

Effects of Lift Date and Seed Lot on Field Performance of Containerized Douglas-fir Seedlings

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Abstract

To determine the effect of nursery lifting dates on field performance of containerized Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) seedlings, we lifted and stored seedlings from 2 seed lots on 9 dates from late October 2019 to early March 2020. We planted the seedlings in a field test and in large pots in April 2020. All seedlings broke bud and grew vigorously. No mortality occurred in the pots, and less than 6 percent mortality occurred in the field test. Bud burst was slightly faster for seedlings lifted after mid-January than for those lifted on earlier dates. We found consistent differences in phenology and growth between seed lots, representing seeds collected from the same seed orchard at different years. Seed lot effects were confounded, however, by differences of sowing time and dormancy induction and must be studied further.

Introduction

In reforestation or afforestation programs, successful plantation establishment is not possible without high-quality seedlings. Delivering high-quality seedlings requires nursery managers to not only understand how to grow seedlings in nursery beds or in containers in the greenhouse, but also how to lift, pack, store, and deliver seedlings while maintaining quality. Although physiological and biological rationales are well established for these processes (Grossnickle et al. 2020, Haase et al. 2016, Ritchie 1984), very little research has been conducted to verify the relationship between chilling hours, subsequent seedling quality, and field performance (Haase et al. 2016). Transferring these rationales to nursery operations will empower nursery managers in making the best decisions for providing high-quality seedlings for forest regeneration programs.

Conifer seedlings require a period of chilling to complete dormancy before they resume growth and are exposed to favorable photoperiods and temperatures (Haase et al. 2016; van den Driessche 1975, 1977; Wommack 1960). Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) needs 1,200 to 2,000 chilling hours to break dormancy (van den Driessche 1977), which dictates the timeline for lifting or packing seedlings at the nursery. Although chilling can sometimes be partially achieved through storage (Lavender and Stafford 1985), Haase et al. (2016) found that seedlings lifted in early to mid-October performed poorly after outplanting. It appears that seedlings may have a threshold of natural chilling hours and photoperiod required before lifting and storage, after which seedlings are less susceptible to handling stresses. Natural chilling hours typically accrue gradually, thereby allowing seedlings to acclimate to cold temperatures. Because cold storage is an abrupt change to a uniformly cold temperature, seedlings may not have adequate time to acclimate to the sudden low temperature in the cooler. Haase et al. (2016) suggested that Douglas-fir requires a minimum of 300 to 400 hours of natural chilling before storage. Ideally, the lift date and the storage temperature are matched to avoid seedling damage before seedlings are cold hardy. Cold hardiness is defined as a minimum temperature at which a certain percentage of a random plant population will survive or sustain a given level of damage (Ritchie 1984). Seedling hardiness in the nursery relates to overall resistance to stresses associated with lifting, packing, storing, and outplanting and to survival and growth in the field (Haase et al. 2016, Landis et al. 2010).

Studies on Douglas-fir seedling quality in northwestern States and the Rocky Mountains identified seed source differences on chilling hours and cold hardiness (Landis et al. 2010, Tinus 1996). For example, most temperate conifer seedlings typically achieve peak dormancy in

October or November (Haase et al. 2016), although they do not reach maximum cold hardiness until January (Timmis et al. 1994). One guideline to determine the lifting window is the “F-date,” which is 30 to 45 days after the average date of the first fall frost (Landis et al. 2010).

Considerable information is available for dormancy, cold hardiness, lifting, and storage (Landis et al. 2010), though data specific to California are limited. Because seedling characteristics vary with seed source and nurseries have varying geographic locations and associated daylength and temperature patterns, it is useful to study specific seed sources and nursery locations. The objective of this study was to evaluate survival and performance in northern California of seedlings from two Douglas-fir seed lots lifted on several dates. The main purpose was to find an effective and efficient way to deliver the best seedlings for reforestation programs.

Materials and Methods

Seedlings and Lift Dates

Douglas-fir seeds were collected from the Sierra Pacific Industries seed orchard near Trinity Lake, California (N 40.8791, W 122.8363; 3,465 ft [1,056 m] elevation). The orchard consists of families from California seed zones 331, 332, and 521 with elevations ranging from 3,000 to 4,000 ft (915 to 1,220 m) in the central interior of northern California. Open-pollinated seeds were collected in 2009 (seed lot S503) and in 2011 (seed lot S349). Seeds were stored in the freezer until they were sown at Cal-Forest Nurseries (Etna, CA; N 41.4746, W 122.8234; 2,845 ft [867 m] elevation). The S349 lot was sown on March 12, 2019 and the S503 was sown on April 29, 2019. These sow dates were based on the nursery’s commercial operations. Seedlings were subjected to artificially reduced daylength (“blackout”) to hasten the hardening process during the first week of July and during the second week of August in 2019 for S349 and S503, respectively.

Seedlings were grown in Styroblock™ containers (412B; 5.8 in³ [95 cm³]; Beaver Plastics, Alberta, Canada) under standard nursery operational practices. Seedlings were lifted and stored on 9 dates during fall-winter 2019-2020: October 23, November 21, December 4, December 18, January 2, January 15,

January 29, February 12, and March 5. On each date, 50 seedlings from each seed lot (except December 18, 2019 when only 30 seedlings were lifted) were pulled from the containers, packed in plastic bags, placed in waxed corrugated boxes, and stored in a cooler with an average temperature of 33.3 °F (0.7 °C) until April 2020.

Environmental Monitoring and Chilling Hours

Hourly temperatures inside the storage cooler, outside at the nursery, and inside the greenhouse were obtained from Argus weather stations (Argus Control, Surrey, BC, Canada). The accumulated chilling hours were calculated from hourly temperatures inside the greenhouse for natural chilling plus accumulated chilling hours inside the storage cooler based on temperatures between 32 and 41 °F (0 and 5 °C) (Bailey and Harrington 2006, Haase et al. 2016, van den Driessche 1975) and were also calculated using the Richardson method (Richardson et al. 1974). Daylength was estimated based on Campbell and Norman (1998).

Field site air temperature and precipitation were obtained from a Remote Automatic Weather Station at Sims, CA (<https://raws.dri.edu/cgi-bin/rawMAIN.pl?-caCSIM>) located 9.3 mi (15 km) northeast of the field site. In addition, we recorded soil temperature and soil water content at a 4-in (10-cm) depth with a HOBO® datalogger (H21-USB; Onset Computer Corporation, Bourne, MA) at 60-minute intervals.

Pot Trial

After storage, we planted 10 seedlings from each seed lot/lifting date combination in two 15-gal (57-L) nursery pots (5 seedlings per pot) on April 24, 2020 (figure 1). The growing medium consisted of 80:20 peat and sawdust and pots were irrigated with a continuous liquid feed irrigation regime with all necessary nutrients. Bud burst was evaluated on May 13, 2020 based on the first six developmental stages used by Malmqvist et al. (2017) who cited Krutzsch (1973): (1) buds slightly swollen; (2) buds swollen, green to grey-green in color, bud scales still closed; (3) burst of bud scales, tips of needles emerging; (4) needles elongated to about double the bud length; (5) first spread of needles, buds now have the appearance of a painter’s brush; and (6) elongation of shoot, basal needles spread. On June 24, 2020, we harvested 5 seedlings from each seed lot/lift



Figure 1. (a) Douglas-fir seedlings growing in 15-gal (57-L) pots at Cal-Forest Nurseries (Etna, CA) on May 13, 2020. (b) Seedlings were removed from the pots, (c) washed, (d) measured, and prepared for dry mass measurements on June 24, 2020. (Photo A by Tom Jopson and Photos B, C, and D by Jianwei Zhang)

date combination, rinsed the growing medium off, and measured each for shoot height, root length, and root collar diameter (RCD). Then, root and shoot dry mass for each seedling were determined after drying them at 176 °F (80 °C) for a constant weight.

Field Trial

On April 23, 2020, we planted 40 seedlings from each seed lot/lifting date combination (except for the December 18 that only had 20 seedlings available) to a field test site (figure 2). The site is located in Shasta County on ground salvaged after the 2018 Delta Fire (N 40.9465, W 122.4698; 2,525 ft [770 m] elevation), approximately 20 mi (32 km) east of the seed orchard. The previous stand was a mixed conifer forest with ponderosa pine (*Pinus ponderosa* Douglas ex C. Lawson), Douglas-fir, white fir (*Abies concolor* [Gordon] Lindley ex Hildebrand), and California black oak (*Quercus kelloggii* Newberry).

During the post-fire salvage operation, logging debris and most slash were left on the ground. The soil is well-drained gravelly loam in a Marpa family based on USDA's Natural Resources Conservation Service (<https://websoilsurvey.sc.egov.usda.gov/App/HomePage.htm>).

We collected soil samples in June 2020 and found an average of 3.60 percent carbon (C) and 0.19 percent nitrogen (N) in the top 4 in (10 cm) soil layer and 2.72 percent C and 0.14 percent N in the 10 to 20 cm soil layer. Seedlings were planted at 6 by 6 ft (1.8 by 1.8 m) spacing. The herbicide GlyStar® (Albaugh, LLC, Ankeny, IA) was spot sprayed on April 27, 2020 at the 1.5 percent rate recommended on the label. Bud burst was evaluated on May 15, 21, and 29, 2020 using the 6 developing states previously described. Final seedling height and ground-level diameter (GLD, 1.5 in [4 cm] aboveground) were measured for all seedlings on December 2, 2020.



Figure 2. (a) Douglas-fir seedlings were outplanted to a field site following storage. Seedlings were from (b) seed lot S349 and (c) seed lot S503. (Photos by Jianwei Zhang, May 15, 2020)

Experimental Design and Data Analyses

The design in the nursery, pot trial, and field trial was a split-plot randomized complete block design with lifting date as the main plot effect randomly assigned to each of two (pot trial) or four (field trial) blocks and two seed lots as the subplot effect assigned to the main plot. The analyses were performed using SAS PROC MIXED (SAS Institute Inc. Cary, NC). For each variable, residuals were examined to ensure that statistical assumptions of normality and homoscedasticity were met. If not, a natural log transformation was applied. Multiple comparisons among treatments were conducted for least squares means using the Tukey-Kramer test by controlling for the overall $\alpha = 0.05$. The full statistical model is:

$$y_{ijkl} = \mu + \alpha_i + \varepsilon_{1ik} + \beta_j + \alpha\beta_{ij} + \gamma_k + \varepsilon_{2ijkl} \quad [1]$$

Where y_{ijkl} is the dependent variable summarized for the i th lift date, j th seed lot, and the k th block, μ is the overall mean, α_i and β_j are the fixed effect of the i th lift date ($i = 1, 2, \dots, 9$) or j th seed lot ($j = 1$ and 2), γ_k is the random effect of the k th block ($k = 1, 2, 3$, and 4), $\gamma_k \sim N(0, \sigma_B^2)$ and ε_{1ik} is an experimental error to test main plot effect, ε_{2ijkl} is an experimental error to test subplot effect and other terms, $\varepsilon_{1ik} \sim iid N(0, \sigma_{e1}^2)$, and $\varepsilon_{2ijkl} \sim iid N(0, \sigma_{e2}^2)$.

For the seedling phenology of budburst, we calculated a bud developmental index for each experimental unit:

$$BDI = \sum n_i S_i \quad [2]$$

where n_i is a percentage of seedlings that are in developing Stage i (S_i). BDI values range between 0 and 6 matching the stage categories. We modelled developmental trends with conventional chilling hours prior to left date as the independent variables (linear and/or quadratic term) and BDI as a dependent variable using SAS GLM procedure. The statistical model is adapted from the full model [1] above.

Results

Environmental Conditions and Chilling Hours

From October 2019 through April 2020, air temperature outside the greenhouse averaged 44.2 °F (6.8 °C) and daylength ranging from 9.1 to 12.7 hours (figure 3). Chilling hours based on the conventional method reached 172 when the first seedlings were lifted on October 23, 2019 and 1,752 when the last seedlings were lifted on March 5, 2020 (figure 4). Except for the first two lifting dates, the Richardson's method yielded fewer chilling hours than the conventional method. By counting chilling hours completed in the storage cooler, seedlings from all lift dates achieved more than 2,500 hours.

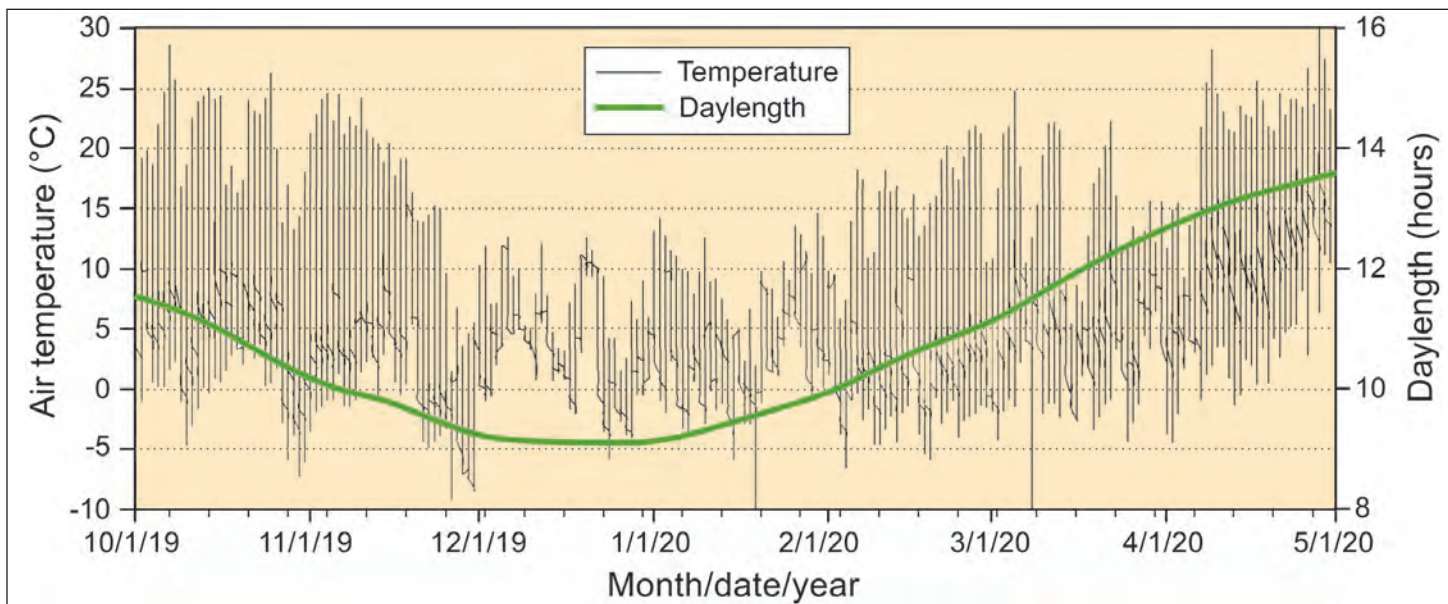


Figure 3. Hourly air temperature and estimated daylength (based on Campbell and Norman 1998) from October 1, 2019 to March 31, 2020 at the nursery.

Pot Trial

Within about 3 weeks after seedlings were potted, most had broken bud and started elongation (figure 5). Using the chilling hours as a quantitative independent variable, we found that the natural chilling hours prior to lifting and storing seedlings significantly affected bud burst phenology, both linearly and quadratically (figure 6). The latest lifted seedlings with more natural chilling hours developed

more quickly than the earlier lifted seedlings, especially for the S503 lot. The S349 lot showed a similar trend throughout all lift dates and developed significantly faster than the S503 lot ($P < 0.001$) (figure 6).

All seedlings in the pot trial survived and grew vigorously (table 1). Except for root length and root mass, the S349 seedlings grew significantly larger than the S503 seedlings. All measured variables varied

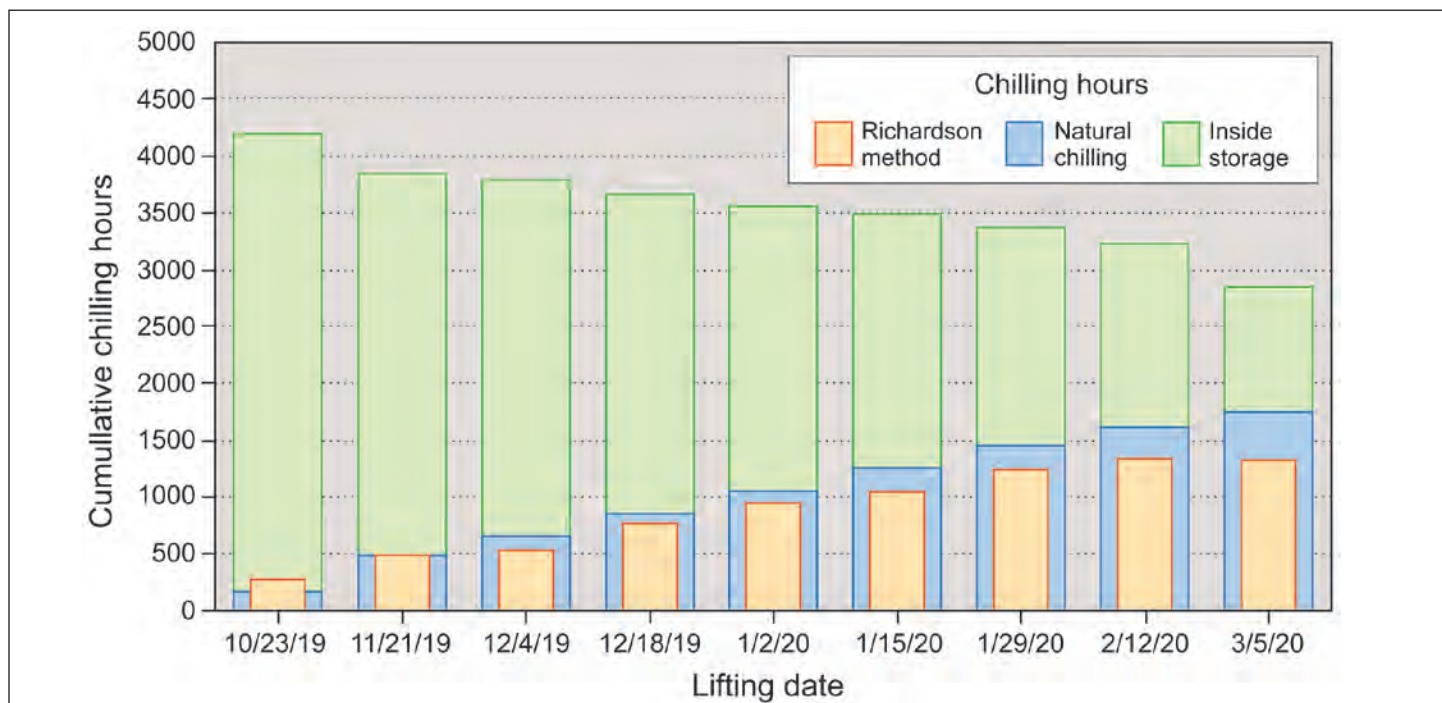


Figure 4. Accumulated natural chilling hours at each lift date calculated using the conventional method (32 to 41 °F [0 to 5 °C]) and the Richardson method (Richardson et al. 1974) plus hours in storage varied by lift date.



Figure 5. Douglas-fir seedlings bud burst varied between two seed lots (S349 and S503) and among lifting dates: (a) October 23, 2019, (b) November 21, 2019, (c) December 18, 2019, (d) January 29, 2020, and (e) March 5, 2020. (Photos by Tom Jopson, May 13, 2020).

significantly among lifting dates with best performance for those lifted in the last 3 dates. Seedling height and root length showed significant interactions between lifting date and seed lot. Regardless of seed lot or lift date, root and shoot mass were strongly correlated ($r^2=0.94$, $P<0.001$) with a R:S ratio of about 0.30 (figure 7).

Field Experiment

Precipitation was low and temperatures were warm compared with normal for March and April 2020 (figure 8). After seedlings were planted on April 23, it rained about 5.35 in (136 mm) from May 10 to May 18 resulting in sufficient soil moisture by the middle

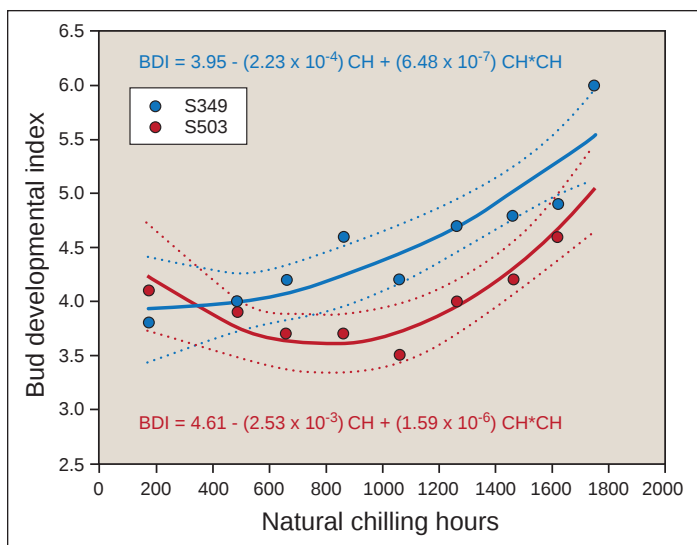


Figure 6. The relationship between bud developmental index (BDI) and chilling hours at the time of lift varied by seed lot for Douglas-fir seedlings grown in the pot trial at Cal-Forest Nurseries. Dashed lines are the 95% confidence intervals for their respective regression lines.

of June (figure 8). Bud burst differed significantly between seed lots ($P < 0.001$). Similar to the pot trial, the S349 seedlings developed faster than the S503 seedlings, especially for seedlings from the later lift dates that had had substantially more natural chilling hours (figure 9). Chilling hour significantly affected bud burst on May 15 and 21 (figures 9a and 9b). By May 29,

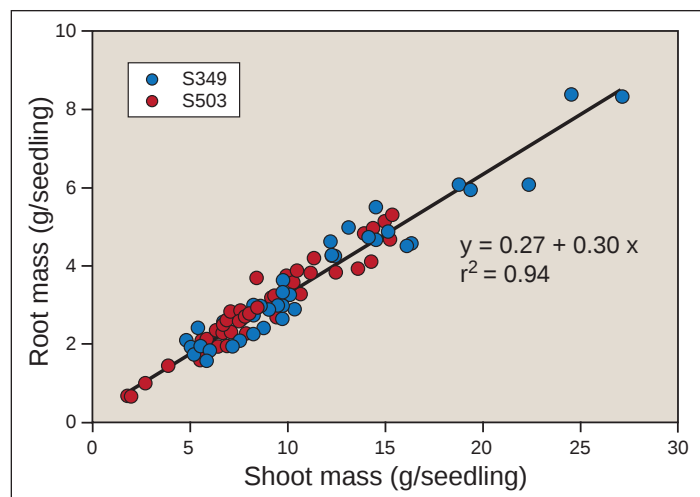


Figure 7. There was a strong correlation between root and shoot dry mass for Douglas-fir seedlings from two seed lots and 9 lift dates 2 months after transplanting into pots.

however, most seedlings had developed beyond our evaluation stages (figure 9c), when neither seed lot nor chilling hour continued to affect bud burst phenology.

Survival was 100 percent on May 29, 2020. By the final measurements, 3.2 percent of seedlings from the S503 lot and 5.3 percent seedlings from the S349 lot died across the lift dates. The difference

Table 1. Mean morphology of Douglas-fir seedlings grown in 15-gal (57-L) pots for 2 months. Seedlings were from two seed lots lifted on 9 different dates. (Note: Seedlings from S349 on January 2 lift date were not sampled.)

	Lift date	Seed lot	Height (cm)		Root collar diameter (mm)		Root length (cm)		Shoot mass (g)		Root mass (g)		Total mass (g)	
			S349	S503	S349	S503	S349	S503	S349	S503	S349	S503	S349	S503
	Oct 23		35.7	42.2	4.8	5.2	38.2	42.2	5.5	7.7	2.2	2.7	8.5	10.5
	Nov 21		44.2	34.0	6.2	4.2	39.4	36.6	10.3	5.2	3.9	2.0	14.2	7.2
	Dec 04		41.7	45.7	5.5	5.8	35.9	37.9	7.8	9.6	2.7	2.8	10.5	12.4
	Dec 18		50.3	41.4	5.7	4.9	36.9	36.1	11.2	6.7	3.1	2.3	14.3	9.0
	Jan 02		-	43.2	-	5.7	-	37.0	-	8.6	-	2.9	-	11.4
	Jan 15		49.8	39.6	6.1	5.4	35.6	34.4	11.2	7.4	3.1	2.7	14.4	10.2
	Jan 29		49.5	44.7	6.3	6.3	40.4	33.8	13.1	9.9	4.5	3.4	17.6	13.4
	Feb 12		52.8	41.4	6.8	5.7	41.0	38.9	13.8	9.7	4.2	3.4	18.1	13.1
	Mar 05		53.3	52.3	6.9	6.6	34.1	39.7	13.5	11.9	4.4	4.1	17.8	16.1
	Mean		47.2	42.7	6.0	5.5	37.7	37.4	10.8	8.5	3.5	2.9	14.4	11.5
Probabilities for treatment effects	Lifting date		0.001		0.004		0.029		0.030		0.034		0.049	
	Seedlot		0.004		0.025		0.771		0.016		0.054		0.018	
	LD*S		0.013		0.120		0.004		0.273		0.590		0.451	

Conversions: 1 cm = 10 mm = 0.39 in; 1 g = 0.035 oz

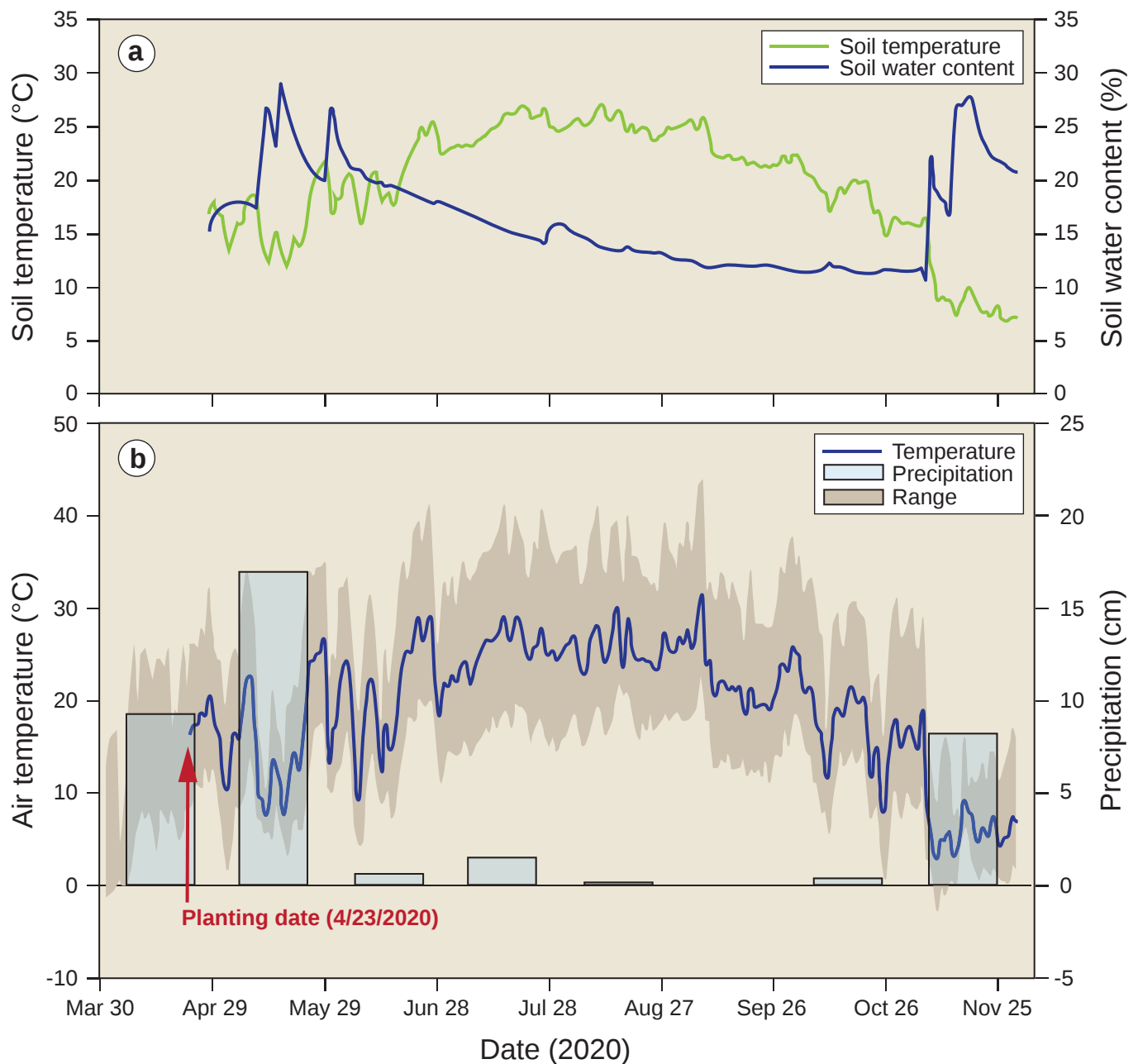


Figure 8. (a) Soil temperature and water content at the field trial site and (b) air temperature and precipitation at a nearby weather station were used to understand seedling responses during the study.

between the seed lots was significant ($P < 0.001$) but not between lift dates ($P > 0.50$).

There was a significant interaction on both height and GLD between seed lot and lift date ($P = 0.004$). Overall, seed lot S349 tended to have greater height and diameter growth with few exceptions (figure 10). Because lift date had little influence on height and GLD, we did not model the chilling hour effect on these growth variables.

Discussion

The purpose of this study was to address some concerns from regeneration foresters on poor survival of Douglas-fir plantings in southeastern Oregon and northern California. Poor seedling quality may be one of possible causes, but the specific reasons for poor performance often cannot be determined. We focused on two factors that may influence seedling quality: (1) lifting date and associated effects on seedling physiology and (2) seed lot.

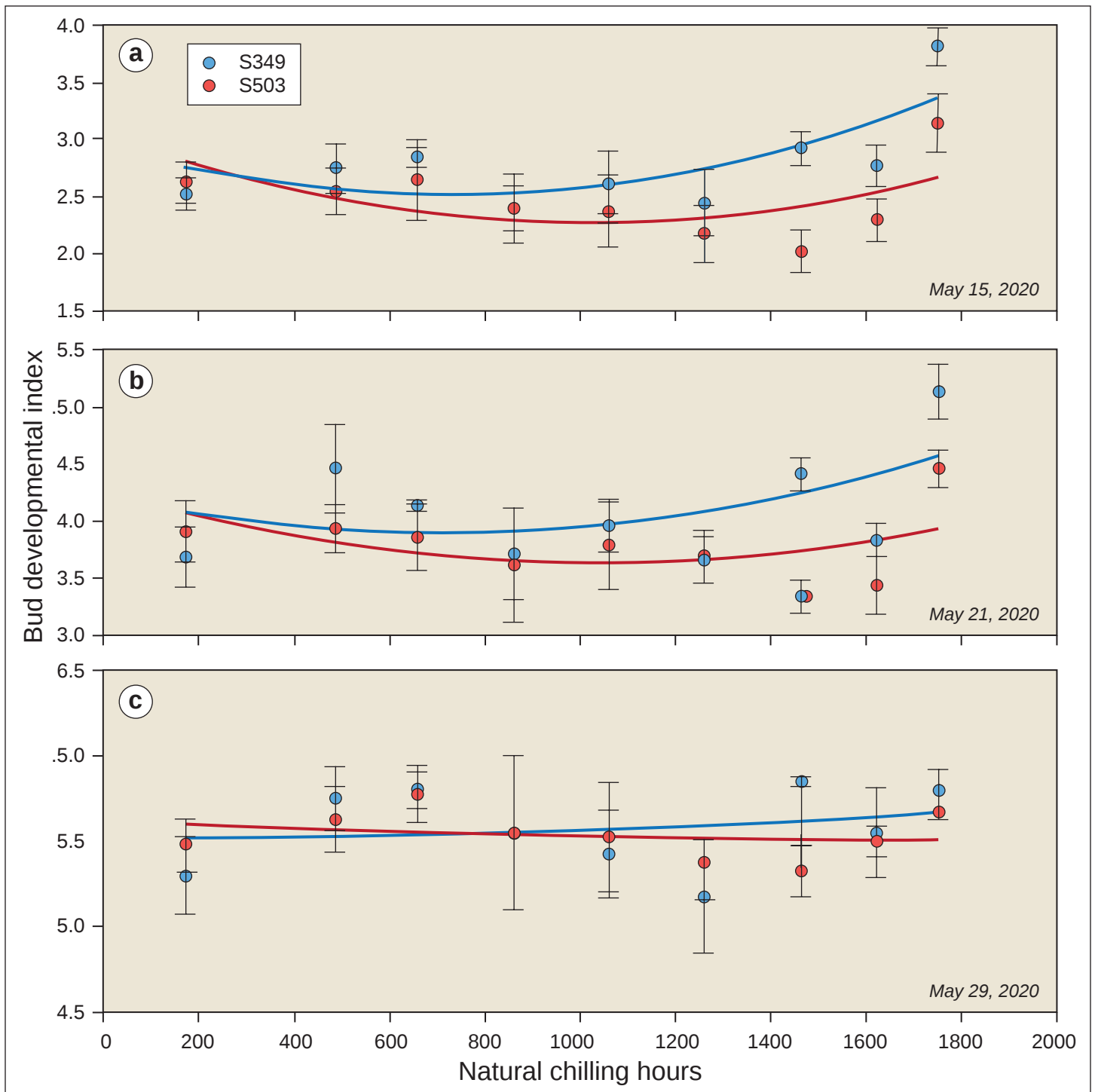


Figure 9. The relationship between bud developmental index (BDI) and chilling hours at the time of lift for Douglas-fir seedlings outplanted to a field site near Redding, CA varied between seedlots on (a) May 15 and (b) May 21 but was no longer evident on (c) May 29 when most seedling buds had developed beyond the index phases.

Chilling Hour Effect

Results from this study indicate that lift dates from late October to early March did not affect seedling survival or growth in the field. Seedlings lifted in February or March, however, could be negatively affected during years with warmer winter conditions in the nursery that cause seedlings to break bud early.

Nursery location is an important factor because each site has its own photoperiod and temperature patterns which affect seedling physiology and phenology (Campbell and Sugano 1975, Ritchie 1989).

Haase et al. (2016) reported that Douglas-fir may benefit from a minimum of 300 to 400 natural chilling hours for optimal stress resistance before being

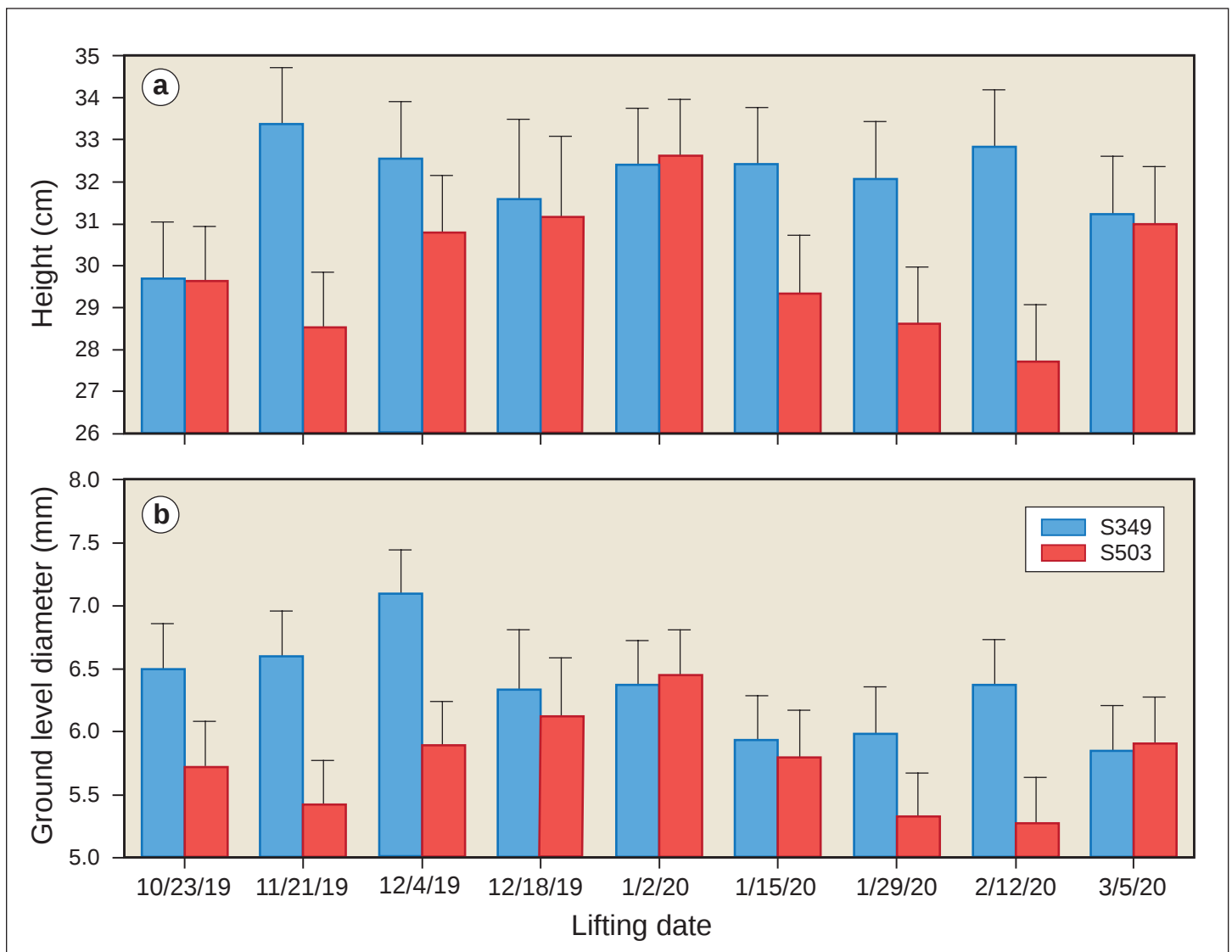


Figure 10. Average (a) height and (b) ground-level diameter of Douglas-fir seedlings after one growing season in the field trial site near Redding, CA. Seed lot S349 tended to have more growth across lift dates.

lifted and stored for completing a threshold of 1,200 chilling hours (Ritchie 1984; van den Driessche 1975, 1977). If these two minimum requirements are true, the first packing date in our study did not meet the former with only 172 chilling hours calculated with the conventional method and 185 with the Richardson method (figure 4). Nonetheless, all seedlings performed well in both the pot and field trials (figures 7, 9, and 10).

In the pot trial, higher root mass was found in seedlings from the later lifting dates compared with those from the earlier dates although shoot-root ratios were unaffected (figure 7). These results differ from studies that found later lifted seedlings were exposed to higher temperatures which stimulated

bud elongation and reduced root growth (Nadel et al. 2020, Ritchie and Dunlap 1980). We believe this discrepancy is due to our study being conducted in the comparatively cooler climate of Etna, CA where considerably more natural chilling hours were accumulated for seedlings lifted from late January to March (figure 4).

Douglas-fir seedlings grown under controlled conditions (Ritchie 1984, van den Driessche 1977) or in a field test with older saplings (Bailey and Harrington 2006) tend to have earlier bud burst with chilling hours beyond the minimum 1,200 hours. This phenomenon was observed in the current study if we only count the natural chilling hours, but not when we include the supplemental chilling hours in the storage cooler.

Seed Lot Effect

Both seed lots were collected from the same seed orchard but in different years. Genetically, they should have been very similar. Douglas-fir is a monoecious species with both male and female cones occurring on the same tree and other pollens coming from surrounding trees. The two seed lots may have been influenced by climatic conditions during their parental reproductive periods. For the S503 lot (collected in 2009), precipitation in 2008 and 2009 was 435 mm and 628 mm, respectively. For the S349 lot (collected in 2011), precipitation in 2010 and 2011 was 1039 mm and 750 mm, respectively. Temperature conditions were similar. Seed sources or families from mesic habitats grow faster than those from xeric sites in common garden studies (Wright 1976). Thus, seeds produced in wetter years may yield larger seedlings than seeds produced in drier years which may explain the seed lot differences in our study.

Differences among seed lots may also be attributed to sowing dates and subsequent blackout timing in the nursery. The earlier sowing and blacking out for S349 seedlings affected photoperiod and dormancy induction. Unfortunately, these confounding effects could not be avoided. Notably, however, the earlier budbreak of S349 seedlings carries the risk of late frost damage (Malmqvist et al. 2018). Also, fast aboveground growth may deplete soil water and cause mortality in the late growing season (Darychuk et al. 2012) on droughty sites. Because our field test was planted relatively later than the usual spring planting time at this elevation, seedlings were not damaged by frost. During the late growing season when high temperatures and drought occurred, S349 seedlings had higher mortality (5.3 percent) than S503 seedlings (3.2 percent) in the field test, though these numbers are still quite low. In an adjacent site, an observational fall planting test (September 2019) had 63 percent mortality of S349 seedlings compared with 10 percent mortality of S503 seedlings. These results require additional research to better understand differences between the seed lots.

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