

Mites and Fungi in Heavily Infested Stores in the Czech Republic

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ABSTRACT Toxigenic and allergen-producing fungi represent a serious hazard to human food and animal feed safety. Ninety-four fungal species were isolated from mite-infested samples of seeds taken from Czech seed stores. Fungi were isolated from the surface of four kinds of seeds (wheat, poppy, lettuce, and mustard) and from the gut and external surface of five species of mites (i.e., *Acarus siro* L., 1758, *Caloglyphus rhizoglyphoides* (Zachvatkin, 1973), *Lepidoglyphus destructor* (Schränk, 1781), *Tyrophagus putrescentiae* (Schränk, 1781) and *Cheyletus malaccensis* Oudemans 1903) separately. Multivariate analysis of fungi complex composition showed that the frequency of fungal was species significantly influenced by the kind of seed. Fungal frequencies differed between mites gut and exoskeleton surface and between the surfaces of mites and seeds. Three groups of fungal species were recognized: 1) mite surface-associated fungi: *Penicillium brevicompactum*, *Alternaria alternata*, and *Aspergillus versicolor*; 2) mite surface- and seed-associated fungi: *Aspergillus niger*, *Penicillium crustosum*, *Penicillium aurantiogriseum*, *Penicillium chrysogenum*, and *Aspergillus flavus*; and 3) seed-associated fungi: *Cladosporium herbarum*, *Mucor dimorphosporus* f. *dimorphosporus*, *Botrytis cinerea*, *Penicillium griseofulvum*, and *Eurotium repens*. Mite-carried species of microfungi are known to produce serious mycotoxins (e.g., aflatoxin B₁, cyclopiazonic acid, sterigmatocystin, ochratoxin A, and nephrotoxic glycopeptides) as well as allergen producers (e.g., *A. alternata* and *P. brevicompactum*). Storage mites may play an important role in the spread of some medically hazardous micromycetes. In addition, these mite-fungi associations may heighten the risk of occurrence of mycotoxins in food and feed stuffs and cause mixed contamination by fungal and mite allergens.

KEY WORDS mites, aflatoxin, vectors, mycotoxins, stored-products

STORAGE PESTS CAUSE ENORMOUS damage to stored commodities worldwide. Recently, the concept of ecosystem approach to manage storage-product systems was proposed (Arbogast and Throne 1997) because the occurrence of various pests in stored seeds is not independent. For example, mites influence the growth of micromycetes by grazing and spore dispersion (Griffiths et al. 1959, Armitage and George 1986, Franzolin et al. 1999). Stored seeds for human consumption are frequently infested by many species of fungi that decrease germination (e.g., malting barley), food quality, and discoloration of grain (Christensen 1957). Both finished food and food raw materials may be contaminated with fungal spores from different environmental sources. Rodents and arthropods are among the most frequently listed vectors (Beti et al. 1995). The detailed understanding of vector ability and capacity of various pests is extremely important because fungal infestation of food may represent an enormous risk to human health. The current literature gives convincing evidence that food-infesting fungi

are able to produce many secondary metabolites, including mycotoxins, that may influence both animal and human health (Miller 1995, Jayas and White 2003). The most important mycotoxin is aflatoxin B₁, a carcinogenic metabolite of *Aspergillus flavus*, which especially affects the liver (Pitt and Hocking 1997, Wild and Hall 2000). Nephrotoxic effects are known to be associated with ochratoxin A and citrinin produced by *Penicillium verrucosum* and with viomellein and nephrotoxic glycopeptides originating from *Penicillium aurantiogriseum* and its allies. A carcinogenic effect is supposed in sterigmatocystin (Frisvad and Samson 1991, Frisvad 1995, Samson et al. 1996). Except mycotoxins, some of storage fungi produce allergens (Larsen 1994).

Although there is documentation of mites as vectors of some species of fungi (Jacot 1930), the relative importance of most species of storage mites as vectors of fungi that infest food grains (cf. Aucamp 1969) still remains unclear. Therefore, the aim of this study was 1) to compare the frequency of various fungal species in the guts and on the surfaces of various species of mites to the seeds to find which species of fungi are associated with mites most frequently; and 2) to evaluate the risk of contamination of heavily infested stored seeds by toxigenic fungi related to mite infes-

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Table 1. Seed species samples and the species of mites recovered

Seed species	Variety	Harvest date	Sampling date	Mite species recovered	Family	Approx. mite density (mites per kg seed)
Wheat	BRUTA	3 Aug. 2000	15 Jan. 2001	<i>Acarus siro</i>	Acaridae	3500
<i>Triticum aestivum</i>				<i>Tyrophagus putrescentiae</i>	Acaridae	1400
			5 Feb. 2001	<i>Caloglyphus rhizoglyphoides</i>	Acaridae	1800
			1 Mar. 2001	<i>Tyrophagus putrescentiae</i>	Acaridae	1520
Lettuce	SORAYA	8 Jul. 2000	24 Feb. 2001	<i>Acarus siro</i>	Acaridae	1100
<i>Lactuca sativa</i> var. Capitata				<i>Lepidoglyphus destructor</i>	Glycyphagidae	2500
			26 Nov. 2000	<i>Acarus siro</i>	Acaridae	1890
Poppy	OPAL	29 Aug. 2000	24 Feb. 2001	<i>Cheyletus malaccensis</i>	Cheyletidae	1250
<i>Papaver somniferum</i>				<i>Acarus siro</i>	Acaridae	2500
				<i>Lepidoglyphus destructor</i>	Glycyphagidae	1850
				<i>Acarus siro</i>	Acaridae	1800
Mustard	VERONIKA	21 Aug. 2000	24 Feb. 2001	<i>Acarus siro</i>	Acaridae	4550
<i>Sinapis alba</i>						

tation in the Czech Republic. This work is a part of long-term research program at the Research Institute of Crop Production aimed at mapping and evaluating pest hazards and risks in the stored grain and food industry in the Czech Republic (Stejskal and Verner 1996; Žďárková 1979, 1998a; Stejskal and Horák 1999; Stejskal et al. 2003).

Materials and Methods

Samples. The motivation of this study was to compare fungal composition between seeds and mites. Therefore, the sampling procedure was not designed to get good representation of all possible stores or to get good representation of a single store. The idea was to get a range of stores with high infestation in which samples suitable for this kind of analysis could be taken, but to keep the sample size relatively small, so that the time-consuming identification of all the fungal species could be done with high accuracy. Based on our previous survey (unpublished data) of stores in Czech Republic (Central Europe), we selected three stores with high infestation of mites and that differed in the type of stored seed to obtain samples. Wheat (w1, w2, and w3) samples were taken from a horizontal flat store in Buštěhrad; poppy (p1 and p2), lettuce (l1), and mustard seeds (m1) samples were collected from a horizontal flat store in Veleliby near Nymburk; and lettuce seeds (l2) originated from the horizontal grain store of the Research Institute of Crop Production (Prague-Ruzyně). Samples were collected just before planting time of the seeds (i.e., 6–9 mo after the harvest; Table 1) to leave maximal time for development of mite and fungal populations. In the stores, the seeds are stored in sacks (50 kg). These sacks are organized into piles, with the base made of 10 sacks. Within each store, we first identified the pile with concentration >1000 individuals kg⁻¹ seed (threshold for tolerance in European stores; Ridgway et al. 1999). If more such piles were found within a store, we randomly selected one of these. The selected pile was used for detailed sampling. The detailed sampling included taking 10 subsamples (500 g per subsample) from all 10 sacks creating the bottom layer of the preselected pile. Only five subsamples

were taken from the store in Prague-Ruzyně because there were only five sacks in the bottom layer of the piles. These subsamples were gently mixed in a large plastic bag from which only one final sample (500 g) was taken with plastic cup sampler for further analysis. Together, eight independent samples were collected. From each of these samples, we analyzed fungal composition separately from seeds, and from each mite species found in the sample. Within each mite species we analyzed separately fungal communities from mite guts and mite surface. Each of samples thus provided a large amount of information, and the eight samples were a good representation of the system of interest.

Samples Extraction. One-half of each sample was used for immediate mite extraction, whereas the other half was kept in refrigerator (8°C) and used for isolation of fungi during the next 1–3 d. Mites were extracted by a Berlese-Tullgren funnel. The time of exposure was 24 h, and the distance of a 25-W bulb from the sieve was 20 cm (i.e., the temperature at extracted sample surface 35°C). Mites were preserved in Oudemans solution (70% ethanol [87 ml]; acetic acid [8 ml], and glycerol [5 ml]), and microscopic slides were done for their determination. The determination of mites was made according to Zachvatkin (1941), Robertson (1959), Samšínák (1962, 1966), Griffiths (1964), Johnston and Bruce (1965), and Hughes (1977). The mites for mycological analysis were sieved out from the rest of samples, and species was determined under a binocular microscope (Carl Zeiss, Jena, Germany). Mites were removed directly using preparation needles and subjected to micromycetes isolation. From every seed sample, ≈24 mites (12 for surface and 12 for gut) per species were taken for fungal isolation. Six subsamples (without mites) of seed samples follow.

Fungal Isolation. Fungi were isolated from three sites: mite surface (MS), mite digestive tract (feces) (MD), and seed surface (SS) (cf. Hubert et al. 2000). For MS fungal isolation, mites were washed individually in 1 ml of resterilized distilled water for 5 min without any sterilization of their surfaces. Twelve replicates per mite species and seed type were usually used for analysis. For MD fungal isolation, the surface sterilization started by washing mites in 8% sodium

hypochlorite with free chlorine-Czech commercial antiseptic (disinfection) preparation (Savo, Tescona) for 2 min and then transferring to 96% ethanol for the next 2 min. The sterilized mites were placed into sterile microcentrifugation tubes and homogenized in 1 ml of resterilized distilled water. For SS fungal isolation, 0.5 g of tested seeds was washed in 10 ml of resterilized distilled water for 120 min. From all three treatments, 2 by 0.2 ml of supernatant was diluted with resterilized distilled water to provide a concentration range of 5, 25, 125, 625, and 3,125 $\times$. Six replicates per seed type were analyzed. From all these dilutions, 0.2 ml of supernatant was placed on petri dishes with malt agar in the first series and on Czapek-Dox agar (Imuna, Slovakia) agar in the second series. Six replicates per one seed sample were taken.

The fungi were incubated at $25 \pm 2^\circ\text{C}$ in the dark. The colony-forming units (CFU) of fungi were counted for orientation after 5 and 7 d, and the fungi present were determined. Fungal presence was based on the occurrence of at least one fungal species in any of the diluted samples. The fungal frequency was evaluated based on presence of fungal species in isolates from MS, MD, and SS on one type of agar. The isolated microfungi were cultivated in 720 petri dishes (288 MS, 288 MD, and 144 SS).

Fungal Identification. Microscopic fungi were identified according their microscopic and macroscopic features; in *Penicillium* and *Eurotium*, physiological features also were used. The Zygomycetes were cultivated on wort agar (Fassatiová 1986) and identified according to Zycha et al. (1969), Schipper (1973, 1976, 1984a,b), and Váňová (1989). For identification of *Penicillium*, Czapek yeast extract agar, malt extract agar, nitrite sucrose agar, yeast extract sucrose agar, and creatine sucrose agar were used (Frisvad 1981, Samson et al. 1996). For *Eurotium*, Czapek yeast extract agar with 20% sucrose after Pitt and Hocking (1997) was used. Majority of other fungi were cultivated on potato carrot agar and malt extract agar and identified according to Domsch et al. (1993) and Pitt and Hocking (1997). Additional literature was used for determination of rather rare fungi (Morton and Smith 1963, Shoemaker 1968, Hoog 1972). Some fungal representatives are maintained in the Culture Collection of Fungi (Department of Botany, Faculty of Science, Charles University, Prague).

Statistical Analyses. To analyze fungal composition of the samples, we used multivariate analysis. To determine the appropriate type of analysis, we first ran detrended correspondence analysis to determine length of gradient in the data. The length of gradient was 3.25. This is in the interval in which use of both unimodal and linear methods are feasible according to Lepš and Šmilauer (2003). We selected the unimodal methods (namely, Canonical correspondence analysis; Jongman et al. 1987) for the analysis because these methods are considered more robust and more appropriate for ecological data (ter-Braak and Verdonschot 1995, Makarenkov and Legendre 2002, Oksanen and Minchin 2002). Data on frequencies of fungal

species in the samples were used as dependent variables in all the analysis.

To analyze differences in fungal composition between mites and seeds, we used mite versus seed as binary independent variable. Codes of the sample (binary coding) were used as covariates. This test was done in three variants, comparing fungal composition between 1) seeds and both mite guts and mite surface, 2) seeds and mite guts, and 3) seeds and mite surface. Furthermore, we compared fungal composition between mite guts and surface. To do this, we used gut versus surface as independent variable and codes for samples as covariates. To compare differences in fungal composition between different mite species, we used mite species as independent variables (five species, binary coding). We used gut versus seed and sample as covariates.

To test differences between the categories in each of the above-mentioned comparisons, we used Monte Carlo permutation test with 499 permutations of the original data. Single subsamples were randomly permuted within the samples as blocks. In this way, we were able to test the pure difference between the variables of interest within one sample, without confounding the comparison by differences between samples.

In all analyses, rare species (as defined by ter-Braak and Šmilauer 2002) were down-weighted, to decrease their impact on the Canonical Correspondence Analysis (CCA) results. The main idea of this study was to determine the most common and the least common fungal species that are transmitted by mites. Therefore, the fungal frequencies were not transformed before the analysis; consequently, common species had much higher influence on the analysis than the rare ones that are not so important in the context of the study. All the analyses were done using Canoco for Windows software, version 4.5 (ter-Braak and Šmilauer 2002).

Results

Together, 94 fungal species were isolated from five species of mites and from four kinds of seeds. Forty-nine species were isolated from gut of mites (MD), 55 from mites surface (MS), and 60 from the seeds (SS). The frequencies of fungal species were significantly correlated (Table 2) to the kinds of the seeds, e.g., wheat, lettuce, mustard, and poppy (Fig. 1). The kind of the seeds was considered as covariates in further analysis. There was a marginally significant difference between mite species (*Acarus siro* L., 1758, *Caloglyphus rhizoglyphoides* (Zachvatkin, 1973), *Lepidoglyphus destructor* (Schränk, 1781), *Tyrophagus putrescentiae* (Schränk, 1781) and *Cheyletus malaccensis* Oudemans 1903) in fungal composition (Table 2). The marginal significance is probably due to very low sample size for each species in each type of seed. Due to the strong difference between kinds of the seed, which was used as a covariate in this analysis, the residual variation was very low.

Table 2. Details on the multivariate analysis used to analyze data on species frequencies

Data used	Independent variable	Covariate	Randomization	Trace	F	P
Mites and grain	Grain type	Seed/mite-surface/mite-gut	Unrestricted permutation within blocks defined by covariables	0.807	2.919	0.002
Mites	Surface/gut		Split-plot, split-plots (gut-surface) freely exchangeable, wholeplots (individual mite samples) not permuted	0.169	1.133	0.034
Mites	Mite species	Grain type, mite/gut	Split-plot, split-plots (gut-surface) not permuted, wholeplots (individual mite samples) freely exchangeable	0.654	1.452	0.088
Mites and grain	Mite/grain	Grain type	Unrestricted permutation	0.203	2.216	0.006
Mite-gut and grain	Mite/grain	Grain type	Unrestricted permutation	0.149	1.034	0.308
Mite-surface and grain	Mite/grain	Grain type	Unrestricted permutation	0.250	1.889	0.024

Each row corresponds to one analysis and shows type of data used, dependent and independent variables and covariates used, type of randomization and results of the test of significance of all canonical axes.

The frequencies of fungal species isolated from the guts (MD) and surface of mites (MS) showed significant differences (Table 2). Position of fungal species on the first ordination axis of the last analysis was used as a criterion of the affinity of various fungi to be isolated more frequently from mites gut (MD) or their surface (MS). The fungi were classified into three groups based on their position (ax1) and frequency values in isolates (Table 3): 1) mite surface-associated $ax1 < -0.26$, 2) mite surface- and gut-associated $ax1 > -0.26$ $ax1 < 0.26$, and 3) mites gut-associated $ax1 > 0.26$. Twenty-nine fungal species belonged to mite surface-associated group; the most important were *Penicillium griseofulvum*, *Scopulariopsis brevicaulis*, *Eurotium repens*, *P. aurantiogriseum* group, and *Penicillium chrysogenum*. Eighteen fungal species were classified as mite surface and gut-associated. *Aspergillus candidus*, *Aspergillus versicolor*, *Penicillium crustosum*, *Penicillium brevicompactum*, *P. aurantiogriseum*, and *Aspergillus niger* belonged to the most important members of this group. Twenty-four species were mite gut-associated. The most important members of this group were *Cladosporium herbarum*, *P. verrucosum*, *A. flavus*, and *Cladosporium cladosporioides*.

Comparison of fungal composition between mite gut (MD) and seeds (SS) was not significant, whereas the comparison between mite surface (MS) and seeds (SS) was significant (Table 2). Position on the first ordination axis of the last (mite surface/seeds) analysis was used as a criterion of the affinity of various fungi to occur more frequently on mites than on the seeds (Table 4). Based on the position of fungal species on the first axis, the species were categorized into the following "ecological" groups: 1) seed-associated fungi $ax1 < -0.20$, 2) mite surface- and seed-associated fungi $ax1 = > -0.20$ $ax1 < 0.24$, and 3) mite surface-associated fungi $ax1 > 0.25$. Thirty-seven fungal species were classified as seed-associated, the most important were *Mucor circinelloides*, *Mucor dimorphosporus* f. *dimorphosporus*, *Rhizopus arrhizus*, *Botrytis cinerea*, *Mycocladium corymbifer*, *Penicillium polonicum*, *C. herbarum*, *Eurotium amstelodami*, *Penicillium griseofulvum*, and *P. verrucosum*. Fourteen fungal species belonged to mite surface and

seed groups. *Cladosporium cladosporioides*, *Eurotium repens*, *Aspergillus niger*, *Penicillium chrysogenum*, *P. crustosum*, *P. aurantiogriseum*, and *Aspergillus flavus* were the most important member of this group. Thirty-two species were mite surface-associated. *Aspergillus candidus*, *Scopulariopsis brevicaulis*, *Aspergillus versicolor*, *Alternaria alternata*, *P. brevicompactum*, and *P. aurantiogriseum* were the most important species in this group (Table 5).

Discussion

Association of Mites and Micromycetes. In many natural and agroecosystems, mites play a role as vectors and hosts of microfungi; however, mycelium of fungi serve as a food for mites. We isolated 94 kinds of fungi from mites occurring on four kinds of stored seeds in the selected Czech seed storages. Except for *C. malaccensis*, all observed mites species (e.g., *A. siro*, *L. destructor*, *T. putrescentiae*, and *C. rhizoglyphoides*) are facultative fungivores (Sinha 1964, 1966; Sinha and Mills 1968; Smrř and Čatská 1987; Parkinson et al. 1991a,b; Žd'árková 1998b). This indicates that the interaction between mites and microfungi may play an important role in their occurrence and distribution also in storage ecosystems (Arbogast and Throne 1997).

Generally, there are two essential mechanisms of association of spores of fungi with mites: either on or within (feces/gut surface) their bodies (Griffiths et al. 1959, Behan and Hill 1978, Armitage et al. 1986). In this study, we found difference of frequency in various fungal species that occurred within versus on the bodies of mites (Table 3). It may be explained by fungi-interspecific differences in spore production, adhesiveness of spores, or spore viability after gut passage (Smrř and Čatská 1989; Williams et al. 1998a,b; Hubert et al. 1999).

Mites are attracted by moist grain microhabitats (Sinha 1979a), which are also suitable for the fungi (Sinha 1979b, Jayas and White 2003). The existence of commonly preferred microhabitats may be prerequisite for purely accidental association of mites and fungal spores via random contact. We suggest that a

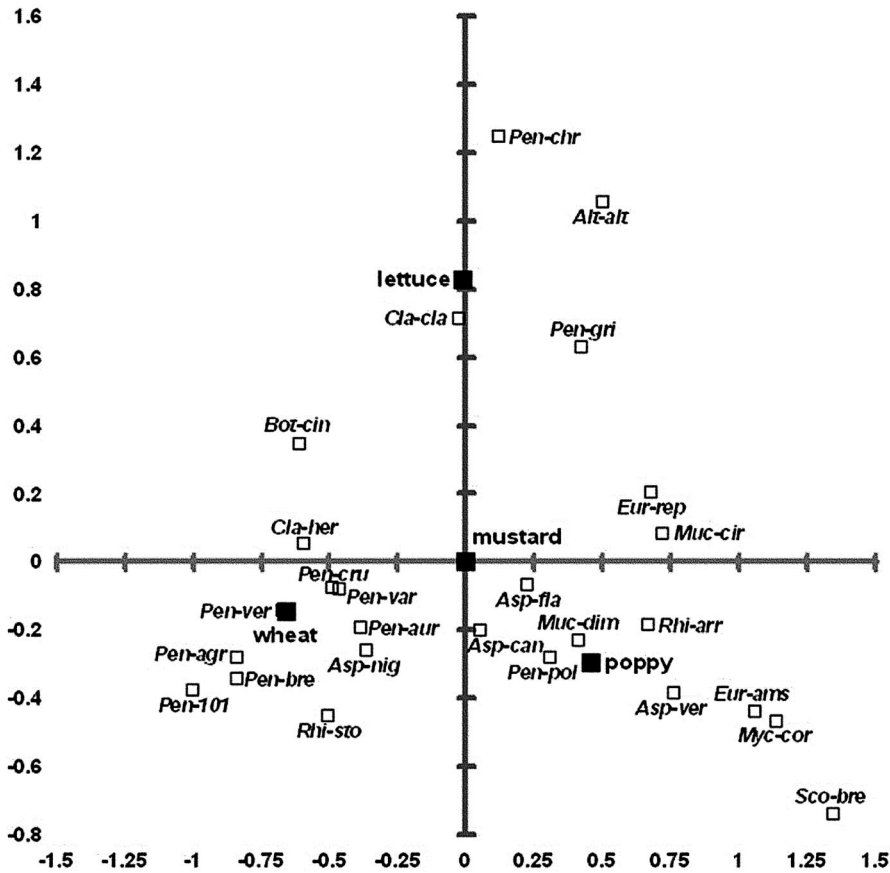


Fig. 1. Results of CCA of the effect of seed type on fungal composition, expressed as frequencies. Type of sample (grain/mite gut/mite surface) was used as a covariate. The first canonical axis explained 10.9% and the second 8.07% of the total variation in the data set. The biplot scores of important fungal species are shown. Alt-alt, *Alternaria alternata*; Asp-can, *Aspergillus candidus*; Asp-fla, *Aspergillus flavus*; Asp-nig, *Aspergillus niger*; Asp-ver, *Aspergillus versicolor*; Bot-cin, *Botrytis cinerea*; Cla-cla, *Cladosporium cladosporioides*; Cla-her, *Cladosporium herbarum*; Eur-ams, *Eurotium amstelodami*; Eur-rep, *Eurotium repens*; Myc-cor, *Mycoclados corymbifer*; Muc-cir, *Mucor circinelloides*; Muc-dim, *Mucor dimorphosporus* f. *dimorphosporus*; Pen-aur, *Penicillium aurantiogriseum*; Pen-arg, *Penicillium aurantiogriseum* group; Pen-bre, *Penicillium brevicompactum*; Pen-cru, *Penicillium crustosum*; Pen-chr, *Penicillium chrysogenum*; Pen-gri, *Penicillium griseofulvum*; Pen-pol, *Penicillium polonicum*; Pen-variety, *Penicillium variable*; Pen-ver, *Penicillium verrucosum*; Pen-101, *Penicillium* sp.; Rhi-arr, *Rhizopus arrhizus*; Rhi-sto, *Rhizopus stolonifer*; Sco-bre, *Scopulariopsis brevicaulis*.

group of such “mite randomly associated fungi” includes *B. cinerea*, *C. sphaerospermum*, *C. herbarum*, *E. amstelodami*, *P. polonicum*, *P. griseofulvum*, *P. verrucosum*, and *Aspergillus fumigatus*, because these species of fungi were almost equally associated with both mites and seed surfaces. However, this work also reveals (Table 4) that the association of the most fungal species with storage mites was not proportional, which indicates that the association, and thus possible vector status, may be selective. What are the mechanisms for disproportionate occurrence of various fungi on or in mites and seeds? It is suggested that it is fungal attractiveness or repellency. Mites are able to perceive fungal odor (vanHaelen et al. 1980, Levinson et al. 1991) and depending on fungal species the odor is attractive (kairomones) or repellent (Pankiewicz-Nowicka and Boczek 1984; Bengtsson et al. 1988, 1991). The attractive fungi lure mites to graze on them

and during grazing their spores get stuck on the surface of the mite. From the fungal species associated with the mite surface (*A. candidus*, *S. brevicaulis*, *A. versicolor*, *Alternaria alternata*, *P. brevicompactum*, and *P. aurantiogriseum* group (Table 4), two species, *Alternaria* spp. *A. candidus*, were reported as attractive (Pankiewicz-Nowicka et al. 1984, Sinha 1984). Mites do not migrate toward the repellent fungal species and do not disperse them or their dispersion is low. The fungal species isolated only from seeds (Table 4), *A. fumigatus* and *M. corymbifer*, are reported to be repellent for storage mites (Sinha 1966).

Medical Hazards of Fungi Associated with Mites. All major mycotoxins reported for the particular species of fungi isolated from our samples to document the current risk of mycotoxin contamination in the Czech grain stores are listed in Table 5. From this review, it is evident that the majority of isolated fungi,

Table 3. Fungi associated with surface and gut of mites

Group 1 - mite surface-associated						Group 2 - mite surface- and gut-associated						Group 3 - Mite gut-associated					
Fungal species	ax1	w	MD	MDrank	MSrank	Fungal species	ax1	w	MD	MDrank	MSrank	Fungal species	ax1	w	MD	MDrank	MSrank
<i>Alternaria alternata</i> (Fr.) Keissl.	-0.81	0.92		8	13-14	<i>Eurotium anstelodami</i> L. Mangin	-0.16	0.49	1	25-29	3	20-25	<i>Cladosporium herbarum</i> (Pers.) Fr.	0.28	2.32	10	6
<i>Penicillium</i> sp. 1	-0.81	0.62		6	16	<i>Eurotium herbariorum</i> Link	-0.16	0.24	1	30-49	1	32-37	<i>Penicillium verrucosum</i> Dierckx	0.30	1.28	6	10-12
<i>Penicillium</i> sp. 1	-0.81	0.40		3	19	<i>Mycocladus corimbifer</i> (Cohn) Vánová	-0.16	0.24	1	30-49	1	32-37	<i>Aspergillus flavus</i> Link; Fr.	0.32	2.22	10	7
<i>Alternaria</i> sp.	-0.81	0.33		3	20-25	<i>Aspergillus candidus</i> Link; Fr.	-0.14	1.85	5	13	10	9	<i>Cladosporium cladosporioides</i> (Fresen.) G. A. de Vries	0.37	1.97	9	8
<i>Penicillium citrinum</i> Thom	-0.81	0.26		3	20-25	<i>Aspergillus versicolor</i> (Vuill.) Trab.	-0.14	4.22	12	5	24	2	<i>Blizopus stolonifer</i> (Ehrenb.) Fr.	0.43	0.63	3	15-16
<i>Scopulariopsis brumptii</i> Salvaet-Duval	-0.81	0.26		3	20-25	<i>Aspergillus sydowii</i> Thom et Church	-0.02	0.54	2	20-24	3	20-25	<i>Geomyces panorum</i> (Link) Sigler et J.W. Carmich.	0.52	0.22	1	25-29
<i>Penicillium polonicum</i> K. M. Zalesky	-0.81	0.22		2	26-31	<i>Penicillium crustosum</i> Thom	0.03	4.68	16	3	23	3	<i>Aspergillus fumigatus</i> Fresen.	0.55	0.25	1	25-29
<i>Ulocladium chartarum</i> (Preuss) Simmons	-0.81	0.22		2	26-31	<i>Penicillium brevicompactum</i> Dierckx	0.06	3.78	13	4	18	6	<i>Botrytis cinerea</i> Pers.; Fr.	0.86	0.9	6	10-12
<i>Mucor plumbeus</i> Bonord.	-0.81	0.16		1	32-37	<i>Penicillium aurantiogriseum</i> Dierckx	0.12	5.65	21	1	26	1	<i>Penicillium</i> sp. 8	1.24	0.68	6	10-12
<i>Mycocladus ramosus</i> (Zopf) Vánová	-0.81	0.08		1	32-37	<i>Penicillium variable</i> Sopp	0.16	0.63	3	17-19	3	20-25	<i>Penicillium olsonii</i> Bainier et Sartory	1.24	0.38	3	15-16
<i>Circinella muscae</i> (Sorokin) Berl. et de Toni	-0.81	0.04		1	38-55	<i>Penicillium viridicatum</i> Westling	0.17	0.48	2	20-24	2	26-31	<i>Acrodonium salmonum</i> de Hoog	1.24	0.22	2	20-24
<i>Emertella nidulans</i> (Eidam) Vuill.	-0.81	0.04		1	38-55	<i>Penicillium expansum</i> Link; Fr.	0.17	0.32	1	25-29	1	32-37	<i>Eurotium rubrum</i> Jos. Koenig Spickermann et Brenner	1.24	0.11	2	20-24
<i>Eurotium</i> spp.	-0.81	0.04		1	38-55	<i>Cladosporium sphaerospermum</i> Penz.	0.17	0.16	1	30-49	1	38-55	<i>Aspergillus ustus</i> (Bainier) Thom et Church	1.24	0.03	1	30-49
<i>Penicillium rugulosum</i> Thom	-0.81	0.04		1	38-55	<i>Penicillium</i> sp. 6	0.17	0.16	1	30-49	1	38-55	<i>Mortierella</i> sp.	1.24	0.04	1	30-49
<i>Penicillium</i> sp. 2	-0.81	0.04		1	38-55	<i>Penicillium</i> sp. 7	0.17	0.16	1	30-49	1	38-55	<i>Mucor circinelloides</i> Tiegh.	1.24	0.04	1	30-49
<i>Penicillium</i> sp. 3	-0.81	0.04		1	38-55	<i>Rhizopus microsporus</i> Tiegh. var. <i>rhizopodiformis</i> Schipper et Stalpers	0.17	0.16	1	30-49	1	38-55	<i>Oldendendron cerealis</i> (Thüm.) Barron	1.24	0.04	1	30-49
<i>Penicillium</i> sp. 4	-0.81	0.04		1	38-55	<i>Doratomyces putredinis</i> (Corda) F. J. Morton et G. Sm.	0.18	0.48	2	20-24	2	26-31	<i>Paeclomyces farinosus</i> (Holmsk.) A. H. S. Br. et G. Sm.	1.24	0.04	1	30-49
<i>Penicillium</i> sp. 5	-0.81	0.04		1	38-55	<i>Aspergillus niger</i> Tiegh.	0.19	4.72	19	2	20	4	<i>Penicillium roqueforti</i> Thom	1.24	0.04	1	30-49
<i>Rhizopus</i> sp.	-0.81	0.04		1	38-55								<i>Penicillium roseopurpureum</i> Dierckx	1.24	0.04	1	30-49
<i>Stemphylium herbarum</i> E. G. Simmons	-0.81	0.04		1	38-55								<i>Penicillium simplicissimum</i> (Oudem.) Thom	1.24	0.04	1	30-49
<i>Trichothecium roseum</i> (Pers.; Fr.) Link	-0.81	0.04		1	38-55								<i>Penicillium spinulosum</i> Thom	1.24	0.04	1	30-49
gray sterile mycelium	-0.81	0.04		1	38-55								<i>Penicillium thomii</i> Maire	1.24	0.04	1	30-49
<i>Penicillium hordei</i> Stolk	-0.54	0.58	1	30-49	4								<i>Verticillium lecanii</i> (Zimm.) Viégas	1.24	0.04	1	30-49
<i>Penicillium griseofitum</i> Dierckx	-0.52	1.07	1	25-29	8								arthrosporic white fungus	1.24	0.04	1	30-49
<i>Scopulariopsis brevicaulis</i> (Sacc.) Bainier	-0.51	2.05	3	17-19	15	7											
<i>Eurotium repens</i> de Bary	-0.38	2.11	4	14	14	8											
<i>Penicillium aurantiogriseum</i> group	-0.36	1.39	3	17-19	9	10-11											
<i>Dendryphon penicillatum</i> (Corda) Fr.	-0.33	0.33	1	30-49	2	26-31											
<i>Penicillium chrysogenum</i> Thom	-0.27	3.28	7	9	20	4											

Scores of single fungal species (ax1) along 1st ordination axis from the CCA analysis of the effect of mite gut versus mite surface. Second column gives weights (w) of the species in the analysis (the higher the weight, the higher contribution of the species to the gut versus surface division of the fungal community). Negative scores correspond to surface associated fungi, positive to gut associated fungi. The first canonical axis explained 4.9% of the total variation in the dataset. ax1, species score; w, weight of species in analysis; MD, frequency (%) of occurrence of fungus in gut isolates; MS, frequency (%) of occurrence of fungus in surface isolates.

Table 4. Fungi associated with surface of mites and the seeds

Group 1 - seed-associated							Group 2 - mite surface and seed-associated							Group 3 - mite surface-associated						
Fungal species	axl	w	MS	MSrank	SS	SSrank	Fungal species	axl	w	MS	MSrank	SS	SSrank	Fungal species	axl	w	MS	MSrank	SS	SSrank
<i>Arctoditium salmoneum</i> de Hoog	-1.15	0.04			2	36-60	<i>Aspergillus flavus</i> Link:Fr.	-0.20	3.17	8	12	24	5-7	<i>Alternaria</i> sp.	0.27	0.50	3	20-25	2	36-60
<i>Actinomyces elegans</i> (Edlm.) C.R. Benj. et Hesselt	-1.15	0.09			2	36-60	<i>Cladosporium cladosporioides</i> (Fresen.) G. A. de Vries	-0.19	2.17	7	15	19	10-11	<i>Doratomyces putredinis</i> (Corda) F. J. Morton et G. Sm.	0.33	0.41	2	26-31	2	36-60
<i>Aureobasidium pullulans</i> (de Bary) Arnaud	-1.15	0.09			2	36-60	<i>Rhizopus stolonifer</i> (Ehrenb.: Fr.) Vuil.	-0.19	0.75	2	26-31	6	23-29	<i>Aspergillus candidus</i> Link: Fr.	0.34	2.08	10	9	9	20-21
<i>Fusarium</i> sp. 1	-1.15	0.09			2	36-60	<i>Mucor plumbeus</i> Bonord.	-0.17	0.50	1	32-37	4	30-35	<i>Penicillium hordei</i> Stolk	0.36	0.83	4	18	4	30-35
<i>Fusarium</i> sp. 2	-1.15	0.09			2	36-60	<i>Rhizopus microsporus</i> Tiegh. var. <i>disparidiformis</i>	-0.14	0.23	1	38-55	2	36-60	<i>Scopulariopsis brevicaulis</i> (Sacc.) Buitier	0.37	3.08	15	7	15	13-15
<i>Penicillium minioluteum</i> Dierckx	-1.15	0.04			2	36-60	Schipper et Stolpers	-0.11	0.83	3	20-25	6	23-29	<i>Aspergillus versicolor</i> (Vuill.) Tirab.	0.50	4.33	24	2	17	12
<i>Syncephalastrum racemosum</i> Cohn ex J. Schrot.	-1.15	0.09			2	36-60	<i>Penicillium variable</i> Sopp	-0.11	0.23	1	38-55	2	36-60	<i>Scopulariopsis brumptii</i> Salvanet-Daval	0.57	0.50	3	20-25	2	36-60
<i>Ulocladium</i> sp.	-1.03	0.46			6	23-29	<i>Emmentella nidulans</i> (Edlm.) Vuil.	-0.11	0.23	1	38-55	2	36-60	<i>Alternaria alternata</i> (Fr.: Fr.) Ketsl.	0.57	1.17	8	10-11	4	30-35
<i>Aspergillus ochraceus</i>	-1.00	0.42			6	23-29	<i>Eurotium</i> spp.	0.02	3.75	14	8	24	5-7	<i>Penicillium brevicompactum</i> Dierckx	0.59	3.00	18	6	9	20-21
<i>Mucor dimorphosporus</i> Lendh. f. <i>sphaerosporus</i> (Hägen) Váňová	-0.93	0.67			7	22	<i>Eurotium repens</i> de Bary	0.05	5.33	20	4-5	35	1	<i>Penicillium</i> sp. 1	0.89	0.64	6	16		
<i>Penicillium olsonii</i> Buitier et Surtory	-0.90	0.46			6	23-29	<i>Mucor ramosus</i>	0.05	0.33	1	32-37	2	36-60	<i>Penicillium</i> sp. 5	0.89	0.04	1	38-55		
<i>Penicillium</i> sp. 8	-0.90	0.26			6	23-29	<i>Penicillium chrysogenum</i> Thom	0.06	4.50	20	4-5	24	5-7	<i>Penicillium</i> sp. 6	0.89	0.04	1	38-55		
<i>Pseudomyces variotii</i> Baitier	-0.90	0.17			4	30-35	<i>Penicillium crustosum</i> Thom	0.12	5.42	23	3	31	2	<i>Ulocladium chartarum</i> (Preuss) Simmons	1.01	0.23	2	26-31		
<i>Clonostachys rosea</i> (Link: Fr.) Schroers, Samuels, Seifert et W. Gams	-0.90	0.09			2	36-60	<i>Penicillium aurantogriseum</i> Dierckx	0.24	5.75	26	1	30	3-4	<i>Penicillium citrinum</i> Thom	1.08	0.27	3	20-25		
<i>Fusarium</i> sp. 3	-0.90	0.09			2	36-60								<i>Penicillium viridicatum</i> Westling	1.14	0.13	2	26-31		
<i>Penicillium solitum</i> Westling	-0.90	0.09			2	36-60								<i>Circinella muscae</i> (Sorokin) Bert. et de Toni	1.14	0.04	1	38-55		
<i>Trichoderma</i> sp. 1	-0.90	0.09			2	36-60								<i>Geomyces panuorum</i> (Link) Sigler et J. W. Carmich.	1.14	0.04	1	38-55		
<i>Verticillium lecanii</i> (Zimm.) Viegas	-0.90	0.09			2	36-60								<i>Penicillium</i> sp. 3	1.14	0.04	1	38-55		
<i>Epicoecium nigrum</i> Link	-0.84	0.33			4	30-35								<i>Penicillium</i> sp. 7	1.14	0.04	1	38-55		
<i>Mucor circinelloides</i> Tiegh.	-0.84	1.33			15	13-15								<i>Aspergillus sydowii</i> Thom et Church	1.16	0.33	3	20-25		
<i>Mucor dimorphosporus</i> Lendh. f. <i>dimorphosporus</i> (Hägen) Váňová	-0.83	2.00			22	8								<i>Penicillium aurantogriseum</i> group	1.19	1.08	9	10-11		
<i>Rhizopus arrhizus</i> A. Fisch.	-0.80	1.33			15	13-15								<i>Europenicillium</i> sp. 1	1.25	0.11	3	19		
<i>Penicillium</i> sp. 9	-0.79	0.09			2	36-60								<i>Dendrothrips penicillatum</i> (Corda) Fr.	1.25	0.25	2	26-31		
<i>Penicillium</i> sp. 10	-0.79	0.09			2	36-60								<i>Eurotium herbariarum</i> Link	1.25	0.17	1	32-37		
<i>Trichoderma harzianum</i> Rifai	-0.79	0.09			2	36-60								<i>Penicillium rugulosum</i> Thom	1.25	0.04	1	38-55		
<i>Botrytis cinerea</i> Pers.: Fr.	-0.77	1.75	1	32-37	20	9								<i>Penicillium</i> sp.	1.25	0.04	1	38-55		
<i>Mucor hiemalis</i> Wehner f. <i>hiemalis</i>	-0.68	0.46			6	23-29								<i>Rhizopus</i> sp.	1.25	0.04	1	38-55		
<i>Arctoditium crateriforme</i> (F. H. Beyma) de Hoog	-0.62	0.09			2	36-60								<i>Strophidium herbarum</i> E. G. Simons	1.25	0.04	1	38-55		
<i>Penicillium</i> sp. 11	-0.62	0.09			2	36-60								<i>Tricholothium roseum</i> (Pers.: Fr.) Link	1.25	0.04	1	38-55		
<i>Mycobolus corymbifer</i> (Cohn) Váňová	-0.50	1.17	1	32-37	11	18-19								<i>Penicillium expansum</i> Link Fr.	1.42	0.09	1	32-37		
<i>Cladosporium sphaerospermum</i> Penz.	-0.49	0.31	1	38-55	4	30-35								<i>Penicillium</i> sp. 2	1.42	0.04	1	38-55		
<i>Penicillium polonicum</i> K. M. Zalesky	-0.48	1.42	2	26-31	13	16-17								gray sterile mycelium	1.42	0.04	1	38-55		
<i>Cladosporium herbarum</i> (Pers.: Fr.) Link	-0.28	3.50	9	10-11	30	3-4														
<i>Eurotium ansteladamii</i> L. Mangin	-0.26	1.33	3	20-25	11	18-19														
<i>Penicillium griseofulvum</i> Dierckx	-0.24	2.58	8	13	19	10-11														
<i>Penicillium verrucosum</i> Dierckx	-0.24	1.75	5	17	13	16-17														
<i>Aspergillus fumigatus</i> Fresen.	-0.23	0.23	1	38-55	2	36-60														

Scores of single fungal species (axl) along 1st ordination axis from the CCA analysis of the effect of seeds versus mite surface. Second column gives weights (w) of the species in the analysis (the higher the weight, the higher contribution of the species to the seeds versus mite-surface division of the fungal community). Negative scores correspond to seed-associated fungi, positive to mite surface-associated fungi. The first canonical axis explained 7.9% of the total variation in the dataset. axl, species score; w, weight of species in analysis; SS, frequency (%) of occurrence of fungus in the seeds isolates; MS, frequency (%) of occurrence of fungus in surface of mite isolates.

Table 5. Fourteen fungal species of medical importance (after Frisvad and Samson 1991; Samson et al. 1996; www.allergen.org)

Species	Allergen-hazards	Mycotoxin-hazards
<i>Alternaria alternata</i> (Fr.: Fr.) Keissl.	(9) Alt a 1-4, Alt a 6-7, Alt a 10-12	Tenuazonic acid, alternariol, altertoxins
<i>Aspergillus flavus</i> Link: Fr.	(1) Asp fl 13	Aflatoxin B ₁ , cyclopiazonic acid, kojic acid, 3-nitropropionic acid
<i>Aspergillus fumigatus</i> Fresen.	(19) Asp f 1-18, Asp f 22w	
<i>Aspergillus niger</i> Tiegh.	(3) Asp n 14, Asp n 18, Asp n?	Malformins, naphthopyrones
<i>Aspergillus versicolor</i> (Vuill.) Tirab.		Sterigmatocystin, nidulotoxin
<i>Cladosporium herbarum</i> (Pers.: Fr.) Link	(7) Cla h1 -6, Cla h12	
<i>Epicoccum nigrum</i> Link	(1) Epi p 1	
<i>Eurotium</i> spp.		Phycion, echinulin
<i>Penicillium aurantiogriseum</i> Dierckx		Penicillic acid, verrucosidin, viomellein, nephrotoxic glycopeptides
<i>Penicillium brevicompactum</i> Dierckx	(1) Pen b 13	Botryodiploidin, mycopenolic acid
<i>Penicillium chrysogenum</i> Thom	(3) Pen ch 13, Pen ch 18, Pen ch20	Roquefortine C, meleagrin, penicillin
<i>Penicillium crustosum</i> Thom		Penitrem A, roquefortine C, terrestrial acid
<i>Penicillium griseofulvum</i> Dierckx		Cyclopiazonic acid, patulin, roquefortine C, griseofulvin
<i>Penicillium verrucosum</i> Dierckx		Ochratoxin A, citrinin

Numbers in parentheses indicate the kinds of allergens.

including those associated with mites, belong to important producers of mycotoxins. Although some of isolated species of fungi, such as *C. herbarum*, *M. dimorphosporus* f. *dimorphosporus*, and *B. cinerea*, are presently not known to produce mycotoxins, they are known to produce allergens (Pitt and Hocking 1997). Currently, the most important producers of allergens are species *A. fumigatus*, *A. alternata*, and *Cladosporium herbarum*. This documentation indicates that association of mites and fungi may lead to mixed contamination of stored products not only by mycotoxins but also mite (Arlian 2002) and fungal allergens with serious health implications.

The fungi associated with both the mite surface and seeds include the common species of fungi such as *A. flavus*, *A. niger*, *P. crustosum*, *P. aurantiogriseum*, and *P. chrysogenum*. These species of fungi are known to produce mycotoxins such as aflatoxin B₁, cyclopiazonic acid, roquefortine C, malformins, penitrem A, verrucosidin, and viomellein (Table 5, after Frisvad and Samson 1991, Samson et al. 1996). *P. chrysogenum* and *A. niger* are allergen producers. The fungi associated solely with the mite surface include fewer fungal species than the previous ecological group (i.e., *P. brevicompactum* and *A. versicolor*). However, even this small group of fungi also produces hazardous mycotoxins such as botryodiploidin, mycopenolic acid, and sterigmatocystin. *A. alternata* and *P. brevicompactum* are allergen producers (Table 5; www.allergen.org).

In summary, we found that many species of medically important fungi may be associated with storage mites in the Czech Republic. This association may play an important role in (1) mixed contamination of infested stored commodity with fungi and mite allergens, and (2) the facilitation of spatial spread of some toxicologically important species of fungi via mites. However, the exact estimation of the mites' role in field studies, and especially manipulative laboratory experiments that describe mites as spore carriers, are needed.

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