



Effects of seed sources and nursery cultural practices on field performance of containerized Douglas-fir seedlings

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Abstract

Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*) is an important species in the Pacific Northwest including California forests. Due to the increasing need for reforestation in this region after widespread disturbances related to changes in climate (i.e., drought, megafires, beetle mortality), it is necessary to examine the factors that contribute to performance and survival of planted seedlings in reforestation projects. While most conifer planting in northern California is done in spring, fall planting is also an alternative practice used. With the recent increase in demand of seedlings for reforestation projects beyond which the current infrastructure is capable of, particularly in spring, expanding the fall planting season has potential to mitigate this and constraints to the spring labor force. Here, we studied the first-year performance of both spring and fall planted Douglas-fir seedlings for different seed sources and nursery cultural timings at a single site in northern California. We found that the fall planting can be successful in October or November, while planting earlier requires immediately favorable temperature and soil moisture conditions. Later sowing and blackout regimes also resulted in increases in height growth and bud development while also reducing damage due to spring freezes. For spring planting, early sow and blackout resulted in earlier bud break, while later sow, blackout, and lift dates benefited the first-year growth of height and diameter.

Keywords Douglas-fir reforestation · Dormancy induction · Fall and spring plantings · Nursery practices · Seed source · Seedling cultures

Introduction

High quality seedlings are necessary for successful reforestation programs. Recent intensified mega disturbances such as wildfires and insect outbreaks have damaged many forest lands and require rapid widespread restoration (SAF 2022). As trees are one of the most

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powerful tools in mitigating the climate crisis, the planting trillion tree campaign is one proposed effort targeted to keep temperature rise below 1.5 °C (<https://www.plant-for-the-planet.org/trillion-trees/>). To achieve this goal, many seedlings are required. Recent evaluations have showed that current seed demands surpass the available seed stores and there is a lack of sufficient nursery facilities to produce large amounts of seedlings in the United States (Fargione et al. 2021). For example, between historic timber harvests and recent increases in disturbance related mortality on private timberland, there is potentially a demand of 28,000 to 44,000 ha of reforestation annually on private land in California alone (Stewart 2022). Therefore, continuing previous research in improving techniques for optimizing success and survival of forest plantations across various outplanting sites is essential. One species that has commercial and ecological value in northern California is Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*) that is interplanted with ponderosa pine (*Pinus ponderosa* Lawson & C. Lawson), incense-cedar (*Calocedrus decurrens* (Torr.) Florin), and other suitable coniferous species as mixed species plantations.

At the recent Joint Annual Meeting of the Forest Nursery Association of British Columbia and the Western Forest and Conservation Nursery Association (five US western States and one Canadian Province) in fall 2019, nursery practitioners and foresters came to a consensus on five topics centered around maximizing plantation success, one of which that strongly relates to the focus of this paper was, “what are the best cultural hardening practices to maintain physiological quality” (Grossnickle et al. 2021). In addition to taking the desired outplanting site conditions into account, nursery cultural practices are specific depending on the desired species, seed source, stock type, outplanting window, and include seedling lift, storage and handling, and hardening techniques (Grossnickle et al. 2021). Regardless of cultural practices used by nurseries, timing at each stage of seedling development and handling can influence ultimate success once planted in the field.

Numerous studies have explored the relationships between nursery practices and outplanting success for Douglas-fir (Jenkinson et al. 1993; Dunsworth 1988; Ritchie 1984; Sloan 1989; Taylor et al. 2011; Sorensen 1996; Kiiskila et al. 2023; Haase et al. 2006; Arnott 1975). However, information from other regions may not be directly transferable to California. A previous study evaluating cultural regime, packing date, and seedlot effects on field performance of containerized Douglas-fir seedlings in northern California showed the consistent differences in phenology and growth between seed sources and between the differences of sowing time and dormancy induction (Zhang et al. 2021). Unfortunately, the effects of culture regime and seed source were confounded for that previous study, which warranted a further study to differentiate genetic (seed source) from physiological effect. This information can help nursery managers and reforestation foresters precisely identify appropriate seed lot and cultural regime for a specific reforestation site.

In an assessment of reforestation needs in the US, another limiting factor across the reforestation pipeline was labor shortages (Fargione et al. 2021; SAF 2022). Although planting times are highly dependent on species and site-specific conditions, one potential way to address this issue in northern California where the primary planting season is spring, is to increase utilization of fall planting when possible. A fall-planting observational trial indicated that an appropriate selection of seed source and/or culture regimes could yield great seedling survival and growth (Zhang et al. 2021). In fact, fall planting is commonly used in areas of higher elevations, when limited access and soil temperatures make it difficult to plant during the spring (Landis et al. 2010a, b, Ch. 6). In a review of the usage of fall planting for reforestation, previous research has found that generally, the survival of fall planted Douglas-fir is comparable to spring planting when drought conditions were avoided (Grossnickle and MacDonald 2021). Fall planting success is also tied to

suitable hardening practices, which have different requirements compared to spring seedlings while simultaneously ensuring that planting is done during favorable environmental conditions (Grossnickle and MacDonald 2021). Due to high demand of reforestation on wildfire-burned land in the western United States, expanding this alternative planting season has potential for the following reasons. First, it mitigates the labor shortage during regular spring planting season and limited seedling overwinter storage space (Fargione et al. 2021; SAF 2022). Second, as roots of Douglas-fir seedlings will grow when temperatures are above 5 °C and reach the rapid root growth near 20 °C (Lavender and Overton 1972; Lopushinsky and Max 1990), soil temperature is usually still good for root growth in the early winter and is not too cold in late winter due to snow cover (Zhang, unpublished data). Third, soil water content is often limiting factor in seedling establishment and growth in the Mediterranean climate in California (Show 1930; Powers and Ferrell 1996). Precipitation occurs in the winter and seedlings planted in fall will not face soil water shortage unless they are planted too early. Last, seedlings are able to grow roots and sometimes shoots even if the site is not accessible in the early spring, although spring frost damage is a risk (Malmqvist et al. 2018).

The primary goal of this study was to fill the information gaps left over from the previous study (Zhang et al. 2021) by separating the effects of seed source from cultural regime (sowing date and dormancy induction) on field performance of Douglas-fir seedlings planted in fall and spring in Northern California. For fall planting specifically, we wanted to examine the interactions between seed source and sowing regimes across three separate planting dates. Additionally, we aimed to determine if lift and packing dates interacted with seed source and cultural regime on seedling performance in spring plantings.

Materials and methods

Seeds and seedlings

Douglas-fir seeds were collected from mother trees representing 3 elevational seed zones within one breeding zone in northern California by Sierra Pacific Industries. Thereafter, these seed sources are referred as three seed lots (Fig. 1). Seed lot S521 consists of seeds collected from some mother trees in a vicinity of the Sacramento River Canyon (Lat. 41.1237, Long. – 122.2916, Elev. 1200 m) in 1997. Seed lots S62L and S63M were collected from Gazelle Seed Orchard (Lat. 41.5527, Long. – 122.5528, Elev. 860 m) in 2020 and represented low (~760 m) and middle (~1000 m) elevations of the seed zone, respectively. Seeds were dried to 7% moisture, placed in sealed plastic bags, and stored at -16 °C in the Nursery's storage freezer, Siskiyou Seed Co., until they were sown in the nursery.

Seedlings were grown in Styro 6 containers (5 cm diameter and 15 cm long, 250 ml volume, Beaver Plastic, Alberta, Canada) at Cal-Forest Nurseries, Etna, California (Lat. 41.4746, Long. – 122.8234, Elev. 867 m). To examine differences between nursery culture regimes, all 3 seedlots were sown and had blackouts started and ended on two different schedules in 2021. Schedule one is called the April Sow; seeds were sown on April 2 and seedling blackout from July 6 to August 1. Sow date was May 6 on schedule two (called the May Sow) and the blackout was from August 27 to September 24. Seedlings used in this study were only a nominal portion of seedlings raised for reforestation with standard seedling production in the nursery beds. One or two seeds sown per container yielded almost 100% germination and thinning was conducted for growing one seedling per container.

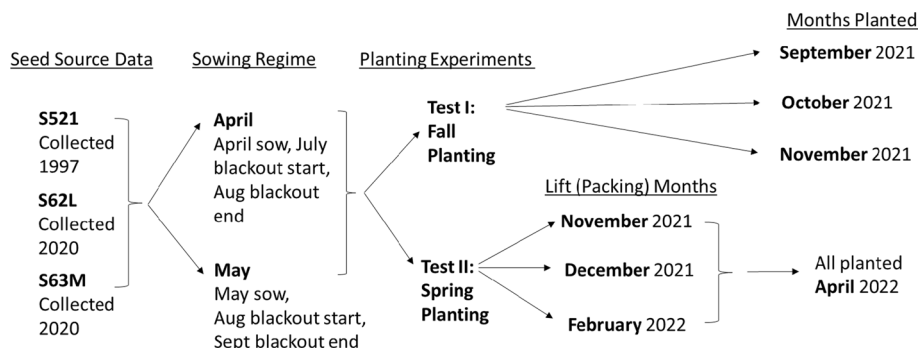


Fig. 1 Douglas-fir seeds and culture regimes to raise seedlings in a commercial nursery for reforestation and also for two tests in this study. Fall planting tested field performance among three planting months and Spring planting tested field performance among three packing months, both with three seed lots and two culture regimes

There was no evidence of negative effect of storage duration on seed germination and seedling vigor. For this study, seedlings were stored on greenhouse benches before they were planted in the fall experiment or packed and stored for the spring planting experiment.

Field tests

Site description

The field site is located in Trinity County, California (Lat. 40.8038, Long. – 122.8703, Elev. 939 m). Climate is a typical Mediterranean climate with hot, dry summer and mild, wet winter. Recent climate estimates using Trinity Camp RAWs (1996–2022) has average temperatures typically peaking in July above 24 °C with December and January averaging below 3–4 °C (Table S1; <https://raws.dri.edu/cgi-bin/rawMAIN.pl?caCTRI>). According to the USDA soil survey (<https://websoilsurvey.sc.egov.usda.gov>), the soil is primarily well drained loam in the Forbes family and well drained gravelly loam in the SoulaJule family.

A natural stand, initiated from a wildfire about 100–115 years ago, consisted of natural mixed-conifers dominated by Douglas-fir, with a mix of ponderosa pine, incense-cedar, sugar pine (*Pinus lambertiana* Douglas), and California black oak (*Quercus kelloggii* Newberry). About 10 ha was operationally logged in late 2020 and remained fallow for the 2021 growing season. Logging was mostly mechanical harvesting and predominantly whole tree logged, aside from the hand falling of oversized trees. Logging debris was scattered as a result of logging operations. All landing piles were burned, which were avoided when research blocks were installed. No additional slash mitigations took place.

The understory consisted of dogwood (*Cornus* spp.), California hazelnut (*Corylus cornuta* var. *californica* (A. DC.) Sharp), Pacific madrone (*Arbutus menziesii* Pursh), deerbrush (*Ceanothus integerrimus* Hook. & Arn.) and bigleaf maple (*Acer macrophyllum* Pursh). Perennial grasses and bracken fern (*Pteridium aquilinum* (L.) Kuhn) were also present and targeted by a pre-harvest treatment. A pre-harvest herbicide treatment was applied in late June of 2018 that consisted of a foliar and broadcast of 2% Accord XRT II (glyphosate) (Corteva Agriscience), 2% Rotary 2SL (imazapyr, Alligare, Inc.), and 3% Super Spread MSO (Wilbur-Ellis Co, LLC), a common prescription for site preparation

for reforestation. There was also a follow-up hack and squirt in the fall of that year with 100% imazapyr 4SL (Alligare, Inc.) to control larger hardwoods. These applications were performed prior to logging operation and planting because it allows for treating nearly all hardwoods, shrubs, and herbaceous competing vegetation prior to planting, rather than wait for various responses by the plants after site disturbance. This approach results in less pesticide applications needed to establish new plantation and eliminates having to spray around the seedlings, which can damage them.

Test I: fall planting

A complete randomized block design was used like that of the previous outplanting trial (Zhang et al. 2021). Forty seedlings from each of 2 culture regimes by 3 seed lots were planted across 4 blocks on September 21, October 14, and November 8, 2021 (Fig. 1). Each block had randomly assigned rows of 10 seedlings from each of the 18 treatment combinations (experimental units) planted at a 1.8×1.8 m spacing. There was a total of 720 seedlings planted across the 4 blocks (180 seedlings per block). Seedlings were planted by industry professional foresters. To mitigate any potential animal damage, all seedlings had rigid mesh seedling (Quadel Industries, Coos Bay, Oregon, USA) protector tubes installed.

Competing vegetation was hand weeded when feasible on these blocks. Then, a release treatment of 2% glyphosate and 0.5% MSO was applied to all blocks along with hand weeding of competition directly against each seedling in June of 2022.

Test II: spring planting

Forty seedlings from each seed lot and sow date regime were pulled from containers ('lifting') and packed in separated plastic bags on November 12, 2021, December 23, 2021, and February 22, 2022 (Fig. 1). This yielded 18 combinations (treatments) with 2 culture regimes by 3 seed lots by 3 packing dates. Seedling bags were immediately placed in the waxed-corrugated boxes and stored in a cooler with an average of 0.7 °C (− 0.3–1.4 °C) from November of 2021 to April of 2022.

Field test blocks were laid out with pin-flags before a pre-plant site prep broadcast of 3.75% Cleantraxx, 2% Roundup Custom, 0.6% Transline, and 1% MSO with total 13.7 L per ha was applied on March 18, 2022. Then, ten seedlings from each of 18 experimental units were planted per block (4 blocks total) on April 1, 2022, similar to the Fall planting test design. Seedlings were planted in Spring by a seasoned contractor crew of 3 under immediate supervision of industry professionals. Further competing vegetation control treatments were applied alongside the Fall planting blocks.

Environmental monitoring

For all stages of seedling development, hourly temperatures were obtained for each environmental stage (open air, greenhouse, and seedling storage cooler) from Argus weather stations (Argus Control, Surrey, British Columbia, Canada) with means from the logged readings every 15 min. These data were used for calculating the natural chilling (0–5 °C) hours for each of the three lift dates of the spring test and combined with outplanting site conditions for the fall test (van den Driessche 1975, Bailey and Harrington 2006). The natural chill hours were then added to the cold storage hours for a cumulative number of chill hours.

Field site hourly soil temperature and water content at a depth of 10 cm was recorded with a HOBO H21-USB datalogger, as well as recording hourly air temperature and relative humidity using a HOBO MX1101 datalogger. Both dataloggers were installed at the site (Fig. S1) and began collecting data on November 30, 2021. Since the dataloggers were installed after all of the fall planting dates, daily weather data from August 1 to November 30, 2021 were downloaded from the nearest RAWS weather station from Trinity Camp (<https://raws.dri.edu/cgi-bin/rawMAIN.pl?caCTRI>). Then, these data were calibrated with the relationships between RAWS and HOBO from December 1, 2021 to October 31, 2022 using regression equations. Study site chilling hours (0–5 van den Driessche 1975) were also summed. It must be noted that all following meteorological data presented are representative only of conditions for the experimental area and nursery and not of the overall region. Late spring freeze events can cause damage to seedlings and occur when temperatures drop below freezing after bud development has begun (Malmqvist et al. 2018; Chamberlain and Wolkovich 2021). We identified freeze events as being at least 4 consecutive hours under below -2°C .

Measurements

Phenology—Bud development for every seedling was assessed on May 3, May 16, and May 24, 2022, across all measurement blocks. Phenology was categorized on a scale from 1 to 6 similar to that used by Malmqvist et al. (2017), with 1 representing dormant buds and 6 representing fully emerged needles and elongated shoot. Additional yes/no phenology categories included frost damage, dead, or missing (i.e., if planted seedling had been removed by animal damage).

Seedling growth and mortality—For fall planted seedlings (Test I), initial (2021) survival rate was tallied on February 23, 2022. For all living seedlings on both tests, height and ground level diameter (GLD) were measured on October 5, 2022.

Statistical analyses

The design is a randomized complete block design with the 18 combinations of 2 culture regimes, 3 seed lots, and 3 either planting months (Test I) or lifting dates (Test II), randomly planted on each of 4 blocks. 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:

$$y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + \alpha\beta_{ij} + \alpha\gamma_{ik} + \beta\gamma_{jk} + \alpha\beta\gamma_{ijk} + \delta_l + \varepsilon_{ijkl} \quad (1)$$

Where y_{ijkl} is the dependent variable summarized for the i th planting date (Test I) or packing date (Test II), j th seedlot, the k th culture regimes, and the l th block, μ is the overall mean, α_i , β_j , and γ_k are the fixed effect of the i th planting or packing date ($i=1, 2, 3$), j th seedlot ($j=1, 2, 3$), and the k th culture regimes ($k=1, 2$), and δ_l is the random effect of the l th block ($l=1, 2, 3$, and 4), $\gamma_k \sim N(0, \sigma_B^2)$, and ε_{ijkl} is an experimental error $\varepsilon_{ijkl} \sim iidN(0, \sigma_e^2)$.

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 is also between 0 and 6 matching the stage categories.

Results

Environmental conditions

Chilling hours

Based on recorded air temperature measurements and estimated temperatures from Trinity RAWS data, seedlings planted in fall reached above ~1300–1400 chilling hours onsite (Table 1).

From September 1, 2021 to March 31, 2022, average temperature for the nursery was 7.5 °C and the average daylength was 10.6 h and ranged from 9.1 to 13.0 h (Fig. S2).

All three lift dates for the spring planting achieved beyond the minimum number of 1200 chill hours required for Douglas-fir. Due to being lifted and put into cold storage the earliest, seedlings from the November lift date had the greatest number of cumulative chill hours, but had the lowest natural chill hours (Table 1). Not surprisingly, subsequently later lift dates had greater natural chill hours and less cold storage hours. February lift date had the lowest amount of cumulative chill hours.

Following spring planting, these seedlings had approximately 200 additional chill hours on site prior to the first phenology observation on May 3, 2022.

Table 1 Number of chilling hours for the three lift dates achieved at the nursery for the spring planting test and for the three planting dates of the fall planting test

Spring Planting	Lift date	Natural Chill (0–5 °C)	Freezing hours (<0 °C)	Cold storage hours (0.7 °C)	Cumulative chill hours (0–5 °C)
	Nov 12, 2021	170	43	3312	3482
	Dec 23, 2021	537	278	2400	2937
	Feb 22, 2022	973	855	912	1885
Fall Planting	Date Planted	Natural Chill (0–5 °C)	Freezing hours (<0 °C)	Cold storage hours (0.7 °C)	Cumulative chill RAWS + HOBO
	Sept 21, 2021	1234	850	N/A	1437
	Oct 14, 2021	1207	850	N/A	1410
	Nov 8, 2021	1108	850	N/A	1311

Natural chill and freezing are the number of chilling and freezing hours, respectively, prior to lift and packing for the spring test, while all chill and freezing hours for the fall test are post planting

Field environmental measurements

Seedlings planted in September 2021 were exposed to some very hot days with estimated maximum temperature as high as 40 °C and vapor pressure deficit (VPD) of ~7 kPa (Fig. 2A), while rainfall was 18 mm (Fig. 2C). However, both temperature and VPD were relatively lower in October and November than in September. More importantly, there was a substantial amount of precipitation with 150 mm in October and 72 mm in November of 2021. In Spring of 2022, temperatures had fluctuated with a few days with frost around March. However, soil temperatures at 10 cm depth were never below 0 °C (Fig. 2B). A comparison of monthly averages for the time frame of this study with long-term monthly averages for temperature and VPD can be found in Table S1. September 2021 was warmer than the 26-year average for the same month with similar average VPD, while October was 2.2 °C cooler and had lower average VPD compared to the long-term average.

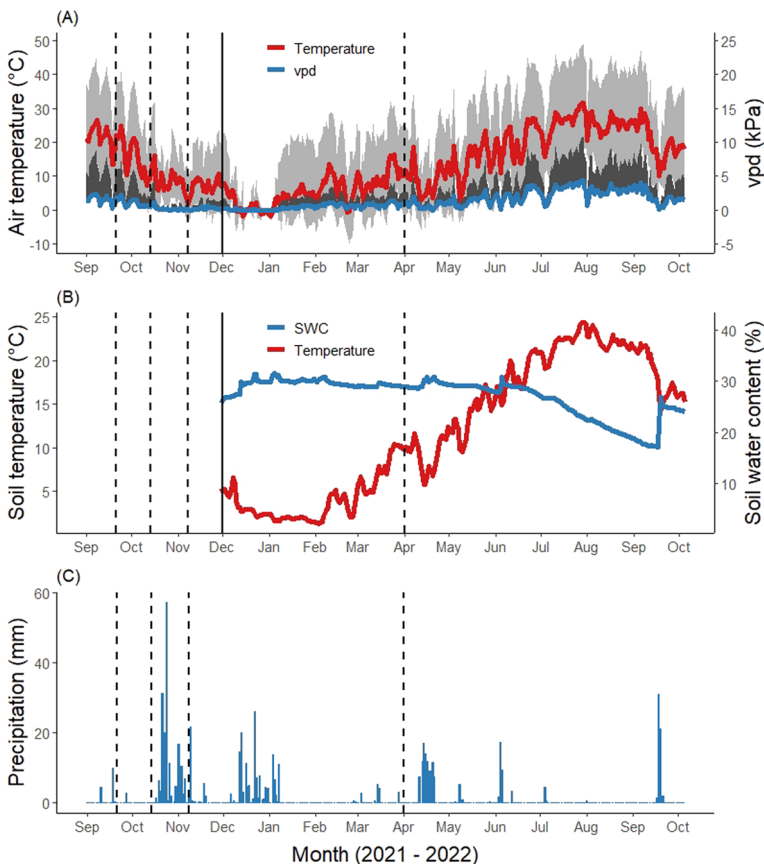


Fig. 2 Field conditions measured by **A** Daily average air temperature and vapor pressure deficit (vpd) at the field site, light gray shading represents air temperature maximum and minimum, and dark shading represents maximum vpd **B** Average daily soil temperature and soil water content at the field site recorded using a HOBO datalogger, and **C** Total daily precipitation collected by Trinity Camp RAWS. Solid vertical line indicates the start of data recording using the HOBO datalogger while all prior values were estimated using regression equations for the Trinity Camp RAWS. Dashed vertical lines represent planting dates

Following spring planting, there were 8 freeze events where temperatures were sustained below -2°C for at least 4 h and the last freeze event was the morning of May 10, 2022 (Chamberlain and Wolkovich 2021; Malmqvist et al. 2018). Soil moisture content was $>25\%$ until middle of July and then depleted gradually till late September when it was replenished. From Oct 1, 2021 to Sept 30, 2022, the total precipitation recorded by the Trinity Camp RAWS was 593.26 mm.

Fall planting test

Bud burst phenology

The bud development phenology for the fall trial showed no difference between seed lots for all three evaluating dates (Table 2). During the first evaluation date, seedlings in the May sow had faster bud development when planted in November compared to seedlings planted in September (Fig. 3A). During all three evaluations dates for seedlings planted in September, the April sow seedlings had faster bud development compared to the May sow (Fig. 3).

While all three fall planting treatments had evidence of frost damage on new shoots during the first phenology measurement period on May 3, 2022, there was no effect due to planting month (Table 2). The percent of live seedlings with frost damage was influenced by seed lot, sow date and the interaction between the two (Table 2, Fig. 4). For the S521 seed lot, the May sow regime had fewer percent live seedlings with frost damage compared to the April sow (Fig. 4). For the April sow regime, frost damage was higher for the S521 seed lot compared to the S62L lot (Fig. 4). Because the frost damaged tissue was only new bud and shoot growth, the following results for phenological development are for undamaged or recovering tissues at the time of observation.

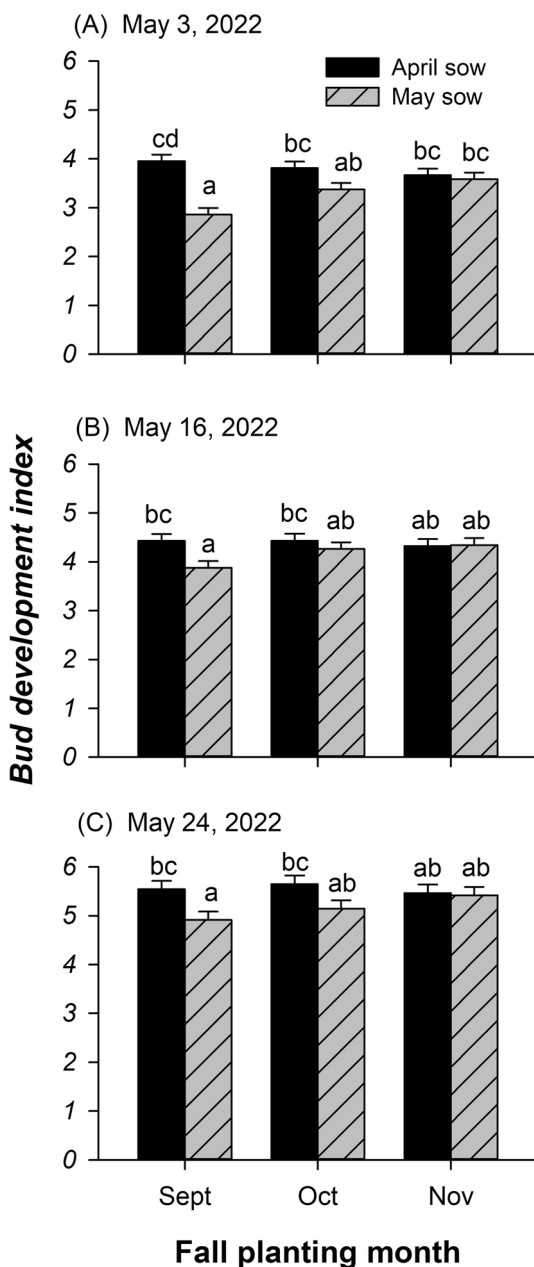
Survival rate and growth

During the first assessment of survival in February 2022, seedlings planted in September had higher mortality compared to the October and November planting months (Fig. 5A). At the time of our fall measurements in 2022, survival was not different across the three

Table 2 Source of variation, numerator (Num) and denominator (Den) degree of freedom (DF), and p-values (bold numbers denote for significance) for the bud development indices observed on May 3 (BDI-1), May 16 (BDI-2), and May 24, 2022 (BDI-3) and for survival, height, and ground level diameter (GLD) in fall planted Douglas-fir seedlings

Fall planted Source of variation	DF		P-values						
	Num	Den	BDI—1	BDI—2	BDI—3	Frost	Survival	Height	GLD
Seed Lot	2	51	0.778	0.816	0.369	0.013	0.787	0.001	0.663
Planting Month (PM)	2	51	0.232	0.209	0.378	0.227	0.002	0.241	0.142
Sow Date (SD)	1	51	<0.001	0.023	0.005	<0.001	0.200	<0.001	0.284
Seed Lot x PM	4	51	0.599	0.443	0.245	0.939	0.624	0.245	0.271
Seed Lot x SD	2	51	0.785	0.326	0.085	0.026	0.947	0.270	0.976
SD x PM	2	51	0.002	0.065	0.171	0.558	0.045	0.249	0.907
Seed Lot x SD x PM	4	51	0.469	0.733	0.621	0.226	0.179	0.174	0.223

Fig. 3 Bud development indices on a scale from 1 to 6 for Douglas-fir seedlings planted in fall recorded on **A** May 3, 2022; **B** May 16, 2022, **C** May 24, 2022. Different letters above the standard error bars indicate significant difference between treatments



planting months for seedlings in the April sow, although it was slightly higher for the October planting (Fig. 5B). For the May sow, survival for seedlings planted in November were higher than in the September planting (Fig. 5B).

Fig. 4 Percent of fall 2021 planted live seedlings with frost damage observed on May 3, 2022. Different letters above the standard error bars indicate significant difference between treatments

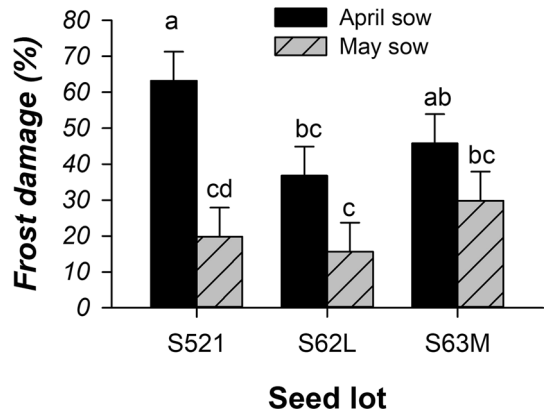
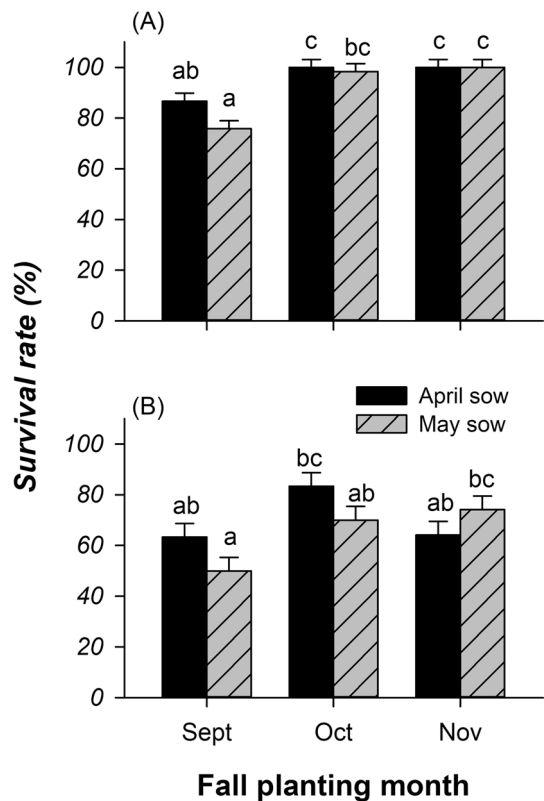
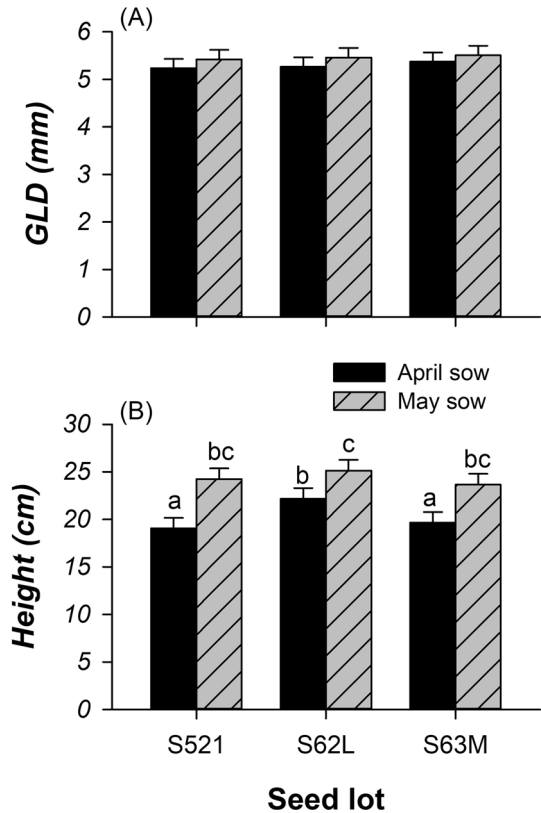


Fig. 5 Survival rate for seedlings planted in fall tallied on **A** February 23, 2022 (overwinter survival); and **B** October 5, 2022. Different letters above the standard error bars indicate significant difference between treatments



No difference was detected in ground level diameter across any of the treatment regimes (Table 2, Fig. 6A). The height of seedlings in the May sow were similar across the 3 seed lots while being taller than seedlings from the same seed lot that were sowed in April (Fig. 6B). The S62L sowed in April was also taller than the other two seed lots sowed at the same time (Fig. 6B). Planting month had no effect on height (Table 2).

Fig. 6 Seedling growth across 3 seed lots and sow dates for **A** ground level diameter (GLD) in mm and **B** height in cm. Different letters above the standard error bars indicate significant difference between treatments



Spring planting test

Bud burst phenology

The effects of sow and lift (or packing) dates had influence of bud development for seedlings planted in spring during the first two observations (Table 3). In the first assessment on May 3, 2022, all seedlings in the May sow were slower in bud development than those sowed in April (Fig. 7A). The February lifted and April sow was also further along in bud development compared to the other two lift dates of the same sow (Fig. 7A). During the second observation, the May sow bud development was again slower than the April sows, except for seedlings lifted in December (Fig. 7B). By the time of the final observation, nearly all seedlings planted in spring, regardless of treatment, had reached beyond our highest score with fully expanded needles (Fig. 7C). There was no frost damage observed for any of the spring planted seedlings.

Survival rate and growth

Survival averaged around 87% for all seedlings planted in spring (Table 4) and was not affected by any of the treatments (Table 3).

Table 3 Source of variation, numerator (Num) and denominator (Den) degrees of freedom (DF), and p-values (bold numbers denote for significance) for the bud development indices observed on May 3 (BDI-1), May 16 (BDI-2), and May 24, 2022 (BDI-3) and for survival (2022), height, and ground level diameter (GLD) in spring planted Douglas-fir seedlings

Spring Planted Source of variation	DF		P-values					
	Num	Den	BDI—1	BDI—2	BDI—3	Survival	Height (log)	GLD (log)
Seed Lot	2	51	0.129	0.003	0.094	0.236	< 0.001	0.204
Lift	2	51	0.001	0.039	0.147	0.433	< 0.001	0.004
Sow Date (SD)	1	51	< 0.001	< 0.001	0.186	0.254	< 0.001	0.106
Seed Lot * Lift	4	51	0.786	0.987	0.334	0.106	0.686	0.174
Seed Lot * SD	2	51	0.550	0.580	0.417	0.261	0.754	0.455
SD * Lift	2	51	0.007	0.004	0.874	0.882	< 0.001	< 0.001
Seed Lot * SD * Lift	4	51	0.058	0.697	0.844	0.641	0.011	< 0.001

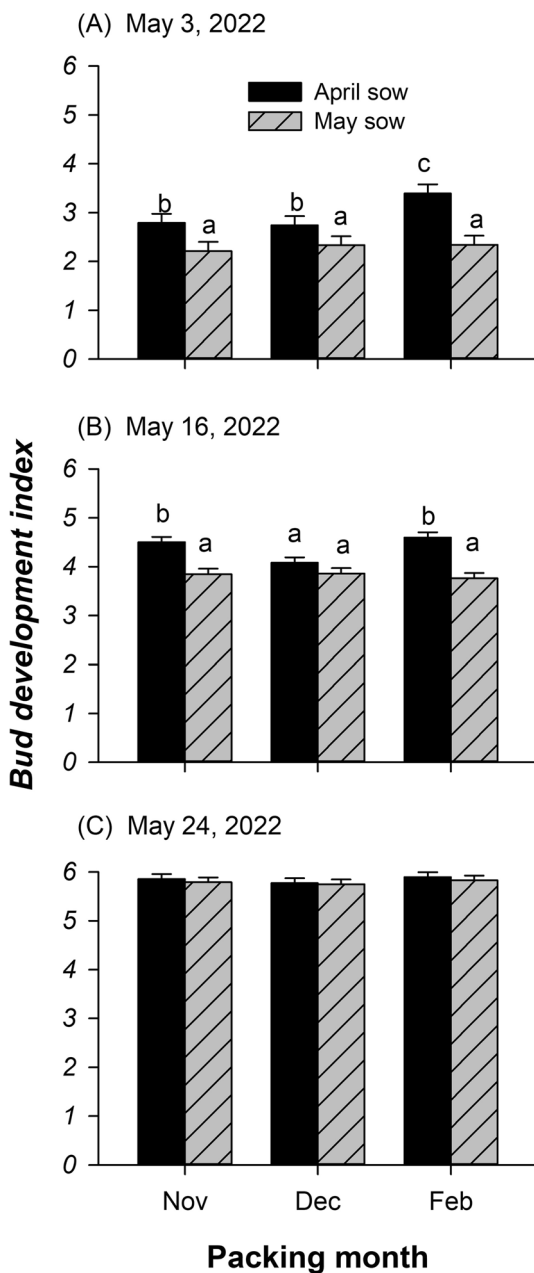
Except for the terms of seed lot * lift and seed lot * sow date, all treatments influenced seedling height (Table 3). The seedlings in the May sow averaged 26.0 cm in height while those sowed in April averaged 22.9 cm (Table 4). Seedlings lifted in February were the tallest at an average 26.2 cm while November and December averaged 23.4 cm and 23.8 cm, respectively. On average, the S62L seed lot was the tallest at 25.8 cm while the S521 was 23.6 cm and S63M were 23.9 cm. For the interactions of sow date * lift on height, April sowed * December lift was the shortest on average at 21.8 cm, while May sowed * February lift was the tallest at an average 29.2 cm with all three seed lots showing their tallest height growth. The tallest of all possible treatment regimes was May sow * February lift * S62L seed lot at 32.1 cm (Table 4).

Ground level diameter (GLD) was influenced by the treatment interactions of lift, sow date*lift, and seed lot*sow date*lift (Table 3). The February lift GLD was the largest at 5.7 mm, while November and December lifts were 5.4 and 5.7 mm, respectively (Table 4). The May sow * November lift and April sow * Dec lift had the smallest average diameters of 5.1 and 5.2 mm, respectively, while the May sow * February lift was once again the largest at 6.1 mm. Like height, the May sow* February lift*S62L seed lot had the largest GLD of 6.7 mm.

Discussion

Results from this study confirmed what we had expected previously (Zhang et al. 2021), that both seed source and the sowing and blackout date affected seedling performance in the field. Genetic differences in seedling growth and survival have been reported for Douglas-fir in the many previous studies (Campbell and Sorensen 1978; Rehfeldt 1989; Bansal et al. 2015) and information has been used in establishment of seed zones or seed transferring guidance in afforestation and reforestation. However, manipulating sowing and blackout date to induce seedling dormancy and hardiness is used to promote seedling quality and successful reforestation (Landis et al. 2010a, b, Ch. 3). Our discussion will focus this treatment as the main factor to explore how it interacted with other treatments influencing seedling establishment for both Fall and Spring plantings.

Fig. 7 Bud development index for spring planted seedlings on a scale from 1 to 6 tallied on **A** May 3, 2022; **B** May 16, 2022, and **C** May 24, 2022. Different letters above the standard error bars indicate significant difference between treatments



Bud burst phenology and frost damage

The results for the fall planting test indicated that different seed sources used in the study did not have a significant impact overall on the timing of bud burst. However, there were significant differences in bud development between the May sow and the

Table 4 Means and standard errors of the combinations of culture regimes, packing date and seed lots for ground level diameter (GLD), height, and survival rate of Douglas-fir seedlings assessed on October 5, 2022, planted on April 1, 2022

Culture regimes	Packing date	Seed lot	GLD (mm)		Height (cm)		Survival rate	
			μ	SE	μ	SE	μ	SE
April sow, July blackout	November	S521	5.6	0.333	23.1	0.489	0.88	0.063
		S62L	5.9	0.514	25.9	0.864	0.85	0.050
		S63M	5.3	0.468	22.3	0.820	0.83	0.085
	December	S521	5.2	0.763	20.5	1.466	0.80	0.082
		S62L	4.9	0.665	22.5	0.439	0.88	0.063
		S63M	5.5	0.367	22.3	0.959	0.90	0.041
		S521	5.4	0.491	23.2	1.104	0.95	0.029
		S62L	5.4	0.332	23.5	0.818	0.88	0.048
		S63M	5.3	0.633	22.5	0.721	0.65	0.119
May sow, August blackout	November	S521	5.3	0.624	22.4	1.346	0.93	0.025
		S62L	5.0	0.140	23.0	1.297	0.83	0.085
		S63M	5.0	0.207	23.6	0.885	0.88	0.025
	December	S521	5.4	0.500	24.9	0.866	0.93	0.048
		S62L	5.7	0.240	27.7	0.551	0.88	0.095
		S63M	5.2	0.580	24.9	0.581	0.95	0.029
		S521	5.2	0.196	27.7	0.208	0.93	0.048
		S62L	6.7	0.653	32.1	1.571	0.80	0.058
		S63M	6.3	0.314	27.9	0.991	0.83	0.118
		HSD	1.11		3.83		0.35	

April sow with expected results; earlier sowings (and earlier blackouts) resulted in faster bud development in both the fall plantings and in the spring planting. These results are consistent with the findings from both nursery ‘pot’ and field tests in the previous study (Zhang et al. 2021). However, the fall plantings had spring frost damage which killed the new shoots much heavier on the April sow seedlings because of their faster bud burst than on the May sow seedlings (Fig. 4). When varieties of Douglas-fir were introduced to southern Sweden, the varieties that had faster bud development also had a greater proportion of seedlings with spring frost damage (Malmqvist et al. 2018).

An early study on the chilling requirements for Douglas-fir seedlings found that chilling above 2000 h results in faster bud development at the same temperature as compared to 1250 chilling hours (van den Driessche 1975). While within the fall planting months the chilling hours were comparable to one another, within May sown seedlings, the latest planting months showed faster initial bud development than the earliest month. This result could have caused by the heavier mortality occurring in the September planted seedlings, that was likely due to the inferred lack of soil moisture and high vapor pressure deficit and temperature after seedlings were planted. As September is usually still hot and dry in this region, especially at lower elevation, foresters must plant seedlings while closely watching the weather patterns (Fredrickson 2003).

In the spring planting test, we saw the same result as the previous study (Zhang et al. 2021) that later lift and packed seedlings went through bud development faster, perhaps due to their going through more chilling hours although all seedlings had surpassed 1200–2000 h chilling threshold for this species (Fig. 6) (van den Driessche 1977). However, Ritchie (1984) had opposite results where Douglas-fir seedlings lifted early lost dormancy faster compared to seedlings lifted later.

No frost damage was observed for any of the spring-planted seedlings. Typically, frost damage events occur more frequently in late spring compared to frost events in fall (Timmis et al. 1994). It was surprising that the April sow had a higher percentage frost damage than what was observed in the May sow because seedlings grown in northern California for fall planting are typically sown earlier for the goal of improved hardening (Fredrickson 2003). Douglas-fir will also naturally begin germinating in the Coastal Range in April (Lavender and Cleary 1974). As we expected, the S521 seed lot sowed in April had the highest overall percent of live seedlings with frost damage as these seeds came from the highest elevation seed source. It has previously been found that when transferring seed sources from high to low elevations, plants tend to break bud earlier, thus increasing risk of damage due to spring frosts (Wright 1976). These findings highlight the importance of considering both seed lot selection and the sowing date to mitigate the risk of frost damage on seedling establishment.

Survival and growth

By Fall 2022, the September 2021 planting month had the lowest survival, while October and November were similar to each other with little difference between sow dates (Fig. 5). A previous report on fall planting in northern California recommends that there should be at least one decent rainfall prior to planting (Fredrickson 2003). While there was one rain event prior, and soil moisture was determined to be suitable at the time of planting, there was little to no precipitation after the September planting (Fig. 2), which was likely a contributing factor for the higher mortality. Similarly, when Douglas-fir seedlings were planted from August to January in western Oregon, the August planting had the lowest survival and those planted in early fall had the greatest height growth after 3 years (Taylor et al. 2011). Adams et al. (1991) also evaluated first year survival of Douglas-fir seedlings planted every two weeks from August to early November in 1988 in northern Idaho and did not see any effect of planting timing on survival which ranged from 95 to 100%. However, our results are hard to compare with theirs because they watered their site before and after planting in late August and fall rains provided sufficient subsequent soil moisture. While late winter or spring planting is more common practice in much of North America, fall planting is also used to meet reforestation objectives, particularly at higher elevation sites (Fredrickson 2003, Landis et al. 2010a, b, ch6, Grossnickle and MacDonald 2021, Jenkinson et al. 1993). As moisture availability is essential for survival of newly planted seedlings, and that this region is a Mediterranean climate that rarely experiences precipitation in summer and often early fall, timing of planting must be considered (Taylor et al. 2009). Findings from this study demonstrated that planting seedlings, especially the May sow regime, in October and November at the similar elevational site could be successful in northern California. The survival rate of seedlings planted in the spring was around 87% (Table 4), and none of the treatments and their interactions had a significant effect on survival (Table 4). This suggests that the factors examined in this study, including sow date, seed source, and lift date, did not significantly impact seedling survival in the spring planting.

As for growth, ground level diameter of fall planting did not differ significantly across any treatment regimes, suggesting that the planting month and seed lot did not have a significant effect on seedling diameter at ground level. However, there were significant differences in seedling height. May-sown seedlings were significantly taller than April-sown seedlings within each seed lot, which might be due to the heavier frost damage on the April-sown seedlings. The lowest elevation seed source sown in April was also

significantly taller than the other two seed lots sown at the same time, which might also relate to its lower frost damage or slower bud burst.

In the spring planting test, we found that later sow and lift dates had the largest height and ground level diameter growth for all seed lots. This contradicts the results from our previous study (Zhang et al. 2021), in which earlier sown seedlings were found to have a faster bud burst and subsequently larger height and diameter. Here, the April sow seedlings did break buds earlier, but grew less. This could be due to the confounding interactions (seed source and sow data) from the previous study and/or slower developing root systems in the April sow seedlings (personal observations) that water stress onsets earlier than the May sown seedlings. Our results also differed from the study implemented by Sloan (1989) which found that Douglas-fir seedlings planted in spring had better height growth when lifted in November compared to early March, both for first year growth as well as 13 seasons later. Dunsworth (1988) also found that Douglas-fir seedlings had a moderate improvement to height growth with later lift dates while diameter growth decreased with later lifts. Additionally, another study found that overall, earlier lift dates also performed worse than later lift dates (Haase et al. 2016). Similar to our results, Haase et al. (2016) also found that low-elevation seedlings had greater height growth compared to seedlings originating from higher elevations. Clearly, there are more factors that control seedling field performance and perhaps, it is the interactions between the nursery culture and handling and field micro habitat environments that determine final seedling growth.

Conclusions

Planting Douglas-fir seedlings with appropriate seed sources and/or culture regimes in Fall can be successful during the first year of growth in northern California. Several caveats must be considered: (1) early fall planting must consider the potential drought and heat shocks that seem to retard root and shoot development and therefore results in higher mortality in the following year; (2) early sow and ‘blackout’ increase bud development and height growth of field planted seedlings, while unfortunately having a higher percentage of live seedlings with frost damage; and (3) for this particular site and years’ planted, field performance of fall-planting seedlings was not as good as the spring planting. To avoid microenvironment induced stresses and early bud breaks in following year, planting seedlings in areas where slower spring soil warming facilitates soil moisture conditions that are more conducive to Douglas-fir survival and growth is recommended.

For the spring planting, early sow and ‘blackout’ seedlings broke buds earlier than later ones. Yet, seedlings in the later sow and ‘blackout’ dates had better height and GLD growth, especially those lifted at the latest date in February. Despite 2022 being a very dry and hot year, seedlings performed very well with an acceptable overall survival rate of 87%. While one year planting and monitoring of establishment could be influenced by seasonal differences, this study provides additional information on how nursery practices can influence survival and growth of Douglas-fir seedlings planted for reforestation in northern California.

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Declarations

Conflict of interest The authors declare that they have no conflicts of interest.

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