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ORIGINAL PAPER

# Population parameters for resistance to *Fusarium graminearum* and *Fusarium verticillioides* ear rot among large sets of early, mid-late and late maturing European maize (*Zea mays* L.) inbred lines

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**Abstract** Infection of maize ears with *Fusarium graminearum* (FG) and *Fusarium verticillioides* (FV) reduces yield and quality by mycotoxin contamination. Breeding and growing varieties resistant to both *Fusarium* spp. is the best alternative to minimize problems. The objectives of our study were to draw conclusions on breeding for ear rot resistance by estimating variance components, heritabilities and correlations between resistances to FV and FG severity and to investigate different inoculation methods. In 2007 and 2008, three maturity groups (early, mid-late, late) each comprising about 150 inbred lines were tested in Germany, France, Italy, and Hungary according to their maturity group. They were silk channel inoculated by FG (early) and FV (all groups). In the late maturity group, additionally kernel inoculation was applied in a separate trial. The percentage of mycelium coverage on the ear was rated at harvest (0–100%). Significant ($P < 0.01$) genotypic variances of ear rot severity were found in all groups. Inoculation was superior to natural infection because of higher disease severities and heritabilities. In early maturing flints and dents, FG caused significantly ($P < 0.01$) higher ear rot severity than FV (61.7 and 55.1% FG vs. 18.2 and 11.1% FV ear rot severity, respectively). FV inoculation in Southern Europe (mid-late, late) resulted in similar means between 10.3 and 14.0%. Selection is complicated by significant ($P < 0.01$) genotype × environment interactions. Correlation between FG and FV severity was moderate in flints and dents ($r = 0.59$ and 0.49, respectively) but lines resistant to both fungi exist. We conclude that chances for selecting improved European elite maize material within the existing germplasms is promising by multi-environmental inoculation trials.

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## Introduction

Maize ear rot in Europe is primarily caused by *Fusarium graminearum* (FG) and *Fusarium verticillioides* (FV) and to a lesser extent by *Fusarium culmorum*, *Fusarium subglutinans* and *Fusarium proliferatum* (Logrieco et al. 2002). FV occurs in whole Europe, whereas FG prevails in Central and Eastern Europe (Bottalico 1998; Logrieco et al. 2002). Ear rot reduces both yield and quality of grain (Presello et al. 2008; Vigier et al. 2001). FG produces among others the mycotoxins deoxynivalenol, zearalenone and their derivatives, whereas FV is a producer of fumonisins (Gelderblom et al. 1992; Logrieco et al. 2002). All these mycotoxins can cause severe diseases in animals and humans (Pestka 2007; Voss et al. 2007) and legal limits for human food exist within the EU.

In Europe no fungicide for control of infection has been released. Lower mycotoxin concentrations of US and Canadian maize due to higher ear rot resistance have been reported for both *Fusarium* spp., (Reid et al. 1996b; Robertson et al. 2006). Therefore, breeding and growing resistant maize varieties is the best alternative to reduce yield loss and mycotoxin contamination. Resistance to FG or FV in maize is quantitatively inherited with a continuous distribution of ratings among $F_1$ progenies (Ding et al. 2008; Pérez-Brito et al. 2001; Robertson-Hoyt et al. 2006). Generation mean analyses after inoculation with FG

indicated additive, dominant, and dominant × dominant effects (Chungu et al. 1996b).

Early maturing hybrids are required in Central and Eastern Europe for silage due to a short growth and grain-filling period. They are frequently attacked by FG and, in warmer and drier years, by FV, primarily by silk infection (Bottalico 1998; Görtz et al. 2008; Munkvold 2003). Mid-late and late maturity groups are predominantly used for grain production in Southern France, Hungary, Italy, Spain, and the Balkan states and are mostly infected by FV. Here, additionally kernel infection by insect attack plays a major role.

Breeding for any trait requests sufficient genotypic variation within the breeding material. Significant genotypic differences in Canadian and US materials were reported for resistance to FG (Reid et al. 1996b; Schaafsma et al. 1997) and FV (Clements et al. 2004). However, population parameters of ear rot resistance in European elite breeding material are lacking. Thus, by inoculating three maturity groups of maize each containing about 150 inbred lines our objectives were to (1) draw conclusions on breeding for ear rot resistance by estimating variance components, heritabilities and correlations between resistances to FV and FG, (2) investigate different inoculation methods (non-inoculation vs. silk inoculation in all maturity groups, silk vs. kernel inoculation in the late group) and (3) determine discrimination (DA) and prediction ability (PA) of location × year combinations.

## Materials and methods

### Plant material and field evaluation

Three maturity groups (early, mid-late, and late) were evaluated for resistance to FV in 2007 and 2008. The early maturity group consisted of 50 flint and 90 dent inbred lines, the mid-late and late group exclusively of 147 and 148 dent lines, respectively. Additionally, the same genotypes of the early maturity group were grown in adjacent but separate trials to evaluate resistance to FG. In the early maturity group, four Canadian inbred lines developed by Reid (CO354; CO430; CO433; CO441) were used as checks (Reid et al. 2001; Reid et al. 2003) but not included in the analysis of variance. All other inbred lines were elite breeding material of the KWS SAAT AG, Einbeck, Germany, not preselected for their resistance to both FG and FV. Prior to their use, lines were genotyped by anonymous molecular markers and chosen in such a way that they cover the whole range of European germplasm of the respective pool/maturity group (M. Ouzunova, personal communication). Locations for evaluation of the early maturity group were Einbeck (EIN, Northern Germany), Gondelsheim (GON, Southern Germany) and Chartres (CHA, Northern France). The mid-late group was tested in Alzonne (ALZ, Southern France) and Murony (MUR, South-East Hungary) and the late maturity group in Monselice (MCE, Northern Italy). Additionally, the same genotypes of the late maturity group were grown near Cremona (CRE, Northern Italy) to evaluate their reaction to natural infection. The experimental design was a randomized complete block design with two replications. Each single-row plot consisted of 20 plants.

### Inoculum production

The isolates used for artificial inoculation were IFA 66 and FV234/1, both kindly provided by M. Lemmens, for FG and FV, respectively. 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 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 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 25°C for 7 days. Afterwards, conidia were concentrated for easier shipment and storage by sedimenting in separating funnels overnight. 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. In the late maturity group, kernel inoculation was additionally used in separate but adjacent trials at MCE. At CRE, the same genotypes were planted without any inoculation to observe natural infection. Inoculations and rating were conducted as described in detail by Reid et al. (1996a). Briefly, silk channel 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 silking date. The primary ears of ten plants per plot having approximately the same stage of silking were marked and inoculated with 1 ml of inoculum with concentrations of $1 \times 10^5$ and $1 \times 10^6$ conidia $ml^{-1}$ for FG and FV, respectively. At MCE, Italy, kernel inoculation was conducted 13–14 days after 50% silk emergence in each plot with the same genotypes. Mean silking date of each row was recorded for inoculated variants. At CRE,

Italy, where usually a high natural infection pressure of *Fusarium* spp. exists (F. Monguzzi, personal communication), no inoculation was performed. This is called "natural infection" throughout this article.

For disease assessment, ten primary ears per row of naturally infected and inoculated plants were dehusked and immediately afterwards visually rated as the percentage of the surface covered with mycelium of each primary ear (0–100%). In addition, eight to ten extra and non-inoculated plants per row of all inoculated variants were rated for ear rot severity and given as "non-inoculated variant" throughout. The infection of this variant results, of course, also from natural infection, but is called different to emphasize the factorial nature of this variant. Thus, we had in total up to three variants (1) natural infection at CRE (FVn), (2) inoculated, and (3) non-inoculated variant at all other locations.

### Statistical analysis

From the individual ratings of each plant, means of single plots were calculated and used for analyses of variance (ANOVA). According to Shapiro–Wilk tests residuals were normally distributed for inoculation by FG but not for FV in all inoculation variants. Therefore, the data of severity of FV inoculation, non-inoculation and natural infection were natural log transformed to achieve variance homogeneity and normal distribution of residuals. The statistical model used for ANOVA was

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

where $\mu$ 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 $\varepsilon_{ijkl}$ the effect of experimental error. All effects were considered to be random except the overall mean. In the early maturity group, flint and dents were analyzed separately and only for the assessment of significant differences among both heterotic pools combined analyses were conducted. For that the genotypes were pooled within the factor $P_m$, the effect of the heterotic group $m$. Each location–year combination was regarded as one environment for convenience. Based on entry means, variance components were estimated as described by Searle (1971) and broad-sense heritabilities were calculated according to Fehr (1987). Standard errors of variance components and heritabilities were calculated as described by Searle et al. (1992) and Knapp and Bridges (1987), respectively. Phenotypic and genotypic correlations among ear rot ratings of inoculated versus non-inoculated variants in all groups, FG versus FV severity in the early group and silk channel versus kernel inoculation in the late group were calculated by standard procedures (Mode and Robinson 1959). To evaluate the DA and PA of the environments, each location–year combination was regarded as one environment. DA is the regression coefficient of genotypic means in an individual environment regressed onto means across environments (Utz 1972). PA is the squared correlation between genotypic means in an environment and means across environments. All computations were performed with the computer packages PLABSTAT (Utz 2004) and SAS (SAS Institute 1996).

## Results

### Means

In the early maturity group, means of the dent lines after inoculation with FG and FV were 55.1 and 11.1% and those of flint lines 61.7 and 18.2%, respectively (Table 1). Significant differences of means between flint and dent lines were found only after inoculation with FV, but not after inoculation with FG and non-inoculation. Mean FG severities were significantly ($P < 0.01$) higher than mean FV severities in early maturing flints and dents. In all maturity groups, FV severities of inoculated genotypes were skewed towards lower trait expression, but FG severities showed no skewness (Fig. 1a–h). The Canadian checks—CO354, CO430, CO433 and CO441—had FG severities of 65, 42, 24 and 35% and FV severities of 26, 3, 5 and 7%, respectively (Fig. 2). In the early maturity group, a lower ear rot severity was observed for FV and FG at GON than at the other two locations (Table 1). In the mid-late maturity group, means and ranges were lower at MUR than at ALZ. Ear rot severities of the non-inoculated variant were consistently lower than the inoculated variant in the early maturity group. In mid-late and late maturity groups, non-inoculated genotypes had similar ear rot severities like the artificially inoculated ones in both years.

Mean temperatures from female flowering to rating were higher at GON than at CHA or EIN in the early maturity group (Table 1). Precipitation was highest at EIN with a great difference between both years. In the mid-late maturity group, temperatures at ALZ were similar to MUR, but precipitation at MUR was higher. Temperatures at MCE were the highest with differences of precipitation between years.

### Genotypic variation, DA and PA

Genotypic variances for ear rot resistance among both inoculated and non-inoculated inbred lines were significant

**Table 1** Means of ear rot ratings of *n* lines in early, mid-late and late maturity groups inoculated with *Fusarium graminearum* (FG) or *F. verticillioides* (FV) in 2 years and means and ranges of series analyses. Early maturity group is divided according to its heterotic pools. Additionally, means of non-inoculated plants rated in the same plot like inoculated plants and corresponding weather data

| Maturity group | Pool | Location | Fusarium | Ear rot severity | | | | | | | Weather[a] | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Inoculated (%) (individual years) | | Inoculated (%) (series) | | | Non-inoculated (%) (individual years) | | Temperature (°C) (individual years) | | Precipitation (mm) (individual years) | |
| | | | | 2007 | 2008 | Mean | Minimum | Maximum | 2007 | 2008 | 2007 | 2008 | 2007 | 2008 |
| Early | Flint ($n = 50$) | CHA | FGs | 80.87 | 75.08 | 77.97 | 2.98 | 100.00 | 4.38 | 6.33 | 15.9 | 16.8 | 49.0 | 37.5 |
| | | EIN | FGs | 94.65 | 78.12 | 86.38 | 33.65 | 100.00 | 13.71 | 0.56 | 16.2 | 16.5 | 101.2 | 68.7 |
| | | GON | FGs | 17.05 | 23.78 | 20.41 | 0.14 | 78.03 | 0.98 | 2.94 | 17.6 | 17.9 | 68.8 | 62.0 |
| | | Mean | FGs | 64.38 | 58.99 | 61.69 | 12.25 | 91.05 | 6.38 | 3.35 | – | – | – | – |
| | | CHA | FVs | 20.27 | 29.94 | 25.11 | 2.30 | 95.17 | 6.22 | 4.19 | 15.9 | 16.8 | 49.0 | 37.5 |
| | | EIN | FVs | 18.04 | 24.23 | 21.13 | 1.45 | 51.57 | 16.78 | 0.89 | 16.2 | 16.5 | 101.2 | 68.7 |
| | | GON | FVs | 7.79 | 8.55 | 8.17 | 1.30 | 47.30 | 0.87 | 1.65 | 17.6 | 17.9 | 68.8 | 62.0 |
| | | Mean | FVs | 15.57 | 20.90 | 18.24 | 3.02 | 57.64 | 8.27 | 2.37 | – | – | – | – |
| | Dent ($n = 90$) | CHA | FGs | 58.13 | 56.61 | 57.37 | 7.07 | 100.00 | 2.97 | 5.58 | 15.9 | 16.8 | 49.0 | 37.5 |
| | | EIN | FGs | 85.13 | 61.08 | 73.11 | 17.50 | 100.00 | 3.10 | 0.73 | 16.2 | 16.5 | 101.2 | 68.7 |
| | | GON | FGs | 34.05 | 35.49 | 34.77 | 3.38 | 91.82 | 1.30 | 2.80 | 17.6 | 17.9 | 68.8 | 62.0 |
| | | Mean | FGs | 59.10 | 51.03 | 55.07 | 14.05 | 96.37 | 2.44 | 3.04 | – | – | – | – |
| | | CHA | FVs | 9.49 | 17.02 | 13.26 | 0.74 | 70.20 | 4.50 | 6.48 | 15.9 | 16.8 | 49.0 | 37.5 |
| | | EIN | FVs | 10.20 | 17.01 | 13.60 | 0.70 | 50.20 | 3.44 | 0.50 | 16.2 | 16.5 | 101.2 | 68.7 |
| | | GON | FVs | 4.81 | 8.12 | 6.46 | 0.85 | 40.15 | 0.87 | 1.78 | 17.6 | 17.9 | 68.8 | 62.0 |
| | | Mean | FVs | 8.17 | 14.05 | 11.11 | 0.87 | 52.34 | 2.93 | 2.90 | – | – | – | – |
| Mid-late | Dent ($n = 147$) | ALZ | FVs | 22.69 | 18.49 | 20.65 | 2.95 | 63.22 | 12.97 | 12.08 | 20.9 | 20.9 | 22.7 | 27.7 |
| | | MUR | FVs | 8.61 | 5.94 | 7.31 | 1.18 | 25.75 | 5.94 | 2.24 | 20.6 | 20.2 | 48.1 | 58.0 |
| | | Mean | FVs | 15.61 | 12.22 | 13.98 | 2.63 | 44.49 | 9.45 | 7.16 | – | – | – | – |
| Late | Dent ($n = 148$) | MCE | FVs | 9.22 | 11.53 | 10.31 | 0.30 | 41.01 | 8.03 | 8.89 | 22.7 | 22.4 | 29.7 | 47.9 |
| | | MCE | FVk | 9.84 | 11.14 | 10.41 | 0.60 | 75.04 | 7.05 | 7.57 | 22.7 | 22.4 | 29.7 | 47.9 |
| | | CRE | FVn | – | – | 5.72 | 0.13 | 31.09 | 6.17 | 5.18 | ND | ND | ND | ND |

*EIN* Einbeck; *GON* Gondelsheim (Germany); *CHA* Chartres; *ALZ* Alzonne (France); *MUR* Murony (Hungary); *MCE* Monselice; *CRE* Cremona (North Italy); *FGs*, *FVs*, silk channel inoculation; *FVk* kernel inoculation; *FVn* natural infection; *ND* not detected

[a] Monthly means of the time interval from first female flowering to rating

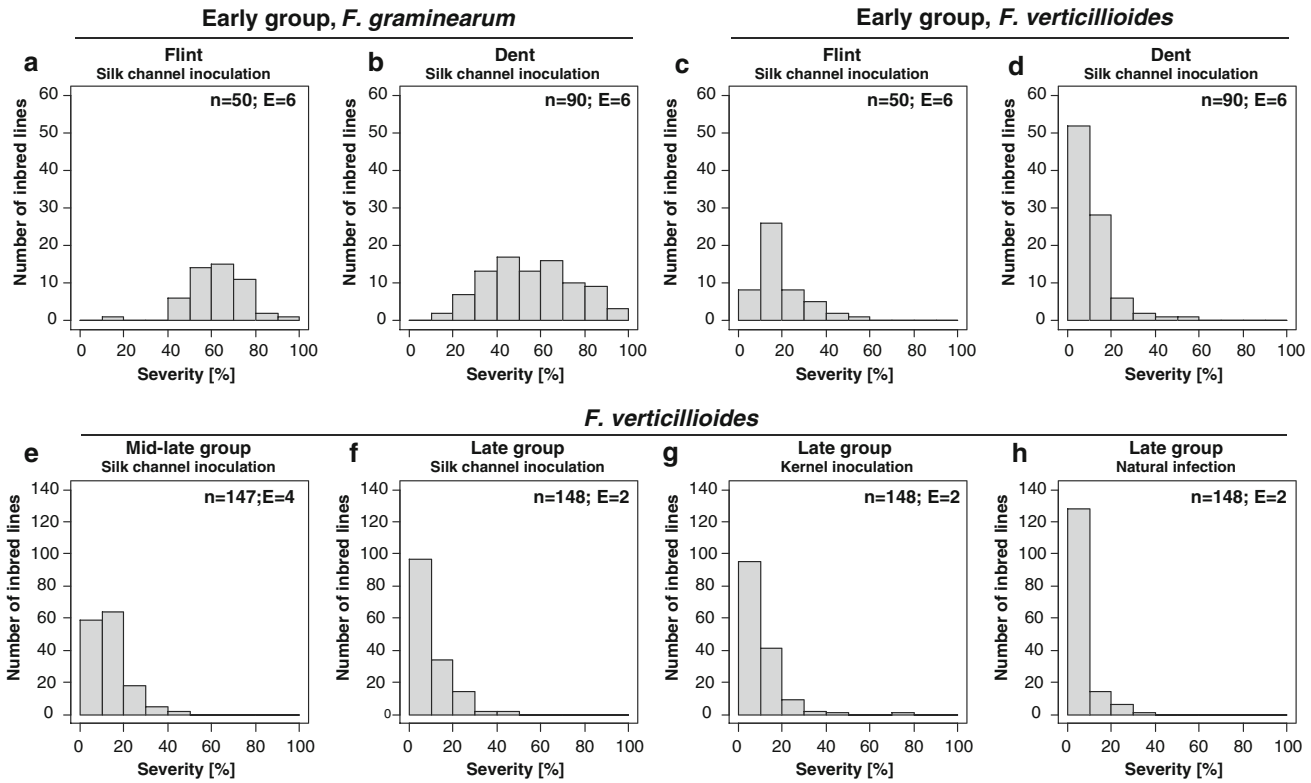


**Fig. 1** Distribution of *n* inbred lines of maturity groups early (**a**–**d**), mid-late (**e**) and late (**f**–**h**) after inoculation with *Fusarium graminearum* or *F. verticillioides* tested in E environments. In the late maturity group different inoculation methods were used

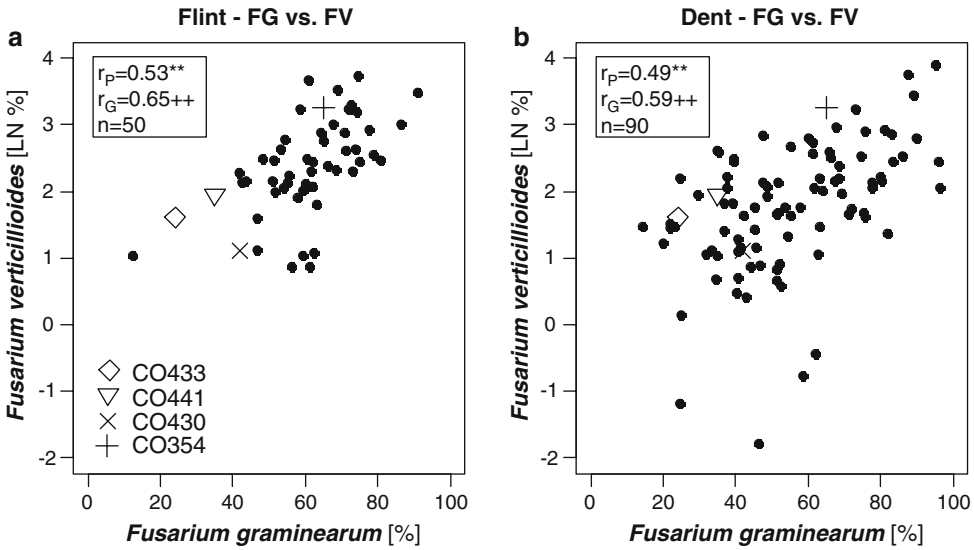


**Fig. 2** Scatter plots of ear rot severities caused by *F. graminearum* or *F. verticillioides* based on entry means of *n* flint (**a**) or dent (**b**) inbred lines evaluated in six environments. $r_P$ and $r_G$ refer to phenotypic and genotypic correlations, respectively (Checks CO354, CO430, CO433 and CO441 were not included in the coefficients of correlation). **Significant at $P < 0.01$. ++ Genotypic correlation exceeded twice its standard error

($P < 0.01$) in all instances (Table 2). In the early and mid-late maturity groups, all genotype × location × year interaction variances were significant for inoculated ($P < 0.01$) and non-inoculated genotypes. In the late maturity group, significant ($P < 0.01$) genotype × year interaction was found only after kernel inoculation. In the non-inoculated variants, the ratio error to genotype variance component ($\sigma_e^2/\sigma_G^2$) was higher than in the inoculated variant. Heritabilities after inoculation and non-inoculation were similar in the late maturity group. In all other maturity groups, heritabilities of the inoculated variant were higher than those of non-inoculation. Natural infection in the late group provided considerably higher genotypic variance than the inoculated variants, but also higher genotype × year interaction and error variances leading to a moderate heritability.

**Table 2** Estimates of variance components, heritabilities and their standard errors (SE) of ear rot severity of early, mid-late and late maturity groups of non-inoculated and inoculated plants either with *Fusarium graminearum* (FG) or *F. verticillioides* (FV) rated in the same plot (natural log transformed except inoculation with FG). Early maturity group is divided according to its heterotic pools

| Group | Pool | Fusarium | Inoculation | Variance components ± SE | | | | | Heritability ± SE |
|---|---|---|---|---|---|---|---|---|---|
| | | | | $\sigma^2_G$ | $\sigma^2_{GL}$ | $\sigma^2_{GY}$ | $\sigma^2_{GLY}$ | $\sigma^2_e$ | |
| Early | Flint | FGs | Inoculated | 100.90 ± 8.54** | 92.75 ± 8.24** | 38.90 ± 6.73** | 58.29 ± 8.81** | 155.21 | 0.58 ± 0.11 |
| | | | Non-inoculated | 6.27 ± 2.16** | –[a] | –[a] | 13.08 ± 4.78** | 45.73 | 0.51 ± 0.17 |
| | | FVs | Inoculated | 0.43 ± 0.28** | 0.04 ± 0.43 | 0.01 ± 0.35 | 0.21 ± 0.39** | 0.31 | 0.85 ± 0.05 |
| | | | Non-inoculated | 5.37 ± 3.05* | 5.17 ± 3.55$^+$ | 6.73 ± 2.89** | 2.83 ± 4.72 | 44.62 | 0.37 ± 0.18 |
| | Dent | FGs | Inoculated | 343.23 ± 7.11** | 39.38 ± 9.30** | 17.18 ± 7.59$^+$ | 94.49 ± 8.85** | 156.69 | 0.87 ± 0.03 |
| | | | Non-inoculated | 10.89 ± 2.31** | 4.25 ± 3.49* | –[a] | 5.78 ± 4.31* | 37.10 | 0.67 ± 0.07 |
| | | FVs | Inoculated | 0.68 ± 0.52** | 0.25 ± 0.65** | 0.09 ± 0.53$^+$ | 0.53 ± 0.57** | 0.65 | 0.72 ± 0.06 |
| | | | Non-inoculated | 9.4 ± 1.76** | –[a] | –[a] | 8.88 ± 4.11** | 33.72 | 0.69 ± 0.07 |
| Mid-late | Dent | FVs | Inoculated | 0.28 ± 0.37** | –[a] | –[a] | 0.27 ± 0.63** | 0.80 | 0.63 ± 0.08 |
| | | | Non-inoculated | 1.90 ± 1.04** | –[a] | –[a] | 3.20 ± 2.10** | 8.86 | 0.50 ± 0.12 |
| Late | Dent | FVs | Inoculated | 0.50 ± 0.51** | –[b] | 0.03 ± 0.70 | –[b] | 0.97 | 0.66 ± 0.06 |
| | | | Non-inoculated | 1.61 ± 1.07** | –[b] | 0.28 ± 1.41 | –[b] | 3.99 | 0.58 ± 0.07 |
| | | FVk | Inoculated | 0.24 ± 0.38** | –[b] | 0.13 ± 0.39** | –[b] | 0.30 | 0.63 ± 0.06 |
| | | | Non-inoculated | 1.50 ± 1.05** | –[b] | 0.09 ± 1.45 | –[b] | 4.22 | 0.58 ± 0.07 |
| | | FVn | _ | 2.49 ± 1.16** | –[b] | 0.21 ± 1.57 | –[b] | 4.96 | 0.65 ± 0.06 |

*FGs*, *FVs*, Silk channel inoculation; *FVk*, kernel inoculation; *FVn*, natural infection

[a] Negative estimator

[b] Not estimated

$^+$ $P < 0.1$, * $P < 0.05$, ** $P < 0.01$

**Table 3** Discrimination and prediction abilities of locations in different years for early and mid-late maturity groups after inoculation with either *Fusarium graminearum* (FG) or *F. verticillioides* (FV)

| Year | Early FG | | | Early FV | | | Mid-late FV | |
|---|---|---|---|---|---|---|---|---|
| | CHA | EIN | GON | CHA | EIN | GON | ALZ | MUR |
| Discrimination ability | | | | | | | | |
| 2007 | 1.29 | 0.65 | 0.81 | 1.57 | 1.17 | 0.80 | 1.72 | 0.90 |
| 2008 | 1.12 | 1.20 | 0.92 | 0.64 | 1.21 | 0.62 | 0.65 | 0.73 |
| Prediction ability | | | | | | | | |
| 2007 | 0.69 | 0.58 | 0.40 | 0.51 | 0.55 | 0.39 | 0.69 | 0.38 |
| 2008 | 0.72 | 0.69 | 0.53 | 0.60 | 0.45 | 0.62 | 0.46 | 0.46 |

*EIN* Einbeck, *GON* Gondelsheim (Germany), *CHA* Chartres, *ALZ* Alzonne (France), *MUR* Murony (Hungary)

For calculation of DA and PA values we did not distinguish anymore between flint and dent lines to get more representative results of each environment. DA values of inoculation varied in a wide range (Table 3). In the early maturity group, the lowest DA values were consistently found at GON except in the year 2007 in the FG trial. High PA values were found at CHA for FV and FG. In the mid-late maturity group, ALZ had the highest DA of all locations in 2007 but the lowest in 2008.

## Correlations

In the early maturity group, phenotypic ($r_P$) and genotypic ($r_G$) correlations of artificial inoculation between FG and FV were medium in flint and dent lines (Fig. 2). In the dent pool, correlations between artificial inoculation and non-inoculation were low for FG ($r_P = 0.11$, $r_G = 0.11$) and moderate for FV ($r_P = 0.38$, $r_G = 0.47$). Low to moderate correlations between inoculation and non-inoculation were found for FG in the flint pool ($r_P = 0.30$; $r_G = 0.58$), but no correlations were calculated for FV due to too many missing data in the non-inoculated variant. Correlations between silking date and ear rot severity were calculated for flint and dent lines together and were significant, but low (FG, $r_P = -0.28$; FV, $r_P = -0.26$). In the mid-late maturity group, phenotypic and genotypic correlations between inoculation and non-inoculation were medium and high, respectively ($r_P = 0.55$; $r_G = 0.83$). In the late maturity group, only phenotypic correlations are presented since genotypic correlations were biased upwards due to only two test environments. Correlation between silk channel and kernel inoculation was moderate (Table 4). In MCE, correlations between artificial inoculation and non-inoculation were medium to high. All correlations between different inoculation variants at MCE and natural infection at CRE were low. In the mid-late and late maturity groups,

**Table 4** Correlations between artificial inoculated (silk channel or kernel) and non-inoculated plants rated in the same plot in Monselice (MCE) and natural infection in Cremona (CRE). Artificial inoculation methods were evaluated in adjacent but separate trials

| | MCE | | | CRE |
|---|---|---|---|---|
| | Silk channel | Kernel | | Natural infection |
| | Non-inoculated | Inoculated | Non-noculated | |
| Silk channel | | | | |
| Inoculated | 0.76** | 0.66** | 0.72** | 0.27** |
| Non-inoculated | – | 0.51** | 0.81** | 0.30** |
| Kernel | | | | |
| Inoculated | – | – | 0.57** | 0.30** |
| Non-inoculated | – | – | – | 0.37** |

** Significant at $P < 0.01$

correlations between flowering date and ear rot severity were not significant and close to zero ($r_P = -0.10$ and $-0.08$, respectively).

## Discussion

### Artificial inoculation versus non-inoculation

Artificial inoculation resulted in significant ($P < 0.01$) ear rot differentiation among the unselected inbred lines at all locations and maturity groups. In the early maturity group, similar heritabilities after non-inoculation and inoculation with FG were found in the flint pool and after inoculation with FV in the dent pool (Table 2) suggesting that inoculation is not necessary to achieve similar precision when natural infection level is high enough. Nevertheless, we suggest to inoculate this material for two reasons: (1) the non-inoculated variant had only low disease severity (Table 1) diminishing visual differentiation, and (2) the *Fusarium* spp. cannot be distinguished by ear rot rating and, thus, a breeder does not know which *Fusarium* resistance is addressed. The latter point is of interest because 13 *Fusarium* species were isolated from naturally infected maize in Germany with the prevalent species changing between years according to weather (Görtz et al. 2008) and because resistances to different *Fusarium* spp. were not highly correlated (Fig. 2; Presello et al. 2004; Schaafsma et al. 2006). In the mid-late maturity group, disease severity of the non-inoculated variant was only high enough for genotypic differentiation at ALZ and heritability of the non-inoculated variant was considerably lower. In conclusion, we suggest using silk channel inoculation for the evaluation of ear rot resistance in these maturity groups to get sufficient ear rot severity for sufficient visual differentiation and to ensure testing for the desired *Fusarium* spp. resistance.

In the late maturity group, similar means of ear rot severity and similar heritabilities after inoculation and non-inoculation indicate a high natural infection pressure. Thus, a breeder could save extra trials meaning less monetary input and less work while the work peak at flowering. However, ear rot data were collected only for the two experimental years and, therefore, it cannot be assumed that sufficient high natural infection pressure occurs each year. Additionally, the ratio $\sigma_e^2/\sigma_G^2$ decreased considerably with inoculation improving the statistical power. The high errors of natural infection might be caused by (1) natural infection caused by other *Fusarium* spp. and/or more aggressive isolates, (2) difficulties in rating due to symptoms occurring randomly on kernels or groups of kernels (Hau and Kranz 1989), and (3) disease escape due to flowering dates outside rainy periods, patchy disposal of inoculum or no inoculation at all. Additionally, we observed that the selection of resistant genotypes based on artificial inoculation (<10% covered ear) resulted in genotypes also resistant to non-inoculation but not vice versa (data not shown). The same was true for silk channel inoculation in MCE and natural infection in CRE, but not for kernel inoculation. Both locations are only about 160 km afar and low correlations among inoculation and non-inoculation in MCE and natural infection in CRE may be explained by different pathogen populations and/or by different weather conditions. The late maturity group was tested in two environments and due to the high error variances of evaluation of ear rot severity, the results of the late maturity might be biased. In conclusion, artificial inoculation should result in more accurate and repeatable data for selection also in the late maturity group.

### Silk channel versus kernel inoculation

Due to the importance of European corn borer (*Ostrinia nubilalis*) damage in Southern Europe, we additionally used kernel inoculation in Italy. Thus, both main ways of entry of FV were addressed (Munkvold 2003): (1) silk infection with growth of mycelium down the silks to the kernels from spores germinating on the silks and (2) kernel infection by secondary infection through wounds. Means and ranges of ear rot severity of both methods were similar (Table 1). Therefore, both methods are able to differentiate between resistant and susceptible genotypes as suggested by Chungu et al. (1996a). A moderate correlation ($r_P = 0.66$, $P < 0.01$) between both inoculation methods was achieved indicating that at least some QTL involved in resistance might be acting against both ways of entry. The correlation between both inoculation methods of this study is similar to correlations found in Argentinean and Canadian

maize (Presello et al. 2004; Schaafsma et al. 2006). In contrast, a low correlation ($r_P = 0.12$) after inoculation of Austrian hybrids with FG evaluated in two environments was reported (Lemmens 1999). Instead of silk channel inoculation, the latter study used spray inoculation which causes less severity (Clements and Kleinschmidt 2003). Consequently, breeding for ear rot resistance requires application of both inoculation methods, particularly if varieties should be developed for areas in which damage by European corn borer occurs more frequently.

### *F. graminearum* versus *F. verticillioides* among early maturing inbred lines

Inoculation with FG resulted in significantly ($P < 0.01$) higher ear rot severity in both heterotic pools than inoculation with FV. The distributions of ear rot rating after FG inoculations were similar to normal distributions but ratings after FV inoculations were generally skewed to lower ear rot severity (Fig. 1a–d). Both are in good agreement with studies from Canadian or US maize for FG and FV, respectively (Clements et al. 2004; Reid et al. 2002; Robertson et al. 2006). The significant ($P < 0.01$) difference between species show that our FG isolate is more aggressive than the FV isolate. This difference is in accordance with other studies (Miller 1994; Presello et al. 2004; Schaafsma et al. 2006). The lower aggressiveness of FV might be caused by its dual nature as an endophyte with symptomless infection or as a pathogen causing symptoms more randomly (Bacon et al. 2008).

Significant ($P < 0.01$) but moderate associations between resistances to FG and FV found in flint and dent pools (Fig. 2) were also reported by Presello et al. (2004). Schaafsma et al. (2006), however, did not find significant associations across years. The moderate genotypic correlation might be explained by some common resistance mechanisms to both species, i.e., unspecific resistance factors like a thick wax layer (Sampietro et al. 2009). Similarly, Reid et al. (2009) recently reported that selection on resistance to FG resulted indirectly in increased resistance to FV and *Ustilago zeae* in Canadian material. Other resistance mechanisms might be species-specific attributed to the different ways of spreading within the ear. Interestingly, genotypes resistant to FG (<30% ear rot severity) also had a low FV severity (<10%, Fig. 2). Thus, if resources are limited (i.e., seeds in early inbred line testing), a pre-testing for resistance to FG should be preferred, followed by a FV resistance test to get a resistance as broad as possible.

### Influence of weather on ear rot severity

Ear rot severities of FV at CHA (North France) and EIN (Central Germany) were higher than at the warmer location GON (Southern Rhine valley, Germany) and at most South European locations like MUR (Hungary) and MCE (North Italy, Table 1). Referring to the higher temperature demands of FV, we expected the opposite (Bottalico 1998; Miller 2001; Shelby et al. 1994). Also FG severities were consistently lower at GON than at CHA and EIN. The lower temperatures at CHA and EIN might result in chilling stress changing the balanced endophytic relationship with FV into a disease (Bacon et al. 2008).

Precipitation also influences disease development. Wet conditions favor growth of FG whereas FV is favored by dry conditions (Bottalico 1998; Miller 2001). In the early maturity group, the higher disease severity in EIN 2007 compared to EIN 2008 might be caused by the much higher precipitation in 2007. At MUR, higher precipitation occurred than at ALZ. Based on the preference of FV for drier conditions this might have caused higher disease severity at ALZ. In conclusion, breeding for higher tolerance to abiotic stress factors might indirectly increase resistance to ear rot by reducing predisposition of maize to diseases.

### Flint versus dent

Flint lines were generally more susceptible to FG and FV than dent lines in the early maturity group (Fig. 2). Nevertheless, significant ($P < 0.01$) differences between heterotic pools could be found only for FV severity. The higher susceptibility of the flint lines might be historically explained by a combination of two factors: (1) low genetic diversity within the flint pool because it was developed by selfing a few European open-pollinated varieties and no further influx of foreign germplasm occurred (Reif et al. 2005) and/or (2) low co-occurrence of FV and flints, and hence no resistance selection, since FV occurs in warm to hot and dry climates and flints have been bred for Northern Europe (Bottalico 1998; Miller 2001; Shelby et al. 1994). In addition, the generally earlier flowering time of flints compared to dents might increase susceptibility. Consequently low, but significantly negative correlations between flowering time and ear rot severity were found in the early maturity group which is in contrast to low but positive correlations found by Robertson et al. (2006). Therefore, the resistance within the flint pool should be improved by introgressing resistance alleles followed by recurrent selection. Sources for alleles of FG resistance might be the CO lines from the breeding program of Eastern Cereal and Oilseed Research Center, Ontario, Canada, if lines are flints or non-Stiff Stalk as proposed by Reif et al. (2009). US cornbelt non-Stiff Stalk lines like the moderately resistant GE440 (Robertson et al. 2006; J. Holland, personal communication) might be a source for FV resistance alleles. They would, however, require

an introgression of QTL for chilling tolerance to be useful in Central Europe. Given the mainly additive nature of most ear rot resistance QTL, both pools should be improved, but breeding for ear rot resistance in the dent pool likely gains faster in highly resistant lines than in the flint pool.

## Conclusions for breeding and selection of appropriate locations for ear rot testing

Broad ranges and significant ($P < 0.01$) genotypic variances after inoculation in all maturity groups and gene pools showed that sufficient genetic variation of ear rot resistance to FG and FV already pre-exists in adapted European breeding material. Weather highly affects ear rot severity and consequently resistance selection. Significant ($P < 0.01$) genotype × environment interaction variances indicate the complexity of selection of resistant genotypes and the need of resistance tests across several locations/years.

Not every location provides the same discrimination among genotypes. To identify optimal environments the indices of DA and PA can be applied (Dhillon et al. 1991). Despite good disease development of FG and FV at EIN 2007 and CHA 2008, respectively, corresponding DAs were low (Table 3). An explanation might be abiotic stress as shown for EIN 2007 (see above). This indicates that under stress conditions differentiation among genotypes likely diminishes. The variation of DA and PA among locations from year to year stresses again the importance of multi-environmental trials for accurate selection. But based on the 2-year analysis, no location could be found to be a better discriminator or predictor than the other location in the mid-late maturity group. In the early maturity group, locations CHA and EIN would be the best discriminator and predictor for FG and FV, respectively, but this should be analyzed further.

In conclusion, selection for resistance to FG and FV among European dents by artificial inoculation is promising. Resistance in flints should be improved by introgression of new resistance alleles from foreign material. Due to high genotype × environment interactions, multi-environmental trials are necessary for reliable selection of resistant genotypes. The resistance to FG explains about 30–40% of the resistance to FV. Since a major problem of ear rot in maize is the contamination with mycotoxins, more needs to be known about the association between ear rot and mycotoxin concentrations in European maize.

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