MICROBIOLOGICAL STUDY OF EXPORTED SOYBEANS

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ABSTRACT

An overseas shipment of bulk soybeans was studied microbiologically both before and after shipment. Samples were collected at four levels (0–3, 3–6, 6–9, 9–12 ft) in three holds of the ship. Each sample was analyzed microbiologically as the original sample, as whole intact beans, and as splits. Bacterial counts on the surface of intact beans did not change generally during shipment. Counts per gram of splits or damaged beans, however, increased from 6,400 to 170,000 before shipment to 36,000 to 2,100,000 after shipment. Fungal counts were generally higher on yeast extract agar than on Czapek's agar. No apparent increase in fungi on sound beans was observed; counts were much higher on the splits, ranging from 17 to 13,000 per gram, but did not increase after shipment. A small but rather uniform actinomycetous flora was found. A variety of fungi was encountered, the two most common forms on the surfaces of the beans were members of the *Aspergillus glaucus* group and species of *Penicillium*. Soybean germination was reduced during shipment except for those held at the lower level. Contamination of the beans by fungi increased markedly at the top three levels but not at the lowest level. The splits from all lots were heavily contaminated (66–98%), and the infection rate went up during shipment at all levels except the lowest. The fungi isolated from the soybeans were represented by many genera, with *A. glaucus* and *Penicillium* species being the ones most often encountered. *A. glaucus* infected whole beans, and splits were about equal. *Penicillium* species occurred three times more often in splits than in whole beans.

A number of reports (1–7) on microorganisms in and on harvested soybeans have been published, usually describing occurrence and numbers of bacterial and fungal pathogens. An exception to these reports is Kennedy's (8) article on the lipolytic microorganisms found in soybean seeds. Soybeans stored at room temperature were examined by plating whole surface-sterilized seeds or by preparing dilution plates with seeds chopped in a Waring Blendor. In making this study, Kennedy had to isolate the microorganisms present in typical lots of soybeans. He reported isolating bacteria, yeasts, and species of *Penicillium, Aspergillus, Alternaria, Fusarium, Rhizopus,* and *Helminthosporium*. Almost all microorganisms isolated were able to modify fatty substrates, but their total numbers were not given. Gangopadhyay *et al.* (1) found some of the same fungi from seed infected with *Macrophomina phaseoli,* and Nicholson and Sinclair (2) likewise reported a number of fungi from soybean seed. The last authors found the order of most to least abundant to be *Chaetomium, Cercospora, Alternaria, Sclerotinia, Aspergillus, Fusarium, Rhizopus, Penicillium, Pestalotia,* and *Thielavia*. Seed lots from Louisiana and Mississippi contained the highest numbers of fungi, while those from Texas, South Carolina, and Illinois had the least. Nicholson and Sinclair (3) found that with *in vitro* tests, soybean seed germination was as high as 96%, even when 8% of the seed was infected with *Pseudomonas glycinea.*

Dhingra *et al.* (4) reported the occurrence of *Aspergillus flavus* in soybeans. Twenty seed lots of Lee soybeans harvested in 1970 in Kentucky, Louisiana,

[1]The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

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TABLE I
Bacterial and Actinomycetous Counts on Soybeans Before Shipment From Toledo and After Arrival in Rotterdam[a]

Sample	Toledo			Rotterdam		
	Bacteria	Actinomycete	Moisture Content (%)	Bacteria	Actinomycete	Moisture Content (%)
Original						
Level 1[b]	810	20	10.5	800	35	8.8
Level 2	1,100	25	10.1	500	5	8.3
Level 3	89,000	40	9.6	880	5	8.6
Level 4	360	0	9.9	750	30	8.3
Whole beans						
Level 1	47,000	30	9.9	520	10	8.5
Level 2	520	15	10.3	480	15	8.4
Level 3	430	15	10.2	500	25	8.0
Level 4	570	15	8.6	550	25	7.9
Splits						
Level 1	9,300	55	8.8	1,100,000	59	8.2
Level 2	17,000	200	10.1	2,100,000	140	7.6
Level 3	170,000	100	9.7	1,800,000	70	8.2
Level 4	6,400	50	9.7	36,000	39	7.9

[a]Counts per gram.
[b]Level 1 was 0–3 ft; level 2, 3–6 ft; level 3, 6–9 ft; and level 4, 9–12 ft.

TABLE II
Fungal Counts of Soybean Surfaces on Yeast Extract Agar and Czapek's Agar Before and After Overseas Shipment to Rotterdam[a]

Sample	Toledo		Rotterdam	
	YXT[b]	Czapek	YXT	Czapek
Original				
Level 1[c]	120	35	65	40
Level 2	750	180	75	20
Level 3	180	120	210	75
Level 4	200	30	60	40
Whole beans				
Level 1	750	850	45	25
Level 2	75	30	55	45
Level 3	45	45	75	45
Level 4	45	10	25	20
Splits				
Level 1	770	130	290	180
Level 2	440	240	1,400	510
Level 3	520	410	490	300
Level 4	13,000	330	17	65

[a]Counts per gram.
[b]YXT = yeast extract agar.
[c]Level 1 was 0–3 ft; level 2, 3–6 ft; level 3, 6–9 ft; and level 4, 9–12 ft.

Mississippi, South Carolina, and Texas were examined for *A. flavus* by placing 400 surface-sterilized and nonsurface-sterilized seeds of each lot on moistened sterile filter paper disks in plates and incubating at 30 and 35° C. This species was found to be seedborne in 17 of the 20 lots. Of the 20% of this fungus recovered at 35° C, 2.7% represented internal contamination and 17.3% surface contamination. Increased incidence of *A. flavus* was significantly correlated with decrease in soybean germination. Kennedy (5) noted that with increased damage (for instance, after frost), the aerobic microflora increased and the percentage of viable seed decreased. The mold flora in soybeans after three to five months of storage included *Alternaria, Fusarium, Aspergillus,* and *Penicillium.* From surface-sterilized seeds, 15% of the seed from 23 samples collected in 1961 were infected, and 12% from 11 samples collected in 1962. Both yeasts and bacteria were prevalent, but kinds and numbers were not enumerated.

TABLE III
Distribution of Fungi Obtained From Dilution Plates From Original Samples, Whole Soybeans, and Splits at the Four Different Levels Within the Hold of the Ship at Time of Shipment and Delivery

	Originals									
	Levels									
	1[a]		2		3		4		1	
Fungi	T[b]	R[c]	T	R	T	R	T	R	T	R
Absidia	+[d]		+	+	+	+	+		⊞	+
Alternaria										
Aspergillus groups										
candidus	+	+	+		+			+		+
clavatus										
flavus			+	+	+	+			⊞	
fumigatus	+	+						+		
glaucus	+	⊞	⊞	⊞	⊞	⊞	⊞	+	⊞	⊞
niger			+						+	
ochraceous			+			+				
sydowii										
terreus	+				+					
versicolor				+						+
Cephalosporium	+									
Cladosporium		+								+
Fusarium	+		+				+			
Mucor					+					
Nonsporulating white mold		+		+						
Paecilomyces				+		+				
Penicillium	⊞	⊞	⊞	⊞	+	+	+	⊞	+	⊞
Rhizopus	+		+	+				+		
Syncephalastrum			+							
Unidentified mold				+		⊞				+
Yeast									+	

[a]Level 1 was 0–3 ft; level 2, 3–6 ft; level 3, 6–9 ft; and level 4, 9–12 ft.
[b]T = Toledo.
[c]R = Rotterdam.
[d]+ = fungus present.
[e]⊞ = predominant fungi.

Two further studies (6,7) on soybeans should be noted. In the first article, Christensen and Dorworth (6) reported that soybeans, even with a moisture as low as 13%, were invaded by *Aspergillus halophilicus* and *A. restrictus*. They also discovered that when Acme and Chippewa variety beans with different moisture contents were mixed together and stored, the Acme variety had a lower moisture content despite a higher initial moisture. Later, they (7) reported on soybeans stored at moisture levels from 12.1 to 18.3% at temperatures of 15–30° C. At 15° C, germination remained above 95% at all moisture levels except 18.3%. The fungi found to invade the seeds were *A. glaucus* and *Penicillium* species. Germination decreased with increasing infection by these fungi.

No studies have been made on the changes in the microbial flora of soybeans during international shipment. Hence, the objectives of our research were to determine a) the bacterial, actinomycetous, and fungal populations on the

Whole Beans						Splits							
Levels						Levels							
2		3		4		1		2		3		4	
T	R	T	R	T	R	T	R	T	R	T	R	T	R
	+		+			+	+	+	+	+	+	+	+
							+					+	
	+	+					+	+	+	+		+	+
		+			+					+			+
+	+		+			+	+	+	+	+	+	+	+
	+	+		+	+		+		+		+	+	
⊞	+	⊞	⊞	⊞	⊞	⊞	⊞	+	⊞	⊞	⊞	⊞	⊞
								+			+	+	
	+				+					+			
					+								
						+		+	+	+			
	+									+			
	+			+			+						
+		+	+			+		+		+		+	
											+		+
				+			+						
										+			+
+	⊞	⊞	⊞	⊞	⊞	⊞	⊞	⊞	⊞	+	+	+	⊞
						+	+	+	+	+	+	+	+
							+						
	+		+				+				+		
											+		

surface of U.S. Grade 2 soybeans before and after shipment, b) the incidence of molds growing from surface-sterilized whole beans as well as broken beans, and c) the extent of damage or deterioration of soybeans in transit.

MATERIALS AND METHODS

Collection of Samples

A test shipment of mixed lots of U.S. Grade 2 soybeans was loaded into three holds of the motor vessel Nanfry, at Toledo, OH, on August 19, 1975. Agricultural Marketing Service (AMS) personnel performed the sampling at the loading site by probing each hold (12-ft depth) at four locations. Layers at each location were sampled at four depths (0–3, 3–6, 6–9, 9–12 ft) with a 12-ft probe and designated from top to bottom level 1, level 2, level 3, and level 4. Samples were then forwarded to AMS for physical determination of splits. Subsequently, samples were forwarded to Northern Regional Research Center (NRRC) where they were combined by level (four total) without regard to hold to yield 50-lb samples. Each level sample was then divided into two fractions; one was maintained as the original, while the second fraction was further separated into whole beans and splits. Foreign material (2–3%) was removed from the split fraction by screening.

Identical sampling was conducted prior to unloading the ship September 2–4 at Rotterdam, The Netherlands. Again, samples were combined according to level. Consequently, for each level, three samples were obtained for analyses: a) original, b) whole beans, and c) split beans, for a total of 24 samples, 12 each at the origin and at the destination.

The moisture content of each sample was determined in a Brabender rapid moisture tester for 30 min at 130° C.

Microbiological Analyses

Surface counts of total aerobic bacteria, molds, and aerobic actinomycetes as well as the percentage of mold-contaminated seeds were determined according to the procedures outlined by Bothast *et al.* (9).

Eleven-gram samples were aseptically weighed into sterile dilution bottles containing 99 ml of 0.1% peptone water and approximately 10 g of sand. From this primary dilution (1:10), serial dilutions (without sand) were prepared. Each was shaken for 2 min, and appropriate dilutions were incorporated into melted and cooled medium plated in duplicate. Plates were incubated at 28° C.

The total aerobic bacteria were counted after three days in plates of standard methods agar (0.5% trypticase peptone, 0.2% yeast extract, 0.1% glucose, and 1.5% agar) to which 100 μg/ml of cycloheximide had been added to inhibit fungal growth.

The total mold population was determined after three days both in plates of yeast extract agar (YXT) (0.4% yeast extract, 1.0% malt extract, 0.4% dextrose, 1.5% agar), plus 30 μg/ml of tetracycline, and in plates of Czapek's agar, plus 20% sucrose (0.3% $NaNO_3$, 0.1% K_2HPO_4, 0.05% $MgSO_4 \cdot 7H_2O$, 0.05% KCl, 0.001% $FeSO_4 \cdot 7H_2O$, 1.5% agar, 20% sucrose) to which 30 μg/ml of tetracycline had been added to inhibit bacterial growth. The predominant sporulating molds were identified by morphological characteristics.

The number of aerobic actinomycetes were obtained from the standard

methods agar plates, which had been incubated a total of 14 days. Sporulating actinomycetous colonies were easily distinguished from the degenerate bacterial colonies and were counted after two weeks' incubation.

From each whole bean sample, 50 beans with unbroken seed coats were selected; from each split bean sample, 50 separated, whole cotyledons were selected. Beans from each sample were surface sterilized with 1% sodium hypochlorite for 1 min, followed by two washings in sterile, distilled water. The individual beans or cotyledons were placed on the surface of YXT plates (without tetracycline), five per plate, and incubated at 28° C for seven days. The fungi that grew out were identified on the basis of morphological characteristics. At the time the seedborne fungal contamination was being determined, the number of seeds germinating was recorded directly from the agar plates.

RESULTS AND DISCUSSION

The average moisture content of the original soybeans dropped from 10.0 to 8.5% during transit. Moisture content ranged from 10.5% in original beans from level 1 at Toledo to 7.9% in whole beans and splits from level 4 at the destination. Such moisture contents are not conducive to growth of microorganisms. Consequently, any changes in microbial counts may reflect a handling effect. As shown in Table I, bacterial counts were usually higher on split beans than on original or whole beans. Bacterial counts on original or whole beans remained constant or decreased during shipment from Toledo to Rotterdam, while counts on split beans increased 10–100-fold. With the exception of the sample from level 4, the bacterial count was over a million per gram of splits at Rotterdam. An actinomycetous flora was present in all samples and in all layers, but the numbers were quite low and appeared to remain relatively constant.

The fungal counts on the soybean surfaces are shown in Table II. In most instances, the counts were higher with YXT medium than with the Czapek agar.

The highest fungal count (13,000/g) was found in splits at the port of loading. Although not in such striking numbers as the bacteria count, more molds were found in the splits both before and after shipment than in the whole beans; there did not appear to be any increase in number during the sea voyage. The mold count at Rotterdam at the lowest level seemed to be less than that at the other

TABLE IV
Soybean Germination and Total Fungal Contamination in Whole Beans and Splits

	Germination of Whole Beans (%)		Internal Contamination			
			Whole Beans (%)		Splits (%)	
Level	Toledo	Rotterdam	Toledo	Rotterdam	Toledo	Rotterdam
Level 1[a]	100	94	6	24	84	98
Level 2	98	88	6	34	68	78
Level 3	95	94	9	20	72	83
Level 4	90	98	50	18	68	66

[a]Level 1 was 0–3 ft; level 2, 3–6 ft; level 3, 6–9 ft; and level 4, 9–12 ft.

levels, especially with the splits. This may have resulted from greatly reduced oxygen levels in the bottom of the hold where reproduction did not occur or death of spores resulted.

Table III summarizes the fungi detected from surface-sterilized beans. The most common species or genera are indicated. With a few exceptions, these were species in the *A. glaucus* group and species of *Penicillium*. In three samples, *A. flavus, Absidia,* and an unidentified mold were the dominant fungi.

The percentage of beans germinating before and after shipment at the various levels is shown in Table IV. There was a slight decrease in germination of beans from the first three levels: germination of beans collected at Toledo was 90–100% and at Rotterdam, 88–98%. The percentage of contaminated whole soybeans, however, was striking. In levels 1, 2, and 3, the percentage of contamination seed before shipment was 6–9%, but after shipment was 20–34%. Contamination of beans at the lowest level in the ship was higher at origin and decreased during shipment. The initial high level of contamination might reflect a sampling problem, since contamination after shipment was close to that observed in levels

TABLE V
Summary of Contamination of Whole Beans and Splits at Four Different Levels in Ships at Toledo and Rotterdam[a]

	Whole Beans								
	Levels[b]								
	1		2		3		4		
Microorganism	T[c]	R[d]	T	R	T	R	T	R	Total
Bacteria[d]	2	1	1	4	3	1	4	2	18
Molds									
Absidia									0
Alternaria		1							1
Aspergillus									
candidus		2					2		4
flavus		1				1	1		3
fumigatus		1						1	2
glaucus		5	2	5		7	15	2	36
niger		1							1
ochraceous									0
terreus				1					1
Chaetomium									0
Fusarium							1		1
Nonsporulating black	1			3			1		5
Nonsporulating white		1		1					2
Penicillium		1		1	1	1	1	4	9
Phomopsis						1			1
Rhizopus				1	1				2

[a]Number of colonies found in 50 kernels.
[b]Level 1 was 0–3 ft; level 2, 3–6 ft; level 3, 6–9 ft; and level 4, 9–12 ft.
[c]T = Toledo.
[d]R = Rotterdam.
[c]Based on 45 kernels.
[l]Based on 35 kernels.

1, 2, and 3. The percentage of contaminated splits before shipment was high (68–82%) and increased during shipment except in the lowest hold level. The level 4 decrease of 2% is probably not significant.

The contaminating microorganisms detected in this study are listed in Table V. The kinds of microorganisms most common in whole beans were members of the *A. glaucus* group, bacteria, and *Penicillium* sp. In the splits, by far the most commonly isolated agents were bacteria, followed by *A. glaucus, Penicillium,* and unidentified nonsporulated white fungal colonies. The latter group does not represent a single species but probably a collection of diverse fungi, all with white mycelium. A few genera such as *Absidia* and *Chaetomium* did not occur in the whole beans but were found in four or more of the surface-sterilized splits.

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Splits								
Levels								
1		2		3		4		
T	R	T	R	T	R	T	R	Total
28[c]	33[c]	25	29	22	28[f]	26	18	209
1	1	1		1	1	1	1	7
								0
								0
	2	2	1	2				7
	1						1	2
1	5	6	5	9		4	4	34
1								1
	1							1
						1		1
	1	1					2	4
				2				2
	2							2
2	1	4		1		3	4	15
7	2	1	6	3	1	1	4	25
								0
1			2				1	4

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