

Whitebark Pine Seed Storage and Germination: A Follow-Up Look at Seedlots from Oregon and Washington

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Abstract

During the spring of 2006, 21 seedlots (families), collected from 1996 through 2005 from across the Pacific Northwest range of whitebark pine, were used in 3 trials with the following objectives: to evaluate effects of storage time on germination success and speed of germination; to evaluate differences in embryo length relative to cavity size on germination success and speed of germination; to evaluate effects of x-rays on germination success and speed of germination; and to compare results of tetrazolium (TZ) testing with x-ray results and actual germination in whitebark pine seeds. Two key findings were: 1) following mechanical sanding, seedlots stored as long as 10 years germinated almost as well as seedlots stored for shorter periods of time; and 2) properly scored X-rays can be a good predictor of germination success.

Introduction

Information on seed viability and seed storage periods for whitebark pine has been variable and limited. Whitebark pine seeds contain higher proportions of lipids than most other conifer seeds (Lanner and Gilbert 1994; Lanner 1998), which could affect viability following periods of freezer storage. Some research has shown that seed viability is significantly reduced following storage of periods greater than 2 to 3 years (Burr and others 2001), with earlier studies showing losses down to 1 to 3% total germination after 11 years (McCaughey and Schmidt 1990). However, work in Oregon found that at least some older Oregon and Washington seeds can be stored without significant damage for longer periods of time (Riley and others, this proceedings).

In recent years, there has been a surge in whitebark pine seed collections to be sown and cultured at various seedling nurseries and testing facilities. Proper collection of whitebark pine cones requires caging of the immature cones in the spring to protect from nutcracker and squirrel damage, then actual collection in the fall. Depending on the location and size of the trees, both of these activities may require climbing of individual trees. In addition, whitebark cones may require special extraction procedures once they are transported to a seed extractory. The result can be extremely expensive and valuable seeds, requiring high seed use efficiency in a nursery.

Several factors enter into seed use efficiency and sowing calculations, with seed viability and germination potential as the most important. In order to determine the best use of seeds available for sowing and eventual white pine blister rust (*Cronartium ribicola*) resistance

testing at USDA Forest Service Dorena Genetic Resource Center (DGRC), a study was implemented to:

- 1) evaluate the effects of storage time on germination success and speed of germination;
- 2) evaluate differences in embryo length relative to cavity size, as a subjective estimate of maturity, on germination success and speed of germination;
- 3) evaluate effects of x-ray analysis on germination success and speed of germination to determine if whitebark pine seeds are damaged during the X-ray process; and
- 4) compare results of tetrazolium (TZ) testing with x-ray results and actual germination.

This study was done to follow-up similar small trials of seedlots from Oregon and Washington at DGRC (Riley and others, this proceedings).

Methods And Materials

A total of 21 seedlots, collected from 1996 through 2005 from across the Pacific Northwest range of whitebark pine, were used in trial 1, with a subset of seedlots used in trials 2 and 3 (Table 1). With the exception of those seedlots collected in 2005, all seeds had been stored at DGRC under standard seed-freezer storage conditions. Seeds were air-dried to approximately 7% moisture content, placed in triple-layer foil seed packages, and stored in a seed freezer at -15 to -17 °C.

Table 1. Seedlots used in whitebark pine Trials 1 through 3.

Seedlot #	Coll Year	Origin	% Filled	Prev Germ	# Seeds	Trial 1	Trial 2	Trial 3
1	2005	Crater Lake NP	34		100	X		
2	2005	Crater Lake NP	43		100	X		
3	2005	Giff Pin NF	55		300	X	X	X
4	2005	Okan/Wen NF	32		100	X		
5	2005	Okan/Wen NF	61		300	X	X	X
6	2005	Okan/Wen NF	29		100	X		
7	2005	Deschutes NF	47		100	X		
8	2005	Deschutes NF	51		100	X		
9	2003	Mt Hood NF	100	72	300	X	X	X
10	2003	Mt Hood NF	98	86	100	X		
11	2003	Umatilla NF	96	83	300	X	X	X
12	2003	Umatilla NF	100	85	300	X	X	X
13	2003	Umatilla NF	96	73	100	X		
14	2001	Deschutes NF	90	44/38/53	100	X		
15	2001	Deschutes NF	96	75/64/58	300	X	X	X
16	1999	Fremont NF	34	53/86	100	X		
17	1999	Fremont NF	21	21	300	X	X	X
18	1997	Colville NF	60	25	100	X		
19	1997	Colville NF	56	34	100	X		
20	1996	Colville NF	73	22	100	X		
21	1996	Colville NF	83	46	300	X	X	X

Trial 1—Storage Time and Ratio of Embryo to Cavity Size

A total of 100 randomly selected seeds from each of the 21 single-tree collections were placed on a template and X-rayed. Seeds were then divided into 4 categories as follows:

- a) embryo fills >75% of cavity (Figure 1A);
- b) embryo fills 50% to 75% of cavity (Figure 1B);
- c) embryo fills <50% of cavity (Figure 1C);
- d) embryo cavity empty (unfilled seed) (Figure 1D).

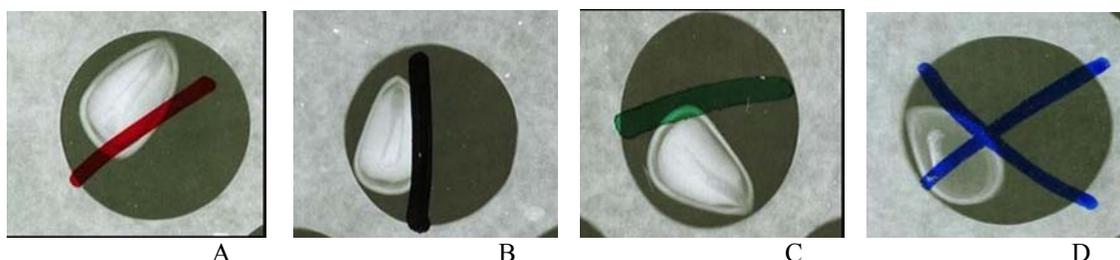


Figure 1. Embryo fills >75% of seed cavity (A); embryo fills 50% to 75% of seed cavity (B); embryo fills <50% of seed cavity (C); unfilled seed (D).

Seeds in each category varied widely by seedlot (Table 2). Seeds in each of categories “A” through “C” were divided into 2 reps for testing.

Table 2. Number of seeds in each category in each seedlot (total 100 seeds/lot).

Seedlot #	Coll Year	Origin	>75% embryo-fill	50% to 75% embryo-fill	<50% embryo-fill	Unfilled embryo
1	2005	Crater Lake NP	15	32	44	9
2	2005	Crater Lake NP	30	28	35	7
3	2005	Giff Pin NF	35	36	20	9
4	2005	Okan/Wen NF	31	31	31	7
5	2005	Okan/Wen NF	24	43	30	3
6	2005	Okan/Wen NF	23	39	31	7
7	2005	Deschutes NF	20	36	40	4
8	2005	Deschutes NF	36	42	19	3
9	2003	Mt Hood NF	29	39	32	0
10	2003	Mt Hood NF	32	49	17	2
11	2003	Umatilla NF	34	24	36	6
12	2003	Umatilla NF	60	26	13	1
13	2003	Umatilla NF	37	22	38	4
14	2001	Deschutes NF	28	36	18	18
15	2001	Deschutes NF	25	39	29	7
16	1999	Fremont NF	0	9	80	11
17	1999	Fremont NF	2	14	68	16
18	1997	Colville NF	32	21	10	37
19	1997	Colville NF	40	18	17	25
20	1996	Colville NF	0	12	53	35
21	1996	Colville NF	11	18	32	39

Trial 2—X-ray Effects

A subset of 8 lots, with either relatively high filled percentages or previous high germination success, were used in trial 2. Seedlots were spread across the geographic range and across collection years. A total of 100 randomly selected seeds that were **not** X-rayed were divided into 2 reps for this trial.

Individual seedlots/rep for trials 1 and 2 were labeled and placed in mesh stratification bags (Figure 2). Using standard protocols for seed stratification at DGRC, seeds were soaked in 1% H₂O₂ for 24 hours, rinsed, and soaked in water for an additional 24 hours. Bags were then placed in covered plastic tubs for stratification at 10 °C for 30 days, then 1 to 2 °C for an additional 90 days. All seeds were rinsed once per week during stratification.

At the end of the stratification period, seeds were abraded, using 100-grit sandpaper, approximately 1 mm back from the radicle end and along the main line dividing the 2 halves of the seedcoat. This process was done to help maximize germination percentage as well as uniformity of germination. Five seedlots had a percentage of seeds that began to crack during stratification. These seeds were not sanded. All seeds were placed on moistened blotter paper in 10 x 10 cm clear plastic boxes (Figure 3), which were then placed in a germinator maintained at 16 °C night/18 °C day with a 12-hour photoperiod.



Figure 2. Individually labeled mesh bags for seed stratification.

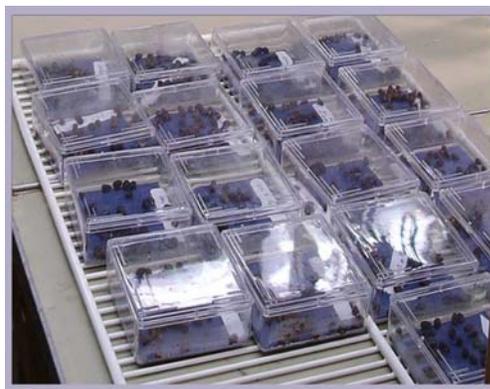


Figure 3. Whitebark pine seeds in individually labeled germination boxes.

All lots were monitored for germination twice per week for 21 days. Seeds were considered germinated when the radicle protruded from the seedcoat to a length of 2 mm and was curved. Germinated seeds were removed from the germination boxes, sown into individually labeled Ray Leach SuperCells® (164 cm³) (Stuwe and Sons, Inc., Corvallis, OR) containing pre-fertilized (180-day Nutricote® control-release fertilizer [18-6-8 with minors]) and pre-moistened media (peat:composted fir bark:perlite:pumice [40:20:20:20]), and covered with nursery grit. Seedlings were cultured under standard protocols for whitebark pine at DGRC (see Riley and others, this proceedings, for further information on seedling culture).

Trial 3—Tetrazolium Testing

A subset of 8 seedlots (same lots as those used in trial 2) were used in this trial. A total of 100 randomly selected seeds were X-rayed and then sent to the USDA Forest Service National Seed Laboratory in Athens, GA for tetrazolium testing to determine viability. (See Grabe (1970) and Peters (2000) for information on tetrazolium testing.)

Results

Trial 1

Germination by collection year

Germination varied widely by seedlot, ranging from 21% to 92%, with an overall average of 62%. Germination percentage was relatively high overall (Figure 4); 81% of the individual seedlots averaged greater than 50% germination, and 67% averaged greater than 70% germination. Although seeds stored for less than 5 years had significantly higher germination rates ($P < 0.05$), it appears that seeds are still viable up to 10 years after collection. Previous germination percentages for most seedlots used in earlier sowings compared favorably to current results, although some varied significantly (Table 3). (Seedlots collected in 2005 did not have previous germination percentages.) Seeds collected in 1999 displayed lower germination presumably due to the large number of immature seeds. Burr and others (2001) found that immature seeds do not store well for long periods of time. Other variations may have been due to early incorrect stratification and seed handling methods (Riley and others, this proceedings).

Table 3. Comparison of germination percentages for seedlots used in previous sowing (no 2005 seedlots).

Seedlot #	Coll Year	Origin	Average Previous germination	Current Study germination
9	2003	Mt Hood NF	72	84
10	2003	Mt Hood NF	86	76
11	2003	Umatilla NF	83	83
12	2003	Umatilla NF	85	76
13	2003	Umatilla NF	73	48
14	2001	Deschutes NF	45	83
15	2001	Deschutes NF	66	68
16	1999	Fremont NF	70	13
17	1999	Fremont NF	21	20
18	1997	Colville NF	25	65
19	1997	Colville NF	34	48
20	1996	Colville NF	22	25
21	1996	Colville NF	46	39

Seeds which were stored less than 5 years (2001 through 2005 collections) germinated more quickly than those stored for longer periods of time (Figure 5). Nearly half of all seeds in these lots germinated within 4 days. However, the majority of seeds for all collection years germinated within 10 days.

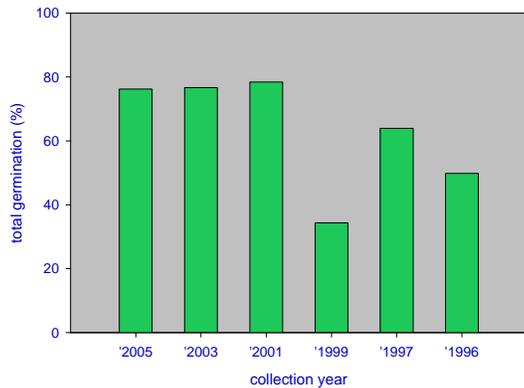


Figure 4. Total germination by collection year (all categories of filled embryos).

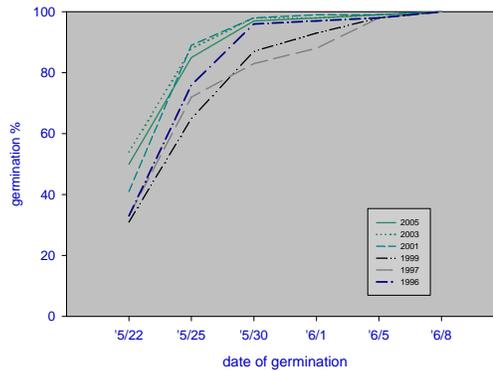


Figure 5. Speed of germination by collection year (all categories of filled embryos).

Germination by embryo-fill category

No significant difference was found in germination percentage between the >75% filled embryo cavity and the 50% to 75% filled embryo cavity categories (Figure 6). Although germination was significantly lower ($P < 0.0004$) in seeds with <50% filled embryo cavity, over half of the seeds in this category germinated.

No significant differences were found in speed of germination between embryo-fill categories (Figure 7).

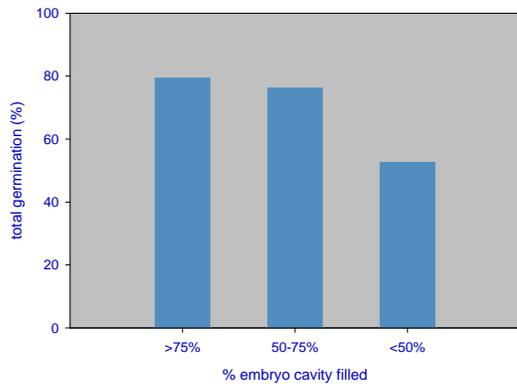


Figure 6. Total germination by embryo-fill category (all collection years).

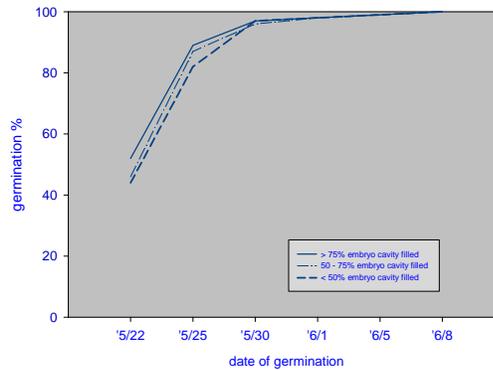


Figure 7. Speed of germination by embryo-Fill category (all collection years).

Trial 2

Effects of X-ray on seed viability

No significant differences were found in germination percentages or speed of germination between X-rayed seeds and seeds which were not X-rayed (Figures 8 and 9).

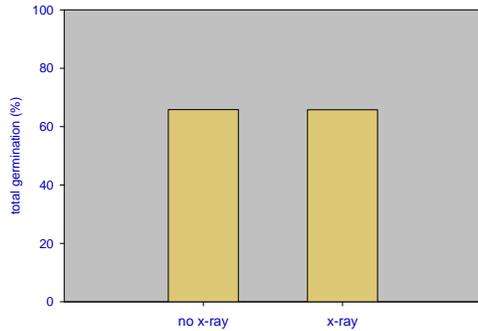


Figure 8. Total germination for X-rayed seeds vs seeds which were not subjected to X-ray (all collection years).

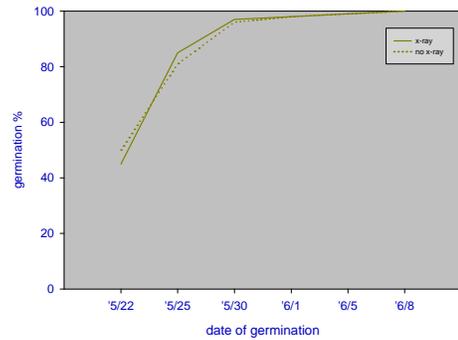


Figure 9. Speed of germination for X-rayed seeds vs seeds which were not subjected to X-ray (all collection years).

Trial 3

Tetrazolium testing vs X-ray and germination

The relationship of tetrazolium testing results with seed X-ray and actual germination percentages varied between seedlots (Figure 10). Older lots (sow numbers 17 and 21) showed more variation between test types than seedlots that had been in storage for shorter periods of time. However, there were no significant differences found overall when test results were averaged over all seedlots.

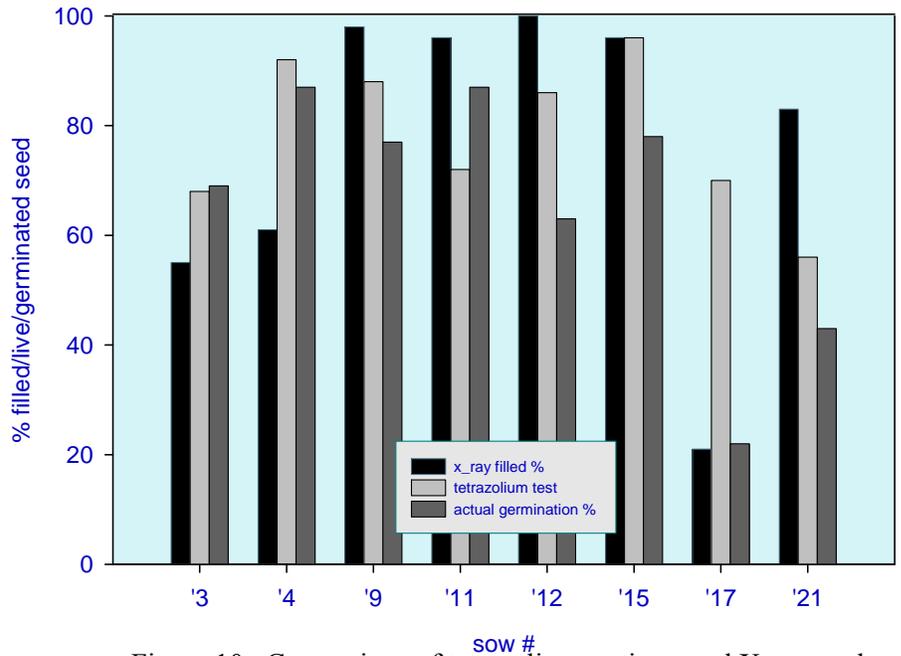


Figure 10. Comparison of tetrazolium testing, seed X-ray, and germination as predictors of seed viability.

Conclusions

The effects of storage time on whitebark pine seed viability has been studied over a number of years, with differing results. Although relatively small numbers of seeds were used in this study, some trends are apparent. Under standard seed storage conditions at DGRC, it appears that whitebark pine seeds from Oregon and Washington can be stored effectively and maintain fairly high viability for up to 10 years. However, it does appear that younger seeds (in storage less than 5 years) do complete germination faster than those stored for longer periods of time.

Seeds in which the embryo fills >50% of the cavity appear to have a high potential for germination. However, even seeds where the embryo cavity has a filled percentage of <50% may still be viable, which is in contrast to seeds of many other conifer species. Leadem (1986) found that maintaining imbibed whitebark pine seeds under warm conditions for 30 to 60 days (warm stratification) effectively promoted the development of immature tissue. That is, seeds with a significant proportion of apparently immature embryos (as compared with other conifer species) may still germinate successfully.

As with other conifer species, X-rays do not appear to affect germination success. In comparison to other testing methods, including laboratory tetrazolium testing or actual germination, properly scored X-rays do appear to be a good predictor of germination potential.

Further study may be needed on the question of storage time, as well as collection date, as further seed collections are completed. Potential future studies could include: 1) the effects of storage time on germination success and speed of germination by testing the same families every 3 years for up to 20 years; 2) the effects of collection year on germination success and speed of germination by collecting from the same trees over time; and 3) the effects of collection date on germination success and speed of germination.

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