Weather-Related Incidence of Aflatoxin Contamination in Late-Harvested Pecans

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ABSTRACT

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In surveys of pecan nuts in metropolitan market channels, 4.6% of samples were contaminated by aflatoxins from 1969 through 1980. In a related sampling of orchard pecans, aflatoxin contamination was found in weevil-damaged samples harvested late in the 1974–1975 season. Incidence in subsequent years has been negligible to nil. Weather records indicate that monthly average temperatures for December through February of each season since 1974–1975 have been below 8.7 C, compared with a normal temperature of 11.2 C. The limiting temperature of aflatoxin production on fresh pecans was determined to be 10 ± 1 C. Thus, monthly average temperatures during the late pecan season in the southeastern United States may be a significant factor in the incidence of aflatoxin contamination.

Pecans, the fruit of *Carya illinoensis* (Wang) K. Koch, are semiperishable nuts that mature on trees in late September and October. The nuts then dehisce from the shuck and are either shaken from the tree or drop naturally to the orchard floor in November. Commercial harvesting operations begin at nut dehiscence and continue until January and February of the next year. Thus, nuts gathered and processed late in the season may have been exposed as long as 4 mo to the microclimate of the orchard floor.

Aspergillus flavus and *A. parasiticus* Speare are ubiquitous in soils of pecan orchards in the southeastern United States (J. M. Wells, *unpublished*). Schroeder (6) demonstrated that under laboratory conditions, these fungi can invade pecans with damaged shells in contact with moist soils and produce detectable levels of aflatoxins. Aflatoxins have been found in commercial samples by Beuchat (1) in discolored packinghouse culls, by Wells and Payne (12) in orchard samples containing nuts damaged by the pecan weevil, *Curculio caryae* Horn, and by the Food and Drug Administration (FDA) as contaminants of pecan samples in commercial marketing channels (8).

Despite evidence that aflatoxin contamination occurs in pecans before they enter market channels, there is little information on the etiology. Observations are not available on such specific environmental factors as harvesting practices, weather conditions, or nut quality that may influence contamination. If such correlations can be made, the information may be used to develop practical control measures for southeastern pecans.

This report presents data on the incidence of aflatoxins in pecans collected in orchards in Georgia during the 1974–1980 harvest seasons and collected in marketing channels during 1969–1980. It also relates factors in the orchard environment to the natural occurrence of aflatoxins in pecans.

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MATERIALS AND METHODS

Market survey, 1969–1980. Pecan nuts were examined as part of the general FDA survey of aflatoxin-susceptible commodities. Total numbers of samples (>5-kg of meats in each) collected per period ranged from a low of 26 during 1975–1976 to a high of 214 during 1977–1979. Samples were obtained from cracking plants, roasters and repackers, and wholesalers throughout the United States.

Orchard survey, 1974–1980. Pecan samples for the orchard survey were obtained from Wilcox, Randolph, and Dougherty counties, the center of a concentrated pecan-growing area in southern Georgia, and from Houston County in central Georgia. The 5-kg samples of mixed cultivars were collected late in the season of each crop year, starting in November and ending in January. Nuts were gathered from the orchard floors or from collection bins at shelling plants.

In the 1974–1975 season, aflatoxins were only detected in samples containing 3–5% nuts damaged by the pecan weevil. Thus, sampling in subsequent seasons was predominantly from orchards or collection bins containing weevil-damaged nuts. In the 1975–1976 season, 69 weevil-damaged samples were collected, and 18–24 samples were collected in each successive season. Samples were stored at −2 C for 1 wk to 5 mo before analysis for aflatoxins.

Climatic data. Weather data for the Dougherty County area county seat in Albany, GA, were obtained from the National Climatic Center (NCC), U. S. Department of Commerce, Asheville, NC 28801. Monthly temperature and precipitation averages were calculated for December, January, and February—the months of the late pecan harvest in which aflatoxin-positive samples were collected in 1974–1975 (12). Cumulative hours exceeding 10, 15.5, and 21.1 C during each 3-mo period were calculated, as well as cumulative precipitation data. Ten-year averages were provided by the NCC. The data for Albany were considered representative for orchards in the general area.

Limiting conditions of aflatoxin production on pecans. Limiting time and temperature conditions for aflatoxin production on pecans were determined in the laboratory with a strain of *A. flavus* isolated from weevil-damaged pecans (ATCC 26944). Twenty-gram portions of fresh comminuted pecans in presterilized 125-ml Erlenmeyer flasks were inoculated with 10 ml of a spore suspension at three inoculum densities: 10^3, 10^4, and 10^5 spores per milliliter. Spores were collected from 12-day-old cultures of *A. flavus* grown on potato-dextrose agar. After incubation of culture flasks at 8, 10, 12, or 16 C for as long as 21 days, flasks were autoclaved at 121 C for 15 min to stop fungal growth and then stored at 2 C until analyzed for aflatoxins. Incubation temperatures were accurate to within ±1 C. Medium in flasks visibly contaminated by molds other than *A. flavus* was discarded. Treatments were replicated three times and replicates were pooled for analyses.

Analytical procedures. Orchard samples of pecans were analyzed for aflatoxins by blending 5-kg samples of whole nuts into a water slurry with a 25-L Hobart vertical cutter-mixer. Slurries were then extracted by the method of Velasco and Morris (11). The extract was cleaned up by the ferric hydroxide gel technique (10) and

analyzed by the minicolumn method of Holaday and Lansden (3). The lower limit of detection for total aflatoxins by this technique was determined to be 5 ng/g (nut meat basis) from experiments conducted with spiked samples.

Market samples of pecans were analyzed by Method II (BF Method) of the Association of Official Analytical Chemists (AOAC), fully described in sections 26.032–26.036 in the AOAC Official Methods of Analysis (4).

Portions of comminuted pecan meal inoculated with *A. flavus*, incubated for 3-day intervals as long as 21 days, and then autoclaved were analyzed by the method of Pons and Goldblatt (5), a method used to study production of aflatoxins on peanuts under controlled conditions (2). Aflatoxin concentrations were based on visual estimation of fluorescence on thin-layer plates. The detection limit by this method for total aflatoxins was about 5 ng/g.

RESULTS

Aflatoxins have been detected in market samples of pecans since 1969, when the FDA surveillance program began. Although the level of incidence has been variable, overall incidence for a 12-yr period has been 4.6% (Table 1). Highest contamination levels occurred before the 1974–1975 season. Aflatoxin levels above the FDA action guideline of 20 ppb (ng/g) were found in 1.6% of the lots sampled, most collected during the first half of the surveillance period.

In the survey of orchard pecans in Georgia, four positive samples were found among 96 sampled in the first year of the survey (1974–1975) (Table 2). The samples happened to be among a group of seven weevil-damaged lots collected late in the season. More intensive sampling of weevil-damaged nuts in subsequent years yielded only one positive sample in the 1975–1976 season and none since then.

A possible correlation between weather data and aflatoxin contamination in orchard pecans was encountered in a search for factors that may be connected with the incidence of aflatoxins in the samples of the 1974–1975 season. Monthly mean temperatures were 11.2 C for the 3-mo period from December 1974 to February 1975, slightly above the average of the preceding 10-yr period for the region (Table 3). In the 1975–1976 season, the 3-mo average was 8.7 C (2.2 C below the 10-yr average). Average mean temperatures for each of the subsequent seasons were at least 2.9 C below the 10-yr average. The cumulative total of hours above 10, 15.5, and 21 C also indicates shorter periods of warmer temperatures during the winters subsequent to the 1974–1975 season. Precipitation data show no significant differences in rainfall during the survey period (*unpublished*).

As a test of the hypothesis of a temperature-related factor in the incidence of aflatoxins in Georgia pecans, the limiting temperatures for significant aflatoxin production were determined on artificially inoculated pecan meal. Aflatoxins were produced by *A. flavus* after growth of 5 days at 16 ± 1 C, 5–9 days at 14 ± 1 C, 9–15 days at 12 ± 1 C, and 15–18 days at 10 ± 1 C (Table 4). At 8 ± 1 C, no aflatoxins above the limits of detection were produced throughout the 21-day test period.

Table 1. Aflatoxins in pecan samples analyzed by the Food and Drug Administration

Fiscal year	Number of samples[a]		Aflatoxin concentration (ng/g)	
	Total	Aflatoxin detected[b]	Mean[c]	Maximum
1969–1971	185	12	156	1,900
1971–1972	55	1	355	...
1972–1973	95	13	42	172
1973–1974	125	11	34	125
1974–1975	101	2	24	38
1975–1976	26	0	...	...
1976–1977	128	1	5	...
1977–1979	214	6	27	126
1979–1980	132	3	8	9

[a] Samples ranging from 2 to 5 kg.
[b] Detection limit for total aflatoxins, 5 ng/g (nut meat basis).
[c] Mean of samples in which aflatoxins were detected.

Table 2. Number of weevil-damaged and undamaged pecan samples with detectable aflatoxins collected in Georgia during November through January of the 1974–1975 to 1979–1980 seasons

Season	Weevil-damaged nuts[a]		Undamaged nuts	
	Samples collected[b]	Aflatoxin detected[c]	Samples collected	Aflatoxin detected
1974–1975	7	4	89	0
1975–1976	65	1	4	0
1976–1977	21	0	3	0
1977–1978	21	0	3	0
1978–1979	21	0	3	0
1979–1980	18	0	3	0

[a] Samples containing 3–5% nuts with weevil damage.
[b] 5-kg samples.
[c] Detection limit for total aflatoxins, 5 ng/g (nut meat basis).

Table 3. Mean temperatures and cumulative hours exceeding various limits for Albany, GA, during the late pecan harvest of 1974–1975 to 1979–1980

Season	Mean temperatures (C)				Cumulative hr exceeding:[a]		
	December	January	February	Average	10.0 C	15.5 C	21.1 C
1974–1975	10.05	11.33	12.33	11.22	1,215	570	130
1975–1976	9.05	6.61	10.39	8.67	1,010	456	112
1976–1977	7.50	3.55	8.05	6.39	636	170	21
1977–1978	9.05	5.11	5.50	6.55	572	231	75
1978–1979	10.55	5.77	7.72	8.00	843	377	97
1979–1980	8.95	4.78	7.01	6.91	523	205	52
1963–1973 Mean	10.50	10.50	11.72	10.89	...[b]	...	...

[a] For January–February of each season.
[b] Not calculated.

DISCUSSION

Aflatoxin contamination is a potential problem in pecans marketed in this country. A contributory factor is considered to be breaks in the integrity of the shell by either mechanical or insect damage. This report extends the correlation in late-season pecans to a weather-related factor. Variations in occurrence of aflatoxins in market pecans during a 12-yr period and the remarkable absence of significant contamination in insect-damaged pecans in recent years could be strongly influenced by prevailing orchard temperatures during the late-harvest season. The uninterrupted succession of cold winters in the southeastern United States since 1974–1975 could have limited aflatoxin production.

The lowest temperature for detectable aflatoxin production by *A. flavus* on fresh pecans held in a relatively high humidity was 10 ± 1 C. This limitation is to be compared with the 12 ± 2 C temperature limit for aflatoxin production on damaged peanut kernels after 21 days (2), with 13 ± 1.5 C after 21 days by *A. parasiticus* on polished rice substrate (9) and with the report of trace levels of aflatoxin produced by a strain of *A.*

Table 4. Relative concentrations of total aflatoxins in fresh pecan meal inoculated with *Aspergillus flavus* and incubated for 21 days at temperatures of 8–16 C

Incubation temperature[a] (C)	Inoculum density[b] (spores/ml)	Incubation time (days)							
		3	5	7	9	12	15	18	21
8	10^3	–	–	–	–	–	–	–	–
	10^4	–	–	–	–	–	–	–	–
	10^5	–	–	–	–	–	–	–	–
10	10^3	–	–	–	–	–	–	+[c]	+
	10^4	–	–	–	–	–	–	–	–
	10^5	–	–	–	–	–	+	+	+
12	10^3	–	–	–	–	–	–	+	+
	10^4	–	–	–	–	–	–	+	+
	10^5	–	–	–	+	+	+	++	+
14	10^3	–	–	–	+	++	+++	+++	+++
	10^4	–	–	–	+	+++	++++	++++	++++
	10^5	–	+	++	++	++++	++++	++++	++++
16	10^3	–	+	++	+++	++++	++++	++++	++++
	10^4	–	++	++	++++	+++	++++	++++	++++
	10^5	–	++	+++	+++	+++	++++	++++	++++

[a] ±1 C

[b] Log dilutions of spores collected from 12-day-old potato-dextrose agar cultures of *A. flavus* with distilled water + 0.05% Tween 20 detergent and counted by hemacytometer.

[c] Ratings based on visual estimation of fluorescence on thin-layer plates from 5 to 50 ng/g (+), 50–150 ng/g (++), 150–500 ng/g (+++), and more than 500 ng/g (++++).

flavus on peanuts held at 10 ± 0.5 C after 10 days (7). Strain variability in *Aspergillus* sp. and differences in substrate undoubtedly account for most of the discrepancies among reports of cardinal temperatures. Our data demonstrate the existence of a strain of *A. flavus* capable of producing aflatoxins in pecans at 10 C but not at 8 C.

Diurnal cycling of temperatures is characteristic in nature. Our data on the lower limits of aflatoxin production were determined in the laboratory under constant temperature conditions. Reported comparisons of aflatoxin biogenesis by *A. parasiticus* under constant and cycling temperature indicated that at the lowest thermal ranges, cycling has no demonstrable effect (9). Thus, cardinal temperatures determined for aflatoxin at the lower ranges can be considered representative of those in nature.

Winter temperatures in pecan orchards in the lower temperature ranges may be critical for aflatoxin production on pecans. This hypothesis should be tested further by continued orchard surveys, particularly in years when producing areas experience unseasonably warm winters. If contributory factors leading to aflatoxin contamination can be confirmed, the industry will have the information required for intensive self-surveillance of selected crops.

LITERATURE CITED

1. Beuchat, L. R. 1975. Incidence of molds on pecan nuts at different points during harvesting. Appl. Microbiol. 29:852-854.
2. Davis, N. D., and Diener, U. L. 1970. Environmental factors affecting the production of aflatoxin. Pages 43-47 in: Toxic Microorganisms. M. Herzberg, ed. UJNR, U.S. Dep. Interior, Washington, DC. 489 pp.
3. Holaday, C. E., and Lansden, J. 1975. Rapid screening for aflatoxin in a number of products. J. Agric. Food Chem. 23:1134-1136.
4. Horwitz, W., ed. 1980. Official Methods of Analysis of the Association of Official Analytical Chemists. 13th ed. AOAC, Washington, DC. 1,019 pp.
5. Pons, W. A., and Goldblatt, L. A. 1965. The determination of aflatoxins in cottonseed products. J. Oil Chem. Soc. 42:471-475.
6. Schroeder, H. R. 1976. Development of aflatoxin in 'Stuart' pecans as affected by shell integrity. HortScience 11:53-54.
7. Schroeder, H. R., and Hein, H., Jr. 1967. Aflatoxins: Production of the toxins in vitro in relation to temperature. Appl. Microbiol. 15:441-445.
8. Stoloff, L. 1976. Occurrence of mycotoxins in foods and feeds. Pages 23-50 in: Mycotoxins and Other Fungal Related Food Problems. J. V. Rodricks, ed. Adv. Chem. Ser. 149. Chem. Soc., Washington, DC.
9. Stutz, H. K., and Krumperman, P. H. 1976. Effect of temperature cycling on the production of aflatoxin by *Aspergillus parasiticus*. Appl. Environ. Microbiol. 32:327-332.
10. Velasco, J. 1970. Determination of aflatoxin in cottonseed by ferric hydroxide gel cleanup. J. Assoc. Off. Anal. Chem. 53:611-616.
11. Velasco, J., and Morris, S. L. 1976. Use of water slurries in aflatoxin analysis. J. Agric. Food Chem. 24:86-88.
12. Wells, J. M., and Payne, J. A. 1976. Incidence of aflatoxin contamination on a sampling of southeastern pecans. Proc. Fla. State Hortic. Soc. 89:256-257.