CHAPTER 1

INTRODUCTION AND BACKGROUND.

1.1 INTRODUCTION

As agriculture has become increasingly mechanised and sophisticated there has arisen a strong demand for improved uniformity within crop stands. Uniformity in size and quality as well as in the time of maturity is desired.

Variability within a crop stand can result from a number of edaphic factors, such as variations in soil type, soil moisture content, microclimate and topography. These causes can be identified in the field and appropriate management practices can be instituted to reduce their effects.

However, it has been shown by a number of workers that a considerable proportion of the variation in attainment of maturity and in plant size results from differences which occur at emergence. In a closely spaced crop, such as carrots, Salter, Currah and Fellows (1981) have shown that differences in time to emergence were further reinforced by competitive growth throughout the development of the crop. In more widely spaced crops, differences in emergence time influenced the attainment of maturity and the size of the harvestable product. For example, Salter and Fradgley (1969) working with cauliflower, Peck and Clark (1973) with broccoli, and Gray (1976) with lettuce have all shown that earlier emerging seedlings produced earlier maturing and higher yielding plants than did later emerging seedlings.

Logically, then, it should be possible to reduce differences in plant size and maturity at harvest by reducing the temporal differences at seedling emergence.

In the search for more reliable, rapid and uniform germination and emergence a number of pre-sowing treatments have been investigated. These include: seed size grading; hydration-dehydration treatments; high humidity hydration; infusion of hormones; pregermination and fluid-sowing; and hydration in an osmotic solution or seed priming.

The fluid-sowing of pregerminated seed has had the most spectacular success in reducing the time from sowing to emergence, but it has often not provided a more uniform germination nor reduced variability in time to emergence (Darby and Salter, 1976). Moreover, there are considerable technical difficulties yet to be overcome, such as the storage problems whilst awaiting suitable sowing conditions and the high capital costs involved (Coolbear, Grierson and Heydecker, 1980). These difficulties may make the sowing of pregerminated seed inappropriate for some crops. Thus there is considerable scope for the development of seed treatments that not only improve germination performance, but allow the use of existing sowing equipment and also the storage of treated seeds. Seed priming, which involves the hydration of seeds in an osmotic solution before sowing, may prove to be this treatment. Moreover, available evidence indicates that this treatment also decreases the time to emergence of seed sown in soil at low temperature, a most important consideration for spring-sown crops where the subsequent growing season is restricted or contains environmentally unfavourable periods.

1.2 THE HISTORY OF SEED-SOAKING TREATMENTS

The oldest extant records of scientific investigations into germination appear to be those of Theophrastus of Eresos (who lived from c.372 to 287 B.C.). He observed many aspects from seed development to dispersal, but those of relevance to this work include his discussion of the differences in germination behaviour: "the causes of such differences must be found in several different circumstances: In the seeds themselves, in the habitat, in the state of the atmosphere and in the season at which each is sown, according as it is cold or clear" (De Historia Plantarum [D.H.P.] Book VII, 1:5).

Theophrastus also recognised that different species had different strategies of germination: "There is . . . a singular feature about beet; the seed does not all germinate at once, but some even in the next or in the third year" (D.H.P. Book VII, 1:6). He reported observations of presoaking treatments: "Some even presoak the seed of cucumber in milk or water to stimulate germination" (D.H.P. Book VII, 1:6) and "Coriander germinates with difficulty; indeed fresh seed will not come up at all until it is moistened" (D.H.P. Book VII, 1:3). But similar presoaking treatments were already known before Theophrastus. Pliny (23 - 91 A.D.) reported that Democritus (5th century B.C.) advised soaking all seeds before they were sown "in the juice of the plant that grows on roof tiles" (Naturalis Historia, Book XVIII, XLV:159).

The next report of a seed-soaking treatment, after the fall of the Roman Empire, is apparently that of Sir Kenelm Digby in 1661 who reported improvements in yield from soaking corn in a

solution of saltpetre (KNO_3). In 1843 Campbell claimed a remarkable improvement in yield from oats, barley, wheat and rye by soaking the seed in NH_4SO_4, NH_4NO_3 and NH_4Cl as well as $NaNO_3$ and KNO_3.

Kidd and West (1919) reviewed the work carried out in this field during the nineteenth century, notably that of Kraus (1880, 1881) and Wollny (1885, 1885) who investigated the effects upon subsequent growth and yield of soaking seeds in water. Kidd and West (1919) concluded that seeds soaked in water and sown still moist germinated more quickly than untreated ones; that the minimisation of the amount of water was important; and that slow drying after soaking maintained the benefits whereas rapid drying caused the seeds to germinate more slowly than untreated ones. Kidd and West (1919) also made reference to investigations into the effects of soaking seeds in salt solutions, but were of the opinion that the results obtained were not appreciably better than those obtained from water-soaked seeds.

Kidd and West's (1918) own experiments showed some beneficial effects from soaking in water of wheat, oats, mustard and broad bean, whereas soaking of dwarf bean seed had only detrimental effects. Tincker (1925) claimed that soaking seed in water caused an increase in yield in various Gramineae, and Jones and Tincker (1926) found that initial leaf growth was improved in many cereals by water-soaking treatments. The effects of water-soaking on grass seed were investigated by Chippendale in 1934 and, like Kidd and West, he found no generalized effects, even in closely related species.

Roberts (1948) soaked seeds of wheat, barley, oats and rye in a range of solutions containing the three potassium orthophosphate salts. The seeds were soaked for a period of 24 hours in an amount of solution equal to one-third of their weight and then air-dried at 22 to 25 C. The solution concentrations used ranged from 1 to 20%. Roberts found that 10 and 20% solutions of all salts killed the wheat seeds, while the lower concentrations were not markedly beneficial to early growth. With barley all concentrations proved beneficial with little difference between compounds except for the 20% dibasic solution which increased early growth four-fold and yield by 48% over that of conventionally fertilized crops in nutrient-deficient soils. All concentrations of the tribasic salt proved beneficial to oats increasing yield by 46%. The monobasic salt proved somewhat beneficial to early growth of rye. Roberts concluded that different salts gave different results, and that for each type of seed it was necessary to determine both the most suitable salt and the optimum concentration. Roberts claimed that it was possible to provide a large proportion of the major plant nutrients required throughout the development of a crop by soaking seeds in an appropriate solution for a short period.

A surge of studies followed the publication of a review of the work of the Russian P. A. Genkel and colleagues on pre-sowing drought-hardening of seeds in the U.S.S.R. (May, Milthorpe and Milthorpe, 1962). A number of workers reported other effects of pre-sowing drought-hardening than that of drought tolerance. Zubenko (1959) found that pre-sowing drought-hardening increased the field emergence of corn. Austin, Longden and Hutchinson (1969) showed that hardened carrot seed had a higher germination rate which led to higher field emergence rates, greater seedling

weights and higher root yields. Hegarty (1970) confirmed these emergence results and showed that soaking in an amount of 0.01M K_2HPO_4 solution which could be absorbed in 16 hours enhanced the effects.

Ells (1963) described a method of treating tomato seeds with a solution of 1% K_3PO_4 and 1% KNO_3 (c. -0.7 MPa). He reported that soaking for six days in this solution at 24 C had an outstanding effect upon the rate of emergence of tomato seeds at suboptimal temperatures. Oyer and Koehler (1966) reported a similar advantage in tomato seeds from a similar salt treatment.

Heydecker (1974) acclaimed the use of high molecular weight polyethylene glycol (PEG) solutions as a successful method of treating seeds to enhance their germination. Heydecker hypothesised that: "it may be possible to split the germination process of any seed neatly in two. An osmoticum of an appropriate potential might conceivably prevent any seed indefinitely from reaching the final phase of germination - namely cell elongation and radicle emergence - while permitting the preliminary processes that prepare the seeds for this final phase to take place . . . ." Heydecker (1974) gave this seed treatment the name "priming" from an analogy with the priming of a pump.

Priming may be considered as part of the continuum of seed-soaking treatments. For this continuum a number of claims have been made, especially the improvement in the germination and establishment of crops and possible improvement in their yield. However, as is evident from all the reports, the soaking effects are dependent upon the solution used, its concentration, the duration and temperature of treatment, the degree and rate of drying and most importantly the species and variety so treated.

1.3 METHODS USED TO PRIME SEEDS

Priming may be defined as:

> The soaking of seeds in a solution of appropriate osmotic potential, such that radicle emergence is prevented, while permitting the activation of many of the preliminary processes of germination.

The aims of seed priming are generally considered to be one or all of the following: a decrease in time to germination; increased uniformity of germination (decreased time-spread); better germination at suboptimal soil temperatures and an increase in final yield. Rarely have empirical measurements been made on all four aims simultaneously and even more occasionally have all four been demonstrated simultaneously.

Some work has been reported of the effects upon germination of various treatments described as priming when, in fact, these treatments are such that they would not satisfy the above definition. Implicit in this is that the duration of a priming treatment be longer than the time required for seed placed in water at the same temperature to germinate. Treatments of shorter duration must be considered as seed-soaking treatments, as distinct from priming treatments.

Seed treatments which allow drying before sowing and hence the use of conventional drilling equipment, offer appreciable economic benefits. Consequently, those treatments which do not improve germination performance after drying are not considered here because of the capital costs of specialised equipment necessary to sow such seeds.

1.3.1 Polyethylene glycol methods

Some of the priming treatments that have been used are presented in Table 1. Most workers have followed Heydecker and used PEG solutions as osmotica. Temperatures used range from 5 to 25 C with 20 C most common. Durations range from 2 to 70 days. In most reports no reasons have been given for the combination of concentration, temperature and duration used. In general, osmotic potentials between -0.75 and -1.00 MPa have been favoured. Temperature and duration combination have been such that longer durations have proved more beneficial when used with lower temperatures than those optimal for germination. The results for longer durations at the higher temperatures indicated that some physiological damage may have ensued from the treatment.

Most workers, who have noted beneficial effects of priming treatments, have reported the advancement of germination time, particularly at suboptimal temperatures. Generally this has been measured as a decrease in time to a particular percentage germination, e.g. time to half the maximum germination ($t_{0.5A}$). Some workers (Coolbear and Grierson, 1979; Szafirowska, Khan and Peck, 1981) have measured the improvement in uniformity of germination of primed seed as the decreased time taken between defined percentages of germination whereas others have either not reported any measure of uniformity or briefly mentioned improvement without presenting data.

Table 1. Priming treatments using polyethylene glycol solutions. Estimated osmotic potential values marked with an asterisk (*). (ns) not stated in reference.

Species	Osmotic pot. (MPa)	Temp. (C)	Time (d)	Reference
Onion	-1.0	10	23	Heydecker et al (1974)
Red beet	-1.0	15	21	v
Celery	-1.0	15	14	Heydecker et al (1975)
Capsicum	-1.44	15	14	v
Tomato	-1.44	15	14	v
Carrot	-0.88	15	7	v
Celery	-1.0	10	5-35	Salter & Darby (1976)
Onion	-1.2	20	27	Peterson (1976)
Cyclamen	-0.8	15	35	Heydecker & Wainwright (1976)
Oat	-1.0	5-25	3-6	Akalehiywot & Bewley (1977)
Wheat	-1.0	5-25	3-6	v
Calabrese	-0.5/-2.0	10	1-21	Hegarty (1977)
Parsnip	-1.0/-1.5	20	5-20	Gray & Steckel (1977)
Capsicum	-0.8	15	5	Yaklich & Orzolek (1977)
Lettuce	-0.97*	15	14	Khan et al (1978)
Onion	-0.93*	9	ns	v
Celery	-0.79*	20	8	v
Soybean	-0.97/-1.56*	15	4-10	v
Pea	-0.97/-1.56*	15	8	v
Corn	-0.97*	15	8	v
Tomato	-0.75	20	15	Rumpel & Szudyga (1978)
Strawberry	-1.0	15	21	Gutteridge & Bright (1978)
Cabbage	-1.2	15	14	Ralph (1978)
Celery	-1.0	18	14	Rennick & Tiernan (1978)
Celery	-1.0	20	14	Tiernan & MacNaeidhe (1979)
Celery	-1.0	10	27	Darby et al (1979)
Antirrhinum	-1.25	15	21	Kepczynski (1979)
Tomato	-0.625	25	12	Coolbear & Grierson (1979)
Carrot	-1.0	20	12	Wiebe & Tiessen (1979)
Carrot	-1.0	20	7	Nakamura & Enohora (1980)
Eggplant	-0.75	25	14	v
Cryptotaenia	-1.0	20	14	v
Impatiens	-0.75	25	10	Simmonds (1980)
Chenopodium	-0.86	15	14	Khan & Karssen (1980)
Tomato	-0.75	25	10	Coolbear et al (1980)
Tomato	-0.50	20	5	Green (1980)
Soybean	-0.86	15	2-14	Knypl & Khan (1981)
Freesia	-0.5	20	7-10	Kepczynski (1981)
Onion	-1.5	15	14	Hatridge-Esh & Orton (1981)
Carrot	-0.86	15	6	Szafirowska et al (1981)
Parsley	-1.2	5-15	7-70	Ely & Heydecker (1981)

1.3.2 Salt solution methods

While most work has reported the advantages to be gained through priming using PEG, only Peterson (1976) has considered the possibility of a commercial scale treatment using a PEG-vermiculite slurry as the medium. Major difficulties were encountered in separating seed and slurry following treatment. However, Darby and Salter (1976) described a technique for the priming of celery seeds using a perspex column containing an aerated solution of K_3PO_4 + KNO_3. Large quantities of small seeds were able to be treated with this technique.

Following Ells (1963) most salt solutions used to prime seeds have contained K_3PO_4 + KNO_3 (Table 2). It is not clear why K_3PO_4 was chosen to combine with KNO_3 and no reason was found in the papers cited - apart from the empirical finding of Roberts (1948) that it worked. The solution has a pH of over 12 which may assist in the control of unwanted micro-organisms; it conceivably may also be toxic to embryos if absorbed.

Although not detailed in Table 2, Sachs (1977) investigated a range of salt solutions including K_3PO_4 + KNO_3 for priming watermelon seeds and found that nitrate salts were more beneficial than other compounds with 0.2M KNO_3 giving the best results. Salter and Darby (1976) and Nakamura and Enohara (1980) have reported the results of experiments investigating the effects of a range of osmotica, concentration, temperature and duration combinations. Their results together with Sachs (1977) have shown, similarly to those of Roberts (1948), that each species has a different response to the different treatment combinations.

Table 2. Priming methods using salt solutions.
Estimated osmotic potential values marked with an asterisk (*).

Species	Solution	Osmotic Potential (MPa)	Temp. (C)	Duration (d)	Reference
Tomato	1%K_3PO_4 + 1%KNO_3	-0.7*	24	6	Ells (1963)
Tomato	1M $MnSO_4$	-2.5*	25	7	Traverse & Reikels (1973)
Celery	KNO_3 + K_3PO_4	-1.0	10	7-27	Darby & Salter (1976)
Tomato	1.5%K_3PO_4 + 1.5%KNO_3	-1.2*	24	5	Bussell & Gray (1976)
Carrot	2% KNO_3	-0.8*	20	6	Wickham & Nicholls (1976)
Radish	2% KNO_3	-0.8*	20	6	v
Watermelon	0.2M KNO_3	-0.8*	20	6	Sachs (1977)
Parsnip	KNO_3 + K_3PO_4	-1.0	20	10	Gray & Steckel (1977)
Tomato	1.5%K_3PO_4 + 1.5%KNO_3	-1.2*	24	5	Rumpel & Szudyga (1978)
Strawberry	KNO_3 + K_3PO_4	-1.0	15	21	Gutteridge & Bright (1978)
Carrot	K_2HPO_4 + KNO_3	-1.0	20	12	Wiebe & Tiessen (1979)
Tomato	1.8%KH_2PO_4 + 1.8%KNO_3	-1.2*	25	5	Maluf & Tigchelaar (1980)
Capsicum	3% KNO_3	-1.2*	20	3-9	Sachs et al (1980)
Onion	0.24M NaCl	-1.1	10	2-8	Furutani et al (1981)
Onion	0.1M KNO_3 + 0.1M K_3PO_4	-1.1	10	2-8	v

1.4 GERMINATION IN RELATION TO WATER SUPPLY

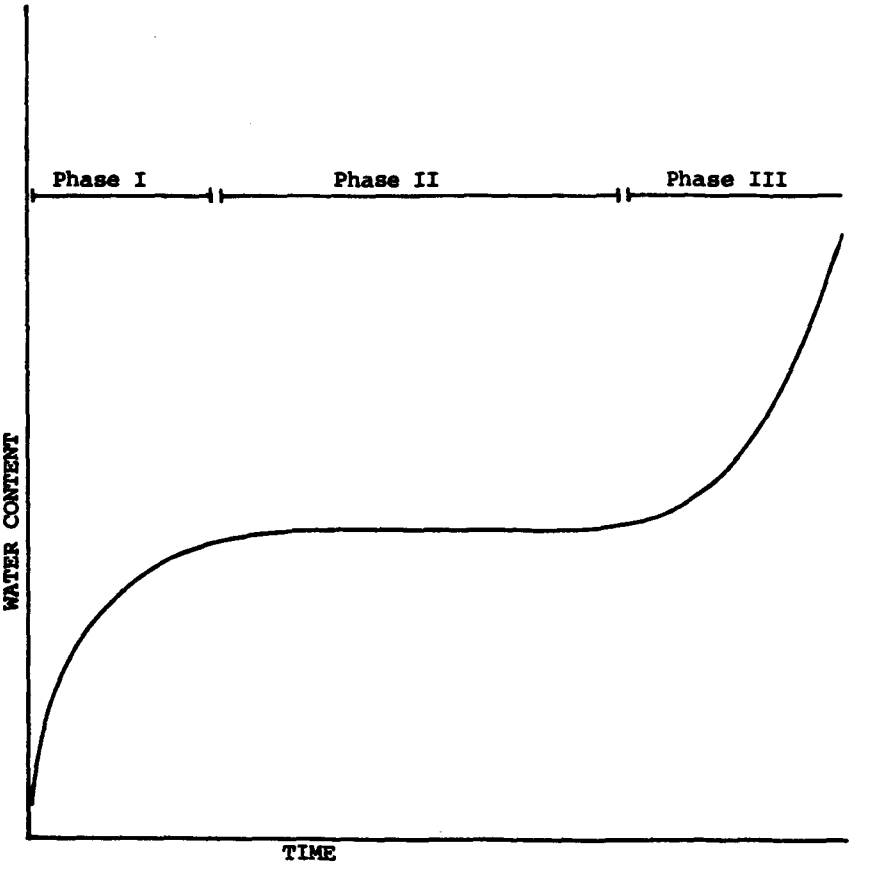
1.4.1 Water Uptake

Water uptake by seeds can be described as a triphasic process, each phase merging gradually into the next (Bewley and Black, 1978) (Figure 1). The initial phase of rapid water uptake is basically the physical process of absorption; however, absoption in the endosperm of cereals, for example, continues long after embryo tissues have effectively moved into the next phase. This lack of any sharp distinction possibly explains why uptake becomes increasingly temperature dependent during its course (Allerup, 1958).

The rate of water uptake is initially dependent on the water potential gradient between the seed and its environment and the permeability of the seed. Differences in the permeability of seed coats, the size and type of storage tissues and the chemical composition of the seeds cause differences in water uptake between species; moreover, the hydration properties of the organs within the seeds result in the hydration of the whole seed being dependent upon the balance between these organs. Stiles (1948) has shown that embryos in maize and cotton imbibe more rapidly than do the other seed structures but differences in whole seed hydration reflect differences in hydration of the endosperm (maize) or the cotyledons (cotton) because of their relatively greater mass.

The membranes of seeds have been shown to be leaky at the beginning of imbibition, but this leakiness is reduced and

Figure 1. The triphasic pattern of water uptake by germinating seeds.


Phase I
Phase II
Phase III
WATER CONTENT
TIME

eventually stops as the imbibition proceeds (Simon and Raja Harun, 1972). Hendricks and Taylorson (1976) demonstrated the temperature dependence of membrane reorganization in imbibing seeds. Membrane phase changes have been shown to greatly influence seed leakiness (Hendricks and Taylorson, 1976; Simon, 1978). Seewaldt, Priestley, Leopold, Feigenson and Goodsaid-Zalduondo (1981) have shown that membranes of soybean seeds possess a lamellar (bilayer) structure in the dry state which altered abruptly through a change in bilayer spacing as seed hydration increased from 17 to 23% (on a fresh weight basis) in the cotyledons. They also showed that no free water (i.e. non-surface-bound water) was available below 17% water content and that the membrane phase change occured at a hydration level just above that at which free water could be detected. However, it has also been demonstrated that rapid water uptake in pea seeds led to areas of cell death on the cotyledons (Powell and Matthews, 1978) and that this damage caused high solute leakage (Powell and Matthews, 1979) resulting in poor field emergence (Powell and Matthews, 1980).

Because temperature affects the viscosity of water imbibition may be dependent upon both the properties of water as well as of the seeds (Mayer and Poljakoff-Mayber, 1975). By examining the effects of temperature on imbibition of radish seeds and excised sugar pine embryos (both of which have small storage organs) Murphy and Noland (1982) were able to demonstrate that the temperature dependence of initial imbibition and solute leakage was primarily related to changes in the viscosity of water. Moreover, it was shown that membrane integrity influenced the rate of water uptake, because heat-killed seeds and embryos exhibited significantly higher rates of imbibition and leakage

than did viable ones. Therefore, the temperature relationships of imbibition and germination may reflect these effects as well as those on the rates of the metabolic processes involved.

The second phase of water uptake is a lag period when matric and osmotic potentials change little, as the initially extremely low matric potentials have reached near-equilibrium values close to zero, little hydrolysis of seed reserves have occurred and little cell division and extension has commenced (Bewley and Black, 1978). During this phase catabolism of the seed reserves begin. The activity and quantity of the protein-synthesizing machinery, mitochondria, glyoxysomes, enzymes, co-factors and substrates increase (Ching, 1972). It is a period during which preparations are made for the rapid hydrolysis of seed reserves and the subsequent growth of the seedling.

The third phase of water uptake is associated with the synthesis of new cells and cellular components and the emergence of the radicle from the seed (Ching, 1972). Water provides the means for cell expansion as well as for the continued hydrolysis of reserves.

Not all seeds clearly exhibit this triphasic pattern of water uptake. Because of differences in seed composition this pattern may be masked when measurements are made of whole seeds. Further, it should be noted that, with seeds which contain large endosperms or have hypogeal cotyledons, water saturation of these organs will have occured during phase II; during phase III hydrolysis will continue supplying substrates for growth of the seedling, but the organs do not increase in volume, decreasing in mass and showing only a small increase in water content as

insoluble substrates are hydrolysed (Bewley and Black, 1978). Therefore, because of the bulk of these organs, their water uptake will mask changes in the rest of the seed. It has been observed that the water content of the embryo of dent corn was 261% on a dry weight basis whilst that of the rest of the seed was 50%, which resulted in an overall water content of 75% after imbibition (Blacklow, 1972).

Despite these reservations, the water uptake in phases I and II (Figure 1) can usually be described by

$$\theta = \theta_E - (\theta_E - \theta')\exp(-at) \qquad (1a)$$

or

$$\theta = \alpha - bt - (\alpha - \theta')\exp(-at) \qquad (1b)$$

where θ is the water content at time t, θ_E the equilibrium water content during phase II if constant and $\alpha - bt$ the asymptote where the water content increases during phase II (Blacklow, 1972). θ' is an estimated water content at time zero assuming the above function; it is always higher than θ_o of dry seeds, reflecting partly the difficulty of completely removing surplus water during uptake measurements and also possibly the more rapid wetting of the seed coats than can be explained by Fick's law (Becker, 1960).

1.4.2 Water Supply from the Germination Medium

The water potential of air dry seeds is extremely low - -50 MPa to -100 MPa (Manohar, 1966; Black, 1968 and Hegarty, 1978) - being determined mainly by the matric potential of the storage materials and cell walls. When seeds are placed in a moist environment water moves along the potential gradient between the medium and the seeds. The rate and degree of movement depends upon the continuing existence of the gradient and the permeability of the seeds.

1.4.2.1 Soil Media

It has been known for some time that water availability affects the rate and maximum percentage of germination. Doneen and McGillivray (1943) have shown that total germination decreased with soil moisture content over the range from field capacity to permanent wilting point in a wide range of vegetable seeds. No germination occured in any species below the permanent wilting point; moreover, in many of those tested only small percentages (less than 25%) germinated at this soil water content. Hunter and Erickson (1952) reported the soil moisture potential which prevented germination of a number of seeds: -1.25 MPa for corn, -0.79 MPa for rice, -0.66 MPa for soybean, and -0.35 MPa for sugar beet. Other studies, notably those of Collis-George and co-workers (1959, 1966, 1968) and Hadas (1970, 1976, 1977) and Hadas and Russo (1974) have also shown that soil water potential affects the maximum percentage germination of a wide range of seeds.

Not only is the matric potential of the soil important, but also the hydraulic conductivity of the soil and the degree of seed-soil contact (Koller and Hadas, 1982). The work of Dasburg and Mendel (1971) illustrates the difficulty of interpretation of soil water potential effects on germination. They were able to demonstrate clear soil water potential effects on the germination of the grass seed Oryzopsis holciformis. In loess soil germination percentage was maximum at a potential of -0.5 MPa whilst in sand the potential at which germination was maximum was influenced by the size of the sand particles used.

1.4.2.2 Osmotic Media

1.4.2.2.1 Osmotic potential effects

In order to avoid some of the difficulties of working with soils other workers have used osmotic solutions such as mannitol, polyethylene gylcol (PEG), and salt solutions. Studies using these osmotica have shown similar results to observations of germination in soil media (Parmar and Moore, 1968; Kaufmann, 1969). Scarascia, Lucia and Mastrorilli (1979) reported that decreases in osmotic potential caused decreases in germination of sorghum seeds from a maximum obtained at zero potential. However, these osmotic potential effects differed between osmotica, with greater effects reported in mannitol solutions than in PEG or sodium chloride. No germination occured at osmotic potentials below -0.71 MPa in NaCl and PEG or below -0.51 MPa mannitol. Taylor, Motes and Kirkham (1982) found that decreasing osmotic potential of PEG solutions caused decreasing maximum percentage germination of tomato seeds and that no germination occured at osmotic potentials below -0.69 MPa at

25 C. They reported, however, that the osmotic potential threshold to germination changed with temperature. At 30 C it was -0.55 MPa and at 35 C it was -0.15 MPa. Heikal, Shaddad, and Ahmed (1982) reported that germination rate and percentage of flax, sesame and onion seeds decreased as osmotic potential decreased in solutions of PEG and NaCl. The effect was more marked in PEG solutions than in NaCl. Although onion seeds treated in -0.8 MPa NaCl showed a large (70-80%) capacity to recover on transfer to water, those from the equivalent PEG solution failed to recover.

1.4.2.2.2 Specific ion effects

Differences in response to different osmotica may reflect differences in permeability to different substances. As pointed out by Uhvits (1946), Prisco and O'Leary (1970) and Redman (1972), there are differences between the osmotic and ionic aspects of certain osmotica, notably NaCl. Redmann (1972) reported that germination of three varieties of alfalfa did not decrease uniformly with decreasing osmotic potential in different osmotica. At -0.5 MPa germination ranged from 13 to 90% whilst at -1.0 MPa it ranged from 0 to 50%. The osmotic potential which reduced germination by 50% ranged from -0.72 MPa (Na_2SO_4) to -1.32 MPa (KCl) for the variety Beaver while the corresponding values for the other two varieties ranged from -0.38 to -1.18 MPa and -0.4 to -0.92 MPa. The corresponding values for PEG and mannitol were about -1.0 MPa for Beaver and about -0.8 MPa for the other varieties. Redmann concluded that a particular combination of ions produced the toxic effects rather than them being attributable separately to specific cations or anions. Other workers with alfalfa seeds similarly reported that osmotic

potential effects on germination depended upon the type of osmoticum used (Uhvits, 1946; Collis-George and Sands, 1962).

Assuming control of water uptake to be the sole requirement, avoidance of ion and other osmoticum effects should be possible using controlled atmospheres with a vapour gap between seeds and liquid (Hegarty, 1978). Differing responses of seeds to saturated atmospheres have been reported by Brewer and Butt (1950) and Griffin (1966) who found deterioration of the seeds whereas Owen (1952) reported germination of wheat over a range of water-vapour concentrations. Levitt and Hamm (1943) and Heydecker (1974) both reported improvements in subsequent germination of seeds of Taraxacum kok-saghyz and leek, respectively. Coleman and Fellows (1925) and James (1967) found equilibrium moisture contents from saturated atmospheres for a range of species. The water contents ranged from 27% for flax to 37% for barley. These water contents were lower than those determined for individual germinating calabrese (Brassica oleracea) seeds, hydrated at -0.75 MPa in PEG solutions, which ranged from 44 to 66%. Moreover, available free water is required for cell expansion and seeds if equilibrated with saturated vapour, may be substantially less hydrated than if imbibed in contact with water (Hegarty, 1978).

Hegarty and Ross (1981) have shown that in many species seedling growth after germination continued at water potentials lower than those which prevented germination. They postulated that this phenomenon may have resulted from water potential effects upon cell elongation and that radicle emergence in many seeds may be due to cell expansion rather than cell division. It has been shown in lucerne (Medicago sativa) that radicle emergence results

from cell elongation and that osmotic potential affected cortical cell length while mitotic activity was maintained (Ross and Hegarty, 1980).

It is clear that water supply has a major influence on germination. The restriction of water supply causes reductions in the rate and maximum percentage of germination. These reductions in germination percentage may be a form of induced dormancy (suspended growth) capable of being released in more favourable conditions or may reflect death of the seeds and seem to depend on both the nature of the stress and of the seed. It is apparent that not only does the environment in which the seeds are placed affect water uptake and germination, but also that the composition and type of seeds affect the way in which they respond and the way in which this response is manifested.

Priming effects; however, especially when salt solutions are used, may reflect more than simply the control of water uptake. Although intact seed coats are often regarded as impermeable, there appears to be no firm evidence on this and it is possible that ions may also be absorbed with effects which may be harmful or beneficial. It is quite clear that Roberts (1948) regarded her results as due to ions absorbed by the seeds and the effects attributable to KNO_3 imply that the salt was absorbed. There are likely to be substantial differences between seeds of different structure; for example, embryos of cereals may be readily accessible to ions in solution whereas those of tomato, located well within the seed coats with an internal cuticle may be not so exposed. These issues greatly complicate the interpretation of priming experiments.

1.5 PHYSIOLOGICAL MECHANISMS INVOLVED IN PRIMING

Only a small number of biochemical studies have been made of primed seed.

Koehler (unpublished, cited in Heydecker, 1974) in 1966 treated tomato seed with a salt solution for six days at 24 C. He found that there was a very high respiration rate during the treatment. This respiration rate was higher than that achieved during subsequent germination.

Hegarty (1977) investigated the effects of PEG solutions on the germination of carrot and calabrese seed. All PEG solutions reduced the rates of water and oxygen uptake below those of seed placed in water. In solutions which prevented germination water content was static after one day while oxygen uptake was constant for a time then decreased after two weeks in the solution. Hegarty postulated that this decrease in oxygen uptake reflected the build-up of respiratory products and a resultant feed-back mechanism rather than being the result of seed reserve exhaustion. Moreover, it must be remembered that the solubility in and diffusion of oxygen through PEG solutions are greatly reduced; considerable care is needed to avoid anoxia when using these solutions.

The rate of RNA synthesis during the germination of primed seeds was higher than that in untreated germinating seeds. More rapid polyribosome formation occured in primed seeds than during imbibition of unprimed seeds. However, no polyribosomes were found in primed seeds following drying (Khan, 1977). RNA levels began to increase after one day's treatment with a linear rise to

achieve a level on the sixth day 88% higher than that attained during germination of untreated seeds (Koehler, unpublished, cited in Heydecker, 1974). Coolbear and Grierson (1979) detailed the changes in nucleic acid content of tomato seed caused by priming. They reported a larger increase in RNA than did Koehler; however, their duration of priming was double that used by Koehler. The increase was in the form of ribosomal RNA, total DNA per seed remained constant, and the primed seeds maintained their advantage through further synthesis of nucleic acids during germination. The rate of synthesis of RNA in primed seed was higher than in untreated seeds. Germination (i.e. radicle emergence) began in primed lettuce seeds at the time when RNA synthesis peaked (Khan, Tao, Knypl, Borkowska and Powell, 1978).

Radicle emergence in carrot seeds only occured when the embryo had attained a specific length (Wiebe and Tiessen, 1979). Imposed treatments, including pre-sowing drought-hardening and priming in both salt and PEG solutions, resulted in an increase in embryo length. The treatment which gave the greatest germination enhancement (priming in salt solution) resulted in an embryo length nearly as long as the seed. They reported a good correlation between the embryo length of the treated seeds and the earliness of emergence. There is a degree of similarity between the response of carrot seed to pre-sowing drought-hardening and to priming treatments as evidenced by the comparability of the observations of Wiebe and Tiessen (1979) and those of Austin et al (1969).

Pre-sowing drought-hardening treatment of carrot seeds led to an increase in embryo length. Although the largest increase in length occured during the first two cycles of pre-sowing drought-

hardening, there were further small increases after six cycles. After three cycles the embryos became curved because they had achieved a length greater than the cavity which they occupied. The increase in embryo length was due mainly to cell division (Austin et al, 1969).

In contrast to the observations of Wiebe and Tiessen (1979) and of Austin et al, (1969) were those of Berrie and Drennan (1971). They reported that a pre-sowing drought-hardening treatment of both oat and tomato seeds was beneficial only when dehydration occurred before the onset of cell division. Not all seeds respond identically to similar treatments. Pre-sowing drought-hardening caused a more rapid water uptake during subsequent exposure to water, supposedly because of changes in the seed coverings (Berrie and Drennan, 1971).

Koehler (unpublished, cited in Heydecker, 1974) found increased soluble protein content of the order of 140% after six days of priming of tomato seeds, but did not determine whether this increase was from synthesis or from release from a membrane-bound state. Both the rate and quantity of protein synthesized increased in primed lettuce seeds; moreover, qualitative differences have been noticed in protein content (Khan, Tao, Knypl, Borkowska and Powell, 1978). The activities of acid phosphatase and of esterase were increased markedly in primed seeds (160% and 380% respectively of that in untreated seeds). Priming did not alter the total enzyme activities of peroxidase, aminoacyl t-RNA synthetase, malate dehydrogenase, nor of 3-phosphoglyceraldehyde dehydrogenase. Further, it was found that priming caused the appearance of a fourth isozyme of acid phosphatase and an increase in activity of some and a decrease in

others of the isozymes of the esterases (Khan, Peck and Samimy, 1978). Protease activity in hardened oat seeds was from 30 to 70% greater than in untreated seed. The activity increased with the duration of the pre-sowing drought-hardening treatment and was retained during dehydration. It was claimed that the seed proteins displayed hysteretic properties and therefore reacted differently to water at subsequent exposures (Berrie and Drennan, 1971).

Although priming caused profound increases in protein synthesis Knypl, Janas and Radziwonowska-Jozwiak (1980) reported that there was no qualitative modification of the electrophoretic protein pattern of soybean seed, and only inconsiderable quantitative modifications of the pattern. This is in marked contrast with the report by Khan et al (1978) of the profound qualitative and quantitative changes in lettuce seed proteins caused by priming.

In a series of experiments Khan and co-workers (Khan, 1981; and Khan et al, 1981) demonstrated that it was possible to enhance germination through priming despite the presence of an RNA synthesis inhibitor, both during the priming treatment and during subsequent germination. However, the presence during priming of the protein-synthesis inhibitor, cycloheximide, inhibited the promotive effects of priming on germination. This inhibitor also prevented the germination of both non-primed and primed seeds. This indicated that radicle emergence and the promotive effects of priming resulted from the synthesis of proteins controlled by the stored mRNA present in the seed. The protein synthesis controlled by newly-made mRNA was not essential for radicle emergence. However, the influence of those enzymes activated during priming cannot be discounted.

While the physiological mechanisms involved in the priming of seeds are still relatively unclear, it is apparent that protein synthesis is of major importance. Some evidence has been presented to indicate a possible role for cell division in some species. The low water potentials involved in priming suggest that the prevention of radicle emergence may result from a lack of turgor to provide the driving force for cell expansion. Because of species differences in response to osmotic and ionic stresses it may not be possible to delineate a single controlling mechanism.

It was the aim of the work reported here to determine the optimal conditions for priming the species investigated. It was hoped that a method of priming would be developed which could readily be scaled up for commercial use. This work has been primarily concerned with the method of priming, it being important to first establish empirical details to give the most desirable responses, before exploring further the more interesting physiological aspects. Only very preliminary experiments in relation to the value of this treatment to crop production are reported here.

CHAPTER 2

THE TIME COURSE OF GERMINATION AND ITS ANALYSIS

The germination of a population of seeds has a number of features. It can vary in the maximum percentage attained, the rate at which germination proceeds, the time to the beginning of germination of the population and the time-spread from the first to the last seed to germinate. Perhaps then the germination of a population of seeds is best presented graphically as a plot of the time course of cumulative percentage germination. Such a curve (Figure 2) shows the maximum percentage achieved (the asymptote), the rate of germination at any particular time (the slope), the time at which the first and last seeds germinated and hence the time-spread between them. However, differences between treatments can be most conveniently and succintly compared, if the observations can be fitted adequately by some standard function. The time course of germination is, like many other biological processes, sigmoidal in nature.

Such data can be described by a generalized logistic function, such as the Richards function:

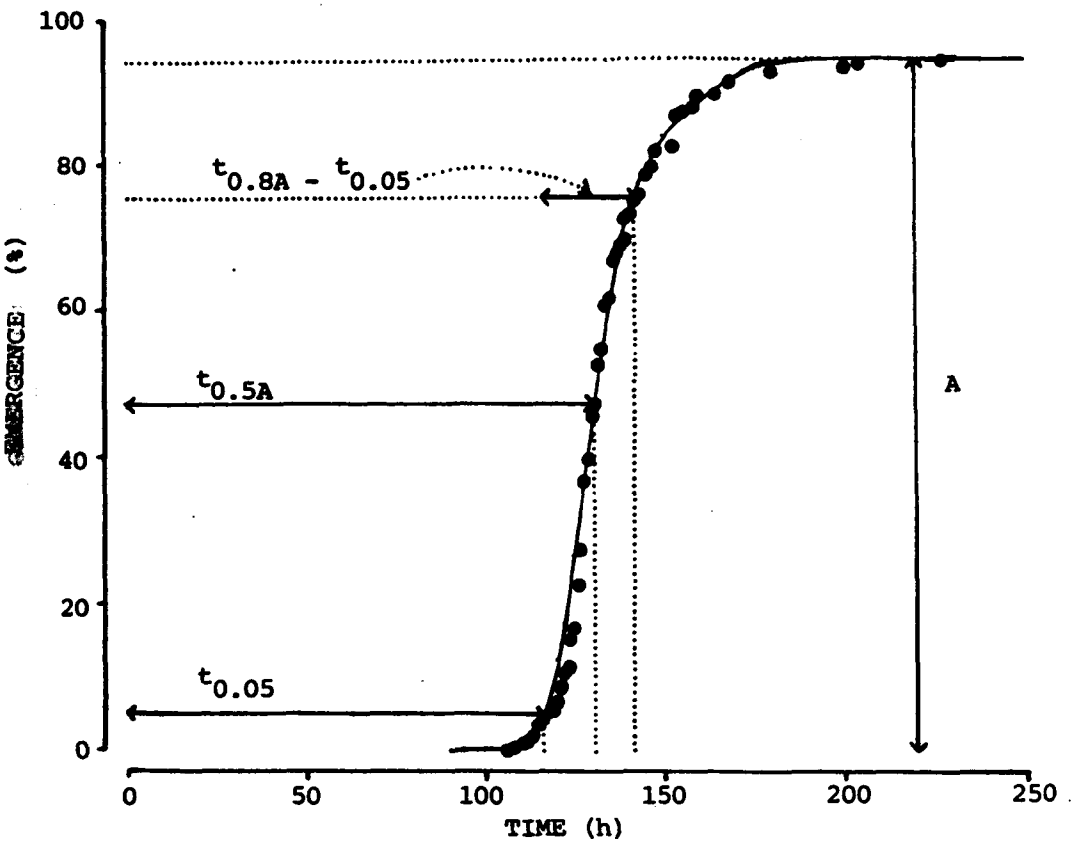
$$p = A[1 \pm \exp(B - kt)]^{-1/n} \qquad (2.1)$$

where p is the percentage germinated at time t, A is the maximum percentage germination (the asymptote), B is the ordinate intercept, k is the slope of the curve and n determines the shape of the curve. The negative alternative is used when n is negative. Although this function is extremely flexible, the

Figure 2. The emergence of unprimed tomato seeds from sand at 20 C. The curve was described by

$$p = A \exp (e^{-12.2 + 0.0957t}) \qquad (r^2 = 97.7)$$

Where p is the percentage germinated at time t and A is the maximum percentage germination. Also shown are the emergence descriptors: time to 5% emergence, $t_{0.05}$; time-spread of emergence, $t_{0.8A} - t_{0.05}$; and the time to the median seedling to emerge, $t_{0.5A}$, the reciprocal of which is the median rate of emergence.


100
80
60
40
20
0
EMERGENCE (%)
$t_{0.8A} - t_{0.05}$
$t_{0.5A}$
$t_{0.05}$
A
0
50
100
150
200
250
TIME (h)

solution for four parameters requires the use of iterative procedures in which selection of starting values close to the final best-fit values are essential and most difficult to estimate. Procedures and programs for this are available (Causton and Venus, 1981). As applied here, experimental values of A may be (and are) taken; even so, iterative procedures for estimating the three remaining parameters are still required.

Simpler functions are particular variants of this generalized function such as the:

monomolecular (n = 1)

$$p = A[1 - e^{-k(t - t_o)}] \qquad (2.2)$$

and the Gompertz ($n \rightarrow 0$) in the form

$$p = A \exp[-e^{(B - kt)}] \qquad (2.3)$$

Which of these most closely approximates to a good fit of any set of observations depends to a large extent on the frequency and timing of the observations; often the early positive curvilinear part of the curve may be missed if observations are widely spaced relative to the rate of germination.

Preliminary examination of time courses covering the sets of observations included in this thesis indicated that the Gompertz usually provided an adequate fit ($r^2 > 0.95$) and its use was adopted as routine to describe the time courses of germination and emergence for each replicate using the following procedure:

The raw observations were transformed to percentages of the number of seeds in the replicate. The percentages were fitted to a Gompertz function, by linear regression of the log logs of the

quotients of A over p against the times of observation by the method of least squares, giving estimates of B and k. The asymptote, A (maximum percentage germination), was taken as the cumulative percentage of seeds germinated during a time course when no further seeds had germinated for a period of three successive days. The paramaters of the Gompertz function were used to derive three descriptors of germination. These were (a) the median rate of germination $[1/(t_{0.5A})]$, (b) the time to 5% germination ($t_{0.05}$), and (c) the time-spread from 5% germination to 80% of the maximum germination ($t_{0.8A} - t_{0.05}$). These descriptors are shown in Figure 2. Evaluation of all experimental results was based upon the three derived descriptors and the observed maximum percentage germination. When maximum percentage germination did not exceed 15% this procedure was not followed and descriptors were not derived.

Statistical analyses of these descriptors were made after transformation to logarithms and of the maximum percentage after transformation to arcsines. These transformations were required to normalize variances. For ease of understanding the transformed data were retransformed back to percentages and hours after analysis for presentation in the body of the thesis. Because of the necessary transformations and retransformations it is not possible to show measures of variability with the retransformed data; therefore transformed data with standard errors are presented in Appendix 1. Values were taken as being significantly different at the $p = 0.05$ level.

As indicated above, time courses of germination are influenced by the time of observation. In order to accurately record the germination of a population of seeds it is necessary to make

observations at intervals frequent enough to ensure a good fit of the function used to describe the observations. Intervals which are too long result in too few observations to describe the first half of the germination of the population of seeds. Moreover, long intervals in conjunction with rapidly germinating seeds result in the first observation recording a relatively high percentage and therefore in a poor estimate of the time to the beginning of germination.

CHAPTER 3

OSMOTIC EFFECTS ON GERMINATION

In order to determine suitable solution(s) in which to prime particular types of seeds, their germination responses to osmotica and osmotic potential must be known. Evaluation of these responses and of germination subsequent to treatment in osmotic solutions will reveal the solution(s) which maximally enhances the germination of the primed seeds. A series of experiments were conducted to discover the germination responses to osmotica and osmotic potential of tomato, carrot,onion and sorghum seeds. These species were chosen because it was thought that improved uniformity of emergence and/or earlier emergence from cold soils would be beneficial to crop establishment. The osmotica used were the di- and tri-potassium phosphate salts and potassium nitrate as well as the inert osmoticum PEG. These osmotica were the main ones reported as favourable for priming solutions (Tables 1 and 2).

3.1 MATERIALS AND METHODS

Throughout the experiments reported in this thesis seeds of commonly used cultivars of tomato, carrot and onion, supplied by Yates Seeds Limited and two cultivars of sorghum, supplied by Dekalb Shand Seed Company Pty Limited were used. The cultivars used were:

tomato	UC 82B	lot no. 826/E (1981 and 1982)
carrot	Yates Baby 242	lot no. 207/E (1981 and 1982)
onion	Creamgold	lot no. ROL609A (1981) 609/LL(1982)
sorghum	E 57	
sorghum	E 55e	

A number of experiments on these species were made, using the one approach which is described below, to explore the effect of osmotica and osmotic potential on germination. Seeds were placed in a range of solutions at the following osmotic potentials: -0.25; -0.5; -0.75; -1.0; -1.25; -1.5; -1.75 MPa at 15 C. The solutions used were: K_2HPO_4; K_2HPO_4 + KNO_3; KNO_3; K_3PO_4; K_3PO_4 + KNO_3 and PEG. In addition a control using water only was included. The concentrations necessary to produce the desired osmotic potentials at 15 C were calculated as follows: K_2HPO_4, K_2HPO_4 + KNO_3, and KNO_3 solutions concentrations were calculated from concentrative properties tabulated in Weast (1968). K_3PO_4 solutions were calculated from measurements of osmotic potential at 15 C made by a Wescor P52 thermocouple psychrometer calibrated against known NaCl solutions. PEG solutions were calculated from the relationships of Michel and Kaufmann (1973) and checked by the Wescor psychrometer. The solutions used are tabulated below

in Table 3. PEG solutions were passed through cation- and anion-exchange resins (Amberlite IR-120 and IRA-400) to remove initial impurities and toxic hydrolytic products (Greenway, Hiller and Flowers, 1968) before use.

Seeds were selected for uniformity of size by eye. These were thoroughly mixed and counted into lots of 50 seeds for each replicate. Each lot of seeds was arranged in a 90mm-diameter petri dish containing one piece of Ekwip U70 paper. Sufficient water or solution was added to give an amount slightly in excess of that needed to saturate the paper. This level of water or solution was maintained by additions of water to replace evaporative losses. Where solutions were used, the seeds were transferred to new petri dishes on a regular basis to maintain concentrations approximately constant and to minimise the risks of anoxia and infection. A seed was considered germinated when its radicle was seen to protrude through the seed coat. Germination of the population of seeds was assumed to be complete when no further germination occurred over three consecutive days. There were four replicates and germination was recorded initially at six-hour intervals, then at twelve-hour intervals until complete.

Table 3. Concentrations of salts and PEG used for osmotic potential solutions.

Osmotic potential (MPa)	Concentration
-0.25	0.048M K_2HPO_4
-0.50	0.096M K_2HPO_4
-0.75	0.144M K_2HPO_4
-1.00	0.190M K_2HPO_4
-1.25	0.244M K_2HPO_4
-1.50	0.300M K_2HPO_4
-1.75	0.352M K_2HPO_4
-0.25	0.022M K_2HPO_4 + 0.029M KNO_3
-0.50	0.044M K_2HPO_4 + 0.058M KNO_3
-0.75	0.066M K_2HPO_4 + 0.087M KNO_3
-1.00	0.090M K_2HPO_4 + 0.118M KNO_3
-1.25	0.117M K_2HPO_4 + 0.154M KNO_3
-1.50	0.132M K_2HPO_4 + 0.173M KNO_3
-1.75	0.160M K_2HPO_4 + 0.210M KNO_3
-0.25	0.039M K_3PO_4
-0.50	0.079M K_3PO_4
-0.75	0.118M K_3PO_4
-1.00	0.161M K_3PO_4
-1.25	0.211M K_3PO_4
-1.50	0.237M K_3PO_4
-1.75	0.289M K_3PO_4
-0.25	0.017M K_3PO_4 + 0.034M KNO_3
-0.50	0.034M K_3PO_4 + 0.068M KNO_3
-0.75	0.051M K_3PO_4 + 0.102M KNO_3
-1.00	0.069M K_3PO_4 + 0.139M KNO_3
-1.25	0.091M K_3PO_4 + 0.181M KNO_3
-1.50	0.102M K_3PO_4 + 0.204M KNO_3
-1.75	0.125M K_3PO_4 + 0.249M KNO_3
-0.25	0.060M KNO_3
-0.50	0.121M KNO_3
-0.75	0.175M KNO_3
-1.00	0.250M KNO_3
-1.25	0.320M KNO_3
-1.50	0.392M KNO_3
-1.75	0.462M KNO_3
-0.25	122g kgH_2O^{-1} PEG
-0.50	184g kgH_2O^{-1} PEG
-0.75	233g kgH_2O^{-1} PEG
-1.00	274g kgH_2O^{-1} PEG
-1.25	310g kgH_2O^{-1} PEG
-1.50	343g kgH_2O^{-1} PEG
-1.75	373g kgH_2O^{-1} PEG

3.2 RESULTS

3.2.1 Responses of Tomato Seeds. (Experiment 1).

3.2.1.1 Germination in Osmotica

Tomato seeds showed the same general response to osmotic potential in all osmotica. Maximum percentage germination was not affected by high osmotic potential in all osmotica except K_3PO_4; however, as osmotic potential decreased maximum percentage germination decreased until a threshold was reached at which no germination occurred within twenty-four days (Table 4, Appendix Table A1.1). This threshold potential differed between osmotica. In K_3PO_4 it occurred at -0.5 MPa, in K_2HPO_4 at -0.75 MPa, in K_2HPO_4 + KNO_3 and in PEG at -1.0 MPa, while in KNO_3 and in K_3PO_4 + KNO_3 it occurred at -1.25 MPa.

The median rate of germination was slower than that of seeds germinated in water at all osmotic potentials in all osmotica. In all osmotica the median rate of germination decreased with lowering of osmotic potential (Table 5, Appendix Table A1.1). There were differences in rate between osmotica at the same osmotic potential. Seeds placed in -0.25 MPa solutions showed a wide range of median rates, the seeds with the slowest rate (K_3PO_4, 0.0023 h^{-1}) germinated at a median rate that was only 42% of the fastest rate (KNO_3, 0.0055 h^{-1}). At -0.5 MPa there was a smaller range in rates, with seeds in PEG the fastest (0.0031 h^{-1}), those in K_2HPO_4 the slowest (0.0017 h^{-1}, 53% of the rate for PEG seeds) while those in K_3PO_4 did not germinate. As osmotic potential was decreased there was a reduction in the range of median rates of germination as few seeds germinated.

Table 4. The effect of osmotica and osmotic potential on the maximum percentage germination of tomato seeds. Data for seeds germinated in water also given. The broken line indicates a boundary below which less than 50% of the seeds germinated.

Osmotic potential	Osmotica					
	K_2HPO_4	K_2HPO_4 + KNO_3	KNO_3	K_3PO_4 + KNO_3	K_3PO_4	PEG
(MPa)	Maximum percentage germination (%) (H_2O: 97.7)					
-0.25	96.3	97.6	98.9	98.2	87.4	97.0
-0.50	66.1	95.4	92.7	90.5	0.0	96.0
-0.75	0.0	76.9	86.8	74.8	0.0	67.1
-1.00	0.0	0.0	88.8	21.5	0.0	0.0
-1.25	0.0	0.0	0.0	0.0	0.0	0.0
-1.50	0.0	0.0	0.0	0.0	0.0	0.0
-1.75	0.0	0.0	0.0	0.0	0.0	0.0

Table 5. The effect of osmotica and osmotic potential on the median rate of germination of tomato seeds. Data for seeds germinated in water also given. The broken line indicates a boundary below which less than 50% of the seeds germinated.

Osmotic potential	Osmotica					
	K_2HPO_4	K_2HPO_4 + KNO_3	KNO_3	K_3PO_4 + KNO_3	K_3PO_4	PEG
(MPa)	Median rate of germination (h^{-1}) (H_2O: 0.0075)					
-0.25	0.0042	0.0047	0.0055	0.0035	0.0023	0.0040
-0.50	0.0017	0.0026	0.0027	0.0019	-	0.0031
-0.75	-	0.0018	0.0021	0.0012	-	0.0019
-1.00	-	-	0.0014	0.0012	-	-

Osmotic potential also affected the time to the beginning of germination of the population of seeds (Table 6, Appendix Table A1.1). Seeds placed in all -0.25 MPa solutions took longer to begin germination than those placed in water, with, again, seeds placed in K_2HPO_4 the least affected (174 hours) and those in K_3PO_4 the most affected (326 hours). At lower osmotic potentials the populations of seeds took increasingly longer to begin germination and the seeds responded differently to osmotica. The seeds which took the longest to begin germination (K_3PO_4 + KNO_3, 704 hours) took nearly twice as long as those which also germinated at the same osmotic potential (KNO_3) and almost seven times as long as those placed in water.

The time-spread of germination was increased by osmotic potential (Table 6, Appendix Table A1.1). This lengthening of the time-spread also increased with lowering of osmotic potential, the longest time-spread (521 hours) being for seeds placed in -0.75 MPa K_3PO_4 + KNO_3, was ten times longer than that of seeds placed in water. Again, there were differences in response to osmotica. The low value for seeds placed in -1.0 MPa K_3PO_4 + KNO_3 (381 hours) is an artefact of the low maximum percentage germination of these seeds (21%, Table 4). From Tables 5 and 6 it can be seen that this population of seeds began germination some time after those in -0.75 MPa K_3PO_4 + KNO_3 and that the median rate of germination was very slow.

Table 6. The effect of osmotica and osmotic potential on the time to 5% germination and on the time-spread of germination of tomato seeds. Data for seeds germinated in water also given. The broken line indicates a boundary below which less than 50% of the seeds germinated.

Osmotic potential	Osmotica					
	K_2HPO_4	K_2HPO_4 + KNO_3	KNO_3	K_3PO_4 + KNO_3	K_3PO_4	PEG
(MPa)	Time to 5% germination (h) (H_2O: 103)					
-0.25	174	152	137	208	326	195
-0.50	431	264	246	366	-	239
-0.75	-	374	330	569	-	371
-1.00	-	-	412	704	-	-
	Time-spread of germination (h) (H_2O: 53)					
-0.25	115	104	82	132	198	93
-0.50	306	201	227	276	-	142
-0.75	-	343	276	537	-	303
-1.00	-	-	521	381	-	-

3.2.1.2 Germination of Treated Seeds

After twenty-four days, seeds from those petri dishes in which no germination had occurred were transferred to petri dishes with water-moistened U70 papers and germination was recorded at six-hour intervals until complete.

Maximum percentage germination of those seeds transferred to water from the solutions in which no germination had occurred during treatment was not affected by the treatment. Seeds from all solutions showed similar values to untreated seeds placed in water (Table 7, Appendix Table A1.2). The median rates of germination of the treated seeds from all solutions were faster than the rate for untreated seeds in water (Table 8, Appendix Table A1.2). There were large difference between the median rates of germination of seeds treated in different osmotica at the same osmotic potential. Of the seeds treated in -1.0 MPa solutions the fastest rate was for seeds from K_2HPO_4 + KNO_3 treatment (0.1303 h^{-1}) which was 2.5 times faster than the seeds from the K_2HPO_4 treatment. Lowering of osmotic potential of the treatment solution caused a slowing of the median rate of subsequent germination of the seeds. With all osmotica the fastest rate was obtained from seeds treated in the highest osmotic potential which prevented germination during treatment. The influence of osmoticum appeared to be greater than osmotic potential for, although the fastest overall rate was for seeds treated in the -0.5 MPa K_3PO_4 solution, seeds from the -1.0 MPa K_2HPO_4 + KNO_3 solution germinated at a rate faster than seeds from any of the -0.75 MPa solutions.

Table 7. The effect of osmotica and osmotic potential of the pretreatment solution on the maximum percentage germination of tomato seeds on subsequent transfer to water. Data for untreated seeds germinated in water also given.

Osmotic potential	Osmotica					
	K_2HPO_4	K_2HPO_4 + KNO_3	KNO_3	K_3PO_4 + KNO_3	K_3PO_4	PEG
(MPa)	Maximum percentage germination (%) (H_2O: 97.7)					
-0.50	-	-	-	-	91.6	-
-0.75	95.7	-	-	-	93.5	-
-1.00	95.9	95.7	-	-	92.3	92.7
-1.25	96.8	96.9	95.7	91.5	93.8	96.6
-1.50	97.2	92.7	90.5	90.0	97.5	96.1
-1.75	95.5	91.9	90.4	90.9	95.5	97.7

Table 8. The effect of osmotica and osmotic potential of the pretreatment solution on the median rate of germination of tomato seeds on subsequent transfer to water. Data for untreated seeds germinated in water also given.

Osmotic potential	Osmotica					
	K_2HPO_4	K_2HPO_4 + KNO_3	KNO_3	K_3PO_4 + KNO_3	K_3PO_4	PEG
(MPa)	Median rate of germination (h^{-1}) (H_2O: 0.0075)					
-0.50	-	-	-	-	0.142	-
-0.75	0.107	-	-	-	0.0797	-
-1.00	0.0530	0.130	-	-	0.0554	0.0718
-1.25	0.0377	0.0862	0.0843	0.0664	0.0206	0.0437
-1.50	0.0266	0.0652	0.0582	0.0569	0.0190	0.0317
-1.75	0.0198	0.0484	0.0382	0.0454	0.0116	0.0258

The time to the beginning of germination for all treated seeds was much shorter than that for untreated seeds (Table 9, Appendix Table A1.2). There was considerable variation in response between treatments with times ranging from one hour (-0.5 MPa K_3PO_4) to 36.1 hours (-1.75 MPa K_3PO_4). Seventeen of the treatments resulted in times to germination of less than ten hours. The shortest time to the beginning of germination was for seeds from -0.5 MPa K_3PO_4 (one hour) which was significantly different from most other times (Appendix Table A1.2).

Despite increasing the rate of germination in all cases, pretreatment did not necessarily result in a reduced time-spread of germination. The time-spread ranged from 8.5 hours for seeds from -1.0 MPa K_2HPO_4 + KNO_3 to 89 hours for seeds from -1.75 MPa K_3PO_4. The shortest time-spread was for seed from -1.0 MPa K_2HPO_4 + KNO_3 and not for seeds from -0.5 MPa K_3PO_4. These times are significantly different (Appendix Table A1.2). The seeds from the -0.5 MPa K_3PO_4 treatment had both the shortest time to germination and the fastest median rate of germination, but did not have the shortest time-spread of germination. Seeds treated in solutions which contained KNO_3 had time-spreads which ranged from 8.5 to 22 hours, a much smaller range than that for any other osmoticum: 16.7 to 51.2 hours for PEG, 12.9 to 60.3 hours for K_2HPO_4 and 10.8 to 89.1 hours for K_3PO_4.

Table 9. The effect of osmotica and osmotic potential of the pretreatment solution on the time to 5% germination and on the time-spread of germination of tomato seeds on subsequent transfer to water. Data for untreated seeds germinated in water also given.

Osmotic potential	Osmotica					
	K_2HPO_4	K_2HPO_4 + KNO_3	KNO_3	K_3PO_4 + KNO_3	K_3PO_4	PEG
(MPa)	Time to 5% germination (h) (H_2O: 103)					
-0.50	-	-	-	-	0.857	-
-0.75	1.10	-	-	-	3.25	-
-1.00	5.95	2.79	-	-	5.42	3.88
-1.25	5.89	4.49	2.37	6.53	11.7	8.27
-1.50	8.47	7.91	7.94	9.13	21.5	10.1
-1.75	15.7	10.5	13.7	12.4	36.1	9.42
	Time-spread of germination (h) (H_2O: 53)					
-0.50	-	-	-	-	10.8	-
-0.75	12.9	-	-	-	16.3	-
-1.00	22.6	8.49	-	-	22.2	16.7
-1.25	35.6	12.0	15.0	15.2	59.6	25.8
-1.50	47.9	13.0	16.5	14.7	54.5	37.2
-1.75	60.3	17.9	22.0	17.2	89.1	51.2

Tomato seeds responded similarly in all solutions. Maximum percentage germination decreased with lowered osmotic potential until a potential was reached at which no germination occurred. Similarly, time to the beginning of germination lengthened, median rate of germination decreased and time-spread of germination increased. There were marked differences in response to the different osmotica. The highest osmotic potential which prevented germination differed between osmotica. In the K_3PO_4 solutions it was -0.5 MPa, in K_2HPO_4 -0.75 MPa, in K_2HPO_4 + KNO_3 and in PEG -1.0 MPa, and in KNO_3 and in K_3PO_4 + KNO_3 -1.25 MPa.

The germination responses in water of those seeds which did not germinate in the osmotic potential solutions were improved over that of untreated seeds. The germination responses of seeds treated at the lower osmotic potentials showed a lessening of improvement with lowering of osmotic potential of treatment solution. The treatment solution which resulted in the shortest time to the beginning of germination and also in the fastest median rate of germination did not result in the smallest time-spread of germination. Seeds from solutions which contained KNO_3 showed much shorter time-spreads of germination than those from solutions which did not. The treatment solution which produced seeds with the most improved germination response was -1.0 MPa K_2HPO_4 + KNO_3.

3.2.2 Responses of Carrot Seeds. (Experiment 2).

3.2.2.1 Germination in Osmotica

Maximum percentage germination of carrot seeds was little affected at high osmotic potentials (Table 10, Appendix Table A1.3). Lowering of osmotic potential resulted in decreased maximum percentage germination. At -1.25 MPa only the K_3PO_4 solution prevented all seeds from germinating, at -1.5 MPa K_2HPO_4, K_3PO_4 + KNO_3 and PEG also prevented germination, while at -1.75 MPa all osmotica prevented germination. There were differences in response to osmotica at potentials which permitted germination, with K_3PO_4 solutions consistently permitting the lowest percentage germination. At -1.0 MPa the maximum percentage germination ranged from 16 (K_3PO_4) to 68.7 (PEG).

Decreased osmotic potential caused a reduction in the median rate of germination from that of seeds in water (Table 11, Appendix Table A1.3). Seeds placed in PEG solutions showed a much smaller reduction in rate than those in salt solution at osmotic potentials below -0.25 MPa. Median rates of carrot seeds in PEG solutions at osmotic potentials from -0.50 to -1.00 MPa were about 0.0016 h^{-1} faster than those of seeds in the salt osmotica. These differences were significant (Appendix Table A1.3). There were small differences in median rates of seeds placed in the salt solutions at any one osmotic potential, the effect of lowering of osmotic potential being dominant over that of the type of salt osmotica.

Table 10. The effect of osmotica and osmotic potential on the maximum percentage germination of carrot seeds. Data for seeds germinated in water also given. The broken line indicates a boundary below which less than 50% of the seeds germinated.

Osmotic potential	Osmotica					
	K_2HPO_4	K_2HPO_4 + KNO_3	KNO_3	K_3PO_4 + KNO_3	K_3PO_4	PEG
(MPa)	Maximum percentage germination (%) (H_2O: 81.2)					
-0.25	81.5	84.1	81.6	80.8	72.1	82.6
-0.50	68.2	85.2	77.1	78.0	67.3	85.4
-0.75	67.1	78.1	72.3	72.9	42.5	78.6
-1.00	44.1	67.2	61.2	45.7	16.0	68.7
-1.25	12.0	40.2	44.0	9.0	0.0	35.3
-1.50	0.0	25.5	19.6	0.0	0.0	0.0
-1.75	0.0	0.0	0.0	0.0	0.0	0.0

Table 11. The effect of osmotica and osmotic potential on the median rate of germination of carrot seeds. Data for seeds germinated in water also given. The broken line indicates a boundary below which less than 50% of the seeds germinated.

Osmotic potential	Osmotica					
	K_2HPO_4	K_2HPO_4 + KNO_3	KNO_3	K_3PO_4 + KNO_3	K_3PO_4	PEG
(MPa)	Median rate of germination (h^{-1}) (H_2O: 0.0065)					
-0.25	0.0056	0.0059	0.0055	0.0055	0.0056	0.0063
-0.50	0.0040	0.0045	0.0044	0.0044	0.0041	0.0063
-0.75	0.0031	0.0034	0.0033	0.0031	0.0026	0.0048
-1.00	0.0024	0.0027	0.0026	0.0023	0.0020	0.0036
-1.25	0.0021	0.0022	0.0022	-	-	0.0025
-1.50	-	0.0020	0.0019	-	-	-

Lowering of osmotic potential caused a lengthening of the time to the beginning of germination (Table 12, Appendix Table A1.3). This effect was less marked in PEG solutions down to -1.0 MPa than in the corresponding salt solutions. There were differences in response to osmotica at the lower osmotic potentials, particularly at -0.75 MPa and at -1.0 MPa, where times ranged from 146 (PEG) to 305 (K_3PO_4) hours and 164 (PEG) to 489 (K_3PO_4) hours, respectively. K_3PO_4 solutions were the most effective in lengthening the time to the beginning of germination at all osmotic potentials down to -1.0 MPa. The time-spread of germination was increased by the lowering of osmotic potential (Table 12, Appendix Table A1.3). Again, this process was least marked in PEG solutions down to -0.75 MPa. Interpretation of Table 12 was complicated by the low maximum percentage germination of carrot seeds in -1.0 MPa K_3PO_4, -1.0 MPa K_3PO_4 + KNO_3, and in all solutions which permitted some germination at -1.25 MPa and at -1.5 MPa. The time-spread of germination of the other solutions ranged from 92 hours (-0.25 MPa PEG) which is similar to that of untreated seeds to 242 hours for seeds in -0.75 MPa K_2HPO_4.

Table 12. The effect of osmotica and osmotic potential on the time to 5% germination and on the time-spread of germination of carrot seeds. Data for seeds germinated in water also given. The broken line indicates a boundary below which less than 50% of the seeds germinated.

Osmotic potential	Osmotica					
	K_2HPO_4	K_2HPO_4 + KNO_3	KNO_3	K_3PO_4 + KNO_3	K_3PO_4	PEG
(MPa)	Time to 5% germination (h) (H_2O: 105)					
-0.25	116	117	113	125	127	107
-0.50	176	153	158	151	170	111
-0.75	188	196	207	213	305	146
-1.00	319	258	269	368	489	164
-1.25	454	369	368	-	-	307
-1.50	-	465	509	-	-	-
	Time-spread of germination (h) (H_2O: 86)					
-0.25	109	96	124	101	94	92
-0.50	140	125	127	135	138	88
-0.75	242	185	171	209	170	113
-1.00	186	216	202	116	34	208
-1.25	101	194	187	-	-	185
-1.50	-	115	81	-	-	-

3.2.2.2 Germination of Treated Seeds

After twenty-eight days, all ungerminated seeds from all petri dishes were transferred to petri dishes with water-moistened U70 papers. Germination was recorded at twelve-hour intervals until complete or until no seeds germinated for three days.

Treatment in osmotic potential solutions for twenty-eight days was detrimental to the maximum percentage of subsequent germination on transfer to water. Seeds from the two PEG solutions (-1.5 and -1.75 MPa) and one of the K_3PO_4 + KNO_3 solutions (-1.75 MPa) which prevented germination during treatment were reduced only by small, but significant, amounts below that of untreated carrot seeds (Table 13, Appendix Table A1.4). One of the remainder of the solutions which prevented germination during treatment (-1.75 MPa KNO_3) produced seeds which attained only 37.5% germination. Some of the ungerminated seeds from the solutions which permitted germination during treatment showed only low (16 to 40%) maximum percentage germination on subsequent transfer to water. Twenty percent of carrot seeds were not viable before treatment, thus a large proportion of those seeds which were ungerminated in solutions which permitted germination were incapable of germination on transfer. The results obtained from the transfer of treated seeds showed that the reductions in maximum percentage germination which occurred during treatment were largely due to inhibition of germination of some or all of the seeds rather than to toxic effects during treatment.

The median rate of germination for all treated seeds was higher than for that of untreated seeds (Table 14, Appendix Table A1.4).

Table 13. The effect of osmotica and osmotic potential of the pretreatment solution on the maximum percentage germination of carrot seeds on subsequent transfer to water. Data for untreated seeds germinated in water also given. Entries below the broken line are those solutions in which no seeds germinated during the priming treatment.

Osmotic potential	Osmotica					
	K_2HPO_4	K_2HPO_4 + KNO_3	KNO_3	K_3PO_4 + KNO_3	K_3PO_4	PEG
(MPa)	Maximum percentage germination (%) (H_2O: 81.2)					
-0.75	-	-	-	-	16.0	-
-1.00	42.6	-	-	21.5	38.0	-
-1.25	40.9	60.4	43.4	44.1	59.2	57.9
-1.50	58.2	58.5	41.0	62.2	62.7	75.1
-1.75	55.1	56.7	37.6	69.1	61.7	71.7

Table 14. The effect of osmotica and osmotic potential of the pretreatment solution on the median rate of germination of carrot seeds on subsequent transfer to water. Data for untreated seeds germinated in water also given. Entries below the broken line are those solutions in which no seeds germinated during the priming treatment.

Osmotic potential	Osmotica					
	K_2HPO_4	K_2HPO_4 + KNO_3	KNO_3	K_3PO_4 + KNO_3	K_3PO_4	PEG
(MPa)	Median rate of germination (h^{-1}) (H_2O: 0.0065)					
-0.75	-	-	-	-	0.111	-
-1.00	0.0752	-	-	0.131	0.0862	-
-1.25	0.0652	0.0584	0.111	0.0991	0.0152	0.0272
-1.50	0.0196	0.0860	0.0849	0.0638	0.0127	0.0316
-1.75	0.0123	0.0642	0.0633	0.0160	0.0120	0.0299

Of the seeds from the solutions which prevented germination during treatment only those from -1.5 MPa K_3PO_4 + KNO_3, -1.75 MPa K_2HPO_4 + KNO_3 and from -1.75 MPa KNO_3 treatments had rates which were comparable to some of those of the seeds transferred from solutions which permitted germination. Seeds from all three PEG treatments had very similar median rates. These were less than half that of seeds from the three salt treatments producing "fast" seeds.

Time to the beginning of germination was greatly reduced by treatment in all solutions (Table 15, Appendix Table A1.4). Eighteen treatments resulted in times that were less than ten hours. Of the solutions which prevented germination during treatment the PEG solutions resulted in the shortest times. Treatment in -1.75 MPa and -1.5 MPa K_3PO_4 resulted in times to the beginning of germination that were only slightly less than half that of untreated seeds. There was prolonging of the time to the beginning of germination when osmotic potential was decreased. This was particularly marked in K_3PO_4 solutions and in K_2HPO_4 solutions.

The time-spread of germination for the treated seeds was reduced by most treatments to below that of untreated seeds (Table 15, Appendix Table A1.4). In many of the solutions which prevented germination during treatment this reduction was small, with seeds from -1.75 MPa K_3PO_4 + KNO_3, -1.75 MPa K_2HPO_4, -1.5 MPa K_2HPO_4, and -1.25 MPa K_3PO_4 solutions the time-spreads of germination were between 80 and 100 hours, i.e. similar to or longer than that of untreated seeds. Of the solutions which prevented germination during treatment the one which produced seeds with the shortest time-spread was -1.5 MPa K_3PO_4 + KNO_3. Similar

time-spreads were obtained for seeds treated in -1.75 MPa K_2HPO_4 + KNO_3 and in -1.75 MPa KNO_3. These last two lots of seeds had lower maximum percentage germination than those from the -1.5 MPa K_3PO_4 + KNO_3 treatment. The short time-spreads for most of the seeds from the treatment solutions which permitted germination probably resulted from the low maximum percentage germination of these seeds.

Table 15. The effect of osmotica and osmotic potential of the pretreatment solution on the time to 5% germination and on the time-spread of germination of carrot seeds on subsequent transfer to water. Data for untreated seeds germinated in water also given. Entries below the broken line are those solutions in which no seeds germinated during the priming treatment.

Osmotic potential	Osmotica					
	K_2HPO_4	K_2HPO_4 + KNO_3	KNO_3	K_3PO_4 + KNO_3	K_3PO_4	PEG
(MPa)	Time to 5% germination (h) (H_2O: 105)					
-0.75	-	-	-	-	3.39	-
-1.00	3.29	-	-	1.82	2.18	-
-1.25	4.12	0.579	1.61	2.02	21.0	6.49
-1.50	8.32	1.53	1.76	5.15	41.0	1.78
-1.75	29.0	2.03	3.46	9.38	46.3	1.37
	Time-spread of germination (h) (H_2O: 86.5)					
-0.75	-	-	-	-	19.3	-
-1.00	19.8	-	-	12.3	18.5	-
-1.25	22.1	30.2	14.7	15.2	83.3	55.6
-1.50	79.8	18.3	20.0	19.7	70.0	54.3
-1.75	90.9	25.0	23.3	96.1	70.2	58.8

Carrot seeds germinated similarly in all solutions. An osmotic potential of -1.75 MPa solutions prevented germination in all solutions. Germination occurred in only KNO_3 and K_2HPO_4 + KNO_3 at -1.5 MPa, whereas at -1.25 MPa only K_3PO_4 prevented germination. In the other solutions there were similar trends in the responses of the carrot seeds. Lowering of osmotic potential caused some decreases in the maximum percentage germination, a decrease in the median rate of germination, lengthening of the time to the beginning of germination, and an increase in the time-spread of germination. There were differences in response to osmotica at the same potential with seeds in PEG solutions showing moderate responses.

Transfer to water after twenty-eight days treatment resulted in an improvement in germination for those seeds from solutions which prevented germination during treatment. Whilst time to the beginning of germination and median rate of germination were greatly improved following treatment, the time-spread of germination of treated seeds was similar to that of untreated seeds in most cases. The solution which produced seeds with the most improved germination was -1.5 MPa K_3PO_4 + KNO_3.

3.2.3 Responses of Onion Seeds. (Experiment 3).

3.2.3.1 Germination in Osmotica

Maximum percentage germination was unaffected by osmotic potential in most osmotica down to -1.0 MPa. Below this potential there were marked differences in response to osmotica (Table 16, Appendix Table A1.5). At -1.25 MPa germination in K_3PO_4 was very low (22.5%) and was also significantly affected in K_2HPO_4 (79%) and in K_3PO_4 + KNO_3 (85%). Further lowering of osmotic potential caused reductions in the maximum percentage attained. At -1.5 MPa germination was inhibited in K_3PO_4 and also at -1.75 MPa in K_2HPO_4. It was reduced in all other osmotica at -1.75 MPa.

The median rate of germination of onion seeds in all osmotica at all osmotic potentials was slower than that of seeds in water. In all osmotica the median rate of germination decreased with lowering of osmotic potential (Table 17, Appendix Table A1.5). There were differences in rate between seeds in different osmotica at the same osmotic potential, with seeds in the K_3PO_4 solutions having median rates that were significantly slower than those of seeds in the other osmotica (Appendix Table A1.5). At -1.0 MPa the median rates of germination ranged from 0.0023 h^{-1} for seeds in K_3PO_4 to 0.0044 h^{-1} for seeds in K_2HPO_4 + KNO_3.

Table 16. The effect of osmotica and osmotic potential on the maximum percentage germination of onion seeds. Data for seeds germinated in water also given. The broken line indicates a boundary below which less than 50% of the seeds germinated.

Osmotic potential	Osmotica					
	K_2HPO_4	K_2HPO_4 + KNO_3	KNO_3	K_3PO_4 + KNO_3	K_3PO_4	PEG
(MPa)	Maximum percentage germination (%) (H_2O: 98.0)					
-0.25	97.6	98.8	99.3	96.1	95.3	94.4
-0.50	94.8	97.8	96.3	97.3	93.0	96.1
-0.75	94.6	97.5	95.5	94.5	91.1	95.3
-1.00	92.7	92.1	97.1	95.4	76.0	93.8
-1.25	79.2	93.3	95.7	85.1	22.5	93.7
-1.50	44.7	91.2	87.0	80.3	0.0	11.5
-1.75	0.0	74.7	76.6	51.7	0.0	20.1

Table 17. The effect of osmotica and osmotic potential on the median rate of germination of onion seeds. Data for seeds germinated in water also given. The broken line indicates a boundary below which less than 50% of the seeds germinated.

Osmotic potential	Osmotica					
	K_2HPO_4	K_2HPO_4 + KNO_3	KNO_3	K_3PO_4 + KNO_3	K_3PO_4	PEG
(MPa)	Median rate of germination (h^{-1}) (H_2O: 0.0091)					
-0.25	0.0081	0.0080	0.0080	0.0082	0.0076	0.0079
-0.50	0.0066	0.0070	0.0066	0.0066	0.0059	0.0067
-0.75	0.0050	0.0055	0.0055	0.0054	0.0042	0.0052
-1.00	0.0038	0.0044	0.0042	0.0039	0.0023	0.0039
-1.25	0.0024	0.0031	0.0032	0.0023	0.0018	0.0026
-1.50	0.0018	0.0025	0.0024	0.0019	-	-
-1.75	-	0.0016	0.0019	0.0012	-	0.0025

Time to the beginning of germination was increased in all osmotica at all osmotic potentials (Table 18, Appendix Table A1.5). At -0.25 MPa this effect was small with times to the beginning of germination in all osmotica being of the order of 7 to 15 hours longer than that of onion seeds in water (77.7 hours). At the lower osmotic potentials there were significantly larger differences between osmotica (Appendix Table A1.5). At a potential of -1.25 MPa times to the beginning of germination ranged from 197 hours (K_2HPO_4 + KNO_3) to 450 hours (K_3PO_4). Seeds in the K_3PO_4 solutions had longer times to the beginning of germination than those of seeds in other osmotica at potentials from -0.5 to -1.25 MPa.

Similarly, lowering of osmotic potential lengthened the time-spread of germination in all osmotica (Table 18, Appendix Table A1.5). At -0.25 MPa there was no significant effect of osmotic potential on the time-spread of seeds in two osmotica; PEG and K_3PO_4 + KNO_3 (Appendix Table A1.5). There were large differences in effect of osmotica at the lower osmotic potentials. At -1.25 MPa time-spreads ranged from 207 hours (KNO_3) to 395 hours (K_3PO_4 + KNO_3). The reduced values for the time-spread of germination of seeds in some of the osmotica at the lower potentials (222 hours for seeds in -1.25 MPa K_3PO_4 and 377 hours for seeds in -1.75 MPa K_3PO_4 +KNO_3) resulted from the low maximum percentage germination of seeds in these solutions.

Table 18. The effect of osmotica and osmotic potential on the time to 5% germination and on the time-spread of germination of onion seeds. Data for seeds germinated in water also given. The broken line indicates a boundary below which less than 50% of the seeds germinated.

Osmotic potential	Osmotica					
	K_2HPO_4	K_2HPO_4 + KNO_3	KNO_3	K_3PO_4 + KNO_3	K_3PO_4	PEG
(MPa)	Time to 5% germination (h) (H_2O: 78)					
-0.25	86	86	85	89	89	94
-0.50	104	91	96	102	112	108
-0.75	133	108	112	126	160	127
-1.00	175	141	143	160	213	141
-1.25	232	197	199	221	450	214
-1.50	344	230	243	330	-	-
-1.75	-	364	318	612	-	373
	Time-spread of germination (h) (H_2O: 55)					
-0.25	66	67	69	58	74	57
-0.50	83	83	97	85	101	73
-0.75	121	133	126	107	145	112
-1.00	164	151	169	173	408	200
-1.25	347	223	207	395	222	300
-1.50	398	307	305	366	-	-
-1.75	-	476	375	377	-	109

3.2.3.2 Germination of Treated Seeds

After twenty-four days, seeds from those petri dishes in which no germination had occurred were transferred onto water-moistened WTB papers in petri dishes and germination was recorded at twelve-hour intervals until complete or until no further seeds germinated for a period of three days.

Treatment in salt solutions which prevented germination during treatment did not greatly affect the maximum percentage of subsequent germination, similarly seeds from the two PEG treatments had high maximum percentages (Table 19, Appendix Table A1.6). Seeds treated in the PEG solutions had median rates of germination which were twice as fast as those of seeds treated in the salt solutions (Table 20, Appendix Table A1.6). All treated seeds began germination within twelve hours of transfer to water and had attained 80% of their maximum percentages within a further 26 hours (Table 21, Appendix Table A1.6). The PEG treated seeds had significantly faster median rates of germination, shorter times to the beginning of germination and shorter time-spreads than did the salt treated seeds (Appendix Table A1.6). Of the seeds treated in salt solutions those from the -1.75 MPa K_2HPO_4 treatment showed the most improvement in germination.

Table 19. The effect of osmotica and osmotic potential of the pretreatment solution on the maximum percentage germination of onion seeds on subsequent transfer to water. Data for untreated seeds germinated in water also given. Entries below the broken line are those solutions in which no seeds germinated during the priming treatment.

Osmotic potential	Osmotica					
	K_2HPO_4	K_2HPO_4 + KNO_3	KNO_3	K_3PO_4 + KNO_3	K_3PO_4	PEG
(MPa)	Maximum percentage germination (%) (H_2O: 98.2)					
-1.50		-	-	-	90.1	95.9
-1.75	91.7	-	-	-	88.3	95.9

Table 20. The effect of osmotica and osmotic potential of the pretreatment solution on the median rate of germination of onion seeds on subsequent transfer to water. Data for untreated seeds germinated in water also given. Entries below the broken line are those solutions in which no seeds germinated during the priming treatment.

Osmotic potential	Osmotica					
	K_2HPO_4	K_2HPO_4 + KNO_3	KNO_3	K_3PO_4 + KNO_3	K_3PO_4	PEG
(MPa)	Median rate of germination (h^{-1}) (H_2O: 0.0091)					
-1.50		-	-	-	0.0668	0.130
-1.75	0.0765	-	-	-	0.0383	0.151

Table 21. The effect of osmotica and osmotic potential of the pretreatment solution on the time to 5% germination and on the time-spread of germination of onion seeds on subsequent transfer to water. Data for untreated seeds germinated in water also given. Entries below the broken line are those solutions in which no seeds germinated during the priming treatment.

Osmotic potential	Osmotica					
	K_2HPO_4	K_2HPO_4 + KNO_3	KNO_3	K_3PO_4 + KNO_3	K_3PO_4	PEG
(MPa)	Time to 5% germination (h) (H_2O: 78)					
-1.50	5.30	-	-	-	6.00	2.36
-1.75		-	-	-	11.9	2.22
	Time-spread of germination (h) (H_2O: 55)					
-1.50	13.9	-	-	-	16.0	9.06
-1.75		-	-	-	25.5	7.54

A threshold osmotic potential at which germination of onion seeds was prevented was reached in only two osmotica: in K_3PO_4 the osmotic potential which prevented germination was -1.5 MPa, in K_2HPO_4 it was -1.75 MPa. Maximum percentage germination of the seeds in the other osmotica was little affected at potentials down to -1.0 MPa and to -1.5 MPa in $K_2HPO_4 + KNO_3$. However, the median rate of germination was decreased, the time to the beginning of germination was increased and the time-spread of germination was lengthened with lowering of osmotic potential in all osmotica.

Treated onion seeds showed improved germination over that of untreated seeds. Treatment greatly increased the median rate of germination, reduced the time to the beginning of germination and decreased the time-spread of germination. Seeds treated in PEG solutions showed no reduction in maximum percentage germination, whereas those treated in salt solutions were somewhat affected.

3.2.4 Responses of Sorghum E 57 Seeds. (Experiment 4).

3.2.4.1 Germination in Osmotica

Maximum percentage germination was not affected at the highest osmotic potential(-0.25 MPa). Lowering of osmotic potential caused reductions in the maximum percentage attained (Table 22, Appendix Table A1.7). There were significant differences in response to the osmotica at osmotic potentials below -0.5 MPa. At -1.0 MPa the maximum percentages ranged from 31.4 (K_3PO_4) to 70.6% (KNO_3). Seeds in KNO_3 solutions at all osmotic potentials were generally least affected, with those in K_3PO_4 or K_3PO_4 + KNO_3 solutions being the most affected. Seeds in PEG solutions with osmotic potentials below -0.75 MPa were equally affected as those in K_3PO_4 + KNO_3 solutions. Reductions in maximum percentage germination continued with further lowering of osmotic potential in all osmotica. In two of the salt osmotica a threshold potential was reached at which no germination occurred. In K_3PO_4 the threshold potential was -1.25 MPa, in K_2HPO_4 it was -1.75 MPa. In all other osmotica maximum percentage germination was very low at -1.75 MPa ranging from 2.5 to 12.5%.

The median rate of germination of sorghum E 57 seeds in all osmotica at all osmotic potentials was reduced below that of seeds in water. The median rates decreased with lowering of osmotic potential (Table 23, Appendix Table A1.7). Seeds in PEG solutions were most affected, with median rates markedly lower than those of seeds in salt solutions. At an osmotic potential of -1.0 MPa seeds in PEG germinated at a median rate of 0.0067 h^{-1} while those in salt osmotica germinated at median rates around 0.0120 h^{-1}. There were small differences between

Table 22. The effect of osmotica and osmotic potential on the maximum percentage germination of sorghum E 57 seeds. Data for seeds germinated in water also given. The broken line indicates a boundary below which less than 50% of the seeds germinated.

Osmotic potential	Osmotica					
	K_2HPO_4	K_2HPO_4 + KNO_3	KNO_3	K_3PO_4 + KNO_3	K_3PO_4	PEG
(MPa)	Maximum percentage germination (%) (H_2O: 91.2)					
-0.25	89.8	93.0	93.7	93.7	87.0	87.5
-0.50	87.1	79.3	88.1	77.6	78.5	89.9
-0.75	71.0	70.7	77.3	56.6	59.1	75.7
-1.00	67.1	59.6	70.6	48.2	31.4	39.7
-1.25	38.1	51.8	58.6	18.6	0.0	13.0
-1.50	19.0	30.5	29.0	8.00	0.0	3.00
-1.75	0.0	3.00	12.5	2.50	0.0	2.50

Table 23. The effect of osmotica and osmotic potential on the median rate of germination of sorghum E 57 seeds. Data for seeds germinated in water also given. The broken line indicates a boundary below which less than 50% of the seeds germinated.

Osmotic potential	Osmotica					
	K_2HPO_4	K_2HPO_4 + KNO_3	KNO_3	K_3PO_4 + KNO_3	K_3PO_4	PEG
(MPa)	Median rate of germination (h^{-1}) (H_2O: 0.0198)					
-0.25	0.0174	0.0169	0.0181	0.0188	0.0176	0.0103
-0.50	0.0160	0.0162	0.0161	0.0160	0.0171	0.0092
-0.75	0.0134	0.0141	0.0142	0.0140	0.0143	0.0083
-1.00	0.0117	0.0123	0.0120	0.0121	0.0116	0.0067
-1.25	0.0090	0.0098	0.0098	0.0103	-	-
-1.50	0.0078	0.0086	0.0076	-	-	-

the median rates of seeds in salt osmotica.

Time to the beginning of germination was increased by lowering of osmotic potential (Table 24, Appendix Table A1.7). At the higher osmotic potentials in the salt osmotica the increases were small. Approximately 5 hours were added to the time to the beginning of germination of seeds in water for each -0.25 MPa step down in osmotic potential to -0.75 MPa. At lower osmotic potentials the increases were larger, up to 20 hours, and there were also greater differences between osmotica. The seeds in PEG soltions were more affected, achieving times at -0.75 MPa (87 hours) which were not reached in the salt osmotica until -1.25 MPa. Similarly, lowering of osmotic potential lengthened the time-spread of germination (Table 24, Appendix Table A1.7). At -0.25 MPa and -0.5 MPa in the salt osmotica the time-spreads of germination were not much longer than that of seeds in water, 26 hours. At lower osmotic potentials the time-spreads increased to 90 hours (seeds in -1.0 MPa PEG and in -1.5 MPa KNO_3). Seeds in PEG solutions were most affected at all potentials at which they germinated.

Table 24. The effect of osmotica and osmotic potential on the time to 5% germination and on the time-spread of germination of sorghum E 57 seeds. Data for seeds germinated in water also given. The broken line indicates a boundary below which less than 50% of the seeds germinated.

Osmotic potential	Osmotica					
	K_2HPO_4	K_2HPO_4 + KNO_3	KNO_3	K_3PO_4 + KNO_3	K_3PO_4	PEG
(MPa)	Time to 5% germination (h) (H_2O: 35.6)					
-0.25	41.5	40.9	40.2	39.5	41.4	71.3
-0.50	44.0	46.0	45.8	43.6	43.8	73.9
-0.75	51.6	53.4	47.2	50.5	51.0	86.8
-1.00	67.2	59.8	60.4	59.6	67.4	101
-1.25	82.6	73.9	73.4	82.7	-	-
-1.50	106	90.5	91.4	-	-	-
	Time-spread of germination (h) (H_2O: 26.1)					
-0.25	28.4	32.5	26.9	24.0	27.2	44.6
-0.50	32.7	28.7	28.9	34.5	26.7	60.8
-0.75	40.5	32.5	40.2	39.5	36.0	62.6
-1.00	34.3	40.9	42.1	43.9	41.0	94.6
-1.25	55.8	51.1	53.1	39.8	-	-
-1.50	60.9	55.9	88.4	-	-	-

3.2.4.2 Germination of Treated Seeds

After fourteen days, ungerminated seeds from all petri dishes were transferred to petri dishes with water-moistened U70 papers. Germination was recorded at twelve-hour intervals until complete or until no further seeds had germinated for a period of three days.

Sorghum E 57 seeds treated in salt osmotica had much reduced maximum percentage germination on transfer to water (Table 25, Appendix Table A1.8). Seeds treated in K_3PO_4 solutions did not germinate on transfer to water. Seeds treated in K_3PO_4 + KNO_3 had very low percentages, less than 5%. Only one salt osmoticum treatment produced seeds capable of more than 50% germination it was -1.75 MPa K_2HPO_4. Seeds treated in PEG solutions had maximum percentages on transfer which were similar to that of untreated seeds, 91%. The median rates of germination of PEG treated seeds were about twice that of untreated seeds (Table 26, Appendix Table A1.8). There was an improvement in median rate with lowering of osmotic potential of the treatment solution.

The PEG treated sorghum E 57 seeds had times to the beginning of germination which were about 12 hours, i.e. one-third of that of untreated seeds (Table 27, Appendix Table A1.8). There were reductions in time to the beginning of germination with lowering of osmotic potential of the treatment solution. However, the time-spread of germination was not affected by treatment in PEG solutions (Table 27, Appendix Table A1.8). Although salt solution treated seeds had shorter time-spreads, these reflect the greatly reduced maximum percentages of germination attained by these seeds.

Table 25. The effect of osmotica and osmotic potential of the pretreatment solution on the maximum percentage germination of sorghum E 57 seeds on subsequent transfer to water. Data for untreated seeds germinated in water also given. Entries below the broken line are those solutions in which no seeds germinated during the priming treatment.

Osmotic potential	Osmotica					
	K_2HPO_4	K_2HPO_4 + KNO_3	KNO_3	K_3PO_4 + KNO_3	K_3PO_4	PEG
(MPa)	Maximum percentage germination (%) (H_2O: 91.2)					
-1.00	0.0	0.0	0.0	0.0	0.0	82.7
-1.25	12.0	12.3	0.0	0.0	0.0	93.0
-1.50	44.2	15.0	10.9	4.0	0.0	86.0
-1.75	54.1	29.4	15.5	2.5	0.0	92.1

Table 26. The effect of osmotica and osmotic potential of the pretreatment solution on the median rate of germination of sorghum E 57 seeds on subsequent transfer to water. Data for untreated seeds germinated in water also given. Entries below the broken line are those solutions in which no seeds germinated during the priming treatment.

Osmotic potential	Osmotica					
	K_2HPO_4	K_2HPO_4 + KNO_3	KNO_3	K_3PO_4 + KNO_3	K_3PO_4	PEG
(MPa)	Median rate of germination (h^{-1}) (H_2O: 0.0198)					
-1.00	-	-	-	-	-	0.0343
-1.25	-	-	-	-	-	0.0344
-1.50	0.0406	-	-	-	-	0.0362
-1.75	0.0330	0.0322	-	-	-	0.0399

Table 27. The effect of osmotica and osmotic potential of the pretreatment solution on the time to 5% germination and on the time-spread of germination of sorghum E 57 seeds on subsequent transfer to water. Data for untreated seeds germinated in water also given. Entries below the broken line are those solutions in which no seeds germinated during the priming treatment.

Osmotic potential	Osmotica					
	K_2HPO_4	K_2HPO_4 + KNO_3	KNO_3	K_3PO_4 + KNO_3	K_3PO_4	PEG
(MPa)	Time to 5% germination (h) (H_2O: 35.6)					
-1.00	-	-	-	-	-	14.9
-1.25	-	-	-	-	-	12.8
-1.50	16.2	-	-	-	-	12.1
-1.75	20.7	23.8	-	-	-	11.3
	Time-spread of germination (h) (H_2O: 26.1)					
-1.00	-	-	-	-	-	25.0
-1.25	-	-	-	-	-	28.8
-1.50	14.6	-	-	-	-	23.2
-1.75	18.4	15.9	-	-	-	24.6

Sorghum E 57 seeds germinated throughout the range of osmotic potential investigated. The maximum percentage germination was reduced, the median rate of germination decreased, time to the beginning of germination lengthened, and the time-spread of germination increased with lowering of osmotic potential. The germination responses to the osmotica differed. Germination was prevented at -1.25 MPa in K_3PO_4 and at -1.75 MPa in K_2HPO_4, whereas in the other solutions germination, although reduced, occured at all osmotic potentials. Further, the salt solutions, particularly K_3PO_4 and K_3PO_4 + KNO_3, proved toxic to the seeds at the lower osmotic potentials, whereas PEG did not, as evidenced by the germination response upon transfer to water. Sorghum E 57 seeds treated in -1.5 and in -1.75 MPa PEG solutions showed improved median rates of germination, reduced time to the beginning of germination, and decreased time-spread of germination in water, to that of untreated seeds. The only salt solution to show similar effects was -1.75 MPa K_2HPO_4, however, the maximum percentage germination of seeds from this solution was reduced to 54.1%, whereas the PEG treated seeds showed similar percentages to that of the untreated seeds (90%).

3.2.5 Responses of Sorghum E 55e Seeds. (Experiment 5).

The results from Experiment 4 revealed that sorghum E 57 seeds germinated at a wide range of osmotic potentials. The salt solutions proved toxic to many of the sorghum E 57 seeds. It was decided to conduct a smaller scale investigation of a different type of sorghum seed, one which, unlike the E 57 hybrid, was unable to osmoregulate and was reportedly less drought tolerant (Snowball, pers. comm.). Sorghum E 55e was chosen.

Sorghum E 55e seeds were placed in three osmotica at a range of osmotic potentials. The osmotica used were: K_2HPO_4 + KNO_3, KNO_3, and K_3PO_4 + KNO_3 at the following osmotic potentials: -0.5, -1.0, and -1.5 MPa. In addition there was a control using water only. There were four replicates of fifty seeds each. Seeds were arranged in a 90 mm-diameter petri dish containing one piece of Ekwip U70 paper and a slight excess of solution. Germination was recorded at six-hour than twelve-hour intervals until complete or until no further seeds had germinated for a period of three days.

Maximum percentage germination was reduced with lowering of osmotic potential (Table 28, Appendix Table A1.9). There were significant differences between osmotica at -0.5 MPa. At -1.0 MPa and at -1.5 MPa seeds in K_3PO_4 + KNO_3 solutions attained lower maximum percentages than those in the other osmotica. The seeds from K_2HPO_4 + KNO_3 and KNO_3 solutions were not significantly different at any osmotic potential. Median rate of germination was reduced in all osmotica by lowered osmotic potential (Table 29, Appendix Table A1.9). Seeds in K_3PO_4 + KNO_3 solutions were most affected at all osmotic potentials, while those in KNO_3 solutions were least affected at -0.5 and -1.0 MPa, but those in K_2HPO_4 + KNO_3 were least affected at -1.5 MPa.

Table 28. The effect of osmotica and osmotic potential on the maximum percentage germination of sorghum E 55e seeds. Data for seeds germinated in water also given. The broken line indicates a boundary below which less than 50% of the seeds germinated.

Osmotic potential	Osmotica		
	K_2HPO_4 + KNO_3	KNO_3	K_3PO_4 + KNO_3
(MPa)	Maximum percentage germination (%) (H_2O: 90.6)		
-0.50	82.1	84.8	84.0
-1.00	73.6	75.6	62.6
-1.50	52.7	55.1	18.5

Table 29. The effect of osmotica and osmotic potential on the median rate of germination of sorghum E 55e seeds. Data for seeds germinated in water also given. The broken line indicates a boundary below which less than 50% of the seeds germinated.

Osmotic potential	Osmotica		
	K_2HPO_4 + KNO_3	KNO_3	K_3PO_4 + KNO_3
(MPa)	Median rate of germination (h^{-1}) (H_2O: 0.0197)		
-0.50	0.0163	0.0171	0.0156
-1.00	0.0119	0.0126	0.0111
-1.50	0.0083	0.0071	0.0067

Time to the beginning of germination was lengthened in all osmotica by lowering osmotic potential (Table 30, Appendix Table A1.9). Again, seeds in K_3PO_4 + KNO_3 were most affected at all osmotic potentials, while those in KNO_3 and in K_2HPO_4 + KNO_3 were not significantly different. Time-spread of germination was not affected in any osmotica at -0.5 MPa. Lowering of osmotic potential below this caused a lengthening of the time-spread. Seeds in -1.5 MPa KNO_3 had the longest time-spread (107 hours); however, the time-spread of seeds in -1.5 MPa K_3PO_4 + KNO_3 was shorter because of the low maximum percentage germination (18.5%) attained by the seeds in this solution.

Table 30. The effect of osmotica and osmotic potential on the time to 5% germination and on the time-spread of germination of sorghum E 55e seeds. Data for seeds germinated in water also given. The broken line indicates a boundary below which less than 50% of the seeds germinated.

Osmotic potential	Osmotica		
	K_2HPO_4 + KNO_3	KNO_3	K_3PO_4 + KNO_3
(MPa)	Time to 5% germination (h) (H_2O: 33.6)		
-0.50	45.5	43.9	47.0
-1.00	56.5	53.8	64.8
-1.50	84.3	84.0	131
	Time-spread of germination (h) (H_2O: 29.9)		
-0.50	28.7	26.3	30.3
-1.00	50.0	45.7	46.8
-1.75	71.0	107	50.2

Germination of sorghum E 55e seeds showed a similar response to the limited range of osmotic potential solutions as that of sorghum E 57 seeds. Maximum percentage germination was reduced with lowering of osmotic potential. This effect was more pronounced in K_3PO_4 + KNO_3 solutions than in the other osmotica. Median rate of germination decreased, time to the beginning of germination lengthened and the time-spread of germination increased with lowering of osmotic potential. No major difference in response was found between the two cultivars of sorghum tested, one of tropical origin and supposedly drought tolerent (E 57) and the other of more temperate origin and more drought sensitive (E 55e).

3.3 CONCLUSIONS

It was clear from the foregoing results that there were marked interactions between species, osmotic potential and the nature of the osmoticum. In terms of the objective of this thesis, i.e. to ascertain the most effective priming treatment, I seek the osmoticum which will prevent germination without being toxic, preferably at the highest osmotic potential. PEG had no detectable toxicity, but a lower osmotic potential was required to prevent germination than with K_2HPO_4 or K_3PO_4 (Table 31). KNO_3, either alone or with either phosphate solution, lowered the osmotic potential at which germination was prevented.

Tomato was the simplest and only species of those tried which could be readily primed in solutions of all osmotica although lower potentials were required with KNO_3 or its mixtures and with PEG than with K_2HPO_4 and K_3PO_4. Carrot appeared to be readily primeable in all solutions, except perhaps KNO_3. Carrot was peculiar in that although germination was prevented in all osmotica within the range of -1.5 to -1.75 MPa, all appeared to be toxic to a proportion of the viable seeds, ranging from about half with KNO_3 to less than 10% with PEG. Onion appeared to be the next most tractable species although very low osmotic potentials of K_2HPO_4, K_3PO_4 and PEG were required, while the presence of KNO_3 either mixed or alone did not prevent germination within the range of potentials investigated. It seemed possible that sorghum may be primed in PEG, but at potentials below the range explored here; however, all salt solutions were extremely toxic. Summarizing, tomato seeds can be primed in any of the solutions tried, carrot in any solution, but

Table 31. Highest osmotic potential (MPa) required to prevent germination in a range of osmotica and the subsequent germination (%) of seeds so exposed when placed in water.

Species	Osmotic Potential (MPa)					
	K_2HPO_4	K_2HPO_4 + KNO_3	KNO_3	K_3PO_4 + KNO_3	K_3PO_4	PEG
Tomato	-0.75	-1.0	-1.25	-1.25	-0.5	-1.0
Carrot	-1.5	-1.75	-1.75	-1.25	-1.5	-1.5
Onion	-1.75	<-1.75	<-1.75	<-1.75	-1.5	<-1.75
Sorghum	-1.75	<-1.75	<-1.75	<-1.75	-1.75	<-1.75
	Subsequent germination of exposed seeds(%) (germination of non-exposed seeds in water in brackets)					
Tomato (98)	96	96	96	92	92	93
Carrot (81)	58	57	38	69	63	75
Onion (98)	92	-	-	-	90	-
Sorghum(91)	54	-	-	-	0	-

with a degree of cost to the maximum percentage germination, onion in K_2HPO_4, K_3PO_4 or PEG, but sorghum only in PEG solutions.

Generally, provided the solution prevented germination during treatment and there was little or no toxicity, then the other desirable features of priming, i.e. earlier and more uniform germination, were also exhibited. However, when less suitable solutions were used or even when too concentrated a solution of an appropriate osmoticum was used only minor improvements were found, particularly in uniformity of germination. Occasionally, priming at too low an osmotic potential in a suitable osmoticum resulted in time-spreads of germination which were longer than that of untreated seeds. The germination responses of the primed seeds showed a decrease with lowering of osmotic potential of the priming solution.

These varied responses raise many issues, which will not be speculated upon at this stage. They also lead to conclusions which may have resulted in changes of design of experiments which are reported subsequently, if the results had been available at the time of planning. However, it will be appreciated that each of the above experiments occupies a very long time-span (about 8 to 10 weeks) and many experiments had to proceed simultaneously, guided to some degree by the experience of previous investigators. This explains what may appear to be unwarranted persistence with K_3PO_4 and K_2HPO_4 plus KNO_3 solutions; moreover, there was appreciable reluctance to give up easily used salt solutions in favour of PEG with all its attendant difficulties, especially with large-scale use in mind. Further, priming in this series was carried out for up to 28 days - a relatively long period - in order to explore full effects of these solutions; different results may well be obtained with shorter durations of priming.

CHAPTER 4

HIGH-HUMIDTY HYDRATION OF SEEDS

(EXPERIMENT 6).

A further attempt to prime sorghum seeds was made by placing seeds in 0.120M K_3PO_4 + 0.204M KNO_3 (-1.7 MPa) solutions at 15, 20 and 25 C for up to 3 weeks. Treatment for longer than 1 week resulted in death of all seeds. Of the seeds treated for 1 week those treated at 15 C had a subsequent germination percentage of 24, those treated at 20 C 11%, those at 25 C 3%. In an attempt to overcome the toxicity problem a vapour gap between seeds and solution was tried, similar to the experiments of Levitt and Hamm (1943) and Heydecker (1974).

4.1 MATERIALS AND METHODS

Sorghum E 57 seeds, selected for uniformity of size, were placed in stainless steel wire baskets suspended in sealed glass jars. The seeds were placed in atmospheres of 81%, 93% and 98% relative humidity (R.H.). These relative humidities were obtained through the use of saturated solutions at 20 C: 81% R.H. above a saturated solution of $(NH_4)_2SO_4$; 93% R.H. above $ZnSO_4$; 98% R.H. above $CuSO_4$ (Weast, 1968). After five, ten and sixteen days four replicates from each treatment were sown in emergence trays at 20 C at a depth of 20 mm. Four replicates of untreated seeds were sown for comparison. Emergence was recorded at twelve-hour intervals until complete.

4.2 RESULTS

The seed water contents (on a dry weight basis) after 5 days were 14.2% in 81% R.H.; 22.0% in 93% R.H.; and 25.1% in 98% R.H. The water content of these seeds was assumed to remain constant throughout the duration of the experiment after initial hydration. Determination of water content at later stages was complicated by respiratory induced changes in weight and microbial infection.

The hydration of sorghum E 57 seeds for five days at any of the high relative humidities or at 81% R.H. for any duration did not significantly reduce the maximum percentage emergence (Table 32, Appendix Table A1.10). Longer treatment at the higher relative humidities caused large reductions in the maximum percentage emergence, from 89% for seeds treated for 5 days to 68% and 58% for seeds treated for 16 days at 93% R.H. and 98% R.H., respectively. These effects were greater for seeds treated at higher relative humidity. Seeds were prone to microbial infection during treatment in the higher relative humidity atmospheres. The median rate of emergence of treated seeds was similar to that of untreated seeds, except for seeds treated at 98% R.H. for 16 days which at 0.0101 h^{-1} were significantly faster than untreated seeds, 0.0096 h^{-1} (Table 33, Appendix Table A1.10). Seeds treated at 81% R.H. and 93% R.H. for 10 days had significantly slower median rates of emergence, 0.0090 h^{-1}.

The time to the beginning of emergence of the treated seeds was the same as that of untreated seeds, 90 hours, except for seeds treated at 81% R.H. and 93% R.H. for 10 days, which emerged 97 hours after sowing (Table 34, Appendix Table A1.10). The time-

Table 32. The effect of hydration of sorghum E 57 seeds in atmospheres of high humidity on the maximum percentage emergence at 20 C. Data for untreated seeds also given.

Relative humidity	Duration (d) 5	10	16
(%)	Maximum percentage emergence (%) (control: 95.5)		
81	87.1	85.7	88.4
93	88.6	74.2	68.2
98	88.8	67.2	57.6

Table 33. The effect of hydration of sorghum E 57 seeds in atmospheres of high humidity on the median rate of emergence at 20 C. Data for untreated seeds also given.

Relative humidity	Duration (d) 5	10	16
(%)	Median rate of emergence (h^{-1}) (Control: 0.0096)		
81	0.0093	0.0090	0.0094
93	0.0094	0.0090	0.0094
98	0.0093	0.0097	0.0101

Table 34. The effect of hydration of sorghum E 57 seeds in atmospheres of high humidity on the time to 5% emergence and the time-spread of emergence at 20 C. Data for untreated seeds also given.

Relative humidity	Duration (d) 5	10	16
(%)	Time to 5% emergence (h) (Control: 89.5)		
81	91.5	97.3	93.8
93	88.3	97.2	89.5
98	89.8	92.0	80.3
	Time-spread of emergence (h) (Control: 26.7)		
81	27.6	25.0	23.0
93	30.7	24.7	27.8
98	31.6	20.8	30.1

spread of emergence was decreased from 27 hours for untreated seeds to 23 hours by treatment at 81% R.H. for 16 days. Treatment at higher relative humidities for 5 days lengthened the time-spread. This effect increased with increase in the relative humidity of the treatment atmosphere (Table 34, Appendix Table A1.10). Reduction in the maximum percentage emergence by treatment at the higher relative humidities for longer periods did not result in reduced time-spreads except for seeds treated at 98% R.H. for 10 days.

4.3 CONCLUSIONS

Hydration of sorghum E 57 seeds in atmospheres of high relative humidity did not enhance their emergence at 20 C. Maximum percentage emergence was similar or much reduced from that of untreated seeds. Of those seeds with similar maximum percentages to the untreated seeds no treatment produced seeds with markedly faster median rates of emergence, shorter times to the beginning of emergence nor reduced time-spreads of emergence. Longer periods of hydration at 93% and 98% R.H. caused large reductions in the maximum percentage emergence. Seeds placed in the higher relative humidity atmospheres were prone to microbial infection, but no experiments were made with seeds dusted with fungicide.