J. Range Manage.
47:485-488 November 1994

Cation concentration in post-imbibed winterfat seeds as influenced by imbibition temperature

D. TERRANCE BOOTH AND MILLER B. MCDONALD, JR.

Authors are range scientist, USDA-ARS, High Plains Grasslands, Research Station, 8408 Hildreth Road, Cheyenne, Wyo. 82009; and Professor, Department of Agronomy, Ohio State University, Columbus 43210-1086. *Pam Freeman and Janet Sullivan provided technical assistance.*

Abstract

Winterfat [*Eurotia lanata* (Pursh) Moq.] is a chenopod shrub often used in range seedings because of its palatability and nutritive value. Environmental conditions influence winterfat germination and seedling vigor and, therefore, seedling success. We tested the hypothesis that seed imbibition at warm temperatures damages seed membranes, resulting in lower post-imbibition concentrations of seed cations. Energy-dispersive x-ray microanalysis of Cl uptake and fresh weight increase over time gave evidence that winterfat seeds imbibe more rapidly at 25 than at 4°C. However, imbibition of seeds at 20°C did not result in consistently lower post-imbibition cation concentrations. Although imbibition is more rapid at warm temperatures, the post-imbibition concentration of cations in seeds imbibed at 20°C does not justify the conclusion that warm imbibition damages winterfat seed membranes.

Key words: *Eurotia lanata, Ceratoides lanata*, seedling vigor, seedbed environment, Ca, Mg, Na, K

Winterfat [*Eurotia lanata* (Pursh) Moq.; Ceratoides lanata (Pursh) J.T. Howell] is a low, chenopod shrub of western North America long valued on winter ranges where it furnishes palatable and nutritious forage for wildlife and livestock (USDA Forest Service 1937). Because of these desirable characteristics, it is commonly used in mine reclamation and range revegetation projects.

The winterfat diaspore consists of 2 connate bracts enclosing a pubescent utricle (Booth 1988). White hairs, 2 to 8 mm long, cover the bracts. The seed is flat, 3 to 4 mm long, 2 mm wide, and 1 mm thick. It has a thin testa and a well developed, peripheral-linear embryo lying obovoid around the perisperm. Bracts average 9,580, and seeds, 1,070 μg/g Ca. Seeds gain a significant quantity (635 μg/g) of Ca from the bracts during imbibition at 0 ± 2°C (Booth 1989). Winterfat and closely related species are favored by cool season seedings (Hilton 1941, Strickler 1956, Haferkamp et al. 1990, Shaw and Haferkamp 1990). Winterfat seedling vigor decreases as the temperature at which the seeds imbibe increases (Booth 1992). Why this occurs is not known. Booth (1992) proposed that rapid imbibition at warm temperatures damaged seed membranes; that as temperature increased, imbibition rate and seed damage also increased and seedling vigor decreased (Booth 1992). Our objective in studying the cation concentration in post-imbibed winterfat seeds was to test the imbibition-rate/membrane-damage hypothesis. The assumption was that damaged membranes would leak cations (Bewley and Black 1978, Duke et al. 1986). Therefore, if winterfat seeds did not imbibe more rapidly at the warmer temperatures, or if the post-imbibition concentration of seed cations was greater than, or equal to, that of seeds imbibed at cooler temperatures, we would reject the hypothesis.

Methods

Imbibition Rate

Material—A thoroughly mixed sample (1.0 g) of diaspores harvested at Cheyenne in 1986 and stored at 5°C was used. Diaspore germination as indicated by radicle protrusion was 55% using methods suggested by Booth (1992).

Treatments—To test the effect of temperature on imbibition rate, and to identify the order of hydration of seed parts, seeds were carefully hand threshed, so that testas were intact, then imbibed in 5% sodium hypochlorite (commercial bleach) at 4 or 25°C for 1, 4, and 8 hours. Seeds were then analyzed for Cl (in ClO^-) uptake by energy-dispersive X-ray microanalysis (EDAX). Since ClO^- is soluble in water and since Cl is not normally found in high concentrations in dry seeds (Cl^- is a toxic anion), seed Cl was an indirect measure of water movement into the seeds. Following soaking, the seeds were fixed to a carbon planchet using silver paint, then viewed and photographed on an ISI 40 Scanning Electron Microscope[1] (SEM) at 30 kV. Seed tissue was analyzed for Cl and P using an EDAX 9900 Series Wave Length Dispersive X-ray Spectrometer mounted on the SEM. Each element emits a characteristic energy pattern when exposed to electrons from the SEM. This allows for spectrometric analysis of very small points of seed tissue. Because the spectrometer data are not absolute, and because P levels in imbibing seeds are unlikely to change, Cl concentration was measured relative to P for each soaking interval. Therefore Cl is expressed as a percentage of the amount of P measured. [Phytin is an insoluble compound of K, P, Mg, and Ca salt of phytic acid and a major phosphate reserve in seeds. Phytic acid breakdown is a late event in the germination of seeds, usually occurring during growth (Bewley and Black 1978, p. 223). Since phytic acid breakdown and the release of P into solution is a late event, the amount of P in a point of tissue is constant even though the concentration on a fresh weight basis may be changing.]

Manuscript accepted 30 Apr. 1994.

[1]Mention of trade names/dealers is for information only and does not imply an endorsement by USDA.

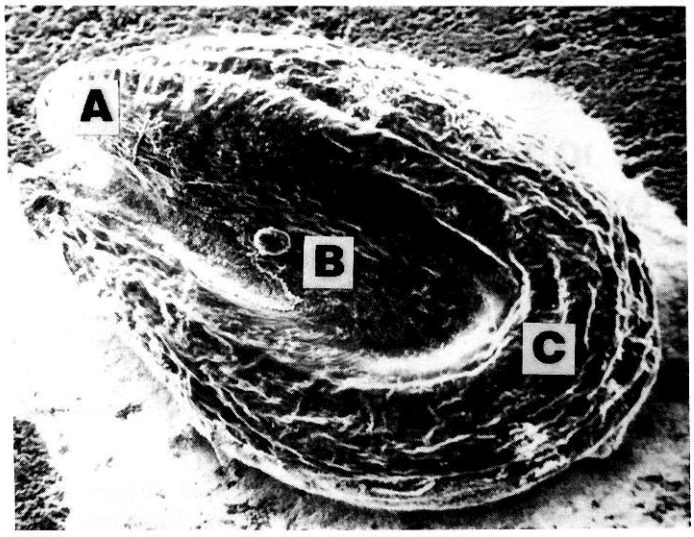

Fig. 1. Scanning electron microscope image of a winterfat seed showing seed parts analyzed by energy-dispersive x-ray microanalysis. A = cotyledon apex, B = perisperm, C = hypocotyl.

Three parts of the seed were evaluated, the cotyledon apex, the perisperm, and the hypocotyl at a point opposite the apex (Fig. 1). Each seed part was evaluated in the center of the structure. Data obtained for the hypocotyl were limited, therefore the values reported for each seed represent the mean of the cotyledon apex and the perisperm.

The EDAX analysis followed a preliminary test of the effect of temperature on imbibition rate. Threshed seeds were placed in water and allowed to imbibe at 4 or 25°C for 1, 4, and 8 hours, then blotted dry and weighed as 1 group of 5 seeds imbibed at each time and temperature.

Experimental Design

A completely random experimental design was used with at least 2, and generally 4 replications. This varied because the seeds placed on the carbon planchet were sometimes inadvertently covered with the silver cementing material and could not be evaluated. An analyses of variance for unequal subclasses (SAS) was used and comparisons made for seed Cl uptake by temperature, and Cl uptake by time and temperature. The fresh weight gain by imbibing seeds is not replicated and is presented as additional information.

Seed Leakage

Seed leakage, an indication of membrane damage, is commonly tested by measuring the conductivity of the water in which the seed is imbibed. Since significant amounts of cations, particularly calcium, are stored in winterfat bracts and utricle, a conductivity test might be confounded by cations stored outside the seed. This is particularly true when testing the effect of imbibition temperature on seeds imbibed as intact diaspores. Therefore we elected to use atomic absorption spectrophotometry to measure cation concentrations in the seed and to determine if imbibition temperature influenced concentration.

Material—Diaspores were harvested in Colorado in 1991 by Wind River Seed, Manderson, WY (Lot #1, stock #737). Diaspore germination was 49% (tag).

Treatments—Diaspores were thoroughly mixed, subsamples randomly collected, and a portion hand threshed as before. Seeds and diaspores were humidified by placing them on filter paper held above distilled water at 2°C for 24 hours for seeds, and 48 hours for diaspores. This increased seed moisture to 20% (dry weight basis), reducing the chance of imbibitional injury (Vertucci and Leopold 1984, Vertucci 1989) and duplicating the procedure followed by Booth (1992). Seeds and diaspores were then soaked in deionized water at 5 or 20°C with agitation. Twenty-five ml water was used for seeds and 100 ml for diaspores. The 2-gram samples of threshed seeds were imbibed for 1, 4, and 8 hours. Diaspore samples weighed 5 g and were imbibed for 8, 16, and 32 hours. Imbibition times differed because of the longer hydration time required for diaspores. After soaking, the material was drained for 30 minutes on Whatman 40 filter paper, then oven dried at 60°C for 24 hours. After drying, seeds were hand threshed from the diaspores. A control treatment consisted of a randomly collected subsample of threshed seeds that was humidified and oven dried, but not imbibed. Cation analysis for Ca, Na, K, and Mg by atomic absorption spectroscopy used standard methods.

Experimental Design—A randomized complete block with 4 replications was used. All treatments within a replication were begun the same day. Replications were separated by 1-week intervals. After the first analysis was completed, a second subsample of each treatment was weighed and the analysis repeated giving 2 observations for each experimental unit. Two analyses of variance were conducted. In the first, the control was not included in the analysis and differences in cation concentrations by imbibition temperature (1 df) and imbibition time (2 df) were tested. The second analysis tested for cation differences among 7 treatments composed of all combinations of temperature and time, plus the control. Dunnett's procedure was used to compare the control with all other treatments. To reduce the chance of a Type II error (accepting the null hypothesis of no difference in cation concentration due to imbibition treatment when there was an effect), we used a significance level of $P< 0.20$ (Steel and Torrie 1980, p 91).

Table 1. Concentrations of cations in winterfat seeds as influenced by imbibition temperature and time. Data on unimbibed seeds are presented for comparison. The observed significance level (OSL) is indicated.

	Ca	Na	K	Mg
		-µg/g		
Unimbibed Threshed				
Mean ± SD	1,137 ± 56	122 ± 28	13,282 ± 263	1,863 ± 96
Imbibed Threshed - Temperature effect				
5°C	1,107	97	11,782	1,929
20°C	1,160	105	12,035	1,919
OSL	0.13	0.50	0.05	0.87
Imbibed as Diaspore - Temperature effect				
5°C	1,198	103	11,677	1,925
20°C	1,278	110	11,201	1,937
OSL	0.12	0.50	<0.01	0.62
Imbibed Threshed - Time effect				
1 hour	1,183	108	12,088	1,906
4 hour	1,115	95	12,016	1,936
8 hour	1,099	100	11,622	1,929
OSL	0.11	0.64	<0.01	0.92
Temp x Time OSL:	0.49	0.77	0.75	0.99
Imbibed as Diaspore - Time effect				
8 hour	1,202	98	11,754	1,896
16 hour	1,201	112	11,358	1,928
32 hour	1,311	110	11,206	1,953
OSL	0.14	0.45	0.01	0.06
Temp x Time OSL:	0.91	0.77	0.43	0.65

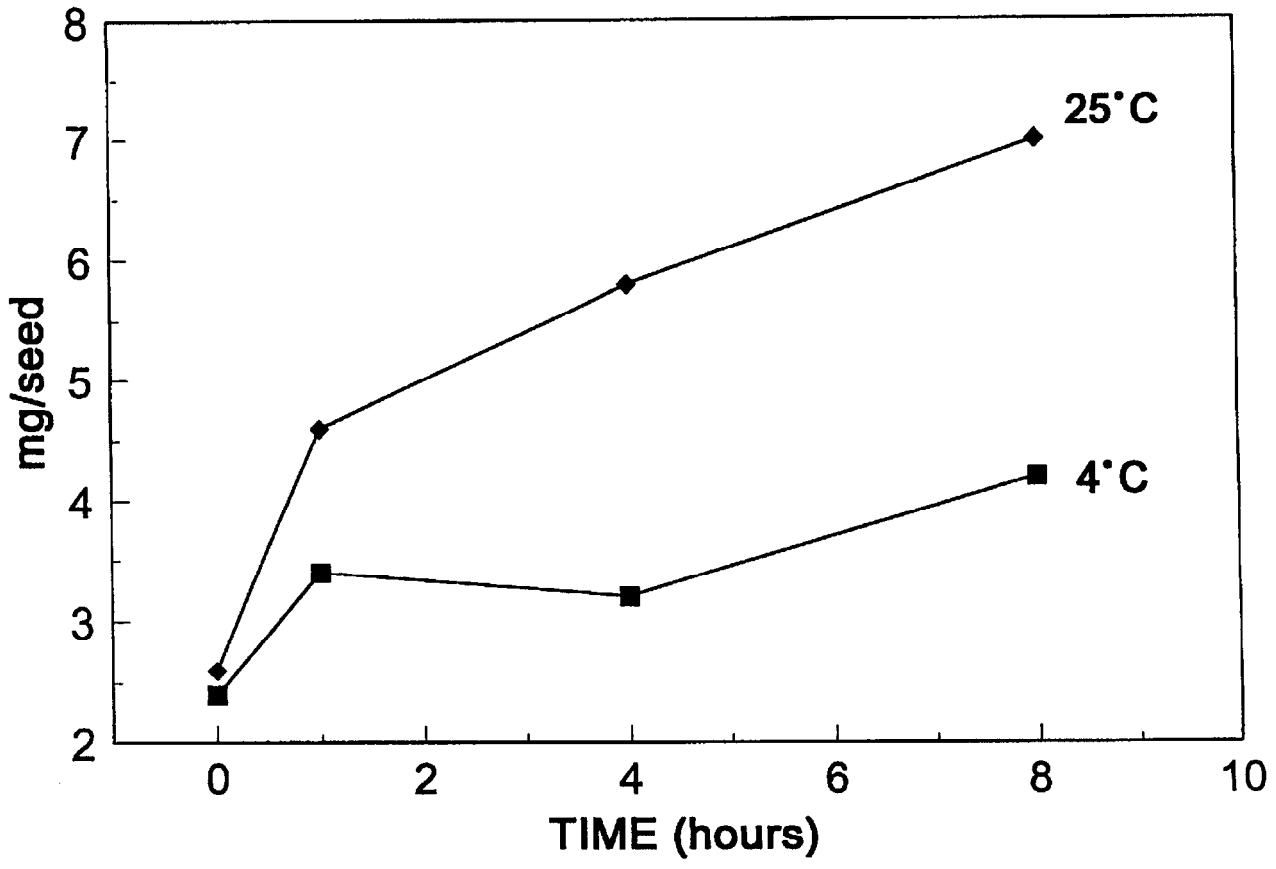

Fig. 2. Fresh weight increase of winterfat seeds at 4 and 25°C imbibition temperatures.

Results

Imbibition Rate

Seeds imbibed at 4°C had less Cl (average percent relative to P) than seeds imbibed at 25°C (71.0 vs 79.7% when averaged over all imbibition times). The observed significance level (OSL) was P=0.057. Fresh weight gain by imbibing seeds also indicated that the imbibition rate at 4°C was less than for seeds imbibed at 25°C (Fig. 2). Chlorine- concentration did not change over time (OSL=0.94), perhaps due to the large amount of uptake which occurred during the first hour, and the time-temperature interaction was not significant (OSL=0.96). The rapid uptake of water by threshed seeds is attributed to the very thin testa of winterfat.

Seed Leakage

Imbibition temperature caused a significant change ($P \leq 0.20$) in the concentration of Ca and K; however, differences due to temperature indicated lower cation concentrations at 5, rather than at 20°C except K in seeds imbibed as diaspores (Table 1). Soaking time influenced Ca and K, and Mg for seeds imbibed as diaspores. Threshed seed Ca and K decreased as soaking time increased, but Ca and Mg increased with soaking time when imbibed as diaspores. This is consistent with previous findings (Booth 1989). Potassium in seeds imbibed as diaspores decreased over time. When imbibed seeds were compared with control seeds in the second analysis of variance, K (all treatments), and Mg (1 treatment) concentrations differed by imbibition treatment (data not shown). Again, there was no consistent evidence of cation loss due to imbibition at 20°C.

Discussion and Conclusions

No convincing evidence of membrane damage due to rapid imbibition at 20°C was obtained. In fact, the lower cation concentrations found for Ca and K (threshed) and Ca (diaspore) at 5, rather than at 20°C, and the lack of differences for Na and Mg (Table 1) is the opposite of what would be expected if rapid imbibition at 20°C damaged seed membranes. We conclude that for winterfat, as with other seeds, warm imbibition (20°C) is more rapid than cold imbibition (Shell 1920, Allerup 1958, Vertucci 1989); however, the cation level in seeds imbibed at 20°C does not suggest excessive loss of cations such as would occur with membrane damage. The greater seedling vigor of winterfat seeds imbibed at low temperatures is most likely due to factors other than membrane damage during warm temperature imbibition.

Revegetation has been a part of range management from the time the discipline was recognized as a separate science. To advance the science, we must do more than document success and failure of the seed sown. We must understand why, and develop innovative management that applies this knowledge. The work reported in this paper does not answer why warm imbibition decreases winterfat seedling vigor. It does quite clearly suggest that membrane damage is not the answer.

Literature Cited

Allerup, S. 1958. Effect of temperature on uptake of water in seeds. Plant Physiol. 11:99-105.

Bewley, J.D., and M. Black. 1978. Physiology and biochemistry of seeds. Vol. I. Springer-Verlag, N.Y.

Booth, D.T. 1988. Winterfat diaspore morphology. J. Range Manage. 41:351-352.

Booth, D.T. 1989. Seedbed ecology of winterfat: cations in diaspore bracts and their effect on germination and early plant growth. J. Range Manage. 45:178-182.

Booth, D.T. 1992. Seedbed ecology of winterfat: Imbibition temperature affects post-germination growth. J. Range Manage. 42:159-164.

Duke, S.H.,G. Kakefuda, C.A. Henson, N.J. Loeffler, and N.M. Van Hulle. 1986. Role of the testa epidermis in the leakage of intracellular substances from imbibing soybean seeds and its implications for seedling survival. Physiol. Plantarum 68:625-632.

Haferkamp, M.R., D.C. Ganskopp, K.L. Marietta and B.W. Knapp. 1990. Environmental influences on germination of utricles and seedling establishment of 'Immigrant" forage kochia. J. Range Manage. 43:518-522.

Hilton, J.W. 1941. Effects of certain micro-ecological factors on the germinability and early development of Eurotia lanata. Northwest Sci. 15:86-92.

Shaw, N.L., and M.R. Haferkamp. 1990. Field establishment of spiny hopsage, p. 193-199. *In*: E.D. McArthur et al. (compilers), Proc.- Symp. on cheatgrass invasion, shrub die-off, and other aspects of shrub biology and management. USDA-Forest Service Gen. Tech. Rep. INT-276. Ogden, U.

Shull, C.A. 1920. Temperature and rate of moisture intake in seeds. Bot. Gaze. 69:361-390.

Steel, R.G.D. and J.H. Torrie. 1980. Principles and procedures of statistics. McGraw-Hill Book Company, NY.

Strickler, G.S.S. 1956. Factors affecting the growth of whitesage Eurotia lanata (Pursh.) Moq. M.S. Thesis, Univ. Nevada, Reno.

USDA Forest Service. 1937. Range plant handbook. U.S. Gov. Printing Office, Washington, D.C.

Vertucci, C.W. 1989. The effects of low water content on physiological activities of seeds. Physiol. Plant. 77:172-176.

Vertucci, C.W. 1989. The kinetics of seed imbibition: controlling factors and relevance to seedling vigor. P. 93-115. *In*: Seed moisture, CSSA Special Pub. 14. Crop Sci. Soc. Amer.., Madison, WI.

Vertucci, C.W. and A.C. Leopold. 1984. Bound water and its relation to respiration and imbibitional damage. Plant Physiol. 75:114-117.