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# Olfaction in the Colorado potato beetle: Ultrastructure of antennal sensilla in *Leptinotarsa* sp

A SEN<sup>\*,†</sup> and B K MITCHELL

Department of Entomology, University of Alberta, Edmonton T6G 2E3, Alta., Canada

\*Present address: Entomology Research Institute, Loyola College, Chennai 600 034, India

†Corresponding author (Fax, 91-44-8265542; Email, alok\_sen@hotmail.com).

Sensillae on the antennae of the Colorado potato beetle, *Leptinotarsa decemlineata* are described using scanning (SEM) and transmission (TEM) electron microscopy and compared with SEM observations of antennal sensilla in *L. haldemani* and *L. texana*. In all the three species, 13 distinct sensillar types were identified with a higher density of sensilla in the more polyphagous species, *L. decemlineata* than in the moderately host specific *L. haldemani* and the highly host specific *L. texana*. Cuticular specializations and the predominance of olfactory sensilla are discussed in relation to host specificity in the three species.

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## 1. Introduction

The Colorado potato beetle, *Leptinotarsa decemlineata* Say, an oligophagous species restricted to feeding on Solanaceous plants, has been extensively studied and often used as a model for comparative studies on insect-plant relationships (see Jermy *et al* 1988; Mitchell 1988; Chapman and Bernays 1989). Host plant selection in this species is based on a complex set of stimuli. Limited distance recognition of host plants in the field has been demonstrated (Hsiao 1993; Jermy *et al* 1988). In wind tunnel experiments, mixtures of common leaf volatiles from *Solanum* species cause upwind orientation and walking (Visser and Ave 1978). Both generalist and specialist types of olfactory sensilla sensitive to these compounds have been described using electrophysiological methods (Ma and Visser 1978; Visser 1979). These attractive blends can be made unattractive by increasing or decreasing the concentration of some constituents (Thiery and Visser 1986, 1987).

Extensive sampling following physical encounter with a plant appears to be the most critical stage of host selection (Harrison 1987; Mitchell 1993). Beetles explore leaves of marginal hosts and non-hosts for a longer time prior to biting, suggesting a combination of boundary layer and leaf surface compounds are an important component in this close range assessment (Harrison 1987). Foraging *L.*

*decemlineata* adults also tap the leaf surface with the antennae, suggesting that antennal gustatory sensilla may also be involved (Harrison 1987; Sen and Mitchell 1995).

The ultrastructure of antennal sensilla that play a central role in the above behaviours however, has never been described for any *Leptinotarsa* spp. except for an scanning electron microscopic (SEM) study by Sinitsina and Kryukova (1990). The purpose of this study was to elucidate the structure and function of sensilla on the terminal five antennal segments of *L. decemlineata* and to compare them with SEM studies on *L. haldemani* and *L. texana*. Such studies coupled with those on behaviour and sensory physiology would contribute to a better understanding of the functional basis of host acceptance and rejection in adults of *Leptinotarsa* genus.

## 2. Materials and methods

Adults of *L. decemlineata* were collected in Edmonton while *L. haldemani* were obtained from south east Arizona and *L. texana* from Mission, Texas. All species were reared in the laboratory on greenhouse grown plants – *L. decemlineata* on leaves of *Solanum tuberosum*; *L. haldemani* on either *Lycopersicon esculentum*, *S. dulcamara* or *S. rostratum* and *L. texana* on *S. elaeagnifolium*.

For SEM, antennae were fixed in glutaraldehyde and dehydrated through a graded series of ethanol. Sub-

**Keywords.** Antenna; *Leptinotarsa*; olfaction; potato beetle; *Solanum*; ultrastructure

sequently, they were cleaned in tetrachloromethane as described by Cuperus (1985). Specimens were air-dried and coated with gold in a Nanotech Semprep 2 and observed using a Cambridge 250 scanning electron microscope. Five samples of each species were observed with the SEM.

For transmission electron microscopy (TEM), antennae were dissected from beetles in chilled 5% glutaraldehyde fixative. They were fixed in glutaraldehyde for 16 h and post fixed in Millonig's 1% osmium tetroxide for 2 h. Specimens were dehydrated in a series of ethanols and finally in propylene oxide, embedded in EPON 812 and sectioned using a Reichert OMU2 ultramicrotome. Sections were stained with a saturated solution of uranyl acetate for 20 m followed by lead citrate for 10 m. Sections were observed using a Philips EM300 electron microscope. Five specimens were sectioned to observe variance, if any.

### 3. Results

The antennae of the adult Colorado potato beetle, *L. decemlineata*, consists of an elongated scape, a short pedicel and a flagellum of 8 segments. The terminal five segments of the antennae in all the three species examined (*L. decemlineata*, *L. haldemani* and *L. texana*) are densely covered with sensilla (figure 1). The terminal segment, contains a variety of sensillar types some of which are not present in the other four segments proximal to it. The terminal segment is about 550  $\mu\text{m}$  long in *L. decemlineata* while it is 470  $\mu\text{m}$  in *L. haldemani* and 420  $\mu\text{m}$  in *L. texana*. About 250  $\mu\text{m}$  from the apical tip, a circumferential band, 30  $\mu\text{m}$  wide, containing some unique sensilla occurs in the terminal segment (figure 2). In both *L. haldemani* and *L. texana*, this band occurs about 170  $\mu\text{m}$  from the apical tip.

#### 3.1 Sensillar types common to the five terminal segments in *L. decemlineata*

From SEM, three types of thick and long sensilla with longitudinal grooves can be distinguished from all other sensilla which are shorter and have smoother surfaces.

Type I sensilla with a pointed tip and longitudinal grooves are 30–35  $\mu\text{m}$  long (see figure 3). These aporous sensilla are fairly abundant and are distributed on all the terminal five segments.

Type II sensilla have longitudinal grooves and an apical pore and arise almost perpendicular from the cuticle surface (figure 3). They are 40–45  $\mu\text{m}$  long with a basal diameter of 6.5  $\mu\text{m}$  and a tip diameter of 1.6  $\mu\text{m}$ . There are 12–15 type II sensilla on the apex of the terminal segment distal to the band with a total of 25–30 such hairs

on the terminal segment (see figure 1). On the remaining four segments, the distribution pattern is repetitive with ten such sensilla occurring as a band 75–80  $\mu\text{m}$  from the intersegmental joint.

Type III sensilla with longitudinal grooves are shorter than type I sensilla. They are 20–25  $\mu\text{m}$  long, uniporous with a basal diameter of 5.25  $\mu\text{m}$  and a tip diameter of 1.6  $\mu\text{m}$  (figure 4). Distal to the band on the terminal segment, there are 15 type III sensilla with a total of 20–25 such sensilla on the entire terminal segment. These sensilla are also present on the other four distal segments.

Type IV sensilla have shallow longitudinal depressions along the shaft of the sensillum (figure 5). These sensilla are about 10  $\mu\text{m}$  in length with a basal diameter of 2.1  $\mu\text{m}$  and a tip diameter of 0.4  $\mu\text{m}$ .

#### 3.2 Sensillar types occurring only on the terminal segment

Type V sensilla are 10  $\mu\text{m}$  long (figure 6). These sensilla have a basal diameter of 2.5  $\mu\text{m}$  and a tip diameter of 0.5  $\mu\text{m}$ .

Type VI sensilla are slender, slightly curved with a rounded apical tip and a large flat plate like socket (figure 7). Their surface cuticle appears rough. They are 6.5  $\mu\text{m}$  long with a basal diameter of 2.7  $\mu\text{m}$  and a tip diameter of 0.75  $\mu\text{m}$ . These sensilla are randomly distributed but they are more abundant on the lateral surface of the terminal segment.

Type VII sensilla are uniporous, 6–8  $\mu\text{m}$  long with a basal diameter of 2.4  $\mu\text{m}$  and a tip diameter of 0.6  $\mu\text{m}$  (figure 8).

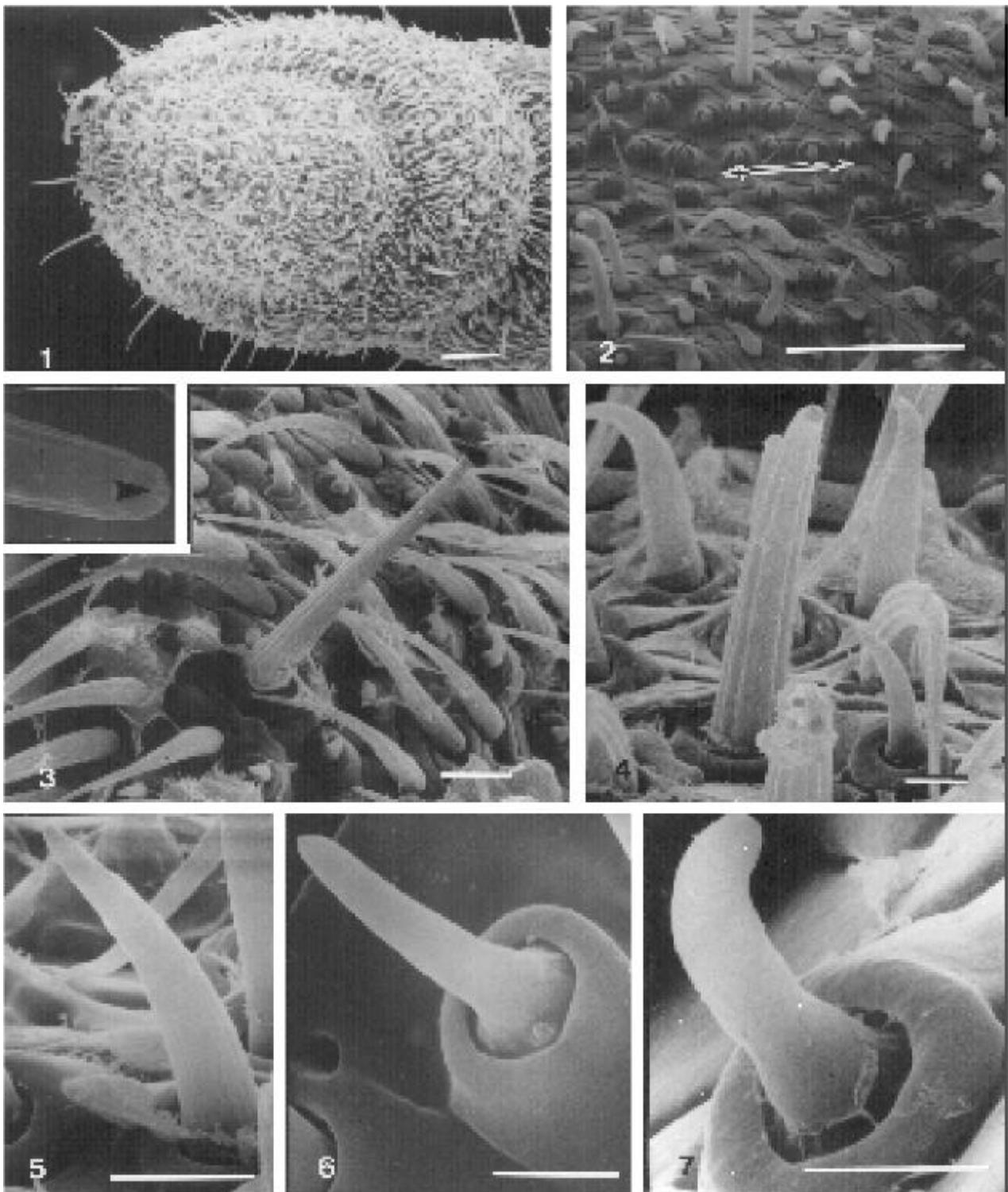
#### 3.3 Sensillar types unique to the ring on the terminal segment

Type VIII sensilla have a cuticular collar in addition to a socket (figure 9). These uniporous sensilla are 2.5  $\mu\text{m}$  long with a basal diameter of 1.8  $\mu\text{m}$  and a tip diameter of 1.6  $\mu\text{m}$ . They are primarily distributed on the ring of the terminal segment but there are also one or two such hairs on the apical tip. The cuticular collar is 3.25  $\mu\text{m}$  in width while the socket is 10.25  $\mu\text{m}$  wide.

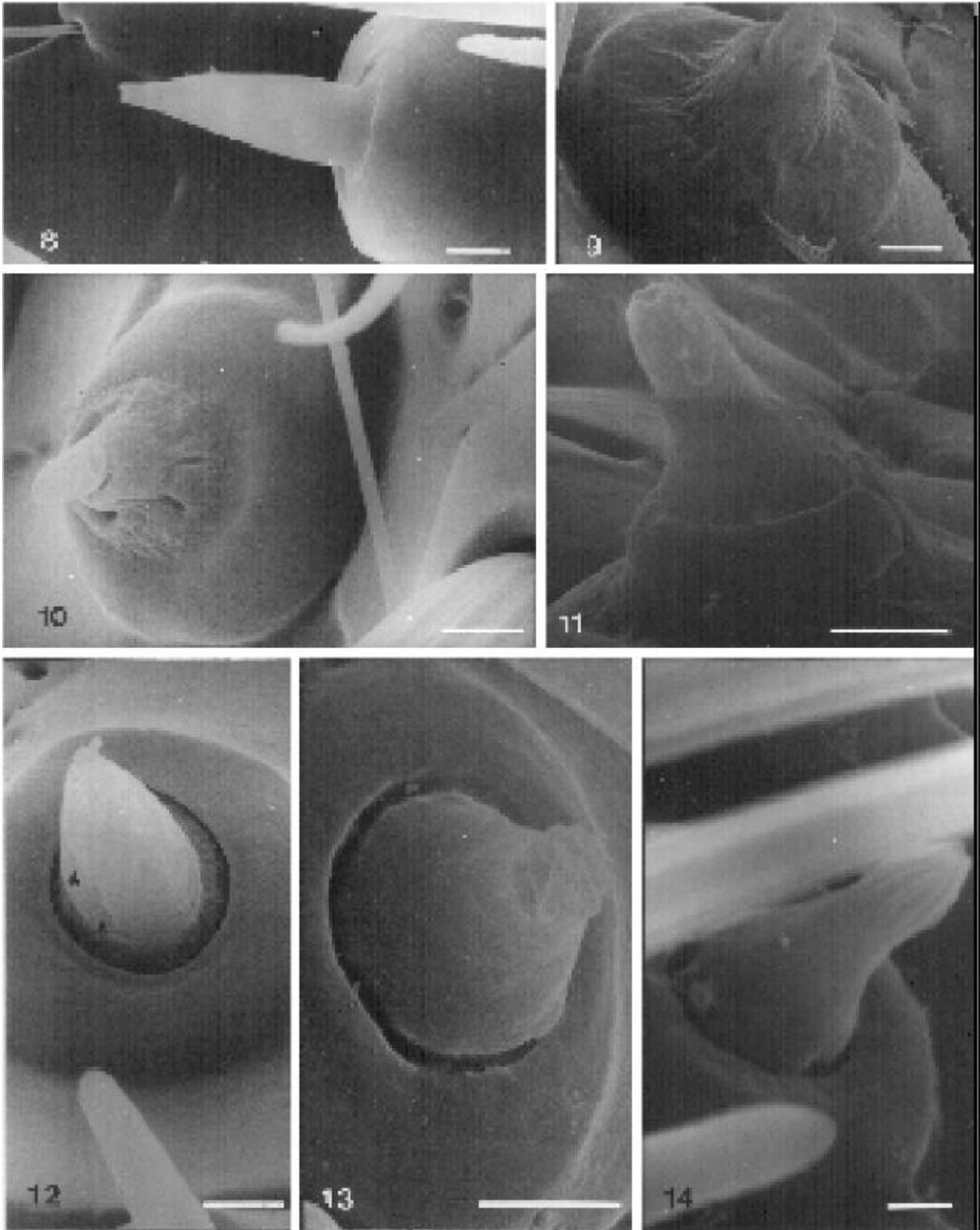
Type IX sensilla have a unique socket. They are 2  $\mu\text{m}$  long with a basal diameter of 1.2  $\mu\text{m}$  and a tip diameter of 0.4  $\mu\text{m}$ . The width of the cuticular collar and socket are 2.1  $\mu\text{m}$  and 8.6  $\mu\text{m}$  (figure 10).

Type X sensilla are uniporous. They are 4.5  $\mu\text{m}$  long, 3.2  $\mu\text{m}$  wide at the base with a tip diameter of 1.0  $\mu\text{m}$ . The width of the socket is 4.1  $\mu\text{m}$  (figure 11).

Type XI sensilla are short, 5  $\mu\text{m}$  long with a basal diameter of 3.25  $\mu\text{m}$  and a tip diameter of 0.4  $\mu\text{m}$  (figure 12). They have indentations on the wall and are primarily distributed on the band in the terminal segment.



**Figures 1–7.** SEM of antennal sensilla in adult *L. decemlineata*. (1) Distribution of sensilla on the distal antennal segment of *L. decemlineata*. (2) The circumferential band (marked by arrows) on the distal segment of the antennae containing some unique sensilla (Bar: 1, 2 = 50  $\mu$ m). (3) Type II sensilla with longitudinal grooves arising almost perpendicular to the surface (*inset*: distal part of sensilla showing apical pore). Numerous type I sensilla with a pointed tip are also seen (Bar = 10  $\mu$ m). (4) Type III sensilla with longitudinal grooves. (5) Type IV sensilla with longitudinal striations (visible at the base of the sensillum) and a pointed tip. (6) Type V sensilla. (7) Type VI sensilla characterized by a slightly curved and blunt apical tip and a flattened socket (Bar: 4–7 = 4  $\mu$ m).



**Figures 8–14.** SEM of antennal sensilla in adult *L. decemlineata*. (8) Type VII sensilla with an apical pore. (9) Type VIII sensilla with a unique socket. (10) Type IX sensilla. (11) Type X sensilla with an apical pore. (12) Type XI sensilla with depressions along the wall. (13) Type XII sensilla with a wide socket and apical finger like projections. (14) Type XIII with apical finger like projections (Bar: 8–14 = 2  $\mu$ m).

Type XII sensilla are 3.5  $\mu\text{m}$  long with a basal diameter of 3.75  $\mu\text{m}$  and a tip diameter of 1.25  $\mu\text{m}$ . The socket is 8.1  $\mu\text{m}$  wide. These sensilla have apical finger-like projections (figure 13). These sensilla are rare being distributed only on the band in the terminal segment.

Type XIII sensilla are similar to type XII but differ in size. These sensilla are 8.25  $\mu\text{m}$  long with a basal diameter of 5.1  $\mu\text{m}$  and a tip diameter of 0.8  $\mu\text{m}$ . The socket is 12.5  $\mu\text{m}$  wide (figure 14).

All the above sensillar types (I through XIII) also occur in *L. haldemani* and in *L. texana* and their distribution patterns are similar in all the three species. Although no attempt has been made to determine the total number of sensilla of each type on the entire antenna, numbers of sensilla per 200  $\mu\text{m}$  on a particular area of the terminal segment were counted to test for possible differences among the three species. In three replicates, *L. decemlineata* had a total of  $127 \pm 6$ , *L. haldemani* had  $61 \pm 4$  and *L. texana* had  $90 \pm 6$  sensilla.

### 3.4 Fine structure of the sensilla

To simplify description and to preliminarily organize the numerous types, sensilla are grouped on the basis of ultrastructural similarity into five groups, A through E.

**Group A:** Type I sensilla are innervated by a single dendrite, the distal segments of which do not extend into the lumen of the sensillum shaft but extend to a socket region where a tubular body is found (figure 15). The dendritic sheath enclosing the distal dendritic segment is thick and convoluted. The apical area of the tubular body is very dense and microtubules run parallel to each other (figure 16).

**Group B:** Uniporous sensilla types II and III are characterized by a thick wall and a narrow dendritic lumen. Wall thickness varies: 1.8  $\mu\text{m}$  in type II (figure 17) and 1.25  $\mu\text{m}$  in type III (figure 20). Six to seven dendrites innervate type II sensilla (figure 18) while 3–4 innervate type III (figure 22). The distal dendritic segments in the above types of sensilla do not branch and are tightly enclosed within an electron dense dendritic sheath. Microtubules are abundant in the dendrites of sensillum types II and III and at the socket region, one of the dendrites is modified into a tubular body (figures 18, 21 and 34). The dendritic sheath extends from the distal part of the ciliary region to the base of the peg. The distal dendritic segments appear to be bound by septate junctions (figure 19). Proximally, the dendrites continue to cell bodies containing an oblong nucleus and the usual complement of other cell organelles (figure 47).

There are four sheath cells associated with each of these sensilla which withdraw to give rise to two sinuses,

the ciliary sinus and the sensillar sinus. The inner sheath cell encases the dendrites around the ciliary region and extends distally to the base of the sensillum (figures 19, 23, 26). At the ciliary region, the inner sheath cell withdraws to form the ciliary sinus and extends proximally to the cell body. An intermediate and outer sheath cell wrap the inner sheath cell successively and continue proximally to the cell body. Distally, they withdraw to form the sensillar sinus (figure 23).

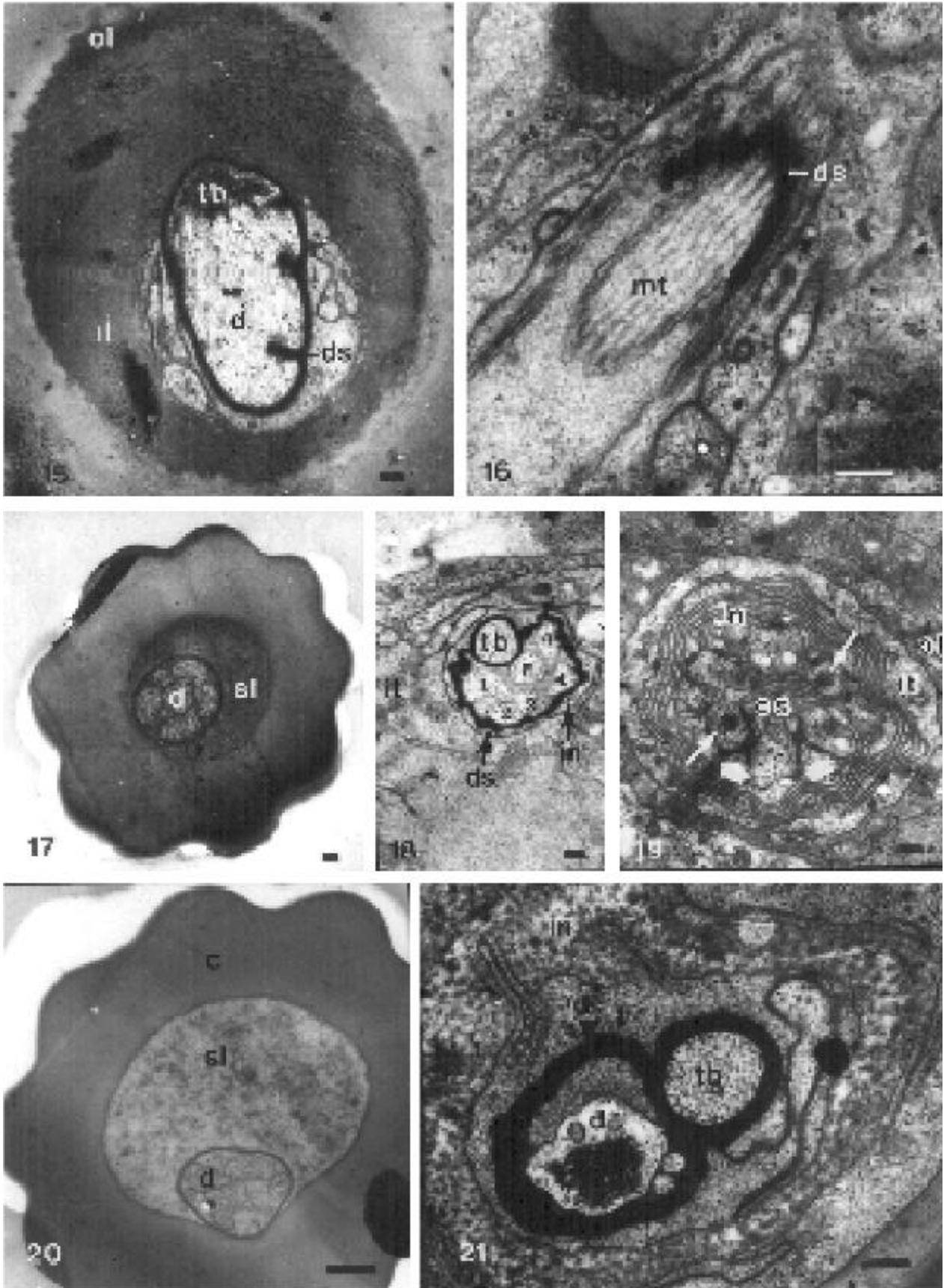
**Group C:** Sensillar types VIII and X with a characteristic raised cuticular collar and type VII are uniporous. Wall thickness varies from 0.7  $\mu\text{m}$  in type VII (figure 33), 0.30  $\mu\text{m}$  in type VIII (figure 35) and 0.48  $\mu\text{m}$  in type X (figure 41). Type VII are innervated by 5–6 dendrites (figure 33), type VIII by 2 dendrites (figures 36, 37) while type X are innervated by 5–6 neurons (figures 42, 43).

**Group D:** Sensillar types IV, V, VI, XI and XII are characterized by thin walls and have wall pores. In type IV sensilla, the thickness of the wall ranges from 0.25  $\mu\text{m}$  to 0.30  $\mu\text{m}$  (figure 24), while it is 0.30  $\mu\text{m}$  in type V (figures 27, 28), 0.45  $\mu\text{m}$  in type VI (figure 30), 0.35  $\mu\text{m}$  in type XI (figure 44), 0.75  $\mu\text{m}$  in type XII (figure 45) and 0.9  $\mu\text{m}$  in type XIII (figure 46). The number of dendrites in type IV are 3 (figure 25) while there are 25–30 dendritic branches in type V (figure 27). Type VI sensilla have 2 dendrites (figures 31, 32) and there are 16–20 dendritic branches in type XI (figure 44) and 2–3 dendrites in types XII and XIII (figures 45, 46). Type V are innervated by 1 dendrite (figure 29). The exact number of dendrites innervating types XI and XII sensilla could not be determined with certainty. The distal dendritic segments are characterized by prominent microtubules and are enclosed within an electron dense dendritic sheath. These sensilla have three sheath cells and from their relation to the dendrites, to the dendritic sheath, to the sensillar sinus and to each other, they correspond to the typical inner, intermediate and outer sheath cells as described for sensilla in group B.

**Group E:** Type IX sensilla are non-porous with an inflexible socket (figure 38). These sensilla are innervated by 3 neurons tightly enclosed within a dendritic sheath (figure 39). Below the level of the socket, one of the dendrites is transformed into a stack of lamellae (figure 40).

## 5. Discussion

The present study demonstrates that the antenna of the Colorado potato beetle, *L. decemlineata*, contains thirteen morphologically distinct sensillum types. Comparing the cuticular specializations and ultrastructure of these



sensilla to those described in other insect species (Hansen 1978; Altner and Prillinger 1980; McIver 1975, 1985; Zacharuk 1980, 1985), these sensilla are capable of responding to various stimuli, viz. olfactory, gustatory, tactile as well as thermo- and/or hygroreception. The distribution patterns of individual sensillar types provides evidence for the importance of the distal five segments reported in behavioural studies (Schanz 1953; de Wilde *et al* 1969; de Wilde 1976; Visser and Ave 1978; Sen and Mitchell 1995) and in particular, the terminal segment, with its diversity of all the sensillar types, demonstrated in electrophysiological studies (Visser and Nielsen 1977). In addition, the three species had similar antennal morphology with regard to the number of segments, types of sensilla and their pattern of distribution on the respective segments of the antenna. However, the density of sensilla on the antennae made it rather difficult to accurately estimate the total number of each type of sensilla in the three species.

Type I sensilla with a thick cuticular wall and the absence of a dendrite within the lumen but with a distal tubular body as the stimulus conducting structure suggests a mechanoreceptive function of these sensilla (McIver 1975, 1985). Regular invaginations of the dendritic sheath probably prevents movements of the dendrite when the hair shaft is moved or it may serve to increase the rigidity of the dendritic sheath thereby increasing the surface area of force transmission to the dendrites. The length of these sensilla allows them to control the pressure with which the antennae touches surfaces and the longitudinal ridges serve to reinforce the wall of these sensilla against mechanical deformation. It also allows them to stand clear of other sensilla increasing the likelihood that they are the first parts of the antennae to contact surfaces. Lewis (1971) suggested that differences in the structure of antennal sensilla are related to their exposure to physical damage.

Sensillar types II and III with unbranched dendrites, lack of wall pores and the presence of a tubular body suggests a combined mechanosensory and gustatory function. The tubular body at the base of the sensillum is a typical mechanosensitive structure (Zacharuk 1980, 1985). The five to six dendrites extending to the tip of the shaft probably respond to host plant chemicals including leaf

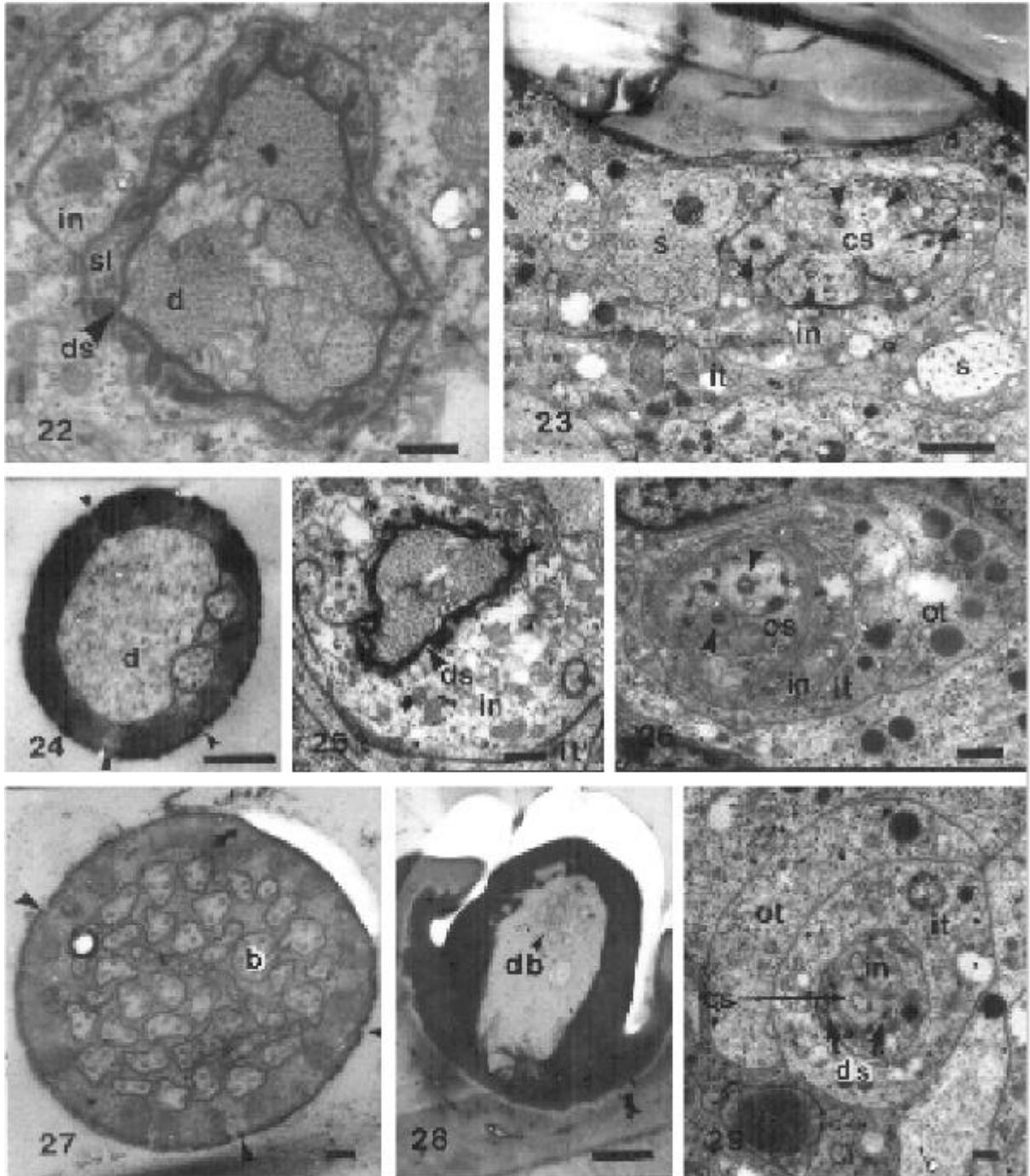
surface chemicals. Alternately, they could also be involved in the perception of contact pheromonal or oviposition deterring substances. Similar gustatory sensilla have been reported in the antennae of several species of beetles including the pine weevil, *Hylobius abietis* (Mustaparta 1973) and in the saw toothed grain beetle, *Oryzaephilus surinamensis* (White and Luke 1986). Antennal tapping of leaf surfaces prior to feeding probably exposes these gustatory sensilla to tactile and chemical stimuli.

Sensillar types IV, V, VI, XI, XII and XIII correspond to the multiporous sensilla of Altner (1977) and Zacharuk (1980, 1985). The presence of pores and pore tubules for conduction of stimulus to the dendrites indicates their ability to perceive air borne stimuli. The dendritic branching within the lumen, as observed in type V and XI increases the surface area resulting in increased sensitivity. Their distribution pattern is such that they are protected from mechanical damage by the longer mechanoreceptors and gustatory sensilla. Such olfactory sensilla have been reported in several species including scolytid beetles (Moeckh 1968; Borg and Norris 1971; Payne *et al* 1973; Mustaparta 1973; Dickens and Payne 1978).

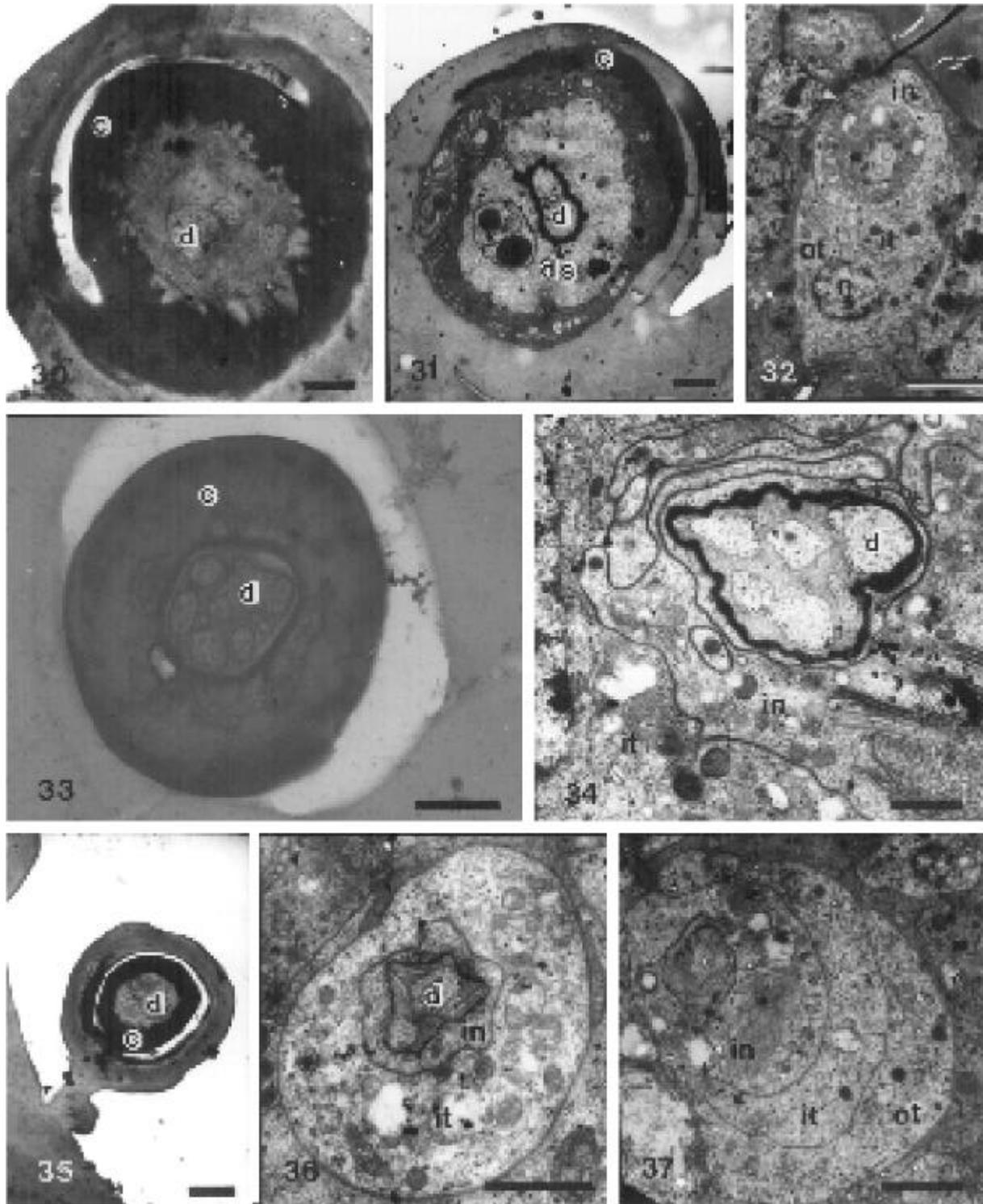
Sensillar types VII, VIII and X are uniporous, the latter two types in addition have a blunt apical tip and a distinct cuticular collar based on which criteria can be designated as gustatory chemoreceptors. However, this is very unlikely due to their location and their size. These sensilla are primarily distributed on the circumferential band in the terminal segment and are surrounded by longer mechanoreceptors (type I) and gustatory chemoreceptors (types II and III) which would prevent these sensilla from access to contact chemical stimuli. Similar sensilla have been reported in the terminal antennal segment of the yellow spotted longicorn beetle, *Psacotha hilaris* (Dai and Honda 1990). Further study is needed for final elucidation of their function.

Type IX sensilla correspond closely to the np-sensilla with inflexible sockets of Altner *et al* (1983). Similar structures have been described as sensilla capitula (Yokohari 1978, 1981), coelocapitular sensilla (Yokohari *et al* 1982), peglike sensilla styloconica (Cave and Gaylor 1987) and have been identified as hygro-thermo receptors in several species (Zacharuk 1985). In *L. decemlineata*, one of the dendrites is modified into a stack of lamellae

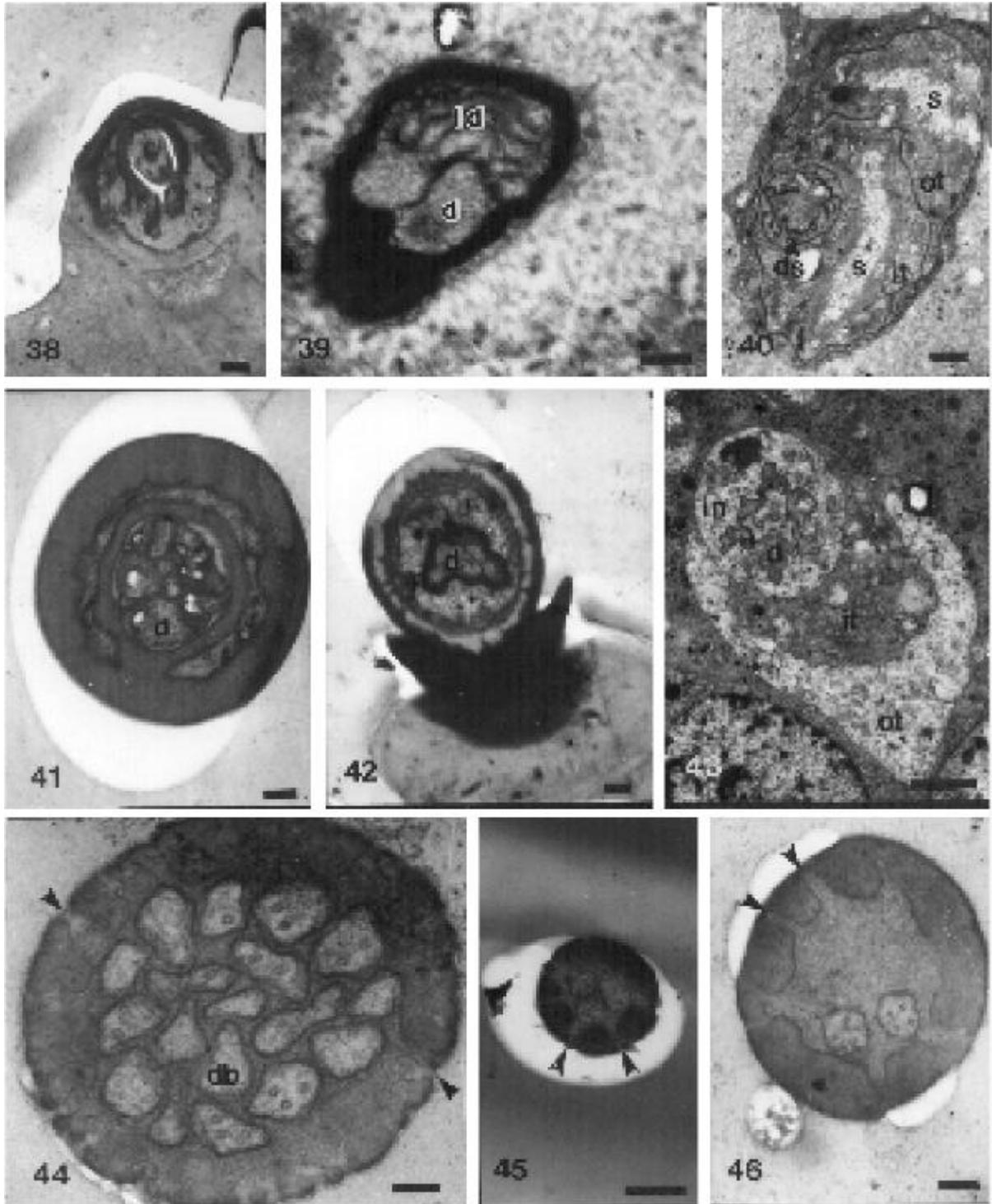
**Figures 15–21.** TEM of antennal sensilla in adult *L. decemlineata*. (15) Cross-section through the socket region of type I sensilla. Invaginations in the dendritic sheath (ds) is seen (Bar = 0.25  $\mu\text{m}$ ). (16) Cross-section of type I sensilla below the socket region. Numerous microtubules (mt) run parallel within the dendrite (Bar = 0.5  $\mu\text{m}$ ). (17) Cross-section through type II sensilla along the hair shaft (Bar = 0.3  $\mu\text{m}$ ). (18) Cross-section through type II sensilla showing dendrites (1–6) enclosed within a dendritic sheath also containing a tubular body (Bar = 0.5  $\mu\text{m}$ ). (19) Cross-section of type II sensilla distal to the ciliary region. Septate junctions occur between the dendritic segments (arrowheads). Two of the dendrites are seen containing the double arrangement of microtubules (arrows) (Bar = 0.75  $\mu\text{m}$ ). (20) Cross section of type III sensilla innervated by 4 dendrites enclosed within a dendritic sheath (Bar = 0.5  $\mu\text{m}$ ). (21) Cross-section of type III sensilla below the level of the socket region. An additional dendrite in the form of a tubular body (tb) is also seen at this level (Bar = 0.20  $\mu\text{m}$ ). (c, Cuticular wall; cs, ciliary sinus; d, dendrite; ds, dendritic sheath; in, inner sheath cell; it, intermediate sheath cell; mt, microtubules; ot, outer sheath cell; sl, sensory lumen; tb, tubular body.)



**Figures 22–29.** TEM of antennal sensilla in adult *L. decemlineata*. (22) Cross-section of type III sensilla with 4 dendrites distal to the ciliary region (Bar = 1  $\mu$ m). (23) Cross-section of type III sensilla at the region of ciliary sinus above the ciliary collar where the dendrites are differentiated as distal basal bodies with the microtubules arranged in a characteristic pattern (arrowheads). Note the disintegration of the dendritic sheath (Bar = 1  $\mu$ m). (24) Cross-section of type IV sensilla along the hair shaft. Pores (arrowheads) are visible (Bar = 0.5  $\mu$ m). (25) Cross-section of type IV sensilla below the level of the socket showing 3 distinct dendrites tightly enclosed within a dendritic sheath (Bar = 0.75  $\mu$ m). (26) Cross section of type IV sensilla at the ciliary region. Note two dendrites have the typical arrangement of microtubules (arrowheads) (Bar = 1  $\mu$ m). (27) Cross-section of type V sensillum along the hair shaft showing numerous dendritic branches with prominent microtubules. Pores along the wall of the sensilla are also seen (Bar = 0.1  $\mu$ m). (28) Longitudinal section of type V sensillum at the socket region showing dendritic branches (Bar = 0.75  $\mu$ m). (29) Cross-section of type V sensillum at the region of ciliary sinus. The dendritic sheath is disintegrating at this stage (arrows)(Bar = 0.75  $\mu$ m). (cs, Ciliary sinus; d, dendrite; db, dendritic branches; ds, dendritic sheath; in, inner sheath cell; it, intermediate sheath cell; ot, outer sheath cell; s, sinus; sl, sensory lumen.)



**Figures 30–37.** TEM of antennal sensilla in adult *L. decemlineata*. (30) Cross-section of type VI sensillum with two dendrites at the socket region (Bar = 0.4  $\mu\text{m}$ ). (31) Cross-section of type VI sensillum below the socket region (Bar = 0.5  $\mu\text{m}$ ). (32) Cross-section of type VI sensillum distal to the ciliary region (Bar = 1  $\mu\text{m}$ ). (33) Cross-section of type VII sensillum with a thick wall (Bar = 0.5  $\mu\text{m}$ ). (34) Cross-section of type VII sensillum distal to the ciliary region showing 6 dendrites (Bar = 0.25  $\mu\text{m}$ ). (35) Cross-section of type VIII sensillum with a double socket (Bar = 0.75  $\mu\text{m}$ ). (36) Cross-section of type VIII sensillum below the socket region (Bar = 1  $\mu\text{m}$ ). (37) Cross-section of type VIII sensillum at the ciliary region with the dendritic sheath disintegrating (Bar = 0.75  $\mu\text{m}$ ). (c, Cuticular wall; cs, ciliary sinus; d, dendrite; in, inner sheath cell; it, intermediate sheath cell; n, nucleus; ot, outer sheath cell.)



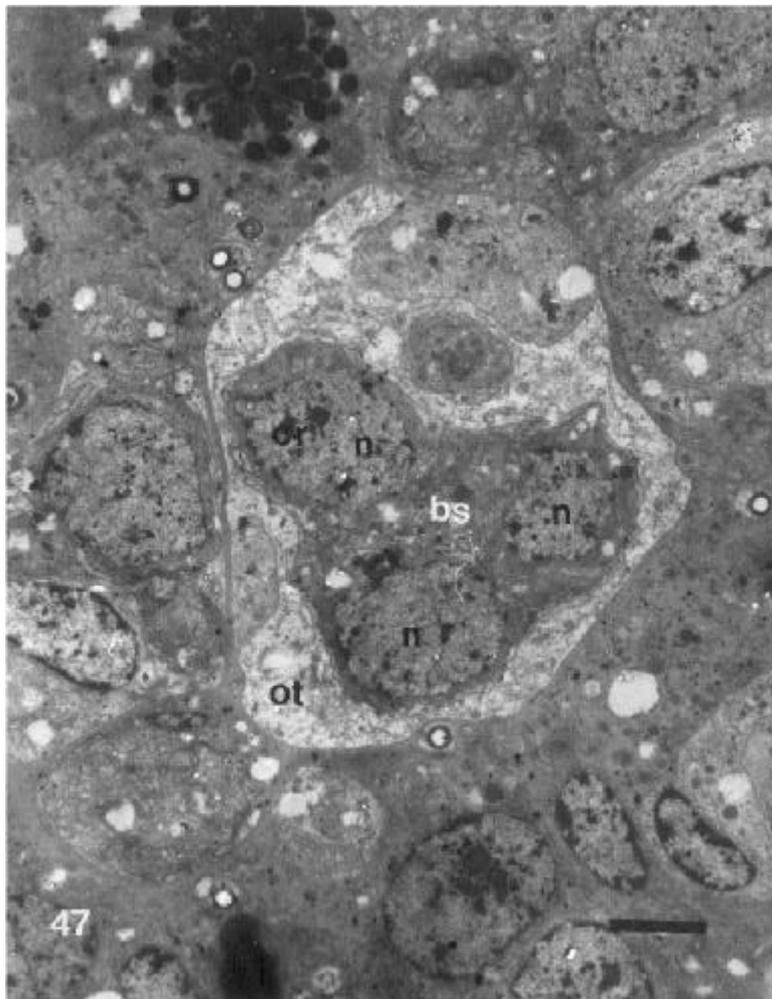
**Figures 38–46.** TEM of antennal sensilla in adult *L. decemlineata*. (38) Cross-section of type IX sensillum at the socket region (Bar = 0.5  $\mu$ m). (39) Cross-section of type IX sensillum distal to the ciliary region. The dendrites are tightly encased within the dendritic sheath and one of the dendrites is modified as a lamellated dendrite (Bar = 0.4  $\mu$ m). (40) Cross-section of type IX sensillum at the ciliary region where the dendritic sheath disintegrates (Bar = 0.5  $\mu$ m). (41) Cross-section of type X sensillum (Bar = 0.4  $\mu$ m). (42) Cross-section of type X sensillum at the region of the proximal dendritic segments wrapped separately by the inner sheath cell (Bar = 0.4  $\mu$ m). (43) Cross-section of type X sensillum proximal to the ciliary region (Bar = 1  $\mu$ m). (44) Cross-section of type XI sensillum showing dendritic branches and pores along the hair wall (Bar = 0.3  $\mu$ m). (45) Cross-section of type XII sensillum (Bar = 1.5  $\mu$ m). (46) Cross-section of type XIII sensillum (Bar = 0.70  $\mu$ m). (cs, Ciliary sinus; d, dendrite; in, inner sheath cell; it, intermediate sheath cell; ld, lamellated dendrite; ot, outer sheath cell; s, sinus.)

below the level of the cuticle. Such lamellated dendrites were first reported by Lewis (1970, 1971) and Corbiere-Tichane (1971) where it was suggested that they have humidity and thermoreceptive functions. Thermoreceptors are generally found in non porous as well as in sensilla with wall pores (Altner *et al* 1978, 1983) while hygrometers are found exclusively in non-porous sensilla. In the 12th antennal segment of *Carausius morosus*, a single aporous sensillum is characterized as a short peg inserted in a pit and surrounded by a broad inflated collar (Altner *et al* 1978). Electrophysiological and ultrastructural studies reveal the presence of 3–4 cells (with only two cells entering the lumen of the shaft) responding to cold, dry and moist air stimuli (Becker 1978; Altner and Prillinger 1980; Loftus and Corbiere-Tichane 1981; Altner *et al* 1983; Steinbrecht 1984; Altner and Loftus 1985). How-

ever, electrophysiological studies on the wall-pore sensilla with lamellated dendrites in *Pieris* sp suggests an olfactory function (Lee *et al* 1985). A similar sensilla with wall pores occurring on the labial palp tip of *Rhodogastria* sp responds best to CO<sub>2</sub> with a moderate response to various other odorous stimuli. Interestingly, the projections of the receptor cell axons in *Rhodogastria* sp were traced to glomeruli in the deutocerebrum which are not innervated by cells from antennal receptors (Bogner *et al* 1986).

Sensilla similar to type XII and XIII have been reported in locusts (Boeckh 1967; Steinbrecht 1969; Waldow 1970), cockroaches (Altner *et al* 1978) and also correspond to the fluted sensilla in *D. frontalis* (Dickens and Payne 1978).

These ultrastructural observations on the antennal sensillar types support earlier behavioural and electrophysio-



**Figure 47.** TEM section below the proximal dendritic region in type III sensilla showing the cell bodies, containing chromatin, of the individual dendrites enclosed within a basal sheath cell which in turn is surrounded by one of the enveloping sheath cells (Bar = 1  $\mu$ m). (bs, Basal sheath cell; c, chromatin; n, nucleus; ot, outer sheath cell.)

logical studies. In an attempt to evaluate the importance of sensilla on the mouthparts and antennae of the Colorado potato beetle, Sen and Mitchell (1995) observed that the ability of adults to perceive chemical stimuli as well as to continue feeding is reduced by the removal of antennae, maxillary and labial palpi and that input from sensilla on these appendages are used to detect leaf surface waxes. The precise ratio of C<sub>6</sub> alcohols, aldehydes and the derivative acetate in potato leaf odour (essentially the components of green leaf volatiles) are decisive in the response of the adult beetle to its host plant (Visser and Ave 1978). Further, electrophysiological responses from the peripheral nervous system demonstrate that only 25 of the 100 cells on the antennae of *L. decemlineata* respond to green leaf volatiles (Ma and Visser 1978) which were classified into two main categories – one with a response spectrum to several compounds and the others with a narrower and different response spectra. Interestingly, the responses are similar at the level of the central nervous system. de Jong and Visser (1988) classified the responses of deutocerebral neurons in the antennal lobe of adult *L. decemlineata* beetles into 2 similar groups – one with neurons that are not highly specific and the other containing highly specific neurons. Based on these observations, the authors suggested the existence of 2 channels involved in the olfactory processing in the antennal lobe, with one channel involved in the detection of the presence of C<sub>6</sub> components and the other for an evaluation of the component ratio.

In his extensive review, Chapman (1982) recognized several selection pressures like insect size and the need for sensitivity affect the number of chemoreceptors. Thus according to him, sensory capabilities in Orthopteroidea and Endopterygota are relatively less specialized and thus have more number of sensilla resulting in across-fibre patterning of response. Insects with specialized feeding habits, on the other hand, have fewer sensilla corresponding to labelled lines. Extending these generalizations to the Colorado potato beetle, we find that the number of sensilla on the antennae (present study), palpi (Sen 1988), galea (Sen and Mitchell 1987), larvae (Chin 1950; Mitchell and Schoonhoven 1974) are high. Gross morphology of chemosensilla on the maxillae and galea (Sen and Mitchell 1987; Haley 1988; Sen 1988) and the antennae (present study) of all the three species is similar. However, differences in antennal olfactory cell responses to individual green leaf components between *L. decemlineata* and *L. haldemani* exist (Visser 1983). Though both species were more responsive towards the C<sub>6</sub> alcohols than the corresponding aldehydes, responses to the latter group of compounds was higher in *L. haldemani*. While *L. decemlineata* is the most polyphagous of the three species, *L. haldemani* is moderately host specific and *L. texana* is highly host specific (Haley 1988). Among

the three species, variation in electrophysiological responses to gustatory stimuli, particularly plant saps, were greatest in *L. haldemani* and the least in *L. decemlineata* probably corresponding to the degree and evolution of feeding specialization (Haley 1988). It thus appears reasonable to conclude that a polyphagous species has a broader fit for the neural template coding for feeding behaviour than restricted feeders and consequently, a behavioural sequence may be elicited even if a series of peripheral signals do not indicate a perfect match for the template for feeding behaviours.

### Acknowledgements

We thank Dr Gary Bernon and his colleagues at USDA, Mission, Texas for providing field caught *L. texana*. Thanks are also due to Dr R L S Whitehouse, Department of Medical Microbiology, University of Alberta, Edmonton, Canada who kindly provided access to the TEM and to Mr George Braybrook, Department of Entomology, for his help and advice, with the SEM. This research was supported by a Natural Sciences and Engineering Research Council of Canada (NSERC) operating grant to BKM.

### References

- Altner H 1977 Insect sensilla: principles of structure and function; *Verh. Dtsch. Zool. Ges.* **70** 130–153
- Altner H and Loftus R 1985 Ultrastructure and function of insect thermo- and hygroreceptors; *Annu. Rev. Entomol.* **30** 273–295
- Altner H and Prillinger L 1980 Ultrastructure of invertebrate chemo-, thermo- and hygroreceptors and its functional significance; *Int. Rev. Cytol.* **67** 69–139
- Altner H, Tichy H and Altner I 1978 Lamellated outer dendritic segments of a sensory cell within a poreless thermo- and hygroreceptive sensillum of the stick insect, *Carausius morosus*; *Cell Tissue Res.* **191** 287–304
- Altner H, Schaller-Selzer L, Stetter H and Wohrab I 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** 279–304
- Becker D 1978 *Electrophysiologische Untersuchungen zur Fencherezeption durch die styloconischen sensillen bei *Mamestra brassicae* L (Lepidoptera: Noctuidae)* (Regensburg: Naturwiss. Diss., Univ.)
- Boeckh J 1967 Reaktionschwelle, Arbeitsbetect und spezifität eines Geruchrezeptors auf de Heuschreck en antennae; *Z. Vgl. Physiol.* **55** 378–406
- Borg T K and Norris D M 1971 Ultrastructure of sensory receptors on the antennae of *Scolytus multistriatus* (Marsh.); *Z. Zellforsch.* **113** 13–28
- Bogner F, Boppre M, Ernst K-D and Boeckh J 1986 CO<sub>2</sub> sensitive receptive receptors on labial palps of *Rhodogastria* moths (Lepidoptera: Arctiidae): Physiology, fine structure and central projections; *J. Comp. Physiol.* **158** 741–749
- Cave R D and Gaylor M J 1987 Antennal olfactory sensilla of male and female *Telonomus reynoldsii* Gdh and Coker

- (Hymenoptera:Scelionidae); *Int. J. Insect Morphol. Embryol.* **17** 121–133
- Chapman R F 1982 Chemoreception: The significance of receptor numbers; *Adv. Insect Physiol.* **16** 247–356
- Chapman R F and Bernays E 1989 Insect behavior at the surface and learning as aspects of host plant selection; *Experientia* **45** 215–222
- Chin C-T 1950 Studies on the physiological relations between the larvae of *Leptinotarsa decemlineata* Say and some Solanaceous plants; *Tidschr. Pflanzk.* **56** 1–88
- Corbiere-Tichane G 1971 Structure nerveuse énigmatique dans l'antenne de la larve du *Speophyes lucidulus* Delar. (Coleoptera Cavernicole de la sous famille de Bathysciinae). Etude du microscope électronique; *J. Microsc.* **19** 191–202
- Cuperus P L 1985 Inventory of pores in antennal sensilla of *Yponomeuta* spp. (Lepidoptera: Yponomeutidae) and *Adoxophyes orana* F. v. R. (Lepidoptera: Tortricidae); *Int. J. Insect Morphol. Embryol.* **14** 347–359
- Dai H and Honda H 1990 Sensilla on the antennal flagellum of the yellow spotted longicorn beetle, *Psacotha hilaris* (Pascoe) (Coleoptera: Cerambycidae); *Appl. Entomol. Zool.* **25** 273–282
- de Jong R and Visser J H 1988 Integration of olfactory information in the Colorado potato beetle brain; *Brain Res.* **447** 10–17
- de Wilde J 1976 The olfactory component in host plant selection in the adult Colorado beetle (*Leptinotarsa decemlineata* Say); *Symp. Biol. Hung.* **16** 291–300
- de Wilde J, Lambers-Surverkrupp H R and van Tol A 1969 Responses to air flow and air borne plant odor in the Colorado beetle; *Neth. J. Plant Pathol.* **75** 53–57
- Dickens J C and Payne T L 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
- Haley J 1988 *Host selection and gustatory chemoreception in three Leptinotarsa spp.*, M.Sc. Dissertation, University of Alberta, Edmonton, Canada
- Hansen K 1978 Insect chemoreception; in *Taxis and behavior* (ed.) G L Hazelbauer (New York: John Wiley) vol 5, pp 233–292
- Harrison G D 1987 Host plant discrimination and evolution of feeding preference in the Colorado potato beetle, *Leptinotarsa decemlineata* (Say); *Physiol. Entomol.* **12** 407–415
- Hsiao T H 1993 Host specificity, seasonality and bionomics of *Leptinotarsa* beetles; in *Biology of Chrysomelidae* (eds) P Jolivet, E Petitpiere and T H Hsiao (Kluwer Acad. Publ.) pp 581–599
- Jermy T, Szentesi A and Horvath J 1988 Host plant finding in phytophagous insects: the case of the Colorado potato beetle; *Entomol. Exp. Appl.* **49** 83–93
- Lee J-K, Selzer R and Altner H 1985 Lamellated outer dendritic segments of a chemoreceptor within wall pore sensilla in the labial palp-pit organ of the butterfly, *Pieris rapae* L. (Insecta, Lepidoptera); *Cell Tissue Res.* **240** 333–342
- Lewis C T 1970 Structure and function in some external receptors; *Symp. R. Entomol. London* **5** 59–76
- Lewis C T 1971 Superficial sense organs of the antennae of the fly, *Stomoxys calcitrans*; *J. Insect Physiol.* **17** 449–461
- Loftus R and Corbiere-Tichane 1981 Antennal warm and cold receptors of the cave beetle, *Speophyes lucidulus* Delar. in sensilla with a lamellated dendrite. Response to sudden temperature changes; *J. Comp. Physiol.* **143** 443–452
- Ma W C and Visser J H 1978 Single unit analysis of odor quality coding by the antennal olfactory receptor system of the Colorado beetle; *Entomol. Exp. Appl.* **24** 520–533
- McIver S B 1975 Structure of cuticular mechanoreceptors of arthropods; *Annu. Rev. Entomol.* **20** 381–397
- McIver S B 1985 Mechanoreception; in *Comprehensive insect physiology, biochemistry and pharmacology* (eds) G A Kerkut and L I Gilbert (Oxford: Pergamon Press) vol 6, pp 71–132
- Mitchell B K 1988 Adult leaf beetles as models for exploring the chemical basis of host plant recognition; *J. Insect Physiol.* **34** 213–225
- Mitchell B K 1993 The chemosensory basis of host plant recognition in Chrysomelidae; in *Biology of chrysomelidae* (eds) P Jolivet, E Petitpiere and T H Hsiao (Kluwer Acad. Publ.).
- Mitchell B K and Schoonhoven L M 1974 Taste receptors in Colorado beetle larva; *J. Insect Physiol.* **20** 255–264
- Moeck H A 1968 Electron microscopic studies of antennal sensilla in the ambrosia beetle *Trypodendron lineatum* (Olivier) (Scolytidae); *Can. J. Zool.* **46** 521–556
- Mustaparta H 1973 Olfactory sensilla on the antennae of the pine weevil, *Hylobius abietis*; *Z. Zellforsch. Mikrosk. Anat.* **144** 559–571
- Payne T L, Moeck H A, Wilson C D, Coulson R N and Humphreys W J 1973 Bark beetle olfaction. II. Antennal morphology of sixteen species of Scolytidae (Coleoptera). *Int. J. Insect Morphol. Embryol.* **2** 177–192
- Schanz M 1953 Der Geruchssinn des kartoffelkafer (*Leptinotarsa decemlineata* Say); *Z. Vgl. Physiol.* **35** 353–379
- Sen A 1988 Ultrastructure of the sensory complex on the maxillary and labial palpi of the Colorado potato beetle, *Leptinotarsa decemlineata*; *J. Morphol.* **195** 159–175
- Sen A and Mitchell B K 1987 Ultrastructure of the galeal sensory complex of the Colorado potato beetle, *Leptinotarsa decemlineata* Say; *Physiol. Entomol.* **12** 81–90
- Sen A and Mitchell B K 1995 The role of maxillary and labial palpi and leaf surface waxes in the feeding behavior of the Colorado potato beetle, *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae); *Phytophaga* **7** 83–94
- Sinitina E E and Kryukova I P 1990 The antennal sensory equipment in *Leptinotarsa decemlineata* (in Russian); *Biol. Nanki (Mosc.)* **12** 36–45
- Steinbrecht R A 1969 Comparative morphology of olfactory receptors; in *Olfaction and taste* (ed.) C Pfaffman (New York: Rockefeller University Press) pp 3–21
- Steinbrecht R A 1984 Chemo, hygro and thermoreceptors; in *Biology of the integument*; Vol I. *Invertebrates* (eds) J Bereiter-Hahn, A G Matoltsy and K S Richards (Berlin: Springer-Verlag) pp 523–533
- Theiry D and Visser J H 1986 Masking of host odor in the olfactory orientation of the Colorado potato beetle; *Entomol. Exp. Appl.* **41** 165–172
- Theiry D and Visser J A 1987 Misleading the Colorado potato beetle with an odor blend; *J. Chem. Ecol.* **13** 1139–1146
- Visser J H 1979 Electroantennogram responses of the Colorado potato beetle, *Leptinotarsa decemlineata* to plant volatiles; *Entomol. Exp. Appl.* **25** 85–97
- Visser J H 1983 Differential sensory perceptions of plant compounds by insects; in *Plant resistance to insects* (ed.) P A Hedin (Washington DC: American Chemical Society) pp 215–230
- Visser J H and Ave D A 1978 General green leaf volatiles in the olfactory orientation of the Colorado potato beetle, *Leptinotarsa decemlineata*; *Entomol. Exp. Appl.* **24** 738–749

- Visser J H and Nielsen J K 1977 Specificity in the olfactory orientation of the Colorado potato beetle, *Leptinotarsa decemlineata*; *Entomol. Exp. Appl.* **21** 14–22
- Waldow U 1970 Electrophysiologische Untersuchungen an Feuchte, Trocken- und Kalterzeptoren auf der antennae der Wanderheuschrecke *Locusta*; *Z. Vgl. Physiol.* **69** 249–283
- White P R and Luke B M 1986 Fine structure, function and distribution of antennal sensilla in the saw toothed grain beetle, *Oryzaephilus surinamensis*; *Physiol. Entomol.* **11** 227–238
- Yokohari F 1978 Hygroreceptor mechanisms in the antennae of the cockroach *Periplaneta*; *J. Comp. Physiol.* **124** 53–60
- Yokohari F 1981 The sensillum capitulum, an antennal hygro- and thermo receptor sensillum of the cockroach, *Periplaneta americana* L.; *Cell Tissue Res.* **216** 525–543
- Yokohari F, Tominaga Y and Tateda H 1982 Antennal hygroreceptors of the honeybee, *Apis mellifera* L.; *Cell Tissue Res.* **226** 63–73
- Zacharuk R Y 1980 Ultrastructure and function of insect chemosensilla; *Annu. Rev. Entomol.* **25** 27–47
- Zacharuk R Y 1985 Antennae and sensilla; in *Comprehensive insect physiology, biochemistry and pharmacology* (eds) G A Kerkut and L I Gilbert (Oxford: Pergamon Press) vol 6, pp 1–69

*MS received 24 January 2001; accepted 17 April 2001*

Corresponding editor: VERONICA RODRIGUES