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Production of Aflatoxins B_1 and G_1 by *Aspergillus flavus* and *Aspergillus parasiticus* Isolated from Market Pecans

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One hundred and forty-eight isolates of *Aspergillus flavus* and *A. parasiticus* were isolated from 5,608 pecans obtained from Chicago and Georgia markets. The percentage of internal contamination by these species was 7.3% in the Chicago market pecans and 1.7% in those from markets in Georgia. Of the 148 isolates, 93% of the *A. parasiticus*, but only 54% of the *A. flavus*, were capable of producing aflatoxin. Overall, 57% of the isolates were potentially aflatoxigenic. *A. parasiticus* isolates generally produced a greater amount of aflatoxins than *A. flavus*.

The production of aflatoxin appears to be limited primarily, if not exclusively, to strains of *Aspergillus flavus* and *Aspergillus parasiticus* (4). Investigators have noted differences in both the types (B_1, B_2, G_1, G_2, etc.) and quantities of aflatoxins produced by *A. flavus* and *A. parasiticus* species. It has been reported (4) that 82% of *A. parasiticus* isolates produced both aflatoxins B_1 and G_1. Among the *A. flavus* strains, the largest group produced only aflatoxin B_1, but a number also formed both B_1 and G_1. Several other reports have suggested that *A. parasiticus* are the most active aflatoxin producers (2, 5).

Numerous instances of isolations of both *A. flavus* and *A. parasiticus* from many agricultural commodities have been reported (4). Aflatoxigenic isolates of *A. flavus* in pecans were first reported in 1970 (7). Escher et al. (3) surveyed mold and aflatoxin contaminations in pecans during the commercial shelling process. These investigators reported that *A. flavus* isolates out-numbered *A. parasiticus* by four to one, but they did not determine the aflatoxin-producing potential of the isolates.

In this study, 148 isolates of *A. flavus* and *A. parasiticus* from 5,608 pecans sampled in Georgia and Chicago markets were identified, and aflatoxin production was quantitatively determined.

MATERIALS AND METHODS

Isolation and identification. Pecan halves or pieces were disinfected before plating by immersion for 2 min in a solution consisting of 20 ml of 5% sodium hypochlorite and 20 ml of 95% ethanol in 60 ml of water. Georgia pecans were plated on rose bengal-streptomycin agar prepared as described by Tsao (8), except that streptomycin sulfate was added at the level of 0.06 g/liter. All of the other pecans were plated on the Botran modification of Martin's rose bengal-streptomycin medium devised by Bell and Crawford (1). All plates were incubated at room temperature and examined periodically for 2 to 3 weeks for the presence of members of the *A. flavus* group. Species of that group were transferred to malt extract agar slants for storage and subsequent identification.

Assay for aflatoxin production. All isolates identified as *A. flavus* or *A. parasiticus* were assayed for ability to produce aflatoxins by inoculating 10^6 conidia of each isolate into 250-ml Erlenmeyer flasks containing 50 ml of yeast extract (2%)-sucrose (20%) (YES) media and incubating at 25 C for 7 days. Aflatoxin was extracted by adding 50 ml of chloroform to each flask and shaking for 15 min in a gyrorotary shaker. After separation in a 250-ml separatory funnel, the lower (chloroform) layer was drained through Whatman no. 1 filter paper into a 500-ml boiling flask, and the upper (media) layer was returned to the original 250-ml Erlenmeyer flask. This extraction procedure was repeated three times. The combined chloroform extracts were evaporated to a small volume on a rotary vacuum evaporator, quantitatively transferred to a volumetric flask, and adjusted to exactly 10 ml for quantitation by thin-layer chromatography. The concentrated extracts or appropriate dilutions were spotted on Adsorbosil-1 (0.25 mm thick; Applied Science Laboratories, State College, Pa.) thin-layer chromatographic plates along with standard aflatoxin solutions of known concentrations (Southern Utilization Research and Development Laboratories, U.S. Department of Agriculture, New Orleans, La.) and developed in chloroform-acetone (88:12). Aflatoxins were estimated by comparing the intensity of the fluorescence of sample spots with standards with a fluorodensitometer (365-nm excitation filter, 425-nm emission filter).

RESULTS AND DISCUSSION

Ninety percent of the 148 isolates of *A. flavus* and *A. parasiticus* collected from market pe-

cans were *A. flavus*, whereas only 10% were *A. parasiticus*. A total of 5,608 pecan halves were plated, 2,758 from Chicago markets and 2,850 from Georgia markets. Of the 148 isolates of *A. flavus* and *A. parasiticus* found, 47 were from samples collected from Georgia markets, whereas 101 isolates were from Chicago market pecans. The percentage of internal invasion by these species was 7.3% in pecans from Chicago markets and 1.7% in those from Georgia markets. Growth and aflatoxin production of all 47 Georgia isolates and 101 Chicago isolates were determined in YES media. No significant differences in growth were observed (Table 1). The average dry weight of the mycelia differed less than 0.1 g between the two species. In addition, the average weight of all the nonproducing isolates (1.53 g) was identical to the producing isolates (1.47 g). Of the 148 *A. flavus* and *A. parasiticus* isolated from pecans, 57% produced one or more aflatoxins when grown 7 days in YES media.

All aflatoxin-producing isolates produced primarily aflatoxins B_1 and G_1. Aflatoxin G_1 production was always associated with the production of aflatoxin B_1 (Table 2). In no case did aflatoxin B_2 or G_2 contribute significantly to the total toxin produced.

Overall, 57% of the isolates produced some aflatoxin on YES media. Over 93% of *A. parasiticus* strains isolated from pecans were capable of aflatoxin production, whereas only 54% of *A. flavus* isolates produced aflatoxin under the same conditions. Aflatoxin G_1 production occurred in a much higher percentage of *A. parasiticus* isolates than *A. flavus*. Eighty-seven percent of *A. parasiticus* isolates produced aflatoxin G_1, whereas only 10% of *A. flavus* did so.

The average amount of aflatoxin B_1 produced by toxigenic *A. parasiticus* isolates was over twice that produced by aflatoxin-producing strains of *A. flavus*. *A. parasiticus* produced larger amounts of aflatoxin G_1, but the difference (1.3×) was not as great as for aflatoxin B_1. The average ratio of aflatoxin B_1 to G_1 production by *A. flavus* (0.3) and *A. parasiticus* (0.5) did not differ significantly due to the wide variation occurring in this ratio. The B_1-G_1 ratio in *A. flavus* species varied from a maximum of 2.8 to a low of 0.1, whereas the ratio in *A. parasiticus* ranged from 1.9 to 0.2.

Although the origins of all of the pecans collected in the Chicago market area were known, the majority of the samples were from only three states, Georgia, Alabama, and Oklahoma. Insufficient numbers of isolates were available from other areas to make any valid comparisons. Pecans originating from Georgia yielded a considerably lower proportion of isolates capable of aflatoxin production than those from the other regions (Table 3). In addition, those Georgia isolates that were aflatoxigenic produced lower average quantities of aflatoxin B_1 (1.7 mg/50 ml) than isolates from the other regions. Oklahoma pecans, whose toxigenic isolates had the highest overall yields of aflatoxin B_1 (3.4 mg/50 ml), included an unusually high percentage of *A. parasiticus* isolates. The 17% rate of isolation of *A. parasiticus* was over three to four times that of other regions. This in itself would tend to cause higher average aflatoxin production, since, generally, *A. parasiticus* isolates are stronger producers than *A. flavus* (Table 1).

Six isolates of *Aspergillus tamarii*, another member of the *A. flavus* group, were isolated from market pecans but none of these produced

TABLE 1. *Growth and aflatoxin production characteristics of A. flavus and A. parasiticus from market pecans*

Isolate	No.	Wt[a] (g)	No. of producers	Aflatoxin	
				B_1 (mg)[b]	G_1 (mg)[b]
A. flavus	133	1.48	72	2.16	7.28
A. parasiticus	15	1.57	14	4.54	9.24
All isolates	148	1.50	86		

[a] Average of all isolates.
[b] Average of all aflatoxin-producing isolates.

TABLE 2. *Production of aflatoxins B_1 and G_1 by A. flavus and A. parasiticus from market pecans*

Isolate	% Producing			
	Aflatoxins	B_1 only	G_1 only	B_1 and G_1
A. flavus	54	43	0	10
A. parasiticus	93	7	0	87
All isolates	57	39	0	18

TABLE 3. *Aflatoxin production by A. flavus and A. parasiticus from market pecans of different origin*

Origin	No.	% Producers	% *A. flavus*	% *A. parasiticus*	B_1 (mg)[a]
Georgia	43	56	95	5	1.7
Alabama	23	74	96	4	2.1
Oklahoma	29	72	83	17	3.4
All isolates	148	58	90	10	2.4

[a] Average of all producing strains.

any aflatoxins when grown on YES media. An *Aspergillus oryzae* strain isolated from pecans also failed to produce any aflatoxins.

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