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Mycotoxin accumulation and corresponding ear rot rating in three maturity groups of European maize inoculated by two *Fusarium* species

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Abstract *Fusarium graminearum* (FG) and *F. verticillioides* (FV) produce the mycotoxins deoxynivalenol (DON), zearalenone (ZEA) and fumonisins (FUM), respectively, in maize. The EU released limits for these toxins in food. Growing resistant varieties is one alternative to fulfill these limits. Quantification of mycotoxin concentrations is expensive and time consuming. If indirect selection based on cost efficient and fast ear rot rating is feasible, this could increase efficiency of selection. The objective of this study was to analyze correlations between mycotoxin concentrations and ear rot rating by inoculating three maturity groups (early, mid-late, late) each comprising about 50 inbred lines tested in Central and Southern Europe. In the early maturity group flint lines were more susceptible in all instances except ZEA than dent lines. Broad ranges and significant ($P < 0.01$) genotypic variances were detected, but also genotype × environment interaction variances were significant ($P < 0.01$). Heritabilities of ear rot rating were similar or higher than those of mycotoxin concentrations (0.61−0.93 and 0.56–0.89, respectively). Although high genotypic correlations between FUM and DON or ZEA were found (0.77; 0.76, respectively), separate testing of FV and FG and corresponding mycotoxins is necessary since genotypes resistant to FV were not necessarily resistant to FG and vice versa. Medium to high heritabilities and high genotypic correlations between ear rot and corresponding mycotoxin concentrations (0.87–0.99) suggest frequent identification of lines with reduced mycotoxin concentrations by ear rot rating. Assuming fixed budgets we conclude that indirect selection by applying cost efficient ear rot rating could increase selection intensity and therefore is more effective than direct selection for reduced mycotoxin concentrations.

Keywords Mycotoxin · *Fusarium graminearum* · *Fusarium verticillioides* · Ear rot · Maize

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Introduction

In Europe, maize was grown on approximately eight million ha in 2007 (FAO 2009). In Central and Eastern Europe early maturing varieties are required for silage due to short growth period. Mid-late and late maturity groups are predominantly used for grain production in Southern France, Hungary and in Italy, Spain and the Balkan states, respectively. The early

and late maturity group correspond approximately to Canadian and US maize, respectively.

Ear rot in maize is caused by different *Fusarium* species. In Central and Eastern Europe *F. graminearum* (FG) is the most predominant, whereas *F. verticillioides* (FV) is ubiquitous across whole Europe, but other *Fusarium* spp. may also occur (*i.e. F. subglutinans, F. proliferatum, F. culmorum*) (Bottalico 1998; Logrieco et al. 2002). Ear rot decreases both yield and quality (Presello et al. 2008; Vigier et al. 2001). Quality reduction is mainly caused by different mycotoxins. Beside other toxins FG produces deoxynivalenol (DON) and zearalenone (ZEA), whereas FV produces fumonisins (FUM). DON can cause immunosuppression and reproductive failure (Pestka 2007) and ZEA causes hyperestrogenism in pigs and may cause premature thelarche in humans (Zöllner et al. 2002). FUM may be the causal agent of esophageal or liver cancer and neural tube defects in humans and equine leukoencephalomalacia and porcine pulmonary edema in animals (Voss et al. 2007). To minimize risk of exposure to these mycotoxins for humans the European Union released limits for FUM (4 mg kg^{-1}), DON (1.75 mg kg^{-1}) and ZEA (0.35 mg kg^{-1}) in unprocessed maize for use as human food in 2007. For feeding animals the recommended levels vary between 2–8 mg kg^{-1} for DON and FUM and 0.25–0.5 mg kg^{-1} for ZEA depending on animal species and its age. These levels can be largely exceeded when natural infection occurs (Logrieco et al. 1995).

In maize fungicide application is not feasible during female flowering due to plant height and no fungicide for control of infection has been released in Europe. Thus, breeding and growing varieties with resistance to mycotoxin accumulation is one alternative to reduce mycotoxins in basic maize products. Resistances to ear rot and FUM accumulation are inherited quantitatively with mainly additive or GCA (General combining ability) effects (Butrón et al. 2006; Chungu et al. 1996; Ding et al. 2008; Pérez-Brito et al. 2001; Robertson-Hoyt et al. 2006). Quantification of mycotoxin concentrations is expensive (~5–7 € per sample laboratory costs without labor for immunotests), laborious and time consuming, in contrast ear rot rating is more cost efficient, less laborious and faster. Therefore indirect selection on reduced toxin concentrations by ear rot rating could gain in higher efficiency of selection. Successful indirect selection requires strong genetic associations between both traits. They have been reported for FUM and DON in US or Canadian maize (Kleinschmidt et al. 2005; Reid et al. 1996b; Robertson et al. 2006; Vigier et al. 2001). No clear association between symptoms and ZEA concentration has been found (Bakan et al. 2002; Cullen et al. 1983; Hart et al. 1984).

Very few information has been published about genetic variation of mycotoxin concentrations and associations with ear rot severity in early European elite maize. With regard to mid-late and late European maize information on these parameters is totally lacking. In a companion study, each of 150 early, mid-late and late maturing inbreds were tested for FG and FV ear rot, but no mycotoxins were determined (Löffler et al. 2009). The objectives of this study were to analyze quantitative-genetic parameters like variance components, heritabilities and correlations between ear rot rating and mycotoxin concentrations in three maturity groups tested across Europe. These parameters were used to estimate the relative efficiency of indirect selection on mycotoxin concentrations by selection on ear rot.

Materials and methods

Plant material and field evaluation

The plant material was separated in three maturity groups (early, mid-late and late maturity group) and was evaluated in 2007 and 2008. The early maturity group consisted of 21 flint and 36 dent inbred lines, the mid-late and late group exclusively of 45 and 49 dent lines, respectively. The lines were taken out of a larger sample of 150 inbred lines per maturity group (Löffler et al. 2009) according to their ear rot resistance in 2007 under the consideration of a similar phenotypic variation. All lines were inoculated by FV, the early maturity group additionally by FG in separate, but adjacent trials. All lines were current breeding lines of the KWS SAAT AG, Einbeck, Germany. Locations for evaluation of the early maturity group were Einbeck (EIN; Central Germany), Gondelsheim (GON; South Germany) and Chartres (CHA; North France). Field trials of the mid-late group were conducted at Alzonne (ALZ; South France) and Murony (MUR; Hungary) and of the late maturity group at Monselice (MCE; North

Italy). Lines were tested in a randomized complete block design with two replications. Each single-row plot consisted of 20 plants.

Inoculum production

The isolates used for artificial inoculation at all locations were FV234/1 and IFA66, both kindly provided by M. Lemmens (IFA, Tulln, Austria), for FV and FG, respectively. FV234/1 is known to be a FUM producer and IFA66 a DON and ZEA producer. Single isolates were used to avoid isolate × isolate interactions. Inoculum was stored as colonized agar plugs on special nutrient poor agar (SNA) in sterile water at 6°C and mass propagation was prepared as described by Reid et al. (1996a). Briefly, the isolates were sub-cultured on SNA under UV light for spore production and the mycelium-spore mixture was washed with sterile water into Erlenmeyer flasks of 2 l containing 600 ml of liquid mineral medium. For conidia production, the mycelium suspension was incubated on rotary shakers (100 rpm) under permanent UV light at about 25°C for seven days. Afterwards, conidia were concentrated for easier shipment and storage by sedimenting in separating funnels overnight at 6°C. Concentrated inoculum was sent cooled in small tubes to the locations, stored frozen (−20°C) until usage and diluted to reach the desired conidia concentrations.

Inoculation and rating

Inoculation and rating procedures were conducted as described by Reid et al. (1996a). Silk channel inoculation was performed by injecting inoculum with a self-refilling syringe into the silk channel four to seven days after 50% silk emergence in each plot. The primary ears of ten plants per plot having approximately the same stage of silking were inoculated with 1 ml of inoculum with concentrations of 1×10^5 and 1×10^6 conidia ml^{-1} for FG and FV, respectively. At MCE, additionally kernel inoculation was conducted with the same plant material in a separate but adjacent randomized complete block design with two replications. Thirteen to fourteen days after 50% silk emergence in each plot primary ears were inoculated by stabbing four nails previously dipped in inoculum into the kernels. At physiological maturity primary ears of inoculated plants in a plot were dehusked and rated. Ear rot severity was visually rated as the percentage (0–100%) of the surface covered with mycelium of each primary ear.

Mycotoxin analyses

Inoculated ears of genotypes were harvested, dried, shelled and then the kernels were milled. After milling with Vorwerk Thermomix® for one and a half minute (10,000 rpm) a representative sample (approx. 100 g) was taken for toxin analyses. Five gram samples were analyzed with the immunoassays RIDASCREEN® FAST DON, ZEA and FUM (R-Biopharm, Darmstadt, Germany). RIDASCREEN® FAST DON detects DON and 3-ADON with a cross reactivity of 213% but has no cross reactivity with other trichothecenes like 15-ADON, nivalenol and fusarenon-X. Fumonisins FB_1, FB_2 and FB_3 were detected with the RIDASCREEN® FAST FUM with cross reactivities of 29–40 and 68–100% of FB_2 and FB_3, respectively. Measurement was conducted with a microtiter plate spectrometer at 450 nm (TECAN SLT Lab Instruments, Crailsheim, Germany). Applying five standard solutions per test the concentrations were calculated with a software package provided by spectrometer manufacturer. To meet the range of the standard solutions, the samples were diluted with distilled water, if necessary.

Statistical analysis

Single plot data were used for analyses of variance (ANOVA). Residuals were normally distributed for inoculation by FG but not by FV and for all mycotoxins. Therefore, the data of severity of FV inoculation and mycotoxins were natural log and fourth root transformed, respectively, to remove heterogeneity of variance. The statistical model used for analysis of variance was

$$X_{ijkl} = \mu + Y_i + L_j + YL_{ij} + R(YL)_{ijl} + G_k + GY_{ik} + GL_{jk} + GYL_{ijk} + \varepsilon_{ijkl}$$

where μ is the overall mean, Y_i the effect of year i, L_j the effect of location j, G_k the effect of genotype k, YL_{ij}, GY_{ik}, GL_{jk} GYL_{ijk} the corresponding interaction effects, $R(YL)_{ijl}$ the effect of replication l within the

year i and location j, and ε_{ijkl} the effect of experimental error. All effects were considered to be random except the overall mean. Based on entry-means variance components were estimated as described by Searle (1971) and broad-sense heritabilities were calculated according to Fehr (1987). Phenotypic and genotypic correlations between ear rot ratings and mycotoxin concentrations were calculated by standard procedures (Mode and Robinson 1959). Standard errors of variance components and heritabilities were calculated as described by Searle et al. (1992) and Knapp and Bridges (1987), respectively. All computations were performed with the computer package PLABSTAT (Utz 2004). Relative efficiency of indirect selection to direct selection was calculated as described in Falconer and Mackay (1996) with the following formula assuming selection intensity to be equal:

$$R_{\text{rot,toxin}}/R_{\text{toxin}} = \frac{h_{\text{rot}} r_{g(\text{rot,toxin})}}{h_{\text{toxin}}}$$

where h_{rot} and h_{toxin} are the square roots of heritabilities of ear rot severity and mycotoxin concentrations, respectively, and r_g the genotypic correlation of both traits.

Results

Naturally infected samples

Non-inoculated ears of each of three resistant, medium susceptible and susceptible lines were analyzed in each maturity group for their mycotoxin concentrations. In Central Europe the frequencies of samples containing DON, ZEA or FUM concentrations above detection limit were 42, 49 and 59% across 2007 and 2008. However, only few samples had DON (18%), ZEA (3%) and FUM (7%) concentrations higher than EU limits. In contrast, most samples from the mid-late and late maturing inbreds grown in Southern Europe exceeded the legal limits of FUM concentrations, particularly those in ALZ (75%) and MCE (82%). At MCE one sample contained up to 150 mg kg^{-1} FUM. Mean FUM concentrations were 13.9 and 28.5 mg kg^{-1} at ALZ and MCE, respectively, whereas the mean at MUR was 2.7 mg kg^{-1}, where samples only of the year 2008 were analyzed.

Means

Ear rot severities and mycotoxin concentrations had broad ranges for single locations and years and across location series analyses (Tables 1, 2). DON and ZEA concentrations were consistently lower in 2008 than in 2007 (Table 1). At GON lowest FG ear rot severities and DON concentrations in both years were observed. High FV severity and FUM concentrations were found at CHA and EIN in the early maturity group (Table 2). The flint lines had consistently higher means of FG and FV severity and also DON and FUM concentrations than dent lines. In the mid-late maturity group FUM concentrations of both locations were similar although FV severity was considerably higher at ALZ than at MUR. In the late maturity group, FV severity and FUM concentrations were higher in 2008 than in 2007. FV severity of both silk channel and kernel inoculation were similar but FUM concentrations were higher with silk channel than with kernel inoculation.

Variances

Significant ($P < 0.01$) genotypic variances were found for both ear rot severity and all mycotoxin concentrations (Table 3). In all maturity groups all traits were affected by significant ($P < 0.01$) genotype × environment interactions except ear rot of kernel inoculation in the late maturing inbred lines. In all instances heritabilities in the early maturity group were high, but heritabilities of the flint lines were lower than those of dent lines. Heritability of FV severity of kernel inoculation was also high whereas heritabilities of all other traits in the mid-late and late maturity groups were medium. Ear rot severities had similar or higher heritabilities than mycotoxin concentrations except in the mid-late maturity group.

Correlations

Phenotypic correlations between ear rot severity and corresponding mycotoxin concentrations were high and significant ($P < 0.01$). Genotypic correlations exceeded the corresponding phenotypic correlations and their standard errors twice (Table 4). Correlations of flint and dent lines were very similar and thus we conducted a combined analysis across flints and dents to get more accurate estimates of genotypic

Table 1 Means of single environments and across environments (series) of ear rot severity and deoxynivalenol and zearalenone concentrations after silk channel inoculation with *Fusarium graminearum* of *n* early maturing flint and dent inbred lines

Pool	Location[a]	Ear rot severity (%)					Deoxynivalenol (mg kg^{-1})					Zearalenone (mg kg^{-1})				
		Years		Series			Years		Series			Years		Series		
		2007	2008	Mean	Minimum	Maximum	2007	2008	Mean	Minimum	Maximum	2007	2008	Mean	Minimum	Maximum
Flint ($n = 21$)	CHA	71.2	69.0	70.1	4.0	99.4	1059.9	417.1	738.5	19.7	3513.5	42.0	13.3	27.7	0.1	92.1
	EIN	91.9	74.3	83.1	33.4	99.8	1517.3	502.5	1009.9	157.4	4065.0	74.0	10.9	42.5	4.3	145.5
	GON	16.9	21.7	19.3	0.1	78.0	43.6	55.4	49.5	1.9	340.7	16.7	16.3	16.5	0.1	79.3
	Mean	60.0	55.0	57.5	12.3	91.1	873.6	325.0	599.3	59.7	1666.7	44.2	13.5	28.9	1.5	77.2
Dent ($n = 36$)	CHA	42.0	46.2	44.1	7.1	100.0	527.3	156.0	341.6	17.2	3540.0	24.2	5.8	15.0	0.7	134.7
	EIN	76.4	45.9	61.2	17.5	99.9	971.0	241.2	606.1	42.0	4140.0	35.7	9.7	22.7	0.7	93.8
	GON	29.6	26.1	27.9	3.4	85.8	110.9	52.4	81.7	6.7	724.9	53.8	23.2	38.5	1.6	428.5
	Mean	49.4	39.4	44.4	14.5	95.2	536.4	152.9	344.7	28.7	2664.6	37.9	13.1	25.5	2.8	219.0

[a] *EIN* Einbeck, *GON* Gondelsheim (Germany), *CHA* Chartres (France)

correlations and relative efficiencies. These estimates were also similar to those of single pools. No genotypic correlation is given for the late maturity group due to only one location across two years. In the early maturity group the genotypic correlation between DON and ZEA based on a combined analysis was close to one, whereas the one between DON and FUM was moderate (Fig. 1). The genotypic correlation between ZEA and FUM was 0.76. Phenotypic correlations between silk channel and kernel inoculation of ear rot severity and between FUM concentrations of these inoculation methods in the late maturity group were 0.77 and 0.66, respectively. Relative efficiencies of indirect selection based on FG ear rot rating to DON and ZEA concentrations were slightly higher than one if calculated across flint and dent lines (1.01; 1.02, respectively). Indirect selection for FUM concentrations based on FV ear rot rating is expected to be less effective than direct selection since efficiencies were 0.97 and 0.82 in the early and mid-late maturity group, respectively.

Discussion

Mycotoxins in naturally infected samples

FUM concentrations of non-inoculated ears above the legal EU limit were found with a high frequency at MCE and ALZ. This and the high FUM concentrations of 150 mg kg^{-1} of one sample are in accordance with Logrieco et al. (1995). In Central Europe only few samples of non-inoculated ears had mycotoxin concentrations above EU limit. Nevertheless, growing resistant varieties in all regions could minimize the risks of animal and human exposure to these harmful mycotoxins and rejection of the harvest by the trader. However, mycotoxin concentrations of the samples in our study might be biased upwards caused by hand harvest of the ears and shelling without an air separator. By machine harvest heavily infected kernels having reduced size and density would have been removed from the grain automatically.

Weather effect on infection and mycotoxin accumulation

Optimal conditions for DON accumulation by FG infection are moderately warm temperatures and wet

Table 2 Means of single environments and means across environments (series) of ear rot severity and fumonisin concentrations after inoculation with *Fusarium verticillioides* in three maturity groups each comprising *n* inbred lines and weather data

Maturity group	Pool	Location[a]	Inoculation[b]	Ear rot severity (%)					Fumonisins (mg kg^{-1})					Temperature (°C)[c]		Precipitation (mm)[d]	
				Years		Series			Years		Series			Years		Years	
				2007	2008	Mean	Minimum	Maximum	2007	2008	Mean	Minimum	Maximum	2007	2008	2007	2008
Early	Flint ($n = 21$)	CHA	Silk	16.5	25.3	20.9	3.8	72.1	74.3	146.1	110.2	4.0	526.4	15.9	16.8	49.0	37.5
		EIN	Silk	15.4	19.5	17.5	1.5	37.0	234.9	92.8	163.9	7.0	597.0	16.2	16.5	101.2	68.7
		GON	Silk	5.2	7.7	6.5	0.8	21.8	24.5	47.9	36.2	2.7	88.6	17.6	17.9	68.8	62.0
		Mean	Silk	12.1	17.5	14.8	3.5	41.5	110.4	95.6	103.0	8.7	383.2				
	Dent ($n = 36$)	CHA	Silk	8.7	17.0	12.8	0.6	70.2	55.6	69.2	62.4	3.1	378.6	15.9	16.8	49.0	37.5
		EIN	Silk	7.8	12.8	10.3	0.4	47.2	53.8	46.3	50.1	2.0	400.2	16.2	16.5	101.2	68.7
		GON	Silk	4.5	6.8	5.6	0.4	40.8	21.4	41.9	31.6	1.9	170.4	17.6	17.9	68.8	62.0
		Mean	Silk	7.0	12.2	9.6	0.7	52.7	43.6	52.5	48.0	5.1	305.8				
Mid-late	Dent ($n = 45$)	ALZ	Silk	12.7	15.5	14.1	4.7	49.1	20.1	23.8	21.9	6.4	81.2	20.9	20.9	22.7	27.7
		MUR	Silk	7.2	4.3	5.8	1.9	16.0	21.0	16.8	18.9	4.3	64.0	20.6	20.2	48.1	58.0
		Mean	Silk	9.9	9.9	9.9	4.0	28.9	20.5	20.3	20.4	7.5	72.6				
Late	Dent ($n = 49$)	MCE	Silk	9.9	13.6	11.7	1.2	41.0	20.7	66.1	43.4	3.5	139.9	22.7	22.4	29.7	47.9
		MCE	Kernel	9.4	13.3	11.3	2.6	75.0	18.1	44.2	31.1	4.4	89.5	22.7	22.4	29.7	47.9

[a] *EIN* Einbeck, *GON* Gondelsheim (Germany), *ALZ* Alzonne, *CHA* Chartres (France), *MUR* Murony (Hungary), *MCE* Monselice

[b] *Silk* silk channel inoculation, *Kernel* kernel inoculation

[c] Monthly mean from 1st inoculation to harvest

[d] Cumulative precipitation from 1st inoculation to harvest

Table 3 Estimates of variance components, standard errors (SE) and heritabilities of three maturity groups containing *n* inbred lines after inoculation with *Fusarium graminearum* (FG) or *F. verticillioides* (FV)

Maturity group	Pool	Fusarium[a]	Trait[b]	Variance components[c] ± SE					Heritability ± SE
				σ^2_G	σ^2_{GL}	σ^2_{GY}	σ^2_{GLY}	σ^2_e	
Early	Flint ($n = 21$)	FGs	Ear rot	244.82 ± 6.84 **	97.25 ± 8.78 **	[f]	85.32 ± 8.29 **	137.51	0.77 ± 0.07
			DON[d]	0.761 ± 0.34 **	0.127 ± 0.51	[f]	0.267 ± 0.50 **	0.495	0.84 ± 0.06
			ZEA[d]	0.186 ± 0.23 **	0.081 ± 0.34 +	[f]	0.152 ± 0.29 **	0.164	0.70 ± 0.10
		FVs	Ear rot[e]	0.643 ± 0.34 **	0.078 ± 0.42	0.070 ± 0.34	0.147 ± 0.45 *	0.398	0.84 ± 0.07
			FUM[d]	0.539 ± 0.35 **	0.150 ± 0.30 **	0.086 ± 0.24 **	0.077 ± 0.32 *	0.202	0.81 ± 0.07
	Dent ($n = 36$)	FGs	Ear rot	437.70 ± 5.40 **	14.67 ± 8.84	[f]	91.49 ± 8.05 **	129.65	0.93 ± 0.02
			DON[d]	0.967 ± 0.34 **	0.132 ± 0.39 +	0.047 ± 0.32 **	0.161 ± 0.38 **	0.293	0.89 ± 0.03
			ZEA[d]	0.248 ± 0.18 **	0.008 ± 0.30	[f]	0.103 ± 0.29 **	0.163	0.88 ± 0.04
		FVs	Ear rot[e]	0.962 ± 0.39 **	0.105 ± 0.51 +	0.060 ± 0.42	0.323 ± 0.44 **	0.388	0.86 ± 0.05
			FUM[d]	0.484 ± 0.24 **	0.035 ± 0.31	0.038 ± 0.25	0.127 ± 0.26 **	0.133	0.90 ± 0.04
Mid-late	Dent ($n = 45$)	FVs	Ear rot[e]	0.174 ± 0.34 **	0.029 ± 0.36	0.066 ± 0.36 +	0.096 ± 0.41 *	0.329	0.61 ± 0.14
			FUM[d]	0.062 ± 0.17 **	[f]	0.024 ± 0.21 +	0.033 ± 0.23 *	0.103	0.68 ± 0.12
Late	Dent ($n = 49$)	FVs	Ear rot[e]	0.530 ± 0.49 **	[g]	0.250 ± 0.48 **	[g]	0.470	0.69 ± 0.09
			FUM[d]	0.113 ± 0.28 **	[g]	0.063 ± 0.31 *	[g]	0.196	0.58 ± 0.12
		FVk	Ear rot[e]	0.413 ± 0.28 **	[g]	[f]	[g]	0.336	0.84 ± 0.05
			FUM[d]	0.063 ± 0.22 **	[g]	0.032 ± 0.26 +	[g]	0.137	0.56 ± 0.13

[a] *FGs*, *FVs* silk channel inoculation, *FVk* kernel inoculation

[b] *DON* deoxynivalenol, *ZEA* zearalenone, *FUM* fumonisin

[c] σ^2_G,σ^2_{GL}, σ^2_{GY}, σ^2_{GLY}, σ^2_e Genotype (G), G × location (L), G × year (Y), G × L × Y and error variance components

[d] 4th root transformed

[e] Natural log transformed

[f] Negative estimator

[g] Not estimated

+, *, ** Significant at $P < 0.1$, 0.05, 0.01, respectively

Table 4 Phenotypic (r_P) and genotypic (r_G) correlations and relative efficiencies between ear rot severity and mycotoxin concentrations of three maturity groups after inoculation with *F. graminearum* (FG) and *F. verticillioides* (FV)

Maturity group	Pool	Ear rot[a]	Toxin[b]	r_P	r_G	Relative efficiency
Early	Flint	FGs	DON	0.95**	1.01 ++	0.97
			ZEA	0.96**	1.06 ++	1.11
		FVs	FUM	0.95**	1.03 ++	1.05
	Dent	FGs	DON	0.95**	0.99 ++	1.01
			ZEA	0.95**	1.00 ++	1.03
		FVs	FUM	0.89**	0.94 ++	0.92
	Across flint & dent	FGs	DON	0.95**	0.99 ++	1.01
			ZEA	0.91**	0.97 ++	1.02
		FVs	FUM	0.91**	0.97 ++	0.97
Mid-late	Dent	FVs	FUM	0.83**	0.87 ++	0.82
Late	Dent	FVs	FUM	0.86**	–[c]	–[c]
		FVk	FUM	0.76**	–[c]	–[c]

[a] *FGs*, *FVs*, silk channel inoculation, *FVk* kernel inoculation

[b] *DON* deoxynivalenol, *ZEA* zearalenone, *FUM* fumonisin

[c] Not estimated

** Significant at $P < 0.01$, ++ Exceeded twice its standard error

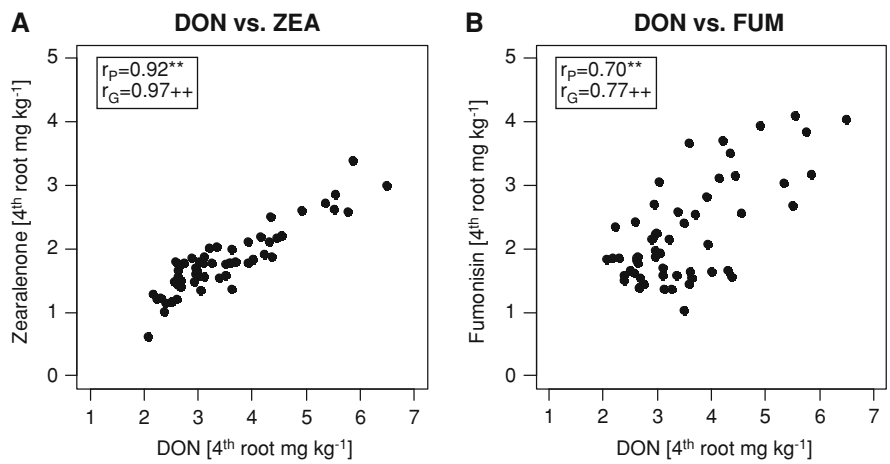

Fig. 1 Scatter plots of deoxynivalenol (DON) and zearalenone (ZEA) (**a**) or fumonisin (FUM) (**b**) based on 57 entry means of the early maturity group. r_P, r_G refer to phenotypic and genotypic correlations, respectively. ** Significant at $P < 0.01$. ++ Exceeded twice its standard error

weather at silking and during maturation (Doohan et al. 2003; Munkvold 2003), whereas ZEA production is fostered by low temperatures with high humidity (Munkvold 2003). DON and ZEA concentrations were, on average, lower in 2008 than 2007 (Table 1) which could be caused by lower temperatures at CHA and GON, particularly in July and August.

The considerably higher means of FG severity than of FV severity in the early maturity group are in accordance with the general observation of Miller (1994). The lower means of FV severity observed in all maturity groups are in agreement with reports of US corn belt material (Kleinschmidt et al. 2005; Robertson et al. 2006) and might be explained by the dual nature of FV either as an endophyte or pathogen (Bacon et al. 2008; Pascale et al. 2002; Yates et al. 1997).

FUM accumulation by FV infection is favored by warm to hot and dry weather at silking and during grain-filling period (Bottalico 1998; Doohan et al. 2003; Miller 2001; Munkvold 2003). However, some of our results do not follow this rule. Highest mean FV severities and FUM concentrations were observed

at the coolest locations CHA and EIN (Table 2). An explanation might be chilling stress weakening plants changing the balanced endophytic relationship of plants and FV into a disease (Bacon et al. 2008). Despite hot and dry conditions at silking at MCE in July 2007 (Temperature 25.6°C; Rainfall 4 mm), FV severity and FUM concentrations were lower than in 2008 for both inoculation methods. FUM concentrations were similar at both locations in the mid-late maturity group, although ear rot severities were considerably higher at ALZ than at MUR, probably due to less precipitation at ALZ. This illustrates that the characteristics of FV as a pathogen and the conditions of FUM production under field conditions are more complex and less understood than that of FG.

Testing *Fusarium* resistance by silk channel or kernel infection

In Central Europe resistance to FG and FV is necessary since both can be found in maize frequently depending on the weather which species is preferred (Görtz et al. 2008). Genotypes resistant to DON or ZEA were not necessarily resistant to FUM accumulation, although moderate genotypic correlations were found (0.77; 0.76, respectively) (Fig. 1b). This corresponds to the correlation of resistance between FG and FV reported previously (Löffler et al. 2009) and is in contrast to Schaafsma et al. (2006). If mycotoxins of FG infection should be assessed it is sufficient to measure DON concentration, because the genotypic correlation to ZEA concentration equals one (Fig. 1a). Separate testing of FG and FV resistance and their corresponding mycotoxins is necessary, but is not necessary for DON and ZEA.

For Southern Europe, both ways of infection (Munkvold 2003), via silk channel and via wounding of kernels, gave similar ear rot severities, but differing FUM concentrations. They were lower with kernel inoculation which is in contrast to Schaafsma et al. (2006). Moderate phenotypic correlations ($r = 0.66$) between both inoculation methods for FUM concentration indicate that at least some QTL involved in resistance might be acting against both ways of entry. But not all genotypes resistant to one inoculation method were resistant to the other (data not shown). In conclusion, reliable selection of genotypes having resistance for both infection pathways requests both silk channel and kernel inoculation, particularly in regions were European corn borer occurs frequently.

Flint vs. dent

Higher means were found for the flint pool than for the dent pool in all instances in the early maturity group except for ZEA concentrations (Tables 1, 2). This is not caused by outliers as shown by similar ranges. Thus, the European flint pool seems to be more susceptible to ear rot, DON and FUM accumulation in general which was found in a previous study for ear rot severity in larger sets (Löffler et al. 2009). The higher susceptibility of the flint lines might have historic causes because the flint pool was created by a few founding populations only. In contrast, in the European dent pools recurrent influx of alleles from other germplasms occurred (Reif et al. 2005). Considering that the inheritance of resistance is mainly caused by additive or GCA effects (Butrón et al. 2006; Chungu et al. 1996), both parents of hybrids need to possess resistance alleles. Consequently, resistances in the flint pool should be improved by introgression of resistance alleles followed by recurrent selection to improve other traits. Regarding heterotic patterns sources of resistance alleles should belong to similar heterotic groups like non-Stiff-Stalk for the flint pool (Reif et al. 2009). Higher variation and lower minima of the dent pool (Tables 1, 2, 3) indicate that breeding for resistance likely gains faster in highly resistant genotypes than in the flint pool.

Indirect selection of low mycotoxin concentrations by ear rot rating

Important factors affecting the response of indirect selection are (i) genotypic variation of ear rot severity and mycotoxin concentrations, (ii) their heritabilities and (iii) association between the two traits. Broad ranges and significant ($P < 0.01$) genotypic variances were found for ear rot severity and mycotoxin concentrations in all maturity groups and sub-pools (Tables 1, 2, 3) indicating that considerable genetic variation among adapted European inbred lines exist. But accurate selection of stable resistance under varying weather conditions is complicated by high

genotype × environment interactions showing that multi-environmental tests are required. The lower heritability of flint lines for ear rot severity and ZEA concentrations after inoculation with FG might be attributed to the lower genetic variation (Table 3). Heritabilities of ear rot severity and FUM concentrations of flints and dents in the early maturity group were similar to those of Robertson et al. (2006) for US maize. Lower heritabilities found in the mid-late and late maturity group might be caused by natural background infection increasing the error variances (Löffler et al. 2009). Correlations between ear rot severity and mycotoxin concentrations in all maturity groups were high (Table 4). This agrees with reports from Canadian maize for FG (Reid et al. 1996b; Vigier et al. 2001) and US maize for FV (Kleinschmidt et al. 2005, Robertson et al. 2006). In contrast, no clear associations between ear rot and ZEA were found in former studies (Bakan et al. 2002, Cullen et al. 1983; Hart et al. 1984). The high genotypic correlations strongly indicate that similar genes/QTL should be responsible for ear rot resistance and tolerance to mycotoxin accumulation as previously shown for the 3B and 5A QTL from 'Sumai 3' in wheat (Lemmens et al. 2005; Miedaner et al. 2006).

Although flint and dent lines should be regarded separately, we additionally calculated correlations and relative efficiencies in a combined analysis because (1) correlations of flints and dents were almost equal (Table 4) and (2) accuracies of estimations of genotypic correlations and consequently relative efficiencies were increased due to higher sample size. Relative efficiencies of indirect selection for DON and ZEA concentrations based on FG ear rot severity were slightly higher than one (Table 4). Thus, indirect selection for reduced mycotoxin concentrations by rating FG ear rot severity is more effective than direct selection. Relative efficiencies of FV ear rot severity and FUM were below one (0.97; 0.82 in early and mid-late maturity group, respectively). Assuming a fixed budget, indirect selection based on ear rot severity would additionally allow testing of more genotypes and/or using more test environments. This would clearly increase response to selection by higher selection intensity and/or heritability making indirect selection based on ear rot severity even more attractive. Another reason supporting ear rot rating is that selection can be conducted prior to sowing the winter nursery additionally saving money and time. The success of indirect selection for reduced DON concentration by FG head blight severity has been shown previously in wheat experimentally (Wilde and Miedaner 2006). Nevertheless, lines with low ear rot ratings should be additionally analyzed for their mycotoxin concentrations, especially after FV infections, because (1) symptomless kernels are reported to occur frequently that still contribute to FUM accumulation (Desjardins et al. 1998) and (2) still fairly high levels of mycotoxins could be found in some lines despite a good ear rot performance (Data not shown). In conclusion, assuming fixed budgets indirect selection for reduced mycotoxin concentrations based on ear rot severity is effective. However, mycotoxin analyses of indirectly selected genotypes are recommended to exclude lines with low ear rot severity but rather high mycotoxin concentrations.

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