

Polyethylene Glycol Solution Contact Effects on Seed Germination

William E. Emmerich* and Stuart P. Hardegree

ABSTRACT

While polyethylene glycol (PEG) solutions have been used to control water potential in numerous seed germination investigations, there is concern that PEG solution-seed contact may reduce seed germination. The PEG solution-seed contact effect on seed germination was evaluated on four grass species: sideoats grama [*Bouteloua curtipendula* (Michaux) Torrey], buffelgrass [*Cenchrus ciliaris* L.], Lehmann lovegrass [*Eragrostis lehmanniana* Nees], and kleingrass [*Panicum coloratum* L.]. A water potential control system for seed germination was developed that controlled PEG (8000 mol. wt.) solution-seed contact. Seeds were germinated on filter paper and cellulose membrane over the water potential range of 0.0 to -1.62 MPa. Buffelgrass and Lehmann lovegrass 21-d germination and germination rate index (GRI) were not affected by PEG solution-seed contact. Sideoats grama and kleingrass showed reduced germination and GRI without PEG solution contact on the cellulose membrane. The reduction was attributed to the hydraulic conductivity at the seed-water-membrane contact point which was influenced by PEG solution water potential and seed size and shape. Therefore, it was concluded PEG solution-seed contact does not reduce seed germination.

POLYETHYLENE GLYCOL solutions are commonly used to control water potential in seed germination studies (Young et al., 1983). The PEG solutions

are used because high molecular weight PEG cannot pass through plant cell walls (Tarkow et al., 1966; Carpita et al., 1979) and a solution water potential control eliminates the confounding hydraulic conductivity effects of the solution-seed contact and the germination medium (Heydecker, 1967). Seeds are germinated in a Petri dish with PEG solution-saturated filter paper as the germination substrate (Sharma, 1973, 1976; Fulbright, 1988; Call and Spoonts, 1989). Filter paper has been shown to exclude high molecular weight PEG and lower the PEG solution water potential (Hardegree and Emmerich, 1990). Water vapor loss from germination containers has also been shown to change significantly the water potential of PEG solutions (Berkat and Briske, 1982).

Besides a water potential change, PEG solution-seed contact may influence germination in other ways that have not been evaluated. The solubility and availability of O_2 in PEG solutions has been shown to decrease as molecular weight of the solute and

USDA-ARS, Aridland Watershed Manage. Res. Unit, Tucson, AZ 85719. Received 7 Feb. 1990. *Corresponding Author.

Published in *Agron. J.* 82:1103-1107 (1990).

concentration increases (Mexal et al., 1975). Hence, seeds in contact with PEG solutions may be subject to O₂ limitations which would influence germination. Polyethylene glycol has been shown to concentrate in a boundary layer adjacent to water-absorbing-plant material (Michel, 1971). This could effectively lower the water potential of the PEG solution to imbibing seeds and reduce germination. Seeds can be separated from the PEG solution with a cellulose membrane. Most studies that have used cellulose membranes also included a soil component, which imparts an unknown soil hydraulic conductivity effect on seed germination (Kaufmann, 1969; McWilliam and Phillips, 1971; Sharma, 1973; Kaufmann and Eckard, 1977; Johnson and Asay, 1978).

The first objective of this study was to develop a simple seed germination system that minimizes water potential changes and can allow or prevent PEG seed contact. The second objective was to compare the germination response of seed in contact with PEG solution to that of seed separated from PEG by a cellulose membrane.

MATERIALS AND METHODS

The germination system was a clear plastic vial (47-mm i.d. by 85 mm high) with a snap-top lid. A polyvinyl chloride (PVC) ring (42-mm o.d., 34-mm i.d. by 35 mm high) was supported inside the vial 38 mm from the bottom by three plastic bars glued to the wall. Whatman¹ no. 1 filter paper or Spectra/Por 3 (Spectrum Medical Industries, Inc., Los Angeles, CA) cellulose membrane with a molecular weight exclusion limit of 3500 was glued with contact cement to the bottom end of the PVC ring. The cellulose membrane-PVC contact area received an additional outside coating of silicon rubber to ensure a complete seal.

The PEG molecular weight 8000 (Union Carbide, Danbury, CT) was mixed with deionized water at six different concentrations ranging from 0 to 0.40 kg PEG/kg H₂O representing water potentials of 0, -0.07, -0.43, -0.81, -1.24, and -1.62 MPa. Solution water potential was determined with an SC-10A (Decagon Devices, Pullman, WA) thermocouple psychrometer at 25 °C. Approximately 60 mL of PEG solution was poured into the vial until it came into contact with the seed-support material (i.e., filter paper or cellulose membrane) attached to the bottom of the PVC ring. The vials were weighed at the initiation and conclusion of the germination experiment and a change in water potential was calculated for water vapor loss.

Seed germination response to PEG solution-seed contact was evaluated with caryopsis of sideoats grama, buffelgrass, Lehmann lovegrass, and kleingrass. These species were selected because they are arid zone plants with seeds of different sizes. Thirty seeds of each species were placed in separate vials on both seed support materials and at all water potentials. To limit fungal growth, seeds were treated with a 50-μL-Daconil (tetrachloroisophthalonitrile) suspension (2.5g/100 mL). The vials were kept in a temperature-controlled room at 25 ± 1 °C with both fluorescent and incandescent light for 12 h/d. Seed germination counts were made on Days 1 through 5, 7, 9, 11, 14, 17, and 21. Seed with a radicle extension of 2 mm or greater were considered germinated and removed. Seeds that developed fungal growth were removed from the vials and considered as nonviable. All vials were opened and aerated on noncount days. Each

species, water potential, and seed-support material combination was replicated six times and conducted simultaneously with a completely random experimental design.

Twenty-one-day-total-percent germination and germination rate index (GRI) were calculated and used to evaluate PEG solution-seed contact effects on germination. The GRI (Maguire, 1962) was calculated from the formula

$$\text{GRI}(\%/d) = \sum [(G_i - G_{i-1})/i]$$

where i is the germination count day, G_i is the percentage of seeds germinated through Day i , and G_{i-1} is the percentage of seeds germinated through the previous count day. These parameters were considered to represent a final germination amount and seed vigor.

Analysis of variance techniques were used to analyze the data. The analysis of variance model used species, seed-support material, and water potential as main effects. Variance homogeneity between species was tested with a F -test (Ott, 1977). Significant species variance differences required that each species be analyzed separately. Analysis of variance was used within species to analyze main effect interactions and to separate seed-support material main effect when there was a nonsignificant interaction ($P = 0.05$).

Germination and GRI were estimated by quadratic regression equations with PEG solution water potential as the independent variable for each species and seed-support material. The regression equation 95% confidence intervals at the measured water potentials were used to evaluate seed-support material differences when there was a seed support material by water potential interaction (Ott, 1977). Confidence interval overlap was considered to indicate that germination was not significantly influenced by PEG solution-seed contact. The zero water potential means (i.e., no PEG) for germination and GRI on the seed support materials were separated with Tukey's Studentized Range (HSD) Test. Percent germination data were analyzed transformed by the arcsin of the square root and untransformed. Conclusions were the same; therefore, analysis of untransformed data are presented.

RESULTS

At the zero water potential treatment, germination and GRI on the seed-support materials were similar ($P = 0.05$), with the exception of buffelgrass GRI (Table 1). Germination ranged from 95% with buffelgrass to 24% for Lehmann lovegrass and GRI from 36 to 6%/d, respectively.

Figures 1 and 2 show that germination and GRI decreased as water potential decreased on both seed-support materials for all species. Sideoats grama germination was 53% on cellulose membrane and 17% higher on filter paper at -1.62 MPa. For buffelgrass and Lehmann lovegrass germination was zero at -1.62 MPa. Kleingrass germination on the cellulose

Table 1. Mean 21-d-total germination and GRI on filter paper and cellulose membrane at zero water potential.

Species†	Germination		GRI	
	Filter paper	Cellulose membrane	Filter paper	Cellulose membrane
	%		%/d	
Sideoats	90	94	35	33
Buffelgrass	93	95	36	32
Lehmann	33	24	9	6
Kleingrass	76	80	19	18

† Only buffelgrass germination rate index significantly different between filter paper and cellulose membrane $P = 0.05$.

¹ Mention of trade names or proprietary products does not indicate endorsement by USDA, and does not imply its approval to the exclusion of other products that may also be suitable.

membrane approached zero at -0.95 MPa while on the filter paper still had 32% germination. The GRI for the four species followed the same trends found for germination (Fig. 2).

The seed-support material by water potential interaction and the seed-support material main effect were not significant for Lehmann lovegrass germination. Therefore, Lehmann lovegrass germination was concluded not to be affected by PEG seed-solution contact. There was no seed-support material by water potential interaction and seed-support material main effect for sideoats grama, buffelgrass, and Lehmann lovegrass GRI. The absence of a seed-support material main effect indicated that PEG seed-contact did not

affect sideoats grama, buffelgrass, and Lehmann lovegrass GRI.

There was a significant seed-support material by water potential interaction for the sideoats grama, buffelgrass, and kleingrass germination and kleingrass GRI. Regression equation confidence intervals were used to evaluate the effect of PEG solution-seed contact on germination and GRI. Sideoats grama and buffelgrass germination and GRI regression equation confidence intervals overlapped at all measured water potentials, with the exception of sideoats grama germination at -1.62 MPa (Fig. 1 and 2). Kleingrass confidence intervals overlapped at -0.07 and -1.62 MPa water potentials. Buffelgrass and kleingrass con-

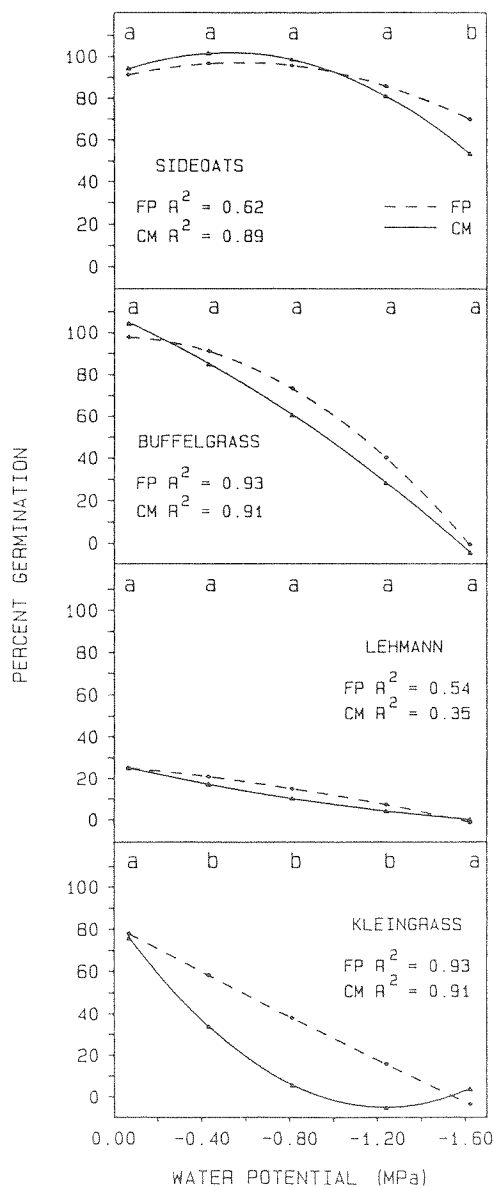


Fig. 1. Estimated 21-d-germination as a function of water potential. Regressions were of the form: germination = $B_0 + B_1\psi + B_2\psi^2$, where ψ is the water potential ($-$ MPa) and B_0 , B_1 , and B_2 are regression coefficients. Letter a indicates overlap of 95% confidence intervals at that water potential and b indicates no overlap. FP = filter paper; CM = cellulose membrane.

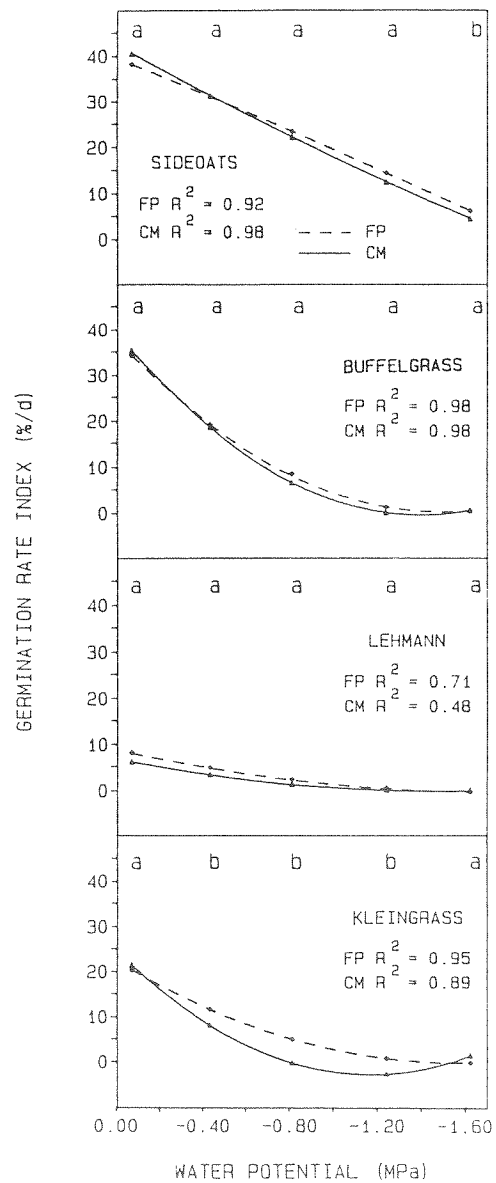


Fig. 2. Estimated 21-d-germination-rate index as a function of water potential. Regressions were of the form: germination rate index = $B_0 + B_1\psi + B_2\psi^2$, where ψ is the water potential ($-$ MPa) and B_0 , B_1 , and B_2 are regression coefficients. Letter a indicates overlap of 95% confidence intervals at that water potential and b indicates no overlap. FP = filter paper; CM = cellulose membrane.

fidence interval overlap at -1.62 MPa was expected because of near zero germination. Confidence interval overlap indicated PEG solution-seed contact did not affect germination and GRI for sideoats grama and buffelgrass species. Higher kleingrass germination and GRI on the filter paper when the confidence intervals did not overlap indicated that they were not affected by PEG solution-seed contact.

DISCUSSION

The simple germination system developed for this study eliminated two problems associated with PEG solution-saturated filter paper in a Petri dish. Organic germination substrates have a PEG exclusion volume that absorbs water and concentrates PEG lowering the solution water potential (Hardegree and Emmerich, 1990). The change in PEG concentration has been shown to be minimal for a PEG solution volume to filter paper weight ratio above 12 L/kg. Therefore, the high PEG solution volume to filter paper ratio used in this system minimizes the change in water potential from PEG exclusion associated with filter paper substrates. Second, the snap-top lid minimizes water potential changes caused by water vapor loss from the germination vials. Berkat and Briske (1982) reported a 0.15-MPa-water-potential change in polyethylene wrapped germination trays over a 12-d period. In our study the 21-d-water-potential change in the vials from evaporation was calculated to be ≤ 0.06 MPa.

The simple germination system has additional advantages. There is a choice of PEG solution-seed contact or separation with a differentially permeable membrane. Other germination systems that use a membrane to separate seed, or seed and soil, from the water potential control solution are bulky and relatively difficult to construct and maintain (McWilliam and Phillips, 1971; Kaufmann and Eckard, 1977; Johnson and Asay, 1978). The simplicity and small size also allow for many replications to be conducted simultaneously.

The hydraulic conductivities through the two seed-support materials were expected to differ because of pore size differences and this could affect germination. The pores remained fully hydrated in both materials at the water potentials of the study. Therefore, the hydraulic conductivities were relatively constant, but they were not equal (Williams and Shaykewich, 1971). Indistinguishable germination and GRI on the two seed support materials at zero water potential indicated the hydraulic conductivities were sufficiently high not to affect seed germination (Table 1).

The reduced sideoats grama and kleingrass germination and GRI on the cellulose membrane when compared to the filter paper were attributed to the hydraulic conductivity at the seed-water-membrane contact point (Fig. 1 and 2). Seed on the saturated filter paper had a high constant hydraulic conductivity at the seed filter paper contact point and maximum solution-seed contact without submerging the seeds (Williams and Shaykewich, 1971). Hydraulic conductivity to the seed on the cellulose membrane was restricted to the water at the seed-membrane contact and a thin layer of adsorbed water surrounding the seed.

Seed-water contact area has been shown to decrease with decreasing water potentials and this reduces seed germination and imbibition rates (Collis-George and Hector, 1966; Hadas and Russo, 1974a,b; Bouaziz and Bruckler, 1989). Sideoats grama and kleingrass were the largest seeds and would have been most affected by a reduction in seed-water contact area at low water potentials (Collis-George and Hector, 1966; Hadas and Russo, 1974a,b). These conductivity and contact effects were concluded to have accounted for decreased sideoats grama and kleingrass germination and GRI on the cellulose membrane.

The simple seed germination system allowed testing of PEG solution-seed contact effects on germination without changes in water potential from organic substrate exclusion of PEG and water vapor loss. There was no distinguishable PEG solution-seed contact effects on germination for the four species evaluated. The reduced sideoats grama and kleingrass germination on the cellulose membrane was attributed to hydraulic conductivity at the seed-water-membrane-contact point. Seed size and shape were hypothesized to have contributed to the observed reduced germination. Therefore, it is suggested that seed germination on a cellulose membrane be restricted to small size seed. Additional investigations are needed to evaluate further the seed size and shape and matrix contact interactions on seed germination in the soil continuum.

REFERENCES

- Berkat, O., and D.D. Briske. 1982. Water potential evaluation of three germination substrates utilizing polyethylene glycol 20,000. *Agron. J.* 74:518-521.
- Bouaziz, A., and L. Bruckler. 1989. Modeling of wheat imbibition and germination as influenced by soil physical properties. *Soil Sci. Soc. Am. J.* 53:219-227.
- Call, C.A., and B.O. Spoons. 1989. Characterization and germination of chasmogamous and basal axillary cleistogamous florets of Texas wintergrass. *J. Range Manage.* 42:51-55.
- Carpita, N., D. Sabularse, D. Montezinos, and D.P. Delmer. 1979. Determination of the pore size of cell walls of living plant cells. *Science* 205:1144-1147.
- Collis-George, N., and J.B. Hector. 1966. Germination of seeds as influenced by matric potential and by area of contact between seed and soil water. *Aust. J. Soil Res.* 4:145-164.
- Fulbright, T.E. 1988. Effects of temperature, water potential, and sodium chloride on Indiangrass germination. *J. Range Manage.* 41:207-210.
- Hadas, A., and D. Russo. 1974a. Water uptake by seeds as affected by water stress, capillary conductivity, and seed-soil water contact. I. Experimental study. *Agron. J.* 66:643-647.
- Hadas, A., and D. Russo. 1974b. Water uptake by seeds as affected by water stress, capillary conductivity, and seed-soil water contact. II. Analysis of experimental data. *Agron. J.* 66:647-652.
- Hardegree, S.P., and W.E. Emmerich. 1990. The effect of polyethylene glycol exclusion on the water potential of solution-saturated filter paper. *Plant Physiol.* 92:462-466.
- Heydecker, W. 1967. Drought hazards to seed germination. *Ann. Arid Zone* 6:23-24.
- Johnson, D.A., and K.H. Asay. 1978. A technique for assessing seedling emergence under drought stress. *Crop Sci.* 18:520-522.
- Kaufmann, M.R. 1969. Effects of water potential on germination of lettuce, sunflower, and citrus seeds. *Can. J. Bot.* 47:1761-1764.
- Kaufmann, M.R., and A.N. Eckard. 1977. Water potential and temperature effects on germination of Engelmann spruce and lodgepole pine seeds. *For. Sci.* 23:27-33.
- Maguire, J.D. 1962. Speed of germination aid in selection and evaluation for seedling emergence and vigor. *Crop Sci.* 2:176-177.
- Mexal, J., J.T. Fisher, J. Osteryoung, and C.P. Patrick Reid. 1975. Oxygen availability in polyethylene glycol solutions and its implications in plant-water relations. *Plant Physiol.* 55:20-24.
- Michel, B.E. 1971. Further comparisons between carbowax 6000 and mannitol as supplants of cucumber hypocotyl elongation.

- Plant Physiol. 48:513-516.
- McWilliam, J.R., and P.J. Phillips. 1971. Effects of osmotic and matric potentials on the availability of water for seed germination. *Aust. J. Biol. Sci.* 24:423-431.
- Ott, L. 1977. An introduction to statistical methods and data analysis. Duxbury Press, North Scituate, MA.
- Sharma, M.L. 1973. Simulation of drought and its effect on germination of five pasture species. *Agron. J.* 65:982-987.
- Sharma, M.L. 1976. Interaction of water potential and temperature effects on germination of three semi-arid plant species. *Agron. J.* 68:390-394.
- Tarkow, H., W.C. Feist, and C.F. Southerland. 1966. Interaction of wood with polymeric materials penetration versus molecular size. *For. Prod. J.* 16:61-65.
- Williams, J., and C.F. Shaykewich. 1971. Influence of soil water matric potential and hydraulic conductivity on the germination of rape (*Brassica napus* L.). *J. Exp. Bot.* 22:586-597.
- Young, J.A., R.A. Evans, B. Roundy, and G. Cluff. 1983. Moisture stress and seed germination. USDA-ARS ARM-W-36. Oakland, CA.