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Imbibition temperature affects winterfat *(Eurotia lanata* (Pursh) Moq.) seed hydration and cold-hardiness response

YUGUANG BAI, D. TERRANCE BOOTH, AND J.T. ROMO

Authors are range ecologist, Agriculture and Agri-Food Canada, Kamloops Range Res. Unit Kamloops, BC, V2B 8A9, Canada, rangeland scientist, USDA-ARS, Cheyenne, Wyo. 82009, USA, and professor, Dept. of Plant Sciences, Univ. of Saskatchewan, Saskatoon, SK, S7N 5A8, Canada. At the time of the research Bai was research scientist, Dept. of Plant, Soil and Insect Sciences, Univ. of Wyoming, Laramie, Wyo. 82071, USA.

Abstract

Winterfat (*Eurotia lanata* (Pursh) Moq.) diaspores harvested from 1 Canadian and 2 USA sites were imbibed at 0, 5, 10, and 20°C. It was hypothesized that imbibition temperature affects seed hydration which is related to cold-hardiness of winterfat. Weight gain was measured at 8-hour intervals until full hydration, and embryo water content was determined. Water content of fully hydrated seeds differed among collections and lower imbibition temperatures were always associated with greater seed water content. Differences in water content of seeds imbibed at different temperatures was related to cold-hardiness. When water content of embryos was measured, differences among imbibition temperatures existed, but were reduced. Differences in seed water content among imbibition temperatures were mainly due to endosperm other than the embryo because the embryo hydrated faster than other seed parts. Suggestions were made for modeling seed water relations based on this study.

Key Words: *Krascheninnikovia, Ceratoides*, embryo, threshold water content.

Resumen

Semillas de "Winterfat" (*Eurotia lanata* (Pursh) Moq.) cosechadas de un sitio canadiense y dos de U.S.A fueron imbibidas a 0, 5, 10, y 20°C. Se hipotetizó que la temperatura de imbibición afecta la hidratación de la semilla la cual esta relacionada con la resistencia al frío del "winterfat". La ganancia de peso se midió a intervalos de 8 horas hasta la hidratación total y se determinó el contenido de agua del embrión. El contenido de agua de semillas completamente hidratadas difirió entre colecciones y las bajas temperaturas de imbibición siempre estuvieron asociadas con un mayor contenido de agua de la semilla. La diferencia del contenido de agua de las semillas imbibidas a diferentes temperaturas fue relacionada a la resistencia al frío. Cuando se midió el contenido de agua de los embriones existían diferencias entre las temperaturas de imbibición, pero eran mínimas. Las diferencias en el contenido de agua de las semillas entre temperaturas de imbibición fueron debidas principalmente al endospermo mas que el embrión, esto porque el embrión se hidrató más rápido que otras partes de la semilla. Basado en este estudio se hicieron sugerencias para modelar las relaciones hídricas de la semilla.

Environmental stress affects plants at all life stages, though dry seeds are most tolerant. As seed water content increases, physiological activities of seeds are increasingly influenced by temperature-water interactions. Many seed studies have dealt with species that are injured by imbibing cold water (Hobbs and Obendorf 1972, Ashworth and Obendorf 1980, Herner 1986). Few have examined basic themes using seeds with unusual tolerance to extremes, such as winterfat (*Eurotia lanata* (Pursh) Moq.), which may be unaffected or even benefited by cool conditions (Booth 1992).

Winterfat seeds can germinate over a wide range of temperatures (Dettori et al. 1984), including 0 or near 0°C (Hilton 1941, Woodmansee and Potter 1971, Dettori et al. 1984, Booth 1987). A recent study indicated that hydrated winterfat seeds were tolerant to cooling as low as –30°C even after the occurrence of the low temperature exotherm; cold-hardiness measured by seed germination percentage and rate depended on imbibition temperature (Bai et al. 1998). Since seed water relations are also related to cold-hardiness, a closer look at hydration processes of winterfat seeds may provide additional information on its cold-hardiness mechanism.

Seeds must reach a threshold water content before germination starts. Water content at the onset of germination was the same among osmotic conditions (Bradford 1986, Gray et al. 1990) or certain temperatures (Gummerson 1986). On the other hand, if different seed parts imbibe water at different rates, then using the whole seed water content as a pre-condition for seed germination may be questionable. In the study reported here, seed hydration of winterfat was tested at temperatures ranging from 0 to 20°C. Objectives of this study were to determine effects of imbibition temperature on seed water in relation to cold-hardiness and to incipient germination in winterfat.

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Materials and Methods

Seed sources and post-harvest handling

Winterfat diaspores (seed-containing dispersal units) were hand-collected in October 1994 from Matador, Saskatchewan, Sterling, Colo., and Pine Bluffs, Wyo. (Table 1). The first 2 sites are located in Mixed Prairie while the third is located in Shortgrass Prairie (based on a map by Dodd; in Tetlyanova et al. 1990). Harvested diaspores were stored in paper bags at room temperature until February 1995. Dry weight of threshed seeds from Matador and Sterling were 38 and 28% heavier than from Pine Bluffs.

Seed water content as affected by imbibition temperature

We placed diaspores of the 3 collections on moistened germination paper (Anchor Paper Corp., St. Paul, Minn.) over plastic slant-boards, and covered them with 1 layer of cellulose tissue (Jones and Cobb 1963). Slant-boards were then placed in closed germination boxes (25 x 40 x 20 cm), which were filled with distilled water to 3-cm depth. These boxes were placed in incubators at 0, 5, 10, and 20°C in darkness. The experimental design was a randomized complete block with 3 replicates arranged over time.

For each experimental unit (slant board), we retrieved 10 diaspores from incubators at 8-hour intervals until full hydration (1 to 8 days, depending on imbibition temperature). Bracts were removed and surface water of seeds was blotted away with tissue paper. Seeds were then sealed in 0.25 ml tin capsules (Leco Corp., St. Joseph, Mich.) and weighed (Booth and Bai 1998) before being oven dried at 80°C for 24 hours and dry weight determined. Weighing was done with a micro-balance to 0.001 mg and seed water content was expressed on a dry weight basis (%DW). We defined full hydration as the stage 8 hours before germination began.

Comparison of water contents of seeds and embryos at full hydration

The above imbibition procedures were repeated in June 1995 (4 months after the above study), with 4 replicates for each collection. At the end of imbibition, embryos of half of the seeds were separated from other seed parts and the water contents of both seeds and embryos were determined as described above.

Data analysis

Data were first analyzed with ANOVA or GLM in a randomized complete block design (Snedecor and Cochran 1980) over the 3 seed collections, and then analyzed within each collection because of the interaction between seed collection and imbibition temperature. Data were further analyzed separately for each imbibition temperature. Statistical significance was assumed at $P \leq 0.05$ and means were separated using LSD.

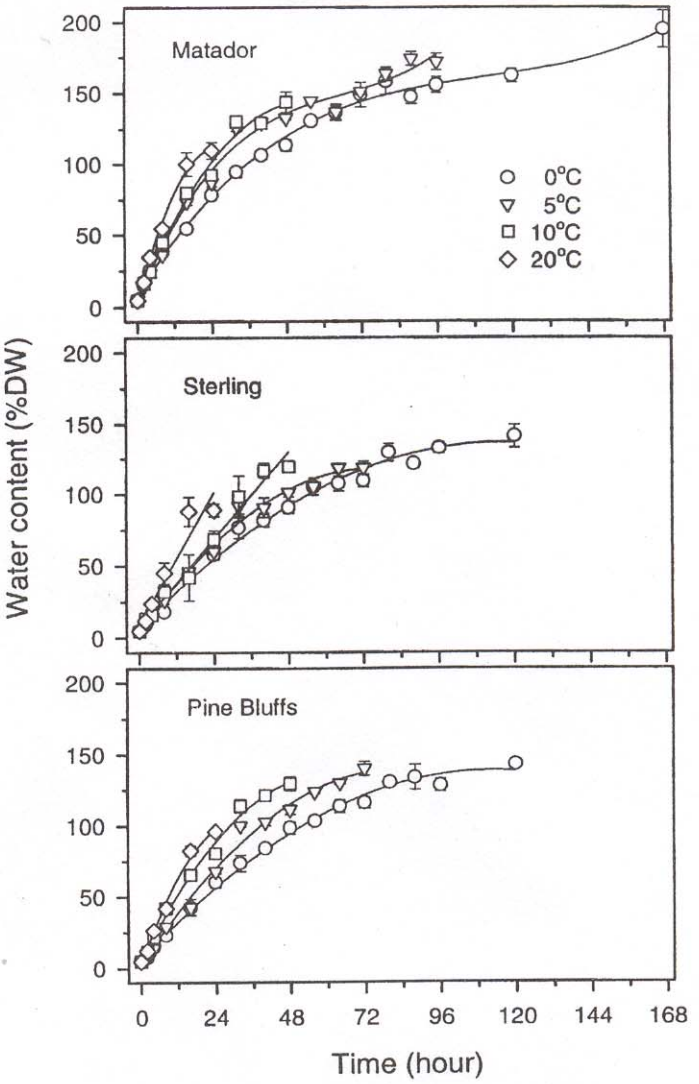

Fig. 1. Water content (mean±SE) of winterfat seeds collected from Matador, Sask., Sterling, Colo., and Pine Bluffs, Wyo. after imbibition at 0, 5, 10, or 20°C. See Table 2 for regression equations.

Results

Seed water content as affected by imbibition temperature

Initial seed water content was similar among seed collections, averaging 4.8% (Fig. 1). However, the rate of water uptake during imbibition, time required to reach full hydration, and seed water content when fully hydrated depended on seed collection and imbibition temperature. Water content of seeds at the time of germination decreased with increasing imbibition temperatures. As expected, the rate of seed water uptake increased, and time required to reach full hydration decreased with increasing imbibition temperature.

Seeds from Matador required more

Table 1. Descriptions of sources and habitats for winterfat seeds used in the study.

Site	Geographical location	Vegetation and dominant species	Seed weight (g 100 seeds^{-1})
Matador	Saskatchewan, Canada 50°42'N, 107°43'W, elev. 685 m	Mixed Prairie *Agropyron dasystachyum* (Hook.) Scribn *Agropyron smithii* Rydb. *Koeleria cristata* Pers. *Stipa viridula* Trin. *Eurotia lanata* (Pursh) Moq.	0.25
Sterling	Colorado, USA 40°37'N, 103°13'W, elev. 1181 m	Shortgrass Prairie *Bouteloua gracilis* (H.B.K.) Lag. *Buchloe dactyloides* (Nutt.) Engelm.	0.23
Pine Bluffs	Wyoming, USA 1°10'N, 104°09'W, elev. 1554 m	Mixed Prairie *Stipa comata* Tri. & Rupr. *Agropyron smithii* Rydb. *Bouteloua gracilis* (H.B.K.) Lag.	0.18

time to reach full hydration, particularly at 0 and 5°C, than those from Sterling or Pine Bluffs (Fig. 1). When seeds were imbibed at 0°C, seed water content at full hydration was greater for the Matador collection than the Sterling or Pine Bluffs collections. At 5, 10, and 20°C, seed water content was highest for the Matador collection and lowest for the Sterling collection.

The relationship between seed water content and time after imbibition were described in linear, quadratic and cubic equations (Fig. 1, Table 2). Phases I and II during imbibition (as defined by Ching 1972, 1973) were more distinct for seeds imbibed at lower temperatures than those imbibed at higher temperatures.

Comparison of water contents of seeds and embryos at full hydration

Water content of seeds and embryos at full hydration was similar among collections (P = 0.26 and 0.17 for seeds and embryos, respectively) and data were pooled (Fig. 2). Seed water content again decreased with increasing imbibition temperatures, ranging from 169 to 231%. Water content of embryo decreased between 0 and 10°C imbibition temperatures, but increased at 20°C, with the range from 94 to 114%.

Discussion

The definition of full hydration of seeds has largely depended on researchers and the intervals used for seed water content determination. Keefe and Moore (1981) used the middle point of Phase II during germination, or 24 hours before germination started. Others imbibed seeds for given durations regardless of the physiological stage, and simply called them "imbibed" or "hydrated" seeds (Ishikawa and Sakai 1982, Gray and Steckel 1983, Cremer and Mucha 1985). In the present study we defined full hydration as 8 hours before germination started, or the end of Phase II. Seed water content at full hydration varied by seed collection, but was always greater with lower imbibition temperatures. The greater water content at 0°C explains the warmer low-temperature exotherm for seeds imbibed at lower temperatures than those imbibed at higher temperatures (Bai et al. 1998).

The fact that embryo water content was much lower than whole seed water content indicates that the rate of hydration, and the water holding capacity, vary among seed parts. Faster hydration in the embryo than in the endosperm was found in several species such as corn (*Zea mays* L.) (Styles 1948, Vertucci 1989). The seed coat of winterfat is membranous and highly permeable (Booth and McDonald 1994), enabling seeds to absorb water quickly and store it in the endosperm. Nevertheless, seed hydration generally increases with increasing temperature (Shull 1920, Allerup 1958) and at warmer temperatures the embryo may hydrate faster than the endosperm. Variation in hydration rate explain the lower whole-seed water content at higher temperatures than at lower temperatures. Therefore, water content of the whole seed may not accurately reflect the true water status related to seed germination. Instead, the water content of an embryo is a better measurement of germination readiness than that of the whole seed, at least for seeds similar to winterfat.

Table 2. Regression equations describing relationships between water content (Y, %DW) and time after imbibition (X, hour) of seeds collected from Matador, Sask., Sterling, Colo., and Pine Bluffs, Wyo. Curves are presented in Fig. 1.

Seed Collection	Imbibition Temperature	Regression equation	R^2	P-value
Matador	0	$Y=8.58+3.49X-0.0286X^2+0.000085X^3$	1.00	0.000
	5	$Y=2.96+5.39X-0.0722X^2+0.000364X^3$	0.99	0.000
	10	$Y=5.94+5.13X-0.0477X^2$	0.99	0.000
	20	$Y=2.85+8.35X-0.1600X^2$	0.99	0.000
Sterling	0	$Y=6.66+2.31X-0.0096X^2$	0.99	0.000
	5	$Y=3.75+3.03X-0.0201X^2$	0.99	0.000
	10	$Y=7.46+2.56X$	1.00	0.001
	20	$Y=2.60+5.37X$	1.00	0.001
Pine Bluffs	0	$Y=5.89+2.36X-0.0105X^2$	0.99	0.000
	5	$Y=2.45+3.30X-0.0198X^2$	0.99	0.000
	10	$Y=4.92+4.38X-0.0367X^2$	0.99	0.000
	20	$Y=2.14+6.29X-0.0958X^2$	0.99	0.001

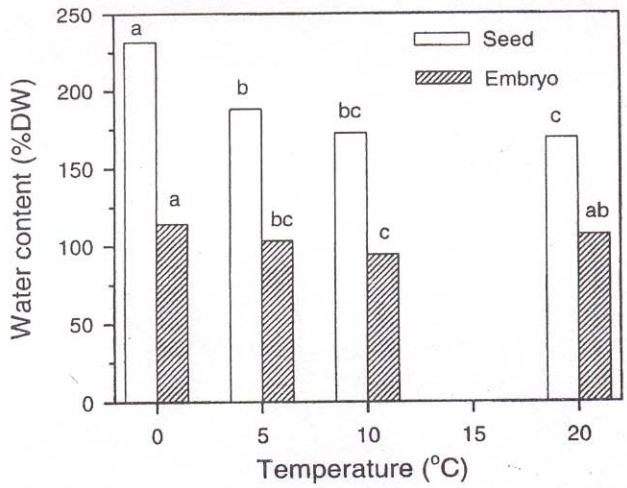

Fig. 2. Water content of seeds and embryos at full hydration at 0, 5, 10, or 20°C imbibition temperature for winterfat seeds collected from Matador, Sask., Sterling, Colo., and Pine Bluffs, Wyo. Data were pooled for collections and means with the same letter within seed or embryo were not significantly different at P≤ 0.05.

The dependence of seed water content at full hydration on imbibition temperature has been observed in seeds of other species (C.W. Walters, personal communication), but has not, to our knowledge, been reported. The finding implies, the critical moisture content concept (Hunter and Erickson 1952) and the hydrothermal time model (Gummerson 1986, Dahal et al. 1993) of seed germination must be modified based on embryo water content, and expanded to a wider temperature range, particularly for temperatures near 0°C . What remains unanswered is the existence of differences in embryo water content among imbibition temperatures. One possibility is that cold cell walls with lower energy level provide greater resistance to cell extension and elongation. Therefore, greater turgor pressure (higher water content) is required for germination to proceed.

In summary, differences in seed water contents were related to cold-hardiness of winterfat. Higher seed water content at full hydration was associated with lower imbibition temperatures, apparently a result of different hydration rates between embryo and endosperm. Further studies using embryos instead of the whole seeds over a wider range of temperatures are needed for modeling water relations for seed germination.

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