Seedling Quality Tests: Stress resistance

by Gary Ritchie and Tom Landis

In the Winter, 2004 issue of Forest Nursery Notes (FNN) we discussed bud dormancy, how it is measured and why it is important (11). We also emphasized that bud dormancy is closely related to stress resistance (SR) and will now discuss this relationship in more detail. From an operational standpoint, we will introduce some techniques that you can use in your nursery to estimate the relative SR of a crop at any point during the lifting-to-outplanting process. As a review of terminology and concepts, we suggest that you reread our article on dormancy in the Winter 2004 FNN.

The concept of stress resistance

We all know that seedlings are subjected to a variety of stresses from the time they are harvested in the nursery to when they are outplanted: mechanical stresses, root exposure, and desiccation to just name a few. Nursery managers use a variety of cultural techniques, collectively termed "hardening-off", to prepare their stock to tolerate these stresses. Realizing its importance and the practical applications, seedling physiologists have been studying SR for almost 40 years. Hermann found that SR seems to be primarily a property of root systems in bareroot stock (3), and Lavender (5) showed that SR varies seasonally, reaching a mid-winter peak after bud dormancy has begun to decline (Figure 1). The data for this seasonal curve came mainly from outplanting trials and that is why it corresponds exactly with the traditional mid-winter lifting season.

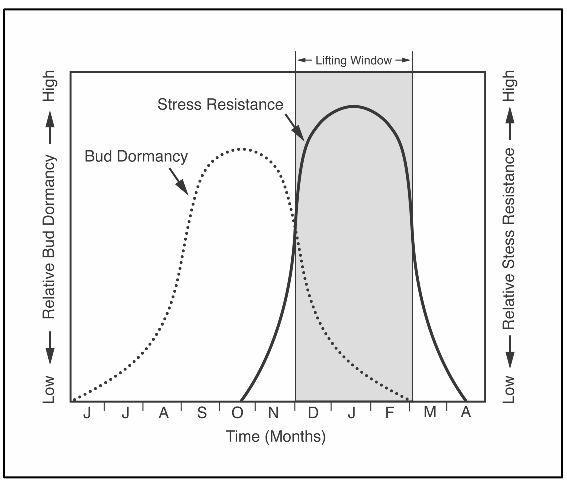

Figure 1 - This classic illustration shows the temporal relationships between bud dormancy, stress resistance and the traditional mid-winter lifting window (modified from 5).

Obviously, nursery managers want to maximize SR in their crops and maintain this condition until they are shipped to their customers for outplanting or transplanted back into the nursery. But how can we measure or estimate SR, and how can we culture our crops to reach this peak?

Measuring stress resistance

A quick and easy way to measure the SR of nursery stock would be an invaluable tool, and there have been many attempts to develop a test to develop this important aspect of seedling quality.

Stress Tests - During the 1970s and 1980s, several attempts were made to develop quick tests of SR. For example, a Stress Test was developed at Oregon State University and consisted of lifting seedlings, potting them, and exposing them to stressful conditions - mainly high temperature, low humidity and low soil moisture (6). After a pre-determined time, the seedlings would then be moved into a greenhouse and assessed for survival, vigor, root growth, bud break and other indicators of vigor. Despite some promising early results, the outcomes of literally hundreds of such tests proved difficult to interpret and not very repeatable. Accordingly, this method was gradually abandoned.

Another more elaborate, but more accurate, method of measuring SR involves a procedure similar to cold hardiness testing (10). It consists of three sequential steps:

1. Exposing plants to a controlled stress treatment. The most commonly used stress treatments employ some sort of controlled trauma to the root systems. This might

involve exposure to high or low temperatures, prolonged drying, or another treatment that simulates rough handling, such as dropping or tumbling (8).

2. Outplanting them into a natural environment where their growth response to the treatment can be expressed. By "natural", we mean the seedlings should be growing in soil and exposed to the ambient outdoor environment but they must be able to express growth potential without confounding effects of browsing, water stress, or weed competition. A nursery bed that is watered regularly and kept weed free is ideal. The test plants are set out in replicated blocks along with unstressed controls of similar initial size from the same seed lots or families.

3. Evaluating the impact of the stress treatment by comparing the performance of the stressed seedlings to that of unstressed controls after a predefined time period - typically one complete growing season. The assessment can be as simple as measuring shoot growth or as complicated as destructively sampling the entire plant and measuring total biomass. We have found that removing the shoots of the seedlings and determining their dry weight is a good basis for comparison. Using this technique, SR is characterized as the difference in growth between the stressed plants and unstressed controls. A helpful way of expressing this difference numerically is by calculating a Stress Injury Index (SII) using the first year shoot growth of the stressed (Gs) and control seedlings (Gc):

$$SII = 100 - (Gs/Gc \times 100)$$

The SII expresses the percent reduction in top growth resulting from stress injury and so, the lower the value, the higher the stress resistance of the test seedlings (12).

Using Cold Hardiness Tests to Estimate Overall Stress Resistance

Decades of nursery experience have shown that, when seedlings are at their maximum state of hardiness, they will show the most resistance to the many stresses of harvesting, handling, storage, shipping, and outplanting. Container nurseries in western Canada are using a "storability test" to determine if seedlings are physiologically ready for lifting, packaging, and cold storage (13). This test is basically a modification of a standard cold hardiness test in which seedlings are placed in a programmable freezer and taken down to the predetermined temperature threshold of 0 °F (-18 °C). After a period of exposure, the seedlings are evaluated for cold injury to the foliage or cambium. A more recent modification uses chlorophyll fluorescence to determine if tissue damage has occurred and produces results up to 6 days earlier than visual evaluation. Because this method tests seedling samples directly, it has proven a reliable predictor of outplanting performance (4).

This storability test shows that cold hardiness testing can also be used to estimate overall stress resistance. Its application should be tested on other species but it only makes sense that, if seedlings are cold hardy, they will be more resistance to other stresses as well. If you will remember from our discussion of Cold Hardiness Testing in the Summer, 2003 issue of FNN, the seasonal stress resistance curve in Figure 1 corresponds very closely to that for cold hardiness. So, cold hardiness tests can give a good indication of overall SR.

Using Chilling Hours to Predict Stress resistance

Research in seedling physiology has revealed that SR is very closely related to dormancy intensity (8, 9, 12). As dormancy intensity weakens through winter in response to chilling, SR gradually increases to a mid-winter high. Then it falls rapidly as dormancy is fully released and spring approaches. This is shown in the gray area of Figure 1. The physiological mechanisms behind this relationship are not fully understood, but it is repeatable from year to year with different crop types (bareroot and container) and species (Douglas-fir, pines, spruces, some hardwoods) and across nurseries (1, 2, 12). This means that if you can track the dormancy status of your crop through winter, you can use this information to estimate SR without measuring it directly.

Calculating Days to Bud Break and Dormancy Release Index - You may recall that in our Winter 2004 FNN article we showed that bud dormancy peaks in fall and is released gradually during winter as plants are exposed to low temperatures (Figure 2). The days to bud break (DBB) can be estimated by accumulating the hours when the air temperature is 41ºF (5 ºC) or below - the "chilling requirement". This relationship is curvilinear so converting it to a linear "Dormancy Release Index" (DRI) makes it much easier to use (Figure 2). The DRI = 0 at peak dormancy in fall, and approaches 1 as dormancy is released in spring.

Research with Douglas-fir has revealed a consistent relationship between DRI and SR (8). In early winter, when DRI is in the range between 0 and about 0.25, SR is low but improving. Between DRI 0.26 and 0.40 (mid-winter) SR is at a seasonal high, but when DRI passes 0.40 (early spring) SR declines and plants become very susceptible to damage. These results lead to the definition of three seedling quality classes based on dormancy intensity and SR (Table 1).

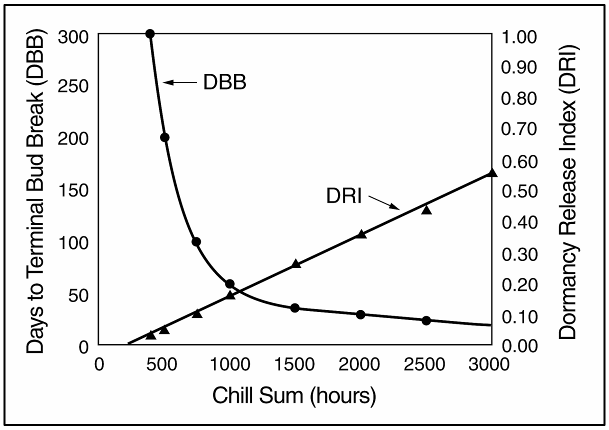

Figure 2 - Because the relationship between chilling hours and days to bud break (DBB) is curvilinear, it is useful to convert to a linear dormancy release index (DRI). In this example, DRI = 10/DBB because Douglas-fir seedlings flushed in 10 days when their full chilling requirement was satisfied (7).

So, once the relationship between chilling and DRI has been established for a given species in a given nursery, it can be used to estimate SR at any point during the winter for subsequent crops at that nursery. Let's say, for example, that it is late December and your nursery chilling sum is about 1,000 hours. Using Figure 2, you would estimate that DRI was approaching 0.2. From Table 1 we see that the stock at this time is in SR class 2 – not yet peaked, but will improve with more chilling. Now, let's say it is February and you have about 2,000 hours of chilling at your nursery. DRI is about 0.38 indicating that SR is in the seasonal high range, but will soon begin to decline.

Adjusting for The Added Effect of Refrigerated Storage - For crops that are "hot planted" without refrigerated storage, this above relationship is very useful. You simply look at your chilling sum at any point and, from it, estimate stress resistance. But many nursery crops are cold or freezer stored from a few weeks to several months before transplanting or outplanting. So, how does that effect SR?

The low temperatures in cold and freezer storage are within the chilling range and contribute to dormancy release. They do so inefficiently, however, because storage temperatures are below the optimum chilling temperature (7,14). Therefore, refrigerated storage *slows the release of dormancy*. This means that seedlings that are lifted and placed into refrigerated storage will pass through SR Classes 2, 1, and 3 more slowly than they would have had they been left in the field or in open container storage. An example: bareroot seedlings that remain in the ground until April will be exhibiting bud swell and will have lost all stress resistance. However, if those seedlings had been lifted in December then placed into storage, when April arrived they would still be dormant and be more resistant to stress.

A summary graph that includes both ambient and storage chilling hours has been developed (Figure 3). The horizontal axis represents the sum of chilling hours to which the seedlings were exposed in the nursery. The vertical axis represents the amount of time the seedlings were held in refrigerated storage (shown in hours on the left and weeks on the right). The roughly parallel curved lines in the graph represent lines of equal DRI, going from about 0 to over 0.50. The three quality classes are shown on the left of the DRI lines and correspond to the values in Table 1.

Here's an example of how to use the graph. Enter the graph on the bottom horizontal axis with total ambient chilling hours from your nursery - for this example, let's use 1,000 hours. At this point, the stock will have a DRI value of about 0.20, placing it in Quality Class 2.

However, if the seedlings are held in refrigerated storage for about 4 weeks, they will enter Class 1 and have even higher SR. However, if these same seedlings had been held in the nursery for several more weeks until they accumulated over 1,300 hours of chilling, they would exceed the DRI limit of 0.25 and enter Class 1 - maximum SR. Then, if they were placed in freezer storage, they could be held for at least 15 weeks (left axis) before their DRI approached 0.40 and their quality dropped to Class 3.

Table 1. Seedling quality classes based on dormancy release index (DRI) and stress resistance (SR) (modified from 9).

Quality class	DRI value	Degree of SR
Class 2	< 0.25	Seedlings are below peak SR, but are improving.
Class 1	0.26 to 0.40	Seedlings are at peak SR.
Class 3	> 0.40	Seedlings are beyond peak - SR is deteriorating.

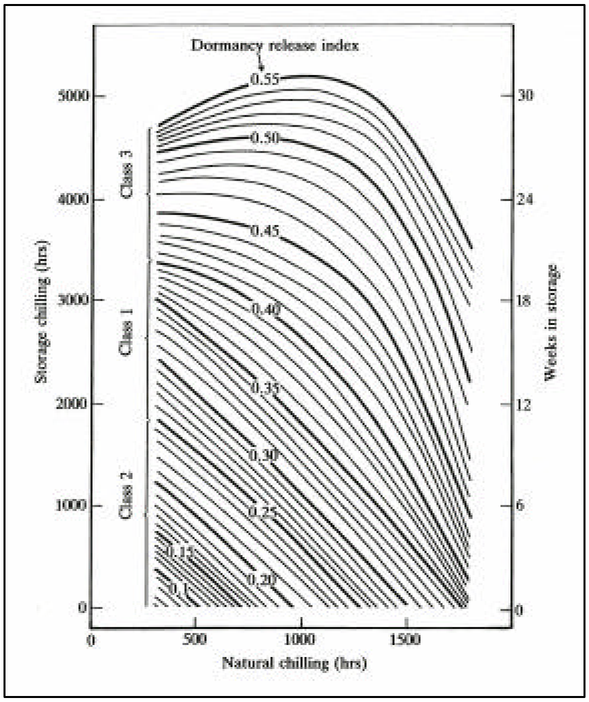

Figure 3. Graph showing how the chill sum at time of lifting, combined with time in cooler or freezer storage, can be used to predict the dormancy intensity (DRI) and stress resistance class (Table 1), of planting stock (9).

So, as you can see, this single graph shows how date of lifting and time in storage interact to control DRI and, hence, stress resistance. If the chill sum at the time of lifting is known, then time in storage can be planned to deliver stock when it is at maximum SR - Class 1. On the other hand, if the outplanting date is known, then lift date and time in storage can be pre-arranged to deliver stock to the outplanting site so it will be in the same Class 1. This graph illustrates the very important point that for late outplanting, early lifting with overwinter storage is preferable to late lifting with no storage.

Application to other species and regions - The data that were used to produce Figure 3 came from coastal Douglas-fir seedlings from four different seed lots (high and low elevations in both Washington and Oregon) that were grown in two different coastal nurseries – one in WA and one in OR. These results have been operationally tested with Douglas-fir crops from other seed lots and during other growing seasons with consistent results. So, for west coast nurseries raising Douglas-fir, Figure 3 is a very handy way of estimating SR from chilling hours.

For interior or northern nurseries, however, the relationship between chilling and DRI may be quite different. This was tested in an interior west Canadian nursery with lodgepole pine and interior spruce (12). The results showed that chilling began to accumulate earlier in the fall and that more chilling accumulated throughout winter. The authors also suggested that these species may have required more chilling hours for full dormancy release than coastal Douglas-fir. Nevertheless, the relationships between DRI and SR and storage were similar to what has been found with Douglas-fir. So, it appears that, before SR can be accurately be predicted from chilling hours, a chilling-DRI "calibration curve" should be developed for other species and nurseries.

Conclusions and Recommendations

Stress resistance (SR) is an important, but elusive, seedling quality attribute that describes a seedling's ability to tolerate the stresses associated with lifting, handling, storing and planting. SR varies seasonally: it is low in fall, high in mid-winter, and low in spring.

SR is very laborious to measure - there is no known "quick test" of SR. However, the seasonal pattern of SR closely coincides with the pattern of cold hardiness (CH). So, CH measurements can be used to estimate SR.

Studies have shown that SR is related to dormancy intensity expressed as a dormancy release index (DRI). When DRI is in a range between 0 and about 0.25, SR is low but improving. Between DRI 0.25 and 0.40, SR is at a seasonal high. Above DRI 0.40, SR is declining. Importantly, this relation tends to be consistent whether or not seedlings have been stored.

Reminders

1. Seedlings destined for overwinter freezer storage need at least 400 hours of chilling to attain sufficient hardiness before lifting and storing.
2. Seedlings requiring more than 6-8 weeks of storage should be freezer, not cooler, stored.

Because cold and freezer storage slow the release of dormancy, they also prolong the period of high stress resistance. These relationships can be used to schedule lifting and storage to deliver stock to the planting site that has very high stress resistance.

To apply these principals in northern or interior nurseries, where winters are longer and colder than in coastal nurseries, it may be necessary to develop calibrations between winter chilling and dormancy intensity for the crop species grown.

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