(12) LEVEL II

AD

TECHNICAL REPORT
NATICK/TR-79/029

AD A074173

EVALUATION OF INSECT-RESISTANT-TREATED FOOD SACKS FOR MOLD RESISTANCE

by
B. J. Wiley
M. Greenberger
and
A. M. Kaplan

DDC
RECEIVED
SEP 25 1979
B

DDC FILE COPY

June 1979

Approved for public release;
distribution unlimited.

UNITED STATES ARMY
NATICK RESEARCH and DEVELOPMENT COMMAND
NATICK, MASSACHUSETTS 01760

79 09 24 012

Food Sciences Laboratory

Approved for public release; distribution unlimited.

Citation of trade names in this report does not constitute an official indorsement or approval of the use of such items.

Destroy this report when no longer needed. Do not return it to the originator.

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER: NATICK/TR-79/029	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle): EVALUATION OF INSECT-RESISTANT-TREATED FOOD SACKS FOR MOLD RESISTANCE		5. TYPE OF REPORT & PERIOD COVERED: Final Report June 1979
		6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s): B. J. Wiley, M. Greenberger, and A. M. Kaplan		8. CONTRACT OR GRANT NUMBER(s)
9. PERFORMING ORGANIZATION NAME AND ADDRESS: Food Sciences Laboratory, DRDNA-YEP, US Army Natick Research and Development Command, Natick, MA 01760		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS: 6.2, 1L162724AH99, Work Unit BD-002
11. CONTROLLING OFFICE NAME AND ADDRESS: US Army Natick Research and Development Command, Food Sciences Laboratory, DRDNA-YEP, Natick, MA 01760		12. REPORT DATE: June 1979
		13. NUMBER OF PAGES: 31
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)		15. SECURITY CLASS. (of this report): Unclassified
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release, distribution unlimited.

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

INSECT-RESISTANCE	AFLATOXINS	MICROBIAL DETERIORATION
PYRETHRINS	OCHRATOXIN	PACKAGING MATERIALS
PIPERONYL BUTOXIDE	ENVIRONMENTAL STUDIES	PACKAGING
MOLD-RESISTANCE	FUNGAL-PENETRATION	MATERIALS
MYCOTOXINS	MICROORGANISMS	FOOD SACKS
		STORAGE

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Insect-Resistant-Treated (IRT) food sacks were evaluated for mold resistance under field conditions, and in the laboratory using plate, tropical chamber and soil burial tests. Penetration of fungal growth through the layers of the multiwall food sacks was also evaluated. Mycotoxin production was determined using known aflatoxin-producing fungus strains as controls. This determination was needed because 15 of the 69 strains of fungi isolated from the food sack samples used in the field study represented two groups of organisms known to produce carcinogenic metabolites during growth.

DD FORM 1473, 1 JAN 73 — EDITION OF 1 NOV 65 IS OBSOLETE

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

PREFACE

Insect-Resistant-Treated (IRT) food sacks are required mostly for storage of wheat flour in Department of Defense (DoD) supply systems. Since DoD uses a relatively small number of these sacks, consideration is being given to using the same type of sack used by the United States Department of Agriculture (USDA) for its Food for Peace program. We thank Dr. Henry Highland of the Stored Product-Insects Research Laboratory, USDA for supplying the standard IRT and experimental test sacks used in our studies, and for the interim report of the USDA field studies conducted in Santo Domingo, Dominican Republic.

ACCESSION for
NTIS White Section ☑
DDC Buff Section ☐
UNANNOUNCED ☐
JUSTIFICATION
BY
DISTRIBUTION/AVAILABILITY CODES
Dist. AVAIL. and/or SPECIAL
A

TABLE OF CONTENTS

	Page
LIST OF FIGURES	4
LIST OF TABLES	4
INTRODUCTION	5
MATERIALS AND METHODS	6
Storage Tests	7
Fungal Resistance Test	8
Soil Burial and Tropical Chamber Exposure	10
Mold Penetration Studies	12
Mold Identification	12
Mycotoxin Production	13
Determination of Aflatoxin Production on IRT Food Sacks	16
RESULTS	16
Storage Tests	16
Fungal Resistance Test	18
Soil Burial and Tropical Chamber Exposure	19
Mold Penetration Studies	21
Mold Identification	21
Mycotoxin Production	26
Determination of Aflatoxin Production on IRT Food Sacks	26
DISCUSSION	27
CONCLUSIONS	29
REFERENCES	30

LIST OF FIGURES

		Page
Figure 1.	Scaled-down Food Sack System	11
Figure 2.	Food Jar System	11
Figure 3.	IRT Sacks Under Test in the Dominican Republic	17
Figure 4.	IRT Sacks after Seven Weeks of Incubation Showing Moldy Patches	18
Figure 5.	SEM of Fungal Growth on Underside of Outer Layer of IRT Sack No. 1	23
Figure 6.	SEM of Fungal Growth on Underside of Outer Layer of IRT Sack No. 3	23
Figure 7.	Sample of the Standard IRT Sack as Received from the Dominican Republic	24
Figure 8.	Sample of the IRT Test Sack as Received from the Dominican Republic	24

LIST OF TABLES

		Page
Table 1.	Construction of IRT Sacks	7
Table 2.	Nutrient Salts Medium	8
Table 3.	Fungi Used in Preparing Mixed Spore Suspension	9
Table 4.	Hay Infusion Agar	13
Table 5.	Glucose-Ammonium Nitrate Medium (GAN)	14
Table 6.	Mycotoxin-Producing QM Strains	14
Table 7.	Microbial Evaluation of IRT Sacks	18
Table 8.	Results from Soil Burial Test	19
Table 9.	Data from Soil and Tropical Chamber Exposed Jar Systems	21
Table 10.	Microbial Penetration Study of IRT Sacks	22
Table 11.	Fungi Isolated from Deteriorated IRT Sacks	25

EVALUATION OF INSECT-RESISTANT-TREATED FOOD SACKS FOR MOLD RESISTANCE

INTRODUCTION

Insect-Resistant-Treated (IRT) food sacks, developed by the United States Department of Agriculture (USDA) are used to store and ship large quantities of a number of processed cereal grains as part of the Food for Peace program of the Agency for International Development (AID).[1]

The IR treatment consists of pyrethrins, synergized with piperonyl butoxide. Although the treatment is applied to the outer kraft layer of the sacks, piperonyl butoxide can migrate through multiwall kraft layers and through the food-enveloping polyethylene (PE) liner.[2] Highland, _et al_. have reported that this migration can be prevented by incorporation of a greaseproof layer in the construction of the sack.[3]

Large quantities of filled sacks are shipped from Great Lakes ports during cool and cold weather. During long shipping periods, the ambient temperature increases and moisture condenses on the sacks which encourages mold growth on spilled materials adhering to the sacks. Consequently, the growth weakens the paper with loss of the insect-resistance and possible formation of toxic fungal metabolic products (mycotoxins).

---

[1] Highland, H.A. 1976. Interim Report on Storage Test with Experimental IRT Mold Resistant Shipping Sacks. (Unpublished).

[2] Highland, H.A., E.G. Jay, M. Phillips and D.F. Davis. 1966. The Migration of Piperonyl Butoxide from Treated Multiwall Kraft Bags into Four Commodities. J. Econ. Entomol. 59: 543-545.

[3] Highland, H.A., M. Secreast and P.A. Merritt. 1970. Packaging Materials as Barriers to Piperonyl Butoxide Migration. J. Econ. Entomol. 63: 7-10.

Preliminary laboratory tests by the sack manufacturer indicated that the antimicrobial agent n-dodecylguanidine hydrochloride is ineffective in preventing mold formation on the surface of the sack, but that a thin PE coating on the outer kraft layer would prevent mold penetration.[4] Dr. Highland reported that a thin exterior PE coating would make the IRT shipping sack mold resistant.[5]

Since IRT shipping sacks are also required for the shipment and storage of wheat flour in Department of Defense (DoD) supply systems, it had been suggested that DoD use the same sack now used by AID.

In December 1975, a study was initiated in our laboratory to evaluate four types of IRT food sacks for their ability to resist microbial growth.

MATERIALS AND METHODS

Construction of four types of IRT sacks manufactured by St. Regis Paper Co. for USDA is shown in Table 1. Sample 1 was the standard sack now in use; samples 2, 3, and 4 were newly developed test sacks protected by the recommended PE coating.[6]

---

[4]See footnote 1.

[5]Highland, H.A. 1978. Insect Resistant Package Design, p. 36, In: Proceedings of Fall 1977 Meeting, Research and Development Associates for Military Food and Packaging Systems, Inc. 4-6 October 1977.

[6]See footnote 1.

TABLE 1. CONSTRUCTION OF IRT SACKS

Sack No.	Construction
1	3-mil (76.2 μm) PE liner/four 50-lb (22.7 kg) plies/ one 60-lb (27.3 kg) IRT ply
2	76.2-μm PE liner/four 22.7 kg kraft plies/one 22.7 kg IRT, PE-coated ply
3	76.2-μm PE liner/three 22.7 kg kraft plies/one 40-lb (18.2 kg) greaseproof ply/one 22.7 kg IRT, PE-coated ply
4	76.2-μm high impact PE liner/three 22.7 kg kraft plies/ one 18.2 kg greaseproof ply/one 22.7 kg IRT, PE-coated ply

Storage Tests

A field test to evaluate the efficacy of the PE coating in preventing fungal damage to the shipping sack was conducted by the USDA Stored Product-Insects Research and Development Laboratory, Savannah, GA at Cooperative for American Remittances to Europe, Inc. (CARE) warehouses in Santo Domingo, Dominican Republic. Sacks filled with corn meal-soy-milk mixture (CSM) were shipped from Milwaukee, WI on December 4, 1975, and arrived in Santo Domingo on December 18, 1975. Two pallets, each containing 60 sacks of two types were set up. A PE sheet separated each type of sack and the sacks were stacked 10 tiers high. A small quantity of CSM and water was sprinkled on each tier. The loaded pallets were then wrapped with PE and allowed to incubate for seven weeks.[7] After that time, samples of the standard IRT sack (Control Bag 7) and of a

[7] See footnote 1.

test sack (Test Bag 3) were sent to the Environmental Protection Group, FSL, NARADCOM, for identification of the molds.

Fungal Resistance Test

Plate testing was done using the American Society for Testing and Materials (ASTM) G21-70 procedure[8] which involves:

1) placing the specimen onto the surface of an agar medium (Table 2) in petri plates,

2) inoculating the specimen with the suitable organisms (Table 3),

3) incubating the inoculated specimens under conditions suitable for growth, and

4) examining and rating for visual growth.

TABLE 2. NUTRIENT SALTS MEDIUM*

KH_2PO_4	0.7 g
K_2HPO_4	0.7 g
$MgSO_4 \cdot 7H_2O$	0.7 g
NH_4NO_3	1.0 g
NaCl	0.005 g
$FeSO_4 \cdot 7H_2O$	0.002 g
$ZnSO_4 \cdot 7H_2O$	0.002 g
$MnSO_4 \cdot H_2O$	0.001 g
Agar	15.0 g
Distilled H_2O	1.0 L

*pH 6.0 to 6.5, autoclaved at 121°C for 20 minutes at 15 psi (1.03 x 10^5 Pa).

---

[8]Lukens, R. P. Managing Standards Editor. 1976. Annual Book of ASTM Standards. Part 35. ASTM Designation: G21-70 (Reapproved 1975). American Society for Testing and Materials. Philadelphia, PA.

TABLE 3. FUNGI USED IN PREPARING MIXED SPORE SUSPENSION

QM 279c	Aureobasidium pullulans
QM 365	Gliocladium virens
QM 380	Aspergillus flavus*
QM 386	Aspergillus niger
QM 391	Penicillium funiculosum
QM 432	Aspergillus versicolor*
QM 459	Chaetomium globosum

*Not required in the specification, but organisms of specific interest may be included.

A spore suspension of each of the organisms listed in Table 3 was prepared by adding 10 milliliters (mL) of sterile distilled water containing 0.05 g/L of a nontoxic wetting agent (Tween 80) to a 2 to 3 week-old culture and scraping the surface growth with a sterile inoculating loop to dislodge the spores. The resultant suspension was then filtered through sterile glass wool to remove hyphal fragments. Sterile distilled water was added to 50 mL. The suspension was centrifuged aseptically and the supernatant solution was discarded. The pellet was washed twice by resuspending in 50 mL of sterile distilled water, centrifuging, and discarding the supernatant solution. The final pellet was diluted with sterile nutrient salts solution so that the resultant spore suspension contained $1 \times 10^6 \pm 2 \times 10^5$ spores/mL determined with a Levy counting chamber. Equal volumes of each spore suspension were blended to obtain the final mixture.

Samples of the six-layered sacks were cut into approximately 5 cm squares which were ethylene oxide (ETO) sterilized, together with filter paper control discs (Whatman #1, 5.5 cm diameter circles). Three specimens and three controls were prepared from samples for each type of sack.

Specimens were placed onto the surface of nutrient salts agar plates, inoculated by spraying with the mixed spore suspension, and incubated at 28° ± 2°C for a minimum of 21 days, the growth being observed and recorded at weekly intervals.

Soil Burial and Tropical Chamber Exposure

Small 2 x 2-inch sacks (5 x 5 cm) were fashioned from the four types of IRT food sacks. Paper from the sack materials was cut into 4 x 2-inch (10 x 5 cm) strips and then hot-glued to form a pouch. The PE inner liner was separately heat-sealed on three sides, filled with CSM, and heat-sealed on the fourth side. This food packet was placed into the paper pouch, and the sack was sealed on its fourth side. These were placed in a soil bed and incubated at 30°C and 95% relative humidity (RH) in a tropical chamber (Figure 1).

A second experiment was designed to direct fungal attack to the outer surface of the sack material. A hole (3.7 cm diameter) was drilled into the center of bakelite-type plastic caps of 2-oz (57 g) glass jars. Test circles, 4.9 cm diameter, were cut from the food sack materials and cemented with silicone rubber adhesive to the inside surface of the caps and allowed to set overnight. A small amount of CSM was placed in each

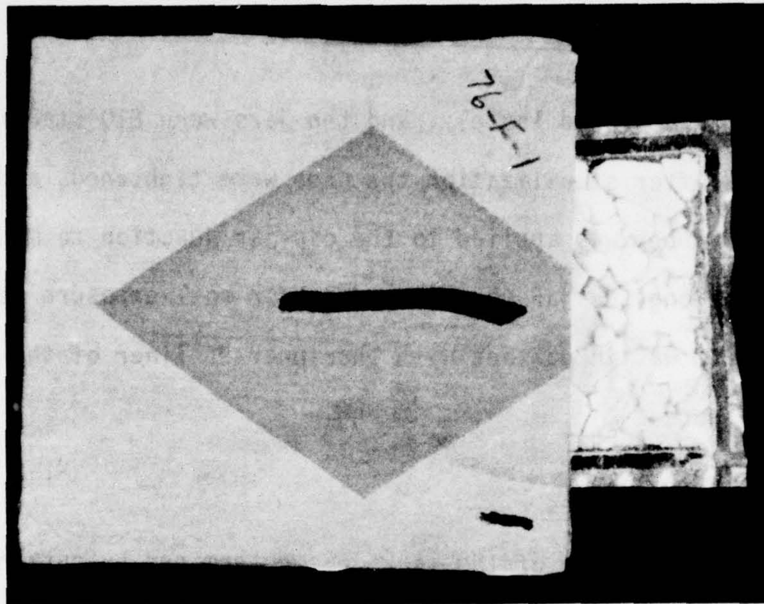

Figure 1. Scaled-down Food Sack System

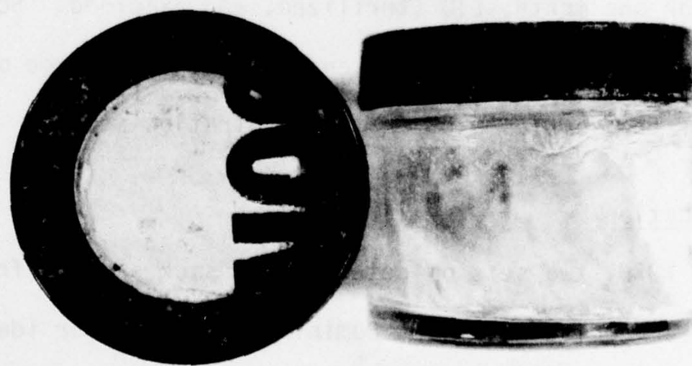

Figure 2. Food Jar System

jar, caps were attached loosely, and the jars were ETO sterilized for five hours. After sterilization, the caps were tightened, and a layer of silicone rubber was applied to the cap-jar junction to minimize penetration along the jar threads. Jars for soil exposure were inverted so that CSM was in contact with the inner PE liner of the food sack material (Figure 2).

Mold Penetration Studies

Fungal penetration of the sacks was determined by cutting circles of the six-layered sacks and using these as liners in Ball jar lids fastened to Mason pint (0.5 L) canning jars. Two sets were prepared for each of the four sacks. The sets were ETO sterilized, and CSM was sprinkled on the top layer of one set. Both sets were then sprayed with the mixed fungus spore suspension, incubated in a small humid chamber at 30°C for one month, ETO sterilized, and examined. Scanning electron photomicrographs (SEM) were taken of the undersurface of the outermost layers of the samples used in the penetration study.

Mold Identification

In March 1976, two sets of deteriorated sack samples from the USDA field test were received from the Dominican Republic for identification of the mold growth. Each set contained a sample of the standard sack and a test sack. The fungi present on the deteriorated sacks were isolated by using three media: potato dextrose agar (PDA), rose-bengal streptomycin agar (RBS), and hay infusion agar (HAY). The PDA and RB

media are available commercially. Streptomycin (8 mL of a 1% solution per L) is added to form the RBS. HAY agar is prepared as shown in Table 4.

TABLE 4. HAY INFUSION AGAR

Distilled H_2O	1.0 L
Dried grasses	50.0 g
Autoclave 30 minutes at 121°C.	Filter
Infusion filtrate	1.0 L
K_2HPO_4	2.0 g

Adjust pH to 6.2 ± 0.2. Sterilize 20 minutes at 121°C.

Mycotoxin Production

Analysis for mycotoxin production involved using a glucose-ammonium nitrate (GAN) medium (Table 5), inoculating 50 mL of the medium per 250 mL flask with the suspected organism, and incubating at 25°C for ten days under static conditions.[9, 10]

Aflatoxin-producing strains of *Aspergillus flavus* and *A. parasiticus*, and ochratoxin-producing *A. ochraceus* were obtained from the Culture Collection of Fungi (QM), Department of Botany, University of Massachusetts, Amherst, MA, for use as controls (Table 6).

---

[9] Brian, P.W., A.W. Dawkins, J.F. Grove, H.G. Hemming, D. Lowe and G.L.F. Norris. 1961. Phytoxin Compounds Produced by *Fusarium equisetti*. J. Expt'l. Bot. 12: 1-12.

[10] Parrish, F.W., B. J. Wiley, E.G. Simmons, and L. Long, Jr. 1965. A Survey of Some Species of *Aspergillus* and *Penicillium* for Production of Aflatoxins and Kojic Acid. Technical Report, Microbiology Series No. 20, US Army Natick Laboratories, Natick, MA. 21 pp.

TABLE 5. GLUCOSE-AMMONIUM NITRATE MEDIUM (GAN)

Glucose	50.0 g
NH_4NO_3	2.4 g
KH_2PO_4	10.0 g
$MgSO_4 \cdot 7H_2O$	2.0 g
Distilled H_2O	1.0 L
Mineral supplement*	2.0 mL

*Mineral supplement

$FeSO_4 \cdot 7H_2O$	0.1 g
$CuSO_4 \cdot 5H_2O$	0.015 g
$ZnSO_4 \cdot 7H_2O$	0.1 g
$MnSO_4 \cdot H_2O$	0.01 g
$K_2MoO_4 \cdot 5H_2O$	0.01 g
0.25 N HCl	100.0 mL

TABLE 6. MYCOTOXIN-PRODUCING QM STRAINS

QM 4780	*Aspergillus flavus*
QM 5828	*Aspergillus ochraceus*
QM 8378	*Aspergillus parasiticus*
QM 9363	*Aspergillus parasiticus* var. *globosus*

Reference standards of aflatoxins and ochratoxin were obtained from Dr. R. D. Stubblefield, Northern Regional Research Center (NRRC), Peoria, IL, for use in thin-layer chromatography (TLC). The reference standard for aflatoxins contained 2.5 micrograms (μg) of B_1/mL, 0.5 μg of B_2/mL, 2.0 μg of G_1/mL, and 0.5 μg of G_2/mL. The standard solution of ochratoxin A contained 5.0 μg/mL.[11, 12]

The GAN medium was extracted with an equal volume of hot chloroform ($CHCl_3$), cooled to room temperature, and filtered through glass wool. The filtered aqueous/chloroform mixture was shaken, the $CHCl_3$ layer was removed, and the aqueous layer was re-extracted with an equal volume of $CHCl_3$. The combined $CHCl_3$ extracts were dried with anhydrous sodium sulfate, filtered, and evaporated to dryness *under reduced pressure*.

The residue remaining after evaporation of the $CHCl_3$ was dissolved in a known amount of $CHCl_3$, microliter quantities were spotted onto activated Silica Gel G thin-layer plates, developed in 3% methanol in $CHCl_3$, and examined under ultraviolet (UV) light.

Aflatoxins, when present, appear as blue or blue-green fluorescent spots at R_f values of 0.4 to 0.5. Ochratoxin appears as a yellow-green fluorescence at an R_f value of 0.3 to 0.4.

---

[11] Windholz, M., Ed. 1976. The Merck Index. Ninth Ed. Merck and Co., Inc., Rahway, NJ.

[12] Stubblefield, R.D. Personal Communication.

Determination of Aflatoxin Production on IRT Food Sacks

Eighteen flasks of NH_4NO_3 medium (GAN, Table 5, without glucose) were prepared, 50 mL/250 mL flask. Four flasks each of IRT food sack samples 1, 2, 3, and Whatman filter paper control discs (16 flasks total), were set up. Half of these (8) received a small amount of CSM. Half of each received no CSM (8). Two additional flasks contained only NH_4NO_3 medium and CSM. Two flasks containing GAN (50 mL/250 mL flask) were used as controls.

A spore suspension of QM 9363 *Aspergillus parasiticus* var. *globosus* was prepared by pouring 10 mL sterile distilled H_2O containing 0.05 g/L of Tween 80 into a 2 to 3 week-old culture of the organism, suspending the spores, and using this as an inoculum. Approximately 0.1 mL of the suspension was used to inoculate each flask. The 20 flasks were incubated under static conditions for six weeks at $28^oC \pm 2^oC$. The same extraction procedure was used as for the mycotoxin analysis.

RESULTS

Storage Tests

The storage tests performed by the USDA found that the mold on the standard IRT sacks ranged from 10% to 80% surface coverage, from 1 to 5 layers had been penetrated, and frequently the paper was digested. Mold on the test sacks ranged from 0% to 90% coverage of the surface area, with test sack type 3 being the most severely affected. Mold had not penetrated to the inner layers of sack types 2 and 4, but had penetrated

from 2 to 5 inner layers of seven sacks of type 3, although the paper was usually intact. The USDA tests also showed that the greaseproof ply incorporated in test sack type 3 had prevented migration of the IR treatment to the inner plies. More than 20% of the synergized pyrethrins on sacks without a greaseproof layer had moved into the inner kraft plies. Tests on empty sacks showed that there was a 40% loss of treatment from the outer ply after one month and a 67% loss after six months.[13]

Figures 3 and 4 show the IRT food sacks during the USDA storage test in the Dominican Republic.

Figure 3. IRT Sacks Under Test in the Dominican Republic

---

[13] See footnote 1.

Figure 4. IRT Sacks after Seven Weeks of Incubation Showing Moldy Patches

Fungal Resistance Test

Evaluation of the four IRT sacks in our laboratory, according to the ASTM procedure, gave the results shown in Table 7.

TABLE 7. MICROBIAL EVALUATION OF IRT SACKS

Sack No.	Evaluation
1	Profuse growth completely covering the outer layer of the sample and penetrating through all layers.
2	Light to medium growth covering the outer layer, with heavy growth on the inner layers.
3	Light to medium growth covering the outer layer, with heavy growth on the inner layers.
4	Light to medium growth covering the outer layer, with heavy growth on the inner layers.

Soil Burial and Tropical Chamber Exposure

Table 8 contains data from the scaled-down food sack systems after 7 to 14 days of soil burial. After 7, 10, and 14 days exposure, sack systems were retrieved from soil burial, and each sack was separated into layers for observation of visible fungal growth. Sack type 1 contained growth between kraft layers 1 and 2, between 4 and 5, and extensive growth adjacent to the food-containing packet after 7 days soil exposure, with growth between all layers after 10 days. Sack type 2 had trace growth adjacent to the food packet after 10 days of soil exposure, and growth between all layers after 14 days.

TABLE 8. RESULTS FROM SOIL BURIAL TEST

Days Soil Exposure	Sack Type	Kraft Layers: PE-Coated 1st IRT	2nd	3rd	4th	5th	PE liner	CSM
7	1[a]	+[b]			+	+		
	2							
	3[c]							
	4[c]	+	+	+	+	+		
10	1	+	+		+	+		
	2							
	3	+			+	+		
	4	+	+	+	+	+		
14	1	+	+	+	+	+		
	2	+	+	+	+	+		
	3	+	+	+	+	+		
	4	+	+	+	+	+		

[a]IRT kraft ply without PE coating
[b]Mold growth between adjacent layers
[c]2nd kraft ply is a greaseproof barrier

Sack type 3 exhibited the same growth pattern between layers after 10 days of soil exposure that type 1 had exhibited after 7 days, and growth between all layers after 14 days. Sack type 4 had growth adjacent to the food packet and between all layers except layers 3 and 4 after 7 days of soil exposure, and growth between all layers after 10 days.

The absence of growth between some inner layers of the sack systems suggests that fungal growth proceeded both from the outer surface and from inner layers, as well as through possible pin-hole leaks in the hot glue seals used to form the paper pouch. The experiment with food jar systems was devised to direct fungal attack to the outer surface of the sack materials.

Table 9 contains data obtained from the food jar systems after soil and tropical chamber exposure. After 35 days in either soil or tropical chamber exposure, jar systems were disassembled, and kraft layers were separated for growth ratings. Trace fungal growth was observed between all kraft layers of all sack types evaluated for both soil and tropical chamber exposure. Growth was also apparent in the moist CSM of sack types 1 and 2 after tropical chamber exposure. PE-coated sack types 2 and 3 supported only trace surface growth in soil or tropical chamber exposure, unlike uncoated sack type 1 which supported heavy surface growth. Nevertheless, the data suggest that the PE coatings of sack types 2 and 3 were no more effective than uncoated sack types in preventing fungal penetration of the kraft layers.

TABLE 9. DATA FROM SOIL AND TROPICAL CHAMBER EXPOSED JAR SYSTEMS

		Kraft Layers						
Exposure	Sack Type	PE-Coated 1st IRT	2nd	3rd	4th	5th	PE liner	CSM
35	1[a]	4*	1	1	1	1	1	0
days	2	1	1	1	1	1	1	0
soil	3[b]	1	1	1	1	1	1	0
35	1	4	1	1	1	1	1	4[d]
days	2[c]	1	1	1	1	1	1	1[d]
TC	3	1	1	1	1	1	1	0

[a] IRT kraft without PE coating
[b] 2nd kraft ply is a greaseproof barrier
[c] Kraft layers became moistened
[d] CSM became moistened

*0 No growth
1 1% - 10% surface area coverage
2 10% - 30% surface area coverage
3 30% - 60% surface area coverage
4 60% -100% surface area coverage

Mold Penetration Studies

The fungal penetration studies gave the results shown in Table 10. SEM of the undersides of the layers of the samples show the fungal growth illustrated in Figures 5 and 6, demonstrating the inability of the thin PE coating to withstand microbial penetration.

Mold Identification

Figures 7 and 8 show the deteriorated IRT food sacks as they were received from the Dominican Republic storage test conducted by the USDA.

Table 11 lists the fungi that were isolated and identified from the deteriorated sacks.

TABLE 10. MICROBIAL PENETRATION STUDY OF IRT SACKS

With Fungal Spray Alone:

Sack No.	Evaluation
1	Heavy fungal growth on the outer layer. This layer had split and separated. Fungal penetration through all layers to the PE liner.
2	Light fungal growth on the outer layer. Fungal penetration through all layers to the PE liner.
3	Light fungal growth in a few scattered areas on the outer layer. The PE coating had cracked and there was heavier growth in these areas. Fungal penetration through all layers to the PE liner.
4	Light fungal growth covering the outer layer. The PE coating had cracked. Fungal penetration through all layers to the PE liner.

With CSM Mixture and Fungal Spray:

Sack No.	Evaluation
1	Very heavy fungal growth on the outer layer. The outer layer had split and curled back. Fungal penetration through all layers to the PE liner.
2	Moderate fungal growth on the surface. A few fractures in the PE coating with heavier fungal growth beneath. Fungal penetration through all layers to the PE liner.
3	Moderate fungal growth covering the surface layer. The PE coating had fractured and curled back in places with heavier fungal growth beneath. Fungal penetration through all layers to the PE liner.
4	Light fungal growth covering the surface. The PE coating had split and curled in places. Fungal penetration through all layers to the PE liner.

Figure 5. SEM of Fungal Growth on Underside of Outer Layer of IRT Sack No. 1

Figure 6. SEM of Fungal Growth on Underside of Outer Layer of IRT Sack No. 3

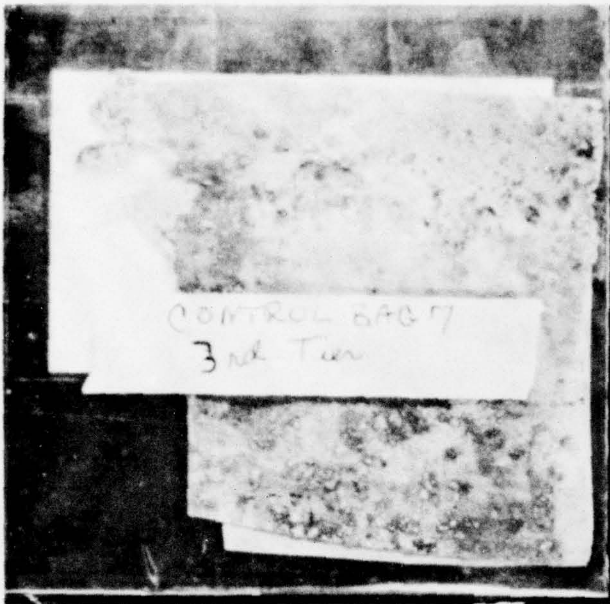

Figure 7. Sample of the Standard IRT Sack as Received from the Dominican Republic

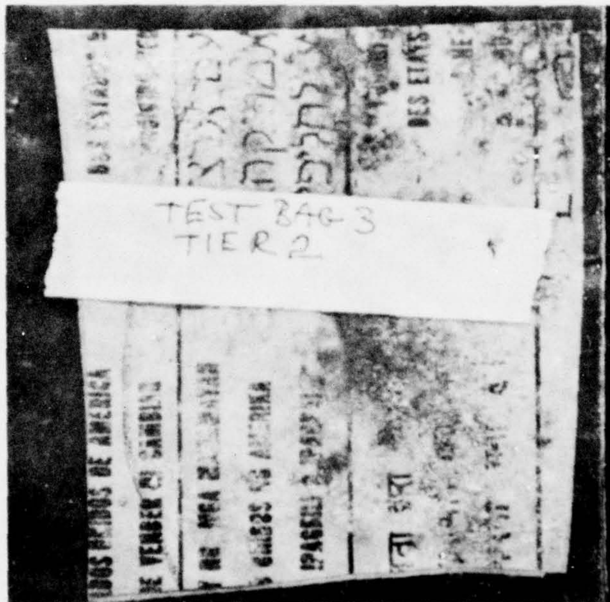

Figure 8. Sample of the IRT Test Sack as Received from the Dominican Republic

TABLE 11. FUNGI ISOLATED FROM DETERIORATED IRT SACKS

Control Bag 7, 3rd Tier

1st Series	2nd Series
Trichoderma sp. (2 different species)	*Trichoderma* sp. (2 sp.)
Aspergillus flavus	*Aspergillus niger*
A. flavus var. *columnaris*	*A. flavus*
A. niger	*A. tamarii*
A. tamarii	*Penicillium* sp.
Penicillium sp. (2 sp.)	*Rhizopus* sp.

Test Bag 3, 2nd Tier

1st Series	2nd Series
Penicillium sp. (2 sp.)	*Penicillium* sp.
Trichoderma sp.	*Aspergillus tamarii*
Mucor sp.	*A. flavus*
Paecilomyces sp. (prob. *P. varioti*)	*A. sydowi*
Rhizopus sp.	*A. oryzae* var. *effusus*
Aspergillus tamarii	*A. fumigatus*
A. flavus	*A. ochraceus*
A. flavus var. *columnaris*	*Trichoderma* sp.
A. niger group	*Mucor* sp.

Mycotoxin Production

A total of 69 fungus strains were isolated from the deteriorated sacks received from the Dominican Republic. Of these, 14 strains were representative of the *Aspergillus flavus*, and one of the *A. ochraceus* groups. These were selected to be checked for mycotoxin production, specifically, aflatoxins and ochratoxin.

Extracts from the four QM cultures used as controls contained large amounts of aflatoxins and ochratoxin when chromatographed with reference standards of these compounds. The 15 IRT test strains contained no detectable amounts of these mycotoxins and were considered to be negative for the production of these carcinogens.

Determination of Aflatoxin Production on IRT Food Sacks

Chromatograms of the extracts of the contents of the flasks containing the IRT food sack samples and the various controls showed aflatoxins B and G were present in large amounts in the GAN control. Faint traces of these mycotoxins were detected in the filter paper controls without CSM and in the NH_4NO_3 controls with CSM. No aflatoxins were detected in the extracts from the flasks containing the IRT food sack samples either with or without the added CSM.

DISCUSSION

Our studies have shown that the PE outer coating of the experimental IRT food sacks did not prevent microbial attack and penetration of the food sack materials in either soil burial or tropical chamber exposure. In penetration studies and in plate tests, fungal hyphae had penetrated through cracks and holes in the PE coating and were growing profusely on the outer surface. Fungal penetration is graphically illustrated in the SEM photographs of the undersides of these PE-coated layers. No penetration of the inner PE liner could be demonstrated.

The presence of mold on these sacks raised the question of whether toxic carcinogenic fungal metabolites were being produced. Analysis for mycotoxin production failed to show the presence of the metabolites on the IRT food sack materials. Growth of mold does not necessarily imply that toxins are produced, since genetic differences, moisture, temperature, substrate, and incubation time are also involved.

For determination of aflatoxin production on IRT food sack samples, we chose six weeks incubation in liquid culture under static conditions at 28°C ± 2°C based on studies by Sorenson, et al. who showed the effect of temperature on aflatoxin production on rice, and Diener and Davis who studied temperature and RH limits for aflatoxin in sound mature peanut kernels.[13, 14]

---

[13] Sorenson, W.G., C.W. Hesseltine and O.L. Shotwell. 1967. Effect of temperature on Production of Aflatoxin on Rice by *Aspergillus flavus*. Mycopath. Mycol. Appl. 33: 49-55.

[14] Diener, U.L. and N.D. Davis. 1970. Limiting Temperature and Humidity for Aflatoxin Production by *Aspergillus flavus* in Stored Peanuts. J. Am. Oil Chem. Soc. 47: 347-351.

Sorenson, et al. found that aflatoxins are produced optimally at 28°C when incubated 4 to 7 days under shaken conditions, but they did not evaluate static conditions. Diener and Davis showed that at a moisture content of 84%, aflatoxin production peaked at 42 days at 30°C. The limiting factor was the moisture content at RH 83% ± 1%.

Our study of aflatoxin production on IRT food sacks indicated that there may be something present in the sacks which inhibits aflatoxin production. A possible inhibitor is the presence of synergized pyrethrins on the outer layer.

Inhibition of aflatoxin biosynthesis has been studied by Hsieh who found that commonly used organophosphorus insecticides such as Parathion and Malathion inhibited aflatoxin production.[15] Dichlorvos (dimethyl 2, 2-dichlorovinyl phosphate) also has been found to inhibit aflatoxin formation in a number of substrates.[16, 17] No references could be located relevant to inhibition of aflatoxin production by synergized pyrethrins.

---

[15] Hsieh, D.P.H. 1973. Inhibition of Aflatoxin Biosynthesis of Dichlorvos. J. Agr. Food Chem. 21: 468-470.

[16] Rao, H.R.G. and P.K. Harein. 1972. Dichlorvos as an Inhibitor of Aflatoxin Production of Wheat, Corn, Rice and Peanuts. J. Econ. Entomol. 65: 988-989.

[17] Schroeder, N.W., R.J. Cole, R.D. Grigsby, and H. Hein, Jr. 1974. Inhibition of Aflatoxin Production and Tentative Identification of an Aflatoxin Intermediate "Versiconal Acetate" from Treatment with Dichlorvos. Appl. Microbiol. 27: 394-399.

CONCLUSIONS

We have shown that the IRT food sacks are susceptible to deterioration, even though a PE coating is included on the outer layer. When breaks or cracks occur in this coating, mold can penetrate through all paper plies to the PE liner. No penetration of the inner liner was detected.

Of the strains isolated from the deteriorated sacks received from the USDA study in the Dominican Republic, 20% represented the Aspergillus flavus and A. ochraceus groups which are known to produce mycotoxins. Although no toxins were shown to be formed in this instance, studies with known toxin-producing strains of fungi used as inocula showed that aflatoxins were produced when the mold grew on the CSM material. None was detected when the organism was grown with CSM on the IRT food sacks. One interpretation of these results is the possibility of suppression of aflatoxin production by the synergized pyrethrins used for insect resistance. This factor needs to be investigated, since the specific conditions required for inhibition of mycotoxin production are not clear.

Further study also is needed in order to produce an acceptable IRT and mold resistant food sack for use in the DoD supply system.

REFERENCES

Brian, P. W., A. W. Dawkins, J. F. Grove, H. G. Hemming, D. Lowe, and G. L. F. Norris. 1961. Phytoxin Compounds Produced by Fusarium equisetti. J. Expt'l. Bot. 12: 1-12.

Diener, U. L. and N. D. Davis. 1970. Limiting Temperature and Humidity for Aflatoxin Production by Aspergillus flavus in Stored Peanuts. J. Am. Oil Chem. Soc. 47: 347-351.

Highland, H. A., E. G. Jay, M. Phillips and D. F. Davis. 1966. The Migration of Piperonyl Butoxide from Treated Multiwall Kraft Bags into Four Commodities. J. Econ. Entomol. 59: 543-545.

Highland, H. A., M. Secreast and P. H. Merritt. 1970. Packaging materials as Barriers to Piperonyl Butoxide Migration. J. Econ. Entomol. 63: 7-10.

Highland, H. A. 1976. Interim Report on Storage Test with Experimental IRT Mold Resistant Shipping Sacks. (Unpublished).

Highland, H. A. 1978. Insect Resistant Package Design, p. 36, In: Proceedings of Fall 1977 Meeting, Research and Development Associates for Military Food and Packaging Systems, Inc. 4-6 October 1977.

Hsieh, D. P. H. 1973. Inhibition of Aflatoxin Biosynthesis of Dichlorvos. J. Agr. Food Chem. 21: 468-470.

Lukens, R. P., Managing Standards Editor. 1976. Annual Book of ASTM Standards. Part 35. ASTM Designation: G 21-70 (Reapproved 1975). Standard Recommended Practice for Determining Resistance of Synthetic Polymeric Materials to Fungi. American Society for Testing and Materials, Philadelphia, PA.

Parrish, F. W., B. J. Wiley, E. G. Simmons, and L. Long, Jr. 1965. A Survey of Some Species of Aspergillus and Penicillium for Production of Aflatoxins and Kojic Acid. Technical Report, Microbiology Series No. 20, US Army Natick Laboratories, Natick, MA. 21 pp.

Rao, H. R. G. and P. K. Harein. 1972. Dichlorvos as an Inhibitor of Aflatoxin Production of Wheat, Corn, Rice and Peanuts. J. Econ. Entomol. 65: 988-989.

Schroeder, H. W., R. J. Cole, R. D. Grigsby, and H. Hein, Jr. 1974. Inhibition of Aflatoxin Production and Tentative Identification of an Aflatoxin Intermediate "Versiconal Acetate" from Treatment with Dichlorvos. Appl. Microbiol. 27: 394-399.

REFERENCES (cont'd)

Sorenson, W. G., C. W. Hesseltine and O. L. Shotwell. 1967. Effect of Temperature on Production of Aflatoxin on Rice by _Aspergillus_ _flavus_. Mycopath. Mycol. Appl. _33_: 49-55.

Stubblefield, R. D. 1976. Personal Communication.

Windholz, M., Editor. 1976. The Merck Index, Ninth Ed. Merck and Co., Inc., Rahway, NJ.