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ORIGINAL PAPER

# Covariation between line and testcross performance for reduced mycotoxin concentrations in European maize after silk channel inoculation of two *Fusarium* species

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**Abstract** *Fusarium* spp. in maize can contaminate grain with mycotoxins harmful to humans and animals. Breeding and growing resistant varieties is one alternative to reduce contamination by mycotoxins. Little is known about the population parameters relevant to resistance breeding. The objectives of this study were to draw conclusions on breeding of reduced mycotoxin concentrations of deoxynivalenol, zearalenone and fumonisins, and resistance to ear rot after silk channel inoculation with *F. graminearum* or *F. verticillioides*, respectively. For that, variation and covariation of line and testcross performance and correlations between both species and between mycotoxin concentrations and ear rot resistance were calculated. Means of ear rot after infection with *F. graminearum* were higher than with *F. verticillioides*. Moderate phenotypic correlations ($r = 0.46$–$0.65$) between resistances to both *Fusarium* spp. implicate the need of separate testing. Analyses of variance revealed significant ($P < 0.01$) differences among lines in line and testcross performance for 30–60 entries per maturity group. Multi-environmental trials for accurate selection are necessary due to significant ($P < 0.1$) genotype × environment interactions. High genotypic correlations between ear rots and mycotoxins ($r \geq 0.90$), and similar heritabilities of both traits, revealed the effectiveness of indirect selection for mycotoxin concentrations based on ear rot rating after inoculation. Moderate genotypic correlations between line and testcross performance were found ($r = 0.64$–$0.83$). The use of one moderately to highly susceptible tester is sufficient since genotypic correlations between testcrosses of different testers were high ($r = 0.80$–$0.94$). Indirect selection for testcross performance based on line performance is less effective than selection based on mycotoxin concentrations. Consequently, selection for resistance to ear rot and mycotoxin accumulation should be started among testcrosses tested first for general combining ability based on ear rot data in parallel with a negative selection for line per se performance.

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## Introduction

Maize (*Zea mays* L.) is grown in Europe with a total acreage of about 14 million hectares in 2009 (FAO 2010), 37% of which is silage maize. In central and north eastern Europe, mainly silage maize is produced, whereas grain maize is used for feeding and as human food (i.e., cornflakes, polenta) in southern Europe. According to the agroclimatical conditions, maize breeding material is divided into different maturity groups: "early" for Denmark, Germany, northern France and The Netherlands (FAO 180–330), "mid-late" for southern France and Hungary (FAO 300–480) and "late" for Spain, Italy and the Balkan states (FAO 400–700). Dents are used in all maturity groups and flints additionally in the early maturity group.

*Fusarium* ear rot in maize is mainly caused by *F. verticillioides* across whole Europe and in central Europe additionally by *F. graminearum* (Bottalico 1998; Görtz et al. 2008; Logrieco et al. 2002). Ear rot causes yield losses, but more important is the contamination of grain with mycotoxins, which are harmful to animals and

humans (Presello et al. 2008; Vigier et al. 2001). *F. verticillioides* produces, besides other toxins, the fumonisins (FUM) that can cause porcine pulmonary edema or esophageal and liver cancer in humans (Voss et al. 2007). *F. graminearum* produces deoxynivalenol (DON) and zearalenone (ZEA). DON causes diarrhea, gastroenteritis, immunosuppression and ZEA symptoms of hyperestrogenism (Pestka 2007; Zöllner et al. 2002). To minimize the risk of exposure to these mycotoxins in 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 that was used as human food in 2007. For animal feed, the recommended levels vary between 2 and 8 mg $kg^{-1}$ for DON and FUM and 0.25–0.5 mg $kg^{-1}$ for ZEA, depending on animal species and age.

In Europe, no effective fungicide for control of infection has been released. Lower mycotoxin concentrations of US and Canadian maize inbred lines due to higher ear rot resistance have been reported for both *Fusarium* spp. (Reid et al. 1996b; Robertson et al. 2006). Thus, breeding and growing resistant varieties has the potential of producing feed and food with reduced toxin concentrations. In companion studies, large genotypic variation for ear rot severity and mycotoxin concentrations was found among inbred lines in three European maturity groups (Löffler et al. 2010a, b). In addition, indirect selection for reduced mycotoxin concentrations based on ear rot rating was shown to be more effective than direct selection in inbred lines (Bolduan et al. 2009; Löffler et al. 2010b).

Hybrid breeding programs are structured in two steps. In the first step, the inbred lines are tested for their per se performance and in the second step promising parental lines are selected on the basis of their testcross performance. The extent of the correlation between line and testcross performance is therefore crucial to determine the efficiency of the indirect selection. Moderate correlations between line and testcross performance for ear rot rating after inoculation with *F. graminearum* and the DON concentrations were found in early maturing maize in Germany (Bolduan et al. 2010). Similar information is lacking for ear rot rating and ZEA concentrations after inoculation with *F. graminearum*, and ear rot rating and FUM concentrations after inoculation with *F. verticillioides* in early maize and additionally in mid-late and late maturing European maize.

The objectives of this study were to investigate the resistance to ear rot and mycotoxin accumulation caused by two *Fusarium* species in inbred lines and their testcrosses and to draw conclusions for breeding resistant varieties. Inbreds and testcrosses obtained from three European maturity groups (early, mid-late, late) were evaluated for (i) their levels of resistance to ear rot and mycotoxin accumulation, (ii) variance components and heritabilities of ear rot and mycotoxin concentration in inbred lines and corresponding testcrosses and (iii) relationships between ear rot and mycotoxin concentrations in testcrosses and associations between line and testcross performance after silk channel inoculation.

## Materials and methods

### Plant material and field evaluation

Three maturity groups of European maize (early, mid-late, late) were evaluated for resistance to *F. verticillioides* in 2008 and 2009. The early maturity group was also evaluated for resistance to *F. graminearum* in adjacent but separate trials. The early and mid-late maturity group were divided into three and the late into two heterotic groups, each comprising 15–22 DH (doubled haploid) or $>S_6$ lines

**Table 1** Means and ranges of mycotoxin concentrations and ear rot for each tester, *n* lines (L) and their *n* corresponding testcrosses (TC) of different heterotic groups in the early maturity group after inoculation with *F. graminearum* (FG)

| Heterotic group | L/TC[a] | Tester number | Deoxynivalenol (mg $kg^{-1}$) | | | Zearalenone (mg $kg^{-1}$) | | | FG (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Tester[a] | Mean | Range | Tester[a] | Mean | Range | Tester[a] | Mean | Range |
| Flint ($n = 22$) | L | – | | 182.4 | 4.5–659.2 | | 10.1 | 2–29.1 | | 40.7 | 0.7–79.3 |
| | TC | 3 | 244.9 | 207.8 | 16.4–457.0 | 41.1 | 29.6 | 9–47.6 | 74.0 | 55.9 | 10.3–79.2 |
| | TC | 4 | 847.6 | 155.2 | 4.8–394.4 | 56.1 | 40.0 | 8–87.6 | 89.2 | 50.6 | 3.4–74.2 |
| Dentl ($n = 22$) | L | – | | 128.1 | 12.3–1027.9 | | 12.0 | 9–50.1 | | 31.3 | 8.5–88.6 |
| | TC | 1 | 249.8 | 202.4 | 30.3–689.8 | 21.9 | 38.0 | 132.5 | 72.6 | 55.9 | 24.9–90.8 |
| | TC | 2 | 198.0 | 80.1 | 11.7–387.9 | 7.8 | 12.3 | 8-38.2 | 51.6 | 33.2 | 11.5–72.7 |
| Dent4 ($n = 16$) | L | – | | 64.9 | 9.1–307.6 | | 7.4 | 1–22.8 | | 28.3 | 6.5–64.9 |
| | TC | 8 | –[b] | 99.7 | 23.1–198.9 | –[b] | 23.9 | 4–45.9 | –[b] | 45.6 | 17.4–61.9 |
| | TC | 15 | –[b] | 124.9 | 43.9–291.1 | –[b] | 29.6 | 9–47.6 | –[b] | 53.1 | 21.1–76.1 |

[a] Tester and L inoculated with 1 ml inoculum, TC with 2 ml

[b] Not calculated due to missing data in 2008

**Table 2** Means and ranges of fumonisin (FUM) concentrations and ear rot for each tester; *n* lines (L) and their *n* corresponding testcrosses (TC) of different heterotic groups in different maturity groups after inoculation with *F. verticillioides* (FV)

| Maturity group | Heterotic group | L/TC[a] | Tester-number | FUM (mg kg$^{-1}$) | | | FV (%) | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Tester[a] | Mean | Range | Tester[a] | Mean | Range |
| Early | Flint ($n = 22$) | L | – | | 65.6 | 4.2–238.4 | | 11.9 | 1.4–35.0 |
| | | TC | 3 | 270.4 | 163.1 | 29.1–311.8 | 43.5 | 18.1 | 6.1–31.4 |
| | | TC | 4 | 94.9 | 120.0 | 14.7–295.5 | 16.5 | 13.2 | 4.2–25.5 |
| | Dent1 ($n = 22$) | L | – | | 52.3 | 5.1–237.0 | | 10.7 | 0.4–47.6 |
| | | TC | 1 | 41.2 | 92.7 | 10.3–244.9 | 15.7 | 10.4 | 2.2–22.9 |
| | | TC | 2 | 62.6 | 71.2 | 18.9–154.7 | 14.5 | 9.5 | 3.6–17.6 |
| | Dent4 ($n = 16$) | L | – | | 30.9 | 5.0–84.3 | | 7.5 | 2.0–24.1 |
| | | TC | 8 | –[b] | 62.3 | 10.3–215.9 | –[b] | 7.1 | 2.5–17.9 |
| | | TC | 15 | –[b] | 96.4 | 24.3–297.9 | –[b] | 10.2 | 4.2–23.9 |
| Mid-late | Dent5 ($n = 16$) | L | – | | 31.1 | 10.8–123.1 | | 9.1 | 3.3–23.4 |
| | | TC | 9 | 31.1 | 34.3 | 13.5–82.9 | 8.1 | 4.7 | 2.8–8.8 |
| | | TC | 10 | 30.8 | 26.3 | 10.3–52.5 | 8.4 | 4.8 | 2.4–8.8 |
| | Dent8 ($n = 17$) | L | – | | 22.5 | 6.4–72.9 | | 7.6 | 2.9–14.6 |
| | | TC | 5 | 48.4 | 27.1 | 13.9–59.4 | 12.8 | 4.3 | 2.6–7.0 |
| | | TC | 6 | 40.9 | 29.4 | 10.9–56.6 | 16.1 | 4.7 | 2.5–7.0 |
| | Dent9 ($n = 17$) | L | – | | 23.1 | 8.9–69.9 | | 7.5 | 4.2–18.9 |
| | | TC | 13 | 151.8 | 133.2 | 21.3–240.9 | 20.1 | 9.4 | 2.8–15.5 |
| | | TC | 14 | 81.0 | 93.2 | 18.3–243.7 | 14.3 | 6.6 | 2.4–15.7 |
| Late | Dent10 ($n = 15$) | L | – | | 53.2 | 13.3–129.8 | | 11.7 | 4.2–36.2 |
| | | TC | 13 | 151.8 | 133.2 | 21.3–240.9 | 20.1 | 9.4 | 2.8–15.5 |
| | | TC | 14 | 81.0 | 93.2 | 18.3–243.7 | 14.3 | 6.6 | 2.4–15.7 |
| | Dent11 ($n = 15$) | L | – | | 49.0 | 3.9–111.1 | | 11.3 | 3.0–29.2 |
| | | TC | 11 | 21.8 | 67.2 | 26.7–201.9 | 5.4 | 5.7 | 2.3–17.7 |
| | | TC | 12 | 113.0 | 61.4 | 14.9–179.2 | 14.9 | 4.7 | 1.9–11.0 |

[a] Tester and L inoculated with 1 ml of inoculum, TC with 2 ml

[b] Not calculated due to missing data in 2008

(Tables 1, 2). These lines were crossed with two unrelated inbred line testers of the opposite heterotic group to produce up to 2 × 22 testcrosses per heterotic group. Testers were evaluated for their levels of resistance within the trials of lines. Lines, testers and testcrosses were elite breeding materials provided by KWS SAAT AG, Einbeck, Germany. The early maturity group was evaluated at Gondelsheim (southern Germany) and Chartres (northern France). The mid-late group was tested in Alzonne (southern France) and Murony (southeast Hungary) and the late maturity group in Monselice (northern Italy). The experimental design of the line performance test was a randomized complete block design with two replications. Testcrosses were evaluated in a split-plot design with testers as main plots and inbred lines as sub-plots with two replications adjacent to the corresponding line tests. Each single-row plot consisted of 20 plants with 0.75 m distance between rows.

## Inoculum production

The isolates used for artificial inoculation were IFA 66 and FV234/1, both kindly provided by M. Lemmens, for *F. graminearum* and *F. verticillioides*, respectively. Single isolates were used to avoid isolate × isolate interactions. The 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 subcultured on SNA and the mycelium was washed with sterile water into Erlenmeyer flasks of 2 l containing 600 ml of a liquid mineral medium with the addition of sugar. For conidia production, the mycelium suspension was incubated on rotary shakers (100 rpm) in liquid medium under permanent UV light at about 23°C for 7 days. Afterwards, conidia were concentrated for easier shipment and storage by sedimenting in separating funnels overnight at 6°C. The concentrated

inoculum was sent cooled in small tubes to the locations, stored frozen (−20°C) until usage, and before usage diluted to reach the desired conidia concentrations.

### Inoculation and disease assessment

Silk channel inoculation was performed in all maturity groups. Inoculations and rating were conducted as described by Reid et al. (1996a). Briefly, inoculation was performed by injection of inoculum with a self-refilling syringe into the silk channel 4–7 days after 50% silk emergence in each plot. Thus, each plot was inoculated separately according to its flowering time. The primary ears of ten inbred lines per plot having approximately the same stage of silking were marked and inoculated with 1 ml of inoculum for the inbred lines, with concentrations of $1 \times 10^5$ and $1 \times 10^6$ conidia $ml^{-1}$ for *F. graminearum* and *F. verticillioides*, respectively, at the same time. The primary ears of testcrosses were inoculated with 2 ml having the same conidia concentrations to account for the higher vigor of testcrosses and longer ears. For disease assessment, the ten inoculated ears per row were dehusked and immediately afterwards visually rated as the percentage of the surface covered with mycelium of each primary ear (0–100%).

### Mycotoxin analyses

Inoculated ears of genotypes were harvested, dried at approximately 36°C until grain moisture was below 14%, shelled and then the kernels were milled. After milling with Vorwerk Thermomix® for one-and-a-half minutes (10,000 rpm), a representative sample (approximately 100 g) was taken for toxin analyses. Samples of 5 g 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 such as 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

Plot means were used for analyses of variance (ANOVA). According to Shapiro–Wilk tests, residuals were normally distributed for inoculation by *F. graminearum* but not for *F. verticillioides* and mycotoxin concentrations. Therefore, data of *F. verticillioides* ear rot rating and mycotoxin concentrations were natural log and fourth root transformed, respectively, to achieve variance homogeneity and normal distribution of residuals. Variance components were estimated by the restricted maximum likelihood estimation (REML), using PROC MIXED of SAS (SAS Institute 1996). Statistical analyses were conducted across heterotic groups within maturity groups to obtain more accurate estimates by reducing biases which might be caused by sampling effects. The statistical model used for ANOVA of the testcrosses was

$$\begin{aligned} Y_{ijklm} = {} & \mu + E_i + R(E)_{ij} + P_k + PE_{ik} + G(P)_{kl} \\ & + GE(P)_{ikl} + T(P)_{km} + TE(P)_{ikm} + RTE(P)_{ijkm} \\ & + GT(P)_{klm} + GTE(P)_{iklm} + \varepsilon_{ijklm} \end{aligned}$$

where $\mu$ denotes the overall mean, $E_i$ the effect of environment $i$, $R(E)_{ij}$ the effect of replication $j$ within environment $i$, $P_k$ the effect of heterotic group $k$, $G(P)_{kl}$ the effect of line $l$ within heterotic group $k$, $T(P)_{km}$ the effect of tester $m$ within heterotic group $k$, $PE_{ik}$, $GE(P)_{ikl}$, $TE(P)_{ikm}$, $GT(P)_{klm}$ and $GTE(P)_{iklm}$ the corresponding interaction effects, $RTE(P)_{ijkm}$ the error effect of main plots within the environment $i$, and $\varepsilon_{ijklm}$ the effect of experimental error. All effects were considered to be random except $\mu$, $P_k$ and $T(P)_{km}$. The model of ANOVA for line performance can be extracted by discarding all parameters with effects of testers of the model of the testcrosses.

Entry-mean heritabilities for line trials were calculated as described by Fehr (1987) with

$$h^2_{\mathrm{L}} = \frac{\sigma^2_{\mathrm{g}}}{\sigma^2_{\mathrm{g}} + \frac{\sigma^2_{\mathrm{ge}}}{E} + \frac{\sigma^2}{ER}}.$$

Testcross heritabilities were calculated as

$$h^2_{\mathrm{TC}} = \frac{\sigma^2_{\mathrm{g}}}{\sigma^2_{\mathrm{g}} + \frac{\sigma^2_{\mathrm{ge}}}{E} + \frac{\sigma^2_{\mathrm{gt}}}{T} + \frac{\sigma^2_{\mathrm{gte}}}{ET} + \frac{\sigma^2}{ETR}}$$

where $\sigma^2_{\mathrm{g}}$ denotes the genotypic variance of inbreds, $\sigma^2_{\mathrm{ge}}$ the genotype × environment interaction variance, $\sigma^2_{\mathrm{gt}}$ the genotype × tester variance, $\sigma^2_{\mathrm{gte}}$ the genotype × tester × environment interaction variance and $\sigma^2$ the error variance. Further, $E$, $T$ and $R$ are the numbers of environments, testers per pool and replication per environment, respectively.

Phenotypic correlations between ear rot and mycotoxin concentrations, between testcrosses of different testers and between line and testcross performances were calculated with least square means of genotypes. For computing the genotypic correlations of the variates, the same statistical model used for ANOVA was fitted simultaneously for

the target variates. An unstructured variance–covariance matrix $\sum_G = \mathrm{var}(G_{i1}, G_{i2})$ was used to estimate the genetic covariances, where $G_{i1}$ and $G_{i2}$ denote the genotypic effects of the $i$th genotype for the two variates. Genetic correlations were computed based on the estimates $\sum_G$. The bivariate linear model was fitted using REML in SAS (SAS Institute 1996).

We wanted to compare the effectiveness of indirect selection based on ear rot rating or line performance for mycotoxin concentrations or testcross performance, respectively. For these comparisons, the relative efficiency (RE) was applied with a constant selection intensity (Falconer and Mackay 1996):

$$RE = \frac{h_1 \times r_{g(1,2)}}{h_2}$$

where $h_1$ refers to the square root of heritability of either ear rot rating or line tests, $h_2$ to the square root of heritability of either mycotoxin concentrations or testcross tests, and $r_{g(1,2)}$ to the genotypic correlation between corresponding traits.

## Results

### Means

In the early maturity group the means of ear rot of lines and testcrosses after *F. graminearum* inoculation were higher than after *F. verticillioides* inoculation (Tables 1, 2). Ranges of lines were larger than of testcrosses for *F. verticillioides* and *F. graminearum* ear rot and DON concentrations, but for ZEA and FUM concentrations it was mainly vice versa. The flint group of the early maturity group had higher line means than the two dent groups for *F. graminearum* ear rot, DON and FUM concentrations. In

**Table 3** Variance components, their standard errors (SE), heritabilities and genotypic correlations between line and testcross performance ($r_G$) for ear rot and mycotoxin concentrations of different maturity groups after inoculation with *F. graminearum* (FG) and *F. verticillioides* (FV)

| Maturity group | Trait | L/TC | Variance components ± SE[a] | | | | | Heritability | $r_G$ |
|---|---|---|---|---|---|---|---|---|---|
| | | | $\sigma^2_g$ | $\sigma^2_{ge}$ | $\sigma^2_{gt}$ | $\sigma^2_{gte}$ | $\sigma^2$ | | |
| Early | FG | L | 425.6 ± 86.0** | 66.0 ± 15.6** | –[d] | –[d] | 128.5 ± 12.0 | 0.93 | 0.77 |
| | | TC | 168.7 ± 39.5** | 47.1 ± 15.2** | 20.9 ± 11.8$^+$ | 43.1 ± 17.1* | 190.7 ± 13.1 | 0.81 | |
| | DON[b] | L | 0.688 ± 0.14** | 0.125 ± 0.03** | –[d] | –[d] | 0.233 ± 0.02 | 0.92 | 0.73 |
| | | TC | 0.209 ± 0.05** | 0.045 ± 0.02* | 0.017 ± 0.01 | 0.054 ± 0.02* | 0.267 ± 0.02 | 0.83 | |
| | ZEA[b] | L | 0.198 ± 0.04** | 0.034 ± 0.01** | –[d] | –[d] | 0.082 ± 0.08 | 0.91 | 0.70 |
| | | TC | 0.089 ± 0.02** | 0.025 ± 0.01* | 0.010 ± 0.01 | 0.034 ± 0.01* | 0.154 ± 0.01 | 0.78 | |
| | FV[c] | L | 1.343 ± 0.26** | 0.260 ± 0.07* | –[d] | –[d] | 0.616 ± 0.06 | 0.90 | 0.64 |
| | | TC | 0.228 ± 0.06** | 0.076 ± 0.02** | 0.067 ± 0.02** | 0.030 ± 0.02 | 0.249 ± 0.02 | 0.76 | |
| | FUM[b] | L | 0.219 ± 0.06** | 0.296 ± 0.04** | –[d] | –[d] | 0.145 ± 0.01 | 0.70 | 0.83 |
| | | TC | 0.190 ± 0.05** | 0.038 ± 0.02* | 0.046 ± 0.02* | 0.065 ± 0.02** | 0.247 ± 0.02 | 0.77 | |
| Mid-late | FV[c] | L | 0.170 ± 0.05** | 0.129 ± 0.04** | –[d] | –[d] | 0.329 ± 0.04 | 0.70 | 0.78 |
| | | TC | 0.077 ± 0.02** | 0.044 ± 0.01** | 0.009 ± 0.01 | 0.010 ± 0.01 | 0.157 ± 0.01 | 0.74 | |
| | FUM[b] | L | 0.064 ± 0.02** | 0.019 ± 0.01$^+$ | –[d] | –[d] | 0.104 ± 0.01 | 0.78 | 0.65 |
| | | TC | 0.058 ± 0.02** | 0.014 ± 0.01$^+$ | 0.006 ± 0.01 | 0.009 ± 0.01 | 0.135 ± 0.01 | 0.79 | |
| Late | FV[c] | L | 0.149 ± 0.14 | 0.389 ± 0.16* | –[d] | –[d] | 0.294 ± 0.06 | –[e] | –[e] |
| | | TC | 0.161 ± 0.06** | 0.011 ± 0.03 | 0.037 ± 0.03 | [f] | 0.285 ± 0.04 | 0.80 | |
| | FUM[b] | L | 0.118 ± 0.07 | 0.078 ± 0.06 | –[d] | –[d] | 0.252 ± 0.05 | –[e] | –[e] |
| | | TC | 0.089 ± 0.04* | 0.014 ± 0.03 | 0.005 ± 0.03 | 0.007 ± 0.04 | 0.268 ± 0.04 | 0.79 | |

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

FG, *F. graminearum* ear rot; DON, deoxynivalenol; ZEA, zearalenone; FV, *F. verticillioides* ear rot; FUM, fumonisin; L, line per se performance evaluation; TC, testcross performance evaluation

[a] $\sigma^2_g$,$\sigma^2_{ge}$,$\sigma^2_{gt}$, $\sigma^2_{gte}$, $\sigma^2$ refer to line, line by environment, line by tester and corresponding triple interaction variance components, respectively

[b] Data transformed by 4th root (mg kg$^{-1}$)

[c] Data transformed by natural log (*ln*) (%)

[d] Not applicable

[e] Not estimated due to missing genotypic variance in lines

[f] Negative estimator

the mid-late and late maturity group means of *F. verticillioides* ear rot of testcrosses were lower than of lines except the testcrosses with tester 13 in the pool dent9. Means of FUM concentrations of testcrosses were generally higher than of lines.

### Variance components and heritabilities

In all maturity groups, significant ($P < 0.01$) genotypic variances among lines in both line and testcross performance were found for all traits except for ear rot and FUM concentrations of lines in the late maturity group (Table 3). Line × environment interactions were significant ($P < 0.1$) in all instances except for the testcrosses and for FUM concentrations of lines in the late maturity group. In the testcrosses of the early maturity group, significant ($P < 0.05$) line × tester interactions were found for *F. verticillioides* ear rot and FUM concentrations. The triple interaction of lines, testers and environments was found to be significant ($P < 0.05$) for *F. graminearum* ear rot and DON, ZEA and FUM concentrations in the early maturity group.

Generally, heritabilities were equal or higher than 0.70 except for *F. verticillioides* ear rot of lines in the late maturity group. In the early maturity group, heritabilities of the lines were higher than of the testcrosses except for FUM concentrations. In the mid-late maturity group, heritabilities of the testcrosses were higher than of the lines.

**Table 4** Phenotypic ($r_P$) and genotypic ($r_G$) correlations between ear rot and mycotoxin concentrations in testcrosses and between testcrosses created with tester 1 (TC1) and tester 2 (TC2) in different maturity groups

| Maturity group | Trait | Ear rot versus toxin | | TC1 versus TC2 | |
|---|---|---|---|---|---|
| | | $r_P$ | $r_G$ | $r_P$ | $r_G$ |
| Early | FG | – | – | 0.52 | 0.91 |
| | DON | 0.93 | 0.97 | 0.61 | 0.94 |
| | ZEA | 0.91 | 0.99 | 0.50 | 0.85 |
| | FV | – | – | 0.67 | 0.80 |
| | FUM | 0.96 | 0.99 | 0.67 | 0.83 |
| Mid-late | FV | – | – | 0.46 | 0.90 |
| | FUM | 0.85 | 0.98 | 0.76 | 0.94 |
| Late | FV | – | – | 0.62 | [a] |
| | FUM | 0.84 | [a] | 0.57 | [a] |

All phenotypic and genotypic correlations were highly significant ($P < 0.01$) or exceeded their standard errors twice, respectively

*FG*, *F. graminearum* ear rot; *DON*, deoxynivalenol; *ZEA*, zearalenone; *FV*, *F. verticillioides* ear rot; *FUM*, fumonisin

[a] Not estimated due to two environments

### Correlations and relative efficiencies

Phenotypic and genotypic correlations between *F. verticillioides* and *F. graminearum* ear rot were 0.60 and 0.74, respectively, in testcrosses. The phenotypic and genotypic correlations between mycotoxin concentrations and ear rots were high (Table 4). In lines, these correlations ranged from 0.83 to 0.98. In the late maturity group, only phenotypic correlations were presented, since genotypic correlations were biased upwards due to only two environments. Relative efficiencies of indirect selection for low toxin concentrations based on ear rot rating ranged from 0.94 to 1.12 in lines and testcrosses.

Phenotypic correlations between testcrosses of different testers were moderate, but the corresponding genotypic correlations were high (Table 4). Genotypic correlations between line and testcross performance were moderate to high (Fig. 1; Table 3). Relative efficiencies of selection among lines for testcross performance, however, varied between 0.69 and 0.83.

## Discussion

Significant differentiation among lines was found after silk channel inoculation with *F. verticillioides* or *F. graminearum* in line and testcross performances of ear rot and mycotoxin accumulations except for line tests of the late maturity group. This indicates the ability of silk channel inoculation to differentiate susceptible from resistant genotypes, which agrees well with other studies (Chungu et al. 1996b; Reid et al. 1996a). Löffler et al. (2010a) found inoculation to be superior to natural infection and, hence, only results after inoculation were considered in this study.

### Means

The considerably higher means of the flints for most traits indicate a higher susceptibility of this heterotic group. Similar results were found in companion studies with larger sets of lines (Löffler et al. 2010a, b). A reason for this higher susceptibility might be a lower influx of resistance alleles from other germplasms (Reif et al. 2005). Additionally, the higher resistance of the dent groups might be triggered by their role as seed parents and, therefore, they may have been indirectly selected for ear rot resistance. Consequently, the flint group should be improved either by using the few resistant lines found in this study for recurrent selection or by introgression of resistance alleles followed by recurrent selection to improve other traits.

Inbred lines of maize are prone to inbreeding depression resulting in less vigourous plants with small ears. Therefore, we inoculated lines by one instead of two milliliter conidia

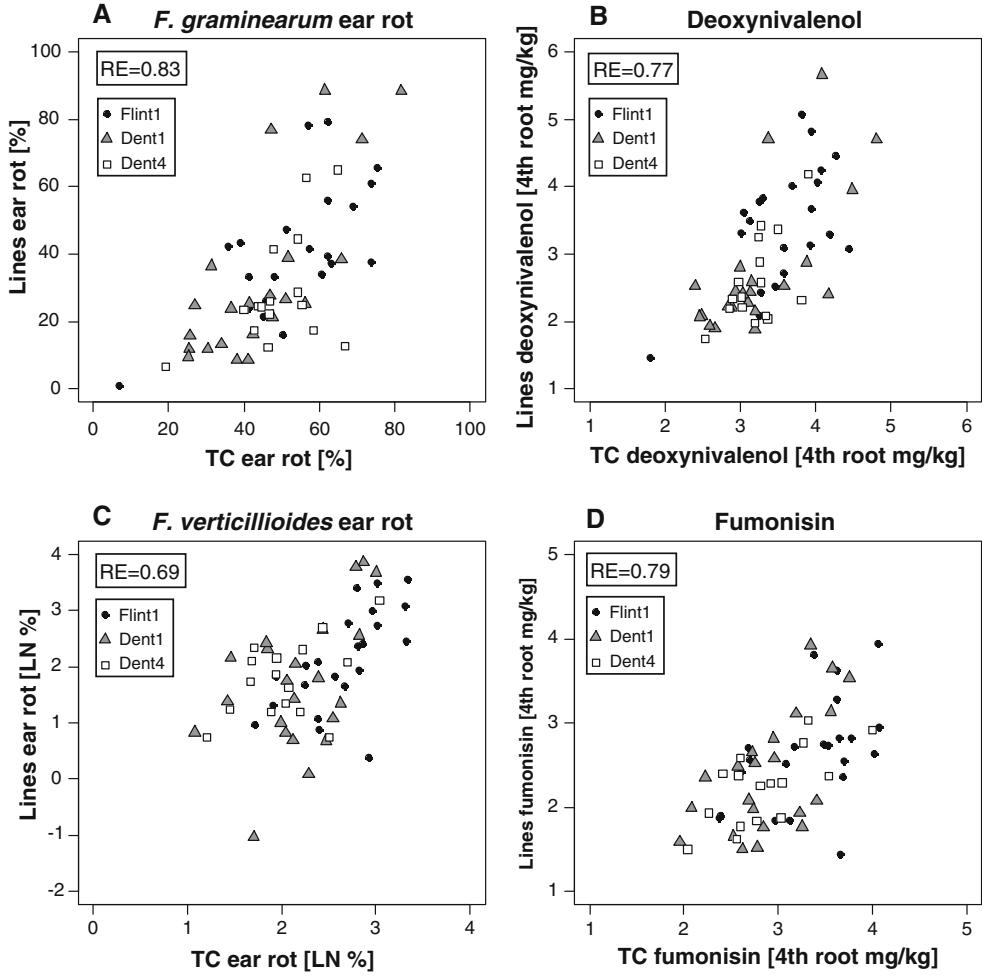


**Fig. 1** Scatter plots of testcross means across both testers (TC) and corresponding lines for the traits *F. graminearum* ear rot (**a**), deoxynivalenol concentration (**b**), *F. verticillioides* ear rot (**c**) and fumonisin concentrations (**d**) based on entry means of different heterotic pools in the early maturity group. RE refers to relative efficiency (Falconer and Mackay 1996)

suspension. Because silk channel inoculation is performed at the tip of the ear, the amount of inoculum must be sufficient to inoculate the full length of the cob as primary infection and to compensate for the effect that the fungus has to grow a longer distance from the inoculation point at the tip to the base of the ear in testcrosses. As a result, however, this may have differentially affected the disease severity in the testcrosses versus inbred lines. Reid et al. (1995) reported significant increases for *F. graminearum* ear rot with increasing inoculum volume due to the high aggressiveness of *F. graminearum,* but also a better differentiation between susceptible and resistant lines for the higher inoculum volume in years with less disease severity. The effect of inoculum volume on DON concentration in our study, however, was not consistently significant over the years. In accordance with Reid et al. (1995), genotypic differences were more pronounced with higher disease severity. This is the main reason why differential doses of inoculum are advantageous in plant breeding experiments. Despite the higher inoculum volume in testcrosses, means and ranges of *F. verticillioides* ear rot of testcrosses were generally lower than in lines except in the flint group. This shows that visual differentiation in testcrosses is more difficult than in lines. But corresponding FUM concentrations of testcrosses were higher than of lines. It is known that FUM can be found in symptomless kernels and ears (Desjardins et al. 1998). An additional explanation for our results might be the dual nature of *F. verticillioides* either as an endophyte with symptomless infection but causing high FUM concentrations, or as a pathogen causing symptoms if the complex host-pathogen relationship is disturbed by stress (Bacon et al. 2008). Generally, conclusions drawn by comparisons between lines and testcrosses should only be done with dimensionless values such as correlations and heritabilities, since different inoculum volumes might affect the means of ear rot and mycotoxin concentrations.

The moderate correlations between both species for ear rots and their corresponding mycotoxins found in

testcrosses are in agreement with correlations reported for lines in other studies (Löffler et al. 2010b; Presello et al. 2004; Schaafsma et al. 2006). Therefore, separate testing of both *Fusarium* spp. is necessary.

## Variances and heritabilities

In Europe, the maize germplasm is divided into three maturity groups to meet the different agroclimatical conditions. Each maturity group consists of an array of heterotic groups (Schmidt 2004). In this study, each maturity group was divided into two to three heterotic groups, each comprising 15–22 lines. Pooled statistical analyses were conducted across heterotic groups within one maturity group to obtain more accurate estimates by reducing biases due to sampling effects in each heterotic group. In this study, all tested inbred lines were either DH lines or $>S_6$ lines. Therefore, no bias due to the use of different selfing generations are expected, since correlations between testcrosses in such late generations equal almost 1 (Bernardo 1991).

Significant genetic variation in dents and flints were found for line per se and testcross performance except in line tests of the late maturity group. The significant genetic variances in lines and testcrosses agree well with other studies on ear rot and mycotoxin concentrations in maize (Kleinschmidt et al. 2005; Löffler et al. 2010a, b; Reid et al. 1996b; Robertson et al. 2006). The significance of genetic variances of testcrosses is likely attributed to the use of moderately susceptible to susceptible testers (Tables 1, 2). Resistant testers can diminish genotypic differences due to the presence of masking dominant alleles. Furthermore, susceptible testers having a low frequency of favorable alleles are expected to be most effective testers from a theoretical, but also an empirical view (Allison and Curnow 1966; Rawlings and Thompson 1962). Therefore, moderate to susceptible testers of the opposite gene pool are suitable for selection of resistant lines by topcross testing.

The entry-mean heritabilities were generally high ($\geq$0.70) when tested in several environments despite high error variances and significant genotype $\times$ environment interaction variances. This indicates that for an accurate selection of resistant genotypes replicated and multi-environmental trials are necessary. This is illustrated by the fact that two environments did not suffice in the late maturity group to significantly differentiate between the lines.

## Mycotoxin concentrations versus ear rot

The high genotypic correlations between toxin concentrations and ear rot found in lines and testcrosses are in accordance with other studies (Bolduan et al. 2009; Kleinschmidt et al. 2005; Löffler et al. 2010b; Reid et al. 1996b; Robertson et al. 2006). Relative efficiencies varied closely around one and, thus, indirect selection was similarly effective as direct selection. The effectiveness is even increased when the high costs of mycotoxin analyses are regarded. The selection intensity can be increased by increasing the number of test entries if only ear rot rating is applied assuming a fixed budget. Another reason supporting ear rot rating is that selection can be conducted prior to sowing the winter nursery, additionally saving resources. Similar conclusions were also drawn from line tests of other studies (Bolduan et al. 2009; Löffler et al. 2010b; Robertson et al. 2006). It might be advisable to test indirectly selected genotypes afterwards for their FUM concentrations, because high FUM concentrations in some visually resistant genotypes could be observed in our study (data not shown) and others (Desjardins et al. 1998; Löffler et al. 2010b). In conclusion, indirect selection based on ear rot rating for low mycotoxin concentrations is effective also in testcrosses.

## Line versus testcross performance

The genotypic correlations between testcrosses of different testers were high ($\geq$0.80) despite a relatively high ratio $\sigma^2_{gt}/\sigma^2_g$ for *F. verticillioides* ear rot and FUM concentrations in the early maturity group. The generally high genotypic correlations indicate that the general combining ability effects are more important than specific combining ability effects for all regarded traits. Additionally, the moderate to high genotypic correlations between lines and testcrosses also indicate a mainly additive gene action (Smith 1986), although non-additive gene actions might be possible in some cases. In generation means and diallel analyses, also mainly additive gene actions were found, but dominant and dominant $\times$ dominant gene actions were reported in some studies (Butrón et al. 2006; Chungu et al. 1996a; Clements et al. 2004; Gendloff et al. 1986; Nankam and Pataky 1996; Williams and Windham 2009). Both parents of a hybrid should have resistance alleles to obtain highly resistant varieties caused by the mainly additive gene action. For evaluation of resistance to ear rot and mycotoxin concentrations, one tester is sufficient for the evaluation of general combining abilities of inbred lines in topcrosses due to the similar ranking of lines in two testcross series.

The relative efficiencies of indirect selection for testcross performance based on line performance were lower than 0.83, and hence indirect selection was not effective. The low relative efficiencies are mainly attributed to the moderate genotypic correlations between line and testcross performance. Additionally, selection based on line

performance in early stages might not be appropriate for practical reasons: (1) directly after creation and propagation of DH lines, sufficient seeds of all genotypes might not be available to conduct separate tests for ear rot resistance and (2) testing of 7,000–10,000 DH lines or even more in each heterotic group per year (Schmidt 2004) would require a high amount of manpower. If ear rot resistance is also considered to be important in lines, i.e., for seed yield and quality in dents, the line performance could be evaluated in parallel to first topcross selection, since parental lines of resistant testcrosses are also resistant, at least moderately (Fig. 1). Inoculating all parental lines but rating only lines of previously selected testcrosses would save resources. This is feasible because testcrosses can be harvested some weeks before rating of lines must be conducted. In conclusion, indirect selection for testcross performance based on per se line performance is not effective.

## Conclusions for breeders

The ear rot resistance of the flint group should be improved since both parents of a hybrid need to be resistant due to mainly additive gene action. It could be improved by introgression of resistance alleles from other germplasm followed by recurrent selection to improve other traits. Our further conclusion for breeding resistance to ear rot and mycotoxin accumulation are implemented in a breeding scheme applying only DH lines (Schmidt 2004). Indirect selection for testcross performance based on first DH line performance is not effective due to the mentioned practical reasons and the low relative efficiencies. At this stage, lines can be selected based on other traits, such as tolerance to abiotic stress and resistances to other biotic stresses, respectively, and seed yield and quality (Schmidt 2004). In the following evaluation of general combining ability in topcross tests, we suggest testing ear rot resistance with one topcross tester being (moderately) susceptible and an inoculum volume of 2 ml to obtain a good genotypic differentiation among lines within testcrosses, particularly for *F. verticillioides* ear rot in the mid-late and late maturity groups. Generally, no mycotoxin analyses are necessary due to the very tight relationship between toxin concentrations and ear rot. Nevertheless, it might be advisable to quantify FUM concentrations by immunotests in indirectly selected genotypes due to some outliers. Additionally, the best selected parental lines can be used to improve resistance in recurrent selection. In the subsequent factorial crosses, resistance to ear rot also should be tested. If a breeder's germplasm already has a good resistance level, then evaluation of resistance also could be delayed to test the factorial crosses in the final stages of variety development. All resistance tests should be conducted in parallel to yield tests in separate but multi-environmental trials for an accurate selection. If resistance to ear rot in lines is important due to seed yield and quality, i.e., in southern Europe where high natural infection regularly occurs, a breeder could inoculate all parental lines in parallel to first topcross tests, but then only parental lines of selected testcrosses would be rated to save resources. It should be noted that we used a silk channel inoculation method. Results with kernel inoculation that are important in regions with high European corn borer pressure may be different because the correlation between silk channel and kernel inoculation is moderate in our material (Löffler et al. 2010a). Hence, this resistance must be tested additionally. In conclusion, with application of these results, phenotypic selection of testcrosses should be effective in reducing mycotoxin concentrations in European maize hybrids by silk channel inoculation.

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