European Journal of Plant Pathology **110:** 299–308, 2004.

The effect of inoculation treatment and long-term application of moisture on Fusarium head blight symptoms and deoxynivalenol contamination in wheat grains

Marc Lemmens[1], Hermann Buerstmayr[1], Rudolf Krska[2], Rainer Schuhmacher[2], Heinrich Grausgruber[3] and Peter Ruckenbauer[1]
[1]*Department of Plant Production Biotechnology, Institute for Agrobiotechnology, Konrad Lorenz Street 20, A-3430 Tulln, Austria (Fax: 43 227266280 203; E-mail: lemmens@ifa-tulln.ac.at);* [2]*Center for Analytical Chemistry, Institute for Agrobiotechnology, Konrad Lorenz Street 20, A-3430 Tulln, Austria;* [3]*Department of Plant Breeding, University of Natural Resources and Applied Life Sciences, Vienna, Gregor Mendel Street 33, Vienna, Austria*

Accepted 20 November 2003

Key words: artificial inoculation, *Fusarium culmorum*, humidity, mycotoxin, scab, *Triticum aestivum*

Abstract

Fusarium head blight (FHB) is an important disease of wheat, which can result in the contamination of grains with mycotoxins such as deoxynivalenol (DON). Artificial inoculation of flowering ears with conidial suspensions is widely used to study FHB diseases. Our goal was to compare four inoculation treatments in which a conidial suspension was sprayed on flowering ears and to study the effect of the application of moisture during kernel setting and filling with a mist-irrigation system. Ten wheat genotypes were inoculated with a DON-producing *Fusarium culmorum* strain. Inoculation treatments varied in time of application of the inoculum (morning or evening) and in the method of controlling humidity during inoculation (bagging or mist irrigation). A wet season was simulated with a mist-irrigation system, keeping the crop canopy wet for at least 26 days after flowering. The severity of FHB symptoms (area under disease progress curve (AUDPC)), yield loss and DON contamination in the grains were determined. AUDPC data obtained with the different inoculation treatments were highly correlated ($r = 0.85$–0.95). Mist irrigation after inoculation resulted in a higher mean disease severity, but in a overall lower toxin contamination as compared to the non-irrigated treatments. Genotypic differences in DON accumulation were present: for one wheat line toxin contamination significantly increased when irrigated, while two genotypes accumulated significantly less toxin. The closest relationships ($r = 0.73$–0.89) between the visual symptoms and the DON content were obtained under moderate mean infection pressure. This relation between visual symptoms and the DON content deteriorated at higher infection levels.

Abbreviations: AUDPC: area under the disease progress curve; DON: deoxynivalenol; FDK: *Fusarium*-damaged kernels; FHB: Fusarium head blight; G_g: genotype g; I_i: inoculation treatment i; M_m: mist-irrigation treatment m; REW: relative ear weight.

Introduction

Fusarium head blight (FHB), or scab, is an important disease of small grain cereals including bread wheat (*Triticum aestivum*). Several *Fusarium* species cause this disease, the most important of which are *F. graminearum* (teleomorph *Gibberella zeae*), *F. culmorum* (teleomorph unknown) and *F. avenaceum* (teleomorph *Gibberella avenacea*) (Parry et al., 1995). At least 12 additional *Fusarium* spp., including *F. poae*, have been reported on wheat ears, but they are generally regarded as less important

(Sutton, 1982; Parry et al., 1995). FHB has negative effects on both grain yield and quality (Bai and Shaner, 1994). The most disquieting factor is the contamination of the grains with mycotoxins such as trichothecenes (e.g. deoxynivalenol (DON)) and zearalenone (Bottalico, 1998).

An integrated approach including agronomic techniques (crop rotation, tillage), chemical control and the growth of resistant cultivars is probably the most effective approach to control this disease. Resistant cultivars are, from both economical and environmental points of view, the best solution for this problem. Large variations in FHB resistance in hexaploid wheat have been reported (Snijders, 1990; Buerstmayr et al., 1996). Introgression of FHB resistance into commercial cultivars is tedious because of the oligogenic to polygenic nature of the resistance trait and the laborious resistance screening methods used in the field.

Several artificial inoculation methods have been reported (Bekele, 1985; Liu, 1985). Specific inoculation procedures to evaluate fungal spread in the ear (Type II resistance) involve the introduction of inoculum into a central spikelet of an ear at early anthesis (Schroeder and Christensen, 1963; Bai and Shaner, 1996). Inoculation methods include a hypodermic syringe, a micropipette or a small tuft of cotton wool soaked in inoculum. These techniques have proved to be reliable (Wang and Miller, 1988b), but are labour-intensive, and important resistance mechanisms contributing to field resistance might not be detected. Inoculation by spreading colonized kernels on the soil surface or by spraying spore suspensions on flowering heads are other commonly used artificial inoculation techniques (Wang and Miller, 1988a; Miedaner, 1997; Mesterházy et al., 1999; Aufhammer et al., 2000). Such methods are suitable for mass screening (Walsh et al., 1998) and approach better the natural infection conditions. However, natural infection processes are dependent on the prevailing environmental conditions. One important environmental factor is the presence of sufficient moisture to promote infection. In general, humidity is controlled by irrigation (Snijders and Perkowski, 1990), bagging of inoculated ears (Mesterházy et al., 1999) or simply by relying on the presence of high relative air humidity or dew building under natural conditions.

Factors triggering, regulating or influencing mycotoxin synthesis and accumulation in the infected host are not well understood and the final toxin concentration in the kernels is probably a result of complex interactions between host, pathogen and environment. This lack of knowledge can lead to unexpected and controversial results. For example, after the application of fungicides a reduction in the amount of visual symptoms was not always correlated with a simultaneous reduction in mycotoxin contamination (D'Mello et al., 1998). It has been shown that moisture during wheat flowering promotes FHB infection (Lacey et al., 1999), but no information is available on the effect of a wet season on disease development and toxin contamination of the grains.

The first aim of the present work was to compare four frequently used artificial inoculation treatments in which a *Fusarium* suspension was sprayed on the ears. The winter wheat lines under investigation were inoculated at 50% anthesis. The inoculation treatments differed in time of application of the inoculum (morning or evening) and in the method of controlling humidity during inoculation (bagging or a computer-controlled mist-irrigation system). The second aim was to investigate the influence of a wet season on FHB. For this purpose, the genotypes under investigation were kept wet with a mist-irrigation system after flowering for an additional 26 days. The development of the disease was assessed in the field. Yield loss and DON contamination of the grains were investigated.

Materials and methods

Locations and wheat lines

The experiments were performed in the seasons 1994 and 1995. In the first season the experiment was in Grossenzersdorf (Location 1) in the pannonic climatic region about 15 km to the east of Vienna (48°12′N, 16°33′E; 9.8 °C mean annual temperature, 572 mm annual rainfall). In 1995, the experiment was repeated in Tulln (Location 2), which is located along the Danube 30 km to the west of the Austrian capital (48°20′N, 16°4′E; 9.2 °C mean annual temperature, 620 mm annual rainfall). Temperature and rainfall during the flowering period of the wheat lines and during the mist-irrigation period at both locations were recorded with a weather station located in the experiment. The data for mean daily temperature and daily rainfall are summarized in Figure 1.

A collection of 10 winter wheat genotypes (*T. aestivum*) was investigated. The lines, described by donor and country of origin, are summarized in Table 1. The seed was treated with bitertanol (Sibutol®) (Bayer, Leverkusen, Germany) at a rate of 2 ml kg^{-1} seed

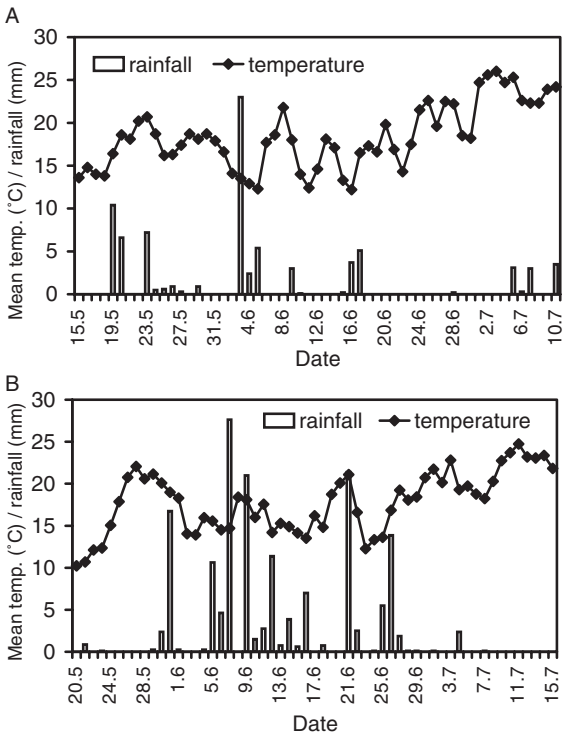

Figure 1. Climatic conditions during the flowering and mist-irrigation period of wheat in the location 'Grossenzersdorf, 1994' (A) and in the location 'Tulln, 1995' (B). In Grossenzersdorf and Tulln the flowering period lasted from May 25 to June 10 and from May 31 to June 16, respectively. In both locations mist irrigation continued for 26 days after the end of the flowering period.

to control seed-borne diseases. The wheat genotypes were sown in 1 m^2 plots in two replicates (each with two different sowing dates, 2 weeks apart, mid- to end-October). In early spring, fertilizer was applied at a rate of (in kg ha^{-1}) 90 N, 14 P, 23 K and 12 Mg respectively at the start of tillering (Zadoks growth stages 20–22) (Zadoks et al., 1974). Weeds were controlled by spraying 15 g ha^{-1} tribenuron (Express®) (Du Pont, Cernay, France) and 0.5 l ha^{-1} fluroxypyr (Starane™) (DowElanco, King's Lynn, UK) in 300 l water ha^{-1} at the late-tillering stage.

Inoculum production

The *F. culmorum* strain used produces DON in large amounts after infection of wheat ears. It was maintained in soil cultures for stable long-term storage (Smith and Onions, 1994). To produce macroconidia with this strain, a mixture of wheat and oat grain (3 : 1 by volume) was soaked in water overnight. The surplus of liquid was decanted and the seeds were autoclaved. After adding the *Fusarium* strain, the seeds were kept for 2 weeks at 25 °C. Subsequently the colonized grain was incubated in the dark at 5 °C for 3 weeks (Snijders and Perkowski, 1990). Storage of the grains occurred under the same conditions.

Before use, conidia were washed-off the seeds with sterile distilled water. The macroconidial concentration was determined with a Buerker–Tuerk haemacytometer. De-ionized water was used to adjust the suspensions to the desired final concentration of 10^5 macroconidia ml^{-1}. The aggressiveness of the inoculum was monitored with a Petridish infection test at the beginning and at the end of the inoculation period as described by Lemmens et al. (1993). This test showed that the aggressiveness of the inocula remained constant during the inoculation period (data not shown).

Table 1. Wheat genotypes described by name, donor institution and country of origin

Wheat genotype	Donorand country of origin
Zombor Szöke	Cereal Research Institute, Szeged, Hungary
204/81/03	State Plant Breeding Institute, Hohenheim, Germany
SVP7200520301	Centre for Plant Breeding and Reproduction Research, Wageningen, the Netherlands
Arina	EFAP Zürich, Switzerland
Copain	Ets. C. Benoist, France
RC 103 (Renan) 81 F3 79	Institut Nationale de Recherche Agronomique, Rennes, France
NR-172/90	Saatzucht Neuhof/Rohrau, Austria
SL 8/80-28 (Justus)	Saatbau Linz, Austria

Inoculation treatments

Inoculations were performed every second day during the flowering period according to the watering regimes described below (see water supply). The inoculation period in Grossenzersdorf and Tulln extended from May 25 to June 10 and from May 31 to June 16, respectively. Treatments of the ears were performed on each plot at 50% anthesis by spraying the inoculum directly onto the wheat heads. The following four inoculation treatments (I) were used:

I_1: inoculation was done in the morning. Separate bunches of about 25 ears were inoculated with

20 ml of the *Fusarium* suspension. The bunches were subsequently covered with a transparent polyethylene bag for about 24 h.

I_2: similar to I_1, but inoculations in the late afternoon or evening and the plastic bags remained on the bunches for about 18 h.

I_3: inoculation in the evening. Separate bunches of about 25 ears were inoculated with 20 ml of the *Fusarium* suspension. No bags used.

I_4: similar to I_3 but inoculation repeated 2 days later.

Water supply

The experiment was sown in two separate blocks, each with two replicates. One block was not irrigated (M_0). The other block was used to simulate a wet period during and after inoculation (M_1) and was treated as follows: an automated mist-irrigation system was used every other day (inoculation days) to apply moisture. An irrigation cycle started at 16.00 h and continued until the next day at 12.00 h (=1 cycle). Water was applied in two pulses of 10 s each at 15 min intervals. The mist-irrigation system was switched on a few days before flowering of the earliest genotype and the application of water continued every second day until the last visible assessment of the disease of the latest genotype in the experiment. In both locations the inoculation period extended for 16 days and watering continued for another 26 days after flowering of the genotypes.

Disease evaluation methods

Disease symptoms were assessed 10, 14, 18, 22 and 26 days after 50% anthesis. Thereafter, ripening made further assessments impossible. In each plot, the percentage of visually infected spikelets of the inoculated ears was estimated according to a linear 0 (no disease) to 4 (100% infected spikelets) scale. Scoring was done between 0 and 1 in 0.1 scale increments and between 1 and 4 in 0.5 intervals (see Lemmens et al., 1993). The area under the disease progress curve (AUDPC) was calculated for each entry according to Equation (1).

$$\text{AUDPC} = \sum_{i=1}^{n} \{[(Y_i + Y_{i-1})/2]^* (X_i - X_{i-1})\}, \quad (1)$$

where Y_i is the score of visually infected spikelets (0–4) on the ith day, X_i is the day of the ith observation and n is the total number of observations (modified from Shaner and Finney, 1977). AUDPC data can theoretically vary from 0 to 84. During the growth season, the non-inoculated part of each plot was visually assessed for FHB symptoms. FHB-infected ears were removed by hand to ensure that the control ears were, essentially, disease-free. At the end of the season, 25 inoculated ears from each inoculation treatment, as well as 25 control ears from the same plot were harvested and weighed. Relative ear weight (REW), expressed as the percentage of the weight of the respective control ears, was calculated. This parameter was taken as a measure for yield reduction and is highly correlated with relative grain weight (Lemmens et al., 1993). Thereafter, the ears were threshed carefully with a single-ear thresher, which was coated with rubber on the inside to prevent damaging the porous *Fusarium*-damaged kernels (FDK). Chaff and kernels were collected in an aspirator and the grains were separated carefully from the chaff to prevent loss of the light FDK.

Mycotoxin analyses

For determination of the mycotoxin content, 15 g kernels from each plot were milled to pass a 1-mm sieve and 10 g was used for further analysis. DON was extracted in 40 ml of an acetonitrile/water (84 + 16) solution on a rotary shaker for 90 min (2.5 Hz). The liquid phase was collected after filtration through a filter paper. Clean-up was performed with the Mycosep–Romer columns, which enabled quick and easy purification of the extract (Krska et al., 2001). About 7.5 ml was pressed through the column and exactly 4 ml of the extract was taken up in a vial. The extract was evaporated to dryness under nitrogen at 50 °C. The residue was dissolved in 500 µl of an acetonitrile/water (5 + 95) solution (mobile phase). The solution was vortexed for 30 s. The solution was transferred in an Eppendorf and centrifuged at 1000*g* for 10 min. The clear supernatant was transferred to HPLC vials, which were subsequently sealed. Identification and quantification was carried out with reversed-phase HPLC (type HP 1050, Hewlett Packard) and UV-detector (220 nm) (type W-VWD-HP 1050) under isocratic conditions (water/acetonitrile 95 : 5, flow-rate 0.4 ml min^{-1}) using an ODS-Hypersil (100*2.1 mm, 5 µm) column. Detection limit of the method was 500 µg kg^{-1} in wheat with a recovery of 81 ± 5.6% (n = 12, 1–50 mg kg^{-1}).

Statistical analyses

Experimental layout was a split-plot design over two irrigation treatments in two replicates with main plot 'genotype' and subplot 'inoculation treatment'. ANOVA analyses were calculated for each location separately. The model for ANOVA was:

$$\begin{aligned}\text{AUDPC}_{mgir} =& M_m + R_{r/m} + G_g + MG_{mg} \\ &+ GR_{gr/m} + I_i + MI_{mi} + GI_{gi} \\ &+ MGI_{mgi} + MGIR_{mgir/m}, \end{aligned} \quad (2)$$

in which AUDPC is defined as above; M_m = mist-irrigation treatments ($m = 1, 2$); $R_{r/m}$ = replication within the irrigation treatment; G_g = genotype ($g = 1, \ldots, 10$); I_i = inoculation treatment ($i = 1, \ldots, 4$); $\text{GR}_{gr/m}$ and $\text{MGIR}_{mgir/m}$ are the main plot and subplot errors, respectively; the other factors are either double or triple interactions of the main factors M, G and I. The same model was used to analyse the REW_{mgir} and the DON_{mgir} data.

The least-squares means (lsmeans) $\text{AUDPC}_{m\cdot i\cdot}$ data were defined as the FHB index. These values calculated in both locations separately ($n = 16$) were taken as a measure of the final disease level in each location/irrigation treatment/inoculation treatment combination.

Statistical analyses were carried out with the SAS/STAT® statistical package. ANOVA analyses and the Fisher's least-significant-difference test ($\text{LSD}_{0.05}$) were calculated with the GLM procedure. Unless mentioned otherwise, lsmeans data are presented. Pearson correlation coefficients and regression analyses were calculated with the CORR and the REG procedures, respectively (SAS® Procedures Guide, 1989).

Results

All artificial inoculation treatments resulted in successful infection. ANOVA analyses were calculated with the data of AUDPC, REW and DON contamination for each location separately (results not shown). The ANOVA analyses with the AUDPC_{mgir} data showed significant effects of all main factors 'mist-irrigation treatments', 'genotypes' and 'inoculation treatments'. Most interactions were not significant, the genotype × irrigation interaction at Location 2 in 1995 being the only exception. The second disease evaluation method was the determination of the REW_{mgir}. Significant differences were present in both locations for the main factors 'genotype' and 'inoculation treatment'. The factor 'mist-irrigation treatment' was significant at Location 2 only. None of the interactions was significant. A similar analysis with the DON_{mgir} contamination data in the grains revealed significant effects for all main factors except for the factor 'genotype' in Location 2. The genotype × irrigation interaction was significant in both locations at the 5% level.

A two-way table was constructed with the factors 'irrigation treatment' and 'inoculation treatment' and the lsmeans of the $\text{AUDPC}_{m\cdot i\cdot}$ as well as the $\text{REW}_{m\cdot i\cdot}$ and $\text{DON}_{m\cdot i\cdot}$ data were calculated. This was done for each location separately (Table 2). In addition, the lsmeans for both irrigation treatments ($\text{AUDPC}_{m\ldots}$) were calculated as well as the grand mean ($\text{AUDPC}_{\ldots\ldots}$) of each location. The grand mean of the Location 2 was approximately twice the level of Location 1 (31.9 and 15.6 AUDPC units, respectively). After application of water, the $\text{AUDPC}_{m\ldots}$ was significantly ($P < 0.0001$) higher than in the non-irrigated treatment in both locations. In the first inoculation treatment (I_1), the ears were covered for 24 h with a bag, while in the second treatment (I_2) they were bagged for only 18 h. This resulted in more disease after using the I_1 treatment (Table 2). This effect was significant ($P \leq 0.05$) in Location 2. The use of bags to cover the ears for 18 h (I_2) increased the infection compared with the inoculation treatment without bags (I_3). This effect was not significant in the irrigated treatment in Location 2. The fourth inoculation treatment (I_4), in which the ears were inoculated twice and double the amount of inoculum was applied, resulted in significantly ($P \leq 0.05$) more disease than the I_3 treatment (Table 2).

For each genotype, the mean $\text{AUDPC}_{mgi\cdot}$ over the two locations was calculated (results not shown). Correlation analyses comparing the results of the 10 genotypes obtained with each inoculation treatment within each irrigation treatment revealed correlation coefficients ranging from 0.85 to 0.95 (mean = 0.89, $n = 6$) and from 0.91 to 0.96 (mean = 0.94, $n = 6$) for the non-irrigated and the irrigated treatment, respectively. With all inoculation treatments, highly correlated FHB resistance data for the genotypes were obtained using AUDPC as a disease parameter, both in the irrigated and in the non-irrigated treatments.

The higher disease pressure in Location 2 was also reflected in a lower REW (Table 2). Yield reduction was greater after mist irrigation than in the non-irrigated treatments. This effect was significant ($P \leq 0.05$) in

Table 2. Summary of the data for AUDPC, REW and DON contamination

	Inoculation treatment	Location 1		Location 2	
		M_0	M_1	M_0	M_1
$AUDPC_{m\cdot i\cdot}$	I_1	15.8	20.1	27.7	40.9
	I_2	14.4	18.3	25.1	36.7
	I_3	10.7	15.8	21.3	36.4
	I_4	14.5	18.9	27.8	39.2
$LSD_{0.05}$		1.9	2.1	2.1	2.6
$AUDPC_{m\cdots}$		13.8	18.3	25.4	38.3
$AUDPC_{\cdots\cdot}$		15.6 ($n = 144$)		31.9 ($n = 160$)	
$REW_{m\cdot i\cdot}$	I_1	61	61	52	41
	I_2	65	63	59	49
	I_3	70	66	62	50
	I_4	62	56	56	48
$LSD_{0.05}$		4	6	6	5
$REW_{m\cdots}$		64	62	57	47
$REW_{\cdots\cdot}$		63 ($n = 144$)		53 ($n = 160$)	
$DON_{m\cdot i\cdot}$	I_1	54.7	43.7	67.6	63.4
	I_2	52.4	36.1	52.2	42.2
	I_3	50.2	39.4	51.0	34.4
	I_4	66.5	41.9	69.0	39.1
$LSD_{0.05}$		12.9	5.2	14.1	8.9
$DON_{m\cdots}$		55.9	40.3	60.2	46.1
$DON_{\cdots\cdot}$		46.8 ($n = 144$)		54.4 ($n = 135$)	

Data are lsmeans over 10 genotypes and two replicates (e.g. $AUDPC_{m\cdot i\cdot}$), lsmeans over 10 genotypes, four inoculation treatments and two replicates (e.g. $AUDPC_{m\cdots}$) or grand means (e.g. $AUDPC_{\cdots\cdot}$) of each location separately. For each location/irrigation treatment combination the LSD for inoculation treatments at the 5% level ($LSD_{0.05}$) were calculated. At Location 1 stormy weather destroyed 16 bagged bunches with inoculated ears ($n = 144$). Due to the high infection level at Location 2, the amount of seeds harvested from 25 bunches was not sufficient for DON analyses ($n = 135$). For explanation of the inoculation treatments I_1–I_4 see text (Inoculation treatments in Materials and methods). (Location 1: Grossenzersdorf, 1994; Location 2, Tulln, 1995; M_0 non-irrigated treatment; M_1, irrigated treatment; AUDPC, area under the disease progress curve; REW, relative ear weight in %; DON in mg kg^{-1}).

Location 2 only. On comparing the lsmean $REW_{m\cdot i\cdot}$ of the individual inoculation treatments, similar tendencies were found as for the AUDPC data described above. Again mean $REW_{mgi\cdot}$ data over both locations were calculated for all genotypes, and the data for each inoculation treatment within irrigation treatment were correlated. The values of the correlation coefficients ranged from 0.82 to 0.94 (mean = 0.88, $n = 6$) and from 0.33 to 0.90 (mean = 0.70, $n = 6$) for the non-irrigated and the irrigated treatment, respectively. In general, the r-values calculated for mist irrigation were smaller than those described for the AUDPC. Again the REW data obtained with each of the inoculation treatments were highly correlated indicating that comparable data were obtained with each of the inoculation treatments under investigation.

There was more DON contamination in the kernels in Location 2 (Table 2). In this respect, DON contamination followed the same tendency as described for the AUDPC data. Inoculation treatment influenced the grain DON content. Application of twice the amount of inoculum (I_4) resulted in a higher DON contamination in the grains than inoculating once in the evening (I_3). This effect was significant ($P \leq 0.05$) in the non-irrigated treatments only. DON concentration in the grains was also influenced by mist irrigation during and after flowering, but in this case, in both locations, DON contamination was significantly ($P < 0.0001$) less with mist irrigation than with the non-irrigated treatments. The mean DON concentration over both locations and replicates were calculated for all genotypes. The data for each inoculation treatment within irrigation treatment were correlated. The values of the correlation coefficients ranged from 0.66 to 0.92 (mean = 0.80, $n = 6$) and from 0.05 to 0.76 (mean = 0.35, $n = 6$) for the non-irrigated and the irrigated treatments, respectively. In general, and especially under irrigation, the r-values were smaller than those calculated for the AUDPC and the REW data.

In Table 3, the lsmeans of the $AUDPC_{mg\cdot\cdot}$, the $REW_{mg\cdot\cdot}$ and the $DON_{mg\cdot\cdot}$ data of each individual genotype investigated are summarized. In both locations the use of irrigation resulted in significantly more infection (AUDPC) for most genotypes. The genotype '204/81/03' did not show an increase in symptoms under irrigation in both locations. In Location 2 some genotypes such as e.g. 'Szöke' and '81 F3 79' reacted with a high increase in symptoms after mist irrigation, explaining the significant genotype × irrigation treatment interaction calculated in the ANOVA. Although in Location 1 a significant increase in AUDPC was observed with mist irrigation, no significant increase in yield loss was present for most genotypes (Table 3). In Location 2, most of the genotypes reacted with a significant decrease in REW with mist irrigation.

In Location 1 the genotypes 'Zombor', 'SVP7200520301', 'NR-172/90' and 'SL8/80-28' reacted with a significant decline in grain DON content after mist irrigation (Table 3). A similar behaviour was observed with the genotypes '204/81/03', 'NR-172/90' and 'SL8/80-28' in Location 2. 'Arina' showed a significant increase in DON content with mist irrigation

Table 3. Summary of the genotypic data for $AUDPC_{mg\cdot\cdot}$, $REW_{mg\cdot\cdot}$ and $DON_{mg\cdot\cdot}$ contamination

Genotype	Location 1			Location 2		
	M_0	M_1	$M_0 - M_1$	M_0	M_1	$M_0 - M_1$
$AUDPC_{mg\cdot\cdot}$						
Zombor	20.2	25.5	**	34.5	52.5	***
Szöke	10.3	14.9	**	19.4	40.2	***
204/81/03	18.4	20.5	ns	31.7	28.3	ns
SVP7200520301	20.8	27.2	***	30.8	38.5	***
Arina	10.5	13.7	ns	16.1	32.6	***
Copain	11.5	15.0	*	21.2	37.9	***
RC103	9.2	14.1	*	29.9	43.6	***
81 F3 79	3.9	6.8	*	13.1	31.2	***
NR-172/90	11.3	17.4	***	25.6	30.0	*
SL 8/80-28	22.0	27.9	**	32.2	48.3	***
$REW_{mg\cdot\cdot}$						
Zombor	48	49	ns	43	41	ns
Szöke	51	53	ns	51	36	***
204/81/03	77	66	*	46	42	ns
SVP7200520301	55	54	ns	60	50	*
Arina	79	72	ns	78	50	***
Copain	64	61	ns	68	54	**
RC103	78	65	ns	51	42	*
81 F3 79	79	75	ns	72	59	**
NR-172/90	65	66	ns	57	60	ns
SL 8/80-28	50	58	ns	47	38	*
$DON_{mg\cdot\cdot}$						
Zombor	110.3	51.2	***	71.2	84.8	ns
Szöke	47.7	33.7	ns	58.6	56.2	ns
204/81/03	43.5	55.3	ns	81.8	28.3	***
SVP7200520301	103.4	65.6	***	57.6	76.5	ns
Arina	17.1	35.6	*	37.1	67.0	**
Copain	44.5	31.9	ns	46.5	33.8	ns
RC103	27.1	35.7	ns	53.4	52.6	ns
81 F3 79	20.5	12.4	ns	40.7	38.2	ns
NR-172/90	43.6	28.0	*	75.9	32.1	***
SL 8/80-28	101.5	53.1	***	79.1	31.8	***

Data are lsmeans over four inoculation treatments and two replicates. Within each location, *t*-tests were carried out to compare the genotypic data of both irrigation treatments. ***, **, * significant at $P \leq 0.001$, $P \leq 0.01$ and $P \leq 0.05$, respectively. ns, not significant ($P > 0.05$) (Location 1: Grossenzersdorf, 1994; Location 2, Tulln, 1995; M_0, non-irrigated treatment; M_1, irrigated treatment; REW, relative ear weight in %; DON in mg kg^{-1}).

compared to the non-irrigated treatment in both locations. For some other genotypes, no significant change was observed ('Szöke', 'Copain', 'RC103' and '81F379'). The significant genotype × irrigation treatment interaction for the DON contamination data was studied in more detail. For the genotypes 'Arina' and 'SL 8/80-28' the lsmean $AUDPC_{mgi\cdot}$ data were plotted against the corresponding lsmean $DON_{mgi\cdot}$ data for each individual location/irrigation treatment/inoculation treatment combination (Figure 2). The data were fitted to a straight line and the model was significant at the 1% level for both wheat genotypes. For 'Arina' the equation was AUDPC $= 2.1 + 0.40 \times$ DON. Coefficient of determination (R^2) equalled 0.79 and the 95% confidence interval for the slope was 0.40 ± 0.12. An increase in DON contamination was observed with increasing AUDPC levels (Figure 2a). For 'SL 8/80-28' an opposite reaction was found (Figure 2b). The straight-line equation was AUDPC $= 48.63 - 0.24 \times$ DON ($R^2 = 0.44$) and the 95% confidence interval for the slope was -0.24 ± 0.15. It was concluded that genotypic reactions in DON accumulation towards mist irrigation were variable.

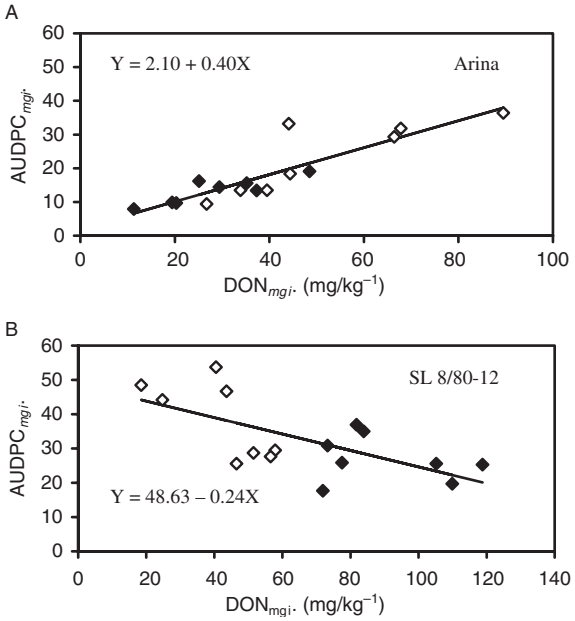

Figure 2. Relationship between the lsmean data for AUDPC$_{mgi.}$ and DON$_{mgi.}$ contamination for each location/inoculation treatment/irrigation treatment combination for the genotype 'Arina' (A) and for the genotype 'SL 8/80-12' (B). ◊ = irrigated and the ♦ = non-irrigated treatments, respectively. Data were fitted with a straight line and the calculated regression lines are illustrated.

The lsmean AUDPC$_{mgi.}$ and DON$_{mgi.}$ data for each location/irrigation treatment/inoculation treatment combination were calculated for the 10 wheat genotypes. For each combination ($n = 16$) the AUDPC data of the genotypes were correlated with the respective DON data. Correlation coefficients varied from 0.08 up to 0.89 (see ordinate in Figure 3). The relationship between these correlation coefficients and the FHB index of the corresponding location/irrigation treatment/inoculation treatment combinations is illustrated in Figure 3. The highest correlation coefficients between the visual data and the grain DON contamination occurred at moderate disease levels. The data further indicated that the relationship between the field score data and the grain DON content deteriorated with increasing disease pressure (FHB index).

Discussion

The major factor that determines the severity of a natural or artificial FHB epidemic is the environment (Parry et al., 1995). The two experiments in this

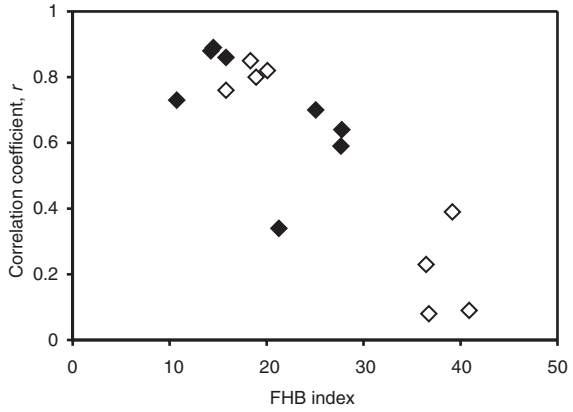

Figure 3. Plot of the correlation coefficients (r) between genotypic AUDPC data and genotypic DON contamination data against the FHB index. The r-values were calculated between the lsmeans AUDPC$_{mgi.}$ and DON$_{mgi.}$ data of the 10 wheat genotypes for each location/irrigation treatment/inoculation treatment combination separately. The FHB indices are the lsmean AUDPC$_{m\cdot i.}$ data over 10 genotypes and two replicates calculated for each location/irrigation treatment/inoculation treatment combination separately. The FHB index is a measure of the disease pressure. ◊ = irrigated and the ♦ = non-irrigated treatments, respectively.

contribution were performed in two different years: in 1995 in Grossenzersdorf and in 1996 in Tulln. Therefore the 'location' and the 'year' effects are confounded. The results showed that in the non-irrigated treatments, the amount of disease (AUDPC), yield reduction (REW) and DON contamination of grain was higher in Location 2 (Tulln, 1995). While during inoculation the mean temperature was similar in both locations (16 and 15.1 °C, respectively), the total precipitation was three times higher in Location 2 (36.6 and 109.0 mm, respectively). The high amount of rainfall in Location 2 could explain these differences. A high level of moisture during flowering was reported to promote FHB infections (Sutton, 1982). Continuous rainfall during the flowering period led to higher grain infection levels and DON content (Birzele et al., 2002).

Providing optimal moisture conditions with bags for a longer time period promoted infection success. This was also previously described by Mesterházy (1988), who found a higher infection level after bagging the ears for 24 h as compared to 17 h. Using bags after inoculation in the evening resulted in more disease as compared to the treatment without bags. This is probably due to an optimal supply of moisture in the bagged ears promoting infection. In the last inoculation treatment (I_4), inoculum was applied a second time 2 days after the first inoculation at mid-anthesis. With this

method we aimed to treat the secondary ears at the most optimal stage. The method probably resulted in higher infection because of the application of double the amount of inoculum and water. The latter factor could also considerably contribute to infection success. In the non-irrigated treatments, moisture could be a restricting factor for infection success, especially under dry and windy climatic conditions. Comparable disease data were obtained for the different wheat lines with the four inoculation treatments. This was the case for all FHB evaluation methods examined (AUDPC, REW and DON) in the non-irrigated treatment. From these data, no convincing evidence was obtained for a preferential inoculation treatment.

A wet season was simulated with a mist-irrigation system. In a repeated cycle of 48 h, the ears and the crop canopy were kept wet during 20 h for at least 26 days after flowering of the wheat lines. The experiment was not irrigated continuously, mainly because of the fear that excess of water could promote other diseases (e.g. those incited by bacteria). Long-term mist irrigation resulted in higher disease and a higher yield reduction, but an overall lower DON contamination in the grains. Lacey et al. (1999) reported that continuous mist irrigation over 3–6 days after inoculation with *F. culmorum* increased the disease. DON contamination in the grains was measured during 3 days of mist irrigation and toxin content continuously increased with increasing wet period. No information is available on the effect of long-term mist irrigation exceeding a period of 3 days after inoculation on DON contamination of the grain (Lacey et al., 1999).

The factors influencing toxin contamination are not well understood and final DON content in the grain is probably a result of complex fungus–plant–environment interactions. DON accumulation starts about 3 days after infection and peaks about 6 weeks later; it then begins to decline and reaches a constant level before harvest maturity (Teich, 1989). Mist irrigation could have influenced either DON accumulation or its decline. DON production by *F. culmorum* depends on moisture. The effect of water activity (a_w) on DON production by *F. culmorum* strains from UK, Italy and Sweden was studied on irradiated wheat grain with conserved germination capacity (Magan et al., 2002). The wheat grain was modified with sterile water to the required a_w ranging from 0.97 to 0.99. DON production decreased with increasing water activity. Hence, at least the theoretical possibility exists that moisture influences DON production of the *F. culmorum* isolate used for artificial inoculation.

Another factor influencing toxin accumulation was the wheat genotype. The data revealed genotypic differences in toxin accumulation under mist irrigation. The genotype 'Arina' reacted with an increase in DON content in the grains. The close relationship between the DON and the AUDPC data for this genotype suggests that DON contamination was influenced by the infection pressure only, irrespective of whether the variation in the amount of disease was caused by mist irrigation. 'SL 8/80-28' showed a consistent and significant decrease in DON contamination under mist irrigation in both locations (Table 3). An intriguing hypothesis to explain this observation is the appearance of premature ears (Snijders and Krechting, 1992; Bai and Shaner, 1994). The fungus can spread from the spikelet to the rachis, kill the tissue, and thereby shut off the water and nutrient supply to distant spikelets of the ear. In premature ears, the upper part of the ear wilts, resulting in an increase in FHB symptoms and yield reduction due to the presence of shrivelled kernels. The wilted part of the ear is not colonized by the fungus. Sinha and Savard (1997) reported that shrivelled kernels contained less DON ($<5\,mg\,kg^{-1}$) than the typical FDK (between 1 and $600\,mg\,kg^{-1}$). In preliminary experiments (results not shown), all genotypes under investigation were tested for resistance towards fungal colonization of the ear (Type II resistance). A single spikelet in the centre of the ear was inoculated. At high infection pressures, the FHB sensitive genotype 'SL8/80-28' reacted intensively with premature ripening of the ears. Premature ears could be already observed 10 days after inoculation. 'Arina' showed a higher FHB resistance level and less premature ears were present. The appearance of premature ears at high infection pressure could simultaneously explain an increased level of head blight symptoms, a reduction in yield and a reduction of overall DON contamination in the grains. Further intensive examinations are required to elucidate the exact cause(s) of the genotypic differences in DON accumulation in the grains.

Toxin analyses are laborious and costly. Investigations have been reported to predict DON content in the grain using other FHB evaluation methods such as AUDPC and REW (Mesterházy et al., 1999; Bai et al., 2001). Our data indicate that good correlations between AUDPC and DON contamination data are present at low to intermediate disease levels. Premature ripening of ears as mentioned above, could also explain why the close relationship between AUDPC and DON data deteriorated at high infection pressures.

References

Aufhammer W, Kübler E, Kaul HP, Hermann W, Höhn D and Yi C (2000) Infection with head blight (*F. graminearum*, *F. culmorum*) and deoxynivalenol concentration in winter wheat as influenced by N fertilization. Pflanzenbauwissenschaften 4: 72–78

Bai G and Shaner G (1994) Scab of wheat: Prospects for control. Plant Disease 78: 760–766

Bai G and Shaner G (1996) Variation in *Fusarium graminearum* and cultivar resistance to wheat scab. Plant Disease 80: 975–979

Bai GH, Plattner R, Desjardins A and Kolb F (2001) Resistance to *Fusarium* head blight and deoxynivalenol accumulation in wheat. Plant Breeding 120: 1–6

Bekele GT (1985) Head scab screening methods used at CIMMYT. In: Villareal R.L. and Klatt A.R. (eds), Wheats for More Tropical Environments. CIMMYT, Mexico, pp. 169–173

Birzele B, Meier A, Hindorf H, Krämer J and Dehne HW (2002) Epidemiology of *Fusarium* infection and deoxynivalenol content in winter wheat in the Rhineland, Germany. European Journal of Plant Pathology 108: 667–673

Bottalico A (1998) *Fusarium* diseases of cereals: Species complex and related mycotoxin profiles in Europe. Journal of Plant Pathology 80: 85–103

Buerstmayr H, Lemmens M, Grausgruber H and Ruckenbauer P (1996) Scab resistance of international wheat germplasm. Cereal Research Communications **24**: 195–202

D'Mello JPF, Macdonald AMC, Postel D, Dijksma WTP, Dujardin A and Placinta CM (1998) Pesticide use and mycotoxin production in *Fusarium* and *Aspergillus* phytopathogens. European Journal of Plant Pathology 104: 741–751

Krska R, Baumgartner S and Josephs R (2001) The state-of-the-art in the analysis of type-A and -B trichothecene mycotoxins in cereals. Fresenius Journal of Analytical Chemistry 371: 285–299

Lacey J, Bateman GL and Mirocha CJ (1999) Effects of infection time and moisture on development of ear blight and deoxynivalenol production by *Fusarium* spp. in wheat. Annals of Applied Biology 134: 277–283

Lemmens M, Buerstmayr H and Ruckenbauer P (1993) Variation in *Fusarium* head blight susceptibility of international and Austrian wheat breeding material. Die Bodenkultur 44: 65–78

Liu ZZ (1985) Recent advances in research on wheat scab in China. In: Villareal R.L. and Klatt A.R. (eds), Wheats for More Tropical Environments. CIMMYT, Mexico, pp. 174–181

Magan N, Hope R, Colleate A and Baxter ES (2002) Relationship between growth and mycotoxin production by *Fusarium* species, biocides and environment. European Journal of Plant Pathology 108: 685–690

Mesterházy Á (1988) Expression of resistance of wheat to *Fusarium graminearum* and *F. culmorum* under various experimental conditions. Journal of Phytopathology 123: 304–310

Mesterházy Á, Bartók T, Mirocha CG and Komoróczy R (1999) Nature of wheat resistance to *Fusarium* head blight and the role of deoxynivalenol for breeding. Plant Breeding 118: 97–110

Miedaner T (1997) Breeding wheat and rye for resistance to *Fusarium* diseases. Plant Breeding 116: 201–220

Parry DW, Jenkinson P and McLeod L (1995) *Fusarium* ear blight (scab) in small grain cereals – a review. Plant Pathology 44: 207–238

SAS Procedures Guide (1989) SAS/STAT® User's Guide, Version 6, Vol 1 and Vol 2, 4th edn., SAS Institute Inc., Cary, NC

Schroeder HW and Christensen JJ (1963) Factors affecting resistance of wheat to scab caused by *Gibberella zeae*. Phytopathology 53: 831–838

Shaner G and Finney RA (1977) The effect of nitrogen fertilization on the expression of slow-mildewing resistance in Knox wheat. Phytopathology 67: 1051–1056

Sinha RC and Savard ME (1997) Concentration of deoxynivalenol in single kernels and various tissues of wheat heads. Canadian Journal of Plant Pathology 198: 8–12

Smith D and Onions AHS (1994) The preservation and maintenance of living fungi. IMI Technical Handbooks No. 2. International Mycological Institute, CAB International

Snijders CHA (1990) Genetic variation for resistance to *Fusarium* head blight in bread wheat. Euphytica 50: 171–179

Snijders CHA and Krechting CF (1992) Inhibition of deoxynivalenol translocation and fungal colonization in *Fusarium* head blight resistant wheat. Canadian Journal of Botany 70: 1570–1576

Snijders CHA and Perkowski J (1990) Effects of head blight caused by *Fusarium culmorum* on toxin content and weight of wheat kernels. Phytopathology 80: 566–570

Sutton JC (1982) Epidemiology of wheat head blight and maize ear rot caused by *Fusarium graminearum*. Canadian Journal of Plant Pathology 4: 195–209

Teich AH (1989) Epidemiology of wheat (*Triticum aestivum* L.) scab caused by *Fusarium* spp. In: Chelkowski J. (ed), Topics in Secondary Metabolism 2. Fusarium Mycotoxins, Taxonomy and Pathogenicity. Elsevier, Amsterdam–Oxford–New York–Tokyo, pp. 269–282

Walsh EJ, Fanning MJ and Bannon E (1998) An evaluation of screening techniques to assess *Fusarium* head blight resistance in spring wheat (*Triticum aestivum* L.). Cereal Research Communications 26: 59–66

Wang YZ and Miller JD (1988a) Effect of *Fusarium graminearum* metabolites on wheat tissue in relation to *Fusarium* head blight resistance. Journal of Phytopathology 122: 118–125

Wang YZ and Miller JD (1988b) Screening techniques and sources of resistance to *Fusarium* head blight. In: Klatt A.R. (ed), Wheat Production Constraints in Tropical Environments. D.F.: CIMMYT, Mexico, pp. 239–250

Zadoks JC, Chang TT and Konzak FC (1974) A decimal code for the growth stages of cereals. Weed Research 14: 415–421