DISSERTATION

DETERMINATION OF OPTIMUM STORAGE CONDITIONS FOR ORTHODOX SEEDS

Submitted by

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY JIAN FANG ENTITLED: DETERMINATION OF OPTIMUM STORAGE CONDITIONS FOR ORTHODOX SEEDS, BE ACCEPTED AS FULFILLING, IN PART, REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

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ABSTRACT OF DISSERTATION

DETERMINATION OF OPTIMUM STORAGE CONDITIONS FOR ORTHODOX SEEDS

Plant genetic resources play a vital role in the breeding of new crop varieties. Consequently, maintenance of plant biodiversity has become a global concern. Storage of orthodox seeds in long-term genebanks is a simple and cost-effective means to conserve germplasm. Seed moisture content (MC) and temperature (T) are considered the most important factors controlling seed longevity during storage. Obtaining a better understanding of the interaction of these two factors, along with storage time, is the principle objective of this study.

Seeds of cucumber, lettuce, maize, onion, pea, and watermelon were equilibrated over H_2SO_4 (>99.5% concentration, 1% RH) and 11 different saturated salt solutions (5.5-93% RH) at temperatures from 5 to 50 °C to determine the interactive effect of relative humidity (RH) and T on MC. Best-fit subset models were selected from a complete third order model having the form $MC = \beta_0 + \beta_1*RH + \beta_2*T + \beta_3*RH^2 + \beta_4*T^2 + \beta_5*RH*T + \beta_6*RH^3 + \beta_7*T^3 + \beta_8*RH*T^2 + \beta_9*RH^2*T$, using Mallows' minimum Cp as the selection criterion. In addition Mallow's graphical method was used

to minimize bias. All six best subset models had the same functional form, $MC = \beta_0 + \beta_1*RH + \beta_2*T + \beta_3*RH^2 + \beta_5*RH*T + \beta_6*RH^3 + \beta_9*RH^2*T$ Coefficients had essentially the same respective values among all species except onion and pea, for which some coefficients were statistically different among species ($P \leq 0.05$). All models indicated that seed MC increased as RH increased and decreased as T increased but RH had the greater influence. The inverse relationship between seed MC and T, although slight, was evident in the response surfaces generated from the selected subset models. The interaction effect of RH and T on MC was significant at $P \leq 0.001$. These results suggest that orthodox seed species respond similarly to T and RH to optimize seed MC. This in turn suggests that a common model could be developed and used for optimizing seed storage environments.

A second experiment involved lettuce and soybean seeds equilibrated over P_2O_5 (1% RH) and 11 different saturated salt solutions (5.5% to 93% RH) in desiccators at T of 5 to 50°C to determine the interaction of storage duration (D), T, and RH on seed quality and to develop models for elucidating over-drying damage, optimum moisture level and accelerated aging at high MC. The best-fit subset models were selected from a complete third order model having the form germination (G) or vigor (V) for lettuce or soybean, $G \text{ (or V)} = \beta_0 + \beta_1*D + \beta_2*T + \beta_3*R + \beta_4*D*T + \beta_5*D*R + \beta_6*T*R + \beta_7*D^2 + \beta_8*T^2 + \beta_9*R^2 + \beta_{10}*D^2*T + \beta_{11}*T^2*D + \beta_{12}*D^2*R + \beta_{13}*D*R^2 + \beta_{14}*T^2*R + \beta_{15}*T*R^2 + \beta_{16}*D*T*R + \beta_{17}*D^3 + \beta_{18}*T^3 + \beta_{19}*R^3$. All best-fit subset models had different

functional forms. The selected models, expressed as three dimensional mesh graphs, showed accelerated deterioration in both species when they were dried to extremely low MC. Relative humidity optima were readily apparent in both species during storage. Reduction in germination and vigor increased with storage duration. Optimum RH for seed storage of both species was about 20-22% resulting in maximum germination and vigor for any storage period. Based on the models and model-generated response surfaces, D, T, RH, and their interaction are all important factors which determine seed longevity and a better understanding of their interaction has been presented.

A third experiment involved lettuce and soybean seeds equilibrated at approximately 1% (over P_2O_5), 25% (over KAc) and 75% (over NaCl) RH at 35 °C to determine the physiological nature of deteriorative reactions occurring at low MC and their effects on seed quality during storage. Five grams of lettuce seeds were stored in gas-tight bottles filled or not filled with N_2 at 35 °C after equilibration. Seed MC, germination percentage, vigor (root length), volatile production, leachate conductivity, and dehydrogenase activity were assayed at approximately two month intervals during storage. This experiment showed maximum seed germination and vigor during storage when lettuce and soybean seeds were stored at optimum MC (4-5%). Storage at high MC caused reduced germination and vigor. Also, storage at extremely low MC resulted in reduced germination and vigor. However, seed germination and vigor were improved greatly at extremely low MC when lettuce seeds were stored in N_2.

In conclusion, greater deterioration was observed not only at high MC but also under over-dry conditions. The detrimental effect was minimized when seeds were stored at 20-22% RH. These equations can be used as a guide in estimating levels of deterioration of orthodox seeds during storage periods given storage T and RH.

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CHAPTER 1

INTRODUCTION

Sustainable agricultural productivity depends on the availability of a broad and diverse source of germplasm for breeding. Consequently, maintenance of plant biodiversity has become a global concern. Long-term preservation of genetic resources is the mission of genebanks such as the USDA, Agricultural Research Services's National Seed Storage Laboratory located in Fort Collins, CO. In a genebank, seeds are stored under carefully controlled environmental conditions. Seed deterioration during storage must be minimized to ensure the genetic integrity of the sample. However, the optimum moisture content for seed storage is still not well known. The theoretical principles that guide the choice of storage conditions for each species continue to be a matter of some debate (Ellis *et al.*, 1988, 1989; Vertucci and Roos, 1990, 1993; FAO/IPGRI, 1994; Vertucci *et al.*, 1994). Moisture content (MC) and temperature (T) are the two most important factors in maintaining seed viability and vigor during storage, but the exact relationship between these variables was not precisely determined (Roberts, 1960; Justice and Bass, 1978). The seed viability equation relating seed survival and storage duration, and T and MC has been quantified by Ellis and Roberts (1980) on the basis of storage experiments under very warm (> 50°C) or very wet

(20% mc) conditions. They found the relationship between water content and the rate of seed deterioration to be logarithmic. Accordingly, there is a low MC limit (critical MC) for seed storage. Drying seeds below the critical moisture level reportedly had no further effect on longevity (Ellis *et al.*, 1989). However, detrimental effects of drying seeds to extremely low water content (below 0.01 g H_2O/g dw) were found in several laboratories (Nutile, 1964; Nakamura, 1975; Carpenter and Ostmark, 1988; Carpenter and Boucher, 1992; Vertucci and Roos, 1990, 1993; Vertucci *et al.*, 1994). Therefore, there is an optimum moisture level for seed storage (Vertucci and Roos, 1990, 1993; Vertucci *et al.*, 1994). Seed vigor is lost more rapidly when MC is above or below the optimum level. The conclusion of this debate awaits a basic understanding of the effects of storage environment on physiological and biochemical processes controlling seed quality.

The purpose of the following study was to determine 1) relative humidity (RH), T, and the interaction of these two factors on seed MC; 2) storage duration (D), T, RH and the interaction of these three factors on seed quality; 3) the nature of deteriorative reactions occurring at low MC and their effect of those reactions on physiological and morphological indices of seed vigor. Models were developed which predict the interaction of storage RH and T on seed MC and interaction of D, T, and RH/or MC on the rate of seed deterioration. The results of these experiments provide insight into the mechanism of damage when seeds are over-dried. Furthermore, understanding mechanisms of deterioration under different storage conditions is necessary in order to optimize seed MC which minimize these reactions. This may form a basis for formulating recommendations for optimum seed storage under a wide array of conditions.

CHAPTER 2

LITERATURE REVIEW

Orthodox and recalcitrant seeds

The most common form of germplasm preservation is storage of seeds. Seeds can be classified as orthodox and recalcitrant depending on their behavior in storage. Successful storage depends upon understanding the two major categories of seeds. Orthodox seeds (desiccation tolerant) can be dried to low moisture content (MC) and tolerate freezing temperatures (T). Recalcitrant seeds (desiccation sensitive) cannot be dried below a critical MC (e.g. 30% MC) and can not tolerate freezing T (Chin and Roberts, 1980).

Orthodox seeds include most of our agricultural plant species such as wheat, rice, and maize and many other wild species. Orthodox seeds have a longevity of several years, decades, or even centuries. The bulk of the species preserved in genebanks have orthodox seeds.

Recalcitrant seeds are often produced by aquatic species, tropical species and some temperate-zone species of trees such as coconut, mango, oak and rubber (Chin, 1988). Seeds are usually large and the longevity of the seeds is only a few days to several months under natural conditions. Four possible factors may contribute to the short longevity of stored

recalcitrant seeds: desiccation injury, chilling injury, microbial contamination, germination during storage (King and Roberts, 1980).

Orthodox seed storage

Orthodox seeds are generally easy to store and thus remain viable for long periods if they are stored at low moisture content and low temperature. The International Board for Plant Genetic Resources (IBPGR) recommends that seeds be stored at a moisture content between 3 to 7% depending on the species (FAO/IPGRI, 1994). In the USDA-ARS National Seed Storage Laboratory, Fort Collins, Colorado, seeds are sealed in moisture proof containers and stored at -18°C. Seed viability can be maintained for more than 25 years under this condition. A large number of orthodox seed species have been shown to survive storage in liquid nitrogen which should further extend their longevity (Stanwood and Bass, 1981; Stanwood, 1985).

Recalcitrant seed storage

Recalcitrant seeds remain viable for only a short period. Some recalcitrant species including rare and endangered plant species, usually from tropical and/or aquatic plants, produce recalcitrant seeds. A list of recalcitrant species and those suspected of having recalcitrant seeds has been complied by King and Roberts (1980).

Research to develop storage technologies for recalcitrant seeds is under way in laboratories around the world. Some recalcitrant plant species can be maintained as clonal material and propagated vegetatively. Clones usually are maintained in greenhouses or field plantings, but field and greenhouse maintenance requires considerable space and high cost.

However, *in vitro* preservation (Engelmann, 1991), embryo rescue, pollen storage and cryopreservation (Stanwood, 1985) are useful for *ex situ* conservation of recalcitrant species

Seed longevity

Most of the world's agriculture depends on seeds. Since many seeds are not used the first year after production, it is important to store seeds for planting the next season. Seed companies store seeds for only one or two years and therefore can sell high quality seeds to farmers. Otherwise, seed companies would have difficulty in balancing supply and demand if they could not carry-over some seeds for a second season. Genebanks, such as USDA's National Seed Storage Laboratory, desire to store seeds for much longer to preserve valued plant genetic resources for future use.

Long-lived seeds

Seed longevity was defined as the period of time that a seed retains any spark of life (Roos, 1989). Viability of seeds over a prolonged period of time is important to plant succession. Several reports have claimed extreme longevity for seeds of many species. Wheat and barley (mummy wheats) seeds from the tomb of Egyptian kings and princes were erroneously claimed to have survived for thousands of years (Barton, 1961; Justice and Bass, 1978). Viable buried lotus (*Nelumbo nucifera*) seeds were dug out of a layer of peat in a naturally drained lake bed near the village of Pulantian in southern Manchuria (Liaoning Province, China) by Ohga (1923). The seeds were initially dated at 1040 ± 210 years using ^{14}C analysis (Libby, 1951); however, a later analysis on these seeds reported the age at 100 ± 60 years (Godwin and Willis, 1964). In a more recent study Shen-Miller et al. (1995) reported a 1288 ± 271 year old seed of sacred lotus (*Nelumbo nucifera*) from the same site

had been germinated and subsequently radiocarbon dated. The six dated ancient sacred lotus fruits ranged in age from 95 to 1288 years (mean age 595 ±380 years) evidently reflecting their production, deposition, and preservation at varying times during the intervening millennium (Shen-Miller et al., 1995).

A well-documented controlled experiment on the longevity of 23 species of seeds subjected to 100 years of burial in soil is still under way (Beal, 1885). The last report (Kivilaan and Bandurski, 1981) showed three species (*Malva rotundifolia*, Malvaceae; and *Verbascum blattaria* and *V. thapsus*, Scrophulariaceae) remained capable of germination. The six remaining bottles will be dug up over the next 60 years.

Even under good storage conditions seeds will eventually deteriorate and, first lose their vigor, then the ability to germinate normally, and finally all viability (Roos, 1989). The storage potential of an individual seed is influenced by the following factors and conditions.

Genetic factors

Longevities of seeds may encompass years, decades, and in some instances centuries depending on the species and storage conditions. Seeds of different plant species vary widely in their longevity under identical or favorable storage conditions. Seeds of some species are genetically and chemically equipped for longer storability than others under similar conditions (Copeland and McDonald, 1985). Most long-lived seeds belong to species possessing hard, impermeable seed coats. Harrington (1972) reported hard seeded genera (*Albizia*, *Cassia*, and *Trifolium*) can germinate after 100 years. Even within the same species, the seeds from different cultivars stored under a stable environment exhibit considerable variation in longevity. Toole and Toole (1953) reported the bean cultivar Black

Valentine stored better than Brittle Wax.

Moisture content and temperature

Seed MC and T are the most important factors in maintaining seed viability and vigor during storage. The effects of MC and T on seed longevity are highly interdependent during storage. Usually, the storage life of orthodox seeds decreases as MC and T increase. Toole (1950) reported that seeds lose their viability more rapidly at high MC (equilibrated at 80% RH) and T of 25 and 30°C than seeds at relatively low MC (equilibrated at 50% RH) and T 5°C or lower.

For orthodox seed species, longevity can usually be increased by adjusting the water content of seeds and by reducing the storage T. According to Harrington's (1972, 1973) rules of thumb, for each 1% decrease in seed moisture the storage life of seeds is doubled and for each 10°F (5.6°C) decrease in seed storage T the storage life of seeds is doubled. However, there is a limitation for these rules. Seed MC must be in the range of 5-14% (FW) to apply the rules. Loss of seed viability increases more rapidly when seeds are stored at MCs above 14%. Below 5% MC, seed deterioration increased due to the breakdown of membrane structure.

More detailed information about the relationship of seed MC and T are discussed in the Chapter 3 and 4.

Mechanical damage

The physical condition and physiological state of seeds greatly influence their life span. As harvesting and threshing machinery and the combine came into general usage, damage to seeds and grain increased (Justice and Bass, 1978). Seeds that have been broken,

cracked, or even bruised deteriorated more rapidly than undamaged seeds. Even in the absence of physical symptoms, seeds may be physiologically impaired and become susceptible to rapid deterioration.

Seed maturity

Mature seeds generally store better than immature seeds. Many factors including temperature, moisture, variety, and nutrient status influence seed maturity. Maximum storage potential is attained at the time of physiological maturity, or maximum dry weight of the seed (Harrington, 1972).

Many crop species flower, produce, and mature seeds over a period of several days or weeks. It is important to know at what stage of maturity the seeds should be harvested. Pollock (1961) indicated harvesting all carrot umbels at the same time resulted in both mature and immature seeds and the level of maturity affected viability. Nutrient status can also influence seed longevity. Harrington (1960) reported that carrot and pepper plants grown in nutrient solutions deficient in nitrogen, potassium, and calcium produced seeds that did not store well during an eight year period.

Seed aging

Aging in seeds is the sum total of the deteriorative processes that eventually lead to death. Whether stored in soil banks, genebanks, or liquid nitrogen; all seeds succumb with time, deteriorate, and eventually die. Most research on quantification of seed deterioration has concentrated on two aspects, loss of viability and loss of vigor. The factors which determine the rate of aging are the T and MC at which seeds are stored.

In general, loss of orthodox seed viability and vigor is slow when seeds are stored

under genebank conditions in which seeds with 3-7% MC are stored at -18°C (FAO/IPGRI, 1994). To shorten the time required to detect deleterious changes, seed can be tested by exposing samples to artificial aging in which RH (85-100%) and T (35-65°C) are high (Delouche and Baskin, 1973; Priestley *et al.*, 1980; Ram and Wiesner, 1988; Roos, 1989). Artificial aging is divided into accelerated aging and controlled deterioration based on aging method.

Accelerated aging

Accelerating aging of seeds using several days of exposure to T at 35-65°C and approximately 85% to 100% RH has been recognized as a good predictor of storability under non-genebank conditions (Delouche and Baskin, 1973; Priestley *et al.*, 1980; Ram and Wiesner, 1988). The more deteriorated the seeds are due to accelerated aging, the shorter the predicted longevity. Accelerated aging was also recognized as a useful vigor test for some species (Roos, 1989; Chaisurisri, *et al.*, 1993).

Controlled deterioration

Controlled deterioration (Matthews, 1980) is similar to accelerated aging except that seeds are partially imbibed to pre-determined moisture levels (Powell and Matthews, 1981, 1984). A weighed sample of a seed lot of known initial moisture content is checked by frequent weighing during imbibition. To ensure an even distribution of moisture, the seeds are held in a sealed container overnight at 10°C before being sealed in foil packages and incubated in a 45°C water bath for 24 hours (Matthews, 1980). Seed germination is then measured at 20°C.

In both accelerated aging and controlled deterioration, high T and RH induce seed

deterioration. Therefore, mechanisms of seed deterioration would appear to be similar. In the study of deterioration mechanism, it is important to know moisture levels which induce deterioration. In accelerated aging, seed moisture content is continuously changing. The rate of water uptake varies depending on the seed or conditions during accelerated aging. In controlled deterioration, seeds with only one moisture level are stored for a certain duration. Therefore, controlled deterioration is a more precise and useful method of deteriorating seeds than accelerated aging (Roos, 1989), since MC is constant.

There are several physiological and biochemical changes during seed aging (high moisture and temperature) (Ching and Schoolcraft, 1968; Leopold and Musgrave, 1980; Matthews, 1985; Francis and Coolbear, 1988; Gitalidas and Sen-Mandi, 1992). These changes mainly occur in macromolecules resulting in injury to cell membranes, peroxidation reactions, and increase in free fatty acid content.

1. Macromolecules involved

Proteins in seeds act both as storage reserves and as enzymes. They can be divided into four classes on the basis of their solubility: albumins, globulins, glutelins, and prolamins (Bewley and Black, 1986). Several investigators have analyzed seed protein characteristics as a function of storage time. Protein contents in seeds changed during natural (uncontrolled) and accelerated aging (Ganguli and Sen-Mandi, 1990; Aguilar *et al.*, 1992; Fujikura and Karssen, 1992). Fujikura and Karssen (1992) reported that protein synthesis was reduced in aged cauliflower seeds. Comparisons of maize grains stored for 13 years with fresh grains showed that protein synthesis was slow in axes of stored grains (Aguilar, *et al.*, 1992). However, Ganguli and Sen-Mandi (1990) reported acid soluble proteins

increased in wheat embryos in both natural and accelerated aging. These inconsistent results may be due to differences in aging conditions, assay used, and proteins studied.

Many enzymes have been studied in an attempt to correlate seed viability with the amount of enzyme activity. Enzyme activity is often considerably reduced in aged seeds. Decreased amylase activity has been reported in aged wheat (Petruzelli and Taranto, 1990). Livesley and Bray (1991) showed that the enzyme was synthesized at a reduced rate by the aleurone layer in aged wheat seeds. Das and Sen-Mandi (1992) found that amylase activity, which was associated with initiation of seed germination, was higher in unaged wheat grains and lower in both natural and accelerated aged grains. Dehydrogenase activity, which is related to respiration, has been used as a measure of seed vigor and viability (AOSA, 1987; Das and Sen-Mandi, 1992; Legesse and Powell, 1992), since the dehydrogenase are not present in dead seed tissues. Das and Sen-Mandi (1992) indicated that dehydrogenase activity was low in aged wheat embryos. Glutamate dehydrogenase isozyme activity in soybean seeds was reduced by 17% after 48 hr of accelerated aging and almost completely lost after 96 hr (Shatters, *et al.*, 1994). Kalpana and Rao (1993) reported lipoxygenase activity in pigeonpea seeds decreased with accelerating aging. Glutamic acid decarboxylases activity also decreased as a consequence of wheat grain deterioration by accelerated aging (Ram and Wiesner, 1988). DNA polymerase activity decreases by around 50% during germination of accelerated deteriorated maize embryo axes when compared with activity in non-deteriorated axes (Coello and Vazquez-Ramos, 1996).

Physiological expression of seed deterioration, such as reduced germinability and seedling growth, suggests low rates of protein synthesis. Protein synthesis, which is usually

evident well within the first hour of imbibition, is reduced in aged seeds (Bray and Dasgupta, 1976). There is good correlation between RNA and protein synthesis in the deteriorative process. SenMandi and Osborne (1977) reported a decline in all classes of newly synthesised RNA that occured in parallel with a decline in protein synthesis, later during imbibition. Accelerated aging of carrot seeds led to significant losses of polyadenylic acid (poly A) RNA and the content declined still further when aged seeds were to germinate (Thompson, *et al.*, 1992).

Seed viability has been inversely correlated with the frequency of aberrations. An abundance of chromosomal aberrations was observed in root tips of deteriorating seeds (Roberts, 1973; Roos, 1982, 1986). Alteration of the cell's DNA resulting from mutations, causes cells to be unable to duplicate, divide and grow in seeds and seedlings.

2. Cell membranes

Cellular membranes include the plasma membranes that surround cells, and intracellular membranes that surround or support the organelles such as the nucleus, plastids, mitochondria, and endoplasmic reticulum. Cell membranes, as selectively permeable barriers, are composed chiefly of proteins and amphipathic lipid molecules assembled in bilayers: the hydrophobic tails point toward the interior of the bilayer, whereas the hydrophilic heads are in contact with the aqueous solution on each surface (Bewley and Black, 1986; Horton *et al.*, 1992). Phospholipids are composed of glycerol esterified with two long-chain fatty acids (saturated or unsaturated). Most molecules and ions, either because they are too large or because they are charged, cannot enter a cell by simple

diffusion (Horton *et al.*, 1992). However, water is able to diffuse relatively freely across a biological membrane.

Membrane structures appear to be particularly sensitive to aging. Heydecker (1972) postulated that the decline in seed vigor is associated with a weakening of cell membranes. Later, many experimenters reported that cell membrane integrity in seeds is disrupted during seed deterioration (Simon, 1974, 1978; Tao, 1978; Duke *et al.*, 1983; Hoekstra, 1984; Powell, 1986; Simon and Mathavan, 1986; Loeffler *et al.*, 1988; Crowe *et al.*, 1989; Hoekstra *et al.*, 1992). Therefore, membrane damage resulted in a decrease in seed viability and vigor (Simon and RajaHarun, 1972; Simon, 1974; Powell, 1986).

Membrane integrity can be predicted by measuring leachate conductivity in the imbibition solutions. The relationship between seed quality and electrolyte leakage has been demonstrated previously; electrical conductivities of the seed leachates have usually been negatively correlated with vigor and viability (Bonner and Vozzo, 1986; Hampton, *et al.*, 1992; Armstrong and McDonald, 1992). In the past, bulked seeds were used for measuring conductivity following a 24 hr soaking (Simon, 1978; Tao, 1978). Now, conductivity measurement of individual seed leachates is practical and data can be summarized in a manner to predict viability (Steere *et al.*, 1981; Moore, *et al.*, 1988). However, the relationship between imbibitional leakage and deterioration cannot be assumed for all species or all types of deterioration (Powell, 1986).

3. Peroxidative reactions

Peroxidative processes are probable causes of deterioration of seeds during aging. Many unsaturated fatty acids found in seeds are highly susceptible to peroxidative

degradation. Lipid peroxidation not only destroys lipids, but generates a variety of potentially toxic products during natural and accelerated aging (Priestley, *et al.*, 1980; Priestley, 1986).

Membrane deterioration may result from the formation of free radicals by peroxidation and changes in the amount of membrane phospholipids (Senaratna *et al.*, 1985, 1987; McKersie *et al.*, 1988; Hendry, 1993). A free radicle is an atom or molecule with an unpaired electron such that it can accept (or donate) an electron from or to an adjacent molecule (McKersie *et al.*, 1988). Peroxidation of an unsaturated fatty acid yields an organic free radicle when a hydrogen atom is removed from a methylene ($-CH_2-$) group adjacent to a double bond (Figure 1). Increased levels of free radicles were observed in aged soybean seeds (Buchvarov and Grantcheff, 1984). Abstractions of hydrogen from fatty acids causes a decline in lipid fluidity, and the changes in structure resulting from peroxidation inactivate membrane-bound proteins, thus changing membrane permeability (Bewley and Black, 1994). The action of free radicles also results in the inactivation of enzymes, denaturation of proteins and disruption of DNA and RNA (Powell, 1986; Hendry, 1993).

Treating seeds with antioxidants may prevent the damaging effects of aging or enhance the viability of aged seeds according to Basu and DasGupta (1978). Aged soybean seeds have a low antioxidant potential in the lipid fraction and low tocopherol (Senaratna *et al.*, 1988). Jeng and Sung (1994) reported that accelerated aging reduced the activity of peroxide-scavenging enzymes in peanut seeds. However, some authors have indicated lipid peroxidation is not a major cause of loss of viability (Priestley and Leopold, 1979; Priestley *et al.*, 1980). Results on the stability of unsaturated fatty acids in soybean seeds indicates

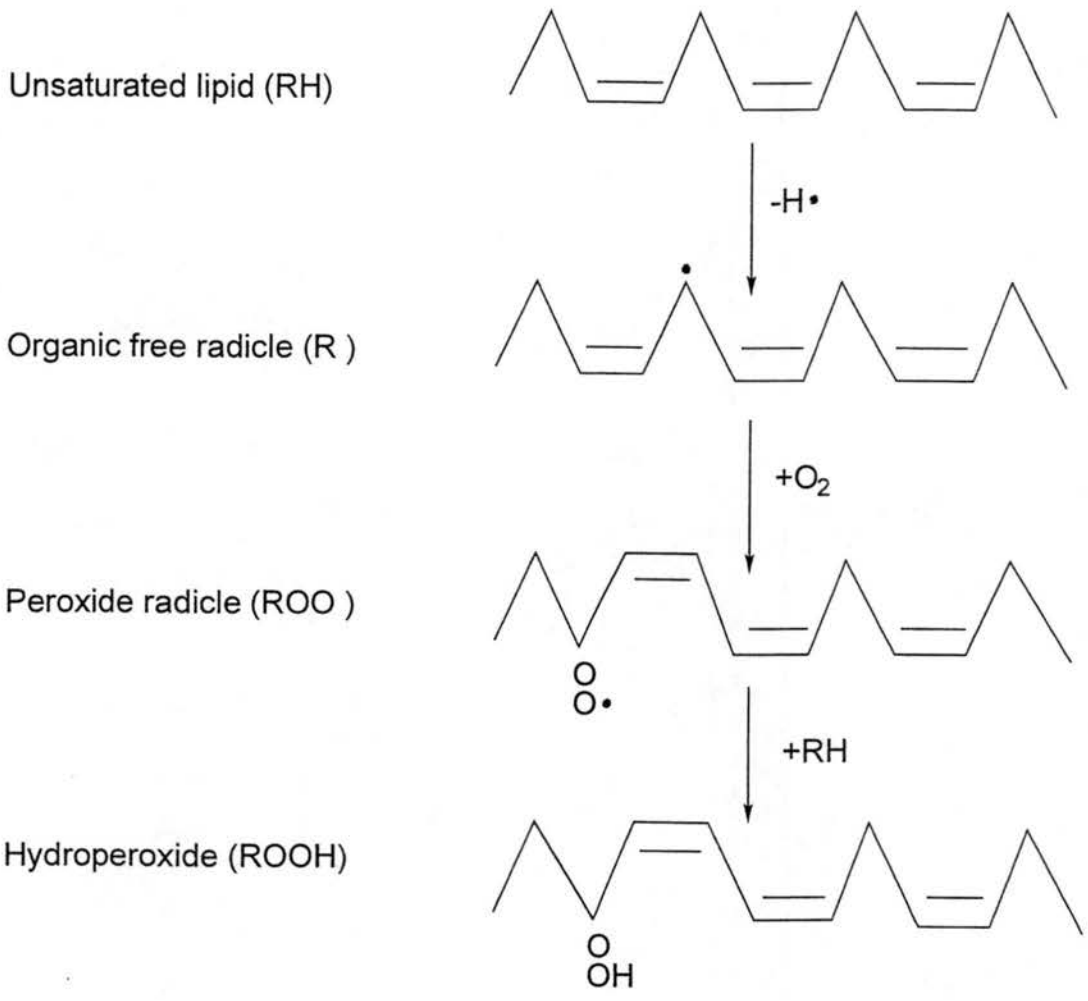


Figure 1. Peroxidation of a polyunsaturated fatty acid (Linolenic) (Priestley, 1986)

that lipid peroxidation is not a significant factor during natural or accelerated aging (Priestley *et al.*, 1980). Vos *et al* (1994) also indicated that lipid peroxidation did not take place since thiobarbituric acid-reactive substances did not increase in aging tomato seeds.

4. Increase in free fatty acid contents

The hydrolysis of esters in seed triacylglycerols yields fatty acids. Elevated levels of free fatty acids are toxic to most cells and are not found in healthy seed tissue. Free fatty acids have particularly deleterious effects on membranes, probably because of their ability to act as detergents (Priestley, 1986). Thus the increase in fatty acids in seeds can be looked at as a symptom of deterioration. Hoffpauir et al. (1947) reported individual cotton seeds containing 1% or more of free fatty acids usually would not germinate. Higher levels of free fatty acids were observed in the microsomal membranes from soybean seeds following aging for 5 years at room T (Senaratna *et al.*, 1988). Elevation of T and MC in stored brown rice grains leads to an increased level of free fatty acids (Hunter *et al.*, 1951).

Fungal invasion is thought to be another cause of breakdown of lipids to free fatty acids (Christensen et al., 1949). Fungal lipases can cause hydrolysis of lipids to fatty acids in stored seeds.

Aging under dry conditions

1. Acquisition of desiccation tolerance during seed development

Seeds are not capable of withstanding desiccation at early stages during development. The acquisition of desiccation tolerance is an important event in establishing the potential autonomy of the seed. Maturation involves drying and is an integral part of the development of most seeds; in fact, development is considered to be complete when the seed has dried.

Subsequent hydration of the mature, nondormant seed leads to germination; hence it is suggested that desiccation plays a role in the switch from developmental processes to those essential for germination (Bewley, 1979; Bewley and Oliver, 1992; Vertucci and Farrant, 1995).

The ability of seeds to tolerate desiccation is a truly remarkable biological phenomenon. The biochemical and physiological mechanism of desiccation tolerance is not well understood. Desiccation tolerance can be induced by osmotic stress during seed development. Wakui *et al.* (1994) reported that osmotic stress caused by high concentrations of sorbitol, induced desiccation tolerance in microspore-derived embryos of Chinese cabbage. Desiccation tolerance may be induced by providing a high level of sucrose (Finkelstein and Crouch, 1987; Anandarajah and Mckersie, 1990). Osmotic stress also caused a decrease in desiccation-induced electrolyte leakage and stimulated the accumulation of LEA (late embryogenic abundant) like proteins (Blackman *et al.*, 1995). Kermode *et al.* (1989) indicated that endogenous abscisic acid (ABA) levels in embryos was increased with osmotic treatment.

The plant growth regulator ABA appears to play an important role in the development of desiccation tolerance (Page-Degivry and Garello, 1991; Farrant, *et al.*, 1993; Ooms *et al.*, 1994a,b; Blackman *et al.*, 1995). ABA has been implicated in the response of vegetative tissues to water stress (Bewley and Oliver, 1992). It is now well-established that ABA levels rise during the mid-stage and decline only during maturation drying of embryogenesis of orthodox seeds (Kermode, 1990; Farrant *et al.*, 1993). This increase in ABA prior to the programmed dehydration of the seed not only prevents the immature embryo from

germinating precociously, but also is apparently involved with the acquisition of desiccation tolerance by the embryo (Kermode, *et al.*, 1989; Kermode, 1990; Page-Degivry and Garello, 1991). ABA levels are negligible in the embryo tissue during maturation of recalcitrant seed of Avicennia marina (Farrant, *et al.*, 1993). Application of fluridone, an inhibitor of ABA synthesis, results in embryos with viviparous characteristics (Xu *et al.*, 1990; Oishi and Bewley, 1992). In addition, double mutants of Arabidopsis which are lacking ABA produce desiccation-sensitive seeds (Ooms *et al.*, 1994b). Furthermore, exogenous application of ABA was effective for the induction of desiccation tolerance in microspore-derived embryos of Chinese cabbage (Wakui, *et al.*, 1994). LEA proteins have been shown to be induced in seedling tissue by ABA (Blackman *et al.*, 1995); thus, it has been suggested that ABA might act as a signal-transducer for the transcription of LEA genes during water stress (Kermode, 1990; Shriver and Mundy, 1990; Ried and Walker-Simmons, 1993).

LEA proteins are produced and accumulated during the late stages of seed development (Blackman *et al.*, 1991; Bradford and Chandler, 1992). These proteins have been implicated in the mechanism of seed desiccation tolerance (Kermode, 1990; Bewley and Oliver, 1992; Vertucci and Farrant, 1995). Blackman *et al.* (1991) indicated the presence of maturation proteins (LEA proteins) was correlated with desiccation tolerance in soybean seeds. Maturation proteins increased at 44 days after flowering, when desiccation tolerance was achieved, and decreased after 18 hours of imbibition, when desiccation tolerance was lost (Blackman, *et al.*, 1991). In addition, the consistent correlation between desiccation tolerance in orthodox seed tissue and an accumulation of LEA proteins suggested that these proteins reduce desiccation-induced cellular damage (Blackman *et al.*, 1995). Farrant *et al.*

(1992) found the patterns of protein synthesis did not change discretely during embryogenesis of recalcitrant seeds of Avicennia marina as was observed in orthodox embryos between the maturation and postvascular separation phase (Galau *et al.*, 1991). However, LEA proteins were detected in recalcitrant seeds of *Acer pseudoplatanus* and *A. saccharinum* (Gee *et al.*, 1994) and intermediate storage behavior seeds of *Zizania palustris* (Bradford and Chandler, 1992). Thus, the expression of LEA proteins alone is not sufficient for tolerance (Blackman *et al.*, 1991; Reid and Walker-Simmons, 1993; Vertucci and Farrant, 1995). It is possible that other factors which interact with LEA proteins are absent from desiccation intolerant tissues (Blackman *et al.*, 1992; Gee *et al.*, 1994; Vertucci and Farrant, 1995).

Carbohydrates might also be involved in membrane protection via glass formation during dehydration (Koster, 1991; Hoekstra *et al.*, 1994; Ooms, *et al.*, 1994a; Sun and Leopold, 1994; Vertucci and Farrant, 1995). Sucrose and larger oligosaccharides were consistently present during the tolerant stage, and desiccation tolerance disappeared as the oligosaccharides were lost in soybean, pea, and corn (Koster and Leopold, 1988). Sucrose may serve as the principal agent of desiccation tolerance, with the larger oligosaccharides serving to keep the sucrose from crystallizing (Koster and Leopold, 1988). Leprince *et al.* (1990) indicated an increase in starch and soluble sugars in relation to the acquisition of desiccation tolerance during maturation of Brassica campestris seeds. Soluble-sugar compositional relationships rather than absolute content may play an important role in membrane stabilization (Chen and Burris, 1990). Loss of desiccation tolerance in the various cauliflower seed parts coincided with an increase in glucose and fructose and the complete

loss of stachyose, but sucrose content was still high (Hoekstra, *et al.*, 1994). The presence of raffinose at certain levels also may be a key factor in protecting maturing seeds from drying damage (Chen and Burris, 1990).

2. Deterioration under dry conditions

The deterioration observed when seeds are dried to extremely low water content has been regarded as an aging phenomenon (Vertucci and Roos, 1990, 1993; Vertucci *et al.*, 1994; Walters, 1998). Seeds aged more rapidly under extremely dry conditions than optimum conditions. However, deteriorative reactions in seeds during storage at very low moisture content are not well known. There have been suggestions that non-enzymic oxidations might occur at low MC and some deteriorative reactions might also be relatively favored by low MC (Vertucci and Farrant, 1995). There is some experimental support for this hypothesis. The strongest is the determination of an optimum MC for seed longevity (Vertucci and Roos, 1990, 1993; Vertucci *et al.*, 1994). That is, seed longevity decreases both at sub- and supra-optimal seed moisture. Numerous experiments have reported the structural and functional importance of water molecules in seeds. Water is a substrate; its concentration affects the equilibrium between reactants and products. It acts as a solvent for enzyme catalytic reactions (Rupley *et al.*, 1983; Leopold and Vertucci, 1989; Vertucci, 1989a, 1990; Bradford, 1994). Altering the water content of tissues is likely to affect the nature and kinetics of these structural changes (Leopold and Vertucci, 1989; Vertucci and Farrant, 1995). These changes mainly result in injury to cell membranes, decreased enzyme activity, increased volatile production, and greater Maillard reaction.

Membrane: Membrane phospholipids are particularly susceptible to structural changes during desiccation and alteration of membrane structure can be particularly damaging. Senaratna *et al.* (1984, 1987) indicated that dehydration prompted a de-esterification of the linkage between glycerol and fatty acid side chains of the phospholipid molecules in the membrane. Thus, an increase in free fatty acid content contributed to dehydration damage. However, Chen and Burris (1991) suggested that alterations in phospholipid molecular species and changes in fatty acid composition to a more saturated composition in maize grain during preconditioning and maturation could be common mechanisms in high-temperature desiccation tolerance.

It is believed that water serves as a "spacer" which limits interactions with highly reactive species such as free radicles or charged moieties. Free radicle induced oxidation may directly cause structural changes of macromolecules or predispose biological materials to structural changes when the environmental conditions are changed (Mckersie *et al.*, 1988; Leprince *et al.*, 1993; Vertucci and Farrant, 1995).

Membrane systems appear to be one of the major sites of desiccation damage (Senaratna and McKersie, 1983). The expression of desiccation damage has been associated with increased free radicle attack and peroxidative damage during water loss (McKersie *et al.*, 1988; Leprince *et al.*, 1993, 1994). The formation of free radicals results from peroxidation (Senaratna *et al.*, 1985, 1987; McKersie *et al.*, 1988; Hendry, 1993; Finch-Savage *et al.*, 1994). In vitro treatment of microsomal membranes from the axes of soybean seeds with free radicles simulates this type of membrane injury (Senaratna *et al.*, 1987). Several significant elements of damage have also been observed in desiccated maize grains

associated with development of desiccation intolerance: increased lipid peroxidation, phospholipid de-esterification, build-up of a stable free radicle, suppression or repression of respiratory enzymes from complex I, II, and IV (Senaratna *et al.*, 1987; Leprince *et al.*, 1994).

Free radical scavenging systems are an important part of desiccation tolerance (Leprince *et al.*, 1993; Vertucci and Farrant, 1995). Scavenging molecules include lipid-soluble antioxidants (tocopherol (vitamin E), β-carotene) or water-soluble ones (Ascorbic acid (vitamin C), glutathione) (Leprince *et al.*, 1993; Vos *et al.*, 1994). In orthodox seeds, free radical scavengers accumulate during maturation and are lost during germination (Koster and Leopold, 1988; Leprince *et al.*, 1990).

Reducing water content increases the likelihood of lipid phase shifts (Crowe *et al.*, 1992; Vertucci and Farrant, 1995). When seeds and pollen are dehydrated, T_m (midpoint of phase transition temperature) for membrane phospholipids increase (Crowe *et al.*, 1989; Hoekstra *et al.*, 1991). As a result, when seeds and pollen are rehydrated, the phospholipids undergo a transition from gel to liquid crystalline phase. Consequently, damage to cellular structure may result (Bramlage *et al.*, 1978, 1979; Woodstock and Tao, 1981; Tilden and West, 1985; Crowe *et al.*, 1989). A rapid leakage into the water occurs when seeds are soaked. Increase in leakage of electrolytes indicates more membrane damage.

Enzyme activity: Reducing water content also increases the denaturation of some proteins including enzymes (Levitt, 1980). The recongnition and repair or metabolism of spontaneously altered proteins may play an important role in limiting the aging process in cells and tissues. One enzyme that appars to play an important role in this process is the L-

isoaspartate (D-aspartate) O-methltransferase active on a variety of peptides and proteins (Mudgett and Clarke, 1993, 1996). Protein carboxyl methyltransferases that catalyze the transfer of a methyl group from S-adenosylmethionine to L-isoaspartyl and D-aspartyl residues in a variety of peptides and proteins are widely distributed in nature. The highest levels of methyltransferase were located in seeds, where the problem of spontaneous protein degradation may become particularly severe upon aging (Mudgett and Clarke, 1993). These enzymes can partcipate in the repair of damaged proteins by facilitating the conversion of abnormal L-isoaspartyl residues to normal L-aspartyl residues. In over dry condition, reduced water content may increase the denaturation of methyltransferases.

Volatile production: Volatile detection provided a powerful tool for monitoring physiological activities occurring in dry seeds (Zhang et al., 1995b). All physiological processes which cause production of volatiles may be monitored with gas chromatography (GC). Meanwhile, water status and physiological reactions in dry seeds were not influenced by headspace detection of volatiles. Volatile detection is a convenient, sensitive and non-destructive technique for detecting seed aging.

Some volatile products of lipid autoxidation and/or peroxidation, such as aldehydes and hydrocarbons, have been used for estimating seed deterioration (Fielding and Goldsworthy, 1982; Hailstones and Smith, 1989; Smith and Adamson, 1989) after lipid autoxidation and /or peroxidation. The free radical reactions were proposed as a mechanism of seed deterioration (Kaloyereas, 1958; Wilson and McDonald, 1986). Other kinds of volatiles, such as ethanol and acetaldehyde, were also proposed to be involved in seed aging

(Zhang et al. 1994, 1995b, 1997). This aging mechanism was suggested to be related to activity of ethanolic fermentation and protein modification by aldehyde-amino reaction.

Methanol, acetaldehyde, ethanol and acetone evolve from seeds during storage causing loss of germinability (Zhang *et al.*, 1994). Furthermore, Zhang *et al.* (1994) indicated that endogenous volatiles, especially acetaldehyde, may be an important factor that accelerates seed deterioration often occurring under lower RHs and/or temperatures throughout long-term storage. In a recent study, Zhang (personal communication) found hexanal production increased at very low seed moisture contents while it was not observed at other moisture levels. However, a reliable relationship between volatile production and seed viability loss was not established (Zhang *et al.*, 1993).

Maillard reaction: The deterioration of seeds during dry storage is a complex phenomenon involving changes in many cellular components. One of these reactions is the Maillard reaction. The Maillard reaction is the non-enzymic condensation of reducing sugars with amino groups of proteins or nucleic acids (Wettlaufer and Leopold, 1991; Baker and Bradford, 1994). Subsequent propagation reactions result in complex and irreversible cross-linked fluorescent structures called "Maillard products". The cross-links impair cell and tissue function by inactivating enzymes and damaging DNA. The accumulation of these products leads to loss of seed viability.

Water controls nature and kinetics of deteriorative reactions

1. Water in seeds

A number of factors affect water uptake of seeds. The concept of water potential (Ψ) as an expression of the free energy status of water can be used to describe imbibition

(Bewley and Black, 1986; Bradford, 1986). The net diffusion of water occurrs down an energy gradient from high (less negative) to low water potential (more negative) and can be expressed by Darcy' law in the following equation

$$\mathbf{Q = K\ (\Delta \Psi_{m\text{-}s})}$$

Where Q is the flow rate in $cm^3\ (cm^2\text{-}s)^{-1}$ or cm s^{-1}, K is the hydraulic conductivity coefficient in cm s^{-1} MPa^{-1}, and $\Delta\Psi_{m\text{-}s}$ is the water potential difference between the seed ($\Psi_{s)}$ and its surroundings (Ψ_m). Ψ_s is the potential of the water in the cell of a seed and can be expressed in the following equation

$$\psi_s = \psi_\pi + \psi_p + \psi_m$$

Where Ψ_π is the osmotic potential or solute potential, Ψ_p is turgor or pressure potential, Ψ_m is matric potential. Values for Ψ_π and Ψ_m are negative and Ψ_p is positive.

The driving force for water uptake is $\Delta\psi_{m\text{-}s}$ (Vertucci, 1989b). Two components comprise the MC gradient: the water concentration or potential exterior to the seed (ψ_m) and the water content or potential (ψ_s) of the seed itself (Vertucci, 1989b). Altering either of these factors will change the driving force for water uptake. The larger the $\Delta\psi_{m\text{-}s}$, the more driving force for water uptake. Dry seeds typically possess an extremely low water potential (-400 MPa) attributed to their osmotic and matric potentials (McDonald *et al.*, 1988). Dry seed provides a large gradient for water uptake when seed is put in pure water (Ψ_m=0). Water uptake decreases when the magnitude of $\Delta\Psi$ decreases during seed imbibition. $\Delta\psi_{m\text{-}s}$ approaches zero when the seed is fully hydrated.

The permeability of the tissue to medium is more important in determining imbibition rates. The hydraulic conductivity coefficient or diffusivity (K) is a component of the flux

equations that account for the permeability of the seed system to water flow (Vertucci, 1989b). This coefficient changes depending on temperature, permeability of the imbibing medium and the seed coat, and seed/medium contact. The contributions of soil permeability and seed/soil contact to the diffusivity coefficient are eliminated if seeds imbibe in water solutions (Vertucci, 1989b) where rate of water uptake is critical to the success of subsequent germination.

Moisture level in seeds can be described as moisture content and/or water activity. Moisture content is a measure of the concentration of water in seeds and is expressed on a relative scale (g water per g fresh or dry weight). Water activity (a_w = RH/100), a proportion that specifies the relative purity of water, measures how many times effective water is at promoting on aqueous reaction at a given MC compared to a standard reference state (Vertucci and Roos, 1993). Water activity describes the tendency for a chemical reaction involving water at a given T and the value of water activity is always ≤ 1 (Vertucci and Roos, 1993).

2. Moisture sorption isotherms

Moisture sorption isotherms describe the relationship between MC and RH for a particular temperature. A method using moisture sorption isotherms to study water binding in seeds has been developed in which seeds are equilibrated under various RH (Leopold and Vertucci, 1986, 1989; Vertucci, 1989a). Isotherms at 5° to 50°C exhibited a reverse sigmoidal shape in pea, soybean, and peanut seeds (Vertucci and Roos, 1993). Equilibrium MC decreased at any given RH as T increased. Water sorption also depended upon seed

composition. Moisture contents are higher in seeds with high starch and lower in seeds with high lipid content (Justice and Bass, 1978).

3. Types of water

Studies of water thermodynamic properties in seeds suggest that there are five types of water (Vertucci, 1990). Vertucci (1990) described five hydration levels in seed tissues by using differential scanning calorimetry to study thermodynamic properties of water in cotyledons of pea and soybean with moisture contents from 1% to 100% (dw). For soybean, the range of moisture contents for the 5 hydration levels were: 0-8%, 8-21%, 22-33%, 33-55%, and above 55% respectively; for pea, they were: 0-12%, 12-24%, 24-35%, 35-58%, and above 58% respectively at ambient temperatures (Vertucci, 1990). The range of MC of seeds changes with T (Vertucci and Roos, 1993; Vertucci *et al.*, 1994).

Various levels of physiological activity are associated with different levels of hydration. Very few enzyme-mediated reactions occur when seeds are dried to within the first hydration level, however, light reactions and reactions occurring in the lipid phase can be measured (Leopold and Vertucci, 1989; Vertucci, 1993b). Some catabolic reactions catalyzed by enzymes occurred in the second hydration level and respiration was detectable in the third (Leopold and Vertucci, 1989, Vertucci, 1989a). Germination was not observed until the seeds reached the MC of hydration level 5, although some metabolism necessary for germination (protein synthesis) occurred in the fourth hydration level (Vertucci, 1993b; Vertucci and Farrant, 1995).

That the mechanism of seed deterioration varies with hydration level is also observed by the kinetics of seed deterioration change according to the MC of storage (Vertucci,

1993b). In the first hydration level (less than 8% water), deterioration was observed (Vertucci and Roos, 1990, 1993). Seed deterioration is slow (within months or years) when seeds are stored at the second hydration level (between 25% and 8% water). However, deterioration usually occurs within several days when seeds are stored at hydration level 3 between 25% and 45% (Delouche and Baskin, 1973; Matthews, 1980; Priestley *et al.*, 1980).

The effect of water content on the kinetics of these reactions may not be straight forward. While reducing water content may increase the likelihood of structural changes in protein and lipid components, reducing the water content may also limit the mobility of cellural constituents, and thus reduce the rate of molecular rearrangements (Leopold and Vertucci, 1989; Roberts and Ellis, 1989; Vertucci and Roos, 1990; Bruni and Leopold, 1991). Therefore, aging damage under dry conditions probably results from structural changes in membranes or proteins (Crowe *et al.*,1992). The kinetics of the aging reaction can be altered by manipulating T because most reactions require molecular motion. Generally the lower the T, the slower the reaction.

Seed viability and vigor tests

Seed viability indicates that a given seed contains structures and substances, including enzyme systems, needed for germination and seedling growth under favorable conditions in the absence of dormancy (Copland and McDonald, 1985). Germination is resumption of active growth by the embryo culminating in the development of a young plant by the seed (Copland and McDonald, 1985). In seed laboratory practice, germination means the emergence and development from the seed embryo of the essential structures that indicate the seed ability to produce a normal plant under favorable conditions (AOSA, 1987). The

AOSA (1987) defined vigor as "those seed properties which determine the potential for rapid uniform emergence and development of normal seedlings under a wide range of field conditions."

Viability is generally measured using the standard germination test. Vigor can be assessed using measurement of root length (AOSA, 1987), level of dehydrogenase activity (ISTA, 1985; AOSA, 1987; Legesse and Powell, 1992), electrical conductivity of the soak water (Matthews and Bradnock, 1968), viability after accelerated aging, controlled deterioration, or cold imbibition (Delouche and Baskin, 1973; Matthews, 1980; AOSA, 1987).

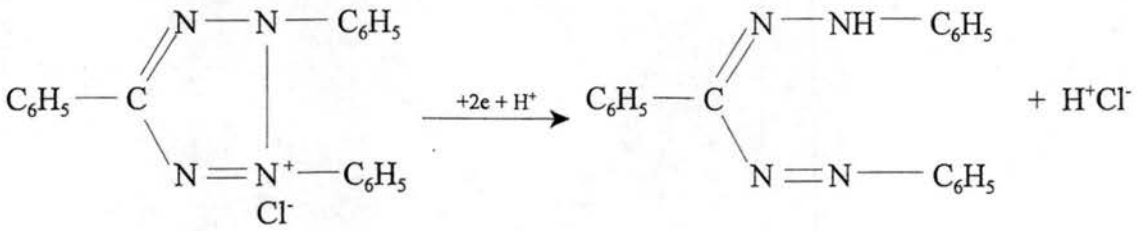
Germination test

The germination test is the most papular method to measure seed viability, but it takes time to obtain results and it can not measure viability of dormant seeds. Tetrazolium, conductivity, and the cold test are other common methods for vigor measurement.

Tetrazolium test

Seed viability can be easily detected by employing the tetrazolium (TZ) test which is based on dehydrogenase activity during respiration. The enzyme responsible for the reduction processes in living tissue provides H+ for reduction of the TZ solution which stains the living tissue red (Figure 2).

To measure seed viability, seeds are usually submerged in a 1% solution of colorless 2, 3, 5- triphenyltetrazolum chloride. Reduction of this compound to red-colored formazan by the dehydrogenase of viable seeds is an indication of living tissue.



2, 3, 5 - triphenyl tetrazolium chloride

formazan (red)

Figure 2. The chemical reaction that changes the colorless tetrazolium solution into formazan (AOSA, 1987).

The tetrazolium test has two advantages vs the regular germination test: 1) TZ is a fast method when speed is important, 2) TZ is not influenced by dormancy. The disadvantages are 1) its difficulty and the experience required to interpret results. 2) Lack of acceptance for the TZ test as an accurate method to estimate seed vigor from many seed companies (Copeland and McDonald, 1985).

Conductivity test

The conductivity test is based on the supposition that cell membrane integrity in seeds is disrupted during seed deterioration (Simon, 1974, 1978; Tao, 1978; Duke *et al.*, 1983; Hoekstra, 1984; Powell, 1986; Simon and Mathavan, 1986; Loeffler *et al.*, 1988; Crowe *et al.*, 1989; Hoekstra *et al.*, 1992). Membrane damage resulted in a higher leachate conductivity and decrease in seed viability and vigor (Simon and RajaHarun, 1972; Simon, 1974; Bonner and Vozzo, 1986; Powell, 1986; Herter and Burris, 1989; Hampton *et al.*, 1992).

The relationship between seed quality and electrolyte leakage has been demonstrated previously: the electrical conductivities of the seed leachates were negatively correlated with vigor and viability (Bonner and Vozzo, 1986; Hampton, *et al.*, 1992; Armstrong and McDonald, 1992). However, the relationship between imbibitional leakage and deterioration cannot be assumed for all species or all types of deterioration (Powell, 1986).

In the past, bulked seeds were used for measuring conductivity following 24 hr soaking (Simon, 1978; Tao, 1978; Loeffler *et al.*, 1988). Now, conductivity measurement of individual seed leachates is practical and data can be summarized in a manner to predict viability (Steere *et al.*, 1981; Moore, *et al.*, 1988; Herter and Burris, 1989; Wilson, 1992).

However, conductivity tests where seeds are soaked for many hours are destructive in that seeds irreversibly change as a consequence of the soak. Seeds cannot be dried back to their initial moisture content for storage and reused at a later date after 24 hours of soaking. Some evidence indicates that 50% or more of the electrolytes are leached out within the first few hours of imbibition (Simon and Mathavan, 1986). Therefore, it may be possible to measure leachate conductivity rate during early imbibition with less distruction.

Measurement of the conductivity of seed leachate during early imbibition may cause less irreversible changes and so might be used as a non-destructive method for seed viability and vigor determination (Davidson, 1993; Davidson, *et al.*, 1994a,b). In this method, individual seeds were soaked for 2 to 8 hours to determine leachate conductivity, and then the seeds were dried back to store for later use. However, non-destructive methods for seed viability determination cannot be used when viability is lost during previous cycles of imbibition and dryback.

The conductivity test has two advantages over the regular germination test: 1) conductivity test is a fast and precise method 2) conductivity has some potential as a non-destructive method for viability and vigor test. The disadvantages are 1) data varies by species, 2). association between germination and vigor is not always accurate.

Cold test

The cold test is one of the oldest methods of stressing seeds and is most often employed for evaluating seed vigor by seed companies (Copland and McDonald, 1995). The method is primarily used for predicting field emergence of maize and soybean seeds. Seeds are usually placed in soil or paper towels and exposed to low temperature for a period of

time. The interaction of low T, microorganisms, imbibition, and seed quality is measured. Seeds are then placed under favorable growth conditions for germination and growth. However, the application for predicting seed storage is minimal. The greatest difficulty for cold test is the lack of uniformity in field soil.

CHAPTER 3

THREE-DIMENSIONAL MODELS REPRESENTING SEED MOISTURE CONTENT AS A FUNCTION OF RELATIVE HUMIDITY AND TEMPERATURE

Introduction

Equilibrium relationships between moisture content (MC) and relative humidity (RH) are revealed by moisture sorption isotherms (Vertucci and Roos, 1990, 1993). Construction of such isotherms is a convenient method for assaying the physical state of water in relatively dry tissues (Rupley et al., 1983; Vertucci and Leopold, 1987). Changes in the physical status of water are associated with changes in the physiological activities of seeds (Leopold and Vertucci, 1989; Vertucci and Leopold, 1986; Vertucci and Roos, 1990).

Mechanisms of seed aging vary with hydration level because the nature of physiological reactions differ at different moisture levels (Leopold and Vertucci, 1989; Vertucci, 1989a, 1993b). Deterioration within hydration level 1 (less than 8%) is the result of molecular interactions because water has been removed from reactive sites (Vertucci and Roos, 1990, 1993; Vertucci and Farrant, 1995). In hydration level 2 (between 25% and 8% water), enzyme activity results in deterioration (Vertucci, 1993b). Seed deterioration under conditions of accelerating aging (Delouche and Baskin, 1973) or controlled deterioration (Matthews, 1980) are usually within hydration level 3 (between 45% and 25% water), where respiration starts (Vertucci, 1993b).

Achieving the optimum seed MC for long-term storage is a goal of genebanks, such as the National Seed Storage Laboratory. Understanding the interaction of temperature (T) and RH on seed MC, and how this varies among species, is an important step in designing storage facilities.

Numerous two-dimensional presentations or response curves have been proposed to describe the relationships between RH and MC (D'Arcy and Watt, 1970; Yang and Cenkowski, 1995). However, a three-dimensional model that includes a T term is needed to generate a response surface to analyze interactions (Fang et al., 1998). The purpose of the experiment was to investigate the interactive effect of RH and T on MC using best-fit models to generate three dimensional response surfaces.

Materials and Methods

Seed storage

Seeds of cucumber ('Straight Eight', Gurney's Seed & Nursery Co., Yankton, S.D.; initial germination, 91%), lettuce ('Black-Seeded Simpson', Gurney's Seed & Nursery Co.,Yankton, S.D.; initial germination, 94%), maize (Hybrid, 'Pioneer 3541', lot 88-10, Pioneer Hi-Bred International Inc., Des Moines, Iowa; initial germination, 95%), onion ('Yellow Sweet Spanish', Henry Field Seed & Nursery Co., Shenandoah, Iowa; initial germination, 93%), pea ('Extra-Early Alaska', Gurney's Seed & Nursery Co., Yankton, S.D.; initial germination, 92%), and watermelon ('Sugar Baby', Henry Field Seed & Nursery Co., Shenandoah, Iowa; initial germination, 91%) were equilibrated over H_2SO_4 (>99.5% concentration, 1% RH) and 11 saturated salt solutions (5.5-93% RH) at T from 5 to 50 °C (Table 1). The experiment continued until a constant MC at each pair of RH and T

Table 1. Relative humidities (%) of various saturated salt solutions and sulfuric acid at different temperatures (Vertucci and Roos, 1993).

Salt	Temperature (°C)				
	5	15	25	35	50
H_2SO_4(>99.5%)	1	1	1	1	1
$ZnCl_2$	5.5	5.5	5.5	5.5	5.5
NaOH	9	8	7	7	6
LiCl	15	14	13	11.5	11
$CaBr_2$	23	22	16.5	15	14
Kac	26	25	25	22	19
$MgCl_2$	33.5	33	32.5	32.5	31.5
K_2CO_3	43	43	43	41.5	41
$Mg(NO_3)_2$	56	54.5	53	50.5	46
NH_4NO_3	72	68	62	62.5	61
NaCl	75.5	75.5	75	75	74.5
KCl	88	86	85	84	80.5
KNO_3	93	92.5	91	90	85

combination was reached, which required from 30 to 45 days.

Determination of seed moisture content

At weekly intervals, two replicates of 10 seeds or 1 gram of seeds each from each of 60 treatments/species were assayed for MC. Fresh weight was measured after seeds were removed from storage. Dry weights were measured after seeds have been heated at 105°C for 24 hours. Moisture content was calculated as following:

$$MC\ (\%) = \frac{(W_1 - W_2)*100}{W_2}$$

where MC is seed moisture content, W_1 is fresh weight, and W_2 is dry weight. Moisture content was expressed on a dry weight basis.

Model selection

Since equilibrium MC of seeds is controlled by two factors, T and RH, interaction of these two factors was examined by comparing surfaces generated by best-fit models and also by analyzing the models. The best-fit subset models for lettuce and soybean germination and vigor were selected from a complete third order polynomial having the form $MC = \beta_0 + \beta_1*RH + \beta_2*T + \beta_3*RH^2 + \beta_4*T^2 + \beta_5*RH*T + \beta_6*RH^3 + \beta_7*T^3 + \beta_8*RH*T^2 + \beta_9*RH^2*T$, where MC is moisture content, R is relative humidity, T is temperature; β_0 (constant or intercept), β_1, β_2, ... β_9 are regression coefficients or parameter. The model from which each best-fit subset model was selected had k=9 independent variables and therefore a size of p=10 since p=k+1 including the constant. A factorial experiment permitted comparison of main and interaction effects on seed MC. Five hundred and eleven total subset models (2^k-1) were available as candidates for each "best-fit" subset.

Cp acts as a gauge of the bias introduced into the estimate of the dependent variable when independent variables are omitted from regression equations. The optimal value of Cp is equal to the number of parameters (independent variables in the subset plus the constant) or

$$Cp = p = k+1$$

The closer the value of Cp is to the number of parameters, the less likely a relevant variable was omitted. Subsets with low orders that also have Cp values close to k + 1 are good candidates for the best subset of variables. Likewise parsimony in the lineal selection is desirable. Mallow's graphical method (Draper and Smith, 1981) finalized each selection.

Results

Figure 3A is presented to illustrate goodness of fit. Response surfaces generated by the best subset models for MC in cucumber, lettuce, maize, onion, pea, and watermelon are presented in figure 3B. The six models having the lowest Cp value and least bias had the same functional form, $MC = \beta_0 + \beta_1*RH + \beta_2*T + \beta_3*RH^2 + \beta_5*RH*T + \beta_6*RH^3 + \beta_9*RH^2*T$.

Coefficients β_0, β_2, β_5, β_9 were the same ($P \leq 0.05$) for all species. Specifically, β_1 onion was different from cucumber and lettuce; β_3 onion and pea was different from cucumber, lettuce, and maize; β_3 pea was different from watermelon; and β_6 pea was different from cucumber (Table 2). Most parameter values among species were essentially the same (Table 2); however, onion and pea β_3 values were larger by an order of magnitude than those of the other four species.

Figure 3. (A) Seed moisture content data clouds where each point is the mean of two observations. (B) Response surfaces for each of six species, generated by using the best-fit models. All had the same form $MC = \beta_0 + \beta_1*RH + \beta_2*T + \beta_3*RH^2 + \beta_5*RH*T + \beta_6*RH^3 + \beta_9*RH^2*T$.

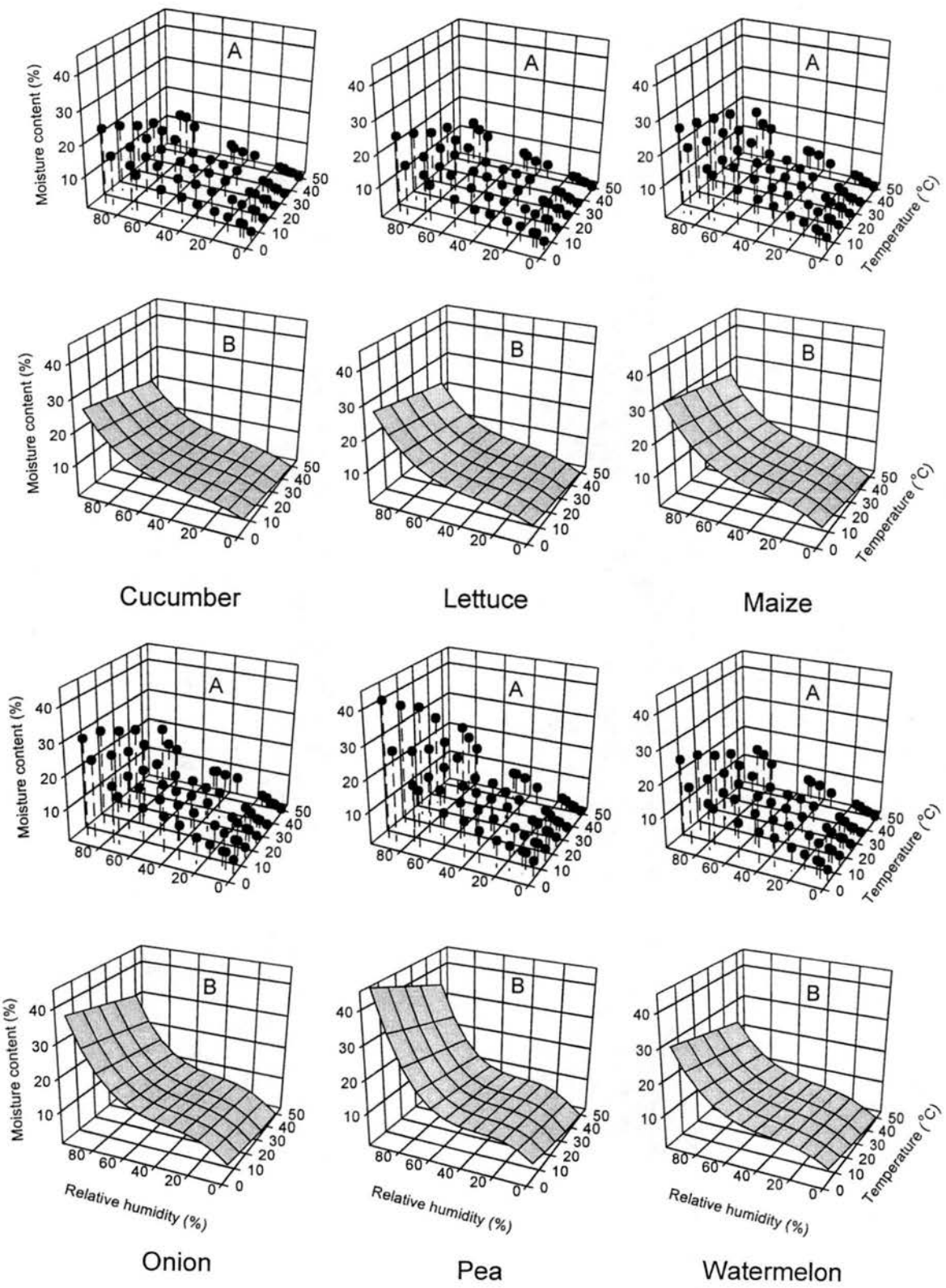

A
B
Moisture content (%)
Relative humidity (%)
Temperature (°C)
Cucumber
Lettuce
Maize
Onion
Pea
Watermelon

Table 2. Regression coefficients of the six models predicting the influence of temperature and relative humidity on seed moisture content. $MC = \beta_0 + \beta_1*RH + \beta_2*T + \beta_3*RH^2 + \beta_5*RH*T + \beta_6*RH^3 + \beta_9*RH^2*T$, was the best fit subset model for all six species.

Species	R^2	β_0	β_1	β_2	β_3	β_5	β_6	β_9
Cucumber	0.98	3.2 a[Z]	0.22 a	-0.072 a	-0.0048 a	0.0014 a	0.000049 a	-0.000019a
Lettuce	0.99	3.4 a	0.22 a	-0.076 a	-0.0052 a	0.0020 a	0.000054 ab	-0.000023a
Maize	0.99	4.0 a	0.31 ab	-0.089 a	-0.0065 a	0.0012 a	0.000061 ab	-0.000014a
Onion	0.99	3.7 a	0.44 b	-0.095 a	-0.0100 bc	0.0017 a	0.000090 ab	-0.000022a
Pea	0.98	4.7 a	0.42 ab	-0.120 a	-0.0120 c	0.0040 a	0.000120 b	-0.000052a
Watermelon	0.98	3.7 a	0.30 ab	-0.089 a	-0.0067 ab	0.0020 a	0.000063 ab	-0.000025a

[Z] Mean separation within columns by 95% confidence limits.

The highest MC estimated by the pea and onion models ranged from 34 to 45% and 30 to 36% at T from 50 to 5 °C respectively, versus 20 to 30 % estimated by the models of the other four species at T from 50 to 5 °C.

Discussion

Vertucci and Roos (1990, 1993) reported the equilibrium relationship between seed MC and RH is revealed by moisture sorption isotherms. A method using moisture sorption isotherms to study physiological activities and water binding in seeds has been developed (Leopold and Vertucci, 1986, 1989; Vertucci, 1989a). Seed hydrational levels can be divided into four hydration levels according to moisture isotherm (Vertucci, 1993b; Vertucci and Farrant, 1995). Seed MC is hydration level 1 at RH<25%; seed MC is hydration level 2 at RH between 25% and 80%; seed MC is hydration level 3 at RH between 80% and 90%; seed MC is hydration level 4 at RH> 90%. Seed physiological activities are different in different hydrational levels.

The generally higher MC estimated by the pea model was attributed to the relatively low lipid content of pea (Vertucci and Roos, 1990). The effect of seed composition in determining seed MC is reflected in the models objectively selected and supported in this study.

Notable was the fact that interaction terms RH*T and RH^2*T were included in the final model, suggesting that interaction was significant. Furthermore, this was supported by the results of the factorial experiment that compared main and interaction effects over

species. These results also suggest that both factors had a greater effect on the MC of pea and onion seeds than on cucumber, lettuce, maize and watermelon seeds (Table 3).

Coefficients of multiple determination were significant at $P \leq 0.01$ and ranged from 0.98 to 0.99. Mallows' graphical method (Draper and Smith, 1981) for determining bias indicated that only the cucumber seed MC model exhibited a degree of bias, and perhaps would not be quite as good a predictor as the other five species. The standardized coefficients (Table 4) support the response surfaces in Fig. 1B, in that RH has a strong positive effect on seed moisture content but T has a slightly negative effect.

Conclusions

Models for cucumber, lettuce, maize, onion, pea, and watermelon seeds had the same functional form. Based on the models and model-generated response surfaces, RH has a greater influence on seed MC than does T. Relative humidity has a strong positive effect while T has a slightly negative effect on MC. The extremely strong interactive effect of RH and T on seed MC is documented by these results. Also, one might justify fitting the Cp selected seven parameter form to a combined species data set, n=6*60 (6 species, 12 RH and five T), thus providing a general model for the purpose of interpolation of the interaction of RH and T on orthodox seed MC.

Table 3. Mean squares for main effects and interaction of relative humidity (RH), and temperature (T), on seed moisture content of six species. All 18 sources of variation, considering main, interaction and species, were significant at $P \leq 0.001$.

Source of variation	d.f.	Cucumber	Lettuce	Maize	Onion	Pea	Watermelon
RH	4	55	52	80	102	146	73
T	11	258	276	402	494	780	314
RH * T	44	1.7	1.5	1.1	2.0	7.7	1.7
Residual	60	0.007	0.007	0.010	0.008	0.011	0.012

Table 4. Standardized[z] regression coefficients β_1 and β_2 associated with linear effects of relative humidity and temperature variables, respectively.

Species	RH	T
Cucumber	1.36	-0.22
Lettuce	1.31	-0.23
Maize	1.53	-0.22
Onion	1.94	-0.21
Pea	1.46	-0.22
Watermelon	1.65	-0.25

[z]Software (Dixon, 1981) computes coefficients from a standardized revision of the model where $\beta_j' = \beta_j(S_j/S_Y)$ j=1, 2, ..., k (Kleinbaum et al., 1988). Column RH is significantly different from column T at $P \leq 0.05$ based on a t test, and variances are equal based on an equal variance test (Fox et al., 1995).

CHAPTER 4

INTERACTION OF STORAGE DURATION, TEMPERATURE AND RELATIVE HUMIDITY ON SEED GERMINATION AND VIGOR

Introduction

Storage of seeds in genebanks is essential to the preservation of biological diversity. Seed viability must be maintained for as long as possible in genbanks in order to preserve the genetic integrity of stored samples. It is very important to predict how long seeds might survive under different storage conditions.

Seed moisture content (MC) and temperature (T) are two of the most important factors in maintaining seed viability and vigor during storage. For orthodox seed species, this can usually be accomplished by adjusting the water content of seeds and by reducing the storage T. However, the exact relationship between seed MC and T has not been clearly determined. The optimum storage conditions are not well known.

The International Plant Genetic Resources Institute (IPGRI) recommends that seeds be dried to about 5±2% water content and stored at -18°C (FAO/IPGRI, 1994). However, many experiments have shown that longevity in seeds was improved when seeds were dried to lower MC than the recommended mean value (Ellis et al., 1988, 1989; Vertucci and Roos, 1990, 1993).

A mathematical model (viability equation) of seed deterioration has been proposed by Ellis and Roberts (1980). The seed viability equation relating seed survival to storage duration, T and seed MC can be used to predict germination after a storage period given T and seed MC

$$v = K_i - p / 10^{(K_E - C_W \log 10\, m - C_H t - C_Q t^* t)}$$

In the equation v is the probit of percent viability, p is the period of storage (days), m is the moisture content (%, fresh weight basis) and t is temperature (°C). The K_i (probit scale) is the initial seed quality constant of a seed lot and K_E, C_W, C_H and C_Q are constants having common values for all seed lots of a given species. The equation was developed by Ellis and Roberts (1980) on the basis of storage experiments under very warm (> 50°C) and/or very wet (20% MC) conditions. The relationship between water content and rate of seed deterioration is logarithmic (Ellis et al., 1986). Accordingly, there is a low MC limit (critical MC) for seed storage. The critical moisture level varies with species and even with cultivars of the same species (Ellis *et al.*, 1988, 1989, 1990). Drying seeds below the critical moisture level reportedly has no further benefit on longevity (Ellis *et al.*, 1989). Based on this concept, they proposed that 'ultra-dry' seeds can substitute for low T in developing countries where electrical power may be lacking or unreliable (Zheng et al., 1998). However, over-drying may cause seed injury (Vertucci and Roos, 1990, 1993; Walters et al., 1998b). The detrimental effects of drying seeds to extremely low water content (below 0.01 g H_2O/g dw) were found in several laboratories (Nutile, 1964; Nakamura, 1975; Carpenter and Ostmark, 1988; Carpenter and Boucher, 1992; Vertucci and Roos, 1990, 1993; Vertucci *et al.*, 1994). Potential risk of over drying seeds must be addressed.

An optimum moisture level concept regarding seed storage has been proposed (Vertucci and Roos, 1990, 1993; Vertucci *et al.*, 1994). Seed vigor is lost more rapidly when moisture is either above or below the optimum level. The optimum water content for seed storage changes as a function of T (Vertucci and Roos, 1993; Vertucci et al., 1994). Because seed water content at a given relative humidity (RH) increases as T decreases, the optimum water content for seed storage would increase as the storage T was lowered. Ellis and colleagues expressed concern that the critical water content might change with storage T (Ellis et al., 1989), but they felt that it would be a minor effect, and experiments storing lettuce and sunflower seeds at T between 35 and 65 °C showed no evidence of a T effect (Ellis et al., 1995).

The conclusion of this debate needs a basic understanding of the effects of storage duration (D), T, and RH on seed quality. The objectives of this experiment were to 1) determine the effects of D, T, and RH and the interaction of these factors on seed quality, 2) develop models for elucidating over-drying damage, optimum storage conditions, accelerated aging at high MC, and the interaction of these factors on seed quality.

Materials and methods

Seeds of lettuce (cv. Black Seed Simpson, Henry Field Seed & Nursery Co., Shenandoah, Iowa, initial germination, 96%) and soybean (cv. Williams 82, Pioneer Hi-Bred International Inc., Des Moines, Iowa; initial germination, 92%) were used in the experiment since high quality seeds are readily available from commercial sources and since the rate at which percentage of germination and growth potential are lost have been documented as a function of storage MC and T.

Seed storage

Lettuce and soybean seeds were equilibrated over phosphorus pentoxide (P_2O_5, 1% RH) and 11 saturated salt solutions (5.5% to 93% RH) in desiccators at T of 5, 15, 25, 35, and 50°C (table 1). At weekly intervals, two replicates of 10 seeds each or 1 gram of seeds from each of 60 seeds aliquots were measured for MC. Moisture content was expressed on a dry weight basis. Seeds were removed from desiccators and stored in gas tight bottles at T of 5, 15, 25, 35, and 50°C after equilibrium.

Germination and vigor assay

Lettuce germination assay involved 50 seeds rolled in two sheets (37 × 26 cm) of germination paper toweling (Anchor Papaer, St. Paul, MN). Rolls (30 per box) were put into crispers (27 × 20 × 15 cm) containing 250 ml distilled water and incubated at 15-30°C (15C, 16 hours, night; 30C, 8 hours, day) for 7 days. After 3 days, an additional 100 ml of distilled water was added to the crisper. Radicle lengths were measured after four days and percentage germination after seven days (AOSA, 1987).

Soybean germination assay involved 25 seeds rolled in two sheets (37 × 26 cm) of germination paper toweling (Anchor Papaer, St. Paul, MN). Rolls (30 per box) were put into crispers (27 × 20 × 15 cm) containing 250 ml distilled water and incubated at 25°C for 7 days. After 3 days, an additional 100 ml of distilled water was added to the crisper. Radicle lengths were measured after four days and percentage germination after seven days (AOSA, 1987).

To avoid differences in imbibitional stress among different treatments, all lettuce and soybean seeds were adjusted to the same initial water content (lettuce, 10% MC; soybean, 12% MC). This was accomplished by equilibrating seeds over a saturated solution of $Ca(NO_3)_2$, 55% RH, for 24 hours on the laboratory bench at room T (22-24°C).

Model selection

Germination and vigor of lettuce and soybean seeds during storage were controlled by D, T and RH in this experiment. This interactive effect on germination and vigor may be explained by selecting a best-fit subset model. The best-fit subset models for lettuce and soybean germination and vigor were selected from a complete third order polynomial using the all-possible-regressions procedure (Kleinbaum et al., 1988). The complete model was a third order polynomial for germination or vigor of each species is G or $V = \beta_0 + \beta_1*D + \beta_2*T + \beta_3*R + \beta_4*D*T + \beta_5*D*R + \beta_6*T*R + \beta_7*D^2 + \beta_8*T^2 + \beta_9*R^2 + \beta_{10}*D^2*T + \beta_{11}*T^2*D + \beta_{12}*D^2*R + \beta_{13}*D*R^2 + \beta_{14}*T^2*R + \beta_{15}*T*R^2 + \beta_{16}*D*T*R + \beta_{17}*D^3 + \beta_{18}*T^3 + \beta_{19}*R^3$, where D is storage duration, R is relative humidity, T is temperature, β_0 is the constant (intercept), and $\beta_1, \beta_2, \ldots \beta_{19}$ are regression coefficients.

Total subset models examined in selecting each of the four best-fit subset models is based on the equation (Kleinbaum et al., 1988). In this case, 524,287 (where k=19) subset models were examined for germination and vigor considering the two species using the Mallows' Cp minimum criterion and Mallow's graphic method to evaluate bias and random error (Daniel and Wood, 1980; Draper and Smith, 1981; Fox et al., 1995).

Results

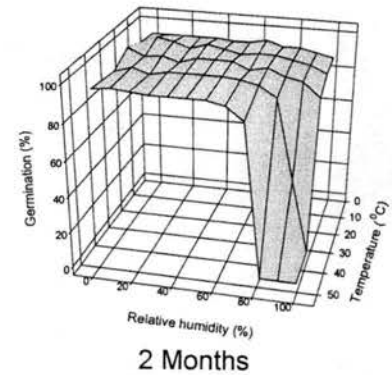
Seed damage under either over-dried or high moisture content conditions

Lettuce seeds were stored at 12 RH and at T from 5 to 50°C for 24 months. Seed germination and vigor were measured every two months. Deterioration in stored seeds was evaluated by reductions in germination percentage and radicle growth. Raw data plots for lettuce germination and vigor are shown in Figures 4 and 5.

Best-fit subset models were selected using Mallows' Cp minimum method to evaluate bias and random error. The best-fit subset models for lettuce germination and vigor are listed in the Table 5. Model, $G = \beta_0 + \beta_1*D + \beta_2*T + \beta_3*R + \beta_4*D*T + \beta_5*D*R + \beta_6*T*R + \beta_7*D^2 + \beta_8*T^2 + \beta_9*R^2 + \beta_{10}*D^2*T + \beta_{11}*T^2*D + \beta_{12}*D^2*R + \beta_{13}*D*R^2 + \beta_{14}*T^2*R + \beta_{15}*T*R^2 + \beta_{16}*D*T*R + \beta_{18}*T^3 + \beta_{19}*R^3$, was selected as a best-fit subset model for lettuce germination. Eighteen variables were selected and only D^3 was eliminated. $V = \beta_0 + \beta_2*T + \beta_3*R + \beta_4*D*T + \beta_5*D*R + \beta_6*T*R + \beta_8*T^2 + \beta_9*R^2 + \beta_{10}*D^2*T + \beta_{11}*T^2*D + \beta_{12}*D^2*R + \beta_{13}*D*R^2 + \beta_{14}*T^2*R + \beta_{15}*T*R^2 + \beta_{16}*D*T*R + \beta_{17}*D^3 + \beta_{18}*T^3 + \beta_{19}*R^3$ was selected as a best-subset model for lettuce vigor. In this model, 17 variables were selected; D and D^2 were eliminated.

The degrees of freedom adjusted coefficient of determination (R_a^2) is a measure of how well a regression model describes the data based on R^2, but takes into account the

Figure 4. Plots of germination data; lettuce seeds were stored at 12 relative humidities and 5 temperatures for 24 months.



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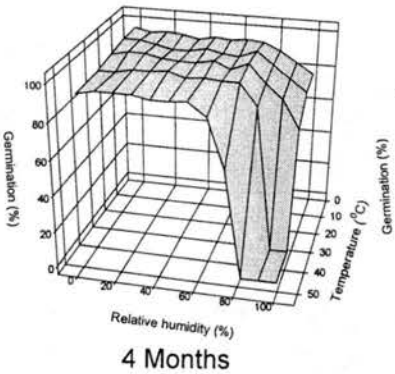

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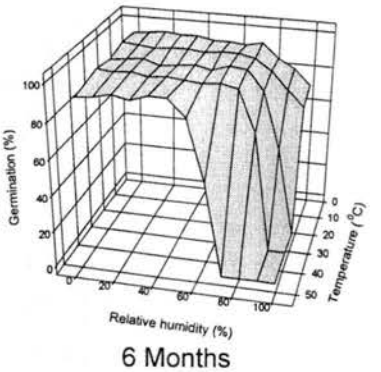

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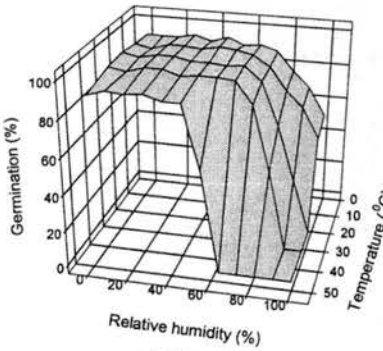

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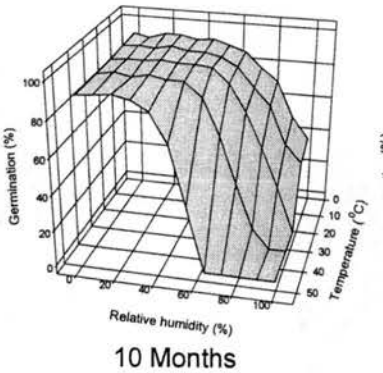

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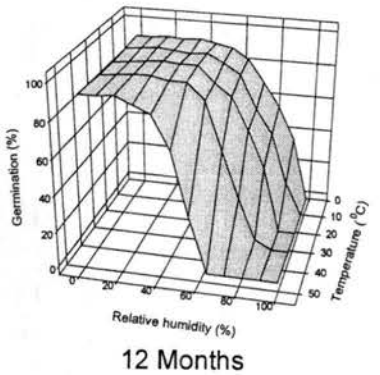

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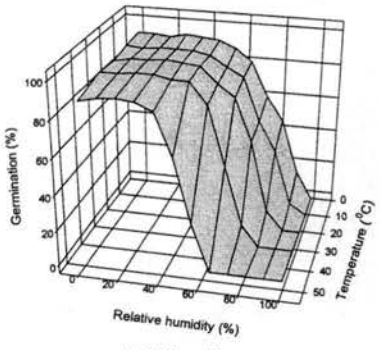

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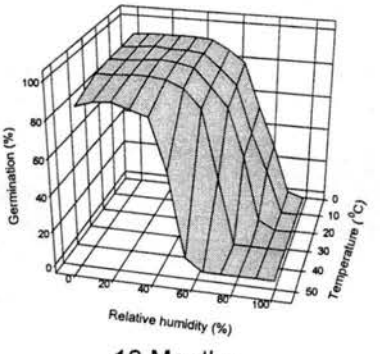

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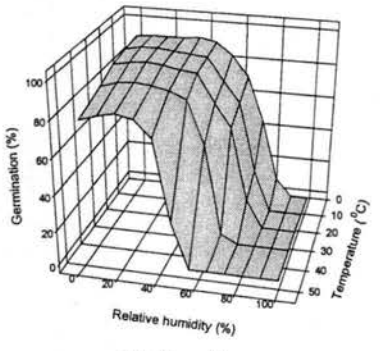

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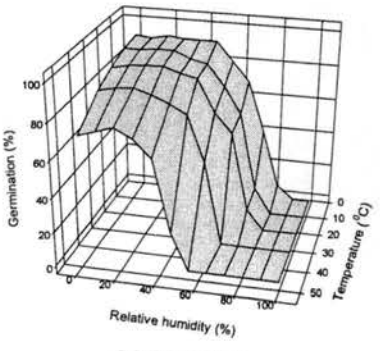

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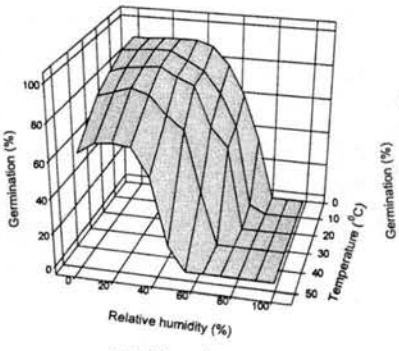

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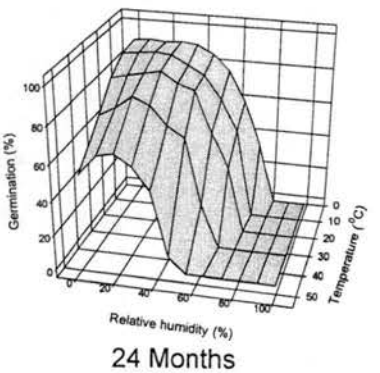
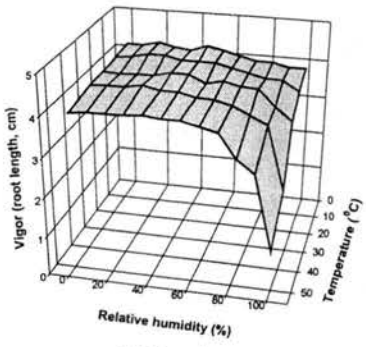


24 Months

Figure 5. Plots of vigor (root length) data; lettuce seeds were stored at 12 relative humidities and 5 temperatures for 24 months.



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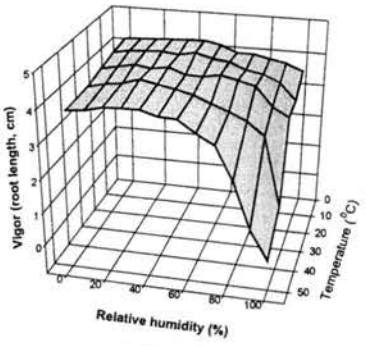

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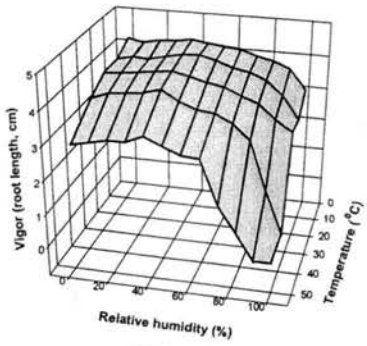

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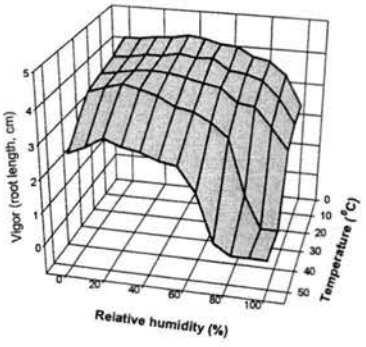

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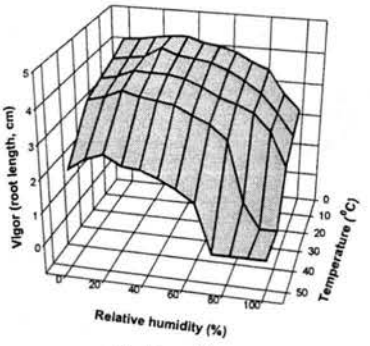

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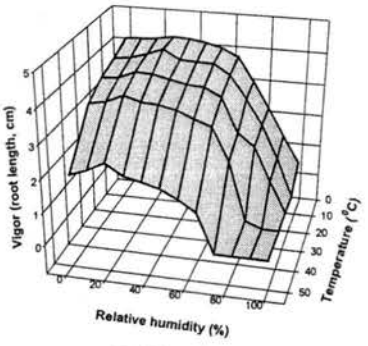

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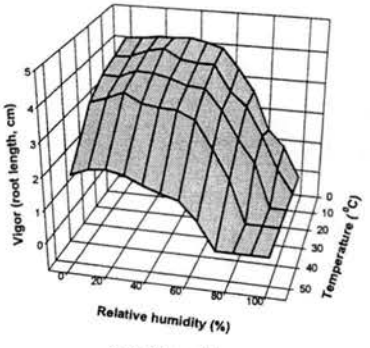

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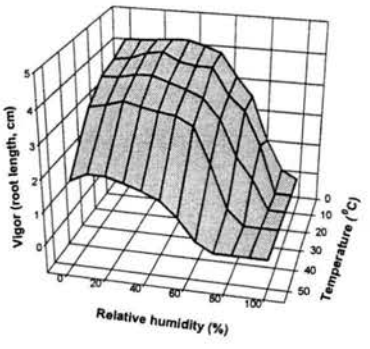

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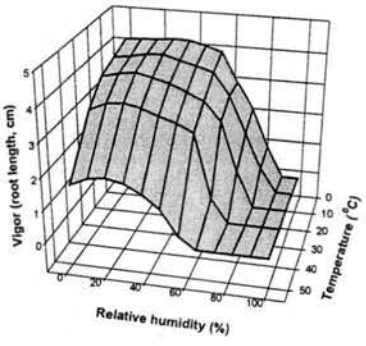

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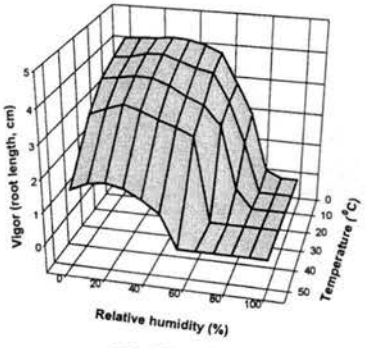

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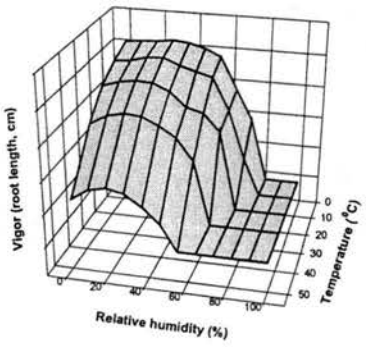

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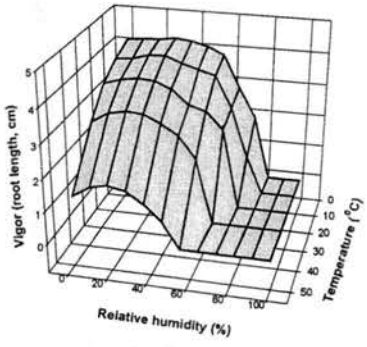


24 Months

Table 5. Best-fit subset models for lettuce and soybean germination and vigor compared to the complete model.

Complete	Lettuce		Soybean	
	Germination	Vigor	Germination	Vigor
D	✓	–	✓	–
T	✓	✓	✓	✓
R	✓	✓	–	–
D*T	✓	✓	✓	–
D*R	✓	✓	✓	✓
T*R	✓	✓	✓	–
D^2	✓	–	–	✓
T^2	✓	✓	✓	–
R^2	✓	✓	✓	–
D^2*T	✓	✓	–	✓
T^2*D	✓	✓	✓	–
D^2*R	✓	✓	✓	✓
D*R^2	✓	✓	✓	✓
T^2*R	✓	✓	–	✓
T*R^2	✓	✓	✓	✓
D*T*R	✓	✓	–	✓
D^3	–	✓	–	✓
T^3	✓	✓	✓	✓
R^3	✓	✓	✓	✓
R_a^2	0.93	0.98	0.98	0.97

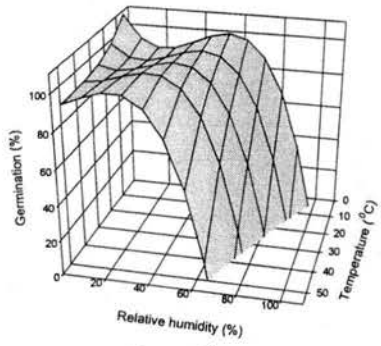
Checkmark (✓) means variables were selected and minus symbol (-) means variables were not selected.

number of independent variables. Values of R_a^2 in the four selected models ranged from 0.93 to 0.98 and were highly significant (Table 5). Large R_a^2 values indicate that the equation provides a good description of the relationship between the dependent and independent variables.

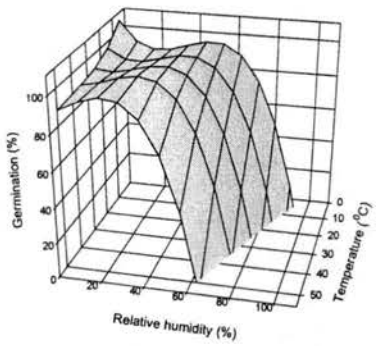
The response surfaces generated by the best-fit subset models for lettuce germination and vigor are presented in Figures 6 and 7. They show lettuce germination and vigor from 2 to 24 months of storage (Figures 6 and 7). Over-drying damage was first observed at 50°C after 2 months of storage and then 35°C after 8 months of storage. For seeds stored at 25 °C, over-drying damage was observed after 12 months of storage. After 18 months of storage, seeds stored at 15°C exhibited damage due to over-drying. After 22 months of storage, over-drying damage was observed at all T. The higher the T, the earlier over-drying damage was observed.

The best-fit subset models for soybean germination and vigor are listed in Table 5. Model, $G = \beta_0 + \beta_1*D + \beta_2*T + \beta_4*D*T + \beta_5*D*R + \beta_6*T*R + \beta_8*T^2 + \beta_9*R^2 + \beta_{11}*T^2*D + \beta_{12}*D^2*R + \beta_{13}*D*R^2 + \beta_{15}*T*R^2 + \beta_{18}*T^3 + \beta_{19}*R^3$, was selected as a best-subset model for soybean germination. In the model, 13 variables were selected. The R, D^2, D^2*T, T^2*R, D*T*R, and D^3 were not selected. Model, $V = \beta_0 + \beta_2*T + \beta_5*D*R + \beta_7*D^2 + \beta_{10}*D^2*T + \beta_{12}*D^2*R + \beta_{13}*D*R^2 + \beta_{14}*T^2*R + \beta_{15}*T*R^2 + \beta_{16}*D*T*R + \beta_{17}*D^3 + \beta_{18}*T^3 + \beta_{19}*R^3$, was selected as a best-subset model for soybean vigor. In this model, 12 variables were selected; D, R, D*T, T*R, T^2, R^2, and T^2*D were not. Optimum RH of 20 to 22% for germination and vigor were observed when seeds were stored at 50°C and 35°C beginning at 12 months

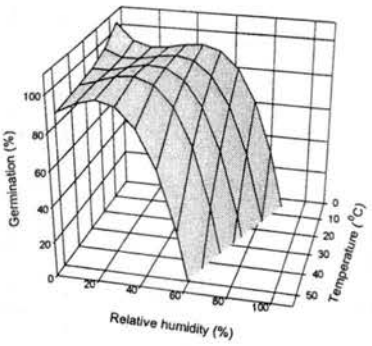
Figure 6. Response surfaces generated by the best-fit seed germination model based on lettuce seeds stored at 12 relative humidities and 5 temperatures for 24 months.



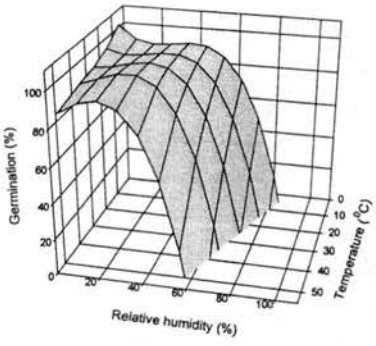
2 months



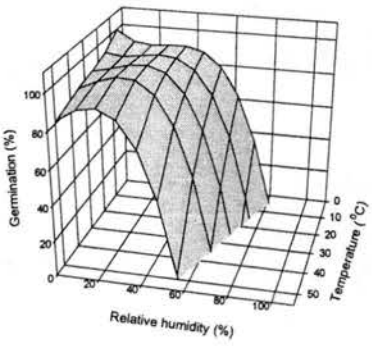
4 months



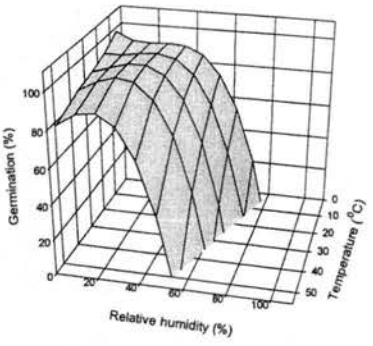
6 months



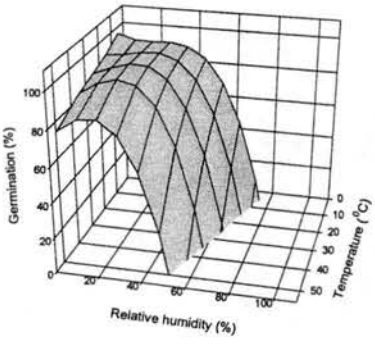
8 months



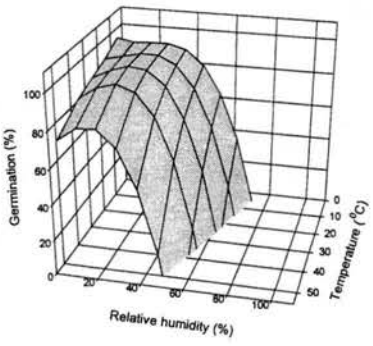
10 months



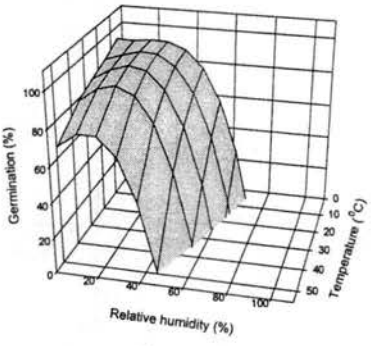
12 months



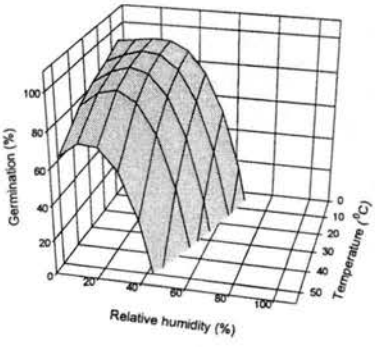
14 months



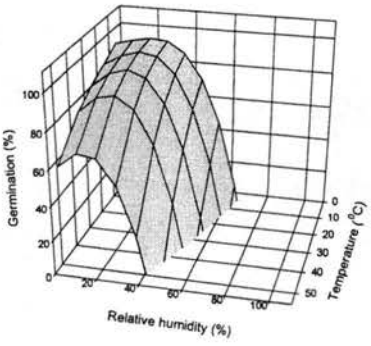
16 months



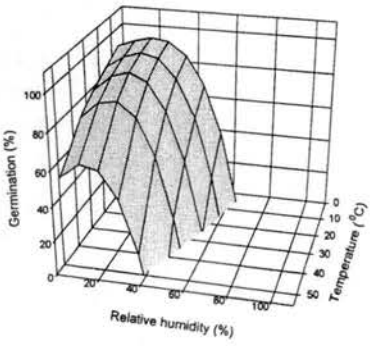
18 months



20 months

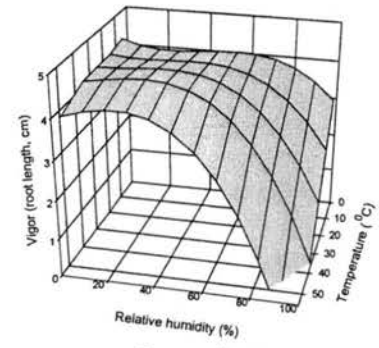


22 months

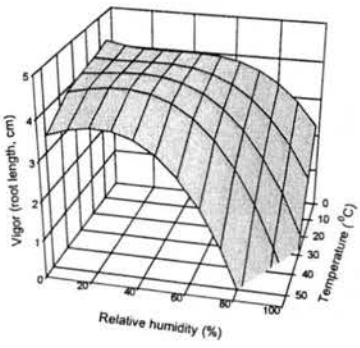


24 months

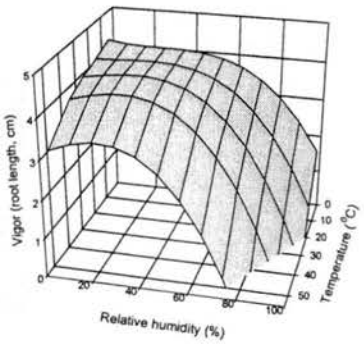
Figure 7. Response surfaces generated by the best-fit seed vigor model based on lettuce seeds stored at 12 relative humidities and 5 temperatures for 24 months.



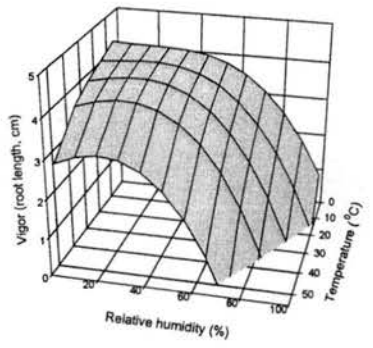
2 months



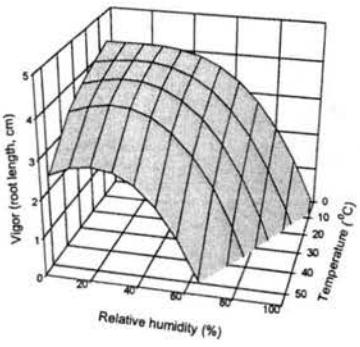
4 months



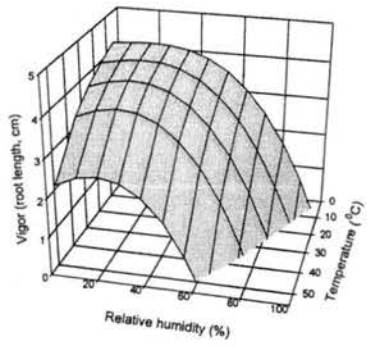
6 months



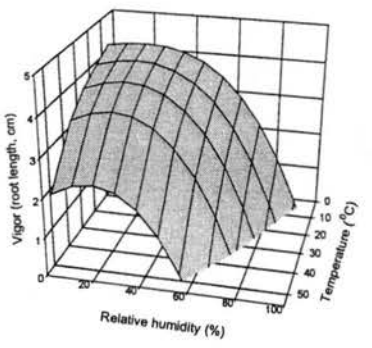
8 months



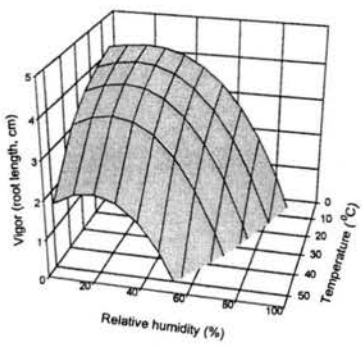
10 months



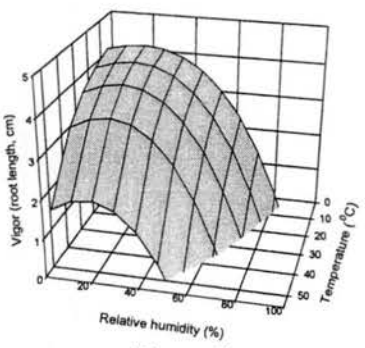
12 months



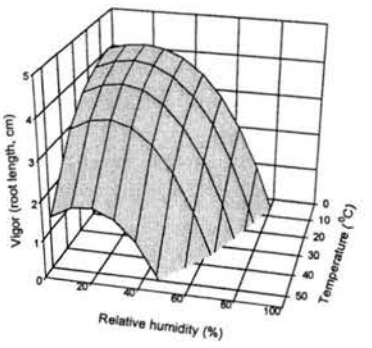
14 months



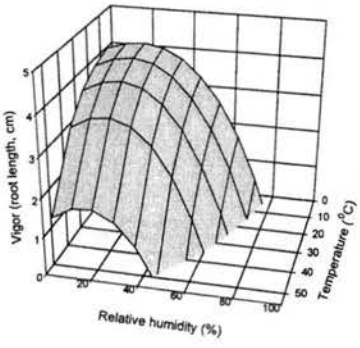
16 months



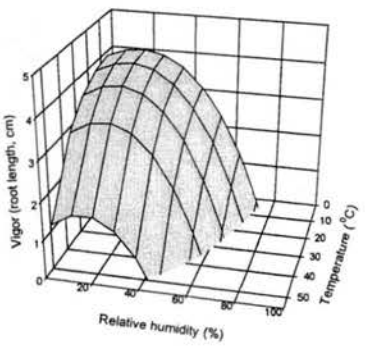
18 months



20 months



22 months



24 months

of storage (Figures 6 and 7). After 24 months of storage, optimum RH were observed at each T from 5 to 50°C. Storage of seeds at RH greater than the optimum resulted in much lower germination and vigor than their drier counterparts. Storage at excessively high MC always resulted in reduced germination and vigor due to accelerated aging.

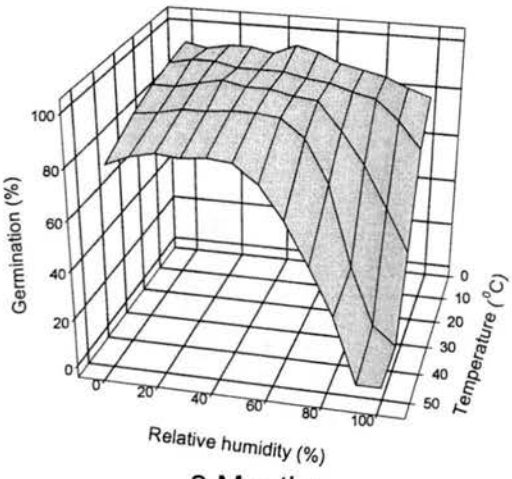
Soybean seeds were stored at 12 RH at T from 5 to 50°C for 12 months. Seed germination and vigor were measured every two months. Deterioration in stored seeds was evaluated as reduction in germination percentage and radicle growth. The raw data for soybean germination and vigor is shown in Figures 8 and 9.

Values of R_a^2 in the four models ranged between 0.97 and 0.98 and were highly significant (Table 5). These large values of R_a^2 indicate that the equations are a good description of the relation between the independent and dependent variables.

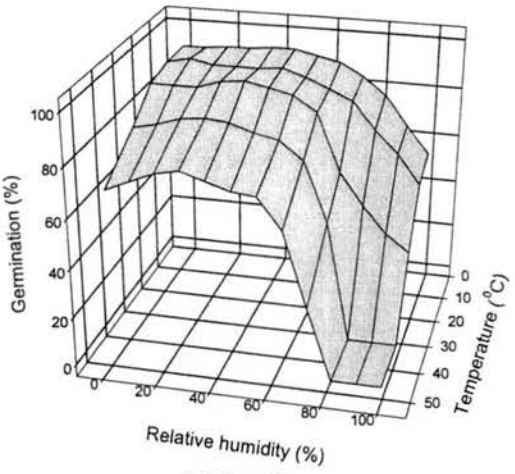
Response surfaces generated by the best-fit subset models for soybean germination and vigor are presented in Figures 10 and 11. Reduction in germination and vigor due to over-drying was first observed at 35 and 50°C after 2 months. Over-drying damage was observed after 8 months of storage for seeds stored at 15 °C. After 10 months of storage, over-drying damage was observed at all T. The higher the T, the earlier in the storage period over-drying damage was observed.

Optimum RH (20-22%) which elicits a maximum germination and vigor were observed after seeds stored at 35°C and 50°C for 2 months (Figures 10 and 11). After 12 months of storage, optimum RH was about 20% at all storage T from 50 to 5°C. Storage of seeds at RH above 20% resulted in much lower germination and vigor than their drier counterparts. Loss of seed germination and vigor at higher RH was due to accelerated aging.

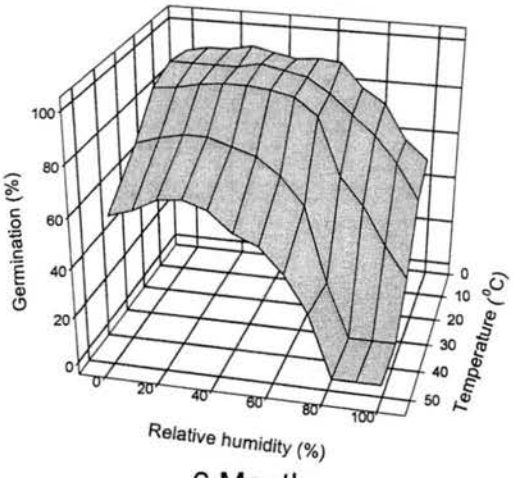
Figure 8. Plots of germination data; soybean seeds were stored at 12 relative humidities and 5 temperatures for 12 months.



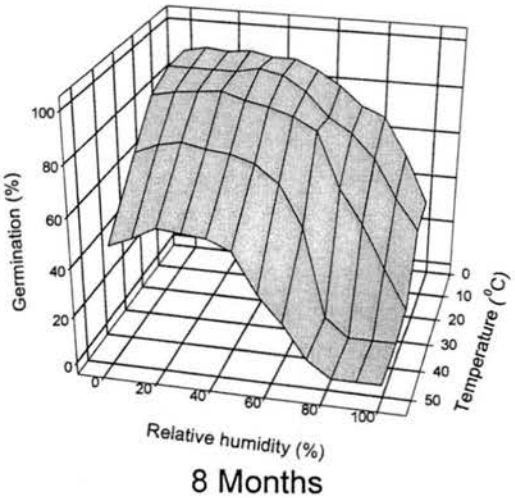
2 Months



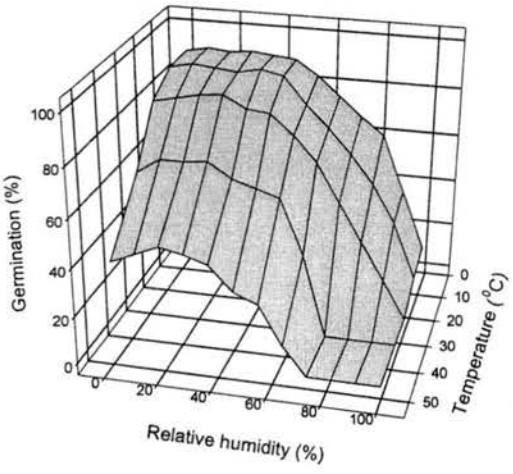
4 Months



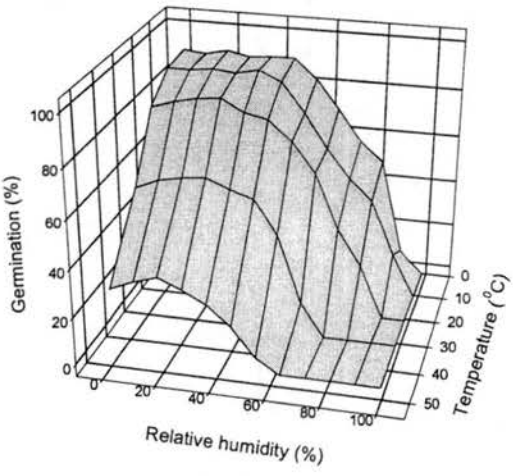
6 Months



8 Months

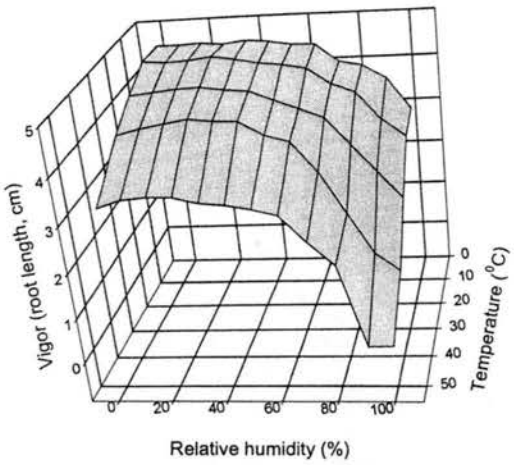


10 Months

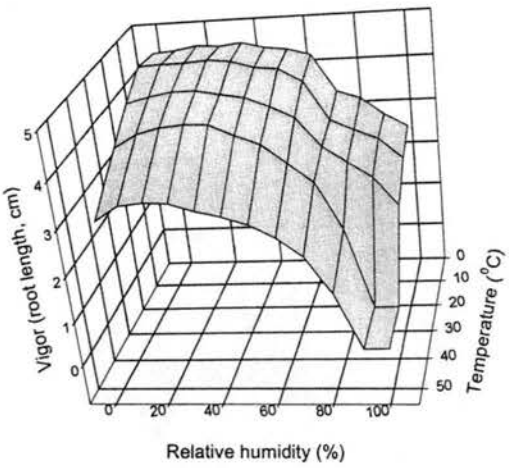


12 Months

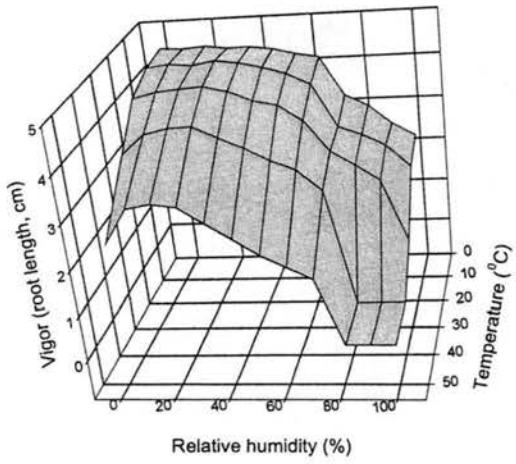
Figure 9. Plots of vigor data; soybean seeds were stored at 12 relative humidities and 5 temperatures for 12 months.



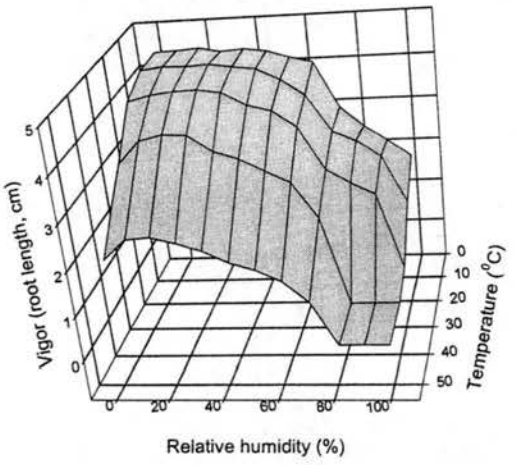
2 months



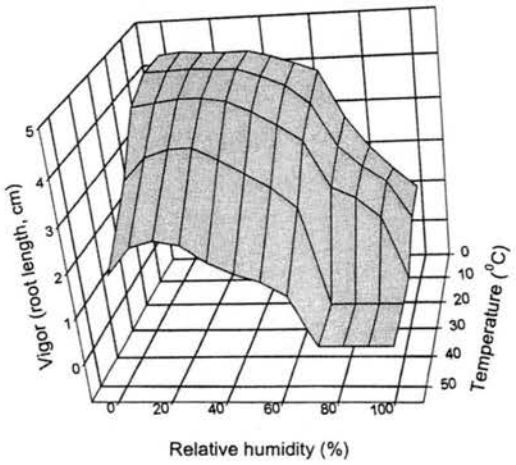
4 months



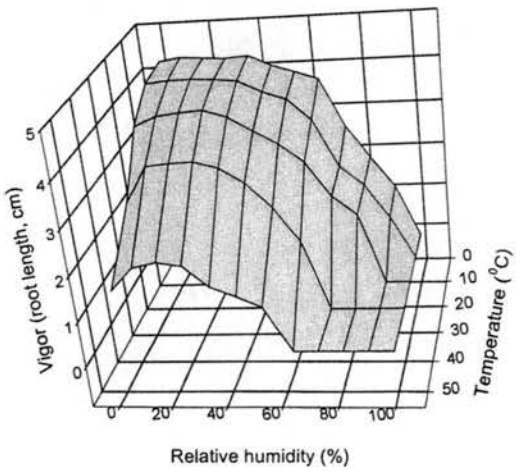
6 months



8 months

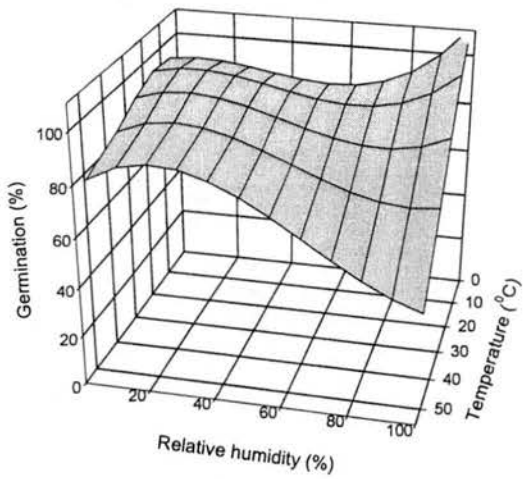


10 months

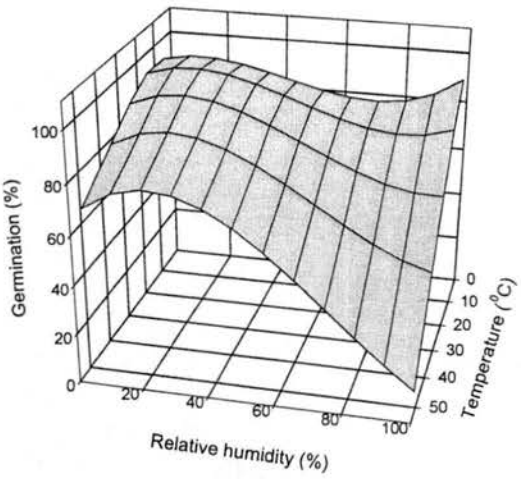


12 months

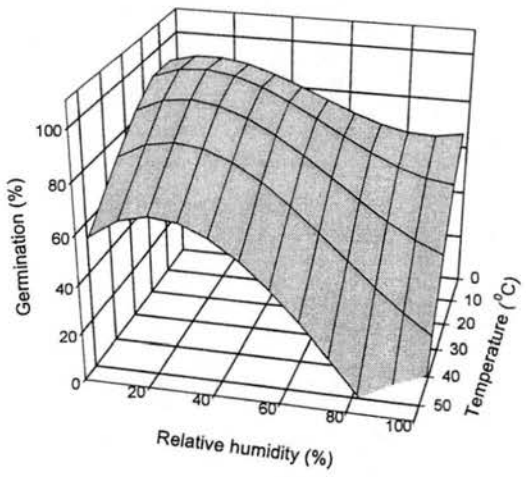
Figure 10. Response surfaces generated by the best-fit germination model based on soybean seeds stored at 12 relative humidities and 5 temperatures for 12 months.



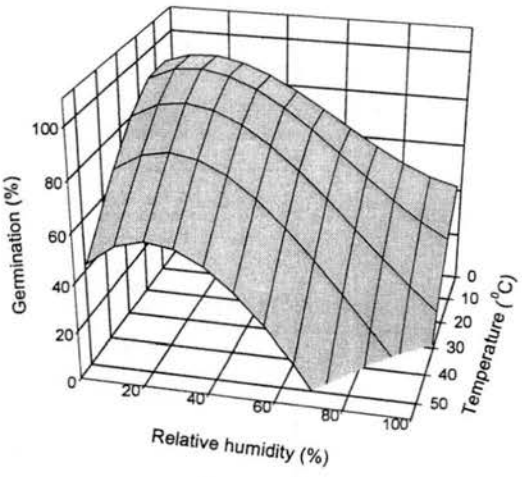
2 months



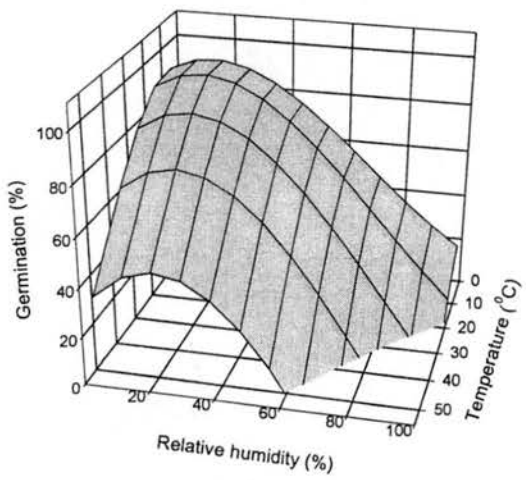
4 months



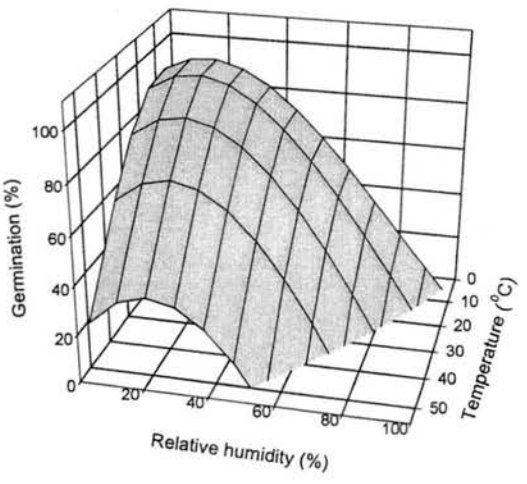
6 months



8 months

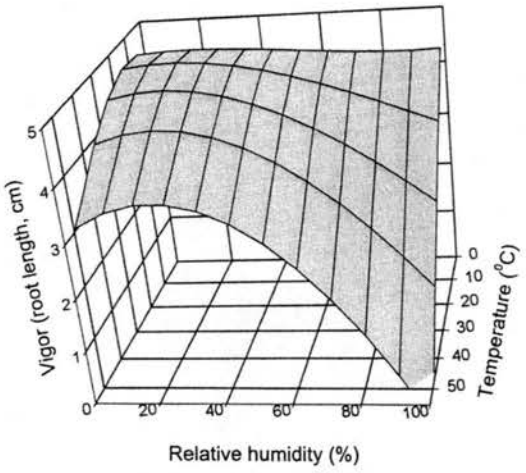


10 months

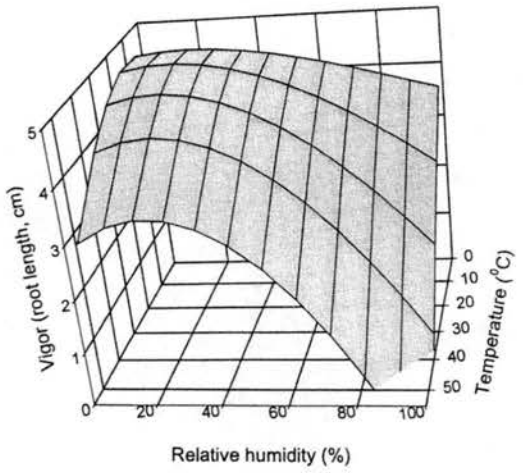


12 months

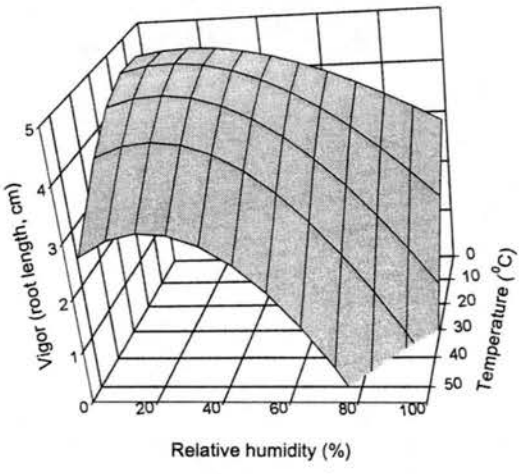
Figure 11. Response surfaces generated by the best-fit vigor model based on soybean seeds stored at 12 relative humidities and 5 temperatures for 12 months.



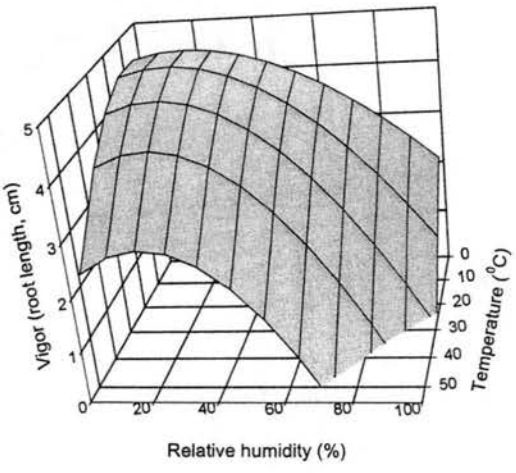
2 months



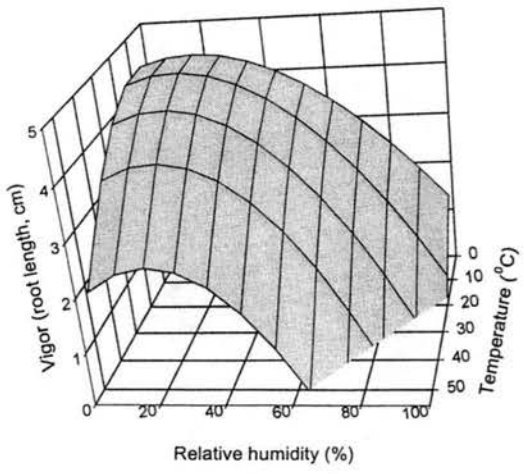
4 months



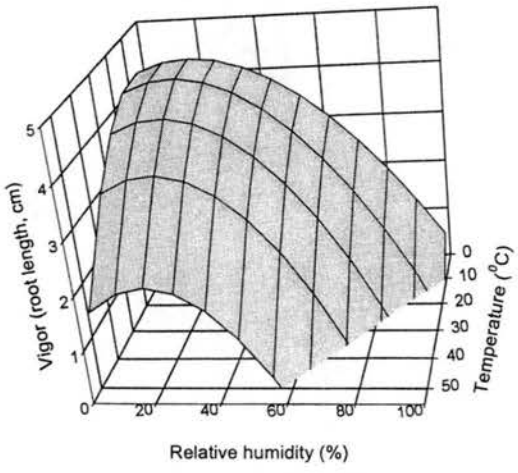
6 months



8 months



10 months



12 months

Discussion

The objective of seed genebanks is to preserve biological diversity of plants by maintaining viability of seed accessions for as long as possible. Strategies for seed storage should be developed in the context of both the required longevity and the efficiency. A major impediment to the development of cost-efficient storage strategies is to predict how long a seed will survive given different storage conditions.

This study considered longevity of lettuce and soybean seeds stored at 12 RH at T from 5 to 50°C to develop the best models for predicting optimum storage conditions. The goal is to find a model that gives the best prediction of dependent variables (germination and vigor) given D, T, and RH.

Several strategies are concerned with determining how many variables should be in the final model. These strategies include all-possible-regression procedure, a forward selection, a backward elimination, and chunkwise methods (Kleinbaum et al., 1988). The all-possible-regressions procedure used in this research is preferred over other variable selection strategies because it is the only method to find the smallest Cp and the largest R_a^2. The all-possible-regressions procedure was impractical in the past because 2^k-1 models must be evaluated. The k is large if the number of variables and/or order of the maximum model is large. As computers became more powerful, the method for all-possible-regressions procedure became practical. A total of 524, 287 subset models were examined for each of the 4 models when k is 19, because the all-possible-regressions procedure requires each possible regression to be evaluated. In the all-possible-regressions procedure, the best-subset model was selected based on the criteria of Cp. Cp is a gauge of the bias introduced

into the estimate of the dependent variable when independent variables are omitted from regression equation. The optimal value of Cp is equal to the number of parameters (the independent variables used in the subset plus the constant). The closer the value of Cp is to the number of parameters, the less likely a relevant variable was omitted. Subsets with low orders that also have Cp values close to k + 1 are good candidates for the best subset of variables.

The best-subset models were developed to determine effects of D, T, RH, and interaction of these three factors on longevity and to better estimate over-drying damage, optimum storage conditions, and accelerated aging at high MC. The results show that drying seeds to very low MC has a detrimental effect on seed longevity. Loss of germination and vigor when seeds were stored at very low MC was apparent at all storage T studied (5-50°C), but was less at lower T. These results are consistent with the finding that over-drying of seeds causes more rapid deterioration (Vertucci and Roos, 1990, 1993).

The capability of many seeds to lose most of their cellular water and not lose their viability is remarkable, but over-drying may cause viability reduction. Sensitivity to drying to very low MC was clear in lettuce and soybean seeds (Figures 6, 7, 10, 11). The reason why detrimental effects were observed from over-drying was probably due to the unstable structure of macromolecules. Vertucci (1990, 1993a) reported the glass structure became unstable if seeds are over-dried. The glass which is disrupted is probably a result of intermolecular interactions because water has been removed from the surfaces of macromolecules (Bruni and Leopold, 1992; Roos et al., 1996). Usually, water protects macromolecules from these interactions. Therefore, the deterioration increased when glassy

water was removed progressively. These results imply that ultra-dry storage technology (FAO/IPGRI, 1994; Zheng et al., 1998) must be used with caution.

One of the primary problems associated with seed preservation is determining the optimum conditions for storing seeds. Traditionally, optimum protocols have been predicted using equations such as Harrington's "rules of thumb" (Harrington, 1963, 1973), or the seed viability equation (Roberts 1973; Ellis and Roberts, 1980), which relies on the extrapolations made at very warm and moist conditions. Vertucci and Roos (1990) have demonstrated optimum moisture levels for orthodox seeds, but optimum MC varies depending on the chemical composition of seeds and the storage T. Optimum moisture levels for storage of any orthodox seed can be easily achieved by equilibrating the seeds at the proper RH (Vertucci and Roos, 1990, 1993). Optimum RH corresponds to the point at which deterioration resulting from aging reactions, desiccation damage, and freezing injury are minimized. Using the models that were developed for germination and vigor in lettuce and soybean seeds clearly indicated an optimum RH of 20-22% for seed storage. Vertucci and Roos (1990) previously proposed that optimum moisture levels could be obtained by equilibrating seeds at 20 to 25% RH. My results were very close to the optimum RH proposed by Vertucci and Roos. Based on these results, germplasm curators must be careful to store seeds at the optimum RH and not to over-dry them (Walters et al., 1998b).

The reason why detrimental effects of over-drying were detected in some experiments and not in others remains unresolved. Data sets that address the question of seed deterioration at low water content are rare since the experiments are logistically difficult. Because aging rates are generally slow under dry conditions, researchers have sought more

sensitive detectors of deterioration than germination assays. Assays measuring changes in radicle growth or time to germination often give the appearance of more rapid deterioration (Vertucci and Roos, 1990; Walters et al., 1998a). Even using similar assays and storage conditions, there can be major differences in deterioration rates among samples from the same species, and these are attributed to differences in seed quality among cultivars and lots.

Conclusions

Models were developed to determine over-drying damage, optimum storage condition, accelerated aging at high MC and interaction of D, T, and RH in lettuce and soybean seeds. Over-drying damage, high MC damage and optimum RH were observed in both lettuce and soybean during storage.

The models showed accelerated deterioration when seeds were dried to extremely low MC. The reduction in germination and vigor increased with D. This deterioration is most probably a result of acceleration of seed aging. This finding suggests that the optimum MC for storage of any orthodox seed can be easily achieved by equilibrating to the proper (optimum) RH. Optimum RH for lettuce and soybean seed storage was about 20 to 22%. Seeds stored at higher RH (higher MC) suffered from accelerated aging resulting in loss viability and vigor.

Based on the models and model-generated response surfaces, all D, T, RH and their interaction are important factors in determining seed longevity.

CHAPTER 5

PHYSIOLOGICAL CHANGES UNDER OVER-DRYING CONDITION

Introduction

Seed moisture has been traditionally viewed as the most important factor determining seed longevity (Justice and Bass, 1978). Numerous experiments have reported the structural and functional importance of water molecules in seeds. Water is a substrate, its concentration affects the equilibrium between reactants and products. It acts as a solvent for enzyme catalyzed reactions (Rupley *et al.*, 1983; Leopold and Vertucci, 1989; Vertucci, 1989a, 1990; Bradford, 1994). However, its role in seed aging and its interaction with other factors has been poorly documented. Water-binding characteristics can be defined into five types according to seed moisture and temperature (T) (Vertucci, 1993b; Vertucci and Farrant, 1995). Physiological activity corresponds to the different types of water present. Consequently the mechanism of seed aging varies with hydration level. Classification by moisture binding has biophysical, biochemical and physiological significance, because there may be different physiological reactions occurring at different moisture regions (Vertucci and Leopold, 1986; Leopold and Vertucci, 1989; Vertucci and Farrant, 1995).

When seeds deteriorate, they lose vigor and become more sensitive to stresses upon germination. Eventually seeds lose the ability to germination. Membranes become leaky,

enzymes lose catalytic activity, and lipid autoxidation/peroxidation when seeds deteriorate (Kaloyereas, 1958; Wilson and McDonald, 1986; Priestley, 1986; Smith and Berjak, 1995).

The most current aging test, is based mainly on germinability, including germination (%), seedling growth or resistance to stress, reflected a final result caused by various deteriorative processes occurring during storage and imbibition (Smith and Berjak, 1995). Causes of early aging can not be determined by germination and/or vigor tests. Methodology for seed aging assays is the major limit for research on seed aging. Seed volatile production may have potential for monitoring aging.

In Chapter 4, the models predicted over-drying damage, optimum storage conditions, and accelerated aging at high moisture content (MC) based on different seed moisture levels. Physiological activities vary at different seed MC. The objective of this experiment was to determine the physiological nature of deteriorative reactions occurring at low seed moisture and their effects on seed quality and to determine the consequence of drying to less than optimum MC. Mechanisms of seed deterioration were investigated on the basis of seed germination, vigor, leachate conductivity, volatile production, and dehydrogenase activity.

Materials and methods

Lettuce (cv. Black Seed Simpson, Henry Field Seed & Nursery Co., Shenandoah, Iowa, initial germination, 96%) and soybean (cv. Williams, Pioneer Hi-Bred International Inc., Des Moines, Iowa; initial germination, 92%) seeds were used in this study since high quality seeds are readily available from commercial sources and since the rate at which germination and growth potential are lost has been well documented as being a function of storage MC and T.

Lettuce

1. Seed storage

Lettuce seeds were equilibrated at approximately 1% RH (over P_2O_5), 22% (over KAc) and 75% (over NaCl) at 35°C (Table 1). Five grams of seeds were put in gas-tight bottles filled or not filled with N_2 after equilibration and stored at 35°C. Seed moisture, germination, vigor (root length), volatile production and leachate conductivity were assayed at approximate two month intervals during storage.

2. Seed leachate conductivity

To measure membrane damage at low moisture content, conductivity of seed leachates were measured using an ASAC-1000 (Automatic Seed Analyzer by Neogen Food Tech. Crop., Lansing, Michigan) at a setting of two volts. Sample size was 100 seeds. Seeds were placed in a white plastic leakage tray (10 seeds per well) containing 2 mls of deionized water. Leakage was expressed as microamps/seed.

3. Volatile production

Volatile production in response to treatments was determined using a gas chromatograph. Seeds were adjusted to equilibrium moisture content at various combinations of relative humidity (RH) and T and then stored in gas-tight bottles. Some were stored in gas-tight bottles filled with N_2 to determine volatile productions. Analysis of lettuce seed volatiles involved 200 ul gas samples removed from a gas-tight bottle using a syringe. Samples were then injected into a gas chromatograph (GC, Perkin-Elmer 8500, Norwalk, CT, USA). Volatiles in the gas sample were separated with a packed column (80/90 Carbopack C/0.2% Carbowax 1500, Supelco, Bellefonte, PA, USA) with nitrogen as

the carrier gas. Separation temperature was programmed from 35°C to 135°C at 5°C/min, and then was set at 135°C for 20 minutes. Volatile components were identified according to the relative retention time vs the standard. Amounts of volatiles were calculated from GC peak area with three replications.

Soybean

1. Seed storage

Soybean seeds were equilibrated at approximately 1% RH (over P_2O_5), 22% (over KAc) and 75% (over NaCl) at 35°C (Table 1). Five grams of seeds were put in gas tight bottles after equilibration and stored at 35°C. Seed moisture, germination, vigor (root length), leachate conductivity and dehydrogenase activity were assayed at approximate two month intervals during storage.

2. Seed leachate conductivity

To measure membrane system damage at low moisture content, conductivity of seed leachates were measured using an ASAC-1000 (Automatic Seed Analyzer Computer by Neogen Food Tech. Crop., Lansing, Michigan) at a setting of two volts. Twenty-five seeds from each treatment were used to measure conductivity. Seeds were placed in a white plastic leakage tray (1 seed per well) containing 2 mls of deionized water.

3. Dehydrogenase activity

The Triphenyl Tetrazolium Chloride (TTC) reduction test was used to measure dehydrogenase activity of soybean seeds for assessing vigor. After seeds were imbibed in distilled water at room temperature for 1 hour, they were cut longitudinally through the midsection and then incubated at 35°C for 3 hours in a solution of 1% (W/V) TTC with a

phosphate buffer (pH 7). Samples were heated in 2 ml 95% ethanol at 55°C for 2 hours to extract the reduced formazan. The cooled extracts were measured using a spectrophotometer at 530 nm for assessing the level of TTC reduction.

To avoid differences in imbibitional stress among different treatments, all lettuce and soybean seeds were adjusted to the same initial water content (lettuce, 10%; soybean, 12%) before germination, vigor (root length), leachate conductivity and dehydrogenase activity were assayed. This was accomplished by equilibrating seeds over a saturated solution of $Ca(NO_3)_2$ (55% RH) for 24 hours on the laboratory bench at room temperature (22-24°C).

Results

Lettuce seeds

1. Germination and vigor during storage

Lettuce seeds with 1, 4, and 10% MC were stored at 35°C for 24 months. Germination and vigor were measured every two months. For seeds with 1% MC, germination with N_2 was higher than without N_2 during storage (Figure 12). Germination of seeds stored in N_2 and air treatment was not different for seeds with 4% MC (Figure 13). Again for seeds with 10% MC, no difference was seen between treatments (Figure 14).

Vigor with N_2 was higher than without it at 1% MC (Figure 15). Vigor with and without N_2 showed no difference in seeds at 4% and 10% MC (Figures 16 and 17).

Damage from over-drying was observed in seeds with very low MC (1%). Germination and vigor were improved considerably when seeds were stored in N_2 at low MC.

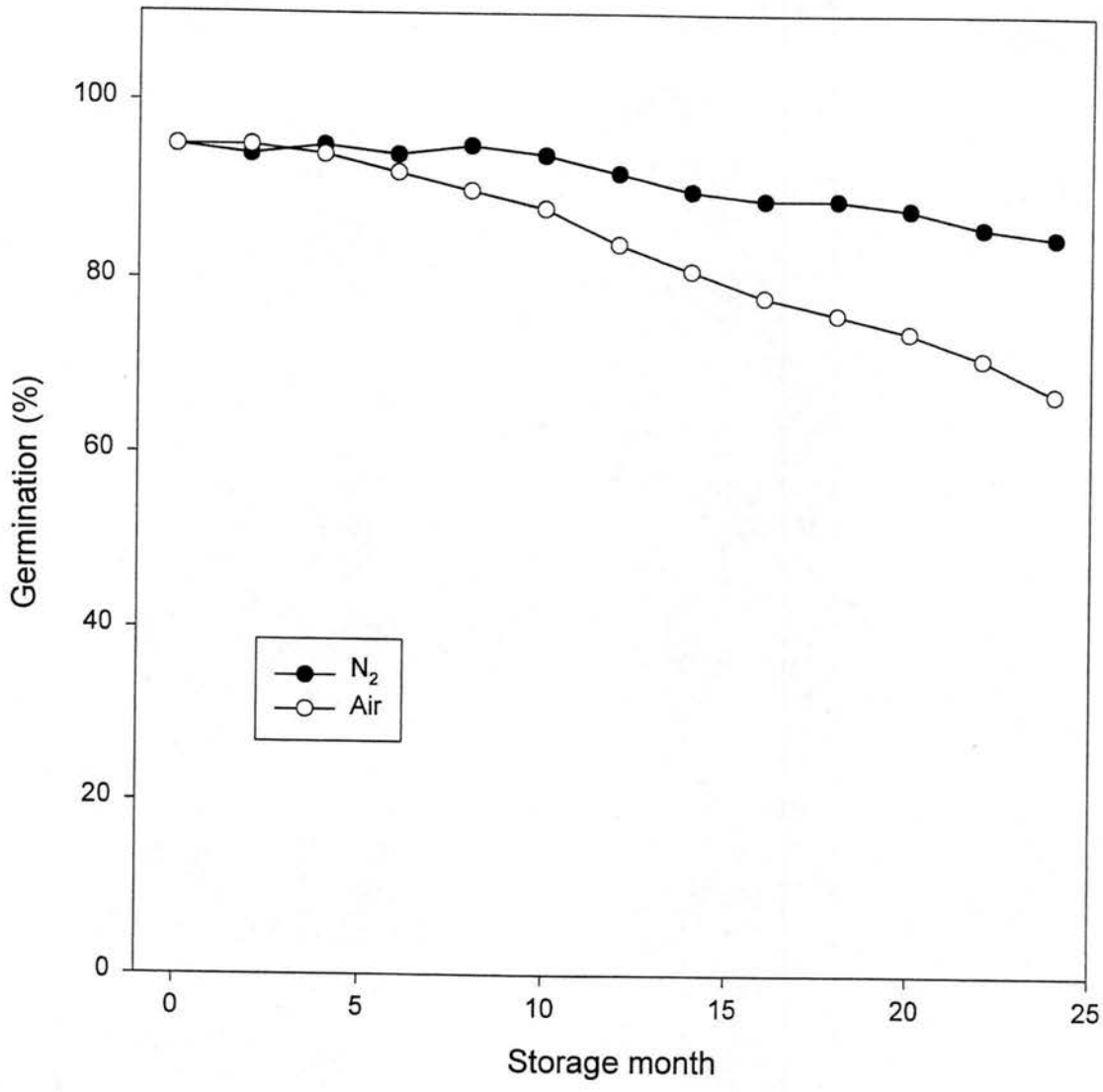


Figure 12. Germination of lettuce seeds (1% MC) with N_2 treatment stored at 35°C for 24 months.

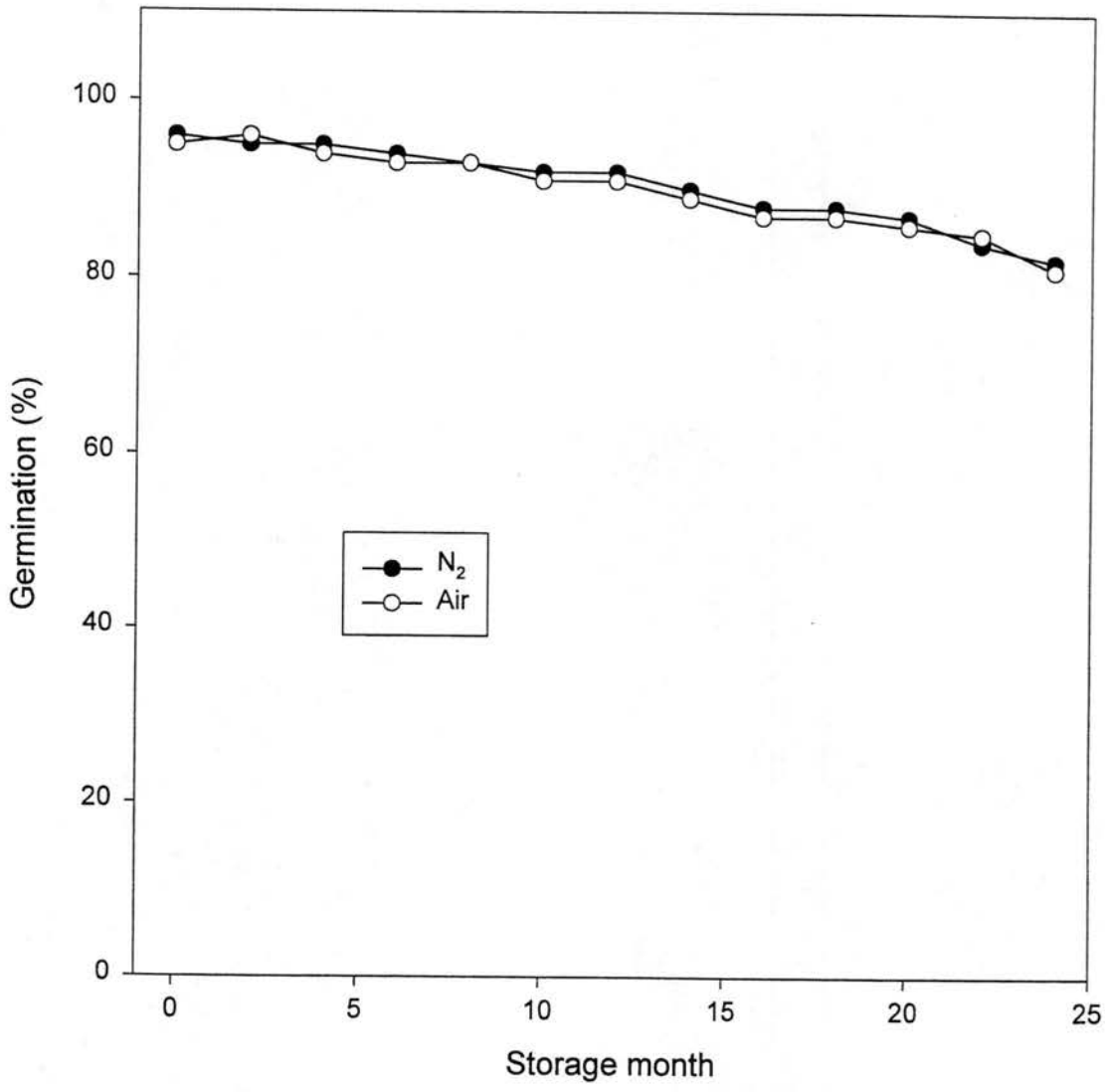


Figure 13. Germination of lettuce seeds (4% MC) with N_2 treatment stored at 35°C for 24 months.

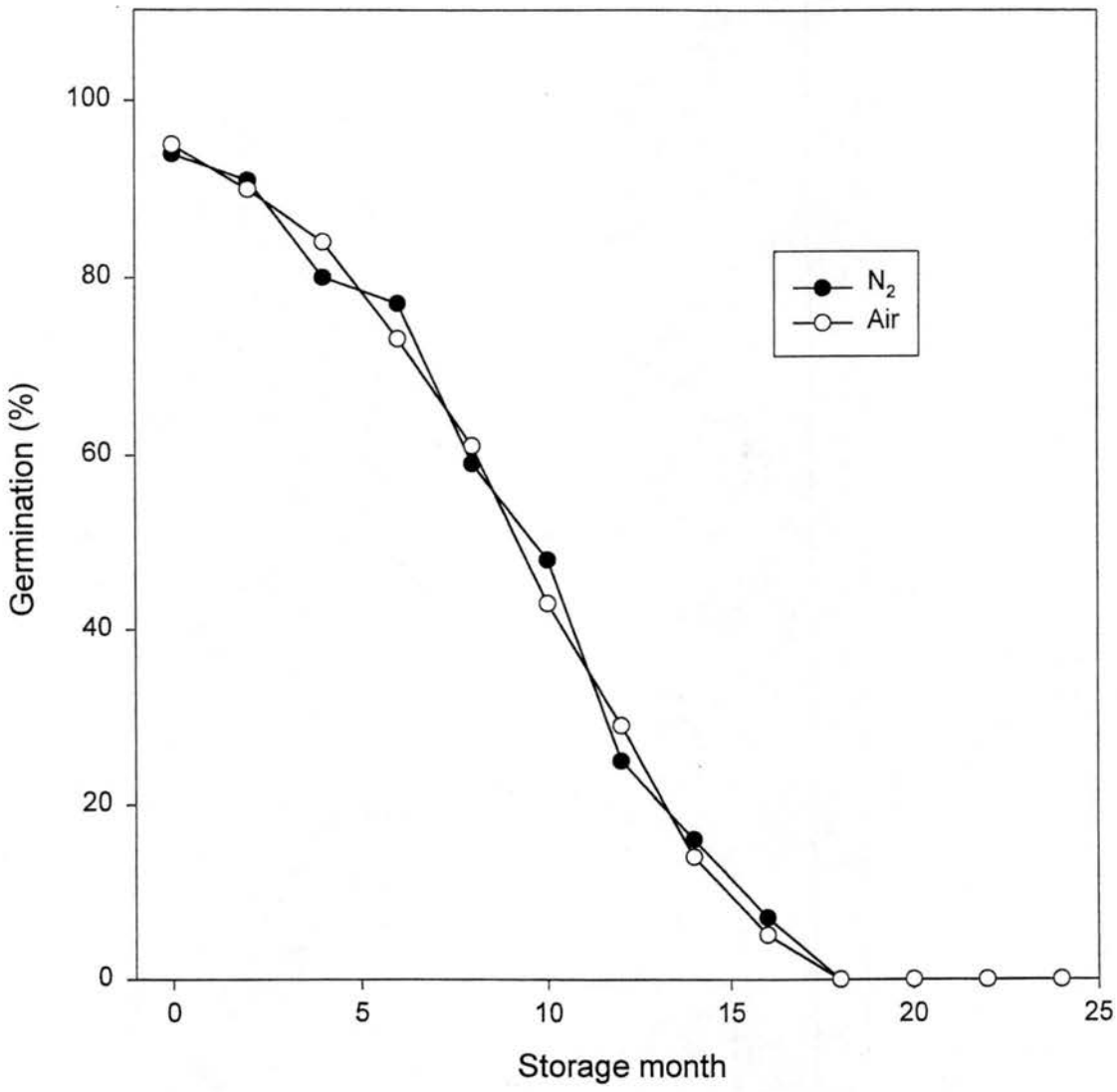


Figure 14. Germination of lettuce seeds (10% MC) with N_2 treatment stored at 35°C for 24 months.

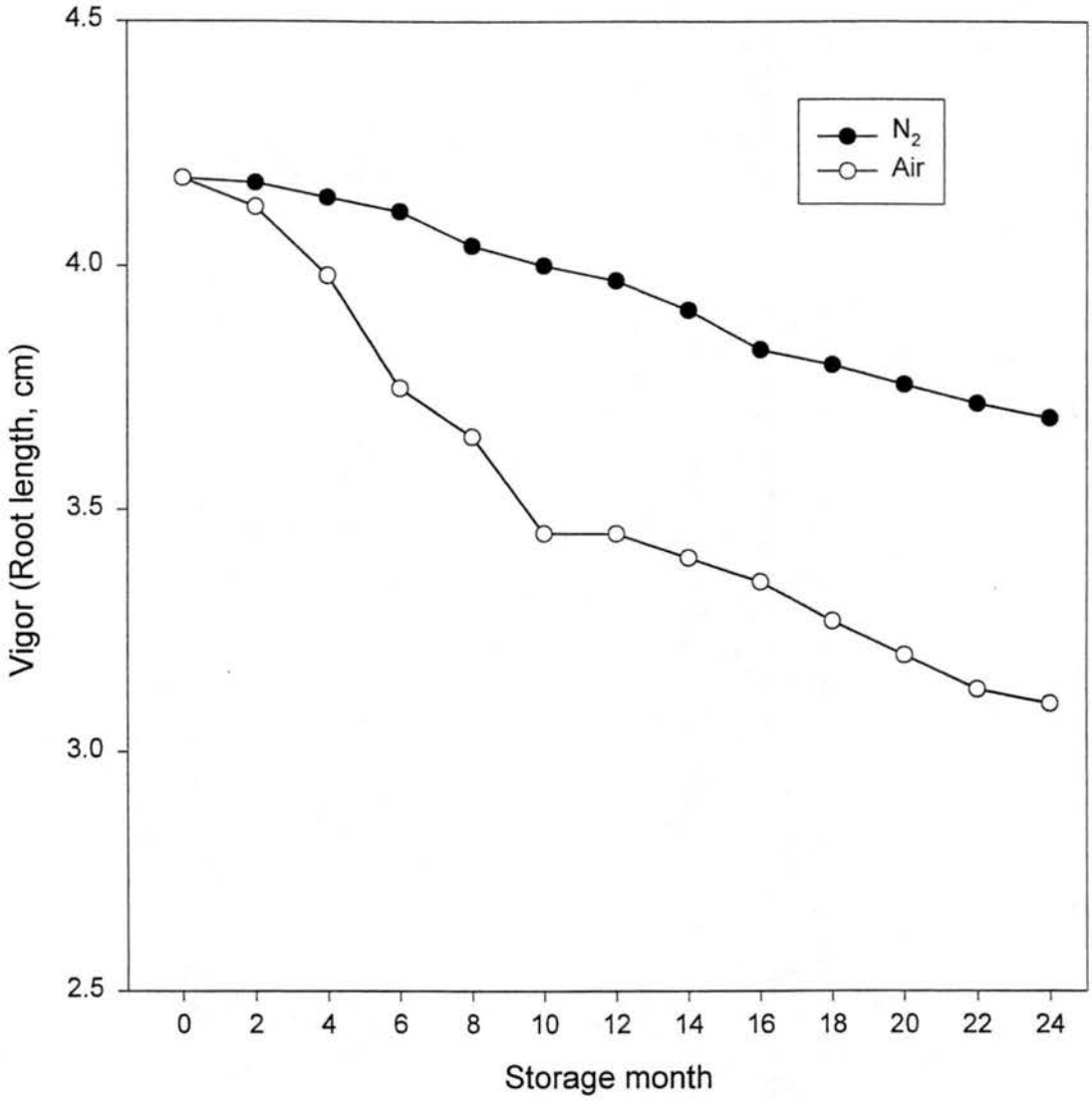


Figure 15. Vigor of lettuce seeds (1% MC) with N_2 treatment stored at 35°C for 24 months.

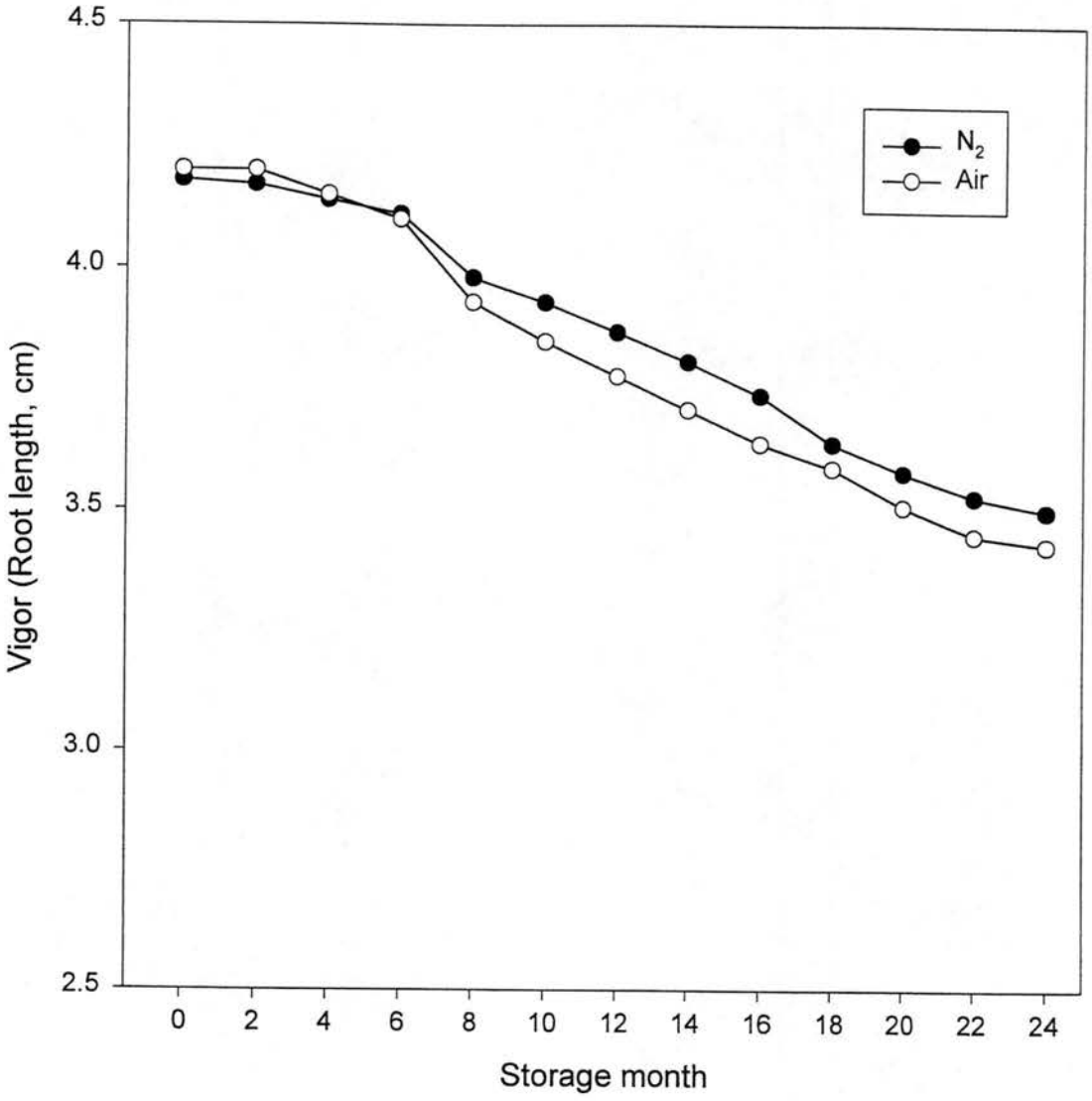


Figure 16. Vigor of lettuce seeds (4% MC) with N_2 treatment stored at 35°C for 24 months.

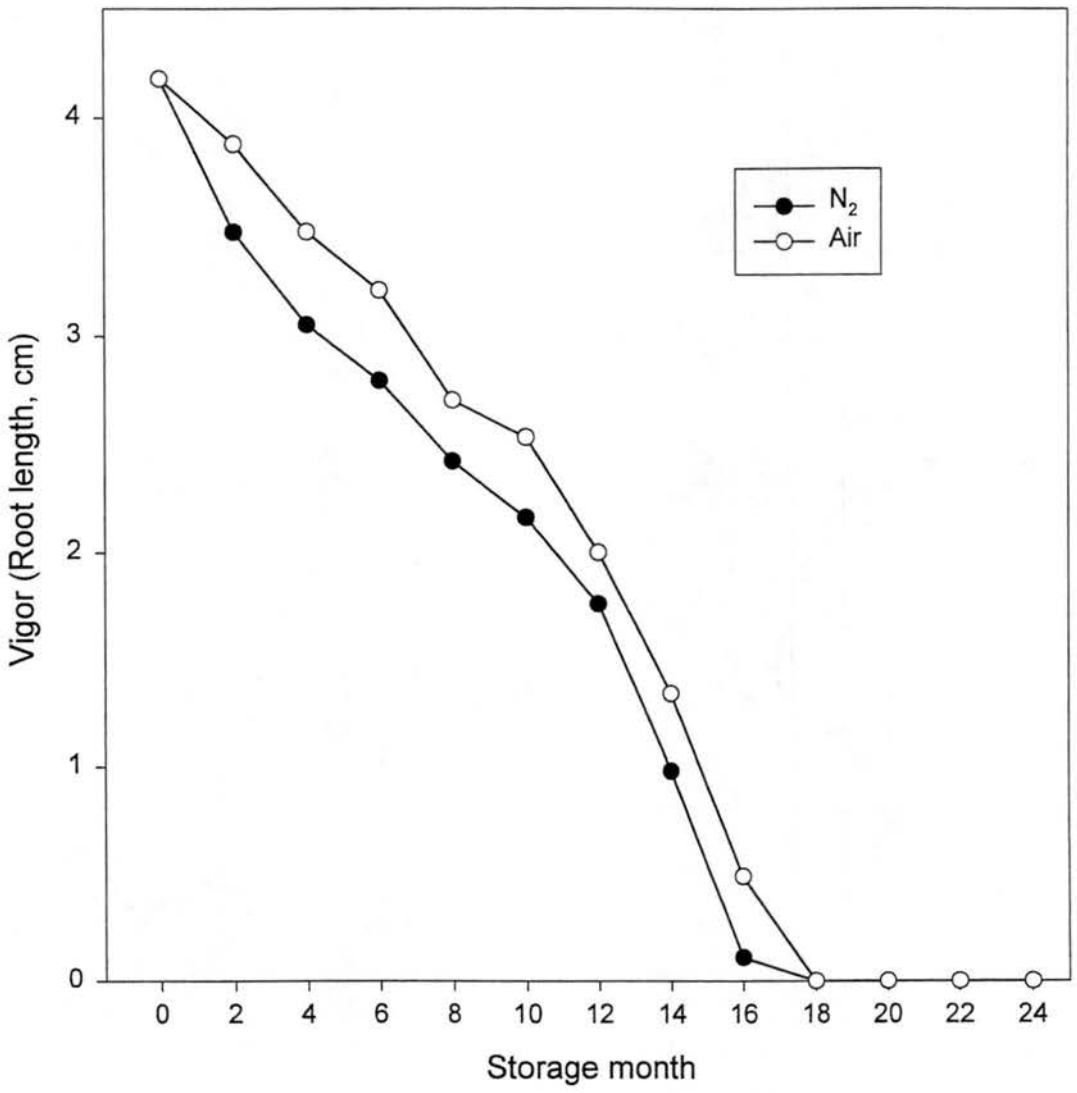


Figure 17. Vigor of lettuce seeds (10% MC) with N_2 treatment stored at 35°C for 24 months.

2. Hexanal production under over-dried conditions

Lettuce seeds with 1, 4, and 10% MC were stored at 35°C for 24 months. Hexanal production was measured every two months during storage. Hexanal production greatly increased in seeds with 1% MC (Figure 18), and slightly increased in seeds with 4% MC (Figure 19), but hexanal production was not observed in seeds having a MC of 10% (Figure 20). However, hexanal production in seeds with N_2 was lower than these without N_2 in seeds stored at 1% MC (Figure 18). The result showed N_2 treatment inhibited hexanal production at very low MC. Hexanal is probably produced from peroxidation reactions at very low MC. N_2 treatment inhibited the peroxidation reaction and therefore improved germination and vigor at very low MC.

3. Leachate conductivity during storage

Lettuce seeds with 1, 4, and 10% MC were stored at 35°C for 24 months. Leachate conductivity was measured every two months during storage. As expected conductivity of seed leachate was negatively related to viability. All leachate conductivity increased in 1%, 4%, and 10% MC seeds (Figures 21, 22, and 23). Leachate conductivity of seeds with 5% and 10% MC, did not differ between N_2 and air treatments (Figures 22 and 23). At very low MC (1%), leachate conductivity of seeds subjected to the N_2 treatment was lower than these having the air treatment (Figure 21). Over-drying caused an increase in leachate conductivity which may have resulted from changes in the membranes in seeds but storage in N_2 treatment reduced conductivity increases at 1% MC.

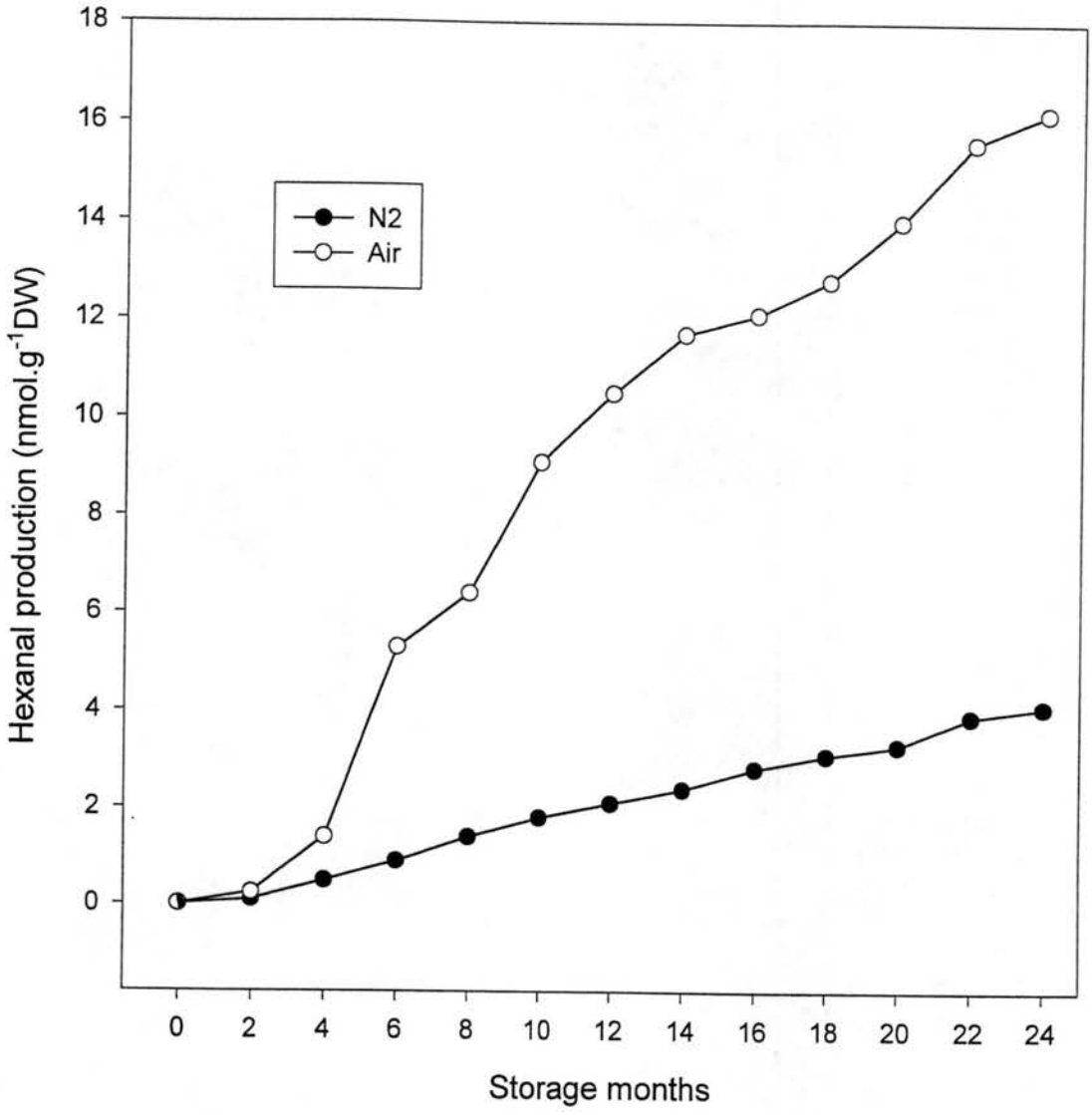


Figure 18. Hexanal production of lettuce seeds (1% MC) with N_2 treatment stored at 35°C for 24 months.

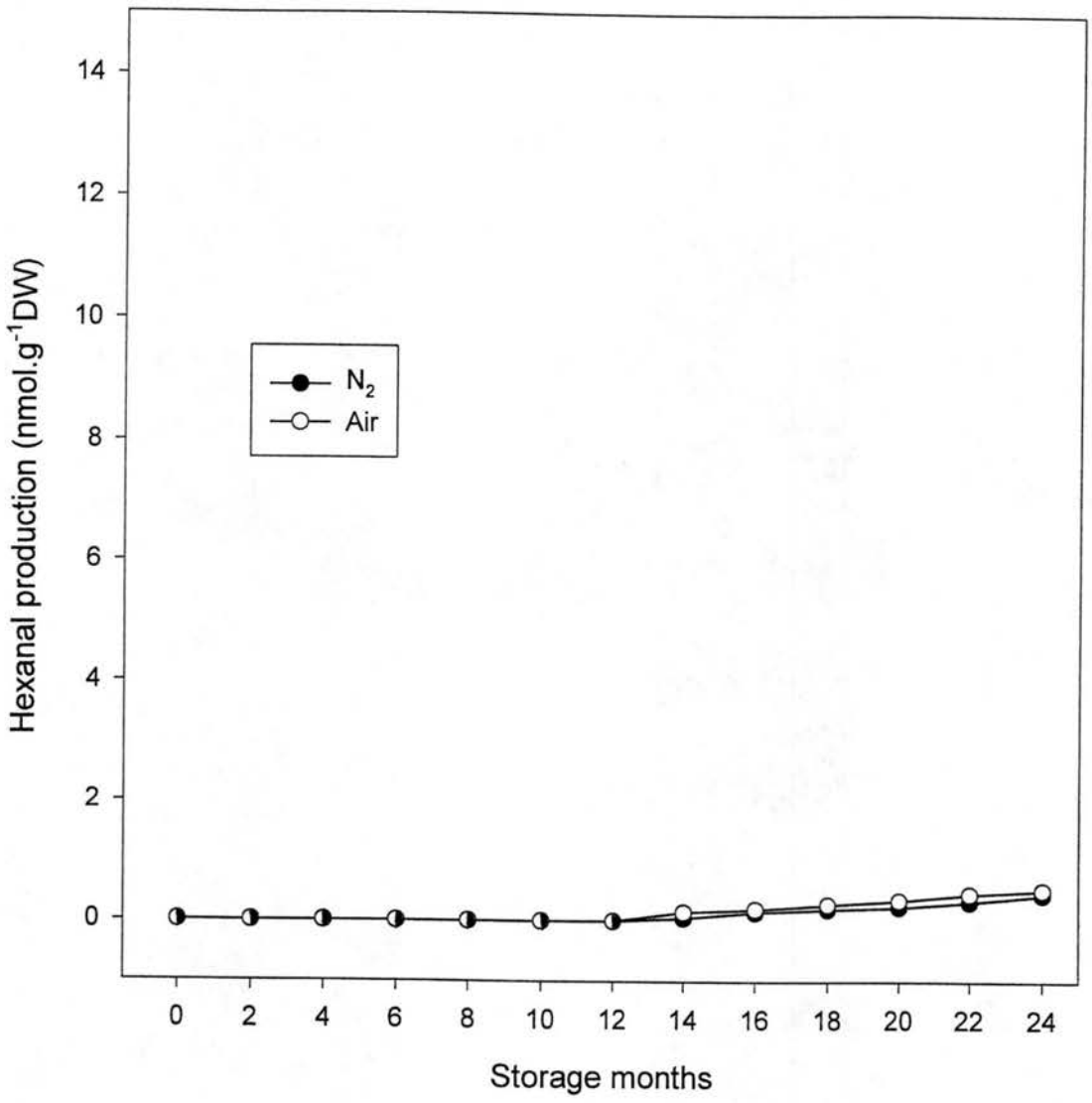


Figure 19. Hexanal production of lettuce seeds (4% MC) with N_2 treatment stored at 35°C for 24 months.

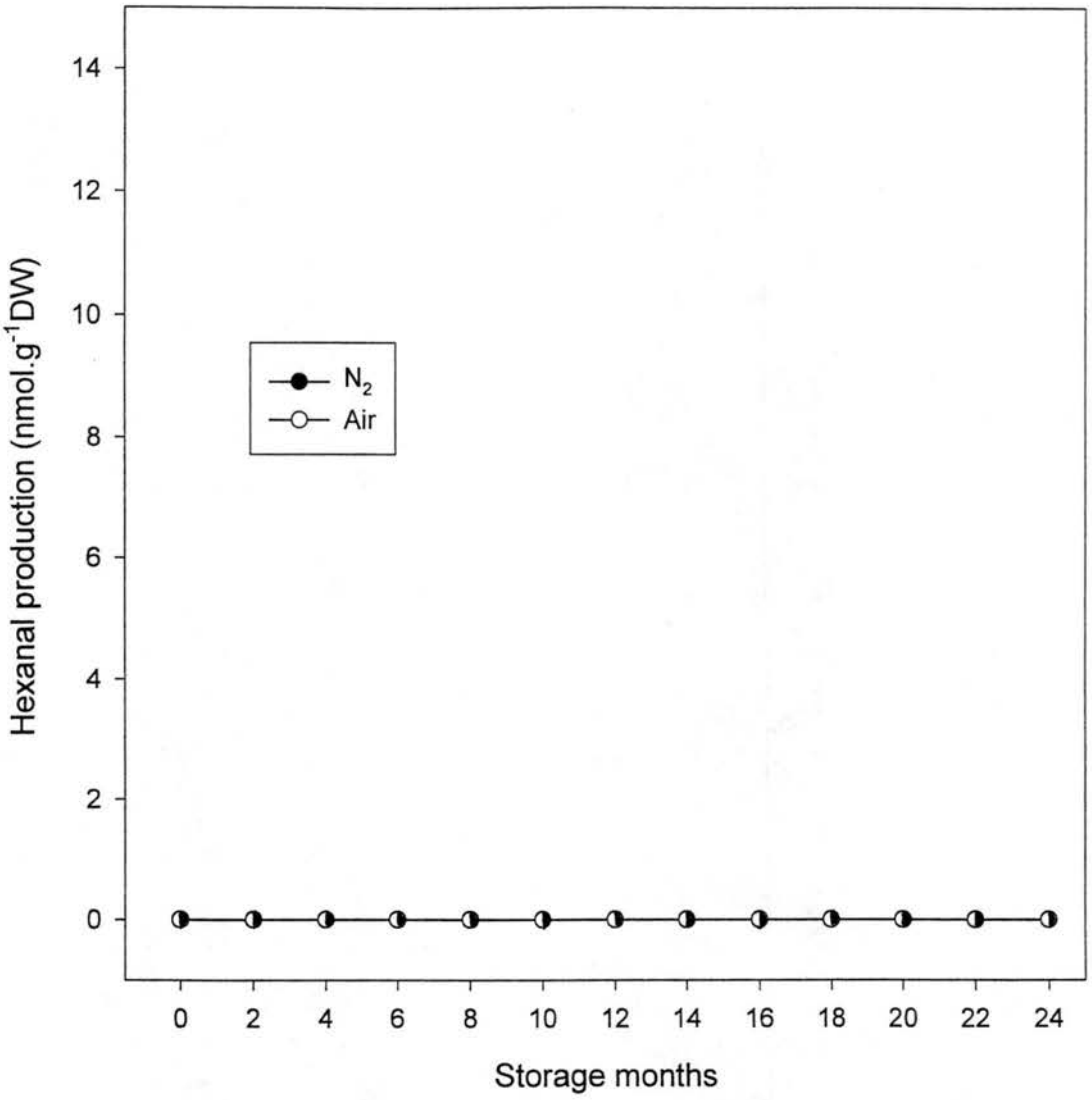


Figure 20. Hexanal production of lettuce seeds (10% MC) with N_2 treatment stored at 35°C for 24 months.

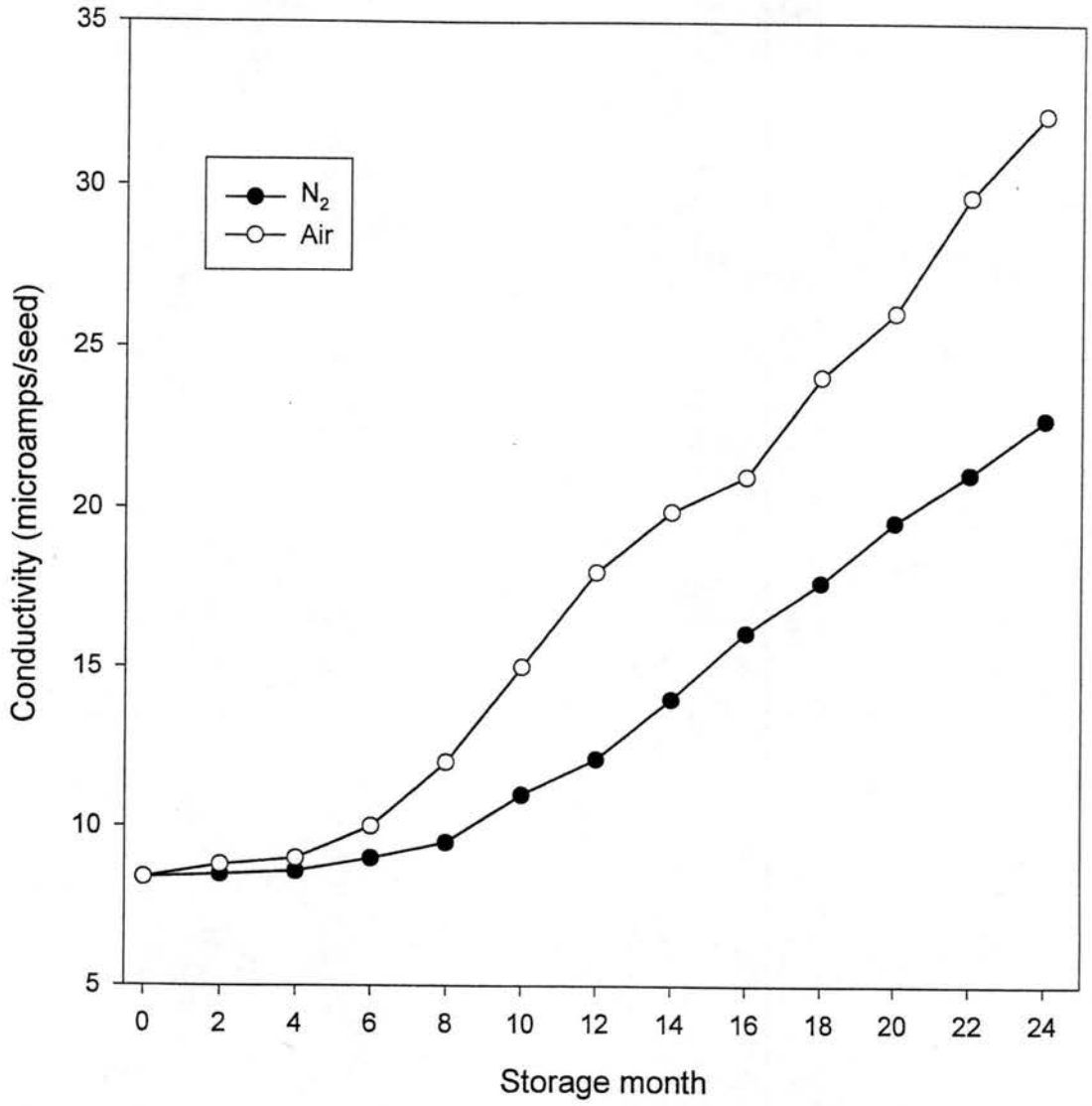


Figure 21. Leachate conductivity of lettuce seeds (1% MC) with N_2 treatment stored at 35°C for 24 months.

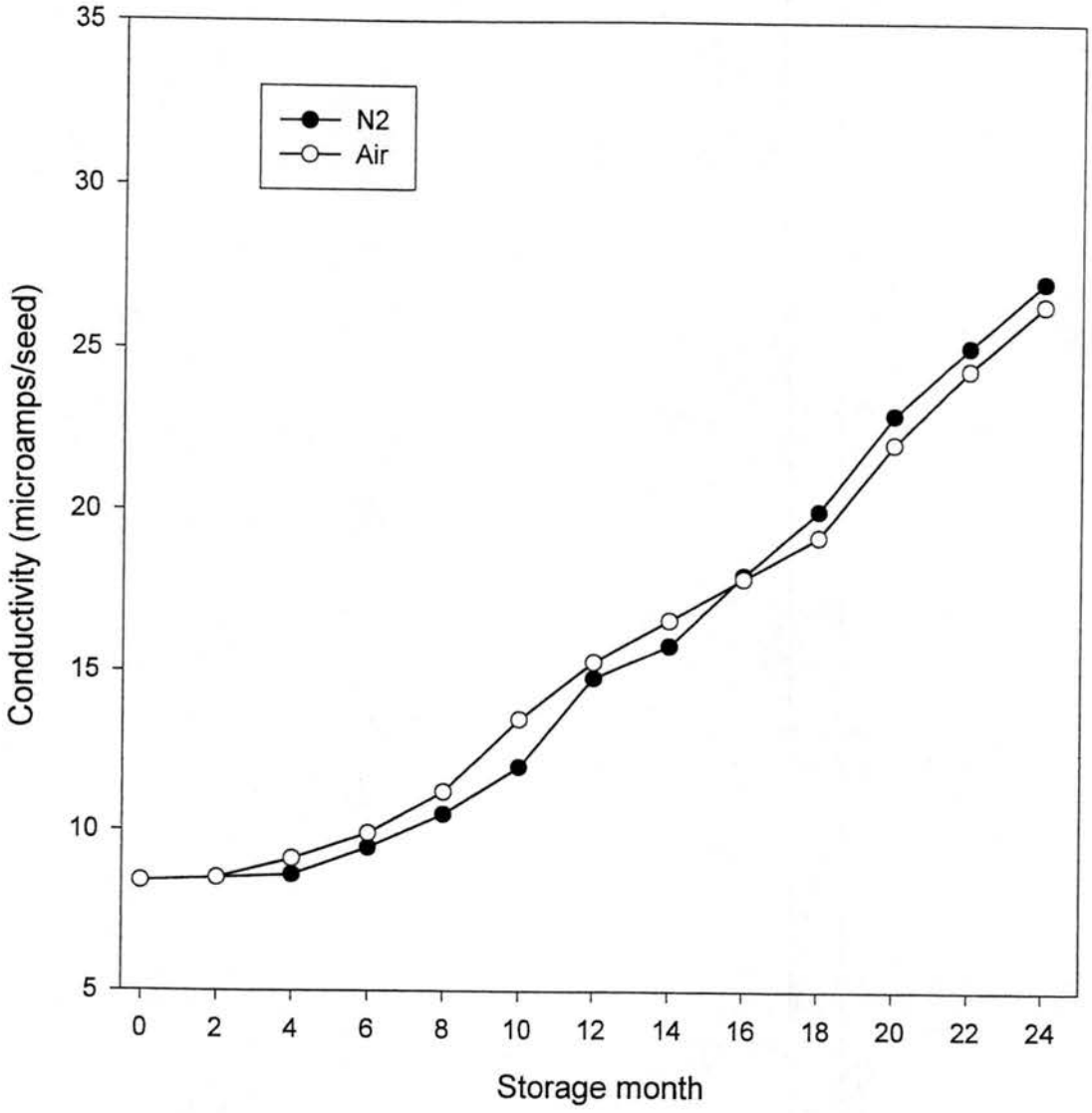


Figure 22. Leachate conductivity of lettuce seeds (4% MC) with N_2 treatment stored at 35°C for 24 months.

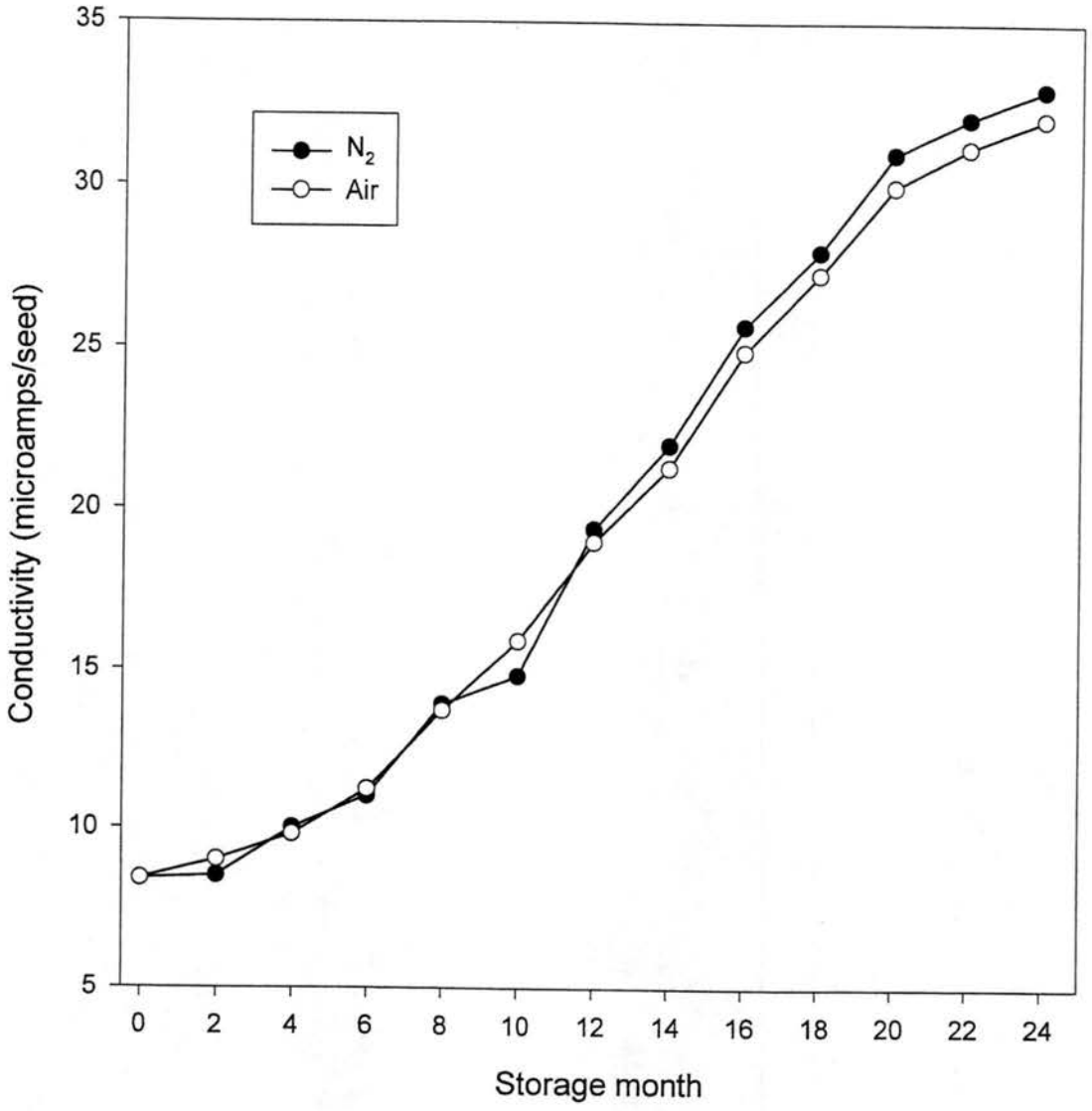


Figure 23. Leachate conductivity of lettuce seeds (10% MC) with N_2 treatment stored at 35°C for 20 months.

Soybean seeds

1. Germination and vigor during storage

Soybean seeds with 1, 5, and 14% moisture content were stored at 35°C for 12 months. Germination and vigor were measured every two months during storage. All germination values decreased during storage (Figure 24). Germination decreased in 14% moisture content seeds was the greatest, followed by seeds with 1% moisture content. Germination decrease in seeds with 5% moisture content was the lowest. Vigor also decreased during storage (Figure 25) and followed a pattern similar to germination with 14%MC showing the greatest decline followed by seeds with 1% and 5% MC.

2. Leachate conductivity during storage

Soybean seeds with 1, 4, and 10% moisture content were stored at 35°C for 24 months. Leachate conductivity was measured every two months. As expected, conductivity increased during storage (Figure 26). Conductivity increased in seeds having a 14% MC the greatest, followed by seeds with 1% MC. Seeds with 5% MC showed less increase in conductivity during storage.

The results showed that seed deterioration in high MC seeds and very low MC seeds was greater than in seeds with optimum MC (5%). Over-drying may cause damage of the membranes in soybean seeds.

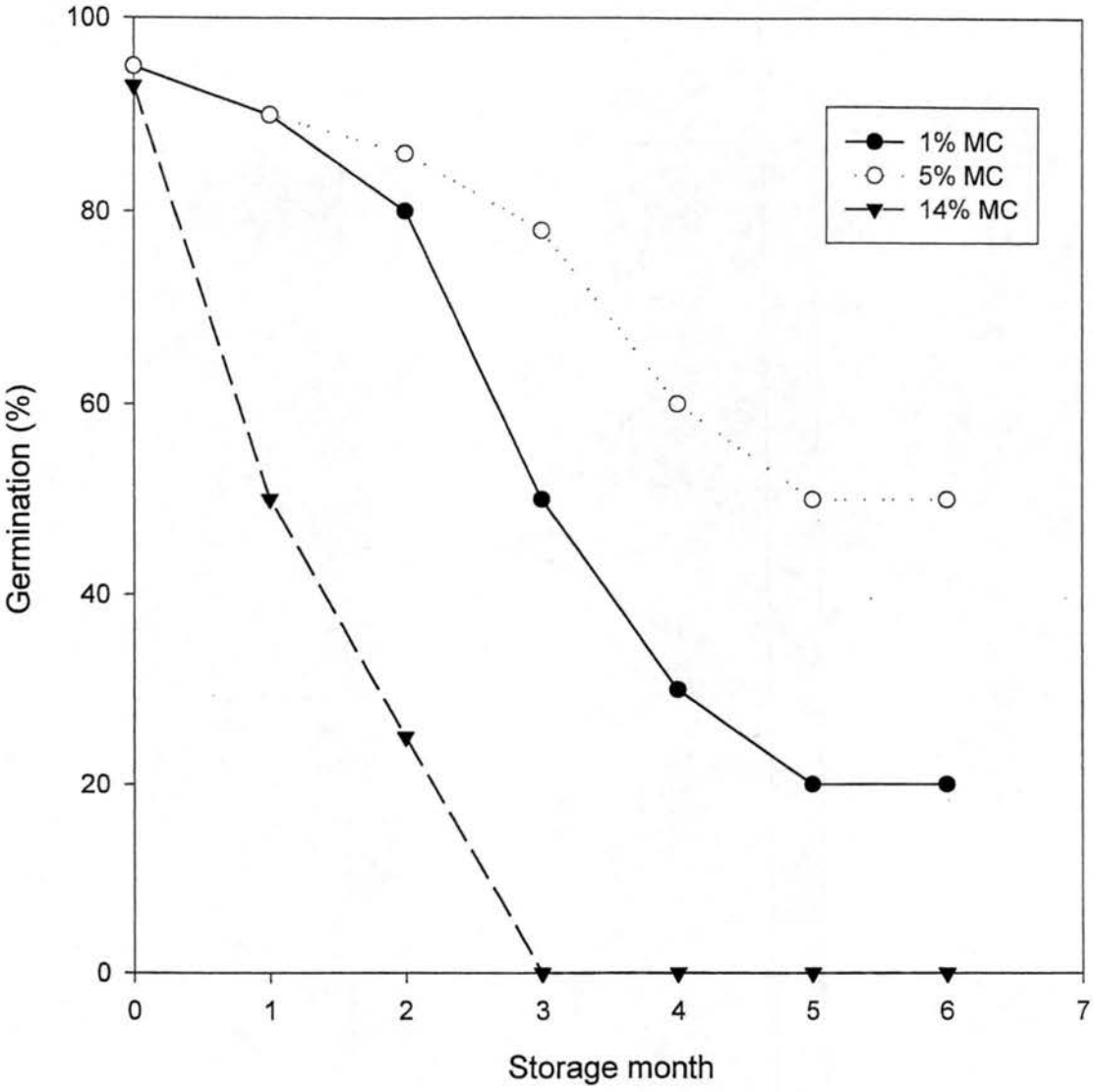


Figure 24. Germination of soybean seeds stored at 1, 5 and 14% MC for 12 months at 35°C.

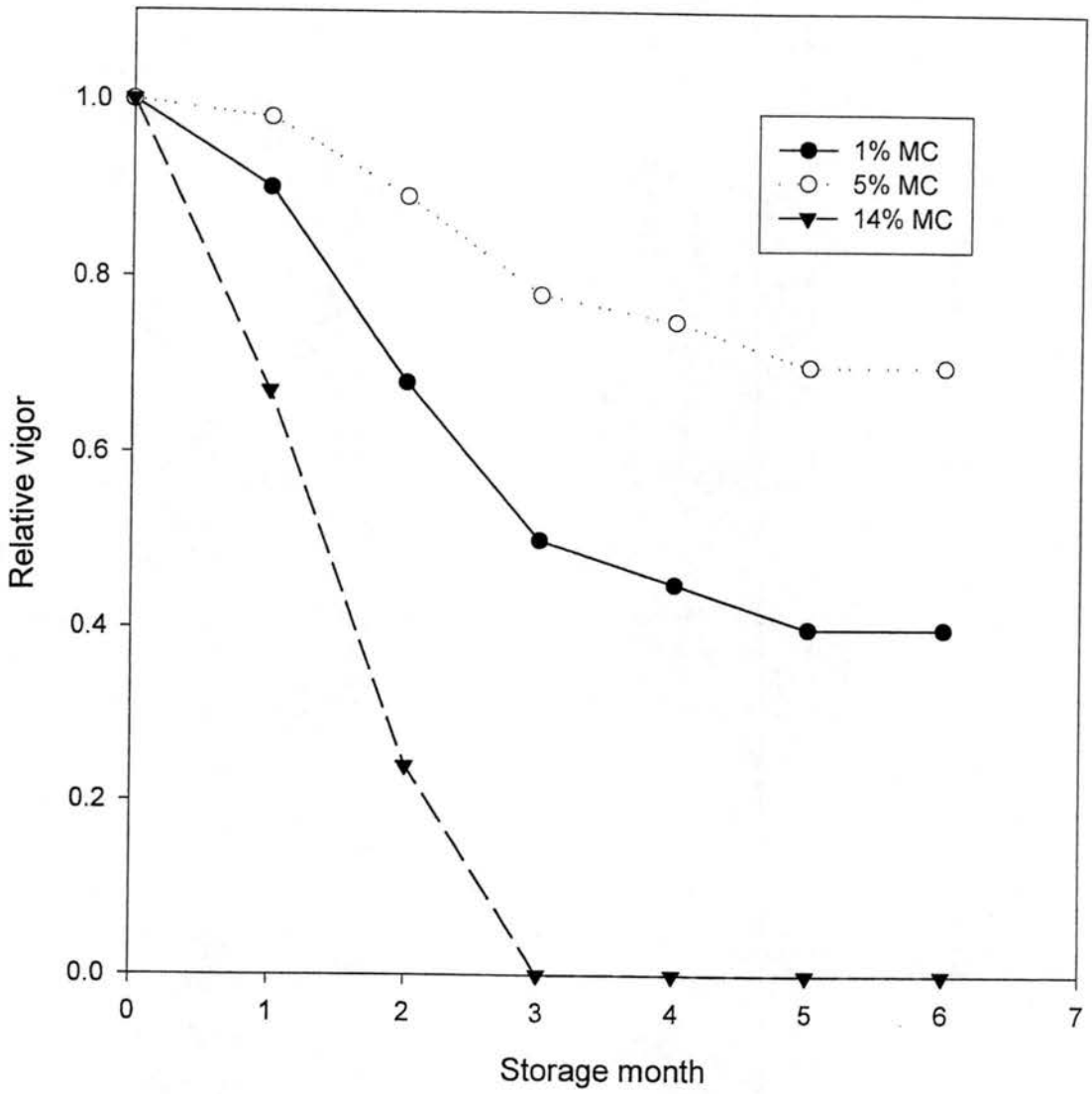


Figure 25. Relative vigor of soybean seeds stored at 1, 5 and 14% MC for 12 months at 35°C.

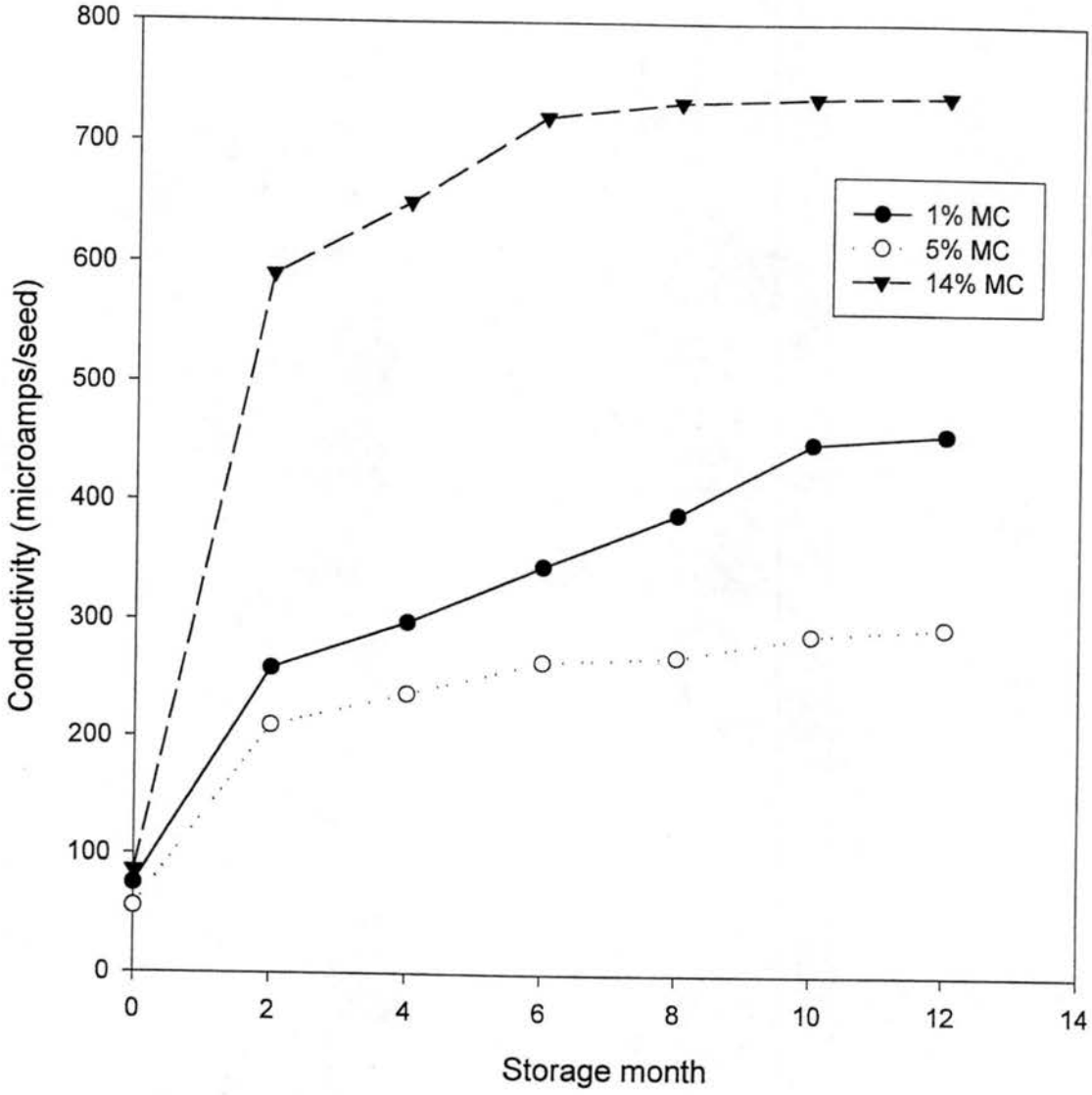


Figure 26. Leachate conductivity of soybean seeds stored at 1, 5 and 14% MC for 12 months at 35°C.

3. Dehydrogenase activity during storage

Soybean seeds with 1, 4, and 10% MC were stored at 35°C for 12 months. Dehydrogenase activity was measured every two months during storage. Dehydrogenase activity decreased during storage (Figure 27). The greatest decrease in dehydrogenase activity was in seeds with 14% MC followed by seeds with 1% MC. Dehydrogenase activity decrease in 5% moisture content was the smallest. Not only did the dehydrogenase activity decrease in seeds with 14% MC but it also decreased in seeds with 1% MC. The latter are considered to be over-dried.

Discussion

All seeds deteriorate and eventually die whether they are stored in soil banks, genebanks or liquid nitrogen. However, seed deterioration is minimized when they are stored at optimum storage conditions. The experiments showed that maximum seed germination and vigor can be gained when lettuce and soybean seeds are stored at optimum MC. Storage at high MC always resulted in reduced germination and vigor. Also, storage at extremely low MC resulted in reduced germination and vigor. In other words, drying to extremely low water content shortened seed longevity. The potential risks of drying seeds to low water content must be addressed.

Non-enzymatic oxidation contributes to seed deterioration in regions of low MC such as type 1 water (Vertucci and Farrarnt, 1995). One of the non-enzymatic reactions is lipid peroxidation. Seeds accumulate an oxidation product, lipid peroxide, when they are stored

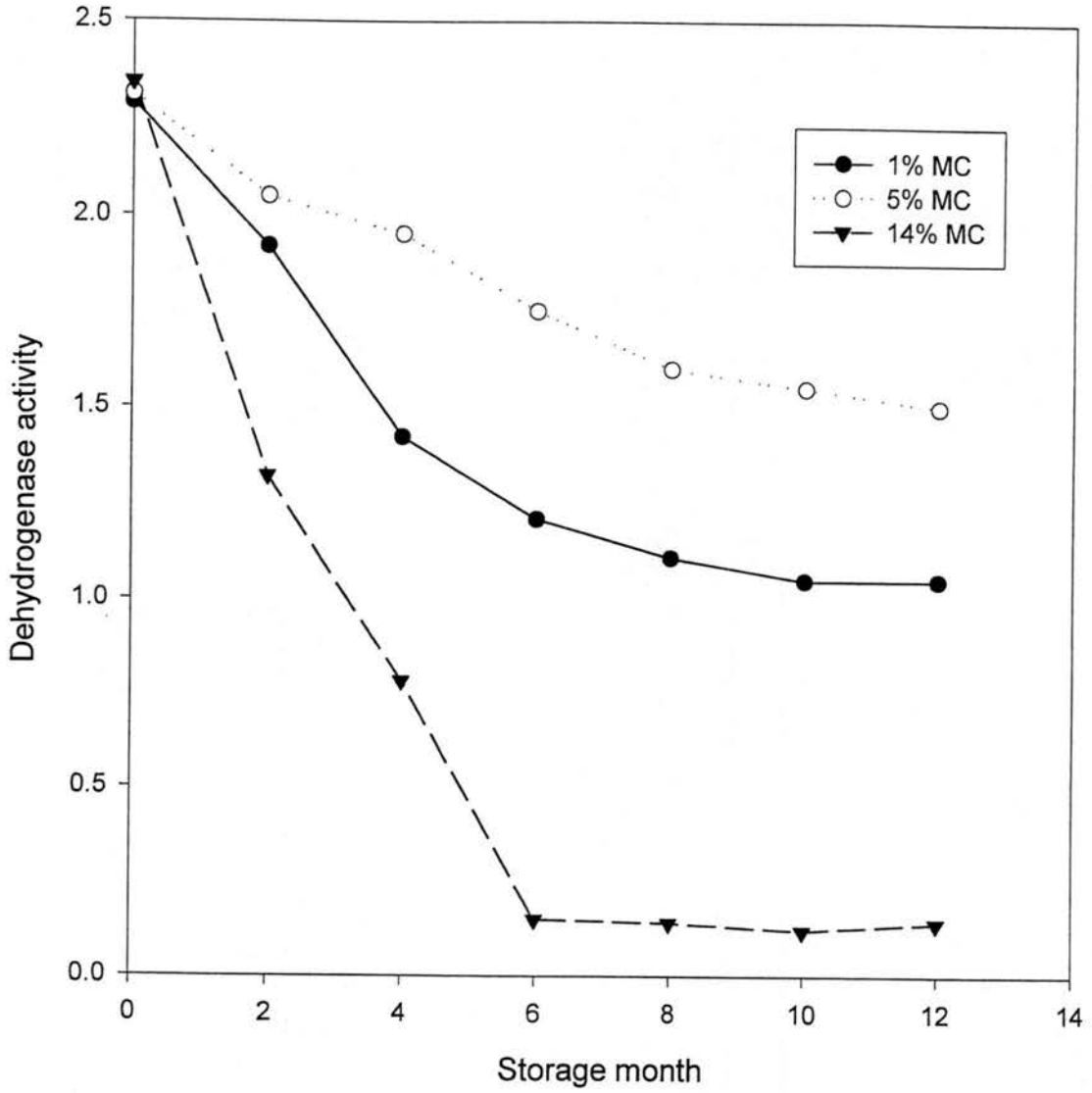


Figure 27. Dehydragenase activity of soybean seeds stored at 1, 5 and 14% MC for 12 months at 35°C.

at low MC. This oxidation product would have a detrimental effect on seeds. Lipid peroxides decompose to hexanal, ethane, several aldehydes, and CO_2 (Priestley, 1986; Frankel et al., 1989; Zhang et al., 1995a, 1995c). Lipid peroxide decomposition products might affect cellular and macromolecular structure and function (Priestley, 1986). Membrane deterioration (changes in lipid fluidity and permeability) and inactivation of membrane proteins might be occurring. Leachate conductivity could increase and energy supply may also be limiting due to damage of membrane systems and enzyme (protein). In this experiment, increases in leachate conductivity were observed when seeds were over-dried.

Lipid peroxidation occurs when a free radical attacks an unsaturated lipid (Priestley, 1986). Lipid reacts with molecular oxygen to form a lipid peroxy radical which abstracts hydrogens from other lipids. Lipid peroxidation was measured by determining hexanal production (Frankel et al., 1989). Peroxidation might be inhibited by eliminating oxygen in the storage environment when storing seeds at low MC. In the experiment, hexanal production increased when seeds were over-dried. The N_2 treatment inhibited peroxidation and thus reduced hexanal production. Seed damage was reduced and germination and vigor were improved when seeds with 1% MC were stored in N_2.

Optimum seed MC changes as a function of T (Vertucci and Roos, 1993; Vertucci et al., 1994). Because the water holding capacity of air at a given RH increases as T decreases, the optimum water content for seed storage would increase as the storage T was lowered. Optimum seed MC level also varies as a function of seed composition (Vertucci and Roos, 1993; Vertucci et al., 1994). For any given RH, the equilibrium water content will

be less in seeds with greater lipid contents (Vertucci and Roos, 1990). In this experiment, the optimum seed MC was 4% for lettuce and 5% for soybean when seeds were equilibrated at a RH of 22% at 35°C because lipid content in lettuce seeds is higher than in soybean seeds.

The optimum moisture level for seed storage should be based on temperature and lipid content. However, the RH at which seeds are equilibrated provides a better means of achieving optimum MC. Optimum moisture levels for storage of any orthodox seed can be easily achieved by equilibrating seeds at the proper RH (Vertucci and Roos, 1990, 1993). The results showed the optimum seed MC can be gained when seeds are equilibrated at 20-22% RH.

Conclusions

These experiments showed maximum seed germination and vigor were maintained during storage when lettuce and soybean seeds were stored at optimum RH which was 20-22%. Over-drying damage was observed when seeds were stored at extremely low MC. Seed storage at high MC always resulted in reduced germination and vigor as expected.

For lettuce seeds, over-drying increased hexanal production and leachate conductivity. Storage of seeds in a N_2 environment reduced hexanal production and leachate conductivity. Seed germination and vigor were improved when seeds were stored at extremely low MC and treated with N_2. Over-drying increased leachate conductivity and decreased dehydrogenase activity for soybean seeds. Germination and vigor decreased when seeds were stored at extremely low MC.

SUMMARY AND CONCLUSIONS

In this dissertation, I attempted to 1) determine relative humidity (RH), temperature (T), and the interactive effect of these two factors on seed moisture content (MC) by using best-fit subset models to generate three dimensional response surfaces 2) determine storage duration (D), T, RH, and the interaction of these three factors on seed quality 3) determine the physiological nature of deteriorative reactions occurring at low moisture and their effects on seed quality during storage.

Best-fit subset models were developed to predict the interaction of storage RH and Ton MC of cucumber, lettuce, maize, onion, pea, and watermelon seeds. These models were selected by using Mallows' Cp minimum method. All the models had the same functional form, $MC = \beta_0 + \beta_1*RH + \beta_2*T + \beta_3*RH^2 + \beta_5*RH*T + \beta_6*RH^3 + \beta_9*RH^2*T$. Bias was also avoided by using Mallow's graphic method. Based on the models and model-generated response surfaces, RH has a greater influence on seed MC than does T. Relative humidity has a strong positive effect while T has a slightly negative effect on MC. The extremely strong interactive effect of RH and T on seed MC is documented by these results. Also, one might justify fitting the Cp selected seven parameter form to a combined species data set, n=6*60 (6 species, 12 RH and five T), thus providing a general model for the purpose of interpolation of the interaction of RH and T on the MC of orthodox seeds.

Longevity of seeds was investigated with regard to three factors, D, T, and RH. Best-fit subset models were developed to predict overdrying damage, optimum storage conditions, and accelerated aging at high MC in lettuce and soybean seeds. The best-fit subset models for lettuce germination and vigor are $G = \beta_0 + \beta_1*D + \beta_2*T + \beta_3*R + \beta_4*D*T + \beta_5*D*R + \beta_6*T*R + \beta_7*D^2 + \beta_8*T^2 + \beta_9*R^2 + \beta_{10}*D^2*T + \beta_{11}*T^2*D + \beta_{12}*D^2*R + \beta_{13}*D*R^2 + \beta_{14}*T^2*R + \beta_{15}*T*R^2 + \beta_{16}*D*T*R + \beta_{17}*D^3 + \beta_{18}*T^3 + \beta_{19}*R^3$ and $V = \beta_0 + \beta_1*D + \beta_2*T + \beta_3*R + \beta_4*D*T + \beta_5*D*R + \beta_6*T*R + \beta_7*D^2 + \beta_8*T^2 + \beta_9*R^2 + \beta_{10}*D^2*T + \beta_{11}*T^2*D + \beta_{12}*D^2*R + \beta_{13}*D*R^2 + \beta_{14}*T^2*R + \beta_{15}*T*R^2 + \beta_{16}*D*T*R + \beta_{17}*D^3 + \beta_{18}*T^3 + \beta_{19}*R^3$. The best-subset models for soybean germination and vigor are $G = \beta_0 + \beta_1*D + \beta_2*T + \beta_3*R + \beta_4*D*T + \beta_5*D*R + \beta_6*T*R + \beta_7*D^2 + \beta_8*T^2 + \beta_9*R^2 + \beta_{10}*D^2*T + \beta_{11}*T^2*D + \beta_{12}*D^2*R + \beta_{13}*D*R^2 + \beta_{14}*T^2*R + \beta_{15}*T*R^2 + \beta_{16}*D*T*R + \beta_{17}*D^3 + \beta_{18}*T^3 + \beta_{19}*R^3$ and $V = \beta_0 + \beta_1*D + \beta_2*T + \beta_3*R + \beta_4*D*T + \beta_5*D*R + \beta_6*T*R + \beta_7*D^2 + \beta_8*T^2 + \beta_9*R^2 + \beta_{10}*D^2*T + \beta_{11}*T^2*D + \beta_{12}*D^2*R + \beta_{13}*D*R^2 + \beta_{14}*T^2*R + \beta_{15}*T*R^2 + \beta_{16}*D*T*R + \beta_{17}*D^3 + \beta_{18}*T^3 + \beta_{19}*R^3$. All had different functional forms and highly significant R_a^2 ranged from 0.93 to 0.98.

Over-drying damage, optimum RH, and accelerated aging at high MC were observed in seeds of both species during storage. The models exhibited accelerated deterioration of seeds when seed were dried to extremely low MC. Reduction in germination and vigor increased with storage duration. Deterioration was probably a result of seed aging. Optimum RH for seed storage was found to be about 20-22% for both lettuce and soybean seeds. Accelerated aging resulted in loss of viability when seeds were stored at high MC. Based on the models and model-generated response surfaces, D, T, RH, and especially their interaction

are important factors which determine seed longevity. Deterioration can be studied by best-fit model generated response surfaces.

The experiments showed that maximum seed germination and vigor occurred during storage when lettuce and soybean seeds were stored at optimum MC. Over-drying damage was observed when seeds were stored at extremely low MC. Seed storage at high MC always resulted in reduced germination and vigor.

Over-drying of lettuce seeds increased hexanal production and leachate conductivity in lettuce seeds. Nitrogen storage reduced hexanal production and leachate conductivity. However, seed germination and vigor were improved when seeds were stored at extremely low MC and stored in a N_2. Over-drying soybean seeds increased leachate conductivity and decreased dehydrogenase activity. Germination and vigor also decreased when soybean seeds were stored at extremely low MC.

More deterioration was observed under both over-dry and over-moist storage conditions. Detrimental effects were minimized when seeds were stored at optimum storage conditions. Models developed for germination and vigor in lettuce and soybean seeds indicated an optimum RH, 20-22%, for perhaps most orthodox seeds. The results provided a better understanding as well as a prediction of damage when seeds are stored at over-dry and over-moist conditions. An even deeper understanding of the nature of the damage might be gained from more physiological studies involving measurement of protein and membrane structure, LEA degradation, and DNA synthesis.

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APPENDIX

```
// Program for seed moisture content prediction based on six species discussed in
// Chapter 3.

#include <iostream.h>
void main()
/*************************************************************************
*                                                                        *
* AUTHOR: Jian Fang,   DATE: 9/26/99                                     *
*                                                                        *
*************************************************************************
Program Description:                                                     *
* This program is to prompt the user for moisture content. The program   *
* assumes relative humidity (RH)and temperature (T) are known.           *
* Then the program caculate moisture content based on RH and T.          *
*************************************************************************
* Const/Type/Variables:                                                  *
* RH          - (float) coefficient                                      *
* T           - (float) coefficient                                      *
* MC          - (float) coefficient                                      *
* species_input  - (char)  species                                       *
*************************************************************************
SubFunction Calls:                                                       *
* None                                                                   *
*************************************************************************/
{
float RH, T, MC;
char species_input;

//prompt the user for a temperature and relative humidity

cout<< "please enter the RH value:  ";
cin>>RH;
cout<< "please enter the T value :  ";
cin>>T;
cout<<endl;

cout<<"  Please enter your species code:  ";
cin>>species_input;
cout<<endl;

switch (species_input)
{
case 'C': cout<<"     The species was:  "<<"  Cucumber  "<<endl;
```

```
MC=3.2+0.22*RH-0.072*T-0.0048*RH*RH+0.0014*RH*T+0.000049*RH*RH*RH
  -0.000019*RH*RH*T;
        break;

case 'L': cout<<"The species was:"<<"Lettuce"<<endl;
MC=3.4+0.22*RH-0.076*T-0.0052*RH*RH+0.0020*RH*T+0.000054*RH*RH*RH
  -0.000023*RH*RH*T;
        break;

case 'M': cout<<"The species was:"<<"Maize"<<endl;
MC=4.0+0.31*RH-0.089*T-0.0065*RH*RH+0.0012*RH*T+0.000061*RH*RH*RH
  -0.000014*RH*RH*T;
        break;

case 'O': cout<<"The species was:"<<"Onion"<<endl;
MC=3.7+0.44*RH-0.095*T-0.0100*RH*RH+0.0017*RH*T+0.000090*RH*RH*RH
  -0.000022*RH*RH*T;
        break;

case 'P': cout<<"The species was:"<<"Pea"<<endl;
MC=4.7+0.42*RH-0.120*T-0.0120*RH*RH+0.0040*RH*T+0.000120*RH*RH*RH
  -0.000052*RH*RH*T;
        break;

case 'W': cout<<"The species was:"<<"Watermelon"<<endl;
MC=3.7+0.30*RH-0.089*T-0.0067*RH*RH+0.0020*RH*T+0.000063*RH*RH*RH
  -0.000025*RH*RH*T;
        break;
}

cout.setf(ios::fixed);
cout.setf(ios::showpoint);
cout.precision(2);

//Write out input RH and T
cout<<endl;
cout<<" ================================== " << "\n";
cout<<"  The input RH was:  " << RH <<" % "<< "\n";
cout<<"  The input T  was:  " << T <<" C "<< "\n";
```

```
//Write out output moisture content (%) based on RH and T

cout<<endl;
cout<<" ================================== " << "\n";
cout<<endl;
cout<<"   Moisture content was   "<<MC<<" % "<<endl;

}
```

Table 6. Raw data for moisture content (%) of cucumber seeds equilibrated over various saturated salt solutions and sulphuric acid at different temperatures (Chapter 3).

Salt[z]	Temperature (°C)				
	5	15	25	35	50
H_2SO_4	2.6	2.1	1.5	1.1	0.4
$ZnCl_2$	4.3	3.8	3.0	2.1	1.3
NaOH	4.4	4.0	2.8	2.2	1.4
LiCl	5.2	4.7	3.9	3.3	2.2
$CaBr_2$	6.0	5.6	4.1	3.7	2.4
$MgCl_2$	6.9	6.5	5.7	5.1	4.5
K_2CO_3	7.9	7.7	7.2	6.1	4.9
$Mg(NO_3)_2$	8.8	8.2	7.6	6.9	5.6
NH_4NO_3	11.4	10.7	9.0	8.0	6.5
NaCl	14.1	12.9	11.6	11.1	10.2
KCl	15.6	14.9	14.1	13.2	12.9
KNO_3	23.5	21.0	17.7	15.2	13.2

z Since the RH variable is dependent upon T, table 1 provides 5 RH values for each salt.

Table 7. Raw data for moisture content (%) of lettuce seeds equilibrated over various saturated salt solutions and sulphuric acid at different temperatures (Chapter 3).

Salt[z]	Temperature (°C)				
	5	15	25	35	50
H_2SO_4	2.7	2.2	1.6	1.1	0.4
$ZnCl_2$	4.5	4.0	3.1	2.1	1.3
NaOH	4.5	4.1	3.0	2.4	1.5
LiCl	5.4	4.8	4.1	3.5	2.4
$CaBr_2$	6.0	5.8	4.6	3.7	2.5
$MgCl_2$	6.9	6.5	5.8	5.4	4.6
K_2CO_3	7.2	6.9	6.5	6.3	5.0
$Mg(NO_3)_2$	8.9	8.4	8.0	6.9	6.2
NH_4NO_3	11.4	10.9	9.0	8.1	7.0
NaCl	14.2	12.9	12.9	11.6	10.2
KCl	15.7	15.0	14.2	13.4	12.0
KNO_3	24.2	21.7	18.6	17.0	13.9

z Since the RH variable is dependent upon T, table 1 provides 5 RH values for each salt.

Table 8. Raw data for moisture content (%) of maize seeds equilibrated over various saturated salt solutions and sulphuric acid at different temperatures (Chapter 3).

Salt[z]	Temperature (°C)				
	5	15	25	35	50
H_2SO_4	3.9	3.2	2.4	1.8	0.6
$ZnCl_2$	5.3	4.4	3.6	2.4	1.6
NaOH	5.8	4.6	3.7	2.6	1.7
LiCl	6.8	5.6	4.6	4.0	2.9
$CaBr_2$	7.8	7.2	5.7	4.9	3.2
$MgCl_2$	9.4	8.5	7.6	6.5	5.2
K_2CO_3	10.4	9.8	9.1	8.2	7.3
$Mg(NO_3)_2$	11.8	11.2	10.7	10.1	7.9
NH_4NO_3	14.4	13.4	11.8	10.9	8.0
NaCl	16.1	15.0	14.5	13.4	12.9
KCl	21.3	19.9	19.2	16.6	14.2
KNO_3	26.6	25.0	23.1	21.4	17.6

z Since the RH variable is dependent upon T, table 1 provides 5 RH values for each salt.

Table 9. Raw data for moisture content (%) of onion seeds equilibrated over various saturated salt solutions and sulphuric acid at different temperatures (Chapter 3).

Salt[z]	Temperature (°C)				
	5	15	25	35	50
H_2SO_4	4.2	3.4	2.5	1.8	0.6
$ZnCl_2$	6.1	4.8	3.8	2.6	1.7
NaOH	6.0	4.8	4.0	2.8	1.8
LiCl	7.1	6.1	5.1	4.3	3.1
$CaBr_2$	8.1	7.8	6.5	5.6	3.6
$MgCl_2$	10.5	10.1	9.2	8.4	7.8
K_2CO_3	12.1	11.4	10.2	9.1	8.0
$Mg(NO_3)_2$	13.1	11.8	10.6	10.4	8.6
NH_4NO_3	14.7	14.0	11.9	11.0	8.4
NaCl	17.5	16.8	15.1	13.5	13.3
KCl	24.1	22.3	20.1	19.1	14.6
KNO_3	30.1	29.0	26.0	23.4	19.3

z Since the RH variable is dependent upon T, table 1 provides 5 RH values for each salt.

Table 10. Raw data for moisture content (%) of pea seeds equilibrated over various saturated salt solutions and sulphuric acid at different temperatures (Chapter 3).

Salt[z]	Temperature (°C)				
	5	15	25	35	50
H_2SO_4	4.8	3.9	3.0	1.9	0.9
$ZnCl_2$	6.7	5.0	4.1	2.9	1.9
NaOH	7.0	5.1	4.3	3.1	2.1
LiCl	7.6	6.4	5.8	4.5	3.2
$CaBr_2$	8.2	8.0	6.6	5.7	3.8
$MgCl_2$	9.8	9.2	8.9	7.2	5.6
K_2CO_3	11.6	10.8	10.4	8.8	8.0
$Mg(NO_3)_2$	12.6	11.9	11.2	10.7	8.8
NH_4NO_3	17.6	14.3	12.4	11.2	8.9
NaCl	18.8	17.6	17.1	16.9	14.8
KCl	27.8	24.6	22.0	20.9	18.1
KNO_3	41.7	37.5	34.3	28.2	21.0

z Since the RH variable is dependent upon T, table 1 provides 5 RH values for each salt.

Table 11. Raw data for moisture content (%) of water melon seeds equilibrated over various saturated salt solutions and sulphuric acid at different temperatures (Chapter 3).

Salt[z]	Temperature (°C)				
	5	15	25	35	50
H_2SO_4	3.4	3.0	2.1	1.5	0.5
$ZnCl_2$	5.1	4.0	3.3	2.3	1.4
NaOH	5.4	4.4	3.4	2.4	1.6
LiCl	6.2	5.2	4.3	3.7	2.6
$CaBr_2$	7.3	6.9	5.4	4.7	3.0
$MgCl_2$	8.8	8.2	7.0	6.3	5.0
K_2CO_3	9.4	8.9	8.4	7.8	6.8
$Mg(NO_3)_2$	10.7	9.6	9.3	8.2	7.4
NH_4NO_3	13.2	12.6	10.2	9.4	8.0
NaCl	14.4	13.8	12.9	11.8	10.8
KCl	18.0	17.1	15.5	14.6	13.5
KNO_3	25.9	24.0	20.6	17.9	14.8

z Since the RH variable is dependent upon T, table 1 provides 5 RH values for each salt.

Table 12. Raw data for germination (%) of lettuce seeds stored at 12 relative humidities and 5 temperatures after 2 months (Chapter 4).

Salt[z]	Temperature (°C)				
	5	15	25	35	50
P_2O_5	97	96	95	94	92
$ZnCl_2$	97	95	96	95	93
NaOH	97	96	94	92	95
LiCl	98	97	95	93	94
$CaBr_2$	98	98	95	95	94
$MgCl_2$	96	95	94	94	94
K_2CO_3	96	93	94	93	95
$Mg(NO_3)_2$	95	94	93	93	90
NH_4NO_3	94	94	91	91	82
NaCl	92	92	90	86	0
KCl	90	90	88	41	0
KNO_3	89	86	80	0	0

z Since the RH variable is dependent upon T, table 1 provides 5 RH values for each salt.

Table 13. Raw data for germination (%) of lettuce seeds stored at 12 relative humidities and 5 temperatures after 4 months (Chapter 4).

Salt[z]	Temperature (°C)				
	5	15	25	35	50
P_2O_5	95	96	95	91	91
$ZnCl_2$	96	96	94	92	91
NaOH	95	95	95	94	92
LiCl	96	96	94	94	93
$CaBr_2$	96	95	94	94	92
$MgCl_2$	94	94	94	94	91
K_2CO_3	94	93	90	91	89
$Mg(NO_3)_2$	94	92	90	89	83
NH_4NO_3	93	90	88	86	65
NaCl	86	84	85	76	0
KCl	84	80	76	0	0
KNO_3	76	72	58	0	0

z Since the RH variable is dependent upon T, table 1 provides 5 RH values for each salt.

Table 14. Raw data for germination (%) of lettuce seeds stored at 12 relative humidities and 5 temperatures after 6 months (Chapter 4).

Salt[z]	Temperature (°C)				
	5	15	25	35	50
P_2O_5	95	94	92	91	87
$ZnCl_2$	95	94	92	93	89
NaOH	95	94	93	94	91
LiCl	95	95	94	94	92
$CaBr_2$	95	94	94	94	92
$MgCl_2$	94	94	92	91	91
K_2CO_3	93	93	91	89	85
$Mg(NO_3)_2$	92	91	90	85	51
NH_4NO_3	89	90	87	80	0
NaCl	84	82	81	67	0
KCl	80	75	50	0	0
KNO_3	70	65	0	0	0

z Since the RH variable is dependent upon T, table 1 provides 5 RH values for each salt.

Table 15. Raw data for germination (%) of lettuce seeds stored at 12 relative humidities and 5 temperatures after 8 months (Chapter 4).

Salt[z]	Temperature (°C)				
	5	15	25	35	50
P_2O_5	95	92	92	90	85
$ZnCl_2$	95	93	93	90	87
NaOH	95	94	94	91	89
LiCl	95	94	94	92	92
$CaBr_2$	95	94	94	94	91
$MgCl_2$	93	92	93	92	88
K_2CO_3	92	90	92	91	56
$Mg(NO_3)_2$	91	89	90	84	0
NH_4NO_3	87	86	85	80	0
NaCl	75	73	61	44	0
KCl	67	64	30	0	0
KNO_3	52	47	0	0	0

z Since the RH variable is dependent upon T, table 1 provides 5 RH values for each salt.

Table 16. Raw data for germination (%) of lettuce seeds stored at 12 relative humidities and 5 temperatures after 10 months (Chapter 4).

Salt[z]	Temperature (°C)				
	5	15	25	35	50
P_2O_5	95	91	90	88	84
$ZnCl_2$	95	92	92	89	85
NaOH	95	92	92	91	87
LiCl	95	94	93	92	89
$CaBr_2$	95	93	93	91	88
$MgCl_2$	94	92	93	89	78
K_2CO_3	92	90	90	81	36
$Mg(NO_3)_2$	88	85	83	62	0
NH_4NO_3	78	74	68	36	0
NaCl	71	64	48	11	0
KCl	50	42	26	0	0
KNO_3	39	32	0	0	0

z Since the RH variable is dependent upon T, table 1 provides 5 RH values for each salt.

Table 17. Raw data for germination (%) of lettuce seeds stored at 12 relative humidities and 5 temperatures after 12 months (Chapter 4).

Salt[z]	Temperature (°C)				
	5	15	25	35	50
P_2O_5	95	91	90	88	81
$ZnCl_2$	95	92	91	89	82
NaOH	95	92	92	90	86
LiCl	96	93	93	92	88
$CaBr_2$	94	92	92	92	89
$MgCl_2$	93	91	90	89	66
K_2CO_3	89	86	83	81	31
$Mg(NO_3)_2$	83	81	76	54	0
NH_4NO_3	70	62	58	28	0
NaCl	56	48	37	5	0
KCl	37	19	0	0	0
KNO_3	0	0	0	0	0

z Since the RH variable is dependent upon T, table 1 provides 5 RH values for each salt.

Table 18. Raw data for germination (%) of lettuce seeds stored at 12 relative humidities and 5 temperatures after 14 months (Chapter 4).

Salt[z]	Temperature (°C)				
	5	15	25	35	50
P_2O_5	94	91	90	84	78
$ZnCl_2$	94	92	92	86	81
NaOH	94	92	92	88	85
LiCl	94	92	93	91	88
$CaBr_2$	94	92	92	90	86
$MgCl_2$	93	90	90	87	62
K_2CO_3	88	84	82	78	27
$Mg(NO_3)_2$	81	79	75	45	0
NH_4NO_3	61	54	51	12	0
NaCl	47	37	11	0	0
KCl	16	6	0	0	0
KNO_3	0	0	0	0	0

z Since the RH variable is dependent upon T, table 1 provides 5 RH values for each salt.

Table 19. Raw data for germination (%) of lettuce seeds stored at 12 relative humidities and 5 temperatures after 16 months (Chapter 4).

Salt[z]	Temperature (°C)				
	5	15	25	35	50
P_2O_5	94	90	88	82	70
$ZnCl_2$	93	91	90	84	73
NaOH	95	92	91	89	80
LiCl	94	93	92	90	84
$CaBr_2$	92	92	91	88	81
$MgCl_2$	92	90	90	85	55
K_2CO_3	87	83	80	75	7
$Mg(NO_3)_2$	80	74	69	38	0
NH_4NO_3	54	47	42	0	0
NaCl	31	25	5	0	0
KCl	3	0	0	0	0
KNO_3	0	0	0	0	0

z Since the RH variable is dependent upon T, table 1 provides 5 RH values for each salt.

Table 20. Raw data for germination (%) of lettuce seeds stored at 12 relative humidities and 5 temperatures after 18 months (Chapter 4).

Salt[z]	Temperature (°C)				
	5	15	25	35	50
P_2O_5	94	90	87	81	65
$ZnCl_2$	94	91	88	83	69
NaOH	95	92	89	86	72
LiCl	94	93	90	87	76
$CaBr_2$	94	92	89	85	74
$MgCl_2$	92	91	85	78	36
K_2CO_3	84	87	70	56	0
$Mg(NO_3)_2$	72	64	58	5	0
NH_4NO_3	46	24	16	0	0
NaCl	10	0	0	0	0
KCl	0	0	0	0	0
KNO_3	0	0	0	0	0

z Since the RH variable is dependent upon T, table 1 provides 5 RH values for each salt.

Table 21. Raw data for germination (%) of lettuce seeds stored at 12 relative humidities and 5 temperatures after 20 months (Chapter 4).

Salt[z]	Temperature (°C)				
	5	15	25	35	50
P_2O_5	93	89	85	79	64
$ZnCl_2$	93	90	87	82	67
NaOH	94	91	88	85	72
LiCl	94	93	91	86	75
$CaBr_2$	93	92	89	84	73
$MgCl_2$	92	85	83	68	33
K_2CO_3	82	76	68	53	0
$Mg(NO_3)_2$	65	62	53	0	0
NH_4NO_3	32	21	4	0	0
NaCl	4	0	0	0	0
KCl	0	0	0	0	0
KNO_3	0	0	0	0	0

z Since the RH variable is dependent upon T, table 1 provides 5 RH values for each salt.

Table 22. Raw data for germination (%) of lettuce seeds stored at 12 relative humidities and 5 temperatures after 22 months (Chapter 4).

Salt[z]	Temperature (°C)				
	5	15	25	35	50
P_2O_5	95	90	82	74	55
$ZnCl_2$	94	91	85	76	59
NaOH	94	92	87	82	63
LiCl	94	92	90	84	68
$CaBr_2$	95	90	88	82	65
$MgCl_2$	90	89	81	66	23
K_2CO_3	79	70	59	31	0
$Mg(NO_3)_2$	62	52	48	0	0
NH_4NO_3	30	5	0	0	0
NaCl	0	0	0	0	0
KCl	0	0	0	0	0
KNO_3	0	0	0	0	0

z Since the RH variable is dependent upon T, table 1 provides 5 RH values for each salt.

Table 23. Raw data for germination (%) of lettuce seeds stored at 12 relative humidities and 5 temperatures after 24 months (Chapter 4).

Salt[z]	Temperature (°C)				
	5	15	25	35	50
P_2O_5	94	87	80	70	45
$ZnCl_2$	94	89	83	74	51
NaOH	94	90	86	78	56
LiCl	94	92	89	83	62
$CaBr_2$	93	90	87	81	59
$MgCl_2$	91	88	80	64	18
K_2CO_3	77	65	54	25	0
$Mg(NO_3)_2$	56	47	37	0	0
NH_4NO_3	12	0	0	0	0
NaCl	0	0	0	0	0
KCl	0	0	0	0	0
KNO_3	0	0	0	0	0

z Since the RH variable is dependent upon T, table 1 provides 5 RH values for each salt.

Table 24. Raw data for vigor (root length, cm) of lettuce seeds stored at 12 relative humidities and 5 temperatures after 2 months.

Salt	Temperature (°C)				
	5	15	25	35	50
P_2O_5	4.43	4.29	4.36	4.30	3.98
$ZnCl_2$	4.33	4.42	4.22	4.22	4.20
NaOH	4.29	4.31	4.32	4.32	4.28
LiCl	4.46	4.23	4.46	4.18	4.12
$CaBr_2$	4.21	4.43	4.37	4.23	4.05
$MgCl_2$	4.30	4.30	4.24	4.12	4.00
K_2CO_3	4.20	4.20	4.03	4.00	3.96
$Mg(NO_3)_2$	4.10	4.10	4.10	3.98	3.90
NH_4NO_3	4.15	4.00	4.00	3.88	3.80
NaCl	4.00	3.90	3.98	3.68	3.21
KCl	3.90	3.89	3.56	3.48	2.89
KNO_3	3.81	3.64	3.23	1.86	1.01

z Since the RH variable is dependent upon T, table 1 provides 5 RH values for each salt.

Table 25. Raw data for vigor (root length, cm) of lettuce seeds stored at 12 relative humidities and 5 temperatures after 4 months.

Salt	Temperature (°C)				
	5	15	25	35	50
P_2O_5	4.21	4.40	4.38	3.66	3.68
$ZnCl_2$	4.36	4.31	4.46	3.89	3.85
NaOH	4.47	4.24	4.27	3.86	3.98
LiCl	4.26	4.46	4.30	3.99	4.06
$CaBr_2$	4.20	4.25	4.10	4.07	4.00
$MgCl_2$	4.25	4.17	4.02	3.65	3.87
K_2CO_3	4.38	4.10	4.00	3.51	3.53
$Mg(NO_3)_2$	4.06	4.10	3.96	3.44	3.32
NH_4NO_3	4.00	3.90	3.85	3.32	3.21
NaCl	3.90	3.80	3.69	3.26	2.45
KCl	3.78	3.59	3.28	2.86	0.87
KNO_3	3.48	3.10	3.01	0.78	0

z Since the RH variable is dependent upon T, table 1 provides 5 RH values for each salt.

Table 26. Raw data for vigor (root length, cm) of lettuce seeds stored at 12 relative humidities and 5 temperatures after 6 months.

Salt	Temperature (°C)				
	5	15	25	35	50
P_2O_5	4.24	4.00	3.84	3.78	2.98
$ZnCl_2$	4.36	4.16	3.95	3.92	3.15
NaOH	4.21	4.25	4.11	3.76	3.22
LiCl	4.32	4.30	4.28	3.94	3.06
$CaBr_2$	4.46	4.15	4.09	4.10	3.15
$MgCl_2$	4.32	4.04	4.00	3.65	3.00
K_2CO_3	4.23	4.11	3.89	3.51	2.59
$Mg(NO_3)_2$	4.13	3.98	3.76	3.45	2.45
NH_4NO_3	4.00	3.79	3.58	3.21	1.56
NaCl	3.80	3.59	3.40	2.76	0.79
KCl	3.60	3.27	2.98	1.29	0
KNO_3	3.10	2.58	2.55	0	0

z Since the RH variable is dependent upon T, table 1 provides 5 RH values for each salt.

Table 27. Raw data for vigor (root length, cm) of lettuce seeds stored at 12 relative humidities and 5 temperatures after 8 months (Chapter 4).

Salt[z]	Temperature (°C)				
	5	15	25	35	50
P_2O_5	4.26	3.89	3.96	3.31	2.65
$ZnCl_2$	4.12	4.07	3.83	3.49	2.85
NaOH	4.29	4.30	3.92	3.75	2.80
LiCl	4.42	4.16	4.12	3.57	2.95
$CaBr_2$	4.18	4.21	4.03	3.62	3.11
$MgCl_2$	4.30	4.11	3.91	3.45	2.79
K_2CO_3	4.06	3.98	3.79	3.17	2.46
$Mg(NO_3)_2$	4.17	3.80	3.70	3.10	1.78
NH_4NO_3	3.90	3.64	3.30	2.70	0.34
NaCl	3.70	3.43	3.20	1.02	0
KCl	3.40	3.10	2.65	0	0
KNO_3	2.55	2.35	2.00	0	0

z Since the RH variable is dependent upon T, table 1 provides 5 RH values for each salt.

Table 28. Raw data for vigor (root length, cm) of lettuce seeds stored at 12 relative humidities and 5 temperatures after 10 months (Chapter 4).

Salt[z]	Temperature (°C)				
	5	15	25	35	50
P_2O_5	4.25	3.75	3.65	3.45	2.23
$ZnCl_2$	4.30	3.89	3.89	3.59	2.34
NaOH	4.45	4.05	3.71	3.81	2.65
LiCl	4.34	4.21	3.98	3.68	2.48
$CaBr_2$	4.27	4.26	4.20	3.61	2.64
$MgCl_2$	4.22	4.12	3.84	3.53	2.11
K_2CO_3	4.06	4.02	3.68	3.27	1.84
$Mg(NO_3)_2$	4.00	3.82	3.42	3.06	1.48
NH_4NO_3	3.75	3.55	3.26	2.64	0
NaCl	3.54	3.21	3.05	0.79	0
KCl	2.83	2.65	2.52	0	0
KNO_3	2.34	1.97	1.48	0	0

z Since the RH variable is dependent upon T, table 1 provides 5 RH values for each salt.

Table 29. Raw data for vigor (root length, cm) of lettuce seeds stored at 12 relative humidities and 5 temperatures after 12 months (Chapter 4).

Salt[z]	Temperature (°C)				
	5	15	25	35	50
P_2O_5	4.11	3.72	3.86	3.35	1.74
$ZnCl_2$	4.31	3.98	3.71	3.51	2.12
NaOH	4.21	4.12	3.90	3.67	2.46
LiCl	4.35	4.28	3.74	3.53	2.64
$CaBr_2$	4.48	4.20	3.98	3.64	2.39
$MgCl_2$	4.13	4.00	3.81	3.28	1.80
K_2CO_3	4.01	3.89	3.58	3.17	1.54
$Mg(NO_3)_2$	3.86	3.68	3.42	2.98	1.21
NH_4NO_3	3.12	3.00	2.39	2.00	0
NaCl	2.31	2.23	2.01	0.21	0
KCl	1.46	1.21	0.96	0	0
KNO_3	0.61	0	0	0	0

z Since the RH variable is dependent upon T, table 1 provides 5 RH values for each salt.

Table 30. Raw data for vigor (root length, cm) of lettuce seeds stored at 12 relative humidities and 5 temperatures after 14 months (Chapter 4).

Salt[z]	Temperature (°C)				
	5	15	25	35	50
P_2O_5	4.28	3.66	3.64	3.22	1.66
$ZnCl_2$	4.49	3.97	3.88	3.49	2.10
NaOH	4.31	4.08	3.74	3.63	2.48
LiCl	4.43	4.25	3.89	3.55	2.36
$CaBr_2$	4.35	4.19	3.98	3.77	2.11
$MgCl_2$	4.15	3.92	3.62	3.31	1.57
K_2CO_3	4.05	3.75	3.58	3.07	1.41
$Mg(NO_3)_2$	3.45	3.12	2.96	2.23	0.84
NH_4NO_3	2.74	2.69	2.10	1.34	0
NaCl	1.51	1.79	1.42	0	0
KCl	0.98	0.75	0	0	0
KNO_3	0	0	0	0	0

z Since the RH variable is dependent upon T, table 1 provides 5 RH values for each salt.

Table 31. Raw data for vigor (root length, cm) of lettuce seeds stored at 12 relative humidities and 5 temperatures after 16 months (Chapter 4).

Salt[z]	Temperature (°C)				
	5	15	25	35	50
P_2O_5	3.73	3.63	3.66	3.54	1.64
$ZnCl_2$	3.98	3.88	3.57	3.35	1.95
NaOH	4.48	4.45	3.83	3.45	1.61
LiCl	4.55	4.35	3.95	3.68	1.96
$CaBr_2$	4.40	4.20	3.72	3.52	2.30
$MgCl_2$	4.26	4.06	3.59	3.21	1.52
K_2CO_3	3.89	3.89	3.35	2.94	0.96
$Mg(NO_3)_2$	3.25	3.06	2.75	1.85	0.29
NH_4NO_3	2.65	2.61	1.63	0.49	0
NaCl	1.24	1.54	0.96	0	0
KCl	0.28	0	0	0	0
KNO_3	0	0	0	0	0

z Since the RH variable is dependent upon T, table 1 provides 5 RH values for each salt.

Table 32. Raw data for vigor (root length, cm) of lettuce seeds stored at 12 relative humidities and 5 temperatures after 18 months (Chapter 4).

Salt[z]	Temperature (°C)				
	5	15	25	35	50
P_2O_5	4.27	3.65	3.56	3.32	1.38
$ZnCl_2$	4.48	3.98	3.75	3.20	1.57
NaOH	4.30	4.40	3.90	3.57	1.64
LiCl	4.12	4.35	3.99	3.48	1.82
$CaBr_2$	4.43	4.18	3.76	3.32	1.50
$MgCl_2$	4.14	3.87	3.55	2.95	1.12
K_2CO_3	3.97	3.71	3.23	2.71	0.36
$Mg(NO_3)_2$	3.03	3.02	2.65	0.73	0
NH_4NO_3	2.21	1.86	1.11	0	0
NaCl	0.98	0.75	0	0	0
KCl	0	0	0	0	0
KNO_3	0	0	0	0	0

z Since the RH variable is dependent upon T, table 1 provides 5 RH values for each salt.

Table 33. Raw data for vigor (root length, cm) of lettuce seeds stored at 12 relative humidities and 5 temperatures after 20 months (Chapter 4).

Salt[z]	Temperature (°C)				
	5	15	25	35	50
P_2O_5	4.10	3.82	3.55	3.00	1.28
$ZnCl_2$	4.26	3.96	3.70	3.52	1.57
NaOH	4.18	4.23	3.68	3.30	1.88
LiCl	4.29	4.12	3.89	3.57	1.52
$CaBr_2$	4.34	4.45	3.75	3.38	1.99
$MgCl_2$	4.12	3.88	3.42	2.85	1.03
K_2CO_3	3.90	3.67	3.11	2.62	0
$Mg(NO_3)_2$	2.99	2.82	2.53	0	0
NH_4NO_3	2.03	1.56	0.61	0	0
NaCl	0.21	0	0	0	0
KCl	0	0	0	0	0
KNO_3	0	0	0	0	0

z Since the RH variable is dependent upon T, table 1 provides 5 RH values for each salt.

Table 34. Raw data for vigor (root length, cm) of lettuce seeds stored at 12 relative humidities and 5 temperatures after 22 months (Chapter 4).

Salt[z]	Temperature (°C)				
	5	15	25	35	50
P_2O_5	4.38	3.59	3.31	2.59	1.08
$ZnCl_2$	4.20	3.73	3.52	2.87	1.49
NaOH	4.23	4.02	3.68	3.02	1.34
LiCl	4.43	4.48	3.42	3.35	1.55
$CaBr_2$	4.30	4.20	3.85	3.19	1.79
$MgCl_2$	3.87	3.75	3.18	2.61	0.79
K_2CO_3	3.47	3.31	2.94	1.96	0
$Mg(NO_3)_2$	2.87	2.64	2.42	0	0
NH_4NO_3	1.93	1.12	0	0	0
NaCl	0	0	0	0	0
KCl	0	0	0	0	0
KNO_3	0	0	0	0	0

z Since the RH variable is dependent upon T, table 1 provides 5 RH values for each salt.

Table 35. Raw data for vigor (root length, cm) of lettuce seeds stored at 12 relative humidities and 5 temperatures after 24 months (Chapter 4).

Salt[z]	Temperature (°C)				
	5	15	25	35	50
P_2O_5	4.24	3.64	3.58	2.52	1.08
$ZnCl_2$	4.45	3.70	3.67	2.98	1.29
NaOH	4.29	4.05	3.46	3.06	1.35
LiCl	4.14	4.48	3.88	3.41	1.52
$CaBr_2$	4.43	4.10	3.62	3.14	1.35
$MgCl_2$	4.06	3.71	2.87	2.42	0.29
K_2CO_3	3.86	3.52	2.41	1.56	0
$Mg(NO_3)_2$	2.83	2.46	2.27	0	0
NH_4NO_3	1.54	0.97	0	0	0
NaCl	0	0	0	0	0
KCl	0	0	0	0	0
KNO_3	0	0	0	0	0

z Since the RH variable is dependent upon T, table 1 provides 5 RH values for each salt.

Table 36. Raw data for germination (%) of soybean seeds stored at 12 relative humidities and 5 temperatures after 2 months (Chapter 4).

Salt[z]	Temperature (°C)				
	5	15	25	35	50
P_2O_5	95	94	84	87	78
$ZnCl_2$	94	95	92	88	79
NaOH	96	97	92	89	83
LiCl	94	94	93	90	86
$CaBr_2$	93	93	94	89	85
$MgCl_2$	92	91	94	88	82
K_2CO_3	90	89	92	85	76
$Mg(NO_3)_2$	89	89	88	79	63
NH_4NO_3	88	86	85	65	46
NaCl	86	84	65	32	27
KCl	82	76	48	8	0
KNO_3	79	65	30	0	0

z Since the RH variable is dependent upon T, table 1 provides 5 RH values for each salt.

Table 37. Raw data for germination (%) of soybean seeds stored at 12 relative humidities and 5 temperatures after 4 months (Chapter 4).

Salt[z]	Temperature (°C)				
	5	15	25	35	50
P_2O_5	94	91	90	79	66
$ZnCl_2$	94	92	90	81	70
NaOH	97	93	91	83	74
LiCl	95	95	94	87	78
$CaBr_2$	95	94	93	86	77
$MgCl_2$	91	92	91	82	72
K_2CO_3	90	90	82	78	69
$Mg(NO_3)_2$	88	86	79	71	57
NH_4NO_3	85	82	71	49	29
NaCl	78	71	48	0	0
KCl	66	62	37	0	0
KNO_3	55	50	30	0	0

z Since the RH variable is dependent upon T, table 1 provides 5 RH values for each salt.

Table 38. Raw data for germination (%) of soybean seeds stored at 12 relative humidities and 5 temperatures after 6 months (Chapter 4).

Salt[z]	Temperature (°C)				
	5	15	25	35	50
P_2O_5	91	90	86	72	55
$ZnCl_2$	92	93	87	75	59
NaOH	93	92	90	77	64
LiCl	94	94	93	79	67
$CaBr_2$	93	94	92	78	66
$MgCl_2$	90	92	91	72	55
K_2CO_3	85	82	80	63	49
$Mg(NO_3)_2$	83	79	74	52	39
NH_4NO_3	80	76	58	23	26
NaCl	74	68	47	0	0
KCl	59	57	31	0	0
KNO_3	51	42	18	0	0

z Since the RH variable is dependent upon T, table 1 provides 5 RH values for each salt.

Table 39. Raw data for germination (%) of soybean seeds stored at 12 relative humidities and 5 temperatures after 8 months (Chapter 4).

Salt[z]	Temperature (°C)				
	5	15	25	35	50
P_2O_5	90	89	83	68	43
$ZnCl_2$	92	90	85	73	46
NaOH	92	91	87	75	52
LiCl	94	93	89	78	56
$CaBr_2$	92	92	88	77	56
$MgCl_2$	88	90	84	69	48
K_2CO_3	80	78	77	60	30
$Mg(NO_3)_2$	78	72	64	43	19
NH_4NO_3	72	68	53	8	7
NaCl	68	58	39	0	0
KCl	52	42	21	0	0
KNO_3	32	29	4	0	0

z Since the RH variable is dependent upon T, table 1 provides 5 RH values for each salt.

Table 40. Raw data for germination (%) of soybean seeds stored at 12 relative humidities and 5 temperatures after 10 months (Chapter 4).

Salt[z]	Temperature (°C)				
	5	15	25	35	50
P_2O_5	89	88	81	60	34
$ZnCl_2$	92	90	84	63	38
NaOH	91	92	87	68	45
LiCl	93	93	88	70	49
$CaBr_2$	92	93	88	69	48
$MgCl_2$	90	90	82	60	32
K_2CO_3	76	74	71	55	26
$Mg(NO_3)_2$	73	63	60	32	19
NH_4NO_3	68	60	48	0	12
NaCl	61	48	32	0	0
KCl	37	32	16	0	0
KNO_3	12	8	0	0	0

z Since the RH variable is dependent upon T, table 1 provides 5 RH values for each salt.

Table 41. Raw data for germination (%) of soybean seeds stored at 12 relative humidities and 5 temperatures after 12 months (Chapter 4).

Salt[z]	Temperature (°C)				
	5	15	25	35	50
P_2O_5	89	89	81	52	21
$ZnCl_2$	90	90	84	57	26
NaOH	96	91	86	60	31
LiCl	95	92	88	64	36
$CaBr_2$	93	92	88	63	35
$MgCl_2$	89	88	80	56	15
K_2CO_3	73	70	68	40	6
$Mg(NO_3)_2$	62	57	51	12	0
NH_4NO_3	58	50	37	0	0
NaCl	50	41	22	0	0
KCl	10	18	0	0	0
KNO_3	0	0	0	0	0

z Since the RH variable is dependent upon T, table 1 provides 5 RH values for each salt.

Table 42. Raw data for vigor (root length, cm) of soybean seeds stored at 12 relative humidities and 5 temperatures after 2 months (Chapter 4).

Salt[z]	Temperature (°C)				
	5	15	25	35	50
P_2O_5	4.67	4.62	4.20	3.80	3.00
$ZnCl_2$	4.80	4.53	4.45	4.14	3.26
NaOH	4.62	4.84	4.60	4.36	3.48
LiCl	4.72	4.68	4.32	4.25	3.31
$CaBr_2$	4.54	4.88	4.65	4.48	3.55
$MgCl_2$	4.68	4.50	4.20	4.10	3.22
K_2CO_3	4.32	4.45	4.10	3.80	3.00
$Mg(NO_3)_2$	4.50	4.25	3.90	3.65	2.86
NH_4NO_3	4.10	4.00	3.70	3.00	2.30
NaCl	4.00	3.80	3.10	2.10	1.80
KCl	3.70	3.20	2.50	1.20	0
KNO_3	3.00	2.80	1.90	0.80	0

z Since the RH variable is dependent upon T, table 1 provides 5 RH values for each salt.

Table 43. Raw data for vigor (root length, cm) of soybean seeds stored at 12 relative humidities and 5 temperatures after 4 months (Chapter 4).

Salt[z]	Temperature (°C)				
	5	15	25	35	50
P_2O_5	4.43	4.40	4.20	3.80	2.78
$ZnCl_2$	4.64	4.32	4.42	3.96	3.10
NaOH	4.57	4.62	4.60	4.48	3.45
LiCl	4.69	4.53	4.40	4.22	3.36
$CaBr_2$	4.44	4.69	4.65	4.45	3.68
$MgCl_2$	4.59	4.45	4.21	3.86	3.21
K_2CO_3	4.45	4.30	4.00	3.65	2.73
$Mg(NO_3)_2$	4.10	4.05	3.75	3.24	2.45
NH_4NO_3	3.46	3.24	3.10	2.82	2.04
NaCl	3.28	3.00	2.75	1.80	1.24
KCl	2.90	2.78	2.40	0	0
KNO_3	2.60	2.34	1.76	0	0

z Since the RH variable is dependent upon T, table 1 provides 5 RH values for each salt.

Table 44. Raw data for vigor (root length, cm) of soybean seeds stored at 12 relative humidities and 5 temperatures after 6 months (Chapter 4).

Salt[z]	Temperature (°C)				
	5	15	25	35	50
P_2O_5	4.62	4.40	4.14	3.45	2.30
$ZnCl_2$	4.42	4.52	4.35	3.87	3.02
NaOH	4.56	4.66	4.40	4.38	3.43
LiCl	4.68	4.49	4.63	4.00	3.21
$CaBr_2$	4.45	4.60	4.45	4.11	3.52
$MgCl_2$	4.13	4.40	4.08	3.50	2.34
K_2CO_3	4.30	4.24	3.96	3.21	1.98
$Mg(NO_3)_2$	4.00	3.98	3.61	3.00	1.71
NH_4NO_3	3.30	3.00	2.91	2.54	1.45
NaCl	3.04	2.81	2.59	0	0
KCl	2.60	2.54	2.24	0	0
KNO_3	2.30	2.05	0.86	0	0

z Since the RH variable is dependent upon T, table 1 provides 5 RH values for each salt.

Table 45. Raw data for vigor (root length, cm) of soybean seeds stored at 12 relative humidities and 5 temperatures after 8 months (Chapter 4).

Salt[z]	Temperature (°C)				
	5	15	25	35	50
P_2O_5	4.38	4.36	4.18	3.26	1.96
$ZnCl_2$	4.20	4.52	4.30	3.74	2.19
NaOH	4.53	4.23	4.36	3.93	2.42
LiCl	4.36	4.53	4.57	3.86	3.44
$CaBr_2$	4.58	4.36	4.19	3.58	3.15
$MgCl_2$	4.29	4.58	4.00	3.28	1.85
K_2CO_3	4.03	4.12	3.75	3.00	1.68
$Mg(NO_3)_2$	4.17	3.75	3.48	2.74	1.43
NH_4NO_3	3.03	2.91	2.52	1.96	0.96
NaCl	2.56	2.67	2.21	0	0
KCl	2.21	2.31	1.93	0	0
KNO_3	1.83	1.24	0.23	0	0

z Since the RH variable is dependent upon T, table 1 provides 5 RH values for each salt.

Table 46. Raw data for vigor (root length, cm) of soybean seeds stored at 12 relative humidities and 5 temperatures after 10 months (Chapter 4).

Salt[z]	Temperature (°C)				
	5	15	25	35	50
P_2O_5	4.20	4.31	4.00	2.92	1.55
$ZnCl_2$	4.42	4.43	4.24	3.41	2.13
NaOH	4.62	4.55	4.41	3.58	2.36
LiCl	4.36	4.37	4.23	3.39	2.58
$CaBr_2$	4.58	4.68	4.46	3.62	2.28
$MgCl_2$	4.28	4.23	3.87	3.00	1.61
K_2CO_3	4.10	4.00	3.57	2.68	1.43
$Mg(NO_3)_2$	3.91	3.59	3.21	2.21	1.11
NH_4NO_3	2.83	2.61	2.15	0	0
NaCl	2.12	2.12	1.89	0	0
KCl	1.58	1.76	1.43	0	0
KNO_3	1.12	0.89	0	0	0

z Since the RH variable is dependent upon T, table 1 provides 5 RH values for each salt.

Table 47. Raw data for vigor (root length, cm) of soybean seeds stored at 12 relative humidities and 5 temperatures after 12 months (Chapter 4).

Salt[z]	Temperature (°C)				
	5	15	25	35	50
P_2O_5	4.21	4.12	3.71	2.51	1.38
$ZnCl_2$	4.35	4.25	3.91	3.19	1.57
NaOH	4.47	4.37	3.78	3.54	1.89
LiCl	4.38	4.18	4.08	3.26	1.78
$CaBr_2$	4.52	4.49	4.22	3.48	1.97
$MgCl_2$	4.12	3.94	3.52	2.87	1.21
K_2CO_3	3.86	3.76	3.22	2.31	0.88
$Mg(NO_3)_2$	3.58	3.29	2.80	1.54	0
NH_4NO_3	2.68	2.21	2.02	0	0
NaCl	1.94	1.76	1.58	0	0
KCl	1.21	0.98	0	0	0
KNO_3	0	0	0	0	0

z Since the RH variable is dependent upon T, table 1 provides 5 RH values for each salt.

Table 48. Constant and regression coefficients of four models predicting lettuce and soybean germination and vigor (Chapter 4).

Complete	Lettuce		Soybean	
	Germination	Vigor	Germination	Vigor
β_0	120.6	4.435	90.47	4.367
β_1	-1.691	–	-0.568	–
β_2	-1.932	-0.027	0.849	0.0400
β_3	-2.715	-0.035	–	–
β_4	0.082	-0.0017	0.0232	–
β_5	0.132	0.0031	0.0786	0.0021
β_6	0.092	0.0014	0.0144	–
β_7	0.023	–	–	0.00032
β_8	0.043	0.0014	-0.041	–
β_9	0.078	0.00090	-0.0081	–
β_{10}	-0.0014	0.00012	–	-0.0018
β_{11}	-0.0012	-0.000065	-0.0026	–
β_{12}	-0.00062	-0.000026	-0.0011	-0.000051
β_{13}	-0.0026	-0.000062	-0.0018	-0.000054
β_{14}	-0.00061	-0.0000069	–	0.0000073
β_{15}	-0.0012	-0.000020	-0.00028	-0.000015
β_{16}	-0.0014	-0.000021	–	-0.000015
β_{17}	–	-0.000039	–	-0.00020
β_{18}	-0.00033	-0.000018	0.00048	0.000011
β_{19}	-0.00059	-0.0000058	0.00013	0.0000011