Cold-temperature Germination of *Elytrigia repens* × *Pseudoroegneria spicata* Hybrids

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Abstract

The successful establishment of perennial grass seedlings on *Artemisia* rangelands may depend on germination in the early spring at cold seedbed conditions. To ascertain the feasibility of selecting for germination at low temperatures in a hybrid population, seeds for 30 RS hybrid [*Pseudoroegneria spicata* (Pursh) Love × *Elytrigia repens* (L.) Nevski] lines were germinated under a wide range of constant or alternating temperatures. The hybrid populations were characterized in 11 different, but related germination responses to incubation temperatures based on discriminate analysis of seedbed temperatures. Germination at very cold incubation temperatures was markedly reduced in all populations compared to germination at more moderate temperatures. A range of 0 to 16% germination existed among the hybrids at very cold temperatures after 4 weeks incubation. At the very cold temperature regime, crested wheatgrass [*Agropryon desertorum* (Fisch.) Schult.] had 1% germination and intermediate wheatgrass [*Thinopyrum intermedium* (Host) Barkworth and D.R. Dewey] had 3% germination. At cold temperatures, germination of one hybrid line exceeded that of the wheatgrasses. At cold fluctuating temperatures, 13 and 22 hybrid lines had higher germination than intermediate and crested wheatgrass, respectively. Results of this study indicate a high potential of selection among these hybrid lines for germination in rangeland seedbeds under cold temperature regimes.

The establishment of seedlings of perennial forage grasses on *Artemisia* rangelands of the western United States is usually difficult because available soil moisture and seedbed temperatures that are conducive to germination are largely out of phase with each other (Hull and Stewart 1948, McGinnies 1959, Evans et al. 1970). When moisture is available in the early spring, temperatures are either too low or the diurnal temperature fluctuation is too great to permit germination. When germination does occur, the perennial grass seedling must develop rapidly before soil moisture is exhausted with the onset of the summer drought. It is logical that germination and seedling development in the early spring under cold seedbed conditions would be of adaptive advantage in surviving summer drought.

Our purposes were (1) to evaluate the germination responses of 30 RS hybrid lines at a wide range of constant and alternate temperatures; and (2) to compare the germination responses of these hybrids at temperature regimes characteristic of early spring or late fall with that of 2 widely used revegetation grasses, 'Nordan' crested wheatgrass and 'Oahe' intermediate wheatgrass.

Methods

Polycross seedlots from 30 RS hybrid lines were obtained from crossing blocks located at Logan, Utah. Seeds were stored in paper envelopes at laboratory temperatures before testing. Four replications of 25 seeds each were incubated in dark germinators at 55 constant or alternating temperature regimes for 4 weeks. The seeds were placed on germination paper in petri dishes and kept wet with tap water. The experimental design was a randomized block. Constant temperatures were 0, 2, 5, 10, 15, 20, 25, 30, 35, and 40° C. Alternating temperature regimes consisted of 16 hr at each lower constant temperature (cold period) and 8 hr at each possible higher temperature (warm period). For example, 0° C was alternated with 2, 5, 10, 15, 20, 25, 30, 35, and 40° C whereas 35° C was alternated with 40° C only. Seeds were considered germinated when the radicle emerged 0.5 cm. Germination counts were made at 1, 2, and 4 weeks.

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Manuscript accepted 4 November 1985.

A quadratic response surface was developed for each line using multiple-regression techniques (Evans et al. 1982). Estimated germination values and their confidence limits ($P \leq .01$) were derived from the quadratic response surface of each series for cold period temperatures through the series of warm period temperatures.

A number of germination parameters were calculated from the germination profiles (Young and Evans 1982). These included optimum germination defined as those means not statistically lower than the maximum observed at the 0.01 level of probability.

The germination response of seeds of the 30 hybrid lines in relation to the 55 constant or alternating temperature regimes were compared using the following breakdown of seedbed temperatures:

a) Very cold, 0/0 (continuous 0° C), 0/2 (0° C for 16 hr and 2° C for 8 hr in each 24 hr), 0/5, 2/2, and 2/5° C.
b) Cold, 0/10, 0/15, 2/10, 2/15, 5/5, and 5/10° C.
c) Cold fluctuating, 0/20 through 0/40° C and 2/20 through 2/40° C.
d) Fluctuating, 5/30 through 15/40° C, 10/35, 10/40° C and 15/40° C.
e) Moderate, 5/15 through 5/25° C, 10/10 through 10/30° C, 15/15 through 15/35° C, 20/20 through 20/35° C, and 25/25 and 25/30° C.
f) Warm, 20/40, 25/35, and 25/40, 30/30 through 30/40° C, 35/35, 35/40, and 40/40° C.

This breakdown of temperature is based on extensive microenvironmental monitoring of seedbed temperature in *Artemisia* communities during the spring germination period in the Great Basin (Evans and Young 1970, 1972).

To evaluate how the mean seed germination of the crosses related to each other by categories of seedbed temperature, means were ranked by germination percentage in each category. Rankings were converted to single digit numbers by the scale: 1 through 5 = 5; 6 through 10 = 4; 11 through 20 = 3; 21 through 25 = 2; and 26 through 30 = 1. The results were then classified into a series of characteristic germination patterns based on the composite ranking in each seedbed category. This system was designed to characterize germination and to determine how seed germination of a given line related to that of the other lines with respect to seedbed temperature categories. This procedure allows the determination of which, if any, relationships are inherent in the physiological systems that control germination at specific temperature regimes. For example, if seeds have high germination at low temperatures, will they have high germination at all other temperature regimes, or is the potential to germinate at high temperatures sacrificed in order to germinate at low temperatures? If all the hybrid lines react the same versus the identification of high and low temperature germintors, high only, low only, etc., the procedure provides insight into the nature of the inherently controlled biochemical pathways governing germination in relation to incubation temperature.

Germination of the RS hybrid lines was compared with that of 3 'Nordan' crested wheatgrass sources and 3 'Oahe' intermediate wheatgrass sources (Young and Evans 1982). Seeds of the 6 wheat-

Table 1. Mean germination of seed of 30 RS hybrid lines incubated at 55 constant or alternating temperatures for 4 weeks.[1]

Cold period 16-hr C	Warm period 8 hr - C 0	2	5	10	15	20	25	30	35	40
					%					
0	0	0	8	23	31	50	52	38	53	41
2		0	10	37	63	61	52	68	67	65
5			69	76	78	80	80	77	79	74
10				80	82	80	81	78	78	74
15					83	81	81	76	75	75
20						80	78	79	78	76
25							80	76	75	74
30							70	70	66	
35								65	61	
40									45	

[1]Underlined means not significantly different from maximum observation defined as optimum germination at 0.01 level of probability.

grass sources were germinated in relation to temperature in the same manner described for the 30 hybrids.

Statistical comparisons among hybrids and wheatgrasses were made with analysis of variance and Duncan's multiple range test at the 0.01 level of probability. Arc sin transformation was computed on germination percentages before analysis.

Results and Discussions

Average Germination Response

The overall mean germination profile for seeds of the 30 hybrid lines had optimum germination at temperatures ranging from 5 through 25° C cold-period alternating with 10 through 35° C maximum warm-period temperatures (Table 1). The maximum observed germination was 83%.

Most of the temperature regimes that supported optimum germination fell into the range of seedbed temperatures that we defined as fluctuating and moderate (Table 2). The seeds of hybrids germinated well over a wide range of temperatures. Germination of cold seedbed temperatures was greater than 60% of that observed at moderate seedbed temperatures (Table 2). Only at very cold temperatures was overall mean germination greatly suppressed.

Variation among Progenies in Germination Response

Considerable variability in germination existed among the hybrid lines at the various seedbed temperatures (Table 2). At the extremes there was a range of 16 and 43% germination at very cold and warm temperature regimes, respectively. The greatest range in germination response occurred under cold-fluctuating temperature regimes. The latter temperature regimes consisted of very cold alternating with warm temperatures.

Table 2. Average germination percentage of seeds of 30 RS hybrid lines at various seedbed temperature categories.

Seedbed temperatures	% Germination Mean	Range
Very cold	4	0–16
Cold	50	24–71
Cold fluctuating	50	24–81
Fluctuating	76	59–82
Moderate	79	66–90
Warm	66	40–83

Considering the range of incubation temperatures tested, the categories of seeded temperatures used to stratify the germination response, and what is known about the germination temperature relations for grass species, 1 or 2 of several types of germination responses were possible from the seeds of these lines. Twelve different types of germination responses, many more than expected, were observed (Table 3).

The first 3 germination responses categoried involved depression of germination by cold incubation temperatures (Table 4). More than one third of the hybrid lines were included in these 3 categories. Categories 4 and 5 are reciprocals of 1, 2, and 3 and resulted in a depression of germination at high temperatures and high fluctuating temperatures. In category 6, germination was lower at extreme fluctuating temperatures compared to lower amplitude fluctuations or constant temperatures.

The largest percentage of the hybrid lines was found in category 7 where germination was depressed at both the cold and warm extremes of the incubation temperatures. Germination responses to temperature were quite well demarked for these hybrid lines with depression in germination at both extremes.

The lower germination observed at extreme fluctuating temperatures (category 6) compared to lower amplitude fluctuating or constant temperature is not common for seeds of most grass species (Young and Evans 1982). Apparently, category 6 was related to category 5 where both extremes depressed germination. Fluctuating temperatures with high or low extremes or a combination of both extremes suppressed germination more than constant extremes. The most restricted germination range was category 8 with depression in germination at all temperatures regimes except

Table 3. Types of germination response in relation to temperatures that were observed for seeds of the 30 RS hybrid lines and mean germination at all temperatures and at cold and warm temperatures for each germination category.[1]

Types of germination response	Percentage of all lines tested	Mean germination: All temperatures	Very cold and cold temperatures	Warm temperatures
1. Depressed at very cold temperature only	17	66 ab	28 b-d	71 ab
2. Depressed at very cold and cold temperature	7	70 ab	33 bc	78 a
3. Depressed at very cold, cold, and cold fluctuating temperatures	14	63 a-c	22 c-f	67 bc
4. Depressed at warm temperatures only	7	56 b-d	33 b	45 e
5. Depressed at warm and fluctuating temperatures	10	71 a	48 a	67 bc
6. Depressed at cold fluctuating and fluctuating temperatures	10	61 a-c	19 df	78 a
7. Depressed at very cold and warm temperatures	20	61 a-c	32 bc	60 cd
8. Depressed at all extra moderate temperatures	3	48 d	16 f	49 de
9. Enhanced at fluctuating temperatures	3	52 cd	26 b-f	47 de
10. Enhanced at cold fluctuating temperatures	3	50 d	17 ef	53 de
11. Enhanced at very cold temperatures	3	56 b-d	28 b-d	56 d
12. Temperature greater than that range tested	3	63 ab	27 b-e	68 a-c

[1]Means within columns followed by the same letter are not significantly different at the 0.01 level of probability as determined by Duncan's multiple range test.

Table 4. Correlation coefficients *(r)* for relation between components of germination profile.

Temperature Regimes	Very Cold	Cold	Cold Fluctuating	Fluct-uating	Warm
Moderate	0.42	0.61	0.60	0.87**	0.80*
Very cold		0.93**	0.86*	0.48	0.10
Cold			0.80	0.52	0.10
Cold fluctuating				0.54	0.14
Fluctuating					0.73*

**, and * indicate significance at 0.01 and 0.05 level of probability, respectively.

those of moderate temperatures.

Categories 9 and 10 are related in that germination at fluctuating temperature regimes was greater than at constant temperatures. Stimulation of grass seed germination by alternating temperatures is rather common and in some cases such temperatures are obligatory for germination to occur (Young and Evans 1982).

The enhancement of germination by very low incubation temperatures (category 11) is symptomatic of cool-moist stratification requirements. As in categories 9 and 10 where enhancement in germination occurred at fluctuating temperatures, the average germination of these lines was markedly lower than that observed for the bulk of the population. These 3 categories apparently reflect the occurrence of dormancy systems where requirements for germination barely are reached by the amplitude and duration of seed incubation treatments.

The last category is very unusual for grass seeds in that it reflects the reciprocal of category 7 where the limits of temperature for germination were clearly defined. The potential germination of seeds of hybrid lines in this category are greater than the temperature range used in this study.

Although the mean germination of the categories of germination differed by more than 20%, in total they represent nearly continuous variation for the characteristics described. In the case of very cold and cold temperatures the range in mean germination was even greater.

Interrelation of Germination at Categories of Seedbed Temperatures

Germination at moderate temperature was positively correlated ($P \leq 0.01$) with germination at fluctuating and warmer temperatures (Table 4). However, high germination at moderate temperatures was not a good indicator of high germination at cold temperatures and decidedly not at very cold temperatures.

Germination at very cold temperatures was positively correlated to germination at cold ($P \leq 0.01$) and cold fluctuating ($P \leq 0.05$) temperatures (Table 4). There was virtually no relation between germination at very cold and warmer temperatures. Germination at fluctuating temperatures was correlated ($P \leq 0.05$) with germination at warmer temperatures. There was little relation between germination at cold fluctuating and fluctuating temperatures. Apparently germination at cold temperatures is an additive biochemical pathway. Selection for germination under cold seedbed conditions improves the chances for germination under very cold seedbed conditions. Selection for germination at warm seedbed conditions has virtually no chance of improving germination at cold or very cold seedbed temperatures.

Germination at cold fluctuating and fluctuating temperatures is very complex because the incubation regimes combine such diurnally contrasting temperatures. The correlation between germination at very cold and cold fluctuating temperatures suggests that the very cold portion of the cold fluctuating temperature regimes (0 to 2° C) is the dominant factor governing germination at these temperatures. Likewise, the positive correlation between germination at moderate and fluctuating temperatures and the lack of correlation between fluctuating or warmer or cold temperature regimes suggest that germination occurs during the moderate portion of the fluctuating temperature regimes.

Table 5. Mean germination of crested and intermediate wheatgrass and of the RS hybrids. RS hybrid lines mean percent germination were significantly higher (0.05) than crested or intermediate wheatgrass.

Temperature Regimes	RS hybrid	Crested wheatgrass	Intermediate wheatgrass
	% Germination		
Very cold	16	1	3
Cold	75	38	53
Cold fluctuating	71	28	45

Comparison of Germination of Hybrids to Revegetation Grasses

Very cold, cold, and cold-fluctuating temperature regimes were selected as representative of spring and fall seedbed temperatures at various times on Intermountain rangelands (Evans et al. 1970; Evans and Young 1970, 1972). At these regimes, germination of the RS hybrid lines was higher than that of crested and intermediate wheatgrass (Table 5). At very cold temperature regimes, germination of 2 hybrid lines exceeded both the wheatgrass species.

At cold temperatures, germination of 1 hybrid line was higher than crested and intermediate wheatgrass. At cold-fluctuating temperatures, germination of 13 and 22 hybrid lines exceeded that of intermediate and crested wheatgrass, respectively.

Conclusions

The variation in the RS hybrid lines for germination at different incubation temperatures, even at very cold incubation temperatures, offers promise for selection. Selection for high germination at moderate incubation temperatures will not necessarily result in enhanced germination at cold and very cold temperatures. Selection for high germination at warmer than moderate temperature will most probably not enhance germination at low temperature, but would probably not result in reduced germination at low temperatures.

The large number of different types of germination responses that were observed for different hybrid lines suggests: (a) that generalizations made from means of the lines may not apply to specific hybrid populations and (b) that the physiological processes governing germination in response to incubation temperature may be manipulated by hybridization and selection.

Germination responses at low temperatures for the RS hybrid lines compared to response of grasses used widely in rangeland revegetation suggests that promise exists for selecting hybrids for early spring or late fall germination. This characteristic would be advantageous in the Intermountain area where soil moisture is often only available for germination and seedling growth under cold conditions.

The next step in evaluation of the RS hybrid lines will be to field test the predicted best and worst lines from this study to confirm laboratory results with those from field trials.

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