

Toxicity to Chicks of *Aspergillus* and *Penicillium* Species Isolated from Moldy Pecans¹

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Isolates of *Aspergillus chevalieri*, *A. flavus*, *A. ochraceus*, *A. repens*, and *Penicillium funiculosum* and complexes of *P. citrinum*-*P. implicatum* isolated from moldy pecan meats were toxic to chicks.

In the spring of 1967, a mold problem in stored pecans (*Carya illinoensis* Koch) was brought to our attention. The pecans involved were harvested in the fall of 1966 and stored in the shell for 4 months at 16 C under relatively humid conditions (>80%). The pecans appeared to be in very good condition externally. When the shells were cracked open, however, clouds of spores arose and fungal growth was evident on the meats. Analysis by thin-layer chromatography revealed that the pecan meats were contaminated with 75 ppb of aflatoxins (B. Doupnik, Jr., unpublished data). Subsequent to this finding, a report was published on aflatoxins in pecans (8).

Because of recent concern over mycotoxins as public health hazards in foods and feeds, we conducted a study to determine the mycoflora of moldy pecans and the toxigenicity of the most prevalent fungi to chicks.

Most of the pecan samples investigated were submitted to us by Ray Worley (Department of Horticulture, Georgia Coastal Plain Experiment Station, Tifton, Ga.). The remaining samples were obtained from various sources by the authors. Upon receiving a given sample, pecans were soaked (with shell intact) for 3 min in a 0.5% (w/v) solution of sodium hypochlorite. The shells were then cracked open, and the meats were removed aseptically. The meats were cut into halves, soaked for 3 min in 0.5% (w/v) sodium hypochlorite, and plated, two halves per petri dish, on either warm rose bengal-streptomycin-agar (9) or high salt-malt-agar (3). After 5 days of incubation at 28 C, fungal colonies growing from the pecan meat halves were enumerated and identified. Several of the *Aspergillus* and *Penicillium* spp. were identified by Dorothy Fennell (Northern Regional Research Laboratory, Peoria, Ill.).

Two or three isolates of each of the 10 most prevalent fungi isolated from the pecan meats were screened for toxigenicity to chicks. Diets for the toxicity tests were prepared as previously described (4, 5). Each isolate was grown singly in 2,800-ml Fernbach flasks containing 500 g of moist, autoclaved, cracked corn at room temperature (approximately 27 C). The flasks were shaken daily to reduce mycelial matting of the corn. After incubation for 2 weeks, each culture (isolate) was dried at 50 C for 15 hr, ground, and singly mixed with a 36% protein supplement (6:4, w/w) to form a diet. Each diet, representing a single isolate, was then fed ad lib to a group of chicks. Each group consisted of 10 1-day-old Babcock B-300 cockerels. A control group of chicks received sterile corn similarly treated and mixed. Water was provided ad lib to all groups. Initial body weights and body weights of surviving chicks were recorded and averaged for each group at 7 and 14 days of age. Each isolate was classified, according to its toxic effect, at the end of the 14-day screening period as follows: none, reduced growth rate, or lethal (Table 1). The reduced growth rate classification arbitrarily included those isolates which depressed gains 20% or more when compared to the controls. Data on gross lesions and histopathology were not taken as this was primarily a study to determine if toxigenic fungi were present in unshelled pecans.

Of the fungi isolated from the pecans, the following 10 species were the most prevalent: *A. chevalieri* (Mangin) Thom & Church, *A. flavus* Link, *A. niger* van Tieghem, *A. ochraceus* Wilhelm, *A. repens* deBary, *Botryodiplodia theobromae* Pat., *Epicoccum nigrum* Link, a mixture (complex) of *P. citrinum* Thom and *P. implicatum* Biourge, *P. funiculosum* Thom, and a *Pestalotia* sp. Isolates of each of the above species were collected for further studies.

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TABLE 1. Toxic effects in chicks fed corn singly infested with isolates of 10 species of fungi isolated from moldy pecans^a

Fungus species	No. of isolates tested	Toxic effects ^b		
		None	Reduced growth rate	Lethal
<i>Aspergillus chevalieri</i>	3		3	
<i>A. flavus</i>	3	1	2	
<i>A. niger</i>	3	3		
<i>A. ochraceus</i>	3			3
<i>A. repens</i>	3	1	2	
<i>Botryodiplodia theobromae</i>	2	2		
<i>Epicoccum nigrum</i>	2	2		
<i>Penicillium citrinum</i> - <i>P. implicatum</i> complex ^c	3			3
<i>P. funiculosum</i>	3	2	1	
<i>Pestalotia</i> sp.....	2	2		

^a Diets for the toxicity tests were prepared by mixing infested corn (6:4, w/w) with a 36% protein supplement.

^b Values are the number of isolates which gave a particular effect.

^c Mixture of *Penicillium citrinum* and *P. implicatum*.

Of 27 isolates screened for toxicity to chicks (Table 1), 13 had no effect, 8 caused reduced growth rates (3 *A. chevalieri*, 2 *A. flavus*, 2 *A. repens*, and 1 *P. funiculosum*), and 6 were lethal (3 *A. ochraceus* and 3 *P. citrinum*-*P. implicatum* complex).

The diets of those isolates producing toxic effects (either reduced growth rate or lethal) were analyzed for aflatoxins and ochratoxins by using the method of Eppley (6). Only the diets of the two toxic isolates of *A. flavus* contained detectable amounts of aflatoxins, and only the diets of the three toxic isolates of *A. ochraceus* contained detectable amounts of ochratoxins. The toxigenicity of the other toxic isolates then is presumably due to other mycotoxin(s).

Tests were also conducted to determine whether autoclaved, moist pecan meats would support ochratoxin and aflatoxin production by known toxigenic isolates of *A. ochraceus* and *A. flavus*. We found that autoclaved, moist pecans would support ochratoxin and aflatoxin production under our experimental conditions. Autoclaved pecans have previously been reported to support aflatoxin production (8) but not ochratoxin production.

The only previous report on toxigenic fungi isolated from pecans is by Lillard et al. (8). Their

report was related to a mold problem involving pecans in bakery products and dealt specifically with *A. flavus* group fungi (*A. flavus* and *A. parasiticus*). Although they undoubtedly encountered other fungi during their mycofloral assays, no mention was made of them. To our knowledge, this is the first report of the isolation of toxic isolates of *A. chevalieri*, *A. ochraceus*, *A. repens*, and *P. funiculosum* and complexes of *P. citrinum*-*P. implicatum* from pecans. All of the above species, however, have previously been reported to be toxic to experimental animals.

Isolates of *A. flavus*, *A. ochraceus*, and *A. repens* have been reported to be toxic to chicks and other animal species by several workers (1, 2, 4, 10, 11). Isolates of *A. chevalieri* and an isolate of *P. funiculosum* have been reported to be toxic to mice (1, 7, 11). Both of the species involved in the *P. citrinum*-*P. implicatum* complex have been shown to be toxic to rats and mice (7, 12). Both species have also been shown to produce citrinin, a potent mycotoxin (12). We did not analyze our toxic diets for citrinin.

It is apparent from this study that pecans can support the growth of fungi which may be toxigenic. Only limited information is available on the proper moisture and temperature conditions for storing unshelled pecans (13), and less is known concerning the relationship of these factors to fungal invasion of meats of unshelled pecans. In view of the possible health hazards, research to determine the proper moisture and temperature conditions for safe storage of unshelled pecans is of vital concern to the pecan industry.

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