

Funicular Sensilla of *Dacus oleae*: Fine Structural Characteristics¹

E. HALLBERG, J.N.C. VAN DER PERS and G.E. HANIOTAKIS²

Department of Zoology, University of Lund, Lund, Sweden

ABSTRACT

The funicular sensilla in *Dacus oleae* (Gmelin) (Diptera: Tephritidae) are found both on the surface and in the single olfactory pit.

The surface sensilla are of three types: two are single-walled, the third is double-walled. The fine structure of these three sensillar types indicates olfactory function capabilities. The single-walled sensilla are, as a rule, innervated by two sensory cells. The long single-walled sensilla have unbranched sensory processes, whereas in the short they are branched. The double-walled sensilla usually possess three sensory cells that send unbranched sensory processes towards the tip of the hair.

The olfactory pit sensilla are of two types: one type is identical to the double-walled type found on the funicular surface. The second type is poreless and found only in the olfactory pit. The poreless sensilla are innervated by three sensory cells, two of which terminate inside the cuticular hair, while the third does not enter inside the hair but terminates freely below it. The functional capabilities of this sensillar type are unknown.

Introduction

The olive fruit fly, *Dacus oleae* (Gmelin) (Diptera: Tephritidae) is a major pest of olives in the Mediterranean region. Current control practices involve the use of chemical insecticides, either as bait or cover sprays. The consequences of prolonged use of insecticides over extended areas have been a concern of government agencies and the public. Entomologists in all interested countries have been working intensively toward developing alternative control methods. The discovery of a sex pheromone produced by both males (Mazomenos and Pomonis 1983) and females (Mazomenos and Haniotakis 1981) and its chemical identification and synthesis (Baker et al. 1980, Mazomenos et al. 1981) has stimulated research aiming at its use for monitoring

and control of this pest. Field studies have shown possibilities for both uses (Haniotakis et al. 1982). For an effective practical use of pheromones in pest management, however, knowledge and understanding of inhibition, synergism and habituation is necessary. For this reason electrophysiological studies of the receptor system were conducted (Van Der Pers et al. 1984). This morphological investigation was undertaken to give a structural basis for the study of the physiological properties of the antennal sensory system of *Dacus oleae*.

Materials and Methods

Recently emerged adults of *Dacus oleae* were used in this study. The pupae were obtained from a laboratory culture maintained at the Biology Department, N.R.C. "Democritos", Aghia Paraskevi, Attiki, Greece.

The funicular segment of the antennae of both sexes was immersed in 6% glutaraldehyde in 0.1 M cacodylate buffer at 4° C for four hours. Postfixation in 1% OsO₄ was carried out for two hours at 4° C.

¹ Received for publication December 19, 1984.

² Department of Biology, "Democritos" Nuclear Research Center, GR-153 10 Aghia Paraskevi, Greece.

Dehydration in an alcohol series was followed by embedding in Vestopal W. The sections were cut with a diamond knife, stained in an LKB 2168 Ultrastainer, and examined in a Zeiss EM10 electron microscope. For scanning electron microscopy the antennae were fixed as above, dehydrated in alcohol/Freon and air-dried. The mounted specimens were coated with gold/palladium (40:60). The antennae were examined in a Nanolab 2000 scanning electron microscope.

Results

The funicular segment of the antennae of *Dacus oleae* is 375 μm long and 160 μm wide. In cross section the shape of the funiculus varies: in the distal parts it is flattened, in the basal parts more rounded. The funiculus possesses two specialized structures: the arista and the olfactory pit. The arista is present on the lateral side of the antenna, whereas the olfactory pit is invaginated basally on the medial side. The funicular surface is covered by a dense array of cuticular spines and three different types of

sensilla. Two types of sensilla are present in the olfactory pit; one of them has a counterpart in one of the surface types, the other occurs only in the olfactory pit.

The general arrangement of the sensillar elements found on the funiculus of *Dacus oleae* conforms to that of other insect sensilla (Altner and Prillinger 1980). However, we found a poreless sensillum that seems to be unlike any that has been described previously. The funiculi and their sensillar types are similar in males and females. For example, unlike other fly species where there is often a sexual dimorphism as regards the development of the olfactory pits (Liebermann 1926), in *Dacus oleae* a single olfactory pit occurs on each funiculus of both sexes.

a. Long single-walled sensilla (LSW)

The LSW sensilla are the most common on the funiculus of *Dacus oleae*. The cuticular hair is stout, having a basal diameter of about 2 μm

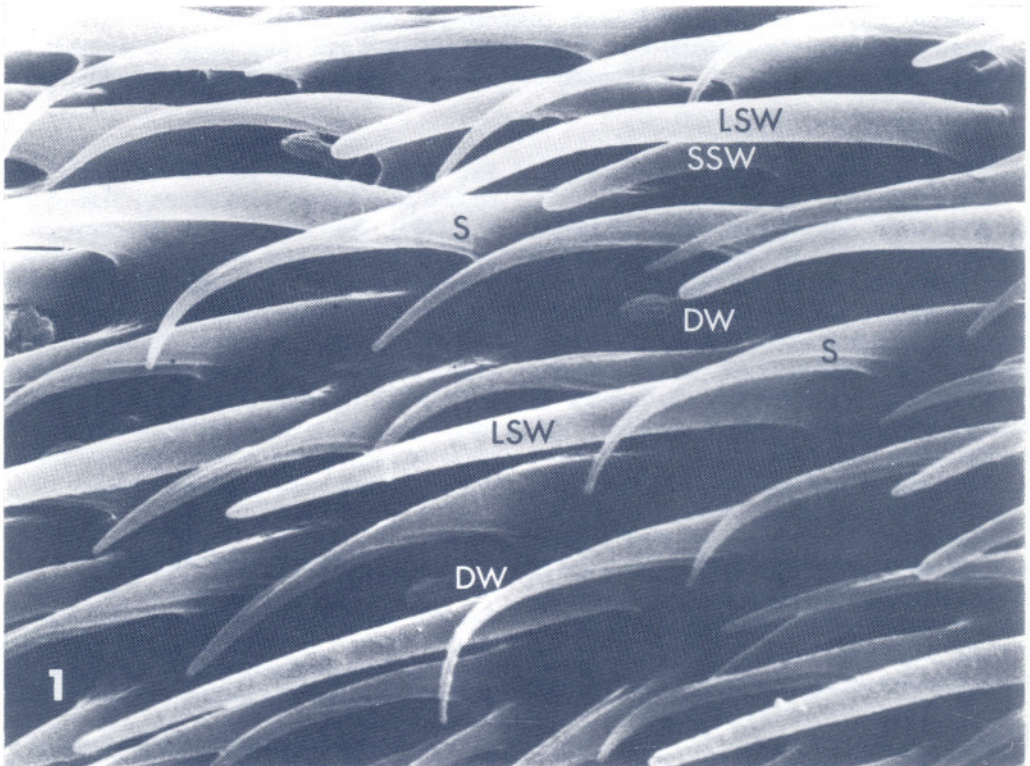


FIG. 1. Sensillar types and non-innervated spines found on the funicular surface of *Dacus oleae* are long single-walled sensilla (LSW) projecting above the spines (S), short single-walled (SSW) and double-walled (DW) sensilla. SEM, $\times 2,460$.

and a length of about 15 μm . The obliquely inserted hairs are straight; they reach above the other sensillar hairs and spines of the funicular surface (Fig. 1). The LSW sensilla are innervated by two sensory cells (Fig. 2) that send unbranched processes through the lymph cavity and cuticular hair. In the terminal part of the cuticular hair there may be some moderate branching of the sensory dendrites. The walls at the mid-part of the cuticular hair are about 0.3 μm thick, and they are penetrated by pores that



FIG. 2. Transverse section through the hair of a long single-walled sensillum. Scale bar, 0.25 μm .

are connected with the fluid-filled interior of the hair through pore-funnels. The pore density is about 10 pores/ μm^2 .

b. Short single-walled sensilla (SSW)

This sensillar type is found on the funicular surface (Fig. 1). The SSW cuticular hair is about 10 μm long and it is curved. The walls of the cuticular hair are about 0.2 μm thick, and the pore density is about 15 pores/ μm^2 . The SSW sensilla are usually innervated by two sensory cells, and these give rise to dendrites that branch profusely in the cuticular hair (Fig. 3).

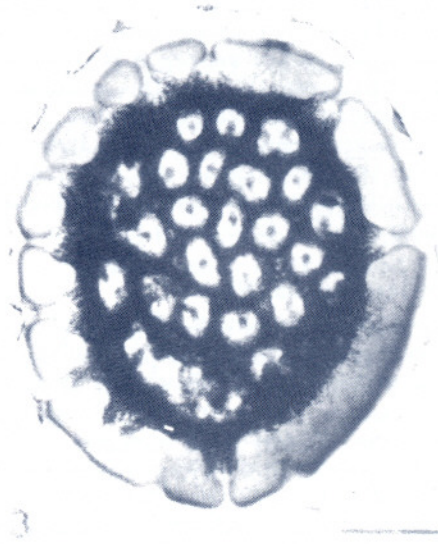


FIG. 3. Transverse section through the cuticular hair of a short single-walled sensillum. Scale bar, 0.25 μm .

c. Double-walled sensilla (DW)

The DW sensilla are found on the funicular surface (Fig. 1). There are also several DW

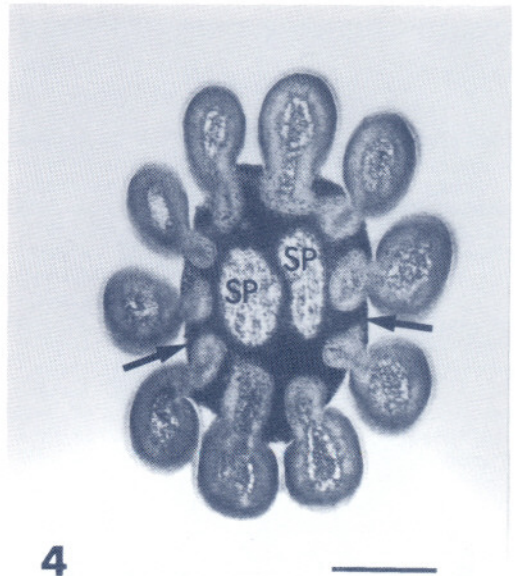


FIG. 4. Transverse section through the apical part of a double-walled sensillum. Although there are two unbranched sensory processes (SP), usually three occur in this sensillar type. The sensory processes are surrounded by an electron dense substance that reaches to the surface (arrows) between the cuticular ribs. Scale bar, 0.25 μm .

sensilla in the olfactory pit. The slender peg is connected to the surrounding cuticle by a socket. The apical part of the peg is provided with radial slit-like pores (Fig. 4). This sensillar type is innervated by three sensory cells that give rise to unbranched sensory processes. Below the peg, the sensory processes are invested by a well-developed cuticular sheath that adheres to the inner cuticular tube of the peg.

d. Poreless sensilla (NP)

These sensilla have a superficial pore system (Fig. 5), but are classified as poreless because the pores do not connect the outside of the hair with the lumen. About 20 poreless sensilla are found in the olfactory pit of each antenna. The cuticular hair is slender, having a length of $6\ \mu\text{m}$, and a basal diameter of $2\ \mu\text{m}$. The surface of the cuticular hair is provided with small knobs, and pores are located between them (Fig. 6). The pores are connected to a system of canals that proceeds towards the base of the hair where it ends on the inside of the cuticle. The NP sensilla are innervated by three sensory cells (Fig. 7) and these give rise to unbranched sensory processes that are invested in a well-developed cuticular sheath. One of the sensory processes

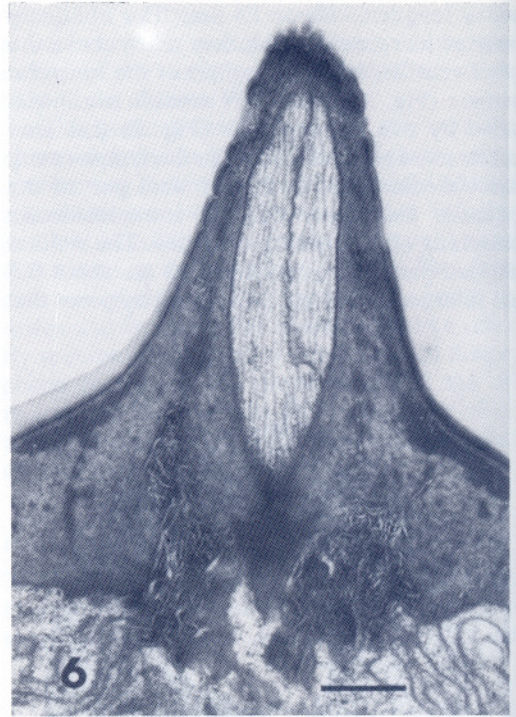


FIG. 6. Oblique section through a poreless sensillum. Scale bar, $0.25\ \mu\text{m}$.

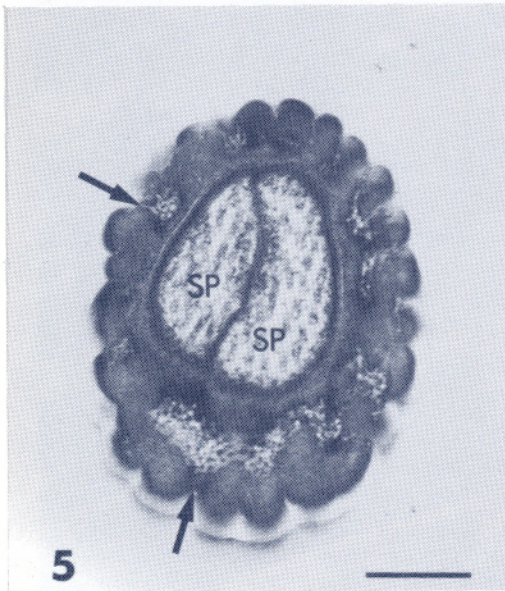


FIG. 5. Transverse section through the peg of a poreless sensillum in the olfactory pit. The cuticle of the hair is penetrated by a system of pores (arrow), but these do not reach the lumen of the hair, which is occupied by two sensory processes (SP). Scale bar, $0.25\ \mu\text{m}$.



FIG. 7. Oblique section through a poreless sensillum below the peg, showing the three sensory processes (SP) that are present at this level. Scale bar, $0.5\ \mu\text{m}$.

terminates freely below the hair, whereas the other two proceed into it (Fig. 6). The sensory processes terminate below the apical part of the hair. The pore system mentioned above does not make contact with the interior of the hair; instead, the sensory processes are surrounded by a homogenous cuticular cylinder. The thickness of the walls of the inner cuticular cylinder is about 70 nm. The sensory processes fill the entire interior of the hair.

Discussion

The funicular surface of *Dacus oleae* possesses three types of sensilla that have a structure indicating an olfactory function (Altner and Prillinger 1980). The olfactory pit, on the other hand, has a few olfactory sensilla of the double-walled type, whereas the majority of the pit sensilla are poreless. Similar sensillar types have been described in other fly species (Bay and Pitts 1976, Honda et al. 1983, Altner et al. 1983). In *Musca autumnalis* (Bay and Pitts 1976) the absence of pores was not established unequivocally. Poreless sensilla, which typically are innervated by three sensory cells, have been shown in other insect species to respond to temperature/humidity (Altner and Prillinger 1980). A similar function for the poreless sensilla in *Dacus oleae* cannot be excluded. Thus, the designation "olfactory pit" seems to be misleading in *Dacus oleae*: in this species only a few of the pit sensilla (the double-walled) have a structure suggesting olfactory function.

The surface of the funiculus in *Dacus oleae* lacks taste mechanoreceptors, which is also the case in other flies (Dethier 1976). The taste mechanoreceptors are generally found on the antennae of most insect species. The short antennae in flies are evidently not a suitable location for sensilla of this type. On the other hand, the taste sensilla are found on the tarsi and labellum, where they can be brought in contact with the substratum more easily (Dethier 1976).

Generally, the sensilla with an established pheromone receptive function among insects have been shown to be single-walled sensilla (Seabrook 1978). In moths, the males usually possess large numbers of long, single-walled sensilla, whereas the females are lacking these. The well-developed antennal receptor

system in the male moths indicates a high sensitivity towards the pheromones emitted by the females (Kaisling 1971). In bark beetles, on the other hand, both males and females respond to the aggregation pheromones, and the antennal receptors are equally well developed in the two sexes (Payne et al. 1973). It has been proposed that two types of single-walled sensilla constitute the pheromone-sensitive receptors in bark beetles (Dickens and Payne 1978).

As regards the pheromone-sensitive sensilla on the funiculus of *Dacus oleae*, these are likely to be represented by either or both of the single-walled types.

Both males and females of *Dacus oleae* emit pheromones, and the reciprocal chemical communication may be reflected in the equally well-developed antennae of both sexes. De Marzo et al. (1978) have shown that *Dacus oleae* males strongly attract females by a sex pheromone. Haniotakis (unpublished data) has observed that for successful mating, a chemical stimulus released by the male and perceived by antennal receptors of the female is required. This stimulus functions as female arrestant or aphrodisiac and in its absence the female strongly rejects the male.

References

- Altner, H. and L. Prillinger. 1980. Ultrastructure of invertebrate chemo-, thermo-, and hygroreceptors and its functional significance. *Int. Rev. Cyt.* 67: 69-139.
- Altner, H., L. Schaller-Selzer, H. Stetter and I. Wohrab. 1983. Poreless sensilla with inflexible sockets. A comparative study of a fundamental type of insect sensilla probably comprising thermo- and hygroreceptors. *Cell Tissue Res.* 234: 279-307.
- Baker, R., R. Herbert, P.E. Howse, O.T. Jones, W. Francke and W. Reith. 1980. Identification and synthesis of the major sex pheromone of the olive fly (*Dacus oleae*). *J.C.S. Chem. Comm.* 1106: 52-53.
- Bay, D.E. and C.W. Pitts. 1976. Antennal olfactory sensilla of the face fly, *Musca autumnalis* Degreer (Diptera: Muscidae). *Int. J. Insect Morphol. and Embryol.* 5: 1-16.
- De Marzo, L., G. Nuzzaci and M. Solinas. 1978. Studio anatomico, istologico, ultrastrutturale e fisiologico del retto ed osservazioni etologiche in relazione alla possibile produzione di feromoni sessuali nel maschio di *Dacus oleae* Gmel. *Entomologica XIV*: 203-266.
- Dethier, V.G. 1976. The etiquette of eating: mechanism and control of ingestion. In, V.G. Dethier: *The hungry fly*. Harvard University Press, Cambridge. pp. 67-118.
- Dickens, J.C. and T.L. Payne. 1978. Structure and function of the sensilla on the antennal club of the southern pine beetle, *Dendroctonus frontalis* (Zimmerman)

- (Coleoptera: Scolytidae). Int. J. Insect Morphol. & Embryol. 7: 251-265.
- Haniotakis, G.E., B.E. Mazomenos and I.M. Hardakis. 1982. Monitoring and control of the olive fruit fly with pheromone traps. Proc. Experts' Meeting E.E.C. on "L'état d'avancement des travaux et échanges d'informations sur les problèmes posés par la lutte intégrée en oléiculture". Antibes, France, Nov. 4-6, 1981, pp. 46-60.
- Honda, I., Y. Ishikawa and Y. Matsumoto. 1983. Morphological studies on the antennal sensilla of the onion fly, *Hylemya antiqua* Meigen (Diptera: Anthomyiidae). Appl. Ent. Zool. 18: 170-181.
- Kaissling, K.E. 1971. Insect olfaction. In, "Handbook of Sensory Physiology. Vol. IV. Chemical Senses. 1. Olfaction". (L.M. Beidler Ed.). Springer Verlag, Berlin. pp. 351-431.
- Liebermann, A. 1926. Correlation zwischen den antennalen Geruchsorganen und der Biologie der Musciden. Z. Morph. Ökol. Tiere. 5: 1-97.
- Mazomenos, B.E. and G.E. Haniotakis. 1981. A multicomponent female sex pheromone of *Dacus oleae* (Gmelin). Isolation and bioassay. J. Chem. Ecol. 7: 137-144.
- Mazomenos, B.E. and G.J. Pomonis. 1983. Male olive fruit fly pheromone: isolation, identification and lab bioassays. Proc. Intern. Sympos. of C.E.C./I.O.B.C. on Fruit Flies of Economic Importance. Athens, Greece, Nov. 16-19, 1982, pp. 96-103.
- Mazomenos, B.E., G.E. Haniotakis, J.H. Tumlinson and N. Ragoussis. 1981. Isolation, identification, synthesis and bioassay of the olive fruit fly pheromones. Abstr., Panhellenic Congress of Agricultural Research. Chalkidiki, Greece, May 5-8, 1981, pp. 96-97.
- Payne, T.L., H.A. Moeck, C.D. Willson, R.N. Coulson and W.J. Humphreys. 1973. Bark beetle olfaction. II. Antennal morphology of sixteen species of Scolytidae (Coleoptera). Int. J. Insect Morphol. & Embryol. 2: 177-192.
- Seabrook, W.D. 1978. Neurobiological contributions to understanding insect pheromone systems. Ann. Rev. Entomol. 23: 471-485.
- Van Der Pers, J.N.C., G.E. Haniotakis and B.M. King. 1984. Electroantennogram responses from olfactory receptors in *Dacus oleae*. Entomologia Hellenica 2: 47-53.

KEY WORDS: *Dacus oleae*, Olive fruit fly, Diptera, Tephritidae, Electron microscopy, Olfactory sensilla, Poreless sensilla, Fine structure

Κορυνικά Αισθητήρια του *Dacus oleae*: Μικροανατομικά Χαρακτηριστικά

E. HALLBERG, J.N.C. VAN DER PERS και Γ.Ε. ΧΑΝΙΩΤΑΚΗΣ

Department of Zoology, University of Lund, Lund, Sweden

ΠΕΡΙΛΗΨΗ

Τα κορυνικά αισθητήρια του εντόμου *Dacus oleae* απαντώνται είτε στην επιφάνεια είτε στην οσφρητική ουλή της κορύνης. Τα αισθητήρια της επιφάνειας είναι τριών τύπων: α) αισθητήρια μεγάλου μήκους με απλά τοιχώματα, β) αισθητήρια μικρού μήκους με απλά τοιχώματα, γ) αισθητήρια με διπλά τοιχώματα. Τα μικροανατομικά χαρακτηριστικά και των τριών ως άνω αισθητηρίων δείχνουν ότι πρόκειται για αισθητήρια οσφρήσεως. Και οι δύο τύποι αισθητηρίων με απλά τοιχώματα περιλαμβάνουν δυο νευρικά κύτταρα. Στα αισθητήρια μεγάλου μήκους οι νευρικές απολήξεις είναι μη διακλαδιζόμενες ενώ στα μικρού μήκους είναι διακλαδιζόμενες. Τα αισθητήρια με διπλά τοιχώματα συνήθως περιλαμβάνουν τρία νευρικά κύτταρα τα οποία καταλήγουν σε μη διακλαδιζόμενες νευρικές απολήξεις στην κορυφή του αισθητηρίου. Τα αισθητήρια της οσφρητικής ουλής είναι δύο τύπων: α) αισθητήρια με διπλά τοιχώματα παρόμοια με εκείνα της επιφάνειας, β) αισθητήρια άνευ πόρων. Τα αισθητήρια άνευ πόρων περιλαμβάνουν τρία νευρικά κύτταρα, δύο από τα οποία καταλήγουν στο χιτινώδες τριχίδιο ενώ το τρίτο κάτωθεν τούτου. Ο τύπος του τελευταίου τούτου αισθητηρίου περιγράφεται για πρώτη φορά και η αίσθηση την οποία εξυπηρετεί είναι άγνωστη.