# Relationships between $C_6$–$C_{12}$ Alkanal and Alkenal Volatile Contents and Resistance of Maize Genotypes to *Aspergillus flavus* and Aflatoxin Production

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An association was found between $C_6$–$C_{12}$ alkanal and alkenal volatile contents in several maize genotypes and aflatoxin contamination. Volatiles generated from untreated, ground, field resistant (R) maize kernels contained in an inverted lid of a Petri dish affected growth and aflatoxin production by *Aspergillus flavus* when spores were inoculated on potato dextrose agar in a closed, sealed Petri dish assay. Volatiles from the ground maize genotypes were purged onto Tenax columns and analyzed by GC/MS; $C_6$–$C_{12}$ aldehydes were more prominent in the GC/MS total ion chromatograms of the R genotypes. Maize genotypes that exhibited a greater inhibition of aflatoxin production in the inverted lid assay also exhibited a larger concentration of linoleic acid in their fatty acid profiles. These results suggest the correlation of decay products of polyunsaturated fatty acids and plant disease resistance and indicate that the lipoxygenase pathway may contribute to this resistance.

**Keywords:** *Lipoxygenase products; volatiles; aldehydes; antifungal; aflatoxin*

## INTRODUCTION

Toxigenic strains of *Aspergillus* produce toxic secondary metabolites called aflatoxins which are carcinogenic to both man and animals (Busby and Wogan, 1979; Groopman *et al.*, 1981). *Aspergillus* spp. infection and aflatoxin contamination are found in many commercially important food/feed crops (corn, cotton, peanuts, and tree nuts). Aflatoxin contamination of maize (*Zea mays* L.) is a serious pre- and postharvest problem, because *Aspergillus flavus* (Link: Fr.) may infect the crop prior to harvest and remain throughout harvest and storage (Lillehoj, 1987; Payne, 1992).

Various lipid breakdown products including small-chain $C_6$–$C_{12}$ aldehydes are formed from polyunsaturated fatty acids such as linoleic and linolenic acids via enzyme steps involving lipoxygenase (EC 1.13.11.12), hydroperoxide lyase, and hydroperoxide dehydrase (Gardner, 1991; Vick and Zimmerman, 1987). The enzymic breakdown of these polyunsaturated fatty acids results in intermediates or final products with structures that resemble the regulatory "eicosanoid" compounds found in animal systems (Anderson, 1989). The term "octadecanoids" was originated to describe polyunsaturated, precursor fatty acids in plants resembling their eicosanoid counterparts from animal systems (Vick and Zimmerman, 1987; Slusarenko *et al.*, 1991; Farmer and Ryan, 1992; Croft *et al.*, 1993). It has been suggested that a similar biological role for these chemical compounds could also exist in the plant world. The suggested bioactivity forms the basis for the theory that lipoxygenase activity might produce signal or regulatory molecules in plants in response to microbial attack.

Field studies of aflatoxin contamination in maize have demonstrated that differences do exist among genotypes in the ability to resist aflatoxin contamination (Zuber *et al.*, 1983; Widstrom *et al.*, 1987). The purpose of the current investigation was to determine if volatiles emitted from ground maize kernels of resistant genotypes could influence the growth and aflatoxin production of *in vitro* cultures of *A. flavus*. We also sought to characterize chemical differences in volatiles emitted among genotypes in an attempt to correlate observed field susceptibility of maize genotypes to *in vitro* results. In this investigation we tested the hypothesis that volatile products of the lipoxygenase pathway may be correlated with aflatoxin resistance in maize.

## MATERIALS AND METHODS

**Fungal Culture and Growth Conditions.** An aflatoxigenic isolate of *A. flavus* (SRRC 1273), obtained from Arizona field grown cotton, was cultured on potato dextrose agar (PDA) Petri plates. After 7 days of incubation at 28–30 °C, the spores were harvested and a spore suspension ($3.5 \times 10^5$ spores/10 $\mu$L of inoculum in deionized water containing 1% Triton X-100) was used as inoculum.

**Sources of Maize Genotypes and Aldehydes.** The resistant breeding population GT-MAS:gk (Widstrom *et al.*, 1987; Brown *et al.*, 1993) was obtained from the USDA/ARS, Insect Biology and Population Management Laboratory, in Tifton, GA. Inbred lines M182, T115, CI2, and 33-16 were obtained from the Department of Plant Pathology of the University of Illinois. T115, CI2, and M182 were identified as potentially resistant as inbreds and as F1 crosses with susceptible inbreds B73 and/or MO17 in field trials, while 33-16 was identified as susceptible (Campbell, 1994). Yellow Creole (R) and Huffman (S) were field tested by Zuber *et al.* (1983) and were obtained from same. Kernels of all genotypes used in this experiment were shelled from maize ears grown in the field in 1993 and harvested at full maturity. The kernels were stored at −20 °C and thawed at 2–4 °C prior to being ground. They were used immediately after being ground.

**Assay of Volatile Activity.** Fifty grams of maize kernels from each maize genotype were milled on a Tekmar analytical mill, Type A 1052 mill, 40 mesh screen, and either 100 or 300 mg of the ground kernels was placed in the inverted lid of a 100 mm × 15 mm Petri plate set. Eighteen milliliters (15.4 g) of PDA was applied to the bottom plate of the Petri plate set; a 10 mm agar plug was extracted with a sterile cork borer from the center section of the solidified PDA. Ten microliters

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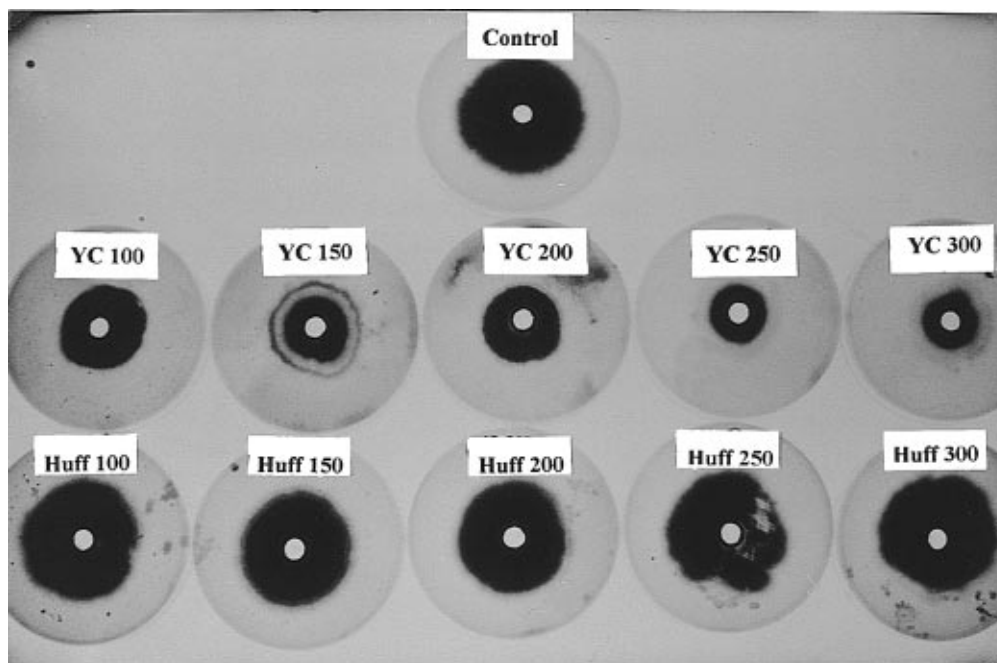
**Figure 1.** Inverted top lid of Petri plate bioassay containing various amounts (100, 150, 200, 250, and 300 mg) of ground Yellow Creole (YC) aflatoxin field resistant and aflatoxin field susceptible Huffman (Huff) genotype. The photograph was taken 5 days after a spore suspension of aflatoxigenic *A. flavus* was inoculated into the center well of the Petri plate assembly.

of a spore suspension containing $3.5 \times 10^5$ spores of an aflatoxigenic strain of *A. flavus* (SRRC 1273) was inoculated in the extracted center well of the PDA. The bottom of the Petri plate set (containing the *A. flavus* inoculated PDA) was then placed in the inverted top (containing the ground kernels of the maize genotype to be tested). The two halves of the plates were sealed together with parafilm. After 5 days of incubation at $30 \pm 0.2$ °C, radial growth and aflatoxin content were determined (Zeringue and McCormick, 1990).

**Assay of Aldehyde Activity.** We modified the 5-day incubation assay described earlier (Zeringue and McCormick, 1990) to assay fungal growth and aflatoxin production in atmospheres of selected aldehydes. A small hole was introduced into the cover of the Petri plate set to inject referenced aldehydes into small glass beakers within the bottom section of the Petri plate pair. This hole was sealed with autoclave tape after the aldehyde injection. After the incubation period, radial growth and aflatoxin determinations were performed as reported earlier (Zeringue and McCormick, 1990).

**Volatile Trapping and GC/MS Procedures.** Fifty milligrams of ground maize kernels were positioned in the center of a glass tube (84 mm × 9 mm, 7 mm i.d.) packed between plugs of glass wool. The tube was placed in an external closed inlet device (ECID) (Legendre *et al.,* 1979), and volatiles were purged at 30 °C with a helium flow of 55 mL/min for 1 h into a similar glass tube containing Tenax GC (0.1 g of Tenax 60/80 mesh) also packed between plugs of glass wool. This procedure separated the trapped volatiles from water in the kernels. In a preliminary test, a similar Tenax GC tube was added in line to the first Tenax tube and was monitored for volatiles not trapped by the first Tenax tube in line. No volatiles were found in this second Tenax-containing tube. The glass tube containing Tenax and the trapped volatiles was then repositioned into the ECID, and trapped volatiles were heat desorbed at 135 °C from the Tenax column with a helium flow of 20 mL/min for 3 min onto a capillary GC column (HP-5, cross-linked 5% phenyl methyl silicon column, 50 m × 0.2 mm with a coating 0.5 $\mu$m thick). Prior to heat desorption from the Tenax column, the GC oven temperature (contained in an HP 5890A-5971 GC/MS system or an HP5890 Series II-59827A GC/MS system) was cryogenically lowered to −30 °C for 3 min and was held at this initial temperature during the desorption of the purged−trapped volatiles onto the GC capillary column. The GC oven was programmed to 30 °C at a rate of 5 °C/min and then to 250 °C at a rate of 15 °C/min. The oven

temperature was held at 250 °C for 5 min. Electron ionization was operated at 70 eV, helium linear velocity was set at 30 cm/s, and the internal injector was adjusted at 200 °C. Identification of the separated aldehydes was based on retention time and mass spectra of authentic samples and by computer matching of the unknown spectra with reference mass spectra in the HP G1035A No. AA9 Wiley 130 K mass spectral databases (Wiley, 1986). Quantitation was accomplished by use of external authentic standards and the HP 1030A computer Chem Station.

**Fatty Acid Profiles of Maize Kernels.** Thirty milliliters of hexane was added to 3 g of each ground lyophilized whole maize genotype kernel tested and was refluxed at 140 °C in a Soxtec System HT6, 1043 extraction unit (Perstory Analytical) for 1 h and then rinsed for 15 min in 30 mL of the same solvent. The solid residue was separated from the liquid hexane extract, 30 mL of a mixture of chloroform/methanol ($CHCl_3$/MeOH, 2:1 by volume) was added to the separated hexane solid residue (to further extract lipid containing materials) and was refluxed in the Soxtec System HT6 at 140 °C for 1 h followed by a 15-min rinse in 30 mL of the same solvent. The liquid extracts from both reflux extractions were dried under $N_2$ and vacuum. The weights of fats or oils were recorded, and the lipid portions were converted to methyl esters (Christie *et al.,* 1984) for GC/MS analysis of fatty acid profiles. Fatty acid methyl esters were identified by GC retention times, by mass spectra from authentic standards, and by the library spectral data bases reported above.

## RESULTS AND DISCUSSION

Volatiles emitted from the ground maize kernels in the inverted lid Petri plate assay suggested that a volatile component or volatile components could be responsible for differences in observed radial growth and aflatoxin production (Figure 1; Table 1). The greatest inhibition of radial growth and aflatoxin production in both the 100 and 300 mg assays (Table 1) was observed with Yellow Creole, GT-MAS:gk, and MI82 maize genotypes. A correlation was observed between the aldehyde content found in the volatile profile of the various maize genotypes and the inhibition of radial growth and aflatoxin production (Table 1). Those genotypes with reported field resistance to aflatoxin

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**Table 1. Emitted $C_6$–$C_{12}$ Aldehydes (Nanograms)[a] from 50 mg of Ground Kernels of Aflatoxin Field Resistant (R)[b] and Aflatoxin Field Susceptible" (S)[b] Maize Genotypes and Percent of Control of Radial Growth (RG) and Aflatoxin Production (AP) of Each Maize Genotype Determined on *in Vitro* 5-Day PDA Solid Cultures**

| maize genotype | emitted $C_6$–$C_{12}$ aldehydes (ng/h) | | | | | | | | total aldehydes | ground kernels (mg) | % of control | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | hexanal | *trans*-2-hexenal | heptanal | *trans*-2-heptenal | octanal | nonanal | decanal | 2,4-do-decadenal | | | RG[c] | AP[d] |
| Yellow Creole (R) | 390 ± 3.5[f] | | 21 | | 36 ± 0.2 | | | | 447 | 100 | 27.9 ± 3.4 | 36.8 ± 2.4 |
| | | | | | | | | | | 300 | 9.2 ± 1.8 | 3.5 ± 1.6 |
| GT-MAS:gk (R) | 199 ± 1.3 | 9 ± 0.5 | | | 136 ± 4.2 | 18 ± 0.6 | | 14 ± 2.6 | 376 | 100 | 38.9 ± 1.8 | 24.5 ± 2.3 |
| | | | | | | | | | | 300 | 19.5 ± 2.5 | 5.8 ± 3.4 |
| T115 (R) | 8 ± 0.4 | | | | | | | | 8 | 100 | 82.3 ± 3.4 | 72.3 ± 3.8 |
| | | | | | | | | | | 300 | 74.9 ± 1.8 | 61.9 ± 1.5 |
| CI2 (R) | 7 ± 0.4 | | | | 134 ± 3.1 | 24 ± 0.3 | 10 ± 0.2 | | 175 | 100 | 97.6 ± 1.5 | 89.9 ± 2.4 |
| | | | | | | | | | | 300 | 93.2 ± 0.6 | 85.8 ± 1.2 |
| MI82 (R) | 100 ± 0.9 | 12 ± 0.2 | | 13 ± 2.1 | 317 ± 5.6 | 26 ± 0.2 | | 8 ± 1.3 | 476 | 100 | 32.4 ± 2.8 | 28.2 ± 1.3 |
| | | | | | | | | | | 300 | 22.9 ± 1.2 | 12.1 ± 1.7 |
| Huffman (S) | 49 ± 1.2 | | | | 26 ± 0.1 | | | | 75 | 100 | 86.4 ± 5.6 | 96.5 ± 3.2 |
| | | | | | | | | | | 300 | 71.4 ± 4.3 | 72.3 ± 1.8 |
| Pioneer 3192 (S) | 10 ± 0.6 | | | | 16 ± 0.2 | | | | 26 | 100 | 83.4 ± 3.6 | 87.6 ± 1.8 |
| | | | | | | | | | | 300 | 91.6 ± 2.4 | 79.4 ± 2.6 |
| 33–16 (S) | 4 ± 0.1 | | | | | | | | 4 | 100 | 98.0 ± 1.6 | 85.6 ± 3.9 |
| | | | | | | | | | | 300 | 81.8 ± 2.3 | 72.1 ± 2.6 |
| | | | | | | | | | | control[e] | | |

[a] See text for description of trapping, elution, and program separation of volatiles into a Hewlett-Packard (HP) 5890A-5971 GC/MS system or an HP 5890 Series II-59827A GC/MS system. [b] Field-grown genotypes that show different levels of resistance or susceptibility to *A. flavus* infection. [c] Mean ± SD for these replicates per tested level. Radial growth measured directly from Petri plate as described in text. [d] Mean ± SD for three per tested level. Aflatoxin $B_1$ was extracted from PDA media/Petri plate purified by thin-layer chromatography, and quantified by fluorescence, as described in text. [e] Radial growth control 33 ± 2 mm ($n = 5$). Aflatoxin $B_1$ control 22 569 ± 286 ng/g PDA ($n = 5$) determined on 5-day cultures. [f] Mean ± SD ($n = 3$).

contamination (Widstrom *et al.*, 1987; Brown *et al.*, 1993; Zuber *et al.*, 1983; Campbell, 1994) also show a higher hexanal and total aldehyde content in the volatile profile of the ground kernels (100–390 ng of hexanal emitted/h, 376–476 ng of total aldehydes emitted/h) compared to susceptible genotypes (4–49 ng of hexanal/h, or 4–75 ng/h total aldehydes) (Table 1). The mean of the total aldehydes (433 ng/h) of those three field resistant genotypes (Yellow Creole, GT-MAS:gk, and MI82) demonstrated increased inhibition of radial growth and aflatoxin biosynthesis in the *in vitro* bioassay compared to the mean (58 ng/h) of the other genotypes (Huffman, Pioneer 3192, 33-16, T115, and CI2) which express lesser resistance in the field. In the *in vitro* assay this represents 7.5 times more aldehyde emitted per hour by the field resistant genotypes.

The fungitoxic properties of hexanal and related $C_6$–$C_{12}$ saturated and unsaturated aldehydes to aflatoxigenic *Aspergillus* species have been previously reported (Gueldner *et al.*, 1985; Zeringue *et al.*, 1989, 1990; Hamilton-Kemp *et al.*, 1992; Andersen *et al.*, 1994). In the current investigation, we found that 50 mg of ground Yellow Creole kernels emitted 447 ng of total aldehydes over a 1-h trapping period and that volatiles emitted by 300 mg of ground Yellow Creole kernels inhibited radial growth by 90.8% and aflatoxin production by 46.5% (Table 1). We exposed aflatoxigenic *A. flavus* in PDA petri plate bioassays to saturated and unsaturated $C_6$–$C_{12}$ aldehydes in lower concentrations than we reported earlier (Zeringue and McCormick, 1990). In Table 2 note that we found 100% reduction in aflatoxin production in 5-day cultures when *A. flavus* was exposed to atmospheres containing 83 μg of hexanal (no fungal growth), 34% reduction when exposed to 8.3 μg of hexanal, 21% reduction when exposed to 4.2 μg of hexanal, and 17% reduction when exposed to 2.1 μg hexanal.

**Table 2. Percent of Control of Radial Growth (RG) and Aflatoxin $B_1$ (AP) Production in 5-Day Solid Cultures of *A. flavus* in Contact[a] with Various Levels of Selected Aldehydes[b]**

| aldehyde | μg of tested aldehyde | % of control[c] | |
| --- | --- | --- | --- |
| | | RG | AP |
| hexanal | 83 | 0 ± 0[d] | 0 ± 0 |
| | 8.3 | 80 ± 2.4 | 66 ± 5.2 |
| | 3.2 | 83 ± 2.3 | 79 ± 3.4 |
| | 2.1 | 89 ± 3.4 | 83 ± 5.0 |
| *trans*-2-hexenal | 66 | 0 ± 0 | 0 ± 0 |
| heptanal | 74 | 0 ± 0 | 0 ± 0 |
| *trans*-2-heptenal | 76 | 0 ± 0 | 0 ± 0 |
| octanal | 65 | 0 ± 0 | 0 ± 0 |
| | 6.5 | 79 ± 2.5 | 60 ± 3.1 |
| | 3.3 | 82 ± 2.0 | 78 ± 6.4 |
| | 1.6 | 85 ± 3.1 | 87 ± 2.1 |
| trans-2-octenal | 67 | 0 ± 0 | 0 ± 0 |
| nonyl aldehyde | 58 | 0 ± 0 | 0 ± 0 |
| | 29 | 84 ± 2.1 | 9.6 ± 0.5 |
| | 14.5 | 83 ± 3.7 | 15 ± 1.3 |
| | 7.3 | 85 ± 2.0 | 46 ± 3.8 |
| *trans*-2-nonenal | 60 | 0 ± 0 | 0 ± 0 |
| *n*-decyl aldehyde | 53 | 100 ± 3.1 | 90 ± 3.2 |
| dodecyl aldehyde | 45 | 100 ± 2.6 | 90 ± 4.8 |

[a] Two 10 mm holes were extracted from PDA Petri plates: one hole was produced in the center of the plate (toxigenic *A. flavus* spores were inoculated in this hole); the other hole was made along the other margin of the PDA plate (a 1-mL glass beaker containing the tested aldehyde was placed in this extracted hole). [b] Authentic aldehydes. [c] Radial growth control = 2.9 ± 2 mm ($n = 3$); aflatoxin $B_1$ control = 28905 ng ± 376 g PDA ($n = 3$) determined on 5-day cultures. [d] Mean ± SD.

In addition to high levels of hexanal produced by two resistant genotypes (GT-MAS:gk and MI82), high levels of octanal (136 and 317 ng, respectively) were also produced by these two genotypes. Resistant CI2 also demonstrated high levels of emitted octanal, but this genotype did not show inhibition of growth or aflatoxin biosynthesis in the inverted plate bioassay (Table 1).

**Table 3. Major Fatty Acid Distributions (Percent) of Hexane and of CHCl₃/MEOH (2:1 by Volume) Extractables from Kernels of Field Resistant and Field Susceptible Maize Genotypes**

| maize genotype | % of total oil | hexane extract | | | $C_{18:2}/C_{18:1}$ ratio | CHCl₃/MEOH extract[a] | | | | $C_{18:2}/C_{18:1}$ ratio |
|---|---|---|---|---|---|---|---|---|---|---|
| | | $C_{16}$[b] | $C_{18:1}$ | $C_{18:2}$ | | $C_{16}$ | $C_{18}$ | $C_{18:1}$ | $C_{18:2}$ | |
| Yellow Creole (R)[c] | 9.7 | 11.47[d] | 24.24 | 64.29 | 2.7 | 18.39 | 4.28 | 19.91 | 57.42 | 2.9 |
| GT-MAS:gk (R) | 9.9 | 12.14 | 25.20 | 62.66 | 2.5 | 19.93 | 6.13 | 26.76 | 47.18 | 1.8 |
| T115 (R) | 10.8 | 16.26 | 41.69 | 39.03 | 0.9 | 19.19 | 11.29 | 32.81 | 36.71 | 1.1 |
| CI2 (R) | 16.1 | 7.6 | 37.41 | 54.99 | 1.5 | 20.36 | 12.58 | 26.58 | 40.48 | 1.5 |
| MI82 (R) | 9.8 | 13.02 | 31.95 | 55.03 | 1.7 | 9.58 | 7.78 | 27.64 | 55.00 | 2.0 |
| Huffman (S)[c] | 9.0 | 11.21 | 43.64 | 45.15 | 1.0 | 12.77 | 6.55 | 36.12 | 44.56 | 1.2 |
| Pioneer 3192 (S) | 11.6 | 17.04 | 42.07 | 40.89 | 0.9 | 18.90 | 5.02 | 39.16 | 36.92 | 0.9 |
| 33−16 (S) | 9.9 | 14.12 | 38.84 | 47.04 | 1.2 | 20.84 | 13.28 | 30.41 | 35.47 | 1.2 |

[a] Chloroform/methanol (2:1 by volume) [b] Methyl esters of fatty acids: 16:0, palmitic; 18:0, stearic; 18:1, oleic, 18:2, linoleic. [c] R, field resistant, S, field susceptible, to aflatoxin B₁ formation. [d] Mean ($n = 2$).

In the 5-day culture assay (Table 2), 65 μg of octanal completely inhibited the growth of *A. flavus* and 1.6 μg of octanal inhibited *A. flavus* growth by 15% and aflatoxin production by 13%.

Table 3 represents the total oil/fat content and the major fatty acid profiles of the ground, lyophilized kernels of the several maize genotypes tested after the kernels were extracted with hexane and CHCl₃/MeOH. The highest area percentages of linoleic acid were found in both the hexane extract and the CHCl₃/MeOH extract in genotypes that exhibited field resistance and *in vitro* aflatoxin inhibition in our bioassay. Table 3 displays the differences in the linoleic acid ($C_{18:2}$)/oleic acid ($C_{18:1}$) ratios in the major fatty acid profiles of the tested maize genotypes. In addition, higher ratios of $C_{18:2}/C_{18:1}$ were observed in those maize genotypes that exhibit field resistance to aflatoxin and those which show an inhibitory effect in our *in vitro* bioassay.

Hexane extracts both the free fatty acids and the triacylglycerol components from the ground maize kernels. The CHCl₃/MeOH mixture apparently extracted more bound fatty acids from the remaining solid residue following the hexane extraction. Maize aleurone contains a high level of triglyceride (Tan and Morrison, 1979), and it is possible that the additional fatty acids extracted with CHCl₃/MeOH may represent bound lipids from endosperm and regions extracted from the aleurone layer, the nonstarch fraction of the starchy endosperm, and the inside of the starch granules (Morrison, 1977; Tan and Morrison, 1979). A difference in the fatty acid compositions of these extracted lipid components among the maize genotypes tested could explain the differences in aflatoxin susceptibility and resistance demonstrated in the field and in the *in vitro* aflatoxin inhibition studies. Hexanal and octanal production can occur from linoleic acid peroxidation by enzymic and nonenzymic means through the action of various active species (singlet oxygen and superoxide anions) and organic free radicals (Thompson *et al.*, 1987). Lipoxygenase will oxidize polyunsaturated fatty acids that have a *cis*-1,4-pentadiene structure such as linoleic ($C_{18:2}$) and linolenic ($C_{18:3}$) acids. Linoleic acid ($C_{18:2}$) is the most abundant fatty acid found in the corn kernel (35−66%) compared to linolenic (<3%) (Bolling and El Baya, 1971) and apparently represents the major precursor of the toxic aldehydes identified.

Lipid enzymes (lipase, phospholipase, lipoxygenase, and peroxidase) of intact cereal grains in the dry state ordinarily are inactive, but they can be activated simply by tissue disruption in the apparent absence of water (Gardner, 1988; Brockmann and Acker, 1977a−c). Damage to maize kernels, either by fungal attack or by grinding (as demonstrated in the current experimentation) initiates lipid peroxidation either by enzymic or

nonenzymic events. Saturated and unsaturated $C_6$−$C_{12}$ fungitoxic aldehydes represent some of the final products of lipid peroxidation in maize kernels generated by enzymic or nonenzymic activities.

In this investigation, we have shown a difference in the levels of fungitoxic aldehydes and their precursors (linoleic acid) in kernels of several maize genotypes that exhibit field resistance or field susceptibility to aflatoxin contamination. We have also demonstrated a correlation between the inhibitory effects of head space volatiles produced from kernels of each of the maize genotypes on radial growth and aflatoxin contamination *in vitro* and the observed level of susceptibility of these maize varieties to aflatoxin contamination in the field. The results suggest an *Aspergillus*/host corn plant interaction that results in the generation of antifungal aldehydes via the lipoxygenase pathway, which may contribute toward the overall disease resistance in those aflatoxin resistant genotypes of maize.

### LITERATURE CITED

Andersen, R. A.; Hamilton-Kemp, T. R.; Hildebrand, D. F.; McCracken, Jr., C. T.; Collins, R. W.; Fleming, P. D. Structure-antifungal activity relationships among volatile $C_6$−$C_9$ aliphatic aldehydes, ketones and alcohols. *J. Agric. Food Chem.* **1994**, *42*, 1563−1568.

Anderson, J. M. Membrane-derived fatty acids as precursors to second messengers. In *Second Messages in Plant Growth and Development*; Boss, W. F., Morré, D. J., Eds.; Liss: New York, 1989; pp 181−212.

Bolling, H.; El Baya, A. W. Investigations on lipids in maize and their importance with respect to maize milling. *Muehle* **1971**, *33*, 480−482.

Brockmann, R.; Acker, L. The behavior of lipoxygenase in low water content systems. I. Effect of water activity on the enzymatic oxidation of lipid. *Lebensm. Wiss. Technol.* **1977a**, *10*, 24−27.

Brockmann, R.; Acker, L. The behavior of lipoxygenase in low water content systems. II. Study of the reaction of enzymic lipid oxidation. *Lebensm. Wiss. Technol.* **1977b**, *10*, 332−336.

Brockmann, R.; Acker, L. Lipoxygenase activity and water activity in systems of low water content. *Ann. Technol. Agric.* **1977c**, *26*, 167−174.

Brown, R. L.; Cotty, P. J.; Cleveland, T. E.; Widstrom, N. W. Living maize embryo influences accumulation of aflatoxin in maize kernels. *Food Prot.* **1993**, *56*, 967−971.

Busby, W. F.; Wogan, G. N. Food-borne mycotoxins and alimentary mycotoxicosis. In *Food Borne Infections and*

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*Intoxications*; 2nd ed.; Reiman, H. P., Bryan, F. L., Eds.; Academic Press: New York, 1979, pp 510−610.

Campbell, K. W. Resistance to *Aspergillus* ear rot and aflatoxin. Ph.D. Dissertation, University of Illinois at Urbana−Champaign, 1994.

Christie, W. W.; Connor, K.; Noble, R. C. Preparation of fatty acid methyl esters from triglycerides and fatty acids. *J. Chromatogr.* **1984**, *298*, 513−515.

Croft, K. P. C.; Jüttner, F.; Slusarenko, A. J. Volatile products of the Lipoxygenase pathway evolved from *Phaseolus vulgaris* (L.) leaves inoculated with *Pseudomonas syringae pv phaseolicola. Plant Physiol.* **1993**, *101*, 13−24.

Farmer, E. F.; Ryan, C. A. Octadecanoid precursors of jasmonic acid activate the synthesis of wound-inducible proteinase inhibitors. *Plant Cell* **1992**, *4*, 129−134.

Gardner, H. W. Lipoxygenase pathway in cereals. In *Advances in Cereal Science and Technology*; Pomeranz, Y., Ed.; American Association of Cereal Chemists: St. Paul, MN, 1988; Vol. 9, pp 161−215.

Gardner, H. W. Recent investigations into the lypoxgenase pathway of plants. *Biochim. Biophys. Acta* **1991**, *1084*, 221−239.

Groopman, J. D.; Croy, R. G.; Wogan, G. N. *In vitro* reactions of aflatoxin $B_1$-addicted DNA. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 5445−5499.

Gueldner, R. C.; Wilson, D. M.; Heidt, A. R. Volatile compounds inhibiting *A. flavus. J. Agric. Food Chem.* **1985**, *33*, 411−413.

Hamilton-Kemp, T. R.; McCraken, Jr., C. T.; Loughrin, J. H.; Anderson, R. A.; Hildebrand, D. F. Effects of some natural volatile compounds on the pathogenic fungi *Alternaria alternata* and *Botrytis cinera. J. Chem. Ecol.* **1992**, *18*, 1083−1091.

Legendre, M. G.; Fisher, G. S.; Schuller, W. H.; Dupuy, H. P.; Rayner, E. T. Novel technique for the analysis of volatiles in aqueous and non-aqueous systems. *J. Am. Oil Chem. Soc.* **1979**, *56*, 552−555.

Lillehoj, E. B. The aflatoxin in maize problem: the historical perspective. In *Aflatoxin in maize: a proceedings of the workshop*; Zuber, M. S., Lillehob, E. E., Renfro, B. L., Eds.; CIMNYT: El Batan, Mexico, 1987; pp 13−32.

Morrison, W. R. Cereal lipids. In *Advances in Cereal Science and Technology*; Pomeranz, Y., Ed.; American Association of Cereal Chemists: St. Paul, MN, 1977; Vol. 2, pp 221−348.

Payne, G. A. Aflatoxin in maize. *Crit. Rev. Plant Sci.* **1992**, *10*, 423−440.

Slusarenko, A. J.; Croft, K. P. C.; Voisey, C. R. Biochemical and molecular events in the hypersensitive response of bean to *Pseudomonas syrungae* pv. phaseolicola. In *Biochemistry and Molecular Biology of Host-Pathogen Interactions*; Smith, C. J., Ed.; Clarendon Press: Oxford, U.K., 1991; pp 126−143.

Tan, S. L.; Morrison, W. R. The distribution of lipids in the germ, endosperm, pericarp and tip of amyomaize, LG-11 hybrid maize and waxy maize. *J. Am. Oil Chem. Soc.* **1979**, *56*, 531−535.

Thompson, J. E.; Legge, R. L.; Barber, R. F. The role of free radicals in senescence and wounding. *New Phytol.* **1987**, *105*, 317−344.

Vick, B. A.; Zimmerman, D. C. Oxidative systems for modification of fatty acids: the lipoxygenase pathway. In *The Biochemistry of Plants, A Comprehensive Treatise: Lipids: Structure and Function*; Stumpf, P. K., Conn, E. E., Eds.; Academic Press: London, 1987; Vol. 9, pp 53−90.

Widstrom, N. W.; McMillan, W. W.; Wilson, D. M. Segregation for resistance to aflatoxin contamination among seeds on an ear of hybrid maize. *Crop Sci.* **1987**, *27*, 961−963.

Wiley. Wiley 130K mass spectral database. John Wiley & Sons, Inc., New York, 1986 (licensed to Hewlett-Packard Co.).

Zeringue, Jr., H. J.; McCormick, S. P. Relationships between cotton leaf-derived volatiles and growth of *Aspergillus flavus. J. Am. Oil Chem. Soc.* **1989**, *66*, 581−585.

Zeringue, Jr., H. J.; McCormick, S. P. Aflatoxin production in cultures of *Aspergillus flavus* incubated in atmospheres containing selected cotton leaf-derived volatiles. *Toxicon* **1990**, *28*, 445−448.

Zuber, M. S.; Darrah, L. L.; Lillehoj, E. B.; Josephson, L. M.; Manwiller, A.; Scott, G. E.; Gudauskas, R. T.; Horner, E. S.; Widstrom, N. W.; Thompson, D. L.; Bockholt, A. J.; Bruebaker, J. L. Comparison of open-pollinated maize varieties and hybrids for preharvest aflatoxin contamination in the southern United States. *Plant Dis.* **1983**, *67*, 185−187.