Fusarioses of Barley with Emphasis on the Content of Trichothecenes

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Abstract

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The infestation of barley grains by fungi of the genus *Fusarium* was studied on malting barley from Kroměříž (360 samples) and one sample from Kojetín in Czech Republic. Most frequent species in the season 1997–1998 were: *F. culmorum* (over 70% of the isolates), *F. poae* (over 20% of the isolates), *F. avenaceum* (over 2%). Much less frequent were: *F. stilboides* v. *stilboides*, *F. aqaeductum* v. *aqaeductum*, *F. merismoides* v. *merismoides* and *F. gigas*. The isolates of *F. culmorum* were highly pathogenic when barley was artificially inoculated; those of *F. poae* had low pathogenity. Five varieties of spring barley at growth stage 1–5 on the Feekes scale reacted to *F. culmorum* as follows: 1) Tolar (most resistant), 2) Akcent, Lumar and Rubín (intermediate), 3) Krona (most susceptible). An application of fungicides on a plot inoculated with *F. avenaceum* and *F. graminearum* increased the yield of grain by 0.46 to 1.71 t/ha. Most effective fungicides against the pathogens were: metconazole, tebuconazole and prochloraz. The effect of the combination tebuconazole + triadimefon + prochloraz was found to be most effective. For determination of six trichothecene mycotoxins in fungal mycelium, macroconidia and spring barley, high resolution capillary gas chromatography with electron capture detection was used. Only toxin T-2 and nivalenol (NIV) were detected in fungal mycelium, and a low level of NIV was found in spring barley, variety Rubín (lower than legislated limit – 2 mg/kg of cereals).

Key words: spring barley; *Fusarium culmorum*; *F. poae*; *F. graminearum*; *F. avenaceum*; inoculation; variety resistance; effectivness of fungicides; mycotoxins

A serious disease of barley is caused by species of the genus *Fusarium* which produces mycotoxins. The determination of the *Fusarium* species is a worldwide problem, and the species concept in *Fusarium* taxonomy has to incorporate morphological, biological and phylogenetic approaches and lately also molecular aspects (BLACKHOUSE *et al.* 1997). Different areas and crops have different spectra of *Fusarium* spp. (ELEN *et al.* 1997; JURKOVIĆ, COSIĆ *et al.* 1997; KOSIAK *et al.* 1997; OBST *et al.* 1997; SCHILLING *et al.* 1997; STACK *et al.* 1997; ŠROBÁROVÁ 1997; TÓTH 1997). ELEN *et al.* (1997) described a field survey of barley, oats and wheat from Norway and listed the most frequent species: *Fusarium avenaceum* Fr. (Sacc.), *Fusarium poae* Peck, *Fusarium culmorum* (W. G. Smith) Sacc. and *Fusarium graminearum Schwabe.*

Diseases caused by *Fusarium* have been a difficult problem particularly in wet years when a wide scale of species, beginning with *F. culmorum* (W. G. Smith) Sacc. with an unknown teleomorphic state, and ending with *F. graminearum* Schwabe (teleomorphic state *Gibberella zeae* Schw.) infect a wide range of host plants. The disease on cereals is partially systemic, the fungus grows throughout the plant, from the roots, stem, single nodes to ears. The disease in ears causes them to turn white and often mycelium (rose or orange coloured) is visible on the surface of glumes, lemmae and paleae. The results of agronomical studies showed that the damages caused by the genus *Fusarium* could be diminished by using proper technologies (the time of sowing, crop rotation, removing plant residues etc.) (CORAZZA *et al.* 1995).

Fusaria are considered to be field fungi since they are primarily plant pathogens, but they can continue to grow on stored crops, provided the environmental conditions are favourable. However, fungal growth and mycotoxin production are not directly linked; visible fungal mycelium does not necessarily indicate high levels of toxins. One of the major groups of mycotoxins produced by some species of the genus *Fusarium* are trichothecenes-potent eukaryotic protein synthesis inhibitors, the structure of which corresponds to tetracyclic sesquiterpenoid compounds with a 12,13-epoxygroup. The name trichothecene

is derived from trichodien, a natural product of *Trichothecium roseum*. They are associated with a variety of mycotoxicoses in humans and animals (MARARAS *et al.* 1984). They can cause acute alimentary toxicoses, in many cases stomach cancer (DE NIJS *et al.* 1997). Deoxynivalenol and acetyldeoxynivalenol caused an acute toxic reaction of the skin and pulmonary tract in workers who were in contact with grass contaminated by *F. culmorum* (SNIJDERS *et al.* 1996). The most often detected mycotoxins were nivalenol (NIV), deoxynivalenol (DON), diacetoxyscirpenol (DAS), HT-2 toxin, T-2 toxin and others. These mycotoxins were not destroyed by the boiling process in the preparation of beer: 80–93% of the deoxynivalenol was still detected in the beer (SCHWARZ *et al.* 1995). At present, over 150 trichothecenes are known, but usually only a few of them are monitored because for most of them no data on toxicity are available and, in addition, their incidence in cereals is not frequent. Nowadays, gas chromatography (GC) with electron capture detection (ECD) (CROTEAU *et al.* 1994) and mass-selective detection (MSD) (BURROWS 1994) or, alternatively, enzyme-linked immunosorbent assay (ELISA) (USLEBER *et al.* 1991) are widely used for determination of trichothecenes in cereals.

MATERIAL AND METHODS

Isolation of *Fusarium* from the Grain of Barley

Seed of spring barley from two sources was used for the tests: from 9 varieties (360 samples) maintained at the Agricultural Research Institute at Kroměříž, and one sample of a mixture of different varieties (harvest 1997) from the malting plant at Kojetín. *Fusarium* was isolated by the following method: the seed was surface-disinfected with 5% sodium hypochlorite (3 min), washed repeatedly in sterile distilled water, and put on solid Komada's medium in Petri dishes. The *Fusarium* species were determined with the use of the keys by BOOTH (1971) and GERLACH & NIRENBERG (1982). The fungi were also cultivated on malt agar. Pure isolates of *Fusarium* from the seeds of barley were prepared by separation of the mycelium and macroconidia from the mixture (by flotation and centrifugation).

Laboratory Experiments – Pathogenicity Tests *in vitro*

A test tube method was used to determine the pathogenicity of *F. culmorum* and *F. poae* on barley. Cultures of the two species originated from the variety Rubín grown at Kroměříž, after isolation on Komada's medium and maintenance on malt agar. The cultures were deposited in the culture collection at RICP, Prague-Ruzyně. Inoculum was prepared by washing fungal mycelium in sterile distilled water. Seed was surface-disinfected, germinated on sterile, moist filter paper. The seedlings were in the age 14 days. The tips of the roots were cut off and the roots were immersed for 3 min in a suspension of conidia (1 million of conidia per 1 ml). The control sample was immersed in distilled water. Inoculated seedlings were placed in test tubes filled to one quarter with pure agar medium. The tubes were closed with cotton plugs and placed into a growth chamber (12 hrs light, 12 hrs dark, 20°C). Artificial infection was done when the plants had three leaves (100 plants were used). The light intensity was about 1500 mmol/m^{2}s. *Fusarium* was often present in the embryo of the seed which negatively influenced the test with inoculation.

Symptoms were first evaluated after 7 days, a second time after 14 days. Disease severity was assessed by an original 4-point-scale, where 0 = without symptoms, 1 = roots or the base of plants with mild beginning brown colour, 2 = half the roots or the base of plants with blackish brown colour, the base of plants to a height of 1 cm blackish brown or covered with mycelium, 3 = plants are dead. The mean level of disease severity was evaluated for every isolate.

Field Trials and the Experiments with Fungicides

Small plot field trials were established (20 March 1998) at Kroměříž (the area with the best conditions to grow spring barley in the Czech Republic) with nine varieties of spring barley, the seed of which had been certified. The plot size was 10 m^2, with 4 replications. The trial was sown in a way suitable for analysis of variance. It was fertilized before sowing by 200 kg NPK per ha, and at the third leaf by 30 kg N per ha. Weed control on 6 May was in form of Granstar + Starane 20 g + 0.4 l/ha. Before flowering, on 1 June, the trial was treated against mildew with Calixine (no influence on *Fusarium* spp.).

In part of the experiment, plots were treated with fungicides (tebuconazole, propiconazole, prochloraz, metconazole, epoxiconazol, triadimefon and spiroxamin). The treatment was done on 10 June with propiconazole and tebuconazole in water at a rate of 292.3 ml a.i./ha, while solid fungicides were spread at 93.2 g a.i./ha (prochloraz, metconazole, epoxiconazol, triadimefon and spiroxamin).

Cultures of *F. avenaceum* and *F. graminearum* were prepared in the laboratory for inoculation of the trials. (both isolates were used for inoculations). The isolates originated from previous experiments at Kroměříž (1997), when *F. avenaceum* had been isolated from the variety Tolar, and *F. graminearum* from the variety Akcent, and both maintained on malt agar. The barley was inoculated in the phase of flowering were infected with *Fusarium* on 12 June during wet rainy weather with morning fog by spraying 150 ml of a suspension (repeatedly two-times a day) with 10^6 of conidia per ml on each plot of 10 m^2. At maturity we evaluated the infection on ears visualy and the grain yield was although measured. The grain was cleaned and graded. Kernels with over 2.5 mm in diameter were used for tests of *Fusarium* occurrence. We took 2 x 200 kernels from every variant, i.e., the control and each of the variants were treated with one of the fungicides. The kernels were surface-sterilized with SAVO (so-

dium hypochlorite) and placed on filter paper saturated with a water solution of iprodion (at a concentration of 50 ppm) and kept for 7 day at 20°C. Then the number of kernels with *Fusarium* was counted and severity of the infection was evaluated.

Methods of Chromatography

Extraction of Fungal Samples: Blended homogenous representative barley sample of 10 g of the kernels or 2 g of malt agar, malt agar with fungi mycelium or fungi mycelium or 0.5 g of macroconidia were extracted with 60 ml of acetonitrile-water (84 : 16, v/v) for 1 h on a rotary shaker (the amount was need for the determination of mycotoxines). The supernatant was filtered into a 100-ml Erlenmeyer flask. Extraction was repeated with 40 ml of acetonitrile-water (84 : 16, v/v), the extracts combined and stored at 4°C (max. two weeks) before purification. The isolates of *F. culmorum* and *F. poae* were the same as those used in the laboratory tests on pathogenicity.

Gas Chromatography – Determination of Trichothecenes (DON, NIV, DAS, T-2 toxin, HT-2 toxin): A HP-5890 series II gas chromatograph (Hewlett-Packard, Avondale, CA., USA) was equipped with a fused silica capillary column HP-35 (30 cm × 0.25 mm × 0.15 μm), an ECD system and an autosampler. The GC-ECD determination was carried out under the following conditions: nitrogen was used as a carrier gas with a flow-rate of 1ml per min and splitless injection mode (1 ml). The temperature of the injection port was 250°C and that of the detector 300°C. Column temperature program was: 80°C held for 2 min, 5°C/min to 150°C and 3°C/min to 207°C and 1.5 °C/min to 250°C held for 5 min.

After cultivation of the fungi, the presence of target analytes (NIV, DON, T-2 tetr., FUS-X, DAS, HT-2 tox. and T-2 tox.) was separately tested in: (i) malt agar with fungal mycelium, (ii) fungal mycelium and (iii) macroconidia. Only two trichothecenes, nivalenol and deoxynivalenol, were employed for quantification of trichothecenes, which are the most common group of mycotoxins produced by *Fusarium* species. GC employing a capillary column offers a high-resolution separation efficiency and good selectivity in the detection process. Due to their relatively polar nature, trichothecenes have to be volatilized prior to a GC run. We used TFAA as derivatization agent for this purpose.

For efficient purification of the crude extract two SPE columns, charcoal/alumina/Celite and MycoSep 225, were used. The recovery of analytes ranged from 76.3% to 88.2% on the first column, and from 16.6% to 80.3% on the latter.

RESULTS

Isolation of *Fusarium* from the Grain of Barley

In 1997 the genus *Fusarium* was very frequent in all tested grain samples (360 samples of 9 varieties) in the mixture with the genus *Drechslera* (*Helminthosporium*) occurred in approximately 30% of all tested samples. In 1998 the genus *Alternaria* in the mixture with *Fusarium* was in 75% of all samples, while *Fusarium* alone was present in the remaining 25% of samples (360 samples of 12 varieties). The proportion of single *Fusarium* species (in %) in the years 1997 and 1998 is shown in Figs 1 and 2.

These results (the occurrence in all samples) showed that the spectrum of fungal genera changed from year to year. The spectrum of species within the genus *Fusarium* was nearly the same in both years. The most frequent *Fusarium* species was *F. culmorum* followed by *F. poae*. These two species were therefore used to inoculate barley seedlings in laboratory tests.

Laboratory Experiments – Pathogenicity Tests *in vivo*

Fig. 3 illustrate the differences between the reaction of the varieties of spring barley to *F. culmorum* and *F. poae*.

It is obvious that the most aggressive isolates of *F. culmorum* caused severe symptoms, while isolates of *F. poae* caused only weak reactions in plants (disease severity 1) while *F. culmorum* had disease severity 3 (the most aggressive isolate was chosen from 20 isolates).

Field Trials and the Experiments with Fungicides

Use of all fungicides increased the yield of spring barley by 0.45 to 1.71 t/ha. This effect was not correlated with the occurrence of *Fusarium* spp. on the grain, but is rather connected with the health state of the seed of single varieties and the spectrum of pathogens each of the chemicals can control.

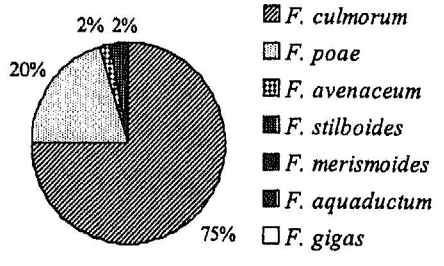

Fig. 1. The species of genus *Fusarium* isolated in 1997

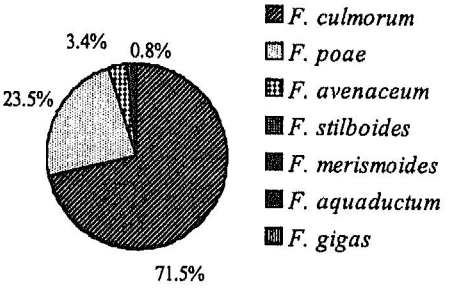

Fig. 2. The species of genus *Fusarium* isolated in 1998

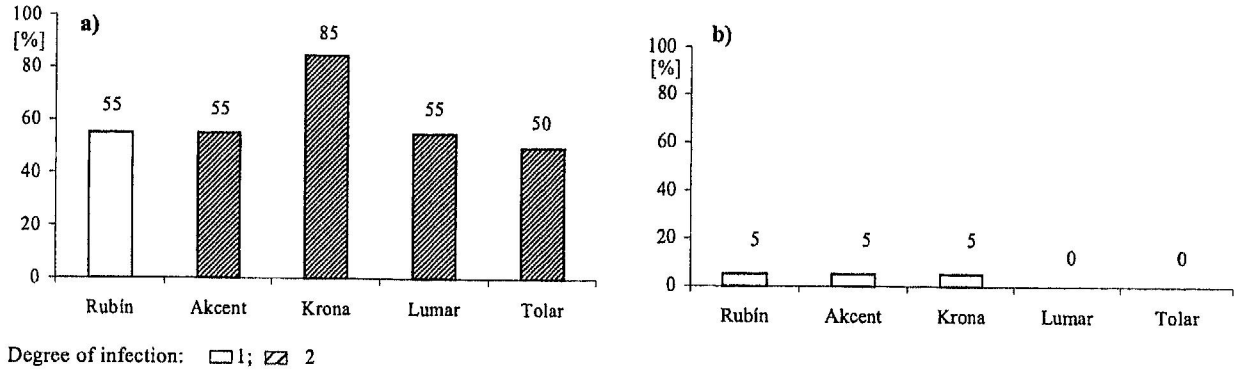

Fig. 3. Percentage of plants attacked with (a) *Fusarium culmorum* and (b) *F. poae* – artificial infection – tube test

In laboratory tests on the harvested grain we found:

1) The lowest disease level was found in variants not inoculated with *Fusarium*. The varieties Krona and Tolar were least infected by natural infection, whereas Olbram and Amulet were most infected.
2) In the variants inoculated with *Fusarium avenaceum* and *F. graminearum* the varieties Scarlett, Krona and Tolar had the lowest disease level, while the varieties Lumar, Kompakt and Olbram were high infested by artificial and natural infection. Artificial infection increased the infestation of all varieties but in different way (Fig. 4).
3) The effect of treatment with fungicides was statistically tested; metconazol and the combination tebuconazole + triadimefon + prochloraz were found to be most effective on the seed infection.

Gas Chromatography – Determination of Trichothecenes (NIV, DON, DAS, T-2 toxin, HT-2 toxin)

The *Fusarium* species most frequent in the field, *F. culmorum* and *F. poae*, were examined for trichothecenes production in culture. Isolates grown on malt agar (for conditions see deoxynivalenol) produced NIV and DON above the limit of quantification (0.05 mg/kg). As shown in Table 1, *F. culmorum* produced the highest level of deoxynivalenol.

In further tests, seed of the spring barley variety Rubín with disease level of *F. culmorum*, and also a mixed sample of barley varieties (from Kojetín) were tested for the presence of the above target analytes. As expected, in the variety Rubín the levels of trichothecenes NIV and DON (Table 2) were significantly below legislated limits set in the Czech Republic (2.0 mg DON/kg for cereals and 0.5 mg DON/kg for baby food). Although the level of DON in fungal mycelium was relatively high (Table 1), that found in Rubín was significantly lower. The mixed sample contained neither NIV nor DON, only trace levels of T-2 tetraol and HT-2 toxin were present. The latter trichothecenes are not produced by *F. culmorum* and *F. poae*, but may have originated from other *Fusarium* species. It should be noted that the experiments with both *F. culmorum* and *F. poae* were conducted under laboratory conditions. Since trichothecene production could change under field conditions, the results apply only to those conditions.

Table 1. The concentration of trichothecenes in fungal samples (after purification of crude extract on a charcoal/alumina/Celite column)

Fungal sample	*F. culmorum* [mg/kg]		*F. poae* [mg/kg]	
	NIV	DON	NIV	DON
Malt agar with mycelium	0.06	0.99	n.d.	n.d.
Mycelium	0.09	0.94	0.05	n.d.
Macroconidia	0.15	n.d.	0.06	n.d.

n.d. – not detected

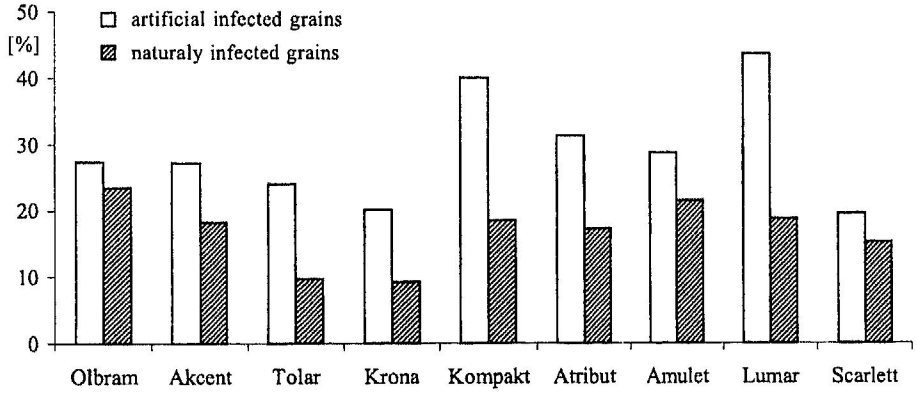

Fig. 4. Percentage of natural attack of barley grains with *Fusarium avenaceum* and *F. graminearum* in comparison with the artificial infection

Table 2. The concentration of trichothecenes in samples of barley grain (after purification of crude extract with a MycoSep 225 column)

Barley	NIV	T-2 tetr.	DON	FUS-X	HT-2 tox.	T-2 tox.
Rubín	0.13 mg/kg	n.d.	< LOQ	n.d.	n.d.	n.d.
1997 Kojetín (mixture)	n.d.	0.05 mg/kg	n.d.	n.d.	< LOQ	n.d.

LOQ – limit of quantification 0.05 mg/kg (the lowest concentration of the standard used for the calibration curve)
n.d. – not detected

DISCUSSION

The species *F. culmorum* and *F. poae* are most frequent in Czech barley; *F. culmorum* occurred in approximately 70% of the population of *Fusarium* isolates and *F. poae* in about 20%. These two species were, therefore, used for inoculation tube-tests. The pathogenicity tests were carried out as tube tests on young plants in Prague-Ruzyně (the origin of the seed was in Kroměříž) and on adult plants in Kroměříž. In many varieties of spring barley the mycelium of *Fusarium* species was found in the seed.

Other species of *Fusarium* were isolated from other regions and crops and the results are as different as sometimes conforming in the comparison with our experiments done on the material from the area of Kroměříž. On the coincidence ELEN *et al.* (1997) showed that the most occurring species of *Fusarium* (on barley, oats and wheat) in Norway were: *F. avenaceum, F. culmorum* and *F. graminearum.* They are big differences between single areas e.g. on winter wheat. SCHILLING *et al.* (1997) found out that in Sersheim (Germany) prevailed *F. graminearum* while in Russia (Nowgorod) *F. culmorum* and in Holland (Groningen) *F. avenaceum.* It seemed that single species of *Fusarium* need special conditions. In northern countries there prevailed species like *F. avenaceum, F. culmorum, F. poae* (which prevailed on the second place also in our experiments) and *F. tricinctum* (ELEN *et al.* 1997; KOSIAK *et al.* 1997), in southern countries there prevailed species like: *F. graminearum* and *F. moniliforme* (JURKOVIĆ, COSIĆ 1997). It was surprising that in Germany prevailed on winter wheat *F. nivale* (*Microdochium nivale*) (it wasn't found us), although this parasitic fungus prevailed in the past time more on the rye (OBST *et al.* 1997). ŠROBÁROVÁ (1997) referred that on the maize there were other species of *Fusarium*: *F. moniliforme* and *F. proliferatum,* different from cereal species of *Fusarium* found in the area of Kroměříž (Czech Republic).

Seed free of *Fusarium* is recommended on the control of the disease. The presence of *Fusarium* sp. could also negatively influence the results from tube tests like those described here. Therefore, we recommended GFP and SPFP plants (Germ-Free Plants, Specified Pathogen-Free Plants) for raising seed without fungal and other pathogens. Such plants must be grown under sterile conditions (sterile soil, water and air) in special isolators, and the procedure must be controlled by microbiological examinations.

In our experiments we studied rather thermostable mycotoxins that constitute a great danger for the health of man and animals (MARARAS *et al.* 1984; SNIJDERS *et al.* 1996; DE NIJS *et al.* 1997). The toxins are also a significant factor in the brewing of beer (SCHWARZ *et al.* 1995). This demonstrates the importance of *Fusarium* diseases. In the literature there are many papers that recommend suitable agronomic technologies to limit them (time of sowing, rotation of crops etc.) (CORAZZA *et al.* 1995) which can't be still studied in our conditions. Protection by fungicide and resistance of varieties are the most applied measures (PROM *et al.* 1997; MC MULLen *et al.* 1997). The use of suitable methods of chemical analysis will enable the selection genotypes with low mycotoxine content (USLEBER *et al.* 1991; SEIDEL *et al.* 1993; BURROWS 1994; CROTEAU *et al.* 1994).

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References

BACKHOUSE D., BURGESS L. W., SUMMERELL B. A., LIDDELL C. M. (1997): A species concept for *Fusarium:* The integration of information. In: MESTERHÁZY A. (Ed.): Cereal Res. Comm. Proc. 5th Eur. Fus. Sem., Szeged, Vol. 25, No. 3/2: 527–532.

BOOTH C. (1971): The genus *Fusarium.* Kew, Surrey (UK). Comm. Mycol. Inst.

BURROWS E. P. (1994): Dimethyl ether chemical ionization Mass spectrometry of trichothecene biotoxins. Biol. Mass Spectrom., **23**: 492–501.

CORAZZA L., CHILOSI G., BALMAS V. (1995): Relazione tra i diversi tipi di ordinamenti colturali e lo sviluppo di patogeni fungini. Agrocoltura-Ricerca, **15**: 255–262.

CROTEAU S. M., PRELUSKY D. B., TRNHOLM H. L. (1994): Analysis of trichothecene mycotoxins by gas chromatography with electron capture detection. Agr. Food Chem., **42**: 928–936.

DE NIJS M., VAN EEGMOND H. P., ROMBOUTS F. M., NOTERMANS S. H. W. (1997): Identification of hazardous *Fusarium* metabolites occurring in food raw materials. J. Food Saf., **17**: 161–191.

ELEN O., LANGSETH W., LIU W., HAUG G., SKINNES H.,

GULLORD M., SUNDHEIM L. (1997): The content of deoxynivalenol and occurrence of *Fusarium* spp. in cereals from field trials in Norway. In: MESTERHÁZY A. (Ed.): Cereal Res. Comm. Proc. 5th Eur. Fus. Sem., Szeged, Vol. 25, No. 3/2: 585–586.

GERLACH W., NIRENBERG H. (1982): The genus *Fusarium* – a Pictorial Atlas. Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft, Berlin-Dahlem, Heft 209.

JURKOVIĆ D., COSIĆ J. (1997): Influence of *Fusarium* species on wheat seed germination. In: MESTERHÁZY A. (Ed.): Cereal Res. Comm. 5th Eur. Fus. Sem., Szeged, Vol. 25, No. 3/2: 761–762.

KOSIAK B., TORP M., THRANE U. (1997): The occurrence of *Fusarium spp.* in Norwegian grain – A survey. In: MESTERHÁZY A. (Ed.): Cereal Res. Comm. Proc. 5th Eur. Fus. Sem., Szeged, Vol. 25, No. 3/2: 595–96.

MARARAS W. F. O., NELSON P. E., TOUSSON T. A. (1984): *Fusarium* Species: Identity and Mycotoxicology. The Pennsylvania State University Press, University Park, P. A.

MC MULLEN M. P., LUKACH J., STOVER R., GREGOITE T. (1997): Studies of fungicide efficacy, application technologies to reduce *Fusarium* head blight and deoxynivalenol. In: MESTERHÁZY A. (Ed.): Cereal Res. Comm. 5th Eur. Fus. Sem., Szeged, Vol. 25, No. 3/2: 699–703.

OBST A., LEPSCHY-VON GLEISSENTHALL J., BECK R. (1997): On the etiology of *Fusarium* head blight of wheat in south Germany – preceding crops, weather conditions for inoculum production and head infection, proneness of the crop to infection and mycotoxin production. In: MESTERHÁZY A. (Ed.): Cereal Res. Comm. 5th Eur. Fus. Sem., Szeged, Vol. 25, No. 3/2: 699–703.

PROM K. L., STEFFENSON B. J., SALAS B., FETCH J. K., CASPER H. H. (1997): Barley accession resistant to *Fusarium* head blight and the accumulation of deoxynivalenol. In: MESTERHÁZY A. (Ed.): Cereal Res. Comm. 5th Eur. Fus. Sem., Szeged, Vol. 25, No. 3/2: 807–808.

SCHILLING A. G., MIEDANER T., GEIGER H. H. (1997): Molecular variation and genetic structure in field populations of *Fusarium* species causing head blight in wheat. In: MESTERHÁZY A. (Ed.): Cereal Res. Comm. 5th Eur. Fus. Sem., Szeged, Vol. 25, No. 3/2: 549–554.

SCHWARZ P. R., CASPER H. H., BEATIE S. (1995): Fate and development of naturally occurring *Fusarium* mycotoxins during malting and brewing. J. Am. Soc. Brew. Chem., **53**: 121–127.

SEIDEL V., LANG B., FRAISSLER S., LANG C., SCHILLER K., FILEK G., LINDNER W. (1993): Analysis of trace levels of trichothecene mycotoxins in Austrian cereals by gas chromatography with ECD. Chromatographia, **37**:191–198.

SNIJDERS C. H. A., SAMSON R. A., HOEKSTRA E. S., QUELLET T., MILLER J. D., ROOIJ-VAN DER GOES P. C. E. M., MAAR A. J. M., DUBOIS A. J. E., KAUFFMAN H. F. (1996): Analysis of *Fusarium* causing dermal toxicosis in marram grass planters. Mycopathol., **135**: 119–128.

ŠROBÁROVÁ A. (1997): Occurrence of *Fusarium* spp. from Slovakia maize kernels. In: MESTERHÁZY A. (Ed.): Cereal Res. Comm. 5th Eur. Fus. Sem., Szeged, Vol. 25, No. 3/2: 617–618.

STACK R. W., FROHBERG R. C., CASPER H. (1997): Reaction of spring wheats incorporating Suma-23-derived resistance to inoculation with seven *Fusarium* species. In: MESTERHÁZY A. (Ed.): Cereal Res. Comm. 5th Eur. Fus. Sem., Szeged, Vol. 25, No. 3/2: 625–627.

TÓTH A. (1997): Dominance conditions of *Fusarium* species occurring in winter wheat kernels in Pest County. In: MESTERHÁZY A. (Ed.): Cereal Res. Comm. 5th Eur. Fus. Sem., Szeged, Vol. 25, No. 3/2: 625–627.

USLEBER E., MÄRTLBAUER E., DIETRICH R., TERPLAN G. (1991): Direct Enzyme Linked Immunosorbent Assay for the detection of the 8-ketotrichothecene mycotoxins deoxynivalenol, the 3-acetyl-deoxynivalenol, 15-acetyl-deoxynivalenol in Buffer Solutions. J. Agr. Food Chem., **39**: 2091–3001.

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HÝSEK J.,VÁŇOVÁ M., HAJŠLOVÁ J., RADOVÁ Z., KOUTECKÁ J., TVARŮŽEK L. (1999): **Fuzariózy ječmene a obsah trichothecenů**. Pl. Protec. Sci., **35**: 96–102.

Bylo sledováno napadení semen sladovnického ječmene rodem *Fusarium* v oblasti Kroměříže (360 vzorků) a jeden směsný vzorek z Kojetína. Nejčastějšími druhy v období 1997–1998 byly *F. culmorum* (více než 70 % izolátů), *F. poae* (více než 20 % izolátů) a *F. avenaceum* (přes 2 %). Mnohem méně časté byly druhy *F. stilboides* v. *stilboides*, *F. aqaeductum* v. *aqaeductum*, *F. merismoides* v. *merismoides* a *F. gigas*. Izoláty *F. culmorum* byly vysoce patogenní při umělé infekci ječmene, izoláty *F. poae* měly patogenitu nízkou. Pět odrůd ječmene jarního v růstové fázi 1–5 podle Feekesovy stupnice reagovalo na *F. culmorum* následovně: 1) Tolar (nejvíce rezistentní), 2) Akcent, Lumar a Rubín (střední rezistence), 3) Krona (nejnáchylnější). Aplikace fungicidů na ploše uměle inokulované s *F. avenaceum* a *F. graminearum* zvyšovala výnos zrna z 0,46 na 1,71 t/ha. Nejvíce účinné fungicidy proti patogenům byly metconazole, tebuconazole a prochloraz a rovněž kombinace tebuconazole + triadimefon + prochloraz. Pro determinaci šesti trichothecenových mykotoxinů (NIV, DON, DAS, FUS-X toxin, T-2 toxin, HT-2 toxin) byla

použita kapilární plynová chromatografie se záchytem elektronu. Pouze toxin T-2 a nivalenol (NIV) byl detekován v houbovém mycéliu a nízká hladina NIV byla nalezena v ječmeni jarním odrůda Rubín (nižší než registrovaný limit – 2 mg/kg obilovin).

Klíčová slova: ječmen jarní; *Fusarium culmorum*; *F. poae*; *F. graminearum*; *F. avenaceum*; umělá infekce; resistence odrůd; účinnost fungicidů; mykotoxiny

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