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# Segregation for aggressiveness and deoxynivalenol production of a population of *Gibberella zeae* causing head blight of wheat

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**Abstract**

Gibberella zeae is a pathogen of wheat and other small-grain cereals, causing yield losses and reducing grain quality by producing the trichothecene deoxynivalenol (DON) which is harmful to animals and humans. One hundred and fifty three progeny from a cross between two European DON-producing isolates of *G. zeae* were analyzed for aggressiveness and DON production in three environments (location–year combinations) in Germany. Aggressiveness, measured as head blight rating and relative plot yield, and DON production showed continuous distribution for each environment and across environments. There was significant ($P = 0.01$) genotypic variation for all three traits. Transgressive segregants occurred for all three traits. Both repeatability estimates within an environment and heritability estimates on an entry-mean basis for head blight rating and DON production were medium to high (0.5–0.7). Progeny–environment interaction accounted for about 29% of the total variance for the two aggressiveness traits and 19% for DON production. The large genetic variation derived from a cross between two rather similar European parents indicates a potential for increasing fungal aggressiveness in the *G. zeae* population.

## Introduction

*Gibberella zeae* (anamorph: *Fusarium graminearum*) is an important plant pathogens worldwide. The fungus infects several major crops, including maize, wheat, barley, rye and triticale. In wheat, *G. zeae* causes seedling blight, crown rot root rot, and head blight. Head blight or scab of wheat causes epidemics in many wheat areas worldwide (Dubin et al., 1997; McMullen et al., 1997). The disease reduces grain quality as well as yield, leading to poor germination of infected grains, reduction in baking quality, and contamination mycotoxins. Deoxynivalenol (DON), and its derivatives 3-acetyl-DON and 15-acetyl-DON, nivalenol (NIV) and zearalenone (ZEA), the major mycotoxins produced by *G. zeae* (Marasas et al., 1984), pose animal and human health risks. In North America and Europe, DON is the most frequently encountered mycotoxin (Müller and Schwadorf, 1993; Placinta et al., 1999; Bottalico and Perrone, 2002).

Aggressiveness of a pathogen is measured by the quantity of disease induced by a pathogenic isolate on a susceptible host (Van der Plank, 1968). Several studies indicate that there is large genetic variation in aggressiveness among *G. zeae* isolates sampled from various parts of the world (Bai and Shaner, 1996; Miedaner et al., 2001), within a country or state (Dusabenyagasani et al., 1999; Walker et al., 2001) and even within

This paper is dedicated to Prof. Dr. H.H. Geiger, Hohenheim, on the occasion of his 65th birthday

populations from individual fields (Miedaner and Schilling, 1996). There was also quantitative variation in mycotoxin production of *G. zeae* among a collection of isolates from winter rye (Miedaner et al., 2000). However, little is known about the genetic basis of aggressiveness and DON production by *G. zeae*. Knowledge of genetics of aggressiveness and DON production of *G. zeae* is important in developing resistant cultivars and in estimating durability of resistance. Resistance to *G. zeae* head blight in wheat is quantitatively inherited. All known cultivars are infected but the degree of infection greatly varies (Bai and Shaner, 1996).

Several studies on the role of trichothecenes in plant diseases suggest that DON production increases aggressiveness of *G. zeae* (Proctor et al., 1995; Desjardins et al., 1996; Harris et al., 1999). Proctor et al. (1995) showed that a DON-deficient isolate of *G. zeae* generated by gene disruption remained pathogenic on wheat and rye but was less aggressive than the wild type. The finding that trichothecenes enhance the aggressiveness of *G. zeae* suggests that it could be possible to reduce head blight of wheat and mycotoxin problems caused by this fungus by breeding trichothecene-resistant crops. Disease-causing capacity of the trichothecene-deficient mutant of *G. zeae*, however, indicates that there are other factors that contribute to aggressiveness, such as cell-wall degrading enzymes (Schwarz et al., 2002; Wanyoike et al., 2002) and other metabolites. Studies on host resistance and genetic modification of the pathogen are required to understand the role of DON in plant pathogenesis. Resistant cultivars will remain the most practical and effective control against head blight of wheat (Martin and Johnston, 1982).

Sexual recombination of *G. zeae* has been demonstrated under laboratory conditions, and should also occur in the field as indicated by high genotypic diversity reported within individual fields (Bowden and Leslie, 1992; Miedaner and Schilling, 1996). In the study reported here, a crossing population of *G. zeae* with two DON-producing parents was tested in three field environments to analyze the inheritance of aggressiveness and amount of DON produced and the effect of environmental variation and isolate–environment interaction on aggressivenesss traits and DON production. A preliminary report of this work concerning one field environment has been published (Cumagun et al., 2002).

## Materials and methods

### *Crossing population and field tests*

Two DON-producing strains of *G. zeae*, FG24 from Szeged, Hungary and FG3211 from Sersheim, Germany served as parents for the crossing population. Both strains belong to lineage 7 (O'Donnell et al., 2000). The parents were chosen from a previous experiment (Miedaner et al., 2000) to represent different aggressiveness and DON production levels. Methods for crossing *G. zeae* were described in detail by Bowden and Leslie (1999). *Gibberella zeae* is homothallic and can outcross or self. Therefore, complementary nitrate non-utilizing (*nit*) mutations were employed as suitable markers. Parents were crossed and single ascospore cultures were isolated in the laboratories of B. Bowden and J. Leslie at Kansas State University, USA. Moderately susceptible German winter wheat cv. Drifter was inoculated in three field environments in southwest Germany: Hohenheim (HOH) near Stuttgart (400 m above sea level, 8.5 °C mean annual temperature, 685 mm mean annual precipitation) in 2001 and 2002 and Oberer Lindenhof (OLI) near Reutlingen (700 m above sea level, 6.6 °C mean annual temperature, 952 mm mean annual precipitation) in 2002.

### *Inoculum production, inoculation, field design, disease and yield assessment*

Conidia from 153 single-ascospore progeny of the cross of *G. zeae* and the two parents were mass produced following the procedure of Miedaner et al. (1996). Wheat grains (~300 g) previously soaked overnight in tap water were placed in 1 milk bottles. Bottles were sealed with aluminum foil and autoclaved twice on successive days, at 121 °C for 20 min at 1 atm. Cooled wheat medium was inoculated with 10–20 ml of the conidial suspension, prepared by flooding 1-week-old SNA (synthetic-nutrient poor agar culture, Nirenberg, 1981) of *G. zeae* with sterile distilled water (SDW), and shaken to distribute the inoculum. After incubation at 18 °C in the dark for 4 weeks, colonized wheat grains were taken out of the bottles,

mixed with SDW, and placed in a thin layer in plastic trays ($40 \times 60$ cm$^2$). Trays were completely covered with plastic sheets and placed about 40 cm below two black light tubes (Philips TLO, 40W/80, Royal Philips Electronics, Amsterdam) for 3 days. Plastic sheets were folded back from part of the tray when wheat grains were colonized by the fungus. Colonized wheat grains were sprayed with SDW while breaking-up clumps of moldy grains by hand and inoculum was air-dried for 2–3 days at room temperature, enclosed in plastic bags, and stored in a cold chamber at 5 °C until inoculation. Conidia concentration was adjusted to 500,000 conidia ml$^{-1}$ for each isolate with the use of a haemacytometer. Plants were inoculated with this concentration at a rate of 120 ml m$^{-2}$ at anthesis. Inoculum suspensions were added with one to two drops of liquid cleansing agent to make sprays more efficient. Inoculation was done in three batches because of the large number of progeny. Batch 1 consisted of progeny numbers 1–51; batch 2, progeny numbers 52–102, and batch 3, progeny numbers 103–153. The progeny of the three batches were sprayed onto host plants in three successive days relying on natural moisture.

A split-plot design was used with the batches as main plots and the progeny as subplots in three replicates. Within the main plots, plots inoculated with the two parental isolates consisted of 10 replicates each to increase accuracy in comparing them with their progeny. Uninoculated plots were also included to calculate relative yield components. Subplots were randomized following a complete block design. Three-row microplots were used (1.2 m length and 0.625 m width) for each isolate and plots were arranged in a chess-cross design, i.e., each plot inoculated with an isolate was surrounded by four border plots of similar size planted with triticale to avoid plot-by-plot interference.

Two aggressiveness traits were assessed: head blight rating per plot and plot yield relative to the non-inoculated control. Head blight was rated by visual estimation (0–100%) of the whole plot when differences in head blight severity among treatments could be observed. This rating includes number of heads infected (incidence) and amount of bleached spikelets per head (severity). Due to artificial inoculation, plots were evenly infected according to the aggressiveness of the respective isolates. Timing of the next rating depended upon the rate of disease development from the previous disease rating. In HOH 2001, disease was rated 18, 20, 25, 32, and 44 days after inoculation and in HOH 2002 and OLI 2002, 14, 16, and 21 days after inoculation. Arithmetic means of the head blight ratings of each assessment date were averaged for further analyses. Grain weight was determined by cutting the whole plot by hand, threshing in a small combine, drying the grain to about 13% moisture, sieving to remove fragments of glumes and rachis, and cleaning again. For relative plot yield, yield of each progeny from the inoculated plots was calculated relative to the respective mean of the control plots and expressed in percentage.

*Mycotoxin analysis*

Wheat grain was ground and mycotoxins were extracted by weighing out 5 g of each sample in 100 ml flasks containing 100 ml double distilled water. Sample suspensions were placed in a rotary shaker (200 rpm) for 5 min and about 1 ml of the suspension was transferred into Eppendorf tubes and centrifuged (14,000 rpm) for 5 min. One milliliter of supernatant served as stock solution for dilution preparations. Dilutions were prepared from the stock solution to optimize ELISA analysis. Mycotoxin content (DON and 3-acetyl-DON) of ground wheat grain samples was analyzed using RIDASCREEN™ FAST DON (R-biopharm GmbH, Darmstadt, Germany), an enzyme immunoassay for the quantitative analysis of DON in cereals, malt, and feed, and a microtiter plate spectrometer (TECAN SLT Lab Instruments, Crailsheim, Germany). Due to the high cost of ELISA, only two replicates from each field experiment were analyzed.

*Statistical analyses*

Plot means were used for analyses of variance. Residuals were independent and followed a normal distribution for head blight rating, but not for relative plot yield and DON production. The latter two trait values were adjusted to normality by square root transformation. The three environments (year–location combinations) were analyzed as a series of random environments according to Cochran and Cox (1957). Estimates of variance components ($\sigma^2$) were calculated as described by

Snedecor and Cochran (1989, p. 322). An appropriate model for a split-plot design was derived to take into account the partitioning into three batches: $\sigma^2_e + \sigma^2_{r:e} + \sigma^2_b + \sigma^2_{be} + \sigma^2_{br:e} + \sigma^2_{p:b} + \sigma^2_{pe:b} + \sigma^2$, where e = environment, r:e = replicate within environment, b = batch, be = batch–environment interaction, br = batch–replicate interaction, p:b = progeny within batch, pe:b = progeny–environment interaction within batch, and $\sigma^2$ = error variance. Coefficient of variation (cv%) of the respective variance components was calculated as square root of the estimate relative to the population mean. This allows direct comparison between trait means of different units. Repeatability estimates were calculated by partitioning the phenotypic variance of spatial replications within one experiment according to the formula $\sigma^2_p/(\sigma^2_p + \sigma^2)$ (Falconer, 1989) where $p$ = progeny. Broad-sense heritabilities ($h^2$) were estimated on an entry-mean basis (Fehr, 1987) as the ratio of genotypic to phenotypic variance using the formula: $h^2 = \sigma^2_p/(\sigma^2_p + \sigma^2_{pe}/E + \sigma^2_e/RE)$, where $R$ = number of replicates and $E$ = number of environments. Confidence intervals of heritability were computed according to Knapp and Bridges (1987). All statistical analyses were computed using the statistical package PLABSTAT (Utz, 2000). All effects were assumed to be random variables.

## Results

Flowering dates of wheat were different in the three environments. Wheat fields in HOH 2002 flowered 1 week earlier than in OLI 2002 due to climatic differences. This situation resulted in a 1-week difference in inoculation period between the two locations. Mean temperatures, relative air humidity, and total precipitation varied considerably among three field environments and even among batches and days of inoculation (Table 1).

Uninoculated plots had a mean head blight rating of 0.9% and mean DON levels of 1.15 mg $kg^{-1}$ across field environments. Several head blight ratings in the field were taken at successive dates from 14 to 44 days after inoculation depending upon disease progress (Table 2). Disease progress was slightly different among environments. First symptoms appeared in 2002 earlier and were about four times more severe 20–21 days after inoculation than in 2001. All head blight ratings showed genotypic differences at the 1% probability level and were highly intercorrelated ($r \geq 0.7$, data not shown). For the further analyses, therefore, the mean of the three ratings shown was used.

Both parents were rather similar, i.e., low to medium in aggressiveness and DON production (Table 3). Significant differences between the parents were only found in head blight rating and DON production in HOH 2002 and DON production in OLI 2002. Genotypic variation was highly significant in each batch and combined across all batches for mean head blight rating, relative plot yield, and DON production. Mean head blight rating and relative plot yield in HOH across years were relatively stable. Differences in disease levels could be attributed in part to differences in temperature and relative humidity, which influenced infection period. However, mean head blight rating in HOH for both years could not be compared directly in terms of disease severity because of large differences in disease progress in 2001 and 2002 (Table 2). The highest head blight infection occurred in HOH 2002, which could be linked to its highest mean temperature among field environments.

Relative plot yields in HOH 2001 and 2002 were similar and slightly lower in OLI 2002 (Table 3). Lower mean relative plot yield in 2002 than in 2001 is an indication of higher disease severity in HOH 2002 because infection occurred earlier. Mean DON production in HOH 2001 was almost three times as much as in HOH 2002 although head blight rating and relative plot yield did not differ much. Batch 1 had the lowest disease and DON level for both years in HOH and batch 3 in OLI 2002. Repeatability estimates for each environment were medium to high for head blight rating and DON production and low to medium for relative plot yield.

All progeny of *G. zeae* cross FG24 × FG3211 caused head blight, reduced grain weight, and produced DON. The progeny differed significantly for these traits (Figure 1). There was a wide range of mean head blight rating, relative plot yield, and DON production across environments. The three traits showed a continuous distribution across environments.

Analysis of variance combined across field environments showed a significant genotypic

*Table 1.* Mean temperature, sum of precipitation, and mean relative air humidity from the first, second, third day after inoculation and from the total incubation period in three field environments

| Environment[a] | Batch | Mean temperature (°C) | | | | Sum of precipitation (mm) | | | | Mean relative air humidity (%) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Day after inoculation | | | Incubation Period | Day after inoculation | | | Incubation period | Day after inoculation | | | Incubation period |
| | | First | Second | Third | | First | Second | Third | | First | Second | Third | |
| HOH 2001 | 1 | 15.6 | 11.5 | 11.6 | 15.4 | 0 | 6.8 | 4.4 | 45.3 | 68.0 | 87.0 | 93.0 | 72.2 |
| | 2 | 11.5 | 11.6 | 11.4 | 15.8 | 6.8 | 4.4 | 6.4 | 45.3 | 87.0 | 93.0 | 94.0 | 71.3 |
| | 3 | 11.6 | 11.4 | 11.2 | 16.2 | 4.4 | 6.4 | 0.7 | 119 | 93.0 | 94.0 | 75.0 | 70.6 |
| HOH 2002 | 1 | 13.2 | 14.5 | 18.4 | 22.0 | 5.6 | 0 | 0 | 91.3 | 73.8 | 73.7 | 64.3 | 70.0 |
| | 2 | 14.5 | 18.4 | 21.3 | 22.4 | 0 | 0 | 0 | 85.7 | 73.7 | 64.3 | 62.7 | 68.7 |
| | 3 | 18.4 | 21.3 | 22.4 | 22.8 | 0 | 0 | 0 | 55.5 | 64.3 | 62.7 | 68.0 | 67.9 |
| OLI 2002 | 1 | 23.8 | 23.1 | 20.6 | 17.8 | 0.4 | 0 | 0 | 55.2 | 46.3 | 49.3 | 52.3 | 54.3 |
| | 2 | 23.1 | 20.6 | 21.3 | 17.1 | 0 | 0 | 0 | 54.8 | 49.3 | 52.3 | 21.7 | 54.3 |
| | 3 | 20.6 | 21.3 | 21.4 | 16.7 | 0 | 0 | 30 | 54.8 | 52.3 | 21.7 | 21.3 | 55.6 |

Incubation period is the time from inoculation until the first head blight rating.
[a] HOH = Hohenheim near Stuttgart; OLI = Oberer Lindenhof near Reutlingen.

*Table 2*. Mean head blight rating (%) on successive dates on wheat cv. Drifter inoculated with 153 progeny of *Gibberella zeae* in three field environments

| Environment[a] | Days after inoculation | | | | | | |
|---|---|---|---|---|---|---|---|
| | 14 | 16 | 18 | 20 | 21 | 25 | Mean |
| HOH 2001 | – [b] | – | 5.1 | 13.5 | – | 34.3 | 33.7[c] |
| HOH 2002 | 28.4 | 36.4 | – | – | 46.9 | – | 37.4 |
| OLI 2002 | 12.9 | 24.6 | – | – | 37.1 | – | 24.9 |

[a] HOH = Hohenheim near Stuttgart; OLI = Oberer Lindenhof near Reutlingen.
[b] Not determined.
[c] Mean of five ratings (18, 20, 25, 32, and 44 days after inoculation) with the latter two ratings not shown here.

*Table 3*. Means, repeatabilities, significance of genotypic variation on wheat cv. Drifter for head blight rating, relative plot yield, and DON production inoculated with 153 progeny of *Gibberella zeae* cross FG24 × FG3211 and their parents in three batches of 51 isolates each in three field environments

| Environment[a] | Batch/parent | Head blight rating (%) | | Relative plot yield (%) | | DON production (mg $kg^{-1}$) | |
|---|---|---|---|---|---|---|---|
| | | Mean | Repeatability | Mean | Repeatability | Mean | Repeatability |
| HOH 2001 | 1 | 29.0 | 55.9** | 69.0 | 30.1** | 17.7 | 50.3** |
| | 2 | 34.6 | 72.1** | 71.1 | 58.2** | 30.0 | 64.6** |
| | 3 | 37.6 | 64.2** | 64.3 | 20.6** | 37.2 | 46.8** |
| | 1-3 | 33.7 | 66.9** | 68.1 | 30.1** | 28.4 | 65.5** |
| | FG24 | 13.9a[b] | – | 80.2a | – | 10.3a | – |
| | FG3211 | 15.0a | – | 90.0a | – | 12.3a | – |
| HOH 2002 | 1 | 30.8 | 45.3** | 68.1 | 45.4** | 9.9 | 53.1** |
| | 2 | 39.3 | 71.9** | 57.2 | 58.7** | 13.1 | 37.9** |
| | 3 | 41.9 | 66.4** | 55.3 | 39.1** | 12.7 | 58.0** |
| | 1-3 | 37.4 | 67.0** | 62.6 | 51.1** | 11.9 | 45.9** |
| | FG24 | 10.2a | – | 98.7a | – | 3.1a | – |
| | FG3211 | 37.2b | – | 65.8a | – | 12.3b | – |
| OLI 2002 | 1 | 27.6 | 57.1** | 55.7 | 56.1** | 12.5 | 78.9** |
| | 2 | 27.5 | 72.6** | 53.1 | 73.7** | 15.7 | 70.0** |
| | 3 | 19.7 | 45.2** | 55.3 | 25.4** | 17.0 | 63.9** |
| | 1-3 | 24.9 | 59.9** | 54.7 | 54.3** | 15.1 | 71.0** |
| | FG24 | 13.4a | – | 71.5a | – | 8.3a | – |
| | FG3211 | 21.6a | – | 58.6a | – | 9.2b | – |

** Significant genotypic variation at probability level $P = 0.01$ ($F$-test).
[a] HOH = Hohenheim near Stuttgart, OLI = Oberer Lindenhof near Reutlingen.
[b] Numbers followed by the same letter are not significantly different at 5% level for comparison of parents.

variation among progeny within batches for head blight rating, relative plot yield, and DON production (Table 4). The effect of the batch was not significant; however, batches reacted differently according to the environment. An important source of variance was the interaction between progeny and environment within batches, but progeny within batch was also significant. These interactions led to medium heritability estimates for aggressiveness. Error variance was similar for head blight rating and DON concentration and lowest for relative plot yield.

Correlations between head blight rating and relative plot yield ($r = -0.9$; $P = 0.01$) head blight rating and DON production were high ($r = 0.7$; $P = 0.01$). Correlation between relative plot yield and DON production was lower ($r = -0.6$; $P = 0.01$).

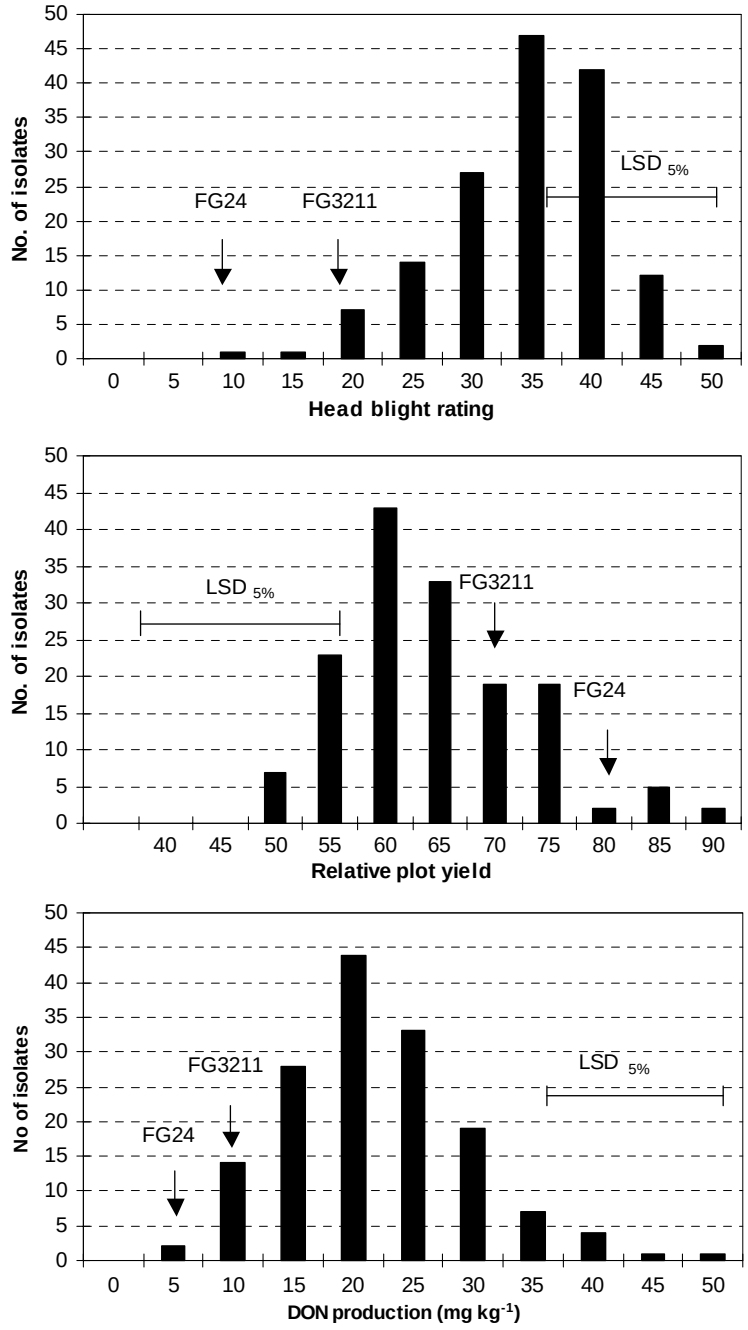


*Figure 1.* Frequency distribution of head blight rating, relative plot yield, and DON production in the susceptible wheat cv. Drifter inoculated with 153 progeny of *Gibberella zeae* cross FG24 × FG3211 combined across three field environments (untransformed data); $LSD_{5\%}$ = least significant difference at probability level $P = 0.05$.

## Discussion

Significant variance in segregation for aggressiveness and DON production was found among the 153 progeny of a cross between two strains of *G. zeae,* FG24 and FG3211. The parents differed little in these phenotypic characters, although they were collected in different areas, southwestern

*Table 4*. Coefficients of variation (%) for head blight rating, relative plot yield and DON production in the susceptible wheat cv. Drifter inoculated with 153 progeny of *Gibberella zeae* cross FG24 × FG3211 in three batches of 51 isolates each, calculated across three (aggressive traits) and two (DON production) replicates, respectively, and three field environments

| Parameter | d.f. | Head blight rating | Relative plot yield (transformed) | d.f. | DON production (transformed) |
|---|---|---|---|---|---|
| Source of variation | | | | | |
| Environment (*E*) | 2 | 16.83 | 4.90 | 2 | 24.84** |
| Replicate (*R*): *E* | 6 | 8.85* | 2.92 | 3 | 5.58* |
| Batch (*B*) | 2 | – [a] | – | 2 | 10.86 |
| B × E | 4 | 15.24** | 1.67 | 4 | 8.82** |
| B × R:E | 12 | 8.52** | 2.39** | 6 | 4.08** |
| Progeny (*P*):*B* | 150 | 14.69** | 4.25** | 150 | 13.31** |
| P × E:B | 300 | 22.66** | 7.59** | 300 | 16.24** |
| Pooled error | 897 | 18.83 | 9.97 | 446 | 17.41 |
| Heritability ($h^2$) | | 0.47 | 0.43 | | 0.67 |
| 90% CI on $h^2$ [b] | | 0.29–0.59 | 0.25–0.57 | | 0.56–0.75 |

Data for relative plot yield and DON were normalized by square root transformation.
[a] Negative estimate.
[b] Confidence intervals (CI) on $h^2$ were calculated using the method of Knapp and Bridges (1987).
*, ** Significant at probability levels $P = 0.05$ and 0.01, respectively (*F*-test).

Germany and southern Hungary. Strains FG24 and FG3211 were DON-chemotype isolates, producing DON in low to medium amounts. Cultural characters were identical, i.e. both were characterized by a pink-white colony and red pigmentation on potato dextrose agar (PDA) and aerial growth habit (data not shown).There was only a slight difference in aggressiveness. Based on a classification proposed by O'Donnell et al. (2000), both isolates belong to lineage 7 of *F. graminearum*.

The handling of 153 progeny in field inoculations, where the spore concentration of each isolate must be adjusted and each isolate must be applied separately, requires much labour and time. To reduce the workload to a manageable level, the progeny were randomly divided into three batches that were inoculated on three subsequent days. This procedure increased the non-genetic variance due to interactions between batches and environment and batches and replicates (Table 4). In central Europe in general, there are daily differences in temperature, rainfall, and humidity that may contribute to this interaction (Table 1). The interaction between progeny and environment within batches was also highly significant. This led to medium-sized heritability for head blight rating, although the repeatabilities of the single batches in the individual environments were medium to high, ranging from 0.5 to 0.7 for the same trait. The latter demonstrates good genotypic differentiation in the individual experiments. Heritability estimate was highest for DON production.

In view of the medium heritabilities obtained, the quantitative variation found for all traits does not necessarily implicate polygenic inheritance. The fact that some progeny were significantly more aggressive, caused lower yields and produced higher DON levels than the most aggressive parent, however, indicates that more than one gene controls these traits, and that these genes act additively. These transgressive segregants, comprising almost 80% of the population, still occurred after square-root transformation of the data on relative plot yield and DON production. This implies genetic effects caused by different unlinked alleles for the traits in both parents that recombined in the progeny. This type of inheritance corresponds to the inheritance of the resistance of wheat to *Fusarium* head blight (Snijders, 1990; Kolb et al., 2001; Miedaner et al., 2003). The pathosystem reveals a similar quantitative inheritance in both host and pathogen. We have demonstrated this for the first time using a segregating population of *G. zeae*. Quantitative variation of aggressiveness has been reported in other plant pathogenic fungi. For instance, aggressiveness of the smut pathogen *Ustilago hordei* in barley (Emara and Sidhu, 1974) and of *Gaeumannomyces graminis var. tritici* in wheat (Blanch et al., 1981) is quantitatively inherited.

In pathosystems with quantitative variation of aggressiveness and resistance, strong interactions with environment are likely to occur (Dusabenyagasani et al., 1997; Campbell and Lipps, 1998). In particular, *G. zeae* and wheat might react differently to the same environment; thus the effect of genotype × environment interaction of each organism will multiply in infection trials. Indeed, the progeny × environment interaction was one of the most important sources of variance, accounting for 29% of total variance for aggressiveness and 19% for DON production. This finding emphasizes the importance of multi-environmental trials. The highest head-blight rating occurred at HOH 2002, but in HOH 2001 the DON production was two to three times higher.

Correlation between head blight rating and DON production was high. For the latter trait, however, covariation with fungal colonization should be further analyzed because isolates are likely to produce different amounts of mycelium within the same host genotype (Miedaner et al., 2000). The large genotypic variation obtained by crossing two parents from the same lineage implicates a high possibility of adaptation of the pathogen to different environments and hosts by sexual recombination (McDonald and Linde, 2002). Indeed, several studies found a high genotypic variation within individual field populations for aggressiveness, DON production, vegetative compatibility groups, and molecular markers (Bowden and Leslie, 1992; Miedaner and Schilling, 1996; Miedaner et al., 2001, Walker et al., 2001). According to O'Donnell et al. (2000), all European isolates are lineage 7. Crosses within a lineage are most common and therefore, of highest practical relevance. Crosses between different lineages might occur through global seed trade. Genetic diversity and the potential of the fungus to shift towards greater aggressiveness or toxin production by hybridization within and between lineages should be appreciated (O'Donnell et al., 2000) and was suggested experimentally by our study. As a consequence, a large amount of genetic variation in *G. zeae* populations might be expected.

The use of aggressive isolates is important in selecting resistant wheat germplasm. Molecular tools permit characterization of genes and quantitative trait loci (QTL) linked to aggressiveness (Hou et al., 2002; Cumagun, et al., 2004). Sexual recombination in nature occurs in *G. zeae* regularly by production of perithecia on wheat and on maize stubble in autumn (Sutton, 1982; Parry et al., 1995). A maize–wheat crop rotation, as frequently used in central Europe and the USA, allows at least one recombination per year and gives the fungus the chance to produce new recombinants. According to population-genetic theory, fungi with mixed recombination, masses of asexually produced conidia, and regularly undergoing sexual recombination (selfing and outcrossing) have the highest risk for adaptation to host resistance (McDonald and Linde, 2002). Because no specific interaction of isolates and host genotypes in *G. zeae* occurs (Van Eeuwijk et al., 1995), directed selection should not play a major role in these populations. In the long term, however, genetic potential for a gradual, unspecific adaptation with increasing aggressiveness levels in populations of *G. zeae* could occur in response to host resistance.

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