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Evaluation of the ability of *Cadra cautella* (Walk.) larvae (Lepidoptera: Pyralidae) to perceive the presence of a food source in flexible biaxially oriented polypropylene packaging

Abstract - The ability of II instar *Cadra cautella* (Walk.) larvae to perceive the presence of food source and to penetrate packaging, containing or lacking food, in biaxially oriented polypropylene, differently treated and with varying thickness, was evaluated. Only packaging containing food source were penetrated and no substantial difference of behaviour between males and females was observed. Co-extruded biaxially oriented polypropylene packaging as well as acrylic coated packaging, in presence or in absence of printing, were perforated to a thickness of 20 μm. Abrasions were observed on the packaging in white expanded polypropylene (40 μm), in presence or absence of printing, but larvae couldn't perforate them.

Riassunto - *Valutazione della capacità delle larve di* Cadra cautella *(Walk.) di percepire un alimento all'interno di confezioni in polipropilene biorientato.*

E' stata valutata la capacità di larve di seconda età di *Cadra cautella* (Walk.) di percepire la presenza di una fonte alimentare e di forare confezioni, contenenti o prive di cibo, in polipropilene biorientato diversamente trattato e a diversi spessori. Sono state forate esclusivamente le confezioni contenenti la fonte alimentare e non si è osservata una sostanziale differenza di comportamento tra maschi e femmine. Le confezioni in polipropilene coestruso biorientato e quelle con laccatura acrilica, in presenza e in assenza della stampa, sono state forate fino allo spessore di 20 μm. Sugli imballaggi in polipropilene bianco espanso (40 μm), in presenza e in assenza di stampa, sono state osservate delle abrasioni ma le larve non sono riuscite a forarli.

Key words: *Cadra cautella,* perceive food, flexible biaxially oriented polypropylene packaging.

INTRODUCTION

Any confectionery product results more or less protected by infestations of *Cadra cautella* (Walk.) larvae depending on the physical features of the packaging used in

terms of allowing aromas to filter through or resistance to the attack of insects' mandibles.

The tendency of larvae to attack packaging depends on their developmental stage. In fact, during the I instars, larvae need a great quantity of nourishment. As time passes, especially when the larvae are close to the maturity stage, they tend to become nymphae precociously, rather searching for an other food source, mostly when the food is inside the packaging.

Many studies underline the attractive ability of foodstuff substrates toward Lepidoptera, while there is little research aimed at evaluating the attraction exerted by packaged food toward different species of insects and their ability to perceive the aroma filtering through the packaging (Bertonazzi & De Beni, 1989; Mullen, 1994).

The possibility of larvae attacking a plastic film depends on their ability to cling to the surface of the material and to climb up it. In fact larvae are facilitated in the perforation by the presence of support points on which to levy, in particular folds and seals (Cline, 1987a). *C. cautella* larvae can cling to and climb up paper, glass, aluminium, cellophane, polyethylene, fluorinated ethylenepropylene, ethylentetrafluoethylene, polyester, polyvinylchloride and polypropylene to an inclination of 90° (Cline, 1978b).

It was observed that seals, folds and cavities present in the parcels are the best places for larvae to attempt to perforate the packaging (Locatelli & Garavaglia, 1994).

C. cautella (Walk.) can perforate different materials used in foodstuff packaging (Highland, 1984; Bowditch, 1997). A polyethylene packaging with a thickness of 127 μm can be perforated by four and five instar larvae (Davey & Amos, 1961). On the contrary packaging of biaxially oriented polypropylene (28 μm), coated on one side with acrylic and on the other one with polyvinylidene chloride are resistant to the attack of I and V instar larvae; generally polyvinylchloride is perforated within 3 days, by V instar larvae (Bowditch, 1997).

Cline (1978a) observed that II instar larvae, without food source, can perforate polyvinylchloride having a thickness of 25.4 μm . II and V instar larvae can perforate: cellophane (25.4 μm), polyethylene (25.4 μm) and kraft paper (114 μm, 81g/m^2). On the contrary, if the packaging is without food source, aluminium foil, polyester and polypropylene can't be perforated.

The aim of this study was to evaluate the ability of II instar *C. cautella* (Walk.) larvae to perceive the presence of a food source and to perforate differently treated biaxially oriented polypropylene packaging with varying thickness.

MATERIALS AND METHODS

The ability of larvae to perceive the aroma filtered through a packaging, to perforate it afterwards, was evaluated using parcels prepared on purpose with film of polypropylene having different features: heat sealable co-extruded biaxially oriented transparent polypropylene (thickness: 17, 20, 25, 30 μm), biaxially oriented transparent 2

sides acrylic coated polypropylene film (thickness: 20, 25, 30 μm) and biaxially oriented white expanded polypropylene (40 μm). Each film was tested in the printed shape as well as in the unprinted one.

The printing was carried out using the rotogravure technique and the used inks are solvent based, that is ethyl acetate, with pigments and composition used for the printing of films normally utilised for foodstuff packaging.

For the preparation of the packaging, shapes of the plastic film were cut out by using a model in plexiglas, (14.5 x 8 cm) covered on one side by a layer of rubber to avoid sliding on the film and facilitate the cutting of the film itself. The film was folded in two parts along the wider dimension and then it was sealed longitudinally. It's a fin-welding, later folded on the envelope. After that a second seal was carried out, the first of the two transversal ones. At this point the bags were formed; half of them filled with 7± 0.1 g of substratum suitable for the development of *Cadra cautella* larvae.

Food, placed inside the packaging(1), is particularly attractive for this species; in fact cocoa and hazelnuts present a vast array of aromas which attract larvae. Dry fruit and cocoa are among the foodstuff which present the greatest infestations by this species (Cox & Bell, 1991). Some cuttings of the same film were placed in the other half of packaging, in order to obtain a volume similar in the 2 packaging. The bags were sealed, making the second transversal seal, to form packaging (6x8 cm). The envelopes thus obtained so-called "pillow shaped", represent a packaging largely used in the field of packaging for single-use baked goods.

All the seals were made with a horizontal bar thermowelding machine (laboratory welder) at 140°C, at a pressure of 4 bar and for a second. Welding shagreened bars with a width of 1 cm, instead of flat ones, were used as they are largely used in the industry of confectionery products.

Tests were carried out in rigid containers of transparent plastic (17x9x8 cm) provided on each lateral side with a circular window (Ø 1.5 cm), closed with a metal wire (120 mesh) to permit air circulation.

Two packages (one with and one without food) were placed in each container so that the longitudinal seal always turned downward.

Male and females were used separately in the test. 10 second instar *C. cautella* larvae were placed at the centre of each container at the same distance from the two packages. The containers were closed with suitable lids and put in an air conditioned cell at 25±1°C and 70%±5 R.H., light 10:14. 6 replies for each sex were carried out.

Therefore the distribution of larvae inside the container was registered over time. The first control was carried out after 5 hours, time considered enough to overcome the shock suffered by the larvae during the drawing and the preparation of the test. The following controls took place after 1, 2, 4 days. Since the individuals did not have access to food and could not survive, no additional controls were made afterwards.

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(1) Composition of food: bran (4 parts), wheat germ (1part), wheat meal (1 part), corn meal (1 part), minced hazelnuts (4 parts), beer yeast ($^1/_2$ part), cocoa (1 part), honey (1 part),glycerine (1 part).

Phenomena of cannibalism for this species are possible, as observed by Bartels & Schliesske (1997).

At the end of the test, packaging was controlled under stereoscopic microscopic 25X in order to locate possible holes or attempts of perforation.

The gathered data were subjected to variance analysis and to Duncan's Test (SPSS, version 9.0 for Windows).

RESULTS

Tables 1-4 show the average number of larvae found out on the packaging (with or without food substrate) or in the surrounding areas; these observations only refer to the tests where no holes of packaging were registered. Tables 5-6 show the significance of larvae location on the packaging and in the surrounding area.

Tables 7-8 indicate the total number of abrasions and holes found out on the packaging after 4 days from the beginning of the test.

The attempts of penetration are generally carried out along the seal, while the penetrations occur more frequently on the flat surface of the packaging.

Only the packaging containing food were perforated and no substantial difference of behaviour between males and females was observed, except for co-extruded polypropylene packaging with a thickness of 20 μm printed – which was perforated only by the female larvae – and 2 sides acrylic coated polypropylene packaging (20 μm printed), which was perforated only by the males.

Co-extruded polypropylene film and 2 sides acrylic coated polypropylene film, in presence or in absence of printing, were perforated to a thickness of 20 μm. On the contrary the white expanded polypropylene was not perforated.

Table 9 shows the total number of packaging of different films, containing the food substrate perforated by *C. cautella*.

The packaging in co-extruded biaxially oriented unprinted polypropylene (of a thickness of 17 and 20 μm), containing food substrate exerted a greater attraction than the empty packaging.

Female larvae perforated the packaging in co-extruded biaxially oriented unprinted polypropylene of 17 μm with food, already at the second day, while for males only one packaging penetrated after 4 days from the beginning of the tests was found out. Larvae of both sexes couldn't perforate the packaging in biaxially oriented co-extruded unprinted (20 μm) within 2 days.

After 5 hours female larvae, not present on the packaging, were significantly fewer than those ones present on the packaging with food in co-extruded polypropylene of 17 μm. With co-extruded polypropylene of 20 μm, the number of larvae distributed in the environment never resulted significantly greater than the number of those found over or under the packaging with food. Moreover, these latter resulted significantly more attractive for male larvae from the first day of tests.

The packaging in co-extruded polypropylene 25 μm filled with food was more attractive for males within the first day of test, while afterwards no significant diffe-

Table 1 - Average number (± E.S.) of male Cadra cautella *(Walk.) II instar larvae found at the time indicated respectively on the packaging, unprinted, in presence (P) and in absence (A) of food source, in the sorrounding area (E) and dead (D).*

Biaxially oriented unprinted polypropylene	5 h				1 day				2 days				4 days			
	P	A	E	D	P	A	E	D	P	A	E	D	P	A	E	D
Co-extruded 17 µm	4,7±1,1 b	0,5±0,2 a	4,8±1,2 b	0,0±0,0 a	3,3±0,7 b	1,3±0,4 a	5,0±0,6 c	0,3±0,2 a	2,8±0,7 b	0,7±0,3 a	5,5±0,6 c	1,0±0,4 a	-	-	-	-
Co-extruded 20 µm	5,3±0,9 c	1,3±0,3 a	3,3±0,9 b	0,0±0,0 a	5,3±0,6 c	1,2±0,4 a	3,2±0,5 b	0,3±0,2 a	3,0±0,7 c	0,7±0,3 a	5,0±1,2 c	1,3±0,5 a	-	-	-	-
Co-extruded 25 µm	4,8±1,0 b	0,8±0,4 a	4,3±1,1 b	0,0±0,0 a	3,5±0,8 b	1,3±0,4 a	4,0±0,5 b	1,2±0,7 a	2,5±0,6 a	1,2±0,5 a	5,0±1,2 b	1,3±0,7 a	2,3±0,3 a	2,0±0,7 a	3,0±0,7 a	2,7±0,7 a
Co-extruded 30 µm	3,7±0,4 b	1,2±0,4 a	5,2±0,7 c	0,0±0,0 a	3,0±0,7 b	1,5±0,4 b	5,5±0,8 c	0,0±0,0 a	3,2±0,5 b	0,8±0,5 a	5,2±0,6 c	0,8±0,3 a	2,0±0,4 b	0,5±0,2 a	3,5±0,5 c	4,0±0,3 c
2 sides acrylic coated 20 µm	3,7±0,8 b	1,5±0,8 a	4,8±0,5 b	0,0±0,0 a	3,8±0,4 b	1,2±0,3 a	4,3±0,6 b	0,7±0,3 a	2,0±0,6 a	1,2±0,5 a	6,0±0,7 b	0,8±0,2 a	-	-	-	-
2 sides acrylic coated 25 µm	3,3±0,6 b	1,2±0,5 a	5,5±0,4 c	0,0±0,0 a	2,3±0,5 b	1,8±0,7 ab	5,8±1,0 c	0,0±0,0 a	1,7±0,6 a	1,3±1,0 a	6,3±0,6 b	0,7±0,3 a	0,8±0,4 a	0,8±0,4 a	4,2±0,7 b	4,2±0,3 b
2 sides acrylic coated 30 µm	3,8±0,6 b	0,8±0,4 a	5,3±0,6 c	0,0±0,0 a	3,5±0,8 b	1,3±0,4 a	4,8±0,7 b	0,3±0,3 a	1,8±0,6 a	0,8±0,5 a	6,3±0,9 b	1,0±0,4 a	1,0±0,3 a	0,3±0,2 a	4,5±0,8 b	4,2±0,8 b
White expanded 40µm	3,5±0,3 c	2,0±0,6 b	4,5±0,3 c	0,0±0,0 a	1,8±0,3 a	1,3±0,5 a	6,5±0,8 b	0,3±0,2 a	2,2±0,4 a	0,8±0,5 a	5,3±0,5 b	1,7±0,7 a	0,5±0,3 a	0,7±0,3 a	2,5±0,4 b	6,3±0,6 c

Table 2 - Average number (± E.S.) of female Cadra cautella *(Walk.) II instar larvae found at the time indicated respectively on the packaging, unprinted, in presence (P) or in absence (A) of food source, in the sorrounding area (E) and dead (D).*

Biaxially oriented unprinted polypropylene	5 h				1 day				2 days				4 days			
	P	A	E	D	P	A	E	D	P	A	E	D	P	A	E	D
Co-extruded 17 µm	6,0±0,7 c	0,3±0,3 a	3,7±0,7 b	0,0±0,0 a	5,0±0,6 b	0,7±0,2 a	4,0±0,7 b	0,3±0,2 a	-	-	-	-	-	-	-	-
Co-extruded 20 µm	4,5±0,5 b	1,0±0,4 a	4,5±0,6 b	0,0±0,0 a	4,3±0,7 b	1,2±0,3 a	4,5±0,7 b	0,0±0,0 a	4,0±0,9 b	1,2±0,5 a	4,3±0,5 b	0,5±0,3 a	-	-	-	-
Co-extruded 25 µm	4,8±0,9 b	0,7±0,5 a	4,3±1,2 b	0,2±0,2 a	3,7±0,8 b	1,7±0,8 a	4,2±0,7 b	0,5±0,3 a	2,8±0,7 b	0,7±0,3 a	5,2±0,5 c	1,3±0,3 a	2,7±0,8 bc	0,7±0,4 a	4,3±0,7 c	2,3±0,4 ab
Co-extruded 30 µm	3,7±1,0 b	1,0±0,5 a	5,3±1,1 b	0,0±0,0 a	2,2±0,7 b	0,3±0,2 a	6,8±0,5 c	0,7±0,2 a	2,2±0,4 a	0,7±0,5 a	6,2±0,7 b	1,0±0,3 a	1,7±0,6 ab	0,3±0,2 a	3,0±0,4 b	5,0±0,8 c
2 sides acrylic coated 20 µm	3,2±0,8 b	0,8±0,3 a	6,0±1,0 c	0,0±0,0 a	2,8±0,7 b	1,5±0,3 ab	5,7±0,7 c	0,0±0,0 a	3,3±0,8 b	0,2±0,2 a	6,2±0,9 c	0,3±0,2 a	-	-	-	-
2 sides acrylic coated 25 µm	2,3±0,6 b	2,3±0,7 b	5,3±0,3 c	0,0±0,0 a	2,2±0,5 b	1,3±0,6 ab	6,2±0,7 c	0,3±0,2 a	1,5±0,2 b	0,5±0,3 a	7,7±0,4 c	0,3±0,2 a	2,8±0,7 b	0,3±0,2 a	4,5±0,8 c	2,3±0,2 b
2 sides acrylic coated 30 µm	4,3±1,0 b	0,8±0,3 a	4,8±0,8 b	0,0±0,0 a	3,5±0,3 c	1,2±0,2 b	5,3±0,3 d	0,0±0,0 a	2,0±0,5 b	0,5±0,2 a	6,2±0,7 c	1,3±0,4 ab	1,8±0,6 ab	0,5±0,2 a	4,5±0,2 c	3,2±0,6 c
White expanded 40µm	3,3±0,6 b	0,7±0,3 a	6,0±0,8 c	0,0±0,0 a	3,2±0,7 b	0,5±0,2 a	6,3±0,6 c	0,0±0,0 a	2,8±0,5 b	0,7±0,3 a	5,3±0,6 c	1,2±0,5 a	1,3±0,5 a	0,5±0,2 a	1,0±0,4 a	7,2±0,5 c

Significance<0,05

Results of tests, where larvae perforated one or more packaging, were not statistically elaborated.

Table 3 - Average number of male Cadra cautella *(Walk) II instar larvae found at the time indicated,respectively on the packaging, printed, in presence (P) and in absence (A) of food source,in the sorrounding area (E) and dead (D).*

Biaxially oriented printed polypropylene	5 h				1 day				2 days				4 days			
	P	A	E	D	P	A	E	D	P	A	E	D	P	A	E	D
Co-extruded 20	4,0±0,4 c	3,5±0,4 bc	2,5±0,7 b	0,0±0,0 a	4,0±0,6 c	2,3±0,6 b	3,5±0,4 bc	0,2±0,2 a	3,8±0,4 b	1,2±0,3 a	4,2±0,5 b	0,8±0,4 a	2,7±0,6 b	1,2±0,5 a	3,0±0,3 b	3,2±0,5 b
Co-extruded 25 µm	2,5±0,3 b	2,2±0,3 b	5,3±0,3 c	0,0±0,0 a	2,7±0,7 a	1,8±0,5 a	4,7±0,8 b	0,8±0,5 a	2,3±0,3 b	1,5±0,3 ab	5,0±0,0 c	1,2±0,5 a	1,5±0,4 a	1,3±0,7 a	1,7±0,6 a	5,5±0,8 b
Co-extruded 30 µm	2,7±0,8 b	1,8±0,3 b	5,5±0,9 c	0,0±0,0 a	2,2±0,7 b	2,8±0,5 b	4,7±0,6 c	0,3±0,3 a	2,5±0,7 a	1,5±0,4 a	5,2±0,7 b	0,8±0,4 a	2,5±0,7 a	1,2±0,3 a	3,0±0,6 a	3,3±0,6 a
2 sides acrylic coated 20 µm	4,0±0,7 c	2,2±0,5 b	3,8±0,5 c	0,0±0,0 a	4,2±0,5 b	2,8±0,9 b	2,7±0,6 b	0,3±0,2 a	4,2±0,9 b	1,0±0,4 a	4,0±0,4 b	0,8±0,3 a	-	-	-	-
2 sides acrylic coated 25 µm	2,2±0,4 b	3,2±0,7 bc	4,7±0,9 c	0,0±0,0 a	1,8±0,3 ab	3,3±1,0 bc	4,7±0,8 c	0,2±0,2 a	1,5±0,2 b	2,2±0,3 b	6,0±0,4 c	0,3±0,2 a	0,7±0,4 a	1,7±0,6 a	4,3±0,8 b	3,3±0,2 b
2 sides acrylic coated 30 µm	1,7±0,8 a	1,5±0,8 a	6,8±0,7 b	0,0±0,0 a	2,5±0,6 b	1,5±0,4 b	6,0±0,4 c	0,0±0,0 a	1,7±0,3 a	1,3±0,3 a	6,3±0,6 b	0,7±0,5 a	1,2±0,3 a	1,3±0,3 a	3,8±0,5 b	3,7±0,4 b
White expanded 40µm	3,8±0,3 b	2,7±0,5 b	3,5±0,6 b	0,0±0,0 a	2,8±0,7 b	2,7±0,6 b	4,2±0,6 b	0,3±0,3 a	2,0±0,5 ab	2,7±0,5 b	4,7±0,5 c	0,7±0,3 a	1,5±0,5 a	1,7±0,5 a	2,3±0,6 a	4,5±0,8 b

Table 4 - Average number of female Cadra cautella *(Walk) II instar larvae found at the time indicated,respectively on the packaging, printed, in presence (P) and in absence (A) of food source,in the sorrounding area (E) and dead (D).*

Biaxially oriented printed polypropylene	5 h				1 day				2 days				4 days			
	P	A	E	D	P	A	E	D	P	A	E	D	P	A	E	D
Co-extrude 20 µm	3,7±0,6 b	2,7±0,5 b	3,7±0,6 b	0,0±0,0 a	4,3±1,0 c	1,8±0,5 ab	3,5±0,9 bc	0,3±0,3 a	-	-	-	-	-	-	-	-
Co-extruded 25 µm	2,2±0,5 b	2,2±0,7 b	5,7±0,9 c	0,0±0,0 a	3,2±0,5 bc	1,8±0,7 ab	4,2±0,8 c	0,8±0,3 a	2,5±0,7 a	2,0±0,5 a	4,5±0,7 b	1,0±0,3 a	2,5±0,6 b	0,8±0,4 a	1,8±0,5 ab	4,8±0,5 c
Co-extruded 30 µm	3,7±0,7 c	1,8±0,5 b	4,5±0,7 c	0,0±0,0 a	4,0±0,7 b	2,8±0,6 b	3,0±0,5 b	0,2±0,2 a	1,8±0,7 a	1,5±0,4 a	5,8±1,0 b	0,8±0,3 a	1,8±0,5 ab	1,2±0,5 a	4,0±0,7 c	3,0±0,4 bc
2 sides acrylic coated 20 µm	3,5±0,6 b	2,7±0,8 b	3,8±0,6 b	0,0±0,0 a	3,3±0,3 c	2,0±0,5 b	4,2±0,5 c	0,5±0,3 a	3,3±0,4 bc	1,8±0,6 ab	4,2±0,7 c	0,7±0,5 a	3,2±0,5 b	1,2±0,3 a	2,8±0,6 b	2,8±0,5 b
2 sides acrylic coated 25 µm	2,8±0,7 b	2,0±0,4 b	5,2±1,0 c	0,0±0,0 a	2,0±0,9 a	2,0±0,3 a	5,8±0,7 b	0,2±0,2 a	2,3±0,6 a	1,2±0,2 a	5,7±0,7 b	0,8±0,4 a	1,8±0,3 ab	1,3±0,4 a	4,0±0,5 c	2,8±0,5 bc
2 sides acrylic coated 30 µm	1,8±0,7 ab	2,2±0,7 b	6,0±1,0 c	0,0±0,0 a	2,3±0,3 b	1,7±0,6 b	5,7±0,3 c	0,3±0,2 a	2,5±0,4 b	1,7±0,4 ab	5,2±0,8 c	0,7±0,4 a	0,5±0,2 a	2,3±0,6 b	4,0±0,7 c	3,2±0,4 bc
White expanded 40µm	3,2±0,5 b	3,7±0,8 b	3,2±0,4 b	0,0±0,0 a	2,5±0,7 b	3,7±0,8 b	3,8±0,9 b	0,0±0,0 a	1,7±0,6 ab	2,5±0,8 b	5,2±0,4 c	0,7±0,3 a	1,3±0,3 a	0,8±0,3 a	3,8±0,7 b	4,0±0,5 b

Significance <0,05

Results of tests, where larvae perforated one or more packaging, were not statistically elaborated.

Table 5 - Significance of the location of Cadra cautella *(Walk.) II instar larvae on packaging in biaxially oriented unprintend polypropylene, with or without food.*

Biaxially oriented polypropylene			Is the number of larvae observed on the packaging containing food significantly higher than the one found on the packaging without food?				Is the number of larvae observed in the environment significantly higher than the one found on the packaging with food?			
Type	µm	Sex	5 hours	1 day	2 days	4 days	5 hours	1 day	2 days	4 days
Co-extruded	17	♂	Yes	Yes	Yes	-	No	Yes	Yes	-
		♀	Yes	Yes	-	-	No*	No	-	-
Co-extruded	20	♂	Yes	Yes	Yes	-	No*	No*	No	-
		♀	Yes	Yes	Yes	-	No	No	No	-
Co extruded	25	♂	Yes	Yes	No	No	No	No	Yes	No
		♀	Yes	Yes	Yes	Yes	No	No	Yes	No
Co-extruded	30	♂	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes
		♀	Yes	Yes	No	No	No	Yes	Yes	No
2 sides acrylic coated	20	♂	Yes	Yes	No	-	No	No	Yes	-
		♀	Yes	No	Yes	-	Yes	Yes	Yes	-
2 sides acrylic coated	25	♂	Yes	No	No	No	Yes	Yes	Yes	Yes
		♀	No	No	Yes	Yes	Yes	Yes	Yes	Yes
2 sides acrylic coated	30	♂	Yes	Yes	No	No	Yes	No	Yes	Yes
		♀	Yes	Yes	Yes	No	No	Yes	Yes	Yes
White expanded	40	♂	Yes	No	No	No	No	Yes	Yes	Yes
		♀	Yes	Yes	Yes	No	Yes	Yes	Yes	No

Table 6 - Significance of the location of Cadra cautella *(Walk.) II instar larvae on packaging in biaxially oriented printed polypropylene, with or without food.*

Biaxially oriented printed polyipropylene			Is the number of larvae observed on the packaging containing food significantly higher than the one found on the packaging without food?				Is the number of larvae observed in the environment significantly higher than the one found on the packaging with food?			
Type	µm	Sex	5 hours	1 day	2 days	4 days	5 hours	1 day	2 days	4 days
Co-extruded	20	♂	No	Yes	Yes	Yes	No*	No	No	No
		♀	No	Yes	-	-	No	No	-	-
Co-extruded	25	♂	No	No	No	No	Yes	Yes	Yes	No
		♀	No	No	No	Yes	Yes	No	Yes	No
Co-extruded	30	♂	No	No	No	No	Yes	Yes	Yes	No
		♀	Yes	No	No	No	No	No	Yes	Yes
2 sides acrylic coated	20	♂	Yes	No	Yes	-	No	No	No	-
		♀	No	Yes	No	Yes	No	No	No	No
2 sides acrylic coated	25	♂	No	No	No	No	Yes	Yes	Yes	Yes
		♀	No	No	No	No	Yes	Yes	Yes	Yes
2 sides acrylic coated	30	♂	No	No	No	No	Yes	Yes	Yes	Yes
		♀	No	No	No	No**	Yes	Yes	Yes	Yes
White expanded	40	♂	No	No	No	No	No	No	Yes	No
		♀	No	No	No	No	No	No	Yes	Yes

*: The number of larvae found near the packaging with food is significantly higher than that of larvae in the environment.

**: The number of larvae found near the packaging without food is significantly higher than that of larvae found near the packaging with food.

-: Results of tests, where larvae perforated one or more packaging, were not statistically elaborated.

Table 7 - Number of abrasions and holes made by male and female Cadra cautella *(Walk.) II instar larvae on the packaging of biaxially oriented unprinted polypropylene, within 4 days. Moreover the distribution of total number of abrasions and holes on the packaging is reported.*

BIAXIALLY ORIENTED POLYPROPYLENE		ABRASIONS				HOLES			
Type	µm	♂	♀	Sealing	Surface	♂	♀	Sealing	Surface
Co-extruded	17	1	3	4	A	1	1	1	1
Co-extruded	20	A	4	4	A	1	1	A	2
Co-extruded	25	A	1	1	A	A	A	A	A
Co-extruded	30	2	2	4	A	A	A	A	A
2 sides acrylic coated	20	5*	5*	10**	A	1	3	A	4
2 sides acrylic coated	25	1	A	1	A	A	A	A	A
2 sides acrylic coated	30	1	A	1	A	A	A	A	A
White expanded	40	5*	1*	5**	1	A	A	A	A

Table 8 - Number of abrasions and holes made by male and female Cadra cautella *(Walk.) II instar larvae on the packaging of biaxially oriented printed polypropylene, within 4 days. Moreover the distribution of total number of abrasions and holes on the packaging is reported.*

BIAXIALLY ORIENTED PRINTED POLYPROPYLENE		ABRASIONS				HOLES			
Type	µm	♂	♀	Sealing	Surface	♂	♀	Sealing	Surface
Co-extruded	17	1	A	1	A	1	3	1	3
Co-extruded	20	1	A	1	A	A	1	1	A
Co-extruded	25	3	1	4	A	A	A	A	A
Co-extruded	30	1	1	2	A	A	A	A	A
2sides acrylic coated	20	1	2	3	A	4	A	4	A
2 sides acrylic coated	25	2	1	3	A	A	A	A	A
2 sides acrylic coated	30	A	A	A	A	A	A	A	A
White expanded	40	2*	3	5*	A	A	A	A	A

*: 1 attempt on the packaging without food was registered.
**: 2 attempts on the packaging without food were registered.
A: Absence of perforated packaging.

rences were noted between that one with and without food. Females were constantly attracted by the packaging of co-extruded polypropylene 25 µm containing food, even if they couldn't perforate it.

The packaging in co-extruded polypropylene 30 µm containing food is not

Table 9 - Number of packaging in biaxially oriented polypropylene, containing food, perforated by Cadra cautella *(Walk.) II instar larvae after 5 hours, 1, 2 and 4 days from the beginning of tests.*

BIAXIALLY ORIENTED POLYPROPYLENE	µm	5 hours	1 day	2 days	4 days
Co-extruded	17	A	A	1	2
Co-extruded	20	A	A	A	2
Co-extruded	25	A	A	A	A
Co-extruded	30	A	A	A	A
2 sides acrylic coated	20	A	A	A	4
2 sides acrylic coated	25	A	A	A	A
2 sides acrylic coated	30	A	A	A	A
White expanded	40	A	A	A	A
Co-extruded*	17	2	3	3	3
Co-extruded*	20	A	A	1	1
Co-extruded*	25	A	A	A	A
Co-extruded*	30	A	A	A	A
2 sides acrylic coated*	20	A	A	A	4
2 sides acrylic coated*	25	A	A	A	A
2 sides acrylic coated*	30	A	A	A	A
White expanded*	40	A	A	A	A

*: printed material.

A: Absence of perforated packaging

constantly attractive toward males, while it resulted more attractive than the empty one within the first day of test toward females.

Larvae of both sexes could perforate the material between the second and the fourth day, even if the packaging in 2 sides acrylic coated polypropylene film of 20 µm containing food isn't sometimes more attractive than the empty one.

Larvae of both sexes, in presence of the packaging of acrylic coated polypropylene of 25 µm, showed a higher tendency to disperse in the environment while with the thickness of 30 µm, the individuals are mostly attracted by the packaging with food compound, than the empty one, within the first day; afterwards the tendency to disperse inside the container became prevailing.

After 5 hours the packaging in white expanded polypropylene of 40 µm containing food, shows on the surface a higher number of males compared to the empty one, while this fact doesn't occur in the next controls. Females, on the contrary, showed the tendency to stay on the packaging with food source longer than on the empty one, but their number results lower than that one of the larvae dispersed inside the containers.

After 2 days, except when the packaging with food was perforated occurred, a tendency to abandon the packaging is registered for both sexes.

As to the types of printed biaxially oriented polypropylene, it was observed that packaging in co-extruded polypropylene printed (thickness: 17 μm) containing food was perforated within the first 5 hours of the test.

After 5 hours larvae are attracted in the same way by the two types of packaging in co-extruded polypropylene 20 μm; afterwards the packaging with food are preferred to those empty and, within the second day, one of the packaging was perforated by female larvae.

The packaging, both full and empty in co-extruded polypropylene, 2 sides acrylic coated polypropylene film, with a thickness of 25 and 30 μm, and also that in white expanded polypropylene (40 μm) always exerted a not significantly different attraction on larvae. Moreover, in the tests carried out with 2 sides acrylic coated polypropylene film of 25 and 30 μm, larvae always showed a higher tendency to disperse inside the containers.

Most of the attraction exerted by packaging with food source in 2 sides acrylic coated polypropylene film of 20 μm, compared to the empty one, was not constant over time. The tendency to disperse wasn't higher than that one to stay on the packaging with food; males perforated the packaging between the second and the fourth day.

CONCLUSIONS

Cadra cautella female and male larvae showed the same ability to perceive aroma filtering through the packaging as both sexes have to feed to complete their development. As far as the adults are concerned, aromas, exhaled by food source, exert a stronger attraction on fecundated females (Gothilf *et al.* 1993).

Cadra cautella second instar larvae don't perforate the packaging without food source; probably the individuals are stimulated to penetrate in a packaging when the coating allows the diffusion of the smell of food outside.

Larvae could perceive the aromas filter through the packaging in co-extruded biaxially oriented transparent polypropylene (printed or not) to a thickness of 20 μm. The individuals could perforate these packaging, showing that a low thickness doesn't offer enough mechanical resistance to protect a foodstuff from the attack of this moth. The packaging was perforated rather rapidly, even in less than 5 hours in the case of co-extruded printed polypropylene of 17 μm. Co-extruded biaxially oriented polypropylene wasn't perforated with a higher thickness (25 and 30 μm), as Cline already observed (1978a). Moreover, the results show that with such thickness larvae were not always attracted by packaging containing food compared to the empty one. This behaviour is more evident in printed materials. This difference could be explained by the fact that print represents an obstacle to aromas or that larvae, in their choice, are induced, besides by an olfactory stimulus, also by a visual one. In fact olometabolous larvae can distinguish a different reflex of the light (Doane & Leonard, 1975; Gilbert, 1994). Tests carried out by Harris *et al.* (1995) placing in a container newly hatched larvae of *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae) and pieces of card

of different colours (blue, green, yellow, orange and red), showed that larvae only approached green, yellow and orange ones in a not significantly different way.

It was observed that by diminishing permeability to aromas of packaging in 2 sides acrylic coated polypropylene film, larvae stay in a casual way on the packaging with or without food inside or in the surrounding area.

The packaging in 2 sides acrylic coated polypropylene film, with or without printing of a thickness of 20 μm, containing food, didn't exert a steady attraction toward larvae, but the material was perforated, confirming a low mechanical resistance provided by this thickness. Kail (1984) says that acrylic coated materials, if manipulated, don't provide any barrier against the aromas filtering. In fact the presence of coated doesn't improve mechanical resistance, but causes a better sealing of the material and reinforces the barrier to aromas filtering from the packaging outward. The protection provided by the coating wasn't enough for the film with the lowest thickness. Even the presence of the printing didn't affect larvae penetration ability; in fact also the packaging printed in 2 sides acrylic coated polypropylene film of a thickness of 20 μm, containing food, was perforated. With a higher thickness (25 and 30 μm) larvae better disperse in the area near the packaging, and this proves that packaged food doesn't exert particular attraction on them.

The white expanded polypropylene of 40 μm should offer a lower barrier to odorous molecules as it presents a scarcely homogenous structure with less compact areas which should facilitate the penetration of the larvae inside this packaging; nevertheless these features, *C. cautella* larvae can't perforate this material but they can only manage to carry out various attempts of penetration. Therefore these researches should be carried out to better clarify the behaviour of larvae on the different types of polypropylene.

During the test, it was observed that in most cases larvae were under the packaging or sheltered under the edge of the longitudinal seal, rather than over the packaging causing therefore a higher number of attempts of penetration on the seals, rather than on the surface of the packaging. The seals, such as folds at the edges of the packaging, offer to the individuals supports on which their mandibles can levy. Also Von Khan (1982) observed that insects prefer attack seals and edges of the packaging. These supports aren't indispensable to the penetration.

Larvae of Lepidoptera, thanks to the production of silk can in fact cling even to smooth and vertical surfaces (Cline, 1978b).

The results show that to protect confectionery products from the attack of *C. cautella* larvae is advisable to use film in coated acrylic polypropylene in both sides, with a thickness higher than 25 μm, as this type of packaging provides a good mechanical resistance and a low permeability to aromas. Several studies evaluate the substances which filter through from the packaging to food while there is little research about the passage of odorous molecules of food to packaging material and outward.

In fact standard processes were set up to evaluate the permeability of plastic materials to gases (such as O_2 and CO_2) and to steam (Tibaldi & Fava, 1999) while an official method doesn't yet exist for organic vapours (Fayoux *et. al.*, 1997; Kail, 1984).

In these last years, in the field of flexible packaging, materials with a very low thickness are preferred to reduce production and disposal costs, in order to avoid the attacks of insects to packaged foodstuff it is important that materials used show a mechanical resistance and a barrier to the passage of odorous substances.

In fact keeping these substances inside a packaging, not only preserves sensorial features of foodstuff but also limits the attraction exerted toward the same infesting insects.

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