2012

Effects of maturity group, seed composition and storage conditions on the quality and storability of soybean (Glycine max L. Merrill) seed

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Effects of maturity group, seed composition and storage conditions on the quality and storability of soybean (Glycine max L. Merrill) seed

by

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A thesis submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Crop Production and Physiology (Seed Science)

Program of Study Committee:
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Ames, Iowa
2012

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ABSTRACT

Soybean seed is usually not carried-over to the next planting season because it deteriorates rapidly in storage, resulting in poor growth when planted. This rapid deterioration is a serious problem in seed production. The short shelf life of soybean seed is the result of its high lipid content and high levels of polyunsaturated linolenic and linoleic acids. The soybean production practice of planting early to maximize yield has led to routine use of seed treatments to protect the seeds and seedlings during the early stages of development. The amount of treated seed is increasing every year as research identifies more effective active ingredients. However, excess treated soybean seed must be disposed of differently from untreated seed, which generates an additional cost for the seed industry. There is a need for prolonging storage life of carry-over treated seeds to minimize seed disposal costs. The objectives were to determine the best storage conditions of temperature and relative humidity that will minimize the deterioration of chemically treated soybean seed from different maturity groups and seed composition. Twenty-four soybean varieties were treated with the fungicides fludioxonil and mefenoxam, or a mixture of these fungicides and the insecticide thiamethoxam, or left untreated as a control. The seeds were packaged and stored under one of three storage conditions: a non-climate controlled warehouse, a climate controlled coldroom (10°C and 59.6±7.3% RH), or a climate controlled warmroom (25°C and 31.2±11.1% RH). The decline in viability and vigor was evaluated at 4, 8, 12, 16 and 20 mo after storage. After 20 mo in storage, the mean moisture content of seed lots in the coldroom ranged between 10.15 to 10.77%, in the warmroom ranged between 5.66 to 5.81 %. The moisture contents for seeds in the warehouse ranged between 11.4 and 12.7%. Soybean genotypes
differed significantly in their rate of decline of seed viability and vigor over time. Seed viability values remained high in seeds stored in the coldroom and warmroom but dropped to almost zero in the warehouse at 20 mo after storage. The loss in viability of untreated seed was significantly greater than that of treated seeds at 16 months in the warehouse while in the coldroom and warmroom the effects were visible at 20 mo after storage. Viability of seeds in the coldroom and warmroom remained higher than 80% at the end of 20 mo of storage. Temperature and relative humidity of the coldroom were best for maintaining seed vigor above 80% for 12 months only. Maturity group and protein content did not affect deterioration. Only 5 to 15% of the decline in seed vigor could be attributed to oil content of the seeds, depending on the storage condition. Treated soybean seeds could be carried-over for two seasons if the temperature of storage is maintained at 10 °C and the relative humidity kept constant at ≤ 40%. Seed treatment would improve storability if seeds are stored in low temperature and relative humidity conditions. Prolonging soybean seed viability and vigor of treated seed in storage could reduce the need for disposal of treated seeds. These results are also important for the crop-protection chemical companies because of the importance of seed treatments to the overall crop-protection strategies.
CHAPTER 1. GENERAL INTRODUCTION

LITERATURE REVIEW

THE SOYBEAN SEED

The soybean \textit{[Glycine max (L.) Merr.]} seed represents the planting unit that is made up of an embryo axis and two cotyledons, and surrounded by a protective covering, the seed coat (Justice and Bass, 1978). The natural openings on the seed are the hilum and micropyle through which water and gases enter or leave the seed. The major food reserve in the soybean seed is stored in the cotyledons which also serve as photosynthetic organs for the growing seedling. A damaged hilum or seed coat could become an entry points for pathogens. During seed development, anabolic reactions predominate resulting in an increase in dry matter. These anabolic reactions change to catabolic processes after seed maturation and will eventually lead to death of the seed in storage (Delouche, 1974).

SEED QUALITY

Seed quality is a complex trait that is determined by the genetics, physical, physiological and health properties of a seed (Delouche and Baskin, 1973; McDonald, 1999; Marco-Filho et al., 1998). These properties are in turn influenced by the agroecological conditions in the seed production field, seed handling and processing, storage conditions and storage period (Vieira et al., 2001; McDonald, 1998). At each stage of the seed production process, great care is taken by the seed producer to ensure optimum quality. Seed quality therefore comprises genetic and mechanical purity, seed germination, vigor and seed health (McDonald, 1998).
The field performance of seed lot is dependent on its quality. Two attributes of seed quality that are often measured are seed viability and vigor (Johnson and Wax, 1976). The viability of a seed refers to its ability to germinate and produce a normal seedling (Delouche and Caldwell, 1960). Viability also refers to the degree to which a seed is metabolically active and contains enzymes capable of catalyzing the reactions needed for germination and seedling growth. Thus, a seed lot usually made up of a mixture of dead and live seeds with the live seeds containing both dead and live tissues (Copeland and McDonald, 2001).

Vigor is defined as those seed properties, which determine the potential for rapid, uniform emergence and development of normal seedlings under a wide range of field conditions (AOSA, 1983). Vigor is therefore a measure of the performance of the seed under unfavorable conditions. Because vigor is directly linked to the quality of the seed, seed vigor is affected by the same factors that influence seed quality (Sun et al., 2007). Soybean seeds attain their maximum vigor at physiological maturity, and seed vigor will decrease irreversibly thereafter (Sun et al., 2007). Seed vigor can be measured through germination rate, seedling length, root length, seedling fresh weight, seedling dry weight, seed longevity, and tolerance to adversity. These vigor-related traits are quantitative in nature and often interact with the environment during seed maturation, harvest and storage to determine the vigor of the seed at any point in time (Sun et al., 2007).

The deposition of the same molecular compounds that prepare the seed for desiccation tolerance is important to seed vigor. At maturation drying the cytoplasm of seeds enter into a state of high viscosity known as the glass state (Buitink and Leprince, 2008). A glass is an amorphous metastable state that resembles a solid brittle material,
but retains the disorder and physical properties of the liquid state (Buitink and Leprince, 2008). Glasses fill spaces in seeds during dehydration and the high viscosity may stop all chemical reactions requiring molecular diffusion (Buitink and Leprince, 2008). The lower the temperature of drying during this period, the higher the cellular water content of the seed at which the cytoplasm becomes glassy. Late embryogenesis abundant (LEA) proteins are produced at maturation drying and accumulate in the cotyledon mesophyll cells (Shih et al., 2004). These proteins interact with high temperature oligosaccharides to form tight glass matrices that protect the seed from cellular damage during maturation drying (Shih et al., 2004). The stability of the seed is thus maintained in the glass state until fluctuations in moisture and temperature during storage destabilizes the glass state and causes rapid deterioration (Buitink and Leprince, 2008).

Uniform field emergence and faster stand establishment are two qualities that are very critical for the realization of the full potential of yield and value (McGee, 1995). However, to produce seed of high quality, many complex technologies are required during the seed production phase. During planting, growth and development, harvesting, processing and storage, careful handling will protect the seed from mechanical injuries, adverse environmental conditions, pests, and diseases (McGee, 1995).

SEED QUALITY TESTS

Tests for viability

According to the Association of Official Seed Analysts (AOSA, 2009) seed germination is ‘the emergence and development from the seed embryo of those essential structures which, for the kind of seed in question, are indicative of the ability to produce a normal plant under favorable conditions’. The standard germination test is used for seed...
labeling and it is conducted under the best conditions possible for seed and seedling growth. However, these conditions are seldom encountered in the field. Thus the standard germination test is a poor predictor of field performance of a seed lot but nevertheless, allows the seed producer to determine and compare the potential germination of the seed lot before it is planted. Many other viability tests have been developed but these tests are seldom used because of the expertise needed as well as the lack of standardization of the protocols among seed testing laboratories. Subtle manifestations of the loss of seed quality are often expressed by an increasing production of abnormal seedlings, which is a component of the standard germination test. The deficiencies of the standard germination test are addressed by seed vigor tests. Soybean seeds with a standard germination score of ≥ 95% were deemed good for predicting field emergence (Egli and Tekrony, 1995).

Tests for vigor

The vigor test is more sensitive than the standard germination test and it is a good measure of the performance of a seed lot under field conditions (Johnson and Wax, 1978). Any physical or physiological event that precedes loss in viability could be used for estimating the vigor of a seed lot. Consequently, membrane integrity, oxygen consumption, level and activity of enzymes could all be used as determinants of vigor (Johnson and Wax, 1978). The cold test, the accelerated aging test, the conductivity test, the brick grit test and the osmotic stress test are some vigor tests adopted by seed analysts (AOSA, 1983). Soybean seed producers usually establish a vigor index for each season before seeds are conditioned, treated and marketed. For each soybean seed lot a series of vigor tests are performed and the results are compiled into a vigor index. However, high
vigor seeds do not guarantee high yields as the conditions beyond planting may affect the yield of the crop. But high vigor in a seed improves its chances of emergence and establishment (AOSA, 1983). Egli and Tekrony, (1995) found that seed lots with an accelerated aging score of ≥80% would produce adequate emergence in what they termed a reasonable range of field conditions. In addition, Byrd and Delouche (1971) considered the accelerated aging test to be superior to other vigor tests in predicting the storage potential of soybean seeds.

The most commonly used vigor test for soybean seed is the accelerated aging test in which seeds are artificially aged by exposing the seeds to high temperature and relative humidity over a relatively short time (Delouche and Baskin, 1973). The test evaluates the physiological potential of soybean seeds (Torres et al., 2004). Delouche and Baskin (1973) showed that accelerated aging responses of seed lots were closely associated with emergence potential of seeds, growth, development and productivity of the subsequent plants. They also reported that the germination potential of seeds after accelerated aging was highly correlated with seed survival in storage under a variety of conditions for up to 3 years. Thus, in addition to predicting field performance, the accelerated aging test is also a predictor of seed deterioration during storage (Delouche and Baskin, 1973; Tekrony et al., 1993).

FACTORS THAT AFFECT SOYBEAN SEED QUALITY

INITIAL SEED QUALITY

Seeds that have high initial viability withstand unfavorable storage conditions better than similar seeds of low initial viability. The predictive ability of any seed quality test of seed deterioration in storage is based on the relationship that exists between the
initial seed quality, seed longevity, seed moisture content and storage conditions of temperature, relative humidity and oxygen concentration (Tang et al., 2000). Several inherent (genetic) factors of the seed such as hybrid vigor, hard-seededness, susceptibility to seed damage, and chemical composition can influence the seed vigor and ultimately, viability (Copeland and McDonald, 2001).

Environment during seed production.

The environment where seed develops can have great influence on viability as well as seed vigor (Sun et al., 2007). The seed production environments are defined by the availability of soil nutrients, soil moisture, and the temperature and relative humidity during seed development and maturation (Sun et al., 2007). Low humidity, minimal rainfall and favorable temperatures during seed maturation give rise to good quality seeds (Copeland and McDonald, 2001). High temperature stress during the later stages of seed development in soybean can result in seeds with significantly lower germination rates than at early seed development stages (Egli et al., 2005; Spears et al., 1997). Adequate soil nutrients usually results in seeds with abundant storage material for use during germination of the seed and seedling until seedling photosynthetic ability is established (Justice and Bass, 1978). An immature seed or seed that has endured weathering conditions in the field may not store well. Warm and wet conditions are known to increase infection by some fungi especially Phomopsis longicolla on soybean (Spears et al., 1997).

Soybean seed composition and genetics

Seed vigor differences exist among species and among cultivars of the same species; and these seed vigor differences are genetically regulated (Yu et al., 1999).
The mature soybean seed is made of very few proteins (Hill and Breidenbach, 1974), which can be structural, enzymatic or storage proteins. Hill and Breidenbach (1974) isolated and characterized soybean seed proteins and obtained protein profile proportions with sedimentation coefficients of 2.2S, 7.5S and 11.8S. The proportion with sedimentation coefficient of 2.2S corresponded to proteins with enzymatic activities such as the trypsin inhibitors. The 7.5S and 11.8S proportions were storage proteins composed of two main fractions, vicillins and legumins, respectively (Hill and Breidenbach, 1974; Thanh and Shibasaki, 1978). The bulk of the soybean storage proteins are the salt-soluble globulins, β-conglycinin and glycinin (Krishnan, 2000). Together, these soybean storage proteins account for about 70% of the total protein content in the seed (Morales and Kokini, 1997). Most of the functional proteins are stress-related proteins. For example, the heat shock proteins protect against heat stress, while the late embryogenesis abundant (LEA) proteins play a role in seed desiccation tolerance and membrane stability (Hundertmark et al., 2011). The chemical composition of the seed determines the optimum seed storage moisture content, which varies among varieties, among species, among cultivars and among tissue types in the individual seed (Vertucci and Roos, 1990).

Twenty percent of the dry weight of soybean seed is made up of oil (Clemente and Cahoon, 2009). The soybean seed contains 14 fatty acids of which palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2), and linolenic (C18:3) are considered essential to human nutrition. The percentage of these five fatty acids in soybean oil averages 10%, 4%, 18%, 55%, and 13%, respectively (Clemente and Cahoon, 2009). Sixteen percent of the total fatty acid is saturated while 84% is unsaturated. This fatty
acid profile results in low oxidative coefficients, which are considered partly responsible for its poor storability.

Vertucci and Roos (1990) observed that the moisture content at which physiological processes change within the seed was highly correlated with the lipid content of the seed. High lipid seeds had lower thresholds for respiration and required lower moisture contents for optimum storage. Oil content also appeared to have a strong correlation with absolute longevity of soybean seeds under open storage conditions (Nagel and Börner, 2010). But the relationship between longevity and oil composition is more complex for legume seeds containing a range of oil compositions such as soybean (Walters et al., 2005).

Soybean seed maturity and maturity groups

Soybean is considered a short-day plant in which flowering occurs when the day length is shorter than the critical photoperiod (Zhang et al., 2007). The time of flowering and maturity in turn determine the geographical adaptation of a variety (Zhang et al., 2007). The main reason for soybean adaptation to different geographical zones is the sensitivity to photoperiod, which is modulated by daily temperatures (Upadhyay et al., 1994). Thus, photoperiod and temperature interact with genotype to control soybean growth and development from germination through onset of flowering, seed development and maturity (Major et al., 1975; Cober et al., 2001). Most of the soybean cultivars will flower if the day length is less than the critical photoperiod, defined as the photoperiod that if exceeded causes a delay in flowering (Roberts and Summerfield, 1987).

Thus depending on this critical photoperiod threshold, soybean varieties are grouped into 13 maturity groups (MG) depending on the climate or latitude for which
they are adapted. MG 0 and I are adapted to Canada and the Northern United States while varieties belonging to higher MG would grow in the Tropics (Rigg et al., 2000). When a variety is grown north of its latitude, it will have delayed maturity because of the long days (Benitez et al., 2004). Conversely, if a variety is grown south of its latitude, it will mature faster because of the shorter days (Scott and Aldrich, 1970). Depending on the geographical location, early and late maturities within a group can differ by as many as two weeks.

The stage of maturity of the seed is also very important in determining its quality and the quality will determine its longevity in storage. The same plant may have seeds at different stages of maturity thus some seeds from the same plant may be more vigorous than others (Harrington, 1973). The increase in germination and vigor is directly associated with accumulation of reserve substances of protein and starch. During acquisition of desiccation tolerance, there is accumulation of late embryogenesis abundant (LEA) proteins, oligosaccharides, hormones such as abscissic acid, and vitamin E. The LEA proteins and oligosaccharides form the glass that maintains the macromolecular structure of the seed while the hormones and vitamin E would function in reduction of antioxidants storage the storage period (Tang et al., 2000; Wang et al., 2000; Zang and Wang, 2005; Sun et al., 2007). The temperature at which the cytoplasm becomes vitrified is the transition temperature (Tg). Calculations of moisture/Tg relationships of some orthodox species have shown that glass will form at 25°C when the embryonic tissues of the seeds reach moisture equilibrium at a relative humidity of 44-49% (Buitink and Leprince, 2008).
LEA proteins do not have tissue-specific expression thus they generally protect the plants from damage induced by abiotic stress (Ban et al., 2008). These proteins are synthesized in abundance at later stages of seed development. LEA proteins induce drought tolerance in the seed through membrane stabilization, acting as molecular chaperones, scavenging reactive oxygen species, sequestering ions, replacing water during maturation drying to form glass that stabilizes the cytoplasm (Ban et al., 2008; Shih et al., 2004). Accumulation of these heat-stable proteins during acquisition of desiccation tolerance can increase seed vigor and resistance to adverse storage conditions (Scott et al., 2004).

*Presence of microflora*

Pathogens found on or in seeds that could potentially be transmitted to a subsequent crop can adversely affect germination and vigor (Kulik and Schoen, 1981; Hepperly and Sinclair, 1981), emergence and seedling vigor. The level of infection of the seed determines the degree to which the seedling is affected. Diseased plants in the field produce shrunken seeds and often times smaller seeds that have reduced germination potentials and low vigor (McGee, 1995). When harvest is delayed due to wet weather seeds experience both physiological and pathological deterioration (McGee, 1995). Fungal invasion of seeds can lead to destruction of cell walls, starch granules, and storage proteins (Nightingale et al., 1999; Lisker et al., 1985). These effects have been documented in most seeds of cereal crops infected with *Fusarium* spp. (Nightingale et al., 1999). Lisker et al. (1985) found that seeds with cracked seed coats had more fungi than seeds with intact seed coats. In addition, the fungi count was correlated with free fatty acids and an increased respiration indicating a reduction in the starch granules within the
seed. The physiological changes caused by fungal infections could lead to considerable reduction in seed quality. Fungi such as *Colletotrichum truncatum* (Schw.) Andrus & W. D. Moore, *Phomopsis sojae* lehman, *Cercospora kikuchii* (Tak. Matsumoto & Tomoy.) M. W. Gardner and *Alternaria* spp. are considered seedborne and usually infect the seed in the field (Manandhar et al., 1987; Schortt et al., 1981; McGee, 1992).

Fungi commonly referred to as storage molds are fewer than 10% and belong to the genera of *Chaetomium* sp., *Rhizopus*, *Mucor*, *Aspergillus* and *Penicillium* spp and increase during storage (Roy et al., 2000). Field fungi will attack developing or mature seeds which contain at least 20% moisture content and are in equilibrium with RH of 90 – 100%; while storage fungi will infect seeds that have a moisture content of 13 – 20% or are in equilibrium with RH of 70 - 90%. Nevertheless, field pathogens can still survive on the seed if storage conditions are favorable. The temperature and RH of the storage environment can greatly influence the infection rate, growth and development of the fungal pathogens on the seed. Infection rate of soybean seeds with *Diaporthe phaseolorum* (Cooke Ellis) Sacc. var. *sojae* was significantly lowered after 2 years of storage in a dry and cool environment (Kulik and Schoen, 1981).

**SEED MOISTURE CONTENT, RELATIVE HUMIDITY AND TEMPERATURE**

Temperature, relative humidity and oxygen content are the three most important environmental factors that affect seed quality in storage. At harvest maturity, the seed contains a hydration dependent glass state that will remain stable at physiological temperatures (Bruni and Leopold, 1990). The stability of the glass state is influenced by the amount and nature of water within the seed and this condition is very important for long-term seed survival. Vertucci and Leopold (1987) identified 5 possible hydration
levels within the seed that corresponded to three water-binding regions that determined the physiological activities in relatively dry systems. Seeds that are subjected to accelerated aging conditions fall within hydration level 3 where mitochondria are functional. In level 3, water is weakly bound and integrated enzymatic systems are functional (moisture content between 0.25 and 0.5 g H₂O/g dw). At hydration level 2, water is bound with intermediate strength and some simple enzyme systems are operable. The fluidity of the system determines the rate of deterioration at hydration level 2 (moisture content between 0.25 and 0.08 g H₂O/g dw). At hydration level 1, all water has been removed from reactive sites and deterioration is a result of intermolecular interactions (moisture content < 0.08 g H₂O/g dw). Damage to the seed could be due to ionic bonding on charged sites on proteins, membrane phase transitions, or free radical attacks on macromolecules. Further removal of water from seeds at hydration level 1 accelerates the deteriorative process because intramolecular, structural water is removed.

The rate of deteriorative reactions in a seed increases with an increase in its moisture content (Delouche, 1968). Vertucci and Roos, (1990) hypothesized that the level of a degradative reaction depends on the size of the substrates and their ability to diffuse through the glass matrix. Seeds with moisture contents in the range of 4-6% to 12-14% are thought to store longer than seeds with moisture contents above and below this range (Harrington, 1973). Consequently, the relative importance of each factor to the rate of deterioration of a seed will depend very much on the moisture content of the seed (Harrington, 1973). Seed moisture content also influences storability through its influence on growth, activity and reproduction of storage molds and insects (Delouche, 1968).
Seeds are hygroscopic so they absorb moisture from or release moisture to the atmosphere until the vapor pressure of the seed moisture and the atmospheric moisture reach equilibrium (Delouche, 1968). The moisture content of the seed at which its vapor pressure is in equilibrium with the atmospheric moisture is the equilibrium moisture content (EMC) (Delouche, 1968). The vapor pressure of atmospheric moisture at a constant temperature is directly related to its relative humidity. The time taken to reach EMC varies with variety, initial moisture content, relative humidity and temperature (Delouche, 1968).

The relative humidity (RH) of a storage environment, therefore, directly influences the moisture content of the seed (McDonald, 1972). The RH at which physiological changes occur within the seed was similar for most orthodox species (Ellis et al., 1991; Vertucci and Roos, 1990). The rates of thermal-chemical reactions within the seed will increase when the RH of its environment was 27% but at a RH of 19%, physiological reactions were slowed down enough for optimum longevity of the seed (Vertucci and Roos, 1990). Thus, the optimum moisture content for seed storage in terms of RH was defined as the moisture level between the RH where reactions become thermodynamically less feasible due to slow diffusion, and the level below which seeds deteriorate more rapidly (Vertucci and Roos, 1990). The moisture content of the seed that is in equilibrium with RH of between 19 and 27% was defined as the optimum for storage (Vertucci and Roos, 1990). However, Ellis et al. (1991) proposed that seeds should be dried to moisture contents in equilibrium with a RH in equilibrium with 11% moisture content at a temperature of 20°C. The moisture content at this RH was defined as the
critical moisture content of a seed lot below which the viability equation was not applicable (Ellis et al., 1988).

Temperature determines the amount of moisture the air can hold in the storage environment. Environments with higher temperatures will hold more moisture in the air than cooler ones. If the relative humidity of the environment is kept constant, the moisture content of a seed will decrease by a magnitude of 1% with every 6.7°C rise in temperature (Delouche, 1968). Temperature can affect the seed aging process by altering the rate of certain reactions through inactivation of enzymes (Vertucci and Roos, 1993). Using temperature isotherms, Vertucci and Roos (1993) showed that optimum moisture content for storage of soybeans seeds varied with storage temperature. Thus critical temperatures for long term storage are those below the glass transition temperature (Sun, 1997). The theoretical optimum moisture content for storage of soybean is within the wide range of 0.035 g H₂O/g dw at 65°C to 0.11g/g at -150°C (Vertucci and Roos, 1993).

Temperature has a significant effect on the thermodynamic properties of seed systems (Vertucci and Roos, 1990). At constant relative humidity, an increase in temperature causes an increase in water activity. Higher temperatures can also cause breaks in intermolecular bonds in a glass (Walters, 1998). The temperature at which the glass state changes to a rubbery state is known as the transition temperature (Tg). The consequence of this transition is an increase in molecular mobility, which can increase the rate of reactions within the seed. There is usually an increase in aging reaction rates when seeds are dried below a critical moisture level. The increase in the rate of reaction can be explained by the removal of water that is closely associated with macromolecules.
thus exposing macromolecular surfaces or metal ions to deleterious reactive species (Walters, 1998).

Vertucci and Roos (1990) reported that when the RH of the environment was held at 20%, oxygen uptake did not decline and viscosity did not increase with further decreases in moisture content. The authors concluded that oxidative reactions continued in a non-aqueous milieu. Thus the seed moisture content, amount of oxygen, temperature and relative humidity of the storage environment are the determinants of the type of deteriorative reactions at any point in storage time. High temperatures accelerate the rate of peroxidation of lipids and the absence of oxygen can inhibit the process (Schultz et al., 1962). Of all the factors that influence the longevity of seed in storage, seed moisture content has the greatest influence (Harrington, 1973).

SEED TREATMENTS

Soybean producers plant early to maximize yields. Early season growing conditions are characterized by cold and wet soils that increase the time required for seed germination, and consequently lengthen the infection period (Broders et al., 2007; Shulz and Thelen, 2008). As a result, soybean seed is treated with chemicals to guard against soilborne pathogens and insects that can potentially reduce yield. Chemical seed treatments ensure against diseases and pests that impair stand establishment and reduce yields (Buehring et al. 2004; Munkvold, 2009). Insecticide-fungicide treatments are applied to soybean seeds to control a complex of early season pests and pathogens, which include thrips, aphids, bean leaf beetle, Pythium and Fusarium species.

The use of fungicide seed treatments is the most common practice for managing soilborne seed and seedling pathogens. Research indicates that commonly used
fungicides are effective against *Fusarium* spp, *Rhizoctonia*, *Botrytis cinerea*, *Alternaria* spp. and *Pythium* spp (Broders et al., 2007; Shulz and Thelen, 2008). The commonly used fungicides include the benimidazole with active ingredient carbendazim; phenylpyrrole (fludioxonil); strobilurins (pyclastrobin and trifloxystrobin); and Phenylamide (mefenoxam). The mode of action of Fludioxonil is through inhibition of the protein kinase enzyme thereby inhibiting growth and development of the fungi through blockage of phosphorylation. Mefenoxam (Apron XL) acts by inhibiting spore production and mycelial growth (Broders et al., 2007).

One of the classes of insecticides commonly used is the neonicotinoids that include compounds such as imidacloprid and thiamethoxam. Thiamethoxam is a systemic insecticide that is translocated rapidly throughout the plant providing complete protection and controls insects both through contact and ingestion.

Other added physiological benefits documented so far include induction of plant defense responses, increased stress tolerance, or improved growth and yield (Bartlett et al., 2002; Munkvold 2009). Maximum soybean yields are often achieved with a combination of seed treatments and foliar application of insecticides later in the season (Bradley, 2008). If the cause of poor germination and vigor of a seed lot is the presence of fungal pathogens, the application of fungicide seed treatments usually improves the germination and vigor of such seed lots. Thus seed treatments are recommended in cases when seed is grown for seed production, when soybean is sown at a reduced seeding rate, when seeds are planted early in cool or cold soil with temperatures below 13°C (55°F) or in dry soil and when only poor quality seed is available for planting (University of Illinois Extension, 1988).
MECHANISMS OF SEED DETERIORATION

Seed deteriorates through normal physiological reactions and changes that occur within the seed over time. These changes result in the accumulation of deleterious byproducts that increase the seed’s vulnerability to external challenges and decrease the ability of the seed to survive (Justice and Bass, 1978). This process is inevitable and irreversible and only its rate can be controlled (Delouche, 1968). The process of deterioration, therefore, involves several physiological and structural changes within the seed. Structural changes involve membrane permeability, proteins, sugars, nucleic acids, fatty acids and volatile substances, while physiological processes involve enzyme activity, respiratory competence, lipid peroxidation and physiological repair mechanisms (Walters, 1998; Sun et al., 2007). According to Vertucci and Roos (1990) optimum protocols for seed storage must take into account the chemical composition of the seed, the physiological status of the seed, and the physical status of water within the seed. Two models of seed deterioration have been proposed (Walters, 1998).

The Mechanistic model

This model uses the premise that changes in water properties of a seed coincide with changes in physiological activities within the seed. Therefore the types of reactions that take place within a seed in storage will depend on the thermodynamic properties of the water within the seed (Leopold and Vertucci, 1989; Vertucci and Roos, 1990). Most importantly, the thermodynamic property of the water is strongly correlated with the hydration level of the seed (Vertucci, 1993).
The Kinetic model

The kinetic model was postulated based on the fact that different reactions are involved in seed deterioration and that the rates (kinetics) of these reactions are differentially controlled by temperature and moisture content of the seed (Walters, 1998). The relative importance of each reaction in the seed deterioration process varies among different storage environments (Walters, 1998). According to this model, lipids are degraded into lipid hydroperoxides or free fatty acids that are then peroxidized. The products of peroxidation then react with proteins and inactivate enzymes. Carbohydrates are also degraded in hydrolytic reactions in a pathway that eventually leads to protein-carbonyl end-products in Amadori-type reactions (Sun and Leopold, 1994). This model was simulated in soybean and chemical degradation rates were predicted using a soybean variety containing 20% lipid, 40% protein and 35% carbohydrate. It was concluded that the relative importance of lipid and carbohydrate degradation varied with moisture content of the seed and temperature of storage. In addition, it was concluded that the critical moisture content for storage for each seed lot would increase with decreasing storage temperature (Walters, 1998).

SEED LONGEVITY

The longevity of a seed lot is the length of time the seeds remain viable after reaching physiological maturity (Delouche, 1968). For seed storage purposes, longevity is used synonymously with storability. To preserve the initial seed quality, seeds must be properly stored between the time of harvest and the planting of a subsequent crop. Delouche (1968) defined the total seed storage period as comprising segments of bulk
storage, which is the period from harvest through packaging including conditioning. Packaged storage was defined as the period between packaging and distribution; and distribution storage covered the period between sales to farmers, including time at wholesalers and retail outlets.

Sun (1997) showed that the glass state is required for long-term seed storage. The glass state is positively correlated with the accumulation of high temperature oligosaccharides such as sucrose, raffinose, stachyose and verbacose (Williams and Leopold, 1995). The amount of high temperature oligosaccharides within a seed influences the stability and magnitude of the glass state. Therefore, the higher the oligosaccharide content, the greater the stability of the glass and the longer the seed could be stored (Bernal-Lugo and Leopold, 1995).

The glass within the soybean seed was shown to effectively reduce the release of free radicals and inhibit sugar hydrolysis (Sun and Leopold, 1994, Sun, 1997). But because respiration in the soybean seed is still high at very low seed moisture contents, the accumulation of toxic substances over a short period of time may be responsible for its poor storability (Vertucci and Leopold 1987). Harrington’s rule of thumb (1972) states that within the normal range of moisture and temperatures for stored seed: each 1% reduction in seed moisture or each 5.6° C reduction in temperature doubles the storage life of the seed. These rules will not hold true for moisture contents greater than 14% because of increase respiration and fungal growth; and will not hold true at less than 5% moisture content because of breakdown of membrane structure due to the reorientation of hydrophilic components within the membranes. And below 0° C, this rule may not hold because biochemical reactions associated with deterioration do not occur.
Because at maturity metabolic activity within a seed is reduced to a minimum, the death of a seed is not as abrupt as in other living organisms. Rather, it is an imperceptible change from a state of very low metabolic activity to non-living state (Walters, 1998). Even under the best storage conditions, the seed is still incapable of repairing the structural and functional changes brought about by the low metabolic activity within the seed in the dry state (Hinton, 1968). Consequently, seed longevity is a quantitative trait that is more of the characteristic of the species or variety and the storage environment can only help maintain it (Delouche, 1968).

RESPIRATION, LIPID PEROXIDATION AND FREE RADICALS: THE CASE OF SOYBEAN SEEDS

Oxidative reactions that occur within stored seeds are considered to be, in part, responsible for seed deterioration. The rate of oxygen uptake in soybean is very strongly correlated with the seed moisture content. Seeds with high moisture content (≥ 24%) had a higher respiratory quotient than those with lower moisture content (≤ 24%) (Vertucci and Leopold, 1987). At very low moisture contents of 0.10 and 0.24 g H₂O/g seed at which respiratory processes are imperceptible in other seeds, Vertucci and Leopold (1986) were able to measure low levels of respiration in the soybean seed. Evidence that aging in soybean is induced by accumulation of respiratory by-products was observed in aged seeds in which there was a decrease in oxygen consumption due to mitochondria injury (Amable and Obendorf 1986). Other observable effect of aging in the mitochondrion was the reduction of the rate of conversion of AMP and ADP to ATP in deteriorated axes of soybean (Amable and Obendorf, 1986). Soybean seeds stored at high temperatures also accumulated reactive oxygen species in their testa (Khan et al., 1996).
The consequences of respiration at all moisture contents is the peroxidation of membrane unsaturated lipids. Lipid peroxidation can be non-enzymatic (autoperoxidation) or enzymatic (by lipoxygenase) and both processes result in seed aging (Nagel and Börner 2010). Autoperoxidation is initiated by oxygen around unsaturated or polyunsaturated fatty acids such as linoleic and linolenic acids found commonly in seed membranes and storage oils (Copeland and McDonald, 2001). In intact seeds, autoperoxidation usually starts in the mitochondria polar lipids of the embryonic axes (Priestley et al., 1985; Stewart and Bewley, 1980). This results in the formation of free radicals that are propagated to other membranes within the seed. Lipid peroxidation occurs in all cells, but in fully imbibed cells, water acts as a buffer between the free radicals generated by autoxidation and the target macromolecules, thereby reducing damage. The choice of the lipid peroxidation reaction is dictated by the moisture content of the seed. Thus, as seed moisture content is lowered, autoperoxidation is more common and is accelerated by high temperatures and increased oxygen concentrations (Trawatha et al., 1995). Autoperoxidation is the predominant cause of seed deterioration at moisture contents below 6%, while above 14% moisture content, lipid peroxidation is stimulated by the activity of hydrolytic oxidative enzymes such as lipoxygenase that become more active with increasing water content (Krishnan, 2000).

According to Nagel and Börner (2010) the chemical composition of the seed affects its sorption properties; the available potential sites for free radical attack and the presence and activity of protective compounds within the seed. In addition to their low threshold for respiration, soybeans have high levels of polyunsaturated fatty acids. The consequence of peroxidation of polyunsaturated fatty acids of the seed membranes is the
destabilization of the membranes, which leads to uncontrolled leakage of solutes (Priestley et al., 1980). In addition, damage to the mitochondria results in decreased oxygen consumption and low levels of oxidative phosphorylation (Amable and Obendorf, 1986). Soybean seeds stored at 4°C and at low humidity for 44 months showed a marked decline in vigor associated with a decrease in the proportion of unsaturated fatty acids (Priestley and Leopold, 1983).

Other by-products of the seed aging process that can lead to seed deterioration are the Amadori and Maillard products. The Amadori and Maillard products are the result of sugar hydrolysis and lipid peroxidation coupled with nonenzymatic protein modification (Murthy and Sun, 2000). Amadori reactions lead to the chemical modification of proteins by reducing sugars to form fructosyl derivatives, or glycosylated proteins within the seed during storage (Wettlaufer and Leopold, 1991). This process gradually reduces the ability to limit free radical damage and hinders the repair of damage during seed germination (Murthy et al., 2003; Murthy and Sun, 2000). Maillard products are formed through subsequent complex interactions between glycosylated Amadori products to form polymeric brown colored products. Maillard products were observed in naturally aged soybean seeds and were associated with the loss of seed viability under long term storage conditions (Sun and Leopold, 1995). The accumulation of Maillard products was observed for soybean axes that were subjected to the accelerated aging conditions also (Sun and Leopold, 1995). At seed moisture contents between 8% and 12%, raffinose and stachyose are hydrolyzed giving rise to reducing sugars that are rapidly used in the Amadori and Maillard reactions (Sun and Leopold, 1995). Also lipid peroxidation may
give rise to secondary products that may degrade protein and DNA in a nonenzymatic way through the Amadori and Maillard reactions (Murthy and Sun, 2000).

Soybean seeds contain a complex system of antioxidants to protect against the harmful consequences of reactive oxygen species. These include the free-radical scavengers such as superoxide dismutase, catalase, ascorbate peroxide, peroxidase, and tocopherols (Harrington, 1972). The location of some of these compounds in the seed is an indication of the importance of their role as reactive oxygen species scavengers in the seed. Storage lipid bodies forms about 90% of the cellular lipid in soybean seeds and are associated with the great majority of tocopherols. A significant proportion of the tocopherols are also associated with membranous fractions (Yamauchi and Matsuchita, 1976). Tocopherol contents in soybean seeds were shown to decline after accelerated aging suggesting it was consumed to protect the seed against free radicals (Seneratna et al., 1988). Superoxide dismutase is believed to be synthesized de novo by the seed during imbibition (Stewart and Bewley, 1980). Experimental evidence that support the involvement of these antioxidants in preventing soybean seed deterioration is sketchy (Stewart and Bewley, 1980; Sung, 1996; Priestley et al., 1985; Priestley et al. 1980). Some reports on the involvement of free radicals in the seed deterioration process are contradictory. The lack of agreement of study results between research laboratories has been explained by the fact that free radical measurements in the seed are not done against a gradient (Hendry, 1993). Indirect evidence that autooxidative mechanisms due to free radical activity was obtained when Pammenter et al. (1974) reverse vigor losses by reducing free radical in the seed with a 300 V charged cathode.
The radicle of the soybean embryonic axis is susceptible to deterioration because of its proximity to the funicular end. This proximity places it directly in contact with both water and oxygen that enters through the hilum. Because the meristems are seats of intense energy production and possess high numbers of mitochondria, lipid peroxidation may be greatest in the radicle than elsewhere in the seed. The presence and amount of antioxidants in the soybean seed may minimize some of the damage caused by free radicals during storage (McDonald 1999). But tocopherol and other antioxidants levels do not increase during aging because these are synthesized during imbibition of the seed. Therefore, the amount and rate of synthesis of antioxidants determines whether the seed is capable of germinating or not (Sun and Leopold, 1995).

Because seed deterioration does not occur uniformly throughout the seed, the presence of cotyledons may mask metabolic activity of embryonic axes (Anderson, 1977; Anderson and Baker, 1982; Hendry, 1993). Therefore, assaying whole seeds for lipid peroxidative and antioxidant activities may lead to erroneous results. The process of lipid peroxidation may be initiated in the embryonic axes, particularly in the radicle in a cryptic way and accelerates to the cotyledons, a tissue that contains 90% of cellular lipids. Stewart and Bewley (1980) estimated the content of linoleic and linolenic acids in the polar lipid fraction of soybean seed axes to be 56.4 and 15.4% in unaged seeds, and 60.2 and 14.9% in low humidity aged seeds, respectively.

In conclusion, soybean seed contains high levels of membrane polyunsaturated fatty acids. Respiration in the mitochondrion within the radicle axis generates free radicals that may uncouple the oxidative phosphorylation process through autoxidation of mitochondrial membrane’s linolenic and linoleic acids (McDonald, 1999; Parish and
Leopold, 1978; Priestly et al., 1985; Wilson and Rinne, 1974; Anderson, 1977). The free radicals generated are trapped in the mitochondrion but may eventually escape as the deteriorative process continues. Less energy is stored and the mitochondrial DNA because of lack of protection is destroyed. As more free radicals are formed, other membranes of the seed are destroyed by lipid peroxidation (Priestley, 1986; Sun and Leopold, 1995). At moisture contents between 6-14%, autooxidation of membranes lipids is not accompanied by peroxidation mediated by hydrolytic lipoxygenases. At moisture contents above 14%, peroxidation is stimulated by hydrolytic lipoxygenases. The uncoupling of oxidative phosphorylation and insufficient production of ATP in the radical axis may be cryptic but enough to cause the death of the embryonic axes (McDonald, 1999). And because levels of tocopherol are not high enough and other free radical scavengers are synthesized only during imbibition, this repair process maybe too slow to be effective for recovery during germination. Death of the embryonic axes may occur earlier and deterioration of cotyledons may occur at a later stage, as evident from the contradictory results with naturally and artificially aged soybean seeds. Therefore, soybean seeds with low or no polyunsaturated fatty acids, with high expression of tocopherol may have a better shelf life. The fact that soybean seeds continue to respire even at moisture contents as low as 0.1 and 0.24 g H₂O/g seed may greatly contribute to its poor longevity in storage (Vertucci and Leopold, 1987).

JUSTIFICATION FOR STUDY

The soybean seed is well known for its short storage life and is currently not carried-over to the next planting season. This problem is presently compounded by the consistent use of seed treatments, which render the left over seed not fit for the feed
market. Consequently, soybean seed deterioration is a major problem in agricultural production systems. Because seed longevity has never been one of the agronomic traits in soybean breeding, the relationship between seed vigor and seed treatments, seed composition, and seed maturity group has not been studied. This study focuses on the importance of seed treatment chemicals, soybean maturity group, seed composition, and initial seed-borne pathogen load on seed aging in a range of storage environments. Our hypothesis is that soybean seeds could be carried-over at least two years if the storage conditions are optimized to reduce seed deterioration (Harrington, 1972). Knowledge of the relationship between seed vigor and longevity with any of the agronomic traits of soybeans is very important for future work in breeding for soybean seeds with high field performance and better storability. The impact of different seed treatments and fungi on germination and vigor of the seed after storage may provide seed companies with useful information to plan for proper storage conditions to maximize shelf-life of the soybean seed.

The objectives of this study were to determine the effect of storage temperature and relative humidity on viability and vigor of chemically treated soybean varieties, the effect of maturity group and seed composition on longevity of soybean seeds; and finally the effect of initial fungi load on the rate of deterioration of soybean seeds.

THESIS ORGANIZATION

This thesis is divided into three chapters. The first chapter comprises a literature review that includes a description of seed quality, the factors that affect seed quality, the mechanisms of seed deterioration and a justification for the research conducted. The second chapter deals with the effect of temperature and relative humidity of the storage
environment on viability and vigor of a range of soybean seed genotypes that have been chemically treated. The third and last chapter summarizes the research and gives a general conclusion.

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CHAPTER 2

EFFECT OF STORAGE TEMPERATURE AND RELATIVE HUMIDITY ON VIABILITY AND VIGOR OF CHEMICALLY TREATED SOYBEAN SEEDS

A paper to be submitted to Crop Science Journal

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ABSTRACT

Seed treatments are routinely applied to soybean (Glycine max L. Merrill) seeds to control early season diseases and insects that are common pest problems in an early soybean production system. However, unsold treated soybean seed at the end of the growing season must be disposed differently than untreated seed. In order to minimize treated seed disposal costs there is a need for improved storage of carry-over seeds. The objective of this study was to determine the best storage conditions that will minimize deterioration of chemically treated soybean seed from different maturity groups and seed composition. Twenty-four soybean varieties were treated either with fungicide, a mixture of fungicide + insecticide or untreated and stored in three storage conditions that differed in temperature and relative humidity. The potential of the three storage conditions to maintain the initial seed viability and vigor was evaluated over time using standard germination and accelerated aging tests. The duration of the storage period was 20 mo. Soybean varieties were significantly different in their rate of decline in viability and vigor over time. Seed viability values remained high for both, coldroom and warmroom, but decreased to almost zero in the warehouse. The advantages of seed treatment were evident at 16 months in the warehouse, while in the coldroom and warmroom the positive
effects were evident at 20 months. Maturity group and protein content did not affect
deterioration while seed oil content accounted for only 5 to 15% of the decline in seed
vigor. Treated soybean seeds could be carried-over for two seasons if the temperature of
the storage environment is maintained at 10 °C and the relative humidity kept constant at
≤ 40%. Seed treatment would improve storability if seeds are stored in low temperature
and constant relative humidity environments but not in other environments.

**Key words:** *Glycine max*, seed treatment, seed viability, seed vigor, storability.
INTRODUCTION

Research has shown that planting early and reducing disease pressure have greater positive impact on soybean (*Glycine max* L.) yield than any other management practice. A positive yield response also may be obtained when seed treatment is applied to the seed before planting into cold and wet soil conditions. Seed treatments also minimize the use of foliar and soil pesticide application because they are applied in small quantities directly to the seed. In addition, seed treatments protect the growing seedling and promote good emergence and uniform stand establishment by eliminating seed-associated pathogens (Schulz and Thelen, 2008). As a consequent, soybean production has evolved into an early soybean production system (ESP) in which soybean producers plant early in order to maximize yield (Smith and Mengistu, 2010) without the risks of yield losses due to seedling pathogens.

An estimated 80% of the soybean planted in the US is treated soybean seed, which translates into more than 71.14 million bags (NASS, 2010). The excess seeds must be discarded at the end of each planting period. In the past, excess non-treated seed was sold in the grain commodity market. But this disposal method is no longer feasible as treated seed must be incinerated, planted at high rates based on label restrictions, or buried (ISTF, 2000). An alternative solution is to carry-over the excess seed for the next cropping season, but soybean seeds store poorly (Delouche et al., 1973; Kreuger et al., 2012). In order to minimize seed disposal costs there is a need for safe and economical storage of carry-over seeds.

Soybeans are not carried over for planting beyond six months because of the fast rate at which the seeds age and eventually lose their ability to germinate (Burris, 1980;
Byrd and Delouche, 1971). Justice and Bass (1978) placed soybean among the group of least storable seeds in the “relative storability index” classification. However, seed longevity in storage is influenced by the initial quality of the seed lot, the moisture content of the seed, and the temperature, relative humidity and gaseous exchanges in the storage environment (Barton, 1943; Vertucci and Roos, 1990; Vertucci and Roos, 1993). Seed longevity in storage is also a genetically regulated process because the accumulation of seed storage substances is the result of a determinant genetic program (Delouche, 1968).

Maximum seed quality, as defined by seed germination and vigor, coincides with the developmental stage of physiological maturity (Bewley and Black, 1994). Beyond this stage the seed starts deteriorating. Seed deterioration, therefore, is an inexorable process that cannot be reversed. Only its rate can be slowed by storage in a controlled environment (Delouche, 1968). Studies have shown that high temperature and relative humidity in the storage environment will speed the rate of deterioration of a seed lot (Harrington, 1973). Also seeds subjected to fluctuating levels of moisture seem to deteriorate faster than seeds held at a constant level (Bass, 1973). Thus, the magnitude of temperature and relative humidity and the duration of storage are important determinants of the rate of deterioration (Delouche, 1968). Storage fungi are also a major cause of quality losses in stored seed (Delouche, 1968), with the extent of deterioration being dependent on the relative humidity of the storage environment.

Harrington (1959) defined the best storage conditions in a set of “rules of thumb” that have become a standard in the seed industry. These rules state that for each 1% decrease in moisture content the storage life of the seed is doubled; for each 5.6°C
decrease in storage temperature the storage life of a seed is doubled; and that the arithmetic sum of temperature in °F and percent relative humidity should not exceed 100 with not more than half contributed by temperature. These rules have been used in seed conservation for short term storage of two to more years (Walters, 1998). Soybean seeds stored for 6 months at a temperature of 15°C maintained high germination and vigor, and the germination rate remained at 95% for 6 months when a cool storage environment also was maintained at 60% relative humidity (Herrera and Rosales, 1987). Other studies showed that seeds stored in controlled temperature of 15-20 °C had higher percentage germination than those stored at ambient temperature (Nattasik et al., 2001). We therefore hypothesized that treated soybeans seeds could be carried-over at least two years if the storage conditions follow Harrington’s rule (1959) of temperature of 10 °C and 50% relative humidity.

While much is known about storage of untreated soybean seed, very little information is available on the effect of seed treatment and seed characteristics on longevity of soybean seeds in storage. This study focuses on the importance of seed treatment chemicals, soybean maturity group, seed composition, and initial seed-borne fungi load on seed aging in a range of storage conditions. The objective of this study was to determine the best storage conditions that will minimize soybean seed deterioration of chemically treated seed from a wide range of genotypes.
MATERIALS AND METHODS

Seed lots

A total of twenty-four soybean varieties were obtained from three seed companies (Monsanto, Pioneer Hi-Bred International Inc., and Stine Seed Company). The varieties were chosen to represent four maturity groups (Maturity groups I, II, III, and IV) and two seed composition extremes within each maturity group, high oil and high protein varieties. Two bags of each soybean variety were used as replications. Each bag of seed was subdivided into three equal parts, and each third was assigned to a seed treatment. A seed weight of 1500 g per variety was treated with fungicide or fungicide + insecticide following the manufacturer’s medium labeled rates. The treatments were applied a day before packaging to allow chemicals to dry on the seed. The seed treatments were a mixture of the fungicides, fludioxonil and mefenoxam, a mixture of these fungicides and the insecticide thiamethoxam, or an untreated control. These seed treatments represent some of the currently available treatments for soybean seed. Standard germination and accelerated aging tests were conducted for all seed lots before storage to determine the initial seed viability and vigor.

Seed storage

Two samples of 100 seeds each per treatment per replicate and per seed lot were placed inside coin envelopes, and the coin envelopes were placed inside a large envelope (Quality Park Products, Minneapolis, MN). One of the samples was used for evaluating seed viability and the other was used for evaluating seed vigor. Twenty-four large envelops representing the 24 varieties of soybean per seed treatment were stored inside a triple-wall seed paper bag (Central Bag Company, Kansas City, MO). The seeds were placed in three storage conditions: a non-climate controlled warehouse, a climate
controlled coldroom (10°C and 59.6±7.3% RH), and a climate controlled walk-in
germinator (25°C and 31.2±11.1% RH). The three storage conditions hereafter will be
referred to as Warehouse, coldroom and warmroom, respectively. Seed viability and
vigor evaluations were carried out at 4, 8, 12, 16 and 20 mo after storage. HOBO model
U-14 temperature and RH data loggers (OnSet Corp., Pocasset, MA) were used in each
storage environment for recording temperature and relative humidity data. The
experimental design was a split split split plot in a randomized complete block design
with two replications. Upon reception, seed samples were evaluated for initial seed
viability and vigor.

*Seed viability test*

The standard germination test was used to evaluate seed viability. The tests were
performed following the Association of Official Seed Analysts Rules for Testing Seeds
(AOSA, 2009). One sample of 100 seeds per replication per treatment were placed on
crepe cellulose paper (Kimberly Clark, Neenah, WI) previously moistened with 840 ml of
water on fiber-glass trays (45 cm x 66cm x 2.54 cm). The trays were placed in
germination carts after planting and the carts were placed in a walk-in germination room
with alternating 4-h of light and 4-h of darkness totaling 12-h of light a day for 7 d.

*Seed vigor test*

Seed vigor was evaluated using the accelerated aging test (AA). The test was
performed according to the AOSA (1983) seed vigor testing rules. One hundred seeds per
replication per treatment were placed in a single layer on a wire mesh in a plastic 10 x 10
x 4 cm box (Hoffman Manufacturing Co. Albany, OR) containing 40 ml of distilled
water. Lids were placed over boxes and the seeds were then subjected to a temperature of
41°C and a RH of 100% for 72 hours in an AA chamber (VWR Scientific, Chicago, IL). Immediately after the aging period, the seeds were removed from the chamber and planted on moist crepe cellulose paper on fiberglass trays and covered with 2.5 cm of moistened sand. The seeds were allowed to germinate for 7 d in a constant 25°C walk-in germination room with alternating 4-h of light and 4-h of darkness for a total of 12-h of light a day.

*Seed composition analysis*

Seed oil and protein content of each seed lot were analyzed in the Grain Quality Laboratory at Iowa State University. The test was conducted on two replicates of 400g of seed of each variety using a whole-grain near infra-red analyzer following protocols established by Rippke and Hurburgh (2006) and the results were standardized to a seed moisture level of 0.13 g H₂O g⁻¹ fw basis.

*Seed fungi assessment*

The blotter test was used to identify and enumerate the initial fungi load on each seed lot before storage. Two blotter sheets were saturated with a solution of 0.05% Botran, active ingredient 2, 6-dichloro-4-nitroaniline (Gowan Company, Yuma, AZ) and placed in plastic boxes. One hundred seeds were plated on the blotter with a planting board and evenly spaced with a pair of forceps. Then boxes were incubated for 10 d inside a dark germination cart in a constant 25°C walk-in germination room. Seeds were examined for fungal growth 3, 5, 7, and 10 d after plating.
Moisture content of seeds in storage

The initial and final moisture content of the seeds were determined for the constant storage conditions (coldroom and warmroom). Triplicate samples of 100 seeds per seed lot were placed in Pyrex petri dishes and weighed using a satorius balance (Satorius Ag, Goettingen, Germany). Weighed samples were placed in an isotemp gravity-convection oven (Thermo fisher Scientific, Hanover Park, Illinois) set at 103 °C for 72 hours. At the end of the drying period, the dishes were removed and weighed. The percentage of moisture (wet basis) was calculated by dividing the loss in weight due to drying by the weight of the original sample, and multiplying by 100. The moisture content of seeds in the warehouse was calculated using the Kews Royal Botanical Gardens moisture content calculator that uses seed oil content, temperature and relative humidity of the storage environment to estimate the seed equilibrium moisture content over time (Cromarty et al., 1982).

Data analysis

The effect of storage conditions and seed treatment on seed viability and vigor as determined by the standard germination and accelerated aging tests were analyzed using the generalized linear mixed model (PROC GLIMMIX) of SAS (SAS Institute, Gary, USA). All factors were treated as fixed effects, while interactions with replication were considered random effects. Means of main effects and interactions were compared with Tukey’s test by using least square mean comparisons. The statistical analysis showed a significant interaction among seed treatment, storage conditions and evaluation time. Consequently, the data were sorted by evaluation time and reanalyzed. The mean effect of seed maturity group, seed oil and protein content and initial fungi load on seed
viability and vigor changes over time were compared and regression analyses were done using PROC REG procedure of SAS. Daily and monthly average temperatures and relative humidity were calculated from measurements taken every three hours at each storage condition.

RESULTS

The initial moisture content of the seed lots before storage ranged between 5.95% and 8% fresh weight basis (fw); and variety 20 had the lowest moisture content of 5.95% fw. The moisture contents of the seed lots and the relative humidity and temperature of the storage conditions measured at the end of the experiment are presented in Table 1. The mean moisture content for each seed lot was averaged over all varieties after 20 months in storage in the coldroom ranged between 10.15 to 10.77%, while the seed lots in the warmroom had lower moisture contents in the range of 5.66 to 5.81% (Table 1). The moisture contents for seeds in the warehouse were calculated according to the fluctuating temperature and relative humidity of the warehouse using the seed moisture content calculator on the website of the Kews Royal Botanical Garden. The calculator was developed by Cromarty et al. (1982) based on the viability equation of Ellis and Robert (1980). The calculator was modified to take into account the oil content of the seed (Eckey, 1954). The calculated ranges of moisture content under these storage conditions were between 11.4 and 12.7% (data not shown). The daily temperatures within the coldroom for most of the duration of the experiment ranged from 9.80 °C to 11.58 °C, and the daily mean was 10.4±0.4 °C. The daily range of relative humidity was 42% – 68.5%, with a mean of 59.6±7.3%. The daily range of temperature for the warmroom was between 24.4 °C and 27 °C and the daily mean was 25.4±0.8 °C. The relative humidity in
the warmroom was in the range of 14.8% and 45% and the daily mean of 31.2±11.1%. In
the warehouse the temperature fluctuated between -7.8 and 28 °C, and the mean daily
temperature was 14.9±8.6 °C. The range of relative humidity in the warehouse was 37 to
74% and the daily mean relative humidity was 59.67±8.9%.

The overall analysis of variance for variety, seed treatment, storage conditions,
and storage time after 16 and 20 mo of storage were very similar for seed viability but not
for seed vigor. This was because after 20 mo of storage, seed lots in the warehouse were
severely deteriorated and the measured seed vigor was zero for all seed lots.
Consequently, the mean square error, variance, and standard deviation for seed vigor
could not be computed. However, the overall analysis of variance for seed viability after
20 mo of storage showed that the four-way interaction between all factors was not
significant (P<0.05). But a significant (P<0.0001) variety x storage condition x storage
time interaction as well as a significant seed treatment x storage conditions x storage time
interactions for seed viability were obtained (table 2).

In addition, overall analysis four months before the termination of the experiment
(16 mo after storage) showed that the interaction among variety x seed treatment x
storage condition for seed viability and vigor of the seed lots was not significant.
However, there were significant interactions (P = 0.0009 for viability; P = 0.0002 for
vigor) among storage condition x seed treatment x storage time for the decline in viability
and vigor (data not shown). Because there was a significant three-way interactions with
storage time at 16 and 20 mo after storage, analysis of variance was done at each storage
time to determine the changes in viability and vigor over time. When data were analyzed
by storage time, variety, seed treatment and storage condition were significant at all
storage times, except at 20 mo where varietal differences had no effect on viability (data not shown). The interaction between storage condition and seed treatment was also significant at the different storage times, except at 12 mo after storage. However, for ease of interpretation, the changes in viability and vigor as affected by seed treatment and storage condition are presented uniformly sorted by storage time (Figure 1).

Seed Viability

Initial seed viability as determined by standard germination test percentages ranged between 95 and 99% (Fig. 1A). Viability for all seed lots were therefore similar. After 4 mo in storage, seed viability within each storage condition was not significantly different \((P<0.05)\) regardless of the seed treatment applied (Fig. 1B) but the rate of deterioration was significantly different between storage conditions. The rate decline in seed viability in treated seed lots was similar among treatments in both, the coldroom and the warmroom 8 mo after storage. However, for seed lots stored in the warehouse for 8, 16 and 20 mo, seed viability was higher for treated seed than for untreated seed (Fig. 1C, E, and F). After 12 mo of storage, there were still no significant differences in seed viability among treatments in the coldroom regardless of seed treatment applied, and standard germination percentages were still very close to 100%. In the warmroom, the viability of fungicide treated seeds was similar to that of the fungicide + insecticide treated seeds but significantly different from that of the untreated seeds. Nevertheless, the viability of fungicide-treated seeds in the warmroom was not significantly different from that of seeds stored in the coldroom after 16 mo of storage.

Even though viability of seeds stored in the warehouse was still above 80%, this value was significantly lower than for seeds stored in the coldroom and warmroom at 12
mo (Fig. 1D). Four mo later the viability of seed lots in the warehouse declined drastically to below 20% while those in the coldroom and warmroom remained high (>80%) (Fig. 1E). Temperature and relative humidity conditions in the warehouse from 12 mo of storage fluctuated from 9°C to 25°C, and from 46% to 69% respectively. While at 16 mo, the temperature and relative humidity readings went from 24°C to 9°C and from 73% to 59% respectively (data not shown). Even though seed viability estimates in the warehouse at 16 mo were very low, treated seeds within this storage condition still had significantly higher standard germination percentage than untreated seeds. The viability of seed lots in the coldroom at 20 mo after storage was still >92% for all seed treatments, while treated seeds in the warmroom maintained a viability of >89% compared to untreated seeds (>78%) for the same storage time (Fig. 1F). The best storage condition was the coldroom which maintained the viability of the seed lots at 95.96% for fungicides-treated seeds, 95.27% for fungicides + insecticides-treated seeds, and 92.17% for untreated seeds, for the entire duration of storage. Only the treated seeds retained viability above 80% in the warmroom while the viability of the untreated seeds declined to levels below 80% at the end of the storage period (Fig. 1F). The least favorable storage conditions for maintaining the viability of the seeds was the warehouse.

*Seed vigor*

Initial seed vigor as measured by AA test ranged between 83 – 97% with fungicide-treated seeds having the lowest seed vigor (Fig. 1G). The vigor of the fungicide + insecticide-treated seeds and the untreated seeds in the coldroom was ≥80% after 4 mo of storage indicating no significant differences between seed treatments (Fig. 1H). Similarly, in the warmroom, the AA percentage for fungicide-treated seeds was higher
than that for fungicide+insecticide-treated seed and untreated seed (Fig. 1H). Seed vigor decrease was similar for all seed treatments for seed lots in the warehouse at this storage time but significantly lower than that in both the coldroom and the warmroom. The mean AA percentage for these seed lots was ≤60% at this storage time. After 8 mo of storage, the seed vigor of fungicide-treated seeds increased (83.75%) compared to the same treatment at 4 mo (78.54%) within the coldroom. Both fungicide- and fungicide+insecticide-treated seeds had higher seed vigor than untreated seeds after 8 mo of storage in the coldroom (Fig. 1I). On the other hand, the seed vigor of seed lots in the warmroom declined to <70%, with the untreated seeds losing more seed vigor (53.19%) than both the fungicide- (66.94%) and fungicide+insecticide-treated (65.60%) seeds (Fig.1I). Even though the vigor of seeds in the warehouse declined to below 40%, treated seeds still maintained higher vigor of >34% compared to 18.52% for the untreated seeds at 8 mo in storage.

Twelve months after storage, there was a distinct difference in seed vigor of treated seeds (>83%) compared to untreated seeds (>68% and >61.19% respectively) in both, the coldroom and the warmroom. But seed vigor of treated seeds in the warmroom (>70%) was similar to that of untreated seeds in the coldroom (>68%) (Fig. 1J). Sixteen months after storage, seed vigor in the three storage conditions was below 80% (Fig. 1K). However, the treated seeds in the coldroom still maintained a vigor of >64% compared to 50.75% for the untreated seeds. In the warmroom, the seed vigor of fungicide-treated seeds was 64.54% and higher than 52.24% for the fungicide+insecticide-treated seeds. The seed lots in the warehouse had a 0% vigor at 16 mo of storage (Fig. 1K). The rate of decline in seed vigor from the 4th mo to the 20th mo after storage, for treated seeds was
slower than the decline for untreated seeds in both the coldroom and the warmroom. For fungicide-treated seeds in the coldroom the decline was from 78.5% to 64% and for fungicide + insecticide-seeds it was 84% to 69%. In the warmroom the decline was from 78% to 64% and from 70% to 52% for fungicide and fungicide + insecticide-treated seeds respectively. While the untreated seeds declined from 85% to 28% in the coldroom and from 70% to 19% in the warmroom. The vigor of the treated seeds in the coldroom was much higher than for the treated seeds stored in the warmroom (>65% compared to >46%) (Fig. 1K).

Effect of oil and protein content

Seed lots were classified into four groups based on their oil content which ranged from 16 to 20%. Mean comparisons showed that the rate of seed viability decline in soybean seed lots was significantly different among groups (data not shown). Seed viability averaged over seed treatment and storage condition, had no relationship to oil and protein content of the seed as observed from plotting the data (data not shown).

A regression analysis of the effect of seed oil and protein content on seed vigor and seed storage condition is presented in Table 3. Seed oil content was important to explain the seed vigor decline of seed lots stored in the coldroom and warmroom. The effect of seed oil content on seed vigor of seed lots stored in the coldroom increased with storage time (Table 3). In the coldroom, the effect of seed oil content on seed vigor increase from 6% to >14% at 16 mo and then decreased to <7% 20 mo after storage. In the warmroom, >15% of the variation in seed vigor could be attributed to the oil content of seed lots (Table 3). There was no relationship between seed oil content and seed vigor decline for seed lots stored in the warehouse (Table 3). The protein content of varieties
ranged between 32 to 37% (Table 3). When the varieties were categorized into 5 groups depending on the protein content, analysis of variance showed significant differences in deterioration rates among the 5 categories (data not shown). A regression analysis showed that the decline in seed vigor over time is independent from seed protein content regardless of storage conditions and storage times (Table 3).

**Variety effect**

An analysis of variance showed that the change in viability of soybean varieties belonging to maturity groups I, III, and IV was similar; but different from that of maturity group II. There was no relationship between maturity group and change in viability (data not shown). There were significant differences between the different maturity groups as far as vigor was concerned (data not shown) but these differences were not important in determining the rate of decline in vigor of the seed lots in storage as revealed by a non-significant regression analysis within storage environment and over time.

**Fungi isolations**

Several fungi were isolated from the seed lots upon reception including *Phomopsis* spp., *Cercospora* sp., *Chaetomium* sp., *Cladosporium* sp., *Alternaria* spp., *Fusarium* spp., *Rhizopus* sp., *Aspergillus* spp., and *Penicillium* spp. A plot of the initial fungi incidence against standard germination values of varieties averaged over seed treatment and storage condition over time showed no relationship between the decline in seed viability or vigor and initial fungi incidence of seed lots.
DISCUSSION

Seed genetics, the environment where seeds are produced and storage environment are the three major factors that influence seed viability and vigor (Sun et al., 2007). In this study we investigated the effect of storage temperature and relative humidity and their effects on the storability of chemically treated soybean seeds. The results strongly suggest that treated soybean seeds store better than untreated seeds if stored under conditions of low temperature and relative humidity as these treatments had higher germination and vigor percentages compared to the untreated seeds. In addition, the soybean varieties in this study could maintain comparatively high standard germination percentage values for up to 12 mo when stored under all three conditions. Beyond this time, the viability of the seeds changes drastically in the warehouse probably due to the rapid accumulation of deleterious compounds compared to the coldroom and warmroom.

The decline in viability is intricately linked to the moisture content of the seed, which is in turn controlled by the relative humidity of the storage environment (Barton, 1943; Vieira et al., 2001). The relative humidity of the warehouse fluctuated within a wide amplitude, thus the seeds adsorbed or desorbed moisture from the air until the moisture content of the seed was in equilibrium with the surrounding air (Barton, 1943). Thus, the moisture content of seeds fluctuated constantly during the length of this storage study according to changes in the storage environment. Seeds stored at alternating relative humidity for periods longer than 12 weeks rapidly lose their viability (Barton, 1943). Seeds stored in environments with low relative humidity would equilibrate at lower moisture contents (Barton, 1973). In the same study, onion seeds placed in
alternating relative humidity of 35 or 55% retained their viability longer than those placed at higher alternating relative humidity of 55 or 76% (Barton, 1943). The relative humidity fluctuations in the warehouse environment from our study resulted in changes in moisture content of seeds thus contributing to a rapid decline in seed viability and vigor. The rate of deterioration is directly proportional to the duration of storage of the seeds in the harmful relative humidity environment (Barton, 1943). Considering that seed lots in the warehouse maintained a standard germination percentage of >80% after 12 mo in storage, it is possible that the higher relative humidity values recorded just before the 16 mo evaluation could have resulted in the increase in moisture contents of the seeds and therefore to an increase in rate of deteriorative reactions. The mean monthly temperatures during this period were also increasing in magnitude. High temperatures are known to increase the rate of reactions by affecting the enzymes that are involved in reactive oxygen species scavenging and repair (Bernal-Lugo and Leopold, 1998).

The decrease in viability over time in the coldroom and warmroom was almost imperceptible up to 16 mo compared to the warehouse. The difference in temperature of these environments might have also played a key role. Lower relative humidity in the warmroom kept the moisture content of the seeds low, which slowed the deterioration process (Barton, 1973; Bernal-Lugo and Leopold, 1998). The effect of higher relative humidity in the coldroom increased the moisture content by 4 to 6 points. However, the lower temperature in this environment could slow the rate of loss in seed viability. Similar results were obtained in studies with 6 soybean varieties where the decrease in germination over time was exponential at higher temperatures and near linear at lower temperatures (Burris, 1980). Vieira et al. (2001) also observed that the electrical
conductivity of seeds transferred from a high temperature environment to low
temperature environment remained unchanged. Because the loss of electrolytes from a
seed is influenced by the integrity of the seed coat, they concluded that at low
temperatures the membranes were somehow stabilized.

On the other hand, changes in seed vigor were observed just 4 mo after storage
and slowed down thereafter to a steady rate across storage environments. It is of
particular interest to note that the vigor of fungicide-treated seeds stored in the coldroom
declined initially and 4 mo later increased to >80%. Other studies have also documented
initial vigor decline and then increases in seed lots stored in continuous low temperature
and low relative humidity (De Vries et al., 2007; Houston, 1973; Krueger et al., 2012;
Moore and Roos, 1982), but the reason for this initial and later increase in seed vigor is
still unknown. However, the decline of seed vigor in all storage environments preceded
the decline in seed viability for the same treatments. This difference between seed
viability and vigor response could be explained by the fact that deteriorated seeds are still
able to have good seed germination percentages if the embryo axes, including the
meristematic cells of the radicle and the plumule, are able to germinate and produce a
seedling under ideal conditions (Byrd and Delouche, 1971; Harrington, 1973). The
standard germination test provides the seed with ideal temperature and moisture
conditions for the germination of the species (AOSA, 2009). Hence, a deteriorated seed
may still produce a normal or weak seedling in the standard germination test even if most
of the cells in the seed are deteriorated. Contrariwise, the AA test is a stress test (AOSA,
2009) and only seeds with little or no cell deterioration can germinate after being
submitted to this stress (Bernal-Lugo and Leopold, 1998). Deteriorated seeds do not
withstand the stressful conditions of the AA test (Delouche and Baskin, 1973). Byrd and Delouche (1971) also observed similar increases in sensitivity to accelerated aging treatment before the loss of seed viability in soybean seeds. Thus the accelerated aging test is more sensitive in detecting seed vigor changes than the standard germination test.

The fungicide and fungicide+insecticide seed treatments may be advantageous in enhancing seed storability as these treatments had higher germination and vigor percentages compared to the untreated seeds. Seed treatments are usually applied to protect the seed from soilborne fungi and insect pests. In addition, some treatments may induce plant defense responses in cases of increased stress and ultimately improve growth and yield (Bartlett et al., 2002; Munkvold, 2009). For example, the application of Captan (fungicide) seed treatment to medium and low vigor soybeans seed which were stored at 40°C and 12.6-13.1% moisture (Edje and Burris, 1971). Nevertheless, to our knowledge our study is the first to assess storability of treated seed, and to show that treated seed can be advantageous for seed survival in storage. This information is of critical importance to the seed industry because most soybean seed lots are treated before storage.

The mechanisms by which seed treatments slowed down deteriorative reactions under all three storage conditions of our study are not known. However, during the periodic evaluations of seed viability and vigor, we observed that treated seed had fewer fungi than untreated seed, especially in seed lots stored in the warmroom and warehouse where temperature and relative humidity conditions were conducive for colonization and growth of storage fungi (data not shown). In addition, the response to seed treatment seemed to depend on the storage temperature and relative humidity. Therefore, low temperature and relative humidity synergistically minimized aging reactions in the treated
seeds (Bernal-Lugo and Leopold, 1998; Bruni and Leopold, 1991; Burris, 1980; Delouche and Baskin, 1973; parish and Leopold, 1978; Walters et al., 2005). Burris (1980) also noted that temperature and relative humidity had both separate and combined influences on vigor and viability of seeds.

The range of moisture content of the seed lots just before storage was between 6 and 8%. Deteriorative reactions in seeds at this moisture contents range are considered at a minimum (Harrington, 1973). The seeds in the coldroom equilibrated to relatively higher moisture contents than those in the warmroom where relative humidity was lower. The lower moisture content of seed in the warmroom was counteracted by the higher temperature, which consequently was responsible for the greater decline in seed vigor (Harrington, 1973). Seed moisture content also influences the level of infection by storage fungi. Fungi such as *Fusarium*, *Cercospora* and *Phomopsis* can degrade storage protein and oil of soybeans (Wilson et al., 1995). Although, the initial fungi load did not significantly contribute to the deterioration process, it is possible that development of storage fungi during the storage period was detrimental to the viability and vigor of the seeds.

The total oil content of soybean seeds did not significantly influence their seed viability in the three storage environments. On the other hand, seed oil content significantly affected seed vigor. However, the effect of seed oil content on the decline in seed vigor was not as strong as expected as demonstrated by the low regression coefficients. Sun et al. (2007) defined seed vigor as a comprehensive trait that is affected by many factors, and that vigor is expressed through individual traits among which are germination, seedling length, root length, seedling fresh weight, and seed longevity. The
physiological process associated with seed oil content is peroxidation of membrane lipids (Harrington, 1973; Walters et al., 2005). This phenomenon has been proposed as the main cause for seed deterioration and is directly linked to membrane integrity of the seeds (Bewley and Black, 1994). Because seed vigor is controlled by multi-gene loci most of which have relatively small effects (Sun et al., 2007), the seed oil content of the varieties used in our study accounted for only ≤ 15% of the decline in vigor of the seeds in the different storage environments. Comparable results were obtained in studies for other quantitative traits associated with seed vigor in rice (Redona and Mackill, 1996).

The effect of the protein content on seed viability and vigor was never more than 5% in all three environments. The lack of relationship between seed protein content and seed viability and vigor was likely because the seed moisture content in the different storage environments was not high enough to initiate sugar hydrolysis, which is the initial step in the Maillard and Amadori reactions that involve protein degradation (Sun and Leopold, 1995). However, this observation is not exclusive as protein degradation maybe associated with more than one degradative process in soybean. Other studies have found that high protein levels in soybean seeds were correlated with lower seed germination percentages irrespective of the moisture contents of the seed in the laboratory (LeVan et al., 2008). Therefore, seed protein content effects on seed viability and vigor might not have had measurable effects.

The choice of storage conditions may depend on the value of the soybean seed to be stored and the duration of storage. Burris (1980) suggested that drying soybean seeds down to 8-10% moisture level before storing at low temperatures and relative humidity could provide acceptable seed quality for at least 3 years. Our results are in support of
this suggestion because 20 mo after storage the viability of seeds stored in the coldroom and warmroom were still very high. However, seed vigor would decline appreciably under the same storage conditions. In practice soybean is harvested at 14 to 16% moisture content in order to reduce the chances of mechanical damage during processing and handling (Burris, 1980). By the time our experiment was set up, the moisture content of seed lots was much lower. The fact that seed viability was still very high in the coldroom and the warmroom at the end of our experiment indicates that viability alone is not a good indicator of seed quality in storage as has been determined by others (Egli and Tekrony, 1995).

Three types of deteriorative reactions take place in seeds that lead to decline in seed quality: nonenzymatic lipid peroxidation, enzymatic lipid peroxidation and sugar hydrolysis (Sun et al. 2007; Harrington, 1973). These three reactions are a function of respiration rate and moisture content of the seed. Even at very low respiratory rates, oxidative free radicals could still be formed within the seed that may increase the rate of nonenzymatic lipid peroxidation (Sun et al., 2007).

In all three storage conditions, deteriorative reactions are occurring but at different rates depending on the moisture content of the seeds which is in equilibrium with the temperature and relative humidity of the storage environment. Because vigor continued to decline even in the coldroom, it is assumed that the predominant degradative reaction in the coldroom was lipid peroxidation. This reasoning derives from the fact that the seed moisture content of the seed lots was below the threshold for activating enzymatic lipid peroxidation and sugar hydrolysis within the seed (Shih et al., 2004; Sun and Leopold, 1995). Thus, seed vigor was maintained at commercially acceptable levels
of ≥80% in the coldroom after 12 mo in storage. These results are critical to the seed
industry since seed vigor of ≥80% is recommended for good seed emergence and stand
establishment in soybeans (Egli and Tekrony, 1995).

REFERENCES


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Table 1. Mean and standard deviation (Stdev) for moisture content of 24 soybean varieties 20 mo after storage in three storage conditions, coldroom, warmroom and warehouse, and with three seed treatments of fungicide, fungicide+insecticide or untreated control; and mean and Stdev for temperature and relative humidity of the storage environments.

<table>
<thead>
<tr>
<th></th>
<th>Moisture content (% fw)</th>
<th>Temp (°C)</th>
<th>Relative humidity (%)</th>
<th>Stdev</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fung</td>
<td>Stdev</td>
<td>Fung+Ins</td>
<td>Stdev</td>
</tr>
<tr>
<td>Coldroom</td>
<td>10.77</td>
<td>0.91</td>
<td>10.54</td>
<td>0.32</td>
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<tr>
<td>Warmroom</td>
<td>5.81</td>
<td>0.15</td>
<td>5.72</td>
<td>0.16</td>
</tr>
<tr>
<td>Warehouse†</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

† Calculated moisture content ranges for the seed lot in the fluctuating temperature and relative humidity conditions of the warehouse are presented in the results section.
Table 2. Analysis of variance for seed viability determined by using the standard germination test and seed vigor determined by using the accelerated aging test of 24 soybean varieties after 20 mo storage in three storage conditions, coldroom, warmroom, and warehouse, differing in temperature and relative humidity, and seeds treated with three seed treatments of fungicide, fungicide+ insecticide or untreated control.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Viability (Standard germination)</th>
<th>Vigor (Accelerated aging)</th>
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</thead>
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<tr>
<td></td>
<td>df</td>
<td>$F$-value</td>
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<tr>
<td>Variety</td>
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<td>Seed treatment (ST)</td>
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<td>ST*Variety</td>
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<td>Storage condition (SC)</td>
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<td>SC*Variety</td>
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<td>5.22</td>
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<tr>
<td>SC*ST</td>
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<td>1.75</td>
</tr>
<tr>
<td>SC<em>ST</em>Variety</td>
<td>92</td>
<td>0.66</td>
</tr>
<tr>
<td>Time in storage (T)</td>
<td>5</td>
<td>3142.03</td>
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<tr>
<td>T*Variety</td>
<td>115</td>
<td>2.97</td>
</tr>
<tr>
<td>T*ST</td>
<td>10</td>
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<tr>
<td>T<em>ST</em>Variety</td>
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<td>0.53</td>
</tr>
<tr>
<td>T*SC</td>
<td>10</td>
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</tr>
<tr>
<td>T<em>SC</em>Variety</td>
<td>230</td>
<td>2.26</td>
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<tr>
<td>T<em>ST</em>SC</td>
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<td>4.04</td>
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<tr>
<td>T<em>ST</em>SC*Variety</td>
<td>461</td>
<td>0.44</td>
</tr>
</tbody>
</table>
Table 3. Regression analysis of the effect of seed oil and protein contents on seed vigor determined by using the accelerated aging test of 24 soybean varieties stored for 20 mo in three storage conditions, coldroom, warmroom, and warehouse, differing in temperature and relative humidity, and seeds treated with three seed treatments, fungicide, fungicide+insecticide or untreated control.

<table>
<thead>
<tr>
<th>Vigor</th>
<th>Oil content</th>
<th>Protein content</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T4</td>
<td>T8</td>
</tr>
<tr>
<td>Coldroom</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R²</td>
<td>0.0678</td>
<td>0.0166</td>
</tr>
<tr>
<td>Pr&gt;F</td>
<td>0.0016</td>
<td>0.1234</td>
</tr>
<tr>
<td>Warmroom</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R²</td>
<td>0.0555</td>
<td>0.0897</td>
</tr>
<tr>
<td>Pr&gt;F</td>
<td>0.0045</td>
<td>0.0003</td>
</tr>
<tr>
<td>Warehouse</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R²</td>
<td>0.0520</td>
<td>0.0090</td>
</tr>
<tr>
<td>Pr&gt;F</td>
<td>0.0060</td>
<td>0.2572</td>
</tr>
</tbody>
</table>
Figure 1. The effect of seed treatment of fungicide, fungicide + insecticide, and untreated control, and storage conditions of coldroom, warmroom, and warehouse on seed viability and vigor of soybean varieties over time. Figure-panels A, B, C, represent seed viability and panels G, H, and I, represent seed vigor upon arrival, and at 4 and 8 months after storage, respectively. Seed viability and vigor are expressed in percentage of normal seedlings according to AOSA Rules for Testing Seed (2009).
Fig. 1 cont’d. The effect of seed treatment of fungicide, fungicide + insecticide and untreated control, and storage conditions of coldroom, warmroom, and warehouse on seed viability and vigor of soybean varieties over time. Figure-panels D, E and F, represent seed viability and panels J, K and L, represent seed vigor at 12, 16 and 20 months after storage, respectively. Seed viability and vigor are expressed in percentage of normal seedlings according to AOSA Rules for Testing Seed (2009).
CHAPTER 3. GENERAL CONCLUSIONS

The results of this study suggest that fungicide or fungicide+insecticide-treated soybean seed stored under coldroom conditions of temperature and relative humidity maintain seed vigor at the recommended levels for successful field emergence for 12 mo. The temperature and relative humidity conditions in the coldroom were not effective in maintaining vigor at the recommended value for longer storage periods. The rate of decline in seed viability and vigor at the higher temperatures in the warmroom was faster even at the lower relative humidity in this environment. Meanwhile, the wide fluctuations in temperature and relative humidity in the warehouse produced a rapid decrease in seed viability and vigor of the seed lots regardless of seed treatment.

Fungicide- and fungicide+insecticide-treated soybean seed maintained their viability and vigor longer than untreated seed. The relationship between the viability and vigor of the treated seed lots was also dependent on the temperature and relative humidity in storage. The advantages of treating seed before storage were more pronounced for seed vigor than for seed viability.

There were significant differences in the rate of decline of seed viability and vigor of the 24-soybean varieties. Total oil content of the seed is a quantitative trait and had little effect on the variation in seed vigor and almost none in seed viability over time. Total seed protein content and soybean maturity group did not affect the rate of decline in viability and vigor of the seed lots. Optimization of the coldroom conditions could result in high soybean seed viability and vigor levels in storage, and longer storage times. Prolonging good soybean seed viability and vigor of treated seed in storage could reduce the need for disposal of treated seeds. These results are also important for the crop-
protection chemical companies because of the importance of seed treatments to the overall crop-protection strategies. It is important to use the best storage environments to prolong seed viability and vigor of treated seeds.
ACKNOWLEDGMENTS

I am very thankful to Dr. Susana Goggi for welcoming me into her lab, for her guidance, patience and support when life got very difficult at times during the program. I am also grateful for the training I received and the exposure to the seed science world. My special thank you to my co-advisor, Dr. Leonor Leandro for all the encouragement without which I would never have finished the program. I also wish to thank Dr. Russell Mullen for his critic and support during this time. Special thanks to Dr. Manjit Misra for providing funds that enabled me to finish this research. Thanks to the Seed Lab staff for their constant support and provision of space for over two years.

Thanks to members of the Seed Physiology lab for the fun times we’ve had together and the cooperative spirit that reigned within the lab. Thanks to all the hourlies from Dr. Leandro’s lab and special thanks to my fellow Plant Pathology colleagues (Miralba, Vijitha, Noor, David) who stepped in to either learn by doing or just to help process the samples.

To Alan Gaul, thanks for your friendship, patience and creativity whenever there was a need. Thanks for allowing us to use your lab as a base for setting up the experiment, and thanks for always being there for me.

Special thanks to Pioneer, Monsanto and Stine Co. for kindly providing the seed for this project. Thanks to my Pastor, and church family who stood by me when I lost everything in the flood.

Finally, I thank the Lord for His grace upon grace and mercies that are renewed every morning.
Table 4. Analysis of variance for the standard germination and accelerated aging tests of fungicide-treated, fungicide+insecticide-treated and untreated seeds from 24 soybean varieties evaluated over time in three storage environments, coldroom, warmroom, and warehouse.

<table>
<thead>
<tr>
<th>Effect</th>
<th>df</th>
<th>F-value</th>
<th>P&gt;F</th>
<th>df</th>
<th>F-value</th>
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Table 4. cont’d. Analysis of variance for the standard germination and accelerated aging tests of fungicide-treated, fungicide+insecticide-treated and untreated seeds from 24 soybean varieties evaluated over time in three storage environments, coldroom, warmroom, and warehouse.

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Table 5. Regression analysis of the effect of seed oil and protein content on seed viability and vigor of 24 soybean varieties treated with fungicide, fungicide+insecticide, or untreated control, evaluated over time in three storage environments, coldroom, warmroom, and warehouse.

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Table 5 cont’d. Regression analysis of the effect of seed oil and protein content on seed viability and vigor of 24 soybean varieties treated with fungicide, fungicide+insecticide, or untreated control, evaluated over time in three storage environments, coldroom, warmroom, and warehouse.

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Table 6. Change in seed vigor over time of 24 soybean varieties treated with fungicide, fungicide+insecticide, and untreated control, stored in three different temperature and relative humidity regimes of coldroom, warmroom, and warehouse.

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Table 6. Cont’d. Change in seed vigor over time of 24 soybean varieties treated with fungicide, fungicide+insecticide, and untreated control, stored in three different temperature and relative humidity conditions of coldroom, warmroom, and warehouse.

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Table 6. cont’d. Change in seed vigor over time of 24 soybean varieties treated with fungicide, fungicide+insecticide, and untreated control, stored in three different temperature and relative humidity conditions of coldroom, warmroom, and warehouse.

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† Means not followed by the same letter are significantly different at $P<0.05$. 
Figure 2. Changes in seed viability (A) and seed vigor (B) over time in three storage conditions, coldroom, warmroom, and warehouse. Bars with the same letters are not significantly different from each other ($P \leq 0.05$).
Figure 3. The effect of temperature and relative humidity of three storage conditions, coldroom, warmroom, and warehouse, on seed viability (A) and seed vigor (B) of soybean seeds. Bars with same letters are not significantly different at $P \leq 0.05$. 
Figure 4. The effect of seed treatment on the mean seed viability (A) and seed vigor (B) of soybean seeds after 16 mo storage in three storage conditions, coldroom, warmroom, and warehouse. Bars with the same letters are not significantly different at $P \leq 0.05$. 

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Figure 5. The effect of seed treatment on mean seed viability (A) and seed vigor (B) of soybeans treated with fungicide, fungicide+insecticide, and warehouse, and stored up to 12 mo in three storage conditions, coldroom, warmroom and warehouse. Bars with the same letters are not significantly different at $P \leq 0.05$. 
Figure 6. The effect of seed treatment on mean seed viability (A) and seed vigor (B) of soybean seeds over time in three storage conditions, coldroom, warmroom, and warehouse. Results are averaged across varieties and storage conditions. Evaluations were done at four-monthly intervals.
Figure 7. Seed viability (SG) and seed vigor (V) changes over time of soybean seeds in three storage conditions, coldroom (C), warmroom (G), and warehouse (W), averaged over varieties and seed treatments of fungicide, fungicide+insecticide, and untreated control. Sampling and seed quality evaluations were done at four-monthly intervals.
Figure 8. The effect of seed oil and seed protein content of soybean varieties on seed viability (A and B) and seed vigor (C and D) 16 mo after storage. Values are averaged over storage conditions and seed treatments.
Figure 9. The effect of initial fungal incidence on the rate of decline in seed viability and seed vigor (A and B) of 24 soybean varieties averaged over seed treatment and storage conditions; and the relationship between soybean seed viability and seed vigor with initial fungal incidence (C and D) 16 mo after storage.
Figure 10. Daily average temperature in degrees Celsius and daily relative humidity in percentages of the three storage conditions for part of the duration of the experiment.