Pre- and Post-harvest Aflatoxin in South Texas Grain Sorghum

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Season-long drought in the Coastal Bend of South Texas resulted in three distinct sorghum cropping seasons, or harvests in 1996. These seasons were; 1. normal season with typical July maturity, except for a few fields harvested in early August, 2. late season of fields sown after failed cotton, or for similar reasons that matured in late-August through mid-September, and 3. fall season of fields either sown, or ratooned in July that matured in late-October to early-November. Many of the normal season, drought-stressed fields throughout the Coastal Bend had problems of mixed maturity, that was also reflected in the harvested grain. Grain of mixed maturity needed more drying at the elevators and it was difficult to accurately assess its moisture content.

Aflatoxin levels > 20 ppb were first detected on 22 July 1996 in current-year grain sorghum from the South Texas area elevators that was intended for shipment through the port of Corpus Christi. Although the rejected grain was moved as rapidly as possible from the elevators and producer-storage facilities to the port, storage for at least several days made it impossible to determine if the aflatoxin contamination was of pre-, or postharvest origin. During July and August 1996, 732 sorghum samples (truckloads) from thirteen counties were rejected at the port because the Corpus Christi Grain Exchange (CCGE) determined them to be aflatoxin-positive (levels > 20 ppb). The bulk of the problem came from four contiguous Coastal Bend counties where aflatoxin-positive samples were 88 from Nueces, 89 from Bee, 99 from Kleberg, and 371 from San Patricio'. Aflatoxin levels were generally low, as the average across all counties was only 49 ppb, but the range for individual loads was from 20 to 630 ppb. Across all counties, only 69 samples had kernel moisture contents above 15% (15-16.5%) and their aflatoxin levels ranged from 23 to 120 ppb. Samples of aflatoxin-positive grain provided by the CCGE all contained immature grain, but it did not consistently have higher aflatoxin contents than other grain fractions. Field samples of grain sorghum representing normal-season maturities were collected on 13-14 Sep 1996 from the Texas A&M Experiment Station nurseries at Corpus Christi and similar samples representing late-season maturities were collected from commercial fields in San Patricio county on 14 Sep and 3 Oct 1996.

Sorghums that had matured in mid-July at Corpus Christi had no measurable aflatoxin despite heavy damage by weathering fungi and some seed germination damage in the panicle. Sorghum samples from producer fields in San Patricio county, that matured in early Sep, generally had preharvest aflatoxin levels ranging from 0 to 110 ppb. One producer's field had 100 ppb in the grain of primary stalks and tillers, and 270 ppb in the more immature, fungal-colonized (primarily by *Fusarium* spp) grain of the secondary tillers. Field samples of the fall-season crop taken in San Patricio county and eastward to Matagorda county on 3, 22, and 26 Oct, and 5 Nov 1996 were free from aflatoxin, despite some germination damage in a few samples. The preharvest origin of aflatoxin. in the normal-season harvest could not be determined, but mixed-maturity grain may have contributed to the high moisture storage problems, including aflatoxin accumulation. Despite the occurrence of preharvest aflatoxin in some late-maturing fields in the Coastal Bend in 1996, there is a low probability of recurrence in most drought-stressed environments, because sorghum has been historically free of preharvest aflatoxin. The 1997 normal-season crop sown during March was not drought-stressed and the crop is not expected to have problems with either pre-, or post-harvest aflatoxin contamination. However, those high moisture conditions also resulted in an extremely large number of late-sown sorghum fields (mid-May and later sowing dates) in some South Texas sorghum production areas. Some fields were blooming, or younger, even as the harvest was starting on the earliest-sown fields at the end of June. These fields will be monitored for pre-harvest aflatoxin as they mature in late August and early September.

Variation in Phenol Content of Sorghum Lines after Inoculation with *Colletotrichum graminicola*

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Phenolic compounds accumulate in numerous plant species following infection by plant pathogens (Kuk 1972). Many of such compounds, or their oxidation products are

toxic to pathogenic and non-pathogenic fungi, and have been considered an important factor in induced resistance to plant pathogens. The variation in phenol content following infection with *Colletotrichum graminicola,* the causal fungus of anthracnose of sorghum was studied using six sorghum lines, resistant (A 2267-2, IRAT204); moderately resistant (IS 3758, IS 8354); and susceptible (IS 3089, IS 18442) to anthracnose. The lines were selected based on their disease reactions in the International Sorghum Anthracnose Virulence Nursery conducted at 10-12 locations in India and Africa during 1992 and 1993. Plants of each genotype were grown in 13-cm square plastic pots in a Vertisol mix (Vertisol, farmyard manure, and sand, 2:1:1 by volume, steam sterilized at 105°C) in a greenhouse at ICRISAT-Patancheru.

Plants at the 4-leaf stage were spray inoculated with conidial suspension (105 conidia mL^{-1}) of C. *graminicola* (ICRISAT isolate Cg 042). The plants were then transferred to a plastic tray lined with pre-wetted blotter sheets to provide high humidity (>95% RH), and covered with another similarly lined plastic tray. The plants were incubated at 25°C with 12-h photoperiod. The covering tray was removed after 24 h. Noninoculated plants of the same genotypes sprayed with sterilized distilled water were used as a control.

Leaf samples were collected 3, 5,7, and 10 days after inoculation. Leaf samples of each genotype were cut into 1-2 cm pieces, boiled separately in ethyl alcohol (96%) for 5-10 min on a steam bath, cooled, and the tissue crushed in a blender for 5 min. The suspension was passed through a double layer of cheese cloth to remove leaf debris, which was re-extracted with 80% ethyl alcohol, and the suspension again passed through the cheese cloth. Both the first and the second extracts were mixed together, and filtered through Whatman no. 41 filter paper. The suspension was evaporated to adjust the volume to 5-10 mL g^{-1} of leaf tissue used.

Folin Coicateau reagent (1 mL) was mixed with 1 mL leaf tissue extract to which 2 mL sodium carbonate solution (20%) was added, and the resulting solution was boiled until it turned blue. After cooling, the optical density of the solution was measured using a spectrophotometer at 650 nm. The readings were compared with a standard curve previously plotted with catechol, and the total phenol content of the leaves was estimated.

The phenol content of leaves from all six genotypes increased progressively with time after inoculation, and the increase was significant at 7 days after inoculation (DAI). The highest increase in phenol content occurred in A 2267-2 from 4.0 mg g^{-1} at 3 DAI to 14.0 mg g^{-1} at 10 DAI, and the lowest increase was in IS 3758, from 5.6 mg g^{-1} at 3 DAI to 7.2 mg g^{-1} at 10 DAI (Fig. 1). The phenol content of noninoculated control plants varied from 3.8 to 9.9 mg g^{-1} in various genotypes. Differences in phenol contents between inoculated and noninoculated controls were not significant at the 3- and 5-day stages, but became significant after 7 days. The phenol content in the resistant genotype A 2267-2 was significantly higher than those in moderately resistant and susceptible genotypes. It was, however, moderate in IRAT 204.

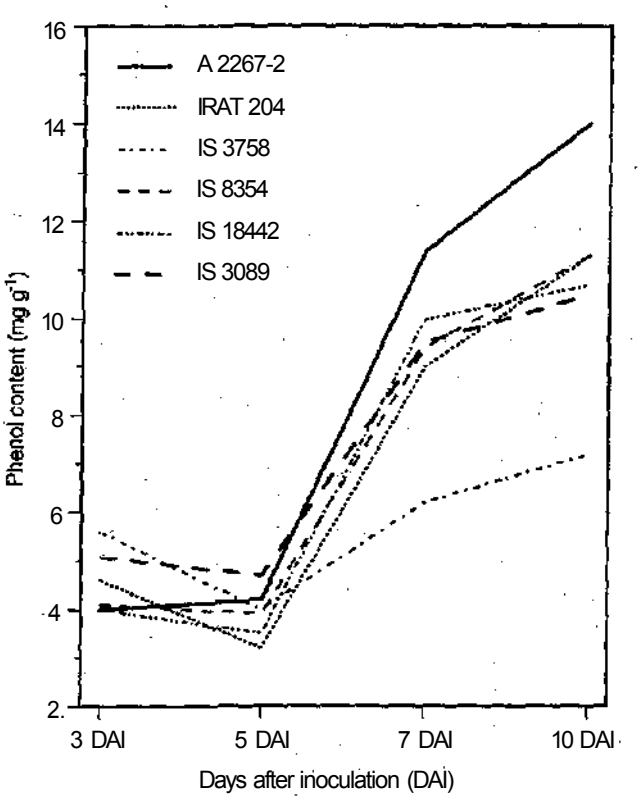

Figure 1. Variation in phenol content in the leaves of sorghum genotypes, resistant, moderately resistant, and susceptible to anthracnose after inoculation with *Colletotrichum graminicola* at ICRISAT-Patancheru, India.

Accumulation of higher than normal quantities of phenolics (phytoalexins) at the site of infection was. reported by Snyder et al. (1991). Many workers (Bhatia et al. 1972, Chiranjeevi and Tripathi 1975, Mishra et al. 1980) have correlated the presence of high amounts of phenolics with resistance to various plant pathogens. The higher phenolic content in response to infection in the resistant sorghum genotypes could be atype of resistance mechanism to anthracnose.

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Stability Analysis of Grain Mold Resistance in Sorghum Using the AMMI Model

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Introduction

Grain molds in sorghum have serious implications for the utilization of the caryopsis for food, or feed. Infected grain is of poor quality due to the breakdown of grain structure, loss of viability, chalky endosperm, and con-. tamination with mycotoxins. These reduce grain quality for processing and lower its nutritional value. Grain molds also reduce yield and the market value of the grain. Grain mold is caused by a complex of fungi including *Curvularia lunata,* and species of *Fusarium, Alternaria, Phoma,* and *Helminthosporium.* Selection for grain mold resistance-is difficult because of the complex inheritance of resistance, the variety of pathogens, and environmental variation. These produce significant genotype by environment (GxE) interactions that reduce the accuracy of methods used to estimate disease resistance and select appropriate germplasm.

Several methods are available to analyze GxE interaction, and to estimate the stability of a genotype across environments. Stabilities are estimated using one of three basic methods; rank, regression, and multivariate analysis. An array of methods has evolved from these basic analysis types. The additive main effect multiplicative interaction (AMMI) model was developed to analyze trials with a focus on GxE interactions (Gauch 1992). AMMI uses analysis of variance to study the main effects of genotypes and environments, and principal component analysis for the residual multiplicative interaction (Zobel et al. 1988). This paper reports the stability of grain mold resistance of 22 lines across 12 environments, and the value of AMMI in the analysis of grain mold incidence in multilocational trials of sorghum.

Material and methods

Twenty two inbred lines of sorghum selected from the Texas A&M sorghum breeding program were tested. These lines were chosen because of their different levels of resistance to grain mold. The evaluated lines are: SC 170-6-17 (1), SC 279-14E (2), SC 630-11E11 (3), SC 650-11E (4), SC 719-11E (5), Tx 2536 (6), Sureno (7), VG 153 (9), 84 BH 5629 (10), R 4317 (11), R 6078 TM (12), Town (13), 90 EON 343 (14), 89 BE 5844 (15), 90 B.2662 (16), 92 B 1941 (17), 90 BE 3533 (30), 90 L 19037 (19), 90 L 19178 (20), 90 CC 549 (21), 92 BD 1982-4 (22), and 90 L 19023 (29). Each experiment was carried out at 12 Texas locations (Corpus Christi (CC93), and Weslaco (WE93) during 1993, Corpus Christi (CC94), Beeville (BV94), and College Station (CS9.4) in 1994, Corpus Christi (CC95), Beeville (B V95), and College Station (CSS95) in 1995, Corpus Christi (CC96), College Station (CSS96, and CSW96) and Weslaco (WE96) during 1996. Each experiment was sown in a randomized complete-block design (RCBD) with two replications. Individual plots consisted of single 5-m rows. Cultural practices at each location were those recommended for the region. At College Station, overhead sprinkle-irrigation (CSS) was used to promote grain mold severity. The nurseries were not inoculated as significant levels of grain mold occurred naturally at all locations. Ten days after physiological maturity, grain mold rating was recorded on a 1 to 5 scale (1 = seed bright, free from mold damage, 5 = very moldy, embryo dead, and endosperm deteriorated). Data on grain mold incidence, based on visual assessment of symptom expression in the experimental field, were analyzed following a combined ANOVA of all locations. Two versions of AMMI were used according to the variability explained, AMMI1