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Resistance of new generation food-packaging films against various species of primary and secondary stored-product pests

Master Thesis

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DECLARATION

I hereby declare that this Master's Thesis titled "Resistance of new generation food-packaging films against various species of primary and secondary stored-product pests" is my own work completed with the expert guidance of my thesis supervisor and consultant. I worked separately under supervisor of the thesis and was using literature and other information.

In Prague, 12/04/2019

Signature

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Summary

Infestation by stored-pests is a world-wide problem, it is responsible for infestation of processed, semi-processed and raw food. It directly influences in food safety as well as food security. Stored-pest can be divided into two groups: penetrator (ability to chew holes directly in packaging materials) and invaders (enter package through the existing opening).

The main aim of this thesis is to evaluate the protective abilities of selected food-packaging foils against infestation of variously stored product beetles. We observed the Penetration test using a Hou's apparatus, where we tested resistance of four films (i.e. PP 20, PE 50, PP 40 and PET/PE 12/70) to penetration was tested against 6 types of pests (*R. dominica, S. oryzae, T. castaneum*, *T. confusum, P. ratzeburgii* and larvae of *T. confusum*).

Another aim of this thesis is to elucidate, invasion test of whole packages of rice (polyethylene mono-layer with thickness 50 μm) under optimum temperature and relative humidity. Usually, there are two ventilation holes on either side of the package of rice, which is made by the manufactures. We performed our experiment in Lock and Lock plastic container of size 180×110×110 mm. Every container containing one 1000 g package of rice and we kept filter paper to provide better movement for the pests.

We found that *R.dominica* can penetrate slightly more holes in the wild spectrum of films then *S. oryzae*. While the numbers of all injury in tested foils was created slightly more by *S. oryzae* then *R.dominica*. Surprisingly we found that *R. dominica* created more fully penetrated holes in PP 40 than in PP20. While in the invasion test, we observed no synergy effects between *R.dominica* and *S. oryzae* while they were kept together. The ventilation holes made by the manufactures were enough for pests to invade (gain entry), but the secondary pests invade only in a few samples.

Key-words : Infestation, stored-pests *R. dominica, S. oryzae, T. castaneum*, *T. confusum, P. ratzeburgii,* penetrator, invaders, packaging- films, ventilation-holes,

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1.Introduction

Since the beginnings of agriculture about 10 000 years ago, growers have had to compete with harmful organisms – animal pests (insects, mites, nematodes, rodents, slugs and snails, birds), plant pathogens (viruses, bacteria, fungi,) and weeds (i.e. competitive plants), collectively called pests – for crop products grown for human use and consumption. Today's food industries are facing a serious problem of insect pest in raw and finished stored commodities. These insect pests can cause serious qualitative and quantitative losses. According to FAO less than 0.5 percentage of the total number of the known insect species are considered pests. Insect pests have been defined by Williams (1947) as organisms that compete with a human about human resources.

Packaging represents the ultimate defense of food products against an insect pest. Even food products come uncontaminated from the production line, this does not guarantee that it will reach the consumer in the same condition. Stored-product insects can enter in food package during transportation, storage in warehouses or in retail stores. Any consumer who comes across contaminated packages, holds the manufacturer responsible for the inconvenient, with severe consequences on the images of the company. Damage caused by insect pests results in annual multi-million dollar losses suffered by the global food industry. Directly it is big problem of food company around the globe, indirectly it is the problem for all humans as it can influence in world food security. Apart from the infestation pests, insects cause additional damage, such as altering temperature, moisture/humidity level and micro-flora of the infested commodity through their metabolism and multiplication. Furthermore, stored-product insect pests are also vectors of several diseases that may endanger human or animal health. The usage of plastic films has been increasing and decreasing the uses of traditional materials such as paper and cardboard. The reason why food industries are attracting towards plastic as packaging materials are they are flow-able and mouldable (under certain conditions), to make sheets, shapes, and structures; they are generally chemically inert, though not necessarily impermeable; they are cost-effective in meeting market needs; lightweight; they provide choices in respect of transparency, color, heat sealing, heat resistance, and barrier properties. The most common types of the package are Polyethylene which was invented in 1933 and was used in packaging from the late 1940s. In Europe, nearly 40% of all plastics are used in the packaging sector. About 50% of Europe's food is packed in plastic packaging (British Plastics Federation (BPF).

2. Hypothesis and objectives

Various food packaging films (plastic or paper) differ in their protective properties against primary and secondary pests. The main aims of the thesis are 1. To evaluate the protective abilities of selected food-packaging foils against infestation of variously stored product beetles. 2. To evaluate the role of compression holes in the packaging in relation to infestation by secondary stored-product pests.

The main objective is to determine and evaluate the penetration abilities of beetles in selective common types of packaging materials. The study is focused on the resistance of selective packaging foils against penetration by insect pests. Some beetles and larva are package penetrators and some of them are invaders. This study subjected to consumer health and save the losses of food industry due to these beetles.

3.LITERATURE REVIEW

3.1 Introduction of Store pests infesting food

There are several disadvantages of stored pests over processed, semi-processed and raw food product such as the food adulteration, loss of consumer trust, potential loss and litigation costs which do not affects only in the economy but also an overall reputation of the product. These losses can happen in several stages such as raw material, during manufacturing, before packaging, and in the retail environment. (Hagstrum and Subramanyam, 2009). Food manufacturer takes multiple preventative measures to secure their product from insects but once the food is out of there sites there reminds a threats of pests infestation generally while transportation (Mullen et al., 2012) and storage in a retail environment because in storehouse contains varieties of store pests (Roesli et al., 2003).

The chances of insects in products can be categorized into two different part: 1. presence of insects before food packages; 2. insects invaded or penetrate the package of food after packaging Athanassiou et al. (2011). So manufacturers should identify the risk of insect resistance and determine the package to get red out of problems and insecurities. We can say this is the key point of designing of a food package.

Based on packaged product stored product pest can be divided into two distinct groups: 1. penetrators and 2. invaders. Penetrators got the ability to chew holes directly in packaging materials examples *Rhyzopertha dominica, Sitophilus oryzae*, etc (Mullen et al., 2012). Not only the adults but the larvae can also penetrate the package (Wohlgemuth, 1979). Those larvae deprived of food showed a greater ability to penetrate packages than those presented with a food source (Cline, 1978).

Whereas the invaders can enter food package through the extension opening holes like rip, tear or holes (Mullen et al., 2012). It has been estimated that 75% of package infestation can occur due to the existing packaging defects or by insect produce entry point by chewing the food package (Collins 1963; Mullen et al., 2012). The larva can cause harm just like the adults since they fit in a small hole as compared to the adult one. The smallest size of the hole through which adults can enter can range from 0.71-2.25 mm (Cline and Highland, 1981). Adult female moths can produce 100-300 eggs and once the egg got hatched the larva are always in search of food (USDA, 1986). Older instars (developmental stage of arthropods) can cause serious damages than the younger one because the former have strong mandibles. Whereas the thickness of film can affects the capacity of penetration (Scheff, Subramanyam, 2014). There are some methods such as insect growth regulator, insect resistance packaging, odor barriers, modified atmosphere, uses of appropriate food additives, etc has been reported to prevent infestation.

In the retail-stores environment the possibilities of insects infestation are higher with a large number of store-pests variety species (Roesli et al., 2003). (Athanssiou et al. 2011) documented two chances of insects inside the packaged product: 1. the insects are already inside the food package before packaging and 2. insects invade or penetrated the food product after packaging. (Scheff D, Subramanyam B et al., 2014) demonstrated that the oldest instar caused more damage than the younger instar, because they have strong mandibles and with increasing the thickness of films decreased the ability of *P. interpunctella* to penetrate.

S.K. Chung et al. (2011) reported that the choice of packaging materials for the food depends on several factors including the resistance against the store- pests. Polyethylene (PE) acts as a strong barrier to the water vapor and moisture but its low barrier to the oils and fats; in addition it has low heat resistance. S.K. Chung et al.(2011) documented, third -instar larvae of *P. interpunctella* nor T. castaneum adults cannot penetrate the films (casted polypropylene (CPP) 20 mm and 25 mm, oriented polypropylene (OPP) 20 mm and 30 mm, linear low-density polyethylene (LDPE) 40 mm and 50 mm, and polyethylene terephthalate (PET)) without the pinhole. While G. Muratore et al. (2008) reported that good resistance of the polypropylene to penetration of insect pests, not depending on the association with other materials in the laminated structure.

In Japan, 2006, 30% of all shipped pasta product sustain damage to the packages heat-sealed before reaching retail stores and in the investigation, the researcher found that *S. oryzae* and *Sitophilus zeamais* invade the pasta packages in polypropylene pouches. In damages product, both species entered through the breaks in the seal and laid their eggs on the pasta (Murata et al, 2006). From 2008- 14, in Czech Republic market mite have been reported pre-packed dried food - *Carpoglyphus lactis* (L) in dried fruit and *Tyrophagus putrescentiae* (Schrank) in dog feed, Hubert et al 2015, documented the infestation had happened due to the lack of adequate sealing of the package.

3.2 Effects of pests on human health

The UN Food and Agriculture Organization has estimated the population will have reached 9.8 Billion in 2050 (within the next 31 years) and increasing population required more food. It is not a problem of some countries, it is a global problem. Alone agriculture production cannot ensure the Human Food Security, effective post-harvest storage and distribution of food commodities should go hand by hand. Integrated Pest Management (IPM) of stored product contribute significant role to preserve global food supply (Hagstrum and Philips, 2017). Pest organism like insects, mites, rodent, birds, viruses, bacteria, fungi are all time problem of post-harvest storage. The problem which is associated with human and animal interaction can be classified into two groups: human health-related and economical. (Hagstrum and Subramanyam, 2009) has reported there are more than 1600 insect species associated with the stored food product. Uses of chemical to avoids from these problems can bring other problems i.e. excessive pesticide residues in food. Some stored arthropods can be helpful to control pests in stores i.e. bioagents but they have negative health problems (Stejskal et. al 2000). The negative effects of the pest are listed below

3.3 Direct effects on Human Health

There are significant negative impacts of storage pests in human health which we cannot avoid some of them are spoilage of food (related to food security); food contamination (related to food safety); pests in home and environment can directly attack human (stings and parasitism).

3.3.1 Store pests negative impact on Food Security

"Food security exists when all people, at all times, have physical and economic access to sufficient, safe and nutritious food that meets their dietary needs and food preferences for an active and healthy life" (World Food Summit, 1996). Even in an unexpected crisis situation such as natural disaster and wartime government are responsible to provide adequate food supply to the civilian so they gather the strategic food resources data (Stejskal et al. 2015).

A) Deterioration of food value malnutrition

According to International agencies FAO, WHO, etc there are more than 900 million people suffering from malnutrition on a worldwide scale. According to the UN, about 1.4 million children are at risk annually from starvation which means many developing countries are facing food security problems. The reason of which cause food security problem are ongoing war, political instability, disaster, environmental fluctuation, lack of economic resources and agricultural practices, etc. Food losses can happen not only in cropping stages but also in processing and storage stage. Pest-damaged cereals if consumed it may possess malnutrition leading to immune system weakening and outbreak of different infectious diseases. On the other hand, the nutritional value of food will decline.

B) Food/ commodity losses in the period of storage

There are different estimated dates related to losses of food due to feeding activities of insects, mites, birds and rodents ranging from 3-60 % in world-wide. While the post-harvest losses in developed countries and developing countries are also significantly differenced i.e. 9 % in a developed country and 20 % in developing county (Philips and Throne 2010). Infestation can be done by the use of Chemical Insecticides and pesticides to preserve the storage losses example malathion. But the proper storage technology and effective chemicals may not available in every country which may skyrocket the figure. (Tefera et al. 2011). In the case of stored agricultural commodities in Asia, the rodents and arthropods are responsible for damaging about 5% than birds 0.8% losses (Ahmed 1983).

3.3.2 Stored pests negative impact on Food Safety

Arthropods are responsible for major contamination source in agricultural commodities. Arthropods pests are major sources of food various food diseases and toxicogenic fungi (molds) bedside that productions of allergens and potential carcinogenic (Gorman 1979; Arlian 2002; Stejskal and Hubert; 2006). Three basic parameters are required to calculate risk are contamination rate; distribution of contaminants and efficacy of disinfection and cleaning treatments. In European Union legislation there is zero tolerance if live pests present in food but it hasn't established action level or threshold. Some impacts of store pests concern with food safety are described below

A) Many larvae (belonging to from *Dermestidae* (*Coleoptera*), if ingested, can cause irritation of the digestive tracts and cause diarrhea and perianal itch (Goddard 2003).

B) Many arthropods can contaminate the foods and impact negatively in human health via pest allergenic protein contains in feces or dead bodies and cause allergic diseases such as asthma, rhinitis, and eczema. Mite-contaminated food if eaten can lead to anaphylactic reactions (Gonzalez- Perez et al.2013).

C) Some insects can produce defensive substances. , They are toxic to human after ingestion. The most dangerous are blister beetles also known as 'Spanish flies' which can found in field crops can appear in storehouse can produce toxin cantharidine (Goddard 2003). It is significantly food safety danger can be used as criminal purposes (Lockwood 2012). T. castaneum can produce 13 different quinones (Howard 1987) which are 'defense and antimicrobial chemicals' if contaminated food it could be responsible for liver and spleen tumors in small vertebrates (El-Mofty et al. 1988).

3.4 Effect on home work place safety and disease issue

Stored product insects and rodents are commonly present in households, supermarkets, commodities stores, mills, bakeries, and farms, etc. Many occupational and professional diseases among cereals workers, bakers and storekeeper have been reported very often. Beside them, scientist, pest management professionals and technician related to pest insects are at risk from occupational diseases.

Physical irritants and arthropod allergens: Respiration allergies may be generated by inhalation of spikes and setae from the larvae of Dermestid beetles. Rhinitis and asthma symptoms after the exposure Dermestidae have been documented (Sheldon and Johnston 1941). The case study involving hypersensitive reaction by different insects have been explained by several authors (e.g. Lunn 1966; Alvarez et al. 1999 etc). While in the meat industry by mites, allergic diseases may cause (Armentia et al. 1994). In addition; protein extract from this mite can cause a skin reaction (Neto et al. 2002).

Unfortunately, there are no worldwide acceptable threshold levels of numbers of arthropods in stored commodities. Exposure of 100 mites/ gram dust can increase the risk of sensitization and clinical symptoms of allergic disease, while 500 mites/ gram of dust could increase the risk of actual asthma attacks (Lau et al. 1989; Platts-Mills et al. 2000).

Parasitoses: Mite is responsible for causes of human acariasis of human internal organs (Li and Wang 200) he added there is a high risk of pulmonary ascariasis among the staff of medical herbs stores and workers of rice mill and storehouse.

Mite and Insect bite and Itches: There are several cases documented by several in different years of mite and insect bite around the world. Some of them are sting dermatitis caused by Cephalonomia gallicola in Japan (Hatsushika et al. 1990); several cases of C. gallicola attacks in the Czech Republic and elsewhere in Europe (Mazanek 2002); Parasitic or predatory mites may cause dermatitis. Similarly in United State skin rashes caused by the outbreak of Pyemotes herfsi was reported (Broce et al. 2006). While after bites of C. malaccensis caused skin lesions (Yoshikawa 1985).

Rodent bites: Bites of rodents at farms and urban area are considered as a public health issue (Battersby et al. 2008). 622 victims of rodents bites have been reported in the USA (Hirschhorn and Hodge 1999).

Psychic health effect caused by pest: Negative emotional effects while insect encounters in food can be defined as psychic health effects caused by the pest. (Baker and Swan 2013). It can be both a medical and social dimension (Anderson 1993). Repeated exposure to arthropods or vertebrates pests can cause entomophobia or pathological anxiety. Nesse and William (1995) documented that the fear or phobia of pests responses can encounter a natural defensive mechanism.

Common injuries on warehouse and retail markets is slip on floor accidents caused by insects and mites contamination

Fire and exposure associated with pests activities in stores and silos (Kimball 1997; Persson 2013). Rodents can chew the electric wirings and insulations and microorganism and insects can increase the moisture.

3.5 Indirect health effects of store pests

It includes transmissions of pathogens, parasites, increases the temperature and moisture of food commodities which results in the productions of mycotoxins or allergen-producing fungi. Secondary exposure to insecticides which contribute to health risks.

Transmission and hosting of Pathogens: Microbial infestations(generally *Aspergillus* and *Penicillium*) is a common problem associated with the stored product which are unsuitable for consumption (Hanuny et al. 2008). Synergistic effects and mutual benefits can be seen between *Tyrophagus putrescentiae* and *Aspergillus flavus* in house mice (Stejskal et al. 2015).

Rodents urine and feces may contain parasites, pathogenic bacteria, and viruses such as *salmonella, staphylococcus aureus, Enterococcus spp, Escherichia coli, Pseudomonas aeruginosa,* etc; which can contaminate food and agricultural commodities (Stejskal and Aulicky 2014). The feces-to-food is the main route of pathogens transmission.

Physical grain destructions and humidity and temperature changes by primary pest: Fungi can enter and grow through the disrupt grains attacked by primary moth and beetles pests.

The increase of temperature and humidity by the activity of storage pests population was experimentally shown for the first time by Cotton et al. (1960); which later become known as 'hot spots'. The reason for water and heat production is respiration of pests populations (Fleurat- Lessard 2002).

3.5.1 Poisoning by toxic Rodenticides and Insecticides

If the level direction is followed on the uses of pesticides then there are fewer chances of health risk; but if not there is a threat of acute or chronic health risk in workers (sometimes death). There are so many stores, farms and food industries facilities rely on the use of pesticides. The fumigant phosphine is very toxin; in some developing countries due to incorrect doses and mis-application, they are facing occupational health risk (Eddleston et al. 2002).

"A maximum residue level (MRL) is the highest level of a pesticide residue that is legally tolerated in or on food or feed when pesticides are applied correctly (Good Agricultural Practice)" European Commission. European Food Safety Authority (EFSA 2015) showed 97.4% of tested food sample is within legal limits and 54.6% sample didn't have quantifiable residues in Europe. Grain Protectants like deltamethrin (half-life 23.9-24.8 days) and pirimiphos-methyl (half-life 23.9-28.9days) are commonly used in the European Union. The residue is expected to be in control limits while these kinds of protectants are used in a storage condition. Pyrethroids are mostly stable to heat while Organophosphate degrades if temperature increases (Arhur et al. 1992,1994). Protective residues generally decrease as the commodity is processed (Fleurat- Lessard et al. 2007).

3.6 Description and biology of frequently occurring stored pests

3.6.1 *Rhyzoperha dominica*

Average minimum life cycle

25 days at 34°C (93.2°F)

Distribution: Worldwide; both larvae and adult are voracious feeder Egg: 300 to 500 per female; laid on the grain surface. The eggs are opaque, white in color with a waxy appearance (freshly laid eggs) (Kucerova' and Stejskal, 2008). The oval-shaped eggs are about 0.5-0.6 mm in length and 0.2-0.25 mm in diameter (Thompson, 1966; LeCato and Flaherty, 1974; Kucerova' and Stejskal, 2008). The oviposition period is 6 days (Thompson,1966) to 15 days (Schwardt, 1933). The oviposition period strongly depends on temperature and humidity i.e. 43 days at 25°C and 70% RH (Howe, 1950) but the data will raise to 4 months when the temperature is increased to 32°C (Birch 1945c).

Larvae: Whitish with brown head; about 5 mm long and eat into the grain and feed into grain dust. There are on average 4 instars in *R. dominica* (Potter, 1935; Howe, 1950; Thompson, 1966). The first instar is about 0.78 mm long and 0.13 mm wide across the head capsule (Potter, 1935). The activity of larvae in grains is very active and moves rapidly. (Winterbottom,1922). The larvae chew the damaged grains made by adults and it stays there until it emerges to an adult (Winterbottom, 1922; Potter, 1935). The second instar is larger in size than the 1st one. The firsts, seconds and third instar look similar in color while the fourth one looks different- the ventral region is white and ahead is light brown (Chittenden,1911; Winterbottom, 1922).

Pupae: Usually from a cell inside grain; stage last 5 to 8 days. The average body length is 3.15mm long whereas the diameter of the head capsule is 0.5mm. The pupa of *R. dominica* is inactive and the moment of a body is limited to the abdomen segment. The young pupa looks whitish and later on eyes and mouth part the brown pigment is laid down (Winterbottom, 1922). At 70% RH and 25°C, the pupa stage is completed in about 8 days whereas at 28 °C in 5-6 days (Howe, 1950).

Adults: Voracious feeder; bullet shaped cylindrical body; clubbed shaped antennae. The young formed adult might go for3-5 days after emergence (Schwardt, 1933). The adults are 2-3 mm long and 0.8 to 1 mm in wide. It is reddish brown to dark in color.

Food: They are mostly found in grains such as rice, wheat, sorghum, oat, millet, malt barley and in legumes like chickpeas, peanuts, and beans (Chittenden, 1911; Potter, 1935). However, they archived maximum reproduction success in wheat (dried grains) (Chittenden, 1911; Schwardt, 1933; Potter, 1935; Bashir, 2002; Edde and Phillips, 2006c).

Types of damage and symptoms: The adult's beetles can fly and can enter grain bin through headspace, gradually it moves through the grain mass in downward progression (Sharangapani and Pingale, 1957; Keever, 1983; Vela-Coiffier et al.,1997; Hagstrum, 2001). R. dominica can go down up to 12 meters in depth which is the deeper in observation from other grain pests (Flinn et al., 2010). Infestation in grain kernels from *R. dominica* can be characterized by irregularly shaped hole (about 1mm diameter). Male can produce aggregation pheromones while infested the grains and it is sweetish in taste (Khorramshahi and Burkholder, 1981). The feeding activity of the adults *R. dominica* can be characterized by a large number of frass, undigested endosperm mixed with finer flour part. Both larvae and adults can feed the endosperm and germ and reduce the kernel of wheat to pericarp (Winterbottom, 1922; Campbell and Sinha, 1976).

They are 2-3 mm long; reddish brown and slim, cylindrical and shiny body; highly destructive insects. It can be easily identified according to its shape. Generally, the head is not visible from above; the head is tucked up under the thorax and the hood-shaped rounded neck shield. They have 10 segment antenna. Moisture content is critical to oviposition and development. If the moisture content is less than 8% there is no it is not suitable for oviposition. The egg is up to 500 per female, are laid outside the whole kernel and young larvae bore inside. Egg development depends on time i.e. 32 days at 18.1°C while only five days at 36°C. 17 days prolong in larvae development in a 3°C rise in temperature (25 to 28°C).

The larvae may complete their development in grain residues while adults usually remain within kernel for few days prior to emergence. Oviposition starts after two weeks and continues for four months in mated females.

Figure 1 : *R. dominica*

3.6.2 *Sitophilus oryzae* (Linnaeus)

Common name : Rice weevil

Average minimum life cycle : 25 days at 29.1°C and 70% RH.

Economical importance: *S. oryzae* is considered as the most destructive primary pest for stored cereals like rice, wheat, barley, and corn. It can even attack the cereals plant of fields but it also does attack pasta and split peas but it doesn't often breed in non-cereal foods. Due to voracious eating habit, it can cause several negative impacts in stored grains such as weight loss, fungal growth, increase in fatty acid which is subjected to quality loss and even completely destroy of stored grains. It is a primary pest and it could cause grain heating and may establish the secondary pests, fungal colony, and mite pest.

Geographical distribution: It is cosmopolitan (found all over the world) but mostly found in the tropical and temperate area; they are the least cold tolerance of all the grain weevils.

S. oryzae are mostly found in countries like Argentina, Australia, China, Czech Republic, Slovakia, India, Egypt, Iraq, Italy, Japan, South Africa, Egypt, USA, Nigeria, etc.

Climate adaptability: *S. oryzae* is moderately cold harder but it required relatively high humidity. It doesn't usually exist in cooler areas and extremely high summer temperature, it is common in the tropical and warm temperate zone. The generation per years depends on climatic factor for example in the mid-western region of USA it product 4-5 generations per year while in Canada only one per generation.

Eggs: Laid in grain in the field and storage. Eggs are shiny, white, opaque and ovoid to pear-shaped. A female lay two or three eggs per day and between 300 to 576 eggs in a lifetime.

Larvae: The larvae are white, stout and legless feed internally within the Kernel of grains. It spends all its instars inside the kernel. Measurement of head capsule widths of a slide- mounted larvae under the microscope is the best way of separating the four larval instars.

Pupae: It is also white but it contains legs, wings, and the snout of the fully grown adult and it is generally found within the Kernel.

Adults: 2.5 to 4.6 mm long, radish brown or dark brown, with long narrow snout containing strong, chewing mouthparts and eight segmented elbowed, club-shaped antenna.

Food: Generally, both larvae and adults feed on whole cereals grains like wheat, corn, barley, rye, oat, rice buckwheat, etc. Although they are whole grain pests they have been reported to feed beans, nuts, processed cereals, pasta, cassava, spaghetti, birdseed, and pet food. Female can lay and develop on a solid product made of cereals like pasta.

Types of damage and symptoms: Larvae usually have strong jaws and it feeds and developed in grains kernels and most of the time they are undetectable from outside. It can chew large irregular holes in the endosperm, germ, and kernel. The adults, which got emerged from the pupa, exists through a circular hole about 1.5 mm in diameter and it can attack new kernels. *S. oryzae* infestation can be characterized from the irregular shaped hole. While development from egg to adult, a single insect can damage up to 30% of wheat kernels- which means it can consume 47 calories out of 126 calories in this process at first 50 days of adult life at 30°C and 70% RH.

Physical limits and optimum rate of multiplication

The maximum oviposition occurs between 25.5 and 29.1°C, while they can lay eggs on temperature between 13.0 to 35.0°C (Birch 1945). Physical limits for successful development are 17- 34°C and 45- 100% RH whereas the optimum conditions are 26-31°C at 70% RH. It multiplies 25 times per lunar month.

Life cycle: They are internal feeders and their entire development within the kernels. Usually, white egg is deposited into the cavity created by a female. After laying the egg, female slowly withdraws the ovipositor, then she fills the cavity with the gelatinous materials that make hard as the plug to protect newly laid eggs. It takes generally five days to hatch eggs and hatchability is about 75% (Arbogast, 1991). It is also affected by moisture content; 5 to 6 days more can be added in lower moisture content (about 11%) (Arbogast 1991).

After hatching, the larvae feed, develops through four instars, and pupates inside a single kernel. Development time of eggs to adults depends on n time i.e. 25 days at 29.1°C to an average time of 35 days at 27°C (Arbogast 1991; Sharifi and Mills 1971). The development larvae instars after developing from the eggs are I- 6 days, II - 13 days, III- 17 days and IV- 24 days while pupa and adult required 34 and 43 days respectively. Adults longevity is about 7-8 month and occasionally up to 2 years at 21°C and 70% RH.

Rice weevils can fly and easily distributed themselves throughout the storage facility. In mild temperature they can infect the grains of a field because of their flight ability; so it is necessary to inspect incoming loads for this pests, even the loads are coming directly from the field.

Ecology: Dispersal behavior of *S. oryzae* is related to the environmental condition. Migration rate is higher from dense population than from light population. In bulk wheat, at 14% moisture content which is stored at 30°C disperse only slightly from the sport from the spot at which it is introduced. But this insects shows high movement when a temperature is more than 32°C. In temperature, RH and light, it response photo-negative when placed in a dark-light environment at 25 °C and 34% RH but when the RH is declined it response towards light i.e it becomes photo-positive.

They are poor flier as compared to *S.zeamais.* In inter-specific condition(29°C and 70% RH) *S. oryzae* is more productive species in wheat whereas the *S.zeamais* in corn. This species becomes more dominating and productive than 30°C and 70% RH in various cultivars. The rising temperature and additional moisture due to metabolism or external factor accelerate both the oviposition rate and development rate, which sky-rocketed the population. But when the temperature is rising more than 40 °C it starts to die.

Figure 2: *S. oryzae* (L.)

3.6.3. *Tribolium Castaneum* Herbst, Rust- Red Flour beetle

Economic importance: *T. Castaneum* is one of the well-known and commonly stored-product insect which can infest cereals, oilseed and other vegetable and animal products in mills, wire-houses, elevators throughout the world. Since it cannot damage the whole grains with less than 12% of moisture so it attacks to grain dust, broken grains, and milled stocks. It is often considered as secondary-pest. Nevertheless, the most important of this species is it can attack store-product, climate adaptability, omnivorous feeding behavior,flying ability, etc.

Geographical distribution: Worldwide but most commonly in a warmer region. It seems to have originated in the Indo-Australian region. It is reported to have invaded human habitats since 2500 B.C. It is mainly found in the USA, Canada, UK, Europe, India, Mexico, Australia, etc.

Climate adaptability: T. castaneum is one of the most well-adapted and successful stored-pest. It is generally found in a cold region and even in low humidity (1%).

Food: It is an omnivorous and cannibalistic insect. Adults and larva are also cannibalistic particular in a crowded situation. Its larva and adults feed on the embryo of see, broken grains and grain dust. It can infest wide-range of food and grains like corn, millet, barley, wheat, rye, oilseed and their product, alfalfa seed, cottonseed, peas, ginger, beans, dried fruits, nuts, chocolate, flour, cocoa-beans and packed all types food of cereals, oilseed, and pulses, etc.

Eggs: Eggs are oblong and white female can lay 360-450. eggs in her lifetime but egg laying capacity got decreases at more than 100 days old female (Sokoloff 1972). Eggs are 0.6-0.7 mm wide white or transparent with a viscous surface. The egg hatch into larvae in 2.6-3.6 days at 30-40°C. The relative humidity does not affect the length of eggs periods. The optimum days for eggs to develop into adults is 20 days under optimum conditions.

Larvae: Larvae are yellowish white with three pair of thoracic legs, they are up to 5 mm. It has an elongated and cylindrical body, with short yellow hairs. T. castaneum larvae can be separated from others by the two-pointed end of the last body segment. The length of first instar larvae is about 1.1 mm and the wide of a head is 0.2 mm.

Pupa: The pupa is white, 3.3-3.4 mm long; 1.1 mm wide and weight is about 2.4 -2.5 mg. The pupa period requires 3.9- 24 days at 20- 37.5°C and 3.9-5.5 days at 30-37.5°C.

Adults: The body of adults looks flat, reddish-brown to blackish, about 2.3-4.5 mm long, thorax slightly darker than the elytra, densely punctuated. The adult's body is relatively flat and the sides of the abdomen are parallel.

Physical limits and optimum rates of multiplication: The optimum temperature and relative humidity for successful development are 32-35°C and 70-75% respectively.

Types of damage and symptoms: Initially damaged cereals grains by primary granivorous like S. oryzae, the larvae of this species can be found inside. Food of food product infested by this species looks many reddish-brown beetles moving over the materials. Cast skins, fecal pellets, and frass could be found in heavily infested flour which looks greyish. Volatile yellow or reddish brown liquid can be produced from the scent glands of adults living on foods which may results in flour turn into pink color and giving out disagreeable taste and odor. In infested flour the level of free fatty acids and the uric acid increases. The baking flour, if infested by T. castaneum over month results in brittleness of gluten, poor flavor and bitter taste of the bread and if infest wheat over 2 months the level of fat acidity will be increased significantly and decrease in germination. During 20 days of development from eggs to adults, the individual can consume 60 calories out of 126- calories.

Ecology: *T. castaneum* can fly up to 90 cm above the ground at a warm temperature and it can spread from one storage area to another. This insect shows more activity at night time.

Unlikely some other store-pest only stay inside it can found in the surface too. Some fungal growth in storage like *Aspergillus glucus* and *A. candidus* favor the accumulation of T. castaneum. In a temperate climate, it can harm farm storage during warm fall harvest weather. Metabolic heat creates by these groups can make hot sport up to 42°C which can cause serious grain spoilage.

Average development from egg to adult ranges from 41.8 days at 25°C to 21.7 days at 35.5 °C (Hagstrum and Subramanyam 2006). Howe (1956a) found that between 35°C and 37.5°C at a relative humidity (RH) more than 70%, development is completed in 19 to 20 days (eggs in three days; larva in 12 to 13 days; pupa in four days). They can live for several months to a year. At 18 to 29°C the average life span of males and females ranges from 130 to 198 days. Adults got capacity to reproduce lifelong. The pre-oviposition period is 8 to 10 days. A mated female can lay 3 to 5 eggs per day for first days and 2 to 3 eggs per day in rest of her life at 25°C and 70% RH. They are largely associated with stored food and feed grains, oilseed, dry fruits and nuts, pulses spices, cottonseed, cocoa and forest product. They do not develop in the sound kernel (Anonymous 1986).

Figure 3: *T. Castaneum*

3.6.4. *Tribolium Confusum*

Economic importance: It has been found associated with human habitat since 2500 B.C. It is highly damageable for prepared and processed cereals products generally in the flour feed mill, warehouses and retails stores. It is secondary grains feeder and it cannot damage whole cereals but it can damage all kinds of broken, damaged, milled product, flour, and grain dust cause millions of dollars damage in every year. It is associated with the place where cereals and bakery products are processed, stored and shipment of grains and the products.

Geographical distribution: (Worldwide) It is found in cooler climate; primarily occur in a flour mill, feed mill, warehouse, retail grocery store, semolina mills and bakery (Cogburn 1973b; Bousquet 1990; Trematerra et al. 2007). *T. confusum* was originated in Africa but due to the transportation with various farinaceous food now it can be found worldwide (cosmopolitan in distribution). It is a relatively high tolerance for low temperature. Comparatively to other species if *Tribolium* it is found in a higher latitude. It is a common flour-mill pest and it can found in a temperate climate in Mediterranean countries like Canada, USA, UK, and Europe. Whereas it is considered as major cereals pest in Cyprus, South America, Australia, China, Spain, and Russia. It is reddish brown in color; looks similar to *T. castaneum*. The two species can be distinguished by the number of segments in a club of the antenna (*T. confusum* has 4 -5 segments and *T. castaneum* has three). Both species adults have well-developed wings but only T. Castaneum have been observed to fly (Arbogast 1991).

Climate adaptability: It is moderately cold-hardy pests species found in a temperate climate. In general, it is climate adaptable stored-product insects.

Food: Both adults and larvae of *T. confusum* feed on wide variety of foods such as wheat flour and wheat feed, rice flour and bran, rye flour and rice flour, cornmeal, barley flour, rolled oat, spices, chocolate, ginger, peas, breakfast cereals, dried fruits and weevil-damaged cereals, cottonseed, sunflower seed, powder milk etc. The larvae can attack the grain if *T. confusum* is present in whole grains.

Types of damage and symptoms: The larvae construct tunnels during their moment through the powdered food and flour, whereas the adults can be easily seen in every moderately infested food. Identifiable, disagreeable and sour smell is produced while gaseous quinones are released from their glands in infested food and food products. The dough from infested by *T. confusum* does not rise, color variations, reduction of load size, offensive better taste and offensive flour.

Eggs: Generally a female can lay 500 eggs in her lifetime and it can lay 1-15 eggs per day. It will take 25 days for an egg to adult development at 35.2°C and 70% relative humidity.

Larvae: The larvae period, which is feed on crushed wheat and kept at 27.5 to 35°C and 70% relative humidity is 18 to 23 days long with 7 to 8 instars

Pupa: The cylindrical and worm-like larvae is 1.1- 1.7 mm long. It is white at first then it turns to yellowish towards the ends during the growth period. The pupal period is 4.9 to 7.7 days between 27.5 -37.5°C and 70% relative humidity.

Adults: The adults of *T. confusum* is shiny, flattened oval, reddish brown 2.6-5.0mm long and 1.2 to 1.3 mm wide. The upper part of the throat and head are densely covered with minute punctures. It has 11 segment antenna. The female is slightly larger than the male. The eyes are white and sticky external surface.

Physical limits and optimum rate of multiplication: The optimum range of growth and development is 30-33°C and 70% relative humidity while the physical limits of developments are 20-38°C and 10-100% relative humidity.

Life cycle: A female *T. confusum* may be egg laying when it is 114-126 hours old. Eggs are hatched between 3.9 to 7.7 days at 25-37°C. The development period varies greatly with both food source and physical factors. When *T. confusum* is fed on crushed wheat, oat and barley it grew faster. At 31°C and 70% relative humidity, the egg to adult development period is 40 days (on some fungal diet). The optimum development of *T. confusum* is 2.5°C lower than the T. castaneum. At 32.5°C and 70% RH the *T. confusum* complete their development in 25 days(i.e. eggs in 4 days, larvae in 16 days and pupae in 6 days). However, development time was 20 days at 35 °C and 56.2 days at 22.5 days (Hagstrum and Subramanyam 2006).

Ecology: The birth and death rate and the outbreak of this species is mainly affected by the factors like temperature, relative humidity, nitration value of food, cannibalism, and crowing. Both adults and larvae feed on their own eggs and pupa. In compare to male, female is more cannibalistic. *T. confusum* do not fly even it does have well-developed wings. It is, however active throughout the year, crawl in large groups, infest all available dry food. Adults can live up to 54 days without food but normally it lives 14-18 days. They can release the quinones which can turn the flour to pink color, and in an airtight and nearly airtight condition with little or no food they can kill all the population. In a heavy infestation, food may be discolored, have unacceptable order and may contain fecal matter. This species can secrete a chemical from its abdominal glands (benzoquinones); which is heat stable making a disagreeable odor to food and it can't be removed by cooking (Hodges et al. 1996).

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Figure 4: *T. Confusum*

3.7 Penetrator and invaders and mechanism of entry

Store-product insects can penetrate or invade the packaged product. Typically, invaders have weakly developed mouth-paths at both larvae and adult stage (Wohlgemuth 1979). They account about 75% or more of total infestation (Collins 1963). Generally, invaders got inside the package from the hole made by other insects, mechanical damage and defects seals (Mullen and Highland 1988). Newly hatched larvae are food demanding and cause the most damage because they can enter packages through small holes from 0.1mm wide (Wohlgemuth 1979). The adult saw-toothed grain beetle seems to enter the package from less than 1mm wide hole and adult red flour beetle through 1.35mm (Cline and Highland 1981). (Cline and Highland, 1981) documented the smallest holes through the adults may gain entry can range from 0.71-2.25 mm.

Penetrator

These groups can chew holes directly into the packaging materials. Those are most dangerous in larvae stage but some of the adults of these groups are also dangerous (Wohlgemuth 1979). *R. dominica* (Fab.); *Lasioderma serricorne* (Fab.); *Trogoderma variabile*; S. oryzae (L.); *Tenebroides mauritanicus*; larvae of *Corcyra cephalonica* are the good examples of the penetrator. They are capable of boring through one or more layer of flexible packaging. It has been reported under certain condition larvae of *Plodia interpunctella* (Hübner), are a good penetrator and could seriously damages packaged food (Mullen and Highland 1988, Mueller 1998). Dry pet food and pasta are often infested by warehouse beetles. These beetles can create serious problems among the customers since cast skin of larvae can cause allergic reactions. *Stegobium paneceum* (L.) are a strong penetrator and they infest a wild variety of foods. (Highland 1991).

Invaders

Those species which are classified as the invaders can enter package through the existing opening. Common invaders include *O. surinamensis*; *T. castaneum; T. confusum; Cryptolestes pusillus* (Mullen and Highland 1988). The most important invaders are larvae of *Tribolium spp.* and *Oryzaephilus spp* (Wohlgemuth 1979). Roughly 75% of package infestation occurs due to the existing packaging defects or by the insects create an entry point from chewing the package (Collins 1963; Mullen et al.,2012).

Although these beetles and larvae are classified as penetrators and invaders which describe the packaging pests group; this is artificial. Under certain condition, invaders can be a penetrator and vice versa. For example, the larvae of Indian meal moth and the almond moth penetrate are classified as invaders but under certain situation, they can be penetrator (Mullen and Mowery 2000). Some invaders can chew weak packages such as paper and cellophane.

Table 1: Classification of pests that generally infest packaged food

Invaders	Penetrators
Red flour beetle (*T. castaneum*)	Red Flour Beetle *(T. castaneum*)
Merchant grain beetle (*Oryzaephilus mercator)*	Warehouse beetle (*Trogoderma glabrum*)
Confused flour beetle *(T. confusum*)	Rice weevil (*Sitophilus oryzae*)
Sawtoothed Grain Beetle *(O. surinamensis*)	Almond moth larvae (Cadra autella)

Almond moth larvae (*C. cautella*)	Indian meal moth larvae (*P. interpunctella*)
Indianmeal moth larvae (*P. interpunctella*)	Lesser grain borer (*R. dominica*)
Squarenecked grain beetle (*Cathartus quadricollis)*	Cadelle (*Tenebrodes mauritanicus*)
Flat grain beetle (*Cryptolestes pusillus*)	Drugstore beetle (*Stegobium paniceum)*
Rice moth larvae (*Corcyra cephalonica*)	

Mechanism of entry

Stored- product insects adults and larvae used to feed to sustain themselves. Both intender and penetrator take advantage of any opening in the packaging to gain entry inside the package. Package opening could be the results of rips, tears or chewing, parachute from normal wear and tear during handling. To allow pressure equalizer and avoid bursting over shipping changing altitude and temperature manufactures package opening or vents. In many cases entry of insects via existence opening which is made by the manufacturer or by the other insects or by poor sealing. Most of the infestation occurs by the invasion through seams and closures then the penetration made by the insects by themselves (Mullen1997). Many insects prefer to lay eggs in tight places like in layer of a multi-wall paper bag and paper-board cardboard.

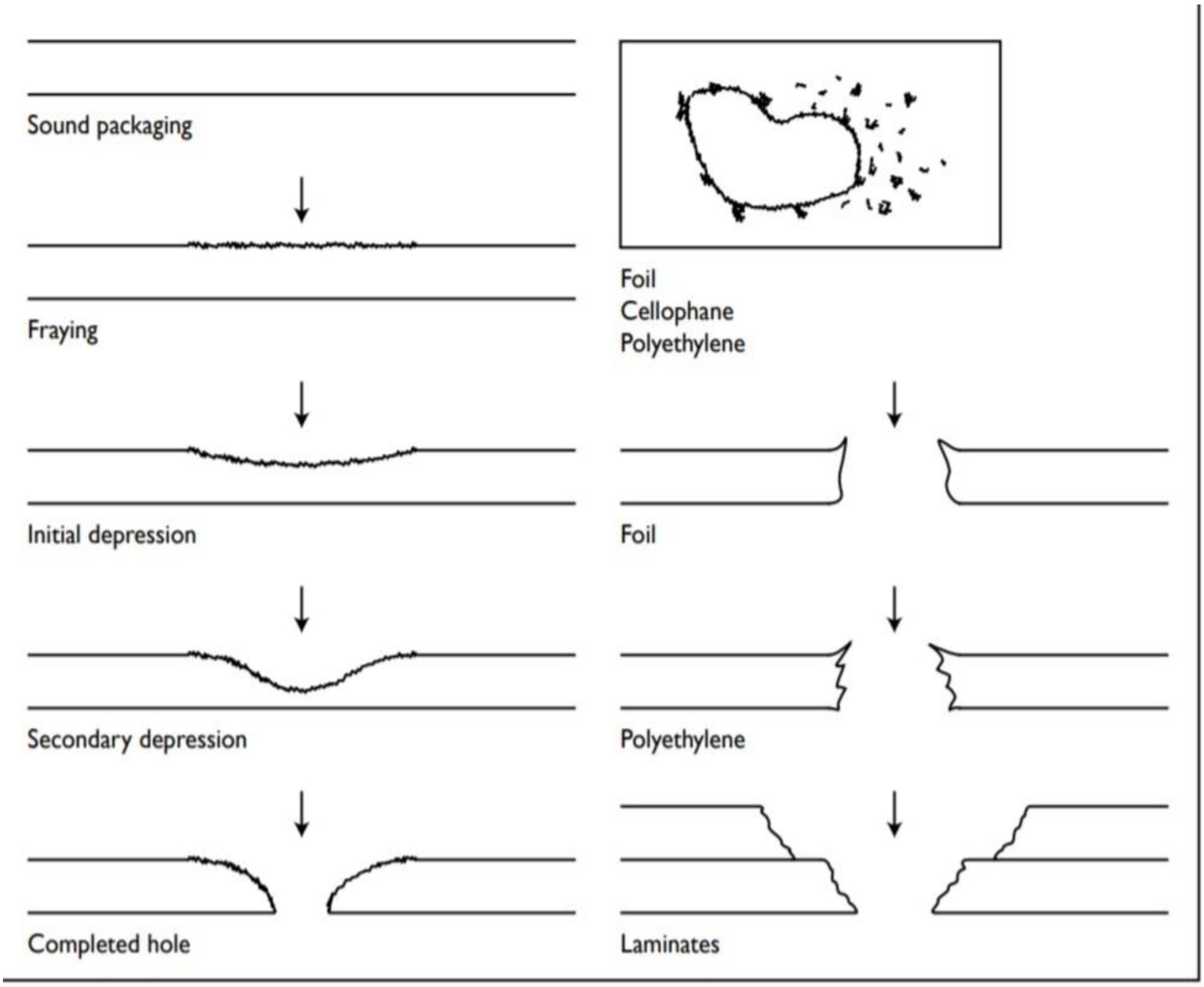


Figure 5:Direction of insect penetration into food packaging adapted from Brickey et al. 1973.

3.8 Biological properties enabling the beetles to infest a commodity

Food order response by the store product pests through consumer packaging films

Food and beverage packaging compromise over $70 billion in USA and over $200 billions in worldwide (Wilkinson 1998). Food manufactures knows if the consumer find any pests in their packaged food that will bring negative long-lasting impacts and they may loose customers. Many stored product insects will infest the package food product to nourish their offspring or themselves, where they are protected from most chemical treatments (Highland 1984). There is a chances of movement of contaminants from one geographical location to other; and these infestation can be spread over the local warehouse or retail store once these contaminated product are placed there. Some products are more susceptible to infestation, and so these product act as reservoirs of insects to other nearby products (Highland 1984). To get entry stored-product insects can take advantage of any sorts of opening in the package. The most important mean through which insect may identify the source to feed and oviposit is olfactory (Bell and Carde´ 1984). Various endogenous factor which can influence the response of insects to food odour, including species, age sex, mating sites and satiation. For example maize weevils, *S. zeamais* Motschulsky about 10 days old were more sensitive to volatile from rice then older one (Honda et al. 1969). Whereas maize weevils, *S. zeamais*, of mixed sex which are kept without food for 2 days shows more sensitivity towards the rice extracts (Ohsawa et al. 1970). Phillips et al. (1993) have reported *S. oryzae* (L.) and *T. castaneum* (Herbst) responds differently to same grains-related volatiles. *T. castaneum* was shown highly attractive towards the WGN (processed consumer food product) which indicates habitat preference of these species to develop in older and damaged grain substrate where as *S. oryzae* was shown lack of responses towards WGN; this indicates not all grains oils are attractive to weevils (Phillips et al. 1993). The sawtoothed grain beetle, *O. surinamensis* (L.), accomplish their entry from through flaws in the packaged commodities (Mullen and Mowery 2002). These beetles are unable to damage sound grains but they can cause serious damage to processed food products (Fraenkel and Blewett 1943). Whereas the adults and larvae are believed to create holes in package. The sawtooth beetles invaded the package is limited by the size of flaws. Adults can fit into the holes > 0.71mm in diameter (Cline and Highland 1981). But the neonate larvae of size 0.3 mm are able to enter the package through small opening (Mowery et al. 2002). Hence if female lay eggs near to the packages have high chances of exploits.

Significantly more sawtoothed beetles infested food packages that have punctured with more than 0.4 mm diameter holes. (S.V Mowery; M.A Mullen 2002) have shown that if the female lay eggs near to the hole of package, it could be highly infested and if there is no food, neither adults and larvae response to the holes of package. They explained when the female beetles cannot enter inside the package, there is still chances of infestation via the eggs of female once got hatched it could chew the package. Female sawtoothed grain beetles do not response to the odour of food (White 1989, Trematerra et al. 2000). One of the interesting finding is when female lay eggs near to the food; the number of laid eggs increases which indicate the combination of tactile, gustatory, and olfactory cues may associate with sawtoothed beetles oviposition (S.V Mowery; M.A Mullen 2002). Virgin female *O. surinamensis* have been shown to have less responses to odours of foods (White 1989). Larvae emerging from eggs laid near to food can locate the sources of food by using volatile clue. (Landolt et al. 1998) documented similar types of clues in the larvae of *Cydia pomonella* (L.).

larvae secretion and oviposition response to source food or grain odours in female (*P. interpunctella*)

This group females orient to odour of food source for oviposition and hexane-extractable semiochemicals are associated with it. (Thomas W. Phillips; Michael R. Strand 1993) shown that more eggs are laid in dishes which contain food than in empty one. They documented that kairomonal factors from mandibular glands also serve as pheromones for oviposition. Contamination resulting by higher numbers of larvae influence the distribution of eggs by female *P. interpunctella*. Low densities of larval contamination were preferred for oviposition when uncontaminated food occurs as an alternative to larval contamination. *P. interpunctalla* upwind response to the volatiles of food have been confirmed by Wind tunnel experiments.

Ephestia cautella

Some store pests beetles like *Sitotroga cerealella* (Oliv.) and *S. zeamuis* attacks cereals before harvest; these have specific mechanism of findings food or grain source (Cotton and winburn 1941; Giles and Ashman, 1997). Furthermore some laboratory demonstration has shown some species like *T. castaneum* (Wills and Roth, 1950); *S. zeamais* (Oshawa et al., 1970) and *Ephestia cuutellu* (Barrer, 1977) respond to food odours. Free flying *Ephestia cautella* strongly response to the odour of Kibbled wheat.

Ability of first instar larvae of the Indian-meal moth, *P. interpunctella* to reach their food.

Larvae of *P. interpunctella* can cause a great hazard in a packaged and bottled food product. (Mallis 1945,1969). Both 4th and 5th instar larvae of this species have the capacity to penetrate 0.03 mm thick polyethylene, and these holes are followed by younger larvae instar and invade the packaged food (Shinoda et al., 1990). Even the 1st instar larvae of Indian-meal moth are capable to invade food containers through the minute pinhole, while they have a poor boring ability. When adults had freely oviposited, first instar larvae of these species easily invade the food container through pinholes of container 0.398mm or greater diameter; whereas only few larvae can enter through 0.345mm and no larvae can enter through 0.173 mm diameter of pinhole (Tsuji 1998). Hatched larvae could reach the pinhole when the eggs are located 6cm or even more in distance from the food container (Tsuji 1998).

Tsuji conducts an experiment, he maintained the distance between the larvae and the food container of 10 cm and 38cm. There was four containers among 2 of them are open and other 2 are closed. The results were 68% of eggs can be developed into larvae and they can reach the food which was in 10 cm distance. This means 100% of hatched larvae could reach their food. Whereas 65% larvae reached the food when the distance was 38cm (Tsuji 2000).

Significance of hermetic seals, controlled ventilation, and wire-mesh screens to prevent the immigration of stored product pests

Pests could enter the storages in two types i.e. 1. infested raw material or 2. Immigration.

In the case of immigration the pest always uses positive chemo-taxis which means the pests moves towards the gradient of attractive volatiles. In a temperate climate such as in Central Europe, storage of harvest agricultural product happens rarely from the field; the main source of infestation is the immigration from the other sources. The store pests occurs in the field can be removed or killed while the post-harvest processing such as drying, threshing or transportation etc. So in tropical or even in subtropical region immigration may be additional sources of infestation. In many countries, the storage houses are made up of a sheet of corrugated metals where the wall and roof are not sealed properly. Old concrete and wooden store house may have a large opening in doors which facilitate infestation. The products such as food need to be pest prevention from the initial phase which is the time of harvest (Council directive 1993/43 EEC and EC regulation 178/2002). That means the HACCP (Hazard Analysis Critical Control Point) is carried out from the first storage sites to avoid any qualitative losses. Process like mechanical drying at high temperature, milling and mixing can kill all the individuals in food industries but the immigration could be a significant sources of infestation in later process for examples *Stegobium* and *Sitophilus* spp may be the attractive towards the fresh dried pasta in pasta factories where as *Tribolium* spp may attractive to raw ingredient semolina.

Physical barriers to prevent from infestation are :

Physical barriers mean any structure made up of metals, plastics, woods or other materials including laving barriers to obstruct or close a passage (Banks 1976).

A)Hermetic seal: Gas-tight seals could enclose the attractive volatiles gas which can prevent the immigration of pests. However, Silos, bins, and warehouse need controlled aeration. The quality of the package which is on the way to consumer determines the level of risk of insects immigration. The risk could be minimized by a hermetically sealed package under a controlled atmosphere.

B)Controlled aeration: Controlled aeration close to the doors and opening could preserve the spray of attractive volatiles in food processing facilities. It is not physical barriers, nevertheless, it could be effective tools to avoid the attracting pests from the environment.

C)Insect proof barriers: Insect proof barriers could be the alternative if controlled aeration and a hermetic seal is not feasible. It has sufficient small opening and consisting of a material to prevent the pests nether the gas-tight. In warehouses and food processing plants, a wire-mesh screen mounted or opening is often used as the barriers to prevents the immigration of pests.

Test of permeability of to wire-mesh screen to stored product insects have been performed by Cornel Adler in 2004. Where adult store product insects were placed in vertical 10-chambered sieves of different pores of 4.5 to 0.05mm. Food culture was placed in the bottom chamber(with smallest pores). The result was the moth sample laid eggs even the food sources was far away. The interesting facts about the female moth are they lay eggs even without food.

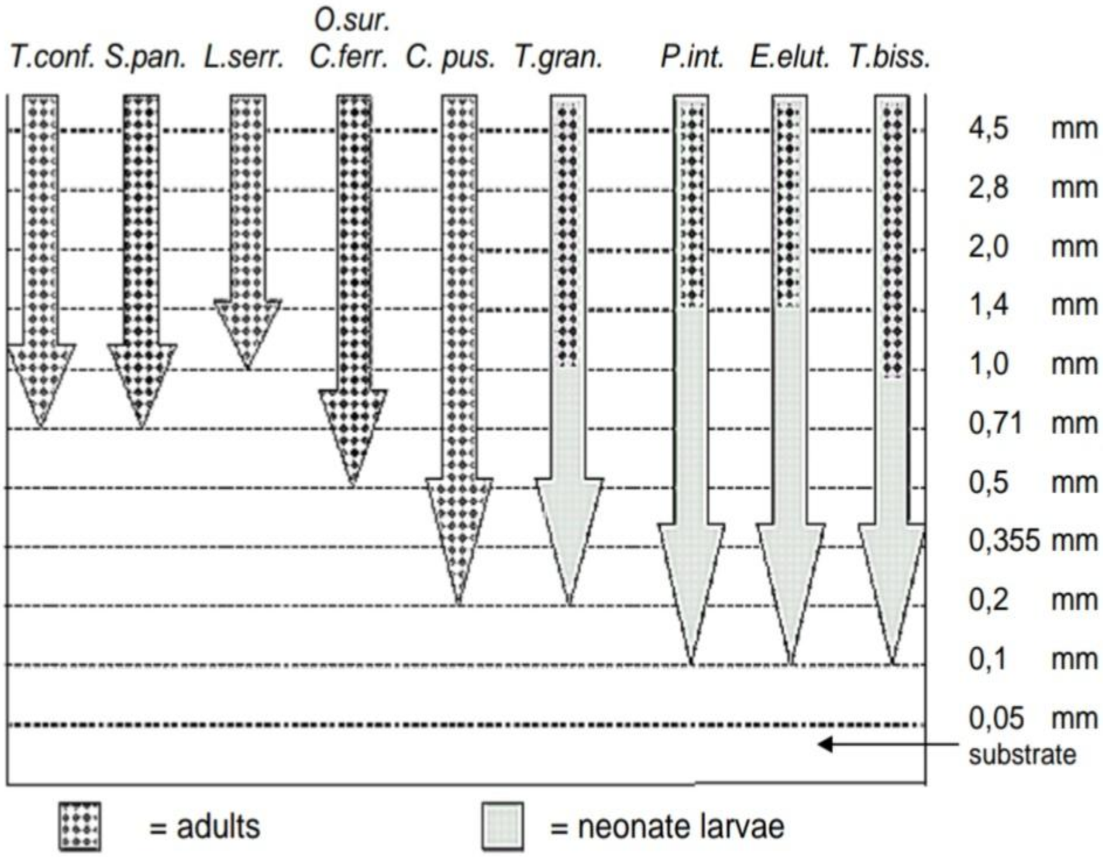


Figure 6: Results on the permeability of wire-mesh screens to various adult stored product insects. Source: Cornel Adler -Federal Biological Research Centre for Agriculture and Forestry, Institute for Stored Product Protection, Berlin, Germany.

In sum up to prevent from infestation insect orientation need to be take more seriously to maintain optimum food quality.

3.9 Testing of food packaging resistance

The selection of Packaging materials determines the package resistance against pests infestation (Highland 1991). Various methods, apparatus, packaging materials and insects have been tested and described in this area. Highland and Wilson (1981) described the apparatus which contain an assembly of five different mechanical parts made up of aluminum tubing to check the penetration of R.Dominica. In 1979 Wohlgemuth mentioned the simple devices in which sample foils can be kept in between two glass rings, and the sample foils separate those two glass rings; Since it doesn't have anchoring point and insect stand free on the foils is considered as the disadvantage of this apparatus. Two methods of penetration test have been studied by Gerhardt and Lindgren in 1954 which was insect penetration against the sample and another one was two small plastic cups consist of screw-types plastic lids. In these methods, food was kept in one cups and insects in another one which was separated by the film to be tested. The various experiment has been conducted to check the resistance of packaging materials against store-pests

SS Batth in 1970 in Canada performed the resistance of aluminum foils which contains dehydrated soups against larvae of *T. inclusum* and *Tenebroides mauritanicus* (L.) and to adults of *O. surinamensis* (L.).

Similarly in 1978; L. Daniel Cline determines the resistivity of cellophane, polyethylene, paper, polyvinyl-chloride, aluminum foil, polyester, or polypropylene against eleven species of stored-product larvae and adults of insects with or without food. He had documented many penetrations occurs within 24 hours and most of occurred in one week. Some larvae didn't penetrate the tested sample, subsequently, they transform into adults and made more penetration.

After the complaints of several consumers in chocolate-based food products experiment was done by Terence Graham Bowditch in the year 1997 against the 1st and 5th instar *E. cautella* Walker and *P. interpunctella* (Hübner) larvae, and adults of *T. confusum*. It was found that Polyvinyl chloride was only resistance of penetration of 1st instar of *E. Cautella* whereas polypropylene was resistance against all species.Different types of a package were tested against the neonate larvae of the Indian meal moth *P. interpunctella* and the rice weevil *S. oryzae* by *Cornel Adel* in the year 2008. It was found that these larvae can enter through the punctures and seams. In Polyethylene, polypropylene, and polyvinyl-chloride. To allow the durable gas exchange at changing temperature and pressure many food companies used to make deliberated puncture; in some cases, some puncture in packages could happen in transportation which increases the chance of pests infestation.

In 2014 Trematerra, P, & Savoldelli, studied the penetration capacity of maize weevil, *S. zeamais* Motschulsky through seventeen different types of commercial pasta packaging in Italy. Folded carton box (tube style) with end flaps tucked and glued; with a clear window of PP film and Plastic pillow pouch with gussets were the packaging materials of tested pasta. It was found that adults of *S. zeamais* are able to respond selectively to the active volatile compound (odor) released from the pasta. Adults were found inside pasta package of barley and buckwheat pasta, durum wheat pasta, egg pasta, spelt and lentil pasta, rice pasta, vitamin enriched pasta, whole wheat pasta, tricolor pasta, spelt pasta. No infestation was found in pasta packages like barley pasta, buckwheat pasta, corn pasta, dietetic pasta, and green pasta. Whereas in folded cartoon boxes, adults gain entry and infested it through the openings not sealed properly by glue.

Muhammad Waqar Hassan (2016) evaluate the stander loose plastic packaging (polypropylene, polyethylene, and polyvinyl chloride) for the management of *R. dominica* (F.) and *T. castaneum* (Herbst). *R.dominica* made more holes in polypropylene then in Polyethylene but the holes, penetrations, and damages done by both species were more in polyethylene. More holes were penetrated by *R. dominica* in less thickness. It is also the time- related process in which *R. dominica* made more holes in lesser time periods whereas another one required more time to make more holes. In sum up, *R.dominica* was more destructive than the *T. castaneum.*

4. Materials and methods

4.1 Experimental Beetles

All beetles used in the study originated from a laboratory culture at the Crop Research Institute, Prague, Czech Republic. Strains of *R. dominica* and *S. oryzae* were reared on wheat, which was dried and subsequently re-moistened to 15% moisture content. Adult beetles were introduced into the rearing medium for three weeks; after this time the beetles were removed and newly emerged adults (emerged approximately two months later) were used for the experiments. The beetles were reared at 25 °C and 60 - 70% relative humidity (r.h.). *T. catsaneum, T. confusum* and *P. ratzeburgii* were reared at 25 °C on a mixture of wheat, oatflakes and yeast. The rearing containers were provided with a piece of moistened filter paper for ensuring a stable relative humidity (60 - 70%). Breeding conditions of the other tenebrionid species were the same, but the diet contained also dried meat in the case of *T. destructor*, fresh apples in the case of *T. molitor* and dried meat and pellets of dry dog food in the case of *A. diaperinus*. The discription of the pest are in 3.6 sections.

Figure 7: Picture of the breeding container with *R. dominica*, which was used in the experiment

4.2Penetration test using a Hou's apparatus:

4.2.1 Packaging films

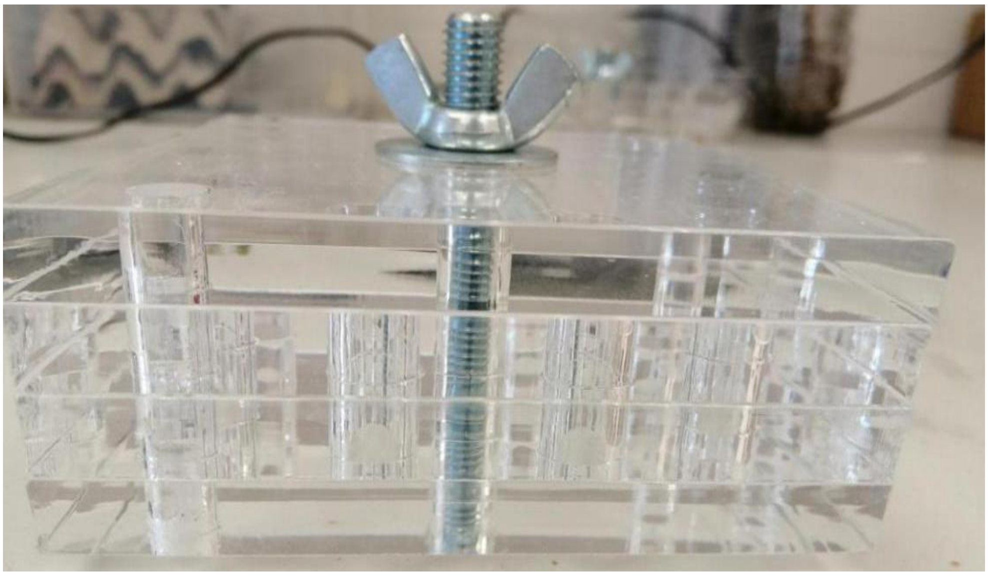
We tested four different types of commercial packaging films (Table 2) in the experiment which are available in European market. The resistance of three films (i.e. PE 50, PP 40 and PET/PE 12/70) to penetration was tested against 6 types of pests (*R. dominica, S. oryzae, T. castaneum, T. confusum, P. ratzeburgii* and larvae of *T. confusum*). To determine the influence of foil's thickness, we tested one of the foil materials (PP) with two different thicknesses (20 and 40 µm, respectively). For this test we used only three pest species i.e R. dominica, S. oryzae, and *T. confusum*. A filter paper was used as a control. All the films were placed on Hou's apparatus (Hou et al., 2004).

Package films material	Thickness (µm)	Description
Polyethylene PE	50	Mono layer
Polypropylene PP	40	Mono layer
Polyethylene terephthalate PET)/Polyethylene (PE)	PET -12 + PE-70	Double layerWhite duplex
Polypropylene PP	20	Unprinted
Filter paper		Control

Table 2: list of packaging films used in the experiment

4.3 Hou's apparatus

The device used for penetration tests was a modified version of that described by Hou et al. (2004). It is a transparent square shaped glass apparatus which is fixed by the metallic nut and bolt in between. The head of the metallic nut is on the bottom and from the upper, it can be fasten. It is designed on the way that even small pests and their larvae cannot escape from the apparatus neither they can shift to another chamber. The two glass slab in the middle has 10 chambers in each slab. The working mechanism is so simple and convenient. The bottom slab (4th slab) is plane surface and it has only one hole to fasten the nut and bolt. The third slab from the top is designed to keep food or attractant. The plastic film is supposed to be placed on the surface of the 3rd slab and to support the movement for insect mesh wire can be installed (Navarro and Navarro, 2018). The 2nd glass slab (from the top) has 10 chambers which are designed for the experimental pests. And the 1st slab encloses the hole.



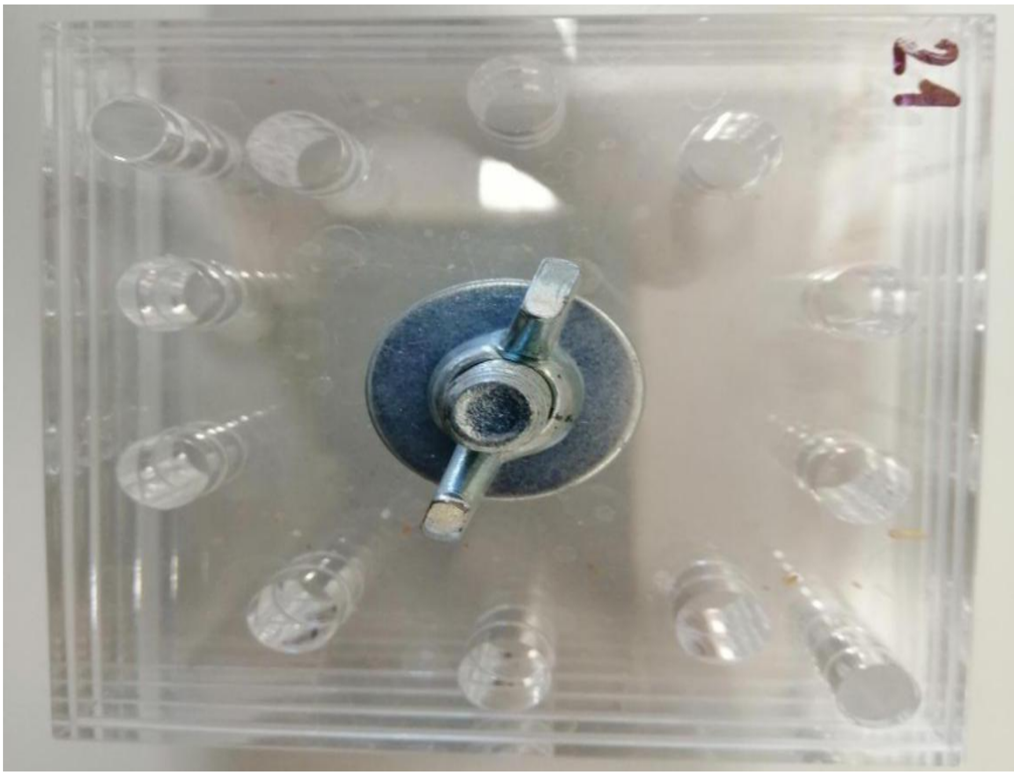


Figure 8 : Hou's apparatus

Three grains of whole wheat per chamber was kept as the attractant for the pests. The experimental films and the mesh wire were kept on the surface of the 3rd glass slab. The back part (i.e. unprinted part) was installed and the pest was kept above it. One individual was placed in each chamber, ten total insects were containing in the apparatus. All apparatus were maintained under laboratory condition in a thermostat at a controlled temperature (25 ± 0.5°C) and Relative humidity 60-70%. Three identical samples i.e. three apparatus for specific films and the insects species were observed. Each chamber was considered as one replicate; thus in total, there were 30 replicates per film/species. Every apparatus was checked after a week and during the inspection, the dead individuals were replaced by new live individuals. Each experimental series was terminated after two weeks and the packaging foils were inspected. During the inspection, the film injuries were checked by a stereoscope microscope. Every data has been observed and recorded carefully.

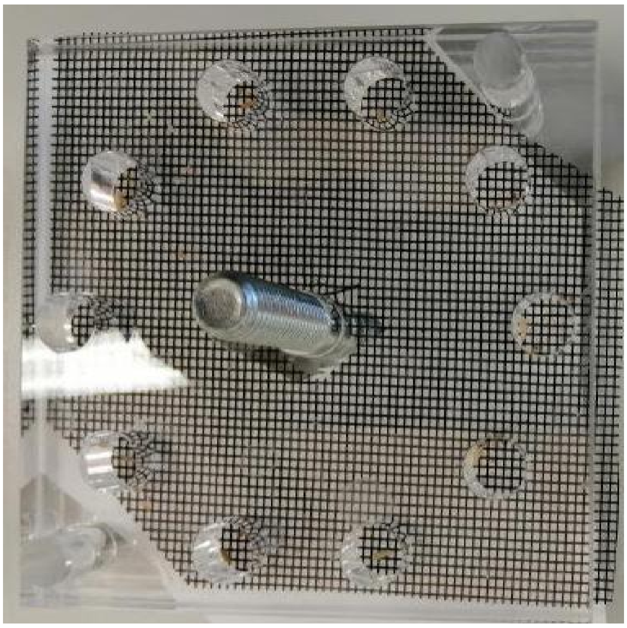



Figure 9: Step-by-step the installation of Hou's apparatus

Regarding to injury we the package were categories into 4 types

1. Type 1 = without injury
2. Type 2 = Injury without holes
3. Type 3 = Small holes without penetration

Type4 = Fully penetrated holes

4.4 Penetration test of whole packages of rice

Infestation tests are designed to stimulate semi- natural conditions, real-world conditions; where pests try to infest stored package under optimum temperature and relative humidity. The aim of this experiment was to elucidate, how the pests deal with a package of rice, i.e. whether they are able to enter into the package of rice (either by already existed ventilation holes or by holes chewed by the insects). The packing film was polyethylene monolayer and its thickness was 50 μm. The experimental design is based on the continual pest exposure to the package of rice in small experimental arena. We performed our experiment in Lock and Lock plastic container of size 180×110×110 mm. Every container containing one 1000 g package of rice was equipped with saturated salt solution (in plastic container and closed with muslin cloth) and filter paper for easier movement of beetles. The experiment has been divided into four sections with 10 replicates. We kept the filter paper in the bottom of the container for their better movement.

1. 100 individuals of *R. dominica* were placed in 10 different containers.

2. 100 individuals of *S. oryzae* were placed in 10 different containers.

3. 50 individuals of *R. dominica* together with 50 individuals of *S. oryzae* were placed in 10 different containers.

4. Mixed species which contains 20 individuals of *T.casteneum*, 20 individuals of *T.confusum*, 10 individuals of *Tenebrie molitor*, 10 individuals of *Alphitobius diaperius*, and 10 individuals of *T. destructors* in five boxes and 10 individuals of *Polorous ratzenburgi* in another five boxes.

The containers were placed in darkness at 25 ± 1°C. *R. dominica* and *S. oryzae* were mixed in one experiment (section No. 3) to observe the ascertain possible synergy effect. The saturated salt solution is placed to regulate the moisture inside the container. The length of the experiment was 14 days, after 14 days the experiment was terminated. The numbers of penetrated holes were observed and counted through the stereoscope microscope. The numbers of individuals which made successful entry inside the package of rice were also counted. The compression/ventilation holes made by manufacturer were measured to ascertain if the pests made any changes. The size of pests was measured to find out if the compression holes made by the manufacturer were enough to gain entry.

4.5. Statistical analysis

The resistance of the tested films was tested by two-way ANOVA followed by Tukey's HSD post-hoc test, where the foil material or thickness, species and their interactions were used as factors. The total numbers of beetles and holes in the invasion test of whole packages of rice were compared by one-way ANOVA. The significance level was set to 0.05.

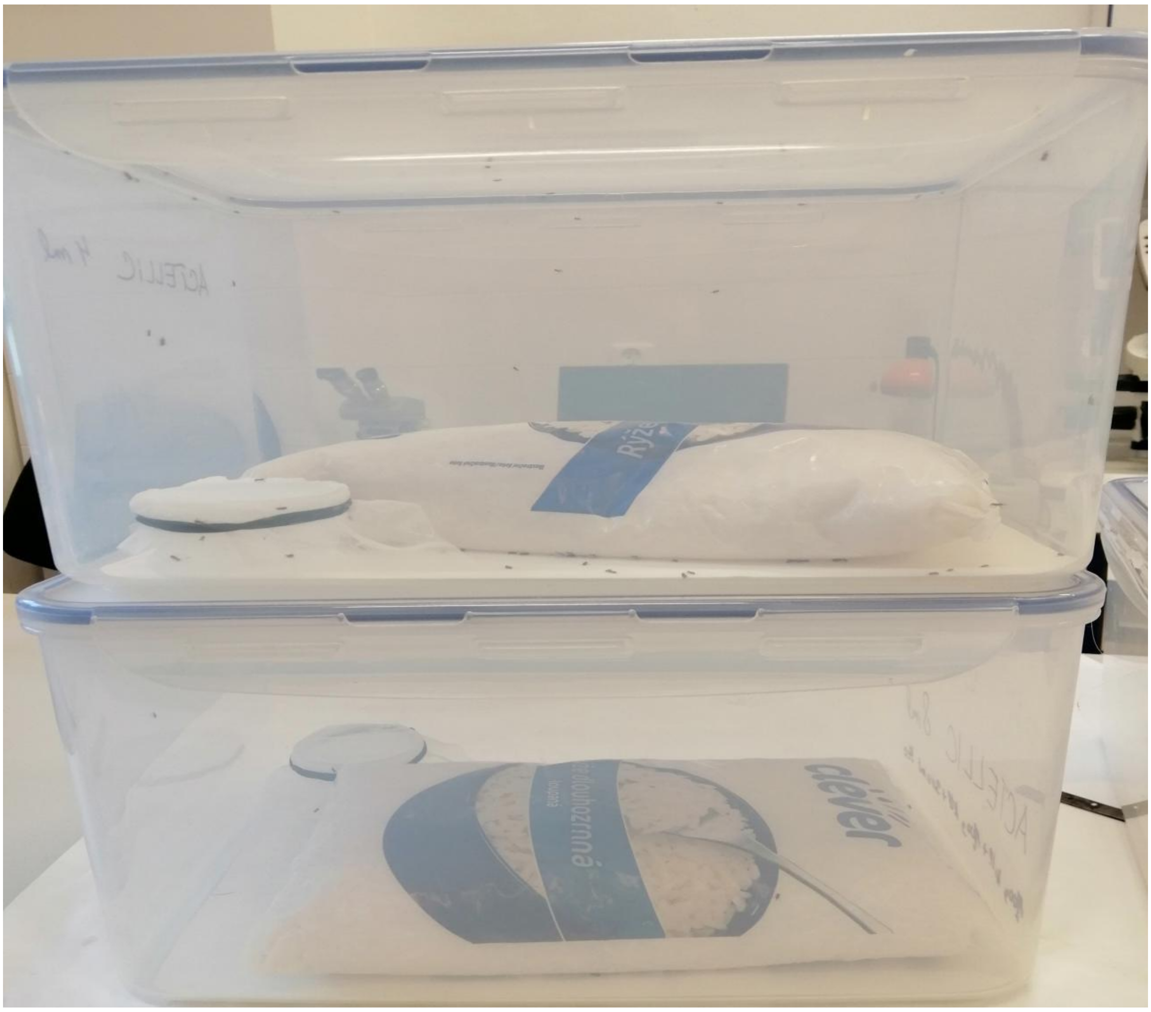


Figure 10: Rice package with beetles inside the lock and lock plastic container

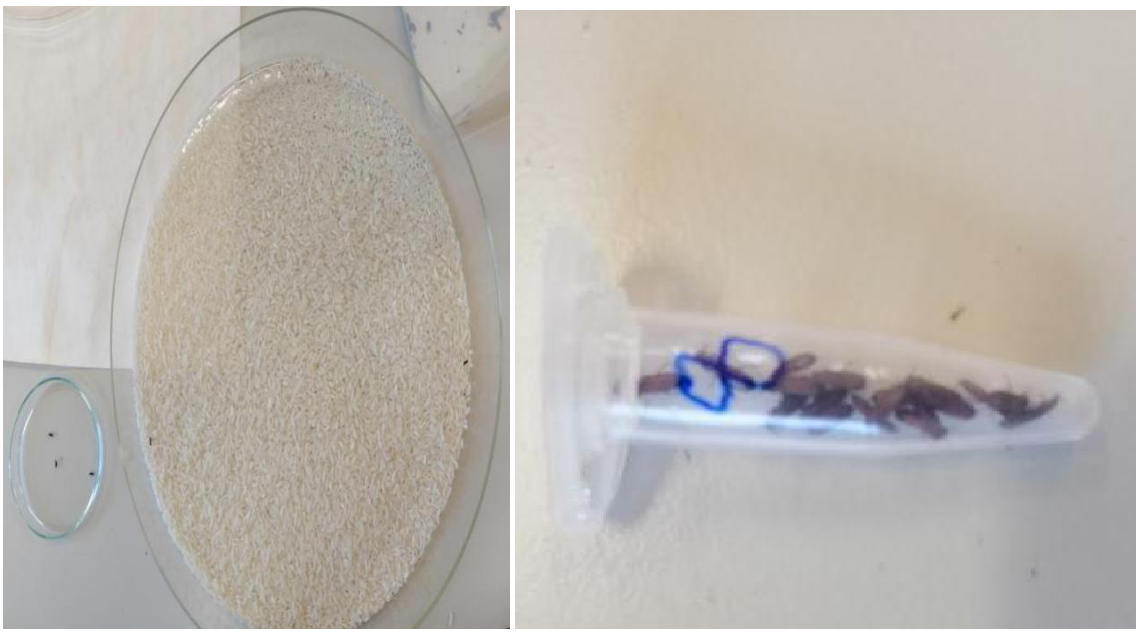


Figure 11: Separation of beetles from the rice

5.Results

5.1 Experiment using Hou's apparatus

5.1.1Comparison of resistance of PP foils with various thickness

Full penetration

There were differences in resistance between the two foils differing in thickness($F_{1,174} = 13.54$, $p < 0.001$) , when the PP foil of thickness 20 µm was (regardless on the species) more susceptible to penetration than the foil of a thickness 40 µm (Fig. 12). Similarly, there was difference between species in its penetrating ability ($F_{2,174} = 26.11$, $p < 0.001$). Interaction of the two variables was also significant ($F_{2,174} = 15.92$, $p < 0.001$), indicating that the foil penetration pattern differed significantly between the two foil thickness, depending on the species. The Tukey's post hoc test revealed that *S. oryzae* penetrated significantly more holes on the foil of a thickness of a 20 µm than of 40 µm. In contrast, in the case of *R. dominica* there was surprisingly no difference between the two foils (Fig. 12). *T. confusum* adults were not able to penetrate any of the tested foils.

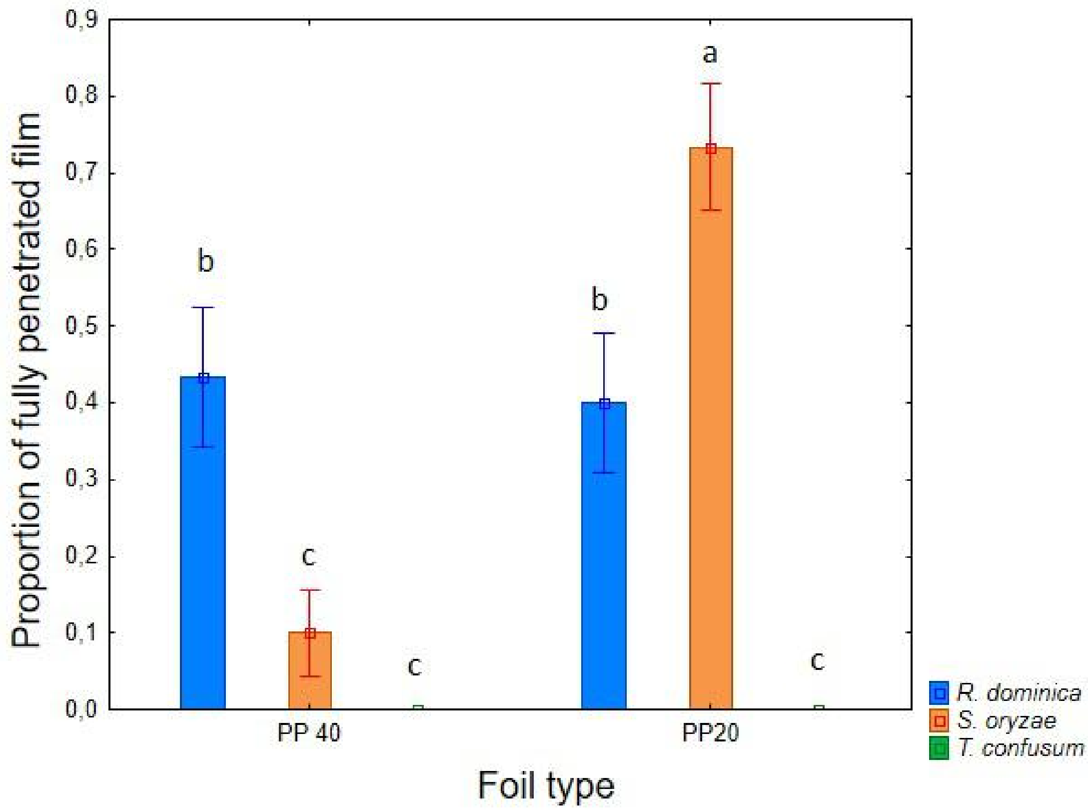


Figure 12 Comparison of proportion of type 4 injuries (i.e. full penetration) in polypropylene foil of a thickness 20 µm and 40 µm. Bars with the same letters are not significantly different ($p \geq 0.5$)

Total number of all injuries

There were no differences in the number of all injuries between the two foils ($F_{1,174} = 0.04$, $p = 0.84$). In contrast, the species differed in number of injuries ($F_{2,174} = 75.96$, $p < 0.001$). Interaction of the two variables was not significant ($F_{2,174} = 2.49$, $p = 0.08$). The difference in the numbers of all injuries was made mainly by *T. confusum* regardless of the thickness of foils. As the graph (Fig. 13) shows, *T. confusum* made significantly less number of all injuries then other two species. The Tukey's post hoc test revealed that S. oryzae caused statistically insignificantly more injuries on foil of a thickness of a 20 µm than 40 µm (Table 2). Surprisingly, in the case of *R. dominica*, it caused slightly more injuries in 40 µm than in 20 µm, but this difference was again non significant *T. confusum* adults were able to make very few injury in both types of foils and the numbers of all injury were nearly similar in both of the tested foils. The main difference from the results on fully penetrated films is in the increased proportion of injuries of PP 40 film by *S. oryzae*, indicating that majority of individuals not penetrating the film were at least able to damage it.5.1.3 Comparison of resistance of several types of foil packaging against stored product beetles.

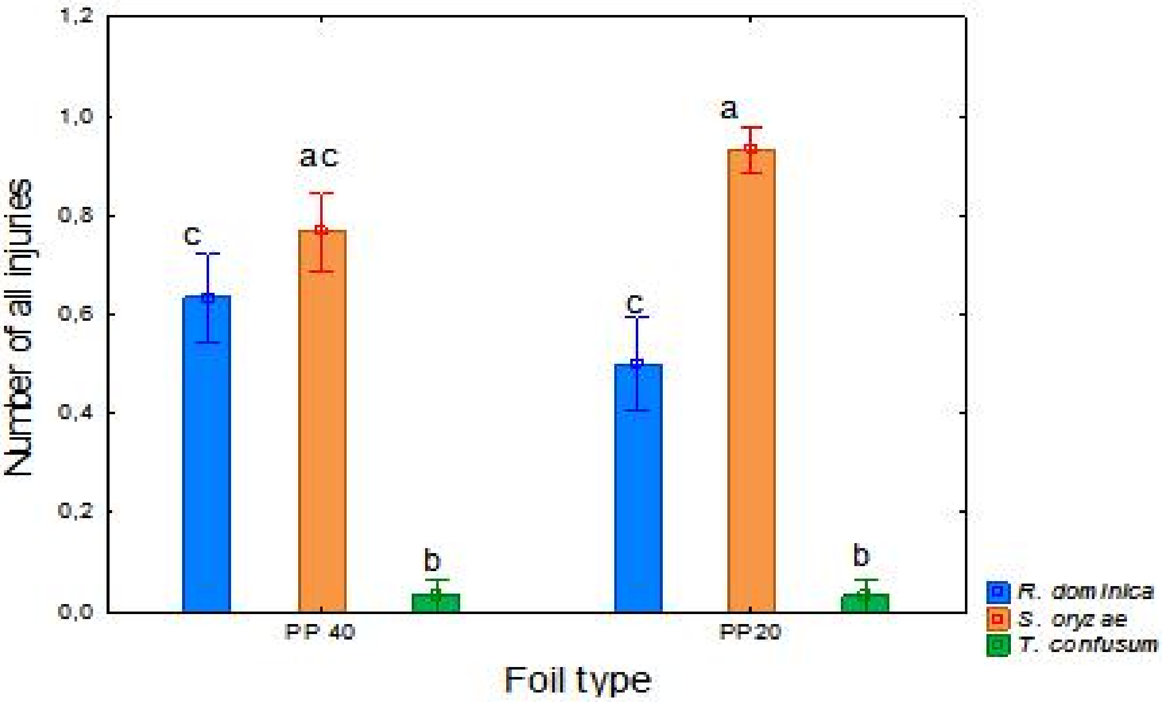


Figure 13: Comparison of all types of injury in PP40 and PP 20

5.1.2 Comparison of resistance of films from various material

Full penetration

The tested foils differed significantly in resistance against pests ($F_{3,696} = 25.41$, $p < 0.001$). There were differences between species ($F_{5,696} = 43.96$, $p < 0.001$) and in interaction of foil type and species ($F_{15,696} = 18.55$, $p < 0.001$). According to Tukey's post hoc test, *R.dominica* caused significantly more fully penetrated holes (type 4 injury) in PP20 and PET50 than in other tested films, while *S. oryzae* was highly capable to penetrate only PP20 film (Fig. 14). PET/PT is highly resistant to all species used in the experiment. Surprisingly, the filter paper (used as a control) was relatively little susceptible to penetration. Other species than *R. dominica* and *S. oryzae* were barely able to penetrate the tested films (Fig. 14). Summary of species' penetrating abilities on different films is provided in Table 3.

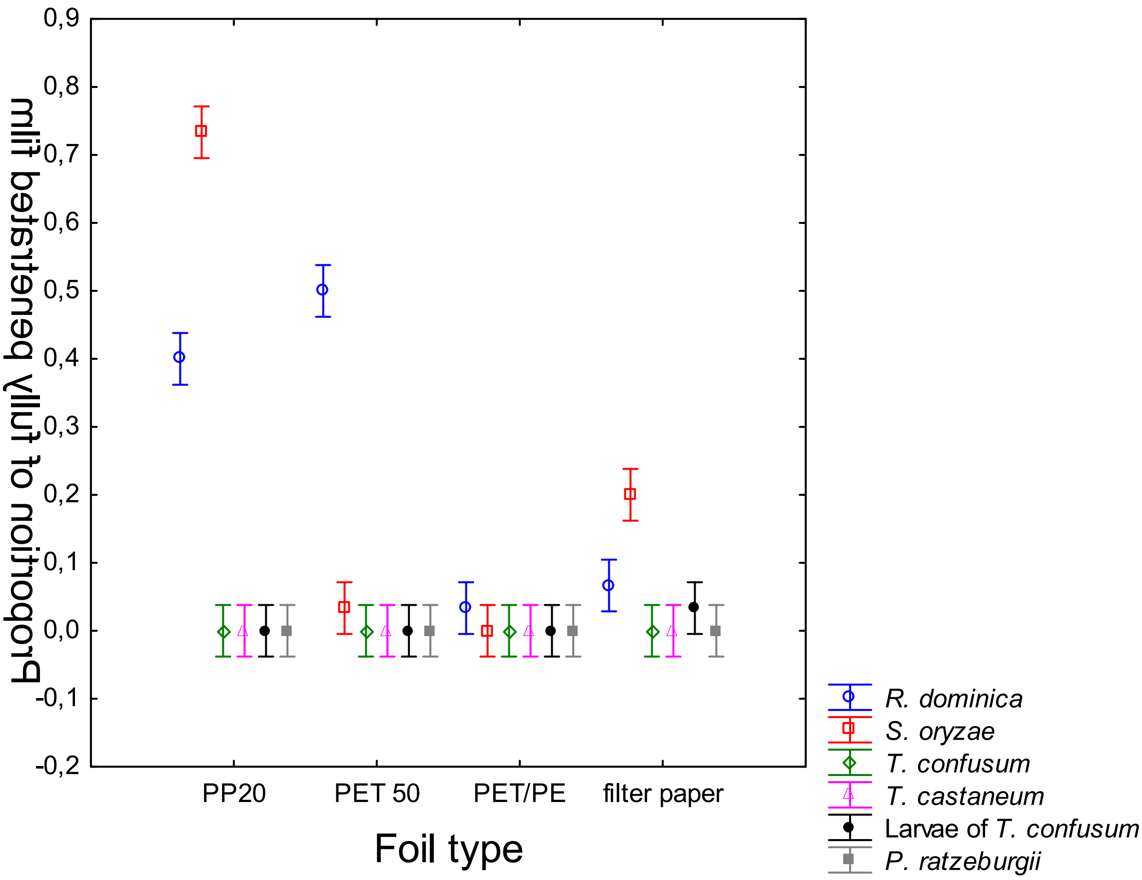


Figure 14: fully penetration films

Table 3:Mean numbers ± SE of fully penetrated foils for each foil type and species. Numbers with the same letters are not significantly different (p≥0.5).

species	foil	Number of type 4 injury %
T.castaneum	PP 20	0 ± 0^{d}
	PET 50	0 ± 0^{d}
	PET/PE	0 ± 0^{d}
	filter paper	0 ± 0^{d}
P. ratzerburgii	PP 20	0 ± 0^{d}
	PET 50	0 ± 0^{d}
	PET/PE	0 ± 0^{d}

	filter paper	0± 0^{d}
L. of T.confusum	PP 20	0± 0^{d}
	PET 50	0± 0^{d}
	PET/PE	0± 0^{d}
	filter paper	3.3 ± 3.3^{cd}
T. confusum	PP 20	0± 0^{d}
	PET 50	0± 0^{d}
	PET/PE	0± 0^{d}
	filter paper	0± 0^{d}
Sitophilus	PP 20	73.33 ± 8.2^{a}
	PET 50	3.3 ± 3.3^{cd}
	PET/PE	0 ± 0^{d}
	filter paper	20 ± 7.5^{c}
R. dominica	PP 20	40 ± 9^{b}
	PET 50	50 ± 9.3^{b}
	PET/PE	3.3 ± 3.3^{cd}
	filter paper	6.67 ± 4.6^{cd}

Total number of all injuries

There were differences in injury resistance between the foils ($F_{3,696} = 3.09$, $p = 0.026$) and also there was difference between species in their penetrating ability ($F_{5,696} = 323.3$, $p < 0.001$). All the mean number ± SD of total numbers of injuries made by specific stored-pests are given below in table 4.

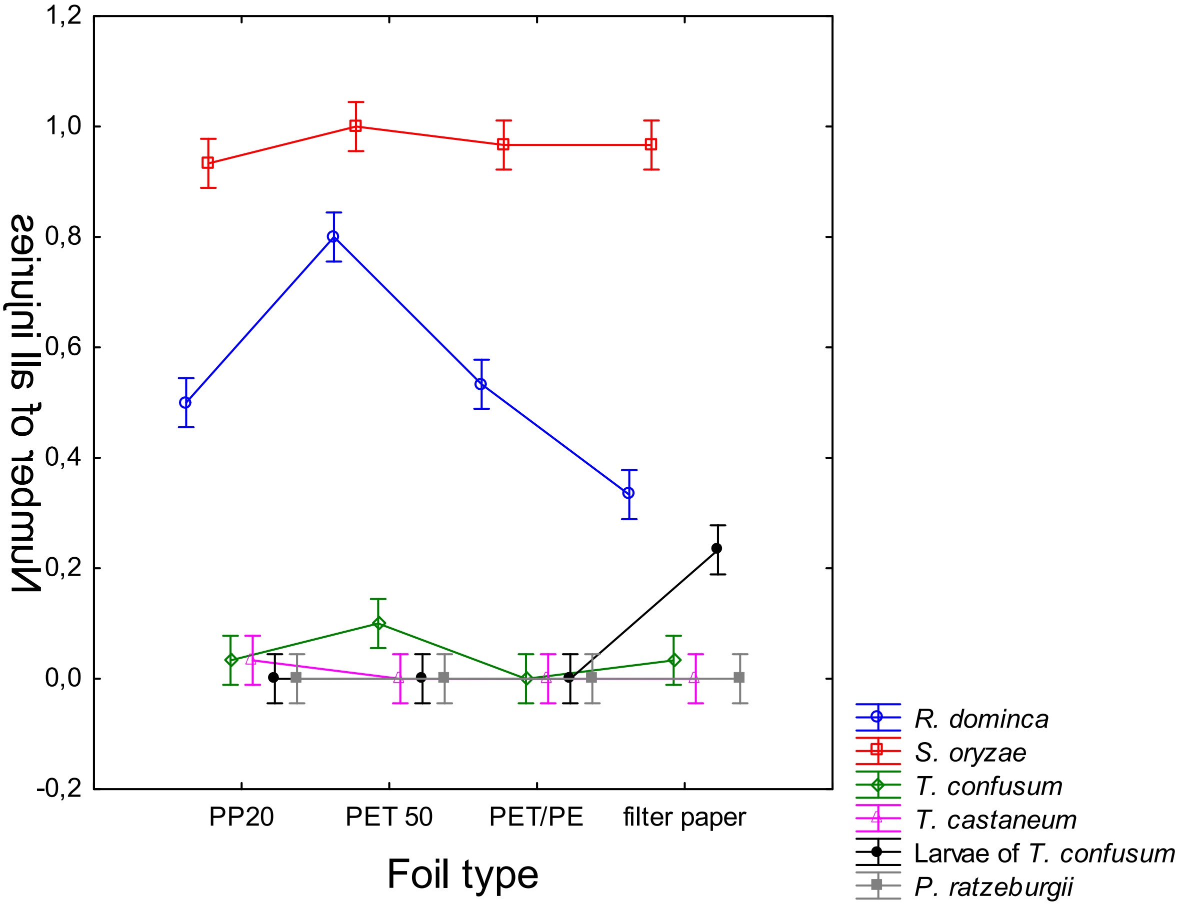


Figure 15: Comparison between foil type and numbers of all injury

Table 4 :Mean numbers ± SD of all injuries for each foil type and species. Numbers with the same letters are not significantly different (p≥0.5).

species	foil	total number of injury (in %)
T.castaneum	PP 20	3.33 ± 3.3^{de}
	PET 50	0 ± 0^{e}
	PET/PE	0 ± 0^{e}
	filter paper	0 ± 0^{e}
P. ratzerburgii	PP 20	0 ± 0^{e}
	PET 50	0 ± 0^{e}

	PET/PE	0 ± 0^{e}
	filter paper	0 ± 0^{e}
L. of T.confusum	PP 20	0 ± 0^{e}
	PET 50	0 ± 0^{e}
	PET/PE	0 ± 0^{e}
	filter paper	23.33 ± 7.9^{cd}
T. confusum	PP 20	3.33 ± 3.3^{de}
	PET 50	10 ± 5.6^{de}
	PET/PE	0 ± 0^{e}
	filter paper	3.33 ± 3.3^{de}
Sitophilus	PP 20	93.33 ± 4.5^{a}
	PET 50	100 ± 0^{a}
	PET/PE	96.66 ± 3.3^{a}
	filter paper	96.66 ± 3.3^{a}
R. dominica	PP 20	50 ± 9.3^{b}
	PET 50	80 ± 7.5^{a}
	PET/PE	53.33 ± 9.3^{b}
	filter paper	33.33 ± 8.8^{bc}

Some images of types of injuries causes by these store pests :

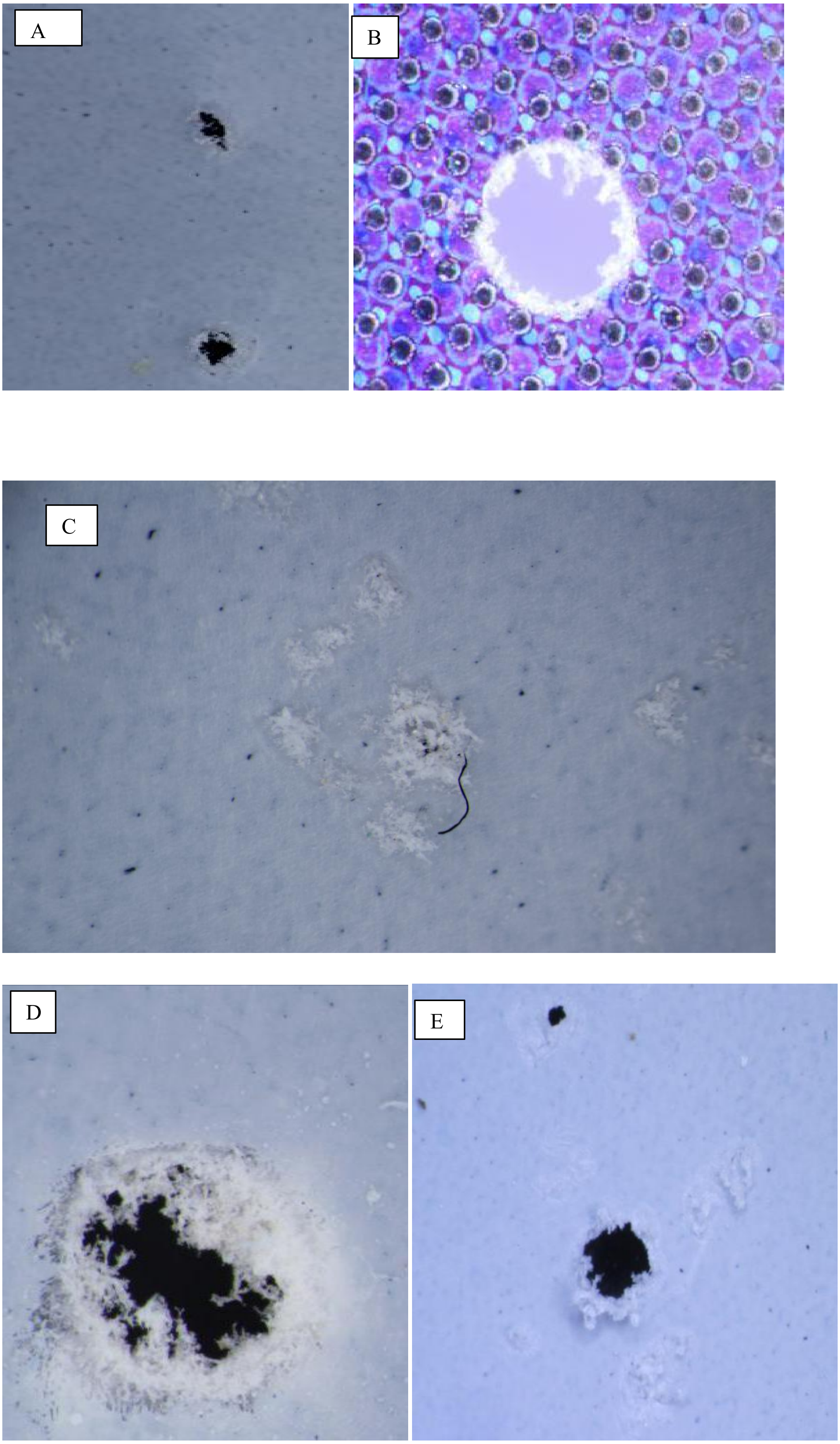
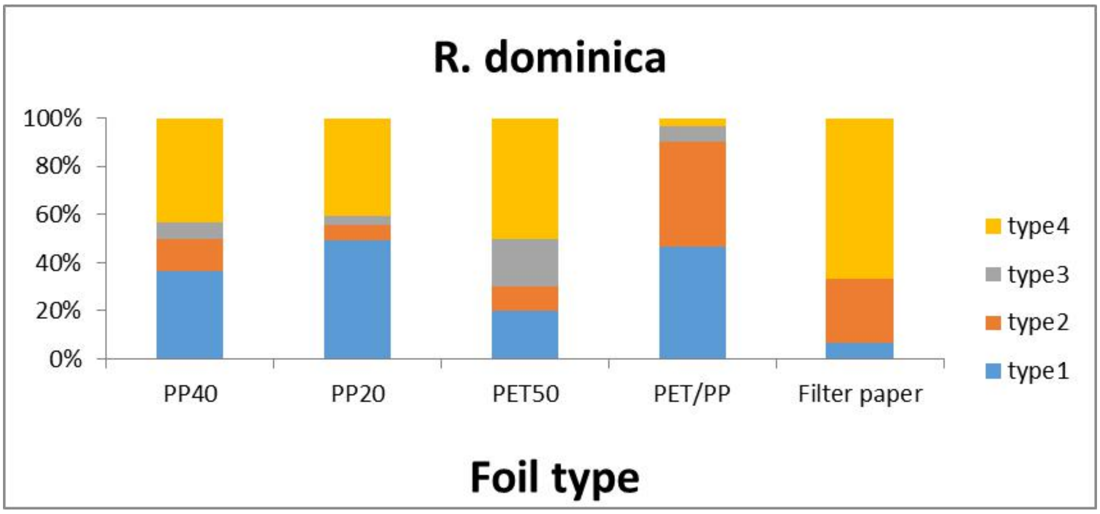
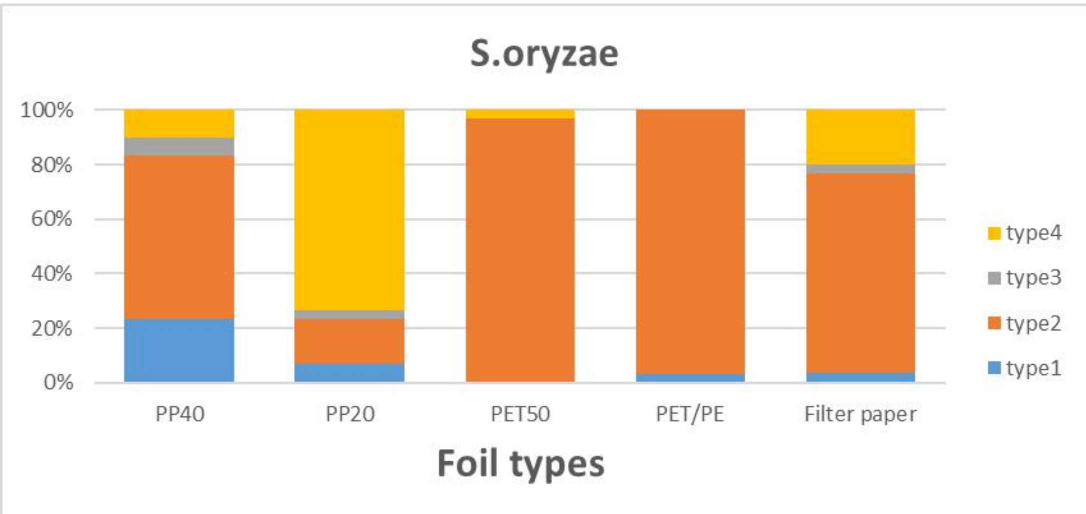
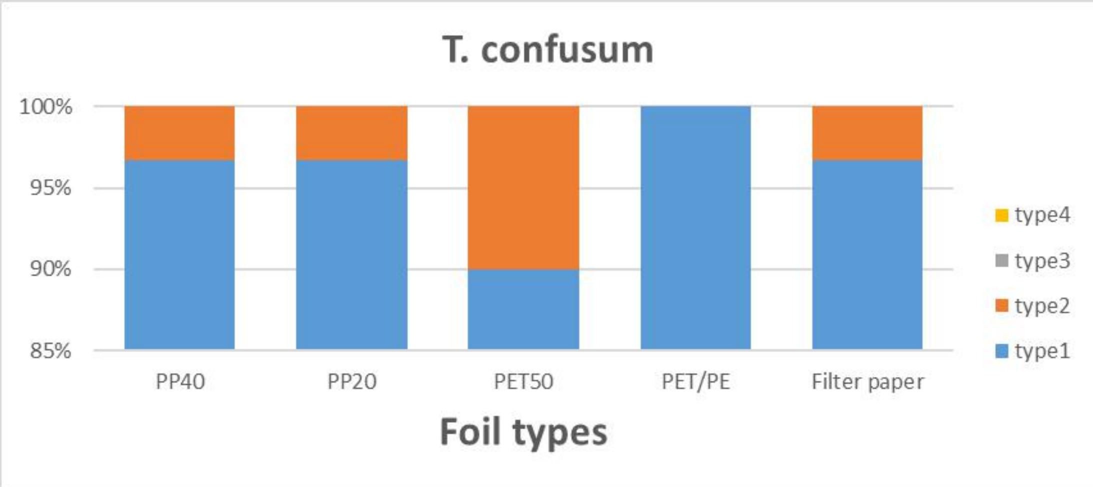

A
B
C
D
E

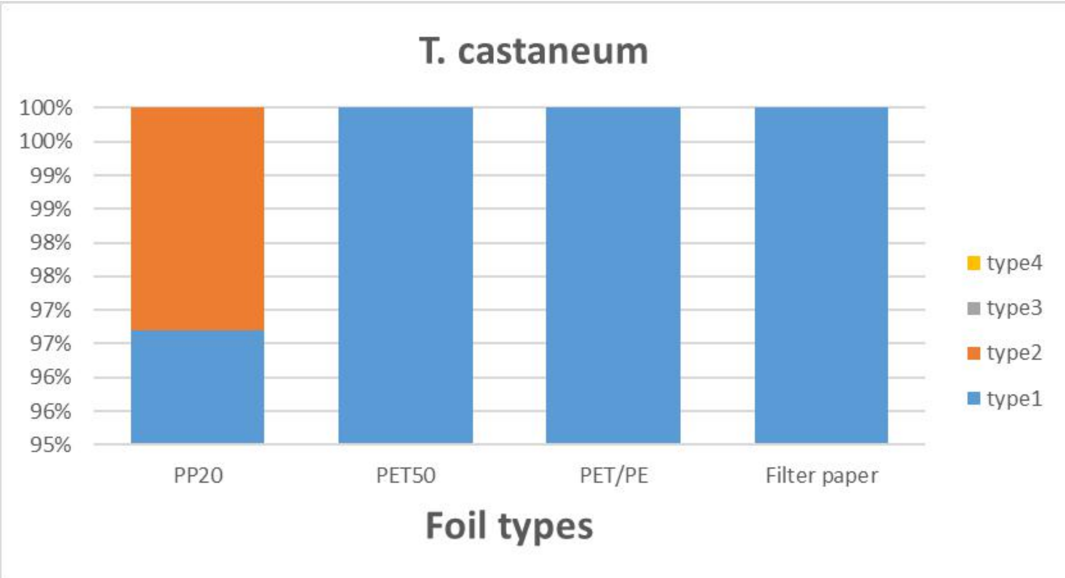
Figure 16 : Film damage from the penetration and invasion tests by stored-product insects; A) small hole without penetration -type 3 injury made by *R. dominica* in PP B)fully penetrated film - type 4 injury damage caused by *R. dominica* in PET having length and width of (L1= 0.93mm and L2=1.03mm) C) Surface injury - type 2 damage caused by *S. oryzae* in PET 50 D) fully penetrated film - type 4 injury damage caused by *S. oryzae* in PET 50 having length of (L1= 1.28mm and L2= 0.91mm) E) fully penetrated film - type 4 injury damage caused by *R. dominica* in PP.

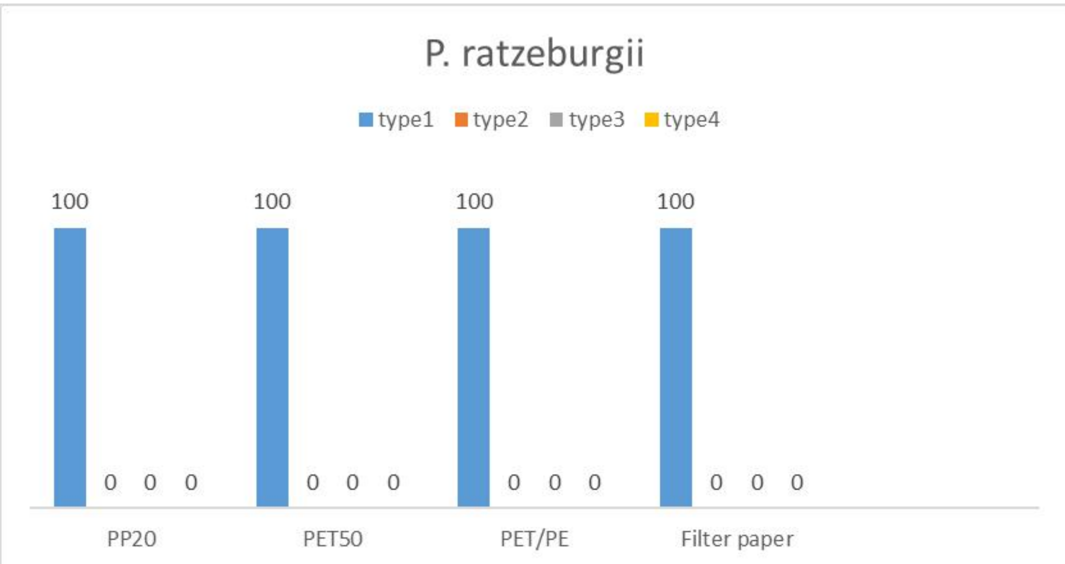
5.1.3 Differential resistance of various packaging materials to the experimental species of stored-product insect

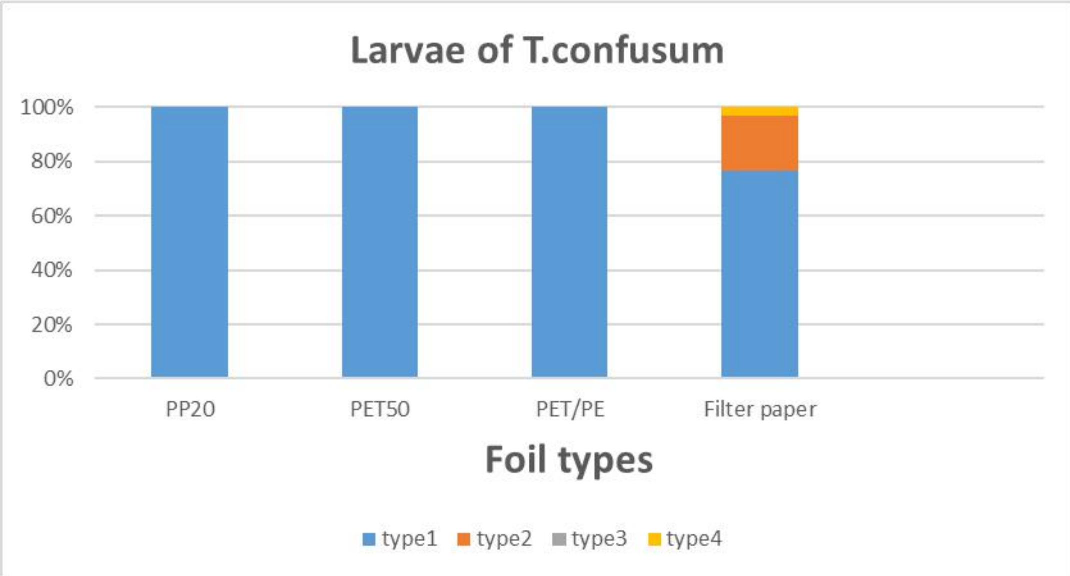





T. confusum
100%
95%
90%
85%
PP40
PP20
PET50
PET/PE
Filter paper
Foil types
type4
type3
type2
type1


T. castaneum
100%
100%
99%
99%
98%
98%
97%
97%
96%
96%
95%
PP20
PET50
PET/PE
Filter paper
Foil types
type4
type3
type2
type1


P. ratzeburgii
type1
type2
type3
type4
100
0 0 0
100
0 0 0
100
0 0 0
100
0 0 0
PP20
PET50
PET/PE
Filter paper



5.2 Invasion test of whole packages of rice:

5.2.1 Comparison of numbers of penetrated holes

R. dominica penetrated on average more holes per rice package (6.3 ±2.44) than *S. oryzae* (only 1.84 ± 0.58 holes per rice package) and when the two species were present together (1.91 ± 0.60), but the difference was on the border of significance ($F = 3.0$, $p = 0.064$). The results suggest that there was no synergy effect between the two species (Fig. 17).

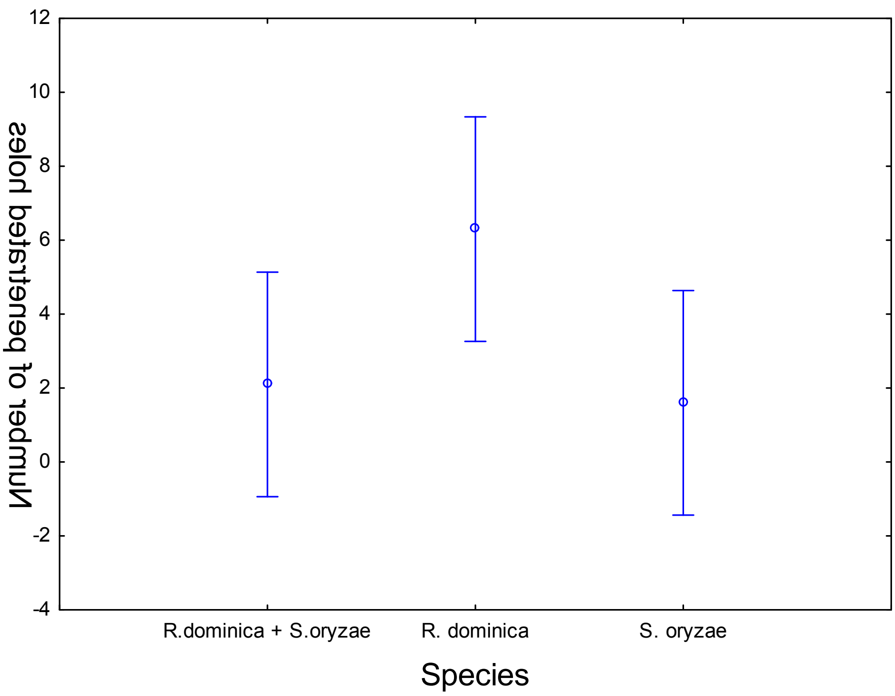


Figure 17: Numbers of penetrated holes in the packages of rice by the stored-pest species

5.2.2 Comparison of numbers of individuals inside the package of rice

Significantly less (t2 = 16.02, p = 0.004) individuals of *R. dominica* (15.9 ± 5.49) gained entry inside rice package than *S. oryzae* (31.9 ± 7.19) when we kept the two species together for possible synergy effects (we kept 50 individuals of both species in the same container). On the other hand in total the number of individuals of both species did not differ from individuals of *R. dominica* (45.4 ± 11.75) nor *S. oryzae* (45.3± 12.39) inside the packages when the species were kept separately (one way ANOVA: $F_{2,27}=0.014$ and $P=0.99$; Fig. 18).

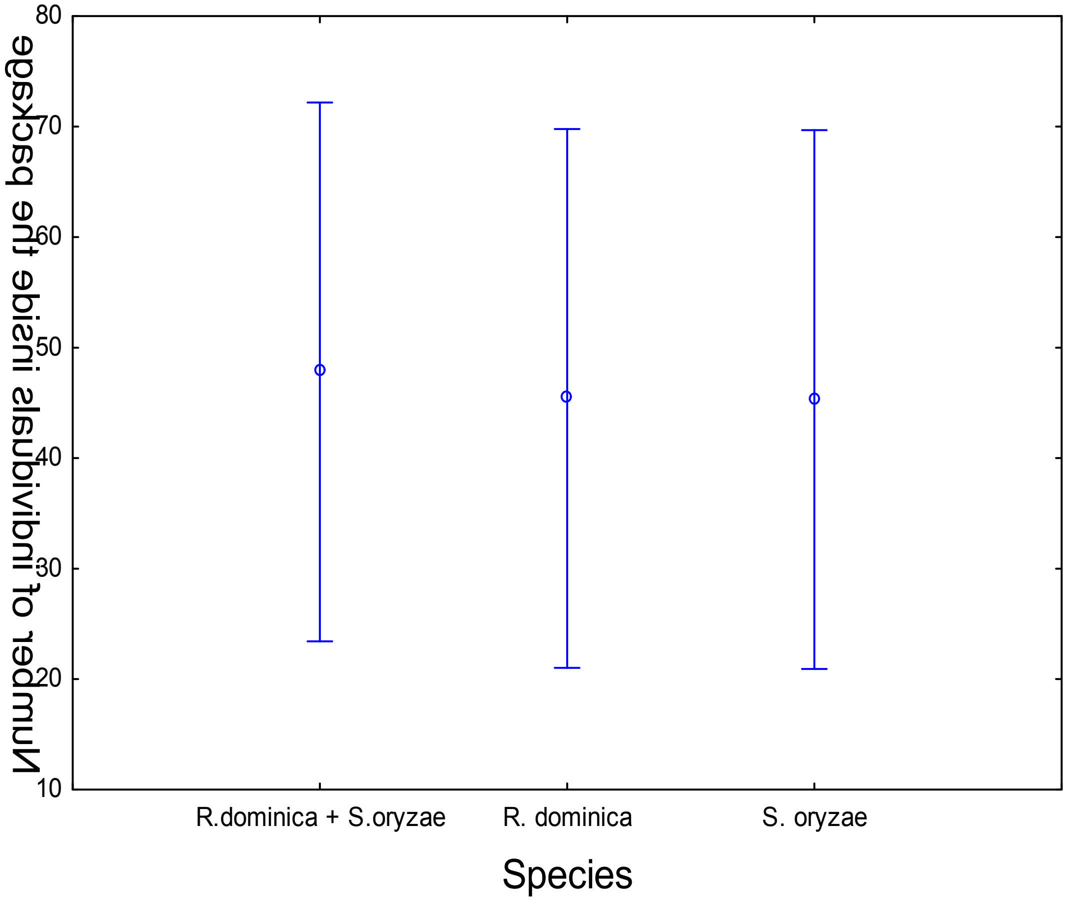


Figure 18 : Numbers of individual pests inside the package of rice

5.2.3 Comparison of numbers of individuals per hole

Significantly more (one-way ANOVA: F2,19 = 4.13, p = 0.03) S. oryzae (42.90 ± 13.3) individuals per hole than *R. dominica* (12.13 ± 2.74) individuals gained entry inside package of rice., which indicates that *S. oryzae* highly used ventilation holes or already penetrated holes.

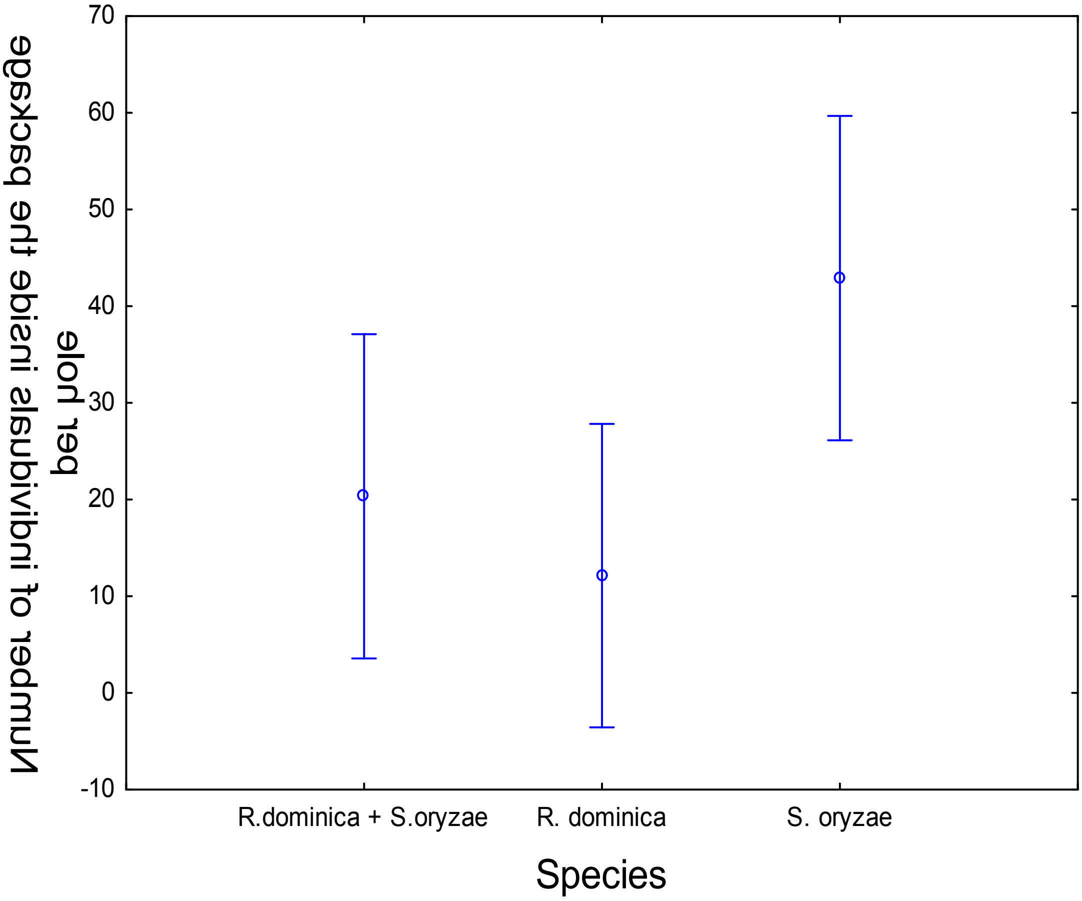


Figure 19: Numbers of individuals store-pests per holes inside the package of rice

5.2.4 Percentage of *R. dominica* inside the package

For enabling to compare numbers of individuals of a particular species when present in the arena alone or together with the other species, we used relative amounts (in percents) rather than absolute numbers (because there were 100 individuals of the species when was present alone, but 50 individuals when present with the other species). On average 45.4% of *R. dominica* was present when it was alone in a container while 31.8% was inside while it was with *S. oryzae* (Fig. 20). Nevertheless, the difference was not significant ($t_{18} = -0.85$, $p = 0.41$)..

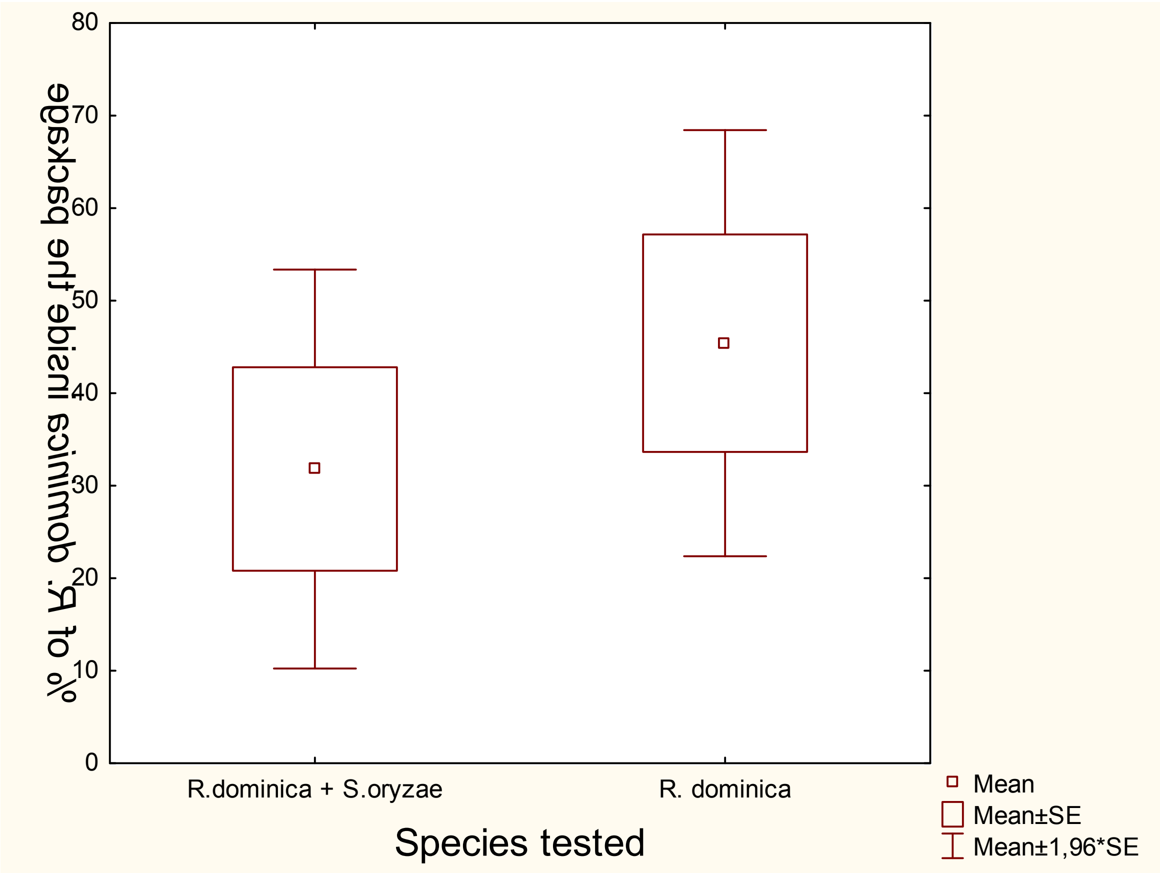


Figure 20: percentage of *R. dominica* inside the package of rice.

5.2.5 Percentage of S. oryzae inside the package

The opposite trend (Fig. 21) was observed in *S. oryzae* where 45.3 % individuals were present in the package when it was alone in a container while 63.8% were inside while it was together with *R. dominica*. Nevertheless, again the difference was not significant ($t = 0.97$, $p = 0.34$).

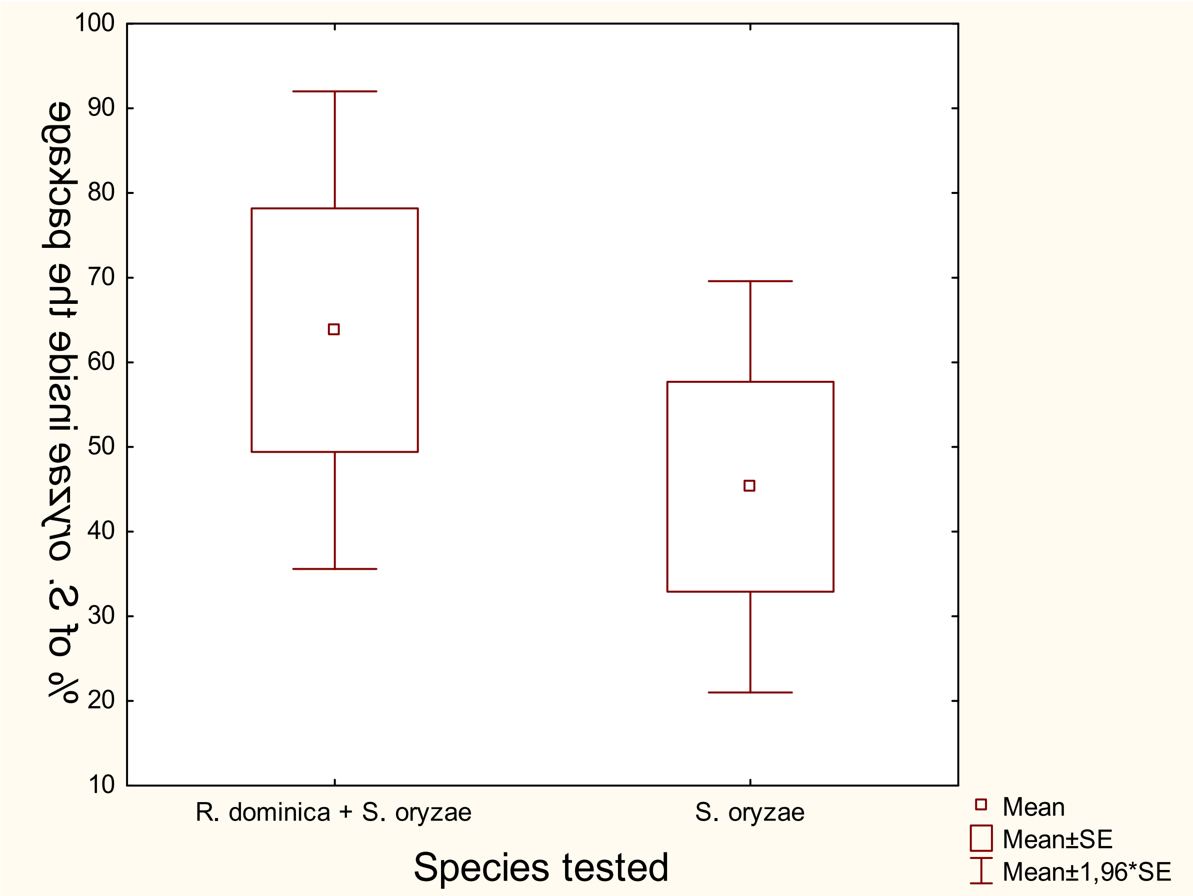


Figure 21: percentage of *S. oryzae* inside the package of rice

5.2. 6 Measurement the size of a ventilation holes of the package of rice

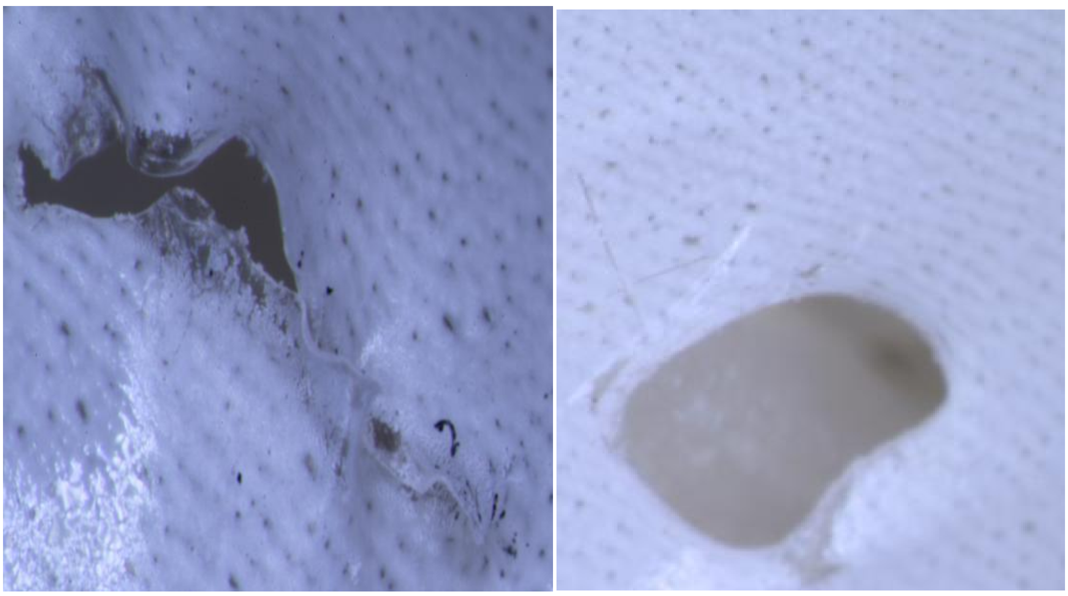


Figure 22 : images of ventilation holes in the package of rice

We took 10 packages of rice in this experiment and measured the ventilation holes (one package had 2 ventilation holes in either side) to ascertain whether they can potentially be used by the beetles to infestation of the commodity.

Table 5: List of size (length = L1; width= L2) of ventilation holes in package of rice made by manufactures

Sample umber	Hole 1 (L1) nm	Hole 1 (L2) nm	Hole 2 (L1) nm	Hole 2 (L2) nm
1	1.21	0.95	2.69	1.08
2	2.99	2.47	2.71	0.75
3	3.34	1.50	4.00	1.47
4	3.8	1.03	2.75	1.11
5	1.92	0.53	1.65	2.22
6	3.70	1.68	2.71	0.54
7	1.89	0.94	2.84	0.65
8	5.50	1.78	3.02	0.51
9	3.18	0.7	2.72	0.42
10	2.81	1.60	2.88	0.60
Mean ± SE	3.03 ± 0.38	1.31 ± 0.19	2.79 ± 0.17	0.94 ± 0.17

In both experiment, it was necessary to find out the size of store pests that we used in our experiment. It can highly influence-able to gain the entry inside the package of rice from ventilation holes. In both cases the beetles can use the ventilation holes to gain entry inside the package of rice, But only few used it.

We measured the size of four important pests which are given below (Table 6). We measured it under a stereoscope microscope with nikon camera. All the below listed size are measured in such a way that size would be enough to gain entry inside the package of rice. Similarly, in the experiment of Hou's apparatus, if the pests were capable to make the hole in package films as much as below -listed they could easily penetrate the films.

R.dominica		S.oryzea		T.confusum	
L1 mm	L2mm	L1mm	L2mm	L1mm	L2mm
0.58	0.52	0.58	0.51	0.63	0.87
0.76	0.89	0.72	0.73	0.75	0.74
0.44	0.36	0.90	0.86	0.82	0.79
0.81	0.70	1.02	0.69	0.91	0.62
0.74	0.69	0.62	0.60	0.91	0.70
0.79	0.59	0.81	1.11	0.62	0.78
0.84	0.56	0.87	0.83	0.91	0.61
0.86	0.47	0.90	0.88	0.74	0.59
0.79	0.65	0.87	0.92	0.77	0.74
0.72	0.77	0.72	0.74	0.87	0.72
0.73± 0.04	0.62± 0.04	0.80± 0.04	0.78 ± 0.05	0.79± 0.03	0.72± 0.03

Table 6: List of size of stored-pests(L1= width; L2= height)

5.2.7. Invasion test using mixed species

The second sample we kept was mixed species which contained 20 individuals of *T.casteneum*, 20 individuals of *T.confusum*, 10 individuals of *Tenebrio molitor*, 10 individuals of *Alphitobius diaperius*, and 10 individuals of Tribolium destructor in five boxes and 10 individuals of *Palorus ratzenburgii* in another five boxes. There was 10 sample (10 boxes of rice and the mixed species of beetles inside the container), but only in three samples, some beetles gained entry. There were no chewing holes which makes clear that these species used the veneration holes to gain entry inside the package of rice. T. molitor (biggest sized beetles in this experiment), *P. ratzenburgi* and *A. diaperinus* were not capable to gain inside the package of rice.

Experimental Chamber no.	*T. confusum*	*T. Castaneum*	*A. diaperinus*	*T.destructor*	*P.ratzenburgi*	*T.molitor*
1	0	0	0	0	0	0
2	4	2	0	1	0	0
3	0	0	0	0	0	0
4	3	3	0	3	0	0
5	0	0	0	0	0	0
6	0	0	0	0	0	0
7	0	0	0	0	0	0
8	16	2	0	0	0	0
9	0	0	0	0	0	0
10	0	0	0	0	0	0
Mean± SE	2.3 ± 1.6	0.7 ± 4	0 ± 0	0.4 ± 3.1	0	0

Table 7 : Beetles gains inside the rice package

6. Discussion

Highland(1984, 1991) classified the store-pests according to the penetration capacity as penetrator and invaders. Stejskal et al (2017) found the area of surface injuries was 10 to 20 times much bigger then the area of fully penetration holes, but this case it was not different significantly. Comparison of fully penetration holes between the PP 20 and PP 40 *T.confusum* cannot penetrate in both cases, while *S. oryzae* penetrate significantly more holes in PP 20. While there were no difference between all number of injuries in PP20 and PP40. In this case, *T.confusum* made some numbers of injuries. As (Mullen and Highland 1988) had classified T.confusum as an invader group. Our experiment fully convinced with their statement. Our results support the observation of Morgan, et al., (2003), *R.dominica* can penetrate more holes in the wild spectrum of films then *S. oryzae.* In real world situation, *R. dominica* have limited ability of infest package then the *S. oryzae* because it has a poor climbing capacity (Cline & Highland, 1976). Our experiment was conducted under the laboratory condition where as in stores, the most important factors are the temperature, which can change the resistivity of the packaging films and the behaviour of the insects (Trematerra & Fleurat-Lessard, 2015). Athanassiou et al., 2011; Chung et al.,(2011) documented films thickness is important factor which can prevent the pest- infection. But in our case, surprisingly the numbers of made by *R. dominica* was slightly more in PP 40 than in PP20 so we do not fully agree to Athanassiou et al., 2011; Chung et al.,(2011).

We found *R. dominica* could make fully penetrated holes in all samples while *S. oryzae* couldn't fully penetrate in PET/PE and comparatively few fully penetrated holes in PET 50, so our experiment supports J. Riudavets et al. (2007). *R.dominica* caused significantly more fully penetrated holes (type 4 injury) in PP20 and PET50 than in other tested films, while *S. oryzae* was highly capable to penetrate only PP20 film. In context of fully penetration by the various species of pests PET/PE showed highly resistivity in our finding. Similarly, *T. catsaneum, T. confusum, P. ratzeburgii* and larvae of *T. confusum* are classified as invaders groups of pests, in our observation also they could not make any type 4 injuries in tested foils except larvae of *T. confusum* made in filter paper (used as a control). We measured some fully penetrated holes (type 4 injury) caused by *R. dominica* in PET having length and width of (L1= 0.93mm and L2=1.03mm) and *S. oryzae* in PET 50 having length of (L1= 1.28mm and L2= 0.91mm)

Stejskal et al. (2017) reported that the porous filter paper (No.11), used as the control was fully penetrated by the S. granarius (L.) and *R. dominica* and 70 % penetrated by *P. interpunctella* larvae, which was kept for 3 month, but in our case it was so resistant against the experimental beetles because the time length of our experiment was 14 days so the beetles had less time to penetrate it. We observed that the thickness of packaging films do not determine the resistivity against the store-pests as G. Muratore et al. (2008) documented similar studies. Stejskal et al. (2017) documented the adults of *R. dominica* were not able to penetrate the printed area but could penetrate the unprinted areas. In our case, we performed the penetration test of packaging materials from the opposite sides (unprinted part of packaging films remains contact with the experimental pests). Under stereoscope microscope we observed the entrance side in the packaging foils is similar to the exist side, as it was mentioned in J. Riudavets et al. (2007).

In our invasion test of whole packages of rice experiment, *R. dominica* penetrated on average more holes per rice package (6.3 ±2.44) than *S. oryzae* (only 1.84 ± 0.58 holes per rice package) and when the two species were present together. But statistically results suggest that there was no synergy effect between the two species. Similarly, in comparison of numbers of individuals inside the package of rice, we found *R. dominica* (15.9 ± 5.49) and *S. oryzae* (31.9 ± 7.19) gained entry inside rice package.

Roughly 75% of package infestation occurs due to the existing packaging defects or by the insects create an entry point from chewing the package (Collins 1963; Mullen et al.,2012), which means 75% of infestation occurs by the invaders. Similarly, on average, 45.4% of *R. dominica* was present inside the package of rice when it was alone in a container while 31.8% was inside while it was with *S. oryzae.* While the opposite trend was observed in *S. oryzae* where 45.3 % individuals were present in the package when it was alone in a container while 63.8% were inside while it was together with *R. dominica*.

In our second invasion test where we kept 20 individuals of *T.casteneum*, 20 individuals of *T.confusum*, 10 individuals of *Tenebrio molitor*, 10 individuals of *Alphitobius diaperius*, and 10 individuals of Tribolium destructor in five boxes and 10 individuals of *Palorus ratzenburgii* in another five boxes, we found only in three samples, some beetles gained entry. There were no chewing holes which makes clear that these species used the veneration holes to gain entry inside the package of rice. T. molitor (biggest sized beetles in this experiment), *P. ratzenburgi* and *A. diaperinus* were not capable to gain inside the package of rice. We measured the ventilation holes from of package of rice and found the size of 1st hole (length 3.03 ± 0.38mm and width 1.31 ± 0.19mm) and 2nd hole having size of (length 2.79 ± 0.17mm and width 0.94 ± 0.17mm), which was sufficient to gain entry.

Most of the store-pests used the damaged part and weak seal to gain entry inside the packaging materials (Mowery, Mullen, Campbell, & Broce, 2002). *S.orazye* infested the pasta in Italy and Japan which was covered by PP films. (Murata et al., 2008;Stejskal, Kucerova, & Lukas, 2004; Trematerra & Savoldelli,(2014) reported their entry was facilitated by the presence of points of improper sealing. In our cases, *T. confusum* used penetration holes to invade the package of rice. It is interesting finding that the more *S. oryzae* than *R. dominica* inside the package of rice, because this species is usually treated as a primary pest penetrating into the packaging - nevertheless, evidently when there is a chance to get into the package without a chewing a hole, the species uses it. It may be connected with the better climbing ability of *S. oryzae* than *R. dominica* (Cline and Highland, 1976)

7. Conclusion

We found that 1) Several store- product pests are capable to penetrate or invade the packages, 2) We did not fully confirmed that the resistivity of the packaging films is strongly dependent to the thickness of it. 3) We probably reported the first observation on *R. dominica* created more fully penetrated holes in PP 40 than in PP20 4) We observed multilayer films are comparatively more resistance then mono-layer films to protect from the store-pests. 5) We observed that there is no synergy effect between *S. oryzae* and *R. dominica* when we kept them together. 6) We found that ventilation holes of the rice package are enough to gain entry for invaders.

8. References

Adler, C. **(2004),** Significance of hermetic seals, controlled ventilation, and wire-mesh screens to prevent the immigration of stored product pests. *IOBC/WPRS Bulletin*, 27(9), 13-16.

Adler, C. 2008: Insect-proof packaging to avoid stored product insects. IOBC/Wprs Bull, 40, 363-369.

Athanassiou, C.G., Riudavets, J. and Kavallieratos, N.G., **(2011)**, Preventing stored-product insect infestations in packaged-food products. *Stewart Postharvest Review*, 3(8), pp.1-5.

Batth, S. S. **(1970)**. Insect penetration of aluminum-foil packages. *Journal of Economic Entomology*, 63(2).

Bell, William J., and Ring T. Cardé **(1984)** Chemical ecology of insects

Brickey, P. M., Gecan, J. S., & Rothschild, A. L. B. E. R. T. **(1973)**. Method for determining direction of insect boring through food packaging materials. *Ass Offic Anal Chem J.*

Bowditch, T. G. **(1996)**, Investigation of factors relating to the insect infestation of chocolate-based consumables *(Doctoral dissertation, University of Tasmania).*

Burks, C. S., Yasin, M., El-Shafie, H. A., & Wakil, W. **(2015).** Pests of stored dates. In Sustainable pest management in date palm: current status and emerging challenges (pp. 237-286).

Cline, T. W. **(1979)**. A male-specific lethal mutation in Drosophila melanogaster that transforms sex. *Developmental biology*, 72(2), 266-275.

Cline, L. D., & Highland, H. A. **(1981)**. Minimum size of holes allowing passage of adults of stored-product Coleoptera. *Journal of the Georgia Entomological Society*, 16(4), 525-531.

Chung, S. K., Seo, J. Y., Lim, J. H., Park, H. H., Kim, Y. T., Song, K. H., et al. **(2011)**. Barrier property and penetration traces in packaging films against Plodia interpunctella (Hubner) larvae and Tribolium castaneum (Herbst) adults. *Journal of Stored Products Research*, 47, 101e105.

Cotton, R. T., & Winburn, T. F. **(1941)**. Field infestation of wheat by insects attacking it in farm storage. *Journal of the Kansas Entomological Society*, 14(1), 12-16.

Davis, R., & Pettitt, G. T. **(1999),** Evaluation of food package resistant to insect invasion. *In 3rd Int. Conf. Urban Pests, Prague, Czech Rep., July* (pp. 19-22).

Daniel Cline, L. **(1978)**. Penetration of seven common flexible packaging materials by larvae and adults of eleven species of stored-product insects. *Journal of Economic Entomology*, 71(5), 726-729.

Edde, P. A. **(2012)**. A review of the biology and control of Rhyzopertha dominica (F.) the lesser grain borer. *Journal of Stored Products Research*, 48, 1-18.

Essig, E. O., Hoskins, W. M., Linsley, E. G., Michelbacher, A. E., & Smith, R. F. **(1943)**, A report on the penetration of packaging materials by insects. *Journal of Economic Entomology*, 36, 822e829

FRAENKEL, Gr, and Marjorie Blewett.**(1943)** ,"The basic food requirements of several insects . *Journal of Experimental Biology* 20.1: 28-34

Gerhardt, P. D., & Lindgren, D. L. **(1954)**. Penetration of various packaging films by common stored-product insects. *Journal of Economic Entomology*, 47(2), 282-287.

Giles, P. H., & Ashman, F. **(1971)**. A study of pre-harvest infestation of maize by Sitophilus zeamais Motsch.(Coleoptera, Curculionidae) in the Kenya highlands. *Journal of Stored Products Research*, 7(2), 69-83.

G. Muratore, **(2008)** Susceptibility of plastic packages to insects pests attracts University of Catania, Italy

Hagstrum, D. W., Phillips, T. W., & Cuperus, G. **(2012),** Stored product protection. *Kansas state university agricultural Experiment station and cooperative Extension Service*

Hagstrum, D. W., & Subramanyam, B. **(2009**). A review of stored-product entomology information sources. *American Entomologist*, 55(3), 174-183.

Hassan, M. W., Ali, U., Ur Rehman, F., Najeeb, H., Sohail, M., Irsa, B., ... & Chaudhry, M. S. *(2016)*. Evaluation of standard loose plastic packaging for the management of *R. dominica* (F.)(Coleoptera: Bostrichidae) and *T. castaneum* (Herbst)(Coleoptera: Tenebriondiae). *Journal of Insect Science*, 16(1).

Highland, H. A. **(1984)**, Insect infestation of packages.

Highland, H. A., & Wilson, R. **(1981),** Resistance of polymer films to penetration by lesser grain borer and description of a device for measuring resistance. *Journal of Economic Entomology*, 74(1), 67-70.

Highland, H. A. **(1991),** Protecting packages against insects. Ecology and Management of Food-Industry Pests. *FDA Technical Bulletin,* 4, 345-350.

Hodges, R. J., Robinson, R., & Hall, D. R. **(1996)**. Quinone contamination of dehusked rice by *Tribolium castaneum* (Herbst)(Coleoptera: Tenebrionidae), 32(1), 31-37.

Hou, X., Fields, P., & Taylor, W. **(2004).** The effect of repellents on penetration into packaging by stored-product insects. *Journal of Stored Products Research*, 40(1), 47-54

Hubert, J., Erban, T., Nesvorna, M., & Stejskal, V. **(2011),** Emerging risk of infestation and contamination of dried fruits by mites in the Czech Republic. F*ood Additives and Contaminants Part A- Chemical Analysis Control Exposure and Risk Assessment,*

28, 1129e1135.

Hubert, J., Nesvorna, M., & Volek, V. **(2015),** Stored product mites (Acari: Astigmata) infesting food in various types of packaging. *Experimental and Applied Acarology*, 65(2), 237-242.

Kirwan, M. J., Plant, S., & Strawbridge, J. W. **(2011),** Plastics in food packaging. *Food and beverage packaging technology*, 157-293.

Mason, L. J., & McDonough, M. **(2012)**. Biology, behavior, and ecology of stored grain and legume insects. *Stored product protection*, 1(7).

Mowery, S. V., Mullen, M. A., Campbell, J. F., & Broce, A. B. **(2002)**. Mechanisms underlying sawtoothed grain beetle *(Oryzaephilus surinamensis* [L.]) (Coleoptera: Silvanidae) infestation of consumer food packaging materials. *Journal of Economic Entomology*, 95(6), 1333-1336.

Mullen, Michael A., Jade M. Vardeman, and Jim Bagwell. **(2012)**, 12 Insect-Resistant Packaging. *Stored product protection* : 135.

Murata, Mika, Taro Imamura, and Akihiro Miyanoshita.**(2008) ,**Infestation and development of Sitophilus spp. in pouch-packaged spaghetti in Japan. *Journal of economic entomology* 101.3 (2008): 1006-1010.

Muratore, Giuseppe, et al. **(2008)**, Susceptablity of plastic packages to insect pests attacks.

Mowery, S. V., Mullen, M. A., Campbell, J. F., & Broce, A. B. **(2002)**. Mechanisms underlying sawtoothed grain beetle *(Oryzaephilus surinamensis* [L.]) (Coleoptera: Silvanidae) infestation of consumer food packaging materials. *Journal of Economic Entomology*, 95(6), 1333-1336.

Newton, J. **(1988),** Insects and packaging. *International Biodeterioration* 24:175-187.

Oshawa, K., Oshima, K., Yamamoto, I., & Yamamoto, R. **(1970)**. Attractant for the rice weevil, Sitophilus zeamais (Motsch.)(Coleoptera: Rhynchophoridae), from rice grains. III.: A new type olfactometer for rice weevils. *Appl. Ent. Zool.*, 5(3), 121-125.

Phillips, T. W., Jiang, X. L., Burkholder, W. E., Phillips, J. K., & Tran, H. Q. **(1993),** Behavioral responses to food volatiles by two species of stored-product coleoptera, Sitophilus oryzae (curculionidae) andTribolium castaneum (tenebrionidae). *Journal of Chemical Ecology*, 19(4), 723-734.

Phillips, T. W., & Strand, M. R. **(1994),** Larval secretions and food odors affect orientation in female Plodia interpunctella. Entomologia Experimentalis et Applicata, 71(3), 185-192.

Roesli, R., Subramanyam, B., Fairchild, F. J., & Behnke, K. C. **(2003)**. Trap catches of stored-product insects before and after heat treatment in a pilot feed mill. *Journal of Stored Products Research*, 39(5), 521-540.

Robert Davis and Timothy Pettitt, Evulation of food packages resistance to insect invasion.

Riudavets, J., Salas, I., & Pons, M. J. **(2007),** Damage characteristics produced by insect pests in packaging film. *Journal of stored products research*, 43(4), 564-570.

Scheff, Deanna, Blossom Sehgal, and Bhadriraju Subramanyam **(2018)**, Evaluating Penetration Ability of Plodia interpunctella (Hübner)(Lepidoptera: Pyralidae) Larvae into Multilayer Polypropylene Packages. *Insects* 9.2 42.

Scheff, D., Subramanyam, B., Sehgal, B., & Dogan, H. **(2014)**. Resistance of three energy bar packages to penetration by Plodia interpunctella (Hübner) larvae. *In 11th International Working Conference on Stored Product Protection, Chiang Mai, Thailand*, pp. 159–165.

Shaw, D. John. "World Food Summit, **(1996)**, World Food Security. *Palgrave Macmillan, London*, 2007. 347-360.

Shlomo Navarro1, Hagit Navarro **(2018),** A device for quick evaluation resistance of packaging films to penetration by storage insects.

Stejskal, V., Hubert J. and Li Z. **(2018)**, Human Health Problems and Accidents Associated with Occurrence and Control of Storage Arthropods and Rodents- Chapter 2. In. Athanassiou, C. G.,Arthur, F. (Eds.) *Recent Advances in Stored Product Protection, Spring*er, pp. 19-43.

Stejskal, V. **(2015),** Pest prevention during storage, transportation and handling of stored products. *Integrated Protection of Stored Products. IOBC-WPRS Bulletin*,111, 171e176.

Stejskal, V., Hubert, J., Aulicky, J., & Kucerova, Z. **(2015),** Overview of present and past and pest-associated risks in stored food and feed products: *European perspective. Journal of Stored Products Research*, 64, 122e132.

Stejskal, V., Bostlova, M., Nesvorna, M., Volek, V., Dolezal, V., & Hubert, J. **(2017)**, Comparison of the resistance of mono-and multilayer packaging films to stored-product insects in a laboratory test. *Food control*, 73, 566-573.

Trematerra, P., & Savoldelli, S. **(2014)**. Ability to penetrate through pasta packaging of the maize weevil, *Sitophilus zeamais* Motschulsky. *Stored-product Protection, Chiang Mai, Thailand* (pp. 89-98). IWCSPP.

Trematerra, P., & Savoldelli, S. **(2014),** Pasta preference and ability to penetrate through packaging of Sitophilus zeamais Motschulsky (Coleoptera: Dryophthoridae). *Journal of stored products research*, 59, 126-132

Trematerra, P., Sciarreta, A., & Tamasi, E. **(2000)**. Behavioural responses of *Oryzaephilus surinamensis, Tribolium castaneum and Tribolium confusum* to naturally and artificially damaged durum wheat kernels. *Entomologia Experimentalis et Applicata*, 94(2), 195-200.

Trematerra, P., & Fleurat-Lessard, F. (2015). Food industry practices affecting pest management. *Stewart postharvest review,* 11(1), 1-7.

TSUJI, H. **(2000),** Ability of first instar larvae of the Indian-meal moth, Plodia interpunctella Hubner, to reach their food. *Medical Entomology and Zoology*, 51(4), 283-287.

Winterbottom, D. C**. (1922).** Weevil in wheat and storage of grain in bags

Wohlgemuth, R. **(1979)**. Protection of stored foodstuffs against insect infestation by packaging. *Chemistry and industry.*

9. List of Abbreviations

CRI	- Crop Research Institute
FAO	- Food and Agriculture Organization
BPF	- British Plastics Federation
LLDPE	-low-density polyethylene
PP	-polypropylene
PET	-polyethylene terephthalate
IPM	- Integrated Pest Management
WHO	-World Health Organization
UN	- United Nations
MRL	-Maximum residue level
GAP	-Good Agricultural Practice
EFSA	- European Food Safety Authority
RH	- Relative Humidity
HACCP	-Hazard Analysis Critical Control Point
ANOVA	-Analysis of variance