toxin. We have proposed that a corn sample containing 1 or more BGY particle/kg should be analyzed for aflatoxin (7). Of the 114 BGY inspections done on 1979 corn samples, 62 revealed less than 1 BGY particle/kg; 3 of the samples in this category had aflatoxin in levels of more than 20 ng/g (21, 22 and 59 ng/g). Of the 52 samples with one or more BGY particle(s)/kg, 29 had less than 20 ng/g aflatoxin and 12 had nondetectable toxin (detection limit is 1-3 ng/g). The BGY test should not be used as the only criterion for rejecting corn lots, but to indicate which lots should be tested further for aflatoxin.

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Tolerance to in vitro Accumulation of Aflatoxins in Pecan Meal as Affected by Factors Associated with Yield (*Carya illinoensis* [Wangenh.] K. Koch)

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ABSTRACT

Unautoclaved pecan (*Carya illinoensis* [Wangenh.] K. Koch) meal from selected trees, 10 each with high or low nut yields, were inoculated with a spore suspension of *Aspergillus parasiticus*. Significantly greater concentrations of aflatoxins ($B_1 + B_2 + G_1 + G_2$) occurred in substrates from high-yielding trees. The data suggest physiological differences associated with yield resulted in tolerance to accumulation of aflatoxins.

INTRODUCTION

Considerable tree-to-tree variation in aflatoxin accumulation occurred within a pecan cultivar (*Carya illinoensis* [Wangenh.] K. Koch) when kernel halves were artificially inoculated with toxin-producing strains of *Aspergillus flavus* or *parasiticus* (H.W. Schroeder, personal communication). Data from the USDA laboratory at Byron, Georgia, indicated significant differences in percentage of oil, refractive index, some minerals and fatty acids in kernels from high- and low-yielding pecan trees. The significance of these relationships are not understood, but they do indicate that concentration of chemical constituents in pecan kernels are affected by the yield of the tree on which they were produced. Yields of pecan trees vary among trees and years because of irregular bearing (1). It is possible that the earlier observed variation in aflatoxin accumulation from trees within the same cultivar were related to yield and, hence, to differences in chemical constituents or their concentrations. The following study was undertaken to determine if aflatoxin accumulation in pecan meal is associated with tree yields and with differences in chemical constituents of the pecan kernel.

MATERIALS AND METHODS

Individual 50-to-60-year-old pecan trees, cv. "Moneymaker," were harvested from a 33-acre block in Nov. 1978. Yields were determined and corrected for moisture content. Nuts were air-dried for 2 weeks, shelled and kernels frozen. Kernels from 10 trees each with the highest (range of 91-120 kg and mean of 104 kg/tree) and lowest (range of 1-2 kg and mean of 1 kg/tree) nut yields were selected for testing. At least 0.5 kg of untreated kernels from each test tree was ground in a Dickens Mill to pass a 3.18-mm screen. Prior to inoculation, meal samples from each tree were analyzed for the presence of aflatoxins. The *A. parasiticus* isolate was obtained from a naturally infested pecan nutmeat. The inoculum was grown for 7 days at 25 C on malt-salt agar. Triplicate 10-g meal samples were placed in 250-mL flasks and inoculated with 10 mL of 0.025% Tween 20 in sterile distilled water containing 10^4 spores/mL. A 10-g check of each sample was inoculated with 10 mL of 0.025% Tween 20 in sterile water. All treatments were incubated for 7 days at 25 C, then autoclaved and frozen. Total aflatoxins ($B_1 + B_2 + G_1 + G_2$) was determined visually by thin layer chromatography (TLC) (2). Total phenolics were determined from duplicate meal samples with gallic acid used as a standard (3,4). Mineral concentrations were determined by atomic absorption. Cold pressed oils were used in the determination of fatty acids by gas liquid chromatography (GLC) (5,6) and refractive index. The percentage of oil was determined by the AOCS method (7).

RESULTS AND DISCUSSION

Aflatoxins were not detected in the samples prior to inoculation and incubation; however, following incubation, trace amounts occurred in all check samples. These trace levels could be due to internal kernel infestation (8,9) or contamination by toxin-producing strains of the *A. flavus* group

TABLE I

Concentration of Nutrients in Pecan Kernels from High- and Low-Yielding Trees, Byron, GA, 1978

Yield (kg)	Magnesium	Calcium (μg/g)	Manganese	Copper	Iron	Zinc
High 104	1165	551	49	10	19	48
Low 1	1028	471	49	9	14	34
LSD 1%	111	62			4	6
5%	–	–	NS[a]	NS		

[a]Not significant.

during processing. The reported aflatoxin concentrations resulted from all sources of inoculum.

There was a significant difference in the mean aflatoxin accumulation of 33.9 compared to 6.6 μg/g for substrates from high-yielding (104 kg) and low-yielding (1 kg) trees, respectively. Considerable variation in aflatoxin accumulation occurred in substrates from high-yielding trees as indicated by a standard deviation of 15.4 μg/g. Aflatoxin variation in substrates from low-yielding trees was not as great with a standard deviation of 2.6 μg/g. Thus, the yields of trees from which the substrates were obtained were associated with significant differences in aflatoxin accumulation.

Several causes for the natural tolerance of aflatoxin accumulation in substrates from low-yielding trees may be suggested. The role of nutrients in aflatoxin formation is not understood, but magnesium (10,11), iron, zinc (10-12) and copper (13) are reported as necessary for aflatoxin production. Concentrations of magnesium, iron and zinc were significantly higher in meals from high-yielding trees (Table I); however, the concentration of these nutrients in substrates from low-yielding trees exceed those reported as necessary for optimal toxin production in chemically defined media (10). There was no significant difference in copper concentration between the 2 substrates (Table I).

Concentrations of 50-100 μg/g calcium in chemically defined media stimulated aflatoxin formation with no additional benefit at 200-300 μg/g (14). The calcium content of the 2 pecan substrates exceeded the 200-300 μg/g, levels that did not promote additional aflatoxin production (Table I). Manganese may either promote (14) or reduce (10) aflatoxin formation at certain concentrations, but there was no significant difference in manganese between the 2 substrate sources (Table I).

The nutrients reported do not appear to be involved in the tolerance to aflatoxin formation in substrates from low-yielding trees. Other minerals are reported to affect toxin formation (10,11,14) but their presence was not determined. Materials such as phytic acid (15) may occur in pecan kernels and limit the availability of nutrients for growth of *A. parasiticus* and aflatoxin production.

The mature pecan kernel contains 3-4% carbohydrate, and 5% glucose was necessary for maximal aflatoxin production on a basal media (12). This suggests that the carbohydrate content of the pecan substrate may have been marginal and that other carbon sources, such as fatty acids (16), may have been used. The percentage of oil was significantly less in substrates from high-yielding trees compared to low-yielding trees, 66 and 73%, respectively (Table II). The percentage of oil present in the substrates does not appear to limit aflatoxin production, as significantly more aflatoxins occurred in the substrates from high-yielding trees that contained the least oil. However, oil quality was also different, as indicated by the significant difference in refractive index (Table II). Myristate, palmitate, oleate, linoleate and linolenate occurred in all samples (Table II) and margarate occurred in some samples from both substrate sources. Laurate was reported to be as effective as sucrose in aflatoxin accumulation, but myristate was only slightly effective and neither palmitate or oleate acted as a carbon source (16). This would suggest that only fatty acids of 14 carbon atoms or less may be effective as a carbon source for accumulation of aflatoxins. Thus, of the fatty acids occurring in cold pressed pecan oil, only myristate may act as a carbon source, but in this case, there was no significant difference in myristate concentration between the 2 substrate sources (Table II). It would seem unlikely that any of the fatty acids occurring in pecan oil accounted for the observed differences in aflatoxin accumulation.

There was a visual difference in growth rate of *A. parasiticus* on the 2 substrates. The fungus was observed growing 3 days after inoculation on substrates from high-yielding trees, but not on low-yielding trees. At termination of the 7-day incubation, there were no final growth differences evident. The initial variation in growth rate may account for the differences in aflatoxin accumulation, but growth rates do not always determine the degree of aflatoxin formation (12,17).

Substrates were not autoclaved and *A. niger* occurred on all samples. Ashworth et al. (18) reported *A. niger* limited growth of *A. flavus* and aflatoxin accumulation on peanuts. The growth rate for *A. niger* did not appear to be affected

TABLE II

Mean Oil Content, Refractive Index and Fatty Acid Content of Cold Pressed Pecan Oil from High- and Low-Yielding Trees, Byron, GA, 1978

Yield (kg)	Total oil (%)	Refractive index	Fatty acids (%)					
			Myristic (14:0)	Palmitic (16:0)	Stearic (18:0)	Oleic (18:1)	Linoleic (18:2)	Linolenic (18:3)
High 104	66	1.46497	0.06	6.9	0.7	53.6	36.6	1.9
Low 1	73	1.46342	0.06	6.3	0.8	69.4	21.9	1.2
LSD 1%	6	1.46115		0.26		3.5	3.2	–
5%	–	–	NS[a]	–	NS	–	–	0.6

[a]Not significant.

by the substrate source and may not be involved in the differences in aflatoxin formation.

It was speculated that phenolic concentrations in the substrates may have been a factor in the observed variation in growth rates of the fungus and aflatoxin accumulation. However, there was no significant difference in total phenolics in the 2 substrates with a mean of 9.1 and 9.5 μg/mg for high- and low-yielding trees. Specific phenolic compounds were not determined, but 7 are known to occur in pecan kernels (19). The different growth rates of *A. parasiticus* may have been due to the concentration of one or more of these phenolic compounds rather than the total concentration of all phenolics present. Juglone, which occurs in pecan leaves and reported as a possible resistant factor to pecan scab (*Fusicladium effusum*) (20), did not occur in the kernels (19).

The data indicate that physiological differences, associated with low-yielding trees, resulted in improved tolerance to accumulation of aflatoxins by one isolate of *A. parasiticus*. Identification of the physiological differences may prove to be a new approach for developing the natural tolerance of pecan nutmeats to members of the *A. flavus* group. The relationship of yield with aflatoxin concentration indicates this variable should be considered in the field evaluation for infection by toxin-producing strains of the *A. flavus* group and the accumulation of aflatoxins.

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Aflatoxin Formation on Whole and Ground Cumin and Anise Seeds

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ABSTRACT

The purpose of this study was to evaluate the potential productivity and growth of *Aspergillus parasiticus* (NRRL 2999) and the resulting toxin production on natural and autoclaved (cooked) cumin and anise spice seed substrates. Both whole and ground seeds were used. Mycelia and sporulation were also noted in this 17-day experiment. Cumin and anise seeds are capable of supporting mycelial growth, sporulation and toxin production when the seeds are moist and maintained at room temperature. Toxin yields were higher on ground sterile seed substrates. Of the commercial samples tested, neither the resulting cultures of natural flora nor dry whole seeds were found to contain aflatoxin or aflatoxin-like producing organisms. The anise substrates were more conducive to mycelial growth, sporulation and aflatoxin production than the cumin. Toxin levels in the various anise substrates ranged from 0.83 to 6.5 μg/g total for the 4 aflatoxins, B_1, B_2, G_1 and G_2. Cumin seed substrates usually showed only B_1 and G_1 at total levels ranging from 0.23 to 0.63 μg/g. Both spice seeds had mycelial growth and sporulation to occur at some time during the experimental period. Both substrates could be considered as low-level-producer substrates for aflatoxins. Anise seeds should be monitored occasionally for aflatoxin contamination when the commodities are purchased and used in large quantities.

INTRODUCTION

Aflatoxins have been found in many agricultural products such as peanuts (1), red peppers (2) and cottonseed (3). Spices such as ginger, mace, cumin seed, dill seed, garlic powder, onion powder and the herbs, marjoram, rosemary, thyme and sage, were examined for aflatoxin with negligible levels reported (4). *Aspergillus flavus* has been isolated from mace, cumin seeds, coriander seeds and other spices (5,6). The potential for aflatoxin contamination of spices is quite evident. Spices are international commodities having widespread usage.

Anise (*Pimpinella anisum*) seeds are used to flavor pastry, cookies, candy and certain cheeses. They have a spicy taste and are used to flavor licorice. The oil is also used in medications, especially for treating children's stomach problems (i.e., flatulence). Oil of anise is volatile and contains anethole, parapropenyl phenyl methyl ether ($C_3H_5C_6H_4OCH_3$). Anethole and its derivatives such as anisole and anisaldehyde are used in perfumes and flavorings. Anise is imported into the U.S. from Europe and the Mediterranean countries (7).