COLD STORAGE INCREASES RESISTANCE TO DEHYDRATION STRESS IN PACIFIC DOUGLAS-FIR ----James L. Jenkinson and James A. Nelson

ABSTRACT: Seedlings of coastal California Douglas-fir grown at the Humboldt Nursery were lifted in January and February and investigated for the effects of a standard dehydration stress applied either before or after cold storage or both. Survival potential and top and root growth capacities of stressed and unstressed seedlings were evaluated in a greenhouse in May, and field survival and growth were determined on a nearby planting site. Survival potential averaged 53 percent for seedlings stressed before storage in January, compared to 97 percent for seedlings stressed after storage. The survival potentials, growth capacities, and field performances of seedlings stressed after storage approximated those of unstressed seedlings. Apparently, seedlings stored in midwinter doubled their resistance to dehydration stress during storage, and seedlings stored in late winter maintained their high resistance. The evidence suggests that, for sources from northern California and southwestern Oregon, stress tests to evaluate survival potentials of Humboldt planting stock should be done after cold storage, shortly before spring planting, if at all.

INTRODUCTION

Field survival and growth of 1-0 and 2-0 bareroot seedlings from the Humboldt Nursery, located near McKinleyville on California's north coast, have proven the efficacy of overwinter cold storage for coastal and inland seed sources of Douglas-fir (pseudotsuga menziesii [Mirb.] Franco) on the Pacific slope (Jenkinson 1984). Storage at 0^0 to 1^0 C (32^0 to 34^0 F) completes the chilling that seedlings lifted in late autumn and early winter need to overcome dormancy and promote shoot growth, and it results in seedling root growth capacities that enable successful plantation establishment (Jenkinson and Nelson 1978, 1983).

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Because operational circumstances sometimes compromise seedling production, harvest, or cold storage, many of Humboldt Nursery's clientele, like those of other nurseries, want independent tests of the physiological quality of planting stock. The aim of such tests is to eliminate the economic losses that result when planted stock fails the minimum first-year survival standard. The present standard in the Pacific Northwest and Southwest Regions is 80 percent (860 seedlings/ha or 348/acre for a spacing of 3 m or 10 ft).

One measure of seedling quality that can be used to estimate survival in the field is root growth capacity (RGC). This test requires three things to predict survival: RGC must be determined after cold storage, near planting time in spring; the critical threshold values of RGC must be known or estimated for the various planting sites; and the seedlings must be planted when the soil is warm enough to permit water uptake and root growth. RGC will change in cold storage, depending on the interaction of seed source and nursery lifting date (Jenkinson 1978), and planting any site when the environment is not conducive to immediate water uptake and root growth will reduce seedling survival (Jenkinson 1980).

Another often used test of the quality of planting stock destined for the Pacific slope assesses the effect of a dehydration stress on subsequent seedling survival in a standard greenhouse or growth room environment (Hermann and Lavender 1979, Lavender and others 1980, Ritchie 1984). This test is used to predict field survival, but the relation of greenhouse survival to field survival is uncertain, and practically unexplored. In one instance where the relation was looked at in southwestern Oregon, survival after one summer was unrelated to survival in the stress test (table 1). Stress tests of Douglas-fir for the Gold Beach Ranger District predicted unacceptable survivals for 5 out of the 7 Humboldt lots evaluated. The general recommendation to not plant lots testing less than 70 percent created a dilemma, because the stress survival of these five lots ranged from 3 to 50 percent. The District chose to plant all lots. Their first-year field survival averaged 84 to 97 percent, and the lowest survival on any planting site was 60 percent.

This failure to predict field survival may be explained if seedling sensitivity to dehydration stress is partly dependent on lifting date and

Table 1.--Stress test and field survivals of Douglas-fir lifted in adjacent nurseries and planted on the Gold Beach Ranger District, Siskiyou National Forest, southwestern Oregon, 1980

Nursery, lifting date, and seed source code	Test survival [1]		Planting [2]		Field survival [3]	
	No stress	Stress	Sites	Times	Mean	Range
Humboldt, Jan 2-3	------Pct-----				-----Pct------	
081.15	100	70	7	Feb 1-Mar 13	84	76-100
081.20 (1)	100	20	2	Jan 14-Feb 12	97	96-98
(2)	100	80	6	Feb 3-Feb 26	86	75-97
(3)	100	43	4	Jan 26-Feb 23	94	85-100
(4)	90	30	5	Feb 22-Mar 13	87	78-96
081.33	93	3	2	Jan 25-Feb 11	95	94-96
081.35	100	50	10	Feb 24-Apr 26	85	60-100
Alder Grove, Feb 6-8						
081.15	100	93	5	Mar 2-Apr 14	94	89-95
081.20	100	100	2	Feb 24-Mar 1	95	95-96
081.25	83	77	2	Mar 24-Mar 28	95	89-100
	Mean	Mean	Total	Range	Mean	Range
Humboldt, Jan 2-3	99	42	36	Jan 14-Apr 26	90	81-98
Alder Grove, Feb 6-8	94	90	9	Feb 24-Apr 14	95	91-97

1 Two-month survivals from stress tests run by Oregon State University.
2 Soil temperatures at planting time were 5° to 10° C (41° to 50° F).
3 First-year surveys run by Gold Beach Ranger District (Sep-Nov).

time in storage. Stock from a transplant nursery (Alder Grove) adjacent to the Humboldt Nursery was lifted and tested 1 month later than the sass or similar sources at Humboldt (table 1). The survival potential of Alder Grove stock after stress treatment averaged more than twice that of Humboldt stock, yet survival on most of the Humboldt planting sites was as high as that on the Alder Grove sites. The earlier sampling and testing of Humboldt stock could account for its lower stress test survival, because Alder Grove has the same climate and soil as Humboldt, and the stock from both nurseries was packed, stored, and shipped by Humboldt.

With this background, we ran three tests to explore the hypothesis that lifting date and cold storage affect resistance to dehydration damage in 2-0 seedlings of Pacific Douglas-fir grown in the Humboldt Nursery. This paper reports the findings and discusses their implications for assessing planting stock quality.

MATERIALS AND METHODS

Seedlings were from a coastal seed source in northern California, near an altitude of 610 m (2000 ft) in seed zone 091 on the Gasquet Ranger District, Six Rivers National Forest. At Humboldt Nursery, 2-0 seedlings were lifted in contiguous plots on January 12 and again on February 9, 1981. For each lifting, seedlings were washed free of soil, culled to a stem diameter of 4 mm, root-pruned 23 cm (9 inches) below the cotyledon node, sorted into 38 sets of 1g, and stored to May 10 in polyethylene bags at 0 to 1° C (32° to 34° F). Sets were randomly drawn for dehydration stress treatments and subsequent tests to determine survival potential, top and root growth capacities (TOC, RGC), and field survival and growth (table 2). The greenhouse tests of survival potential and TGC and RGC were started on Hay 10. The field test was planted on May 11.

TEST	Stress before storage (Jan 12 or Feb 9)	Stress before and after storage	Stress after storage (May 10)	No-stress control
	Number of 10-seedling sets			
Greenhouse survival (May 10 to Jul 9)	3	3	3	3
RGC and TGC (May 10 to Jun 7)	0	0	3	3
Field survival and growth (May 11 to Oct 15)	0	0	10	10

Table 2.--Seedling treatments and sets per lifting date (Jan 12, Feb 9) in tests of a coastal California seed source of Douglas-fir (Gasquet 091.20) grown in the Humboldt Nursery

Stress treatments.--Before cold storage, 6 sets per lifting were dehydrated for 15 minutes at 32° C (90° F), the exposure used in stress tests (Ritchie 1984). The seedlings were individually blotted with toweling to remove surface water, and separately placed on hole-punched stainless steel racks in a thermostatic, vented laboratory convection oven. Immediately after treatment, the seedlings were rehydrated by immersing the roots in tap water at 10 to 15 C (50° to 60 F) for 5 minutes. On May 10, after cold storage, 3 treated and 16 untreated sets per lifting were given the same stress and recovery treatments as applied before storage.

Testing survival Potential.--Three sets of 10 seedlings per lifting date and treatment, that is, stressed before cold storage only, before and after storage, after storage only, and not stressed, were planted with a moist mix of fine sandy loam, sand, perlite, and shredded redwood (1:1:1:1) in plastic pots measuring 30 cm in diameter by 30 cm deep (12 x 12 inches). Each set of 10 seedlings was planted in a separate pot, and the pots were randomly arrayed on a table in an air-conditioned greenhouse covered with a 53 percent shade screen. Air temperature wag held above 17° C (63 F) at night and below 26 C (78 F) during the day. Day length was extended to 16 hours by operating an overhead bank of mercury-phosphor lamps from 6 to 8 a.m. and 4 to 10 p.m. The seedlings were watered three times weekly.

Survival and shoot growth were determined after 2 months (July 9). After 4 months (September 2), the seedlings were washed free of soil to assess the season's root growth.

Testing growth caoacity.--For each lifting, 3 sets of seedlings stressed after storage and 3 sets not stressed were evaluated for top and root growth capacities (TGC, RGC). Each set was divided into 2 groups of 5 seedlings, and each group was planted with the soil mix (1:1:1:1) in a separate stainless steel container measuring 7.5 x 37.5 x 30 cm deep (3 x 15 x 12 inches). The containers were irrigated, drained overnight, weighed, randomized in complete blocks, sealed with rubber stoppers, and immersed to the rim in thermostatically controlled stainless steel water baths located in the greenhouse. The root medium was held at 20 C (68" F), and was watered weekly to full capacity (Jenkinson and Nelson 1978).

New growth of the seedling tops and roots was determined 28 days after planting. Each seedling was evaluated for extension of the leader or longest shoot, new length of all roots elongated 1.5 cm or more, and total number of elongating roots.

Testing field Performance.--For each lifting, 10 sets of seedlings stressed after storage and 10 sets not stressed were planted on a cleared south slope of unused land at Humboldt Nursery. The planting layout consisted of 10 replications of a randomized complete block of split plots, with lifting date split for stress treatment. Each of the treatment plots contained a single row of 10 seedlings (a set). The seedlings were planted with a powered soil auger, and were spaced 0.6 m (2 ft) apart in rows that were I m (3 ft) apart.

First-year survival, tree height, leader length, and stem diameter were determined 5 months later (October 15), after rains had ended the summer drought. Fourth-year survival and tree height were determined in 1984 (June 26).

statistical analyses.--The effects of lifting date and stress treatment on seedling performance were assessed by analyses of variance (greenhouse tests, Jennrich and others 1981; field test, Jennrich and Sampson 1981). Means in the greenhouse tests were contrasted by Tukey's method (Steel and Torrie 1960), and in the field test, by Bonferroni's procedure (Miller 1981).

RESULTS

Survival potential.--Seedlings that were lifted and stressed in January showed a major reduction in greenhouse survival, but the same treatment a month later did not have a significant effect (fig. 1, table 3). Survivals of the seedlings lifted and stressed in January averaged 53 percent. Survival was 100 percent for seedlings that were not stressed, and 97 percent for seedlings stressed only in May, after cold storage. Survival of the seedlings lifted and stressed in February was not significantly different from that of unstressed seedlings and seedlings stressed after storage only. Survivals of seedlings stressed after 4 months or 3 months of cold storage were essentially as high as the survivals of unstressed seedlings.

Table 3.--Analysis of variance for greenhouse survival of Douglas-fir exposed to dehydration stress before or after cold storage or both [1]

Source of variation	Degrees freedom	Mean square	F	Tail prob.
Lifting date, L	1	0.042	0.02	0.887
Stress tmt, S	3	12.486	6.24	.005**
LS	3	10.153	5.08	.012*
Error	16	2.000		

[1] Seedlings of a coastal California seed source (Gasquet 091.20) were lifted at Humboldt Nursery in winter, stored to May 10 at 1 °C, and evaluated after 2 months (n = 30, in 10-seedling sets).

Table 4.--Top and root growth capacities (TGC, RGC) and field survival and growth of Douglas-fir exposed or not exposed to dehydration stress after cold storage [1]

Seedling trait	Nursery lifting date				HSD [4]
	Jan 12		Feb 9		
	No stress	Stress	No stress	Stress	
TGC [2]					
Bud burst, %	91.0	92.5	100.0	96.7	14.6
Leader extension, cm	3.2	2.3	3.3	3.0	1.8
RGC [2]					
Roots elongating, number	115	130	183	159	68.6
Root elongation, cm	110	120	179	130	74.6
Field Performance [3]					
1-yr Survival, %	92	89	97	95	12.0
Tree height, cm	35.0	32.2	31.8	36.4	4.0
Leader length, cm	6.4	6.6	6.4	6.5	1.3
Stem diam, mm	4.7	4.4	4.4	4.6	0.7
4-yr Survival, %	92	86	93	95	13.3
Tree height, cm	90.7	88.4	83.3	86.1	14.6

[1] Seedlings of a coastal California seed source (Gasquet 091.20) were lifted at Humboldt Nursery and stored to May 10 at 1 °C.
[2] Evaluated after 28 days in a greenhouse (n = 30, in 5-seedling sets).
[3] Evaluated on a nearby coastal site (n = 100, in 10-seedling sets).
[4] Tukey's or Bonferroni's significant difference at the 5 percent level.

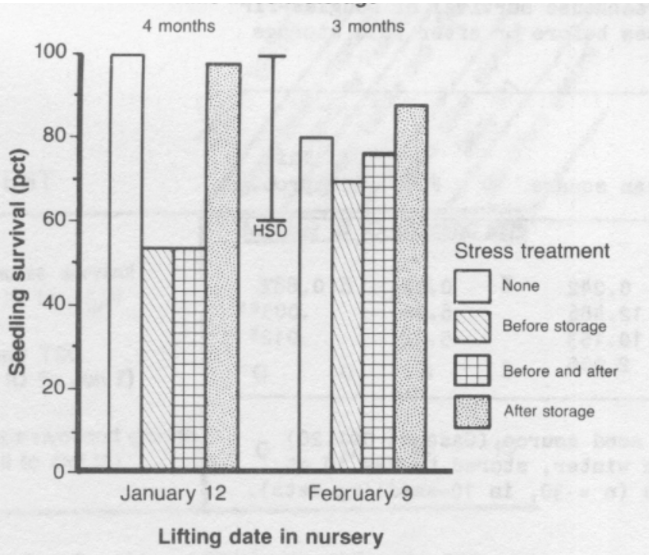

Figure 1.--Lifting date and cold storage affect resistance to dehydration stress in Pacific Douglas-fir. Seedlings of a coastal California seed source (Gasquet 091.20) were lifted at the Humboldt Nursery, stored to May 10 at 1° C, and evaluated after 2 months in a greenhouse. HSD denotes Tukey's honestly significant difference at the 5 percent level (n = 30; see table 3).

Table 5.--Effect of stress test date on estimated survival potentials of 2-0 Douglas-fir grown for the Siskiyou National Forest in southwestern Oregon[1]

Ranger District, seed source code, and test date	Test survival[2] No stress	Stress
	---------Pct-------	
Coastal		
Powers 072.25		
Dec 7	100	47
Mar 8**	100	77
Gold Beach 081.30		
Dec 7	100	10
Feb 8*	100	77
Chetco 082.10		
Dec 7	100	27
Feb 8*	90	23
Chetco 082.20		
Dec 30	100	57
Mar 27**	95	67
Interior		
Illinois Valley 512.40		
Dec 7	100	37
Feb 8*	100	73

1 Seedlings were lifted at Humboldt Nursery and stored 3 days, 1 month (*), or 2 months (**) before testing, 1982-83 and 1983-84 (Chetco 082.20).

2 Two-month survivals from stress tests run by Oregon State University.

The surviving seedlings showed normal shoot growth and substantial root growth. About 20 percent of the seedlings that did not survive flushed the first month, but grew few or no roots.

TGC. RGC. and field Performance.--Dehydration stress after cold storage did not significantly affect seedling TGC, RGC, or field performance (table 4). Overall, TGC, RGC, and field survival seemed to be higher for the February than the January lifting, but the differences were not significant and were not reflected in field growth.

DISCUSSION

Resistance to dehydration stress in Pacific Douglas-fir apparently increases with chilling in the nursery and in cold storage. For the coastal California seed source at Humboldt Nursery, the survival potential of planting stock stressed shortly after lifting increased 21 percent with an additional 28 days of natural cold exposure in late winter (fig. 1). For the same source, the survival potential of stock lifted in January and stressed after cold storage was 97 percent, 44 percent more than for stock stressed at the time of lifting. This increase indicates that cold storage may greatly improve seedling resistance to dehydration damage. The increase depends on the combination of lifting date and storage time, however, because the survival potential of stock lifted in February and stressed after cold storage was only 14 percent more than for stock stressed at lifting.

Survival potential has increased markedly with later stress testing dates for both coastal and interior seed sources of Humboldt planting stock for the Siskiyou National Forest (table 5). Survival potentials of seedlings lifted in January and stored 1 or 2 months were 10 to 67 percent higher than for seedlings tested in December. The exception, a low stress resistance for stored seedlings of Chetco 082.10, may reflect mechanical damage during lifting and sorting in the 1982-83 harvest season.

For any particular seed source, it is likely that the earlier seedlings are lifted and stored within their source lifting window (Jenkinson 1984), the greater will be their increase in resistance to dehydration stress between lifting and spring planting. The California source used in our tests of stress effects has a lifting window that opens in December and closes in March at Humboldt Nursery (fig. 2). Seedlings lifted within their source window have high survival and growth potentials at planting time in spring, regardless of the intervening combination of lifting date and time in storage. Cold storage of seedlings lifted within their source window may promote the development of stress resistance much as it completes the chilling required for dormancy release and rapid shoot growth.

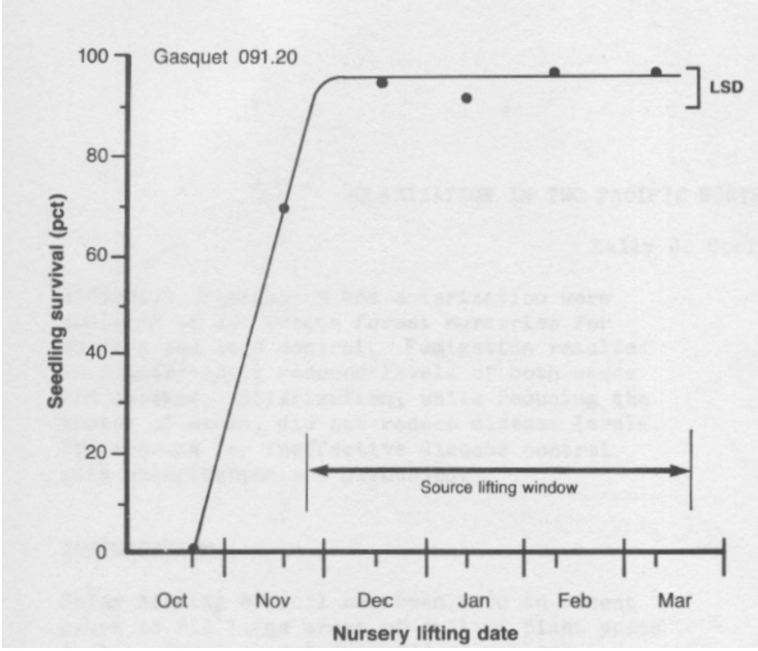

Figure 2.--Effect of lifting date in the Humboldt Nursery on field survival of seedlings from a coastal California seed source of Douglas-fir (Jenkinson 1984). Arrow shows the safe time to lift stock of this source for cold storage and spring planting. LSD denotes least significant difference at the 5 percent level (n = 100).

Attempts to predict field survival and growth by testing seedlings 2 or 3 months before their optimum planting times ignore the fact that physiological conditioning continues in the nursery and in cold storage, and ultimately affects stock performance in the field. Seed source and conditioning environment interact to fix the lifting window, TGC, and RGC, and affect both frost and drought resistance. These traits dictate the timing and limit the duration of tests to predict field performance. When sources of Douglas-fir from western Oregon and northern California are properly lifted and stored until spring begins on the planting site, there is barely enough time to squeeze in the 4-week test of RGC (Jenkinson and Nelson 1978) before summer weather closes the planting window (Jenkinson 1980), much less the 2 months required to score a stress test (Ritchie 1984).

RGC at the time of planting has explained 90 to 99 percent of the variation in first-year field survival of almost every source tested (Jenkinson and Nelson 1978, 1983). Because source lifting windows are stable across years (Jenkinson 1984), spring RGC tests of seedlings stored at monthly intervals through one lifting season can be used to refine the nursery's future lifting schedule. On the other hand, results of stress tests can not be extrapolated; they apply only to the particular seedlings tested.

CONCLUSION

Evidence from our tests and the field suggests that early sampling and stress testing is of little value for predicting field survival of Pacific Douglas-fir. Stress tests to evaluate survival potentials of Douglas-fir from Humboldt Nursery should be done after cold storage, and shortly before spring planting, if they are done at all. For most seed sources, the time is too short to complete a stress test before planting ends.

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