Toxigenic Fungi from Cotton

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ABSTRACT

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Forty-seven cultures of fungi (representing 14 genera and 25 species) isolated from cotton by investigators in Alabama, Georgia, Louisiana, and Mississippi were bioassayed for toxicity to brine shrimp, chick embryos, and rats. Eleven isolates (seven species) were highly toxic to brine shrimp. Twenty-three isolates (16 species) were highly toxic and five (three species) were moderately toxic to chick embryos. Thirty-three isolates (21 species) were bioassayed for toxicity to rats. Extracts of *Fusarium oxysporum* and two species of *Alternaria* caused mortality in some rats, and extracts from seven other fungal isolates caused reduced weight gain by at least 10%. Gross pathological changes observed in treated rats included hemorrhages, kidney abnormalities, and shedding of hair. This research has shown the potential of these fungi to produce toxic substances, but whether they occur naturally in cottonseed remains to be determined.

Cottonseed meal is a potentially important source of protein in human diets. The annual production of cottonseed meal in the United States could yield about 625,000 tons of high-quality, edible flour containing 65% protein by the Liquid-Cyclone-Process (14). However, the presence of mycotoxins in cottonseed meal would preclude its use for human food. Aflatoxin in cottonseed meal was discovered to be the cause of liver cancer in hatchery trout (8). Although aflatoxins in excess of 30 μg/kg occurred in 7% of 1,293 samples of cottonseed meals from the 1965-66 crop (15), inactivation of aflatoxins in large quantities of peanut and cottonseed meals has been accomplished by ammoniation in commercial-scale equipment (5). Only *Aspergillus flavus* and *A. parasiticus* of the *A. flavus* group of species produce aflatoxins. However, cottonseed is invaded before harvest by other fungi, which potentially may produce mycotoxins. Several of these fungi, associated with boll rot and with seed and lint invasion of cotton (1, 2, 7, 12, 13), produce mycotoxins causing such disorders as acute toxicity and various estrogenic, hemorrhagic, and tremorgenic responses in animals (3, 6, 9, 10). This paper reports the toxigenicity of fungi (other than the *Aspergillus flavus* group) isolated from cottonseed and bolls to brine shrimp, chick embryos, and rats as the first step in evaluating their potential for mycotoxin production.

MATERIALS AND METHODS

Fungi that had been isolated from cotton bolls and seed were obtained from H. S. Bagga and R. G. Davis, Delta Branch Experiment Station, Stoneville, MS; R. W. Roncadori, University of Georgia, Athens, GA; R. Y. Mayne, Southern Regional Research Center, U.S. Department of Agriculture, New Orleans, LA; and J. A. Pinckard, Louisiana Agricultural Experiment Station, Baton Rouge, LA.

Fungi were also isolated by the authors from cotton bolls and seed from fields of cotton near Marvyn and Auburn, Alabama, in both 1973 and 1974. Random samples were taken on six different dates as early as October 1 and as late as January 7, representing both loose bolls high on the plant and tight bolls low on the plant. Tufts of lint from seed and acid-delinted seed were plated with and without surface sterilization [3-minute soak with 5.25% NaClO:5% ethanol (1:1, v/v) drain, no rinse] on yeast extract medium (YE) consisting of 50 g dextrose, 7.0 g Difco yeast extract, 5.0 g KH_2PO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, and 20 g agar per liter. Cultures were incubated at 25-27 C and isolations were made after 3, 5, and 10 days. Fungi were transferred to YE test tube slants and to either Czapek-Dox or potato-dextrose agar in petri dishes for identification. Cultures that failed to sporulate in 7 days were placed 20.3 cm (8 inches) beneath a 20-W black light fluorescent lamp (Westinghouse F20T12/BLB) to induce sporulation (15 hours black light, 9 hours white light).

Fungi were grown in 1-liter flasks on nutrient-amended shredded wheat, which had been autoclaved for 15 minutes at 121 C twice in 24 hours (11). Each flask contained 50 g of spoon-size, shredded wheat and 100 ml of nutrient solution consisting of 10% sucrose, 4% dextrose, 2% yeast extract (Difco), and 1% soytone. After 14-21 days of incubation at 25 C, moldy substrates were extracted, filtered, evaporated under an airstream, and prepared for brine shrimp and chick embryo bioassays by methods described previously (4). Fungal extract in peanut oil emulsion was similarly prepared with the contents of two flasks for the rat bioassay. The extract was dried on a hot water (93 C) bath under a gentle

airstream to about 20-25 ml, transferred to a 50-or 125-ml Erlenmeyer flask containing 6 ml of peanut oil, returned to the hot water bath to remove most of the solvents, and then placed in a vacuum oven at 50 C for 24 hours to ensure complete removal of solvent. The fungal extract-peanut oil emulsion was usually less than 7 ml.

Six Charles River (CD), 21-day-old weanling rats (three male, three female) were marked and caged for each treatment and given Purina rat chow and water ad libitum. Extracts were administered per os (po) using an intubation needle with ball tip dipped in peanut oil for smooth entrance into the esophagus. Each rat was fed 0.5 ml of extract on days 1 and 2. Control rats were fed extracts of uninoculated, nutrient-amended shredded wheat. Rats were weighed before treatment and at demise, or at the end of the 14-day experiment. Mean average weight gain or loss relative to the control group was calculated for each group of rats. Observations were made for several hours after intubation, daily, and on autopsy at demise or after the 14th day. Autopsy data included hemorrhaging, stomach ulcers, abnormalities of liver, spleen, heart, kidneys, and other pathological changes in treated rats in comparison to control animals. Date of death and notes on physical appearance, nervous, reactions, etc., were recorded.

RESULTS

Toxicity of 47 fungal isolates, representing 14 genera and 25 species to brine shrimp, chick embryos, and rats was investigated. In the text that follows the three-digit numbers in parentheses that follow fungus species names are Auburn University culture collection numbers. Extracts of 11 isolates (six genera) or 24% of all isolates were highly toxic to brine shrimp; they caused 60-100% mortality in 4 hours. Extracts of eight isolates (seven genera) were moderately toxic and 28 were nontoxic. Forty-seven of the isolates were bioassayed with chick embryos. Extracts of 23 isolates (10 genera) were highly toxic to chick embryos, causing 60-100% mortality in 4 days. This represented approximately 49% of the isolates bioassayed with chick embryos. Thirty-three of the 47 isolates were bioassayed with rats. Extracts of *Fusarium oxysporum* (973) and two species of *Alternaria* caused mortality in two or more of six rats, while extracts of four other fungi each killed one of six rats in their group. Rats fed extracts from eight of the 33 isolates showed reduced weight gain of 10% of more in comparison to the control group. Pathological examination of treated rats revealed that extracts of seven isolates caused hemorrhaging, and that extracts of three fungi caused kidney abnormalities. The extracts of *F. oxysporum* (973) caused the shedding of the hair in one group of rats, the first observance of this symptom in these tests.

Alternaria alternata (584) and *Penicillium steckii* (846) were highly toxic to all three bioassay systems. Highly toxic to two of the three bioassay systems were: *A. alternata* (582), *A. tenuissima, Botryodiplodia theobromae* (586, 561), *Colletotrichum gossypii* (589), *Epicoccum purpurascens* (890), *F. equiseti, F. moniliforme* (587), *F. oxysporum* (973) and *Myrothecium roridum*. Extracts of *Alternaria* sp., *B. theobromae* (580, 586), and *C. gossypii* (581) were toxic only to brine shrimp. Toxic only to chick embryos were: *A. alternata* (938), *Aspergillus versicolor, B. theobromae* (896), *Cladosporium herbarum, F. lateritium* (844, 845), *F. moniliforme* (573, 893), *F. roseum* (588), *Geotrichum candidum, P. notatum,* and *P. steckii* (855). *Aspergillus foetidus, B. theobromae* (837), *C. gossypii* (937), *F. roseum* (564), *Mucor racemosus, Nigrospora sphaerica* (1002), and *Pestalotiopsis* sp. were toxic only to rats as measured by weight gain reduction, pathological abnormalities, or mortality. *E. purpurascens* (886), *F. episphaeria, F. lateritium* (903), and *F. roseum* (579) were nontoxic in the brine shrimp and chick embryo bioassays. However, *C. gossypii* (563), *C. gleosporioides, F. heterosporum, F. oxysporum* (573), *Helminthosporium* sp., and *N. sphaerica* (1036) were not highly toxic in any of the three bioassay systems which were employed.

DISCUSSION

Besides the aflatoxins produced by *Aspergillus flavus* and *A. parasiticus,* no other mycotoxins have been reported in cottonseed, although several mycotoxin-producing fungi have been repeatedly isolated from cottonseeds and bolls. Notable in this study has been the high toxigenicity of several species of *Alternaria, Fusarium,* and *Penicillium*. All three genera contain species, including some evaluated in this investigation, that cause such well-known mycotoxicoses in man and animals as alimentary toxic aleukia and yellowed rice toxicosis associated with liver tumors and acute cardiac beriberi as well as the estrogenic syndrome, emetic, and refusal factors in swine, and fescue foot in cattle (3,9). These findings, and the toxicity of other fungi in these bioassays, justify continuing research to isolate the toxic compounds, to identify their structure, and to determine whether they are known toxic metabolites or new mycotoxins. Ultimately, fungal production of the toxic metabolites on cottonseed and surveys of commercial cottonseed meals for any mycotoxins, known or new, should be carried out to determine the hazard to human health that would result from consumption of cottonseed meal and processed protein obtained from cottonseed meal.

These data showed that some of the fungi (other than the *A. flavus* group) that are associated with cotton bolls and seed produce substances toxic to brine shrimp, chick embryos, and/or rats in the laboratory, but it was not demonstrated in this research that any of these unknown substances occur naturally in cottonseed meal or other cottonseed products.

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