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Effect of Soybean Lipoxygenase on Volatile Generation and Inhibition of *Aspergillus flavus* Mycelial Growth

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Volatiles generated from lipoxygenase (LOX) normal and LOX deficient soybean (*Glycine max*) varieties with and without added lipase inhibited *Aspergillus flavus* mycelial growth and aflatoxin production. Soybean volatiles were analyzed using a solid phase microextraction (SPME) method combined with gas chromatography−mass spectrometry (GC-MS). Twenty-one compounds, including 11 aldehydes, three alcohols, four ketones, one furan, one alkane, and one alkene were detected in the LOX normal soybean line. However, only nine volatile compounds were observed in the LOX deficient soybean variety. The antifungal aldehydes hexanal and (*E*)-2-hexenal were observed in both LOX normal and LOX deficient lines and were detected at significantly higher amounts in soybean homogenate with added lipase. These aldehydes may be formed through alternate pathways, other than the LOX pathway, and may account for the inhibition of *A. flavus* growth observed. Other volatiles detected, particularly the ketones and alcohols, may contribute to the antifungal activity observed in both LOX normal and LOX deficient soybean lines. These results suggest that other factors, other than LOX activity, may better explain why soybeans are generally not as severely affected by *A. flavus* and aflatoxin contamination as other oilseed crops.

KEYWORDS: Aflatoxin; soybean; SPME; volatiles; *Aspergillus flavus*

INTRODUCTION

The fungus *Aspergillus flavus* produces the contaminant aflatoxin in some economically important oil-rich crops such as corn, cotton, peanut, and treenuts (*1*). Soybeans can be infected by *A. flavus* (*2*); however, when compared to other oilseed crops, the contamination is not as severe. Soybeans are a rich source of seed oil and can support the growth of *A. flavus* on damaged seeds. However, when *A. flavus* contamination occurs, generally *A. flavus*-infected soybeans produce little or no aflatoxins (*3*−*5*). A specific mechanism behind *A. flavus* growth inhibition in soybean has not yet been identified; however, recent experimental data have shown that seed coat integrity is critical (*6*). Other reports have suggested that volatiles emitted from plants may be responsible for fungal inhibition (*7*−*10*). Research has shown that atmospheric volatiles emitted from corn (*7*, *8*) and cotton (*9*, *10*) can considerably inhibit the development of mycelia and processes involved in sporulation and toxin production by *A. flavus*.

Research by Doehlert et al. (*11*) provided evidence that growth and aflatoxin production can be disrupted in vitro when *A. flavus* is exposed to volatiles emitted by soybean. Volatiles released from the lipoxygenase (LOX) pathway in soybean cotyledons homogenized with lipase were able to inhibit in vitro growth of *A. flavus* (*11*). A key volatile detected from headspace in experimental chambers was identified as hexanal, an aldehyde known for antifungal activity (*7*, *9*).

Volatile carbonyl compounds (mainly C_6 alkyl- or alkenyl-aldehydes) from soybean are enzymatically derived from hydroperoxides of unsaturated fatty acids (*12*). LOX (EC 1.13.11.12) catalyzes the hydroperoxidation of linoleic acid and other polyunsaturated lipids that contain a *cis*,*cis*-1,4-pentadiene moiety. Soybeans are rich in LOX consisting of three isozymes (LOX-1, LOX-2, and LOX-3), which are active under different conditions and different states of the substrate to yield hydroperoxides, and subsequent cleavage by hydroperoxide lyase gives C_6 aldehydes (*13*−*15*). Soybeans lacking various LOX isozymes were discovered and were shown to produce limited amounts of C_6 aldehydes and alcohols (*16*).

In the present study, we investigated whether *A. flavus* growth and aflatoxin production could be disrupted by volatiles from two soybean varieties and to identify the volatiles produced using solid phase microextraction (SPME) gas chromatography−mass spectrometry (GC-MS). The two soybean varieties chosen for this research were Asgrow 5902 (+LOX-1,2,3) and Iowa 2025 (−LOX-1,2,3). The LOX deficient soybean variety was selected in order to further test the hypothesis that LOX is a factor in volatile generation inhibiting *A. flavus* growth. Soybean homogenate was analyzed with and without added

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lipase, which was found to be a triggering factor for the generation of volatile aldehydes by the LOX pathway.

MATERIALS AND METHODS

Biological Materials. Asgrow 5902 was provided by Helena Chemical Co. (Thibodaux, LA). The soybean cultivar without LOX activity, IA 2025, was provided by Dr. Walter Fehr (Iowa State University). *A. flavus* (SRRC 13) for inoculation was grown in the dark on potato dextrose agar (PDA). Petri dishes contained 20 mL of PDA overlaid with a membrane (prepared from dialysis tubing) to create a barrier between *A. flavus* and the media.

Inoculum Preparation. *A. flavus* was cultured on PDA in a 100 mm × 15 mm Petri dish for 7 days at 30 °C in dark conditions. Conidia were collected from the culture plate flooded with 10 mL of sterile water with 3 drops of Tween 20 and then decanted into a 50 mL conical tube. The conidia suspension was diluted to 1×10^6 spores/mL. Experimental chambers were inoculated with 250 μL of conidial suspension, which was distributed evenly across the membrane surface with a glass rod.

Soybean Homogenate Preparation. Dry soybean seeds (1 g) were surface sterilized with 70% ethanol for 3 min followed by two water rinses at 2 min each and then soaked for 5 h in 12 mL of water in a 50 mL conical tube. *N*-2-Hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES) was prepared at pH 7.5 using KOH. Imbibed seeds were homogenized with a Tekmar tissuizer (Tekmar Co., Cincinnati, OH) for 1−2 min with either 4 mL of lipase (500 units/mL in HEPES buffer) or 4 mL of HEPES buffer only. Lipase (Sigma, St. Louis, MO) was derived from *Rhizopus arrhizus*.

Assay to Determine the Effect of Soybean Volatiles on *A. flavus* Growth. Soybean homogenate was transferred into an inverted dish lid, the bottom containing inoculated PDA was put back so that the dish was upside down, and then the chambers were sealed with a single layer of Parafilm and incubated for 24 h at 30 °C. Samples were collected after 24 h to monitor mycelial/conidial development and aflatoxin produced by *A. flavus* exposed/not exposed to soybean volatiles.

Measurement of Mycelial Growth. Growth was determined by measuring fungal dry mass (mg). The fungal mat was scraped from the membrane surface, transferred to a small glass jar, and dried for 24 h in a forced air-drying oven at 40 °C. The fresh weight before oven drying and dry weights were recorded. The remaining agar and membranes were processed to extract aflatoxin. Each treatment was replicated three times.

Aflatoxin Extraction. Aflatoxin was extracted using a modified method previously described (*10*). Agar (20 mL) was transferred to a beaker (150 mL) and chopped into very small pieces, intact membrane was added, and then the beaker contents were soaked, covered, and left undisturbed for 30 min at room temperature in 50 mL of acetone and water (70:30, v/v). The extract was decanted into a separatory funnel to which was added 50 mL of dichloromethane. After separation, the lower layer was passed through sodium sulfate into a 150 mL beaker. The extract was allowed to dry by passive evaporation overnight and then washed down to the bottom of the beaker with dichloromethane. The extract was transferred to a 1 mL vial and evaporated to dryness with nitrogen purging and a steam bath. Next, the extract was resuspended in 200 μL of chloroform, and the aflatoxins were separated on 20 cm × 20 cm and 250 μm silica gel 250F thin-layer chromatography plates (J. T. Baker, Phillipsburg, NJ), developed in diethyl ether−methanol−water (96:3:1, v/v/v). The aflatoxin extract and standard quantitation were determined with a model CS9000U fluorescent scanning densitometer (Shimadzu Scientific Instruments, Tokyo).

SPME Analysis for the Identification of Soybean Volatiles. Soybean homogenate was placed in 10 mL glass vials. 3-Hexanol, a compound not identified to be present in soybean, was used as an internal standard. The 3-hexanol standard was dissolved in methanol and injected onto the soybean sample (50 ng/kg final concentration). Vials were sealed with a steel crimp cap fitted with a Teflon/silicone septum that was conditioned at 100 °C and mixed by agitation. Each sample was analyzed 90 min after preparation and run in triplicate. Samples for time course experiments were prepared in a similar fashion.

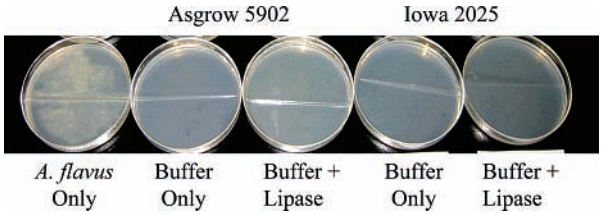

Figure 1. Inverted top lid of Petri plate bioassay containing both Asgrow 5902 (LOX normal) and Iowa 2025 (LOX deficient) soybean homogenate with and without lipase. The photograph was taken 24 h after a spore suspension of *A. flavus* was inoculated into the Petri plate assembly. Measurable mycelial growth was only observed in experimental chambers with no soybean homogenate.

The SPME fiber utilized for this work was divinylbenzene/carboxen/poly(dimethylsiloxane) (DCP, 50/30 μm). Vials containing samples were placed in an aluminum heating block at 60 °C with the SPME fiber inserted into the headspace above the soybean sample. The adsorption was timed for 1 h. SPME fibers were desorbed at 230 °C for 2 min in the injection port of a model 6950N GC-MS (Agilent, Palo Alto, CA) equipped with a MPS2 autosampler (Gerstel, Baltimore, MD). GC-MS runs using a 50 m × 0.2 mm i.d., 0.5 μm, HP-5 cross-linked 5% phenyl methyl silicone column (Hewlett-Packard, Palo Alto, CA) were 40 min, and the fiber remained in the injection port for 20 min after each run. The injection port was operated in splitless mode with a helium inlet flow pressure of 42 psi. The initial oven temperature was 40 °C, held for 3 min, ramped at 10 °C/min to 60 °C; then ramped at 3 °C/min to 150 °C; ramped at 20 °C/min to 250 °C and held for 5 min. The quadrupole mass spectrometer was operated in the electron ionization mode at 70 eV, a source temperature of 200 °C, quadrupole temperature of 100 °C, interface temperature of 200 °C, with a continuous scan from *m*/*z* 40 to 500.

Positive identification of a component was performed by comparison of its retention time and mass spectrum with that of an authentic compound. Specific compounds were integrated on selected ions: 3-octanone and pentane, *m*/*z* 43; pentanal, *m*/*z* 44; (*E*)-2-hexenal, (*E*)-2-octenal, (*E*)-2-nonenal, 1-pentanol, and 1-penten-3-one, *m*/*z* 55; hexanal and 1-hexanol, *m*/*z* 56; octanal, nonanal, 1-octen-3-ol, and 3-heptanone, *m*/*z* 57; 2-heptanone, *m*/*z* 58; 1,3-pentadiene, *m*/*z* 67; heptanal, *m*/*z* 70; (*E*,*E*)-2,4-heptadienal, (*E*,*E*)-2,4-nonadienal, and 2-pentyl-furan, *m*/*z* 81; and (*E*)-2-heptenal, *m*/*z* 83. Samples were run in triplicate, and integrated areas based on selected ions were normalized on 3-hexanol and averaged. For time course experiments, peak areas were calculated from selected ions for each compound.

RESULTS

***A. flavus* Growth and Aflatoxin Production.** Initial experiments examined the effect of volatiles from the two soybean varieties on *A. flavus* growth and aflatoxin formation. The effect of exposure of *A. flavus* to soybean volatiles was readily apparent upon visual examination of each treatment group after 24 h (**Figure 1**). Primary conidia had germinated and formed a thin, cream-colored, even fungal mat across the media surface in chambers containing no soybean treatment. In contrast, in both buffer and buffer with lipase treatments using the soybean variety Asgrow 5902 expressing normal LOX activity, *A. flavus* growth was sporadic, very thin, and was closer to white. A second soybean variety lacking LOX activity, Iowa 2025, was examined for antifungal volatile activity. Using the variety Iowa 2025, a similar inhibition of *A. flavus* growth after 24 h was observed as shown in **Figure 1**. In chambers after 24 h with no soybean homogenate, aflatoxin levels were 60.6 ± 6.4 μg and mycelial growth was 67.2 ± 3.4 mg (dry weight). In both buffer alone and buffer with lipase chambers, aflatoxin levels were below 1 μg and no measurable mycelial growth was detected from either soybean varieties (LOX normal and LOX deficient).

Table 1. Relative Peak Areas for Selected Compounds Recovered in Cv. Asgrow 5902 (LOX Normal) and Cv. IA 2025 (LOX Deficient) Soybean with Buffer and Lipase at 90 min ($n = 3$)[a,b]

		Asgrow 5902		IA 2025	
	RT[c] (min)	buffer[d] ($\times 10^5$)	lipase[d] ($\times 10^5$)	buffer[d] ($\times 10^5$)	lipase[d] ($\times 10^5$)
		aldehydes			
hexanal	11.12	317.8 ± 49.9**	1577.4 ± 96.4**	91.7 ± 23.4**	781.7 ± 86.1**
pentanal	7.28	5.9 ± 2.3**	92.5 ± 4.7**	N/D	N/D
(*E*)-2-hexenal	13.46	33.5 ± 2.9**	116.6 ± 10**	24.2 ± 8.4***	360.1 ± 62.7***
(*E*)-2-heptenal	18.47	5.9 ± 949*	164.7 ± 2.8*	N/D	N/D
(*E*)-2-octenal	23.66	5.0 ± 1.3**	88.8 ± 9.0**	N/D	N/D
heptanal	15.65	0.9 ± 0.6***	7.1 ± 1.1***	N/D	N/D
octanal	20.72	N/D*	5.3 ± 0.8*	N/D	N/D
(*E*,*E*)-2,4-heptadienal	20.57	N/D*	28.9 ± 4.3*	N/D	N/D
nonanal	24.53	3.4 ± 0.4	12.8 ± 8.4	N/D	N/D
(*E*)-2-nonenal	25.75	2.1 ± 0.6	5.1 ± 1.8	N/D**	5.0 ± 0.4**
(*E*,*E*)-2,4-nonadienal	26.73	N/D*	9.6 ± 1.7*	N/D	N/D
		alcohols			
1-pentanol	9.89	19.9 ± 6.4***	51.2 ± 3.6***	N/D	N/D
1-hexanol	14.12	533.1 ± 102.7	528.8 ± 40.2	194.8 ± 18.1***	831.4 ± 116.0***
1-octen-3-ol	19.52	156.5 ± 4.4	200.1 ± 26.1	274.8 ± 32.4	181.4 ± 23.1
		ketones			
1-penten-3-one	6.97	N/D*	207.4 ± 58.7*	N/D	N/D
3-octanone	20.28	19.3 ± 5.2	20.3 ± 10.2	24.1 ± 1.7***	69.0 ± 9.6***
3-heptanone	15.17	4.9 ± 1.9	1.6 ± 2.3	3.9 ± 0.3**	N/D**
2-heptanone	15.27	17.7 ± 8.0	11.0 ± 2.2	14.0 ± 0.8**	3.7±0.6**
		miscellaneous			
1,3-pentadiene	4.21	5.1 ± 1.0***	18.9 ± 2.2***	N/D	N/D
2-pentyl furan	20.18	7.2 ± 1.3***	40.9 ± 7.6***	N/D	N/D
pentane	3.96	38.7 ± 3.5***	107.4 ± 9.6***	N/D**	3.0 ± 0.3**

[a] Recovery of each compound based on specific MS target ions (*m*/*z*) used for quantification. [b] Specific fragment used for calculation of relative peak area of a specific compound. [c] Retention time. [d] Significant differences between treatments within each variety for each compound at $P = <0.001$ (*), <0.01 (**), and <0.05 (***), respectively (Student's *t*-test). N/D, not detected.

Headspace Soybean Volatiles. Twenty-one volatiles were detected using SPME combined with GC-MS analysis in the headspace from the LOX normal soybean line. Several different classes of volatile compounds were observed as follows: 11 aldehydes, three alcohols, four ketones, one furan, one alkane, and one alkene. A total of nine volatile compounds were detected in the LOX deficient line: three aldehydes, two alcohols, three ketones, and one alkane (pentane). Several known antifungal volatiles were detected in both seed varieties. The aldehyde, hexanal, was detected in both buffer-treated and lipase-treated samples in the two seed varieties but increased significantly in samples containing lipase. **Figure 2** displays time course recoveries of hexanal in both buffer- and lipase-treated seeds expressed as peak area (SIM peak area) for both soybean varieties with and without LOX activity. A significantly higher amount (>2×) of hexanal was observed in lipase-treated seeds when compared to buffer alone treatments for LOX normal homogenate shown in **Figure 2A**. At about 18 h, the hexanal amount decreased significantly in samples with buffer alone; however, the hexanal amount remained stable in lipase-treated homogenate. Lower levels of hexanal were observed in LOX deficient soybean homogenate as shown in **Figure 2B** for both treatments. In the lipase-treated homogenate, the hexanal amount dropped significantly between 16 and 21 h.

(*E*)-2-Hexenal was detected in both LOX normal and LOX deficient soybean varieties. A significantly higher amount (>3×) of (*E*)-2-hexenal was observed in lipase-treated LOX normal homogenate at 90 min (**Table 1**). A similar significantly higher amount (>14×) of (*E*)-2-hexenal was detected using the LOX deficient line when comparing lipase treatments to buffer alone. **Figure 3** displays the time course recovery of (*E*)-2-hexenal in both buffer- and lipase-treated homogenate expressed as peak area (SIM peak area) for both LOX normal and LOX deficient

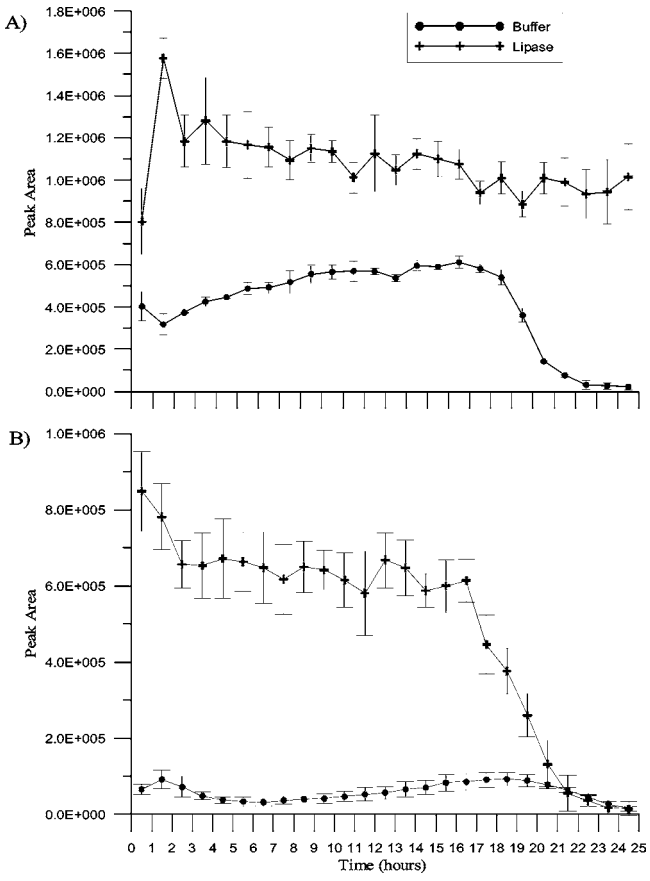

Figure 2. Time course for the generation of hexanal in (**A**) Asgrow 5902 (LOX normal) and (**B**) Iowa 2025 (LOX deficient) soybean homogenate with and without lipase.

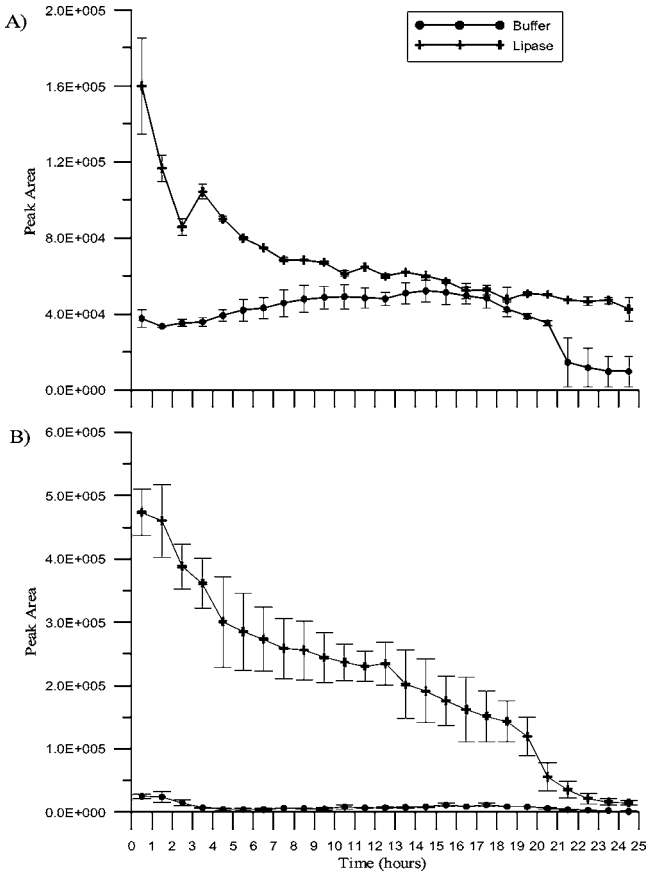

Figure 3. Time course for the generation of (*E*)-2-hexenal in (**A**) Asgrow 5902 (LOX normal) and (**B**) Iowa 2025 (LOX deficient) soybean homogenate with and without lipase.

soybean varieties. Initially, significantly higher amounts of (*E*)-2-hexenal were observed in lipase treatments vs buffer alone treatments for LOX normal soybean homogenate shown in **Figure 3A**. However, the amounts of (*E*)-2-hexenal in the lipase-treated homogenate decreased steadily, and the amounts observed between treatments approached similar levels at 15−18 h. Higher levels of (*E*)-2-hexenal were observed in the LOX deficient soybean homogenate as shown in **Figure 3B** for the lipase treatments, and the amount of (*E*)-2-hexenal decreased steadily with a steep decrease occurring at 20 h. The LOX deficient homogenate from buffer alone treatments displayed the least amount of (*E*)-2-hexenal over the 24 h period.

Several aldehydes were detected in the LOX normal variety but were missing in the LOX deficient line, including pentanal, (*E*)-2-heptenal, (*E*)-2-octenal, heptanal, octanal, (*E*,*E*)-2,4-heptadienal, nonanal, and (*E*,*E*)-2,4-nonadienal. Higher amounts of all of the aldehydes were observed in lipase treatments when compared to buffer alone treatments for the LOX normal line. For the LOX deficient variety, the aldehydes hexanal, (*E*)-2-hexenal, and (*E*)-2-nonenal showed increased amounts in lipase treatments vs buffer alone.

Several alcohols were detected in both LOX normal and LOX deficient soybean varieties. 1-Hexanol and 1-octen-3-ol were detected in both varieties using buffer alone and lipase treatments. No significant difference in the amount of 1-hexanol between treatments was observed in the LOX normal soybean variety, but the amount of 1-hexanol was significantly higher (>4×) for the LOX deficient soybean variety. Also, 1-pentanol was found at higher concentrations in lipase-treated seeds of LOX normal Asgrow 5902 but not detected in the LOX deficient variety IA 2025.

Several ketones were also detected in both buffer- and lipase-treated homogenates from both soybean varieties. 3-Octanone and 2-heptanone were found in both buffer- and lipase-treated samples from both varieties. 1-Penten-3-one was found only in lipase treatments of LOX normal homogenate. 3-Heptanone was found in both buffer and lipase treatments in the LOX active variety but was only found in buffer treatments for the LOX deficient variety.

Several other volatile compounds were also detected in both soybean varieties examined. 2-Pentyl furan and 1,3-pentadiene were found only in the LOX normal variety in both buffer and lipase treatments. Also, pentane was observed in both treatments of the LOX normal variety, with significantly higher amounts in lipase treatments, but only observed in low amounts using the LOX deficient variety.

DISCUSSION

Research by Doehlert et al. (*11*) determined that exogenous lipase applied to soybean cotyledons resulted in the generation of the volatile aldehydes hexanal and (*E*)-2-hexenal that inhibited *A. flavus* spore germination over a period of 16 h. Conversely, volatile production was reduced when a LOX inhibitor was applied, which implies that the LOX pathway is involved in the generation of fungistatic volatiles. In this study, the fungistatic activity of volatiles generated from two soybean varieties with and without LOX activity was compared. We found inhibition of *A. flavus* growth from volatiles generated by both LOX normal and LOX deficient soybean varieties. Also, the soybean homogenate without the addition of lipase caused inhibition of *A. flavus* growth and aflatoxin reduction. These results indicate that other pathways or processes other than those related to LOX may lead to volatile generation inhibitory to *A. flavus* growth and aflatoxin formation.

Various lipid breakdown products, particularly the small chain C_6-C_9 aldehydes, are formed from polyunsaturated fatty acids via LOX, hydroperoxide lyase, and hydroperoxide dehydrase. These C_6-C_9 aldehydes have been shown to inhibit the growth of *A. flavus* and inhibit aflatoxin formation (*7*). From earlier work by Zeringue et al. (*10*), it was shown that the compounds showing the highest fungal inhibition were C_6-C_9 alkenals. In this report, to identify volatiles involved in the inhibition of *A. flavus* growth, the SPME technique combined with GC-MS was utilized. Several volatile aldehydes, ketones, and alcohols were detected in both the LOX normal and the LOX deficient varieties.

It is difficult to determine which component in mixtures of volatiles emitted from soybean homogenate are responsible for the inhibition of fungal growth. The aldehydes hexanal and (*E*)-2-hexenal were detected in both soybean varieties with and without LOX activity, and these aldehydes may account for the antifungal activity observed considering their known inhibition of *A. flavus* growth (*7*, *9*). Soybean contains three types of LOX isozymes, and Matoba et al. (*17*) found that LOX-2 was predominately responsible for the formation of hexanal. Considering that hexanal was detected in the soybean line lacking all three LOX isozymes, although at lower amounts when compared to the LOX normal soybean variety, an alternate pathway other than the LOX pathway may lead to the hexanal formation observed. Hexanal production can occur from linoleic acid peroxidation by nonenzymatic means through the action of various active species (singlet oxygen and superoxide anions) and organic free radicals (*18*). Also, (*E*)-2-hexenal has been shown to greatly increase in concentration due to mechanical damage of plant tissues (*19*). Low amounts of both hexanal and

(*E*)-2-hexenal were observed in buffer alone treatments from the LOX deficient variety; however, inhibition of *A. flavus* growth and aflatoxin formation was observed. The amounts of these two antifungal aldehydes in the headspace of soybean homogenates may be sufficient to inhibit the growth of *A. flavus*. For the LOX normal soybean homogenate, several other aldehydes detected may also contribute to the antifungal activity observed. Heptanal, octanal, nonanal, (*E*)-2-octenal, and (*E*)-2-nonenal have reduced the radial growth and aflatoxin production of *A. flavus* when tested individually in bioassays (*7*, *9*).

Other volatiles observed in LOX deficient buffer alone treatments, including the alcohols 1-hexanol and 1-octen-3-ol, may also contribute to the inhibition of *A. flavus* mycelial growth. Previous work by Zeringue et al. (*9*, *10*) examined the antifungal activities of several alcohols and found that, in general, alcohols inhibited *A. flavus* growth and aflatoxin formation but were weakly active when compared to aldehydes. Interestingly, the amounts of 1-octen-3-ol observed in both buffer and lipase treatments from both LOX normal and LOX deficient soybean varieties remained stable over a period of 24 h (data not shown). Biosynthesis studies indicated that 1-octen-3-ol is formed from linoleic acid directly (*20*); the oxidative formation of 1-octen-3-ol in LOX deficient soybean oils and milk has been described (*21*). In soybeans, the addition of water enhanced the formation of 1-octen-3-ol when compared to dry samples (*22*). In this study, both buffer and lipase treatments contained the same volume of water; therefore, the same amount of 1-octen-3-ol formed would be expected. 1-Octen-3-ol was determined to inhibit the mycelial growth of *Penicillium expansum* PP497A (*23*), a common food spoilage organism; however, little is known about the biological activities of 1-octen-3-ol against *A. flavus* growth and aflatoxin formation.

Besides aldehydes and alcohols, several volatile ketones were observed in the tested soybean varieties. The ketones 2-heptanone and 3-heptanone were observed in both buffer and lipase treatments from LOX normal and LOX deficient soybean lines; however, both were inactive and did not inhibit *A. flavus* growth and aflatoxin formation (*10*). Another ketone, 3-octanone, observed in this study in both LOX normal and LOX deficient soybeans from both buffer and lipase treatments reduced aflatoxin levels below that found in control samples (*10*). 3-Octanone may contribute to the antifungal activity observed in this study, particularly the reduced *A. flavus* mycelial growth found in the LOX deficient buffer alone treatments.

Data obtained when examining the antifungal activity of volatiles generated by soybean lines with and without LOX activity suggest that LOX activity does not play a determinant role in the inhibition of *A. flavus* growth and aflatoxin formation. The antifungal aldehydes hexanal and (*E*)-hexenal were observed in both buffer treatments and lipase treatments of LOX normal and LOX deficient soybeans. These aldehydes may be formed through alternate pathways, other than the LOX pathway, or other processes, and may account for the inhibition of *A. flavus* growth observed. Other volatile compounds were also detected in both LOX normal and LOX deficient varieties, including several aldehydes, ketones, alcohols, and several miscellaneous volatile compounds. These other volatiles, particularly the ketones and alcohols, may contribute to the antifungal activity observed in both LOX normal and LOX deficient soybean lines. These results suggest that other factors, other than LOX activity, may better explain why soybeans are generally not as severely affected by *A. flavus* and aflatoxin contamination as other oilseed crops.

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