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Effect of northern corn leaf blight severity on Fusarium ear rot incidence of maize

Northern corn leaf blight (NCLB) caused by *Exserohilum turcicum* and Fusarium ear rot caused by *Fusarium verticillioides*, are economically important maize diseases in South Africa. The effect of induced plant stress by NCLB on *F. verticillioides* ear rot and fumonisin production is unknown. Four field trials were conducted during 2016/2017 and 2017/2018 (November and December planting dates) at the Agricultural Research Council – Grain Crops in Potchefstroom (South Africa). Three maize cultivars with varying resistance levels to NCLB were selected (IMP50-10B – susceptible, BG3292 – moderately susceptible, DKC 61-94BR – resistant). NCLB severities were created through eight treatments: TMT1 – maximum control (three fungicide applications); TMT2 – standard control (two fungicide applications) and TMT3 – natural control (not inoculated or sprayed). The remaining treatments were inoculated with a cocktail of five NCLB races (Race 3, 3N, 23, 23N and 13N): TMT4 (five weeks after planting / WAP); TMT5 (five and six WAP); TMT6 (five, six and seven WAP); TMT7 (six and seven WAP); and TMT8 (seven WAP). Maize ears were naturally infected with *F. verticillioides*. Fifteen random plants were labelled at dent stage and NCLB severity (%), area under the disease progress curve, ear rot diseased area, ear rot severity (%), ear rot incidence (%) and total fumonisins ($FB^1+FB^2+FB^3$; ug/kg) were established. Low levels of cob rot severity and fumonisins were obtained in all four trials. NCLB severity did not affect ear rot related parameters measured. Mean fumonisin levels were below the South African tolerance levels. Fumonisin concentrations differed significantly between cultivars but was not affected by NCLB severity or the cultivar x treatment interaction.

Significance:

- This is the first study to investigate the effect of NCLB severity as a predisposing factor of ear rot incidence and severity of maize.
- The study confirmed that ear rot incidence and severity are not impacted by secondary stressors induced by NCLB, and that the cultivation of NCLB-resistant varieties would not bring about lower ear rot incidences.

Introduction

Northern corn leaf blight (NCLB), caused by *Exserohilum turcicum* (Pass.) K.J. Leonard and E.G. Suggs, is one of the most prominent leaf diseases of maize (*Zea mayze*) in South Africa. This disease occurs predominantly in the KwaZulu-Natal production areas and is particularly severe under irrigation systems.[1] Typical yield losses attributed to the disease generally range between 15% and 30%, but yield losses of up to 50% have been documented.[2,3] A potential yield reduction of 2–8% exists for every 10% increase in disease severity.[4,5]

Internationally, reference has been made to the development of secondary complications in maize due to severe leaf desiccation owing to infection by foliar pathogens. Latterell and Rossi[6] reported severe lodging and up to 100% yield loss due to stalk deterioration of maize brought about by grey leaf spot (*Cercospora zeae-maydis* Tehon & E.Y. Daniels). Stalk deterioration was attributed to the covering of the photosynthetic surfaces of the plant by lesions, which led to extreme water loss, but no report was given on whether stalk rot pathogens were conversely responsible for the stalk deterioration. NCLB has similarly been shown to potentially predispose maize plants to attack by both stalk[7,8] and root rot pathogens[9] when severe enough, by inducing sufficient stress in plants to weaken their natural defence mechanisms.

Despite the presence of Fusarium ear rot over the whole maize production area, the disease only gained importance when the mycotoxin-producing capabilities of its causal organism became evident.[10] Fusarium ear rot caused by *Fusarium verticillioides* (Sacc.) Nirenberg (syn. *Fusarium moniliforme* J. Sheldon, *Fusarium* section Liseola)[11], negatively affects crop yield and quality. The species can produce secondary metabolites (fumonisins) associated with a wide range of noxious effects on humans and livestock upon ingestion.[12] Locally, high natural infection rates of *F. verticillioides* and resulting fumonisin concentrations were reported in warmer production areas including the Northern Cape, North-West and Free State Provinces of South Africa.[13] South African regulations stipulate a tolerance of 4000 μg/kg for fumonisins in maize grain intended for further processing, while processed products that are ready for human consumption may not contain more than 2000 μg/kg of fumonisins.[14]

High temperatures, drought, poor fertilisation and stiff competition for nutrients are some of the conditions known to weaken the plant's natural defence, which predisposes the plant to increased ear rot infections.[15,16] These conditions can promote colonisation by mycotoxigenic *Fusarium* spp. in maize grain during the growing season. Although it is commonly accepted that severe leaf diseases can potentially result in an increase in stalk rot incidence, it is not yet established whether a similar association could be drawn for ear rot infections (such as *F. verticillioides*) and subsequent fumonisin production in maize grain.

In the course of 2016, the Agricultural Research Council – Grain Crops, initiated a project in which field trials were conducted over a 2-year period to ascertain to what extent NCLB severity would impact on the manifestation of secondary diseases in maize cultivars with differing NCLB resistance statuses. Key to these trials was that NCLB would be the only disease introduced artificially, whilst the response of the cultivars pertaining to the development of secondary diseases through natural infection would be monitored. Of interest in the current study was whether NCLB-resistant varieties would assist in minimising the risk associated with ear rot infections and

subsequent severity, and whether such a cultivar trait could be utilised in an integrated pest management strategy to not only reduce inoculum pressure, but also to minimise input costs. Weighing the cost associated with fungicide applications against the benefit of both natural resistance of NCLB-resistant varieties and the additional benefits of reduced ear rot infections potentially provided by NCLB-resistant varieties, will be useful to producers, allowing for informed decisions to be made regarding which cultivars to plant.

The current study reports on the observed influence of northern corn leaf blight severity on *F. verticillioides* ear rot infection and fumonisin production in the grain of three South African maize hybrids with varying NCLB disease resistance in the field.

Materials and methods

Inoculum preparation, field trials and treatment application

The five *E. turcicum* races (Race 3, 3N, 23, 23N and 13N) used in this study were ascertained through replicated growth chamber studies by means of differential sets of varying backgrounds.[17] NCLB races were inoculated into maize seedlings and re-isolated from lesions. Mycelial plugs of each race were grown on potato dextrose agar for 2 weeks before mycelial plugs were transferred to autoclaved maize kernels in fruit flasks prepared according to Flett and McLaren[18]. Flasks were incubated at room temperature and shaken daily. After 2 weeks, the contents of the flasks were dried for 3 days after which the maize kernels were ground in a standard maize mill.[19] The races were kept separate at all times and the mill was thoroughly cleaned after each isolate batch. After milling, equal amounts of each of the 10 isolates were added and thoroughly mixed to obtain an inoculation mixture.

Four field trials were conducted during 2016/2017 and 2017/2018. Two trials were planted during November and December, during each growing season, on the grounds of the Agricultural Research Council – Grain Crops (ARC-GC), Potchefstroom (North West Province; 26.743594.27 S, 27.069491 E). Three maize cultivars with varying resistance levels to NCLB were selected based on their performance in the national cultivar evaluation trials of ARC-GC under natural NCLB infection and included IMP50-10B (susceptible), BG3292 (moderately susceptible) and DKC 61-94BR (resistant). Various levels of NCLB were created through the application of eight treatments, including three control treatments: TMT1 – maximum control (three fungicide applications); TMT2 – standard control (two fungicide applications); TMT3 – natural control (not inoculated or sprayed). The remaining treatments were inoculated at various dates with the cocktail consisting of five NCLB races that included Race 3, 3N, 23, 23N and 13N: TMT4 – inoculated five weeks after planting (WAP); TMT5 – inoculated five and six WAP; TMT6 – inoculated five, six and seven WAP; TMT7 – inoculated six and seven WAP and TMT8 – inoculated 7 WAP. Each plant was inoculated with approximately 6 g inoculum placed in the whorl. TMT1 and TMT2 received two foliar fungicide formulations used in rotation every season i.e. Abacus® (pyraclostrobin/epoxiconazole – 1L/ha, BASF SA, Johannesburg, South Africa) and Sparta SC (flusilazole/carbendazim – 500 mL/ha, Villa Crop Protection, Johannesburg, South Africa) together with an adjuvant Picanta (150 mL/ha, Villa Crop Protection). Fungicides were applied at 3-week intervals, with TMT1 receiving its first fungicide application at V8 leaf stage and TMT2 at flowering. Fungicides were applied using a CO_2 gas operated knapsack sprayer and a four-nozzle (flat fan; 0.9 m spaced) boom. The knapsack sprayer was calibrated to a spray volume of 78 L/ha.

Each trial was planted in a split-plot design with treatment as the main plot and cultivar as the sub-plot, replicated three times. Each sub-plot consisted of two border rows flanking four rows per cultivar with 0.9-m inter-row spacing, 15 m in length. Intra-row spacing was 30 cm, with two kernels planted per hill. Four weeks after planting the plants were thinned out to one plant per hill. Fertiliser was applied according to soil analysis (150 kg/ha 3:2:1, 200 kg/ha LAN top dressing – 6 weeks after planting). Callisto (mesotrione – 480 g/L Syngenta SA, Centurion, South Africa) and Dual (s-metolachlor – 915 g/L, Syngenta SA) were applied pre-emergence and Basagran® (bendioxide – 480 g/L, BASF SA) was applied post-emergence to prevent weed encroachment. Directly after inoculation, approximately 15 mm water was applied through overhead irrigation over a 4-h period. Thereafter irrigation was supplied supplementary to rainfall as needed throughout the season to ensure that the trials received water weekly. Maize ear rot was initiated from natural infection by *F. verticillioides*. Weather data were captured by the ARC weather station situated on the Potchefstroom research farm.

Screening and sampling

Fifteen randomly selected plants were labelled in the first of the four middle rows of each plot and screened for NCLB development at V12, flower, milk, soft dough and dent stage.[20] Disease was quantified as the percentage infected leaf material per plant per plot using a modified scale of 0.0, 0.5, 1.0, 5.0 10.0, 25.0, 50, 70 and ≥85%.[19,21] Area under the disease progress curve (AUDPC) was determined for each plot.

At physiological maturity, ears from the 15 marked plants were harvested separately from the remaining plants in the allocated row and screened for ear rot severity. Ear rot incidence and area affected (cm^2) were established. Area affected (cm^2) was established by using a 1 cm x 1 cm transparent plastic grid placed over the ear and the number of squares in which diseased areas could be observed, were counted. The ears were threshed and the kernel weight determined. A representative milled sample from each plot was stored at -20 °C until determination of total fumonisin concentrations. Fumonisins were analysed using the HPLC-VICAM method.[22] Fumonisin standards were obtained from the Cape Peninsula University of Technology. A standard curve was generated by evaporating standards and reconstitution with a calibration standard solution ranging from 0.31 to 5 μg/kg. Fluorescence was performed at excitation and emission wavelengths of 335 nm and 440 nm, respectively, using a Waters 2475 multi λ fluorescence detector equipped with a Symmetry C18 (5 μm 3.9 x 150 mm) analytical column (Waters, Milford, USA). The LOD of the method used was 16 μg/kg and R^2 values were ≥99%. Total fumonisins were determined as the sum of FB1+FB2+FB3.

The remainder of the plants in the allocated row were harvested and yield established per plot by combining the kernel weight of the 15 marked plants and the remainder of the plants in the designated row. Yield was calculated at 12.5% moisture (t/ha).

Statistical analysis

Each trial was designed as a randomised block design with three replicates. The treatment design was a split-plot with the eight treatments and four cultivars randomised within each whole plot. Data of the various parameters measured from each trial were subjected to a split-plot analysis of variance to test for significant differences between treatments, cultivars and the interaction. Means of significant source effects were separated using Fisher's protected t-least significant difference (LSD) at a 5% significance level. In cases in which the interaction effect was non-significant, but either of the main effects indicated significant differences, treatment x cultivar interaction means were separated using Fisher's unprotected t-LSD.[23] All the analyses were conducted using GenStat for Windows 18th edition. Regression analyses were performed to ascertain whether a relationship (linear or non-linear) existed between NCLB disease and ear rot parameters measured. Regressions were performed per cultivar, per trial.

Results

As environmental conditions during the flowering period determine the potential for ear rot development, reigning conditions during this period were of interest in the current study. Temperature and rainfall data during January (2017 and 2018) coincided with the general flowering period of the November planting dates (2016 and 2017), whilst February (2017 and 2018) coincided with that of the December planting dates (2017 and 2018) (Table 1). The 2016/2017 season experienced higher rainfall (658 mm) than that of 2017/2018 (414.27 mm), with the majority recorded during the month of February (2017). Average maximum temperatures

were slightly higher during both January and February of 2018 than the same period during 2017. Temperatures for the remainder of the months for both seasons were very similar with the exception of December 2016, which was in general warmer than December 2017.

Ears of the 15 marked plants were inspected for all types of ear rot. *Fusarium verticillioides* ear rot was, however, the only type of ear rot present in all four trials. No Gibberella ear rot (*Gibberella zeae*) or Diplodia ear rot (*Stenocarpella maydis*) was observed.

Table 1: Weather data for the period October to July of the 2016/2017 and 2017/2018 seasons

		Temperature (°C)				Rainfall (mm)		
		Maximum		Minimum				
		2016/2017	2017/2018	2016/2017	2017/2018		2016/2017	2017/2018
October	Average	30.17	26.4	11.86	11.4	Total	55.12	56.13
	Highest	36.86	32.4	17.8	17.1	Highest	26.92	23.88
	Lowest	21.7	18	4.95	5.1			
November	Average	29.67	29.1	15.48	12.7	Total	94.74	69.34
	Highest	36.01	34.5	20.87	17.3	Highest	28.19	18.54
	Lowest	19.79	17.1	10.92	4.5			
December	Average	32.62	29.3	16.97	15.7	Total	73.3	62.48
	Highest	36.48	33.4	18.98	19.1	Highest	6.84	13.72
	Lowest	29.69	15.8	13.5	10.3			
January	Average	28.42	31	16.46	16.1	Total	53.33	47.24
	Highest	32.46	36.6	19.57	20.3	Highest	5.73	12.45
	Lowest	21.05	24.4	11.12	9.3			
February	Average	26.51	27.7	16.82	15.6	Total	225.55	68.33
	Highest	30.42	31.5	19.81	17.7	Highest	80.26	14.99
	Lowest	19.6	20.5	14.53	11.8			
March	Average	27.93	27.5	14.69	14.6	Total	33.78	58.93
	Highest	31.12	31.1	18.81	19.2	Highest	27.69	21.84
	Lowest	18.9	17.6	9.13	10.2			
April	Average	25.42	25.3	10.37	11.1	Total	46.23	35.56
	Highest	32.44	29	16.27	16.1	Highest	14.73	10.67
	Lowest	17.32	19.7	3.35	5.6			
May	Average	22.51	22.8	4.85	4.9	Total	10.67	11.18
	Highest	25.67	26.4	9.89	12.1	Highest	8.64	9.91
	Lowest	12.18	16.4	-1.14	1.3			
June	Average	21.91	21.6	3.15	1.5	Total	65.71	0
	Highest	25.47	25.6	8.43	4.6	Highest	2.55	0
	Lowest	14.19	17.5	-4.06	-2			
July	Average	22.19	19.3	3.47	1.1	Total	0.25	5.08
	Highest	26.1	26.3	9.73	7.8	Highest	0.25	2.03
	Lowest	16.88	14.2	-3.35	-6			
						Total seasonal rainfall (mm)	**658.68**	**414.27**

Northern corn leaf blight severity and AUDPC

Aside from the November planting of 2017/2018, the various treatments allowed for a range of NCLB severity levels to be produced within each trial (Tables 2–5) that allowed a comprehensive view of the possible impacts that different severity levels have on ear rot development. During 2017/2018, untimely or continuous rainfall was experienced, which resulted either in fungicide applications not being applied at the optimum time or fungicide that was applied being washed off after application. This resulted in little to no control in TMT1 and TMT2, especially in the November 2017/2018 planting (Table 4). Although DKC61-94BR was included as the resistant cultivar, the use of a mixture of NCLB races lead to similar NCLB severities in this hybrid compared to that of the more susceptible hybrids (BG3292 and IMP50-10B). Average NCLB disease severities realised within the eight treatments accordingly were in the ranges of 0.7–70.7% (Table 2), 6.7–60.1% (Table 3), 38.5–61.3% (Table 4) and 16.7–55.5 % (Table 5) in the various trials. Both cultivar and treatment differed significantly in all four trials, with the cultivar x treatment interaction differing significantly in the December (2016/2017) and November (2017/2018) trials. Of the three cultivars included, DKC61-94BR consistently gave the lowest NCLB severity, whist TMT5 yielded the greatest NCLB severities in three of the trials. The general trend for AUDPC data generated mirrored that of NCLB severities achieved at dent stage. With the exception of the November 2016/2017 trial (Table 2), cultivar differences were observed in the AUDPC data. In all three trials, DKC61-94BR produced significantly lower AUDPC values (Tables 3–5). Similarly to the NCLB severity, TMT5 yielded the highest AUDPC in three of the trials (Tables 2, 4 and 5). Average AUDPCs achieved within the eight treatments in the various trials were in the ranges 24–1465 (Table 2), 227–1005 (Table 3), 703–1198 (Table 4) and 103–771 (Table 5). Sufficient ranges of AUDPCs were generated to effectively evaluate the potential impact of NCLB on ear rot severity.

Ear rot affected area

In general, low levels of area affected were observed in all four trials. Cultivar differences were observed in three of the four trials (Tables 2–4). BG3292 attained significantly greater ear rot affected areas in all three trials, which varied between 3.7 cm^2 (November, 2016/2017 planting; Table 2) and 10.7 cm^2 (November, 2017/2018 planting; Table 4). The remaining two cultivars had similar ear rot affected areas in all three trials. Only in one trial (November, 2016/2017 planting; Table 2) did the treatments result in significant differences, with TMT2 yielding a significantly greater average ear rot affected area (2.9 cm^2) over the three cultivars included. A significant cultivar x treatment interaction was observed in the December 2017/2018 season, with TMT8, TMT1, TMT5, TMT2 and TMT6 of BG3292 achieving the highest area affected (Table 4).

Ear rot severity

Ear rot severity, similar to ear rot affected area, was very low in all four trials with trial means of 1.1%, 0.6%, 3.6% and 2.62%, respectively (Tables 2–5). Cultivar differences were observed in both the 2016/2017 trials as well as the November 2017/2018 planting trial, with BG3292 yielding significantly greater ear rot severity in all three trials (2.4%, 1.4% and 6.3% respectively; Tables 2–4). Neither the treatment effect nor the cultivar x treatment interaction was significant.

Ear rot incidence

Cultivar differences were observed in both the 2016/2017 trials as well as the November 2017/2018 trial. In all cases, BG3292 gave significantly greater ear rot incidence, which varied from 31.7% of the ears having some degree of ear rot (November 2017/2018 planting; Table 4) to 51% of the ears in the November 2016/2017 planting (Table 1).

Fumonisin

The average fumonisin concentration detected per trial in the sampled material ranged between 2 μg/kg (December 2016/2017 planting; Table 3) and 235 μg/kg (November 2017/2018 planting; Table 4). Cultivar differences occurred in the two 2016/2017 trials (Tables 2 and 3) as well as the November 2017/2018 planting (Table 4). BG3292 achieved the highest average fumonisin concentration in the grain in all three trials (3.8, 2.9 and 381 μg/kg, respectively). Significant differences between treatments in terms of fumonisin concentrations in the grain were only observed for the 2016/2017 November planting (Table 2), with TMT1 (5.3 μg/kg) followed by TMT8 (4 μg/kg). No significant cultivar x treatment interaction was observed. Fumonisin concentrations measured did not exceed 1407 μg/kg (Table 5) in any of the trials.

Regression analyses

Regression analyses were initially conducted against NCBL severity (at dent stage) and AUDPC for each of the ear rot related parameters. This was done per cultivar per season. As none of the regression analyses (either linear or non-linear) was significant (data not shown), the possibility was considered that external factors (other than NCLB severity) had contributed to the random effects observed over seasons. Data were accordingly pooled across the trials for each treatment, as pooling of data aids in minimising any effect that external factors, not linked to NCLB severity, might have had on the ear rot parameters measured. Linear, exponential and polynomial regression analyses were again conducted. Ear rot incidence was the only parameter that demonstrated a potential relationship with NCLB severity ($R^2 = 0.67$; Figure 1a) and AUDPC ($R^2 = 0.65$; Figure 2a) for IMP50-10B; however, the relationship was not significant in either circumstance.

Discussion

The objective of this study was to establish whether the ear rot severity observed in three maize cultivars with varying degrees of NCLB resistance, would be impacted by NCLB severity suffered during the growing season. Multiple season trials were conducted together with an intensive *E. turcicum* inoculation approach to ensure that different degrees of NCLB were created to assess whether NCLB would predispose the maize plant to greater ear rot infections and subsequent fumonisin production in maize grain. Despite the fact that high levels of NCLB were achieved in all four trials, very low levels of ear rot (less than 11% obtained in the November 2017/2018 planting) were nonetheless observed. Fumonisin levels detected in the grain were also well below the accepted 2000 μg/kg concentration for grain. The averages in the trials varied between 2 μg/kg and 235 μg/kg.

Internationally, it is accepted that *F. verticillioides* gains access to the ear by one or more of three main access pathways: (1) fungal spores germinating on the silks and then fungal mycelia growing down the silks to infect the kernels and the ear (rachis); (2) systemic infection of the ear through infected stalks that generate infected seeds and (3) through wounds on the ear generated by insects, birds or hail damage.[11,24] It is also common knowledge that ear rot incidence and severity as well as associations with mycotoxins vary with environmental conditions, genotype, and location.[11,25] In general, higher temperatures and drier weather during flowering (26 °C and higher), higher temperatures during kernel maturation, more rainfall before harvest, drought stress as well as insect damage stress are factors known to increase ear rot severity and fumonisin content at harvest.[11,26,27] Weather conditions during flowering are, however, considered critical for primary infection as well as for toxin synthesis in grain.[28-30] For the current study, it was imperative that moist conditions were maintained throughout the duration of trials to ensure effective NCLB infection and subsequent high NCLB disease severity. Although leaf blight data indicate high and variable levels of disease, the extremely low ear rot levels raised the question of whether these low levels were due to the absence of epidemiologically competent inoculum, the absence of predisposition or possibly the end result of inherent cultivar resistance.

Table 2: Northern corn leaf blight (NCLB) and ear rot related data generated for the first planting trial during 2016/2017

	TMT	Cultivar						*TMT mean*	
		BG3292		DKC61-94BR		IMP50-10B			
NCLB severity (%)*	1	2.0	j	2.3	j	1.5	j	*1.9*	*e*
F prob Treatment < 0.001	2	0.9	j	1.2	j	0.1	j	*0.7*	*e*
LSD Treatment $_{(P=0.05)}$ = 7.49	3	18.0	i	19.7	hi	40.9	ef	*26.2*	*d*
F prob Cultivar < 0.001	4	56.9	cd	58.0	cd	73.3	ab	*62.8*	*b*
LSD Cultivar $_{(P=0.05)}$ = 5.03	5	68.1	bc	64.4	bcd	76.9	ab	*69.8*	*ab*
F prob Cultivar x Treatment = 0.189	6	65.0	bcd	65.3	bcd	81.9	a	*70.7*	*a*
LSD Cultivar x Treatment $_{(P=0.05)}$ =13.47	7	52.8	de	34.0	fg	58.3	cd	*48.4*	*c*
	8	24.9	ghi	26.4	ghi	32.9	fgh	*28.1*	*d*
Cultivar mean		*36.1*	*b*	*33.9*	*b*	*45.7*	*a*	*38.6*	
AUDPC	1	8.6	g	149.1	fg	19.5	g	*59.1*	*d*
F prob Treatment <0.001	2	13.5	g	32.0	g	25.7	g	*23.7*	*d*
LSD Treatment$_{(P=0.05)}$ = 3.28	3	395.6	efg	311.9	fg	588.6	def	*432.0*	*c*
F prob Cultivar =0.48	4	1504.0	a	1287.3	abc	1544.0	a	*1445.1*	*a*
LSD Cultivar $_{(P=0.05)}$ =159.7	5	1568.3	a	1420.7	ab	1405.3	ab	*1464.8*	*a*
F prob Cultivar x Treatment = 0.983	6	1424.3	ab	1294.7	abc	1293.3	abc	*1337.4*	*a*
LSD Cultivar x Treatment $_{(P=0.05)}$ 447.8	7	964.2	bcd	848.9	cde	925.8	cd	*913.0*	*b*
	8	430.1	efg	208.9	fg	252.3	fg	*297.1*	*cd*
Cultivar mean		*789.0*		*694.0*		*757.0*		*746.5*	
Ear rot diseased area (cm²)	1	4.5	ab	0.3	d	0.5	cd	*1.7*	*b*
F prob Treatment = 0.047	2	5.1	a	0.1	d	3.4	ab	*2.9*	*a*
LSD Treatment $_{(P=0.05)}$ = 1.033	3	3.7	ab	0.6	cd	0.2	d	*1.5*	*b*
F prob Cultivar < 0.001	4	3.5	ab	0.3	d	0.3	d	*1.4*	*b*
LSD Cultivar $_{(P=0.05)}$ = 0.86	5	2.6	bc	0.5	cd	0.3	cd	*1.1*	*b*
F prob Cultivar x Treatment =0.786	6	3.1	ab	0.0	d	0.0	d	*1.1*	*b*
LSD Cultivar x Treatment $_{(P=0.05)}$ = 2.193	7	3.5	ab	0.8	cd	0.3	d	*1.5*	*b*
	8	3.5	ab	0.1	d	0.5	cd	*1.4*	*b*
Cultivar mean		*3.7*	*a*	*0.7*	*b*	*0.3*	*b*	*1.6*	
Ear rot severity (%)	1	2.7	ab	0.2	ef	0.3	def	*1.1*	*b*
F prob Treatment = 0.013	2	2.8	ab	0.1	ef	4.1	a	*2.3*	*a*
LSD Treatment $_{(P=0.05)}$ =0.802	3	2.6	ab	0.5	def	0.1	ef	*1.1*	*b*
F prob Cultivar < 0.001	4	1.7	bcdef	0.2	ef	0.2	ef	*0.7*	*b*
LSD Cultivar $_{(P=0.05)}$ = 0.702	5	2.3	bc	0.6	cdef	0.3	ef	*1.1*	*b*
F prob Cultivar x Treatment = 0.19	6	1.8	bcde	0.0	ef	0.0	ef	*0.6*	*b*
LSD Cultivar x Treatment $_{(P=0.05)}$ = 1.773	7	2.0	bcd	0.6	cdef	0.3	def	*1.0*	*b*
	8	3.1	ab	0.0	f	0.6	cdef	*1.2*	*b*
Cultivar mean		*2.4*	*a*	*0.7*	*b*	*0.3*	*b*	*1.1*	
Ear rot incidence (%)	1	50.9	a	21.5	bc	8.1	cd	*26.9*	
F prob Treatment = 0.577	2	50.0	a	5.1	cd	8.1	cd	*21.1*	
LSD Treatment $_{(P=0.05)}$ = 11.85	3	63.1	a	15.5	bcd	3.7	cd	*27.5*	
F prob Cultivar < 0.001	4	49.2	a	12.3	bcd	4.2	cd	*21.9*	
LSD Cultivar $_{(P=0.05)}$ = 6.91	5	27.7	b	16.9	bcd	7.2	cd	*17.3*	
F prob Cultivar x Treatment = 0.212	6	54.6	a	2.8	cd	0.0	d	*19.1*	
LSD Cultivar x Treatment $_{(P=0.05)}$ = 19.29	7	51.3	a	15.5	bcd	4.3	cd	*23.7*	
	8	60.9	a	6.0	cd	5.7	cd	*24.2*	
Cultivar mean		*51.0*	*a*	*12.0*	*b*	*5.2*	*b*	*22.7*	
Fumonisin (μg/kg)	1	9.6	a	0.8	e	5.5	abc	*5.3*	*a*
F prob Treatment = 0.007	2	1.0	de	0.8	e	2.0	cde	*1.3*	*c*
LSD Treatment $_{(P=0.05)}$ = 2.193	3	3.2	bcde	0.8	e	1.4	de	*1.8*	*c*
F prob Cultivar =0.002	4	5.0	bcd	0.3	e	0.3	e	*1.9*	*bc*
LSD Cultivar $_{(P=0.05)}$ =1.51	5	1.6	cde	0.3	e	4.1	bcde	*2.0*	*bc*
F prob Cultivar x Treatment = 0.124	6	0.9	e	0.3	e	0.6	e	*0.6*	*c*
LSD Cultivar x Treatment $_{(P=0.05)}$ = 4.014	7	2.3	cde	2.1	cde	0.3	e	*1.6*	*c*
	8	7.1	ab	2.9	bcde	2.1	cde	*4.0*	*ab*
Cultivar mean		*3.8*	*a*	*2.1*	*b*	*1.0*	*b*	*2.3*	

**at dent stage*
AUDPC, area under disease progress curve; LSD, least significant difference

Table 3: Northern corn leaf blight (NCLB) and ear rot related data generated for the second planting trial during 2016/2017

	TMT	Cultivar						*TMT mean*	
		BG3292		DKC61-94BR		IMP50-10B			
NCLB severity (%)*	1	9.1	g	1.9	g	9.0	g	*6.7*	*e*
F prob Treatment < 0.001	2	29.7	de	6.2	g	15.3	fg	*17.1*	*d*
LSD Treatment $_{(P=0.05)}$ = 9.15	3	58.6	c	26.1	def	60.0	bc	*48.2*	*c*
F prob Cultivar < 0.001	4	66.9	abc	38.3	de	75.6	a	*60.3*	*a*
LSD Cultivar $_{(P=0.05)}$ = 5.1	5	73.3	ab	40.0	d	66.9	abc	*60.1*	*a*
F prob Cultivar x Treatment = 0.033	6	64.7	abc	35.6	de	75.6	a	*58.6*	*ab*
LSD Cultivar x Treatment $_{(P=0.05)}$ = 14.46	7	61.1	abc	25.0	ef	65.6	abc	*50.6*	*bc*
	8	64.7	abc	32.5	de	67.2	abc	*54.8*	*abc*
Cultivar mean		*53.5*	*a*	*25.7*	*b*	*54.4*	*a*	*44.5*	
AUDPC	1	293.2	ef	41.4	g	345.7	e	*226.8*	*c*
F prob Treatment <0.001	2	604.1	d	101.4	fg	747.5	d	*484.3*	*b*
LSD Treatment $_{(P=0.05)}$ = 197.4	3	1115.0	bc	190.8	efg	1300.6	ab	*868.8*	*a*
F prob Cultivar < 0.001	4	1265.7	bc	247.1	efg	1501.3	a	*1004.7*	*a*
LSD Cultivar $_{(P=0.05)}$ = 67.5	5	1222.8	bc	317.7	ef	1279.3	abc	*939.9*	*a*
F prob Cultivar x Treatment < 0.001	6	1203.3	bc	205.6	efg	1259.0	abc	*889.3*	*a*
LSD Cultivar x Treatment $_{(P=0.05)}$ = 243.6	7	1216.3	bc	209.0	efg	1210.3	bc	*878.6*	*a*
	8	1223.3	bc	346.1	e	1047.7	c	*872.4*	*a*
Cultivar mean		*1018.0*	*b*	*207.4*	*c*	*1086.4*	*a*	*771.0*	
Ear rot diseased area (cm^2)	1	5.0	abcde	0.0	e	0.0	e	*1.7*	
F prob Treatment = 0.253	2	3.8	bcde	1.7	de	8.0	abc	*4.5*	
LSD Treatment $_{(P=0.05)}$ = 3.936	3	3.6	bcde	0.0	e	0.0	e	*1.2*	
F prob Cultivar < 0.001	4	7.2	abcd	2.0	cde	0.0	e	*3.1*	
LSD Cultivar $_{(P=0.05)}$ = 2.119	5	10.4	a	1.7	de	3.7	bcde	*5.3*	
F prob Cultivar x Treatment = 0.405	6	8.4	ab	2.3	cde	4.7	abcde	*5.1*	
LSD Cultivar x Treatment $_{(P=0.05)}$ = 6.089	7	3.9	bcde	0.0	e	2.0	cde	*2.0*	
	8	9.0	ab	0.8	e	0.0	e	*3.3*	
Cultivar mean		*6.4*	*a*	*1.1*	*b*	*2.3*	*b*	*3.3*	
Ear rot severity (%)	1	0.7	bcd	0.0	d	0.0	d	*0.2*	
F prob Treatment =0.43	2	0.7	bcd	0.0	d	1.1	bcd	*0.6*	
LSD Treatment $_{(P=0.05)}$ = 0.8203	3	0.9	bcd	0.0	d	0.0	d	*0.3*	
F prob Cultivar < 0.001	4	2.7	a	0.2	d	0.0	d	*0.9*	
LSD Cultivar $_{(P=0.05)}$ = 0.4138	5	1.6	abc	0.1	d	0.3	d	*0.7*	
F prob Cultivar x Treatment = 0.094	6	1.6	ab	0.1	d	0.4	cd	*0.7*	
LSD Cultivar x Treatment $_{(P=0.05)}$ = 1.2198	7	0.6	bcd	0.0	d	0.1	d	*0.2*	
	8	2.5	a	0.1	d	0.0	d	*0.9*	
Cultivar mean		*1.4*	*a*	*0.1*	*b*	*0.2*	*b*	*0.6*	
Ear rot incidence (%)	1	17.8	de	0.0	f	0.0	f	*5.9*	
F prob Treatment = 0.121	2	33.2	bcd	3.3	ef	10.0	ef	*15.5*	
LSD Treatment $_{(P=0.05)}$ = 9.12	3	46.3	ab	0.0	f	0.0	f	*15.4*	
F prob Cultivar < 0.001	4	40.0	abc	5.6	ef	0.0	f	*15.2*	
LSD Cultivar $_{(P=0.05)}$ = 5.78	5	26.0	cd	3.3	ef	6.7	ef	*12.0*	
F prob Cultivar x Treatment = 0.234	6	40.6	abc	5.8	ef	7.5	ef	*18.0*	
LSD Cultivar x Treatment $_{(P=0.05)}$ 15.75	7	31.0	bcd	0.0	f	3.3	ef	*11.4*	
	8	49.9	a	9.1	ef	0.0	f	*19.7*	
Cultivar mean		*35.6*	*a*	*3.4*	*b*	*3.5*	*b*	*14.1*	
Fumonisin (μg/kg)	1	1.1	abc	0.4	abc	0.8	abc	*0.8*	
F prob Treatment = 0.683	2	4.2	abc	0.5	ac	6.0	ab	*3.6*	
LSD Treatment $_{(P=0.05)}$ = 4.229	3	0.3	abc	0.1	c	0.1	c	*0.2*	
F prob Cultivar = 0.014	4	4.9	abc	0.3	abc	0.2	abc	*1.8*	
LSD Cultivar $_{(P=0.05)}$ = 1.854	5	5.2	abc	0.2	c	3.5	abc	*3.0*	
F prob Cultivar x Treatment = 0.613	6	2.2	abc	0.2	abc	1.5	abc	*1.3*	
LSD Cultivar x Treatment $_{(P=0.05)}$ = 5.821	7	1.1	abc	0.9	abc	6.0	a	*2.7*	
	8	4.3	abc	0.1	c	2.8	abc	*2.4*	
Cultivar mean		*2.9*	*a*	*0.3*	*b*	*2.6*	*a*	*2.0*	

**at dent stage*

AUDPC, area under disease progress curve; LSD, least significant difference

Table 4: Northern corn leaf blight (NCLB) and ear rot related data generated for the first planting trial during 2017/2018

	TMT	Cultivar						*TMT mean*	
		BG3292		DKC61-94BR		IMP50-10B			
NCLB severity (%)*	1	47.2	cdef	38.3	ghijkl	42.8	defghij	*42.8*	*c*
F prob Treatment = 0.003	2	46.7	cdefgh	39.7	fgijkl	46.4	cdefgh	*44.3*	*c*
LSD Treatment $_{(P=0.05)}$ = 9.216	3	50.5	bcde	34.7	jkl	51.3	bcd	*45.5*	*bc*
F prob Cultivar < 0.001	4	43.1	defghij	41.0	efghijkl	44.4	cdefghij	*42.8*	*c*
LSD Cultivar $_{(P=0.05)}$ = 2.036	5	64.7	a	59.3	ab	59.8	ab	*61.3*	*a*
F prob Cultivar x Treatment = 0.046	6	54.5	bc	48.5	def	57.6	ab	*53.6*	*ab*
LSD Cultivar x Treatment $_{(P=0.05)}$ = 10.105	7	41.5	defghijk	34.8	jl	39.3	fghijkl	*38.5*	*c*
	8	45.0	cdefghi	41.4	defghijkl	46.8	cdefg	*44.4*	*bc*
Cultivar mean		*49.1*	*a*	*42.2*	*b*	*48.6*	*a*	*46.6*	
AUDPC	1	1114.0	abcde	1064.0	acdef	1288.0	ab	*1155.0*	
F prob Treatment = 0.183	2	721.0	ef	680.0	f	707.0	ef	*703.0*	
LSD Treatment $_{(P=0.05)}$ = 410.1	3	1011.0	abcdef	916.0	abcdef	989.0	abcdef	*972.0*	
F prob Cultivar = 0.002	4	1168.0	abcd	1071.0	abcdef	1179.0	abcd	*1139.0*	
LSD Cultivar $_{(P=0.05)}$ = 63.5	5	1168.0	abcd	1111.0	bcde	1314.0	a	*1198.0*	
F prob Cultivar x Treatment = 0.775	6	1160.0	abcd	1063.0	abcdef	1212.0	abc	*1145.0*	
LSD Cultivar x Treatment $_{(P=0.05)}$ = 429.1	7	928.0	abcdef	756.0	def	805.0	cdef	*829.0*	
	8	1003.0	abcdef	932.0	abcdef	1018.0	abcdef	*985.0*	
Cultivar mean		*1034.0*	*a*	*949.0*	*b*	*1064.0*	*a*	*1016.0*	
Ear rot diseased area (cm²)	1	16.1	a	4.5	de	2.6	e	*7.7*	
F prob Treatment = 0.057	2	12.8	abc	5.9	cde	1.6	e	*6.8*	
LSD Treatment $_{(P=0.05)}$ = 4.232	3	4.2	e	3.0	e	3.2	e	*3.5*	
F prob Cultivar < 0.001	4	0.2	e	7.7	bcde	1.9	e	*3.3*	
LSD Cultivar $_{(P=0.05)}$ = 3.161	5	15.8	ab	1.5	e	1.6	e	*6.3*	
F prob Cultivar x Treatment = 0.036	6	12.6	abcd	7.1	cde	7.0	cde	*8.9*	
LSD Cultivar x Treatment $_{(P=0.05)}$ = 8.24	7	4.3	e	1.9	e	4.3	e	*3.5*	
	8	19.4	a	2.1	e	2.3	e	*7.9*	
Cultivar mean		*10.7*	*a*	*4.2*	*b*	*3.1*	*b*	*6.0*	
Ear rot severity (%)	1	8.9	abc	3.4	cde	0.9	e	*4.4*	
F prob Treatment = 0.127	2	4.2	bcde	6.9	abcd	1.4	de	*4.2*	
LSD Treatment $_{(P=0.05)}$,= 3.422	3	1.9	de	1.0	de	2.4	de	*1.8*	
F prob Cultivar < 0.001	4	3.2	cde	2.0	de	1.1	de	*2.1*	
LSD Cultivar $_{(P=0.05)}$ = 2.208	5	10.6	a	0.6	e	1.4	de	*4.2*	
F prob Cultivar x Treatment = 0.259	6	9.7	ab	4.6	bcde	4.9	abcde	*6.4*	
LSD Cultivar x Treatment $_{(P=0.05)}$ = 5.979	7	2.7	de	1.0	de	2.1	de	*1.9*	
	8	9.4	ab	2.0	de	0.6	e	*4.0*	
Cultivar mean		*6.3*	*a*	*2.7*	*b*	*1.9*	*b*	*3.6*	
Ear rot incidence (%)	1	35.6	abc	8.9	cf	13.3	cdef	*19.3*	
F prob Treatment = 0.134	2	33.3	abcde	24.4	bcdef	13.3	cdef	*23.7*	
LSD Treatment $_{(P=0.05)}$ = 10.07	3	22.2	bcdef	15.6	cdef	4.4	f	*14.1*	
F prob Cultivar < 0.001	4	15.6	cdef	13.3	cdef	4.5	f	*11.1*	
LSD Cultivar $_{(P=0.05)}$ = 9.53	5	44.4	ab	6.7	f	6.7	f	*19.3*	
F prob Cultivar x Treatment = 0.431	6	35.6	abcd	22.2	bcdef	15.6	cdef	*24.4*	
LSD Cultivar x Treatment $_{(P=0.05)}$ = 23.79	7	15.6	cdef	17.8	cdef	11.1	ef	*14.8*	
	8	51.1	a	2.2	f	4.4	f	*19.3*	
Cultivar mean		*31.7*	*a*	*13.9*	*b*	*9.2*	*b*	*18.2*	
Fumonisin (μg/kg)	1	262.3	bcd	154.1	d	71.8	d	*163.0*	
F prob Treatment =0.071	2	152.4	d	200.8	cd	127.0	d	*160.0*	
LSD Treatment $_{(P=0.05)}$ = 232.9	3	64.5	d	67.0	d	143.1	d	*92.0*	
F prob Cultivar = 0.02	4	665.8	abc	728.4	ab	54.0	d	*483.0*	
LSD Cultivar $_{(P=0.05)}$ = 183.5	5	406.5	abcd	116.8	d	189.1	d	*237.0*	
F prob Cultivar x Treatment = 0.493	6	428.4	abcd	158.4	d	187.3	d	*258.0*	
LSD Cultivar x Treatment $_{(P=0.05)}$ = 472.8	7	300.9	abcd	110.9	d	117.9	d	*177.0*	
	8	767.6	a	101.6	d	56.2	d	*308.0*	
Cultivar mean		*381.0*	*a*	*204.7*	*ab*	*118.3*	*b*	*235.0*	

**at dent stage*
AUDPC, area under disease progress curve; LSD, least significant difference

Table 5: Northern corn leaf blight (NCLB) and ear rot related data generated for the second planting trial during 2017/2018

	TMT	Cultivar						*TMT mean*	
		BG3292		DKC61-94BR		IMP50-10B			
NCLB severity (%)*	1.0	21.9	jklmno	11.3	lmnp	16.8	klmnop	*16.7*	*c*
F prob Treatment = 0.006	2.0	25.3	ghijklm	15.5	lnop	25.6	ghijklm	*22.1*	*c*
LSD Treatment $_{(P=0.05)}$ = 19.323	3.0	44.9	abcdefg	38.8	abcdefghij	42.3	abcdefghi	*42.0*	*ab*
F prob Cultivar < 0.001	4.0	47.8	abcde	40.4	abcdfghij	46.6	abcdef	*44.9*	*ab*
LSD Cultivar $_{(P=0.05)}$ = 2.203	5.0	58.1	a	49.9	bc	58.5	a	*55.5*	*a*
F prob Cultivar x Treatment = 0.595	6.0	52.9	ab	42.1	acefghi	48.4	abcd	*47.8*	*ab*
LSD Cultivar x Treatment $_{(P=0.05)}$ = 19.8	7.0	42.5	abcdefgh	28.7	defgijl	36.4	bcdefghijk	*35.8*	*bc*
	8.0	24.7	hijklmn	20.9	jklmnop	21.7	jklmnop	*22.4*	*c*
Cultivar mean		*39.7*	*a*	*31.0*	*c*	*37.0*	*b*	*35.9*	
AUDPC	1.0	128.0	hi	67.0	i	114.2	i	*103.0*	*d*
F prob Treatment = 0.002	2.0	166.3	ghi	99.7	i	163.0	ghi	*143.0*	*d*
LSD Treatment $_{(P=0.05)}$ = 265.46	3.0	342.4	efghi	281.5	efghi	320.0	efghi	*314.6*	*bcd*
F prob Cultivar < 0.001	4.0	543.7	bcde	341.0	fghi	534.2	bcde	*473.0*	*bc*
LSD Cultivar $_{(P=0.05)}$ = 41.15	5.0	803.2	ab	685.6	c	823.3	a	*770.7*	*a*
F prob Cultivar x Treatment = 0.21	6.0	640.9	abcd	413.6	cefg	472.5	cef	*509.0*	*ab*
LSD Cultivar x Treatment $_{(P=0.05)}$ = 277.77	7.0	399.4	defgh	222.7	fgi	285.0	efghi	*302.4*	*bcd*
	8.0	248.4	fghi	209.9	fghi	192.8	ghi	*217.0*	*cd*
Cultivar mean		*409.0*	*a*	*290.1*	*c*	*363.1*	*b*	*354.1*	
Ear rot diseased area (cm^2)	1.0	7.8		7.7		4.2		*6.6*	
F prob Treatment = 0.571	2.0	0.9		10.3		5.7		*5.6*	
LSD Treatment $_{(P=0.05)}$ = 5.611	3.0	2.7		5.6		1.5		*3.3*	
F prob Cultivar =0.463	4.0	2.4		2.8		3.8		*3.0*	
LSD Cultivar $_{(P=0.05)}$ = 2.767	5.0	1.6		0.4		2.0		*1.3*	
F prob Cultivar x Treatment = 0.526	6.0	8.0		1.2		1.8		*3.7*	
LSD Cultivar x Treatment $_{(P=0.05)}$ = 8.233	7.0	1.1		5.6		0.6		*2.4*	
	8.0	5.5		3.4		3.7		*4.2*	
Cultivar mean		*3.7*		*4.6*		*2.9*		*3.8*	
Ear rot severity (%)	1.0	6.3		7.5		2.6		*5.5*	
F prob Treatment = 0.475	2.0	0.4		5.8		2.8		*3.0*	
LSD Treatment $_{(P=0.05)}$ = 4.695	3.0	2.5		5.1		0.6		*2.7*	
F prob Cultivar = 0.273	4.0	0.8		2.0		1.2		*1.4*	
LSD Cultivar $_{(P=0.05)}$ = 2.268	5.0	0.7		0.2		0.7		*0.5*	
F prob Cultivar x Treatment = 0.491	6.0	7.4		0.4		0.6		*2.8*	
LSD Cultivar x Treatment $_{(P=0.05)}$ = 6.808	7.0	0.4		3.9		0.3		*1.5*	
	8.0	4.1		2.7		4.4		*3.7*	
Cultivar mean		*2.8*		*3.5*		*1.7*		*2.7*	
Ear rot incidence (%)	1.0	20.0		11.1		15.6		*15.6*	
F prob Treatment = 0.44	2.0	4.4		22.2		20.0		*15.6*	
LSD Treatment $_{(P=0.05)}$ = 10.05	3.0	8.9		8.9		4.4		*7.4*	
F prob Cultivar = 0.961	4.0	15.6		11.1		13.3		*13.3*	
LSD Cultivar $_{(P=0.05)}$ = 6.94	5.0	6.7		2.2		13.3		*7.4*	
F prob Cultivar x Treatment = 0.551	6.0	17.8		4.4		8.9		*10.4*	
LSD Cultivar x Treatment $_{(P=0.05)}$ = 18.43	7.0	11.1		24.4		6.7		*14.1*	
	8.0	13.3		6.7		8.9		*9.6*	
Cultivar mean		*12.2*		*11.4*		*11.4*		*11.7*	
Fumonisin (*μ*g/kg)	1.0	55.0		69.0		15.0		*46.0*	
F prob Treatment = 0.437	2.0	58.0		42.0		35.0		*45.0*	
LSD Treatment $_{(P=0.05)}$ = 5.37	3.0	37.0		27.0		45.0		*36.0*	
F prob Cultivar = 0.466	4.0	817.0		35.0		37.0		*296.0*	
LSD Cultivar $_{(P=0.05)}$ = 337.5	5.0	84.0		32.0		27.0		*48.0*	
F prob Cultivar x Treatment = 0.522	6.0	153.0		20.0		11.0		*62.0*	
LSD Cultivar x Treatment $_{(P=0.05)}$ = 920.5	7.0	98.0		415.0		28.0		*180.0*	
	8.0	37.0		1407.0		195.0		*546.0*	
Cultivar mean		*167.0*		*256.0*		*49.0*		*158.0*	

**at dent stage*
AUDPC, area under disease progress curve; LSD, least significant difference

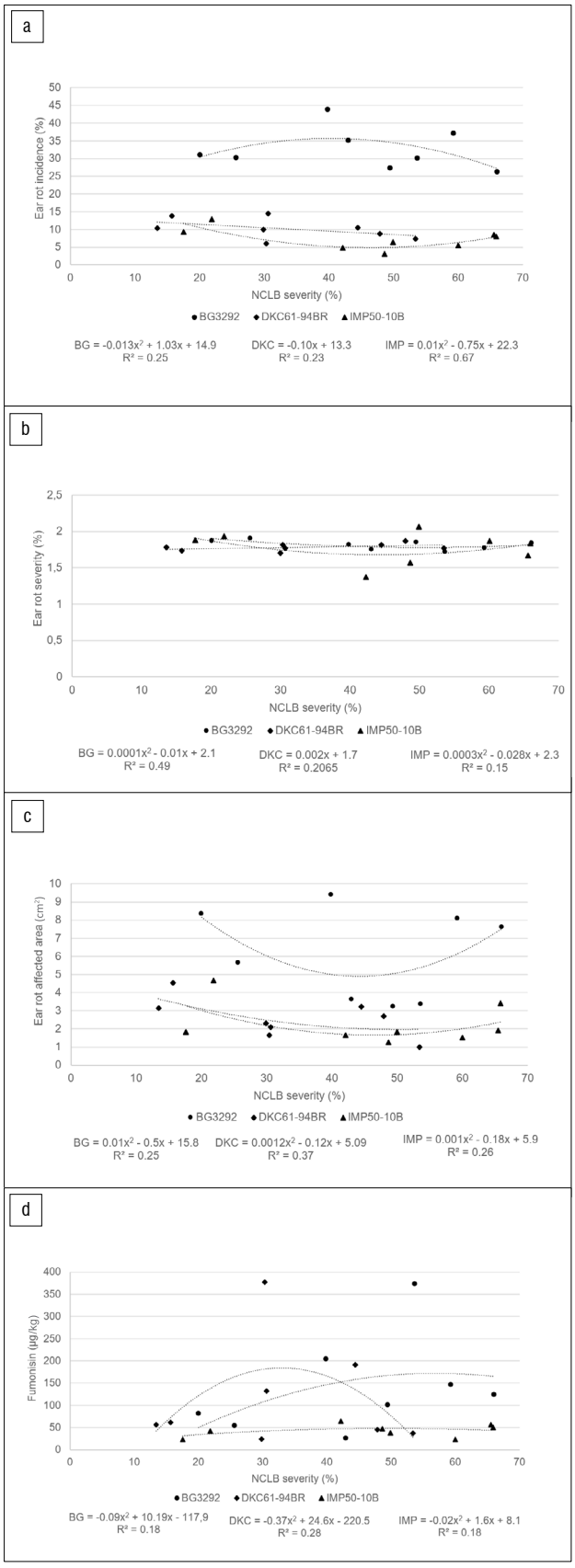

Figure 1: Average obtained over four trials for (a) ear rot incidence (%), (b) ear rot severity (%), (c) ear rot affected area (cm²) and (d) fumonisin concentration (µg/kg) in the grain, regressed against northern corn leaf blight (NCLB) severity achieved at dent stage of eight applied treatments.

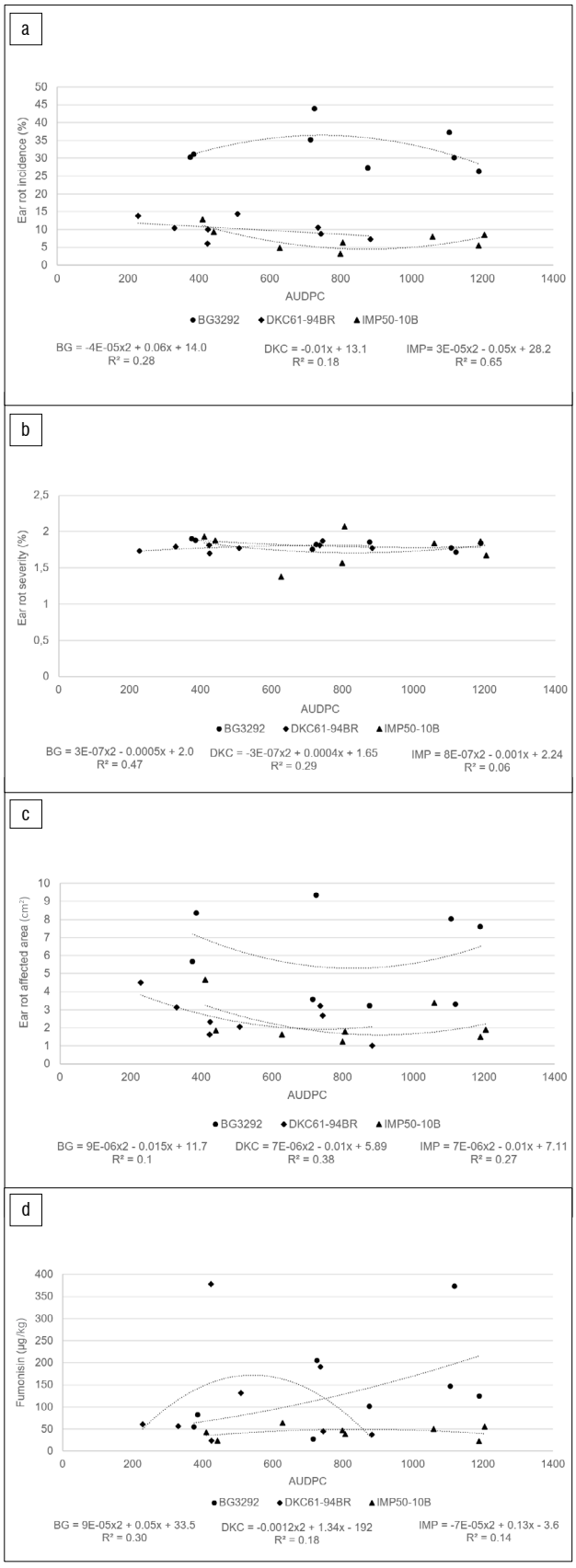

Figure 2: Average obtained over four trials for (a) ear rot incidence (%), (b) ear rot severity (%), (c) ear rot affected area (cm²) and (d) fumonisin concentration (µg/kg) in the grain, regressed against area under the disease progress curve (AUDPC) achieved at dent stage of eight applied treatments.

As all four trial sites of the current study were situated in the same area where ear rot related field experiments have been regularly conducted over numerous seasons, and entailed artificial inoculation with multiple *F. verticillioides* isolates[22,31], it was assumed that present-day isolates at the trial site area would be more than capable of infecting maize ears, provided environmental conditions were conducive for ear rot infection and development. Although maximum temperatures during all four trials were in the required range for Fusarium ear rot development, drier conditions (which would have enhanced ear rot development)[26,27] did not occur during flowering due to the irrigation applied to ensure NCLB development. The question to be addressed was whether NCLB severity would place the plant under sufficient stress to induce a water stress associated situation[6] in the plant, which would unlock a similar response in the plant as would drought stress. One way in which this could happen is if NCLB infection results in stalk rot develoment[7], which would hamper the plant's ability to access water and nutrients. NCLB severity at flowering stage was low with average NCLB severities of between 3% and 14% over the four trials (data not shown). Desiccation due to NCLB was accordingly most likely not severe enough at this critical stage to induce a form of water loss[6] that would aid colonisation by the *F. verticillioides* pathogen and result in fumonisin production[26].

Even though heritable resistance has been identified in maize[32,33], Small et al.[34] were the first to report potentially resistant maize inbred lines locally adapted to southern African production conditions. Very little is, however, known regarding the adoption rate of such lines by local breeding companies, especially as Fusarium ear rot resistance has been established to be a quantitative trait determined by polygenes.[35,36] The respective seed companies could not confirm the Fusarium ear rot resistance of the three cultivars included. Based on what is known internationally, it would nevertheless be highly unlikely that these cultivars would pose such high levels of resistance that could be linked to limited ear rot infection observed over multiple seasons for all three cultivars, as no highly resistant genotypes suited to the production regions in southern Africa exist.[37] A form of indirect resistance through the presence of the Bt gene, which would reduce damage by insects and subsequent infection by the pathogen, might have contributed to lower ear rots being observed. Of the three cultivars included, only DKC 61-94BR contains MON89034. BG3292, which accordingly does not contain Bt genes, consistently had the highest degree of ear rot, but never exceeded levels greater than 10.6% severity in any of the trials (Table 4). Irrespective of how the fungus infected, one would expect that – should stress induced by NCLB create favourable conditions for ear rot infection and growth – greater ear rot infections should have been observed in a cultivar such as BG3292, which consistently had high average NCLB severity over four trials.

Regression analyses conducted over multiple seasons and cultivars point to no significant association between NCLB and natural *F. verticillioides* infection. The possible exception is the fact that BG3292, which consistently had high NCLB severity over four trials, was identified as the cultivar with the highest degree of ear rot and fumonisin concentration observed in the ears (albeit at very low levels). The latter observation nevertheless speaks more to the hybrid's ability to cope with both the diseases individually, than to the link between the two diseases. In essence, the higher levels of NCLB in BG3292 did not result in an increase in ear rot or related parameters in any of the trials conducted.

It has lastly already been established that *F. verticillioides* can also infect through wounds on the ear[11,31]; hence artificial inoculations which make use of techniques which inject the pathogen into the ear are commonly used[22,31]. Although it has been established with the current study that NCLB severity was not able to induce greater ear rot incidence or severity under natural infection of *F. verticillioides*, follow-up research which includes artificial inoculation of *F. verticillioides* would shed additional light on the ability of NCLB to predispose the plant to greater ear rot infection in situations in which ears are damaged by insects, hail or birds.

Conclusion

In the current study, natural ear rot development was monitored in an area in which numerous field studies have been conducted in the past with epidemiological competent *F. verticillioides* ear rot isolates. Very low levels of ear rot severity were nonetheless obtained in all four trials. Without artificial interference, the local *F. verticillioides* isolates were not able to naturally infect the ears, most likely because conditions were too wet during flowering, which was a necessity to ensure sufficient NCLB development. Environmental conditions during flowering are determinant for ear rot development. Although high and variable degrees of NCLB severity were achieved in the current study, blight severity at flowering was not severe or sufficient enough to induce a stress response in the plants, which would simulate water stress conditions that would allow for greater ear rot development. Additional studies which include artificial inoculation of the ears, would aid in clarifying the potential effect of NCLB severity in scenarios in which ear rot development is brought about by insect, bird or hail damage. Based on fitted regression models, NCLB severity did not, however, affect natural ear rot development in three maize cultivars with varying NCLB resistance levels.

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Competing interests

We declare that there are no competing interests.

Authors' contributions

M.C.: Conceptualisation, methodology, data collection, writing – initial draft, funding acquisition. L.M.: Data analyses, validation, data curation. A.A.: Data collection, sample analyses, writing – revision, project leadership. H.N.: Data collection, sample analyses, writing – revision, project leadership. B.J.v.R.: Data collection, sample analyses, writing – revision.

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