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Role of Chitinase and Sormatin Accumulation in the Resistance of Sorghum Cultivars to Grain Mold

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Experiments were conducted to determine the association between resistance to grain mold and the accumulations of chitinase and sormatin. Eight sorghum lines were treated at 50% bloom with *Fusarium thapsinum*, *Curvularia lunata*, a mixture of the two fungi, and a water-sprayed control. At maturity, percent disease severity, seed germination rates, and kernel weight were recorded. Chitinase and sormatin content (mg/g of dry weight) were measured in seed samples taken at 30 and 50 days after treatment (DAT). Seed chitinase content was moderately affected by sorghum line ($P = 0.10$) and significantly affected by the developmental stage of the kernels ($P = 0.05$). Cultivars Sureno, 98LB650, and 98LB723 exhibited larger negative changes in chitinase content at 50 DAT over water-sprayed control treatment at 30 DAT than the susceptible cultivars Dorado, RTx2536, and RTx430. In 2000, significant negative correlations were observed for percent disease severity and chitinase content at 30 DAT, seed germination and sormatin content at 50 DAT, and between seed germination and kernel weight. There also was a significant positive correlation between germination and chitinase content at 30 DAT. No association between disease severity and changes in chitinase content at 50 DAT was observed. Sormatin content also was significantly affected by the stage of kernel development. Sorghum cultivars inoculated with fungal pathogens responded differently as indicated by the significant sorghum line × treatment interaction for sormatin content in 2000. In both years, larger increases in sormatin content over the water-sprayed control treatments were observed on moderately susceptible to susceptible cultivars such as 98LB650, 98LB723, 98LB789, RTx430, and RTx2536 than on Sureno. Except for percent disease severity and germination, there was no significant association among all of the other parameters measured in 2001. The results of this study did not clearly demonstrate a strong association between resistance to grain mold and the accumulation of sormatin and chitinase. Thus, there is the possibility that certain moderately resistant to resistant sorghum cultivars, such as Sureno, may employ other strategies to eschew or restrict fungal invasion either before or after physiological maturity.

KEYWORDS: ***Fusarium thapsinum*; *Curvularia lunata*; antifungal proteins**

INTRODUCTION

One of the major constraints to sorghum productivity and profitability is grain mold, a disease caused by many fungal species, of which *Fusarium thapsinum* and *Curvularia lunata* are considered to be the most common. The disease is most severe in areas where moist conditions occur late in the growing season (*1*, *2*). Although management strategies such as avoidance and chemical seed treatment have been shown to reduce the impact of the disease, the use of resistant cultivars is the most practical method for controlling grain mold. However, grain mold resistance involves several mechanisms, and it is quantitatively inherited (*3*−*5*). These mechanisms include hardness of the kernel, kernels with red pericarp, endosperm texture, high tannins, high concentrations of flavan-4-ol, and plants with the pericarp intensifier (*I*) gene (*5*−*7*). Recent studies have shown that antifungal proteins (AFPs) such as sormatin, chitinases, glucanases, and ribosome-inhibiting protein may play a role in grain mold resistance (*8*−*11*). This resistance is confounded because the disease response by a sorghum line may depend on the fungal species present and the environment (*12*).

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As resistance mechanisms, antifungal proteins are relatively new sources that play a role in plant resistance to disease. During in vitro assays, the use of a combination of these AFPs extracted from sorghum seeds was found to inhibit spore germination but was less effective in inhibiting hyphal elongation (*13*). Chitinase and sormatin contents in sorghum kernels increased from the period after anthesis to physiological maturity and thereafter decreased when 17 sorghum cultivars and hybrids naturally infected with grain mold were evaluated (*8*). Rodriguez-Herrera et al. (*10*) detected higher contents of sormatin, β-1,3-glucanase, and chitinase in grain mold resistant cultivars than in susceptible cultivars in naturally infected fields. In contrast, Bueso et al. (*11*) noted higher contents of sormatin and chitinase in seed caryopsis across susceptible cultivars than across resistant and moderately resistant cultivars that were naturally infected with grain mold. Bueso et al. (*11*) also noted increased sormatin and chitinase contents in seed caryopsis at 30 days after anthesis (DAA) in two out of five resistant cultivars inoculated with a mixture of *F. moniliforme* and *C. lunata*. However, the content of these two AFPs decreased in most susceptible cultivars at 30 and 50 DAA, respectively. Thus, previous studies show no consistent AFP accumulation in floral tissue among the resistant and susceptible sorghum cultivars, even when infected with a mixture of two fungal pathogens.

Whereas Bueso et al. (*11*) used a mixture of *F. moniliforme* and *C. lunata* as inoculum, all of the other studies evaluating the relationship between grain mold resistance and the accumulation of antifungal proteins relied on naturally infected grain. The use of visual rating as the only method for assessing grain mold resistance makes the data in these studies less reliable. This is due to factors such as kernel color and human error, and in some cases, this method tends to overestimate the importance of mold growth late in the growing season (*14*). More reliable parameters used to assess the extent of damage or resistance of a sorghum line to grain mold by researchers include kernel weight and seed germination (*3*, *4*). Thus, the objective of this study was to determine the association between the accumulations of chitinase and sormatin in sorghum lines inoculated with *F. thapsinum*, *C. lunata*, or a mixture of the two fungi and grain mold resistance measured as percent disease severity, germination rate, and kernel weight.

MATERIALS AND METHODS

Field Trial. A split plot design with lines as whole plot and treatments as subplot was used in both years. Treatments were (1) *F. thapsinum*, (2) *C. lunata*, (3) a mixture of the two fungi, and (4) a water-sprayed control. Experiments were established at the Texas A&M Agricultural Research Farm near College Station, TX, in 2000 and 2001. Eight cultivars, Sureno, Dorado, 98LB650, 98LB711, 98LB723, 98LB789, RTx430, and RTx2536, were used. Sureno is considered to have moderate resistance, whereas RTx430 and RTx2536 are rated as susceptible. Dorado and the remaining lines, which are derived from the cross of Sureno/RTx430 (*10*), have moderate to susceptible reactions to grain mold. Seeds were planted in 6 m rows with 31 cm row spacing. Standard cultural and fertilization practices for grain sorghum production were used. Each treatment consisted of three panicles per sorghum line in 2000 and four panicles per sorghum line in 2001. Each treated panicle was considered to be a replicate.

Inoculation Protocol. Single-spore isolates of *F. thapsinum* and *C. lunata* were cultured separately in Petri plates containing one-fifth strength potato dextrose agar (PDA) medium. Plates were incubated at 25 °C for 10−14 days under a 12 h photoperiod provided by F40CW/RS/EW-11 fluorescent lights plus a blacklight that emits light in the visible and UV wavelengths. Fungal spores were harvested by flooding the plates with 10 mL of sterilized water and then scraping the agar surface with a rubber spatula to dislodge the spores. The conidial suspensions were filtered through four layers of sterile cheesecloth into two separate beakers and diluted with sterile water to final concentrations of 1×10^6 and 2×10^4 conidia/mL for *F. thapsinum* and *C. lunata*, respectively. For the mixture, equal volumes (1:1 v/v) of *F. thapsinum* and *C. lunata* suspensions were combined and thoroughly agitated in a flask before inoculation.

Sorghum panicles at 50% bloom were randomly selected and tagged. Due to differences in the rate of development, cultivars at 50% flowering were inoculated on different dates in June and July for both 2000 and 2001 experiments. Panicles were inoculated using a hand-held spray bottle with one of the four treatments previously described. Panicles were sprayed until runoff. Both inoculated and water-sprayed control panicles were covered with paper bags for 24 h to facilitate infection by the two pathogens. To enhance disease development, treated and water-sprayed control panicles were misted with sterile distilled water twice a day for seven consecutive days.

Parameters Measured. At maturity, the treated and water-sprayed control panicles were hand harvested and threshed using a single-head thresher (Almaco Plant and Head Thresher, Allan Machine Co., Ames, IA). Visual disease assessment was employed, and seed germination rates were determined according to the procedure described by Prom et al. (*12*). Seed weight was defined and measured as the weight of 1000 kernels from each panicle.

Antifungal Protein Analysis. Seed samples used for the determination of AFP content were collected from both treated and untreated sorghum panicles at 30 and 50 days after treatment (DAT). The same panicle was sampled at 30 and 50 DAT. Samples were taken from the middle of the panicle. These samples were ground into a fine powder with a coffee grinder and stored in a freezer until ready for analysis. Sormatin and chitinase were extracted using the protocol modified by Seetharaman et al. (*13*). Briefly, a 0.1 g sample of ground seed was mixed with 1.0 mL of Colorado extraction buffer (25 mM sodium phosphate, 50 mM NaCl, and 5 mM EDT, pH 7) and shaken for 1 h. Tubes containing the mixtures were then centrifuged at 10000 rpm for 5 min. The supernatants were collected in Eppendorf tubes and then stored in a freezer at −20 °C until ready to assay. AFP content was determined according to the method described by Bueso et al. (*11*), except that the Dot blot technique was employed instead of SDS-PAGE or Western blot. The use of chitinase and thaumatin as standards followed the procedure described by Seetharaman et al. (*8*) and Bueso et al. (*11*).

Statistical Analysis. Data analysis was performed using the analysis of variance (ANOVA) procedure of the Statistical Analysis System (SAS) software version 8.1 (SAS Institute, Cary, NC). Data were log and square transformed for the kernel weight and the AFP content, respectively, before the analysis of variance was performed. Due to heterogeneity of error, data for the two years were analyzed separately and not combined. Percent reduction in kernel weight and changes in chitinase and sormatin contents at 50 DAT compared to the water-sprayed control at 30 DAT were determined using Abbott's formula (http://embark.tripod.com/idpline/habbott.htm). Correlation coefficients among percent disease severity, germination, kernel weight, chitinase, sormatin, and changes in chitinase and sormatin contents at 50 DAT compared to the water-sprayed control treatment at 30 DAT were calculated across the eight sorghum lines.

RESULTS

Kernel Weight. The main effects of sorghum line and sorghum line × treatment (Trt) interaction for kernel weight were highly significant ($P < 0.01$), but treatment effects were not significant in either year (**Table 1**). The nonsignificant treatment effect indicates that inoculation with the fungal pathogens did not affect the kernel weight. Highly significant sorghum line × Trt interaction indicates that the sorghum cultivars responded differently when treated with *F. thapsinum*, *C. lunata*, or a mixture of the two fungi. When challenged with *C. lunata*, 98LB789 and RTx2536 exhibited 10 and 5% reductions in kernel weight over the water-sprayed control, respectively, in 2000 (**Figure 1**). When treated with *F. thapsi-*

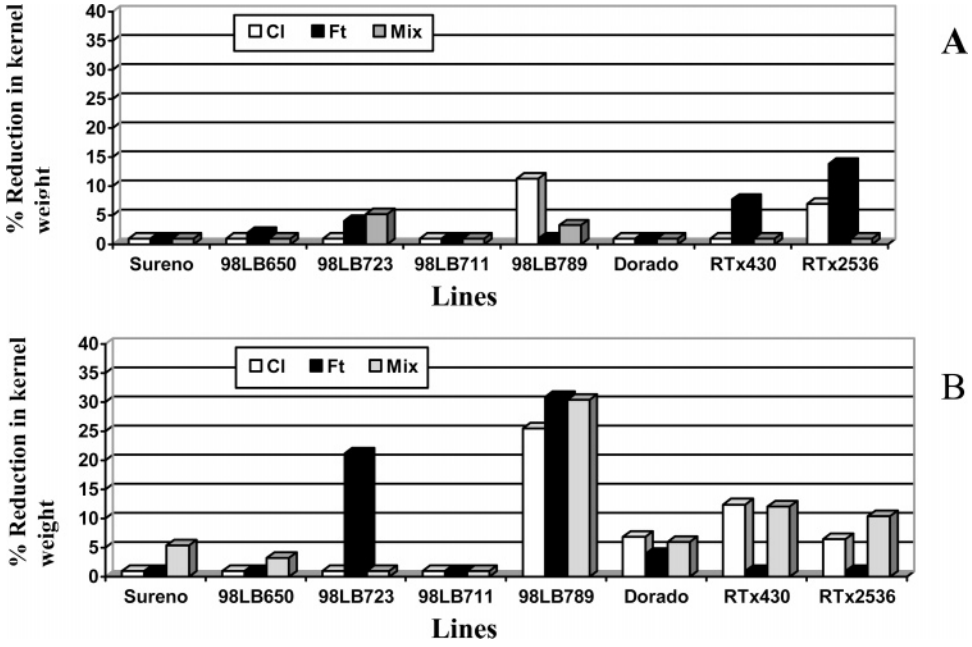

Figure 1. Percent reduction in kernel weight (in grams of 1000 kernels per replicate, $N = 3$ in 2000 and $N = 4$ in 2001) due to inoculation with *C. lunata* (CI), *F. thapsinum* (Ft), and a mixture of the two fungi over the water-sprayed controls of eight sorghum lines grown in College Station, TX, in (**A**) 2000 and (**B**) 2001. Minimum significant difference in 2000 was 1.0 and in 2001, 1.1.

Table 1. Analysis of Variance[a] for Kernel Weight of Eight Sorghum Lines Inoculated at 50% Anthesis with *F. thapsinum, C. lunata*, a Mixture of the Two Fungi, or a Water-Sprayed Control in College Station, TX, during the 2000 and 2001 Growing Seasons

	2000		2001	
source	df	MS[b]	df	MS[b]
replication	2	0.001^{NS}	3	0.0007^{NS}
line	7	0.152***	7	0.2082***
error (A)	14	0.001	21	0.0003
treatment	3	0.005^{NS}	3	0.0482^{NS}
line × treatment	21	0.011***	21	0.0317***
error (B)	48	0.001	72	0.0003
total	95			

[a] Log transformed data. [b] NS, nonsignificant; ***, significant at the 1% probability level.

num, RTx2536 and RTx430 had 14 and 7% reductions, respectively, in their kernel weights. In 2001, 98LB789 had a reduction in kernel weight when treated with the fungal species. Dorado, RTx430, and RTx2536 exhibited 5% or more reductions in kernel weight when treated with the individual fungal species. Sureno, the resistant to moderately resistant line, showed reduction in kernel weight only in 2001 and when inoculated with a mixture of the two fungi.

Antifungal Proteins. The caryopsis stage for sormatin accumulation was significant in 2000 ($P < 0.05$) and in 2001 ($P < 0.01$) (**Table 2**). Sorghum lines responded differently to fungal treatments as indicated by the significant ($P < 0.10$) sorghum line × Trt interaction in 2000. Chitinase content across the sorghum cultivars was moderately affected by sorghum line ($P < 0.10$) in 2000 and significantly affected ($P < 0.01$) in 2001. **Table 3** shows the actual means across treatments for chitinase and sormatin contents of the eight sorghum lines. There were increases in sormatin levels at 50 DAT over the water-sprayed control at 30 DAT for the sorghum line 98LB723 in both years (**Figure 2**). *F. thapsinum* treatment on 98LB723 exhibited the largest increase in sormatin content in 2001.

Table 2. Analysis of Variance[a] for Chitinase and Sormatin Levels across Eight Sorghum Lines Inoculated at 50% Anthesis with *F. thapsinum, C. lunata*, a Mixture of the Two Fungi, or a Water-Sprayed Control in College Station, TX, during the 2000 and 2001 Growing Seasons

		2000		2001	
source	Df	chitinase, MS[b]	sormatin, MS[b]	chitinase, MS[b]	sormatin, MS[b]
replication	2	0.01^{NS}	0.006^{NS}	0.006^{NS}	0.032^{NS}
line	7	0.017*	0.026^{NS}	0.014^{NS}	0.071^{NS}
error (A)	14	0.007	0.015	0.027	0.039
treatment (Trt)	3	0.019^{NS}	0.007^{NS}	0.010^{NS}	0.041^{NS}
line × Trt	21	0.018^{NS}	0.023*	0.011^{NS}	0.051^{NS}
error (B)	48	0.019	0.014	0.017	0.054
DS[c]	1	0.003^{NS}	0.054**	0.380***	0.258***
line × DS	7	0.015^{NS}	0.012^{NS}	0.009^{NS}	0.046^{NS}
treatment × DS	3	0.011^{NS}	0.006^{NS}	0.032^{NS}	0.091^{NS}
line × Trt × DS	21	0.014^{NS}	0.013^{NS}	0.014^{NS}	0.056^{NS}
error (C)[d]		0.014	0.011	0.019	0.049

[a] ANOVA of squared transformed data. [b] NS, nonsignificant; *, **, ***, significant at the 10, 5, and 1% probability levels, respectively. [c] Stage of caryopsis, i.e., samples collected at the two stages of development, 30 and 50 days after inoculation. [d] Df error (C) was 64 in 2000 and 96 in 2001.

Sureno, the resistant check, showed a decrease in sormatin content when treated with *F. thapsinum* and *C. lunata*, whereas with RTx2536, inoculation with the two pathogens caused an increase in sormatin content at 50 DAT over the water-sprayed control treatment in 2001. Dorado, a moderately susceptible sorghum line, showed a decrease in sormatin content across all treatments at 50 DAT in both years. In 2001, increases in the sormatin content of inoculated panicles over the water-sprayed control for sorghum line 98LB789 were observed at both 30 and 50 DAT.

In 2000, increases in chitinase content over the water-sprayed control treatment at 30 DAT were observed in 98LB789 treated with a mixture of *F. thapsinum* and *C. lunata* and on RTX 2536 sprayed with water at 50 DAT (**Figure 3**). In 2001, increases in chitinase content were fournd for Dorado and RTx430 treated

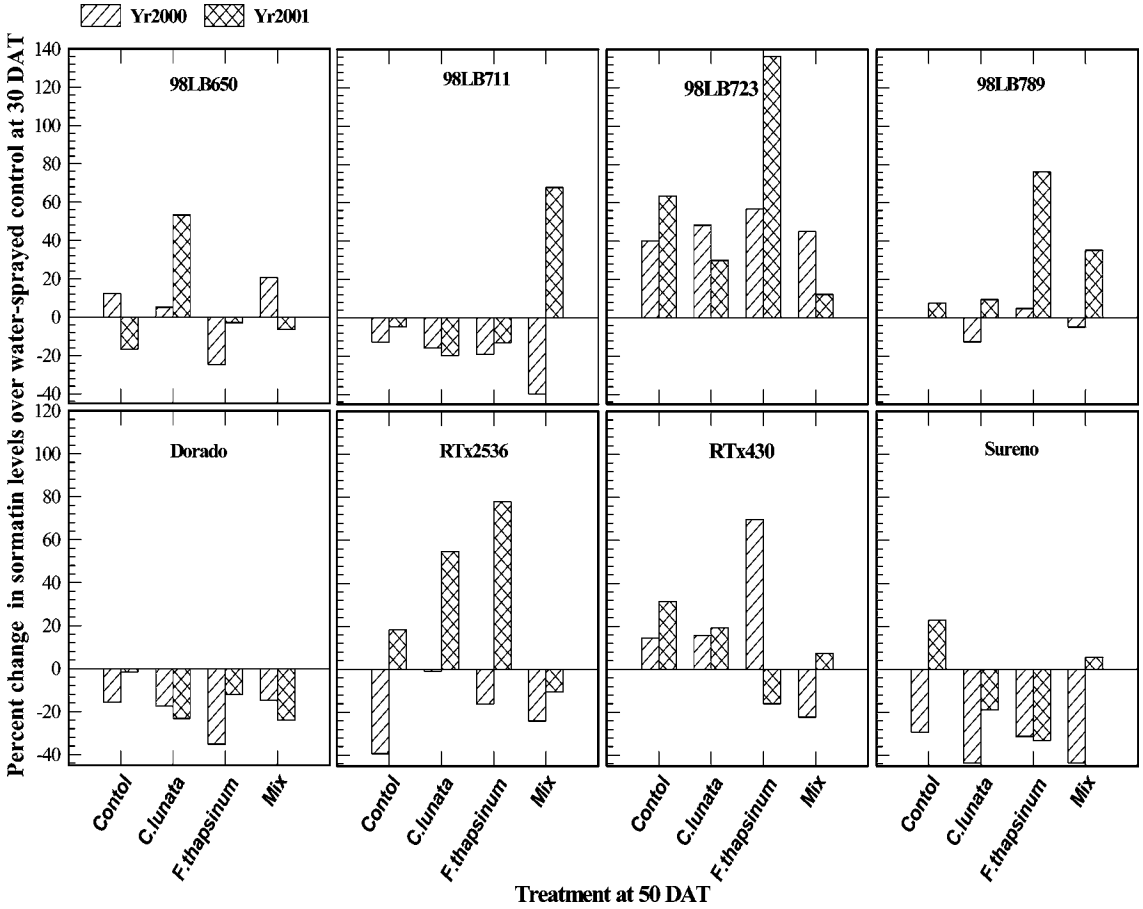

Figure 2. Percent change in sormatin levels between the water-sprayed control of eight sorghum lines grown in College Station, TX, during the 2000 and 2001 seasons 30 DAT and samples collected at 50 DAT.

Table 3. Chitinase and Sormatin Content at Two Stages of Grain Development of Eight Sorghum Lines Inoculated at 50% Anthesis with *F. thapsinum*, *C. lunata*, a Mixture of the Two Fungi (Mix), or a Water-Sprayed Control in College Station, TX, during the 2000 and 2001 Growing Seasons

	chitinase and sormatin content of seed (μg/g)							
	2000				2001			
sorghum line	Chi-30[a,b]	Chi-50	Sor-30	Sor-50	Chi-30	Chi-50	Sor-30	Sor-50
Sureno	0.67	0.48	0.80	0.71	0.73	0.62	1.49	1.20
98LB650	0.78	0.49	1.08	1.25	0.91	0.58	1.51	1.46
98LB723	0.61	0.70	0.87	0.89	0.66	0.62	1.09	1.72
98LB711	0.76	0.72	1.05	0.99	0.76	0.63	1.24	1.65
98LB789	0.71	0.74	0.99	1.01	0.80	0.48	0.93	1.39
Dorado	0.65	0.72	1.10	0.86	0.73	0.65	1.60	1.06
RTx430	0.60	0.62	0.95	0.91	0.74	0.72	1.59	1.79
RTx2536	0.51	0.58	1.03	0.90	0.80	0.55	1.08	1.41

[a] Chi, chitinase; Sor, sormatin content of seed. [b] Chi-30, Chi-50, Sor-30, and Sor-50 are mean chitinase and sormatin contents across treatments measured in seed samples collected at 30 and 50 days after fungal and water-sprayed treatments, respectively.

Table 4. Correlation Coefficients for Percent Disease Severity (SEVR), Germination (GERM), Kernel Weight (KW), Sormatin, Chitinase, and Change in both Chitinase and Sormatin across Eight Sorghum Lines Grown at College Station, TX, during the 2000 and 2001 Growing Seasons[a]

	SEVR	GERM	KW	Sor-30[b]	Sor-50[c]	Chi-30[d]	Chi-50[e]	ΔChi[f]	ΔSor[g]
2000 Growing Season									
SEVR	1.00	–0.59***	–0.13	0.35**	0.26	–0.31*	0.01	0.08	0.09
GERM		1.00	–0.34	–0.27	–0.34	0.38**	0.21	–0.16	–0.18
KW			1.00	0.09	0.16	–0.01	0.05	0.23	0.12
2001 Growing Season									
SEVR	1.00	–0.75***	0.15	0.09	0.19	–0.29	–0.10	–0.11	–0.01
GERM		1.00	–0.17	0.13	–0.10	0.20	0.16	–0.01	–0.05
KW			1.00	0.12	0.01	0.27	0.07	0.11	0.02

[a] *, **, and ***, significant at 10, 5, and 1% probabilities, respectively. [b] Sor-30, sormatin (μg/g) in seed collected at 30 days after treatments (DAT). [c] Sor-50, sormatin (μg/g) in seed collected at 50 DAT. [d] Chi-30, chitinase (μg/g) in seed collected at 30 DAT. [e] Chi-50, chitinase (μg/g) in seed collected at 50 DAT. [f] ΔChi, percent change in chitinase over control at 50 DAT. [g] ΔSor, percent change in sormatin over control at 50 DAT.

with *C. lunata*. Except for sorghum lines 98LB711, 98LB789, and RTx2536 in 2000 and Dorado and RTx430 in 2001, all other sorghum lines tested in this study exhibited negative or small increases in chitinase content at 50 DAT. Sureno and 98LB650 showed decreases in chitinase content across all treatments at 50 DAT in both years.

Correlation Coefficients. In both years, highly significant negative correlations between percent disease severity and seed germination rates were observed (**Table 4**). In 2000, moderately significant negative correlations were observed for percent disease severity and chitinase content at 30 DAT, seed germination, and sormatin content at 50 DAT and between seed germination and kernel weight. There also was a significant positive correlation between germination and chitinase content at 30 DAT. Except for the significant negative association between percent disease severity and seed germination, there was no significant relationship between the other parameters measured in 2001.

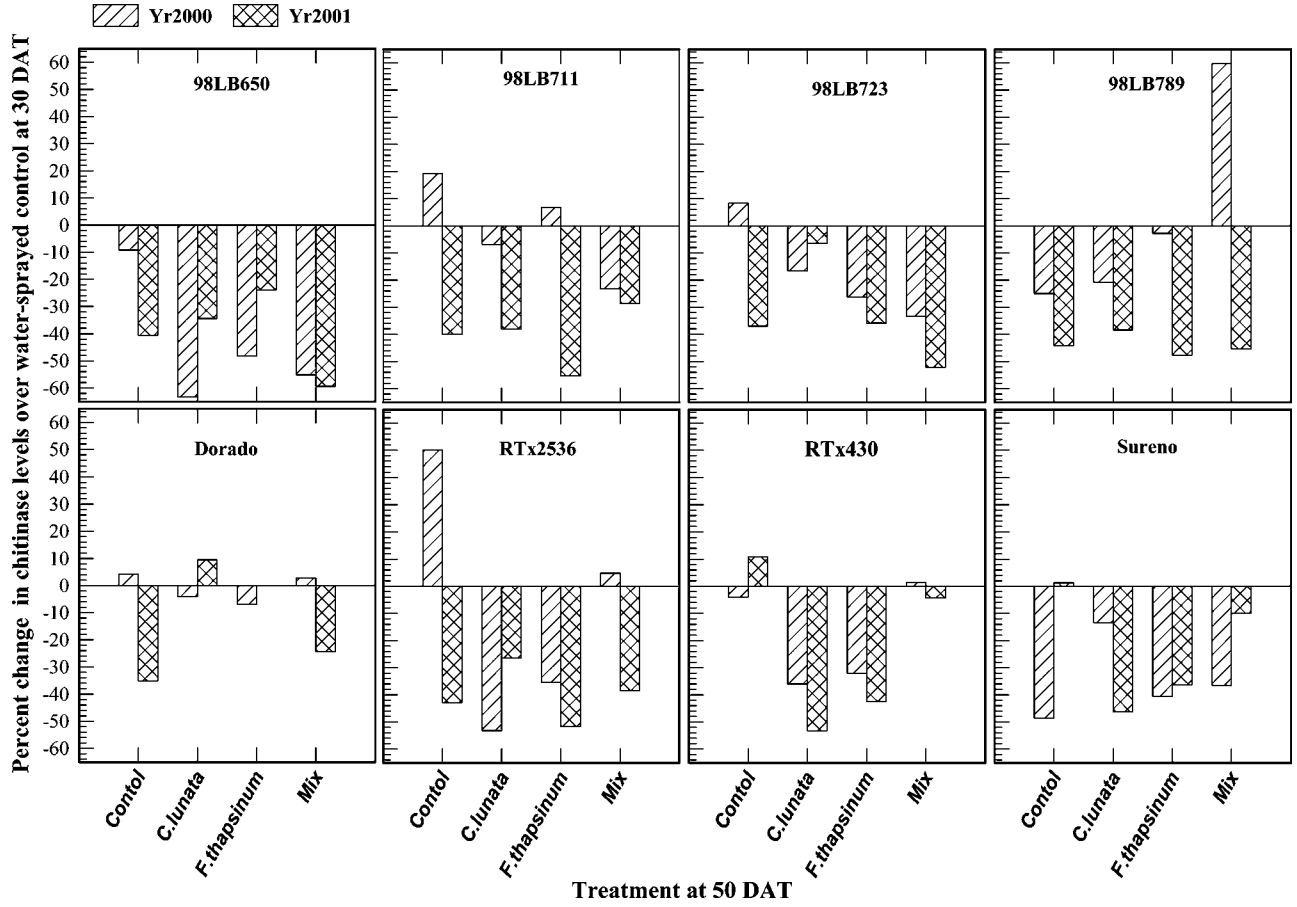

Figure 3. Percent change in chitinase levels between the water-sprayed control of eight sorghum lines grown in College Station, TX, during the 2000 and 2001 seasons 30 DAT and samples collected at 50 DAT.

DISCUSSION

Prom et al. (*12*) have reported the disease response and seed germination rates of the eight lines inoculated with *F. thapsinum*, *C. lunata*, and a mixture of the two fungi. Environmental conditions were more conducive to grain mold severity in 2001 than in the same period in 2000 (*12*). In the present study, a highly significant sorghum line × Trt interaction for kernel weight was observed, indicating that the cultivars responded differently when inoculated with these fungi. Some of the sorghum lines exhibited significant reductions in kernel weight when treated with the fungal pathogens (**Figure 1**). In this study, recovery of *F. thapsinum* from seeds obtained from panicles inoculated with the same species was 33−78% in 2000 and 31−77% in 2001 (*15*), whereas the frequency of isolation of *C. lunata* from panicles inoculated at anthesis with *C. lunata* ranged from 68 to 95% in 2000 and from 47 to 89% in 2001. Mycoflora analysis of control panicles sprayed with water showed *Alternaria* spp., *Fusarium semitectum*, and *C. lunata* as the most frequently isolated fungal species.

Although sorghum line × Trt interaction was moderately significant in only one instance, that is, the sormatin content for the year 2000, results obtained in this study show a wide variation in chitinase and sormatin content in the different sorghum cultivars when treated with the fungal species. For instance, the use of *F. thapsinum* induced appreciable increases in sormatin content on sorghum line 98LB723, a moderately susceptible line, whereas *C. lunata* caused high positive changes in the content of sormatin at 50 DAT in line 98LB650. In both years, more positive changes in sormatin content were observed on moderately susceptible to susceptible sorghum lines such as 98LB650, 98LB723, 98LB789, RTx430, and RTx2536 than on Sureno, a moderately resistant sorghum line. Bueso et al. (*11*) also noted little or no increase in sormatin content in the developing kernel when Sureno was inoculated with a mixture of *F. moniliforme* and *C. lunata*. However, Bueso et al. (*11*) may have included several *Fusarium* spp. such as *F. thapsinum*, which was recently named as a new species (*16*). One possible explanation is that Sureno may have employed mechanisms other than the accumulation of AFPs to resist grain mold infection. Other studies have linked grain mold resistance to the accumulation of sormatin (*9*−*11*, *17*). Our present study did not show a strong association between disease responses and sormatin content, indicating that sormatin may either play a secondary role or act in synergism with other AFPs in the grain mold resistance mechanism. In the present study, the levels of infection during 2000 and 2001 were considered to be moderate.

In contrast to the susceptible cultivars Dorado, RTx2536, and RTx430, Sureno and the moderately susceptible lines 98LB650 and 98LB723 exhibited larger decreases in chitinase content when compared to the water-sprayed control treatment at 30 DAT. This is consistent with the fact that in 2002, Bejosano et al. (*17*) noted in a mold-conducive environment greater mean loss in chitinase levels from 30 to 50 days after anthesis in susceptible sorghum cultivars that were naturally infected with mold than in mold-resistant cultivars. Also, in the present study, there was a significant negative correlation between disease severity and chitinase content at 30 DAT in 2000 but not in 2001 and no relationship between disease severity and changes in chitinase content at 50 DAT. On the other hand, Bueso et al. (*11*) and Bejosano et al. (*17*) reported significant negative

correlations between mold ratings and changes in sormatin and chitinase content at 50 DAT. There is uncertainty as to whether higher accumulations of AFPs in the caryopsis are most effective earlier in the infection process or later when the resistance to invasion of the kernel is at its lowest, that is, at maturity. Castor (*3*) and Forbes (*18*) have noted that fungal colonization of the floral tissues occurs a few days after inoculation, well before physiological maturity, although visual symptoms are more conspicuous after physiological maturity. If this is the case, then grain mold resistant cultivars will have to maintain higher levels of sormatin and chitinase during the early stages of kernel development. In addition, the use of a visual rating system as the only method for assessing disease resistance seems to be unreliable due to factors such as kernel color, human error, and, in some cases, a tendency to overestimate the importance of mold growth late in the growing season (*14*).

Furthermore, in this and previous studies, a reductionist approach to determining the role of antifungal proteins in the grain mold resistance mechanism was used (*9*−*11*, *17*). These studies have shown that the individual AFPs, such as sormatin or chitinase, may play a minor role in grain mold resistance. However, one can assume that sormatin and chitinase in combination with other AFPs that are induced or constitutively mobilized during invasion may play a more significant role in the resistance mechanism. In addition, Seetharaman et al. (*8*) observed high levels of AFPs during sorghum seed germination and postulated that these AFPs may play a significant role in protecting the germinating seed from fungal invasion. The results of our study did not clearly demonstrate a strong association between resistance to grain mold and the accumulation of sormatin or chitinase. This may be attributed to several factors such as the environment, sorghum line, fungal species used, and the fact that sormatin and chitinase may not act individually but synergistically to impart resistance to grain mold. Also, there is the possibility that certain sorghum cultivars, such as Sureno, may employ other strategies to eschew or restrict fungal invasion either before or after physiological maturity.

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LITERATURE CITED

(1) Bandyopadhyay, R.; Chandrashekar, A. Biology and management of sorghum grain mold. In *Proceedings of Consultative Group Meeting on Technical and Institutional Options for Sorghum Grain Mold Management*, May 18−19; Chandrashekar, A., Bandyopadhyay, R., Hall, A. J., Eds.; International Crops Research Institute for the Semi-Arid Tropics: Patancheru, India, 2000; Abstract.

(2) Garud, T. B.; Sawant, L. V.; Ismail, S. Development of grain mold in sorghum-pigeonpea intercropping systems. *Int. Sorghum Millets Newsl*. **2000**, *41*, 55.

(3) Castor, L. L. Grain mold histopathology, damage assessment, and resistance screening within *Sorghum bicolor* (L.) Moench lines. Ph.D. Dissertation Texas A&M University, College Station, TX, 1981; 177 pp.

(4) Forbes, G. A.; Bandyopadhyay, R.; Garcia, G. A review of sorghum grain mold. In *Sorghum and Millets Diseases: A Second World Review*; de Milliano, W. A. J., Frederiksen, R. A., Bengston, G. D., Eds.; International Crops Research Institute for the Semi-Arid Tropics: Patancheru, India, 1992; pp 253−264.

(5) Waniska, R. D.; Venkatesha, R. T.; Chandrashekar, A.; Krishnaveni, S.; Bejosano, F. P.; Jeoung, J.; Jayaraj, J.; Muthukrishnan, S.; Liang, G. H. Antifungal proteins and other mechanisms in the control of sorghum stalk rot and grain mold. *J. Agric. Food Chem.* **2001**, *49*, 4732−4742.

(6) Mukuru, S. Z. Breeding for grain mold resistance. In *Sorghum and Millets Diseases: A Second World Review*; de Milliano, W. A. J., Frederiksen, R. A., Bengston, G. D., Eds.; International Crops Research Institute for the Semi-Arid Tropics: Patancheru, India, 1992; pp 273−285.

(7) Esele, J. P.; Frederiksen, R. A.; Miller, F. R. Importance of plant colour and modifier genes in grain mould resistance in sorghum. *J. East African Agric. For.* **1995**, *61*, 31−37.

(8) Seetharaman, K.; Waniska, R. D.; Rooney, L. W. Physiological changes in sorghum antifungal proteins. *J. Agric. Food Chem.* **1996**, *44*, 2435−2441.

(9) Rodriguez-Herrera, R. Grain mold resistance in sorghum (*Sorghum bicolor* (L) Moench): Genetic, physical and biochemical analysis. Ph.D. Dissertation, Texas A&M, University, College Station, TX, 1999; 143 pp.

(10) Rodriguez-Herrera, R.; Waniska, R. D.; Rooney, L. W. Antifungal proteins and grain mold resistance in sorghum with nonpigmented testa. *J. Agric. Food Chem.* **1999**, *47*, 4802−4806.

(11) Bueso, F. J.; Waniska, R. D.; Rooney, W. L.; Bejosano, F. Activity of antifungal proteins against mold in sorghum caryopses in the field. *J. Agric. Food Chem.* **2000**, *48*, 810−816.

(12) Prom, L. K.; Waniska, R. D.; Kollo, A. I.; Rooney, W. L. Response of eight sorghum cultivars inoculated with *Fusarium thapsinum*, *Curvularia lunata*, and a mixture of the two fungi. *Crop Prot.* **2003**, *22*, 623−628.

(13) Seetharaman, K.; Whitehead, E.; Keller, N. P.; Waniska, R. D.; Rooney, L. W. *In vitro* activity of sorghum seed antifungal proteins against grain mold pathogens. *J. Agric. Food Chem.* **1997**, *45*, 3666−3671.

(14) Forbes, G. A.; Frederiksen, R. A.; Seitz, L. M. Assessment of sorghum grain mould: disease severity and crop loss. *Seed Sci. Technol.* **1989**, *17*, 297−307.

(15) Prom, L. K. The effects of *Fusarium thapsinum*, *Curvularia lunata*, and their combination on sorghum germination and seed mycoflora. *J. New Seeds* **2004**, *6*, 39−49.

(16) Klittich, C. J.; Leslie, J. F.; Nelson, P. E.; Marasas, W. F. O. *Fusarium thapsinum* (*Gibberella thapsina*): A new species in section Liseola from sorghum. *Mycologia* **1997**, *89*, 643−652.

(17) Bejosano, F. P.; Waniska, R. D.; Rooney, W. L. Antifungal proteins in commercial hybrids and elite sorghums. *J. Agric. Food Chem.* **2003**, *51*, 5911−5915.

(18) Forbes, G. A. Characterization of grain mold resistance in sorghum (*Sorghum bicolor* (L.) Moench). Ph.D. Dissertation Texas A&M University, College Station, TX, 1986; 75 pp.

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