Preharvest Fungal Invasion of Sorghum Grain[1]

L. M. SEITZ,[2] H. E. MOHR,[2] R. BURROUGHS,[3] and J. A. GLUECK[4]

Cereal Chem. 60(2):127-130

ABSTRACT

Sorghum grain from Kansas and Texas was analyzed for fungal invasion by ergosterol and whole seed plating assays. Five sorghum hybrids grown near Manhattan, KS, in 1977 were harvested at 12 weekly intervals. Fungal invasion began at or very soon after physiological maturity. Ergosterol content increased rapidly two to three weeks after physiological maturity and then relatively slowly the next seven or eight weeks as the grain weathered and discolored. The five hybrids differed little in susceptibility to fungal invasion. Species of *Alternaria* and *Fusarium* were found in all of the hybrids, with the former predominant. When *Alternaria* spp. began invading the grain, ergosterol contents began to increase. Eight sorghum lines, grown in 1976 at College Station, TX, were harvested on two dates 29 days apart. Due to wet weather, fungal invasion was already extensive at the first harvest and was considerably greater in all lines, especially in TAM428 and TX2536, at the second harvest. Although visual ratings of weathering and discoloration in the field correlated significantly and positively with ergosterol contents, the ratings did not adequately indicate extent of fungal invasion. Concentrations of ergosterol and another fungal metabolite, ergosta-4,6,8(14),22-tetraen-3-one, in the College Station samples correlated. Grain from Dallas, TX, was not weathered or discolored, but differences in extent of fungal invasion were detectable by ergosterol and whole seed plating assays.

Effects of location (weather), type of hybrid, and harvest date on extent of fungal invasion or deterioration in sorghum grain before harvest were studied by measuring *Alternaria* metabolites, discoloration, and physical properties of the grain (Glueck 1979, Seitz et al 1975). Though useful, these measurements are not sensitive indicators of incipient fungal invasion. Fungal invasion is already extensive by the time *Alternaria* metabolites and changes in physical properties, such as bulk density and kernel weight, are measurable. Discoloration at the surface of the kernel may not indicate the amount of internal fungal invasion.

We previously showed that the ergosterol assay is a sensitive measure of total fungal growth in grains (Seitz et al 1979). During early evaluation of the ergosterol assay, we noted that ergosterol content and fungal invasion in sorghum after physiological maturity depended on harvest date even though the grain showed little evidence of fungal growth or discoloration (Seitz et al 1977). Here we describe more completely the relationships among grain maturity, harvest date, kernel discoloration, hybrid type, and fungal invasion, the last of which was measured by ergosterol and whole seed plating assays. Extensively invaded samples also were analyzed for ergosta-4,6,8(14),22-tetraen-3-one (ETO), a metabolite common to many fungi (Seitz and Paukstelis 1977).

MATERIALS AND METHODS

Sorghum Samples

Grain from Kansas was grown on Kansas State University's Agronomy Farm near Manhattan, KS. Hybrids RS610, RS671, RS702, and ACCO 1019R were planted on May 18, 1977, and Pioneer 8790 on May 5. At each harvest, about six heads of each hybrid that best represented the plot were selected, placed in plastic bags, and brought directly to our laboratory. For moisture analyses, a 30-g sample from each hybrid consisting of seeds removed from top, middle, and bottom portions of the selected heads was dried for 20 hr at 120° C in a forced-draft oven. Then the heads were dried in a forced-air oven for 24 hr at 70° C. Dried heads were threshed manually with aid of a rub board. A McGill sheller (H. T. McGill, Inc., Houston, TX) was used to remove glumes and for final cleaning.

Grain from Texas was grown near College Station and Dallas and included lines SCO103-*12*, 74PR759, SCO566-*14*, SCO279-*14*, BTX398, SCO748-*5*, TAM428, and TX2536. Lines from College Station were planted April 26, 1976, and harvested September 22 and October 21. Those grown near Dallas were planted on March 20, 1976, and harvested on July 20.

Characteristics of the grain from sorghum hybrids and lines grown in Kansas and Texas are compared in Table I.

Field Ratings of Grain Deterioration

Extent of visible grain deterioration in sorghums from College Station was rated before harvest on a scale of 1 to 5, with 1 for bright, sound grain and 5 for chalky, discolored grain with apparent low viability. Reported ratings were usually averages of ratings by two or more persons.

Analytical

Previously described methods were used to determine ergosterol (Seitz et al 1979) and ergosta-4,6,8(14),22-tetraen-3-one (Seitz and Paukstelis 1977).

Percentages of seeds invaded by fungi were determined by

TABLE I
Characteristics of Grain from Sorghum Hybrids and Lines Grown in Kansas and Texas

Location	Hybrid or Line	Mesocarp Thickness	Pigmented Testa[a]	Endosperm Texture[b]	Appearance
Kansas					
	RS610	thick	A	I	red
	RS671	thick	A	I	red
	RS702	thick	A	I	red
	ACCO 1019R	thick	A	I	light red
	Pioneer 8790	intermediate	A	I	dark red
Texas					
	SCO103-*12*	thin chalky	P	Fl	reddish brown
	74PR759	intermediate	A	I	red
	SCO566-*14*	thin pearly	A	I	bright red translucent
	SCO279-*14*	thin pearly	A	I	bright red translucent
	BTX398	intermediate	A	I	red
	SCO748-*5*	thin	A	I	lemon yellow
	TAM428	thin	A	I	white
	TX2536	thin pearly	A	I	yellow

[a] A = Absent, P = present.
[b] I = Intermediate, Fl = floury.

[1] Contribution No. 82-61-J, Kansas Agricultural Experiment Station, Manhattan, KS 66502.
Mention of trademarks or proprietary products does not constitute a guarantee or warranty of the product by the USDA and does not imply its approval to the exclusion of other suitable products.

[2] U.S. Grain Marketing Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Manhattan, KS 66502.
[3] Department of Grain Science and Industry, Kansas State University, Manhattan 66506.
[4] Department of Soil and Crop Sciences, Texas A&M University, College Station 77843. Present address: Director of Research, George Warner Seed Company, Hereford, TX 79045.

surface-sterilizing the grain in 2% NaOCl (Clorox brand) for 1 min, rinsing in sterile water, and plating 50 kernels on Difco malt agar (Difco Laboratories, Detroit, MI) containing 4% NaCl and 200 μg/g Tergitol NPX (Union Carbide Corp., New York, NY). Plates were incubated at 25° C until fungi could be identified and counted.

RESULTS AND DISCUSSION

Kansas Samples

Figure 1 shows ergosterol contents of grain from five sorghum hybrids harvested at 12 weekly intervals near Manhattan from August 3 to October 19, 1977. A logarithmic scale was used on the ordinate to emphasize the large increase (34- to 55-fold, excluding Pioneer 8790) in fungal invasion indicated by ergosterol content between the second and fifth harvests (August 10 and 31). As discussed below, the grain was maturing during this period, and moisture contents were still high (Fig. 2). After the fifth harvest, ergosterol content increased relatively slowly (Fig. 1).

At the first harvest (August 3), grain from all hybrids except Pioneer 8790 was clearly immature, consisting of green kernels in milk and early dough stages, with moisture contents of 55–73%. Kernel weights were only about half those of mature grain. Some of the grain from Pioneer 8790, which was planted 13 days earlier than the other four hybrids, was already changing to mature color by the first harvest. Ergosterol content of Pioneer 8790 grain at first harvest showed little variation with degree of grain maturity indicated by kernel color.

By the second harvest (August 10), kernels on the upper parts of the heads were changing to mature color, whereas kernels on the lower parts of the heads were still fairly green and doughy. Kernel weights nearly equaled those of mature grain, except that RS702 kernels were only 60% of mature weight. Again, maturity of Pioneer 8790 was more advanced than the other four hybrids; more of its grain was changing color, and some appeared mature. Change to mature color was nearly complete, and maximum dry kernel weights were achieved by the third harvest (August 17). Thus, physiological maturity of RS671, RS610, RS702, and ACCO 1019R was reached between August 10 and 17. Observation of black-layer formation as an indicator of grain maturity was not attempted.

Percentages of kernels invaded by field fungi were low at the first harvest and generally increased until the tenth harvest (October 6), when all kernels from each hybrid were invaded (Table II). *Alternaria alternata,* the predominant fungus, and *Fusarium* spp. (predominantly *F. moniliforme, F. equiseti,* and *F. semitectum*) were the fungi most consistently present in all hybrids; however, neither was identified at the first harvest. By the second harvest, *Alternaria* spp. had invaded some kernels of most hybrids whereas *Fusarium* spp. invasion was not widespread until the fifth harvest. Other fungi present in low percentages of kernels (2–10%) were species of *Chaetomium, Cladosporium, Curvularia, Epicoccum, Helminthosporium* and several Sphaeropsidales. We previously reported that *Alternaria* spp. and *Fusarium* spp. were the predominant field fungi in sorghums grown in eastern Kansas (Seitz et al 1975).

The weather at Manhattan during August, September, and October, 1977, was unusually wet, with rainfall about half of the

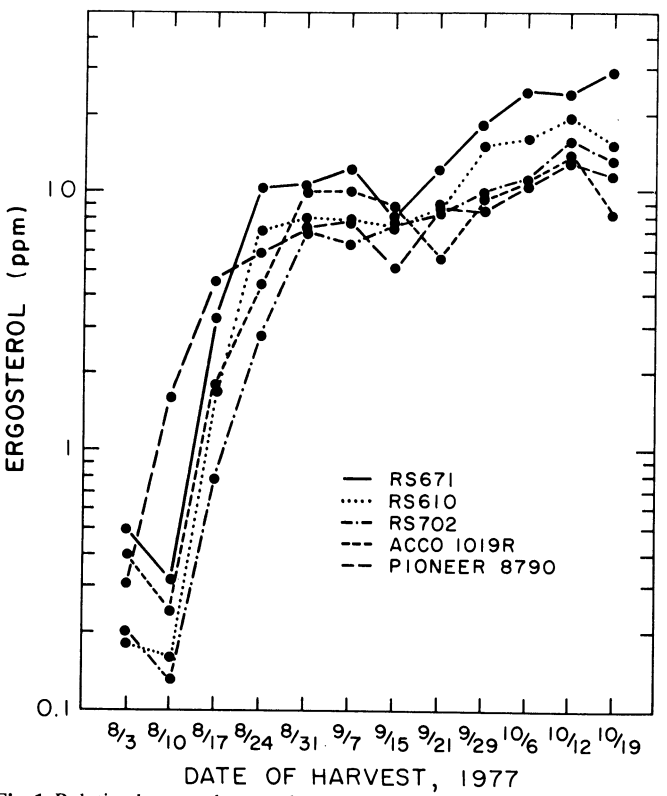

Fig. 1. Relation between harvest dates and ergosterol contents of grain from five sorghum hybrids harvested near Manhattan, KS, in 1977. Each point is an average of duplicates. Coefficient of variation of duplicates averaged 8.2%.

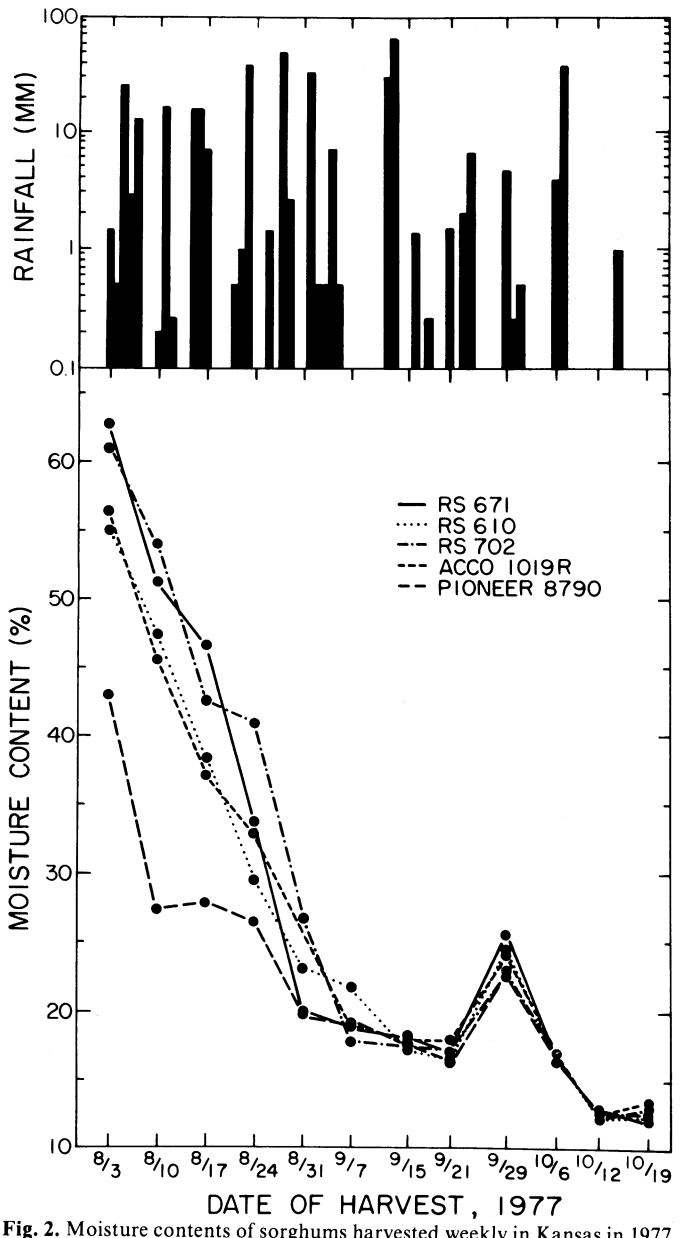

Fig. 2. Moisture contents of sorghums harvested weekly in Kansas in 1977 (lower graph) and rainfall during the harvesting period (U.S. Department of Commerce 1977). Values shown for moisture contents on August 3 represent kernels in the early dough stage. Kernels in the milk stage had moisture contents as high as 73%.

TABLE II
Invasion Percentages by Predominant Fungi of Sorghums Harvested Weekly in Kansas in 1977

Harvest Date[a]	*Alternaria* spp.					*Fusarium* spp.					No Fungi				
	RS610	RS671	RS702	ACCO 1019R	Pioneer 8790	RS610	RS671	RS702	ACCO 1019R	Pioneer 8790	RS610	RS671	RS702	ACCO 1019R	Pioneer 8790
August 3	0	0	0	0	0	0	0	0	0	0	96	92	93	94	92
August 10	10	0	6	8	16	0	0	0	0	2	76	98	92	90	66
August 17	24	36	0	8	16	0	8	0	0	0	72	38	98	88	80
August 24	34	30	2	14	20	2	4	0	0	0	58	48	98	82	72
August 31	28	28	10	16	18	2	2	6	2	2	58	52	78	70	76
September 7	38	52	14	44	96	2	2	10	12	4	54	30	66	34	4
September 29	40	42	6	38	46	14	8	6	4	14	36	36	82	40	...
October 6	44	98	100	96	90	28	34	28	14	26	36	0	0	4	0
October 12	100	100	98	100	94	38	24	30	12	36	0	0	0	0	...
October 19	98	100	92	100	96	28	16	26	20	20	0	0	0	0	0

[a] Data from grain harvested September 15 and 21 were not included because field fungi apparently were killed during temporary storage of freshly harvested grain in a freezer before drying and plating.

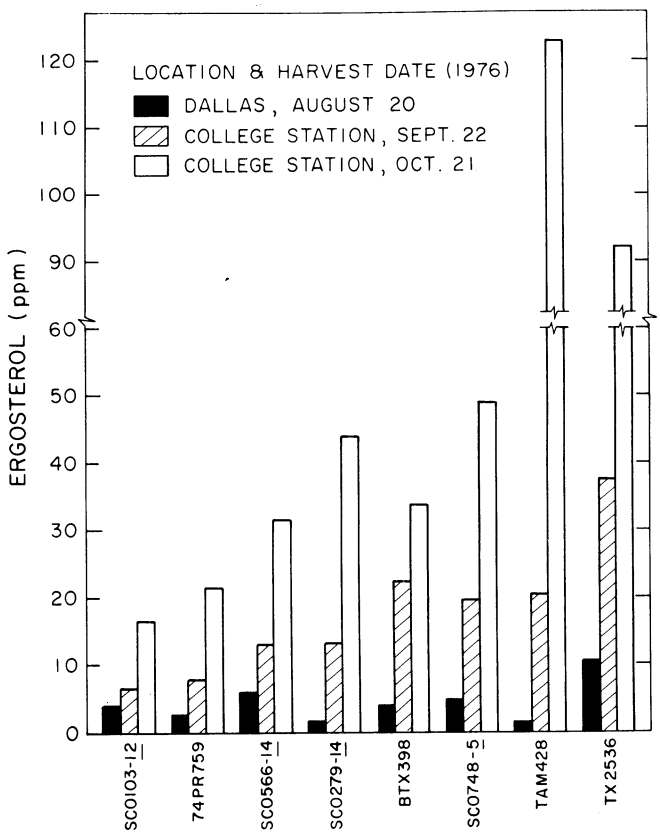

Fig. 3. Ergosterol contents of grain from eight sorghum lines harvested in Texas in 1976. Each bar is an average of duplicates. Coefficient of variation of duplicates averaged 13%.

days and several periods of two to five consecutive rainy days (Fig. 2). Temperatures were near normal, with average minimum to maximum temperatures of 66–87, 60–81, and 46–70° F for August, September, and October, respectively (U.S. Department of Commerce 1977). Generally, the grain dried little between August 31 and October 6. Instead we recorded an increase in grain moisture content at harvest on September 29, midway between September 21 to October 6, when fungal invasion, as indicated by ergosterol content, increased in all hybrids (Fig. 1). September 29 and 30 were particularly foggy, misty days.

Relatively high concentrations of ergosterol in grain harvested in October, 1977, at Manhattan reflect that weather favored preharvest fungal invasion. The samples also appeared weathered and discolored. Sorghums harvested near Manhattan in October, 1976, after the grain matured during dry weather, showed little evidence of extensive fungal growth or discoloration and had ergosterol contents of only 1.8–3.4 ppm (Seitz et al 1977). Sorghum grain that matured during dry weather near Dallas in 1976 had low

TABLE III
Fungal Invasion Percentages, Field Ratings, and Ergosta-4,6,8(14),22-Tetraen-3-One (ETO) Contents of Sorghums Grown in Texas in 1976

Line	Location[a] Harvest Date	Percent Kernels with *Alternaria* spp.	*Fusarium* spp.	Other[b]	No Fungi	Field Rating	ETO (ppb)
SCO103-*12*	D 8/20	72	18	16	8	...	...
	CS 9/22	81	58	32	0	1.6	13
	CS 10/21	89	80	20	0	1.8	39
74PR759	D 8/20	58	0	18	24	...	...
	CS 9/22	84	57	15	0	1.9	11
	CS 10/21	79	83	18	0	2.2	43
SC0566-*14*	D 8/20	82	20	18	0	...	...
	CS 9/22	97	77	14	0	1.9	25
	CS 10/21	87	94	12	0	2.3	104
SC0279-*14*	D 8/20	74	0	10	18	...	...
	CS 9/22	97	43	47	0	1.7	19
	CS 10/21	95	68	37	0	1.9	147
BTX398	D 8/20	82	12	18	4	...	...
	CS 9/22	79	54	46	0	2.1	69
	CS 10/21	85	80	45	0	2.9	176
SC0748-*5*	D 8/20	54	16	28	16	...	...
	CS 9/22	93	58	14	0	2.3	25
	CS 10/21	97	77	13	0	2.5	111
TAM428	D 8/20	26	16	4	56	...	...
	CS 9/22	88	62	20	0	2.9	32
	CS 10/21	88	87	18	0	3.9	410
TX2536	D 8/20	60	12	34	2	...	...
	CS 9/22	97	63	15	0	3.2	43
	CS 10/21	92	89	12	0	3.8	348

[a] D = Dallas, CS = College Station.
[b] Species of *Cladosporium, Curvularia, Epicoccum, Helminthosporium, Cochliobolus, Peyronellaea* and *Phoma.*

ergosterol contents. Weather at Manhattan in the fall of 1977 was generally similar to that in 1973 when weathered, discolored sorghum was common in central and eastern Kansas (Seitz et al 1975). The 1977 sorghums contained *Alternaria* metabolites (alternariols) as in 1973, but amounts were not quantified.

Texas Samples

Ergosterol contents of eight sorghum lines harvested in 1976 near College Station and Dallas varied greatly depending on type of hybrid, location, and harvest date (Fig. 3). For each hybrid, the extent of fungal invasion, indicated by ergosterol analysis and plating (Table III), was much lower in grain from Dallas than in grain from College Station. The weather at Dallas while the

sorghums were maturing was hot and dry with only one or two light showers and low relative humidity (38–75%) during a 30-day period before harvest (U.S. Department of Commerce 1976). The grain did not weather, even though it stood in the field for about 20 days past physiological maturity, as indicated by appearance of the grain.

In contrast, the weather at College Station favored invasion of the grain by field fungi. Rain fell five of 20 days preceding the first harvest (September 22) and 9 of 29 days between the first and second harvest (October 21). Humidity (56–89% in September and 65–89% in October) also was high (U.S. Department of Commerce 1976). Grain from all of the hybrids was already invaded quite extensively by the first harvest (Fig. 3 and Table III), indicating that kernels were past physiological maturity. Ergosterol contents of all hybrids at College Station, especially TAM428 and TX2536, increased considerably between the first and second harvests (Fig. 3). Invasion by *Fusarium* spp. increased significantly between the two harvests (Table III) and may have contributed to, but probably was not the sole cause of, the rise in ergosterol content.

During analyses of College Station samples for ergosterol, we noticed significant amounts of ETO, which is produced by species of *Alternaria, Fusarium,* and other fungi (Seitz and Paukstelis 1977). ETO contents in those samples (Table III) correlated well with ergosterol contents ($r = 0.96^{**}$). We did not quantify ETO in samples from Dallas or Manhattan. Concentrations of ETO in the Dallas samples probably would have been low and difficult to measure.

Grain from TAM428 and TX2536 harvested at College Station October 21 was moldy, with ergosterol and ETO contents (Fig. 3) the highest we have found in sorghum at harvest. Some kernels were so friable that they could be crushed between the fingers. Alternariols produced by *Alternaria* spp. were present, but zearalenone produced by *Fusarium* spp. was not. Schroeder and Hein (1975) found zearalenone in two samples of *Fusarium* "blighted" grain sorghum at harvest in 1973. Even though field ratings and ergosterol contents correlated significantly ($r = 0.84^{**}$), ratings for TAM428 and TX2536 from the second harvest at College Station (Table III) should have been much closer to 5, the top of the scale.

Among sorghum lines from Texas, TX2536 was the most susceptible to preharvest fungal invasion, as indicated by high ergosterol contents in all three samples (Fig. 3). At College Station, ergosterol content of TAM428 was similar to that in several other lines at the first harvest and then increased to a level higher than in any other line by the second harvest. However, among the Dallas samples, TAM428 was the least invaded as indicated by both ergosterol (Fig. 3) and plating (Table III). Line SCO103-*12*, the only one we tested that contained a pigmented testa layer, was the least invaded at College Station (Fig. 3), but median compared with other lines from Dallas (Fig. 3 and Table III). Glueck (1979), using germination, field ratings of visible grain deterioration, and physical measurements (bulk density, kernel weight, and seed size and hardness), identified TX2536 as susceptible, TAM428 as moderately resistant, and all others resistant to preharvest grain deterioration.

CONCLUSIONS

Large increases in ergosterol contents of sorghum grain harvested at weekly intervals indicated that fungi began invading the grain at or very soon after physiological maturity. Much of the total fungal invasion measured by ergosterol assay occurred two to three weeks after physiological maturity. When comparing different sorghum hybrids for resistance or susceptibility to preharvest fungal invasion, it is important to make sure the sorghums are maturing at the same time, or at least to know the relation between harvest date and physiological maturity.

Ergosterol contents in sorghum grain from College Station, TX, showed that fungal invasion in the grain can increase substantially during wet weather. Visual ratings of grain deterioration correlated significantly and positively with ergosterol contents, but the ratings did not adequately indicate extent of fungal invasion. It was most apparent in the ergosterol data that TAM428 and TX2536 from the second harvest at College Station were invaded significantly more than the other sorghum lines we analyzed. Ergosterol and whole seed plating assays identified differences in fungal invasions among lines from Dallas that appeared bright and sound.

ACKNOWLEDGMENTS

We wish to acknowledge the assistance of Lloyd Rooney of the Department of Soil and Crop Sciences, Texas A&M Univ., College Station.

LITERATURE CITED

GLUECK, J. A. 1979. Identification and characterization of *Sorghum bicolor* (L.) Moench lines with resistance to preharvest grain deterioration. Ph.D. dissertation, Texas A&M University, College Station, TX.

SCHROEDER, H. W., and HEIN, H. 1975. A note on zearalenone in grain sorghum. Cereal Chem. 52:751.

SEITZ, L. M., MOHR, H. E., BURROUGHS, R., and SAUER, D. B. 1977. Ergosterol as an indicator of fungal invasion in grains. Cereal Chem. 54:1207.

SEITZ, L. M., and PAUKSTELIS, J. V. 1977. Metabolites of *Alternaria alternata:* Ergosterol and ergosta-4,6,8(14),22-tetraen-3-one. J. Agric. Food Chem. 25:838.

SEITZ, L. M., SAUER, D. B., BURROUGHS, R., MOHR, H. E., and HUBBARD, J. D. 1979. Ergosterol as a measure of fungal growth. Phytopathology 69:1202.

SEITZ, L. M., SAUER, D. B., MOHR, H. E., and BURROUGHS, R. 1975. Weathered grain sorghum: Natural occurrence of alternariols and storability of the grain. Phytopathology 65:1259.

U.S. DEPARTMENT OF COMMERCE. 1976. Climatological Data, Texas, Vol. 81, National Climatic Center, Asheville, NC.

U.S. DEPARTMENT OF COMMERCE. 1977. Climatological Data, Kansas, Vol. 91, National Climatic Center, Asheville, NC.

[Received January 11, 1982. Accepted September 13, 1982.]