Silk Maysin Content and Resistance of Commercial Corn [Maize] Hybrids to Kernel Contamination by Aflatoxin

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Abstract: Several phenols and related compounds have been shown to have detrimental effects on insects while others have antibiotic activity against fungi which attack higher plants. Insects have also been implicated as contributors to preharvest contamination of corn [maize], Zea mays L, by aflatoxin. The objectives, therefore, were to determine (a) if commercial corn hybrids vary in their silk maysin content and aflatoxin contamination of the grain, and (b) if grain aflatoxin contamination is correlated with maysin and related compounds. During 4 years of testing, 16 corn hybrids varied significantly for silk maysin content and grain aflatoxin contamination. Based on correlations, grain aflatoxin content of the hybrids tested was not significantly associated with maysin, chlorogenic acid, and 3'-methoxymaysin contents. It was concluded that other untested phenolic compounds in the category of compounds analyzed in the present study could be involved in resistance to aflatoxin formation, and that other classes of compounds should also now be assessed to locate major chemical resistance components.

Key words: Aspergillus flavus, plant resistance, Helicoverpa zea, antibiosis.

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INTRODUCTION

Protection of the corn [maize], Zea mays L, crop from ravages by insects and disease is an important objective of farmers in most tropical and subtropical regions. Contamination of the grain by aflatoxin, a metabolite of Aspergillus flavus (Link ex Fries) and A. parasiticus (Speare) is also a major concern of farmers in the same corn-growing areas. Ear damage by insects has been demonstrated to be closely associated with the aflatoxin problem in corn (Widstrom 1979; McMillian 1983), especially by corn earworm (Helicoverpa zeae, Boddie), in the southeastern USA (McMillian et al. 1985; McMillian 1987). Control of ear-feeding insects, therefore, also contributes to a reduction in aflatoxin contamination of the crop.

A compound detrimental to the corn earworm has been isolated from corn silks and identified as maysin, a flavone glycoside (Waiss et al. 1979). Corn populations and inbreds have been shown to vary widely in their flavone glycoside contents (Snook et al. 1993), but few commercial hybrids have been tested for their maysin content.

The existence of anthocyanins and flavonoid compounds in maize has been known for many years, but the functions of the compounds have been reported only infrequently (Waiss et al. 1979). Flavonol-like compounds produced by plants have been reported that influence fungal growth (Jambunathan and Kherdekar 1991; Bécard et al. 1992). In addition, many of the compounds found in plants that show biological activity against insects are in the flavonoid and anthocyanin biosynthetic pathways. The flavone glycoside maysin, which has detrimental effects on the corn earworm (Waiss et al. 1979) is closely related to the C-glycosylflavones which were shown to serve as feeding stimulants to plant hoppers in rice, Oryza sativa (Kim et al. 1985).

Compounds in both insects (Leal et al. 1990) and plants (Neucere and Godshall 1991; Neucere et al. 1991) may have inhibitory effects on fungi. Some of these, such as the ketone β-ionone, seem to have deleterious effects on the development of Aspergillus flavus group of fungi (Wilson et al. 1981; Gueldner et al. 1985). Additionally, some plant flavonols stimulate fungal growth (Bécard et al. 1992), while others contain flavones or flavan-4-ols that inhibit the growth of fungi (Jambunathan and Kherdekar 1991). The possibility was then considered that maysin and its related compounds detrimental to corn earworm might also be influential in determining resistance in the corn plant to kernel invasion by A. flavus and subsequent contamination by aflatoxin.

One objective of this study was to determine if differences exist for silk-maysin content and aflatoxin contamination of grain among commercial hybrids. A second objective was to determine the correlation between silk-maysin and aflatoxin contamination of the grain.

MATERIALS AND METHODS

Sixteen commercial hybrids were chosen for testing in this study based on their popularity among farmers in 1980 and 1981, and also on their representing the range of maturities for hybrids grown in the southeastern USA. Eight were hybrids that had been previously evaluated for aflatoxin contamination under heavy infestation by corn earworm (Widstrom et al. 1978). Commercial hybrids were chosen because little (LaPrade and Manwiller 1977) or no information is available on the differences among them for resistance to aflatoxin production and/or maysin content. Some information is available on experimental hybrids (Lillehoj et al. 1983) and various endosperm mutants (Gardner and Wallin 1980; Widstrom et al. 1984), but commercially available hybrids have not been proposed as a source for resistance to aflatoxin production.

The hybrids were evaluated in a randomized complete block experiment with six replications in 1983 and 1984. Plots were single rows, 6-1 m long and 76 cm wide with plants spaced 20 cm apart within the row. One-half of the plants in each plot were knife-inoculated (stabbed on the abaxial side of the ear) at 20 days after silking (Widstrom et al. 1981) with a 10⁵ spore ml⁻¹ suspension of A. flavus. A bulk of unpollinated silks was collected from the remaining plants in each plot, frozen immediately, submerged in methanol and shipped to Western Regional Research Center (USDA, Albany, CA) for spectrophotometric analysis (Waiss et al. 1979). Ten inoculated ears were collected at physiological maturity, dried at 60°C, ground and analyzed for aflatoxin by high-performance liquid chromatography (HPLC) procedures (Thean et al. 1980). With the advent of a new procedure for determination of silk-maysin content (Snook et al. 1989), the same hybrids were planted in 1989 in an eight-replication test and evaluated for maysin and aflatoxin production. In 1989, the capability to quantify two other compounds (chlorogenic acid and 3'-methoxyoxymaysin) by the new HPLC procedure was developed at Richard Russell Research Center (Athens, GA). These compounds, although they each elicit detrimental effects to corn earworm, are of lesser importance than maysin in corn we have tested, since their values both quantitatively and qualitatively are reduced to one-third or less when compared with maysin (Gueldner et al. 1991, 1992). The new procedure provided us with the opportunity to re-examine the interpretation of our results from spectrophotometric analysis (Widstrom et al. 1991). Therefore, a randomized complete block experiment with two replications was evalu-
ated in 1990 for maysin, chlorogenic acid, and 3'-methoxymaysin. All aflatoxin data were transformed to ln(x + 1), where x = ng g⁻¹ aflatoxin, for analysis. The experiments were subjected to analyses of variance and were combined when errors tested homogeneous. Hybrid means were correlated for all traits to determine possible relationships, and mean separation was accomplished by the Waller-Duncan Bayesian k-ratio t-test.

RESULTS AND DISCUSSION

Hybrid silk-maysin values obtained from spectrophotometric absorbance at 352 nm in 1982 and 1983 were first analyzed separately from the HPLC-derived values in 1989 and 1990. Differences between years and among hybrids were significant at P < 0.01 for both 2-year sets (Table 1). The interaction of years with hybrids was also significant in both cases. The correlation between hybrid silk-maysin means obtained by spectrophotometry and by HPLC was 0.66 (P < 0.01), while correlations between 1982 and 1983 maysin means and HPLC chlorogenic acid and 3'-methoxymaysin means in 1990 were 0.53 (P < 0.05) and −0.19 (P = 0.48). It was concluded that maysin was the major contributor to the 1982 and 1983 means, but that chlorogenic acid was also contributing to those values. When the maysin analyses were combined over the 4 year period, years and hybrids were again significant, but a slight reduction in the interaction between these main effects occurred (Table 1).

Silk-maysin means for hybrids (Table 2) measured spectrophotometrically (1982–1983) are nearly four times as large as those determined by reversed-phase HPLC. Even when average values for chlorogenic acid (1.8 mg g⁻¹) and 3'-methoxymaysin (1.7 mg g⁻¹) are added to the mean maysin content of 5.6 mg g⁻¹, less than half the average for 1982–1983 (20 mg g⁻¹) is attained. When the 1990 sums of chlorogenic acid, 3'-methoxymaysin, and maysin were correlated with 1982–1983 maysin determinations, a value of r = 0.64, P < 0.01 was obtained. This suggests that other compounds such as chlorogenic acid and 3'-methoxymaysin exist in hybrid silks that absorb at 352 nm. Maysin must be considered the dominating compound involved with resistance to ear-feeding insects in view of its biological activity (Waiss et al 1979). Maysin, however, is possibly not an important resistance component of the silks in these hybrids since 20 mg g⁻¹ is generally considered to be a minimum level required for significantly reducing larval weight of the corn earworm (Gueldner et al 1991; Snook et al 1993). On the other hand, the fact that hybrids do vary significantly in their silk-maysin content suggests that minimal levels for antibiosis might be obtainable in hybrids developed from existing adapted germplasm, using maysin as a marker.

Aflatoxin contamination was similar in 1982 and 1983, but the 16 hybrids differed significantly as they did in 1989 (Table 1). Years differed greatly in the combined analysis since contamination was 2.5 times greater in 1989 than in previous years. In contrast to maysin evaluations, no interaction occurred between years and hybrids for aflatoxin concentration in mature grain. The aflatoxin contamination values had been determined in 1974 and 1975 under heavy insect infestation for eight of the hybrids (Widstrom et al 1978). The correlation of these values with those obtained under inoculated conditions in the present tests was 0.73 (P < 0.05), confirming the consistency of ranking for aflatoxin contamination of hybrids from year to year.

The demonstration of repeatable differences among commercial corn hybrids is important in that a general opinion exists that all hybrids are uniformly ultra-susceptible to aflatoxin contamination. The differences shown may be largely attributable to factors other than husk coverage, since the inoculation procedure penetrates through the husk directly into the kernels. Some of the hybrids tested have excellent husk coverage but were among the most severely contaminated. The consistency of aflatoxin values from year to year is probably attributable to the reduction of husk effects made possible by the inoculation procedures. The result was

<table>
<thead>
<tr>
<th>Source</th>
<th>Maysin (mg g⁻¹ dry wt)</th>
<th>Aflatoxin [ln(ng g⁻¹ + 1)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Years (Y)</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>Hybrids (H)</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>H × Y</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>CV (%)</td>
<td>55.8</td>
<td>36.6</td>
</tr>
</tbody>
</table>

* * * Significant at P = 0.05 and 0.01, respectively, NS, not significant.

Aflatoxins were not determined in 1990.
TABLE 2
Means for maysin content of silks and aflatoxin concentration in grain of commercial hybrids grown during a 4 year experiment

<table>
<thead>
<tr>
<th>Hybrid</th>
<th>Maysin (mg g(^{-1}) dry wt)</th>
<th>Aflatoxin concentration (ng g(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asgrow RX140A</td>
<td>15.4</td>
<td>2.1</td>
</tr>
<tr>
<td>Big D 6986</td>
<td>27.0</td>
<td>10.6</td>
</tr>
<tr>
<td>Dekalb 1214</td>
<td>9.3</td>
<td>1.5</td>
</tr>
<tr>
<td>Dekalb XL80</td>
<td>13.5</td>
<td>4.0</td>
</tr>
<tr>
<td>Dekalb XL395A</td>
<td>24.7</td>
<td>6.5</td>
</tr>
<tr>
<td>Funk G-4507</td>
<td>33.4</td>
<td>6.6</td>
</tr>
<tr>
<td>Jacques SX247</td>
<td>22.7</td>
<td>11.0</td>
</tr>
<tr>
<td>McCurdy 67-14</td>
<td>6.3</td>
<td>1.0</td>
</tr>
<tr>
<td>McNair X300</td>
<td>14.3</td>
<td>5.2</td>
</tr>
<tr>
<td>McNair 508</td>
<td>12.4</td>
<td>3.7</td>
</tr>
<tr>
<td>O's Gold SX5509</td>
<td>22.1</td>
<td>11.1</td>
</tr>
<tr>
<td>Paymaster VC8951</td>
<td>34.8</td>
<td>5.7</td>
</tr>
<tr>
<td>Pioneer 3030</td>
<td>12.1</td>
<td>3.2</td>
</tr>
<tr>
<td>Pioneer 3147</td>
<td>11.8</td>
<td>4.4</td>
</tr>
<tr>
<td>Pioneer 3369A</td>
<td>26.4</td>
<td>6.0</td>
</tr>
<tr>
<td>Ring Around RA1501</td>
<td>33.3</td>
<td>7.9</td>
</tr>
<tr>
<td>Average</td>
<td>20.0</td>
<td>5.6</td>
</tr>
<tr>
<td>LSD(_{0.05})</td>
<td>9.0</td>
<td>3.0</td>
</tr>
</tbody>
</table>

\(a\) The values for 1982–1983 were obtained spectrophotometrically while those for 1989–1990 were obtained using HPLC procedures.

\(b\) Aflatoxin concentrations are given as geometric means, or the antilogarithm of the logarithmic mean.

\(c\) LSR, least significant ratio and is used instead of LSD when testing significance of differences among geometric means.

no hybrid \(\times\) year interaction and a more reliable evaluation.

Since the hybrids we tested vary significantly for both silk-maysin content and grain aflatoxin contamination, these values should be correlated if there is a common or related resistance factor. When hybrid values for maysin, chlorogenic acid, 3'-methoxymaysin, and the sum of these three were correlated with hybrid means for aflatoxin contamination, within and combined over years, none of the correlations were significantly different from zero. No trends were detected in that among more than a dozen correlations \(r\) values varied over the range of \(-0.31\) to \(0.44\). It must be concluded that maysin and chlorogenic acid, compounds detrimental to insects, and the related 3'-methoxymaysin were not directly involved in resistance to contamination by aflatoxin of the 16 hybrids evaluated. It might be advisable to look for aflatoxin resistance factors among other classes of compounds as well as the phenols.

REFERENCES


Maysin content and resistance to aflatoxin contamination


