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# Genetic variation of aggressiveness in individual field populations of *Fusarium graminearum* and *Fusarium culmorum* tested on young plants of winter rye

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## Abstract

*Fusarium graminearum* and *F. culmorum* are capable of infecting winter cereals at all growth stages. From natural field epidemics of wheat head blight and rye foot rot, three fungal populations were collected with 21, 38 and 54 isolates, respectively; their aggressiveness was analyzed in comparison to collections of *F. graminearum* (25 isolates) and *F. culmorum* (70 isolates) that represent a wide range of geographical locations and host species. All isolates were tested for aggressiveness on young plants of winter rye in the greenhouse and scored for disease severity on a 1–9 scale. Disease ratings of individual isolates ranged from 1.5 to 5.7 indicating quantitative variation of aggressiveness. Genotypic variance was highest in the two *Fusarium* collections. No substantial difference was found in the amount of genotypic variation between *F. graminearum* and *F. culmorum*. Individual field populations revealed 57–66% of the total genotypic variation of the collections. This implies a high degree of diversity of aggressiveness within single field populations of *F. graminearum* and *F. culmorum* causing natural epidemics.

## Introduction

*Fusarium graminearum* Schw. (teleomorph *Gibberella zeae* (Schw.) Petch) and *F. culmorum* (W.G. Smith) Sacc. (teleomorph unknown) occur world-wide as plant pathogens with a broad host range (Gerlach and Nirenberg, 1982). Winter rye (*Secale cereale* L.) can be infected at different stages of host development leading to seedling blight, foot rot or head blight (Chelkowski, 1989). Seedling blight may be caused by seed- or soil-borne inoculum. Severity of infection ranges from individual necrotic lesions at the shoot base to total seedling death. Seedlings with less severe infection mostly show foot rot at later growth stages (Duben and Fehrmann, 1980). In Central Europe, *F. culmorum* is mainly found in the northern, maritime regions, while *F. graminearum* dominates in the southern and continental areas, like southern Germany, Austria and Hungary (Parry et al., 1995). At present, no fungicides are available to control *Fusarium* diseases efficiently. In wheat and rye, plant resistance to *F. culmorum* is highly correlated with resistance to *F. graminearum* (Parry et al., 1995 and Miedaner et al., 1993, respectively) and varies quantitatively at all tested growth stages (Miedaner, 1988; Mesterházy, 1995; Miedaner et al., 1993, 1995).

Morphological variability in culture is high and has been described for both species (Puhalla, 1981; Oswald, 1949). Sources of genetic variability are sexual recombination in *F. graminearum* and mutation, somatic recombination by heterokaryosis in both species (Puhalla, 1981). Additionally, genetic variation in fungal populations can result from genetic drift, gene flow, and selection (McDonald and McDermott, 1993). However, little is known about the extent of genetic variation and the genetic mechanisms that are most significant in both species. The species *F. graminearum* and *F. culmorum* are closely related as determined by analysis of ribosomal proteins (Partridge, 1991), restriction

fragment length polymorphisms of mitochondrial DNA (Kodo et al., 1995), and polymerase chain reaction-based fingerprints (Schilling et al., 1996). Both species are highly aggressive compared to other *Fusarium* species of small grains (Stack and McMullen, 1985; Wilcoxson et al., 1988). According to Vanderplank (1984) aggressiveness designates the quantity of disease caused by a pathogenic isolate on a susceptible host while the isolate does not interact differentially with a host cultivar. In fact, no differential interaction has been shown for *Fusarium* head blight in wheat (Snijders and van Eeuwijk, 1991; van Eeuwijk et al., 1995) and rye (Miedaner et al., 1996b). Previous studies on wheat indicated that genetic variation of aggressiveness exists in both *Fusarium* species (Mesterházy, 1984; Snijders and van Eeuwijk, 1991). In these investigations only a limited number of isolates from different geographic regions were used. In field experiments on head blight of winter rye caused by *F. culmorum*, significant quantitative genotypic variation with high heritability was found among a collection of 42 isolates suggesting that aggressiveness is inherited as a complex trait (Miedaner et al., 1996a). However, it is unclear, whether the extent of variability in aggressiveness was due to the diverse collection of isolates from different geographic origins and host species or whether it occurs also among isolates from a single field. The objective of this study was to determine, on a quantitative basis, the range of aggressiveness within *Fusarium* populations that have caused natural epidemics in individual fields. Furthermore, these were compared to the range of aggressiveness present in collections of *F. graminearum* and *F. culmorum* isolates sampled world-wide.

## Materials and methods

*Fungal isolation and inoculum production.* The collections of *F. graminearum* and *F. culmorum* isolates used in this study are located at our laboratory. Isolates were obtained from naturally infected plant material or from soil collected in different countries (Table 1). With only a few exceptions, isolates were collected from 1975 to 1991. The isolates were stored from the date of isolation until 1991 in sterilized soil tubes (Schneider, 1958) and from 1991 onwards in liquid nitrogen as described below. Some isolates from Germany were contributed by Dr. H. Nirenberg, Berlin; isolates from The Netherlands, Hungary, Italy, and Australia were kindly provided by Dr. C.H.A. Snijders, Wageningen, Dr. A. Mesterházy, Szeged, Dr. A. Visconti, Bari, and by Dr. L.W. Burgess, Sydney, respectively. All *F. graminearum* isolates originating from Australia belong to Group 2 (L.W. Burgess, *pers. comm.*) and the isolates sampled in Middle Europe are assigned to the same group. Following to the isolation and identification of *Fusarium* spp. according to Nirenberg (1981) or receipt by mail, pure cultures were derived from all isolates by monoconidial transfers. The cultures were typed again and preserved in liquid nitrogen from 1991 onwards according to a modified procedure of Hoffmann (1989). Isolates were cultured on SNA (synthetic nutrient-poor mineral agar) according to Nirenberg (1981) supplemented with 5 $mlL^{-1}$ glycerol. Sterilized polyvinylchloride "straws" with a diameter of about 1 mm and a length of 25 mm were used to cut out plugs of mycelium and agar. Fifty straws per isolate were preserved in liquid nitrogen within cryotubes each containing up to 12 straws.

For the present study, all isolates obtained from one field are regarded as a population, i.e. a group of

*Table 1.* Geographic origin, plant organ and host species of a collection of 25 *F. graminearum* and 70 *Fusarium culmorum* isolates

| *Fusarium* species/ Country | Host organ | Host species | | |
|---|---|---|---|---|
| | | Wheat | Rye | Other[1] |
| *Fusarium graminearum* | | | | |
| Germany | Foot | – | 1 | – |
| | Head | 4 | 4 | 2 |
| Other[2] | Foot | – | – | 3 |
| | Head | 5 | 2 | 4 |
| Total | | 9 | 7 | 9 |
| *Fusarium culmorum* | | | | |
| Germany | Foot | 4 | 8 | 2 |
| | Head | 8 | 5 | 7 |
| The Netherlands | Head | 12 | 1 | – |
| Scandinavia (Norway, | Foot | – | – | 1 |
| Sweden, Finland) | Head | – | 4 | 1 |
| Other[3] | Foot | 2 | 2 | 3 |
| | Head | 10 | – | – |
| Total | | 36 | 20 | 14 |

[1] Host origin and numbers of *F. graminearum* isolates: barley (1), durum wheat (2), triticale (1), maize (3), rye grass (1), *Paspalum* sp. (1); *F. culmorum* isolates: barley (2), durum wheat (2), emmer wheat (1), spelt wheat (2), triticale (2), maize (2), soil (3).
[2] Including Australia (5 isolates), Croatia (1), Hungary (4), Italy (1), Poland (2), USA (1).
[3] Including Australia (3 isolates), Canada (1), Hungary (4), Italy (2), Poland (2), Switzerland (3), UK (1), USA (1).

*Table 2.* Host species, host organ, sampling size and structure of *Fusarium* populations isolated from three fields in South Germany (EWE = Eckartsweier near Kehl, SER = Sersheim near Stuttgart) in 1992 and 1993

| Population | *Fusarium* species | Host | | Number of | | Total |
|---|---|---|---|---|---|---|
| | | Species | Organ | Sample points | Isolates per point | |
| EWE92 | *F. graminearum/F. culmorum* | Rye | Foot | 5 | 4–5 | 21 |
| SER92 | *F. graminearum* | Wheat | Head | 9 | 6 | 54 |
| EWE93 | *F. graminearum* | Wheat | Head | 19 | 2 | 38 |

individuals sharing a common gene pool and which are present in a limited geographical area (McDonald and McDermott, 1993). For sampling isolates from natural field populations, plant tissue with visual symptoms was collected arbitrarily from three commercial fields in the South of Germany with a size of about one hectare each (Table 2). The samples were taken from a rye field at Eckartsweier near Kehl/Rhine and a wheat field at Sersheim near Stuttgart in 1992, and from a wheat field at Eckartsweier in 1993 different from the former. In the following, the three populations are abbreviated EWE92, SER92, EWE93. In each field, the sampling was conducted on two to three transects and at about 25-m intervals along each transect. At each sampling point, several infected plants were collected and from these two to six isolates were made. Samples were taken exclusively from heads with symptoms because we were interested in the pathogenic *Fusarium* populations of these fields only. After sampling, the plant tissue was stored immediately at −20 °C. To initiate fungal cultures, pieces of tissue were surface sterilized (Burgess et al., 1994) and placed on SNA medium. Cultures were grown at 18 °C under permanent UV light (Philipps TLO, 40 W/80) for 14–18 days until massive sporulation occurred. Pure cultures were derived by single-spore transfers. To preserve the isolates, several mycelium plugs with a diameter of 5 mm were cut out from pure cultures growing on SNA. The plugs were placed in 1.5 ml tubes containing sterile distilled water and stored at 6 to 8 °C in the refrigerator.

Mass propagation of each *Fusarium* isolate was performed on wheat grain medium as previously reported by Miedaner et al. (1995): 1000 ml flasks were filled half with autoclaved wheat grain and inoculated with conidia from an abundantly sporulating culture produced on one SNA plate (60 mm diameter) and incubated at 18 °C in the dark for four weeks. The colonized grain was air-dried for 2–3 days and stored in plastic bags in a refrigerator at 5 °C until usage.

*Plant material and greenhouse test.* Aggressiveness was tested on young plants of a synthetic population of winter rye (SYN 8392) in the greenhouse established by crossing two self-fertile inbred lines (L283 and L292) of the Carsten gene pool by hand-emasculation in the greenhouse. The resulting $F_1$ hybrid was propagated under open pollination in isolation chambers commonly used for inbred line development in hybrid rye breeding.

Seeds were sown in plastic trays and cultivated in a greenhouse. After approximately 10 days, healthy and vigorous seedlings were transplanted in groups of four into plastic pots (7 × 7 $cm^2$); they were cultivated in a non-heated greenhouse at temperatures of 2–16 °C and without artificial light. The inoculation tests were conducted between November and March in a growth chamber with filtered induction and exhaust air. Conditions were kept at 16–18 °C (day) and 13–15 °C (night), and at a daylength of 10 h with additional light (OSRAM Power Star Day Light HQT-T, 400 W/DH). Plants were inoculated at the beginning of tillering (GS21–22, according to Zadoks et al. (1974)) and incubated for four weeks as described by Höxter et al. (1992). Ten volumes of vermiculite (KLEIN, Zellertal, sieve size 2–4 mm) saturated with SNA solution (without agar) were mixed with one volume of colonized wheat grain medium crushed in a mill (CYCLOTEC, pore size 0.5–1 mm). A layer of 5 mm of the colonized grain-vermiculite mixture was spread uniformly over the soil surface of each pot. During incubation, the pots were placed on a sand layer that was watered regularly. A solution of 2% commercial liquid fertilizer (WUXAL 8-8-6 Super, Schering) was applied once per week. To maintain a relative high humidity during incubation and to avoid any spread of inoculum, each pot was covered by a transparent cellophane bag of 145 mm width and 235 mm height with a punctured top (6 punctures of 5 mm diameter each) secured at the base using an elastic band. The use of cellophane

*Table 3.* Description of the disease rating scale

| Score | Description |
|---|---|
| 1 | No lesion visible, plant fully vigorous, ≥3 tillers, |
| 2 | Lesion of pinpoint size at shoot base, plant fully vigorous, ≥3 tillers, |
| 3 | Lesion covering less than 1/4 the circumference of the shoot base, plant fully vigorous, about 3 tillers, |
| 4 | Lesion covering about 1/4 to 1/2 the circumference of the shoot base, plant still vigorous with 2–3 tillers, |
| 5 | Lesion covering 1/2 to 3/4 the circumference of the shoot base, plant less vigorous with 2–3 tillers per plant, |
| 6 | Lesion covering more than 3/4 the circumference of the shoot base, plant weakened and plant canopy beginning to brown, 1–2 tillers, |
| 7 | Shoot base fully discolored, tissue beginning to soften, about 50% of the plant canopy brown, not more than 1 tiller, |
| 8 | Plant extremely weakened, shoot base totally softened, only some green leaf tissue remaining, no tillering, |
| 9 | Plant dead. |

enabled air exchange and prevented condensation of water within the bags.

*Disease assessment.* Disease symptoms were assessed after four weeks of incubation by rating the plants individually on a 1–9 scale. The extent of lesions at the shoot base and the vitality of the whole plant were taken into account when disease ratings were assigned because seedling blight by *Fusarium* spp. is not restricted to specific plant organs. The non-linear rating scale is shown in Table 3.

*Statistical analyses.* The statistical analyses were based on the disease ratings of single pots, i.e. on the arithmetic mean of four individual plants. Each isolate was inoculated in 10 pots as replicates. Isolates were arranged according to a completely randomized block design. Due to limited space in the greenhouse chamber, collections and populations were tested in three sets with the collections of *F. graminearum* and *F. culmorum* isolates in one set, the isolates of population SER92 in one set, and EWE92 and EWE93 in another set. This incomplete randomization was possible because different experiments conducted in the greenhouse did not show any significant genotype-experiment interactions (Höxter et al., 1992). Even when the experiments were conducted during autumn and spring, no significant effects of season or genotype-season interaction occurred (Höxter et al., 1992). Estimates of genotypic ($\sigma_t^2$) and error ($\sigma_e^2$) variance components were calculated as described by Snedecor and Cochran (1989) for factorial designs. The coefficient of genotypic variation ($CV_t$ %) was estimated as follows: ($\sigma_t$/Mean) × 100. Repeatability estimates were calculated by partitioning the phenotypic variance of spatial replications within one experiment according to the formula $\sigma_t^2/\sigma_t^2 + \sigma_e^2$ (Falconer, 1989). For testing deviation from the normal distribution the procedure of Shapiro and Wilk (1965) was applied using the statistical program SAS (SAS Institute Inc., 1988). All analyses of variance were computed with the statistical package PLABSTAT (Utz, 1994). The effects of isolates and replicates were assumed to be random variables.

## Results

The mean disease severity was approximately of the same magnitude in all tested populations and ranged from 2.8 to 3.4 on the 1–9 scale (Table 4). The isolates of the *F. graminearum* and *F. culmorum* collections did not differ in their mean aggressiveness from those isolated freshly from infected plant tissue. The population EWE92 collected from winter rye revealed a similar disease severity in the tests on young plants of rye as the populations isolated from winter wheat (SER92, EWE93). Although the populations were tested in different sets, repeatabilities were similar. Genotypic variances were significant for all populations ($P = 0.01$). The coefficients of genotypic variation ($CV_t$) were similar in both collections of *F. graminearum* and *F. culmorum*, although *F. culmorum* was represented by more isolates. For both collections, the coefficients of genotypic variation were greater than those observed in the three field populations. Among the field populations, the coefficients of variation were very similar. The population EWE92 consisted of 8 isolates of *F. graminearum* and 13 of *F. culmorum* but did not show a substantially greater genotypic variance compared to the other populations. For each of the two collections and the three populations, continuous variation was found for the aggressiveness of *F. graminearum* and *F. culmorum* (Figure 1). The phenotypic range of the disease ratings was smaller in the three field populations than in the two collections. Each frequency distribution did not significantly ($P = 0.05$) deviate from the normal distribution.

*Table 4.* Means, genotypic variance components ($\sigma_t^2$), coefficients of genotypic variation ($CV_t$%), and repeatabilities of disease ratings for two collections of *Fusarium graminearum* and *F. culmorum* isolates and *Fusarium* populations from three fields (EWE = Eckartsweier near Kehl, SER = Sersheim near Stuttgart) sampled in 1992 and 1993

| Population | *Fusarium* species | Mean (1–9)[1] | $\sigma_t^2$ | $CV_t$ (%) | Repeatability |
|---|---|---|---|---|---|
| Collection | *F. graminearum* | 2.8 | 0.552 ** | 26.5 | 0.84 |
| Collection | *F. culmorum* | 3.4 | 0.770 ** | 25.8 | 0.84 |
| EWE92 | *F. graminearum/F. culmorum* | 2.9 | 0.265 ** | 17.8 | 0.69 |
| SER92 | *F. graminearum* | 3.2 | 0.246 ** | 15.5 | 0.78 |
| EWE93 | *F. graminearum* | 3.2 | 0.236 ** | 15.2 | 0.77 |

** Genotypic variance component significant at probability level $P = 0.01$.
[1] Disease rating: 1 = visually healthy, 9 = plant dead, for full scale see Table 3.

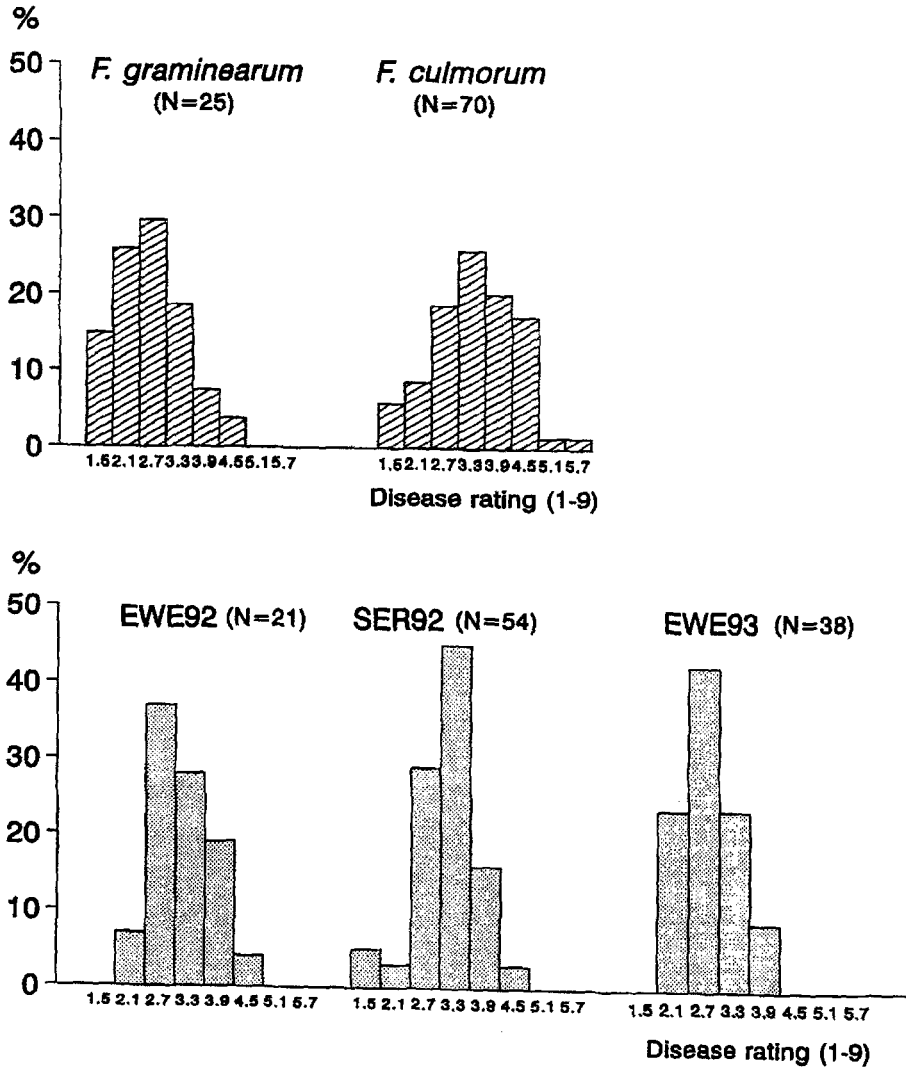


*Figure 1.* Frequency distributions of disease severity (1–9 scale with 1 = visually healthy, 9 = plant dead) from single isolates of the *Fusarium graminearum* and the *F. culmorum* collection, and three field populations. Details on the isolates are given in Tables 1 and 2.

## Discussion

In natural populations of *F. graminearum* and *F. culmorum*, quantitative variation of aggressiveness was demonstrated by a greenhouse test on winter rye in the young-plant stage. This finding is in good agreement to the results of field experiments with artificial inoculation of winter rye heads using 42 isolates of *F. culmorum* (Miedaner et al., 1996a) and to wheat seedling tests with *F. graminearum* reported previously (Mesterházy, 1984). Furthermore, our data show a low level of pathogenic host specialization of *F. graminearum* and *F. culmorum* populations. Although most of the isolates of the collections and two out of three populations were not collected from winter rye, they caused a very similar disease severity on winter rye than isolates of the population EWE92 isolated from this crop. Similarly, the high repeatabilities obtained for the different sets of materials demonstrate that the greenhouse test showed good genotypic differentiation in all instances. This, and the similar mean disease ratings of the populations allow a comparison of statistical parameters without confounding effects by scaling. The population EWE92 was isolated from stem bases of winter rye and revealed the presence of both *F. graminearum* and *F. culmorum* isolates. These isolates were not analyzed separately according to species because mixed infections of the two species at the stem base of winter rye and winter wheat are commonly observed (Duben and Fehrmann, 1979; Miedaner et al., 1995). Therefore, the results from the mixed population are most likely reflect the situation that exists in nature. Indeed, the coefficients of genotypic variation differed only slightly between the mixed *F. graminearum*/*F. culmorum* and the pure *F. graminearum* populations.

Genetic variation of aggressiveness was highest within the collections of *F. graminearum* and *F. culmorum*. Both species showed virtually no difference in levels of genetic variation although *F. graminearum* is able to reproduce sexually. However, *F. graminearum* Group 2 isolates are homothallic (Burgess et al., 1994) and in random mating populations selfings may occur regularly limiting the amount of genetic diversity. The isolates of the collections originated from different geographic regions and host species and this may partly explain their great genetic variance. In the individual field populations, however, about 60% of the total variation of the respective isolate collections was found. This high degree of genetic diversity of aggressiveness within an individual field could result from any of several factors. For *F. graminearum*, outcrossing might be a major source of genetic variation due to genetic recombination during meiosis. Somatic recombination by heterokaryosis and migration of wind-borne ascospores from adjacent fields could also increase variation. Additionally, the strong saprophytic ability of both *Fusarium* species (Burgess and Griffin, 1967) could enable even low aggressive isolates to propagate on decaying host debris and/or on organic matter in the soil. The relative importance of any of these factors in determining the degree of variation in aggressiveness has not been studied in the two *Fusarium* species investigated in our study. Among field isolates of *F. graminearum* from wheat heads, Bowden and Leslie (1994) described a high level of genetic diversity for vegetative compatibility groups (VCG). The authors concluded that sexual recombination must occur frequently because on most wheat heads they found different VCGs. In other plant pathogenic fungi that are known as facultative saprophytes, genetic variation assessed by molecular markers, is also distributed on a fine geographic scale. For instance, in natural populations of *Mycosphaerella graminicola* (anamorph *Septoria tritici*), and *Stagonospora nodorum* (anamorph *Septoria nodorum*) abundant genetic variation has been found within individual fields (McDonald and Martinez, 1990; McDonald et al., 1994).

To assess the durability of host resistance, the structure of natural pathogen populations is highly important. If a fungal population of an individual field exhibits a high level of genetic variation, then the pathogen might rapidly adapt to resistance genes of the host (McDonald and McDermott, 1993). For populations of *F. graminearum* and *F. culmorum*, however, other factors have also to be considered. In wheat and rye, quantitative host resistance is thought to be inherited in a complex way (Geiger and Heun, 1989). So far, no host genotype has been identified uninfected by either species (Snijders, 1990) and no races of the pathogen have been differentiated (van Euwijk et al., 1995). Moreover, since *F. graminearum* and *F. culmorum* are successful saprophytes and are only weakly specialized as pathogens on a particular host species, the selection pressure by the host is expected to be of minor importance. Therefore, host resistance to both *Fusarium* species is most likely durable despite the high variation in aggressiveness that we have found within individual field populations. Accordingly, a high ecological stability has been shown for the resistance of some wheat genotypes to *F. graminearum* head blight across 16 environments (Mesterházy, 1995).

Our results may have some implications for the type of inoculum used in breeding for resistance. Due to the lack of host genotype-isolate interactions in this pathosystem (Miedaner et al., 1996b), any isolate or isolate mixture that is sufficiently aggressive could be used (van Eeuwijk et al., 1995). However, a mixture of isolates differing in aggressiveness should be preferred for artificial inoculation, as this would more closely reflect the genetic variability that we have observed within single field populations. For achieving adequate differentiation between host genotypes, isolates that have been shown to be moderately to highly aggressive when used individually should be mixed to give a medium level of aggressiveness in the mixture. Rather than using a single isolate for screening breeding materials, the suggested procedure should reflect the conditions that operate in natural epidemics more closely.

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