Evaluation of Maize Inbred Lines for Resistance to Fusarium Ear Rot and Fumonisin Accumulation in Grain in Tropical Africa

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ABSTRACT

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Fusarium ear rot and fumonisin contamination is a major problem facing maize growers worldwide, and host resistance is the most effective strategy to control the disease, but resistant genotypes have not been identified. In 2003, a total of 103 maize inbred lines were evaluated for Fusarium ear rot caused by *Fusarium verticillioides* in field trials in Ikenne and Ibadan, Nigeria. Disease was initiated from natural infection in the Ikenne trial and from artificial inoculation in the Ibadan trial. Ear rot severity ranged from 1.0 to 6.0 in both locations in 2003. Fifty-two inbred lines with disease severity ≤ 3 (i.e., $\leq 10\%$ visible symptoms on ears) were selected and re-evaluated in 2004 for ear rot resistance, incidence of discolored kernels, and fumonisin contamination in grain. At both locations, ear rot severity on the selected lines was significantly ($P < 0.0020$) higher in 2004 than in 2003. The effects of selected inbred lines on disease severity were highly significant at Ikenne ($P = 0.0072$) and Ibadan ($P < 0.0001$) in 2004. Inbred lines did not affect incidence of discolored kernels at both locations and across years except at Ikenne ($P = 0.0002$) in 2004. Similarly, significant effects of inbred lines on fumonisin concentration were observed only at Ikenne ($P = 0.0201$) in 2004. However, inbred lines 02C14585, 02C14593, 02C14603, 02C14606, 02C14624, and 02C14683 had consistently low disease severity across years and locations. Fumonisin concentration was significantly correlated with ear rot only at Ikenne ($R = 0.42$, $P < 0.0001$). Correlation between fumonisin concentration and incidence of discolored kernels was also significant at Ikenne ($R = 0.39$, $P < 0.0001$) and Ibadan ($R = 0.35$, $P = 0.0007$). At both locations, no significant inbred $\times$ year interaction was observed for fumonisin concentration. Five inbred lines, namely 02C14585, 02C14603, 02C14606, 02C14624, and 02C14683, consistently had the lowest fumonisin concentration in both trials. Two of these inbred lines, 02C14624 and 02C14585, had fumonisin levels < 5.0 $\mu\text{g/g}$ across years in trials where disease was initiated from both natural infection and artificial inoculation. These lines that had consistently low disease severity are useful for breeding programs to develop fumonisin resistant lines.

Additional keywords: corn, mycotoxin

Fusarium verticillioides (Sacc.) Nirenberg is a common pathogen of maize causing root, stalk, and ear rots worldwide (25) and is more widespread in tropical and subtropical regions. Ear rot is a major economic concern to maize (*Zea mays* L.) producers and the processing industry due to the losses in grain yield and quality. Generally, Fusarium ear rot is favored by warm and dry conditions (30,39). However, warm and wet conditions following inoculation at silking have been reported to be conducive for disease development (8,32). Intact or split kernels covered with

white or pinkish white mold are typical symptoms of ear rot infection by *F. verticillioides*. The fungus can cause disease at all developmental stages of the plant, in some cases without causing symptoms (27). In addition to its effects on yield and seed quality, *F. verticillioides* can produce several toxic metabolites, of which fumonisins are the most prevalent (21). This situation is further complicated by the common occurrence of fumonisins in symptomless infected kernels (5,27). Previous studies have reported *F. verticillioides* to be the most predominant *Fusarium* species in West Africa (2,3,12).

Fumonisin is a group of structurally related mycotoxins that are common contaminants of maize-based foods and feeds worldwide, and the most abundantly found fumonisins in naturally contaminated foods and feeds are FB₁, FB₂, and FB₃ (34). Although a cause-and-effect relationship has not been clearly demonstrated (9),

evidence exists of potentially serious human and animal health problems associated with consumption of maize products contaminated with fumonisin. These problems include human esophageal cancer (7), neural tube defects in newborns (37), leukoencephalomalacia in horses (15), liver cancer in rats (13), and neurodegeneration in mice (31). Due to these inherent risks to human and animal health through the consumption of contaminated grains, guidelines for fumonisin content in maize products intended for food and animal feed have been recommended (6,38).

In Africa, no formal guidelines for fumonisin content are available (38). However, high levels of fumonisin contamination in maize intended for food have been reported from several West African countries such as Nigeria (3), Benin (12), Ghana (18), Cameroon (28), and Burkina Faso (29). Therefore, a substantial portion of the maize crop in many parts of Africa could be affected when environmental conditions favor fumonisin accumulation in grain. There is thus 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 minimize the risk of fumonisin accumulation in grain is the development of host-plant resistance (10,32). Resistance will provide producers with environmentally sound and economical control of fumonisins, particularly in Africa where use of fungicides is very limited.

Heritable resistance has been identified in maize (14,16), but no highly resistant genotypes are known. Adaptation requirements of maize are unique in West Africa due to a myriad of biotic and abiotic factors that are much different and more severe than those in other parts of the world. The International Institute of Tropical Agriculture (IITA) has developed maize inbred lines that are high yielding and adapted to West African agroecological zones. These inbred lines are generally developed under high disease pressure in tropical Africa and possess a higher level of aflatoxin resistance (4,23) than material found elsewhere. However, the level of resistance in IITA inbred lines to ear rot caused by *F. verticillioides* and fumonisin accumulation in grains has not been de-

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terminated. The potential problems associated with fumonisin can be averted by a proactive strategy to screen these inbreds for ear rot resistance and fumonisin accumulation prior to their release for commercial production. Therefore, the objective of this study was to evaluate IITA's maize inbred lines for resistance to *F. verticillioides* ear rot and fumonisin accumulation in grain under artificial and natural infection. Inbreds possessing resistance to ear rot and fumonisin accumulation would be valuable breeding stock for use as parents of hybrids and synthetics that can be grown by farmers.

MATERIALS AND METHODS

Planting material and field sites. A total of 103 maize inbred lines and two hybrids were planted to evaluate their resistance to Fusarium ear rot and fumonisin accumulation due to *F. verticillioides* in two agroecological zones in Nigeria. The study was conducted during the rainy seasons in 2003 and 2004 at IITA field sites at Ikenne and Ibadan. The Ikenne site is located in the rain forest belt, 6° 52' N latitude and 3° 43' E longitude, where soils are typically entric nitosols. The field site at Ibadan is located in the derived savanna region, 7° 30' N latitude and 3° 54' E longitude, where soils are typically ferric luvisols (24). Under natural infection, disease severity is usually higher at Ikenne and lower at Ibadan field sites. In both years, the field site at Ikenne was used to evaluate inbred lines for resistance under natural infection. Weather conditions at Ibadan are usually variable, and to ensure that disease epidemics develop consistently during the study period, plants were artificially inoculated in both years. The two commercial hybrids (Oba super 1 and Oba super 2) were included as resistant checks in both trials.

In 2003, fields were manually planted (2 seeds per hill) in single-row plots 5 m long with 21 plants per row on 6 June in Ikenne and 19 June in Ibadan. At 2 weeks after planting, plants were thinned to one plant per hill and a compound fertilizer was applied to the plots at the rate of 60 kg/ha NPK. An additional 30 kg N/ha (urea) was applied as top dressing approximately 4 weeks after planting. Plots were spaced 0.75 m between rows and 0.25 m between hills in an augmented (unreplicated) experimental design (22).

From the initial total of 103 inbred lines, 52 inbreds were selected (see below under disease assessment) for field re-evaluation in 2004 in rotated plots. Seed of selected lines was sown on 8 June at Ikenne and 15 June at Ibadan, in single-row plots 5 m long with 21 plants per row. Plots were spaced 0.75 m between rows and 0.25 m between hills arranged in a randomized complete block design with two replications. Cultural practices at the two locations were similar in both years except that

fields at both sites were irrigated with a sprinkler device three times a week for 3 h during the dry month of August 2004.

Inoculum and inoculation. Inoculum was prepared from cultures of *F. verticillioides* (ATCC MYA 836) for field inoculation at Ibadan. This *F. verticillioides* isolate was obtained from a naturally infected ear in Ibadan and routinely maintained on modified Czapek-Dox complete medium (20) and stored in 15% glycerol at -80°C. Prior to inoculation, cultures were grown on modified Czapek-Dox complete medium at approximately 25°C under 12 h of diurnal fluorescent light for 7 days. Conidia were washed from the surface of the agar media with sterile distilled water, and the resulting propagule suspension was strained through two layers of cheesecloth. Sterile distilled water was used to adjust the conidial concentration to 1×10^6 conidia/ml as determined using a hemacytometer, and amended with Tween 20 surfactant (polyoxyethylene 20-sorbitan monolaurate; Fisher Biotech, Fairlawn, NJ) at 0.2 ml/liter. Inoculum suspension was used within 2 h of preparation.

Ears were inoculated using a syringe (Cornwall Luer-Lok) fitted to a continuous pipetting outfit (Becton, Dickinson and Company, Rutherford, NJ) in 21 August to 6 September 2003 and 17 to 31 August 2004. Conidial suspension (4 ml) was injected down the silk channel of primary ears of all plants at the blister (R2) growth stage (36). Ears were covered after inoculation with waxed paper shoot bags for 2 days to maintain high humidity and to protect the inoculum from being drained by rain or dried by excessive heat.

Disease assessment. In 2003, ears were manually harvested at maturity on 4 and 16 October at Ikenne and Ibadan, respectively. At each harvest date, 10 ears in an experimental unit (plot) were hand-picked, dehusked, and evaluated for severity of ear rot symptoms. Disease severity was assessed by determining the percentage of each ear covered by symptoms using a 7-class rating scale in which 1 = no infection, 2 = 1 to 3%, 3 = 4 to 10%, 4 = 11 to 25%, 5 = 26 to 50%, 6 = 51 to 75%, and 7 = 76 to 100% of the kernels exhibiting visible symptoms of infection, such as rot and pinkish or white mycelial growth (33). This scale has been tested for accuracy when assessing severity of Fusarium ear rot by Chungu et al. (8). Based on this scale, 52 inbred lines (from a total of 103 lines) with disease severity ≤ 3 (i.e., with less than 10% visible symptoms on ears) were selected from both sites for re-evaluation in 2004.

Ears were sun-dried after disease assessment to approximately 14% grain moisture content. Grain was hand-shelled and bulked by plots. Two hundred grains from bulked samples of each plot were separated into symptomless and symptomatic fractions (discolored kernels). The

latter fraction contained kernels that were visibly moldy, darkened, streaked, or chalky in appearance. The incidence of discolored kernels was determined by expressing the number of visibly discolored kernels as a proportion of 200 grains from bulked samples of each plot multiplied by 100.

In 2004, ears of the 52 inbred lines were manually harvested at maturity on 29 September at Ikenne and 6 October at Ibadan and assessed for ear rot severity and incidence of discolored kernels as described above.

Weather information for Ibadan and Ikenne locations was obtained from IITA's Geo-Spatial Unit, Ibadan, Oyo State, and Nigeria Meteorological Agency, Federal Secretariat, Abeokuta, Ogun State, respectively.

Fumonisin analysis. Bulk grain from each plot was ground, and a 20-g sample was used for fumonisin extraction. Ground samples were extracted with 100 ml of 70% methanol using a high-speed blender (Waring Commercial, Springfield, MO) for 3 min. The extract was filtered through a Whatman no. 4 filter paper (Whatman International Ltd., Maidstone, England) and the filtrate collected for evaluation. Concentration of total fumonisin (FB₁ + FB₂ + FB₃) in extracted filtrate was quantified using a competitive direct enzyme-linked immunosorbent assay (ELISA) in a microwell format (EZ-Quant Fumonisin Test Kit, Diagnostix Limited, Mississauga, Canada) according to the manufacturer's instructions as described below.

A 100- μ l aliquot of each fumonisin standard (0, 0.3, 1.5, and 6.0 μ g/g) was dispensed separately into four mixing wells, and 100 μ l of each sample extract was added to all the remaining mixing wells. To each mixing well, 100 μ l of enzyme conjugate (horseradish peroxidase) was dispensed in the well, and after mixing, 100 μ l was transferred to the antibody-coated wells and incubated for 10 min at room temperature (~27°C). The contents of the antibody wells were removed, and after a wash step, 100 μ l of substrate 3-3'-5-5' tetra methyl benzidine (TMB) was added and incubated for 5 min at 28°C. Reactions were stopped by adding 100 μ l of stop solution (HCl) to each well, and optical densities were measured with Bio-Tek EL301 TM Microwell Strip Reader (Bio-Tek Instruments, Inc., Vermont, USA) fitted with a 450-nm filter. Fumonisin concentrations of samples were determined by plotting the optical densities on the standard curve of known fumonisin concentrations. Samples with fumonisin concentration higher than the highest standards were diluted with the extraction solvent, and results obtained were multiplied by the dilution factor. All samples were run in duplicates, and the minimum detection limit was 0.2 μ g/g. The recovery rate was determined as described by Afolabi et al. (1) and ranged between 78 and 80%.

Statistical analysis. Effects of years and selected inbred lines on severity of *Fusarium* ear rot, incidence of discolored kernels, and fumonisin concentration in grain for each location were analyzed using PROC MIXED procedure of SAS as described by Wolfinger et al. (40). In the statistical model, years were considered random while inbred lines were considered as fixed effects. Data from individual years were analyzed and presented separately if a significant inbred $\times$ year interaction was detected for either fumonisin concentration or ear rot severity. Differences between inbred lines within years were determined with Fisher's protected least significant difference (LSD) test. Data on discolored kernels were transformed by the equation $y = \log_{10}(1 + \text{incidence of discolored kernels})$, to normalize residuals. Linear correlation coefficients were determined for the

relationship among ear rot severity, percentage of discolored kernels, and fumonisin concentration. Linear correlations were performed using the CORR procedure in SAS based on untransformed means separately for each location, using combined data due to nonsignificant inbred $\times$ year interaction for fumonisin concentration. All statistical analyses were performed in SAS (version 9.1, SAS Institute, Cary, NC).

RESULTS

Evaluation under natural infection at Ikenne. In 2003, *Fusarium* ear rot severity on the 103 inbred lines ranged from 1.0 to 6.0 with a mean of 1.8. In 2004, mean disease severity for the 52 selected inbred lines was 2.1. For the selected inbred lines, disease severity was significantly ($P = 0.0020$) higher in 2004 than in 2003. The

effects of selected maize inbred lines on disease severity, incidence of discolored kernels, and fumonisin contamination differed between years. In 2003, no significant differences in *Fusarium* ear rot severity ($P = 0.7106$) were observed among the selected inbred lines (Table 1). However, highly significant ($P = 0.0072$) differences in ear rot severity among the inbred lines were observed in 2004. Inbred lines 02C14585, 02C14683, 02C14624, and 02C14593 were among the lines that had lower disease severity (<1.8) when compared with the resistant checks in 2004 when higher disease severity levels were observed (Table 1). The incidence of discolored kernels due to *Fusarium* ear rot ranged from 2.7 to 47.5% in 2003, and no significant differences ($P = 0.6500$) were observed among the selected inbred lines. The incidence of discolored kernels was

Table 1. *Fusarium* ear rot severity and incidence of discolored kernels for some selected maize inbred lines naturally infected by *Fusarium verticillioides* in field trials at Ikenne, Nigeria, in 2003 and 2004

Inbred line	2003				2004			
	Ear rot severity ^x	Discolored kernels (%)	Discolored kernels (%) ^y	Fumonisin ($\mu\text{g/g}$)	Ear rot severity ^x	Discolored kernels (%)	Discolored kernels (%) ^y	Fumonisin ($\mu\text{g/g}$)
02C14582	2.2	16.9	1.3	1.8	2.6	50.0	1.7	25.5
02C14583	1.8	5.1	0.8	1.0	2.7	31.9	1.5	17.7
02C14585	2.0	10.1	1.0	2.6	2.0	30.0	0.7	4.0
02C14588	2.0	15.7	1.2	5.7	2.4	29.7	1.5	11.0
02C14589	2.2	17.7	1.3	3.4	2.2	38.8	1.6	5.2
02C14593	1.0	13.3	1.2	0.2	1.5	29.3	1.5	1.1
02C14595	1.4	5.9	0.8	0.7	1.6	21.4	1.3	0.5
02C14601	1.0	41.4	1.6	7.3	1.9	56.3	1.8	4.3
02C14603	1.2	2.9	0.6	3.9	1.8	7.7	0.9	7.1
02C14606	1.8	22.7	1.4	3.3	1.9	27.8	1.5	5.3
02C14607	1.0	10.0	1.0	8.8	1.9	27.7	1.5	3.3
02C14611	1.2	8.3	1.0	3.5	2.5	35.9	1.6	9.5
02C14613	1.0	45.8	1.7	0.5	1.8	36.4	1.6	7.6
02C14617	2.0	10.1	1.0	8.4	2.1	45.8	1.7	7.4
02C14618	2.4	18.7	1.3	18.3	1.6	24.1	1.4	7.2
02C14619	2.0	3.3	0.6	5.4	1.4	18.2	1.2	8.7
02C14621	1.8	20.2	1.3	5.1	1.9	29.4	1.5	8.2
02C14622	1.4	18.7	1.3	1.2	2.4	4.1	0.7	9.9
02C14624	1.6	2.7	0.6	4.7	1.7	4.7	1.5	4.2
02C14626	2.0	9.9	1.0	2.7	2.2	32.4	1.5	7.2
02C14628	1.2	3.6	0.7	1.5	2.1	31.5	1.5	15.8
02C14629	2.6	20.3	1.3	4.8	1.9	42.0	1.6	15.7
02C14637	2.0	7.7	0.9	12.0	1.7	32.7	1.5	16.9
02C14640	2.2	12.0	1.1	3.4	2.3	38.1	1.6	5.5
02C14643	1.2	24.9	1.4	18.6	2.1	26.3	1.4	3.0
02C14645	1.6	28.1	1.5	2.0	2.7	22.4	1.3	11.4
02C14646	1.7	45.5	1.7	3.1	2.6	53.3	1.7	62.3
02C14649	2.2	15.9	1.2	26.9	1.2	15.3	1.2	2.6
02C14651	1.8	25.3	1.4	25.4	1.0	30.0	1.5	10.2
02C14654	1.3	7.8	0.9	82.4	2.2	42.2	1.6	26.6
02C14655	2.5	27.7	1.5	13.5	2.5	45.5	1.7	14.3
02C14656	1.0	24.5	1.4	9.5	1.9	43.8	1.6	22.4
02C14661	3.0	47.5	1.7	123.0	3.0	31.1	1.5	27.7
02C14671	1.0	10.6	1.1	2.5	2.3	59.3	1.8	9.4
02C14675	1.6	10.8	1.1	0.6	2.3	45.8	1.7	7.8
02C14677	1.2	3.9	0.7	1.9	1.8	31.4	1.5	7.0
02C14678	1.0	6.4	0.9	2.1	2.1	37.6	1.6	11.1
02C14680	1.0	30.5	1.5	2.2	2.1	64.2	1.8	16.3
02C14683	2.0	18.0	1.3	7.8	2.4	30.2	1.7	7.7
Oba super 1	1.6	14.4	1.1	0.4	2.0	16.4	1.2	6.3
Oba super 2	1.3	3.8	0.7	0.2	2.2	32.3	1.5	6.9
LSD ^z	10.7	...	6.8	128.5	0.9	...	0.4	21.3

^x *Fusarium* ear rot severity was visually assessed at harvest using a 7-class rating scale (33) where 1 = no symptoms, 2 = 1 to 3%, 3 = 4 to 10%, 4 = 11 to 25%, 5 = 26 to 50%, 6 = 51 to 75%, and 7 = 76 to 100% of the ear affected by symptoms.

^y Data transformed to the $\log_{10}$ of the percentage of discolored kernels, i.e., $y = \log_{10}[1 + \text{discolored kernels} (\%)]$.

^z LSD = least significant difference at $\alpha = 0.05$.

Fig. 1. Total fumonisin (FB₁ + FB₂ + FB₃) concentrations in kernels for some selected maize inbred lines planted at the International Institute of Tropical Agriculture experimental site at Ikenne, Nigeria, during the rainy seasons in 2003 and 2004. Fusarium ear rot was initiated from natural infection, and depicted inbred lines are selected to illustrate consistency in fumonisin production across the 2 years.

Fig. 2. Linear correlations of Fusarium ear rot severity and incidence of discolored kernels with fumonisin concentration (**A** and **B**, respectively) from naturally infected maize inbred lines at the International Institute of Tropical Agriculture experimental site at Ikenne, Nigeria, during the rainy seasons in 2003 and 2004. Disease severity is based on the percentage of each ear with symptoms using a 7-class rating scale in which 1 = no infection, 2 = 1 to 3%, 3 = 4 to 10%, 4 = 11 to 25%, 5 = 26 to 50%, 6 = 51 to 75%, and 7 = 76 to 100% of kernels exhibiting visible symptoms of infection. In each year, each point represents data from an individual maize inbred line.

significantly ($P < 0.0001$) higher in 2004 than in 2003. Highly significant ($P = 0.0002$) differences in incidence of discolored kernels among inbred lines were observed in 2004, with incidences ranging from 4.1 to 64.2% (Table 1). Inbred lines 02C14603, 02C14683, 02C14593, and 02C14622 had lower incidences of discolored kernels compared with the resistant checks.

Total fumonisin concentration was significantly ($P = 0.0201$) affected by selected inbred lines in 2004, but no significant ($P = 0.7410$) differences were observed in 2003 (Table 1). Maize inbred lines with low levels of total fumonisins in 2003 included 02C14593, 02C14675, and 02C14585, with concentrations of 0.2, 0.6, and 2.6 µg/g, respectively. Total fumonisins in grain for the check hybrids Oba super 1 and Oba super 2 were 0.4 and 0.2 µg/g, respectively (Table 1). In 2004, compared with the two resistant checks, inbred lines 02C14585, 02C14606, 02C14607, 02C14624, 02C14683, 02C14593, and 02C14640 had lower fumonisin concentrations in harvested grain. Fumonisin concentration for the two check hybrids was about 6.8 µg/g. There was no significant ($P = 0.9300$) inbred $\times$ year interaction for fumonisin concentration. Inbred lines 02C14585, 02C14603, 02C14606, 02C14624, 02C14683, 02C14593, and 02C14640 had consistently lower levels of fumonisins across the 2 years (Fig. 1). Levels of contamination in grains of these inbred lines with low fumonisin concentrations ranged from 0.2 µg/g for 02C14593 to 7.7 µg/g for 02C14683. Fumonisin concentration was significantly correlated with severity of Fusarium ear rot ($R = 0.42$, $P < 0.0001$) and incidence of discolored kernels ($R = 0.39$, $P < 0.0001$) for combined data over years (Fig. 2).

Evaluation under artificial inoculation at Ibadan. In 2003, Fusarium ear rot severity for all 103 inbred lines ranged from 1 (no infection) to 6 (i.e., 51 to 75% ear rot symptom), and about 50% of the entries had severity levels $>10\%$. Maximum disease severity level observed in 2004 on the selected inbred lines was 7 (i.e., $>75\%$). For the selected lines, disease severity was significantly ($P < 0.0001$) higher in 2004 than in 2003, with means of 2.6 and 5.1 in 2003 and 2004, respectively. Selected maize inbred lines did not have significant ($P = 0.3600$) effect on disease severity in 2003, but inbred lines significantly ($P < 0.0001$) affected disease severity in 2004. Among the selected inbred lines, 02C14603, 02C14618, 02C14606, 02C14675, and 02C14683 had disease severity levels lower than those of the resistant checks (Table 2). Of the inbred lines selected in 2003 for re-evaluation, 02C14585, 02C14603, 02C14624, and 02C14683 and had significantly lower levels of disease severity in 2004 compared with the resis-

tant check, Oba super 2 (Table 2). In both years, inbred lines did not have any significant effect on the incidence of discolored kernels in harvested grain (Table 2). However, the incidence of discolored kernels was significantly ($P = 0.0148$) higher in 2004 than in 2003. Inbred line 02C14624 had the lowest incidence of discolored kernels in 2003 with a value of 2.4%. Inbred lines with low incidence of discolored kernels and correspondingly lower ear rot severity were 02C14624, 02C14683, and 02C14651, with incidence levels of 2.4, 13.6, and 15.6%, respectively. In 2004, inbred lines with lower incidence of discolored kernels and correspondingly lower ear rot severities were 02C14603 and 02C14683 with 24.5 and 22.8%, respectively. Incidence of discolored kernels for Oba super 1 and Oba super 2 were 45.2 and 60.2%, respectively (Table 2).

Inbred lines did not significantly ($P > 0.1743$) affect fumonisin contamination in grain in both years. Further, there was no significant ($P = 0.0741$) inbred $\times$ year interaction on total fumonisins. Inbred lines 02C14585, 02C14603, 02C14606, 02C14624, and 02C14683 had consistently lower levels of fumonisin contamination in harvested grain across the 2 years (Fig. 3), with concentration ranging from 0.8 $\mu\text{g/g}$ for inbred line 02C14624 to 7.3 $\mu\text{g/g}$ for the inbred line 02C14606. The corresponding range of total fumonisins for the checks was between 2.4 and 16.2 $\mu\text{g/g}$. Fumonisin concentration in grain was not linearly correlated with *Fusarium* ear rot but was significantly ($R = 0.35$, $P = 0.0007$) linearly correlated to incidence of discolored kernels (Fig. 4).

Weather data. Mean monthly temperatures were generally similar across the 2 years at Ikenne during the experimental

period between June and October, with a range between 25.8 and 27.5°C (Fig. 5A). Monthly rainfall amounts during the same period were consistently lower in 2003 and higher in 2004 except during the month of June in 2003, when up to 346 mm of rain was recorded compared with 249.5 mm in 2004. Rainfall decreased from June to August and increased in September before slightly dropping at harvest in October (Fig. 5A). Mean monthly temperatures at Ibadan were also generally similar across the 2 years during the experimental period between June and October, with a mean of 24.9°C except during the month of July when the mean temperature dropped to 23°C in 2003 (Fig. 5B). Monthly rainfall amounts during the same period were consistently lower in 2003 and higher in 2004 except during the month of September in 2003, when up to 387 mm of rain was recorded compared with 87.8 mm in 2004.

Table 2. *Fusarium* ear rot severity and number of discolored kernels for some selected maize inbred lines artificially inoculated with *Fusarium verticillioides* in field trials at Ibadan, Nigeria, in 2003 and 2004

Inbred line	2003				2004			
	Ear rot severity ^x	Discolored kernels (%)	Discolored kernels (%) ^y	Fumonisin ($\mu\text{g/g}$)	Ear rot severity ^x	Discolored kernels (%)	Discolored kernels (%) ^y	Fumonisin ($\mu\text{g/g}$)
02C14582	2.0	15.3	1.2	25.9	5.5	19.7	1.3	6.1
02C14583	3.0	46.4	1.7	32.4	5.3	45.0	1.7	37.1
02C14585	3.0	36.3	1.6	3.3	4.2	54.5	1.5	3.5
02C14588	3.0	17.9	1.3	20.8	7.0	...	...	...
02C14589	3.0	30.7	1.5	27.6	5.9	37.9	1.6	35.3
02C14593	2.8	31.3	1.5	43.5	4.2	34.1	1.5	25.4
02C14595	2.8	14.3	1.2	30.0	5.6	13.9	1.2	0.5
02C14601	3.0	4.7	0.8	1.1	5.2	88.4	2.0	26.1
02C14603	1.7	29.2	1.5	4.7	3.0	24.5	1.4	3.7
02C14606	2.8	11.8	1.1	3.5	4.9	24.8	1.7	7.3
02C14607	2.8	12.6	1.1	1.3	6.2	24.3	1.4	14.9
02C14611	3.0	11.1	1.1	...	5.5	66.3	1.8	18.7
02C14617	1.8	13.3	1.2	24.3	6.0	57.8	1.8	21.5
02C14618	1.8	18.5	1.3	63.5	4.2	48.7	1.7	29.2
02C14619	2.0	10.6	1.1	55.0	5.5	48.6	1.7	0.8
02C14621	3.0	31.2	1.5	8.2	5.6	44.0	1.7	19.5
02C14624	1.8	2.4	0.5	2.5	4.4	16.0	1.0	0.8
02C14626	2.6	30.9	1.5	22.2	4.9	35.5	1.5	24.2
02C14628	3.0	9.9	1.0	15.0	4.8	38.8	1.4	25.0
02C14629	2.6	66.4	1.8	102.0	5.6	23.6	1.2	0.2
02C14637	2.4	62.7	1.8	87.2	4.5	40.0	1.4	12.6
02C14640	2.4	36.9	1.6	62.0	5.6	52.5	1.7	58.6
02C14643	3.0	42.6	1.6	24.9	4.5	8.9	0.9	12.6
02C14645	2.6	21.9	1.4	...	5.3	27.6	1.5	13.1
02C14646	2.8	20.9	1.3	24.8	5.3	16.3	1.2	2.6
02C14649	2.2	11.8	1.1	3.0	4.4	35.4	1.6	11.0
02C14651	1.6	15.6	2.0	104.0	5.0	57.1	1.8	5.1
02C14654	2.8	38.5	1.6	54.0	4.9	17.7	1.3	24.3
02C14655	2.8	58.0	1.8	51.0	5.1	43.7	1.7	61.2
02C14656	2.8	27.2	1.5	15.4	5.4	25.4	1.3	24.5
02C14661	3.0	37.2	1.6	25.0	6.6	56.5	1.7	2.3
02C14670	2.8	11.2	1.1	8.9	6.1	71.0	1.9	23.2
02C14675	1.8	23.5	1.4	9.9	5.2	38.0	1.6	6.7
02C14677	2.6	14.7	1.2	9.1	3.6	31.9	1.5	8.1
02C14678	2.8	9.1	1.0	7.9	4.9	39.6	1.6	1.0
02C14680	2.6	51.9	1.7	26.7	4.4	41.8	1.6	3.7
02C14683	1.6	13.6	1.2	5.9	3.9	22.8	1.4	6.5
Oba super 1	2.0	24.0	1.4	4.1	3.6	45.2	1.7	2.4
Oba super 2	2.6	5.0	0.8	5.6	6.9	60.0	1.8	16.2
LSD ^z	7.1	...	7.1	115.8	2.5	...	1.2	67.4

^x *Fusarium* ear rot severity was visually assessed at harvest using a 7-class rating scale (33) where 1 = no symptoms, 2 = 1 to 3%, 3 = 4 to 10%, 4 = 11 to 25%, 5 = 26 to 50%, 6 = 51 to 75%, and 7 = 76 to 100% of the ear affected by symptoms.

^y Data transformed to the $\log_{10}$ of the percentage of discolored kernels, i.e., $y = \log_{10} [1 + \text{discolored kernels (\%)}]$. In 2004, ears of inbred line 02C14588 were completely damaged, and no data on incidence of infected kernels were available.

^z LSD = least significant difference at $\alpha = 0.05$.

Fig. 3. Total fumonisin ($FB_1 + FB_2 + FB_3$) concentrations in kernels for some selected maize inbred lines planted at the International Institute of Tropical Agriculture experimental site at Ibadan, Nigeria, during the rainy seasons in 2003 and 2004. Primary ears of plants at the blister (R2) growth stage were artificially inoculated with *Fusarium verticillioides* by injection of a spore suspension through the silk channel. Depicted inbred lines were selected to illustrate consistency in fumonisin production across the 2 years. Asterisk (*) indicates that 02C14651 was not re-evaluated in 2004, and hence no fumonisin concentration data was available.

Fig. 4. Linear correlations of *Fusarium* ear rot severity and incidence of discolored kernels with fumonisin concentration (A and B, respectively) from artificially inoculated ears at the International Institute of Tropical Agriculture experimental site at Ibadan, Nigeria, during the rainy seasons in 2003 and 2004. Disease severity is based on the percentage of each ear with symptoms using a 7-class rating scale in which 1 = no infection, 2 = 1 to 3%, 3 = 4 to 10%, 4 = 11 to 25%, 5 = 26 to 50%, 6 = 51 to 75%, and 7 = 76 to 100% of kernels exhibiting visible symptoms of infection. In each year, each symbol represents data from an individual maize inbred line.

DISCUSSION

In this study, maize inbred lines were evaluated in two agroecological zones in Nigeria to identify inbred lines that are resistant to *Fusarium* ear rot and fumonisin accumulation in grain across the two agroecological zones. Inbred lines with low disease severity and consistently low fumonisin levels in grain were identified across years and locations. This is the first study in Africa to report identification of maize genotypes with resistance to *Fusarium* ear rot and fumonisin accumulation. Inbred lines identified in this study can be useful sources of resistance to these grain yield and quality reducing factors in maize.

The 52 inbred lines did not differ in susceptibility to *Fusarium* ear rot in 2003 but exhibited significant differences in 2004. The lack of differences in ear rot severity can be attributed to the criteria adopted for re-evaluation of selected lines in the subsequent season. Since only 52 inbred lines with disease severity ≤ 3 were selected from among 103 lines, this level of disease severity was not adequate to distinguish relative differences in resistance of inbred lines to *Fusarium* ear rot. When higher disease severity occurred in the subsequent season, differences in susceptibility among the selected inbred lines were apparent. Similar inconsistencies in disease severity and the inability of low disease severity to differentiate genotypes with different levels of resistance has been reported previously (9,17) in evaluation of corn hybrids for resistance to *Fusarium* ear rot and fumonisin contamination.

Despite the differences in disease severity over the 2 years, inbred lines 02C14585, 02C14589, 02C14593, 02C14624, 02C14603, and 02C14683 had consistently low disease severity in both locations. Several inbred lines with low or high disease severity in 2003 did not have a correspondingly similar level of disease severity when re-evaluated in 2004, irrespective of the nature of field infection. This poor reproducibility of severity of *Fusarium* ear rot in maize has been reported previously (17,32). The higher disease severity in 2004 compared with 2003 may be attributed to environmental conditions that favored disease development. Temperatures during this period were similar and suitable for disease development (33) in both years, but more rainfall was recorded for all months during the study period in 2004 except for August, when more rainfall was recorded in 2003 at Ibadan. A further examination of the Ibadan weather data indicated that all days except one during the 2-week inoculation period in August were warm and cloudy in 2004 as opposed to 7 days out of the 2-week inoculation period in 2003. Thus, high rainfall and cloudy conditions during silking and grain formation may have favored disease establishment and

progress observed in 2004. Although dry conditions during silking have been reported to be favorable for disease development (39), our results are similar to previous reports in which wet conditions during silking were reported to be conducive for *Fusarium* ear rot development (8,32).

Total fumonisins and disease severity were positively correlated across years when disease was initiated from natural infection. Similar correlations between fumonisin and ear rot severity have been reported previously (9,26). However, although this relationship describes a general trend, a number of inbred lines (e.g., 02C14654 and 02C14661) with low ear rot severity had very high levels of fumonisin concentration in grain. This observation is similar to previous reports that identified high levels of fumonisins in apparently asymptomatic kernels (5,9) and underpins the need to select for resistance to both fumonisin in grain and ear rot in maize breeding programs. Nonetheless, five inbred lines, 02C14585, 02C14603, 02C14606, 02C14624, and 02C14683, had consistently low fumonisin concentration in grain across years and locations. For example, when disease was initiated from artificial inoculation, inbred lines 02C14624 and 02C14585 had fumonisin concentrations of <2.5 and <4.8 $\mu\text{g/g}$, respectively. Of these five inbred lines, 02C14585, 02C14603, 02C14624, and 02C14683 were among those that also had consistently low ear rot severity in both locations across years. The inbred line 02C14640 had consistently low total fumonisins when evaluated under natural infection but had very high levels of fumonisins when it was evaluated under artificial infection. Such disparities among maize genotypes in fumonisin production under natural and artificial conditions have been reported previously (17). These IITA inbred lines originate from different crosses and thus represent diverse genetic backgrounds. Of the above five inbred lines that had consistently low fumonisin concentration, 02C14624 and 02C14683 are white and their days to silking are 70 and 61, respectively; 02C14585, 02C14603, and 02C14606 have yellow grains with 71, 66, and 62 days to silking, respectively. Inbred lines such as 02C14629, 02C14651, and 02C14661 that had high levels of total fumonisins have similar days to silking as the five inbred lines that had low fumonisin contamination. This may suggest that factors other than days to silking are responsible for the low fumonisin contamination in inbred lines 02C14585, 02C14603, 02C14606, 02C14624, and 02C14683.

The lack of positive correlation between fumonisin concentration and severity of ear rot when disease was initiated from artificial inoculation is inconsistent with earlier studies (9,26). However, our results

are similar to those of Clements et al. (10), who also did not observe consistent correlation between fumonisin production and ear rot severity. It was suggested that factors affecting kernels infection might operate independently from those affecting fumonisin production, which in turn may affect the relationship between these two variables (9,10). Thus, since no differences in fumonisin concentration were observed between the 2 years, while differences in ear rot severity existed between years, no meaningful correlation was observed between fumonisin concentration and severity. In addition, a number of maize inbred lines evaluated under artificial inoculation with very low disease severity had very high total fumonisins, and this further contributed to the lack of correlation between fumonisin concentration and disease severity.

In our study, although no differences in the incidence of discolored kernels were observed among inbred lines across years and locations, total fumonisins in grain was positively correlated with the incidence of discolored kernels across years in

each location. Our observations are similar to those of Desjardins et al. (11), who reported a very high correlation between fumonisin concentration and visible symptoms, with fumonisin levels being higher in symptomatic kernels and lower in symptomless kernels, respectively. Thus, removal of diseased or damaged kernels in grain destined for human consumption or production of livestock feed should significantly reduce fumonisin contamination in maize infected by *F. verticillioides* (1). However, not all diseased and damaged kernels contain higher levels of fumonisin, in part because ear rot symptoms can be caused by *Fusarium* spp. that do not produce fumonisin (19,35). In addition, the low correlation coefficients observed in this study indicate that fumonisin concentration in grain cannot be determined by the severity of *Fusarium* ear rot or incidence of discolored kernels. Therefore, grain must be tested chemically to determine the extent of fumonisin contamination.

This study identified maize inbred lines with very high levels of resistance to Fusa-

Fig. 5. Monthly rainfall and mean temperatures during the experimental period in 2003 and 2004 at International Institute of Tropical Agriculture research sites at Ikenne (A) and Ibadan (B), Nigeria.

rium ear rot and fumonisin accumulation. Two of these inbred lines, 02C14624 and 02C14585, had consistently low fumonisin concentrations of <5 µg/g. These inbred lines can be used directly for the development of hybrids with good levels of resistance to *Fusarium* ear rot adapted to Central and West Africa and as sources of resistance genes in maize breeding programs. Since the inbred lines were derived from different crosses, they may represent different resistance genes that are useful in breeding maize for resistance to *Fusarium* ear rot. These inbred lines are currently being evaluated in Nigeria for resistance to *Aspergillus* ear rot and aflatoxin accumulation.

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