POOR FIELD EMERGENCE OF LATE-MATURING PEANUT CULTIVARS (*Arachis hypogaea* L.) DERIVED FROM PI-203396

By

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TABLE OF CONTENTS

page

LIST OF TABLES

LIST OF FIGURES

Abstract of Dissertation Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Doctor of Philosophy

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Late-maturing peanut cultivars DP-1, C-99R, Hull, and Florida MDR-98 (*Arachis hypogaea* L.) have superior resistance to leafspot (*Cercosporidium personatum*, Berk & Curt.), white mold (*Sclerotium rolfsii*, Sacc.), and tomato spotted wilt virus. The improved resistances are primarily derived from PI 203396. The cultivars are high yielding. They provide the grower the opportunity to reduce fungicide applications and variable costs without reducing yields. Because of poor field emergence, commercial seed companies have stopped producing Florida MDR-98, DP-1, and Hull. Official towel germination tests usually show acceptable seed quality. Reduced field emergence seldom occurs when the seed peanuts have been grown, harvested, and stored in small batches in research storage facilities. The poor field emergence occurs when seed production is through commercial channels with large volumes being harvested, stored in bulk bins, and treated with fungicides. The problem may be related to the commercial practice of storing seed peanuts in large piles with inadequate ventilation.

Four cultivars from two different field origins were stored in four environments and then tested for field emergence. Field origin did not affect field emergence, but storage environment did. Peanuts stored in bulk in a traditional peanut warehouse at elevated temperatures and

relative humidity had reduced field emergence. There was a genotype by storage environment interaction. Field emergence was maintained when seed was stored at < 16^{o}C and < 70% relative humidity. Standard towel germination tests were not reliable indicators of field emergence. Electrolyte conductivity tests and seed vigor tests were highly correlated with field emergence. The increased electrolyte conductivity and decreased rate of growth of the hypocotyl-radicle indicated that cellular membranes were damaged during storage at elevated temperatures and relative humidity. The literature suggests that peroxidation of lipids occurred resulting in the production of free radicals and autoxidation. The antioxidant capacity of seed varied by cultivar and year of production.

Field emergence could be improved by reducing temperature and relative humidity in the storage environment. Since standard towel germination tests were not reliable indicators of field emergence for these late-maturing cultivars, an alternative method of evaluating peanut seed quality should be adopted.

CHAPTER 1
INTRODUCTION

Cultivars

The University of Florida Agricultural Experiment Station (FAES) at the North Florida Research and Education Center (NFREC), Marianna, Florida, has released several late-maturing peanut cultivars, namely Florida MDR-98 (Gorbet and Shokes, 2003), C-99R (Gorbet and Shokes, 2002), DP-1 (Gorbet, 2003), and Hull (Gorbet, 2003). These cultivars have superior resistance to late leafspot (*Cercosporidium personatum,* Berk & Curt.), white mold (*Sclerotium rolfsii,* Sacc.), and tomato spotted wilt virus **(**genus *Tospovirus*; family *Bunyaviridae*) (Table 1-1). The improved pathogen resistances were derived primarily from lineage to PI 203396 through a common parent or grandparent UF81206. DP-1 has 50% genetics inherited from PI 203396, Florida MDR-98 and Hull have 38%, and C-99R has 25%. The cultivars are high yielding with good grades and acceptable flavor and processing characteristics. They provide the grower the opportunity to reduce fungicide applications during the growing season and, therefore, to reduce variable field costs without reducing yields. Because of the indeterminant flowering of peanut, late-maturing cultivars have the ability to fill pods longer and can compensate for unfavorable weather or pathogen damage.

Poor Field Emergence

Florida MDR-98 was released for commercial production in 1998. Within two years, seed production of Florida MDR-98 was terminated because of poor field emergence. DP-1 and Hull were released in 2002. Field emergence was poor and seed is no longer commercially available for either cultivar. Southern Runner, a cross of PI 203396 and Florunner, is a parent to both Florida MDR-98 and DP-1 and, like Florida MDR-98 and DP-1; Southern Runner had germination problems (Gorbet *et al.*, 1987). Unpublished studies found that Southern Runner

immature seed was sensitive to cold soil temperatures. The University of Florida recommendation was to delay planting Southern Runner as long as soils were cool, and to increase screen size in grading to an 18/64 mesh to screen out small and immature seed (Gorbet, 2006. Per. Comm.). Southern Runner is a parent of Georgia Green, the current dominant variety grown in the Southeast. Georgia Green is medium maturity and has no major field emergence problems. C-99R with only 25% PI 203396 was released in 2000. Field emergence problems for C-99R have been less frequent and less severe and seed is commercially produced. Prasad *et al.* (2006) reported a genotype difference in field emergence response to cool soil temperatures. Florida MDR-98 was the most sensitive, followed by Southern Runner and then C-99R. Georgia Green was the least sensitive to cool soil temperature. This ranking of sensitivity to cool soils coincides with the percent genetic lineage of these cultivars to PI 203396.

Official towel germination tests usually show acceptable seed quality for these late-maturing cultivars (Tillman, 2004, Per. Comm.). Research data from NFREC show that reduced field emergence of these cultivars seldom occurs when the seed has been grown, harvested, and stored in research storage facilities (Tillman, 2004, Per. Comm.). The poor field emergence occurs when seed is produced in commercial channels with large volumes being harvested, stored in bulk in-shell, shelled, and treated with fungicides. The problem may be related to commercial storage of in-shell seed peanuts in piles in large warehouses with no humidity control, temperature control, or forced ventilation.

Seed deterioration is inexorable and not uniform (McDonald, 2004). Many factors contribute to seed deterioration including genetic composition, seed moisture content, mechanical and insect damage, pathogen attack, seed maturity, and relative humidity and temperature of the storage environment. Of these factors relative humidity and temperature are

the most important (McDonald, 2004). Relative humidity directly influences seed moisture. Increasing temperature increases the amount of moisture air can hold and the rate of cellular metabolism. Harrington (1972) concluded that for seed storage the sum of the temperature in degrees Fahrenheit and the percentage relative humidity should not exceed 100.

Seed Handling and Storage

Seed peanuts in the southeast USA are harvested starting in early September by digging the pods and vines, inverting the biomass to air dry, and then 3–5 days after digging, machine combining to separate pods from stems. With forced heated air, pods and seeds are dried in wagons to approximately 9.5% moisture content (Stadsklev, 2004, Per. Comm.). The peanut pods are stored in large bulk bins at ambient atmospheric temperatures that may exceed 32^{o}C and relative humidity that may exceed 95%. Dimensions of the bulk pile may be very large, often exceeding 7,000m ^{3}. Ventilation usually is accomplished by circulating surface air through opened doors and exhausting through roof vents. Shelling of seed peanuts begins as early as December. Shelled peanuts are stored in a warehouse at ambient temperature and relative humidity in solid cardboard containers on pallets until treated with fungicides, bagged, and ready for delivery to the farm producer. During storage in the bulk bins, the peanut pile surface frequently shows substantial fungal growth and the hulls may become dusty from spores.

Hypotheses

C-99R, DP-1, and Hull have important disease resistances for peanut production and PI 203396, the primary source of these resistances, appears in the pedigree of many lines in the University of Florida peanut breeding program. This research was conducted to identify the factors in production and storage of these peanut cultivars that contribute to poor field emergence. The research examines the following four hypotheses:

- High temperature or high relative humidity in commercial storage conditions may reduce field emergence.
- There is an interaction of cultivar by storage environment on seed germination and field emergence.
- Reduced field emergence may be caused by storage pathogens.
- Potential field emergence can be measured by testing for seed vigor and the electrolyte conductivity of the leachate of germinating peanuts.

Table 1-1. Performance of runner market-type peanut cultivars in two or three Florida locations over four years (2002–2005). Entries are sorted by maturity and the four year average yield (in descending order) (Tillman, 2007).

		YIELD			TSMK			TSWV***		
		(lbs./acre)			(%)			(1-10)		
Name	Maturity*	2005	3-YR†	4-YR††	2005	3-YR	4-YR	2005	3-YR	4-YR
AP-3	M	3177	4094	4162	71.6	73.0	73.9	3.3	2.1	2.2
C-99R	L	4107	4458	4349	75.3	76.5	77.2	2.8	2.2	2.2
DP-1	L	3320	3983	3953	73.6	74.7	75.3	2.7	1.9	2.0
Hull**	L	3134	3780	3579	73.6	74.9	75.6	4.2	2.9	2.8
C.V.		16	13	13	2.4	1.8	1.9	23.1	22.4	20.7
LSD		351	239	209	2.1	0.9	0.8	0.9	0.4	0.3

*E = early, M = medium, L = late; **High oleic oil chemistry. †3 YR = average of 2003, 2004 and 2005; ††4 YR = average of 2002, 2003, 2004 and 2005. ***Tomato Spotted Wilt Virus ratings (1-10, 1 = no disease).

CHAPTER 2
LITERATURE REVIEW

Effect of Temperature and Relative Humidity on Germination

Ideal conditions for storage of peanut seed are 10°C and 65% relative humidity (Ketring, 1992). The resulting seed moisture content is approximately 6%. Maintaining these conditions is difficult when peanuts are stored in warehouses without climate control. Navarro *et al*. (1989) studied the interaction of temperature and moisture concentration on the germination of peanut seeds. Germination of peanuts stored in-shell at 15°C and 79–83% relative humidity (RH) remained above 80% for 150+ days. In the same peanut cultivars stored at 15°C and 85–89% RH, germination decreased dramatically to 30% in 80 days. If the storage temperature increased to 20°C and RH was at 79–83%, germination steadily decreased to 80% at 80 days and, if stored at 20°C and 85–89% RH, germination dropped to 20% in 80 days. Navarro et *al*. (1989) found an interaction of the tested cultivars with the temperature and RH. The cultivar Congo (Valencia type) tolerated higher storage temperatures and RH better than the cultivar Hanoch (Virginia type). Ketring (1992) reported peanut seed tolerated temperatures of 44°C when relative humidity was low, but that genotypes varied in tolerance of high temperatures within seasons and across seasons, showing the effect of genetic variation and environmental conditions during seed maturation. Hypocotyl-radicle length was more adversely affected than germination.

Germination, Seed Vigor, and Field Emergence

Germination tests are required for the commercial sale of peanut seed and are intended to be an indicator of the seed's potential for field emergence. Germination is variously defined. For the producer, it is the appearance of the seedling at the soil surface. For the peanut seed tester, it is the initial protrusion of the radicle from the seed and germination is reported as the percent of normal seedlings at the conclusion of the test (Association of Official Seed Analysts,

1970). Germination is triphasic (Black and Bewley, 1994). In phase I when water is imbibed rapidly, leakage of ions and soluble sugars occurs until the organelle membranes are repaired. In phase II, the lag phase, organelle membranes are organized, mitochondria increase in size and number, enzymes are produced *de novo*, and the seed becomes prepared for rapid growth. Phase III begins when the radicle protrudes from the seed and continues until the plant has emerged from the soil, produced leaves, and commenced photosynthesis. This is the phase when food reserves are mobilized and cell elongation and cell division commence. The producer accessing field emergence observes the conclusion of phase III; whereas, the seed tester observes the commencement of phase III.

The *Seed Vigor Testing Handbook* (Association of Official Seed Analysts, 2002) states "Seed vigor comprises those properties which determine the potential for rapid, uniform emergence and development of normal seedlings under a wide range of field conditions." Vigor testing quantifies the vitality of the seed and places a premium upon uniform emergence. Ketring (1993) developed a Vigor Index for peanut that takes into account germination and rate of seedling growth. Peanut seed exposed to short periods of elevated temperature had reduced seedling vigor, but no reduction in germination. Repeated exposure to adverse temperatures resulted in additional loss of vitality and eventual loss in germination. The Vigor Index provides a numerical value for statistical analysis of seed vitality resulting from genotype interactions with storage conditions. Field emergence was positively correlated with rapidly growing seedlings. At 63 days after planting (DAP), plants originating from seeds with low vigor had shorter main stems, narrower plant width, and reduced ground cover. They produced lower yields than those plants from seeds with high seed vigor. Ketring concluded that Vigor Index is a better indicator of potential field emergence and final plant stand than germination tests.

Deterioration of Seed Quality

Seed deterioration is the result of changes within the seed that decrease the ability of the seed to survive. It is distinct from seed development and germination and it is cumulative (McDonald, 2004). Peanut seed quality is highest just prior to physiological maturity, but can deteriorate rapidly during storage (Perez and Arguello, 1995). McDonald (2004) points out that a seed is a composite of tissues that differ in their chemistry and proximity to the external environment and that seed deterioration does not occur uniformly throughout the seed. The embryonic axis is more sensitive to aging than the cotyledons. In the axis, the radicle is more sensitive to deterioration than the shoot. In soybean during imbibition, the radicle absorbs water more rapidly than the cotyledons (McDonald *et al*., 1988). In maize, water uptake begins in the radicle followed by the scutellum and then the shoot axis and coleoptile (McDonald *et al*., 1994). McDonald suggested that water present in the atmosphere may be attracted by the same matric forces as soil water resulting in higher water content in the radicle compared to the storage reserves (McDonald, 1998). The higher moisture content could selectively accelerate seed deterioration in the axis. He concluded that studies of seed deterioration should focus on the seed part that deteriorates first.

Wilson and McDonald (1986) reviewed the literature concerning lipid peroxidation in plants, animals, and *in vitro* and proposed a model of ageing mechanisms that explains seed deterioration, especially the tendency of oilseeds to deteriorate rapidly. Lipid peroxidation is the result of either autoxidation or the action of lipoxgenases. In either process, fatty acid chains become oxidized producing highly reactive free radical intermediates termed hydroperoxides. In the divinyl methane structure of the polyunsaturated fatty acids, the hydrogen atoms are easily removed and hydroperoxides are formed (Mead, 1976). Once a free radical is produced, usually involving oxygen attack, a chain reaction is initiated producing additional free radicals. The

susceptibility of fatty acids to peroxidation increases exponentially with increasing unsaturation. Antioxidants, such as α-tocopherol, reduce autoxidation by scavenging free radicals thereby breaking the chain reaction cycle (Kaloyereas *et al.*,1961 in Wilson and McDonald, 1986).

The effects of peroxidation are extensive and include biomembrane degradation, protein denaturation, interference with protein and DNA synthesis, accumulation of toxic materials, and destruction of the electron transport system of oxidative phosphorylation (Wilson and McDonald, 1986). Membrane degradation is evident as increased electrolyte leakage from the hydrated seed and can be measured with conductivity tests (Dey *et al.*, 1999). In addition, hydroperoxides may decompose into volatile aldehydes such as malondialdehyde (MDA). The volatile aldehydes produce a wide array of cytotoxic effects including reaction with sulfhydryl groups causing inactivation of proteins. Harman *et al.* (1982) demonstrated an association between volatile aldehyde production during early germination and low soybean seed vigor.

Harman and Mattick (1976) studied the effect of free radicals on biomembranes. The phospholipids of membranes, especially the inner membranes of mitochondria, have a larger surface area, are oxygen sinks, and are usually more unsaturated than storage lipids (Moreau, 1978). Lipid peroxidation within the membrane results in polar bridges across the hydrophobic barrier of the membrane leading to an increase in permeability and a decrease in respiratory competence (Simon, 1974). The changes in membrane properties result in increased leakage of sugars, amino acids, and inorganic salts; reduced phosphorylative capacity; reduced activity of enzymes such as cytochrome oxidase and malic and alcohol dehydrogenases; and reduction in protein and carbohydrate synthesis (Abdul-Baki and Baker, 1973).

Perez and Arguello (1995) found in aging tests that germination tests by International Seed Testing Association Rules (ISTA) were not a sensitive assay for detecting the degree of

deterioration in peanut. To analyze the poor membrane structure that results in leaky cells and subsequent low seed vigor, they used electrical conductivity tests to measure the amount of electrolyte leakage. Their study analyzed leakage from the whole seed, the cotyledons, and the embryonic axes of the seeds. The conductivity tests using accelerated aging showed that the axis was the structure most sensitive to deterioration. In addition, using MDA as an indicator of hydroperoxides and lipid peroxidation in peanut, they determined that MDA increase was most pronounced in the axis. Perez and Arguello (1995) concluded that biochemical changes which take place in the membranes of peanut seed as they age may be detected best in the embryonic axes, either through changes in the leakage of electrolytes or in MDA content and that the embryonic axis may be the active center in relation to vigor.

Water in Seeds

Although lipid peroxidation occurs in all cells, in fully imbibed cells water acts as a buffer between the autoxidatively generated free radicals and target macromolecules, thereby reducing damage from the free radicals (McDonald, 2004). Between 6% and 14% moisture, lipid peroxidation may be minimal because sufficient water is available to serve as a buffer against autoxidation, but is insufficient to activate lipoxygenase-mediated free radical production (McDonald, 2004). Below 6% moisture, lipid autoxidation may be the prime factor in seed deterioration as water is unavailable to buffer the free radicals. Above 14% moisture, the increasing water content increases the activity of oxidative enzymes, such as lipoxygenase, and the production of free radicals. As seed moisture increases, autoxidation increases and is further accelerated if temperature increases. During imbibition seed moisture increases dramatically and lipoxygenase-mediated free radical production increases, thus creating additional damage. Antioxidants can suppress additional free radical damage. Concurrently, hydration increases anabolic enzyme activity and cellular repair of the damage created by free radicals. Seed vigor

and ultimately seed field emergence depends upon the extent of cellular damage and the seed's ability to repair the damaged membranes, enzymes, and DNA.

Water concentration is a measure of water in seeds but does not measure the thermodynamic properties of seed water. Water in a system exists in a continuum of energy states. Walters (1998) reviewed the mechanisms and kinetics of seed ageing. Her central hypothesis is that the nature of chemical reactions and/or the kinetics of these reactions change at critical water concentrations. Such critical water concentrations may be related to the viscosity of the aqueous milieu, for example, a glass versus a rubber amorphous state. Drying of seeds reduces the viscosity of seed water to a glass state. The high intracellular viscosity typical of glasses slows molecular diffusion and decreases the probability of chemical diffusion (Krishnan *et al*., 2004). At a certain temperature (Tg), the glass undergoes a state change to an amorphous rubber. Rubbers differ from glasses by greater fluidity and free volume changing the nature of chemical reactions and making the seed more susceptible to ageing reactions. For each seed and its genetics and maturation environment, optimum moisture concentration will vary and may correspond to the point of saturation of strong binding sites (Walters, 1998). With an increase in temperature the amorphous structure becomes increasingly fluid allowing reactions to occur more rapidly. Thus, temperature above Tg may have a disproportionate effect on the stability of biological materials.

A dry seed is metabolically incompetent and chemical aberrations go unrepaired. Over time, damage from a suite of degrading reactions will accumulate. A change occurs from strong viability to a weaker seed to a non-viable seed. Walters (1998) developed a "kinetic model of chemical degradation in seeds" to predict seed ageing time-lines. The model demonstrates that different reactions are involved in seed deterioration, and that, if the kinetics of these reactions

are differentially controlled by temperature and water concentration, the relative importance of each reaction in the overall loss of seed viability is likely to vary among different storage regimes. Comparing soybean seed, 20% oil content, with wheat seed, 1–5% oil content, Krishnan *et al*. (2004) reported a sudden change in thermodynamic properties of water for soybean at 1 and 8 days of storage at temperatures of 45^{o} and 35^{o}C, respectively, and for wheat at 4 and 11 days at 45^{o} and 35^{o}C, respectively. They concluded that soybean seeds have a higher water activity and are more sensitive to changes in water status compared to wheat seeds.

Seed Protection Mechanisms

Antioxidants suppress autoxidation and limit the damage from lipid peroxidation. Enzymatic antioxidants, such as superoxide dismutase, catalase, and glutathione peroxidase, act to neutralize activated oxygen species (McDonald, 1998). Although enzyme activity is limited in the quiescent seed, these enzymes are vital during imbibition as autoxidation increases with the increasing fluidity of the cytoplasm. The nonenzymatic antioxidants include glutathione, vitamin E (tocopherol), and vitamin C (ascorbic acid). These antioxidants function as free radical scavengers and react with the free radicals to block the propagation of free radical chain reactions. One tocopherol molecule can protect several thousand fatty acid molecules (Bewley, 1986). Soybean seed subjected to ageing had reduced content of tocopherol, suggesting that the tocopherol was consumed while protecting the seed from free radical attack. The seed antioxidant content may reduce the extent of cellular damage resulting from free radical attack during seed storage (McDonald, 2004). Talcott *et al.* (2005b) found that antioxidant capacity varied with genotype and oleic acid concentration and Talcott *et al.* (2005a), in analysis of oxidative stability of polyphenolics, reported that, in dry roasted peanut, rates of lipid oxidation are directly proportional to the degree of fatty acid unsaturation. Hashim *et al.* (1993) suggested

that natural antioxidants should always be considered as a parameter when predicting peanut seed stability.

Some seed degradation is inevitable (McDonald, 2004). In addition to the protective action of antioxidants, repair enzymes and repair pathways exist specifically to fix damage caused by free radicals. During imbibition the pathways can repair DNA by removing damaged bases and oxidative lesions and correct misincorporation of nucleotides during DNA replication. If the damage is not severe, the cellular mechanisms are quickly repaired and the seed germinates into a vigorous seedling.

Accelerated Aging

Accelerated aging (AA) tests expose seeds for short time periods to elevated temperature and relative humidity, the two environmental factors most influential in seed quality loss (TeKrony, 2005). The AA test is used to evaluate seed vigor in crops and has been successfully correlated to field emergence and stand establishment in soybean (Egli and TeKrony, 1995). The AOSA *Seed Vigor Testing Handbook 2002* (AOSA, 2002) provides standards for using AA to test peanut seed vigor. Accelerated-aged peanuts had increased MDA in the axes and cotyledons and the axes had greater lipid peroxidation and accumulated more peroxides than the cotyledons (Jeng and Sung, 1994). The AA peanuts tended to have less soluble protein and reduced activity of peroxide-scavenging enzymes. Germination decreased and the number of weak seedlings increased compared to the control. These effects are consistent with increased lipid peroxidation in the axes and cotyledons. The AA test is easy to conduct and relatively fast. However, researchers debate whether AA produces the same biochemical events as occur in natural aging (McDonald, 1999). Liklatchev *et al*. (1984) concluded that biochemical changes during accelerated aging were the same as those in natural aging; the only difference was the rate at which they occur.

Mode of Action of *Aspergillus* spp.

Aspergillus spp. are facultative saprophytes capable of producing a large quantity of extra cellular enzymes, which probably enhance their ability to utilize a broad assortment of organic resources to produce mycotoxins (van der Hondel *et al.*, 1994). Mycotoxins are a sub-set of secondary metabolites that can be synthesized from primary metabolites, such as proteins, fatty acids, and sterols (Moss, 1994). Synthesis involves several precursor steps resulting in the production of inducible enzymes, in particular cytochrome oxygenases that may be involved in hydroxylations, oxidative cleavages, and rearrangements leading to a remarkable diversity of secondary metabolites.

Effect of *Aspergillus* on Peanut Seed Germination

Neergaard (1977) summarized the categories of storage fungi and their temperature and relative humidity required for active growth. Most of the storage flora are species of *Aspergillus* and *Penicillium*, which are active at humidity ranging from 70 to 90%. *Aspergillus* spp. each have a characteristic temperature range and minimum and optimum water activity (Lacey, 1994). *A. halophilus* Sartory & Mey is active at 70 to 73% relative humidity; *A. flavus* Link is active at 85-95 percent relative humidity. Damage to the pod predisposes the seed to invasion. Invasion can occur at seed moisture concentration levels as low as 13.2 percent. Seed water concentration determines the species of *Aspergillus* and a small change in the water concentration may result in colonization by a different species. Neergaard (1977) pointed out that there is great variation of the moisture level within a bulk seed storage unit. The problem of unequal distribution of moisture in a bulk storage mass occurs where no forced aeration system is available. For seed susceptibility to storage fungi, Neergaard prefers the term water activity (a_w). The development of storage fungi depends on the water activity of the seed rather than its relative moisture concentration. Water activity differs for species and, assuming that lipid is non-miscible, the

critical moisture concentration should be computed on the non-lipid portion of the seed. For example, in soybean (*Glycine max* L.), which has lipid concentration of ≈18%, fungi will grow at a moisture concentration 1.5 percentage points lower than in cereal grains, which have lipid concentration of < 5%. Seed may be affected internally without showing any external evidence. Infected seed can lose ability to germinate within a few weeks or months. *Aspergillus* spp. eventually kills peanut seeds or seedlings (Lopez in Neergaard 1977).

Dhingra *et al.* (2001) studied the effect of *Aspergillus ruber* Thom & Church on soybean seed stored at 25^{o}C and water activity varying from 0.66 to 0.86 (moisture concentration 11.3% to 17%). Seedling emergence rate in sand began to decline for all treatments within 20 days of storage and continued to decline significantly with increased storage time. The decline differed with water activity (a_w) and was slower in the samples of lower a_w and increased as a_w increased. Concurrently, free fatty acids (FFA) increased significantly. At a_w of 0.66 (11.3% moisture), emergence decreased to zero by 140 days while free fatty acids increased approximately 0.5 units. The proportion of normal seedlings decreased. All abnormal seedlings exhibited some degree of negative geotropism and the radicle tips were necrotic. They concluded that loss of seed viability during storage was directly dependent upon the amount of fungal growth as measured by FFA content. Storage fungi lipases in maize incite production of free fatty acids (Neergaard, 1977). Trawatha *et al.* (1995) concluded that increased FFA disrupts membranes resulting in seed deterioration that is evident in high electric conductivity of seed leachates.

During germination of oilseeds, fats are converted to sucrose, which is the energy molecule transported to growing sites for biosynthetic processes. Singh *et al.* (1974) reported that the rate of formation of sucrose, at all stages of germination, decreased with increases in the concentration of aflatoxins. Aflatoxin B has been reported to affect the osmotic behavior of

mitochondria and inhibit tissue respiration (Bassir and Bababunmi 1972). Ratnavathi and Sashidhar (2000) looked at the effect of aflatoxin on enzyme activity within germinating seed of sorghum (*Sorghum bicolor* L.). Although sorghum is not a high oil content seed, increasing aflatoxin reduced the activity of lipase. The normally very high ∂-amylase and ß-amylase activity in germinating seeds was also significantly less in infected grains. However, protease activity was observed to be higher in the infected grains. They hypothesized that the increase in proteases may be attributed to new fungal proteins. Christensen (1957) observed that fungal infection associated with stored grain caused under-development of the plumule and radicle. Singh *et al.* (1974) found lower sucrose concentration and lower percent germination as result of *Aspergillus* infection of seed.

Hypotheses and Objectives

Poor field emergence of late maturing, disease resistant peanut cultivars occurs when seeds are produced in commercial channels with large volumes being harvested, stored in bulk in-shell, shelled, processed, and treated with fungicides. The problem may be related to commercial storage of in-shell seed peanuts in large piles with no humidity control, temperature control, or forced ventilation. Based on the review of literature, the materials and methods of this study were designed to test the following four hypotheses:

- The high temperatures and high relative humidity in commercial storage conditions may reduce field emergence of peanut.
- There is an interaction between storage environment and cultivar relative to seed germination and field emergence.
- Reduced field emergence may be caused by storage pathogens.
- Potential field emergence can be measured by testing for seed vigor and the electrolyte conductivity of the leachate of germinating peanuts.

CHAPTER 3
MATERIALS AND METHODS

Cultivars and Storage Locations

To study the interaction of cultivars and storage environment, four cultivars were selected: C-99R, DP-1, Hull, and AP-3. C-99R, DP-1, and Hull are late-maturing cultivars with parentage tracing to PI 203396, the primary source of their resistance to tomato spotted wilt virus (genus *Tospovirus*; family *Bunyaviridae*), late leafspot (*Cercosporidium personatum*, Berk & Curt.), and white mold (*Sclerotium rolfsii*, Sacc.). These cultivars consistently produce high yields and good grades (Table 1-1). However, field emergence has been unreliable after storage in commercial bulk peanut bins. AP-3 was chosen as the control, mainly because AP-3 has no lineage of PI 203396 and has good field emergence after storage in commercial bulk bins. AP-3 is medium maturity and yields well, has resistance to tomato spotted wilt virus and white mold, but is susceptible to leafspot and rust. For seed origin comparison, seed was obtained in October 2004 from two different field-growing environments: Florida Foundation Seed (FFSP), Marianna, Florida, and the University of Florida North Florida Research and Education Center (NFREC) near Marianna, Florida. Pods were dried with forced heated air (≈33^{o}C) to a seed moisture content of 8 to 10%. The pods of each cultivar within a seed source were thoroughly mixed to minimize variation and bagged in burlap sacks for placement in four storage locations. Samples of each seed origin and cultivar were frozen to preserve seed condition prior to the storage treatment. All peanuts were stored in-shell, unless otherwise stated. Bags of in-shell peanuts of each cultivar from each seed origin in each year were placed into storage treatment locations as follows:

- NFREC temperature and relative humidity controlled storage facility (UNICOOL),
- Approximately 1.5 meters into the base of the bulk in-shell peanut pile within the FFSP commercial peanut storage barn (STACKS),

- In bags on pallets in the FFSP warehouse (WHSE),
- In the center of bulk peanuts being stored in wagons housed in open-sided sheds at FFSP (WAGON).

A HOBO® Pro Series temperature/relative humidity recorder manufactured by Onset Computer Corp., Bourne, Mass., was placed adjacent to the stored samples at each treatment location. UNICOOL location provided uniform air temperatures of 12–15^{o}C and relative humidity at 60–70% throughout October to December. In the STACKS piles varied in height to 15 meters and had a volume of 7,000m^{3}. Ventilation was accomplished through open bin doors that allowed ambient air to move across the face of the pile and exhaust by fans through roof openings. No forced air or intra-pile ventilation was provided. Peanut bags in WHSE were exposed to daily fluctuating temperatures and relative humidity without being deeply buried under other in-shell peanuts. The WAGON storage site was in drier wagons under open sheds and provided a fourth storage condition. Temperature and relative humidity data were recorded at 15 minute intervals in each storage location. Ambient temperatures and relative humidity outside the storage facilities were recorded by the Florida Automated Weather Network (FAWN) substation located at Latitude 30.850 and Longitude -85.165 approximately 50m from the FFSP storage facilities. In December, FFSP began shelling peanuts. When a bin became empty, the treatments were ended; the peanut bags were removed from their storage places and placed into controlled atmospheric conditions at 12–13^{o}C and 66–68% relative humidity to hold condition until tested for germination or field emergence (April and October).

In crop production year 2004, placement of bagged in-shell peanut samples was delayed and bagged peanuts could not be placed within the center of STACKS or WAGON. For comparison of storage effects deeper within the STACKS and WAGON, a second set of samples designated Bulk Stored Seed was collected at the end of the storage period from pods deep

within the bulk piles of STACKS and the center of WAGON. This sample set is similar to the bagged peanut samples in all aspects except that the peanuts during storage were located deeper within the bulk piles of the STACKS or the bulk peanuts of the WAGON. In the analysis of towel germination and field emergence tests of bulk stored seed, data for the controlled environment treatment was the same data as UNICOOL in the bagged seed analysis.

All seed was stored in-shell. Bagged seed was shelled in small lots using a pod separator, a standard Federal/State grade sheller, a seed sizer, and sized, keeping sound mature kernels (SMK) that rode a 16/64 screen. Bulk seed was shelled by FFSP as follows: A front-end payloader scoops the peanuts out of the bin, loads the peanuts into a bulk wagon, and peanuts are dumped into an elevator, lifted to the top of the elevator leg, dropped to the sheller and then sized using a 16/64 screen. The seed was stored in large cardboard boxes in the warehouse.

Germination Tests for Seed Viability

Germination tests were conducted according to the *Rules for Testing Seeds* published by the Association of Official Seed Analysts (2004). Seeds were treated with Vitavax® PC for control of fungi. Active ingredients in Vitavax® by weight are Captan 45.0%, pentochloronitro benzene 15%, and Carboxin 10%. Samples of 50 seeds of each treatment were evenly spaced on double germination towels, covered with a third towel, the lower edge of the towels was folded to retain seeds, and the towels were rolled into a cylinder shape and set on end in sealed plastic containers. Unless otherwise noted, germination tests were conducted in a Model I-35LVL germination chamber manufactured by Percival Mfg. Co., Boone, Iowa. Temperature was set at 25^{o}C and seedlings with $\geq$ 1-cm radicle were counted 10 days after imbibition. Each year towel germination tests were run just prior to sowing field emergence tests. In April 2005 the towel germination test of seed from crop production year 2004 included four replications of 50 seeds each of four cultivars, C-99R, DP-1, Hull, and AP-3, from two field origins, NFREC and FFSP,

stored in the four treatment locations. In April 2006 the towel germination test of seed from crop production year 2005 included the same four cultivars plus three new cultivars McCloud, Florida-07, and York. For comparison, at the conclusion of the storage period, seed from these same cultivars was tested for germination by Tallahassee Seed Testing, LLC, located at 1510, Capital Circle SE Suite E1, Tallahassee, Florida, 32301.

Seedling Emergence Field Tests

Four replications of 50 seeds each of the treatments derived either from the bagged peanuts or the bulk stored peanuts were sown on successive days in a Randomized Block Design (RBD) in Millhopper fine sand in field research plots located at the University of Florida, Gainesville, Florida. Soil temperatures exceeded 15.5^{o}C at the sowing depth of 5 cm. All seeds were treated with Vitavax®. Seeds were placed in twin rows spaced 15 cm apart with in-row spacing between seeds of 8 cm. The plots were watered by overhead irrigation and all emerged plants were counted 14 days after sowing (DAP). No fertilizer was applied. All subsequent seedling emergence field tests followed this procedure unless otherwise noted. For production year 2004, sown seed in April came from both the bagged seed (FFSP and NFREC origins) and bulk stored seed (FFSP origin only). In the October seedling emergence test, the seed sown came from the bulk stored seed (FFSP origin) that had been stored from the time of the April sowing until October in UNICOOL at 12–13^{o}C and 66–68% relative humidity. The additional storage time was 5.5 months. Samples sown consisted of the four cultivars selected at the following time periods from the specified storage locations: UNICOOL-Jan, STACKS-Jan, WAGON-Jan, UNICOOL-Mar, STACKS-Mar, WHSE-shelled-Mar, and WAGON-Mar. Treatments were sown October 4, 2005, in a polyethylene-covered hoop greenhouse located at the University Research Plots in Gainesville. Replications were sown at five-day intervals.

For the 2005 production year seed, a seedling emergence field test was sown May 5, 2006. Three additional cultivars, McCloud, Florida-07, and York, were added to the previously selected four cultivars. Seed origin was FFSP only; since prior year tests showed no effect of seed origin. Storage treatment locations were: 1) UNICOOL, 2) STACKS, and 3) WHSE. Four replications were sown on successive days in the same field site as the prior year's field emergence test.

Storage Pathogen Assays

Pathogen assays were conducted using Difco Sabouraud Maltose Agar mixed thoroughly at the rate of 65 grams of agar suspended in one liter of de-ionized water. Suspension of the agar was accomplished with constant agitation and the suspension was dissolved by boiling the water for one minute. The solute was autoclaved at 121^{o}C for 15 minutes and poured into Petri dishes for cooling. The approximate formula per liter of solute (Tuite, 1969) was 10g of enzymatic digest of casein, 40g of maltose, and 15g of agar resulting in a final pH of 5.6 ± 0.2. Peanut seeds were disinfected with 1% Clorox brand of sodium hypochlorite for 2 minutes, rinsed in sterile water for 2 minutes, and then, using aseptic techniques, the seeds were split in half and the cotyledon containing the embryo was placed flat-side down on the maltose agar. The specimens were incubated at 32^{o}C for 84 hours and then pathogens were identified. The assay consisted of 20 seeds for each of the four cultivars, AP-3, C-99R, DP-1, and Hull, from three storage treatments in years 2004 and 2005. The samples from the 2004 crop production year were 1) seed frozen at harvest, 2) seed stored in UNICOOL, and 3) seed stored in the STACKS. The samples from the 2005 crop production year were: 1) seed frozen at harvest, 2) seed stored in UNICOOL, and 3) seed stored in the STACKS.

Seed Vigor Tests

Seed vigor tests were conducted in accordance with the guidelines presented in the *Seed Vigor Testing Handbook* published by the Association of Official Seed Analysts (2002) and a "vigor index" developed by D.L. Ketring (1993). Seed vigor is difficult to standardize from test to test. Moisture and temperature have profound effects upon rate of growth and the measurement technique may vary among seed analysts. Vigor tests in this research were conducted to test cultivar effect and the interaction between cultivar and storage location. The results of the vigor tests are reported as "comparative vigor index" (CVI) for the specific purpose of comparing seedling growth within a specified towel germination test and differs from the Ketring (1993) Vigor Index, in which he adds a factor of total percent germination. CVI tests were conducted using the standard towel germination test procedure for peanut (Association of Official Seed Analysts, 2004). Seedling growth rate was measured as linear growth of the hypocotyl/radicle axis and seedlings were separated into four classes: no growth, radicle < 3cm, radicle 3-6cm, and radicle > 6cm. Numerical values were assigned to the classes to reflect relative value of the seedlings: 0 for no growth, 1 for growth < 3cm, 2 for growth 3-6cm, and 3 for growth > 6cm. The CVI for the treatment equaled the sum of the products of number of seedlings per class times the value assigned to the class. Percent germination was computed by counting all seeds with protruding radicles ≥1cm.

For the Seed Vigor Tests #1 and #2, the seed source was from production year 2005. For Vigor Test #1, the treatments were four cultivars, AP-3, C-99R, DP-1, and Hull, from each of four storage locations. Two replications of 25 seeds were held for 44 hours at 40^{o}C and 100% relative humidity in an accelerated ageing chamber. Two replications of 25 seeds were placed into the controlled storage at 13^{o}C and 67% relative humidity for 44 hours. All four replications of 25 seeds were germinated at 30/20^{o} for 7 days in the University of Florida Seed Laboratory

germination chamber. In Vigor Test #2 the treatments were seven cultivars, AP-3, C-99R, DP-1, Hull, McCloud, Florida-07, and York, each stored in three locations, UNICOOL, STACKS, and WHSE. Four replications of 25 seeds were germinated at 25^{o}C for 7 days in the University of Florida Seed Laboratory germination chamber.

Electrolyte Conductivity Tests

The procedure for determining the electrolyte conductivity of leachate of germinating peanut seed followed the guidelines suggested by McDonald and Copeland (1989). Sample size was 50 seeds. Each sample was weighed and then placed into 200mL de-ionized water in beakers in a controlled temperature chamber at 30/20^{o}C for 24 hours. After 24 hours, 10 mL of leachate per treatment were withdrawn from the leachate beakers to test for electrolyte conductivity. The leachate was agitated by swirling gently and electrolyte conductivity was determined using a Fisher Scientific Digital Conductivity Meter (Fisher Scientific, Pittsburgh, PA). Data were expressed as µmhos and were divided by the seed g weight to obtain µmhos g^{-1}. Unless otherwise stated, electrolyte conductivity tests followed this procedure.

All electrolyte conductivity tests were accompanied with a companion CVI test. There was a leachate conductivity test of seed from production year 2004 and a second leachate conductivity test as part of the accelerating aging test on seed from production year 2005. To determine CVI and percent germination, the seeds, after the 24 hours of imbibition, were wrapped in moist towels and incubated at 30/20^{o}C for an additional 5 days. In the leachate conductivity tests, each year the seed source was from excess seed stored at temperature < 0^{o}C since the April field emergence tests. The cultivars were AP-3, C-99R, DP-1, and Hull. Storage locations were UNICOOL, STACKS, WHSE, and WAGON.

Accelerated Ageing Tests

Accelerated ageing treatments were used to simulate the storage environment of the STACKS. The seeds were first subjected to ageing treatments and then evaluated for electrolyte conductivity, seedling vigor, germination, and field emergence. The seed for this test came from seed of production year 2005 stored in WHSE until March 2006 and then stored in UNICOOL at 12-13^{o}C and 66-68% relative humidity. Total sample size per treatment was 250 seeds of each of the seven cultivars: AP-3, C99, DP-1, Hull, McCloud, Florida-07, and York. The treatment time period was 5 weeks. The treatments were (1) control, in the UNICOOL at 13^{o}C and 67% relative humidity, (2) accelerated ageing chamber at 32^{o}C and 100% RH (EAA5wks), and (3) the University germination chamber at 32^{o}C and relative humidity < 10% (HT/LRH). All seeds were treated with Vitavax®. For the EAA5wks treatment, seeds were placed in a single layer on a copper screen above a water surface in small plastic germination boxes sealed with lids (Association of Seed Analysts, 2002). The boxes were placed in an accelerated ageing chamber containing water and a submerged electrical heating element. For the HT/LRH treatment, the seeds were placed in plastic cups without lids in a larger sealed plastic box containing desiccants and held in the germination chamber. The 250 seeds of each cultivar of each treatment were divided into two lots, one of 200 seeds for subsequent field planting and one of 50 seeds to be used for percent moisture measurements, electrolyte conductivity tests, seedling vigor tests, and germination tests**.** Upon completion of the 5 week test period, samples of 20 seeds from each treatment were placed in 100 mL de-ionized water at 30^{o}C for 24 hours. The electrolyte conductivity of the resulting leachates was recorded. After 24 hours of imbibition, the samples of 20 seeds were wrapped in moist towels and returned to the germination chamber at 30^{o}C for 96 hours for determination of comparative seed vigor and percent germination. The lots of 200 seeds selected for the seedling field emergence test were divided into four replications of 50

seeds each and sown into the field plots at the University of Florida beginning September 20, 2006. Soil temperatures exceeded 22°C at the sowing depth of 5 cm. The number of vigorous plants was counted at 8 DAP and 12 DAP. A plant was considered vigorous if the trifoliate leaves were open, horizontal, and the plant was green and healthy. The 8 DAP counts indicated relative vigor of the treatment sample and the 12 DAP counts provided final percent field emergence.

Antioxidant Capacity Assay

Antioxidant capacity of seeds was evaluated using a method designed to assay antioxidants in animal tissue (Glavind, 1963). The cultivars were AP-3, C-99R, DP-1, and Hull. The seed sample sources were seed from the 2004 harvest, the 2005 harvest, the 2006 harvest and seed from 2004 production year stored in STACKS. Peanuts from 2004 harvest were stored in UNICOOL at 12–13°C until January 31 and then stored at temperature < 0°C. Seed from 2005 harvest and 2006 came from samples stored at temperature < 0°C since harvest. Seed from STACKS was stored at temperature < 0°C upon removal from STACKS. There were three replications. For this assay, the testa was removed and the seeds were trimmed to approximately uniform weights of 0.400 g. Peanuts were ground individually with mortar and pedestal and soaked in 5mL of methanol for 15 minutes. The mixture was centrifuged at 2500 rpm for 10 minutes at room temperature. A sample of 150µL of methanol extract was drawn from each sample and placed into a small test tube containing 850µL of DPPH at 0.25mM. DPPH is 90% 1-Diphenyl-2-picryl-hydrazyl ($C_{18}H_{12}N_5O_6$). DPPH at 0.25mM is prepared from 0.0098 g DPPH in 100 mL methanol. This procedure was replicated three times per seed. The combination of peanut methanol extract and DPPH was allowed to react for a minimum of 15 minutes. Standards for comparison were prepared using Trolox, a Vitamin E equivalent. Trolox is ±-6-hydroxy 2,5,7,8-tetra-methychromane-2-carboxylic acid ±-6-hydroxy-2,5,7,8-tetra-methyl-

chroman-2- carbonsaure. Trolox at 1.0mM was prepared from 0.0048g Trolox placed into 20 mL of methanol and then diluted with additional methanol to create one mL standards containing 5µM, 10µM, 25µM, 50µM, 75µM, and 100µM of Trolox. A blank standard consisted of zero Trolox made from 100µL of methanol and 900µL DPPH. The resulting total number of samples was three replicates x four cultivars x four storage treatments with each sample assayed three times. Samples were pipetted into 96-well Corning Costar plates and assayed for antioxidant capacity using a Spectra Max 340 PC manufactured by Molecular Devices Corporation, Union City, CA. Spectra Max 340 PC is a visual range spectrophotometer applicable for evaluating ELISA assays. The software program was Softmax pro 4.8. The assay was conducted at a wave length of 517 nm.

Experimental Design and Data Analysis

Laboratory tests and field emergence trials were set up as Randomized Block Designs (RBD). Analyses of Variance (ANOVA) was accomplished by the procedures in SAS System Release #9.1 (SAS Institute). Least Squares Means and Duncan's Multiple Range Tests were generated using the general linear model (PROC GLM). Pearson correlation coefficients were generated using the correlation procedures (PROC CORR). Regression analyses were conducted using PROC REG. Unless stated otherwise, differences reported were significant at alpha of less than or equal to 0.05.

CHAPTER 4
RESULTS AND DISCUSSION

Effect of Seed Storage Environment on Germination and Field Emergence

Prior Germination Tests of Cultivars at NFREC

Peanut cultivars Florida MDR-98, C-99R, DP-1, and Hull were released by the University of Florida for commercial production in 1999 - 2002. Poor field emergence and unacceptable stands occurred (Tilllman, 2004, Per. Comm.). The 2002 and 2003 NFREC field emergence trials included limited entries of seeds produced by Florida Foundation Seed Producers (FFSP) (Tillman and Gorbet, 2004). A comparison of the entries showed that FFSP seed had lower germination than NFREC seed and that the cultivars were not equally affected. Germination of AP-3, C-99R, DP-1, and Hull seed produced by FFSP was 10.7%, 13.4%, 33.1%, and 32.4% lower than germination of seed produced and stored by the NFREC peanut breeding program.

Germination Tests of Seeds from NFREC and FFSP Stored in Bags in Various Locations

These towel germination and field emergence tests were designed to identify (1) the process and factors in commercial seed production that affected seed vigor and field emergence, (2) the reliability of germination tests in predicting field emergence, and (3) year to year variation in field emergence.

At the conclusion of the storage seasons, mean towel germination for crop production year 2004 was 93.6% and was superior to the mean towel germination of 87.1% for production year 2005 ($P < 0.0001$) (Tables 4-1 and 4-2).

Mean towel germination for seed from FFSP origin was 94.4% and was superior to the mean germination of 92.8% of the NFREC origin ($P = 0.0321$) (Tables 4-1 and 4-3). The lower mean germination of seed from NFREC resulted from the weaker germination of NFREC seed of C-99R and DP-1. C-99R and DP-1 of NFREC origin germinated at 88.4% and 85.8% compared

to 95.3% and 93.1% for C-99R and DP-1 from FFSP origin. The low towel germination was not expected and may have resulted from residual dormancy or cultural conditions during seed production.

The main effects of cultivar and storage location for germination tests and field emergence tests of peanuts stored in bags in various locations for crop years 2004 and 2005 are presented in Figures 4-1 and 4-2. In both 2004 and 2005, cultivar differences were evident in towel germination tests (2004, $P<0.0001$; 2005, $P=0.0442$; combined, $P=0.0239$) (Table 4-1). In 2004, towel germination of AP-3 and Hull was superior to C-99R and DP-1; C-99R was superior to DP-1 (Figure 4-1**)**. In 2005, towel germination of AP3 and C-99R was similar and greater than that of Hull; towel germination of DP-1 was intermediate. Combined across both years, towel germination of C-99R was 92.6%, which was greater than both DP-1 and Hull. Towel germination of AP-3 was intermediate.

Storage environment/location affected towel germination (2004, $P=0.0493$; 2005, $P=0.0498$; combined, $P=0.0847$ (Table 4-1). For the 2004 crop, towel germination of seed from the warehouse (WHSE) and wagon (WAGON) storage locations was superior to the University of Florida controlled storage facility (UNICOOL); germination of seed stored in the bulk bin facilities of FFSP (STACKS) was intermediate (Figure 4-2). For the 2005 crop, towel germination of seed stored in STACKS was superior to UNICOOL; germination of seed from WHSE and WAGON was intermediate. In the combined analysis, towel germination of seed stored in the WAGON was superior to UNICOOL; germination of seed from STACKS and WAGON was intermediate.

The towel germination tests showed interaction of cultivars with storage locations for crop production year 2004 ($P=0.0021$), but not for crop production year 2005 ($P =0.6526$) (Table 4-1).

Germination of AP-3 and Hull was consistent across storage environments, but germination of DP-1 and C-99R was not consistent across storage environments (Figure 4-3). Germination of C-99R stored in UNICOOL was inferior to C-99R stored in STACKS (P=0.0003), WHSE (P=0.0474), and WAGON (P=0.0191). Germination of DP-1 stored in UNICOOL was inferior to DP-1 stored in WAGON (P=0.0627) and WHSE (P=0.0007).

In summary, the towel germination tests indicate that seed quality in crop year 2004 was superior to seed quality in crop year 2005 (Table 4-2). Contrary to expectations, FFSP seed origin was not inferior to NFREC seed origin (Table 4-3). UNICOOL storage location was not superior to other storage locations. Germination of cultivars was differentially affected by storage location (Table 4-1).

Field Emergence of Seeds from NFREC and FFSP Stored in Bags in Various Locations

In 2004 seed origin did not affect field emergence (P=0.9104) (Table 4-1). Based on this finding and the fact that in the towel germination tests, seed of FFSP origin was not inferior to seed of NFREC origin, and the fact that poor seed emergence occurred frequently with peanut produced and stored by FFSP and only infrequently with peanut produced and stored by NFREC, the decision was made to limit all subsequent seed tests to seed of FFSP origin.

Year of production affected field emergence (P=0.0082) (Table 4-1). Mean emergence for crop production year 2004 was 88.3% and was superior to the mean emergence of 85.1% for crop year 2005 (Table 4-2).

For both 2004 and 2005 crop years, cultivar affected field emergence (2004, P<0.0001; 2005, P=0.0043; combined P<0.0001) (Table 4-1). For the 2004 crop year, field emergence of AP-3 was superior to DP-1 and Hull, and similar to C-99R (Figure 4-1**)**. Field emergence of DP-1 was less than AP-3, C-99R, and Hull. For the 2005 crop year, field emergence of AP-3 and C-

99R was similar and greater than that of DP-1 and Hull. Combined across both years, field emergence of AP-3 and C-99R was about 89%, which was greater than both DP-1 and Hull.

Storage location affected field emergence (2004, P=0.0208; 2005, P=0.0957; combined, P=0.0063) (Table 4-1). From the 2004 crop, field emergence of seeds stored in the STACKS was lower than that from UNICOOL and WAGON, but similar to that from WHSE (Figure 4-2). For the 2005 crop year, field emergence of seeds stored in the STACKS was less than those stored in the WHSE and emergence of seeds stored in UNICOOL and WAGON was intermediate. In the combined analysis, field emergence of seeds stored in the STACKS was less than all other locations (P=0.0711).

In summary, field emergence tests confirmed that seed origin was not a significant factor in poor field emergence; that the seed quality of crop production year 2004 was superior to the seed quality of crop production year 2005; and that cultivars AP-3 and C-99R had superior field emergence, compared to DP-1 and Hull. In contrast to towel germination tests, field emergence of seed stored in STACKS had lower field emergence than all other storage locations.

Towel Germination and Field Emergence of Bulk Stored Seed from Production Year 2004

Bagged seed in-shell samples were derived by hand mixing peanuts from several wagons and placing the bags as deep as possible into STACKS and WAGON. This depth was approximately 1.5m. In contrast to the bagged samples, the bulk stored seed samples were collected from the same STACKS and WAGONS either by probing into the bulk pile or as the peanuts were being emptied at the conclusion of the storage season. Since the bulk stored seed samples came from deeper within the piles than the bagged seed, they should be more representative of the effects of interaction of cultivar and storage environment and provide a comparison to the towel germination and field emergence of the shallower buried bagged peanuts.

The main effects of cultivar and storage location for towel germination tests and field emergence of bulk stored in-shell peanuts sampled from various locations for production year 2004 are presented in Figures 4-4 and 4-5**.** Seed origin was FFSP. Cultivar differences were evident in towel germination tests, and in field emergence tests in April and October (towel, P=0.0002; April field emergence, P<0.0001; October field emergence P<0.0001) (Table 4-4**).** In towel germination and the April field emergence, cultivars AP-3 and C-99R were superior to DP-1 and Hull and in the April field emergence, Hull was superior to DP-1 (Figure 4-4). In October field emergence, AP-3 was superior to C-99R, DP-1, and Hull; C-99R was superior to DP-1 and Hull.

Storage location also affected towel germination and April and October field emergence (towel, P=0.0309; April field emergence, P<0.0001; October field emergence, P<0.0001) (Table 4-4**).** Towel germination of seeds stored in UNICOOL and WAGON was superior to those stored in STACKS (Figure 4-5). In April field emergence, UNICOOL was superior to STACKS and WAGON, and WAGON was superior to STACKS. In October field emergence, UNICOOL was superior to both STACKS and WAGON.

The interaction of cultivar and storage location is presented in Figures 4-6 and 4-7. The P-values for interaction of cultivar and storage location for towel germination, April field emergence, and October field emergence were P=0.5447, P<0.0001, and P<0.0001 respectively (Table 4-4). Field emergence of AP-3, C-99R and DP-1 was similar in the UNICOOL, but when stored in either the WAGON or STACKS, field emergence of DP-1 was inferior to that of AP-3 or C-99R (Figure 4-6). In October field emergence, the interaction of cultivar and storage environment was more pronounced (Figure 4-7). For all cultivars, storage in UNICOOL was superior to storage in STACKS. Comparing UNICOOL to STACKS, October field emergence

of AP-3 decreased from 89% in UNICOOL to 69%, C-99R from 75% to 57%, DP-1 from 79% to 26%, and Hull from 71% to 37%. AP-3 stored as well in the WAGON as in the UNICOOL, but C-99R, DP-1, and Hull had greatly reduced field emergence when stored in WAGON as compared to UNICOOL. Note that field emergence in October of AP-3 stored in STACKS was less than field emergence of AP-3 stored in UNICOOL (P=0.0003), indicating that seed vigor of AP-3 while stored in STACKS decreased. The implication is that STACKS storage may reduce vigor for all cultivars and, therefore, in conditions of stress, reduce seedling populations in the field.

Comparison of Treatment Effects on Bagged Seed Samples and Bulk Stored Seed Samples

In comparing the April field emergence of bagged seed and the April field emergence of bulk stored seed from production year 2004, there was no interaction between cultivar and storage location among the bagged seed (P=0.1355). However, among the bulk stored seed the P-value for interaction of cultivar and storage location was <0.0001 (Tables 4-1 and 4-4). A comparison of field emergence of bagged seed and bulk stored seed (both in STACKS) is presented in Table 4-5. Bulk stored seed had reduced field emergence when compared to the field emergence of bagged seed. The extreme example is the decrease of 37.5% in field emergence of bulk stored seed of DP-1.

The effect of interaction between cultivar and storage environment/location was very pronounced for the field emergence test in October (Figure 4-7). Field emergence was greatly reduced except when cultivars were stored in UNICOOL. Seed deterioration is a result of changes within the seed that decrease the vigor of the seed (McDonald, 2004). Over time, damage from a suite of degrading reactions accumulates and a change occurs from strong viability to a weaker seed to a non-viable seed (Walters, 1998). Seed vigor declines faster than germinability (Figure 4-8). Although the towel germination tests indicated acceptable seed

quality in April, the interaction of cultivar and storage environment/location may have greatly reduced seed vigor, as seen in the differences in April field emergence of bagged seed versus the April field emergence of bulk stored seed (Table 4-5). The storage period for all treatments from April field emergence to October field emergence was 5.5 months in UNICOOL at 12–13^{o}C and 66–68% relative humidity. The additional stress was uniform and minimal for all samples, and, yet, in October field emergence, the number of emerged seedlings was dramatically lower (Table 4-6). The greatly reduced field emergence in October indicates that vigor of seed not stored in UNICOOL was marginal in April and that seed germinability and seed vigor had reached the points of steep descent indicated by the Ys on the viability and vigor curves of the seed deterioration graph (Figure 4-8**)**.

Correlation of Towel Germination and Field Emergence

Towel germination tests were completed one week preceding sowing peanut seed in April. A comparison of towel germination data, the germination tests conducted by Tallahassee Seed Testing, LLC, and the field emergence in April of bulk stored seed is presented in Table 4-7. Germination in the Tallahassee Seed Tests and the germination in the towel germination tests of this research are similar. Field emergence of AP-3, C-99R, and DP-1 stored in UNICOOL had final seedling stands similar to the towel germination tests. However, field emergence of seed of AP-3, C-99R, and DP-1 not stored in UNICOOL had diminished final seedling stands (Figure 4-6). For example, the difference for DP-1 was -38.5% for seed stored in STACKS and -33.5% for seed stored in WAGON. Towel germination was not correlated with April field emergence (P= 0.6763) or with October field emergence (P=0.4507) (Table 4-8). Similarily, April field emergence was not correlated to October field emergence (P=0.1519). The insignificant P-values for correlation of towel germination tests and field emergence support the concept that

vigor decreases at a rate different from the rate of decrease of seed viability and that towel germination tests do not reflect seed vigor and are not reliable for estimating field emergence.

Summary of Results of Towel Germination Tests and Field Emergence Tests

The data from the crop year 2004 and 2005 towel germination and field emergence tests is in accord with the antidotal reports that poor field emergence and stand failures vary from year to year and that failures are more frequent with DP-1 and Hull. Researchers and growers have depended upon standard towel germination tests to evaluate the effect of winter storage and to estimate seedling population stands. Progress in peanut breeding programs has been hampered by the poor correlation of towel germination tests to seed vigor and the failure of towel germination tests to identify loss of seed vigor when peanuts are stored in unventilated bulk bins at elevated temperatures and relative humidity. The results of these studies support the conclusions that:

- Seed production origin is not a significant factor in eventual field emergence (Tables 4-1 and 4-3).
- There may be an effect of season upon seed vigor and field emergence (Table 4-1).
- Across years the field emergence of AP-3 and C-99R cultivars was superior to DP-1 and Hull (Figure 4-1).
- Field emergence of seed was maintained when seed was stored in UNICOOL at temperatures < 16^{o}C and relative humidity < 70% (Figures 4-2, 4-6 and 4-7).
- Field emergence of seed may decrease when seed is stored in large bulk bins (STACKS) or drying wagons (WAGON) (Figures 4-2, 4-6 and 4-7).
- There was a cultivar by storage environment/location interaction for field and laboratory germination, based on the two year analysis of bagged seed and the 2004 bulk stored seed (Tables 4-1, 4-4 and Figures 4-6 and 4-7). Compared to storage in UNICOOL, field emergence of DP-1 and Hull stored in STACKS and WAGON declined more than that of AP-3.
- Towel germination tests are not a reliable measure of field emergence for peanut (Tables 4-7 and 4-8).

Storage Environment Characteristics as Possible Factors in Declining Seed Vigor

External atmospheric temperature and relative humidity data at 2m height for 2004 and 2005 were recorded at the Florida Automated Weather Network (FAWN) adjacent to the storage facilities. Daily mean temperatures for the period September 16 to January 24 are presented in Figure 4-9. The mean of the daily temperatures was 15.8^{o}C in 2004 and 15.7^{o}C in 2005. Although there is no meaningful difference in the mean daily temperatures of the external air for ventilation, the temperatures within the center of STACKS October 11 to January 24 for the 2004 crop year averaged 22.8^{o}C compared to 15.8^{o}C for the 2005 crop, a difference of 7.0^{o}C (Figure 4-10). Conceivably the timing of warm and cool weather fronts for ventilation will affect the relative rate of cooling of peanuts in STACKS. In 2005 from October 23 to November 11, external air temperatures averaged 13.4^{o}C, which was 8.6^{o}C cooler than the average of 22.0^{o}C for the same period in 2004. During that time interval, temperature in STACKS in 2005 decreased 5^{o}C and temperature in STACKS in 2004 increased 3^{o}C. However, the 11-day period of higher temperatures in 2004 by itself does not account for more than a part of the higher temperatures throughout the storage period in 2004. Although external air temperature may change rapidly, sometimes as much as 10^{o}C within 24 hours, the temperatures at the center of the STACKS changed slowly, usually less than 2^{o}C per day. The temperature and relative humidity for the DP-1 in WAGON storage fluctuated with the external atmospheric conditions and that temperature was cooler than in STACKS for the same time period (Figure 4-11**).** A comparison of temperature in STACKS to the outside temperatures recorded at FAWN and by sensors in WAGON under sheds suggests that the temperature in STACKS in 2004 is unexpectedly higher, possibly associated with heating in STACKS by peanut respiration resulting from insufficient drying or a climate event in 2004 that may have affected maturity of the seed prior to drying.

Temperatures in UNICOOL varied between 10 and 15°C in 2004 and fluctuated from 7 to 21°C in 2005 (Figure 4-12). The UNICOOL 2005 seed was stored in an inadequately insulated building and temperatures could not be held constant. Mean temperature in UNICOOL was 12.3°C in 2004 and 14.4°C in 2005. Compared to STACKS, in 2004 temperature in the center of STACKS exceeded that in UNICOOL by 5–15°C until the end of December (Figure 4-13). By contrast, in 2005 the mean temperature of 15.8°C in the center of STACKS exceeded the UNICOOL mean temperature of 14.4°C by only 1.4°C. For 2005, STACKS environment was cooler and UNICOOL environment was warmer; a fact that may explain the differences in effect of year in the towel germination and field emergence tests.

Harrington (1972) and McDonald (2004) stated that relative humidity and temperature were the two most important factors affecting the rate of seed deterioration and that seed moisture and temperature interact. Relative humidity in all storage conditions varied throughout the storage period (Figures 4-11, 4-14, and 4-15). In the UNICOOL the relative humidity fluctuated between 58% and 79% in 2004 and between 44% and 77% in 2005. In the STACKS the relative humidity decreased from 86% to a range fluctuating between 52% and 62% in 2004 and relative humidity decreased from 86% to 73% in 2005. The differences in relative humidity across years and storage locations is minor, thus indicating that relative humidity may have been a contributing factor to loss of seed vigor, but was not the single factor causing loss in seed vigor.

The temperature and relative humidity data support the conclusions:

- In 2004, temperatures in STACKS averaged 7.0°C higher than temperatures in STACKS in 2005 and 10.5°C higher than temperatures in UNICOOL in 2004 (Figures 4-10 and 4-13).
- Temperatures at the center of the STACKS during the first months of storage may range from 20°C to >30°C (Figure 4-13).

- The internal temperature in STACKS changes much slower than external air temperature (Figures 4-9 and 4-10).
- Temperature in STACKS may differ from year to year (Figure 4-10).
- Differences in relative humidity were present but not enough to be the only factor in poor field emergence of peanut (Figure 4-15).
- The literature states that relative humidity and temperature are the two most important factors affecting the rate of seed deterioration and that seed moisture and temperature interact. In the STACKS in 2004 relative humidity and temperatures were elevated and probably interacted to reduce the seed vigor during the storage of the 2004 seed.

Effect of Storage Pathogens

Five storage fungi were isolated; *Aspergillus flavus* Leek, *Aspergillus niger* Thom & Raper, *Rhizopus* spp., *Fusarium* spp. and *Penicillium* spp. (Table 4-9). In 480 seeds assayed, contamination by *A. Flavus* was 0.4%, *A niger* was 2.5%, *Rhizopus* spp. was 1.5%, and *Fusarium* spp. was 2.7%. *Penicillium* spp. were the most numerous fungi isolated and were found on 5.8% of the seed assayed. Seed stored in the stacks had more incidences of fungi than seed at harvest or seed stored in UNICOOL. The highest incidence of storage fungi was *Penicillium* spp. present in seed stored in the stacks during the winter of 2005. The very low incidence of storage fungi and the random distribution of fungal contamination support the conclusions:

- Storage fungi were not an important factor contributing to poor field emergence in 2004 and 2005.
- Storage environmental conditions did not cause excessive growth of *Aspergillus* spp.

Measures of Seed Quality

Comparative Vigor Index Tests

Seed vigor is reported here as "comparative vigor index" (CVI). The hypocotyl-radicles of germinating seeds were measured and placed into classes with numerical values. The CVI for a seed sample equals the sum of the products of number of seedlings per class times the value

assigned to the class. A photograph presented in the appendix demonstrates the variation in rate of growth of germinating peanuts. There are 11 seedlings with excellent vigor (value 3), 10 seedlings with medium vigor (value 2), 3 seedlings with low vigor (value 1), and one seed counted as zero vigor (value 0). The CVI computes to 56**.**

In vigor tests #1 and #2, the seed source was from crop production year 2005. Vigor Test #1 consisted of cultivars AP-3, C-99R, DP-1, and Hull representing the four storage environment locations; half of the seed was subjected to accelerated ageing (AA) at 40^{o}C and 100% humidity for 44 hours and the other half was the control. Vigor Test #2 consisted of the same four cultivars plus McCloud, Florida-07, and York, each stored in three locations, UNICOOL, STACKS, and WHSE.

Cultivar had a significant effect on CVI Test #1 (P=0.0058) and in CVI Test #2 (P=0.0083) (Table 4-10). In Vigor Test #1, AP-3 and C-99R were more vigorous than DP-1 (Table 4-11). In Vigor Test #2, AP-3, C-99R, Florida-07, and York were more vigorous than Hull and McCloud; but only York was superior to DP-1 (Table 4-12). The relative ranking of cultivars by seed vigor in these two vigor tests correlates with the April and October field emergence (Table 4-8). P-values for correlation of Vigor Test #1 with field emergence were 0.1084 for April and 0.0003 for October. In Vigor Test #2, P-values for correlation were 0.0027 for April and 0.0220 for October. The data support the conclusion that seed vigor tests can be used as reliable indicators of potential final field population stands. This is in agreement with the conclusions of Ketring (1993).

Location effect was significant (0.0003) in CVI #1 (Table 4-10). UNICOOL was inferior to all other storage locations (Table 4-11). The low vigor of seeds in this vigor test reflects the low towel germination tests of seed stored in UNICOOL (Figure 4-2). In contrast, field

emergence of seed stored in UNICOOL was similar to all storage locations and superior to STACKS (Figure 4-2). Surprisingly, seeds of the accelerated ageing treatments were intermediate in vigor (Table 4-11). It was expected that peanut seed subjected to accelerated ageing temperatures of 40^{o}C and 100% relative humidity for 44 hours would have greatly reduced vigor and that the mean vigor index would be low. Instead, the mean vigor index for accelerated-aged seed was 54.7 compared to 39.5 for the control treatment (Table 4-11). In a physical examination of the accelerated-aged seed, it was obvious that the seeds had absorbed water. The unintended consequence of this accelerated ageing was seed priming. The increased moisture concentration of the seed enabled the seed to commence germination earlier than seeds in the control treatment. With a head start in germination, hypocotyl/radicle growth was measured as increased comparative vigor index.

In Vigor Test #2 at day 7, storage environment/location had no effect upon seed vigor (P=0.5031) (Table 4-10). Since storage location had no effect, then Vigor Test #2 may reflect genotype differences in rate of germination of the cultivars. AP-3, C-99R, Florida-07, and York may inherently germinate faster and in field plantings, seedling emergence would be faster than seedling emergence of Hull and McCloud, but final population stands may be similar.

Electrolyte Conductivity and Comparative Vigor Tests

An electrolyte conductivity test measures the electrical conductivity of the leachate of germinating seed. A higher electrolyte conductivity value indicates greater leakage of electrolytes through the cellular membranes and decreased seed vigor (McDonald, 1998).

The seed samples were AP-3, C-99R, DP-1, and Hull from crop production year 2004 stored in UNICOOL, STACKS, WHSE, and WAGON. The data for leachate conductivity and seed vigor along with the means from April and October field emergence are presented in Table 4-13**.** Seed stored in STACKS had higher electrolyte conductivity than seed stored in

UNICOOL, WHSE or WAGON. The mean electrolyte conductivity by storage location was 1.39 for STACKS, 0.88 for WAGON, 0.63 for UNICOOL, and 0.59 for WHSE. In the subsequent seed vigor test, the comparative mean seed vigor for STACKS was 1.5; much lower than the CVI of 34.5 for UNICOOL, 47.0 for WHSE, and 27.8 for WAGON. In the April field emergence, the corresponding means for field emergence were 69.6% for STACKS, 91% for UNICOOL, 88.5% for WHSE, and 77.7% for WAGON. In the October field emergence, the corresponding means were 47.0% for STACK, 78.3% for UNICOOL, 48.5% for WHSE, and 44.5% for WAGON.

The Pearson Correlation Coefficient comparing electrolyte conductivity to comparative seed vigor was -0.7638 (P=0.0009), as shown in Table 4-14. Correlation of electrolyte conductivity and April field emergence was -0.8150 (P= 0.0002) and with October field emergence was -0.5022 (P=0.0564) (Table 4-14). Comparative vigor index (CVI) correlated with the April field emergence. During the intervening 5.5 months, vigor declined so rapidly that by October the minimal residual vigor only correlated marginally at alpha = 0.05 with the CVI test conducted in early April. Figure 4-16 presents a regression graph demonstrating the relationship between electrolyte conductivity and seed vigor. As the electrolyte conductivity of the leachate increases, seed vigor decreases as represented by the slower rate of hypocotyl/radicle elongation. Figure 4-17 shows the negative regression of April field emergence as electrolyte conductivity increases.

The electrolyte conductivity test and the subsequent vigor test confirm that, for seed stored in STACKS in 2004, seed quality deteriorated. Seed quality of DP-1 and Hull deteriorated more than the seed quality of AP-3 and C-99R. The significant correlations of electrolyte

conductivity, seed vigor test, and April field emergence confirm that both the electrolyte conductivity test and the seed vigor tests are reliable indicators of potential field emergence.

Extended Accelerated Ageing Tests

In the extended accelerated ageing test (EAA), the treatments of 5-week duration for seed from 2005 were: (1) the UNICOOL at 13^{o}C and 67% relative humidity, (2) the accelerated ageing chamber (EAA5wks) at 32^{o}C and 100% RH, and (3) the laboratory germination chamber (HT/LRH) at 32^{o}C and relative humidity < 10%. Seeds were evaluated for electrolyte conductivity, seedling vigor, towel germination, and field emergence at 8 DAP and 12 DAP. Seed moisture changed with seed treatment (Table 4-15). Seed moisture in UNICOOL remained constant. Seed moisture in the EAA5wks increased from an average of 5.6% to a final moisture average of 38.2%. In HT/LRH, moisture of seeds decreased from 5.6% to the final moisture average of 1.4%.

P-values for effect of cultivar on seedling field emergence at 8 DAP and 12 DAP were <0.0001 (Table 4-16). More seedlings of AP-3 and C-99R emerged at 8 DAP than in Florida-07, DP-1, York, and Hull (Table 4-17). Hull had the lowest percent emerged seedlings. AP-3, C-99R, Florida-07, and York at 12 DAP were similar in percent emerged seedlings, and exceeded the percent emerged seedling of DP-1 and Hull. Hull had the lowest percent emerged seedlings. The differences between seedlings emerged at 8 DAP and 12 DAP may indicate that AP-3 and C-99R have the potential for more rapid initial establishment than Florida-07, and York (Table 4-17).

Seedling field emergence at 8 DAP and 12 DAP of UNICOOL and HT/LRH treatments was superior to EAA5wks (P<0.0001) (Tables 4-16 and 4-17). The EAA5wks treatment of 32^{o}C and 100% relative humidity resulted in high average seed moisture of 38.2%, low electrolyte conductivit**y,** high vigor index, and high towel germination, but low seedling emergence in the

field (Table 4-17). The EAA test was designed as an attempt to duplicate possible post-harvest storage conditions in bulk bins. The temperature was 2–3^{o}C above the expected initial high storage temperatures. The increased temperature and 100% relative humidity increased moisture absorption by the radicle (Table 4-15). The increased moisture would have allowed cellular metabolic rates to increase sufficiently for the seed to begin phases I & II of germination and repair DNA, enzyme and membrane damage, causing the seed to be primed. In the seed vigor test, the EAA5wks treatment had a head start and the hypocotyl-radicle elongated faster than seeds from the other treatments. At the time of sowing, the EAA5wks treatments appeared fully imbibed with radicles protruding. The extent of priming varied by cultivar and within cultivar. The seed was fragile and field emergence of EAA5wks was reduced possibly by damage to the radicle during sowing, or possibly by direct ageing effects.

For interaction of treatment and cultivar, P-values at 8 DAP and 12 DAP were <0.0001 for field emergence. Field emergence of C-99R, Hull, and York from the EAA5wks treatment 12 DAP was low compared to the treatments of UNICOOL and HT/LRH (Figure 4-18). Seed priming was an unintended consequence in the EAA5wks treatment. Both the vigor test and the field emergence results are not representative here and should be discounted, because vigor index was inflated by priming effect of EAA5wks but was reduced by HT/LRH treatment, while field emergence was reduced by EAA5wks as expected and not reduced by warm, dry treatment (HT/LRH). Table 4-17 summarizes the mean values of the EAA tests sorted by both treatment and cultivar. As µmhos g^{-1} increased, vigor index, towel germination, and field emergence decreased. This pattern is uniform throughout the table, except for the field emergence of EAA5wks, which was confounded by the unintended priming of EAA5wks seed samples. The

pattern is in agreement with Table 4-8, which shows the strong negative correlation of electrolyte conductivity of leachate with seed vigor and field emergence.

The data from electrolyte conductivity tests, seed vigor tests, and field trials support the conclusions:

- The cultivars were affected differentially by temperature and relative humidity in the bulk storage bins (Figures 4-4, 4-16, and 4-17).
- Electrolyte conductivity is negatively correlated to seed vigor and field emergence (Table 4-8).
- Electrolyte conductivity tests and seed vigor tests correlate with field emergence and are reliable indicators of seed quality (Table 4-8).
- In the accelerated ageing tests, the elevated temperature and relative humidity primed the accelerated aged seed, resulting in good towel germination but in poor field emergence (Table 4-17).

Antioxidant Capacity Assay

A preliminary test was conducted to compare the seed antioxidant capacity of AP-3, C-99R, DP-1, and Hull. The seed sources were from the 2004 harvest, the 2005 harvest, the 2006 harvest, and seed from the 2004 production year after storage for 4 months in STACKS. Peanut seed from production years 2005 and 2006 after harvest were placed in storage at temperature < 0^{o}C. Peanuts from production year 2004 at harvest were stored in UNICOOL at 12–13^{o}C until January 31 and then stored at temperature < 0^{o}C. Seed from the STACKS of 2004 production seed year was placed in < 0^{o}C in April 2005 at the conclusion of the 4 month storage period.

The antioxidant capacity in peanut seed differed by cultivar (P=0.0016) and by date/environmental storage conditions (P=0.0003) (Table 4-18). Antioxidant capacity of Hull was superior to C-99R and DP-1; AP-3 was superior to DP-1; and for C-99R and DP-1 antioxidant was similar (Table 4-19). Antioxidant capacity at harvest for all years was greater than antioxidant capacity of seed stored in STACKS in 2004 for four months (Table 4-20).

Listed by year of sampling, the mean µequivalents/g peanut was 73.7 at harvest 2006, 63.6 at harvest 2005, and 58.4 at harvest 2004. After storage in STACKS in 2004, the mean µequivalents/g peanut was 43.5. The variation in antioxidant capacity by year may reflect different growing conditions during the crop year, or, for harvest 2004, the lower antioxidant capacity of seed may reflect loss of antioxidant capacity during the four month period preceding the freezing of the peanuts.

In 2004 antioxidant capacity of AP-3 was 68.4 at harvest and decreased to 32.9 during storage in the STACKS (Figure 4-19). For DP-1 and Hull, the antioxidant capacity decreased only minimally; DP-1 from 38.3 to 36.2 and Hull, a high oleic cultivar, from 76.8 to 71.9. The decrease in antioxidant capacity of AP-3 may indicate that antioxidants were used to protect the seed from peroxidation during storage; whereas, the poor field emergence of DP-1 and Hull may have resulted from low antioxidant activity, and thus, low protection from autoxidation by the antioxidants. The minimal antioxidant activity and the elevated temperatures of the stacks may have allowed the production of free radicals resulting in increased cellular membrane and enzyme damage, causing the loss of seed vigor which was evident in the comparative vigor, leachate conductivity, and field emergence tests of DP-1 and Hull.

This antioxidant data is preliminary. Subsequent assays of antioxidant capacity may support the observations that:

- Antioxidant capacity varies by cultivar and by year of production **(**Table 4-19**).**
- Antioxidant capacity of peanut seed may decrease during storage in bulk bins which are similar to STACKS (Figure 4-19).
- Antioxidant capacity is an important factor for preserving seed vigor and cultivar antioxidant capacity should be evaluated in peanut breeding programs.

Table 4-1. ANOVA for towel germination and April field emergence of peanut seed as affected by year (Y), cultivar (C), origin of seed (O), and storage environment/location (L) for crop production years 2004 and 2005

		P-values					
		2004		2005		2004-05	
Source	df	Towel Germination	April Field Emergence	Towel Germination	April Field Emergence	Towel Germination	April Field Emergence
Year (Y)	1					<.0001	0.0082
Rep	3	0.3340	0.0370	0.8065	0.0005	0.8931	0.0013
Cultivar (C)	3	<.0001	<.0001	0.0442	0.0043	0.0239	<.0001
Origin (O)	1	0.0321	0.9104				
Location (L)	3	0.0493	0.0208	0.0498	0.0957	0.0847	0.0063
Y*C	3					0.2213	0.1370
Y*L	3					0.0002	0.4231
C*L	9	0.0021	0.1355	0.6526	0.0814	0.0188	0.0711
C*O	3	<.0001	<.0001				
O*L	3	0.1792	0.2222				
C*O*L	9	0.0601	0.1917				

Table 4-2. Means of towel germination and field emergence tests of peanut seed from crop production years 2004 and 2005

Crop Year	Germination (%)	Field Emergence (%)	Number of Samples
2004	93.6	88.3	128
2005	87.1	85.1	64

Table 4-3. Means of cultivar and seed origin in towel germination tests and April field emergence test of peanut seed produced in crop year 2004

Cultivar	Seed Origin	Towel Germination Means	Standard Deviation	April Field Emergence Means	Standard Deviation
AP-3	FFSP	94.8	4.7	91.3	4.9
AP-3	NFREC	99.0	1.8	93.4	6.4
C-99R	FFSP	95.3	3.6	91.1	9.5
C-99R	NFREC	88.4	6.3	88.8	6.5
DP-1	FFSP	93.1	5.5	88.9	6.4
DP-1	NFREC	85.8	5.8	78.6	5.4
Hull	FFSP	94.3	4.5	81.9	9.0
Hull	NFREC	98.1	1.5	92.9	5.0
Mean	FFSP	94.4		88.3	
Mean	NFREC	92.8		88.4	

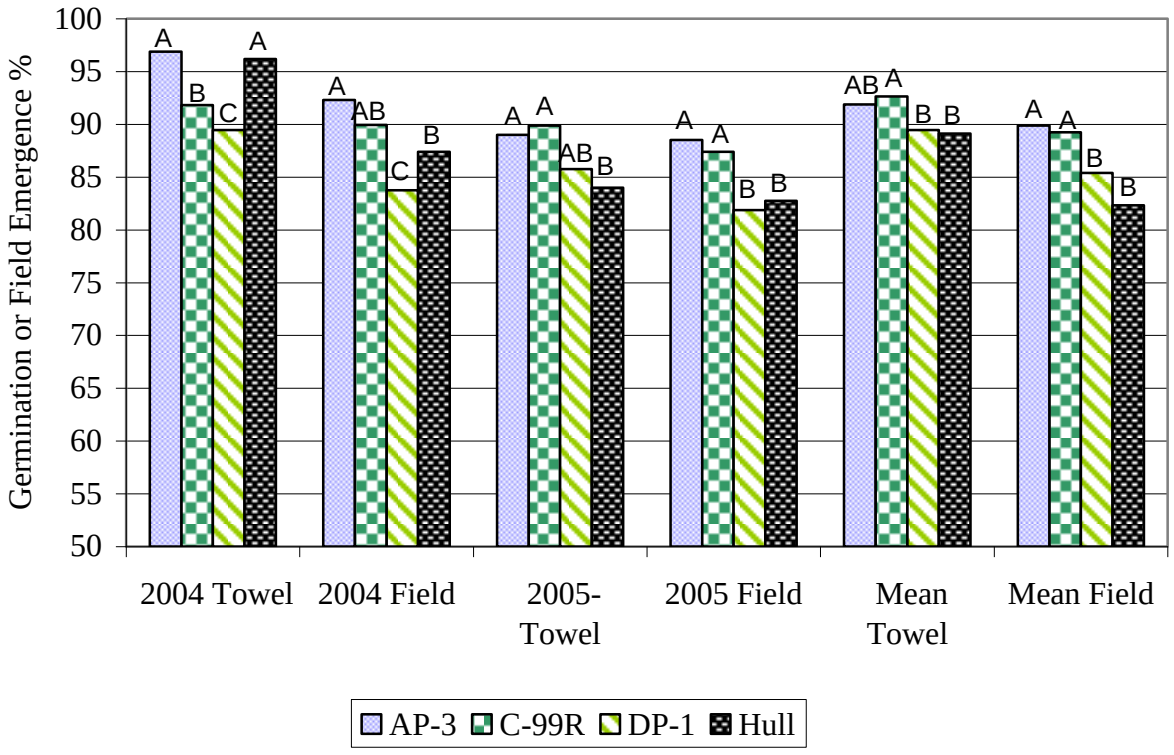

Figure 4-1. Effect of cultivar on moist towel germination and field emergence at the end of winter storage of seed peanut from crop production years 2004 and 2005. Within a grouping, means with the same letter are not significantly different.

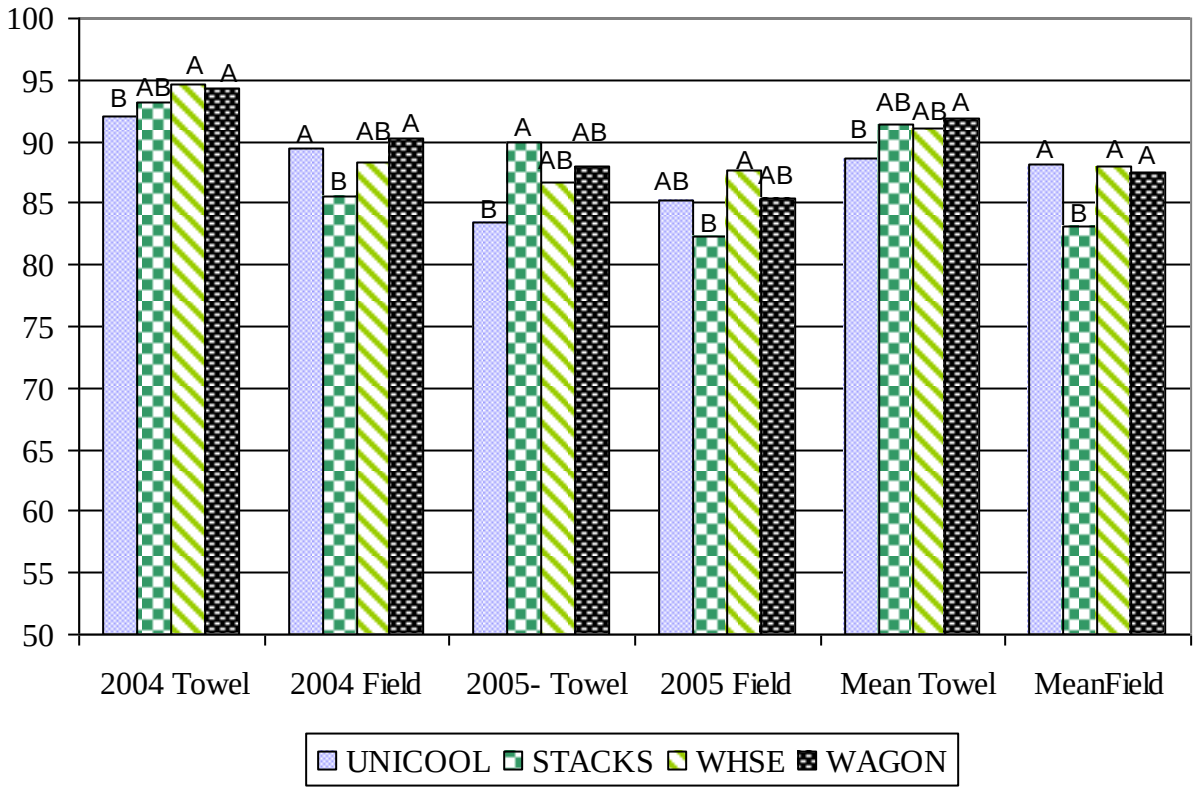

Figure 4-2. Effect of environment/storage location on moist towel germination and field emergence of seed peanut from crop production years 2004 and 2005. Within a grouping, means with the same letter are not significantly different.

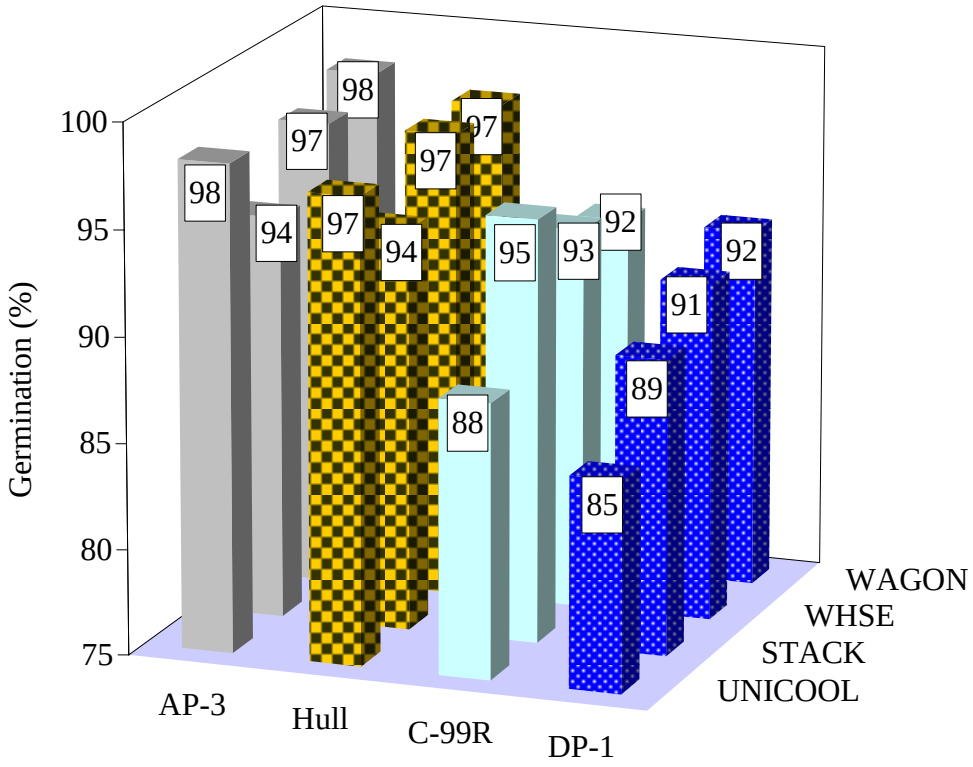

Figure 4-3. Moist towel germination of bagged seed produced in crop year 2004 as affected by cultivar and storage environment/location.

Table 4-4. ANOVA P-values for towel germination and field emergence of bulk stored peanut as affected by cultivar and storage location for crop production year 2004.

Source	df	Towel Germination	April Field Emergence	Oct Field Emergence
Rep	3	0.0915	0.4016	0.0078
Cultivar (C)	3	0.0002	<0.0001	<0.0001
Location (L)	2	0.0309	<0.0001	<0.0001
C*L	6	0.5447	<0.0001	<0.0001

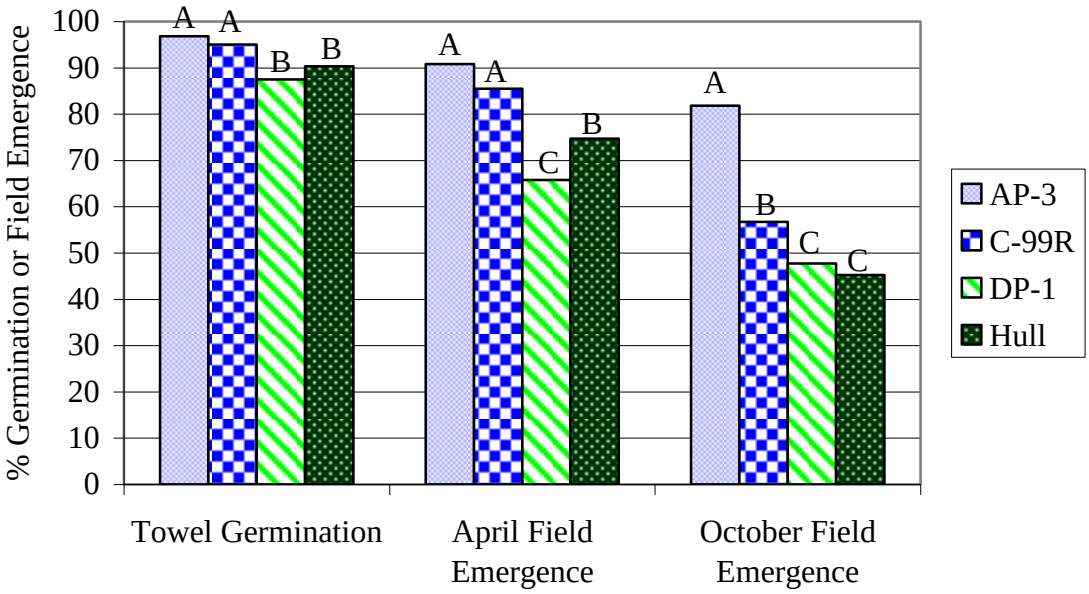

Figure 4-4. Effect of cultivars on moist towel germination and field emergence of bulk stored peanut produced in crop year 2004. Within a grouping, means with the same letter are not significantly different.

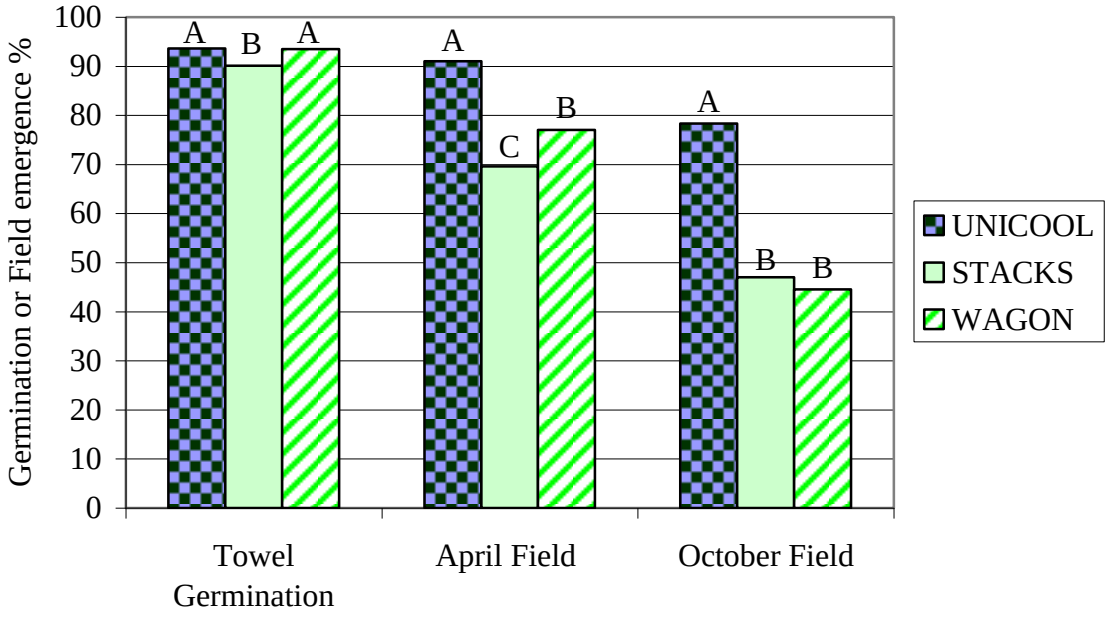

Figure 4-5. Effect of storage environment/location on moist towel germination and field emergence of bulk stored peanut produced in crop year 2004. Seed from UNICOOL is a second sample from bagged seed and not truly bulk seed. Within a grouping, means with the same letter are not significantly different.

Table 4-5. Effect of cultivar and storage location on April field emergence of bagged seed versus bulk stored seed of peanut for crop production year 2004. Seed from UNICOOL is a second sample from bagged seed and not truly bulk seed.

Cultivar	Location	Bagged Seed (%)	Bulk Stored Seed (%)	Difference (%)
AP-3	UNICOOL	92.0	94.0	2.0
AP-3	STACKS	90.3	81.5	8.8
AP-3	WAGON	94.5	97.0	2.5
C-99R	UNICOOL	93.3	85.5	2.2
C-99R	STACKS	85.5	76.0	9.5
C-99R	WAGON	92.3	85.0	7.3
DP-1	UNICOOL	85.5	94.5	9.0
DP-1	STACKS	84.0	46.5	37.5
DP-1	WAGON	85.5	56.5	29.0
Hull	UNICOOL	86.8	80.0	6.8
Hull	STACKS	82.0	74.5	7.5
Hull	WAGON	88.5	69.5	19.0

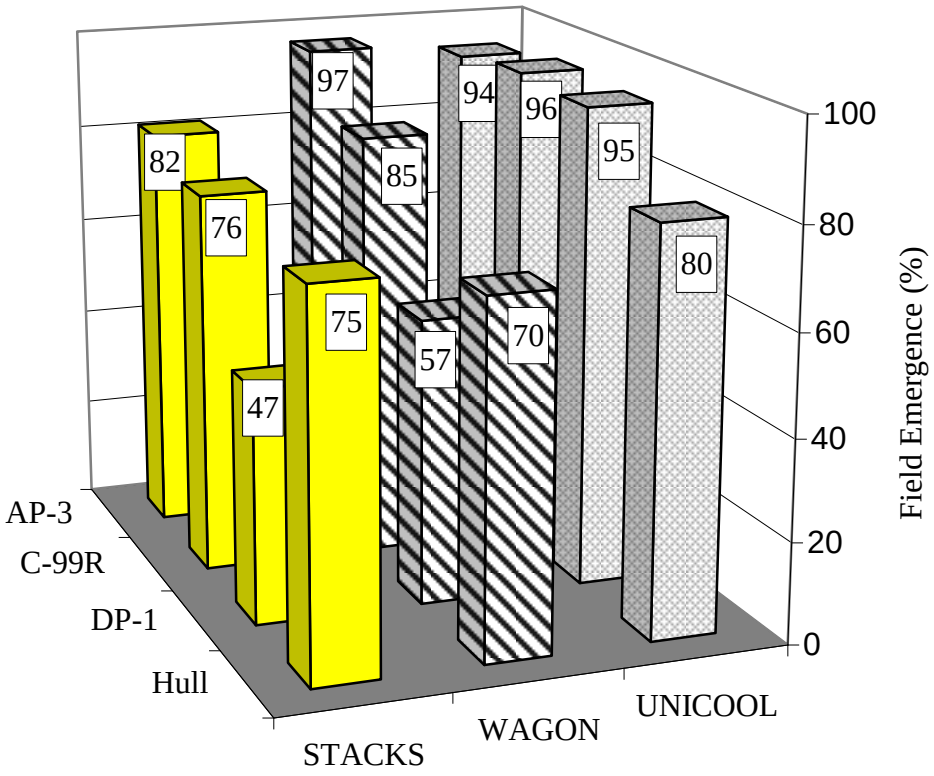

Figure 4-6. Field emergence in April 2005 of bulk stored peanut as affected by cultivar and storage environment/location of seed peanut produced in crop year 2004. Seed from UNICOOL is a second sample from bagged seed and not truly bulk seed.

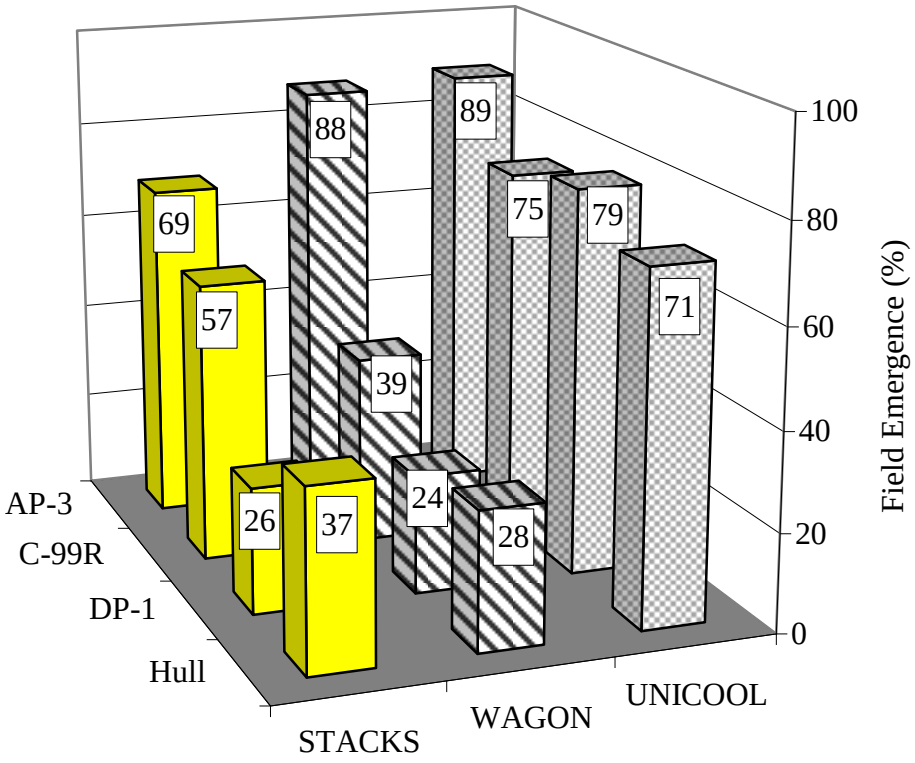

Figure 4-7. Field emergence in October 2005 of bulk stored peanut as affected by cultivar and storage environment/location of seed peanut produced in crop year 2004. Seed from UNICOOL is a second sample from bagged seed and not truly bulk seed.

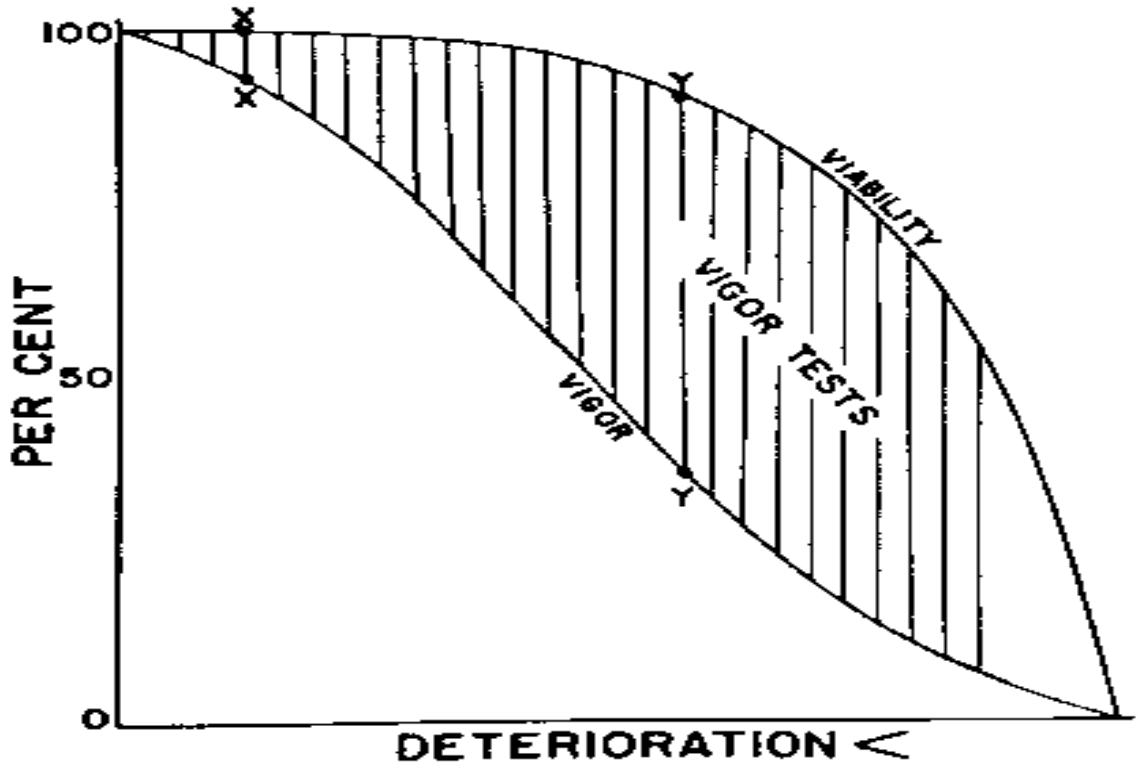

Figure 4-8. Representation of differences in rate of deterioration of seed viability and vigor showing decrease from 100% to 0% over time from J.C. Delouche and W.P. Caldwell, (1960) Seed vigor and vigor tests, Proceedings of the AOSA 50(1):136.

Table 4-6. Comparison of April field emergence to October field emergence of bulk stored peanut as affected by cultivar and storage location for crop production year 2004. Seed from UNICOOL is a second sample from bagged seed and not truly bulk seed.

Cultivar	Location	April Field Emergence(%)	October Field Emergence(%)	Difference (%)
AP-3	UNICOOL	94.0	89.0	-5.0
AP-3	STACKS	81.5	69.0	-12.5
AP-3	WAGON	97.0	87.5	-9.5
C-99R	UNICOOL	95.5	74.5	-21.0
C-99R	STACKS	76.0	57.0	-19.0
C-99R	WAGON	85.0	38.5	-46.5
DP-1	UNICOOL	94.5	78.5	-16.0
DP-1	STACKS	46.5	25.5	-21.0
DP-1	WAGON	56.5	24.0	-32.5
Hull	UNICOOL	80.0	71.0	-9.0
Hull	STACKS	74.5	36.5	-38.0
Hull	WAGON	69.5	28.0	-41.5

Table 4-7. Comparison of towel germination to field emergence of bulk stored peanut as affected by cultivar and storage location for crop production year 2004. Seed from UNICOOL is a second sample from bagged seed and not truly bulk seed.

Cultivar	Location	Towel Germination (%)	Seed Lab Germination Test (%)	April Field Emergence (%)	Towel Germination Minus Field Emergence (%)
AP-3	UNICOOL	97.5		94.0	-3.5
AP-3	STACKS	96.5	91.0	81.5	-15.0
AP-3	WAGON	96.5		97.0	0.5
C-99R	UNICOOL	94.5		95.5	1.0
C-99R	STACKS	92.5	88.0	76.0	-16.5
C-99R	WAGON	98.0		85.0	-13.0
DP-1	UNICOOL	87.5		94.5	-7.0
DP-1	STACKS	85.0	86.0	46.5	-38.5
DP-1	WAGON	90.0		56.5	-33.5
Hull	UNICOOL	95.0		80.0	-15.0
Hull	STACKS	86.5	89.0	74.5	-12.0
Hull	WAGON	89.5		69.5	-20.0

Table 4-8. Correlation of towel germination, field emergence, comparative vigor index, and leachate conductivity of peanut as affected by cultivar and storage location for production years 2004 and 2005.

Pearson Correlation Coefficients
Prob > |r| under H0: Rho=0
Number of Observations

	Towel Germination Test	April Field Emergence	October Field Emergence	Vigor Test #1	Leachate Conductivity Test
April Field Emergence	0.0532 0.6763 64				
October Field Emergence	-0.13819 0.4507 32	0.25924 0.1519 32			
Vigor Index Test #1	-0.28554 0.0491 48	0.23467 0.1084 48	0.60126 0.0003 32		
Leachate Conductivity Test	0.12429 0.4 48	-0.22936 0.1168 48	-0.78774 <.0001 32	-0.77338 <.0001 48	
Vigor Index Test #2	-0.00246 0.9928 16	0.69661 0.0027 16	0.56697 0.0220 16	0.55237 0.0265 16	-0.68839 0.0032 16

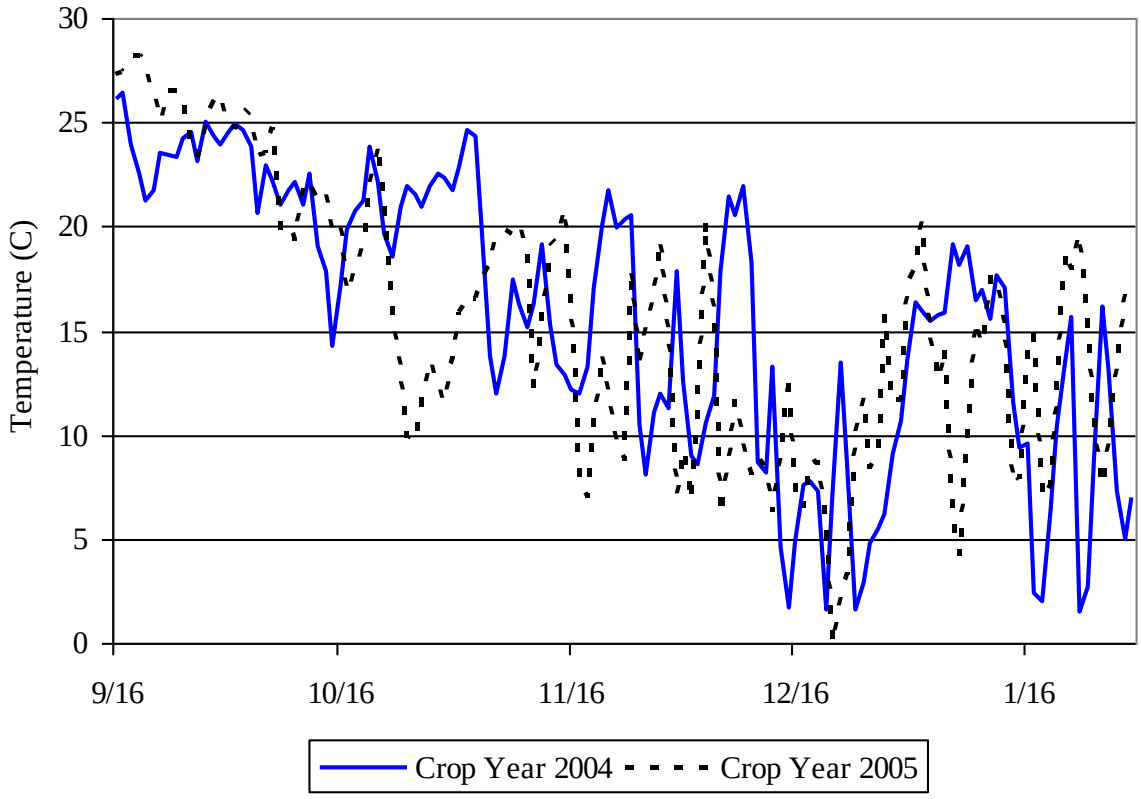

Figure 4-9. Mean daily air temperature at 2 meters above ground level as measured at the Florida Automated Weather Network (FAWN) substation, Marianna, Florida, September 16 to January 24 for crop years 2004 and 2005.

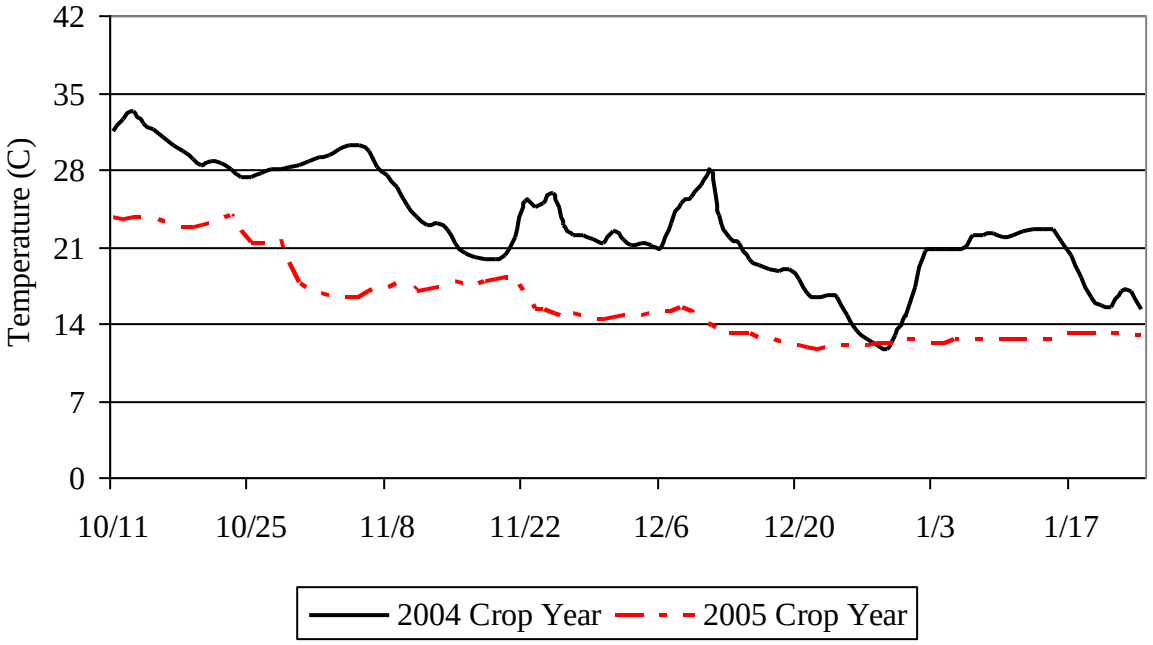

Figure 4-10. Mean daily temperature within the bulk pile of peanut cultivar AP-3 stored in a traditional storage bin at the Florida Foundation Seed Producers (FFSP) for October 15 to January 24 for crop years 2004 and 2005.

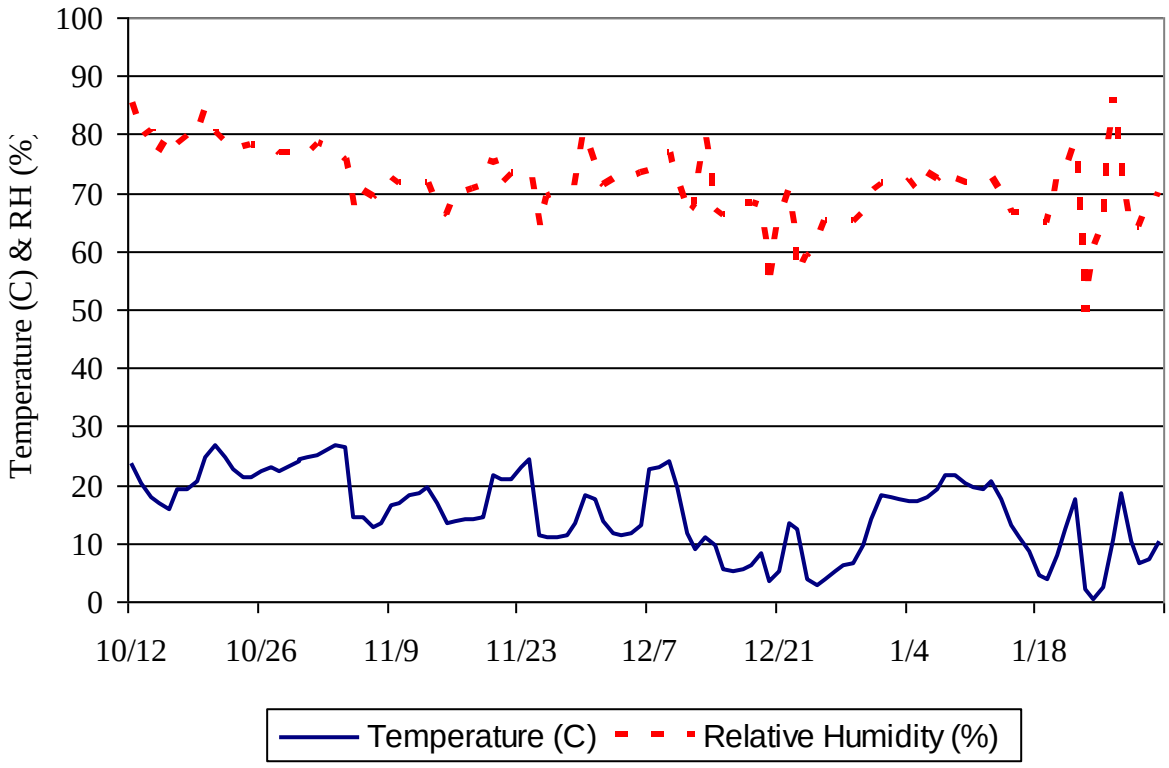

Figure 4-11. Mean daily temperature and relative humidity within the bulk pile of peanut cultivar DP-1 stored in a peanut wagon at the Florida Foundation Seed Producers (FFSP) for the period October 15, 2004 to January 31, 2005.

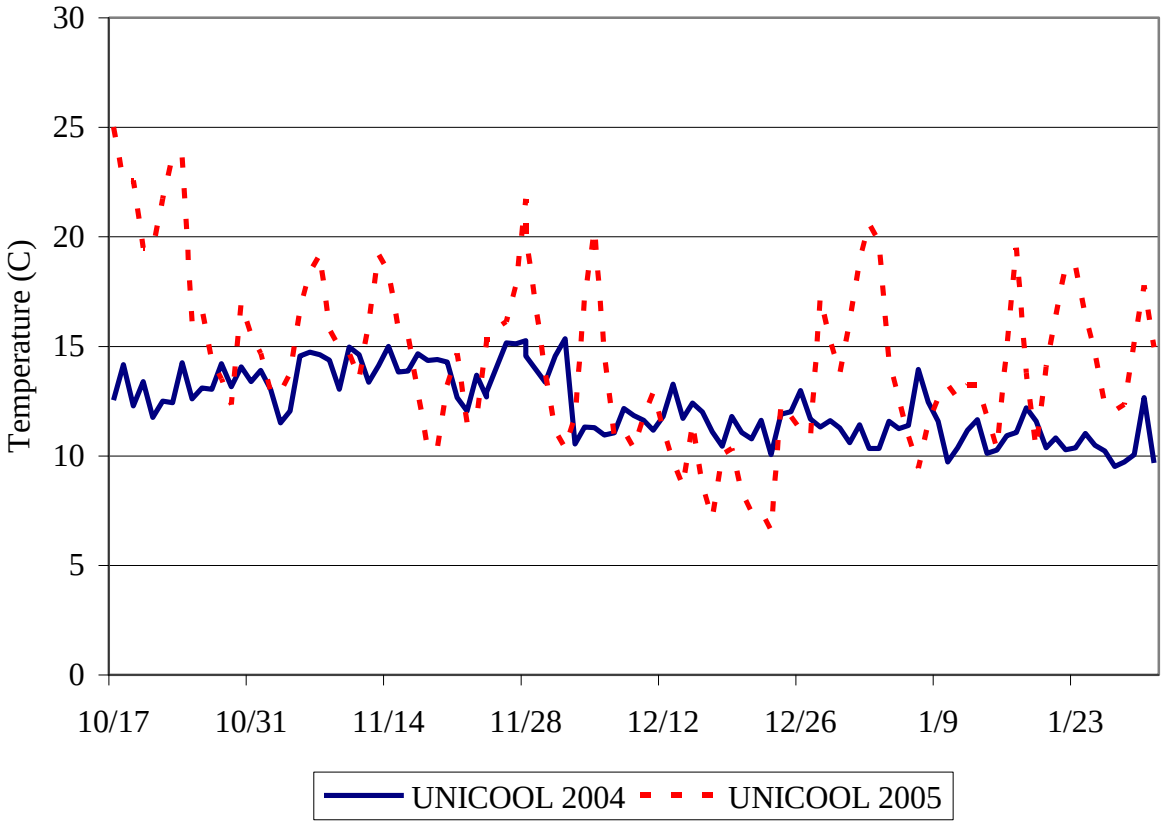

Figure 4-12. Mean temperature of the seed storage room located at the University of Florida Research and Education Center (NFREC), Marianna, Florida for the periods October 15 to January 31, 2004 and 2005.

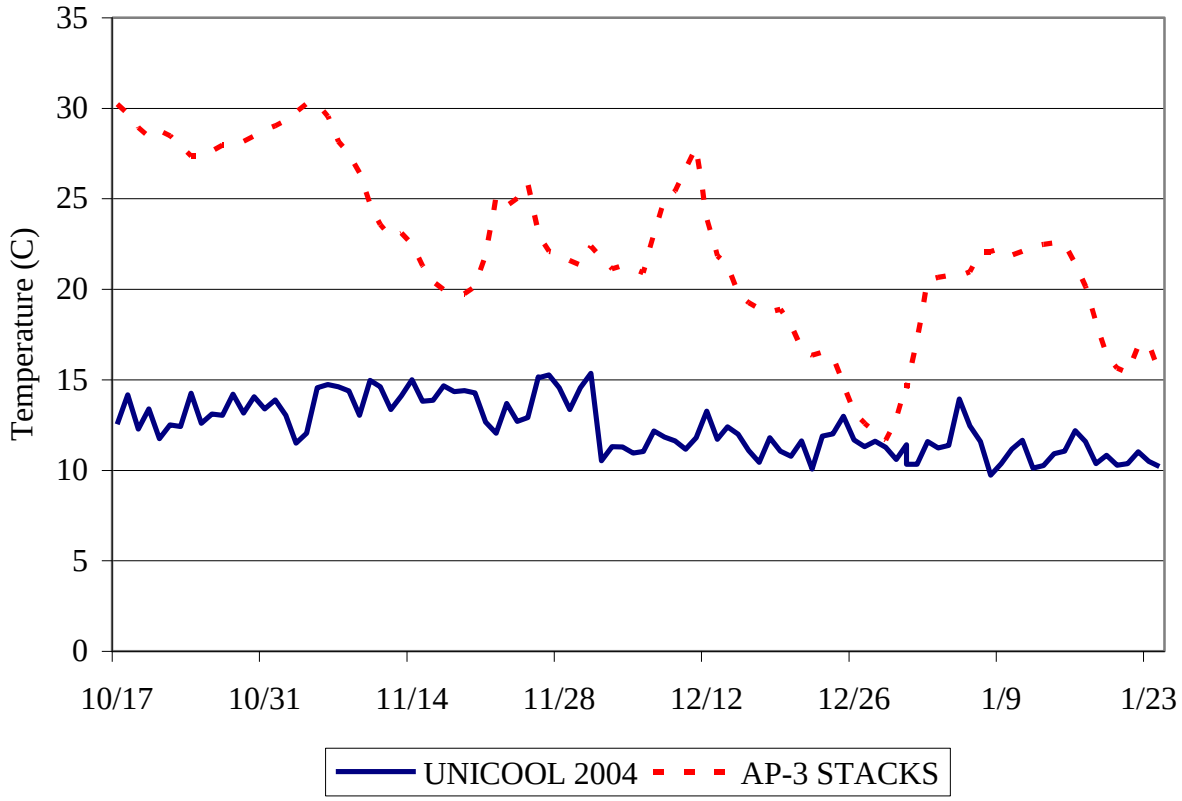

Figure 4-13. Comparison of mean daily temperature in the seed storage room located at the University of Florida Research and Education Center (NFREC) and the mean daily temperature within the bulk pile of peanut cultivar AP-3 stored in a traditional storage bin at the Florida Foundation Seed Producers (FFSP) for the period October 17, 2004 to January 24, 2005.

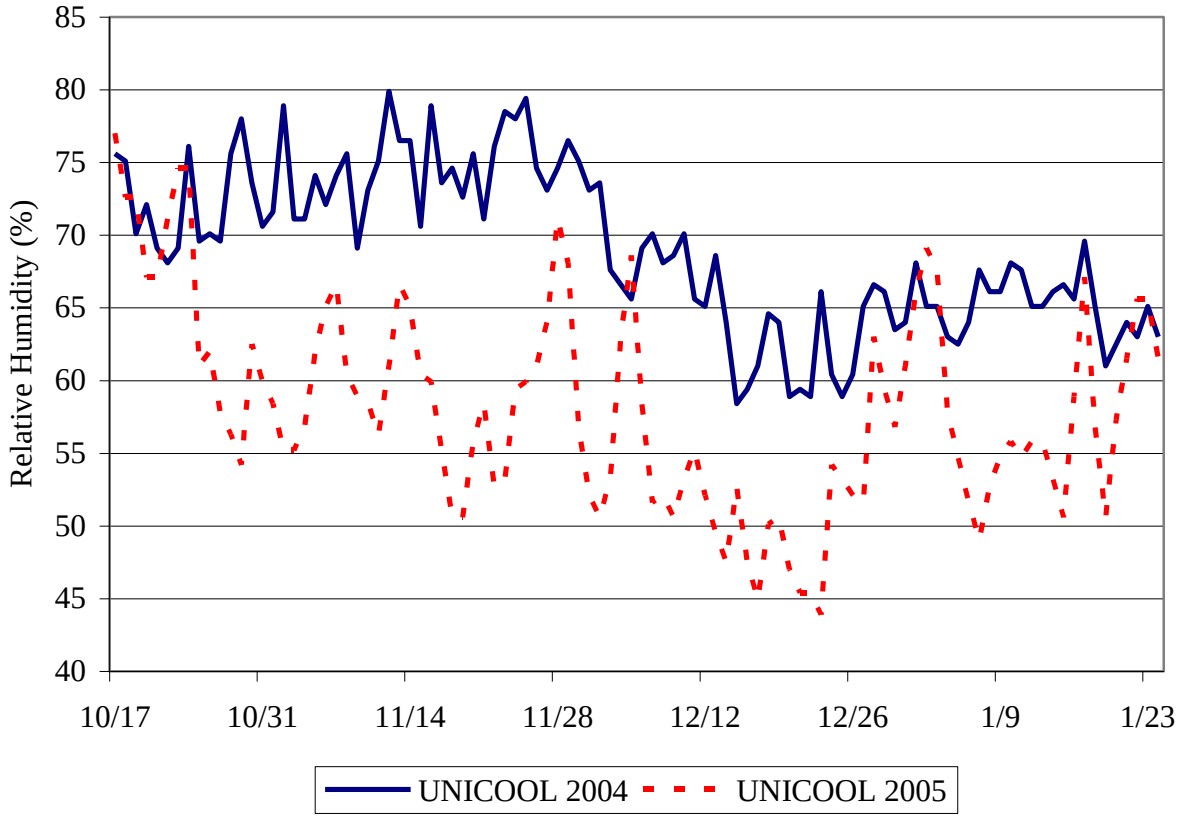

Figure 4-14. Comparison of relative humidity (RH) in the seed storage room at the University of Florida Research and Education Center (NFREC) for October 15 to January 31 for crop years 2004 and 2005.

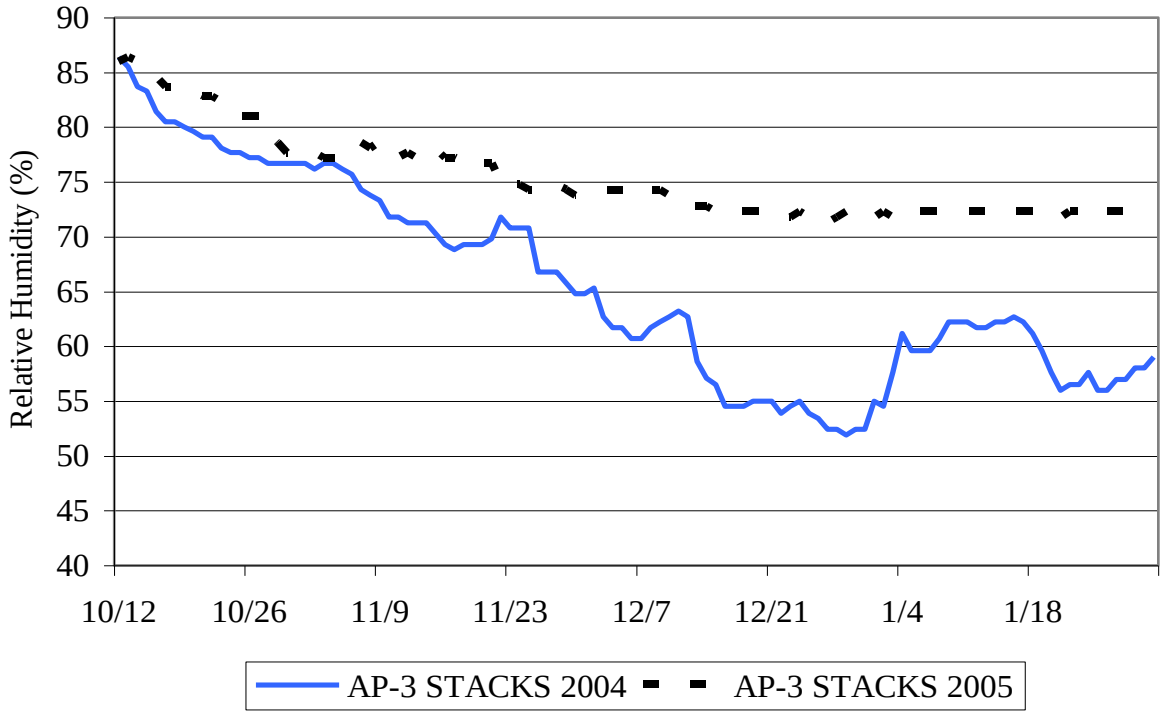

Figure 4-15. Comparison of relative humidity (RH) within the bulk pile of peanut cultivar AP-3 stored in a traditional storage bin at the Florida Foundation Seed Producers (FFSP) for October 15 to January 31 for crop years 2004 and 2005.

Table 4-9. Incidence of fungi per 20 seeds per storage location in bulk stored peanut for crop production in 2004 and 2005.

Year	Cultivar	Treatment	Penicillium	A. flavus	A. niger	Rhizopus
2004	AP-3	Harvest	1	.	.	.
2004	C-99R	Harvest	.	.	.	.
2004	DP-1	Harvest	.	.	.	.
2004	Hull	Harvest	1	.	.	.
2004	AP-3	Unicool	.	.	.	.
2004	C-99R	Unicool	1	.	.	.
2004	DP-1	Unicool	.	.	.	.
2004	Hull	Unicool	3	.	.	.
2004	AP-3	Stacks	.	.	3	.
2004	C-99R	Stacks	2	.	2	.
2004	DP-1	Stacks	.	.	.	.
2004	Hull	Stacks	1	.	1	.
2005	AP-3	Harvest	2	.	3	.
2005	C-99R	Harvest	.	.	1	.
2005	DP-1	Harvest	.	.	.	.
2005	Hull	Harvest	1	.	.	2
2005	AP-3	Unicool	.	.	.	1
2005	C-99R	Unicool	.	1	1	2
2005	DP-1	Unicool	.	.	.	.
2005	Hull	Unicool	6	.	.	.
2005	AP-3	Stacks	2	.	.	1
2005	C-99R	Stacks	1	1	.	1
2005	DP-1	Stacks	2	.	.	.
2005	Hull	Stacks	5	.	1	.
Total in 480 seeds			28	2	12	7
Percent seeds with pathogen present			5.8	0.4	2.5	1.5

Table 4-10. ANOVA for vigor tests #1 and #2 of peanut germination as affected by cultivar and storage environment/location for crop production in 2005.

	Vigor Test #1		Vigor Test #2	
Source	df	P-value	df	P-value
Rep	1	0.9453	3	<.0001
Cultivar (C)	7	0.0058	6	0.0083
Location (L)	3	0.0003	2	0.5031
C*L	21	0.3248	12	0.2930

Table 4-11. Effect of cultivar, storage environment/location and accelerated ageing (AA) on seedling vigor (Vigor Test #1) for peanut seed from 2005 crop production year. Within a grouping, means with the same letter are not significantly different.

Cultivar	Means	Duncan Grouping	N
AP-3	58.3	A	8
C-99R	56.6	A	8
AP-3_AA	55.1	A	8
C-99R_AA	55.1	A	8
DP-1_AA	54.5	A	8
Hull_AA	54.0	A	8
Hull	50.1	AB	8
DP-1	42.8	B	8

Location	Means	Duncan Grouping	N
WAGON	56.9	A	16
STACKS	56.6	A	16
WHSE	54.1	A	16
UNICOOL	45.7	B	16
Test Means	53.3		64
Control_no AA	39.5		32
AA Means	54.7		32

Table 4-12. Effect of cultivar and storage environment/location on seedling vigor (Vigor Test #2) for peanut seed from 2005 crop production year. Within a grouping, means with the same letter are not significantly different.

Cultivar	Means	Duncan Grouping	N
York	61.0	A	12
Florida-07	59.9	AB	12
AP-3	59.8	AB	12
C-99R	59.7	AB	12
DP-1	55.3	BC	12
McCloud	54.4	C	12
Hull	54.3	C	12

Location	Means	Duncan Grouping	N
STACKS	58.6	A	28
WHSE	57.9	A	28
UNICOOL	56.8	A	28

Table 4-13. Electrolyte conductivity of leachate, comparative vigor index (CVI), April field emergence, and October field emergence as affected by cultivar and seed storage environment/location of seed peanuts produced in crop year 2004.

Location	Cultivar	µmhos g-1	CVI	April Field Emergence	October Field Emergence
UNICOOL	AP-3	0.607	25.0	94.0	89.0
UNICOOL	C-99R	0.663	41.0	95.5	74.5
UNICOOL	DP-1	0.458	68.0	94.5	78.5
UNICOOL	Hull	0.791	4.0	80.0	71.0
	Means	0.630	34.5	91.0	78.3
STACKS	AP-3	1.159	5.0	81.5	69.0
STACKS	C-99R	1.218	0.0	76.0	57.0
STACKS	DP-1	1.531	1.0	46.5	25.5
STACKS	Hull	1.638	0.0	74.5	36.5
	Means	1.390	1.5	69.6	47.0
WHSE	AP-3	0.546	40.0	88.5	71.0
WHSE	C-99R	0.522	73.0	90.5	62.0
WHSE	DP-1	0.554	68.0	86.0	24.0
WHSE	Hull	0.733	7.0	89.0	37.0
	Means	0.590	47.0	88.5	48.5
WAGON	AP-3	0.520	72.0	97.3	87.5
WAGON	C-99R	0.614	35.0	86.7	38.5
WAGON	DP-1	1.159	4.0	60.0	24.0
WAGON	Hull	1.261	0.0	66.7	28.0
	Means	0.880	27.8	77.7	44.5

Table 4-14. Pearson correlation of electrolyte conductivity of leachate, comparative vigor index (CVI), April field emergence, and October field emergence as affected by cultivar and seed storage environment/location of seed peanuts produced in crop year 2004.

	Pearson Correlation Coefficients, N=15 Prob > |r| under H0: Rho=0		
	Conductivity (µmhos g-1)	CVI	April Field Emergence
Comparative Vigor	-0.76378		
Index (CVI)	0.0009		
April Field	-0.81504	0.63036	
Emergence	0.0002	0.0118	
October Field	-0.5022	0.32572	0.67772
Emergence	0.0564	0.2361	0.0055

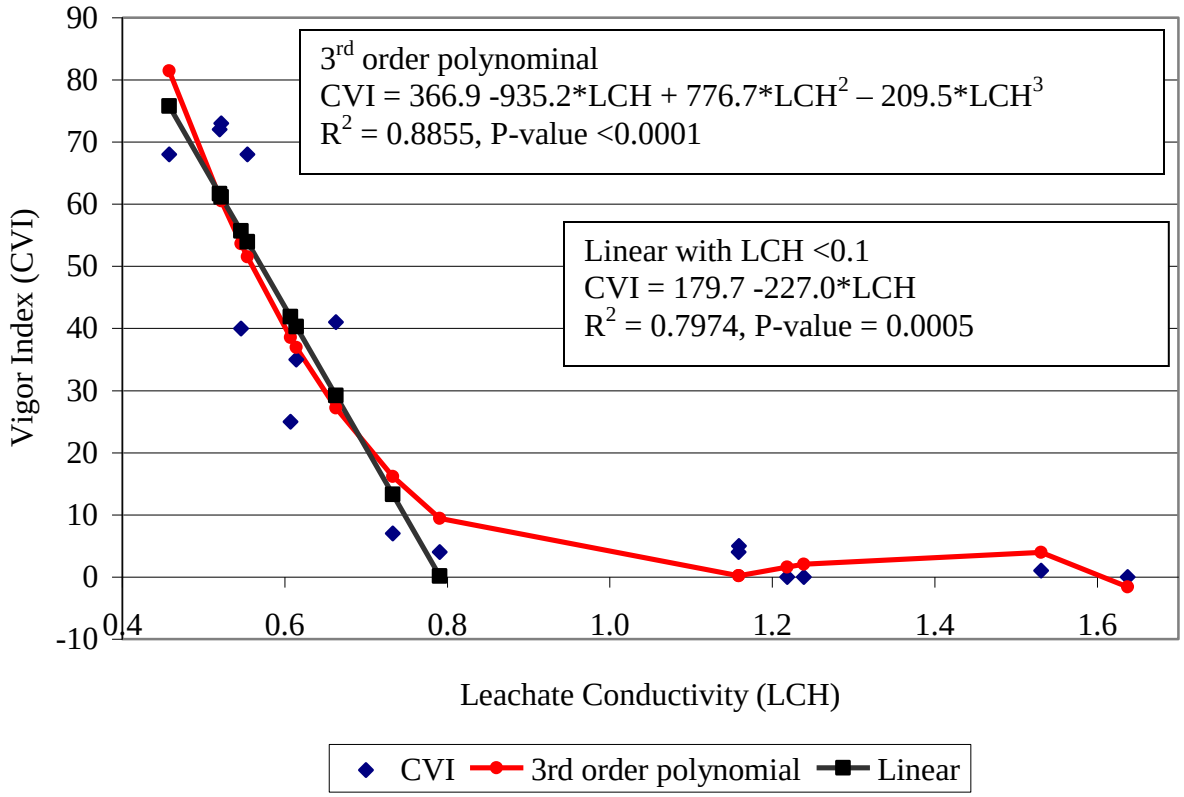

Figure 4-16. Comparative vigor index (CVI) versus electrolyte conductivity of leachate (LCH) from germinating peanut seed over all cultivars and storage environments for seed from crop production year 2004. Seed with a LCH > 0.1 were considered to be non-viable.

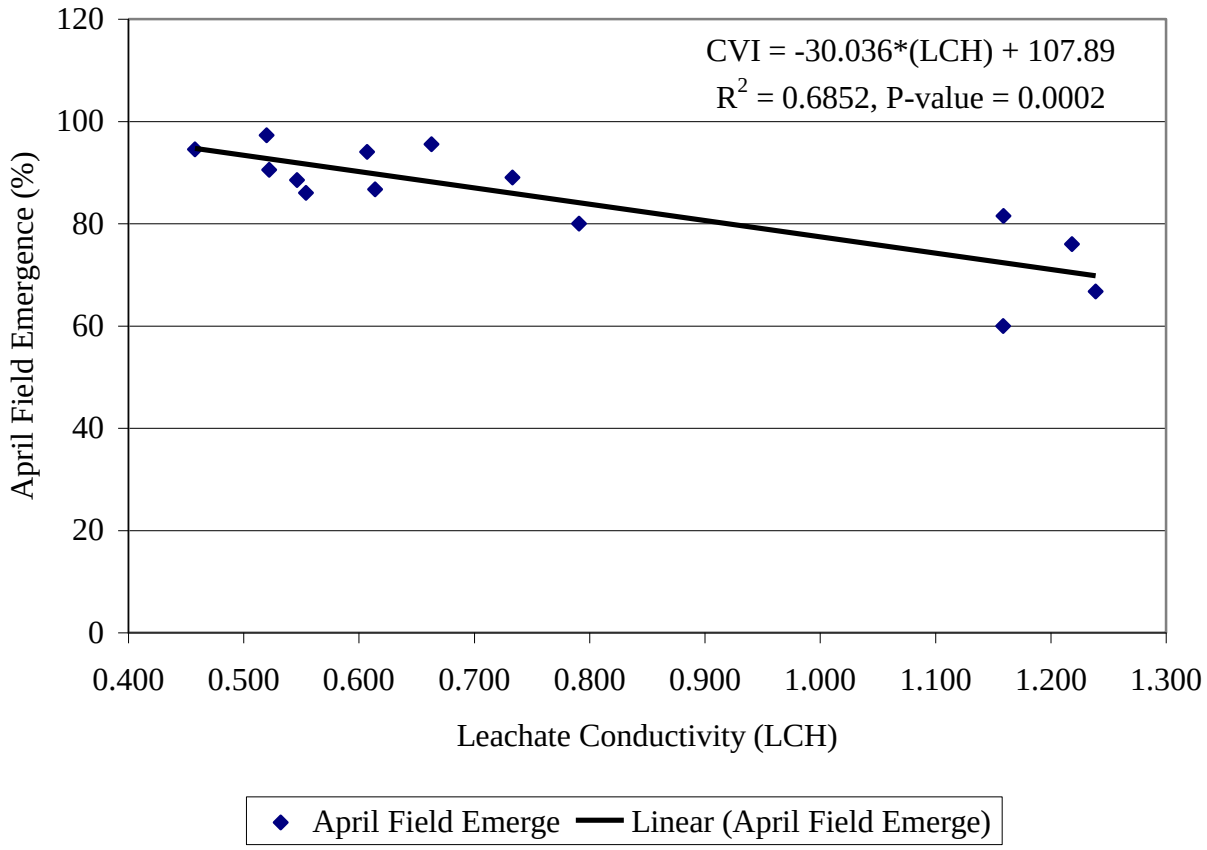

Figure 4-17. April field emergence versus electrolyte conductivity of leachate (LCH) from germinating peanut seed over all cultivars and storage environments for seed from crop production year 2004.

Table 4-15. Changes in seed moisture in 5-week accelerated ageing test of peanut cultivars stored in 3 environments: 1) 13°C and 67% relative humidity (UNICOOL), 2) 32°C and 100% relative humidity (EAA5wk), and 3) 32°C and <10% relative humidity (HT/LRH). Seed source was crop year 2005 seed that was stored in WHSE location.

Treatment	Cultivar	Initial Seed Weight (g)	Five Week Weight (g)	Change in Weight (g)	Ave. Initial Moisture (%)	Moisture Range (%)	Ave. Final Moisture (%)	Change in Moisture (%)
UNICOOL	AP-3	30.39	30.41	0.02	5.5	4.6-6.5	5.9	0.4
UNICOOL	C-99R	31.23	31.29	0.06	5.2	4.0-6.2	5.7	0.5
UNICOOL	DP-1	35.04	35.07	0.02	5.8	4.9-6.5	6.2	0.4
UNICOOL	Hull	34.89	34.88	-0.01	6.1	4.6-7.1	6.5	0.4
UNICOOL	FL-07	36.59	37.78	1.19	5.3	4.3-6.3	9.0	3.7
UNICOOL	York	30.23	29.98	-0.25	5.2	4.1-6.0	4.6	-0.6
Average		33.06	33.23	0.17	5.5		6.3	0.8
EAA	AP-3	32.28	43.54	11.26	5.5	4.6-6.5	42.7	37.2
EAA	C-99R	extensive fungal growth						
EAA	DP-1	35.61	45.05	9.44	5.8	4.9-6.5	34.3	28.5
EAA	Hull	34.10	43.81	9.71	6.1	4.6-7.1	36.8	30.7
EAA	FL-07	34.42	44.66	10.24	5.3	4.3-6.3	37.0	31.7
EAA	York	29.04	38.58	9.54	5.2	4.1-6.0	40.1	34.9
Average		33.09	43.13	10.04	5.6		38.2	32.6
HT/LRH	AP-3	32.42	30.99	-1.43	5.5	4.6-6.5	1.2	-4.3
HT/LRH	C-99R	32.84	31.44	-1.41	5.2	4.0-6.2	1.0	-4.2
HT/LRH	DP-1	34.43	33.00	-1.43	5.8	4.9-6.5	1.8	-4.0
HT/LRH	Hull	38.66	36.95	-1.71	6.1	4.6-7.1	1.8	-4.3
HT/LRH	FL-07	38.15	36.26	-1.89	5.3	4.3-6.3	0.4	-4.9
HT/LRH	York	29.14	28.21	-0.93	5.2	4.1-6.0	2.1	-3.1
Average		34.27	32.81	-1.47	5.5		1.4	-4.2

Table 4-16. ANOVA for field emergence of peanut seed as affected by 3 treatments: 1) 13°C and 67% relative humidity, 2) 32°C/100% relative humidity, and 3) 32°C <10% relative humidity in 5-week accelerated ageing test Seed source was crop year 2005 seed that was stored in WHSE location.

Days after Planting		8 Days	12 Days
Source	df	Pr > F	Pr > F
Rep	3	<.0001	0.6505
Treatment (T)	2	<.0001	<.0001
Cultivar (C)	5	<.0001	<.0001
T*C	10	<.0001	<.0001

Table 4-17. Electrolyte conductivity of germinating peanut leachate, comparative vigor, towel germination, and field emergence at 8 and 12 days after planting (DAP) as affected by cultivar and by three simulated storage treatments for 5 weeks: 1) 13°C and 67% relative humidity (UNICOOL), 2) 32°C and 100% relative humidity (EAA5wk), and 3) 32°C and <10% relative humidity (HT/LRH). Within a grouping means with the same letter are not significantly different.

Treatment	umhos g-1	Vigor Index	Towel Germination (%)	8 DAP Field Emergence (%)		12 DAP Field Emergence (%)	
EAA5wks	0.278	53.2	97.5	43.8	B	66.2	B
UNICOOL	0.393	49.5	89.2	68.2	A	84.3	A
HT/LRH	0.658	25.0	61.7	63.0	A	82.0	A
Cultivar							
FL-07	0.240	50.0	95.0	57.2	B	82.3	A
C-99R	0.332	45.1	Error	67.4	A	79.2	A
York	0.356	45.0	83.3	50.2	BC	82.8	A
AP-3	0.463	44.3	81.7	72.8	A	85.2	A
DP-1	0.573	40.0	75.0	56.4	B	72.5	B
Hull	0.769	23.0	56.7	46.4	C	63.3	C

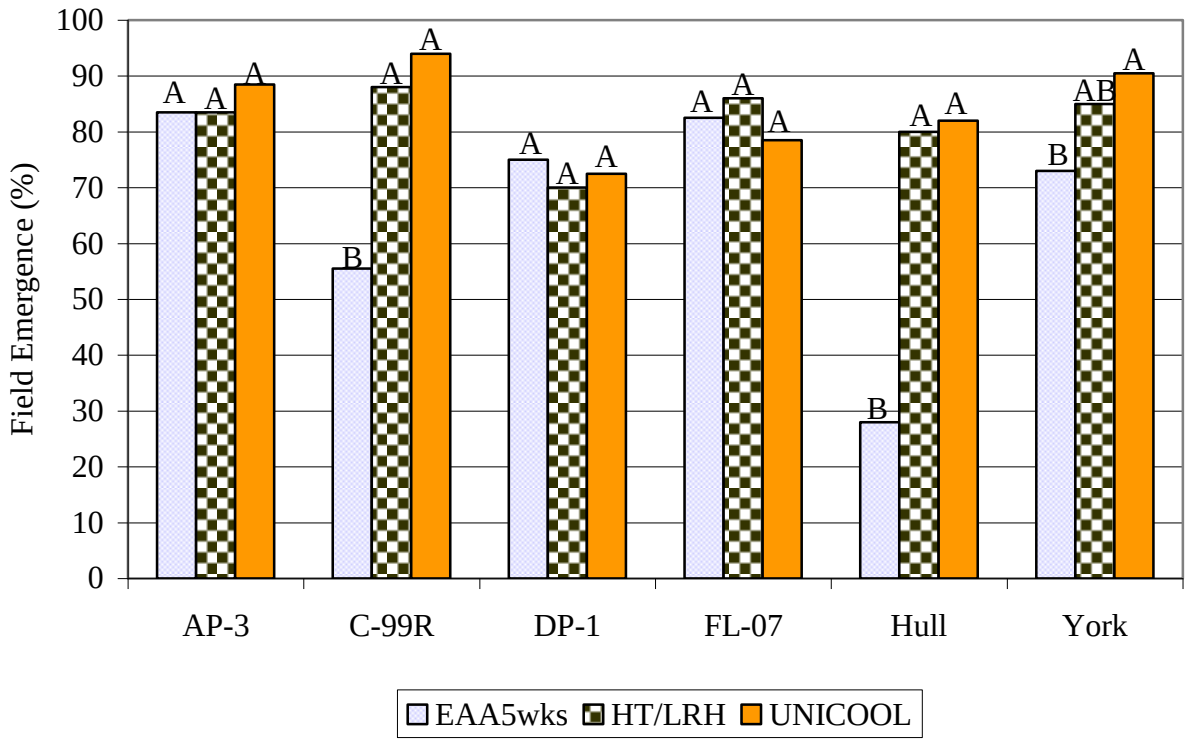

Figure 4-18. Field emergence at 12 DAP of peanut cultivars from crop production year 2005 as affected by treatment in an extended accelerated ageing test (EAA). The treatments of five week duration were: 1) seed stored in a University of Florida seed storage room (UNICOOL) at 13^{o}C and 67% relative humidity, 2) seed placed in an accelerated ageing chamber (EAA5wks) at 32^{o}C and 100% relative humidity, and 3) seed placed in a University of Florida germination chamber (HT/LRH) at 32^{o}C and relative humidity < 10%. Within a grouping, means with the same letter are not significantly different.

Table 4-18. ANOVA for antioxidant capacity of peanut seed as affected by cultivar and date/year of sample.

Source	df	F value	P-value
Rep	2	11.63	0.0002
Cultivar (C)	3	6.63	0.0016
Date (D)	3	8.95	0.0003
C*D	8	1.41	0.2365

Table 4-19. Antioxidant capacity of peanut seed as affected by cultivar over three sampling years. In 2006 seed of Hull was not produced. Means with the same letter are not significantly different.

Cultivar	Means	Duncan Grouping	N
Hull	73.3	A	9
AP-3	66.0	AB	12
C-99R	54.6	BC	12
DP-1	45.2	C	12

Table 4-20. Antioxidant capacity of peanut seed as affected by seed storage environment/location and year of crop production. In 2006 seed of Hull was not produced. Means with the same letter are not significantly different.

Year	Location	Means	Duncan Grouping	N
2006	Harvest	73.7	A	9
2005	Harvest	63.6	AB	12
2004	Harvest	58.4	B	12
2004	Stacks	43.5	C	12

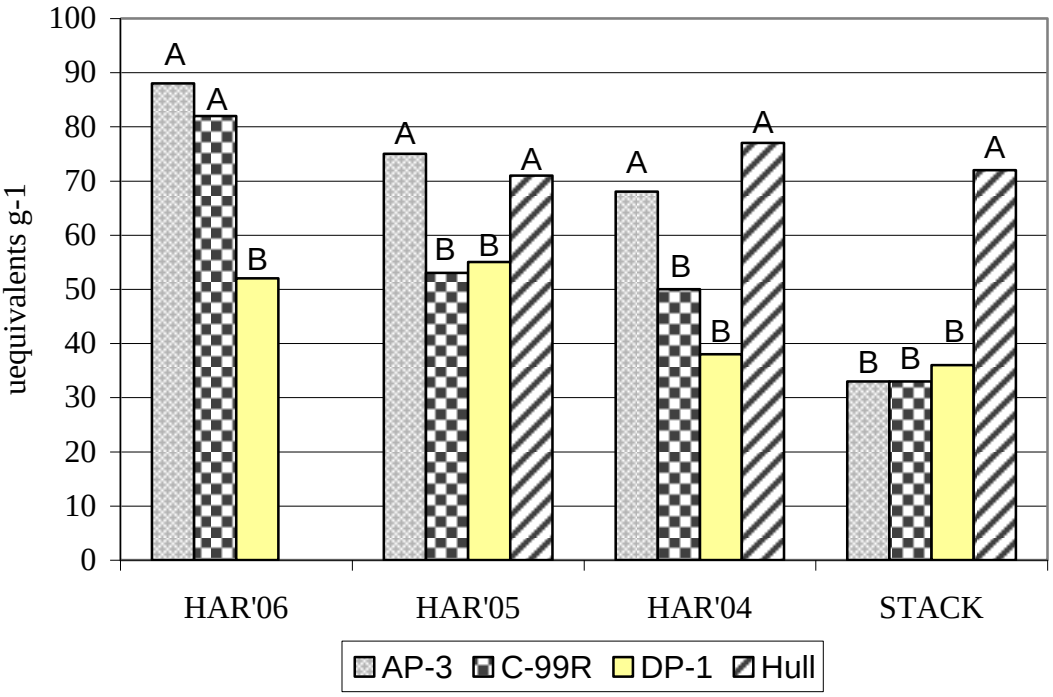

Figure 4-19. Antioxidant capacity of seed peanut measured in µequivalents g^{-1} as affected by year of production, cultivar, and sample date. Sample dates were harvest 2006 (HAR'06), harvest 2005 (HAR'05), harvest 2004 (HAR'04), and March 15, 2005 of peanuts stored in stacks (STACKS) from crop production year 2004. Within a grouping, means with the same letter are not significantly different.

CHAPTER 5
CONCLUSIONS

Seed Deterioration and Poor Field Emergence

Late-maturing peanut cultivars with genetics related to PI 203396 frequently have poor field emergence after storage in commercial bulk peanut bins. The cultivars with PI 203396 lineage include Florida MDR-98, C-99R, DP-1, Hull, and Southern Runner. Because of their pathogen resistance and yield potential, these cultivars are important for peanut production. This research looked at effects of storage environment on three of these cultivars compared to the AP-3 check cultivar, as revealed by seed vigor and field emergence. Analysis of data from the 2004 and 2005 crop storage treatments show:

- The field of origin in crop 2004 year was not a factor in field emergence.
- The seed storage environment/location was a factor affecting leachate conductivity, seedling vigor, and field emergence in 2004 and 2005.
- The bulk storage environment differed in 2004 and 2005. Temperatures within the peanut stack piles from October 11 to January 24 in 2004 averaged 7.0^{o}C higher than temperatures within the stack piles for the same period in 2005.
- Temperature within the peanut stack piles from October 11 to January 24 for crop production year 2004 averaged 10.5^{o}C higher than temperatures within the University climate controlled storage room for the same period.
- There was a cultivar by seed storage environment (location) interaction affecting seedling vigor and field emergence.
- Cultivars stored in the bulk bin location had reduced seed vigor and reduced field emergence.
- When stored in elevated temperatures and relative humidity, field emergence of DP-1 and Hull was less than field emergence of AP-3 and C-99R.
- Seed vigor and field emergence were maintained when seeds were stored at < 16^{o}C and < 70% relative humidity.
- Storage fungi were not an important contributing cause to poor field emergence in 2004 and 2005.

- Standard towel germination tests were not reliable indicators of seed vigor or field emergence.
- Electrolyte conductivity tests and seed vigor tests were highly correlated with seed quality as indicated by field emergence.
- At harvest the antioxidant capacity of peanut seed varied by cultivar and year of production.

The *Seed Vigor Testing Handbook* (2002) states "Seed vigor comprises those properties which determine the potential for rapid, uniform emergence and development of normal seedlings under a wide range of field conditions." The genetic constitution of seed establishes its maximum physiological potential. Cultural and climatic factors during seed maturation may limit the seed from developing maximum vigor potential. Regardless of the level of initial vigor, at maturity seed vigor begins to deteriorate. Seed deterioration is the result of changes within the seed that decrease the ability of the seed to survive. It is distinct from seed development and germination and it is cumulative (McDonald, 2004). Of the many factors that can reduce seed quality, elevated temperature and relative humidity are the most important (McDonald, 2004).

Tests for vigor of bulk stored seed showed increased electrolyte conductivity of peanut leachate and decreased rate of seedling growth for seed stored in the bulk bin (stack) and wagon locations, but not for seed stored in the controlled environment location. Although the towel germination tests indicated acceptable seed quality, DP-1 emerged poorly in the April 2005 field emergence test. After the seed from the same samples was stored for an additional 5.5 months at $< 16^{o}C$ and < 70% relative humidity and sown in October, seedling emergence was poor for seed which had been stored during the winter months in the stack and wagon locations. Field emergence generally was good for seed stored in the University of Florida cool storage location. Seed vigor declines faster than germinability (Figure 4-8). As indicated by conductivity and seed vigor tests, seed vigor by April had deteriorated. An additional 5.5 months of cool storage,

despite being only a minor additional stress, resulted in greatly reduced field emergence in the October 2005 field test. The field emergence test in October 2005 demonstrated that seed vigor in April 2005 was marginal and that the seed vigor had reached the point of steep descent indicated by 'Y' on the seed deterioration graph of Figure 4-8.

Scenario of Seed Quality Deterioration

The data, in conjunction with the literature review, supports the following possible scenario of seed quality deterioration:

- The elevated temperature and increased relative humidity during a warm storage period may allow the exposed peanut radicle to absorb moisture (McDonald, 1998).
- Because of the high lipid content (>45%) of peanut, seed moisture is concentrated within the embryo axis. With the increased moisture and temperature, the glass state of water can reach Tg and water changes to an amorphorous state (Walters, 1998).
- The consequence is an increase in the rate of molecular diffusion within the cytoplasm, so that reactive oxygen species are able to attack the unsaturated lipids of cellular membranes, especially the lipids of mitochondrial membranes. The free radicals produced create a chain reaction of autoxidation. The cellular membranes become porous and, at imbibition, there will be increased leakage of solutes. In addition, the roaming free radicals damage proteins, DNA, and the electron transport system (Wilson and McDonald, 1986).
- Although the seed water exists in an amorphorous state and autoxidation may occur, the seed is still quiescent and there is insufficient water for the metabolic reactions necessary for repair of the cellular machinery (Walter, 1998).
- Damage accumulates and seed quality deteriorates. In the initial phase of imbibition, the cell rapidly repairs damaged membranes, proteins and DNA. The greater the damage, the longer the repair phase, as is evident in the reduced rate of seedling growth and increased number of non-viable seeds in the seed vigor tests. If damage is too severe, the seed becomes non-viable (McDonald, 1998).
- Since germination tests are based on radicle protuberance and do not evaluate differences in elongation of the hypocotyle-radicle, germination tests may over-estimate seed vigor and field emergence.

The difference in field emergence of seed from production years 2004 and 2005 may be related to the 7.0^{o}C higher temperature within the peanut piles (STACKS) during the common period of October 11 to January 24, 2005 compared to the 2005 production year. The cause for

this temperature difference has not been determined, but, is speculated to be associated with heating inside the pile; outside ambient temperatures during this period were similar over years. The pile temperature difference could alternatively be related to crop maturity or seed moisture at harvest going into storage. However, the environment within the STACKS in 2004 consisted of elevated temperatures and high relative humidity, an environment which would allow interaction of seed moisture and temperature and increase the incidence of lipid peroxidation. Data from the seed vigor and leachate conductivity tests of 2004 suggest that increased peroxidation of lipids occurred and damaged cellular membranes, especially in the seeds of Hull and DP-1. The data from 2005 seed vigor and leachate conductivity tests suggest that with the cooler temperatures in the pile in 2005, peroxidation may have been less than in 2004.

Seed deterioration is an individual seed event (McDonald, 2004). As peanuts are removed from bin storage with a front-end loader, the face of the pile keeps sliding down so that peanuts close to the pile surface and perhaps of higher quality become mixed with peanuts of perhaps lower quality from the center of the pile. As a result, the peanuts bagged for sale to the grower are a composite of peanut quality and may not emerge as a uniform and vigorous stand.

Antioxidants have the ability to scavenge free radicals and suppress autoxidation (McDonald, *et al*., 1988). In the analysis of antioxidant capacity for the four principal cultivars, antioxidant capacity at harvest varied by cultivar, oleic acid content, and year. This is in agreement with Amaral *et al.* (2005) and Talcott *et al.* (2005b). For example, the antioxidant capacity of AP-3 at harvest, measured in µequivalents g^{-1}, was 68.4 in 2004, 75.0 in 2005, and 87.6 in 2006. The antioxidant capacity of Hull, a high oleic peanut, at harvest measured in µequivalents $g^{-1}$was 76.8 in 2004 and 71.2 in 2005. During subsequent storage in stacks of seed produced in 2004, antioxidant capacity of AP-3 decreased from 68.4 to 32.9, C-99R from 50.0 to

33.1, DP-1 from 38.3 to 36.2, and Hull from 76.8 to 71.9. In AP-3 the lost antioxidant capacity may have been consumed in suppressing autoxidation, thus protecting the seed from free radical attack. For C-99R, DP-1, and Hull, antioxidant capacity reduction was substantially less and suppression of autoxidation may have been insufficient to protect the seed from free radical attack, thus resulting in the cellular membrane damage and poor seed vigor, as evidenced in increased leachate conductivity, reduced seed vigor, and poor field emergence of C-99R, DP-1, and Hull. At this time there is no explanation for the failure of DP-1 and Hull antioxidant capacity to suppress autoxidation nor conclusive evidence that for AP-3 antioxidant capacity was instrumental in protecting its seed from autoxidation.

CHAPTER 6
RECOMMENDATIONS

Recommendations for Continued Research

AP-3, C-99R, DP-1, and Hull can be ranked by percentage of lineage to PI 203396, which relates to degree of intolerance to elevated temperature and relative humidity during storage. Perhaps decreased antioxidant capacity is linked to PI 203396. The literature reports that in many species antioxidant capacity depends upon year of production and genotype (Amaral *et al*., 2005) and that it may be possible in a breeding program, to select for elevated α-tocopherols by selecting for altered response to temperature (Britz and Kremer, 2002). Based on the data from this research, storage quality of peanut may be improved by including antioxidant capacity as a standard in cultivar evaluation. Additional research is necessary to refine the evaluation of antioxidant capacity and verify the relationship of antioxidant capacity to seed vigor and field emergence.

Recommendations for Improving Quality of Seed Peanut

Field emergence of peanut seedlings is affected by many factors, including seed maturity, seed size within the cultivar, seed damage during harvesting and processing, loss of viability during seed storage, and soil tilth, temperature, and moisture at planting time (McDonald 2004). The following suggestions may improve seed quality and field emergence:

Improve Ventilation of Storage Facilities

Peanut seed quality may be improved by developing a low capital investment, low operating cost, and fully automated forced air ventilation system for reducing temperature and relative humidity within the peanut pile during the October-February storage period. Butts *et al.* (2006) published research comparing four possible ventilation methods for peanut barns. Peanut Company of Australia (PCA) found that lowering the percent moisture in seed with high oil

content helped to maintain field emergence (PCA, 2006. Per. Comm.). Alternatively, effective storage may require refrigeration/air conditioning capacity, especially for these storage-sensitive cultivars.

Replace Towel Germination Tests with a Test that Measures Seed Vigor

Develop a method of evaluating vigor of peanut seed at the time of bagging the seed for sale to the grower. One method is an electrolyte conductivity test of seed leachate derived by soaking a sample of peanuts in water for 18 hours. The test can be completed within 24 hours, takes very little space, requires an inexpensive conductivity meter, and can be accomplished accurately with minimal training of technicians. A second method is to use seed vigor tests. These tests require 5 days, more space, germination chambers, and the data can reflect the bias of the technicians measuring the linear length of hypocotyl-radicles.

APPENDIX
SEED VIGOR DIFFERENCES IN GERMINATING PEANUT SEED

An example of seed vigor differences as evident in variation of hypocotyl/radicle length of seeds germinating in a moist towel test.

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BIOGRAPHICAL SKETCH

Barry Morton earned a Bachelor of Arts in English literature at Brown University and a Master of Science in agronomy from Pennsylvania State University. His master's thesis was a "Study of Pollination and Pollen Tube Growth in Buckwheat (*Fagopyrum sagittatum* Gilib.)". His dissertation "Poor Field Emergence of Late-Maturing Peanut Cultivars (*Arachis hypogaea* L.) Derived from PI 203396", completes his doctoral program toward a Doctor of Philosophy in agronomy from the University of Florida in May 2007. Mr. Morton's experience is a unique combination of farm owner/operator of a cash crops and swine farrowing operation, agricultural lender, agricultural consultant, and teacher at the secondary and university level. As a commercial farm owner/producer, he raised corn, alfalfa, small grains, and potatoes in Southeastern Pennsylvania. The swine farrowing operation, with a total of three employees and an on-farm feed mill, produced 9000 pigs/year. As an agricultural loan officer, Mr. Morton analyzed credit, appraised collateral, made cash flow projections, and tracked loan progress of farm operating loans and mortgages for a regional bank in Pennsylvania. As an agricultural consultant, Mr. Morton negotiated bank financing and provided crop/livestock planning, cash flow projections, and on-farm management. Clients included a 2500 acre Manor Farm located in Maryland producing corn, soybeans, and hay using sewage sludge to supplement fertilizer. Mr. Morton taught biology at York College of Pennsylvania and secondary level English classes at Desert Pride Academy located at the border of New Mexico and Mexico. He was instrumental in the initial phases of establishment of a program for returning students and co-authored guidelines and curriculum for the program.