Resistance in Maize Inbred Lines to *Fusarium verticillioides* and Fumonisin Accumulation in South Africa

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Abstract

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Fusarium ear rot of maize, caused by *Fusarium verticillioides*, is an important disease affecting maize production worldwide. Apart from reducing yield and grain quality, *F. verticillioides* produces fumonisins which have been associated with mycotoxicoses of animals and humans. Currently, no maize breeding lines are known with resistance to *F. verticillioides* in South Africa. The objective of this study, therefore, was to evaluate 24 genetically diverse maize inbred lines as potential sources of resistance to *Fusarium* ear rot and fumonisin accumulation in field trials at Potchefstroom and Vaalharts in South Africa. After artificial silk channel inoculation with *F. verticillioides*, *Fusarium* ear rot development was determined at harvest and fumonisins B₁, B₂, and B₃ quantified. A significant inbred line by location effect was observed for *Fusarium* ear rot severity ($P \leq 0.001$), although certain lines proved

to be consistently resistant across both locations. The individual inbred lines also differed considerably in fumonisin accumulation between Potchefstroom and Vaalharts, with differentiation between susceptible and potentially resistant inbred lines only being possible at Vaalharts. A greenhouse inoculation trial was then also performed on a subset of potentially resistant and highly susceptible lines. The inbred lines CML 390, CML 444, CML 182, VO 617Y-2, and RO 549 W consistently showed a low *Fusarium* ear rot (<5%) incidence at both Potchefstroom and Vaalharts and in the greenhouse. Two of these inbred lines, CML 390 and CML 444, accumulated fumonisin levels <5 mg kg⁻¹. These lines could potentially act as sources of resistance for use within a maize breeding program.

Fusarium spp. are known as common fungal pathogens of maize, causing ear, stalk, and root rots (19). The most important species responsible for *Fusarium* ear rot are *Fusarium verticillioides* (Sacc.) Nirenberg, *F. proliferatum* (Matsush.) Nirenberg, and *F. subglutinans* (Wollenw. & Reinking) P.E. Nelson, Toussoun and Marasas, while *F. graminearum* Schwabe is responsible for Gibberella ear rot (18). *F. verticillioides* is the predominant *Fusarium* sp. associated with maize grain in South Africa (21,24) and can infect the maize plant at all developmental stages, in some cases without causing symptoms (20). When infection develops into *Fusarium* ear rot, the disease becomes of particular concern to maize producers and the processing industry, not only because it reduces grain quality (1) but also because *F. verticillioides* produces toxic secondary metabolites in maize kernels, called fumonisins (15).

Fumonisin s compose a family of related metabolites that consists of at least 28 analogues. The most common analogues that contaminate maize-based foods and feeds and that result in mycotoxicoses of animals and humans (15,34) are fumonisins B₁, B₂, and B₃ (26). Due to their risks to human and animal health, guidelines for fumonisin content in maize products intended for food and animal feed have been implemented in a number of countries (35).

In southern Africa, legislation on tolerable levels for fumonisins generally does not exist or is not enforced (31,36). Therefore, a

substantial portion of the maize crop in many parts of southern Africa could be affected when environmental conditions favor fumonisin accumulation in grain. Thus, there is a need to control ear rots of maize to obtain good quality grain and to reduce the potential health effects associated with consumption of fumonisin-contaminated grain. The most effective approach to controlling *F. verticillioides* ear rots and minimizing the risk of fumonisin accumulation in grain is the development of host resistance (6). Host resistance would provide producers with environmentally sound and economical control of *Fusarium* ear rot and fumonisins.

Genetic variation for resistance to *Fusarium* ear rot has been investigated for a number of decades (4,6,8,11,12,14,23,25,29). Heritable resistance has been identified in maize (1,6) but no highly resistant genotypes suited to the production regions in southern Africa, where maize is the preferred staple food, are known (2,10).

The potential problems associated with *Fusarium* ear rot and fumonisin contamination of maize could be averted by proactively screening maize cultivars for resistance to *F. verticillioides* and fumonisin. In addition, sources of resistance should be identified in maize germplasm, preferably in locally adapted inbred lines, for use in breeding programs. Existing cultivars in South Africa are not known to have sufficient resistance to *Fusarium* ear rot or fumonisin contamination (25), and limited information is available for inbred lines used in South Africa. The objective of this study, therefore, was to evaluate maize inbred lines used by the South African Agricultural Research Council's Grain Crops Institute (ARC-GCI) for resistance to this pathogen and the fumonisins it produces. Inbred lines possessing resistance to *Fusarium* ear rot and fumonisin accumulation would be valuable in a breeding program to develop resistant maize cultivars that can be grown by farmers.

Materials and Methods

Plant material evaluated. In total, 24 inbred lines selected by plant breeders from the ARC-GCI for their genetic diversity and positive agronomic traits were evaluated for resistance to *Fusarium*

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ear rot and fumonisin accumulation caused by *F. verticillioides*. The inbred lines consisted of both yellow and white maize, as well as normal and high-lysine maize (Table 1). The inbred lines were planted at two field sites in maize-producing regions of South Africa. Based on their resistance or susceptibility to Fusarium ear rot in the 2007–08 field trials, 12 lines were chosen for further evaluation under controlled greenhouse conditions at Welgevallen experimental farm, Stellenbosch University (Table 1).

Preparation of fungal inoculum. Inoculum for the artificial inoculation of field and greenhouse trials was prepared using a culture of *F. verticillioides* (Medical Research Council [MRC] 826). This isolate was originally obtained from infected maize in the former Transkei region of South Africa, and is known as a well-characterized, prolific producer of fumonisin B₁ (26). Isolate MRC 826 was grown in Armstrong liquid medium (3) in Erlenmeyer flasks incubated at 25°C in a rotary shaker at 100 revolutions min⁻¹. After 4 days, the fungal spores were collected by pouring them through two layers of sterile cheesecloth into a centrifuge bottle. The suspension was then spun at 3,500 rcf for 10 min, the supernatant was removed, and the conidia were washed twice in deionized, autoclaved water. The conidial pellet was suspended in sterile distilled water and adjusted to a final concentration of 2×10^6 conidia ml⁻¹. Tween 20 surfactant (polyoxyethylene 20-sorbitan monolaurate; Fischer Biotech, Fairlawn, NJ) was added to the conidial suspension before inoculation of maize ears, as a surfactant, at a rate of 3 drops liter⁻¹.

Field evaluation. A field trial, consisting of 24 inbred lines, was planted in the 2006–07 season at Potchefstroom, Northwest Province in South Africa (grid reference 26°44'S, 27°05'E; altitude, 1,355 m), and natural infection by *F. verticillioides* was allowed to take place. Because the trial was situated within a high infection area for Fusarium ear rot, it was decided not to artificially inoculate the trial. In 2007–08, the field trials were repeated at two locations, one at Potchefstroom and the other at Vaalharts (grid reference 27°57'S, 24°50'E; altitude, 1,180 m), in the Northern Cape Province of South Africa. This time, however, the trials were artificially inoculated because of insufficient disease development in the previous planting season. The Potchefstroom and Vaalharts sites have different climatic conditions and, therefore, weather data was obtained from the ARC's weather stations located at the respective field sites.

Agricultural practices at the Potchefstroom and Vaalharts locations were similar. The land was treated with pre-emergence herbicide bendioxide at 480 g liter⁻¹ (Basagran) and post-emergence with halosulfuron-methyl at 750 g kg⁻¹ (Servian 75 WG) to control weeds. The trials were fertilized with 2:3:2 N:P:K at a rate of 200 kg ha⁻¹ prior to planting. Maize seeds were planted manually, two seeds per planting hole, in double-row experimental units (plots) that were 5 m long, with 17 plants per row, and later thinned to one plant per planting hole. The intra-row spacing was 0.3 m with inter-row spacing of 1 m in a randomized complete block design with four replications per inbred line. The Potchefstroom trial was conducted under dryland conditions, with moisture stress being monitored and supplemental irrigation applied for the duration of the trial. In contrast, the trial in Vaalharts was irrigated on a weekly basis via flood irrigation. The trials were top-dressed with LAN 28, by broadcast method, at the eighth leaf stage (V8; 27). At approximately the 10th leaf stage, both trials were treated for stalk-borer infestation with β -cyfluthrin at 0.5 g kg⁻¹ (Bulldock 0.05 GR). Application of 40 g per 100-m row was made directly into the whorl of the maize plants by hand.

Greenhouse evaluation. An artificially inoculated, hydroponic greenhouse trial was conducted to evaluate 12 inbred lines (Table 1) under conditions where nutrient and water availability could be controlled. Temperature and humidity were not strictly controlled in the greenhouse but a pad and fan cooling system was used to prevent the temperature exceeding 30°C. Before the onset of the trial, the greenhouse was surface sterilized by spraying all surfaces with dimethyl didecyl ammonium chloride at 120 g liter⁻¹ (Spore-kill) at a rate of 1 ml liter⁻¹. Seed from the inbred lines was soaked in distilled water for 4 h before undergoing a hot water treatment (60°C for 5 min) in order to disinfect the seed (13). The seed was then placed on sterile paper towels and incubated at 27°C. After 5 days, seed that had successfully germinated with no sign of fungal infection were planted directly into 15-liter greenhouse plant bags filled with coconut coir growth medium (Vegetech 2000, Cape Town, South Africa). The coconut coir medium was prepared by soaking the dry blocks in water amended with CaNO₃ (1 kg per 1,000 liters), MgS (0.3 kg per 1,000 liters), and dimethyl didecyl ammonium chloride (120 g per 1,000 liters) for 3 days before being used to fill the plant bags. The plastic plant bags were black on the interior and white on the exterior to reduce heat absorption.

Table 1. Description and identities of maize inbred lines evaluated for resistance to Fusarium ear rot and fumonisin accumulation in field and greenhouse trials in South Africa

Inbred line number	Identity of inbred line	Source ^y	Kernel color	Protein content
1 ^z	CML 390	CIMMYT-Zimbabwe	White	Normal
2 ^z	CML 395	CIMMYT-Zimbabwe	White	Normal
3	CML 442	CIMMYT-Zimbabwe	White	Normal
4 ^z	CML 444	CIMMYT-Zimbabwe	White	Normal
5	CML 488	CIMMYT-Zimbabwe	White	Normal
6	CZL 03012	CIMMYT-Zimbabwe	White	Normal
7 ^z	CML 182	CIMMYT-Zimbabwe	White	High lysine
8	CML 176-2	CIMMYT-Zimbabwe	White	High lysine
9	KO 800Y	ARC-GCI-South Africa	Yellow	High lysine
10 ^z	VO 617Y-2	ARC-GCI-South Africa	Yellow	High lysine
11	UO 718Y-1	ARC-GCI-South Africa	Yellow	High lysine
12 ^z	RO 424 W	ARC-GCI-South Africa	White	High lysine
13	RO 450 W	ARC-GCI-South Africa	White	High lysine
14 ^z	RO 544 W	ARC-GCI-South Africa	White	High lysine
15 ^z	RO 549 W	ARC-GCI-South Africa	White	High lysine
16	U 2540	ARC-GCI-South Africa	White	Normal
17	K64R-22	ARC-GCI-South Africa	White	Normal
18 ^z	L116W	ARC-GCI-South Africa	White	Normal
19 ^z	I 137tnW	ARC-GCI-South Africa	White	Normal
20	R119W	ARC-GCI-South Africa	White	Normal
21 ^z	M162W	ARC-GCI-South Africa	White	Normal
22	CBS 3-11-3-B	ARC-GCI-South Africa	White	Normal
23 ^z	R2565Y	ARC-GCI-South Africa	Yellow	Normal
24	I-B	ARC-GCI-South Africa	Yellow	Normal

^y ARC-GCI: Agricultural Research Council's Grain Crops Institute.

^z Inbred lines evaluated in greenhouse trials at Stellenbosch University.

Before planting, the medium in the plant bags was rinsed with water until the electrical conductivity of the drainage water was below 0.7. The trial layout was a completely randomized design with four replicates and seven plants per replicate for each line.

The seedlings were irrigated in their plant bags using an automated hydroponic nutrient irrigation system. Initially, the seedlings were each given a daily irrigation of approximately 500 ml of non-fertilized water for a period of 1 week. From weeks two to five, a modified Steiner nutrient solution (33), as described by Combrink (7), with an electrical conductivity (EC) of 1.5 at a pH of 6.5 was used to irrigate the seedlings. The plants received a minimum of five daily irrigations, sufficient to result in a total of 30% drainage over the entire day, to prevent the accumulation of salts in the medium. From week five onward, the nutrient solution was adjusted to an EC of 2.5 at a pH of 6.5. The drainage was monitored on a regular basis and the irrigations adjusted to account for changing weather conditions.

Inoculation of maize ears. Ears were inoculated using a cattle inoculator fitted with an 18 G $\times$ 1.5-in. (1.20-by-38-mm) Terumo needle (sterile, nontoxic, and nonpyrogenic). Conidial suspensions (2 ml) were injected down the silk channel of primary ears of all plants at the blister (R2) growth stage (27) based on the inoculation method used by Afolabi et al. (1). Inoculated ears were marked with nylon twine for identification at harvest. The inoculation procedure and timing were identical for both field and greenhouse trials.

Disease assessment. The inoculated maize ears (marked with nylon twine) were handpicked when grain was dry (between 12 and 18% kernel moisture), dehusked, and evaluated for severity of ear rot symptoms. Disease severity was assessed by estimating the percentage of each ear covered by visible symptoms of infection, such as brown, pink, or reddish discoloration of kernels, and pinkish or white mycelial growth (6).

Grain processing. After disease assessment, the ears collected in the field were dried in a ventilated glasshouse to approximately 14% grain moisture content, while those collected in the greenhouse were dried to 12% in a forced-air oven at between 50 and 60°C. Several hours at temperatures between 50 and 60°C have been shown to have no significant effect on fumonisin concentration in grain (9). Grain was hand shelled and bulked by plot. A sample of 250 g was taken from each bulked sample and ground using a Cyclotech sample mill (Foss Tecator, Hoganas, Sweden) to pass through a 1-mm mesh. Ground samples were then stored at -20°C for fumonisin analysis.

Fumonisin analysis. A 25-g sample of the stored ground grain from each plot was used for fumonisin extraction using the method developed by Shephard et al. (32). The dried sample residue was stored at -20°C for no longer than 1 week before analysis.

Liquid chromatographic tandem mass spectrometry (LC-MS/MS) was performed on a Quattro Micro triple quadrupole mass spectrometer from Waters/Micromass (Manchester, UK), equipped with an Alliance 2695 high-performance liquid chromatography system (Waters). The electrospray ionization source was used in the positive mode and the settings were optimized for best sensitivity. The mass spectrometer was operated in MRM mode and a cone voltage of 25 V was used for all transitions. The following transitions were used: for fumonisin B₁, 722.4 > 352.2 and 722.4 > 334.4; and, for fumonisin B₂ and B₃, 706.0 > 318.4 and 706.0 > 336.4, respectively, at a collision energy of 40 eV. Separation was achieved on a SunFire C18 analytical column (3.5 μ m, 150 by 4.6 mm, with guard column; Waters, Milford, MA). The mobile phase consisted of 1% formic acid (solvent A) and methanol (solvent B) and a flow rate of 1 ml min⁻¹ was used. After an isocratic step of 62% B for 3 min, it was linearly increased to 100% B over 2.1 min and held constant for 1 min followed by reequilibration for 7.9 min. The retention times under these conditions were 2.65 min for fumonisin B₁, 5.98 min for fumonisin B₂, and 4.61 min for fumonisin B₃.

Standards of fumonisin B₁ (10 mg), B₂ (10 mg), and B₃ (1 mg), >95% pure, were obtained from the MRC Program on Mycotoxins

and Experimental Carcinogenesis, Tygerberg, South Africa. Fumonisin calibration standards ranged between 0.05 and 20 mg kg⁻¹ for FB₁ and FB₂, and between 0.005 and 2 mg kg⁻¹ for FB₃. Purified sample residue was reconstituted in 1 ml of ACN/water (50/50 [vol/vol]) and 5 μ l of the solution was injected into the LC-MS/MS system. Purified sample residue was reconstituted in ACN/water because fumonisins are more stable in this solvent than in methanol mixtures (5). The limits of quantification for FB₁, FB₂, and FB₃ were 0.02, 0.002, and 0.02 mg kg⁻¹, respectively. The extraction recovery rate ranged between 60 and 65% for each compound from naturally contaminated samples.

Statistical analysis. Effects of field location and inbred lines on severity of Fusarium ear rot and fumonisin concentration in grain were analyzed using STATISTICA (data analysis software system, version 8.0; StatSoft, Inc., Tulsa, OK). A mixed-model approach was used, where replications were considered random terms and location and inbred lines were considered fixed effects. Results for individual locations were presented separately if a significant inbred-location effect was detected for either fumonisin contamination or ear rot severity. Significant differences between inbred lines were determined with Fisher's protected least significant difference test. To investigate the relative performance of inbred lines across locations, nonparametric Spearman rank correlation coefficients were determined for both ear rot severity and fumonisin concentration using STATISTICA based on untransformed means. Relationships among ear rot severity and fumonisin concentration were studied using Pearson as well as nonparametric Spearman rank correlation coefficients based on untransformed means.

For the greenhouse trial, effects of inbred lines on Fusarium ear rot, fumonisin concentration in grain, and yield were analyzed using a one-way analysis of variance in STATISTICA. In the statistical model, replications were considered random terms and inbred lines were considered fixed effects. The relationships among ear rot severity and fumonisin concentration were investigated as for the field trial.

Results

Field evaluation. In the 2006-07 maize planting season, *F. verticillioides* infection took place under natural conditions, with mean Fusarium ear rot symptom ratings of 0.1 $\pm$ 0.1% to a maximum of 4.2 $\pm$ 8.3%, which prevented the differentiation of resistant from susceptible inbred lines (*results not presented*). Fumonisin data from the trial supported this conclusion.

Sufficient Fusarium ear rot developed and fumonisin was produced in maize kernels in the 2007-08 planting season following artificial field inoculation with *F. verticillioides*. The mean Fusarium ear rot severity for the 24 inbred lines was 0.1 $\pm$ 0.1 to 26.5 $\pm$ 7.8% at Potchefstroom and 0.2 $\pm$ 0.2 to 24.0 $\pm$ 9.2% at Vaalharts (Table 2). Mean total fumonisin content (B₁, B₂, and B₃) in maize inbred lines was 0.5 $\pm$ 0.1 to 7.5 $\pm$ 5.2 mg kg⁻¹ at Potchefstroom and 2.7 $\pm$ 0.8 to 61.1 $\pm$ 33.5 mg kg⁻¹ at Vaalharts. A significant inbred line-location interaction effect was observed for both Fusarium ear rot severity and fumonisin contamination ($P \leq 0.001$).

The average visual rating of Fusarium ear rot infection of maize ears at Potchefstroom and Vaalharts was 6.7 and 5.0%, respectively, suggesting similar levels of disease development at the two locations (Table 2). Although most of the inbred lines showed comparable responses to Fusarium ear rot across locations, as supported by a Spearman correlation value of $r = 0.61$ ($P \leq 0.001$), some lines, such as lines 5, 14, 19, 21, and 23, showed significantly different responses to the disease ($P \leq 0.05$). For instance, the mean visual rating for line 5 at Potchefstroom was significantly higher (11%) than at Vaalharts (2.7%), exemplifying the highly significant inbred line-location interaction effect ($P \leq 0.001$). Significant differences in response of inbred lines to Fusarium ear rot also occurred within field locations. At Potchefstroom, six inbred lines (lines 1, 4, 7, 10, 13, and 15) had significantly ($P \leq 0.05$) less Fusarium ear rot (0.1 to 2.4%) than the lines with the highest disease severity (lines 3, 5, 9, 14, 21, and 23; 9.6 to 26.5%) (Table 2). At Vaalharts, the 12 inbred lines that showed the lowest ear rot

severity (lines 1, 4, 5, 6, 7, 8, 10, 13, 15, 17, 20, and 22; 0.2 to 2.9%) differed significantly ($P \leq 0.05$) from those displaying the highest severities (lines 3 and 19; 11.7 to 24%) (Table 2). Maize inbred lines 1, 4, 7, 10, 13, and 15 were the only lines that consistently grouped into the low Fusarium ear rot severity group for both field locations, with mean visual ratings for these lines of 0.1 to 2.4%.

The individual inbred lines differed considerably in their fumonisin accumulation between Potchefstroom and Vaalharts, as indicated by a significant but poor Spearman rank correlation ($r = 0.43$, $P = 0.03$). For instance, at Potchefstroom, line 2 accumulated significantly less fumonisin (0.7 mg kg^{-1}) than at Vaalharts (27.6 mg kg^{-1}) (Table 2). This location effect was also indicated by the lower average fumonisin concentration at Potchefstroom (2.7 mg kg^{-1}) compared with Vaalharts (17.2 mg kg^{-1}) ($P = 0.002$; Table 2). At Potchefstroom, no significant differences between inbred lines for mean fumonisin content were observed. Some of the inbred lines at Vaalharts, however, differed significantly from each other in fumonisin content, with inbred lines 1, 3, 4, 6, 16, 17, and 20 containing significantly lower levels of fumonisin (2.7 to 5.3 mg kg^{-1}) than lines 2, 9, 12, 18, 19, 23, and 24 (22.7 to 61.1 mg kg^{-1}) (Table 2).

A significant correlation was observed between Fusarium ear rot and fumonisin content at Potchefstroom (Pearson correlation of $r = 0.54$, $P \leq 0.01$; Spearman correlation of $r = 0.59$, $P \leq 0.01$) and Vaalharts (Pearson correlation of $r = 0.72$, $P \leq 0.01$; Spearman correlation of $r = 0.62$, $P \leq 0.01$). This finding is exemplified by line 1, which had a disease severity of 0.1% and fumonisin content of 0.7 mg kg^{-1} at Potchefstroom, or line 19, with a disease severity of 24% and fumonisin content of 61.1 mg kg^{-1} at Vaalharts. Some lines did, however, record relatively high disease severity and low fumonisin content, such as line 3 at Vaalharts, which had a visual rating of 12% and fumonisin contamination level of 5 mg kg^{-1} ; or low disease severity and high fumonisin content, exemplified by line 24 at Vaalharts, which had a visual rating of 4% while still containing 34 mg kg^{-1} of fumonisin (Table 2; Fig. 1).

Mean monthly temperatures at the two field locations during the 2007–08 season were similar, with a slightly higher average tem-

perature at Vaalharts during the growing season (December to July; Fig. 2). The monthly rainfall was higher at Potchefstroom at the start of the growing season (December) and over the inoculation period (February and March). At the Vaalharts location, there was significantly lower rainfall during grain filling (March) when compared with Potchefstroom and slightly higher rainfall during the months of April and May when the grain was drying (Fig. 2).

Greenhouse evaluation. Significant differences in Fusarium ear rot development were found between inbred lines in the greenhouse trial. Lines 1, 2, 4, 7, 10, 12, 14, and 15 were considered as having low mean Fusarium ear rot severity (0.4 to 10.3%) that differed significantly from lines 18, 19, 21, and 23 (17.6 to 46%) (Table 3; Fig. 3). Fumonisin analysis showed that a group of lines (lines 1, 2, 4, 7, 10, 12, 14, 15, 21, and 23) which had lower fumonisin content (0.1 to 8.9 mg kg^{-1}) differed significantly from the two lines (18 and 19) that had the highest fumonisin content (28.5 to 31.2 mg kg^{-1}) (Table 3; Fig. 4).

The response of the inbred lines following artificial inoculation with *F. verticillioides* in the greenhouse correlated well with the response of the same lines when artificially inoculated in the field. Visual rating results of the respective lines correlated well between the greenhouse and Potchefstroom field trial ($r = 0.75$, $P \leq 0.01$) and between the greenhouse and Vaalharts field trial ($r = 0.89$, $P \leq 0.001$). Toxin rating results for the lines in the greenhouse correlated well with the Potchefstroom field trials ($r = 0.79$, $P \leq 0.001$) and moderately well between the greenhouse and Vaalharts field trial ($r = 0.58$, $P \leq 0.05$).

In the greenhouse, a significant correlation was found between Fusarium ear rot severity and fumonisin content (Pearson correlation of $r = 0.94$, $P \leq 0.01$; Spearman correlation of $r = 0.92$, $P \leq 0.01$). Interestingly, however, line 23 accumulated low levels of fumonisin in the greenhouse despite being highly susceptible to Fusarium ear rot in the greenhouse and field trials.

Discussion

Maize inbred lines with potential resistance to Fusarium ear rot and fumonisin accumulation, caused by *F. verticillioides*, were

Table 2. Evaluation of 24 maize inbred lines for resistance to Fusarium ear rot and fumonisin accumulation in field trials at Potchefstroom and Vaalharts in the 2007–08 planting season

Inbred line number	Fusarium ear rot severity (%) ^{x,y}		Fumonisin (mg kg^{-1}) ^{x,z}	
	Potchefstroom	Vaalharts	Potchefstroom	Vaalharts
1	0.1 a	0.2 a	0.7 a	2.7 a
2	6.4 a-i	4.7 a-h	0.7 a	27.6 ef
3	9.6 ghi	11.7 ij	1.2 a	5.1 ab
4	2.4 a-f	1.0 abc	1.7 a	4.1 ab
5	11.0 hi	2.7 a-f	2.5 a	13.6 a-e
6	5.0 a-h	0.7 ab	2.2 a	4.3 ab
7	0.7 ab	1.5 a-e	1.0 a	8.6 abc
8	4.7 a-h	1.2 a-d	5.4 ab	9.4 abc
9	11.0 hi	8.1 f-i	2.8 a	46.0 gh
10	1.4 a-e	2.7 a-f	1.1 a	10.8 a-d
11	3.2 a-g	6.9 b-i	3.5 ab	20.4 b-f
12	3.3 a-g	6.2 a-i	1.4 a	26.7 def
13	2.2 a-f	2.4 a-f	0.9 a	12.0 a-e
14	26.5 l	7.7 e-i	5.5 abc	16.6 a-e
15	2.0 a-f	0.7 ab	0.9 a	6.4 abc
16	3.6 a-g	4.4 a-h	0.5 a	5.1 ab
17	4.1 a-g	2.9 a-f	3.8 ab	5.2 ab
18	4.3 a-g	6.2 a-i	6.0 abc	22.7 c-f
19	7.7 d-i	24.0 kl	7.5 abc	61.1 h
20	6.9 b-i	1.7 a-f	1.8 a	5.3 ab
21	18.8 k	7.5 c-i	5.2 ab	6.3 abc
22	4.6 a-h	2.8 a-f	1.2 a	13.0 a-e
23	17.7 jk	7.9 e-i	5.2 ab	45.4 gh
24	4.7 a-h	4.2 a-g	2.5 a	34.2 fg
Mean	6.7	5.0	2.7	17.2

^x For each variable (Fusarium ear rot severity or fumonisin content), means followed by the same letter do not differ significantly according to Fisher's least significant difference test ($P \leq 0.05$).

^y Percentage of maize ear showing visual symptoms of Fusarium ear rot; mean of disease severity (%) for four field plots.

^z Fumonisin concentration = total of $\text{FB}_1 + \text{FB}_2 + \text{FB}_3$; mean of fumonisin concentration for four field plots.

identified from well-adapted breeding material used in South Africa. This is of great significance, because *F. verticillioides* is known to be the most common fungal pathogen associated with Fusarium ear rot in the country and because existing cultivars are not known to have sufficient resistance to Fusarium ear rot or fumonisin contamination (25). Maize genotypes adapted to Central and West Africa with resistance to Fusarium ear rot and fumonisin contamination have been identified by Afolabi et al. (1). These genotypes, however, are better adapted to tropical conditions and are not necessarily suited to the dry temperate conditions in Southern Africa.

None of the inbred lines evaluated in this study were completely resistant to *F. verticillioides* or the fumonisins it produces, which is in agreement with previous studies (6,22). However, several inbred lines, including 1, 4, 7, 10, and 15, were found to have consistently low Fusarium ear rot at both the Potchefstroom and Vaalharts field locations and in the greenhouse. Additionally, certain lines were identified that consistently accumulated low fumonisin levels, such as lines 1 and 4. These lines could potentially act as sources of resistance for use within a maize resistance breeding program.

Significant genotype–location interactions were observed for the inbred lines evaluated. Genotype–location interactions for resistance to Fusarium ear rot and fumonisin have been previously recorded (28,30). Due to the nature of plant diseases, successful infection of the host and subsequent production of mycotoxins is dependent on prevailing environmental conditions, susceptibility of the plant host to the pathogen, as well as insect vector activity under some circumstances (17). Further, it has been documented that maize genotypes grown outside of their normal production zones are likely to be more susceptible to Fusarium ear rot and fumonisin contamination (30). For these reasons, it is important that locally adapted material be used in breeding programs, and that the potentially resistant lines are further evaluated over an extended range of conditions, representative of the localities where they will eventually be grown, to further confirm their resistant status.

A significant correlation between Fusarium ear rot rating and fumonisin concentration in the grain was observed in the field trials. Some of the inbred lines, however, were recorded as having severe ear rot but low fumonisin concentrations. Two possible explanations can be provided: (i) in some plots containing severely rotten

ears, much of the kernel tissue was so badly destroyed that it barely contributed to the ground grain sample from which the fumonisin concentration was measured; and (ii) the visual rating was unable to discriminate between visual disease symptoms caused by non-fumonisin-producing fungal species, resulting in high visual rating and low fumonisin concentration. Conversely, outliers were ob-

Fig. 2. Weather data recorded from Agricultural Research Council (ARC) weather stations for the 2007–08 season at A, Potchefstroom and B, Vaalharts in South Africa. Line and bar series show mean monthly temperature and total monthly rainfall, respectively, for the growing season.

Fig. 1. Comparison of mean visual Fusarium ear rot ratings with mean fumonisin content for 24 maize inbred lines grown at Vaalharts in South Africa.

served with low ear rot rating but high fumonisin concentrations. Munkvold and Desjardins (19) previously suggested that fumonisin could accumulate in kernels with minimal ear rot. The occurrence of such outliers suggests that fumonisin analyses are necessary when evaluating maize resistance. The cost and time involved in laboratory analyses of fumonisin concentration, however, is prohibitive when conducting large-scale trials. Robertson-Hoyt et al. (28) studied the heritability and correlation of *Fusarium* ear rot resistance and fumonisin contamination resistance in two maize populations and found that, due to the moderate to high heritability and strong genetic correlation between ear rot and toxin resistance, selection for resistance to ear rot should result in lines with greater

resistance to fumonisin contamination. Nonetheless, later generation lines selected for reduced ear rot should be evaluated for low fumonisin concentration directly to verify that resistance has been identified.

A higher average fumonisin concentration was observed at the Vaalharts than at the Potchefstroom location in spite of similar average *Fusarium* ear rot ratings. At the Vaalharts location, there was significantly lower rainfall during grain filling (March) when compared with Potchefstroom, with dry periods before or during grain filling known to favor more severe *Fusarium* ear rot and higher levels of fumonisins (30). The low rainfall at Vaalharts, however, is unlikely to have resulted in plant stress because these trials were flood irrigated weekly. In vitro studies have shown that optimal conditions for *F. verticillioides* growth and FB₁ production are 30°C and a water activity of 0.97, with a dramatic reduction in germination and fumonisin production below a water activity of 0.92 (16). This would suggest that infection may be favored by dry conditions before or during grain filling but that fumonisin production would also be favored in the grain for as long as the grain remains above a water activity of 0.92. Grain would have a suitable water activity for fumonisin production during the initial drying period in the field, and additional rain after physiological maturity would extend the period suitable for fumonisin production. Slightly higher rainfall at Vaalharts during the months of April and May, when the grain was drying, combined with increased relative humidity due to flood irrigations, may have allowed extended fumonisin accumulation at Vaalharts. Alternatively, the possibility of naturally higher fumonisin-producing inoculum having infected the Vaalharts trial cannot be excluded.

A good correlation was shown between the results from the artificially inoculated greenhouse trial with those of both field locations. The similar responses of the inbred lines evaluated under greenhouse conditions with those of the field trials suggests that greenhouse trials could be used to accelerate progress in the selection of resistant germplasm. In addition, the ability to manipulate environmental conditions, as well as nutrient and water supply in the greenhouse provides an ideal opportunity to investigate the impact of these factors on the resistance of the maize plant and on the ability of the fungus to cause *Fusarium* ear rot and produce fumonisin.

Table 3. Evaluation of 12 maize inbred lines for resistance to *Fusarium* ear rot and fumonisin accumulation in a greenhouse trial^x

Inbred line number	<i>Fusarium</i> ear rot severity (%) ^{x,y}	Fumonisin (mg kg ⁻¹) ^{x,z}
1	1.2 a	0.1 a
2	3.3 a	1.1 a
4	0.4 a	0.5 a
7	2.9 a	1.0 a
10	2.8 a	1.1 a
12	8.4 ab	5.6 a
14	10.3 ab	5.1 a
15	3.1 a	1.5 a
18	36.7 d	28.5 b
19	46.0 d	31.2 b
21	17.6 bc	8.9 a
23	22.0 c	2.6 a
Mean	12.9	7.3

^x For each variable (*Fusarium* ear rot severity or fumonisin content), means followed by the same letter do not differ significantly according to Fisher's least significant difference test ($P \leq 0.05$).

^y Percentage of maize ear showing visual symptoms of *Fusarium* ear rot; mean of disease severity (%) for four experimental replications (containing seven plants each).

^z Fumonisin concentration = total of FB₁ + FB₂ + FB₃; mean of fumonisin concentration from four experimental reps (grain from seven plants pooled for each replication).

Fig. 3. Mean visual rating of *Fusarium* ear rot severity for selected inbred lines evaluated in field trials at Vaalharts and Potchefstroom, as well as in greenhouse trials at Stellenbosch, South Africa.

Fig. 4. Mean fumonisin (FB₁ + FB₂ + FB₃) concentrations in maize samples from selected inbred lines evaluated in field trials at Vaalharts and Potchefstroom, as well as in greenhouse trials at Stellenbosch, South Africa.

This study is the first to report potentially resistant maize inbred lines locally adapted to southern African production conditions. These inbred lines will not only be useful for the development of resistant commercial cultivars adapted to Southern African production conditions but also may allow insight into the mechanisms involved in resistance to *Fusarium* ear rot and fumonisin contamination. Similarly, the highly susceptible inbred lines could be used for comparative functional genomics or proteomics, or as susceptible controls in future experiments.

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