Disease Severity and Susceptibility of Sorghum [*Sorghum bicolor* (L.) Moench.] to Infection by *Claviceps africana* Frederickson, Mantle and de Milliano in Mexico and the United States of America

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Abstract. Experiments were carried out at College Station, USA, and Rio Bravo and Celaya, Mexico, under irrigated conditions during 2002 and 2003. Six sorghum hybrids and three male-sterile lines were planted at each location. *Claviceps africana* isolates were applied every other morning. Panicles were inoculated using a hand atomizer until runoff with a suspension of 1.6 x 10^6 conidia mL^{-1}. Ergot severity was measured at milk stage. Bartlett's test was performed to determine homogeneity of variances among years. The results showed variability in susceptibility to ergot among genotypes at a single planting date, at planting dates within a year, locations and years. Ergot severity was statistically greater in 2002 in both hybrids and A-lines. Celaya had the greatest amount of ergot on hybrids, followed by College Station and Rio Bravo. In general, A-lines had the greatest severity. ATx2752 had the lowest ergot severity (22.8%), while ATx635 and ATx623, had 27.4% and 36.2%, respectively. Sorghum hybrid AP2233 was the most susceptible, and NC+8R18 showed the least amount of ergot severity.

Additional keywords: Ergot, hybrids, male-sterile lines.

Resumen. Los experimentos se llevaron a cabo en College Station, EUA, Río Bravo y Celaya, México, bajo condiciones de riego durante 2002 y 2003. Seis híbridos de sorgo y tres líneas andro-estériles se sembraron en cada localidad. los aislados de *Claviceps africana* se aplicaron cada dos días. Las panojas se inocularon usando un atomizador manual hasta escurrimiento total con una suspensión de 1.6 x 10^6 conidios mL^{-1}. La severidad del cornezuelo fue evaluada en el estado lechoso. La prueba de Bartlett se utilizó para definir homogeneidad de varianzas entre años. Los resultados indicaron variabilidad en la susceptibilidad a la enfermedad entre genotipos en una misma fecha de siembra, entre fechas de siembra dentro de un mismo año, entre localidades y años. La severidad del cornezuelo fue estadísticamente mayor en 2002, tanto en híbridos como líneas andro-estériles. Celaya mostró el mayor valor de severidad en híbridos, seguida de College Station y Río Bravo. En general, las líneas andro-estériles mostraron la mayor severidad de la enfermedad. ATx2752 presentó la menor severidad (22.8%), mientras que ATx635 y ATx623, mostraron 27.4 y 36.2%, respectivamente. El híbrido AP2233 fue el más susceptible y NC+8R18 mostró la menor severidad a la enfermedad.

Palabras clave adicionales: Cornezuelo, híbridos, líneas androestériles.

Ergot has been observed in the majority of sorghum [*Sorghum bicolor* (L.) Moench.] production areas in the world. In Asia, sorghum ergot caused by *Claviceps sorghi* B.G.P. Kulk. (Kulkarni *et al*., 1976) was observed for the first time in India around 1915 (McRae, 1917). In Africa, the disease was first observed in Kenya in 1924, and later, in other countries (De

Milliano *et al*., 1991). It was in Africa where Frederickson *et al*. (1991) identified a different species, *Claviceps africana* Frederickson, Mantle and de Milliano. In 1988, ergot was observed in Thailand (Boon-Long, 1992) and in 1991 in Taiwan (Cheng *et al*., 1991), although the pathogen was not identified to species. In Japan, another sorghum ergot species, *Claviceps sorghicola* Tsukib, was discovered (Tsukiboshi *et al*., 1999). Since early 1995, sorghum ergot caused by *C. africana* was observed for the first time outside Africa and Asia, in Brazil (Reis *et al*., 1996), Australia (Ryley *et al*., 1996), and in North America (Aguirre *et al*., 1997; Isakeit *et al*., 1998). Losses due to ergot in seed production fields can be high. In India, losses up to 80% have been reported, whereas in Zimbabwe annual losses are between 12 and 25% and sometimes up to 100% (Bandyopadhyay *et al*., 1998). In 1997, nearly 45% of the hybrid seed production fields in the Texas Panhandle had ergot with varying degrees of severity (Workneh and Rush, 2003). In Mexico, losses have been reported up to 100% in seed production and 30% in grain production fields. Losses of seed quality can be an issue, because of honeydew contamination of healthy sorghum grain, increasing colonization by saprophytic fungi. McLaren (1992) found that such seed had reduced germination. In addition, honeydew stickiness can interfere with harvest. Genetic diversity among isolates of *C. africana* (Komolong *et al*., 2002) has been analyzed by random amplified microsatellite (RAM) and amplified fragment length polymorphism (AFLP) (Pazoutová *et al*., 2000; Pecina *et al*., 2007; Tooley *et al*., 2000; Tooley *et al*., 2002). Isolates from Australia, India, and Japan were grouped into one cluster, and isolates from the USA, Mexico, and Africa into another (Tooley *et al*., 2002). This supports the results obtained by Pazoutová *et al*. (2000), who found american isolates to be similar to isolates from South Africa. Pecina *et al*. (2007), using AFLP analysis found that isolates from Texas and Tamaulipas, Mexico (northern Mexico) were similar, and they were different from another group from Celaya and Ocotlan, Jalisco, Mexico (central Mexico), suggesting that maybe the pathogen has evolved to adapt to different weather conditions in those areas. According to Frederickson *et al*. (1993), spread by wind-borne secondary conidia is a factor in long distance dispersal. The initial ergot observation in Mexico was on sorghum plants that were planted out of the 1996 normal fall planting dates in San Fernando, Tamaulipas, and flowered in January-February period in 1997. During September 1998, high amounts of rainfall throughout south Texas supported ratooning of plants, which were exposed to low temperatures from October to December. The extended flowering period associated with a cool and wet environment caused a fast development and spread of the pathogen on forage sorghum, grain sorghum along roads and johnsongrass [*Sorghum halepense* (L.) Pers.] plants (Odvody *et al*., 1999b). During 1999 ergot was observed in several sorghum winter nurseries located on the west coast of Mexico and Puerto Rico, and south Texas. During November-December, the disease was observed in the Bajio area and Tamaulipas, and in early 2000 in Puerto Vallarta, Mexico, with disease incidence up to 35% and severity up to 100% in commercial hybrids. The epidemic in 2000 showed that *C. africana* is well established in the major sorghum production areas of Mexico and the United States, and has shown its capability to overwinter and survive hot and dry weather conditions. The objective of this study was to determine ergot susceptibility among sorghum hybrids and three widely-utilized male-sterile lines exposed to variable environments at multiple locations.

MATERIALS AND METHODS

The experiments were carried out in College Station, USA (30° 25' N, 96° 40' W), and Mexico (25° 58' N, 98° 00' W and 20° 34' N, 100° 50' W) under irrigated conditions 2002 and 2003. At all locations, population density used was around 125,000 plants ha^{-1}. Weeds were controlled by hand hooded sprayer with Roundup® (Glyphosate) at 25 mL ha^{-1} per 12 L of water. Sorghum midge (*Contarinia sorghicola* Coquillett) was controlled as needed with Asana XL (70 mL ha^{-1}). Sorghum hybrids AP 2233 (Syngenta®), KS 310 (Sorghum Partners®), NC+7W97 (NC+ seeds®), GARST 5664 (Syngenta®), ATx399 x Tx430 (Texas A&M University), NC+8R18 (NC+ seeds®), and A-lines ATx635, ATx2752, and ATx623 (all Texas A&M University) were planted every month at each location. Hybrids were chosen based on previous ergot reaction studies conducted during the fall in Weslaco, Texas (Isakeit *et al*., 1999), and similarities in flowering pattern. The number and planting dates (on the figures, Julian day is the planting date) at each location depended upon weather conditions. At Rio Bravo (RB), there were ten planting dates, at College Station (CS) six, and five at Celaya (CEL). Plants were grown at each location in a completely randomized block design with four replications. The experimental unit was one row of 5 m length, with a row spacing of 0.81 to 0.91 m (32" to 36"). In each row, five panicles of similar maturity were selected and tagged (total genotype sample = 20) at bloom initiation. Inoculation was conducted using local *C. africana* isolates at each location. Inoculum was increased under greenhouse conditions and applied every other morning between 8:00 am and 10:00 am. Targeted panicles were marked and inoculated using a hand atomizer until runoff with a suspension of 1.6 x 10^6 conidia mL^{-1}. The period from initial and final bloom dates for each inoculated panicle was recorded (the period ranged from 4 to 13 days depending on weather conditions and type of plant, *i.e*. A-lines showed a wider period than hybrids), as well as ergot severity (percentage of infected florets observed in each inoculated plant) measured at milk stage (10-12 days after 50% flowering). Disease severity was transformed using arcsine of the square root of ergot severity to satisfy assumptions of normality. Data were analyzed taking into consideration the chronological variation (dates) nested in location by year (loc x year), instead of the spatial variation due to replications (blocks) in each planting date. Because male-sterile sorghum lines are highly susceptible to *C.*

africana in comparison with hybrids, data were analyzed in two different groups (A-lines and hybrids). Bartlett's (X^2) test of homogeneity was performed to determine homogeneity of variances among years. If the variances were homogeneous, data were combined. Analysis of variance was conducted for each location considering genotypes and locations as fixed effects, and years and dates as random effects. PROC GLM (SAS Institute, Cary, NC) procedure was used to estimate total variance, means of genotypes, dates and years. Genotype and planting date means were compared using Tukey's ($P < 0.01$) at each year and location.

RESULTS

In general, sorghum ergot severity was statistically greater ($P < 0.01$) in 2002 in both hybrids and A-lines, compared with 2003. The CEL location had the greatest amount of ergot severity on hybrids (12.5%), followed by CS and RB, which was 9.6% and 7.2% respectively. Locations as CEL and CS had greater ($P < 0.01$) ergot severity in A-lines compared with RB. In general, A-lines had the greatest ergot severity that was 66% more than the average severity observed in hybrids (Table 1). According to the average severity presented across years, locations, and dates, male-sterile line ATx2752 had the lowest ergot (22.8%), while ATx635 and ATx623 had 27.4% and 36.2%, respectively. Sorghum hybrid AP2233 was the most susceptible to ergot followed by GARST 5664, ATx399 x RTx430, and NC+7W97. In general, hybrid NC+8R18 showed the least amount of ergot (4.7%), which was 32% of the severity of the most susceptible one (Table 1). The ANOVA for hybrids showed a highly significant ($P < 0.01$) difference among genotypes and dates at all locations (Table 2). The ANOVA for A-lines showed a highly significant effect of genotype, date and the interaction (genotype x date) in all the locations (Table 3) with the exception of CEL, where date and interaction were not significant. The differences in hybrid response at each location are shown in Figure 1. At RB and CS disease had similar trends, ranging from almost zero infection during the spring and summer, to almost 50% ($P < 0.1$) infection during the fall. Meanwhile, the CEL location showed an inverse relationship, where the highest ergot level was during the summer and the lowest during the fall, maybe due to complete sterility including the ovaries and/or poor pathogen development. With A-lines, ergot was almost identical at CS and CEL with 95 to 98% more infection than RB during the spring (Fig. 2). The reverse was seen at RB, where the highest infection (66% more than CS) was during the fall.

Table 1. Sorghum ergot (*Claviceps africana*) severity (%) observed in sorghum (*Sorghum bicolor*) hybrids and A-lines evaluated at three locations in Mexico and the USA.

Genotype	Rio Bravo	College Station	Celaya	Average
Hybrids				
KS 310	9.8 bc[z]	2.1 c	13.1 ab	8.3 d
ATx399 X Tx430	9.0 bcd	9.6 ab	13.6 ab	10.7 bc
AP 2233	13.7 a	11.9 a	17.8 a	14.4 a
GARST 5664	10.4 b	8.1 b	14.9 a	11.1 b
NC+7W97	7.8 cd	9.7 ab	10.4 ab	9.3 cd
NC+8R18	6.9 d	1.9 c	5.2 b	4.7 e
Average	9.6	7.2	12.5	9.8
Male-steriles lines				
ATx 2752	21.3 b	22.8 c	24.1 b	22.8 c
ATx 634	34.2 a	30.4 b	17.6 b	27.4 b
ATx 623	21.3 b	43.8 a	43.7 a	36.2 a
Average	25.6	32.3	28.5	28.8

[z]Genotype type showing same letter at each location are statistically similar (Tukey, $p < 0.01$).

DISCUSSION

The results show great variability in susceptibility to sorghum ergot among genotypes that are evaluated at a single planting date, and also the variation in susceptibility of a single genotype evaluated at different planting dates within a year, locations and years. Therefore, sorghum ergot resistance studies should use all these components to assure that the disease resistance is genetic and not a type of avoidance mechanism. These results corroborate McLaren (1992), who found that ergot reaction of a specific line is quantified by comparing observed ergot severities associated with different flowering dates with the ergot potential of those dates. The hybrid NC+8R18 showed the least amount of ergot over

Table 2. Mean squares and test of significance of factors generated in the ergot (*Claviceps africana*) severity ANOVA for sorghum (*Sorghum bicolor*) hybrids evaluated at several locations.

Variation	Rio Bravo		College Station		Celaya	
Factor	2002	2003	2002	2003	2002	2003
Genotype	0.15[z]	0.41[z]	1.18[z]	0.16[z]	0.21[z]	0.02[y]
Date	14.4[z]	3.76[z]	7.24[z]	0.45[z]	1.42[z]	0.11[z]
Gen x Date	0.23[z]	0.21[z]	0.58[z]	0.13[z]	0.18[z]	0.01 ns
Error	0.021	0.013	0.0135	0.013	0.0191	0.0093

[y]Significant (Tukey´s, $p < 0.01$), [z]highly significant (Tukey's, $p < 0.05$), and ns = not significant.

Table 3. Mean squares and test of significance of factors generated in the ergot (*Claviceps africana*) severity ANOVA for A-lines evaluated at several locations.

Variation	Rio Bravo		College Station		Celaya	
Factor	2002	2003	2002	2003	2002	2003
Genotype	4.08[z]	1.12[z]	0.70[z]	2.79[z]	1.75[z]	0.32[z]
Date	7.50[z]	6.71[z]	0.35[z]	5.14[z]	0.12 ns	1.19[z]
Gen x Date	0.45[z]	0.62[z]	0.48[z]	0.77[z]	0.05 ns	0.12[z]
Error	0.045	0.038	0.033	0.037	0.035	

[z]Highly significant according to Tukey's $P < 0.01$; ns = not significant.

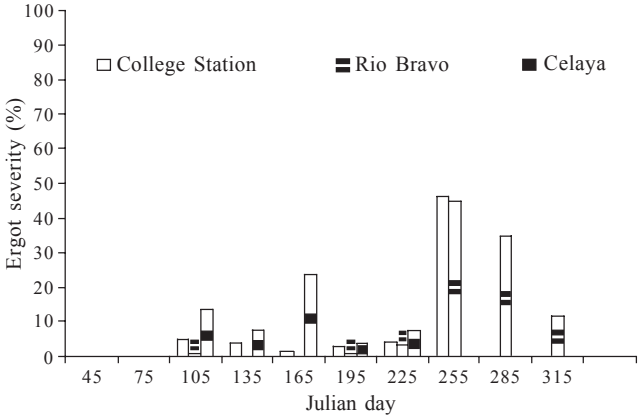

Fig. 1. Average ergot (*Claviceps africana*) development on sorghum (*Sorghum bicolor*) hybrids observed at three locations.

planting dates, years and locations. However, data showed that none of the hybrids and A-line genotypes had genetic resistance. This confirms the observations of Bandyopadhyay *et al*. (1996), who found that variations in ergot incidence and severity in sorghum lines is related to partial genetic resistance (Hernández *et al*., 2005), tolerance of lines to ergot favorable conditions (McLaren and Flett, 1998; Montes *et al*., 2003b; Montes-Belmont *et al*., 2002; Workneh and Rush, 2002; 2006) such as pre-flowering cold stress (Brooking, 1976; McLaren and Wenher, 1992; Montes *et al*., 2003a), and the ability to escape infection by ensuring

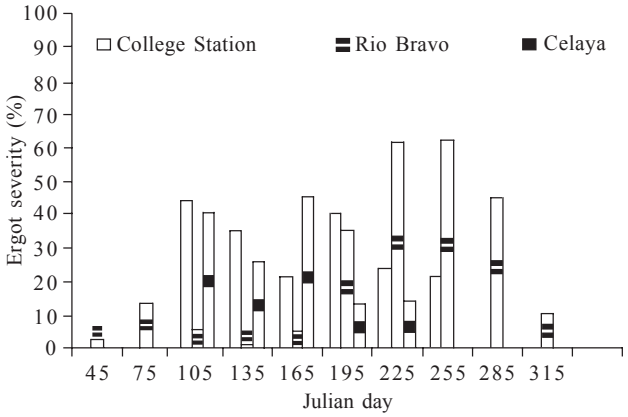

Fig. 2. Average ergot (*Claviceps africana*) development on sorghum (*Sorghum bicolor*) A-lines at three locations.

rapid and effective pollination or reducing the stigma receptivity period (Moran, 2000). It is clear that the hybrid seed production system is at high risk for ergot, because it can develop on A-lines at all locations during the normal growing season. Ergot infection in CS and CEL locations during the fall season did not occur due to frost that reduced flower development and cool temperatures that inhibited the pathogen. Situations like this and that at RB, where there are no commercial sorghum fields planted after September, result in no new infections during the winter, so new infections in the next growing season could arise from overwintering of inoculum (Odvody *et al*., 1999a; 2003; Prom *et al*., 2005b) or secondary conidia coming from infected hosts elsewhere or transmitted by insects that have been feeding in infected plants (Prom and López, 2004). The observed ergot severity values were attributable to the *C. africana* inoculum and the inoculation method that were used. Inoculum used at these locations came from fresh infected panicles from which the spore suspension was prepared. The hand sprayer inoculation technique has shown to be the best method in evaluating disease resistance (Prom *et al*., 2005a) or in chemical control studies (Montes *et al*., 2003c). The recommendation to farmers will be to plant sorghum hybrids in periods in which plants can avoid exposure to low minimum temperatures before flowering (Brooking, 1976; McLaren and Wenher, 1992; Montes *et al*., 2003a). For seed production fields, the recommendation is to apply chemical control (Montes *et al*., 2005) and crop management (Komolong *et al*., 2003) to reduce ergot. The analysis of historical weather data can give a better idea of the possible *C. africana* impact in sorghum hybrids and male-sterile lines.

CONCLUSIONS

A-lines ATx2752, ATx634, and ATx623 had greater ergot susceptibility than hybrids KS310, ATx399 x Tx430, AP 2233, NC+7W97, GARST 5664 and NC+8R18. Nevertheless, differences between A-lines suggests that breeding for resistance is a possible control option. Sorghum hybrids are at great risk for ergot at Celaya, due to the low minimum temperatures that are present throughout the year that can cause pollen sterility.

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