PHYSIOLOGICAL CHANGES IN PEA SEEDS DURING STORAGE

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ABSTRACT

Pea seeds stored in humid or dry conditions showed deteriorative changes before loss of viability. This occurred over a period of weeks in 93% rh at 25°C and 1% rh at 10°C, or days in 94% rh at 45°C. Deterioration was seen in an increase in solute leakage from seed which maintained complete vital staining, and later, a further increase in solute leakage, the development of dead tissue on the cotyledons, and loss of viability. In early deterioration, the decline in the ability of seeds to retain solutes was attributed to changes in living cells, possibly, deterioration of cell membranes. This was presented as good evidence that membrane deterioration is one of the first stages of ageing. Analyses of seed phospholipid content following storage in 94% rh at 45°C, when increased leakage occurred from living seeds, showed changes in the proportions of different phospholipid classes. These changes were discussed as possible factors leading to increased membrane permeability.

Seed lots stored for two years in conditions simulating warehouse storage showed deterioration, with increased leakage, reduced staining, and in some lots a decline in viability. Deterioration also occurred in commercially stored seed which remained viable, suggesting that one cause of vigour differences in pea seed lots could be prolonged storage. The similarity of deterioration in short and long term storage suggested that short term storage accelerated natural ageing.

The response of seeds to storage was related to their initial embryo condition, seeds with good embryo condition deteriorated less rapidly. The response of seeds to accelerated ageing gave a

more accurate prediction of seed storeability, rapid deterioration in accelerated ageing was associated with rapid deterioration during prolonged storage. A predictive test of pea seed storeability, based on solute leakage following accelerated ageing for 1d in 94% rh at 45°C was suggested.

Vital staining of seeds imbibed minus the testa showed damage to outer layers of cells of the cotyledons within two minutes of contact with water. Damage was caused by rapid imbibition, possibly as a result of disruption of membranes by the inrush of water. The time course of early leakage from pea embryos was explained by membrane damage to outer cells and reformation of membranes of inner cells, during hydration.

The different levels of solute leakage observed in seed lots of intact peas were ascribed to two causes: (1) the rate of imbibition, which was influenced by testa condition - seeds that imbibed rapidly had high leakage; (2) embryo condition, which influenced response to a given imbibition rate.

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INTRODUCTION

Extensive use has been made in plant physiology of the garden pea plant (*Pisum sativum* L.). The choice of this species as an experimental plant has arisen for two reasons: it provides convenient experimental material for work on a dicotyledon species, and is also an important vegetable crop, occupying, according to the latest figures, 90,000 hectares in the United Kingdom alone (Anon., 1976). Pea seeds in particular have been the subject of much research work, sometimes because of practical problems associated with the production of the crop, but more often because they are convenient for basic work on seed physiology. The work described in this thesis began with an investigation into a production problem in peas, out of which emerged both information of significance to seeds in general, and specific practical propcsals concerning pea seed storage.

A problem frequently associated with pea seeds is the variability in the field emergence of seed lots with equally high laboratory germinations, a seed lot being the term used in seed testing and the seed trade to denote a uniform batch of seeds within one cultivar from which samples are drawn for testing. Different seed lots may differ in their place of production and subsequent handling. Poor emergence of peas was first recognised by Hiltner (1903), and since then there have been several reports in Great Britain of the lack of correlation between field emergence and laboratory germination tests (Eastham, 1925; Wellington, 1962; Perry, 1967, 1970; Matthews and Bradnock, 1967; Matthews and Whitbread, 1968; Bradnock and Matthews, 1970), with similar reports

coming from Denmark (Stahl, 1936), Sweden (Gadd, 1936), North America (Munn, 1926) and the U.S.A. (Clark and Little, 1955). Failure to emerge has been associated with the infection of the seeds by the facultative soil borne parasite Pythium spp and in particular with Pythium ultimum (Baylis, 1941; Flentje, 1964; Matthews and Whitbread, 1968). The predisposition of seeds to infection, and their ability to emerge well in the field has been said to reflect the vigour of the seed, seed with high vigour resisting infection and emerging well, low vigour seed being highly susceptible to infection and frequently failing to emerge. The leaching of solutes from seeds into steep water has been used to detect low vigour seeds with unsatisfactory field emergence potential (Matthews and Bradnock, 1967, 1968; Matthews and Whitbread, 1968; Bradnock and Matthews, 1970); low vigour seeds usually have high levels of leaching and steep water conductivity.

The term vigour is not only used to describe this particular problem of variable field emergence in peas, but is more generally used to describe "the sum total of those properties of any seed which determine the potential level of activity and performance of the seed or seed lot during germination and seedling emergence" (Perry, 1977). Thus, this quality of vigour may express itself at several stages in the life of the seed: its survival in the non-active state, survival on sowing in the field, the ability to establish a plant, and subsequently, the ability to grow well (Heydecker, 1972). Synonyms which have been used to describe seed vigour are the integrity, efficiency, adaptability or sturdiness of the seed (Heydecker, 1972).

In peas, the possible causes of low vigour are associated with

periods before harvest, the timing of harvest, and periods after harvest (Perry, 1969). Reductions in pea seed viability and vigour between fertilisation and harvesting may result from environmental conditions. Rainfall immediately prior to harvest reduces viability and the emergence in soil of viable seeds (Flentje, 1964; Matthews, 1973a). Moore (1965) has suggested that the alternate wetting and drying caused by rain on the maturing crop, causes the development of physical stresses within the seed and the crushing of tissue, resulting in damage to the embryo. If a period of high rainfall is followed by exposure to strong sunlight while the seed is still on the parent plant, bleaching of the embryo can result (Maguire, 1973) and may cause a reduction in germination and a decline in vigour, which is reflected in increased electrolyte leakage and reduced respiration. The time of pea seed harvest is also critical. On the basis of both field (Matthews, 1973a) and laboratory (Perry, 1969; Matthews, 1973b) investigations, it has been suggested that one cause of low vigour in seeds may be the harvest of immature seeds, that have not developed the ability to withstand desiccation (Matthews, 1973a, b). The vigour of pea seeds may also be impaired by the way in which seeds are handled following harvesting. For instance, excessively rapid drying at high temperatures (Perry, 1969) or mechanical injury in handling (Moore, 1972) may cause reduced vigour.

It has also been suggested that in many crops, differences in seed vigour may result from physiological changes which result in deterioration of the seed during storage (Heydecker, 1972), and ultimately lead to loss of viability (Delouche, 1969). Compared with other crop seeds, peas have been considered particularly

tolerant of storage conditions with respect to the retention of high levels of viability. Their longevity has been illustrated by the retention of high viability over periods of long term storage (Haferkamp, Smith and Nolan, 1953; James, Bass and Clark, 1967) and shorter periods of storage in simulated adverse conditions (Toole, Toole and Gorman, 1948; Sijbring, 1963; James, Bass and Clark, 1967). After storage in uncontrolled environmental conditions for 31 years, the viability of several pea cultivars varied from 61% to 78%, whereas the viability of most cultivars of wheat had declined to below 15%, and that of rye to zero (Haferkamp, Smith and Nolan, 1953). In favourable storage conditions, James, Bass and Clark (1967) showed that viability was retained for at least five years. In more adverse conditions (55% relative humidity at 30°C) for 16 months, peas retained a high viability, whereas the viability of rye declined to 19%, and red fescue to 36% in eight months (Sijbring, 1963). Toole et al (1948) measured the time for complete loss of viability of ten species in 80% relative humidity (rh) at 26.6°C; peas retained viability longest along with cucumber, the time taken for complete loss of viability being 36 weeks, compared to 12 and 21 weeks for onion and lettuce respectively. Although a 30% loss of viability was recorded for peas by James et al (1967) after storage at 90°F for three months in 90% rh or 12 months in 70% rh, other species, namely bean, cucumber, tomato and sweetcorn, showed a much greater decline in viability. The retention of pea seed viability in storage may explain why changes in the vigour of peas during storage have not been investigated as a possible cause of the vigour differences observed between seed lots.

Most of the work on the storage of seeds has concentrated on the effects of a variety of storage conditions on the viability of different species. The major factors which influence seed viability in storage are oxygen, moisture content, and temperature (Crocker, 1948; Owen, 1956; Barton, 1961; James, 1967). Early work on the influence of oxygen on seed viability was conflicting, partly due to lack of control of temperature and seed moisture content (Owen, 1956; Roberts, 1961). More recent work (Harrison, 1966; Roberts, Abdalla and Owen, 1967; Roberts and Abdalla, 1968) suggests that the higher the oxygen pressure of the atmosphere, the shorter the storage life. Most attention has, however, been given to the influence of seed moisture content and temperature on viability, and since the work in this thesis arises out of a problem which exists in seed drawn from commercial warehouse storage where the partial pressure of oxygen in the atmosphere will be fairly constant, only the effects of moisture content and temperature will be considered in detail.

The moisture content of seed in storage is related to the relative humidity (rh) of the storage atmosphere (James, 1967; Roberts, 1972), since seeds are hygroscopic, and take up or lose water until the moisture content is in equilibrium with the ambient relative humidity. There are many records of moisture equilibria values for seeds, including those of Harrington (1960) and Roberts (1972). Tenperature affects the moisture content at equilibrium only slightly; in general, the lower the temperature, the greater the seed moisture content at a given relative humidity (James, Bass and Clark, 1967; Roberts, 1972). Seed moisture content may have adverse effects on the seed during storage, both through being too low, and more commonly, too high.

In some seeds, only a slight reduction in moisture content may have an adverse effect on viability, even though the moisture content is still high (12 - 31% depending on the species). These species, which include citrus, coffee, cocoa, and many large seeded hardwoods have been termed recalcitrant species (Roberts, 1973). These species are, however, exceptional, as most seeds require some reduction in moisture content in order to retain viability, and have been described as orthodox species (Roberts, 1973). The garden pea is an orthodox species, and therefore the effects of seed moisture content and storage temperature will be discussed for orthodox species only.

Extreme drying of orthodox seed, either before storage, or through equilibration with a low relative humidity, to low moisture contents may result in a reduction in viability (Ewart, 1895; Harrington and Crocker, 1918; Evans, 1957; Kosar and Thompson, 1957; Ching, Parker and Hill, 1959; Roberts, 1959; Nutile, 1964). The moisture content at which drying reduces viability appears to vary with the species. Timothy (Phleum pratense) showed reduced viability after three years at 5% moisture content compared to 7% (Roberts, 1959), and retention of viability was not improved in Lolium perenne by reducing the moisture content from 8.3% to 6% (Ching et al, 1959). Viability of celery, tomato, pepper, carrot and several grasses remained high after five years storage at 4% moisture content, but reduction of the moisture content to 1% and 0.4% resulted in viability being seriously impaired over this storage period (Nutile, 1964). Similarly, Harrington (1970) noted that seeds dried below 4% moisture content showed more deterioration than those between 4 - 7%.

In the work summarised above, it appears that 4 - 7% moisture content is the limit below which viability declines. However, work on other species suggests that the critical moisture content is lower. The moisture content of Chewings fescue has been reduced to 0.5% over phosphorus pentoxide with no loss of viability after 25 days storage (Smith and Gane, 1938/39). Seeds of wheat, barley, and several grasses whose moisture contents have been reduced to less than 1% (Harrington and Crocker, 1918) have been stored for 10½ months without loss of viability. Lowering the moisture content in the case of lettuce, cabbage, cucumber and onion to 0.3 - 0.4% also had no effect on final germination after five years (Nutile, 1964). Severe drying of peas over sulphuric acid (Ewart, 1895) was detrimental and resulted in a viability of only 15% after three weeks storage; that these seeds had themselves deteriorated was indicated by the abnormal growth of most of the seedlings they produced. Recently, Nakamura (1976) has classified seeds into three groups according to the relative humidity conditions in which they maintain viability best, which were dependent on the seeds' tolerance to dry conditions. From his work, he classified the garden pea amongst seeds which deteriorate rapidly in atmospheres below 10% rh and maintain viability best at 25 - 30% rh.

Low moisture contents which occur as a result of dry storage may have another effect, the development of hardseededness, which does not appear to be detrimental to viability. One proposed reason for the longevity of many leguminous seed is their possession of hard seeds, which fail to imbibe due to an impermeable seed coat. This condition has long been regarded as one of the causes of dormancy (Crocker, 1916; Owen, 1956; Wareing, 1962), and is a

genetic characteristic in many species (Crocker, 1948; Quinlivan and Millington, 1962). However, the proportion of such seed in a sample may also be influenced by environmental conditions during seed development (Quinlivan and Millington, 1962) such as daylength (Evenari, Koller and Gutterman, 1966) and weather conditions during seed dehydration on the plant (Aitken, 1939; Crocker, 1948; Quinlivan, 1965). Dormancy is naturally broken when the testa becomes permeable, as a result of the activity of micro-organisms (Wareing, 1963), or after subjection to extremely low (Busse, 1930) or fluctuating temperatures (Aitken, 1939; Quinlivan and Millington, 1962; Quinlivan 1965; Barret-Lennard and Gladstones, 1963). Hard seeds have been observed in many legumes, including many clover species, lupin, lucerne, tufted vetch, and laburnum. In an extensive study of the occurrence of hardseededness in Pisum sativum, Gloyer (1932) found that the percentage of hard seeds varied in different varieties, ranging from 0% to 79%, and the average for 242 varieties was 19%. There was no correlation between the type of pea and the occurrence of hard seed, although wrinkle seeded peas tended to have the least hard seed.

The development of hard seeds has been associated with a reduction in seed moisture content, and they usually develop once a certain low moisture content is reached (Aitken, 1939; Gladstones, 1958; Barrett-Lennard and Gladstones, 1964). The seed moisture content may be reduced by artificial drying, which Gloyer (1932) suggested accentuates the hardseeded character of peas. Jones (1928) has associated low storage humidities with the development of hard seeds, which may be due to a decline in seed moisture content in these storage conditions. This could explain the

development of impermeable seed coats which occurred during storage of Phaseolus vulgaris (Nutile and Nutile, 1947; Harrington, 1949), subterranean clover (Aitken, 1939), sweet clover (Helgeson, 1932), red clover (Stahl, 1937), Urena lobata (Garrard, 1955), crimson clover and perennial ryegrass (Ching, Parker and Hill, 1959), at low relative humidities.

High relative humidities in which the seed equilibrates to a high moisture content are more commonly recognised as the conditions leading to loss of viability. Boswell, Toole, Toole and Fisher (1940) found that although the rate of the deteriorative response depended on the type of seed, all species tested showed most rapid loss of viability at high humidity and temperature. Toole (1950) noted that most crop seeds which maintained viability for 10 years in 45-50% rh at 5°C, lost viability rapidly in relative humidities around 80% at 25-30°C. Lettuce seed stored at constant temperature over a range of relative humidities for four years (Kosar and Thompson, 1957) retained their high viability in 38-58% rh, but were dead after four years in 67% and 75% rh, and after two years in 88% rh. In conditions of high relative humidity (90% rh at 70°F), the viability of pea seeds declined to 4% in 12 months, but was maintained at 99% in less humid conditions (50% rh at 50°C) after five years (James et al, 1967). Roberts and Abdalla (1968) did not compare seeds stored in different relative humidities, but equilibrated peas to different moisture contents, before examining changes in viability in hermetic storage; the rates of deterioration were compared by noting the time for viability to drop to 50%. The viability of seeds at the highest moisture content (18%) declined to 50% after only 15 weeks, whereas

at lower moisture contents (15.4% and 12.5%), a similar decrease only occurred after 35 and 100 weeks, respectively.

In most of the examples quoted where reduced viability after storage in high relative humidities was observed, the temperature of storage was also high. Early work only considered the effect of temperature on viability (Groves, 1917) and provided evidence that with increased temperature, viability declined. Groves (1917) however noted that at any temperature, if the moisture content of the seeds was increased, viability decreased. In later investigations, the effects of the two factors of temperature and moisture content on the viability of wheat (Hutchinson, 1944; Roberts, 1960, 1961a), soya-beans (Toole and Toole, 1963), rice (Roberts, 1961b), oats (Roberts, 1960), and peas, broad beans and barley (Roberts and Abdalla, 1968), have been considered together. These investigations have shown that the effects of temperature and moisture content are constant and independent of each other; there is no mathematical interaction between them. Harrington (1960) expressed the relationship of seed moisture content and temperature to loss of viability by his "rule of thumb" of seed storage, that a 10°F (5.6°C) reduction in temperature, or a 1% reduction in seed moisture content doubles the storage life of the seed. The relationship between viability and environmental factors has been more precisely described in terms of an age index (Hukill, 1963), which uses the concept of the seeds physiological age under different storage conditions to predict viability, and in viability equations (Hutchinson, 1944; Roberts, Abdalla and Owen, 1967; Roberts and Abdalla, 1968; Roberts, 1972, 1973). Roberts and Roberts (1972) have presented the information derived from these equations in the form of nomo-

graphs, from which it is possible to estimate the time taken for viability to fall to a given level in various conditions of temperature and relative humidity, and also the combination of temperature and moisture content necessary to maintain viability above a given level for a certain period.

In much of the predictive work on storage described above, a uniform initial condition of the seeds was assumed. The initial condition of the seed may however influence response to storage. Barton (1961) noted that the effect of mechanical injury during threshing, was an immediate reduction in germination capacity and accelerated loss of viability in storage, and scarification of the seed coat of hard seeds to ensure rapid germination on removal from storage,has been shown to result in a decline in the viability of clover (Brett, 1953), sweet pea (Harrington, 1963) and alfalfa (Battle, 1948) during storage. Roberts (1972, 1973) recognised that such severe mechanical damage could influence the application of viability equations, and emphasised that seed viability nomographs should only be regarded as a useful guide to seed response to storage. Another factor which may influence the initial seed condition, in addition to mechanical damage, is the embryo condition. Embryo injury usually accompanies mechanical damage and may influence viability in storage through acting as a site for fungal infection or by initiating accelerated ageing in surrounding tissue (Moore, 1972). Although Grabe (1964) suggested that the physiological condition of the seed influences response to storage, and Crocker and Barton (1957) noted rapid deterioration during storage of seed in which deterioration had already been initiated, the influence of embryo condition on response to storage has seldom been investi-

gated. The variation in response of peas harvested in different years, to storage under a variety of temperatures and relative humidities (James et al, 1967), was attributed to possible damage either during seed development, which is likely to affect embryo condition, or during processing, which may cause mechanical injury. The influence of initial embryo condition on response to storage is therefore not at all clear, and requires further investigation.

Several of the investigations on the loss of viability in storage in a range of species, make reference to a decline in seed vigour before loss of viability. AbuShakra and Ching (1967) showed reduced phosphorylative efficiency of old as compared to new soya-bean seeds, and Abdul Baki and Anderson (1970) noted reduced protein and polysaccharide synthesis in aged wheat and barley well before loss of viability. In addition there is evidence for changes in enzyme activity. Increases in hydrolytic enzymes such as phosphatase, phytase and protease in aged barley were suggested by Abdul Baki (1969), and Berjak and Villiers (1972c) observed increased phosphatase activity in aged maize embryos, although Ching (1972) noted a decrease in the activity of such enzymes in crimson clover. A decrease in the activity of glutamic acid decarboxylase (Grabe, 1964; Gill, 1970) and cytochrome oxidase (Ching, 1972) has also been observed in aged seeds. These observations all indicated a reduction in the overall metabolic efficiency of aged, but viable seeds, which suggests reduced vigour. This reduced efficiency may explain the decline in vigour which was seen in the form of a decrease in the initial growth rates of lettuce, parsnip, tomato and Phaseolus vulgaris (Harrison, 1966), and in barley, broad beans and peas (Abdalla and Roberts, 1969).

Thus, even though pea seeds retain viability in storage for long periods (Toole et al, 1948; Haferkamp et al, 1953; Sijbring, 1963; James et al, 1967), a decline in seed vigour may occur before loss of viability.

If such a change in vigour does occur in peas, it may well be of practical significance as a factor contributing to the variability in vigour found in seed peas. Perry (1969) did not think this to be a possible cause of vigour differences, since, due to the longevity of peas, he considered that differences in vigour observed in seeds stored for only one or two years in commercial storage, were unlikely to result from deterioration. However, no evidence is available from storage work on peas under normal warehouse conditions to substantiate this view, and, in fact the data of Harman and Granett (1972) on pea seed deterioration under moist warm storage in laboratory conditions, suggests that a decline in vigour, in the form of impaired solute retention, occurs before a loss in viability. If a decline in vigour during storage is to be of relevance to the practical problem of variable seed vigour in commercially available pea seeds, then investigations of vigour during both laboratory and warehouse storage should be considered.

The physiological changes that have been described in the work on deteriorative changes in storage before loss of viability, much of which has already been summarised, have been drawn together by Delouche (1969) and presented in a sequence of deteriorative changes during ageing, representing progressive reductions in vigour until finally viability is lost. Some of the earliest changes, which represented a reduction in the overall metabolic efficiency of the seed (p.12), could be associated with, if not

due to, deterioration of cell membranes, which Delouche (1969) suggested was possibly the first deteriorative change occurring in seeds. Three lines of evidence in the literature indicate possible membrane deterioriation: increased solute leakage from aged seed, ultrastructural observations of membranes, and biochemical analyses of membrane composition.

Solute leakage from aged seeds has been measured by the electrical conductivity of water in which seeds have been soaked. Increases in the leakage of sugars and amino acids have been correlated with age, and the development of weak, deteriorating and dead seed in crimson clover (Ching and Schoolcraft, 1968; Ching, 1972), although there was no correlation between sugar leakage and age in perennial rye grass (Ching and Schoolcraft, 1968). Ching and Schoolcraft (1968) suggested that increased protease activity might be associated with ageing, degrading the structural proteins of the membranes, with the resultant decline in solute retention. However, more recently, Ching (1972) noted a decline in the activity of enzymes such as proteases, and suggested that impaired membrane integrity results from chemical breakdown such as auto-oxidation and hydrolysis. The increased conductivity associated with deterioration and loss of viability in cotton seed after rapid deterioration in high relative humidity and temperature (Presley, 1958) has also been explained by alteration of the semipermeable properties of the membranes. Similar increases in leachate conductivity were observed by Abdul Baki and Anderson (1970) in aged barley, in which sugar leaching correlated with a decline in viability during natural ageing. They explained their results by attributing the increased leakage levels of aged seeds to a lower

rate of glucose utilisation in old compared to new seed; however, they did not rule out the possibility that changes in membrane permeability could account for the increase in leaching.

In each of these examples of reduced solute retention with age, leaching was measured not for individual seeds, but for bulk samples, and in all cases, the viability of the aged seeds had declined. Solute leakage from dead seed is greater than that of living seeds (Takayanagi and Murakami, 1968, 1969), therefore increases in conductivity over a period when viability declines may reflect an increase in dead seed in a sample rather than membrane damage and a reduction in the ability to retain solutes. Even if viable seeds show increased leakage, this may not necessarily arise as a result of deteriorating membranes in living cells, since increases in conductivity in peas have been observed in viable seed which show an increase in the amount of dead tissue on the cotyledons (Matthews and Rogerson, 1976). This emphasises that increases in solute leakage from aged seeds can only be attributed to membrane damage as an early stage of deterioration if these increases are seen from completely living tissue. The interpretation of the evidence which has been used to indicate that membrane damage is the first stage of deterioration which occurs in ageing seeds, does not fulfil this requirement.

Further support for membrane deterioration before the loss of viability comes from ultrastructural investigations (Berjak and Villiers, 1972a, b; AbuShakra and Ching, 1967). In their work on root cap cells of maize,Berjak and Villiers (1972a, b) divided the aged embryos into three groups according to severity of damage; embryos in two of these groups showed severe disorganisation of

organelles and membrane deterioration, and were not viable; embryos in the third group with less damage were considered viable. These embryos showed membrane damage in cells with otherwise organised cytoplasm: the external membranes of mitochondria were irregularly shaped and a peculiar internal membrane bound space was thought to represent the disorganisation of the internal membrane, and the internal membranes of plastids were distorted. In addition, other membrane systems such as the endoplasmic reticulum and dictyosomes showed disorganisation, and lyosomes were precociously developed. Mitochondria from aged soya bean cotyledons showed similar membrane damage, with distorted and dilated cristae, and in some cases, rupture of the outer membrane (AbuShakra and Ching, 1967). However, in neither investigation could the possibility that dead cells were being examined, be completely eliminated.

Biochemical analyses of membrane composition have formed the third approach to the examination of membrane deterioriation during ageing. Before examining the biochemical evidence, some consideration of membrane composition and structure is appropriate. Membranes have two main components, proteins and lipids; the main emphasis here will be on the phospholipids, which have long been considered the more important molecules as regards membrane structure (Simon, 1974). The phospholipids are diglycerides, consisting of glycerol esterified with two long chain fatty acids, which may be unsaturated or saturated, and bearing on the third carbon position of the glycerol molecule, an alcohol group according to which the phospholipid molecule is given a specific name, for example, if the alcohol group is choline, the phospholipid is phosphatidyl choline. The whole molecule is amphipathic, the

fatty acid chains being non-polar and hydrophobic, the head group polar and hydrophilic; it is this amphipathic nature which determines the molecules arrangement in the membrane. The basic unit of the membrane is a lipid bilayer, in which the hydrophilic groups line up at the outside with the hydrocarbon chains stretched out alongside one another, at right angles to the plane of the membrane (Fig 1, B).

Since phospholipids are a major component of membranes (Benson, 1964; Kates, 1970; Kates and Marshall, 1975), and there is a case for supposing that all phospholipids are located in membranes (Chapman, 1965; Getz, 1970), the total amount of phospholipids could be taken as an approximate measure of the amount of membrane present. As a result, phospholipid extractions of aged material have been used to illustrate membrane deterioration. Koostra and Harrington (1969) noted decreases in the total phospholipid extracted from naturally aged cucumber seeds, and also changes in the proportions of the different classes of phospholipid and their degradation products; these changes were enhanced in seeds in which ageing was accelerated. They attributed these changes to lipid oxidation which resulted in membrane deterioration, but did not relate them to any other features of ageing which might indicate membrane damage. In an investigation of deteriorative changes in pea seeds during storage, Harman and Mattick (1976) have focussed attention on another part of the phospholipid molecule, the fatty acid chain. There was a reduction in the proportion of unsaturated fatty acids in both the complete embryo, and axis during storage, and the decrease in the axis paralleled seed vigour loss as measured by the growth rate of the seedling. Again, there was no correla-

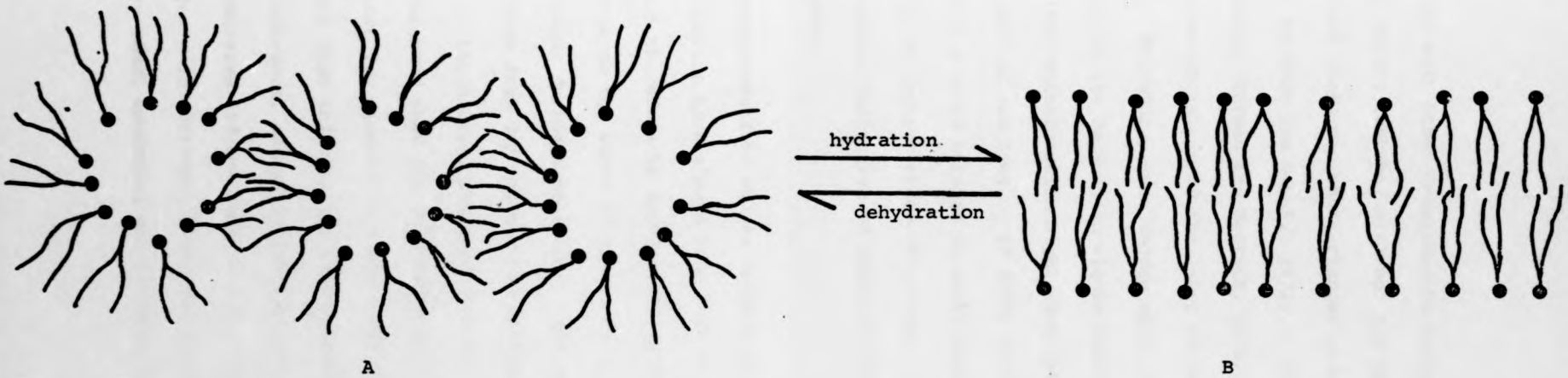


Fig. 1 Orientation of membrane phospholipids.

A: the hexagonal configuration adopted under relatively dry conditions, with narrow water-filled cylinders running through a dry matrix.

B: the lamellar configuration, as in the membranes of hydrated cells. After Luzatti and Husson (1962).

tion made with other measurements indicating membrane deterioration, such as solute leakage, although the degree of saturation of the fatty acid chain may cause changes in membrane permeability (Van Deenen, De Gier and Demel, 1972). Vigour was assessed in these experiments (Harman and Mattick, 1976) by changes in shoot fresh weight, which is one of the later stages of deterioration (Delouche, 1969). Therefore, even though small changes in fatty acids accompanied the decline in vigour which occurred before loss of viability, deterioration might have been well advanced even at the first time of sampling. If fatty acids, or total phospholipid changes are to be related to early membrane deterioration, measurements at an earlier stage of ageing, and comparison with other measurements indicative of membrane damage, such as solute leakage are needed.

Measurements of solute leakage as an indication of membrane deterioration have always been made by placing dry seeds in water. It would therefore be appropriate to consider solute leakage with reference to the state of membranes in the dry seed, and the changes which occur during imbibition. Larson (1968) noted that leakage of solutes from dry embryos was initially high over the first few hours of imbibition, and more recently Simon and Raja Harun (1972) have observed that the high level of leakage declines rapidly within the first few minutes to a low constant level. Larson (1968) proposed that this initial rapid leakage was due to disruption of cell membranes, caused by the rapid inrush of water; as a result the membranes did not form an effective barrier against solute leakage. In contrast, Simon and Raja Harun (1972) argued that in the dry seed, membranes are incomplete, since the water content is

below the 20-30% necessary for maintenance of the lipoprotein membrane structure (Chapman, Williams and Ladbroke, 1967; Finean, 1969), and that at such water contents, the phospholipids would take up the hexagonal array of water filled channels or pores (Fig 1, A) described by Luzzati and Husson (1962). This would be a relatively porous structure, so that on placing a dry seed in water, there would be a rapid loss of solutes from the cells. They suggested that further rapid leakage was restricted due to rapid rehydration of the membranes, and reformation of the normal lipid bilayer structure of the membrane (Fig 1, B). Electron microscopy has shown changes in membrane appearance during the first minutes of imbibition in maize scutellum (Buttrose, 1973) and in pea cotyledons (Swift and Buttrose, 1973), which may be due to hydration of the membranes. Thus, the decline in leakage from dry seed could be explained by a cessation of leakage from the outer cells, with slow leakage beginning from the inner cells (Simon and Raja Harun, 1972; Simon, 1974); this would be slow by virtue of the increased length of the diffusion path, and may be further reduced due to reabsorption of solutes by the outer cells, or solute accumulation in the cell walls as they diffuse to the outside (Simon, 1977). However, a decline in leakage following an initial rapid leakage due to membrane disruption (Larson, 1968), could also be explained by the increased length of the diffusion path.

Failure to reduce leakage by reducing the rate of imbibition through lowering the imbibition temperature (Short and Lacy, 1976) appeared to support the hypothesis of Simon and Raja Harun (1972). It was claimed that if the large initial leakage was the result of a physical phenomenon as Larson (1968) suggested, a reduction in

the rate of imbibition would reduce leakage. If the time course of leakage does in fact reflect membrane rehydration, any changes in membrane condition during ageing which influence leakage might be reflected in the form of the pattern of leakage during the early stage of imbibition.

The literature suggests that the decline in seed vigour, and ultimately in viability during storage occurs in all seed, although species show widely different tolerance to storage conditions and the length of the storage period (Boswell et al, 1940; Toole et al, 1948; James et al, 1967). Differences in storage potential also occur between genotypes within species. Under similar storage conditions, differences have been noted in the storage life of cultivars of lettuce (Harrison, 1966), bean (Barton, 1941; Toole and Toole, 1953), cereals (Haferkamp et al, 1953; Mackay and Tonkin, 1967), herbage seeds (Mackay and Tonkin, 1967) and peas (Haferkamp et al, 1953; James et al, 1967). Moore (1963) has suggested that further differences in the maintenance of vigour and viability may even exist between seed lots within a cultivar; however evidence in support of this suggestion is lacking. If such differences do exist between seed lots, as they do between cultivars, it is clearly important to determine which seeds would have the best storage potential, that is, will deteriorate least, over a long period in storage.

The criteria for assessing the quality of seed are laboratory germination tests, purity analyses, and seed moisture content, and in peas, measurements of leachate conductivity and the occurrence of the condition of hollow heart. Such information is frequently insufficient to provide the basis for sound decisions on which seed

lots are the most suitable for storage. The germination test in particular has received considerable criticism for its inadequacies in measuring the potential of seed to withstand storage (Delouche and Caldwell, 1960; Gill, 1969; Baskin, 1970; Byrd, 1970).

The storage potential of seeds has been related to prestorage levels of glutamic acid decarboxylase activity (Grabe, 1964, 1965), and Moore (1963) proposed that tetrazolium staining might predict seed storeabiiity. However, the most commonly used approach in predicting storage potential is accelerated ageing, which accelerates the changes occurring in normal storage by subjecting the seeds to conditions of high temperature and relative humidity; in these conditions, deterioration occurs within days. Seed germination after accelerated ageing has been shown to reflect the storage potential of crimson clover (Helmer, Delouche and Lienhard, 1962), peanut (Baskin, 1970), soya-bean (Byrd, 1970), and ten other vegetable species (Delouche and Baskin, 1973). The accelerated ageing response also predicts field emergence of crimson clover (Helmer et al, 1962) and peanut (Baskin, 1970) following storage. Since field emergence may reflect seed vigour, this observation could indicate that vigour differences that develop in storage before loss of viability may be predicted from a storage test. The long storage life of peas (Toole et al, 1948; Haferkamp et al, 1953; Sijbring, 1963) may explain the lack of attention given to prediction of the storage potential of peas. However cultivar differences in pea seed longevity have been noted in storage (James et al, 1967), and a significant correlation was found between their viability after three months storage in adverse conditions and viability after up to five years storage in more

favourable conditions. This correlation was however not considered sufficiently significant for the application of a predictive test for storeability of peas to be investigated any further.

The present work examined some of the physiological changes occurring during the ageing of pea seeds in both commercial and laboratory storage. These changes were related to the ageing of seeds in general, and more specifically to the practical problem of vigour differences observed between pea seed lots.

The influence of adverse storage conditions, producing both moist and excessively dry seeds, on the leaching of solutes from living cells was investigated, with the aim of providing good evidence for membrane damage during early stages of seed deterioration. The leakage of solutes was also related to membrane composition by analysis of seed phospholipid content. The importance of storage of pea seeds in laboratory and commercial storage as one possible cause of the vigour differences between seed lots was evaluated, and the role of the initial condition of the embryo was considered as a factor which might influence seed response to storage. The development of a test to predict the storage potential of different cultivars and seed lots of peas was initiated. The effects of the imbibition process on the leakage of solutes was examined and related to the frequently observed, and practically significant phenomenon of differing solute retention in seed lots of peas.

MATERIALS AND METHODS

Seeds

Seed (cv Kelvedon Wonder) were produced in glasshouses in Stirling in 1974 and 1975. In addition, a further 13 seed lots from six cultivars were obtained from Charles Sharpe and Co. Ltd. Sleaford, Lincolnshire in 1974. Seed samples were also drawn from commercial storage in the warehouses of Charles Sharpe and Co. in 1974, 1975 and 1976.

Short term storage conditions

A variety of storage conditions were produced in desiccators in which the well was filled with a saturated salt solution to give the required relative humidity, and maintained at constant temperature. An atmosphere of 93% relative humidity (rh) at 25°C was obtained over ammonium dihydrogen orthophosphate; 94% rh at 45°C over lead nitrate (Winston and Bates, 1960); approximately 1% rh over calcium chloride fused flakes at 10°C (Winston and Bates, 1960); and 45% rh by allowing the seeds to equilibrate in a room at 45% rh before sealing the desiccator. All seeds were surface sterilised in a solution of sodium hypochlorite containing 0.5% available chlorine for 15s and washed in two baths of sterile distilled water, before being put into storage.

Long term storage conditions

Conditions simulating warehouse storage were attempted by storing seeds in hessian bags on a shelf in a brick outhouse with no control of temperature or relative humidity.

Seed samples were also obtained after commercial storage for different periods in the warehouses of Charles Sharpe and Co. Ltd., Sleaford, Lincs. These seeds were stored in hessian or woven polyethylene fibre sacks and stacked on pallets. The storage environment of the warehouses was not controlled.

The seed moisture contents before and after storage were measured by drying at 105°C for 48h and expressing the moisture content as a percentage of the oven dry weight.

Viability

Seeds were surface sterilised in sodium hypochlorite containing 0.5% available chlorine for 15s and washed in two baths of sterile distilled water before planting either in trays of moist sand at 11% moisture content, or between sheets of damp towelling; after being set to germinate, seeds were incubated at 20°C. Seeds were regarded as viable after the appearance of both the radicle and plumule.

Leaching of electrolytes

Leaching of electrolytes into 20ml deionised water at 20°C from weighed single seeds, was determined by measuring the electrical conductivity of the soak water after 24h (Matthews and Rogerson, 1976). The electrical conductivity of bulk samples - 25 weighed seeds in 200ml deionised water - was also measured after 24h at 20°C.

Time course of leakage

Seeds were imbibed for 6h in deionised water, the testa removed

using fine forceps, and the embryos dehydrated slowly over calcium chloride to their original dry weight. Fifty pea embryos minus the testa were placed in a 100ml beaker and 50ml deionised water added. After 1 min., the supernatant was decanted and retained, and a fresh 50ml of deionised water added. This was repeated for 30 min., when a final 50ml water was added, and the embryos were imbibed for 24h. The electrical conductivity of the supernatants was used as a measure of electrolyte leakage.

Vital staining

Testae were removed from imbibed seeds, the cotyledons separated and the embryos placed in a 1% solution of 2, 3, 5 triphenyl tetrazolium chloride (TTC) for 3h at 20°C (Matthews, 1971) and the staining on the abaxial surfaces visually assessed. A quantitative assessment of staining was obtained by extraction of the red formazan in 10ml of 95% (v/v) ethanol at 80°C for 5 min. After cooling, the absorbance of the extract was measured in a spectrophotometer at 540nm (Steponkus and Lanphear, 1967).

Formazan extraction as a measurement of TTC penetration

Extraction of formazan was used as a quantitative measurement of the reaction of dehydrogenases with TTC. The two procedures used were modifications of the method used by Byrd (1970) in which seeds were stained with TTC either before they were ground up (post-staining) or after grinding (pre-staining).

In both methods, intact seeds were first imbibed for 24h at 20°C and the testae removed. In the post-staining method, the seeds were then stained in TTC (5ml g^{-1}) for 3h at 20°C, the embryo axis removed, and the seeds dried overnight at 35°C before

grinding in a pestle and mortar. In pre-staining, the embryo axis was removed from the imbibed seeds, which were ground up in TTC (5ml g^{-1}). The slurry was incubated for 3h at 20°C, centrifuged, the TTC discarded and the residue dried overnight at 35°C.

Further extraction followed the same procedure for both methods. One gramme of the dried samples (± 0.001g) was extracted in 25ml acetone by continuous shaking for 30 min. The extract was ground to ensure that the formazan went into solution, centrifuged, the supernatant decanted and made up to 25ml, and the absorbance read at 520nm in a spectrophotometer.

Extraction of sugars and potassium

Sugars and potassium were extracted by soaking five replicates of five seeds each in 10ml deionised water for 6h at 4°C, removing the testae, and grinding in the imbibing medium in an ice cooled mortar. The slurry was centrifuged at 20,000g at 4°C for 15 min, the supernatant decanted and the precipitate washed with 10ml of deionised water and centrifuged again. The supernatant and washing were combined to form the extract, and made up to 25ml.

The potassium concentration in the extract was measured by flame emission spectrophotometry. Total sugars were measured by a modification of the anthrone method (Loewus, 1952). Two millilitres of the extract were put in a pyrex test tube and 0.5ml anthrone solution (2% anthrone in ethyl acetate w/v) added. Five millilitres 95% sulphuric acid was layered onto the mixture, the test tube shaken, and after 10 min, the colour intensity was measured at 620nm in a Spectronic 20 spectrophotometer. The sugar concentration in the extract was calculated from a calibration

curve obtained by conducting the test on glucose solutions of various concentrations.

Field emergence

Emergence experiments were conducted in the early spring of 1975, 1976 and 1977. The seeds were sown by hand in thirteen 3m rows, 50 seeds per row at a depth of approximately 3.5cm. A replicate block consisted of one row of each seed lot, 0.3m apart, four replicate blocks being sown on each sowing date. Seed lots were randomised within the blocks.

In 1975, 13 seed lots of six cultivars were sown on 20th February and emergence counts taken periodically over a period of nine weeks after which emergence counts were constant. In 1976 and 1977, the same 13 seed lots were sown after periods of storage. Two sowing dates were used in 1976 (21st April and 18th May), and emergence counts were made until constant, which took six and four-and-a-half weeks after sowing, respectively. Seeds were sown on only one date in 1977 (22nd March) and emergence counts taken until constant after six weeks.

Meteorological records of earth temperature at 5cm and rainfall, for the week preceding and after sowing, were obtained from a meteorological station within 100m of the sowing area.

Rate of imbibition

a) In aqueous solution

Individual weighed seeds were placed in 20ml of an aqueous solution. At predetermined intervals, they were removed, blotted dry, weighed, and returned to the imbibing solution. After the

final weighing, the electrical conductivity of the solution was measured. Changes in weight due to imbibition were expressed as the percentage weight increase compared to the original air dry weight.

In some experiments, the imbibing solution was one of polyethylene glycol in water, obtained by use of Carbowax (George Gurr Ltd) with an average molecular weight of 4000.

b) In sand

Individual weighed seeds were planted in sand equilibrated to 22% moisture content. Seeds were removed at intervals, the sand brushed off, and the seeds blotted dry and weighed, before returning to the sand. Weight increases were expressed as a percentage of the original air dry weight.

Growth tests

Seeds were surface sterilised for 15s in sodium hypochlorite with 0.5% available chlorine, followed by two washings in sterile distilled water, before planting in trays of sand at 11% moisture content. Trays were placed in polythene bags in the dark at 20°C. Seeds were removed from each treatment after 3, 5, 7 and 10 days, and the dry weights of cotyledons, plumule and radicle of each seed determined by drying in an oven at 105°C for 48h.

Respiration measurements

The respiration rate of seeds was measured by oxygen uptake at 20°C in a Gilson differential respirometer. Carbon dioxide was absorbed by 10% potassium hydroxide in the centre well of each

reaction flask. Samples of three seeds were placed in 17ml reaction vessels with 0.5ml distilled water. Two flasks containing potassium hydroxide, and a volume of water equivalent to the volume of three pea seeds plus 0.5ml, were included in each measurement, following the recommendations of Carver and Gloyne (1971). Seeds were allowed to equilibrate for 1h at 20°C, after which readings were taken at 15 min intervals for a further hour.

Assessment of testa condition

The condition of the testa was examined in intact seeds that had been allowed to imbibe 24h in water at 20°C. Seeds were stained in 1% fast green for 5 min, then washed in tap water and examined for damage to the testa. All cracks in the testa were revealed by deep green staining of the broken edges. The length of cracks was measured using calipers.

Phospholipid extraction, separation and determination

All seeds were imbibed 24h at 20°C before extraction, and the testae and embryo axes removed. Three replicates of each treatment were extracted, each made up of five cotyledons, and each cotyledon coming from a different seed; thus a total of 15 seeds were sampled. Tissues were extracted with 30 parts by weight of chloroform-methanol (2:1 v/v) according to the Folch procedure (Folch, Ascoli, Lees, Heath and LaBoran, 1957), with modifications by Christie (1973). An antioxidant, 4 methyl, 2,6 di-t-butyl phenol was added to solvents to prevent lipid oxidation. Lipid extracts were stored under nitrogen at - 20°C to await further analysis.

The phospholipids in the extracts were separated by thin layer chromatography (T.L.C.) of the phospholipid extracts. Thirty microlitres of each phospholipid extract were applied to the base of a T.L.C. Plate (Merck, Silica Gel 60 F_{254}, 20cm × 20cm, layer thickness 0.25mm) along with samples of known phospholipids, each spot being dried under nitrogen. The plates were run in a solvent mixture of chloroform : acetone : methanol : acetic acid : water (10 : 4 : 4 : 2 : 1) (Rouser, Kritchevsky, Galli and Heller, 1965; Siakotos and Rouser, 1964; Rouser, Kritchevsky and Yamamoto, 1967) until the solvent front was approximately 1-2cm from the top of the plate. Plates were dried under nitrogen, developed in iodine vapour, and the phospholipid spots outlined using a needle. Areas of silica gel bearing phospholipids were removed and stored in a desiccator before subsequent determination of the phosphorus content.

The concentrations of the phospholipids present in the extracts were measured by determining the phosphorus content of the phospholipids removed from the T.L.C. plate. Phosphorus determinations were made using a spectrophotometric analysis according to the method of Bartlett (1959). The amount of phosphorus in the samples was read from a calibration curve prepared by performing the reaction on known amounts of a 0.5mM sodium dihydrogen phosphate solution.

RESULTS

The Effects of Short Term Storage

The moisture contents of five replicates of five seeds were determined after different periods of storage in three conditions of relative humidity : 45% rh at 10°C, 93% rh at 25°C and 1% rh at 10°C. There was little change in the moisture content of seeds held in 45% rh at 10°C during eight weeks of storage (Table 1) indicating that the moisture content of these seeds before storage was close to the equilibrium for these conditions. The moisture content of seeds held in 93% rh at 25°C increased to 24.6% after six weeks and was maintained at a level in excess of 20% up to the end of the 15 week experimental period. Seeds in 1% rh at 10°C showed a progressive decline from 10.4% to 5.7% over the whole 15 week storage period. Thus, one effect of the different relative humidities was to change the seed moisture content either by the uptake (in 93% rh) or release (in 1% rh) of water.

TABLE 1

Moisture contents of seeds after storage in humid and excessively dry conditions

Each figure is the moisture content expressed as the percentage of the oven dry weight. Each percentage is a mean of 20 seeds.

Time in storage weeks	93% RH 25°C	1% RH 10°C	45% RH 10°C
0	10.4	10.4	10.4
6	24.6	8.5	9.6
9	21.8	7.9	9.8
12	20.9	6.5	-
15	22.7	5.7	-

In the first experiment in this series, the effects of storage in 93% rh at 25°C and 45% rh at 10°C, on the viability and leachate conductivity of a batch of seed (glasshouse grown in Stirling, 1975) were determined. Seeds held in 45% rh at 10°C retained 100% viability for the eight week storage period (Fig 2). Over the same period of time there was little change in the leaching of electrolytes from the seeds, as indicated by the electrical conductivity of the seed soak water (Fig 2). In contrast, seeds stored in 93% rh at 25°C showed a slight decrease in viability after three weeks (100% to 94%) followed by a rapid decline in germination to 56% after six weeks. The conductivity of the seed soak water increased throughout the storage period, even in the first three weeks, when there was an increase from 294 to 423 μmho cm^{-1} g^{-1} seed.

In a second experiment using a different batch of seed (glasshouse grown in Stirling, 1973; Table 8), the storage period was extended to 15 weeks, and a dry storage treatment (1% rh at 10°C) replaced the conditions of 45% rh at 10°C. Seeds stored in 93% rh at 25°C again showed a decline in germination and an increase in leakage of electrolytes from the seed (Fig 3). The fall in germination after six weeks storage was small, 96% to 95%, but over the same period, the mean conductivity showed a large increase from 290 to 400 μmho cm^{-1} g^{-1} seed. Both viability and leachate conductivity changed little over the period of 15 weeks storage in dry conditions (Fig 3). Thus, in both experiments, large increases in electrolyte leaching were observed over the first few weeks of storage in 93% rh at 25°C, when only a small decline in viability had occurred.

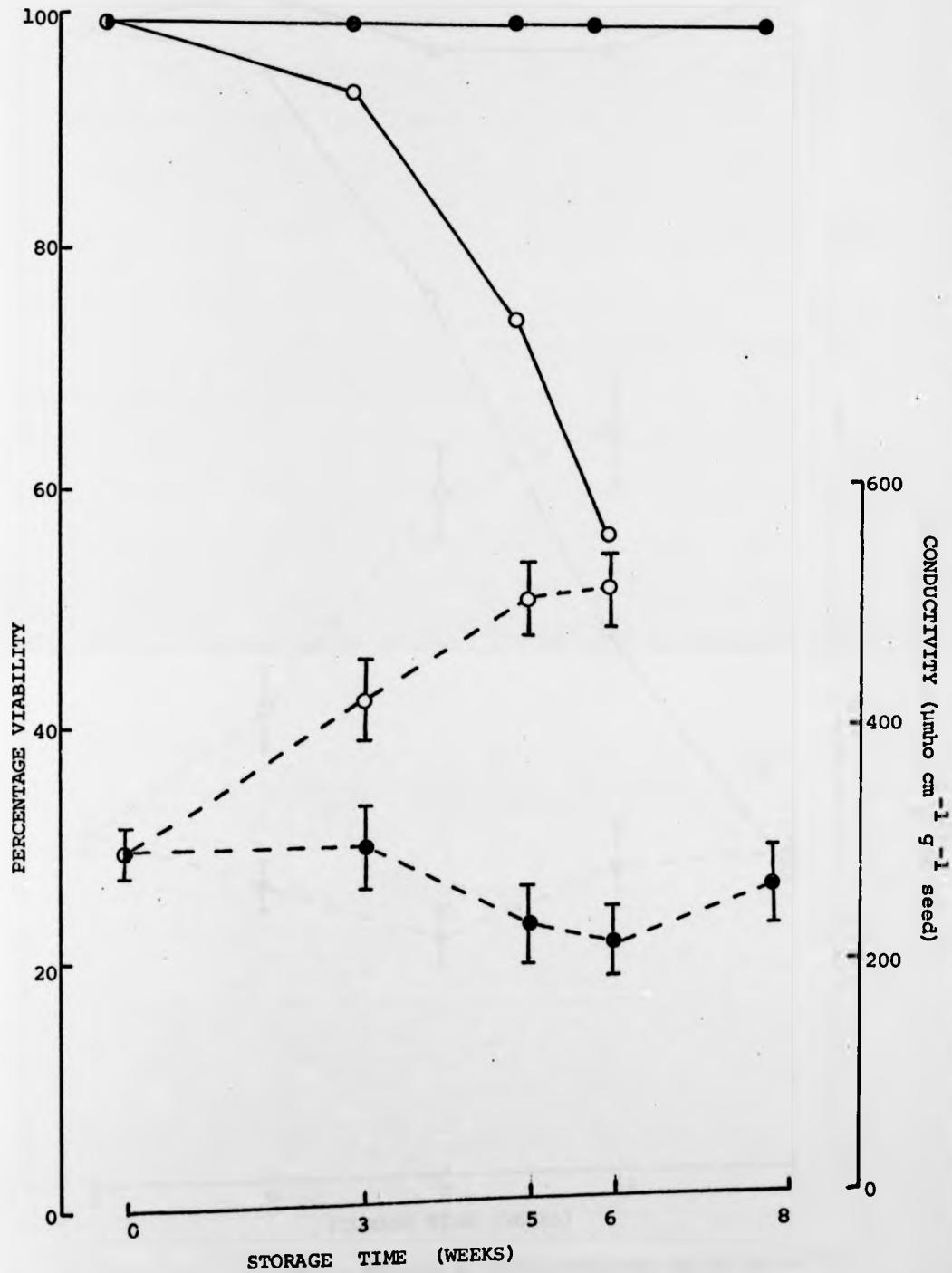


Fig.2 Effect of storage in 93% rh at 25°C (—O—) and 45% rh at 10°C (—●—) on viability (——) and leaching (---). Each conductivity reading is the mean of 20 seeds. Vertical lines represent ± standard error.

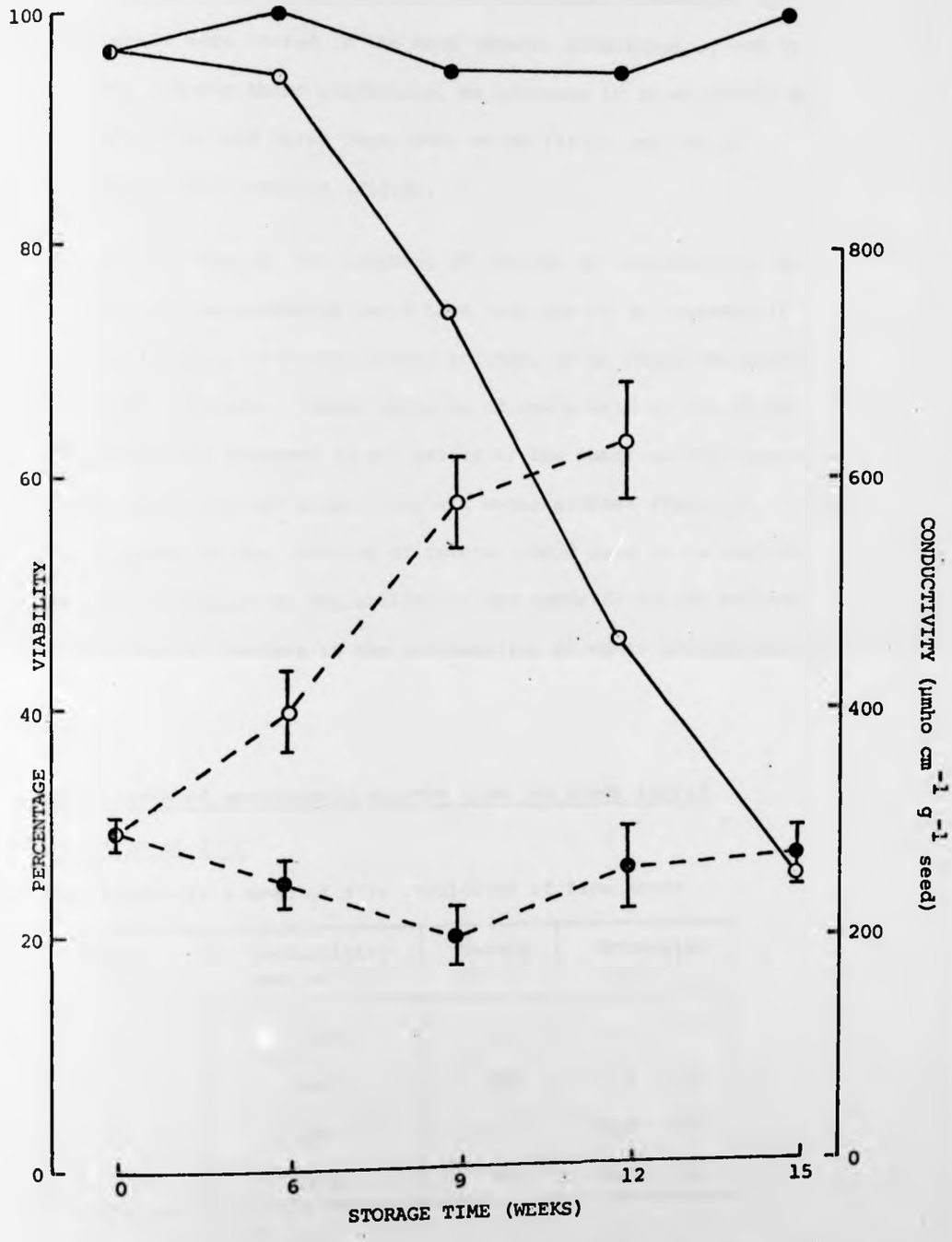


Fig.3 Effect of storage in 93% rh at 25°C (-O-) and 1% rh at 10°C (-●-) on viability (——) and leaching (---). Each conductivity reading is the mean of 20 seeds. Vertical lines represent ± standard error.

A similar observation was made in a further experiment in which seeds were stored in the more adverse conditions of 94% rh at 45°C. Under these conditions, an increase in conductivity was seen after two and three days, when no or little decline in germination had occurred (Fig.4).

The increase in the leaching of solutes as indicated by the conductivity measurements could have been due to an increase in the availability of water soluble solutes, or an impaired ability to retain solutes. Water extracts of seeds held in 93% rh at 25°C showed no increase in the levels of the water soluble electrolytes, potassium and sugar, over six weeks storage (Table 2). Thus, the increase in the leaching of solutes would seem to be associated with a decline in the ability of the seeds to retain solutes, and not to an increase in the availability of water soluble solutes.

TABLE 2

Comparisons of extractable solutes from pea seeds stored in 93% RH at 25°C

Each figure is a mean of five replicates of five seeds

Weeks Storage	Conductivity $\mu mho\ cm^{-1}g^{-1}$	Sugars $mg\ g^{-1}$	Potassium $ppm\ g^{-1}$
0	1100	101	6.2×10^3
3	1263	106	6.6×10^3
5	1045	104	6.0×10^3
6	1120	92	6.1×10^3

Although an increase in leaching was seen for seed showing little or no reduction in germination after storage, a large proportion of dead tissue can develop on pea cotyledons before

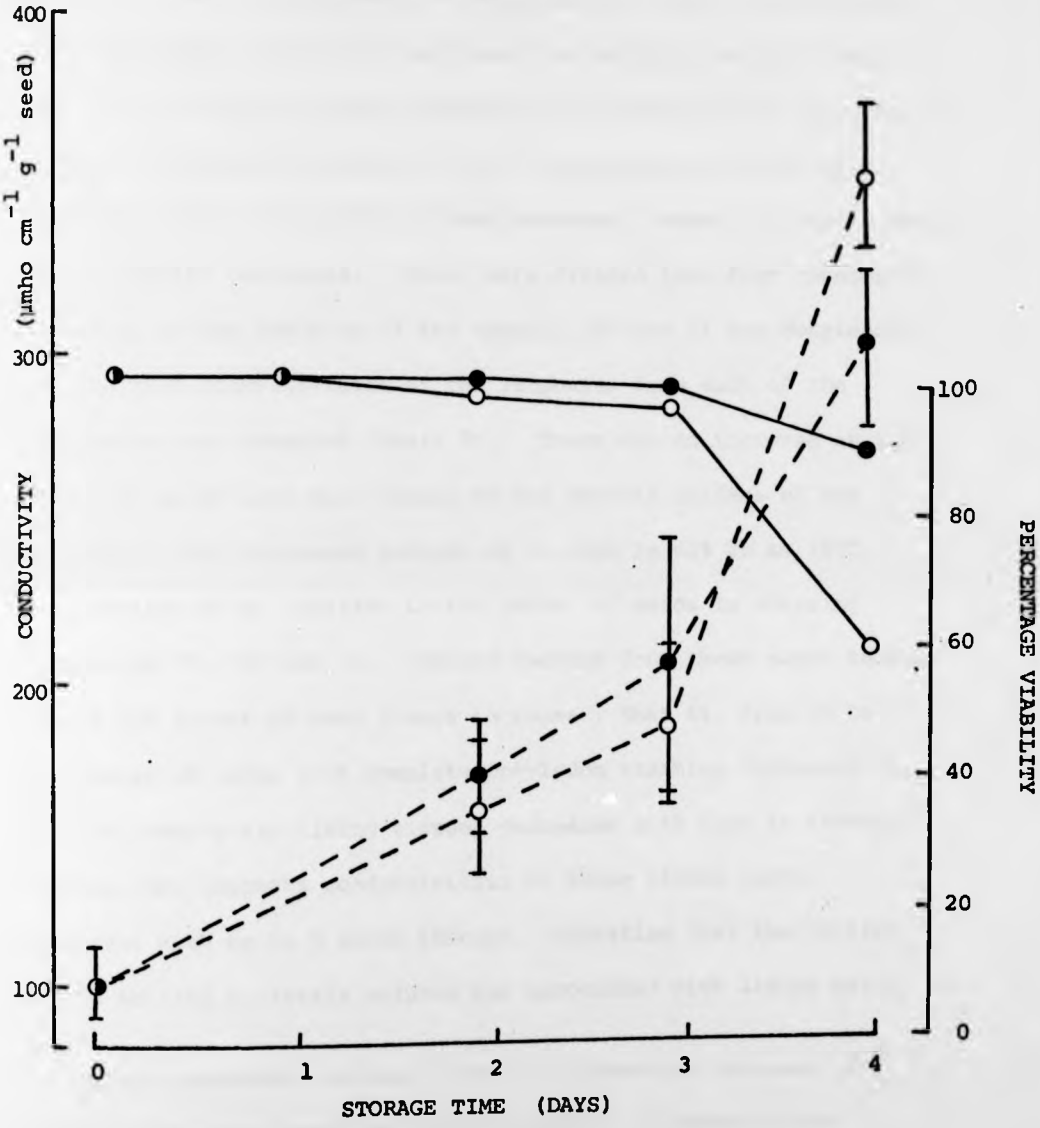


Fig.4 Effect of storage in 94% rh at 45°C on viability (——) and leaching (---). Replicate 1 (●); replicate 2 (○). Each conductivity reading is a mean of 20 seeds. Vertical lines represent ± standard error.

viability is lost (Matthews and Rogerson, 1976). The reduced ability to retain solutes could therefore have been associated with the development of dead tissue on the cotyledons. This possibility was investigated by comparing the loss of electrolytes from individual seeds with their tetrazolium chloride (TTC) staining. This staining method indicates live tissue by the production of the red formazan (Cottrell, 1948), through the reaction of tetrazolium chloride with cell dehydrogenases (Roberts, 1951); dead tissue remains unstained. Seeds were divided into four categories according to the staining of the abaxial surface of the cotyledons, and the mean conductivities of the leachates from each of the categories were compared (Table 3). There was an increase in the number of seeds with dead tissue on the abaxial surface of the cotyledons with increased periods of storage in 93% rh at 25°C, as indicated by an increase in the number of seeds in staining categories II, III and IV. Solute leakage from these seeds increased as the extent of dead tissue increased, that is, from II to IV. The number of seeds with complete cotyledon staining (category I), that is, completely living tissue, decreased with time in storage. However, the leachate conductivities of these living seeds, increased with up to 9 weeks storage, indicating that the decline in the ability to retain solutes was associated with living cells.

These completely stained seeds also showed an increase in staining intensity over the storage period. A quantitative measurement of staining intensity was obtained by measuring the absorbance at 530nm of the formazan ethanol extracts of seeds that had stained completely with TTC. The absorbance of the extract increased over the 12 week storage period from 0.11 to 0.42 (Table 4).

TABLE 3

Leachate conductivity (μmho cm^{-1} g^{-1} seed) of pea seeds for each of the four TTC staining categories after periods of storage in two conditions

Staining category of abaxial surface of cotyledons;

I: completely stained; II: less than 50% unstained;

III: more than 50% unstained; IV: no staining.

The number of seeds in each category is shown in parenthesis.

Storage Condition	Storage Time (weeks)	Staining Category I	II	III	IV
93% rh 25°C	0	277(20)	0(0)	0(0)	0(0)
	6	369(15)	480(5)	0(0)	0(0)
	9	500(8)	595(9)	853(2)	1217(1)
	12	375(4)	650(13)	891(1)	0(0)
	15	655(1)	592(10)	939(8)	784(1)
1% rh 10°C	0	277(20)	0(0)	0(0)	0(0)
	6	251(15)	261(3)	213(2)	0(0)
	9	202(19)	125(1)	0(0)	0(0)
	12	237(11)	303(6)	-(2)	-(1)
	15	262(14)	245(6)	0(0)	0(0)

TABLE 4

The absorbance of formazan ethanol extracts from seeds, after storage in 93% rh at 25°C and in 1% rh at 10°C

Storage treatment	Storage Time (weeks) 0	6	9	12	15
93% rh 25°C	0.11	0.23	0.37	0.42	-
1% rh 10°C	0.11	0.10	0.21	0.30	-

Seeds from 1% rh storage at 10°C also showed the development of dead areas, more seeds being placed in staining categories II to IV with increasing time in storage (Table 3). There was also an increase in staining intensity, the absorbance of ethanol extracts increasing from 0.11 to 0.30 (Table 4). This suggested that some damage had occurred despite the fact that it was not seen in the form of an increase in conductivity, which was at first sight difficult to explain. However, it was noticed that with increasing time in storage, these relatively dry seeds showed a decrease in the amount of water taken up after 24h imbibition, and an increase in the proportion of hard seed (Table 5). This observation prompted the suggestion that a slower rate of water uptake in seeds from dry storage was responsible for the lower conductivity readings. Possibly, the time of contact between water and cell solutes over the 24h soaking period had been shorter than had been the case for seeds that had previously been held in 93% rh, resulting in less leaching during 24h soaking. The time courses of imbibition of 10 individual seeds after periods of dry storage were therefore compared.

TABLE 5

Imbibition characteristics of seeds stored in 1% rh at 10°C

Storage time (weeks)	Moisture content (%) after storage	% wt increase after 24h imbibition	Percentage hard seed
0	10.4	141	0
3	8.7	110	3
5	7.5	102	15
6	8.5	119	10

The rate of imbibition of seeds held for three weeks in dry conditions was unaffected by storage, but after five and six weeks dry storage, the rate of imbibition was slower. This difference between the imbibition rate after three weeks storage and the longer storage periods cannot be explained solely by differences in moisture content, since the moisture content had fallen within three weeks storage (Table 5). Slower imbibition seen after five and six weeks storage could have been related to the development of an increased proportion of hard seeds (Table 5), which characteristically have slow rates of imbibition.

During measurements of the rate of imbibition, considerable variation was noted between seeds. The rates of imbibition of individual seeds were compared to their 24h conductivities, after five weeks storage in 1% rh at 10°C (Table 6). Two groups of seeds could be distinguished in Table 6. Seeds 1 to 4 showed a slow rate of water uptake, increases in weight only becoming apparent after 24h imbibition; the mean 24h conductivity of these seeds was less than 49 μmho cm^{-1} g^{-1} seed. The second group, consisting of seeds 5 to 10, imbibed water more rapidly and produced a high 24h conductivity reading (234 μmho cm^{-1} g^{-1} seed). This group was similar to the 93% rh stored seeds in both rate of imbibition and conductivity. The mean conductivity of the whole sample removed from dry storage included both these extremes, and thus when used as a description of the sample as a whole was misleading. The development of these two groups of seeds was confirmed by the fact that the range of conductivities of seeds from dry storage increased with time in storage (Table 7). The variation in rate of imbibition between individual seeds might

TABLE 6

Percentage increase in weight of seeds during imbibition after five weeks storage in 1% rh at 10°C

Replicate	Time of imbibition (h)											24h conductivity µmho cm^{-1} g^{-1} seed
	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	5.0	12	24	
1	0	0	0	0	0	0	0	0	0	0	1	28.7
2	0	0	0	0	0	0	0	0	0	0	6	33.8
3	0	0	0	0	0	0	0	0	0	1	27	47.9
4	0	0	0	0	0	0	0	0	0	3	61	86.9
5	1	2	4	6	8	12	15	23	59	108	129	118.3
6	0	0	0	1	1	3	4	9	15	65	142	157.8
7	5	11	27	40	40	55	62	75	85	103	112	152.3
8	2	5	9	20	20	59	76	104	128	169	181	280.5
9	6	12	18	24	24	36	41	51	71	121	129	289.86
10	6	13	18	23	28	32	45	67	97	127	140	404.38

TABLE 7

The effect of increasing time in storage in 1% rh at 10°C on the range of conductivity readings (µmho cm^{-1} g^{-1} seed) for the soak water from around single pea seeds which contribute to the mean conductivity

Conductivity	Storage time (weeks)				
	0	6	9	12	15
Lowest	190	145	26	4	5
Highest	313	354	398	349	530

account for the higher mean rate of imbibition observed in seeds after three weeks dry storage as only samples of ten seeds were used on each occasion.

It was possible that the damage to seeds from dry storage did not occur during storage, but during subsequent imbibition in water. Seeds were therefore placed in 55% rh at 20°C (Nutile, 1964) to hydrate them slowly, and to observe their condition after dry storage without the possibility of damage occurring during imbibition in water. The seeds failed to hydrate after three months storage in 55% rh at 20°C and therefore this possibility was not eliminated.

The intense staining with TTC observed in the early stages of deterioration might be produced as a result of the stain penetrating deeper into the tissues, and thus more layers of cells producing formazan and contributing to the observed red colour (Byrd, 1970). Extraction of the formazan produced by reaction of TTC with cell dehydrogenases was used to investigate the possibility that the penetration of TTC into the seed after storage in 93% rh at 25°C led to intense staining. The absorbance of the formazan extract was measured for seeds macerated before staining (pre-staining) and for seeds macerated after staining (post-staining). Byrd (1970) suggested that the absorbance after pre-staining should give a measure of the ability of the seed to reduce TTC if all dehydrogenases are freely available for the reaction, since maceration of the seed should release the dehydrogenases from the cells. The absorbance after post-staining would depend on the number of layers of cells producing formazan, and should therefore reflect the degree of TTC penetration into the tissue.

After pre-staining, the absorbance of the formazan extract increased slightly after three weeks storage, and declined sharply after five weeks (Fig.5) indicating decreased dehydrogenase activity. This would be expected in deteriorating seeds that were beginning to develop dead tissue on their cotyledons. Absorbance after post-staining changed little up to five weeks storage, when intense staining was observed, which may indicate that there was no increase in the penetration of TTC into the seed. Thus, as far as these observations were concerned, intense staining of the cotyledons seen after five weeks could not be explained by increased layers of cells contributing to the reduction of TTC.

The reduced ability to retain solutes in seeds from 93% rh at 25°C was not associated with dead tissue or availability of solutes. This suggested that changes within living cells were allowing leakage. One possible change was an increase in membrane permeability. The observation that there is a rapid early release of solutes from dry pea embryos (that is, seeds with their testae removed), followed by a decline to a low constant rate of leakage within the first minutes of imbibition has been explained in terms of a rehydration and reformation of cell membranes during imbibition (Simon and Raja Harun, 1972). If changes in membrane permeability occurred during storage, resulting in increased leakage, the reformation of the membranes may be disrupted and the form of the time course of leakage might also be affected. Therefore the time course of leakage was examined before and after storage in 93% rh at 25°C. Seeds were imbibed, their testae removed, and the embryos then dehydrated to their original moisture content; the time course of leakage was measured for these dry

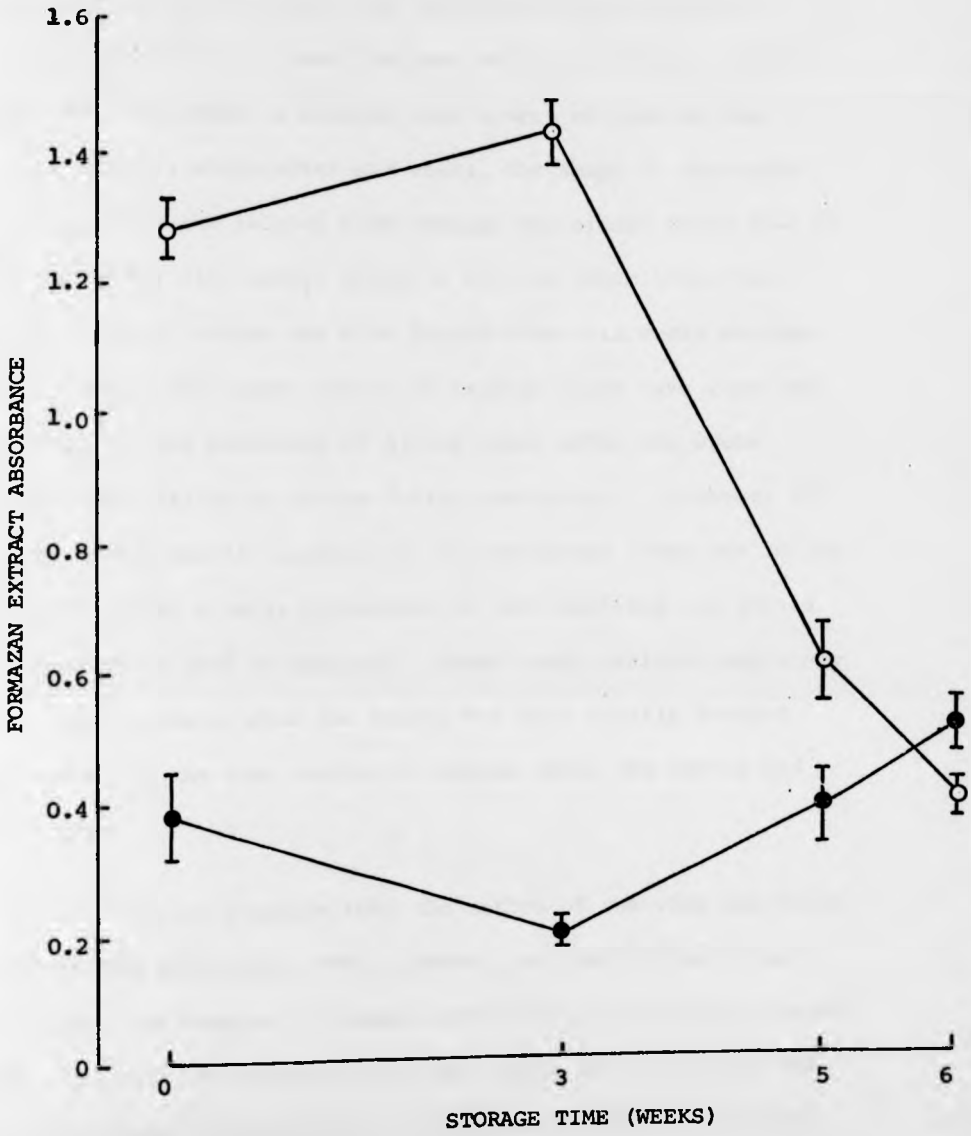


Fig.5 Changes in the absorbance of formazan extracts from seeds following storage in 93% rh at 25°C, using prestaining (-O-) and post-staining (-●-) extraction methods. Each figure is the mean of 4 replicates of 5 seeds. Vertical lines represent ± standard error.

embryos. Unstored seed showed an initially high leakage of solutes which declined rapidly in the first five minutes of imbibition (Fig.6) to a lower constant rate of leakage. After storage for five weeks, a similar time course of leakage was obtained (Fig.6), while after six weeks, the shape of the curve was the same but the initial high leakage was almost twice that of seeds stored for five weeks; after 30 minutes imbibition, the constant rate of leakage was also higher after six weeks storage. In these seeds, the higher levels of leakage might have resulted from damage to the membranes of living cells after six weeks storage, which failed to reform during imbibition. However, TTC staining of the abaxial surface of the cotyledons after 24h imbibition, showed that a large proportion of both unstored and stored seed were either dead or damaged. These seeds stained completely when imbibed normally plus the testa, but were clearly damaged after measuring the time course of leakage after the testae had been removed.

It was thought possible that the method of removing the testa, which involved imbibition, testa removal, and dehydration, might have caused this damage. Several alterations to this method were tried: the rate of dehydration of the embryo after removing the testa was reduced and increased; seeds were imbibed for only 6h before the testa was removed, this being the minimum time necessary to facilitate testa removal without mechanical damage to the embryo; and both the imbibition and dehydration steps were left out by chipping the testa off the dry seed. Damage in the form of incomplete staining was still observed after imbibition, when the testa was removed in all these different ways. Since all of the

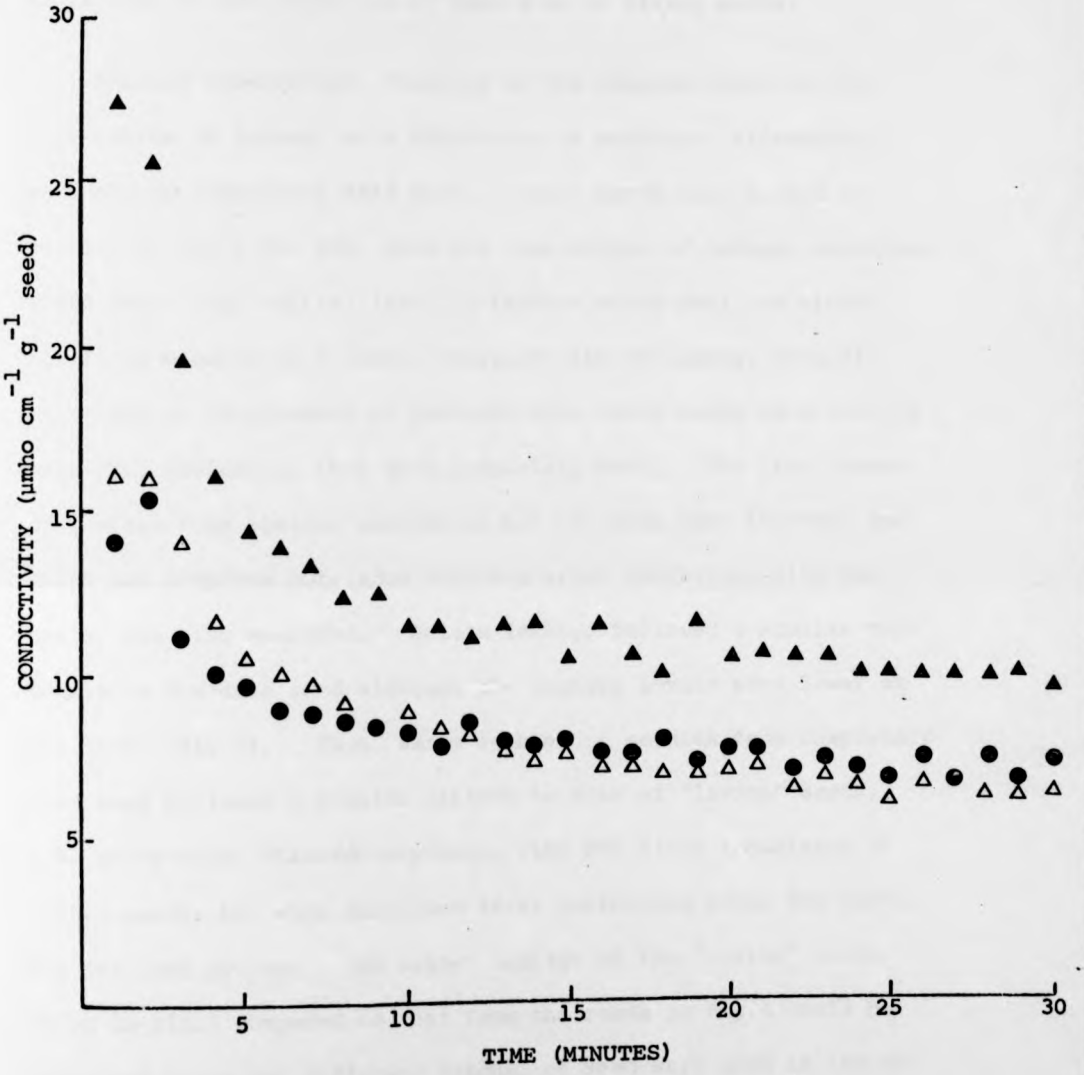


Fig.6 The time course of leakage from unstored seeds (-●-) and from seeds stored in 93% rh at 25°C for 5 (-Δ-) and 6 (-▲-) weeks.

time courses of leakage after storage were from damaged seed, with incomplete cotyledon staining, it was considered invalid to relate these observations of leakage during early stages of imbibition to the condition of membranes in living seeds.

Another observation, relevant to the consideration of the time course of leakage as a reflection of membrane reformation, was made on completely dead seed. These seeds were killed by heating at 105°C for 48h, then the time course of leakage measured. There was a high initial level of leakage which declined within the first minutes to a lower, constant rate of leakage (Fig.7). There was no development of formazan when these seeds were stained with TTC, indicating they were completely dead. The time course of leakage from similar seed which had not been heat treated, and which had complete cotyledon staining after imbibition plus the testa, was also measured. Solute leakage followed a similar time course to the dead seed although the leakage levels were lower at all times (Fig.7). Thus, early leakage of solutes from completely dead seed followed a similar pattern to that of "living" seeds, i.e. seeds which stained completely with TTC after imbibition of intact seeds, but were unstained after imbibition minus the testa for the time course. The higher leakage of the "living" seeds shown in Fig.7 compared to that from the seeds in Fig.6 could be explained since two different batches of seed were used in the two experiments.

The response of seed to storage in extreme humid conditions was influenced by the origin of the seed. Table 8 shows the response of three batches of seed representing different years of

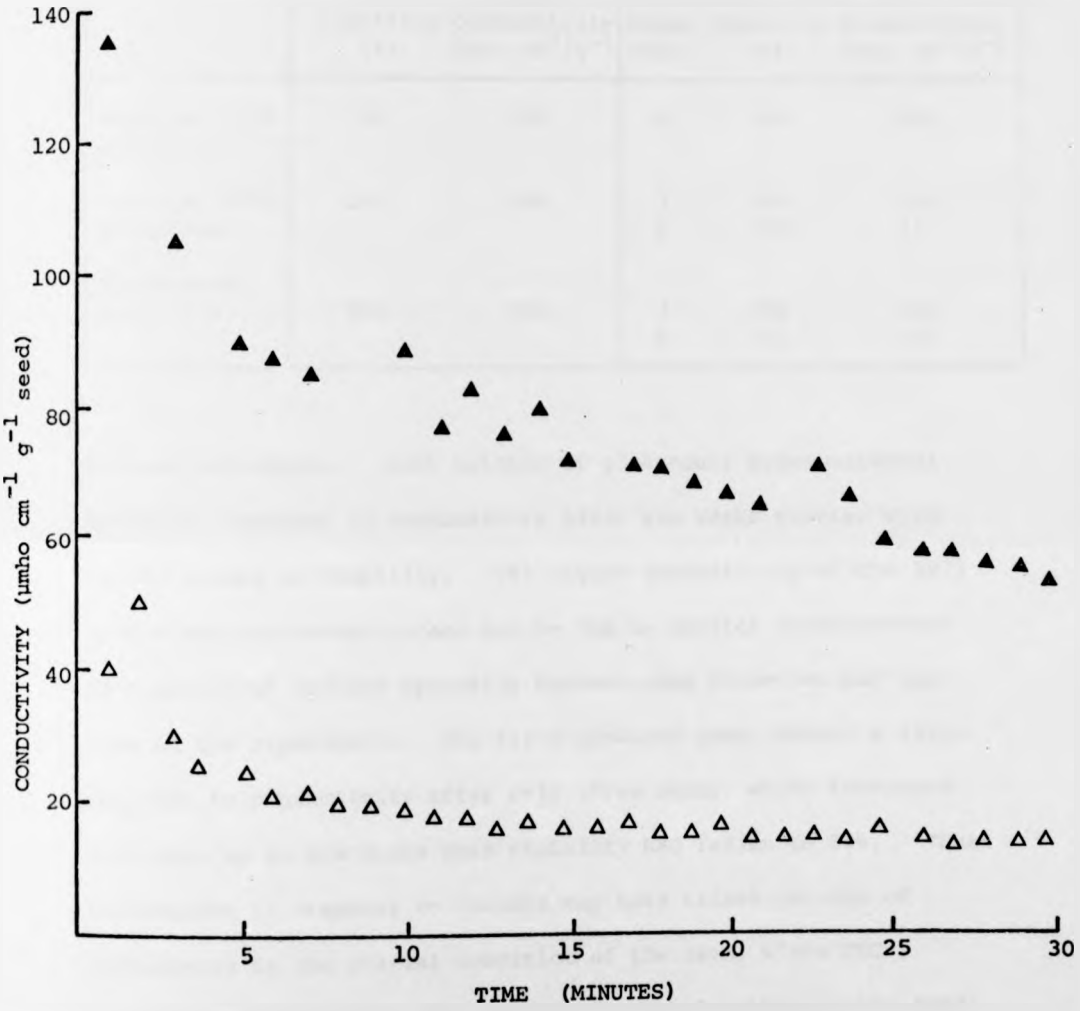


Fig.7 The time course of leakage from living (-Δ-) and dead (-▲-) seeds.

TABLE 8

Effect of storage in 93% rh at 25°C on pea seeds (cv Kelvedon Wonder) of different harvest date and origin

	Before storage		After storage		
	Viability (%)	Conductivity μmho $cm^{-1}g^{-1}$	Weeks stor.	Viability (%)	Conductivity μmho $cm^{-1}g^{-1}$
Stirling 1973 glasshouse	96	295	6	95	400
Stirling 1975 glasshouse	100	104	3	94	126
			6	98	153
Commercial seed 1974	100	295	3	94	430
			6	54	570

harvest and origin. Both batches of glasshouse grown material showed an increase in conductivity after six weeks storage with little change in viability. The higher conductivity of the 1973 seed after six weeks storage may be due to earlier deterioration as a result of factors operating between seed formation and the time of the experiment. The field produced peas showed a large increase in conductivity after only three weeks, which increased even more up to six weeks when viability had fallen to 54%. These differences in response to storage may have arisen because of differences in the initial condition of the seed, since TTC staining before storage was complete in the glasshouse grown seed (Table 9), whereas the field produced peas included seed with patchy and deeply stained cotyledons.

Storage of pea seeds in extreme dry and humid conditions resulted in deterioration of the seed in weeks (93% rh at 25°C; 1% rh at 10°C) or in days (94% rh at 45°C). The first evidence

TABLE 9

The initial condition of seed of different age and origin, revealed by the percentage of seeds in different staining categories after TTC staining

	Staining Category I	II	III	IV
Stirling glasshouse 1973	100	0	0	0
Stirling glasshouse 1975	100	0	0	0
Commercial seed 1974 field grown	17	38	25	21

For staining categories see Table 3

of deterioration was an increase in solute leakage from seeds into soak water, and an increase in the intensity of TTC staining, both of which occurred in completely living seed before loss of viability. These changes over a short period of storage in extreme conditions may reflect an acceleration of the normal processes of ageing. Examination of the response of a larger number of cultivars to short term storage would indicate whether such deterioration is of a general nature, and could therefore possibly be due to a common phenomenon such as ageing. Comparisons of short term deterioration with changes occurring during long term storage in less severe conditions, would illustrate whether the deteriorative changes observed, do in fact reflect an acceleration of natural ageing. This would also allow examination of the influence of initial embryo condition on seed behaviour in long term storage, since in the extreme conditions used, seeds in poor condition deteriorated rapidly in storage in comparison to seeds in initially good condition.

The evidence of increased solute leakage from living seeds in which there was no change in the availability of water soluble solutes, indicated changes within cells which influenced solute retention. One possible change was an increase in the membrane permeability, which could occur as a result of changes in the chemical composition of the membranes. It is possible, for example, that changes in the amount of phospholipids and in the classes of phospholipids present could be altered.

The examination of the time course of leakage, as an expression of membrane reformation during imbibition, after periods of storage was unsuccessful, since seeds were damaged after measurement of the time course. This raises the question of why seeds which were living after imbibition plus the testa, were dead after imbibition minus the testa. In addition, the pattern of leakage from completely dead seed, in which cell membranes are unlikely to be restricting leakage, was similar to that of "living" seeds. This might throw some doubt on the role of membranes in determining the pattern of early leakage from seeds.

In summary, the results of the effects of short term storage in adverse conditions on pea seeds has focussed attention on three further lines of investigation: the relation of short to long term storage, changes in seed phospholipid content during deterioration, and the effect of water on dry embryos.

The Effects of Long Term Storage

Short term storage of seed resulted in seed deterioration which was reflected initially by increased electrolyte leakage from living seed, followed by the development of dead tissue on the abaxial surfaces of the cotyledons, and eventually resulted in the loss of viability. The investigation of these changes was extended to 13 seed lots, covering six different cultivars (Table 10). The deteriorative changes occurring during short periods of storage in extreme conditions were compared to the changes that occurred during long term storage in conditions more closely resembling those of commercial practice. In addition, the responses of the seed lots to storage were compared, and the possibility that the short term deterioration of a lot could be used to predict long term storage performance was examined.

Thirteen seed lots of six cultivars that were commercially available for sale in 1975 were used for comparisons between short and long term storage. The initial condition of the seed lots before storage was assessed by measuring the leachate conductivities of individual seeds, followed by TTC staining, and showed considerable variation (Table 10). The extent of complete staining (category I) within a seed lot varied from 90% in DSP2 to only 25% in Sp2. Although only two lots included seeds with no staining (J1 and Sp2), there were five lots with more than 30% of the seeds showing incomplete staining (categories II and III). This variation in initial condition was also reflected in the conductivity of seed leachates, which ranged from 195 μmho cm^{-1} g^{-1} seed in Sp1 to 586 μmho cm^{-1} g^{-1} seed in Sp2 (Table 10).

TABLE 10

Initial condition of seed lots before storage, illustrated by leachate conductivity and TTC staining (percentage of seeds in each of four staining categories)

Staining category of abaxial surface of the cotyledons;

I: completely stained; II: less than 50% unstained;

III: more than 50% unstained; IV: no staining.

Each conductivity reading is the mean of 20 single seeds.

Seed lot		Conductivity µmho cm^{-} g^{-} seed	Staining category I	II	III	IV
Kelvedon Wonder (KW)	KW1	322.9	85	15	0	0
	KW2	380.9	70	30	0	0
Puget (P)	P1	255.6	80	20	0	0
	P2	425.2	80	15	5	0
Scout (Sc)	Sc1	326.7	65	30	5	0
	Sc2	333.3	80	20	0	0
	Sc3	382.6	80	20	0	0
Jade (J)	J1	560.3	30	50	15	5
	J2	329.9	50	40	10	0
Sprite (Sp)	Sp1	195.4	80	20	0	0
	Sp2	586.2	25	60	10	5
Dark Skinned	DSP1	212.1	85	15	0	0
Perfection (DSP)	DSP2	244.8	90	10	0	0
LSD ($p \leq 0.05$)		88.1				

The seed lots were placed in storage in a brick outhouse in May 1975 for 21 months, seeds being removed for testing in February and May 1976 (9 and 12 months storage, respectively), and in February 1977 (21 months storage). The seed moisture contents before and after storage were determined for five replicates of five seeds, and are shown in Table 11. The initial moisture contents of all lots were similar, all being around 9%. The moisture content of seeds increased in storage, indicating uptake of water from the surrounding atmosphere. The outhouse had been chosen to simulate warehouse storage conditions with the expectation that the relative humidity was low. The increase in moisture content showed that the relative humidity was perhaps higher than had been anticipated and therefore less favourable for storage. The seed moisture content fluctuated during the year (Table 11), being higher in both February samples compared to the May 1976 sample, probably due to changes in the relative humidity of the atmosphere. Variability in the moisture contents of the seed lots increased in storage, the range being greatest in the February samples; e.g. February 1976 varied from 24.3% in J2 to 34.4% in Sc3, compared to differences of from 16.3% in Sc3 to 19.7% in Sc2 in May 1976.

The viability, determined in germination tests in moist tissue at 20°C, of all seed lots declined over the 21 month storage period (Table 12). However, viability declined more rapidly in some lots than others, for example the germination of Sc.3 decreased from 100% to 30% in 12 months, whereas other lots (for example DSP2, KW2, P1) maintained their viability above 80% after 21 months storage.

TABLE 11

Moisture contents of seed lots (percentage oven dry weight) before and after outhouse storage

Each figure is a mean of five replicates of five seeds

Seed lot	Unstored seed (May 1975)	9 months storage (Feb 1976)	12 months storage (May 1976)	21 months storage (Feb 1976)
KW1	9.3	29.7	17.5	29.5
KW2	9.4	25.9	17.3	28.1
P1	9.8	31.7	17.0	30.6
P2	9.5	27.8	17.7	32.0
Sc1	9.5	26.4	16.9	26.4
Sc2	9.5	31.6	19.7	31.7
Sc3	9.6	34.4	16.3	27.5
J1	9.2	23.2	16.4	31.0
J2	9.3	24.3	16.1	30.0
Sp1	9.5	30.1	16.1	32.1
Sp2	9.3	27.4	17.8	36.3
DSP1	9.4	30.4	17.7	31.6
DSP2	9.5	26.8	16.8	31.8

TABLE 12

Viability and field emergence (as percentage of viability) of seed lots after 12 and 21 months outhouse storage

Seed Lot	Viability Months storage			Field emergence Months storage			
	0	12	21	0	12:sowing I	12:sowing II	21
KW1	96	80	70	44	75	59	8
KW2	92	92	82	36	92	74	9
P1	90	98	86	39	60	49	6
P2	93	74	68	23	75	55	3
Sc1	89	84	76	28	66	42	2
Sc2	95	42	16	31	100	55	2
Sc3	100	30	30	28	98	32	0
J1	93	68	26	13	65	23	8
J2	96	76	42	15	53	40	0
Sp1	97	90	72	33	60	44	1
Sp2	97	56	26	12	64	51	1
DSP1	98	86	66	67	86	70	23
DSP2	99	96	80	42	84	72	0

At each time of sampling, the field emergence was determined - for two sowing dates after 12 months storage, and for one sowing date before storage, and after 21 months storage. The emergence of seeds at the beginning of the storage period and after 12 months in store varied between different seed lots, even between those of similar viability (Table 12). Seed lots DSP1 and J2 had similar viabilities before storage, but in the first emergence trial, they had strikingly different field emergences of 67% and 15%, respectively. Similar contrasts in field emergence between lots of similar viability were also seen after 12 months storage, for example DSP1 and Sc1. Differences in field emergence of lots that possessed similar levels of viability, indicated vigour differences between them. After 21 months storage, the field emergence of all lots was very low, only exceeding 20% in one lot, DSP1 (Table 12). This suggested that even though viability was still greater than 80% in three seed lots, the seeds had deteriorated so much that the vigour of the seeds was very low, with a resultant low field emergence.

Comparisons of the field emergence of a lot after different periods of storage could not be fairly made because sowing conditions differed markedly between years and sowing dates. In 1975, an average of 4.2 mm of rain fell each day of the week preceding sowing, although only 0.7 mm per day fell in the week after sowing (Table 13). This resulted in a soil moisture content of 34% in soil which had a field capacity of 44%; the soil moisture content was therefore high during this period and in addition was associated with low temperatures (Table 13). These were the most severe weather conditions encountered in any field

TABLE 13

Soil temperatures (°C at 5cm) and rainfall (mm d^{-1}) in seven days immediately before and after sowing dates

	Soil Temperature °C at 5cm				Rainfall (mm per day)			
	Feb. 1975	Apr. 1976	May 1976	Mar. 1977	Feb. 1975	Apr. 1976	May 1976	Mar. 1977
Mean max 7 days before sowing	3.4	7.5	9.9	5.9	4.2	0	7.0	3.4
Day of sowing	3.2	7.1	13.1	4.5	1.5	0	1.1	0
Mean max 7 days after sowing	4.0	10.1	13.0	4.0	0.7	0	6.4	0.9

sowing and may account for the very low emergence of seed which had high viability (Table 12). The effect of high rainfall on field emergence was further illustrated in the two 1976 sowings. In periods immediately before and after the sowing dates, the soil temperature was higher in May than in April, which might have been expected to favour emergence. However, rainfall was very high in May, an average of 7.0mm per day falling before, and 6.4mm per day falling after sowing (Table 13). In contrast there was no rain over the equivalent period about the time of the April sowing. The very high rainfall in May would have produced a higher soil moisture content than occurred in April. The prime importance of soil moisture was exemplified by the fact that field emergence of all lots was lower in May (Table 12), and the variability observed between seed lots was greater (74% to 23%) compared to that in April (100% to 60%).

Measurements of seed leachate conductivity (Table 14) and of the TTC staining of the cotyledons (Table 15) were used to

TABLE 14

Leachate conductivities of seed lots after periods in outhouse storage

Each figure is a mean of 20 single seeds

Seed lot	Conductivity 0 storage	12 month storage	21 month storage
KW1	322.2	1044.6	1126.8
KW2	380.9	889.2	901.2
P1	255.6	772.1	836.5
P2	421.2	990.3	1422.4
Sc1	326.7	1075.9	1569.0
Sc2	333.3	1622.1	1675.9
Sc3	382.6	2180.9	1694.0
J1	560.3	1298.1	1529.0
J2	329.9	1213.2	1499.5
Sp1	195.4	646.4	1115.2
Sp2	586.2	1376.5	1915.1
DSP1	212.4	855.3	697.5
DSP2	244.8	625.9	984.9
LSD ($p < 0.05$)	88.1	320.1	358.7

TABLE 15

Percentage of seed in different TTC staining categories after outhouse storage

Months Storage / Seed lot	Staining Category											
	I			II			III			IV		
	0	12	21	0	12	21	0	12	21	0	12	21
KW1	85	65	40	15	25	20	0	10	40	0	0	0
KW2	70	40	35	30	50	35	0	10	30	0	0	0
P1	80	60	40	20	30	55	0	10	5	0	0	0
P2	80	50	20	15	40	40	5	10	20	0	0	20
Sc1	65	20	0	30	65	30	5	10	30	0	5	40
Sc2	80	10	0	20	50	0	0	30	0	0	10	50
Sc3	80	10	5	20	35	30	0	25	15	0	30	50
J1	30	20	10	50	65	40	15	15	40	5	0	10
J2	50	35	10	40	50	40	10	15	40	0	0	10
Sp1	80	60	15	20	40	50	0	0	10	0	0	25
Sp2	25	15	0	60	70	30	10	15	35	5	0	35
DSP1	85	60	35	15	25	50	0	15	10	0	0	5
DSP2	90	90	10	10	10	55	0	0	20	0	0	5

For staining categories see Table 10

to assess seed quality periodically during storage. Seed leachate conductivity increased in all seed lots with time (Table 14). Some lots had a large increase after 12 months storage, with little further change after 21 months (for example, KW2, P1, Sc2), whereas others showed a large increase at both times (P2, Sc1, Sp2).

The number of seeds with complete TTC staining (Table 15, category I) had declined in all seed lots after 12 and 21 months storage. The reduction in complete staining was rapid in some lots (Sc2 and Sc3), but others, for example DSP2, maintained a high percentage of completely stained seed after 12 months storage. There was a corresponding increase in the number of seeds with incomplete staining (categories II and III), although after 21 months, in some seed lots (Sc2, Sc3, Sp2), the number of seeds in these categories also declined. These lots had developed seeds that failed to stain on the abaxial surface of the cotyledons (category IV). The development of dead tissue on the cotyledons of all seed lots would explain the increase in leakage of solutes from the seeds during storage. Those seed lots which developed incompletely stained and dead seed early in storage, were those showing the greater increases in conductivity after both 12 and 21 months (for example, Sc1 and Sp2).

Seed in good initial condition with a large proportion of completely stained seed (Table 10) generally showed smaller conductivity increases and less development of dead tissue after long term storage than did those in poor initial condition. For example, DSP2 maintained a high level (90%) of completely stained seed after 12 months storage and had a relatively small conductivity increase (244 to 625 μmho cm^{-1} g^{-1} seed), whereas the percentage of completely

stained seed in Sc1 which was in poor condition initially, declined from 65% to 20%, and consequently conductivity showed a much larger increase from 326 to 1075 μmho cm^{-1} g^{-1} seed over the same period. This suggested that the initial seed condition may be a factor which influences seed response to storage. The percentage of completely stained seed before storage was taken as an indication of initial seed condition and correlated with viability after storage. The initial seed condition was positively correlated with viability after 12 and 21 months storage ($r = 0.58$ and 0.76, $p \leq 0.05$ and 0.01 respectively). Thus seeds with good initial staining retained high viability after prolonged storage, whereas the viability of seeds with poor initial condition declined. However, some seed lots which had similar conductivities and staining before storage (Table 10: KW1 and Sc2; KW2 and Sc3), showed different patterns of deterioration, the two Scout lots deteriorating more rapidly than the Kelvedon Wonder seed lots (Tables 14 and 15). Apparently similar seed lots may therefore differ in their tolerance to storage. These different responses to storage did not seem to be related to the different moisture contents of the seed lots after storage (Table 11).

Therefore, over a period of 21 months storage, all seed lots showed deterioration, reflected in increased solute leakage, the development of dead tissue on the cotyledons, and a reduction in viability, although the rate of deterioration varied between seed lots.

Changes in leachate conductivity and TTC staining of these seed lots were also measured after storage for six weeks in 93% rh at 25°C (Table 16) and for up to three days in 94% rh at 45°C

TABLE 16

Leachate conductivity and TTC staining (percentage seeds in each staining category) of seed lots after six weeks storage in 93% rh at 25°C

Each conductivity reading is the mean of 20 individual seeds

Seed lot \ Weeks storage	Conductivity µmho cm^{-1} g^{-1} seed		Staining Category							
			I		II		III		IV	
	0	6	0	6	0	6	0	6	0	6
KW1	322.2	1028.0	85	3	15	70	0	23	0	6
KW2	380.9	938.5	65	3	35	68	0	20	0	9
P1	255.6	836.2	80	20	20	65	0	15	0	0
P2	425.2	1321.8	80	10	15	53	5	25	0	12
Sc1	326.2	1702.4	65	0	30	0	5	38	0	62
Sc2	333.3	1726.8	80	2	20	10	0	40	0	48
Sc3	382.6	1724.1	80	0	20	15	0	15	0	70
J1	560.3	1770.6	30	0	50	8	15	18	5	74
J2	329.9	1547.5	50	0	40	12	10	25	0	63
Sp1	195.4	1686.9	80	0	20	35	0	40	0	25
Sp2	586.2	1606.6	25	0	60	18	10	45	5	37
DSP1	212.1	911.2	85	28	15	22	0	35	0	15
DSP2	244.8	939.1	95	2	5	38	0	43	0	17
LSD ($p \leq 0.05$)	88.1	245.8								

For staining categories see Table 10

TABLE 17

Leachate conductivity of seed lots after storage in 94% rh at 45°C

Each conductivity reading is the mean of 20 individual seeds

Seed lot	Days storage in 94% rh at 45°C			
	0	1	2	3
KW1	322.2	611.6	1080.7	1508.7
KW2	380.9	874.1	1242.2	1573.0
P1	255.6	522.2	1155.5	1631.0
P2	425.2	1000.8	1207.9	1526.6
Sc1	326.7	917.6	1455.6	1749.3
Sc2	333.3	934.1	1447.3	1725.1
Sc3	382.6	1117.1	1691.4	1785.2
J1	560.3	1213.0	1560.9	2020.2
J2	329.9	1021.3	1625.1	1874.4
Sp1	195.4	609.5	1196.6	1869.9
Sp2	586.2	960.7	1390.1	1944.4
DSP1	212.1	647.8	953.6	1135.5
DSP2	244.8	494.8	766.2	1137.5
LSD ($p \leq 0.05$)	88.1	208.1	112.7	168.8

TABLE 17

Leachate conductivity of seed lots after storage in 94% rh at 45°C

Each conductivity reading is the mean of 20 individual seeds

Seed lot	Days storage in 94% rh at 45°C			
	0	1	2	3
KW1	322.2	611.6	1080.7	1508.7
KW2	380.9	874.1	1242.2	1573.0
P1	255.6	522.2	1155.5	1631.0
P2	425.2	1000.8	1207.9	1526.6
Sc1	326.7	917.6	1455.6	1749.3
Sc2	333.3	934.1	1447.3	1725.1
Sc3	382.6	1117.1	1691.4	1785.2
J1	560.3	1213.0	1560.9	2020.2
J2	329.9	1021.3	1625.1	1874.4
Sp1	195.4	609.5	1196.6	1869.9
Sp2	586.2	960.7	1390.1	1944.4
DSP1	212.1	647.8	953.6	1135.5
DSP2	244.8	494.8	766.2	1137.5
LSD ($p \leq 0.05$)	88.1	208.1	112.7	168.8

TABLE 18

The percentage of seeds of different seed lots in four TTC staining categories after storage in 94% rh at 45°C

Seed lot \ Days storage	Staining Category															
	I				II				III				IV			
	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3
KW1	85	50	0	0	15	40	55	5	0	10	30	15	0	0	15	80
KW2	70	25	5	0	30	40	15	0	0	30	40	5	0	5	40	95
P1	80	45	0	0	20	50	50	5	0	5	30	15	0	0	20	80
P2	80	15	0	0	15	40	40	0	5	25	30	20	0	20	25	80
Sc1	65	10	0	0	30	55	20	0	5	25	15	5	0	10	60	95
Sc2	80	20	0	0	20	30	10	0	0	25	25	0	0	20	65	100
Sc3	80	10	0	0	20	30	0	0	0	35	35	0	0	20	65	100
J1	30	0	0	0	50	45	5	0	15	30	25	5	5	25	70	95
J2	50	5	0	0	40	40	5	0	10	35	20	5	0	20	75	95
Sp1	75	20	0	0	20	55	25	0	0	15	35	0	0	10	40	100
Sp2	25	10	0	0	60	50	5	0	10	35	50	15	5	5	45	85
DSP1	90	55	8	10	10	15	30	30	0	25	40	35	0	5	20	25
DSP2	85	65	20	0	15	30	50	0	0	5	20	65	0	0	10	35

For staining categories see Table 10

(Tables 17 and 18). After both storage treatments, seed leachate conductivity increased (Tables 16 and 17), and the number of completely stained seed (category I) declined (Tables 16 and 18), whilst the proportion of seed with incomplete staining (categories II and III) increased. After six weeks in 93% rh at 25°C and three days in 94% rh at 45°C, most of the seeds of each seed lot had no staining (category IV; Tables 16 and 18).

The response of seed to both these extreme storage environments was again related to initial seed condition, lots with poor initial staining deteriorating most rapidly in storage (for example, J1 and Sp2). However, assessments of initial condition, such as cotyledon staining, were not always accurate indicators of a seed lot's tolerance of short term storage in adverse conditions: the staining and conductivity of KW1 and Sc2, which were similar before storage (Table 10) were considerably different after six weeks in 93% rh at 25°C (Table 16) and one day in 94% rh at 45°C (Tables 17 and 18).

Thus, both long and short term ageing resulted in similar deteriorative changes, and as the changes during long term storage are essentially those of natural ageing, the extreme short term storage conditions could be described as accelerated ageing. The relationships between the changes in the two situations were compared for the 13 seed lots, using leachate conductivity as a measure of deterioration in accelerated ageing, and conductivity, viability and field emergence as a measure of changes in long term storage.

The leachate conductivities after natural and accelerated

ageing were positively correlated (Table 19, Fig.8), which suggested that seed lots responded similarly under both sets of conditions. The viability of the seed lots after natural ageing in long term storage was negatively correlated (12 months, $r = -0.71$; 21 months, $r = -0.69$) with the seed response to two days accelerated ageing (Fig.9, Table 19). This relationship indicates that seed lots which responded to accelerated ageing with a high conductivity showed reduced viability after 12 or 21 months storage, that is, they had poor storage potential; a low conductivity after accelerated ageing was associated with high viability after storage. Seed response to accelerated ageing for one day on 94% rh at 45°C and six weeks in 93% rh at 20°C showed similar correlations with viability to that after 2d in 94% rh at 45°C (Table 19).

The conductivity of unstored seed did not relate to the seed lots viability, conductivity or field emergence after 12 months, although there was a positive correlation of this conductivity measurement with viability and conductivity after 21 months. Thus, the initial leachate conductivity could not be used to predict seed lot response to 12 months storage; this response could only be predicted after accelerated ageing. Seed lot storage potential over a longer period could perhaps be predicted by initial conductivity. However, there is an increased correlation of the conductivity after accelerated ageing with viability after long term storage. Thus, even long term storage potential might be predicted more usefully by conductivity after accelerated ageing than by initial conductivities. The absence of a correlation between initial conductivity of unstored seed, and that

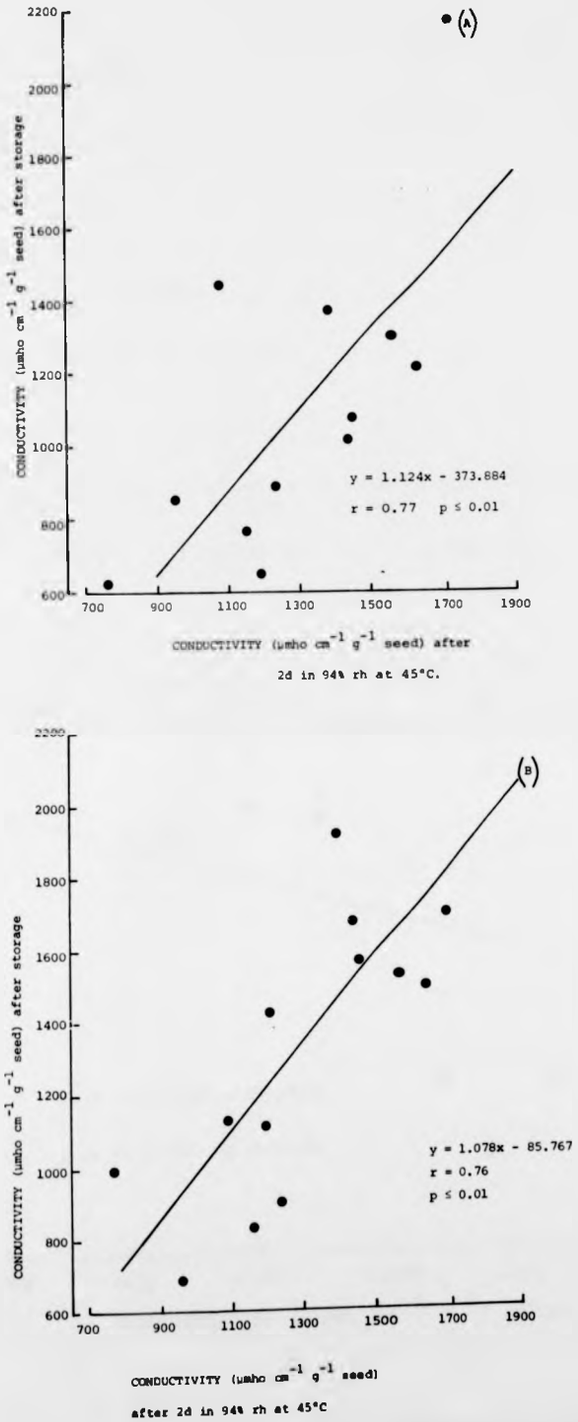


FIG.8 The relationship between the solute leakage from seeds after storage for 2d in 94% rh at 45°C (accelerated ageing) and that after prolonged storage for (A) 12 months and (B) 21 months

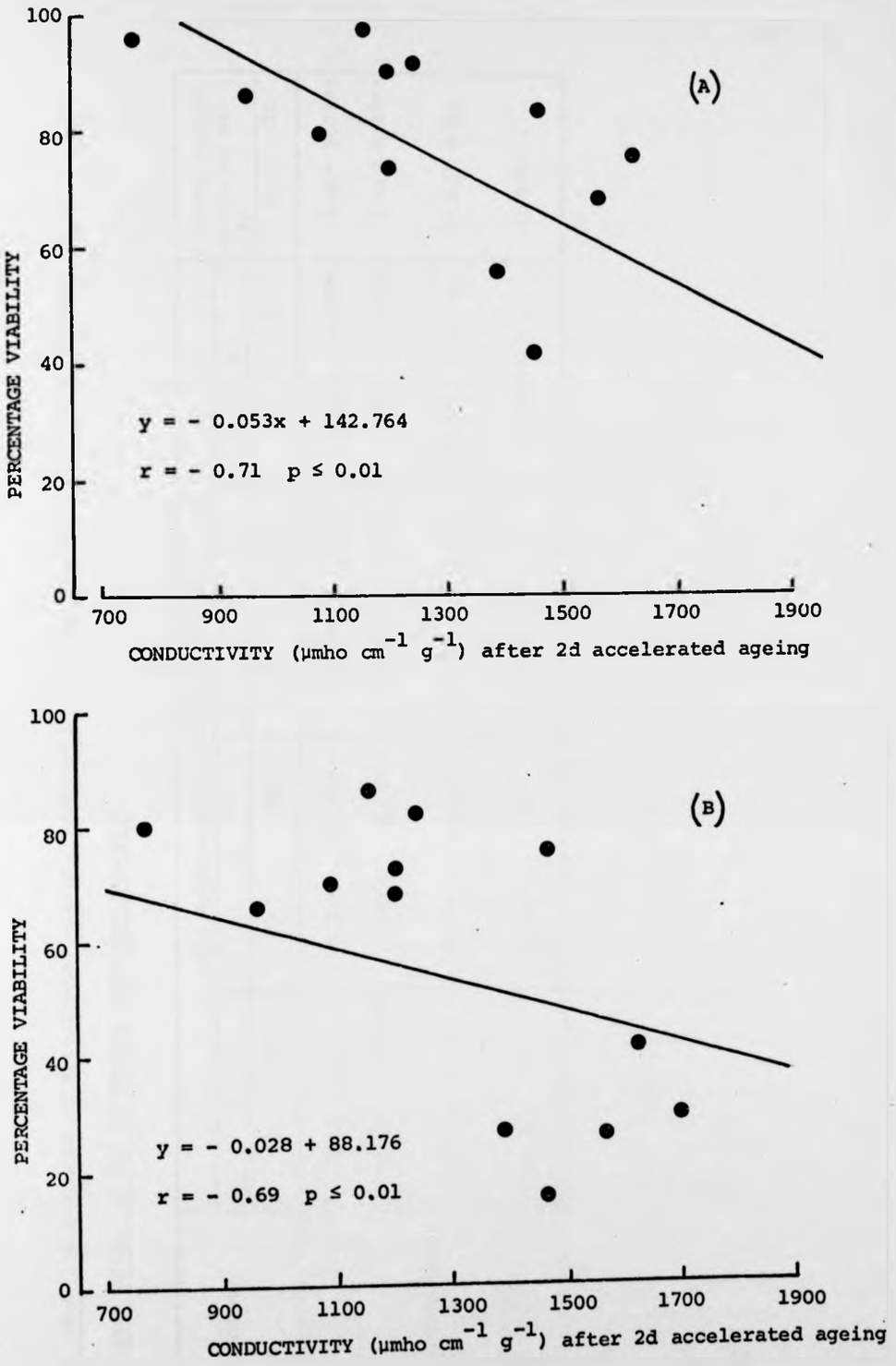


Fig.9 The relationship of seed lot response to accelerated ageing (2d in 94% rh at 45°C) to viability after long term storage for (A) 12 months and (B) 21 months.

TABLE 19

Correlation of seed lot response to accelerated ageing, measured by leachate conductivity, with various measurements taken after 12 and 21 months outhouse storage

Months storage	Days accelerated ageing in 94% rh at 45°C								6 weeks ageing in 93% rh at 25°C	
	0		1		2		3			
	12	21	12	21	12	21	12	21	12	21
Viability	-0.52	-0.60*	-0.74**	-0.71**	-0.71**	-0.69**	-0.46	-0.57*	-0.66*	0.71**
Conductivity	0.49	0.69**	0.72**	0.75**	0.77**	0.76**	0.47	0.71*	0.61*	0.85**
1st planting emergence (as % viability)	-0.08	-0.20	-0.07	-0.23	-0.10	-0.39	-0.43	-0.49	-0.13	0.52
2nd planting emergence (as % viability)	-0.41	-	-0.62*	-	-0.79***	-	-0.81***	-	-0.77**	-

* $p \leq 0.05$

** $p \leq 0.01$

*** $p \leq 0.001$

after 12 months long term storage might be explained by the smaller variation in the conductivity of the seed lots in unstored seed. No stress had been placed on these seeds which would draw out vigour differences which could lead to different storage potential.

Conductivity after accelerated ageing treatment for three days also did not correlate with long term storage response (Table 19). Most seeds were dead after 3 days accelerated ageing, and therefore differences in solute retention between seed lots, which reflected vigour differences would be unlikely.

Seed lot response to accelerated ageing was also negatively correlated with field emergence after the May 1976 sowing (Fig.10, Table 19). After two and three days accelerated ageing, the correlation between conductivity and field emergence at the May 1976 sowing was highly significant, indicating that lots with a high conductivity after accelerated ageing had low field emergence after storage. It was interesting to note that after the April 1976 sowing, emergence was not related to accelerated ageing conductivity (Table 19). Weather conditions immediately before and after this sowing date were more favourable to field establishment, since for all seed lots emergence was higher in April than it was after the May 1976 sowing (Table 12). The lack of correlation between emergence and conductivity suggested that under favourable conditions for emergence, vigour differences have less influence on field emergence. This leads to less variability between seed lots than under more stressful conditions, such as after the high rainfall seen in the May 1976 sowing when greater variability in field emergence occurred. The absence of any correlation between the 1977 sowing and accelerated ageing could also be explained by

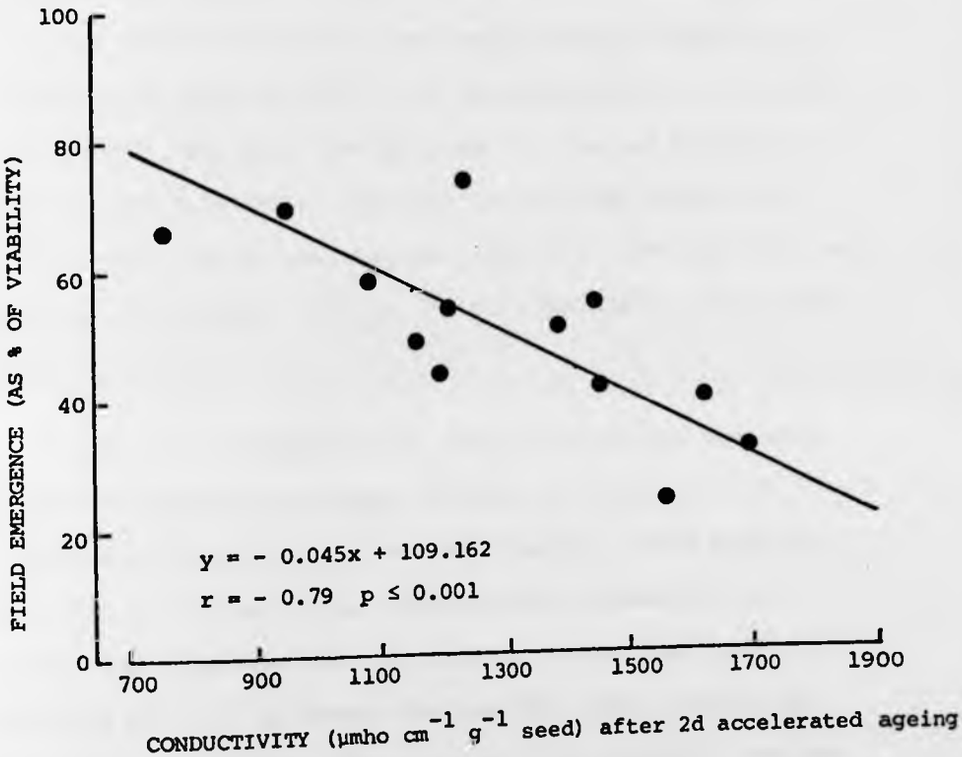


Fig.10 The relationship of seed lot response to accelerated ageing (2d in 94% rh at 45°C) to field emergence after storage for 12 months.

lack of variability in the seed lots. In this case however, this was the result of low field emergence in all lots due to low viability and vigour.

The response of thirteen seed lots to prolonged storage was examined following storage in commercial warehouses. Of these lots, only four, namely KW2, J1, P1 and Scl, were also used in the storage experiments in Stirling. Conductivity measurements of bulk seed samples were made on seed from commercial storage in November 1974, 1975 and 1976. All thirteen lots were sampled in 1974 and 1975, but only three of these were carried over by the seed merchant into 1976. The seed lots had been harvested in 1973 and 1974, and in each sampling year (1974, 1975 and 1976) were commercially available for sale, having a germination greater than 80%.

There was an increase in the conductivity of the soak water of twelve samples after storage for one year (Fig.11). The conductivity of one lot, J1, declined slightly. Some seed lots harvested in 1974 had higher conductivities, indicating lower vigour, than other seed lots of the same cultivar that had been harvested in 1973, for example DSP7 and KW7 (1974 harvest) had higher conductivities than DSP5 and KW4 (1973 harvest). Further storage of three seed lots (DSP4, DSP6 and KW3) to 1976 resulted in an even greater conductivity increase (Fig.11).

The TTC staining of the abaxial surfaces of the cotyledons of the thirteen seed lots held in commercial storage showed an increase in the percentage of seeds with incomplete staining (Table 20) after storage from 1974 to 1975, more seeds appearing in

Fig. 11

Conductivity of the soak water of bulk pea seed samples measured in 1974 ( ) and, following commercial storage, in 1975 ( ) and 1976 ( ).

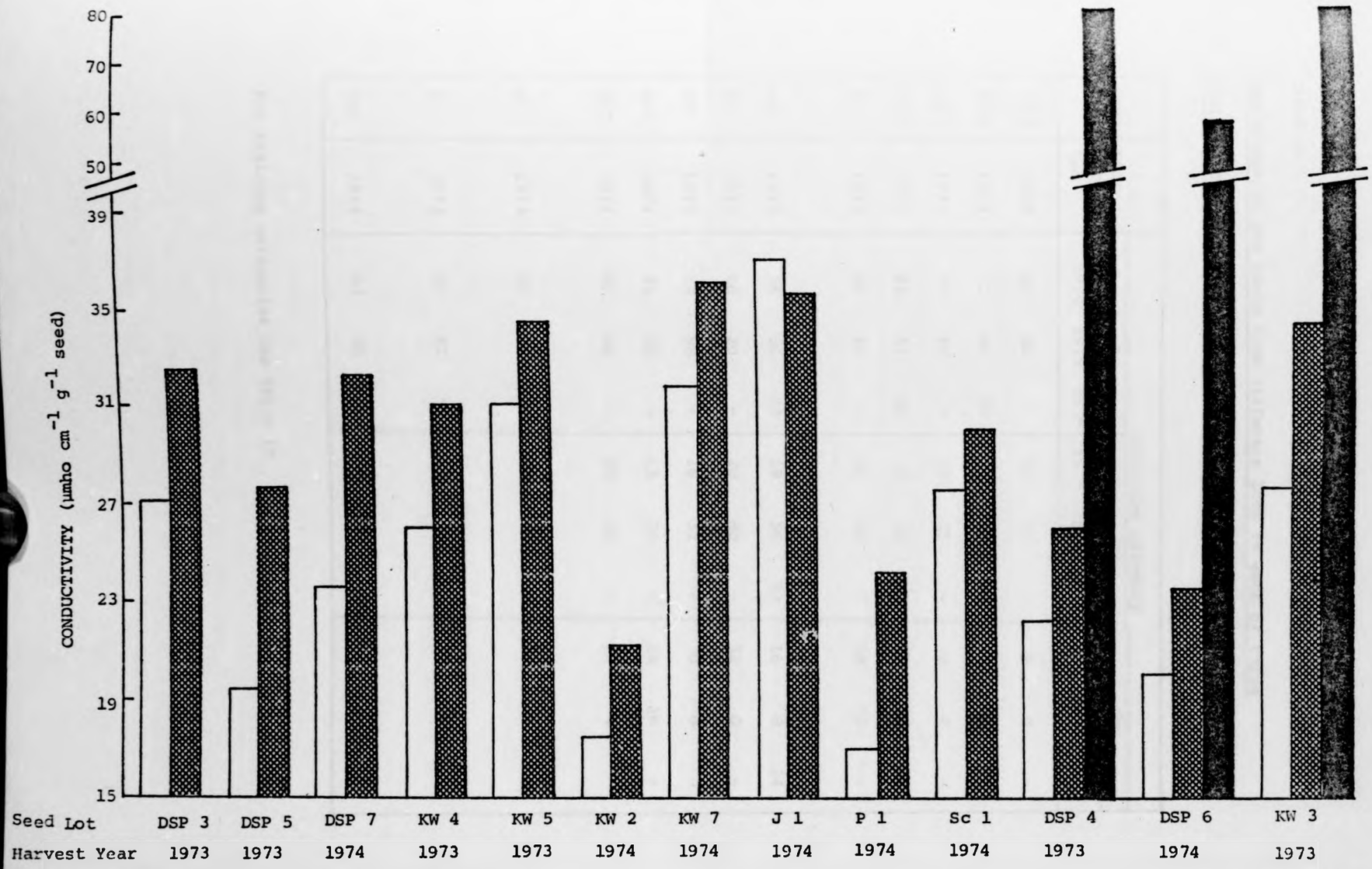

80
70
60
50
39
35
31
27
23
19
15
CONDUCTIVITY (µmho cm⁻¹ g⁻¹ seed)
Seed Lot
Harvest Year
DSP 3 1973
DSP 5 1973
DSP 7 1974
KW 4 1973
KW 5 1973
KW 2 1974
KW 7 1974
J 1 1974
P 1 1974
Sc 1 1974
DSP 4 1973
DSP 6 1974
KW 3 1973

TABLE 20

Percentage of pea seeds from different lots in each of three staining categories after storage for up to three years

Seed lot	Year harvested	Staining category								
		I			II			III		
		1974	1975	1976	1974	1975	1976	1974	1975	1976
DSP3	1973	40	48	-	52	52	-	8	0	-
DSP4	1973	72	46	51	20	24	25	8	20	24
DSP5	1973	72	40	-	20	56	-	8	4	-
DSP6	1974	84	52	45	8	32	43	8	16	12
DSP7	1974	60	40	-	16	48	-	24	12	-
KW3	1973	56	36	33	28	56	33	16	8	34
KW4	1973	60	20	-	28	80	-	12	0	-
KW5	1973	84	68	-	16	32	-	0	0	-
KW2	1974	72	56	-	12	28	-	16	16	-
KW7	1974	56	66	-	28	30	-	16	4	-
J1	1974	60	44	-	28	47	-	12	9	-
P1	1974	60	62	-	16	29	-	0	9	-
Sc1	1974	64	48	-	36	40	-	0	12	-

For staining categories see Table 1

categories II and III. These increases in incomplete staining and increased conductivity of seed soak water were indicative of reduced seed vigour, although laboratory germination was greater than 80%. In some cases, the 1973 seed showed initially more complete staining, that is, more seed in category I, than did seed produced in 1974, which was surprising since newly harvested seed might have been expected to be in better condition, for example KW4 and DSP5 (1973 harvest) had more complete staining than KW7 and DSP7 (1974 harvest) respectively. Therefore, seed lots entering storage were in poorer condition than was found in other lots at the end of one year storage. This suggested that the lots harvested and put into store in 1973 had been in very good condition to start with, and retained this condition one year later in 1974.

Thus, increased conductivity and reduced staining were seen in seed lots after periods in commercial stores. There was considerable variability between lots in both conductivity and staining despite the fact that all lots retained viability of greater than 80%.

The deterioration of four seed lots (KW2, J1, P1 and Sc1) in commercial storage could be compared to their deterioration in both short and long term storage in Stirling. In commercial storage, although the viability of all four lots remained above 80%, the seed lots could be divided into two pairs with respect to their extent of deterioration, based on conductivity and staining. Two lots, KW2 and P1 retained good staining after one year commercial storage, and although conductivity increased, it was still lower than many other lots (Fig.11). The other two lots,

Scl and Jl had poor staining after a year storage, and also high conductivities. Thus, Pl and KW2 deteriorated slowly, and Jl and Scl rapidly in commercial storage. Rapid deterioration of Jl and Scl had also been noted in prolonged outhouse storage, with larger increases in conductivity (Table 14) and more incompletely stained seeds (Table 15) than Pl or KW2 after storage for 12 and 21 months. The response of seed lots to short term adverse storage conditions also pointed to more rapid deterioration in Scl and Jl, whether deterioration occurred over weeks (Table 16) or days (Tables 17 and 18). The similarity in the response of these seed lots to prolonged storage in commercial warehouses and to long term storage in Stirling, suggested that the different rates of deterioration observed in the more adverse conditions of outhouse storage reflected those in commercial conditions, even though deterioration was occurring more rapidly. The observations of similar deteriorative responses to short term storage and prolonged commercial storage, added further support to the suggestion that storage in adverse conditions for short periods, accelerates the natural ageing process.

The response to accelerated ageing after one day storage in 94% rh at 45°C was measured in the autumn of 1974 for seed lots that had been harvested in the summer of 1974, that is, seeds which would not have undergone much deterioration during commercial storage. Conductivity increased after accelerated ageing (Table 21), and this increase was significantly correlated ($r = 0.80$, $p \leq 0.05$, Fig.12) with the increase in conductivity which occurred after one year commercial storage.

Thus, deteriorative changes reflected in increased leachate conductivities, reduced TTC staining, and a decline in viability,

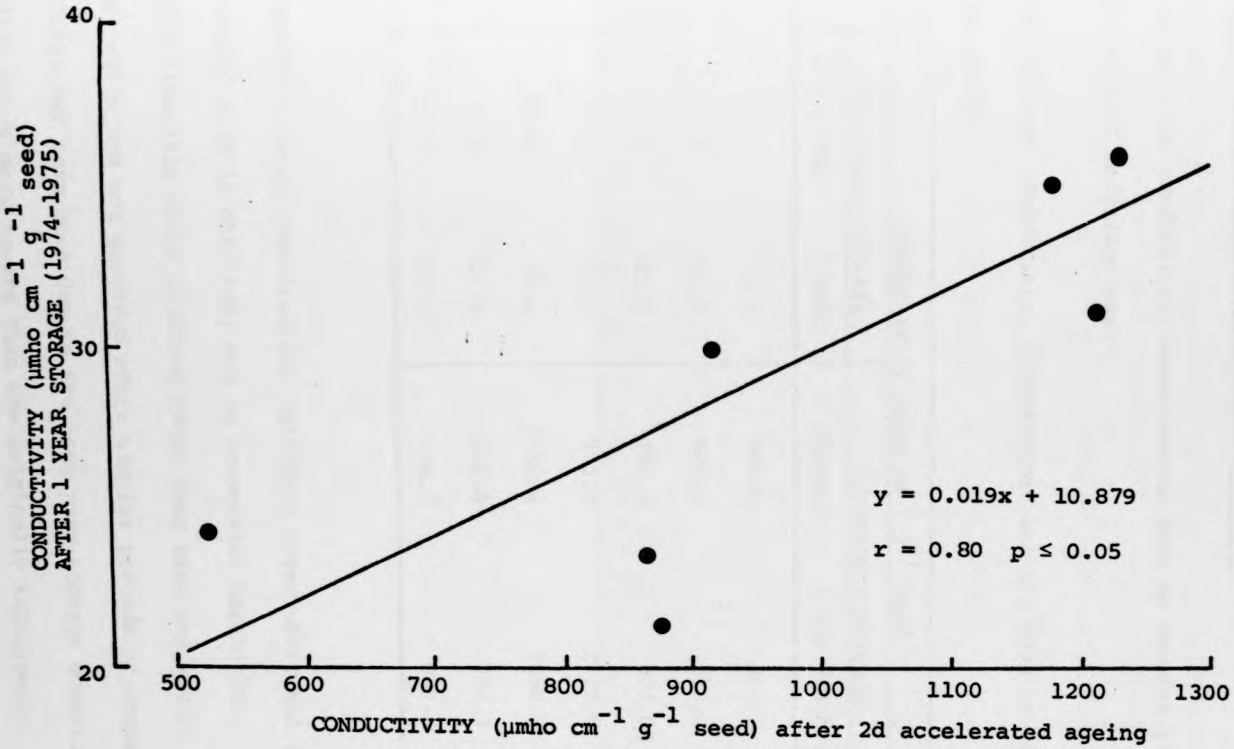


Fig.12 The relationship between seed lot response to accelerated ageing (2d in 94% rh at 45°C) before storage, and leachate conductivity following 1 year in commercial storage.

TABLE 21

Leachate conductivity before and after both laboratory and commercial storage of seed lots harvested in 1974

Commercial storage conductivity measurements made on samples of 25 seeds in 200ml deionised water.

Laboratory storage conductivity measurements are the mean of 20 individual seeds

	Conductivity μmho cm^{-1} g^{-1} seed			
	Commercial storage		Laboratory storage	
	0 storage	1 year	0 storage	1 day 94%rh 45°C
DSP7	20.2	23.6	900.8	862.8
DSP6	23.5	32.7	843.0	1213.4
KW2	17.6	21.3	380.9	874.06
KW7	31.8	36.1	753.3	1226.6
J1	36.9	35.6	1060.8	1180.9
P1	16.8	24.4	255.6	522.2
Sc1	27.7	30.3	326.7	917.6

were observed in seed lots from six cultivars after short and long term storage, both in Stirling, and in commercial warehouses. The decline in viability which occurred after long term storage in Stirling, which was not observed after similar periods of commercial storage, may have been due to the long term storage conditions in Stirling being more severe than was originally anticipated. A significant positive correlation between conductivities determined after periods of short and long term storage suggested that the same deteriorative changes were occurring in both situations,

and that short term storage in extreme conditions was accelerating the natural ageing process. The viability and field emergence of seed lots after storage were related to their response to accelerated ageing before storage, those with low viability and field emergence after storage, having high conductivities after accelerated ageing. Although initial seed condition was to some extent related to storage potential, seed response to accelerated ageing gave a more accurate prediction of storage potential.

Deteriorative changes indicative of reduced vigour occurred before loss of viability in commercially stored seed. Thus, seed lots which are commercially available for sale (with germinations greater than 80%) may have reduced vigour as a result of periods of commercial storage.

The Effects of Storage on Phospholipids

Short term storage of seeds in warm moist conditions led to reduced solute retention in living cells which was reflected in increased leakage of electrolytes from seeds. It was suggested (p.45) that this increased leakage after accelerated ageing was caused by an increase in membrane permeability as a result of the deterioration of cell membranes. An increase in membrane permeability may result from changes in the chemical composition of the membranes, either in the total amounts of the components present, or in the proportions of the different chemical constituents. Phospholipids are one of the major components of membranes, therefore, the phospholipid composition of seeds during accelerated ageing was examined to see if changes in this particular component of membranes could result in increased membrane permeability.

The conditions of 94% rh at 45°C were used to age the seeds. These conditions had been shown to result in increased leakage from viable seeds (Fig.4) before dead tissue developed on the cotyledons. Thus, any increases in leakage observed, would have occurred from living cells. In order to avoid making phospholipid comparisons between seeds composed of different proportions of living cells, the effects of the storage treatment on the condition of the seed were also examined by TTC staining, along with the phospholipid analyses.

Seeds from four seed lots were aged for one, two and three days in 94% rh at 45°C. One seed lot was produced in glasshouses in Stirling in 1975 (KW75) and was initially in good condition, having a low 24h conductivity (104 μmho cm^{-1} g^{-1} seed) and 95% of the seed with complete TTC staining. The other three

seed lots were commercial lots from three cultivars (Spl, DSP2, KW1) all of which were in good condition (Table 10) but varied in their rate of deterioration in long term storage (Tables 12, 14 and 15).

Tetrazolium staining after 24h imbibition was used to examine the extent of deterioration of the seed lots after periods of storage in 94% rh at 45°C. The four seed lots could be divided into two pairs with respect to the rate of deterioration (Fig.13); KW75 and KW1 retained complete staining over a longer period and therefore deteriorated more slowly than DSP2 and Spl. After one day of the storage treatment, both KW75 and KW1 had similar staining to that before storage, with 95% and 70% of the seed respectively, having complete staining. After two days, there was a reduction in the extent of complete staining although the majority of the seeds were still completely stained in both lots. Only 20% of KW1 seeds were completely stained after three days storage, i.e. 80% had developed dead tissue on the cotyledons, and although most KW75 seed still had complete staining, this was very faint indicating that only a small proportion of the cells were reacting with TTC, therefore it was considered that many of the cells of these seeds were dead. Although both lots of Kelvedon Wonder had similar patterns of deterioration, the percentage of KW1 seeds with complete staining was always lower than that of KW75. This may indicate that KW1 seeds were in poorer condition to start with; possibly some deterioration had already taken place. Few of the seeds of the other two seed lots, Spl and DSP2, were completely stained after only one day storage (Fig.13), that is, deterioration was rapid and dead tissue had developed on the seed

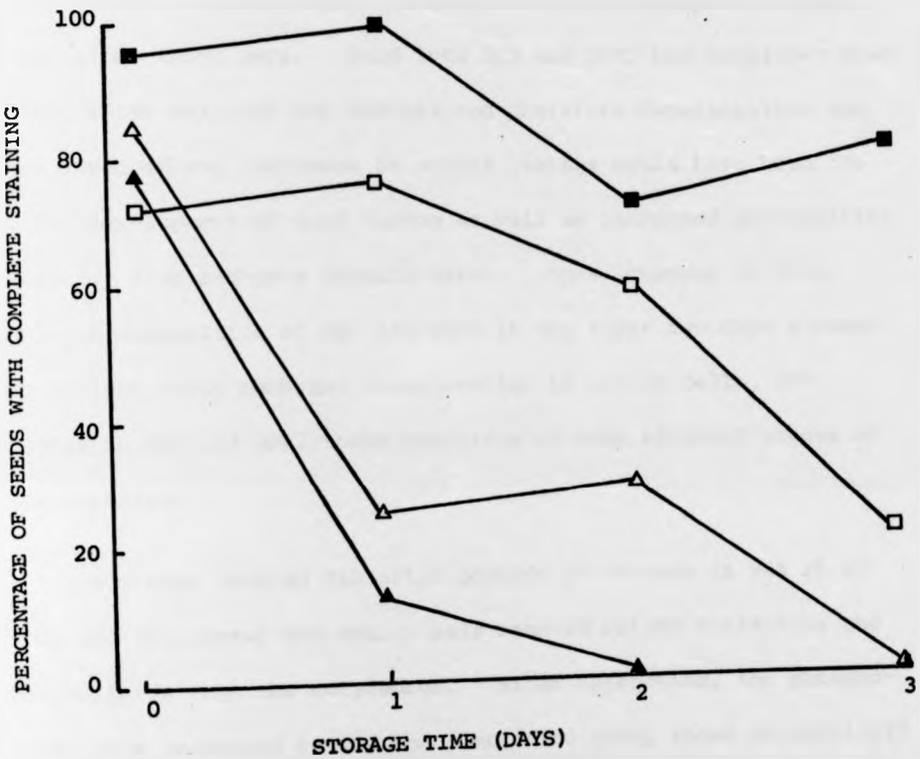


Fig.13 Changes in the percentage of seeds from four seed lots with complete TTC staining after storage in 94% rh at 45°C.

KW 75 (-■-); KW 1 (-□-);

DSP 2 (-Δ-); Sp 1 (-▲-).

within one day. The percentage of seeds with complete staining had declined to zero after two days storage of Spl and three days storage of DSP2 (Fig.13).

Changes in phospholipid composition could be attributed to changes within living cells in seed lots KW1 and KW75 only during the first two days storage, since dead tissue developed on these seeds after three days. Seed lots Spl and DSP2 had developed dead tissue after only one day storage, and therefore deterioration was more advanced and increases in solute leakage could have been due to the development of dead tissue as well as increased permeability resulting from membrane deterioration. Thus, changes in phospholipid composition of KW1 and KW75 in the first two days storage may reflect early membrane deterioration in living cells, and changes in Spl and DSP2 those occurring at more advanced stages of deterioration.

Seeds were imbibed 24h after periods of storage in 94% rh at 45°C, and the testae and embryo axis removed before extracting the phospholipids from the cotyledons. After extraction, the phospholipids were separated by TLC and identified using known phospholipid standards. The standards used were phosphotidylcholine (PC), phosphatidyl inositol (PI), phosphatidic acid (PA), phosphatidyl glycerol (PG) and phosphatidyl ethanolamine (PE). Phosphatidyl ethanolamine ran at the top of the TLC plate followed by PA, PG, PC and PI. The concentration of phospholipids present was measured by scraping the phospholipid spots off the TLC plate, and determining the phosphorus content of each spot. Phosphorus determinations were also conducted on the site of application of the phospholipid extract at the origin of the TLC plate.

No consistent sequence of change was seen in the total phospholipids extracted from the four seed lots (Fig.14) and few of the changes observed were statistically significant. Changes in the pattern of total phospholipids in KW75 were different to those in the other three lots (DSP2, Sp1, KW1). The most notable difference was the large increase in total phospholipids of the three lots after two days storage; this increase seemed contrary to what appeared to be a decrease in the total phospholipid after one and three days storage. Phospholipid extractions of these three lots were done at the same time, whereas extractions from KW75 were done separately. Some feature of extraction may have resulted in this increase in total phospholipid in three seed lots which did not occur in KW75.

Identification of the phospholipid classes which made up the total showed that five phospholipids, which corresponded with the known phospholipids on the TLC plate, occurred in all seed lots (Fig.15 and 16), that is, in descending order of amounts present, PC, PI, PG, PA and PE. In addition to the identifiable phospholipids, three other spots (U1, U2 and U3) developed on the TLC plates after storage, which did not correspond to any of the standards used.

The effects of the first two days storage on KW75 and KW1 was especially interesting as any changes in phospholipids must have occurred in living cells. Both these lots showed many similarities in the changes occurring in the proportion of the phospholipid classes. The amount of phosphorus occurring at the origin of the TLC plate showed little change during the first two days storage, which would suggest that there was no increase in

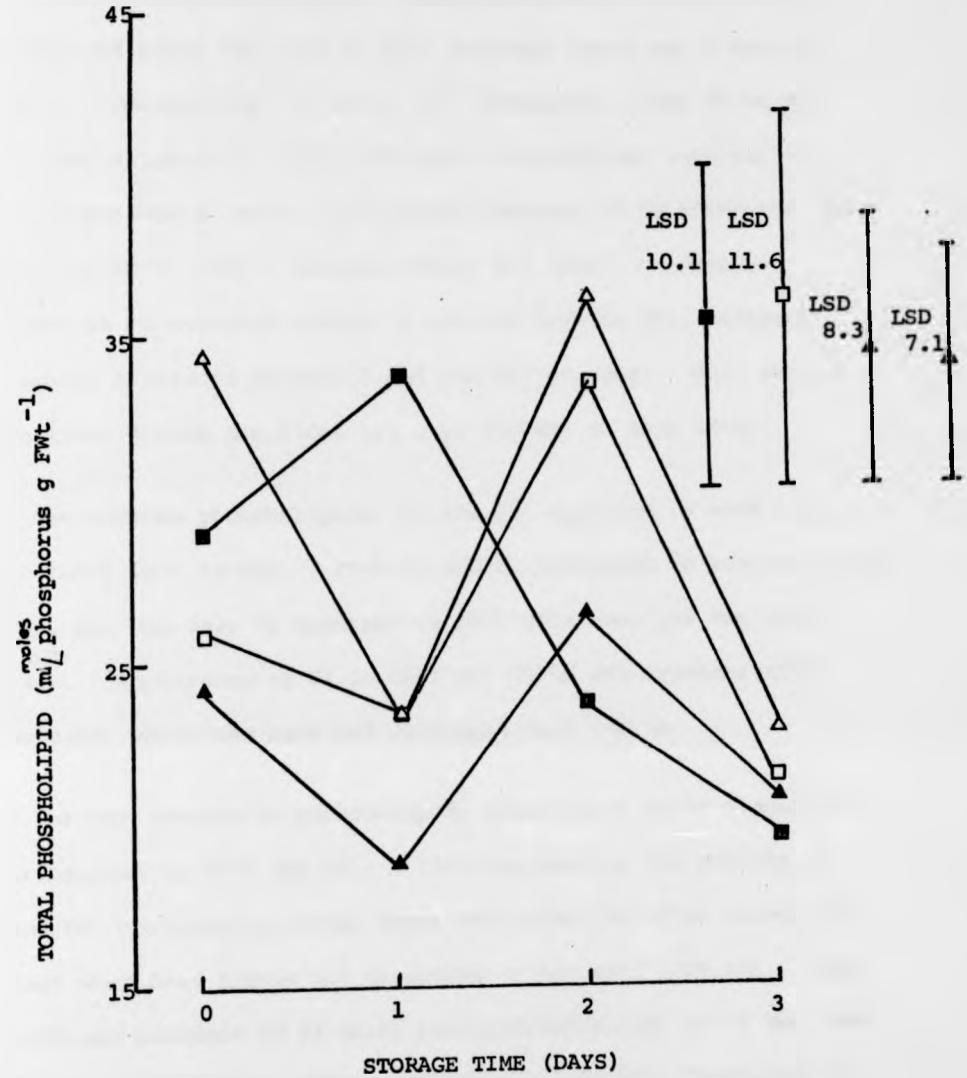


Fig.14 Changes in the total phospholipid extracted from four seed lots following storage in 94% rh at 45°C.

KW 75 (-■-); KW 1 (-□-);

DSP 2 (- Δ-); Sp 1 (- ▲-).

the polar lipids present. The amount of PC present increased in both KW1 and KW75 after one day storage (Fig.15). This increase was enhanced after two days in KW1, although there was a decrease in KW75. Phosphatidyl inositol (PI) increased in the first two days, only slightly in KW75, but with a significant increase in KW1. There was a large, significant increase in PG after one day storage of KW75, with a decrease after two days. A smaller increase in PG occurred between 0 and two days in KW1, although the amount decreased between 0 and one day storage. Both PE and PA declined during the first two days storage of both lots.

Two unknown phospholipids (U1 and U2) appeared in both lots, and a third (U3) in KW1. Both U1 and U2 developed in living tissue in KW1, whereas only U2 appeared in KW75 after one and two days storage. Appearance of U1 in KW75 and U3 in KW1 occurred after three days, when the seed had developed dead tissue.

Further changes in phospholipids occurred at later stages of deterioration in KW75 and KW1. The decreases in the amounts of PC and PG, observed in living seeds were enhanced after three days storage when dead tissue had developed on the seed (Fig.15). The significant increase in PI which had occurred in KW1 after two days, now appeared in KW75. The occurrence of a similar change one day later in KW75 than in KW1 might imply that deterioration was occurring more slowly in KW75 than KW1. The less complete staining of KW1 at all times during storage, lends support to this view. After three days storage, a small increase in the amount of phosphorus at the origin, suggested that more polar lipids were present as dead tissue developed. Such phospholipid changes in seeds which had developed dead tissue on their cotyledons, might

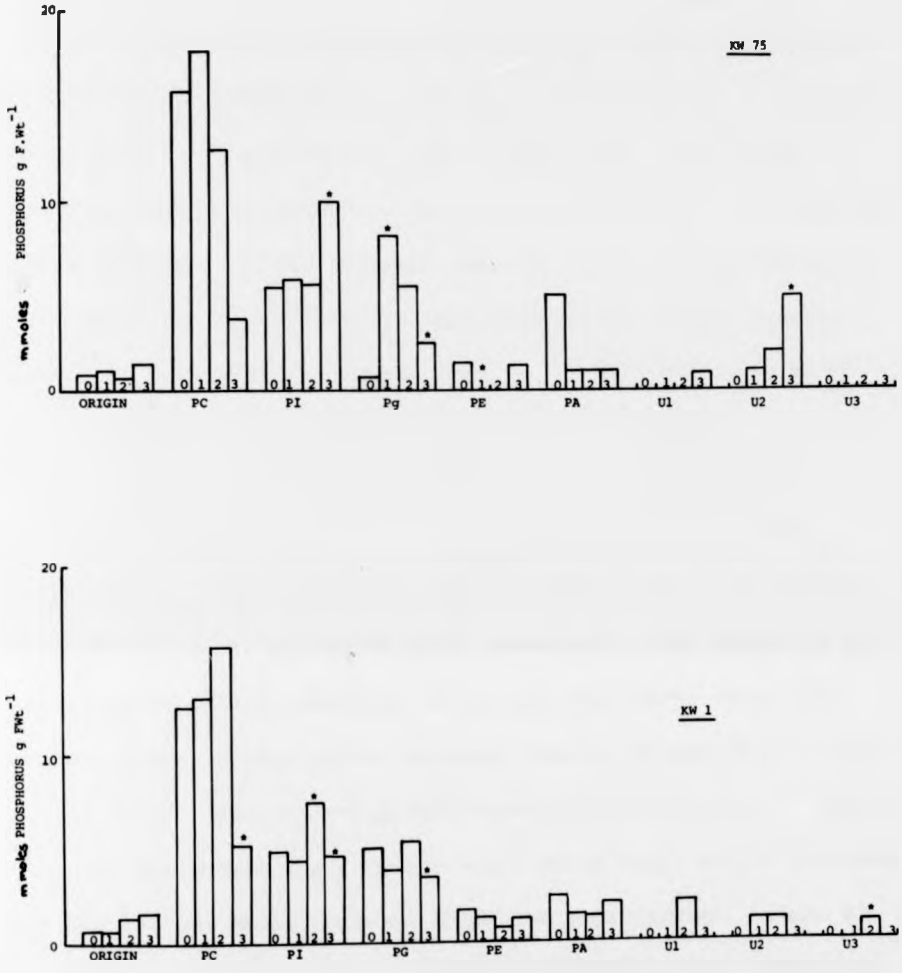


FIG.15 Changes in the proportions of different phospholipids in two seed lots, KW75 and KW1, following 0, 1, 2 and 3 days storage in 94% rh at 45°C.

PC = phosphatidyl choline; PI = phosphatidyl inositol; PG = phosphatidyl glycerol; PE = phosphatidyl ethanolamine; PA = phosphatidic acid; U1, U2 and U3 = three unidentified phospholipids

* = significant at $p \leq 0.05$

suggest further disorganisation of membrane composition at later stages of ageing.

Few seeds of the other two seed lots (DSP2 and Spl) had complete staining after only one day storage, therefore all phospholipid changes observed occurred at an advanced stage of deterioration, when increased solute leakage from seeds could not be solely attributed to membrane damage in living cells. The similar pattern of deterioration in these two lots (DSP2 and Spl) revealed by TTC staining (Fig.13) was further illustrated by very similar patterns of phospholipid changes (Fig.16). Although these seeds showed advanced deterioration, the amount of phosphorus at the origin (Fig.16) was similar to that in the less deteriorated Kelvedon Wonder seed lots. The most notable changes in the phospholipid classes that occurred in Spl and DSP2, were in PC which decreased in amount, and in PA which increased. The amount of PC present showed a large decrease in Spl and DSP2 after three days storage; prior to this large increase, smaller decreases in PC had occurred in Spl whereas levels fluctuated in DSP2 (Fig.16). Levels of PA were low before the increase after three days, with a decrease after one day and small increase after two days storage. Both PI and PG had similar patterns of changes over three days. The levels of PE were low in both DSP2 and Spl, although showing a small decrease followed by an increase. Two unidentified phospholipids (U1 and U3) appeared after storage, and a third (U2) was present before storage.

The fact that there was little change in the total phospholipid extracted from seeds stored in 94% rh at 45°C suggested that deterioration did not involve gross degradation of phospholipids at

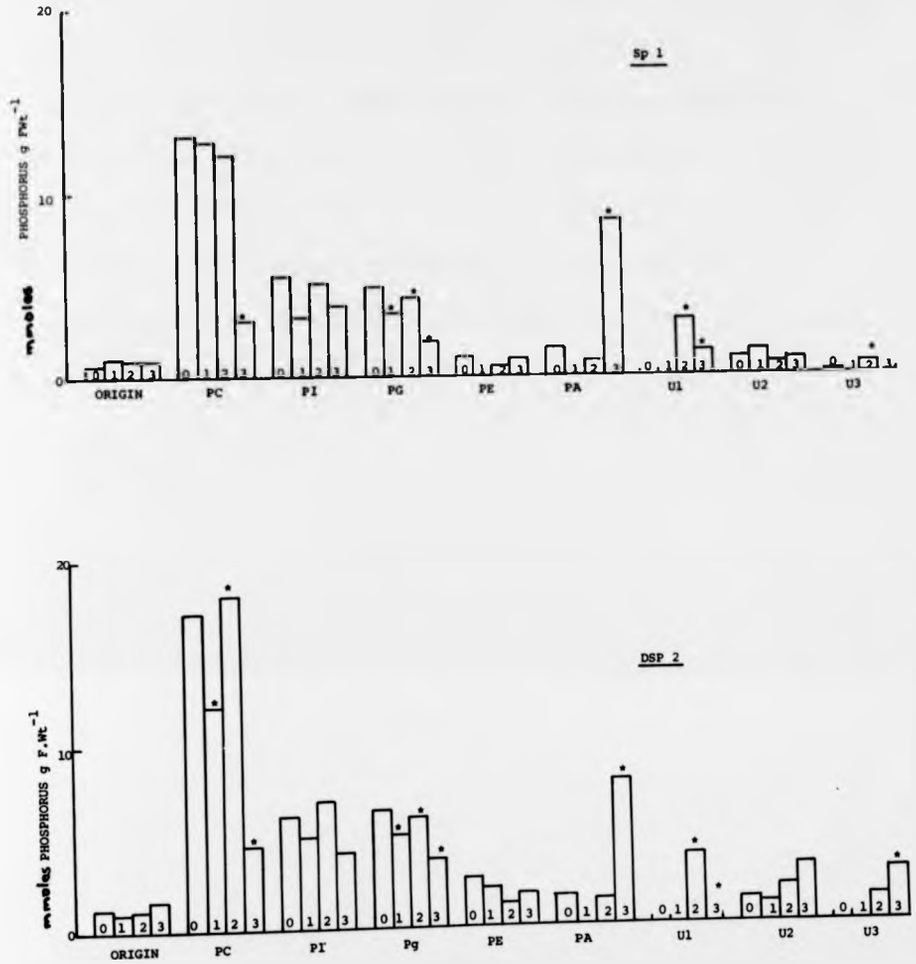


FIG.16 Changes in the proportions of different phospholipids in two seed lots, Spl and DSP2, following 0, 1, 2 and 3 days storage in 94% rh at 45°C.

PC = phosphatidyl choline; PI = phosphatidyl inositol; PG = phosphatidyl glycerol; PE = phosphatidyl ethanolamine; PA = phosphatidic acid; U1, U2 and U3 = three unidentified phospholipids

* = significant at $p \leq 0.05$

either early or advanced stages. This was supported by the observation that there was no increase in the amount of polar lipids present at the origin of the TLC plates. The main changes observed during deterioration were in the proportions of the different phospholipid classes present. Small changes were associated with early deterioration in two of the lots (KW75 and KW1) in which little development of dead tissue had taken place, and membrane deterioration may have occurred. These changes involved increases in the amount of PC and PI present, and decreases in PG, PE and PA.

The Effect of Water During Imbibition

When the time course of leakage was determined for dry embryos in the short term ageing experiments, the abaxial surfaces of the cotyledons showed incomplete vital staining with TTC (p.41). These seeds had been imbibed with their testae removed. The same seed produced complete staining of the abaxial surface of the cotyledons when they were imbibed intact, that is, plus the testa. This observation raised the question of what was causing the damage seen in seeds after measurements had been taken of the time course of leakage in the absence of the testa.

In order to examine the influence of the testa on water uptake, the rates of imbibition of seeds, plus and minus the testa, were compared. The seeds used in this, as in all subsequent imbibition experiments were glasshouse grown in Stirling in 1975 (Table 8). The testa was removed from the seed by chipping it off the dry embryo. Intact seeds took up little water in the first 4h, after which the rate of imbibition showed a more rapid and linear rate of increase up to 24h (Fig.17). In contrast, the embryos imbibed minus the testa took up water very rapidly from the first 30 minutes in water, showing a linear increase up to 9h, when imbibition was almost complete (Fig.17). After 24h, the percentage weight increases of 140% in embryos, and 100% in intact seeds suggested that the intact seeds had not completed water uptake at 24h.

The staining of the embryo with TTC showed little development of the red formazan on the abaxial surfaces of the cotyledons (Table 22), 80% of the seeds showing no staining (category IV). Failure to produce formazan indicated dead cells, since formazan is formed through the reduction of TTC by cell dehydrogenases (Roberts, 1951;

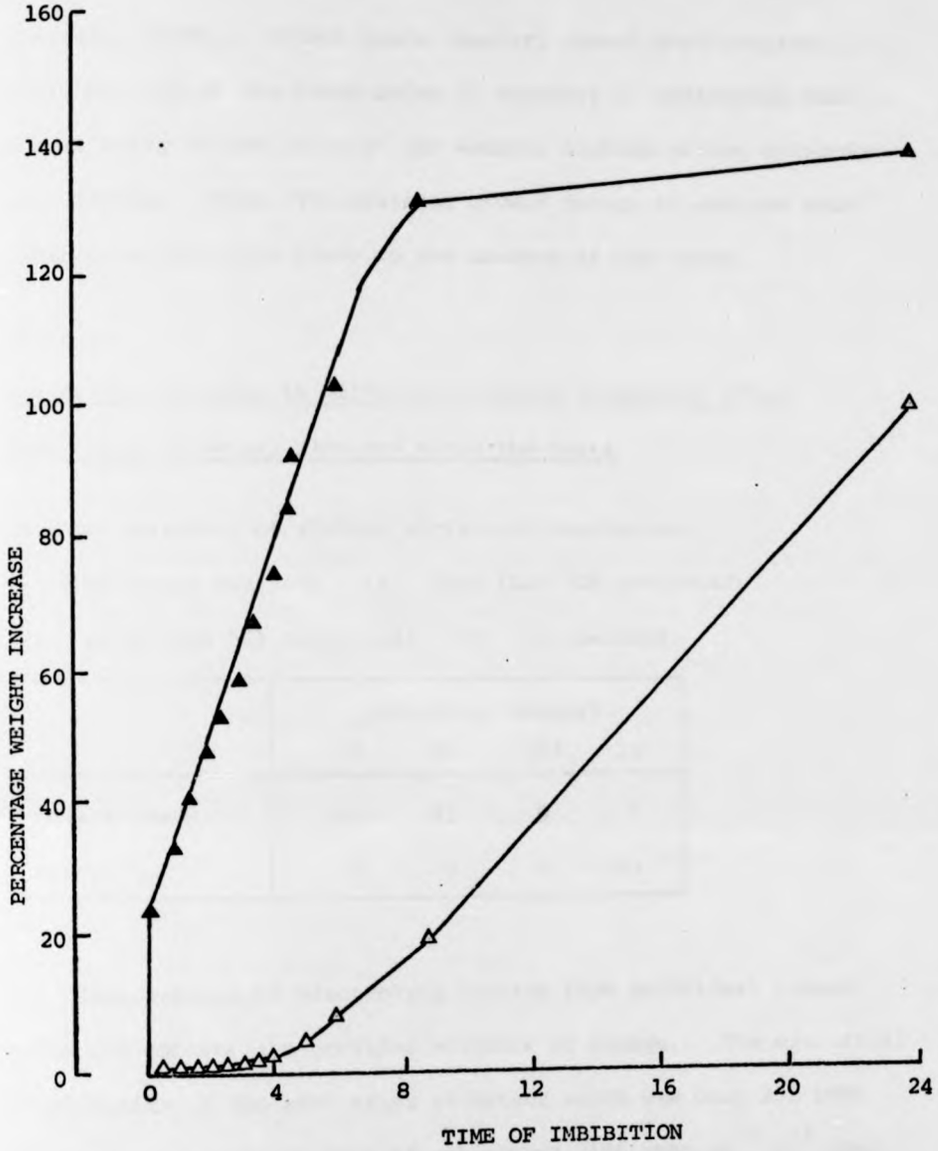


Fig.17 The rate of imbibition of intact seeds (-Δ-) and embryos with the testa removed (-▲-).

Cottrell, 1948). Intact seeds however, showed more complete staining, 63% of the seeds being in category I, indicating that the majority of the cells on the abaxial surface of the cotyledon were living. Thus, TTC staining showed damage to embryos when imbibition had taken place in the absence of the testa.

TABLE 22

Percentage of seeds in different staining categories after imbibition in water, plus and minus the testa

Staining category of abaxial surface of cotyledons;
I: completely stained; II: less than 50% unstained;
III: more than 50% unstained; IV: no staining.

	Staining Category			
	I	II	III	IV
Intact seeds	63	32	0	5
Embryo	0	0	10	80

Measurements of electrolyte leakage from individual intact seeds and embryos also provided evidence of damage. The electrical conductivity of the soak water of intact seeds was only 271 µmho cm^{-1} g^{-1} seed, whereas that of embryos was 2106 µmho cm^{-1} g^{-1} seed; such high levels of solute leakage from seeds have been associated with dead or damaged tissue (Matthews, 1971; Matthews and Rogerson, 1976). Although the testa was not removed from the intact seed before the conductivity was measured, it has been shown (Simon, 1974), that following imbibition, dissection of the testa amd embryo apart in fresh water does not result in a great release of electrolytes. Simon (1974) suggested that the testa did not just act as

a barrier to solute release, but reduced the extent of leakage from the embryo itself. The large difference in leakage observed here between intact seeds and bare embryos was therefore unlikely to be due to a restriction of electrolyte loss by the testa; the damage may have resulted from the effect of imbibition itself.

Damage was also revealed by differences in the respiration rates of intact seeds and embryos. After 24h imbibition, the testae were removed from intact seeds, and the oxygen uptake of seeds, that had been imbibed plus the testa, and of embryos, that had been imbibed minus the testa, was measured. The respiration rate was reduced in embryos to only half that of intact seeds (Table 23). This reduced respiration was further evidence of lower enzyme activity, in addition to that provided by reduced TTC staining.

TABLE 23

Respiration rates ($\mu l\ O_2\ g^{-1}\ h^{-1}$) of peas after imbibition in water, plus and minus the testa

Each replicate is made up of three seeds

Replicate	Plus testa	Minus testa
1	551.2	255.2
2	551.7	247.9
3	550.0	211.9
4	416.3	185.2
5	632.3	315.6
Mean	540.3***	205.2

*** Means significantly different ($p \leq 0.001$) as indicated by t test

Imbibition minus the testa seemed to be associated with damage to the embryo which was expressed in several forms: reduced TTC staining, increased electrolyte leakage, and reduced oxygen uptake.

The association of damage with the absence of the testa during imbibition was investigated further by removing half the testa from seeds, so that during imbibition, half the embryo was in direct contact with water, and half covered by the testa. After 24h in water, the remaining half of the testa was removed before staining with TTC for 3h. The half of the embryo that was not covered by the testa during imbibition showed little staining (Plate 1), whereas the half that had been covered by the testa was well stained. This suggested that damage occurred only when the embryo was in direct contact with water during imbibition. The damage to the embryo may have been caused by testa removal from dry seed. However, mechanical damage was clearly discernible (Plate 1), and differed from the overall damage observed to the half of the embryo which had been imbibed minus the testa. Mechanical damage was eliminated by removing the testae from mature seeds, which had not dehydrated in the pod, and slowly dehydrating the embryos over calcium chloride. The dry embryos produced in this way were imbibed 24h in water and stained with TTC; the same overall damage observed after imbibition of embryos from which the testa had been removed when dry, still occurred.

The possibility that the rapid water uptake occurring in embryos imbibed minus the testa (Fig.17), was responsible for the damage to these embryos was tested by reducing the rate of water uptake during imbibition using solutions of a polyethylene glycol, namely Carbowax 4000. Thirty, 20, 15, 10 and 5% solutions of

PLATE 1. Tetrazolium chloride staining of seeds following imbibition with the testa removed from the lower half of each seed.

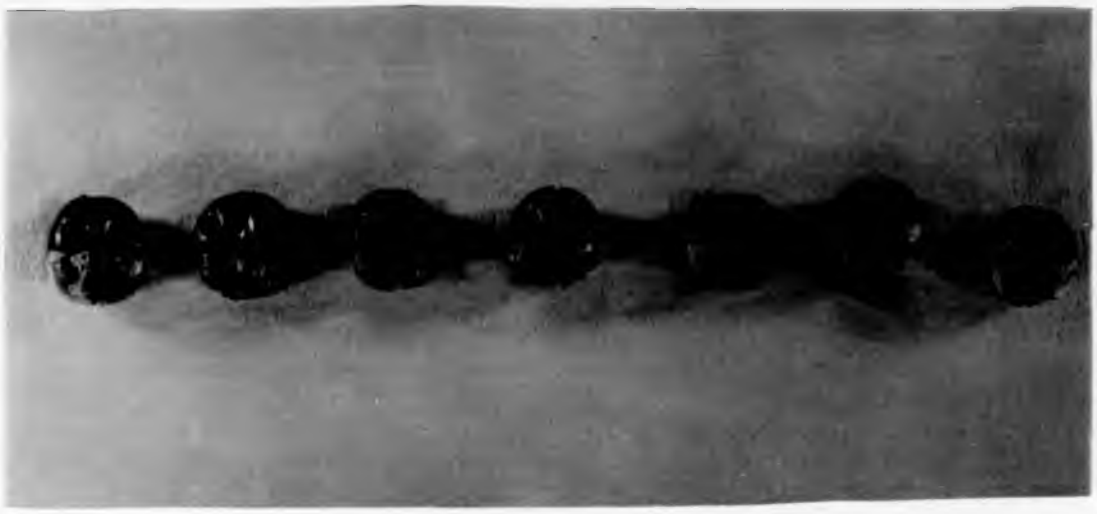


PLATE 1. Tetrazolium chloride staining of seeds following imbibition with the testa removed from the lower half of each seed.

Carbowax 4000 in water were used to give solutions of -19.0, -7.3, -4.5, -2.7 and -1.5 bars respectively (Mexal, Fisher, Osteryoung and Reid, 1975). Embryos were imbibed in solutions of Carbowax for 6h, during which time most of the imbibition of embryos has usually taken place (Fig.17), before transfer to water for 24h. Two Carbowax treatments were used, either 30% Carbowax for 6h, or 30% Carbowax for 2h, followed by 1h in each of 20%, 15%, 10% and 5% Carbowax, that is, in solutions with gradually ascending water potentials. Imbibition in 30% Carbowax was much slower than in water, with only a 60% weight increase after 6h compared to 140% in water (Fig.18). After transfer to water from the 30% Carbowax solution, embryos showed a large rapid weight increase of 60% within the first hour. When the decreasing Carbowax concentrations were used, the rate of imbibition during the first 3h was the same as that in 30% Carbowax, and then increased to a similar rate to that of embryos in water (Fig.18). After transfer to water at 6h, there was a 38% increase - the same increase as in the previous hour in Carbowax. Thus, the rate of water uptake in water after transfer of embryos from 5% Carbowax to water was no faster than it was in 5% Carbowax. When seeds were imbibed in solutions of ascending water potential, a sharp increase in rate of water uptake such as that occurring in the embryos imbibed in water and after transfer from 30% Carbowax to water, was to some extent avoided.

Damage to embryos imbibed in different water potentials was assessed using vital staining rather than loss of electrolytes, because of the effect of Carbowax on leakage (Appendix I). Tetrazolium staining of embryos which had been given different imbibition treatments during the first 6h imbibition, was examined

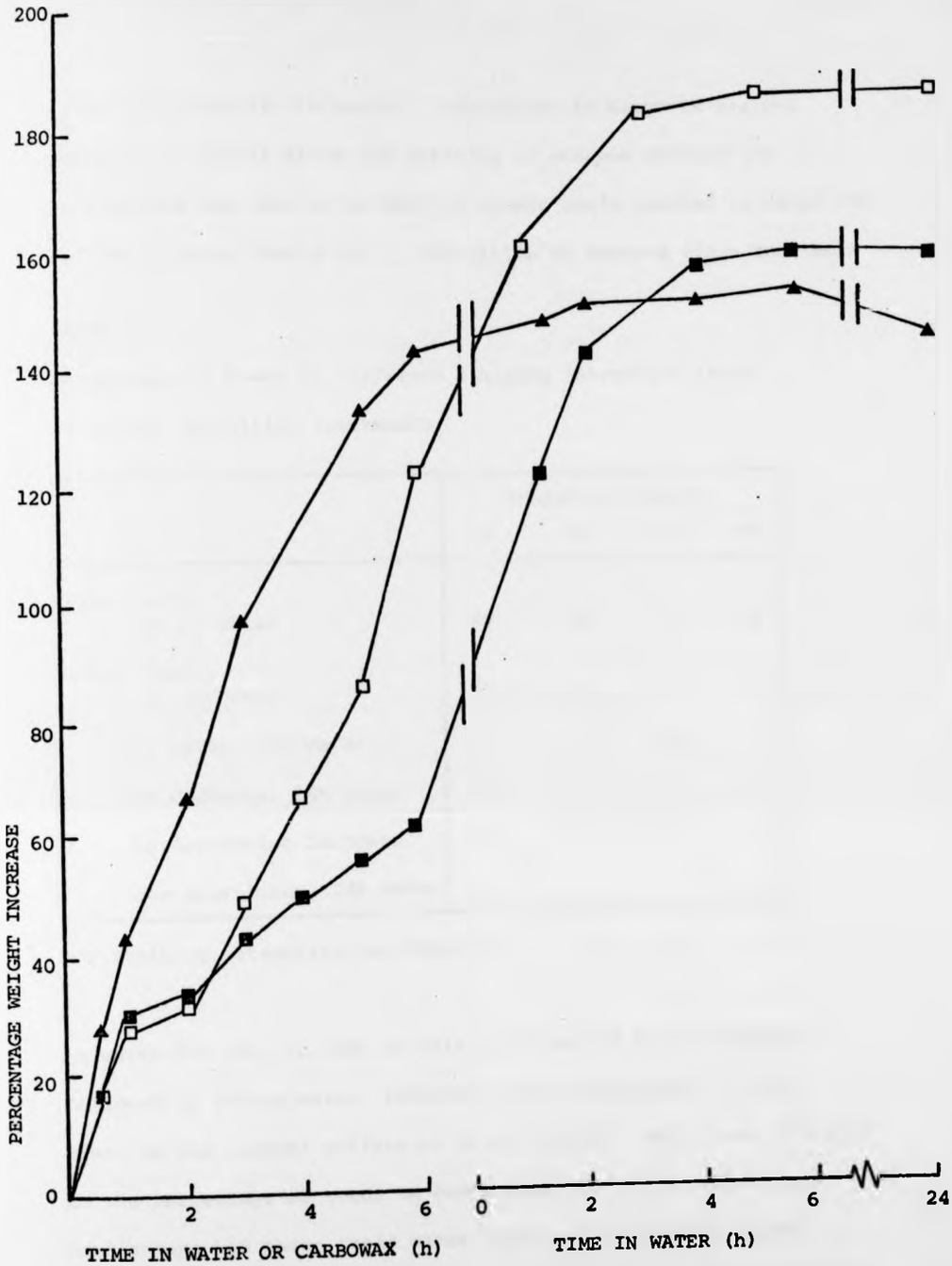


Fig.18 The rate of imbibition of embryos in water (-▲-), 30% Carbowax (-■-), and decreasing concentrations of Carbowax (-□-) for 6h, followed by 24h in water.

after a further 24h in water. Imbibition in Carbowax was not damaging in itself since the staining of embryos imbibed 24h in Carbowax was similar to that of intact seeds imbibed in water 24h before staining (Table 24). Imbibition of embryos minus the testa

TABLE 24

Percentage of seeds in different staining categories after different imbibition treatments

	Staining Category			
	I	II	III	IV
Plus testa:				
24h in water	80	20	0	0
Minus testa:				
24h Carbowax	75	25	0	0
6h water, 24h water	0	45	55	0
6h Carbowax, 24h water	0	50	50	0
6h decreasing Carbowax concentrations, 24h water	50	40	10	0

For staining categories see Table 22

in water for 30h, or with an initial 6h period in 30% Carbowax, followed by 24h in water, resulted in the development of dead areas on the abaxial surface of the cotyledons, seen in an increase in the percentage of seeds in categories II and III (Table 24). Both groups had shown rapid water uptake at some point during imbibition. The treatment of decreasing Caarbowax concentrations, compared to water and 30% Carbowax treatments, to some extent avoided sharp increases in the rate of imbibition. In these embryos, staining was more complete, with 50% of the embryos in

category I. Therefore, damage to the embryos during imbibition minus the testa appeared to be associated with rapid water uptake, and was reduced, if not completely prevented, by slowing the rate of uptake. Damage did not appear to result from the loss of ions necessary for maintenance of membrane integrity since damage also occurred following imbibition in a calcium chloride solution (Appendix II).

Imbibing pea seeds at low temperatures has been shown to reduce the rate of imbibition (Shull, 1920), which presents the possibility of another way in which the influence of the rate of water uptake on the dry embryo can be tested. The influence of temperature on the rate of imbibition and the occurrence of damage was examined for intact seeds, and embryos (seeds minus the testa) at 7°, 10°, 20°, and 30°C, and damage was assessed after 24h imbibition by TTC staining. The rate of imbibition became progressively lower as the temperature of the imbibing water was reduced (Fig.19). Differences in the rate of imbibition at the different temperatures were particularly marked between 7° and 10°C in intact seeds, and between 10° and 20°C in embryos (Fig.19).

There was no damage to intact seeds after imbibition at all temperatures (Table 25), most seeds staining completely. This uniformity of staining at all temperatures showed that temperature itself, and in particular, low temperatures, did not cause damage. Imbibition of embryos with the testa removed, resulted in damage and the development of dead tissue on the abaxial surface of the cotyledons at all temperatures, a greater proportion of embryos being placed in categories II and III (Table 25) than was the case for the intact seeds. At 7°C and 10°C however, a larger proportion

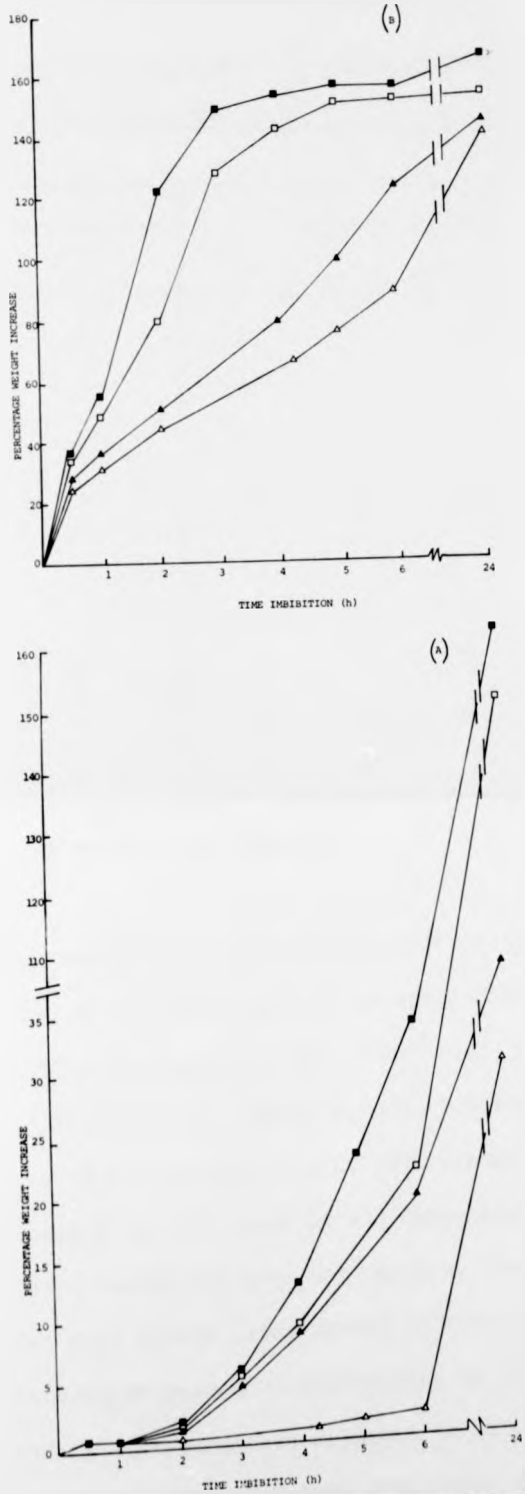


FIG.19 Rate of imbibition of intact seeds (A) and embryos (B) at 7°C (-Δ-), 10°C (-▲-), 20°C (-□-) and 30°C (-■-).

TABLE 25

Percentage of seeds in different staining categories after imbibition plus and minus the testa at four temperatures

	Temperature °C	Staining Category I	II	III	IV
Plus testa	7	83	9	8	0
	10	100	0	0	0
	20	95	5	0	0
	30	85	10	0	5
Minus testa	7	0	5	35	60
	10	0	10	25	65
	20	20	50	30	0
	30	65	35	0	0

For staining categories see Table 22

of the embryos occurred in category IV, that is there was no staining on the abaxial surfaces of the cotyledons. Therefore, although the rate of imbibition was considerably reduced at low temperatures (Fig.19B), the damage occurring during imbibition without the testa was greater than at higher temperatures. Similar staining of intact seeds at all temperatures had shown that low temperatures themselves were not damaging (Table 25), which suggested that even though water uptake occurred more slowly at 7° and 10°C, the embryos were more susceptible to damage during imbibition at these temperatures compared to 20° and 30°C. Thus, although damage to the embryo during imbibition could be reduced

by slowing the rate of water uptake by imbibition in Carbowax, when a slower rate of imbibition was achieved by lowering the imbibition temperature, damage increased, because of greater sensitivity to water damage at low temperatures.

Rapid water uptake had been seen to result in damage when it occurred both at the beginning of imbibition in water, and also after transfer to water following a 6h period of slow imbibition in 30% Carbowax (Fig.18, Table 24). The rapid water uptake in the Carbowax imbibed seeds, occurred in embryos which had already increased their weight by 60% as a result of imbibition. It was possible that embryos need to achieve a larger weight increase as a result of slow imbibition, before they can tolerate rapid water uptake without damage occurring. Alternatively, rapid imbibition may not occur once the embryos have reached a certain stage of imbibition. In order to examine both these possibilities, the weight increases of embryos imbibed for different periods in 30% Carbowax before transfer to water, and the subsequent weight increases after 1h in water were measured; damage to the embryos was assessed by TTC staining after a total of 30h imbibition. With increased time in Carbowax, the weight increases resulting from water uptake increased from 60% of the air dry weight after 6h to 113% after 24h (Table 26). After transfer to water for 1h there was a weight increase similar to that found in the earlier experiment (Fig.18), which decreased from 60%, to 59%, and then to 55% after imbibition in Carbowax for 6h, 10h and 12h respectively. Tetrazolium staining showed that these embryos were damaged (Table 26) with some embryos having incomplete staining (categories II and III). After 18h in Carbowax there was a larger decrease in

TABLE 26

Changes in seed weight after imbibition minus the testa in 30% Carbowax followed by transfer to water, and condition of the imbibed seeds revealed by TTC staining

Time imbibed in Carbowax	Percentage weight increase in Carbowax	Percentage weight increase after 1h in water	Staining Category			
			I	II	III	IV
6	65	60	66	22	11	0
10	85	59	85	15	0	0
12	96	55	60	25	15	0
18	111	45	60	40	0	0
24	113	40	95	5	0	0

For staining categories see Table 22

the percentage of water taken up in the first hour in water (55% to 45%), but the embryos had similar damage to those which had been imbibed in Carbowax for 6, 10 and 12h (Table 26). Increasing the time in Carbowax from 18 to 24h before transfer to water, reduced the uptake in water by a further 5%, to 40%. Although this 40% weight increase in 1h was still a rapid water uptake, 95% of these embryos were completely stained (category I), indicating little damage.

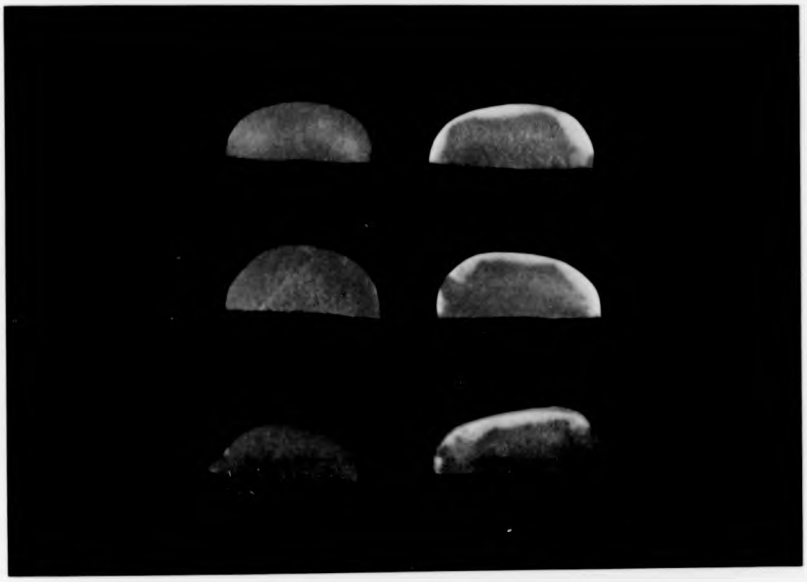
The absence of damage in embryos imbibed minus the testa for 24h in Carbowax could be explained in two ways; in terms of a critical weight increase required to cause damage, or the development of a tolerance to rapid imbibition. Although the weight

increase due to water uptake was only 5% lower than that of embryos imbibed 18h in Carbowax, the absence of damage may indicate that there is a critical weight increase, which occurs as a result of rapid imbibition, between 40% and 45%, above which, damage to the embryo occurs. The weight increase after transfer to water of embryos imbibed for 18h in Carbowax was above this critical value, and therefore the embryos were damaged. After 24h in Carbowax before transfer to water, the critical weight increase due to rapid imbibition was not reached, and therefore these embryos were not damaged. A second explanation of the absence of damage in embryos imbibed for 24h in Carbowax before rapid imbibition could be that the embryos imbibed for 24h in Carbowax were more tolerant of rapid water uptake than those imbibed for 18h in Carbowax.

The extent to which the damage observed on the abaxial surfaces of the cotyledons extended within the cotyledons after imbibition plus and minus the testa was examined. After TTC staining, sections of the cotyledons were cut by hand from the abaxial to the adaxial surface, and the staining pattern observed (Plate 2). This showed that the cotyledons of seeds imbibed plus the testa had uniform even staining throughout, whereas those seeds imbibed minus the testa had a thin layer of unstained dead cells over the abaxial surface of the cotyledon (Plate 2). The central tissue of these embryos was stained, and therefore living. The cotyledons of some seeds that had been imbibed minus the testa tended to separate although they were still connected by the embryo axis; as a result, the adaxial surfaces of the cotyledons were no longer pressed together and were also in direct contact with water. Where this occurred, the outermost layer of tissue of the adaxial surface

A B

PLATE 2. Tetrazolium chloride staining of cotyledons sectioned from the abaxial to the adaxial surface after imbibition plus (A) and minus (B) the testa



A B

PLATE 2. Tetrazolium chloride staining of cotyledons sectioned from the abaxial to the adaxial surface after imbibition plus (A) and minus (B) the testa

showed no staining. Thus, wherever cotyledon tissue was in direct contact with water during imbibition, cell death was observed.

The minimum time required for seeds minus the testa to be in contact with water for damage to occur was investigated. Dry seeds with the testa removed (that is embryos) were imbibed in water for different periods of time before transfer to 30% Carbowax for 24h, after which time the extent of damage was examined by TTC staining. After one minute imbibition in water, embryos showed no damage, all had uniform staining (Table 27).

TABLE 27

Percentage seeds in four staining categories after different times of imbibition in water and Carbowax

	Time in:		Staining Category			
	Water	Carbowax	I	II	III	IV
Plus testa	0	24h	100	0	0	0
	24h	0	95	0	0	0
Minus testa	0	24h	100	0	0	0
	1 min	24h	100	0	0	0
	2 min	24h	20	45	30	5
	4 min	24h	10	50	30	10
	6 min	24h	0	40	60	0
	10 min	24h	0	15	75	10
	20 min	24h	0	35	65	0
	30 min	24h	15	50	35	0
	24h	0	5	85	10	0

For staining categories see Table 22

After two minutes however, the percentage of embryos with complete staining (category I) had decreased from 100% to 20%, 80% with incomplete staining (categories II and III). Damage was therefore apparent after two minutes imbibition in water; no further increase in the extent of damage occurred after imbibition in water for up to 24h (Table 27).

The effects of damage resulting from rapid imbibition on germination and seedling growth were examined following three treatments: 1. imbibition of intact seeds for 30h in water (undamaged after imbibition); 2. the same but minus the testa (damaged after imbibition); 3. imbibition minus the testa in decreasing Carbowax concentrations for 6h, followed by 24h in water. This last treatment was used as an intermediate condition between damaged and undamaged embryos since although such a treatment reduced damage, it was not entirely prevented (Table 24). Germination was measured in sand, and the appearance of the plumule above the sand within the test period (10d) was the criterion considered as germination. The germination of seeds imbibed intact was 100%, and for the Carbowax treatment, 80%; the germination rate was slower in the Carbowax treatment (Fig.20). Germination after imbibition minus the testa in water was 70%, and the rate of germination slower than the other two treatments.

Measurements indicating the rates of growth of the seedlings produced from seeds after the imbibition treatments showed that the increase in dry weight of the plumule and radicle was greater in the intact seeds imbibed in water than in either of the other two treatments (Fig.21). Plumule growth was similar in the intermediate and more completely damaged seeds, and radicle growth was

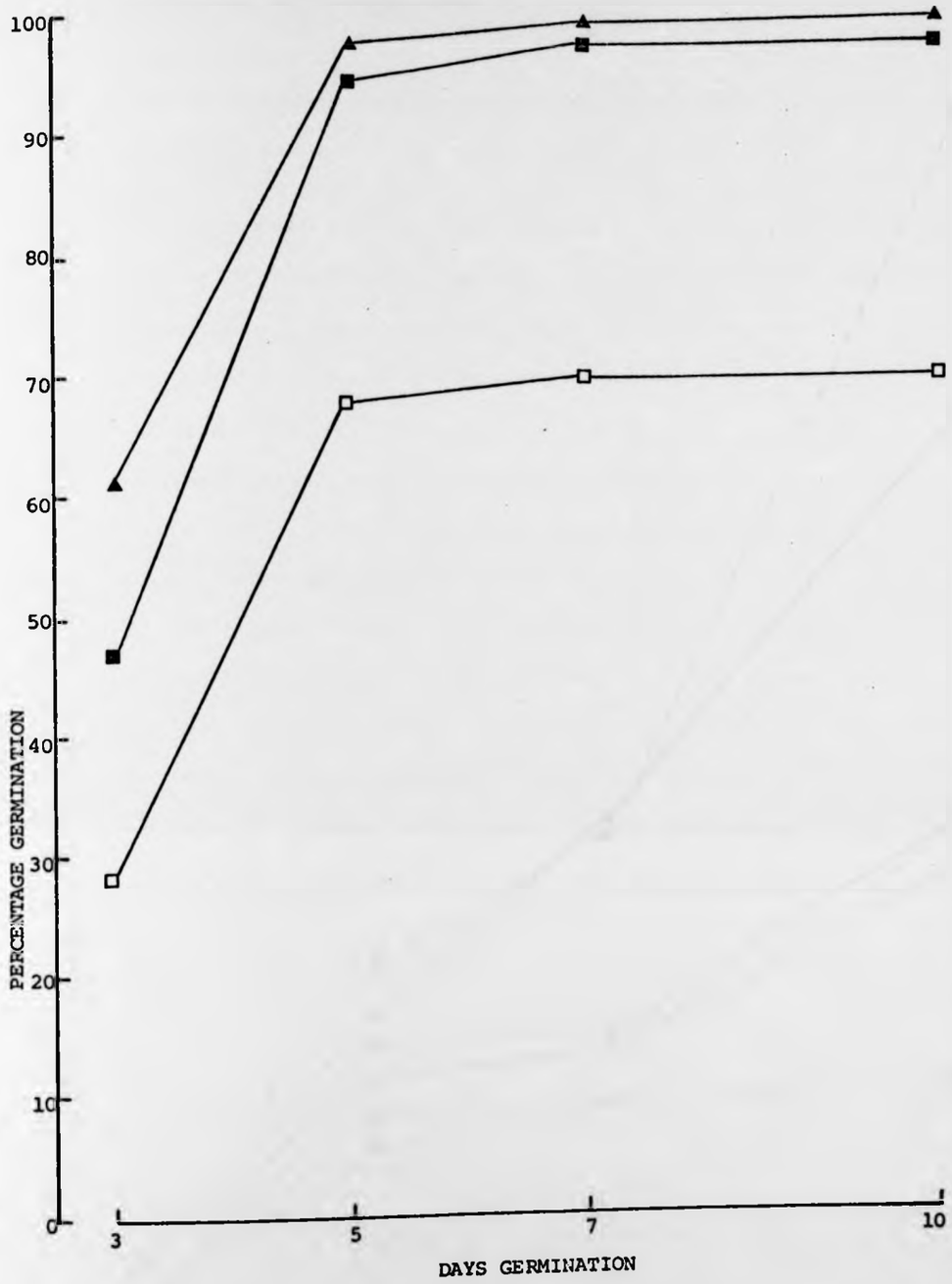


Fig. 20 The rate of germination after three imbibition treatments: plus testa in water (-▲-); minus testa in water (-□-); minus testa, 6h in decreasing concentrations of Carbowax, followed by 24h in water (-■-).

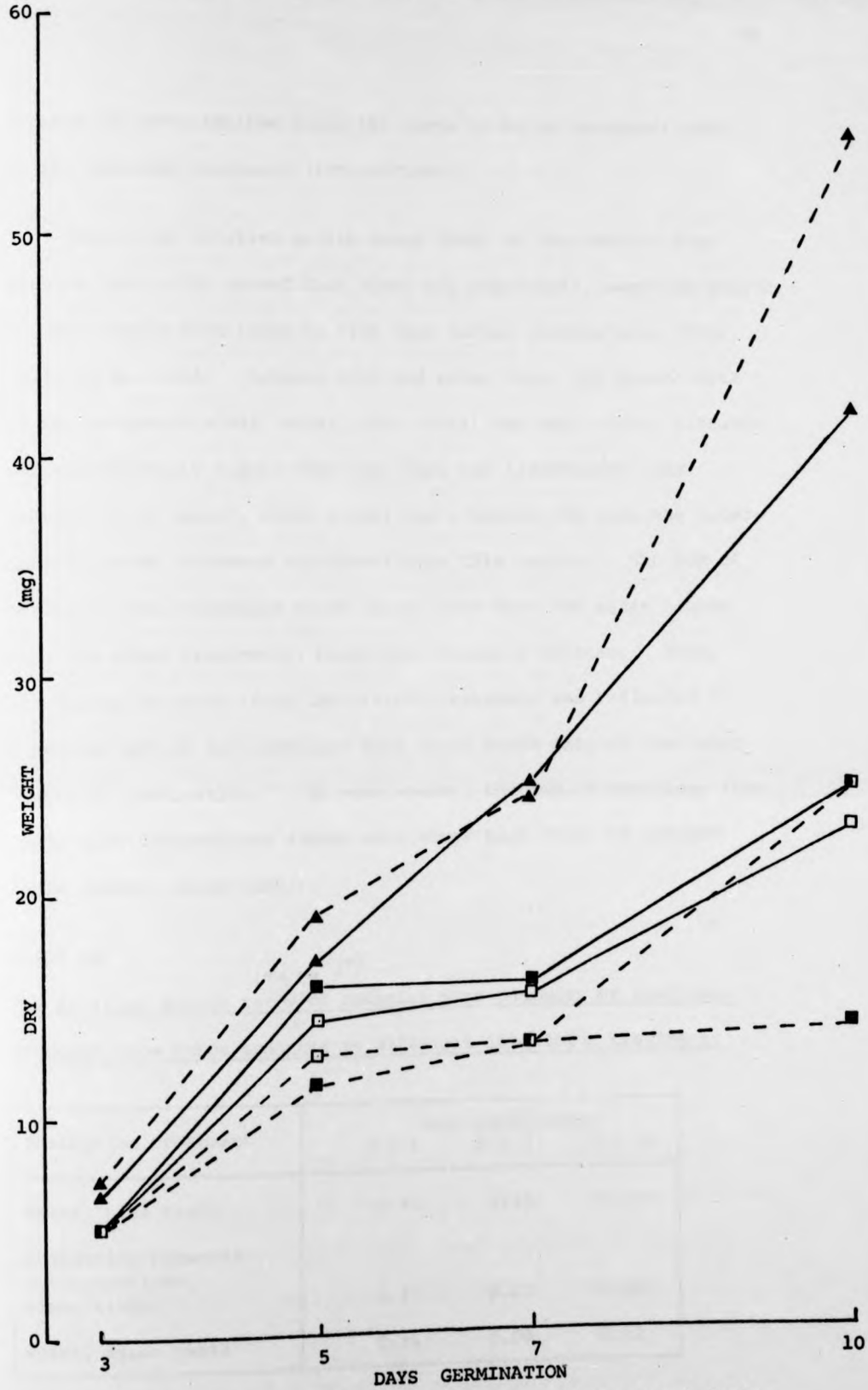


Fig. 21 Dry weights of plumules (——) and radicles (---) during germination after different imbibition treatments: plus testa in water (—▲—); minus testa in water (—□—); minus testa, 6h in decreasing concentrations of Carbowax, followed by 24h water (—■—).

greater in seeds imbibed minus the testa in water (damaged) than in the Carbowax treatment (intermediate).

Calculated relative growth rates (RGR) of the radicle plus plumule (Table 28) showed that after all treatments, seedling growth was most rapid from three to five days during germination, after which it declined. Between five and seven days, the growth rate of the undamaged seeds (water, plus testa) was most rapid, although not significantly higher than the other two treatments; the damaged seeds (water, minus testa) had a higher RGR than the intermediate seeds (Carbowax treatment) over this period. The RGR of seedlings from undamaged seeds after seven days was again higher than the other treatments, being significantly greater. Thus, the damage to seeds after imbibition treatments was reflected in a reduced RGR of the seedlings from these seeds only at the later stages of germination. For some reason, the RGR of seedlings from seeds with intermediate damage were lower than those of damaged seeds (water, minus testa).

TABLE 28

The relative growth rates ($mg\ mg^{-1}\ d^{-1}$) of radicles plus plumules of seedlings produced from seeds subjects to different imbibition treatments

Imbibition treatment	Days germination 3 - 5	5 - 7	7 - 10
Water, plus testa	0.47	0.16	0.23*
Decreasing Carbowax concentrations, minus testa	0.47	0.03	0.09
Water, minus testa	0.35	0.09	0.12

* $p \leq 0.05$

Reduction in the cotyledon dry weight during germination reflected the rate of food reserve transfer from the cotyledons to the growing axis. Movement of reserves over the first five days germination was most rapid in seeds imbibed minus the testa in water (Fig.22), which may have been due to these seeds being at a more advanced stage of imbibition before planting. After five days the rate of decline in cotyledon dry weight increased in both the intact seeds, and embryos imbibed in Carbowax, such that it was greater than in the damaged seed. The intact seeds sustained this rate of weight loss from five days to the end of the experiment at ten days. In the Carbowax treatment, the rate of decline in cotyledon dry weight decreased slightly after seven days, to become equal to that of the embryo imbibed in water.

Increased leakage of seeds in the early stages of deterioration has been linked with the decreasing ability of membranes to retain solutes (p.45). Although the observed high levels of leakage from embryos damaged as a result of rapid imbibition has been associated with dead cells, the possibility that there were also changes in the composition of membranes was investigated by extracting the phospholipids from the seeds. Seeds were imbibed for 24h in water, either intact or minus the testa, and the testae removed from the seeds before further extraction. An analysis of variance revealed that there was no significant difference in the total phospholipid extracted from the two groups of seeds (Table 29). Small increases occurred in the amounts of phosphatidyl glycerol (PG) and phosphatidyl ethanolamine (PE) extracted from the seeds imbibed minus the testa (Table 29), although these were not significant increases, and there was little change in the amount of

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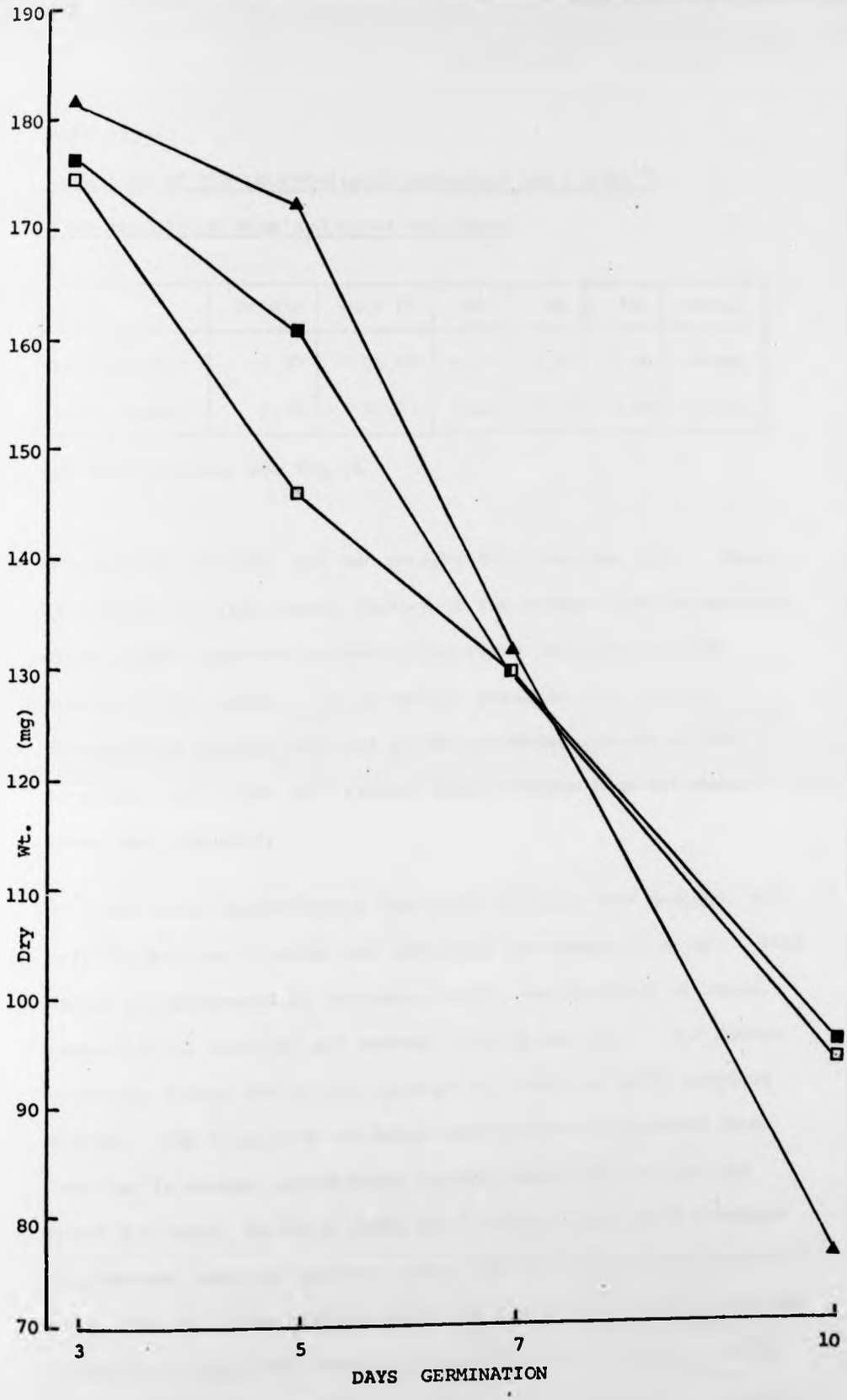


Fig.22 Changes in cotyledon dry weight during germination following three imbibition treatments: plus testa in water (—▲—); minus testa in water (—□—); minus testa, 6h in decreasing concentrations of Carbowax, followed by 24h in water (—■—).

TABLE 29

Comparison of the phospholipids extracted (mM g F.Wt^{-1}) after imbibition plus and minus the testa

	Origin	PC + PI	PG	PE	PA	Total
Plus testa	0.77	21.05	0.57	1.40	5.05	28.84
Minus testa	1.50	21.83	1.46	3.17	5.55	33.51

For abbreviations see Fig.15

phosphatidyl choline (PC) and phosphatidyl inositol (PI). Thus, in summary, no significant changes in the phospholipid composition of the seeds appeared to result from rapid imbibition in the absence of the testa. It is however possible that crucial phospholipid changes occurred in the outermost tissues of the cotyledon which were not revealed when extracts from the whole seeds were compared.

Pea seeds imbibed minus the testa in water were damaged, and only two minutes in water was necessary for damage to occur. This damage was expressed by increased solute leakage from the seeds, reduced vital staining and reduced respiration rate. The damage occurring during imbibition was also reflected in early seedling growth. The first sign of damage was seen in a treatment which resulted in damage intermediate between seeds imbibed plus and minus the testa, in which there was a reduced rate of germination and reduced seedling growth. Seeds with more severe damage than this, that is, those imbibed minus the testa in water,had a similar reduction in seedling growth, and in addition, a reduction in the final level of germination.

Imbibition of Different Seed Lots

Variability in solute leaching from different seed lots within cultivars of peas has been reported several times (Matthews and Bradnock, 1967, 1968; Matthews and Whitbread, 1968; Bradnock and Matthews, 1970; Perry, 1970). In the preceding section of this thesis, high levels of leaching were found from dry seeds from which the testa had been removed. The possibility that rapid imbibition occurring in intact seeds of some seed lots may be a contributory cause of high levels of leaching in these seed lots (e.g. Sp2, J1, Sc3: Table 30) was examined.

The time course of imbibition of intact seeds of 13 seed lots was measured over 24h. The imbibition curves of three seed lots showing contrasting time courses of imbibition are shown in Figure 23; Sp2 took up water most rapidly, P1 the most slowly and Sc2 is an example of an intermediate uptake pattern. All other seed lots had imbibition curves within the range covered by these lots (Table 30), and the characteristics of the three imbibition curves can be considered as representative of all lots.

All three seed lots had a phase during imbibition in which the weight increase due to water uptake was linear, although this phase began at different times after the start of imbibition. In Sp2, the linear phase of imbibition appeared to begin as soon as the seeds were immersed in water (Fig.23), whereas there was a short lag period of 0.5-1h before Sc2 entered this phase, with a longer lag of $1\frac{1}{2}$-2h in P1. The rates of imbibition during the linear phase were clearly different (Table 30), ranging from 13% weight increase h^{-1} in P1 to 23% in Sp.2.

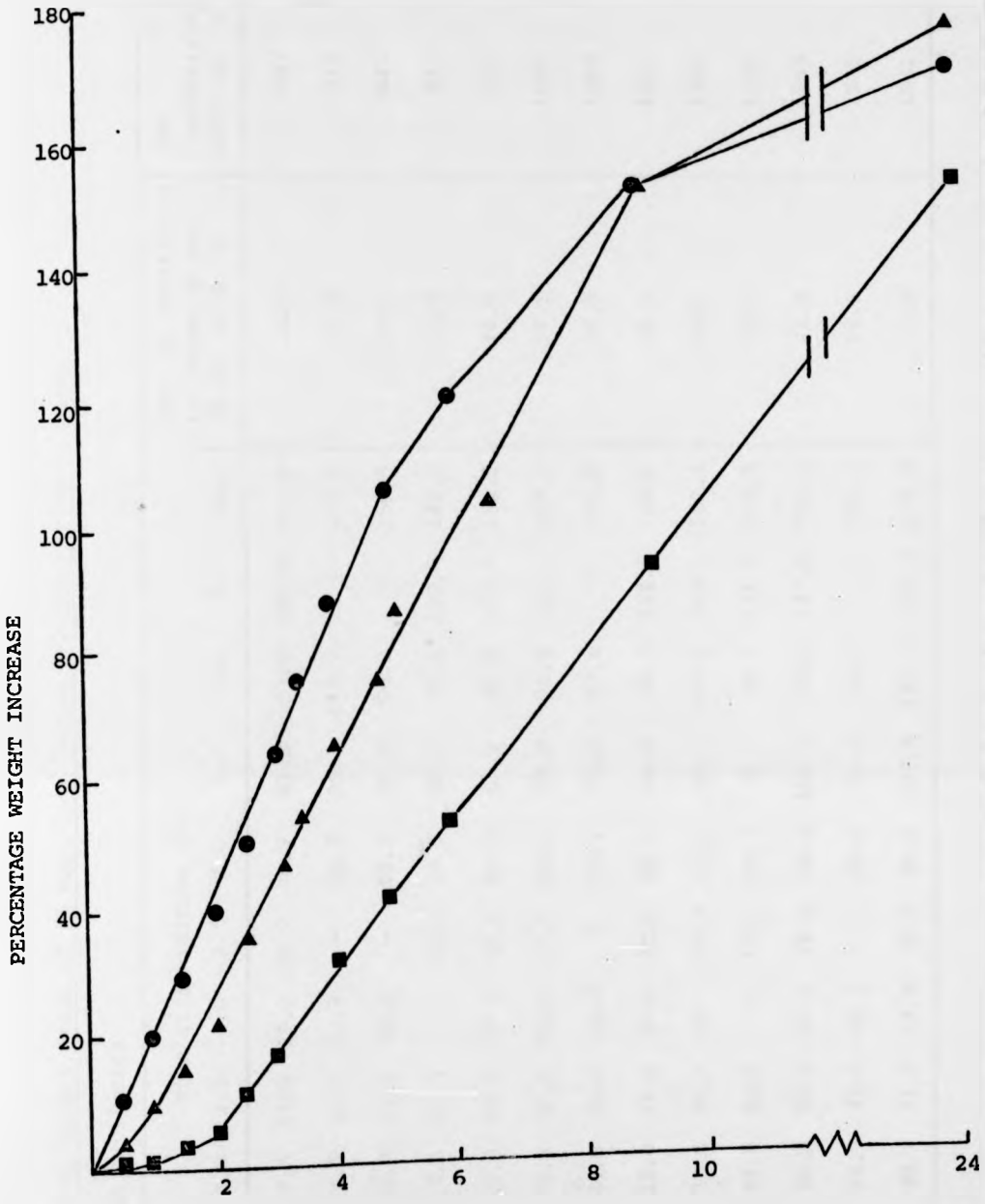


Fig.23 The rates of imbibition in water of intact seeds of three seed lots.

Pl (-■-); Sc 2 (-▲-); Sp 2 (-●-).

TABLE 30

Percentage weight increases of intact seeds of 13 seed lots during 24h imbibition in water

Each figure is a mean of ten replicates

Seed lot	Time of imbibition (h)												Rate of imbibition in linear phase (% wt inc h^{-1})	24h Conductivity $\mu mho\ cm^{-1}\ g^{-1}$
	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	5.0	6.0	9.0	24.0		
P1	0.3	2.2	4.3	7.5	11.9	18.0	24.7	30.9	42.8	53.8	91.3	162.9	13.0	647
DSP1	9.9	21.8	30.6	38.7	57.0	67.9	-	84.5	99.7	114.2	-	154.9	21.0	711
DSP2	1.1	5.1	11.2	19.3	27.1	38.9	-	59.3	80.6	96.2	-	156.5	19.0	811
KW1	0.3	2.0	5.9	17.3	22.3	-	41.1	48.9	58.7	76.1	100.7	145.2	15.0	873
Sp1	3.7	7.8	14.5	20.4	27.7	33.9	42.1	50.2	62.7	83.9	117.2	151.5	14.0	937
P2	5.3	13.8	21.3	31.3	40.4	48.9	57.5	64.1	69.8	94.6	125.3	159.2	17.5	1082
J2	3.5	12.0	-	24.0	32.9	43.4	-	59.9	73.6	87.4	-	148.5	16.0	1167
Sc1	1.4	3.5	9.7	15.0	22.8	30.8	40.6	48.5	66.9	89.5	122.7	183.6	19.0	1231
Sc2	5.0	10.5	17.1	23.6	36.8	45.7	54.3	65.8	80.7	104.1	153.7	182.4	18.0	1245
KW2	8.7	17.2	27.0	34.0	42.7	-	59.6	65.7	72.2	86.1	121.8	161.2	16.0	1246
Sc3	8.9	22.7	34.5	46.0	56.2	65.2	75.0	84.8	102.1	118.8	147.2	168.2	21.0	1563
J1	7.4	13.5	-	24.7	33.4	40.3	-	56.6	75.9	91.3	-	176.1	15.5	1801
Sp2	10.8	21.2	30.3	39.7	51.0	64.8	76.5	88.9	107.8	121.2	153.3	173.8	23.0	1842

The linear phase lasted for different lengths of time in the three seed lots, continuing for 5h in Sp2 (Fig.23), and for 7h in Sc2 (up to 8h within imbibition). In P1, the linear phase may have continued for more than 22h; seeds only began to imbibe rapidly after 2h in water, and still appeared to be within the linear phase when the final reading was taken at 24h.

After 8h in water, the imbibition of seed lots in which early imbibition was most rapid, with no lag period (Sp2) was almost complete (Fig.23), and the rate of imbibition had begun to decline. However, seed lots which had a longer lag period before imbibition began (for example, P1) were still within the phase of rapid imbibition and therefore they were imbibing more rapidly than the other lots.

All the seed lots showed differences in the levels of solute leakage from the seeds (Table 30), the 24h conductivities ranging from 647 μmho cm^{-1} g^{-1} seed in P1 to 1842 μmho cm^{-1} g^{-1} seed in Sp2. When conductivities were compared with the rates of imbibition (Table 30), it was found that seed lots which had rapid rates of imbibition, e.g. Sc3 and Sp2, had high levels of solute leakage (conductivities of 1563 and 1842 μmho cm^{-1} g^{-1} seed respectively), whereas lots with slow imbibition (P1 and KW1) had lower levels of leakage (647, and 873 μmho cm^{-1} g^{-1} seed). Examples of intermediate rates of imbibition occurred in J2 and Sc2, both of which had conductivities lying between the two extremes (1167 and 1245 μmho cm^{-1} g^{-1} seed). The rate of imbibition thus seemed to be related to the level of leakage from the seeds. The 24h conductivity was correlated with the imbibition rate, expressed by the

percentage weight increase at different times during imbibition, and by the rate of imbibition during the linear phase (Table 31). The percentage weight increase after imbibition, for 0.5, 1.5, 3.5, 9 and 24h was positively related to leaching (Table 31), being highly significant after 3.5h ($p \leq 0.001$) and 9h ($p \leq 0.01$). At 3.5h and 9h, the weight increases of two lots, DSP1 and J1, were not recorded. When the 24h conductivity was plotted against the percentage weight increase after 0.5 and 1h, and the rate of imbibition during the linear phase of imbibition (Figs. 24 and 25), these two lots (DSP1 and J1) deviated from the general trend of high solute leakage associated with rapid imbibition. This suggested that perhaps the accidental exclusion of their weight increases after 3.5 and 9h had resulted in the high correlation coefficients of 0.91 and 0.81. The correlations of the weight increases at all times during imbibition with the 24h conductivity were recalculated excluding these lots (Table 31); all correlations were highly significant up to 9h imbibition. The influence of these seed lots on this correlation indicated that these lots were responding differently during imbibition. Further evidence to suggest that these seed lots are exceptional is provided later in this section.

Therefore, the 24h conductivity of most seed lots could be related to their rate of imbibition; lots imbibing rapidly having high conductivities, lots imbibing more slowly having low conductivities. The different rates of imbibition of these lots could be due to features of the embryo or testa. When the rates of imbibition of the seed lots was measured with the testa removed (i.e. embryos), there was less variability between lots (Table 32),

TABLE 31

The correlations between leaching of solutes from intact seeds of different seed lots (24h conductivity), with their percentage weight increases at different times during imbibition and the rate of imbibition in the linear phase

	Time of imbibition (h)												Rate of imbibition in linear phase (% wt inc h^{-1})
	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	5.0	6.0	9.0	24.0	
Correlation coefficient with 24h conductivity (includes all lots)	* 0.59	NS 0.52	* 0.60	NS 0.50	NS 0.44	NS 0.40	*** 0.91	NS 0.48	NS 0.53	NS 0.53	** 0.88	* 0.59	NS 0.41
Correlation coefficient with 24h conductivity (excludes J1 and DSP1)	*** 0.87	* 0.60	** 0.84	** 0.83	*** 0.86	*** 0.89	*** 0.91	*** 0.89	*** 0.87	*** 0.85	*** 0.81	* 0.51	*** 0.82

NS = not significant

* $p \leq 0.05$

** $p \leq 0.01$

*** $p \leq 0.001$

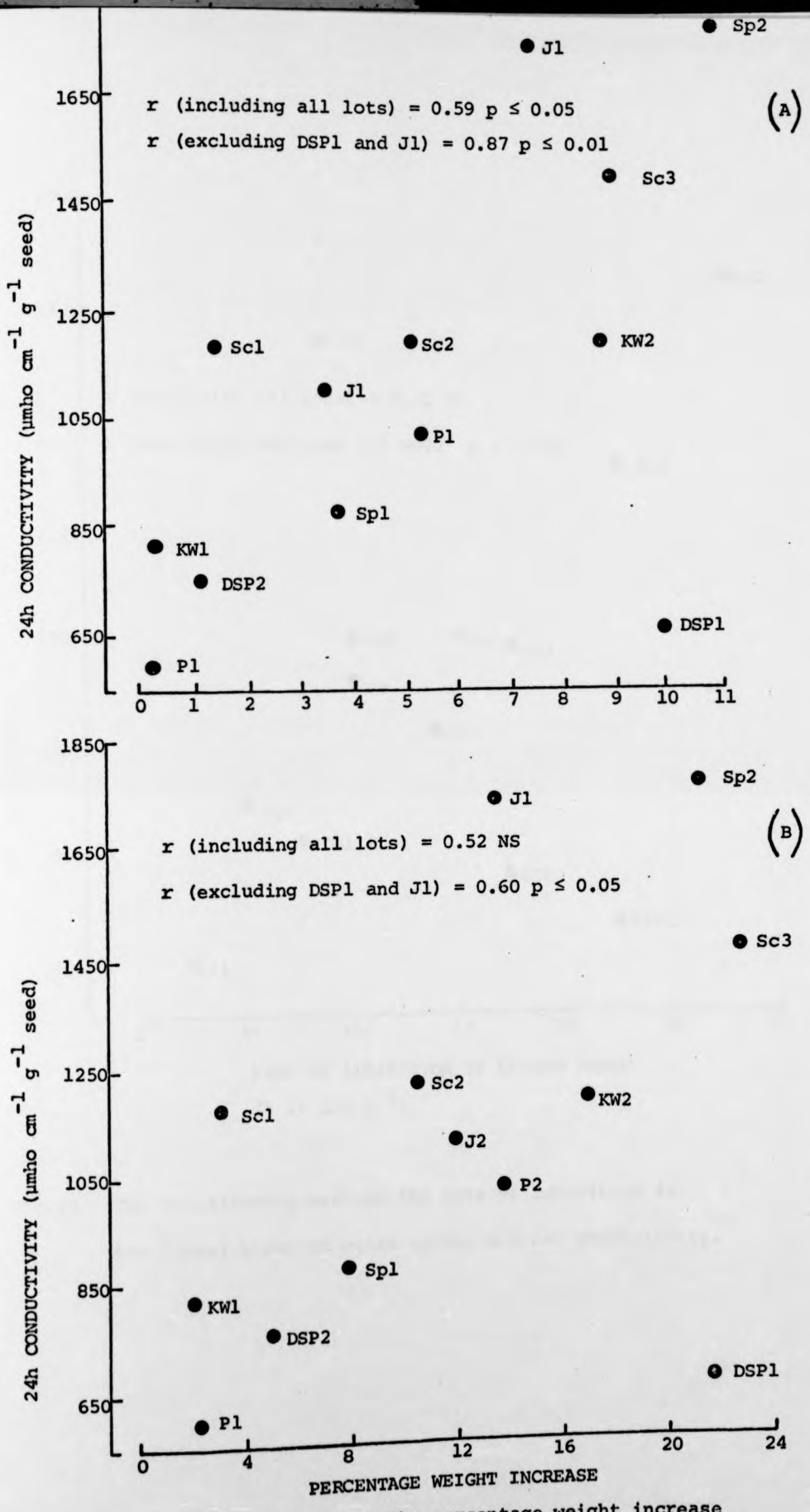


Fig.24 The relationship between the percentage weight increase after (A) 0.5h and (B) 1h inbibition and 24h conductivity of diffcrent seed lots.

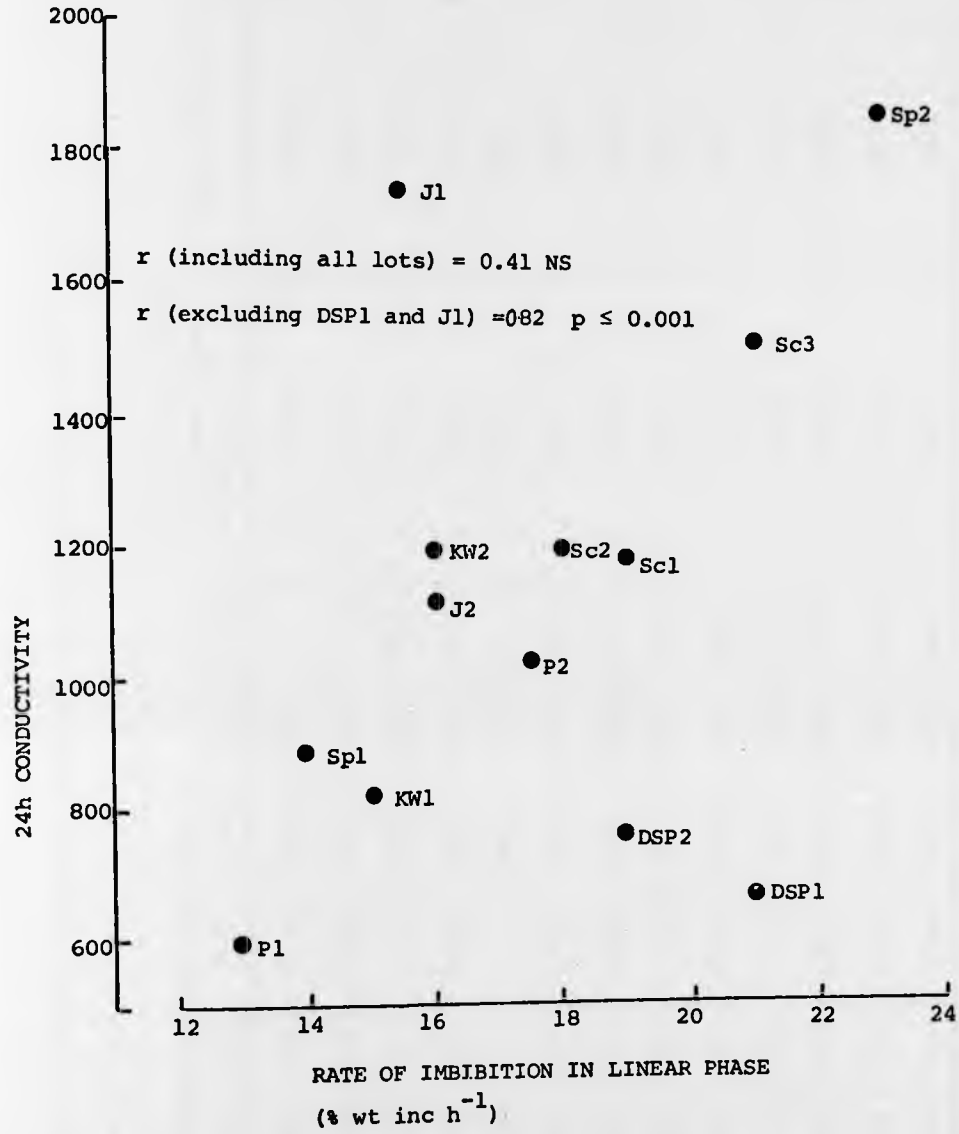


Fig.25 The relationship between the rate of imbibition in the linear phase of water uptake and 24h conductivity.

TABLE 32

Percentage weight increases of 13 seed lots during imbibition minus the testa for 24h in water

Each figure is a mean of ten single seeds

Seed lot	Time of imbibition (h)								Rate of imbibition in linear phase (% wt inc h^{-1})	24h conductivity (µmho cm^{-1} g^{-1})
	0.5	1.0	2.0	3.0	4.0	5.0	6.0	24.0		
DSP2	28.7	39.5	57.6	76.8	94.3	118.2	126.4	-	20.0	1995
DSP1	26.8	40.6	65.6	101.4	125.4	137.0	140.0	-	28.0	2045
KW1	27.3	37.8	55.7	-	-	118.5	129.5	136.5	20.5	2275
Sp1	32.2	43.4	59.9	76.7	96.5	117.6	125.3	134.5	21.0	2633
KW2	29.4	41.1	59.2	-	-	126.0	143.9	151.0	20.0	2681
J2	29.4	41.8	60.5	78.0	99.1	114.7	126.3	154.4	21.0	2727
P2	29.5	41.0	59.8	76.7	98.0	119.0	132.6	137.0	21.0	2809
Sc1	34.6	47.1	68.9	90.4	115.0	146.5	157.1	165.3	23.5	2827
J1	32.7	44.8	62.5	83.2	109.6	136.1	150.6	160.3	22.0	2921
P1	31.2	44.0	63.9	82.4	101.5	130.0	138.5	145.7	21.0	2951
Sc2	39.0	51.7	73.3	93.2	116.5	147.1	159.9	177.9	23.0	3003
Sp2	35.3	47.6	66.4	82.7	102.7	118.9	147.5	159.9	20.0	3137
Sc3	34.8	45.0	65.3	83.1	99.6	118.8	136.9	148.5	18.0	3248

with eleven lots having percentage weight increases during the linear phase of 20-23% h^{-1}, compared with a range from 13-23% h^{-1}, in intact seeds (Table 30). This indicated that the testa may have a role in determining the rate of imbibition of these lots.

Leakage of solutes from embryos of all lots imbibed minus the testa was higher than from intact seeds (Table 32). Differences in solute leakage occurred between seed lots, and were positively correlated with the weight increases occurring in the embryos during the first hour of imbibition (Table 33). The previous section of this thesis showed that high levels of solute leakage from imbibed embryos resulted from damage occurring in the first two minutes of imbibition. The high leakage from these embryos was therefore probably due to damage occurring early in imbibition, and would be more likely to correlate with the imbibition rate at the time of damage than later. Thus, differences in the rate of imbibition and leakage, seen earlier in intact seeds, were also noted during imbibition minus the testa; such differences might be attributed to properties of the embryo.

Since testa removal resulted in similar rates of imbibition in the different seed lots during the linear phase of imbibition, imbibition and subsequent leakage could be influenced by the condition of the testa. However, the differences in water uptake of embryos within the first hour, and their significant correlation with leaching suggested that properties of the embryo might also be involved.

The importance of the testa in influencing imbibition rates was investigated further by staining for mechanical damage since

TABLE 33

The correlations of solute leaching from embryos of 13 seed lots (24h conductivity) with their percentage weight increases at different times during imbibition and the rates of imbibition in the linear phase

	Time of imbibition (h)								Rate of imbibition in linear phase (% wt inc h^{-1})
	0.5	1.0	2.0	3.0	4.0	5.0	6.0	24h	
Correlation coefficient	**	**	NS	NS	NS	NS	NS	NS	NS
with 24h conductivity	0.77	0.72	0.51	-0.15	-0.14	0.11	0.47	0.50	0.41

NS = not significant

** $p \leq 0.01$

such damage might enable water to pass through the testa more rapidly compared with normal water uptake. Dry seeds in which gross cracks were observed in the testa were not included in these measurements, as they were always excluded from conductivity measurements in which they would have caused an artificially high mean. The proportion of these seeds was not high in any seed lot. After imbibition and fast green staining, the percentage of seeds in each lot which had damage to the testa was assessed. The percentage of seeds showing testa damage in the form of measurable cracks (< 1mm - 15mm) varied (Table 34), ranging from only 16% in KW1 to 55% in Sc2, and was positively related to the rate of imbibition of the seed lots (Fig.26).

TABLE 34

The percentage of seeds in different seed lots with small cracks (< 15mm) in the testa

Seed lot	KW1	P2	Sp1	P1	KW2	Sc3	Sc1	DSP2	J1	J2	Sp2	DSP1	Sc2
Percentage seeds with cracks	16	17	21	27	30	37	38	41	44	45	47	48	55

Apparently, seed lots in which a large proportion of the seeds showed testa damage, had a rapid rate of imbibition. This would seem to suggest that the condition of the testa can influence the rate of imbibition, which in turn can eventually influence solute leakage through facilitating more rapid imbibition which causes cell death.

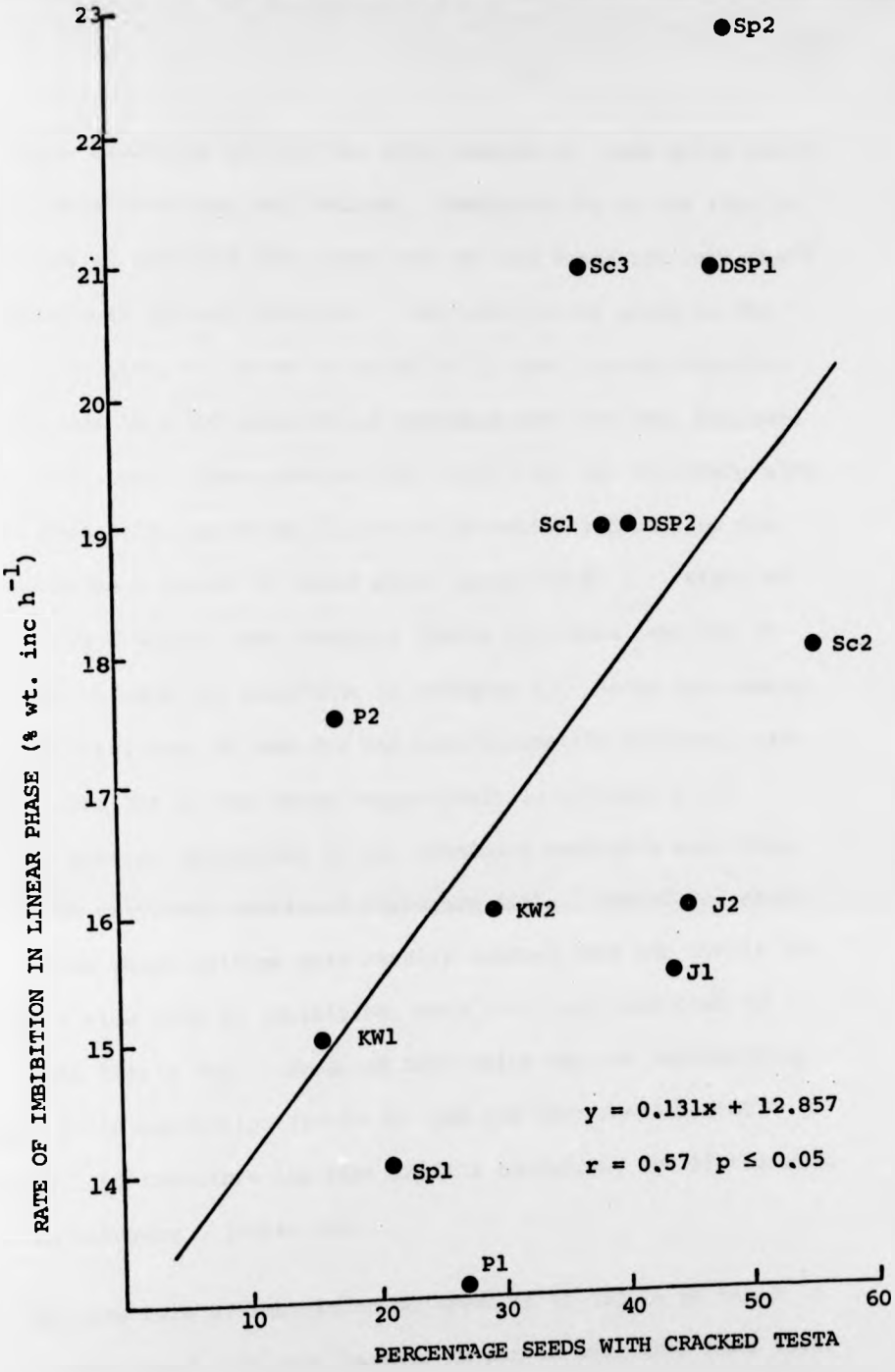


Fig.26 The relationship between the extent of testa damage in seed lots and their rate of imbibition.

Testa condition was not the only feature of seeds which could affect imbibition rate and leakage. Measurements of the rate of imbibition of two seed lots (DSP1 and J1) had suggested that there are additional factors involved. One such factor might be the embryo condition, which was examined in 13 seed lots by imbibing intact seeds in a 30% solution of Carbowax 4000 for 24h, followed by TTC staining. This ensured that imbibition was uniformly slow in all seed lots, and would therefore prevent any damage to the cotyledons as a result of rapid water uptake (p.82). Eight of the seed lots showed good staining (Table 35), more than 80% of the seeds in each lot occurring in category I. Among the remaining seed lots, two, J1 and Sc2 had more incomplete staining, with only 58% and 55% of the seeds respectively in category I, J1 having a greater proportion of the remaining seed with more than 50% of the cotyledon unstained (category III). Therefore, seeds of J1, from which solutes were readily leached over 24h (Table 30) despite a slow rate of imbibition, were in a poor condition to start with (Table 35). Seeds of DSP1 which had low conductivity despite rapid imbibition (Table 30) had the most complete TTC staining, and therefore the best initial condition, 90% of the seed being in category I (Table 35).

The slow rate of imbibition observed in J1 (15.5% wt inc h^{-1}) was not associated with high leakage in any of the other seed lots. It is possible that the poor embryo condition of J1 makes the embryos more sensitive to damage, even at relatively slow rates of imbibition, with a resulting high leakage. Solute leakage from DSP1 however was low despite a rapid rate of imbibition (Table 30). The good initial embryo condition of this lot might make the embryos

TABLE 35

Percentage of seeds in each of four TTC staining categories after slow imbibition in Carbowax

Staining category of abaxial surface of the cotyledons;

I: completely stained; II: less than 50% unstained;

III: more than 50% unstained; IV: no staining

	24h conductivity after imbibition minus testa $\mu mho\ cm^{-1}\ g^{-1}$	Staining Category			
		I	II	III	IV
DSP2	1995	93	2	0	0
DSP1	2045	90	8	2	0
KW2	2681	86	14	0	0
KW1	2275	86	14	0	0
P2	2809	84	13	3	0
J2	2727	82	18	0	0
Sp1	2633	82	13	0	5
Sc3	3248	80	15	0	5
P1	2951	79	21	0	0
Sc1	2827	75	15	5	0
Sp2	3137	65	27	5	4
J1	2921	58	36	6	0
Sc2	3003	55	45	0	0

more tolerant of rapid imbibition, so that less damage occurred during imbibition. Other lots with poorer initial condition than DSP1 (Sc1 and Sc3) were damaged by similar rates of imbibition (19% and 21% wt inc h^{-1}). Thus, in these seed lots, embryo condition appeared to influence the response of the seeds to different rates of imbibition.

The possible influence of embryo condition on leakage had also been suggested by the correlation between the early rate of imbibition of seeds minus the testa (embryos) with solute leakage (Table 33). Comparison of the initial embryo condition with the conductivity after imbibition of embryos showed that seeds with poor initial condition tended to have more rapid imbibition and higher conductivities (Table 35). Hence, although increased leaching indicated that seeds of all lots were damaged when imbibed minus the testa, the initial embryo condition might influence the seed lots sensitivity to rapid imbibition. Alternatively, the rapid imbibition of these lots may be due to the poor condition of the embryos, water uptake being facilitated in seeds which contain a large proportion of dead tissue.

The effect of the embryo on response to rate of imbibition was further illustrated using two seed lots (KW1 and Sp1) which had similar rates of imbibition in water (15% and 14% weight increase h^{-1} in linear phase, respectively) and similar testa damage (16% and 21% of seeds with cracks). Seeds were given three imbibition treatments: 1. imbibed with intact testa for 24h in 30% Carbowax; 2. the same for 24h in water; 3. imbibed with scarified testa for 24h in water. The testa was scarified by making two scratches in the testa approximately 2mm long using pointed forceps, one scratch over each cotyledon. These three treatments would result in increasing rates of water uptake, seeds imbibing most slowly in Carbowax and most rapidly with the testa scarified. The condition of the seeds after imbibition was then assessed by the TTC staining of the abaxial surfaces of the cotyledons.

After imbibition in Carbowax, 85% and 82% of the seeds were completely stained in KW1 and Spl respectively, that is, they were in good condition after slow imbibition (Table 36). The cotyledons

TABLE 36

The percentage of seeds of two lots in each of four staining categories after three imbibition treatments

Seed lot	Imbibition treatment	Staining Category I	II	III	IV
KW1	Intact testa, 24h 30% Carbowax	85	15	0	0
	Intact testa, 24h water	86	14	0	0
	Scarified testa, 24h water	39	44	17	0
Spl	Intact testa, 24h 30% Carbowax	82	18	0	0
	Intact testa, 24h water	65	27	5	4
	Scarified testa, 24h water	0	50	40	10

For staining categories see Table 35

of KW1 had similar complete staining after imbibition in water with the testa intact, whereas only 65% of the cotyledons of Spl had complete staining although the rate of imbibition of Spl was similar to that of KW1 (Fig.27). However, water uptake in the first two hours in water was slightly greater in Spl than KW1. It may be that this slightly faster rate of imbibition was sufficient to account for the difference in the extent of damage. Scarification of the testa increased the rate of imbibition equally in both lots (Fig.27). Both lots had fewer seeds with complete staining after this treatment, indicating that neither lot

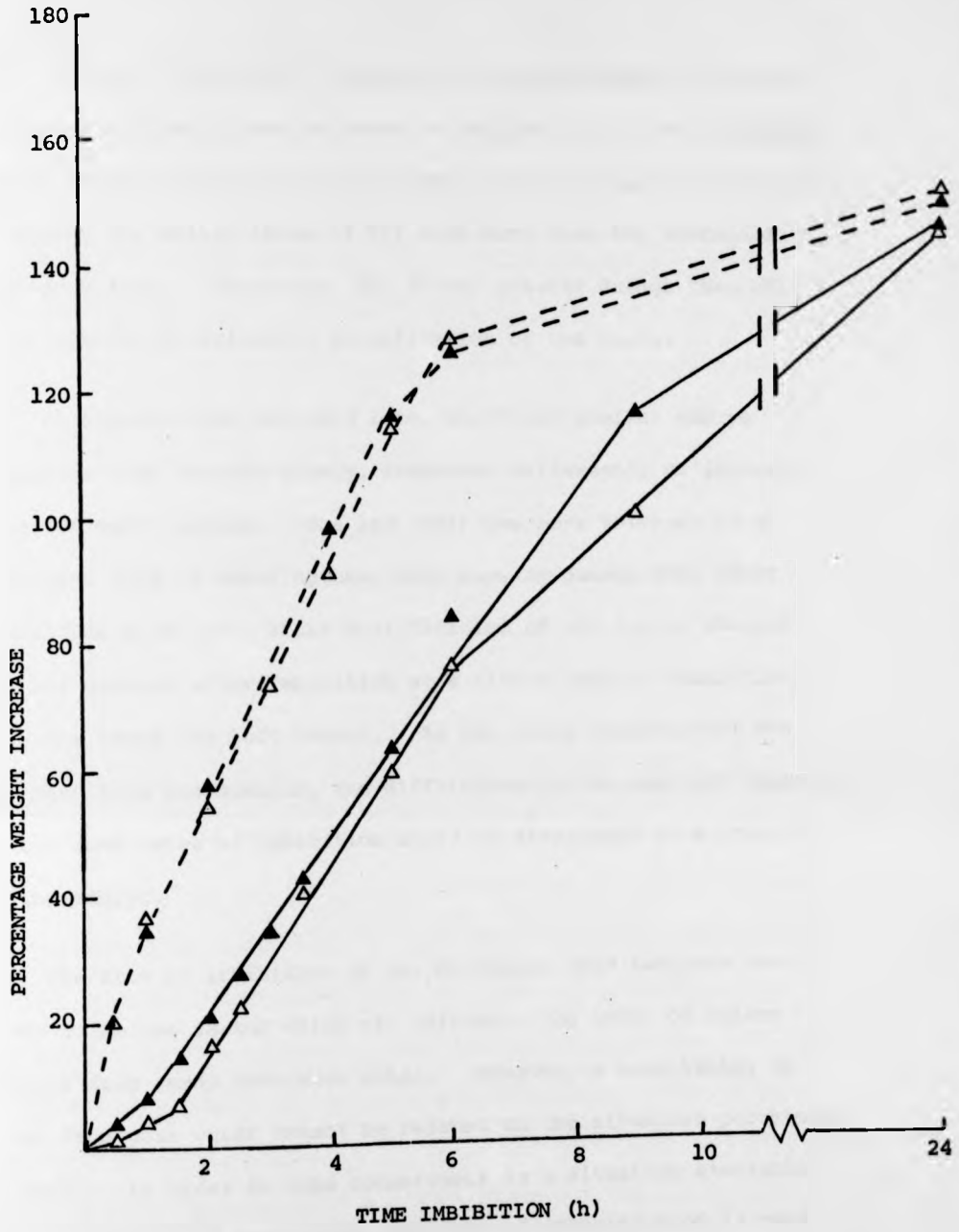


Fig.27 The rate of imbibition in water of two seed lots, Sp 1 (-▲-) and KW 1(-Δ-), with the testa intact (——) and with the testa scarified (---).

could tolerate this rate of imbibition without damage. Both lots had a similar percentage of seeds in category II (< 50% unstained) but the majority of the remaining KW1 seed were completely stained (category I), whilst those of SP1 were more than 50% unstained (category III). Therefore, Sp1 showed greater damage than KW1 after imbibition following scarification of the testa.

It appears that two seed lots, which had similar embryo condition when imbibed slowly, responded differently to increasing rates of water uptake. One lot (KW1) was more tolerant of an increased rate of water uptake, only showing damage when water uptake was most rapid after scarification of the testa, whereas Sp1 was damaged after imbibition at a slower rate of imbibition, when the testa was left intact. As the testa condition of the two seed lots was similar, the differences in the seed lot response to the same rates of imbibition could be attributed to a property of the embryo.

The rate of imbibition of the different seed lots has been shown to be one factor which can influence the level of solute leakage from seeds into soak water. However, a seed taking up water from soak water cannot be related to the situation pertaining in soil. In order to make comparisons in a situation simulating more closely field conditions, the rate of imbibition of 13 seed lots was measured in sand at 22% moisture content. This moisture content (22%) was used rather than the 11% of germination tests, since differences in the field emergence of lots are usually revealed in the more stressful condition of high moisture, than in the 11% moisture content used in germination tests which provide

the optimum conditions for growth. It might particularly be the case that differences in the rate of imbibition would be important at high soil moisture contents.

The imbibition curves in moist sand of the three seed lots used earlier as representative of all seed lots are shown in Fig.28. Differences in the percentage weight increase due to water uptake could be seen between lots at each time during imbibition. After 48h, germination of the seeds had occurred, and weight increases at this time could have been due to growth, therefore only weight increases up to 28h were considered to be imbibition. In the earlier work on imbibition in water, differences in the rate of imbibition were noted at all time intervals during imbibition, and also during the linear phase. In most seed lots, imbibition was almost complete after the end of the linear phase, when an average weight increase of 90% had occurred. A similar linear phase of imbibition in sand may occur between 6 and 24h (Fig.28) and could be used for comparison with earlier work, in addition to the time taken to reach a 90% weight increase. These two measurements, and the weight increases occurring at different times during imbibition, were used as measures of the rate of imbibition of seeds in moist sand, and correlated with the 24h conductivity of the seed leachates. The weight increases after 6 and 28h (Table 37) and the time to reach a 90% weight increase were positively related to the 24h conductivity (Table 37, Fig.29). Thus, seed lots which imbibed rapidly in sand had high conductivities. The correlation between the rate of imbibition in the linear phase, and the 24h conductivity however was not significant (Table 37).

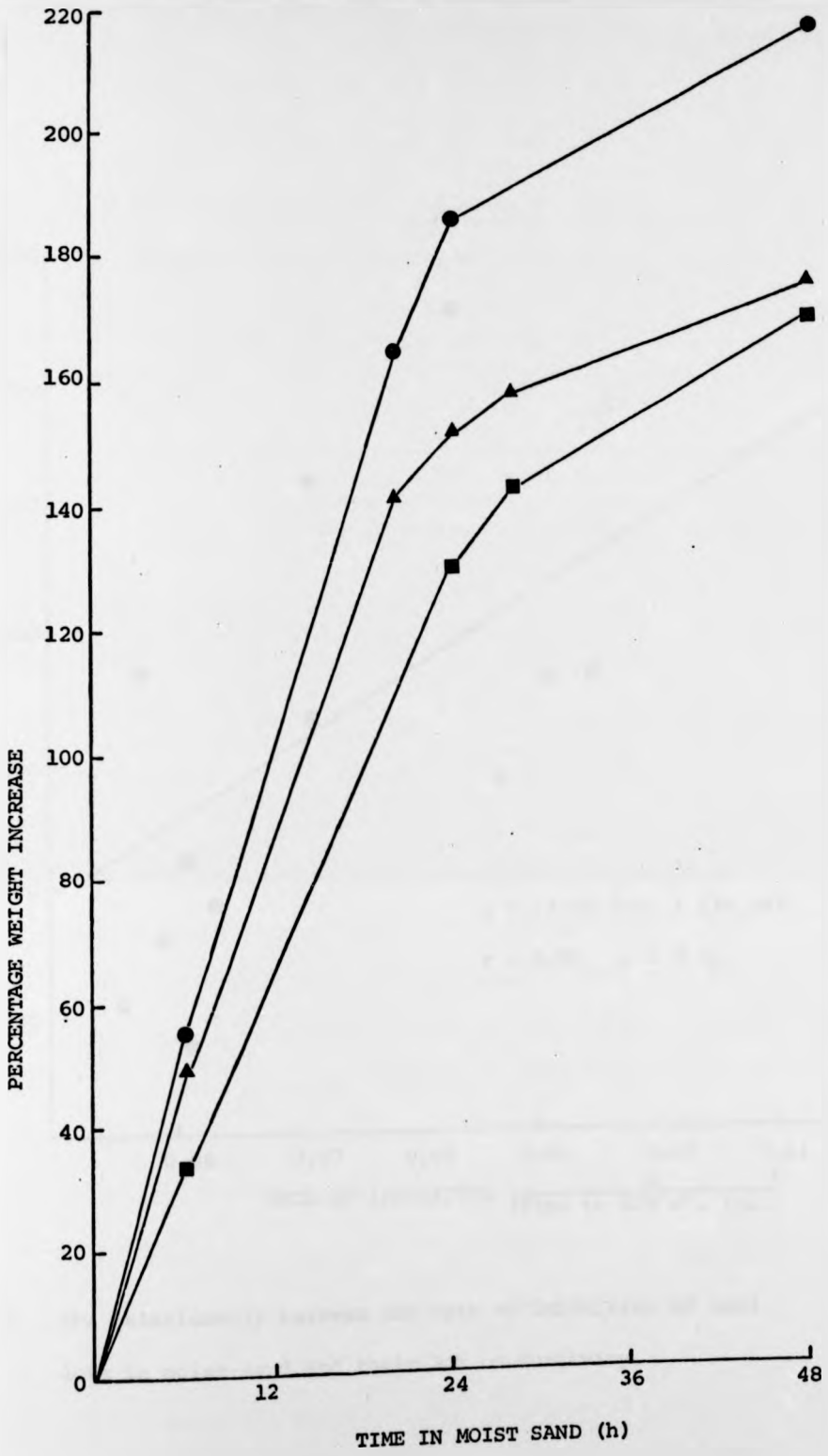


Fig.28 The rate of imbibition of three seed lots in moist sand.

P1 (-■-); Sc2 (-▲-); Sp2 (-●-)

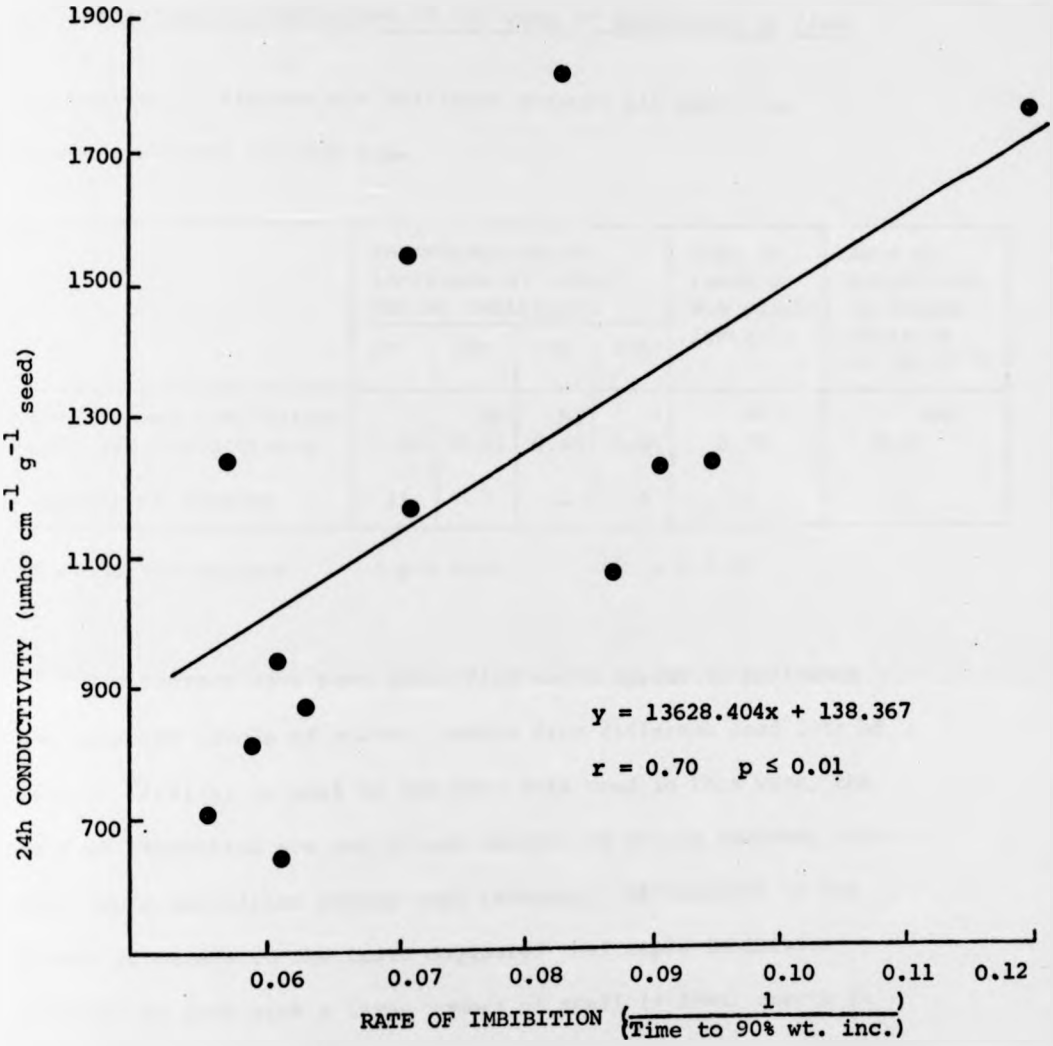


Fig.29 The relationship between the rate of imbibition of seed lots in moist sand and their 24h conductivity.

TABLE 37

Correlation coefficients of the 24h conductivity of intact seeds with measurements indicative of the rate of imbibition in sand

The degrees of freedom are different because all seed lots were not weighed at each time.

	Percentage weight increases at times during imbibition				Time to reach a 90% weight increase	Rate of imbibition in linear phase (% wt inc h^{-1})
	6h	20h	24h	28h		
Correlation coefficient with 24h conductivity	* 0.56	NS 0.51	NS 0.45	* 0.80	** 0.70	NS 0.10
Degrees of freedom	11	7	11	6	11	11

NS = not significant * $p \leq 0.05$ ** $p \leq 0.01$

Two factors have been identified which appear to influence the relative levels of solute leakage from different seed lots of peas. Firstly, in most of the seed lots used in this work, the rate of imbibition was positively related to solute leakage, lots with rapid imbibition having high leakage. Differences in the extent of damage to the testa suggested that rapid imbibition occurred in lots with a large number of small (< 15mm) cracks in the testa.

Secondly, the initial condition of the embryos before imbibition appears to influence both leakage and the rate of imbibition. After imbibition minus the testa, all seed lots showed damage reflected in increased solute leakage. In addition, differences in the level of leakage could also be related to initial embryo condition, high leakage, arising as a result of greater damage

occurring in seeds with poor initial embryo condition. Seeds with poor initial condition appeared to be less tolerant of rapid imbibition minus the testa than seeds in good condition. It may also be possible that the rapid imbibition of these seeds may itself be a result of embryo condition.

DISCUSSION

The work in this thesis has concentrated on two main areas: the storage of pea seeds, and their imbibition in water. Physiological aspects of seed response to storage in either humid or dry storage, provided evidence that membrane deterioration was an early stage of seed ageing, which was confirmed by phospholipid analyses. In addition, response to laboratory storage drew attention to the practical significance of prolonged storage as a possible cause of differences in vigour observed in seed lots of peas. The study of imbibition revealed that the testa has an important function in regulating the rate of water uptake. The testa condition, along with embryo condition was related to the differences in leaching of seed lots that was associated with pea seed vigour. Thus, both work on storage and imbibition have provided information of significance to seeds in general, and to a practical problem in pea seed production.

Physiological changes in storage

High levels of solute leakage from deteriorating but viable seeds, and from dead seeds of crimson clover (Ching and Schoolcraft, 1968; Ching, 1972) cotton (Presley, 1958) and barley (Abdul Baki and Anderson, 1970) have been regarded by the investigators as the result of degradation of cellular membranes and subsequent loss of their retentive ability. On the basis of this evidence, membrane deterioration has been suggested as the first stage of seed ageing (Delouche, 1969). However, increased solute leakage can occur from dead seeds (Takayanagi and Murakami, 1968, 1969), and also

from viable seeds which have dead tissue on their cotyledons (Matthews and Rogerson, 1976). Therefore, these observations of increased leakage from deteriorating and dead seeds, may not be explained solely on the basis of membrane deterioration.

It is clearly important to relate increased leakage to living cells if membrane deterioration is to be suggested as a possible cause of increased leakage, and a knowledge of the extent of deterioration of the seed and the development of dead tissue is essential. In the present work, vital staining was used as one means of assessing seed deterioration, and in this way other deteriorative changes could be related to living or dead tissue.

Storage of pea seeds in either humid or dry conditions resulted in increased solute leakage from the seeds. These increases in leakage were observed from living cells, which did not have higher levels of solutes available for leakage. These observations suggest subcellular damage in living cells which adversely affects solute retention, and provided sound evidence that loss of membrane integrity could be the first of a sequence of deteriorative changes leading to a loss of viability (Delouche, 1969).

An increase in membrane permeability might also have been indicated by the increase in the intensity of vital staining observed in living seeds before the development of dead tissue. Similar staining has been reported previously in damaged cotyledon tissue (Moore, 1972) and has also been found to be a feature of pea seeds from which solutes are readily leached (Matthews, 1971). An increase in membrane permeability would facilitate the penetration

of the stain to inner layers of cells so that more cells could react with the stain, producing formazan, and thus staining intensity would increase (Byrd, 1970). In the present work extraction of formazan from deteriorated pea seeds did not confirm that any increase in stain penetration occurred. In contrast Byrd (1970) showed that there was an increase in the amount of formazan produced in aged soya beans indicative of increased staining intensity, as a result of increased stain penetration.

Although seed deterioration occurred during storage in both humid and dry conditions, viability declined only in humid storage. Loss of viability of pea seeds in conditions of high relative humidity and temperature has previously been noted by James, Bass and Clark (1967), and by Roberts and Abdalla (1968). The response of seeds to increased temperature and relative humidity followed that which would be predicted by Harrington's (1960) "rule of thumb" and the predictive equations of Roberts and Abdalla (1968). Although in the present work, viability of pea seeds was retained in dry storage, a loss of viability has been reported after both short and long term storage in dry conditions (Ewart, 1895; Nakamura, 1976). The loss of viability in short term storage was rapid, severe drying conditions resulting in a decline in pea viability down to only 15% in three weeks (Ewart, 1895). Following prolonged storage for five years in conditions of either 30% rh, or less than 10% rh, the viability of two cultivars of peas declined (Nakamura, 1976). In the drier conditions, viability declined to only 3% and 10% compared to 66% and 58% in 30% rh.

However, pea seeds from dry storage did show evidence of

deterioration, in the form of increased leaching and reduced staining. This suggested that perhaps deterioration was occurring, but more slowly than in humid conditions. Similarities in the effects of humid or dry storage on several species have also been noted by Nutile (1964), although at a later stage of deterioration. Seedlings from seeds stored in both conditions showed injury, and the viability of all seeds eventually declined.

The development of hard seeds in dry storage

A second group of seeds developed in dry storage in addition to those which showed deterioration in the form of increased solute leakage; these were hard seeds, showing little or no imbibition. The failure of these seeds to imbibe was due to the impermeability of the testa, since scarified seed took up water at the site of scarification until fully imbibed and thereafter germinated normally.

The reduction in seed moisture content which occurred as a result of equilibration with the low storage humidity may be the cause of the increase in the proportion of hard seed. Similarly, Gloyer (1932) suggested that severe drying during production of pea seeds was responsible for increasing their hardseeded character, and Jones (1928) has associated low storage humidities with the development of hard seeds. Hyde (1954) described the development of an impermeable testa simultaneously with the desiccation of the seed interior in two Trifolium species and Lupinus arboreus. Impermeability developed at 14% moisture content, and thereafter drying took place only at the hilum which acted as a hygroscopic valve, opening at low relative humidity and allowing further drying of the seed, and closing at high relative humidities preventing rehydration. Thus the moisture content of seeds would be in equilibrium with the lowest relative humidity to which they were exposed. Closure of the hilum after transfer of seeds from 1% to 55% rh would explain the failure of the seeds from dry storage to rehydrate in these conditions of higher relative humidity. Similar reductions in moisture content and increased proportions of hard seeds during dry storage have been reported in Phaseolus

vulgaris (Nutile and Nutile, 1947; Harrington, 1949), subterranean clover (Aitken, 1939), sweet clover (Helgeson, 1932), red clover (Stahl, 1937), *Urena lobata* (Garrard, 1955), crimson clover and perennial ryegrass (Ching, Parker and Hill, 1959).

In this investigation and many others, the failure of hard seed to imbibe was ascribed to the presence of an impermeable testa. Many workers have examined the anatomy of the testa in order to ascertain the site of impermeability (Raleigh, 1930; Hamly, 1932; Stevenson, 1937; Aitken, 1939; Corner, 1951; Ballard, 1973). As a result many suggestions have been made as to the site and cause of testa permeability, including the cuticle (Nobbe, 1876; Pammel, 1899; White, 1908), the Malpighian cells (Rees, 1911; Lute, 1928; Hamly, 1932), and the chemical substances in the light line (Coe and Martin, 1920). At present, the most popular view of the cause of impermeability is the suberisation of the Malpighian cells (Ballard, 1973). All of these causes are chemical in origin, whereas Corner (1951) suggested that impermeability was caused by the physical contraction of the walls of the Malpighian cells. He was supported in this suggestion by Gladstones (1958) who proposed that as impermeability is often associated with the degree of seed hydration, this indicates that purely physical processes might be involved in restricting water entry. The development of increased numbers of hard seeds during dry storage of peas could be more easily explained as a result of physical contraction of layers of cells due to dehydration so that they are more tightly packed, than by changes in their chemical constituents. Furthermore, the association of tightly packed cells with impermeability has been noted in the seed coat of sweet clover, in which cells were more

compactly arranged in impermeable areas than in permeable areas (Stevenson, 1937). In addition, Stevenson (1937) suggested that hard seed from normally permeable lines of sweet clover are in fact different to those from impermeable lines. This would suggest that the cause of hardseededness which develops in seeds which initially had permeable testae might be different to that in seeds which have impermeable testae at the time of harvest, and would provide an explanation for the role of both chemical and physical factors influencing testa impermeability.

The proposal that possession of an impermeable seed coat may be a favourable condition for seed longevity has been made by many workers (including Groves, 1917; Crocker, 1948; Crocker and Barton, 1957). At low moisture contents, enzyme catalysed reactions would occur slowly, and provided the seed coat is impermeable to gases, respiration would be limited. As a result, it has been suggested that deteriorative processes would occur slowly. The condition of hardseededness may therefore explain the long storage life of many legumes. Present work showed that dry storage can result in pea seed deterioration as well as the development of hard seeds. It is possible that the development of a hard seed coat in dry conditions may have evolved as a means of avoiding deterioration in these conditions.

Membrane changes in storage

Seeds were analysed for their phospholipid content over the period in humid storage in which an increase in solute leakage occurred in living seeds, in an attempt to relate an expression of ageing, that is, increased leakage, with membrane deterioration during the early stages of ageing. The main component of the phospholipids extracted from pea seeds was phosphatidyl choline (PC), which is the major phospholipid in most membranes, in particular the mitochondrial and plasma membranes (Benson, 1964; Kates, 1970; Donaldson, Tolbert and Schnarrenberger, 1972; Kates and Marshall, 1975). The other major plant phospholipids are phosphatidyl inositol (PI) and phosphatidyl ethanolamine (PE), (Kates, 1970; Donaldson et al, 1972; Kates and Marshall, 1975). Both of these were extracted from pea cotyledons, the level of PI being second largest after PC; thus PC and PI contributed a similar proportion to the total phosphorus as they did in the work of Quarles and Dawson (1969) on pea cotyledons, although the amounts of all phospholipids extracted from peas in this work were higher than those extracted by Quarles and Dawson (1969). Phosphatidyl ethanolamine (PE) only made a minor contribution to the total phospholipid, although it is a major phospholipid in leaves, algae and photosynthetic bacteria (Benson, 1964; Kates, 1970; Donaldson et al, 1972; Kates and Marshall, 1975) and has been found in pea seeds (Quarles and Dawson, 1969) in the third largest amount after PC and PI. The four seed lots from which phospholipids were extracted differed greatly in the amount of phosphatidic acid (PA) in the seeds before storage. The two lots which went on to deteriorate little in storage contained a larger proportion of PA, although PA is usually considered a minor phospholipid, whereas

the two lots which later deteriorated rapidly contained comparatively little PA. Low levels of PA were also found in peas by Quarles and Dawson (1969). The levels of phosphatidyl glycerol (PG) previously extracted from pea cotyledons (Quarles and Dawson, 1969) were found to be very low, but in this investigation it occurred in amounts similar to that of PI in three of the four lots; in the fourth lot, only low levels were detected.

It is difficult to explain the significance of such differences in the phospholipids of unstored seeds from different seed lots, since only a limited amount of work was done on phospholipids. As different cultivars were used, it may be that there are cultivar differences in phospholipid levels, or alternatively, as the lots differed in vigour, the differences in phospholipids may reflect the condition of the seed.

There were no consistent changes in the total phospholipids extracted from pea seeds during short term storage, which suggested that there was no net degradation of these compounds. Since total phospholipid was unchanged, it would beunlikely that changes in membrane permeability could result from the development of incomplete membranes due to a decrease in the total membrane area in a cell. This was supported by the absence of any change in the amount of phospholipid occurring at the origin of the TLC plate following storage. An accumulation of phosphorus at the origin has been attributed to the development of more polar lipids (Koostra and Harrington, 1969) arising from oxidation of the phospholipids. In the present work, as an increase in polar lipids did not develop during short term ageing, any changes in phospholipids did not appear to result from breakdown due to lipid oxidation.

Changes in the amounts of the different phospholipid classes present did occur. The charge and size of the polar head group of the phospholipid can influence permeability (Van Deenen, De Gier and Demel, 1972), therefore such changes in the amounts of the phospholipid classes may influence permeability. The changes in the phospholipids could only be related to leakage from living cells in two seed lots (KW75 and KW1), which retained complete cotyledon staining over a period of short term storage. In these seed lots there were changes in phospholipids before dead cells developed, with further, often greater changes in composition as the proportion of dead tissue increased.

In the earliest stages of deterioration in seed lots KW75 and KW1, after one day storage, there was an increase in PC and decreases in PA and PE in both lots. In one lot (KW75) a large increase in PG also occurred and an unknown phospholipid (U2) appeared. The development of this phospholipid early in deterioration, and other unknowns (U1 and U3) at later stages, suggested that there was some breakdown of the major phospholipids to another form which was not identified. Such breakdown could result from the action of phospholipase A_1 which degrades phospholipids, and in particular PC and PE, to their lysoderivatives (Benson, 1964; Kates, 1970; Moreau, Dupont and Lance, 1974). The changes in phospholipids after two days storage also occurred in living cells, although there was less similarity in the two lots. This might have reflected the slightly more rapid rate of deterioration in one of the lots.

These changes in the phospholipid composition of totally living cells may result in a change in the overall charge of the lipid

component of the membrane. Alternatively as a result of differences in the size of the polar head groups, the membrane may become more, or less tightly packed. A change in either of these factors may alter membrane permeability (Van Deenen et al, 1972), therefore, the alteration of the phospholipid composition may possibly explain the increase in solute leakage from living cells.

Phosphatidic acid, which declined early in the deterioration of KW75 and KW1, is an intermediate in the formation of all other phospholipids (Beevers, 1975; Kates and Marshall, 1975) and frequently occurs as an artefact in extracts due to the degradative action of phospholipase D (Benson, 1964; Kates, 1970; Moreau et al, 1975; Wilson and Rinne, 1976, a). In the present work, PA increased after three days in two out of three seed lots which were extracted at the same time. If a high level of PA occurred as an artefact due to extraction, it would be expected to occur in all three of the lots extracted at the one time, since conditions for extraction would be the same. Only two out of the three lots had high levels of PA after three days storage, there was no increase in the third lot. This would suggest that PA did not occur as an artefact. A decline in PA, observed in two seed lots (Sp1 and DSP2) on deterioration, might indicate its utilisation in the synthesis of other phospholipids, due to its role as an intermediate in their synthesis.

Changes in the occurrence of phospholipid classes have also been observed in cucumber seeds after both natural and artificial ageing (Koostra and Harrington, 1969); these changes were small before loss of viability, and were enhanced as viability was lost. Harman and Mattick (1976) in work on pea seeds, focussed attention

on a different part of the phospholipid molecule, the fatty acid chain, and noted small decreases in the unsaturated acids, linoleic and linolenic acid before loss of viability, with larger decreases after viability declined. Since the decline in linolenic acid in the axis paralleled vigour loss, they concluded that free radical formation may be an important factor in early seed deterioration. Fresh weight changes in the growing axis were used as a measurement of vigour (Harman and Mattick, 1976), however a decline in fresh weight is one of the later stages of deterioration (Delouche, 1969). Present evidence suggests that similar changes occur at early and late stages of deterioration although of different magnitude, and therefore the saturation changes observed by Harman and Mattick (1976) at a late stage of deterioration might also occur at earlier stages. Since changes in the degree of saturation of fatty acid chains can alter membrane permeability (Van Deenen et al, 1972), such saturation changes may be involved in allowing increased solute leakage from aged but living cells. Further analysis of unsaturated and saturated fatty acids should be done during early stages of deterioration to confirm this suggestion.

The changes occurring in the amounts of the different phospholipids during short term storage might be explained by the occurrence of interconversions between the phospholipids, with alcohol moieties being released into and incorporated from the cytoplasm. Interconversion of phosphatidyl serine (PS) and PE has been noted (Mazliak, 1973; Kates and Marshall, 1975), and the three step methylation of PE to PC is well known (Chapman, 1965; Kates and Marshall, 1974; Mazliak, 1973; Beevers, 1975; Moore, 1976). Phosphatidyl serine was not identified in this work, and

the decline in PC levels along with the fairly constant low levels of PE meant that interconversions of these were unlikely. An alternative explanation for the changing levels may lie in the synthesis, degradation, and recycling of the phospholipids, all of which constantly occur (Kates, 1970; Wilson and Rinne, 1976a, b). Deterioration may cause changes in the rate at which one or more of these processes occurs, and result in the fluctuating phospholipid levels. The changing levels of PA in particular, which is an intermediate in the synthesis of other phospholipids, suggests that changes in the rates of synthesis may be occurring. Seeds were imbibed for 24h before extraction, and so changes in phospholipids may not be due to changes in the phospholipids present after deterioration of the seed, but reflect the effect of deterioration on the de novo synthesis of phospholipids.

This evidence therefore, appears to suggest that the early stages of seed ageing do not result from degradation of chemical constituents of the membranes, but from changes in their proportions. In contrast (Ching, 1972) provided evidence from aged crimson clover seeds suggesting hydrolysis and breakdown of macromolecules during ageing as result of chemical degradation, by for example auto-oxidation and spontaneous hydrolysis. Thus, the deterioration in crimson clover appeared to be related to degradation of chemical constituents. However, this evidence does not allow the early stages of membrane deterioration to be unequivocably attributed to chemical degradation, since although breakdown of protein, carbohydrate, and phosphate esters appeared to occur in viable seeds in which ageing was indicated by increased leakage, the precise extent of deterioration was unknown. This was another

situation in which the results could be complicated by the presence of dead tissue in viable seeds. Similar analyses on seeds which are known to be totally living, would clarify whether membrane deterioration could occur as a result of chemical degradation.

Chemical analyses therefore, suggest that membrane deterioration occurs during ageing. Support for membrane deterioration in aged, but viable seeds has come from ultrastructural investigations on aged maize embryos (Berjak and Villiers, 1972a, b) and soya beans (AbuShakra and Ching, 1967). In their work on the root cap cells of maize, Berjak and Villiers (1972, a, b) observed membrane damage in viable embryos in cells with otherwise organised cytoplasm, the membrane systems of the mitochondria, plastids, endoplasmic reticulum, and dictyosomes being disorganised. AbuShakra and Ching (1967) noted similar damage to the mitochondria of aged soya beans. Viable embryos may however include large proportions of dead tissue, and therefore the possibility that dead cells were being examined could not be entirely eliminated.

These preliminary investigations of phospholipid changes over a period of short term storage in moist conditions when membrane deterioration was indicated by increased solute leakage from living cells, have underlined the dynamic nature of membranes in imbibed seeds. Impaired solute retention in living cells may have resulted from the observed change in the proportions of phospholipids. The dynamic nature of these changes strongly implicated the involvement of enzyme action.

Seeds from dry storage had low moisture contents in which enzyme catalysed reactions would occur only slowly. Thus, although

the expression of damage in these seeds was similar to that of seeds from humid storage, the mechanism of deterioration may be different. Three suggestions have been put forward to explain the damage observed in seeds from dry storage: lipid autoxidation, physical damage to molecules due to water loss, or damage during imbibition.

The first explanation, that of lipid autoxidation was proposed by Harrington (1965), who theorised on the basis of a comparison of deterioration in dehydrated foods with that of seeds in dry storage. Deterioration of dehydrated foods is associated with lipid oxidation in areas of unsaturated bonds (Stuckey, 1962), and Harrington (1965) proposed that at low moisture contents, the loss of viability of seeds is due to autoxidation of unsaturated lipids in the cells of the embryo, leading to the formation of free radicals. As many of the membrane lipids contain unsaturated fatty acids, such autoxidation may cause membrane damage, with a resultant increase in leakage. Autoxidation may be facilitated in dry storage by the destruction of the monomolecular layer of water which normally surrounds the macromolecules (Schultz, Day and Sinnhuber, 1962). Free radical formation is not enzyme controlled, and so this reaction could take place at the low moisture contents of the seeds from dry storage, and membrane damage due to autoxidation could therefore explain a decrease in membrane integrity and the increased leakage of electrolytes from living seeds.

Lipids are situated close to proteins in membranes, and thus lipid autoxidation may have secondary effects as a result of damage to membrane proteins and enzymes (Tappel, 1962); damage to these molecules could affect enzyme reactions and subsequent growth.

A reduction in enzyme activity and growth are two of the later stages of seed ageing (Delouche, 1969), therefore deterioration due to lipid autoxidation in dry storage could explain both early leakage, and the later stages of ageing.

If lipid autoxidation was responsible for the deterioration of the seeds in dry storage, the slower deterioration of these seeds may be explained by the initial protective effect of tocopherols. Tocopherols are naturally occurring antioxidants, e.g. Vitamin E, which occur in most seeds and may protect the seeds from deterioration. A prestorage treatment of okra and onion seed with α tocopherol or starch phosphate (a synthetic tocopherol) has been shown to reduce deterioration, and thus increase the storage life of the seeds (Kaloyereas, Mann and Miller, 1961). Their protective action lies in their reaction with the free radicals produced in autoxidation, rendering them harmless (Tappel, 1962) and preventing damage. Harrington (1973) suggested that in dry storage, once the inherent tocopherol was utilised, deterioration would result, as synthesis of tocopherol could not take place at low moisture contents. Free radicals would therefore accumulate, reacting with other lipids and proteins, and resulting in membrane damage, enzyme inactivation and chromosome abnormalities (Harrington, 1973). The initial protection afforded by tocopherols would result in a lag period before deterioration began in the seeds in dry storage, so that at any time they would be at an earlier stage of deterioration than seed from humid storage.

A second explanation for the deterioration observed in seeds from dry storage could be that physical, rather than chemical, damage to the membranes occurred as a result of the low moisture

contents of these seeds. Recent work by Buttrose and Swift (1975) on desiccation injury in pea roots has suggested that damage due to drying is a physical effect on protein molecules. They suggested that the contraction of membrane systems occurring during drying, may destroy bonds between structural protein molecules which may not be reconstituted on rehydration. As a result of the disorganisation of proteins, the membrane would lose its continuity during rehydration, and an increase in solute leakage could result.

Thirdly, it has been suggested that the damage observed after dry storage occurs during imbibition, and not during storage itself (Nutile, 1964). It has been suggested (Ching, 1963; Nutile, 1964) that severe drying results in the removal of the monomolecular water which is an integral part of the chemical configuration and constitution, so that on rehydration, water molecules might not attach to the correct places, and thus, damage would result due to disruption of normal metabolic processes. Woodstock, Simkin and Schroeder (1976) have also suggested that the damage seen in reduced germination and vigour of seeds dried over synthetic desiccants compared to freeze-dried seeds was due to the removal of the monomolecular water in these seeds. In the present work, the increased leakage of solutes from seeds following dry storage, might have resulted from an alteration in the configuration of the membranes due to severe drying, which caused an increase in their permeability. However, the previous observation of damage to seeds from dry storage (Nutile, 1964) was associated with rapid imbibition. The pea seeds from dry storage imbibed more slowly than unstored seed, and therefore it seems unlikely that rapid imbibition was the cause of the damage observed. The attempt to eliminate the possibility

of damage during imbibition by hydrating seeds slowly in a high relative humidity was unsuccessful. Thus, although damage due to rapid imbibition was unlikely, it was not possible to establish for certain whether the damage in dry stored seed was due to storage in dry conditions, or rapid imbibition in a very dry seed.

The practical significance of deterioration in laboratory storage

The extension of the examination of seed deterioration in short term storage to long term storage in both commercial warehouses and in conditions simulating warehouse conditions, revealed two practical implications of this work. Firstly, evidence was obtained regarding the influence of storage on pea seed vigour, and secondly a predictive test of pea seed storeability was suggested.

The evidence of deterioration in short term storage, in the form of an increased leachate conductivity and reduced vital staining, also occurred in long term storage. This was most significant from a practical point of view because both these features are indicative of a decline in seed vigour, and occurred in seeds from commercial warehouses which retained viability above 80% and were commercially available for sale. Similar deterioration of viable seeds was also seen in seeds from outhouse storage, although in these more adverse conditions, the viability of many seed lots declined over the period of storage. Prolonged storage has not previously been considered as a possible cause of vigour differences between seed lots (Perry, 1969). However, these observations of a decline in vigour of pea seeds during a period of long term storage suggested that one possible source of the differences in seed vigour commonly found in commercially available lots of pea seeds could well arise from different lengths of time in storage.

The second practical implication which arose, developed from the observation that there was a similarity in the response of seeds to both short and long term storage. This suggested that short term storage in extreme conditions was accelerating the normal ageing process. Koostra and Harrington (1969) have also observed

similar changes in naturally and accelerated aged tissue, but cautioned against the assumption that the ageing processes leading to such changes are the same in both tissues. Abdul Baki and Anderson (1970) found that the leaching of sugars from naturally aged barley increased, whereas there was little change in leaching after accelerated ageing. This suggested that the processes of natural and accelerated ageing are in fact different.

Although the response of seeds to both storage conditions was similar, seed lots with uniformly high initial viability, deteriorated in storage at different rates, an observation previously noted between different varieties of peas (James et al, 1967). The rate at which the seed lots deteriorated over a period of days under accelerated ageing conditions, as measured by solute leaching, was positively correlated with their rate of deterioration over a period of 21 months. Thus, high conductivities after accelerated ageing occurred in lots in which vigour and viability declined rapidly in prolonged storage. This suggested that a seed lot's response to accelerated ageing might be used to predict its storage potential. In this way, if prolonged storage of pea seeds is necessary, seed lots which show minimal deteriorative changes could be selected for storage. Using less severe short term storage conditions, James et al (1967) have shown a similar significant relationship between the viability of peas after three months short term storage, and viability after up to four years storage. However, they concluded that their correlations were "not of sufficient magnitude to use germination decline at 90°F and 90% rh as an indication of storeability under more favourable conditions." More recently however, accelerated ageing has been shown to be the most

efficient test for predicting the rate of deterioration in storage of soya bean (Byrd, 1970) and peanuts (Baskin, 1970), and Delouche and Baskin (1973) have outlined the accelerated ageing conditions most suitable for predicting the relative storeability of a number of species.

In the earlier work on predicting seed storeability, germination tests have been used after accelerated ageing to determine the extent of seed deterioration. Since a decline in germination is recognized as one of the final stages of ageing (Delouche, 1969), germination tests may not be a very sensitive measurement of the extent of deterioration. Many deteriorative processes may have occurred before germination declines, which could reduce seed performance in the field. Leachate conductivity was found to be predictive of seed storeability after accelerated ageing, and since it has been found that an increase in leachate conductivity can reflect an early stage of deterioration, that is, membrane damage, measurements of leaching after ageing would reveal early seed deterioration and thus give a more sensitive measurement of seed storeability. Conductivity measurements are also more rapidly obtained than germination data and could be easily developed into a routine test. Thus, an accelerated ageing test for the storeability of peas could be completed in two days, involving one day accelerated ageing in 94% rh at 45°C, followed by measurement of the 24h seed leachate conductivity.

Conductivity measurements of unaged material also gave a significant correlation with the viability and conductivity of seeds after 21 months storage, suggesting that perhaps the leachate conductivity of unaged material might be used for predicting

storeability over a longer period. However, the necessity for a storage test was emphasised by the observation that some seed lots with similar initial conductivities showed different responses to storage, therefore their initial conductivity would have given misleading estimates of their relative storage potential.

The accelerated ageing response of seed lots before storage also predicted the field emergence of the seeds after 12 months storage, an association which has also been found in soya beans (Byrd, 1970). The field performance of highly viable seed reflects the seed vigour, which suggests that in situations where viability is retained in storage, the accelerated ageing response will predict the vigour of different seed lots after storage. Even so, caution should always be applied when predicting field emergence, due to the influence that the weather conditions at sowing may have on performance. In the present work differences in vigour of lots from commercial storage which had high viability were also indicated by different levels of solute leaching. In this case, the accelerated ageing response before storage was positively correlated with leaching after 12 months storage, and since viability was retained, could be said to be related to the vigour of these seed lots. Thus, seed vigour after storage could be related to accelerated ageing response which might be used to predict vigour levels after storage.

The response of all seeds to storage whether short or long term, was related to their initial embryo condition, adding support to the suggestion by Grabe (1964) that seed longevity in storage is governed by the physiological condition of the seed before storage.

Hence, seed lots with initially good condition retained high levels of vigour and viability whereas those in poor condition deteriorated more rapidly. Rapid deterioration of seed with poor initial condition, in which deterioration had already been initiated before storage, was also noted by Crocker and Barton (1957). Moore (1963) suggested that initial deterioration of seeds in storage usually results from localised areas of damage, which suggests that more rapid deterioration in storage might be expected in seeds damaged before storage and therefore in poor initial condition.

Previous consideration of the influence of seed condition on the storeability of seeds has emphasised the detrimental effect of mechanical damage to the seed on the retention of viability (Battle, 1948; Brett, 1952; Barton, 1961; Harrington, 1963; Moore, 1972). The extent of mechanical damage to the testa varied in the seed lots used, but was only significantly correlated with the viability of the seeds after 21 months storage ($r = -0.63$, $p \leq 0.05$). However, while embryo condition also varied, the extent of complete cotyledon staining was positively related to viability after both 12 and 21 months storage. This indicated that both mechanical and physiological damage to the seed can influence storeability; however the higher correlation of embryo condition with viability suggests that embryo condition may have the more important role in determining seed storage potential.

The influence of seed physiological condition on response to storage also draws attention to the necessity for a knowledge of seed condition in any physiological experiments. One feature of seed condition which is particularly important, and is not frequently considered, is the extent of dead tissue which is contained within

the seed. The importance of this fact was emphasised earlier when considering solute leakage from dead and deteriorating seeds. The assumption that high viability is indicative of living tissue is an error that is frequently made, with the result that conclusions drawn from such experiments can be misleading.

The effects of water during imbibition

One experimental approach which was to be used to examine the influence of deterioration in storage on the condition of cell membranes, drew attention to the process of seed imbibition. This approach was based on the time course of early leakage from seeds, and its relationship to membrane rehydration and reformation (Simon and Raja Harun, 1972). Simon and Raja Harun (1972) proposed that in the dry seed, membranes are incomplete, thus high levels of leakage occur when the seed is first placed in water. They suggested that these membranes rapidly rehydrate and reform, with the result that leakage declines rapidly in the first minutes of imbibition to a lower constant rate. If the time course of leakage could be explained as a result of reformation of membranes, any changes in membrane condition during ageing, indicated by increased leakage from living cells, could be reflected in the form of the time course. In the present investigation, vital staining after measurements of the time course revealed that the tissue on the abaxial surface of the cotyledons was either partially or completely dead. As a result of this observation, the association of the time course of leakage from apparently dead or damaged seeds with membrane reformation seemed to be invalid.

The damage to the embryo resulted from rapid water uptake during imbibition, since damage was reduced, if not entirely prevented, by reducing the rate of water uptake. Cell death occurred in cells which were in direct contact with water, within two minutes of the embryos being placed in water, the inner tissues of the embryo remained undamaged. There did however appear to be a critical point that was reached after 24h slow imbibition, when rapid water uptake ceased

to cause damage. Two possible explanations could account for this, either the existence of a critical rate of imbibition below which damage does not occur, or the development of tolerance to rapid imbibition.

If there is a critical rate of imbibition, below which damage does not occur, it may be expected to fall between a 40% and 45% weight increase due to water uptake in one hour, as embryos showing these increases were undamaged and damaged respectively. In experiments in which slow imbibition was followed by a more rapid uptake of water, partial or complete damage was observed wherever a weight increase in excess of 45% occurred. Damage was also observed after imbibition of dry embryos in water when smaller weight increases of approximately 30% h^{-1} occurred. In this case, rapid imbibition took place in air dry embryos, whereas the critical value of 40-45% h^{-1} was noted for embryos which were partially imbibed. Investigations on chilling injury in soya beans (Obendorf and Hobbs, 1970; Hobbs and Obendorf, 1972), lima beans (Pollock and Toole, 1966; Pollock, 1969), cotton, (Christiansen, 1968) and garden beans (Pollock, Roos and Manalo, 1969) have all led to the suggestion that sensitivity to rapid imbibition at low temperatures increases below a certain moisture content. Therefore, the greater sensitivity of dry embryos to damage during rapid imbibition may not be unexpected.

A second explanation for the absence of damage in embryos imbibed slowly for 24h before experiencing rapid water uptake, could be that these embryos were more tolerant of rapid imbibition. Villiers and Edgcumbe (1975) proposed that the longevity of hydrated seeds in the soil was due to the operation of repair mechanisms in the

hydrated seed which could not occur in dry storage. Thus, any damage due to ageing was repaired in the hydrated seed, whereas seeds in dry storage deteriorated. The seeds imbibed for 24h before rapid imbibition may have reached a state of hydration at which such repair mechanisms could operate, and could therefore tolerate rapid imbibition without apparent damage. This explanation is dependent on the degree of hydration of the seed, that is, its moisture content. Simon and Wiebe (1975) found that a moisture content of 30% or more is sufficient to suppress leakage from pea embryos, and suggested that at this moisture content, enough water was available to stabilise the membrane bilayers. Embryos imbibed slowly for 24h may have reached this state of hydration, and since membranes play an important role both in cell organisation and in many metabolic processes, their stabilisation may facilitate the repair mechanisms proposed by Villiers and Edgcumbe (1975).

If seed moisture content is important in determining the tolerance of pea embryos to rapid imbibition, an analogy may again be drawn between this situation and chilling injury. Above certain moisture contents, the tolerance of soya beans (Obendorf and Hobbs, 1970; Hobbs and Obendorf, 1972), lima beans (Pollock and Toole, 1966; Pollock, 1969), cotton (Christiansen, 1968) and garden beans (Pollock, Roos and Manalo, 1969) to chilling injury, increases. Most of these workers have explained chilling injury as a result of disruption of cell membranes, although Christiansen (1968) proposed that it resulted from blockage of a metabolic system. Even though the critical moisture of these seeds was lower (12-16%) than it would be in the pea embryos which did not show imbibition damage, a similar situation of membrane organisation and repair mechanisms may exist

above these moisture contents which prevent the expression of chilling injury.

The damage that occurred as a result of rapid imbibition was first observed by reduced vital staining of the embryo. Other expressions of damage were revealed in increased solute leakage, reduced respiration, slower germination of the embryos, and reduced seedling growth. Similar consequences of rapid imbibition have been noted in both embryos and intact seeds by a number of other workers. The rapid rate of imbibition which was observed here in pea embryos in comparison to intact seeds, has also been associated with a higher level of solute leakage by Larson (1968), and similar observations have been made on intact pea seeds (Kidd and West, 1918, 1919; Eyster, 1940). Such high levels of leakage have been associated with dead or damaged tissue (Takayanagi and Murakami, 1968, 1969; Matthews, 1971; Moore, 1972; Matthews and Rogerson, 1976), which was confirmed here by the reduced vital staining. High levels of leakage have been associated with low pea seed vigour (Matthews and Whitbread, 1968; Bradnock and Matthews, 1970), thus the damage occurring as a result of rapid imbibition might be expected to have consequences in subsequent growth.

Measurements of seed germination and seedling growth did in fact suggest a reduction in the vigour of damaged embryos, as not only were the rate and final level of germination reduced, but growth of the radicle and plumule of the seedlings were also lower than in undamaged seeds. These differences were not revealed in the relative growth rates of the seedlings until ten days after the beginning of germination. A reduction in germination following rapid imbibition of intact seeds has been observed in both peas

(Barton, 1950, 1952; Perry and Harrison, 1970; Rowland and Gusta, 1977) and Phaseolus (Barton, 1950, 1952; Orphanos and Heydecker, 1968). Harrison (1973) has also noted a decline in germination of intact peas following rapid imbibition in water at 1°C, and although the low temperature was itself damaging, the decline in germination following rapid imbibition in water was double that after slow imbibition between blotters. This decline in germination was associated with the death of the meristematic areas of the radicle and plumule, no damage to cotyledons was observed. In addition, chilling injury in lima beans (Pollock and Toole, 1966; Pollock, 1969), soya beans (Obendorf and Hobbs, 1970; Hobbs and Obendorf, 1972) and cotton (Christiansen, 1968) has been associated with rapid hydration, and results in a decline in seed germination.

The reduction in the growth of seedlings from damaged seed was also linked with a slower rate of food reserve transfer from the cotyledons to the growing axis, an observation which has also been made by Larson (1968) using round seeded peas. The growth rates of damaged and undamaged seeds may be related to the pattern of food reserve mobilisation in the cotyledons during germination and the localisation of the damage occurring as a result of seed imbibition minus the testa. Mobilisation of food reserves would be reduced in tissues in which dead cells occurred, because enzyme activity would be absent. Differences in growth rates of seedlings from damaged and undamaged embryos would be expected when reserves were mobilised from the abaxial surface of the cotyledon, where cell death occurred in damaged embryos. Significant differences in seedling growth rate occurred only after ten days growth, and in

consequence, the pattern of food reserve mobilisation might be predicted to occur from the adaxial to abaxial surface of the cotyledons. However, reserve mobilisation in Pisum sativum occurs from the abaxial to adaxial surface (D.L. Smith, personal communication) in a similar manner to P. arvense (Smith and Flinn, 1967), so that any differences in the seedling growth rates as a result of reserve mobilisation would be expected to occur early in germination.

Seeds which sustained intermediate damage during imbibition showed that damage to seeds may be quite extensive before it is revealed by a decline in germination, since although their final level of germination equalled that of undamaged seeds, their rate of germination was reduced, and the growth rate of the seedlings was similar to that of damaged seeds. Berjak and Villiers (1972a) have suggested that the delay in germination of old, but viable seed may be due to a period in which damaged organelles are repaired and replaced, while failure to germinate is a result of irreparable membrane damage. Seeds with intermediate damage may still possess the capacity to repair organelles which have been damaged during imbibition and therefore remain viable, although the rates of germination and growth are reduced.

There have been many discussions of the nature of the damage observed after rapid water uptake, whether it is physical, causing damage to cell membranes (Kidd and West, 1917, 1919; Eyster, 1940; Pollock, 1969; Obendorf and Hobbs, 1970), or chemical with disruption of cell metabolism (Christiansen, 1968). Pollock (1969), however, emphasised the close association between physical and

metabolic damage, pointing out that since membranes are important features of cell organisation, their physical disruption would have a secondary influence on metabolic events.

Two features of the damage occurring in pea embryos after imbibition suggested that this damage was physical. Firstly, damage occurred very rapidly, within two minutes contact with water. It seems unlikely that chemical modifications could occur so quickly as to account for the damage. Furthermore, phospholipid analyses confirmed that little change occurred in the chemical nature of the membranes. Some caution is necessary in considering this evidence of phospholipids, as whole seeds, and not the damaged area alone, were extracted. Secondly, damage was complete at the first time it occurred and, if damage resulted from the disruption of metabolic systems, accumulative damage would develop over a period of time (Christiansen, 1968). This evidence seems to suggest that physical rather than chemical damage occurs as a result of rapid imbibition. Similar damage in round seeded peas, detected by increased solute leakage and reduced growth (Larson, 1968), was also attributed to the disruption of cell membranes as a result of the rapid water uptake which occurred in the absence of the testa, and failure to establish subcellular co-ordination after rapid imbibition has been suggested as the cause of the death of low vigour pea seeds (Perry and Harrison, 1970).

Short and Lacy (1976) however, disputed that high levels of leakage during early imbibition resulted from rapid water uptake and physical damage to membranes, since reducing the rate of imbibition by lowering the imbibition temperature failed to reduce leakage. They maintained that if a rapid rate of water uptake

caused physical damage, a reduction in the rate of imbibition should reduce damage and therefore leakage. Their failure to reduce damage may however be explained by the greater sensitivity to imbibition damage which was observed at low temperatures. Although hydration occurs more slowly at low temperatures, the change of the phospholipid architecture from the dry hexagonal to the imbibed lamellar state may also occur more slowly (Simon, 1974), and therefore, the advantage of a reduced rate of imbibition is minimised, and imbibition damage occurs.

Therefore, one explanation for the apparently physical damage to the outer cells of the cotyledon due to rapid imbibition may be that the inrush of water into the dry embryo during early imbibition is so rapid that phospholipid molecules are swept far from their original position, and the cells cannot achieve the reorganisation of membranes from the dry hexagonal to the imbibed lamellar state which is necessary for the structural and metabolic co-ordination of the cell. The rate of water movement into the inner tissues will be slower due to diffusive resistance, and therefore damage is unlikely, and it would be possible for the membranes of these cells to rehydrate and reform in the manner proposed by Simon and Raja Harun (1972).

An alternative explanation for damage may be that normally, in intact seeds, the testa offers mechanical resistance to cell swelling during imbibition (Ballard, 1973), and therefore in its absence the cells may rapidly swell up and burst. The inner cells of the cotyledon would however hydrate normally, as restrictions to rapid swelling would be provided by the surrounding cells.

As a result of these observations, an alternative explanation to that of Simon and Raja Harun (1972) of the sequence of events leading to the time course of early leakage from pea embryos, can be offered. The initial high leakage in the first minutes of imbibition could be due to damage to the cells of the abaxial surface of the cotyledons as a result of membrane damage or cell bursting. The decline in the rate of leakage in the first five minutes after the beginning of imbibition would result from the cessation of leakage from these damaged cells, and the beginning of slow leakage from the inner cells as they hydrate. Leakage from the inner cells will be slow since they are living, and leakage will be restricted by the cell membranes. The increase in the length of the diffusion path for solutes leaching out of these inner cells, reabsorption of solutes by the outer cells, or solute accumulation in the cell walls as they diffuse to the outside (Simon, 1977) may also contribute to the decline in the rate of leakage. The decline in leakage observed during measurement of the time course from dead seeds could also be explained in terms of cessation of leakage from outer cells, and slower diffusion from inner cells due to length of the diffusion path. In this case however, membrane reformation in inner cells is unlikely to restrict leakage, which would explain the final higher rate of leakage observed from the dead seeds compared to living seeds. The time course of early leakage from pea embryos can now be explained in terms of physical damage to outer cells during imbibition, and membrane reformation of the inner cells, that is, by a combination of the proposals of Larson (1968) and Simon and Raja Harun (1972).

The observation of damage to cotyledons as a result of imbibition minus the testa, suggested that the testa normally has a protective role in the seed, preventing the rapid imbibition which is damaging. As early as 1917 Denny stressed the importance of non-living membranes such as the seed coat as a factor influencing both water uptake and leaching of material from seeds, although no association was made between these events. Pollock and Toole (1966) demonstrated in soya beans, a protective mechanism against rapid imbibition at low temperatures and high moisture stress which acted through the testa. The seed coats of stressed seeds tended to become impermeable, reducing the rate of imbibition and minimising the damage to the stress conditions. Fayemi (1957) has reported a similar seed coat avoidance mechanism for alfalfa and clover, and Gaspar, Xhaufflaire and Lacoppe (1969) recognised the protective effect of seed coats against the osmotic processes occurring between the soaking water and the embryo. Perry and Harrison (1970) have also suggested a protective role of the testa in peas, although they restricted its importance to the early stages of imbibition. Present observations indicate that protection is necessary over a longer period, as damage due to rapid imbibition occurred even at advanced stages of hydration.

The importance of the testa to seed vigour

In the further investigation of imbibition in a number of seed lots, two factors were identified which appeared to influence the relative levels of solute leakage from different seed lots of peas. Firstly, the rapid imbibition which was associated with damage in embryos, was also associated with high levels of leakage found in seed lots of intact peas. Seed lots with slow imbibition had low leakage. Since rapid imbibition has been associated with damage and high levels of leakage, this association of the rate of imbibition and leakage suggests that the level of leakage of different seed lots may be determined by the rate of imbibition and therefore the extent of damage to the cotyledons. The high levels of leakage in some lots may be the result of damage to cotyledons occurring after rapid imbibition. Thus, the observation made in beans by Schroth and Cook (1964), that seeds which swelled more slowly generally exuded less, would be explained by less damage occurring during slow, compared to rapid imbibition. This relationship between the rate of imbibition and leaching also occurred after imbibing seeds in moist sand, which suggested that the rate of imbibition may be a factor which is important in determining the vigour differences observed between lots in the field situation.

The rate of imbibition of the lots appeared to be influenced by the extent of mechanical damage to the testa. The testae of seeds from lots which had rapid imbibition and high leaching, were generally more damaged than those with low leachate conductivity. High levels of leakage have been previously associated with testa damage in peas (Flentje and Saksena, 1964) and beans (Schroth and Cook, 1964), and with pericarp damage in corn (McKeen and

MacDonald, 1976), although the cause of the increased leakage has not been suggested. In peas, imbibition normally occurs through the micropyle (Manohar and Heydecker, 1964), the chalazal region (Spurny, 1973), and the testa as a whole. Mechanical damage to the testa may facilitate rapid uptake of water, which results in cotyledon damage and subsequent leakage. Consequently, seed lots with severe testa damage would be expected to have high leakage as a result of damage caused by rapid imbibition.

Both high levels of leakage (Flentje and Saksena, 1964; Matthews and Bradnock, 1967: Matthews and Whitbread, 1968; Bradnock and Matthews, 1970; Perry, 1970), and damage to the testa (Hulbert and Whitney, 1934; Schuster, 1943; Flentje and Saksena, 1964; Schroth and Cook, 1964) have been associated with pea seeds showing poor field emergence. Infection by Pythium spp. is frequently the cause of emergence failure (Baylis, 1941; Flentje, 1964; Matthews and Whitbread, 1968), and Perry (1973) observed that primary sites of infection are cracks in the testa. Perry (1973) suggested that the cracks allowed increased diffusion of soluble material out of the seed which would increase the inoculum of Pythium either due to an increase in the rhizosphere effect, or the attraction to a high carbohydrate source. The present work suggests that a higher availability of carbohydrates may occur at the sites of cracks in the testa as a result of damage to the cotyledons produced by rapid imbibition. This would explain the increased leakage and infection which has been observed in pea seeds (Flentje and Saksena, 1964) following increased testa damage.

This evidence of higher levels of leakage from seeds with

greater testa damage suggested that it might be possible that the increases in leakage and reduced staining observed in seeds after prolonged storage, could have resulted from the development of increased numbers of cracks in the testa during storage which led to rapid imbibition and therefore damage. An increase in the number of cracks might result at weak areas of the testa following small increases in moisture content and therefore in seed size. The percentage of seeds in a lot with cracks in the testa was only noted before storage, but some lots were already one year old, and there was no evidence to suggest that the testae of seeds from these lots had more cracks than newly harvested seeds. Thus it seems unlikely that increased testa damage could explain increases in solute leakage from seeds following storage.

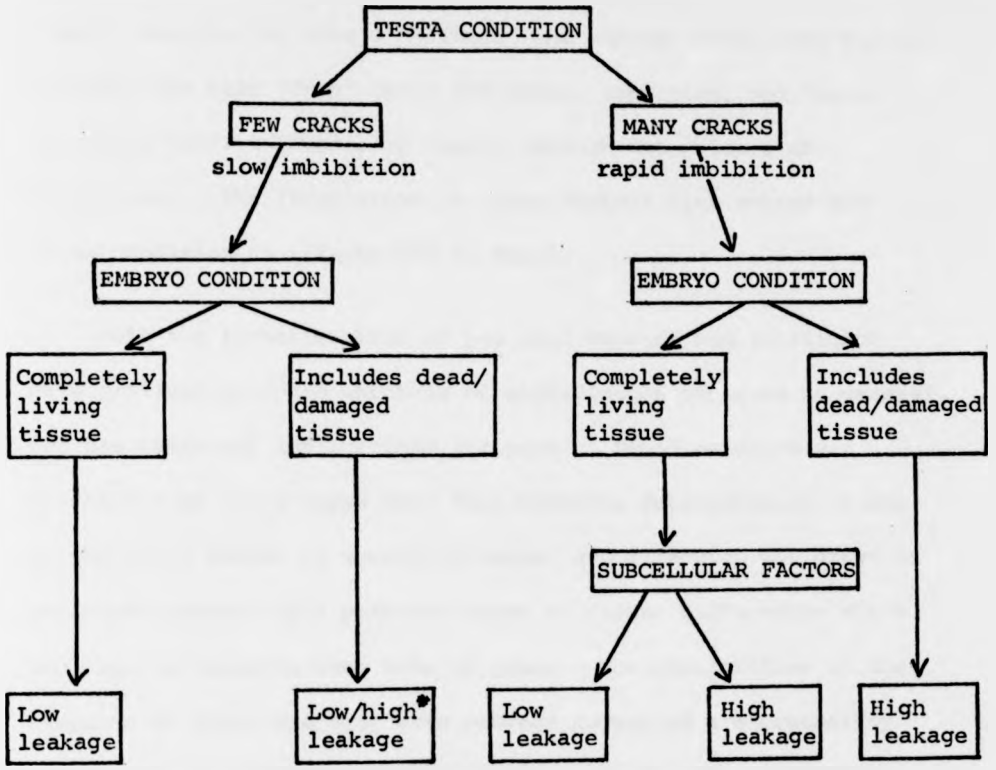
If the condition of the testa can influence the extent of damage to the cotyledons and thus solute leakage from the seeds and fungal infection of the seeds in soil, it is clearly an important feature of the seed which should be given some attention. It might be possible, through a consideration of features of seed production, to determine at what stage of production most testa damage occurs, and how it could be eliminated. One means of reducing testa damage may be in careful control of seed moisture during handling, as very dry seeds are more susceptible to mechanical damage (Moore, 1972). Recent work (N.E. Rogerson, personal communication) has suggested the possibility of early harvesting of peas at moisture contents higher than those at which they are currently harvested. It may be that, if this is possible, much of the mechanical damage occurring to the testa of pea seeds could be avoided.

A second factor, that of embryo condition, also appeared to influence leakage, through its modification of a seed lots response to a given rate of imbibition. Seed lots in exceptionally good condition appeared to be more able to tolerate rapid imbibition without increased damage than seed lots with very poor condition. This was so, whether imbibition occurred in intact seeds or in the absence of the testa. This variation in the tolerance of seed lots with different embryo condition, to similar rates of imbibition, supported the suggestion by Matthews (1971) that the leachate conductivity of pea seed lots was associated with embryo condition. This association of embryo condition and rate of imbibition could also have been explained by the poor condition of some lots actually facilitating more rapid water uptake than good embryo condition. This was unlikely since some seed lots which had very good embryo condition showed rapid imbibition; a slow rate of water uptake would have been expected in these seeds if the embryo condition governed the rate of imbibition.

There also appeared to be another property of the embryo, not revealed by vital staining, which influenced the seed lots tolerance of increased rates of imbibition, because seed lots with initially similar, good embryo condition responded differently to increased rates of imbibition. Increasing the rate of water uptake put the seeds under conditions of increased stress, and the different responses of seed lots produced a good example of what a good stress test might be expected to show, that is, differences between apparently similar seed lots in their response to stress. Perhaps the response observed could have been considered as a further indication of the vigour of the seed lots involved, and introduces the concept of a subcellular aspect to vigour.

Thus, both testa and embryo condition appear to be factors which influence the rate of imbibition and subsequent leakage from seeds, the testa through determining the rate of imbibition, and the embryo through influencing the seeds response to a given rate of imbibition. The following sequence of events might be expected on placing a dry seed in water. First of all, the rate at which water enters the embryo will be determined by the condition of the testa. The embryo condition will then determine whether or not that rate of imbibition is damaging, with resultant high leakage, seeds with poor embryo condition being susceptible to damage at slower rates of imbibition than seed with good embryo condition. In seed lots with good embryo condition, additional subcellular factors may also influence tolerance to imbibition, and therefore affect the final level of leakage. The levels of leakage that might be expected to result from different combinations of testa and embryo condition are summarised in Fig.30.

The differences in leakage from seed lots of peas which give an indication of the vigour of these lots, can therefore be explained in terms of their testa and embryo condition. The earlier work on storage showed that leakage from seeds increased, and therefore vigour declined following prolonged storage. Storage also resulted in a decline in embryo condition, with first of all damage to living cells of the embryo, followed by the development of dead tissue on the cotyledons. In the light of the observation that embryo condition influences response to the rate of imbibition, the increased leakage after storage may be due to the greater sensitivity of seeds with poor embryo condition to damage resulting from water uptake. Other factors known to influence pea seed



* Actual level of leakage will depend on extent of damage to the embryo before imbibition and rate of imbibition.

Fig.30 The levels of solute leakage which would result from pea seeds, given different condition of the testa and embryo.

vigour, such as the time of harvest, and weather conditions before harvest, may also affect testa and embryo condition, and therefore have their influence on vigour through the process of imbibition. The interaction of these factors with embryo and testa condition is illustrated in Fig.31.

Both the investigations of pea seed storage and imbibition have provided evidence which is of significance to seeds in general, and has practical implications for peas. Sound evidence was obtained from the storage work that membrane deterioration is one of the early stages of ageing in seeds, and attention was drawn to prolonged storage as a possible cause of vigour differences which are observed between seed lots of peas. The similarities in the response to short and long term storage suggested a storeability test for peas. The observation of damage to embryos following imbibition minus the testa suggested a role for the testa which has previously been neglected, that of protecting the embryo from damage during imbibition. The imbibition work also revealed that the actual reason for the differences in leaching which indicate vigour differences in pea seed lots, may be ascribed to the condition of both the testa and embryo.

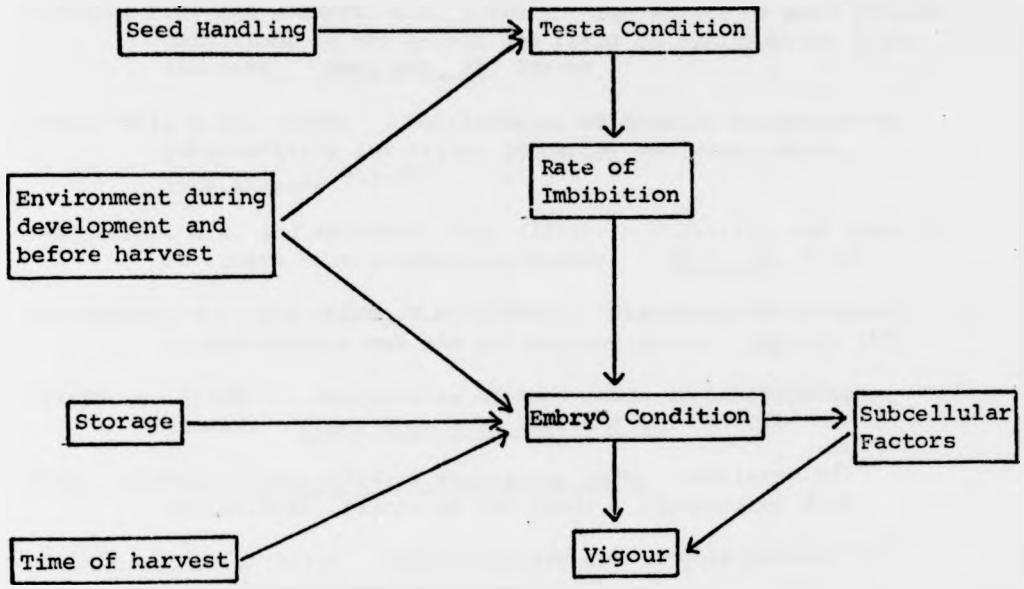


Fig.31 The interaction of factors, known to influence pea seed vigour, with testa and embryo condition.

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APPENDIX I

The influence of Carbowax on leakage

Initial experiments using Carbowax solutions (15% Carbowax 4000 in water) showed only a slight decrease in the rate of imbibition minus the testa in Carbowax, whereas leaching for seeds imbibed in Carbowax was much less than from seeds imbibed in water (Table i).

TABLE i

Rate of imbibition (% wt inc. h^{-1}) and 24h conductivity after imbibition in water of 15% Carbowax

Treatment	Percentage weight increase after different times imbibition (h) 1	2	3	4	5	6	7	24h Conductivity $\mu mho\ cm^{-1} g^{-1}$
Minus testa in water	36	50	63	79	96	107	143	2106
Minus testa 15% Carbowax	33	47	70	73	86	104	140	652

Although Carbowax had reduced the rate of imbibition, it was felt that the very small reduction in imbibition rate could not account for the large differences in conductivity. Therefore, seeds were imbibed minus the testa for 30 minutes in water before transfer to either 15% Carbowax or water for 24h. The 24h conductivity of the Carbowax solution and water were measured. Since the initial treatment in water was the same, cotyledon damage was similar in both groups of seed, therefore conductivity differences would not be due to differences in dead tissue on the cotyledons. Thus, any influence of Carbowax on leakage would be seen.

Seed weight increases after 6h and 24h imbibition were similar in both treatments (Table ii). However, the 24h conductivity of

TABLE ii

The effect of Carbowax on leakage

Treatment	% wt inc after 6h	% wt inc after 24h	24h conductivity
6h water 24h Carbowax	157.3	159.4	620.3
6h water 24h water	157.3	162.9	1156.3

the seeds imbibed in Carbowax was only 53% of the conductivity of seeds imbibed in water. Since initial treatment and damage were similar in both treatments, it would appear that Carbowax reduced the leakage of electrolytes from the seed. As a result, leakage from seeds was not used as a measurement of damage following imbibition in Carbowax.

APPENDIX II

Imbibition of embryos in a solution of calcium chloride

Although the damage observed after imbibition of embryos minus the testa in water appeared to be due to rapid imbibition, it was possible that damage occurred as a result of the leaching of calcium ions, essential for the maintenance of membrane structure, out of the embryo. Therefore, the rate of imbibition of embryos was examined at 20°C in a 0.5 mM calcium chloride solution over 24h, and damage to cotyledons assessed by TTC staining.

The rate of water uptake of embryos in calcium chloride was similar to that in water (Table iii), although slightly slower during the first 3h imbibition, and slightly faster from 4 to 24h. Tetrazolium staining showed that the abaxial surface of most of the cotyledons from both imbibition treatments were unstained (Table iii: category IV); thus, both treatments resulted in damage. Observation of damage after imbibition in a solution in which calcium was freely available suggested that the damage observed after imbibition of embryos in water was not due to leaching from the embryo of the calcium ions essential for maintaining membrane structure.

TABLE iii

The rate of imbibition (% wt inc h^{-1}) of embryos and the percentage of cotyledons in each of four staining categories after imbibition in water or 0.5mM calcium chloride

Imbibition treatment	Time of imbibition (h)								Staining category			
	0.5	1	2	3	4	5	6	24	I	II	III	IV
0.5mM Ca Cl_2	22.6	33.7	48.5	61.4	83.6	118.7	137.8	153.0	0	0	30	70
Water	26.2	35.9	50.1	62.6	78.5	107.1	135.5	142.6	0	10	10	80