Variation in the Production of Trichothecene Mycotoxin Deoxynivalenol (DON) in Spring Barley Varieties after Treatment with the Fungicides Azoxystrobin and Tebuconazole

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Abstract

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Eight varieties of spring barley (*Hordeum vulgaris* Lin.) were artificially inoculated with a *Fusarium culmorum* (W.G. Smith) Saccardo – isolate and naturally infected in the middle of the flowering period, and 2 d later treated with the fungicides azoxystrobin or tebuconazol at a dose of 1 l/ha in 250 l of water. In both control and treated samples of grain the content of deoxynivalenol (DON), the main trichothecene mycotoxin produced by *F. culmorum,* was determined by gas chromatography (GC-ECD). The treatment with either fungicide resulted in elevated levels of DON, an effect that was more pronounced with azoxystrobin.

Keywords: deoxynivalenol; *Fusarium culmorum*; azoxystrobin; tebuconazole

The influence of fungicides on the production of the trichothecene mycotoxin deoxynivalenol (DON) has been fairly intensively studied. Magan *et al.* (2002) examined the *in vitro* efficacy of fungicides to control *Fusarium* species in cereals and the effect in the field on both *Fusarium* infection of ripening ears and mycotoxin production. The field studies suggested that fungicides such as tebuconazole and metconazole provide good control of both *Fusarium* infection of ears and DON production. Azoxystrobin and related fungicides were less effective and grain from treated crops sometimes had an increased concentration of nivalenol and deoxynivalenol. Studies with *F. culmorum* isolates from different parts of Europe showed a complexity of interactions occurring between environmental factors, the type of fungicide and isolate in relation to growth inhibition and DON production. These studies confirmed the ineffectiveness of azoxystrobin and suggested that environmental stress

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factors, particularly water availability and suitable temperature, and low fungicide doses may stimulate mycotoxin production by *Fusarium*. D'Mello *et al*. (1998) induced a four-fold increase in the mycotoxin concentration by 0.1 g/ml tubuconazole. Similar results were obtained with *F. culmorum* and difenoconazole, which had no effect on fungal growth but increased the production of deoxynivalenol at 25°C but not at 11°C. The authors also compared strains of *F. culmorum* that were either resistant or sensitive to difenoconazole. Overall, both strains produced 3-Ac deoxynivalenol in the presence of difenoconazole at 0.1 g/ml (in the culture medium), although the production was slower in resistant strains. Hope *et al*. (2000) had applied the fungicides azoxystrobin and propiconazol to wheat grain and found significantly increased production of DON in the presence of the fungicides. Siranidou and Buchenauer (2001) stated that treatment of the plants with azoxystrobin reduced the disease index of spikes, but the DON content of the grain was higher than that of the untreated control. Bauer (2000) applied the fungicide Amistar (azoxystrobin) on wheat. He emphasised that weather conditions during flowering that favour *Fusarium* infection have to be considered as a risk factor leading to higher toxin levels.

Azoxystrobin, the active ingredient in the fungicide Amistar (at 0.4 l/ha), was used to control quality and quantity of *Triticum durum* in Italy; the foliar treatment safeguarded the quality of the grain (Lanzellotti 2002). Azoxystrobin (Amistar F) was also used in a mixture with Agat-25 K (*Pseudomonas aureofaciens*) against septoriose and rusts in winter wheat. Pirgozliev *et al*. (2002) and Jorgensen (2001) studied the effect of azoxystrobin and metconazole on *Fusarium* head blight and on the accumulation of DON in wheat grain. There was no evidence that fungicide applications directly increased the concentration of DON of the grain. Azoxystrobin in two full dosages was used by Jorgensen and Olesen (2002) for the protection of wheat against fungal diseases. The increase in straw yield as a result of fungicide treatment was relatively low. Azoxystrobin (250 g/ha) was also successfully used as a spray against take-all in wheat caused by *Gaeumannomyces graminis* var. *tritici* (Jenkyn & Gutteridge 2002), and for the protection of spring malting barley (Overthrow 2002) and to improve grain quality. Azoxystrobin (strobilurin) reduced the occurrence of *Rhynchosporium secalis* on winter barley (Cooke *et al*. 2002).

The aim of our work was to show the differences between DON concentration after azoxystrobin's treatment of spring barley and after any treatment.

MATERIAL AND METHODS

Azoxystrobin was purchased from Syngenta Ltd. (GU 27 3JE Guildford, UK) and tebuconazol from Bayer AG (Leverkusen, FRG). Their purities were confirmed by gas chromatography (GC). We used eight varieties of spring barley for monitoring the contamination of grain by the trichothecene mycotoxin deoxynivalenol (DON) after artificial and natural infection. The varieties (Forum, Amulet, Tolar, Olbram, Akcent, Nordus, Kompakt and Jersey) had approximately the same date of maturity; the seed was certified. The barley (*Hordeum vulgare* L.) was sown after the three different pre-crops sugar beet, winter wheat and maize, the grain was harvested and crop residues were ploughed shallowly into the soil. The experiments were performed for 3 years. The experimental variants were sown into Latin quarters (at a size of 5 × 4 m with three replications). Every fungicide treatment had a control. The weather at time of inoculations was rainy. The symptoms on the seed of barley were estimated from the increasing degree of dark discolouration.

The strain of *Fusarium culmorum* (W.G. Smith) Saccardo used by us was high DON producing. Cultures to prepare inoculum for the trials were grown in laboratory conditions, on Czapek-Dox agar at 20°C. Barley plants were inoculated at flowering (yearly in 2000–2003) two times during one day with wet rainy weather. The isolate had the origin in the barley cultivated in Prague. The suspension of inoculum contained 1 million conidia/1 ml. This concentration appeared sufficient without being too heavy. Two days after inoculation the plots were treated by spraying (100 ml/5 m^2) with the fungicides tebuconazole (formula, 250 g tebuconazole/1 l, in a dose 1 l/ha, 250 l of water) or azoxystrobin (formula, 250 g azoxystrobin/1 l, 1 l/1 ha, 250 l of water), a second application followed a day later. The size of analysed sample was 5 g. Solid phase extraction (SPE) using MycoSepTM≠225 column was used for the clean-up of acetonitrile-water (84:16, v/v) sample extracts. Volatilisation of analytes prior to the determinative step was performed by trifluoroacetic acid anhydride (TFAA). Trifluoroacyl

derivatives of the trichothecenes were separated by high resolution capillary gas chromatography employing electron capture detector (GC/ECD). The detection limit of the target analyte was 5 µg/kg.

The statistical programme Statgraphics +4.0 was used for the evaluation of differences in the content of deoxynivalenol as dependent on fungicide treatment, pre-crops and varieties of spring barley.

RESULTS AND DISCUSSION

Grain from the varieties of spring barley grown after different pre-crops (sugar beet, winter wheat, maize) and after artificial inoculation with *F. culmorum* strongly differed in the level of deoxynivalenol (DON). The levels were significantly higher after maize as a pre-crop than after winter wheat and sugar beet. Disease symptoms were also much higher when maize was the previous crop. This may have been caused by natural infection. We do not know whether *F. graminearum* was frequent in the grains of barley after maize. Our study is in fact more one of the interaction between artificial inoculation by *F. culmorum* and natural infection through the effect of the previous crop rather than only the effect of inoculation by *F. culmorum.*

The concentration of DON in the seed after the maize pre-crop rose from 200 µg/kg in untreated controls of the variety Amulet to 2900 µg/kg of the variety Akcent treated with azoxystrobin. With winter wheat as a pre-crop the content of DON in the untreated varieties Olbram, Akcent and Jersey was 0, but when treated with azoxystrobin it was 90 µg/kg in the variety Tolar. After sugar beet, DON content was 0 in the untreated varieties Forum and Jersey and the variety Jersey treated by tebuconazole, while the variety Tolar treated by azoxystrobin contained 150 µg/kg. Figure 1 summarises the data on DON levels in grain of the eight cultivars of spring barley after different pre-crops (maize, winter wheat and sugar beet). Maximum values were found with maize as a pre-crop, while only a slight increase occurred after winter wheat and sugar beet. The mycotoxin content after maize is probably due to very high natural infection from the large amount of plant residue and the strong incidence of *Fusarium* in this material.

Multiple Range Tests of DON levels showed that the differences between azoxystrobin and tebuconazol treatment and also between azoxystrobin and the non-treated control were statistically significant, while the differences between tebuconazol and the control were small and statistically not significant. The level of DON in the grain of spring barley was much higher after maize as pre-crop, and was also significantly higher in the variety Kompakt than in the other varieties, including Jersey, Olbram and Amulet. With the statistical method used there is a 5% risk of calling each pair of means significantly different although the actual difference may be 0.

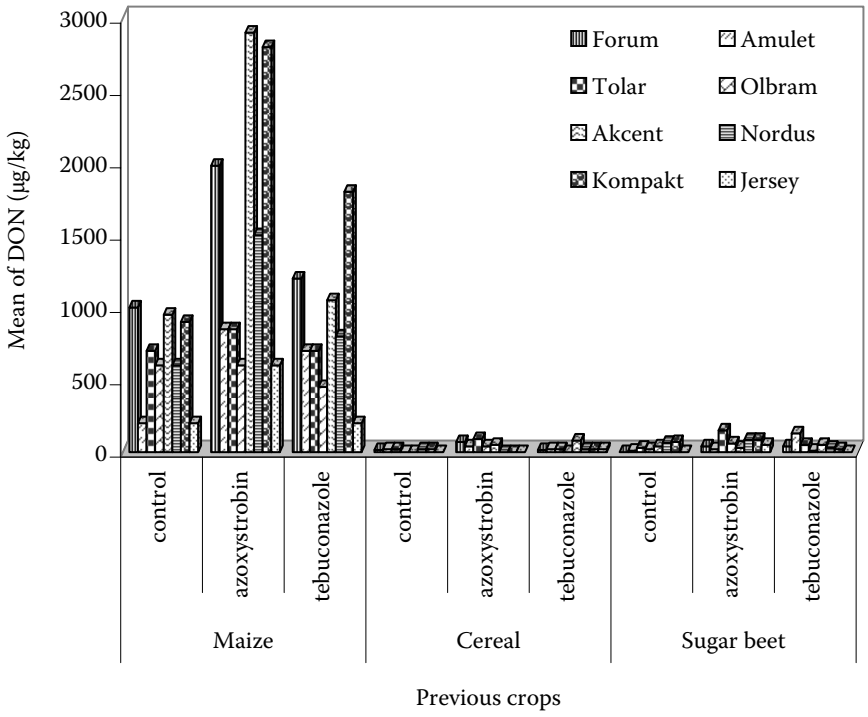

Figure 1. The mean level of deoxynivalenol in different varieties after artificial infection with *Fusarium culmorum* and fungicide treatments

Variances are probably not homogenous between previous crop groups.

Magan *et al*. (2002), who had examined the *in vitro* efficacy of selected fungicides (tebuconazole and metconazole), proved that they influenced the levels of DON in the substrate. Our present study, on grain from spring barley treated with a fungicidal preparation with tebuconazole as active ingredient, yielded similar results but the increase of DON levels was even more pronounced than in Magan's study. In other studies (Hope *et al*. 2000; Siranidou & Buchenauer 2001), the levels of DON were also higher after treatment with a formulation containing azoxystrobin that was reported to be very effective in protecting *Triticum durum* against fusarioses (Lanzellotti 2002). In addition, azoxystrobin very effectively protected wheat (Jenkyn & Gutteridge 2002), spring barley (Overthrow 2002) and winter barley (Cooke *et al*. 2002) against take-all disease. Besides the compounds tested in our experiments, other fungicides, e.g. the chemically similar tubiconazole, also increased the production of DON (D'Mello *et al*. 1998). On the other hand, it should be noted that in some studies azoxystrobin formulations did not enhance the production of DON (Bauer 2000; Dovgalenko 2002; Pirgozliev *et al*. 2002), probably due to different experimental conditions and/or different strains of pathogens. In our opinion, the higher levels of DON in grain after the application of tebuconazole or azoxystrobin formulations may be the consequence of stress put on the fungi by these chemicals, a stress that was absent in the infected control samples with no fungicidal spray.

When comparing the reactions of individual barley varieties, in some cases their infestation by *Fusarium* was high (e.g. varieties Olbram and Tolar) and the level of mycotoxin was also high; in other cases (e.g. varieties Kompakt and Nordus) the infection level was low but the production of mycotoxin was still relatively high. Thus, we grouped the varieties by considering two criteria: (*i*) the reaction of the variety to infection by *Fusarium* as judged by the appearance of the grain of the untreated controls, with S = susceptible and R = resistant; and (*ii*) the level of mycotoxin (DON) found in the grain, which could either be substantially higher than that found in the untreated control (Toxin high), or was about equal to that found in the control (Toxin low). Applying these criteria the varieties could be divided into four groups (Table 1).

Two groups contain varieties that could be characterised as being susceptible to fungal infection. While in the first the levels of DON in the varieties after treatment with the fungicide were increased typically and sometimes drastically above those found in the untreated controls, in varieties of the second group the levels of mycotoxin had remained close to or essentially equal to that of the controls. The second pair of groups encompasses varieties that are more resistant to the pathogen. Yet again, there was a group of varieties with an increase of mycotoxin, and in another group its level had stayed about the same.

Thus, in spring barley the level of accumulation of DON appears to be independent from the reaction of the variety to the fungus that produces the mycotoxin. The chemical composition of cereal tissues is determined by many genetic factors which form the basis of variety. Generally, the metabolism of the plants can be influenced by e.g. a high level of nitrogen and other nutritive elements. The influence of mycotoxins on the structure of plant tissues, mostly surface structures, could depend on the age of the plants and also on the variety. Some varieties (Kompakt, Forum) accumulated toxin quite strongly, others (Tolar, Olbram) accumulated it rather weakly. The latter varieties, though relatively susceptible to the infestation by *Fusarium*, are resistant to accumulation of the mycotoxin. This lower accumulation could possibly be due to degradation in the plant. In some varieties artificially infected with *F. culmorum* and treated with azoxystrobin the increase in DON was higher than after treatment with tebuconazole. This may be due to the stress caused by both pathogen and

Table 1. Combinations of some characteristics in eight varieties of spring barley infected with *Fusarium culmorum*

Reaction to fungal infection	Change in level of mycotoxin	
	toxin level high	toxin level low
S (susceptible)	var. Akcent, Amulet	var. Olbram, Tolar
R (resistant)	var. Forum, Kompakt	var. Jersey, Nordus

fungicide treatment. Finally it has to be stressed that inoculation with *F. culmorum* was not the only factor, but natural inoculum from the pre-crop maize also played a serious role.

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Abstrakt

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Různé odrůdy jarního ječmene byly uměle infikovány *Fusarium culmorum* ve fázi kvetení po ošetření fungicidy obsahujícími azoxystrobin a tebuconazol v dávce 1 l/ha v 250 l vody. Obsah deoxynivalenolu (DON) byl stanoven plynovou chromatografií v kontrolách i v ošetřených variantách. Ošetření jarního ječmene fungicidními preparáty obsahujícími azoxystrobin nebo tebuconazol v některých případech vedlo ke zvýšení hladin deoxynivalenolu v zrnu. Tento efekt byl výraznější u azoxystrobinu.

Klíčová slova: deoxynivalenol; *Fusarium culmorum*; azoxystrobin; tebuconazol

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