

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

Jan Hubert, Vaclav Stejskal, Tomas Erban, Marta Nesvorna

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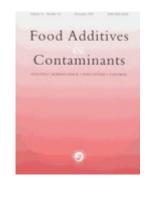
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Food Additives and Contaminants



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 Carpoglpyhus lactis L. (Acarina: Carpoglyphidae) 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 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

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fa a ir n a p	apricots, dates and plums at 25 °C and 85% of relative humidity ollowing exposures of 21 days. The experiment found that mites are able to enter to every dried fruit packing material tested, ncluding polypropylene and aluminum foils. This indicates that nites 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. actis contamination exists in dried fruit.
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20 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 *Carpoglpyhus 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.

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

40 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 interpunctela* (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).

63 Saleh et al. (1987) suggested a consequence of arthropod contamination is a 60 64 decrease in the nutritional values of the fruits. However, recently the risk of the

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arthropod contamination is due to their health impact, being producers of allergens and the vectors of microorganisms. Because mites are well-known allergen producers (Colloff 2009), house dust mites are a primary concern because of their proximity to humans. However, recently, there has been an increasing number of reports describing the sensitization of humans not only to *Dermatophagoides* mites but also to stored-product mites (Fernandez-Caldas 1997). The consumption of pest mites may cause allergic reaction and also direct infestation in the form of intestinal acariasis (Li et al. 2003). Allergy risk is heightened by the limited options available for effective mite chemical control using traditional pesticides (Hubert et al. 2007). The second risk is associated with the vectoring of mycotoxin-producing fungi. The association of mites and mycotoxin-producing fungi such as Aspergillus spp. and Penicillium spp. has been reported (Aucamp 1969; Franzolin et al. 1999; Hubert et al. 2004). The contamination of dried fruit by aflatoxins and ochratoxin is a common event (Trucksess and Scott 2008). During their migration, mites can disseminate the fungal spores. The risks associated with C. lactis are possible allergen production and vectoring of mycotoxin-producing fungi. Although there is no evidence of this for *C. lactis*, evidence does exist for related species of mites. The contamination of dried fruit is directly correlated with the risk of direct consumption of hazardous mites.

In this study, we analyzed the mite contamination of 180 samples of dried fruit obtained in Czech markets. To compare the risks of contamination of the fruit, we analyzed in laboratory the growth rate of the mites on various dried fruit. This analysis of growth rate simulates the risk of contamination of dried fruit in supermarkets or in consumers' kitchens. This study is part of a broader pest-risk study related to arthropod contamination of stored food and commodities in the

Central Europe (Stejskal and Hubert, 2008; Hubert et al. 2009; Trematerra et al.
2011).

92 Material and Methods

93 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.

- 48 108
 - 109 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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of dog food (Ontario-pet, Placek, Podebrady, CZ), wheat germ, Aqua-tropic dried fish
food (LonBio, Aqua Tropic Lonsky, Praha, CZ), Pangamin and gelatin (Serva
Electrophoresis GmbH, Heidelberg, Germany) (10:10:3:2:1 w/w). The diet was
powdered using blender and sieved. The size of opening of the sieves was 0.5 mm.
After sieving the diet was heated to 70 °C for 0.5 h, then powdered and sieved again.
The chambers were placed into Secodar desiccators (P-Lab) at 85% r.h. and kept at
25 °C in darkness.

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

As only the initial and the final mite population densities were known, we assumed that the populations grew exponentially (McCallum 2000). The differential densityindependent model ($N_t = N_0 e^{rt}$) was used to estimate growth rate *r*. In this equation , N_0 is the initial density of mites (=10), N_t is the final mite density, and *t* is the duration of the experiment (21 days). Data showed normal distribution and were analyzed using analysis of variance (ANOVA) with Tukey's honest significant differences using analysis of variance (ANOVA) with Tukey's honest significant differences

(HSD). Analyses were performed in XLSTAT 2007 (Addinsoft USA, New York, NY,USA).

141 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±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.

53 159

 Results

161 Levels of contamination of dried fruit in the markets

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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).

171 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.

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184 Infestation of dried apricot in bags under laboratory conditions

185 Mites were found on the exterior of the packing during inspection, which indicates
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 60 186 that they migrated from the inside of the infested packing to the surface. The packing

differed in its protection to the mite infestation and decreased from polypropylene
back with aluminum foil, cellophane sac, polypropylene bag to plastic bag. However,
after six months, the mites penetrated into all types of packing. The highest numbers
of mites were observed in plastic bags packing (see Table 3).

13 191

192 Discussion

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

To date, only a few reports have described the direct mite contamination of food for human consumption. For instance, Thind and Clarke (2001) reported the occurrence of mites in several ready-to-use cereal-based foods that included baby food, biscuits and breakfast cereals, among others. Aygun et al. (2007) reported Tyrophagus putrescentiae contamination in 10% of samples of traditional Turkish cheese Surk. Quintero and Acevedo (1991) found C. lactis contamination in 33 and 12% of traditional fermented liquid samples of pulpe and tepache, respectively. Our results confirmed previous findings (Thind and Clarke 2001), that there is an

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212 opportunity to ingest mites in food. These findings are considered unsavory and 213 undesirable and do not conform to the known medical importance of mites. In 214 medical literature, reports of mite oral anaphylaxes after ingestion of mite-215 contaminated food were described (Sanchez-Machin et al. 2010, Iglesias-Souto et al. 216 2009; Sanchez-Borges et al. 2005). The levels of mites in such contaminated food, 217 which provokes anaphylaxes, started from 1,000 individuals per g of food (Sanchez-218 Machin et al. 2010).

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

Our field data from supermarkets showed a contamination density with a maximum of 650 individuals per g of dried food. The simulation model indicates that such levels of contamination can be reached quickly during storage of contaminated dried fruit in supermarkets or consumers' kitchens. From a hypothetical initial mite population of 10 individuals, the risk level observed in supermarkets (650 individuals) is reached very quickly: 42 days of population multiplication on dried figs, 49 days on

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 (Emecki et al. 2004). Zdarkova and Voracek (1993) found that mite

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exposure to extremely low temperature (-15 °C) or low barometric (95mm Hg for 96
hours) pressure completely kills the mites in 80 hours.

A recent study by Thind and Clarke (2001) documented a high risk of mite contamination of cereal-based foods, including sensitive baby food in Great Britain. However C. lactis is not present in cereal product and has not been reported. In addition C. lactis has never been subjected to the immunological analyze and the information about production of allergens are missing. C. lactis is reported as the pest on the Swedish farm, when the farmers showed allergy symptoms to the mites (van-Hage-Hamsten et al. 1991). In the website for the systematic allergen nomenclature that is approved by the World Health Organization and International Union of Immunological Societies (WHO/IUIS) Allergen Nomenclature Sub-committee new description of allergens in stored product mites appears in those, which has been previously described in house dust mites.

Our study has showed the elevated risk of *C. lactis* to the final consumer of dried fruits in Central Europe. We have reviewed all storage mite management options and shown that effective *C. lactis* detection and control tools are missing. An unanswered question remains what was the reason for the increased infestation and contamination of imported dried fruits to Czech republic from Turkey, Greece and Moldova. It should be interesting to tests hypotheses whether this new mite risk could be some how associated with the worldwide deregistration of the most powerful guarantine fumigant methyl bromide in 2006. We also recommend that large-scale surveys for infestation of packaged dried fruit by C. lactis should be established and conducted in other European countries where no data exist in order to evaluate the overall risk of this mite to potential consumers in the EU market.

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2 3 4	381	Figures
5 6	382	Figures 1. Massive infestation of dried apricot by Carpoglyphus lactis.
7 8 9	383	
10 11	384	Figure 2. Simulation of the contamination/population growth of Carpoglyphus lactis
12 13 14	385	on dried fruit from a hypothetical initial number of 10 pest individuals.
13 14 15 16 17 18 90 12 23 24 25 27 28 90 12 33 35 36 78 90 41 23 44 56 78 90 12 34 56 78 90 41 23 44 56 78 90 12 34 55 57 55 55 55 55 55 55 55 55 55 55 55	386 387	
56 57 58 59 60		

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 contamined	N total (individuals)	N (in	dividuals.g ⁻¹)
		sample		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

 meantst.

 40
 104±17t.

 104
 104±17t.

 2,926
 28±40.

 23,163
 100±28.

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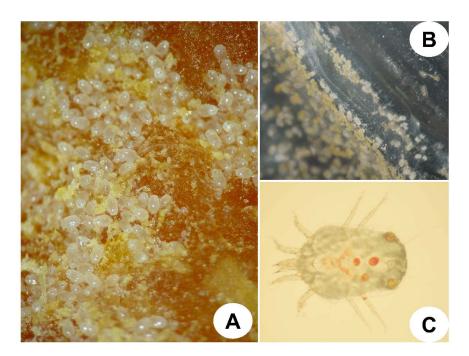
 0±0.003

Table 2. Multiplication of *Carpoglpyhus lactis* on various dried fruit under laboratory conditions. The starting population was 10 individuals; final numbers of contaminants N (means ± 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	ŕ	Ν
Appricots	24	0.071±0.014b	46±13
Dates	24	0.076±0.006b	50±6
Figs	12	0.109 <mark>±0.00</mark> 4d	100±8
Pine apples	12	0.093±0.004c	71±6
Plums	24	0.072±0.007b	46±7
Raisins	24	0.064±0.007a	39±6
Rearing diet*	10	0.103±0.019	93±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	Time	Ν	Non
name	(month)	(individuals.g	contaminated
	ι <i>γ</i>	· 1)	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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