

The Effect of Scarification and Stratification Treatments on the Germination of *Danthonia californica* Seed from Three Populations

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Danthonia californica (California oatgrass) is an important species for habitat restoration and revegetation in the Pacific Coast states, USA.

Introduction

Danthonia californica Bolander (California oatgrass) is an important cool season perennial grass for prairie restoration as well as rangeland and wildlife habitat improvement in Pacific Coastal States USA. However, establishment is often confounded by delayed germination attributed to one or more types of seed dormancy. Dormancy varies from low to high among populations and among and within seed lots of the same population. Hulling/scarification (Trask 1996) or the use of concentrated sulfuric acid to erode the pericarp (Laude 1949) have been among the most successful methods promoting germination, but cold moist stratification or a combination of treatments have worked as well. This suggests the possibility of combined dormancy in some seed lots (Darris et al. 2008). In order to break dormancy, a series of four experiments were conducted to compare the effects of single and multiple treatments on the germination of *Danthonia californica*, including cold moist stratification (moist chilling), warm moist stratification, hulling, and acid or mechanical scarification of the seed coat.

Methods

Seed from three natural populations of *Danthonia californica* [Polk Co, OR (9040747=747), Douglas Co., OR (9079415=415), and California (9083030=030)] were used in a series of four germination experiments. All experimental samples consisted of a random mix of terminal inflorescence seed and cleistogenes, typical of machine harvested *Danthonia* seed lots that have been aggressively threshed. Seed was stored under ambient air conditions in the office until used. All tests used standard germination boxes and paper placed in a growth room with a photoperiod of 16 hr light/8 hr dark at 20°C/15°C (Tables 1-2) or 25°C/20°C (Tables 3-4). Germination was recorded weekly for 21 days. Experimental design consisted of a completely randomized design with four replications and 50 seeds per replication. All data were transformed with an angular transformation and means compared using analysis of variance and LSD test at the alpha 0.05 level of significance.

Cold moist stratification occurred for 90 days at 3°C (Table 1) or 60 days at 3°C plus 30 days at 11°C (Table 3). Warm moist stratification was conducted in the dark by wrapping germination boxes in aluminum foil and placing them in a growth room at 25°C/20°C for 14 or 28 days prior to being uncovered (Table 4). Any germination that occurred in the dark was recorded. Seeds were manually hulled by rubbing and peeling off the lemma and palea by hand to minimize any damage to the pericarp, and mechanically hulled by moving the seed between two rubber belts travelling at slightly different speeds (called a belt thresher). Scarification of the pericarp was accomplished with concentrated sulfuric acid (sp. gr. 1.84, as per Laude 1949), with a 50% dilute solution of sulfuric acid, or with a Forsberg seed scarifier using 320 grit sandpaper, all at varying time intervals (Tables 1-2). Seed was "nicked" by making a cut with a scalpel over the endosperm on the dorsal side of manually hulled seed (Laude 1949) (Tables 2-3).



Basket Slough National Wildlife Refuge is the origin of 9040747 California oatgrass used in the experiments. In the Willamette Valley of western Oregon, the species is a common native component of remnant wetland and upland prairies but such plant communities now occupy less than 1% of their original (pre Euro-American settlement) range.

Results and Discussion

Means with the same letter are not significantly different ($P=0.05$) (Tables 1-4). Manually hulled then nicked seed germinated within 2% of total viability as determined by tetrazolium test (Tables 2-3), but manually hulling alone did not significantly improve germination over the controls for two of three populations. This suggests that for at least some populations, delayed germination is the result of a single, seed coat imposed dormancy. This result concurs with work by others (Laude 1949, Trask 1996), and does not indicate a combined dormancy mechanism. While the hull may still impose partial dormancy in some seed lots, manual removal is impractical and mechanical removal did not improve germination over no treatment. Mechanical hulling also increased the number of abnormal seedlings (Table 2). The fact that the embryo is in a vulnerable position and subject to physical injury (Laude 1949) probably explains the highly reduced germination from all sandpaper scarification treatments by a machine. In sharp contrast to Laude (1949), concentrated sulfuric acid treatments did not improve germination but were instead detrimental, if not lethal to the seed. While reported that light is required for germination (Maslovat 2001) a limited number of seeds germinated in the dark (less than 1% for 747 and 415 but 4.8% for 030) indicating the requirement is not absolute for all seeds. In this study, 90 days of cold moist stratification alone or 28 days of warm moist stratification in the dark were virtually equivalent. Rather than promoting the leaching or breakdown of inhibitors over time, the moist treatments may involve biochemical degradation or physical changes to the pericarp akin to a simple nick or abrasion, thus allowing for a higher percent germination. A change in total moisture imbibition is not likely the reason as both untreated and nicked seed uptake similar amounts of moisture (Laude 1949). A more precise explanation is still needed. Cold moist or warm moist stratification are the simplest and most practical means to maximize germination of some populations of *Danthonia californica* without undue damage to the seed.

Table 2. Effect of hulling and scarification treatments on germination of *Danthonia californica* (population 030)

Treatment	% germination ¹	
	abnorm	normal
Total viability (TZ test)		96
Manually hulled + nicked	2	97 a
Manually hulled	0	82 b
20 min 50% dilute acid	0	80 bc
5 min 50% dilute acid	0	76 bcd
Machine hulled	7	71 cd
None (control)	0	65 de
Mach. hulled + 5 sec sand	21	57 ef
5 min acid	5	48 f
Mach. hulled + 20 sec sand	24	19 g
Mach. hulled + 5 min acid	3	18 gh
20 min acid	8	10 h
Mach. hulled + 20 min acid	2	0.5 i

Table 3. Effect of manual hulling and moist chilling on germination of *Danthonia californica*

Treatment	% germination by population ¹		
	030	415	747
Total viability (TZ test)	96	96	93
Man. hulled + nicked coat	—	95 a	91 a
Man. hulled + 90 d cold/cool	85 a	—	—
90 d cold/cool	70 b	83 b	59 b
Manually hulled	—	23 c	20 c
None (control)	53 c	15 c	14 c

¹For all tables, means with the same letter do not significantly differ ($P=0.05$, LSD test.)

Table 4. Effect of warm moist stratification on germination of *Danthonia californica*

Treatment	% germ. by population ¹		
	030	415	747
Total viability (TZ test)	96	96	93
28 d warm	72 a	88 a	81 a
None (control)	68 a	75 b	47 b
14 d warm	44 b	42 c	46 b
Demonstration (nonrepl.)			
7 d warm	50	50	32
7 d warm + 7 d dehydrate	26	4	58
21 d warm	84	66	90
21 d warm + 7 d dehydrate	50	56	58

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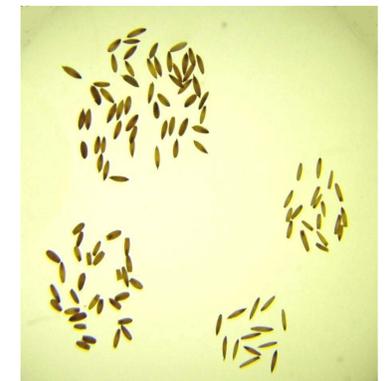


A growth room and germination boxes were used to conduct the experiments.

Table 1. Effect of mechanical hulling, moist chilling, and scarification on germination of two populations of *Danthonia californica*

Treatment	% germination by population ¹	
	415	747
Total viability (TZ test)	96	93
90 d	88 a	81 a
Hulled + 90 d	53 b	52 b
None (control)	52 bc	39 c
90 d + 15 sec sand	50 bc	47 bc
Hulled	44 c	50 bc
90 d + 5 min acid	31 d	18 e
Hulled + 15 sec sand	17 e	8 f
Hulled + 90 d + 5 sec sand	8 f	28 d
Hulled + 90 d + 10 sec sand	8 fg	7 f
Hulled + 90 d + 15 sec sand	4 fgh	1 g
Hulled + 90 d + 20 sec sand	4 gh	7 f
20 min acid	2 h	20 de
Hulled + 90 d + 5 min acid	0 i	0 g
Hulled + 90 d + 10 min acid	0 i	0 g
Hulled + 90 d + 20 min acid	0 i	0 g
Hulled + 90 d + 30 min acid	0 i	0 g
Hulled + 90 d + 40 min acid	0 i	0 g
Hulled + 20 min acid	0 i	0 g
90 d + 20 min acid	0 i	0 g

¹For all tables, means with the same letter do not significantly differ ($P=0.05$, LSD test.)



For California oatgrass, the embryo end of the caryopsis is very vulnerable to damage from mechanical hulling or seed coat scarification. Clockwise from top left: hulled, uninjured chasmogenes, hulled injured cleistogenes, hulled uninjured cleistogenes, hulled injured chasmogenes.



Germination (91-97%) of all 3 populations of California oatgrass was rapid and equal viability (93-96%) only when the seed was manually hulled and nicked with a scalpel.



Spikelets of California oatgrass are flattened with 5-10 flowers (florets) each. Photo by Steve Matson used with permission.



Small seed production field of California oatgrass at the USDA Natural Resources Conservation Service, Plant Materials Center, Corvallis, Oregon.



Chasmogenes (left) and cleistogenes (right) of California oatgrass with hulls (lemma and palea) still intact. Chasmogenes are seeds born in the exposed terminal inflorescence (panicle) that result from open pollination. Cleistogenes form at the lower nodes of the flowering stem, typically remain enclosed in the leaf sheath, and result from closed pollination (obligate self-fertilization). All germination experiments used a random, roughly equal mix of both types, typical of seed lots resulting from machine harvesters such as a combine that dislodge the seed from the stems.

Conclusions

- Manual hulling then weakening or penetrating the seed coat (pericarp) by nicking was the most effective means of eliminating dormancy and improving germination without damaging the seed; however, it is impractical for field scale work.
- Results indicate that for at least some seed lots, there is no combined or double dormancy but only a single, seed coat imposed one. However, dormancy contributed in part by the hull cannot be ruled out in other populations.
- Contrary to other published work, concentrated sulfuric acid to scarify, erode, or weaken the seed coat was far too detrimental to the seed, at least for the three populations in this study. However, soaking seed in 50% dilute sulfuric acid for 20 minutes may benefit germination in some seed lots.
- Mechanical hulling alone, even with a machine as gentle as a belt thresher, did not improve germination over no treatment and is not recommended.
- For short intervals (as little as 5 seconds) machine sanding of the seed coat was too injurious and substantially reduced germination in two of three seed lots. For the third lot it provided no benefit. Sanding the seed coat with a machine such as a seed scarifier is not recommended.
- As a practical method, cold moist stratification of the seed for 90 days provided the highest germination with little or no apparent injury to the embryo. Presumed biodegradation or associated physical changes in the seed coat under high moisture conditions for 90 days may be akin to an immediate physical weakening, nicking, or erosion of the seed coat. Cold moist stratification is recommended. Fall sowing and over-wintering the seed outdoors will probably provide a similar benefit. This is already the recommended practice for establishing California oatgrass from seed.
- Warm moist stratification for 28 days in the dark produced virtually the same high germination results as 90 days of cold moist stratification. This method is an alternative where shorter time frames are needed. It might be achieved outdoors by summer sowing in warm soil, provided there is frequent irrigation. Field confirmation is needed.
- Light appears to be required for most California oatgrass seeds to germinate, but not all. This finding supports the recommendation that seed should be sown no deeper than 6 mm or ¼ inch.

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