Seed Germination in Polyethylene Glycol Solution: Effects of Filter Paper Exclusion and Water Vapor Loss

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ABSTRACT

Seed germination under controlled water potentials is commonly investigated in petri dishes containing polyethylene glycol (PEG) solution–saturated filter paper. Filter paper exclusion of PEG and water vapor loss from unsealed containers change the solution water potential from the initial PEG solution potential. The influence of water potential change attributable to PEG exclusion and vapor loss were evaluated with sideoats grama [*Bouteloua curtipendula* (Michaux) Torrey], buffelgrass [*Cenchrus ciliaris* L.], Lehmann lovegrass [*Eragrostis lehmanniana* Nees], and kleingrass [*Panicum coloratum* L.]. Exclusion of PEG by filter paper substrates significantly influenced total germination and germination rate for three of the four species. The absence of a measurable effect on Lehmann lovegrass was attributed to low germination at lower water potentials. Oscillating substrate water potentials originating from water vapor losses and subsequent additions produced significantly greater total germination and faster germination rate when compared to a constant water potential. Polyethylene glycol filter-paper exclusion and water vapor loss from PEG solutions are significant influences that must be considered when conducting seed germination investigations.

POLYETHYLENE GLYCOL (PEG) solution–saturated filter paper is a commonly used substrate to control water potential at a constant value in petri dish seed germination studies (Macke and Ungar, 1971; McWilliam and Phillips, 1971; Sharma, 1973, 1976; Redmann, 1974; Kaufmann and Eckard, 1977; Thill et al., 1979; Everitt et al., 1983; Bhatt and Rao, 1987; Rasmussen and Wright, 1988). Water vapor loss from the germination container must be minimized, to maintain a constant water potential of the germination substrate. Many studies have been conducted without taking steps to prevent or account for vapor loss from the germination containers. Berkat and Briske (1982) measured the water potential change from vapor loss in sealed and unsealed germination trays and found decreases of 0.15 and 1.85 MPa between sealed and unsealed trays during a 12-d period. Numerous techniques have been employed to prevent or compensate for this water vapor loss. Containers have been sealed against vapor loss (Macke and Ungar, 1971; Potter et al., 1986, Fulbright, 1988), maintained in a high-humidity environment (McWilliam and Phillips, 1971; Sharma, 1973, 1976), and periodically rewatered (Redmann, 1974). Water potential changes due to water vapor losses that could influence seed germination compared with a constant initial water potential have not been evaluated.

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Berkat and Briske (1982) reported a significant water potential decrease when a PEG solution was added to substrates containing paper. The change in water potential was attributed to water vapor loss despite precautions taken to seal the trays. Osmotic stability of PEG solutions could not explain the change, since PEG solutions have been found to be stable over time (Greenway et al., 1968; Thill et al., 1979). Hardegree and Emmerich (1990) demonstrated that filter paper absorbs water and excludes PEG (8000 mol. wt.). This phenomenon concentrates PEG when a solution is poured onto filter paper. The water potential change from PEG filter-paper exclusion has been quantified for PEG 8000 and Whatman no. 1 filter paper and is a function of PEG concentration and the ratio of solution volume to filter paper weight (Hardegree and Emmerich, 1990). The magnitude of the change in substrate water potential could be as large as 0.3 MPa for a −1.70 MPa PEG solution.

Both water vapor loss and filter paper exclusion decrease the water potential of the germination substrate in comparison with the initial water potential of the PEG solution. We hypothesized that these two effects would cause the seed germination response to vary from that of a constant initial water potential. Our first objective was to determine if there is a significant difference in the germination response from an initial water potential to that of a changed water potential from filter paper exclusion of PEG. The second objective was to determine if there is a difference in the germination response from a constant initial water potential and a 24-h oscillating water potential caused by water losses and additions to a PEG solution.

MATERIALS AND METHODS

Seed germination was conducted on Whatman no. 1 filter paper disks (70 mm diam.) in clear plastic petri dishes (80 mm diam. by 13 mm deep). Solution volume to filter-paper weight ratios (FPR, mL g^{-1}) of 3, 5, and 14 were produced by adding 4, 6.5, and 9 mL of PEG solution to 4, 4, and 2 sheets of filter paper weighing a total of 1.29, 1.29, and 0.65 g, respectively. A filter paper ratio (FPR) of 3 is indicative of moist filter paper, free solution is visible at a FPR of 5, and at a 14 FPR the filter paper is floating in free solution. The filter paper in all treatments was supported by a plastic screen 2 mm thick, to prevent the seed and filter paper from floating in the higher FPR treatment. The upper filter paper sheets (3 or 1) had a 10-mm-diam. hole punched in the center; the bottom sheet had a 3-mm hole. The area inside the large hole and outside the small one was used to add deionized water to the filter papers, to replace the water that had evaporated. The small hole allowed excess water to drain away from the seeds and to storage below the filter paper. The petri dishes were weighed daily and deionized water was added to bring them back to the original weight. Half the petri dishes were sealed with petroleum jelly on the in-

side lip of the lids. The sealed petri dishes were used to evaluate the FPR effect on seed germination and were compared to the unsealed dishes for the oscillating water potential effect.

Polyethylene glycol (8000 mol. wt.) was mixed with deionized water at six different concentrations ranging from 0 to 0.40 g PEG g^{-1} H_2O. Solution water potential was determined with an SC-10A (Decagon Devices, Pullman, WA)[1] thermocouple psychrometer at 25 °C without filter paper in the sample chamber (Hardegree and Emmerich, 1990). A regression equation relating water potential and PEG concentration at 25 °C was developed for use in modeling PEG solution water potential changes from vapor loss and filter paper exclusion:

$$\text{Water potential (MPa)} = -11.2[\text{PEG}]^2 + 0.59[\text{PEG}] - 0.07 \quad [1]$$

with a R^2 of 0.99. Initial solution water potentials were 0, −0.07, −0.52, −0.90, −1.31, and −1.68 MPa. Water potential changes from filter paper exclusion and daily water vapor loss were calculated using Eq. [1], FPR, water loss, and the relationships between water potential and PEG exclusion volume of Whatman no. 1 filter paper (Hardegree and Emmerich, 1990). The water potential changes from filter paper exclusion and daily water vapor loss were calculated values and designated as predicted water potential values because regression equations were used in their calculation. Measurements of the actual water potential with the experimental conditions of this study were not possible.

The seed germination response was evaluated with caryopses from sideoats grama, buffelgrass, Lehmann lovegrass, and kleingrass. Thirty seed of each species were placed in sealed and unsealed petri dishes at all filter paper ratios and water potentials. The seeds were treated with 50 μL of Daconil (tetrachloroisophthalonitrile, 2.5g 100 mL^{-1}) suspended in the corresponding PEG water potential solution to limit fungal growth. The fungicide was included as part of the total volume of PEG solution added to the petri dishes. The petri dishes were incubated in a temperature-controlled room at 25 ± 1 °C with fluorescent light for 12 h d^{-1}. Seed germination counts were made on Days 1, 2, 3, 4, 5, 7, 9, 11, 14, 17, and 21 of the study. Seed with a radicle extension of ≥2 mm were considered germinated and removed. Seeds that developed fungal growth were removed from the dishes and considered nonviable. Each treatment combination of water potential, FPR, and petri dish seal (seal) was replicated six times and conducted simultaneously with a completely random design.

Twenty-one day total percent germination and mean germination time (MGT) were used to evaluate the seed germination response. Mean germination time (Ellis and Roberts, 1978) was calculated from the formula

$$\text{MGT (d)} = \frac{\Sigma\, G_i i}{\Sigma\, G_i} \quad [2]$$

where i is the germination count day and G_i is the number of seeds germinated on Day i. The MGT was not calculated when the mean 21-d total germination was <10% and more than one replication had zero germination, because of the potential for bias.

Analysis of variance techniques were used to analyze the data with species, FPR, seal, and initial water potential as main effects. Variance homogeneity between species was tested with an F-ratio test (Ott, 1977). A significant variance difference was found, requiring that the species be analyzed separately. Main effects were either pooled or separated, depending on the significance of the interactions ($P = 0.05$). Filter-paper ratio and seal-effect means were separated with a least significant difference test (LSD). Evaluation of the FPR effect on germination and MGT required its separation, if possible, from interaction with the seal effect; therefore, the sealed dishes were used when necessary to evaluate the FPR effect by itself. The LSD values were calculated for each species using the error term from the complete model. Percent germination data were analyzed as arcsine transformed and untransformed. The conclusions were the same, and therefore only the untransformed data analyses are presented.

RESULTS

The PEG filter-paper exclusion decreased the substrate water potential in the petri dishes from the initial measured water potential (Table 1). The change in water potential from PEG exclusion was very small at the high water potential. As PEG concentration increased, the effect of filter paper exclusion on water potential increased. The change at the low water potential was 0.34 and 0.07 MPa for the 3 and 14 FPR, respectively. The change at the 14 FPR was small and considered to be the same as the initial solution potential.

The sealed dishes were considered to have a constant water potential because of the small change from evaporation. The predicted water potential after 24 h of evaporation from the PEG solution decreased by 14 to 60% in the unsealed dishes, compared with 1 to 5% in the sealed containers (Table 1). As the water potential and/or FPR increased, the 24-h water potential oscillations decreased. The combination of unsealed petri dish, at the lowest water potential, and a FPR of 3 produced an average 1.21 MPa 24 h water potential oscillation for the six replications.

Sideoats grama germination and MGT had a significant three-way main-effect interaction. With main effects separated, FPR significantly influenced germination and MGT below −0.07 MPa initial water potential in the unsealed petri dishes and −0.90 MPa in

Table 1. Predicted PEG solution water potential as affected by filter paper ratio (FPR)† and evaporation.

Initial water potential	Seal‡	FPR: Water potential with filter paper 3	5	14	FPR: Mean water potential after 24-h evaporation 3	5	14
−MPa		−MPa			−MPa		
0.0	no	0.0	0.0	0.0	0.0	0.0	0.0
0.0	yes	0.0	0.0	0.0	0.0	0.0	0.0
0.07	no	0.09	0.08	0.08	0.10	0.09	0.09
0.07	yes	0.09	0.08	0.08	0.09	0.08	0.08
0.52	no	0.69	0.62	0.56	1.00	0.83	0.88
0.52	yes	0.69	0.62	0.56	0.71	0.63	0.58
0.90	no	1.13	1.03	0.94	1.79	1.49	1.49
0.90	yes	1.13	1.03	0.94	1.17	1.07	0.98
1.31	no	1.61	1.48	1.37	2.26	2.24	2.11
1.31	yes	1.61	1.48	1.37	1.65	1.53	1.42
1.68	no	2.02	1.88	1.75	3.23	2.91	2.74
1.68	yes	2.02	1.88	1.75	2.08	1.90	1.80

† FPR = ratio of solution volume to weight of filter paper, mL g^{-1}. FPR 3 = moist filter paper, 5 = free solution visible, and 14 = filter paper floating on free solution.

‡ No = unsealed petri dish, yes = sealed petri dish.

[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.

Table 2. Sideoats grama 21-d germination percent and mean germination time (MGT) as affected by water potential, seal, and filter paper ratio (FPR).†

Initial water potential	Seal‡	FPR Germination 3	5	14	MGT 3	5	14
−MPa		%			d		
0.0	no	88a§	90a	93a	2.4a	2.6a	2.3a
0.0	yes	93a	93a	94a	2.2a	2.3a	2.3a
0.07	no	97a	88a	89a	2.3a	2.5a	2.5a
0.07	yes	96a	91a	95a	2.5a	2.4a	2.6a
0.52	no	74a	87ab	93b	5.6a	3.6b	3.3b
0.52	yes	92a	92a	95a	3.8a	3.3a	2.9a
0.90	no	59a	77b	92c	5.7ab	6.9b	4.7a
0.90	yes	85a	88a	94a	5.7a	4.4a	4.4a
1.31	no	56a	38b	74a	8.5a	9.4a	8.5a
1.31	yes	64a	70ab	82b	9.4a	9.1a	6.7b
1.68	no	38a	55b	42ab	7.4a	11.3b	13.6c
1.68	yes	12a	26a	55b	15.8a	14.0a	11.8b
LSD			15.1			1.92	

† FPR = ratio of solution volume to weight of filter paper, mL g^{-1}. FPR 3 = moist filter paper, 5 = free solution visible, and 14 = filter paper floating on free solution.
‡ No = unsealed petri dish, yes = sealed petri dish.
§ Within rows and within germination percent or time, means followed by the same letter are not significantly different at the 0.05 probability level.

Table 3. Buffelgrass 21-d germination percent and mean germination time (MGT) as affected by water potential, seal, and filter paper ratio (FPT).†

Initial water potential	Seal‡	FPR Germination 3	5	14	MGT 3	5	14
−MPa		%			d		
0.0	no	84a§	91a	95a	3.0a	2.8a	2.7a
0.0	yes	91a	88a	93a	2.7a	2.7a	2.8a
0.07	no	80a	82a	84a	2.7a	2.9a	2.8a
0.07	yes	80a	84a	81a	3.2a	2.7a	2.9a
0.52	no	58a	72ab	89b	5.2a	5.4a	4.4a
0.52	yes	79a	80a	86a	5.3a	4.8a	4.6a
0.90	no	46a	58a	81b	6.0a	8.2b	7.4ab
0.90	yes	40a	57b	72b	13.9a	9.4b	7.0b
1.31	no	36ab	52a	29b	8.7a	10.8b	14.6c
1.31	yes	17a	29a	26a	15.4a	15.2a	16.2a
1.68	no	44a	14b	2b	5.4	−¶	—
1.68	yes	3a	1a	1a	—	—	—
LSD			16.5			1.89	

† FPR = ratio of solution volume to weight of filter paper, mL g^{-1}. FPR 3 = moist filter paper, 5 = free solution visible, and 14 = filter paper floating on free solution.
‡ No = unsealed petri dish, yes = sealed petri dish.
§ Within rows and within germination percent or time, means followed by the same letter are not significantly different at the 0.05 probability level.
¶ Insufficient germination to calculate MGT.

the sealed (Table 2). Lower FPR reduced germination and increased MGT when the ratios had an effect. Sideoats grama MGT at −1.68 MPa water potential in the unsealed petri dish was the exception, as there was a decreasing MGT with decreasing FPR.

Buffelgrass germination and MGT also had a significant three-way main-effect interaction. The effect of FPR on buffelgrass germination and MGT was similar to that on sideoats grama. The lower ratios reduced germination and increased MGT when there was an effect (Table 3). At the lower water potentials, the FPR had the opposite effect, as germination increased and MGT decreased with lower FPR in the unsealed dishes. This change in the effect of FPR was notable for buffelgrass germination, because at −1.68 MPa treatment germination was near zero but in the unsealed petri dish at a 3 FPR it was still 44%.

Table 4. Lehmann lovegrass 21-d germination percent as affected by water potential and seal.

Initial water potential	Seal†	Germination
−MPa		%
0.0	no	41a‡
0.0	yes	71b
0.07	no	30a
0.07	yes	57b
0.52	no	20a
0.52	yes	30b
0.90	no	9a
0.90	yes	7a
1.31	no	5a
1.31	yes	1a
1.68	no	1a
1.68	yes	0a
LSD		5.4

† No = unsealed petri dish, yes = sealed petri dish.
‡ Within a single water potential, means followed by the same letter are not significantly different at the 0.05 probability level.

Table 5. Kleingrass 21-d germination percent as affected by water potential and filter paper ratio (FPR).†

Initial water potential	FPR 3	5	14
−MPa	%		
0.0	88a†	85a	88a
0.07	81a	85a	80a
0.52	57a	59a	74b
0.90	40a	33a	51b
1.31	8a	20b	18b
1.68	11a	9a	3a
LSD		8.9	

† FPR = ratio of solution volume to weight of filter paper, mL g^{-1}. FPR 3 = moist filter paper, 5 = free solution visible, and 14 = filter paper floating on free solution.
‡ Within rows, means followed by the same letter are not significantly different at the 0.05 probability level.

Lehmann lovegrass germination and MGT three-way main-effect interaction, the two-way main effects involving FPR, and the FPR main effect were all nonsignificant. Hence, there was no detectable FPR influence on Lehmann lovegrass germination or MGT. The water potential × seal interaction was significant for germination, but not for MGT. Seal-effect means were separated for germination within water potentials (Table 4). The seal effect reduced germination in the unsealed dishes at the higher water potentials. Analysis of variance indicated water potential and seal main effects were significant for MGT. Mean germination time was shorter in the unsealed dishes (data not shown).

Kleingrass germination and MGT had no three-way main-effect interaction. The two-way FPR × water potential interaction for germination was significant.

Table 6. Kleingrass 21-d germination percent and mean germination time (MGT) as affected by water potential and seal.

Initial water potential	Seal†	Germination	MGT
−MPa		%	d
0.0	no	86a‡	3.6a
0.0	yes	88a	3.5a
0.07	no	82a	4.0a
0.07	yes	81a	3.7a
0.52	no	56a	6.4a
0.52	yes	71b	5.6a
0.90	no	38a	7.7a
0.90	yes	44a	9.0b
1.31	no	17a	10.8a
1.31	yes	14a	14.9b
1.68	no	12a	−§
1.68	yes	3b	−
LSD		7.3	1.14

† No = unsealed petri dish, yes = sealed petri dish.
‡ Within a single water potential, means followed by the same letter are not significantly different at the 0.05 probability level.
§ Insufficient germination to calculate MGT.

Hence, seal effects were pooled and the FPR effect means separated within water potentials (Table 5). Once again, a lower FPR reduced germination when there was an influence. There was an indication at −1.68 MPa initial water potential that germination was higher at the lower FPR, but not at a significant level. There was no FPR × water potential interaction for MGT, but the FPR main effect was significant. Hence, water potential and seal data were pooled for separation of FPR effects on MGT. Mean germination time at the 3 FPR was significantly shorter than that at the 14 FPR, while at a 5 FPR the MGT was similar to the other two FPR (data not shown).

There was a water potential × seal interaction for germination and MGT in kleingrass, necessitating the seal-effect means being separated within water potential treatments (Table 6). Germination was reduced in the unsealed dishes when influenced by the seal at the higher water potentials. At the lower water potential, germination was greater in the unsealed dishes. Mean germination time was shorter at the lower water potentials in the unsealed dishes.

DISCUSSION

We hypothesized that a change in water potential from filter paper exclusion and water vapor loss would significantly influence seed germination, compared with germination at the initial solution water potential. It was accepted, as many studies have shown, that a decreasing water potential reduces germination and increases MGT. The FPR and seal main effects on germination and MGT were attributed to PEG concentration changes influencing solution water potential.

The hypothesis is supported by the data from sealed petri dishes for sideoats grama and buffelgrass (Tables 2 and 3). The FPR effect on kleingrass germination was difficult to assess because of the two-way main-effect interaction (water potential × FPR), which required that the seal treatment data be pooled for FPR mean separations (Table 5). Nonetheless, a separation of the seal main effect without pooling the data indicated that the FPR affected germination at the −0.52 MPa water potential in the sealed dishes. The absence of a FPR effect on Lehmann lovegrass germination or MGT was attributed to its low total germination at water potentials below −0.52 MPa, where FPR effect is greater (Tables 1 and 4).

The three-way main effect interaction for sideoats grama and buffelgrass prevented mean separation of both FPR and seal effects (Table 2 and 3). An expected decrease in germination and increase in MGT from the seal effect was still observed in the data, with interactions complicating the analysis (Tables 2, 3, 4, and 6). The increase in germination and decrease in MGT for sideoats grama, buffelgrass, and kleingrass in the unsealed dishes over the sealed at the lower water potentials and FPR was unexpected. We hypothesized that the water potential oscillations may have a stimulating effect at the lower water potentials. The stimulation of germination from oscillating water potentials could be related to a seed-priming response. Many seed species osmoconditioned to a low water potential and then germinated at a higher water potential have shown increased germination and rate (Hegarty, 1978; Khan et al., 1980–1981). The water-potential oscillations in this study displayed a broad range, sufficient for osmoconditioning to occur. An oscillating water potential is indicative of field germination conditions and may allow germination at much lower potentials than would be indicated by a constant water potential germination test.

The Lehmann lovegrass germination and MGT response to oscillating water potential from water vapor loss was not only a water potential effect. The seal effect was observed at zero water potential; hence, there was another factor influencing germination and MGT (Table 4). Seed dormancy in Lehmann lovegrass has been reported and alleviated by a number of treatments (Wright, 1973; Haferkamp and Jordan, 1977). Ethylene is produced by germinating seeds and has been shown to break dormancy and stimulate germination in lettuce (*Lactuca sativa* L.) seed (Mayer, 1974; Negm and Smith, 1978; Saini et al., 1989). In the sealed dishes, ethylene would accumulate between daily water additions and may have stimulated germination in dormant seeds.

Filter paper exclusion of PEG has undoubtedly occurred in previous germination studies and lowered the water potential of the initial PEG solution as it was added to the substrate. Our results showed that PEG exclusion could change 21-d germination and MGT by 43% and 6.9 d, respectively, from what would have occurred at the initial water potential. Our results also have implications for other germination studies that compared germination under constant isopotential conditions and used filter paper as a germination substrate (Macke and Ungar, 1971; McWilliam and Phillips, 1971; Sharma, 1973, 1976; Kaufmann and Eckard, 1977; Thill et al., 1979). A comparison of germination with salts, PEG, and other osmotica-induced water potentials could induce errors resulting from the exclusion effect lowering the PEG solution water potential. Toxicity and osmoregulation have been used to explain the differences in germi-

nation. Direct contact between PEG solution and the seed does not appear to influence germination (Emmerich and Hardegree, 1990). Water potential variations caused by PEG filter paper exclusion was most likely the factor affecting seed germination. Evidence for a water potential explanation comes from the observation that PEG water potentials generally produced lower germination when compared to what was thought to be isopotential water potentials from other sources (Macke and Ungar, 1971; Sharma, 1973, 1976). Also, water vapor loss from sealed containers cannot be ignored as an explanation. Our data showed that there was a 0.05 MPa change in water potential in the 14 FPR sealed petri dishes at an initial potential of −1.68 MPa and this small change added over 21 d would equal 1 MPa (Table 1).

Berkat and Briske (1982) and Hardegree and Emmerich (1990) have shown that organic germination substrates interact with a PEG solution and change the initial solution water potential. Polyethylene glycol exclusion and water vapor loss changes in water potential produced significant differences in seed germination when compared to germination at the initial water potential. A large PEG solution reservoir that produces a FPR >14 is recommended when filter paper is used as a germination substrate, to minimize the exclusion effect on water potential. The use of a vapor seal coupled with a solution reservoir will minimize the oscillation of substrate water potential associated with water loss and additions.

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