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Aggressiveness and mycotoxin production of eight isolates each of *Fusarium graminearum* and *Fusarium verticillioides* for ear rot on susceptible and resistant early maize inbred lines

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Abstract *Fusarium graminearum* and *F. verticillioides* are among the most important pathogens causing ear rot of maize in Central Europe. Our objectives were to (1) compare eight isolates of each species on two susceptible inbred lines for their variation in ear rot rating and mycotoxin production across 3 years, and (2) analyse two susceptible and three resistant inbred lines for potential isolate x line interactions across 2 years by silk-channel inoculation. Ear rot rating, zearalenone (ZEA) and deoxynivalenol (DON) concentrations were evaluated for all *F. graminearum* isolates. In addition, nivalenol (NIV) concentrations were analysed for two NIV producers. Fumonisin (FUM) concentrations were measured for all *F. verticillioides* isolates. Mean ear rot severity was highest for DON producers of *F. graminearum* (62.9% of the ear covered by mycelium), followed by NIV producers of the same species (24.2%) and lowest for *F. verticillioides* isolates (9.8%). For the latter species, ear rot severities differed highly among years (2006: 24%, 2007: 3%, 2008: 7%). Mycotoxin concentrations among isolates showed a broad range (DON: 100–284 mg kg^{-1}, NIV: 15–38 mg kg^{-1}, ZEA: 1.1–49.5 mg kg^{-1}, FUM: 14.5–57.5 mg kg^{-1}). Genotypic variances were significant for isolates and inbred lines in all traits and for both species. Isolate x line interactions were significant only for ear rot rating ($P<0.01$) and DON concentration ($P<0.05$) of the *F. graminearum* isolates, but no rank reversals occurred. Most isolates were capable of differentiating the susceptible from the resistant lines for ear rot severity. For resistance screening, a sufficiently aggressive isolate should be used to warrant maximal differentiation among inbred lines. With respect to *F. verticillioides* infections, high FUM concentrations were found in grains from ears with minimal disease symptoms.

Keywords Gibberella ear rot · Fusarium ear rot · Maize · Mycotoxins · Resistance · Deoxynivalenol · Nivalenol · Zearalenone · Fumonisins

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Introduction

Fusarium graminearum (Schwabe, teleomorph *Gibberella zeae* (Schw.) Petch)) and *Fusarium verticillioides* (Sacc.) Nirenberg (syn. *F. moniliforme* Sheldon, teleomorph *G. fujikuroi* (Sawada) Wr.) both

cause ear rot in maize, called Gibberella ear rot (or red ear rot) and Fusarium ear rot (or pink ear rot), respectively. For Gibberella ear rot, symptoms initiate from the tip and cover the ear with a red or pink mold. In contrast, Fusarium ear rot occurs as a white or light pink mold on randomly infected kernels, groups of kernels, or physically injured kernels (Munkvold 2003). *F. graminearum* is worldwide the most important pathogen for Fusarium head blight in small-grain cereals. In Northern Europe, maize is predominantly infected by *F. graminearum*, but *F. verticillioides* occurs frequently (Görtz et al. 2008). In Southern Europe, *F. verticillioides* is the predominant species (Logrieco et al. 2002) and may occur on maize as a seedborne endophyte or infect the plant after emergence (Munkvold et al. 1997). Plant and fungus can coexist without visible disease symptoms. Interestingly, *F. graminearum* shows no host specialisation among cereals infecting wheat, rye, triticale, barley, and maize whereas *F. verticillioides* mainly occurs on maize (Leslie and Summerell 2006).

Both *Fusarium* species are capable of producing mycotoxins. The mycotoxins deoxynivalenol (DON), nivalenol (NIV), and zearalenone (ZEA) are produced by *F. graminearum* and the fumonisins (FUM) by *F. verticillioides* (Leslie and Summerell 2006) of which FB1, FB2 and FB3 are generally present in naturally contaminated maize (Nelson et al. 1993). The EU has released legally enforceable limits for DON, ZEA, and FUM in cereal-based food (Verstraete 2008).

In wheat, numerous studies have shown a wide range of variation among *F. graminearum* isolates for their capability to produce disease on a susceptible host in a non-race specific pathosystem (*i.e.*, aggressiveness *sensu* Van der Plank 1968) and their mycotoxin production in wheat (for review see Miedaner et al. 2008). Isolate aggressiveness is a heritable trait and has a quantitative inheritance governed by several genes with mainly additive effects (Cumagun and Miedaner 2004). This is similar to the inheritance of host resistance in wheat (Löffler et al. 2009) and maize (Gendloff et al. 1986) where all levels from resistant to highly susceptible occur. There is no substantial isolate x host genotype interaction of *F. graminearum* in wheat (van Eeuwijk et al. 1995; Bai and Shaner 1996; Mesterházy 2003), but the severity of the disease depends on the aggressiveness of an isolate, the degree of host resistance, and to a large extent on environmental factors. Deoxynivalenol is not a pathogenicity factor, but it affects the aggressiveness of an isolate (Nicholson 2009).

Compared to wheat, much less is known about the aggressiveness and mycotoxin production of *F. graminearum* in maize ear rot (Hart et al. 1982; Atlin et al. 1983; Gendloff et al. 1986). For aggressiveness of *F. verticillioides* isolates in maize, information is even more scarce and restricted to stalk infections in the greenhouse (Jardine and Leslie 1999). To standardise resistance tests and to detect differences among maize genotypes reliably, inoculation of a conidial suspension of sufficiently aggressive isolates into the ear is indispensible (Reid et al. 1993). Our objectives were to (1) establish the variation for aggressiveness (ear rot rating) and mycotoxin production of eight isolates each of *F. graminearum* (DON or NIV and ZEA concentrations) and *F. verticillioides* (FUM concentration) using silk inoculations of two susceptible maize inbred lines across 3 years and (2) analyse isolate x inbred line interaction of these isolates on two susceptible and three resistant inbred lines for the mentioned traits across 2 years. The overall aim is to select reliable isolates for inoculations and to obtain information on the stability of resistance incorporated in maize inbred lines.

Materials and methods

Plant materials and fungal isolates

Two experiments were carried out to evaluate differences in aggressiveness and mycotoxin production between isolates of *F. graminearum* and *F. verticillioides* in two susceptible maize inbreds (Exp. 1) and to compare the same isolates for their ability to infect five maize inbred lines varying in their resistance (Exp. 2).

In Experiment 1, six (2006) and eight (2007, 2008) isolates of each of *F. graminearum* (Fg1-6, Fg1-8) and *F. verticillioides* (Fv1-6, Fv1-8) were inoculated on two susceptible maize inbred lines (D171 and UH303) in 3 years. In Experiment 2, the same eight isolates were tested on the former two susceptible and additionally three quantitatively resistant maize inbred lines (UH6, UH7, and FV7) in 2007 and 2008. This implies that data of some of the isolate x line

combinations were used twice for analyses. All lines are early-maturing commercial inbreds developed by the University of Hohenheim. They were chosen for this experiment according to their diverse disease reaction to inoculation with the respective *Fusarium* species in a pilot study evaluating 42 inbreds towards *F. graminearum* and 21 inbreds towards *F. verticillioides* (Bolduan et al. 2009).

The *F. graminearum* isolates were collected from various geographic regions in Europe, mainly from wheat (Table 1), and tested for their aggressiveness and mycotoxin production. Two isolates of *F. graminearum* (Fg7, Fg8), previously known for their ability to produce NIV, were tested additionally in 2007 and 2008.

Each of three isolates of *F. verticillioides* from Italy (Fv1–Fv3) and France (Fv4–Fv6) were tested for their aggressiveness and production of FUM. In 2007 and 2008, two extra isolates of *F. verticillioides* were analysed originating from South Africa (Fv7) and the USA (Fv8). Isolates were stored as plugs of special nutrient-poor agar (SNA) in sterile water in 2 ml Eppendorff tubes in the refridgerator (6°C) as stock collections. Each year the respective isolates from the same Eppendorff tube were transferred to a fresh SNA plate and sub-cultured for production of inoculum in the field experiment.

Field experiments and inoculum production

The field experiments were conducted in Eckartsweier near Kehl/Rhine (141 m above sea level, 9.9°C mean annual temperature, 726 mm mean annual precipitation). Mean temperatures from silking to harvest were 1.5°C cooler in 2007 than in the other years (16.5°C in 2007 vs. 18.0°C and 17.6°C in 2006 and 2008, respectively) as measured by the weather stations at the respective locations. We used a

Table 1 Geographic origin, host, and donor of a collection of each of eight isolates of *Fusarium graminearum* and *F. verticillioides*

Code	Location/country	Host	Year of collection	Original code	Donor[a]
F. graminearum					
Fg1	Tulln/Austria	Maize	1990	IFA66	M. Lemmens
Fg2	Szeged/Hungary	Wheat	1991	Fg24	T. Miedaner
Fg3	Zagreb/Croatia	Wheat	1987	Fg07	S. Tomasovic
Fg4	Sersheim/Germany	Wheat	1992	Fg3211	T. Miedaner
Fg5	Brasov/Romania	Wheat	1996	Fg96	M. Ittu
Fg6	Hohenheim/Germany	Wheat	1999	Fg153	T. Miedaner
Fg7	Sersheim/Germany	Wheat	1992	Fg1111	T. Miedaner
Fg8	ND[b]/Japan	Wheat	2002	Fg136	M. Yoshida
F. verticillioides					
Fv1	Piacenza/Italy	Maize	2003	Fv216/1	P. Battilani
Fv2	Piacenza/Italy	Maize	2003	Fv234/1	P. Battilani
Fv3	Piacenza/Italy	Maize	2003	Fv259/1	P. Battilani
Fv4	Alzonne/France	Maize	2005	Fv-IFA420	M. Lemmens
Fv5	Alzonne/France	Maize	2005	Fv-IFA425	M. Lemmens
Fv6	Alzonne/France	Maize	2005	Fv-IFA429	M. Lemmens
Fv7	ND/South Africa	Maize	ND	FM20	P. Karlovsky
Fv8	ND/USA	Maize	ND	FM8114	G.P. Munkvold

[a] Affiliation of donors: M. Lemmens, Institut of Biotechnology in Plant Production, Tulln, Austria; S. Tomasovic, Bc Institute for Breeding and Production of Field Crops, Zagreb, Croatia; Mariana Ittu, National Agricultural Research-Development Institute Fundulea, Calarasi, Romania; Megumi Yoshida, National Agricultural Research Center for Kyushu Okinawa Region, Koshi (Kumamoto), Japan; Paola Battilani, Faculty of Agriculture, Università Cattolica del Sacro Cuore, Piacenza, Italy; P. Karlovsky, Department of Molecular Phytopathology, University of Goettingen, Germany; G.P. Munkvold, Iowa State University Department of Plant Pathology, Ames, USA

[b] ND=not determined.

split-plot design with maize inbreds as main plots (=whole plots) and *Fusarium* spp. isolates as subplots with three replications in each environment. The inbreds were randomly assigned to the main plots using a separate randomisation scheme. The levels of the subplots were randomly arranged within the main plots using a randomized complete block design. As experimental units, one-row plots were used 4 m in length with 0.75 m between rows. Rows were overplanted and thinned to approximately 30 plants.

Isolates were cultured on SNA and conidia were produced in liquid medium according to Reid and Hamilton (1996). Inoculum was prepared by diluting conidia of *F. graminearum* and *F. verticillioides* with sterile water to approximately 1×10^5 and 1×10^6 conidia ml^{-1}, respectively. Inoculation of rows was done 5–7 days after flowering of each row when silks were still green but started to dry out from the tips. At this physiological stage, maize plants show the best differentiation among genotypes when inoculated *via* the silk channel (Reid and Hamilton 1996). Silk-channel inoculation was performed by injection of 1 ml inoculum into the silk channel of the primary ear of each plant. In each row the ears of 10 plants were inoculated, showing the greatest conformity in the physiological stage of silk development.

Symptom and mycotoxin assessment

Disease severity of Gibberella ear rot (GER) and Fusarium ear rot (FER) was rated at physiological maturity as the percentage of visibly infected kernels on the ear surface after dehusking. Rating was done on a single-ear basis and averaged for each row. For mycotoxin analyses, the inoculated ears of each row were hand harvested from two replications due to economical constraints. All inoculated ears per row were bulked and dried with forced air (30–40°C) to approximately 15% grain moisture. After drying, ears were shelled and grain was ground using a Retsch mill (ZM200-DR100, Haan, Germany) to pass through a 1-mm mesh. In both experiments, DON, ZEA, and FUM concentrations were determined for each environment (= location x year combination) in rows inoculated by *F. graminearum* and *F. verticillioides*, respectively. In addition, NIV concentration was determined for the two NIV-producing isolates (Fg7, Fg8).

A 5 g sample of ground material was analysed using commercially available enzyme immunoassays (RIDASCREEN™ FAST DON, RIDASCREEN™ FAST Zearalenon, and RIDASCREEN™ FAST Fumonisin, R-Biopharm GmbH, Darmstadt, Germany). The DON assay detects DON and 3-acetyl DON (cross reactivity 213%) and has no cross reactivity to other trichothecenes such as 15-acetyl DON, triacetyl DON, nivalenol, triacetyl nivalenol, and fusarenon-X. The FUM assay detects the fumonisins FB1, FB2 and FB3. The test plates were measured at 450 nm with the aid of a microtiter plate spectrometer (TECAN SLT Lab Instruments, Crailsheim, Germany) and mycotoxin contents were calculated using a software package provided by the spectrometer manufacturer. Calculation was based on the extinction of five standard solutions (0, 0.222, 0.666, 2, and 6 mg kg^{-1} for DON and FUM; 0, 0.050, 0.100, 0.200, and 0.400 mg kg^{-1} for ZEA) provided by the immunoassay kit. Due to high infection severity following artificial inoculation, dilution of samples with distilled water was conducted to allow measurements in the range of standards given by the test kit.

For samples inoculated with the NIV producers Fg7 and Fg 8 (see Table 3), DON and NIV concentrations were determined by high performance liquid chromatography (HPLC) at the Bavarian State Research Center for Agriculture, Freising, Germany. The quantification followed the method described in detail by Sano et al. (1987), the solid-phase extraction column cleanup of the sample with acetonitrile — water (84+ 16) was done after Yoshizawa (2001).

Statistical analyses

Single plot data were used for analyses of variance (ANOVA). To normalize distribution of residuals and remove heterogeneity of error variances, DON, ZEA, and FUM data were transformed by their fourth root ($\sqrt[4]{x}$). The linear model for both experiments was:

$$X_{ijkl} = \mu + Y_i + R(Y)_{il} + L_j + YL_{ij} + R(YL)_{ijl} + I_k + IY_{ik} + IL_{jk} + IYL_{ijk} + \varepsilon_{ijkl}$$

where μ is the overall mean, Y_i the effect of year *i*, L_j the effect of line *j*, I_k the effect of isolate *k*, YL_{ij}, IY_{ik},

IL_{jk} IYL_{ijk} the corresponding interaction effects, $R(Y)_{il}$ and $R(YL)_{ijl}$ the effect of replication l within the year i and within the year x line interaction effect ij, respectively, and ε_{ijkl} the effect of experimental error (Cochran and Cox 1957). For simplicity, the $R(Y)_{il}$ and $R(YL)_{ijl}$ effects have been pooled to error a and ε_{ijkl} is represented by error b. All effects in the model were considered as fixed except the overall mean. Therefore, we give in the ANOVA tables mean squares only. The Tukey test controls the Type I error rate and was used for multiple comparisons of means. The Tukey honestly significant difference (HSD) method is based on a variation of the t distribution that takes into account the number of means being compared (Steel and Torrie 1960). All computations were performed using the statistical software PLABSTAT (Utz 2004).

Results

In Experiment 1, inoculation generally resulted in high disease severity by the six DON-producing isolates across the two susceptible maize inbreds in all 3 years (Table 2). Across years, GER ranged from 37 to 73% with all isolates being similarly aggressive except Fg2. DON concentrations were lower for Fg2 and Fg3, but similarly high for the other isolates. ZEA concentrations did not necessarily go in parallel with the DON concentrations.

Disease severity caused by the two NIV-producing isolates of *F. graminearum* (Table 3) was among the lowest in the respective years and could be compared only to the low aggressive DON-producer Fg2. NIV concentrations were remarkably high ranging from 7 to 42 mg kg^{-1} and DON concentrations in these samples were negligible.

Mean disease severity of *F. verticillioides* isolates ranged from 8 to 16% and mean FUM concentration from 15 to 58 mg kg^{-1} across 3 years (Table 4). In 2006, FER was high and in 2007 extremely low. In 2008, isolates from South Africa (Fv7) and USA (Fv8) were among the most aggressive isolates, the latter producing a high amount of FUM. Isolates causing the same ear rot severity might, however, differed considerably in their FUM production.

Significant differences among lines and isolates were detected in the ANOVA across years for both *Fusarium* species and for all traits except ZEA concentration, mostly on a high probability level (Table 5). Isolate x line interactions were not significant ($P>0.1$). Interactions with year were generally of lower importance but significant in some instances.

In Experiment 2, comparison of means for the individual line-isolate combinations clearly showed that *F. graminearum* isolates were more aggressive than those of *F. verticillioides* (Table 6). Again, the NIV-producing isolates Fg7 and Fg8 were together with the DON-producing isolate Fg2 significantly less aggressive than the other isolates of *F. graminearum*. The susceptible inbred lines were, on average, significantly different from the resistant ones with

Table 2 Means and least significant differences (LSD, $P<0.05$) of Gibberella ear rot rating (GER), deoxynivalenol (DON), and zearalenone (ZEA) concentration of six DON-producing isolates of *F. graminearum* across two susceptible maize inbred lines in three years (Experiment 1)

Isolate	2006			2007			2008			Overall mean		
	GER (%)	DON ($mgkg^{-1}$)	ZEA ($mgkg^{-1}$)	GER (%)	DON ($mgkg^{-1}$)	ZEA ($mgkg^{-1}$)	GER (%)	DON ($mgkg^{-1}$)	ZEA ($mgkg^{-1}$)	GER (%)	DON ($mgkg^{-1}$)	ZEA ($mgkg^{-1}$)
Fg1	78.8	256.5	26.0	72.4	339.3	22.4	48.4	144.8	21.3	66.5	246.9	23.2
Fg2	65.5	221.9	15.3	26.4	156.3	5.0	18.6	32.5	16.1	36.8	136.9	12.1
Fg3	78.7	182.5	58.4	58.6	131.0	16.8	57.3	152.8	24.1	64.8	155.4	33.1
Fg4	86.3	375.3	43.9	68.4	178.3	42.4	60.5	120.4	62.1	71.7	224.7	49.5
Fg5	89.7	325.7	29.8	64.4	188.9	16.2	64.0	337.2	56.1	72.7	283.9	34.0
Fg6	82.7	407.1	54.2	69.5	179.2	40.8	41.6	121.5	61.4	64.6	235.9	52.1
Mean	80.3	294.8	37.9	59.9	195.5	23.9	48.4	151.5	40.2	62.9	214.0	34.0
$LSD_{5\%}$	10.2	104.6	17.1	18.6	120.9	55.7	19.9	286.2	70.5	9.3	100.2	27.2

Table 3 Means of Gibberella ear rot rating (GER), deoxynivalenol (DON), zearalenone (ZEA), and nivalenol (NIV) concentrations of two NIV-producing isolates of *F. graminearum* across two susceptible maize inbred lines in two years (Experiment 1)

Isolate	2007				2008				Overall mean			
	GER (%)	DON[a] ($mgkg^{-1}$)	ZEA[b] ($mgkg^{-1}$)	NIV[a] ($mgkg^{-1}$)	GER (%)	DON[a] ($mgkg^{-1}$)	ZEA[b] ($mgkg^{-1}$)	NIV[a] ($mgkg^{-1}$)	GER (%)	DON[a] ($mgkg^{-1}$)	ZEA[b] ($mgkg^{-1}$)	NIV[a] ($mgkg^{-1}$)
Fg7	32.7	0.36	21.5	33.1	30.4	0.09	21.6	41.8	31.5	0.23	21.6	37.5
Fg8	7.9	1.41	ND[c]	6.7	25.8	0.86	1.1	23.1	16.9	1.14	1.1	14.9
Mean	20.3	0.89	21.5	19.9	28.1	0.47	11.3	32.5	24.2	0.68	18.7	26.2

[a] Analysed by HPLC.

[b] Analysed by ELISA

[c] Not determined.

the exception of D171 for *F. verticillioides*. Additionally, UH303 was less diseased by Fg8 than expected and Fg5 caused in UH6 considerably higher disease than expected. There were, however, no rank reversals in the reaction of lines across isolates.

In the ANOVA, variances among lines and isolates were significant ($P<0.01$) throughout, isolate x line interactions were significant for GER ($P<0.01$) and DON concentration ($P<0.05$) only, but not for ZEA concentration and *F. verticillioides*-related traits (Table 7). Interactions of lines or isolates with year were significant for GER ($P<0.01$), FER ($P<0.05$), and FUM concentration ($P<0.01$).

For all isolates of each *Fusarium* species, the resistant inbred lines contained the lowest mycotoxin contents compared with the susceptible lines (Figs. 1, 2). However, the susceptible line UH303 was less stable across isolates in its reaction for *F. graminearum* mycotoxins. Moreover, the susceptible line D171 had similar low FUM contents than some resistant lines. Three isolates of *F. verticillioides* (Fv1, Fv7, Fv8) produced very high FUM concentrations in UH303 (Fig. 2). For both species, isolates producing higher mycotoxin concentrations differentiated the resistant *vs*. susceptible lines better than those isolates causing only low mycotoxin concentrations.

Table 4 Means and least significant differences (LSD, $P<0.05$) of Fusarium ear rot rating (FER), and Fumonisin (FUM) concentration of six European (Fv1–Fv6), one South African (Fv7), and one US (Fv8) isolate of *F. verticillioides* across two susceptible maize inbred lines in three and two years, respectively (Experiment 1)

Isolate	2006		2007		2008		Overall mean	
	FER (%)	FUM ($mgkg^{-1}$)	FER (%)	FUM ($mgkg^{-1}$)	FER (%)	FUM ($mgkg^{-1}$)	FER (%)	FUM ($mgkg^{-1}$)
Fv1	31.2	72.1	4.3	16.7	12.9	59.2	16.1	49.3
Fv2	32.8	138.2	3.0	14.4	6.9	19.8	14.2	57.5
Fv3	24.8	22.8	1.6	6.0	6.6	14.5	11.0	14.5
Fv4	15.3	25.2	5.2	13.0	4.4	17.2	8.3	18.5
Fv5	19.5	34.8	1.9	7.9	4.6	14.3	8.7	19.0
Fv6	19.0	46.7	2.1	7.6	4.4	10.1	8.5	21.5
Mean	23.8	56.6	3.0	10.9	6.6	22.5	11.1	30.0
$LSD_{5\%}$	9.7	45.1	3.4	8.5	5.9	42.0	3.9	19.7
Fv7	ND[a]	ND[a]	2.9	7.3	12.2	50.4	7.5	28.9
Fv8	ND[a]	ND[a]	2.0	9.9	17.1	98.3	9.5	54.1
Mean	ND[a]	ND[a]	2.5	8.6	14.7	74.4	8.5	41.5

[a] Not determined.

Table 5 Degrees of freedom (df) and mean squares from the analysis of six isolates each of *Fusarium graminearum* and *Fusarium verticillioides* for Gibberella ear rot rating (GER), deoxynivalenol (DON), zearalenone (ZEA) concentrations, Fusarium ear rot rating (FER), and fumonisin (FUM) concentration, respectively, across three years tested on two susceptible maize inbred lines (Experiment 1, transformed data)

Source	*Fusarium graminearum*						*Fusarium verticillioides*			
	GER		DON		ZEA		FER		FUM	
	df	Mean square	df	Mean square	df	Mean square	df	Mean square	df	Mean square
Year (Y)	2	9370**	2	4.07*	2	0.85	2	4435**	2	3.43*
Line (L)	1	8376**	1	4.51*	1	0.03	1	2864**	1	2.02*
L x Y	2	1855**	2	0.76	2	0.69	2	1057**	2	1.42
Error a	6	126	3	0.38	3	0.21	6	41	3	0.34
Isolate (I)	5	3147**	5	1.09**	5	0.86**	5	199**	5	0.76**
I x L	5	333	5	0.47	5	0.28	5	35	5	0.04
I x Y	10	361	10	0.48*	10	0.13	10	89**	10	0.19*
I x L x Y	10	241	10	0.28	10	0.27	10	33	10	0.13
Error b	60	195	30	0.21	29	0.20	58	33	28	0.08

*, ** Significant at $P<0.05$ and $P<0.01$, respectively.

Table 6 Mean ear rot ratings (%) of eight isolates each of *Fusarium graminearum* and *F. verticillioides* inoculated on two susceptible and three resistant maize inbred lines across two years (Experiment 2)

Species/isolate	Susceptible inbreds		Resistent inbreds			Mean
	UH303	D171	FV7	UH6	UH7	
F. graminearum						
Fg8[a]	7.4	26.4	4.9	6.5	7.4	10.5 a[b]
Fg2	16.5	28.5	1.2	8.1	7.8	12.4 a
Fg7[a]	33.6	29.5	9.5	1.5	14.4	17.7 a
Fg6	58.2	52.9	11.6	5.9	30.4	31.8 b
Fg3	44.4	71.5	15.7	11.9	28.6	34.4 b
Fg1	56.0	64.8	22.4	15.2	26.5	37.0 bc
Fg5	60.4	68.1	15.0	38.0	37.7	43.8 c
Fg4	49.7	79.2	12.6	14.3	35.3	38.2 bc
Mean	41.3c	54.4d	11.4a	13.2a	24.5b	
F. verticillioides						
Fv6	4.7	1.7	0.7	1.2	4.1	2.5 ab
Fv5	4.5	2.1	0.9	0.7	2.5	2.1 a
Fv3	7.3	1.8	0.7	4.5	1.5	3.2 ab
Fv4	6.5	2.9	0.5	2.6	1.4	2.8 ab
Fv2	7.0	2.9	1.8	2.5	2.5	3.3 ab
Fv7	10.1	5.0	2.0	2.5	4.9	4.9 ab
Fv1	12.6	4.6	1.3	2.0	3.2	4.7 ab
Fv8	14.1	5.0	1.7	2.4	3.2	5.3 b
Mean	8.3 b	3.2 a	1.2 a	2.3 a	2.9 a	

[a] NIV-producing isolate.

[b] Different letters within one row or one column are designating significantly different values at $P<0.05$ (Tukey test).

Table 7 Degrees of freedom (df) and mean squares from the analysis of eight isolates each of *Fusarium graminearum* and *Fusarium verticillioides* for Gibberella ear rot rating (GER), deoxynivalenol (DON), zearalenone (ZEA) concentrations, Fusarium ear rot rating (FER), and fumonisin (FUM) concentration, respectively, across two years tested on two susceptible and three resistant maize inbred lines (Experiment 2, transformed data)

Source	*Fusarium graminearum*						*Fusarium verticillioides*			
	GER		DON[a]		ZEA[a]		FER		FUM	
	df	Mean squares	df	Mean squares	df	Mean squares	df	Mean squares	df	Mean squares
Year (Y)	1	5285**	1	3.85*	1	1.40	1	450.4**	1	1.07
Line (L)	4	15441**	4	16.30**	4	9.88**	4	354.0**	4	4.79*
L x Y	4	1034	4	1.20	4	1.02	4	182.9*	4	0.69
Error a	16	356	8	0.66	8	0.70	16	43.7	8	0.67
Isolate (I)	7	4892**	5	2.57**	5	1.42**	7	44.0**	7	1.18**
I x L	28	536**	20	0.47*	20	0.33	28	16.2	28	0.22
I x Y	7	762**	5	0.40	5	0.38	7	29.4	7	0.88**
I x L x Y	28	237	20	0.26	20	0.17	28	21.7	28	0.23*
Error b	135	152	49	0.23	46	0.24	135	14.8	64	0.14

*, ** Significant at $P<0.05$ and $P<0.01$, respectively.

[a] DON- and ZEA-producing isolates only.

Discussion

Silk-channel inoculations resulted in visible disease symptoms in all instances and high mycotoxin concentrations. All eight *F. graminearum* isolates were capable of infecting maize although seven of them were isolated from wheat. Fg1 originated from maize but did not result in higher GER or mycotoxin production than the wheat isolates. This again illustrates the low pathogenic specialisation of *F. graminearum* (Miedaner et al. 2008). Accordingly, the low aggressive isolate Fg2 was among the less aggressive isolates for wheat as already shown by Cumagun and Miedaner (2004). The two NIV producers were among the least aggressive isolates. This is in accordance with results from wheat

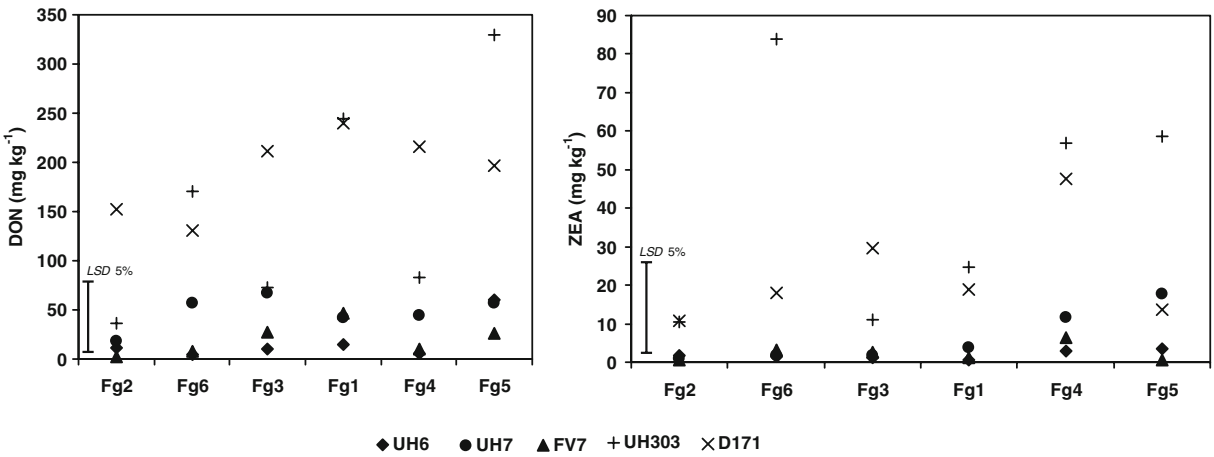

Fig. 1 Differences in deoxynivalenol (DON), and zearalenone (ZEA) concentrations among six DON-producing isolates of *F. graminearum* tested on two susceptible (UH303, D171) and three resistant (UH6, UH7, FV7) maize inbred lines in two years. Isolates are arranged with respect to increasing mean of GER of susceptible inbreds (Experiment 2, LSD=least significant difference at $P<0.05$)

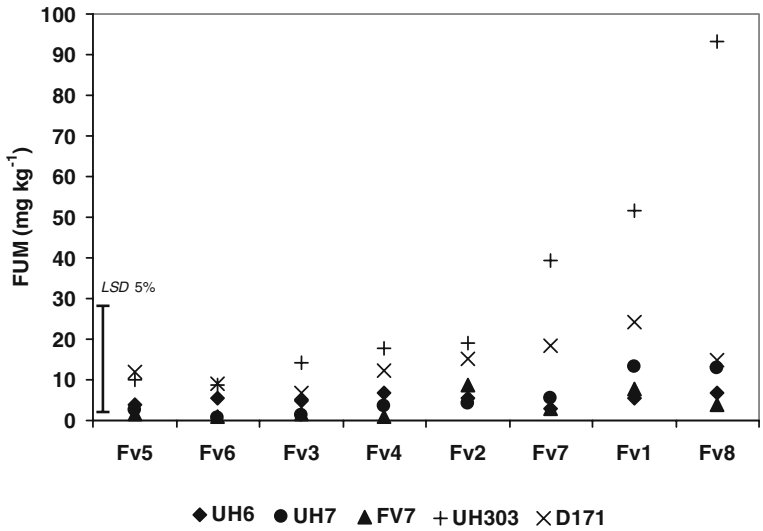

Fig. 2 Differences in fumonisin (FUM) concentrations among eight isolates of *F. verticillioides* tested on two susceptible and three resistant maize inbred lines in two years. Isolates are arranged with respect to increasing mean of FER of susceptible inbreds (Experiment 2, LSD=least significant difference at $P<0.05$)

(Miedaner et al. 2000) and does not substantiate a special prevalence of NIV producers to maize as stated earlier (Carter et al. 2002; Maier et al. 2006). All isolates were capable of producing high concentrations of ZEA contradicting the study of Hart et al. (1982), where only one out of three isolates could produce ZEA in maize ear rot. This mycotoxin, known for its oestrogenic behavior in swine and humans, should also be considered in future analyses.

Isolates of *F. verticillioides* showed a much lower level of ear rot than *F. graminearum* isolates. Mean disease severity obtained in this study corresponds to reports from the US corn belt using inoculations with *F. verticillioides* (Robertson et al. 2006; Kleinschmidt et al. 2005) and might be explained by the dual nature of *F. verticillioides* either as an endophyte or pathogen (Munkvold 2003; Bacon et al. 2008). Our results, however, clearly demonstrate that all *F. verticillioides* isolates used could reliably infect maize when inoculated into the silk channel although no additional abiotic stress occurred (Munkvold 2003). FUM concentrations were much higher than expected from the low level of visible infection, especially in 2007. Fv5 for example produced only 1.9% FER in this year, but FUM concentration was around 8 mg kg^{-1}. This clearly shows that visual inspection of maize grain alone is not a reliable predictor for concentrations of this toxin. The cause might be that symptomless kernels frequently contribute to FUM concentration (Munkvold et al. 1997; Desjardins et al. 1998). All isolates of both *Fusarium* species produced high amounts of mycotoxins. The high DON levels reported here for susceptible inbreds demonstrate that maize generally supports the production of this mycotoxin even more than the already susceptible wheat.

With respect to the non-genetic variation in the ANOVA, the year was more important for *F. verticillioides* than for *F. graminearum*. In 2007, for example, symptom development of *F. verticillioides* was minimal ranging from 1.6 to 5.2% FER, whereas in 2006 FER ratings were about ten times higher. Infection and FUM accumulation by *F. verticillioides* is usually favoured by warm to hot and dry weather at silking and during the grain-filling period (Bottalico 1998; Munkvold 2003) Indeed, mean temperatures from silking to harvest were in 2007 cooler than in the other years. In *F. graminearum*, the least aggressive isolate Fg2 proved to be most sensitive to the year. This underlines the need to use environmentally stable, highly aggressive isolates for resistance trials (Reid et al. 1993) and to conduct experiments across several environments. The inoculations with isolates of *F. verticillioides* from other geographic regions (Italy, USA, South Africa) resulted in satisfactory infection levels in Germany and mycotoxin contaminations that allowed us to distinguish between susceptible and resistant inbred lines. Similarly in *F. graminearum*, Fg5 from Romania was as aggressive as Fg4 from Germany.

Differences among susceptible and resistant lines were considerably lower for the less aggressive isolates Fg2, Fv5, and Fv6. The other similarly aggressive isolates did not differ much in this respect.

The significant isolate x line interaction variance for GER was mainly caused by the reaction of the inbred line UH6 to Fg5 and UH303 to Fg8. Despite this, no change in ranking of host genotypes occurred. Thus, the earlier results of Gendloff et al. (1986) and Atlin et al. (1983) could be confirmed for early European maize. In our study, the resistant inbred lines consistently had the lowest ear rot ratings and mycotoxin concentrations. The susceptible lines showed a lower stability across isolates. Because the tested maize inbred lines have been pre-selected for their ear rot reaction to *F. graminearum* and *F. verticillioides* as well (Bolduan et al. 2009), they provide a maximum of variation among the current early maturing maize inbreds and, consequently, the results should be representative for modern German flint maize germplasm. Our results support the conclusion of Reid et al. (1993) that genotypic differences in silk resistance to *F. graminearum* can be detected with almost any aggressive isolate. This is further substantiated by a study from Naef and Défago (2006), where *F. graminearum* isolates from one maize field showed a similar genetic variation than 16 isolates of lineage 7 (syn. *F. graminearum sensu strictu*) collected worldwide. Hence, due to this high degree of genetic variation within an extremely small spatial scale, even randomly chosen isolates are appropriate for inoculation purposes as far as they are aggressive enough. Accordingly, for ear rot severities and FUM concentrations caused by *F. verticillioides* no isolate x line interactions occurred, a fact that has not been reported previously. Because each line was ranked similarly with each isolate, resistance of maize inbreds to *F. graminearum* and *F. verticillioides* should be fairly stable irrespective of the composition of the pathogen population. Quantitative resistances are obviously capable of controlling even highly aggressive isolates.

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