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The Effect of Nitrogen Fertilization on *Fusarium* Head Blight Development and Deoxynivalenol Contamination in Wheat

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With one figure

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Abstract

The impact of nitrogen (N) fertilization on the development of *Fusarium* head blight (FHB) in wheat and the resulting deoxynivalenol (DON) contamination in the kernels was studied. In a first experiment, the disease was assessed on two locations under natural infection pressure. Five different types of nitrogen fertilizer (both organic and mineral) were investigated, each applied at five input rates from 0 to 160 kg N/ha. With all fertilizers, a significant increase of disease intensity was observed with increasing N input, while the type of N fertilizer had poor or no effects on FHB. Depending on the fertilizer used, the percentage of diseased spikelets increased from 2.2% at zero N rate up to 6.6% at 160 kg N input per hectare. In a second series of trials, three spring wheat varieties including one Durum wheat line were artificially inoculated with a *Fusarium graminearum* and a *F. culmorum* strain, known producers of DON. A mineral N fertilizer was applied at five input levels from 0 to 160 kg N/ha. A significant increase in FHB intensity and DON contamination in the grain was observed with increasing N from 0 to 80 kg/ha. At higher input rates, relevant in contemporary crop husbandry, disease intensity and toxin contamination remained at constant levels. It is concluded that adaptation of N fertilization represents no relevant tool in managing FHB in practical wheat cultivation.

Introduction

Fusarium head blight (FHB or scab) is a fungal disease of small grain cereals including wheat (*Triticum aestivum* L.). The primary causal organisms in Europe are *F. graminearum* (Schwabe) (teleomorph: *Gibberella zeae* (Schw.) Petch) and *F. culmorum* (Wm. G. Smith) Sacc. (perfect state unknown). *Fusarium graminearum* is prevalent in warmer regions, whilst *F. culmorum* predominates in the cooler climates of north-western Europe (Parry et al., 1995). Other important *Fusarium* spp. isolated from FHB damaged wheat ears in Austria are *F. avenaceum* (Corda ex Fr.) Sacc. [perfect state: *G. avenacea* (Cook)] and *F. poae* (Peck) Wolenw. (perfect state not known). FHB occurs in most wheat-growing areas of the world (Parry et al., 1995). Severe outbreaks of FHB on wheat have been reported in the north-central United States and southern Canada. In the latter regions, the problems are mainly caused by the widespread use of minimum tillage in combination with the cultivation of susceptible wheat genotypes (McMullen et al., 1997; Campbell and Lipps, 1998; Windels, 2000).

FHB has negative effects both on grain yield and quality (Bai and Shaner, 1994). Early infections can completely inhibit kernel formation or result in *Fusarium*-colonized kernels, which are smaller than normal and white to pale pink in colour. Further yield reductions originate from the presence of shrivelled kernels where *Fusarium* infection results in the destruction or clogging of the vessels in the rachis. Upper parts of the ear are, therefore, not supplied with sufficient water and nutrients resulting in premature ripening of the kernels. FHB reduces seed germination and plant vigour and causes seedling blight and poor stand (Bai and Shaner, 1994). Negative effects on the baking quality have also been reported (Meyer et al., 1986; Seitz et al., 1986). Scab can cause additional quality loss because of grain contamination with mycotoxins. The most important toxins produced by *F. graminearum* and *F. culmorum* are the trichothecene deoxynivalenol (DON) and the oestrogenic mycotoxin zearalenone (Bottalico, 1998). These mycotoxins have been linked to livestock toxicoses and feed refusal.

An integrated approach, including the growth of resistant varieties and cultural control techniques such

as crop rotation, tillage, chemical control and the appropriate use of fertilizers, is probably most effective to control FHB. Optimizing the use of nitrogen (N) might contribute to the control of FHB. Martin et al. (1991) observed that increasing N from 70 to 170 kg/ha significantly increased the incidence of *Fusarium*-infected grain in wheat, barley and triticale. N fertilization did not generally stimulate head blight infection or toxin production (Aufhammer et al., 2000). Teich and Hamilton (1985) and Fauzi and Paulitz (1994) could not find any effect of N on the incidence and severity of FHB. Parry et al. (1995) came to the conclusion that the effect of N on FHB remains unclear and that further work is needed to clarify this issue.

The aim of this study was to investigate the influence of N input on (1) the development of FHB in wheat and on (2) the resulting DON contamination in the kernels. During initial experiments, FHB was assessed under natural infection conditions. Five different types of N fertilizer (both organic and mineral) were used to investigate a possible influence of the type of fertilizer on FHB symptoms. As the disease in these experiments was mainly caused by *F. avenaceum* (a moniliformin producer), DON could not be detected. Therefore, further experiments were undertaken where wheat plots were artificially inoculated with *F. graminearum* and *F. culmorum* strains known to produce DON in high quantities. N input was set at 5 levels between 0 and 160 kg/ha with a mineral N fertilizer, and three wheat genotypes were investigated over two seasons.

Materials and Methods

Experiments under natural infection pressure

The experiment was grown in 1996 at the locations 'Schönering' (48°17′N, 14°09′E, altitude 270 m) and 'St Georgen' (48°12′N, 13°06′E, altitude 390 m). Both locations were situated in Upper Austria close to a river in a region known for heavy natural FHB epidemics. Preceding crops were potatoes in 'Schönering' and soya bean in 'St Georgen'. Conventional plough-based soil tillage was performed at both locations. The experiment in 'St Georgen' was destroyed by flooding after the visual assessment of the disease but before the ears could be harvested. The Austrian winter wheat cultivar 'Hubertus' (Saatbau Linz) was selected for the experiment, as this genotype is very sensitive for FHB (Buerstmayr et al., 1996). The trial design was that of a split-plot with main plots consisting of the amount of N input and subplots consisting of the type of N fertilizer applied. Each treatment had four replications. Main plots were 1.2 m apart and the area of each subplot was 10 m^2. Five different types of fertilizers, made available by Agrolinz Melamin GmbH, were used (see Table 1). Five N rates with 0, 40, 80, 120 and 160 kg N/ha were tested. All fertilizers except nitramoncal (NAC) were applied at growth stage 12 (2-leaf stage) according to Zadoks' scale of cereal growth stages (modified by Tottman and Makepeace, 1979). Application of the mineral N fertilizer NAC was split as described in Table 2. Because of different accompanying nutrients (P, K) in the organic fertilizers and split of NAC, there was a confounding of these factors with the N effects.

Table 1
Description of the nitrogen (N) fertilizers applied under natural infection conditions

Fertilizer	Type	N (%)	P_2O_5 (%)	K_2O (%)
Ammonium-nitrate-urea solution (ANU)	Mineral	27	0	0
Nitramoncal (NAC)[1]	Mineral	27	0	0
Colza cake	Organic	5.3	2.7	1.8
Animal tankage	Organic	9	5	0
Molasses	Organic	5.2	0.2	11.2

[1]Pure N fertilizer with 13.5% ammonium and 13.5% nitrate.

Table 2
Splitting of the nitrogen (N) fertilizer nitramoncal (NAC) (in kg N/ha) in the experiments both under natural infection conditions and after artificial inoculation

Total N rate	First application ZGS 12[1] (2-leaf stage)	Second application ZGS 30[1] (pseudo stem erect)	Third application ZGS 51[1] (first spikelet of ear just visible)
40	20	20	–
80	40	40	–
120	40	40	40
160	50	60	50

[1]According to Zadoks' scale of cereal growth stages, modified by Tottman and Makepeace (1979).

Experiments with artificial inoculation

These experiments were carried out in 1997 at the location 'Linz' (48°18′N, 14°17′E, altitude 250 m) and in 1998 at 'Lambach' (48°45′N, 13°53′E, altitude 250 m). Both locations are situated in Upper Austria. Each year by location combination was considered as a separate environment: 'Linz' in 1997 and 'Lambach' in 1998 were defined as Environment 1 and Environment 2, respectively. Conventional plough-based soil tillage was performed at both locations. Further information on the preceding crop, the amount of soil mineral N present in the soil and climatic data in both environments is summarized in Table 3. The total amount of soil mineral N at the location 'Linz' was higher than at 'Lambach'.

Two spring wheat varieties, 'Eta' and 'Kadett', were chosen for the experiments. In addition, 'Extradur', a *T. durum* variety, was included. All varieties were spring wheat genotypes of Austrian origin (Probstdorfer Saatzucht). 'Kadett' and 'Extradur' have been investigated during previous FHB resistance studies and are regarded as medium resistant and very sensitive, respectively (Buerstmayr et al., 1996). Genotypes were sown in plots of 12 m^2. The experimental design was that of a split-split-plot design with main plots being the amount of N input, subplot being genotype and sub-subplot being the *Fusarium* inoculation treatment

Table 3
Description and climatic data of the environments used for the experiments under artificial inoculation

	Environment 1	Environment 2
Location/year	Linz/1997	Lambach/1998
Preceding crop	Oats	Wheat
SMN[1] (0–30 cm)	21.6	18.9
SMN (30–60 cm)	29.5	20.2
SMN (60–90 cm)	16.1	9.4
SMN (total)	67.1	48.5
Mean temperature in June (°C)	18.2	18.5
Mean temperature in July (°C)	18.3	19.4
Total precipitation in June (mm)	78	151
Total precipitation in July (mm)	206	218

[1]SMN, soil mineral nitrogen in kg N/ha.

(see below). In the first and second environment, two and three replications were used, respectively.

NAC was used as a mineral N fertilizer. It was applied manually. In the first environment, three treatments with 0, 80 and 160 kg N/ha were tested. In the second environment, two additional treatments with 40 and 120 kg N/ha were investigated. Application of N was split as summarized in Table 2. P_2O_5 and K_2O were applied to all variants at the same rate of 15 and 30 kg/ha, respectively with the mineral fertilizer DC45 ($P_2O_5 : K_2O = 1 : 2$).

Inocula and artificial inoculation

The *F. culmorum* and the *F. graminearum* isolates used for artificial inoculation were stored in soil cultures for long-term storage (Smith and Onions, 1994). Both isolates differ in aggressiveness and are known to produce high quantities of DON in the field. Inoculum of the *F. culmorum* isolate was prepared on a mixture of wheat and oat kernels according to Snijders and Perkowski (1990). Before use, conidia were washed off the seeds with sterile distilled water. Inoculum of *F. graminearum* was produced in a liquid mung bean [*Vigna radiata* (L.) Wilczek] medium with the bubble breeding method (Mesterhazy, 1978). The mung bean medium was prepared by cooking mung beans (20 g/l water) for 22 min and the supernatant was used as the medium. Spore concentration was determined with a Buerker-Tuerk haemacytometer (Sondheim, Germany). Deionized water was used to adjust the conidial suspension to the desired final concentrations. To obtain a range of final disease levels not only two fungal isolates differing in aggressiveness were selected, but each isolate was also applied at two different conidial concentrations. Hence, four inoculation treatments (I_1 to I_4) were used: *F. culmorum* was applied at 1×10^4 and 4×10^4 macroconidia/ml representing inoculation treatment I_1 and I_2, respectively. *Fusarium graminearum* was applied at 2×10^4 and 5×10^4 macroconidia/ml (inoculation treatment I_3 and I_4, respectively). During the field inoculation period, which lasted for 12 days, the *Fusarium* suspensions were stored in the refrigerator at 2–4°C.

Inoculation of each wheat genotype was carried out at 50% anthesis. Time point of 50% flowering not only differed from genotype to genotype but also within a single genotype depending on the N input. High N application postponed anthesis up to 3 days. Before inoculation, each subplot (genotype) was divided in four equal sub-subplots of approximately 3 m^2 with a protective shield. The sub-subplots were at random treated with the four inoculation treatments described in the previous paragraph, i.e. each sub-subplot was treated with a different inoculation treatment. The fungal suspension (200 ml/m^2) was sprayed on the heads in the evening with a backpack sprayer. After inoculation, the plants were watered with a backpack sprayer (2 l/m^2) to keep a high humidity during the night in order to improve infection. Two days later the whole inoculation procedure was repeated on each plot.

Visual scoring of the disease and sampling

In all experiments, visual scoring (VS) of the disease was performed on 20 days after 50% anthesis. The percentage of diseased spikelets was determined on a linear 0 (0% bleached spikelets) to 4 (100% bleached spikelets) scale. Scoring was carried out between 0 and 1 in 0.1 scale increments, between 1 and 4 in 0.5 intervals (see Lemmens et al., 1993). Between 0 and 10% bleached spikelets, the scale was refined (steps of 1%) to be able to evaluate lower infection pressures under natural conditions.

In all trials, kernel samples were obtained by harvesting all ears within 1 m^2 of the experimental plot by hand to keep disturbance of the plot minimal. Border rows were neither considered for disease evaluation nor for sampling. Under natural infection pressure, ears were harvested 60 days after anthesis (for the location 'Schönering' only). The ears were hand threshed carefully in order to avoid the loss of heavily infected seeds, which are porous and light. Protein content as well as DON contamination of the grains was determined as described below. Because no DON was detected in the seeds and the dominant FHB causing fungus under natural infection conditions was *F. avenaceum* (see below), the grain samples were analysed for moniliformin. For the artificially inoculated experiments, ears from a 1 m^2 area within each sub-subplot ('inoculation treatment') were harvested at 20, 40 and 60 days after inoculation. Harvested ears were dried (24 h at 80°C) and stored at 15°C for further use. Dried ears were then hand threshed carefully. Protein content and DON contamination of the grains harvested 60 days after inoculation were determined. At 'Linz' in 1997, the weight of recovered grains of the highly susceptible genotype 'Extradur' was too low for further analyses because of the high FHB infection pressure. In 'Lambach' (1998), in addition, the DON contamination was investigated in the kernels of the variety 'Eta' harvested 20 and 40 days after inoculation with the I_2 and I_4 inoculation treatments. The experimental design of this additional trial was that of a split-split-plot design with main plots being the amount of N input, subplot being the inoculation treatments and sub-subplot being the harvest time point.

Fusarium taxonomy

Grain samples harvested 60 days after anthesis at 'Schönering' were investigated for the *Fusarium* spp. present. Grains originating from plots with the same N input rate were bulked and samples of 100 seeds of each N input level were further examined. The seed surface was sterilized with a 6% H_2O_2 solution and the seeds were plated on a Pepton pentachloronitrobenzene (PCNB) Agar. The *Fusarium* species were identified according to Nirenberg (1980).

Determination of soil mineral nitrogen and protein content in the grains

Determination of the soil mineral N was performed according to OENORM L 1091, 1993. Grain protein content was determined with a LECO CN 2000 according to the official AOAC (1995) method 992.23.

Toxin analyses

For the determination of the toxin content (DON and moniliformin), kernel samples were milled to pass through a 0.5 mm sieve. To analyse the DON content, 10 g flower was extracted in 100 ml of an acetonitrile/water (84 + 16) solution on a rotary shaker for 120 min. After filtration through a filter paper, the liquid phase was collected. Clean-up was performed with the Mycosep-Romer® no. 227 columns (Herzogenburg, Austria), which enabled quick and easy purification of the extract (Romer, 1986). Approximately 6 ml was pressed through the column and exactly 2 ml of the extract was taken up in a vial. The extract was evaporated to dryness in a rotary evaporator at 40°C. The residue was dissolved in 250 μl water. Identification and quantification was carried out with reverse-phase high-performance liquid chromatography (RP-HPLC) with a linear gradient of 0–10% acetonitrile in water and a UV-detector (220 nm). The liquid chromatograph was a HP 1050 with a diode-array detector HP 1040 M (Waldbronn, Germany). The column was a LiChrospher 100, RP18e, 250 × 4 mm, 5 μm. Water was used as a mobile phase for 5 min, followed by a linear gradient from 0 to 10% acetonitrile in 15 min. The flow rate was 2 ml/min and the retention time of DON was approximately 17 min. The injection volume was 0.1 ml and the detection limit was 0.5 mg/kg. Except from the cultivar 'Eta' at 'Lambach', the DON results presented were determined in the samples harvested 60 days after inoculation (anthesis). Each sample under investigation was examined three to four times. The mean DON content was used for further statistical analyses.

Moniliformin was determined by ion-pair HPLC and diode array-detection (Lew et al., 2001) with a detection limit of <0.1 mg/kg.

Statistical analyses

Statistical analyses were carried out with the SAS/STAT® statistical package (SAS-Institute Inc., 1989). Analyses of variance and *t*-tests were calculated with the General Linear Models (GLM) procedure. Pearsons' correlation coefficients were calculated with the correlation (CORR) procedure.

Results

Effect of N under natural infection pressure

Effectiveness of N application was checked by determination of the protein content in the kernels. Data were available for the location 'Schönering' only. Significant effects of the amount of N, the type of fertilizer as well as the interaction were observed following ANOVA ($P < 0.0001$ in all cases, data not shown). All fertilizers, except molasses, had a significant effect on grain protein content. The largest increase in protein content was reached with the ammonium-nitrate-urea (ANU) solution and NAC. The latter fertilizer increased grain protein content from 9.5% at 0 kg N input to 12.7% at 160 kg N input. It was concluded that N fertilization was effective for most fertilizers.

FHB data were available for both locations. Significant differences in FHB infection pressure were present for all the main factors, i.e. locations, the amount of N input as well as the type of fertilizer (see ANOVA in Table 4). Increasing total N rate progressively increased the amount of FHB symptoms for all types of fertilizer tested (Table 4). This was the case at both locations (data not shown). Increasing N input with NAC, ANU and colza cake had a greater effect on FHB intensity than the other fertilizers. It was concluded that N input influenced the amount of naturally occurring *Fusarium* infections in the ears of wheat.

Table 4
Mean visual *Fusarium* head blight (FHB) scores and ANOVA table for the experiments with the cultivar 'Hubertus' under natural infection

Nitrogen (N) rate (kg/ha)	Visual scores[1] Type of fertilizer				
	ANU[3]	NAC[3]	Colza cake	Tankage	Molasses
0	2.2a[2]	2.2a	2.2a	2.2a	2.2a
40	3.1ab	2.7a	3.1b	3.1b	2.5a
80	3.9bc	4.5b	3.3b	2.7ab	3.4b
120	4.8cd	4.7b	3.8b	4.4c	4.4c
160	6.1d	5.9c	6.6c	4.7c	4.8c

Source	df	MS[4]
Location (L)	1	5.470*[5]
Replication within L	6	0.965 ns
N rate (W)	4	17.431**
L*W	4	1.250 ns
Error A	24	0.612
Type of N fertilizer (F)	4	1.071**
F*W	16	0.385*
F*W*L	17	0.245 ns
Error B	111	0.155

[1]Visual scoring (VS, in percentage bleached spikelets) are mean values over the two locations 'Schönering' and 'St Georgen'.
[2]Within each type of fertilizer, the mean values with the same character are not significantly different at the 5% level.
[3]ANU, ammonium-nitrate-urea solution; NAC, nitramoncal.
[4]Mean squares. For the ANOVA analysis the data were transformed using a square root transformation [$VS_{sqr} = (VS + 0.5)^{1/2}$] to normalize the data.
[5]**, *Significant at $P \leq 0.001$ and $P \leq 0.01$, respectively; ns, not significant ($P > 0.05$).

In kernel samples harvested from the 'Schönering' site, DON could not be detected. Microbiological investigations revealed that the percentage of *Fusarium*-colonized kernels was 25, 27, 41 and 42% at 40, 80, 120 and 160 kg N input ha^{-1}, respectively. *Fusarium avenaceum* was the most prevalent *Fusarium* species (58% of the isolates), followed by *F. graminearum* (27%), *F. poae* (9%) and *F. equiseti* (6%). These ratios were independent from N rate. *Fusarium avenaceum* produces moniliformin, but also this mycotoxin could not be detected in the grain samples. Based on these results it was decided to (1) carry out artificially inoculated field trials using well characterized DON-producing *Fusarium* strains at low-risk locations and use other methods to assure a high-air humidity during infection, and to (2) use NAC for further experiments.

Effect of N after artificial inoculation

In order to determine whether the crop was able to utilize the NAC applied, kernel protein content was taken as a parameter for successful N uptake. ANOVA analysis of the protein data showed that the main effects, environment, amount of N fertilization and genotype were highly significant ($P < 0.0001$ in all cases, data not shown). In the first environment ('Linz', 1997), protein content in the grains increased from 12.0 to 15.3% at 0 and 160 kg N rate/ha, respectively. In the second environment ('Lambach', 1998) protein content increased from 9.8 (0 kg N input) to 14.9% (160 kg N rate). Inoculation treatment had no significant effect on the protein content of the kernels.

The amount of NAC application not only influenced protein content but also the FHB scores (see Table 5). Following ANOVA, significant effects of all main factors, environment, N input rate, genotype and inoculation treatment were observed. Except for zero N input, the FHB intensity in the second environment was higher than in the first one (see Table 5). In both environments, a significant increase in visual disease symptoms was observed with increasing N application. The N rate by environment interaction was significant. As N input increased from 0 to 160 kg/ha, the visual FHB scores increased by 25% and almost 300% in the first and second environment, respectively. In both environments, the visual scores seemed to reach a plateau at 80 kg/ha N and beyond. Highly significant differences in resistance of the genotypes and in FHB disease levels of the inoculation treatments were recorded. The increase of the genotypic FHB symptoms as a function of the N rate is summarized in Table 5. The data confirm the high susceptibility of the *T. durum* cultivar 'Extradur'. The visual score for 'Eta' and 'Kadett' reached a constant level at 80 kg N input/ha, while a further significant increase in FHB symptoms was observed for the genotype 'Kadett' at an N rate of 160 kg/ha. The mean visual scores for the inoculation treatments I_1 to I_4, were 15.0, 25.1, 28.3 and 32.3% infected spikelets, respectively (mean across three genotypes and two environments with either two or three replications and three or five N application rates). The genotype by environment interaction was significant. Comparing the visual scores in both environments revealed that for the cultivars 'Eta' (11% vs. 21%) and 'Kadett' (11% vs. 26%) disease was higher in the second environment and that this was contrasting for 'Extradur' (47% vs. 38%). The very high infection level for the latter genotype in the first environment was responsible for an almost complete yield loss.

Both *Fusarium* species used for artificial inoculation are known DON producers. The toxin was detected in all samples investigated. The results of an ANOVA analysis with the DON data in the grain samples harvested at 60 days post inoculation showed significant differences for all main factors (see Table 5). In the first environment, DON contamination was higher than in the second one. As with visual disease scores, DON concentration increased significantly as N rate was increased up to 80 kg/ha (see Table 5). Above 80 kg/ha, a small but significant decrease was observed in the first environment, whereas in the second environment, toxin contamination remained constant at around 10 mg/kg. DON contamination in the individual genotypes followed the same pattern (see Table 5). The largest increase in toxin contamination was observed upon increasing N rates from 0 to 80 kg/ha. At higher N input rates, toxin content did not significantly increase any further. DON contamination also depended on the inoculation treatment. The mean grain DON content for I_1 to I_4 was 5.1, 9.0, 9.4 and 15.2 mg/kg, respectively. The genotype by inoculation treatment interaction was significant. Upon increasing the macroconidial concentration of the *F. culmorum* isolate from 1×10^4 (I_1) to 4×10^4 (I_2), the DON content in the grains from 'Eta' and 'Kadette' increased from 2 to 6 mg/kg and from 3 to 9 mg/kg, respectively. The grains from 'Extradur' on the contrary, were already heavily contaminated with 10 mg/kg DON after inoculation with I_1, and the mycotoxin concentration merely increased to 11 mg/kg after treatment with I_2.

Time course of DON accumulation

In the artificially inoculated experiments, wheat samples were harvested at 20, 40 and 60 days after inoculation. DON contamination was analysed in the grains from the genotype 'Eta' treated with inoculation treatment I_2 and I_4. The results of the ANOVA showed, that the amount of N fertilizer applied ($P = 0.0002$) as well as the sampling dates ($P < 0.0001$) were significant (results not shown). DON content varied significantly between the inoculation treatments tested ($P = 0.0189$). DON concentration significantly increased from 20 to 40 days post inoculation (see Fig. 1A). This was observed at all N input rates. The highest increase in DON contamination in this period of time was measured after applications of 40 kg/ha of N. Sixty days after inoculation, a slight but non-significant decrease in DON content was observed for all fertilizer rates except at 40 kg/ha. DON production was dependent on

Table 5
Fusarium head blight (FHB) scores and deoxynivalenol content in the grains and ANOVA table from field experiments with artificial inoculation

Nitrogen (N) rate (kg NAC[9]/ha)	Visual scores[1] (in percentage diseased spikelets)		DON[9] content[1] (in mg/kg grains)	
	Environment 1[2,9]	Environment 2	Environment 1[3]	Environment 2
0	20.6a[4]	12.5a	13.2a	5.8a
40	nt[9]	26.9b	nt	8.7b
80	22.6b[4]	33.8c	16.6b	9.7c
120	nt	34.1c	nt	10.2c
160	25.5b[4]	37.5c	15.0c	10.9c

	Visual scores[6]			DON content[6]		
N rate[5]	'Eta'	'Kadett'	'Extradur'	'Eta'	'Kadett'	'Extradur'[7]
0	8.8a[4]	10.9a	27.6a	5.9a	7.9a	9.9a
80	20.5b[4]	22.2b	44.6b	9.7b	10.5b	15.6b
160	19.7b[4]	29.1c	46.2b	10.8b	9.9b	16.7b

	Visual scores[8]		DON content	
Source	df	MS	df	MS
Environment (E)	1	0.55**[10]	1	1367.14***
Replication within E	3	0.02 ns	3	34.13*
N rate (W)	4	1.01***	4	97.23***
W*E	2	0.66**	2	11.31 ns
Error A	10	0.05	10	6.34
Genotype (G)	2	3.96***	2	1540.11***
G*W	8	0.06 ns	8	16.74*
G*E	2	0.78***	1	0.60 ns
G*W*E	4	0.05 ns	2	9.89 ns
Error B	26	0.03	23	4.95
Inoculation treatment (I)	3	1.94***	3	591.15***
I*G	6	0.01 ns	6	127.40***
I*W	12	0.02 ns	12	5.22 ns
I*E	3	0.02 ns	2	42.82***
I*G*W	24	0.02 ns	24	4.67 ns
I*G*E	6	0.01 ns	2	30.28**
I*W*E	6	0.02 ns	4	1.60 ns
I*G*W*E	12	0.02 ns	4	10.65*
Error C	108	0.02	93	4.10

[1]Data are mean values over three genotypes ('Eta', 'Kadett' and 'Extradur'), four inoculation treatments (I_1–I_4) and three replications.
[2]Only two replications available.
[3]Only two replications and two genotypes ('Eta' and Kadett') available.
[4]Mean values with the same character are not significantly different at the 5% level.
[5]Only N rates investigated in both environments are presented.
[6]Data are mean values over two environments, four inoculation treatments and two (Environment 1) or three (Environment 2) replications.
[7]DON data for 'Extradur' were available for the second environment only.
[8]A log transformation of the visual scores (VS) [$VS_{log} = \log_{10}$ (VS)] was used to normalize the data for ANOVA.
[9]NAC, nitramoncal; DON, deoxynivalenol; nt, not tested; Environment 1, location 'Linz' 1997; Environment 2, location 'Lambach' (1998).
[10]***, **, *Significant at $P \leq 0.001$, $P \leq 0.01$ and $P \leq 0.05$, respectively; ns, not significant ($P > 0.05$).

the inoculation treatment used. For example, the increase in DON from day 20 to day 40 was greater for I_4 than it was for I_2 (see Fig. 1B).

Discussion

This work indicates that supplementary N, in the form of mineral and organic fertilizer, can have a significant impact on FHB intensity. The role of supplementary N in FHB infections is unknown (Martin et al., 1991). N did not change the inherent susceptibility of wheat ears to *F. graminearum* (Fauzi and Paulitz, 1994). N rate may increase FHB intensity by changing crop characteristics, notably by increasing crop density and altering the microclimate of the canopy. For example, in the first environment the number of ears/m^2 for the genotype 'Kadett' increased from 333 to 525 at 0 and 160 kg N input/ha, respectively. This canopy could remain humid over a longer period of time after rain or after dew periods. Moist conditions are required for formation of both macroconidia and ascospores (Sutton, 1982). This may promote inoculum production on crop debris on the soil surface (Fauzi and Paulitz, 1994). Changes in canopy characteristics resulting from the use of N may also enhance infections of the ears. Moisture is necessary for successful infections of the wheat ears (Sutton, 1982; Parry et al., 1995). Wheat is most susceptible to infection during anthesis, and after successful infection colonization of the ears proceeds

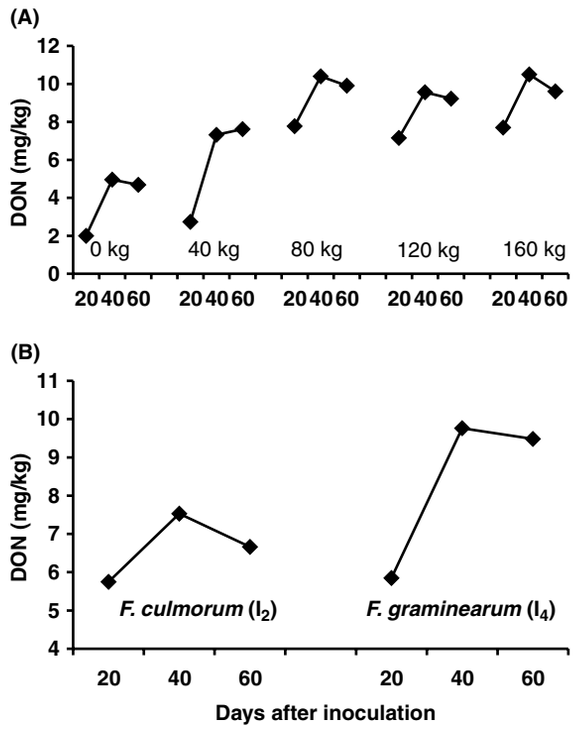

Fig. 1 Deoxynivalenol contamination of kernels of genotype 'Eta' 20, 40 and 60 days after inoculation at different nitrogen rates in kg/ha (A) and after inoculation with *Fusarium culmorum* (I_2) and *F. graminearum* (I_4) (B). The data in Fig. A are mean values of two inoculation treatments (I_2 and I_4) and three replications. The data in Fig. B are mean values across all nitrogen (N) input levels and three replications. The increase in deoxynivalenol (DON) contamination from day 20 to day 40 was significant (5% level) at all N rates and in both inoculation treatments

until ripening of the crop. Higher N inputs extend the stages during which wheat is susceptible to infection and a longer flowering period and later ripening was reported (Weinert and Wolf, 1995). Aufhammer et al. (1999) reported that FHB infection severity was generally correlated with the duration of flowering.

The form of N was reported to play a role in the FHB response (Teich, 1987). In our trials under natural infection conditions, the effect of the organic fertilizers on grain protein content was lower than with the mineral fertilizers. This is probably because of slow release of the N administered in organic form. This hypothesis is supported by the results of the soil mineral N analyses conducted 3 days after harvesting at 'Schönering'. After application of NAC and ANU, 24–26 kg N/ha were measured at the highest N input rate. After the application of molasses at the same N input rate, only 18 kg of mineral N/ha were measured. All N fertilizers affected FHB development. For molasses no significant increase in grain protein content was observed at 'Schönering', but nevertheless FHB symptoms doubled at higher N inputs. This may indicate that higher N or protein concentrations in kernels and/or ear tissue *per se* are not responsible for the higher FHB infection level.

Teich (1987) reported that FHB incidence was lower in wheat fertilized with urea than in wheat fertilized with ammonium nitrate. In our experiments, FHB responses after application of ANU and of NAC were not significantly different. *Fusarium* inoculum may have dispersed from neighbouring plots with the same amount of N input but a different type of fertilizer. In a study over two seasons, Fernando et al. (2000) showed that highest density of *G. zeae* spores were sampled 1.5 m from the inoculum source with fewer spores 5 m away. Macroconidia from *F. culmorum* and *F. avenaceum* can be splash-dispersed as high as 45–60 cm vertically and 90–100 cm horizontally from the source (Jenkinson and Parry, 1994).

In the artificially inoculated experiments, the inocula were sprayed directly on the flowering ears. The high amount of precipitation in the second environment during inoculation (June, see Table 3) might explain the differences in disease level between the environments. The major effect of N in these trials probably is on ear infection. This includes germination of macroconidia, infection and colonization of the ears. In general, FHB symptoms and DON content of the grains increased with increasing N input. As described above, changes in canopy density resulting in more optimal microclimatic conditions for infection (moisture) or a longer flowering period might explain these results.

The relationship between visual scores and DON contamination at different fertilizer rates as presented in Table 5 was weak in the first environment (Pearsons' correlation coefficient $r = 0.44$, $n = 3$) but stronger in the second environment ($r = 0.99$, $n = 5$). Based on the percentage of visually infected spikelets, no prediction of the absolute amount of DON contamination can be made. If the visual scores and the DON contents of both environments are compared at a N input rate of, e.g. 160 kg/ha, a lower DON level (10.9 mg/kg) was observed in the second environment although the visual score was higher (37.5%) than in the first environment (25.5%, see Table 5). 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. The appearance of premature ears might explain, at least in part, these data (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 is wilting, resulting in an increase in FHB symptoms and yield reduction as a result of 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) than the typical *Fusarium* damaged kernels (between 1 and 600 mg/kg). FHB sensitive genotypes such as 'Extradur' expressed extensive 'wilting' symptoms upon increasing the environmental infection pressure. This may result in a higher visual score to DON ratio.

The results further indicate that DON contamination during kernel development reached a maximum

around 40 days post inoculation. At 60 days after flowering, a small but non-significant decrease in DON content was observed. Such a decrease of DON concentration has been reported before. Snijders and Krechting (1992) compared the DON content in kernels harvested 28 and 58 days post inoculation. The susceptible genotype showed a decrease in mycotoxin content. The physiological mechanism is not known. A decrease in toxin content could be caused by breakdown of the toxin (catabolism) (Miller and Arnison, 1986), or conjugation of the mycotoxin to other cell components (Miller and Arnison, 1986; Gareis et al., 1990). Glucoside conjugation is the most common xenobiotic conjugation reaction in plants. Gareis et al. (1990) used the term 'masked mycotoxins' because the toxins are not detected with routine analytical procedures. This could lead to an apparent reduction of the toxin concentration.

In all the trials on four locations, N input rate had a significant effect on FHB development in wheat. After artificial inoculation, it was demonstrated that also DON contamination of the grains increased at fertilizer rates from 0 to 80 kg/ha. It is concluded, that FHB cannot be sufficiently controlled only by manipulation of the N input in practical crop husbandry.

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