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Functional morphology of *Bactrocera oleae* Gmel. (Diptera: Tephritidae) tarsal chemosensilla involved in interactions with the host-plant

ABSTRACT

The Olive fruit fly, *Bactrocera oleae* (Gmel.), is the principal insect pest insect on olives in the Mediterranean Region. Observations through scanning and transmission electron microscopy evidence contact-chemosensilla trichodea on the ventral side of each 2nd to 5th prothoracic tarsomeri. These sensilla look very similar to one another, although rather varied in size, and show uniporous, bluntly tipped hair-shaft. Also the sensillum cellular components display almost the same features in all sensilla: 5 sensory neurons per sensillum, one of which ending with a tubular body at the hair-shaft base (hence representing a mechanosensory element), whereas the other 4 neurons send each an undivided dendrite into the dendritic channel of the biluminal hair-shaft. On the 5th tarsomere the mentioned sensilla are 8-10 distributed as follows: a pair medial at the tarsomere distal margin, and the rest in two subdistal, sublateral groups of 3-4 elements each. Detailed observations and relative illustrations of cuticular and cellular components of the 2 medial sensilla ("C") are herein reported. Direct contacts between sensory neuron somata have been observed, which might be indicative of possible peripheral interactions between sensory neurons. Behavioural bioassays confirm oogenesis and oviposition stimulation in *B. oleae* female through tarsal contact with host-plant substances such as Oleuropein and its demolition products (e.g., Pyrocatechin). Electrophysiological bioassays on "C" sensilla evidence a response to Oleuropein and Pyrocatechin, and confirm the above mentioned possibility of interactions between the sensory neurons.

Key words: behaviour, contact chemoreceptor, electrophysiology, Oleuropein, olive fruit fly, oogenesis stimulants, oviposition, Pyrocatechin, sensory neurons, sheath cells, somata contacts, ultrastructure.

1. INTRODUCTION

The Olive fruit fly, *Bactrocera oleae* (Gmel.), is the principal insect pest insect on Olives in the Mediterranean Region. It is known that chemoreception is crucial in host-plant finding and selection by the Fruit-flies (LEVINSON &

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LEVINSON, 1984; DREW, 1987; DREW and FAY, 1988). Also, it has been reported that the Olive juice released from the olive Fly oviposition wound and by the Fly normally spread on the olive surface, prevents further ovipositions on the same fruit (CIRIO, 1971). The deterrent activity on oviposition are mostly linked to the oily fraction of olive juice but hydrosoluble phenols are also implicated (GIROLAMI et *al.*, 1981). Non volatile olive compounds such as the glycoside Oleuropein stimulate the Fly oogenesis most probably by contact with tarsi (GIROLAMI et *al.*, 1989; GIROLAMI & COIUTTI, 1991, 1994).

Thus, it is likely that such insect-plant interactions may be mediated by the Fly tarsal contact chemosensilla as it has been proved in other Tephritidae (CRNJAR & PROKOPY, 1982; STÄDLER *et al.*, 1994) and Anthomyiidae (ROESSINGH *et al.*, 1997). In fact, it is interesting that the female Olive fruit fly just landed on an olive-fruit preliminarily rubs prothoracic tarsi (as it were washing hands: fig. 1) next it proceeds with examining and probing the fruit and finally it ends (or not) with oviposition.

Our aim was to evidence a mediation of the tarsal contact chemosensilla in

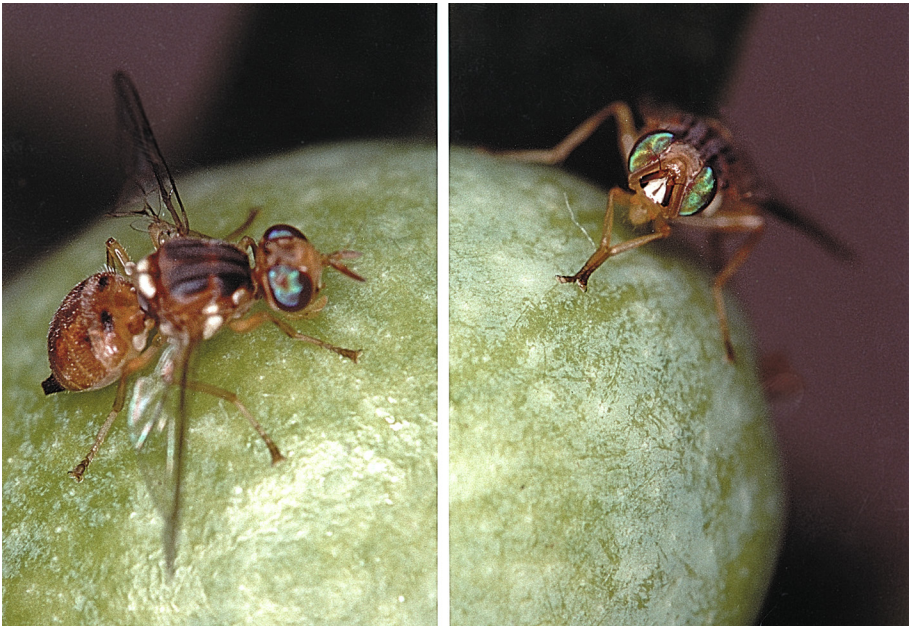

Fig. 1 - *Bactrocera oleae* (Gmel.). Female on an olive, exploring the fruit surface (on left), and rubbing prothoracic tarsi (as it were washing hands).

the mentioned interactions between the Fly and the host-plant. All that, through behavioural bioassay using the above mentioned substances (especially Oleuropein or its demolition products), as well as through electrophysiological bioassay using the same plant-compounds on the contact-chemosensilla trichodea present on the ventral side of each prothoracic 5th tarsomere, in particular the so called "C" sensilla, on the basis of ultrastructural and morpho-functional investigations.

2. MATERIALS AND METHODS

2.1. Morphology

For SEM observations, excised foreleg tarsi of *B. oleae* females were dehydrated in graded ethanol series, critical point dried in a Balzers Union CPD 020 unit, gold coated in a Balzers Union SCD 040 sputter unit, and viewed-micrographed through a Philips XL30.

For TEM observations, foreleg tarsi from newly emerged, CO_2 anesthetized *B. oleae* females, collected as pupae from the fields, were excised, immediately immersed in Karnovsky's (1965) fixative with 2% Acrolein and left for 3 h at 4°C. Then the tarsi were washed overnight in cacodylate buffer with 5% sucrose, postfixed in 1% Osmium tetroxide for 1 h 15 min, rinsed in the same buffer, dehydrated in graded ethanol series, block stained with 1% Uranyl acetate in 95% ethanol solution for 1 h (during dehydration process) and finally embedded through propylene oxide in Epon-Araldite. Thin sections obtained by an L.K.B. "Nova" ultramicrotome, sequentially stained with Uranyl acetate and Lead citrate, were examined through a Philips EM 400T.

2.2. Electrophysiological bioassay

For electrophysiological experiments were used *B. oleae* mated, gravid females (20-30 days old), obtained from olives (cv "Gentile di Larino") collected in the fields in Central Italy (Campobasso) during October and November, kept in Plexiglas cages (30x25x28 cm) at 20±2°C, 60±10% R.H., L:D cycle 12:12 h, and fed with a diet based on sucrose, casein and yeast (ratio 1:1:1), supplied on a wet cotton ball.

Electrophysiological responses from "C" sensilla on the foreleg 5th tarsomere were recorded combining different equipments and techniques previously used to study single chemosensory (gustatory or olfactory) sensilla (Hodgson *et al.* 1955, Den Otter, 1992; De Cristofaro, 1995; Den Otter *et al.*, 1996; De Cristofaro *et al.*, 1998).

The indifferent electrode, i.e., a glass micropipette with tip diameter of 1 mm, filled with a Beadle-Ephrussi saline, was applied to the proximal end of a prothoracic leg, excised at the coxo-femural joint. The recording electrode, i.e., a glass micropipette with tip diameter of 2-4 µm, containing one of the test stimuli (i.e., 10 mM Oleuropein, Pyrocatechin or Elenoic acid, in 100 mM NaCl solution) or 100 mM NaCl alone (control), was contacted to the sensillum tip. Higher concentrations of the test solutions induced deformations in the spike shape and amplitude so that single cell responses were hardly analysable, while lower concentrations showed spike frequency not dissimilar to the control solution. Electrical connection was obtained by silver wires inserted into the glass pipettes and attached to an electrophysiological equipment (INR-01®, Syntech®, The Netherlands). Since our aim was just to find out cells sensitive to our stimuli, concentrations were chosen according to preliminary tests.

The test solutions were put in the micropipette 10 s before the experiment. Single sensillum recordings were carried out at 22±2°C and 70±10% R.H. Electrical activity was recorded for 1 s after stimulus onset and 5 min was allowed to elapse between presentation of successive stimuli to the same sensillum. Test and control solutions were applied, in a random series on the same sensillum. Action potentials (spikes) were recorded on a magnetic tape (Sony® CditII, IEC II/Type II, High Bias 70 ms EQ, position chrome) by a double channel recorder (Sony® TC-D5M) and successively analysed with the programme AutoSpike™ 3.1 (Syntech®, The Netherlands).

Sensilla which failed to respond to the tested solutions were considered not-functioning and discarded (Crnjar & Prokopy, 1982). Responses of the sensory cells were evaluated as spike frequency (spikes/s) during the first second of stimulation, 100 ms after stimulus onset. Firing frequencies were compared by means of the Student's *t*-test.

2.3. Oogenesis and oviposition behaviour bioassays

The experimental *B. oleae* females emerged in the laboratory from pupae collected with infested olives (cv. "Bianchera" or "Casaliva") from the field in September- December, or from the ground of an oil-press factory in January - March. Pupae were kept at laboratory conditions or stored at 11-12°C for 30 days at most. The females were kept in Plexiglas boxes with tulle's bases (6x12x27 cm), in a room at 23±2°C, 55±15% R.H., and photoperiod of 14 hours of light (L:D = 14:10), and fed with saturated sucrose water solution

(GIROLAMI, 1979).

As oviposition beds were used either olives (cv. "Bianchera" or "Pendolino") harvested in September in Trieste province (Noth-East Italy) and stored in refrigerator at 4°C until utilization or hemispheric, artificial oviposition beds, sized like an olive, made of 4% agar and 0.05% benzoic acid water solution, and chlorophyll (just to give green colour), and finally covered with paraffin.

The test solutions (fig. 8), i.e., 0.1% Oleuropein, Pyrocatechin or Elenoic acid in ethanol, were spread on the artificial beds at a rate of 10 µl per each one.

For testing oogenesis stimulation by the above mentioned substances, three experimental sets have been arranged, each consisting of at least 3 cages containing 10 newly emerged females each: - the females of the first set were presented with 2 artificial beds per cage, both sprayed with test solution; - the females of the second set were presented with same number of artificial beds not sprayed; - the females of the third set were presented with 2 olives per cage.

With each cage, at detection of the first oviposition punctures on the substrate, several females were dissected to ascertain the degree of egg maturation; the rest of females were dissected almost 3 days later on the same purpose.

The ovaries, according to the maturation degree of the eggs, were divided into three developmental stages, i.e., previtellogenesis, vitellogenesis and mature eggs (FLETCHER *et al.*, 1978). The G-test of independence (SOKAL & ROHLF, 1981) was applied to results.

For testing oviposition stimulation by the test solutions, mated females 10-40 days old were used. The experimental protocol was similar to the above reported, but at least 100 females, divided into at least 16 cages, per experimental set were employed; furthermore, of the 2 artificial oviposition beds per cage, one was treated with the test solutions, whereas the other not; and of the 2 olives per cage, one was replaced by a non treated artificial bed. To compare the number of eggs (counted at stereomicroscope) laid on the different oviposition substrates the Student *t*-test after normalization of the data was used.

3. RESULTS

3.1. Morphology

3.1.1 Tarsal functional morphology concerning ventral chemoreceptors

The tarsi of *B. oleae* consist of 5 articles (fig. 2,a), the first of which is the longest but normally does not come in contact with the substrate on which the Fly walks, whilst the fifth (fig. 2,a,b) is the widest and it always touches the olive surface during the Fly walking or exploration; and tarsomeres 2nd to 4th may or may not touch the substrate according to the Fly behaviour.

Observations through scanning and transmission electron microscopy evidence contact-chemosensilla trichodea (sensu Schneider, 1964; Altner and Prillinger, 1980) symmetrically distributed in two sublateral groups on the ventral side of each 2nd to 5th tarsomeres. These sensilla look very similar to one another, although rather varied in size, and show uniporous (fig. 2,a), bluntly tipped hair-shaft bearing fluted walls (fig. 2,d). Also the sensillum cellular components display almost the same features in all sensilla. In fact, there are always 5 sensory neurons per sensillum, one of which ending with a tubular body at the hair-shaft base (hence representing a mechanosensory element), whereas the other 4 neurons send each an undivided dendrite into the dendritic channel of the biluminal hair-shaft.

On the 5th tarsomere, in particular, there are 8-10 of the sensilla in question (fig. 2,c): a pair medial (the "C" sensilla according to Grabowski & Dethier's terminology, 1954) at the tarsomere distal margin (fig. 2,c,d), and the rest in two subdistal, sublateral groups of 3-4 elements each.

3.1.2 Fine morphology of "C" sensilla

The sensillum cuticular apparatus consists of a hair-shaft having fluted walls (fig. 2,d), quite rigid, anteroventral oriented and gently bent at distal third, about 40 µm long, about 3 µm in diameter at base and gradually tapering to a rounded tip about 0.5 µm wide and bearing a simple apical pore (fig. 3,a) naturally concealed by viscous material that condenses outside (as it appears in SEM observations) and inserted in a specialised, flexible socket (figs 3,d,e; 4,a). The shaft lumen is longitudinally partitioned (figs 3,b,c) almost to tip, into two compartments or channels, i.e., the "inner" (posterior, or better proximal) and the "outer" (anterior, or distal), also called "dendritic channel" and "sensillar channel" respectively, for the former is continuous with the inner sheath cell space (ciliary sinus) and encloses the dendrites, and the latter is an extension of the outer sheath cells' space (sensillar sinus). The dendritic channel terminates with the apical pore

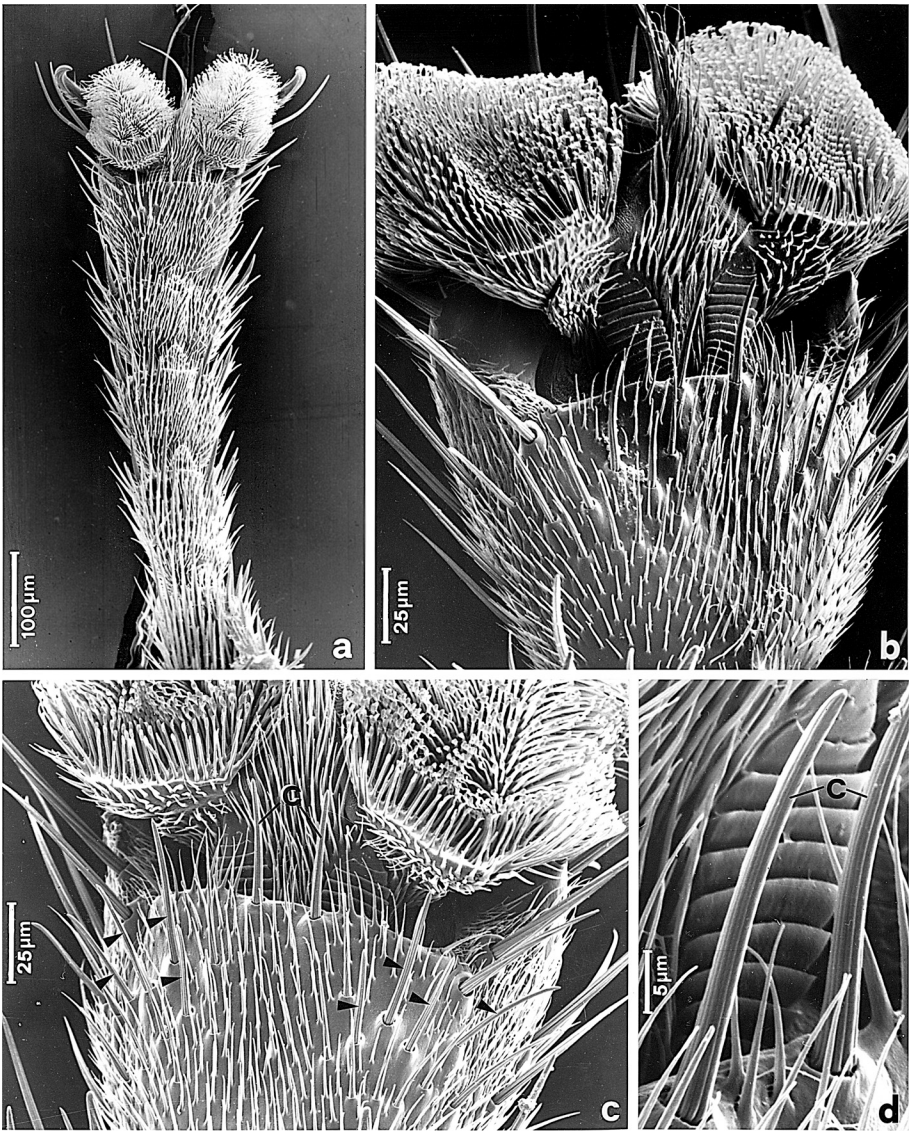

Fig. 2 - Scanning electron micrographs of a female prothoracic tarsus, ventral view: a, 1st (distal portion) to 5th tarsomeres and pretarsus; b, 5th tarsomere with pretarsus; c, detail of b showing the whole set of ventral chemosensilla (C, and arrow heads); d, detail of c displaying "C" sensilla.

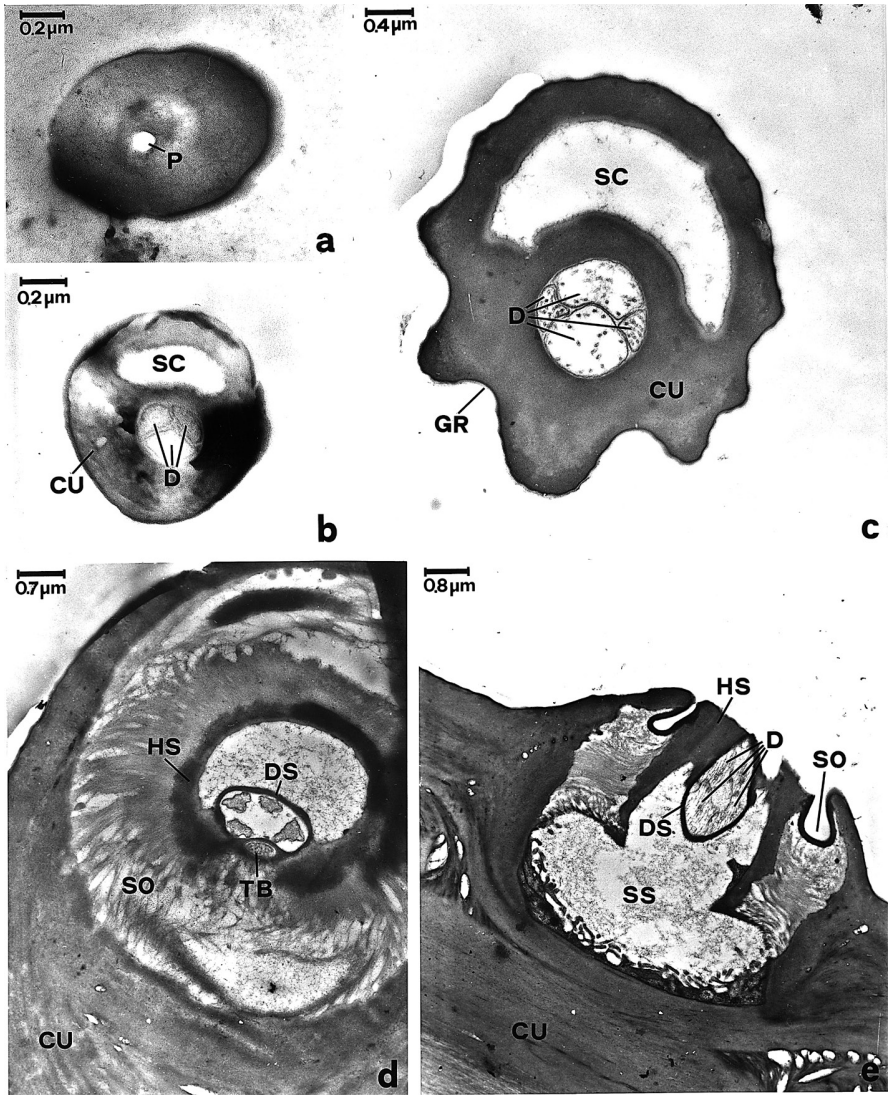

Fig. 3 - Transmission electron micrographs of "C" sensillum. Hair-shaft cross sections: a, almost tangential to the tip, showing apical pore (P); b, subdistal, showing dendritic channel with three of the four chemoreceptive dendrites (D), and sensillar channel (SC); c, intermediate, with all four dendrites (D) filling the dendritic channel; d, through socket region, showing the mechanosensory element ending with tubular body (TB) at hair-shaft base (HS); e, oblique section of the socket region, displaying the four chemosensory elements entering the dendritic channel in the hair-shaft (HS). CU, cuticle; DS, dendritic sheath; GR, wall groove; HS, hair-shaft; SO, socket; SS, sensillar sinus.

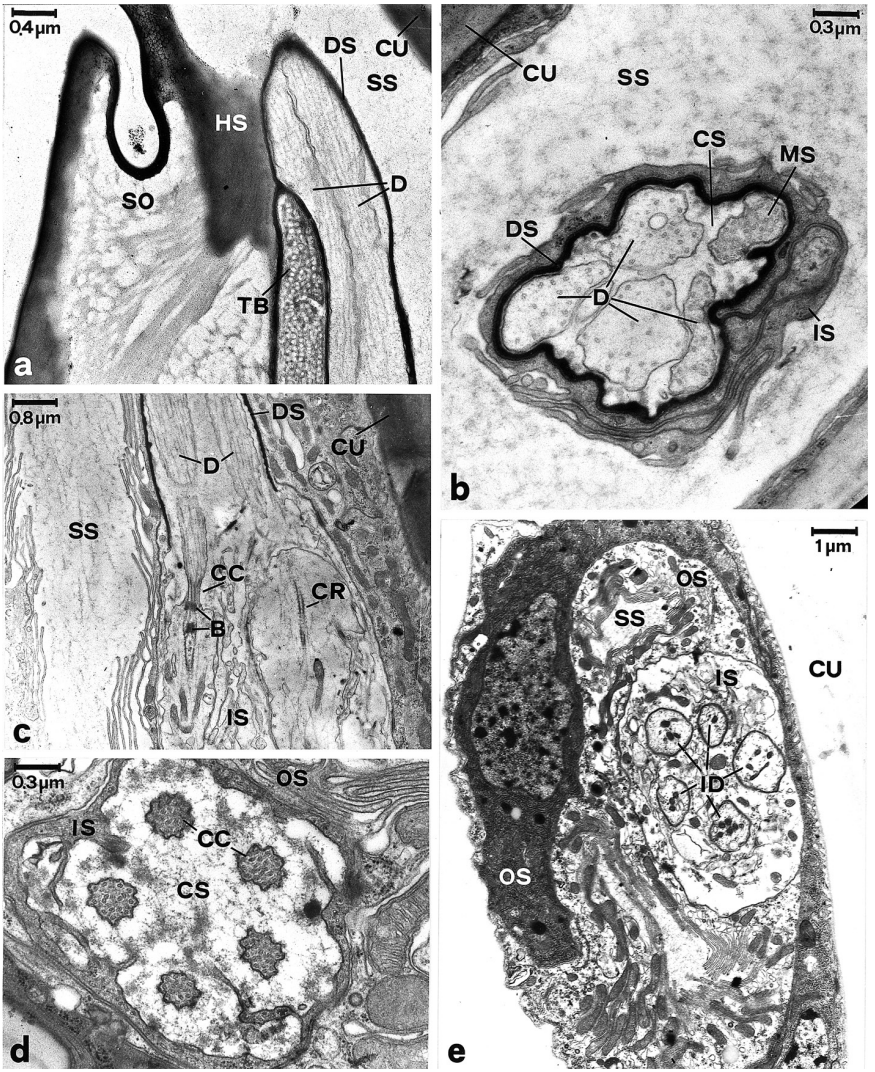

Fig. 4 - "C" sensillum cellular components: a, sublongitudinal section through socket region (SO), showing the mechanosensory element termination (TB) at hair-shaft base (HS) and two chemoreceptive elements (D) entering the hair-shaft lumen still encased in a thick dendritic sheath (DS); b, cross section through ciliary sinus (CS), showing the five outer dendritic segments of which the mechanosensory element (MS) is already distinct from the chemosensory ones (D); c, sublongitudinal section through ciliary sinus region, displaying two of the five sensory elements; d, cross section at ciliary constriction (CC) level; e, cross section general aspect at inner dendritic segments (ID) level. B, basal bodies; CR, ciliary rootlets; CU, cuticle; IS, inner sheath cell; OS, outer sheath cell; SS, sensillar sinus.

whereas the sensillar channel ends, just proximally to that (fig. 3,a), apically closed by a thin cuticular *septum*.

The cellular components are represented by five sensory neurons and three auxiliary or sheath cells.

The sensory cell somata lie just beneath the integument, all together held by the innermost (thecogen) sheath cell (IS, fig. 4,b,c,d,e) without being completely isolated from one another so that direct contacts between sensory cell somata take place (fig. 5). The sensory neurons send inner dendritic segments roughly parallel to the tarsomere ventral wall (fig. 4,c). The outer dendritic segments cross the sensillar sinus (SS, Figs 3,e; 4,a,b,c), enclosed in a thick dendritic sheath (DS, Figs 3,d,e; 4,a,b,c). One of them terminates with a conspicuous tubular body (TB, Figs 3,d; 4,a) at the shaft base, thus forming a mechanosensory element; whereas the other four dendrites enter the dendritic channel of the hair-shaft (fig. 3,d,e) and, naked and unbranched, run it almost to the tip (fig. 3,b,c), thus representing four chemosensory elements.

The auxiliary cells typically consist of an innermost sheath cell (thecogen) and two outer sheath cells (trichogen and tormogen). The thecogen cell (IS, Figs 4, 5) is quite large, envelops the sensory somata (see above) and the inner dendritic segments to whose distal portion it is connected by extensive septate junctions (fig. 4,e). This cell forms the inner boundary of the ciliary sinus (CS, fig 4,b,d) and a conspicuous labyrinth (LA, fig. 5,a) which is continuous with the sinus and extends down almost to the level of the sensory somata. The thecogen cell cytoplasm (IS, fig. 5,b,c,d) displays conspicuous multivescicular bodies (lysosomes ?), rough endoplasmic reticulum and moderate numbers of mitochondria. The thecogen cell secretes a quite thick dendritic sheath that encases the outer dendritic segments (see above) from the ciliary sinus (fig. 4,c) to the shaft base where it terminates fusing itself with the inner walls of the dendritic channel (Figs 3,d,e; 4,a). The trichogen and tormogen cells (OS, fig. 4,d,e) are ultrastructurally very similar to one another, thus they only can be distinguished by their typical position in the sensillum. They display a cytoplasm relatively rich in rough endoplasmic reticulum and ribosomes scattered in groups, in mitochondria (especially accumulated close to the very extensive apical cell membrane) and in lysosome-like structures. The apical membranes of both outer sheath cells together form a conspicuous sensillar sinus (SS, Figs 3,e; 4,b,c,e) lined with dense microvilli and microlamellae, and extended down beyond the level of the ciliary constrictions (fig. 4,c,e) but well separated from the ciliary sinus by the thecogen cell and the dendritic sheath (see above).

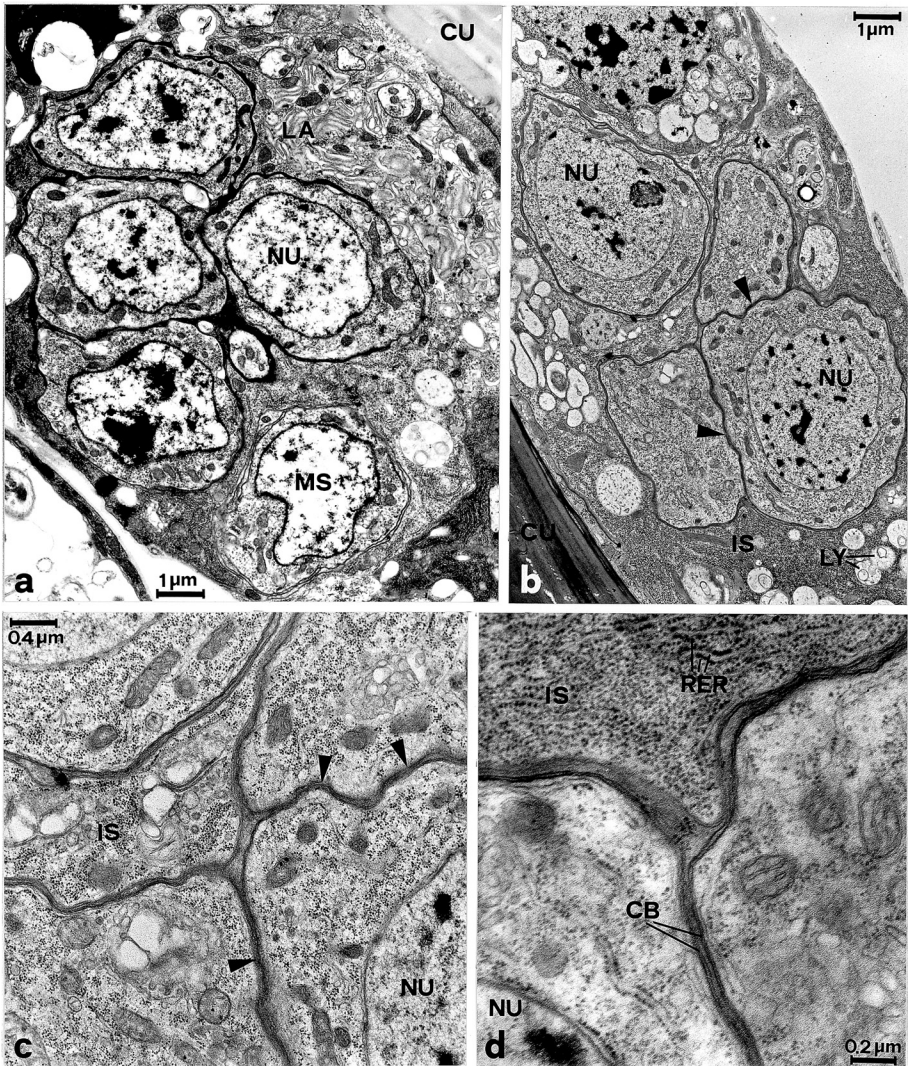

Fig. 5 - "C" sensillum cellular components, cross sections at sensory cell somata level, displaying: a, general view with mechanosensory element (MS) obviously apart from the chemosensory ones which are comparatively closer or adjacent to one another; b, another similar section showing two pericarions both in direct contact (arrow heads) with a third one; c, detail of the latter; d, detail of direct contact between two pericarions showing the cell boundaries (CB) running tight and parallel to one another, while the inner sheath cell (IS) remains well apart. CU, cuticle; LA, labyrinth of the ciliary sinus; LY, lysosome-like structures; NU, sensory neuron nucleus; RER, rough endoplasmic reticulum.

3.2. Electrophysiological bioassay

On electrophysiological bioassay 20% of the contacted "C" sensilla allowed to record spike activity.

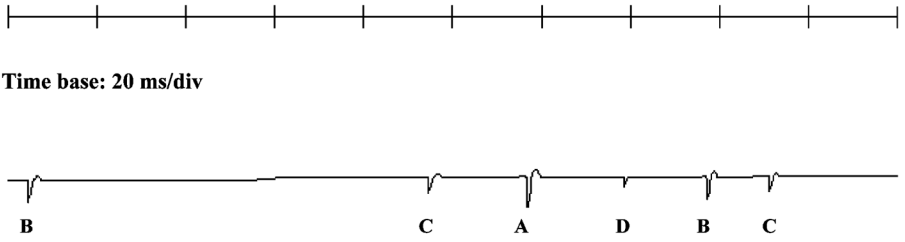

Fig. 6 - Action potentials with decreasing amplitude (A, B, C, D) elicited by the chemosensory cells of a *B. oleae* "C" sensillum.

Through applications of the control solution (100 mM NaCl), 4 action potentials were recorded, characterized by different amplitudes referred (in decreasing order, fig. 6) to A, B, C, and D eliciting neurons; while the spike frequencies from the same neurons show an opposite trend (tab. 1).

The neurons evoking the highest (A) or the lowest (D) spike amplitudes did not respond to test solutions with significant increases in action potential frequency (tab. 1). Responses from the cells B and C, both eliciting action potentials of intermediate amplitude, were hardly distinguishable (fig. 7) all the more when higher concentrations of test solutions were applied.

Cell C responded to Pyrocatechin, while cell B responded to both Oleuropein and Pyrocatechin solutions, with a significant increase ($P \leq 0.01$)

Tab. 1 - Spike frequency (spikes/s ± DS) recorded from four cells (A, B, C, D) with different action potential amplitude of a *B. oleae* "C" sensillum (n=30) on stimulation with Oleuropein and Pyrocatechin (10 mM) in NaCl solution (100 mM).

Cell	NaCl	Oleuropein	Pyrocatechin
A	3.4±1.8 a	6.0±2.3 a	3.9±1.8 a
B	5.6±1.6 a	9.7±2.6 b	12.8±3.6 b
C	6.5±2.1 a	8.5±2.2 a	14.6±2.8 b
D	8.7±2.1 a	9.1±1.9 a	10.6±2.2 a

Different letters on the same line show significant differences ($P \leq 0.01$).

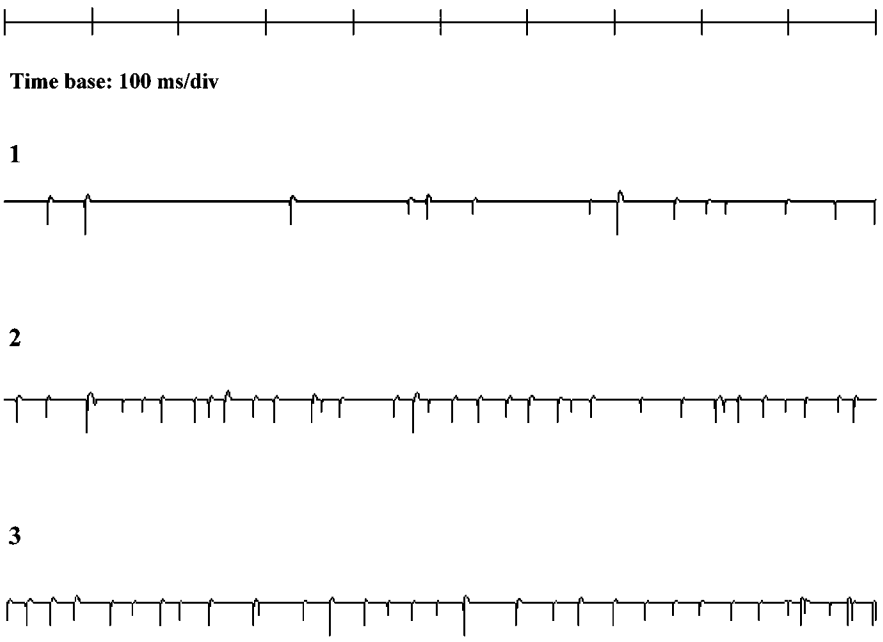

Fig. 7 - Action potentials recorded from a "C" sensillum stimulated by different solutions: 1, NaCl 100 mM; 2, NaCl 100 mM + Oleuropein 10 mM; 3, NaCl 100 mM + Pyrocatechin 10 mM.

in spike frequencies (tab. 1).

Elenoic acid solution did not produce spike frequencies dissimilar from those obtained with control solution.

3.3. Oogenesis and oviposition behaviour bioassays

3.3.1. Oogenesis stimulation

The experimental data clearly show (fig. 9) oogenesis stimulation on *B. oleae* by contact with olives or Oleuropein. These results from dissection tests confirm previous results from behavioural observations (Girolami *et al.,* 1981;

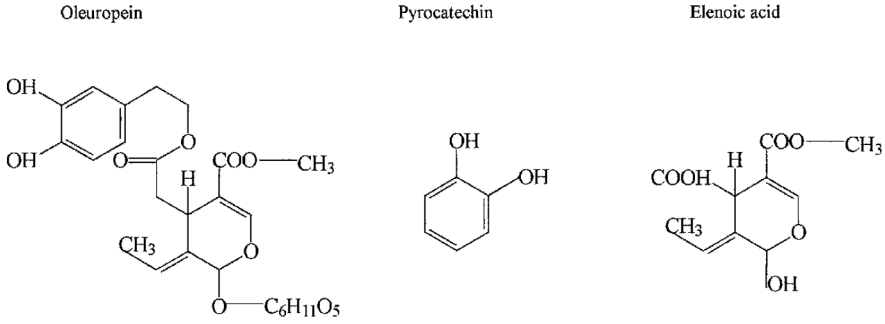

Fig. 8 - Chemical structure of tested substances.

GIROLAMI, *et al.* 1989; GIROLAMI & COIUTTI, 1991 and 1994).

In the laboratory, oogenesis stimulation produced by Oleuropein sprayed on artificial oviposition beds has proven to be similar to that produced by olive fruits.

Pyrocatechin stimulated oogenesis at a less extent than Oleuropein.

Elenoic acid sprayed on artificial oviposition beds gave not different results from untreated artificial beds.

It is noteworthy that several tests with spreading the cage walls with Oleuropein solutions, or adding the latter to the diet, did not produce any

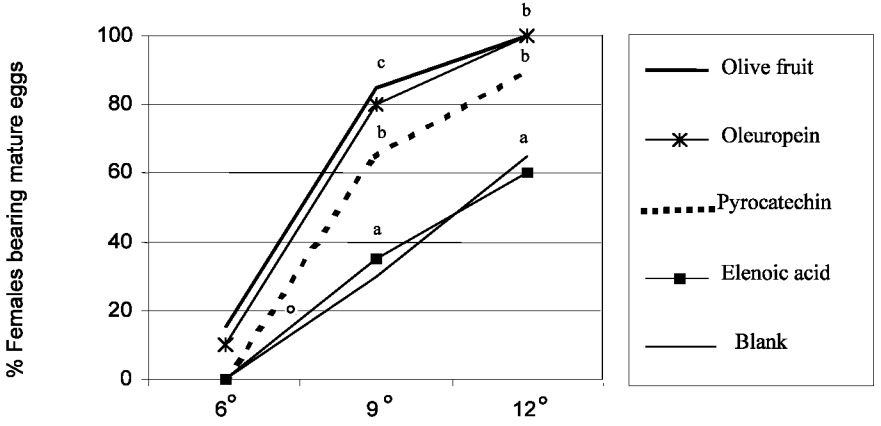

Fig. 9 - Oogenesis stimulation activity of tested substances. Different letters indicate statistic significant differences to G test ($P < 0.05$).

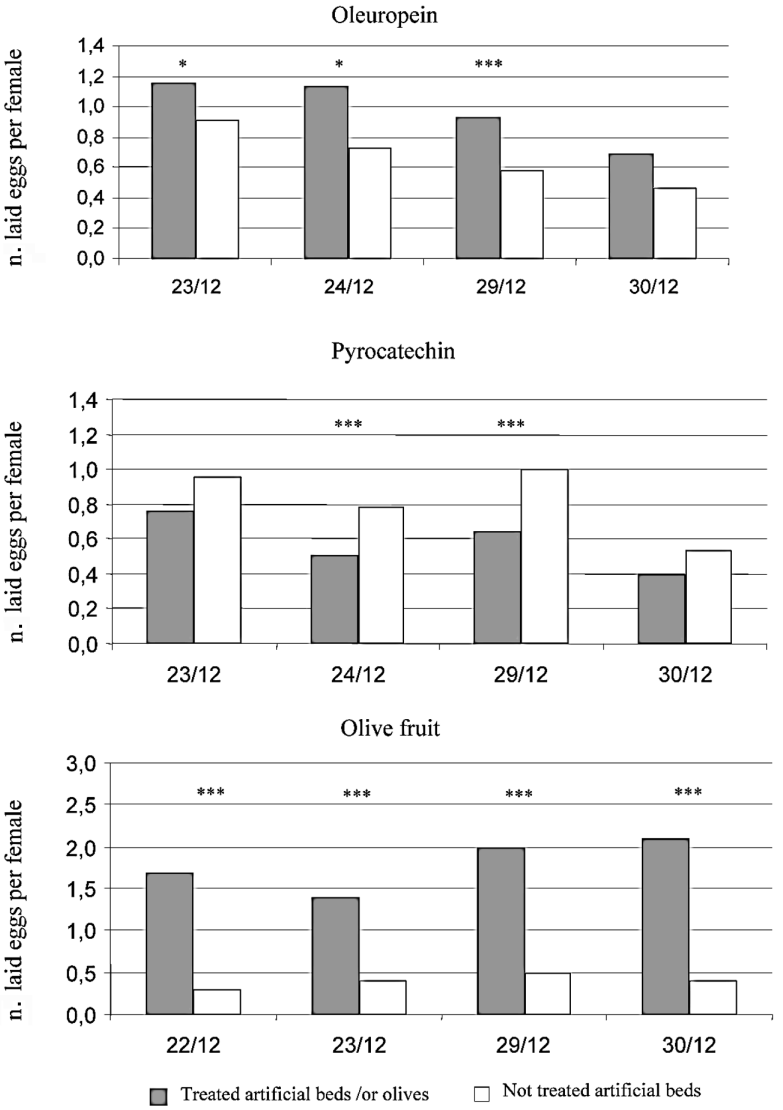

Fig. 10 - Oviposition stimulation activity of the tested substances. The asterisks indicate statistic significant differences to Student *t*-Test (* P<0.05, *** P<0.01).

oogenesis stimulation on *B. oleae*.

3.3.2. Oviposition stimulation

Global analysis of the data (fig. 10) suggests that Oleuropein, sprayed on

the artificial beds, stimulates oviposition in *B. oleae*. However, oviposition stimulation by Oleuropein can't be comparatively evaluated with the olive's because the Flies presented with artificial beds sprayed with Oleuropein in the presence of olives clearly preferred the latter.

In the same way (fig. 10), Pyrocatechin sprayed on the artificial beds, inhibited oviposition in *B. oleae*, confirming previous results (Girolami *et al.*, 1981; Capasso *et al.*, 1994).

It is notable that results from the experiment replications, both for oogenesis or oviposition stimulation, were influenced by several factors such as: female density in the cages (Coiutti, 1994; Toic, 1995), male presence (Tzanakakis, 1967; Cavalloro & Delrio, 1971), learning (Di Bernardo, 1997), as well as by the diet (especially by starvation of females), and the time elapsed in the fridge at 11-12°C by the pupae until the emergence of females.

4. DISCUSSION

Our choice to investigate the "C" sensilla with first priority, was motivated by the fact that in other similar cases, e.g., *Delia radicum* L., these sensilla have proven to be specifically sensitive to host-plant leaf surface extracts (Isidoro *et al.*, 1994) which also proved to stimulate *D. radicum* oviposition (Roessingh *et al.*, 1997).

"C" sensillum is a very typical contact chemosensory (gustatory) sensillum, consisting (according to conventional definition, Altner & Prillinger, 1980; McIver & Siemicki, 1978; Zacharuk, 1980) of: a) an uniporous (or terminal-pore) rather rigid hair-shaft set in a flexible specialised socket and having two lumina which are morphologically isolated from each other but possibly allow for ion exchange through the dendritic sheath at hair-shaft base (Keil & Thurm, 1979), as well as through the apical *septum* at hair-shaft tip; b) five sensory neurons of which one terminates at the hair-shaft base as mechanosensitive element while the other four ones invade the hair-shaft lumen almost to the tip pore, as chemosensitive units; c) and three accessory cells, i.e., a thecogen, a trichogen and a tormogen cell.

As far as we know, this is the second time that a "C" sensillum has been investigated ultrastructurally. The previous investigation concerns *D. radicum* (the Cabbage root fly), a relatively close related insect of *B. oleae* (the olive fruit fly) but living in a quite different environment. Thus a comparison of some morpho-functional features between "C" sensilla of these species might be interesting. In *B. oleae* we have found two particularly noteworthy morpho-functional features in common with *D. radicum*. Firstly, direct contacts between

sensory cell somata, previously described in other contact chemoreceptors and also in thermo-/hygrosensitive sensilla, contacts interpreted (Steinbrecht, 1989, 1991 and references therein) as possible structural basis of peripheral interactions between individual receptor neurons of a sensillum. Secondly, a very intensive activity (from abundance of active cell organelles) of the accessory cells, as previously observed in the thecogen cell of maxillary contact chemosensilla (Seidl, 1992), but in *B. oleae* involving the outer sheath cells as well.

The electrophysiological bioassay confirmed the presence in "C" sensilla of 4 chemosensory neurons (fig. 6) of which two (B and C) specifically respond to host-plant compounds. Precisely, cell B has showed sensitivity to both Oleuropein and its demolition product, Pyrocatechin; while cell C is significantly sensitive to the latter compound only. However, clear distinction between these cells according to impulse amplitude was not usually possible. Furthermore, it was not always possible to determine the exact number of individual impulses of B and C cells simultaneously responding to the same substance (Pyrocatechin), since the frequency distributions of the different spike types may considerably overlap, as evidenced in tarsal hairs of *Calliphora erythrocephala* Meig., even when a high-input impedance preamplifier was used to avoid the electrolyte interference (Den Otter & Van Der Starre, 1967). In *B. oleae* this result might be a confirmation of possible interactions between the sensory neurons of "C" sensilla, as above hypothesized according to morphological observations.

Oleuropein is a glycoside having a relatively large, non-volatile and hydrosoluble molecule (fig. 8), hence it cannot be considered the substance that directly regulates reproductive behaviour in *B. oleae*. The glycoside is probably the chemical precursor of one or more unknown liposoluble compounds that can reach the olive fruit surface and remain in the superficial waxes. There is a large amount of Oleuropein (1-2% of fresh weight) in the olive pulp (Amiot *et al.*, 1989).

The inactivity of Elenoic acid on both oogenesis and oviposition, and the modest activity of Pyrocatechin on oogenesis let suppose that the semiochemicals derived from Oleuropein and involved in *B. oleae* reproductive behaviour should be phenolic compounds.

Since oogenesis is not stimulated by the mere contact with Oleuropein sprinkled on the cage walls or supplied to females with diet, it is likely that some influence on oogenesis stimulation might be due to Oleuropein being presented to female flies on a spherical surface suitable for oviposition. At any rate, the latter was the best place in the cage for Oleuropein to come into contact with tarsal chemosensilla.

Electrophysiological bioassays confirm that “C” tarsal sensilla perceive Oleuropein stimulation but it may be that the contemporaneous perception of both Oleuropein and sphericity of fruits stimulates oogenesis and oviposition as well.

5. CONCLUSIONS

From the above reported results and discussion, the following conclusions may be drown:

- Oleuropein, the phenolic glucoside of olive fruit, spread on artificial oviposition beds, stimulates both oogenesis and oviposition in *B. oleae*;
- the ortodiphenol Pyrocatechin, product of cleavage of Oleuropein, stimulates oogenesis but inhibits oviposition; while Elenoic acid, another product of degradation of Oleuropein, results inactive both on oogenesis and (data not reported herein) oviposition;
- the tarsal sensilla called “C” sensilla are contact chemosensilla (gustative) having 4 chemosensory neurons, one of which shows direct contacts with other two of them at somata level, what represents a morphological basis for possible interactions between the three cells in question;
- electrophysiological bioassay with “C” sensilla evidences 4 distinct action potentials elicited by the 4 chemosensory neurons (A, B, C, D, ordered according to decreasing spike amplitudes);
- B and C neurons specifically respond to the host-plant compounds Oleuropein and Pyrocatechin, thus confirming the hypothesized mediation of the tarsal “C” sensilla in the mentioned interactions between *B. oleae* and its host-plant;
- the role of direct contacts between the receptor cell somata cannot be explained yet; however it will be the basis for further investigations both on the ultrastructural and physiological level; dose-response curves to Oleuropein and Pyrocatechin might solve this problem.

ACKNOWLEDGMENTS

We are very grateful to: Mr. A. Mommi for film processing and photographic printing; Mr. C. Dentini for technical assistance in fixing-embedding specimens. This research is financially supported by the Italian MURST ex 40% 1998-‘99 Project “Chemoreceptors and Semiochemicals involved in Interactions between Insects or Acari and relative Host-plants”, Co-ordinator Prof. Mario Solinas.

The third author thanks Prof. C.J. Den Otter, Department of Animal Physiology, University of Groningen, who introduced him to the Single Cell Recording and Single Sensillum Recording techniques.

RIASSUNTO

MORFOLOGIA FUNZIONALE DEI CHEMIOSENSILLI TARSALI DI *BACTROCERA OLEAE* GMEL. (DIPTERA: TEPHRITIDAE) IMPEGNATI IN INTERAZIONI CON LA PIANTA OSPITE.

La Mosca delle olive (*Bactrocera oleae* Gmel.) è notoriamente il principale insetto dannoso alle olive nella regione mediterranea. Prove comportamentali hanno dimostrato che sostanze contenute nelle drupe, come il glucoside Oleuropeina, provocano per contatto nella Mosca un aumento della oogenesi. È presumibile che in natura detta interazione insetto-pianta possa avvenire mediante i chemiorecettori tarsali della Mosca durante l'azione esplorativa della medesima sulle olive.

Osservazioni al microscopio elettronico a scansione e a trasmissione mettono in luce la presenza di sensilli tricoidei gustativi sulla faccia ventrale di ciascuno dei tarsomeri protoracici dal 2° al 5°. Detti sensilli appaiono esteriormente molto simili tra loro, benché di varie dimensioni, nel senso che presentano tutti un pelo sensoriale con apice arrotondato e provvisto di un poro. Anche le componenti cellulari risultano molto simili, presentando, ciascuno dei sensilli, 5 neuroni sensoriali, dei quali uno terminante alla base del pelo con un corpo tubulare (elemento meccanorecettore), mentre gli altri 4 invadono indivisi il canale dendritico del pelo medesimo. Nel 5° tarsomero i sensilli in questione sono in numero di 8-10, un paio mediali al margine distale del tarsomero, e gli altri in due gruppi subdistali e sublaterali di 3-4 elementi ciascuno.

Si riportano in dettaglio i risultati di osservazioni morfologiche fini e relativa documentazione elettronmicrografica dei due sensilli apicali menzionati (i sensilli "C"). In questi ultimi sono stati messi in luce anche contatti diretti tra somata dei neuroni sensoriali, i quali potrebbero rappresentare la base morfologica di interazioni periferiche tra neuroni dello stesso sensillo.

Biosaggi comportamentali hanno confermato l'azione stimolante l'oogenesi e l'ovideposizione sulla Mosca, per contatto tarsale, da parte di sostanze contenute nelle olive, quali Oleuropeina e relativi prodotti di demolizione (es. Pirocatechina). Mentre parallelamente, biosaggi elettrofisiologici hanno evidenziato che i sensilli "C" rispondono al contatto con Oleuropeina e Pirocatechina, confermando inoltre la possibilità di interazioni periferiche tra i neuroni sensoriali del medesimo sensillo.

Parole chiave: cellule avvolgenti, comportamento, contatti tra somata, elettrofisiologia, Mosca delle olive, neuroni sensoriali, Oleuropeina, Pirocatechina, sensilli gustativi, stimolazione oogenesi, ultrastruttura.

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