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Analysis of the relationship between parameters of resistance to *Fusarium* head blight and *in vitro* tolerance to deoxynivalenol of the winter wheat cultivar WEK0609®

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Abstract

Fusarium head blight (FHB) symptom development, relative spikelet weight (RSW), fungal DNA (FDNA) and deoxynivalenol (DON) content of grain was assessed in the FHB resistant winter wheat cv. WEK0609 and the FHB susceptible cv. Hobbit 'sib', and among doubled haploid progeny lines (DHLs) developed from a cross between these cultivars. In addition, the relationship between FHB resistance traits and germination on DON-containing medium (*in vitro* DON tolerance (IVDT)) was also investigated to assess the possibility of using this test as an *in vitro* method of screening for FHB resistance in this cultivar. Analysis indicated that WEK0609 resistance significantly reduced symptom development, yield loss and the FDNA and DON content of grain relative to Hobbit 'sib'. Although both the DON and FDNA content were greater in susceptible than in resistant progeny lines, the ratio of DON to FDNA decreased with increasing susceptibility. The resistance derived from WEK0609 appears to have a greater effect on colonisation of the grain by the fungus than on the accumulation of DON within the grain. *In vitro* tolerance to DON does not appear to relate to FHB resistance in WEK0609 and thus does not provide a means of selecting for FHB resistance derived from this cultivar.

Introduction

A number of *Fusarium* species cause *Fusarium* head blight (FHB) or scab in wheat (Parry et al., 1995). Each species within the *Fusarium* complex tends to have a preferred climatic range within which, and under conditions favourable to development, it can produce economically significant reductions in yield and seed quality (Dill-Macky, 1996). *Fusarium culmorum*, *F. poae* and *F. avenaceum* generally predominate in cooler maritime regions such as the UK and northern Europe, while *F. graminearum* is the main pathogen in warmer areas including USA, China and Central Europe (Parry et al., 1995). In addition to yield and seed quality losses, FHB is of particular concern because of the potential of many *Fusarium* species to produce toxic secondary metabolites (mycotoxins). Previous studies indicate that trichothecenes, particularly deoxynivalenol (DON), are the most frequently identified mycotoxins in samples of *Fusarium*-contaminated grain worldwide (Placinta et al., 1999). *Fusarium* mycotoxins are potent inhibitors of eukaryotic protein synthesis and have been associated with acute and chronic toxicosis in both humans and animals (Placinta et al., 1999).

Authors of field-based genetic studies have concluded that FHB resistance is generally conditioned by the additive effect of several (Snijders, 1990a) or a few (Van Ginkle et al., 1996) genes of minor effect. For example, recent quantitative trait loci (QTL) analyses of the FHB resistant cv. Sumai-3 suggests that resistance is oligogenic, conditioned by the additive effect of a major QTL on the short arm of chromosome 3B plus a few additional QTL of lesser effect (Anderson et al., 2001; Buerstmayr et al., 2003).

Several types of FHB resistance have been postulated (reviewed in Ban, 2000); resistance to initial infection (type I) (Shroeder and Christensen, 1963), resistance to colonisation (type II) (Shroeder and Christensen, 1963), the ability to degrade DON mycotoxin (type III) (Miller et al., 1985), tolerance to high mycotoxin concentrations (type IV) (Wang and Miller, 1988), resistance to kernel infection (type V) (Mesterhazy, 1995) and tolerance of yield to infection (Mesterhazy et al., 1999). Studies of the relationship between resistance parameters have indicated a medium to high level of correlation between visual disease and DON content in wheat and rye (Miedaner and Perkowsky, 1996; Mesterhazy et al., 1999; Mesterhazy, 2002). More variable levels of correlation have been reported between DON content and the extent of fungal colonisation inferred by quantification of the fungal sterol, ergosterol (Snijders and Krechting, 1992; Miedener and Perkowski, 1996; Miedaner et al., 2001). Indeed variability for DON accumulation and DON/fungal biomass ratio among FHB resistant wheat, rye and maize genotypes has been suggested by several authors as evidence for the existence of host resistance mechanisms that reduce the production of and, or, degrade DON (Snijders and Perkowski, 1990; Miedener and Perkowski, 1996).

The phytotoxicity of DON has been investigated as a means of predicting levels of FHB resistance in wheat. Shimada and Otani (1990) reported that seedlings of FHB resistant cultivars were significantly more tolerant to DON than susceptible cultivars but correspondence between DON tolerance and FHB resistance was inconsistent. Bruins et al. (1993) could find no correspondence between *in vitro* tolerance to DON and FHB resistance in the adult plant. However, significant coefficients of correlation between FHB resistance and DON tolerance were reported by Lemmens et al. (1994) using an *in vitro* technique termed the 'Petritox' test which monitors the rate of germination in the presence of DON or *Fusarium* culture filtrate.

The aim of the current study was to analyse the FHB resistance of the north American winter wheat cultivar WEK0609 and determine the relationship between individual parameters using a population of doubled haploid lines (DHLs) developed from a cross between Hobbit 'sib' and WEK0609. Visual disease (area under the disease progress curve, AUDPC), relative spikelet weight (RSW), fungal DNA (FDNA) and DON content of grain was assessed for each DHL in 2 years. In addition, tolerance to DON was assessed using the 'Petritox' test and the results were compared to those for the FHB resistance traits.

Materials and methods

Plant materials

The source of FHB resistance used was WEK0609, a winter wheat cultivar supplied by Pioneer Hi-Bred International Inc. Previous field and controlled environment trials had indicated that WEK0609 possesses both type I (reduced incidence of infection) and type II (reduced spread within the spike) resistance (*sensu* Schroeder and Christensen, 1963). The FHB susceptible cultivar was Hobbit 'sib' which has been reported to have no appreciable resistance to head blight (Buerstmayr et al., 1999). A doubled haploid (DH) population of 53 lines was created from a cross between Hobbit 'sib' and WEK0609 using a method based upon that of Laurie and Reymonde (1991).

The DH lines and the parent cultivars were phenotyped in two experiments during the summer of 1999 and 2000. Seeds were sown in 24 cell trays (Plantpak™ Cookson Plantpak Ltd.) of a peat and sand mix in October and vernalised in an unheated glasshouse. Seedlings received natural vernalisation throughout. In February, seedlings were re-potted singly into 1 pots of John Innes compost No. 2 (pH adjusted to 8.0) and transferred to an unheated polytunnel with side ventilation (National Polytunnels Preston UK). Water was supplied via a ground based irrigation system. Non-target fungal pathogens were controlled during the seedling and pre-ear emergence stages using the commercial fungicides 'Fortress' ai. Quinoxifen (Bayer) and 'Tern' ai. Morpholine (Ciba Agriculture) neither of which have activity against *F. culmorum*.

Origin and production of fungal inoculum

A highly aggressive DON-producing isolate of *F. culmorum* (Fu42) was cultured on potato dextrose agar (PDA) (Difco) and incubated at 20 °C. Conidia were washed from plates using sterile water and the titre was estimated using a

haemocytometer. Inoculum was stored at −20 °C and amended by addition of 0.05% Tween 20 prior to inoculation.

Head blight resistance

Two separate trials were carried out using a randomised complete block design with 16 plants per genotype arranged in four blocks of four plants. Two ears per plant were inoculated to run-off at mid-anthesis (growth stage (GS) 59 (Zadoks et al., 1974)) with conidial suspension (1×10^5 conidia ml^{-1}) using a hand-held pump action sprayer. Ears were covered with a cellophane 'crossing' bag for 72 h to maintain high humidity. A third ear on each inoculated plant was sprayed with 5 ml of sterile water amended with 0.05% Tween 20 and covered with a cellophane bag for use in the calculation of relative spikelet weight (RSW). Disease was measured as the percentage of diseased spikelets per ear (more than one floret affected) 7, 14, 21 and 28 days post-inoculation and expressed as AUDPC. Ears from inoculated and control plants were harvested at GS 90 freeze-dried, weighed and spikelets counted to determine RSW of inoculated ears compared to those of non-inoculated control spikes on the same plant.

DNA extraction and quantitative PCR

For each genotype, grain from inoculated ears of the four plants within each block was bulked for DNA extraction. Grain was weighed and ground to a fine powder in a ball mill (Glen Creston Ltd Stanmore; mixer/mill 8000). DNA was extracted and standardised for PCR (Nicholson et al., 1996). DNA was quantified in a Titretec Fluoroscan II plate reader (INC Biomedicals Ltd UK) using SYBR Green (Hopwood et al., 1997). DNA concentration was assessed by comparison with a standard curve generated from a dilution series (0–1.0 ng μl^{-1}) of DNA (*Hind* III cut λ DNA) that was run on each plate. Quantitative PCR analysis using *F. culmorum*-specific primers was carried out in volumes of 50 µl (Nicholson et al., 1998). Following amplification, the PCR products were separated by electrophoresis through 2% agarose gel stained with ethidium bromide and viewed under UV light on a 'Gel Doc 1000' system (Bio-Rad UK). Gels were analysed using Molecular Analyst software (Bio-Rad UK). The amount of fungal DNA in each sample was determined by reference to a standard curve. Quantitative PCR assays were repeated at least twice and the amount of fungal DNA was expressed as a percentage of the total DNA content of the sample.

Deoxynivalenol (DON) content

In order to avoid underestimating DON levels, samples were hand-harvested from inoculated spikes and manually threshed and winnowed to ensure retention of small highly infected kernels which can contain a high concentration of mycotoxin (Sinha and Savard, 1997). DON was extracted from milled samples of grain using 10% methanol (10 ml g^{-1}). Tubes were agitated vigorously for 3 min in an orbital shaker set at maximum speed and centrifuged at 2600 g for 10 min. The supernatant was removed and stored at −20 °C prior to analysis. A 1:40 (DH trial 1) or 1:60 (DH trial 2) dilution in 10% methanol was made from each sample and 50 µl aliquots assessed for DON content using the Ridascreen® Fast DON™ (R-Biopharm Rhône Ltd.) enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions. Absorbance was measured at 450 nm using a Wallec 400 plate reader and the sample data converted to DON concentration by reference to a standard curve generated from DON standards provided in the kit.

DON media preparation and in vitro *selection for mycotoxin resistance*

The assessment of DON tolerance was based on the 'Petritox test' of Lemmens et al. (1994) with the following modifications. DON medium was prepared according to Lemmens et al. (1994) except that DON (Sigma-Aldrich) alone was added to a final concentration of 7 ppm. Seeds of each line (120) were used at a rate of 20 seeds per replicate on a 90 mm Petri-dish with three replicates exposed to DON and three used as controls. The degree of germination retardation was expressed as the area under the germination response curve (AUGRC) calculated from seven measurements made over 10 days. The AUGRC of seed exposed to DON was expressed as a percentage of the AUGRC of controls.

Statistical analysis

In all cases, results from competitive PCR analysis were subjected to a logarithmic transformation prior to analysis due to the non-independence of mean and variance. Analysis of variance (ANOVA) was carried out to assess variability attributable to genotype and PCR replicates for competitive PCR data and to partition the variance attributable to genotype, replicates, disease severity and environment for visual score (AUDPC) and RSW data. Statistical analysis for cultivar differences was carried out by one-way analysis of variance incorporating Dunnett's intervals for treatment means tests (Mead et al., 1994). Heritability was calculated according to the method quoted in Gervais et al. (2003), in which, over the two experimental years, heritability was estimated from the ANOVA using the formula: $h^2 = \sigma^2_G/[\sigma^2_G+(\sigma^2_{GE}/E)+ (\sigma^2_e/rE)]$, and within years, using the formula: $h^2 = \sigma^2_G/[\sigma^2_G + (\sigma^2_e/r)]$, with σ^2_G is the genetic variance; σ^2_{GE} the genotype × environment interaction variance; σ^2_e the residual variance; E the number of environments; r the number of replicates per line. All analyses were carried out using Minitab release 12 (1994 Minitab Incorporated USA).

Results

Fusarium *head blight symptoms*

In 1999, the average incubation period was 4 days for Hobbit 'sib' and 10 days for WEK0609. Disease progress was slower in 2000 and the average incubation period was 7 days for Hobbit 'sib' and 2 weeks for WEK0609. Over both years WEK0609 had significantly ($P < 0.001$) lower mean AUDPC, DON and FDNA content compared to Hobbit 'sib' with no overlap between the trait ranges (Table 1). WEK0609 also had a significantly higher RSW and *in vitro* DON tolerance than Hobbit 'sib' in both years. However, WEK0609 had a significantly ($P \leq 0.001$) higher DON content/FDNA ratio than Hobbit 'sib' in both 1999 and 2000 (Table 1).

The mean disease levels among DHLs was significantly greater ($P \leq 0.001$) in 1999 than in 2000 while the RSW was significantly ($P \leq 0.001$) lower in the first year (Figure 1). However, the mean level of DON was similar in both years ($P \geq 0.05$) (Figure 1). Comparison of absolute FDNA levels between years was not possible due to the use of different batches of competitor template in the 2 years.

Table 1. Means and ranges of visual score, relative spikelet weight, deoxynivalenol (DON); content of seed, fungal DNA content of seed and *in vitro* tolerance to DON of the parental cultivars WEK0609 and Hobbit 'sib' in 1999 and 2000

Genotype	Trait[a]	1999		2000	
		Mean	Range[b]	Mean	Range
Hobbit 'sib'	AUDPC	1706.19	1229.41–2215.14	1569.64	1139.79–2663.58
WEK0609	AUDPC	419.08	121.17–759.24	157.15	74.74–273.67
Hobbit 'sib'	RSW	40.54	22.67–57.59	55.70	41.28–85.07
WEK0609	RSW	66.48	45.18–91.88	89.36	60.89–107.69
Hobbit 'sib'	DON	188.45	151.93–229.66	203.43	159.42–238.38
WEK0609	DON	81.19	44.98–128.64	73.74	44.90–113.35
Hobbit 'sib'	FDNA	16.07	9.95–18.69	35.75	30.93–44.92
WEK0609	FDNA	2.42	1.84–3.89	2.68	1.60–3.49
Hobbit 'sib'	D/FDNA	14.47	9.65–23.83	5.92	3.54–7.59
WEK0609	D/FDNA	59.46	44.3–75.55	29.06	13.74–48.74
Hobbit 'sib'	DON tolerance	19.92	19.12–20.56	21.77	17.05–25.04
WEK0609	DON tolerance	51.95	45.80–57.58	47.28	45.79 – 49.35

[a] Traits: AUDPC – visual score as area under the disease progress curve; RSW – relative spikelet weight; DON – deoxynivalenol (DON) content of grain (ng mg^{-1}); Fungal DNA – percent fungal DNA in grain; D/FDNA – DON content/fungal DNA ratio; DON tolerance – area under the germination response curve assessed using the 'Petritox' test of; Lemmens et al. (1994).
[b] Range – Range of replicate mean.

The distributions for most traits, except for DON content of grain, were skewed in at least one year (Figure 1). In 1999 AUDPC values were compressed, relative to values in 2000, while in 2000 the distribution was skewed towards lower values; this was mirrored by the RSW distributions in which the distribution was skewed towards lower values in 1999 while in 2000 the distribution and mean of RSW values was greater. The distributions for FDNA were similar to those for RSW in both years. ANOVA incorporating Dunnett's intervals for treatment means (DITM) tests (at 5% significance), with the parents as standards, indicated that there was no significant transgressive segregation for any of the resistance traits in either year (Figure 1, Table 1). Heading among the DHLs occurred over 10 and 11 days in 1999 and 2000, respectively. There was no significant correlation between any of the FHB traits and heading date in either 1999 or 2000. ANOVA indicated that over both years genotypic variation was significant ($P < 0.001$) for all resistance traits (Table 2). Genotype by year interactions were also significant for all traits but were considerably lower than the corresponding genotypic variances. Over years, the heritability estimate was medium to high for all the resistance traits.

Correlation between years was high for AUDPC ($P < 0.001$; $r = 0.63$), moderate for DON and fungal DNA content ($P < 0.01$; $r = 0.51$ and 0.42, respectively) and low for RSW and DON content/FDNA ratio ($P < 0.05$; $r = 0.38$ and 0.37, respectively). The pattern of correlation among traits was very similar across years (Table 3). In 1999, AUDPC had a significant negative relationship with RSW and a significant positive relationship with DON and FDNA content. The order of coefficient significance was

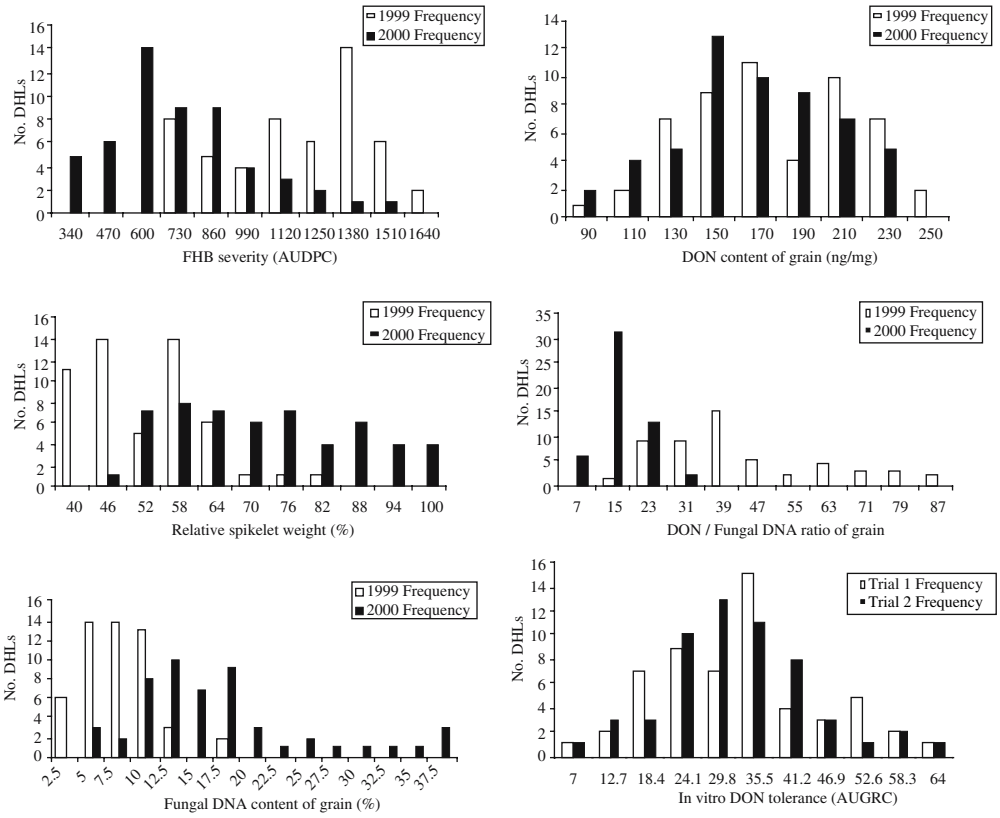

Figure 1. Frequency distribution of visual score (area under the disease progress curve (AUDPC)), spikelet weight relative to non-inoculated control heads on the same plant (%), fungal DNA content of grain as a percentage of total DNA, deoxynivalenol (DON) content of grain (ng mg^{-1} sample dry weight), DON content/fungal DNA ratio of grain and *in vitro* DON tolerance (area under the germination response curve (AUGRC)) assessed using the 'Petritox' test of Lemmens et al. (1994).

Table 2. *F* values and heritability from the analysis of variance (ANOVA) for FHB resistance traits and *in vitro* tolerance to deoxynivalenol (DON) of 53 doubled haploid progeny lines from a cross between Hobbit 'sib' and WEK0609 over 2 years

Trait[a]	Genotype (G)	G × Year	h^2
AUDPC	27.16***	6.14***	0.79
RSW	18.70***	9.59***	0.63
DON	8.54***	3.91***	0.76
Fungal DNA	6.48***	2.49***	0.65
D/FDNA	4.64***	2.18***	0.59
DON tolerance[b]	6.65***	1.28	0.74

*** $P < 0.001$.
[a] Traits: AUDPC – visual score as area under the disease progress curve; RSW – relative spikelet weight; DON – deoxynivalenol (DON) content of grain (ng mg^{-1}); Fungal DNA – percent fungal DNA of grain; D/FDNA – DON content/fungal DNA ratio; DON tolerance – assessed using the 'Petritox' test of Lemmens et al. (1994).
[b] Variance components for genotype by experiment, not genotype by year.

Table 3. Correlation analysis of FHB resistance traits[a] and *in vitro* tolerance to deoxynivalenol (DON) in 53 doubled haploid progeny lines from a cross between Hobbit 'sib' and WEK0609 over 2 years

Correlation	1999	2000
AUDPC vs. RSW	−0.60**	−0.69***
AUDPC vs. FDNA	0.56**	0.73***
AUDPC vs. DON	0.78***	0.76***
AUDPC vs. D/FDNA	−0.32	−0.54**
AUDPC vs. DON tolerance	−0.01	−0.14
RSW vs. FDNA	−0.38	−0.45
RSW vs. DON	−0.66**	−0.53**
RSW vs. D/FDNA	0.20	0.31
RSW vs. DON tolerance	0.26	0.03
FDNA vs. DON	0.54**	0.82***
FDNA vs. D/FDNA	−0.86***	−0.90***
FDNA vs. DON tolerance	−0.04	−0.15
DON vs. D/FDNA	−0.24	−0.48
DON vs. DON tolerance	−0.06	−0.15
D/FDNA vs. DON tolerance	−0.01	−0.10

** $P < 0.01$; *** $P < 0.001$.
[a] Traits: AUDPC – visual score as area under the disease progress curve; RSW – relative spikelet weight; DON – deoxynivalenol (DON) content of grain (ng mg^{-1}); Fungal DNA – percent fungal DNA of grain; D/FDNA – DON content/fungal DNA ratio; DON tolerance – assessed using the 'Petritox' test of Lemmens et al. (1994).

DON ($r = 0.78$) then RSW ($r = -0.60$) and FDNA ($r = 0.56$). DON content was significantly correlated with RSW ($r = -0.66$) and FDNA ($r = 0.54$) while DON/FDNA ratio was significantly correlated with FDNA ($r = -0.86$). The relationships between DON/FDNA ratio, AUDPC, RSW and DON content were non significant. In 2000, AUDPC had a significant relationship with RSW, DON, FDNA and DON/FDNA ratio (Table 3). The order of coefficient significance was DON ($r = 0.76$), FDNA ($r = 0.73$) and RSW ($r = -0.69$). In 2000, AUDPC also had a significant negative relationship with DON/FDNA ratio ($r = 0.54$). Again RSW correlated significantly with DON ($r = 0.53$) but not with FDNA or DON/FDNA ratio. FDNA had a significant positive correlation with DON content ($r = 0.82$) and a significant negative relationship with DON/FDNA ratio ($r = -0.9$).

Plots of the trait relationships indicated that DHLs with the lowest levels of symptom development also tended to have the lowest levels of fungal DNA and DON in grain and a linear trend line provided the best fit for the relationship between the traits (Figure 2). In general, as susceptibility increased, so did the FDNA and DON content of

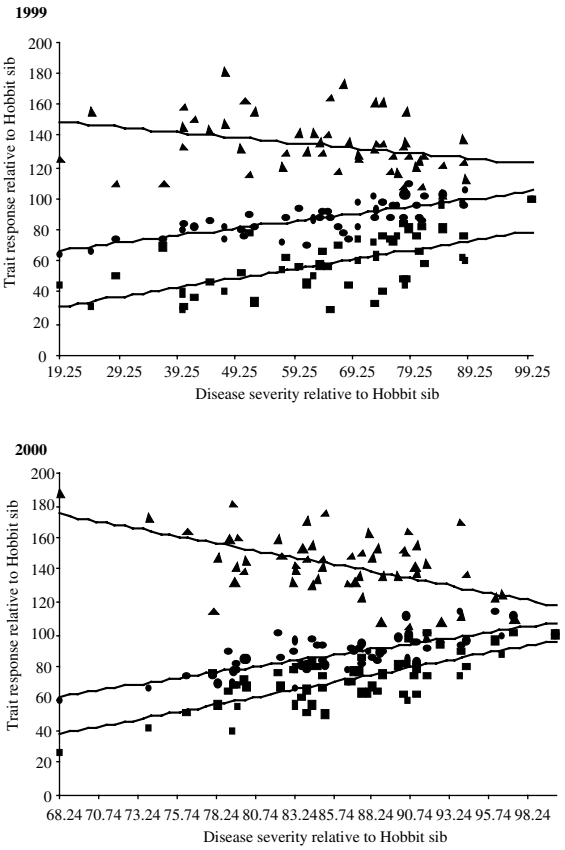

Figure 2. DON content of grain (●), fungal DNA content of grain (■) and DON/fungal DNA ratio (▲) plotted against disease severity (area under the disease progress curve (AUDPC)) in 1999 and 2000. All values are relative to Hobbit 'sib' (%). In 1999, the R^2 values of the linear relationship between disease severity and DON, FDNA and DON/FDNA ratio were, 0.61, 0.36 and 0.10, respectively. In 2000, the R^2 values of the linear relationship between disease severity and DON, FDNA and DON/FDNA ratio were 0.57, 0.53 and 0.29.

the grain. However, DON/FDNA ratio decreased as susceptibility increased, being more pronounced in 2000 when the disease pressure was moderate. The R^2 value of the relationship between DON content and disease severity was consistently high over both years. In contrast, the R^2 of the relationship between disease symptoms and FDNA varied over years and that between symptoms and DON/FDNA ratio was consistently low.

In vitro *DON tolerance*

Two independent assessments of DON tolerance were made on seed from DHLs harvested in 1999 (trials 1 and 2). Average AUGRC was lower than the mid-parent value in both trials (Table 1 and Figure 1). ANOVA indicated that genotypic variance was highly significant ($P < 0.001$) and genotype by trial interactions were non significant (Table 2). In addition, the coefficient of correlation across experiments was high ($P < 0.001$; $r = 0.68$) indicating that the levels of DON tolerance among DHLs were highly reproducible. Over the two experimental replicates, the heritability estimate was high (0.74) and comparable with that of AUDPC and DON content of grain (Table 2). The AUGRC was averaged over trials 1 and 2 and used in correlation analyses to assess the relationship between toxin tolerance and FHB resistance in 1999 and 2000. None of the FHB resistance traits correlated significantly in either year with DON tolerance (AUGRC) (Table 3). Phenotypic frequency distributions for DON tolerance were continuous in both trials (Figure 1 and Table 1). ANOVA incorporating the Dunnett's intervals for treatment means test with the parents as standards indicated that significant ($P < 0.05$) low transgressive segregation was present in both trials.

Discussion

Parameters of FHB resistance (AUDPC, RSW, DON and FDNA) of WEK0609 were investigated in a doubled haploid population following spray inoculation of individual spikes at the same physiological stage (mid-anthesis). The study of multiple parameters was undertaken in order to improve phenotypic differentiation among progeny and provide insight into the characteristics of the FHB resistance of WEK0609. This analysis revealed that WEK0609 resistance significantly reduced symptom development (AUDPC), yield loss (RSW), fungal colonisation (FDNA) and DON content of grain relative to the susceptible parent, Hobbit 'sib' (Table 1). However, the ratio of DON content to FDNA was significantly greater in WEK0609.

Symptom development (AUDPC) among DHLs was more significantly correlated with DON in both years and with fungal biomass (FDNA) in 2000 than with yield reduction (Table 3). Similarly, Snijders and Krechting (1992) reported that symptom development was closely

related to the amount of fungal biomass and DON accumulation in wheat kernels. These findings contrast with those from a study of winter rye single cross hybrids (Miedaner and Perkowski, 1996). These workers reported that visual disease and relative grain weight often did not reflect levels of DON in grain. They concluded that, in FHB caused by *F. culmorum*, DON accumulation in grain might be affected by a different set of environmental factors than those that influence symptom development and fungal colonisation. Overall these results may reflect differences in the relationship between disease symptoms, fungal colonisation and DON accumulation in wheat and rye.

While grain of more susceptible DHLs contained more DON and FDNA than more resistant DHLs, the amount of DON per unit of fungus (DON/FDNA ratio) decreased with increasing susceptibility (Figure 2). In addition, a striking relationship was observed between the FDNA content of grain and the DON/FDNA ratio in which higher levels of colonisation were strongly associated with a reduced level of DON per unit of fungal DNA (Table 3). These results contrast with those of Miller et al. (1985), who found that FHB resistant cultivars of wheat, rye and triticale accumulated less DON per unit of fungal biomass than susceptible cultivars. However, the mycotoxin to fungal biomass ratio is thought to be dependent upon the host genotype and similar amounts of fungal biomass may be associated with different mycotoxin contents or *vice-versa* (Miedaner and Perkowski, 1996). The present study involves a population of DHLs rather than a collection of unrelated cultivars. These findings may reflect an intrinsic and specific aspect of the FHB resistance of WEK0609 compared to that of other cultivars but the mechanisms responsible for this phenomenon are not known. Membrane-associated inhibition of translocation of DON into the grain has been hypothesised to account for such relationships in some cultivars (Snijders and Krechting, 1992), while it is suggested that, in other cultivars, the effect is due to factors that prevent synthesis or promote degradation of DON (Miller et al., 1985). Further studies are required to elucidate whether such factors are present in WEK0609.

There was considerable phenotypic variation among DHLs for visible disease severity and for RSW, FDNA and DON content. Although ANOVA indicated that much of the variance was due to genotype, there was also a large environment ($G \times E$) interaction component (Table 2). This was reflected by inter-annual coefficients of correlation that were significant for all traits. However symptom development (AUDPC) and DON content were more reproducible over years than RSW, FDNA and DON/FDNA ratio. Large $G \times E$ interaction variance has been reported by numerous authors of field-based studies of wheat and rye (Mesterhazy et al., 1999; Miedaner et al., 2001; Miedaner and Perkowski, 1996). It is widely acknowledged that there is an interaction between physiological spike maturity and FHB susceptibility (Miedaner, 1997; Miedaner et al., 2001). In the present study all ears were individually inoculated at mid-anthesis to minimise this effect, and no significant interaction was observed.

Phenotypic frequency distributions of DHLs were continuous for all traits over both years supporting a quantitative model of inheritance for FHB resistance (Figure 1). In the present study no significant transgressive segregation was found for any of the traits over the 2 years indicating that all resistance to FHB in this population was derived from WEK0609, in agreement with previous reports that found Hobbit 'sib' to be highly susceptible to FHB (Buerstmayr et al., 1999). Other studies of wheat using recombinant inbred populations derived from Frontana and Sumai-3 have reported high and low transgression suggesting that the susceptible parent used in each case may have contributed some unique resistance genes (Van Ginkel et al., 1996; Waldron et al., 1999).

Frequency distributions for visual disease and RSW in 1999 and DON/FDNA ratio in 1999 and 2000 were skewed towards the susceptible parent (Table 1 and Figure 1). This probably reflects the combination of moderate resistance of many DHLs and differing disease pressures between years. It has been reported that moderate resistance to FHB is heavily influenced by the environment and results in an unstable reaction over years (Mesterhazy, 1995; Mesterhazy et al., 1999). The apparent lower average FDNA in 1999 compared to 2000 (Figure 1) is probably due to differences in absolute concentration of the competitor DNA stocks used in the two years and prevents direct comparison of FDNA for the two trials.

The heritability of *in vitro* DON tolerance (AUGRC) within the WEK0609/Hobbit 'sib' DH population was investigated using the 'Petritox' test (Lemmens et al., 1994). Whilst heritability for DON tolerance was comparable with that for visual symptoms (Table 2), correlation analysis revealed no significant relationship with any of the FHB resistance traits in either 1999 or 2000 (Table 3). These results contrast with those of field-based tests in which moderate correlation between visual disease (Lemmens et al., 1994), kernel DON accumulation (Lemmens et al., 1997) and 'Petritox' DON tolerance was observed. In addition, Wang and Miller (1988) reported that coleoptile segments of FHB-resistant cultivars were 10–100 times more tolerant to DON than those of FHB susceptible cultivars. However, experiments by Bruins et al. (1993) and Snijders (1990b) indicated that the seedling response to DON was unrelated to FHB resistance in the adult plant. Recently Ruckenbauer et al. (2001) referred to results of unpublished studies that also failed to find significant correlations between FHB symptom development and DON tolerance using the Petritox test.

The lack of evidence for a link between FHB resistance and DON sensitivity found in the current study does not necessarily demonstrate that toxin resistance has no role in FHB resistance in WEK0609. It may indicate that DON tolerance plays only a minor role in FHB resistance in WEK0609, or that DON tolerance may be tissue specific. However, it is also conceivable that the Petritox test does not measure DON tolerance. The lack of correlation between FHB resistance and *in vitro* DON tolerance, coupled to that of previous unpublished reports of similar findings with other germplasm (Ruckenbauer et al., 2001), indicates that alternative tests may be required to investigate the potential role of mycotoxin tolerance in FHB resistance.

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