EFFECT OF OSMOTIC AND MATRIC POTENTIALS ON THE AVAILABILITY OF WATER FOR SEED GERMINATION

By J. R. McWilliam* and P. J. Phillips*

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Abstract

Under special conditions where soil-moisture diffusivity and seed–soil contact are non-limiting, the osmotic and matric potentials of the substrate were found to be equivalent in their effect on the germination of seeds of ryegrass and dehulled phalaris over a range of water potentials from 0 to −15 bars. However, with intact phalaris seeds it appears that the seed coat constitutes a large resistance to the absorption of soil water, and under these conditions the equivalence between osmotic and matric potential no longer holds, and results of germination under osmotic stress must be used with caution in predicting the germination behaviour of seeds in dry soil.

Germination of both species declined with decreasing potentials, although ryegrass was more tolerant of stress, and there was significant germination of this species at potentials about 6 bars below that which prevented germination of phalaris.

While this tolerance of moisture stress during germination may be one of the factors contributing to the relative ease of establishment of this species under field conditions, it does not provide a reliable index of drought tolerance in mature plants.

I. Introduction

The effect of the total potential of the soil moisture on an organism can be reduced to those of matric and osmotic potentials in a non-swelling soil, because differences in gravitational and pressure potentials are negligible in the vicinity of the organism. From thermodynamic considerations the two potentials would be expected to be equivalent, and therefore to be additive in their effect on the supply of water to plants, provided the plant behaves as a perfect osmometer.

Generally, however, soil solutes enter the plant to a significant extent (Bernstein 1961; Slatyer 1961) and therefore to test the hypothesis of equivalence it is necessary to use a non-permeating solute such as a high molecular weight polyethylene glycol.

In this study we have investigated the equivalence of osmotic and matric potentials in relation to seed germination of two grass species. Also some of the factors responsible for differences in germination response and their implications for establishment under field conditions have been considered.

II. Materials and Methods

(a) Species

Seeds of two perennial grasses, phalaris (*Phalaris tuberosa* L.) and ryegrass (*Lolium perenne* L.), were used to compare germination over an equivalent range of osmotic and matric potentials. The choice of two grasses was influenced by previous studies (McWilliam, Clements, and Dowling 1970; McWilliam and Dowling 1970) in which the germination response of these species, when subjected to various forms of water stress, has been described. Seed was of high quality (> 90% germination), less than 1 year old, and harvested at full maturity from plants grown under favourable conditions.

* Division of Plant Industry, CSIRO, P.O. Box 109, Canberra City, A.C.T. 2601.

(b) *Seed Treatments*

Treatments applied to samples of phalaris seed before imbibition designed to increase water uptake were as follows:

(1) Control (untreated).
(2) Seeds treated with a wetting agent [1% solution of polyvinyl alcohol (PVA)].
(3) Outer seed covering (seed coat), made up of the palea and lemma which form a closely fitting envelope around the caryopsis, removed by dissection without damage to the caryopsis (dehulling).

(c) *Germination*

The experiments were carried out in the dark in a room maintained at 23 ± 1°C unless otherwise stated. A seed was considered to have germinated when the length of the protruding radicle exceeded the small dimension of the seed. The results are expressed as percentage germination after a period of 14 days, based on four or five replicate samples of 50 seeds.

(d) *Osmotic Potentials*

Seeds were exposed to a range of osmotic potentials from 0 to -15 bars during germination by placing them in Petri dishes on filter paper saturated with an aqueous solution of polyethylene glycol (PEG, mol. wt. *c.* 20,000, Union Carbide Chemicals Co., U.S.A.). Good aeration was maintained by limiting the depth of the solution to approximately 1 mm. To prevent evaporation, and hence a change in osmotic potential, the dishes were kept in containers under high humidity. The PEG was dialysed to remove contaminants (Lawlor 1970), and the concentrations required to produce the various solution potentials were read directly from a calibration curve drawn from values obtained with a hanging-drop osmometer. There was no evidence that the high molecular weight PEG used entered the seed or that it restrained or inhibited germination by any process other than by reducing the availability of water (Manohar 1966; McWilliam, Clements, and Dowling 1970).

(e) *Matric Potentials*

These were generated using germination chambers of the type illustrated in Figure 1. The seeds were embedded in a thin layer of fine soil which was separated from a solution of PEG by a thin semipermeable membrane. The membrane used (a brackish water reverse osmosis membrane, T119, Aerojet-General Corporation, U.S.A.) was chosen because it is highly permeable to water but excludes all large molecules including PEG. The exclusion of PEG by the membrane was confirmed in these experiments by analysing soil samples after a 10-day exposure on the membrane, using the method described by Shaffer and Critchfield (1947). The soil (*c.* 1 cm deep) was a mixture of fine sandy loam and peat (3:1 mix), ground to pass a 150-μm screen, leached, and heat-sterilized before use. The possible effect on germination of reduced soil water diffusivity with increasing moisture stress was minimized by

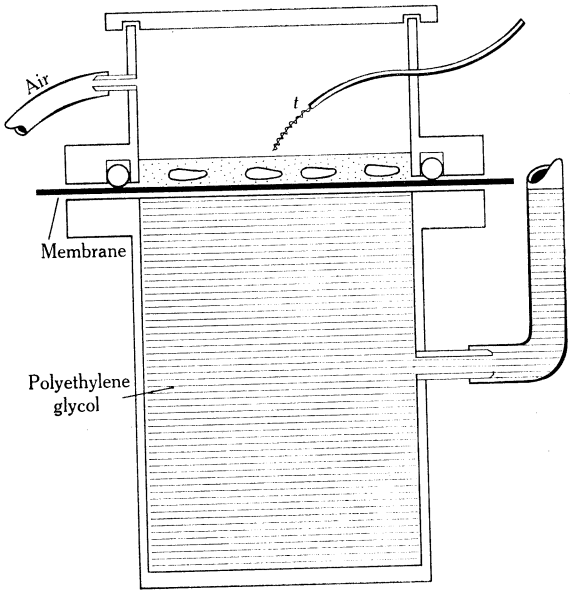

Fig. 1

restricting the path of water diffusion to a layer (*c.* 2 mm) of fine soil between the membrane and the seed, and also by permitting adequate time for equilibration. Also, the extremely fine soil mixture ensured good contact between seed, soil matrix, and the supporting membrane.

A range of matric potentials was produced in the soil surrounding the seed by varying the osmotic potential of the PEG below the membrane from 0 to −15 bars. Dry soil and seed were placed on the membrane at the commencement of each run, and it was sealed by means of an "O" ring to the germination chamber above and to the reservoir of PEG below as shown in Figure 1. The sealed germination chamber was also connected to a larger vessel containing air in equilibrium with PEG at the same potential as that in the apparatus to provide a supply of oxygen and an outlet for the respired carbon dioxide. The reservoir also had a side-arm to maintain a constant level of PEG at the undersurface of the membrane. The thermocouple (*t*) recorded temperature at the soil surface. Measurements of soil moisture indicated that an equilibrium was reached in the germination chamber within 24 hr.

Under the isothermal conditions in these experiments, it was assumed that, at equilibrium, the matric potential of the soil was equivalent to the osmotic potential of the PEG solution as has been shown by Zur (1966) and Kaufmann and Ross (1970) using similar equipment over a range of potentials and soil types.

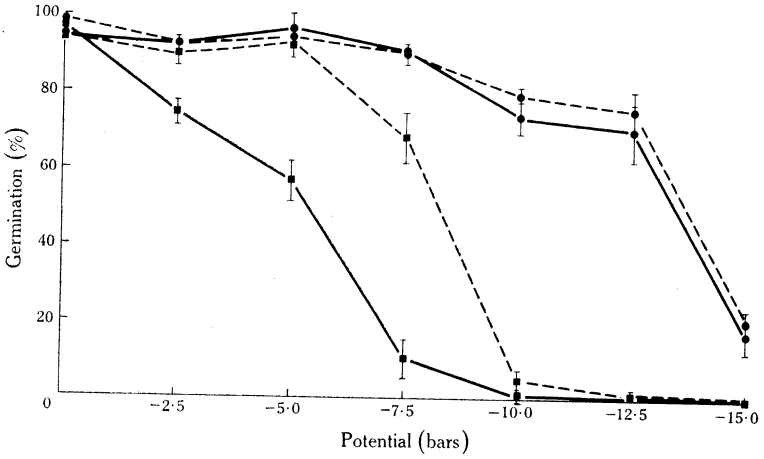

Fig. 2.—Germination of ryegrass (●) and phalaris (■) after 14 days exposure to a range of equivalent osmotic (– – –) and matric (—) potentials. Confidence intervals of twice the standard error are shown for each mean value.

(*f*) *Simulated Surface Sowing*

A cabinet, in which temperature, relative humidity, and light intensity were controlled, was used to simulate the conditions at the soil–air interface experienced by surface-sown seed in the field following substantial falls of rain. Dry seed of ryegrass and phalaris (including PVA and dehulled treatments) were placed on a level uniform soil surface composed of a fine sandy loam (particle size < 200 μm) subirrigated to maintain a constant water potential (either 40 or 80 mm of water tension) at the soil surface. Two levels of evaporative stress were imposed by varying the relative humidity and temperature of the air passing over the soil surface. These were designated either as low stress (18°C, 95% R.H.) or medium stress (20°C, 91% R.H.). Both treatments were applied under conditions of continuous light (1000 f.c. fluorescent source).

(*g*) *Structure of Seed Coat*

Seeds of ryegrass and phalaris were fixed in formalin–acetic acid–alcohol (5:5:95 v/v), embedded in either wax or Epon, and sectioned on a rotary microtome to examine the structure of the outer seed covering (seed coat) and the spatial relationship between it and the pericarp of the grain.

III. Results

(a) *Comparison of Osmotic and Matric Potentials*

The germination of ryegrass and intact phalaris seed over a range of equivalent osmotic and matric potentials is illustrated in Figure 2. In both species the rate and total germination decreased with decreasing water potentials, but the pattern of response differed markedly between the species.

There was no significant difference in the rate or total germination of ryegrass at equivalent osmotic and matric potentials over a range of 0 to −15 bars, but with phalaris low matric potentials led to a much greater reduction in germination than osmotic potentials of similar magnitude. This difference between osmotic and matric potentials was apparent at all levels from −2·5 to −12·5 bars, which represented the lower limit for germination in phalaris.

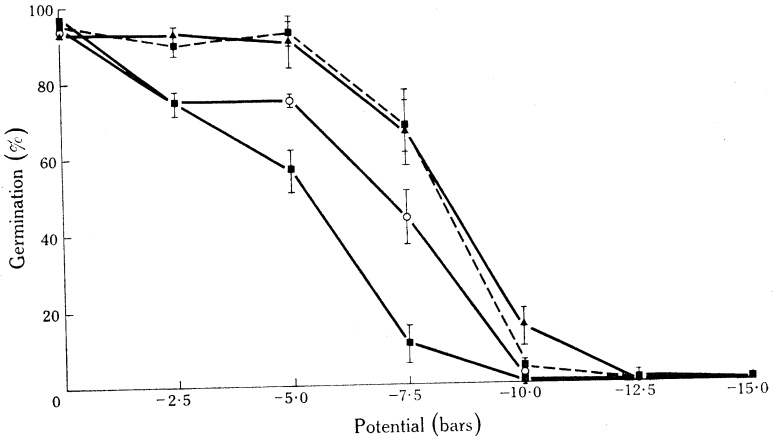

Fig. 3.—Germination of dehulled (▲), PVA-treated (○), and intact (■) phalaris seed after 14 days exposure to a range of matric (—) potentials, compared with the germination of intact phalaris seed for the same period over an equivalent range of osmotic (– – –) potentials. Confidence intervals of twice the standard error are shown for each mean value.

In an attempt to minimize this difference in the germination behaviour of phalaris under these two types of potential, seeds were either dehulled or treated with a wetting agent (PVA). PVA treatment was slightly inhibitory under conditions of osmotic stress (not shown), but increased germination at intermediate matric potentials (Fig. 3). Dehulling seed made no difference to the germination of phalaris when exposed to osmotic stress (not shown), but it had a striking effect on seeds

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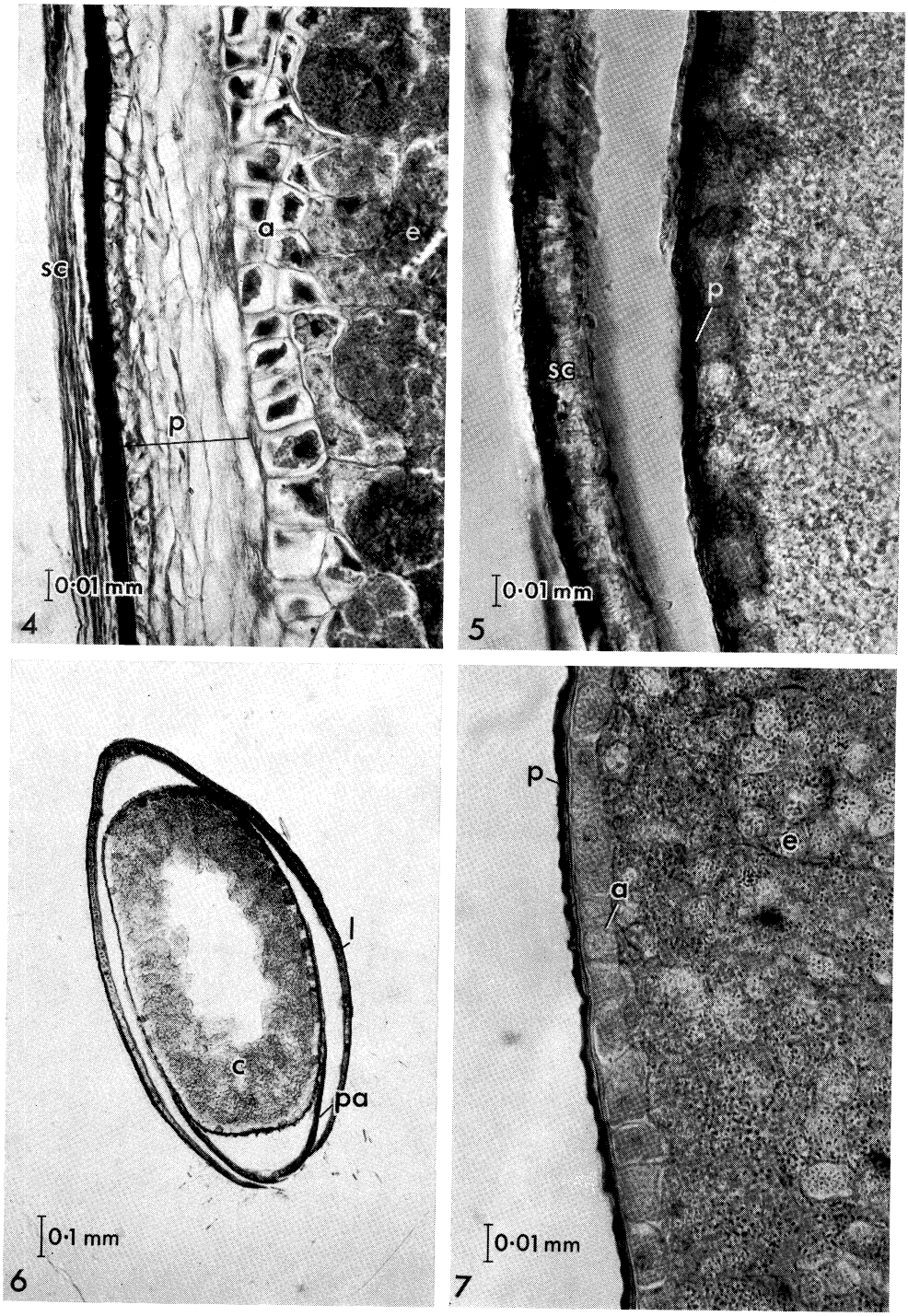
pericarp (*p*) which surrounds the multilayered aleurone layer (*a*) of the endosperm (*e*); *5*, transverse section of phalaris seed, showing the gap between the seed coat (*sc*) and the pericarp (*p*); *6*, cross-section of phalaris seed, showing the relationship between the seed coat (*l*, lemma, *pa*, palea) and the caryopsis (*c*); *7*, transverse section of phalaris seed, showing the detail of the thin pericarp (*p*) surrounding the aleurone layer (*a*) of the endosperm (*e*).



Figs. 4–7.—Longitudinal and transverse sections of seed of ryegrass and phalaris showing the relationship between the seed coat (palea and lemma) and the pericarp of the seed: *4*, transverse section of ryegrass seed, showing the close association between the seed coat (*sc*) and thick

exposed to matric potentials, and increased germination to the level obtained under equivalent osmotic potentials over the range from 0 to −12·5 bars (Fig. 3).

(b) *Nature of the Seed Coat*

Because of the obvious importance of the seed coat in germination at reduced water potentials, its structure was studied in both species to examine its possible role in influencing water uptake. In ryegrass the lemma and palea which make up the seed coat are closely associated with the thick-walled pericarp, and both structures form a continuous layer of tissue around the caryopsis (Fig. 4). In phalaris the palea and lemma are separated from the thin-walled pericarp over most of its surface and form a loosely fitting envelope around the caryopsis, except at the distal tip where they often fail to close completely (Figs. 5–7). The dimensions of the air gap between the two structures vary between seeds, but average 0·02±0·01 mm on the flattened sides and 0·07±0·03 mm on the edges of the seed.

A further difference exists in the surface characteristics of the seed coat in these two species. The seed coat in phalaris has a smooth polished appearance and is covered by fine hairs which make it difficult to wet, whereas in ryegrass this structure is devoid of hairs and has a slightly granular surface texture which wets readily.

TABLE 1

GERMINATION OF PHALARIS AND RYEGRASS ON A SOIL SURFACE UNDER TWO LEVELS OF MOISTURE STRESS

Species	Treatment	Germination (%)	
		Medium Stress*	Low Stress†
Ryegrass	Untreated	46·4±3·3	83·2±2·3
Phalaris	Untreated	3·6±2·2	34·8±1·6
Phalaris	+PVA	1·6±0·4	32·0±4·4
Phalaris	Dehulled	6·4±1·5	85·6±2·1

* 20°C, 91% R.H., 80 mm of water tension.

† 18°C, 95% R.H., 40 mm of water tension.

(c) *Germination under Simulated Field Conditions*

An attempt was made to extrapolate from the results obtained with controlled soil matric potentials to a simulated field situation in which seeds were set out to germinate on a moist soil surface maintained in a controlled environment. The results obtained for ryegrass and phalaris at the two levels of stress are given in Table 1. Under medium stress conditions only ryegrass gave any effective germination; however, under the more favourable low stress conditions germination of both species was significantly increased. Of special note was the high germination of the dehulled phalaris which was more than double the level of the untreated controls, and equivalent to ryegrass under these conditions. In this situation PVA treatment of phalaris seeds was without effect.

IV. Discussion

In the experiments reported, substrate osmotic and matric potentials were found to be equivalent in their effect on the germination behaviour of seeds of ryegrass and dehulled phalaris. This applied over the full range of potentials for germination of both species, and it indicates that both these components of water stress are equivalent in their effect on the germination process. These results were achieved under conditions where the soil's capacity to supply water to the seed at a given potential was not limiting, and where good seed–soil contact was achieved. However, where other factors impose additional resistance to water movement between the soil and the imbibing surfaces of the seed, such as was found for the intact seeds of phalaris, this equivalence no longer holds, and performance under osmotic stress cannot be used to predict the behaviour of seeds in dry soil. A similar conclusion has been reached by Kaufmann and Ross (1970), and such evidence should be taken into consideration in interpreting the results of germination experiments in which osmotic solutions have been used to simulate moisture stress (Uhvits 1946; Collis-George and Sands 1962; Parmar and Moore 1966; McWilliam, Clements, and Dowling 1970).

In the case of intact phalaris seeds, the presence of a vapour gap between the seed coat and the pericarp, which remains air-filled at reduced water potentials, can drastically reduce the rate of water flow (Bernstein, Gardner, and Richards 1959), and it probably constitutes the main resistance to water movement, although the non-wetting properties of the seed coat may also contribute a further resistance.

The effects of the seed coat on germination in phalaris were also obvious with seeds sown on a moist soil surface under simulated field conditions, where the ability to germinate is determined by the balance between water uptake and water loss by evaporation. The substantial increase in germination achieved by dehulling seed clearly demonstrates the importance of maintaining good contact between seed and soil under these conditions.

The ability of ryegrass to germinate on the soil surface under conditions of moisture stress confirms the results obtained under both osmotic and matric potentials, and highlights the difference between ryegrass and phalaris in this respect.

Although differences of this order have been reported previously for seeds of a number of crop plants (Hunter and Erickson 1952; Parmar and Moore 1966; Tadmor, Cohen, and Hartaz 1969), there are few reports of seeds which are capable of germinating at potentials as low as those reported for ryegrass (−15 bars) which is in the vicinity of the permanent wilting percentage for most plants (Doneen and MacGillivray 1943; Owen 1952; Hadas 1970). These results also support the suggestion of Hunter and Erickson (1952) that there is a characteristic soil water potential below which seeds of a given species will not germinate.

This tolerance of low water potentials during germination may be one of the factors contributing to the ease of establishment of ryegrass under field conditions, particularly when sown on the soil surface where moisture conditions for germination are more likely to be limiting (Campbell 1968; McWilliam and Dowling 1970). Similarly, poor establishment of phalaris under these same conditions can be attributed to its greater sensitivity to water stress and to the seed coat characteristics which apparently restrict the rate of absorption of soil water by the seed.

In this connection, it has been suggested (Vasudevan and Balasubramanium 1965) that the ability to germinate under moisture stress may provide a reliable index of drought resistance in the mature plant. However, the studies of Younis, Stickler, and Sorenson (1963) suggest that there is no such association. Furthermore, the cultivar of ryegrass used in the present study (originally of northern European origin) has shown poor survival under drought conditions (McWilliam and Dowling 1970), whereas phalaris (originally from the Mediterranean region) was substantially more drought-tolerant. To provide additional evidence on this point, the germination behaviour of two native perennial grasses, wallaby grass (*Danthonia caespitosa*) and Mitchell grass (*Astrebla squarrosa*), from semi-arid regions of Australia was also examined under a range of osmotic potentials. The results (not presented) are similar to those obtained for phalaris with practically no germination above $-10\cdot0$ bars.

It may well be that failure to germinate under moisture stress has adaptive significance in grasses from dry environments by restricting germination to periods of the year when adequate moisture is available.

V. Acknowledgments

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