

Pest Control Strategies and Damage Potential of Seed-Infesting Pests in the Czech Stores – a Review

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Abstract

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This work reviews the historical and current pest risks and research concerning seed storage in the Czech Republic (CR). Stored seed pests (i.e. animals causing injuries to the germ and endosperm) represent a high risk of economic damage due to the high value of seeds coupled with long-term seed storage in small storage units (e.g., boxes, satchels). Rodents represent a significant risk to all types of seeds, especially seeds stored in piles or bags. Mites, psocids, and moths are the main pests of stored grass and vegetable seeds: mites can decrease seed germinability by 52% and psocids caused 9.7% seed weight loss in broken wheat kernels after 3 months of infestation under laboratory conditions. Although beetles (*Sitophilus* sp., *Tribolium* sp., *Oryzaephilus* sp.) and moths (*Plodia* sp.) are common pests of grain seeds (e.g., wheat, barley, maize), two serious seed pests, *Sitotroga cerealella* and *S. zeamays*, are rare in the CR. *Bruchus pisorum* is a common pest of pea seeds, while other Bruchids are rare in the Czech legume seed stores. Currently, the control of seed pests is becoming difficult because the efficient pesticides (e.g., methylbromide, dichlorvos, drinking anticoagulant rodent baits) for seed protection have been lost without the development of adequate substitutes. New research on seed protection in the CR using biological control (mite predators *Cheyletus* sp.), low pressure, modified atmospheres, and hydrogen cyanide is overviewed.

Keywords: seed; arthropods; seed stores; pest risk

HOWE (1973) was the first to systematically review the negative impacts of various insect and mite pests on stored seeds. The recent book by HAGSTRUM and SUBRAMANYAM (2009) provided an excellent and comprehensive review of the association of storage pests with host seeds on the worldwide scale. The worst damages to stored commodities and seeds occur in the tropical countries (e.g. MCFARLANE 1982; STEJSKAL *et al.* 2006, 2007). However, serious economic damages resulting from infestation may occur in developed countries due to the high added value of seeds and low economic injury level (STEJSKAL 2003). Stored seeds are vulnerable to pest attack because of their prolonged period of storage (often more than one year) and because seed varieties are stored in relatively small quantities in separate packages that usually exclude the possibility of active

ventilation and of regular inspection and monitoring. Seeds are marketed and transported over long geographical distances (e.g. almost 100% of maize seeds in the Czech Republic are imported), which promotes repeated introductions of invasive stored seed pests and their global spread.

Stored-grain infesting pests are classified into two groups according to the grain-injury pattern: secondary pests and primary pests. “Secondary pests” are externally feeding groups of stored-product arthropod (mites and insects) pests that cause injury mainly to the seed germ. There are two patterns of seed injury executed by secondary pests: (i) large species (e.g. *Plodia* sp., *Ephestia* spp., *Tribolium* sp.) completely remove the germ from the outside (Figure 1f), while (ii) small or microscopic arthropod species (e.g. *Cryptolestes* spp., mites) make cavities

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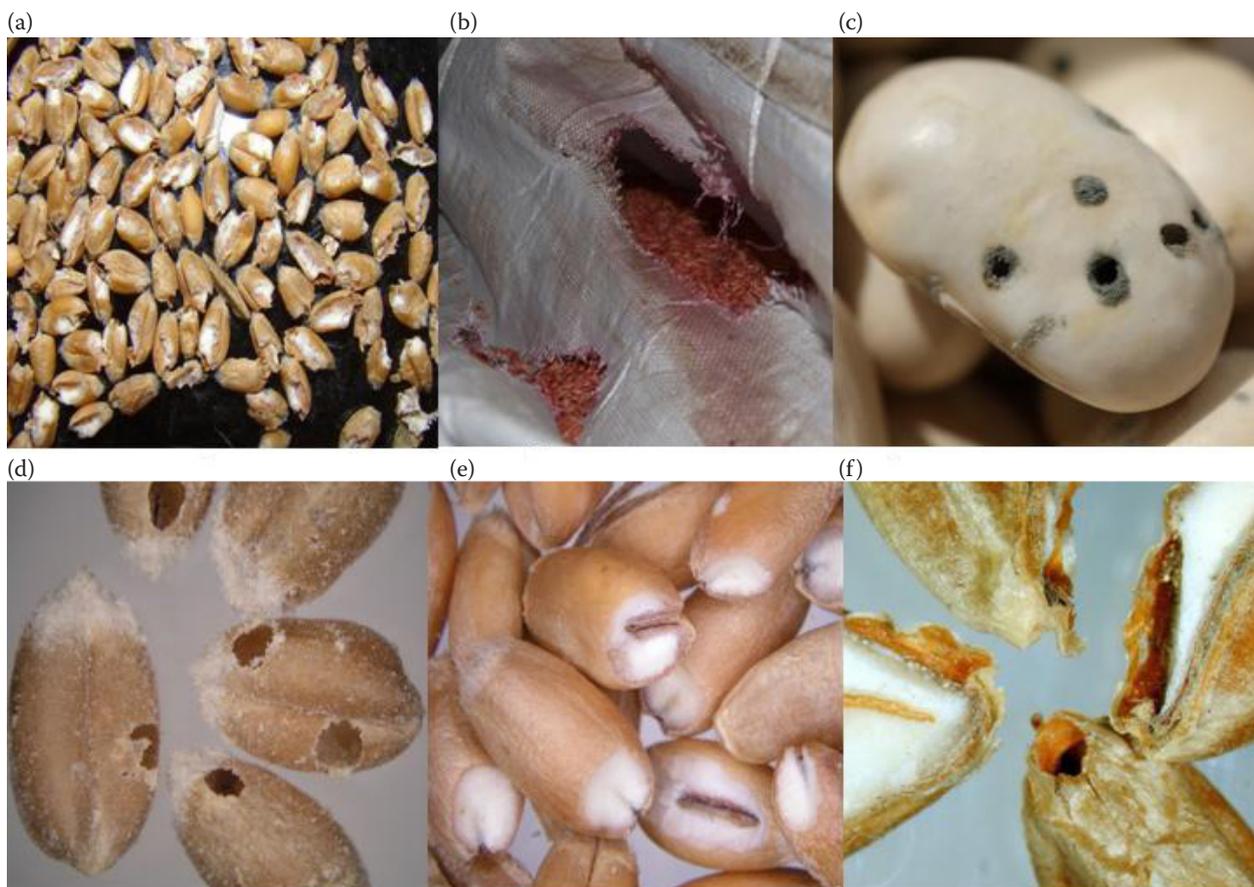


Figure 1. Types of injury to seeds caused by stored seed pests: (a), (b) – rodents cause multiple cracks, crevices and injuries to seeds (b – demonstrates that chemical seed pickling does not deter rodents from feeding); (c) – primary legume pests make cavities in the seeds (e.g. Bruchids); (d) – primary cereal pests make cavities in the endosperm (e.g. *Sitophilus* sp., *Rhyzopertha* sp., *Sitotroga* sp.); (e) – “macroscopic secondary pests” (e.g. large arthropods such as *Plodia* sp., *Tribolium* sp.) completely remove the germ from the outside; (f) – “microscopic secondary pests” (e.g. small arthropods such as *Cryptolestes* spp., psocids, and mites) remove the germ through the small hole on germ cover

in the germ after penetrating into the grain via the germ area. Psocids make characteristic small holes (KUCEROVA 1999; GAUTAM *et al.* 2013) in the seed coat to gain access to the germ (Figure 1e), which they can then completely consume. They can also continue to damage the endosperm depending on the duration and the degree of infestation. Nevertheless, many secondary pests, including beetles, mites, and psocids (KUCEROVA 2002b; ATHANASSIOU *et al.* 2010; GAUTAM *et al.* 2013), prefer to feed on mechanically cracked seeds. Primary pests are internally feeding beetles (e.g. *Sitophilus* sp., Figure 1d; *Rhyzopertha* sp.; *Acanthoscelides* sp., Figure 1c) and moths (*Sitotroga* sp.) causing hidden infestation inside seed kernels. In many cases, they remove the endosperm while leaving the germ uninjured (Figure 1d). Due to their internal development in seeds, primary pests are mainly responsible for filth-contamination of finished

cereal products (TREMATERRA *et al.* 2011a). A specific pattern of seed injury is produced by rodent gnawing (Figures 1a,b). In grain and pulses, stored for feeding and fodder purposes, secondary pests are considered less economically harmful than primary pests. Therefore, STEJSKAL and HUBERT (2008) found that in the Czech Republic, most chemical treatments of mercantile stored-food grain are targeted at primary pests and not at secondary pests. In contrast, different risk perceptions regarding primary and secondary pests can be found in seed producing companies. The two groups of pests are equally economically important for these companies because secondary pests may cause a serious drop in stored seed germination.

Recently, protecting seeds against pests has become more difficult due to the elevated worldwide incidence of secondary pests (e.g., ARTHUR 2012), increasing pesticide resistance, and decreasing pesticide active

ingredients. Mostly keenly missed in seed protection against pests are fumigant methyl-bromide, aerosols and sprays based on organophosphates, and dichlorvos evaporating strips recently banned in the European Union.

Due to their high economic importance, this article reviews the current risks caused by seed pests in the Czech seed stores and the seed-pest related research activities performed in the Czech Republic.

Seed storage systems and pest risk in the Czech Republic

Seeds are stored either in silos or in flat stores in the Czech Republic (CR). Silos are mainly used for short-term storage after the harvest and before the seeds proceed to drying, cleaning, pickling, packaging, and storage in flat stores. Silos are not commonly used for long-term seed storage, with some exceptions for high-volume commodities such as wheat, barley, and rape seeds (Figure 2a). Most commonly, seeds are stored in flat stores (Figure 2b); varieties and cultivars are separated in small bulk containers or enclosed in various packages (boxes, bags, and large or small bags) (Figures 2c–f). This type of storage

inherently includes a high risk of pest infestation because flat stores and hangars are less protected against attack, and cooling is more difficult because most flat stores are not insulated. Packaging materials may attract many pests as pupation sites. Seed packages (paper boxes and bags) can be covered by the silken filaments and pupal cocoons spun by the larvae of Pyralid moths and Ptinid beetles. Silky pest-cocoons (usually located on the outside surface and folds of the infested bags) are easily observed by a potential customer, and the seed products are then penalized or returned even without infestation of the seeds inside the bags.

We documented (STEJSKAL *et al.* 2003) a significantly higher infestation of stored wheat and barley by arthropod pests in flat stores than in silos in the CR. In the flat stores, the main risks for infestation are spillage and seed residues inside or in the vicinity of the storage buildings (KUCEROVA *et al.* 2003, 2005). According to personal communication from several anonymous Czech storekeepers, seeds stored in bags, large bags, bulk containers, and boxes are also sensitive to rodent (mice, rat) (Figures 1a,b) and bird (pigeons, sparrows) attacks. However, even grain stored in silos can be infested by roof rats (*Rattus*



Figure 2. Most of the commonly used seed stores and packages are sensitive to attack of stored pest arthropods and rodents: (a) seeds in silos, (b) seed heaps in flat stores; (c) seeds in wooden boxes; (d) seeds in big bags (1000 kg); (e) seeds in medium bags (30–50 kg); (f) seeds in small bags (100 g to 5 kg) protected in paper boxes

rattus) (STEJSKAL & AULICKY 2014) due to their enormous climbing ability.

Pests of seeds in the Czech Republic

Pests of cereal seeds. Several studies have been published documenting the pest species composition infesting stored cereals in the CR. Approximately 100 species of stored-seed infesting arthropods have been found, including Coleoptera, Psocoptera, Lepidoptera, and Acari. The most important pests of stored cereal seeds (wheat, barley, oats, and maize) are beetles (*Sitophilus granarius*, *S. oryzae*, *Rhyzopertha dominica*, *Tribolium* sp., *Oryzaephilus surinamensis*), psocids and mites (STEJSKAL *et al.* 2003). Moths (*Ephestia elutella* and *Plodia interpunctella*) occur less frequently and less abundantly than beetles. Two serious seed-infesting pests, *Sitophilus zeamays* and *Sitotroga cerealella*, are almost missing in seed and commercial grain stores. However, no one has performed a survey of pests imported to the CR with maize seeds from warm and dry areas where *S. zeamays* and *S. cerealella* are prominent. Seed-infesting Gelechid moth *S. cerealella* and Dermestid beetle *Reesa vespulae* were recorded in high numbers from a stored seed gene resources collection in Prague (Ruzyně) (STEJSKAL & KUCEROVA 1996a). Adults of *R. vespulae* were found in light-traps near the breeding site, showing flight capability in Czech conditions (NOVAK & VERNER 1990). Interestingly, it was found that the Czech population of webbing clothes moth (*Tineolla bisselliella*), originally considered textile pests, can also injure sound wheat, maize, sunflower, and lettuce seeds and cracked legume seeds (STEJSKAL & HORAK 1999).

Some work has been performed to evaluate the effect of stored psocids, mites, moths, and beetles on germination seed weight losses of stored seed cereals in the CR. KUCEROVA (2002a) correlated the weight of cereal loss with increasing populations of booklice. The average grain weight loss (broken wheat kernels) was 9.7% after 3 months of psocid infestation under laboratory conditions. ZDARKOVA (1996) estimated a rapid decrease in the germination of wheat, maize, and oat seeds after 3 and 6 months of storage due to infestation by 2 mite species (*A. siro*, *T. putrescentiae*).

Rodents and arthropods transfer fungi and bacteria to seed stores that may affect their germination. Mouse populations were sampled in three grain stores in the CR and their faeces subjected to mycological analysis (STEJSKAL *et al.* 2005). The analysis isolated 11 genera and 35 species of fungi. The highest spe-

cies diversity was found in the genera *Penicillium* sp. (13 species), *Aspergillus* sp. (5 species), and *Mucor* sp. (5 species).

Pests of legume (pulse) seeds. Many grani-vorous pest species are unable to develop on legume seeds due to their high content of digestion inhibitors (e.g. HUBERT *et al.* 2007a). Thus, most of the Czech pests infesting stored legumes are from the legume-specialised Coleoptera subfamily Bruchinae (family Chrysomelidae). Although imported legume seeds are occasionally infested by *Acanthoscelides* sp., *Callosobruchus* sp., and *Zabrotes* sp., there is no current documentation that these species are commonly found infesting stores of commercial Czech seed companies. Unlike the neighbouring Germany (SCHOELLER 2013), the main pest of legume seeds is currently not *A. obtectus* but *Bruchus pisorum*, which begins the seed infestation not in stores but in the field. There is historical documentation (JUHA 1926) of high seed infestation (30%) and dramatic losses of germination (ca. 60–90%) on the old Czech pea (*Pisum sativum*) cultivar (i.e. Rychlik, Telefon, and Viktoria). This species still remains pestilential because of uncertainty about its field control, even with the modern generation of insecticides (SEIDENGLANZ *et al.* 2011). Additionally, the fumigation of the Bruchid infested pea seeds by phosphine in stores must be performed with care and under hermetic conditions to ensure full efficacy (WILLIAMS & WHITTLE 1996). GUPTA and KASHYAP (1995) reported malicious effects of phosphine on seed germination and the seedling vigour index with repeated fumigations; at 12% moisture content, the deterioration of seed quality was rapid even when the seed was exposed to the normal recommended (0.250 mg/l) phosphine doses.

Pests of grass, vegetable, herb, and spice seeds. The main pests of small seeds are mites, psocids, moths, and secondary pest beetles, which cause a serious decrease in germination. ZDARKOVA (1996) studied under laboratory conditions (20°C; 75%) how the mites *Acarus siro* and *Tyrophagus putrescentiae* reduce the viability of seed stocks (beet, carrots, kohlrabi, lettuce, radish, and spinach). She observed a rapid decrease in the germination of seeds after 3 and 6 months of storage due to infestation with two mite species (*A. siro*, *T. putrescentiae*). The two species of mites had similar effects on seed germination. The average decrease in grain germination was 21.1% after 3 months and 52.5% after 6 months. The decrease in vegetable seed germination was 11.3% after 3 months and 35.8% after 6 months.

KUCEROVA and HORAK (2004) analysed 201 commercial seed samples containing 21 types of seeds (mainly vegetable and grass seed). The samples were obtained from anonymous, big, Czech seed companies during a 5-year period. The seeds of beet, grass, onion, radish, and lettuce were the most sensitive to infestation. It was reported that, of the 201 samples, 60% were positive for arthropod infestation: Acarina (14 species) and Psocoptera (5 species). They documented that mites were three times more abundant than psocids in vegetable and oil seeds and 1.4 times more abundant in grass seeds. *A. siro* was a dominant mite pest in all aspects (frequency, abundance, and diversity of seed infestation), followed by *T. putrescentiae*, *Tarsonemus granarius*, and *Lepidoglyphus destructor*. *Cheyletus eruditus* was a dominant predatory mite. *Lepinotus patruelis* was the most frequent psocid pest.

Current strategies and methods to control pests of stored seeds in the Czech Republic

There are limited strategies for protecting seeds against pest attack. Major Czech seed-producing companies are well equipped with sieving and aspirating machines that are used to remove dust, impurities, and pests before accepting new seed batches into the facility for further processing (dry or moist sowing seed pickling) and storage. Freezing chambers are available and used for the treatment of infested seeds in companies for ecological production (Probio CZ manager, personal communication). Deep freezing stores and facilities (-22°C) are used only for long-term seed collection storage in agricultural genetic resource banks in the Czech Republic (e.g. the Crop Research Institute in Prague); this type of seed conservation and protection would not be possible without the direct economic support of the Czech Ministry of Agriculture due to the high costs associated with its enormous energy consumption. Cooling options are limited for seed protection and long-term storage in the CR; active ventilation is available only for grain in silos, but not for cooling seeds in bags and boxes located in flat stores. Even in the companies that possess insulated stores, the practical usage of cooling machinery is limited due to operational economic constraints. Chemical residual sprays, aerosols, and smoking apparatus are therefore more or less regularly applied in the flat stores. Currently, several formulations of pyrethrin (pyrethrum), pyrethroids (deltamethrin, cypermethrin), and organophosphates (chlorpyrifos,

pirimiphos-methyl) are available in the CR. However, the ban on DDVP vaporisation strips (dichlorvos, Vapona) in 2013 due to EU legislative restrictions is felt to be a serious problem, as no alternative insecticide formulation that provides a slow release of insecticide to ensure long-term protection against moth infestation is available in the CR or the EU. Although phosphine (PH_3)-based fumigants are the most commonly used procedure to control seed infestation by internally feeding pests, the direct admixture of grain protectant (K-obiol, deltamethrin) is increasing. Empty stores and mills can be fumigated with hydrogen cyanide (HCN), which is the only fumigant produced in the Czech Republic (STEJSKAL *et al.* 2014). Although research activities in this field have only been initiated (AULICKY *et al.* 2012; KUCEROVA *et al.* 2013), very limited storage techniques based on modified atmospheres (e.g. CO_2 , N_2 , O_2 absorbers, vacuum) are currently used by seed companies for seed protection in the CR in practice.

Research on seed pest monitoring and control in the Czech Republic

Research on seed pest monitoring

Detection. The laboratory efficacy of various methods for the detection and removal of seed pests (mites, moths, and beetles) has been evaluated by various Czech authors (e.g. KRIZKOVA-KUDLIKOVA *et al.* 2007; HUBERT *et al.* 2009b). LUKAS *et al.* (2009) compared the precision of mite counts in cereal samples when performed by a human technician or by an automatic optical image analysis in the laboratory. STEJSKAL *et al.* (2008) described how the combination of various detection and extraction techniques can lead to different results in real-world grain storage conditions. Pheromone trap data can also be difficult to interpret, as captures may be affected by the presence of food or shelters (e.g. STEJSKAL 1995).

Identification. Original keys, based on SEM photography for the egg identification of bostrichid, anobiid, silvanid, and laemophloeid beetles (KUCEROVA & STEJSKAL 2002, 2008, 2010), psocids (KUCEROVA 2002b), and mites (KUCEROVA & STEJSKAL 2009) were published. In collaboration with Beijing Agricultural University, Oklahoma State University, and the University of Strossmayer of Osijek, the new molecular methods for identifying various species and geographic strains of psocids have been described (e.g. QIN *et al.* 2008; ARIF *et al.* 2012; YANG *et al.* 2012).

Research on seed pest control

Temperatures for control of seed pests. ASPALY *et al.* (2007) and HUBERT *et al.* (2013) showed that for most of the year, pest mites can be controlled in the Czech stores via low temperatures that are maintained by active ventilation in flat stores and silos.

Plant and seed resistance. Plant resistance belongs among the important preventive methods of stored seed protection (THRONE *et al.* 2000). HUBERT *et al.* (2009a) found that stored barley seed is more seriously infested by mites than wheat in the Czech stores. KUCEROVA and STEJSKAL (1994) compared the varietal resistance of Czech winter wheat seeds against the infestation of stored grain. Of the eleven tested cultivars, no cultivar was completely resistant to the infestation of *S. granarius*, although there were significant differences in pest progeny production among the tested cultivars. According to the value of index of susceptibility (IS), the cultivar S-d-211 was the most resistant (IS = 7.46), followed by cvs Vlada and Hana. The cv. Viginta was the most susceptible (IS = 9.09). The size of *S. granarius* adults was positively correlated with the size of the seeds (STEJSKAL & KUCEROVA 1996b). KUCEROVA (1999) compared the susceptibility of 10 wheat cultivars to infestation with psocids (*L. bostrychophila*). The cv. Linda was the most resistant, and cv. Viginta was the most susceptible. HUBERT *et al.* (2013) compared the sensitivity of 6 barley cultivars – Calgary, Diplom, Jersey, Prestiz, Sebastian, and Tolar to the infestation of 3 storage mites under various humidity and temperature condition. They found that all of the tested factors, including variety, influenced the rate of population increase of the tested mites (*A. siro*, *L. destructor*, and *T. putrescentiae*).

Chemical control. Efficacy of chemical control of *B. pisorum* was assessed under field conditions on the area of Moravia (SEIDENGLANTZ *et al.* 2011). A comparative study on the efficacy of the current residual sprays on stored-product pest mites in the Czech Republic is available (e.g. HUBERT *et al.* 2007b), while no relevant data on stored-product pest beetles and moths have been published. However, there are new data on the efficacy of the protection of pallets and wooden packages using HCN (STEJSKAL *et al.* 2014).

Effects of fumigation on seed germination. HORÁK (1959) found that methylbromide (CH_3Br) and phosphine (PH_3) have no adverse effect on most tested seeds; hydrogen cyanide (HCN) did not decrease or increase germination of the treated seeds, while dichlorethane decreased germination dramatically.

HORÁK and STROSOVÁ (1962a,b) confirmed that the label doses of 3 tested fumigants (PH_3 , CH_3Br , HCN) generally had no adverse effects on dry seed germination, with the exception of seeds containing aromatic compounds, such as caraway, chive, and onion seeds. They also found an unexplained sensitivity to fumigation in various seed cultivars. Therefore, they recommended the preliminary fumigation of seed samples and germination testing prior to the fumigation of the whole seed volume. They warned that a risk of decreased germination may occur if the seeds are wet, if high fumigant doses are used, or if the fumigants are used repeatedly.

Modified atmospheres and pressure. AULICKY *et al.* (2012) tested the efficacy of modified 100% gaseous nitrogen (N_2) atmospheres on brown-legged mite (*Aleuroglyphus ovatus*) adults' survival. They found that 100% nitrogen caused 100% imago mortality after 33 h; $\text{LT}_{50} = 8.88$ h. ZDARKOVA and VORACEK (1993) estimated that mites from the family Acaridae were killed by a low pressure of 95 mm Hg after an exposure of 48 hours. KUCEROVA *et al.* (2013, 2014) used a vacuum packing machine for laboratory testing of the effectiveness of vacuum packaging on the mortality of 2 species of grain infesting beetle-pests (*T. castaneum*, *S. granarius*) at different temperatures and exposure times. There were significant differences between the adult insects of the tested species in their susceptibility to low pressure (vacuum): *T. castaneum* was approximately 10 times more susceptible to low pressure than *S. granarius*. A higher temperature significantly shortens the vacuum exposure time necessary to reach 100% mortality in the tested beetles under a constant low-pressure value (1 kPa). The lethal times (LT_{99}) for adult *T. castaneum* were 15.1 h at 25°C and 30.8 h at 15°C. LT_{99} for adult *S. granarius* were 160.1 h at 25°C and 274 h at 15°C.

Mating disruption. Mating disruption (MD) is a new biorational method for stored product protection. It is based on using high doses of sexual pheromone to confuse males as they try to find and mate with females. MD was concurrently evaluated in the Czech Republic and 2 other countries (TREMATERRA *et al.* 2011b). Although it was found to be a promising method, the MD efficacy showed significant variability among the tested buildings, most likely caused by the specific local conditions.

Biological control. It was documented that four *Cheyletus* species naturally occur in the Czech stores (PULPAN & VERNER 1959; ZDARKOVA 1998). However, it seems that non-manipulated occurrence can rarely provide efficient control (LUKAS *et al.* 2007). There-

fore, an original method for mass production of the predator *Cheyletus* has been developed, along with strategies for the protection of seeds using predatory mites (PULPAN & VERNER 1959; ZDARKOVA & PULPAN 1973). The predators have been mass reared (ZDARKOVA 1986) and sold in paper bags, each containing 2000–3000 live specimens of *C. eruditus*, under the commercial name CHEYLETIN®. Biological control can be used preventively (in empty stores) or repressively on grain or seeds. Biological control has specifics that include the following: (a) it takes time, sometimes several months; (b) the predator can develop at temperatures above 12°C (this lower temperature threshold must be exceeded); (c) the initial infestation by Acaroid mites must not exceed 1000 mites per kg; (d) recommended predator-prey ratios are 1 : 10 or 1 : 100, depending on the moisture content and temperature. When the infestation of Acaroid mites is higher than 1000 specimens per kg of material or when other insect pests are also present, it is necessary to suppress the population of mites (or insects) chemically (ZDARKOVA 1994). Both fumigation and contact insecticides can be used. After the treatment, a new sampling was necessary because the mortality of the pest mites was not necessarily 100%. Predatory mites can be applied as early as one week after the chemical treatment.

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