Seedling Quality Tests: Bud Dormancy

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In the Winter 2002 issue of Forest Nursery Notes, we initiated a series of articles on seeding quality testing with a discussion of the popular root growth potential test. In the Summer 2003 issue we reviewed aspects of the cold hardiness test. In this third installment, we will discuss dormancy - what it means, how it can be measured, and how it can be practically used in forest and conservation nurseries.

The Concept of Dormancy

Dormancy is one of the oldest concepts in nursery science. Foresters learned by observation and trial and error that plants could be transplanted and outplanted most successfully when they were not actively growing. In the temperate zone, this occurs in winter so nurseries have traditionally harvested their stock during this period. The concept of the "lifting window" was developed by harvesting and outplanting seedlings from late fall through early spring and measuring survival and growth. These trials supported the traditional practice of harvesting during mid-winter, and people interpreted these results to mean that seedlings were most "dormant" during this period. However, as we will show, the concept of a mid-winter dormancy peak is not correct.

Defining dormancy. Okay then, what do we mean by dormancy in forest and conservation nurseries? Dormancy has a couple of common definitions: a state of minimal metabolic activity; or any time that a plant tissue is predisposed to grow, but does not (6). In other words, dormancy is that condition in which plant growth – cell division and enlargement – is not occurring. In horticulture, dormancy can refer to either seed dormancy or plant dormancy. In the published literature, seed dormancy has been much more studied than plant dormancy but it is the latter that we are concerned with here.

Two kinds of plant dormancy are recognized: 1) imposed dormancy, also known as "quiescence", occurs when environmental conditions (*e.g.* severe water stress) will not support growth (6). Plants exhibiting imposed dormancy will resume growth when these unfavorable conditions are relaxed (*e.g.* it rains). 2) Internal or deep dormancy is a condition in which plants will not resume growth until they have experienced a long period of exposure to low temperatures (8). This article will focus on deep dormancy and how this physiological condition affects nursery culture.

Dormancy Refers to Tissues, Not Plants - In everyday nursery jargon we talk about seedlings, or even entire crops, being dormant. While this is common terminology, it is important to understand that plant dormancy refers to a specific meristematic tissue –

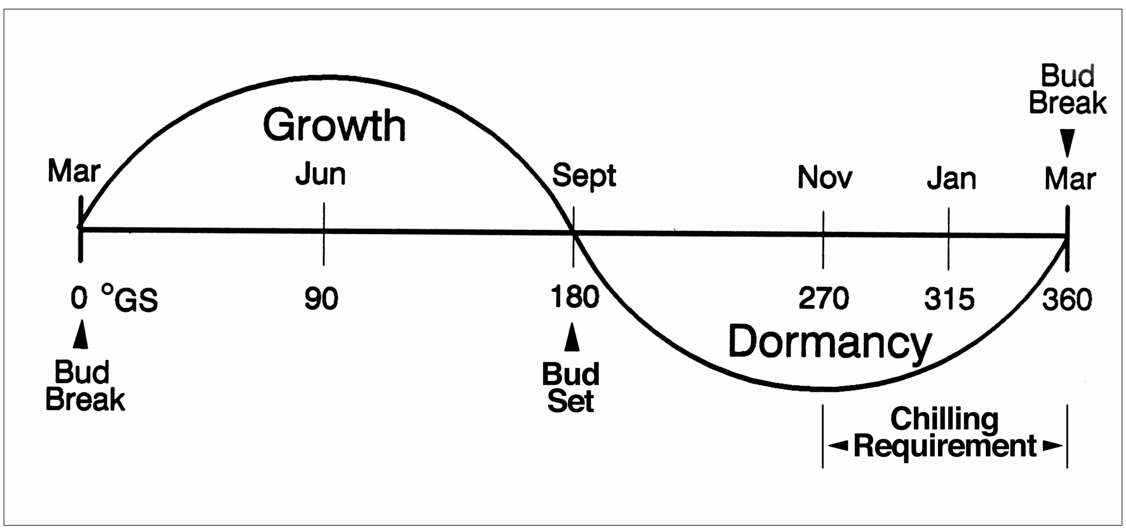

Figure 1. The shoots of all perennial plants, including forest and conservation nursery stock, undergo a seasonal cycle of shoot growth and dormancy. Note that peak dormancy occurs in late fall instead of mid-winter as is popularly believed. Bud dormancy is released by cumulative exposure to cold temperatures - the chilling requirement (modified from 2).

usually buds. In the same seedling, buds may be dormant while the lateral meristem may not. Root meristems never truly go dormant and will grow anytime that environmental conditions, especially temperature, are favorable. So, since we are concerned with seedling quality testing, we will be discussing bud dormancy, which is most clearly observed in the behavior of terminal buds.

The Dormancy Cycle **-** Perennial plants that grow in temperate regions exhibit a pronounced seasonal "cycle of dormancy" (Figure 1). In spring, as day length and temperature increase, plant buds begin to exhibit dimensional increases reflecting both cell division and expansion **-** in other words, they begin to grow. Shoot growth persists through spring and into summer. In early summer, as the day length (photoperiod) begins to shorten, the increasing length of the dark period is perceived by the phytochrome system in the leaves as a signal to begin preparing for winter. At this point shoot growth slows, and winter bud development proceeds (2, 3).

The Chilling Requirement **-** Through late summer, plant shoots enter the condition of imposed dormancy. As summer surrenders to autumn, imposed dormancy gradually gives way to deep dormancy and buds reach maximum dormancy in late fall (270 degrees in Figure 1). As we just discussed, the only way this dormant condition can be released is by exposure of the plants to an extended period of low temperature (this is known as a "chilling requirement") that is sensed by the buds. This evolutionary adaptation ensures that plants will not break bud during a mid-winter warm spell only to be killed by a return of cold weather. Once this chilling requirement is satisfied, warm spring temperatures and, to a lesser extent, lengthening photoperiod, will trigger and sustain a resumption of shoot growth (4). Although temperatures in the range of about 3°C to 5°C (37°F to 41°F) are most efficient at releasing bud dormancy (1), temperatures above and below this range also are effective to a lesser degree (Figure 2).

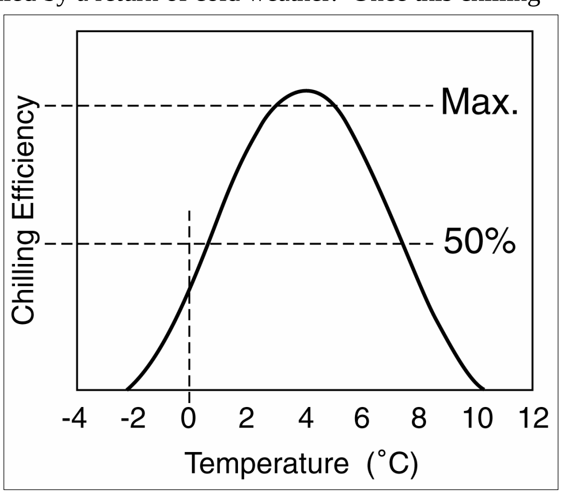

Figure 2. A range of chilling temperatures is effective at breaking bud dormancy (modified from 1). Note that temperatures in the range of refrigerated storage [30 to 33°F (-1°C to +1°C)] are relatively inefficient at releasing dormancy (modified from 1).

Orchardists, and other horticulturalists, have developed elaborate models to predict the date of flower bud opening in cold sensitive crops such as peaches (9). These models take into account the efficiency of chilling and the fact that warm interruptions during late fall can negate some chilling that has occurred up to that time. In forest nurseries, however, this level of precision is not warranted, and a simplified process involving calculating chill sums or chilling hours has been effective. The details are given in the following section.

Measuring Dormancy

Because of its tremendous importance to nursery management, there have been many attempts to develop a simple way to measure dormancy. As we will now discuss, this objective has been elusive:

1. Dormancy meters. In the 1970s, researchers observed that changes in electrical resistance of plants provided a useful way to determine whether tissues were injured or dead. Building on these observations, they constructed a "dormancy meter" (Figure 3) with the objectives of measuring dormancy in the fall and telling nursery managers when it was safe to lift their stock. Unfortunately, subsequent tests showed that these meters were not reliable (13). The idea of a simple "black box" is still attractive but it is doubtful that any equipment or technique will be able to instantaneously measure bud dormancy.

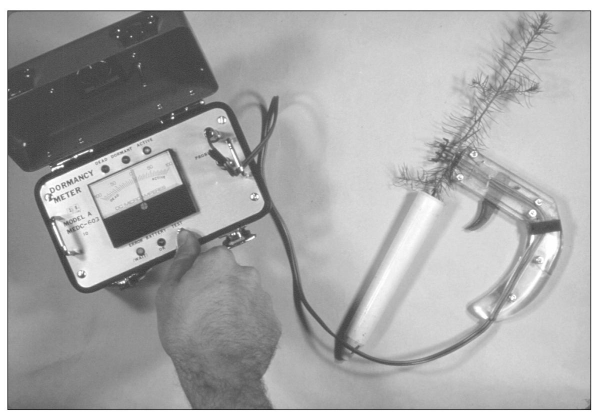

Figure 3. The "dormancy meter" was an attempt to find a simple and easy way to measure dormancy and determine when seedlings were ready for harvest. It didn't work, perhaps because they weren't even measuring bud tissue!

Table 1—An Example of How to Calculate Chilling Sums Using Degree Days, Calculated from an Average of Daily Maximum and Minimum Temperatures and a 40 ºF (4.5 ºC) Base Temperature

Day	Base Temp. (ºF)	Daily Temperatures (ºF)			Degree Days	Chilling Sum (Days)
		Maximum	Minimum	Average		
One	40	40	20	30	10	10
Two	40	45	35	40	0	10
Three	40	50	40	45	0	10
Four	40	40	30	35	5	15

2. Chilling Sums. The easiest and most practical method for estimating the intensity of bud dormancy as it weakens during winter is based on the chilling requirement that we just discussed. The concept is logical enough - the cumulative exposure of seedlings to cold temperatures controls the release of dormancy. So, by measuring the duration of this exposure, it is possible to estimate the intensity of dormancy indirectly. In practice, chilling hours, or degree-hardening-days (DHD) have been used.

The process involves measuring the temperature each day and calculating the amount of time below some reference temperature. There are several different formulas for calculating chilling sums. One short cut method is to record the daily maximum and minimum temperatures, average them, and subtract this average from the base temperature. Note that, when calculating chilling sums, only negative values are recorded (Table 1).

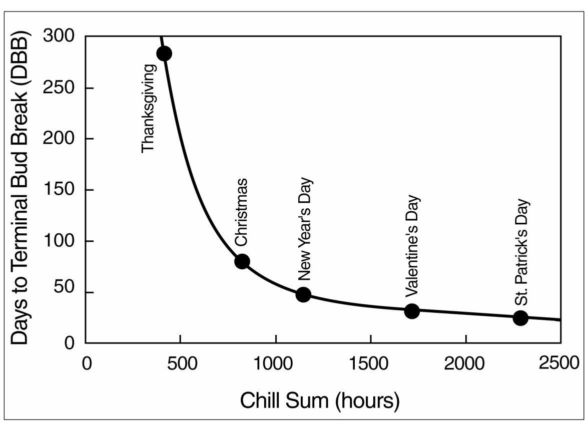

Figure 4. The only reliable test for bud dormancy intensity is a bud break test which can be performed by bringing seedlings into a greenhouse at various times during winter. As they break bud, the number of days to bud break (DBB) is plotted against the chilling sum for each lift date.

3. Bud Break Test. The deeper their dormancy, the slower the terminal buds will break. This phenomenon forms the basis of the only direct way of measuring dormancy intensity – the bud break test. If you have access to a greenhouse or other growth-promoting structure where you can maintain ideal growing environment through the winter, then you can measure intensity of dormancy in your nursery stock by observing days to bud break (DBB).

The procedure is relatively simple. Grow your plants to shippable size in seedbeds or containers and harden them to the fully dormant condition. Container stock should be kept outside or fully exposed to ambient conditions. At this point, it will be fall and your seedlings should have formed a winter bud and exhibit the other morphological changes such as increased needle waxes in conifers and leaf color change and abscission in hardwood stock. Place your temperature-recording device at seedling height in the bareroot seedbeds or within the containers. Check temperatures at least weekly and record them to compute your chilling sums (Table 1).

Set the environmental controls in the greenhouse to maintain spring "forcing" conditions with warm days, cool nights, and long photoperiods using lights. Then, beginning around Halloween, harvest a sample of seedlings and bring them into the greenhouse at regular intervals through the winter. With each sample group, pot them if they are bareroot stock and label them. Then, keep the sample seedlings watered, and count the number of days required for the terminal buds to break (Figure 4). Repeat this process at every major holiday: Thanksgiving (late November), Christmas (late December), New Years Day (early January), Valentine Day (mid-February) and St. Patrick's Day (late March). When you have finished, plot the DBB values over the chilling sums. The number of days required for the terminal buds to break is a direct measure of dormancy intensity. (Note: the Halloween seedlings may never break bud). You will likely obtain results similar to those shown in Figure 4, which came from coastal Douglas-fir in western Washington and Oregon (10) and are in agreement with the general curve proposed by Lavender (6). As the chilling sum accumulates during winter, the number of days to bud break will shorten dramatically. Similar experiments have been done with many tree species, always yielding similar results (12). Once this curve has been developed for a species or response group, it can be used subsequently to estimate the dormancy intensity from chilling sums.

From this experiment, it is clear that bud dormancy intensity is very high in the fall and drops sharply in early winter, in contrast to the common misconception that deepest dormancy occurs in mid-winter. In addition, this test illustrates that there is no simple "chilling requirement" for any species. Rather, there is a curvilinear relationship between chilling and dormancy in which more chilling will result in more rapid bud break. For example, seedlings with only 800 hours of chilling will eventually break bud, but not nearly as rapidly as those exposed to 2,000 hours of chilling (Figure 4).

4. Calculating The Dormancy Release Index. Now that the days to bud break for your crop have been estimated, how can you use this information? If you were to measure bud break rate on a group of seedlings that were fully released from dormancy (*i.e.*, the chilling requirement was completely fulfilled) you would find that buds would break in about ten days. Taking this number as the denominator, you can then calculate an

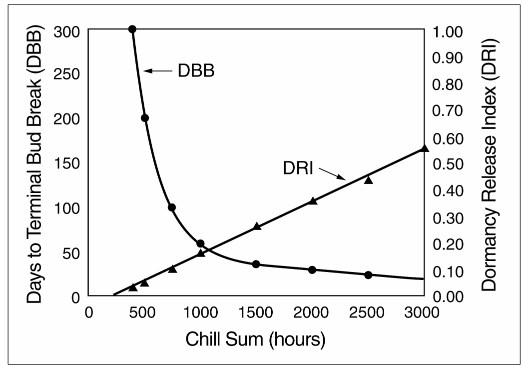

Figure 5 - Because days to bud break (DBB) is a curvilinear relationship, it is useful to convert to a linear dormancy release index (DRI). In this example, DRI = 10/DBB because Douglas-fir seedlings flushed in 10 days when their full chilling requirement was satisfied (10).

index that expresses the dormancy intensity on a linear scale:

$$\text{Dormancy release index (DRI)} = 10/\text{DBB} \qquad (1)$$

where DBB is the days to bud break of a test group of seedlings as described in the experiment above.

Seedling buds at peak dormancy have a DRI value near zero (e.g., DRI = 10/300 = 0.03). As dormancy weakens, DRI approaches one (e.g., DRI = 10/15 = 0.67). This relationship is shown in Figure 5. DRI is useful because it linearizes the response of dormancy intensity to the chilling sum. Hence it can be used to provide a benchmark and for normalizing the response of stock lots to chilling.

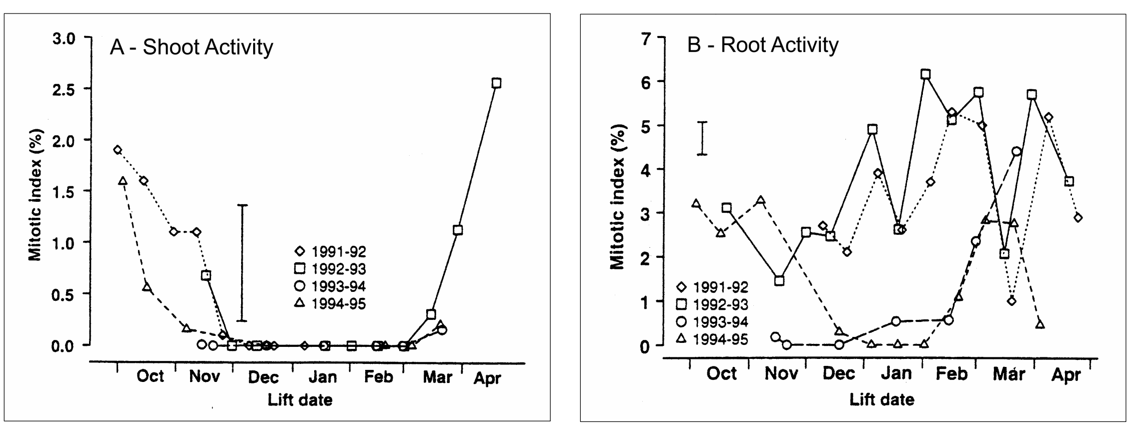

Figure 6. Measuring cell division rates is a laboratory measure of dormancy. Shoot activity over four years shows a characteristic pattern of inactivity during winter (A), but roots (B) continue to grow whenever conditions are favorable (7).

The Dormancy Release Index has been particularly useful as an indicator of seedlings stress resistance – a key seedling quality attribute. In a sequel to this article we will discuss this relationship and how it is used.

5. Measuring Mitotic Index. In our definition of dormancy, we stressed that dormancy could refer only to buds or other plant meristems. A technique has been developed to determine the percentage of bud cells that are dividing at any given time. This is a laboratory test and has been used only for research purposes, but the results help illustrate dormancy patterns. For example, the tips of terminal shoots and long roots of bareroot Douglas-fir seedlings were excised and a mitotic index was calculated under microscopes at 400X for four successive winters (7). The results indicate that terminal bud activity shows a definite seasonal pattern - cell division slows gradually in the fall and stops completely during the winter. With the warmer temperatures and longer days of late winter and early spring, cell division begins to increase rapidly (Figure 6A). This is in direct contrast to the patterns of root meristem activity over the same four seasons, showing that roots never do go truly dormant but will grow whenever soil temperatures permit (Figure 6B). So, although measuring the mitotic index in buds can mark the beginning and end of deep dormancy, this technique has little practical value for tracking dormancy release through winter.

6. Bud Size and Development. While bud size and development are not, in themselves, indicative of the intensity of bud dormancy, they have traditionally been viewed by nursery managers as a measure of seedling quality. As part of their seedling quality testing, the Ontario Ministry of Natural Resources developed a bud dissection and evaluation service. The process involves cutting the buds in half and counting the needle primordia. At the end of the hardening phase, low numbers of primordia were interpreted to indicate stressful conditions and increased susceptibility of overwinter damage. On the other hand, seedlots having buds with large numbers of needle primordia were rated as being of higher quality (5). The Ontario Ministry of Natural Resources has terminated their seedling quality testing program but KBM Forestry Consultant Laboratories in Thunder Bay, ON now offer bud assessment on a fee basis:

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FAX: 807.345.3440
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Conclusions and Recommendations

Although the term "dormant plants" is common in nursery jargon, dormancy refers only to meristematic tissues - buds, lateral cambium, and root tips. Of these, bud dormancy has been studied most often and is of major interest to nurseries.

Forest and conservation nursery seedlings, like all perennial plants, undergo an annual cycle of activity. In late summer, shortening photoperiods trigger plants to begin the bud dormancy process, which culminates in late fall. This condition is known as deep dormancy and can be broken by gradual exposure to low temperatures. This process is known as satisfying the chilling requirement, and temperatures in the range of about 37°F to 41°F (3°C to 5°C) are most efficient. By late winter, the chilling requirement has been met and buds will break whenever temperatures permit.

Unfortunately, there is no quick and easy way to measure bud dormancy. The only reliable method is to conduct a bud break test by bringing samples of seedlings into a forcing greenhouse at regular intervals throughout the winter, and recording the days required for the buds to break (DBB). Once the relationship between DBB and chilling has been established, it can be used to estimate the dormancy intensity during subsequent winters.

References

1. Anderson JL, Seeley SD. 1993. Bloom delay in deciduous fruits. IN: Janick J, ed. Horticultural Reviews 13. New York: John Wiley and Sons: 97-144

2. Burr KE. 1990. The target seedling concepts: bud dormancy and cold hardiness. IN: Rose R, Campbell SJ, Landis TD, eds. Target seedling symposium: proceedings, combined meeting of the Western Forest Nursery Associations, General Technical Report RM-200. Ft. Collins, CO: USDA Forest Service, Rocky Mountain Forest and Range Experiment Station: 79-90.

3. Burr KE, Tinus RW, Wallner SJ, King RM. 1989. Relationships among cold hardiness, root growth potential and bud dormancy in three conifers. Tree Physiology 5: 291-306.

4. Campbell RK. 1978. Regulation of bud burst timing by temperature and photoperiod regime during dormancy. In: Hollis CA, Squillace AE, eds. Proceedings of Fifth North American Forest Biology Workshop. Athens, GA: USDA Forest Service, Southeastern Forest Experiment Station: 19-34

5. Colombo SJ, Sampson PH, Templeton CWG, McDonough TC, Menes PA, DeYoe D, Grossnickle SC. 2001. Assessment of nursery stock quality in Ontario. IN: Wagner RG and Colombo SJ, eds. Regenerating the Canadian forest: principles and practice for Ontario. Markham, ON: Ontario Ministry of Natural Resources and Fitzhenry & Whiteside Ltd: 307-323.

6. Lavender DP. 1984. Bud dormancy. In: Duryea, ML. Evaluating seedling quality: principles, procedures, and predictive abilities of major tests. Corvallis, OR: Oregon State University, Forest Research Laboratory: 7-15.

7. O'Reilly C, McCarthy N, Keane M, Harper CP, Gardiner JJ. 1999. The physiological status of Douglas fir seedlings and the field performance of freshly lifted and cold stored stock. Annals of Forest Science 56: 297-306.

8. Perry TO. 1971. Dormancy of trees in winter. Science 171: 29-36.

9. Richardson EA, Seeley SD, Walker DR. 1974. A model for estimating the completion of rest for “Redhaven” and “Elberta” peach trees. HortScience 9: 331-332.

10. Ritchie GA. 1984. Effect of freezer storage on bud dormancy release in Douglas-fir seedlings. Canadian Journal of Forest Research 14: 186-190.

11. Romberger JA. 1963. Meristems, growth and development in woody plants. Washington, DC: USDA Forest Service, Technical Bulletin No. 1293. 214 p.

12. Sorensen FC. 1983. Relationship between logarithms of chilling period and germination or bud flush rate is linear for many tree species. Forest Science 29: 237-240.

13. Timmis KA, Fuchigami LH, Timmis R. 1981. Measuring dormancy: the rise and fall of square waves. HortScience 16: 200-202.