Seedling Ecophysiology: Five Questions To Explore in the Nursery for Optimizing Subsequent Field Success

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Abstract

This paper is based on questions from an audience participation discussion with the author S. Grossnickle during the Joint Annual Meeting of the Forest Nursery Association of British Columbia and the Western Forest and Conservation Nursery Association (Sidney, BC, September 30-October 2, 2019). The five question topics presented herein were, by consensus, the most discussed questions presented by the audience of nursery practitioners and foresters. Topics explored in this paper relate to nursery hardening practices, irrigation management to promote stress resistance, cultural strategies to promote vigorous root growth, storage practices for hot-lifted seedlings, and storage length for overwinter stored seedlings. The following answers to these specific topics are the authors' combined views on these nursery cultural practices.

Introduction

Nursery cultural practices have a direct impact on seedling quality and subsequent field performance (Dumroese et al. 2016, Grossnickle 2012, Grossnickle and MacDonald 2018a, Mattsson 1997, Sutton 1979). Culturing seedlings requires specialized knowledge and skill to produce adequate quantities of high-quality plants from appropriate genetic seed sources in a timely manner. This process starts with a partnership between the client and the nursery manager to determine plant specifications that are matched to the outplanting site (Dumroese et al. 2016). These plant specifications include species, seed source, and stocktype, as well as particular morphological and physiological characteristics that will maximize the seedling potential to survive and thrive after outplanting (Haase 2008).

For any given seedling crop, it is important to define and refine the path required to go from start to finish. Plants are biological organisms and must be treated as such; they are not widgets in a factory. Morphology is relatively easy to see and measure, and most target specifications are based on these measures. Nonetheless, physiological function must also be considered because seedling physiological responses to the environment determine their survival and morphological development (Grossnickle 2000).

Plants' physiological function is ever changing and responding to their external environment. Thus, constant monitoring of seedling development in the nursery is essential, especially for identifying and addressing any problems (Duryea 1985, Grossnickle and MacDonald 2018b). Throughout the process, growers must manage risks to maximize yield and performance. Without good quality upon leaving the nursery, seedlings with the best genetics cannot do well in the field.

This paper explores five questions about seedling ecophysiology and nursery culturing raised during the 2019 Joint Annual Meeting of the Forest Nursery Association of British Columbia and the Western Forest and Conservation Nursery Association.

Question 1 – What Are the Best Cultural Hardening Practices To Maintain Physiological Quality?

Cultural hardening practices that improve seedling "physiological quality" have long been considered important for increasing survival and growth potential after field planting for both bareroot (Wakeley 1948, 1954) and container-grown (Landis et al. 2010, Lavender and Cleary 1974, Tinus 1974) seedlings. This is because hardened seedlings usually have quality attributes necessary to become established after planting on restoration sites (Grossnickle 2012, Grossnickle and MacDonald 2018a). As nursery-grown seedlings reach a desired morphological size, cultural practices to modify daylength, temperature, watering, and fertilization can be applied to harden seedlings (Landis et al. 1999, Landis 2013, Tinus and McDonald 1979).

Stress resistance is not considered to be related to plant age (e.g., freezing resistance [Sakai and Larcher 1987]; drought resistance [Teskey et al. 1984]), but rather to its morphological, physiological, and phenological state (Fuchigami et al. 1982, Lavender 1985). Changes in phenological and physiological parameters are known to occur in parallel (Fuchigami et al. 1982, Fuchigami and Nee 1987, Lang et al. 1985) with stress resistance varying seasonally with plant development (Bigras 1996, Burr 1990, Grossnickle 2000) in temperate zone tree species (figure 1). In addition, root growth is related to seasonal shoot dormancy patterns, decreasing as shoot endodormancy (regulated by internal factors) intensifies in the fall and increasing as seedlings move toward ecodormancy (regulated by environmental factors) (Ritchie and Dunlap 1980, Ritchie and Tanaka 1990). This knowledge of plant acclimation in relation to the phenological state can be used for scheduling hardening practices during the last stages of a nursery cultural program, thereby improving seedling quality and enhancing subsequent field performance (Landis et al. 2010, Lavender and Cleary 1974, Tinus 1974).

Acclimation of seedlings is based on the concept of "slowly increasing stresses to induce physiological adjustments in plants" (Kozlowski and Pallardy 2002); thus, cultural practices that enhance stress tolerance or avoidance can help seedlings develop morphological and physiological protection from potentially limiting field site conditions (Landis et al. 1999, Lavender and Cleary 1974, Tinus 1974, Wakeley 1954). The following sections describe cultural practices of modified daylength (photoperiod), temperature, and fertilization to promote seedling hardening while maintaining

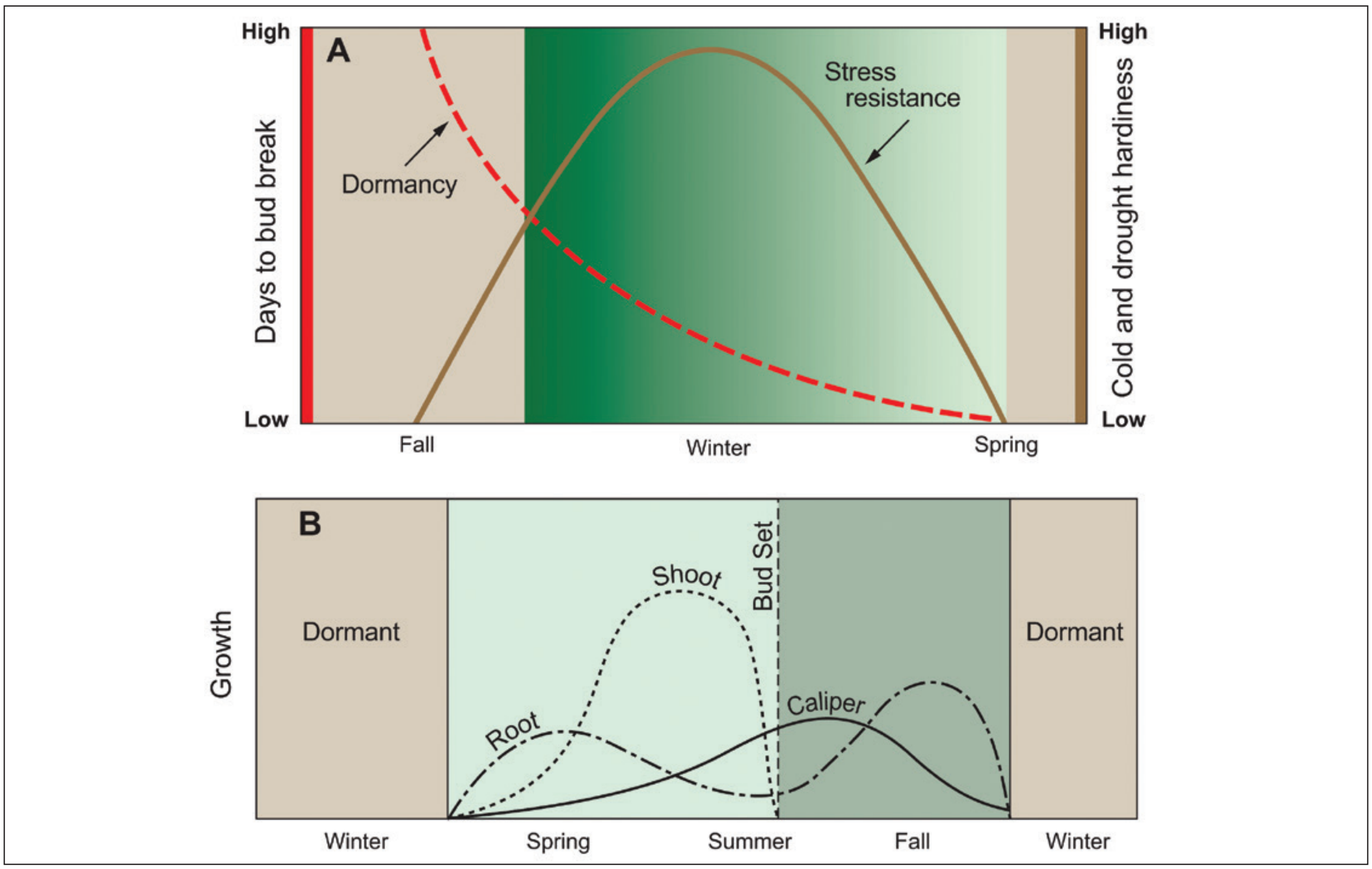

Figure 1. Seedlings have distinct phenological cycles which can vary somewhat based on species, geographic seed source, and weather patterns. (a) Bud dormancy (measured as days to budbreak) is high in the fall and declines through the winter and early spring, while stress resistance and cold hardiness peak in winter. (b) Root and shoot growth follow different patterns. (a - adapted from Landis et al. 2010; b – adapted from Landis et al. 1999)

physiological quality. A detailed discussion on watering as a seedling hardening cultural practice is described in the answer to Question 2.

Daylength

After the summer solstice, daylength shortens, promoting development of endodormancy. With northern-latitude tree species, the end of shoot elongation and development of terminal buds is considered to be the first stage of fall acclimation to low temperatures (Weiser 1970) and an overall increase in stress resistance (Levitt 1980). Seedlings normally enter the first stage of fall acclimation to low temperatures (Grossnickle 2000, Lang et al. 1985, Levitt 1980, Weiser 1970) and develop increased drought resistance (Abrams 1988, Teskey et al. 1984) in the latter half of summer, when shoot elongation has ended and terminal buds are developing (Burr 1990). As seedlings develop a "hard bud," they are considered endodormant and will not break bud even if they are exposed to optimal environmental conditions (see Temperature section). In this state, they continue to grow roots, though root growth is declining (Ritchie and Dunlap 1980, Ritchie and Tanaka 1990).

Because temperate tree species respond to seasonal decreases in daylength, short-day treatments have been developed in northern-latitude container nurseries to induce shoot growth cessation and bud formation (Landis et al. 1999, Tinus and McDonald 1979). The typical short-day treatment for spring-planted seedlings is initiated in August with an 8- to 10-h day and 14- to 16-h night treatment for 10 to 12 days, with variations depending on species and genetic sources (Grossnickle 2000, Landis et al. 1999). Seedlings are then placed under a cultural regime to maintain budset (i.e., moderate water stress, shortened photoperiod, and low N fertilization; Landis et al. 1999, Lavender and Cleary 1974). During hardening, the reduction of N fertilization is an optional practice that brings N levels below their optimum range, with N levels returned to their optimum range when limiting seasonal environmental conditions ensure seedlings remain endodormant and will not reflush. These practices are then maintained until they are lifted for storage in late fall or early winter. For summer-planted (Grossnickle and Folk 2003, Luoranen et al. 2006) and fall-planted (Luoranen and Rikala 2015; MacDonald and Owens 2006, 2010) seedlings, short-day treatment (as defined above) is initiated approximately 2 months before seedlings are lifted and shipped to the field to allow for a 5- to 6-week exposure to seasonal shortening photoperiods and ambient temperatures. This approach recognizes the annual seedling phenological and physiological cycles (figure 1), and utilizes them to promote budset development, dormancy, freezing tolerance, and drought resistance (Colombo et al. 2001, Grossnickle 2000, Landis et al. 2010), thereby producing hardened seedlings. The advantage of using photoperiod manipulation is that it allows for the application of a uniform cultural treatment over the entire crop (Landis et al. 1999).

Temperature

As seedlings are exposed to cold fall temperatures and accumulate chilling hours, they move through the endodormancy phase, with maximum days to budbreak in late summer and early fall decreasing through the fall and into winter (Burr 1990, Fuchigami et al. 1982) and increasing stress resistance (Grossnickle 2000) peaking in winter (figure 1). When seedlings complete the endodormancy phase and move into the ecodormancy phase, root growth potential increases (Burr 1990, Ritchie and Tanaka 1990) and seedlings only remain inactive as long as environmental conditions are unfavorable for growth (Burr 1990, Fuchigami et al. 1982, Lang et al. 1985).

Chilling hours, rather than calendar date, are used by nursery practitioners to track fall acclimation because temperate conifers require a period of chilling to move through endodormancy and become ready for overwinter storage. Chilling hours are quantified based on specific temperature ranges. For example, in the Pacific Northwest and Canada, chilling hours are often recorded from 0 to 4.4 °C (32 to 40 °F) (Timmis et al. 1994, van den Driessche 1977), or to 10 °C (50 °F) (Burdett and Simpson 1984, Ritchie et al. 1985), while in the southern United States, chilling hours are typically reported within the range of 0 to 8 °C (32 to 46.5 °F) (Carlson 1985, Garber 1983). In some instances, temperatures above or below a certain level are given partial or negative chilling hours (Haase et al. 2016, Harrington et al. 2010). Chill days (O'Reilly et al. 1999), degree-hardening-days (Landis et al. 2010), or hardening degree days (Carles et al. 2012) are sometimes reported when hourly data are not available. As chilling hours increase, the days to budbreak decrease and stress resistance increases for a wide range of temperate tree species (Grossnickle and South 2014) (figure 1).

Fertilization

Reduction, reformulation, or withdrawal of fertilizer toward the end of the growing season is an effective means to slow growth and induce bud formation (Landis et al. 1999, Tinus and McDonald 1979). This practice is sometimes done in concert with short-day treatments at container nurseries. Typically, N fertilization is reduced by 50 to 90 percent from rates used during the rapid growth phase of seedling development (Landis et al. 1989). Fall fertilization regimes, applied after the hardening fertilization treatment, have been developed to result in optimum nutrient levels available for growth after outplanting (Dumroese 2003, Hawkins 2011, Landis 1985), while fall nutrient loading after the completion of budset is designed to increase seedling nutrient reserves to luxury consumption levels, thus increasing field performance potential (Dumroese 2003, Grossnickle 2012, Grossnickle and MacDonald 2018a, Hawkins 2011, Timmer 1997).

Question 2 – How Can Irrigation Management Be Used To Promote Stress Resistance?

Modifying irrigation practices to create water stress events at the end of the growing season affects plant development and can be used to increase stress resistance and hardening. These water stress events result in "physiological adjustments" in plants (Kozlowski and Pallardy 2002), increased drought resistance (Teskey et al. 1984), and induction of bud formation (Calme´ et al. 1993, Lavender and Cleary 1974, Macey and Arnott 1986, Timmis and Tanaka 1976, Young and Hanover 1978). Drought resistance is a combination of drought avoidance and drought tolerance (Abrams 1988, Teskey et al. 1984). Drought avoidance (i.e., postponement of plant dehydration through reduction in water loss) includes cuticular development (Grossnickle 2000), stomatal sensitivity (Folk and Grossnickle 1997, Timmis 1980), morphological balance (Mexal and Landis 1990, Thompson 1985), increased water absorption by roots (Carlson and Miller 1990), and improved root growth capacity (van den Driessche 1991). Drought tolerance (i.e., capacity to undergo dehydration without irreversible injury) includes osmotic and cell wall elasticity adjustment (Joly 1985, Lopushinsky 1990, Ritchie 1984, Timmis 1980) and chloroplast drought resistance (Timmis 1980).

Exposing seedlings to water stress, in combination with reduced photoperiod and fertilization, is used to harden seedlings (Landis et al. 1999). Successful implementation of this cultural practice requires an understanding of necessary water stress levels for the development of seedling drought resistance. For example, loblolly pine (*Pinus taeda* L.) seedlings developed drought resistance during a 5-week reduced irrigation regime (figure 2a) with a 50-percent increase in drought avoidance (cuticular transpiration declined from 3.8 to 2.3 percent water loss h-1 after stomatal closure) and a 100-percent increase in drought tolerance (osmotic potential at turgor loss point that declined from

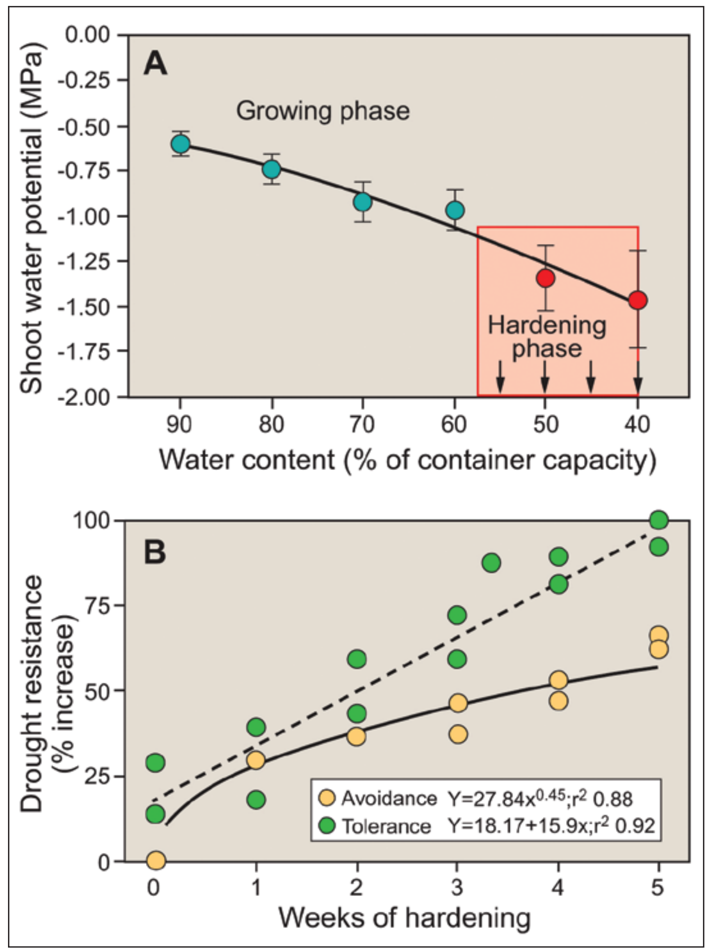

Figure 2. (a) Mid-day shoot water potential of loblolly pine (*Pinus taeda* L.) seedlings changes in relation to the water content container capacity percentage (CC%). The arrows along the X-axis are hardening targets to progressively lower the CC% to 40 percent over a series of weeks. (b) Drought resistance is measured by drought avoidance (cuticular transpiration that declined from 3.8- to 2.3-percent water loss per hour after stomatal closure) and drought tolerance (osmotic potential at turgor loss point that declined from -1.0 to -2.0 MPa) during nursery hardening (i.e., reduced fertilization and watering) (Grossnickle unpublished).

-1.0 to -2.0 MPa) (figure 2b). Other studies have also found that restricted watering hardens loblolly pine seedlings (Bongarten and Teskey 1986, Hennessey and Dougherty 1984, Seiler and Johnson 1985). As loblolly pine seedlings proceed through this drought-hardening event, their shoot and root systems stop growing, needle cuticular development occurs resulting in tactile changes from a feather-like to a stiff feel when moving one's hand across the foliage, needle color changes from lush green to light green, and root suberization occurs resulting in a color shift from white to brown (figure 3). These visual cues allow the nursery practitioner a means to track seedling changes during the drought hardening process.

For a water-stress cultural practice to be successful, one needs to increase water stress in a stepwise

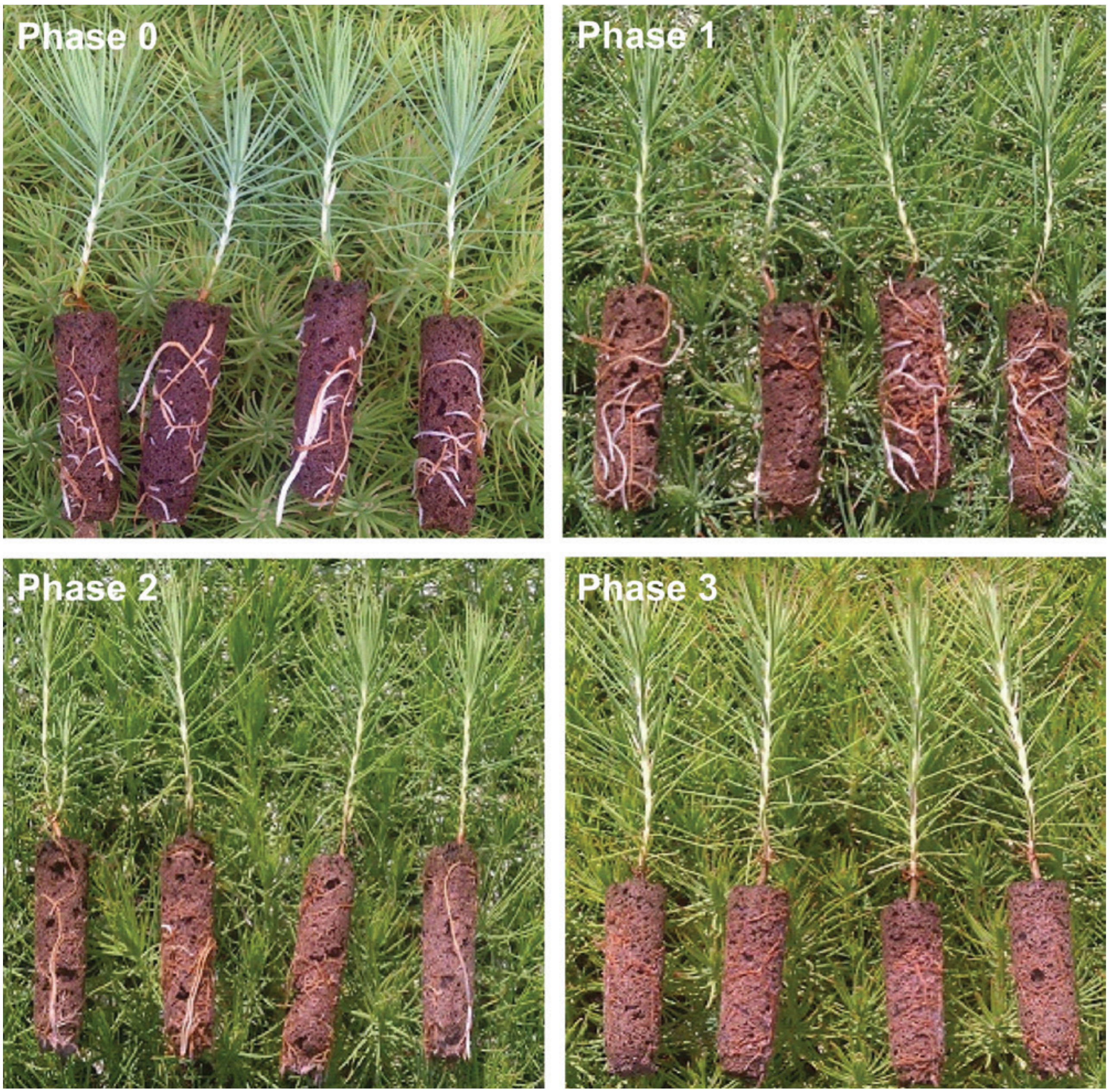

Figure 3. Loblolly pine (*Pinus taeda* L.) seedling morphological development during drought hardening. Phase 0 (onset of hardening, week 0) is an actively growing seedling with needles exhibiting a lush, green color, feather-like feel when moving one's hand across the foliage, and more than 50 percent of the root system is unsuberized with a white color. In Phase 1 (occurring by week 2), seedling needles start to lose their green luster and roots show initial stages of suberization on the upper portions of the plug. Phase 2 (occurring by week 3 to 4) is characterized by light green needles that exhibit initial cuticle development and have a slightly stiff feel; also, less than 25 percent of the root system shows an unsuberized white color. In Phase 3 (occurring by week 5), needles are light green and exhibit full cuticle development with a stiff feel, plus 100 percent of the root system shows brown, suberized roots. (Photos by Steven C. Grossnickle)

progression as seedlings transition from the growing phase into the hardening phase. For example, container-grown loblolly pine seedlings typically go through a series of drying cycles (i.e., watered to saturation and allowed to dry to a defined container weight) with an initial dry down to 60-percent container capacity, followed by progressively lower levels over 3 to 5 weeks until reaching 40-percent container capacity and a mid-day shoot water potential of -1.5 MPa (figure 2a). These drying cycles are intended to expose seedlings to drought stress that comes near, but does not exceed, the shoot wilting point (Landis et al. 1999). A standard operational monitoring practice for certain species is to wait until 10 percent (Kiiskila, personal communication), 25 percent (Grossnickle et al. 1991), or even up to 40 percent (Grossnickle, personal communication) of the crop has shoot tip wilting before rewatering. A minimum predawn water potential of -1.0 MPa (Lavender and Cleary 1974) or daytime readings between -1.2 and -1.5 MPa (Cleary 1978), or even as low as -1.5 to -1.7 MPa (Landis et al. 1999, Tinus 1982) over a series of stress events was sufficient to terminate shoot growth and develop stress resistance in conifer species. When seedlings are fully hardened, the crop will not show shoot system wilt during a drought event (Grossnickle, personal communication). If water stress is too severe or too rapid during these drying cycles, it impedes the physiological development of drought resistance (Cleary 1978). Avoiding rapid development of water stress is critical to ensure this is an effective cultural practice.

Vapor pressure deficit is another environmental variable related to the plant-water balance and can be used to harden seedlings. Seedlings harden with exposure to the combination of lower available soil water and higher vapor pressure deficit (Larcher 1995). These conditions will cause moderate plant water stress and reduced photosynthesis (Grossnickle 2000, Kozlowski et al. 1991) which can slow or stop seedling growth (Grossnickle 2000, Kozlowski 1982) and help harden seedlings for reforestation site conditions (Landis et al. 1999).

The use of water stress is not always successful in hardening seedlings within an operational nursery environment (Landis et al. 1989). First, there is difficulty in implementing a uniform drought treatment due to differences in irrigation coverage and variation in individual seedling water use. Second, when standard peat-based growing media dry, they can become hydrophobic, making it difficult to rewet and thereby causing uneven exposure to the drying regime. To avoid or overcome media becoming hydrophobic, it is important to overwater after a drought-stress treatment to ensure all cavities are fully saturated (Kiiskila, personal communication). Third, species differ in development of drought resistance (Abrams 1988), making it difficult to apply water stress as a universal hardening treatment across all species. Thus, it is important to monitor water stress treatments to ensure they are applied uniformly and result in successful hardening.

Question 3 – What Nursery Cultural Strategies Promote Vigorous Root Growth?

A well-developed, functional root system is critical for outplanting success (Grossnickle 2005, 2012; Grossnickle and MacDonald 2018a). Quality root systems readily uptake water and nutrients and give structural support to the seedling. Measures of root quality include mass, shoot-to-root ratio, form, length, fibrosity, root growth potential, and nutrient/carbohydrate content (Davis and Jacobs 2005, Haase 2011a). Although the root system is not easily observed compared with the shoot due to its belowground nature, attention to root morphology and physiology in the nursery are imperative to help ensure good field performance. When working with growers, Landis (2008) often referred to seedlings as a “root crop” to emphasize the importance of good-quality root systems.

For the most part, nursery strategies for developing vigorous seedling root systems are inextricably linked with strategies for promoting overall plant quality. For instance, root vigor is tied to the transfer of photosynthates from the shoots (Binder et al. 1990, Philipson 1988, van den Driessche 1987). To achieve target specifications, the grower must consider the phenological cycle for the species and seed source (figure 1), along with environmental patterns at the nursery. As such, growing regimes must be tailored to stocktype (i.e., container type, size, depth, and density, seedling age, and outplanting season) and its associated target specifications for the outplanting site conditions. For example, some species (e.g., pine) are strongly taprooted and tend to not generate lateral roots in the upper part of the root system. In a nursery setting, however, development of lateral roots and numerous root tips is a primary goal for ensuring root egress and vigor after outplanting (figure 4). In studies with overwintered spruce (*Picea* spp.) seedlings, root hydraulic conductivity increased with new root growth because newly developed roots have low root resistance and high water uptake capability during the first few weeks after thawing (Colombo and Asselstine 1989, Grossnickle 1988). Thus, alleviation of planting stress depends on the number of new roots a seedling develops just after planting (Grossnickle 2005).

To encourage a quality seedling with well-developed roots, the grower must sow seed into a well-drained container growing medium (or bareroot seedbed) with adequate aeration (Landis et al. 1990) during temperature and moisture conditions suitable for germination and rapid root elongation. Irrigation is one of the most useful culturing tools in any nursery and can make all the difference between the production of high-quality or low-quality plants. Irrigation based on the plant's transpirational demands, target water content, and seedling growth phase is far more effective and efficient than irrigation on a set schedule (Dumroese et al. 2015). The best irrigation programs always involve watering to saturation and then allowing a dry down sufficient to ensure good root aeration. High irrigation levels tend to result in higher shoot-to-root ratio (Moser et al. 2014) and proliferation of pathogens and other pests (Dumroese and Haase 2018). Similarly, excessive fertilizer, especially nitrogen, promotes excessive shoot growth and an unbalanced shoot-to-root ratio (Landis et al. 1989).

Figure 4. Good quality seedlings have vigorous roots that egress rapidly after outplanting, such as the Douglas-fir container seedling. (Photo by Diane L. Haase 2013)

Proper timing of nutrient and water deprivation to induce budset and hardening correlates with the push to generate stem diameter and root growth in the fall before temperatures drop and all growth ceases. This phase is critical for achieving target height-to-diameter and shoot-to-root ratios. Quality container-grown seedlings have root plugs with good integrity such that the plug is readily extractable and stays together during, lifting, handling, storage, and planting. Root development should be adequate to fill the plug and hold the growing medium, but care must be taken to not create a rootbound condition (South and Mitchell 2006). After outplanting, rootbound seedlings may have poor root egress, root deformation, slowed growth, instability, and/or reduced survival. This issue can be avoided with careful attention to sow date, container size, irrigation, and fertilization.

Root pruning is another tool to manipulate root architecture and function. For container seedling production, the use of containers with copper-coated walls chemically prunes elongating roots and increases the proliferation of a fibrous root system within the plug (Sword-Sayer et al. 2009, Tsakaldimi and Ganatsas 2006). For bareroot seedling production, nursery growers prune roots horizontally (i.e., undercutting or wrenching) or vertically (sidecutting) (Landis 2008, Riley and Steinfeld 2005). When applied and timed properly, bareroot root culturing results in a more compact, fibrous root system at the time of lifting for both conifer (Dierauf et al. 1995) and hardwood (Schultz and Thompson 1997) seedlings. This practice is also used to create a mild stress event to control height growth (Buse and Day 1989), induce bud formation (van Dorsser and Rook 1972), and mitigate soil compaction (Miller et al. 1985).

Question 4 – When and How Long Can Storage Be Used For "Hot-Lifted" Seedlings?

Hot-lifted seedlings used for summer or fall planting have usually developed a "hard bud" that will not break even if the seedlings are exposed to optimal environmental conditions (MacDonald and Owens 2006, 2010), although they are still growing roots (Ritchie and Dunlap 1980, Ritchie and

Tanaka 1990) and developing drought resistance (Abrams 1988, Teskey et al. 1984) and freezing tolerance (Weiser 1970). Thus, hot-lift seedlings are still physiologically active at planting and require unique handling procedures.

In Western Canada and the United States, hot-lifted seedlings are commonly planted in two distinct periods: the first being late June through July (summer planting), and the second being mid-August through early October (fall planting). Seedlings for both summer and fall planting programs are subject to the same cultural hardening practices at the nursery (see Question 1) and are in a similar phenological state at the time of planting. Thus, physiological hardiness is similar between summer- and fall-planted seedlings and any field performance differences are generally associated with environmental conditions during and after planting (Pikkarainen et al. 2020).

Handling and storage practices can affect quality of hot-lifted seedlings (Binder and Fielder 1995, DeYoe 1986, Landis et al. 2010). In particular, temperature conditions will influence maintenance respiration; each 10 °C (18 °F) increase approximately doubles the respiration rate (Kramer and Kozlowski 1979). The temperatures inside closed boxes can quickly increase, causing hot-lift seedlings to use more of their stored carbohydrates (Landis et al. 2010). Thus, hot-planted seedlings must be kept cool to maintain their vigor. After harvest, hot-lifted seedlings should be kept in a nursery cooler and/or refrigerated trailer at 2 to 10 °C (35 to 50 °F) prior to shipment, with 2° C (35 °F) being the ideal short-term storage temperature (Grossnickle personal communication). Depending on seed source, species, and nursery hardening regime, seedlings in both summer and fall planting programs develop some degree of cold hardiness after budset (Bigras et al. 2001) and can easily withstand cold storage temperature conditions.

Properly hardened summer/fall planted seedlings can be safely cold stored for approximately 4 weeks prior to outplanting without any chilling requirement (Jackson et al. 2012). Cultural practices to induce hardening (i.e., water stress and low N) resulted in container-grown loblolly pine seedlings being able to withstand 4 to 6 weeks of cold storage without prior chilling hours (Grossnickle and South

Figure 5. Refrigerated trailers are required for transporting large quantities of hot-lift seedlings from the nursery and are ideal for short-term cool seedling storage prior to planting. (Photo by Steven B. Kiiskila 2010)

2014). While it is possible to safely hold hot-lifted seedlings for a maximum of 4-weeks, it is contingent on maintaining a 2 °C (35 °F) storage temperature; safe storage duration decreases with increasing storage temperature (Paterson et al. 2001).

Shipping hot-lifted seedlings to the outplanting site occurs in refrigerated trailers with temperatures below 10 °C (50 °F) (Dunsworth 1997, Stjernberg 1997). Upon arrival at the planting site, seedlings may be kept in a refrigerated trailer (figure 5) or transferred to a field cache in a shady location and/or under a suspended tarp with boxes opened to prevent heat buildup (Kiiskila 1999, Landis et al. 2010). During summer and fall months, moisture stress might occur; therefore, seedlings need to be monitored and irrigated if required (Landis et al. 2010). Under these conditions, only enough seedlings for 1 day of planting should be transported to the site. Alternatively, hot-lifted seedlings have been stored at 4 to 21 °C (40 to 70 °F) in refrigerated trailers on the planting site for up to a week (Dumroese and Barnett 2004), although lower temperatures between 2 to 4 °C (36 to 39 °F) are recommended (Landis et al. 2010). The full storage duration for hot-planted seedlings includes time spent in the nursery's cold storage facility and time spent in storage away from the nursery (e.g., in refrigerated trucks or other off-site holding areas). The combined length and care for all of these handling and storage steps is critical to ensure quality seedlings are outplanted.

Question 5 – How Long Can Seedlings Be Overwintered in Refrigerated Storage?

Overwintered spring plant seedlings are harvested in the fall once dormant and most commonly held in refrigerated storage until shortly before planting. Refrigerated storage is differentiated by temperature into cooler (1 to 2 °C) or freezer (–2 to –4 °C) storage, with the storage practice dependent on species' tolerance to freezing temperatures, available facilities, and expected storage duration (Grossnickle and South 2014, Landis et al. 2010).

Properly hardened and dormant seedlings (see Question 1) can be lifted in late fall and early winter and stored well into the spring for planting (Camm et al. 1994, Ritchie 1987). A dark and cold or frozen environment, however, is an unnatural environment for seedlings. The lack of light in storage prevents seedlings from replenishing carbohydrates lost through respiration (Ritchie 1987) and interrupts the seedling's circadian rhythm (Camm et al. 1994, Lavender 1985). Seedlings lifted and stored correctly are rarely damaged by cold or frozen storage, though some plant deterioration can occur as storage time lengthens (McKay 1997). Both cold and frozen storage conditions, when managed properly, allow properly hardened seedlings to maintain their physiological integrity required for good seedling quality (Landis et al. 2010). This is critical because quality seedlings typically have vigorous rooting at planting, which is required to overcome planting stress (Grossnickle 2005), thus increasing chances for successful seedling establishment (Grossnickle 2012).

Cold storage is a cultural practice where seedlings are held at 1 to 2 °C (35 to 36 °F) for no longer than 2 months (Landis et al. 2010, Ritchie 2004). Increasing cold storage can result in decreases in days to bud break (DBB), root growth potential (RGP), freezing tolerance, and carbohydrates (Grossnickle and South 2014). Extended exposure to cold temperatures and high humidity during cold storage creates conditions for storage molds (Camm et al. 1994, Hocking 1971, Landis et al. 2010, Ritchie 2004). Treating seedlings with appropriate fungicides prior to storage can improve seedling storability (Barnett et al. 1988), though their beneficial effects diminish as cold storage lengths reach 2 or more months (Grossnickle personal communication). Managers holding seedlings in cold storage should monitor stored seedlings regularly to detect problems.

Frozen storage is a cultural practice where seedlings are held at -2 to -4 °C (25 to 28 °F). This below-freezing storage temperature further slows physiological changes in the seedlings, thereby allowing them to be stored longer compared with cold storage. Frozen storage temperatures should not drop below -5 °C (23 °F), however, because some species are susceptible root damage at lower temperatures (Bigras et al. 2001, Kooistra 2004). Seedlings harvested at the correct phenological stage, and thus in a state of maximum stress resistance, are usually freezer stored for 4 to 6 months (Grossnickle 2000, Kooistra 2004), though seedlings have been successfully freezer stored for up to 8 months (Helenius et al. 2005, Luoranen et al. 2012). Once planted, seedlings are in a state of ecodormancy whereby the chilling requirement has been met and buds will break after exposure to favorable temperatures and begin the yearly cycle of growth (Burr 1990, Haase 2011b, Lavender 1985).

Two issues should be considered with regard to freezer storage effects on seedling quality. First, seedlings are still physiologically active (albeit at a low level), which is reflected in the decrease in DBB, RGP, freezing tolerance, and carbohydrates as the storage duration lengthens (Grossnickle and South 2014, Landis et al 2010). Second, the low humidity in freezer storage prevents storage molds (Haase and Taylor 2012, Hansen 1990, Trotter et al. 1991) but can desiccate seedlings with excessive storage duration, which may lead to reduced root growth potential (Deans et al. 1990). Packaging frozen-stored seedlings in a plastic bag or a poly-lined paper bag inside a waxed box minimizes seedling desiccation (Kooistra 2004), although seedlings may still lose up to 10 percent water content after 5 or more months in frozen storage (Lefevre et al. 1991).

The thawing process prior to planting should also be considered in conjunction with frozen storage duration (figure 6). While frozen seedlings were originally thawed slowly over a period of weeks, it has been shown that slow thawing causes seedling quality to decrease with increasing thawing duration (Silim and Guy 1997), and rapid thawing within a matter of days maintains seedling quality (Rose and Haase 1997). Thus, thawing seedlings as quickly as possible is now recommended (Landis et al. 2010). Rapid thawing is also preferred because it prevents

Figure 6. Rapid thawing of frozen seedlings in closed boxes can be done by spacing stacked boxes in a warm location without direct sunlight and rotating the boxes top to bottom. (Photo by Steven B. Kiiskila 2009)

storage molds (Rose and Haase 1997), minimizes depletion of carbohydrates (Silim and Guy 1997), and results in seedlings having later bud break and greater frost hardiness at time of planting (Camm et al. 1995). Seedlings can also be planted frozen without thawing, but must be individually wrapped at harvest such that seedling plugs can be separated from one another while frozen (figure 7). Eliminating the thawing process requires more effort in the nursery at lifting but offers operational flexibility during the busy planting window. Research to date has shown no deleterious physiological effects of planting frozen seedlings (Camm et al. 1995, Kooistra and Baaker 2005), although limiting site environmental conditions at the time of planting such as dry cold soils can have a negative effect (Helenius 2005).

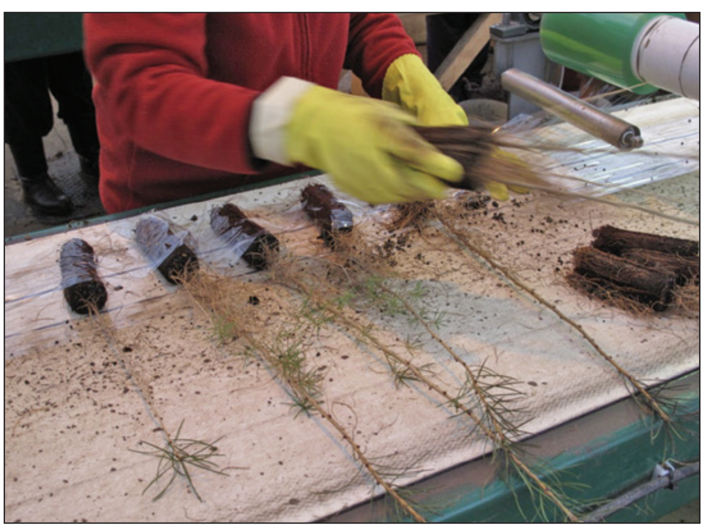

Figure 7. One method to enable separation of frozen seedlings from one another without damaging their roots is to place poly wrap around each seedling's root plug when bundled after lifting. (Photo by Steven B. Kiiskila 2010)

Long-term frozen storage for "late" spring planting may result in seedlings being initially out of sync with the annual growth rhythms of the planting site. That is, planted seedlings may have budbreak patterns that do not reflect that of natural seedlings on the planting site (Grossnickle 2000). Seedlings require sufficient time to complete the growth processes initiated with budflush and begin development of hardiness before the onset of fall frosts. The potential risk of fall frost damage to seedlings at different planting dates can be estimated through analysis of long-term climatic data (Hänninen et al. 2009). Delaying spring planting into early summer increases the likelihood of bud break and shoot elongation when the site environment has warm temperatures, high vapor pressure deficits, and dry soils (Grossnickle 2005, Mitchell et al. 1990). As such, the site may not be suitable for planting until late summer/fall and may be more appropriate for planting with hot-lifted seedlings (see Question 4).

Conclusions

Seedlings are not widgets; they are biological organisms that respond to their surrounding environment. Nursery cultural practices have a direct influence on the seedling environment, thereby influencing seedlings' physiological function and subsequent morphological development. This discussion shows that all nursery cultural decisions, from hardening practices, to strategies that promote vigorous root growth, to storage practices affect seedling development. Understanding how cultural practices affect seedling performance will ensure that the nursery practitioner develops sound practices that enhance seedling quality and subsequent success of forest restoration programs.

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