Fungal Infection of Malt Barley Kernels in Slovak Republic

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Abstract

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The influence of agro-environmental factors (year, cultivar and climatic factors) on the occurrence of fungi on kernels of malt barley, species spectrum and seedling viability were evaluated during 2004 and 2005. The seeds (asymptomatic, fraction above 2.5 mm) originated from different cultivars and locations of the Slovak Republic. Surface sterilisation of the kernels before isolation of the fungi was the key factor for objective results on the occurrence of *Fusarium* species. Screening of non-sterilised kernels gave a different spectrum of fungal species and their frequency and may lead to distorted results. The most frequent species isolated from barley kernels were *Alternaria* spp., *Cochliobolus sativus*, *Epicoccum nigrum, Fusarium* spp. and *Pyrenophora teres*. The results confirmed that agro-environmental factors (mostly year and microclimatic conditions) had a major influence on infection by and population structure of fungi in malt barley kernels. The total sample infection by *Fusarium* spp. was significantly higher at localities with higher altitude and in 2005. The infection level varied from 0% to 20%, in some localities in 2005 it exceeded 20%. The widest fungal species spectrum was recorded in the locations with high level of kernel infestation. In localities with lower infection, the species spectrum was narrower.

Keywords: malt barley; kernels; fungal infection; agro-environmental factors

The infection of barley seeds before and after maturity is greatest at high relative humidity (Mathre 1997; Rabie *et al.* 1997). Several seed-borne fungi, including species of the genera *Fusarium*, *Alternaria*, *Aspergillus* and *Penicillium* have been considered as important pathogens of cereal grains (Hassan 1999; Doohan *et al*. 2003). The infection can reduce grain yield, seed vigour and germination (Šarić *et al.* 1997). The malting quality is lowered by negative changes in the protein, colour, taste and volume of the extract. Infected malt barley is discounted in the market and severely discoloured grain is not used for malting industry but used as animal feed (Mathre 1997). The intercellular and intracellular growth of the fungi is usually accompanied by the production of enzymes and mycotoxins (Kang & Buchenauer 2002). Infection of the grain by *Fusarium* decreased germination (Brennan *et al*. 2003) and quality of kernels, accompanied by contamination with mycotoxins (Visconti *et al*. 2000). The *Fusarium* mycotoxins are dangerous for human (Marasas *et al*. 1984) and animal health if the *Fusarium* species are associated with feed mixtures made from infected cereals (Labuda *et al*. 2003). The most important fusariotoxins are deoxynivalenol (DON), diacetoxyscirpenol (DAS), 3-acetyldeoxynivalenol (3-ADON), 15-acetyldeoxynivalenol (15-ADON), T-2 toxin, HT-2 toxin, nivalenol and others (Snijders & Krechting 1992; Bottalico & Perrone 2002).

Environmental factors have a significant influence on the occurrence of fungal species and disease severity (Vigier *et al*. 1997). Temperature and rainfall affect the production and dispersal of inoculum and the infection of wheat spikes and stem bases, which in turn determine the occurrence and severity of diseases (Pettitt *et al*. 1996). The prevalence of *Fusarium* and other fungal species is different in each plant tissue and agroclimatic condition (Corazza *et al*. 2001).

The aim of this work was to evaluate the influence of the location, year and cultivar on fungal contamination and structure of the *Fusarium* population in asymptomatic malt barley kernels.

MATERIALS AND METHODS

Sample collecting. The samples of malt barley kernels (asymptomatic grains, fraction above 2.5 mm) were collected at five locations with different climatic conditions of Slovak Republic in 2004 and 2005. Seeds of three cultivars – Expres, Madonna and Nitran were collected at Bučany, Sládkovičovo, Vígľaš-Pstruša, Trebišov, and Malý Šariš after standard harvest. The locations are State Experimental Stations, representing different climatic conditions of the Slovak Republic. Details of the locations are shown in Table 1.

Isolation and identification of fungi. Two samples with 300 kernels per sample from each location were used for isolation of fungal species: one sample with surface sterilised kernels (SK), and one with surface non-sterilised kernels (NSK). The samples with SK grains were surface sterilised by shaking for 2 min in 1% NaOCl solution and then rinsed in redistilled water. The SK grains were placed in Petri dishes with potato-dextrose agar (PDA) and incubated at 23°C under 12/12 photoperiod (4000 lux, no UV light). The NSK were placed on PDA directly, without prior sterilisation. Developing fungal colonies were isolated, purified and identified according to manuals (Gerlach & Nirenberg 1982; Simmons & Roberts 1993; Samson *et al*. 2002), by visual and microscopic observation of cultures. The isolation frequencies (IF) of the species were evaluated for each location and cultivar, according to González *et al*. (1999) – IF (%) = (number of kernels (samples) with occurrence of species/total number of evaluated kernels (samples)) × 100. The numbers of germinated and ungerminated kernels in a Petri dish during isolation of fungi were expressed as germination activity (GA, in %).

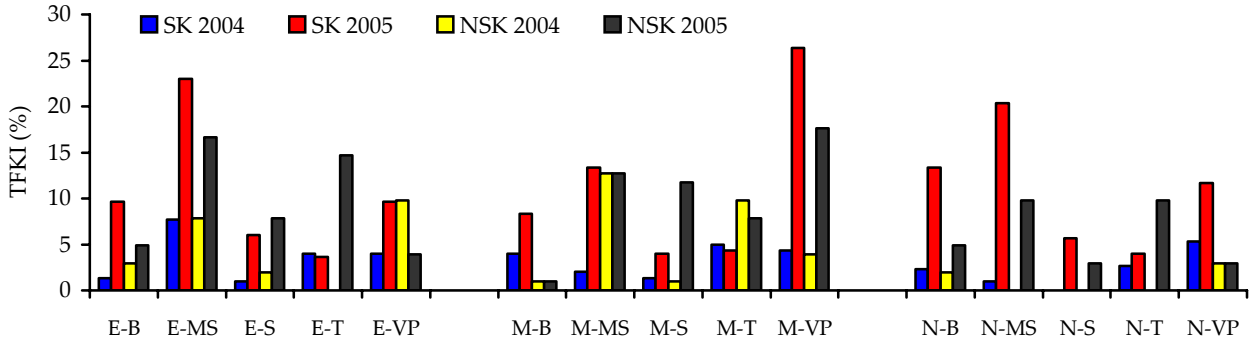
RESULTS

The following fungal species were isolated from malt barley kernels: *Acremonium strictum, Alternaria* spp., *Aspergillus niger*, *A. flavus*, *Botrytis cinerea*, *Cladosporium herbarum*, *C. cladosporioides, Cochliobolus sativus* (*Helminthosporium sativum*), *Epicoccum nigrum*, *Fusarium* spp., *Chrysonilia sitophila*, *Microdochium nivale*, *Mucor* sp., *Nigrospora sphaerica*, *Penicillium* sp., *Phaeosphaeria nodorum* (*Septoria nodorum*), *Pyrenophora teres* (*Helminthosporium teres*), *Rhizoctonia* sp., *Rhizopus nigricans*, *Stemphylium* sp., *Tanatephorus cucumeris* (*Rhizoctonia solani*), *Trichoderma* sp., *Verticillium albo-atrum*.

Occurrence of *Fusarium* spp. on surface sterilised and non-sterilised kernels is presented in Figure 1. High total infection of kernels by *Fusarium* (TFKI) was recorded in the samples from colder and more humid locations (Malý Šariš and Vígľaš-Pstruša), medium infection at Bučany, and the lowest level of infection at the locations Trebišov and Sládkovičovo. In 2005, the TFKI was higher (in both the SK and NSK samples – Figure 1) than in 2004. The TFKI was not significantly influenced by cultivar in 2004, but large differences in TFKI among cultivars were recorded in 2005. The GA of most cultivars at all locations was lower in 2005 than in 2004 (Figure 2).

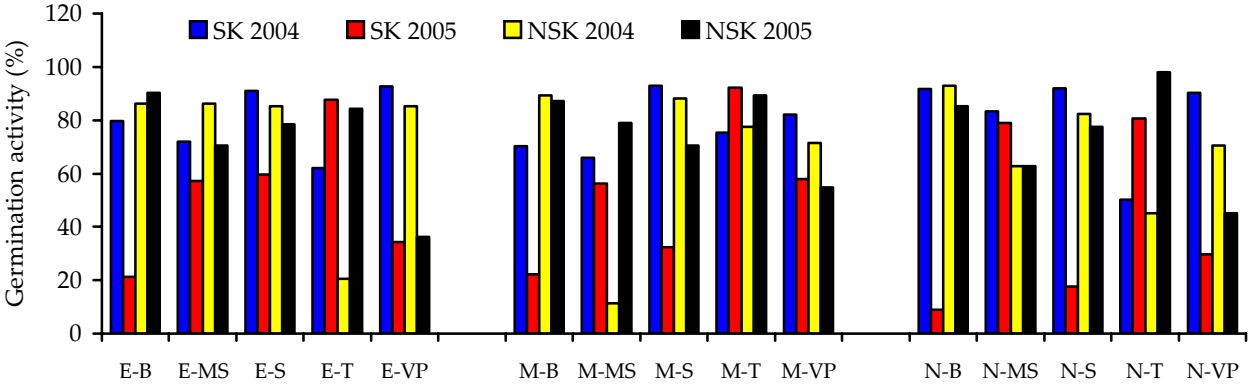
Table 1. Characterisation of evaluated localities

Locality (part of Slovak Republic)	Altitude (m)	Mean annual rainfall (mm)	Mean annual temperature (°C)
Sládkovičovo (south)	122	497.2	10.46
Bučany (south-west)	165	570.0	9.40
Malý Šariš (east)	295	599.3	7.86
Vígľaš-Pstruša (central)	375	640.0	7.90
Trebišov (south-east)	109	552.0	9.00



TFKI – total infection of kernels by *Fusarium* species; E – cv. Expres, M – cv. Madonna, N – cv. Nitran; B – locality Bučany, MS – locality Malý Šariš, S – locality Sládkovičovo, T – locality Trebišov, VP – locality Vígľaš-Pstruša; SK – surface sterilised kernels, NSK – surface non-sterilised kernels

Figure 1. Total infection of kernels by *Fusarium* spp. and *Microdochium nivale* (%)



E – cv. Expres, M – cv. Madonna, N – cv. Nitran; B – locality Bučany, MS – locality Malý Šariš, S – locality Sládkovičovo, T – locality Trebišov, VP – locality Vígľaš-Pstruša; SK – surface sterilised kernels, NSK – surface non-sterilised kernels

Figure 2. Germination activity of kernels on agar plates (%)

The isolation frequency (IF) of the most frequent fungal species (IF > 5%), except *Fusarium* spp. and *M. nivale,* is shown in Table 2. *Alternaria* spp. were the dominant species on SK kernels, followed by *Cochliobolus sativus*, *Epicoccum nigrum* and *Pyrenophora teres* (Table 2). The dominance of certain species depended on whether the kernel surface had been sterilised. While *Alternaria* spp. were predominant and *Aspergillus* spp. occurred less on SK kernels, the reverse was observed on NSK where *Aspergillus* spp. occurred in higher frequency.

On NSK kernels, the most frequent species were *Alternaria* spp. The IF of the other most frequent species (*Aspergillus* spp., *E. nigrum*, *C. sativus*, *Cladosporium* spp., *P. teres*) depended mostly on locality and year. The most frequent species associations (several fungi isolated from one kernel) in NSK kernels were *Aspergillus niger* + *Alternaria* spp., *Alternaria* spp. + *Cladosporium* spp., *Alternaria* spp. + *Cladosporium* sp. + *Helminthosporium sativum*. On SK kernels the following species associations were the most frequent: *Alternaria* spp. + *Cochliobolus sativus*, *Epicoccum nigrum* + *C. sativus*, *Alternaria* spp. + *E. nigrum*.

DISCUSSION

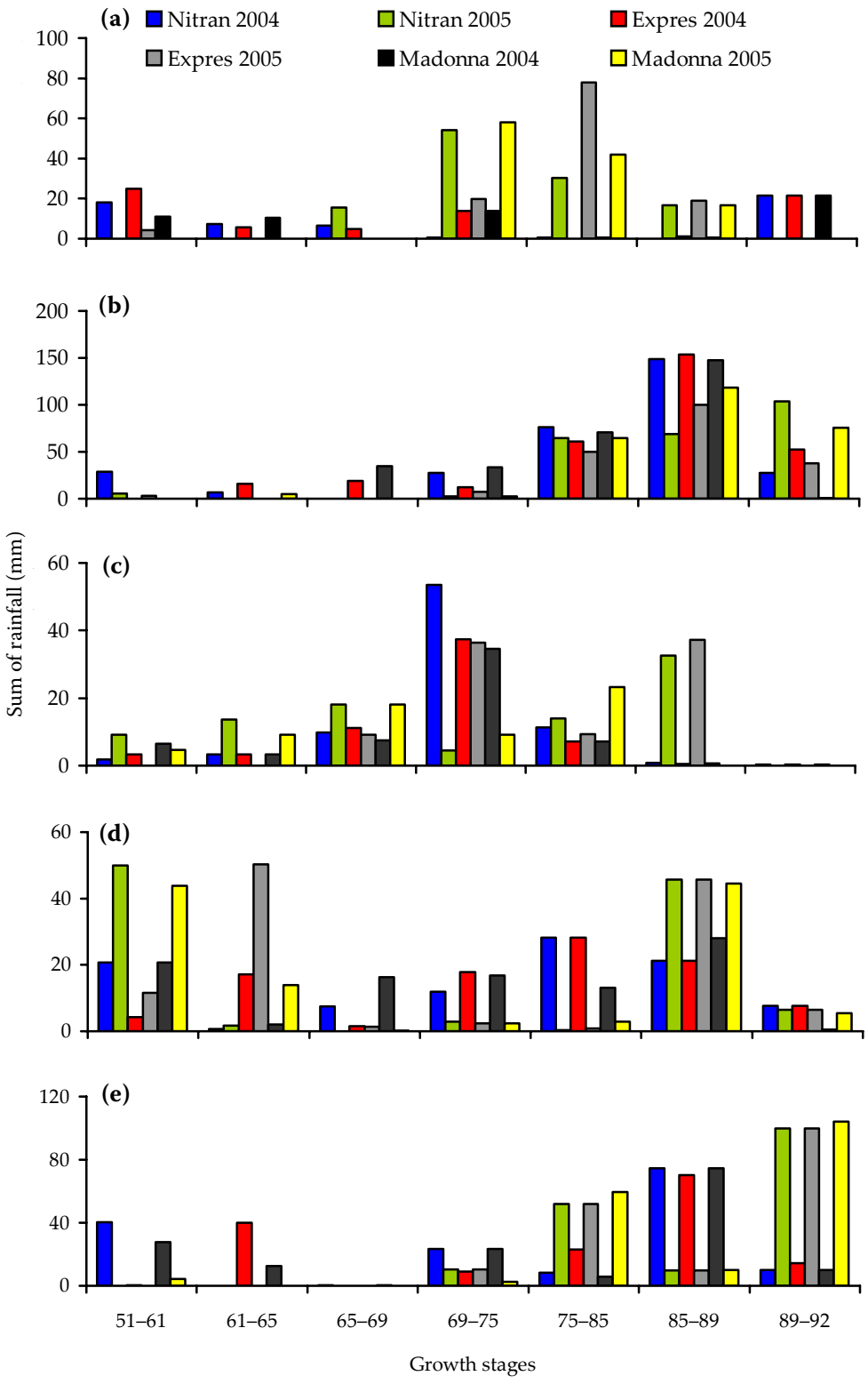
The evaluation of fungal infection and symptoms formation on malt barley kernels is useful for quality control and general recommendations for agricultural practice. The species spectrum isolated from the barley kernels is in agreement with results of Prokinová (1999) and Clear *et al.*

Table 2. Occurrence of the most frequent fungal species (IF above 5%) in barley kernels, except of *Fusarium* species and *Microdochium nivale*

Locality	Year	Cv.	Alt	Asp	EN	CS	Csp	PT
B	2004	E	79*(87.3**)	17(2.9)	13(20.6)	8.7(3.9)	0(0.9)	0.3(2.9)
		M	84(96.1)	2(2.9)	11.3(15.7)	12(20.6)	0(76.5)	0(0)
		N	57.3(87.2)	3.7(0)	7.3(8.8)	34(19.6)	13.7(34.3)	0.3(7.8)
	2005	E	89.3(92.2)	2(0)	34.3(22.6)	9.3(9.8)	20.7(95.1)	0(0)
		M	88.7(98.1)	0(0)	29(1.9)	14(7.8)	17.3(90.2)	0(0)
		N	82(84.3)	3.7(0.9)	38.7(34.3)	17(8.8)	31(95.1)	0.3(0.9)
MŠ	2004	E	73.7(69.6)	0.3(4.9)	14.7(13.7)	6.3(2.9)	0(0)	9.3(26.5)
		M	64.3(75.5)	(10.8)	(34.3)	(17.7)	(0)	(7.8)
		N	59(60.8)	2.3(62.8)	4.7(6.9)	25.7(7.8)	0(0)	26.7(21.6)
	2005	E	77.7(38.2)	1.7(89.2)	10.3(9.8)	17(8.8)	0.3(0)	2(0)
		M	82.7(17.7)	0.7(89.2)	18(15.7)	28(8.8)	0(0)	1.7(0)
		N	61(12.8)	2(82.4)	13(8.8)	33.3(12.8)	0.7(0)	5(0)
S	2004	E	88(92.2)	0(57.8)	4(0.9)	13.7(5.9)	9.3(12.8)	0.3(3.9)
		M	92.3(87.3)	8(80.4)	6(0.9)	14.7(2.9)	4(20.6)	0(0)
		N	64(71.6)	39(87.3)	3(5.9)	22.7(1.9)	1.7(65.7)	0.3(0.9)
	2005	E	97.7(79.4)	3(1.3)	24.7(16.3)	1.7(0.7)	34(41.2)	0.3(0)
		M	94.3(74.5)	5.7(6.9)	23.7(55.9)	7(2.9)	40(50.9)	0.3(0)
		N	93.3(93.1)	8.3(3.9)	19.7(20.6)	15(0.9)	32.3(100)	1(0.9)
T	2004	E	69.3(62.8)	2.7(0)	13(0.9)	4.3(2.9)	1.7(13.8)	19.7(22.6)
		M	73(70.6)	1.3(28.4)	12(10.8)	14.6(18.6)	0.7(9.8)	4(3.9)
		N	70.3(25.5)	6.7(97.1)	8(0.9)	26.7(11.8)	1.7(0)	1.7(5.9)
	2005	E	91.7(91.2)	5.7(14.7)	8.7(31.4)	17.3(13.7)	1.7(0.9)	0.7(0)
		M	86(71.6)	4.3(36.3)	13.3(35.3)	14.7(2.9)	0.7(0)	7.8(0)
		N	92(81.4)	2.9(32.4)	10.3(30.4)	17(11.8)	1.7(1.9)	1(0.9)
VP	2004	E	32(87.3)	6(0)	21.7(25.5)	33.7(30.4)	1.3(3.7)	12(4.9)
		M	58.3(75.5)	5(18.6)	17.7(20.6)	37.3(40.2)	5(9.8)	8.3(2.9)
		N	54.7(46.1)	4(79.4)	13.3(5.9)	49(27.5)	0(15.7)	14(6.9)
	2005	E	89.7(93.1)	6(3.9)	23.7(40.2)	8.3(2.9)	18(92.2)	1.7(1.9)
		M	77(95.1)	4.3(0.9)	19(42.2)	19.3(11.8)	1.7(91.2)	1.3(3.9)
		N	89.3(96.1)	4(0)	38.7(14.7)	8.7(3.9)	14.3(89.2)	3(3.9)

B – Bučany, MŠ – Malý Šariš, S – Sládkovičovo, T – Trebišov, VP – Vígľaš-Pstruša; Cv. – cultivar: E – Expres, M – Madonna, N – Nitran; Alt – *Alternaria* spp., Asp – *Aspergillus* spp., EN – *Epicoccum nigrum*, CS – *Cochliobolus sativus*, Csp – *Cladosporium* spp., PT – *Pyrenophora teres*; * isolation frequency on surface sterilised kernels (%); (**) isolation frequency on surface non-sterilised kernels (%)

(2000) who described similar species spectra. The fungi isolated from infected seeds can co/parasitise seed, but they differ widely in aggressiveness. Without competition from other organisms, each tends to dominate the substrate (Wiese 1991). The highest frequency of *Alternaria* spp. in SK and NSK kernel categories suggests that this species has a most important role in the population structure of the microflora of kernels, which is in agreement with the results of Andersen *et al.* (2001). In oat and wheat seeds, *Alternaria* spp. are also the most frequent species (Clear *et al.* 2001). The elimination of *Aspergillus* spp. in the SK category by surface sterilisation suggests



Nitran, Expres, Madonna – evaluated cultivars of barley; 51–61 – sum of rainfall (mm) between growth stage 51 to 61 BBCH (beginning of heading–beginning of flowering), 65 – flowering, 69 – end of flowering, 75 – milk ripeness, 85 – wax ripeness, 89 – full ripeness, 92 – harvest

Figure 3. Rainfall at certain growth stages (a) locality Bučany, (b) locality Malý Šariš, (c) locality Sládkovičovo, (d) locality Trebišov, (e) locality Vígľaš-Pstruša

that the species are only surface contaminants of barley kernels. A higher frequency of *P. teres* and *C. sativus* was recorded at some locations, which is connected with the affinity of these species to barley kernels and their association with specific symptoms of black point. This agrees with results of Prokinová (1999) who confirmed that species of *Alternaria* and *C. sativus* play a big role in the occurrence of black point symptoms on barley kernels. It is also in agreement with Wiese (1991) who mentioned high black point occurrence in a humid environment during rainfall at the time of seed formation, maturation and premature seed senescence. The high black point severity and higher incidence of *C. sativus* on the barley kernels might have been due to cool, wet weather conditions and frosts during seed development that delayed ripening (Fernandez *et al.* 2000), which in our tests were observed more in 2005 than in 2004 (Figure 3). The isolated pathogenic and saprophytic fungi associated with barley kernels induce variable symptoms and reduction of seedling viability. Although such damage to seed begins before harvest, it may increase if grains are harvested late or stored under moist or wet conditions (Wiese 1991). According to our results, the germination activity in the majority of cultivars was affected more in 2005, which was a year of higher occurrence of saprophytic and phytopathogenic fungi. In general, fungi of the genera *Alternaria*, *Penicillium*, *Aspergillus* and *Fusarium* and *Cochliobolus sativus* are capable of killing or reducing a seedling's vigour and have the ability to produce their respective toxins (Hassan 1999). *Alternaria alternata* as a facultative parasite is a frequently occurring species of particular interest because it produces a number of mycotoxins, including alternariol (AOH), alternariol monomethyl ether (AME), altenuene (ALT), altertoxins I, II and III (ATX-I, -II, and -III) and L-tenuazonic acid (TeA) (Li *et al.* 2001; Scott 2001).

Our evaluation of the influence of locality, year and cultivar on TFKI has important implications for the general recommendations for agricultural practice. The significantly higher TFKI at colder and moister locations should be considered in practical plant protection measures. The average levels of TFKI correspond with results of Yli-Mattila *et al.* (2002), who reported a 4–20% wheat grain infection. In our study the infection level by *Fusarium* spp. was higher than 20% only at some locations in 2005. This was probably related with higher rainfall between growth stages BBCH 85–89 (wax ripeness–full ripeness) because the highest rainfall in 2005 was recorded between these growth stages at all locations except Vígľaš-Pstruša (VP) and Malý Šariš (MŠ) (Figure 3). These two locations received a higher rainfall between the growth stages full ripeness and harvest. It suggests that high precipitation from full ripeness to harvest is also important for *Fusarium* infection of malt barley. The presumption of a higher incidence of fungal pathogens at moist locations (Elen *et al.* 2000) is confirmed by our results. The infection of asymptomatic kernels proceeded during grain formation, long after classical Fusarium head blight infection (during flowering and spike formation). The saprophytic mycoflora on the surface of the kernels was eliminated by sterilisation before they were plated out (Christensen & Kafmann 1969); thus, the *Fusarium* species that formed colonies had been present underneath the surface tissues of the evaluated grains. Surface sterilisation of kernels played an important role when determining the species spectrum and the frequency of species isolated from kernels. According to the presented results, the surface sterilisation of kernels before culture and isolation (as widely practiced in laboratories) is better for objective determination of *Fusarium* spp. in kernels than analysis of non-sterilised kernels. During surface sterilisation, the frequent saprophytic surface contaminant *Aspergillus* spp. is removed from the isolation process. The species spectrum obtained after sterilisation is also more objective.

Our results indicate that asymptomatic malt barley seeds may be an important source of infection by *Fusarium,* and for further infections and its spread in the field (Wiese 1991; Gilbert 2001) or during the malting process. Therefore, a myco-analysis done before sowing would give important information on fungi present in the seeds (Jurković *et al.* 2001). The results suggest that wet localities are favourable for *Fusarium* infestation of malt barley. However, there was a higher incidence of FHB in both wheat and barley in 2004 than in 2005 at all locations evaluated in this work (unpublished information). Yet the TFKI was higher in 2005 (Figure 1), suggesting that high occurrence of FHB on spikes is not automatically followed by high incidence of *Fusarium* species in kernels.

The differences of the TFKI between evaluated cultivars and locations have important practical

meaning. The differences between the TFKI of certain cultivars increased in a year with higher *Fusarium* incidence. The results highlighted that the microclimate of a locality is more important for the level of the TFKI than the general agroclimatic characteristics of a specific year and the used cultivars. Thus, in our country, susceptible cultivars of malt barley should be grown at dry and warm locations.

In conclusion, the results show that infection by and population structure of fungi in malt barley grains depend on the year and microclimatic conditions. High precipitation from growth stage milk ripeness to wax ripeness, and from full ripeness to harvest favours infection, which is higher at locations at higher altitude. The fungal species spectrum gets wider by rising rainfall rate and altitude. Surface sterilisation of kernels is important for objective screening of *Fusarium* and other fungal species in kernels; conversely, analysis of surface non-sterilised kernels leads to distortion of frequency and species spectrum, in *Fusarium* spp. especially.

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