Associated-Food-Hazards: Storage Fungi and Mites in Poppy, Mustard, Lettuce and Wheat

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Abstract

Storage fungi and mites frequently cause injury of crops and contamination of crop agro-products (= "sensitive food ingredients") by allergens and toxins. This may have serious practical consequences since currently the food safety is one of the most important priorities of EU-agricultural policy. However, the risk of occurrence of biotic-hazard in various agricultural product and food ingredients is not equal since they differ in their sensitivity to infestation/contamination by various fungi- and mite-hazards. Therefore, the goal of our study was to identify and review the fungi-hazards connected with occurrence of 5 key-species of mite-hazards, in 4 kinds of "sensitive food ingredients" that include poppy, mustards, lettuce and wheat grain. Different numbers of fungal-hazards (wheat: 44, poppy: 37, mustard: 13, lettuce: 31) were isolated from the tested 4 kinds of crop agro-product. This indicates that their sensitivity to mite-associated fungal infestation/contamination increases in the following order: mustard, lettuce, poppy, and wheat. Mite-hazards differ in their vector-capacity of various fungi-hazards. Generally, predatory mites (i.e. *Cheyletus* spp.) represent lower risk than fungivorous and herbivorous species of mites (i.e. *Acarus siro*, *Tyrophagus putrescentiae, Lepidoglyphus destructor, Caloglyphus rhizoglyphoides)* in terms of vectoring fungi hazards. Many of the mites and fungi hazards rarely occurred independently. We therefore propose that (i) such pest-hazard-systems (i.e. fungi-mite-hazard-systems) should be called "associated-hazards" (ii) the new and specific approaches to risk assessment of "associated hazards" should be developed and implemented into practice.

Keywords: fungi; mites; transport; vector; food safety; sensitive ingredients; poppy; mustard; wheat; mycotoxins

INTRODUCTION

The organisms directly affecting the food safety via contamination or spoilage of raw or finished foodstuffs materials are termed "hazards", because the Codex Alimentarius Commission, defined food hazard as: "A biological, chemical or physical agent in or condition of, food with potential to cause an adverse health effects" (SPERBER 2001). Stored seeds for human consumption are frequently infested by many species of fungi that cause not only various kinds of crop injury (i.e. decrease in germination of malting barley and discolouration of grain – CHRISTENSEN 1957) but also a contamination of food agricultural products by mycotoxins and allergens (FRISVAD & SAMSON 1991; FRISVAD 1995; SAMSON *et al*. 1996; MILLER 1995). The infestation of food agro-products by fungi is frequently connected with presence of mites, which may be dangerous vectors of many species of fungi (GRIFFITHS *et al*. 1959; SINHA 1966; ARMITAGE & GEORGE 1986; PARKINSON *et al*. 1991; FRANZOLIN *et al*. 1999). However, it is important to realize that also fungi "provide food" for many species of fungivorous pest-mites (SINHA 1968). In other words, the presence of fungi is a primary cause and thus also a limiting condition for the infestation of food agro-product by mites. This is important from the food-safety point of view since not only fungi but also mites contaminate food agro-products by allergens (ARLIAN 2002). In the past decade, it was documented that the consumption of cereal-grain contaminated by storage-mite allergens may even result in human anaphylaxis (CASTILLO *et*

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al. 1995; SCALA 1995; SÁNCHEZ-MONGE *et al*. 1996). The associated multi-infestation of food by allergenic fungi and mites may be even more dangerous to public health than the single infestation either by mite or fungi hazards.

In the pest risk assessment and "Hazard Analysis and Critical Points" (HACCP) -plan, a hazard analysis is the collection and evaluation of information and data of contaminants and conditions leading to food safety risks (HOORNSTRA *et al*. 2001). SPERBER (2001) stressed that the major method of "hazard and risk analysis" is the identification and review of known hazards for each sensitive ingredient (i.e. food agro-products in this case) where sensitive ingredient is defined as "a material that has been historically associated with a known hazard". For hazard and risk analysis is thus important to construct a complete list of hazards.

Various agricultural product and food ingredients differ in their sensitivity to various biotic-hazards. Therefore, the goal of our study was the identification and review of "associated-hazards" (i.e. fungi-hazards associated with five key mite-hazards) in four sensitive ingredients that include poppy, mustard, lettuce and wheat grain.

MATERIAL AND METHODS

The micromycetes were isolated from samples infested by storage mites. The samples were collected in the flat horizontal stores near Prague (Czech Republic) from November 2000 to March 2001. Four kinds of samples were observed: wheat grain and seeds of poppy, lettuce and mustard.

Wheat grain was sampled in Buštěhrad and was infested by *Acarus siro*, *Tyrophagus putrescentiae* and *Caloglyphus rhizoglyphoides*. Mustard samples were collected in multiple flat store in Veleliby and infesting species was *Acarus siro,* only. The poppy seeds were also sampled in Veleliby and were infested by *Acarus siro* and *Lepidoglyphus destructor*. The lettuce samples originated from Prague and *Acarus siro*, *Lepidoglyphus destructor* and *Cheyletus malaccensis* were infesting species. The weight of each sample was about 500 g, a part (200 g) of each sample was used for extraction and determination (method see in ŽĎÁRKOVÁ 1998) of mites, the rest of the samples was stored in freezer and used for isolation of micromycetes.

Mites were separated individually under binocular microscope using preparation needles from small part of sample placed into sterile Petri dishes. To isolate fungi from mites surface mites were washed individually in 1 ml of re-sterilized distilled water for 5 minutes without any sterilization of their surfaces. To isolate fungi from mite digestive tract their surface was sterilized by washing in 8% sodium hypochlorite (Savo) for 2 minutes, and then in 96% ethanol for next 2 minutes (HUBERT *et al*. 2000). The sterilized mites were placed into sterile micro-centrifugation tubes and homogenized in 1 ml of re-sterilized distilled water. 0.2 ml of supernatant from every sample was placed on Petri dishes with malt agar and on Czapek-Dox agar (Imuna®, Slovakia). Altogether it was analysed 300 mite samples.

The fungi were incubated at 25 ± 2°C in dark condition. The CFU (colony forming units) of fungi were counted for orientation after 5 and 7 days and present fungi were inoculated to identification media. Determination of microfungi was made according to MORTON and SMITH (1963); SHOEMAKER (1968); DOMSCH *et al*. (1980); VÁŇOVÁ (1989); SAMSON *et al*. (1996); PITT and HOCKING (1996). Some of the interesting fungal strais (*Acrodontium salmoneum* CCF 3220, *Circinella muscae* CCF 3187, *Clonostachys rosea* CCF 3222, *Dendryphion penicillatum* CCF 3190 and 3191, *Doratomyces putredinis* CCF 3221, *Eurotium repens* CCF 3305, *Oidiodendron cerealis* CCF 3193, *Penicillium verrucosum* CCF 3215 and 3216, *Penicillium viridicatum* CCF 3213 and 3214, *Scopulariopsis brumptii* CCF 3192, *Stemphylium herbarum* CCF 3189) were freeze-dried and deposited in the Culture Collection of Fungi (CCF), Department of Botany, Charles University, Prague, Czech Republic.

RESULTS AND DISCUSSION

Fungi and mites as "associated-food-hazards". Altogether 71 fungal-hazards (i.e. fungal species) were associated with the 5 most important mite-hazards that include *Acarus siro*, *Tyrophagus putrescentiae, Lepidoglyphus destructor, Caloglyphus rhizoglyphoides* and *Cheyletus malacccensis*. Mite- and fungi-hazards rarely occurred independently and therefore should be assessed together as"associated-hazards". Forty-four fungal-hazards were isolated from wheat, 37 from poppy, 13 from mustard and 31 from lettuce (Table 1). Thus our study indicates that the sensitivity of food ingredients increases in the following order mustard, lettuce, poppy, and wheat, in terms of occurrence of mite-associated fungi-hazards. Most frequent fungi-hazards associated with mite-hazards include *Aspergillus niger*, *Penicillium crustosum*, *Penicillium aurantiogriseum*, *Aspergillus versicolor*, *Eurotium repens*, *Cladosporium cladosporioides*, *Aspergillus candidus*,

Penicillium chrysogenum, *Penicillium brevicompactum*, and *Penicillium verrucosum* (Table 1). Most of micromycetes recorded are typical for stored grain (CHELKOWSKI 1991; PITT and HOCKING 1996). Notably, xerotolerant fungi such as *Eurotium* spp. growing at low water activity were often found.

Transfer of fungi via predatory vs. fungivorous mites. Mite-hazards differ in their vector-capacity of various fungi-hazards (Figure 1). The highest number of fungi-hazards was associated with *Acarus siro*, while the lowest number of fungi-hazards was associated with *Cheyletus malaccensis*. This indicates that predatory mites (i.e. *Cheyletus* spp.) represent lower risk than fungivorous and herbivorous species of mites (i.e. *Acarus siro*, *Tyrophagus putrescentiae, Lepidoglyphus destructor, Caloglyphus rhizoglyphoides)* in terms of vectoring fungi-hazards (BOWMAN 1984).

Poppy as a sensitive ingredient. The most frequent fungi-hazards in poppy where *Aspergillus versicolor*, *Scopulariopsis brevicaulis*, *Aspergillus niger*, *Aspergillus candidus*, *Eurotium repens*, *Penicillium crustosum*, *Eupenicillium* sp., *Scopulariopsis brumptii*, *Dendryphion penicillatum*, and *Aspergillus sydowii*. Among the most important toxigenic fungi belong after FRISVAD and SAMSON (1991) *A. versicolor* (producent of sterigmatocystin), *A. candidus* (tephenyllin and xanthoascin), *P. crustosum* (penitrem *A, roquefortine* C and terrestric acid).

Mustard as a sensitive ingredient. The most frequent fungi-hazards in mustard where *Penicillium aurantiogriseum*, *Penicillium* sp. 102/01, *Penicillium expansum*, *Eurotium repens*, *Penicillium aurantiogriseum* group, *Penicillium verrucosum*, *Penicillium* sp. 100/01, *Penicillium chrysogenum*, and *Penicillium griseofulvum*. *P. aurantiogriseum* is potential producent of nephrotoxic glycopeptides, viomellein and xanthomegnin. The following fungi are great mycotoxin producents: *P. aurantiogriseum* (viomellein and xanthomegnin), *P. expansum* (patulin), *P. verrucosum* (ochratoxin A), *P. chrysogenum* (roquefortine C and xanthocillin X) and *P. griseofulvum* (cyclopiazonic acid, patulin and roquefortine C) – see in FRISVAD and SAMSON (1991).

Lettuce as a sensitive ingredient. The most frequent fungi-hazards in mustard where *Penicillium chrysogenum*, *Aspergillus niger*, *Penicillium crustosum*, *Cladosporium cladosporioides*, *Eurotium repens*, *Aspergillus versicolor*, *Alternaria alternata*, *Penicillium* sp. 36/01, *Penicillium aurantiogriseum*, and *Penicillium griseofulvum*. Most notable toxigenic fungi are *P. chrysogenum*, *P. crustosum, A. versicolor, P. griseofulvum* (see above), and *A. alternata*, producer of tenuazonic acid, alternariol and altertoxins.

Wheat as a sensitive ingredient. The most frequent fungi-hazard in wheat where *Penicillium aurantiogriseum*, *Aspergillus niger*, *Penicillium crustosum*,

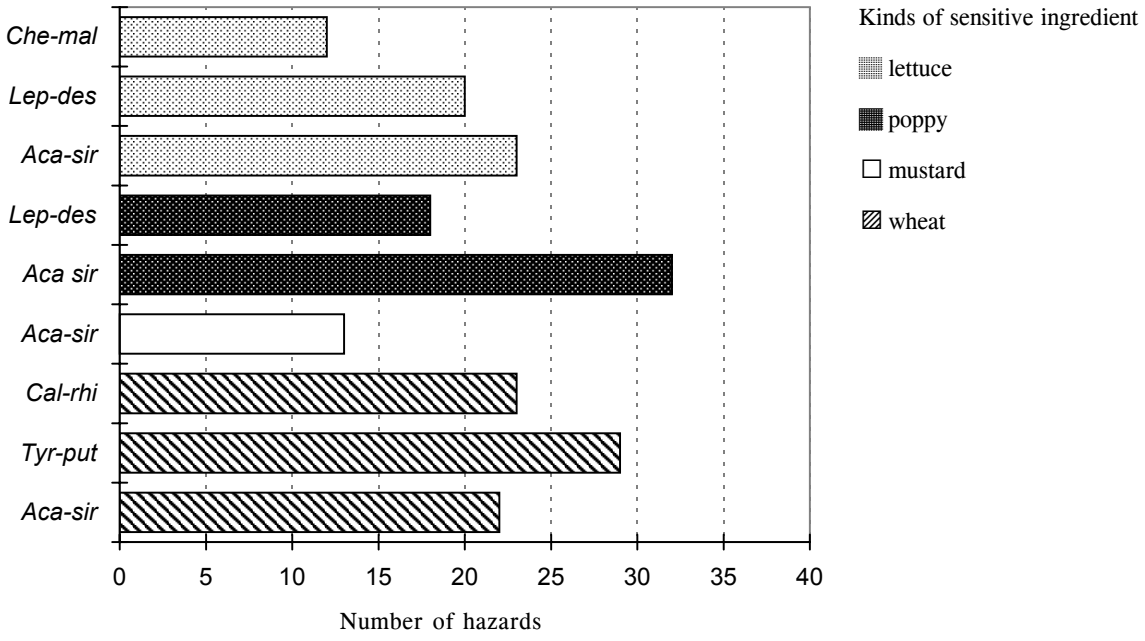

Figure 1. Total number of fungal hazards (species) associated to mites and various kinds of grain. Legends: Aca-sir – *Acarus siro*, Cal-rhi – *Caloglyphus rhizoglyphoides,* Che-mal – *Cheyletus malaccensis*, Lep-des – *Lepidoglyphus destructor*, Tyr-put – *Tyrophagus putrescentiae*

Table 1. The list of fungal hazards associated to mites

Type of sensitive ingredient hazards	Lettuce						Mustard		Poppy				Wheat					
	Acarus siro		*Cheyletus malaccenis*		*Lepidoglyphus destructor*		*Acarus siro*		*Acarus siro*		*Lepidoglyphus destructor*		*Acarus siro*		*Caloglyphus rhizoglyphoides*		*Tyrophagus putrescentiae*	
	F (%)	F_i	F (%)	F_i	F (%)	F_i	F (%)	F_i	F (%)	F_i	F (%)	F_i	F (%)	F_i	F (%)	F_i	F (%)	F_i
Acremonium sp.															4	14		
Acrodontium salmoneum			8	7											4	14		
Alternaria alternata	8	5			16	3			4	12					4	14		
Alternaria sp.	2	14			8	8			2	17								
Arthrosporic white fungus															4	14		
Aspergillus fumigatus	2	14	4	10											4	14		
Aspergillus candidus	2	14			4	12	4	6	12	5	15	4	32	5			4	14
Aspergillus avus			13	4					6	7			12	12	46	4		
Aspergillus niger	4	8	13	4	24	2	4	6	18	3	19	2	24	7	83	1	27	3
Aspergillus sydowii	2	14							2	17	15	4					2	18
Aspergillus ustus																	2	18
Aspergillus versicolor	4	8	4	10	16	3			46	1	27	1	40	3	4	14	8	8
Botrytis cinerea	2	14	8	7	4	12									25	5	2	18
Circinella muscae																	2	18
Cladosporium cladosporioides	12	2	25	1	4	12			4	12			8	18	13	9	8	8
Cladosporium herbarum	4	8	13	4									4	21	54	3	19	6
Cladosporium sphaerospermum					4	12									4	14		
Dendryphion penicillatum					4	12			6	7	4	11						
Doratomyces putredinis															21	7	2	18
Emericella nidulans									2	17								
Eurotium amstelodami					4	12			2	17	12	6						
Eurotium herbariorum									6	7								
Eurotium repens	10	3	4	10	16	3	12	2	14	4	12	6	8	18			2	18
Eurotium rubrum													12	12				
Eurotium spp.											4	11						

Type of sensitive ingredient hazards	Lettuce						Mustard		Poppy				Wheat					
	Acarus siro		*Cheyletus malaccenis*		*Lepidoglyphus destructor*		*Acarus siro*		*Acarus siro*		*Lepidoglyphus destructor*		*Acarus siro*		*Caloglyphus rhizoglyphoides*		*Tyrophagus putrescentiae*	
	F (%)	F_i	F (%)	F_i	F (%)	F_i	F (%)	F_i	F (%)	F_i	F (%)	F_i	F (%)	F_i	F (%)	F_i	F (%)	F_i
Geomyces pannorum													12	12				
Grey sterile mycelium							4	6										
Mucor ramosus					8	8												
Mycocladus corymbifer	2	14							4	12								
Oidiodendron cerealis									2	17								
Paecilomyces farinosus																	2	18
Penicillium aurantiogriseum	4	8	8	7	8	8	40	1	4	12	4	11	100	1	13	9	42	1
Penicillium aurantiogriseum group							8	5					20	8			19	6
Penicillium brevicompactum	4	8					4	6	2	17			80	2			25	4
Penicillium citrinum					4	12									13	9		
Penicillium crustosum	8	5	21	2	8	8			12	5	8	8	16	11	83	1	29	2
Penicillium expansum							12	2				19	4	21				
Penicillium griseofulvum	8	5			12	6	4	6	2	17	4	11	12	12				
Penicillium hordei									2	17			12	12			6	12
Penicillium chrysogenum	31	1	21	2	44	1	4	6			8	8			21	7	2	18
Penicillium olsonii															8	13	6	12
Penicillium polonicum													8	18			2	18
Penicillium roqueforti	2	14																
Penicillium rugulosum									2	17								
Penicillium simplicissimum											4	11						
Penicillium sp. 100/01							4	6										
Penicillium sp. 101/01													20	8			8	8
Penicillium sp. 102/01							12	2					36	4			21	5
Penicillium sp. 164/01																	2	18
Penicillium sp. 33/01									6	7	8	8						

Type of sensitive ingredient hazards	Lettuce						Mustard		Poppy				Wheat					
	Acarus siro		*Cheyletus malaccenis*		*Lepidoglyphus destructor*		*Acarus siro*		*Acarus siro*		*Lepidoglyphus destructor*		*Acarus siro*		*Caloglyphus rhizoglyphoides*		*Tyrophagus putrescentiae*	
	F (%)	F_i	F (%)	F_i	F (%)	F_i	F (%)	F_i	F (%)	F_i	F (%)	F_i	F (%)	F_i	F (%)	F_i	F (%)	F_i
Penicillium sp. 34/01									2	17								
Penicillium sp. 36/01	10	3			12	6												
Penicillium sp. 41/01	2	14																
Penicillium sp. 50/01	2	14			4	12												
Penicillium sp. 94/01																	4	14
Penicillium spinulosum											4	11						
Penicillium thomii															4	14		
Penicillium variabile															25	5	4	14
Penicillium verrucosum	2	14					4	6	4	12			32	5			8	8
Penicillium viridicatum													20	8			2	18
Rhizopus microsporus var. *rhizopodiformis*									2	17							2	18
Rhizopus sp.									2	17								
Rhizopus stolonifer											4	11	12	12	13	9	4	14
Scopulariopsis brevicaulis									38	2	19	2						
Scopulariopsis brumptii									6	7	4	11						
Stemphylium herbarum									2			19						
Trichothecium roseum									2			19						
Ulocladium chartarum	4	8							2			19						

F – frequency, i – rank

Aspergillus versicolor, *Penicillium brevicompactum*, *Cladosporium herbarum*, *Penicillium* sp. *102/01*, *Rhizopus stolonifer*, *Cladosporium cladosporioides*, and *Penicillium verrucosum*. Among them, *P. aurantiogriseum*, *P. crustosum*, *A. versicolor*, *Penicillium brevicompactum* (producent of botryodiploidin and mycophenolic acid) and *P. verrucosum* are considered as important toxigenic fungi.

CONCLUSIONS

Many kinds of mite-fungi-hazards regularly occurred infested the four sensitive food-ingredients (i.e. mustard, lettuce, poppy, and wheat) in the Czech Republic. It is to note that similar storage condition for food-agro product can be found throughout whole Europe and other temperate geographical areas of the world. This indicates that our results may be of general importance.

The frequent presence of biotic-hazards in food-agro products may have serious implications for agricultural and food industry practice since the food safety is one of the most important priorities of agricultural policy in EU. In this political climate, the presence of any hazard in food may lead to the dramatical penalization of the afflicted food producer and its devastating negative publicity. Therefore, mustard, lettuce, poppy, and wheat grain should be regularly checked for presence of the above presented associated fungi-mite hazards and, most importantly, these sensitive commodities should be stored and transported under conditions that prevent occurrence and growth of population of both types of hazards (i.e. low temperature and moisture). In addition, it should be beard on mind that many mite- and fungi-hazards rarely occurred independently. We therefore propose that (i) such pest-hazard-systems (i.e. fungi-mite hazard systems) should be called "associated-hazards" (ii) the new and specific approaches to risk assessment of "associated hazards" should be developed and implemented into practice.

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References

ARLIAN L.G. (2002): Arthropod allergens and human health. Annu. Rev. Entomol., **47**: 395–434.

ARMITAGE D.M., GEORGE C.L. (1986): The effect of three species of mites upon fungal growth on wheat. Exp. Appl. Acarol., **2**: 111–124.

BOWMAN C.E. (1984): Comparative enzymology of economically important astigmatid mites. In: GRIFFITHS D.A., BOWMAN C.E. (eds): Acarology 6, Vol. 2, Ellis Horwood, Chichester: 993–1001.

CASTILLO S., SANCHEZ-BORGES M., CAPRILES A., SUAREY-CHACON R. (1995): Systematic anaphylaxis after ingestion of mite-contaminated flour. J. Allergy Clin. Immunol., **95**: 304.

CHELKOWSKI J. (ed.) (1991): Cereal Grain. Mycotoxins, Fungi and Quality in Drying and Storage. Elsevier, Amsterdam etc.

CHRISTENSEN C.M. (1957): Deterioration of stored grain by fungi. Bot. Rev., **23**: 108–134.

DOMSCH K.H., GAMS W., ANDERSON T.-H. (1993): Compendium of Soil Fungi. Vol.1. Eching, IHW-Verlag.

FRANZOLIN M.R., GAMBALE W., CUERO R.G., CORREA B. (1999): Interaction between toxigenic *Apergillus flavus* Link and mites (*Tyrophagus putrescentiae* Schrank) on maize grains: effects on fungal growth and aflatoxin production. J. Stored Products Res., **35**: 215–224.

FRISVAD J.C. (1995): Mycotoxins and mycotoxigenic fungi in storage. In: JAIAS D.S., WHITE N.D.G., MUIR W.E. (eds): Stored-Grain Ecosystems. Marcel Dekker, Inc., New York: 251–288.

FRISVAD J.C., FILTENBORG O. (1989): Terverticillate penicillia: chemotaxonomy and mycotoxin production. Mycologia, **81**: 837–861.

FRISVAD J.C., SAMSON R.A. (1991): Mycotoxins produced by species of *Penicillium* and *Aspergillus* occurring in cereals. In: CHELKOWSKI J. (ed.): Cereal Grain. Mycotoxins, Fungi and Quality in Drying and Storage. Elsevier, Amsterdam: 441–476.

GRIFFITHS D.A., HODSON A.C., CHRISTENSEN C.M. (1959): Grain storage fungi associated with mites. J. Econ. Entomol., **52**: 514–518.

HOORSNTRA E., NOTERSMANS N., BARENDSZ A.W. (2001): The use of quantitative risk assessment. Food Control., **12**: 229–234.

HUBERT J., KUBÁTOVÁ A., ŠÁROVÁ J. (2000): Feeding of *Scheloribates laevigatus* (Acari: Oribatida) on different stadia of decomposing grass litter (*Holcus lanatus*). Pedobiologia, **44**: 627–639.

MILLER J.D. (1995): Fungi and mycotoxins in grain: Implications for stored product research. J. Stored Products Res., **31**: 1–16.

MORTON F.J., SMITH G. (1963): The genera *Scopulariopsis* Bainier, *Microascus* Zucal, and *Doratomyces* Corda. Mycol. Pap., **8**: 1–96.

PARKINSON D., JAMIESON N., EBORALL J., ARMITAGE D.M., (1991): Comparison of the fecundity of three species of grain store mites on fungal diets. Exp. Appl. Acarol., **12**: 297–302.

PITT J.I., HOCKING A.D. (1996): Fungi and Food Spoilage. London etc.

SAMSON R.A., HOEKSTRA E.S., FRISVAD J.C., FILTENBORG O. (1996): Introduction to Food-Borne Fungi. Baarn et Delft.

SÁNCHEZ-MONGE R., GARCÍA-CASADO G., BABER D., SALCEDO G. (1996): Interaction of allergens from house dust mite and from cereal flours: *Dermatopahgoides pteronyssinus* alpha-amylase (Der p 4) and wheat and rye alpha amylase inhibitors. Allergy, **51**: 176–180.

SCALA G. (1995): House-dust mite ingestion can induce allergic intestinal syndrome. Allergy, **50**: 517–519.

SHOEMAKER R.A. (1968): Type studies of *Pleospora calvescens, Pleospora papaveracea*, and some allied species. Can. J. Bot., **46**: 1143–1150.

SINHA R.N. (1966). Feeding and reproduction of some stored-product mites on seed-borne fungi. J. Econ. Entomol., **59**: 1227–1232.

SINHA R.N. (1968): Adaptive significance of mycophagy in stored-product Athropoda. Evolution, **22**: 785–798.

SPERBER W.H (2001): Hazard identification: from quantitative to a qualitative approach. Food Control., **12**: 223–228.

VÁŇOVÁ M. (1989): Revize československých zástupců vybraných čeledí *Mucorales* (*Absidiaceae, Cunninghamellaceae, Mucoraceae, Mycotyphaceae, Syncephalastraceae and Thamnidiaceae*). [Disertační práce.] Univerzita Karlova, Praha.

ŽĎÁRKOVÁ E. (1998): Fauna roztočů v skladovaném obilí v ČR. Ochr. Rostl., **34**: 49–52.