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Ability of *Plodia interpunctella* (Hbn.) (Lepidoptera: Pyralidae) larvae to find food and flavours

Abstract - The ability of I and III instar larvae of *Plodia interpunctella* to find food was evaluated in a polypropylene one way olfactometer. As attractants an artificial diet, corn meal, almonds and raisins were used. The attraction of artificial flavours used in the bakery industry was tested on III instar larvae: almond, hazelnut, Sicily lemon, chocolate and croissant. The higher number of I instar larvae was observed on artificial diet and corn meal as far as 3 m distance. III instar larvae are indifferently attracted by the substrates as far as 4 m; the artificial diet and corn meal resulted the most attractive ones as far as 5 and 6 m distance. Only few larvae were attracted by flavours.

Riassunto - *Capacità delle larve di* Plodia interpunctella *(Hbn.) (Lepidoptera: Pyralidae) di individuare alimenti e aromi.*

In un olfattometro di polipropilene a una via è stata valutata la capacità di spostamento di larve di I e III età di *Plodia interpunctella* alla ricerca di cibo. Sono stati utilizzati come attrattivi dieta artificiale, farina di mais, mandorle e uvetta passa. Su larve di III età è stata saggiata l'attrattività di alcuni aromi impiegati nell'industria da forno: mandorla, nocciola, limone di Sicilia, cioccolato e croissant. Il numero maggiore di larve di I età è stato osservato su dieta artificiale e farina di mais; su questi substrati sono stati trovati alcuni individui fino a 3 m di distanza. Le larve di III età non mostrano preferenza per i diversi substrati fino a 4 m; a 5 e 6 m la massima attrattività si riscontra per dieta artificiale e farina di mais. Solo poche larve sono state attratte dagli aromi.

Key words: *Plodia interpunctella*, larvae, mobility, attraction, foods, flavours.

INTRODUCTION

Stored product moths lay eggs preferably on food attracted by flavours (Barrer & Jay, 1980); sometimes eggs are not laid directly on the food and I instar larvae are obliged to seek a proper nourishment.

Insects identify food by odour that can attract or repel according to the insect's

stadium (Chapman, 1969). In fact olfactive identification is sufficient in the first phase of approach, when insect uses the odour to head for the source, afterwards the sense of taste can confirm the choice (Dethier, 1937; Kennedy, 1965; Levinson & Levinson, 1978). After a first tasting, if the food is recognized, insect keeps on eating, otherwise it will stop after a few bites.

Olfactive attraction, especially in stored product insects, can be induced by a blend of volatile flavour substances emitted from food sources like wheat or other cereals, while the ability to gather on food and to eat can be stimulated by components such as salts, sugars and fats (Kennedy, 1965).

Moth larvae present little developed antennae and the few sensilla have a very simple structure (Hansson, 1995). In this work the food searching ability of larvae of *Plodia interpunctella* (Hbn.) was evaluated.

MATERIALS AND METHODS

Olfactive ability of *Plodia interpunctella* larvae was evaluated using a polypropylene one way olfactometer (thickness 30 μ, diameter 19 cm, length variable from 1 to 8 m). The choice of the material was made according to features of flexibility, transparency, gas permeability and its resistance to insects' perforation; besides larvae of this species are not able to climb polypropylene film (Cline, 1978a, b).

Each olfactometer has 30% of the surface provided with net covered opening (120 mesh) to allow gas exchange and the two ends of each opening were covered with a net to insert insects and food substrates. At one of the ends insects were placed while at the other end 20 g of the test substrate were placed. The olfactive response of larvae to corn meal, almond, raisins and artificial diet, placed one at a time at variable distance, was observed. Ingredients of the artificial diet are: bran (4 parts), corn meal (2 parts) , wheat meal (2 parts), glycerin (1,5 parts), wheat germ (1 part), honey (1 part) and yeast (1/2 part). In order to verify possible movements when there is no food, tests were carried out placing an adhesive cardboard instead of food.

Newly hatched larvae were obtained from 100 eggs 24 to 48 hours old. Controls were carried out after 10 days, in order to allow eggs to hatch, that happens in 3-4 days in test condition, and larvae to reach food. Food was analysed under a stereomicroscope for larvae presence.

Twenty III instar larvae were used for each replicate. After 2 days the cardboard with the food was analysed under a stereomicroscope for larvae presence.

In the same condition as the previous test, at a 2 m distance, attraction of some artificial flavours[1] used in the bakery industry was tested on III instar larvae made of synthetic flavours, natural flavours and different components according to the flavour: almond: vegetal oil (max. 10%), safrole (max. 0.011%); hazel nut: vanillin (max. 0.1%), benzyl alcohol (max. 0.1%); Sicily lemon: butyl hydroxyanisole (max.

[1] Supplied by Metroz Essences, via Andrea Doria 40 - I-20093 Cologno Monzese (MI)

0.03%); chocolate: 1,2-propylene glicol (max 65%), ethyl vanillin (max 1%); croissant: glycerol triacetate (max. 35%), ethanol (max. 15%), butyl hydroxyanisole (0.03%). A paper (1x1cm) imbued of the artificial flavour to test (50 µl) was placed in the middle of a rectangular adhesive surface (12x6 cm). After 2 days the number of larvae on the adhesive cardboard was verified.

For each test 4 replicates were carried out at 26±1°C and a photoperiod of 13L:11D. Results were analysed using ANOVA and Duncan test.

RESULTS AND DISCUSSION

The mean number of eggs hatched observed during the tests was 95.1±0.4. Only I instar larvae that reach the substrate were counted at the end of the tests because it was difficult to find them in the olfactometer. III instar larvae remain viable during the tests.

Without food I instar larvae were not attracted to the adhesive substrate (Table 1), while only few III instar individuals reached a distance of 1 to 3 m (Table 2). In the absence of food larvae do not perceive food and volatiles emitted by flavours, therefore, they dispersed without orientation.

The mean number of I and III instar larvae on the different substrates was inver-

Table 1 - Mean number (±S. E.) of I instar larvae of Plodia interpunctella *(Hbn.) able to reach different foods at variable distances.*

Substrate	Distance (m)				
	1	2	3	4	5
No substrate	0±0a	0±0a	0±0a	0±0a	-
Artificial diet	27.5±6.28c	11.7±1.65c	2±0.82ab	0.7±0.48a	0
Raisins	7.5±1.04b	0.5±0.29a	00±0a	0±0a	-
Almond	9.0±0.91b	3±0.7b	0.7±0.48a	0±0a	-
Corn meal	31.7±2.87c	11.2±1.75c	3.2±1.18b	0.2±0.25a	0

Table 2 - Mean number (±S.E.) of III instar larvae of Plodia interpunctella *(Hbn.) able to reach different foods at variable distances.*

Substrate	Distance (m)							
	1	2	3	4	5	6	7	8
No substrate	2.7±0.75a	1.7±0.75a	0.2±0.25a	0±0a	0±0a	0±0a	0±0a	-
Artificial diet	16.5±0.87b	14.5±1.04bc	12.7±1.1b	8.2±0.63b	7.7±1.55b	3.5±1.04c	0.5±0.29a	0
Raisins	16.5±1.32b	16.7±0.48c	10.7±0.71b	8.2±1.11b	2±0.82ab	1.0±0.7b	0±0a	-
Almond	16.0±0.91b	13.2±1.25b	12.7±1.1b	8.7±0.75b	1.7±0.63ab	1.0±0.4b	0±0a	-
Corn meal	16.0±1.29b	14.5±0.65bc	11.5±0.64b	11±1.08b	5.5±1.04b	2.0±0.58bc	0±0a	-

sely proportional to the increase of the olfactometer length (Tables 1-2). A few III instar larvae move 6 m in order to reach the artificial diet.

The highest number of I instar larvae was observed on artificial diet and corn meal (Table 1). At a distance of 4 m just one I instar larva was found on artificial diet and corn meal, while no individuals were observed on the other substrates.

III instar larvae don't show any preference for the different substrates as far as 4 m; the highest attraction was noticed for artificial diet and corn meal at a distance of 5 and 6 m (Table 2).

I and III instar larvae prefer artificial diet and corn meal; the latter is one of the main ingredients of the rearing substrate. Crombie (1941) observed that *Rhyzopertha dominica* F. larvae, in front of different foods, prefer the one that previous generations were developed. Moreover these foods contain high percentage of carbohydrate and fat acids. Baker & Mabie (1973) observed that foods with glucose, fructose and maltose are more attractive and the association of saccarose with linoleic, oleic and palmitic acids has synergic effect on feeding of *P. interpuctella* larvae.

III instar larvae were attracted indifferently by various foods as far as 3 m, while I instar larvae were mainly attracted to artificial diet and corn meal. In the present work whole almonds and raisins were used, while it was shown that newly hatched larvae prefer crumbly or broken food (Candura, 1932; Zacker, 1945; Johnson *et al.*, 1992; 1995). In addition ground food has apparently a higher emission capacity of volatiles of flavour substance than the whole one, this due to the increased surface area of the product (Locatelli & Süss, 2003).

Only one or three individuals of III instar larvae are attracted by the different artificial flavours at a distance of 2 m (Table 3). A similar result was observed in absence of food.

Table 3 - Mean number (±S.E.) of III instar larvae of Plodia interpunctella *(Hbn.) able to reach different foods and flavours at a distance of 2 m.*

Substrate	Mean ± S. E.
No substrate	1.7±0.75a
Artificial diet	14.5±1.04bc
Raisins	16.7±0.48c
Almond	13.2±1.25b
Corn meal	14.5±0.65bc
Almond flavour	1.5±0.86a
Hazelnut flavour	1.7±0.86a
Sicily lemon flavour	2.2±0.63a
Chocolate flavour	0.5±0.29a
Croissant flavour	0.5±0.29a

CONCLUSIONS

Plodia interpunctella (Hbn.) larvae were able to perceive different food substrates even at considerable distance and III instar ones can move as far as 6 m. Newly hatched larvae, with limited energy sources, are less mobile than III instar larvae, which have a sufficient energy reserve.

The flavours, used as ingredients in the bakery industry, tested on III instar larvae were not found significantly different in attraction; in fact, comparing, at the same distance, the attraction of almond flavour with almonds, food particularly appreciated by moth (Cox & Bell, 1991; Gothilf *et al.*, 1993; Meaney, 1998), only the seed is infested. Baker & Mabie (1973) observed that natural extracts of wheat, corn and peanuts contain positive sensory stimuli that are involved in short-range orientation. It's worth considering that artificial flavours, used in the bakery industry, are made of numerous components not present in the natural flavour, some could act as repellents, as some ingredients of synthetic flavours. Moreover only few components of the natural flavours can be used in the artificial flavours, or their quantity can be different and, for this reason, they do not result in attraction for the insect.

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