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Deoxynivalenol, nivalenol and moniliformin in wheat samples with head blight (scab) symptoms in Poland (1998–2000)

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Abstract

Fusarium head blight (scab) epidemics of wheat occurred in Żuławy (Northern Poland) during 1998 and in Wielkopolska (West) and in Southern regions of Poland in 1999. Four species were identified in wheat heads with scab symptoms: *Fusarium culmorum*, *Fusarium graminearum*, *Fusarium avenaceum* and *Microdochium nivale*. A significant increase in the frequency of *F. graminearum* (between 23% and 38%), was observed, compared to about 10% during the previous decade. The mycotoxins deoxynivalenol (DON), nivalenol (NIV) and moniliformin (MON) in amounts up to 24.3, 14.2 and 1.72 mg kg^{-1}respectively, were identified in kernels samples.

Introduction

Fusarium head blight (scab) of small grain cereals, particularly wheat and barley, was recently found to be a re-emerging disease with significant reduction of yield and contamination of grain with *Fusarium* mycotoxins (Bai and Shaner, 1994; Bottalico, 1998; Chełkowski, 1998; Jones and Mirocha, 1999; McMullen et al., 1997; Parry et al., 1995). Damage from head scab is multifold: reduction in seed quality, contamination with several mycotoxins (mostly deoxynivalenol (DON)), reduced yield, and the formation of discoloured and shrivelled kernels. A salmon-pink to red fungal growth with sporodochia may be seen along the edge of the glumes or at the base of the spikelet.

Scab has been reported during the 20th century and since epidemics on wheat in 1980–1982 in USA and Canada, DON has been recognized as a *Fusarium* mycotoxin contaminating scabby kernels (Chełkowski, 1998; Jones and Mirocha, 1999; McMullen et al., 1997). Later, *Fusarium* head blight caused significant losses during 1991–1995, both in United States and in Canada (McMullen et al., 1997; Jones and Mirocha, 1999).

The last high incidence of small grain cereal scab in Poland was observed in 1974 (Chełkowski, 1998). Since 1985, our research group has evaluated the percentage of heads affected with the disease. Samples of infected heads were collected and examined to identify species of *Fusarium* sporulating in sporodochia on chaff surfaces as well as colonizing kernels. Mycotoxins have been identified and quantified in chaff and kernels (Chełkowski, 1998; Goliński et al., 1996; Perkowski et al., 1991; 1997; Sharman et al., 1991; Visconti et al., 1986; Wakuliński and Chełkowski, 1993).

In previous studies (Chełkowski, 1998; Wakuliński and Chełkowski, 1993) during 1985–1989, the percentage of individual species in affected wheat heads was: *F. culmorum* 30%, *F. avenaceum* 30%, *F. graminearum* 10% and *M. nivale* 30%. In diseased kernels, trichothecene mycotoxins were most frequently present, with DON as the dominating contaminant, followed by its acetylated derivatives and nivalenol (NIV) (Chełkowski, 1998; Perkowski et al., 1991;

Perkowski et al., 1997; Visconti et al., 1986). Occurrence of NIV was of lower significance than DON. Moniliformin (MON) has been frequently found in wheat grain in Poland, since its first detection in 1990 (Goliński et al., 1996; Sharman et al., 1991). *Fusarium* head blight epidemics during 1998, in Żuława (North of Poland) encouraged us to examine the fungal species causing the disease and to analyse occurrence of mycotoxins (DON, NIV, MON) in kernels in the following three years.

Materials and methods

Samples

Fields of wheat were inspected in July and August in 1998–2000. A short time before harvest, samples of wheat heads were collected: 61 in 1998, 68 in 1999 and 45 in 2000. They were placed in paper bags and transported to the laboratory. In heads containing pink sporodochia, *Fusarium* species were identified using light microscopy according to the manuals of Nelson et al., 1983 and Kwaśna et al., 1991. Heads were threshed using a laboratory threshing machine. DON and NIV were analysed in samples of kernels from heads infected with *F. culmorum* and/or *F. graminearum*, while MON concentrations were analysed in heads infected with *F. avenaceum*.

Chemical analyses

Finely-ground kernels were extracted with acetonitrile–methanol–water (16 : 3 : 1 v/v/v) using 5 ml of the solvent per 1 g of samples. Extracts were de-fated with n-hexane (3 × 50 ml), and were then concentrated. Half of the extract was used for MON analysis while the remaining part was used to analyse DON and NIV. The extract for MON was purified on Florisil columns (Kostecki et al., 1995). Moniliformin content was estimated on Merck 5554 silica-gel thin-layer chromatography plates with developing solvent 2-propanol–butanol–water–ammonium hydroxide (12 : 4 : 1 : 1). After spraying with 3-methyl-2-benzothiazolinonehydrochloride (MBTH), the intensity of the dark spots on the chromatogram was compared with that of the metabolite standard. To analyse the trichothecenes (DON, NIV), extracts were purified by filtration on a column (Celite 545–charcoal Darco G-60–neutral alumina 3 : 9 : 5 w/w/w) conditioned with acetonitrile-water (82 : 18 v/v). DON and NIV were eluted with the same solvent. Moniliformin, DON and NIV were analysed by HPLC using Waters-501 apparatus with C-18 Nova Pak column (3.9 × 300 mm) and a Waters-486 UV detector (λ_{max} = 224 nm for DON and NIV; λ_{max} = 229 nm for MON). DON and NIV were eluted with 25% water solution of methanol (flow rate 0.7 ml/min) after retention times of 11.72 and 7.46, respectively. Detection limits were: DON and NIV = 0.01 mg/kg. Regarding MON, acetonitrile–water (15 : 85; v/v) buffered with 10 ml of 0.1 M K_2HPO_4 in 40% t-butyl-ammonium hydroxide per 1 litre of solvent was applied as mobile phase (flow rate 0.6 ml/min). Retention time of MON was 11.5 min, detection limit 0.01 mg/kg. Positive results (on the basis of retention times) were confirmed by HPLC analysis with internal standards. Recoveries for NIV, DON and MON were: 75, 87 and 90% respectively.

Sequence characterised amplified regions (SCAR) method of identification of Fusarium *species*

Sporodochia from the chaff surface of one head, after the species had been identified by microscopy, were scratched into an Eppendorff tube. DNA was extracted using a modified CTAB method (Doohan et al., 1998). After grinding the material with a pestle, 500 µl of CTAB buffer with 0.4% 2-mercaptoethanol were added, followed by adding 50 µl of chloroform-octanol (24 : 1/vol.). Samples were incubated for 25 min in a water bath set at 65 °C. Subsequently, after addition of 50 µl of chloroform-octanol, samples were mixed, left at room temperature for 10 min and centrifuged 10 min at 11.000 rpm. The aqueous upper phase was transferred to a new Eppendorff tube. DNA was precipitated with ice-cold ethanol (99.98%) and left in the refrigerator for 2 h. The precipitate was centrifuged at 11.000 rpm for 10 min, supernatant was removed and DNA was washed carefully with cold ethanol (96%) and again centrifuged 5 min at 11.000 rpm. DNA was dried and re-dissolved with 100 µl of TE buffer pH = 8.0. The extracts were stored at −20 °C until used.

Species-specific primers and PCR conditions for all three species were described by Schilling et al., 1996 and Chełkowski et al., 1999. Each sample contained 1 U of Taq DNA polymerase (Finnzymes), 2.5 µl of PCR buffer, 12.5 pmol of forward/reverse

primers, 2.5 mM of each dNTP and about 10 ng of fungal DNA. Amplification products were electrophoresed in 1.5% agarose gel (Amersham Biotech) with ethidium bromide at 50 V for about 3 h and visualised under UV light and photographed (Syngen UV visualiser).

Results and discussion

In 1998, *Fusarium* head blight was observed in significant number of fields in the Żuława region, with about 20% of ears exhibiting visible disease symptoms: pink, abundant sporodochia on chaff, shrivelled and discoloured kernels (*Fusarium* damaged kernels = FDK). The following frequencies of fungi were observed in affected heads: *Fusarium graminearum* 20%, *F. culmorum* 16%, *F. avenaceum* 31% and *Microdochium nivale* 33% (Table 1). The identification of the three *Fusarium* species by microscopy was confirmed by SCAR (Table 1). Furthermore in 9 samples, where *Fusarium* species were not identified by using microscopy (because abundant mycelium contained no macroconidia), the SCAR method was used successfully to identify species in infected heads. In the Wielkopolska region, *Fusarium* head blight incidence in 1998 was below 1% of affected heads on most of the fields. However, the frequency of *Fusarium* species in infected wheat heads was similar to that in the Żuława region (Table 1).

In 1999, epidemics of *Fusarium* head blight occurred in the Wielkopolska region, 25 years after the previous epidemics observed in 1974 (Chełkowski, 1998), with about 20% of affected heads in most of wheat fields. The frequencies of species infecting heads in this region were the following: *F. graminearum* 38%, *F. culmorum* 7%, *F. avenaceum* 52% and *M. nivale* 3% (Table 1). The incidence of FHB in 1999 at Żuława was low – about 1% of heads were infected with up to 5% in some fields. The frequency of species infecting ears was the following: *F. graminearum* 26%, *F. culmorum* 10 %, *F. avenaceum* 29% and *M. nivale* 35% (Table 1). The significant increase of the frequency of *F. graminearum* in Wielkopolska (38%) and Żuława (26%) should be emphasised both. In the previous decade, *F. graminearum* had not been identified in any samples originating from the northern part of the country (Chełkowski, 1998; Perkowski et al., 1991; Visconti et al., 1986; Wakuliński and Chełkowski, 1993). DON, NIV and MON were present in kernels from all regions in both years in amounts (Tables 1–3).

In wheat grain samples in Poland, DON and 3-acetyldeoxynivalenol (3AcDON) were, for the first time, identified in 1985 (Visconti et al., 1986), while zearalenone had been found in 1980 (Chełkowski et al., 1983). The frequency and amounts of zearalenone were found to be low (Perkowski et al., 1990; 1991; 1997). A high correlation was found between the percentage of *Fusarium* damaged kernels (FDK) and the amounts of DON in wheat grain samples in consecutive years 1986–1989 (Perkowski et al., 1991).

Moniliformin was identified for the first time in Polish cereals in 1990 (Sharman et al., 1991) and in each subsequent year it was a frequently occurring metabolite in wheat samples, although at low concentrations – significantly below 1 mg kg^{-1} (Chełkowski, 1998; Goliński et al., 1996). In the present survey, the highest amounts of MON were detected in samples originating from the Żuława region – up to 0.72 mg kg^{-1} in 1998 and 1.72 mg kg^{-1} in 1999. High levels of MON (above 10 mg kg^{-1}) were found only in samples of *Fusarium* damaged kernels (Sharman et al., 1991). Acetylated derivatives of DON 3- and 15-AcDON, detected in previous survey in wheat samples at very low amounts (Perkowski et al., 1990) were not analysed in samples collected at 1998 and 1999.

Taking into consideration their frequency and concentration, the most important mycotoxins detected in field samples of wheat kernels was DON, occurring in 60–65% of samples in 1998 and in 89–95% of samples in 1999 (Table 1). NIV was detected at high concentrations, up to 14.2 mg kg^{-1} only in 1998, when this metabolite was present in 80–85% of samples. Simultaneous co-occurrence of the three mycotoxins was found in a high percentage of positive samples (33% and 35%), during 1998 and 1999.

The results of this survey confirm the possibility of high contamination of wheat kernels with *Fusarium* mycotoxins under epidemic conditions in Poland. Samples collected from fields not affected with scab contained low amounts of mycotoxins – significantly below 1 mg kg^{-1} (Goliński et al., 1996). High frequency of *M. nivale* infecting heads was associated with low concentration of DON, NIV and MON. In 2000 *Fusarium* scab was not observed in the fields because of very dry weather conditions.

Table 1. Natural contamination of wheat samples with *Fusarium* species and *Fusarium* mycotoxins during seasons 1998 and 1999

Year	Region	Distribution of fungal species (%)				Percentage of positive samples and *Fusarium* mycotoxins concentrations								
		*Fusarium** *graminearum*	*Fusarium** *culmorum*	*Fusarium** *avenaceum*	*Microdochium nivale*	DON			NIV			MON		
						Percentage	Concentration (mg kg^{-1})		Percentage	Concentration (mg kg^{-1})		Percentage	Concentration (mg kg^{-1})	
							Range	Average		Range	Average		Range	Average
1998	Żuławy	20	16	31	33	65	0.01–0.76	0.19	85	0.01–0.63	0.18	66	0.01–0.72	0.12
1998	Wielkp.	23	25	25	26	60	0.02–15.3	3.71	80	0.02–14.2	1.08	20	0.01–0.13	0.08
1999	Żuławy	26	10	29	35	95	0.03–14.01	2.27	57	0.04–0.32	0.15	100	0.01–1.72	0.34
1999	Wielkp.	38	7	52	3	89	0.05–24.29	2.61	30	0.02–0.21	0.08	66	0.02–0.85	0.19

*In all 30 head samples, affected by *Fusarium,* identification by microscopy was confirmed using SCAR.

Table 2. Percentage of samples contaminated with three mycotoxins (DON+NIV+MON), two mycotoxins (DON + NIV or DON + MON or NIV + MON) and one *Fusarium* mycotoxin in wheat kernels samples collected in 1998 and 1999

Year	Toxins detected							
	DON, NIV, MON	DON, NIV	DON, MON	NIV, MON	DON	NIV	MON	ND
1998								
Number of samples	20	17	0	8	2	6	3	5
Percentage (%)	32.8	27.9	0	13	3.3	10	5	8
1999								
Number of samples	24	1	25	1	12	0	2	3
Percentage (%)	35.3	1.5	36.8	1.5	17.6	0	2.9	4.4

Table 3. DON, NIV and MON concentrations in three groups of samples collected in 1998 and 1999

Year	Toxins detected and concentration range								
	<1 mg kg^{-1}			1 mg kg$^{-1} \leq \geq 5$ mg kg^{-1}			≥ 5 mg kg^{-1}		
	DON	NIV	MON	DON	NIV	MON	DON	NIV	MON
1998									
Number of samples	55	60	61	3	0	0	3	1	0
Percentage (%)	90.2	98.4	100	4.9	0	0	4.9	1.6	0
1999									
Number of samples	30	68	66	29	0	2	9	0	0
Percentage (%)	44.1	100	97.1	42.7	0	2.9	13.2	0	0

References

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

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

Chełkowski J, Goliński P, Mańka M, Trojanowska K, Wiewiórowska M and Szebiotko K (1983) Mycotoxins in cereal grain. Part IX. Zearalenone and *Fusaria* in wheat, barley and corn kernels. Die Nahrung (Food) 27: 526–531

Chełkowski J (1998) Distribution of *Fusarium* species and their mycotoxins in cereals grains. In: Sinha KK and Bathnagar (eds) Mycotoxins in Food and Agricultural Safety (pp 45–64) Marcel Dekker, New York

Chełkowski J, Bateman GL and Mirocha CJ (1999) Identification of toxigenic *Fusarium* species using PCR assays. Journal of Phytopathology 147: 307–311

Doohan FM, Parry DW and Jenkinson P (1998) The use of species-specific PCR-based assays to analyse *Fusarium* ear blight of wheat. Plant Pathology 47: 197–205

Goliński P, Perkowski J, Kostecki M, Grabarkiewicz-Szczçsna J and Chełkowski J (1996) *Fusarium* species and *Fusarium* toxins in wheat in Poland – comparison with neighbour countries. Sydowia 48: 12–22

Jones RK and Mirocha CJ (1999) Quality parameters in small grains from Minnesota affected by Fusarium head blight. Plant Disease 83: 506–511

Kostecki M, Grabarkiewicz-Szczçsna J, Chełkowski J and Wiśniewska H (1995) Beauvericin and moniliformin production by Polish isolates of *Fusarium* subglutinans and natural co-occurrence of both mycotoxins in maize samples. Microbiologie-Aliments-Nutrition 13: 67–70

Kwaśna H, Chełkowski J and Zajkowski P (1991) *Fusarium* (Sierpik) Vol XXII MYCOTA, Institute of Botany, Polish Academy of Sciences, Warszawa-Kraków

McMullen M, Jones R and Gallenberg D (1997) Scab of wheat and barley: A re-emerging disease of devastating impact . Plant Disease 81: 1340–1348

Nelson PE, Toussoun TA and Marasas WFO (1983) *Fusarium* species. An illustrated manual for identification. The Pennsylvania State University Press. University Park and London

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

Perkowski J, Plattner RD, Goliński P, Vesonder RF and Chełkowski J (1990) Natural occurrence of deoxynivalenol, 3-acetylodeoxynivalenol and zearalenone in polish wheat. Mycotoxin Research 6: 7–12

Perkowski J, Chełkowski J, Błażczak P, Snijders CHA and Wakuliński W (1991) A study of correlations between the amount of deoxynivalenol in grain of wheat and triticale and percentage of *Fusarium* damaged kernels. Mycotoxin Research 7A: 102–114

Perkowski J, Stachowiak J, Kiecana I, Goliński P and Chełkowski J (1997) Natural occurrence of *Fusarium* mycotoxins in Polish cereals. Cereal Research Communication 25: 483–487

Schilling AG, Möller EM and Geiger HH (1996) Polymerase chain reaction-based assays for species-specific detection of *Fusarium culmorum*, *F. graminearum* and *F. avenaceum*. Molecular Plant Pathology 86: 515–522

Sharman M, Gilbert J and Chełkowski J (1991) A survey of the occurrence of the mycotoxin moniliformin in cereal samples from sources worldwide. Food Additives and Contaminants 8: 459–466

Visconti A, Chełkowski J and Bottalico A (1986) Deoxynivalenol and 3 acetyldeoxynivalenol – mycotoxins associated with wheat head fusariosis in Poland. Mycotoxin Research 2: 59–64

Wakuliński W and Chełkowski J (1993) *Fusarium* species causing scab of wheat, rye and triticale in Poznań. Hodowla Roślin, Aklimatyzacja i Nasiennictwo 37(4): 137–142