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Effect of seed moisture on Wyoming big sagebrush seed quality

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Abstract

Seed germination and seedling vigor of Wyoming big sagebrush (*Artemisia tridentata* Nutt. ssp. *wyomingensis*) were evaluated following manipulation of seed moisture, a practice benefiting many species. At the time of harvest, seed moisture ranged from 2.3 to 9.0% for 5 collections tested and seeds with moisture between 5 to 6% had the highest and most rapid germination. Seed moisture changed during storage, but germination percentage was not affected by post-harvest seed moisture change, indicating that germination is related more to habitat or genetic variations than the initial moisture content. Seedling vigor increased after storage, suggesting that after-ripening may be required. Seeds of 2 commercial collections were subsequently humidified at 2, 5, 10, and 15°C for up to 15 days, or to 60% moisture content. Seed moisture increased most gradually at 2°C and seeds held at 10°C attained a higher moisture level than at other temperatures. Germination percentage, germination rate, and seedling vigor were similar between treatments and controls regardless of seed moisture change. Imbibition temperature did not affect germination percentage or seedling vigor, but the time to 50% germination decreased with increasing imbibition temperature. We conclude that artificial seed moisture management did not affect germination percentage, germination rate, or seedling vigor of this species when tested under optimum moisture conditions. Germination is more related to habitat or genetic variables than initial seed moisture content.

Key Words: *Artemisia tridentata* ssp. *wyomingensis*, germination percentage, germination rate, seedling vigor, seed weight, seed dispersal

Restoring native shrubs to post-mining plant communities in a cost-efficient manner has remained a challenge to western reclamationists. The inherent low seedling vigor of shrubs generally makes direct seeding difficult. A better understanding of the seed physiology before planting can provide a basis for improving restoration technology. Advances in seed physiology, particularly in the area of seed rehydration, suggest that managing seed moisture and imbibition can enhance the transition from seed to seedling. Controlled imbibition has been used to improve seedling vigor and synchronize seedling emergence in a variety of species (Coolbear and McGill 1990, Heydecker and Coolbear 1977, Khan 1992, Roos et al. 1976, Taylor and Harman 1990). The early imbibition processes are generally considered reversible and seeds are desiccation tolerant as long as the radicle has not emerged (Bewley and Black 1985, Koller and Hadas 1982). During that time many changes, such as macromolecular repair, will occur in seeds (Bray 1995).

The influence of seed moisture on seed germination and seedling vigor of sagebrush is unknown. Given the studies discussed above, we hypothesized that increasing sagebrush seed moisture would improve germination percentage, germination rate, and/or seedling vigor. The purpose of this study was to test the effect of humidification on germination and seedling vigor of Wyoming big sagebrush (*Artemisia tridentata* Nutt. ssp. *wyomingensis* Beetle & Young), and relate this to initial seed moisture. Our objectives were to determine: 1) the relationship between initial seed moisture after harvest and seed germination or seedling vigor; 2) the rate of seed water uptake under humidification treatments as a function of temperature, and; 3) the effect of humidification treatment and the consequent imbibition temperature on seed germination and seedling vigor of Wyoming big sagebrush.

Materials and Methods

Experiment 1: Effects of seed moisture at harvest on seed quality

Seeds of Wyoming big sagebrush were collected from 5 locations in Wyoming in February 1994, when air temperature was below 0°C (Table 1). Twenty plants were selected randomly at each location and 7 seeds from each plant were sealed in an aluminum container in the field at the time of harvest. The seed moisture content of sealed seeds and the seed weight per 100 seeds were determined on a dry weight basis after drying for 24 hours at 80°C. The remaining seeds were put in paper bags, hand cleaned, and stored in the laboratory for approximately 2 weeks before the first germination test.

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Table 1. Site descriptions and seed characteristics for the 5 seed collections of Wyoming big sagebrush.

Collection	Date	Site	Moisture	Seed weight
			(%)	(g 100^{-1} seeds)
1	10 Feb. 94	S. of Hanna, Wyo.	9.0 a[1]	0.024 bc
2	11 Feb. 94	E. of Ft. Steele, Wyo.	5.6 b	0.027 b
3	10 Feb. 94	S. of Rock River, Wyo.	5.2 b	0.032 a
4	4 Feb. 94	Med. Bow Forest, Wyo.	3.3 c	0.022 c
5	4 Feb. 94	Roger Canyon Rd., Wyo.	2.3 c	0.022 c
Mean			5.3 ***[2]	0.025 ***

[1]Means with the same letter within a parameter are not significantly different at $P \leq 0.05$.
[2]The probability of significance among collections ≤ 0.005.

Twenty seeds from each collection were placed on 1 mm thickness germination paper on slant boards. Seeds were imbibed at 5°C in an incubator for 4 days, then incubated under 12-hour light for 14 days at 20°C because seedling vigor of some sagebrush seeds tends to be enhanced by low imbibition temperatures (Booth and Bai 1996). Germinated seeds were counted daily and were considered germinated if the radicle length was ≥ 1 mm. Germination rate was determined by the time required to reach 50% germination based on total germinated seeds. Seedling vigor was determined by measuring the axial length at the end of the germination test using a digitizing tablet (Booth and Griffith 1994). A completely randomized design with 4 replicates was used to test germination, germination rate, and vigor for each collection. Seed moisture content, seed weight, germination percentage, germination rate, and seedling vigor were analyzed with a one-way ANOVA to test differences among seed collections (Snedecor and Cochran 1980). Means were separated using LSD (Snedecor and Cochran 1980). Seed germination was also tested at 6 months and 2 years after seed harvest with seed moisture measured as described above.

Experiment 2. Seed water uptake during humidification and its influence on seed germination and seedling vigor

Two different seed collections were obtained from a commercial supplier. These lots were collected in late October 1993, from Lincoln County, Wyo., at 2,044 m elevation (Collection 1) and from a site near Casper, Wyo., at 1,624 m (Collection 2). Seed weight was 0.022 ± 0.001 and 0.023 ± 0.001 g per 100 seeds (mean ± S E) for Collections 1 and 2, respectively. After harvest, seeds were put in large woven polypropopylene bags and stored in an unheated warehouse for approximately 4.5 months before being processed with a 48" Simon-Day debearder for 10 min (for details see Booth et al. 1997).

Twenty seeds from each of the above 2 collections were put in 0.25 ml tin capsules weighing 0.16163 g. A pill plate with 4 columns and 22 rows (88 cells) was filled with 4 seed-containing capsules per cell. The columns served as blocks. Two of the 4 capsules in each cell were sealed as controls while the remaining 2 were left open for humidification. A 32 × 19 × 18 cm plastic box was filled to 10 cm depth with distilled water and a pill plate was placed 1 cm above the water surface. These boxes were then sealed inside clear plastic bags and placed in incubators at 2, 5, 10, or 15°C. This was a split-plot design within randomized complete blocks. Four replications were arranged over time as blocks.

Samples of both humidified and untreated seeds were collected at 0, 2, 4, 6, 8, 16, and 24-hour and 2, 5, and 15-day intervals. Immediately after being removed from incubators, open capsules were sealed to prevent seed moisture loss. These capsules were left for 1 hour at room temperature to allow the evaporation of surface water from the capsule exterior before weighing. Seed moisture content was measured with a CAHN-31 microbalance (6-place digital) after 24 hours of drying at 80°C. Seeds from all treatment intervals were imbibed and incubated immediately after humidification as described in Experiment 1, and seedling axial length was measured at the end of the germination test. Data were analyzed with ANOVA corresponding to the experiment design and means were separated with LSD (Snedecor and Cochran 1980).

Experiment 3. Effects of imbibition temperature

The same 2 seed collections used in Experiment 2 were humidified at 10°C for 0, 8, 24, 48, or 96 hours and seed moisture content was determined as described above. After humidification, seeds were imbibed at 2, 10, or 20°C in darkness for 4 days. Seeds were incubated for an additional 14 days at 20°C under 12-hour light. Seedling axial length was measured at the end of germination test. This was also a split-plot design within randomized complete blocks. Four replications were arranged over time as blocks and data were analyzed with ANOVA accordingly.

Results

Experiment 1: Effects of seed moisture at harvest on seed quality

The moisture level of seeds harvested in February ranged from 2.3 to 9.0% and seed weights ranged from 0.022 to 0.032 g per 100 seeds (Table 1). Germination percentage was highest and seeds germinated most rapidly (or required the least time for 50% germination) for Collections 2 and 3 (Table 2), which had the highest seed weight and intermediate moisture content. Seedling vigor was similar among collections.

Seed moisture content of the 5 collections ranged from 4.9 to 5.9% after 6 months of storage and from 3.9 to 4.5% after 24 months (data not shown). Interactions between storage duration and collection were significant for germination percentage and time to 50% germination, but not for seedling vigor.

Germination percentage increased after 24 months of storage for Collection 4, decreased after 6 months for Collections 2 and 3, and did not change for Collections 1 and 5 (Table 2). Time to 50% germination increased after 24 months of storage for Collections 2 and 3 with no change for the other 3 collections. Seedling vigor increased after 6 months, but decreased after 24 months.

Experiment 2. Seed water uptake during humidification and its influence on seed germination and seedling vigor

Seed moisture before humidification was similar for the 2 seed collections (Fig. 1). Seed water uptake during humidification was also similar. However, interactions among humidification, temperature, and time were significant. Seed moisture increased with humidification time at all temperatures. Significant moisture increase occurred after 16 hours at 2°C, after 4 hours at 5°C, and after 2 hours at 10 and 15°C; and seed moisture did not increase significantly after 5 days. Seed moisture was higher under 10°C humidification than under other temperatures after 5 days of humidification. There was no difference in moisture change for

Table 2. Germination percentage, time to 50% germination (T50), and seedling vigor of 5 collections of Wyoming big sagebrush at 3 testing times after harvest.

Collection	Germination	T50	Seedling vigor
	(%)	(day)	(mm)
0.5 months after harvest			
1	23 c[1] A[2]	6.5 a A	15.1 a B
2	87 a A	1.0 c B	16.2 a B
3	89 a A	1.0 c B	17.9 a A
4	43 b B	3.3 b A	16.7 a B
5	16 c A	4.5 ab A	20.8 a A
Mean	52 ***[3]	3.3 ***	17.3 NS
6 months after harvest			
1	26 c A	2.8 b A	22.9 a A
2	76 a A	1.5 b B	22.3 a A
3	61 ab B	1.3 b B	21.6 a A
4	48 b B	2.5 b A	22.4 a A
5	25 c A	5.3 a A	24.8 a A
Mean	47 ***	2.7 ***	22.8 NS
24 months after harvest			
1	32 c A	3.8 b A	21.1 ab A
2	52 b B	3.3 a A	14.2 c B
3	53 ab B	2.0 a A	19.4 b A
4	70 a A	2.8 a A	23.6 a A
5	30 c A	3.5 a A	24.7 a A
Mean	47 ***	3.0 NS	20.6 ***

[1]Means with the same letter within a testing time are not significantly different at $P \leq 0.05$.
[2]Means with the same letter within a collection are not significantly different at $P \leq 0.05$.
[3]The probability of significance among collections is ≤ 0.005.

non-humidified seeds sealed in capsules during humidification except after 15 days.

Germination percentage, germination rate, and seedling vigor were not affected by humidification and temperature (data not shown). Germination percentage was higher for Collection 2 than Collection 1 throughout the experiment, averaging 77 versus 65% (data not shown). The time to 50% germination was similar for the 2 collections, averaging 1.9 days. Vigor was higher for Collection 1 than Collection 2, averaging 23.5 vs. 22.5 mm.

Experiment 3. Effects of imbibition temperature

Seed moisture content after humidification was similar between collections. It was 4.1 ± 0.1, 19.8 ± 2.4, 33.3 ± 1.0, 41.8 ± 2.3, and 47.6 ± 1.2% after 0, 8, 24, 48, and 96 hours of humidification at 10°C, respectively, for Collection 1; it was 4.2 ± 0.2, 17.4 ± 0.4, 34.9 ± 1.2, 44.8 ± 1.9, and 49.4 ± 2.2% for Collection 2. Germination percentage was similar among humidification treatments and among imbibition temperatures, but it was higher for Collection 2 than Collection 1, averaging 73 and 78% (data not shown). Seedling vigor was higher for Collection 1 than for Collection 2, averaging 24.1 and 23.2 mm, but was similar among imbibition temperatures. The time to 50% germination decreased with increasing imbibition temperature (Fig. 2).

Discussion

The moisture content of Wyoming big sagebrush seeds in February varied among sites. Differences in seed moisture among collections were not the effect of weather conditions immediately before collection because temperatures were near or below 0°C and there was no snow on the plants at the time of harvest. Some

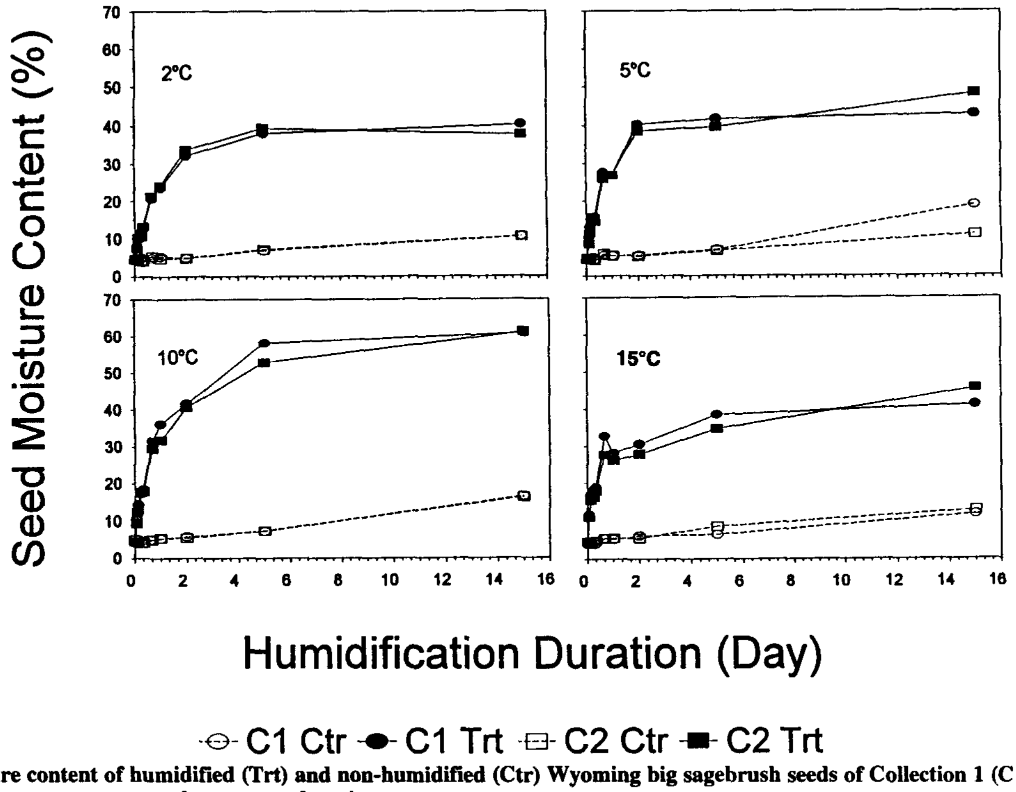

Fig. 1. Seed moisture content of humidified (Trt) and non-humidified (Ctr) Wyoming big sagebrush seeds of Collection 1 (C1) and Collection 2 (C2) at different temperatures and treatment durations.

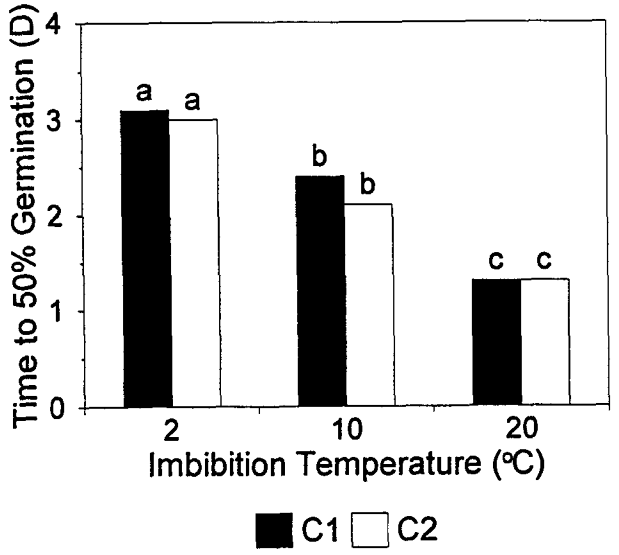

Fig. 2. Time to 50% germination for Wyoming big sagebrush seeds of Collection 1 (C1) and Collection 2 (C2) imbibed at 2, 10, and 20°C. Means with the same letter within a collection are not significantly different at $P \leq 0.05$.

other environmental factors such as topography, wind, relative humidity, and air temperature, as well as genetic variability and seed maturity, may have contributed to the varying seed moisture.

Seed moisture content near 5% at harvest was related to high and rapid germination when seeds were tested shortly after harvest. Germination percentage and germination rate, but not seedling vigor, were positively correlated with seed weight. The fact that germination percentage was not affected by post-harvest seed moisture change during storage indicates that germination is related more to habitat or genetic variations than the initial moisture content and that manipulating seed moisture of this species may not be beneficial. Germination percentage and seedling vigor increased in some seed collections during storage, reflecting a possible after-ripening requirement of Wyoming big sagebrush seeds as observed by Booth et al. (1997).

We observed during seed harvest in February that at least a portion of seeds were capable of staying on mother plants during the winter. Previous studies suggest that basin big sagebrush (*Artemisia tridentata* ssp. *tridentata* L.) generally shed seeds rapidly and completed dispersal before winter (Young and Evans 1989). However, there are large variations in the dispersal phenology of seeds among individuals within a population of fringed sagebrush (*A. frigida* Willd.) (Bai and Romo 1997). Therefore, variations in seed dispersal among and within populations of Wyoming big sagebrush should be considered in studies on seed dispersal phenology.

Seeds of Wyoming big sagebrush absorbed water quickly during humidification, hydrating faster at 10°C than at higher or lower temperatures, indicating an optimal temperature for maximum water uptake. Even though the seed moisture content reached as high as 60% after humidification, total germination percentage, time to 50% germination, and seedling vigor did not change. This is inconsistent with results from legume species such as soybean and snap bean, in which germination was enhanced by seed moisture manipulation (Roos et al. 1976, Obendorf and Hobbs 1970, Hobbs and Obendorf 1972). The difference may be due to seed size, because larger seeds are known to suffer mechanical stress as tissues hydrate (Spaeth 1989). In addition, germination in the present study was conducted under optimal water condition (no water stress). Germination percentage and germination rate of Lehmann lovegrass (*Eragrostis lehmanniana* Nees.) and side-oats grama (*Bouteloua curtipendula* (Michx.) Torr.) were not affected by increased seed moisture through priming when germinated at optimal conditions, but performance was improved when germinated under water stress (Hardegree and Emmerich 1992). Whether increasing moisture will affect seed germination or seedling vigor of Wyoming big sagebrush under environmental stress is unknown and will be addressed in future studies.

We conclude that seedling vigor of Wyoming big sagebrush is not influenced by increasing seed moisture before sowing. Seed moisture content at harvest reflects the plant environment, but environmental influences on seed quality can not be separated from those of ecotype. Seed weight is an important seed quality variable in sagebrush as in other species, and further study on this aspect may produce guidelines to help seed collectors select high quality seed lots.

Literature Cited

Bai, Y. and J.T. Romo. 1997. Seed production, dispersal, and the soil seed bank of fringed sagebrush. J. Range Manage. 50:151–155.

Bewley, J.D. and M. Black. 1985. Seeds: Physiology of development and germination. Plenum Press, N.Y.

Booth, D.T. and Y. Bai. 1996. Seedling vigor of eleven crops and shrubs related to imbibition temperature. Seed Sci. Res. (submitted).

Booth, D.T. and L.W. Griffith. 1994. Measuring post-germination growth. J. Range Manage. 47:503–504.

Booth, D.T., Y. Bai, and E.E. Roos. 1997. Preparing sagebrush seed for the market: Effects of debearder processing. J. Range Manage. 50:51–54.

Bray, C.M. 1995. Biochemical processes during the osmopriming of seeds. p 767–789. *In*: J. Kigel and G. Galili (eds.), Seed development and germination. Marcel Delcker, N.Y.

Coolbear, P. and C.R. McGill. 1990. Effects of a low-temperature presowing treatment on the germination of tomato seed under temperature and osmotic stress. Sci. Hort. 44:43–54.

Hardegree, S.T. and W.E. Emmerich. 1992. Seed germination response of four southwestern range grasses to equilibration at subgermination matric-potentials. Agron. J. 84:994–998.

Heydecker, W. and P. Coolbear. 1977. Seed treatments for improved performance—survey and attempted prognosis. Seed Sci.Technol. 5:353–425.

Hobbs, P.R. and R.L. Obendorf. 1972. Interaction of initial seed moisture and imbibition temperature on germination and productivity of soybean. Crop Sci. 12:664–667.

Khan, A.A. 1992. Preplant physiological seed conditioning. p 131–181. *In*: J. Janick (ed.), Horticultural Reviews. John Wiley and Sons, N.Y.

Koller, D. and A. Hadas. 1982. Water relations in the germination of seeds. p. 401–431. *In*: D.L. Lang, P.S. Nobel, C.B. Osmond, and H. Ziegler (eds.). Encyclopedia of plant physiology. New Ser. Vol. 12B. Springer-Verlag, Berlin.

Obendorf, R.L. and P.R. Hobbs. 1970. Effect of seed moisture on temperature sensitivity during imbibition of soybean. Crop Sci. 10:563–566.

Roos, E.E., J.R. Manalo, and B.M. Pollock. 1976. Effect of initial seed moisture on snap bean emergence from cold soil. J. Amer. Hort. Sci. 101:321–324.

Snedecor, G.W. and W.C. Cochran. 1980. Statistical methods (7th ed.). Iowa State Univ. Press. Ames, Iowa.

Spaeth, S.C. 1989. Extrusion of protoplasm and protein bodies through pores in cell walls of peas and faba bean cotyledons during imbibition. Crop Sci. 29:452–459.

Taylor, A.G. and G.E. Harman. 1990. Concepts and technologies of selected seed treatments. Ann. Rev. Phytopathol. 28:321–339.

Young, J.A. and E.A. Evans. 1989. Dispersal and germination of big sagebrush seed (*Artemisia tridentata*). Weed Sci. 37:201–206.