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Competition effects among isolates of *Fusarium culmorum* differing in aggressiveness and mycotoxin production on heads of winter rye

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Abstract

Fusarium culmorum is a phytopathogenic, toxigenic fungus causing seedling diseases, foot rot and head blight of cereals. For estimating competition effects in mixtures of two single-spore isolates, two winter rye single crosses were tested with either four isolates individually or four 1 : 1 mixtures of the same isolates in six field environments. Two isolates (FC46, FC64) were highly aggressive deoxynivalenol (DON) and 3-acetyl DON-producers, the other two (FC30, FC71) were medium aggressive nivalenol-producers. Rye heads were inoculated during flowering with conidia of pairs of isolates expressing similar (FC46 + FC64, FC30 + FC71) or contrary (FC46 + FC71, FC30 + FC64) levels of aggressiveness and similar or different concentrations and chemotypes of mycotoxins, respectively. Head blight rating and yield components relative to the non-inoculated plots were recorded as aggressiveness traits. Additionally, mycotoxin concentrations were measured in the rye grain. Random pathogen samples were re-isolated from heads at the onset of symptom development and analysed by molecular markers (RAPD–PCR) in one environment. Aggressiveness of the isolate mixtures was significantly lower than that of the isolates applied individually on both rye genotypes. Similarly, mycotoxin concentrations were significantly lower in the mixtures in seven out of eleven comparisons. Among the re-isolates, the component genotypes of a mixture significantly deviated from the inoculated 1 : 1 ratio when a particular isolate (FC46) was present in the mixture. This isolate displayed a superior competitive ability irrespective of the aggressiveness or mycotoxin profile of the mixing partner illustrating that pathogenic fitness is caused by additional factors that have not, as yet, been identified.

Abbreviations: DON – deoxynivalenol; 3-ADON – 3-acetyl deoxynivalenol; FC – *Fusarium culmorum*; NIV – nivalenol; PCR – polymerase chain reaction; RAPD – random amplified polymorphic DNA.

Introduction

Fusarium culmorum infects seedlings, stem-bases and heads of winter rye (*Secale cereale*) and other small-grain cereals (Parry et al., 1995). Natural epidemics of head blight lead to severe yield and quality losses and contamination with mycotoxins. The type B trichothecenes deoxynivalenol (DON) and nivalenol (NIV) are the most frequently observed *Fusarium* toxins (Placinta et al., 1999). Most isolates of *F. culmorum* produce either DON or NIV in high amounts (Gang et al., 1998; Muthomi et al., 2000). DON and NIV cause feed refusal and decreased weight gain in swine (D'Mello et al., 1999). *Fusarium culmorum* is a soilborne pathogen and a good saprophytic competitor in the natural soil habitat (Burgess and Griffin, 1967). It infects cereal heads by splash-dispersed macroconidia shown to be spread by wind

and rain up to 1 m in a vertical and 0.6 m in horizontal direction (Jenkinson and Parry, 1994). Successful infections result in prematurely bleached spikelets. The proportion of visibly diseased spikelets is a reproducible measure of aggressiveness of isolates in inoculation experiments (Miedaner et al., 1996a).

Fusarium culmorum exhibits a large molecular and phenotypic diversity. Aggressiveness, mycotoxin profiles, and concentrations vary among isolates (Miedaner et al., 1996a; Gang et al., 1998; Muthomi et al., 2000). Random amplified polymorphic DNA (RAPD) analyses of 41 isolates from a single wheat field revealed 23 different multi-locus haplotypes (Miedaner et al., 2001a). This result is comparable to studies in *Fusarium graminearum* that also detected a high genetic diversity at extremely small spatial scales by analyses of vegetative compatibility groups (VCG) and RAPD markers (Bowden and Leslie, 1994; Miedaner et al., 2001a). In both species, isolates belonging to different multi-locus haplotypes or VCGs were detected frequently in the same heads. The different haplotypes most probably originate from different infection events. No data are available, however, as to whether differences exist between isolates in pathogenic fitness when these are competing on the same head in the complex field environment. This question is of fundamental interest for understanding the dynamics of *Fusarium* populations in time and space. The objectives of this study were (1) to compare disease severity and mycotoxin concentration of single *versus* mixed inoculations and (2) to investigate competition effects between isolates of *F. culmorum* differing in aggressiveness and mycotoxin profile (DON/NIV) under field conditions. Changes in the mixtures of two isolates were monitored by molecular fingerprinting (RAPD markers) of the pathogens re-isolated from visibly infected heads.

Materials and methods

Isolates, isolate mixtures, and inoculum production

Four single-spore isolates of *F. culmorum* (FC30, FC46, FC64, FC71) already described in detail for their aggressiveness (Miedaner et al., 1996a) and mycotoxin production (Gang et al., 1998) were used (Table 1). Isolates originated from infected host materials from four countries and were identified as *F. culmorum* by their conidial morphology (Gerlach and Nirenberg, 1982) and by a species-specific polymerase chain reaction (PCR)-based DNA analysis (Schilling et al., 1996). The following combination of treatments were established in equal proportions: two highly aggressive DON-producers (FC46 + FC64), two medium aggressive NIV-producers (FC30 + FC71), and two mixtures of a highly aggressive DON and a medium aggressive NIV-producer (FC46 + FC71, FC64 + FC30). Long-term storage of isolates was in liquid nitrogen. Inoculum was produced on wheat-grain medium (Miedaner et al., 1996a). One-litre glass flasks containing 400 ml of boiled and sterilized wheat grain were incubated with a spore suspension of the respective isolate in the dark at 16–18 °C for 4 weeks. Grain was removed and incubated in plastic boxes ($40 \times 60\,cm^2$), which were covered with polyethylene foil under permanent black light for 6–14 days to allow extensive spore production. During incubation, the development of mycelial mats was prevented by thoroughly mixing the wheat grain by hand every day. Wheat-grain medium and conidia were rapidly air-dried and stored at 6 °C in plastic bags until usage. On the day of inoculation, conidia were washed from the wheat grain with tap water and filtered through metal sieves. With this method, pure conidial suspensions were produced without mycelial fragments.

Table 1. Origin and characteristics of the isolates of *F. culmorum*

Isolate		Aggressiveness[1]	Major type of mycotoxin	Origin		
Designation	Code[1]			Location/country	Host	Collector
FC46	40	High	DON	Wageningen/NL[2]	Wheat	C.H.A. Snijders
FC64	35	High	DON	Svalöf/S[2]	Rye	Authors
FC30	09	Medium	NIV	Vaihingen/D[2]	Emmer wheat	Authors
FC71	06	Medium	NIV	Crookwell/AUS[2]	Corn	L. Burgess

[1]According to Miedaner et al. (1996a); Gang et al. (1998).
[2]AUS = Australia, D = Germany, NL = The Netherlands, S = Sweden.

Spore concentration of all isolates was adjusted to 1×10^6 conidia ml^{-1}. The mixtures of two isolates were produced by mixing spore-adjusted conidial suspensions of the individually propagated isolates in a 1 : 1 ratio. Finally, Tween 20 (final concentration of 0.01%) was added to the suspension.

Plant materials and field design

Two homogeneous single crosses (L7 × L6 and L12 × L05) of self-fertile winter rye inbred lines were used. They were known to be highly and moderately susceptible to *F. culmorum* head blight (Miedaner et al., 2001b). Experiments were conducted over two years (1996 and 1997) at three locations in Southwest Germany: Hohenheim (HOH) near Stuttgart (400 m above sea level, 8.5 °C mean annual temperature, 685 mm mean annual precipitation), Eckartsweier (EWE) near Kehl/Rhein (141 m above sea level, 9.9 °C mean annual temperature, 726 mm mean annual precipitation), and Oberer Lindenhof (OLI) near Reutlingen (700 m above sea level, 6.6 °C mean annual temperature, 952 mm mean annual precipitation). The six location–year combinations (environments) will be abbreviated by HOH96, EWE96, OLI96, HOH97, EWE97, and OLI97. Plants were grown on three-row microplots (0.75 m^2) laid out as a complete randomized block design with 18 treatment combinations: four individual isolates, four mixtures, and a non-inoculated control for each of the two rye genotypes in four replicates. Each plot was surrounded on its four sides in a chessboard-like manner by border plots grown to a tall, non-inoculated winter rye variety ('Danko') to minimize interplot interference. To avoid complex interactions between infections of *F. culmorum* and other pathogens, all plots were sprayed to control *Pseudocercosporella herpotrichoides* (Sportak Alpha (Prochloraz 450 g l^{-1} ha^{-1} + Carbendazim 120 g l^{-1} ha^{-1}, Aventis) at stem elongation) and *Blumeria graminis* and *Puccinia recondita* (Opus Top (Epoxiconazol 126 g l^{-1} ha^{-1} + Fenpropimorph 375 g l^{-1} ha^{-1}, BASF), shortly before heading).

Inoculation and disease assessment

These were carried out as described previously (Miedaner et al., 1996a). Each treated plot was inoculated once with about 100 ml of the spore suspension at the mid-flowering stage by a portable sprayer. At HOH96 and HOH97, mist-irrigation was applied in the morning (05:30–12:00 h) after each inoculation date for 15 s every 15 min to further increase and maintain air humidity.

Head blight severity was assessed plotwise three times on a nine-class rating scale in which 1 = no symptoms visible, 2–9 = ≤5%, 6–15%, 16–25%, 26–45%, 46–65%, 66–85%, 86–95%, and >95%, respectively, of all spikelets visually diseased. Rating commenced with the onset of symptom development until the beginning of yellow ripening (EC77) (Zadoks et al., 1974). Arithmetic means of three head-blight ratings were used for further analyses. At the yellow-ripening stage, 60 arbitrarily selected ears per plot were harvested by hand and carefully threshed in a single-head thresher with a minimum of forced air to save highly infected, shrivelled, and degenerated kernels. From these samples, grain weight per spike and kernel weight were assessed. All yield components were expressed as percentages of the corresponding means of the non-inoculated treatment of the same rye genotype.

Re-isolation of fungal genotypes and marker analyses

From each HOH 96 field plot inoculated with an isolate mixture, 10 visibly infected heads were sampled randomly at the beginning of symptom development. From each of these heads two infected spikelets were disinfected in 1% sodium hypochlorite and 0.1% Tween 20 for 10 min, then rinsed three times in sterile distilled water and plated on agar. From each of the resulting colonies, one single-spore isolate was derived according to Schilling (1996). In total, 54–66 isolates from each of the four mixtures were finger-printed by RAPD–PCR.

Four out of 151 RAPD primers (C19, D05, D13, UBC31) were selected that together differentiated the four isolates. These decamer primers were purchased from the University of British Columbia (UBC), Vancouver, Canada, or synthesized commercially. Total genomic DNA was extracted from mycelium grown in continuously shaken liquid culture (SNA according to Nirenberg (1981) without agar plus 0.1% yeast extract) by a microextraction protocol (Möller et al., 1992) including pretreatment with RNase A. DNA was quantified by comparing DNA standards using agarose gel electrophoresis and amplified by PCR. RAPD–PCR was conducted under

conditions described in detail elsewhere (Schilling, 1996). Amplicons were cooled immediately to 15 °C and stored at 4 °C until gel electrophoresis. A gel-loading solution was added and half of the PCR was resolved on 1.5% agarose in 1× TAE buffer (0.04 M Tris–HCl, 0.002 M EDTA, adjusted to pH 8.0 with 98% acetic acid) for 3 h at 2 V cm^{-1} including a 100-bp ladder (Pharmacia, Germany) as the size standard. Gels were stained in ethidium bromide, and images were recorded using Polaroid film 667. Banding patterns of re-isolates from mixed inoculations were compared with those of the two respective components and the frequency of re-isolates was determined for each head sample.

Toxin analysis

From the grain harvested in HOH96, the type and content of mycotoxins was determined by gas chromatography with mass spectrometry (GC–MS). Grain was ground to a particle size of about 1 mm with a laboratory mill, mixed and stored at −20 °C. Extraction of toxins from grain was carried out according to Tanaka et al. (1985) and sample clean-up and determination of toxins according to Schollenberger et al. (1998). Seven trichothecenes could be determined in one step (DON, 3- and 15-acetyl deoxynivalenol (3-ADON, 15-ADON), NIV, fusarenon-X, T-2 toxin, and HT-2 toxin), but only DON, 3-ADON, and NIV were found in amounts above the detection limit of 70, 50, and 120 µg kg^{-1}, respectively. For samples from non-inoculated plots, a lower sample dilution was used, the respective detection limits being 7, 5, and 12 µg kg^{-1}. Due to limited laboratory facilities, the samples taken from each of two replicates of the field experiment were blended in equal amounts, i.e. two samples were analysed for toxins from each inoculation treatment.

Statistical analyses

Data were analysed on a plot basis. Residuals in individual environments followed a normal distribution for all traits, and error variances were homogeneous across environments according to Bartlett's test (Snedecor and Cochran, 1989). The six location–year combinations were analysed as a series of random environments (Cochran and Cox, 1957). Estimates of variance components were calculated as described by Snedecor and Cochran (1989). Repeatabilities achieved in individual environments were estimated on an entry-mean basis as the ratio of the genotypic to the phenotypic variance (Fehr, 1987). All analyses of variance were performed with the computer package PLABSTAT (Utz, 2000). The effects of replicates and environments were assumed to be random variables and the effects of inoculation treatments, inocula, and rye genotypes were considered as fixed because of the low number of degrees of freedom and the pre-selection of specific host and pathogen genotypes for the experiment.

Results

Moderate disease severities were observed on the inoculated plots in all environments: head blight ratings varied from 3.2 to 5.6 (Table 2). Low relative grain weight per spike indicated high grain losses in all environments

Table 2. Means of inoculations with four single isolates and four mixtures of two isolates of *F. culmorum* for head blight rating and yield components relative to the non-inoculated plots in six environments across two rye genotypes

Environment[2]	Head blight rating (1–9)[1]			Relative grain weight per spike (%)			Relative kernel weight (%)		
	Single	Mixture	Sig.[3]	Single	Mixture	Sig.	Single	Mixture	Sig.
EWE96	4.66	3.95	**	34.0	44.2	**	56.7	61.2	**
HOH96	5.55	4.92	**	25.5	30.9	**	57.4	58.9	
OLI96	3.21	3.29		61.3	62.8		68.5	70.7	*
EWE97	4.37	3.79	**	35.9	43.7	**	43.8	49.2	**
HOH97	4.42	3.70	**	22.5	36.1	**	50.7	55.3	**
OLI97	5.38	4.66	**	14.1	18.0	**	49.3	51.4	

[1] 1 = no symptoms visible, 2–9 = ≤5%, 6–15%, 16–25%, 26–45%, 46–65%, 66–85%, 86–95%, and >95%, respectively, of all spikelets visually diseased.

[2] EWE = Eckartsweier near Kehl/Rhine, HOH = Stuttgart–Hohenheim, OLI = Oberer Lindenhof near Reutlingen; 96 and 97 designate the year.

[3] *,** Significant difference between inoculations of single isolates and mixtures of two isolates at $P = 0.05$ and 0.01, respectively (F-test).

Table 3. Means of head blight rating and yield components relative to the non-inoculated plots of two winter rye single crosses inoculated with four isolates of *F. culmorum* individually (single) or in four mixtures of two isolates across six environments

Inoculation treatment	Rye genotype	Head blight rating (1–9)[1]	Relative grain weight per spike (%)	Relative kernel wt. (%)
Single	L7 × L6	5.26 a[2]	27.5 a	48.3 a
	L12 × L05	3.93 c	36.9 b	66.4 c
Mixture	L7 × L6	4.61 b	34.4 b	58.1 b
	L12 × L05	3.50 d	44.2 c	75.6 d

[1]1 = no symptoms visible, 2–9 = ≤5%, 6–15%, 16–25%, 26–45%, 46–65%, 66–85%, 86–95%, and >95%, respectively, of all spikelets visually diseased.
[2]Means with the same letter within a column are not significantly different at $P = 0.01$ (Tukey test).

Table 4. Means of head blight rating and yield components relative to the non-inoculated plots of inoculations with four isolates of *F. culmorum* individually (single) or in four mixtures of two isolates across two rye genotypes and six environments

Inoculation treatment	Isolate(s)	Head blight rating (1–9)[1]	Relative grain weight per spike (%)	Relative kernel wt. (%)
Single	FC46	5.02 a[2]	26.47 a	48.33 a
	FC64	5.13 a	23.70 a	45.28 a
	FC30	4.20 b	37.84 b	61.04 b
	FC71	4.04 b	40.76 b	62.90 b
Mixture	FC46 + FC64	4.44 a	32.88 a	50.42 a
	FC30 + FC71	3.62 c	47.05 c	66.27 c
	FC46 + FC71	4.09 b	38.68 b	57.75 b
	FC64 + FC30	4.06 b	38.60 b	56.73 b
Single vs. mixture	Observed	4.05	39.30	57.79
	Expected[3]	4.60	32.13	54.37
	Significance	**	*	**

*,**Significant difference at $P = 0.05$ and 0.01, respectively (t-test).
[1]1 = no symptoms visible, 2–9 = ≤5%, 6–15%, 16–25%, 26–45%, 46–65%, 66–85%, 86–95%, and >95%, respectively, of all spikelets visually diseased.
[2]Means with the same letter are not significantly different at $P = 0.01$ (Tukey test).
[3]From the arithmetic mean of the respective pairs of isolates tested individually.

except OLI96. Relative kernel weight was less affected. Repeatabilities were generally very high (around 0.9). The isolates caused a significantly higher disease severity when inoculated individually than in a mixture in all environments except OLI96. Rye single cross L7 × L6 was significantly more susceptible than single cross L12 × L05 for all traits (Table 3). On both genotypes, isolate mixtures were less aggressive than individual isolates. Isolates FC46 and FC64 were both significantly more aggressive than FC30 and FC71 when inoculated individually (Table 4). Accordingly, the mixtures with highly (FC46 + FC64) and moderately aggressive isolates (FC30 + FC71) differed significantly ($P = 0.01$) in aggressiveness. Observed and expected means also differed significantly. This was also true when the four mixtures were compared individually to the mean aggressiveness of their component isolates (data not shown). According to the analysis of variance, aggressiveness was significantly ($P = 0.01$) affected by the environments, inoculation treatment (single vs. mixture), the fungal, and rye genotypes (Table 5). Interactions between these factors were often significant, but of small magnitude relative to the main effects.

Table 5. Estimated components of variance for head blight rating and yield components relative to the non-inoculated control for single-isolate and mixed *F. culmorum* inoculations (treatment) tested with different inocula, two rye genotypes and in six environments (df = degrees of freedom)

Source of variation	df	Head blight rating[1]	Relative grain weight per spike	Relative kernel weight
Environment (E)	5	51.77**	239.06**	64.58**
Replicate within E	18	18.65*	5.50**	3.95**
Rye genotype (R)[2]	1	73.44**	44.81**	—[3]
Inoculation treatment (T)[2]	1	14.12**	23.71*	5.45**
Inocula (I) within T[2]	6	21.00**	51.57**	60.08**
R × T[2]	1	1.05**	—	—
R × I within T[2]	6	—[3]	1.44	—
R × E	5	3.61**	6.77**	8.09**
I × E	19	1.35**	1.97*	0.68
T × E	5	4.44**	8.54**	0.59
Error	308	9.94	36.17	22.97

*,**Significant at $P = 0.05$ and 0.01, respectively (F-test).
[1]Multiplied by 100.
[2]Factor with fixed effects (variance component estimate stands for variance of estimated effects).
[3]Negative estimate.

The frequencies of the re-isolates collected in HOH96 did not deviate significantly from a 1 : 1 ratio in two mixtures (FC30 + FC71, FC30 + FC64, Figure 1); however, in the two remaining mixtures the frequency of recovery of isolate FC46 amounted to more than

80% independently from the aggressiveness of the respective mixing partner.

All isolates tested produced moderate to high mycotoxin concentrations when inoculated individually (Table 6). FC46 and FC64 produced DON and 3-ADON only. In plots inoculated with FC30 and FC71, NIV was found, DON and 3-ADON concentrations were similar to non-inoculated plots. FC64 and FC30 produced individually 1.4 times more mycotoxin than FC46 and FC71. The mixed inoculations resulted in the type of mycotoxin profile (DON vs. NIV) that was expected from the single inoculations. In seven out of eleven pairwise comparisons the concentrations, however, were significantly lower in the mixtures than expected from the weighted single-inoculation treatment means. Only with the mixture of FC46 + F64 was more DON found than expected, and in three instances the difference was not significant.

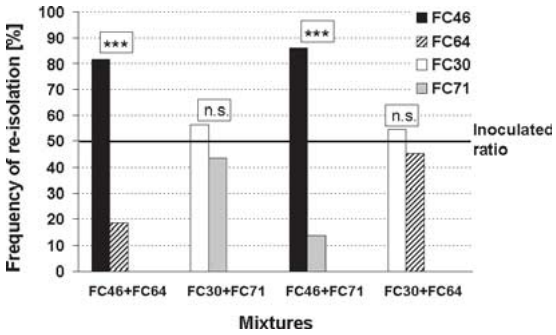

Figure 1. Frequency of re-isolated fungal genotypes from four mixtures of two isolates in HOH96 as determined by molecular fingerprinting; boxes indicate significance of deviation from a 1 : 1 ratio (n.s. = not significant; *** = significant at probability level $P = 0.001$ [χ^2 test]).

Discussion

The four isolates used were genetically distinct according to their RAPD banding patterns and differed with regard to aggressiveness and the type and concentration of mycotoxins produced. This study shows that the aggressiveness of a mixed inoculum may be significantly lower than that of single-isolate inoculations independent of the environment and the resistance level of the host. This was even found when two highly aggressive isolates (FC46 + FC64) were mixed. Furthermore, large differences were observed in the competitive ability of the tested isolates. In both mixtures, FC46 out-competed FC64 and FC71, isolates of high and moderate aggressiveness, respectively.

Competition between plant-pathogenic fungi infecting the same host organ may be due to three factors: competitive exploitation, interference competition (Wicklow, 1981), and parasitic fitness (Reid et al., 1999). Competitive exploitation describes the ability of fungi to use nutrients provided by the host faster than other species occurring on the same substrate without direct interaction of the fungi. An important component

Table 6. Means for head blight rating, concentration of DON, 3-ADON, and NIV in the grain of two winter rye single crosses inoculated with four isolates of *F. culmorum* individually or in four mixtures of two isolates (observed); expected concentrations of the mixtures based on the weighted means of the component isolates according to Figure 1; data from HOH 1996

Isolate(s)	Head blight rating (1–9)[1]	DON (mg kg^{-1})		3-ADON (mg kg^{-1})		NIV (mg kg^{-1})	
		Observed	Expected	Observed	Expected	Observed	Expected
Non-inoculated	1.25	2.3		0.2		0.3	
FC46	6.32 a[2]	49.4	—	12.6	—	nd[3]	—
FC64	6.30 a	71.4	—	15.1	—	nd	—
FC30	4.96 b	1.8	—	0.38	—	47.0	—
FC71	4.63 b	1.8	—	0.28	—	32.1	—
FC46 + FC64	5.52 a	60.8	53.4	10.8	13.1	nd	—
FC30 + FC71	4.09 c	1.8	1.7	0.3	0.3	33.4	40.6
FC46 + FC71	5.25 a	34.4	42.8	6.8	10.9	6.9	4.5
FC64 + FC30	4.83 b	32.0	39.9	6.5	8.3	15.5	21.4
$LSD^4_{5\%}$			4.56		1.05		3.80

[1] 1 = no symptoms visible, 2–9 = ≤5%, 6–15%, 16–25%, 26–45%, 46–65%, 66–85%, 86–95%, and >95%, respectively, of all spikelets visually diseased.
[2] Means with the same letter within a column are not significantly different at $P = 0.01$ (Tukey test).
[3] nd = not detected, i.e. below detection limit.
[4] Least significant difference between observed and expected toxin concentration of the mixtures at $P = 0.05$ (t-test).

is the response of fungi to temperature or water activity of the substrate. Reid et al. (1999) concluded from inoculations of mixtures of isolates of *F. graminearum* and *Fusarium moniliforme* on maize ears that the latter species out-competed the first mainly by better adaptation to a broader temperature range. Interference competition involves a chemical compound that limits the access of the competing fungus to the substrate. The type and concentration of mycotoxins have been discussed as a competitive factor that might be ecologically significant (Cuero et al., 1988; Ramakrishna et al., 1996). Therefore, we mixed DON and NIV chemotypes of *F. culmorum*. The isolates of each chemotype (FC46 vs. FC64 and FC30 vs. FC70) had a similar aggressiveness when inoculated individually. The DON-producing FC46, however, dominated when it was mixed with FC64 although the latter isolate produced 1.4 times more DON in the single-inoculation treatment. FC46 also out-competed the NIV-producing isolate FC71. On the other hand, the DON-producing FC64 was re-isolated in a 1 : 1 ratio when mixed with the less aggressive NIV-producer FC30. The same result was achieved when both NIV-producing isolates were mixed. This illustrates that neither the type of mycotoxin nor the concentration of the mycotoxin, lead to a competitive advantage in two-isolate mixtures. It should be noted that the fungi were re-isolated at the onset of symptom development about 10 days after infection. The competition effects were therefore analysed during the invasion and early colonization of the host, because these early events might be important in determining aggressiveness. Mycotoxins, however, could not be analysed in such early stages but were determined at ripening. The mycotoxin concentrations produced by the different isolates might be different in the early stages of pathogenesis. Their chemotype (DON vs. NIV), however, should not change, because it remained stable even under *in vitro* conditions in *F. culmorum* (Gang et al., 1998). A further indication that the mycotoxins were of minor importance for competition is the fact that in the mixtures lower concentrations were found than expected from the concentrations in the single-isolate inoculation treatment. Similar results were obtained by Reid et al. (1999) when maize ears inoculated with mixtures of *F. graminearum* and *F. moniliforme* were analysed for DON and fumonisin B_1. If the amount of mycotoxin production constitutes a competitive advantage for fungal isolates the opposite would be expected as long as the isolates or species are not degrading the mycotoxin of their competitor.

An important factor of parasitic fitness is aggressiveness, measured in our study as head blight rating and relative reduction of yield components. Among isolates of *F. culmorum,* aggressiveness is a genetically stable trait with high heritability (Miedaner et al., 1996a). The isolates used were selected from an infection experiment across five environments and there was an excellent agreement between the former and the present ranking of aggressiveness. However, aggressiveness did not account for the outstanding competitive ability of FC46, because FC46 was not significantly different from FC64 in the single-inoculation treatment for inducing head blight and reducing relative spike and kernel weights (Table 4). Furthermore, mixing the highly aggressive isolate FC64 with the significantly less aggressive isolate FC30 did not result in a reduced frequency of FC30 in the mixture. These data indicate that isolate FC46 displays fitness characters other than mycotoxin profile or aggressiveness as measured by disease severity. Such characters might involve a faster initial colonization of the host tissue, a broader response to environmental factors and/or secondary metabolites not analysed here, such as other mycotoxins or antibiotics.

The large competition effects obtained with FC46 indicate that it may be more difficult to predict the disease severity level caused by an isolate mixture than a single isolate. For resistance screening purposes with isolate mixtures, competition between isolates should be of minor importance as long as the aggressiveness level of the mixture is high enough and no serious host by isolate interaction occurs. It has been shown previously, that the ranking of wheat and rye genotypes is the same irrespective of the inoculated isolate of *F. culmorum* (van Eeuwijk et al., 1995; Miedaner et al., 1996b). If an extremely high disease severity is required, e.g. in unfavourable environments or for discriminating highly resistant genotypes, the use of single isolates might be advantageous to maximize aggressiveness.

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