Annals of Botany 66, 587–595, 1990

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Partitioning Water Potential and Specific Salt Effects on Seed Germination of Four Grasses

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Accepted: 23 May 1990

ABSTRACT

In this study water potential (ψ_w) and the salt contribution to the osmotic component of $\psi_w(\psi_s)$ of the germination solution were controlled separately to determine the interaction between specific salt and ψ_w effects on seed germination of *Bouteloua curtipendula* (Michx.) Torr., *Cenchrus ciliaris* L., *Eragrostis lehmanniana* Nees and *Panicum coloratum* L. Seeds were germinated in contact with solutions of NaCl, $CaCl_2$, K_2SO_4, polyethylene glycol 8000 (PEG), and salt–PEG mixtures over the ψ_w range of 0 to −1·2 MPa. Ranking of specific salt effects on seed germination was not constant at different levels of ψ_w. $CaCl_2$ was found to have the least detrimental, and often a positive, effect on total germination in all species and at all water potentials. The relative ranking of NaCl and K_2SO_4 effects was changed for two species when rank was based on germination response to isopotential salt–PEG mixtures.

Key words: Seed germination, water potential, specific salt effects, *Bouteloua curtipendula* (Michx.) Torr., *Panicum coloratum* L., *Cenchrus ciliaris* L., *Eragrostis lehmanniana* Nees.

INTRODUCTION

Salts in the soil solution induce water potential (ψ_w) and specific salt effects on germinating seeds (Redmann, 1974; Ryan, Miyamoto and Strochlein, 1975). The relative magnitude of ψ_w and specific salt effects is dependent upon the degree of salt uptake (Sharma, 1973). Passive or active salt uptake raises the effective osmotic potential (ψ_s) of the soil solution and increases water availability to the seed (Collis-George and Sands, 1962). Salt toxicity, however, may overshadow any beneficial osmotic effects (Collis-George and Sands, 1962; Redmann, 1974).

If specific salt effects are defined as any osmotic or toxic effect resulting from seed uptake of a salt, then ψ_w and specific salt effects can be partitioned by comparing seed germination response in a salt solution to that in an isopotential solution of a non-penetrating osmoticum (Sharma, 1973; Redmann, 1974). Polyethylene glycol (PEG) with a molecular weight greater than 4000 is the most common osmoticum used for this purpose because it does not penetrate plant cell walls (Tarkow, Feist and Southerland, 1966; Carpita *et al.*, 1979). Individual salts can also been ranked for specific effects by comparison of seed germination response in isopotential solutions of the respective salts (Redmann, 1974; Ryan *et al.*, 1975).

A simple comparison of germination response in isopotential salt solutions, however, may not reflect the ranking of specific salt effects under natural germination conditions. Under natural conditions, ψ_s of soil water co-varies with changes in soil water content and, therefore, soil matric potential (ψ_m; Roundy, 1984). Roundy, Young and Evans (1985) and Hao and de Jong (1988) germinated seeds in soils at different ψ_s and ψ_m and found a significant interaction between ψ_s and ψ_m effects on germination. Their results, however, may have been confounded by other environmental variables that co-vary with soil water content (Collis-George and Williams, 1968). Romo and Haferkamp (1987) combined salt and PEG solutions to measure the interaction between salt concentration and ψ_w effects on seed germination but limited their investigation to a narrow range of salt concentrations and could not distinguish ψ_s differences between salt treatments.

The objective of this study was to determine the

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0305-7364/90/110587+09 $03.00/0

TABLE 1. *Target ψ_s, ψ_p and ψ_w for salt, PEG and salt–PEG solutions used for osmotic control of the germination medium*

ψ_s(MPa)	0	−0·2	−0·4	−0·6	−0·8	−1·0	−1·2
ψ_p (MPa)			ψ_w of Mixtures (MPa)				
0	0	−0·2	−0·4	−0·6	−0·8	−1·0	−1·2
−0·2	−0·2	−0·4	−0·6	−0·8	−1·0	−1·2	
−0·4	−0·4	−0·6	−0·8	−1·0	−1·2		
−0·6	−0·6	−0·8	−1·0	−1·2			
−0·8	−0·8	−1·0	−1·2				
−1·0	−1·0	−1·2					
−1·2	−1·2						

magnitude of specific salt effects on the germination characteristics of four grass species under conditions where ψ_w and ψ_s were separately controlled. Ranking of seed germination response in pure salt solutions was compared to the ranking in isopotential salt solutions that were amended with a non-penetrating osmoticum.

MATERIALS AND METHODS

Bouteloua curtipendula (Michx.) Torr., *Panicum coloratum* L., *Cenchrus ciliaris* L and *Eragrostis lehmanniana* Nees seeds were germinated on single sheets of Whatman No. 1 filter paper in contact with a 65-ml reservoir of osmotic solution inside a clear plastic vial with a snap top lid. A 3·8-cm diameter sheet of filter paper was glued to the bottom of a 12-mm length of polyvinyl chloride (PVC) pipe. The PVC segment was supported above the osmotic solution by plastic rod supports glued to the inside of the germination vials. Osmotic solution was poured into the vials so that the level of solution just reached the top of the support rods and saturated the filter paper attached to the bottom of the PVC. The large solution reservoir, relative to filter paper weight, was expected to limit the effects of PEG solution concentration by filter-paper fibres (Hardegree and Emmerich, 1990). The large solution volume and tight-fitting snap-top lid were also designed to limit concentration changes caused by water loss over the course of the experiment.

Solutions of NaCl, $CaCl_2$, K_2SO_4, PEG 8000 and salt–PEG mixtures were mixed according to the experimental design outlined in Table 1. Salt solutions were mixed according to standard tables for predicting ψ_s (Young *et al.*, 1983). PEG contribution to the osmotic potential (ψ_p) was predicted by eqn (4) of Michel (1983) as suggested by Hardegree and Emmerich (1990). Salts induce a synergistic effect on ψ_p when mixed with PEG in solution (Michel and Kaufmann, 1973; Michel, 1983), therefore Table 1 represents only the targeted ψ_s, ψ_p and ψ_w values for the salt–PEG mixtures. Actual ψ_w of each solution was measured three times, in random order, without filter paper (Hardegree and Emmerich, 1990), in an SC-10a thermocouple psychrometer (Decagon Devices, Pullman, WA)[1]. The psychrometer was calibrated with standard NaCl solutions (Lang, 1967) after every fourth sample.

Thirty seeds of a given species were placed on filter paper in contact with each of the osmotic solutions outlined in Table 1. Each seed group was treated with a 50-μl fungicide suspension (Daconil; tetrachloroisophthalonitrile) to restrict fungal growth. Seed germination vials were placed in a controlled-temperature room at 25 ± 1 °C, under illumination from both fluorescent and incandescent lights for 12 h d^{-1}. Each treatment was replicated six times with internally randomized replicate blocks placed in different areas of the controlled-temperature room. The large number of germination vials and the size limitation of the controlled-temperature room made it impossible to evaluate all species simultaneously. *B. curtipendula* and *C. ciliaris* were measured in the first germination trial and *P. coloratum* and *E. lehmanniana* in the second. The number of germinated seed was recorded on days 1–5, 7, 9, 11, 14, 17 and 21. Vials were opened for aeration during all non-count days. Seeds with a radical extension of $\geqslant 2$ mm were considered germinated and removed. A seed was considered to have abnormal germination if shoot growth occurred in the absence of radical extension $\geqslant 2$ mm. Seeds that developed fungus or exhibited abnormal germination were removed and recorded but treated as non-viable.

Three germination indices were calculated for each germination vial: total percent germination after 21 d divided by the mean value for germi-

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[1] Mention of a trademark name or proprietary product does not constitute endorsement by the USDA and does not imply its approval to the exclusion of other products that may also be suitable.

TABLE 2. *Calculated values for total percent germination* (G) *as a function of ψ_w for the seed of four grasses germinated in pure solutions of PEG, NaCl, K_2SO_4 and $CaCl_2$*

Species (solute ranking)	Solute	ψ_w (MPa) 0	−0·2	−0·4	−0·6	−0·8	−1·0	−1·2
B. curtipendula	PEG	101(4)*	103(3)	104(3)	104(3)	102(3)	99(3)	95(3)
(PEG > C ≫ N = K)	NaCl	110(12)	77(7)	50(7)	29(8)	14(7)	6(8)	3(12)
	K_2SO_4	107(8)	75(5)	48(5)	27(6)	12(5)	3(5)	−1(8)
	$CaCl_2$	103(8)	98(6)	92(5)	87(4)	81(5)	76(6)	70(8)
P. coloratum	PEG	99(8)	103(5)	98(6)	84(5)	66(5)	46(5)	27(7)
(C ⩾ PEG ≫ N > K)	NaCl	105(16)	90(11)	65(11)	37(10)	13(11)	1(11)	7(15)
	K_2SO_4	105(8)	81(6)	51(6)	23(5)	1(6)	−8(6)	0(8)
	$CaCl_2$	99(9)	99(6)	95(6)	87(6)	74(5)	57(6)	36(9)
C. ciliaris	PEG	96(8)	101(5)	101(5)	95(5)	83(5)	65(5)	41(6)
(C ⩾ PEG ≫ N ⩾ K)	NaCl	100(8)	81(5)	56(6)	29(5)	7(6)	−5(6)	−1(8)
	K_2SO_4	105(8)	79(5)	50(6)	23(5)	3(5)	−7(5)	−2(7)
	$CaCl_2$	99(8)	96(6)	97(6)	98(5)	93(6)	78(6)	48(9)
E. lehmanniana	PEG	113(18)	80(11)	52(10)	31(11)	15(11)	6(10)	2(14)
(C ⩾ PEG > N > K)	NaCl	93(13)	63(8)	40(8)	22(8)	10(8)	4(8)	3(12)
	K_2SO_4	105(12)	53(8)	21(8)	5(7)	−1(8)	0(8)	2(11)
	$CaCl_2$	93(16)	78(12)	62(9)	47(8)	32(9)	16(12)	1(15)

* Values in parentheses represent one-half of the width of calculated confidence intervals ($P \leqslant 0{\cdot}05$). Solute ranking was based upon degree of confidence interval overlap between regression models. Higher rank indicates large *G* in isopotential solution.

nation in the pure water treatment for the same species (*G*), days to 50% of *G* (D_{50}), and percent abnormal germination (G_a). Total percent germination was divided by the mean of the pure-water treatment in order to adjust for differences in seed viability between species. Cubic regression equations were first calculated to characterize *G*, D_{50} and G_a as a function of ψ_w for just the pure-salt and pure-PEG solution treatments. Cubic response surfaces were then calculated for the salt–PEG mixtures (Table 1) to characterize the interaction between ψ_s and ψ_w effects on seed germination. Two cubic response surfaces were calculated for each species–salt combination using *G* and D_{50} as dependent variables and ψ_w and ψ_s as independent variables (Evans *et al.*, 1982). All regression equations were first calculated using linear, quadratic and cubic terms. The regression equations were then recalculated, deleting first cubic, then quadratic, then linear terms that were not significant ($P \leqslant 0{\cdot}10$). Lower-order terms that were not significant were left in the equation if a higher-order term was significant. For D_{50}, only values in treatments with an average *G* of greater than 10% were used in the regression model.

G, D_{50} and 95% confidence limits were calculated from the cubic regression models for each of the target solutions listed in Table 1. Ranking of specific salt effects was based on a comparison of the relative position of modelled regression lines and the degree of confidence interval overlap. For purposes of ranking, the relative effects of any two solutes were not considered to be significantly different if the confidence limits of one model overlapped the predicted mean value of an isopotential point from the other model. Germination response was first ranked by a comparison of modelled germination response in isopotential pure solutions. Specific salt effects were then re-ranked by comparing modelled germination response in the PEG–salt solution mixtures.

RESULTS

Mean total percent germination for a given vial was divided by that in the pure-water treatment to calculate *G*. Mean total percent germination and standard errors for the pure-water treatments were: *B. curtipendula*, 90 ± 2; *P. coloratum*, 86 ± 7; *C. ciliaris*, 93 ± 5; *E. lehmanniana*, 36 ± 6.

Germination in isopotential pure solutions

Germination responses in isopotential pure solutions of PEG, NaCl, K_2SO_4 and $CaCl_2$ are shown in Tables 2 and 3. All regression models of *G* and D_{50} for the pure-salt and pure-PEG treatments were significant ($P \leqslant 0{\cdot}01$) except for the models of D_{50} for *E. lehmanniana* in the K_2SO_4 and PEG solutions.

Of the three salts, $CaCl_2$ had the least detrimental

TABLE 3. *Calculated values for days to 50% of* G (D_{50}) *as a function of* ψ_w *for the seed of four grasses germinated in pure solutions of PEG, NaCl,* K_2SO_4 *and* $CaCl_2$

Species (solute ranking)	Solute	ψ_w (MPa) 0	−0·2	−0·4	−0·6	−0·8	−1·0	−1·2
B. curtipendula	PEG	1·7(0·2)*	1·8(0·2)	2·0(0·1)	2·4(0·2)	2·9(0·2)	3·6(0·1)	4·4(0·2)
(PEG = C > N = K)	NaCl	1·7(0·5)	2·4(0·3)	2·8(0·4)	2·9(0·4)	2·5(0·6)	†	†
	K_2SO_4	1·7(0·6)	2·2(0·4)	2·7(0·4)	3·2(0·6)	†	†	†
	$CaCl_2$	1·7(0·3)	2·0(0·2)	2·2(0·2)	2·4(0·2)	2·7(0·2)	3·3(0·2)	4·2(0·3)
P. coloratum	PEG	2·6(1·0)	3·2(0·7)	3·5(0·7)	3·9(0·7)	4·9(0·7)	7·0(0·7)	10·7(0·9)
(PEG = N = K ⩾ C)	NaCl	2·7(0·4)	3·0(0·2)	3·5(0·3)	4·1(0·3)	4·9(0·4)	5·8(0·5)	†
	K_2SO_4	2·7(0·2)	3·3(0·2)	3·9(0·2)	†	†	†	†
	$CaCl_2$	2·8(0·9)	3·4(0·6)	4·4(0·6)	5·8(0·6)	7·5(0·6)	9·5(0·6)	11·9(1·0)
C. ciliaris	PEG	2·2(1·2)	1·9(0·9)	2·9(0·8)	4·8(0·7)	7·2(0·8)	9·7(0·9)	11·8(1·1)
(C ⩾ PEG > N ⩾ K)	NaCl	1·9(0·4)	3·2(0·4)	3·6(0·4)	5·1(0·4)	†	†	†
	K_2SO_4	1·8(0·4)	3·1(0·2)	4·4(0·4)	†	†	†	†
	$CaCl_2$	2·1(0·6)	2·2(0·3)	2·7(0·3)	3·6(0·4)	4·8(0·4)	6·4(0·4)	8·3(0·6)
E. lehmanniana	PEG	3·9(0·5)	3·9(0·5)	3·9(0·5)	3·9(0·5)	3·9(0·5)	†	†
(K > PEG > N = C)	NaCl	3·0(1·5)	4·8(1·2)	4·8(1·0)	4·8(1·3)	6·8(1·6)	†	†
	K_2SO_4	3·3(0·4)	3·3(0·4)	3·3(0·4)	†	†	†	†
	$CaCl_2$	3·5(0·8)	4·3(0·6)	5·1(0·5)	5·9(0·5)	6·7(0·6)	7·4(0·8)	†

† Treatments with a measured mean value of $G < 10$ were not included in the regression model.

* Values in parentheses represent one-half of the width of calculated confidence intervals ($P \leqslant 0·05$). Solute ranking was based upon degree of confidence interval overlap between regression models. Higher rank denotes a more rapid germination rate (lower D_{50}).

TABLE 4. *Cubic response surface for total percent germination* (G) *of* Bouteloua curtipendula *as a function of* ψ_w *and* ψ_s *based on calculated values*

ψ_s (MPa)	Salt	ψ_w (MPa) 0	−0·2	−0·4	−0·6	−0·8	−1·0	−1·2
0	NaCl	105(8)*	107(5)	108(7)	107(4)	105(6)	101(4)	96(5)
	K_2SO_4	104(6)	106(5)	106(6)	106(4)	106(6)	101(4)	98(4)
	$CaCl_2$	99(5)	102(5)	103(4)	103(4)	102(4)	99(6)	94(4)
−0·2	NaCl	—	83(6)	79(4)	74(4)	66(4)	58(4)	48(6)
	K_2SO_4	—	79(6)	76(4)	73(3)	70(4)	67(3)	64(4)
	$CaCl_2$	—	100(5)	101(4)	100(3)	98(3)	95(3)	90(4)
−0·4	NaCl	—	—	56(6)	49(4)	40(4)	30(4)	19(6)
	K_2SO_4	—	—	53(6)	48(4)	43(3)	40(3)	38(5)
	$CaCl_2$	—	—	97(5)	97(3)	95(3)	92(3)	88(4)
−0·6	NaCl	—	—	—	29(5)	22(4)	14(4)	4(5)
	K_2SO_4	—	—	—	29(5)	23(4)	20(4)	18(5)
	$CaCl_2$	—	—	—	90(4)	90(3)	89(3)	87(4)
−0·8	NaCl	—	—	—	—	8(6)	5(4)	1(6)
	K_2SO_4	—	—	—	—	10(5)	6(4)	5(5)
	$CaCl_2$	—	—	—	—	82(5)	84(3)	85(5)
−1·0	NaCl	—	—	—	—	—	−1(7)	3(7)
	K_2SO_4	—	—	—	—	—	−1(6)	−1(4)
	$CaCl_2$	—	—	—	—	—	73(5)	78(5)
−1·2	NaCl	—	—	—	—	—	—	8(9)
	K_2SO_4	—	—	—	—	—	—	0(8)
	$CaCl_2$	—	—	—	—	—	—	66(8)

* Values in parentheses represent one-half of the width of calculated confidence intervals ($P \leqslant 0·05$).

TABLE 5. *Cubic response surface for total percent germination* (G) *of* Panicum coloratum *as a function of* ψ_w *and* ψ_s *based on calculated values*

		ψ_w (MPa)						
ψ_s (MPa)	Salt	0	−0·2	−0·4	−0·6	−0·8	−1·0	−1·2
0	NaCl	106(9)*	103(6)	96(8)	85(4)	71(5)	51(4)	28(6)
	K_2SO_4	107(6)	105(6)	98(7)	87(4)	72(6)	53(4)	29(4)
	$CaCl_2$	101(6)	101(6)	96(5)	86(4)	71(4)	51(5)	26(4)
−0·2	NaCl	—	91(7)	85(5)	75(4)	60(4)	42(4)	19(5)
	K_2SO_4	—	84(6)	82(4)	76(4)	66(4)	52(4)	33(5)
	$CaCl_2$	—	100(5)	94(4)	83(4)	67(3)	46(4)	20(5)
−0·4	NaCl	—	—	65(6)	58(4)	47(4)	32(4)	12(5)
	K_2SO_4	—	—	53(5)	52(4)	46(4)	36(4)	22(5)
	$CaCl_2$	—	—	95(5)	86(3)	71(3)	52(3)	27(5)
−0·6	NaCl	—	—	—	35(6)	30(4)	21(4)	8(6)
	K_2SO_4	—	—	—	21(5)	20(4)	16(4)	6(5)
	$CaCl_2$	—	—	—	86(5)	75(3)	60(3)	40(4)
−0·8	NaCl	—	—	—	—	10(7)	10(4)	6(5)
	K_2SO_4	—	—	—	—	−3(6)	−3(4)	−7(6)
	$CaCl_2$	—	—	—	—	73(6)	64(4)	50(5)
−1·0	NaCl	—	—	—	—	—	−2(7)	6(5)
	K_2SO_4	—	—	—	—	—	−11(5)	−10(6)
	$CaCl_2$	—	—	—	—	—	56(6)	52(6)
−1·2	NaCl	—	—	—	—	—	—	8(10)
	K_2SO_4	—	—	—	—	—	—	6(9)
	$CaCl_2$	—	—	—	—	—	—	37(9)

* Values in parentheses represent one-half of the width of calculated confidence intervals ($P \leqslant 0{\cdot}05$).

effect on *G* for all four species (Table 2). *B. curtipendula* is the only species for which $CaCl_2$ had a negative specific salt effect on *G* over the entire ψ_w range (Table 2). Isopotential solutions of NaCl and K_2SO_4 had a negative specific salt effect on *G* for all species over most of the ψ_w range (Table 2). For three of the species, isopotential pure solutions of K_2SO_4 had a more negative effect on *G* than solutions of NaCl over most of the ψ_w range (Table 2). *B. curtipendula* was the only species for which NaCl and K_2SO_4 could not be distinguished under the criteria for ranking isopotential pure solution effects.

Specific salt and PEG solution effects on D_{50} were ranked in order of decreasing germination rate (increasing time required for germination). The overall ranking by D_{50} was about the same as for *G* for *B. curtipendula* and *C. ciliaris* (Table 3). For *E. lehmanniana* and *P. coloratum*, specific salt ranking of D_{50} was roughly the reverse of the ranking for *G* (Table 3). Specific salt effects on D_{50} were not as clear-cut as for *G* because the differences between treatments were smaller and relative ranking between salts was not consistent for all species (Table 3). D_{50} could not be compared for all solutes at every level of ψ_w because some treatments exhibited zero germination. Some treatments were also excluded from the regression models for D_{50} if mean *G* for the treatment was less than 10%. D_{50} in treatments with a mean *G* of less than 10% was highly variable because in some cases, a single seed defined the germination rate for a whole treatment replicate.

Germination in salt–PEG mixtures

Total germination response in salt–PEG solution mixtures is shown in Tables 4–7. All regression models in Tables 4–7 were significant ($P \leqslant 0{\cdot}01$).

There was a clear interaction between ψ_s and ψ_w effects on *G* (Tables 4–7). For *B. curtipendula*, $CaCl_2$ had a negative specific salt effect for *G* but the detrimental effect of a given concentration of salt was less at lower ψ_w (Table 4). For *P. coloratum* (Table 5) and *C. ciliaris* (Table 6), some of the $CaCl_2$–PEG treatments had greater *G* than in isopotential pure solutions of either PEG or $CaCl_2$. *E. lehmanniana* germination increased with any increase in $CaCl_2$ concentration (Table 7).

TABLE 6. *Cubic response surface for total percent germination* (G) *of* Cenchrus ciliaris *as a function of* ψ_w *and* ψ_s *based on calculated values*

		ψ_w (MPa)						
ψ_s (MPa)	Salt	0	−0·2	−0·4	−0·6	−0·8	−1·0	−1·2
0	NaCl	98(8)*	102(5)	101(6)	94(3)	82(4)	63(4)	39(5)
	K_2SO_4	100(5)	105(5)	105(5)	98(4)	85(6)	65(4)	40(3)
	$CaCl_2$	95(4)	101(5)	101(3)	95(4)	83(3)	66(4)	43(3)
−0·2	NaCl	—	84(5)	70(3)	55(3)	40(4)	24(4)	7(4)
	K_2SO_4	—	80(5)	77(3)	69(3)	58(3)	43(3)	23(3)
	$CaCl_2$	—	95(5)	99(4)	96(4)	88(3)	74(3)	55(4)
−0·4	NaCl	—	—	60(6)	38(3)	19(4)	5(4)	−5(4)
	K_2SO_4	—	—	53(5)	45(3)	35(3)	24(3)	10(4)
	$CaCl_2$	—	—	97(5)	97(4)	91(3)	80(3)	63(4)
−0·6	NaCl	—	—	—	33(5)	12(3)	0(3)	−3(5)
	K_2SO_4	—	—	—	24(4)	17(3)	9(3)	1(4)
	$CaCl_2$	—	—	—	97(5)	93(3)	82(3)	66(4)
−0·8	NaCl	—	—	—	—	9(5)	−1(4)	3(5)
	K_2SO_4	—	—	—	—	2(4)	−2(3)	−3(4)
	$CaCl_2$	—	—	—	—	92(6)	82(3)	65(4)
−1·0	NaCl	—	—	—	—	—	−5(6)	6(6)
	K_2SO_4	—	—	—	—	—	−9(5)	−4(4)
	$CaCl_2$	—	—	—	—	—	78(6)	60(5)
−1·2	NaCl	—	—	—	—	—	—	−4(8)
	K_2SO_4	—	—	—	—	—	—	−1(6)
	$CaCl_2$	—	—	—	—	—	—	51(7)

* Values in parentheses represent one-half of the width of calculated confidence intervals ($P \leqslant 0{\cdot}05$).

NaCl and K_2SO_4 had a detrimental effect on *G* in both pure salt and salt-PEG solution mixtures. The negative specific salt effect on *G* of a given concentration of NaCl, however, became greater at lower ψ_w for *B. curtipendula* and *C. ciliaris* (Tables 4 and 6). In contrast, the negative specific salt effect on *G* of a given concentration of K_2SO_4 became proportionally less negative at lower ψ_w for these two species (Tables 4 and 6). The relative effect of NaCl and K_2SO_4 in the different salt-PEG mixtures was so large that it changed the ranking of specific salt effects on *G* for *B. curtipendula* from NaCl = K_2SO_4 to K_2SO_4 > NaCl and for *C. ciliaris* from NaCl > K_2SO_4 to K_2SO_4 > NaCl (Tables 4 and 6).

The interaction between ψ_w and ψ_s was less clear for D_{50} because, as with the pure solutions, differences between treatments were relatively small (data not shown).

Abnormal germination

Abnormal germination response was highly species- and salt-specific. There was very little abnormal germination in pure PEG, pure $CaCl_2$ and $CaCl_2$-PEG mixtures for any species, and *E. lehmanniana* had very little abnormal germination in any solution treatment (data not shown). Calculated values for G_a and 95% confidence limits for isopotential solutions of NaCl and K_2SO_4 are shown for *B. curtipendula*, *P. coloratum* and *C. ciliaris* in Table 8. All regression models represented in Table 8 were significant at the $P \leqslant 0{\cdot}01$ level except for *B. curtipendula*-K_2SO_4 and *C. ciliaris*-K_2SO_4 which were significant at the $P \leqslant 0{\cdot}02$ level.

Interpretation of regression models

Confidence limits are included in Tables 2–8 as an estimate of model variability and to provide criteria for ranking specific salt effects. Confidence-interval overlap, however, is invalid as a statistical test for distinguishing the regression models or for distinguishing different levels of a quantitative factor within the regression models (Chew, 1976).

The regression models were not constrained to pass through the mean of the control treatment as

TABLE 7. *Cubic response surface for total percent germination* (G) *of* Eragrostis lehmanniana *as a function of* ψ_w *and* ψ_s *based on calculated values*

ψ_s (MPa)	Salt	ψ_w (MPa) 0	−0·2	−0·4	−0·6	−0·8	−1·0	−1·2
0	NaCl	110(11)*	77(6)	50(8)	29(4)	14(5)	5(4)	2(7)
	K_2SO_4	116(7)	81(6)	52(8)	30(4)	15(8)	6(4)	3(5)
	$CaCl_2$	107(6)	80(6)	56(4)	36(4)	20(4)	8(6)	0(5)
−0·2	NaCl	—	65(8)	43(4)	25(4)	12(4)	4(4)	0(5)
	K_2SO_4	—	55(7)	35(4)	20(4)	9(4)	3(4)	1(5)
	$CaCl_2$	—	82(8)	58(5)	39(5)	23(5)	11(5)	3(7)
−0·4	NaCl	—	—	36(8)	22(4)	11(4)	3(4)	−1(6)
	K_2SO_4	—	—	19(7)	11(4)	5(4)	1(4)	0(5)
	$CaCl_2$	—	—	61(6)	41(5)	25(5)	13(4)	5(6)
−0·6	NaCl	—	—	—	19(7)	10(5)	3(5)	−2(6)
	K_2SO_4	—	—	—	2(6)	1(4)	0(4)	0(5)
	$CaCl_2$	—	—	—	43(6)	28(5)	16(4)	8(6)
−0·8	NaCl	—	—	—	—	10(7)	4(5)	−1(6)
	K_2SO_4	—	—	—	—	−2(6)	0(5)	0(6)
	$CaCl_2$	—	—	—	—	30(6)	18(5)	10(6)
−1·0	NaCl	—	—	—	—	—	6(7)	0(6)
	K_2SO_4	—	—	—	—	—	0(7)	1(5)
	$CaCl_2$	—	—	—	—	—	21(7)	13(8)
−1·2	NaCl	—	—	—	—	—	—	2(10)
	K_2SO_4	—	—	—	—	—	—	2(9)
	$CaCl_2$	—	—	—	—	—	—	15(9)

* Values in parentheses represent one-half of the width of calculated confidence intervals ($P \leqslant 0{\cdot}05$).

TABLE 8. *Calculated values for abnormal percent germination* (Ga) *as a function of* ψ_w *for the seed of three grasses germinated in pure solutions of NaCl and* K_2SO_4

Species	Solute	ψ_w (MPa) 0	−0·2	−0·4	−0·6	−0·8	−1·0	−1·2
B. curtipendula	NaCl	−1(9)*	14(6)	24(6)	29(6)	29(6)	23(6)	13(9)
	K_2SO_4	1(6)	6(4)	9(4)	11(4)	10(4)	8(4)	3(5)
P. coloratum	NaCl	−2(12)	6(9)	23(9)	42(8)	54(9)	52(8)	29(12)
	K_2SO_4	−6(9)	18(6)	26(7)	22(5)	12(6)	3(6)	−2(8)
C. ciliaris	NaCl	−3(6)	8(4)	15(4)	17(4)	16(4)	10(4)	0(6)
	K_2SO_4	0(3)	3(2)	4(2)	5(2)	4(2)	2(2)	0(3)

* Values in parentheses represent one-half of the width of calculated confidence intervals ($P \leqslant 0{\cdot}05$).

this would have masked the variability between individual vials at $\psi_w = 0$. The unconstrained models provided the best fit for all of the data, but introduced a seeming discrepancy between models at $\psi_w = 0$ (Tables 2–7). According to the criteria for ranking, however, there was significant overlap of calculated confidence intervals and, therefore, little difference between models at $\psi_w = 0$.

The regression models were also unconstrained with respect to the absolute possible minimum value for *G*, resulting in some negative *G* estimates at low ψ_w. This generally occurred when a few seeds germinated at very low ψ_w in the absence of germination at adjacent, higher values of ψ_w.

DISCUSSION

ψ_m and ψ_s are the thermodynamic components of soil water availability for seed germination. Germinating seeds, however, respond to other environmental variables that co-vary with soil water content and the type of salt present. Soil water content determines ψ_m but also affects hydraulic conductivity, aeration, soil strength, and the area of seed/soil-water contact (Collis-George and Williams, 1968). Salts determine ψ_s of the germination medium but also induce specific salt effects if taken up by the seed (Redmann, 1974; Ryan *et al.*, 1975). The effect of ψ_m and ψ_s *per se* should be identical and therefore additive, if all other soil variables remain constant and if seed membranes are impermeable to salts in the soil solution (Sharma, 1973). In this experiment, PEG solution was used in place of soil to eliminate the germination-confounding effects of unsaturated porous matrices (Collis-George and Williams, 1968). It was therefore assumed that all differences between isopotential treatments could be attributed to the effects of specific salt uptake.

Ranking of specific salt effects on seed germination was more complex than indicated by a simple comparison of germination response in isopotential solutions of salt and PEG (Tables 2 and 3). The most dramatic change in specific salt ranking occurred in the comparison of NaCl and K_2SO_4 effects. The detrimental effects of NaCl and K_2SO_4 in isopotential pure-salt solutions could not be distinguished for *B. curtipendula* (Table 2), but the interaction of ψ_s and ψ_w was enough to clearly separate NaCl and K_2SO_4 in the salt–PEG solution mixtures (Table 4). The larger detrimental effect of NaCl at lower ψ_w in salt–PEG mixtures was enough to reverse the relative ranking of NaCl and K_2SO_4 for *C. ciliaris* (Tables 2 and 7).

Ranking of specific salt effects in this experiment was not the same as for many previous experiments. Sharma (1973) and Romo and Eddleman (1985) found that total germination in an NaCl solution was greater than germination in an isopotential solution of PEG. PEG exclusion from the organic germination matrices used by these workers may have lowered ψ_w of the PEG solutions below expected values (Hardegree and Emmerich, 1990), but the PEG exclusion effect is unlikely to account for the very large difference between NaCl and PEG noted by Sharma (1973). Other workers have found that NaCl has the same effect on germination (Chaudhuri and Wiebe, 1968) or a less detrimental effect on germination than $CaCl_2$ (Hyder and Yasmin, 1972). The general benefits of $CaCl_2$, for germination, however, have been demonstrated in mixtures with other salts (Marcar, 1986).

Ranking of specific salt effects in isopotential pure solutions was not consistent between germination indices. Dividing specific salt effects into osmotic and non-osmotic effects may explain the differences in the pure solution ranking of G and D_{50} (Tables 2 and 3). Both active and passive salt uptake would result in osmotic specific salt effects that would tend to increase seed germination rate by increasing the ψ_w gradient between seed and soil. Non-osmotic specific salt effects result from enhancement of, or interference with, metabolic processes in the seed and would more likely show up as a difference in total germination. Specific salts, however, may also interfere with germination rate-limiting metabolic processes. Discrepancies between rankings of total germination and germination rate effects (Tables 2 and 3) indicate that osmotic and non-osmotic specific salt effects may be manifest to different degrees in different species. If the decrease in D_{50} caused by NaCl and K_2SO_4 for *E. lehmanniana* and *P. coloratum* indicates greater salt uptake relative to $CaCl_2$, then the detrimental effect of these salts on G may be caused by higher internal concentrations rather than a higher specific toxicity of these ion pairs. $CaCl_2$ may also be less toxic to *B. curtipendula* than is indicated by G if, as the lower D_{50} indicates, more of this salt is taken up. This experiment was not designed to measure the amount of salt uptake by germinating seeds, but it is clear that an understanding of the mode of action of specific salts will require information on the amount of salt taken up, as well as the amount available to be taken up.

C. ciliaris is the only species for which both G and D_{50} were more favourable in solutions of $CaCl_2$ than in solutions of either PEG, NaCl or K_2SO_4 over most of the ψ_w range. The addition of $CaCl_2$ to soil may, therefore, enhance germination for this species. Our study, however, was not designed to evaluate intraspecific variability in seed germination: thus, the significance of individual species response to specific salts cannot be assessed.

The percentage of seeds exhibiting abnormal germination was highly species-specific but was usually greater in solutions of NaCl (Table 8). Abnormally germinated seeds generally had shoot growth and radicle extension but the radicle aborted before achieving a length of 2 mm. At lower ψ_w, G_a in pure solutions of NaCl and K_2SO_4 was greater than G for *B. curtipendula*, *P. coloratum* and *C. ciliaris*. Interpretation of seedling emergence data may therefore be affected by the timing of emergence measurements. If seedlings are considered emerged as soon as leaf elongation is observed, non-viable seedlings may be counted as normal, even though they would otherwise die from lack of root growth if observed for a longer period. Our definition of normal germination, however, was similarly arbitrary in that we did not determine whether seeds with radicale extension ⩾ 2 mm exhibited subsequent shoot growth.

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