Occurrence of Aflatoxins and Aflatoxin-Producing Strains of Aspergillus spp. in Soybeans

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Above average rainfall in Maryland during August, September, and October 1971 resulted in heavy mold growth in soybeans while still in the field. Of 28 samples of soybean seed, aflatoxins were found in 14, 2 of which had been used in poultry feed. Aflatoxins were identified by thin-layer chromatography, spectrophotometry, and chicken embryo bioassay. Aspergillus spp. were isolated from 11 samples, and 5 of these isolates produced aflatoxins when grown in liquid culture.

Aflatoxins are not considered a serious problem on soybeans (6). Although aflatoxin-producing strains of Aspergillus flavus will grow on soybean seed in the laboratory, the amount of aflatoxins produced is much lower than on other commodities (5). The occurrence of both A. flavus and aflatoxins in soybeans under field conditions is generally very low. During 1965 and 1966, A. flavus could not be isolated from more than 3,100 samples of soybean seed examined (5), and Shotwell et al. (9) reported that only 2 of 866 soybean samples contained aflatoxins and the samples were in "sample grade." A number of reasons have been proposed for the resistance of soybeans to A. flavus such as unfavorable moisture conditions for the fungus at the time of soybean maturity, development of seeds in a closed pod, or the possibility that soybean seed contains an inhibitor which prevents growth of the fungus (5).

An unusually wet growing season in Maryland during 1971 resulted in heavy mold growth in soybeans. Soybean samples were collected and examined to detect either the occurrence of aflatoxins or the presence of aflatoxin-producing strains of Aspergillus spp.

MATERIALS AND METHODS

Twenty-eight samples of soybean seed were examined; 24 were collected in experimental field plots from different locations in Maryland. Included were the three cultivars "Wayne," "Callard," and "Cutler"; the remainder were experimental lines. The percentage of moldy seed in each sample was determined. Four soybean samples to be used in poultry feed were obtained from the Poultry Department, University of Maryland. Samples were composed of non-moldy soybeans.

To isolate Aspergillus spp., soybeans showing mold damage were surface sterilized with sodium hypochloride, split in half, and placed on Difco potato dextrose agar (PDA) containing 6.0% NaCl and incubated at 30 C. When colonies of Aspergillus spp. developed on the PDA, they were transferred to SMKY liquid medium (3) and the cultures were incubated at 25 C for 10 days prior to extraction of aflatoxin. The Aspergillus spp. were not identified as to species.

The extraction and identification of aflatoxins were by the procedures of Pons and Goldblatt (8). Fifty grams of moldy seed, hand-selected from each sample, and the culture filtrate from the Aspergillus spp., isolated growing in liquid medium, were examined. Soybeans were ground in a Waring Blender, shaken for 30 min with 70% acetone, and filtered to remove solid particles. Pigments were precipitated with lead acetate and centrifuged. The centrifuged filtrate and the liquid culture filtrates were each extracted with chloroform to remove aflatoxins.

The chloroform extracts were evaporated under nitrogen and spotted on Adsorbosil I thin-layer chromatography plates along with pure aflatoxin standards obtained from L. A. Goldblatt (U.S. Department of Agriculture, New Orleans, La.). The plates were developed with chloroform-methanol (97:3, v/v) and then were dried and examined under ultraviolet (UV) irradiation. The fluorescent zones corresponding to the Rf values of aflatoxin standards were eluted from the plates and re-chromatographed as before.

The fluorescent zones, including standard, were removed from the plates, and the aflatoxins were dissolved in methanol. The concentrations were de-
RESULTS AND DISCUSSION

Although 24 field samples were collected, 7 were discarded including 3 samples each of Callard and Cutler because they contained less than 1% moldy grain which was an insufficient amount for extraction of aflatoxins. The percentage of moldy grain in each sample, the amount of aflatoxins present, the occurrence of Aspergillus spp., and whether or not those strains were aflatoxin producers are presented in Table 1. Results of the study on soybean samples to be used in poultry feeds are summarized in Table 2, and in Table 3 are the results of the chicken embryo test.

The percentage of moldy grain ranged from less than 1% to a maximum of 72%. The highest percentages of moldy grain occurred in experimental lines; the 3 cultivars Wayne, Callard, and Cutler all had between 2 to 7% moldy grain. The highest amounts of aflatoxins recorded were samples from the cultivar Wayne, even though the percentage of moldy grain was relatively low. Sample 11 had 68% moldy grain yet contained only an average amount of aflatoxins, indicating that there was little correlation between percentage of moldy grain and the amount of aflatoxin. Samples 18 and 19, both of cultivar Wayne, were from two different locations and differed in aflatoxin level, whereas samples 10 and 15 (aflatoxin and no aflatoxin, respectively) were from replications of the same experimental line in the same location so that great differences in aflatoxin levels can occur according to cultivar, location, or even rows of the same cultivar in the same field, or perhaps even to pods on a plant.

Aspergillus spp. were also isolated from moldy grain, and again there was little correlation between the occurrence of Aspergillus spp. with either amount of moldy grain or level of aflatoxin present. Furthermore, some isolates produced aflatoxins whereas others did not, indicating the difficulty in detecting potential aflatoxin problems by only isolating Aspergillus spp.

Table 1. Aflatoxins and aflatoxin-producing strains of Aspergillus spp. from soybean samples

| Sample | Moldy grain (%) | Aflatoxins (µg/kg) | No. of Aspergillus spp. isolates | Aflatoxin producer |
|--------|----------------|--------------------|---------------------------------|-------------------|
| 3      | 72             | 2                  | 1                               | -                 |
| 4a     | 2              | 48                 | 1                               | +                 |
| 7      | 23             | 81                 | 1                               | -                 |
| 8      | 3              | 2                  | 1                               | +                 |
| 9*     | 2              | 68                 | 2                               | -                 |
| 10     | 2              | 2                  | 1                               | -                 |
| 11     | 2              | 4                  | 2                               | -                 |
| 15     | 1              | 15                 | 1                               | -                 |
| 16     | 3              | 7                  | 1                               | -                 |
| 17*    | 3              | 7                  | 1                               | -                 |
| 18*    | 7              | 2                  | 2                               | -                 |
| 19*    | 7              | 1                  | 2                               | -                 |
| 20     | 7              | 1                  | 2                               | -                 |
| 21     | 3              | 1                  | 2                               | -                 |
| 22     | 2              | 2                  | 2                               | -                 |
| 23     | 1              | 2                  | 2                               | -                 |
| 24*    | 1              | 2                  | 2                               | -                 |

* Cultivar "Callard;"
* Cultivar "Wayne;"
* Cultivar "Cutler;"

Table 2. Aflatoxins and aflatoxin-producing strains of Aspergillus spp. in soybean samples used in poultry feeds

| Sample | Aflatoxins (µg/kg) | Aspergillus isolates/soybeans cultured | Aflatoxin producer |
|--------|--------------------|---------------------------------------|-------------------|
| Soybeans, nonmoldy | 0 | 0/42 | - |
| Soybean mix, nonroasted | 0 | 3/31 | + |
| Moldy mix, roasted | 310 | 7/32 | + |
| Moldy mix, nonroasted | 210 | 7/40 | + |

* Soybeans, nonmoldy, had no moldy grain.
* Soybean mix, nonroasted, had less than 1% moldy grain.
* Moldy mix, roasted and nonroasted, had more than 10% moldy grain.

Table 3. Toxicity of soybean-derived aflatoxins in the chicken embryo

| Sample | Moldy grain (%) | Aflatoxin/egg (µg) | Hatch (%) |
|--------|----------------|--------------------|-----------|
| Aflatoxin standard | 0.025 | 33 |
| 9      | 2              | 0.015             | 53        |
| 17     | 3              | 0.010             | 66        |
| 11     | 68             | 0.005             | 13        |
| 19     | 7              | 0.002             | 66        |
| 15     | 3              | 0.001             | 40        |
| Control (70% EToH) |       |                    | 73        |
We also found that even though aflatoxin-producing isolates of *Aspergillus* spp. could be identified in the soybean samples to be used in poultry feed, detectable amounts of aflatoxins did not always occur (Table 2). The nonmoldy soybean sample was devoid of *Aspergillus* spp. and aflatoxins, as expected, whereas the two samples designated moldy mix yielded both aflatoxin-producing isolates of *Aspergillus* spp. and relatively high levels of aflatoxins. According to Hintz et al. (4) corn and soybeans having up to 50 \( \mu g \) of aflatoxin/kg would not be injurious to swine.

The detection of aflatoxins using the chicken embryo test is presented in Table 3. The aflatoxins used in this test were extracted directly from the sample by chloroform, reduced in volume, dissolved in ethanol, and after being quantitated spectrophotometrically were injected directly into the eggs without first being purified by thin-layer chromatography. This may explain the high mortality in embryos injected with extracts from sample 11 and the low level of correlation between aflatoxin level and mortality. Other chloroform-soluble mycotoxins may have been present in this sample which could have increased the percentage mortality of embryos.

Our studies indicate that soybeans can be a suitable substrate for growth of aflatoxin-producing strains of *Aspergillus* spp. especially if heavy rainfall occurs during maturation. Future routine examination of soybeans for the presence of aflatoxins is justifiable especially in areas of high rainfall or where soybeans will be used in poultry or livestock feeds.

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