A NOTE ON ZEARALENONE IN GRAIN SORGHUM

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Mirocha *et al.* (1,2) reviewed the research leading to the isolation and identification of zearalenone, an estrogenic mycotoxin produced by *Fusarium* spp, that is a problem primarily in corn in the U.S. Corn Belt. It has also been found in England in moldy hay (3). Although *Fusarium* spp attacks and "blights" maturing heads of grain sorghum in the field under conditions of high humidity and temperature, possible contamination of grain sorghum with zearalenone has not been investigated.

In 1973, we received two samples of "head blighted" grain sorghum (a combine-harvested grain sample and blighted culms). Isolation of fungi from the grain established that all of the kernels were heavily infected by *Fusarium* spp. Representative cultures were identified as *Fusarium roseum* 'Gibbosum' and *F. roseum* 'Semitectum.' Subsamples (50 g) of the grain were extracted with 70% aqueous acetone. The extracts were compared by thin-layer chromatography (tlc) with an authentic sample of zearalenone, providing putative evidence that the grain was contaminated with zearalenone. Contamination of grain from selected blighted heads was about double that of the combined grain.

Eight *Fusarium* isolates from the grain sorghum were each grown on autoclaved grain sorghum for 19 days. Comparison of these extracts by tlc indicated that all isolates produced zearalenone but in varying amounts. The extracts were then bulked and absorbed on silica gel (particle size 0.05–0.20 mm), placed in a Butt tube, and eluted first with hexane followed by diethyl ether. The diethyl ether fraction (containing the zearalenone) was applied to a 25-mm × 10-cm silica gel (particle size < 0.063 mm) column. The column was packed and developed on 3% acetone in chloroform. Fractions containing zearalenone were detected by tlc, bulked, evaporated to dryness, then dissolved in methylene chloride. Hexane was added to the incipient precipitation point and then the solution was set aside to crystallize. Recrystallization gave colorless crystals that were dissolved and compared with an authentic sample of zearalenone by tlc. The R_f and fluorescent characteristics of the two materials were identical. A sample of the crystals was sent to C. J. Mirocha, University of Minnesota, and its identity was verified by mass spectrometry and gas chromatography.

The highest yielding isolate, S-74-1c, was selected and grown on cracked corn or grain sorghum at a constant temperature of 25°C for 19 days, or at 25°C for 6 days, followed by 13 days at 10°C. The yields of zearalenone were obtained by weighing the purified crystals. In contrast to previous studies of zearalenone production by corn isolates (4,5), our isolate S-74-1c produced considerably more zearalenone when incubated at a constant 25°C than when changed to 10°C after growth was established at 25°C (Table I). Also at 25°C, more zearalenone was produced on sorghum than on cracked corn. These results suggest that zearalenone contamination may be a significant factor when

TABLE I
Yield of Zearalenone from 19-Day-Old Cultures of Isolate *Fusarium roseum* S-74-1c

Treatment	Substrate	Zearalenone Yield mg/kg
25°C	Cracked corn	1593
25°C	Sorghum	3030
25° + 10°C[a]	Cracked corn	499
25° + 10°C	Sorghum	300

[a]Six days at 25°C followed by 13 days at 10°C.

Fusarium head blight is severe in maturing grain sorghum during warm, highly humid weather.

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