APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Sept. 1978, p. 533-535
0099-2240/78/0036-0533$02.00/0

Vol. 36, No. 3
Printed in U.S.A.

Evaluation of *Sclerotinia sclerotiorum* as a Potential Mycotoxin Producer on Soybeans

A. CIEGLER, K. A. BURBRIDGE, J. CIEGLER,† AND C. W. HESSELTINE*

Northern Regional Research Center, Federal Research, Science and Education Administration, U.S. Department of Agriculture, Peoria, Illinois 61604

Received for publication 22 June 1978

Solvent extracts of *Sclerotinia sclerotiorum* sclerotia were nontoxic to mice and chicken embryos; psoralens were not detected. Solvent extracts of soybeans inoculated with 10 strains of *S. sclerotiorum* were toxic on injection but nontoxic on per os administration to mice. The presence of chlorinated hydrocarbons in the soybeans may partially help explain toxicity by intraperitoneal injection.

Sclerotinia sclerotiorum can produce in diseased celery several phototoxic compounds such as 5-methoxypsoralen, 8-methoxypsoralen, and 4,5,8-trimethylpsoralen that cause a blistering cutaneous disease on the skin of people handling the contaminated vegetable (1, 7). On soybeans, this soil-borne fungus causes stem rot and may produce large black sclerotia which are not separated from the seeds during harvest; beans are not invaded normally. The presence of sclerotia in shipments of soybeans intended for human consumption results in rejection of the shipment at foreign entry ports (4). Toxicological safety of contamination by sclerotia has been questioned but definitive answers have not been reported (2, 6).

In 1977 two large shiploads of American soybeans were rejected at a foreign entry port. We analyzed sclerotia and soybeans from these two contaminated shiploads for the presence of psoralens and other potential toxins by using chromatography and biological assays to determine if their consumption could result in a mycotoxicosis.

A total of 60 g of sclerotia was extracted successively with 800 ml each of hexane, methylene chloride, ethyl acetate, and methanol. These extracts were concentrated and analyzed for 5-methoxypsoralen, 8-methoxypsoralen, and 4,5,8-trimethylpsoralen by thin-layer chromatography, using the procedure of Wu et al. (9); reference standards were included in all assays. Developed plates were observed under long- and shortwave ultraviolet light and also sprayed with *p*-anisaldehyde reagent (8). All extracts were negative for the psoralens. The same extracts (in vegetable oil) were injected into mice and chicken embryos and applied, in ethyl acetate, onto rabbit skin, again with negative results.

The two soybean samples and the sclerotia after surface sterilization with 2% sodium hypochlorite to eliminate adventitious microbial contamination were plated under a variety of conditions conducive to growth of *S. sclerotiorum* (9). Primarily, *Penicillium* and *Aspergillus* spp. grew out of the soybeans, but lack of growth of the sclerotia indicated nonviability.

Subsequently in a preliminary experiment, 10 isolates of *S. sclerotiorum* from the Agricultural Research Services Culture Collection were inoculated onto soybeans obtained locally (300 g of soybeans plus 150 ml of water per Fernbach flask; autoclaved for 30 min). Samples of the rejected soybeans were not inoculated in the initial experiment because of the limited quantity in our possession, but they were included as uninoculated controls. Cultures were incubated

TABLE 1. *Toxicity of extracts from soybeans inoculated with various strains of S. sclerotiorum*

Sample	Lethality to:		
	Mice		Chicken embryo
	i.p.	p.o.	
Local soybeans inoculated with *S. sclerotiorum*, 10 strains	+	−[b]	+
Uninoculated control	+	−	+
Uninoculated soybeans, R-1, R-2[c]	−	−	−
R-1 inoculated with two strains of *S. sclerotiorum*	+	−	+
R-2 inoculated with two strains of *S. sclerotiorum*	+	−	+

[a] i.p., Intraperitoneal; p.o., per os.
[b] Six strains intubated.
[c] R-1 and R-2 represent the two rejected soybean samples.

† Present address: Food Safety Quality Service, U.S. Department of Agriculture, Peoria, IL 61604.

TABLE 2. *Chlorinated hydrocarbons detected by gas chromatography[a] in soybean samples before and after fermentation with S. sclerotiorum*

Chlorinated hydrocarbon	Sample (ppm)					
	R-1[b]	R-2[b]	Uninoculated control[c]	A-23532[c]	A-23533[c]	A-23534[c]
Dieldrin	0.08	0.08	1.60	0.34	0.27	0.28
Heptachlor	0.12	0.19	ND[d]	ND	ND	ND
1,1,1-Trichloro-2,2-bis(*p*-chlorophenyl)ethane	0.27	0.27	ND	ND	ND	ND
Hexachlorobenzene	ND	ND	0.01	0.01	0.01	0.01

[a] Hewlett-Packard GC model 5710-A. Method 1. Column: 1 m by 4 mm ID packed with 4% OV-210 and 1% OV-17 on Gas Chrom Q. Gas: 5% methane, 95% argon; pressure, 40 lb/in^2; flow rate, 60 cm/min. Detector: electron capture. Temperatures: oven, 210°C; injection port, 203°C; detector, 303°C. Method 2. Column: 1 m by 4 mm ID packed with 3% XE-60 on Gas Chrom Q. Gas: nitrogen, 40 lb/in^2. Temperatures: oven, 163°C; injection port, 220°C; detector, 285°C. Internal standards of 14 chlorinated hydrocarbons were added to each run, and samples were quantitated by programmed computer.

[b] Represents lots of rejected soybeans.

[c] Soybeans purchased locally were used for the uninoculated control and for fermentation by *S. sclerotiorum* strains.

[d] ND, Not detected.

without agitation for 2 weeks at 25°C and then extracted in a blender with ethyl acetate. Solvent was removed by flash evaporation, and the oily residue was analyzed for psoralens with negative results. Intraperitoneal injection (0.1 ml) of the oily residues into mice and 0.05 ml into the air-changed sac of chicken embryos gave unexpected results (Table 1). All extracts except those from the two uninoculated rejected soybean samples were lethal. Gas chromatographic analyses of the oils on two different columns indicated that toxicity in the uninoculated local soybeans could possibly be attributed to the presence of chlorinated hydrocarbons (Table 2). This is a factor that previously has not been given cognizance by those investigating mycotoxins in agricultural products, although their occurrence in soybeans has been noted (3) and may help explain some of the anomalies encountered by various mycotoxin investigators. Since this aspect was not within our current experimental design, we did not further pursue this point.

In a following experiment, two of the cultures were inoculated onto 300 g each of the two rejected soybean samples (R-1, R-2). These were incubated and extracted as noted above. The oily extracts previously nontoxic to mice or chicken embryos now proved lethal to the two test systems. Analysis of the oils by gas chromatography and infrared spectroscopy revealed only the presence of the chlorinated hydrocarbons and linoleic acid; none of the other fatty acids normally present in soybeans was detected. Intraperitoneal injection of 0.1 ml of pure linoleic acid proved lethal to mice. Jeffrey et al. (5) have shown that linoleic acid hydroperoxide destroys cytochrome P-450 in hepatic microsomes. If it is assumed that the hydroperoxide is formed from the injected linoleic acid, loss of the P-450 system, particularly in the presence of toxic substances such as the chlorinated hydrocarbons, could result in death of the test animals. This aspect will require additional investigation. However, when 0.5 ml of these oily extracts was subsequently intubated into duplicate mice along with seven of the samples from the previous experiment plus the uninoculated local soybean control, no overt toxic effects were observed after 3 months of observation (Table 1). Therefore, based on the lack of toxicity of the sclerotia, the unlikelihood of *S. sclerotiorum* invasion of the soybean per se, and the lack of toxicity of extracts from deliberately contaminated soybeans on oral administration to mice, it would appear doubtful that *S. sclerotiorum* contamination of soybeans poses the threat of a potential serious mycotoxicosis.

LITERATURE CITED

1. **Birmingham, D. J., M. M. Key, G. E. Tubich, and V. B. Perone.** 1961. Phototoxic bullae among harvesters. Arch. Dermatol. **83**:73.
2. **Brown, J. C.** 1937. Relation of livestock to the control of sclerotinosis of lettuce. Phytopathology **27**:1045–1050.
3. **Chaudry, M. M., A. I. Nelson, and E. G. Perkins.** 1976. Distribution of aldrin and dieldrin in soybeans, oil, and by-products during processing. J. Am. Oil Chem. Soc. **53**:695–697.
4. **Compendium of Soybean Diseases.** 1977. Sclerotinia (Whetzelinia) stem rot. Am. Phytopathol. Soc. Monogr., p. 12.
5. **Jeffrey, E. H., D. Nerland, R. El-Azhary, and G. J. Mannering.** 1976. Destruction of cytochrome P-450 by

linoleic acid hydroperoxide, p. 323–330. *In* V. Ullrich (ed.), Microsomes and drug oxidations, Pergammon Press, New York.

6. **Ruddick, J. A., and J. Harwig.** 1975. Prenatal effects caused by feeding sclerotia of *Sclerotinia sclerotiorum* to pregnant rats. Bull. Environ. Contam. Toxicol. **13**:524–526.
7. **Scheel, L. D., V. B. Perone, R. L. Larkin, and R. E. Kupal.** 1963. The isolation and characterization of two phototoxic furanocoumarins (psoralens) from diseased celery. Biochemistry **2**:1127–1131.
8. **Scott, P. M., J. W. Lawrence, and W. van Walbeek.** 1970. Detection of mycotoxins by thin-layer chromatography: application to screening of fungal extracts. Appl. Microbiol. **20**:839–842.
9. **Wu, C. M., P. E. Koehler, and J. C. Ayres.** 1972. Isolation and identification of xanthotoxin (8-methoxypsoralen) and bergapten (5-methoxypsoralen) from celery infected with *Sclerotinia sclerotiorum*. Appl. Microbiol. **23**:852–856.