Southern Forest Nursery Management Cooperative

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LIFTING DATES, CHILLING HOURS AND STORAGE DURATION ON ROOT GROWTH POTENTIAL (RGP), GROWTH, AND SURVIVAL

by
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INTRODUCTION

The United States annually produces more than 1.2 billion seedlings for reforestation, with more than a billion produced in the southeastern United States (Haase et al. 2019). The majority are conifers, produced as bareroot seedlings and grown in a similar manner to that of regular agricultural crops (Starkey et al. 2015, Haase et al. 2019). Seedlings are typically grown in native soils within open fields for approximately 9 months before they are removed from the soil during harvesting, or what is called lifting (Starkey et al. 2015). They may be planted in areas that have recently been harvested or into fields, converting land back into forests.

Lifting usually occurs between late November and late February, the optimum time when seedlings are dormant. These seedlings are packed in boxes, bags or bundles and placed in cold storage for a two to three week period before shipped to the field where they are replanted in areas prepared for reforestation (Carlson 1991, Johnson and Cline 1991, Starkey et al. 2015). Seedlings are stored for this time interval to avoid issues of increased mold and decay, that if outplanted, has been shown to decrease seedling survival (Landis and Haase 2008, Grossnickle and South 2014). However, weather conditions are not always favorable for the outplanting of seedlings once lifted, and seedlings are sometimes stored for longer time intervals than recommended. With fluctuating freezing and above normal temperatures occurring more often across the southern US during the winter months of December, January and February, there is a concern as to the impact for optimum lifting time, their storability once lifted and their growth and survival after outplanted (Harrington and Gould 2015).

Environmental conditions during seedling growth in the nursery has an impact on the seedling quality and physiological readiness for storage and outplanting (Carlson 1991). Seedling quality can be defined as a seedling that can survive periods of environmental stress and produce vigorous growth following outplanting (Grossnickle and South 2017, Grossnickle and MacDonald 2018). Seedlings have to be physiologically ready to grow and the importance of photoperiod and to environmental cues during the growing season within the nursery is fundamental (Haase et al. 2016). Seedling dormancy, cold hardiness and the accumulation of carbohydrate reserves (Deligöz 2013) are the main contributing physiological processes that ensures that a seedling is physiologically ready to withstand the stress of lifting, handling and planting so after outplanting optimal root and shoot growth can occur (Burdett and Simpson 1984).

Dormancy and Cold Hardiness
Environmental factors affect both the dormancy and cold hardiness of tree seedlings, however, they are two distinct processes that are difficult to separate in the field (Burr 1990). The plant tissues affected (buds versus the entire seedling) in addition to the occurrence, are a method to differentiate the two processes (Ritchie and Landis 2004, Haase 2011). These processes of dormancy and cold hardiness usually occur sequentially commencing in mid fall (Nov – Jan) with bud dormancy and cold hardiness following later in early winter (Jan – Mar) with the tissues of the whole seedling (Burr 1990, Haase 2011). One should note that the physiological process of dormancy and cold hardiness are complex and not well understood for southern pine species (Johnson and Cline 1991).

Dormancy
Physiologically, during the active phenological phase of seedling growth, bud dormancy arises whenever stressful environmental conditions occur. Examples of such conditions that lead to bud dormancy include drought, high/low temperatures or nutrition limitations (Ritchie and Dunlap 1980). Bud dormancy during this active phenological growth stage is, however, reversible with the resumption of renewed growth with the removal of the environmental stress (Ritchie and Landis 2004). During late spring and early summer (June) the active phenological growth stage is slowed coinciding with the initiation of quiescence (external dormancy) phase of the seedling (Burr 1990). The initiation of this phenological stage is typically characterizes by the formation of buds, however, quiescence occurs with decreasing temperatures in early fall (September) and for some of the northern provenances the shortening of photoperiod also plays an important role (Haase 2011, Cooke et al. 2012). During this period, if favorable conditions occur for seedling growth, quiescence is reversible. Beginning late fall (October/ November), however, true internal dormancy (seedling rest) starts and continues into December characterized by the seedlings inability to grow even when favorable conditions and temperatures for growth are present. Studies have shown that at this phenological stage, growth can only occur when seedlings experience a certain period of low temperatures referred to as 'meeting chilling hour requirements'. During this seedling rest (internal dormancy) phenological stage, once the chilling requirements are met, the seedling re-enters quiescence (external dormancy), such that, if warmer temperatures and favorable conditions were present, seedling growth would resume (Ritchie and Landis 2004). It is thus during this quiescence (external dormancy) period that only low temperatures are inhibiting seedling growth.

For southern pine species little is known about the acquisition of bud dormancy and role of seedling rest (internal dormancy) related to changing photoperiod and temperature. Although there is evidence that both nutrition and water availability can influence the timing of bud development (Ritchie and Dunlap 1980, Larsen et al. 1986, Cooke et al. 2012, Harrington and Gould 2015). Increased photoperiod (day length) was found to partially substitute for chilling temperatures. While, southern pine species show a need to obtain certain chilling requirements, the quantity is still unclear when compared to that of the northern pine species that remain dormant in winter until they fulfill certain chilling requirements for bud elongation (Kolb et al. 1985, Larsen et al. 1986, Hallgren and Tauer 1989, Johnson and Cline 1991, Cooke et al. 2012). For southern pines they appear to respond to chilling quantitatively, the more chilling received the faster the rate of bud break up to a maximum (Larsen et al. 1986, Johnson and Cline 1991, Harrington and Gould 2015). Exposure to low (above freezing) temperatures once the chilling requirements have been fulfilled enhanced height growth in subsequent growing seasons. Cold storage can also affect seedling physiology by partially satisfying the chilling requirements to break dormancy, obtaining additional chilling hours while in cold storage is known to enhanced budbreak (Harrington and Gould 2015).

Cold Hardiness

Cold hardiness results in physiological changes throughout all seedling tissues following the suspension of rapid cell expansion. For southern pine species, cold temperature acclimation is referred to as hardening, initiating when the air temperatures decrease. Thus, the cold hardiness process lags behind the bud dormancy process (Johnson and Cline 1991). Several studies have shown that for southern pines species, tissues within the seedling acclimate differently (Kolb et al. 1985, Hallgren and Tauer 1989). With increasing temperature acclimation (accumulating chilling hours) once a sufficient amount of chilling hours are met (maximum usually achieved in winter) we assume there is a corresponding increase in seedling freeze tolerance (Burr 1990, Haase 2011, Grossnickle and South 2017). However, the level of freeze tolerance for a species is genotype specific (Grossnickle and South 2014).

In contrast to bud dormancy, cold hardiness can decrease or "be lost" if seedlings are exposed to increasing temperatures. Seedlings have been shown to de-acclimate with as little as 3 to 7 warm nights (South et al. 2008, South et al. 2009). These nights of warm temperature encourage bud break and shoot elongation as the chilling requirements have already been met and seedlings are only in a state of quiescence (external dormancy) awaiting favorable conditions for rapid growth and bud elongation (South et al. 2009).

Chilling Hours and Seedling Storage

The cumulative number of hours of exposure of temperatures between 32 – 46°F are referred to as chilling hours. There is currently no universally accepted temperature range to define a chilling hour and reference temperatures are often been species and nursery dependent (Burdett and Simpson 1984, Carlson 1991, Johnson and Cline 1991). South (2012) and Grossnickle and South (2014) both stated that several researchers in the southeastern US count chilling hours within the range of 32 – 46°F (the range they prefer and used for this manuscript) and do not count temperatures below 32°F. South (2012) further showed the importance of defining temperature range in determining chilling hours as demonstrated that from 13 different definitions of a chilling hour for a given date, the number of chilling hours calculated might be as low as 250 hours or more than 800 hours dependent on the definition used.

Using this preferred method of chilling hour calculation, loblolly pine (dependent of geographic location of the tree) require between 200 – 400 chilling hours to overcome rest (internal dormancy), although this is merely a commonly used indicator of dormancy intensity (Ritchie and Dunlap 1980, Johnson and Cline 1991). For the southeastern US, this chilling hour requirement is usually met in early to mid-December, resulting in the seedling entering a state of quiescence (external dormancy)(Johnson and Cline 1991). At this time, seedlings are waiting for warmer temperatures and sufficient moisture to start rapid root and shoot growth.

The topic of bud and seedling dormancy, chilling hours and freeze tolerance should not be mistaken with the impact on seedling storage (Carlson 1991). Successful long-term storage of seedlings requires seedlings being able to tolerate extended periods in dark storage while maintaining seedling quality and physiological integrity (Grossnickle and South 2014). Short term storage is often used once seedlings have been lifted until outplanting occurs. Often a 2 to 3 week supply of seedlings are kept in the cooler, however, chilling hours are not necessary for short-term cooler storage (Grossnickle and South 2014). For many years it has been incorrectly believed that seedlings, regardless of stock type, require 400 chilling hours before storage can successfully occur. Several studies had shown that container seedlings could be stored for a month without any chilling hours. Other studies using bareroot seedlings exposed to 113 chilling hours were capable of tolerating 4 weeks and others exposed to 223 chilling hours were successfully stored for 11 weeks (South and Donald 2002, South 2012).

Inadequate number of chilling hours has occasionally been used to explain low outplanting survival that follows a hard freeze. South (2012) highlighted certain myths and facts related to chilling hours that include:

- "**Myth**: Southern pines reach maximum dormancy at 400 chilling hours. This is a myth as not all genotypes of a given pine species respond the same to chilling."
- "**Fact**: natural-light chilling increases freeze tolerance. It is commonly accepted that chilling is required for plant tissues to become freeze tolerant." He highlighted the fact that loblolly, slash and shortleaf pine, could develop a tolerance to 21°F freeze due to natural light chilling in comparison to usually only being able to tolerate a 28°F freeze when no chilling had been received.
- "**Myth**: placing a bareroot loblolly seedling in a refrigerator will increase freeze tolerance".
- "**Myth**: loblolly seedlings cannot survive cold storage unless they are hardened off or dormant before being place in cold storage."

Chilling hours is known as being beneficial to pine seedlings as increasing chilling hours increases seedlings' freeze tolerance. The successful storage of seedlings, however, is not related to the number of chilling hours. The impact of chilling hours on seedling storability and their subsequent growth needs to be studied further. To ascertain the relation of chilling hours on seedling storability, seedling growth and seedling survival after outplanting, this three-year study was conducted to partly address this issue.

MATERIALS AND METHODS

Site Information

For this study, a single seedlot (genotype) of *Pinus elliottii* (slash pine) were used over 3 seedling production and lifting seasons (2016 – 2017; 2017 – 2018; 2018 – 2019). Seedlings were grown and lifted from a commercial bareroot forest tree nursery in Georgia using their standard operating systems. For the outplanting component of the study, seedlings were planted at the Auburn University trophotron, where they grew without supplementation of water, weed control, or nutritional supplements for 12 months before being lifted and measured.

An essential component of this study was to determine the impact that chilling hours exposure, prior to being lifted, had on seedlings. Chilling hours were calculated for each lifting period commencing from 1 November until each lifting date (**Table 1**). Chilling hours were calculated using the Utah chill hour model. The Utah chill hour model is a weighted function assigning different chilling efficiencies to different temperature ranges, including negative contributions for high temperatures.

Trial Design

For each lifting season, at trial initiation and for each lifting period (6 in total), 1000 seedlings were removed from the nursery bed and placed into the cooler (temperature of 33 – 37°F) (**Figure 1**). From this 1000 seedlings removed from the nursery bed, 15 seedlings were kept aside, measurements collected (RCD and seedling root and shoot lengths) and then 10 of them outplanted in the Trophatron with the remaining 5 seedlings placed in the root growth potential (RGP) tanks (**Figure 1)**.

Storage period -Time in cooler

The remaining seedlings (985) were put in standard nursery shipping boxes and placed in a nursery refrigerator for storage (33 – 37°F) and be labelled $Time_0$. At two-week interval periods (for a 14-week period) 15 seedlings were randomly removed from the cooler stored seedlings at the nursery for measurements and outplanting as described above. These seedlings were $Time_1$ – $Time_5$, respectively (**Figure 1**).

Lifting period

At two week intervals an additional 1000 seedlings from the same seedlot (genotype) and nursery bed were lifted from the nursery for each treatment as described above and place into the cooler as well ($Time_1$ – $Time_5$) (**Figure 1**). Fifteen of these seedlings were kept aside for measurement, root growth potential and outplanting as described above with the remaining 985 seedlings placed in boxes and placed in then nursery cooler for storage.

Treatments

Lifting period: There were 6 different lifting times per season, separated by a two-week intervals ($Time_0$, $Time_1$, $Time_2$, $Time_3$, $Time_4$, $Time_5$)

Storage period (time in cooler): Once lifted from the nursery bed seedlings were stored in standard shipping boxes in their storage cooler for 0, 2, 4, 6, 8, 10, 12 and 14 weeks

Measurements

Root growth potential (RGP) is a measure of a seedlings ability to rapidly produce new growth (Ritchie and Dunlap 1980). For each sampling date ($Time_0$ – $Time_5$) RGP was determined on 5 seedlings for each sample group (lifting and storage period). These seedlings were placed in aquarium tanks filled with continually aerated water. After 30 days, the numbers of new white root tips formed (length of > 0.5 cm) for each seedling was recorded.

Root collar diameter (RCD) and height (Ht) were measured on all seedlings collected (15 for each lifting and storage period) seedling parameters measured included. For the seedlings designated for the outplanting study, at three time periods (at planting, June and December) the growth (RCD and Ht) and survival of the seedlings were measured. On conclusion of the study in December of each year (when seedlings were presumed to be dormant) the total number of surviving seedlings were calculated. Seedlings were removed from the ground following growth measurements with seedling biomass and root weight ratios determined

Analyses

In December of each year (2017-2019) root collar diameter (RCD) and total seeding height (Ht) measurements were taken on all living seedlings. Shoots and roots were separated and pooled by lifting date and storage period (weeks in cooler) and placed in drying oven until constant mass was achieved. Mean shoot and root mass calculated from total mass of pooled seedlings divided by number of living seedling. Root weight ratios were calculated as:

$$RWR\ (\%) = \frac{dry\ root\ weight}{(dry\ root\ weight\ +\ dry\ shoot\ weight)} \times 100$$

Percent seedling survival was calculated at the end of each year as the number of surviving seedlings divided by the number of seedlings outplanted for each lifting time and time in cooler combination.

Growth models for RCD and Ht were developed for each year across all measurement dates by either the number of weeks in the cooler or the date of lifting from the nursery bed. Exponential growth models ($y = a + e^{(bx)}$) were generated using PROC NLIN in SAS (SAS v9.4, Cary, NC) where y is either RCD or Ht, x is Julian day, and a and b are fit parameters.

RESULTS

Root Growth Potential

The relationship between seedling RGP and time stored in cooler differed between years (**Figure 2**). For 2017, there was a decrease in RGP with an increase in storage time. This was different to the RGP for the 2018 season that remained relatively constant for the storage periods, however, the 2019 season revealed an increase in RGP with that of storage time (**Figure 2**).

Growth

For both the 2017 and 2019 growing season, the shoot and root mass decreased with that of increase in storage time for each lifting period (**Figure 3**). In contrast, there was an increase in shoot and root mass with an increase in storage period in 2018 for the each lifting period. In 2018 for lifting period $Time_1$, seedlings stored for 2 weeks in the cooler had higher root and shoot mass compared to those that were stored for a period of 4 weeks (**Figure 3**).

Shoot:Root ratios for growing year 2017 were the highest ratios (>3) across storage periods compared to the lower ratios in 2018 (<2) (**Table 2**). Following a year of outplanting growth, results reveal that for each year the root weight ratio (RWR) tended to increase with time in cooler (**Table 2**). For 2017, the RWR trend of increase with time in storage occurs until 6 weeks in cooler thereafter the RWR decreased (**Table 2**). For both the 2018 and 2019 RWR, there was and trend of an increasing RWR with that of storage time with a maximum occurring for seedlings that had been stored in the cooler for 12 weeks (**Table 2**).

At $Time_0$, there was an increase in all RCD seedling growth for all storage periods for the growing year 2017, with 0 weeks in cooler having the highest with 14 weeks in cooler the lowest (**Figure 4**). For the 2018 growing season, there was a positive increase in RCD growth with storage periods 2, and 4 weeks in a cooler being the highest. A constant to slightly negative trend for RCD growth occurred in 2019, except for an increase in seedlings that had not been stored in the cooler (**Figure 4**).

For all lifting periods in 2017, there was an increase in RCD with $Time_0$ and $Time_3$ having the highest with $Time_4$ and $Time_5$ the lowest (**Figure 5**). In 2018, $Time_1$ had the highest increasing trend in RCD although $Time_3$ that showed a negative trend. All lifting periods for the 2019 period had similar increasing trends in RCD (**Figure 5**).

For the height growth model, for the 2017 season, across all storage periods there was an increase in growth with the storage period 0 weeks in cooler the highest and 12 weeks in cooler the lowest (**Figure 6**). In comparison the 2018 season showed had a very weak increase in growth with storage period of 2 weeks in the cooler having the highest compared to the negative growth trend for seedlings that had been stored in the cooler for 6 weeks prior to planting (**Figure 6**). An increase trend in growth was present for all storage times for seedlings lifted at $Time_0$, with 0 weeks in the cooler being the highest and 12 weeks in the cooler the lowest (**Figure 6**).

When comparing the lifting periods and its impact for seedling height, seedlings not stored for 2017 $Time_3$ had the highest followed by $Time_0$ and $Time_1$ respectively with $Time_4$ showing the lowest height growth (**Figure 7**). In 2018, lifting periods $Time_1$ was highest positive trend followed by $Time_2$ and $Time_0$, the remaining had negative growth trends (**Figure 7**). Positive height growth trends were present for the 2019 season with $Time_1$ having the highest followed by $Time_0$ (**Figure 7**).

<u>Survival</u>

Seedling survival showed a decreasing trend with an increase in storage time in a cooler for growing season 2017 and 2018 (**Figure 8**). For 2019, there was good survival across all storage periods (**Figure 8**).

For seedlings that had been stored in the cooler for 0, 2, and 4 weeks respectively, before being outplanted, had a decrease in survival for increasing lifting times from $Time_0$ to $Time_5$ for all years (**Figure 9**). There was also a decreasing trend in survival with that of lifting time for of all seedling stored in a cooler prior to being outplanted (**Figure 9**).

DISCUSSION

The relationship between RGP and increasing storage time differed between years (**Figure 2**). Previous studies on other southern pine species, have similarly shown increases, decreases and constant RGP with that of increasing storage time and related these increases, decreases and constant RGP to lifting date, storage temperature and duration (Ritchie and Dunlap 1980, Hallgren and Tauer 1989, Deligöz 2013, Grossnickle and South 2014). Root growth potential is linked to the bud dormancy cycle, peaking in mid- late winter just before bud break when seedling shoots are still dormant (Ritchie and Dunlap 1980, Carlson 1991, Deligöz 2013). With increasing temperatures, the RGP of seedlings usually decreases as the competition for resources (moisture and nutrition) for bud elongation instead of root growth occurs (Ritchie and Dunlap 1980, Deligöz 2013). It is for this reason that studies on using loblolly pine found increasing chilling hours increased RGP, and seedlings with a high proportion of quiescent buds at planting had higher RGP (Ritchie and Dunlap 1980, Larsen et al. 1986, Carlson 1991). This relationship was also observed in this study with the highest RGP occurring in 2019 and 2018, which had almost double the RGP of that found in 2017 when seedlings had no chilling hours and thus no dormancy (**Table 1; Figure 2**). Also evident is that with an increase in storage time, for seedlings that were dormant when lifted (2018 and 2019 years only) there was a further increase in chilling hours in storage and thus enhanced RGP (**Figure 2**). The level of seedling dormancy measured using chilling hours differed between years, thus an explanation for annual differences in RGP observed.

For different lifting periods ($Time_0$ – $Time_3$) there was a decrease in both shoot and root mass as storage time increased (2017 and 2019) when compared to an increase over the 2018 lifting period (**Figure 3)**. This decreasing relationship in 2017 was also noted with outplanting survival for all growing years (**Figure 9**). Decreases in both root and shoot growth, based on lifting periods, have been shown in other studies on conifers (Deligöz 2013). Roots and shoots compete within a plant for the access of carbohydrates, with the onset of bud break and shoot elongation there is a subsequent decrease in root growth (Ritchie and Dunlap 1980). By lifting the seedlings later the dormancy of the seedling is likely to be reduced with time, as it is being held back only by temperature (Ritchie and Dunlap 1980). Lifting later in the growing season usually occurs along with increases in temperature, which stimulates bud elongation and root growth reduction (Ritchie and Dunlap 1980). It is likely that the increase in the shoot and root growth for the 2018 growing season is that the seedlings were still dormant or just coming out of dormancy (**Table 1**). The ability for seedling to grow roots shortly after planting is related to improved seeding survival after outplanting (Mena-Petite et al. 2001, Grossnickle 2005). The correlating trends for both shoot and root mass is likely due to the balance of the two and the ability to grow and survive to avoid the stress of planting (Grossnickle 2005). The results observed at the end of the 2018 growing season are likely showing the impact that the lifting period had on root growth related to their survival and resulting shoot growth.

There was an increasing trend of increasing RWR with that of storage for the 2018 and 2019 growing period (**Table 2**). Sufficient root growth is important for seedling survival and successful establishment (Carlson 1986, Larsen et al. 1986, Mena-Petite et al. 2001, Grossnickle and South 2017). Greater root mass indicates that there is a greater absorptive surface for moisture and nutrient uptake (Mena-Petite et al. 2001, Grossnickle 2012). Seedlings without sufficient root development can lead to moisture stress and negative impact on seedling survival. Seedlings that had been stored for longer periods in 2017 (**Table 2**), had significantly lower RWR compared to 2018 and 2019 growing season which could explain the steeper negative survival curve as storage times increased (**Figure 8**). One explanation for the increasing RWR as storage increased in 2018 and 2019 is a result of the seedling dormancy when placed in the cooler (**Table 1**). As with other studies, seedling survival has been reported as being greater in years in which a greater number of chilling hours are acquired prior to being placed in the cooler (Larsen et al. 1986). Since cold storage can also partially satisfying the chilling requirements to break dormancy, obtaining additional chilling hours is known to enhanced root development with other conifers (Mena-Petite et al. 2000, Harrington and Gould 2015). Root weight ratios correlate well with seedling survival, greater root system size indicated that seedlings have an increase surface area to uptake water and essential nutrients to sustain shoots and thus enhancing the ability to overcome both water and planting stress.

Shoot:Root (S:R) ratios for seedlings were greatest for the 2017 growing season across all storage periods when compared to the lower ratios in 2018 (**Table 2**). Shoot:Root ratios are commonly used as measurement of drought avoidance potential. For example, if the ratio is unbalanced there can be too many transpiring shoot tissues compared to the moisture absorbing root tissues, hence impacting drought tolerance (Grossnickle 2012). Seedling survival after outplanting is noted as being negatively correlated with an increasing S:R ratio for loblolly pine (Larsen et al. 1986). It has therefore, been suggested that with higher S:R ratios (>3) will negatively impact seedling survival when compared to S:R ratios between 1 – 3, thereby increasing chances of survival (Grossnickle 2012). For this study, in 2017 the S:R were >3 for all storage periods compared to 2018 and 2019 lower values, resulting in the steeper negative curves in terms of seedling survival (**Figure 8**). When it comes to seedlings at the time of outplanting, survival is negatively correlated with S:R ratios but positively correlated with that of the RWR.

In 2017, there was an increase in RCD across the season with the 0 weeks in cooler having the highest and 14 weeks in cooler the lowest that decreased with lifting date. Previous studies on conifers have shown that as times increase in cold storage, a corresponding reduction in RCD can occur (South and Donald 2002, Deligöz 2013). As RCD is correlated with that of root mass, an increase in RCD usually indicates improved chances of seedling survival during drought and temperature increases as larger root mass is capable of obtaining more moisture and nutrients (Carlson 1986, Mena-Petite et al. 2001, Grossnickle 2012, Grossnickle and South 2017). Root growth occurs year-round, with seasonal peaks taking place in early spring (Nov – Mar) before bud break, and reductions in root growth linked with an increase in shoot growth (Ritchie and Dunlap 1980). Root growth potential has been shown to be a good indicator of potential seedling growth and survival and used as a quick measure as to the impact of storage (Feret and Kreh 1985, Simpson and Ritchie 1997). It is for this reason that one needs to examine the RGP data to better understand the seasonal growth observed (**Figure 2**). Looking at the RGP for this 3-year trial on seedlings (**Figure 2**) it is clear that root growth was affected by the level of bud dormancy. For example, in 2017, seedlings were not dormant and actively growing when outplanted, seedlings planted having no cold storage (0 weeks in cooler) were thus larger in both RCD and height than those planted 12 weeks later as they had more time to grow.

In contrast, seedlings planted in 2018 and 2019 had chilling hours when lifted and prior to being stored, thus affecting their growth after outplanted. It is known that cold storage can also affect seedling physiology by partially satisfying the chilling requirements to break dormancy, obtaining additional chilling hours while in cold storage in known to enhanced budbreak (Harrington and Gould 2015). This effect is evident with data from the 2018 growing season when dormant seedlings (**Table 1**) were stored for 2 to 4 weeks in cooler had larger RCD and height (**Figure 4 and 6**) than those that were not stored. Being stored in the cooler for 4 weeks resulted in larger growth compared to those stored for 2 weeks, which was greater than those with no cold storage.

This trend was also shown when looking at the effect of lifting date as seedlings lifted later in the season (for those lifting times with increased chilling hours Table 1). Results indicate that enhanced bud break due to increased chilling hours resulted in better growth as seen when comparing growth in 2018 and 2019 growing season. For 2018 RCD and height $Time_1$ >$Time_2$>$Time_0$ with seedlings having achieved higher chilling hours prior to being lifted were larger than those that received less chilling hour (**Figure 5, Table 1**). This same trend can be seen only for height in the 2019 growing season with $Time_1$ >$Time_2$ with same trend occurring with chilling hours (**Figure 7, table 1**). The ability for seedlings to increase chilling hours before being outplanted results in improved survival and growth than those with fewer chilling hours.

MANAGEMENT IMPLICATIONS

Chilling hours are not required for successful storage of seedlings with the success of seedling storage being species and genotype dependent. Improved survival once outplanted was partly related to the level of seedling bud dormancy impacting root growth. The growth and survival of seedlings once outplanted is, however, strongly affected by planting conditions (Grossnickle 2012). Under optimal temperature and site conditions with minimal moisture and nutrient stress, outplanted seedling survival is likely to be high compared to a more stressful environment when low survival is expected (Landis and Haase 2008, Grossnickle 2012). For this reason root growth at planting is essential and thus the need to plant early in the planting season when root growth can be maximized due to bud dormancy. Lifting and planting seedlings early improves both the growth and survival compared to seedlings that had been lifted later in the lifting season.

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Table 1. Chilling hours calculated for each seedling-lifting period over three lifting seasons.

YEAR	Number of chilling hours at time of lifting					
	$Time_0$	$Time_1$	$Time_2$	$Time_3$	$Time_4$	$Time_5$
2017	-302	-426	-428	-636	-820	-1016
2018	97	142	121	-103	-91	-152
2019	118	135	-6	-67	-142	-262

Table 2. The shoot and root mass parameters for seedlings that had different storage periods within a cooler prior to outplanting

		Weeks in Cooler at Lifting Time 0							
	Year	0	2	4	6	8	10	12	14
Shoot Mass (g)	2017	36.32	26.42	15.48	13.85	12.45	16.98	8.7	7.93
	2018	6.35	6.47	8.5	4.61	7.01	5.36	5.79	.
	2019	7.51	3.65	4.84	4.35	2.08	3.08	2.7	.
Root Mass (g)	2017	8.83	8.34	4.64	5.31	3.24	4.01	1.69	2.26
	2018	3.63	4.12	4.88	3.09	3.82	3.25	4.96	.
	2019	2.35	1.31	1.56	1.27	0.93	1.02	1.22	.
shoot: root	2017	4.1	3.2	3.3	2.6	3.8	4.2	5.1	3.5
	2018	1.7	1.6	1.7	1.5	1.8	1.6	1.2	
	2019	3.2	2.8	3.1	3.4	2.2	3.0	2.2	
Root weight ratio (%)	2017	19.6	24.0	23.1	27.7	20.7	19.1	16.3	22.2
	2018	36.4	38.9	36.5	40.1	35.3	37.7	46.1	
	2019	23.8	26.4	24.4	22.6	30.9	24.9	31.1	

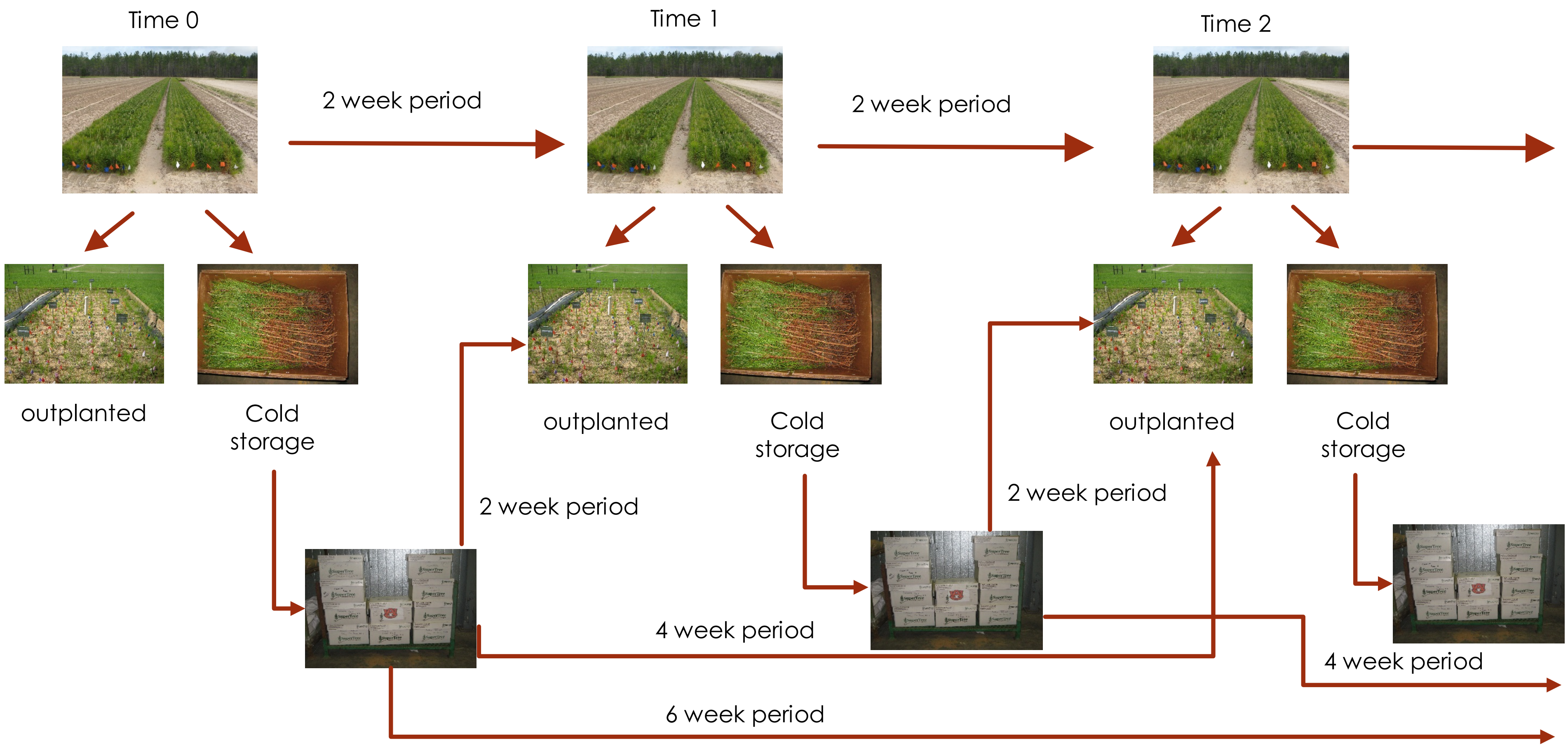

Figure 1. A graphical representation of the overview of the methodology used for this study

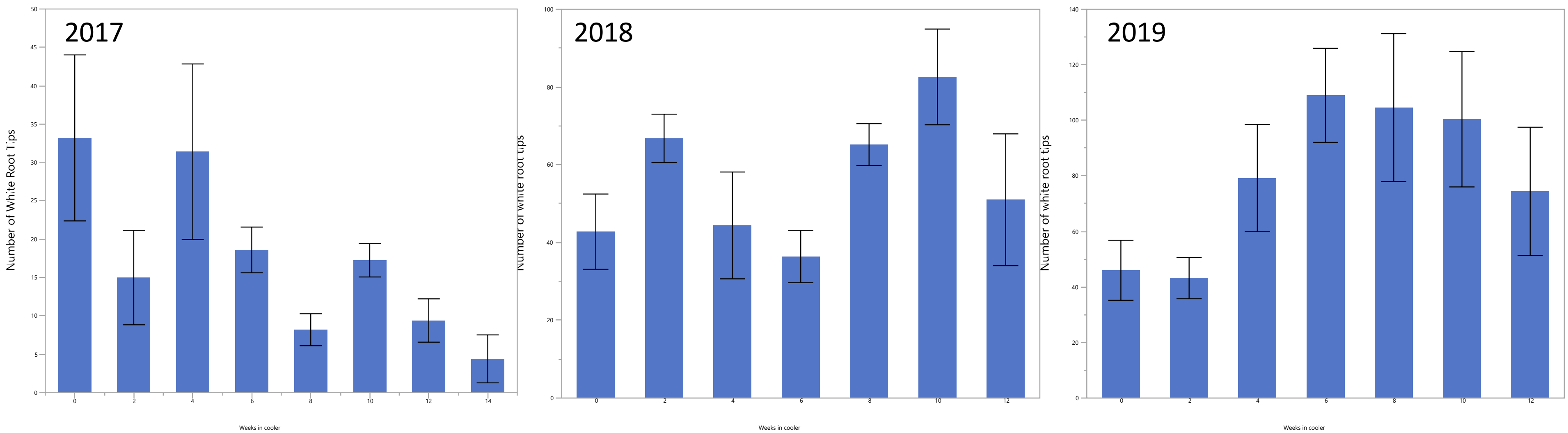

Figure 2. The root growth potential (RGP) of tree seedlings stored for within a cooler over a twelve week period, replicated over three lifting seasons (2017; 2018; 2019)

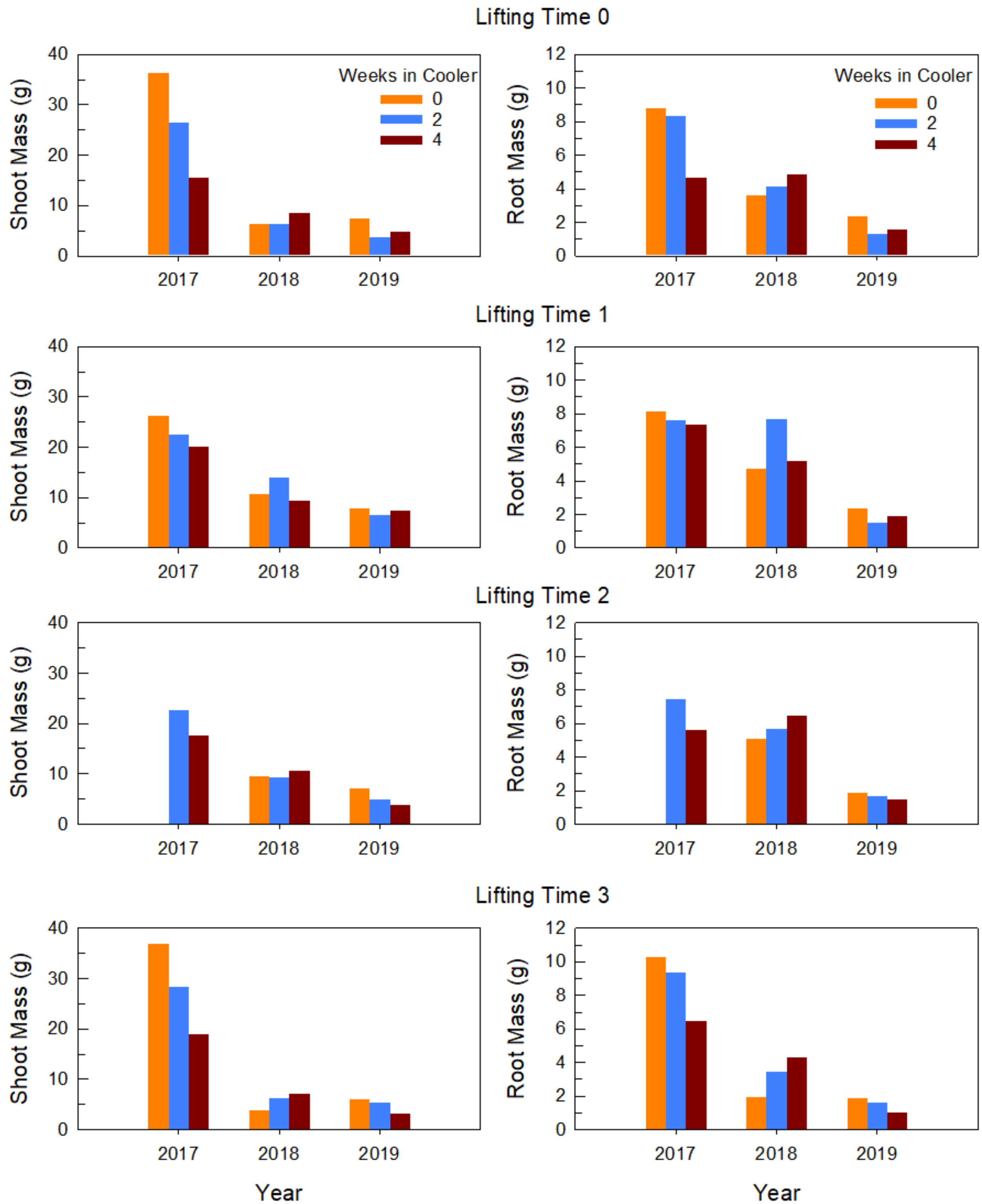

Figure 3. The final root and shoot mass (g) for seedlings outplanted for a given year (2017, 2018, 2019), comparing lifting dates (Time $_0$, Time $_1$, Time $_2$, Time $_3$) and storage period (0, 2, 4 weeks in cooler).

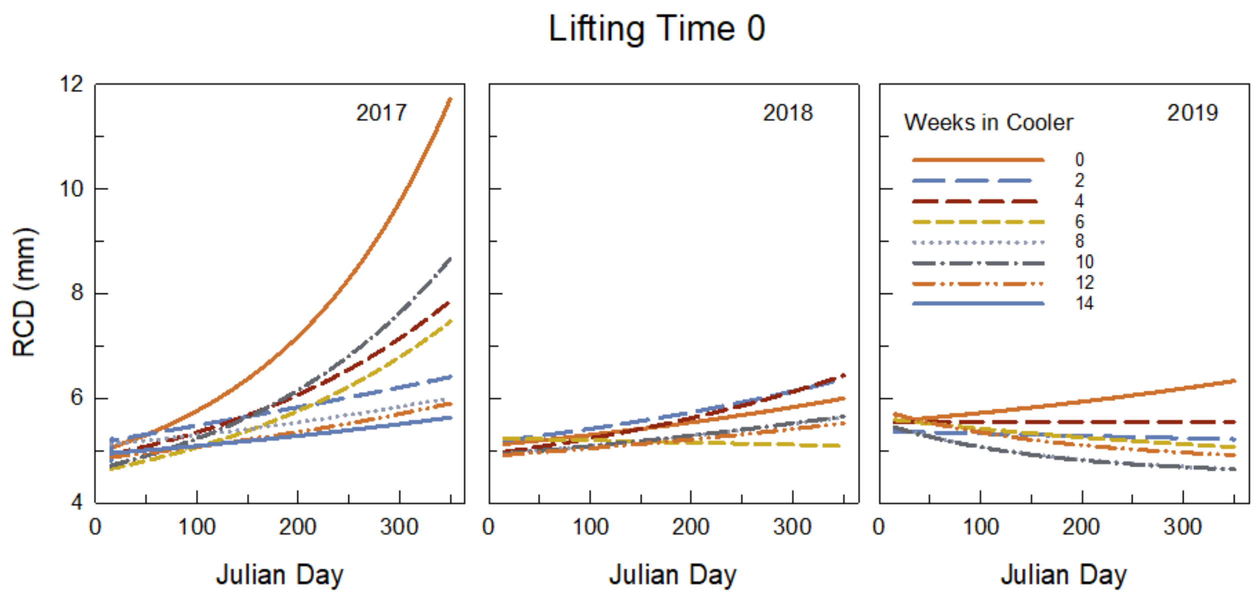

Figure 4. Exponential root collar diameter (RCD) growth models looking at the impact of storage period (weeks in cooler) of seedlings at $Time_0$ lifting period for each year.

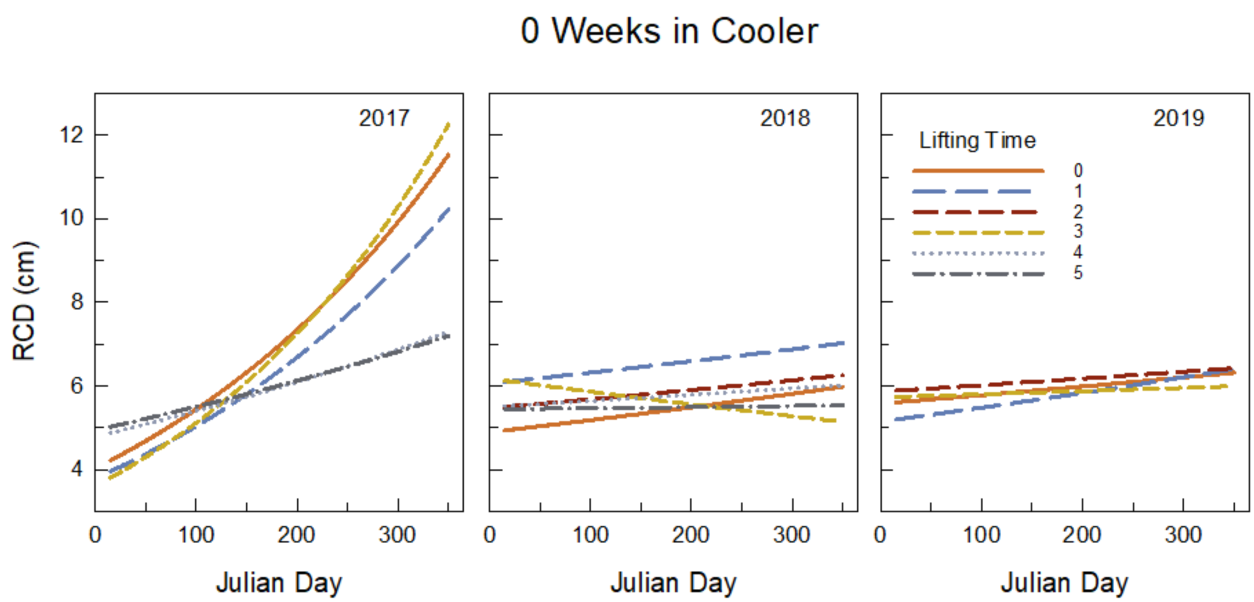

Figure 5. Exponential root collar diameter (RCD) growth models looking at the impact of lifting period ($Time_0$ - $Time_5$) on seedlings not stored in a cooler for each year.

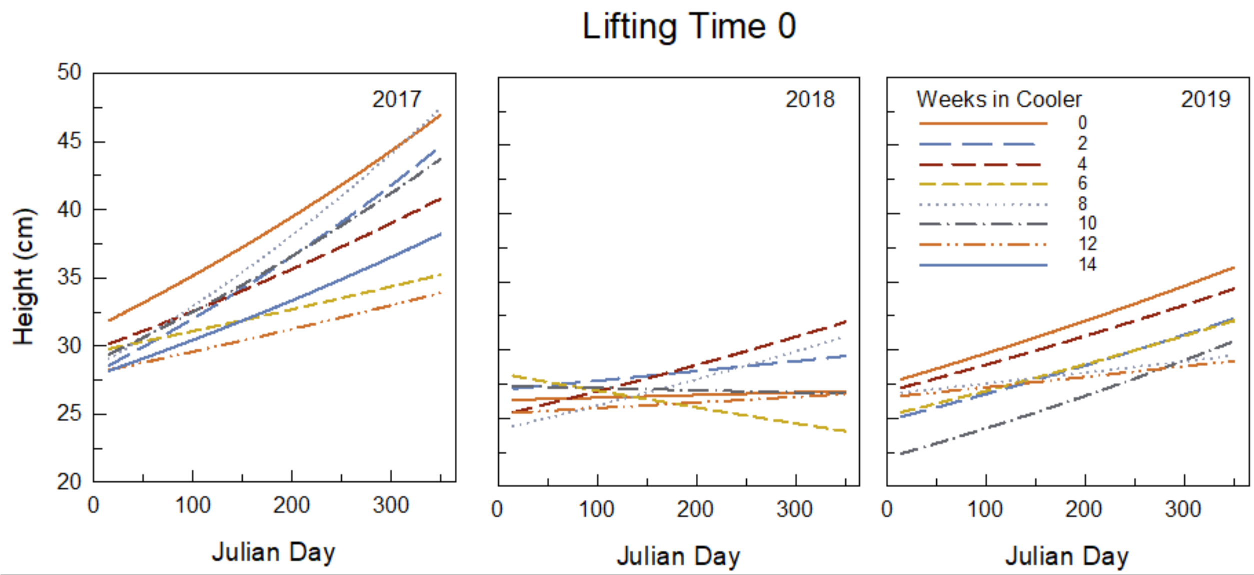

Figure 6. Exponential height growth models looking at the impact of storage period (weeks in cooler) of seedlings at $Time_0$ lifting period for each year.

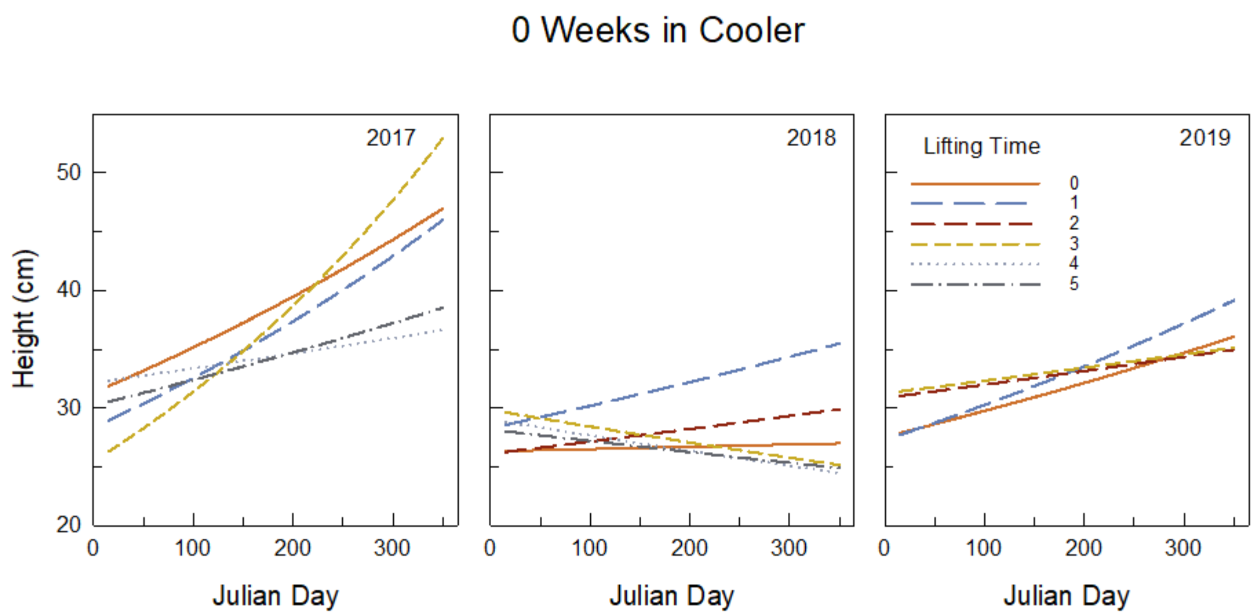

Figure 7. Height growth models looking at the impact of lifting period ($Time_0$ - $Time_5$) on seedlings not stored in a cooler for each year.

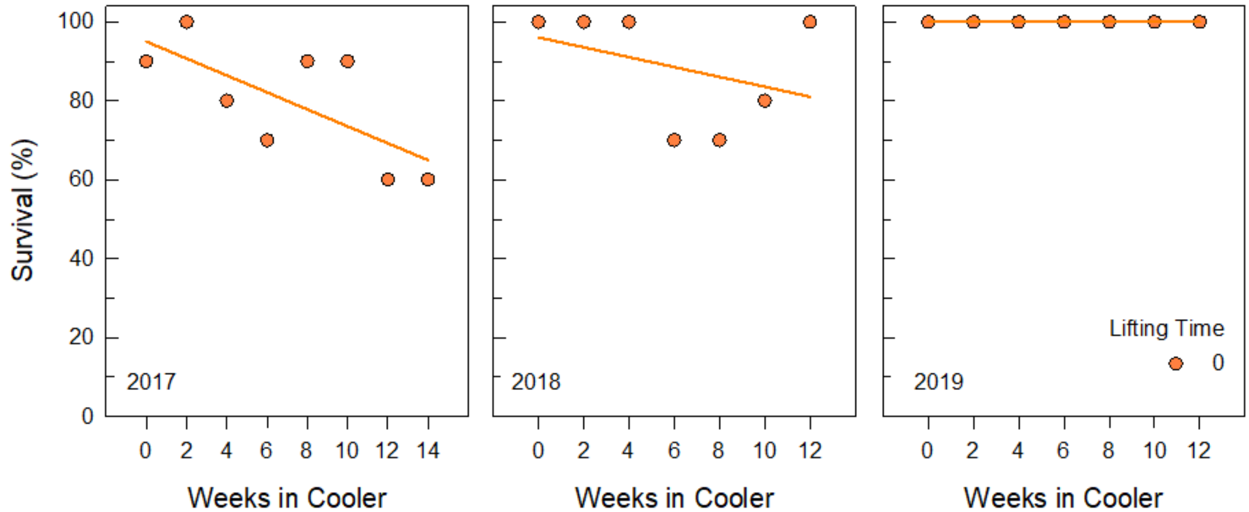

Figure 8. For each year the percentage survival for seedlings collected over two lifting periods (Time$_0$ and Time$_1$) that had been stored in a cooler for up to 12 weeks prior to being planted.

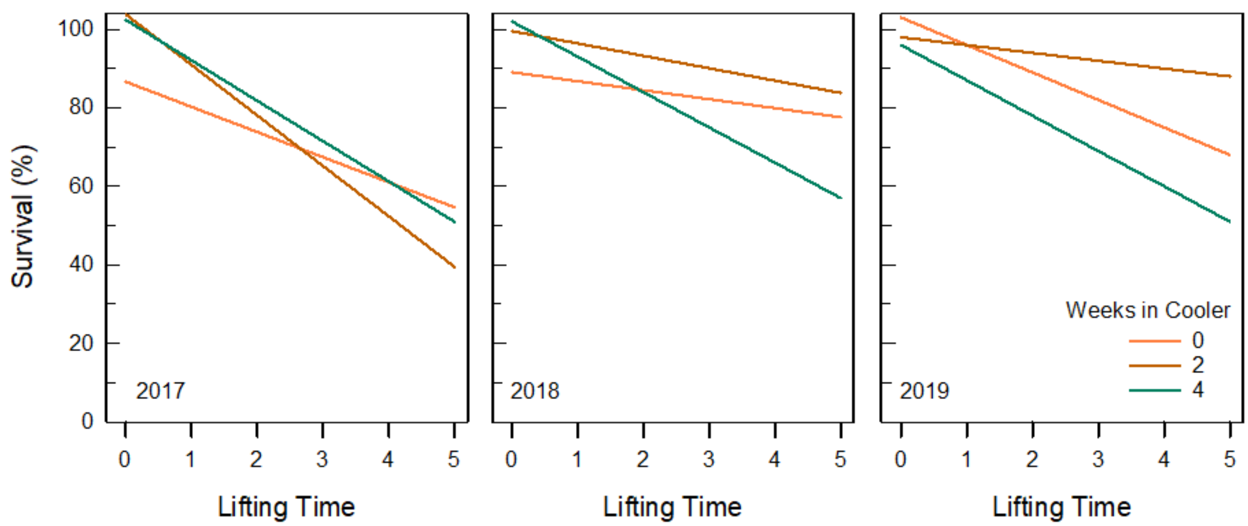

Figure 9. For each year the percentage survival for seedlings collected over 5 lifting periods that had been stored for different time periods (0, 2, 4 weeks in a cooler) prior to being planted.