Resistance test for different pasta packagings to the massive and repeated attack by *Plodia interpunctella* larvae (Lepidoptera: Pyralidae) and *Sitophilus oryzae* adults (Coleoptera: Dryophthoridae)

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ABSTRACT

Pasta infestation discovered by the consumers is often caused by stored-grain pests that manage to enter in the packaged product on the shelves of the retail outlets, breaking through the package wall or passing through existing openings.

In this study 2 types of pasta packages by 3 different brands, with different characteristics in terms of construction material (cardboard, plastic and plastic-coated paper) and presence/absence of micropores, were exposed to the massive and repeated attack by *Plodia interpunctella* (Hbn.) larvae and *Sitophilus oryzae* (L.) adults, in order to evaluate their resistance to penetration.

The results showed that packaging with micropores were on average more susceptible to the penetration of *P. interpunctella* and *S. oryzae* compared to those without. The cardboard cases resulted more resistant than the others, while the more susceptible material was plastic-coated paper.

1. Introduction

Pasta is one of the most important foods in the world, both for its nutritional values and volume of production, and its demand is gradually increasing. According to the data of UN.A.F.P.A (Union des Associations de Fabricants de Pates Alimentaires de l'U.E.), the European Union area is globally the main producer, with Italy being the Country with the biggest production (over 3 million tonnes in 2015) and consumption (23,5 kg per capita/year). Pasta dough, mainly composed of semolina obtained from durum wheat, is shaped in different sizes and lengths and then dried. The drying process is the final step of industrial pasta production, and it is fundamental to greatly prolong product shelf life.

In Italy packaging solutions differs for dried and fresh industrial pasta, also according to the pasta length. Short-cut pasta is often let to dry in silos before the packaging, while the long-cut one is packed right after the production and let to dry in cases with small openings or air vents.

The type of material used for packaging can be different; cases of 0.5 or 1.0 kg in capacity normally used for retail are mostly made of plastic (poly-propylene), cardboard or plastic-coated paper and they are usually tailored to fit the product being protected.

Most food products available on the market are packed to prevent infestation, although contamination by insects during the storage period, both on the shelves of the retail outlets and in the households, is still frequent. Packages have several points of weakness and almost every packaging material can be perforated by insects, although different materials have different resistances to the penetration.

The widespread use of susceptible packaging materials, like paper and cardboard, for food products should be carefully evaluated, because losses from insect infestation are important consequences of problems encountered in growing, harvesting, transportation, processing and packaging of the raw material.

The most destructive insect pests of stored products belong to the Orders *Coleoptera* (beetles) and *Lepidoptera* (moths) (Robertson, 1993) and can be subdivided into "penetrators" and "invaders" (Highland, 1984). Penetrators can bore holes through packaging materials; an example is the Indian meal moth, *Plodia*

interpunctella (Hübner) (*Lepidoptera: Pyralidae*), which is considered responsible for 98% of food infestation worldwide (Süss and Locatelli, 1999). Invaders enter packages through existing holes, such as holes in defective seals (i.e. thermosealing for plastic cases or gluing for cardboard), holes caused by mechanical damage, or holes made by penetrators. Example of invaders are *Sitophilus oryzae* (L.) and *Sitophilus zeamais* Motschulsky (*Coleoptera: Dryophthoridae*); these were the main insects found in a screening of packaged pasta products from several countries (>90% of cases examined).
In order to prevent insect contamination, it is important to understand how insects invade food products.

In this study we tested the resistance of six different pasta packaging to the attack of *P. interpunctella* larvae and *S. oryzae* adults, representing respectively penetrator and invader species. The packaging examined differs in term of construction material (cardboard, plastic and plastic-coated paper) and for the presence/absence of air vent micro-holes. Long and short-cut pasta packaging were tested. We evaluated their performance against the massive and repeated attack from these two species of insects in a 14 days test, simulating the presence of an infestation on the shelves of a G.D.O. supermarket.

2. Materials and methods

2.1. Insects

All the insects used for the tests were reared in Agroblu Laboratory of Applied Entomology (LEAA) and maintained at 28 ± 2 °C, 50%± 5% RH and a photoperiod of 12:12 (L:D). For the tests were employed *Sitophilus oryzae* adults reared on barley and rice, and *Plodia interpunctella* larvae reared on a mixture of bran, cornmeal, semolina, honey and glycerin.
For infestation tests unsexed, mixed-age vital adults from *S. oryzae* culture and unsexed II-III instar larvae from *P. interpunctella* culture were randomly chosen.

2.2. Pasta and packaging material

The pasta products tested are made of durum wheat flour, packaged in cases of 0.5 kg. Sixty pasta packages were randomly selected from the shelves of a G.D.O supermarket, checking for the absence of mechanical damages that could affect their structural integrity.
The pasta packages were sorted into three categories, depending on packaging material: plastic (PP5, polypropylene) pillow pouch (A); plastic-coated paper (monogloss kraft) pillow pouch with clean windows of PP film (B) and folded carton box with end flaps tucked and glued, with a clean window of PP film (C). For each of these categories, packaging containing long (.L) and short-cut (.S) pasta were tested (Table 1).
Two separate studies were performed to test the packaging resistance against the attack of *P. interpunctella* larvae and *S. oryzae* adults, in 5 experimental units for each packaging type and insect.

Table 1. Packaging description and characteristics.

	Pasta characteristics		**Package characteristics**				
Code	**Type**	**Shape**	**Dimensions mm***	**Thickness (µm)**	**Material**	**n. Air vent micro-pores (n. lines)**	**Air vents dimensions (mm, mean of 9 measurements)**
A.L	Long	Spaghetti	324 x 91	60	Plastic pillow pouch (PP)	38 (2)	1,49 ± 0,12
A.S	Short	Penne	278 x 104	80	Plastic pillow pouch (PP)	0	NA
B.L	Long	Spaghetti	340 x 100	100 50 (only PP film)	Plastic-coated paper pillow pouch (monogloss kraft)	20 (1)	1,14 ± 0,04
B.S	Short	Penne	287 x 100	100 50 (only PP film)	Plastic-coated paper pillow pouch (monogloss kraft)	0	NA
C.L	Long	Spaghetti	269 x 70 x 31,5	450 30 (only PP film)	Cardboard box (PAP21) with plastic window (PP film)	0	NA
C.S	Short	Penne	151,5 x 129 x 77	500 30 (only PP film)	Cardboard box (PAP21) with plastic window (PP film)	0	NA

* Depth x width x length for board boxes. Width x length for pouches, measured flat and within the seals. NA: not applicable.

2.3. Experimental units

5 packages of the same type were singularly enclosed with 10 insects in translucent plastic container with lid (50x40x25 cm). Each plastic container has been previously modified with the creation of a window (10x10 cm) covered with net, in order to allow an adequate ventilation, confine the infestation and avoid the contamination by alien organisms. Teflon® oil was applied to the upper part of the container walls to prevent the insects from escaping.

After insects placement, the experimental units were covered with the lid and held at controlled condition at 28 ± 2 °C, 50%± 5% RH and 12:12 (L:D) photoperiod.

2.4. Assessments

At the first two assessments, after 3 and 8 days, the plastic containers were opened and the insects (either dead or alive) found outside each pasta packaging were counted and removed, while 10 vital individuals were added and then experimental units were closed. 14 days after the test start, this procedure was repeated, then all the pasta packages were carefully examined to check for the presence of holes or damages due to insect activity. Each pasta package was finally opened and the insects inside were counted.

2.5. Data analysis

The number of missing insects in each assessment was used to determine the alleged starting time of the infestation (Table 2). The infestation rate for each packaging type was calculated as percentage of infested cases on 5 packages.

Table 2. Infestation rate, infestation detection and penetration point in different pasta packaging, for *P. interpunctella* larvae and *S. oryzae* adults.

		Sitophilus oryzae (adult)			***Plodia interpunctella*** (larvae)		
Code	**Air vent micro-holes**	**Infestation rate (%)***	**Infestation detection (days)**	**Penetration point**	**Infestation rate (%)***	**Infestation detection (days)**	**Penetration point**
A.L	Yes	0	NA	None	100	3	Enlarged air vents
A.S	No	0	NA	None	20	3	Holes in plastic film
B.L	Yes	20	14	Enlarged air vents	40	3	Enlarged air vents
B.S	No	0	NA	None	40	3	Holes in plastic window and in correspondence of a mechanical tearing of paper
C.L	No	20	8	Openings due to poor glue sealing	20	3	Openings due to poor glue sealing
C.S	No	20	3	Openings due to poor glue sealing	20	8	Openings due to poor glue sealing

*calculated as percentage of infested cases on the 5 packages tested

3. Results

Both *Sitophilus oryzae* adults and *Plodia interpunctella* larvae have proven able to penetrate into several kinds of pasta packaging. The pasta cases exposed to the attack of *P. interpunctella* larvae show a lower resistance compared to those exposed to *S. oryzae* adults; all the six type of packaging analyzed were successfully penetrated by *P. interpunctella*, while only three type of packaging resulted vulnerable to *S. oryzae*.

Comparing the two packaging types with air vent micro-holes, plastic-coated paper pillow pouch (B.L) was susceptible to *S. oryzae* adults attack, with a successful penetration in 20% of the cases, while A.L. packaging was not penetrated by this insect. Conversely, *P. interpunctella* larvae were successfully

penetrated into all the plastic pillow pouch cases with micro-holes (A.L.), while only 40% of the B.L. cases was found infested.

In the packages with micro-holes every penetration was performed through the pre-existing openings. *S. oryzae* attack was detected after 14 days from the test start, thanks to the presence of signs of jaw attacks, creating by pulling the edges of air vents. *P. interpunctella* larvae penetrated already from the third day, by chewing the edges of air-vents in order to enlarge the opening and reach the substrate.

The results show that *S. oryzae* adults were not able to enter into the two types of film pouch without air vent micro-holes considered (A.S and B.S.). *P. interpunctella* larvae managed to penetrate in both A.S. and B.S., respectively in 40% and 20% of the cases, thanks to the ability of this insect to bore holes even through plastic films.

The two types of pasta packaging folded in carton boxes were successfully penetrated by both the insects tested, in 20% of cases and within 8 days from the test start. There were no mechanical holes in the carton packages but the insects entered inside exploiting the openings due to poor glue sealing.

4. Discussion

In this study we tested the resistance of different pasta packaging types to the massive and repeated attack of *P. interpunctella* larvae and *S. oryzae* adults, simulating the presence of an infestation on the shelves of a G.D.O. supermarket.

P. interpunctella larvae and *S. oryzae* adults belongs to different categories of package pests, respectively penetrators and invaders (Highland, 1991). Insects classified as penetrators are those that can chew holes directly into packaging materials, while invaders are insects that enter packages through existing openings. Usually, penetrators are most dangerous at the larval stage, although some beetle species can also be dangerous as adults.

Despite this distinction, mechanical holes or other openings can be used by both penetrators and invaders to reach a food product. When pasta packages are exposed to their attack, the insects will take advantage of any sort of opening in the packaging material in order to gain entry. These openings may form as rips, tears or punctures resulting from normal wear and tear throughout the handling process, while rarely are the result of the chewing of penetrators. According to Mullen et al. (2012) most infestation are the results of invasion through seams and closures.

Opening in packaging may be made deliberately by the manufacturer in the form of “vents” to allow pressure equalization. In this way, the bursting or shrinking of food packages during shipment can be avoided. In polypropylene pasta packages with one or two rows of mechanical air vent micro-holes on the back, the holes facilitate the entry of insects.

The air vents micro-holes present in two of the packaging types considered (plastic film and plastic-coated paper) were large enough (1,49 ± 0,12 mm and 1,14 ± 0,04 mm respectively) to allow the entrance of *P. interpunctella* larvae. This results consist with the observations made by Adler (2008), according to which first instars larvae of the common stored-grain pests are able to invade packages of different materials through holes of minimal size, often less than 0.5 mm in diameter.

In most of plastic film and plastic-coated paper cases with air-vents, *S. oryzae* infestation was not detected, because the diameter of the holes wasn’t large enough to allow their entrance. Only in one case adult *S. oryzae* have managed to penetrate, nibbling around the air vent micro-holes in plastic-coated paper, in order to enlarge one of them.

In addition to the diameter, also the number of micro-holes present in the packaging material is an important factor that influence the probability of infestation; in this study the plastic pillow pouch allowed a greater rate of infestation by *P. interpunctella* larvae compared to the plastic-coated paper pillow pouch (100% and 40% respectively), thanks to the greater diameter and number of air vents.

The results show that *S. oryzae* adults weren't able to enter in pillow pouch cases without air vents, as observed for *S. zeamais* in Trematerra (2014), both through plastic film and plastic-coated paper. *P. interpunctella* larvae, instead, could penetrate even into packages of commercial pasta without micro-holes.

P. interpunctella larvae proved their capability to bore opening in thin layers of plastic (80 µm) and plastic-coated paper. In the latter case the larvae choose to attack areas of the packaging where the material is thinner, in correspondence to the plastic window or where the paper kraft was damaged. This insect penetrated more efficiently in plastic coated paper, probably because the plastic layer in this packaging was thinner than the plastic film used in plastic pillow pouch (50 µm and 80 µm respectively).

In the case of folded carton boxes, both *S. oryzae* and *P. interpunctella* entered inside through openings not well sealed by glue; if overwraps are not completely sealed, insects can easily gain entry at the corners of the folded flaps. The infestation rate of intact carton pasta cases was the same for the two insects and there were no differences between long and short pasta cases, since there were no air vents micro-holes in this packaging type. Although the carton is a material more porous than the plastic film and can let food odor pass more easily, its greater thickness (450-500 µm) seems efficient in discourage the creation of new openings through the packaging.

For this kind of packaging the integrity of the pasta case is crucial to prevent infestations and the reinforcement of seals and closure, by changing of glue patterns or the type of glue used, can improve significantly the resistance to insect attack.

VanRyckeghem (2011) listed several packaging materials and their resistance to penetration by insects, pointing out the importance of the material used: impervious to attack (vacuum sealed jar and tin cans); insect proof (poly-carbonate, polyethylene terephthalate, polyester, nylon plastics); insect resistant (cellulose acetate, polyamide, polyethylene 250 µm, polypropylene, polyvinyl chloride); susceptible to attack (acrylonitrile, polylactic acid, polyethylene 125 µm); no protection from attack (ethylene vinyl acetate, kraft paper, corrugated paperboard, paper/foil/polyethylene, polyethylene 25-100 µm, polyvinylidene chloride).

5. Conclusions

This study demonstrates that all the six different types of pasta packaging tested are susceptible to the attack of insects during their shelf-life in a G.D.O. supermarket in experimental conditions. Larvae of *P. interpunctella,* a penetrator species, are more efficient than adults of *S. oryzae,* an invader species, in penetrating pasta cases. Different types of packaging apparently have different resistance to infestations, according to the material used (cardboard, plastic and plastic-coated paper) and the presence/absence of air vents micro-holes. The infestation rate of packages with air vents depends also from diameter and number of the micro-holes. Packages without micro-holes are not penetrated by *S. oryzae* adults, while *P. interpunctella* larvae have the capability to bore holes through thin plastic layers.

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