Resistance of three energy bar packages to penetration by *Plodia interpunctella* (Hübner) larvae

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Abstract

Stored-product insect pests are a common invaders or penetrators of packaged food products. Roughly 75% of package infestations occur as a result of insects entering a package due to an existing product defect such as a tear or seam failure. The remaining infestations result from insects chewing through the packaging material. Larvae of the Indian meal moth, *Plodia interpunctella* (Hübner), have the ability to infest stored products by either mechanism. Larvae of *P. interpunctella* have been reported to infest 179 different stored commodities. Manufacturers can take all possible precautions to prevent infestations in their facility, but have little control of their product during transportation and storage in the retail marketplace. The objective of this study was to evaluate three packaging variations to determine which provided the greatest protection against penetration by *P. interpunctella* larvae. The three packaging variations were challenged with 50 first, 50 third, and 50 fifth instars of *P. interpunctella* at 28°C and 65% r.h., and held for 21 and 42 days. Packaging samples were observed for number and size of holes created by larvae, amount of damage sustained to the energy bar, and number of life stages present inside the packages. Results showed all larval stages of *P. interpunctella* were able to penetrate and infest all three types of energy bar packages resulting in varying degrees of damage. First instars, on average, penetrated fewer packages than third and fifth instars. Among package types, the control treatment packages were most resistant to penetration. Energy bar manufacturers need to invest in improving packaging designs by implementing thicker gauge or odor barrier films to prevent penetration and infestation by *P. interpunctella* larvae.

Keywords: *Plodia interpunctella*, Indian meal moth, packages, insect penetration

1. Introduction

Infestation of ready-to-eat packaged products by stored-product insect pests results in economic losses due to product loss, product adulteration, loss of consumer confidence, loss of potential markets and litigation costs. Stored-product insects are common contaminants of grain-based products, pet foods, and birdseed (Hagstrum et al., 2013). Infestation by stored-product insects can occur in raw ingredients, during manufacturing, prior to packaging, during transportation, in retail environments, and in homes of consumers (Hagstrum and Subramanyam, 2009). Food processors take multiple precautions to ensure that their products are insect-free when they leave the facility but have little control of the product during shipping, storage, or display in retail environments (Mullen et al., 2012). Generally, pest infestation is often the result of problems during transportation or storage (Mullen et al., 2012).

Insect infestation of packaged products is common in the retail environments because retail stores are infested with large numbers of a variety of stored product insect species (Roesli et al., 2003). Athanssiou et al. (2011) proposed two scenarios to explain the presence of insects in packaged products: (1) insects are present in the product before packaging, or (2) insects invaded or penetrated the product after packaging. Determining which type of infestation is more likely to occur will enable manufacturers to tailor their insect-resistant packaging designs.

Stored-product insects can penetrate or invade a packaged product. Penetrators have the ability to chew holes directly into packaging materials (Mullen et al., 2012). Common insect species are the adults of the lesser grain borer, *Rhyzopertha dominica* (F.); rice weevil, *Sitophilus oryzae* (L.); larvae of the warehouse beetle, *Trogoderma variabile* Ballion; and larvae of the Indian meal moth, *Plodia interpunctella (*Hübner) (Mullen et al., 2012). Among the developmental stages, the active larval and adult stages contribute to package infestations (Wohlgemuth, 1979). The penetration ability of seven common stored-product insect larvae showed that 50% or more of penetrations occur near a fold in packaging materials (Cline, 1978). In addition, those larvae deprived of food showed a greater ability to penetrate packages than those presented with a food source (Cline, 1978).

Invaders enter packages through existing openings such as a rip, tear, or hole (Mullen et al., 2012). Roughly 75% of package infestations occur due to an existing packaging defect or by insects creating an entry point from chewing through the package (Collins 1963; Mullen et al., 2012). Common invaders include adults and larvae of the red flour beetle, *Tribolium castaneum* (Herbst); confused flour beetle, *Tribolium confusum* Jacquelin du Val; and larvae of *P. interpunctella* (Mullen et al., 2012). Like insect penetrators, the young larvae can cause the most damage since they can fit through smaller holes compared to adults (Wohlgemuth, 1979). Generally, first instars have a greater ability to invade packages due to their small size, and have been shown to invade holes less than 0.5 mm (Adler, 2008). The smallest holes through which adults may enter can range from 0.71-2.25 mm (Cline and Highland, 1981). Insect growth regulators, insect resistant packaging, odor barriers, use of approved food additives, and modified atmospheres have all been documented as practical methods to prevent infestation of packaged products.

Larvae of *P. interpunctella* can attack a wide range of stored products (Allotey and Goswami, 1990), including 179 different commodities in 48 different countries in six continents (Hagstrum et al., 2013). Adult female moths are capable of laying 100-300 eggs (USDA, 1986). Once the eggs hatch, young larvae will wander in search of food. Larvae produce sticky silken threads (USDA, 1986), and the webbing often contains fecal material and cast skins. The first instars can invade holes less than 0.39 mm in size (Tsuji, 1998). The second to fifth instars possess the ability to penetrate kraft paper, polyethylene (25.44-µm thick), cellophane (25.4-µm thick), and aluminum foil (16.5-µm thick) (Cline, 1978). Bowditch (1997) and Cline (1978) demonstrated that older instars show a greater propensity to penetrate packages than younger instars. The objective of this research was to evaluate susceptibility of three types of polypropylene energy bar packages, varying in thickness, to penetration and infestation by *P. interpunctella* larvae at the request of a major energy bar manufacturer.

2. Materials and Methods

2.1. Insects

Cultures of *P. interpunctella* have been in rearing since 1999 in the Department of Grain Science and Industry at Kansas State University, Manhattan, KS. Insects were reared on a diet consisting of 1,000 g poultry mash, 150 ml glycerol, 150 ml honey, and 75 ml distilled water (Subramanyam and Cutkomp, 1987) at 28°C, 65% r.h., and 14:10 L:D photoperiod.

2.2. Properties of films

Three dual layer packaging film types, each containing a single energy bar, were obtained from the energy bar manufacturer. Packaging films included a control treatment of 15.24 µm oriented polypropylene/30.48 µm metalized cast polypropylene; test 1, of 15.24 µm oriented polypropylene/25.40µm metalized cast polypropylene; and test 11 of 15.24 µm oriented polypropylene/27.90 µm metalized oriented polypropylene. Packaging samples were visually similar in appearance and contained the same type of energy bars. All packages were evaluated for pre-existing rips, tears, and seam integrity before use in tests.

2.3. Package susceptibility against first, third and fifth instars

Male and female moths were collected from cultures and introduced into 0.95-liter glass jars fitted with a mesh screen. Glass jars were inverted over a 9-cm glass Petri dish and moths were allowed to mate and oviposit. Eggs collected within 6 h were added to 500 g of poultry mash diet and maintained in growth chambers at 28°C and 65% r.h. to facilitate larval development.

Fifty eggs, ≤24 h old, were counted under a stereomicroscope and added to 0.45-liter glass jar fitted with mesh screen containing a package type and held in a growth chamber at 28°C and 65% r.h. Egg hatchability was determined according to procedures described by Huang et al. (2004). Three replicates of 100 eggs each of *P. interpunctella* were collected and placed in glass Petri dishes. Dishes were placed in a growth chamber at 28°C and 65% r.h.; dishes were examined daily for 7 d. The percentage of eggs that hatched out of the total (100) was calculated (Huang et al., 2004). The mean ± SE hatchability was 87.0 ± 1.7%.

When the larvae in culture jars reached third or firth instars, as measured by head capsule width (Allotey and Goswami, 1990), 50 third or 50 fifth instars were added to separate 0.45-L glass jars fitted with mesh screens containing a package type. All jars after insect introduction were held at 28°C and 65% r.h. All infested jars with energy bar packages were examined after 21 and 42 d, and separate jars were observed at these two times. Each larval age and package was replicated five times.

After 21 and 42 d, packages were removed from jars and examined for the number and size of holes in each package. Hole sizes were measured by stereomicroscope fitted with a calibrated ocular micrometer. Each package was opened and the number of larvae, pupae, and adults inside the package were recorded. The energy bar was examined and the amount of damage sustained to the bar was quantified on a 0-4 scale. A score of “0” represented no visible damage to the energy bar. A score of “1” represented a bar that had 25% of its surface covered with larval webbing, fecal material, larvae or pupae cast skins, and dead insects. A score of “2” represented similar damage to 50% of the bar. A score of “3” and “4” represented 75 and 100% damage to the bar, respectively.

2.4. Data analysis

A completely randomized design was used for all experiments. The means and standard errors were calculated and reported (SAS Institute, 2008). The number of larvae, pupae, or adults found inside energy bar packages after 21 and 42 d were transformed to $\log_{10}(x+1)$ scale for analysis. Data on the number of holes in packages, hole size, and damage score were not transformed for analysis. All data were subjected to a two-way analysis of variance (ANOVA) and the two factors tested were packaging treatment and instar age and their interaction.

3. Results and Discussion

3.1. Packaging penetration

Older instars of *P. interpunctella* penetrated more packages than the first instars, irrespective of the package type (Table 1). As expected, the number of packages penetrated increased from 21 to 42 d exposure periods. The control treatment had the least number of packages penetrated by first and third instars after 21 and 42 d of exposure. In tests with fifth instars, test 11 had the least number of packages penetrated. Among all packaging types, test 1 had the most packages penetrated. The increase in packages penetrated from 21 to 42 d can be associated with the increase in exposure time and the increase in age of the instars. Stronger mandibles of older instars may enable them to more easily chew through the packaging material. Bowditch (1997) demonstrated that fifth instars of *P. interpunctella* penetrated more polyvinyl chloride packages (25 µm) than first instars. With the exception of fifth instars and test 11, increase in larval age resulted in an increase of packages penetrated at 21 d. Both Cline (1978) and Bowditch (1997) demonstrated that the ability of *P. interpunctella* to penetrate packaging materials varies among instars.

Table 1 Number of packages penetrated by first, third, and fifth instars of *P. interpunctella*.

Treatment	Instar	Observation time (d)	
		21	42
Control	1	0	1
Test 1	1	3	4
Test 11	1	1	3
Control	3	2	3
Test 1	3	4	4
Test 11	3	3	4
Control	5	4	*
Test 1	5	5	*
Test 11	5	0	*

*Data not collected as adults emerged within the 21 d period.

3.2. Observations after 21 d

A two-way ANOVA of 21 d data, showed that the number of larvae found inside packages were not significantly different among the packaging types (F = 2.25; df = 2, 36; P = 0.1196), but were significantly different among instars (F = 4.78; df = 2,36; P = 0.0144) (Table 2). Test 1 had the most larvae present inside the package and the control had the least. The package type and instar interaction was not significant (F = 2.48; df = 2, 36; P = 0.0613). The number of pupae found inside the package was significantly different among package types (F = 6.23; df = 2, 36; P = 0.0047), instars (F = 17.99; df = 2, 36; P < 0.0001) and package type and instar interaction (F = 7.13; df = 2, 36; P = 0.0002). The number of adults found inside the package was significant among package types (F = 2.75; df = 2, 36; P = 0.0332), and instars (F = 9.50; df = 2, 36; P = 0.0005). However, the package type and instar interaction was not significant (F = 2.20; df = 2, 36; P = 0.0880). The number of pupae and adults found inside each packaging type increased with an increase in instar age. Fifth instars had the most pupae and adults compared with third instars because of greater survival.

Table 2 Life stages of *P. interpunctella* present inside energy bar packages.

		Number of larvae		Number of pupae		Number of adults	
Treatment	Instar	21 d	42 d	21 d	42 d	21 d	42 d
Control	1	0 ± 0	N	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Test 1	1	21.6 ± 9.3	Y	0 ± 0	0.4 ± 0.2	0 ± 0	8.0 ± 3.3
Test 11	1	15.4 ± 7.1	Y	0 ± 0	0.2 ± 0.2	0 ± 0	3.6 ± 2.7
Control	3	1.8 ± 1.4	Y	0.6 ± 0.4	0 ± 0	2.8 ± 1.8	2.6 ± 1.5
Test 1	3	0.8 ± 0.8	Y	2.4 ± 0.7	0.8 ± 0.6	6.0 ± 2.3	3.4 ± 1.9
Test 11	3	0.8 ± 0.4	Y	2.6 ± 1.3	0 ± 0	3.4 ± 2.1	0.6 ± 0.6
Control	5	0.2 ± 0.2	*	2.6 ± 0.9	*	4.6 ± 2.1	*
Test 1	5	1.6 ± 1.0	*	4.4 ± 0.6	*	7.4 ± 3.1	*
Test 11	5	0 ± 0	*	0 ± 0	*	0 ± 0	*

Each mean is based on $n = 5$.
N = no; Y = Yes. At 42 d there were larvae in varying stages of development from eggs laid by F_1 adults. Therefore, their presence or absence was noted.
*Data not collected as adults emerged within the 21 d period.

As expected, the number of holes, size of holes, and amount of damage to the energy bar increased with increasing larval age (Table 3). Test 1 packages had the most holes per package and the most damage sustained to the energy bar for all instars tested. The number of holes varied significantly by packaging type ($F = 9.37$; df = 2, 36; $P = 0.0005$) but the effect of instar age was not significant ($F = 2.40$; df = 2, 36; $P = 0.1055$). The interaction of the main effects was significant ($F = 2.88$; df = 2, 36; $P = 0.0361$). The hole size was significantly different among the instars ($F = 4.76$; df = 2, 36; $P = 0.0147$). However, the average hole size across packaging types was not significant ($F = 2.91$; df = 2, 36; $P = 0.0675$). The interaction of the main effects was significant ($F = 5.09$; df = 2, 36; $P = 0.0023$). As instar age increased, the amount of damage to the energy bars increased. Test 11 was most susceptible to penetration by *P. interpunctella* and therefore had the highest average damage score among all packaging types. Damage among packaging type was significantly different ($F = 6.54$; df = 2, 36, $P = 0.0038$), but damage was not different among instars ($F = 2.18$; df = 2, 36; $P = 0.1283$). The package type and instar interaction was significant ($F = 4.25$; df = 2, 36; $P = 0.0064$).

Table 3 Packaging and energy bar integrity.

		Number of holes		Hole size (mm)		Damage score	
Treatment	Instar	21 d	42 d	21 d	42 d	21 days	42 d
Control	1	0 ± 0	0.2 ± 0.2	0 ± 0	0.2 ± 0.2	0 ± 0	0.2 ± 0.2
Test 1	1	1.0 ± 0.6	0.8 ± 0.2	0.3 ± 0.2	1.2 ± 0.3	1.6 ± 0.7	2.6 ± 0.7
Test 11	1	0.8 ± 0.2	0.6 ± 0.4	0.7 ± 0.4	0.6 ± 0.4	1.4 ± 0.6	1.4 ± 0.9
Control	3	0.4 ± 0.2	0.6 ± 0.2	0.4 ± 0.3	0.8 ± 0.3	1.0 ± 0.6	1.2 ± 0.6
Test 1	3	2.0 ± 0.6	2.8 ± 1.1	1.2 ± 0.3	1.0 ± 0.3	2.2 ± 0.6	2.6 ± 0.7
Test 11	3	1.2 ± 0.4	0.2 ± 0.2	1.1 ± 0.3	0.3 ± 0.3	2.2 ± 0.6	0.2 ± 0.2
Control	5	1.2 ± 0.4	*	1.0 ± 0.2	*	1.8 ± 0.6	*
Test 1	5	2.8 ± 0.7	*	1.3 ± 0.2	*	3.2 ± 0.2	*
Test 11	5	0 ± 0	*	0 ± 0	*	0 ± 0	*

3.3. Observations after 42 d

Observations at 42 d only included data for first and third instars. Fifth instars emerged as adults within 21 days so we do not have any data for 42 d. Additionally, the number of larvae present inside the package was recorded as present or not present because at 42 d there were larvae in various stages of development from eggs laid by F_1 adults, Table 2. A two-way ANOVA showed that the number of pupae found inside packages were not significant among package types, instars, or their interaction ($F_{\text{range among instars}}$ = 0.00-3.23; df_{range} = 1 (instar) or 2 (for package types and interaction), 24; P_{range} = 0.0572-1.000). The same trend was seen in the number of adults found inside the packages ($F_{\text{range among instars}}$ = 0.23-3.13; df_{range} = 1 or 2, 24; P_{range} = 0.0619-0.6373).

Among packaging types, test 11 had the most holes in the package after 42 d (Table 3). Yet, the results were not significantly different based on packaging type, instar, or their interaction (F_{range} = 0.05-3.17; df_{range} = 1 or 2, 24; P_{range} = 0.0602-0.8290). Test 11 had the largest hole size and damage score between the three packaging variations tested. The effect of packaging type was significant (F = 5.20; df = 2, 24; P = 0.0124). The effect of instar age was not significant (F = 2.70; df = 1, 24; P = 0.1132). The interaction of the main effects was significant (F = 3.30; df = 2, 24; P = 0.0673). The damage score followed the same trend. Damage scored varied by packaging type (F = 5.97; df = 2, 24; P = 0.0079) but was not significant among instars (F = 0.02; df = 1, 24; P = 0.8962). However, the interaction between packaging type and instar was significant (F = 1.58; df = 2, 24; P = 0.2261).

4. Conclusions

The results of this study demonstrate that older instars cause more damage compared to younger instars, because the former have stronger mandibles. However, it should be noted that all three instars are capable of penetrating packages. Increasing the film thickness decreased the ability of *P. interpunctella* to penetrate, but further testing needs to be conducted on thicker packages in order to prevent infestation of *P. interpunctella.* Additional tests are underway to determine the effect of package thickness, and incorporating repellents and growth regulators to packaging on insect resistance of packages.

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