Evaluation of contamination of packages containing cereal-fruit bars by eggs of the pest Indian meal moth (*Plodia interpunctella*, Lepidoptera) due to perforations in their polypropylene foil packaging

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Abstract The quality and type of food packaging affect the level of food protection against pests. This work first evaluated the effect of package perforations on the infestation of cereal-fruit bars by the eggs of the Indian meal moth (*Plodia interpunctella*). We measured the differential oviposition of moths on the unpackaged bars, empty packages, bars in packages and bars in perforated packages in choice and no-choice experiments. Almost 100% of the laid eggs were laid directly on the bars when they were placed in the enclosure without packaging. A low proportion of the eggs (0.4–3%) were laid on either the empty or non-perforated polypropylene foil packages. *Plodia interpunctella* efficiently located and infested the bars with eggs when 5 mm package perforations were present in no-choice test. In choice test *P. interpunctella* preferred to oviposit on open bars than on the packaged or perforated bars. After deposition on the bars the egg hatchability ranged between 40 and 74%. For the food industry, the most significant practical conclusions of this study are that non-perforated packages provide protection against oviposition of *P. interpunctella*, but small perforations enable the moth to infest the resource with eggs.

Keywords Food · Stored products · Risk assessment · Infestation · Moths · Oviposition

Introduction

A recent analysis of food safety by the European RASFF (the Rapid Alert System for Food and Feed) notifications (Djekic et al. 2017) revealed that the top three reported foreign materials/bodies in food were arthropod pests (54.6%), glass fragments (17.4%), and metals (11.5%). Storage- and food-infesting arthropods pose the highest product contamination risk since they can infest a vast array of both unprocessed food commodities (Stejskal et al. 2014) and finished products along the whole food production chain, literally “from-field-to-fork” (Stejskal et al. 2015). Arthropod related food filth contaminants are not restricted to developing countries and may be a frequent problem even in developed countries (e.g., Hubert et al. 2011, 2018; Trematerra et al. 2011). Although food infestations may be caused by many species of arthropods, pyralid storage moths, including the Indian meal moth *Plodia interpunctella*, are among the most important food infesting pests, since they are frequent causes of customer complaints, resulting in multimillion dollar recalls to packaged food at a global scale. The customer complaints are generally based on finding of adults, larvae and/or their products (faeces, exuviae), but the problem often starts already by the micro-contamination of the product by eggs, mainly because the adults, in contrast to larvae, have considerable dispersal capacity. Cereal products, nuts and dried fruits are listed among the most attractive food products for *P. interpunctella* (Mohandass et al. 2007); hence, cereal-fruit bars are at a high risk of infestation. Food production facilities mitigate this risk by integrated pest management (IPM) (Trematerra 2013) and protective packaging (Athanasios et al. 2011; Kavallieratos et al. 2017). However, food packaging materials differ in their level of food protection against pests (Riudavets et al.

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2007). Some packages may be prone to rupturing during manipulation, and some may be intentionally penetrated by producers in order to decrease their storage volume or internal pressure. In addition, it was recently found (Stejskal et al. 2017) that some packages made from multilayer films, which are otherwise extremely resistant to direct penetration by insect mandibles, showed a low resistance to invasion through crevices caused by sealing imperfections. Even tiny microperforations potentially make the packages vulnerable to the invasion of early pest stadia after their emergence from eggs of microscopic size (Kucerova and Stejskal 2002, 2008).

To address the above described pest infestation problems associated with food package perforation, we studied and quantified the risk of how quickly and efficiently *P. interpunctella* females were able to locate packed (polypropylene foil), unpacked or perforated cereal-fruit bars for oviposition to ensure food sources for their offspring, i.e., if the package provides protection against egg infestation and how the perforation influences the protective quality of the package. Based on daily monitoring over an entire moth's life-span, we also studied how the presence of the cereal bar influenced the temporal dynamics and total number of laid eggs (i.e., if the presence of food stimulates higher oviposition in females and how the oviposition changes over time).

Materials and methods

Insects

Larvae were reared on a mixture of wheat flour, oatmeal, dried yeast and glycerol (10:10:2:5) at 26 °C, 60–70% relative humidity (RH), and 16:8 light:dark cycle (L:D). After emergence, 1–2 days old adults were sexed and randomly grouped, and 9 females and 3 males were simultaneously introduced into an experimental arena.

Exp. no. 1: no-choice setup

The experiment was conducted in boxes (50 × 50 × 100 cm; volume 0.25 m³) made from transparent Plexiglas. The top side of the arena was covered by nylon mesh. The arena floor (2500 cm²) was covered with black paper to make the detection of laid eggs easier. In the centre of the arena, there was a 19-cm diameter (298.5 cm²) glass dish with “food” presented in the following four states: (1) packed cereal bar, (2) cereal bar with a perforated package (the perforated hole was circular, 5 mm in diameter made by a single slit and covered by a nylon mesh to prevent physical contact but permit the perception of olfactory cues), (3) cereal bar without a

package (i.e., unpacked bar) as a positive control, and (4) empty package without a bar (the package was made from “clear” material provided by a manufacturer and arranged to form of a “regular” packaged bar) as a negative control. The tested “food” was a 45 g cereal-fruit bar; the ingredients according to manufacturer are: oatmeal, glucose syrup, maize, sweetened cereal blend, rice, fructose, cranberries, almonds, strawberries, soybean, citric acid, and aroma. The package was made from biaxially oriented polypropylene (BOPP) duplex (20 µm printed + 20 µm transparent) film. A total of 5 replicates for each food state were conducted.

The eggs were inspected by a stereomicroscope every 24 h, and the numbers of newly laid eggs were counted. The inspected eggs were removed. After each replicate, the paper covering the arena floor was replaced by a new one. The experiment was terminated after all females died. The boxes were kept at a 2:22 L:D photoperiod at 26 °C and 50% RH.

Exp. no. 2: two-choice setup

The two-choice experiment was conducted in the same experimental arena and under the same external conditions as in the case of the no-choice experiment. The arena floor was covered with black paper. One piece (14 × 20 cm) of paper from the same material was placed on both sides of the arena. In the centre of each piece of paper was placed a cereal bar and the arrangement of the experiment was as follows: (1) perforated and packed cereal bar in the arena, and (2) perforated and unpacked cereal bar in the arena. A total of 10 replicates for each experimental setup were conducted. After 72 h after moths' introduction, each piece of paper with the cereal bar was removed and a number of eggs both on the cereal bar and on the paper were counted.

To get an estimate of *P. interpunctella* eggs' viability after egg deposition on bars we placed 5 unpacked cereal bars (with precisely counted number of eggs) into 500 ml plastic boxes which were kept in a climate chamber at a 30 °C and 50% RH. The boxes were checked every other day and all larvae (in wandering phase) were recorded. The experiment was terminated when no larva emerged within 1 week period.

Statistical analysis

The effect of the “food” state on the spatial distribution of the eggs was tested by two-way ANOVA followed by the Tukey's HSD post hoc test, where the egg location (on the cereal bar or on the floor), “food” state (unpacked bar, packed bar, perforated package or empty package) and their interactions were used as the factors. The effect of the “food” state on the temporal pattern of the oviposition was

tested by repeated measure ANOVA followed by the least significant difference (LSD) post hoc test. Because of violation of the sphericity assumption, we used the Greenhouse-Geisser correction. In the two-choice experiment, the egg numbers on the bar states were compared by the Wilcoxon signed-rank test.

Results

Exp. no. 1: no-choice setup

There were no significant differences ($F_{3,16} = 1.55$, $p = 0.24$) in the total number of eggs laid between the different bar states, although the amount of eggs was slightly higher in the presence of the unpacked bar (2556 eggs) than in the presence of the perforated package (1950 eggs), packed bar (1904 eggs) and empty package (1904 eggs) states.

In contrast, ANOVA revealed that bar state had a significant effect on the spatial distribution of eggs (bar state * eggs location: $F_{3,32} = 51.82$, $p < 0.001$). As predicted, in the presence of the unpacked bar, the vast majority of eggs (> 98%) were allocated directly on the bar. It was considerably higher than the perforated package (32%), packed bar (3%) and empty package (0.4%) percentages (Table 1).

Irrespective of bar state, the egg production changed over time ($F_{6,96} = 6.44$, $p < 0.001$). Overall, the oviposition peak occurred on the third day, and from the fourth day onward, it started to decline. However, we found a significant interaction between the bar state and the time ($F_{18,96} = 2.18$, $p = 0.008$), indicating that oviposition changed differentially over time depending on which type of “food” was present. The LSD post hoc test revealed that in the case of the unpacked bar, the females oviposited significantly more eggs during the first 3 days than in the rest of their lifespan (Fig. 1). In contrast, for all other bar

states, the oviposition rate was more evenly distributed over time (Fig. 1). This conclusion is supported by the fact that in the presence of unpacked bar the females oviposited 73% of their lifespan egg production during the first 3 days, whereas in presence of perforated, packed and empty bar states it was only 50, 51 and 47%, respectively.

Exp. no. 2: two-choice setup

There was significantly larger number of eggs laid on the perforated cereal bar compared with the packed one and, similarly, on the unpacked cereal bar compared with the perforated one (Table 2). Smaller amount of eggs laid during experimental setup 1 (i.e. in the presence of packed and perforated cereal bars) than during experimental setup 2 (i.e. presence of unpacked and perforated cereal bars) was probably caused by factors indicated in the no-choice experiment: (1) larger proportion of eggs are deposited directly on the unpacked cereal bar in contrast with other cereal bar states, where relatively large amount of eggs is placed on floor outside the bar, and (2) there could be possibility that in the presence of unpacked bar the females produce larger amount of eggs. Egg hatchabilities from the unpacked cereal bars ranged between 40 and 74% (mean $62.14\% \pm 6.04$ SE).

Discussion

Stored-product pest moths (e.g., *Plodia* sp., *Cadra* spp., *Ephestia* sp.) are a group of pests causing many millions of dollars in feeding damages on packaged food at a global scale. Unlike their larvae, adults of storage pest moths cannot cause direct feeding injury to food or packing due to the functional limitations of their mouth parts; they are adapted only for sucking fluid food. However, adults represent serious risks due to their ability to infest food by loads of eggs coupled with their high mobility and ability to locate food resources. Therefore, the evaluating the risks and patterns of oviposition, as well as understanding how effectively various food packages can be recognized by pest moths as food for their progeny (and consequently infested by their eggs), are essential steps for damage prevention or mitigation in the food production practice. In this work, we studied the effect of small perforations (that may be caused by producers to decrease the internal package pressure or as accidental ruptures during manipulation) on the *P. interpunctella* infestation risk by measuring the egg loads of Indian meal moth (*P. interpunctella*) placed on cereal-fruit bars packages or in their vicinity.

Indian meal moth females are attracted to and ovipositionally stimulated by a variety of food sources and

Table 1 Oviposition (mean $\pm$ SE) of *P. interpunctella* females in the presence of cereal-fruit bars with different package states in no-choice test

Package state	Number of eggs laid on	
	Cereal bar	Floor
Unpacked	504.80 $\pm$ 50.14 ^a	6.4 $\pm$ 3.0 ^d
Perforated	125.59 $\pm$ 59.99 ^{cd}	264.40 $\pm$ 33.30 ^{bc}
Packed	11.99 $\pm$ 6.96 ^d	368.80 $\pm$ 58.83 ^{ab}
Empty	1.6 $\pm$ 0.68 ^d	379.20 $\pm$ 46.79 ^{ab}

Means followed by the same letter are not significantly different (Tukey's HSD post hoc test; $p \geq 0.05$)

Fig. 1 Temporal dynamics and distribution of *P. interpunctella* eggs in the presence of cereal-fruit bars with different package states in no-choice test. Bars with the same letters are not significantly different (least significant difference post hoc test; $p \geq 0.05$)

Table 2 Differences in number of eggs (Median, [LQ, HG]) deposited on the cereal-bars in the two-choice test (Wilcoxon signed-rank test)

Two-choice combination	Number of eggs	<i>p</i>	<i>z</i>
Packed versus Perforated	10.5 [4–12]	0.005	2.80
Unpacked versus Perforated	45.5 [28–61]	0.005	2.80
Unpacked versus Packed	215.5 [158–245]	0.005	2.80
Unpacked versus Empty	3 [2–11]		

volatiles (Phillips and Strand 1994; Olsson et al. 2005). In addition to the odour component of food source, there is evidence that tactile and gustatory qualities are also important for oviposition stimulation (Mowery et al. 2002; Nansen and Phillips 2003). It has previously been shown that females are able to discriminate between individual odour stimuli (Nansen and Phillips 2003; Sambaraju and Phillips 2008), and that cereal products, nuts and dried fruits are among the most attractive food products for *P. interpunctella* (Mohandass et al. 2007). For this reason, it is not surprising that unpacked cereal-fruit bars stimulated females to an oviposition, as in the no-choice test the vast majority (> 98%) of eggs laid by females were placed on the bar and only small fraction was placed on the floor. Because the females oviposited higher amount of eggs on bars with package holes compared with regularly packaged bars, it is probable that they were able to locate—by some cue—the unpacked cereal bars. However, presence of a potential visual or/and chemical cues associated with cereal

bar and response of *P. interpunctella* females should be investigate in further studies.

Although Mullen (1994) found that Indian meal moth females are able to find and locate packaged food, in the present study there were no differences in the total number of laid eggs or in their spatial distribution between the packaged bar and the empty package. This suggests that the type packaging used in this study (duplex BOPP film of a thickness $20 + 20 \mu\text{m}$) provided an effective odour barrier. The discrepancy between this result and those of other studies suggests that different packages have different odour permeability and susceptibility to pest detection. Earlier, there has been recorded variability between several types of materials in resistance against feeding penetration from stored-product insects; polypropylene film was found to be one of the most resistant (Bowditch 1997; Stejskal et al. 2017). Nevertheless, some literature records can be found (Scheff et al. 2018) claiming that this type of package is not completely resistant to penetration by moth larvae. The present study suggests that the polypropylene type film may also inhibit the release of specific attractive odours from the packaged food into surrounding atmosphere. The unperforated polypropylene packaging thus seems to provide partial protection to cereal-fruit food products from being detected by stored-product moths. In contrast to unperforated packaging, it has been found that a 5 mm hole in the package was sufficient to cause the localization of the food source by the females. The females allocated a relatively large portion (32%) of eggs on the bar with the perforated packaging, although the number of eggs was lower than those for the unpacked bar. It is not clear

how the size and position of the holes influence the females' behaviour, but it is reasonable to assume that most packaging flaws are smaller than 5 mm or are in seals and do not allow direct contact with the food. Regarding the eggs that were not laid on the bar, there was a tendency for the eggs to be located either in the vicinity of the bar or on the arena walls and their vicinity (Fig. 2). A similar inclination of the females to lay eggs along the edge of the arena was recorded in the case of the packaged bar and the empty package. This egg distribution pattern may be related to the fact that the females use the walls as resting places and spend most of their time there (Sambaraju et al. 2016). This work indicates the various risk levels of product contamination by eggs only. However, it should be taken into account that number of eggs does not necessarily equal number of larvae/adults so the infestation risk resulting from our results may be to some extent overestimated. The present data indicate that on average only

62% of eggs hatch and reach maturity. On the other hand, because larvae are able to locate and reach food sources as distant as 38 cm (Tsuji 2000), the cereal bar with perforated package could be colonized by larvae hatching from eggs out of the bar.

The present study indicates that unprotected food products are at a very high risk of quick infestation by *P. interpunctella* eggs shortly after their introduction to the grocery store or market shelves and are thereby exposed to pest oviposition. For example, based on the data, females can place up to 120 (1st exposure day) and 140 (2nd exp. day) eggs on a single unprotected cereal bar. An interesting finding of this study was the variation in the temporal distribution of eggs: there were clear differences between the egg production of the unpacked bar state and all other bar states over time. In the presence of the unpacked bar, oviposition was considerably increased during the first three days, and from the fourth day onward, it declined. On

Fig. 2 Visualization of distributions of *P. interpunctella* eggs in the presence of **a** an unpacked cereal bar, **b** a cereal bar with a perforated package, **c** a packed cereal bar, and **d** an empty package. A rectangle

in the centre of the arena represents a position of cereal bar or an empty package, the colour and points indicate location and time of deposited eggs, and size of the point indicates egg load size

the other hand, for the other bar states, the oviposition rate was much more evenly distributed during the females' lifespans. Although most studies on the oviposition behaviour of *P. interpunctella* focus on a period of a few days, there is some evidence corroborating our finding that (at least in some circumstances) females lay most of their eggs (in our case, 73%) during the first 3 days (Hamlin et al. 1931). This fact has a practical impact on pest protection, as it suggests that pest control treatments are important shortly after the adult emergence. Because mating occurs during the first 24 h and oviposition occurs shortly thereafter (Hamlin et al. 1931; Silhacek et al. 2003), 5–6 days after emergence, the female may have already produced most of the eggs in its lifetime, so it is important to treat the infested space before this time.

Conclusion

In conclusion, the present study shows the adaptive behaviour of pests that increase the pest potential and food product infestation risk during storage and distribution. For the food industry, the practical conclusions from the study are: (1) unprotected bars can be found by *P. interpunctella* and infested by its eggs relatively quickly and are thus very likely vulnerable to infestation shortly after their introduction to stores or shop shelves; (2) non-perforated packages (duplex BOPP film of a thickness $20 + 20 \mu\text{m}$) provide protection against oviposition; (3) even small perforations enable *P. interpunctella* to find a resource and infest it with eggs.

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Compliance with ethical standards

Conflict of interest Authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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