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RESEARCH

Effects of Storage Temperature and Relative Humidity on Viability and Vigor of Treated Soybean Seeds

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ABSTRACT

Seed treatments are applied to soybean [*Glycine max* (L.) Merr.] seeds to control early season diseases and insects. Unsold, treated soybean seed must be disposed in a different manner than untreated seed. To minimize treated seed disposal costs, it is necessary to improve seed storage. The objective was to determine the best storage environments that would minimize deterioration of treated soybean seed. Twenty-four soybean varieties, different in lipid and protein contents and from four maturity groups, were treated either with fungicide or a mixture of fungicide plus insecticide or were untreated and were stored in three storage environments differing in temperature and relative humidity: a cold storage (CS) (10°C), a warm storage (WS) (25°C), and a warehouse (WH). Seed viability and vigor were evaluated each 4 mo for 20 mo using standard germination and accelerated aging tests. Seed viability remained high throughout the study for seeds stored in CS (>92%) and moderate in the WS (>78%) but decreased to almost 0% after 20 mo in the WH. The seed viability of treated seed was significantly higher than that of untreated seed after 16 mo in the WH while in the CS and WS the positive effects lasted for 20 mo. Seed vigor was affected by only seed lipid content for seeds stored for 12 mo, regardless of storage environment. Treated soybean seeds could be carried over for two seasons if the storage temperature is maintained at 10°C and the relative humidity is below 40%.

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Abbreviations: AA, accelerated aging; AOSA, Association of Official Seed Analysts; CS, cold storage; RH, relative humidity; WH, warehouse; WS, warm storage.

Planting early and reducing disease pressure have a greater positive impact on soybean yield than other management practices (Heatherly and Spurlock, 1999). A positive yield response may also be obtained when seed treatment is applied before planting in either cold or wet soil conditions (Munkvold, 2009). Seed treatments also minimize the use of foliar and soil pesticide applications because they are applied in small quantities directly to the seed. In addition, seed treatments promote good seedling emergence and uniform stand establishment and protect the germinating seed by eliminating seed-associated pathogens (Schulz and Thelen, 2008). Consequently, soybean production has evolved into an early soybean production system, in which soybean producers plant early to maximize yield (Smith and Mengistu, 2010) without the risks of yield losses due to seedling diseases and insects.

An estimated 80% of the soybean seed planted in the United States is chemically treated, which translates to more than 71.14 million bags of seed (USDA-NASS, 2010). The excess treated seeds must be discarded at the end of each planting period. In the past, excess nontreated seed was sold in the grain commodity market. However, this disposal method is no longer feasible, as treated seed

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must be incinerated, planted at high rates based on label restrictions, or buried (ISTF, 2000). Consequently, the increase in soybean carryover stock has been of great concern to the seed industry (Edje and Burris, 1971). An alternative solution is to carry over the excess seed for the next cropping season, but this option may pose the risk that soybean seeds stored under certain conditions may deteriorate rapidly (Delouche and Baskin, 1973; Krueger et al., 2012). To minimize seed disposal costs, safe and economical storage of carry-over treated seeds is needed.

Justice and Bass (1978) placed soybean among the group of least storable seeds in the "relative storability index" classification. Furthermore, research has shown that the length of time a seed lot remains viable in storage (seed longevity) is influenced by the initial quality of the seed lot, its moisture content, temperature, relative humidity, and gaseous exchanges in the storage environment (Barton, 1943; Vertucci and Roos, 1990, 1993). Due to the fact that accumulation of seed storage substances is genetically predetermined, seed longevity in storage is a genetically regulated process (Delouche, 1968). Maximum seed quality, as defined by seed germination and vigor, is reached at physiological maturity (Bewley and Black, 1994); beyond this stage, the seed deteriorates. Seed deterioration is defined as an inexorable process that cannot be reversed. Only its rate can be slowed by controlling the conditions of the storage environment (Delouche, 1968).

Harrington (1959) defined the best storage environments in his "rules of thumb," which have become standard in the seed industry. These rules state that for a 1% decrease in seed moisture content, the storage life of the seed is doubled; for a 5°C decrease in storage temperature, the storage life of a seed is doubled; and that the arithmetic sum of temperature in °F and percent relative humidity should not exceed 100, with not more than half contributed by temperature. These rules have been used in seed preservation for short-term storage of two or more years (Walters, 1998). Studies have shown that high temperature and relative humidity in the storage environment increase the rate of deterioration of a seed lot (Harrington, 1973). Seeds subjected to fluctuating levels of moisture deteriorate faster than seeds held at a constant level (Bass, 1973). Therefore, the magnitude of temperature fluctuation and relative humidity and the duration of storage are important determinants for the rate of deterioration (Delouche, 1968). Storage fungi are a major cause of quality losses in stored seed as well, with the extent of deterioration being dependent on the relative humidity of the storage environment (Delouche, 1968).

While much is known about storage of untreated soybean seed, very little information is available on the effect of seed treatment and seed chemical composition on the longevity of soybean seeds in storage. Soybean seeds stored for 6 mo at a temperature of 15°C maintained high germination (95%) and vigor, when a cool storage environment was maintained at 60% relative humidity (Krittigamas et al., 2001). Additional work showed that seeds stored in controlled temperature of 15 and 20°C had higher rate of germination than those stored at an ambient temperature.

This study focused on the influence of seed chemical treatments, maturity groups, seed composition, and initial seed-borne fungi loads on seed deterioration three storage environments. We hypothesized that treated soybean seed could be carried over at least 20 mo if the storage environments follow Harrington's rule (1959) of temperature, 10°C, and 50% relative humidity.

The objective of this study was to determine the best storage environment that would minimize soybean seed deterioration of chemically treated seed from a wide range of genotypes.

MATERIALS AND METHODS

Seed Lots

A total of 24 soybean varieties were obtained from three seed companies (Monsanto, Pioneer Hi-Bred International Inc., and Stine Seed Company). The varieties were chosen to represent four maturity groups (maturity groups I, II, III, and IV) and two seed composition extremes within each maturity group, high oil and high protein contents. Two bags of each variety were obtained from the seed companies and used as replications, and seed treatments were applied to each bag separately. These bags of seed or replications belonged to a different seed lots or to different stacks of the same seed lot to allow for true statistical replications when analyzing variety effect. For the purpose of this study, therefore, each bag is referred to as a seed lot. Upon reception each seed lot was subdivided into three equal parts of 1500 g and then evaluated for initial seed viability, vigor, and moisture content before each third was assigned a seed treatment. Seed treatments consisted of (i) fungicide, (ii) fungicide plus insecticide following the manufacturer's labeled medium rates, and (iii) untreated control. The seed treatments were a mixture of the fungicides fludioxonil (4-(2,2-difluoro-1,3-benzodioxol-4-yl)-1*H*-pyrrole-3-carbonitrile), applied at a rate of 3.6 mL per 45.4 kg of seed (Syngenta Crop Protection), and mefenoxam ((R,S)-2-[(2,6-dimethylphenyl)-methoxyacetylamino]-propionic acid methyl ester), applied at rate of 11.8 mL per 45.5 kg of seed (Syngenta Crop Protection), or a mixture of these fungicides and the insecticide thiamethoxam (3-(2-Chloro-5-thiazolylmethyl) tetrahydro-5-methyl-N-nitro-4H-1,3,5-oxadiazin-4-imine), applied at a rate of 37.9 mL per 45.4 kg of seed (Syngenta Crop Protection). These seed treatments represent some of the current available treatments for soybean seed in today's market. The treatments were applied a day before packaging to allow chemicals to dry on the seed. Standard germination and accelerated aging (AA) tests were conducted for all seed lots before storage to determine the initial seed viability and vigor.

Seed Storage

Two replicates of 100 seeds per seed treatment per seed lot were placed inside 8 by 14 cm coin envelopes (Quality Park Products, Minneapolis, MN), and these coin envelopes were then placed inside a 23 by 30 cm envelope (Quality Park Products,

Minneapolis, MN). One of the 100 seed samples was used for evaluating seed viability, and the other was used for evaluating seed vigor. Twenty-four large envelopes representing each of the 24 varieties of soybean (per seed treatment per replicate) were stored inside a triple-wall seed paper bag (Central Bag Company). The seeds were placed in three storage environments: a nonclimate controlled warehouse (WH), a climate controlled cold storage (CS) (10°C and 59.6 ± 7.3% relative humidity [RH]), and a climate controlled walk-in germinator or "warm storage" (WS) (25°C and 31.2 ± 11.1% RH). The number of triple-wall bags per seed treatment per storage environment corresponded to the number of evaluation times. Seed viability and vigor evaluations were conducted at 4, 8, 12, 16, and 20 mo after storage. Temperature and RH data loggers (HOBO model U-14; OnSet Corp.) were used in each storage environment to record temperature and RH data. The experimental design was a split-split-split plot in a randomized complete block design with two replications.

Seed Viability Test

The standard germination test was used to evaluate seed viability. The tests were performed following the Association of Official Seed Analysts (AOSA) Rules for Testing Seeds (AOSA, 2012). One sample of 100 seeds per variety per replication per treatment were placed on crepe cellulose paper (Kimberly Clark) previously moistened with 840 mL of water on fiberglass trays (45 by 66 by 2.54 cm). The trays were placed inside sealed germination carts after planting and the carts were placed in a walk-in germination room with alternating 4 h of light and 4 h of darkness, totaling 12 h of light d^{-1} for 7 d at a constant temperature of 25°C.

Seed Vigor Test

Seed vigor was evaluated using the AA test. The test was performed according to the AOSA (2009) Seed Vigor Testing handbook. One hundred seeds per variety per replication per treatment were placed in a single layer on wire mesh in a 10 by 10 by 4 cm plastic box (Hoffman Manufacturing Co.) containing 40 mL of distilled water. Lids were placed over boxes, which were then placed inside an AA chamber (VWR Scientific) at a temperature of 41°C and a RH of approximately 100% for 72 h. Immediately after the aging period, the seeds were removed from the chamber and planted on crepe cellulose paper moistened with 840 mL of water on fiberglass trays and covered with 2.5 cm of moistened sand. The trays were placed inside sealed germination carts after planting, and the carts were then placed inside a constant 25°C walk-in germination room, alternating 4 h of light and 4 h of darkness, for a total of 12 h of light d^{-1}. The seeds were allowed to germinate for 7 d.

Seed Composition Analysis

Seed oil and protein contents of each seed lot were analyzed in the Grain Quality Laboratory at Iowa State University. Tests were conducted on two replicates of 400 g of seed of each variety using a whole-grain, near-infrared analyzer, following protocols established by Rippke and Hurburgh (2006), and the results were standardized to a seed moisture level of 0.13 g H_2O g^{-1} fresh wt. basis.

Seed Fungi Assessment

A blotter test (ISTA, 1999) was used to identify and enumerate the initial fungal load on each seed lot before storage. Two blotter sheets were saturated with a solution of 0.05% Botran, active ingredient 2, 6-dichloro-4-nitroaniline (Gowan Company), and placed in plastic boxes. One hundred seeds were placed on the blotter and were evenly spaced using forceps. Seeds were placed in boxes and incubated for 10 d inside a dark germination cart in a constant 25°C walk-in germination room. Seeds were examined for fungal growth 3, 5, 7, and 10 d after plating.

Moisture Content of Seeds

The initial moisture content of seed, before storage, and the final moisture content, after storage, were determined for the constant storage environments, that is, CS and WS. Triplicate samples of 100 seeds per seed lot were placed in Pyrex petri dishes and weighed using a balance (Satorius Ag). Weighed samples were placed in an Isotemp gravity-convection oven (Fisher Scientific) set at 103°C for 72 h (ISTA, 2012). At the end of the drying period, the dishes were removed and weighed. The percentage of moisture (wet basis) was calculated by dividing the loss in weight, due to drying by the weight of the original sample, and multiplying by 100. The moisture contents of seeds in the WH were calculated using the Kews Royal Botanical Gardens moisture content calculator that uses seed oil content, temperature, and RH of the storage environment to estimate the seed equilibrium moisture content over time (Cromarty et al., 1982).

Data Analysis

The effects of storage environments and seed treatments on seed viability and vigor, as determined by the standard germination and AA tests, were analyzed using the generalized linear mixed model (PROC GLIMMIX) of SAS (SAS Institute, 1994). All factors were treated as fixed effects while interactions with replication were considered random effects. Means of main effects and interactions were compared using Tukey's test with least square mean comparisons. The statistical analysis showed significant interactions among seed treatments, storage environments, and storage period. The data were sorted by storage periods and were then reanalyzed. The mean effects of seed maturity group, seed lipid, protein content, and initial fungi load on seed viability and vigor were compared, and regression analyses were calculated using PROC REG procedure of SAS. Daily and monthly average temperatures and RH were calculated from measurements taken every 3 h at each storage environment.

RESULTS

The initial moisture content of the seed lots before storage ranged between 5.95 and 8.00% fresh wt. basis. Variety 20 had the lowest moisture content of 5.95% (data not shown). The mean moisture content of the seed lots and the RH and temperature of the storage environments measured at the end of the experiment are presented in Table 1. The mean moisture content for each seed lot was averaged over all varieties after 20 mo in storage in the CS and ranged between 10.15 and 10.77% while the seed lots in the WS had lower moisture contents, in the 5.66

Table 1. Mean and SD for moisture content of 24 soybean varieties after 20 mo of storage in three storage environments, cold storage (CS), warm storage (WS), and warehouse (WH), and with three seed treatments of fungicide (Fung), fungicide plus insecticide (Fung+Ins) and untreated control, and mean and SD for temperature and relative humidity of the storage environments.

	Moisture content† (% fresh wt.)						Temperature (°C)	SD	Relative humidity (%)	SD‡
	Fung	SD	Fung+Ins	SD	Untreated	SD				
CS	10.77	0.91	10.54	0.32	10.15	0.39	10.40	0.40	59.60	7.30
WS	5.81	0.15	5.72	0.16	5.66	0.18	25.40	0.80	31.20	11.10
WH†	–	–	–	–	–	–	14.90	8.60	59.70	8.90

†Calculated moisture content ranges for the seed lots in the fluctuating temperature and relative humidity conditions of the WH are presented in the results section.

‡WH: min. and max. temperature: –10 and – 27°C; relative humidity (RH): 38 to 75%; min. and max. RH in CS: 45 to 68%; WS: 15 to 50%.

to 5.81% range (Table 1). A seed moisture content calculator accessed on the website of the Kews Royal Botanical Garden was used to estimate the moisture content of seed lots in the WH at the end of the experiment. The calculator was developed by Cromarty et al. (1982) based on the viability equation of Ellis and Roberts (1980). It takes the oil content of seed lots into account (Eckey, 1954). The calculator required entry of the mean monthly temperature and RH values recorded in the WH during seed storage and was used to estimate the seed moisture contents. The calculated ranges of moisture content under the three storage environments were between 11.4 and 12.7% (data not shown). The daily temperatures within the CS for most of the duration of the experiment ranged from 9.80 to 11.58°C, and the daily mean was 10.4 ± 0.4°C. The daily RH range was 42 to 68.5%, with a mean of 59.6 ± 7.3%. The daily temperature range for the WS was between 24.4 and 27°C, and the daily mean was 25.4 ± 0.8°C. The RH in the WS ranged from 14.8 to 45%, with a daily mean of 31.2 ± 11.1%. In the WH, the temperature fluctuated between –7.8 and 28°C, and the mean daily temperature was 14.9 ± 8.6°C. The RH range in the WH was 37 to 74% and the daily mean RH was 59.67 ± 8.9%.

Table 2 shows the overall analysis of variance for seed viability after 20 mo storage and seed vigor after 16 mo storage. The seed lots stored for 20 mo in the WH were severely deteriorated and seed vigor from all seed lots and seed treatments reached 0%. Hence, the analysis of variance for seed vigor at 20 mo could not be calculated and results are presented only for 16 mo of storage. A significant three-way interactions for variety × storage environment × storage period ($P < 0.0001$) was observed for seed vigor. Also, a significant interaction among seed treatment × storage environments × storage period ($P < 0.0001$) was observed for seed viability (Table 2). Consequently, the data are presented by storage period to allow for comparisons between seed viability and seed vigor at all storage periods (Fig. 1).

Table 2. Analysis of variance for seed viability, determined by the standard germination test, and seed vigor, determined by the accelerated aging test, of 24 soybean varieties after 20 mo (seed viability) and 16 mo (seed vigor) of storage in three storage environments, cold storage, warm storage, and warehouse, of seeds treated with fungicide, fungicide plus insecticide, and untreated control.

	Seed viability			Seed vigor†		
	Standard germination			Accelerated aging		
Effect	df	*F*-value	*P* > *F*	df	*F*-value	*P* > *F*
Variety	23	1.94	<0.0001	23	42.99	<0.0001
Seed treatment (ST)	2	1.85	<0.0001	2	63.31	<0.0001
ST × variety	46	0.95	0.9999	46	1.71	0.0027
Storage condition (SC)	2	283.47	<0.0001	2	1916.1	<0.0001
SC × variety	46	1.51	<0.0001	46	12.03	<0.0001
SC × ST	4	1.39	0.0013	4	6.81	<0.0001
SC × ST × variety	92	0.96	0.9998	92	0.95	0.625
Time in storage (T)	4	184.8	<0.0001	4	797.05	<0.0001
T × variety	92	1.41	<0.0001	92	5.35	<0.0001
T × ST	8	1.17	0.0129	8	11.50	<0.0001
T × ST × variety	184	0.98	1.0000	184	0.89	0.8503
T × SC	8	158.96	<0.0001	8	134.75	<0.0001
T × SC × variety	184	1.05	<0.0001	184	2.64	<0.0001
T × ST × SC	16	0.99	0.0009	16	2.83	0.0002
T × ST × SC × variety	368	0.95	1.0000	368	0.49	1.0000

†Analysis of seed vigor after 20 mo of storage could not be computed because all data points from seed stored in the warehouse environment were zero.

Seed Viability

Initial seed viability, as determined by standard germination test percentages, ranged between 95 and 99% (Fig. 1A). After 4 mo in storage, seed viability within each storage condition was not significantly different ($P < 0.05$) regardless of the seed treatment applied (Fig. 1B). However, the rate at which seeds deteriorated was significantly different among storage environments. The seed viability decline for treated and untreated seed lots in CS and the WS after 8 mo storage was not significantly different. After 12 mo of storage, there were still no significant differences in seed viability among treatments in the CS regardless of the seed treatment applied, and the germination percentages remained close to 100%. In the WS, the viability of fungicide-treated seeds (98%) was similar to that of the fungicide plus insecticide-treated seeds (95%) but was significantly higher than the viability of untreated seeds. Moreover, the viability of fungicide-treated seeds

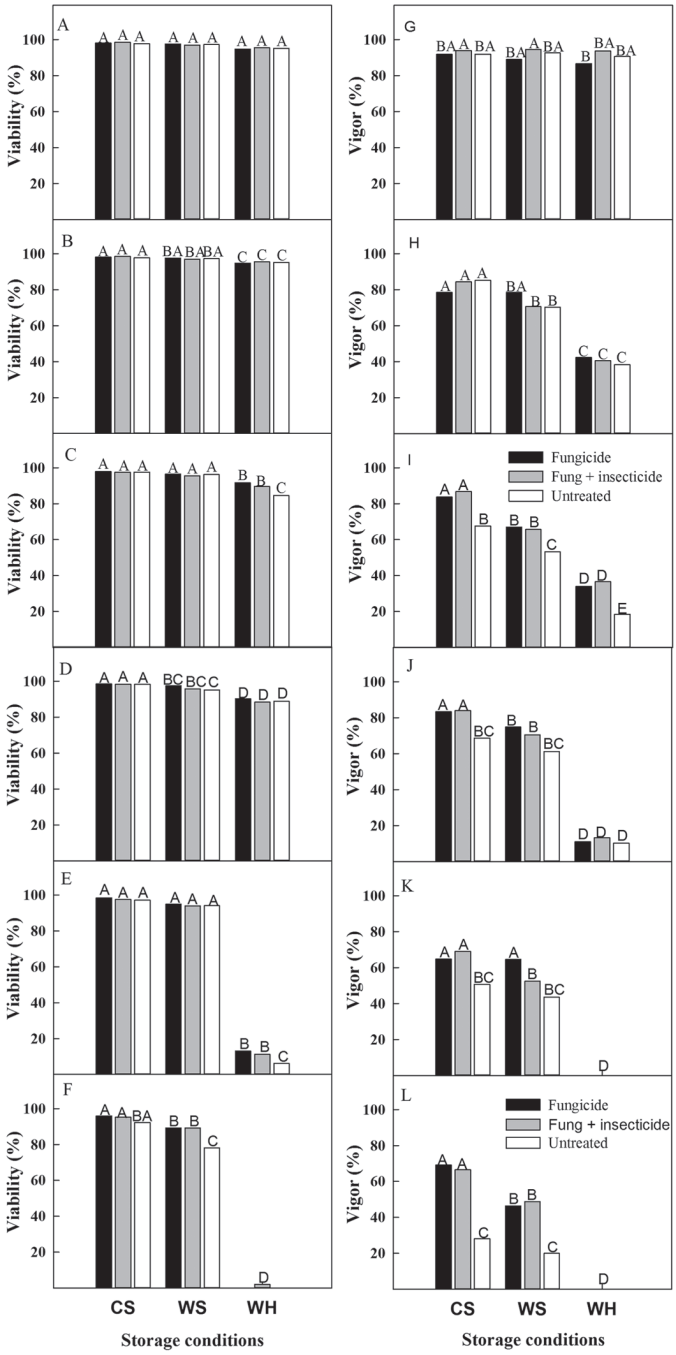

Figure 1. The effect of seed treatments with fungicide, fungicide plus insecticide, and untreated control and storage environments of cold storage (CS), warm storage (WS), and warehouse (WH) on seed viability and vigor of 24 soybean varieties over time. Figure panels A, B, and C represent seed viability, and panels G, H, and I represent seed vigor on arrival, after 0, 4, and 8 mo of storage, respectively. The effect of seed treatment with fungicide, fungicide plus insecticide, and untreated control and storage environments of CS, WS, and WH on seed viability and vigor of 24 soybean varieties over time. Figure panels D, E, and F represent seed viability, and panels J, K, and L represent seed vigor at 12, 16, and 20 mo after storage, respectively. Different letters above the columns in the graph denote significant mean differences ($P \leq 0.05$) as determined by Tukey test.

in the WS was not significantly less than that of all the seeds stored in the CS after 16 mo.

Seed viability of seed lots stored in the WH for 8, 16, and 20 mo was significantly higher for treated seed than for untreated seed (Fig. 1C, 1E, and 1F). Even though viability of seed lots stored in the WH was still above 80% at 12 mo, this value was significantly lower than those of seed lots stored in the CS (98%) and WS (96%) (Fig. 1D). Four months later (16 mo of storage) the viability of seed lots in the WH declined drastically to below 20% while those in the CS and WS remained high (>90%) (Fig. 1E). Temperature and RH conditions in the WH during the initial 12 mo of storage fluctuated from 9 to 25°C and from 46 to 69%, respectively. At 16 mo, the temperature and RH readings ranged from 9 to 24°C and from 59 to 73%, respectively (data not shown). Even though seed viability for seed lots stored in the WH at 16 mo were very low, treated seeds within this storage condition had significantly higher germination than untreated seeds. The viability of seed lots in CS at 20 mo after storage was still >92% for all seed treatments while treated seeds in WS maintained a viability of >89% compared to the untreated seeds (>78%) for the same storage period (Fig. 1F).

When comparing seed viability for the three storage environments, the best storage condition was the CS. The CS maintained the viability of the seed lots at 96% for fungicides-treated seeds, 95% for fungicide plus insecticides-treated seeds, and 92% for untreated seeds, for the entire duration of storage. Only the treated seeds retained viability above 80% in the WS while the viability of the untreated seeds declined to levels below 80% at the end of the storage period (Fig. 1F). The WH was the least favorable storage environment for maintaining seed viability.

Seed Vigor

Initial seed vigor as measured by the AA test ranged from 83 to 97% (Fig. 1G). In CS, vigor values of the fungicide plus insecticide-treated seeds and the untreated seeds were ≥80% after 4 mo of storage and did not differ significantly between seed treatments (Fig. 1H). Similarly, the AA percentage for fungicide-treated seeds stored in the WS was significantly higher (79%) than that of fungicide plus insecticide-treated seed (71%) and untreated seed (70%) (Fig. 1H). At 4 mo, seed vigor was similar (≤60%) for all seed treatments and seed lots stored in the WH and significantly lower than seed lots stored in the CS (82%) and the WS (73%).

After 8 mo of storage in CS, the seed vigor values of fungicide-treated seeds were higher (84%) compared to the vigor values of the same treatment at 4 mo (79%). The fungicide- (84%) and fungicide plus insecticide-treated seeds (87%) had higher seed vigor than untreated seeds (68%) after 8 mo in CS (Fig. 1I). Seed vigor of seed lots in WS declined to <70% after 8 mo; seed vigor of the untreated seeds in this environment was lower (53%) than the fungicide- (67%) and

Table 3. Regression analysis of the effect of seed oil and protein contents on seed vigor, determined by the accelerated aging test, of 24 soybean varieties stored in three storage environments, cold storage (CS), warm storage (WS), and warehouse (WH), using seeds treated with fungicide, fungicide plus insecticide, and untreated control. Results are presented for each storage period with T4, T8, T12, T16, and T20 representing 4, 8, 12, 16, and 20 mo after storage.

	Oil content					Protein content				
	Months after storage									
Vigor	T4	T8	T12	T16	T20	T4	T8	T12	T16	T20
CS										
R^2†	0.0678	0.0166	0.1344	0.1453	0.0643	0.0081	0.0025	0.0278	0.0281	0.0051
$P > F$‡	0.0016	0.1234	<0.0001	<0.0001	0.0023	0.2828	0.5503	0.0458	0.0447	0.3987
WS										
R^2	0.0555	0.0897	0.1531	0.1541	0.0912	0.0111	0.0231	0.029	0.0575	0.0495
$P > F$	0.0045	0.0003	<0.0001	<0.0001	0.0002	0.2081	0.0687	0.0412	0.0041	0.0074
WH										
R^2	0.0520	0.0090	0.1005	–	0.0050	0.0002	0.0009	0.0586	–	0.0109
$P > F$	0.0060	0.2572	0.0001	–	0.4014	0.8826	0.7143	0.0035	–	0.2121

†R^2, coefficient of determination depicting the proportion of vigor variance explained by seed oil and protein contents.

‡$P > F$, P-value associated with the F-statistics; significant when $P > F$ is less than 0.05.

fungicide plus insecticide-treated (66%) seeds (Fig. 1I). Even though the vigor of seed lots in the WH declined to below 40% after 8 mo in storage, treated seeds maintained seed vigor of >34% compared to the 19% of the untreated seeds.

Twelve months after storage, there was a distinct difference in seed vigor of treated seeds (>83%) compared to untreated seeds (69%) in CS. Seed vigor was slightly lower in WS and treated seeds maintained a higher vigor than untreated seeds (72% compared to 61%, respectively). Seed vigor of treated seeds in WS (72%) was comparable to that of untreated seeds in CS (69%) (Fig. 1J). Sixteen months after storage, seed vigor of all seed lots in the three storage environments was below 80% (Fig. 1K). The treated seeds in CS maintained a vigor of >64% compared to 51% of the untreated seeds. In WS, the seed vigor of fungicide-treated seeds was significantly higher (65%) than the fungicide plus insecticide-treated seeds (52%). The vigor of seed stored in the WH was 0% after 16 mo of storage (Fig. 1K). The vigor decline for treated seeds from 4 to 20 mo in storage was less than the rate of decline for untreated seeds in both CS and WS. Seed vigor decline from 4 to 20 mo in CS was from 79 to 64% for fungicide-treated seeds and from 84 to 69% for fungicide plus insecticide-treated seeds, respectively. In WS, the vigor declined from 78 to 64% and from 70 to 52% for fungicide and fungicide plus insecticide-treated seeds, respectively. The seed vigor of untreated seeds declined from 85 to 28% in CS and from 70 to 19% in WS for 4 and 20 mo, respectively.

Effect of Oil and Protein Content

The oil content of seed lots ranged from 16 to 20%. Seed lots were classified into four groups based on their oil contents from 16.0 to 16.9, from 17.0 to 17.9, from 18.0 to 18.9, and from 19.0 to 20%. Mean comparisons showed that the decline in seed viability among the different seed oil groups was similar. Seed viability averaged over seed treatments and storage environments had no relationship to oil and protein contents (data not shown). A regression analysis of the effect of seed oil and protein contents on seed vigor in storage environments is presented in Table 3. The results showed that seed oil content was important to explain the vigor decline of seed lots stored in the CS or WS. In both the CS and WS, the R^2 values were 15% of the variation observed in seed vigor (Table 3). There was no clear relationship between seed oil content and seed vigor decline for seed lots stored in the WH (Table 3). Figure 2 represents the significant positive effect of oil content on seed vigor of the seed lots at 12 mo, regardless of storage environment.

The protein content of soybean varieties ranged between 32 and 37% (Table 3). When the varieties were categorized into five groups depending on the protein content, analysis of variance showed no significant differences in seed viability decline among the five categories (data not shown). However, the five categories differed significantly in seed vigor decline. A regression analysis showed that the decline in seed vigor over time was independent of seed protein content regardless of storage environments and storage periods (Table 3).

Variety and Maturity Group Effect

The analysis of variance for seed viability showed a significant variety effect after 20 mo in storage ($P \leq 0.0004$); however, the viability of all maturity groups, I, II, III, and IV, was similar (data not shown). The seed vigor of all varieties after 20 mo of storage in WH was zero (data not shown). The varieties Var1, Var4, Var5, Var11, and Var13 maintained seed vigor levels of more than 80% before decreasing to below 80% at 20 mo after storage in the CS and WS (data not shown). Varieties that belong to maturity group IV presented lower seed vigor levels, but not significant, compared to those of maturity groups I, II, and III (data not shown). Even though mean comparison of seed vigor

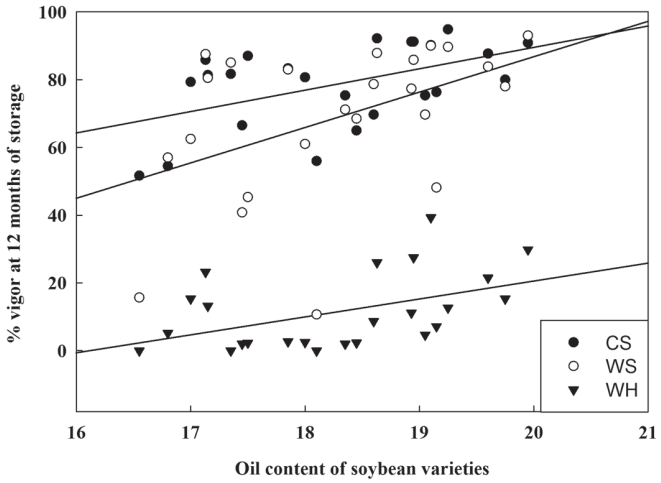

Figure 2. Linear regression models fitted to total oil content in percentage and the vigor test percentages of 24 soybean varieties at 12 mo of storage in cold storage (CS), warm storage (WS), and warehouse (WH) environments. The coefficient of determination was significant for all regression models. For CS: R^2 = 0.26, P = 0.0119, and y = 6.31x – 36.8; for WS: R^2 = 0.21, P = 0.0242, and y = 10.45x – 122.12; and for WH: R^2 = 0.22, P = 0.0257, and y = 5.17x – 82.7.

showed significant differences among the various maturity groups (data not shown), these differences were not important in determining the decline in vigor of the seed lots in storage, as revealed by a nonsignificant regression analysis within each storage environment, and over time.

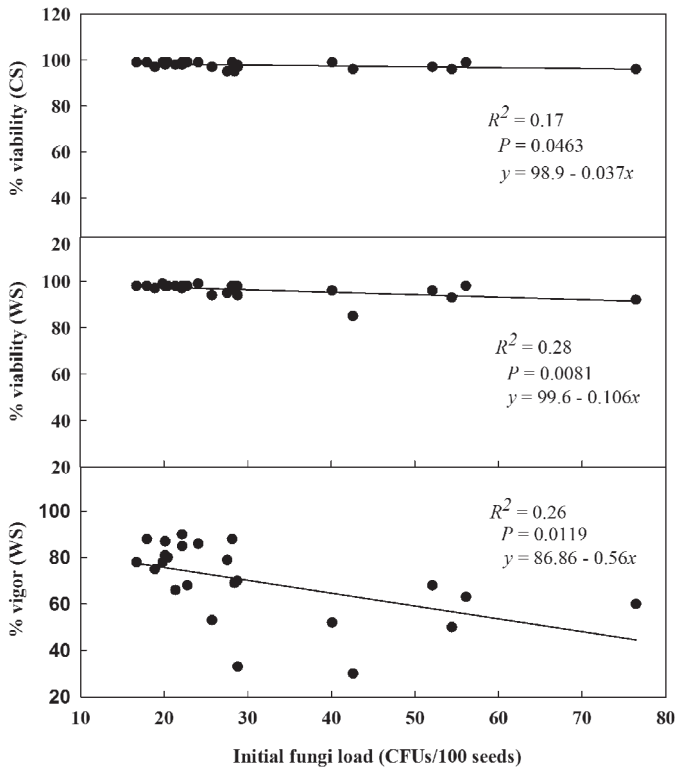

Figure 3. The relationship between initial fungi load in colony forming units (CFU) and viability and vigor of 24 soybean varieties averaged over seed treatment in cold storage (CS) and warm storage (WS). The relationship was nonsignificant in warehouse (WH) environment.

Fungi Isolations

Several fungi were isolated from the seed lots on reception, including *Phomopsis phaseolorum* var. *sojae* (S. G. Lehman) Wehmeyer and *Phomopsis longicolla* T. W. Hobbs, *Cercospora kikuchii* (Tak. Matsumoto & Tomoy.) Gardner, *Chaetomium* spp., *Cladosporium* spp., *Alternaria* spp., *Fusarium* spp., *Rhizopus* spp., *Aspergillus* spp., and *Penicillium* spp. A plot of the initial fungi load against standard germination values of varieties averaged over seed treatment and storage environments over time showed no significant relationship between the decline in seed viability or vigor and initial fungi load of seed lots. However, a more detailed analysis showed the initial fungi load was important in accounting for 17 and 28% of the variability in viability of the seed lots at 12 mo of storage in CS and WS, respectively. The initial fungi load also accounted for only 26% of the variability in vigor of the seeds in the WS (Fig. 3). Irrespective of the method of analysis used, no significant relationship was found between the initial fungi load and the change in viability and vigor of seed lots stored in the warehouse.

DISCUSSION

Seed genetics, the environment where seeds are produced, and storage environment are the three major factors that influence seed longevity, viability, and vigor (Sun et al., 2007). In this study we investigated the effect of storage temperature and relative humidity on the storability of chemically treated soybean seeds. The results strongly suggested that treated soybean seeds stored better and maintained higher viability and vigor than untreated seeds under low temperature and relative humidity. In addition, the soybean varieties in this study maintained high viability, as measured by standard germination tests, for up to 12 mo under all storage environments. Beyond this time, the viability of the seeds declined drastically in the WH compared to a slower deterioration rate in CS and WS.

The decline in seed viability is intricately linked to the moisture content of the seed, which depends on the relative humidity of the storage environment (Barton, 1943; Vieira et al., 2001). The relative humidity of the WH fluctuated within a wide amplitude; the seeds adsorbed or desorbed moisture from the air until the moisture content of the seed was in equilibrium with the surrounding air. Thus, the seed moisture content of soybean seeds fluctuated constantly during the length of our storage study. Barton (1943) reported similar findings, in which seeds of tomato (*Solanum lycopersicum* L.), dandelion (*Taraxacum officinale* F. H. Wigg.), onion (*Allium cepa* L.), and eggplant (*Solanum melongena* L.) stored in environments with fluctuating relative humidity for periods longer than 12 wk rapidly lost their viability. Seeds stored in environments with low relative humidity equilibrated at lower moisture contents (Barton, 1943). In the same study,

onion seeds placed in constant relative humidity of 35 or 55% retained their viability longer than those placed at a higher alternating relative humidity of 55 or 76% (Barton, 1943). The relative humidity fluctuations in the WH in our study resulted in changes in moisture content of seeds contributing to a rapid decline in seed viability and vigor. The rate of deterioration is directly proportional to the duration of storage of the seeds in this high relative humidity environment (Barton, 1943). Considering that seed lots in the WH maintained a standard germination percentage of >80% after 12 mo in storage, it is possible that the higher relative humidity values recorded just before the 16 mo evaluation could have increased the moisture contents of seeds and therefore increased the rate of deteriorative reactions resulting in a lower germination percentage (<20%) at 16 mo. The mean monthly temperatures during this period also increased in magnitude. High temperatures are known to increase the reaction rates by affecting enzymes that are involved in reactive oxygen species scavenging and repair (Bernal-Lugo and Leopold, 1998). Another explanation for this sharp decline in seed viability at 16 mo of storage could be the accelerated progression of seed deterioration in the WH environment. After the sharp decline in seed vigor, recorded in soybean seed, stored in the WH for 12 mo (Fig. 1J), seed viability declined rapidly soon after.

The decrease in seed viability in CS and WS was almost imperceptible up to 16 mo. The difference in temperature and relative humidity in these two environments likely played a key role in the rate of seed deterioration and in the loss of seed viability. The lower relative humidity in the WS kept the moisture content of seeds low, which thus slowed the deterioration process (Barton, 1943; Bernal-Lugo and Leopold, 1998; Harrington, 1973). The effect of higher relative humidity in CS increased the seed moisture content from 4 to 6% points. However, the lower temperature in this environment slowed the rate of loss in seed viability. Similar results were obtained in studies with six soybean varieties, in which the decrease in germination over time was exponential at higher temperatures and near linear at lower temperatures. However, the relative humidity was not stated (Burris, 1980). Vieira et al. (2001) observed that the seed vigor determined by electrical conductivity of seeds, transferred from a high temperature environment to low temperature environment, remained unchanged. However, the authors did not consider the relative humidity in the storage environment. Because the loss of electrolytes from a seed is influenced by the stability of the membranes, they concluded that the lower temperatures somehow stabilized the membranes.

Changes in seed vigor were observed 4 mo after storage, and seed vigor decline continued at a steady rate in all storage environments. The vigor of fungicide-treated seeds stored in the CS declined initially and 4 mo later increased to >80%. Other studies have also documented similar initial seed vigor decline and a subsequent seed vigor increase for seed lots stored in continuous low temperature and low relative humidity environments (De Vries et al., 2007; Houston, 1973; Krueger et al., 2012; Moore and Roos, 1982). The reason for this fluctuation is still unknown.

The decline of seed vigor in soybean seeds from all storage environments preceded the decline in seed viability for the same environment. Prior research has demonstrated that deteriorated seed lots can have high seed germination percentages if the embryo axes, including the meristematic cells of the radicle and the plumule, are able to germinate and produce a seedling under ideal conditions (Byrd and Delouche, 1971; Harrington, 1973). The standard germination test provides the seed with ideal temperature and moisture conditions for the germination (AOSA, 2012). Hence, a deteriorated seed may still produce a normal or weak seedling in the standard germination test even if most of the cells in the seed are deteriorated. In contrast, the AA test is a stress test (AOSA, 2009) and only seeds with little or no deterioration can germinate after being subjected to this stress (Delouche and Baskin, 1973; Bernal-Lugo and Leopold, 1998). Byrd and Delouche (1971) also observed soybean seed sensitivity to AA treatment, before any loss of seed viability. Therefore, the AA test is more sensitive in detecting seed vigor changes than the standard germination test.

The fungicide and fungicide plus insecticide seed treatments may be advantageous in lengthening seed storability, as treated seeds had higher germination and vigor percentages than the untreated seeds. Seed treatments are usually applied to protect the seed from soilborne and seedborne fungi and insect pests. In addition, some treatments may induce plant defense responses, in cases of increased stress, and ultimately improve growth and yield (Bartlett et al., 2002; Munkvold, 2009). For example, the application of captan (N-trichloromethylmercapto-4-cyclo-hexene-l,2-dicarboximide) (fungicide) as a seed treatment to medium and low vigor soybean seed, stored at 40°C and 12.6 to 13.1% moisture content, significantly increased germination compared to high vigor seeds (Edje and Burris, 1971). However, the storability of treated seeds was not evaluated. To our knowledge, our study is the first to assess storability of treated seed and to show that seed treatments can be advantageous for seed survival in storage. This information is of critical importance to the seed industry because most soybean seed lots are treated before storage.

The mechanisms by which seed treatments slowed down deteriorative reactions under all three storage environments of our study are not known. However, during the periodic evaluations of seed viability and vigor, we observed that treated seed had fewer fungi than untreated seed, especially in seed lots stored in the WS and in the WH, where temperature and relative humidity conditions were conducive for colonization and growth of storage fungi (data not shown). In the future, to better access the effect of seed treatments on seed health during storage, it

will be necessary to collect data on fungal incidence. In addition, the response to seed treatments seemed to depend on the storage temperature and relative humidity. Therefore, low temperature and relative humidity synergistically minimized aging reactions (Bernal-Lugo and Leopold, 1998; Bruni and Leopold, 1991; Burris, 1980; Delouche and Baskin, 1973; Parish and Leopold, 1978; Walters et al., 2005) and fungal colonization in the treated seeds. Burris (1980) also noted that temperature and relative humidity had both separate and combined influences on soybean seed vigor and viability. Seed moisture content influences the level of infection by storage fungi as well. Fungi such as *Fusarium*, *Cercospora*, and *Phomopsis* can degrade storage protein and oil of soybeans (Wilson et al., 1995). Although the initial fungi load did not significantly contribute to the deterioration process, it is possible that the rate of development of storage fungi during the storage period was detrimental to the viability and vigor of the soybean seeds. Future work could address this problem.

The total oil content of soybean seeds did not significantly influence their seed viability in the three storage environments. On the other hand, seed oil content significantly affected seed vigor. The effect of seed oil content on the decline in seed vigor, however, was not as strong as expected as demonstrated by the low coefficients of determination. The effect of oil content on seed vigor was evident across the three environments at 12 mo of storage. Interestingly, the relationship was positive implying that higher oil content seeds were more vigorous than low oil content seeds. These results were surprising as high oil content in seeds is commonly associated with poor seed storage. However, the computed confidence interval for the three slopes was found to be significantly positive (data not shown). A positive relationship between seed viability and oil content in soybean seed was observed 1 yr in seed lots were grown in two different growing season (LeVan et al., 2008). The authors reported that this relationship was inversed the following growing season indicating strong genotype × environment effect for seed viability and oil content in seeds. An evaluation of the fatty acid profile may reveal the real response to vigor over time as the ratio of saturated and unsaturated fatty acids is very important in the genesis and maintenance of lipid peroxidation (Bewley and Black, 1994). Sun et al. (2007) defined seed vigor as a quantitative trait that is affected by many factors, and that vigor is measured through individual traits among which are germination, seedling length, root length, seedling fresh weight, and seed longevity. The physiological process associated with seed oil content is peroxidation of membrane lipids (Harrington, 1973; Walters et al., 2005). This phenomenon has been proposed as the main cause for seed deterioration and is directly linked to membrane integrity of the seeds (Bewley and Black, 1994). Because seed vigor is controlled by multigene loci, most of which have relatively small effects (Sun et al., 2007), the seed oil content of the varieties used in our study accounted for only ≤15% of seed vigor decline in the different storage environments. Comparable results were obtained in studies for other quantitative traits associated with seed vigor in rice (Redona and Mackill, 1996).

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

The choice of storage environments may depend on the value of the soybean seed to be stored and the duration of storage. Burris (1980) suggested that drying soybean seeds down to 8 to 10% moisture level before storing at low temperatures and relative humidity could maintain acceptable seed quality for at least 3 yr. Our results are in support of this suggestion because after 20 mo, the viability of seeds stored in the CS and the WS was still >92 and >78%, respectively. However, seed vigor declined sharply under the same storage environments. The fact that seed viability was still very high in the CS and the WS at the end of our experiment indicates that seed viability alone is not a good indicator of seed quality in storage (Egli and Tekrony, 1995).

In all three storage environments used in our study, deteriorative reactions were occurring at different rates, depending on the moisture content at which seeds equilibrated, based on the temperature and relative humidity of each storage environment. Seed vigor continued to decline even in the CS. Presumably, the predominant degradative reaction of the seed stored in the CS was nonenzymatic lipid peroxidation, as the seed moisture content of the seed lots was below the threshold for activating enzymatic lipid peroxidation and sugar hydrolysis within the seeds (Shih et al., 2004; Sun and Leopold, 1995). Therefore, seed vigor was maintained at commercially acceptable vigor levels of ≥80% in the CS after 12 mo in storage. Optimization of the CS conditions could result in high soybean seed viability and vigor levels in storage and longer storage times. Furthermore, prolonging good soybean seed viability and vigor of treated seed in storage could reduce the need for disposal of treated seeds (Krueger et al., 2012). It is important to use the best storage environments to prolong seed viability and vigor of treated seeds. These results are critical to the seed industry since seed vigor of ≥80% is recommended for good seed emergence and stand establishment in soybeans (Egli

and Tekrony, 1995). If seed companies are storing seeds in an uncontrolled environment or WH they might consider treating the seed before storage as our results indicated that treated seed exhibited an advantage in storage longevity.

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