SHORT COMMUNICATION

Progression of Deoxynivalenol Concentrations in Spikes and Kernels of Winter Wheat Cultivars after Inoculation with *Fusarium culmorum*

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Abstract: Progression of deoxynivalenol (DON) concentrations in spikes and kernels was studied in relation to Fusarium head blight (FHB) symptoms in five winter wheat cultivars, differing in resistance to FHB, after single floret inoculation with an aggressive isolate of *Fusarium culmorum*. After inoculation in field conditions the spikes were detached from the plant and kept in the greenhouse under controlled conditions. High concentrations of DON were detected in susceptible cultivars at an early stage of pathogenesis (7 days after inoculation). Over the whole examined 21-day period and also at maturity spikes contained more DON than kernels. While differences between cultivars in the accumulation of DON were highly expressed already 7 days after inoculation, differences in symptomatic reactions were not clear until day 21. Owing to the reported crucial role of DON at early stages of pathogenesis, the importance of appropriate timing of fungicide application is highly stressed.

Keywords: Fusarium head blight; symptoms; accumulation of deoxynivalenol; resistance; winter wheat

Fusarium head blight (FHB) is a devastating disease in most wheat-growing countries resulting in yield and quality loss, and contamination by mycotoxins. Deoxynivalenol (DON), belonging to trichothecene mycotoxins, is the most frequent toxin reaching the highest concentration levels also in the conditions of Central Europe. Tebuconazole- and metconazole-based fungicides were reported to suppress FHB and accumulation of mycotoxins, however, cereal protection by these products was not sufficient under all conditions (Mesterházy *et al.* 2003; Šíp *et al.* 2004). Though more effective prothioconazole fungicides are now available, evidently the control of this complicated disease cannot rely only on a single measure and should consider different factors that influence FHB progression and accumulation of mycotoxins. Kang & Buchenauer (2002) documented that the accumulation of secondary metabolite DON in host tissues might start very soon (36 h after inoculation). The trials of Ludewig *et al.* (2005) in growth chambers revealed the maximum rise of the DON content in the examined wheat cultivars as early as three days after inoculation. Kernels contained less DON than the rachis and chaff. Progression of DON and zearalenone (ZON) concentrations in straw of wheat infected with *Fusarium culmorum* was analyzed by Brinkmeyer *et al.* (2005). DON was produced in higher concentrations and at earlier stages, whereas ZON was

Supported by the Ministry of Agriculture of the Czech Republic, Project No. MZE 0002700602.

formed later and in smaller amounts. Sidorov *et al.* (1996) found the highest DON concentration in rachides 14 days after inoculation. DON concentrations in kernels of susceptible cultivars increased between 14 and 26 days after inoculation and then decreased at maturity.

The objective of our study was to analyze the progression of contamination by DON in spikes and kernels of winter wheat cultivars after inoculation with *Fusarium culmorum* in relation to the development of FHB symptoms.

MATERIAL AND METHODS

Progression of DON concentrations and FHB symptoms was studied in 5 winter wheat cultivars with varying levels of resistance to FHB. On the basis of long-term studies the cultivar Arina could be classified as resistant to medium resistant, Šárka and Ebi as medium resistant to medium susceptible and Saskia and Siria as susceptible to FHB. The used isolate B (Stupice) of *Fusarium culmorum* can be characterized as an isolate with medium to high aggressiveness (Šíp *et al.* 2002a, b). The plants were grown in field conditions. At full anthesis (Zadoks scale 65) one floret of the central spikelet was inoculated with 40 µl of pathogen conidial suspension (0.8×10^7/ml) using a pipette (Finnpipette® – Thermo Electron Corporation). The spikes were covered with polythene bags immediately after inoculation and then detached from the plants at the second internode from the top, sterilized with a spirit solution (70%) and placed in the greenhouse. Detached spikes were kept in pans with water upgraded with Floralife following the manufacturer's instructions (Floralife® Cut Flower Food – Europe GmbH), which guaranteed optimal development of plant units. The solution of water with Floralife was changed every three days. The spikes were kept at 100% humidity for 3 days after inoculation (in polythene bags) and then at 90–95% humidity for 18 days at the air temperature of 22–25°C. Ripening took place at 25°C and at 60–75% humidity till the 45th day after inoculation. The spikes and kernels were analysed for DON content in 7, 14, 21 and 45 (at maturity) days after the inoculation. The examined sample of each variety contained 20 spikes for the analysis of DON content in dry matter of the whole spike (rachis, chaff, kernels) and 15 spikes for the analysis of DON content in dry matter of excised kernels. Drying was carried out at 45°C for 18 h. A routine reference method was used to determine moisture content (ČSN ISO 712 Cereals and Cereal Products – Determination of Moisture Content). The content of DON was determined by ELISA on RIDASCREEN® FAST DON kits from R-Biopharm GmbH, Darmstadt, Germany (Šíp *et al.* 2004). DON content in each sample was adjusted according to moisture content in ripe kernels, which was 9%. The visual scoring of FHB symptoms (VSS) was based on estimates of the percentage of infected spikelets in a spike in 7, 14 and 21 days after inoculation.

RESULTS AND DISCUSSION

Single floret inoculation is considered as suitable for studies of host resistance to an infection spread within a spike (Type II) and to accumulation/ degradation of DON in kernels (Type III) (Wisniewska *et al.* 2004). It follows from data presented in Table 1 that the described method enabled to reach high concentrations of DON in

Table 1. Average data on DON content in spikes and kernels (mg/kg) and % of infected spikelets (VSS) in 7, 14, 21 and 45 days after inoculation with *F. culmorum* obtained from 5 winter wheat cultivars

	Days after inoculation							
	7		14		21		45	
	average	%	average	%	average	%	average	%
DON content in spikes	50.7	59.2	68.3	79.7	77.1	90.0	85.7	100
DON content in kernels	41.3	70.6	42.1	72.0	58.4	99.8	58.5	100
% of infected spikelets	7.6	16.5	18.0	39.0	46.0	100		

Columns '%': DON content: % of the value obtained in 45 days after inoculation; % of infected spikelets: % of the value obtained in 21 days after inoculation

spikes and kernels, which was not common when the single floret inoculation technique was used in field conditions (Šíp *et al.* 2002b). However, it is necessary to mention that DON concentrations were probably influenced by kernel development in detached spikes. Relatively lower kernel weight was obtained using this technique, but all the cultivars were similarly affected. The conditions (temperature, relative humidity, light) in the greenhouse could be considered as favourable for disease development. It is a great advantage that similar conditions can be created for further studies that will undoubtedly be needed.

Table 1 clearly shows that the progression of DON concentrations in spikes and kernels differed from the development of symptoms. It is documented that a high amount of DON can be produced very soon, during the first week after inoculation, when symptoms usually occur only on spikelets in the close proximity of the infection site and the percentage of infected spikelets in a spike is generally low (in these experiments below 10%). 70.6% of DON content in ripe kernels was detected in dry matter of kernels excised 7 days after inoculation. A high increase occurred in these experiments between day 14 and day 21 (up to 99.8%). Over the whole examined period (since the 7th day after inoculation) average concentrations of DON were relatively higher in dry matter of spike (rachis, chaff and kernels) than in kernels, similarly like in the experiments of Ludewig *et al.* (2005). These authors suggested that DON might play its role especially during earlier and mid stages of pathogenesis and enhance fungal aggressiveness (play a role as a virulence factor) (Desjardins *et al.* 1996). The finding that trichothecene production contributes to the virulence of *Fusarium graminearum* (Proctor *et al.* 2002) led to a conclusion that it may be possible to generate plants that are resistant to this fungus by increasing their resistance to trichothecenes.

Differences in reactions of the studied winter wheat cultivars to the infection with isolate B of *Fusarium culmorum* are shown in Figure 1. It is obvious that relatively lower accumulation of DON in spikes and also a lower percentage of infected spikelets in a spike during the whole examined period were characteristic of the moderately resistant cultivar Arina. Relatively higher resistance to DON accumulation (particularly at an early stage of pathogenesis) was also detected in the cultivar Šárka, similarly like in previous experiments in field conditions when this inoculation technique was used (Šíp *et al.* 2002b). On the contrary, susceptible reactions were detected in cultivars Ebi, Siria and Saskia in 7 days after inoculation. Though differences in DON accumulation were

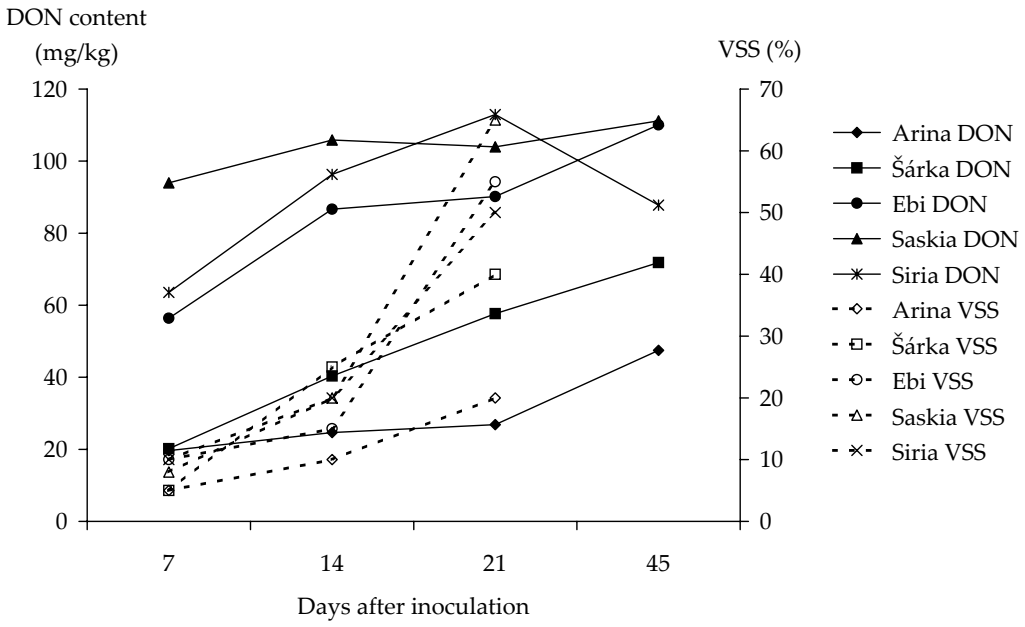

Figure 1. Average DON content in spikes of 5 winter wheat cultivars in 7, 14, 21 and 45 (maturity) days after inoculation with *Fusarium culmorum* and corresponding data on the percentage of infected spikelets in the spikes (VSS)

evident as early as 7 days after inoculation, differences in symptomatic reactions between cultivars were not very pronounced until day 21. The drop in DON content in kernels at maturity, described e.g. by Sidorov *et al*. (1996), and the ability of the cultivar to degrade trichothecene mycotoxins in kernels (resistance Type III – Mesterházy 1995) could not be evaluated in these experiments because the period between day 21 after inoculation and maturity was not examined on this study. Ludewig *et al.* (2005) reported a rapid increase in the DON content in wheat spikes during the first 20 days of pathogenesis. Then DON values increased more slowly and reached a maximum in about 50 days after inoculation.

It is suggested that studies of this type may improve the knowledge of resistance mechanisms and have also implications for disease control. These experiments, similarly like the other studies cited here, showed very early contamination of spikes and kernels by DON particularly in susceptible wheat cultivars and therefore the importance of appropriate timing of fungicide application in agricultural practice is stressed. A survey of DON content in grain samples collected from different districts of the Czech Republic, performed in 2003–2005, clearly showed the ineffectiveness of delayed fungicide applications (Chrpová *et al.* 2006). The experiments also indicate the superiority of DON accumulation prediction. Hooker *et al.* (2002) demonstrated the importance of short time periods around heading (4–7 days before heading and 3–6 days after heading) for the prediction of DON content. Models based on weather variables in these periods can be considered as desirable for decisions on the use of fungicides.

Acknowledgements. The authors express thanks to Mrs. M. Vlčková, M. Berová and Š. Bártová for technical assistance.

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Received for publication October 18, 2006
Accepted after corrections November 9, 2006

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