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Functional morphology of the antennal chemosensilla of *Ceutorhynchus assimilis* Payk. (Coleoptera: Curculionidae)

ABSTRACT

The cabbage seed weevil (*Ceutorhynchus assimilis* Payk.) is a serious pest of oilseed rape (*Brassica napus* L.) in Europe. Previous EAGs and behavioural investigations suggest an essential role of the antennal olfaction and taste in the chemical ecology of the weevil, but morpho-functional knowledge of its antennal sensilla is still lacking. To fill the gap, an anatomo-ultrastructural study of *C. assimilis* antennal chemosensilla has been carried out and presented here. The sensilla in question have been found on the antennal club (9^{th} + 10^{th} + 11^{th} antennomeres) only. Three types of chemosensilla have been identified and illustrated: (i) sensilla chaetica (CH), the most prominent, arranged in three whorls, ultrastructurally gustative; (ii) peg-like sensilla (P), the most numerous but difficult to detect, ultrastructurally olfactive; (iii) hair-like (H) or sickle shaped sensilla, moderately numerous, ultrastructurally olfactive. The behavioural meaning of the mentioned sensilla is discussed, and concluding suggestions are given to a fuller understanding of electrophysiological studies.

Key words: (in addition to those in the title): Insect, olfactory, gustative, sensory cell contacts, ultrastructure.

INTRODUCTION

The cabbage seed weevil (*Ceutorhynchus assimilis* Payk., syn.: *fallax* Schultze, *brassicae* Foc.), is a serious pest of oilseed rape (*Brassica napus* L.) in Europe (BROMAND, 1988), and it is also present in North Africa, Middle East, and North America (BALACHOWSKY, 1963).

To develop biorational control methods based on the use of pheromones, host-plant attractants, deterrents, etc., a sound knowledge of the weevil antennal chemosensory biology is essential. Thus far, selective responsiveness of the antennal olfactory system to host-plant volatiles has been proven through EAGs (KOZLOWSKI *et al.,* 1983; KOZLOWSKI, 1984; BLIGHT *et al.*, 1989; EVANS & ALLEN-WILLIAMS, 1992), and behavioural observations confirm this (BARTLET *et al.*, 1992; EVANS, 1992) and suggest (FERGUSON & WILLIAMS, 1989 and 1991) that the weevil oviposition-deterring pheromone is perceived by antennal contact chemoreception and/or olfaction of compounds with a low vapour pressure. But, to a fuller understanding of the mentioned sensory biology, morpho-functional data on the antennal chemoreceptors are needed.

Our aim was to fill the gap through an anatomo-ultrastructural study of *C. assimilis* antennal chemosensilla.

2. MATERIALS AND METHODS

For TEM observations, antennal clubs from CO_2 anesthetized *C. assimilis* females, collected in the fields near Perugia and reared in laboratory, were excised, immediately immersed in Karnowsky's (1965) fixative with 2% Acrolein and left for 2 h at 4°C. Then the specimens were washed overnight in cacodylate buffer with 5% sucrose, postfixed in 1% Osmium tetroxide for 1 h, rinsed in the same buffer. After dehydration in graded ethanol series, the clubs were finally embedded through propylene oxide in Epon-Araldite. Thin sections obtained by a L.K.B. "Nova" ultramicrotome, sequentially stained with Uranyl acetate and Lead citrate, were examined through a Philips EM 400T.

For SEM observations, excised antennae 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 XL20.

Abbreviations used in the figures: BB = basal body; dBB = distal basal body; pBB = proximal basal body; CC = ciliary constriction; CH = sensillum chaeticum; CR = ciliary rootlet(s); CS = ciliary sinus; CU = cuticle; D = outer dendritic segment(s); iD = inner dendritic segment(s); DB = dendritic branches; DE = desmosomes; DS = dendritic sheath; H = hair-like sensillum; He = haemocoele; LA = labyrinth; M = mitochondrion; MT = microtubules; ML = microlamellae; N = sheath cell nuclei; P = peg-like sensillum; Po = pore(s); iSC = inner sheath cell; oSC = outer sheath cell; SJ = septate junctions; SL = scolopale-like structure(s); SN = sensory neuron nuclei; SO = socket; SS = sensillar sinus; TB = tubular body; I, II, III = first, second and third clavomeres, respectively.

3. RESULTS AND DISCUSSION

3.1 ANTENNAL TOPOGRAPHICAL ANATOMY CONCERNING CHEMOSENSILLA

C. assimilis antennae are geniculate, not sexually dimorphic (externally), formed by 11 antennomeres: scape (1st), pedicell (2nd), and flagellum consisting of a funiculum (3rd to 8th) and a relatively big club made of 3 subsegments (I, II, III clavomeres, i.e., 9th to 11th antennomeres).

There are 3 types of chemosensilla situated on the club only, in number and distributed as follows: (i) "sensilla chaetica" (CH, Figs 1, 2), 16-17 (n = 10) altogether, of which 7-8 around I clavomere distal third, 5 around the distal end of II clavomere, and 4-5 near the club tip; (ii) "peg-like sensilla" (P, Figs 1, 2), several dozen, most of which confined in two bands around the distal margin of I and II clavomeres and scattered on III clavomere, whilst few of them are isolated here and there on the rest of the club; (iii) "hair-like sensilla" (H, Figs 1, 2), sickle-shaped, easy to detect only when projecting from the antennal outline (thus difficult to count precisely), confined to an irregular ring just above the mentioned band of peg-like sensilla (sometimes individually located among these), apparently much less numerous than these here, but abundantly scattered on III clavomere (Fig. 2a).

Most of the club lumen is filled with the cellular components of the above mentioned chemosensilla whose sensory neuron somata (SN, Fig. 2) lie in a rather central mass apparently invaded by a very narrow haemocoele in the I and to the proximal third of the II clavomeres only (Fig. 2b).

3.2 SENSILLA FINE MORPHOLOGY

Sensilla chaetica (CH). So called *sensu* Schneider (1964), they show a cuticular apparatus consisting of an outstanding hair shaft (Figs 1, 3a), 35-40 μm long (n = 10) gradually tapering from the base (ca 2 μm in diameter) to a blunt, porous tip, with fluted aporous walls (Fig. 3a, b, c), and inserted in a specialized, flexible socket (Fig. 3a, d).

The cellular components are represented by 6 sensory neurons and 3 auxiliary cells. The sensory cell somata lie deep in the antennal lumen (see 3.1) held together by the inner sheath cell (iSC) without being completely separated from one another (Fig. 4c), so that direct contacts between sensory cell somata are possible. The sensory neurons send relatively short inner dendritic segments that sometimes come into contact to one another (Fig. 4b, arrow head), show well developed ciliary rootlets (CR) and abundant organelles, especially mitochondria (Fig. 4b, d). The outer dendritic segments, much longer (Fig. 4c, d) and enclosed in a thick dendritic sheath, cross the sensillar sinus (Fig. 3f, SS). One of them, the thickest and distally isolated by individual sheath (Fig. 3f), terminates with a conspicuous tubular body (Fig. 3e, TB) at the shaft base, thus forming a mechanosensitive element, whereas the other 5 dendrites enter the shaft lumen still enclosed in the common sheath (Fig. 3c, DS) and run unbranched almost to the shaft tip (Fig. 3b), thus representing 5 chemosensitive elements.

The auxiliary cells typically consist of an innermost sheath cell (thecogen) and 2 outer sheath cells (trichogen and tormogen). The thecogen cell (Figs 3f, 4b, d) is quite large, envelops the sensory somata and the inner dendritic segments to whose distal portions it is connected by extensive septate junctions as well as desmosomes (Fig. 4b, DE). This cell, produces the dendritic sheath (Fig. 4c, DS), and forms the boundary of the ciliary sinus (Fig. 4d, CS) and a

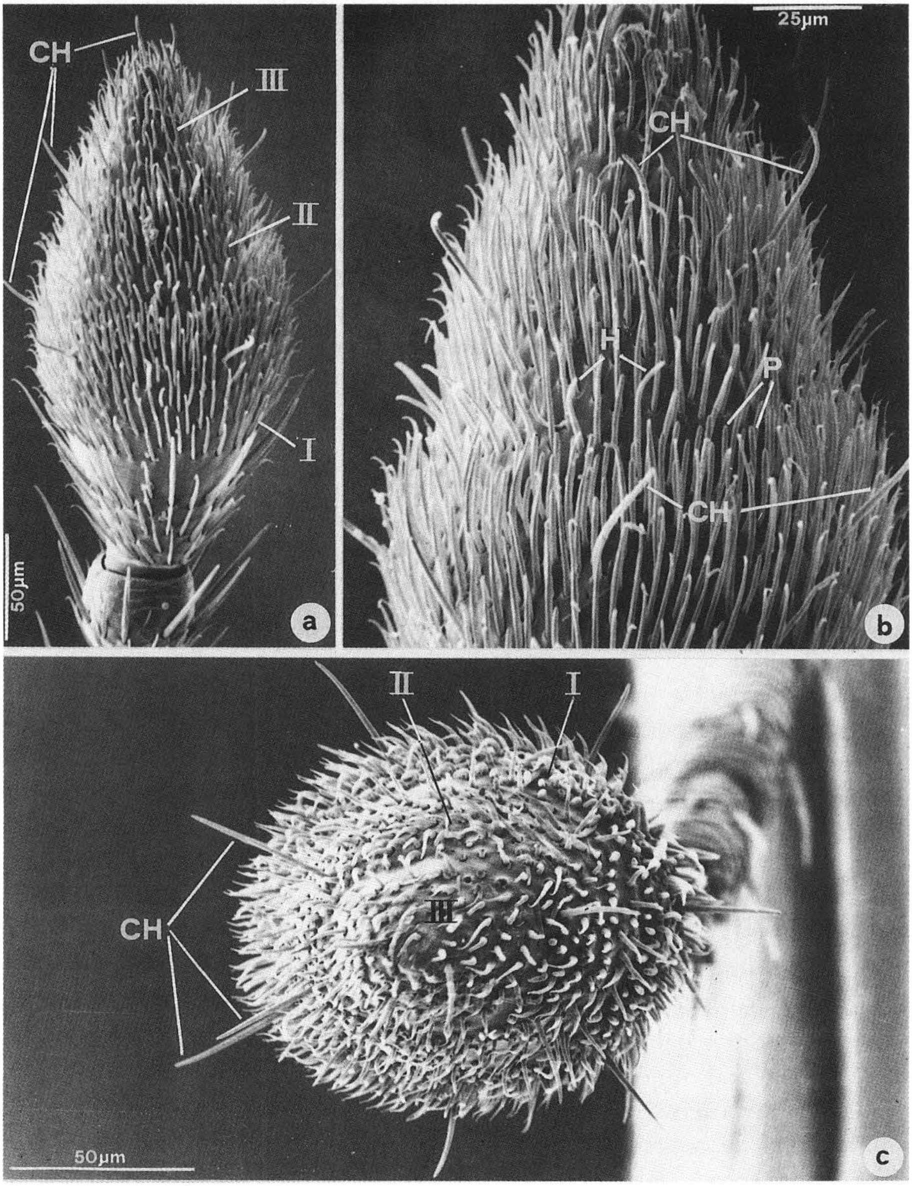

Fig. 1. *Ceutorhynchus assimilis* female. SEM micrographs showing antennal details: (a) club lateral view; (b) lateral detail; (c) club apical view.

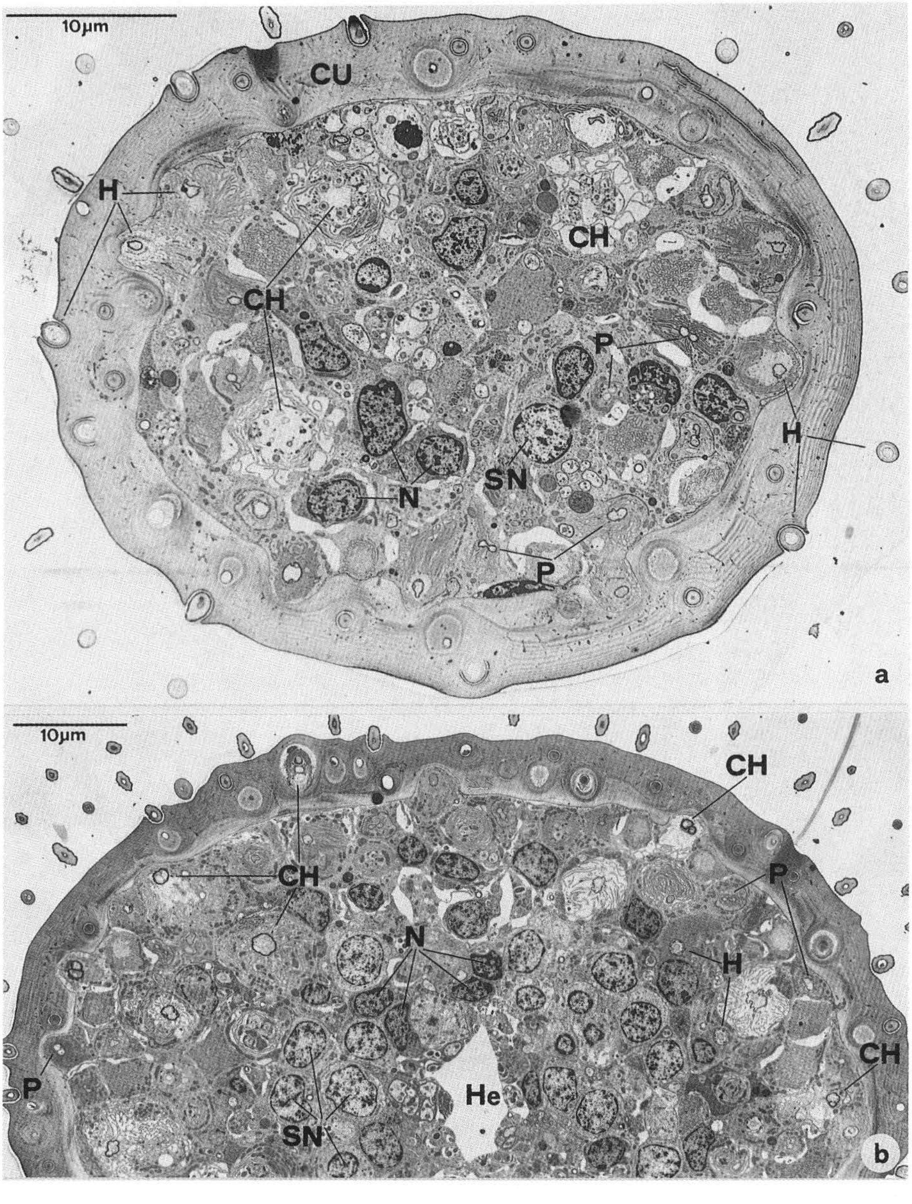

Fig. 2. Club cross sections at about half length of third (III) clavomere (a), and (b) at about half length of the second (II) clavomere.

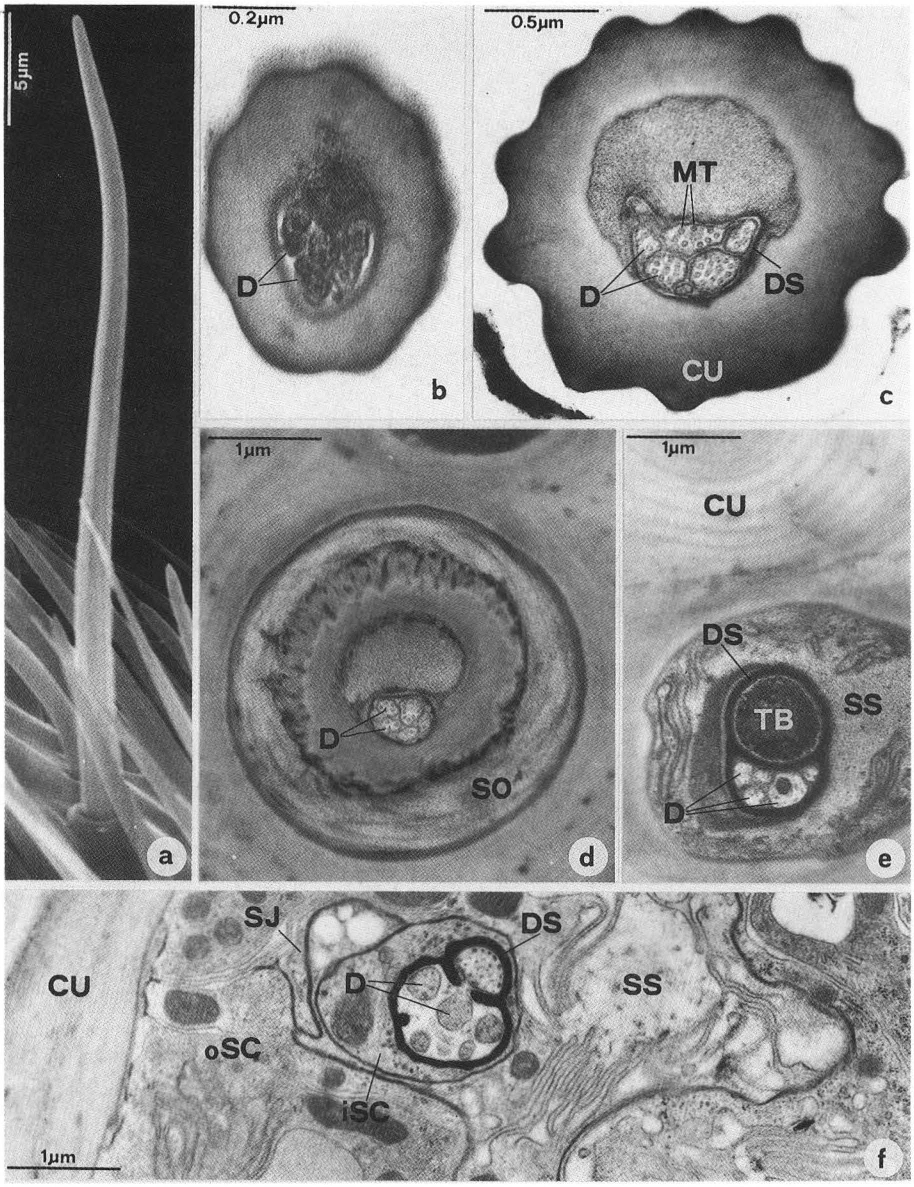

Fig. 3. Sensillum chaeticum. (a) SEM micrograph showing whole outer aspect; subdistal (b) and intermediate (c) cross sections of hair shaft; (d) cross section through the socket; cellular parts' cross sections at antennal wall level (e) and somewhat deeper (f).

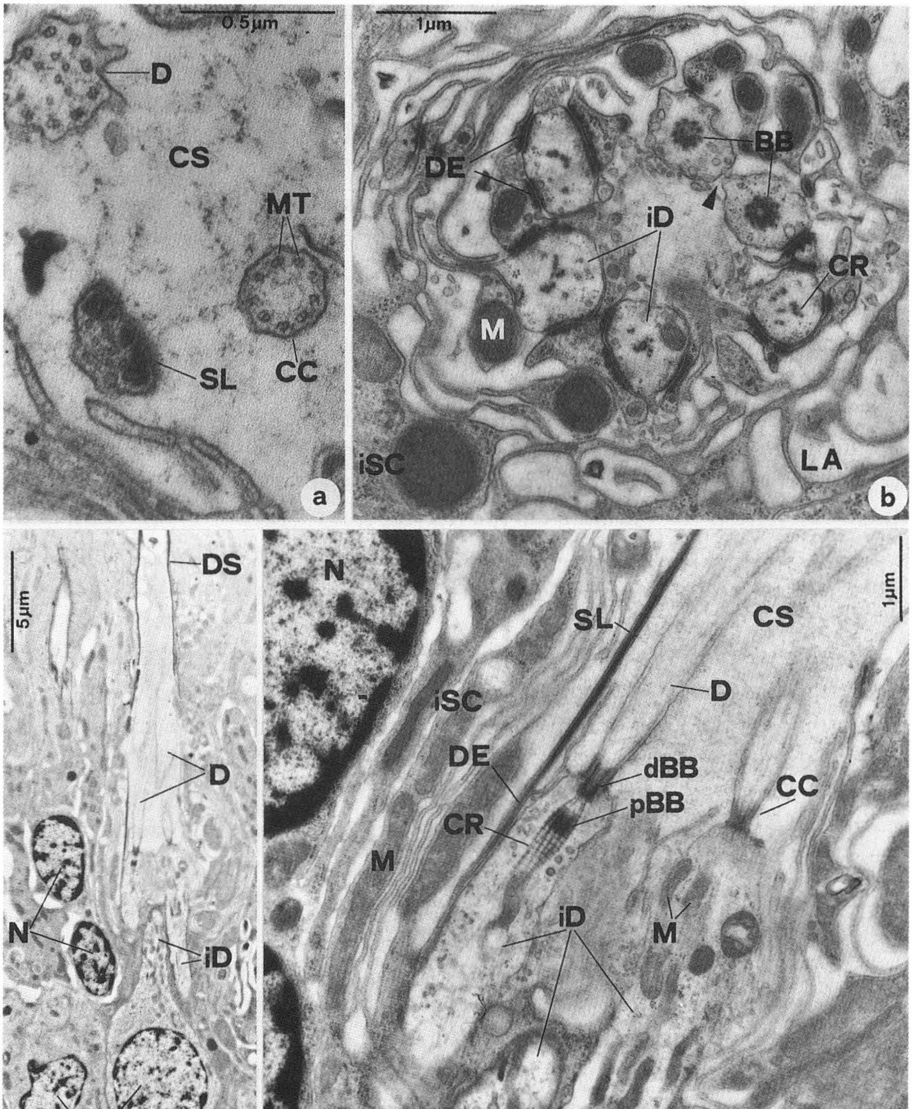

Fig. 4. Sensillum chaeticum cellular parts. Cross sections' details through dendrite ciliary constrictions (a) and ciliary rootlets (b); roughly longitudinal sections through sensory cell somata (c) and dendrite ciliary constrictions (d).

conspicuous labyrinth (Fig. 4b, d, LA) which is continuous with the sinus and extends almost to the level of the sensory somata (Fig. 4c, d). The thecogen cell cytoplasm displays numerous, conspicuous mitochondria (Figs 3f, 4b, d), rough endoplasmic reticulum and ribosomes scattered in groups, and numerous, longitudinally oriented microtubules also forming scolopale-like structures (Fig. 4a, SL) that to some extent parallel the outer dendritic segments apparently rising on the above mentioned desmosomes (Fig. 4d, DE - SL). The trichogen and tormogen cells are ultrastructurally very similar to one another as well as to the thecogen cell (except for the last mentioned structures). Their apical membranes together form a sensillar sinus (Fig. 3f, SS) lined with microvilli and microlamellae, and extended down in a wide labyrinth (Fig. 2b, CH).

This kind of sensillum, morphologically typical gustative, must be very common on weevils' antennae. It may be easily identified with MUSTAPARTA'S (1973) "type IV hairs" of *Hylobius abietis* L., HATFIELD'S (*et al.*, 1976) "sensilla trichodea type II" of *Curculio caryae* (Horn), SMITH'S (*et al.*, 1976) "sensilla trichodea type I" of *Hypera postica* (Gyllenhal), and ALM & HALL'S (1986) "hair type V" of *Conotrachelus nenuphar* (Herbst).

Peg-like sensilla (P). They are sensilla basiconica (*sensu* Schneider, 1964) with hair shaft 14-19 μm long (n = 18), blunt tipped, peg-shaped (Fig. 5a, b), rising on a rigid socket (i.e., without any articular membrane, Fig. 5b, f), with proximal third having aporous, thicker walls (Fig. 5b, e), and the rest having abundantly porous walls to the tip (Fig. 5b, c, d).

The cellular components consist of 2 sensory neurons and 3 auxiliary cells. The sensory cell somata lie deep in the antennal lumen, i.e., in the above mentioned central mass (3.1), held together by the thecogen cell without being isolated to one another so that wide direct contacts between them apparently take place (Fig. 6e, arrow heads). The 2 neurons send inner dendritic segments relatively short and rich in organelles, like in the previous sensilla, running parallel and widely coming into contact to one another (Fig. 6c, d, e). The 2 outer dendritic segments run parallel and, first enclosed in the thecogen cell to some extent (Fig. 6a, iSC) and then in a peculiar common dendritic sheath (Fig. 5f, DS), cross the sensillar sinus, enter the shaft lumen remaining entire to the shaft proximal third (Fig. 5b), and finally divide into numerous branches (Fig. 5c, d, DB) that fill the rest of the shaft lumen (Fig. 5b).

There are typically 3 auxiliary cells, i.e., an innermost or thecogen (Fig. 6, iSC) and 2 outers (Fig. 6a, oSC) or trichogen and tormogen. The former is relatively large, envelops the couple of sensory neurons from somata (see above) to some extent of the outer dendritic segments. This cell shows extensive septate junctions and desmosomes along the boundaries with inner dendritic segments (Fig. 6c, DE), while its apical membrane forms the boundary of a wide ciliary sinus (Fig. 6b, CS) and a conspicuous labyrinth (Fig. 6c, d, LA,) that is continuous with the sinus and extends down to the level of the inner dendritic segments. The thecogen cell cytoplasm shows numerous mitochondria, abundant rough endoplasmic reticulum as well as scattered groups of ribosomes, and numerous longitudinally oriented microtubules and scolopale-like structures (Fig. 6b, d, SL), in a very similar fashion as in the previous sensilla (see above). The 2 outer auxiliary cells are ultrastructurally very similar to one another and also to the thecogen cell. Their apical membranes together form a sensillar sinus (Fig. 5f, SS) lined with microvilli and microlamellae, and wide extended in a labyrinth (Fig. 2, P).

Comparing our observations with the literature, we may identify our peg-like sensilla with MUSTAPARTA'S (1973) "type IB hairs" of *Hylobius abietis* L., HATFIELD'S (*et al.*, 1976) "sensilla basiconica type I" of *Curculio caryae* (Horn), BLAND'S (1981) "sensilla basiconica Type II" of *Hypera postica* (Gyll.) ALM'S (*et al.*, 1986) "hairs type II" of *Conotrachelus nenuphar* (Herbst). These sensilla have been proven to be olfactory in *Hylobius abietis* (MUSTAPARTA, 1975).

It is worth remarking that direct contacts between sensory cell somata (consequently, the possibility of peripheral interactions) have so far only been shown in contact chemosensilla (MOULINS, 1968; MOULINS & NOIROT, 1972; ISIDORO *et al.*, 1993) and in thermo-hygrosensitive sensilla (STEINBRECHT, 1989, and references therein). Hence, our case represents the first observation of direct contacts between sensory cell somata and between inner dendritic segments in olfactory sensilla.

Hair-like sensilla (H). Sensilla basiconica (sensu SCHNEIDER, 1964) having a hair shaft 23-27 μm long (n=10), proximally straight and distally sickle-shaped (Fig. 7a), the former portion with aporous and thicker walls (Fig. 7b) whereas the latter with moderate number of pores on the walls (Fig. 7b, c).

The cellular components are represented by a single sensory neuron and 3 auxiliary cells. The sensory neuron cell body lies in the above described mass (3.1) and sends a dendrite, ultrastructurally very similar to those of the peg-like sensilla (compare Fig. 8 with 6) but with outer dendritic segment comparatively thicker (Fig. 2, 7e) and producing fewer branches in the shaft lumen (compare Fig. 7b, c with Fig. 5c, d, e). Also the auxiliary cells show the same ultrastructural features like those of the peg-like sensilla (compare Figs 7e, f and 8 with 5f and 6).

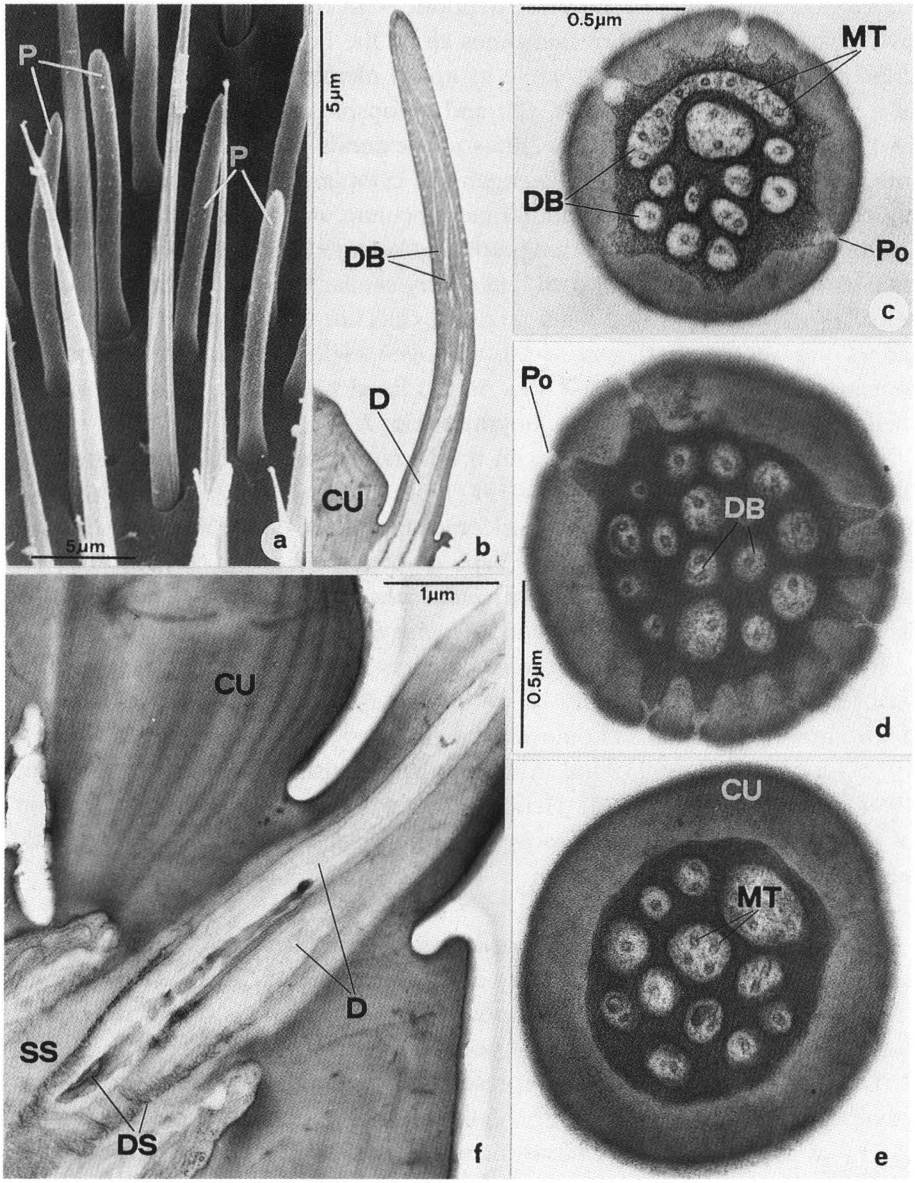

Fig. 5. Peg-like sensillum. (a) SEM micrograph displaying a detail of subdistal portion of the first clavomere; (b) shaft longitudinal section; shaft cross sections at subapical (c), intermediate (d) and proximal third (e) levels; (f) longitudinal section through the socket. (c, d and e at the same magnification).

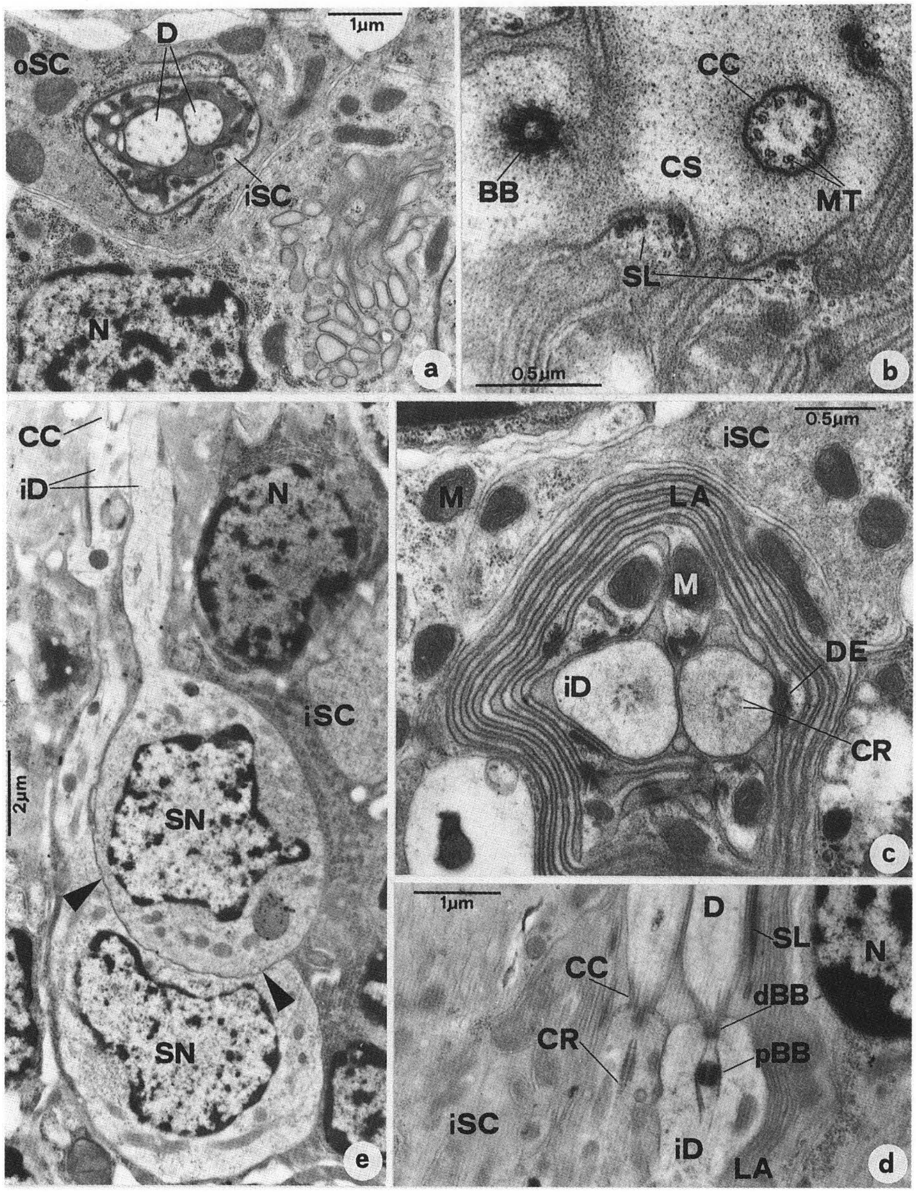

Fig. 6. Peg-like sensillum cellular parts. Details of cross sections through: outer dendritic segments (a), ciliary constriction and basal body (b), and inner dendritic segments (c); longitudinal section details through sensory cell bodies (e) (arrow heads, direct contacts between cell somata) and ciliary constrictions (d).

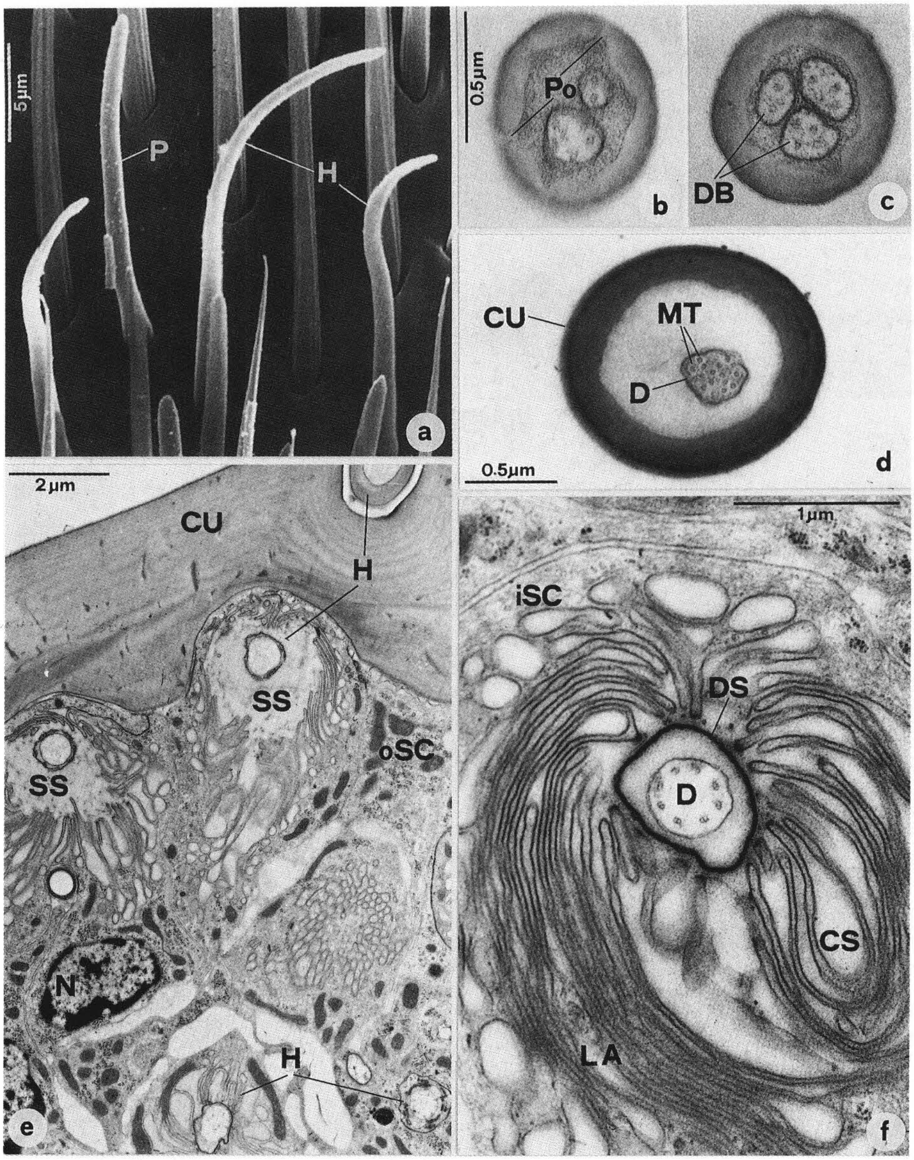

Fig. 7. Hair-like sensillum. (a) SEM micrograph exhibiting a detail of subdistal portion of the first clavomere; hair shaft cross sections through subdistal (b), intermediate (c), and subproximal (d) portions; (e) detail of first clavomere cross section through subdistal portion; (f) sensillum cellular parts' cross section, just above the dendritic ciliary constriction.

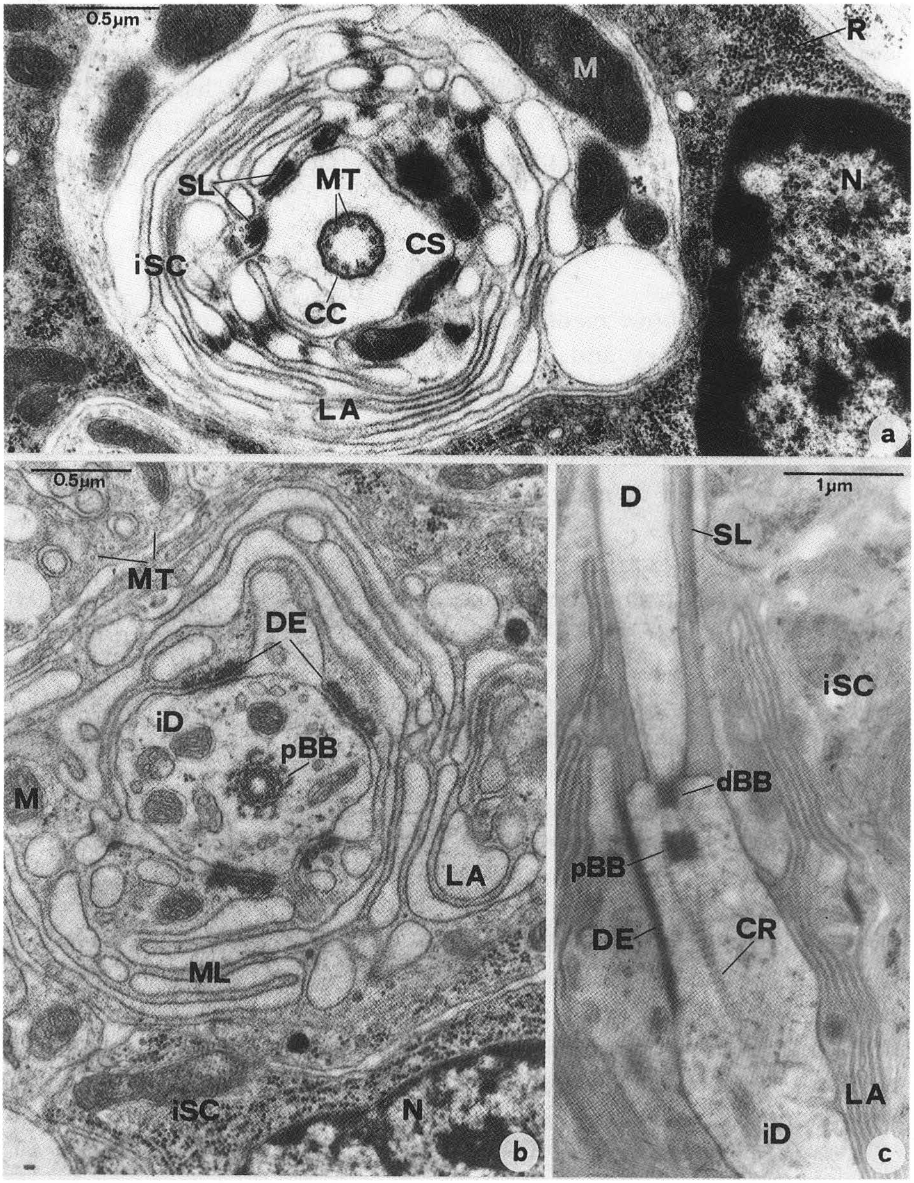

Fig. 8. Hair-like sensillum, inner cellular parts. Cross sections' details through the dendritic ciliary constriction (a) and proximal basal body (b); (c) detail of longitudinal section showing dendritic ciliary constriction.

Our hair-like sensilla may be identified with MUSTAPARTA'S (1973) "type II hairs" of *Hylobius abietis* L., HATFIELD'S (*et al.*, 1976) "sensilla basiconica type II" of *Curculio caryae* (Horn), and ALM & HALL'S (1976) "hairs type IV" of *Conotrachelus nenufar* (Herbst). Also these sensilla have been proven to be olfactory (mainly sensitive to plant odours) in *Hylobius abietis* (MUSTAPARTA, 1975).

4. CONCLUSIONS

Combining the above mentioned previous results on both the antennal chemosensory biology and host-plant finding-acceptance behaviour of *Ceutorhynchus assimilis* with the present morphological observations, the following conclusions may be drawn.

a) Antennal chemosensory biology essentially mediate *C. assimilis* host-plant finding as well as pheromone reception (including oviposition-deterrent).

b) All the sensory structures involved are situated on the antennal club, i.e., 9th+10th+11th antennomeres, and consist of 3 types of chemosensilla, namely, sensilla chaetica (CH), peg-like sensilla (P), and hair-like sensilla (H).

c) Sensilla chaetica, externally represented by the most projecting setae from the antennal surface, are ultrastructurally (both for cuticular and cellular features) typical contact chemoreceptors, strategically located to easily touch and taste host-plant surface, and/or oviposition-deterrent pheromone.

d) Peg-like sensilla, the most numerous chemosensilla, are ultrastructurally (both for outer and inner features) typical olfactory, comparatively most suitable to detect environmental volatiles present in low concentrations such as pheromones.

e) Hair-like sensilla, distally sickle-shaped, simplest chemoreceptors (being innervated by a single sensory neuron), are ultrastructurally (both for outer and inner features such as few pores on the hair shaft and few dendritic branches produced in the shaft lumen, respectively) typical olfactory of moderate efficiency, suitable for detecting environmental volatiles present in relatively high concentrations such as host-plant odours.

f) It is worth remarking that, for a fuller interpretation of electrophysiological results, both the sensillum sensory neuron number and the possibility of peripheral interactions between sensory neurons must be considered with sensilla chaetica and peg-like sensilla.

5. ACKNOWLEDGMENTS

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6. RIASSUNTO

MORFOLOGIA FUNZIONALE DEI CHEMIOSENSILLI ANTENNALI DI *CEUTORHYNCHUS ASSIMILIS* PAYK. (COLEOPTERA: CURCULIONIDAE)

Il punteruolo delle silique del colza (*Ceutorhynchus assimilis* Payk.) è un fitofago seriamente dannoso alla oleaginosa in tutta Europa. Studi preliminari comportamentali ed elettrofisiologici hanno messo in evidenza un ruolo essenziale dell'olfatto e del gusto antennali nella ecologia chimica dell'insetto. Una base morfo-funzionale, indispensabile allo sviluppo di detti studi e tuttora mancante, viene qui fornita mediante ricerche anotomo-ultrastrutturistiche sui sensilli implicati nel ruolo in questione.

Tali sensilli, presenti esclusivamente sulla clava antennale (antennomeri 9°+10°+11°), risultano essere di 3 tipi: 1) "sensilla chaetica" (CH), i più vistosi, sistemati in tre verticilli, ultrastrutturalmente gustativi; 2) sensilli "peg-like" (P; esternamente a forma di piolo), i più numerosi ma difficili da individuare tra la folta peluria di rivestimento, ultrastrutturalmente olfattivi; 3) sensilli "hair-like", distalmente falciformi, moderatamente numerosi, ultrastrutturalmente pure olfattivi.

Viene discusso il significato etologico dei sensilli menzionati e vengono fatte considerazioni conclusive utili, in particolare, ad ottimizzare la comprensione di ulteriori studi elettrofisiologici.

7. REFERENCES

ALM S.R., HALL F.R., 1986 - Antennal sensory structures of *Conotrachelus nenuphar* (Coleoptera: Curculionidae). *Ann. Entomol. Soc. Am.*, 79: 324-333.

BALACHOWSKY A.S., 1963 - Entomologie appliquée a l'agriculture, Tome I, second vol., pp. 1028-1034.

BARTLET E., WILLIAMS I.H., BLIGHT M.M., HICK A.J., 1992 - Response of the oilseed rape pests, *Ceutorhynchus assimilis* and *Psylliodes chrysocephala,* to a mixture of isothiocyanates. *Proc. 8th Int. Symp. Insect-Plant Relationships, Dordrecht: Kluwer Acad. Publ. SBJ Menken, J.H. Visser & P. Harrewijn (eds).* 103-104.

BLAND R.G., 1981 - Antennal sensilla of the adult alfalfa weevil, *Hypera postica* (Gyllenhal). *Int. J. Insect Morphol. & Embryol.* 10: 265-274.

BLIGHT M.M., PICKETT J.A., WADHAMS L.J., WOODCOCK C.M., 1989 - Antennal responses of *Ceutorhynchus assimilis* and *Psylloides chrysocephala* to volatiles from oilseed rape. *Aspects Appl. Biol.* 23: 329-334.

BROMAND B., 1988 - Diversities in Oilseed Rape Growing within the Western Palaearctic Regional Section. *Bull. SROP\WPRS - Proc. OILB Meeting Malmo,* Sweden, 21-22 March, 1988, 13 (4): 7-31.

EVANS K.A., 1992 - The olfactory and behavioural response of seed weevils, *Ceutorhynchus assimilis,* to oilseed rape volatiles. *Proc. 8th Int. Symp. Insect-Plant Relationships, Dordrecht: Kluwer Acad. Publ. SBJ Menken, J.H. Visser & P. Harrewijn (eds).* 107-108.

EVANS K.A., ALLEN-WILLIAMS L.J., 1992 - Electroantennogram responses of the cabbage seed weevil, *Ceutorhynchus assimilis*, to oilseed rape, *Brassica napus* ssp. oleifera, volatiles. *J. Chem. Ecol.*, 18 (9): 1641-1659.

FERGUSON A.W., WILLIAMS I.H., 1989 - Oviposition-deterring pheromone of the cabbage seed weevil (*Ceutorhynchus assimilis* Payk.). *Aspects Appl. Biol.* 23: 339-342.

FERGUSON A.W., WILLIAMS I.H., 1991 - Deposition and longevity of oviposition deterring pheromone in the cabbage seed weevil. *Physiol. Entomol.* 16: 27-33.

HATFIELD L.D., FRAZIER J.L., COONS L.B., 1976 - Antennal sensilla of the pecan weevil, *Curculio caryae* (Horn) (Coleoptera Curculionidae). *J. Insect Morphol. & Embryol.* 5 (4/5): 279-287.

ISIDORO N., SOLINAS M., BAUR R., ROESSINGH P., STAEDLER E., 1993 - Ultrastructure of a tarsal sensillum of *Delia radicum* L. (Diptera; Anthomyiidae) sensitive to important host-plant compounds. *Int. J. Insect Morphol. & Embryol.* (in press).

KOZLOWSKI M.W., LUX S., DMOCH J., 1983 - Oviposition behaviour and pod marking in the cabbage seed weevil, *Ceutorhynchus assimilis. Entom. exp. et appl.* 34: 277-282.

KOZLOWSKI M.W., 1984 - Selectively responsiveness of the antennal olfactory system in the cabbage seed weevil, *Ceutorhynchus assimilis* towards host plant volatiles. *Acta Physiol. Pol.* 35: 5-6.

MOULINS M., 1968 - Les sensilles de l'organe hypopharingien de *Blabera craniifer* Burm (Insecta, Dictyoptera). *J. Ultrastruct. Res.* 21: 474-513.

MOULINS M., NOIROT C., 1972 - Morphological features bearing on transduction and peripheral integration in insect gustatory organs. pp. 49-55. In D. Schneider (ed.), olfaction and taste IV. Wissensch. Verlagsges., Stuttgart.

MUSTAPARTA H., 1973 - Olfactory sensilla on the Antennae of the Pine Weevil, *Hylobius abietis. Z. Zellforsch.* 144: 559-571.

MUSTAPARTA H., 1974 - Responce of the Pine Weevil, *Hylobius abietis*, L. (Col.: Curculionidae) to Bark Beetle Pheromones. *J. comp. Physiol.* 88: 395-398.

MUSTAPARTA H., 1975a - Responses of single olfactory cells in the Pine Weevil, *Hylobius abietis* (Col.: Curculionidae). *J. comp. Physiol.* 97: 271-290.

MUSTAPARTA H., 1975b - Behavioural Responses of the Pine Weevil, *Hylobius abietis* L. (Col.: Curculionidae), to Odours Activating Different Groups of Receptor Cells. *J. comp. Physiol.* 102: 57-63.

SCHNEIDER D., 1964 - Insect antennae. *Annu. Rev. Entomol.* 9: 103-122.

SMITH C.M., FRAZIER J.L., COONS L.B., KNIGHT W.E., 1976 - Antennal sensilla of the clover head weevil *Hypera meles* (F.) (Coleoptera: Curculionidae). *Int. J. Insect Morphol. & Embryol.* 5 (6): 349-355.

STEINBRECHT R.A., 1989 - The fine structure of thermo-/hygrosensitive sensilla in the silkmoth *Bombyx mori:* Receptor membrane substructure and sensory cell contacts. *Cell Tissue Res.* 255: 49-57.