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Morphological and Physiological Evaluations of Seedling Quality

Diane L. Haase

Diane L. Haase is Associate Director, Nursery Technology Cooperative, Department of Forest Science, Oregon State University, Corvallis, OR; Tel: 541.737.6576; E-mail: diane.haase@ oregonstate.edu.

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Abstract: Seedling quality and subsequent field performance can be influenced by various stress factors. Measuring seedling quality can help to identify possible crop problems in order to make informed decisions for culturing, lifting, storing, and planting. In addition, seedling quality data can help seedling growers and users to better understand annual patterns among species, stock-types, seed lots, and cultural treatments. This paper describes stresses that can reduce seedling quality and summarizes several common morphological and physiological measurements that have been linked to seedling field performance.

Keywords: height, stem diameter, shoot:root, cold hardiness, plant moisture stress, root growth potential

Introduction

Each year, millions of seedlings are sent to the forest for outplanting. However, many of those do not survive or grow well. Often, mortality and poor field performance are attributed to poor seedling stock quality. Each year's crop quality can vary due to several factors. Measuring seedling quality can create a basis for nursery and buyer understanding of a crop at lifting and after outplanting.

Testing seedling quality is especially useful when there is a concern about stock performance due to a weather event or disease in the nursery, when there is a customer request for the information, or when a nursery is forced to harvest outside the normal lifting window. Data can then be used to make outplanting and storage decisions. Seedling quality data can also be gathered for needed information on a variety of species. For example, there is a near absence of literature on sugar pine (Pinus lambertiana Dougl.) seedlings, despite the fact that this is a high-value species with a limited seed supply. Additionally, annual data is very useful for species such as Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) and ponderosa pine (Pinus ponderosa Doug. ex Laws) to track developmental differences based on new stocktypes, cultural treatments, seed lots, and annual environmental patterns. Testing can also be used to link seedling morphological and physiological characteristics with subsequent field performance.

Factors Influencing Seedling Quality

Seedlings are living, perishable plants and can be profoundly impacted by stresses introduced during culturing, lifting, packing, grading, handling, pruning, storage, transport, and outplanting. In general, nursery growers and field reforestation personnel can expect seedlings to be of high quality when they leave the nursery. However, environmental extremes or mishandling can lead to reductions in seedling quality. The three primary types of stress that influence seedling quality are moisture, temperature, and physical stress. The cumulative effect of these stresses can be greater than the sum of separate effects. As stress increases, physiological functions are impaired and the seedling's energy shifts to damage repair. As a result, survival and growth can be significantly reduced. These effects are exacerbated further when seedlings are outplanted to harsh sites.

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Moisture Stress

Of all types of stress, moisture stress can be the most damaging. Water loss during handling and planting can have a profound effect on subsequent seedling survival and growth. Because plant water potential influences many basic physiological processes, these effects can be apparent for several seasons after outplanting. Roots are especially vulnerable to desiccation because, unlike leaves/needles, they have no waxy coating or stomata to protect them from water loss. Once fine roots appear dry, they are probably already dead. Compared to bareroot seedlings, container seedling roots are protected somewhat from moisture stress by the growing medium; however, if the plug is allowed to get too dry, desiccation damage can be severe. Once roots have dried, rewetting has been shown to be ineffective in preventing growth reductions, even when shoot water potential recovers (Balneaves and Menzies 1988). Dormant conifer seedlings are more vulnerable to damage from root exposure than dormant hardwood seedlings.

Temperature Stress

Temperature stress can also affect seedling quality. Survival and growth can be decreased following exposure to high or low temperatures. The level of susceptibility varies with seedling conditioning and phenology. For example, a succulent seedling moved from a greenhouse to an outdoor area may experience sunscald if temperatures are much greater than the greenhouse environment. Similarly, a seedling that is not hardened off is likely to be damaged when exposed to a freeze event. Genotype and species also influence the degree of vulnerability to temperature stresses.

Physical Stress

During nursery processing and field planting, potential for physical damage to seedlings includes dropping, crushing, vibrating, abrasion, and root stripping. Too often, bags and boxes of seedlings are carelessly tossed off the back of trucks onto loading docks or planting sites; the higher the drop, the more the damage. Studies have shown that dropping seedlings may increase fine-root electrolyte leakage, reduce root growth potential, decrease height growth, and increase mortality (Tabbush 1986; Sharpe and others 1990).

Seedling Quality Parameters

Stresses to seedlings are usually not readily apparent by simple observation. Without further testing, it is often difficult to determine whether seedling quality has been affected by stress. This may result in dead or dying seedlings being outplanted, which is a waste of time and money.

Morphological Quality

Morphology is defined as the form or structure of an organism. Although morphological assessments do not provide direct information about a seedling's current physiological condition, they can be considered a physical manifestation of a seedling's physiological response to the growing environment (Mexal and Landis 1990). Seedling shoot height and stem diameter are the most common measures used for growing and grading standards in forest nurseries. There are many additional morphological parameters that can be assessed as well. Morphological standards vary greatly by species, seed zone, and stocktype. No single factor has been shown to provide a perfect prediction of outplanting success, but each of them has been linked with seedling performance potential in some way.

Shoot Height-Shoot height is defined as the distance from the cotyledon scar to the base of the terminal bud on dormant seedlings or to the tip of the shoot on growing seedlings. Because height is correlated to the number of needles on the shoot, it is a good estimate of photosynthetic capacity and transpirational area. This suggests a positive relationship with subsequent growth, but an unpredictable relationship with survival, especially on droughty sites. Taller seedlings may have a competitive advantage on sites with severe weed competition and may be indicative of superior genetics. Smith (1975) found 11-year height growth of 3+0 Douglas-fir seedlings was highly correlated with initial height. On the other hand, taller seedlings with greater transpirational area may have a disadvantage on dry sites; and exceptionally tall seedlings may be difficult to plant, out of balance (poor shoot-to-root ratio), and subject to wind damage (Ritchie 1984).

Stem Diameter-Stem diameter (often referred to as root collar diameter or caliper) is defined as the diameter of the main stem of a seedling at or near the cotyledon scar. Diameter has often been considered the best single predictor of field survival and growth (Thompson 1985). Of 14 independent nursery seedling characteristics, Omi and others (1986) found that Douglas-fir stem diameter and root weight had the highest correlation with first-year height growth. Blake and others (1989) reported increased survival with increas ing stem diameter for Douglas-fir. South and others (1988) examined 30-year growth of loblolly pine (Pinus taeda L.) and found average tree volume was highly correlated with seedling diameter at the time of planting. In another study, planting Douglas-fir seedlings 2 mm larger in initial basal diameter resulted in fourth-year stem volume gains of 35% and 43% on two diverse sites (Rose and Ketchum 2003).

Height:Diameter-Height: diameter is a unitless sturdiness ratio calculated from height and stem diameter. A high ratio indicates a relatively spindly seedling while a lower ratio indicates a stouter seedling. Roller (1977) found that black Spruce (Picea mariana [P. Mill.] B.S.P.) seedlings with high sturdiness quotients were more susceptible to damage from wind, drought, and frost exposure. Figure 1 shows relative differences for height, stem diameter, and height:diameter for a sample of bareroot (1+1) and container (Styroblock^m Styro-15) seedlings.

Bud Length- Bud length is measured from the base of the bud to the tip of the bud. Bud length is correlated with the number of needle primordia in many species and therefore gives an indication of seedling vigor and field growth potential. Seedling bud length has been found useful for predicting subsequent shoot length (Kozlowski and others 1973).

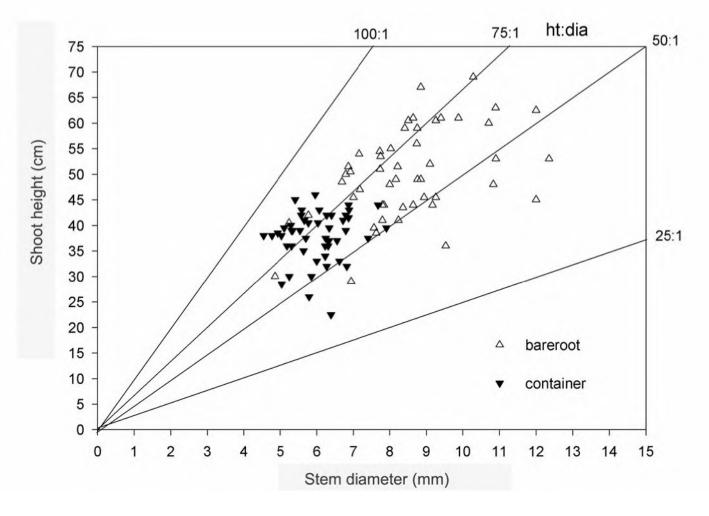


Figure 1-Height and stem diameter data for a sample of bareroot (1+1) and container (Styroblock™ Styro-15) Douglas-fir seedlings. The diagonal axes indicate the height:diameter sturdiness ratio.

Root and Shoot Volume-Root and shoot volume are both measured via the water displacement method (Harrington and others 1994). Root volume includes all root mass below the cotyledon scar while shoot volume includes all shoot mass above the cotyledon scar. Root volume, however, does not always reflect root fibrosity, because a seedling with many fine roots can displace the same volume as a seedling with a large tap root. Blake (1989) reported that the relationship between stem diameter and survival was affected by seedling root mass. Seedlings with good root mass consistently survived better than those with poor root mass. Even seedlings that would normally be considered culls (<3 mm stem diameter) had high survival (>70%) if they had good root mass. However, large seedlings (>5 mm) had good survival even with a poor root mass. These data suggest that nurseries should provide large stem diameters regardless of root mass or incorporate root grading into the sorting process. Rose and others (1997) showed that Douglas-fir and ponderosa pine seedlings with larger root volumes at the time of outplanting had significantly greater field growth and survival than those with smaller root volumes. Similarly, Jacobs and others (2005) showed that second year height and diameter were greater for hardwood seedlings with greater initial root volume.

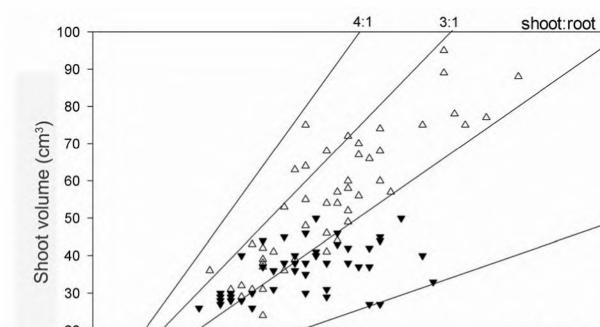
Weights—Fresh weight is the weight of the seedling or its parts on a fresh (operational water content) basis. Dry weight is the weight of the seedling or its parts after drying for a minimum of 48 hours at 68 °C (154 °F). Weight is commonly measured on whole seedlings or root, shoot, and foliage separately. Because water content in the tissue can vary greatly, dry weight tends to provide a more consistent measurement than fresh weight. Not surprisingly, shoot and root volumes are strongly correlated with shoot and root dry weights. There is a strong relationship between seedling dry weight and stem diameter (Ritchie 1984); thus it correlates to field survival and growth similarly.

Shoot:Root—Shoot:rootis aunitless ratio of seedling balance calculated from root and shoot volumes or dry weights. Shoot:root measures the balance between the transpirational area (shoot) and the water absorbing area (root) of the seedlings. Generally, quality bareroot seedlings have shoot:root of 3:1 or less and quality container seedlings have shoot:root of 2:1 or less. Figure 2 shows relative differences for shoot volume, root volume, and shoot:root for a sample of bareroot

(1+1) and container (Styroblock $\ensuremath{^{\rm TM}}$ Styro-15) seedlings.

2:1

1:1



20 Δ bareroot 10 container 0 25 30 0 5 10 15 20 35 45 50 40 Root volume (cm³)

Figure 2-Root and shoot volume data for a sample of bareroot (1+1) and container (Styroblock™ Styro-15) Douglas-fir seedlings. The diagonal axes indicate shoot:root balance.

Color, Form, and Damage—Seedling foliar color is a general indicator of seedling quality and can vary by species and time of season. Yellow, brown, or pale-green foliage indicate lower vigor and/or chlorophyll content than dark green foliage. Existence of multiple shoots, stem sweep, root deformity, stiff lateral roots, physical damage, and any other noticeable characteristics that can affect seedling performance are also important factors to evaluate when determining seedling quality.

Physiological Quality

Cold Hardiness— Cold hardiness is defined as a minimum temperature at which a certain percentage of a random seedling population will survive or will sustain a given level of damage (Ritchie 1984). Cold hardiness develops in an annual pattern similar to dormancy with roots being much less hardy than shoots. The LT_{50} (lethal temperature for 50% of a population) is commonly used to define the cold hardiness level. Changes in LT _{50 are} strongly linked to the seedling dormancy cycle and stress resistance and are influenced by seed source, nursery practices, and environment (Faulconer 1988; Burr 1990). Simpson (1990) found that LT ₅₀ at lifting correlated well with first-year survival and shoot growth.

Cold hardiness can be evaluated using whole plant freeze testing (WPFT) in which the buds, cambium, and foliage are examined for freeze damage 6 days after freezing (Glerum 1984; Tanaka and others 1997). Figure 3 is an example of data generated from a WPFT. Another method is to evaluate freeze-induced electrolyte leakage (FIEL) of stem, needles, roots, or buds (Burr and others 1990).

Root Growth Potential-Root growth potential (RGP) is defined as the ability of a tree seedling to initiate and elongate roots when placed into an environment optimal for root growth (Ritchie 1985). RGP is usually measured in late winter or early spring by either potting a sample of seedlings or placing them into a hydroponic tank. After 3 weeks, root growth is quantified. RGP is influenced by stocktype, species, seedlot, and physiology. Despite its popularity, there is debate over whether RGP can be accurately linked to field performance (Simpson and Ritchie 1997). It can predict actual field performance when trees are dead or when water uptake is dependent on new growth. However, because RGP only represents the potential to grow new roots in a favorable environment, that potential may or may not be expressed when the seedling is outplanted to field conditions. Root growth after outplanting rarely occurs immediately because soil temperatures are below optimal for RGP to be fully expressed.

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budbreak under favorable growth conditions is an index of a seedling's dormancy state and stress resistance (Lavender 1985; Burr 1990). The speed with which buds resume growth in the spring is a function of the physiological state of the bud and is dependent on the number of chilling hours (<5 $^{\circ}\mathrm{C}$ [41 . , to which a seedling is exposed after budset. Mitotic index (MI) is another measure of bud dormancy and, unlike days to budbreak, it does not require a long period of time to assess. MI is defined as the percentage of cells in mitosis at a given time. Owens and Molder (1973) termed Douglas-fir buds to be dormant when mitotic activity in the bud cells is zero, a condition that generally occurs from December through February. MI is measured by placing the shoot tips in a fixative, then squashing and staining the apical meristem on a microscope slide. MI is determined within a gridded area under a microscope. Actively dividing cells are identified and counted as a percentage of the total number of cells within a counting grid.

Foliar Nutrient Concentration — Plant nutrient condition is well known to play a crucial role in determining seedling quality and subsequent outplanting performance. Seedling nutrient status can affect seedling physiological factors which are related to outplanting survival and growth (Landis 1985). Seedling size has been shown to be positively correlated with foliar nitrogen, which was, in turn, directly related to nursery fertilization (van den Driessche 1980). Haase and others (2006) showed that increased seedling nutrient content due to fertilization with slow-release fertil. izers had a significant effect on seedling size at the time of outplanting and for several subsequent field seasons.

Plant Moisture Stress-Plant moisture stress (PMS) measurement indicates seedling water potential and reflects interactions among water supply, water demand, and plant regulation. PMS is often used to schedule irrigation and monitor water stress during lift and pack operations (Lopushinsky 1990). The most common method for determining plant

moisture stress is by using a pressure chamber (Cleary and Zaerr 1980). PMS can be affected by time of day, species, plant age, level of dormancy and stress resistance, and environment. Moderate water deficit results in stomatal closure, decreased photosynthesis, and growth reductions. Severe water deficit can result in permanent damage to the photosynthetic system as well as other physiological processes in the plant, which will impact growth or result in mortality.

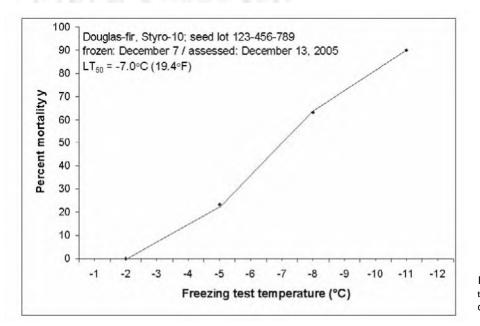
Testing Options

While some companies do in-house seedling quality evaluations on some of their seed lots, others perform no tests at all or rely on limited data, such as height and stem diameter, to assess their stock quality. There are only a few testing facilities in the northwestern United States. The Nursery Technology Cooperative established a regional forest seed ling quality testing facility at Oregon State University in Corvallis, OR. Those who use the service are pleased to have an objective third party available to provide the requested data in a timely manner. Currently, available services include morphological evaluation (height, stem diameter, height: diameter, root and shoot volume, root:shoot) and cold hardiness determination (whole plant freeze test). Further information can be found online at the Cooperative's internet site (URL:http:llntc.forestry.oregonstate.edu).

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Figure 3—Sample cold hardiness report with the estimated LT50 based on mortality at each of four test temperatures.



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