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CONTAMINATION OF WHEAT GRAINS WITH SPECIES OF GENERA *FUSARIUM* IN DIFFERENT LOCALITIES OF SLOVAKIA IN 2006–2008

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The frequency and relative density of occurrence of *Fusarium* spp. was evaluated on 112 wheat grain samples from different agro-ecological localities in Slovakia. The samples were collected in 2006, 2007 and 2008 from the same farmers and from the same localities every year immediately after harvest. In 2006, contamination was 95.2%, in 2007 it was 64.3%, and 71.4% in 2008. The highest average frequency of occurrence was found in *Fusarium graminearum* in 2006 – 65%. The prevalence of *Fusarium poae* was ascertained in 2007 and 2008. The highest frequency of *Fusarium* spp. occurrence was revealed in locality Turčiansky Ďur in 2008 – 53.9%. The highest identified amount of *Fusarium* species (12) was from the area of Turčiansky Ďur in 2007. *Fusarium graminearum*, *Fusarium sporotrichioides*, *Fusarium poae* and *Fusarium oxysporum* were the most frequent in 2006, while *Fusarium poae*, *Fusarium sporotrichioides*, *Fusarium graminearum* and *Fusarium semitectum* dominated in 2007. *Fusarium poae* dominated in 2008, then followed *Fusarium sporotrichioides*, *Fusarium graminearum*, *Fusarium oxysporum* and *Fusarium avenaceum*. Other identified species, such as *Fusarium equiseti*, *Fusarium tricinctum* and *Microdochium nivale*, were in population structure in a relatively low density. Grains contaminated with *Fusarium* spp. are unsuitable for both human and animal consumption because of the adverse health effects of fusariotoxins..

Key words: *Fusarium*, frequency of occurrence, year, locality

Several Fungi of *Fusarium* genus, as important plant pathogens, attack wheat during growth and storage, if the grain is not dried sufficiently (Stenglein 2009). The intensity of disease incidence is heavily dependent on weather conditions during vegetation, forecrop and tillage method. In the stage of flowering and grain development wheat is most susceptible to Fusarium Head Blight (FHB) and the key role is played by weather conditions. Wheat grains are colonized by several *Fusarium* species and form a complex with other species of microscopic fungi of *Alternaria, Epicoccum, Botrytis* or *Aspergillus* genera (Tančinová et al. 2009). In Europe, the prevaling species are *F. culmorum* (W. G. Smith) Saccard, *F. graminearum* Schwabe, (*Gibberella zeae* Schwein (Petch), *F. avenaceum* (Fries) Saccardo (*Gibberella avenaceae*) and *F. poae* (Peck) Wollenweber (Nicholson et al. 1997, cit. Vogelgsang et al. 2008; Zemánková & Lebeda 2001; Bottalico & Perrone 2002). Species composition is different not only in crops, but it is also related to climatic conditions of the locality. The prevailing species in colder areas are *F. culmorum*, *F. avenaceum*, *F. poae*, in warmer areas dominate *F. graminearum*, *F. solani* (Martius) Appel & Woll. emend. Snyder & Hansen (*Nectria haematococca*), *F. equiseti* (Corda) Sacc., *F. oxysporum* Schltdl. Emend Snyder & Hansen. The frequency of occurrence and the spectrum of species are not stable, they vary depending on the year, varietal composition and development of weather in the time of infection and vary also in different countries. In the period from 1976 to 1981 the most frequent species on wheat was *F. avenaceum* in Estonia. Results of the analysis of the

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years 2002–2003 revealed a change in species composition, the most frequently occurring species was *F. semitectum* Berkely & Ravenel, followed by F. *poae, F. culmorum* and *F. avenaceum* (Lőiveke et al. 2003). Changes in the structure of species were observed in wheat samples in the Netherlands between 1980 and 1990, prevailing species was *F. culmorum*. Analyses from the years 2000 and 2001 confirmed the prevalence of *F. graminearum* before *F. culmorum* and *M. nivale* (*F. nivale*, *Monographella nivalis* (Schaffnit) E. Műll.) (Waalwijk et al. 2003). In Germany in 2003, occurred mainly *F. graminearum* (52%), *F. poae, F. avenaceum, F. equiseti, F. tricinctum* (Corda) Sacc. (*Gibberella pulicaris* (Fr.) Sacc.). Since 1990, the main species in Poland was *F. poae* (64%), followed by *F. tricinctum* (15%), *F. avenaceum* (8%), *F. culmorum* (6%) and *F. graminearum* (4%) (Goliński et al. 1997). Overall, the level of FHB before 2000 was low in the south of Poland, in 2005 and 2006 five times higher frequency of *F. graminearum* and reduced frequency of *F. culmorum* were recorded (Tomczak et al. 2002; Stepień et al. 2008). In the years 2003–2005, 92.1% of wheat and barley samples collected from different localities in Lithuania were contaminated by fungi of *Fusarium* (Mačkinaitë et al. 2006). The spectrum of species varied depending on climatic conditions and type of cereals. Contamination of barley grains was higher than contamination of wheat grains and reached 97.1%. Changes of species spectrum were also found between the years, in 2003 prevalent species isolated from the surface of wheat grains were *F. moniliforme* J. Sheld (*Gibberella moniliformis* Wineland), *F. avenaceum*, *F. graminearum* and *F. oxysporum*, in 2004 *F. poae*, *F. sporotrichioides* Scherb. and *F. equiseti*, in 2005 *F. graminearum* was dominating, while *F. equiseti* has not been isolated. According to the study of Hýsek et al. (1999) *F. culmorum* was the dominant species in the Czech Republic. Weather changes start also to affect the spectrum of *Fusarium* species, *F. graminearum* began to prevail (Ostrý et al. 2004) and recent studies show the increasing representation of *F. poae*. Harmfulness of *Fusarium* fungi lies not only in deterioration of technological, nutritional and hygienic quality of production. Conditions suitable for the development of *Fusarium* spp. are also suitable for the production of mycotoxins in grain (Doohan et al. 2003; Xu 2003). Limitation of *Fusarium* spp. incidence and hence also mycotoxins incidence in grain can be partially eliminated by compliance with agro-technical measures, timely application of fungicides yet at a stage when the disease is on the leaves as well as by proper storage of grain. Monitoring of *Fusarium* spp. occurrence and prediction of disease incidence by phytopathological and molecular analyses should be important factors. Wheat products are an indispensable part of human and animal nutrition and production of healthy food must be an essential part of a healthy environment.

The goal of the study was to evaluate contamination of wheat grain from different agro-ecological localities of Slovakia in the years 2006 to 2008 and find prevalence of *Fusarium* spp. in the studied localities and years.

MATERIAL AND METHODS

Grain samples

Grain samples of commercial cultivars of winter wheat, a total of 112, came from 10 localities of Slovakia situated in different agro-ecological conditions. Samples were taken directly from the producers immediately after harvest in 1 kg quantities from the land, where the standard procedures were used, including application of fungicides. In the years studied wheat grain samples were always supplied by the same producer, from the same locality but from different fields in accordance with crop rotation. In the studied years the number of samples varied: in 2006, 21 samples were processed, in 2007, 42 samples and in 2008, 49 samples.

Isolation and identification of fungi of Fusarium genus

Grains of wheat samples were surface-sterilized in 1% NaOCl solution for 2 minutes. They were subsequently rinsed three times in re-distilled water and cultured in Petri dishes (Ø 8 cm) on potato-dextrose agar (PDA) in a biological thermostat at 23°C for 5–7 days. For each sample, 100 randomly selected grains were tested. From the developed colonies mycelium was re-isolated and re-cultured in Petri dishes on synthetic nutrient medium (SNA) under UV-light, photoperiod 12 hours by day/12 hours by night, temperature of 24°C. To determine the species the classical identification based on microscopic characteristics according to the laboratory manuals Gerlach and Nirenberg (1982) and

Leslie and Summerell (2006) were used.

The frequency of occurrence and relative density of Fusarium spp.

Studied indicators were calculated according to formulas by Gonzáles et al. (1996):

Frequency of occurrence (Fr %) = (ns / N) × 100

ns = number of samples in which genus or species were detected,
N = total number of samples.

Relative density (RD %) = (ni / Ni) × 100

ni = number of isolates of genus or species,
Ni = total number of detected isolates.

Relative density in percentage (arcsin transformation) were statistically evaluated by analysis of variance (ANOVA) using SPSS software.

RESULTS AND DISCUSSION

The determination of *Fusarium* spp. was made from 112 analyzed samples of wheat grain. In the period from 2006 to 2008, contaminated samples formed 73.2%. The highest contamination was found in 2006 (95.2%), the lowest in 2007 (64.3%). Totally 12 *Fusarium* species have been identified: *F. graminearum*, *F. poae*, *F. sporotrichioides*, *F. culmorum, F. tricinctum, F. semitectum, F. avenaceum, F. sambucinum, F. oxysporum, F. equiseti, F. compactum* and *M. nivale*. Due to contamination by other microscopic fungi not all the species of *Fusarium* have been identified in some samples, in different years their frequency was from 0.3 to 1.3%. In 2006, on average, the highest frequency of occurrence was recorded in *F. graminearum*, followed by *F. sporotrichioides* and *F. poae* (Fig. 1). The same order of the species was in the relative density of occurrence (Table 1). Regarding the localities, the highest average frequency of occurrence was in Sládkovičovo in 2006 (52.8%, Fig. 2), seven *Fusarium* species have been identified. The 100% frequency was in *F. graminearum, F. poae, F. sporotrichioides, F. semitectum, F. oxysporum* and *F. compactum*, the species producing trichothecenes of the groups A and B. The location Veľký Meder follows with the average occurrence frequency of 48.8%, Spišské Vlachy (45.8%) and Abrahám (33.3%). The area in which Veľký Meder is located is the area with higher temperatures and in 2006 there was also relatively high rainfall (Table 4). These

Table 1

The relative density [%] of *Fusarium* species in 2006 from wheat grain surface in Slovakia

Locality	*Fusarium* species												Locality RD [%]*
	gram	poae	sporo	culm	tric	semit	aven	samb	oxys	equi	com	Mn	
Abrahám	14.8	0.0	8.8	0.0	0.0	1.8	0.0	0.0	8.8	0.0	5.3	0.0	3.2
Veľký Meder	5.3	0.0	5.3	4.0	2.7	2.7	0.0	0.0	2.7	0.0	1.3	0.0	2.0
Želiezovce	5.3	2.6	2.6	0.0	0.0	2.6	0.0	0.0	0.0	0.0	4.0	0.0	1.4
Sládkovičovo	4.6	16.3	9.3	0.0	4.6	2.3	0.0	0.0	2.3	0.0	2.3	0.0	3.4
V. Ripňany	5.6	0.0	5.6	0.0	0.0	0.0	0.0	0.0	1.9	0.0	0.0	0.0	1.0
Turčiansky Ďur	7.6	5.5	2.5	0.0	0.0	0.0	0.0	0.0	0.0	5.5	0.0	7.6	2.3
Malý Šariš	0.0	9.3	2.6	0.0	0.0	0.0	0.0	0.0	2.6	0.0	0.0	1.3	1.3
Spišské Vlachy	9.0	5.4	5.4	0.0	3.6	0.0	7.3	0.0	5.4	5.4	0.0	0.0	3.4
Spišská Belá	7.1	7.1	2.3	0.0	0.0	0.0	7.1	0.0	0.0	4.7	0.0	7.1	2.9
Vranov nad Topľou	7.2	5.4	3.6	0.0	0.0	0.0	3.6	0.0	5.4	0.0	0.0	0.0	2.1
Average	6.6	5.2	4.8	0.4	1.1	0.9	1.8	0.0	2.9	1.6	1.3	1.6	2.3

gram – *F. graminearum*; poae – *F. poae*; sporo – *F. sporotrichioides*; culm – *F. culmorum*; tric – *F. tricinctum*; semit – *F. semitectum*; aven – *F. avenaceum*; samb – *F. sambucinum*; oxys – *F. oxysporum*; equi – *F. equiseti*; com – *F. compactum*; Mn – *M. nivale*; * not significant differences between the means (ANOVA, Duncan test, p=0.05)

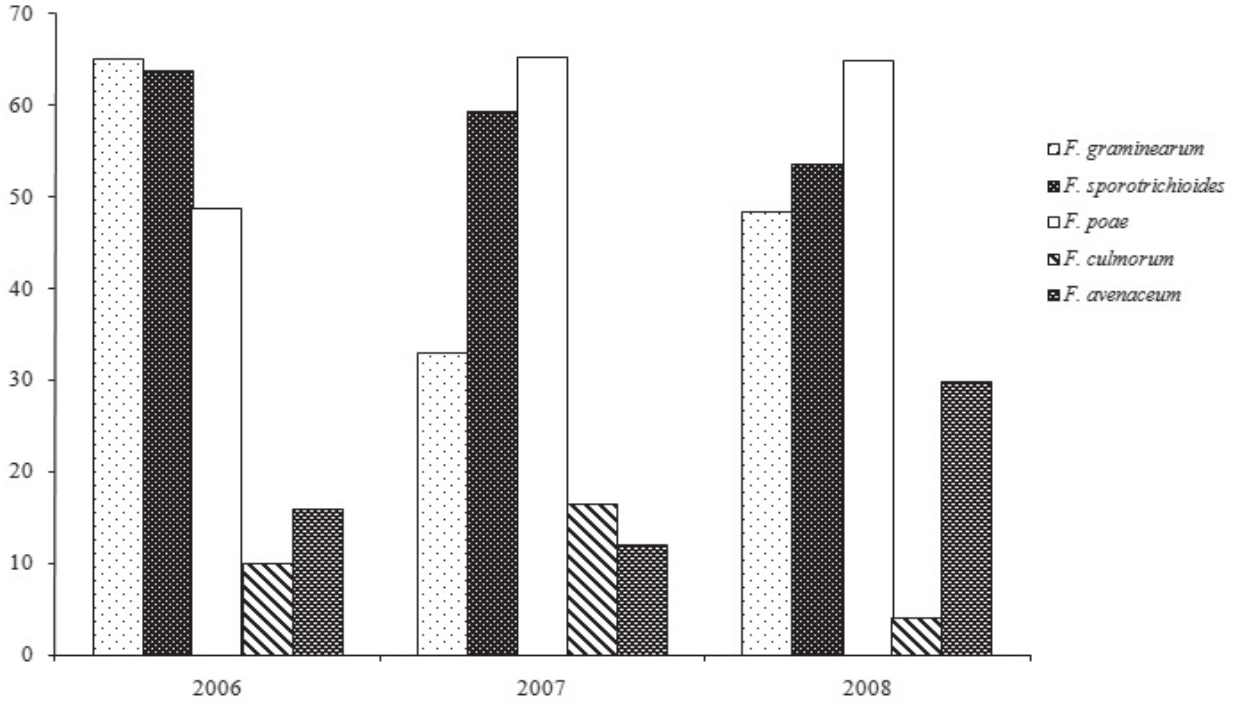
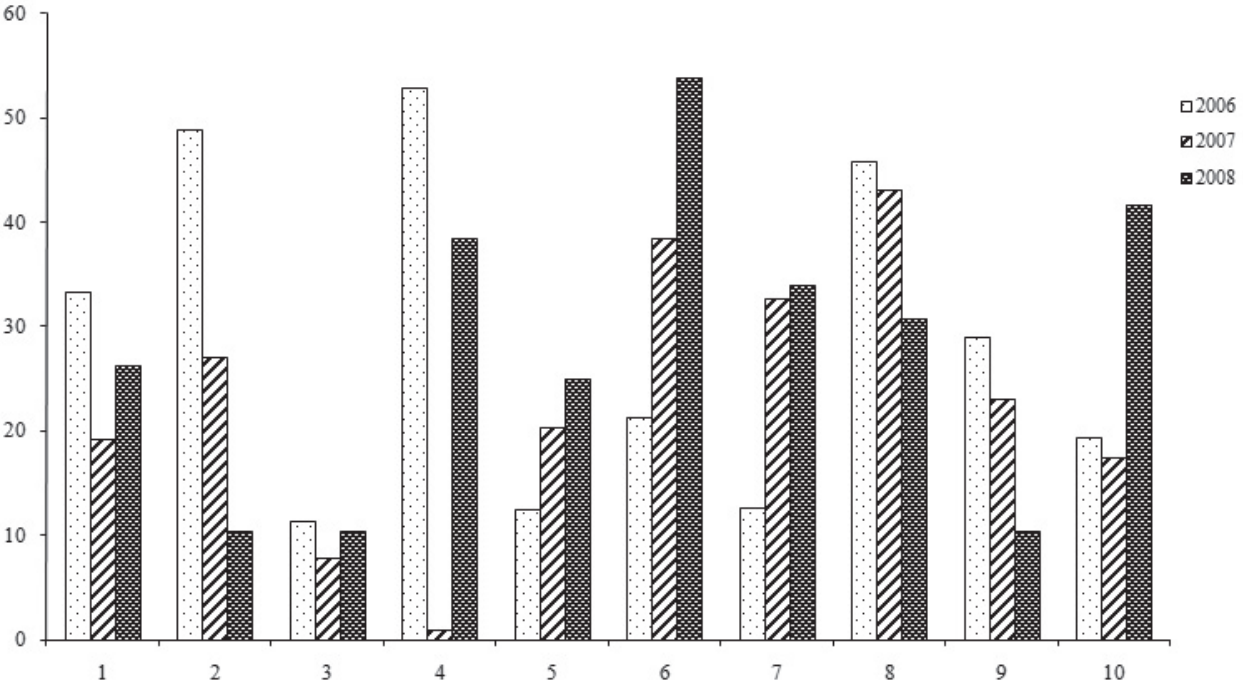


Fig. 1. The average frequency (%) of occurrence of *Fusarium* species in 2006–2008 from wheat grain surface re-isolated on SNA medium in Slovakia (without minor species)



1–Abrahám; 2–Veľký Meder; 3–Želiezovce; 4–Sládkovičovo; 5–Veľké Ripňany; 6–Turčiansky Ďur; 7–Malý Šariš; 8–Spišské Vlachy; 9–Spišská Belá; 10–Vranov n/Topľou

Fig. 2. The frequency (%) of occurrence of *Fusarium* spp. in 2006–2008 from wheat grain surface re-isolated on SNA medium in localities of Slovakia

weather conditions were favourable for the occurrence of thermophilous species such as *F. graminearum* and *F. semitectum*, but also *F. culmorum*, considered to be psychrophilous species, was identified. On the contrary, in the localities Spišské Vlachy, Vranov nad Topľou, Spišská Belá and Turčiansky Ďur (localities with a colder climate), *F. graminearum* in frequency from 37.5% to 100% was identified. In these locations

T a b l e 2

The relative density [%] of *Fusarium* species in 2007 from wheat grain surface in Slovakia

Locality	*Fusarium* species												Locality RD [%]*
	gram	poae	sporo	culm	tric	semit	aven	samb	oxys	equi	com	Mn	
Abrahám	7.5	2.5	2.5	0.0	2.5	7.5	0.0	2.5	5.0	0.0	7.5	0.0	3.1^{abc}
Veľký Meder	1.3	7.6	7.6	5.7	0.0	5.7	0.0	7.6	0.0	0.0	0.0	0.0	2.9^{ab}
Želiezovce	0.0	16.7	3.3	0.0	0.0	0.0	0.0	0.0	3.3	0.0	0.0	0.0	1.9^{ab}
Sládkovičovo	0.0	2.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2^{a}
Veľké Ripňany	8.5	2.8	0.0	2.8	0.0	5.7	0.0	0.0	2.8	0.0	8.5	0.0	2.5^{ab}
Turčiansky Ďur	9.0	25.4	21.8	3.6	3.6	7.2	3.6	1.8	7.2	1.8	3.6	1.8	7.5^{cd}
Malý Šariš	13.5	43.7	15.4	0.0	6.4	5.7	4.6	0.0	3.8	1.9	0.0	3.8	8.2^{d}
Spišské Vlachy	19.2	28.0	15.8	5.3	5.3	8.8	7.0	0.0	1.7	3.5	1.7	3.5	8.3^{d}
Spišská Belá	5.3	10.5	3.5	0.0	1.7	7.0	5.3	0.0	0.0	7.0	0.0	0.0	3.3^{bc}
Vranov nad Topľou	0.0	17.8	10.6	0.0	6.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.9^{ab}
Average	6.4	15.8	8.1	1.7	2.6	4.8	2.1	1.2	2.4	1.4	2.1	0.9	4.1

gram – *F. graminearum*; poae – *F. poae*; sporo – *F. sporotrichioides*; culm – *F. culmorum*; tric – *F. tricinctum*; semit – *F. semitectum*; aven – *F. avenaceum*; samb – *F. sambucinum*; oxys – *F. oxysporum*; equi – *F. equiseti*; com – *F. compactum*; Mn – *M. nivale*; * differences between values designated by the same letter are not significant (ANOVA, Duncan test, p=0.05)

T a b l e 3

The relative density [%] of *Fusarium* species in 2008 from wheat grain surface in Slovakia

Locality	*Fusarium* species												Locality RD [%]*
	gram	poae	sporo	culm	tric	semit	aven	samb	oxys	equi	com	Mn	
Abrahám	14.2	42.4	29.3	0.0	0.0	2.8	2.8	0.0	3.3	2.8	0.0	1.8	8.2^{cd}
Veľký Meder	0.0	10.0	6.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.3^{a}
Želiezovce	0.0	10.0	10.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.6^{a}
Sládkovičovo	2.5	2.5	2.5	0.0	0.0	2.5	2.5	5.0	7.5	0.0	5.0	2.5	2.7^{abc}
Veľké Ripňany	20.0	6.6	13.3	0.0	0.0	10.0	3.3	3.3	20.0	0.0	10.0	13.3	8.3^{cd}
Turčiansky Ďur	29.2	12.3	9.2	15.4	3.0	3.0	6.1	4.6	6.1	0.0	1.5	9.2	8.3^{d}
Malý Šariš	5.0	6.6	6.6	1.6	0.0	3.3	3.3	0.0	3.3	0.0	0.0	3.3	2.7^{abc}
Spišské Vlachy	7.7	5.8	3.8	3.8	1.9	3.8	5.8	0.0	3.8	3.8	5.8	9.6	4.6^{bcd}
Spišská Belá	3.1	6.2	6.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.1	1.5^{a}
Vranov nad Topľou	12.5	18.7	14.6	4.2	0.0	12.5	8.1	6.2	4.2	4.2	4.2	10.2	8.3^{d}
Average	9.4	12.1	10.2	2.5	0.5	3.8	3.2	1.9	4.8	1.1	2.7	5.3	4.7

gram – *F. graminearum*; poae – *F. poae*; sporo – *F. sporotrichioides*; culm – *F. culmorum*; tric – *F. tricinctum*; semit – *F. semitectum*; aven – *F. avenaceum*; samb – *F. sambucinum*; oxys – *F. oxysporum*; equi – *F. equiseti*; com – *F. compactum*; Mn – *M. nivale*; * differences between values designated by the same letter are not significant (ANOVA, Duncan test, p=0.05)

the third decade of June was the warmest, with the average temperatures from 20°C to 24°C. These temperatures together with sufficient rainfall create optimal conditions for development and spreading of *Fusarium* species. Considering the sites with higher altitude, there was in these sites probably the shift in flowering, which took place at these higher temperatures. In earlier publications was the occurrence of individual species presented according to temperature and rainfall conditions (Šrobárová 1995; Mesterházy 1997; Bottalico & Perrone 2002). Climatic changes recorded in recent years have changed the geographical and altitudinal distribution of individual *Fusarium* species (Hudec 2006; Stepień et al. 2008). In the years 2007 and 2008, *F. poae* became the dominant species with the average frequency of 65.1%, and 64.9%, respectively (Fig. 1). The composition of species was not changed, only the dominance of species was changed. In 2007, the highest relative density was found in *F. poae* in the locality Malý Šariš (43.7%, Table 2). This species maintained its dominant position also in 2008, when the relative density reached 42.4% in the Abrahám location and the average relative density was 12.1%. The prevalence of this species in Slovakia was also recorded in the works of Roháčik & Hudec (2005), and Mašková et al. (2009). Regarding localities, the highest average frequency of *Fusarium* species in 2007 was recorded in Turčiansky Ďur (Fig. 2), 12 *Fusarium* species were identified. The frequency of occurrence of individual *Fusarium* species in this area was from 20% to 80%, the highest frequency was in *F. poae* (80%). In the species *F. graminearum, F. oxysporum, F. sporotrichioides* and *F. semitectum* the frequency of occurrence was 60% and 40% in *F. culmorum, F. equiseti, F. compactum* and *F. tricinctum*. The highest average relative density was found in *F. poae* (15.8%, Table 2), and in the area of Malý Šariš it also reached the highest relative density (43.7%). In 2008, the highest average occurrence frequency of *Fusarium* spp. was recorded in the locality Turčiansky Ďur (53.9%, Fig. 1), where 11 *Fusarium* species were identified, and *F. poae* had the frequency of 100%. *F. graminearum, F. culmorum, F. semitectum* and *F. sambucinum* had identically 80% frequency of occurrence. The highest relative density was recorded in *F. poae* in Abrahám locality (42.4%, Table 3). In the locality Turčiansky Ďur *F. graminearum* had the relative density of 29.2% and *F. culmorum* 15.4%, which is for this type in our observations

Table 4

The average of rainfall and air temperature in June in 2006–2008 from ten localities of Slovakia

Locality	Meteorological observatory	2006		2007		2008	
		[mm]	[°C]	[mm]	[°C]	[mm]	[°C]
Abrahám	Žihárec	123	20.1	81	21.2	101	20.9
Veľký Meder	Podhájska	74	19.8	82	21.3	94	21.0
Želiezovce	Mochovce	107	18.9	75	20.5	97	20.0
Sládkovičovo	Žihárec	123	20.1	81	21.2	101	20.9
Veľké Ripňany	Topoľčany	65	19.8	59	20.6	55	21.0
Turčiansky Ďur	Turčianske Teplice	67	16.5	127	17.5	144	17.8
Malý Šariš	Jakubovany	111	17.2	69	19.0	55	18.2
Spišské Vlachy	Spišské Vlachy	248	16.1	62	18.2	44	17.8
Spišská Belá	Spišské Vlachy	248	16.1	62	18.2	44	17.8
Vranov nad Topľou	Kamenica nad Cirochou	121	17.6	75	19.8	126	18.5
Average		128.7	18.2	77.3	18.3	86.1	19.4

mm – sum of rainfall

°C – average daily temperature

Resource: Slovak hydrometeorological institute Bratislava

Agrometeorological and fenological informations 2006, 2007, 2008

the highest value, although the overall decline of occurrence was recorded (Fig. 1). A similar trend was observed in Poland (Stepień et al. 2008), Czech Republic (Nedělník et al. 2007), Austria (Adler et al. 2002), the Netherlands (Waalvijk et al. 2003). One explanation could be mono-cultivation of wheat, minimizing tillage (Lukanowski & Sadowski 2002) and growing of maize as a forecrop, crop residues of maize are the main source of *F. graminearum*.

CONCLUSIONS

Slovakia is a country with diverse geographical division and fluctuating weather conditions. The frequency of occurrence and spectrum of Fusarium species are not stable. In 2007 and 2008 a change was observed in species prevalence, when the prevalence of *Fusarium poae* was ascertained. Higher number of *Fusarium* spp. (12) was identified in locality Turčiansky Ďur. Dominant species, as so as *F. poae, F. graminearum* and *F. sporotrichioides* maintained a higher frequency and relative density in all localities and years. Less important species as *F. sambucinum, F. equiseti. F. tricinctum* and *Microdochium nivale* achieved low relative density in population structure also in areas with favourable climatic conditions.

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