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Determination of toxigenic Fusarium spp. in the domestic wheat – using the ICFM methodological recommendation

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Fifty one food wheat samples from three production regions in the Czech Republic have been mycologically examined in this study. *Fusarium* species were isolated by the method of grain rinse with sterile 0.1 % pepton in water and by the method of direct plating of grains after their surface sterilization. Czapek Dox Iprodione Dichlorane Agar (CZID) was used for cultivation. The methodological procedures used issued from the recommendation of the International Commission of Food Mycology (ICFM). The identification of the isolated strains has been done according to Nelson et al. (1983) and by comparing with collection strains of the genus *Fusarium* (Czech Collection of Microorganisms in Brno – CCM). Standardization of the above-mentioned mycological methods in food mycology is necessary for making collaborative studies and also for possibilities of comparison of results obtained in different time. *Fusarium* spp. isolated from food wheat samples of showed the greatest frequency in the following order: *Fusarium graminearum, F. avenaceum, F. sporotrichioides, F. reticulatum and F. solani*. The methodological procedure is recommended for determination of toxigenic *Fusarium* spp. in wheat and other cereals after a harvest and for comparison of results obtained both between individual production regions and in individual years.

Key words: *Fusarium* spp., wheat, isolation, identification, mycotoxins, food mycology

Ostrý V., Ruprich J. a Kožíšek J. (1998): Stanovení toxinogenních mikroskopických hub rodu Fusarium v tuzemské pšenici – použití metodického doporučení ICFM. – Czech Mycol. 50: 313–321

V studii bylo mykologicky vyšetřeno 51 vzorků potravinářské pšenice ze tří produkčních oblastí v České republice na přítomnost toxinogenních fusárií. Druhy rodu *Fusarium* byly izolovány metodou oplachu zrn sterilní peptonovou vodou a metodou přímé kultivace zrn po jejich povrchové sterilizaci. Byla použita kultivační živná půda – Czapek Dox Iprodione Dichloran Agar (CZID). Použité metodické postupy vycházely z doporučení International Commision of Food Mycology (ICFM). Identifikace izolovaných kmenů byla provedena dle Nelsona et al. (1983) a porovnáním se sbírkovými kmeny rodu *Fusarium* z České sbírky mikroorganismů v Brně. Standardizace uvedených kultivačních metod v oblasti mykologie potravin je v současné době nezbytná pro provádění kolaborativních studií i možnosti srovnání získaných výsledků v různém čase. S největší frekvencí byly izolovány ve vzorcích potravinářské pšenice druhy rodu *Fusarium* v následujícím pořadí: *Fusarium graminearum, F. avenaceum, F. poae, F. sporotrichioides, F. reticulatum a F. solani*. Použitý metodický postup můžeme doporučit pro stanovení toxinogenních fusárií v pšenici a ostatních obilovinách po sklizni a pro srovnání získaných výsledků mezi jednotlivými produkčními oblastmi i v jednotlivých letech.

INTRODUCTION

Fusarium species are significant phytopathogens of cereals and other cultivated plants in our country (Šíp and Stuchlíková 1997), in the neighbouring countries (Mesterházy 1984, Chelkowski et al. 1984, Chelkowski et al. 1989, Rintelen 1985, Jimenez et al. 1993), and also in the world (Wilcoxson et al. 1988, Sayer and Lauren 1991). Many of them rank to the significant producers of mycotoxins (trichothecenes, zearalenone, moniliformin, fusarin C, fumonisins and others) (Ueno 1983, Chelkowski 1989, Miller et al.1991) which are hazardous for health of man, farm and wild animals.

From the historical point of view, there are many approaches of dividing the genus Fusarium into sections (groups), species and varieties. There is the conception of Wolenweber and Reinking (1935) with 26 species, through the approach of Snyder and Hansen (1945) with 9 species, Bilai (1955) with 26 species, Booth (1971a) with 44 species, Joffe (1974) with 33 species, Nelson et al. (1983) with 30 species, to Gerlach and Nirenberg (1982) with 78 species. The chemotaxonomic methods, which use dividing on the basis of detection and determination of secondary metabolites, have been developed and applied recently (Thrane 1990, Miller et al. 1991).

A qualitative and quantitative determination of the individual *Fusarium* spp. in cereals is made by means of procedures which are based on different methodics of mycological treatment of samples and on a type of culture medium used Wolenweber and Reinking (1935), Snyder and Hansen (1945), Bilai (1955), Booth (1971b), Joffe (1974), Nirenberg (1976), Gerlach and Nirenberg (1982), Nelson et al. (1983), Andrews and Pitt (1986) and Abildgren et al. (1987). Pitt et al. (1992) recommended methods for mycological examination of foodstuffs in food mycology.

The goal of this study was in particular testing of methodological approaches for isolation and determination toxigenic *Fusarium* spp. in food wheats according to the recommendation of the 1st – 3th International Workshops on Standardization of Methods for the Mycological Examination of Foods and the International Commission of Food Mycology (ICFM). These approaches can be used in practical activities of the National Reference Centre in evaluation of food safety of vegetable origin.

MATERIAL AND METHODS

A) Source of samples

In the course of August 1993 food wheat samples (*Triticum aestivum* L.) were taken in collaboration with the workers of purchasing and supplying agricultural

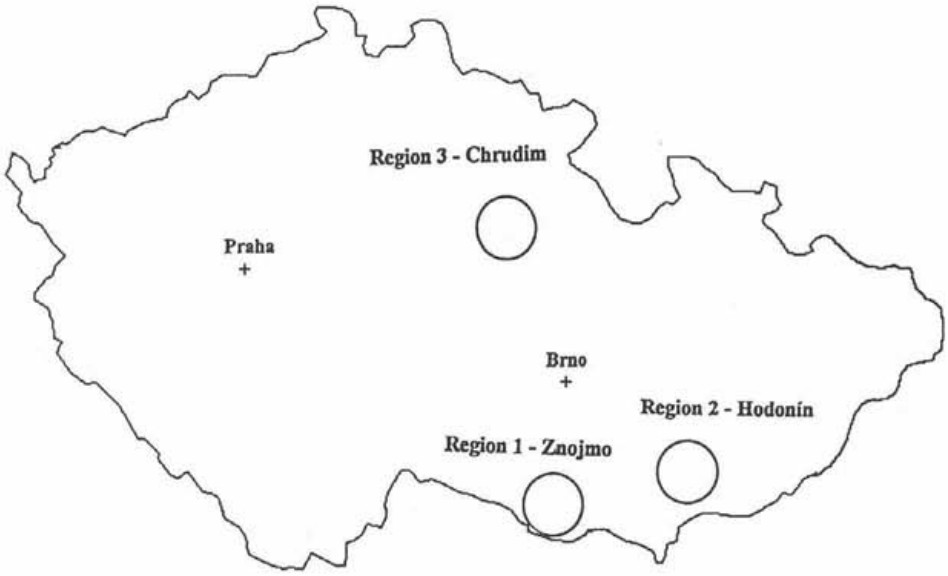

Fig. 1. Sampling regions

firms of shareholding companies a.s. Znojmo (18 samples), Hodonín (13 samples), and Chrudim (20 samples) (for localization see Fig. 1).

B) Mycological study

1. Determination of qualitative representation of *Fusarium* spp. in a rinse

Working procedure:

A rinse from the 30 g sample is performed with 270 ml sterile 0.1 % pepton in water in the 500 ml Erlenmeyer flask on a laboratory shaker for 5 minutes, and then the sample is let to stand for about 1 minute, and it will be used for other dilution. Samples were then serially diluted 1:10 (1+9). Spread plates are recommended over pour plates. Serially diluted samples (0.1 ml) were spread on CZID plates.

Culture medium used:

Czapek Dox Iprodione Dichlorane Agar (CZID) (Oxoid components), 20 ml per plate (Abildgren et al., 1987)

Incubation: 5 days at 25 °C in dark.

2. Determination of internal contamination of wheat grains with the *Fusarium* spp.

Working procedure:

100 wheat grains of each sample are surface-sterilized by immersion into 5 % NaClO (0.6 % chlorine) for the time of 2 minutes, and then it is rinsed three times with sterile distilled water. 100 surface – sterilized grains are placed on the Petri dishes (10 cm) with Czapek Dox Iprodione Dichlorane Agar per 10 grains on each Petri dish.

Incubation: 5 days at 25 °C in dark.

3. Identification of the isolated *Fusarium* spp.

Culture medium used:

(PDA) – Potato Dextrose agar with chloramfenicol selective supplement (Oxoid) (Booth 1971b)

(CLA) – Carnation-leaf agar (Fischer et al. 1982).

Young carnation (*Dianthus caryophyllus* L.) leaves are cultivated in laboratory conditions and are harvested from plants free from pesticide residues.

Identification of the isolates has been made according to Nelson et al. (1983) and by comparison with the collection strains *Fusarium* spp. of Czech Collection of Microorganisms in Brno (only supplemental method).

4. Statistical evaluation

A single-factor analysis of dispersion – ANOVA was applied with the use of the Borland Company software Quattro – Pro for Windows of the version 5.

RESULTS AND DISCUSSION

Fig. 1 represents a percentual internal contamination by the representatives of *Fusarium* spp. in food wheat samples from three production regions. This indicator characterizes an extent of grain internal contamination. It is assumed that the samples of grain with a higher internal contamination by the representatives *Fusarium* spp. may contain also a higher contamination of wheat samples by *Fusarium* mycotoxins (Chelkowski 1989). In the testing food wheat samples from the 1987 harvest in the FRG it was determined internal contamination of grain in the range of 60–100 % in more than 80 % of the tested samples (Muller and Schwadorf 1993), and in testing of wheat samples from the 1987–1989 harvests in New Zealand it was determined internal contamination of grain in the range of 20–100 % in about 7 % of the tested samples (Sayer and Lauren 1991). The above-mentioned examples illustrate a considerable difference in the results achieved, which are dependent on the whole line of factors – climatic conditions, measures of farming technology, variety, region, etc. (Chelkowski 1989), but also

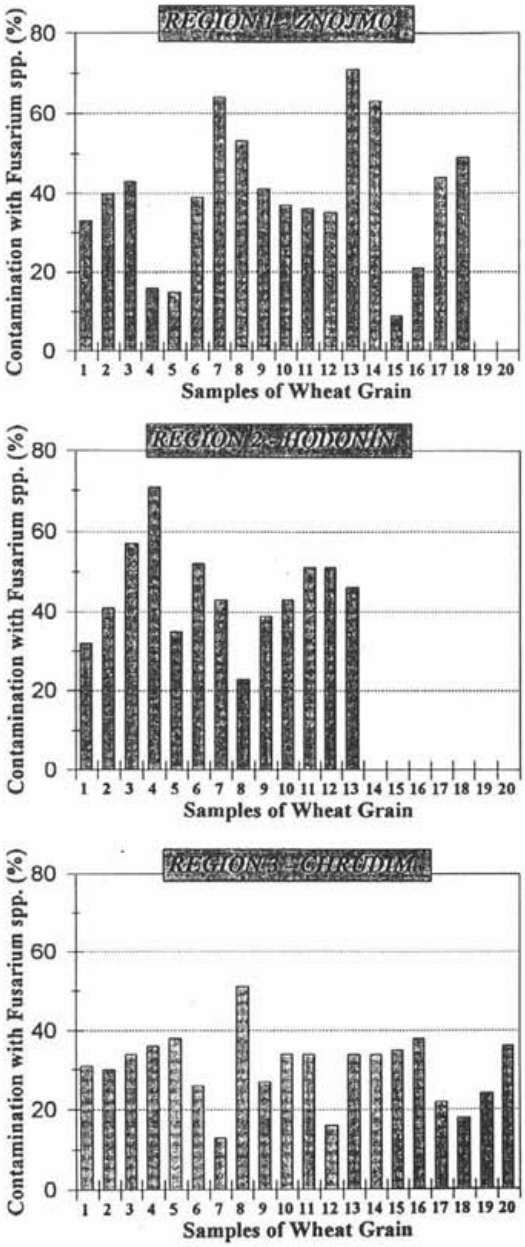

Fig. 1. Internal contamination of wheat grain with *Fusarium* spp.

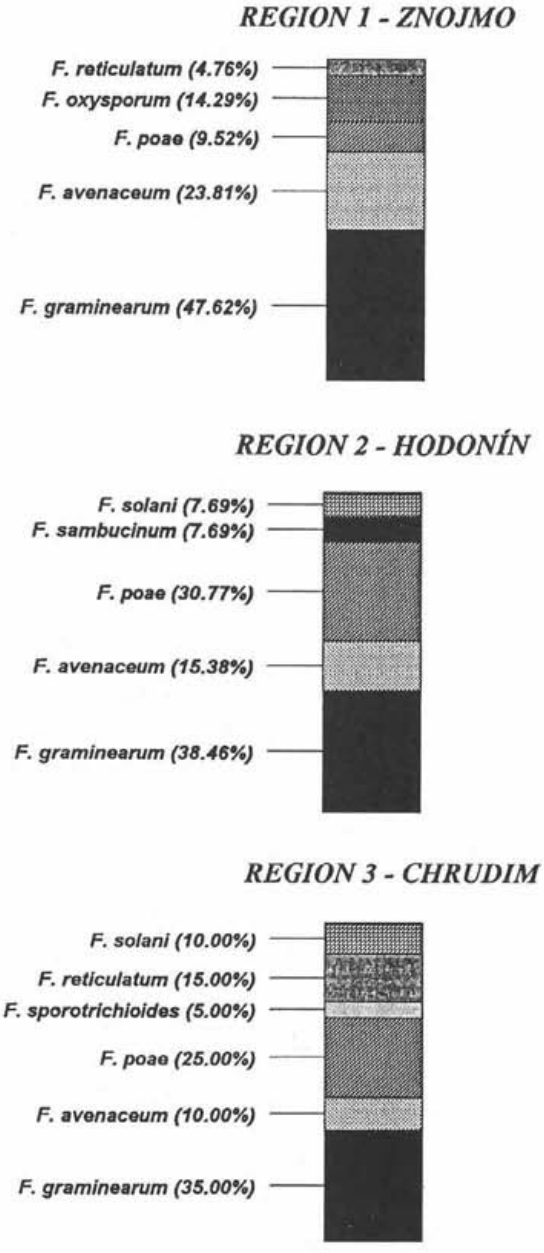

Fig. 2. Qualitative determination of *Fusarium* spp. from the surface of wheat grain from 3 regions

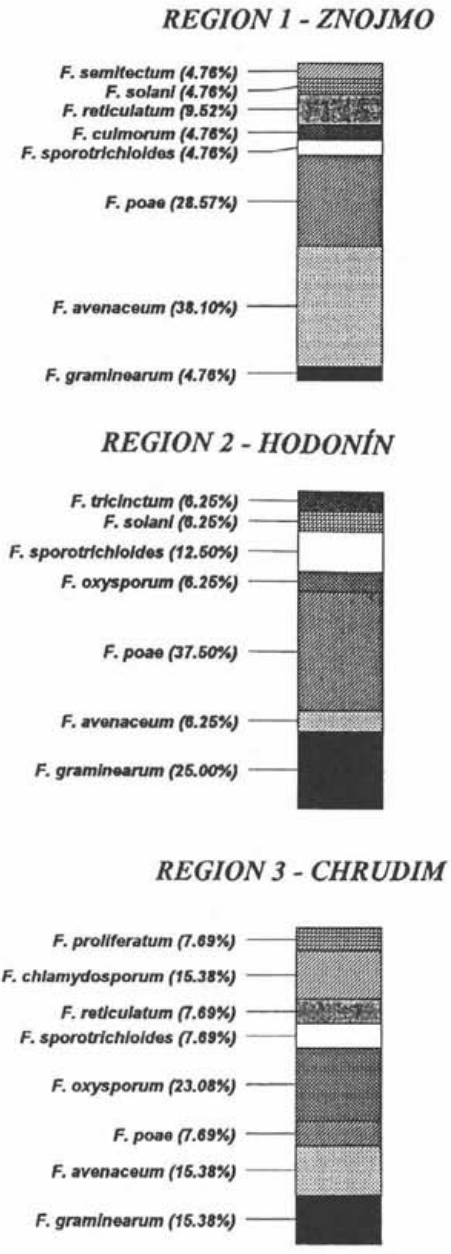

Fig. 3. Qualitative determination of *Fusarium* spp. after surface sterilization of wheat grain from 3 regions

on sampling and methodological approaches to determination of grain internal contamination, which may significantly affect a result of laboratory examination. Only for illustration it is stated that in the German treatise a 5 % solution of NaClO was used for surface sterilization for the time of 2 minutes, in the paper from New Zealand a 10 % solution of the preparation Janola (31.5 g NaClO for 1 l) was used for the time of 3 minutes; similarly, culture media were different as well. In our case, we have detected average % of grain internal contamination in food wheat in the region of Znojmo, which is 39.4 %, in the region of Hodonín 45 %, and in the region of Chrudim 30.6 %. With regard to a great dispersion of the results in individual sets, statistically significant differences between the regions were not, however, detected.

Fig. 2 shows a percentual representation of the *Fusarium* spp. from a rinse of food wheat samples from three production regions. These results are important mainly from the view of comparison of qualitative representation of the genus *Fusarium* in a rinse, and in grains after surface sterilization.

Fusarium spp. in internal contaminated food wheat grains from three production regions is presented in Fig. 3. A qualitative representation of the isolated *Fusarium* spp. is important especially from toxicological view (possible production of *Fusarium* mycotoxins) and the phytopathological view (pathogenicity and invasivity).

Fusarium spp. isolated from food wheat samples showed the greatest frequency in the following order: *Fusarium graminearum, F. avenaceum, F. poae*. The potential toxigenic *Fusarium* spp. can produce the next mycotoxins (Table 1).

In the qualitative mycological examination of the internal contamination of wheat grain from the 1990–91 harvests in Hungary the following *Fusarium* spp. were determined, including their percentage representation (the names are presented as they appear in the original publication): *Fusarium equiseti* (Corda) Sacc. (35 %) and *Fusarium poae* (Peck) Wollenw. (35 %), *Fusarium graminearum* Schwabe (11.7 %), *Fusarium sporotrichioides* Sherb. (11.7 %), *Fusarium semitectum* Berk. et Rav. (5.85 %), *Fusarium culmorum* Sacc. (5.36 %), the other isolated *Fusarium* spp. (less than 5 %) (Toth et al. 1993). In the previous study (Mesterházy 1984) the prevalent strains of *Fusarium graminearum* and *Fusarium culmorum* formed 85 % of all isolated strains of the genus *Fusarium*, while *Fusarium equiseti* and *Fusarium poae* occurred only sporadically. In the qualitative mycological examination of internal contamination of wheat grain in Austria (Adler et al. 1990) nine *Fusarium* species were isolated: *Fusarium culmorum, Fusarium avenaceum, Fusarium graminearum, Fusarium poae, Fusarium equiseti, Fusarium sambucinum, Fusarium nivale, Fusarium oxysporum* and *Fusarium solani*. In the qualitative mycological examination of internal contamination of wheat grain from the 1987 – 1989 harvests in New Zealand four species with the greatest frequency of occurrence were isolated: *Fusarium graminearum*

Table 1 The isolated *Fusarium* spp. and their potential mycotoxins.

Producers	Mycotoxins*
Fusarium graminearum	DON, 3-acetyl DON, NIV, DAS, ZEA, FUS C, FUS X, BUT
Fusarium avenaceum	MON, FUS C
Fusarium poae	T-2 toxin, FUS C, DAS, NIV
Fusarium sporotrichioides	T-2 toxin, DAS, MON, FUS C, ZEA
Fusarium culmorum	DON, NIV, ZEA, MON, FUS C, FUS X, 3-acetyl DON, BUT
Fusarium oxysporum	MON, FUS C, WORT, ISOVER, ZEA
Fusarium reticulatum	?
Fusarium solani	NTCH, FUS A, TRICH
Fusarium proliferatum	MON, FUM
Fusarium tricinctum	FUS C, BUT
Fusarium semitectum	FUSP, DEOFUSP
Fusarium sambucinum	FUS C, ZEA, TRICH, BUT
Fusarium chlamydosporum	?

(*El-Banna et al. 1984, Frisvad 1988, Frisvad and Thrane 1993, Marasas 1994)

3-acetyl DON	3 – acetyl deoxynivalenol	NIV	nivalenol
DON	deoxynivalenol	MON	moniliformin
DAS	diacetoxyscirpenol	FUM	fumonisins
FUS C	fusarin C	ZEA	zearalenon
FUS X	fusarenon X	BUT	butenolide
FUSP	fusapyrone	DEOFUSP	deoxyfusapyrone
FUS A	fusaric acid	TRICH	trichothecenes
WORT	wortmannin	ISOVER	isoverrucarol
NTCH	naphtochinones		

Fusarium avenaceum, *Fusarium culmorum*, and *Fusarium poae* (Sayer and Lauren, 1991).

A fundamental problem inherent in *Fusarium* identification is that members of the genus vary widely in morphological and nonmorphological characteristics, including virulence and toxigenity and these criteria are used in taxonomic systems. Nomenclatural problems dealing with synanamorphs and generic typification and neotypification of species are very often discussed, so identification of the isolates has been made according to Nelson et al. (1983) in this study (Windels 1991).

Quantitative and qualitative finds of *Fusarium* spp. in cereals are dependent on climatic conditions, measures of farming technology, variety, sampling, methodics of mycological treatment of samples, and type of culture medium used. A standardization of the aforesaid mycological methods and introducing the principles of

the Quality Assessment / Quality Control (QA/QC) into a laboratory work also in the field of food mycology is necessary for making collaborative studies and for possibilities of testing capability of laboratories for food mycology.

The selective substrate for *Fusarium* spp. Czapek Dox Iprodione Dichlorane Agar (CZID) was developed for use in cereal industry. This tested culture medium and selected methodological procedure of surface sterilization of grains are according to our practical experience applicable for quantitative determination of the "field" microscopic fungi *Fusarium* spp. in wheat and other cereals after a harvest with a following cultivation of isolated strains on media – Potato Dextrose agar (PDA) and Carnation-leaf agar (CLA).

Interpretation of the results obtained in collaborative studies, and possibility of comparison of the results with other studies (e.g. with phytopathological ones) are bound to similar methodological approaches and use of the same culture media for cultivation.

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REFERENCES

ABILDGREN M. P., LUND F., THRANE U. and ELMHOLT S. (1987): Czapek-Dox agar containing iprodione and dichloran as a selective medium for the isolation of Fusarium species. – Lett. Appl. Microbiol. 5: 83–86.

ADLER A., LEW H. and EDLINGER W. (1990): Vorkommen und Toxicität von Fusarien auf Getreide und Mais aus Östereich. – Bodenkultur. 41: 145–152

ANDREWS S. and PITT J. I. (1986): Selective medium for the isolation of Fusarium spp. and dematiaceous Hyphomycetes from cereals. – Appl. Environ. Microbiol. 51: 1235–1238.

BILAI V. I. (1955): The Fusaria (Biology and Systematics) (In Russian). Akad. Nauk. Ukr. SSR, Kiev. 320 pp.

BOOTH C. (1971a): The genus Fusarium. Commonwealth Mycological Institute, Kew, Surrey, UK. 237 pp.

BOOTH C. (1971b): Fungal culture media. In: Booth, C. (ed.) Methods in Microbiology, London: Academic press.: 49–94.

CHELKOWSKI J., VISCONTI A., SOLFRIZZO M. and BOTTALICO, A. (1984): Formation of mycotoxins by Fusarium species from cereals in Poland. – Phytopathol. Medit. 23: 43–46.

CHELKOWSKI J., MANKA M., KVASNA A., VISCONTI A. and GOLINSKI P. (1989): Fusarium sporotrichioides Sherb., Fusarium tricinctum (Corda) Sacc. and Fusarium poae (Peck) Wollenw. – Curtural characteristics, toxinogenity and pathogenicity towards cereals. – J. Phytopath. 124: 155–161.

CHELKOWSKI J. (1989): Fusarium, mycotoxins, taxonomy and pathogenicity, topics in secondary metabolites, Vol. 2, Elsevier, Amsterodam, 492 pp.

EL-BANNA A. A., SCOTT P. M., LAU P. Y., SAKUMA T., PLATT H. W. and CAMPBELL V. (1984): Formation of trichothecenes by Fusarium solani var. coeruleum and Fusarium sambucinum in potatoes. – Appl. Environ. Microbiol. 47 (5): 1169-1171.

FISCHER N. L., BURGESS L. W., TOUSSOUN T. A. and NELSON P. E. (1982): Carnation leaves as a substrate and for preserving cultures of Fusarium species. – Phytopathology. 72: 151–153.

FRISVAD J. C. (1988): Fungal species and their specific production of mycotoxins. – In: Samson R. A. (ed.) Introduction to food – borne fungi. Baarn, CBS: 239–249.

FRISVAD J. C. and THRANE U. (1993): Liquid column chromatography of mycotoxins. – In: Betina V. (ed.). Chromatography of mycotoxins, techniques and applications. Amsterodam, Elsevier: 253–372.

GERLACH W. and NIRENBERG H. (1982): The genus Fusarium – A pictorial atlas. – Mitt. Biol. Bundesanst. Land – Forstwirtsch. Berl. – Dahlem. 209: 406 pp.

JIMENEZ M., LOGRIECO A. and BOTTALICO A. (1993): Occurence and Pathogenicity of Fusarium Species in Banana Fruits. – J. Phytopathology. 137: 214–220.

JOFFE A. Z. (1974): A modern system of Fusarium taxonomy. – Mycopathol. Mycol. Appl. 53: 201–228.

MARASAS W. F. O. (1994): Fusarium.– In: HUI Y. H. et al. (ed.) Foodborne disease handbook, Diseases caused by viruses, parazites, and fungi. Marcel Dekker, Inc., New York – Basel, Hong Kong,: 521–573.

MESTERHÁZY A. (1984): Fusarium sp.. of wheat in South Hungary, 1970–1983. – Cereal. Res. Comm. 12: 167–170

MILLER J. D., GREENHALGH R., WANG Y. and LU M. (1991): Trichothecene chemotypes of three Fusarium species. – Mycologia, 83: 121–130.

MULLER H. M. and SCHWADORF K. (1993): A survey of the natural occurence of Fusarium toxins in wheat grown in a southwestern area od Germany. – Mycopathologia 121: 115–121.

NELSON P. E., TOUSSOUN T. A. and MARASAS W. F. O. (1983): Fusarium species – An Illustrated Manual for Identification. The Pennsylvania State University Press, University Park and London. 193 pp.

NIRENBERG H. I. (1976): Untersuchungen ber morphologische und biologische Differenzierung in der Fusarium – Sektion Liseola. – Mitteilung aus der Biologischen Bundesanstalt für Land– und Forstwirtschaft Berlin-Dahlem. 169: 1–117.

PITT J. I., HOCKING A. D., SAMSON R. A. and KING A. D. (1992): Recommended methods for mycological examination of foods, 1992. – In: Samson R. A. et al. (ed.) Modern methods in food mycology. Elsevier Amsterodam – London – New York – Tokyo.: 365–368.

RINTELEN J. (1985): Fusarien Befall an eingelagertem Futtergetreide der Ernten 1982 bis 1984 in Bayern. – Bayer. Landw. Jahrb. 62: 1019–1024.

SAYER S. T. and LAUREN D. R. (1991): Fusarium infection in New Zealand grain. – New Zealand J. of Crop and Horticul. Sci. 19: 143–148

SNYDER W. C. and HANSEN H. N. (1945): The species concept in Fusarium with reference to Discolor and other sections. – Am. J. Bot. 32: 657–666.

ŠÍP V. and STUCHLÍKOVÁ E. (1997): Evaluation of winter wheat varieties response to the artifical infection with Fusarium culmorum in field conditions. – Cer. Res. Comm. 25: [in press]

THRANE U. (1990): Grouping Fusarium section Discolor isolates by statistical analysis of quantitative high performance liquid chromatografic data on secondary metabolite production. – J. Microbiol. Methods. 12: 23–40.

TOTH A., BARNA-VETRO I., POMAZI A., SZECSI A. and HORNOK L. (1993): Prevalence and Mycotoxin Production of Fusarium Species Isolated from Wheat Grains in Hungary. – Acta Phytopathol. et Entomol. Hungar. 28: 3–12.

UENO Y. (1983): Trichothecenes, – Chemical, biological and toxicological aspects, development in food science, Vol.4, Elsevier, Amsterodam, 313 pp.

WILCOXSON R. D., KOMMEDAHL T., OZMON E. A. and WINDELS C. E. (1988): Occurrence of Fusarium species in scabby wheat from Minnesota and their pathogenicity to wheat. – Phytopathology 78: 586–589.

WINDELS C. E. (1991): Current status of Fusarium taxonomy. – Phytopathology 81: 1048–1051.

WOLLENWEBER H. W. and REINKING O. A. (1935): Die Fusarien, ihre Beschreibung, Schadwirkung und Bekämpfung. Paul Parey. Berlin. 355 pp.