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Antennal sensilla of *Mycodiplosis erysiphes* Ruebs. (Cecidomyiidae, Diptera) (*) (**)

Introduction

Cecidomyiidae antennae ever aroused Entomologists' attention, since they started being studied (Kieffer, 1900; Ruebsaamen & Hedicke, 1925-1939), because of their peculiar equipment of bristles, hairs and circumfila. Most Dipterologists studied those integumental features for taxonomic purposes and some tried to understand their biological meaning and morphogenesis, particularly for circumfila (Felt, 1925; Janet, 1895), though with modest results due to the obvious technical limits before the advent of electron microscopy. Only recently, morpho-functional interpretation of the said structures has been facilitated by Slifer & Sekhon's (1971) work on *Contarinia sorghicola* (Coq.).

Nevertheless some important aspects still need to be elucitated. For this reason we thought it was opportune to study in detail all types of antennal (flagellomeral) sensilla in a species, like *Mycodiplosis erysiphes* Ruebs, where they are particularly abundant and well developed in both sexes. Furthermore, to contribute more useful information about those features for taxonomic purposes, we have taken into consideration the third flagellomere especially.

Materials and Methods

Newly emerged male and female Midges from larvae collected in the field on *Cucurbita* sp. leaves infected by *Erysiphe cichoriacearum* DC. were used for all purposes.

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Isolated heads were fixed in cold solution of 4% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) containing 5% sucrose, for three hours, rinsed overnight in the same buffer, and post-fixed in 2% Osmium tetroxide in the same phosphate buffer for two hours, rinsed in the same buffer, dehydrated in ethanol and: for T.E.M. observations, specimens (only females) were embedded through propylene oxide in Araldite 502, and thin sections sequentially stained with uranyl acetate and lead citrate were examined in a Zeiss EM 109; for S.E.M. observations, specimens (males and females) were gold coated in an Edwards S 150 A sputter coater and viewed in a Cambridge Stereoscan 100 and/or a Philips 501 B; in addition, one specimen was treated with tetrachloromethane only (Cuperus, 1985) and another one with a protease only, before dehydrating in ethanol and gold coating, to make visible possible present tip pore(s) on the grooved pegs.

SYMBOLS USED IN THE FIGURES

AN: antennal nerve
BB: basal bodies
CF: circumfila
CL: ciliary region
CR: ciliary rootlets
CS: ciliary sinus
CU: cuticle
DB: dendrite branches
DD: distal dendritic segment
DN: dendrite
DS: dendritic sheath
FL: circumfilum lumen
GP: grooved peg
HS: hair shaft
MC: microtrichia
MV: microvilli
PD: proximal dendritic segment
PE: peg
PT: pore tubules
RD: ridges
SB: sensory bristles
SC: sheath cell
SH: sensory hairs
SL: shaft lumen
SO: socket
SS: sensory sinus
ST: circumfilum stalk
TB: tubular body
TH: thecogen cell
TO: tormogen cell
TR: trichogen cell
WP: wall pore

Results and Discussion

Low magnification SEM observations show, on each flagellomere of both sexes, the five types of integumental appendages like they are known and familiar to Cecidomyiidologists, namely (Figs. I and II): a) long and strong bristles inserted on rigid mucronate sockets, hereafter called « sensory bristles » (SB); b) long, clear and flexible hairs inserted on horseshoeshaped sockets, hereafter called « sensory hairs » (SH); c) circumfila (CF); d) isolated, minute, clear hairs inserted on rimmed bases, previously known as « sensory pores » (Panelius, 1965), « sensilli isolati » (Solinas, 1965), « stubby pegs » (Slifer et al., l.c.), « sensory spines » (Roskam, 1977 and 1979), hereafter called « grooved pegs » (GP); and e) microtrichia (MC).

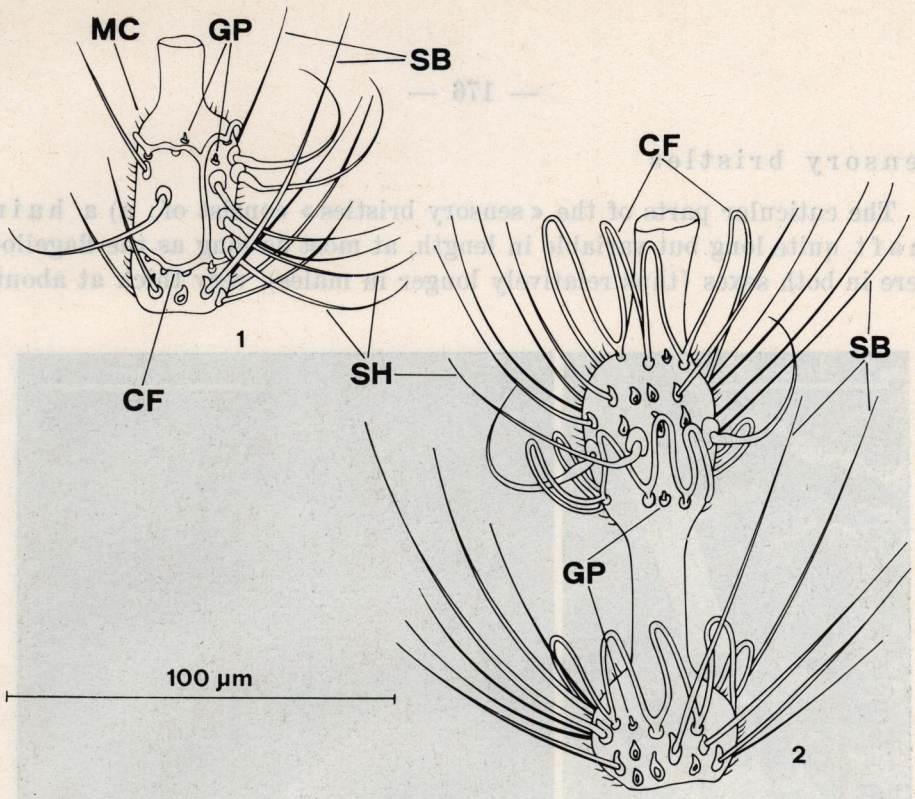

FIG. I

Mycodiplosis erysiphes. Female (1) and male (2) third flagellomere (semi-schematic).

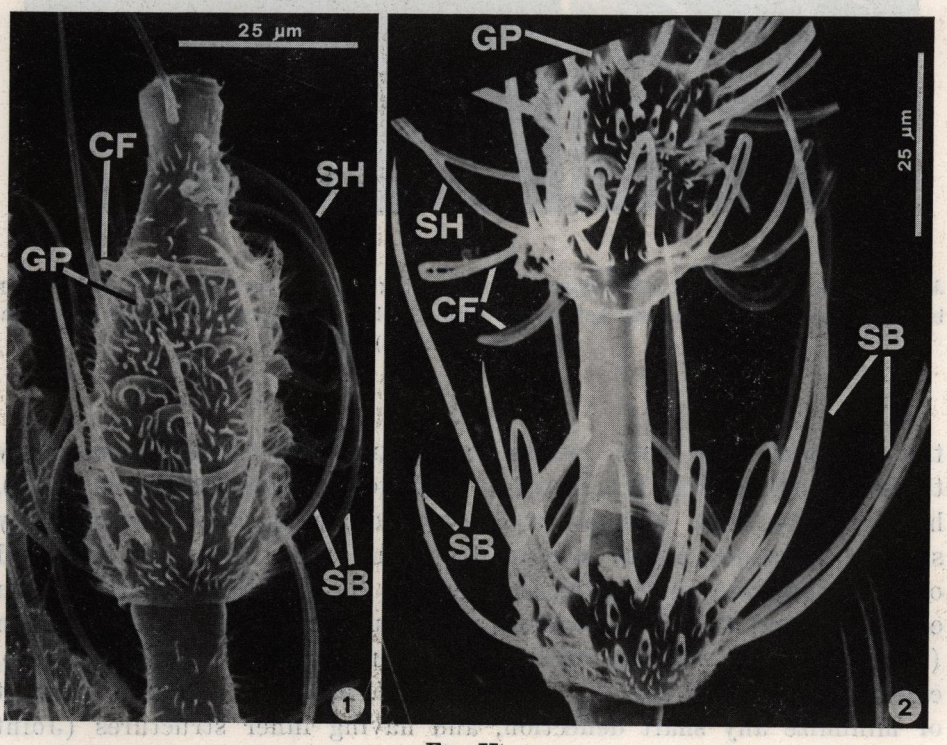

FIG. II

M. erysiphes. Female (1) and male (2) third flagellomere (S.E.M. micrographs).

Sensory bristles

The cuticular parts of the « sensory bristles » consist of: a) a hair shaft quite long but variable in length, at most as long as the flagellomere in both sexes (thus relatively longer in males), very thick at about

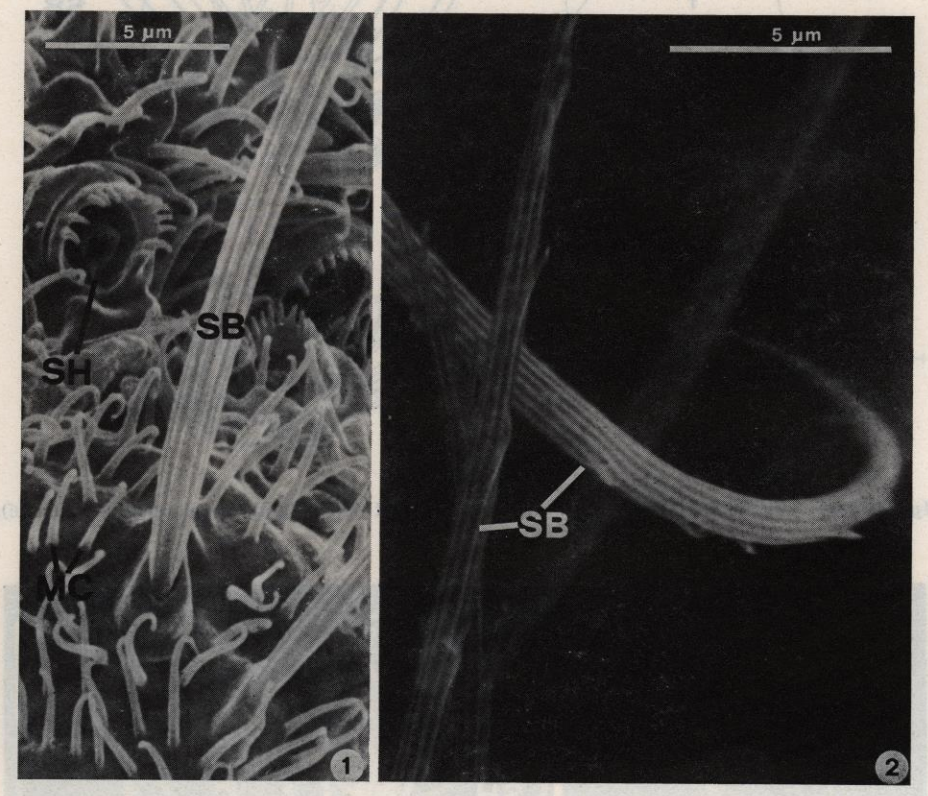

Fig. III

M. erysiphes. Details of a third flagellomere showing: (1) the basal portion of « sensory bristles » (sockets of « sensory hairs » are also visible); (2) apical portion of the same. (SEM micrographs).

the proximal third level (width to 1.5 µm) but tapering moderately towards the base (Fig. III, 1) and strongly towards the apex (Fig. III, 2), having quite thick aporous walls with outer surface longitudinally striated by strong ridges starting from just above the shaft base and often ending like short pointed branches, on the distal shaft portion especially, and with lumen quite narrow and devoid of any innervation (Fig. V, 2, 3); b) a strong, rigid socket upwards tapering to a mucro, embracing the shaft base tightly (Figs. III, 1; V, 1, 2) so as to prevent or minimize any shaft deflection, and having inner structures (Joint

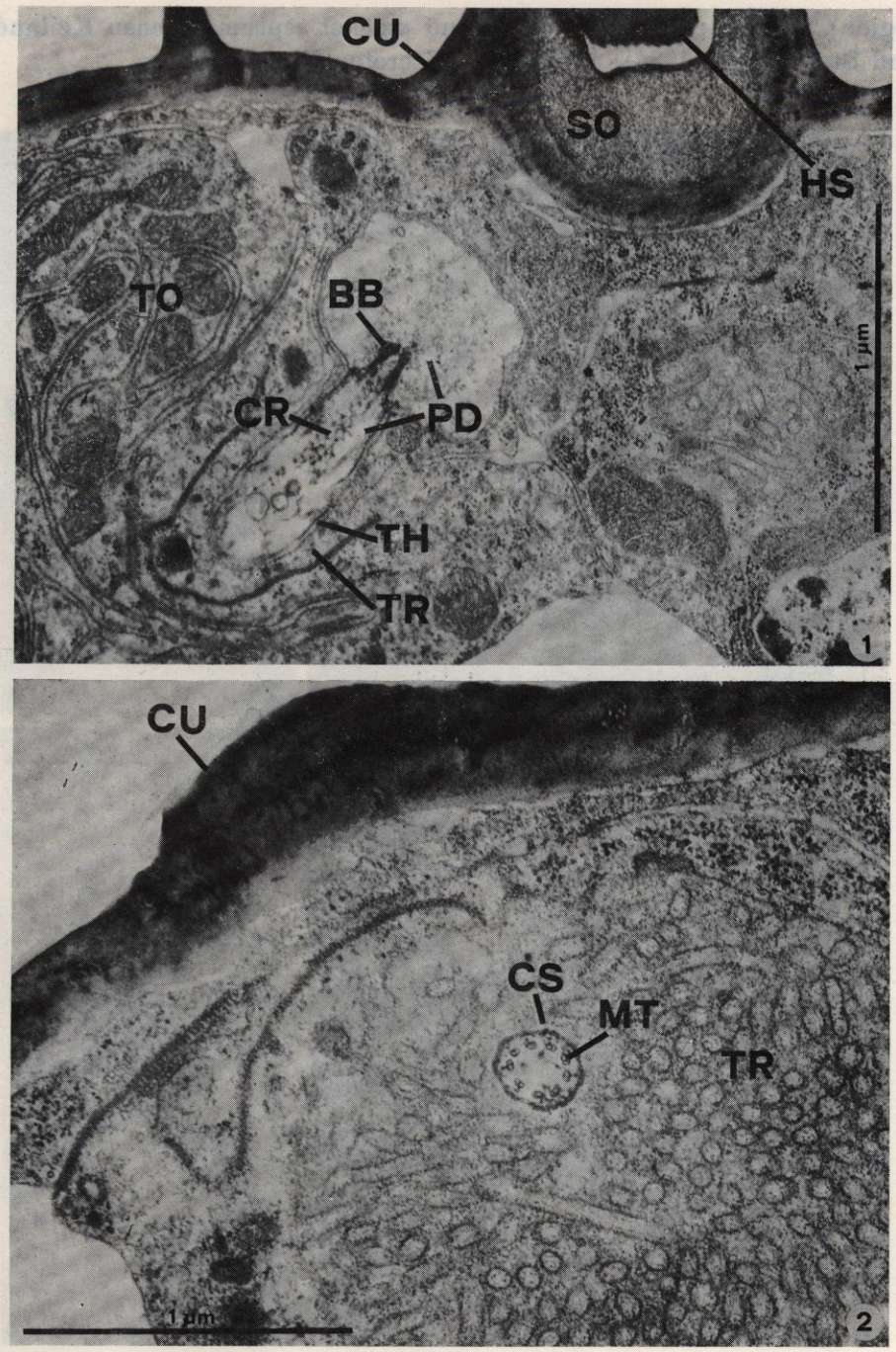

FIG. IV

M. erysiphes. Female. Details of a flagellomere cross section showing, of a sensory bristle: (1) a longitudinal section of dendritic proximal segment and ciliary region of the single sensory neuron; (2) a cross section of ciliary region of the same sensory neuron. (T.E.M. micrographs).

region), namely «joint membrane» and «socket septum» (sensu Keiland) and Steinbretch, 1984), not clearly discrete (Fig. V, 1, 2).

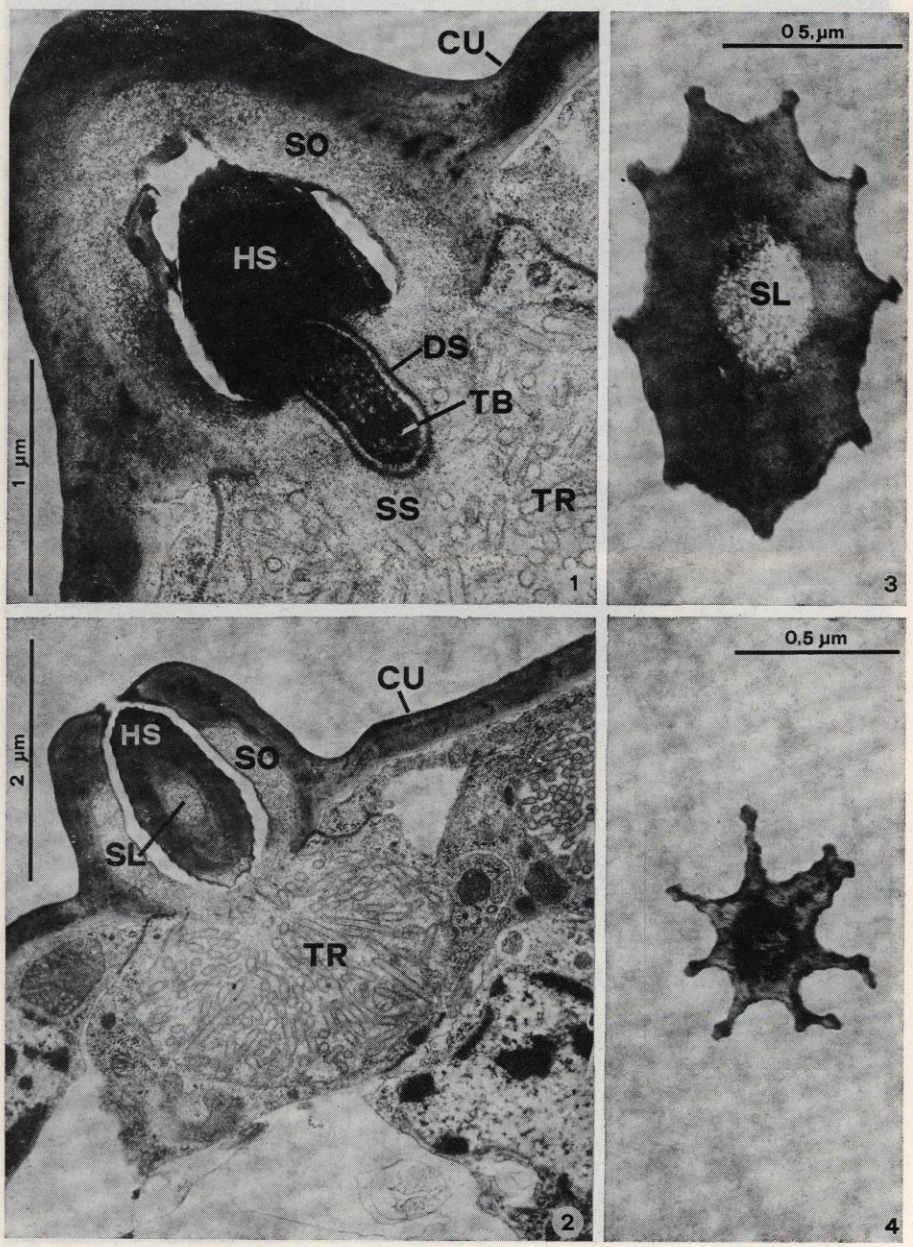

FIG. V

M. erysiphes. Ditto, showing: (1) the ending of dendritic distal segment (with tubular body) attached to the hair shaft base; (2) hair shaft cross section just above insertion (no dendrite within the lumen); (3) shaft cross section at about half length level; (4) ditto, near the hair tip. (T.E.M. micrographs).

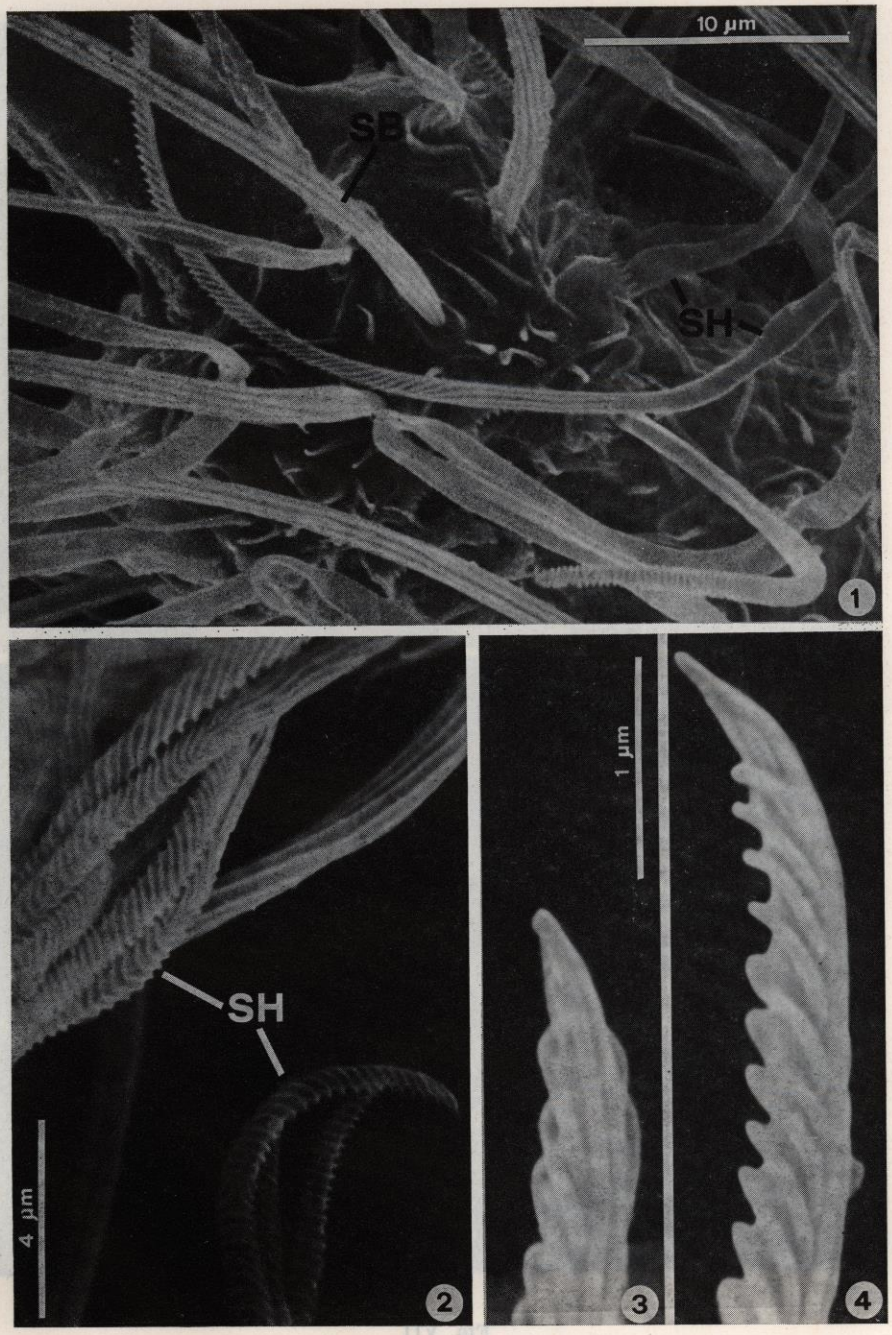

FIG. VI

M. erysiphes. (1) Detail of the distal node of a male third flagellomere showing several « sensory hairs »; (2), (3), (4) different aspects of sensory hair shafts. (3 and 4 at the same magnification). (S.E.M. micrographs).

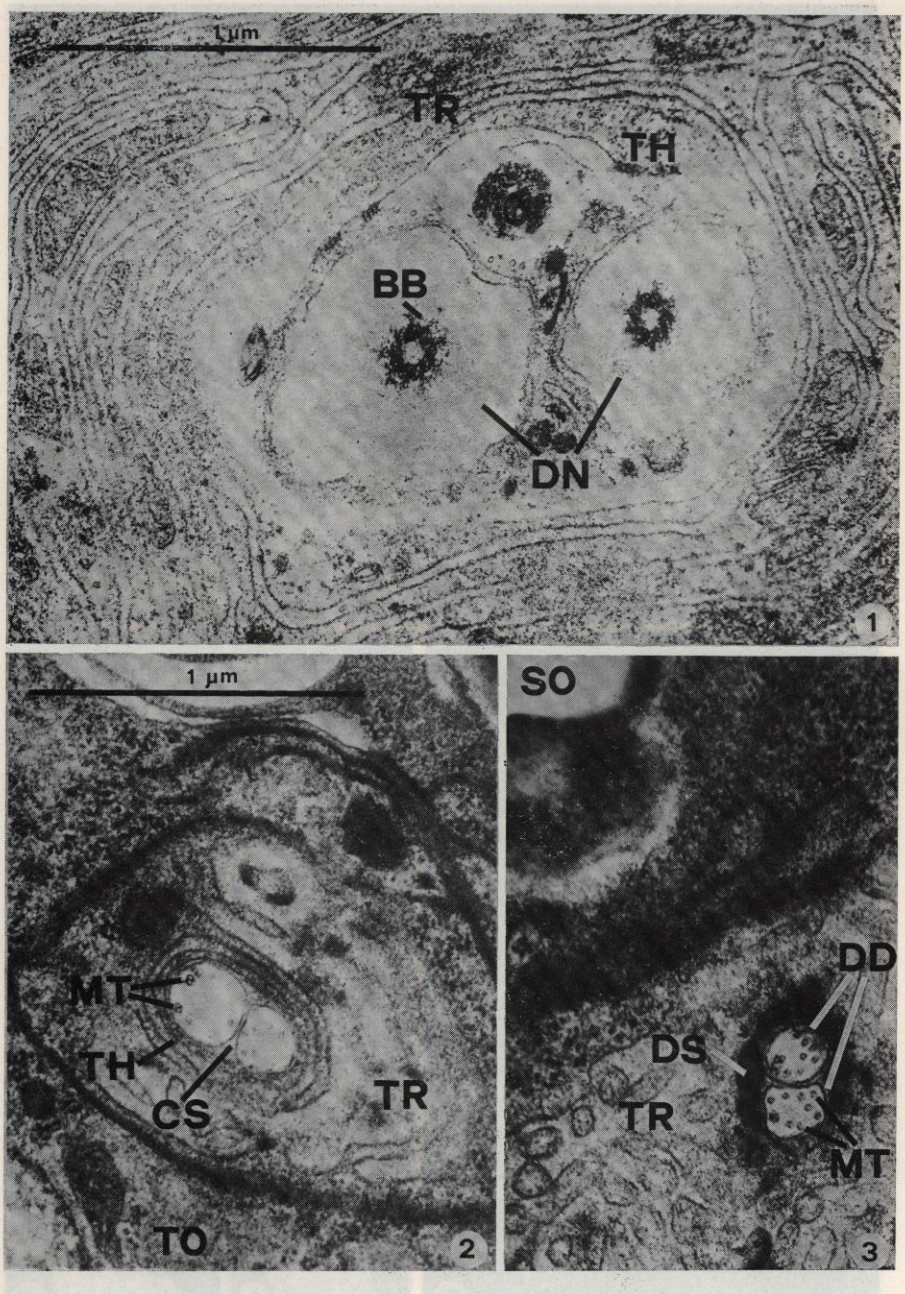

FIG. VII

M. erysiphes. Female. Details of a flagellomere section showing cross sections of the two sensory neuron dendrites of a « sensory hair », made at the level of: (1) ciliary basal apparatus; (2) just above the ciliary region; (3) close to the hair-shaft insertion. (T.E.M. micrographs).

The cellular components are represented by: a) a single sensory neuron (Fig. IV) whose dendrite ends with a tubular body upon the base of the hair shaft where this interacts with inner socket portion (Fig. V, 1); and b) three accessory cells, called sheath cells, namely: a thecogen cell encasing the proximal dendritic segment and bordering the ciliary sinus (Fig. IV, 1); a trichogen cell and a tormogen, both (Figs IV, V) partially encasing the distal dendritic segment and bordering the sensillar sinus with packed microvilli.

Given the above described functional structures, both cuticular and cellular ones, the « sensory bristles » must be considered mechanoreceptors, most probably tactile.

Sensory hairs

The cuticular parts of the « sensory hairs » consist of: a) hair shaft relatively longer in female than in male flagellomera (Figs. I, II), quite thinner than sensory bristles but bearing a remarkable upper swelling (Fig. VI, 1) at the base, where it displays a smooth outer surface, while further up it shows a longitudinal series of ridges running almost parallel on the shaft proximal third (Fig. VI, 1) and becoming like an irregular coil (Fig. VI, 2) on the rest where only two ridges keep running parallel to the tip (Fig. VI, 3, 4), and having multiporous thin walls (Fig. VIII, 2, 3); and a big horseshoeshaped socket (Figs. II, VI, 1) remarkably convex, turned downwards and widely loose around the shaft base (Fig. VIII, 1).

The cellular components are represented by: a) two sensory neurons (Fig. VII) whose dendritic distal segments run encased in a common thick sheath to just under the socket and then, free from any sheath, enter the shaft lumen (Fig. VIII, 1) and keep running unbranched to the hair tip (Fig. VIII, 2, 3); and b) three accessory cells, namely: thecogen, trichogen and tormogen (Fig. VII).

On the base of the above reported cuticular and cellular functional structures, the « sensory hairs » must be considered olfactory sensilla and, given the relatively greater length and number in females (Figs. I, II) than in males, their involvement is conceivable in finding oviposition sites on host-plants and/or fungi.

Circumfila

The cuticular parts of circumfila consist of: a) a threadlike hair shaft typically encircling the flagellomeres, quite differently in males and females (Figs. I, II, XIII), connected to the antennomere by short stalks displaying a smooth outer surface, whereas on the rest of the shaft this shows low irregular ridges in both sexes (Figs. IX, XIII), and having very thin multiporous walls (Figs. XI, XII) with pores

laying at the bottom of shallow depressions and each leading into numerous pore-tubules (Fig. XII, 2, 3); and a relatively weak, roundish

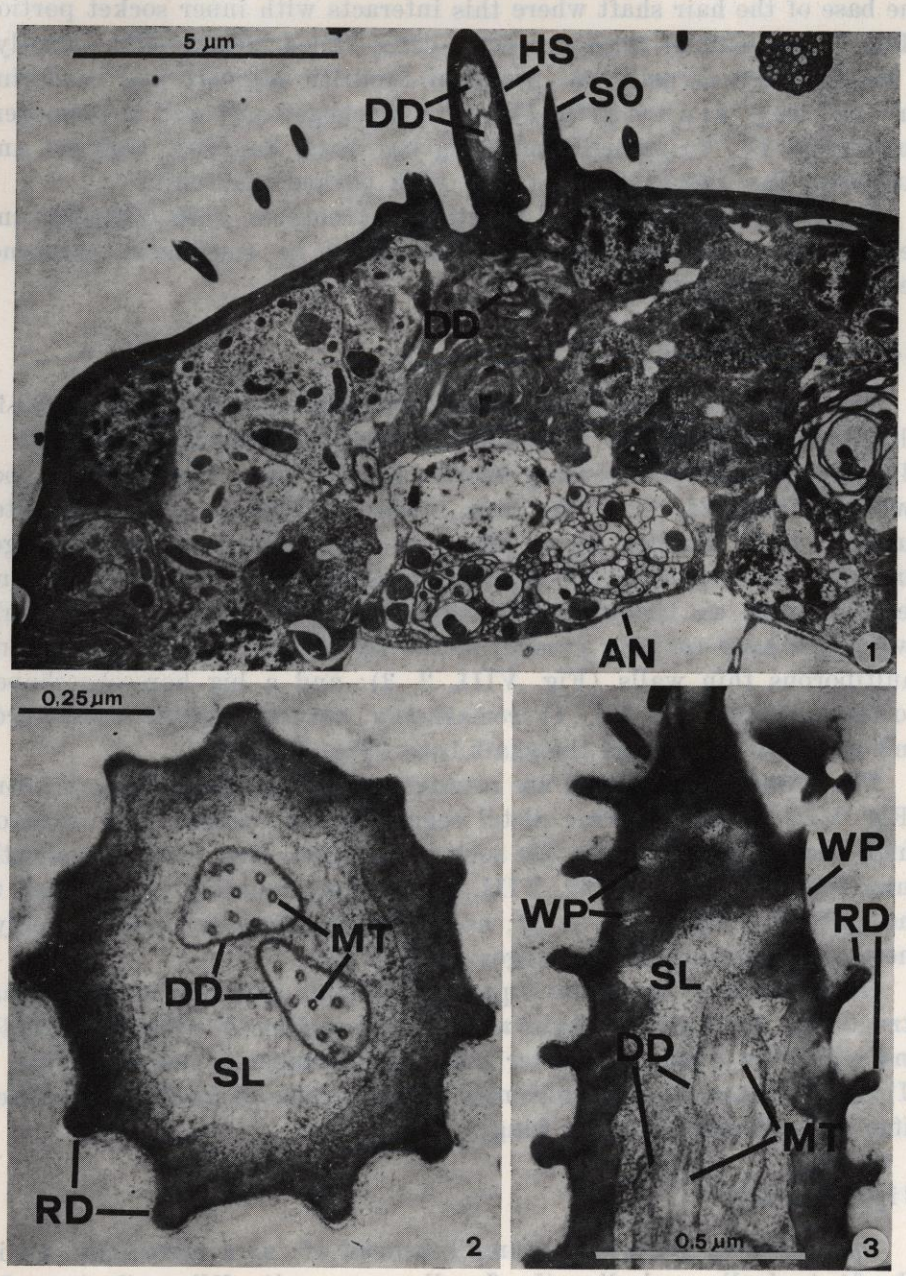

FIG. VIII

M. erysiphes. Female: (1) detail of a flagellomere cross section showing both sensory neuron dendrites of a « sensory hair » entering the shaft lumen; (2) hair shaft cross section of a « sensory hair » made at about 1/3 length level; (3) hair shaft sub-distal longitudinal section of ditto. (T.E.M. micrographs).

socket (Figs. II, IX, XIII) whose rim is often interrupted downwards (Fig. XIII, 2).

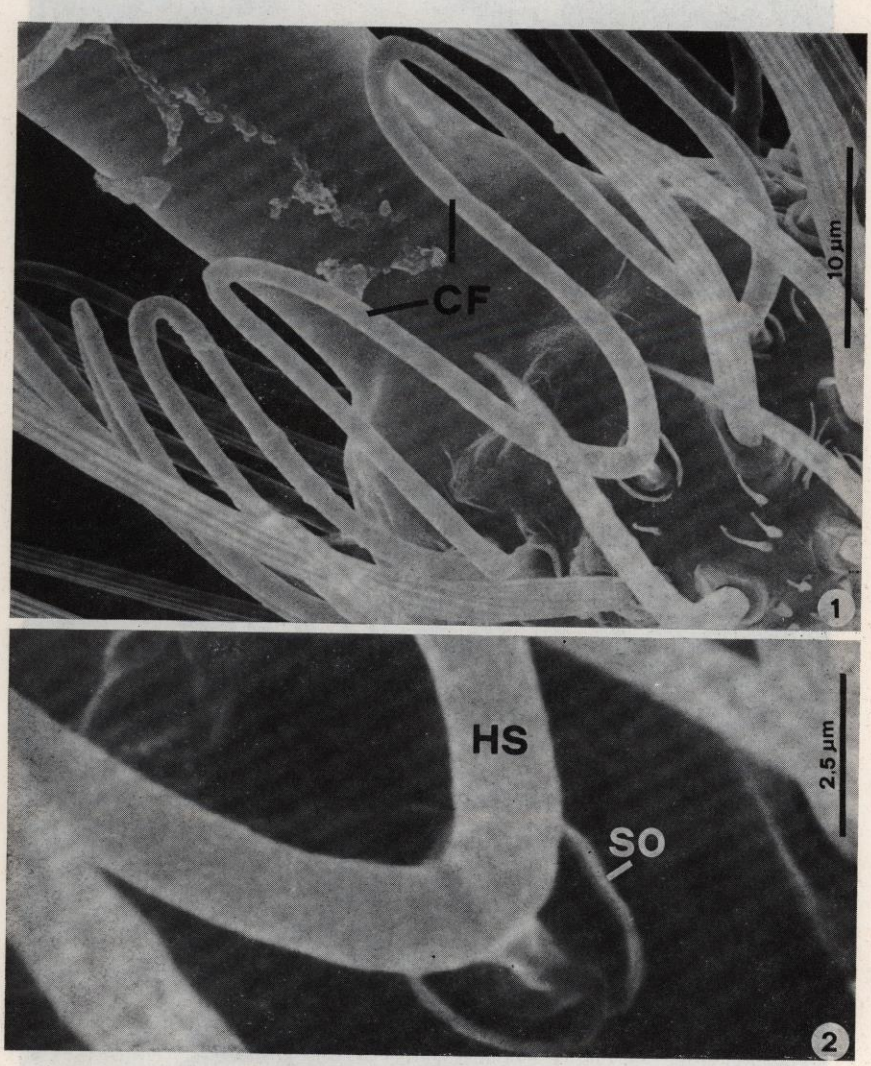

Fig. IX

M. erysiphes. Details of a male third flagellomere distal node showing circumfila. (S.E.M. micrographs).

There are two sensory neurons having perikarion within the hypoderm and each sending both axon and dendrite towards the flagellomere centre where the axon joins the antennal nerve while the distal dendritic segment bends (Fig. X, 2) and returns outwards to reach the socket. The two distal dendritic segments run encased in a common sheath only

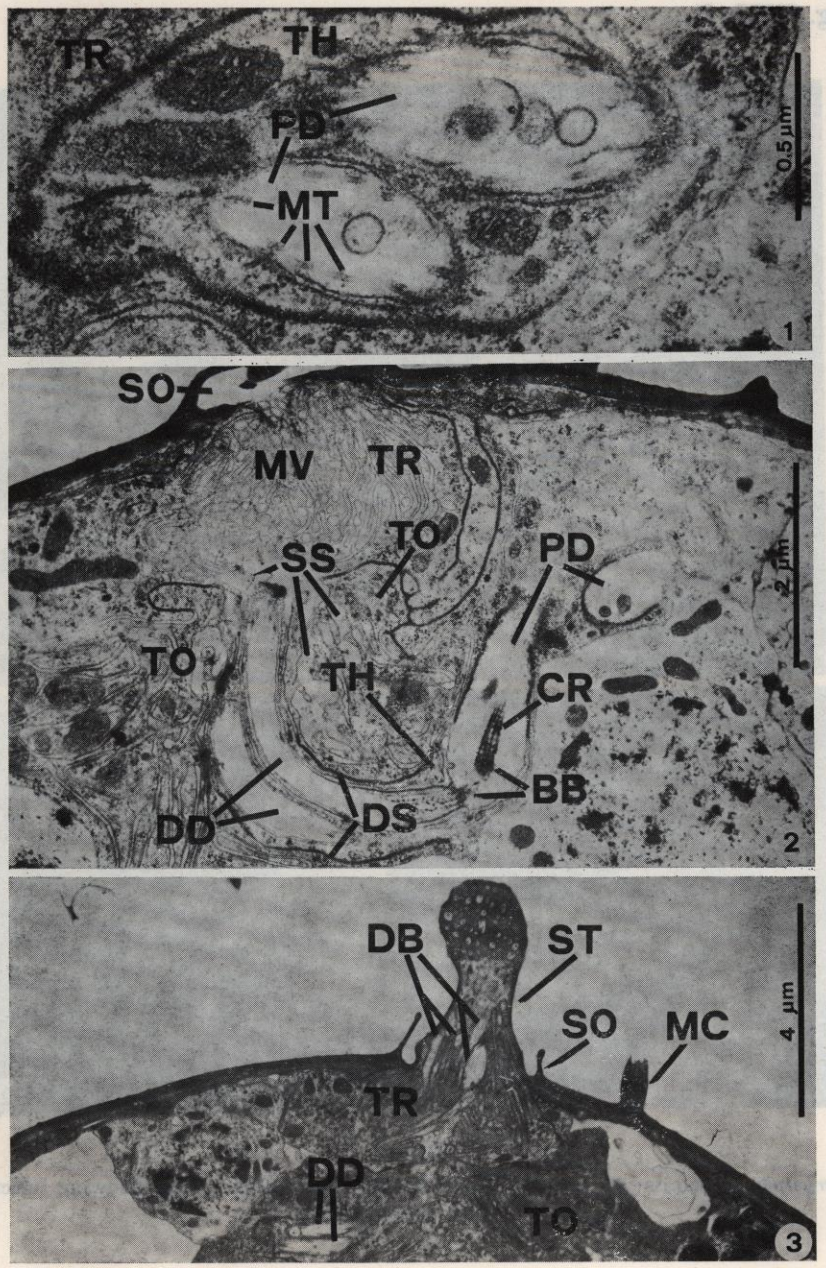

FIG. X

M. erysiphes. Female. Details of a flagellomere cross section showing: (1) an oblique section of the two sensory neuron dendrites of a circumfilum, at their proximal segment level; (2) an almost longitudinal section of both sensory neuron dendrites (partially visible); (3) an oblique section of a circumfilum insertion displaying dendrite branches entering the shaft together with sheath cell microvilli. (T.E.M. micrographs).

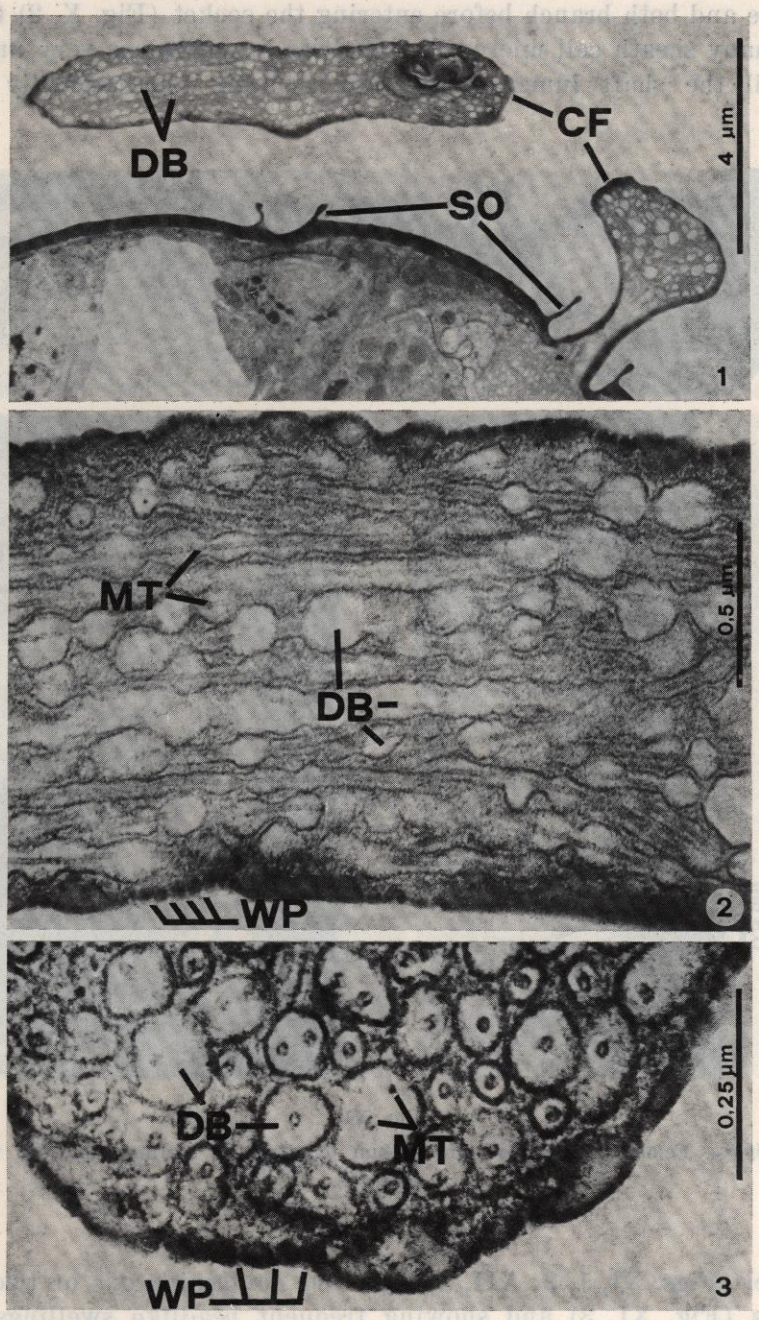

FIG. XI

M. erysiphes. Female. Details of circumfilum sections showing: (1) longitudinal general aspect: (2) dendritic branches running within an intermediate circumfilum portion between two insertions (longitudinal); (3) ditto *cross* section.

proximally (Fig. X, 2), whereas within the sensory sinus they are free and both branch before entering the socket (Fig. X, 2) together with many sheath cell microvilli. Dendrite branches are very numerous and fill the shaft lumen completely, running longitudinally almost

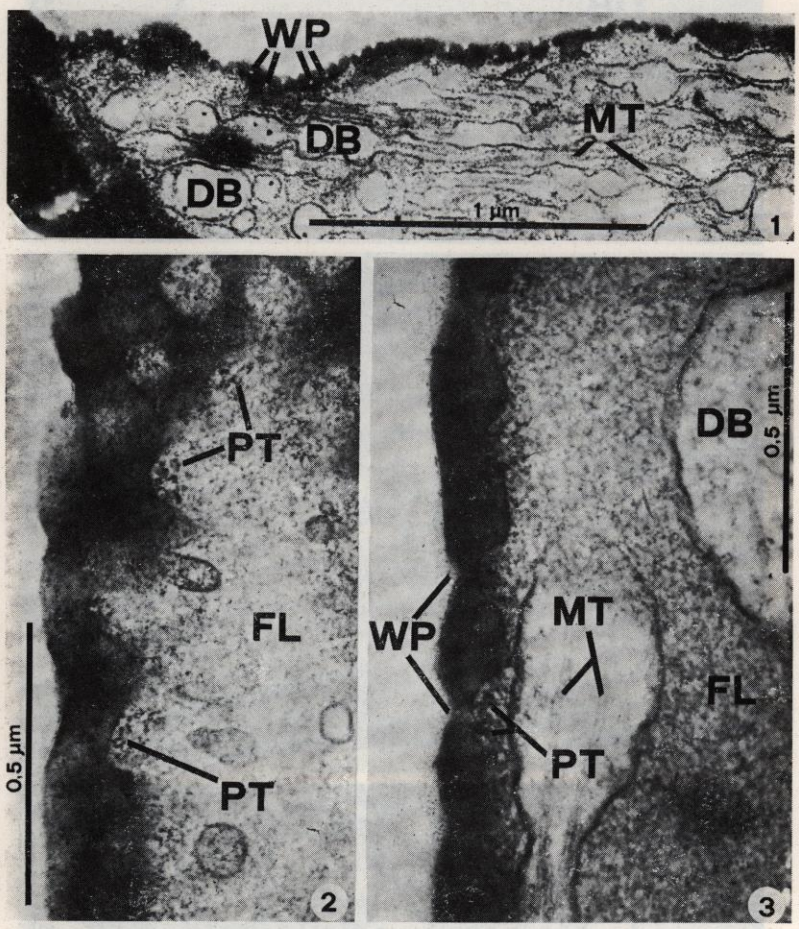

Fig. XII

M. erysiphes. Female. Details of circumfilum longitudinal sections showing wall pore features.

parallel (Figs. XI, 1, 2; XII, 1), each one containing one or two microtubules (Fig. XI, 3) and showing frequent beadlike swellings (Figs. XI, 2; XII, 1, 3).

There are three accessory cells, namely: a thecogen cell (Fig. X, 1, 2) encasing the two proximal dendritic segments to the ciliary sinus; a trichogen and a tormogen cell encasing both distal dendritic segments

to the sensory sinus and also penetrating the circumfilum stalk by numerous microvilli (Figs. X, 3; XI, 2).

The circumfila thus have the features commonly found in olfactory receptors and, given their quite different development between the sexes (Figs. I, II, IX, XIII), it is conceivable that they may be sex pheromon receptors.

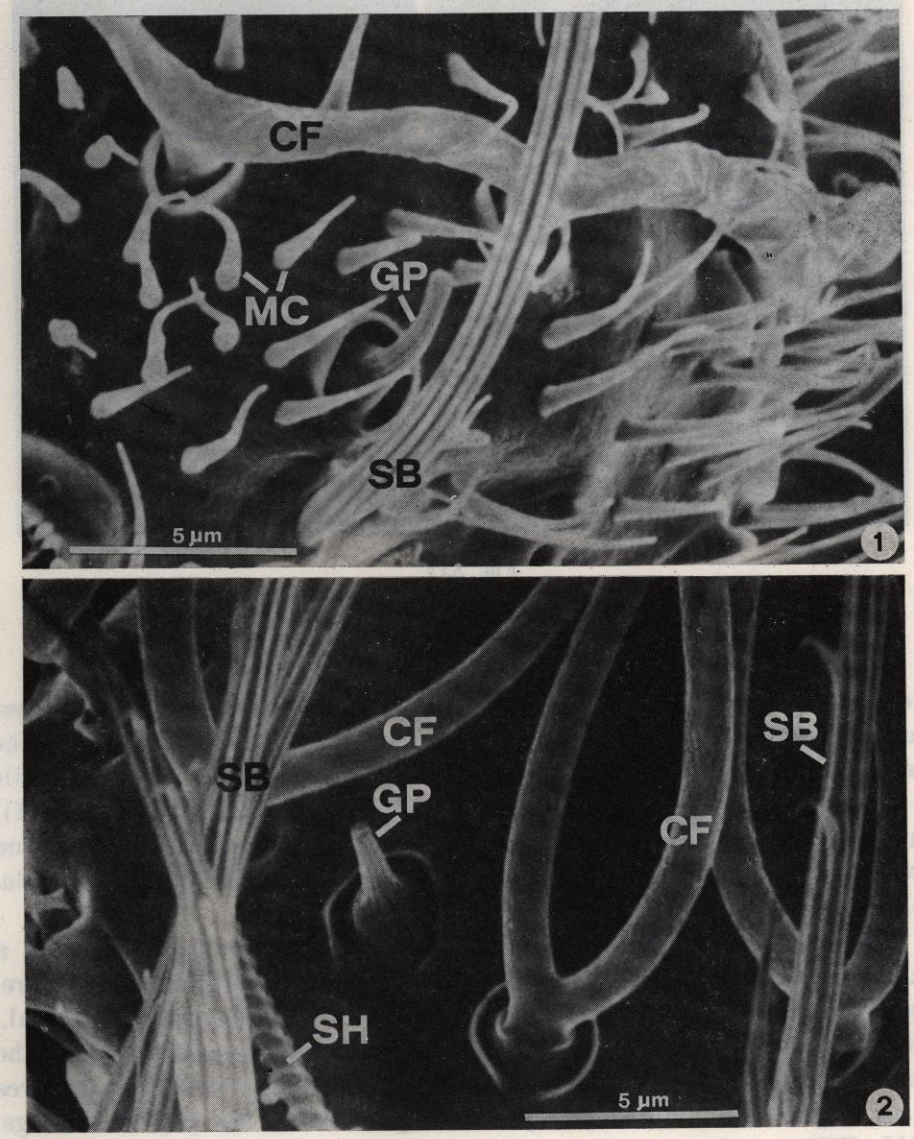

FIG. XIII

M. erysiphes. Details of female (1) and male (2) third flagellomeres showing « grooved pegs ». (S.E.M. micrographs).

Grooved pegs

These are very minute, isolated structures difficult to detect among the thick microtrichia but easily separable from these for they do have a socket which the microtrichia lack (Figs. II, 1; XIII, 1). As a rule

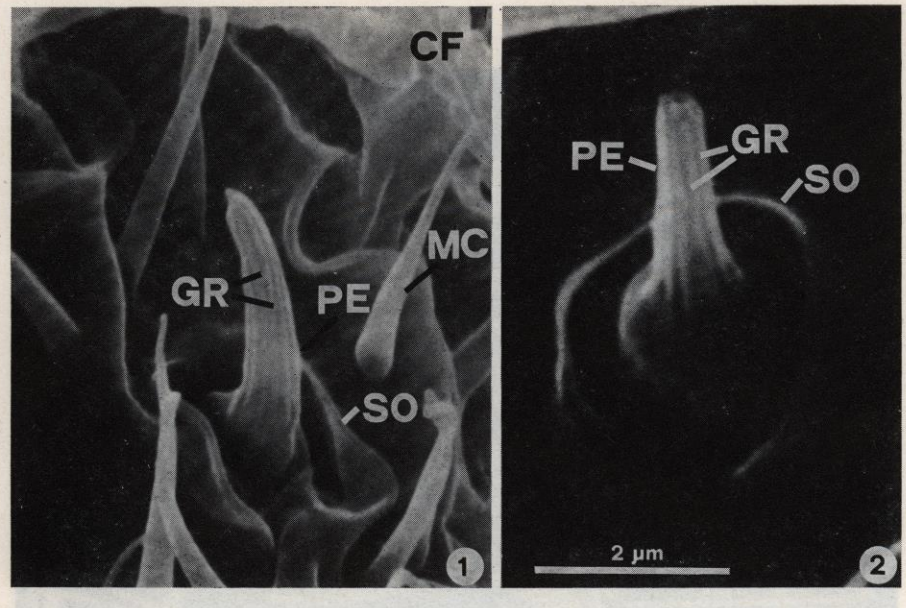

Fig. XIV

M. erysiphes. « Grooved pegs » from female (1) and male (2) flagellomeres.

they are present in number of three per flagellomere in both sexes, located usually (not always) on the mesad antennal side (two near the distal circumfilum whorl and one near the proximal, in females, while in males they are situated one near each circumfilum whorl: Fig. I); but reductions in the number frequently occur in any flagellomere, and exceptionally either none or four grooved pegs can be found on a flagellomere.

The cuticular components of the « grooved pegs » consist of: a) a hair shaft, or peg, very variable in length (at most 3 µm long), frequently shorter than microtrichia, having « double » (sensu Keiland et al., 1984), aporous walls (Fig. XVI) with outer surface, from near the base to the tip of the peg, longitudinally striated by eleven deep grooves (and ridges, obviously) running almost parallel to the peg axis (Figs. XIII, XIV); and a socket very similar to circumfila sockets as to shape and size, both outer (Fig. XIII) and inner (Figs. XVI, 1, 2; X, 3; XI, 1) parts.

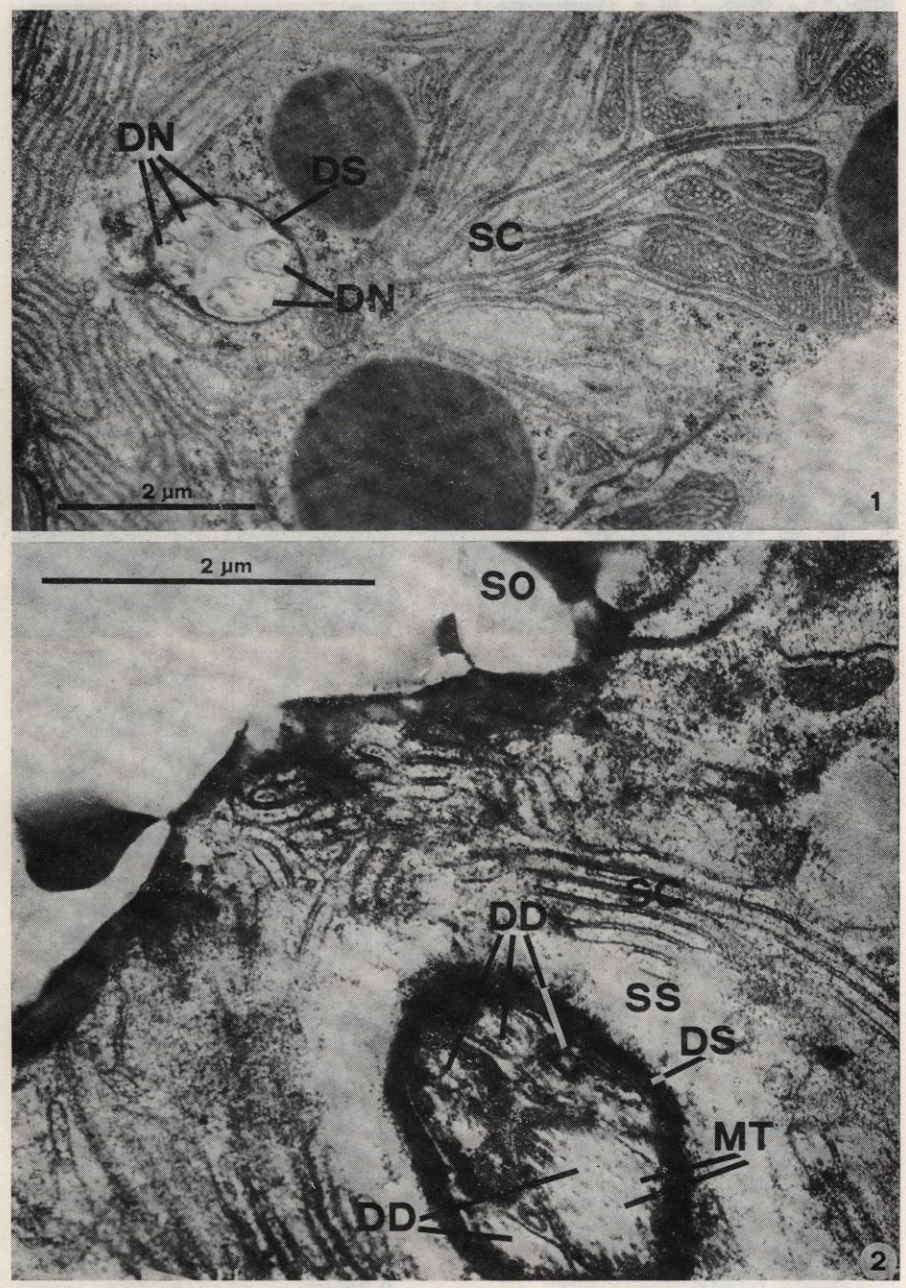

FIG. XV

M. erysiphes. Female. Details of a flagellomere section showing cross sections of the five sensory neuron dendrites (typically surrounded by a common strong sheath), made near the ciliary region (1) and just below the socket (2). (T.E.M. micrographs).

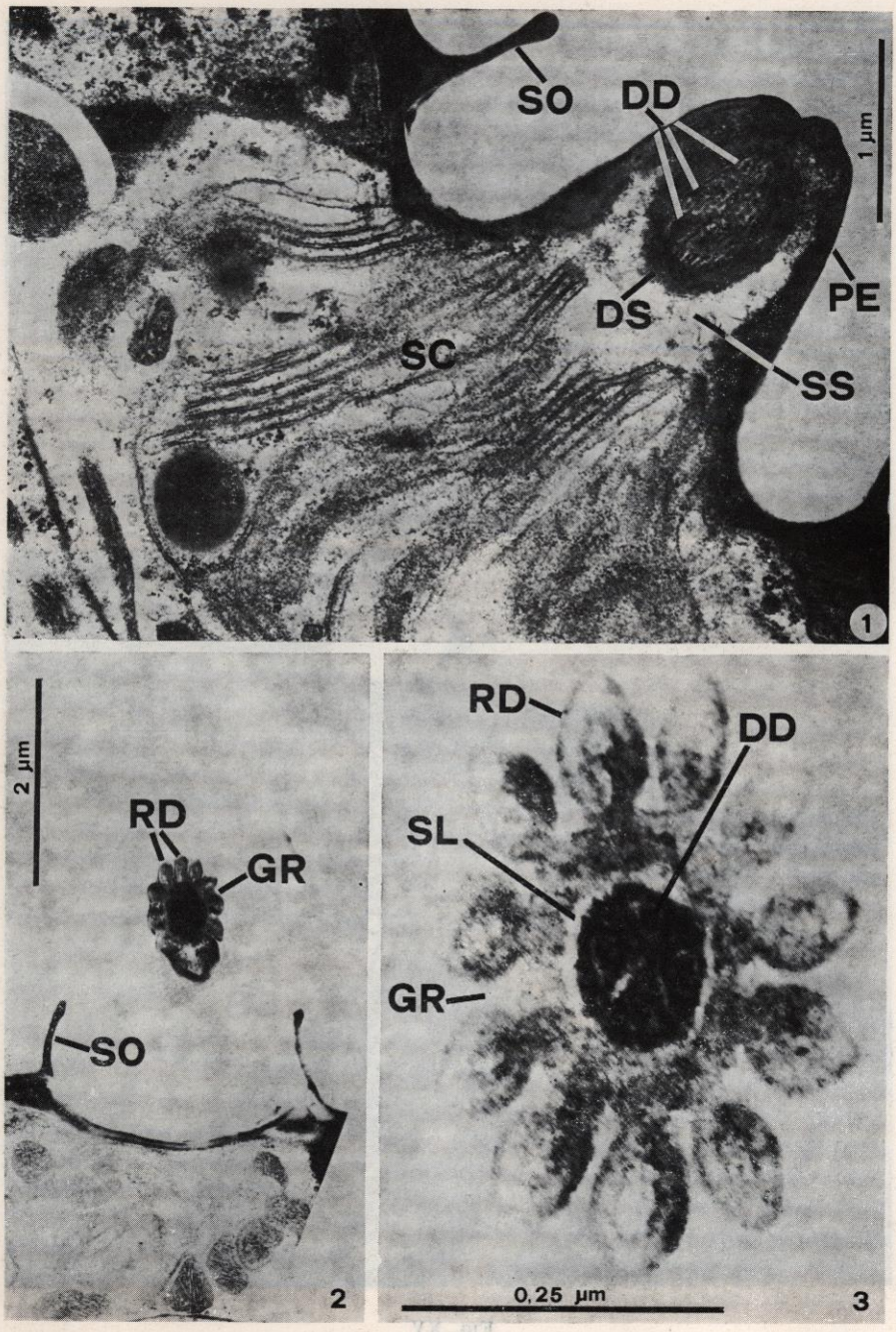

FIG. XVI

M. erysiphes. « Grooved peg » from female flagellomere: (1) oblique section through insertion; (2) cross section of the peg at an intermediate level; (3) ditto, near the peg apex.

There are five sensory neurons whose distal dendritic segments (Fig. XV), encased in a common thick sheath (which becomes ever thicker going upwards: Figs. XV, XVI, 1) and unbranched, enter the peg central lumen filling it completely up to nearly the tip, whereas only four of them reach this (Fig. XVI, 3). It is remarkable that microtubules encrease very much in number from the ciliary region to the peg lumen (Figs. XV; XVI, 1), where they are very packed and as a whole resemble a tubular body (Fig. XVI).

There are three sheath cells: a thecogen, a trichogen and a tormogen, very similar to their homologous cells above described (Figs. XV, XVI).

The « grooved pegs » share many features (small size, low numbers, position on flagellomeres, aporous peg walls, unbranched dendrites distally covered by a thick common sheath up to the peg tip, outer dendritic segments completely filling the peg lumen and having densely packed microtubules) with antennal « nonporous sensilla » of *Rodnius prolixus* interpreted by Mc Iver & Siemicki (1985) as thermo/hygro receptors. This interpretation thus seems to be valide also for the grooved pegs, even more when considering the great importance of environmental temperature and humidity for so delicate insects such as Cecidomyiides.

Conclusions

From the above reported morphological investigations the following conclusions can be drawn:

— The flagellomeres of male and female *Mycodiplosis erysiphes*, as normal in most Cecidomyiidae, bear five different types of integumental appendages, one of which (microtrichia) is only cuticular, whereas the others, herein called « sensory bristles », « sensory hairs », circumfila and « grooved pegs », represent the outer cuticular parts of sensilla.

— The sensory bristles have cuticular (peculiar hair shaft and socket) and cellular (one sensory neuron whose dendrite ends on the shaft base with a tubular body, and three sheath cells) components typical of mechanoreceptors: they are most probably tactile sensilla.

— The sensory hairs display cuticular (hair shaft having thin multiporous walls) and cellular (two sensory neurons whose distal dendritic segments extend into the shaft lumen, and three sheath cells) components typical of olfactory sensilla: they may be involved in host plant and/or fungus finding.

— The circumfila show cuticular (hair shaft having thin multiporous walls) and cellular (two sensory neurons whose distal dendritic segments extend into the shaft lumen, and three sheath cells) components

characteristic of olfactory sensilla: they may be sex pheromone receptors.

The grooved pegs have cuticular (hair shaft with aporous walls) and cellular (five sensory neurons whose distal dendritic segments extend into the peg lumen completely filling it and showing densely packed microtubules within it, and three accessory cells) parts very much similar to « nonporous sensilla » of *Rhodnuis prolixus* interpreted as thermo/hygro receptors: they may be involved in resting sites location.

Acknowledgments

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ABSTRACT

Investigations carried out by scanning and transmission electron microscopy on female and male antennae of *Mycodiplosis erysiphes* Ruebs. have confirmed on each flagellomere (particularly the third one) the presence of the following structures: sensory bristles, previously known to light microscopists as « setae inserted on mucronate sockets» ; sensory hairs, already called « setae inserted on horseshoeshaped sockets »; the well known circumfila ; grooved pegs, quite minute, also named « sensorial spines », « sensory pores », « sensilli »; and microtrichia (not innervated). Moreover, the said investigations contribute new functional-morphological information which allows to infer that: sensory bristles are tactile sensilla; sensory hairs are olfactory sensilla, conceivably involved in host-plant and/or fungus finding; circumfila are olfactory, most probably sex pheromone receptors; grooved pegs may be thermo/hygro sensilla

RIASSUNTO

I sensilli antennali di *Mycodiplosis erysiphes* Ruebs. (Diptera, Cecidomyiidae).

Indagini condotte in microscopia elettronica a scansione e a trasmissione sulle antenne di ambo i sessi di *Mycodiplosis erysiphes* Ruebs. hanno confermato l'esistenza, nei singoli flagellomeri (particolarmente nel terzo), delle seguenti strutture: setole

sensoriali, già note ai microscopisti ottici come «setole inserite su base mucronata»; peli sensoriali, già noti come «setole inserite su base conformata a piede di cavallo»; i ben noti circumfila o «filetti» caratteristici dei Cecidomiidi; sensilli conici, molto piccoli, già noti in microscopia ottica come «pori sensoriali», «spine sensoriali» o «sensilli»; e microtrichi, spinette semplicemente cuticolari. Inoltre, dette ricerche apportano nuove conoscenze morfofunzionali che inducono a considerare: le «setole sensoriali» come sensilli tangorecettori; i «peli sensoriali» come sensilli olfattivi, presumibilmente impegnati nella ricerca della pianta e/o del fungo ospite; i «filetti» come altri sensilli olfattivi, verosimilmente recettori di feromoni sessuali; i «sensilli conici» come possibili recettori di segnali igro-termici.

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