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## Emerging risk of infestation and contamination of dried fruits by mites in the Czech Republic

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Abstract:	The introduction of live insects into human food has been rare in developed countries. This work documents, for the first time, an emerging risk that exists from dried fruit in Central Europe. Recently, massive and frequent infestation of dried fruit imported from the Mediterranean region by the mite <i>Carpoglyphus lactis</i> L. (Acarina: <i>Carpoglyphidae</i> ) has been found. In 180 analyzed samples taken from supermarkets, 13% were contaminated; the contamination levels ranged from 0 to 660 mites per g of dried fruit. The contamination was found in dried apricots, figs, plums and raisins. To estimate the risks and food preferences of <i>C. lactis</i> , its growth rate was examined under laboratory conditions. Starting with a hypothetical population of 10 mites per g of dried fruit, the risk level of 1,000 mites per g of dried fruit is reached at 42 days for dried figs, 49 days for dried pineapple and 63 days for dried

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	apricots, dates and plums at 25 °C and 85% of relative humidity following exposures of 21 days. The experiment found that mites are able to enter to every dried fruit packing material tested, including polypropylene and aluminum foils. This indicates that mites can disperse from package to package in supermarkets. Mites are known as allergen producers and vectors of mycotoxin-producing fungi. These findings indicate that an increased risk of C. lactis contamination exists in dried fruit.

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**Emerging risk of infestation and contamination of dried fruits  
by mites in the Czech Republic**

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**Abstract**

The introduction of live insects into human food is rare in developed countries. However we report, for the first time, an emerging risk that exists from dried fruit in Central Europe. Recently, massive and frequent infestation of dried fruit imported from the Mediterranean region by the mite *Carpoglyphus lactis* L. (Acarina: Carpoglyphidae) has been found. In 180 samples taken from supermarkets, 13% were contaminated; the contamination levels ranged from 0 to 660 mites per g of dried fruit. The contamination was found in dried apricots, figs, plums and raisins. To estimate the risks and food preferences of *C. lactis*, its growth rate was examined under laboratory conditions. Starting with a hypothetical population of 10 mites per g of dried fruit, the risk level of 1,000 mites per g of dried fruit is reached at 42 days for dried figs, 49 days for dried pineapple and 63 days for dried apricots, dates and plums at 25 °C and 85% of relative humidity following exposures of 21 days. We found that mites are able to enter every dried fruit packing material tested, including polypropylene and aluminum foils. This indicates that mites can move from package to package in supermarkets. Mites are known as allergen producers and vectors of mycotoxin-producing fungi. These findings indicate that an increased risk of *C. lactis* contamination exists in dried fruit.

**Key words:** *Carpoglyphus lactis*, dried fruits, contamination, allergy

## Introduction

The presence of insects and mite contaminants in food is unacceptable not only for esthetic but also for health reasons. The pre-harvest infestation of fruits in the field has been widely documented. Several arthropods can become serious post-harvest pests in contaminated dried fruit. Among them, the *Plodia interpunctella* (Hubner) moth, *Carpophilus hemipterus* (L.) beetles, *Oryzaephilus surinamensis* (L.) and the dried food mite *Carpoglyphus lactis* L. are concerns in food production (Johnson et al 2000). Dried apricots are especially sensitive to mite contaminations; the highest reported contamination is 15,000 mites per kg (Cobanoglu 2009). However, little data is available on the direct infestation of finished food products in supermarkets. One reason for this is that most food production companies and marketing chains consider data on pest infestation of finished products market-sensitive and therefore strictly confidential. The second reason is that, in the past three decades, the direct introduction of live arthropods in dried fruit marketed for human food has been considered rare due to the fact that most fruits have been preventively treated prior to export using the multispectral fumigant methyl-bromide. However, this fumigant has been banned for environmental reasons by the Montreal protocol treaty with no effective and reliable acting substitute for it (Fields and White 2002). Consequently, this may be the cause of a newly emerging risk from dried fruit in Central Europe. In fact, a high level of contamination of dried fruit has recently been found in products in Czech markets. For example in 2007, nine out of 76 RAFF (the rapid alert system for food and feed) notifications given by the Czech Republic were caused by mites or insect pests in dried fruit (Anonymous 2008).

Saleh et al. (1987) suggested a consequence of arthropod contamination is a decrease in the nutritional values of the fruits. However, recently the risk of the

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3 65 arthropod contamination is due to their health impact, being producers of allergens  
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5 66 and the vectors of microorganisms. Because mites are well-known allergen  
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8 67 producers (Colloff 2009), house dust mites are a primary concern because of their  
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10 68 proximity to humans. However, recently, there has been an increasing number of  
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12 69 reports describing the sensitization of humans not only to *Dermatophagoides* mites  
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15 70 but also to stored-product mites (Fernandez-Caldas 1997). The consumption of pest  
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17 71 mites may cause allergic reaction and also direct infestation in the form of intestinal  
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19 72 acariasis (Li et al. 2003). Allergy risk is heightened by the limited options available for  
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21 73 effective mite chemical control using traditional pesticides (Hubert et al. 2007). The  
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23 74 second risk is associated with the vectoring of mycotoxin-producing fungi. The  
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25 75 association of mites and mycotoxin-producing fungi such as *Aspergillus* spp. and  
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27 76 *Penicillium* spp. has been reported (Aucamp 1969; Franzolin et al. 1999; Hubert et al.  
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29 77 2004). The contamination of dried fruit by aflatoxins and ochratoxin is a common  
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31 78 event (Trucksess and Scott 2008). During their migration, mites can disseminate the  
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33 79 fungal spores. The risks associated with *C. lactis* are possible allergen production  
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35 80 and vectoring of mycotoxin-producing fungi. Although there is no evidence of this for  
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37 81 *C. lactis*, evidence does exist for related species of mites. The contamination of dried  
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39 82 fruit is directly correlated with the risk of direct consumption of hazardous mites.  
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46 83 In this study, we analyzed the mite contamination of 180 samples of dried fruit  
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48 84 obtained in Czech markets. To compare the risks of contamination of the fruit, we  
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50 85 analyzed in laboratory the growth rate of the mites on various dried fruit. This  
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52 86 analysis of growth rate simulates the risk of contamination of dried fruit in  
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54 87 supermarkets or in consumers' kitchens. This study is part of a broader pest-risk  
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56 88 study related to arthropod contamination of stored food and commodities in the  
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Central Europe (Stejskal and Hubert, 2008; Hubert et al. 2009; Trematerra et al. 2011).

## Material and Methods

### *Contamination of dried food*

The analyses of 180 samples of dried fruit included apricots, pineapple, plums, figs, raisins and dates. The samples were obtained in supermarkets from January to June 2010; three samples of each type of dried fruit were obtained from each market inspected (if available). Altogether we visited 5 different trade networks including 10 markets. The samples were transferred to the laboratory and analyzed immediately. The dried fruits were weighed, and the mite infestation (all visible developmental stadia) was counted on a STEMI 2000 C dissection microscope (C. Zeiss, Jena, Germany). The mite infestation was recalculated per g of dried fruit. The mites were sorted out, preserved and mounted on microscopic slides for species determination. Mites were preserved in Oudemans solution (70% ethanol (87 ml); acetic acid (8 ml), glycerol (5ml)) and microscopic slides were done for their determination (Žďárková 1967). Zuzana Kucerova (Crop Research Institute, Prague) determined mites. The specimens are stored in the collection of "Small organisms and microorganisms" at Crop Research Institute Prague, Czech R.

### *Risk analyses of mite growth on dried fruit.*

*Carpoglyphus lactis* (Linnaeus 1758) originated from laboratory stock cultures stored in the Crop Research Institute in Prague. The mites were reared at the plastic chambers derived from IWAKI tissue cell cultures with filter cap plugs (P-Lab, Praha, Czech Republic/CZ). The mites were reared from a fish food-derived diet composed



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3 114 of dog food (Ontario-pet, Placek, Podebrady, CZ), wheat germ, Aqua-tropic dried fish  
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5 115 food (LonBio, Aqua Tropic Lonsky, Praha, CZ), Pangamin and gelatin (Serva  
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7 116 Electrophoresis GmbH, Heidelberg, Germany) (10:10:3:2:1 w/w). The diet was  
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10 117 powdered using blender and sieved. The size of opening of the sieves was 0.5 mm.  
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12 118 After sieving the diet was heated to 70 °C for 0.5 h, then powdered and sieved again.  
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15 119 The chambers were placed into Secodar desiccators (P-Lab) at 85% r.h. and kept at  
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17 120 25 °C in darkness.

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20 121 The risk analyses included samples of dried apricots, pineapple, raisins, apples,  
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22 122 figs, and dates. The dried fruit samples were observed and no visible contamination  
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24 123 was found. To confirm that the dried fruit used had no hidden infestation, we  
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26 124 incubated the fruits without mites. In all cases we did not observe any mite infestation  
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28 125 after 21 days. The experiments were carried in IWAKI tissue cell cultures with filter  
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30 126 cap plugs (P-Lab). At the beginning of the experiment, pieces of dried fruit were  
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32 127 placed in the chamber and 10 unsexed mite adults were added. The chambers were  
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34 128 incubated in Secodar desiccators at 85% r. h. and 25 °C, in the dark for 21 days. The  
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36 129 experiment was terminated by the addition of 10 mL of 80% ethanol to the chambers.  
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38 130 Mites were directly counted under a dissection microscope. In the experimental  
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40 131 design, 12 or 24 replicates per type of dried fruit were performed.

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43 132 As only the initial and the final mite population densities were known, we assumed  
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45 133 that the populations grew exponentially (McCallum 2000). The differential density-  
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47 134 independent model ( $N_t = N_0 e^{rt}$ ) was used to estimate growth rate  $r$ . In this equation ,  
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49 135  $N_0$  is the initial density of mites (=10),  $N_t$  is the final mite density, and  $t$  is the duration  
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51 136 of the experiment (21 days). Data showed normal distribution and were analyzed  
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53 137 using analysis of variance (ANOVA) with Tukey's honest significant differences  
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(HSD). Analyses were performed in XLSTAT 2007 (Addinsoft USA, New York, NY, USA).

#### *The penetration of mite into dried fruit packing*

Mite transfer in dried fruits and other commodities in supermarkets has been simulated under laboratory conditions. Therefore, it is important to show that packing materials used are mite proof. To test the various packing materials, we obtained 16 packages of each fruit type, at the same time from a single supermarket. We examined four packages and if there was any infestation, we assumed that the rest of the twelve packages were also without contamination and used them for subsequent experiments. The closed packages (200 - 400 g) of dried apricots were placed in plastic containers. Four different packing types of dried apricots were compared: (1) Plastic bag; (2) Polypropylene bag; (3) Polypropylene bag with aluminum foil and (4) Cellophane sac. One container contained 12 packs of one type of packaging including one contaminated package. The contaminated package contained dried plums and contamination had to be visible without opening the package. The containers were incubated in laboratory conditions ( $25 \pm 10$  °C) for 6 months. After one, three and six months, four packages were randomly selected per container. The mite infestation of dried apricot was analyzed as was described above. The presence of mites on the surface of the bags was also verified. We inspected four packs at the beginning of experiments to confirm that they are free of mites.

## **Results**

### *Levels of contamination of dried fruit in the markets*

The contamination of dried fruits was caused by the presence of *C. lactis* mites in stored products (Figure 1). The level of contamination ranged from 0 to 660 individuals per g of dried fruit (Table 1). The highest frequency of contamination occurrence was found in apricots, followed by a lower infestation in plums, figs and raisins. With the exception of apricots and one plum sample, the maxima were lower than 100 contaminants per g of dried fruit. We did not find any contamination in dates and dried pineapples. The infested fruits were produced and imported from Turkey (apricots, figs), Greece (figs, raisins) and Moldova (plums).

*Growth of mites under laboratory conditions*

All types of dried fruit were suitable for *C. lactis* population growth. The growth of mites was influenced by the type of dried fruit ( $F_{(5,114)} = 63$ ;  $P < 0.0001$ ). Dried figs were the most suitable for mite growth, followed by dried pineapples, dates, plums and apricots; raisins were the least suitable for mite growth (Table 2). For simulation according to the formula ( $N_t = N_0 e^{rt}$ ),  $N_t$  values were calculated from observed growth rate ( $r$ ) (Table 2) and a hypothetical population of 10 mites ( $N_0$ ) in dried fruits for the periods ( $t = 21, 28, 35, 42, 49, 56, 63, 70, 77, 84$  and  $91$  days) (Figure 2). The simulation illustrated that the increase in contamination was influenced by the type of dried fruit. The hypothetical increase is approximately 200,000 contaminants on dried figs; followed by 50,000 contaminants on dried pineapple; 8,000 on dried apricots, plums and dates; and 3,000 on raisins.

*Infestation of dried apricot in bags under laboratory conditions*

Mites were found on the exterior of the packing during inspection, which indicates that they migrated from the inside of the infested packing to the surface. The packing

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3 187 differed in its protection to the mite infestation and decreased from polypropylene  
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5 188 back with aluminum foil, cellophane sac, polypropylene bag to plastic bag. However,  
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8 189 after six months, the mites penetrated into all types of packing. The highest numbers  
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10 190 of mites were observed in plastic bags packing (see Table 3).  
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## 14 15 192 **Discussion**

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17 193 Public health inspectors in the Czech Republic discovered dried fruit mite  
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19 194 infestation and reported the risk to the European food alert system (see reports  
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22 195 RAFS - cf. Anonymous 2008) via Czech food safety focal points and the Scientific  
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24 196 Committee on Phytosanitary and Environment (Stejskal and Tlustoš 2009).  
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27 197 Consequently, we conducted this study with the aim of documenting the extent of  
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29 198 mite population density and contamination of dried fruits for human consumption in  
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32 199 Czech hypermarkets. The data obtained illustrated the alarming high contamination  
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34 200 of imported dried fruits by *C. lactis*. With the exception of apricots and dates, we  
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36 201 found infestation in plums, raisins and figs. Although we found contamination in 16%  
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38 202 of samples (n= 180), it is difficult to estimate the size and frequency of contamination  
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41 203 because the study is not a definite survey and sampling was not random.

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43 204 To date, only a few reports have described the direct mite contamination of food  
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45 205 for human consumption. For instance, Thind and Clarke (2001) reported the  
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47 206 occurrence of mites in several ready-to-use cereal-based foods that included baby  
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50 207 food, biscuits and breakfast cereals, among others. Aygun et al. (2007) reported  
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52 208 *Tyrophagus putrescentiae* contamination in 10% of samples of traditional Turkish  
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54 209 cheese Surk. Quintero and Acevedo (1991) found *C. lactis* contamination in 33 and  
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56 210 12% of traditional fermented liquid samples of pulpe and tepache, respectively. Our  
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59 211 results confirmed previous findings (Thind and Clarke 2001), that there is an  
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3 212 opportunity to ingest mites in food. These findings are considered unsavory and  
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5 213 undesirable and do not conform to the known medical importance of mites. In  
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8 214 medical literature, reports of mite oral anaphylaxes after ingestion of mite-  
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10 215 contaminated food were described (Sanchez-Machin et al. 2010, Iglesias-Souto et al.  
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12 216 2009; Sanchez-Borges et al. 2005). The levels of mites in such contaminated food,  
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15 217 which provokes anaphylaxes, started from 1,000 individuals per g of food (Sanchez-  
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17 218 Machin et al. 2010).

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20 219 Compared to insects, the stored product *C. lactis* mites have a short  
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22 220 developmental time, which is approximately 19 days (Chmielski 1971) under optimal  
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24 221 conditions (relative humidity 85% and 25 °C). Such conditions represent the usual  
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26 222 microclimate for dried fruit storage in hypermarkets or in consumers' kitchens.  
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28 223 Therefore, we suggest that during the storage of these products in supermarkets or  
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30 224 consumers' homes, the mites are able to migrate from one contaminated commodity  
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32 225 into another. If the food that is secondarily infested is suitable for mite population  
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34 226 growth the resulting mite multiplication and food contamination is extremely rapid.  
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36 227 Acaridid mites are known to thrive on a variety of food products present in household  
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38 228 pantries and stores, including dried yeasts, milk products, fruit and honey (Hughes  
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40 229 1975).

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45 230 Our field data from supermarkets showed a contamination density with a  
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47 231 maximum of 650 individuals per g of dried food. The simulation model indicates that  
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49 232 such levels of contamination can be reached quickly during storage of contaminated  
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51 233 dried fruit in supermarkets or consumers' kitchens. From a hypothetical initial mite  
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53 234 population of 10 individuals, the risk level observed in supermarkets (650 individuals)  
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57 235 is reached very quickly: 42 days of population multiplication on dried figs, 49 days on  
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dried pineapple and 63 days on dried apricots, dates and plums (Figure 2) in hypothetical temperature 25 °C.

The labeled time for consumption is half a year or longer; however, mites are not detected by buyers, consumers, or inspectors in this time frame because the mites are not visible to the human eye due to their small size. Similar observations have been made in grain stores, in which farmers seriously underrated the risk of infestation by mites and psocids (Stejskal and Hubert 2008) because mite contamination was not visible due to the microscopic size of the mites.

In the hypermarkets, mites are able to infest other packages of dried fruit as was simulated in our experiment. We found that mites are able to penetrate every packaging material for dried fruit that was tested, including polypropylene and aluminum foils. In addition, they are able to develop in them (see Table 3).

Insect and mite presence is traditionally considered an aesthetic rather than a health problem (Thind and Clarke 2001). Codex alimentarius recommended that “dried fruit be prevent by insect and mite contamination” (CAC/RCP 3-1969). Fumigation is required to be used as a preventive treatment. The mites are sensitive to magnesium phosphide treatment (Meyvaci et al. 2010). However, the limitation of fumigation is due to *C. lactis* forming hyppopus stadia with almost zero physiological and breathing activity, which enables them to survive fumigation. Manipulation of temperature and relative humidity is not practical because hyppopus mite developmental stage can survive unfavorable moisture and temperature conditions (Chmielski 1967; 1973). Among the promising control methods of physiologically active stadia are extreme physical conditions or long-term exposure to modified atmospheres (Emeck et al. 2004). Zdarkova and Voracek (1993) found that mite

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3 260 exposure to extremely low temperature (-15 °C) or low barometric (95mm Hg for 96  
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5 261 hours) pressure completely kills the mites in 80 hours.  
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8 262 A recent study by Thind and Clarke (2001) documented a high risk of mite  
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10 263 contamination of cereal-based foods, including sensitive baby food in Great Britain.  
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12 264 However *C. lactis* is not present in cereal product and has not been reported. In  
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14 265 addition *C. lactis* has never been subjected to the immunological analyze and the  
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16 266 information about production of allergens are missing. *C. lactis* is reported as the pest  
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18 267 on the Swedish farm, when the farmers showed allergy symptoms to the mites (van-  
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20 268 Hage-Hamsten et al. 1991). In the website for the systematic allergen nomenclature  
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22 269 that is approved by the World Health Organization and International Union of  
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24 270 Immunological Societies (WHO/IUIS) Allergen Nomenclature Sub-committee new  
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26 271 description of allergens in stored product mites appears in those, which has been  
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28 272 previously described in house dust mites.  
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34 273 Our study has showed the elevated risk of *C. lactis* to the final consumer of dried  
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36 274 fruits in Central Europe. We have reviewed all storage mite management options and  
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38 275 shown that effective *C. lactis* detection and control tools are missing. An unanswered  
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40 276 question remains what was the reason for the increased infestation and  
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42 277 contamination of imported dried fruits to Czech republic from Turkey, Greece and  
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44 278 Moldova. It should be interesting to tests hypotheses whether this new mite risk  
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46 279 could be some how associated with the worldwide deregistration of the most powerful  
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48 280 quarantine fumigant methyl bromide in 2006. We also recommend that large-scale  
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50 281 surveys for infestation of packaged dried fruit by *C. lactis* should be established and  
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52 282 conducted in other European countries where no data exist in order to evaluate the  
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54 283 overall risk of this mite to potential consumers in the EU market.  
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## Figures

**Figures 1.** Massive infestation of dried apricot by *Carpoglyphus lactis*.

**Figure 2.** Simulation of the contamination/population growth of *Carpoglyphus lactis* on dried fruit from a hypothetical initial number of 10 pest individuals.

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**Table 1.** The only contaminant was *Carpoglyphus lactis* in samples of dried fruit obtained in Czech supermarkets. Legend: nd. – not determined, n –number of samples analyzed.

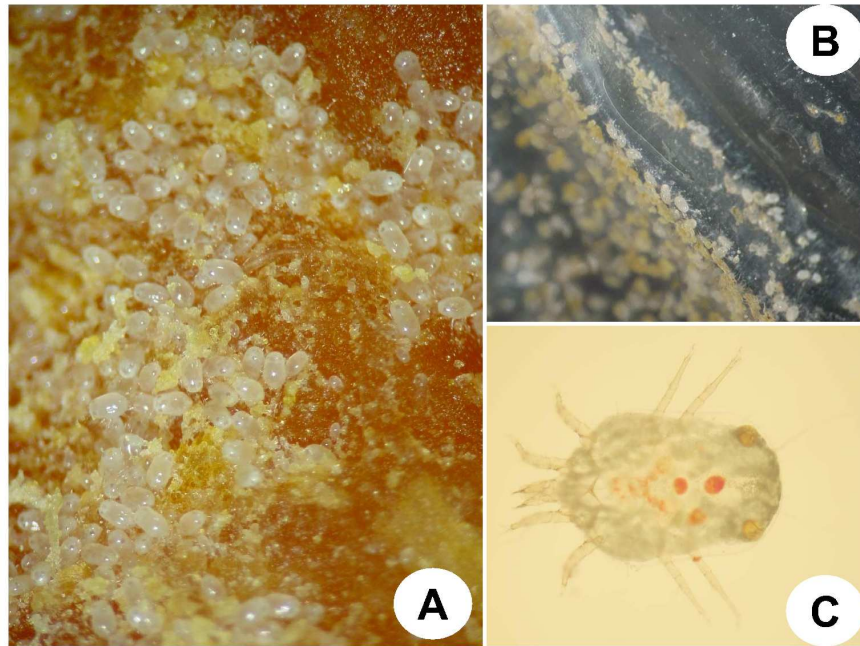
Dried fruit	n	Number of contaminated sample	N total (individuals)	N (individuals.g <sup>-1</sup> )		
				mean±std.dev.	maximum	minimum
Apricot	30	18	93,440	104±176	650	0
Dates	30	0	nd.	nd.	nd.	nd.
Figs	30	6	2,926	28±40	0	89
Plum	30	8	23,163	100±228	663	0
Pine apple	30	1	57	3	nd.	nd.
Raisins	30	3	6	0±0.003	0	0

**Table 2.** Multiplication of *Carpoglyphus lactis* on various dried fruit under laboratory conditions. The starting population was 10 individuals; final numbers of contaminants N (means  $\pm$  standard deviation) and growth rate r are shown, n is the number of replicates; \* - indicates that the rearing diet data was not included in ANOVA test. . The letters indicate differences among the groups based on Tukey's honest significant difference.

Dried fruit	n	r	N
Appricots	24	0.071 $\pm$ 0.014b	46 $\pm$ 13
Dates	24	0.076 $\pm$ 0.006b	50 $\pm$ 6
Figs	12	0.109 $\pm$ 0.004d	100 $\pm$ 8
Pine apples	12	0.093 $\pm$ 0.004c	71 $\pm$ 6
Plums	24	0.072 $\pm$ 0.007b	46 $\pm$ 7
Raisins	24	0.064 $\pm$ 0.007a	39 $\pm$ 6
Rearing diet*	10	0.103 $\pm$ 0.019	93 $\pm$ 32

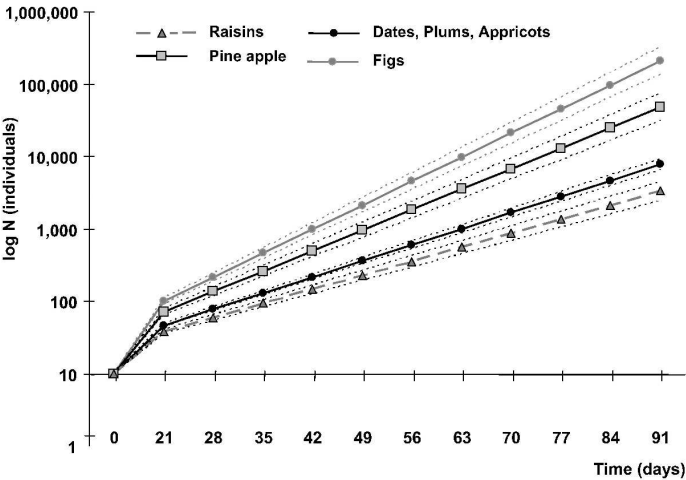
**Table 3.** Effects of type of dried fruit packaging material and exposure period on infestation of packages by *Carpoglyphus lactis* under laboratory experiment. The packages of fruit without mites were stored in containers together with one contaminated package. At different exposure periods, the number of mites in packages was counted. Four packages were used to determine the mean number of mites infesting a particular type of packaging material during each exposure period.

Product name	Time (month)	N (individuals.g <sup>-1</sup> )	Non contaminated sample
Plastic bag	1	0	0
	3	0.04±0.02	4
	6	0.14±0.09	4
Polypropylene bag	1	0.04±0.04	4
	3	1.46±1.41	4
	6	1.68±1.53	4
Cellophane sac	1	0	0
	3	0	0
	6	0.02±0.01	4
Polypropylene bag with aluminum foil	1	0	0
	3	0.01±0.01	2
	6	0.02±0.01	3



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