Five-Year Study of Mycotoxins in Virginia Wheat and Dent Corn

ODETTE L. SHOTWELL and CLIFFORD W. HESSELTINE
U.S. Department of Agriculture, Agricultural Research Service, Northern Regional Research Center, Peoria, IL 61604

Every year during the 5-year period 1976-1980, approximately 100 samples each of corn and wheat from trucks delivering the grains at elevators in Virginia were collected by personnel of the Federal Grain Inspection Service and shipped to NRRC. Samples were analyzed as soon as possible for aflatoxin, zearalenone, and ochratoxin A. The 3 mycotoxins were not detected in any wheat sample. Zearalenone and ochratoxin A were not found in any corn sample; however, aflatoxin was detected in at least 25% of the corn samples from every crop year. In 1976-1980, the incidence of aflatoxin at levels of 20 ng/g or more (the Food and Drug Administration guideline) ranged from 18 to 61%; aflatoxin incidence above 100 ng/g was 5-29%. The average aflatoxin levels in corn samples collected in the 5 years varied from 21 to 137 ng/g. Moisture content of the samples was not determined, so aflatoxin levels given may be higher than they were at harvest. However, there are obviously differences from year to year. In freshly harvested corn samples collected by fieldmen of the Statistical Reporting Service in yield surveys in 1978 and 1979, aflatoxin incidence above the FDA guideline was 10 and 13%, and above 100 ng/g was 4 and 7%. The average aflatoxin level in all samples collected in 1978 was 13 ng/g and in 1979, 36 ng/g. Some aflatoxin can be expected yearly in Virginia corn, but the incidence and levels vary from year to year.

In 1975, the occurrence of aflatoxin, zearalenone, and ochratoxin A was studied in wheat grown in areas where aflatoxin has been a problem (1). Of 42 wheat samples collected in Virginia, 19 had zearalenone, but none had aflatoxin or ochratoxin A. Virginia weather was unusually cold and rainy in 1975; wheat fields were infected with Fusarium species that produce zearalenone. Graders of the Federal Grain Inspection Service (FGIS) observed a large number of “tombstone” wheat samples with the grey pallor characteristic of wheat invaded by Fusarium. Fusarium outbreaks in wheat (“scabby wheat”) are said to be associated with the type of weather conditions experienced in 1975, and these conditions might occur about once every 5 years in the weather cycles (1). Because of the high zearalenone incidence, it was decided to study wheat over a period of years to determine how often such outbreaks could be expected. At the same time, wheat would be analyzed for aflatoxin and ochratoxin A, and the investigation would be extended to corn. A reliable screening procedure is available to detect the 3 mycotoxins in agricultural commodities (2).

We now are reporting the results of a 5-year study of wheat and corn grown in Virginia for the occurrence of aflatoxin, zearalenone, and ochratoxin A. The samples were collected by FGIS personnel. We also are reporting the aflatoxin occurrence in freshly harvested corn samples collected by fieldmen of the Statistical Reporting Service (SRS) in 1978 and 1979.

Materials and Methods

Sample Collection and Handling

About 100 samples each (ca 1.3 kg) of wheat and shelled dent corn were collected each year during the 5-year period 1976-1980 by FGIS personnel from farm trucks delivering at Virginia elevators. The corn and wheat were grown in the same year that samples were collected, and the county in which they were grown was recorded. The samples were shipped to Peoria as soon as collected and were assayed upon arrival in Peoria. Moisture levels were not determined.

In 1978 and 1979, one 15 ft row of mature dent corn was harvested by SRS fieldmen from each field to obtain about 100 samples of shucked ear corn. The collection was made as SRS personnel were conducting yield surveys and was representative of the corn grown in Virginia in that year. Samples were shipped to Peoria in corrugated cardboard boxes surrounded by newspapers and were in transit about 7 days. When received in Peoria, they were shelled and dried (overnight 80°C). The shelled dried samples averaged 2.2 kg.

Preparation of Subsamples for Analysis

Each sample of shelled corn was coarsely ground in a Straub disc mill and then finely ground to pass a No. 20 sieve in a 6 in. Raymond
hammer mill fitted with a screen containing \( \frac{1}{6} \) in. perforations. Wheat samples could be ground rapidly in the Raymond hammer mill to pass a No. 20 sieve without being coarsely ground in the disc mill first. Each finely ground sample of wheat or corn was blended 15 min in a Hobart planetary mixer.

**Analysis for Mycotoxins**

Subsamples (50 g) of corn or wheat collected by FGIS personnel were analyzed by the Eppley procedure for aflatoxin, zearalenone, and ochratoxin A (2). Samples are extracted with chloroform-water and extract aliquots representing 10 g of the subsample are placed on a silica gel column. Zearalenone, aflatoxin, and ochratoxin A are eluted separately from the column and eluates are prepared for thin layer chromatography (TLC). Solvents for TLC on Absorbosil-1 plates are as follows: for zearalenone, ethanol-chloroform (5 + 95); for aflatoxin, water-acetone-chloroform (0.5 + 10 + 90); and for ochratoxin A, glacial acetic acid-benzene (10 + 90). The detection limits of the analysis as carried out were 100 ng/g for zearalenone, 1-3 ng/g for aflatoxins, and 30 ng/g for ochratoxin A. If a sample was positive for ochratoxin A by TLC, the result was tested by the confirmatory test approved by the Association of Official Analytical Chemists (AOAC) (3). If a sample contained aflatoxins, levels were determined by the method designated as the CB (Contaminants Branch) method approved for corn by both the AOAC (3) and the American Association of Cereal Chemists (AACC) (4).

Subsamples (50 g) from corn collected by SRS fieldmen were assayed for aflatoxins by the CB method approved for corn by the AOAC (3) and AACC (4).

**Results and Discussion**

Zearalenone, aflatoxins, and ochratoxin A were not detected in any of the wheat samples collected in the period 1976–1980. The high incidence (45%) of zearalenone in 1975 wheat from Virginia must have been unusual (1). Aflatoxins were detected in corn samples collected in every year by FGIS personnel (Table 1). The percent incidence of samples with detectable aflatoxin varied from 29% (1979) to 82% (1980). The incidence of samples with aflatoxin levels equal to or greater than 20 ng/g ranged from 18% (1979) to 61% (1980). Average aflatoxin levels in all samples collected in a year during the period 1976–1980 were between 21 ng/g in 1978 and 137 ng/g in 1980. In 1980, the aflatoxin incidence in the FGIS samples above the guidelines was unusually high—61%. Of the 1980 samples, 29% had more than 100 ng/g. Aflatoxin levels in corn were high in southeastern corn in 1980 (7). In fact, the situation was so serious that the Food and Drug Administration (FDA) granted limited exemptions to the states of North Carolina and South Carolina and the Commonwealth of Virginia from its prohibition of interstate shipment and blending of corn containing aflatoxin in excess of the agency’s action level of 20 ng/g (8). The FDA allowed corn containing up to 100 ng/g to be shipped in interstate commerce under plans developed by the 3 states and approved by the FDA. The exemption applied only to corn from the crop year 1980. Some toxin could have formed after samples were collected, because some of the samples were noticeably damp when received (9).

Corn samples were collected by the FGIS in 53 counties in Virginia in the 5-year period. However, from some counties samples were received in only 1 or 2 years. There were 8 counties from which samples were collected in every year, 1976–1980. Inspection of aflatoxin levels in corn samples in the 18 counties where 10–55 samples were collected in the 5-year period did not reveal obvious differences in aflatoxin occurrence among counties. There were not enough samples collected in each county to evaluate statistically the likelihood of an aflatoxin outbreak in any one county or area.

At first, aflatoxin levels and incidences appeared to be lower in freshly harvested corn samples collected by SRS fieldmen than in the shelled samples collected by FGIS personnel from trucks delivering at elevators (Table 1). In 1978, aflatoxin incidence in FGIS corn samples was 36% compared with 12% in SRS corn samples. However, in 1979, the toxin incidence was 29% in FGIS samples compared with 21% in SRS corn samples. The average aflatoxin level in SRS samples was 13 ng/g in 1978 and 36 ng/g in 1979; in FGIS samples, 21 ng/g in 1978 and 34 ng/g in 1979.

Of the 3 mycotoxins studied in wheat and
### Table 1. Aflatoxin levels in dent corn grown in Virginia, 1976–1980

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<tbody>
<tr>
<td></td>
<td>No. of samples (%)</td>
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<tr>
<td>ND&lt;sup&gt;a&lt;/sup&gt;</td>
<td>77 (63)</td>
<td>52 (51)</td>
<td>63 (64)</td>
<td>81 (71)</td>
<td>18 (18)</td>
<td>79 (88)</td>
<td>93 (79)</td>
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<tr>
<td>&lt;20</td>
<td>13 (10)</td>
<td>17 (17)</td>
<td>10 (10)</td>
<td>13 (11)</td>
<td>20 (20)</td>
<td>2 (2)</td>
<td>9 (8)</td>
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<tr>
<td>20–100</td>
<td>21 (17)</td>
<td>18 (18)</td>
<td>21 (21)</td>
<td>8 (7)</td>
<td>32 (32)</td>
<td>5 (6)</td>
<td>7 (6)</td>
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<tr>
<td>101–500</td>
<td>9 (7)</td>
<td>10 (10)</td>
<td>5 (5)</td>
<td>10 (9)</td>
<td>26 (26)</td>
<td>4 (4)</td>
<td>7 (6)</td>
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<td>501–1000</td>
<td>1 (1)</td>
<td>1 (1)</td>
<td>2 (2)</td>
<td>1 (1)</td>
<td>—</td>
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<tr>
<td>&gt;1000</td>
<td>2 (2)</td>
<td>3 (3)</td>
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<td>—</td>
<td>2</td>
<td>2</td>
<td>—</td>
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<tr>
<td>Total</td>
<td>123</td>
<td>101</td>
<td>99</td>
<td>114</td>
<td>99</td>
<td>90</td>
<td>117</td>
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<tr>
<td>% Incidence</td>
<td>37</td>
<td>49</td>
<td>36</td>
<td>29</td>
<td>82</td>
<td>12</td>
<td>21</td>
</tr>
<tr>
<td>% ≥ 20 ng/g</td>
<td>27</td>
<td>32</td>
<td>26</td>
<td>18</td>
<td>61</td>
<td>10</td>
<td>13</td>
</tr>
<tr>
<td>% &gt; 100 ng/g</td>
<td>10</td>
<td>14</td>
<td>5</td>
<td>11</td>
<td>29</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>Av. level (ng/g), all samples</td>
<td>48</td>
<td>91</td>
<td>21</td>
<td>34</td>
<td>137</td>
<td>13</td>
<td>36</td>
</tr>
<tr>
<td>Av. level (ng/g), pos. samples</td>
<td>130</td>
<td>187</td>
<td>58</td>
<td>118</td>
<td>167</td>
<td>110</td>
<td>176</td>
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<sup>a</sup> ND = not detected.
corn, only aflatoxin was identified as a continuing problem in corn. From our investigations in 1975 wheat, we learned that zearalenone could cause problems when weather conditions were favorable for *Fusarium* invasions of wheat (scabby wheat). However, the fact that zearalenone was not detected in wheat harvested in 1976-1980 indicates that one outbreak of scabby wheat does not necessarily lead to infestation of crops in subsequent years. This information became more important in 1982, when scab-damaged wheat from a few areas was found to contain deoxynivalenol and to a lesser degree zearalenone-2 mycotoxins produced by *Fusarium* (10). The weather was unusually rainy in the areas where scabby wheat occurred in 1982.

**Acknowledgment**

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**REFERENCES**


(4) *American Association of Cereal Chemists Method 45-05, 1972 Revisions to AACC Approved Methods*


