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Barrier property and penetration traces in packaging films against *Plodia interpunctella* (Hübner) larvae and *Tribolium castaneum* (Herbst) adults

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The barrier property of different types of plastic film (with or without pinholes) against two insects, *Plodia interpunctella* (Hübner) larvae and *Tribolium castaneum* (Herbst) adults, and the morphology of damage produced in these insects were investigated. Using a penetration apparatus, four types of plastic films varying in thickness were used for insect-penetration tests: casted polypropylene, 20 μm and 25 μm (CPP20 and CPP25); oriented polypropylene, 20 μm and 30 μm (OPP20 and OPP30); linear low-density polyethylene, 40 μm and 50 μm (LLDPE40 and LLDPE50); and polyethylene terephthalate 12 μm and 16 μm (PET12 and PET16). After being fixed and tested in the penetration apparatus, each film was cut into a disc shape and 10 holes (200 μm diameter) were made by a pin. The shape of film damage and the mouthparts of insects were observed using scanning electronic microscopy. *Plodia interpunctella* larvae could penetrate all films with pinholes, while *T. castaneum* adults were unable to penetrate any of the films tested, even those with pinholes. The penetration-percentages by *P. interpunctella* larvae were 38% (LLDPE40), 3% (LLDPE), 53% (CPP20), 37% (CPP25), 63% (OPP20), 43% (OPP30), 83% (PET12) and 63% (PET16). The elongation value, tensile strength and thickness of film were important factors in the penetration test. LLDPE, which has the highest elongation value and the lowest tensile strength value, was the film that best protected against insect penetration. In CPP and LLDPE films, there were many scratches and tears around the holes. In comparison, much less damage was observed around the holes in OPP and PET films. By observing the mouthparts of insects, it was determined that *P. interpunctella* larvae had sharper mandibles than those of *T. castaneum* adults.

1. Introduction

Several types of plastic films are used as packaging materials in the food industry where they serve as a significant barrier in reducing insect infestation. In this context, insect species are grouped into penetrators that can penetrate packages and invaders that enter packages only through existing holes (Highland, 1984; Newton, 1988). Adults of *Rhyzopertha dominica* (*F.*), *Sitophilus oryzae* (*L.*) and *Lasioderma serricorne* (*L.*) (Riudavets et al., 2007) as well as larvae of *Ephestia cautella* (Walker) and *Plodia interpunctella* (Hübner) (Browditch, 1997) are known as penetrators. Adults of *Tribolium* species, *Oryzaephilus* species and *Cryptolestes ferrugineus* (Stephens) are invaders (Highland, 1991). *Oryzaephilus surinamensis* (*L.*) adults can enter through holes with diameters of more than 0.71 mm, and larvae are capable of invasion through even smaller openings (Cline and Highland, 1981).

Most penetration-testing methods use small pouches of experimental material with food. That is sealed inside and the pouches exposed to insects. In another method, insects are put into the pouches with or without a small quantity of food. In these methods, folds and seams are the major routes through which insects enter or exit packages, either by piercing or by finding weak points in the seams. But whilst tiny scratches or pinholes on the film surface can be exploited for insect penetration a film surface is typically not penetrated by insects because they are unable to bite the smooth film.

The aim of this study was to evaluate the barrier properties of different types of plastic films of varying thickness, with and

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without pinholes against *Plodia interpunctella* (Hübner) larvae, and *Tribolium castaneum* (Herbst) adults. A secondary aim was to describe the morphology of damage induced by these two insects and to relate this to the structure of their mouthparts.

2. Materials and methods

2.1. Insects

Adult *T. castaneum* and third-instar larvae of *P. interpunctella* were used in this study. *Plodia interpunctella* larvae and *T. castaneum* adults were reared in the laboratory at 27 ± 1 °C, 68 ± 2% relative humidity and a light: dark photoperiod of 16:8. *Plodia interpunctella* larvae were reared on diets of mixed rice bran, glycerine and yeast (8:3:2 by weight). *Tribolium castaneum* adults were reared on wheat flour.

2.2. Mechanical properties of films

Films of casted polypropylene (CPP) 20 μm and 25 μm, oriented polypropylene (OPP) 20 μm and 30 μm, linear low-density polyethylene (LLDPE) 40 μm and 50 μm, and polyethylene terephthalate (PET) 12 μm and 16 μm (Lotte Aluminium, Seoul, Korea) were tested. Tensile strength (TS) and elongation (*E*) at breaking point were measured using a model 5566 universal testing machine (Instron, Canton, MA, USA) according to the ASTM Standard Method D882-88 (ASTM, 1989). Five samples of each film type (10 × 2.54 cm) were cut into the machine direction. Initial grip separation and cross-head speed were set to 5 cm and 500 mm/min, respectively. Tensile strength was calculated by dividing the maximum load by the cross-sectional area of the film. Elongation value was calculated and expressed as a percentage of change of the original specimen length between grips.

2.3. Penetration test

The device used for penetration tests was a modified version of that described by Hou et al. (2004), and consisted of four acryl resin plates with dimensions of 15 × 15 cm (Fig. 1). The A and D plates were 2 cm thick, and the B and C plates were 1 cm thick. Each plate had 10 holes each of 1 cm diameter that formed the insect cages. The outer plates were covered to prevent insects from escaping and the covering was perforated to allow for insect respiration. Two pieces each 15 cm in diameter were cut from each film pieces and in one piece a 0.4 mm-diameter pin was used to create 10 pinholes having a diameter of 200 μm. The other piece was left un-perforated. The un-perforated piece was clamped between the C and D plates then one *T. castaneum* adult or *P. interpunctella* larva was put into each hole of the B and C plates. Next, the test film was fixed between the A and B plates. An attractant of mixed rice bran, glycerine and yeast (8:3:2 by weight) was placed in the A and D plates. The plates were placed together and kept for five days in a 26 °C chamber with 70% relative humidity. Each experiment was replicated six times. The percentage penetration was calculated as [number of penetrated insects ÷ initial number of insects added] × 100.

2.4. Observation of film damage and insect mouthparts

Scanning electron microscopy (SEM) was used to visualize film damage caused by third-instar larvae of *P. interpunctella*. Each film was cut to 8 × 8 mm, placed on a brass stub using double-sided adhesive tape and coated using a JSC-1100E gold/osmium Coater (JEOL, Tokyo, Japan) at 10 mA for 5 min. The model JSM-5410LV scanning electron microscope (JEOL) was set to an accelerating voltage of 20 kV.

Third-instar *P. interpunctella* larvae and *T. castaneum* adults were fixed with 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) for 4 h and washed with sodium cacodylate buffer. Specimens were dehydrated in mixtures of ethyl alcohol with increasing concentrations of isoamylacetate (ethanol: isoamlyacetate of 3:1, 1:1, 1:3) for 20 min at each step, and two 20 min exposures to 100% isoamlyacetate. Finally, specimens were dried using a model RW-0525G critical point dryer (Lab.companion, Seoul, Korea) and coated with gold in a SEM coating system sputter coater (Bio-Rad, Hercules, CA, USA). Morphological characteristics of heads were observed using a model JSM-5300 scanning electron microscope (JEOL).

2.5. Data analysis

Data on insect penetration percentage, TS and *E* at break point in eight types of films were analyzed via the non-parametric Kruskal–Wallis test method with the Statistical Analysis System to determine significant differences in the percentage of penetration and mechanical properties between types and thickness of films (SAS Institute, 2008).

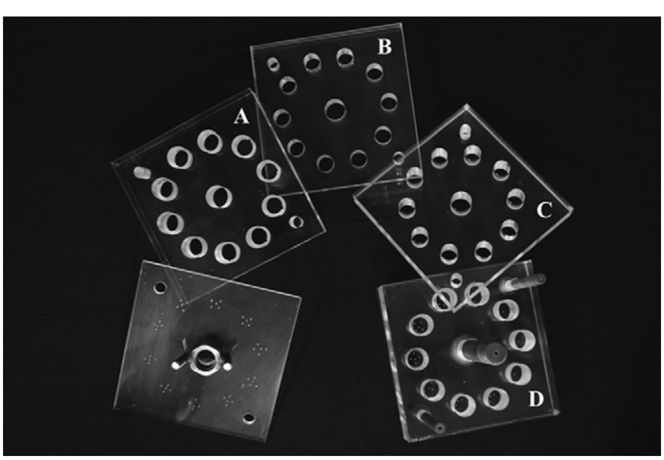

Fig. 1. Device used for the penetration test of *P. interpunctella* larvae. A, B, C and D denote the plates. Ten holes were drilled into each plate and contained cages for test insects. Films with pinholes were between plates A and B and films without pinholes were between plates C and D.

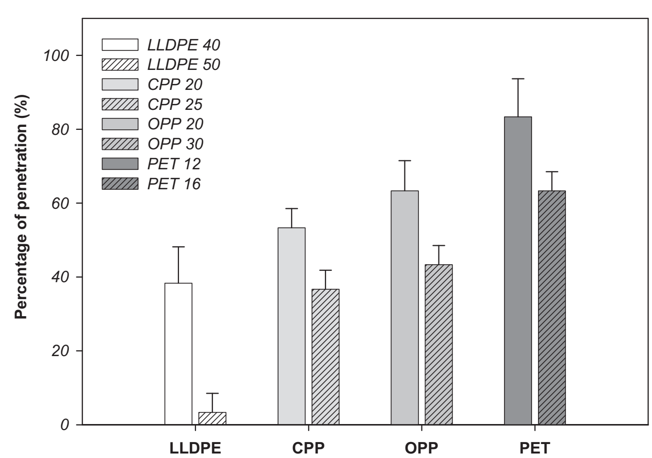

Fig. 2. Percentage of third-instar *P. interpunctella* larvae penetrating (mean ± SD; $n = 6$) polyethylene terephthalate (PET), oriented polypropylene (OPP), casted polypropylene (CPP) and linear low-density polyethylene (LLDPE) films containing pinholes.