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OCCURRENCE OF *FUSARIUM MONILIFORME* AND FUMONISINS IN KERNELS OF MAIZE HYBRIDS IN VENEZUELA

Claudio Mazzani[1]*; Orángel Borges[1]; Odalís Luzón[1]; Venancio Barrientos[2]; Pablo Quijada[1]

[1]Facultad de Agronomía, Universidad Central de Venezuela, Mararay, Venezuela. [2]Fundación para la Investigación Agrícola-DANAC, San Javier, Venezuela

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ABSTRACT

Fusarium moniliforme (FM) incidence and fumonisins (FUM) content were determined in white kernel samples of maize hybrids, harvested in farms of Guárico and Portuguesa states, Venezuela. FM incidence was estimated in subsamples placed on a malt-salt-agar medium. Values in the range of 0-15 % were considered low, from 15-30% intermediate, and more than 30% high. FUM content was quantified through specific immunoassay (Fumonitest, VICAM Sci. Tech., USA) and values above 1 ppm were considered high. The hybrids were compared using a completely randomized design and results were subjected to analysis of variance and mean comparisons. The correlation between FUM content and FM incidence was also estimated. FM and FUM were detected in all of the 89 samples. Mean incidence of FM among hybrids ranged from 26.0 to 72.0% in Portuguesa state (PS) and from 28.7 to 55.7 in Guárico state (GS). Significant differences among hybrids were determined in farms of El Playón and Turén, PS, and El Sombrero and El Socorro, GS. The incidence of FM was high in all hybrids. FUM contents ranged from 0.80 to 10.00 ppm in PS, and from 1.50 to 13.00 ppm in GS. Significant differences among hybrids were found in El Playón, PS, and in both locations of GS. Only in Ospino, PS, the content of FUM was correlated with FM incidence. This indicates the natural occurence of FM strains with different toxigenic capabilities. The results show that Guárico and Portuguesa states are hazardous regions regarding FM and FUM in maize kernels.

Key words: *Fusarium moniliforme*, fumonisins, venezuelan maize, hybrids.

INTRODUCTION

Maize (*Zea mays* L.) is a major crop in Venezuela. It is extensively grown in fifteen states, of which Guárico and Portuguesa contribute with more than fifty percent of the national production (27). Maize is also the main energy source in the Venezuelan daily food allowance, specially among poor people.

Fusarium species are common maize pathogens. Among them, *Fusarium moniliforme* Sheldon has high specialization on this crop, causing stem and ear rot (18). It is worldwide distributed, mainly in tropical and subtropical areas. It has a high survival and a great toxicity (1,24). Fumonisins production by *F. moniliforme* is associated with different animal toxicosis such as equine leucoencephalomalacia, porcine pulmonary edema, and causes lower weight gain, body abnormalities and immunosuppression in poultry, as well as hepatoxicity, nefrotoxicity and carcinogénesis in rodents (1,8,19). Unusual incidence of human esophageal cancer related to the consumption of maize infected with *F. moniliforme* and fumonisins was reported in South Africa and China (19,21).

The distribution of fumonisins is wide and their presence has been confirmed at least in twentyfive countries (7,24,29). In Latin America the high frequency of fumonisins in maize has been associated to frequent intoxications of animals (4,9,11,28). In Venezuela, a high mold contamination has been found on maize under field and storage conditions (3,13) particularly with *F. moniliforme* and fumonisins (15,16,17).

* Corresponding author. Mailing address: Facultad de Agronomia. Universidad Central de Venezuela, Apartado 4579, Mararay 2101-A, Venezuela. E-mail: mazzabic@cantv.net

Extensive colonization of maize kernels by *F. moniliforme* and contamination with fumonisins take place in the field and their magnitude depends, largely, on the plant genotype (5,6,12,16,17,20,23).

This research was conducted to evaluate the occurrence of *F. moniliforme* and fumonisins in maize hybrids grown in commercial fields of Guárico and Portuguesa states, Venezuela.

MATERIALS AND METHODS

Origin of the samples and hybrids evaluated

During November 1998, farms in Guárico and Portuguesa States were selected to evaluate incidence of *F. moniliforme* and fumonisins in the most widely cultivated white kernel maize hybrids. Some of the hybrids had been included as checks in previous experiments (16,17). Commercial maize fields close to each other, but with different hybrids, and under uniform climatic and edafic conditions were selected. The number of samples from each field and hybrid varied according to the field size. Sampling size was 10 m long rows, with approximately 40 plants per sample. All of the ears in each row were blended to conform a compound sample for the mould and toxin analysis. Identification of the hybrids by location and the number of observations are presented in the Table 1.

Incidence of *Fusarium moniliforme* (FM)

Intact desinfected kernels with no mechanical or insect damages were placed on a malt-salt-agar medium with pH 5.8 at a rate of 10 to 12 kernels per plate. After seven days of incubation at 24 ± 2ºC, kernels with sporulating colonies or at least one conidiophore emerging from the seed coat or from the peduncle were counted. The results were expressed as percentage of colonized grains (25). Values of FM incidence in the range from 0 to 15 % were considered low, from 15 to 30% intermediate, and more than 30% high (2,12,14,16).

Fumonisins quantification (FUM)

Total fumonisins (B1 + B2) content in kernels was evaluate by specific immunoassay (Fumonitest-VICAM Science Technology, EE.UU.) (10, 26). Values of FUM content above 1 ppm were considered high.

Statistical analyses

FM incidences and FUM contents were analyzed using a completely randomized design with unequal number of replications applying SAS statistical package v.6.12 (22). Duncan's multiple range test was used for both traits. It was necessary to use arcsen and log transformation for FM incidences and FUM contents, respectively, to accomplish the normality of the data. Single correlation analysis was carried out between those variables.

Table 1. Identification and origin of white kernels corn hybrids harvested at farms of Portuguesa and Guárico States in 1998.

State	Location	Hybrid	Observations
Portuguesa	El Playón	Danac 2002	3
		HFP 2	3
		FP 02B	3
		CARGILL 101	3
	Turén La Colonia	Danac 2002	3
		HFP 2	3
		FP 02B	3
		CARGILL 114	3
	Turén	HFP 2	3
		Pioneer 3086	3
		FP 02B	3
	Ospino	HFP 2	3
		FP 02B	3
		HIMECA 2000	3
Guárico	El Sombrero	Pioneer 3086	3
		HFP 2	3
		FP 02B	3
		SEFLOARCA	3
		FM 6	3
		TOCORON 127	4
	El Socorro	Pioneer 3086	3
		HFP 2	3
		Pioneer 3001W	3
		FP 02B	3
		HS 56	3
		HS 7	3

RESULTS AND DISCUSSION

Portuguesa State Farms (PS)

Fusarium moniliforme (FM) was detected in the 52 analyzed samples. Differences among hybrids were significant at Turén and El Playón, and no significant at Ospino and Turén La Colonia (Table 2). The incidence of FM was higher in El Playón and Ospino than in Turén La Colonia and Turén. Means comparisons in El Playón showed that only hybrids with highest and smallest colonization, 'Cargill 101' and 'HFP 2', respectively, were significantly differents. In Turén, the most colonized hybrid, 'HFP 2', was significantly different from 'Pioneer 3086' and 'FP 02B' Colonization was intermediate in 'Cargill 114' in Turén La Colonia and high in the remaining hybrids in all other locations (Table 3). Some hybrids were consistent for incidence of FM. 'HFP 2', which had intermediate colonization in a previous trial in the Guárico State (16), was the most colonized hybrid in the four locations of PS. 'FP 02B' presented the lowest incidence in Ospino and Turén. FM incidence in the farms of PS (26.0 to 72.0

Table 2. Significance of mean squares for *Fusarium moniliforme* (FM) incidence and fumonisins (FUM) contents in white kernels corn hybrids from farms of Portuguesa and Guárico States in 1998.

State	Location	Source of variation	df	FM (1)	FUM (2)
Portuguesa	El Playón	hybrids	3	0.0330 +	0.5346 +
		error	8	0.0104	0.1728
		C.V. %		11.42	20.79
	Ospino	hybrids	2	0.0094 n.s.	0.1645 n.s.
		error	6	0.0377	0.0982
		C.V. %		19.82	11.79
	Turén La Colonia	hybrids	3	0.0079 n.s.	0.2782 n.s.
		error	8	0.0042	0.1393
		C.V. %		10.68	20.52
	Turén	hybrids	2	0.0111*	0.2791 n.s.
		error	6	0.0013	0.1692
		C.V. %		5.27	24.20
Guárico	El Sombrero	hybrids	5	0.0241*	1.5418 **
		error	13	0.0063	0.1076
		C.V. %		10.61	14.32
	El Socorro	hybrids	5	0.0197*	1.1389 *
		error	12	0.0053	0.3571
		C.V. %		10.91	38.73

(1): values transformed into arcsen $\sqrt{\% / 100}$; (2): values transformed into log ppm; (+): significant at 10%. (*): significant at 5%. (**): significant at 1%. (n.s.): non significant.

Table 3. *Fusarium moniliforme* (FM) incidence and fumonisins (FUM) content in white kernels corn hybrids from farms of Portuguesa State in 1998.

Location	Hybrid	FM (1)	FUM (2)
El Playón	HFP 2	70.0 B (3)	1.15 AB
	FP 02B	65.3 AB	0.80 A
	Danac 2002	59.0 AB	1.25 B
	Cargill 101	47.0 A	0.85 AB
Ospino	HFP 2	72.0 (4)	10.00 (4)
	Himeca 2000	67.7	6.15
	FP 02B	63.7	6.50
Turén La Colonia	HFP 2	37.3 (4)	3.15 (4)
	FP 02B	33.7	2.00
	Danac 2002	32.7	4.15
	Cargill 114	26.0	4.15
Turén	HFP 2	48.0 B	2.85 (4)
	Pioneer 3086	38.3 A	4.00
	FP 02B	37.0 A	2.15

(1):% of colonized grains. (2): ppm; (3): means followed by the same letter are not significantly different by Duncan's multiple range test (p =0.05); (4): mean comparisons were not carried out.

percent) and means above 37.0 , showed that this is an area of high incidence (2,20). This incidence was greater than in a Guárico State trial where environmental conditions excited the fungus occurrence (16).

The analysis of variance (ANOVA) for fumonisins content showed significant differences among hybrids at El Playón and not significant at Ospino, Turén La Colonia and Turén (Table 2). All of the hybrids were contaminated and at El Playón those with the lowest and highest contamination, 'FP 02B' and 'Danac 2002', respectively, were significantly different. FUM contamination was highest at Ospino and high at Turén La Colonia and Turén (Table 3).

FUM levels at Ospino, Turén and Turén La Colonia are high if compared with their natural occurrence in other regions of the world. Doko *et al.* (7), in nine african countries, found that largest concentration of $FB_1 + FB_2 + FB_3$ in a sample from Zimbabwe was 2.74 ppm. Amounts of FB_1 from 2.72 to 10.59 and of FB_2 from 2.34 to 10.31 ppm were determined by Hirooka *et al.* (11) in maize samples from Brazil. All of the hybrids harvested in Ospino and five hybrids from Turén and Turén La Colonia fell in that range. Viquez *et al.* (28) detected mean levels from 1.81 up to 3.58 ppm and a maximum of 6.32 ppm in Costa Rica. This value was overcome in 'HFP 2' from Ospino and five of the hybrids from PS surpassed the maximum mean level. Low fumonisins levels were found by Yoshizawa *et al.* (29) in maize from Thailand where the average was 1.79 ppm of FB_1 and 0.25 ppm of FB_2 , and only one sample presenting 18.8 ppm of FB_1. Likewise, low fumonisins levels (*ca*. 1 ppm) were found by Chulze *et al.* (4) in argentinean maize. Many hybrids evaluated in farms of PS surpassed these values.

Doko *et al.* (6) found a high fumonisins frequency in maize samples from Italy, Portugal, Zambia and Benin, with a maximum mean content (4.45 ppm) in maize from Portugal. Excluding El Playón, the mean contents found at PS were similar to or higher than that value. FUM contents similar to those found at PS were determined in commercial hybrids under artificial inoculation (23), and contamination with FUM was more extensive in our commercial farms (from 0.80 to 10.0 ppm) than those observed by Mazzani *et al.* (16) under experimental conditions at Guárico state (from 1.0 to 6.3 ppm). Positive and significant correlation between FM incidence and FUM content was found at Ospino ($r = 0.73$; $p = 0.02$). Correlation among these variables was no significant at El Playon and negative at Turén La Colonia and Turén. This situation was probably due to different toxigenic ability of the strains under natural occurrence.

Guárico State Farms (GS)

F. moniliforme (FM) was detected in all samples from GS. Significant differences among hybrids were found at El

Sombrero and El Socorro for FM incidence (Table 2). Values were high in all hybrids at both locations, except for 'HFP 2' in El Socorro, which was intermediate. 'Tocorón 127' was significantly less colonized than 'Sefloarca', 'FP 02B', 'HFP 2' and 'FM 6'. The remainig hybrids did not differ among them. The hybrid 'HFP 2' was significantly less colonized than 'FP 02B', 'Pioneer 3086' and 'HS 56' at El Socorro. The latter was also different from 'HS 7' (Table 4).

FM incidences in farms of GS varied from 28.7 to 55.7 percent. GS is also an area of high incidence of FM and the evaluated hybrids are susceptible (2,20). This incidence was greater than in a previous test at El Sombrero, GS (16). The ranges of 'Pioneer 3086' and 'FP 02B' were similar at El Sombrero and El Socorro.

Some genotypes evaluated in farms of GS, had been included as checks in previous experiments and others evaluated in farms of PS. 'FP 02B' was very susceptible to FM in farms of GS and PS. 'HFP 2' presented high incidence at El Sombrero and in farms of the PS, and intermediate at El Socorro and El Sombrero (16). This hybrid was very susceptible to FM under favorable conditions. The behavior of 'Pioneer 3086' was uniform at El Sombrero, El Socorro and Turén (Tables 3 and 4). Some hybrids evidenced environmental effects in the reaction to FM.

All of the hybrids were contaminated with FUM, some of them highly, in both locations of GS. FUM contents showed highly significant differences among hybrids at El Sombrero and significant at El Socorro (Table 2). 'Pioneer 3086' presented the lowest mean contamination, significantly different from 'Sefloarca', 'Tocorón 127' and 'HFP 2' at El Sombrero. Conversely, 'HFP 2' was the most contaminated hybrid, significantly different from the other genotypes. At El Socorro, the hybrid 'HFP 2' was the most contaminated and significantly different from 'FP 02B' and 'Pioneer 3086', while the rest of the cultivars did not show differences among them (Table 4).

The average levels of FUM content were high in most of the hybrids as compared to the natural occurrence of these micotoxins in other countries. Many hybrids at PS and GS overcame the maximum level found by Doko *et al.* (7) in nine african countries. The range found in GS was similar to the one determined by Hirooka *et al.* (11) in Brazil. Most of the hybrids overcame the maximum level found by Viquez *et al.* (28) in Costa Rica, and by Doko *et al.* (6) in Italy, Portugal, Zambia and Benin, and all of them surpassed the average level determined by Yoshizawa *et al.* (29) in Thailand and by Chulze *et al.* (4) in Argentina. These high levels observed in GS are just comparable to those obtained by Shelby *et al.* under experimental inoculation (23).

Some genotypes had a consistently similar behavior at El Sombrero and El Socorro. 'HFP 2' was the most contaminated hybrid in both locations, 'Pioneer 3086' the least contaminated one at El Sombrero and the second at El Socorro, and 'FP 02B' the least contaminated at El Socorro and intermediate at El Sombrero. Some hybrids were inconsistent at different locations probably due to environmental variations. No correlation was found among FM incidences and FUM contents at El Sombrero ($r = 0.02$; $p = 0.928$), while a negative one at El Socorro. These results suggest the co-occurrence of FM strains with different toxigenic capabilities in each locality and each field. Emphasis should be placed on the importance of evaluating the toxin besides the incidence of the fungus, because low incidences do not always correspond to low fumonisins levels.

Table 4. *Fusarium moniliforme* (FM) incidence and fumonisins (FUM) content in white kernels corn hybrids from farms of Guárico State in 1998.

Location	Hybrid	FM [1]	FUM[2]
El Sombrero	FM 6	55.7 B[3]	4.35 AB
	FP 02B	51.3 B	3.85 AB
	Sefloarca	50.3 B	7.65 B
	HFP 2	48.0 B	13.00 C
	Pioneer 3086	44.7 AB	1.50 A
	Tocorón 127	32.8 A	6.65 B
El Socorro	HS 56	49.0 C	4.15 AB
	Pioneer 3086	43.0 BC	1.65 A
	FP 02B	42.3 BC	1.50 A
	Pioneer 3001W	37.0 ABC	3.15 AB
	HS 7	30.7 AB	3.65 AB
	HFP 2	28.7 A	5.85 B

(1):% of colonized grains; (2): ppm; (3): means followed by the same letter are not significantly different by Duncan's multiple range test (p=0.05).

The high frequency and incidence of *Fusarium moniliforme* and of fumonisins contents in the Portuguesa and Guárico states qualify them as areas of high risk, confirming the necessity to find hybrids resistant to FM and to FUM. Those FM incidences and FUM contents are unacceptable in maize for human consumption, even though no referential values have been established in Venezuela. In fact, the FUM analysis is not a routine in the maize industry in this country and therefore it is not a factor of rejection.

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RESUMO

Ocorrência de *Fusarium moniliforme* e de fumonisinas em grãos de milho híbridos na Venezuela

A incidência de *Fusarium moniliforme* (FM) e o conteúdo de fumonisinas (FUM) foram determinados em amostras de grãos brancos de híbridos de milho, coletados em fazendas dos estados Guárico e Portuguesa, Venezuela. Incidência de FM foi calculada em submostras colocadas em ágar malte-sal. Valores na faixa de 0-15% foram considerados baixos, de 15-30% intermediários, e acima de 30% altos. O conteúdo de FUM foi quantificado através de imunoensaio específico (Fumonitest, VICAM Sci. Tech., USA.) e valores acima de 1 ppm foram considerados altos. Os híbridos foram comparados utilizando um esquema completamente aleatório. A correlação entre conteúdo de FUM e incidência de FM também foi calculada. A presença de FM e de FUM foi detectada em todas as 89 amostras. A incidência média de FM nos híbridos variou de 26,0 a 72,0% no estado Portuguesa (PS) e de 28,7 a 55,7% no estado Guárico (GS). Diferenças significativas entre híbridos foram determinadas em fazendas de El Playón e Turén (PS) e El Sombrero e El Socorro (GS). A incidência de FM foi alta em todos os híbridos. Os teores de FUM variaram de 0,80 a 10,00 ppm em PS e de 1,50 a 13,00 ppm em GS. Diferenças significativas entre híbridos foram achadas em El Playón (PS) e em ambas localidades de GS. Somente em Ospino (PS), o teor de FUM correlacionou-se com a incidência de FM. Isto indica a ocorrência natural de cepas de FM com capacidades toxigenicas diferentes. Os resultados mostraram que os estados Guárico e Portuguesa são regiões de risco em relação à FM e FUM em grãos de milho.

Palavras-chave: *Fusarium moniliforme*, fumonisinas, milho, híbridos.

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