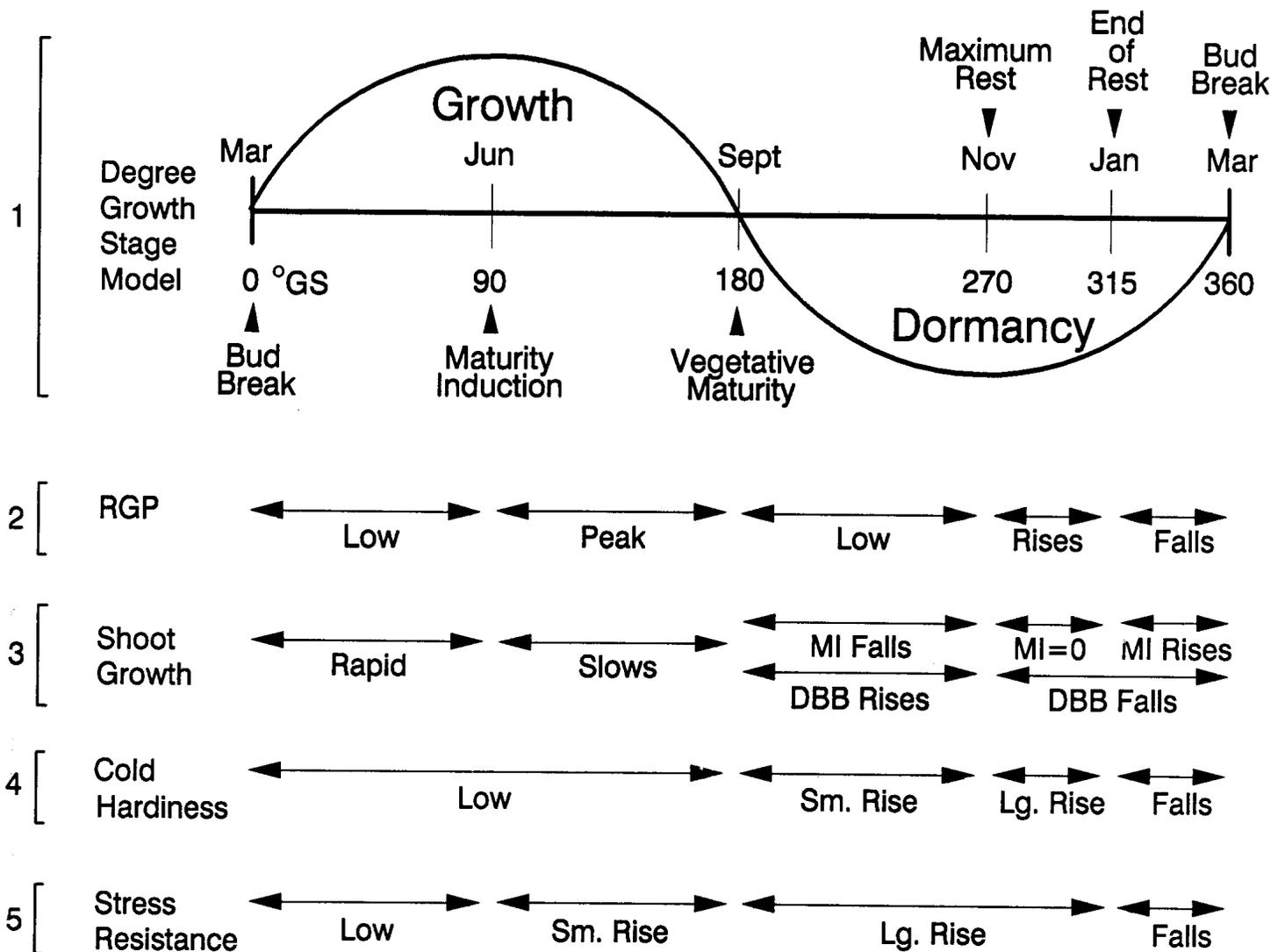The Degree Growth Stage model (Fuchigami and Nee 1987, Fuchigami et al. 1982) is a useful tool for visualizing the annual changes in bud dormancy and cold-hardi ness and their relationship to changes in other physiological attributes (Figure 7.1). Use of such a model can improve communication about the annual growth cycle by offering a standard framework and terminology to serve as a foundation for integrating related aspects of whole-plant physiology.

Figure 7.1 is divided into five sections. Section 1 illustrates a modified Degree Growth Stage model, representing one complete annual cycle for a temperate zone woody plant growing under ambient conditions. Sections 2 through 5 describe the changes in root growth potential (RGP), shoot growth, cold-hardiness, and stress resistance during the cycle. These are idealized patterns for typical conifer seedlings, also under normal climatic conditions.

7.2.1 Degree Growth Stage model defined

The Degree Growth Stage model represents the annual growth cycle as a sine wave from 0 to 360°, with bud break at 0 and at 360° as the cycle begins again (Figure 7.1, Section 1). A sine wave is used rather than a straight line because, as mentioned, physiological changes do not proceed linearly through time. There are five specific phenological "point events" at specific degrees along the sine wave: bud break (0°), maturity induction (90°), vegetative maturity (180°), maximum rest (270°), and end of rest (315°). The months assigned to the point events were established for coastal Oregon, but will vary with location (Ritchie and Tanaka 1990). The five point events delineate the five "segment events" of the model, which will be denoted by the range in degrees over which they occur.

The model is divided into two halves—growth and dormancy—which refer to the condition of the aboveground, vegetative portion of the plant, especially the shoot meristems. **Growth** can be interpreted to mean that the shoot is getting bigger, as by elongation and production of new foliage, for example. **Dormancy** can be loosely defined as the opposite, when shoot growth is not visible, such as during the existence of terminal buds. Note that growth is not synonymous with meristematic activity, however, because there may be activity in the lateral cambium or apical meristems during dormancy. Dormancy can be divided into rest and quiescence, based on internal or external control of growth resumption, respectively (Lavender 1985). A bud is in rest when dormancy is maintained by agents within the bud itself (Romberger 1963). This occurs prior to the meeting of chilling requirements for bud break in late autumn or early winter. A resting bud will not elongate under favorable environmental conditions. A bud is **quiescent** when dormancy is imposed by the environment, such as by continued low temperatures after chilling requirements have been met in late winter (Samish 1954). The transition from rest to quiescence occurs under natural conditions in response to exposure to chilling temperatures (Lavender 1981).

1 Degree Growth Stage Model

Growth

Maximum Rest ▼ Nov

End of Rest ▼ Jan

Bud Break ▼ Mar

Mar — Jun — Sept

0 °GS — 90 — 180 — 270 — 315 — 360

▲ Bud Break

▲ Maturity Induction

▲ Vegetative Maturity

Dormancy

2 RGP ◄──► Low ◄──► Peak ◄──► Low ◄►Rises ◄►Falls

3 Shoot Growth ◄──► Rapid ◄──► Slows — MI Falls / DBB Rises — MI=0 / DBB Falls — MI Rises

4 Cold Hardiness ◄──────► Low ◄──► Sm. Rise ◄─► Lg. Rise ◄─► Falls

5 Stress Resistance ◄──► Low ◄──► Sm. Rise ◄──► Lg. Rise ◄─► Falls

Figure 7.1—A Degree Growth Stage model (Fuchigami and Nee 1987, Fuchigami et al. 1982) representing one complete annual cycle, with changes in root growth potential (RGP), shoot growth (MI = mitotic index, DBB = days to bud break), cold-hardiness, and stress resistance during the cycle.

The point and segment events are described from left (0°) to right (360°). Bud break (0°) is the point at which new foliage becomes visible in the spring. Between bud break and maturity induction (0-90°), trees are temperature sensitive in that the rate of growth and development is generally temperature controlled. Growth is not inhibited by a short photoperiod. At approximately maturity induction (90°), buds are initiated. Between maturity induction and vegetative maturity (90-180°), trees are primarily photoperiod sensitive, with short days promoting budset and long days preventing or retarding budset. Drought can also be a major factor promoting budset, and may cause trees to enter a summer quiescent condition during this period (Lavender 1981, 1985). Vegetative maturity (180°) marks the onset of rest. Overwintering buds are well developed at this point.

Dormancy is maintained internally and intensifies between vegetative maturity and maximum rest (180-270°). The dormancy peak at maximum rest (270°) is characterized by an almost total absence of growth anywhere on the plant, and a chilling requirement which must be met before buds will resume rapid development (Ritchie 1984). Between maximum rest and the end of rest (270-315°), dormancy decreases in intensity as chilling requirements are met. At the end of rest (315°), buds are quiescent, with dormancy then imposed by the environment. An extended period of quiescence follows as long as environmental conditions remain unfavorable. When favorable environmental conditions for growth resume (315°), bud development renews, followed by bud break at 360°, completing the annual cycle.

Table *7.1*—*Comparison of the Degree Growth Stage model segments with the four phases of dormancy presented by Lavender (1984).*

Degree Growth Stage model segment	Phase of dormancy	Physiological Condition
90-180°	I – Dormancy initiation	Buds initiated and developing
180-270°	II – Deep dormancy	Dormancy intensity increasing
270-315°	III – Dormancy lifting	Dormancy intensity decreasing
315-360°	IV - Postdormancy	Quiescence to bud break

Dormancy theory has also been presented in the forestry literature in terms of four phases by Lavender (1984). The Degree Growth Stage model is quite compatible with this alternative approach (Table 7.1).

7.2.2 Root growth potential pattern

Root growth potential (RGP) is the ability of a tree seedling to initiate and elongate roots when placed into an environment favorable for root growth (Figure 7.1, Section 2) (Ritchie 1985, Ritchie and Dunlap 1980). Changes in RGP during the annual growth cycle are related to shoot dormancy and sink strength, defined as the relative ability of a plant part to compete for current photosynthate (Ritchie and Tanaka 1990). Peaks in RGP are evident when dormancy intensity is weak, but the sink strength of the shoot is also weak because rapid shoot growth is not occurring. Thus, RGP is low from 0-90° when shoots are the primary sink. From 90-180°, RGP peaks when shoot growth slows and roots become the primary sink. This corresponds to the typical surge and subsequent decline of root growth in the autumn (Lavender 1984). RGP remains low from 180-270° while dormancy intensifies. As dormancy weakens from 270-315°, RGP rises. This is the increase in RGP used to indicate fall lifting windows. RGP declines again from 315-360° as shoots regain their sink strength, with the resumption of environmental conditions favorable for growth.

7.2.3 **Shoot growth pattern**

Shoot growth is rapid following bud break (0-90°) with elongation and production of new foliage (Figure 7.1, Section 3). As buds develop from 90-180°, shoot growth slows. Elongation ceases by vegetative maturity (180°). During the dormant period (180-360°), the mitotic index (MI) and days to bud break (DBB) are two important measurements that indicate changes in dormancy intensity.

Mitotic index refers to the percentage of dividing cells in an apical meristem (Grob 1 990b, Hawkins and Binder 1990, Owens and Molder 1973). Mitotic index falls as dormancy intensifies between 180-270°. By maximum rest (270°), the mitotic index is approximately zero, indicating that no cell divisions are occurring. This lack of mitotic activity continues until the end of rest (315°) With the onset of favorable environmental conditions assumed to occur at 315°, the mitotic index rises again between 315-360°, as buds resume development.

Days to bud break refers to the number of days required for terminal buds to break under optimum growing conditions. The seedlings must be taken from ambient conditions and placed under optimum conditions to determine days to bud break. Days to bud break increases as dormancy intensifies from 180-270°. At maximum rest (270°), seedlings require the maximum number of days under favorable conditions to break bud, or buds will not break at all, depending on species. After maximum rest, days to bud break decreases as chilling requirements are met and dormancy intensity weakens. A stable number of days to bud break will be maintained if a quiescent period occurs after the end of rest. But when bud development resumes with exposure to warm temperatures, days to bud break will continue decreasing to reach zero by 360°.

7.2.4 Cold-hardiness pattern

Cold-hardiness refers to the ability of a plant or plant tissue to survive or resist injury from exposure to freezing temperatures (Figure 7.1, Section 4). The level of cold-hardiness is frequently defined by a lethal temperature value, such as the LT_{50}, which represents the minimum temperature at which 50 percent of a group of seedlings, or 50 percent of a specified tissue, is killed.

Cold-hardiness is low during the growth period (0-180°), with trees generally unable to withstand exposure to temperatures below about -3°C without sustaining injury (Glerum 1985). Cold-hardiness increases somewhat from 180-270°, the amount varying with species. For example, a change in the LT_{50} from -5°C at vegetative maturity (180°) to -15°C at maximum rest (270°) has been observed in Southwest conifers (Burr, unpublished data). The majority of the cold hardening occurs from 270-315°, with a change in the LT_{50} from -15 to -40°C or lower, depending on the species (Sakai and Larcher 1987). After the end of rest (315-360°), with exposure to warm temperatures, cold-hardiness is rapidly lost arid returns to growth period levels ($LT_0 = -3°C$).

7.2.5 Stress resistance pattern

There are several stresses to which temperate zone trees have developed cyclic annual patterns of increasing and decreasing resistance, such as drought stress (Lavender 1985), low temperature stress (Glerum 1985), mechanical stress (Tabbush 1986), and root exposure stress (Hermann 1 967). Although these stresses affect the tree in different ways, trees develop a general stress resistance that varies

throughout the annual growth cycle (Figure 7.1, Section 5).

Stress resistance is lowest during rapid shoot growth (0-90°) and increases some as shoot growth slows (90-180°), especially with regard to drought resistance, which may parallel the development of summer quiescence (Lavender 1985). The major increase in stress resistance occurs during dormancy, with a maximum reached by the end of rest (315°) (Lavender 1985, Ritchie 1986a). Stress resistance falls with renewed development and growth after the end of rest (315-360°).

7.3 Relationships Among Physiological Attributes

The potential for highest seedling performance results when seedlings are harvested and outplanted when their resistance to stress is highest (Lavender 1985). Thus, the period of maximum stress resistance (290-315°) is the tar-

get period for fall lifting and storing. It has been proposed that one of the reasons RGP is such an effective seedling quality test is because the rise in RGP during dormancy identifies the period of maximum stress resistance (Ritchie 1985, Ritchie and Tanaka 1990). This period is approximately from December to February in the coastal Northwest. Given that the indicated relationships among these attributes exist (Figure 7.1), this period can also be identified by an extended period of low mitotic activity, by decreasing days to bud break, and by rapidly increasing cold-hardiness, as well as by the rise in RGP.

There are data in the literature to support the existence of these relationships (Cannell et al. 1990, Colombo 1990, Faulconer 1988, Glerum 1982, Ritchie 1986a). The following example illustrates the relationships among bud dormancy, cold-hardiness, and RGP in an Arizona seed source of Douglas-fir (Figure 7.2) (Burr et al. 1989). Greenhouse-cultured, container-grown, nine-month-old Douglas-fir seedlings, which had set bud and entered the
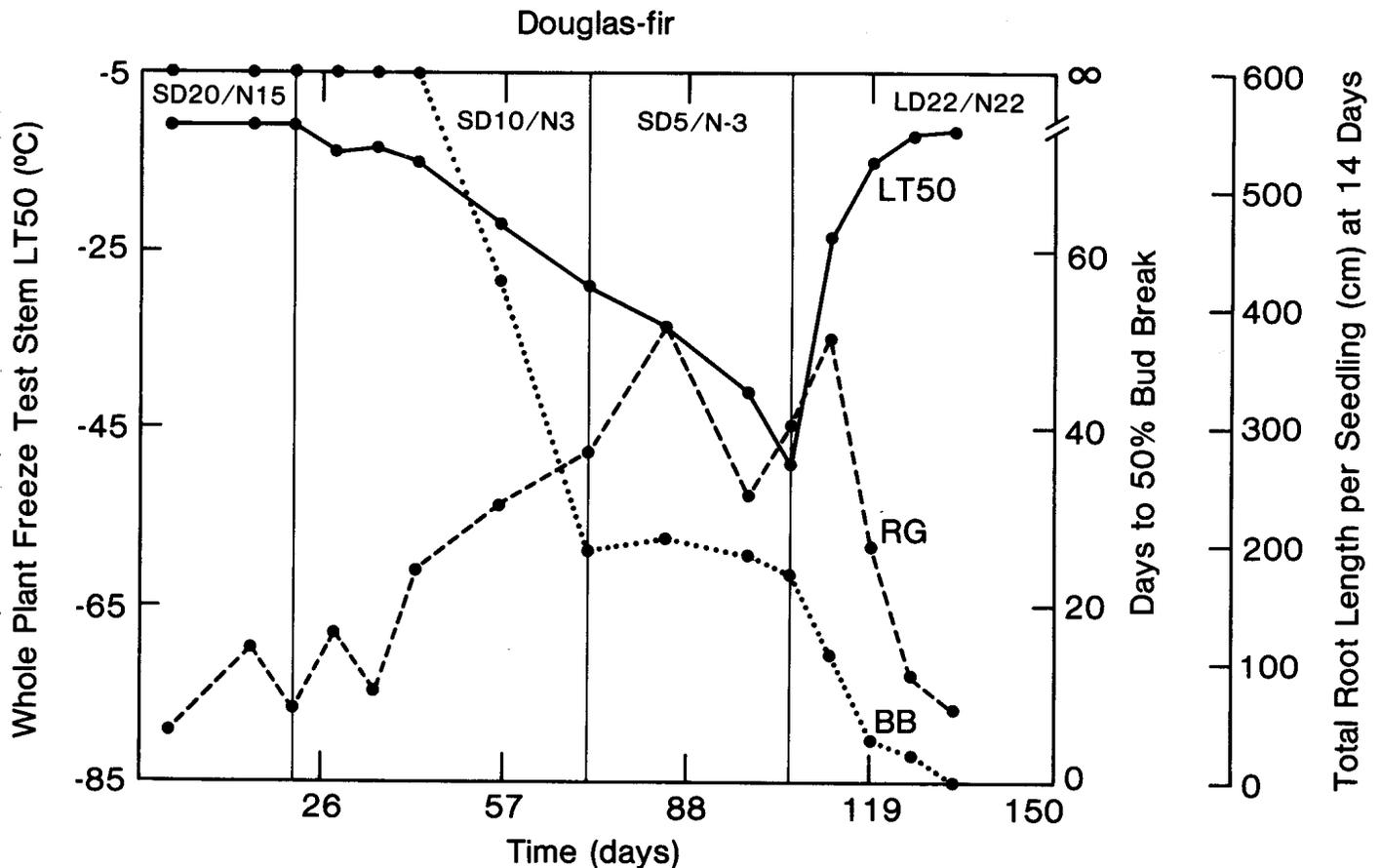
Figure 7.2 --*Interior Douglas-fir stem cold-hardiness (LT$_{50}$), root growth potential (RG), and number of days to 50% bud break (B as a function of time under a 4-stage, growth chamber regime (Burr et al. 1989 from Tree Physiol. 5:301). Growth chamber conditions, indicated across the top of the graph, are as follows: SD20/N15 = 10 hr (Short), 20%C Day/15° C Night; SD10/N3 = 10 hr, 1 Day/3° C Night; SD5/N-3 = 10 hr, 5° C Day/-3° C Night; LD22/N22 = 16 hr (Long), 22° C Day/22° C Night*

dormant period, were placed in growth chambers for a four-stage cold acclimation and deaccilmation regime designed to simulate seasonal development from autumn to spring. The first three stages acclimated seedlings to cold under a short (10 hour) photoperiod and progressively colder temperatures, and the fourth stage deacclimated seedlings under a long (16 hour) photoperiod and warm temperatures. The point events on the Degree Growth Stage model can be identified from the days to bud break curve (BB). During the first 42 days of the experiment, buds did not break after 150 days under forcing conditions, indicated by infinity on the bud break axis. Following day 42 of the experiment, days to bud break declined. Thus, day 42 was the maximum rest point (270°). During the third stage, days 72 to 105 of the experiment, days to bud break stabilized at 24 to 28 days. Since the third stage chilling did not reduce the number of days to bud break, this was a period of quiescence resulting from the continued exposure to cold temperatures after chilling requirements were met. Thus, day 72 of the experiment was the end of rest point (315°). With exposure to a long photoperiod and warm temperatures in the fourth deacclimating stage, beginning day 106 of the experiment, days to bud break continued to decrease rapidly to zero on day 130, which was the bud break point at 360°.

Changes in cold-hardiness (LT_{50}), as well as RGP (RG), were related to the timing of the above sequence of changes in bud dormancy (Figure 7.2). Prior to maximum rest, day 42 of the experiment, RGP was low and cold-hardiness only increased from -11 to –15° C. (Note that an increase in cold-hardiness is represented by a decrease in the LT_{50}.) This is the small rise in cold-hardiness referred to earlier (Figure 7.1, Section 4). Hardening from about -5 to -11° C occurred prior to data collection. After maximum rest, all three attributes changed rapidly; days to bud break decreased, and RGP and cold-hardiness increased. During the quiescent period of the third stage, RGP remained high though fluctuating, and cold-hardiness continued to increase In the deacclimating fourth stage, all three attributes changed rapidly again; days to bud break decreased as bud development resumed, and RGP and cold-hardiness declined. Similar relationships among bud dormancy, cold-hardiness, and RGP have also been observed for Arizona seed sources of ponderosa pine and Engelmann spruce (Burr et al. 1989), and are presented in Ritchie and Tanaka (1 990).

If bud dormancy and cold-hardiness targets for fall lifting were set to indicate the period of rapid change during cold acclimation, such as at day 57 of the experiment, it is expected—given our present state of knowledge—that chilling requirements will be completed and rapid cold hardening will continue in storage. It can also be inferred that RGP will be rising rapidly at lifting (barring any unforeseen detrimental climatic or cultural events)

because of the relationships among the attributes. In addition, the rapid approach to the period of maximum stress resistance will be identified. Lifting and storing at this time will result in improved storability, as well as improved survival and growth in the field. Similarly, outplanting targets should be set such that a decline in any of the three attributes from their levels at the end of rest will indicate resumption of development and the associated loss of stress resistance.

The above discussion does not imply that rapidly increasing cold-hardiness and decreasing days to bud break at lifting will predict good field performance, nor that maximum cold-hardiness and rapid uniform bud break at out-planting will predict good field performance. It means only that, all things being equal, these characteristics will be positively correlated with performance because they are correlated with stress resistant, high quality seedlings. Seedling quality test results only provide part of the equation needed to predict field performance. Outplanting site conditions must also be included in the equation because it is the interaction between seedling physiological condition and the field environment which will ultimately determine performance.

7.4 Relationships Between Physiological Attributes and Performance

There are data in the literature that support the hypotheses that bud dormancy and cold-hardiness are positively correlated with aspects of performance. An example dealing with each attribute follows.

7.4.1 Bud dormancy

The correlation between bud dormancy at lifting and field survival is illustrated in an experiment by Larsen, South, and Boyer (I986) (Figure 7.3). Twenty loblolly pine lots of the same seed source location, produced at 20 southern forest nurseries, were lifted in early December, stored briefly, and then outplanted in late December. At out-planting, an RGP test was conducted on a sample of 20 seedlings from each lot to determine the speed and uniformity of bud break (number of active buds), as well as RGP. Each data point in Figure 7.3 represents one lot and indicates the number of active terminal buds in the sample after 23 days in the RGP test, versus the survival of the outplanted lot 11 months after planting. Rapid, uniform bud break in the RGP test, which reflected the proportion of quiescent buds in each lot at planting, was positively correlated with increased field survival of the lot. Consistent with the relationships indicated in Figures 7.1 and 7.2, samples with a high proportion of active buds, i.e., which had reached the end of rest (315°) at outplanting, also had higher RGP than samples which had not received their full chilling requirement.

Percent Survival

$$Y = 39.9 + 1.96(X)$$
$$R^2 = 0.36$$

Number of Active Buds
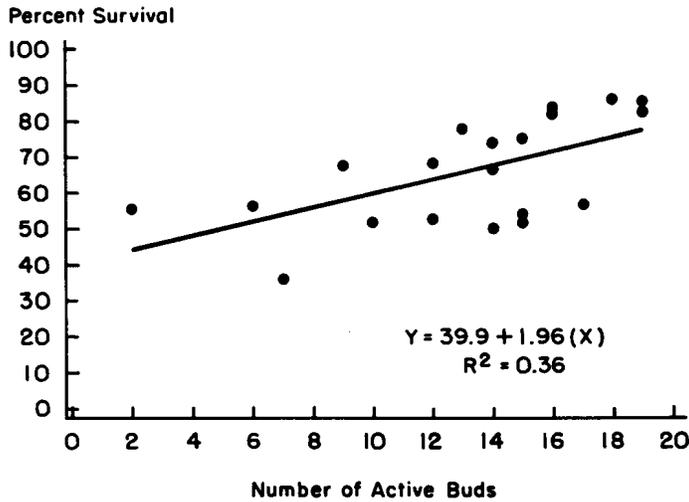
Figure 7.3 --*Relationship between percent survival of 20 lots of a loblolly pine seed source 11 months after planting and terminal bud activity of samples (n = 20) taken from each lot at outplanting, after a 23-day ROP test (Larsen et al. 1986 from Tree Physiol. 1:258).*

it is not possible from the above experiment to conclude that rapid bud break directly resulted in better survi val. However, the combination of quiescence, high RGP, and the inferred high stress resistance contributed to the high- er performance of some lots. It should also be noted that the speed of bud break was an indicator of dormancy intensity in this experiment, and as such, was correlated with field survival. The speed of bud break does not always indicate vigor or future performance in seedlings which have all had chilling requirements fully met prior to exposure to conditions favorable for growth (Lavender 1985).

7.4.2 Cold-hardiness
The correlation between cold-hardiness and performance is illustrated in an experiment by Burden and Simpson (1984) (Figure 7.4). There was a close relationship between cold-hardiness (frost hardiness) at lifting and storability, defined as the ability of 2+0 seedlings to main- tain or improve their RGP during storage. Seedlings lifted with an LT_{50} of about -22° C had the same, or 100 per- cent, of the RGP after storage as at lifting, while seedlings with greater cold-hardiness had greater RGP after storage than at lifting. Even though cold-hardiness at lifting was not necessarily the sole factor affecting storability, a cold- hardiness target of -22° C could be set for these species to minimize loss of physiological quality in storage.

Lodgepole Pine

White Spruce

Frost Hardiness
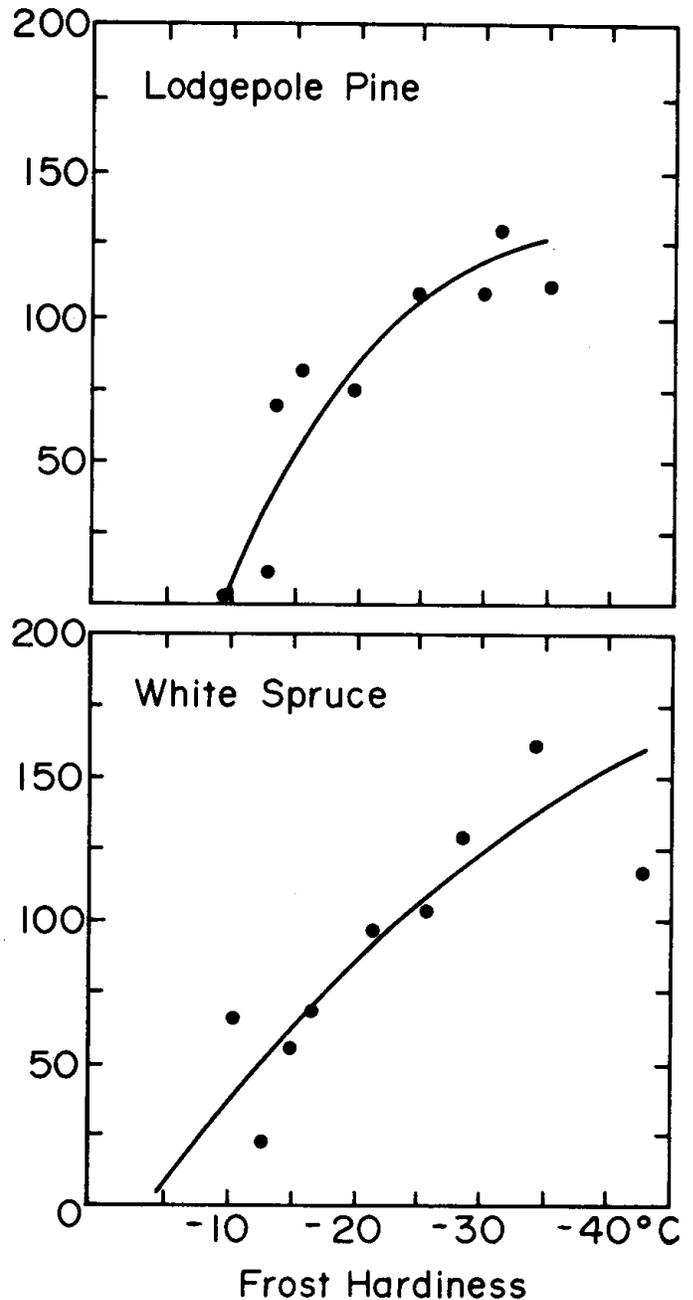
7.5 General Considerations for Establishing Targets
While it is beneficial to monitor bud dormancy and cold- hardiness because of their correlations with various aspects of performance, and the inferences which can be made about other physiological attributes, there are a number of items to consider when using bud dormancy and cold-hardiness data to establish lifting or outplanting targets.

Lifting targets that indicate the period of rapidly decreas- ing days to bud break and rapidly increasing cold-hardi- ness are most informative. Similarly, monitoring bud

dormancy and cold-hardiness at outplanting in such a way that will detect rapid changes in these attributes in the opposite directions will be most informative. This is because the stable minimum number of days to bud break, as well as the maximum level of cold-hardiness, will vary from year to year, and from nursery to nursery, for the same seed source, when seedlings are quiescent, following the end of rest (315°) (Figure 7.2, Stage 3). For example, in an experiment in which one-season-old, container-grown, interior Douglas-fir seedlings were cold acclimated under three different day/night temperature regimes, three different stable values for days to bud break (range: 18-32 days), and three different maximum levels of cold-hardiness (LT_{50} range: -23 to -38° C) were attained (Burr and Tinus, unpublished data). Given the infinite number of nursery environments, combined with yearly differences in climate, setting discrete targets of a minimum number of days to bud break or a maximum level of cold-hardiness, is not as helpful. Instead, rates of change are more relevant.

A second concern is the uncertainty involved in predicting seedling physiological quality following storage because bud dormancy and cold-hardiness can improve, deteriorate, or remain constant in storage, as can other physiological attributes (Ritchie 1986b). Even if improvement occurs, the rate of physiological development in storage may not be the same as if the seedlings had been left in the field, which adds further difficulty to estimating the final effect of storage on physiological condition (Burr and Tinus 1988, Arnott et al. 1988). Consequently, establishing and monitoring for both lifting and outplanting targets is especially important when storage is an intermediate step. Additionally, the storage environment can also be used to advantageously alter seedling physiological development when storage itself is not the primary goal. For example, substantial reacclimation to cold (2° C/week) was possible when deacclimating (315-360°) interior Douglas-fir seedlings were placed in 1° C storage for 4 weeks (Burr and Tinus, unpublished data). Thus, when a loss in cold-hardiness is premature from a management perspective, it is possible to reverse the loss with skill in environmental manipulation of this attribute (Fuchigami et al. 1982, Sakai 1966).

As a final consideration, a single test, measuring one physiological attribute, is not necessarily enough to ensure physiological quality, especially if it requires inferring too much from bud dormancy and cold-hardiness information and the relationships among physiological attributes. For example, it is possible for RGP to be very different from that expected, based on the relationships indicated in Figures 7.1 and 7.2, when bud dormancy and cold-hardiness targets for lifting and outplanting have been met. Fully cold-hardy seedlings, as determined by a needle browning test for cold-hardiness, may produce no new roots in an RGP test. Also, seedlings may break bud

rapidly in an RGP test, but produce no new roots. Both situations can occur because of the delayed response of the shoot to root system damage. Consequently, thoughtful application and interpretation of physiological quality tests is essential.

7.6 Observing and Measuring Targets

An ability to determine seedling physiological status is prerequisite to setting and meeting physiological targets. In this section, observing and measuring bud dormancy and cold-hardiness are discussed, with practical implications for use in nursery monitoring programs.

7.6.1 Bud dormancy

Bud dormancy status can be observed directly at some of the point and segment events on the Degree Growth Stage model (Figure 7.1, Section 1). Obviously, bud break at 0 and 360° would be examples. Maturity induction (90°) can be approximated by examination of the shoot apex for bud initiation. Accurate determination of maturity induction requires testing for photoperiod sensitivity (Fuchigami et al. 1982). The definitive test for vegetative maturity (180°) is most applicable to deciduous species. When defoliation no longer results in bud break (i.e., correlative inhibition has ended), vegetative maturity has been reached (Fuchigami et al. 1982). With conifers, the stop in height growth and the development of overwintering buds can be observed. From 180-270°, the decline in growth to an almost complete absence anywhere on the tree at maximum rest (270°), can be observed, for example, in the decline in root activity as indicated by decreasing numbers of white root tips. However, maximum rest can be estimated with greater precision by testing under forcing conditions to determine the point at which the most time is required for bud break. During the critical 270-315° segment, the change in dormancy intensity cannot be directly observed, nor can resumption of development between 315-360°, until bud swell.

Measurement of mitotic index (Grob 1990b, Hawkins and Binder 1990) can identify maximum rest, as well as the resumption of development after the end of rest (Figure 7.1, Section 3). Monitoring the decline in mitotic activity to zero after vegetative maturity (180°) indicates the maximum rest point (270°). Monitoring for the subsequent increase in mitotic activity indicates the conclusion of any quiescent period and the renewed apical meristem development. However, mitotic index is not useful for assessing the decrease in dormancy intensity as chilling requirements are met between maximum rest and the end of rest (270-315°) because of the complete absence of mitotic activity in most species during this period. A quick test measuring the speed of resumption of mitotic activity under forcing conditions, while the mitotic index is zero under actual conditions (270-315°), is under development to indicate this change in dormancy intensity (Grob

1990a). This test may prove to be a very useful tool to monitor for bud dormancy targets because it provides timely, accurate results by measuring the ability to resume rapid mitotic development, rather than bud break (the result of that development).

Currently, days to bud break tests are the most reliable way to monitor the change in dormancy intensity from 270-315° (Ritchie 1984). This test can be easily conducted as an extension of an RGP test by maintaining seedlings under the optimum root growth conditions until bud break, but the length of time before results are available may range from weeks to months, making days to bud break testing impractical for routine monitoring of dormancy intensity. If the relationship between the decrease in days to bud break and the increase in cold-hardiness were established for this period (270-315°), as was done for interior Douglas-fir (Figure 7.2), inferences about dormancy intensity could be made from the level of cold-hardiness. Setting a cold-hardiness target would also be setting a bud dormancy target. The status of both could then be determined quickly because of the relative speed with which cold-hardiness can be measured.

7.6.2 Cold-hardiness

There are a number of excellent testing procedures available for measuring cold-hardiness. The method of choice for nursery monitoring is the whole-plant freeze test (WPFT), also known as the browning test (Glerum 1985, Ritchie 1984). Entire plants, with root systems insulated, are exposed to a series of sub-freezing temperatures at defined rates of cooling and rewarming. The plants are then maintained under optimum growing conditions until visible evidence of injury develops in about 7 to 14 days (Rietveld and Tinus 1987). Exposure to a range of test temperatures will permit determination of the actual level of cold-hardiness. Once a cold-hardiness target has been set, repeated testing at that temperature until no injury results is a time-saving modification to the standard procedure. Use of stem sections, with needles and buds attached, can be useful if plant material is limited. Root system cold-hardiness can also be measured.

The great advantage of the WPFT is its accuracy resulting from the exposure of entire plants to actual stress temperatures. The test is also easy and inexpensive to conduct, and injured tissue is readily distinguishable. There are disdvantages, however. A week is usually required before the low-temperature injury is evident, though this is much less time than typically required for bud dormancy testing. Additionally, the WPFT does not estimate cold-hardiness with precision because of variability between seedlings. Thus, it may be difficult to detect small (1-2°C) changes in cold-hardiness, and sample sizes of 50-60 are often recommended (Burr et al. 1990, Owston 1988).

Once cold-hardiness targets are set, and seedlings at the target can be identified with the WPFT, it is not difficult to minimize the disadvantages of the WPFT by converting to a faster, more precise, tissue test. Such tests assess cold-hardiness by indirect methods and often use only a single tissue, e.g., electrolyte leakage from needles, differential thermal analysis of buds, and electrical impedance of stems (Glerum 1985). To convert to one of these quick tests, both the WPFT and the tissue test should be conducted on seedlings at the target cold-hardiness level. The tissue test results can then be calibrated to the WPFT results to determine the correct target tissue test result. This is necessary because actual cold hardiness may be inaccurately estimated by a tissue test, depending on the methodology used (Burr et al. 1990).

The tissue test of preference is the freeze-induced elecrolyte leakage test because results are available in 2 days. The test is very precise, it requires less plant material than the WPFT, and it has been operationally tested (Burr et al. 1990, Glerum 1985, Hawkins and Binder 1990). Though the cost of equipment is higher than for the WPFT, a great many samples can be processed at once. Procedures for using this test in nursery applications (Colombo et al. 1984) and in research (Burr et al. 1990) are available.

7.7 Practical Approach

Though there may be a diversity of opinion on how and when to use physiological targets, the following discusion presents one approach for incorporating bud dormancy and cold-hardiness targets into an existing forest nursery operation. The intent is not to suggest that the hisorical lifting and outplanting schedules should be abanoned, but rather that they be supported and enhanced by the additional information physiological monitoring proides.

The first step toward incorporation of physiological targets into nursery practice is the establishment of a solid foundation of data to use in setting appropriate targets (Owston 1988, Rietveld et al. 1987). By routine, periodic monitoring of bud dormancy, cold-hardiness, RGP, root activity, etc., as well as morphological, cultural, storage, and climatic variables, the relationships among the physiological attributes and the nursery environment can be determined. Ideally, this should be done intensively over several years, from sowing through outplanting and establishment, with a spectrum of genotypes representative of the stock produced at a given nursery. While only continal tracking of physiological attributes provides the detailed level of understanding desired, considerable progress can be achieved by measuring physiological staus at lifting and outplanting with simple procedures and inexpensive equipment, depending on the resources of the individual nursery (Burr et al. 1987, Faulconer 1988, Rietveld and Tinus 1987, Ritchie 1984, Simpson 1986).

Additionally, physiological measurements can be made by the nursery, or by sending seedling samples to organiations offering testing services (Munson 1986). In any case, the status of the physiological attributes can then be compared with the successful lifting and outplanting schedules in order to set target values.

Annual air and soil temperature patterns, compiled during intensively monitored years, can be compared with the historical climate at the nursery and with the climate in future years. These comparisons will indicate how representative the monitored years were, and aid in determining the patterns of physiological attributes in future years with considerably less intensive monitoring. Additionally, the probability of damaging low-temperature events on any given date can be determined from historical weather data. This information can aid in making freeze protection decisions in the nursery (James Bryan, Weyerhaeuser Mima Forest Nursery, 1990, pers. comm.).

Targets can be tested by lifting seedlings at several times, before and after the actual lift date. The performance of those seedlings varying in physiological quality at lifting can be monitored at outplanting, and at intervals thereafter in the field. This provides an opportunity to compare quality and refine the target values at both lifting and outplanting.

The physiological attribute(s) best used for lifting and outplanting targets must be decided once information is available on the relationships among physiological attributes, morphological development, and climate. For example, bud dormancy or cold-hardiness targets could be used in a seedling monitoring system, or these could be omitted in favor of RGP testing. An excellent approach is the two-part testing program in use in British Columbia forest nurseries (Simpson 1990). The lifting target is a specific level of cold-hardiness, defined as an LT_{25} measured with a WPFT to -18°C. The outplanting target is a minimum RGP level, tailored to species, stock type, and planting site.

The idea of a monitoring program in which cold-hardiness is measured at the time of lifting, and RGP is measured immediately before planting, is not a new one (Duryea 1985, Johnson 1986). There are many advantages to this testing program.
1. Both a lifting and an outplanting target are used to allow for changes taking place in storage.
2. Two different physiological attributes are used in the event that one test is not enough to ensure quality.
3. Cold-hardiness is ideal as a lifting target attribute because it reflects physiological development well, permits inference about bud dormancy and stress resistance, fluctuates minimally while increasing, and is quick and easy to measure (Faulconer 1988).
4. RGP is ideal as an outplanting target attribute because

it best reflects whole-plant performance potential (Ritchie and Tanaka 1990), and is also easy to measure.
5. Two strong relationships are used in series, the first between cold-hardiness at lifting and post-storage RGP, and the second between post-storage RGP and field performance.
6. The cold-hardiness lifting target is set to indicate the period of rapidly increasing cold-hardiness, rather than maximum cold-hardiness.

7.8 Conclusions and Recommendations
1. A considerable amount of information is known about the annual growth cycle of temperate zone trees and the associated patterns of change in various physiological attributes. The Degree Growth Stage Model is an effective tool for communicating this information.
2. Relationships among physiological attributes such as bud dormancy, cold-hardiness, and RGP permit inferring the status of one from another. The period of maximum stress resistance during which lifting, storage, and outplanting procedures should be conducted can thus be identified by measuring any of these attributes.
3. Lifting targets should be set to identify the period of rapid change in bud dormancy, cold-hardiness, and RGP during cold acclimation, while outplanting targets should be set to detect a decline in the three from their levels at the end of rest.
4. Bud dormancy and cold-hardiness status are **correlated** with performance, but this information must be combined with data on field conditions to **predict** performance.
5. Both lifting and outplanting targets are necessary when storage is an intermediate step.
6. During much of the annual growth cycle, bud dormancy status can be observed or quickly measured by determining the mitotic index. However, to measure the decline in dormancy intensity during the critical lifting period between maximum rest and the end of rest, lengthy bud break tests must be performed. With knowledge of the relationship between bud dormancy and cold-hardiness, cold-hardiness targets will incorporate bud dormancy status, and cold-hardiness is much quicker to measure.
7. Cold-hardiness can be measured with the accuracy of the whole-plant freeze test, or faster and with greater precision using the electrolyte leakage test, once results of the two tests are calibrated.
8. Annual base-line data on the relationships among physiological attributes, morphological parameters, and cultural and climatic conditions are needed to establish why lifting and outplanting schedules are (or are not) successful so that appropriate targets can be set for the many nursery-genotype combinations.
9. A practical approach to pursue is a testing program

with lifting and outplanting targets based on solid relationships among physiological attributes and measured by quick, straightforward, non-labor-intensive testing procedures.

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