Physiological Testing of Plants as a Management Tool

Richard W. Tinus
USDA Forest Service Research, 700 S. Knoles Drive, Flagstaff, Arizona 86001

Practical techniques have been developed for measuring root growth potential, cold hardiness, and other physiological attributes that are important to successful nursery practice and reestablishment of woody nursery stock. The techniques are described and their use in management illustrated. Woody plants are routinely grown in nurseries, shipped to a remote location, and then reestablished where they will remain for the rest of their life. Lifting, storage, shipping, and outplanting are traumatic events in the life of trees or shrubs; they must be in good physiological condition to withstand these treatments if they are to become reestablished and grow.

REQUIREMENTS FOR SUCCESSFUL LIFTING, STORAGE, AND REESTABLISHMENT

For fall lifting and cold storage, woody plants must be fully dormant or in "deep rest." In this condition they are best able to tolerate loss of roots, mechanical handling, and moisture and temperature stresses. The condition of deciduous trees and shrubs can be gauged by the normal abscission of their leaves, but evergreens do not show readily visible changes.

Plants need a certain degree of cold hardiness, at least enough to withstand cold storage and the conditions they will encounter on the site where they are outplanted. Equally important, cold hardiness is a good indicator of overall hardiness and stress resistance.

After outplanting, the plants must grow new roots to become established. How quickly this is necessary depends on the moisture stress at the planting site, which includes not only soil moisture but humidity, air temperature, and wind. Note that this applies to horticulture as much as it does to forestry. For example, it is usually possible to water horticultural plants after outplanting, and yet a plant's access to water is still limited, if its roots are not actively growing.

WHAT TO MEASURE AND HOW

The requirements described above suggest four physiological tests that will indicate the ability of the plant to perform as desired. These are bud dormancy, root growth potential, cold hardiness, and heat stress tolerance. As the plant progresses through its annual growth cycle, these attributes change according to a regular pattern. They are related one to another, fortunately, because some attributes, such as bud dormancy, cannot be measured quickly. However, by knowing the relations between the various attributes one can use a quickly measurable one such as cold hardiness to estimate the others (Tinus et al., 1986).

Bud Dormancy. Bud dormancy is usually measured by placing the plants under favorable growing conditions and observing the number of days until bud break (Lavender, 1985). Most temperate and boreal zone woody plants require a

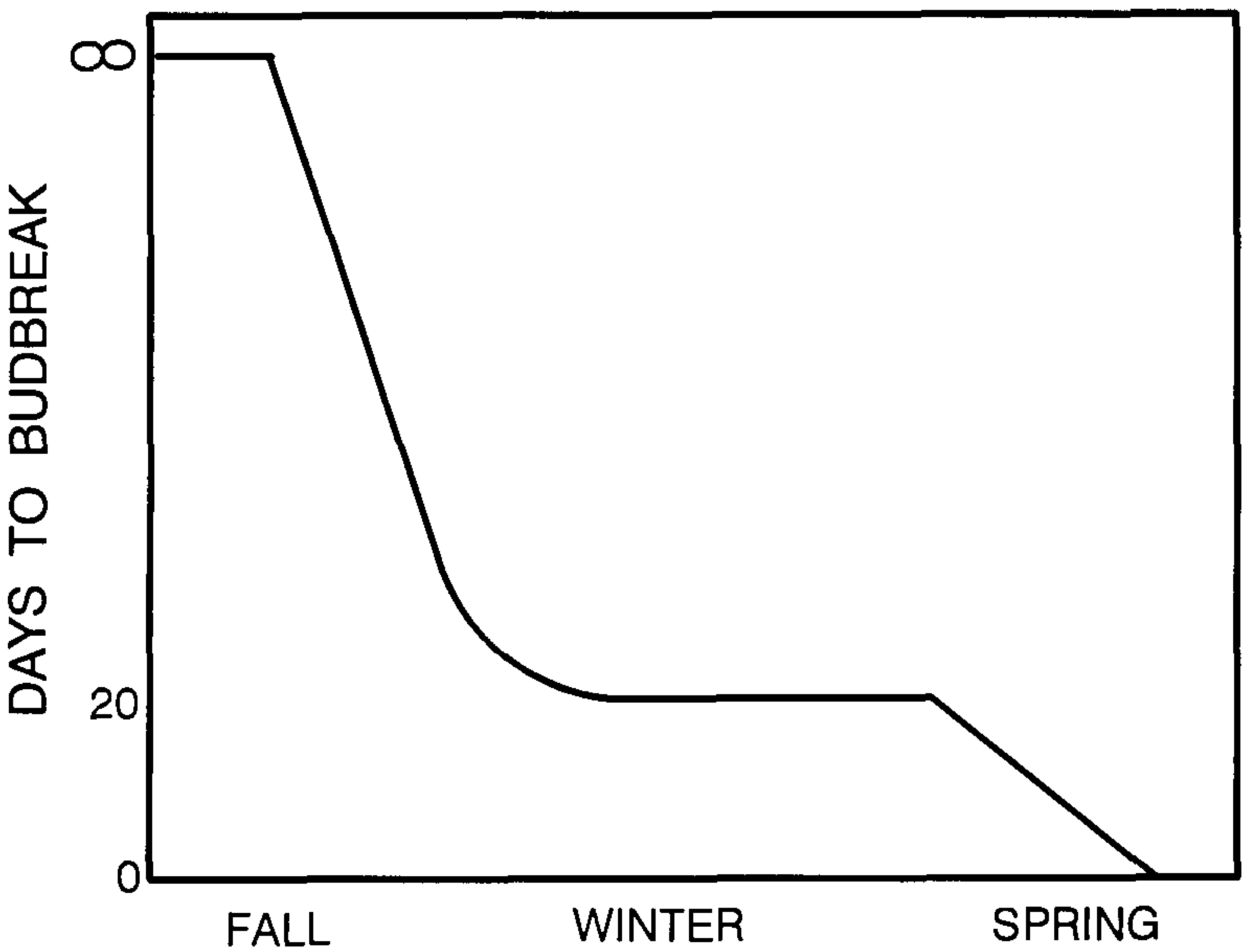

Figure 1. General pattern of days to bud break when woody plants are moved from outside into forcing conditions.

characteristic period of chilling before they will break bud. At first, buds typically take a long time to break, if they break at all, but as chilling progresses, days to bud break declines until it reaches a plateau (Fig. 1) at which there is no further progress toward bud break until warm weather comes. Maximum rest in southwestern conifers (Burr, 1990) occurs about when the chilling requirements have just been met. Although useful in research, measuring days to bud break takes too long to be a useful management tool.

Root Growth Potential. Root growth potential is measured by the number of new roots produced by a plant in a given time under favorable conditions (Ritchie, 1985; Rietveld and Tinus, 1987). Although there are three methods in use, the best is the mist chamber technique (Burr et al., 1987), which in its simplest and most versatile version is a chest freezer with its lid removed. The plants to be tested are suspended from tree holders with their roots exposed to the air in the chest, which contains about 15 cm of water in the bottom. An impact sprinkler mounted on a sump pump resting in the water and controlled by a timer periodically splashes water against the sides of the freezer, scattering small droplets of water throughout the chest, keeping the roots moist and the air at 100% relative humidity. Aquarium heaters in the bottom and the cooling system of the freezer control the temperature.

The root growth potential test is usually run for 7 or 14 days, at which time the new roots are counted on each plant. Absolute numbers of roots vary with species and size of plant, but within these limitations root growth potential will vary dramatically with stage in the annual growth cycle and the condition of the plant. Typically, root growth potential is low in late summer and rises in the fall. In Douglas-fir the rise is gradual and steady, but in Engelmann spruce it is abrupt;

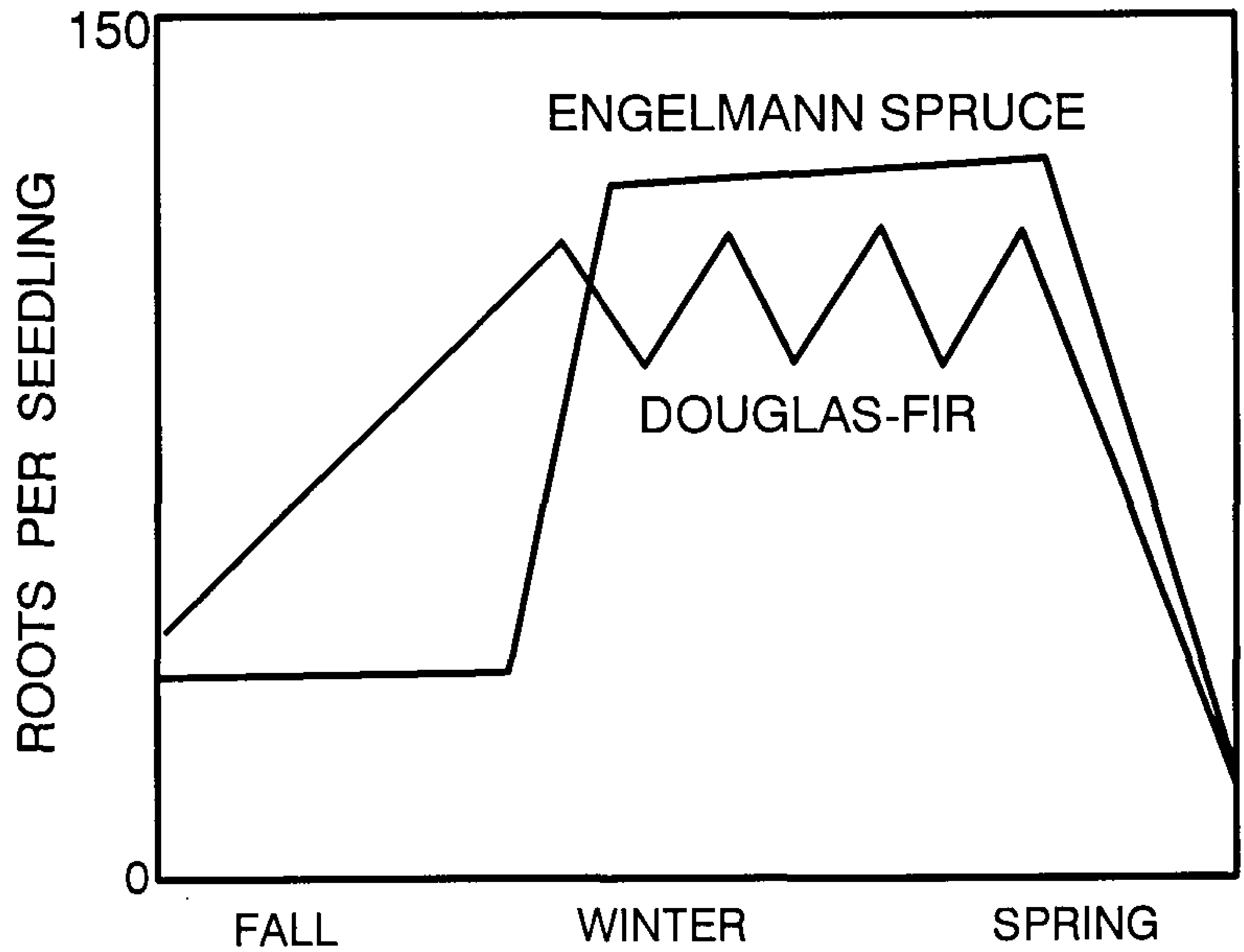

Figure 2. Engelmann spruce and Douglas-fir show different patterns of root growth potential during the dormant season.

it may rise by a factor of four in one week. Root growth potential remains high throughout the winter, may peak in the late winter, and falls to a low level as bud break approaches (Fig. 2).

How does root growth potential affect nursery management practices? It needs to be high when trees and shrubs are outplanted, so (1) it must be high going into storage, and (2) it must not be lost before the plants are back in the ground. The time to begin lifting in the fall is after root growth potential has risen. Normally root growth potential will remain high in cold storage, but it is more likely to decrease than increase (Burr and Tinus, 1988).

Cold Hardiness. Maximum cold hardiness of well adapted vegetation is almost always more than enough to prevent damage from cold during the winter. The critical times are more likely to be in the fall when the plant must harden in a timely manner, and in late winter and spring when it must not lose its hardiness prematurely. Although cold hardiness per se is usually not a problem, it can be measured quickly and, besides being a good measure of overall hardiness, can be used to estimate bud dormancy and root growth potential when a quick answer is important. Several good tests are available (Burr et al., 1990). In the "whole-plant freeze test" potted plants are placed in the bottom of a warm chest freezer, the roots insulated with vermiculite, and the freezer turned on. Once below freezing, the temperature should not fall more than 5°C per hour. At a series of successively lower benchmark temperatures a sample (usually one pot) is removed and placed in a cooler to thaw. After all of the samples have been removed and thawed, they are placed in a warm room or greenhouse. After 7 days the plants are examined for damage and the LT_{50} estimated. Actually, if you have a sensitive nose and with a little training, you can smell the damage on conifers about 30 min after the plants are brought into a warm room.

The whole-plant freeze test is the one against which all others are calibrated, but a faster and more quantitative test is "freeze-induced electrolyte leakage". Foliage samples are placed in vials with a small amount of distilled water and frozen to a series of successively lower temperatures. At each benchmark temperature samples are removed and thawed. After incubation the conductivity of the solution is measured and an index of injury calculated. The greater the damage to the tissue, the more electrolytes leak out, and the greater the conductivity of the solution. This test can be completed in two days and does not destroy whole plants, but needs to be calibrated against the whole-plant freeze test.

Other tests are available and under development, but the two mentioned above are probably the best for management use now. Cold hardiness is least in the late spring, low throughout the summer, and begins rising (the LT_{50} temperature declines) in the early fall, reaching a maximum in early winter. It remains high until warm weather comes in the spring when it declines rapidly (Fig. 3).

Probably the greatest value of cold hardiness testing is in what it can tell about seedling quality. For example, when the LT_{50} in Rocky Mountain ponderosa pine, Douglas-fir, and Engelmann spruce has reached -22°C in the fall, the chilling requirements for bud break have been met, and root growth potential has doubled or more from its late summer low level. In the spring, when two thirds of maximum cold hardiness has been lost, root growth potential peaks and then declines rapidly as bud break approaches. In this case, cold hardiness is an excellent "leading indicator" because it changes measurably weeks before root growth potential is lost and long before there is any sign of bud break.

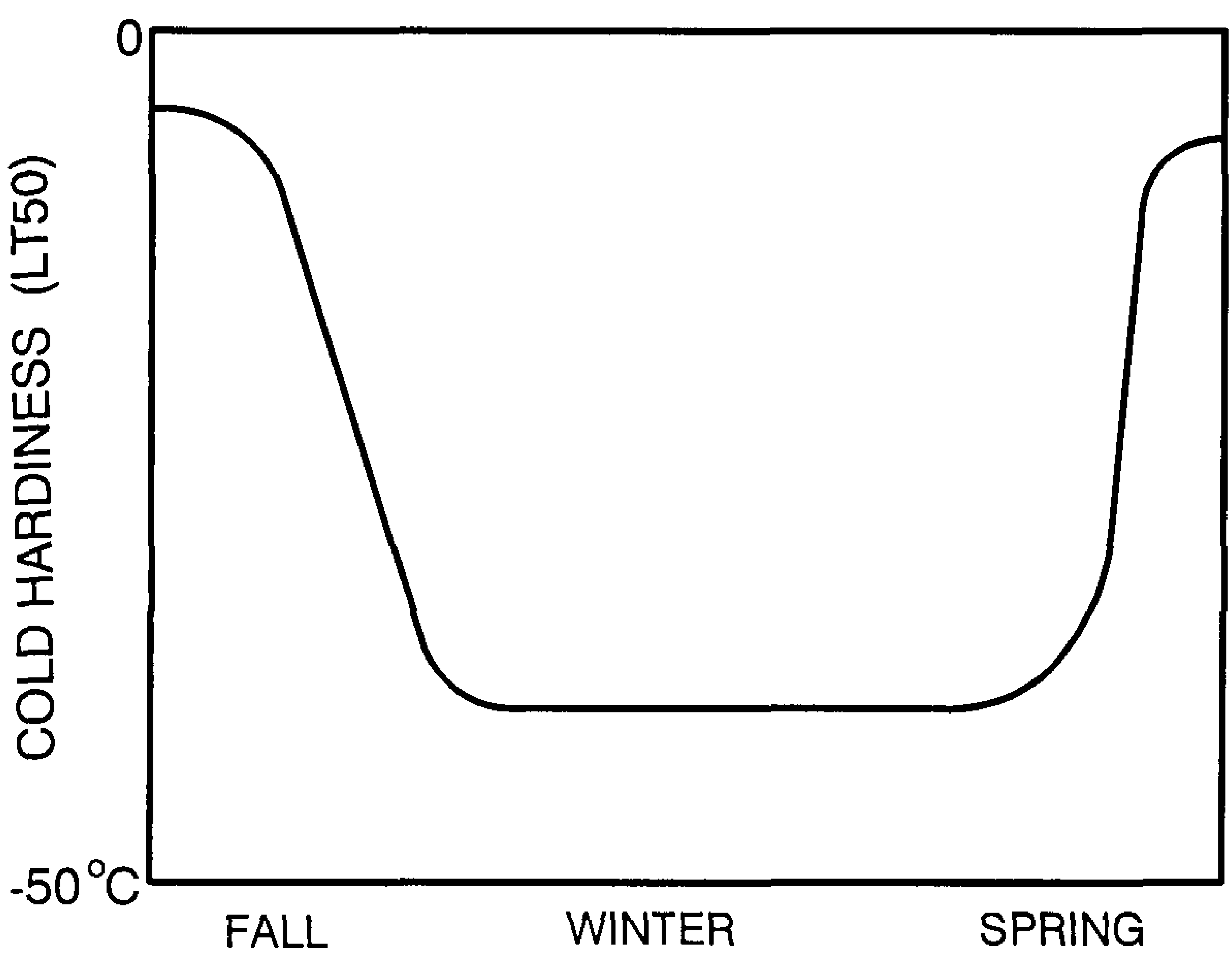

Figure 3. Normal pattern of cold hardiness during the dormant season. However, warm periods during the winter may cause premature de-hardening.

HEAT STRESS TEST

Although good nursery managers do everything possible to avoid high temperatures and desiccation, it is useful to know how much plants will withstand. In the "heat stress test" bare root plants are placed in a forced air drying oven at 32°C and relative humidity of 30% for 15 min. Then they are potted and placed in a greenhouse for several weeks along with some that have not been stressed. Survival and growth of both groups are noted and the difference is a measure of the tolerance for heat and desiccation stress (Duryea, 1985).

THE CASE OF THE FROZEN TRUCK

Usually, physiological tests are used to time cultural practices, monitor plant quality, and provide baseline information, but sometimes they can provide hard data needed for management decisions in a crisis.

In February 1991 a semi-truckload of tree seedlings was received at a National Forest District in Flagstaff—solidly frozen. The refrigeration on the truck apparently had stuck on. The question was: Were these trees damaged and should they still be planted? At stake was about $28,000 worth of trees plus at least that much to plant them. A go or no go decision had to be made in a matter of weeks.

Fortunately, one of the boxes of trees contained an electronic temperature recorder. During the three-day trip from Idaho the temperature in the box declined rapidly, reaching a low of -28°C, which was probably low enough to damage the shoots of the ponderosa pine and certainly low enough to kill the roots. The nursery had tested the seedlings before shipment, and cold hardiness was adequate and root growth potential high.

We retrieved samples of seedlings from two nearby districts that received trees in the frozen truck and from two districts that received trees from a different truck that functioned normally. We tested the four lots for root growth potential and cold hardiness, and the results were as follows:

District	Roots per seedling (mean ± std. error)	Mean % of root system dead
Truck OK		
A	18.4 ± 2.9	0
B	10.2 ± 2.5	0
Truck frozen		
C	1.3 ± 0.7	66
D	0.0 ± 0	86

Clearly, based on the root growth potential test, the trees in the frozen truck were badly damaged and were considered not worth planting.

The benchmark temperatures in the whole-plant freeze test were from -7°C to -23°C and should have shown any lack of adequate cold hardiness. After 14 days in the greenhouse, all of the "frozen truck" trees from all five test temperatures showed about equal damage to the stems and needles, indicating that the damage was preexisting and not caused by the freeze test.

As a result of these tests, the entire truckload of 160,000 trees was dumped. Six years ago, before we had these tests available, the dead trees probably would have been planted, because managers would not have been willing to take responsibility for destroying the trees without good evidence that they were not viable. In addition, the nursery equipped that truck with a whole new $11,000 refrigeration system, something that would not have been done if there were any doubt about what the problem was and how serious it was.

In conclusion, physiological testing can provide valuable information for management decisions about the condition of woody plants and their prospects for survival and growth, especially during the dormant season when their condition is not obvious by inspection.

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