Grain Mold Resistance and Polyphenol Accumulation in Sorghum

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Ten sorghum [*Sorghum bicolor* (L) Moench] genotypes with differences in phenolic compound concentrations and grain mold resistance were evaluated at West Lafayette, IN, over three crop seasons (1989, 1990, and 1992) to assess changes in phenolic compounds during seed development and how these changes influence grain molding. Samples were collected for 9 weeks at 7-day intervals starting 7 days after anthesis. Acidified methanol extracts of the seeds were assayed to determine concentrations of 3-deoxyanthocyanidins, flavan-4-ols, and proanthocyanidins. Seeds were also plated on biological media to observe the level of seed infection by mold-causing fungi. Flavan-4-ol concentrations were high and similar for both the mold-resistant and mold-susceptible genotypes at early stages of seed development. In susceptible genotypes, the flavan-4-ol concentration dropped by 67% between the third and the last sampling dates compared with a 20% decline for the resistant genotypes in the same period. In addition, the resistant genotypes (P954255, P932062, IS15346, IS7822, P013931) had high concentrations of proanthocyanidins throughout the season compared with susceptible lines, which lacked or had negligible amounts of this material. Although significant differences occurred among genotypes for 3-deoxyanthocyanidins, the presence of these pigments did not differentiate mold-resistant and mold-susceptible genotypes. The results also showed that the highest incidence of seed infection by fungi occurred between 25 and 35 days after anthesis. *Alternaria*, *Fusarium* (especially *F. moniliforme*), *Cladosporium*, and *Epicoccum* species were the major fungi isolated from the seeds.

Keywords: *Sorghum; polyphenol; 3-deoxyanthocyanidin; flavan-4-ols; proanthocyanidin; apigeninidin; luteolinidin; grain mold*

INTRODUCTION

Grain mold is a major constraint of sorghum production in areas where the crop flowers and matures under warm and humid weather conditions (Williams and Rao, 1981). Different genera of fungi are associated with molding in different parts of the world (Castor and Fredericksen, 1980; Feliciano et al., 1986; Seitz et al., 1983; Williams and Rao, 1981). Most mold-causing fungi are saprophytic and/or facultative parasites on sorghum (Castor and Fredericksen, 1980; Williams and Rao, 1981). These fungi reduce the quality and quantity of grain yield and may produce mycotoxins that could cause health problems to animals and humans if the infected grain is used for feed or food (Charmley et al., 1994). Development of sorghum germplasm with resistance to grain molding is the most effective and economical means of alleviating this production problem (Williams and Rao, 1981). Because grain mold is caused by diverse groups of fungi, identification of genotypes that are resistant to all of these organisms under all conditions is difficult. Different mechanisms have been implicated in conferring resistance to grain mold. These mechanisms include morphological characteristics, such as seed hardness, pigmented testa, and red pericarp (Jambunathan et al., 1992; Esele et al., 1993); secondary metabolites, like flavan-4-ols and tannins (Jambunathan et al., 1992; Harris and Burns, 1973; Waniska et al., 1989); and antifungal proteins in the seed endosperm that have antimicrobial activity and play an important role as defense mechanisms against pathogens (Vigers et al., 1991; Kumari and Chandrashekar, 1994).

High concentrations of certain phenolic compounds, particularly flavan-4-ols, have been found to correlate strongly with grain mold resistance (Butler, 1989; Jambunathan et al., 1990). This strong association was confirmed by screening 240 diverse sorghum landraces from a collection of sorghum germplasm maintained at Purdue University (Menkir et al., 1995). However, whereas there is a strong association between mold resistance and levels of flavan-4-ols, certain genotypes have high levels of flavan-4-ols but are susceptible to grain mold, and some are low in flavan-4-ols yet resistant to grain mold. This discrepancy suggested that other mechanisms are the basis for grain mold resistance in those genotypes, or that the concentration of flavan-4-ols at certain stages of kernel development is more critical, or that flavan-4-ols are merely indicators of other chemical factors that better determine genetic resistance to grain mold infection. The objectives of this study were (*1*) to assess changes in concentration of flavan-4-ols, 3-deoxyanthocyanidins, and proanthocyanidins (condensed tannins) in mold-resistant and mold-susceptible genotypes during seed development and maturation; (*2*) to determine if the fungal population shifts with changes in accumulation of these secondary metabolites; and (*3*) to determine if decline in flavan-4-ol concentration in certain genotypes during seed development renders them susceptible to grain mold infection.

MATERIALS AND METHODS

Genetic Material. Ten sorghum genotypes with different reactions to mold and with varying concentrations of flavan-4-ol were carefully selected for this study. The 10 genotypes

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were classified into the following four groups based on data from a number of years of analysis of flavan-4-ol concentrations in the seed (Butler and Ejeta, unpublished results): group I, three lines (IS15346, P932062, and P954255) that are high in flavan-4-ols and resistant to mold (HFR); group II, four lines (IS8687, IS0339, P955011, and IS1232) that are high in flavan-4-ols and susceptible to mold (HFS); group III, two lines (IS7822 and P013931) that are low in flavan-4-ols and resistant to mold (LFR); and group IV, one line (IS10354) that is low in flavan-4-ols and susceptible to mold (LFS). Among these genotypes, the HFS and LFR group are relatively rare and those in the HFR and LFS group are very common; therefore, there is uneven distribution of genotypes in the four groups. These genotypes were grown in unreplicated single-row plots in 1989, 1990, and 1992, at the Purdue University Agronomy Research Center near West Lafayette, Indiana. Several randomly selected plants from each genotype were tagged at 50% anthesis. Two tagged panicles were harvested from each genotype at 7-day intervals for 9 consecutive weeks starting 7 days after anthesis and analyzed for concentration of phenolic compounds and for fungal infection.

Phenolic Compounds Assay. Twenty-five seeds from each sample were weighed into 50-mL glass tubes, 10 mL of H+MeOH(1% HCl in methanol, v/v) was added to each tube, and the seeds were homogenized with a Polytron PT-10-35 homogenizer with a PT 20 probe generator (Brinkman Instruments, Westbury, NY) at a setting of 4−6, in repetitive short bursts to minimize heating. The homogenate was centrifuged at 3000 rpm for 5 min in a clinical bench-top centrifuge. The supernatant was decanted, the residue was re-extracted with H+MeOH, and the two extracts were pooled for analysis. The extract was assayed for 3-deoxyanthocyanidins, flavan-4-ols, and proanthocyanidins as previously described (Bate-Smith and Rasper, 1969; Butler et al., 1982; Watterson and Butler, 1983). For determining 3-deoxyanthocyanidins, the H+MeOH extract was diluted three- to fourfold with MeOH, and the absorbance was read at 475 nm (apigeninidin) and 495 nm (luteolinidin) on a Spectronic 20 colorimeter. To determine flavan-4-ols, 0.5 mL of the H+MeOH extract was added to 7 mL of 30% (v/v) HCl in 1-butanol or 7 mL of 15% (v/v) 0.1 N acetic acid, 15% (v/v) methanol, and 70% 1-butanol. No anthocyanidin formation occurs in the latter solvent, which serves as a blank to correct for pigments present in the extract. After mixing by vortex, the samples were left to stand at room temperature for 1 h to allow formation of the unstable anthocyanidin pigment characteristic of flavan-4-ols. After the absorbance was read at 550 nm, the sample but not the blank, was heated in a boiling water bath for 2 h. Under these conditions, flavan-3-ol oligomers are converted to anthocyanidin pigments, and the unstable pigment formed from flavan-4-ols is destroyed. After cooling, anthocyanidins were measured by their absorbance at 550 nm.

Fungi Isolation from Seeds. Within 24 h after the samples were collected, 100 representative seeds from each genotype were surface disinfected with 2% NaOCl solution for 3 min, rinsed with sterile water, blotted dry on sterile paper towel, plated in Petri plates (10 seeds/plate) containing 10 mL of acidified potato dextrose agar (APDA), and allowed to stand at room temperature. After 7 days, colonies of fungi growing out of the seeds were identified and counted.

Grain Mold Damage Assessment. The warm and humid (70−85 °F and 75−100% RH) midsummer and early fall weather at the Purdue Agronomy Research Center is conducive to grain mold development. A uniform and satisfactory level of natural infection is obtained without artificial inoculation. Kernels on unprotected panicles of each genotype were visually evaluated 10 days after the final sampling date on a 1 to 5 scale, where a rating of 1 indicates highly resistant, with no sign of molding on any kernel, and 5 indicates susceptible, with all kernels heavily infected (Bandyopadhyay and Mughogho, 1988). Statistical analyses were performed with procedures outlined by SAS (SAS Institute, 1985).

RESULTS AND DISCUSSION

Significant differences were observed among genotypes for 3-deoxyanthocyanidins, flavan-4-ols, and proanthocyanidins concentration (Table 1). Apigeninidin and luteolinidin in the seeds changed significantly for the different genotypes in the resistant and susceptible groups as the seeds matured (Figure 1). The cultivar by date interaction was significant for flavan-4-ols and proanthocyanidins, indicating that the relative ranking of genotypes for flavan-4-ol and proanthocyanidin concentration was different for the different sampling dates. In both the high flavan-4-ol-resistant (HFR) and high flavan-4-ol-susceptible (HFS) groups, some genotypes had high concentrations of flavan-4-ol at an early (milk-to-soft dough) stage of seed development, but significantly reduced amounts at physiological grain maturity, and vise versa (data not shown). The change in proanthocyanidins (tannin) concentration was different from that of flavan-4-ols in that the mold-resistant genotypes (HFR) had significantly higher concentrations of proanthocyanidins from the first to the last sampling date, whereas the concentrations of this material in the mold-susceptible genotype groups (HFS and LFS) were negligible throughout the study period. However, ranking of individual genotypes changed for proanthocyanidin concentration within the resistant and susceptible groups at different sampling dates.

Table 1. Mean Squares from ANOVA for Selected Sources of Variation for Apigeninidin (A475), Luteolinidin (A495), Flavan-4 ols (FF), and Proanthocyanidin (PRAC)

		mean square			
source	DF	A475	A495	FF	PRAC
cultivar	9	2.11*	1.99*	6.62**	3.78**
date	8	0.90**	1.22**	0.66	0.19*
linear	1	6.83*	9.58**	4.46**	0.56*
quadratic	1	0.01	0.00	0.01	0.02
residual	5	0.04	0.03	0.14	0.15
cultivar*date	72	0.06	0.09	0.08**	0.05*

*,** Significantly different from zero at $p = 0.05$ and $p = 0.01$ levels, respectively.

Apigeninidin and Luteolinidin. The 3-deoxyanthocyanidin concentrations of the four sorghum genotype groups at each sampling time are shown in Figure 1. Apigeninidin and luteolinidin concentrations showed very high correlations ($r = 0.98$, $p = 0.001$) and followed the same trend in all four groups in this study (data not shown). Thus, only apigeninidin concentration is presented in Figure 1. Genotypes in the high flavan-4-ol-resistant group (HFR) had the highest concentration of 3-deoxyanthocyanidins and those in the high flavan-4-ol-susceptible (HFS) group, showed the second highest concentration of these pigments (Table 2; Figure 1.). Three of the four genotype groups (HFR, HFS, and LFS) had higher concentrations of apigeninidin and luteolinidin at physiological maturity than at the soft dough stage. The concentrations of these pigments were different for all four groups and reached their maximum between the fifth and the last sampling date (Figure 1).

Mean fungal colonies per 100 seeds of the four genotype groups at different sampling times are shown in Figure 2. The incidence of the different fungal species at different sampling dates is shown in Table 3. These results showed that seed infection by fungi, especially by the major groups like *Alternaria*, *Fusarium*, and *Epicoccum* species, increased substantially beginning with the third sampling date. Total fungal colonies observed per 100 seeds was especially high for the HFS and LFS groups compared with the HFR and LFR groups (Figure 2). For most genotypes, increased seed

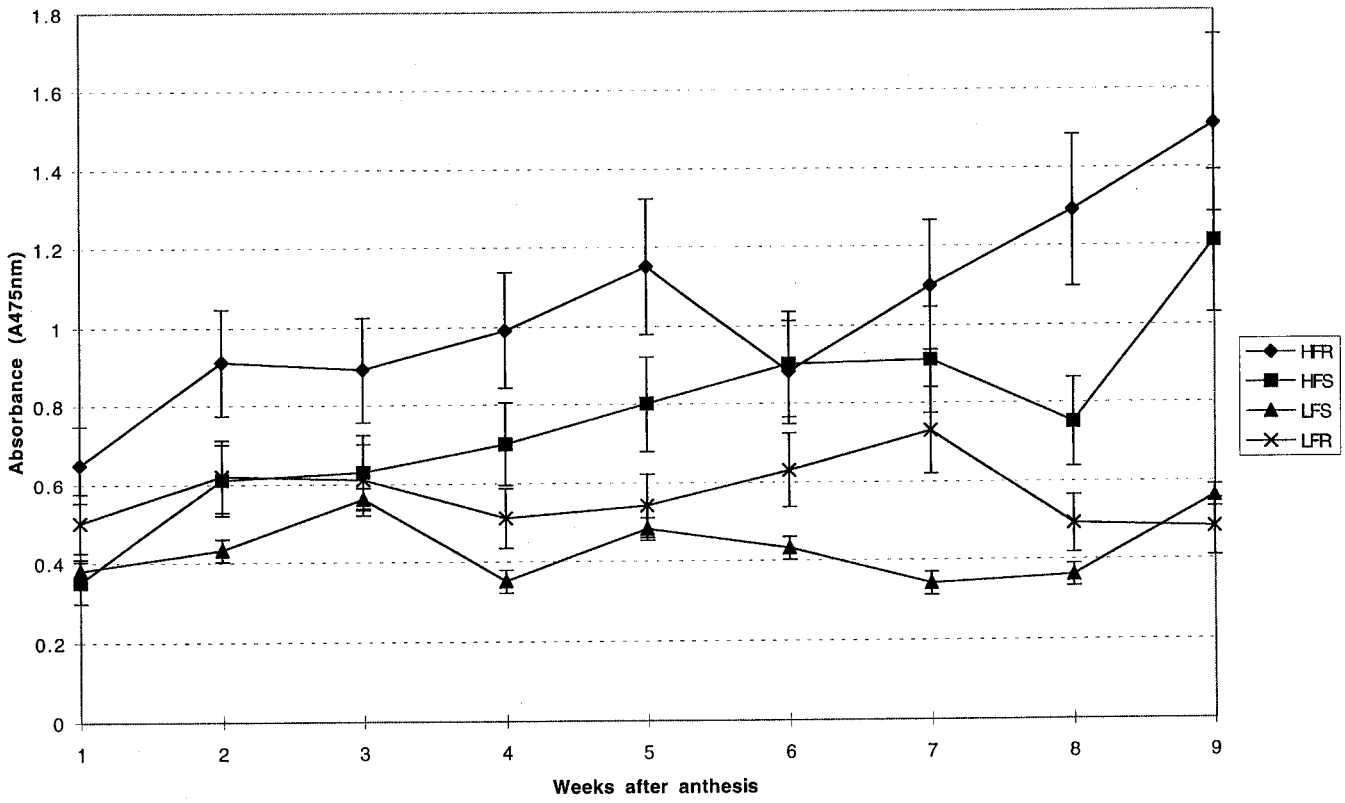

Figure 1. Mean apigenindin concentration in mold-resistant and mold-susceptible sorghum genotypes at different stages of seed development. (HFR) high flavan-4-ol resistant; (HFS) high flavan-4-ol susceptible; (LFR) low flavan-4-ol resistant; (LFS) low flavan-4-ol susceptible. Vertical bars indicate standard error.

Table 2. Mean Apigeninidin, Luteolinidin, Flavan-4-ols, Proanthocyanidin Concentrations and Visual Molding Scores (VS) of 10 Sorghum Genotypes in Different Groups

variety	A475 apigeninidin	A495 luteolinidin	flavan-4-ols	proantho-cyanidin	VS
HFR[a]					
P954255	0.69	0.60	0.98	0.89	1.5
P932062	1.07	0.93	1.54	1.06	1.0
IS15346	1.17	0.98	0.77	1.16	1.0
HFS[b]					
IS8687	0.65	0.58	0.96	0.07	4.0
IS0339	1.28	1.23	0.79	0.08	4.8
P955011	1.39	1.34	0.72	0.07	4.0
IS1232	0.75	0.69	1.01	0.09	4.0
LFR[c]					
IS7822	0.57	0.53	0.08	0.69	1.5
P013931	0.53	0.47	0.06	0.58	1.5
LFS[d]					
IS10354	0.44	0.37	0.02	0.01	4.0
CV	0.27	0.35	0.41	0.47	0.26

[a] HFR = High flavan-4-ol-resistant genotype. [b] HFS = High flavan-4-ol-susceptible genotype. [c] LFR = Low flavan-4-ol resistant genotype. [d] LFS = Low flavan-4-ol susceptible genotype.

infection by fungi coincided with the time of higher levels of 3-deoxyanthocyanidin accumulation (Figures 1 and 2). High flavan-4-ol-susceptible (HFS) genotypes with high concentrations of apigeninidin and luteolinidin showed high visual mold damage (Table 2). Correlations between the 3-deoxyanthocyanidin (apigeninidin and luteolinidin) concentration and number of fungi colonies per 100 seeds were not significant (Table 4). These results suggest that these pigments may not play a significant role in sorghum grain mold resistance. Apigeninidin and luteolinidin accumulated in sorghum seedlings in response to pathogenic fungi infection (Nicholson et al., 1987), and these pigments were active against different fungi in *in vitro* studies (Schutt and Netzly, 1991). Although the accumulation of these pigments in seeds was not measured in response to infection by mold-causing fungi, their presence in developing seeds starting as early as 7 days after anthesis did not stop grain molding in the field.

Flavan-4-ols. Significant differences were found among genotypes for flavan-4-ol concentration (Table 1). The overall flavan-4-ol concentration and visual molding scores of the 10 genotypes are shown in Table 2. The four genotypes in the HFS group had high flavan-4-ol concentrations and also high field visual molding scores, compared with genotypes in the HFR and LFR groups. Flavan-4-ol concentrations of the seeds of HFR and HFS genotype groups were similar and high at the early milk-to-soft dough stage (7−21 days after anthesis). Between the third and fourth sampling dates (mid-dough stage), flavan-4-ol concentrations in both HFR and HFS genotype groups dropped by 20 and 25%, respectively. The flavan-4-ol concentration of the HFS group continued to decline further and, by the last sampling (physiological grain maturity), it had dropped by 67% from its highest level at the third sampling date (Figure 3). In comparison, the seed concentration of flavan-4-ols in the HFR genotypes group remained consistently higher at the level of the fourth sampling, with minor increases at later sampling dates. These results indicated that the high flavan-4-ol resistant (HFR) genotypes, but not the high flavan-4-ol-susceptible (HFS) genotypes, maintained high levels of flavan-4-ol throughout seed development and maturation, especially at the time when fungal infection of seeds was at its highest (Figure 2). Thus, the significant decline in flavan-4-ol concentrations in the HFS group starting the third week after anthesis through physiological grain maturity and the increased infection pressure about the same time (Figure 2) would explain why these

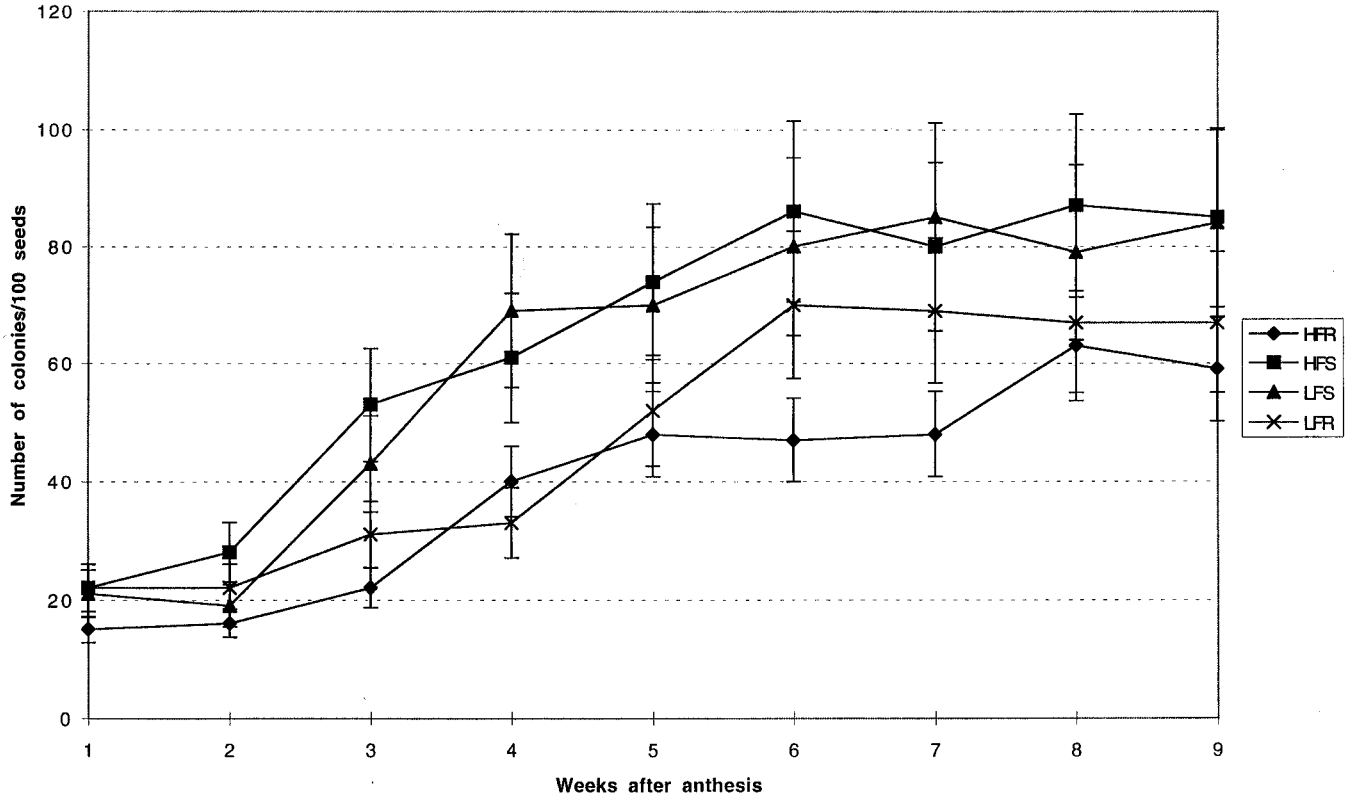

Figure 2. Total number of fungal colonies from seeds of different sorghum genotype groups at different sampling times. Vertical bars indicate standard error.

Table 3. Mean Incidence of Different Fungal Species[a] per 100 Seeds at Different Sampling Times

days after anthesis	fungal species						
	ALT	EPI	CLAD	FUS	FMON	NIG	OTH
7	20.3	4.8	14.5	1.0	3.2	2.3	23.4
14	22.9	3.3	10.8	1.7	7.1	11.2	14.3
21	32.9	11.8	8.3	2.1	3.7	2.6	6.3
28	29.5	10.6	3.2	3.3	8.3	4.0	4.4
35	32.6	11.7	1.2	5.4	14.3	1.1	7.0
42	33.5	8.6	1.0	4.2	15.3	1.9	5.7
49	38.7	11.1	2.1	7.8	13.8	1.4	5.1
56	38.1	8.2	2.4	6.1	14.9	1.0	5.2
63	36.4	8.0	2.9	6.0	11.4	1.5	4.1
CV	0.27	0.69	1.47	1.0	0.69	1.5	1.41

[a] ALT, *Alternaria* spp.; EPI, *Epicoccum* spp.; CLAD, *Cladosporium* spp.; FUS, *Fusarium* spp. other than *F. moniliforme*; FMON, *F. moniliforme*; NIG, *Nigrospora* spp.; OTH, others.

Table 4. Correlation Coefficients between Fungi Colonies per 100 Seeds and Flavan-4-ols (FF), Proanthocyanidins (PRAC), Apigeninidin (A475), and Luteolinidin (A495)

fungal species	FF	PRAC	A475	A495
Alternaria spp.	−0.78**	−0.85**	0.14	0.21
Epicoccum spp.	−0.61	−0.20	−0.12	−0.09
Cladosporium spp.	0.65*	0.45	0.24	0.17
Fusarium spp.	−0.51	−0.73*	0.47	0.50
F. moniliforme	−0.33	−0.95**	0.48	0.54
Nigrospora spp.	0.11	0.11	0.06	0.07
others	0.29	−0.19	0.47	0.48

*,** Significant at $p = 0.05$ and $p = 0.01$ levels, respectively.

genotypes are susceptible to molding. Note that the visual molding scores (Table 2) were taken at the mature kernel stage. By that time, the flavan-4-ol level of the HFS group had already dropped substantially, and any effect this compound might have had on the mold-causing fungi could not have been observed. The results also showed that some genotypes with low flavan-4-ol concentration (LFR group) had low visual molding scores and lower infection by fungi compared with the HFS group (Tables 2 and 5, Figures 2 and 3). As shown later, these genotypes had a higher concentration of proanthocyanidins compared with HFS and LFS groups. Thus, the decline in flavan-4-ol concentration of HFS genotypes when infection by the most serious mold causing fungi (*Fusarium* spp; Castor and Fredericksen, 1980) was high and the low percent seed infection by fungi in genotypes with high proanthocyanidin and low flavan-4-ol concentration (LFR) would explain why there were no significant correlations between flavan-4-ol concentration and the prevalence of *Fusarium* spp, as was the case with proanthocyanidins (Table 4). This explanation supports previous results (Butler, 1989; Jambunathan et al., 1990, 1992) that high flavan-4-ol concentration indicated mold resistance in sorghum if present at a high level throughout seed development and maturation, as shown for the HFR group.

Proanthocyanidin. The overall proanthocyanidin concentrations of the 10 genotypes are given in Table 2. The levels of proanthocyanidins during seed development for the four sorghum genotype groups are shown in Figure 4. High flavan-4-ol-resistant (HFR) and low flavan-4-ol-resistant (LFR) genotype groups had the first and second highest concentration of proanthocyanidins throughout the study period, respectively. The other two genotype groups (HFS and LFS), had negligible amounts of this material (Table 2, Figure 4). The changes in the proanthocyanidins concentration of HFR genotypes were similar to the changes in flavan-4-ol concentration. The proanthocyanidin concentrations of HFR and LFR genotype groups were high in the milk-to-soft dough stage, then declined steadily for both groups throughout much of the season and increased in the last one or two sampling dates. The significant

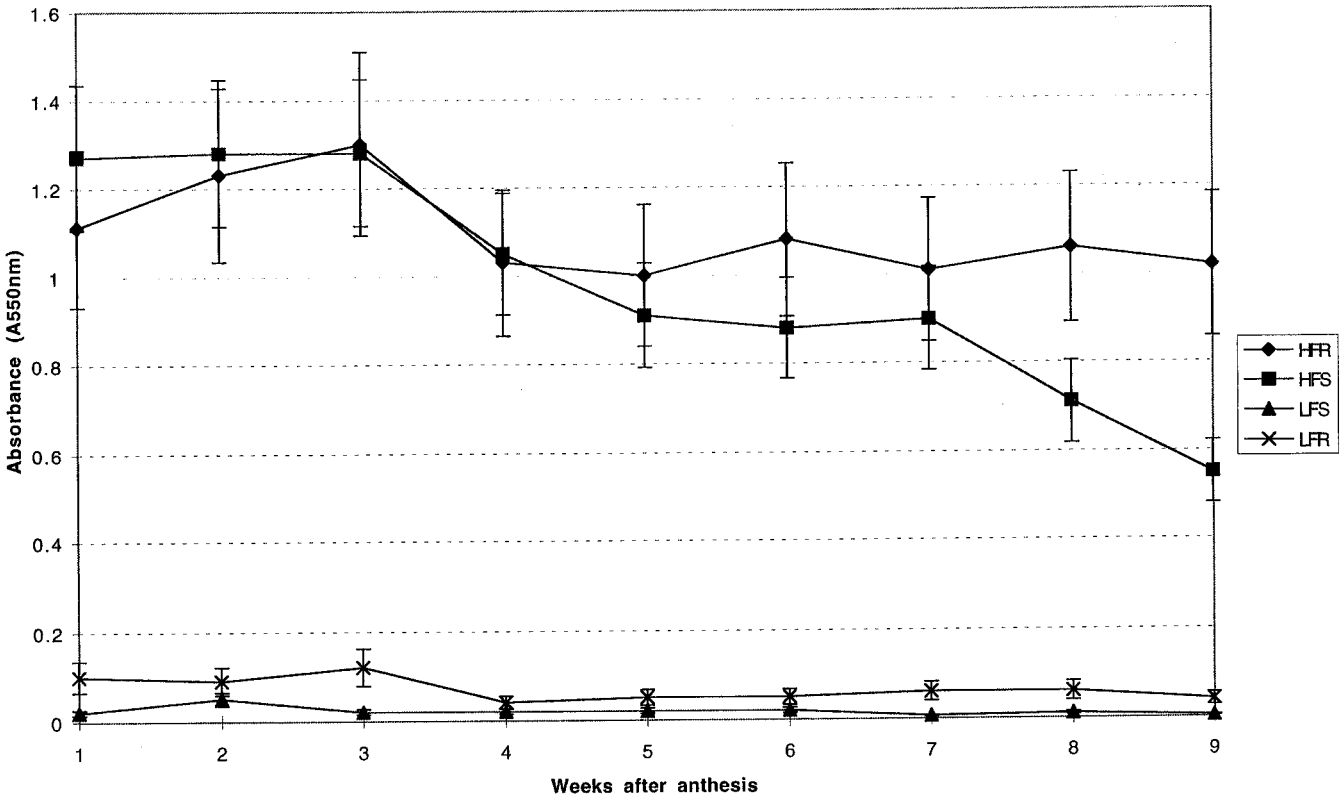

Figure 3. Flavan-4-ols accumulation in seeds of different sorghum groups at different stages of seed development. Vertical bars indicate standard error.

Table 5. Mean Number of Fungal Colonies[a] per 100 Seeds of Different Sorghum Genotypes Averaged over the Study Period

	fungal species							
variety	ALT	EPI	CLAD	FUS	FMON	NIG	OTH	VS
HFR								
P954255	31.3	6.6	4.17	0.3	6.4	1.9	8.7	1.5
P932062	10.7	5.9	5.50	5.7	4.1	2.6	4.2	1.0
IS15346	20.9	3.5	9.33	5.7	4.2	2.1	3.7	1.0
HFS								
IS8687	40.3	10.8	8.61	3.8	10.8	2.6	7.7	4.0
IS0339	37.4	10.7	1.78	6.1	20.2	4.7	12.5	4.8
P955011	34.8	11.6	4.17	4.6	22.1	2.7	15.4	4.0
IS1232	40.2	9.1	5.17	2.2	15.4	3.4	10.8	4.0
LFR								
IS7822	26.5	9.2	3.89	6.7	5.2	2.9	6.7	1.5
P013931	33.1	8.6	5.61	2.7	5.0	4.1	8.1	1.5
LFS								
IS10354	41.1	10.8	3.22	3.8	8.6	2.9	5.9	4.0
CV	0.30	0.69	1.47	1.04	0.68	1.50	1.41	0.26

[a] ALT, *Alternaria* spp.; EPI, *Epicoccum* spp.; CLAD, *Cladosporium* spp.; FUS, *Fusarium* spp.; FMON, *F. moniliforme*; NIG, *Nigrospora* spp.; OTH, others; VS, visual mold score.

and negative correlations between proanthocyanidin concentration and the major mold-causing fungi species (*Alternaria* spp. and *Fusarium* spp.) that were isolated from the seeds (Table 4), along with a very high negative correlation coefficient with visual molding score ($r = 0.95$, $p = 0.001$), indicated that this substance plays an important role in grain mold resistance in sorghum genotypes that produce it. This result is consistent with previous reports (Harris and Burns, 1973) that sorghum lines with high tannin concentration had better mold resistance. However, there are genotypes that are mold resistant and yet have no detectable flavan-4-ol or proanthocyanidins in their seeds (Jambunathan et al., 1992). Recent reports indicated that proteins from sorghum endosperm have *in vitro* antifungal activity that might play a role in mold resistance (Kumari and Chandrashekar, 1994; Vigers et al., 1991).

Fungi Isolated from Seeds. The major fungi isolated from the seeds of all four groups of sorghum genotypes and their distribution across the nine sampling dates are shown in Tables 5 and 3, respectively. Early in the season, *Epicoccum*, *Cladosporium*, and *Alternaria* were the predominant species isolated. Starting at ~4 weeks after anthesis, *Alternaria*, *Epicoccum*, *Cladosporium*, and *Fusarium* spp (especially *F. moniliforme*) were isolated more frequently than other species. A high visual molding score was consistently better correlated with *Alternaria spp* and *Fusarium moniliforme* than with the others (Table 6). Genotypes P932062 and IS15346 had the lowest percentage of seed infection by any of the fungal species isolated (Table 5). Both of these genotypes belong to the HFR group and, as shown in Figures 3 and 4, these genotypes had very high levels of both flavan-4-ols and proanthocyanidins throughout seed development and maturation. Genotypes in the HFS (IS8687, IS0339, P955011, IS1232) and LFS groups (IS10354) had a higher incidence of *Alternaria* spp, *F. moniliforme*, and *Epicoccum* spp than the other two groups (Table 5). The highest increase in percent fungi infection in all groups of sorghum was from 21 to 35 days after anthesis (Figure 2). The *Fusarium* spp. increased substantially starting 4 weeks after anthesis and beyond, mostly on the HFS and LFS groups. Other workers reported a similar trend in fungi buildup on sorghum seeds in the field (Jambunathan et al., 1990; Seitz et al., 1983).

As the flavan-4-ol and proanthocyanidin concentrations of the seeds of the different genotype groups started to decline (Figures 3 and 4), percent seed infection on those genotypes showed proportional increases (Figure 2). This observation suggests that both flavan-4-ols and proanthocyanidins play a significant

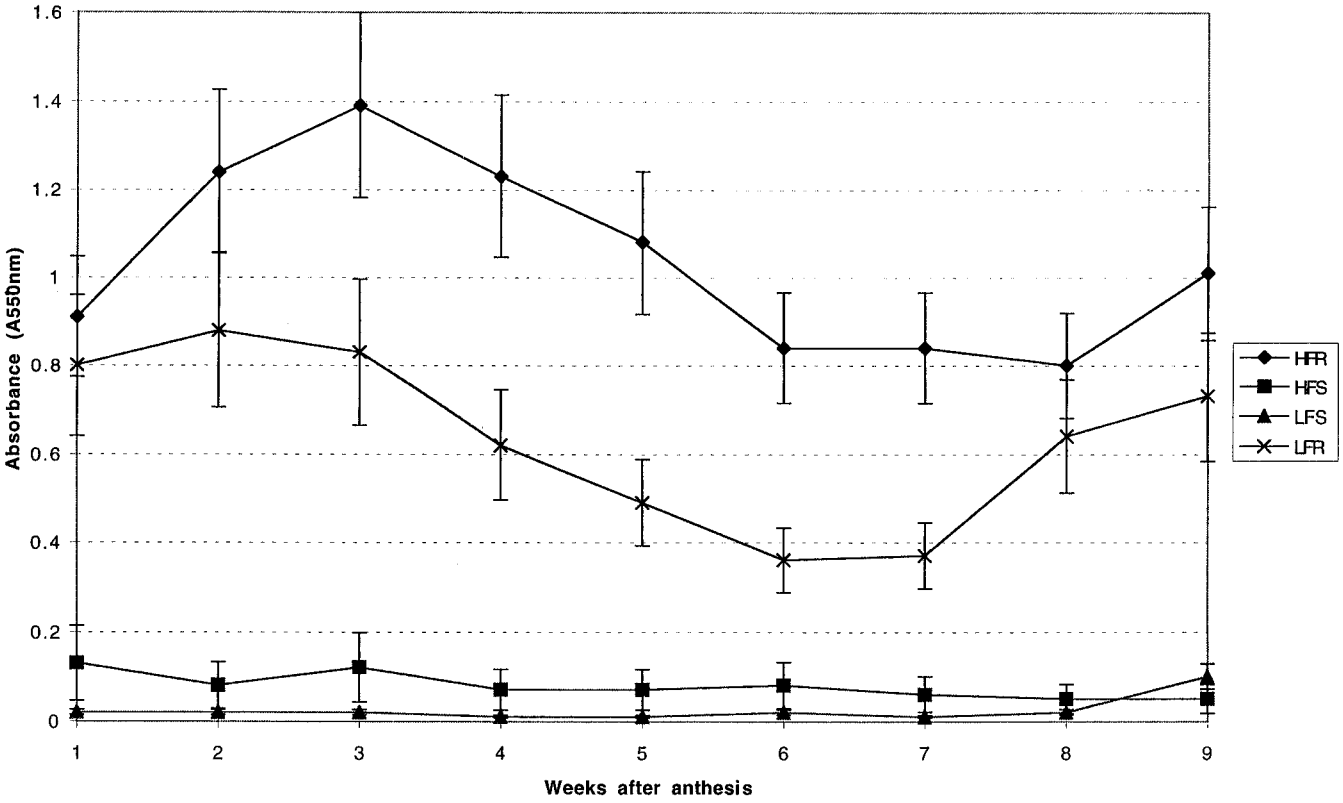

Figure 4. Mean proanthocyanidin concentration in different sorghum genotype groups at different sampling times. Vertical bars indicate standard error.

Table 6. Correlation Coefficients between Visual Mold Score and Number of Fungi Colonies per 100 Seeds at Different Sampling Dates

	correlation coefficients								
fungi	7DAA[a]	14DAA	21DAA	28DAA	35DAA	42DAA	49DAA	56DAA	63DAA
ALT	0.63*	0.32	0.93**	0.80**	0.91**	0.49	0.60	0.66*	0.78**
EPI	−0.16	0.46	0.58	0.78**	0.65*	0.72**	0.45	0.29	0.09
CLAD	−0.17	−0.29	0.17	−0.08	0.13	−0.55	−0.24	−0.65*	−0.27
FUS	0.27	−0.37	−0.30	−0.24	−0.44	0.18	−0.11	0.27	0.78**
FMON	0.38	0.44	0.63	0.79**	0.79**	0.70**	0.81**	0.87**	0.94**
NIG	0.22	0.63	−0.64*	0.38	0.07	−0.52	−0.02	−0.11	0.03
OTH	0.67*	0.15	0.76*	0.15	0.45	0.35	0.18	−0.39	0.13

*,** Significance level of correlation coefficient at $p = 0.05$ and $p = 0.01$, respectively. [a] DAA, days after anthesis.

role in mold resistance in sorghum. It is also possible that at an early stage of development the seed might contain other secondary metabolites that have activity against fungi. It has been reported that sorghum (Ferreira and Warren, 1978) and cotton (*Gossypium hirsutum* L.) (Howell et al., 1976; Bell and Stipanovic, 1978) plants that are resistant as seedlings become susceptible at a later growth stage to the same pathogenic organisms. Whether a similar mechanism is also involved in the resistance of immature sorghum seed to fungal infection is unknown, because genotype IS10354 (LFS) showed a low infection rate similar to the resistant genotypes at early seed development stage (Figure 2) and yet had a negligible amount of these phenolic compounds.

Grain mold is caused by a diverse and dynamic group of fungi from different genera (Castor and Fredericksen, 1980; Williams and Roa, 1981; Feliciano et al., 1986), and it is unlikely that one or two mechanisms of resistance would be totally effective against all of them under all environmental conditions. Accumulation of phenolic compounds in sorghum is influenced by environmental factors (Waniska et al., 1989; Woodhead, 1981). Thus, the effectiveness of these compounds as a mold-resistance mechanism is also influenced by environmental factors, as is the dispersal of fungi and infection of hosts. Identifying different mechanisms of resistance to grain mold and incorporating them into agronomically important genotypes could provide a more lasting and effective solution to this major sorghum disease.

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