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Expression of Susceptibility to Fusarium Head Blight and Grain Mold in A_1 and A_2 Cytoplasms of *Sorghum bicolor*

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ABSTRACT

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Panicle diseases are among the major constraints to sorghum (*Sorghum bicolor*) production in the northern Great Plains; host plant resistance is the primary management option. However, essentially all commercial sorghum hybrids contain A_1 cytoplasm, which raises the concern about increased disease risk as a result of cytoplasmic genetic uniformity. To determine the influence of cytoplasmic background on the expression of susceptibility to panicle diseases, F_1 hybrids with four nuclear genotypes in each of two cytoplasms (A_1 and A_2) were planted in three environmentally diverse geographic locations in Nebraska. Fusarium head blight ranged in incidence from 13 to 100% across locations. Grain mold, caused primarily by species of *Alternaria*, *Fusarium*, and *Cladosporium*, ranged in incidence from 5 to 100% across locations. There was a significant effect of nuclear genotype on the incidence and severity of both head blight and grain mold across the three locations. Cytoplasm had no effect on head blight incidence or severity, or on grain mold severity. Cytoplasm had a significant effect on grain mold incidence, with A_1 exhibiting slightly lower incidence than A_2 (64 versus 70%). Although the cytoplasm effect for grain mold incidence was statistically significant, most of the variation in grain mold incidence was attributable to nuclear genotype. The slight increase in grain mold incidence attributable to A_2 cytoplasm should be overcome easily by selection of nuclear genotypes with grain mold resistance. The use of A_2 cytoplasm to incorporate genetic diversity into grain sorghum hybrids should not increase the risk of head blight or grain mold in commercial grain production.

More than 97% of commercial grain sorghum (*Sorghum bicolor* (L.) Moench) hybrids are based on a single cytoplasmic (A_1) background (15,16), which has raised serious concerns with respect to stability of production and vulnerability to disease of the majority of the commercial sorghum crop (9,11,15,20). Cytoplasmic isolines have been developed and registered to increase genetic diversity for developing commercial grain sorghum hybrids.

These cytoplasmic isolines have been evaluated extensively for agronomic traits (9,15,16,21). In some cases, no differences were reported among hybrids in different cytoplasms with respect to stigma receptivity (2); agronomic traits including flowering, plant height, and yield parameters (3,15); pollen fertility and dry matter accumulation (9); or panicle size and grain moisture (20). In other cases, differences among hybrids in different cytoplasms were reported for days to flowering (5), pollen fertility and seed set (17), and correlation among yield parameters (18). From these studies, it was concluded that the A_2 cytoplasm would be acceptable for developing commercial hybrids in order to increase genetic diversity and decrease vulnerability to disease.

However, little information is available concerning the potential influence of the A_2 cytoplasmic background on the expression of resistance or susceptibility to disease development in sorghum. Susceptibility to rust (*Puccinia purpurea* Cooke), zonate leaf spot (*Gloeocercospora sorghi* Bain & Edgerton ex Deighton), and leaf blight (*Exserohilum turcicum* (Pass) K. J. Leonard & E. G. Suggs) was reported to be lower in hybrids with A_2 cytoplasm than in hybrids with A_1 cytoplasm (14). Susceptibility to sorghum head smut (*Sporisorium reilianum* (Kuhn) Langdon & Fullerton) was reported to be higher in hybrids with A_2 cytoplasm than in hybrids with A_1 cytoplasm (13). These studies indicate differential effects of A_1 and A_2 cytoplasms on the susceptibility to certain diseases in sorghum. Although symptoms of head smut develop in the sorghum panicle, infection and disease establishment occur in seedlings; therefore, susceptibility to head smut is expressed prior to panicle formation. There are no published reports on the influence of the A_2 cytoplasm on susceptibility to pathogens that attack the sorghum panicle.

Panicle and grain diseases are major constraints to sorghum production throughout the world, including the northern Great Plains of the United States (19). Commercial hybrids vary in susceptibility to this family of diseases that affects both grain yield and grain quality. For several reasons, including low profitability, management of these diseases usually is restricted to host plant resistance and cultural practices. Recent widespread epidemics of Fusarium head blight and grain mold affected the sorghum crop over much of Nebraska and established a need for additional management options for these diseases. Multiple *Fusarium* spp. (including *F. thapsinum* Klittich, Leslie, Nelson & Marassas sp. nov., *F. semitectum* Berk. & Ravenel, and *F. proliferatum* (T. Matsushima) Nirenberg) are associated with Fusarium head blight in Nebraska (J. P. Stack, *unpublished*). Grain mold of sorghum is caused by several fungal species, the most prevalent in Nebraska being species of *Alternaria*, *Cladosporium*, and *Fusarium* (J. P. Stack, *unpublished*). The objective of this study was to determine the influence of A_1 and A_2 cytoplasms on susceptibility to sorghum panicle diseases.

MATERIALS AND METHODS

Sorghum genetic materials. Isocytoplasmic hybrids were produced by crossing A_1KS57, A_2N181 (synonym, and hereafter referred to as A_2KS57) (12), A_1Tx3042, and A_2N184 (synonym, and hereafter referred to as A_2Tx3042) (12) to two R-lines, ROKY10 and IA28, known to restore fertility in both A_1 and A_2 cytoplasm. Although IA28 officially is described as having darkly pigmented seed but no testa layer (1), the IA28 accession maintained in Nebraska and used in this study consistently tests positive for a high-tannin testa layer using a bleach test (8).

Experimental design. During the 2001 growing season, experiments were conducted at three locations in Nebraska: Mead, Clay Center, and Hayes Center. These locations were selected to represent the wide range of environments in which grain sorghum is commercially grown in Nebraska and varied greatly in climate during 2001 (Fig. 1). Weather data were

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collected from the High Plains Regional Climate Center weather station in each county; proximity of the weather station to the field ranged from 300 m at Clay Center to 4 km at Hayes Center. The design at each location was a split plot with four replicates. Nuclear genotypes were whole plots and cytoplasms were subplots. Each plot comprised two 9-m-long rows spaced 0.76 m apart. Seed were planted at a 2.5-cm depth and at a rate that was equivalent to 296,400 seed/ha. At Mead and Clay Center, the sorghum was planted into fields that were planted to soybean the previous year. At Hayes Center, the sorghum was planted into a field that was planted to corn the previous year. Planting dates were 22 May, 12 June, and 31 May 2001 at Mead, Clay Center, and Hayes Center, respectively. Standard commercial management practices (e.g., fertility, weed and pest management, and so on) were employed without supplemental irrigation.

Disease assessments. At all three locations, disease developed from natural inoculum; panicle diseases are common in Nebraska and have been epidemic in recent years. Assessments of disease incidence and severity were made at each location at physiological maturity. Ten plants per plot were arbitrarily selected and the presence of head blight and grain mold was recorded. Disease incidence was calculated for both head blight and grain mold as the percentage of diseased plants. Head blight severity measurements were based on the following rating scale: 0 = no disease, 1 = scattered lesions on rachis branches, 2 = lesions on rachis branches over upper half of panicle, 3 = lesions on most rachis branches and minor panicle distortion, 4 = extensive lesions on rachis branches and moderate panicle blight, and 5 = panicle severely blighted. Grain mold severity measurements were based on the following rating scale: 0 = no grain mold observed, 1 = surface mold detected on less than 10% of seed, 2 = surface mold detected on approximately 10 to 20% of seed, 3 = surface mold detected on approximately 20 to 30% of seed, 4 = surface mold detected on approximately 30 to 40% of seed, and 5 = surface mold detected on 50% or more of seed.

Data analysis. Disease incidence percentages were transformed (arcsin square root) prior to analysis to normalize the data. Analyses of variance were performed using the PROC MIXED procedure of SAS (version 8; SAS Institute, Cary, NC). Location, nuclear genotypes, and cytoplasm were considered fixed effects in the model. Disease severity ratings were ordered categorical responses. Nonparametric and parametric statistical analyses were conducted as suggested by Conover (4) by first using analysis of variance, then using the same procedure on the rank transformed data. The model and analysis were otherwise the same as for disease incidence. The two analyses gave nearly identical results. Consequently, disease severity data were treated and will be presented as parametric data for the readers convenience.

RESULTS

Head blight incidence. There was no significant difference (P = 0.717) in the incidence of head blight between the A_1 and A_2 cytoplasms (Table 1). There was a significant effect (P = 0.0001) of nuclear genotype and of location (P = 0.0001) on the incidence of head blight (Table 1). There was also a significant (P = 0.0001) location–nuclear genotype interaction. At Mead, head blight incidence was over

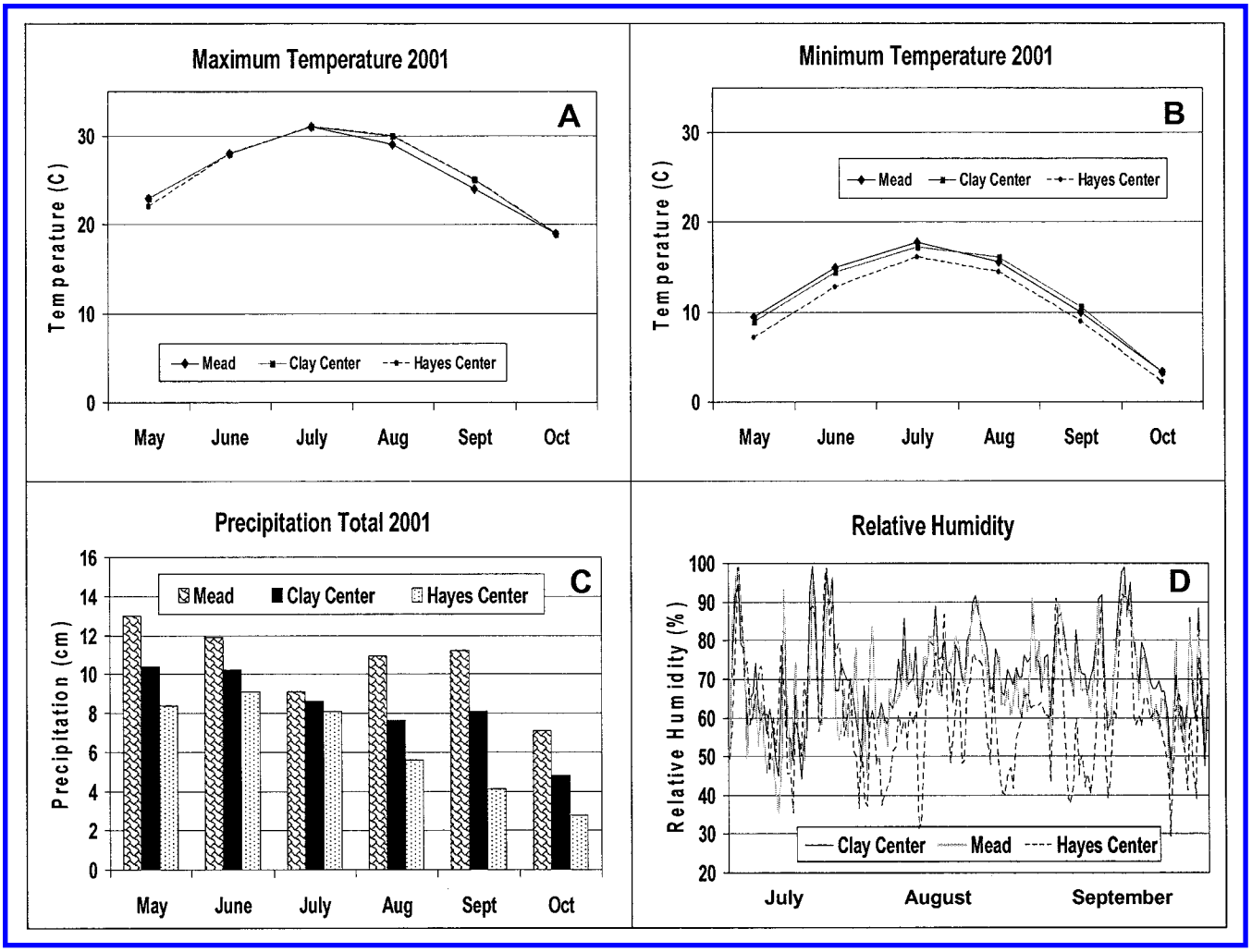

Fig. 1. Prevailing weather conditions (mean monthly maximum temperature, mean monthly minimum temperature, monthly rainfall totals, and relative humidity) during the 2001 growing season at Mead, Clay Center, and Hayes Center, NE.

twice as high in nuclear genotypes containing Tx3042 (disease incidence [DI] = 99%) than in nuclear genotypes containing KS57 (DI = 42%) (Fig. 2). This relative difference did not hold at Clay Center or Hayes Center (Fig. 2). The Tx3042–IA28 nuclear genotype was the most susceptible to Fusarium head blight; it had the highest incidence of disease at all three locations (Fig. 2). Head blight developed earlier and was more severe at Mead than at the other locations, allowing for more dramatic differences among hybrids. Mead had the highest monthly rainfall totals and the highest relative humidity profile (Fig. 1), perhaps accounting for the different disease dynamics.

Head blight severity. There was no significant difference (P = 0.648) in the severity of head blight between the A_1 and A_2 cytoplasms (Table 1). There was a significant effect (P = 0.0001) of nuclear genotype on the severity of head blight (Table 1). There was also a significant (P = 0.0001) location–nuclear genotype interaction. At Mead, head blight severity was over twice as high in nuclear genotypes containing Tx3042 (disease severity [DS] = 5) than in nuclear genotypes containing KS57 (DS = 2) (Fig. 2). This relative difference did not hold at Clay Center or Hayes Center (Fig. 2). As stated above, this may have been due to a more favorable environment at Mead allowing for more dramatic differences to be expressed. As with head blight incidence, the Tx3042–IA28 nuclear genotype had the highest head blight severity at all three locations (Fig. 2).

Grain mold incidence. When compared across all nuclear genotypes and locations, there was a significant effect (P = 0.047)

Table 1. Analysis of variance table for the incidence and severity of Fusarium head blight and grain mold in sorghum grown at three locations in Nebraska during 2001[a]

		Head blight				Grain mold			
		Incidence		Severity		Incidence		Severity	
Source of variation	df	ms	Prob f	ms	Prob f	ms	Prob f	ms	Prob f
Location	2	1.9749	0.0001	1,498	0.2501	0.4748	0.0927	236	0.3736
Nuclear genotype	3	1.9157	0.0001	8,671	0.0001	6.1488	0.0001	14,899	0.0001
Location–nuclear genotype	6	0.6767	0.0001	2,979	0.0001	0.3855	0.0850	902	0.0023
Cytoplasm	1	0.0052	0.7176	35	0.6480	0.0413	0.0470	95	0.1215
Location–cytoplasm	2	0.0171	0.6469	155	0.4017	0.0042	0.6764	27	0.5143
Nuclear genotype–cytoplasm	3	0.0159	0.7473	286	0.1776	0.0032	0.6097	21	0.6150
Location–nuclear genotype–cytoplasm	6	0.0672	0.1423	65	0.8775	0.0107	0.3810	23	0.6972
Replicate (location)	9	0.0500	0.4003	923	0.0102	0.1690	0.5155	215	0.3973
Replicate–nuclear genotype (location)	27	0.0458	0.3195	294	0.0531	0.1841	0.0001	198	0.0001
Residual	36	0.3886	…	165	…	0.0093	…	31	…

[a] Percentage incidence data were normalized using arcsin square root transformation prior to analysis of variance; ms = mean square.

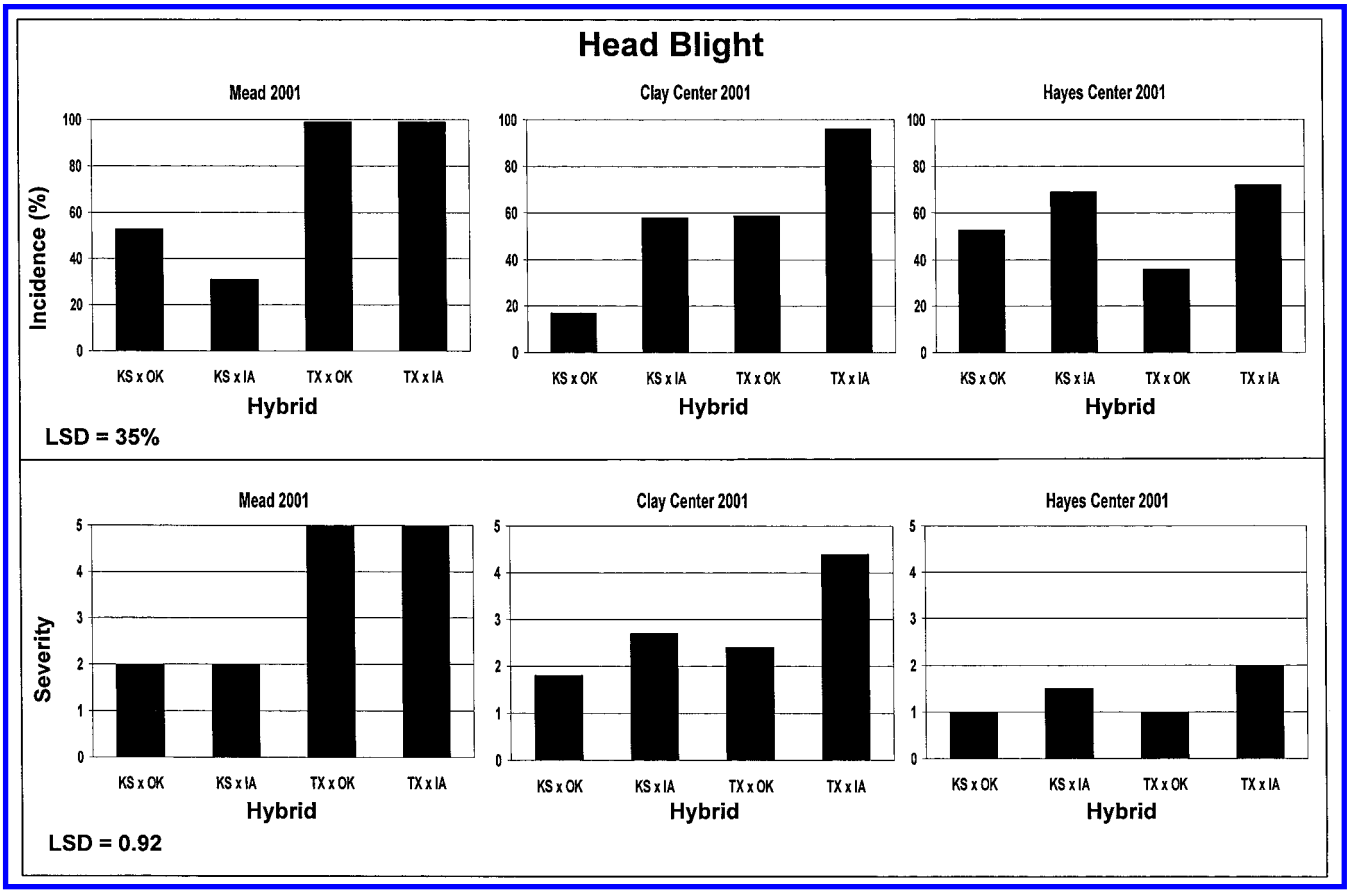

Fig. 2. Incidence and severity of Fusarium head blight on four sorghum nuclear genotypes in the A_1 and A_2 cytoplasmic backgrounds at Mead, Clay Center, and Hayes Center, NE during the 2001 growing season. **A–C,** Least significant difference (LSD) = 35% for head blight incidence; **D–F,** LSD = 0.92 for head blight severity. These are conservative LSD values based on the maximum possible variability for the character.

of cytoplasm on the incidence of grain mold (Table 1); grain mold incidence in the A_2 cytoplasm (DI = 70%) was slightly greater than in the A_1 cytoplasm (DI = 64%). There were no significant interactions among cytoplasm, nuclear genotype, or location. There was also a significant effect ($P = 0.0001$) of nuclear genotype on the incidence of grain mold (Table 1). Grain mold incidence was significantly higher in nuclear genotypes containing OKY10 (DI = 87 to 100%) than in nuclear genotypes containing IA28 (DI = 4 to 64%) across all three locations (Fig. 3). There was no location effect. Despite environmental differences across locations, grain mold developed comparably at all three locations.

Grain mold severity. There was no significant effect ($P = 0.121$) of cytoplasm on the severity of grain mold (Table 1). There was a significant effect ($P = 0.0001$) of nuclear genotype on the severity of grain mold (Table 1). Grain mold severity was significantly higher in nuclear genotypes containing OKY10 (DS = 2.4 to 4.3) than in nuclear genotypes containing IA28 (DS = 0.2 to 1.0) at all three locations (Fig. 3). There was also a significant nuclear genotype–location interaction ($P = 0.0023$); the magnitude of the differences between nuclear genotypes containing OKY10 and nuclear genotypes containing IA28 varied by location but the relative rankings were the same.

DISCUSSION

The hybrids used in this experiment varied dramatically in degree of susceptibility to head blight and grain mold, allowing for assessment of cytoplasmic affects over a range of disease incidence and severity. At three environmentally diverse sites in Nebraska, we demonstrated that, among four sorghum hybrids, no differences existed between A_1 and A_2 cytoplasms for susceptibility to Fusarium head blight. The incidence and severity of Fusarium head blight was determined by nuclear genotype and not by cytoplasm. Head blight symptoms at the site with lowest relative humidity and lowest rainfall were qualitatively different than at the other sites. Possible explanations include altered disease development under less than optimal environmental conditions, the interaction of different *Fusarium* spp. or races in the different environments, or the involvement of other pathogens in the etiology of sorghum head blight in Nebraska. Isolations of pathogenic fungi were made from diseased panicles collected at the three locations in Nebraska (*unpublished data*); however, greenhouse inoculations to complete Koch's postulates were inconclusive.

A_1 and A_2 cytoplasms did differ significantly with respect to the incidence of grain mold. Although statistically significant, the difference in grain mold incidence between the two cytoplasms (64 versus 70%) may not be of practical significance. As was the case with head blight, most of the variation in grain mold incidence was attributable to nuclear genotype. Examination of the mean squares for these main effects reveal 149 times more variation in grain mold incidence attributable to nuclear genotype than to cytoplasm.

With respect to economic impact of grain mold on sorghum production, disease severity is more important than disease incidence (6). In this study, grain mold severity was determined by nuclear genotype and not by cytoplasm. At least five quantitative trait loci have been associated with grain mold severity in sorghum (7). Environment and environment–genotype interactions determined the ability to detect those loci, indicating a correlation between environment and disease phenotype. This may explain, in part, the variation across environments and the nuclear genotype–environment interaction observed in this study. The effect of nuclear genotype on grain mold susceptibility is well established and was confirmed in this study. The hybrids with a tannin-containing testa layer had the lowest incidence

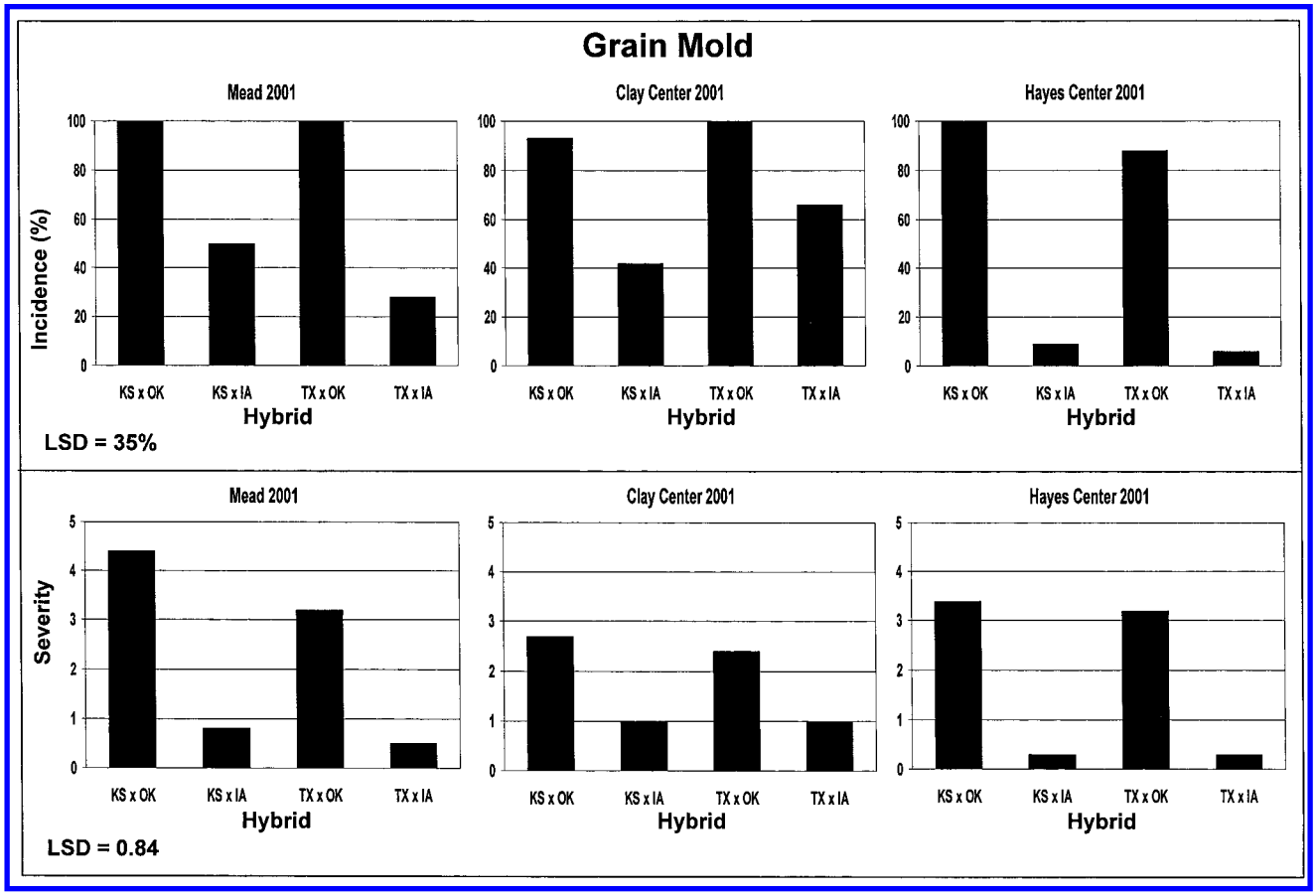

Fig. 3. Incidence and severity of grain mold on four sorghum nuclear genotypes in the A_1 and A_2 cytoplasmic backgrounds at Mead, Clay Center, and Hayes Center, NE during the 2001 growing season. **A–C,** Least significant difference (LSD) = 35% for grain mold incidence; **D–F,** LSD = 0.84 for grain mold severity. These are conservative LSD values based on the maximum possible variability for the character.

and severity of grain mold. Tannins have been associated with resistance to many plant diseases, including sorghum grain mold (10). Whether the tannin content was the primary contributing factor to less grain mold in IA28-containing hybrids or other mechanisms of disease resistance were responsible was not determined.

Compared with A_1 cytoplasm, A_2 cytoplasm did not affect Fusarium head blight incidence and severity or grain mold severity. Cytoplasm had little impact on grain mold incidence compared with the effect of nuclear genotype. Consequently, using A_2 cytoplasm to increase cytoplasmic genetic diversity of grain sorghum hybrids should not increase risk to Fusarium head blight. Selection of nuclear genotypes with grain mold resistance should overcome any slight increase in grain mold incidence attributable to A_2 cytoplasm. Although head blight is an economically important disease of sorghum, there is limited information concerning resistance to this disease. In this study, we demonstrated that susceptibility to head blight varies significantly among hybrids. Considering the importance of Fusarium head blight as a constraint to grain sorghum production worldwide, greater effort should be put into germ plasm evaluation to identify genes for Fusarium head blight resistance.

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