J. Range Manage.
51:709–713

Developmental stages of winterfat germinants related to survival after freezing

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Abstract

Diaspores of winterfat (*Eurotia lanata* (Pursh) Moq.) collected from 2 locations in the USA and 1 in Canada were imbibed at 10° C and grown to 4 different developmental stages (2, 3, 6, and 14 days of incubation), then subjected to cooling temperatures as low as -30°C. Differential thermal analysis was used to detect exotherms associated with ice crystal formation in germinants. The temperature at which exotherms occurred was recorded, and the subsequent growth and mortality of germinants were determined. Only 1 exotherm was observed, and that occurred in the low-temperature exotherm range (usually <-10°C). Changes in the freezing tolerance of germinants from seed to seedling was a gradual process as indicated by increases in exothermic temperature and mortality with increasing developmental stage. Whether the exotherm indicated a lethal event depended on the developmental stage of the germinant. Germinant survival was also affected by cooling below the exotherm temperature.

Key Words: *Eurotia lanata*, seedbed ecology, exotherm, freezing tolerance.

Winterfat,(*Eurotia lanata* (Pursh) Moq.) is a common half-shrub in the Mixed Prairie of North America (Coupland 1950). This species provides excellent forage to livestock and wildlife in this region, and is being used for wildlife habitat reclamation. One problem associated with the revegetation of this species is the high mortality of first year seedlings (Hodgkinson 1975, J.T. Romo, personal observation). Because winterfat seeds are capable of germinating near 0°C in the fall or spring (Hilton 1941, Woodmansee and Potter 1971, Booth 1992), freezing events after seed germination may contribute to high seedling mortality.

Two distinct freezing events may occur during the cooling of plant tissues. As freezing is an exothermic reaction, these events can be detected by the released heat of fusion which causes a sharp temperature rise (Burke et al. 1976, Ashworth 1982). The initial temperature rise (high temperature exotherm) usually occurs at temperatures between 0 and -10°C with the second rise (low temperature exotherm) at temperatures below -10°C. Freezing stress reduces germination rate and seedling vigor of hydrated winterfat seeds, but total seed germination is not affected even after the occurrence of a low temperature exotherm (Bai et al. 1998). Seedlings from germinated seeds incubated at 15/5°C up to 28 days were sensitive to cooling temperatures as high as -6.5°C (Hou and Romo 1997). However, the freezing tolerance of winterfat at stages between radicle emergence and seedling establishment remains unknown.

Stushnoff and Junttila (1978) reported that the low temperature exotherm for lettuce (*Lactuca sativa* L.) seeds disappears after radicle emergence, and the high temperature exotherm represents the killing point of the seed. The moisture content, cell size, and the rate of physiological activities of germinating seeds increase gradually after radicle emergence (Booth 1989, Wesley-Smith et al. 1995). Ice crystal formation in cells is related to their moisture content (Stushnoff and Junttila 1978). We hypothesized that decreased freezing tolerance of winterfat germinants during development is a gradual process, and that this decrease contributes to the death of young seedlings in the field. Specific objectives were to determine: 1) temperatures at which exotherms occur; 2) the relationship between exotherm occurrence and the survival and growth of winterfat germinants after cooling; and 3), how freezing tolerance is related to developmental stage.

Materials and Methods

Seed Sources and Habitats

Winterfat diaspores (seed-containing dispersal units) were hand-collected in October, 1994, from Matador, Saskatchewan, Canada; Sterling, Colo., USA and Pine Bluffs, Wyo., USA. The first 2 sites are located in the Mixed Prairie while the third is located in Shortgrass Prairie (Tetlyanova et al. 1990). The Matador site is located approximately 70 km north of Swift Current, Saskatchewan within a glacial lake near the northern edge of the Mixed Prairie (Coupland 1950). The soils are Rego Brown and Calcareous Brown (Coupland et al. 1974). The climate is cold and semiarid, and soil moisture is the limiting factor for plant growth (Fig. 1). Annual mean temperature is 3° C with precipitation totalling 327 mm. The Sterling, Colo., site is approximately 155 km east of Fort Collins, Colo., and Pine Bluffs, Wyo., is approximately 63 km east of Cheyenne, Wyo. The climate is semiarid with the high elevation and dry air contributing to the wide seasonal and diurnal variation in temperature

This research was supported by funds from Saskatchewan Agric. Development Funds, Canada-Saskatchewan Agric. Green Plan Agreement and Ducks Unlimited Canada to JTR. Authors thank Dr. G. V. Richardson for statistical assistance, and Drs. J. Hou, Z. Ristic, and J. Walker for reviewing this manuscript. Mention of trade names is for information and does not imply an endorsement.

Manuscript accepted 3 Jan. 1998

(Stevenson et al. 1983, Fig. 1). The soil is Brown and Dark Brown. Annual precipitation averages 411 and 414 mm and the annual mean temperature is 9.5 and 7.9°C for Sterling and Pine Bluffs, respectively. Diaspores were stored in paper bags at room temperature until June 1995. Hand-cleaned seeds were used in all experiments.

Moisture Content of Germinants in Relation to Developmental Stage

Threshed seeds from the 3 collections were placed on moistened germination paper (Anchor Paper Co., St. Paul, Minn.) over plastic slant-boards, and covered with one layer of cellulose tissue (Jones and Cobb 1963). Slant-boards were then placed in closed germination boxes (25 × 40 × 20 cm), which were filled with distilled water to a 3-cm depth. These boxes were placed in incubators at 10° C under 12 hour light and seeds were incubated for 2, 3, 6, or 14 days to obtain germinants of different developmental stages. The experimental design was a randomized complete block with 4 replicates arranged over time by 1-day intervals.

Germinants of different stages were defined for this study as follows: Stage 1: 2 days of incubation (DI) with radicles 2 to 5 mm long; Stage 2: 3 DI with radicles 10 to 15 mm long and cotyledons folded in seed coats; Stage 3: 6 DI with cotyledons unfolded; and, Stage 4: 14 DI with growth beyond Stage 3 but the first true leaf not emerged.

Ten germinants, as an experimental unit, were retrieved from incubators after 2, 3, 6, and 14 DI. After surface water was blotted away with tissue paper, germinants were sealed in 0.25-ml tin capsules (Leco Co., St. Joseph, Mich.) and weighed. Germinants were then dried at 80°C for 24 hours and the dry weight determined. Weighing was done with a 6-place digital micro-balance. The moisture content of germinants was expressed on a dry weight basis.

Differential Thermal Analysis

Ten germinants from each developmental stage were retrieved from an incubator and surface water was blotted away with tissue paper. Germinants were then sealed in a tin capsule with a thermocouple contacting the germinant surface and another thermocouple placed outside the capsule. These germinants were placed in an incubator at 0°C for 1 hour before being moved into a freezer which was programmed at a cooling rate of 2.5°C hour^{-1} from 0 to –30°C over a 12 hour period. A randomized complete block design with 4 replicates arranged over time was used. Temperatures of germinants and the air inside the freezer were recorded with a CR7 Campbell Scientific datalogger at 1-minute intervals. Temperatures at which exotherms occurred were obtained by comparing the difference in temperatures inside and outside the capsules. The cumulative percentage of germinants with exotherms occurring between 0 and –6°C, 0 and –10°C and 0 and –30°C were calculated.

Subsequent Growth and Mortality of Germinants after Cooling

Twenty germinants, as an experimental unit, were retrieved from the freezer at 0, –6, –10 and –30°C and put into an incubator at 0°C for 24 hours. Seedling axial length was then obtained using a digitizing tablet (Booth and Griffith 1994) and the germinants were subsequently incubated at 10°C under 12 hour light for 7 days. Seedling axial length was measured again at the end of the experiment and the growth after cooling was calculated as the difference in seedling length before and after incubation. The mortality of germinants was visually determined at the end of the experiment.

Data Analysis

The analysis of variance was first carried out using the General Linear Model (GLM) (Snedecor and Cochran 1980) over the 3 seed collections, and then within each collection because of the interaction between seed collection and treatment. Data were further analyzed separately for imbibition temperature or cooling temperature. Statistical significance was assumed at $P \leq 0.05$ and means were separated by using LSD. A normal approximation was used to calculate the confidence limits for percentage mortality (Steel and Torrie 1980).

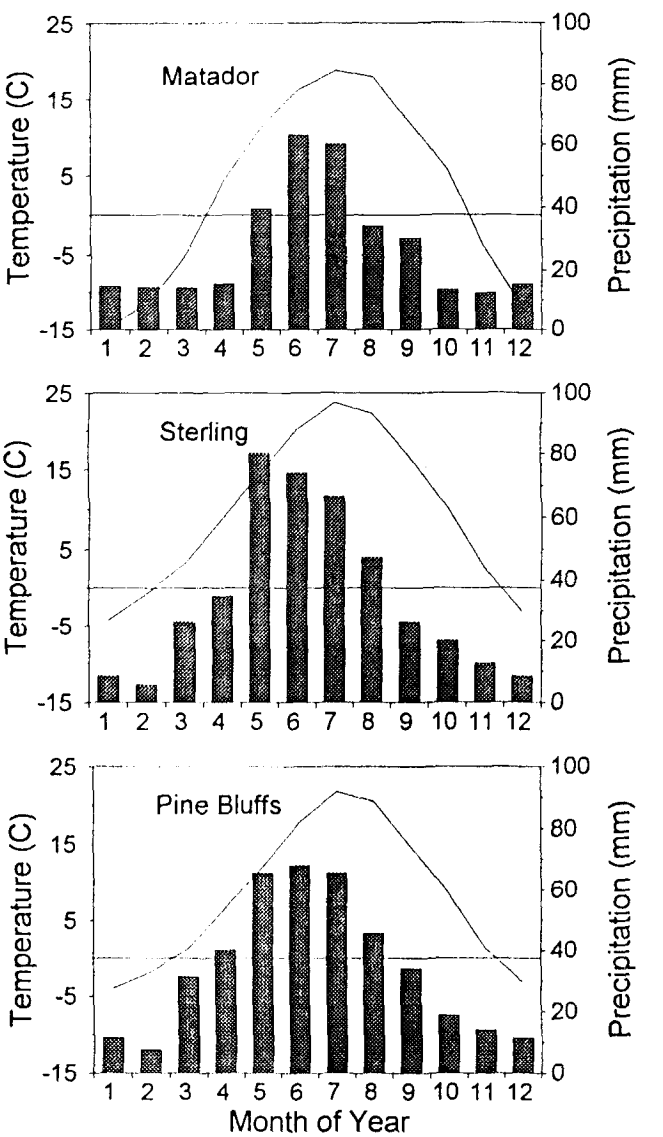

Fig. 1. The long-term monthly precipitation (bar) and mean air temperature (line) at Matador, Saskatchewan; Sterling, Colo.; and Pine Bluffs, Wyo. (based on data of Owenby and Ezell 1992a, 1992b; and Environment Canada 1982).

Results

Axial Length and Moisture Content of Germinants in Relation to Developmental Stage

The axial length of germinants was significantly different among developmental stages, averaging 4.3 ± 0.2, 11.3 ± 0.5, 28.8 ± 0.8, and 45.3 ± 2.2 mm for germinants of 2, 3, 6, and 14 DI, respectively (mean ± SE). The moisture content increased with DI, averaging 185 ± 7, 290 ± 11, 709 ± 29 and 1650 ± 921 for germinants of the 4 developmental stages. There were no differences among seed collections in seedling length or moisture content within any developmental stage.

Exotherm Distribution by Cooling Regime and Developmental Stage

Only 1 exotherm was observed for each germinant. There were no differences in exotherm temperature among seed collections within any developmental stage, therefore, data were pooled (Table 1). All germinants exhibited exotherms before temperatures dropped to –30°C. Temperatures at which the exotherm occurred increased with DI, from an average of –13.3 to –7.6°C.

Growth of Germinants after Cooling

Growth of germinants after cooling was affected by the interaction of seed collection, developmental stage, and degree of cooling. Within any seed collection and cooling temperature, growth decreased with DI (Table 2). Growth of germinants at 2 and 14 DI were not affected by cooling temperature in any seed collection (Table 2). Germinants were killed or growth was reduced after –30°C for all collections at 3 and 6 DI. The only other difference was that germinants of the Pine Bluff collection at 6 DI had greater growth at 0°C than at other temperatures.

Germinant Mortality after Cooling

Mortality of germinants after cooling was interactively influenced by seed collection, developmental stage, and cooling temperature. Most germinants at 2 DI survived cooling to –30°C, except for the Pine Bluffs collection, which had a higher mortality rate at –30°C than at other temperatures (Table 3). In all collections, the survival of germinants at 3 and 6 DI was not affected by cooling temperatures as low as –10°C, but few, if any, survived after –30°C. The mortality of germinants after 14 DI was high and similar among treatments, though the Matador collection had less mortality after cooling to –6 and –10°C than occurred in other treatments.

Table 1. Cumulative percentage of exotherm occurrence at different cooling intervals and, mean exotherm temperature for winterfat germinants incubated at 10° C for 2 to 14 days then cooled between 0 and -30° C.

	Days of incubation			
Colling intercal	2	3	6	14
(°C)				
0 – 0	0.0	0.0	0.0	0.0
0 – –6	5.6b[1]	5.1b	5.1b	22.2a
0 – –10	24.8c	41.3b	43.0b	88.0a
0 – –30	100.0	100.0	100.0	100.0
	----------(°C)----------			
Mean exotherm temperature	-13.3d	-11.5c	-10.7b	-7.6a

[1]Means with the same letter within a cooling interval or exotherm temperature are not significantly different at P ≤ 0.05. Means with no letter are not different among days of incubation.

Table 2. Growth of winterfat germinants incubated at 10° C for 2 to 14 days after different cooling treatments. Note that 0°C does not cause freezing stress.

	Days of incubation			
Seed collection/ cooling temperature	2	3	6	14
(°C)	----------(mm)----------			
Matador:				
0	36.6aA[1]	30.5aB	8.3aC	1.8aD
–6	41.2aA	22.2aB	7.6aC	0.7aD
–10	37.2aA	23.8aB	7.9aC	2.0aD
–30	31.6aA	2.9bB	---	---
Sterling:				
0	30.2aA	17.9aB	9.5aB	1.6aC
–6	26.5aA	18.4aB	7.6aC	0.3aD
–10	26.6aA	15.8aB	6.6aC	1.1aD
–30	26.1a	---	---	---
Pine Bluffs:				
0	26.8aA	18.4aB	8.9aC	1.1aD
–6	33.3aA	16.7aB	6.5bC	0.9aD
–10	29.4aA	18.7aB	6.3bC	0.0aD
–30	27.2a	---	---	---

[1]Means with the same lower case letter within a column (days of of incubation) or those with the same capitalized letter within a row (cooling temperature) are not significantly different at P ≤ 0.05.

Discussion

Sakai and Larcher (1987) reported that the resistance of dormant seeds to freezing is rapidly lost at the beginning of germination. Both a high and a low temperature exotherm occurred in hydrated lettuce seed, but only one exotherm, in the high temperature exotherm range, was observed after radicle emergence (Stushnoff and Junttila 1978). Hydrated winterfat diaspores also exhibited high and low temperature exotherms and these occurred between –4 to –5 and –14 to –19°C, respectively (Bai et al. 1998). Results from the current study show only 1 exotherm after radicle emergence. This exotherm was not the result of a merging of high and low temperature exotherms because it fell in the low temperature exotherm range. The high temperature exotherm in hydrated winterfat seeds is the result of ice formation between pericarp and seed, as evident by the single exotherm from seed with pericarp removed (Bai et al. 1998).

The low temperature exotherm is thought to represent ice formation within embryo cells. In this study, the temperatures at which the exotherm occurred gradually increased with the developmental stage of germinants from –13.3 to –7.6°C, indicating a gradual process of changes in freezing susceptible characteristics as development proceeds from seed to seedling. Booth (1989) suggested that changes in freezing tolerance of winterfat at different developmental stages were associated with increasing cell size, increasing development of vascular cylinder, and the development of root hairs. As a cell grows, the surface area of the plasma membrane increases by the square of cell diameter, but the

Table 3. Percentage mortality of winterfat germinants incubated at 10°C for 2 to 14 days after different cooling treatments. The confidence limit[1] for the 50% mortality point is 30 to 70%. Treatments are significantly different from 50% if the mortality percentage falls outside this range.

Seed collection/ cooling temperature	Days of incubation 2	3	6	14
(°C)	(%)			
Matador:				
0	0.0	0.0	2.5	76.5
-6	0.0	0.0	2.5	55.0
-10	0.0	0.0	5.0	52.5
-30	0.0	90.3	100.0	100.0
Sterling:				
0	0.0	4.2	0.0	70.0
-6	0.0	0.0	2.7	81.7
-10	7.8	0.0	15.7	95.0
-30	0.0	100.0	100.0	100.0
Pine Bluffs:				
0	0.0	2.5	2.5	60.0
-6	2.7	0.0	0.0	90.5
-10	0.0	0.0	2.5	95.0
-30	20.0	100.0	100.0	100.0

[1]Calculated using the normal approximation for a percentage (Steel and Torrie 1980) and a sample size of 20.

cell volume increases cubically. If the permeability of the plasma membrane remains constant, the larger cell has less capacity to dehydrate and is more at risk to damage from intracellular ice as the temperature decreases.

The freezing resistance of seedlings usually decreases gradually in early stages until the cotyledons develop, such as in silver fir (*Abies alba*) (unpublished data of G. Bendetta as cited in Larcher 1985). This resistance increases after the lignified xylem develops. For common beet (*Beta vulqaris*), frost resistance decreased after seed germination, and increased after cotyledons were exposed to light and the first 2 leaves developed (from Gary 1975 as cited in Sakai and Larcher 1987). The freezing tolerance of winterfat germinants decreased with development before the first true leaves emerged (the current study), and continuously decreased in seedlings grown at 15/5°C up to 28 days (Hou and Romo 1997). Therefore, despite its high freezing tolerance in the seed stage (Bai et al. 1998), winterfat is vulnerable to freezing after radicle emergence and before lignification.

Freezing damage, measured by subsequent growth and mortality of germinants after cooling, increased with developmental stage. However, the occurrence of an exotherm was not always lethal to winterfat germinants. A majority of early stage germinants survived the ice crystal formation implied by exotherm occurrence. Some late stage germinants also had post-freezing survival, but low light levels in growth chambers and germination boxes may have restricted post-cooling growth. High mortality (> 50%) of seedlings that were not cooled below 0°C, and the consistent decrease in growth with DI suggest that data for germinants at 14 DI (Tables 2 and 3) may reflect light limitations more than the effects of cooling.

An increase in germinant mortality was also found where temperatures were reduced below the exotherm temperature, indicating that the low temperature exotherm is not the single critical measure for freezing damage in winterfat germinants. Events that happen at lower temperatures may reduce their survival, possibly due to freezing desiccation. For species with deep cooling mechanisms, such as maple (*Acer rubrum* L.) and white ash (*Fraxinus americans* L.) (Lindstrom et al. 1995), southern magnolia (*Magnolia grandiflora*) and evergreen azaleas (*Rhododendron* spp.)(Anisko and Lindstrom 1995), the lowest survival temperature was higher than the low temperature exotherm, indicating that damage may have occurred at a higher temperature. Therefore, the relation between the low temperature exotherm and plant survival is species specific.

In conclusion, we accept our hypothesis that freezing tolerance of winterfat germinants decreases as the development progresses. Warm temperatures increase the rate of plant development, leading to increases in plant size and water content. These changes also increase the probability that plants will be injured by a subsequent freezing event. Thus, freezing events preceeded by extended periods of warm temperatures will likely reduce winterfat recruitment. There are conditions, yet to be studied, which may affect application of our current findings to winterfat revegetation and population ecology. For example, seed germination and germinant development under a diurnal cycle that includes regular exposure to freezing conditions, may produce seedlings that are more tolerant of freezing.

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