SEED QUALITY AND STORAGE PERFORMANCE OF WHEAT (*Triticum aestivum*.) AND SOYBEAN (*Glycine max* (L) Merrill)

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ABSTRACT

Five seedlots of wheat (*Triticum* spp.)cvs. Norseman, Otane, Karamu and two unknown cultivars, and four seedlots of soybean (*Glycine max* (L) Merrill)cv. Davis, two seedlots of cv. CH187 and one unknown cultivar were assessed for prestorage quality by using different laboratory methods ie purity, thousand seed weight, seed moisture content, germination, accelerated ageing, conductivity and seed health. The results of this study showed quality differences between seedlots of both wheat and soybean. Using seed germination and vigour data, three lots of wheat with high quality, two seedlots of soybean with high quality and one seedlot with low quality were chosen and adjusted to two different seed moisture contents (10% and 14% in wheat ,and 8% and 12% in soybean). Seed samples of both species were stored in open storage (muslin bags) or sealed storage (aluminium foil packets) at 20°c 75%RH or 30°c 50%RH for 8 months. All wheat seedlots and two soybean seedlots were also stored under open storage at 30°c 95%RH. Seed quality was assessed at intervals of 1, 2, 4, 6 and 8 months.

The seed moisture content of both species in open storagechanged to reach equilibrium moisture content (EMC) with the prevailing relative humidity. At 30°c 95%RH moisture content of wheat and soybean seeds increased up to 18.5-20.5% and 22-23%, respectively while at the same temperature but lower RH (50%), SMC fell to 8.2-8.5% and 5.2-5.5%, respectively. Both low and high initial SMC of seed stored at 20°c 75%RH either increased or decreased to reach an EMC of 12.8-13.6% for wheat and 9.8-10.1% for soybean. Under sealed storage at different storage temperatures and relative humidities SMC did not change from initial levels.

At 20°c 75%SMC the type of storage container had no significant effect on germination percentage or conductivity in wheat and soybean after 8 months. At 30°c, however, the germination percentage of wheat and soybean with high initial SMC in sealed storage and in open storage high RH declined more rapidly during storage than the other treatments. Germination percentage correlated reasonably well with conductivity, with conductivity readings increasing as vigour decreased. At 30°c 95%

both open and sealed storage at high initial SMC resulted in seed showing a conductivity value increase with longer storage time, indicating seedlot deterioration.

All field fungi were eliminated from seed open stored at 30°c 95% but storage fungi developed rapidly in all seedlots after two months. The main genus involved was *Aspergillus* spp. but Penicillium spp. were also found at low levels in soybean. However, under 30°c 50%RH and 20°c 75%RH storage conduction field fungi levels in wheat and soybean were reduced during storage and seed was either disinfected or remained infected at only low levels after 8 months storage. The main field fungus present in wheat was *Fusarium* spp.. In soybean both *Fusarium* spp. and *Alternaria* spp. survived well along with low levels of *Colletotrichum* spp..

The implications of pre-storage seed quality, seed moisture levels and storage environment and their effects on seed deterioration rate and extent are discussed. The role of field and storage fungi in affecting loss of seed viability in storage and the possibility of exploiting the storage environment to obtain pathogen free seed for planting is also considered.

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INTRODUCTION

Seeds are normally stored sale for some time prior to sale or planting. It is therefore very desirable that they are of guaranteed high quality when they reach the growers. It is impossible to improve seed quality by storage as seed deterioration occurs naturally and continuously after reaching its highest potential usually attained at physiological maturity (Delouche,et al.1973). However, the rate of deterioration loss can be minimised. Obviously it is no use storing worthless seed. Testing is therefore important to determine whether a seedlot should be distributed or discarded or whether it should be sold first based on its storability relative to other seedlots. Test results can be used to predict which seedlots are most suitable for short term or long term storage and also to detect cause of poor quality. Seed testing and predict on helps the seedsman manage and control storage to reduce seed quality loss.

Wheat (*Triticum* spp.) is one of the main cereal crops in term of volume and weight in the worldwide trade of foods and feeds. Nowadays there is an increasing interest in domestic production of wheat in tropical countries such as Thailand. Wheat is grown in the dry winter season and seeds must be stored until the next winter's planting approximately 5 - 8 moths. However seeds still deteriorate continuously. Therefore, storage condition is important. If the storage condition is unsuitable, deterioration accelerates rapidly. Unfortunately, in Thailand seed storage facilities are often poor. In addition, the hot and humid tropical climatic is also a problem, since both temperature and relative humidity are often too high for seed viability maintenance.

According to Bewley and Black (1985), wheat contains approximately 10% protein, nearly 2% of fat and 70% starch. Although fat and protein content in wheat are lower than those of soybean (37% protein, 17% fat and 26% starch), it is also a species which is tolerant to adverse storage conditions.

Soybean (*Glycine max* (L.) Merrill) is one of the worlds most important legume crops, and Thailand is currently encouraging farmer production. It is normally planted after the rice harvest and hence the seed must be stored before being sown in the next planting season (approximately 9 months). Soybean's high oil content often

results in it deteriorating rapidly (Burris,1980) especially under the hot and humid tropical storage conditions. When soybean seed is stored at high temperature or high seed moisture level, germination declines more rapidly than if it is stored under cooler, drier conditions (Toole and Toole,1946; Burris,1980). The provision of such storage conditions in the tropics may be expensive and difficult to control but the ability to control seed moisture content and storage temperature, possibly by the use of sealed packaging, has the potential to provide successful seed storage with the maintenance of both high germination and vigour for an extended period. Also, accurate quality testing at frequent intervals and prediction of likely deterioration can both help to avoid the loss of inventory.

The objectives of this study were to determine:

1. the usefulness of different laboratory methods in assessing prestorage history in soybean and wheat seedlots.
2. the effects of different storage conditions on seed deterioration rate.
3. the differental rates of deterioration between soybean and wheat.

CHAPTER 1

REVIEW OF LITERATURE

A. SEED QUALITY

1.Factors that affect seed quality

1.1 Genetic Effects

Variations in seed quality occur among species or cultivars due to genetic effects. Seed of some cultivars have the ability of germinate and emerge under marginal conditions of temperature and moisture, and even under stress levels of toxic minerals including salinity (Delouche,1992). There may also be genetically influenced resistance to weathering and adverse storage conditions. For example Honeycutt et al.(1989) and Franca Neto et al.(1991) reported that under the same drought stress conditions the level of occurrence of shrivelled seeds in soybean were different depending on the genotype In addition, Vieira et al.(1994) reported that seed processing can increase seed quality depending on the cultivar and the initial seed quality, and the cultivars showed differences in susceptibility to mechanical damage, which can be influenced by seed moisture content.

Differences between cultivars suggest that some mechanism that limits fungal invasion of seeds is also associated with genotype that show a low incidence of infected seeds. Ploper et al.(1992) who studied a diverse group of soybean cultivars, found that only susceptible genotypes showed a rapid increase in seed infection at or near pod maturity.

1.2 Maturation

Seed is physiologically mature when it attains maximum dry weight (Harrington,1972a). At this stage (PM) the seed reaches its maximum viability and vigour (Andrews ,1966; Austin,1972; Delouche,1974; Knittle and Burris,1976). Rubel et al.(1972) suggested that PM occurs in soybean when the seeds start to turn yellow and 75% of the leaves have senesced (Major et al.1975). The seed cannot be harvested at this growth stage due to high moisture content - 50% to 60%. Therefore the plants should be left to stand in the field until seed moisture falls to an acceptable level (harvest maturity)

depending on species. The general recommendation is to harvest at a level of approximately 10-14% SMC (Hill,1995). The time interval from physiological maturity to harvest maturity is about 10-20 days, where seed viability and seed vigour remain high (TeKrony et al.1980). However, a loss of seed number may occur due to shattering or loss of seed quality due to weathering if the plant is left standing too long.

Not all seeds on a plant mature at the same time, thus, each seedlot will contain both immature and mature seeds. If the seed is harvested too early, immature seed level increases, resulting in an increase in abnormal seedlings and a reduction in germination and seed vigour. In addition, Egli and TeKrony (1993) reported that immature soybean seeds need to take up more water before they germinate than mature seeds.

Storability of immature seeds is usually lower than mature seeds. Since the moisture content of immature seeds is generally high this can result in increased micro-organism damage (Bass,1980) and in bruising injury during threshing.

1.3 Weathering Damage

Seed deterioration occurs naturally and continuously after seed reaches physiological maturity and can accelerate rapidly under adverse climatic conditions (Tyagi,1992). Environmental factors such as temperature, mineral nutrients, rainfall and soil moisture may all affect viability during seed development (Austin,1972).

Climatic conditions during the post-maturation/ pre-harvest period have a great influence on the quality of the seed harvested. Frequent rainfall associated with high temperatures and high humidity result in rapid loss of viability and vigour of seed in standing crops, because drying in the open under these conditions is difficult. The incidence and severity of fungal invasion of seed is also increased. The resulting quality of seed produced is generally low and deterioration will continue at a rapid rate during storage (Chin and Wong,1993). Balducchi and McGee (1987) reported that in soybean at least three continuous days of high relative humidity at a temperature around 25°c were required for extensive seed infection to occur, particularly of *Phomosis longicolla*, and that the higher the temperature the greater the rate of seed infection. Under conditions of high temperature and low rainfall during seed filling of soybean, Franca-Neto et al.(1993) found that these stresses resulted in the production of shrivelled seed, with a consequent reduction in seed quality as the level of shrivelling increased. Drought and high

temperature stress reduced seed size, viability and vigour more than drought stress alone (Dornbos and Mullen,1987; Dornbos,1988).

In addition, nutrient deficiencies in the parent plant can affect seed development and maturation resulting in a decrease in seed vigour and can decrease the potential for survival of subsequent generations (Welch,1986). For example, Ozanne and Asher (1965) report seeds containing low levels of potassium, when germinated in potassium-deficient soil, produce seedlings with delayed emergence. The proportion of seedlings capable of emerging was reduced, and also seeds produced from potassium-deficient plants deteriorated faster than did seeds from control plants (Harrington,1960).

1.4 Mechanical Damage

Seed processing included threshing, drying and cleaning can cause mechanical damage which is one of the main factors responsible for reducing seed quality. In addition to the direct effects of machine threshing, seed moisture content and relative humidity are the most important factors affecting susceptibility of seed to mechanical damage. The incidence of mechanical damage to seed causes accelerated seed deterioration when seed is stored (Justice and Bass,1978).

1.4.1 Threshing Damage

The mechanical injury during threshing is dependent on the interaction of several effects such as drum speed of the threshing machine, seed moisture content, free-fall dropping and seed variety. However, small and hidden injuries in seeds, including bruises, may not cause immediate loss in vitality, but they can become increasingly critical with ageing of the seed, particularly when seed is injured to vital organs (ie. various parts of the embryo) (Moore,1972). Injury may be confined to either the embryo or food storage tissue, or include both types of tissue. Injury to plumule or radicle is generally lethal but damage to food storage tissues may vary greatly in seriousness. The most intensive injuries reduce viability immediately.

Hassan Ali Said(1991) studied the effects of threshing speed and "free-fall" dropping on seed quality, and found that increasing the threshing speed of the drum increased the percentage of split and broken seed and "dross". The amount of damage was closely correlated to seed moisture content. Wet seed tended to bruise during impact, while dry seed tended to fracture. The drier the seed and the higher the threshing speed the

greater the amount of damage. The effect of free-fall dropping of pea seeds was less dramatic. Thus the combination of high speed threshing followed by increased dropping height of the seed would have a more seriously damaging and interactive effect.

The presence of a greater degree of seed coat cracking due to machine threshing, results in fungal infection much earlier than hand-threshed seed (Sangakkara,1988). Similarly, Neegaard (1979) reported that mechanical threshing develops conditions that substantially favour infection by fungi, if not carried out properly, resulting in accelerated deterioration.

1.4.2 Drying Damage

After harvesting and threshing, seed moisture content is often too high for safe storage. Thus, drying is an important part of seed production to reduce seed moisture to an appropriate level. Seeds should be dried as soon as possible to avoid damage due to heat produced from the respiration of seed with high moisture content particularly when stored in mass (Hill and Johnstone,1985).

Seed drying requires the transfer of heat, because a seed can be dried only by evaporating moisture from its surface. When water evaporates from the surface of the seed into the atmosphere, a moisture gradient is set up inside the seed that causes internal moisture to move towards the surface. Artificial drying of seed is simply a way of accelerating the rate of natural movement (diffusion) of water from the inside of the seed to the surface where can be removed by evaporation into the air. Evaporation rate is a function of the humidity and temperature of the surrounding air and the air flow rate (Hill,1982). If evaporation from the surface of seed occurs too rapidly, it damages the embryo and causes loss of viability (Justice and Bass,1978).

Hill and Johnstone (1985) commented that wet seeds are much more susceptible to damage at higher temperature, so temperature level must be kept low during the early stages of drying, in order to minimise seed damage. However, no single temperature is safe to heat seed during drying as there are many factors involved such as the type of seed, maximum heat tolerance, seed moisture content, duration of temperature rise, rate of drying and the purpose for which seed is to be used.

Large seeds are more easily damaged than small seeds. Oil seeds will lose germination if dried at high temperature (above 40°c) (Hill,1995). Too much heat reduces

seed quality and too little heat results in slow drying (Justice and Bass,1978). Desirable air-heat ratios vary with the dryer and the kind of seed.

Higher temperature will increase drying rate but will also reduce seed quality. Tunner and Hume (1978) indicated that rapid removal of moisture at high temperature caused stress effects on seed coats, resulting in cracking. and also providing potential sites for fungal infection (Sangakkara,1988). In addition, rapid drying caused the seed coat of some seeds to shrink and become impermeable to moisture. This is known as "case hardening", a condition which can prevent further drying and produce dormant seeds (Harrington,1972b). If seed of high moisture content is dried too slowly this can also lead to mould growth and subsequent loss of vigour. Deterioration can also occur due to sprouting, weathering and respiration heating.

In addition, position in the dryer is another important factor affecting drying damage in a deep bed dryer. Ting et al.(1980) reported that when soybean seed was dried in a deep bed dryer, the initial moisture content, air flow rate, air temperature and location within the bed had a significant effect on drying damage.

1.4.3 Cleaning Damage

The cleaning process may begin with "scalping" which removes material that is coarse enough to be easily separated by screens. In addition, different kinds of seed can be separated when they differ in one or more characteristics. The flow of seed is also affected by seed moisture content. For example, moist seed is often sticky and the flow rate through various machines and augers is often retarded and can cause seed damage (Hill,1995).

In addition, Brezina et al.(1991) also found that damage was caused to wheat seed during passage through the cleaning line. As the seeds passed along the line, the proportion of damage increased from 39 to 64% and subsequently resulted in a significant reduction in field emergence compared with undamaged seeds.

1.5 Fungal Infection

Fungi are universally known as one of the major causes of seed deterioration. They not only cause total spoilage of grain, but may also damage seed quality and mycotoxin contamination (Calderon,1975). Fungi invade seed in both the field and during storage. The former can invade seed during their development on plants in the field right up to

harvest time. Field fungi are infective under conditions where seeds fail to follow their normal pattern of maturation drying. Thus, a period of high rainfall at harvest time can result in extensive grain deterioration. The main fungal species found associated with wheat or barley in the field are *Alternaria, Fusarium* and *Helminthosporium spp.* (Bewley and Black,1985). Fungal pathogens such as (*Diaporthe phaseolorum*, *Phomopsis longicolla* and *Cercospora kikuchii*) enter soybean during seed development (Kmetz et al., 1978; McGee,1983) and can cause a serious quality decline (Pathan, Sinclair and McClary,1989). Velicheti et al.(1992) found that soybean seeds infected with *Cercospora kikuchii* caused purple seed stain, while *Phomopsis longicolla* caused phomosis seed decay as a result of degradation of structural and functional proteins which affect both seed quality and viability. Fungal hyphae penetrate soybean seed coats through coat defects and the hilum region (Hill and West,1982; Singh and Sinclair,1986), and produce abnormal seedlings (Zorrilla, Knapp and McGee, 1994).

The fungi which infect seeds during storage can be divided into two groups, primarily on the basis of their behaviour, ie field fungi and storage fungi (Christensen and Kaufmann,1969; Christensen and Sauer,1982).

Field Fungi are those that invade seeds on plants growing on the field, or after the crop is cut or swathed but before it is threshed (Neegaard,1979). This group comprises a wide range of species. Among these fungi *Alternaria* spp. and *Fusarium* spp. are most commonly found in storage. Hanson and Christensen (1953) found that the presense of *Alternaria* did not affect seed germination percentage but infection by *Fusarium* resulted in reduced germinability. *Fusarium* may invade and kill the developing or mature embryo without causing obvious discolouration of either the pericarps or the embryo - the seeds appear sound, but actually are diseased or even dead. Conversely, although the pericarp may be heavily discoloured as a result of invasion by *Alternaria*, this fungus does not usually invade embryo (Christensen,1973).

Storage fungi develop on seeds during storage and are commonly species of *Aspergillus* and *Penicillium*. The growth of storage fungi is affected by high seed moisture content and high storage temperature. The effects of fungal infection are decreased viability and increased seed decay, resulting in the production of abnormal seedlings and dead seed.

Storage fungi are a major cause of quality losses - including germination in stored grain and seed (Christensen and Kaufmann,1969).

Saprophytic and parasitic seedborne fungi remain dormant during seed storage unless seed moisture content increase greatly (Kulik and Justice,1967). The evidence indicates that all storage fungi are completely inactive below 62%RH (Semenuik,1954) and that there is very little activity below about 75%RH (Milner and Geddles,1954). Nevertheless, storage fungi can grow under limited moisture contents where field fungi and other organisms cannot.

Harrington and Douglas (1970) stated that grain with moisture content above 13% and soybean at moisture content above 12% are readily damaged by storage fungi, especially species of *Aspergillus*. At higher moisture levels in particular, the growth of fungi and loss of germination can be very rapid.

Table 1 Lower seed moisture content limits for common storage fungi in a variety of species . (Christensen and Kaufmann, 1974)

Species	%SMC	
	Wheat	Soybean
Aspergillus restrictus	13.5	12.0
A. glaucus	14.0	12.5
A. candidus	15.0	14.5
A. flavus	18.0	17.0
Penicillium spp.	16.5	16.0

Storage of seed at relative humidities between 65% and 90% provides seed moisture contents favourable for the growth of storage fungi - mainly from species of *Aspergillus* and *Penicillium* (Justice and Bass,1978).

Christensen and Kaufmann (1974) list the lower limits of relative humidity and approximate minimum, optimum and maximum temperatures for the growth of common storage fungi as follows Table2.

Christensen (1973) reported that seeds of pea, barley, corn and wheat stored at moisture contents and temperatures favourable for the growth of storage fungi

germinated 95% or higher after several months storage if kept free of fungi, but germination of seeds inoculated with storage fungi was reduced to near zero during the same period.

Seeds are almost never entirely free of organisms (Priestley,1986). For best control of storage fungi, seeds should not be stored at temperatures above 30°c and relative humidities above 65% (Christensen,1973).

Table 2 Minimum relative humidity and approximate minimum, optimum and maximum temperatures for the growth of common storage fungi.

Fungus	Minimum RH at optimum temperature	Temperature for growth (°c) Minimum	Optimum	Maximum
Aspergillus restrictus	70	5 - 10	30 - 35	40 -45
A. glaucus	73	0 - 5	30 - 35	40 - 45
A. candidus	80	10 - 15	45 - 50	50 - 55
A. flavus	85	10 - 15	40 - 45	45 - 50
Penicillium (depending on species)	80 - 90	-5 - 0	20 - 25	35 - 40

1.6 Insect Damage

Insects and mites can be a serious problem as they destroy plants and provide sites for fungal infection of both plant and seed. Some insects attack the seeds in the field by feeding on immature or mature seeds resulting in loss of viability. For example lygus bugs and caterpillars of the Heliothis moth can penetrate legume seeds and feed internally damaging the embryo and resulting in seedlings with missing structures (Neergaard,1977). Shrivelling of soybean seed is not only due to the effect of stress conditions, but also to seed damage by sucking bugs such as green stink bug (*Nezara spp.*) during seed and pod development (Franco Neto and Krzyzanowski,1990 as cited by Franco Neto et al.,1993).

During the storage stage, beetles, moths and mites are important storage insect pests. In tropical and subtropical environments, seeds in storage warehouses can be damaged by such insects as bean weevil (*Acanthoscelides obtectus*), rice weevil (*Sitophilus oryzae*) saw-toothed grain beetle (*Oryzaephilus spp.*) and moths (*Ephestia elutella*) etc. Weevils puncture the seed coat and destroy the endosperm, but other insects attack the almost exclusively embryo (Justice and Bass,1978). Seeds that have been infested with insects may produce seedlings which lack essential parts or structures or the seedlings may be severely stunted or weakened (Justice,1972).

Seeds may also become infested from contaminated storage bins or sacks or in the field prior to harvest. Therefore insect control measures include thorough cleaning and fumigation of all seed-handling equipment, seed containers, and storage areas and, when required, an insecticide application (Justice and Bass,1978).

The insect pests of different species have certain temperature moisture and food requirements which directly affect their abundance and hence their ability to cause damage (Cotton and Wilbur,1974)

2. Changes associated with seed deterioration

Deterioration as a common phenomenon involves irreversible degenerative changes in seed quality after it has reached its maximum quality level (Abdul-Baki and Anderson,1972). The rate of deterioration is influenced by genotype, pre-storage history conditions (eg. mechanical damage, maturity) and storage conditions (Roberts and Ellis,1978). Moisture and temperature are regarded as two of the most important factors cause the deterioration of seeds during storage (Roberts,1972; Priestley,1986). The lower the storage humidity and temperature, the greater is seed longevity (Cromarty,1990). The symptoms of seed deterioration result from physiological and biochemical changes of aged seed which lead to loss of viability and vigour (Bewley and Black,1982).

Membranes are comprised of lipid and protein. Abdul-Baki and Anderson (1972), Roberts (1972) and Roos (1980) found that changes in the structure of lipid, lead to a loss in integrity of the membrane. General symptoms loss of membrane integrity include both compositional and structural changes. It is possible that free radicals are the primary cause of deterioration.

Free radical damage to cell membranes is mediated through the process of lipid peroxidation which may make membrane susceptible to hydrolytic attack by enzymes, especially proteases and phospholipases (Coolbear,1994).

Loss of lipid from membranes has often been associated with seed deterioration during ageing. Phospholipids (PL) are the major component of membrane lipid (Bewley,1986) and thus, losses of PL can determine membrane damage. Damage of cell membranes can be detected by the conductivity test which has become a routine test for seed vigour (AOSA,1983). A decrease in phospholipid as associated with increasing leakage of all membranes, resulting in a deteriorating seedlot (Priestley and Leopold,1983). Petruzzelli and Taranto (1984) suggested that phospholipid damage and consequent membrane deterioration are a primary event in seed deterioration.

Changes in the ultrastructure of cell organelles are another indicative phenomenon of membrane damage due to ageing. Studies of the ultrastructure of dry soybean seeds have shown plasma membrane infolding and discontinuity (Webster and Leopold,1977). The assumption is that the ultrastructure of the partially imbibed cell is not significantly different from that of dry seeds. However, as water is imbibed by seeds, rapid and extensive changes in physiology and structure organisation occur (Chabot and Leopold,1982).

Chromosome aberrations indicate genetic damage in many species (Marata and Vig,1985). Many chromosome aberrations may be lethal to the cells, if it increases over a critical level, resulting in loss of seed viability (Roberts et al,1967). Chromosomal abnormalities in aged seeds may arise due to either chromosomal or individual chromatid breaks (Murata et al,1984). Although total DNA does not change much during ageing it has been shown to fragment markedly (Harrington,1973). In addition, defective enzyme of DNA metabolism results in longer germination timing or germination failure (loss of seed viability) (Vazquez et al,1991).

3. Seed testing methods for assessing seed quality

3.1 Purity Tests

Purity analysis is an important seed testing method to determine physical quality of a seedlot. It describes how much of the material in the seedlot is intact seed of the species named on the label (Thomson,1979).

According to the ISTA Rules (1993) the aim of the purity analysis is to determine

1. the percentage composition by weight of the sample being tested and by inference the composition of the entire seedlot.
2. the identity of the various species of other seeds and inert particles making up the sample.

The minimum weight of a working sample taken to determine the purity of seed is prescribed in the ISTA Rules (1993). After weighing, the working sample is separated into the following three component parts: pure seed, other seeds and inert matter as defined by the ISTA Rules (1993). The percentage of each part is determined by weight.

Pure seed refers to and includes all varieties and cultivars of the species being analysed and stated by the sender or found to predominate in the test. It includes immature, undersized, shrivelled, diseased, sprouted and mechanically damaged seeds providing the seed is more than half the original size. Seeds of some families *(Fabaceae (Leguminosae), Brassicaceae (Cruciferae)* and some tree seed families) with the seed coat entirely removed are regarded as inert matter. Separated cotyledons of *Fabaceae (Leguminasae)* are regarded as inert matter, irrespective of whether or not the radicle - plumule axis and/or more than half of the testa may by attached. Free caryopses of grasses and cereals removed from the glumes, lemma and palea are considered pure seed.

Other seeds

Other seeds include crop and weed seed from other plants. The classification of these seeds is the same as that for pure seed.

Inert matter

Inert matter includes all other matter and structures that are not defined as pure seed or other seeds. This includes broken or damaged seed half or less than half the original size; unattached sterile florets, empty glumes, paleas, chaff, stems, leaves, cone scales, wings, bark, flowers, nematode galls, fungus bodies such as ergot, sclerotia and

smut balls; soil, sand, stones, caryopses of *Gramineae* replaced by insect larvae and all other non seed matter.

The pure seed separation can be made by visible or mechanical means provided there is no damage to germination capacity (ISTA,1993). The working sample is drawn and placed on a clean surface of a work board for ease of handling and separated by hand, with the aid of forceps, scalpel or spatula having straight smooth edges (Justice,1972). The operation is continued until the entire sample is separated. After separation is completed, the names and numbers of other seeds in order of number present and inert matter composition are recorded.

The percentage by weight of each of the component parts is calculated to one decimal place. Percentages are based on the sum of the weights of the component, not on the original weight of the working sample. If the sum of the weights of the components is 5% more or less than the original weight, a retest must be made.

3.2 Seed Weight

Seed size is usually expressed as the weight of a thousand seeds (Thomson,1979). The thousand seed weight is an easily determined parameter which is often taken as indicative of seed quality and is used as a possible predictor of percentage field emergence (Naylor,1993). There are many reports of the influence of seed size or weight on germination. Kalakannavar et al.(1989), for example, reported that heavy seeds are superior to light seeds in percentage germination and seed vigour. Seedlots with heavy seeds is an indication of high vigour. Such comparisons of seedlots usually demonstrate that seedlots of higher mean weight will produce emerged seedlings faster than those with lower seed weight (eg Kneebone and Cremer,1955; Maranville and Clegg,1977).

Seedlots having different production histories may also produce seed of different mean seed weight (Ellis and Kirby,1980). Thus seed weight depends on variety and on the conditions during the growing season of a seed crop.

3.3 Seed Moisture Content Tests

Seed moisture content is one of the most important factors influencing the length of time that seed will maintain its viability. The amount of water present in seed has a major effect on seed quality, particularly in relation to the amount of damage to seed

which can occur during threshing, cleaning and storage (Harrington,1972a). It is therefore important to ensure that the moisture content of the seed is reduced to a safe level because seeds are living material and respire, producing both heat and water. If the amount of heat or water present is too high the seed will be susceptible to insect attack, mould growth and seed death (Hill,1995).

Hill (1995) defined two ways in which water is held in a seed:

1. as free water capable of moving freely from the inside of the seed to the seed surface
2. as bound water which is held tightly inside the cells and is therefore difficult to remove without causing oxidation, breakdown of cell structure, or loss of other volatile materials such as oil.

It is important when testing seed for moisture content that only the free water is removed. All the methods used for testing seed moisture content, as prescribed by the ISTA Rules (1993), are designed to measure or remove only the free water without altering the chemical structure of the seed.

According to Hill (1995), methods of moisture determination may by classified as follow:

1. Primary methods, where the water is removed from the seed and its amount estimated. These methods use basically two ways: heat and extraction.

2. Secondary methods which determine quantitatively some physical or chemical properly or characteristic of seeds which is related to its moisture content.

Despite the wide range of moisture testing methods, including portable meters, the ISTA Rules (1993) recommend the use of the air oven method which is divided into

1. the low constant temperature oven method. Seed is dried at 103°c for 17 hours $\pm$1 hours. It is suitable for seeds which lose volatile constituents at 130°c eg. peanut, soybean and cotton.

2. the high constant temperature oven method. Seed is dried at 130°c for 1, 2, or 4 hours depending on the species. It is used with seeds which do not lose volatile constituents other than water at that temperature eg. cereals, grasses (including maize) and peas.

3.4 Germination Tests

Germination is usually tested to assess viability of a seed lot in order to reduce the risk of crop failures which result from sowing poor seed. The results of germination tests are used to determine the suitability of a seed lot for sowing and to compare the value of different lots. Germination in a laboratory test is defined, according to the International Rules for Seed Testing (1993), as follows:

1. Normal seedlings. These show the capacity for continued development into a normal plant when grown in good quality soil under favourable conditions of moisture, temperature and light. There are three categories of seedlings classified as normal:

- Intact seedlings
- Seedlings with slight defects
- Seedlings with secondary infection

2. Abnormal seedlings. These do not have the capacity to develop into a normal plant when grow in soil under favourable conditions because one or more of the essential structures is permanently defective.

3. Hard seeds. These are seeds which do not absorb moisture and in consequence fail to swell and germinate. Hard seeds are not found in grasses but are common in many species of legumes.

4. Fresh ungerminated seeds. This category includes seeds which remain firm and apparently viable even after the appropriate treatment for breaking dormancy is applied. The embryo does not appear discoloured and is fresh and firm with a good colour.

5. Dead seeds. These seeds usually are soft, discoloured, frequently mouldy and show no sign of seedling development.

The percentage germination is based on the number of normal seedlings, excluding abnormal seedlings and dead seeds. Differences in the evaluation of normal and abnormal seedling depend on seed structure (monocotyledonous, dicotyledonous) and type of germination (hypogeal, epigeal). The essential structures are root, hypocotyl and cotyledon in dicotyledons, and root and shoot in monocotyledons. If one or more of these structures are irreparably damaged, producing deformed or decayed seedlings, they are classified as abnormal seedlings which result from several causes such as mechanical injury, adverse environment, and harvesting time and method and pests diseases.

The categories of abnormal seedlings resulting from distinctive causes may also be distinguished (ISTA,1979):

1. Damaged Seedlings

Seedlings are classed as abnormal when one or more of the essential structure fail to develop normally because of previous damage to the seed embryo. The resulting abnormalities are, for example, cotyledons or shoots cracked or completely separated from other parts of the seedling; cracks and splits in hypocotyls, epicotyls or cotyledons; stunted or missing primary roots.

Seeds are exposed to various mechanical actions or forces such as impact, abrasion, shear and compression as they pass through handling operations like threshing, separating, conveying and scarifying as well as inappropriate harvesting and transportation. Damaged seeds may show external cracks and breaks, or they may appear sound but have internal breakage or other damage. They may not be entirely dead but only partly dead or cracked so they germinate and produce abnormal seedlings.

Moore(1972) reported that symptoms of mechanical damage in standard growth tests are variable. They included detached seed structures, breaks within structures, abnormally shaped structures, scar tissues, infections, restricted growth, uneven placement of cotyledons, unnatural shrinkage of cotyledons, and splits or otherwise abnormally developed hypocotyls and primary roots. Injured roots often appear dwarfed and twisted and the tips are often blunt and dull in appearance. Similarly, Prakobboon(1982) mentioned that the abnormal seedlings found in mechanically threshed soybean seed, included seedlings with short, thick hypocotyls or with open splits or constrictions interfering with conduction tissue; and seedlings which were short and weak or with unbalanced development of main structures.

In addition to mechanical damage, the producture of abnormal seedlings in the germination test can result from water, drought, heat and insect damage. Disturbances resulting from water damage, which occur by alternate moistening and drying of mature seeds and associated accelerated deterioration, are revealed in growth tests by extensive scar tissue, diseased areas on hypocotyls and cotyledons, diseased seedlings and diseased seeds (Moore,1971).

Drought conditions (low humidity and high temperature), may also cause seed and seedling abnormalities such as short and twisted hypocotyls with longitudinal cracks and

splits, primary leaves that are small and deformed and cotyledons with fractures that cause loss of sections, or differential shrinkage (Kietreiber,1969).

One characteristic of heat damage (high air temperature above 43%) occurs as cracking and splitting in the seed coat, cotyledons and embryonic axis, and results in abnormal seedlings with broken structures, and restricted roots without root hairs and plumule (Mackey, 1972).

Insect damage results from insects capable of attacking the seeds during their development in the field and in the storage warehouse. When such seeds germinate, abnormal seedlings appear with missing structures (Neergaard,1977).

2. Deformed or Unbalanced Seedlings

Seedlings are classed as abnormal when development as a whole is weak or unbalanced. The characteristic abnormalities include retarded or spindly primary roots; short and thick, looping, twisted or spiralled hypocotyls, epicotyls or mesocotyls; curled, discoloured or necrotic cotyledons; inverted direction of growth (shoots bending downward, roots with negative geotropism); chlorophyll deficiency (yellow or white seedlings); spindly or glassy seedlings (ISTA,1979). Seedlings with short leaves extending less than half way up the coleoptile, or the coleoptile split more than 1/3 rd or short and deformed or looping twisted or spiralled are also abnormal.

An abnormal seedling may be caused by internal disturbances of a physiological-biochemical nature. Such disturbances however, are often due to earlier external influences, such as environmental effects on seed before harvest or indirectly on them through the parent plant, poor ripening conditions for the seed, premature harvesting, effect of herbicides or pesticides, poor cleaning procedures or inappropriate storage conditions - types of abnormals found under poor storage include seedlings with a thin and weak primary root, with more than half of the total area of cotyledons broken off or covered with darkened areas and in a few cases without a terminal bud (Prakobboon,1982).

Environmental factors such as high temperature during drying, particularly if the seed has a high moisture content, may produce deformed, weak and brown seedlings with no development of root hair and plumules which fail to develop (Mackey,1972; Neergaard,1977). Low temperature can cause frost damage to mature seed which produce seedlings with necrotic spot on the cotyledons (Neergaard,1977).

Effects of high seed moisture content or high relative humidity during storage can cause seed deterioration, decreased germinatibility and deformed abnormal seedlings which can be seen as stunted, curled or spindly growth with negative geotropism roots and hypocotyl, or seedlings forming a loop spiral or twisted and small, retarded or poorly developed seedlings (Roos,1980).

Koolkaew (1991) found that soybean seedlings with thickened and shortened hypocotyl tissue, and with stunted, stubbed root were the result of chemical damage from prior seed exposure to vapour of 2-4 (dichlorophenoxyacetic acid).

3. Decayed Seedlings

Seedlings are classed as abnormal when any of the essential structures is so diseased and badly decayed as a result of primary infection that normal development would be prevented. This may result from attack by fungi or bacteria, often as a consequence of external damage or internal weakness (ISTA,1979). Fungi invade seeds in both the field and during storage. The main fungal species such as *Alternaria spp. and Fusarium spp.* etc. are commonly found in the field. Storage fungi such as (*Aspergillus spp., Penicillium spp.*) infest seeds only during storage and can damage the cotyledons and hypocotyl causing seedlings to become water-soaked and turn light brown and in the case of *Penicillium spp.* cause yellowing of the leaves (Neergaard,1977).

Abnormal seedlings are produced by infection causing essential structures to be damaged. Seedlings with more than half the original tissue of the cotyledons decayed are abnormal. Primary root or hypocotyl, epicotyl or stem decay results in an abnormal seedling.

Seedlings infected by bacteria such as *Xanthomonas spp.* may appear as water-soaked injury areas on cotyledons and can also lead to dead or necrotic lesions and final decay (Neergaard,1977).

3.5 Seed Vigour Tests

Vigour testing is accepted as an important seed quality component. In addition to the standard germination test, vigour tests are needed to satisfactorily identify the potential emergence of a seedlot under field conditions which are often suboptimal compared with tests carried out under optimal laboratory conditions. McDonald (1980a) noted that the germination test does not provide a complete evaluation of seedlot deterioration or quality.

In some cases, high germination values observed from the standard germination test are inadequate for assessing and detecting quality differences among seedlots which may differ substantially in field emergence, after storage or after transport (Hampton and Coolbear,1990). Vigour test results provide more precise estimates of actual field emergence for growers to make decisions on the quantity of seed to sow and the time of planting.

The definition of seed vigour adopted by the ISTA Congress (Perry,1978) is : Seed vigour is the sum of those properties which determine the potential level of activity and performance of the seed or seedlot during germination and seedling emergence. The definition of seed vigour adopted by McDonald (1980b) is quite similar.

There are three types of vigour test which can be readily distinguished (Hampton and Coolbear,1990):

1. Single tests based on some aspect of germination behaviour. Methods include measuring the rate of germination, seedling growth and seedling evaluation, cold tests, the Hiltner test, accelerated ageing and controlled deterioration tests. These methods are direct tests where an environmental stress which is expected in the field is reproduced in the laboratory (AOSA,1983).

2. Physiological and biochemical tests for vigour which are indirect tests. These methods are based on the potential of physiological or biochemical properties of seeds acting as the indices of seed vigour. The most familiar of these are the electrical conductivity test, the tetrazolium (Tz) test (Perry,1981; AOSA,1983), the measurement of respiratory capacity, ATP content and glutamic acid decarboxylase activity (GADA).

3. Multiple testing and the search for an absolute vigour score. Workers have developed vigour tests which involve assessments based on more than one technique and/or the use of a more detailed interpretation of data.

Researchers believe that no single test can adequately measure seed vigour and field performance across a wide range of seed quality and field conditions. Therefore, they have suggested that improving the accuracy of predicting field performance of seed lots should combine physiological and biochemical tests (Ching et al.1977; Edje and Burris,1970; Egli and TeKrony,1979; Johnson and Wax,1978; Kim, Bin and Choe,1989; Mark and Makee,1968 as cited by Kim et al.,1994). TeKrony (1973) suggested that a

combination of the standard germination test with one or more vigour tests can provide useful information for evaluating soybean seed vigour.

3.5.1 Accelerated ageing

Accelerated ageing (AA) is used to detect seed vigour in high germinating lots. AA responses are also closely associated with emergence potential of seeds, and with growth, development and productivity of plants. Soybean, especially, has been found to have good predictive value for field emergence (Fiala,1987) and AOSA have also recommended this test for soybean seed. The AA test simulates the two most important environmental stresses which influence seed deterioration under controlled conditions of temperature (41°c to 45°c) and relative humidity (greater than 90%).

The results of the accelerated ageing test estimate the longevity of seed in storage (Delouche and Baskin,1973) and have been used to predict the storability or planting potential of each seedlot by using the vigour score to rank seedlots. After the ageing period, seedlots of poor vigour which are intolerant of the stress conditions produce abnormal seedlings or dead seed in the germination test. The AA results should be compared with the results of a standard laboratory germination test before ageing and may be similar to or below germination test result. Seedlots with high initial moisture content deteriorate more rapidly than those with lower initial moisture (McDonald,1977; Tao,1979). However, differences of one to two percent seed moisture will not significantly bias test results (AOSA,1983). Samples should be either treated or not treated with fungicides and results from untreated and treated lots should not be compared. Temperature control, sample size and ageing time used and recommended will vary for each species as variation in final seed moisture and /or germination will limit the acceptance of the vigour test.

3.5.2 Conductivity test

Poor membrane structure and cell leakage are usually associated with deteriorating, low vigour seed. This results is a greater loss of electrolytes, such as amino acids and organic acids, from imbibing seeds and increases the conductivity of the "soak" water, therefore, indicating low vigour and potentially poor field emergence. For wheat the conductivity results from both bulk and single seed samples are well related to field emergence (Heslehurst,1988). Conductivity values for soybean do not differ at seed moisture contents between 10% and 22% (Tao,1978; Loeffler et al.,1988; Eua-

umpon,1991). However Tao (1988) observed a moisture content of 8.8% caused cracking following rapid imbibition of soybean and, as a consequence, conductivity increased. AOSA (1983) suggested that provided seed moisture content is between 10% and 14%, no adjustment is required before conductivity testing.

Conductivity reading are significantly increased when the soak temperature is increase (Tao,1978; Loeffler et al.,1988). Therefore, it is important that conductivity be measured at the same temperature as the soak water (Tao,1978; Matthews and Powell,1987; Loeffler et al.,1988).

Physical injury to the seedcoat of large-seeded legumes allows rapid water uptake leading to imbibition damage and high levels of electrolyte leakage. Thus, the sample should be drawn from the pure seed fraction of the seedlot as prescribed by ISTA (1993) to provide an unbiased sample for testing.

3.5.3 Seedling growth test

Seedlings are judged by their rate of growth provided that all the essential structures show balanced development. Differences in these characteristics between seedlots were observed by Nobbe (1876) and formed the bases of his original definition of vigour. This test is a simple one which measures both germination and seed vigour.

Germ (1949) first suggested measuring plumule growth as a vigour test for cereals, sugar beet and wheat. The rate of plumule growth indicates the level of activity and co-ordination of the metabolism of the germinating seed. He showed that the results of a plumule growth test were usually more closely correlated with grain yields than those of the germination test and showed that both the numbers of seedlings emerging and their vigour may influence final yield figures (Perry,1977). Although seed germination may, on occasions, be high, it is possible that seed vigour may be reduced because of low levels of metabolic activity and short plumule length.

3.6 Ferric Chloride Tests

Seed damage resulting from adverse mechanical or forces during seed processing may not always be apparent but may cause internal damage (Brandenburg,1983) to the seed. Such damage can made conspicuous and visible by placing seeds in a 20% ferric chloride solution for 5 minutes at room temperature and separate black staining seeds within 15 minutes. This technique is particularly useful for enhancing the visibility of

surface seedcoat damage, especially in large dicotyledonous seeds such as soybean (Lankford, personal communication).

3.7 Seed Health Testing

Health of seed refers primarily to the presence or absence of disease - causing organisms, such as fungi, bacteria and viruses, and animals pests, such as eelworms and insects, but physiological conditions such as trace element deficiencies may also be involved.

According to the ISTA Rules (1993), Health testing of seed is important for the following reasons:

1. Seed-borne inoculum may give rise to progressive disease development in the field and reduce the commercial value of the crop.
2. Imported seedlots may introduce diseases into new regions. Tests to meet quarantine requirements may therefore be necessary.
3. Seed health testing may elucidate seedling evaluation and causes of poor germination or field establishment and thus supplement germination testing.

There are many seed health testing methods which can be used for seeds maintained in an environment favourable for the development of pathogens or symptoms. The choice of method depends not only on the properties of the seed and type of disease infection but also on the purpose of the test (De Tempe and Binnerts,1979), ie whether the seeds are to be tested for quarantine, certification, seed treatment etc.. For seeds to be certified by an exporting country as "substantially free" from a particular pathogen, the testing produce must be sufficiently sensitive to reveal even traces of infection (Hampton,1994).

Seed health is a complicated matter. Seeds may be seriously infected and develop into abnormal seedlings in the laboratory but in the field these seedlings may disappear (De Tempe and Binnerts,1979). Therefore routine methods for seed health testing should provide reliable information relating to field performance and quarantine requirements; be reproducible within statistical limits; use time, labour and equipment within economic limits and show results quickly (Hampton,1994). The agar test and blotter test are the most commonly used methods in routine laboratory seed health testing.

B. SEED STORAGE

It is no use producing good quality seed if it because worthless before it can be planted. The principle purpose of storing seeds of economic plants is to preserve planting stocks from one season to the next and maintain their physiological quality by minimising the rate of seed deterioration (Owen,1956; Delouche,1968a,1968b; Justice and Bass,1978). Deterioration can progress during storage to the extent that seeds are essentially worthless for planting purposes although germination percentage remains relatively high (Grabe,1965; Delouche,1969). The best of seed storage conditions can only maintain pre-storage viability and vigour levels of seed, as quality is not improved by storage (Delouche et al,1973).

According to Justice and Bass (1978), there are several factors affecting seed storage life. These factors can be classified into prestorage factors and storage conditions. "Prestorage factors" apply to seeds that are still in the plant or after harvest but before storage but in most cases can an effect on have affecting seed viability during storage and contribute to determining the quality of the seed before storage. "Storage conditions" apply to those environmental conditions that of major importance maintaining seed viability during storage.

Factors that affect storability

1 Prestorage factors

Large variations in seed longevity occur between genera and species due to genetic effects. Seeds of some species are genetically and chemically equipped for longer storability than other under similar conditions (Copeland and McDonald,1995). Seed storage life of some kinds of seed are inherently long-lived, others are short-lived, while others have an intermediate life-span (Harrington,1972a). For example soybean seeds are inherently short-lived while wheat seeds are intermediate (Haferkamp et al.,1953 as cited by Justice and Bass,1978). General, seed species possessing high oil content do not store as well as those with low oil content. Several studies have shown that the inheritance of seed longevity is not only effective at species level but also at cultivar level (ie Bass,1980; Agrawal,1988; Delouche,1992).

In addition, mechanical damage influences the life-span of seed. Seed production practices such as harvesting, cleaning and handling inevitably lead to mechanical damage. For example, seeds which are broken, cracked or bruised. These damaged seed deteriorate more rapidly than undamaged seed (McDonald,1985; Priestley,1986). Seeds with small mechanically damaged areas may later show deterioration of vital embryonic tissue, resulting in poor quality (Moore,1972). Direct injuries to embryonic tissues are much more detrimental to seed longevity than are large injuries to nonembryonic tissues. Mechanical damage also promotes invasion by storage fungi, which can enter the seed through cracks in the seed coat (Mamicpic and Caldwell,1963).

Weathering and seed maturity also influence seed storability. Several kinds of environmental stresses such as temperature, mineral nutrients, rainfall and soil moisture during seed development, and prior to physiological maturity influence seed maturity. For example, rain just before harvest can cause wheat to sprout in the head (Moss et al.,1972) and cause delays in harvesting which create problems with seedborne fungi (Bass,1980). Immature and small seeds within a seedlot do not store as well as mature and large seeds (Wien and Kneneman,1981; Minor and Paschal,1982).

2 Storage Conditions

2.1 Seed moisture content and relative humidity

Moisture content is the most important factor affecting the rate and extent of seed deterioration. Seed deterioration increases as moisture content is increased (Barton,1961; Justice and Bass,1978). Seed moisture content is controlled by the relative humidity of the air, the hygroscopicity of seed being affected by the relative humidity of storage environment (Harrington,1973). His "rule of thumb" states that each 1% decrease in seed moisture content doubles the storage potential of seed when seed moisture content is between 5% and 14%. Drying below 5% moisture may be damagerous to some species, because of damage from lipid autoxidation. At 12% - 14% seed moisture level storage fungi destroy the capacity for seed germination while in the range of 18% - 20% heating may occur and, if oxygen is present, micro-

organism will also contribute to the rapid death of seed. At 40% - 60% SMC, germination occurs (Harrington,1972a).

Seeds are hygroscopic and absorb or lose moisture from the atmosphere until the vapour pressure of seed moisture and atmospheric moisture reaches equilibrium during the storage period (Delouche,1968b) as shown in Table 3. Hard seeds of many legume species are the exception (Harrington,1973). The equilibrium moisture content of different types of seed at the same relative humidity will be different because of chemical composition of seed. For example, proteins can absorb most water per unit of weight, cellulose and starch absorb less while lipids do not absorb water at all (Harrington,1973; Neegaard,1977).

The moisture content of seed in a seed container of small volume will remain essentially constant because the water content of the air in the container is less than the water content of the seeds. Hence, depending on the relative quantities of water available either the seed moisture content or the relative humidity of the air will determine the equilibrium values reached (Harrington,1973). Only low moisture seed can be stored in sealed containers, while humidity must be controlled at a suitable level to maintain safe seed moisture content in open storage.

Table 3 Equilibrium moisture contents of a range of wheat and soybean at various relative humidities and approximately 25°c (Data from Harrington,1960; Delouche,1973; Justice and Bass,1978; Hall,1980; Witte,1986).

Species		Moisture content at indicated relative humidity (%)						
		15	30	45	60	75	90	100
Wheat	Hard	6.4	8.5	10.5	12.5	14.5	19.7	25.0
	Soft	6.3	8.6	10.6	11.9	14.6	19.7	25.6
	White	6.7	8.6	9.9	11.6	15.0	19.7	26.3
		Moisture content at indicated relative humidity(%)						
	20	30	40	50	60	70	80	90
Soybean	5.5	6.5	7.1	8.0	9.3	11.5	14.8	18.8

2.2 Temperature

Storage temperature is the second most important factor in determining seed longevity (Roberts,1972; Harrington,1972a; Justice and Bass,1978). According to Harrington (1972a), seed viability and vigour are reduced with increasing temperature, time of exposure and seed moisture content. In general the higher the temperature, the more rapid the deterioration at a given level. Conversely the lower the temperature, the less the deterioration. Harrington's "rule of thumb", states that for each 5°c decrease in seed storage temperature, the life of seed is doubled at least between 0°c and 50°c. Seed with low moisture content maintained at 5°c to 10°c has a longer storage life than the same seed maintained at ordinary room temperature.

Aruluandhy and Herath (1987) reported that when soybean seed was stored in paper bags in ambient conditions (max. temperature 26 - 30°c, min. temperature 18 - 26° and mean RH of 76.2 ± 5.9 %), seed viability and vigour declined slowly during the first 3 months and rapidly thereafter. Seed vigour also decreased with increasing length of storage.

The effect of temperature on the storage of soybean seed was reported by Toole and Toole (1946). They found that lifespan is reduced as temperature and seed moisture content is increased or alternatively lifespan is increased as temperature and seed moisture content is decreased. The cooler the temperature the more slowly seed viability declines. This rule applies even at temperature below freezing. For maximum longevity 'orthodox' seeds must be stored dry at a low temperature. Most seeds that can be dried live longer at sub-freezing temperatures than at above-freezing temperatures but some cannot. Seeds of barley, rye and wheat are sensitive to cold injury at high moisture content. A temperature of 10°c or lower will prolong seed life (Roberts,1972; Bass,1980).

Interaction of moisture content / relative humidity and temperature

Moisture content (or relative humidity) is directly related to temperature and can have an effect on stored seed viability. However these factors can reinforce and compensate each other in their effects. Both parameters influence seed metabolism. High relative humidities result in increasing seed moisture content, and consequently in biochemical changes such as increased hydrolytic enzyme activity, enhanced

respiration and increases in free fatty acids. High temperatures serve to enhance the rate at which many enzymic and metabolic reactions occur, resulting in a more rapid rate of deterioration.

Harrington (1958) defined safe storage conditions as those that maintain seed quality without loss of vigour for 3 years. But in many cases such conditions simply cannot be economically justified.

Delouche et al. (1973) have provided guideline for safe storage conditions for a wide range of seed in subtropical and tropical countries. Three levels of storage conditions are generally relevant:

1. Short Term Storage. Good quality seed of the major subtropical and tropical crops can be stored satisfactorily from harvest to the next planting season 1 to 9 months - under the following storage conditions:

- 30°c 50%RH (seed moisture contents ranging from maximum of 12% for cereals seed to 8% for oil seeds.
- 20°c 60%RH (seed moisture content ranging from maximum of about 13% for cereals, seed to 9.5%for oil seeds).
- Other combinations of temperature and relative humidity as favourable as these above.

2. Intermediate Term Storage. Successful carry-over storage (18 months) of the seed of major field crops in the subtropics and tropics can be accomplished under conditions that do not exceed the following:

- 30°c 40%RH (seed moisture content ranging from maximum of approximately 10% for the cereals to 7.5% for oil seeds).
- 20 °c 50%RH (maximum seed moisture contents ranging from 12%for cereals to 8%for oil crops).
- 10°c 60%RH (maximum seed moisture contents ranging from about 12% for cereals to 9% for oil seeds).
- Other combinations of temperature and humidity as favourable as these above.

3. Long Term Storage. Cold and dry conditions will maintain the quality of seed for many years in storage. For 3 to 5 years storage, conditions of 10°c and 45%RH are satisfactory for most kinds of field crop seed. Successful storage for 5 -

15 years can be achieved under conditions of 0°c to 5°c and 30 - 40%RH (James,1967).

2.3 Packaging

Packaging seed is to facilitate convenient storage and transport, but it must also preserve seed germination and vigour (Harrington,1973). Seed packaging uses a range of materials which are described as open or sealed containers.

In open storage, seeds are usually packed in porous containers like jute sacks, paper bags, cloth bags etc. The economic storage of large quantities of seed may often be in open storage containers such as bins, silos or in warehouses without covering. Because open storage containers are porous and are easily penetrated materials they permit a free exchange of moisture with the environment, so seeds quickly gain or lose moisture depending on the environment around them (Harrington,1973). As a result, the moisture content of seed in storage is high in humid areas but may be quite low in dry areas irrespective of the initial seed moisture content. Therefore, the maintenance of a safe seed moisture content in open storage requires an average level of atmospheric relative humidity no higher than that in equilibrium with the desired storage seed moisture content (James,1967).

In sealed storage the packages are normally moisture proof, including tins glass jars with gasketed screw top lids or aluminium foil laminated to Mylor or polyethylene. All these packaging types maintain seed moisture content and relative humidity of the microenvironment in the package at a favourable level. If seed moisture is low enough in the package, the seed will store satisfactorily even under the warm ambient temperatures of tropical environments (Delouche et al.,1973). If the seed moisture is too high, seed respiration and the activity of micro-organisms will be so high that the seed will increase in moisture, water being one of products of respiration. This moisture cannot diffuse out of a sealed container. The seed increases in moisture content which in turn increases its respiration and the cycle continues until seed is killed. It is therefore likely that seed of high moisture content in sealed containers will deteriorate faster than seed packaged at the same moisture content in open storage (Harrington,1973; Justice and Bass,1978).

On the other hand, if the seed is kept by in moisture resistant containers at extremely low SMC, it will rapidly die from over drying due to the breaking of the

protective monomolecular layer of water around macromolecules, exposing them more readily to damage from free radicals. Cell membranes are disrupted and the DNA of the chromosomes is inactivated under such condition (Harrington,1972a). The desirable seed moisture range for packaging seed in sealed containers is between 6 and 12% for starchy seeds and between 4 and 9% for oily seeds (Harrington,1973).

Decisions about appropriate packaging are crucial. The choice of packaging will depend on factors such as the kind of seed, duration of storage, storage environment, seed moisture content, cost of packaging material and the geographical area where the seed will be stored.

3 Storage Organism and Insect

In addition to its direct effect on seed moisture content, the relative humidity of the storage environment also indirectly affects seed viability through the development of organism in storage (Delouche et al.,1973).

The types of organisms attacking seed in storage include bacteria, fungi, mites, insects and rodents. The activity of all these organism can lead to damage resulting in loss of vigour or viability and are directly related to temperature, moisture content and gaseous environment (Robert,1972) - except in the case of rodents. Rodents can cause a complete loss of seed, since their activity is not dependent on seed or storage conditions.

4 Respiration and Heating

Respiration is an oxidation process by which oxygen is absorbed from the atmosphere, and carbon dioxide and water vapour formed together with energy, much of which is evolved in the form of heat (Kreyger,1963). Under favourable storage conditions the heat of respiration is of little or no concern for practical seed storage. However, at higher moisture levels the heat of respiration can produce much damage to stored seed (Justice and Bass,1978).

The apparent respiration of seedlots and the associated heat may arise from the seed's metabolism, and/or from micro-organisms on the seed and insects especially at seed moisture contents in equilibrium with approximately 75% relative humidity or higher.

As seeds deteriorate respiration become progressively weaker and ultimately leads to loss of germination. However, prior to loss of germinability the respiration level during the early stages of germination has been correlated with subsequent seedling vigour (Woodstock and Feeley, 1965).

Previously Ramstad and Geddes (1942) also found that the lowest moisture content of soybean seed at which respiration heat is produced when stored at 25° - 26°c was 15.6%. They also reported that under the same conditions cracked and broken seed of soybean respired more rapidly than the whole sound seed.

The respiration rate of soybean with an initial moisture content of 18.5% also increases rapidly as the temperature increase from 30°c - 40°c. Above 40°c there is marked respiration inhibition, the rate at 45°c being less than one-tenth that at 40°c.

CHAPTER 2
MATERIALS & METHODS

SEED SAMPLES USED

Four seedlots of soybean and five seedlots of wheat were used for tests on the initial seed quality by using various laboratory techniques.

Two seedlots of soybean (*Glycine max* (L) Merrill) cultivar 'Davis' harvested in 1988 and 1990, respectively, two seedlots of cultivar 'CH187' and one unknown were from Seedbank (NZ) Ltd. Palmerston North. Wheat (*Triticum spp.)* cultivars 'Norseman', 'Otane', 'Karamu', and two unknown cultivars seedlots were also used.

SAMPLING

The purpose of seed sampling is to remove a portion of seed from the lot which accurately represents the whole lot. It is essential that the sample be taken with care and in accordance with the methods prescribed in the ISTA Rules for Seed Testing (1993).

Each seedlot of soybean or wheat was subsampled using Boerner conical divider or a soil divider, respectively, seeds were passed through it into approximately equal parts. Submitted samples were mixed by passing them through the divider, recombining two parts and passing the whole samples through a second time to ensure complete mixing of the samples. The samples were then reduced in size by passing the seed through repeatedly and removing one half on each occasion until the sample obtained was approximately 600 grams for soybean or 150 grams for wheat. These samples were used to for conduct the initial quality tests.

MEASUREMENT

Purity Test

According to the International Rules for Seed Testing (1993), the weight of the working sample for soybean and wheat are 500 and 120 grams respectively. These working samples were examined carefully seed by seed and separated into the following component parts : pure seeds, other seeds, inert matter. Each component was weighed separately and the percentage composition was calculated.

Thousand Seed Weight

According to the International Rules for Seed Testing (1993), four replicates of 100 pure seeds were counted and weighed. Thousand seed weight was calculated to two decimal places.

Seed Moisture Content Determination

From the pure seed fraction of soybean and wheat approximately 10 g. were ground, weighed, placed in duplicate aluminium containers and dried in an air oven maintained at 103°c for 17 hours for soybean and at 130°c for 2 hours for wheat. After drying, the samples were cooled in a desiccator for 30 minutes and reweighed. Moisture content was calculated on a fresh weight basis, where the amount of water lost in drying was divided by the initial weight of the sample, using the following formula:

M.C. = (M2 - M3)/(M2 - M1) x 100

M1 =the weight in grams of container and cover.

M2 =the weight in grams of container, cover and contents before drying.

M3 =the weight in grams of container, cover and contents after drying.

Germination Test

Using the method prescribed in the ISTA Rules(1993), seed germination was determined by using the between paper method of germination. Four replicates of 50 seeds of soybean and wheat were placed between moist paper towelling. The bottom edge of the paper was turned up before they were loosely rolled and secured with a rubber band. Samples were placed vertically in wire baskets, each being covered with a large plastic bag to prevent loss of moisture and germinated at 25°c for soybean and 20°c for wheat. Seedling evaluation and percentage germination was assessed at 5 and 8 days for soybean and at 4 and 8 days for wheat.

At the first count, only the normal seedlings were counted and removed. Dead seeds which were found to be mouldy, (which could affect the germination of other seeds) were also removed at the first count and numbers recorded. At the end of the test seedlings were evaluated and recorded in appropriate categories : normal seedlings,

abnormal seedlings and dead seeds. The results were calculated as a percentage by number.

The percentage of normal germination as defined in the ISTA Rules (1993) included all seedlings which showed the potential for development into normal plants. Abnormal seedlings are those which do not showthe capacity to develop into a normal plant when grown in soil under favourable conditions of moisture, temperature and light (ISTA,1993). Seedlings with the following defects were classified as abnormal :

1. Damaged seedlings

Seedling with any missing or badly damaged essential structures. Damage resulting from external causes such as mechanical, handling, heat, drought or insect damage. The types of abnormal seedlings in this category include : cotyledons or shoots cracked or completely separated from other parts of the seedling; cracks or splits in hypocotyl, epicotyls or cotyledons (legume seedlings); coleoptiles with damaged or broken tips; split (cereal seedlings), stunted or missing primary roots.

2. Deformed or unbalanced seedlings

Includes seedlings with weak or unbalanced development which may be caused by internal disturbances of a physiological - biochemical nature due to earlier external influences, such as unfavourable growing conditions on the parent plant, poor seed ripening conditions, premature harvesting, effect of herbicides or pesticides, poor cleaning procedures or inappropriate storage conditions. Characteristic abnormals in this category include : retarded or spindly primary roots; short, thick, looping, twisted or spiralled hypocotyl, epicotyl or mesocotyl; curled, discoloured or necrotic cotyledons; short and deformed, split, looping twisted or spiralled coleoptiles; inverted direction of growth; chlorophyll deficiency (white seedlings); spindly or glassy seedlings.

3. Decayed seedlings

Included seedlings with any of the essential structures so diseased or decayed as a result of primary infection which may result from attack by fungi or bacteria,often as a consequence of external damage or internal weakness.

Vigour Test

Accelerated ageing

Accelerated ageing treatments are carried out by holding seed samples in greater than 90% RH. The seed moisture of the sample is tested and if >14% (fresh weight basis) the seed was dried to below 14% before testing. About 50 g of soybean seed or 20 g of wheat seed was used and placed one layer deep on a wire mesh tray, inside a 11x11x35 cm. plastic box with 40 ml. distilled water. Tomes et al.(1988) reported that the actual RH under such conditions during 24-72 hours is in the range of 90-95%. The boxes were covered with lids and then placed in a temperature controlled incubator. The oven was then closed and maintained at a temperature at 42°c for 72 hours for soybean or 42°c for 48 hours for wheat. The incubator was not opened during the aging period. After aging, seeds were tested for germination. The conditions for the germination test were conducted according to the ISTA Rules for Seed Testing (ISTA,1993)as previously described. Prior to planting for standard germination, 10 grams of soybean or wheat seed from each sample was tested for seed moisture using the oven method (ISTA,1993) as previously described. If seed moisture were lower or higher than the range of 28-30% for wheat or 27-30% for soybean, the results were not considered accurate and the sample was retested.

Seedling Growth Test

This test was conducted on four replicates of 25 wheat seeds. Seeds of each replicate were placed plumule upward along a line drawn across the centre the germination paper. The preparation of the rolls was completed as for a standard germination test and incubated at 20°c for 7 days. After the test period, the number of plumule tips which lay between each of the paralled lines was counted. Pairs of lines were given the value of the distance of the mid-point from the centre line (ie 1,3,5,9,11 cm.). Mean plumule length was calculated by the following formula:

$$L = 2\,\frac{(NX_1+NX_3+...+NX_{11})}{25}$$

where L = mean plumule length (cm)

N = number of plumule tips within the pair of parallel lines

X = mid-point distance from centre line.

Abnormal seedlings were not included in the length calculation.

Conductivity Test

Prior to measuring conductivity if seed moisture of each seedlot was lower or higher than 10% - 14%, seeds were adjusted to a moisture content within that range (AOSA, 1983). Four replicates of 50 seeds were sampled from each seedlot and weighed (to two decimal places). Each replicate (50 seeds) was the placed in a 500 ml. flask containing 250 ml. of deionised water which had been equilibrated at 20°c for 24 hours. The neck of each flask was covered with parafilm and then placed in a germinator at 25°c for 24 hours for soybean or at 20°c for 24 hours for wheat. After 24 hours soaking, the conductivity was measured immediately using a conductivity meter (CDM-83 Radiometer) and swirled 10-15 seconds before measurement.The conductivity results are expressed in micro siemens per gram of seed (µs/cm./gm.seed). The dip cell was rinsed in distilled water after measurement of each sample. The conductivity reading of sample was subtracted from the conductivity reading of distilled water as a control and used to calculate the conductivity value. The conductivity reading of distilled water was checked to ensure it was less than the prescribed maximum of 5 ms $cm^{-1}g^{-1}$.

$$\frac{\text{conductivity (ms) for each flask}}{\text{weight (g) of dry seed sample}} = \mu s\ cm^{-1}g^{-1}$$

Ferric Chloride Test

A ferric chloride ($FeCl_3$) test was used to visualise mechanical damage on seeds. A 20% (w/w) solution of ferric chloride was prepared in distilled water by adding 4 parts water to 1 part $FeCl_3$ by weight. Four replicates of 100 soybean seeds were soaked in the 20% ferric chloride solution for up to 15 minutes. Separation of black staining seeds was done within 5 - 15 minutes after soaking ensuring that the stain was black and not a natural dark brown. Seeds with black stain are considered to be mechanically damaged. The black staining on the testa indicated the sites of mechanical damage penetrated by the salt which is subsequently oxidised (Sakunnarak,1992).

Fungal Tests

Potato dextrose agar (PDA) was the media used for field fungus defection and potato dextrose agar with salt for storage fungus detection. In each case 39 g. of dehydrated PDA and 0.05 g. of Chloramphenicol were dissolved in a litre of water and for storage fungi 75 g. of salt was also added. The media was sterilised in a autoclave at 121°c at 15 psi for 20 minutes.

Twenty seeds from each sample were surface sterilised by placing them in muslin cloth bag tied with a rubber band and soaking in a 1% sodium hypochlorite solution (1 part of 'Janola': 3 parts of water) for 5 minutes. The bags were then rinsed for 3 minutes in running water and placed on a towel to remove excess moisture. Ten seeds per plate for each sample were placed onto the agar surface under aseptic conditions in a Lamina Flow Cabinet and incubated at 25°c for 5-7 days. The number of infected seeds was counted when colonies were sufficiently developed for identification and the percentages of seeds infected by field or storage fungi was recorded.

After the initial quality of the seedlots was determined. Three seedlots of soybean and wheat with high germination and vigour were selected for the storage experiment.

All seedlots were stored at one of two levels of seed moisture content selected to be lower or higher than the "safe" seed storage moisture content for each species (8% and 12% for soybean and 10% and 14% for wheat).

Each seedlot was divided into 4 replicates using a Boerner conical divider for soybean and the soil divider for wheat, respectively. Seed moisture content was adjusted to the higher level by exposure to high relative humidity (greater than 90%) at 5°c. Seeds were placed on a wire basket in a plastic box with water. The boxes were covered with lids and placed at 5°c, for approximately 4-7 hours for wheat and 15-20 hours for soybean. Seeds with high seed moisture content were placed on muslin cloth in a desiccator over silica gel for 17-22 hours for wheat and 12-14 hours for soybean at room temperature (25°c) to reduce moisture content. During rehydration and dehydration, seeds were placed in a single layer in a wire basket or muslin cloth.

The time varied depending on the initial moisture content and kind of seed. Seeds were tested for moisture content every 2 hours until they were shown to reached the

required level. Samples were weighed and then recorded to calculate according to the formular:

$$D_{WT} = F_{WT} - \frac{SMC * (F_{WT})}{100}$$

$$SMC = \frac{Fwt_t - D_{WT}}{Fwt_t} * 100$$

D_{WT} = Dry weight (gm.)

F_{WT} = Sample weight before adjusting (gm.)

Fwt_t = Sample weight after adjusting (gm.)

After attaining the desired moisture content, seeds were placed in muslin cloth bags (open storage) or aluminium foil packets (sealed storage) and stored at one of two temperatures (20°c or 30°c) for eight months.

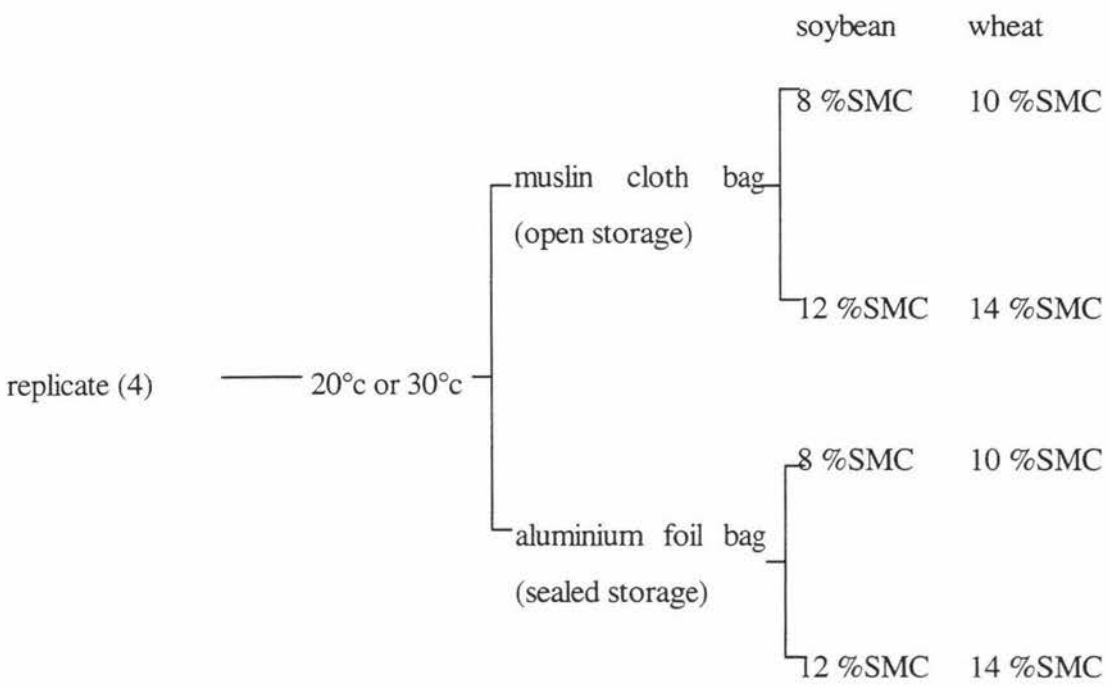
Samples were randomized in storage and were progressively removed for testing for SMC, germination, conductivity and fungal infection after 0, 1, 2, 4, 6 and 8 months of storage.

EXPERIMENTAL DESIGN

A completely randomized design with four replicates in each test was used in initial seed quality tests.

The storage experiment was a randomized complete block design with 2 %SMC x 2 storage temperatures x 2 containers x 6 sampling times x 4 replicates. A schematic design chart for each species is shown in Figure 1.

Figure 1 Schematic diagram of storage experiment design for soybean and wheat.



CHAPTER 3

RESULTS

WHEAT SEED

Initial seed quality

Data on the intial seed quality of the five seedlots used in this study is presented in Table 4

Table 4 Initial quality of wheat seedlots

Laboratory Test	Lot 1	Lot 2	Lot 3	Lot 4	Lot 5
Purity (%)	100	99.91	99.85	99.5	99.75
T.S.W. (g)	35.75	43.24	34.10	38.70	42.11
SMC (%)	13.5±0.15	12.9 ± 0.06	13.3 ± 0.1	15.8 ± 0.17	15.9 ± 0.22
Germination (%)	96±3.65	95 ± 2.52	92 ± 4	45 ± 4.43	12 ± 6.53
- Abnormal seedlings (%)	3.5 ± 0.15	3 ± 1.91	3 ± 2.52	11 ± 5.29	10 ± 2.83
- Dead seeds (%)	1 ± 1	2 ± 2.31	5 ± 1.91	44 ± 9	78 ± 6.32
Germination after AA (%)	90 ± 5.74	86 ± 2.31	85 ± 7.39	5 ± 3.83	0
- Abnormal seedlings (%)	5 ± 3	7 ± 1	7 ± 5.26	3 ± 2.58	0
- Dead seeds (%)	5 ± 3.46	7 ± 3	8 ± 4.12	92 ± 5.89	100
Conductivity Test ($\mu scm^{-1} g^{-1}$)	10.96 ± 0.71	10.01 ± 0.42	6.22 ± 0.84	8.92 ± 1.25	5.7 ± 0.9
Seedling growth (cm)	6.4 ± 0.68	7.16 ± 0.27	6.25 ± 1.1	3.05 ± 0.74	0.4 ± 0.23
Field fungi (%)	27.5 ± 9.57	57.5 ± 5	60 ± 14.14	47.5 ± 17.08	10 ± 14.14
Storage fungi (%)	0	0	5 ± 10	95 ± 10	0

All seedlots were high in purity, with small amounts of inert matter consisting mainly of chaffy matter and pieces of seed half size or less.

Thousand seed weight (T.S.W) of seedlots 2 and 5 were higher than other seedlots (43.24 g. and 42.11 g. respectively). Seedlot 3 had a lowest T.S.W. (34.1 g.).

The initial moisture content in seedlots 4 and 5 (15.8% and 15.9%, respectively) were higher than in seedlots 1, 2 and 3 (13.5%, 12.9% and 13.3% respectively).

The five seedlots showed major differences in germination percentage. Seedlots 1, 2 and 3 had high germination (92 - 96%) whereas seedlots 4 and 5 were lower than an acceptable quality (45% and 12% respectively). Abnormal seedling levels were lower (3 - 3.5%) in high quality seedlots but rose to 10 - 11% in poorer quality seedlots.

Germination after accelerated ageing of seedlots 1, 2 and 3 retained their high germination (90%, 86% and 85% respectively) even under severe stress conditions while seed from seedlots 4 and 5 failed to survive. These results indicate that seedlots 1, 2 and 3 have high potential storability and that loss of germination in seedlots 4 and 5 is well correlated with their initial low standard germination. In addition, seedling growth results were also well related to the results of standard germination with seedlings from seedlot 5 showing a 95% lower seedling growth than seedlings from seedlot2. Seedling growth of seedlots 1, 2 and 3 were high while seedlots 4 and 5 produced poor seedling growth.

The results of the conductivity test were not well correlated with initial germination values. For example, seedlot 5 which had the lowest germination (12%) also showed the lowest conductivity value (5.7 µs/cm/g). Similarly seedlots 1 and 2 with high germination produced provided high conductivity values (10.01 - 10.96 µs/cm/g).

Seeds from seedlots 2, 3 and 4 were most highly infected by field fungi (57.5%, 60.0% and 47.5% respectively). Field fungal infestation was lowest in seedlot 5.

Seeds from seedlot 4 were severely infected by storage fungi (95%) while storage fungi were absent or at very low levels in all others seedlots.

The performance of different wheat seedlots during storage

Since the results of initial quality tests showed that only seedlots 1, 2 and 3 had high viability these were selected for studies on storage performance. SMC is one factor which can have a dramatic effect on seed storability. The SMC results on these seedlots were all at a level considered to be safe for the storage of wheat (12.9-13.3%). Seeds from seedlots 1, 2 and 3 were therefore adjusted to approximately 10% or 14% before storage to provide what might be considered to be extremely'safe' (10%) and marginally 'unsafe' (14%) storage SMC's.

Germination

The percentage germination of all seedlots under different storage conditions after different storage periods is presented in Table 5

Open storage at different temperatures did not affect seed viability in any of the three seedlots which maintained high germination throughout the storage period provided relative humidity was 75% or less. However, in open storage at 30°c 95%RH the decline in germination of all seedlots was dramatic and all seeds were dead after 4 months storage. This was obviously an accelerated influence of storage relative humidity SMC seeds stored at 30°c50%RH produced high germination even after 8 months storage (93 - 97%).

Sealed storage at 30°c, however, had a significant detrimental effect in terms of germination with seed of all seedlots being dead after of 8 months storage. Germination percentage of seed with higher initial SMC (14%) deteriorated more rapidly over time while seed with lower initial SMC (10%) retained high initial germination levels.

There was no great effect of packaging on germination capacity of seed stored at 20°c although there was a suggestion that wet seed of lot 3 in sealed storage may have begun to deteriorate after 8 months storage. Despite this, the results suggest that the effect of packaging and moisture content are more important at higher temperatures (eg 30°c). Although initial germination levels were high (92 - 96 %) in

Table 5 Effect of seedlot, SMC, packaging, temperature and relative humidity on germination of wheat seed after 1, 2, 4, 6 and 8 months storage

Lot	Target SMC level	Initial quality	% Germination																							
			30°c 95%RH		30 °c 50 %RH										20 °c 75%RH											
			Open		Open					Sealed					Open					Sealed						
			2	4	1	2	4	6	8	1	2	4	6	8	1	2	4	6	8	1	2	4	6	8
1	10%	96	4	0	95	96	95	95	96	97	98	97	94	97	97	97	97	95	98	95	97	96	94	97
	14%	96	4	0	95	95	98	95	97	95	93	87	53	1	97	94	97	96	96	93	96	98	95	94
2	10%	95	7	0	94	93	97	93	95	96	96	98	98	97	97	94	97	95	96	92	93	97	94	95
	14%	95	4	0	93	96	95	95	96	91	93	64	15	0	97	92	97	97	97	91	95	95	94	94
3	10%	92	6	0	95	95	97	92	93	91	92	92	92	93	95	94	97	93	96	95	94	94	91	95
	14%	92	8	0	94	92	96	91	94	91	86	27	3	0	89	94	97	93	96	93	94	94	92	86

Table 6 Effect of seedlot, SMC, packaging, temperature and relative humidity on seed moisture content of wheat seed after 1, 2, 4, 6 and 8 months storage

Lot	Target SMC level	Initial quality	% Seed Moisture Content																							
			30°c 95%RH		30 °c 50 %RH										20 °c 75%RH											
			Open		Open					Sealed					Open					Sealed						
			2	4	1	2	4	6	8	1	2	4	6	8	1	2	4	6	8	1	2	4	6	8
1	10%	10	18.5	19.8	8.9	10.1	9	8.4	8.4	8.9	10.2	10.4	10.4	10	12	12.9	13	13.3	13.1	9	10.2	10.3	10.4	10.2
	14%	14.4	18.6	20	9	10.1	9	8.3	8.5	13.1	14.4	14.5	14.6	14.1	12.3	13.4	13.2	13.5	13.2	13.4	14.4	14.5	14.6	14.4
2	10%	10.3	20.1	20.5	8.7	10	8.9	8.3	8.2	9.2	10.5	10.5	10.5	10.1	11.6	13.1	12.8	13.2	12.8	9	10.4	10.5	10.6	10.4
	14%	14.5	18.8	18.9	8.8	10	8.7	8.3	8.2	13.3	14.5	14.6	14.6	14.2	12	13.1	13.1	13.3	13.1	13.2	14.3	14.5	14.5	14.4
3	10%	10.1	19.7	18.8	9.2	10.3	9.2	8.4	8.6	9.4	10.3	10.5	10.5	10.2	12.2	13.2	13.1	13.3	13.2	9.4	10.3	10.4	10.5	10.5
	14%	14	20.1	18.5	9.3	10.2	9.2	8.5	8.5	13.6	14.6	14.8	14.8	14.6	12.6	13.5	13.5	13.7	13.6	13.9	14.7	14.8	14.9	14.8

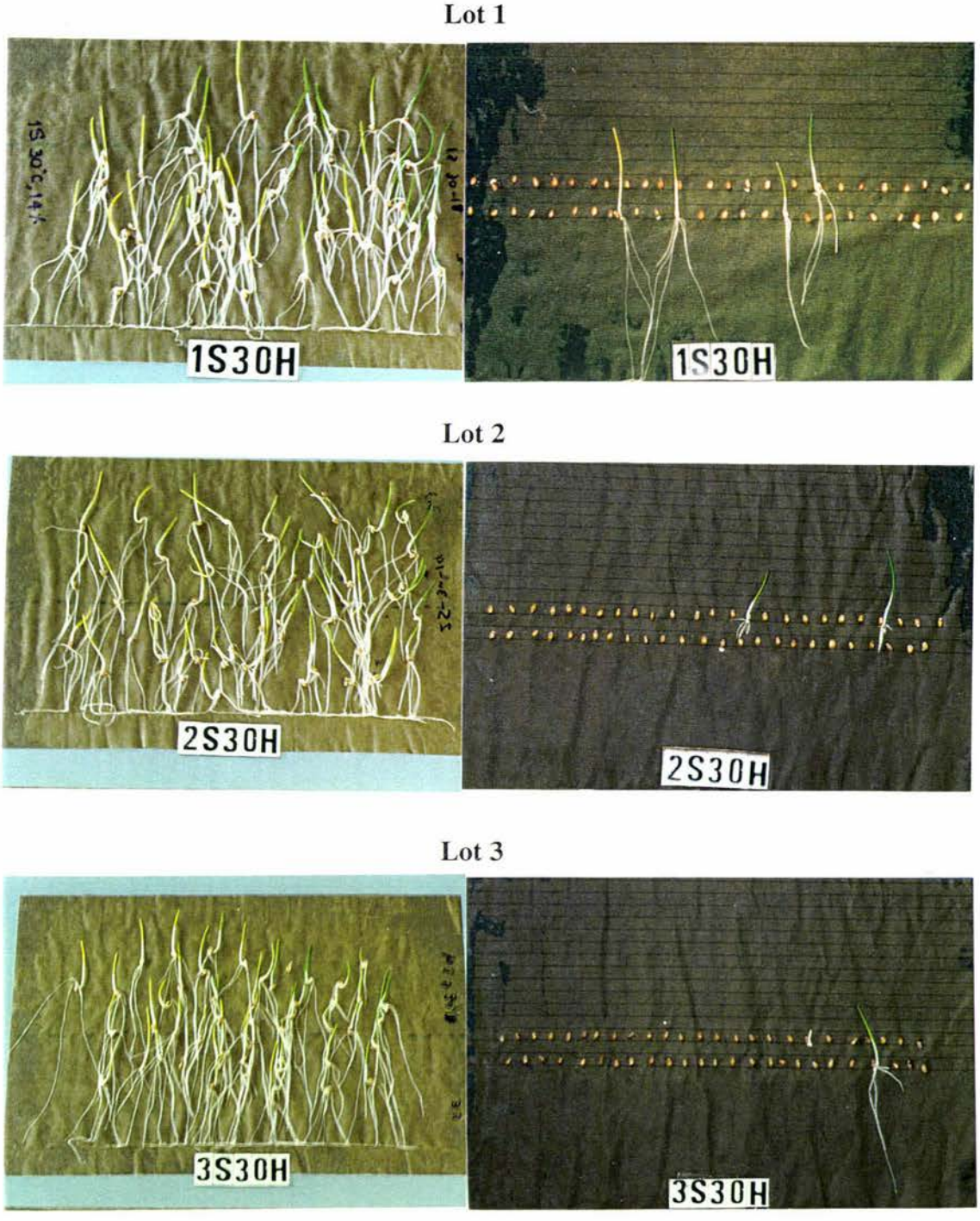


Plate 1 Differences in germination of 3 lots of wheat with high initial SMC (14 - 14.5%) in sealed packaging after 1 and 8 months storage at 30°c 50%RH

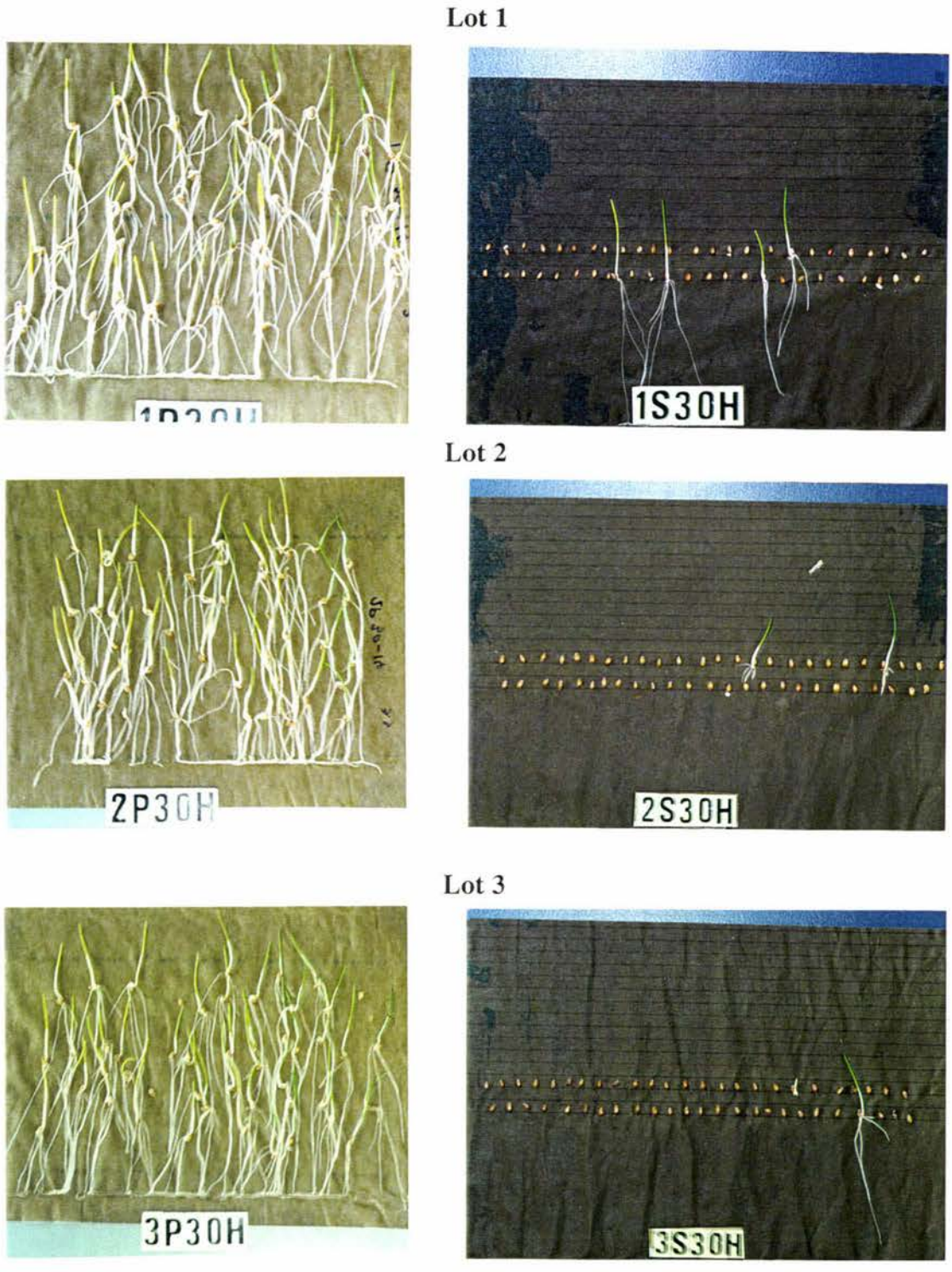


Plate 2 Comparison of the germination of 3 wheat seedlots with high initial SMC (14 - 14.5%) in open and sealed packaging after 8 months storage at 30°c 50%RH

all seedlots before storage, under unfavourable storage (30°c sealed 14%SMC) seeds of lot 3 began to lose germination earlier in the storage period than lot 1. Lot 2 was intermediate.

Seed moisture content

In open storage the SMC at both initial levels (10% or 14%SMC) changed due to moisture movement between seeds and surrounding air to equilibium moisture content (Table 6). At 30°c 95%RH the SMC of both initial levels increased rapidly to 18.5% - 20.5% with most of this change occurring in first 2 months. However, at 30°c 50%RH all seedlots lost moisture to 8.2% - 8.5% after 8 months. Low initial SMC of seed stored in open storage at 20°c 75%RH increased to 12.8% - 13.2% while high initial SMC seed slightly reduced to 13.1% - 13.6%. Sealed storage at different storage temperatures had no effect on SMC, with initial levels being essentially maintained throughout the storage period.

Field fungal infection

Changes in the level of field fungal infestation of three wheat seedlots during 0 - 8 months storage are presented in Table 7. Prior to storage, the initial infection by field fungi varied between seedlots (28 - 60%).

However, after only 2 months open storage at 30°c 95%RH all field fungi were eliminated. In all other open storage conditions, however, field fungi survived, although at lower levels during the entire storage period. Generally there was a tendency for levels of field fungi to be lower in initially drier samples after 8 months storage.

In sealed storage dry (10%) seed held at 30°c showed a higher percentage of fungal infestation in seedlots 1 and 2 after two months storage although levels then declined, while seeds from seedlot 3 were fully disinfected after 8 months storage. At 20°c seeds in sealed storage were increasingly infected after one month. Thereafter changes varied according to seedlot. In fact, levels of field fungi in drier seed of seedlot 1 were

Table 7 Effect of seedlot, SMC, packaging, temperature and relative humidity on field fungi of wheat seed after 1, 2, 4, 6 and 8 months storage

Lot	Target SMC level	Initial quality	% Field Fungi																					
			30°c 95%RH		30 °c 50 %RH										20 °c 75%RH									
			Open		Open					Sealed					Open					Sealed				
			2	4	1	2	4	6	8	1	2	4	6	8	1	2	4	6	8	1	2	4	6	8
1	10%	28	0	0	35	45	15	20	15	53	40	18	15	15	55	30	30	18	13	60	38	43	40	48
	14%	28	0	0	45	38	25	23	20	33	20	0	0	8	75	38	33	28	20	38	18	20	13	0
2	10%	58	0	0	45	38	35	30	10	63	40	28	25	8	55	45	30	23	8	68	43	50	60	43
	14%	58	0	0	43	43	30	38	13	45	5	0	3	3	53	25	38	25	15	60	15	35	13	0
3	10%	60	0	0	63	35	18	15	10	45	48	30	33	0	53	33	20	13	18	68	43	30	50	5
	14%	60	0	0	61	28	15	20	15	28	15	0	0	0	63	48	15	20	5	68	18	5	8	8

Table 9 Effect of seedlot, SMC, packaging, temperature and relative humidity on storage fungi of wheat seed after 1, 2, 4, 6 and 8 months storage

Lot	Target SMC level	Initial quality	% Storage Fungi																					
			30°c 95%RH		30 °c 50 %RH										20 °c 75%RH									
			Open		Open					Sealed					Open					Sealed				
			2	4	1	2	4	6	8	1	2	4	6	8	1	2	4	6	8	1	2	4	6	8
1	10%	0	100	100	0	3	0	0	0	3	10	0	0	0	0	3	0	0	0	0	8	0	0	0
	14%	0	100	100	0	0	0	0	0	0	0	5	0	5	0	0	3	0	0	5	0	0	0	0
2	10%	0	98	100	0	3	10	0	0	0	0	0	5	0	3	0	13	0	0	8	0	3	0	0
	14%	0	100	100	0	3	8	0	0	0	0	0	3	3	3	0	0	0	0	3	8	3	3	0
3	10%	8	100	100	0	5	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0
	14%	8	100	100	0	0	0	0	0	3	0	8	0	0	3	0	0	3	0	0	0	0	0	0

higher than initial levels after 8 months storage while initially wetter seed of the same seedlot was disinfected. Although not as extreme, a similar situation occurred in seedlot 2. Surprisingly however both initially wet and dry samples of seedlot 3 both showed low levels of field fungi after 8 months.

At 30°c the percentage of field fungal infection was lower after 8 months and the effect of initial SMC was less obvious. However, at this storage temperature seed of seedlot 3 was disinfected after 8 months irrespective of initial SMC and levels of field fungi in the other two seedlots were low (3 - 15%).

The main genera of field fungi found were identified as *Fusarium* spp. *and Alternaria* spp. as presented in Table8. Prior to storage, seed from all seedlots was found to be heavily infected by *Fusarium* spp. 25 - 60% and to a lesser extent with *Alternaria* spp. (3-20%) although seedlot 3 was free from *Alternaria* spp.. During open storage at 30°c 50%RH infection of *Fusarium* spp. slowly reduced (to 3 - 8%) whereas seeds infected by *Alternaria* spp. increased after one month, particularly in seedlots 1 and 2 before declining to 5 - 12% after 8 months. Nevertheless seedlot 3, which was initially free of *Alternaria* spp. still showed 7% infection after 8 months storage.

Under open storage under both storage conditions (30°c 50%RH and 20°c 75%RH) infection of *Fusarium spp.* decreased rapidly after eight months but fungal infection was not eliminated.

The effect of initial moisture content on field fungal levels in sealed storage was more clear. After 8 months storage initially wetter samples showed generally lower levels of field fungi, which were eliminated in seedlot 3 at 30°c and in seedlots 1 and 2 at 20°c. Although *Fusarium* spp. (0 - 10%) and *Alternaria* spp.(0 - 5%) levels were low after 8 months sealed storage at 30°c initially drier samples of seedlots 1 and 2 stored in sealed containers at 20°c still showed high levels of both fungal species. In fact *Fusarium* levels in seedlot 1 were the same as initial levels after 8 months storage and level of *Alternaria* spp. was shown to have increased under these conditions by the end of the trial.

Table 8 The percentage of wheat seed infected by *Fusarium spp. and Alternaria spp.* after 1 and 8 months storage

Storage Condition	Lot	Target SMC	Initial			Open						Sealed					
						1 month			8months			1 month			8months		
			F.	*Al.*	Total	*F.*	*Al.*	Total	*F.*	*Al.*	Total	*F.*	*Al.*	Total	*F.*	*Al.*	Total
30°c 50%RH																	
	1	10%	25	3	28	25	10	35	3	12	15	35	18	53	10	5	15
		14%	25	3	28	20	25	45	8	12	20	23	10	33	3	5	8
	2	10%	38	20	58	28	17	45	5	5	10	40	23	63	5	3	8
		14%	38	20	58	30	13	43	3	10	13	35	10	45	0	3	3
	3	10%	60	0	60	35	28	63	3	7	10	33	12	45	0	0	0
		14%	60	0	60	33	28	61	8	7	15	15	13	28	0	0	0
20°c 75%RH																	
	1	10%	25	3	28	40	15	55	8	5	13	33	27	60	25	23	48
		14%	25	3	28	50	25	75	12	8	20	28	10	38	0	0	0
	2	10%	38	20	58	33	22	55	3	5	8	33	35	68	13	30	43
		14%	38	20	58	38	15	53	5	10	15	40	20	60	0	0	0
	3	10%	60	0	60	40	13	53	8	10	18	48	20	68	3	2	5
		14%	60	0	60	38	25	63	2	3	5	55	13	68	8	0	8

F. = *Fusarium* spp. *Al.* = *Alternaria* spp.

Storage fungal infection

The development of storage fungi in different treatments after 8 months storage is presented in Tables 9 and 10. Initially all seedlots were free of storage fungi, except for a low level of infection (8%) of *Penicillium* spp. in seedlot 3. This situation was generally retained during storage at 30°c 50%RH or 20°c 75%RH in both open and sealed storage conditions despite the sporadic low occurrence (3 - 8%) of *Aspergillus glaucus* and (more rarely) *Aspergillus flavus* and *Aspergillus candidus* in some treatments. Initial SMC and storage system (open or sealed) appeared to have little if any effect on this situation.

However, under open storage at 30°c 95%RH storage fungi developed rapidly in all seedlots after 2 months. The main species of storage fungus found was *Aspergillus ochraceus* although *Aspergillus flavus* was also important initially (2 months). This species, however, was not found after 4 months storage although *A. ochraceus* remained important (100%) and *A. glaucus*, which was not defected until after 2 months storage was present at lower levels after 4 months storage (Table 10). In addition, seeds damaged by mite were also found under this condition after one month but not in any other treatments.

Conductivity test

Prior to storage, the three seedlots had different conductivity values. Conductivity readings from seedlots 1 and 2 were highest (10.96 and 10.01 µs/cm/g) while seedlot 3 had the lowest value of conductivity (6.22 µs/cm/g) (Table11).

Under open or sealed storage at both 30°c 50%RH and 20°c 75%RH the level of leachate of all seedlots fluctuated only minimally during storage, although there was the suggestion of a slight increase in conductivity in wet seed samples stored in sealed containers at 30°c 50%RH towards the end of the storage period in all seedlots. Under 30°c 95%RH storage condition , however, conductivity values increased rapidly within the first 2 months. Again, however this effect occurred irrespective of initial SMC and was not greatly different between seedlots.

Table 10 The percentage of storage fungal infection on wheat seed under different storage condition after 2, 4 and 8 months

Storage Condition	Lot	Target SMC	% of storage fungal infection																																					
			Initial			Open 2 months						Open 4months						Open 8months		sealed 2 months						sealed 4months						sealed 8 months									
			gl.	*P.*	Total	*gl.*	*fl.*	*oc.*	*ca.*	*P.*	Total	*gl.*	*fl.*	*oc.*	*ca.*	*P.*	Total	Total	*gl.*	*fl.*	*oc.*	*ca.*	*P.*	Total	*gl.*	*fl.*	*oc.*	*ca.*	*P.*	Total	*gl.*	*fl.*	*oc.*	*ca.*	*P.*	Total
30°c 95%RH																																								
	1	10%	-	-	-	-	40	100	8	-	148	-	-	100	-	-	100																							
		14%	-	-	-	-	48	100	5	-	153	15	-	100	-	-	115																							
	2	10%	-	-	-	-	53	98	-	-	161	8	-	100	-	-	108																							
		14%	-	-	-	-	50	100	-	-	150	-	-	100	-	-	100																							
	3	10%	-	8	8	-	48	100	-	-	148	28	-	100	-	-	128																							
		14%	-	8	8	-	38	100	10	-	148	-	-	100	-	-	100																							
30°c 50%RH																																								
	1	10%	-	-	-	3	-	-	-	-	3	-	-	-	-	-	-	-	8	3	-	-	-	11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		14%	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	3	-	6	5	-	-	-	-	5				
	2	10%	-	-	-	3	-	-	-	-	3	5	-	-	-	5	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		14%	-	-	-	3	-	-	-	-	3	3	5	-	-	-	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	3			
	3	10%	-	8	8	5	-	-	-	-	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		14%	-	8	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8	-	-	-	-	8	-	-	-	-	-	-				
20°c 75%RH																																								
	1	10%	-	-	-	-	3	-	-	-	3	-	-	-	-	-	-	-	5	3	-	-	-	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		14%	-	-	-	-	-	-	-	-	-	3	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
	2	10%	-	-	-	-	-	-	-	-	-	10	3	-	-	-	13	-	-	-	-	-	-	-	-	3	-	-	-	3	-	-	-	-	-	-				
		14%	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8	-	-	-	8	-	-	-	3	-	3	-	-	-	-	-	-				
	3	10%	-	8	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
		14%	-	8	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			

gl. = *Aspergilus glaucus* *fl.* = *Aspergillus flavus* *oc* = *Apergillus ochraceus* *ca* = *Aspergillus candidus* *P* = *Penicillium*

Table 11 Effect of seedlot, SMC, packaging, temperature and relative humidity on electroconductivity of wheat seed after 1, 2, 4, 6 and 8 months storage

Lot	Target SMC level	Initial quality	Conductivity Reading (µs/cm/g)																					
			30°c 95%RH		30 °c 50 %RH										20 °c 75%RH									
			Open		Open					Sealed					Open					Sealed				
			2	4	1	2	4	6	8	1	2	4	6	8	1	2	4	6	8	1	2	4	6	8
1	10%	10.96	20.2	22.7	10.5	10.9	10.7	10.5	10.7	11	10.5	11.3	10.8	11.2	10.6	10.3	10.1	10.4	10.1	11.8	10.7	11	10.4	10.4
	14%	10.96	20.1	21.9	11	10.6	11	10.6	10.9	10.7	10.7	11.6	13	13.9	10.9	10.3	10.7	10.3	10.3	10.1	10.8	10.4	9.78	10.3
2	10%	10.01	18.7	26.8	10.9	10.2	9.21	10.2	11.2	10.3	10.3	11.4	10.2	11.3	11.1	9.63	9.94	9.41	10.1	10.6	11	10.6	10.2	10.2
	14%	10.01	18.7	26.3	11.4	10.6	10	10.8	10.6	9.86	10.4	11.6	13	14.6	11.4	8.97	10.1	10.4	10	10	10.4	9.88	9.66	10.4
3	10%	6.22	14.8	21.3	6.01	6.18	6.47	6.27	6.12	5.95	5.99	5.96	5.91	6.2	5.54	5.91	5.99	5.91	6.16	5.09	7.37	8.06	6.51	7.21
	14%	6.22	16.2	19.6	6.36	6.55	5.96	5.53	6.32	5.97	7.69	9.22	9.58	9.61	5.64	5.7	6.59	5.51	6.45	5.49	6.07	6.05	5.64	6.65

SOYBEAN SEED

Initial seed quality

Data on the initial seed quality of the five soybean seedlots used in this study are presented in Table.

Table12: Initial quality of soybean

Laboratory Test	Lot1	Lot2	Lot3	Lot4
Purity (%)	99.5	99.3	99.8	99.5
T.S.W (g)	163	179.1	218.3	218.2
SMC (%)	10.1 ± 0.22	10.4 ± 0.5	10.5 ± 0.08	10.3 ± 0.08
Germination (%)	81 ± 4.16	23 ± 4.12	85 ± 2.58	84 ± 1.91
- Abnormal seedlings (%)	13 ± 4.16	25 ± 7.72	14 ± 2.52	16 ± 1.91
- Dead seeds (%)	6 ± 3.27	52 ± 9.93	1 ± 1	0
Germination after AA (%)	79 ± 4.76	0	80 ± 5	83 ±3
- Abnormal seedlings (%)	15 ± 3.46	2 ± 4	19 ± 5.03	16 ± 4.12
- Dead seeds (%)	6 ± 1.63	98 ± 4	1 ± 1	1 ± 2
Conductivity test ($\mu scm^{-1}g^{-1}$)	29.62 ± 1.83	73.49 ± 6.33	23.8 ± 0.64	29.38 ± 4.46
Ferric Chloride test (%)	23.25 ± 1.26	30 ± 4.08	29 ± 3.65	26.75 ± 3.5
Field fungi (%)	30 ± 21.6	27.5 ± 22.17	2.5 ± 5	2.5 ± 5
Storage fungi (%)	2.5 ± 5	25 ± 23.8	0	0

All seedlots were high purity (99.3 - 99.8%). The small amount of inert matter found in all seedlots included pieces of seed half size or less and seed coat fractions. The only other seed found was pea seed which occurred only in seedlot 3.

Thousand seed weight (T.S.W) of seedlots 3 and 4 were high (218.2 g and 218.3 g. respectively) and that of seedlot 1 was lower (163 g). Seedlot 2 had intermediate seed weight.

Seed moisture content levels between seedlots were not significantly different and were all at a suitable level for short term soybean storage (10.1% - 10.5%).

Seedlots 1, 3 and 4 produced a high percentage of normal seedlings (81 - 85%) but seedlot 2 had low germination (23%) and an associated high level of abnormal seedlings (25%). Abnormal seedling percentage in the other 3 seedlots were similar (13% - 16%).

Germination after accelerated ageing of seedlots 1, 3 and 4 was relatively high (79% - 83%) even under adverse conditions whereas seed from seedlot 2 was dead. These results show that seedlots 1, 3 and 4 have good potential storability since AA results correlated well with standard germination percentage.

The results of conductivity tests showed differences in levels of electrolyte leakage from soybean tissues of three seedlots. Although the conductivity value for seedlots 1, 3 and 4 were similar (23 - 29 µs/cm/g) the value for seedlot 2 was much higher (73 µs/cm/g). This reinforces the low vigour status of this seedlot, compared with the expression of high vigour which correlated well with the results of accelerated ageing test in seedlots 1, 3 and 4..

The ferric chloride test was used to visualise mechanical damage on seed. Although the results showed only small differences between seedlots (23 - 30%) they did show that in seedlot 2 had a reasonably high level of damage (30%), whereas seed from seedlot 1 was least damaged (23%). The extent of damage in lot 2 was also related toa high abnormal seedling percentage (25%) and extremely high dead seed level (52%) in a standard germination test..

Seeds from seedlots 1 and 2 were heavily infected by field fungi (30% and 27.5% respectively) while seedlots 3 and 4 showed very low infection levels (2.5%). The main fungal genera present were *Alternaria* spp., *Fusarium* spp. and *Colletotrichum* spp.. Similarly, high levels of storage fungi were also found in lot 2

Plate 3 Response of initial soybean seed samples to 20% ferric chloride soaked for 5 - 15 minutes. The black staining on the testa indicates sites of mechanical damage penetrated by the salt which is subsequently oxidised.

while in other seedlots storage fungi were either absent (seedlots 3 and 4) or occurred at very low levels (seedlot 1). The mainstorage fungi detected were *Aspergillus* spp. and *Penicillium* spp.

The performance of different soybean seedlots during storage

From the results of initial quality tests it was found that seedlots 1, 3 and 4 had highest potential storability but the quantity of seed in seedlot 4 was insufficient to allow its use in a further experiment. For this reason seedlot 4 was replaced by seedlot 2. These three seedlots were adjusted to moisture contents of 8% or 12% to provide storage samples which were lower and higher than the recognised safe SMC storage for soybean.

Seeds at each initial SMC level were open or sealed stored in three different environments (30°c 95%RH, 30°c 50%RH and 20°c 75%RH) for up to 8 months, except for seedlot 3, which due to insufficient seed was stored only at 30°c 50%RH and 20°c 75%RH.

Germination

The mean effects of seed moisture level and storage environment on the percentage of normal germination of three soybean seedlots is shown in Table 13. Since seedlot 2 consistently performed poorly due to its low initial quality, seedlots 1 and 3 showed more interesting and important reaction to the storage environment.

Under 30°c 50%RH storage conditions, both packaging and SMC had a interactive effect on seed viability. The germination of initially wet seeds (12%SMC) of all seedlots in sealed containers gradually decreased to 0 - 5% with increasing time storage up to 8 months. However, initially dry seeds (8%SMC) in sealed containers and seed at both initial SMC levels in open containers maintained high germination (74 - 81% excluding seedlot 2). At 20°c 75%RH storage condition packaging SMC did not affect seed viability after 8 months. Even seedlot 2 under these conditions retained reasonal germination capacity despite its obvious prestorage deterioration.

Open storage at 30°c 95% RH rapidly lost seed viability. Under unfavourable storage conditions at high RH (95%), seeds from all seedlots had lost much of their germination after as little as one month, and all seed was dead after 2 months storage

Table 13 Effect of seedlot, SMC, packaging, temperature and relative humidity on germination of soybean seed after 1, 2, 4, 6 and 8 months storage

Lot	Target SMC level	Initial quality	% Germination																							
			30°c 95%RH		30 °c 50 %RH										20 °c 75%RH											
			Open		Open					Sealed					Open					Sealed						
			1	2	1	2	4	6	8	1	2	4	6	8	1	2	4	6	8	1	2	4	6	8
1	8%	81	12	0	84	88	83	74	81	85	84	87	79	79	87	84	89	84	80	85	89	83	87	84
	12%	81	12	0	83	87	86	78	81	82	84	59	12	5	87	89	88	89	81	82	88	87	84	77
2	8%	23	0	0	25	25	14	10	11	27	23	20	11	12	31	31	28	15	12	31	30	27	20	13
	12%	23	0	0	25	24	14	9	7	19	7	1	0	0	32	25	26	17	12	24	28	15	12	10
3	8%	85	-	-	85	83	83	79	74	88	85	87	79	76	88	89	94	92	86	83	86	90	92	91
	12%	85	-	-	82	78	83	73	77	83	72	15	2	0	82	90	92	86	88	89	88	92	88	81

Table 14 Effect of seedlot, SMC, packaging, temperature and relative humidity on seed moisture content of soybean seed after 1, 2, 4, 6 and 8 months storage

Lot	Target SMC level	Initial quality	% Seed Moisture Content																							
			30°c 95%RH		30 °c 50 %RH										20 °c 75%RH											
			Open		Open					Sealed					Open					Sealed						
			1	2	1	2	4	6	8	1	2	4	6	8	1	2	4	6	8	1	2	4	6	8
1	8%	7.7	19.3	22.8	6.2	7	6	5.6	5.3	7.6	7.6	7.8	7.6	7.5	10.2	9.7	10.6	9.6	9.9	7.6	7.6	7.9	7.7	7.6
	12%	11.2	18.7	22	6.2	6.2	6	5.5	5.5	11.6	12	11.7	11.8	11.6	10.2	9.7	10.5	9.9	10	11.7	11.7	11.9	11.6	11.7
2	8%	7.3	18.3	22.5	6	6	5.7	5.3	5.2	7.7	7.3	7.5	7.4	7.1	10	9.5	10.4	9.6	9.9	7.6	7.4	7.7	7.5	7.6
	12%	11.2	19.5	23	6	5.9	5.8	5.3	5.2	11.3	11.4	11.3	11.6	11.4	10.1	9.5	10.3	9.6	9.9	11.6	11.4	11.7	11.5	11.4
3	8%	8.6	-	-	6	6.4	5.8	5.3	5.3	8.6	8.4	8.6	8.5	8.3	10	9.5	10.4	9.6	9.8	8.2	8.5	8.7	8.6	8.5
	12%	11.9	-	-	6	6	5.8	5.4	5.3	12	12.2	12.2	12.1	12.1	10.1	9.6	10.5	9.7	10.1	12.1	12.2	12.3	12.3	12.1

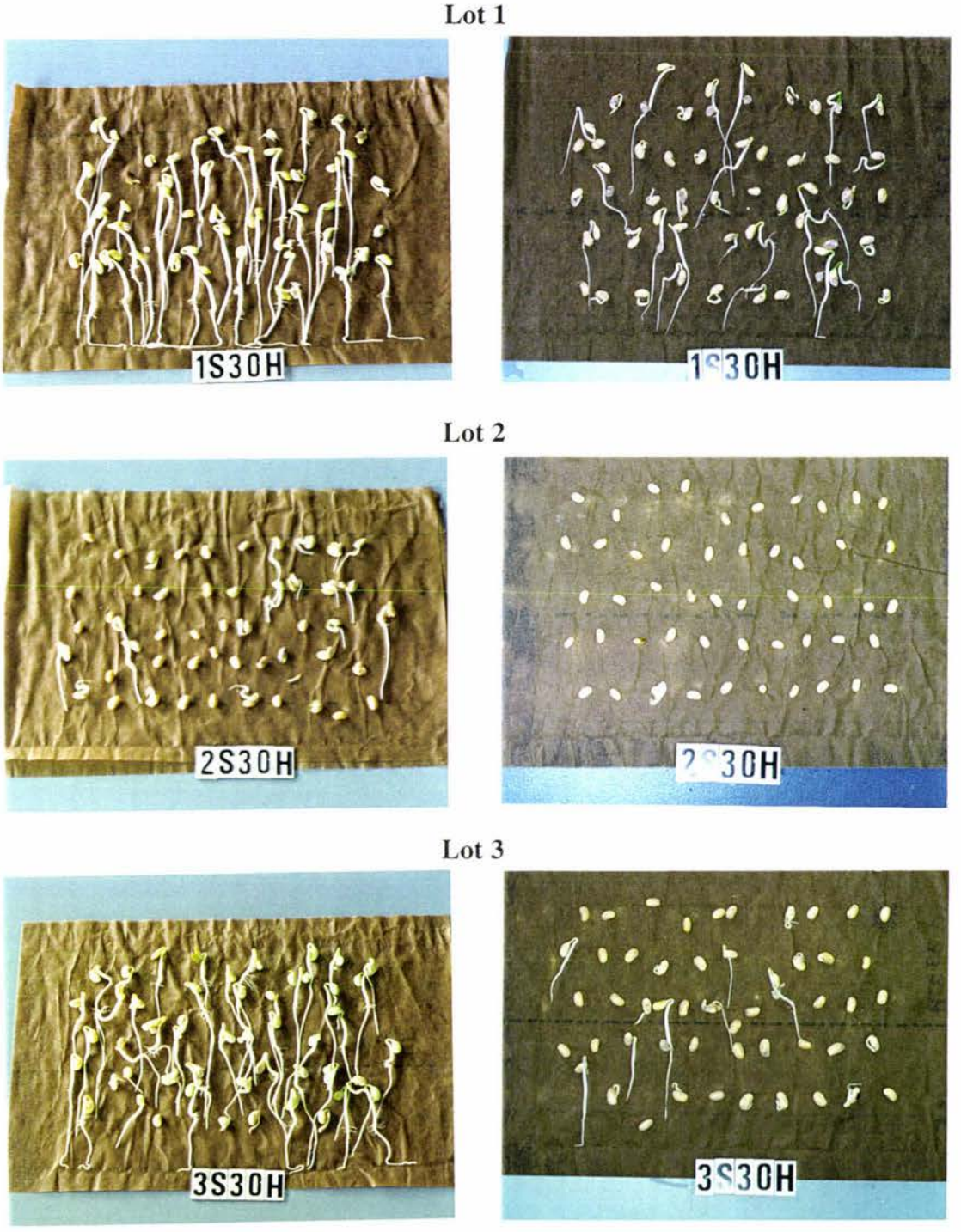


Plate 4 Differences in germination of 3 lots of soybean with high initial SMC (11.2 - 11.9%) in sealed packaging after 1 and 6 months storage at 30°c 50%RH

Plate 5 Comparison of the germination of 3 soybean seedlots with high initial SMC (11.2-11.9 %) in open and sealed packaging after 6 months storage at 30°c 50%RH

Plate 6 Seedlings showing bruised cotyledon deterioration of soybean seed with high initial SMC (11.2 - 11.9%) in sealed packaging after 8 months at 20°c 75%RH (Normal seedlings on right hand side)

Plate 7 Types of abnormal seedlings found in soybean seed with high initial SMC (11.2 - 11.9%) in sealed packaging after 8 months at 20°c 75%RH

Under all storage conditions, seedlot 2 deteriorated most rapidly obviously over time. This suggests that low initial germination percentage seed is susceptible to loss of seed viability even under favourable storage conditions.

Seed moisture content

In open storage there was a change in SMC as gained or lost water to reach equilibrium moisture content with the relative humidity of each particular storage environment (Table 14).

Open storage at 30°c 95%RH had the most major effect on seed moisture content. Seed gained water rapidly to 22 - 23%SMC after two months storage. However, seed open stored at 30°c 50%RH lost water to reach an equilibrium (5.2 - 5.5%SMC) level after 8 months.

In open storage at 20°c 75%RH initially low SMC seed increased SMC to 9.8 - 9.9%, while the SMC of seed at an initially high SMC decreased to 9.9 - 10.1% by the end of the storage time. In sealed storage there was no change in SMC under any storage conditions, confirming the effectiveness of the moisture proof barrier provided by aluminium foil containers.

Field fungal infection

Changes in the level of field fungi infestation of soybean seed over 8 months storage is presented in Table 15.

Prior to storage, seedlots 1 and 2 were found to be moderately infected (23 - 30%) while seedlot 3 showed a very low infection level (3%). The predominant genus of field fungus present in all seedlots was *Alternaria* spp. (Table 16).

At 30°c 95%RH no field fungi were found after 1 month storage whereas at 30°c 50%RH and at 20°c 75%RH field fungal infection level were reduced during storage and seed was either disinfected or supported only low infestation levels (3 - 8%) in all storage condition at both levels of initial SMC. Despite this, field fungus survival was better at 20°c than 30°c in sealed storage.

As shown in Table 16, the main fungal genus detected was *Alternaria* spp. with *Fusarium* spp. and *Colletotrichum* spp. being present sporadically at low levels. *Alternaria* spp. infection levels decreased during storage and did not survive after 8

months open storage at 30°c 50%RH or 20°c 75%RH except in seedlot 2, albeit at a low level (3%). *Alternaria* spp. declined with increasing storage time but did survive after 8 months in sealed storage conditions at 30°c 50%RH and at a very low level at 20°c 75%RH. Levels of *Colletotrichum* spp. and *Fusarium* spp. infected seeds were low but the results suggest that both these species could survive for up to 8 months in these storage environments.

Storage fungal infection

The level of storage fungal infection of soybean seed found during 8 months storage is presented in Table 17.

Prestorage seed health test results showed seedlot 2 was infected with higher levels of *Aspergillus glaucus* (20%) and occasional *Penicillium* spp. (up to 5%) when compared to seedlot 1 which had low fungal infection levels and seedlot 3 where storage fungi were not detected.

At 30°c 95%RH, storage fungi severely infected seeds from all seedlots but fungal infection decreased to low levels under open storage at 30°c 50%RH. Packaging had little apparent effect on storage fungal infection levels.

The storage fungi developing at 30°c 95%RH were dominated by *A. glaucus* and to a lesser extent *A. flavus* and *A. candidus* (Table 18). All three persisted in storage for 8 months. However, the development of *A. ochraceus* after one month storage was short lived, since this species was not detected one month later. Neither *A. ochraceus* nor *A. candidus* were found in other storage treatments and *A. flavus* was only found occassionally and at low levels. *Penicillium* spp., although defected initially in seedlot 2 was also of low occurrence (maximum 3%) throughout the storage period.

Initially dry seed (8%SMC) from seedlot 2 showed an increase in levels of *A. glaucus* after one month storage at 20°c 75%RH and at 30°c 50%RH. However, *A. glaucus* levels fell to low levels (or zero) in all seedlots by the end of the storage period.

Table 15 Effect of seedlot, SMC, packaging, temperature and relative humidity on field fungi of soybean seed after 1, 2, 4, 6 and 8 months storage

Lot	Target SMC level	Initial quality	% Field Fungi																							
			30°c 95%RH		30 °c 50 %RH										20 °c 75%RH											
			Open		Open					Sealed					Open					Sealed						
			1	2	1	2	4	6	8	1	2	4	6	8	1	2	4	6	8	1	2	4	6	8
1	8%	30	0	0	10	8	3	3	3	8	8	5	3	3	13	5	8	3	5	8	8	5	13	8
	12%	30	0	0	3	3	3	0	3	0	0	3	0	0	10	8	5	0	3	8	5	3	3	3
2	8%	23	0	0	3	5	5	10	5	8	5	3	0	0	0	5	3	8	8	0	3	3	3	5
	12%	23	0	0	8	5	5	8	8	3	0	0	5	0	0	8	0	3	5	8	3	15	0	3
3	8%	3	-	-	8	5	0	5	0	0	3	0	3	0	0	0	3	0	0	5	5	3	0	0
	12%	3	-	-	0	3	0	10	0	0	5	3	0	3	5	3	0	0	0	15	8	0	3	3

Table 17 Effect of seedlot, SMC, packaging, temperature and relative humidity on storage fungi of soybean seed after 1, 2, 4, 6 and 8 months storage

Lot	Target SMC level	Initial quality	% Storage Fungi																							
			30°c 95%RH		30 °c 50 %RH										20 °c 75%RH											
			Open		Open					Sealed					Open					Sealed						
			1	2	1	2	4	6	8	1	2	4	6	8	1	2	4	6	8	1	2	4	6	8
1	8%	3	100	100	3	0	5	0	3	0	18	5	0	0	8	0	5	3	0	0	5	0	3	3
	12%	3	98	100	3	0	5	0	0	8	3	5	3	0	0	10	5	0	0	0	0	8	5	3
2	8%	25	100	100	15	5	13	15	5	20	38	23	13	5	30	10	23	20	5	10	20	25	13	3
	12%	25	100	100	8	15	10	18	5	13	15	18	5	3	10	30	20	8	8	20	33	18	20	10
3	8%	0	-	-	5	8	0	0	0	3	5	3	0	0	3	10	5	0	0	0	20	5	3	3
	12%	0	-	-	0	8	3	3	0	3	3	10	25	5	5	3	3	0	0	3	8	5	0	8

Table16 The percentage of soybean seed infected by *Fusarium spp.*, *Alternaria spp.* and *Colletotrichum spp.* after 1 and 8 months storage

Storage Condition	Lot	Target SMC	Initial				Open								Sealed							
							1 month				8months				1 month				8months			
			F.	*Al.*	*C.*	Total	*F.*	*Al.*	*C.*	Total	*F.*	*Al.*	*C.*	Total	*F.*	*Al.*	*C.*	Total	*F.*	*Al.*	*C.*	Total
30°c 50%RH																						
	1	8%	-	30	-	30	-	10	-	10	3	-	-	3	3	5	-	8	3	-	-	3
		12%	-	30	-	30	3	-	-	3	3	-	-	3	-	-	-	-	-	-	-	-
	2	8%	-	23	-	23	3	-	-	3	-	3	3	6	-	8	-	8	-	-	-	-
		12%	-	23	-	23	-	8	-	8	5	3	-	8	-	3	-	3	-	-	-	-
	3	8%	-	3	-	3	-	8	-	8	-	-	-	-	-	-	-	-	-	-	3	3
		12%	-	3	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20°c 75%RH																						
	1	8%	-	30	-	30	-	13	-	13	3	3	-	6	-	8	-	8	3	5	-	8
		12%	-	30	-	30	-	10	-	10	-	3	-	3	3	5	-	8	3	-	-	3
	2	8%	-	23	-	23	-	-	-	-	3	5	-	8	-	-	-	-	-	3	3	6
		12%	-	23	-	23	-	-	-	-	-	3	3	6	-	8	-	8	3	-	-	3
	3	8%	-	3	-	3	-	3	3	6	-	-	-	-	-	5	-	5	-	-	3	3
		12%	-	3	-	3	-	-	-	-	-	-	-	-	5	10	-	15	-	-	-	-

F. = *Fusarium* spp. *Al.* = *Alternaria* spp. *C.* = *Colletotrichum* spp.

Table 18 The percentage of storage fungal infection on soybean seed under different storage conditions after 1 and 8 months storage

Storage Lot	Target	% of storage fungal infection																											
		Open															Sealed												
Condition	SMC	Initial			1 months						8 months						1 month						8 months						
		gl.	*P.*	Total	*gl.*	*fl.*	*oc.*	*ca.*	*P.*	Total	*gl.*	*fl.*	*oc.*	*ca.*	*P.*	Total	*gl.*	*fl.*	*oc.*	*ca.*	*P.*	Total	*gl.*	*fl.*	*oc.*	*ca.*	*P.*	Total
20°c 75%RH																													
1	8%	3	-	3	3	-	-	-	5	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	3
	12%	3	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	3
2	8%	20	5	25	23	-	-	-	8	31	3	-	-	-	3	6	5	5	-	-	3	13	3	-	-	-	-	3
	12%	20	5	25	8	-	-	-	-	8	5	3	-	-	-	8	18	3	-	-	-	21	5	3	-	-	-	8
3	8%	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	3	-	-	-	-	3	3
	12%	-	-	-	5	-	-	-	-	5	-	-	-	-	-	-	-	-	-	-	-	-	8	3	-	-	-	11
30°c 50%RH																													
1	8%	3	-	3	-	-	-	-	3	3	3	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-
	12%	3	-	3	3	-	-	-	3	6	-	-	-	-	-	-	8	-	-	-	-	8	-	-	-	-	-	-
2	8%	20	5	25	13	-	-	-	3	16	3	-	-	-	3	6	15	3	-	-	3	21	3	-	-	-	3	6
	12%	20	5	25	8	-	-	-	-	8	8	3	-	-	-	11	13	-	-	-	-	13	3	-	-	-	-	3
3	8%	-	-	-	5	-	-	-	-	5	-	-	-	-	-	-	-	-	-	-	3	3	5	3	-	-	-	8
	12%	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	3	-	-	-	-	-	-
					1 month						2 months																		
30°c 95%RH																													
1	8%	3	-	3	88	68	10	15	-	181	98	40	-	55	-	193													
	12%	3	-	3	95	65	13	3	-	176	100	50	-	75	-	225													
2	8%	20	5	25	100	53	3	18	-	174	100	50	-	30	-	180													
	12%	20	5	25	100	33	15	18	-	166	93	43	-	50	-	186													

gl. = *Aspergilus glaucus* *fl.* = *Aspergillus flavus* *oc* = *Apergillus ochraceus* *ca* = *Aspergillus candidus* *P* = *Penicillium spp.*

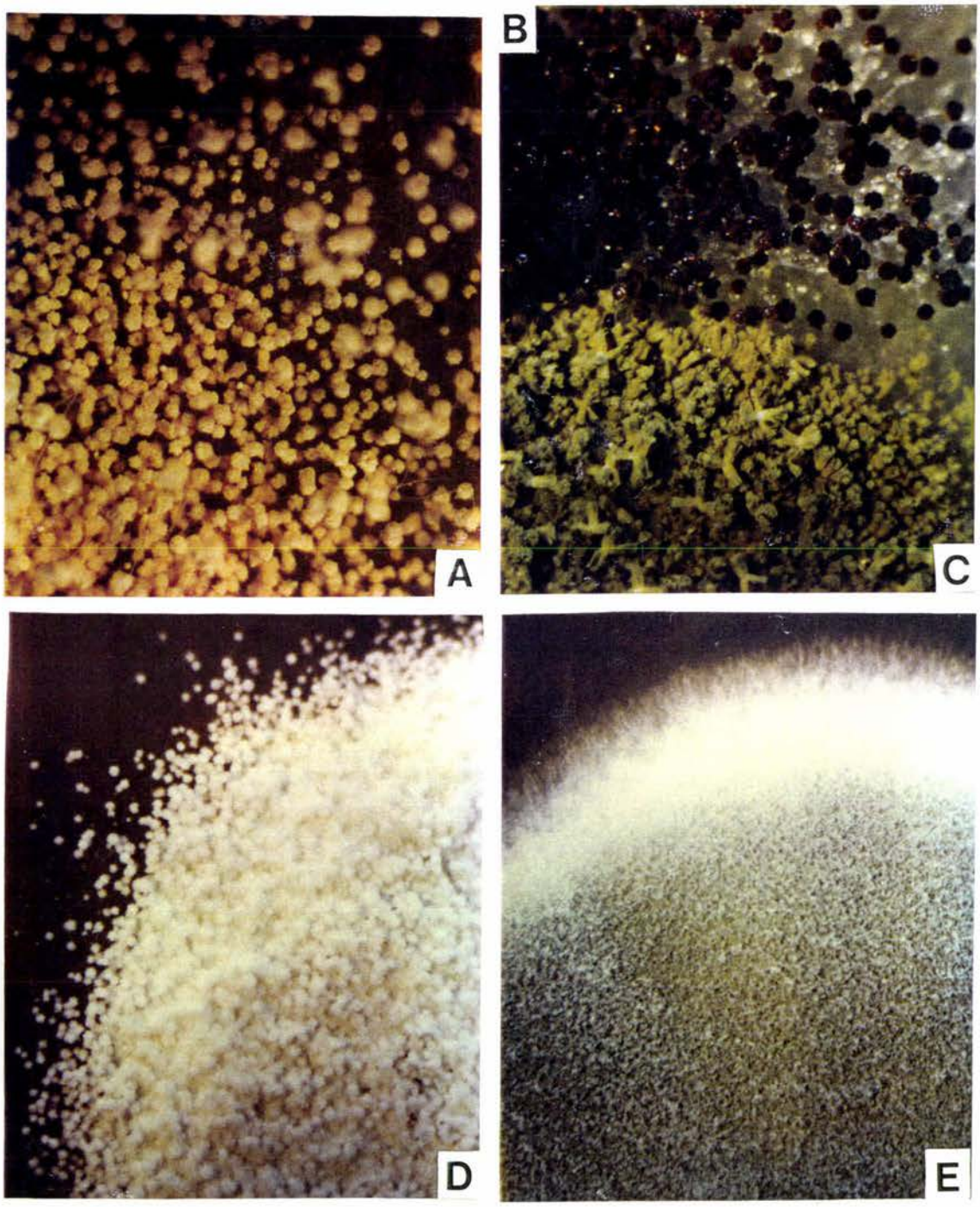


Plate 7 Storage fungi on wheat and soybean seed. (A) *Aspergillus flavus*, (B) *A. niger*, (C) *A. glaucus*, (D) *A. candidus*, (E) *Penicillium* spp.

Conductivity value

The effect of seedlot, initial SMC, packaging and storage conditions on electroconductivity results of three soybean seedlots are presented as Table 19.

The three seedlots had different conductivity values prior to storage, with seedlots 1 and 3 having lower conductivity readings than seedlot 2 (29.62, 23.8 and 73.49 μs/cm/g, respectively). This indicated that seed vigour in seedlots 1 and 3 was higher than in seedlot 2.

In all storage conditions, conductivity values after one month were observed to be generally higher than in any other month of storage. This is likely to be due to imbibition damage since seed moisture level was not adjusted with caution to 10 - 14% SMC prior to testing. It is therefore suggested this data for 1 month storage should be ignored or treated.

Under storage at 30°c 95%RH in open conditions conductivity values rose rapidly to high levels after 1 month storage. This was obviously a humidity response since this effect did not occur at the same temperature but lower humidity (30°c 50%RH).

Under the 30°c 50%RH storage conditions, there was an effect of packaging and SMC on electroconductivity in all soybean seedlots with increasing time of storage. The level of leachate of seeds stored under sealed storage (ignoring 1 month results) increased over time particularlly in initially wet seed samples. Conductivity values for seeds stored in open storage and for seeds with low initial SMC stored in sealed storage showed fluctuating conductivity values with time. However, the general trend of conductivity increase was small.

Under 20°c 75%RH in open storage conductivity readings fluctuated. Again, however, there was a slight increasing trend with time, in both initially dry and wet seed samples. Nevertheless the results suggest that initial seed vigour levels are likely to be maintained only under cooler (20°c) sealed storage conditions where seed is of initially low (8%) moisture content.

Table 19 Effect of seedlot, SMC, packaging, temperature and relative humidity on electroconductivity of soybean seed after 1, 2, 4, 6 and 8 months storage

Lot	Target SMC level	Initial quality	Conductivity Reading (µs/cm/g)																					
			30°c 95%RH		30 °c 50 %RH										20 °c 75%RH									
			Open		Open					Sealed					Open					Sealed				
			1	2	1	2	4	6	8	1	2	4	6	8	1	2	4	6	8	1	2	4	6	8
1	8%	29.62	86.6	79.7	62.3	30	31.8	30.6	30	39.7	24.7	25	26.7	30.8	42.9	35.4	36.6	34.5	37.7	51.3	27.5	25.4	24	23.4
	12%	29.62	129	120	48.1	26.5	32.4	25.6	27.9	59.5	39.7	64.3	87.7	98.7	34.9	33.6	30.9	32.1	34.1	31	28.8	30.2	32.2	34.8
2	8%	73.49	117	137	130	72.7	74.7	79.5	86.6	105	71.2	80.4	73.9	92.3	91.5	71.2	76.5	81.3	91.5	98.2	60.4	72.7	68.2	67.2
	12%	73.49	122	132	120	76.1	84.9	81	82	103	97.5	116	126	132	88.3	71.2	76.9	81.9	84.1	84.4	75.3	80.3	88.7	94.7
3	8%	23.8	-	-	57.2	34.8	36.2	35.1	34.6	44.6	30	31.4	30.4	34.3	40	31.8	34.2	36.5	37.1	41.6	24.4	26.1	24.2	23.5
	12%	23.8	-	-	49	31.6	37.2	32.8	33.7	37.1	54.9	86	97.4	106	36.3	29.9	30.1	30.7	32.7	31.1	24.5	29.7	35.1	41.6

CHAPTER 4

DISCUSSION AND CONCLUSION

DISCUSSION

Initial Quality

All of the wheat and soybean seedlots used in this study had high purity. Despite this all seedlots contained varying amounts of pure seeds which were immature, undersized, shrivelled, diseased, sprouted and/or mechanically damaged but which nevertheless met the pure seed definition defined by the ISTA Rules (1993). All seedlots had been processed efficiently as shown by low levels of other seed. Inert matter levels were low, but included small amounts of chaffy matter and pieces of seed half size or less in all wheat seedlots and included seed coat fractions in all soybean seedlots presumably resulting from seed processing (threshing and cleaning) operations. Only the soybean seedlot 3 contained other seeds (*Pisum sativum*) which was presumably due to mechanical admixture.

Seed weight is a indicator of seed quality and is used as a possible predictor of field emergence (Naylor 1993). Seedlots containing heavy seeds are usually considered to be of high vigour, since it has been demonstrated that seedlots with higher mean weight will produce faster seedling emergence than those with lower seed weight (Kneebone and Cremer 1955; Maranville and Clegg 1977). The seed weight results for wheat showed that seedlots 2 and 5 were heavier than other seedlots with thousand seed weight of seedlot 3 being the lowest. These results do not support the high seed weight, high seed vigour, relationship. According to the results of germination and seed vigour tests (accelerated ageing test), the 'low' weight seedlot 3 had both high germination and vigour while the 'heavy' seedlot 5 exhibited both a low germination percentage and low vigour. This clearly suggest that in some cases there are factors other than seed weight which influence seed performance. Ellis and Kirby (1980) have shown that seedlots having different production histories may provide seed with different mean seed weight and that seed weight varies with variety. Also, it is well known that post harvest operations such as rapid and hot drying systems can seriously reduce both germination and vigour without influencing mean

seed weight. The positive relationship between help seed weight and high germination and vigour may well be true, but only in seedlots where quality has not been reduced by improper handling or processing.

Seed moisture content is one of the most important factors influencing seed storage life (Harrington 1972a). The accurate measurement of moisture in a seedlot becomes important. It is necessary to ensure that the moisture content of the seed is reduced to a safe level for storage, since moisture content of seed which is too high results in greater seed susceptibility to damage by moulds and seed death (Hill 1995). Harrington (1973) stated that in the range from about 12% SMC for oil seeds or about 13% SMC for starchy seeds up to about 18%, storage fungi grow actively and destroy seed viability.

In this study seed moisture content of wheat seedlots 4 and 5 was 15.8-15.9% SMC. During subsequent storage seeds are likely to be susceptible to fungal growth resulting in rapid deterioration. The moisture content of wheat seedlots 1, 2 and 3 (12.9-13.5%) and all soybean (10.1-10.5%) seedlots were considered to be relatively safe for appropriate storage. Christensen and Kaufmann (1969) suggest that seeds whose moisture content is below that in equilibrium with a relative humidity of 65% (about 12.5-13.5% for wheat and 9.3-11.2% for soybean) should be safe from invasion by storage fungi.

Seeds should be tested using the standard germination before sowing in order to reduce the loss of income and time which will result from seeds with low viability. Seeds with high germination percentage have high capacity to develop into normal plants when grow in soil under favourable conditions (ISTA,1993). The percentage germination, associated with high levels of abnormal seedlings and/or dead seeds. These seedlots are worthless for sowing and storage.

Seedlings are classed as abnormal when any of the essential structures are irreparably damaged, producing deformed or decayed seedlings, and can occur as a result of mechanical injury, adverse environment, poorly timed and executed and disease (ISTA,1979). The characteristic appearance of abnormal seedlings can often be used to detect the type of pretesting treatment seed has received and to provide some information on likely seed storage potential. In this study the abnormal seedlings found in seedlots with high germination percentage may have resulted from seed

processing damage. Large seeds such as soybean are particularly susceptible to mechanical damage resulting in the occurrence of higher levels of damaged (abnormal) seedlings. Most of the abnormal seedlings found in wheat seedlot 4 showed primary root decay which may indicate fungal infection as a result of relatively high SMC whereas seedlot 5 produced abnormal seedlings which showed no root decay but were deformed as a result of seed ageing

McDonald (1980a) has noted that the germination test does not indicate complete seed deterioration or quality while the standard germination test can provide useful information when it is combined with one or more vigour tests TeKrony (1973) has suggested that because the results of vigour tests provide more precise estimates of likely field emergence they may often provide more meaningful information to growers by allowing them to make more informed decisions on seedlot selection and time of planting.

In this study the accelerated ageing test and the conductivity test were used to test seed vigour levels for wheat and soybean. The seedling growth test was also used for wheat.

The accelerated ageing (AA) test is used to measure seed vigour in high germinating lots under induced environmental stress (high temperature and high relative humidity) which influence seed deterioration. Seeds are placed in an incubator maintained at 41°c 72 hours for soybean or 42°c 48 hours for wheat and 100%RH. Temperature and durations vary with seed type. This test estimates the likely longevity of different seedlots in storage (Delouche and Baskin 1973). Low vigour seeds are more sensitive than high vigour seeds to stress factors and the predictability of plant population from calculated seed rates becomes uncertain. In this study those wheat seedlots and soybean seedlots, with high germination also provided high vigour performance under AA. This suggests such seedlots are likely to be more tolerant of adverse field environments and are the lots which should be selected for sowing in poor soil conditions as suggested by Tomer and Maguire (1990). After the ageing period, wheat seedlots 4 and 5, and soybean seedlot 2 were intolerant of the stress

conditions imposed by AA and produced large numbers of abnormal seedlings and dead seeds, clearly revealing their low vigour status.

The conductivity test measures the levels of electrolyte leakage from seed tissue when seeds are steeped in water. Poor membrane structure and solute leakage cell are usually associated with deteriorating, low vigour seed. The amount of leachate in soybean seedlot 2 was much higher than from other seedlots, reinforcing the low vigour status of this lot shown in the AA test. This supports the statement by Matthews and Bradnock (1967) suggesting that low vigour seeds are weak seeds which generally possess poor membrane integrity and structure resulting in greater electrolyte loss and higher conductivity measurements.

In this study the conductivity readings for wheat was not always well correlated with germination and AA results. For example seedlot 5, showing low germination also provided a lower electrical conductivity of seed than seedlots 1, 2 and which had high germination. These results agree with findings by Rudrapal and Basu (1982) who showed a high negative correlation between germinability and electrical conductivity of seed leachate under both natural and accelerated ageing condition in this species. Similarly, evidence from the study by Nath (1991) suggests that electrical conductivity changes may not be a reliable test of vigour in wheat seeds.

Seed coat damage, which cannot be seen by visual examination, can be revealed by staining seed in ferric chloride solution. In this study the ferric chloride test was used for soybean. Staining seeds from all seedlots provided an indication that mechanical damage was present in all seedlots. Despite this, damaged seeds may produce normal seedlings or abnormal seedlings depending on the part of seed structure which has been damaged and the extent and severity of damage.

Seed health testing is important to determine the health status of seed before sowing or storage in order to provide information on the need to control disease or avoid diseased seeds being stored (Neegaard,1975). Seed health tests on wheat and soybean seed in this study was for fungal detection. Field fungi were observed to be common in both species, particularly *Fusarium* and *Alternaria*. Common species of *Aspergillus* and *Penicillium* fungi were also found in both species but levels of

occurrence varied between lots and storage conditions. However seedlots of both species which were heavily infected by field fungi generally maintained high viability and vigour while the quality of seedlots heavily infected by storage fungi was low with the exception of wheat seedlot 5. Soybean seedlot 2 was invaded by high levels of both field fungi and storage fungi. Again, however the effect of storage fungal infection on seed viability and vigour was much more severe than field fungal infection.

Effect of storage performance on the wheat and soybean

Levels of relative humidity and temperature in the storage environment are the most important factors affecting the maintenance of seed viability (Owen 1956; Barton 1961; Christensen and Kaufmann 1969; Delouche et al.1973 and Justice and Bass 1978). In this study, seed stored in open storage at high temperature (30°c) and high relative humidity (95%) rapidly and drastically deteriorated and all seed died after 4 months for wheat and 2 months for soybean under lower relative humidity or lower temperature storage conditions, however, seeds retained survival. This clearly suggests that storage of seeds under high temperature and relative humidity rapidly increases the rate of deterioration which agrees with similar reports by Copeland and McDonald (1995) that high relative humidities increase seed moisture content, which results in intensified deteriorative biochemical events. High temperature also hastens the deterioration of high-moisture seeds by increasing the metabolic activity of hydrolysed substrates and enzymes, causing rapid loss of viability. Similarly, Delouche et al.(1973) found that interactive effects of high temperature and relative humidity in storage cause rapid deterioration in seed quality and consequent storability in subtropical and tropical environments.

Seed moisture content in open storage changed according due to the relative humidity. Seeds are hygroscopic and lose or gain moisture from the atmosphere until they reach an equilibrium point (Delouche 1968b). The moisture content of seed in open storage (muslin cloth bag) rapidly increased under 95%RH and slowly decreased under 50%RH to reach equilibrium moisture content. At 75%RH seed with low initial moisture content gained moisture from the ambient air while seed with high initial moisture content lost moisture until it reach equilibrium moisture content. The results also support and similar findings by Harrington (1972a) who reported that at lower

storage temperatures the SMC in equilibrium with a given RH is slightly higher because the energy of the water molecules is less at lower temperature and fewer escape the attractive forces of the macromolecule. It is therefore evident that the SMC in equilibrium with a given RH may have two values, one when the seed absorbs moisture and another when it is drying out (Harrington 1972a; Hunt and Pixton 1974).

The rate of increase in seed moisture content under 95%RH in open storage was fast. This is because of the decreasing magnitude of differences in vapour pressure existing between the seed and the storage environment. The level of equilibrium moisture content of both wheat and soybean seed stored at 95%RH in the present study was higher than the equilibrium value given by Justice and Bass (1978) at this level of RH (18.8%). This elevated equilibrium moisture content may be due to the metabolic water produced by fungi and increased seed respiration at higher moisture contents as shown by Harrington (1963) and Christensen (1972). All seeds in open storage under high humidity were heavily infested by fungi.

Wheat seed absorbed more water than soybean seed. This is not surprising since Harrington (1972a) and Neegaard (1977) have both stated that equilibrium moisture content of different types of seed will be different at the same relative humidity due to differences in their chemical composition. A seed of low lipid content (eg wheat) will therefore have a higher moisture than a high lipid seed (eg soybean) when kept in equilibrium with the same level of relative humidity.

The moisture content of both wheat and soybean seed stored in sealed containers remained essentially constant. This proves that aluminium foil pouches are efficient moisture proof containers when heat sealed. The air around the seed inside a sealed container contains a very small amount of water compared with that present in a larger volume of seed. As a result although relative humidity and seed moisture content the seed moisture come to equilibrium the seed moisture essentially does not change even though the storage temperature changes (Harrington 1973).

Under open storage at 30°c 50%RH and 20°c 75%RH equilibrium seed moisture content of wheat seed was 8.2 - 8.5% and 12.8 - 13.6% respectively . These results are in the range acknowledged as being suitable short term storage of this species. Delouche et al (1973) have recommended a maximum safe moisture content

of 13%SMC for cereals and 9.5%SMC for oily seeds for successful short term storage (1 - 9months). SMC in both wheat and soybean seeds stored at 20°c 75%RH slightly higher than this range. However, even this storage environment did not greatly affect viability and vigour. Under these conditions seeds maintained high germination during 8 months storage.

Initial wet seeds stored in moisture-proof containers at 30°c gradually deteriorated over time whereas initially dry seeds in moisture proof containers maintained high germination. These results show that wet seeds in moisture-proof containers remain high in moisture. Particularly if they are stored at high temperatures, enhanced seed respiration rate and the activity of micro-organism, create a more rapid rate of seed deterioration (Harrington 1973; Justice and Bass 1978; Copeland and McDonald 1995). Conversely, the germination results for dry seeds stored in sealed containers with findings by Delouche et al. (1973) and Harrington (1973) that the 'safe' seed moisture range for packaging seed in sealed containers is between 6 and 12% for starch seeds such as wheat and between 4 and 9% for oil seeds such as soybean. They agree that seed in these moisture ranges will store satisfactorily even at warm ambient temperatures.

Temperature is the second most important factor affecting seed performance in storage. The effect of temperature on seed viability was most clearly evident in sealed storage treatments where seed moisture content did not alter despite difference in storage temperature (30°, 20°c). Although soybean seed stored at 30°c at initially high and low moisture contents lost viability faster than seeds stored at 20°c - the rate of deterioration was slightly faster in initially wetter samples. In wheat stored at only initially wet seeds lost viability whereas other treatments retained high germination. These results show the interaction effect which occurs between SMC and temperature, and supports previous statements that seed deterioration leading to loss of germination progresses more rapidly under higher water content and temperature (Harrington 1973; Nandi, Mondal and Nandi 1982).

Seed deterioration generally begins immediately after seed has reached physiological maturity. Symptoms of deterioration seed can be observed in their

increased leachate content when soaked in water which is measured for electrical conductivity. In open storage under high temperature and humidity (30°c 95%RH), the conductivity values obtained for both wheat and soybean seeds increased rapidly. Under lower humidity at the same temperature, however, the level of seed leachate did not obviously increase. In sealed storage at high temperature leachate conductivity of seeds with high SMC gradually increased with storage time. These results show that deterioration rate in both species is accelerated by high SMC/RH relationships. Other workers eg Koosta and Harrington (1969); Petruzzelli and Taranto (1984) have reached a similar conclusion and reported that the decline in phospholipids (the major membrane component) occurs only under conditions of high humidity because phospholipids are hydrolytic enzymes which function most actively under high SMC conditions. A decrease in phospholipid is associated with increasing leakage of all membranes, resulting in seed deterioration (Priestley and Leopold 1983). Similarly, in soybean seed stored under open storage the conductivity increase was comparatively greater at 20° c 75%RH than at 30°c 50%RH despite temperature being higher in the latter. This was obviously a SMC response, supports the previous findings of Stewart and Bewley (1980). They found that the higher the seed moisture content the faster the phospholipid damage. However, in soybean there was a slightly increase in conductivity values with increasing time of storage in initially high moisture content seed stored under cooler (20°c) sealed storage conditions. This conductivity increase shows deteriorating membranes of seedlots are an early event in seed viability loss as also suggested (Petruzzelli and Taranto 1984). However, the conductivity results for wheat seed did not clearly show seed vigour differences, a fact similarly reported by Nath (1991).

In soybean seed in sealed storage at high temperature, increases in the permeability of cellular membranes are likely to have occurred faster than at low temperature, particularly in seeds stored at a high initial moisture level. This is likely since it is well known that high temperature accelerates seed deterioration rate, particularly in combination with high SMC.

Conductivity values of seeds stored under high temperature and RH were high. In addition to the direct effects of adverse storage conditions, the presence of

microbial toxins may also cause membrane damage (Wheeler 1978), resulting in an increase leachate. In this study the results agree with observations by Harman and Granett (1972) and Keeling (1974) who have all observed that fungal deterioration of seeds increases solute concentration of seed leakage, apparently as a result of damage to cell membranes.

Conductivity results obtained after one month storage of soybean seed were higher than in other months. Since seed moisture contents were not adjusted to higher levels these results should be treated with caution. Unreliably high results are likely to have been obtained at this time since low initial seed moisture (<10%) has been shown to significantly increase conductivity readings in soybean seedlots due to imbibition damage. For this reason seed moisture must be determined prior to conductivity and, if necessary, increased to above 10%SMC (Loeffler et al.1988; Eua-umpon 1991; Hampton et al 1992). This procedure was followed in subsequent tests. In addition to SMC, factors known to directly influence conductivity test results include ion content of the soak water, seed soak temperature, length of the soak period, temperature at evaluation and seed size (Matthews and Bradnock 1968; Bradnock and Matthews 1970; Tao 1978; Loeffler et al 1988).

From the results of both germination and conductivity tests it is obvious that the rate of seed deterioration in soybean seed is faster than in wheat seed during storage. According to Delouche et al (1973) and TeKrony et al (1993) soybean seed has a reputation for being an inherently poor storer and is notorious for losing viability rapidly when stored under ambient conditions in the humid tropics.

In this study difference seedlots performed differently during storage. For example, soybean seedlot 3 deteriorated faster than seedlot 1 despite the fact that these two lots were of similarly high initially quality. This may reflect genotypic differences or differences in pre-storage history. This is a feature of seed storage performance which has been previously highlighted by Ellis et al (1982).

Field fungi in both species were eliminated in storage under high temperature (30°c) and high humidity (95%RH) conditions. This does not agree with Christensen's (1972) suggestion that field fungi are only capable of growing in seed

with a moisture content in equilibrium with a relative humidity of at least 90 - 95%. Under these storage conditions, however, high levels of storage fungi developed. Possibly in the presence of storage fungi, field fungi are eliminated because of competitive relationships between the microorganisms involved.

The presence of storage fungi was deletious to seed viability in open storage at high humidity and temperature. This was not surprising since Harrington (1973) has reported that seed storage at higher humidities causes rapid decay of most seeds through fungal infection and accelerated metabolic processes. Also storage fungi have been known to synthesis which kill the living tissues of seed (Copeland and McDonald 1995). The main species of storage fungi found under high temperature and humidity storage conditions were *Aspergillus ochraceus* and *A. flavus* in wheat and including *A. glaucus* in soybean. Moisture content of both wheat and soybean seeds rose to above 18% in response to the storage environment. According to Christensen and Sauer (1982), this moisture content level is higher than the lower limit of moisture needed for the growth of these storage fungi in seed.

Wheat seeds without initial storage fungal infection and stored under open storage at 30°c 50%RH or 20°c 75%RH remained essentially free from storage fungi during storage. This situation also occurred in soybean where seedlots 1 and 3 were initially infected with low levels of storage fungi and were subsequently disinfected in storage. Soybean seedlot 2, however, had a higher initial storage fungal infection level than the other seedlots and deteriorated more rapidly. This suggests that seedlots with low initial quality are more susceptible to storage fungi infection. Christensen and Kaufmann (1969) have reported that storage fungi cannot grow and reproduce on seed in equilibrium with a relative humidity of less than 65 - 70% and drying seed to a moisture content in equilibrium this range of humidity and maintaining moisture content at that level during will overcome potential storage fungi problems. In the present study, storage fungi did not appear to have an important role in loss of seed viability other than at 30°c 95%RH. The loss of viability of seeds stored under drier humidities (50 - 75%) appears to have occurred due to physiological and biochemical changes rather than as a response to microbial effects.

At 30°c 50%RH or 20°c 75%RH the gradual death *Fusarium* during storage was faster than that of *Alternaria*. Perhaps this reflects different thermal sensitivity and dessication tolerance in these two field fungal species.

In sealed storage a general reduction in fungal infection in both wheat and soybean seeds occurred with increasing time of storage. In 14%SMC wheat seeds stored at 30°c 50%RH , field fungi died after 4 months, presumably as a result of anoxia and an accumulation of carbon dioxide in the sealed storage container. The fungi found in this study are all aerobes and their reaction to sealed storage was identical to that found by Loewer et al (1994) who noted that in a hermitically sealed space aerobic microorganisms do not grow under oxygen-free (anaerobic) conditions. The same situation occurred in initially wet wheat seeds kept in sealed containers at 20°c 75%RH but the rate of death of field fungi was slower than at the higher temperature possibly because seed respiration rate was lower and anaerobic conditions did not occur until later in the storage period.

CONCLUSION

The storage potential of seed is affected by seed quality at the time of entry into storage, depending on its pre-storage history. It is therefore necessary to determine pre- storage quality by using different laboratory methods. The results show that high quality seed stores better than low quality seed (particularly in soybean).

In the storage study the results show that the interactive effect of high humidity or SMC and high temperature enhance deterioration in both wheat and soybean, but at a more rapid rate in soybean.

Under favourable storage conditions of 20°c - 75%RH, neither SMC nor packaging method affected seed viability during storage. At 20°c - 75%RH it is not necessary to store either wheat or soybean seed in sealed packages with an accompanying cost saving.

Seeds are hygroscopic. It is therefore only seed storage under high ambient relative humidity conditions that make sealed containers necessary to prevent seed moisture increases. Although under sealed storage at high temperatures the moisture content of seed should be low, storage temperature does not have a significant effect on seed viability under these conditions. This makes moisture content a much more significant priority in seed storage system than the control of temperature.

Loss of viability in both wheat and soybean seed at 30°c and high moisture content correlated well with increased conductivity readings, suggesting that viability loss is related of membrane ability to re-organise during imbibition.

Field fungi such as *Fusarium* spp. which dominated in wheat seed and *Alternaria* spp. were found to be unimportant in affecting seed longevity in storage. Storage fungi, which developed during storage were mainly *Aspergillus* spp. and a few of *Penicillium* spp.. In this study fungi were not main determinants of seed deterioration. However, they can become particularly abundant in storage environments which combine the high temperature and humidity conditions which favour their development and survival.

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Appendix 1 Effect of seedlot, SMC, packaging, temperature and relative humidity on abnormal seedlings of wheat seed after 1, 2, 4, 6 and 8 months storage

Lot	Target SMC level	Initial quality	% Abnormal Seedling																						
			30°c 95%RH		30 °c 50 %RH										20 °c 75%RH										
			Open		Open					Sealed					Open					Sealed					
			2	4	1	2	4	6	8	1	2	4	6	8	1	2	4	6	8	1	2	4	6	8
1	10%	4	0	0	4	4	4	5	3	3	1	2	5	3	2	2	2	5	2	3	2	4	5	3
	14%	4	2	0	5	3	2	5	2	4	7	8	23	4	3	6	3	4	3	6	4	2	4	5
2	10%	3	2	0	6	5	0	6	3	3	2	2	2	1	2	2	2	2	2	6	4	2	5	2
	14%	3	3	0	7	3	3	5	2	7	5	19	15	2	2	5	1	3	2	6	4	3	4	4
3	10%	4	4	0	4	3	1	4	2	5	4	2	3	2	2	5	0	3	2	3	3	3	4	2
	14%	4	1	0	3	4	2	4	1	3	8	12	0	0	9	2	1	3	2	4	2	3	2	9

Appendix 2 Effect of seedlot, SMC, packaging, temperature and relative humidity on dead seed of wheat seed after 1, 2, 4, 6 and 8 months storage

Lot	Target SMC level	Initial quality	% Dead Seed																						
			30°c 95%RH		30 °c 50 %RH										20 °c 75%RH										
			Open		Open					Sealed					Open					Sealed					
			2	4	1	2	4	6	8	1	2	4	6	8	1	2	4	6	8	1	2	4	6	8
1	10%	0	96	100	1	0	1	0	1	0	1	0	1	0	1	1	1	0	0	2	1	0	1	0
	14%	0	94	100	0	2	0	0	1	1	0	5	24	95	0	0	0	0	1	1	0	0	1	1
2	10%	2	91	100	1	2	3	1	2	1	2	0	0	2	1	4	1	3	2	2	3	1	1	3
	14%	2	96	100	0	1	2	0	2	2	2	17	70	98	1	3	2	0	1	3	1	2	2	2
3	10%	4	90	100	1	2	2	4	5	4	4	6	5	5	3	1	3	4	2	2	3	3	5	3
	14%	4	91	100	3	4	2	5	5	6	6	61	97	100	2	4	2	4	2	3	4	3	6	5

Appendix 3 Effect of seedlot, SMC, packaging, temperature and relative humidity on abnormal seedlings of soybean seed after 1, 2, 4, 6 and 8 months storage

Lot	Target SMC level	Initial quality	% Abnormal Seedling																						
			30°c 95%RH		30 °c 50 %RH										20 °c 75%RH										
			Open		Open					Sealed					Open					Sealed					
			1	2	1	2	4	6	8	1	2	4	6	8	1	2	4	6	8	1	2	4	6	8
1	8%	13	16	0	16	12	15	26	17	14	9	13	19	19	13	15	11	16	20	14	11	14	12	15
	12%	13	10	0	17	12	12	19	18	17	15	19	13	5	13	11	11	10	20	16	12	12	15	22
2	8%	25	0	0	19	19	16	24	19	16	15	17	21	12	23	22	25	26	24	18	23	17	19	23
	12%	25	0	0	17	16	18	20	16	22	22	2	0	0	16	20	19	21	20	21	21	22	26	13
3	8%	15	-	-	14	15	16	20	21	12	15	12	19	21	12	10	5	8	13	16	14	10	8	9
	12%	15	-	-	18	20	15	26	18	17	24	27	2	0	18	10	8	14	11	11	12	8	10	15

Appendix 4 Effect of seedlot, SMC, packaging, temperature and relative humidity on dead seed of soybean seed after 1, 2, 4, 6 and 8 months storage

Lot	Target SMC level	Initial quality	% Dead Seed																						
			30°c 95%RH		30 °c 50 %RH										20 °c 75%RH										
			Open		Open					Sealed					Open					Sealed					
			1	2	1	2	4	6	8	1	2	4	6	8	1	2	4	6	8	1	2	4	6	8
1	8%	6	72	100	0	0	2	0	2	1	7	0	2	2	0	1	0	0	0	1	0	3	1	1
	12%	6	78	100	0	1	2	3	1	1	1	22	75	90	0	0	1	1	0	2	0	1	1	1
2	8%	52	100	100	56	56	70	66	70	57	62	63	68	76	46	47	47	59	64	51	47	56	61	64
	12%	52	100	100	58	60	68	71	77	59	71	97	100	100	52	55	55	62	68	55	51	63	62	77
3	8%	0	-	-	1	2	1	1	5	0	0	1	2	3	0	1	1	0	1	1	0	0	0	0
	12%	0	-	-	0	2	2	1	5	0	4	58	96	100	0	0	0	0	1	0	0	0	2	4