Germination Response of Greasewood (*Sarcobatus vermiculatus*) to Temperature, Water Potential and Specific Ions

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Abstract

Seeds of greasewood *(Sarcobatus vermiculatus* (Hook.) Torr.) were germinated at 5° to 40° C in 5-degree increments to determine temperature response. Seeds were also germinated in solutions of polyethylene glycol 6,000 (PEG), NaCl, and Na_2SO_4, each at osmotic potentials of 0 to -4.2 MPa in -0.3 MPa decrements at 10, 20 and 30° C to determine moisture stress, specific ion, and temperature interaction. Germination was high at all temperatures, 5° C through 25° C being optimal. A direct linear relationship existed between total germination and osmotic potential of each solution at each temperature. Mean germination at 30° C was significantly different for each osmotica with NaCl highest and PEG lowest. Mean germination at 10° C and 20° was not different within osmotica; however, total germination was significantly lower in PEG than in NaCl and Na_2SO_4, indicating the difference between macromolecular PEG and ions (Na^+, Cl^- and $SO_4^=$). Significant difference was observed in the coefficient of rate of germination between ions of Cl^- and $SO_4^=$, with SO_4 = being more stressful.

Greasewood *(Sarcobatus vermiculatus* (Hook.) Torr.), a halophytic shrub, is widely distributed on medium to heavy textured saline or saline-alkali soils in the western United States. Greasewood is perhaps best known for its toxicity to livestock, and communities in which it dominates have been viewed as having low economic value and being difficult to reclaim (Rollins et al. 1968). As a forage plant, however, it can be fairly palatable and nonpoisonous in mixtures with other forage plants. We observed a high percentage of individual plants grazed by cattle in native communities in southeastern Montana.

Greasewood is classified as a phreatophyte, rooting nearly 13 meters deep (Harr and Price 1972); and as an osmophyte, possibly utilizing ions concentrated in leaves (as much as 9.5% sodium by dry weight) for osmoregulation (Rickard 1982). Adult plants are known to concentrate certain ions, particularly sodium salts, beneath the canopy. Rickard (1965) found exchangeable sodium as high as 7.3 meq/100 g in surface soils under greasewood plants. We observed many greasewood seedlings beneath adult plants. Germination apparently took place in what may have been a very stressful environment. Although mature plants tolerate considerable soil salt, germinating seeds are likely much less tolerant. Seeds germinating in salt-affected soils may be responding to dissolved salt concentration, as well as to particular salts or ions involved. Additionally, temperature may interact with salinity, producing a significant but highly variable environmental sieve.

The mechanism by which seeds of greasewood compensate and are thereby able to germinate in salt-affected soils is unknown. The mechanism may be external to the seed, such as a dilution effect by rainfall or flooding; it may be internal osmoregulation as postulated for adult plants; or it may be a combination of the two plus other environmental factors. We examined germination response to temperature, as well as salt concentrations and moisture stress in combinations with three temperatures to ascertain the overall effect of salt concentration, specific ions, and their temperature interaction.

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Materials and Methods

Seeds of greasewood (utricles) were collected approximately 18 km west of Hardin, Mont., in November 1978. The collection area is a dense-clay-clayey-saline upland range site at an elevation of 1,100 m in a 30 to 36-cm annual precipitation zone. Dominant species on the site are greasewood, Wyoming big sagebrush *(Artemisia tridentata wyomingensis),* and western wheatgrass *(Agropyron smithii).* Seeds were transported to the laboratory, dried at room temperature, cleaned, and stored. A seedblower was used to remove empty seeds and provide uniform seed weight.

Germination trials were conducted using dewinged utricles in early 1980. Prior to incubation, utricles were dusted with N-[(trichloromethyl)-thio]-4-cyclohexene-1,2-dicarboximide to control fungi. In all experiments incubation was at constant temperatures without light. Germination was recorded at 2-day intervals through 12 days and at 6-day intervals thereafter through 30 days. Seeds were considered germinated when embryos were completely uncoiled and cotyledons reflexed.

Germination response to temperature was evaluated at 5-degree increments from 5 through 40° C. Seeds were placed in covered petri dishes on a 5-cm square of germination paper and 30 ml of distilled water added, which saturated the paper. During incubation distilled water was added to maintain the germination paper near saturation. Experimental design was a randomized block with 4 replicates of 25 seeds each.

The combined effect of temperature and osmotic potential on germination was evaluated at constant temperatures of 10, 20, and 30° C using solutions of polyethylene glycol 6,000 (PEG), sodium chloride (NaCl) and sodium sulfate (Na_2SO_4). Osmotic potentials of PEG and NaCl were prepared as described by Michel and Kaufman (1973) and Lang (1967), respectively. Concentration-osmotic potential relationships of Na_2SO_4 were determined with a sample chamber psychrometer. Osmoticas were prepared for each temperature in -0.3 MPa decrements to -4.2 MPa. Distilled water was used as control. Petri dishes were prepared as described above and 30 ml of osmotic solution was added. Covered petri dishes were placed on trays on a water-saturated layer of germination paper, and the trays were further enclosed and sealed in plastic sacks. Treatments were factorially applied to 5 replicates of 25 seeds each arranged in a randomized block design.

Seed viability was ascertained by imbibing seeds 48 hours at 10° C, puncturing the pericarp, applying a 0.1% tetrazolium solution, and evaluating at 8 and 12 hours. Data reported herein is corrected for seed viability as determined by this procedure.

Germination rate (CRG) (Maguire 1962) and germination percentage were evaluated with analysis of variance. All percentage

values were subjected to arcsin ($\sqrt{\bar{p}}$) transformation prior to statistical analysis. Untransformed data are reported herein. Mean responses to temperature were segregated with Tukey's W-procedure, and regression analysis was used to characterize germination response in osmotic solutions (Steele and Torrie 1980). All tests were made at the 0.05 probability level.

Results

Viability

Although greasewood utricles with apparently normally developed embryos were used in germination trials, maximum germination attained was 62%. Subsequent tetrazolium tests indicated 64% (s.e. 1.9) embryo viability. We also imbibed seeds with distilled water and examined embryos for evidence of viability. Rapid and accurate determination of greasewood seed viability was attained by examining radicle tips of imbibed embryos. The radicle tip, 1-1.5 mm, of nonviable embryos were consistently brown, whereas they were light colored in viable embryos.

Temperature

Total germination was highest between 5 and 25° C (Table 1) and seeds germinated most rapidly (CRG) in the mid-range temperatures of 15, 20, and 25° C. Over the 5 to 40° C temperature range, 50% of final germination was at day two and germination was 93% complete by the tenth day.

Even though seeds germinated well at all temperatures, seedling vigor was poor at warm temperatures. Temperatures above 25° C resulted in imperceptible hypocotyl and radicle growth and seedlings were limp and decomposing 1 to 2 days after germinating. At cooler temperatures (10–25° C), hypocotyl and radicle elongation was substantial, root hairs were abundant, and seedlings were turgid and erect. Although seedling elongation appeared limited at 5° C, seedlings were turgid and root hairs were well developed.

Osmotic Potential and Temperature

Total germination percentage was directly related to osmotic potential of each osmotica at all temperatures (Fig. 1-3). When incubated at NaCl solutions at 10 and 20° C, seeds actually germinated to -3.6 MPa osmotic potential, while at 30° C seeds germinated to -3.0 MPa. Germination in Na_2SO_4 solutions occurred when the osmotic potential was -2.4 MPa or greater.

Seeds germinated in PEG solutions with osmotic potentials higher than -2.7 MPa at all temperatures. Total germination in PEG solutions was not significantly different at 10° and 20° C, while germination was lower at 30° C (Fig. 3). As temperature increased, regression slope coefficients decreased. Increasing temperature may thus function as a finer sieve and eliminate low-vigor seeds or secondary dormancy may be induced in a portion of the seed population by osmotic or temperature stress. However, in our work seeds that did not germinate at 30° C were rapidly infected and destroyed by fungi.

Germination was significantly higher over a wider range of osmotic potentials in NaCl and Na_2SO_4 than PEG. Within temperatures, relationships between total germination and osmotic potential were not dissimilar between NaCl and Na_2SO_4 (Figures 1 and 2). Although regression slope coefficients at 10, 20 and 30° C did not change, maximum germination (y intercept) was lower at 30° C.

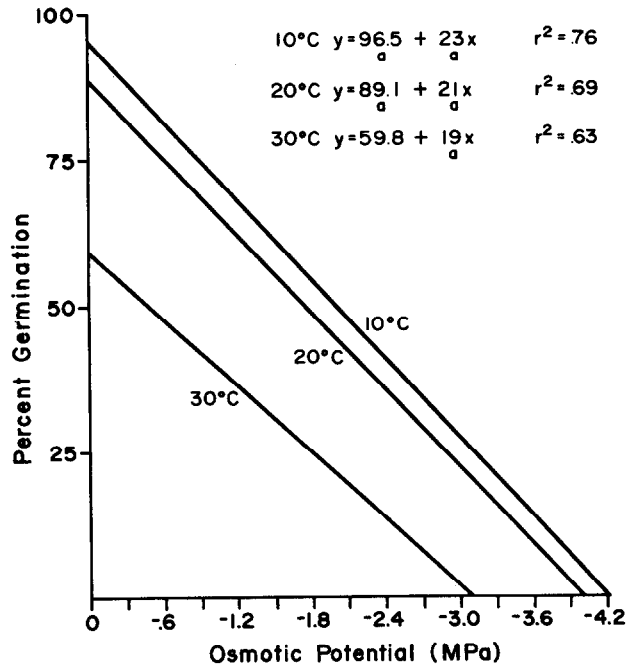

Fig. 1. *Greasewood germination response to osmotic potential of sodium chloride solutions at 10, 20 and 30° C. Similar letters below coefficients indicate nonsignificant differences.* P = .05.

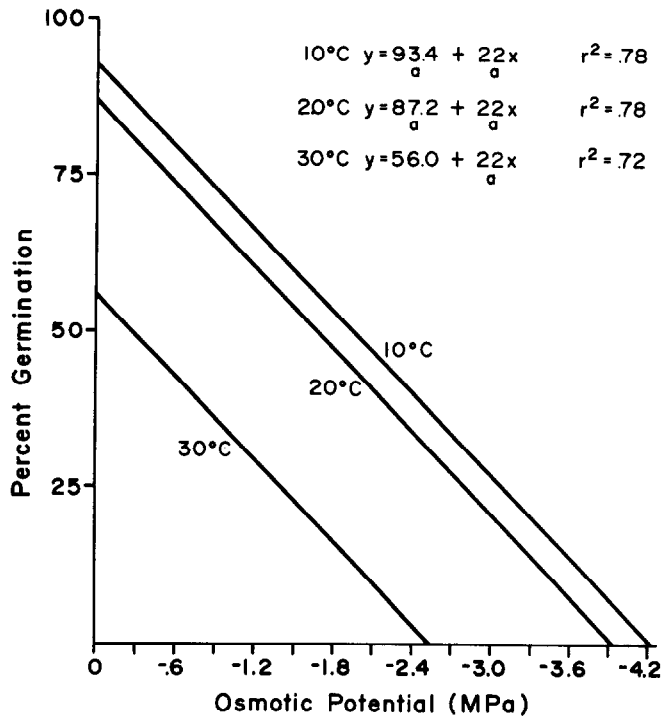

Fig. 2. *Greasewood germination response to osmotic potential of sodium sulfate solutions at 10, 20, and 30° C. Similar letters below coefficients indicate nonsignificant differences.* P = .05.

Table 1. The effect of temperature on total germination percent and coefficient of rate of germination (CRG) of viable greasewood seeds.

	Temperature C							
Parameter	5	10	15	20	25	30	35	40
Germination %	80.1ab	97.7a	91.3a	76.9abc	93.0a	60.9bcd	64.1bcd	53.0d[1]
CRG	3.1d	4.2bcd	5.4ab	5.2abc	6.5a	3.4d	4.4bcd	3.5d

[1]Means followed by the same letter within each parameter are not significantly different at the .05 probability level.

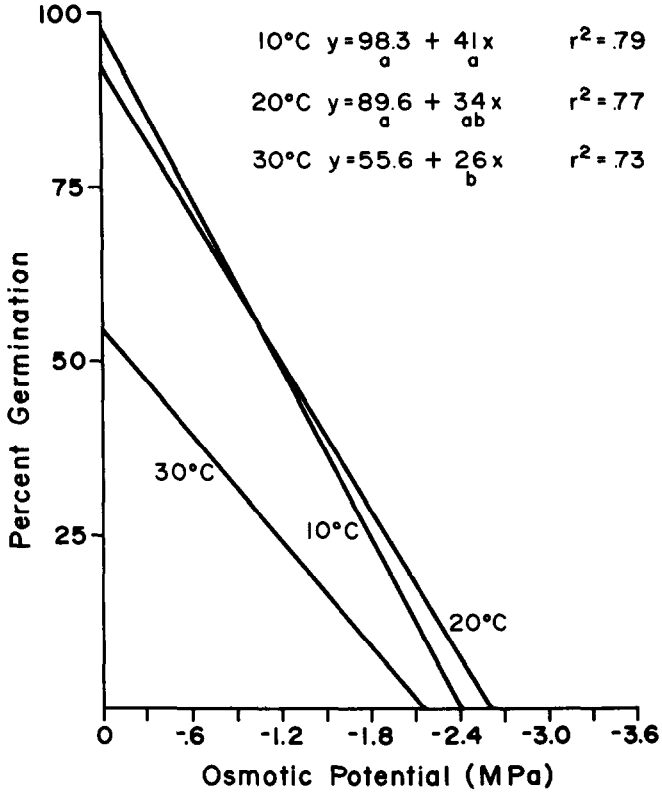

Fig. 3. *Greasewood germination response to osmotic potential of polyethylene glycol 6,000 solutions at 10, 20 and 30° C. Similar letters below coefficients indicate nonsignificant differences.* P = .05.

Coefficient of rate of germination (CRG) has a positive linear relationship to osmotic potential of each osmotica (Figures 4-6). Seeds germinated most rapidly when incubated in NaCl and slowest in PEG. Differences were significant at 10° and 20° C, but not

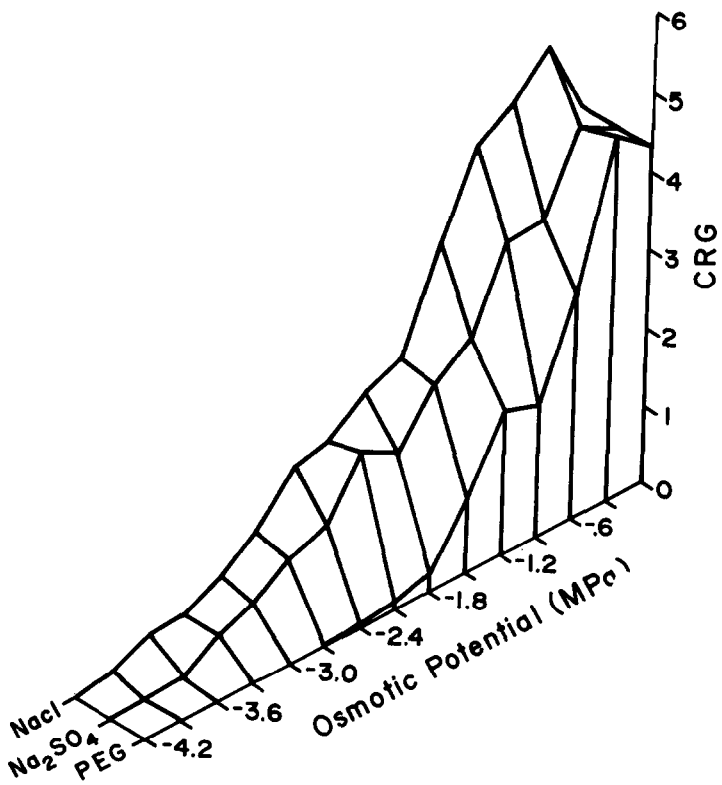

Fig. 4. *Coefficient of rate of germination (CRG) response surface of greasewood in various osmoticas at 10° C.*

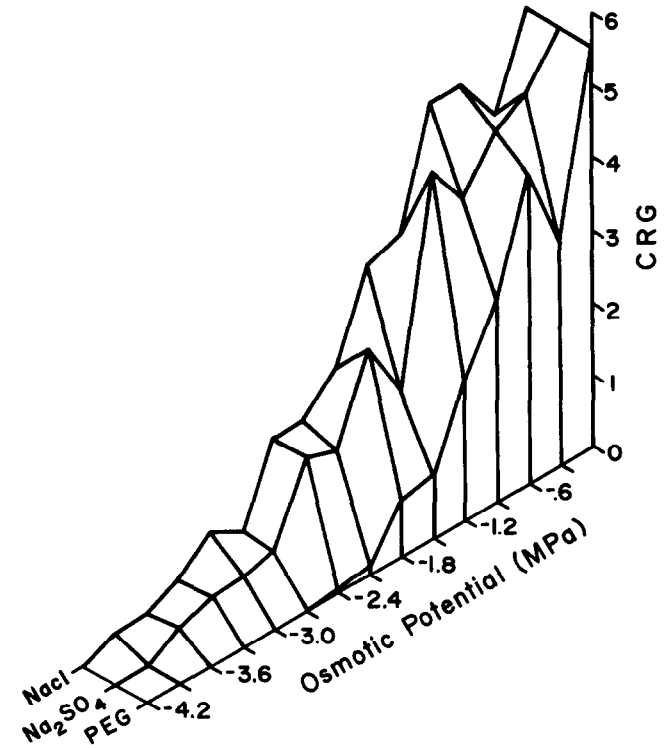

Fig. 5. *Coefficient of rate of germination (CRG) response surface of greasewood in various osmoticas at 20° C.*

at 30° C. Mean days to 50% of final germination across all osmotica and osmotic potentials were 3.5 days at 30° C, 5.8 days at 20° C and 7.1 days at 10° C. By the twelfth day germination was essentially complete in all osmoticas and temperatures. Mean percent of

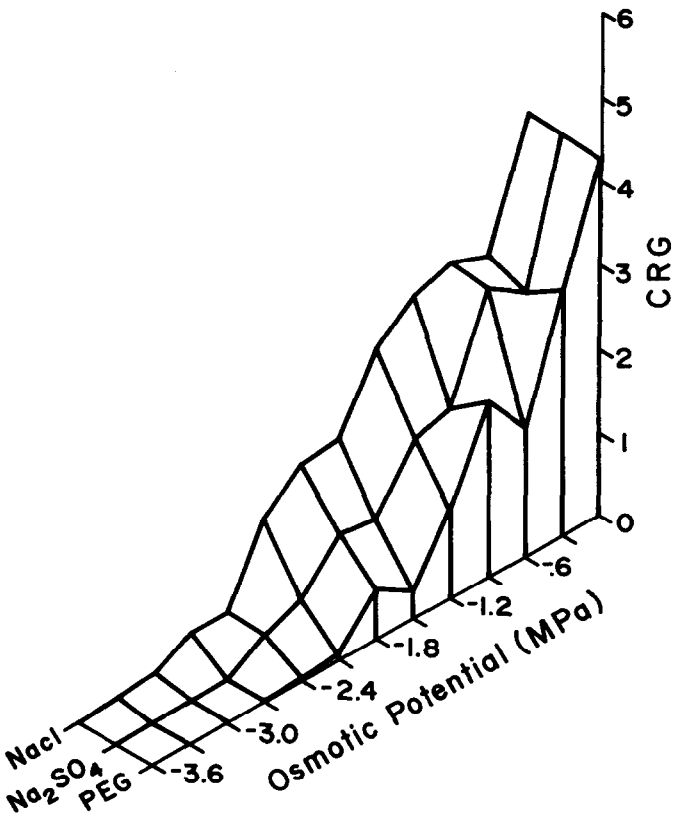

Fig. 6. *Coefficient of rate of germination (CRG) response surface of greasewood in various osmoticas at 30° C.*

total germination completed by day 12 at 10, 20, and 30° C were 85%, 86%, and 96%, respectively. Thus, although CRG was reduced by declining osmotic potential and temperature, greasewood seeds that germinated did so quite rapidly.

Discussion

Seed germination in greasewood from southeastern Montana appears to be opportunistic since most seeds germinate over a broad range of temperature and osmotic potentials. Optimal temperatures for germination appear to be in the 10 to 20° C range when total germination, germination rate, stress response, and seedling vigor are considered. This is consistent with findings of Eddleman (1979) and Sabo et al. (1979).

This southeastern Montana source germinated over a broader range of temperatures than the New Mexico source of Sabo and coworkers (1979). Their seeds did not germinate at constant temperatures above 29° C, while we found 53% germination at 40° C. Conversely, they obtained higher germination at lower water potentials—80% at -1.6 MPa using PEG 4,000, while germination had dropped below 80% at -0.9 MPa for our source using PEG 6,000.

Optimal temperatures tend to reduce adverse effects of moisture or salt stress on germination (Springfield 1968 and Tadmor et al. 1969). In this experiment 10 and 20° C markedly influenced total germination and seedling vigor as compared to 30° C. Therefore, optimal temperatures appear to partially compensate for salt and moisture stress.

Promotion of germination by NaCl and Na_2SO_4 over PEG 6,000 at equal water potentials occurred consistently at lower water potentials. Presence of these salts in solution also resulted in germination at much lower water potentials. This phenomenon has been reported for steppe species (Ghoudhuri 1968) and for several wild safflower *(Carthamnus oxycantha)* ecotypes (Bassiri et al. 1977).

Greasewood plants are known accumulators of high concentrations of sodium (Rickard 1965, Wallace et al. 1973) which is presumably used for osmoregulation. Germinating embryos may function similarly by utilizing sodium sequestered in tissues or by concentrating sodium through uptake from solution to establish a water potential gradient favorable for germination. Actual determination of water and osmotic potential in seeds germinated under experimental conditions would resolve which mechanism is operating. In future research, this point should be clarified.

A short after-ripening period is necessary before greasewood seeds exhibit significant germination at temperatures of 4 to 10° C (Eddleman 1979); however, by spring of the year following seed production, these same cool temperatures are optimal for germination. This adaptation likely coincides with flushes of fresh water which would dilute salts concentrated in surface soil. We postulate that adaptations for germination at cool to cold temperatures and in high salt concentrations act in concert with a dilution effect of spring rainfall to produce successful regeneration of greasewood.

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