Hardegree, S. P. and Emmerich, W. E. (1994). *Seed Sci. & Technol.*, **22**, 1–7

Seed germination response to polyethylene glycol solution depth

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(Accepted August 1993)

Summary

Osmotic solutions of polyethylene glycol (PEG) are commonly used to control water potential in seed germination studies. PEG reduces the water potential of osmotic solutions but may limit oxygen availability to germinating seeds. The purpose of this study was to determine whether seed immersion in PEG solution had a detrimental effect on total germination percentage and germination rate of six grass species. *Bouteloua curtipendula*, *Cenchrus ciliaris*, *Panicum coloratum*, *Eragrostis lehmanniana*, *Pseudoroegneria spicata* and *Leymus cinereus* seeds were germinated over the water potential range of 0 to −1.5 MPa at PEG solution depths of 0, 1, 3, or 5 mm. Total germination percentage and germination rate were generally greatest at 1 mm depth even in the pure water treatments. Reduced germinability at 0 mm depth may have been caused by the lower hydraulic conductivity of the medium. Reduced germinability at 3 and 5 mm depth may have been caused by decreased oxygen availability to the seeds. A reduction in germination was also apparent at 3 and 5 mm depth in pure water treatments, indicating that oxygen diffusion between the seeds and the air/solution interface was more important to oxygen availability than was the presence or absence of PEG *per se*.

Introduction

Osmotic solutions of polyethylene glycol (PEG) are often used to control water potential in seed germination studies (Young, Evans, Roundy and Cluff, 1983). A common experimental protocol is to equilibrate seeds on PEG solution-saturated filter paper inside a petri dish. Filter paper has been shown to contain a volume fraction that excludes high-molecular-weight PEG, absorbs water from the solution, and lowers the water potential of the medium (Hardegree and Emmerich, 1990). One strategy for reducing this effect is to increase the ratio of solution volume to filter paper weight (Hardegree and Emmerich, 1990; Emmerich and Hardegree, 1991). Supersaturation of filter paper, however, may result in seed immersion which could limit oxygen availability to germinating seeds (Mexal, Fisher, Osteryoung and Reid, 1975). Emmerich and Hardegree (1990) determined that PEG-seed contact *per se* has little effect on germination response but did not measure immersion effects. The objective of this experiment was to determine the degree to which seed immersion in PEG solution affects total germination percentage and germination rate of selected grass species.

Materials and methods

Bouteloua curtipendula (Mich.) Torr., *Cenchrus ciliaris* L., *Panicum coloratum* L., *Pseudoroegneria spicata* (Pursh) Löve, and *Leymus cinereus* (Scribn. and Merr.) A. Love seeds were obtained from the Soil Conservation Service, Plant Materials Centers in Tucson, AZ and Aberdeen, ID. *E. lehmanniana* Nees seeds were obtained from a field collection in southern Arizona. These species were selected because they occur over large areas of rangeland in the western United States.

Seeds were germinated using the water potential control system described by Hardegree and Emmerich (1992). This system consists of a membrane-bottom germination cup, the bottom of which is in contact with a solution reservoir of PEG, inside a clear plastic snap-top vial. The cellulose membrane has a molecular weight exclusion limit of 3500 which is sufficient to prevent the PEG from crossing the membrane but does not restrict the movement of water. In previous studies, this system was used to create a matric-potential control surface on top of the membrane that was in equilibrium with the osmotic-potential of the solution under the membrane (Hardegree and Emmerich, 1992). In the current study, PEG solution was also present on top of the membrane, inside the germination cup. Initial solution depth was established by depositing a known volume of PEG solution on top of the membrane. Solution concentration and depth on top of the membrane were maintained by equilibration with the iso-osmotic solution reservoir under the membrane. The 65 mL solution reservoir buffered any changes in solution concentration and depth on top of the membrane that may have resulted from seed imbibition.

PEG was mixed with water to yield osmotic solutions with a water potential of −0.1, −0.3, −0.6, −0.9, −1.2 or −1.5 MPa (Hardegree and Emmerich, 1990). An iso-osmotic solution was deposited on top of the membrane to a depth of 1, 3, or 5 mm. Five replicate samples of 35 seeds each were germinated under all treatment combinations of species, water potential and solution depth. A set of replicate samples were also germinated at all water potentials with 0 mm solution depth (bare membrane) and at all depths in a pure water (0 MPa) treatment. Solution depth in the pure water treatments were initially set at 0, 1, 3, or 5 mm but could not subsequently be controlled in the same manner as the PEG solutions. The level of water in the solution reservoir was adjusted for the 0 MPa treatment to approximately coincide with the depth of water inside the germination cup.

Germination vials were randomly arranged within 5 blocks in a controlled temperature room which was maintained at 25 ± 1 °C under both fluorescent and incandescent lights (10.5 W m^{-2}) which were on for 12 h d^{-1}. Seeds were checked for germination after 1, 2, 3, 4, 5, 7, 9, 11 and 14 days. Seeds were considered germinated and were counted and removed if the exhibited radicle extension of ≥ 2 mm. Seeds that developed fungal growth were removed and considered non-viable.

Two germination indices were calculated for each germination vial: total germination percentage (G) and days to reach 50% of G (D_{50}) as an index of germination rate. Water potential and solution depth were both continuous variables, therefore, a regression

technique was used to describe variability in treatment response (Chew, 1976). Cubic response surfaces were calculated to relate G and D_{50} to water potential and solution depth for each species following the procedure outlined by Evans, Easi, Book and Young (1982). Regression equations were recalculated deleting first cubic, then quadratic, then linear terms that were not significant ($P \leq 0.10$). Lower order terms that were not significant were left in the equation if a higher order term was significant. Germination index values were estimated from the regression equations and model confidence intervals ($P \leq 0.05$) determined for each treatment combination of species, water potential and solution depth.

Germination rate could not be calculated for treatments that had zero germination. Treatments with near zero total germination also exhibited very low variability among treatment replicates which would artificially reduce confidence band width. Treatments with more than two replicate samples showing zero germination were, therefore, not included in the regression analysis.

Results

Solution depth influenced G at all PEG concentrations but the magnitude of the effect varied with species (Table 1). G was highest in the 1 mm depth treatments and lowest in the 5 mm depth treatments for *L. cinereus*, *B. curtipendula*, *P. spicata* and for most water potential treatments of *C. ciliaris* (Table 1). *P. coloratum* showed peak levels of G in the 1 mm treatment but exhibited lowest G values in the 0 mm depth treatment over most of the measured water potential range (Table 1). G values of *E. lehmanniana* increased with solution depth between 0 and 3 mm (Table 1).

D_{50} decreased (germination rate increased) as solution depth increased from 0 to 1 mm solution depths for *L. cinereus*, *C. ciliaris*, *B. curtipendula* and *P. spicata* (Table 2). *P. coloratum* and *E. lehmanniana* showed very little change in D_{50} between the 0 and 1 mm depth treatments (Table 2). Response of germination rate to solution depths greater than 1 mm was highly variable among species.

A reduction in water potential had a fairly consistent effect on both G and D_{50}. Total germination percentage and germination rate were usually lower in the more negative water potential treatments, especially at the more shallow solution depths (Tables 1 and 2).

The regression models were not constrained with respect to minimum possible values of G. Unconstrained models provided the best fit for the data but resulted in some seemingly erroneous (negative) predicted values for treatments having near zero total germination (Table 1). In all cases, however, the confidence limits of the model overlapped the minimum possible value (zero) for G.

Actual treatment means are included in the tables for comparison to predicted values. Model confidence limits, however, provide the best estimate of treatment variability because they take into account both within and between-treatment variability. All regression models were significant at the $P \leq 0.01$ level. Regression R^2 values are included in the tables as an additional index to model fit. R^2 values for the regression models ranged between 0.74 and 0.88 for G and between 0.63 and 0.93 for D_{50}.

Table 1. Predicted values for total germination percentage (G) as a function of water potential and PEG solution depth, and one-half confidence interval widths ($P \leq 0.05$) calculated from the regression model. Numbers in parentheses represent measured mean treatment values.

Species Adj. R^2	Solution Depth (mm)	Water Potential (MPa) 0	−0.1	−0.3	−0.6	−0.9	−1.2	−1.5
	0	96,10 (95)	95,9 (97)	91,7 (96)	82,7 (93)	69,8 (74)	50,8 (39)	27,12 (16)
L. ciner.	1	99,9 (93)	100,8 (89)	100,7 (94)	97,7 (91)	89,7 (94)	77,7 (84)	59,10 (75)
0.74	3	37,10 (48)	41,9 (39)	47,7 (61)	51,7 (49)	51,8 (44)	46,8 (40)	37,11 (31)
	5	–	6,12 (5)	13,9 (11)	20,8 (17)	21,8 (22)	19,9 (20)	11,12 (160)
	0	77,7 (81)	74,5 (82)	65,5 (73)	46,5 (35)	24,6 (10)	5,6 (5)	−4,8 (2)
P. color.	1	94,6 (79)	93,5 (92)	88,5 (82)	72,5 (84)	53,5 (59)	36,5 (45)	26,7 (23)
0.86	3	79,6 (84)	81,5 (78)	80,5 (81)	69,5 (78)	52,5 (55)	34,6 (29)	22,7 (14)
	5	77,7 (79)	81,6 (83)	82,5 (79)	72,6 (62)	53,6 (56)	30,6 (30)	11,9 (16)
	0	83,5 .(85)	82,4 (85)	76,4 (75)	60,4 (58)	40,4 (34)	13,6 (13)	–
E. lehma.	1	86,4 (85)	85,3 (87)	81,3 (76)	68,3 (73)	49,3 (58)	22,5 (24)	–
0.88	3	87,5 (88)	88,4 (89)	87,3 (82)	78,4 (80)	58,4 (60)	30,6 (20)	–
	5	87,6 (90)	89,4 (88)	89,4 (90)	79,5 (74)	56,5 (60)	22,7 (24)	–
	0	82,9 (81)	86,7 (92)	88,6 (87)	79,7 (86)	55,7 (45)	17,9 (16)	–
C. cilia.	1	87,7 (80)	91,6 (87)	93,6 (93)	88,7 (90)	70,6 (77)	40,6 (36)	−2,10 (5)
0.76	3	67,8 (81)	69,7 (66)	70,6 (63)	67,6 (60)	57,6 (62)	39,7 (45)	14,9 (5)
	5	57,9 (63)	56,7 (53)	54,6 (44)	48,7 (46)	40,7 (50)	29,7 (28)	16,11 (16)
	0	89,7 (94)	89,6 (93)	90,5 (92)	86,5 (86)	79,6 (82)	67,6 (57)	51,9 (47)
B. curti.	1	96,6 (89)	96,6 (89)	97,5 (90)	93,5 (89)	85,5 (89)	73,5 (87)	57,7 (63)
0.77	3	82,7 (87)	82,6 (83)	80,5 (85)	73,5 (80)	62,6 (56)	46,6 (42)	26,8 (18)
	5	88,8 (86)	86,7 (84)	79,5 (82)	64,5 (65)	46,6 (40)	23,6 (21)	−4,9 (3)
	0	83,9 (86)	85,7 (84)	81,7 (85)	64,7 (62)	38,7 (29)	11,8 (4)	−10,11 (2)
P. spica.	1	80,8 (84)	84,7 (78)	86,7 (84)	77,7 (78)	59,6 (66)	41,8 (47)	28,11 (17)
0.81	3	13,9 (9)	20,7 (22)	28,7 (25)	28,7 (34)	18,7 (21)	9,9 (7)	–
	5	–	9,11 (14)	15,8 (12)	13,7 (9)	10,7 (4)	–	–

Discussion

Mexal et al. (1975) measured reduced oxygen availability in solutions of PEG but could only infer potential negative effects on plant growth and metabolism. Osmotic seed-priming research has confirmed that PEG solutions must be aerated to prevent deterioration of immersed seeds (Bujalski and Nienow, 1991). The most common use of PEG, however, is the routine analysis of seed germination response to reduced water potential. It is not feasible to aerate a PEG solution contained in a petri dish and it is generally assumed that oxygen will not be limiting in such a small volume. Data from the cur-

Table 2. Predicted values for days to 50% of G (D_{50}) as a function of water potential and PEG solution depth, and one-half confidence interval widths ($P \leq 0.05$) calculated from the regression model. Numbers in parentheses represent measured mean treatment values.

Species Adj. R^2	Solution Depth (mm)	Water Potential (MPa) 0	−0.1	−0.3	−0.6	−0.9	−1.2	−1.5
	0	2.1, 0.6 (1.7)	2.2, 0.5 (2.0)	2.6, 0.5 (2.4)	3.8, 0.4 (3.7)	5.4, 0.5 (5.0)	7.0, 0.5 (8.5)	8.4, 0.7 (7.9)
L. ciner.	1	1.7, 0.5 (2.2)	1.6, 0.4 (2.1)	1.8, 0.5 (2.4)	2.6, 0.4 (2.7)	3.9, 0.4 (3.3)	5.2, 0.5 (4.6)	6.2, 0.6 (5.9)
0.73	3	3.0, 0.6 (2.9)	2.8, 0.5 (2.5)	2.7, 0.5 (2.7)	3.2, 0.4 (3.1)	4.0, 0.4 (3.1)	4.9, 0.5 (5.5)	5.6, 0.7 (6.5)
	5	–	2.4, 0.7 (2.0)	2.3, 0.5 (2.7)	2.9, 0.4 (3.2)	3.8, 0.5 (4.0)	4.8, 0.6 (5.1)	5.5, 0.8 (4.8)
	0	2.2, 0.7 (2.2)	2.4, 0.5 (2.2)	2.7, 0.5 (2.7)	3.5, 0.5 (3.4)	4.5, 0.6 (4.1)	5.9, 0.6 (6.8)	7.5, 0.9 (7.5)
P. color	1	2.2, 0.6 (2.5)	2.4, 0.5 (2.5)	2.8, 0.4 (3.2)	3.6, 0.5 (3.5)	4.6, 0.5 (4.8)	5.8, 0.5 (5.3)	7.3, 0.7 (7.0)
0.67	3	3.0, 0.5 (3.0)	3.3, 0.5 (3.0)	3.8, 0.4 (3.3)	4.7, 0.5 (4.2)	5.6, 0.5 (6.3)	6.6, 0.5 (7.0)	7.7, 0.6 (7.9)
	5	2.6, 0.7 (3.2)	3.0, 0.6 (3.0)	3.6, 0.5 (3.2)	4.6, 0.6 (4.3)	5.4, 0.6 (5.8)	6.2, 0.5 (6.2)	6.9, 0.9 (6.5)
	0	1.6, 0.3 (1.5)	1.4, 0.3 (1.5)	1.5, 0.2 (1.6)	2.5, 0.3 (2.5)	4.9, 0.3 (4.1)	8.4, 0.4 (8.5)	–
E. lehma.	1	1.7, 0.3 (1.5)	1.5, 0.2 (1.6)	1.6, 0.2 (1.6)	2.7, 0.2 (2.6)	5.1, 0.2 (5.5)	8.6, 0.3 (8.7)	–
0.93	3	1.8, 0.3 (1.6)	1.6, 0.2 (1.6)	1.8, 0.2 (2.1)	3.0, 0.2 (2.9)	5.5, 0.2 (5.7)	9.2, 0.3 (9.5)	–
	5	1.9, 0.4 (1.7)	1.8, 0.3 (2.1)	2.0, 0.3 (2.0)	3.4, 0.3 (3.4)	5.9, 0.3 (5.6)	9.7, 0.5 (9.6)	–
	0	1.8, 0.8 (1.4)	1.4, 0.6 (1.5)	1.6, 0.6 (1.7)	3.5, 0.7 (2.6)	6.2, 0.7 (5.3)	8.4, 0.8 (11.0)	–
C. cilia.	1	1.3, 0.7 (1.5)	0.8, 0.6 (1.6)	0.8, 0.6 (1.6)	2.6, 0.6 (2.2)	5.3, 0.6 (4.7)	7.7, 0.6 (8.9)	8.5, 1.0 (6.6)
0.83	3	2.2, 0.7 (1.7)	1.5, 0.6 (1.6)	1.1, 0.6 (1.6)	2.6, 0.6 (2.6)	5.5, 0.6 (4.2)	8.4, 0.6 (9.4)	10.0, 0.9 (10.5)
	5	2.3, 0.9 (1.6)	1.3, 0.7 (1.5)	0.6, 0.7 (1.5)	1.9, 0.7 (1.9)	4.9, 0.7 (4.6)	8.3, 0.8 (7.6)	10.7, 1.1 (11.3)
	0	0.6, 0.4 (0.6)	0.7, 0.3 (0.8)	1.1, 0.3 (1.0)	1.8, 0.3 (1.5)	2.7, 0.3 (3.1)	3.9, 0.3 (3.7)	5.3, 0.4 (5.5)
B. curti.	1	0.4, 0.3 (0.6)	0.4, 0.3 (0.6)	0.6, 0.3 (0.7)	1.2, 0.3 (1.2)	1.9, 0.3 (1.7)	2.9, 0.3 (2.5)	4.0, 0.4 (4.1)
0.77	3	0.8, 0.4 (0.6)	0.8, 0.3 (0.6)	0.8, 0.3 (1.0)	1.1, 0.3 (1.1)	1.6, 0.3 (1.8)	2.3, 0.3 (2.0)	3.3, 0.5 (3.8)
	5	0.7, 0.4 (0.6)	0.7, 0.3 (0.7)	0.7, 0.3 (0.8)	1.0, 0.3 (1.2)	1.5, 0.3 (2.0)	2.2, 0.4 (1.3)	3.1, 0.6 (3.1)
	0	2.4, 0.8 (1.7)	2.9, 0.7 (2.7)	3.8, 0.6 (3.1)	5.2, 0.7 (6.8)	6.6, 0.7 (8.0)	8.0, 0.7 (6.1)	9.4, 1.0 (9.8)
P. spica.	1	1.2, 0.7 (2.1)	1.6, 0.6 (1.9)	2.4, 0.6 (2.4)	3.6, 0.7 (3.0)	4.8, 0.6 (4.1)	6.0, 0.6 (5.8)	7.1, 0.9 (7.2)
0.63	3	2.3, 0.8 (2.8)	2.6, 0.7 (2.1)	3.2, 0.6 (2.6)	4.0, 0.7 (3.7)	4.8, 0.7 (4.9)	5.6, 1.0 (6.4)	–
	5	–	2.6, 1.1 (2.8)	2.9, 0.9 (2.6)	3.3, 0.9 (3.8)	3.7, 1.1 (3.3)	–	–

rent experiment show that immersion in a relatively shallow solution of PEG significantly affects germination response.

Increasing solution depth beyond 1 mm tended to lower G for most treatments and all species except *E. lehmanniana* (Table 1). This phenomenon supports the hypothesis of decreased oxygen availability with increased solution depth. Oxygen availability has two components, solubility and diffusivity, which have been shown to decrease as a function of PEG solution concentration (Mexal et al., 1975). A PEG-induced reduction in oxygen availability, however, does not appear to be the limiting factor to germination response. Our data show that increasing solution depth beyond 1 mm generally reduced G in both the PEG solutions and pure water treatments. The critical aspect of oxygen availability may simply be the distance between the seeds and the air/water interface over which oxygen would have to diffuse.

Presence or absence of PEG seems to have little effect on germination other than in reducing solution water potential. Detrimental effects from PEG uptake or toxic salt contamination (Lagerwerff, Ogata and Eagle, 1961; Jackson, 1962; Lawlor, 1970) are unlikely as germination response was often enhanced at –0.1 MPa relative to the pure water treatment. Emmerich and Hardegree (1990) previously found that PEG solution contact *per se* had no detrimental effect on germination response of four of the species tested in the current study.

Four species showed an increased germination rate going from the 0 mm to the 1 mm depth treatment (Table 2). This increase may be associated with an increase in hydraulic conductivity of the medium. The imbibition path of the bare membrane treatment is limited to the solution volume associated with the capillary interface between the seed and the membrane surface. Immersed seeds are in contact with the solution over the entire seed surface, therefore, hydraulic conductivities of the 1, 3, and 5 mm depth treatments were identical. *Eragrostis lehmanniana* was the only species that consistently showed a decrease in germination rate going from 0 to 1 mm solution depth (Table 2). Increased hydraulic conductivity from immersion may be relatively minor for this species which has very small seeds (less than 2 mm diameter) and a mucilaginous seed coat.

We conclude, that for most germination studies, highest germinability can be obtained in treatments where the seeds are in contact with, but are not immersed within, a PEG-solution-saturated germination medium. There is no reason to separate the seeds from the PEG solution with a cellulose membrane except for special cases where solution contact may cause problems in subsequent determination of seed water content (Hardegree and Emmerich, 1992).

References

Bujalski, W. and Nienow, A. W. (1991). Large-scale osmotic priming of onion seeds: a comparison of different strategies for oxygenation. *Scientia Horticulturae*, **46**, 13–24.

Chew, V. (1976). Comparing treatment means: a compendium. *HortScience*, **11**, 348–357.

Emmerich, W. E. and Hardegree, S. P. (1990). Polyethylene glycol solution contact effects on seed germination. *Agronomy Journal*, **82**, 1103–1107.

Emmerich, W. E. and Hardegree, S. P. (1991). Seed germination in polyethylene glycol solution: effects of filter paper exclusion and water vapor loss. *Crop Science*, **31**, 454–458.

Evans, R. A., Easi, D. A., Book, D. N. and Young, J. A. (1982). Quadratic response surface analysis of seed-germination trials. *Weed Science*, **30**, 411–416.

Hardegree, S. P. and Emmerich, W. E. (1990). Effect of polyethylene-glycol exclusion on the water potential of solution-saturated filter paper. *Plant Physiology*, **92**, 462–466.

Hardegree, S. P. and Emmerich, W. E. (1992). Effect of matric-priming duration and priming water potential on germination of four grasses. *Journal of Experimental Botany*, **43**, 233–238.

Jackson, W. T. (1962). Use of carbowaxes (polyethylene glycols) as osmotic agents. *Plant Physiology*, **37**, 513–519.

Lagerwerff, J. V., Ogata, G. and Eagle, H. E. (1961). Control of osmotic pressure of culture solutions with polyethylene glycol. *Science*, **133**, 1486–1487.

Lawlor, D. W. (1970). Absorption of polyethylene glycols by plants and their effects on plant growth. *New Phytologist*, **69**, 501–513.

Mexal, J., Fisher, J. T., Osteryoung, J. and Reid, C. P. P. (1975). Oxygen availability in polyethylene glycol solutions and its implications in plant-water relations. *Plant Physiology*, **55**, 20–24.

Young, J. A., Evans, R. A., Roundy, B. and Cluff, G. (1983). Moisture stress and seed germination. *USDA Agricultural Research Service ARM-W-36*, Oakland, CA, USA.