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Epidemiology of *Fusarium* infection and deoxynivalenol content in winter wheat in the Rhineland, Germany

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Abstract

Details of our long-term research programme concerning the epidemiology of *Fusarium* spp. and mycotoxin production are summarized. Evaluation of the occurrence of *Fusarium* spp., mainly on winter wheat (*Triticum aestivum*), was carried out by investigating *Fusarium* infection and mycotoxin contamination. Two to 15% of grains were infested during 1995–1998 at three climatologically differing localities of the Rhineland, Germany. Disease progress was accelerated by rainfall during the flowering season. The species most frequently isolated were *Fusarium avenaceum*, *F. poae*, *F. culmorum* and *F. graminearum*. The mean deoxynivalenol (DON) content varied from 19 $\mu g\,kg^{-1}$ (1995) to 310 $\mu g\,kg^{-1}$ (1998) and was not always correlated with disease severity. Organic farming systems showed lower rates of infection with ear blight and lower mycotoxin contamination than conventional farming systems.

Introduction

Since the last decade, research work has been conducted on epidemiological aspects of ear blight in wheat and barley. Infection methods under controlled conditions were carried out to study the effect of different inoculum sources (Engels and Krämer, 1996), the climatological environments for disease intensity and the distribution of the fungi in plants during the different growth stages (Meier et al., 2000). Furthermore, different cultivars were studied concerning their susceptibilities to *Fusarium* infection (Stähle et al., 1998; Lienemann et al., 2000). Artificial infection experiments on barley with *Fusarium culmorum* and *F. avenaceum* indicated that infection rates on grains were highest when the inflorescence was inoculated, while lower rates were obtained when seeds and soil were infected during sowing time (Hindorf, 1995). Identification of different genera and species occurring on tissues of the wheat crop has been extended from morphological and biochemical characteristics to immunological characteristics by means of ELISA (Schwabe et al., 1993a) and latex agglutination tests (Schwabe et al., 1992; 1993b; 1994). Furthermore, molecular characteristics were used for identification with PCR (Schütze et al., 1997; Muthomi et al., 2000; Birzele et al., 2000a,b). The increased demand for data on mycotoxin contamination of grains led to the application of different methods of mycotoxin quantification, e.g. ELISA and chromatographic methods (Schwabe and Krämer, 1995; Berleth et al., 1998; Meier et al., 1999; Birzele et al., 2000a). The impact of suboptimal storage conditions on mycotoxin contamination and *Fusarium* biomass has also been investigated (Berleth et al., 1998; Backes and Krämer, 1999; Birzele et al., 1999, 2000a).

Methods and field sites

Investigations of the *Fusarium* mycoflora were carried out during the period 1995–1998 at three localities of

the Rhineland, Germany. To compare different farming systems, field experiments with organic and conventional farming systems at two neighbouring sites at Hennef, Sieg (altitude: 65 m, rainfall: 700–750 mm, mean temperature: 9.5 °C) were established. These sites differed in agricultural practices, fertilizers and pesticide input, but not in environmental conditions such as climate and soil. The site at Velbert, Bergisches Land (altitude: 240 m, rainfall: 1200 mm, mean temperature: 9.3 °C) is situated in a wet area and was under an organic farming system. The third site at Blankenheim, Eifel mountains (altitude: 500 m, rainfall: 860 mm, mean temperature: 7.3 °C) with marginal conditions for wheat growing was also chosen for investigations on organic farming.

Plant material and grains were taken for isolating *Fusarium* spp. Shrivelled and broken grains were investigated separately. The infection rate of grains, certified seeds and plant material was determined by incubating 200 grains/certified seeds/plant material pieces per cultivar on selective media. In order to determine the inoculum potential in the soil, organic particles of the soil were fractionated by size (Häni, 1979), surface disinfected and also incubated on selective media (Abildgren et al., 1987; Nirenberg, 1976). *Fusarium* species were differentiated microscopically according to Nelson et al. (1983). The deoxynivalenol (DON) content was quantified by a competitive ELISA, HPLC and also liquid chromatography with tandem mass spectroscopy (LCMSMS).

Results

The occurrence of *Fusarium* spp. in wheat ears depended mostly on the climatic conditions during flowering (Figures 1 and 2). Figure 2 shows rainfall and temperature at Hennef in June 1997 and 1998. In 1998, continuous rainfall during and after flowering, with lower temperatures and a longer period of open inflorescences, led to grain infection levels of 15% and an average DON content of 310 $\mu g\,kg^{-1}$ at Hennef (Figure 3). Compared to 1998, the days during and after flowering remained dry in 1997, apart from two instances of rain on the first two days of flowering (Figure 2). The average infection with *Fusarium* species in 1997 was lower (3.5–6.5%) and so was the mean DON content (115 $\mu g\,kg^{-1}$). For all three organic farming localities, infection rates were 6.5–15% during 1998 (Figure 1). In the very dry year of 1995, infection rates did not exceed 2–4% of the total harvested crop

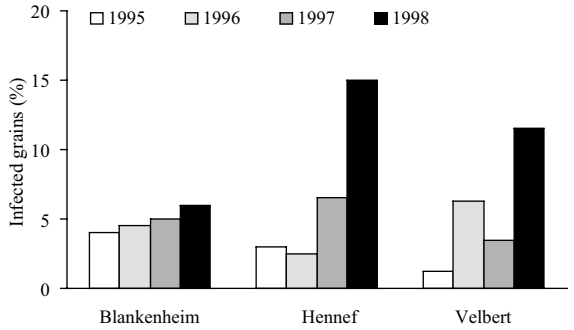

Figure 1. Occurrence of *Fusarium* spp. on winter wheat grains from 1995 to 1998 at three organic farming sites (Blankenheim, Hennef, Velbert).

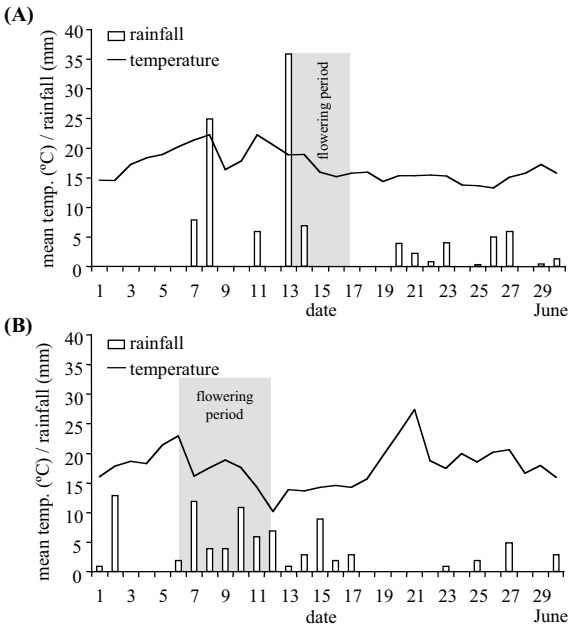

Figure 2. Climatic conditions during the flowering period of wheat at Hennef; (A) 1997, (B) 1998.

(Schade-Schütze, 1999) and the mean DON content was 19 $\mu g\,kg^{-1}$ (Backes and Krämer, 1999).

The comparison of infection rates of *Fusarium* species and DON contents showed that they were not always correlated. In 1998, for example, the content of DON was highest at Velbert, with a mean of 395 $\mu g\,kg^{-1}$ compared to 310 $\mu g\,kg^{-1}$ at Hennef. However, greatest *Fusarium* infection in that year was observed at Hennef, with 15% compared to 11.5% at Velbert (Figure 3). At Blankenheim, where conditions for wheat growing are marginal, *Fusarium* infection

and DON contents were lowest in 1998. This could be linked to the dry period during flowering, but also to comparatively low temperatures leading to low disease severity of *Fusarium* species and also other plant pathogens.

The different *Fusarium* species were scored and identified at all localities. The distribution of *Fusarium* species for example at Hennef, Rhineland, is shown in Figure 4. Out of the total *Fusarium* spp. infection rate of 6.5% (1997) and 15% (1998), *F. avenaceum* occured in both years at a frequency of 30% (1997) and 51% (1998). The percentage of both *F. culmorum* and *F. graminearum* was similar with 35% in 1997 and 37% in 1998. However, a shift of the population from *F. culmorum* to a higher percentage of *F. graminearum* was observed.

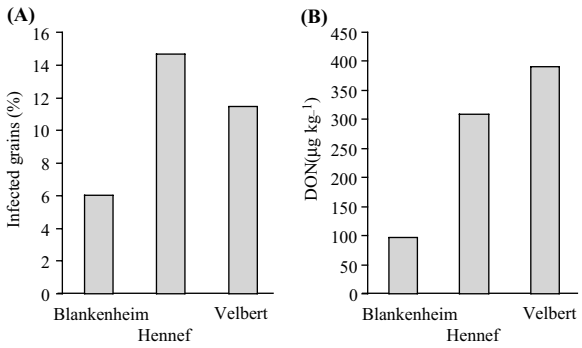

Figure 3. Comparison of (A) *Fusarium* grain-infection and (B) DON content of wheat grains in 1998 in organic farming systems at three localities (Blankenheim, Hennef, Velbert).

The low occurrence of plant pathogens and the dry weather conditions during flowering influenced the yield at Blankenheim in 1998, which was highest with 60 dt ha^{-1} in that specific year. Table 1 shows the yield figures of the organic farming system from 1995 to 1998: no conclusions on the influence of *Fusarium* ear blight on the yield could be made. Besides other parameters, rain, temperature and soil composition at Hennef were optimal for wheat growing, and therefore the mean yield was highest. At Velbert, low yields varying from 30 to 46 dt ha^{-1} with a mean of 41 dt ha^{-1}, were constantly achieved probably because of the high rainfalls. Yields at Blankenheim varied from 26 to 62 dt ha^{-1} showing high uncertainty for stable yields.

Investigations on certified seeds, soil, stem-bases, leaves of different growth stages, as well as grains, for the presence of *Fusarium* spp. (Figure 5) showed that *F. avenaceum* was detected in all samples, but to higher amounts in soil, leaves and grains. *F. graminearum* and *F. culmorum* were observed in soil, leaves and grains, but not in the certified seeds. This indicates that the infection of grains with *Fusarium* species primarily

Table 1. Average wheat yield at three localities (Blankenheim, Hennef, Velbert) in organic farming systems from 1995 to 1998

Localities	Yield (dt ha^{-1})				
	1995	1996	1997	1998	Average
Blankenheim	42	62	26	60	48
Hennef	58	75	61	57	63
Velbert	43	46	44	30	41

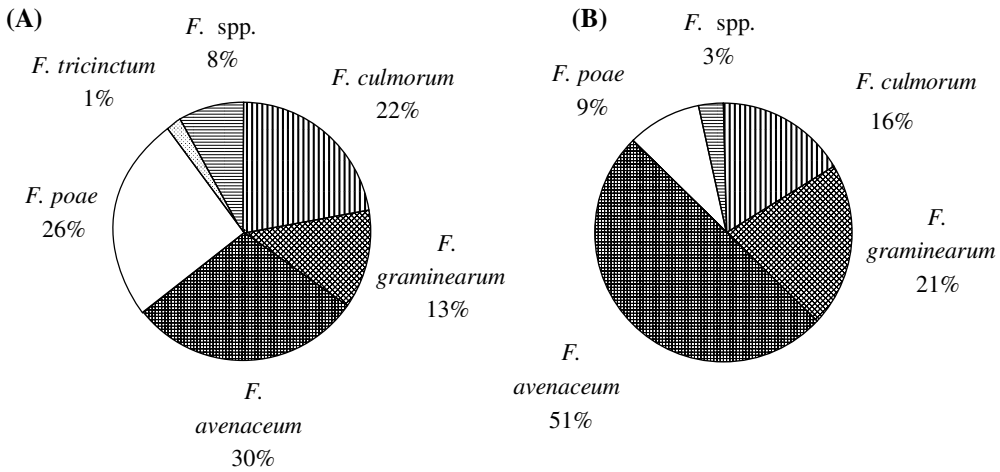

Figure 4. Occurrence of *Fusarium* spp. on wheat grains at Hennef (A) in 1997 and (B) in 1998.

originated from the soil ($r = 0.74$; $P \leq 0.01$), but not from certified seeds ($r = 0.24$; $P \leq 0.01$), from which wheat plants descended. Unlike *Fusarium* spp., *M. nivale* was not isolated from soil. Infection rates for *M. nivale* were highest at the stem base (89%), followed by grains (38%), leaves of GS 75-85 (26%) and certified seeds (6%). *M. nivale* infection on grains and certified seeds correlated ($r = 0.88$; $P \leq 0.01$) confirming that infection with *M. nivale* starts on the certified seeds.

Comparing the occurrence of *Fusarium* and the content of DON in organic and conventional farming systems at Hennef (Figure 6), these were higher in the conventional system, where no specific fungicide was used to control ear blight in the years 1997 and 1998. Application of fungicides during flowering, e.g. tebuconazole, reduced the disease intensity of ear blight to 2/3 of the untreated fields. The organic farming field site showed a much lower occurrence of *Fusarium* and DON content than the conventional farming sites.

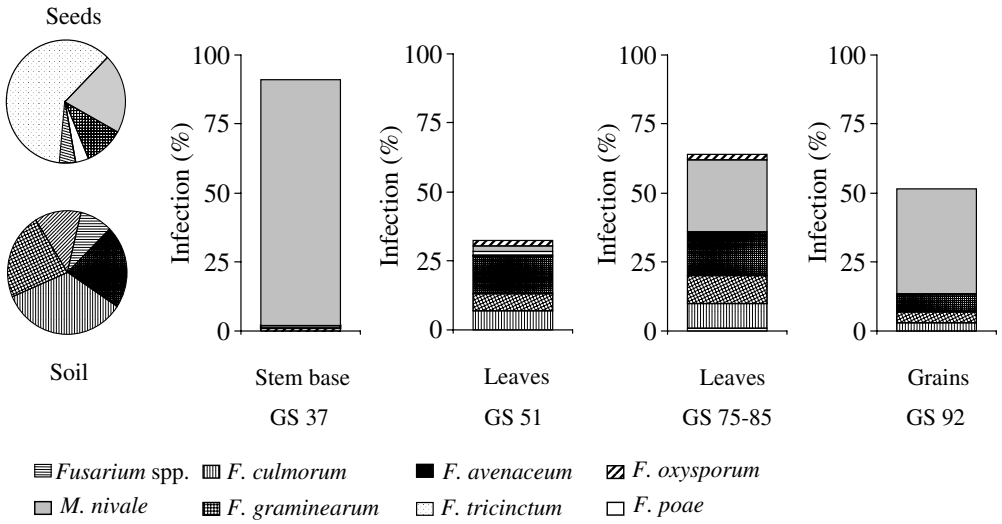

Figure 5. *Fusarium* species identified from soil, certified seeds, plant tissues and grains of wheat at Hennef in 1998.

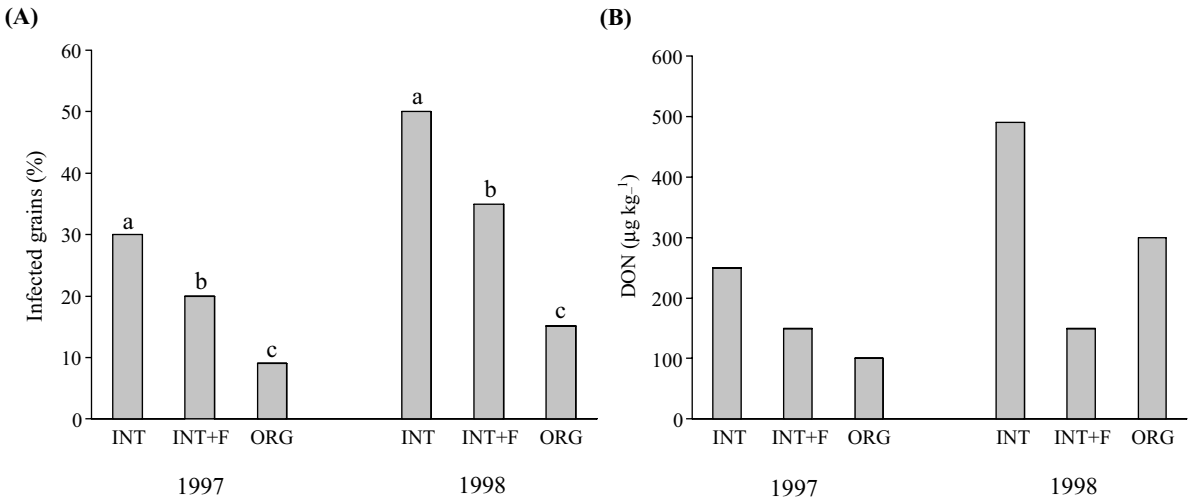

Figure 6. Occurrence of (A) *Fusarium* spp. on wheat grains and (B) DON content of grains in an organic and a conventional farming system at Hennef in 1997 and 1998 (INT = conventional without fungicide control, INT + F = conventional with fungicide control, ORG = organic farming); Tukey test, $P \leq 0.05$.

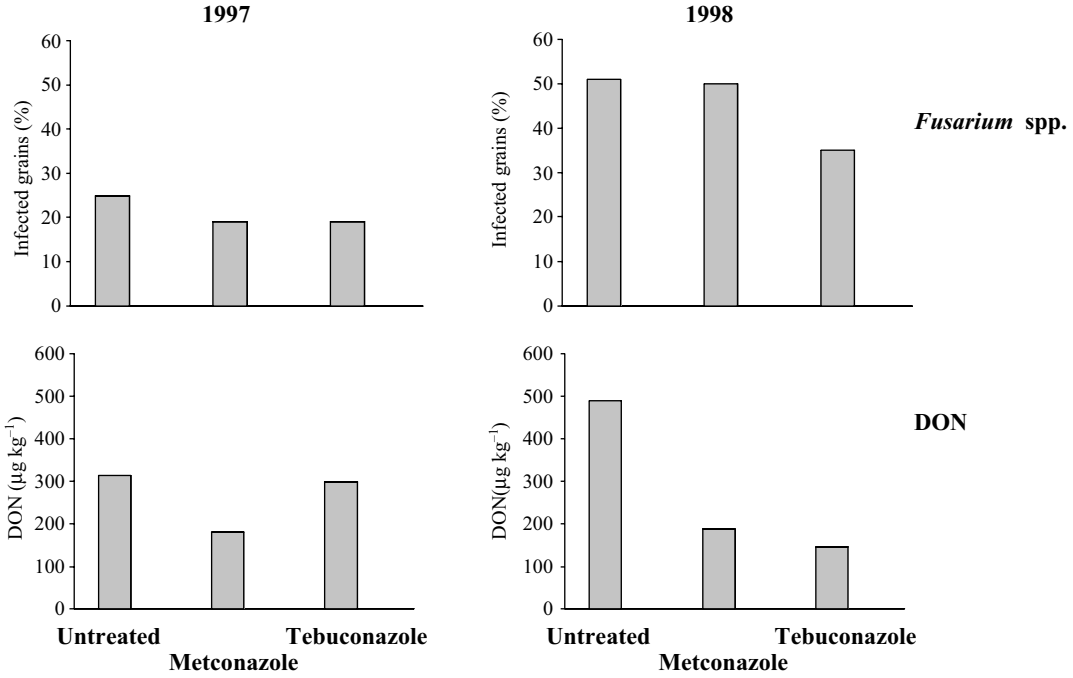

Figure 7. Effect of fungicides on the infection of wheat grains with *Fusarium* spp. and the grain DON content at Hennef in 1997 and 1998.

However, in 1998, when *Fusarium* infection of grains reached 50% in the conventional farming site, fungicide application led to lower DON contents compared to the organic farming site.

In conventional farming systems, the ability of modern fungicides to control ear blight was assessed. During the two years of experiments, metconazole and tebuconazole were applied at the recommended doses at flowering. A certain success was achieved in reducing ear infection at harvesting time (Figure 7). In 1998, high overall infection rates of wheat grains with *Fusarium* spp. were reduced by the application of tebuconazole. DON content of the grains was reduced with both metconazole and tebuconazole. Interestingly, with the lower grain infection levels in 1997 the effectiveness of the fungicides for reducing disease and reducting DON content was lower.

Discussion

At the organic farming sites, *Fusarium* infection of grains varied between 2% and 15% during 1995 and 1998. The occurrence of *Fusarium* spp. in the ear depended mostly on climatic conditions during flowering. In 1998, at Hennef, constant rainfall during and after flowering resulted in an average infection rate of grains of 15% and a mean DON content of 310 µg kg^{-1}. Conclusions on the influence of *Fusarium* ear blight on yield could not be made at infection levels of grains between 2% and 15%. Whereas no correlation was found between the incidence of *Fusarium* spp. on certified seeds and grains ($r = 0.24$; $P \leq 0.01$), there was a strong correlation between the incidence of *M. nivale* on certified seeds and grains ($r = 0.88$; $P \leq 0.01$). A correlation was also determined for the incidence of *Fusarium* spp. on crop residues in the soil (organic particles) and the grains ($r = 0.74$; $P \leq 0.01$). This indicates that the infection of grains mainly originates from the soil, while infection with *M. nivale* originates from the certified seeds.

Comparing the organic farming with the conventional farming system, *Fusarium* infection of grains was significantly higher in the conventional farming system irrespective of fungicide treatment. In years such as 1998, with extremely high infection levels of grains (50%) in the conventional farming system, fungicide treatment reduced the DON levels in the grain below those from the organic farming site. In 1997, when average infection rates of grains occurred, DON contents remained lowest in the organic farming system. Further research on the influence of different

fungicides on *Fusarium* infection and DON contents is needed.

Our long-term research work on the occurrence of *Fusarium* spp. on wheat and their mycotoxin production will continue. Further work will study regional effects on the intensity of *Fusarium* spp. Methods will be improved for the detection of the fungi and their mycotoxin production. Besides microscopy, molecular methods such as PCR and quantitative PCR, have been developed for the precise diagnosis of different species. Other points of interest are the epidemiology of the infection process during flowering and the penetration of the fungus into the plant. Furthermore, sources of inoculum and their distribution as well as the influence of agricultural practices are of particular interest. As a result of the field experiments carried out in organic and conventional farming systems in the Rhineland, the influences of crop rotation, cultivar susceptibility, soil preparation (tillage), weed populations as alternate hosts for *Fusarium* spp., the use of fungicides on the infection rate of *Fusarium* spp. and contamination with DON have been investigated.

The influence of sub-optimal storage conditions on the infection rate of *Fusarium* spp., *Penicillium* and *Aspergillus* spp. will be further investigated and common mycotoxins such as DON, nivalenol (NIV), T-2 toxin and ochratoxin A (OTA), produced by *Penicillium* and *Aspergillus* species, will be analysed in relation to fungal biomass. Furthermore, investigations on the influence of fungi, mycotoxins and sub-optimal storage of wheat on the baking quality and rheological properties of contaminated flours will be continued as part of our research on food quality and food safety.

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