

Changes in Viability and Microflora of Soybean Seeds Stored under Various Conditions in Puerto Rico^{1, 2}

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ABSTRACT

Woodworth soybean (*Glycine max*) seed lots were stored under tropical conditions at two locations of the University of Puerto Rico, in four types of containers at three moisture levels, arranged in a completely randomized factorial design. Germination overall factors decreased with time. Germination decreased with increased length of storage and was accompanied by a concomitant increase in the occurrence of *Bacillus subtilis*. After 9 mo, the seeds with an initial moisture content (dry basis) of 8.6% had a higher germination and lower occurrence of seed-borne microorganisms than those stored with 10.7% and 12.5%. Seeds stored in 200-g sealed food cans maintained germination longer than those stored in nonsealed cans with a plastic lining, plastic-lined fertilizer bags, or in cloth bags. Seeds stored in cloth bags had a greater incidence of *B. subtilis* than those from the other storage containers. Whether or not there were significant differences between storage location for any main effects or interactions depended upon the parameter measured.

INTRODUCTION

Soybean (*Glycine max* (L.) Merr.) seed viability measured by in vitro germination is reduced by storage in temperate regions by several factors, including microorganisms (7, 9, 10, 11, 13, 14, 17), temperature (1, 13), moisture level (8, 10, 13), field history before harvest (7), and storage container (8).

Kennedy (9), Ramstad and Geddes (10), and Tervet (17) reported the association of *Aspergillus* spp. with reduced viability of soybean seeds in storage. *Penicillium* spp. also was found to invade soybean seeds (7). Tedia (13) showed that the microorganisms most frequently associated with the decline in vigor of soybean seeds stored at high temperature (35° C) and moisture (13%) were *Aspergillus* spp. and *Bacillus subtilis*. *B. subtilis* has been shown to be seed-borne in soybean (15). Soybean seed viability decreases with increased moisture levels from 9.4% to 19.1% at room temperature (10).

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Baciu (1) showed that soybean seeds stored in glass containers sealed with paraffin or in double polyethylene bags at 10 to 13° C had higher germination and seedling vigor than seeds stored in more air- and moisture-permeable containers. He concluded that this was due to fluctuations in seed moisture. Harrington (8) found that the storage of seeds at low moisture levels in moisture-proof packages prolonged seed viability.

The effect of various containers and storage conditions on viability and on the microorganisms associated with soybean seeds is reported in this paper. The work was carried out under tropical conditions at the University of Puerto Rico, Mayagüez (UPRM) and at the agricultural experiment station in Isabela.

SEED STORAGE EXPERIMENTAL DESIGN

LOCATION					
Mayagüez			Isabela		
STORAGE CONDITIONS					
Ambient			Insulated		
CONTAINERS					
C1		C2		C3 C4	
MOISTURE CONTENTS					
8.6%		10.7%		12.5%	
STORAGE PERIODS					
3 months		6 months		9 months	
REPLICATIONS					
1		2		3	

FIG. 1.—Experimental design for storage experiment conducted in Puerto Rico at the Mayagüez Campus and the Agricultural Experimental Substation in Isabela. C1 = sealed metal can; C2 = metal can with plastic lining (not sealed); C3 = fertilizer bag with plastic lining; and C4 = cloth bag. MCDB = moisture content dry basis.

MATERIALS AND METHODS

The study was designed for factorial analysis, using 432 200-g samples to determine the effect of three levels of seed moisture (8.6, 10.7, and 12.5% dry basis), four storage containers (a sealed 200-g metal food can, a nonsealed can with 1 mil plastic lining, a fertilizer bag with 1 mil plastic lining, or a cloth bag), two storage conditions (open shelves or packed in rice hulls in cardboard containers), and three storage times (3, 6, or 9 mo) (14). There were 144 samples for each storage time (fig. 1).

Two kinds of assays were made on each seed lot after each storage period. All seeds were surface disinfected by soaking in 0.5% sodium hypochlorite (Clorox)⁴ for 4 min, 70% ethanol for 2 min, and rinsed in

⁴ Trade names are used in this publication solely for the purpose of providing specific information. Mention of a trade name does not constitute a guarantee or warranty of equipment or materials by the Agricultural Experiment Station of the University of Puerto Rico or an endorsement over other equipment or materials not mentioned.

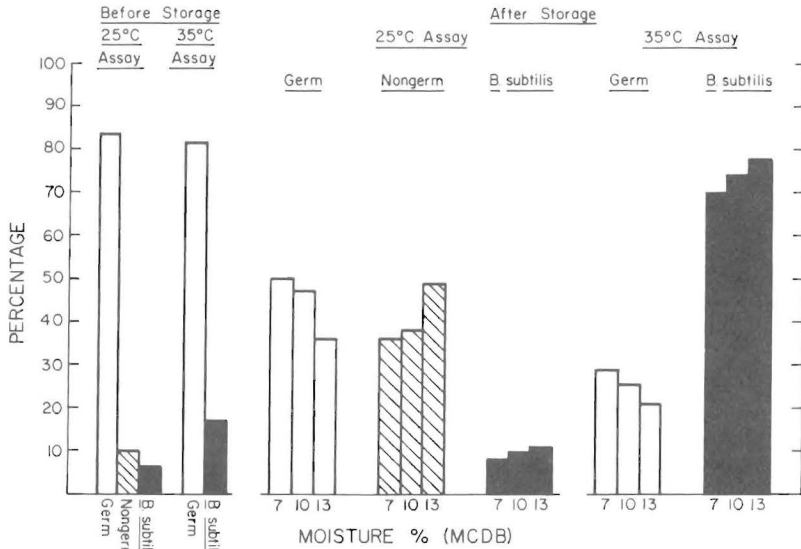


FIG. 3.—Mean percentage germination, nongermination, and occurrence of *Bacillus subtilis* from Woodworth soybean seeds assayed after harvest at 25 and 35° C after 9 mo storage for seeds stored initially at 7, 10, and 13% moisture content dry basis at ambient conditions in Puerto Rico.

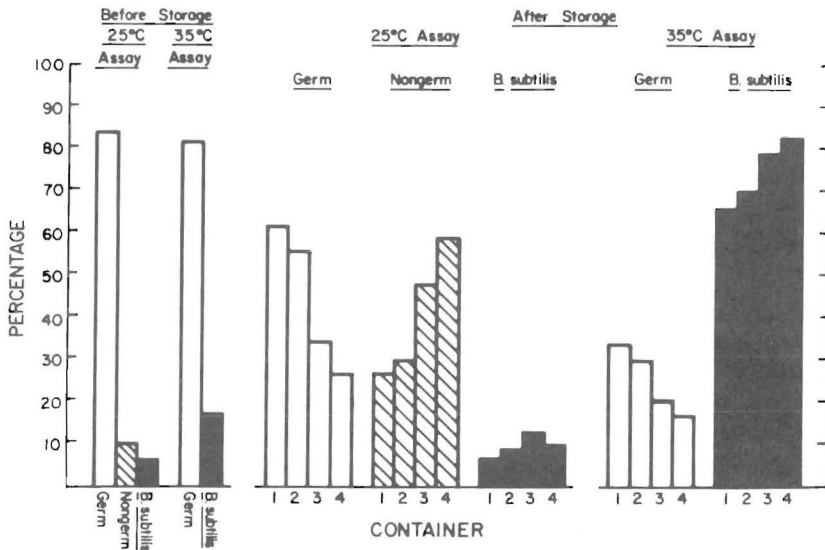


FIG. 4.—Mean percentage germination, nongermination, and occurrence of *Bacillus subtilis* from Woodworth soybean seeds assayed in Puerto Rico after harvest at 25 and 35° C before and after 9 mo storage in the containers of: 1) sealed metal can; 2) unsealed metal can with plastic lining; 3) fertilizer bag; and 4) cloth bag.

cultures; and size of cells and spore production. All isolates from the affected soybean seeds reacted identically to the ATCC isolate of *B. subtilis*.

RESULTS

The nontreated (not stored) seed lots assayed on PDA germinated 84%. Ten percent of the nongerminated seeds contained no microorganisms and 6% contained *B. subtilis*. Seed placed on cellulose pads ger-

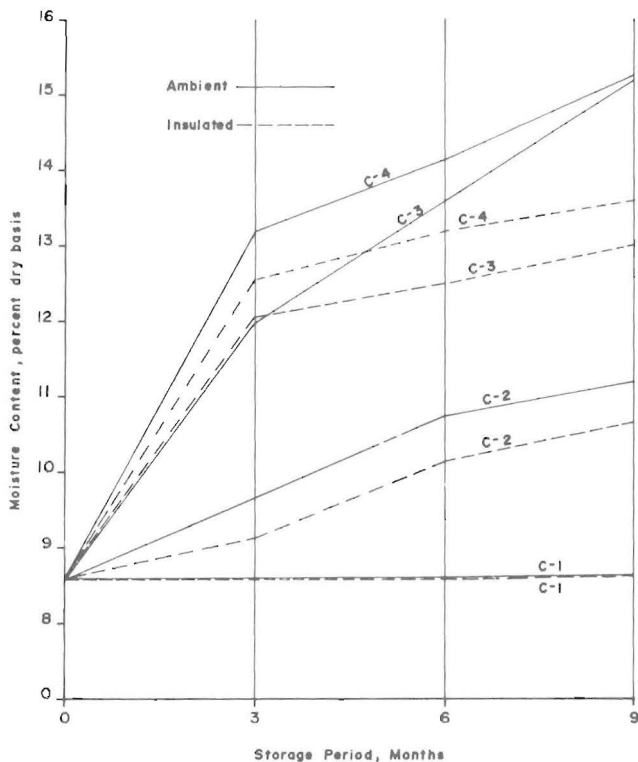


FIG. 5.—Changes in moisture content (dry basis) of Woodworth soybean seeds stored for 3, 6, or 9 mo under ambient conditions in sealed metal food cans (C₁); metal cans with plastic lining (not sealed) (C₂); fertilizer bags with plastic linings (C₃); and cloth bags (C₄).

minated 86.6%, and 14.4% of the nongerminated seeds had *B. subtilis* associated with them.

Results from both assays showed that, as storage time increased, there was concomitant decrease in germination and an increase in the recovery of *B. subtilis* (fig. 2). There was a high incidence of nongerminating seeds without microorganisms at the 25° C assay, while at 35° C, there was a high percentage of *B. subtilis* associated with nongerminated seeds. This

TABLE 1.—The main effects for the factors of location, storage condition, container, seed moisture content, and storage time, and all their possible interactions, for surface-disinfected seeds for the parameters of germination without microorganisms, nongermination without microorganisms, and with *Bacillus subtilis*, on potato-dextrose agar for 7 days at 25° C, and germination without microorganisms and nongermination with *Bacillus subtilis* on cellulose pads for 5 days at 35° C

Source	Degrees of freedom	Potato-dextrose agar			Cellulose pads	
		Germination	Nongermination		Germination	Nongermination
		Without microorganisms	Without microorganisms	With <i>Bacillus subtilis</i>	Without microorganisms	With <i>Bacillus subtilis</i>
A ¹	1	0.71 NS ²	19.56**	33.13**	67.57**	67.57**
B	1	11.65** ³	.63 NS	24.71**	.21 NS	.21 NS
C	3	254.70**	140.20**	9.13**	83.49**	83.49**
D	2	64.21**	43.03**	4.00**	31.85**	31.85**
E	2	1257.76**	613.14**	144.52**	1768.71**	1768.71**
A × B	1	1.64 NS	10.56**	40.00**	2.61 NS	2.61 NS
A × C	3	10.78**	7.27**	3.76*	41.26**	41.26**
A × D	2	.45 NS	1.56 NS	8.87**	11.11**	11.11**
A × E	2	6.41**	37.64**	31.90**	94.61**	94.61**
B × C	3	1.22 NS	1.77 NS	7.89**	1.04 NS	1.04 NS
B × D	2	.80 NS	1.46 NS	3.82*	.12 NS	.12 NS
B × E	2	9.97**	11.29**	4.64**	.35 NS	.35 NS
C × D	6	12.72**	7.53**	2.77*	6.13**	6.13**
C × E	6	66.79**	38.64**	6.01**	27.96**	27.96**
D × E	4	14.57**	6.85**	2.80*	11.86**	11.86**
A × B × C	3	.30 NS	1.28 NS	4.97**	.24 NS	.24 NS
A × B × D	2	1.79 NS	2.66 NS	.36 NS	4.00*	4.00*
A × B × E	2	3.39*	42.84**	47.39**	.44 NS	.44 NS
A × C × D	6	.81 NS	.86 NS	1.26 NS	3.13**	3.13**
A × C × E	6	4.12**	4.36**	6.81**	18.21**	18.21**

A × D × E	4	2.90*	1.22 NS	6.52**	6.61**	6.61**
B × C × D	6	2.28*	3.84**	5.64**	.52 NS	.52 NS
B × C × E	6	.95 NS	6.38**	9.34**	.82 NS	.82 NS
B × D × E	4	1.91 NS	2.16 NS	4.81**	.21 NS	.21 NS
C × D × E	12	7.31**	2.81**	2.87**	3.97**	3.97**
A × B × C × D	6	.82 NS	1.81 NS	3.92**	.47 NS	.47 NS
A × B × C × E	6	1.20 NS	4.20**	9.39**	2.23*	2.23*
A × B × D × E	4	1.11 NS	1.52 NS	.65 NS	.63 NS	.63 NS
A × C × D × E	12	1.61 NS	.81 NS	2.68**	2.29**	2.29**
B × C × D × E	12	2.58**	2.99**	4.92**	.65 NS	.65 NS
A × B × C × D × E	12	1.11 NS	3.31**	4.70**	.89 NS	.89 NS

¹ A = locations, B = storage conditions, C = containers, D = initial moisture contents, and E = storage periods (time).

² Calculated F values.

³ ** = Significant at the 1% level; * = significant at the 5% level; NS = nonsignificant.

illustrates the selectivity of the two assay temperatures for the development of the bacterium on soybean seeds.

Seeds with an initial moisture level of 8.6% had the highest percentage germination and lowest percentage of occurrence of *B. subtilis*, while those with an initial moisture level of 12.5% had the lowest percentage germination and the highest percentage of occurrence of *B. subtilis* at both assay temperatures (fig. 3). More nongerminated seeds contained microorganisms at 35° C than at 25° C assay.

Germination decreased and the percentage of *B. subtilis* increased as the container varied from sealed metal food cans to nonsealed metal food cans with plastic lining, fertilizer bag with plastic lining, and cloth bag, in that order (fig. 4).

Changes in germination and population of *B. subtilis* for various storage conditions investigated in this study seemed strongly associated with the changes in moisture content while in storage (fig. 5).

Location, containers, and initial moisture level of seeds were major factors which most influenced final seed quality. Interactions between these factors were measurable. The *F* values for the main effects of all experimental factors and their interactions are summarized in table 1.

DISCUSSION

This is the first report on the role of microorganisms in the deterioration of soybean seeds stored under tropical conditions. With the beginning of soybean production in the tropics, the development of proper storage conditions for the crop is an important consideration. The results show that microorganisms, particularly *B. subtilis*, can cause serious problems (figs. 2, 3, and 4).

Schiller et al. (11), Tedia (13), and Tenne et al. (16) reported similar results from studies in the temperate regions. *B. subtilis* is seed-borne in soybeans (15, 16), occurring in association with roots, stems, and leaves of soybean cultivars sensitive to high concentrations of phosphorus (4). It has been reported associated with soybean seeds in India (12) and six other countries, including the United States (16).

Low moisture content is a key factor in maintaining seed viability in storage. The results suggest that the ability of the container to minimize moisture gain during storage is as important as low initial moisture content in preserving seed viability in the tropics. Low moisture contents are unfavorable for the growth of microorganisms. Unprotected seeds absorb moisture from the air until equilibrium is reached. This is especially critical in the tropics where wide daily fluctuations of relative humidity occur, ranging from as low as 60% in the afternoon to a high of nearly 100% in the early morning. The key to preserving seed viability under the humid conditions in the tropics suggested by the results of this

study is to dry the seed to about 8.6% moisture content dry basis and store it in containers which minimize moisture gain from the atmosphere while the seed is in storage. These studies show that the best procedure for soybean seed was storage in sealed metal cans at 8.6% moisture. Similar results were reported by Tedia (13).

RESUMEN

En las condiciones tropicales de Puerto Rico, en dos campos de la Universidad de Puerto Rico (Mayagüez e Isabela) se almacenaron semillas de sojas de la variedad Woodworth. Se usaron cuatro tipos de envases a tres niveles de humedad en un diseño factorial distribuido completamente al azar. De todos los factores, la germinación fue el que más disminuyó con el tiempo. La germinación disminuyó según aumentó el tiempo de almacenamiento; al mismo tiempo aumentó la incidencia del *Bacillus subtilis*. A los 9 meses, las semillas con un contenido de humedad inicial de 8.6% tuvieron una germinación mayor y una incidencia menor de organismos portados por la semilla que las almacenadas con 10.7 y 12.5% de humedad. Semillas almacenadas en latas selladas de 200 gramos retuvieron la germinación por más tiempo que las almacenadas en latas sin sellar con revestimiento plástico o en sacos de tela. Semillas almacenadas en sacos de tela tuvieron una incidencia mayor de *B. subtilis* que las de otros tipos de envases. Para cualquiera de los efectos o interacciones mayores, dependiendo de la característica medida, hubo o no hubo diferencias significativas entre las localizaciones.

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