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Ultrastructure of sensory receptors on the labium of the cassava mealybug, *Phenacoccus manihoti* Matile Ferrero

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Abstract

The ultrastructure of the sensory receptors located on the labium of the cassava mealybug *Phenacoccus manihoti* Matile-Ferrero (Homoptera, Pseudococcidae) was studied with scanning and transmission electron microscopes. Trichoid hairs of probable mechanoreceptive function are distributed over the labium. Uniporous chemosensilla which possess a mechanoreceptive dendrite, multiporous chemosensilla and mechanoreceptive pegs are present on the tip of the labium. The presence of contact and olfactory chemoreceptors on the labial tip of *P. manihoti* suggests that tapping it on the cassava leaf provides the pest with information about the chemical nature of the leaf surface.

Introduction

The study of the different components of resistance of cassava, *Manihot esculenta* Crantz (Euphorbiaceae) to the mealybug *Phenacoccus manihoti* Matile-Ferrero (Homoptera: Pseudococcidae), has identified varieties of cassava with different degrees of antixenosis (non preference) to the pest (Tertuliano *et al.*, 1993). This type of resistance, which has a role during plant identification, initially involves the physical and chemical characteristics of the surface of plants. To determine the role of these characteristics in plant selection by the mealybug, a detailed study of the mealybug behaviour on the leaf surface was made (Renard, 1993). It was found that, before probing, simply walking on the leaf surface allows the insect to differentiate between plants with varying levels of antixenosis. When the mealybug walks about, the antennae are held pointing forward and the labium quickly taps the leaf surface (contact is brief); when it stops, it rubs the surface with the tip of its legs and slowly taps it with the lower side of the last antennal segment and with the tip of the labium (contact is more prolonged). This last pattern of behaviour is very similar to the one described in many Hemiptera (Backus, 1988) and especially in other Homoptera Sternorrhyncha, such as aphids, Aphididae (Ibbotson & Kennedy, 1959; Klinghauf, 1970) and whiteflies, Aleyrodidae (Noldus *et al.*, 1986; Walker, 1987).

Tapping the leaf surface with the tip of the labium suggests that this organ is involved in plant identification. To determine how host plants are identified, it is necessary to know what type of sensory information the mealybug can detect. To our knowledge, the ultrastructure of sensory receptors on the labium of Coccoidea has not been investigated before. Three studies have described the sensory receptors on the labium in Homoptera Sternorrhyncha and some degree of variability has been recorded; thus, the tip of the labium in aphids (one species studied: *Brevicoryne brassicae* L.) has only mechanoreceptors (Wensler, 1977; Tjallingii, 1978) and in whiteflies (one species studied: *Parabemisia myricae* Kuwana), the labium is equipped with chemoreceptors or with mechano-chemoreceptors (Walker & Gordh, 1989). The aim of the present study was to describe the morphology and ultrastructure of

sensilla present on the tip of the labium of *P. manihoti* and to discuss their probable function in relation to their structure and innervation.

Materials and methods

Insects. *P. manihoti* reproduces by thelytokous parthenogenesis. The clone we used in this study was initially collected on cassava from a local garden in Brazzaville, Congo in 1992. Since then, a culture of *P. manihoti* has been maintained in the laboratory in Rennes (France) on cassava (*M. esculenta*, variety: M'pembe) at 25 ± 2 °C and L12:D12 photoperiod. In a previous study, Renard (1993) found that all four instars of the cassava mealybug had the same sensory equipment on the labium. In this study, we used the fourth instar only.

Scanning electron microscopy. Specimens of *P. manihoti* were killed and dehydrated in ethanol just after moulting in order to limit the amount of wax on their bodies. Wax deposits were removed by 2 methods; the method of Walker & Gordh (1989) slightly modified: chloroform and xylene used alternately (at ca. 60 °C) for 4 or more hours or the method of van Baaren (1994) with modifications: treatment with aluminum sulfate (5 or 10%, 2 or 4 h) and dehydration in ethanol and then in acetone. Samples were either air dried or critical-point dried, gold coated and examined with a JEOL JSM 6400 scanning electron microscope (SEM).

Transmission electron microscopy. Heads of mealybugs were fixed in sodium cacodylate buffer (ph 7,4) containing 2,5% glutaraldehyde for 2 h at 4 °C. After three successive rinses in cacodylate buffer for 30 min. each, tissues were postfixed for 1 hour in a 1% osmium tetroxide solution and rinsed again in the cacodylate buffer. They were then dehydrated in acetone and embedded in an Epon Araldite mixture (Resin E.F. Fullau). Ultrathin sections on film-coated grids were stained with uranyl acetate and lead citrate (Reynolds, 1963) and viewed in a Philips CM-12 microscope.

Results

The labium of *P. manihoti* (Figs. 1, 2) has 30 sensilla of 4 distinct types which belong to 2 morphological classes as follows:

class A. Sensilla trichodea or trichoid hairs (Fig. 1)

class B. Sensilla chaetica or pegs

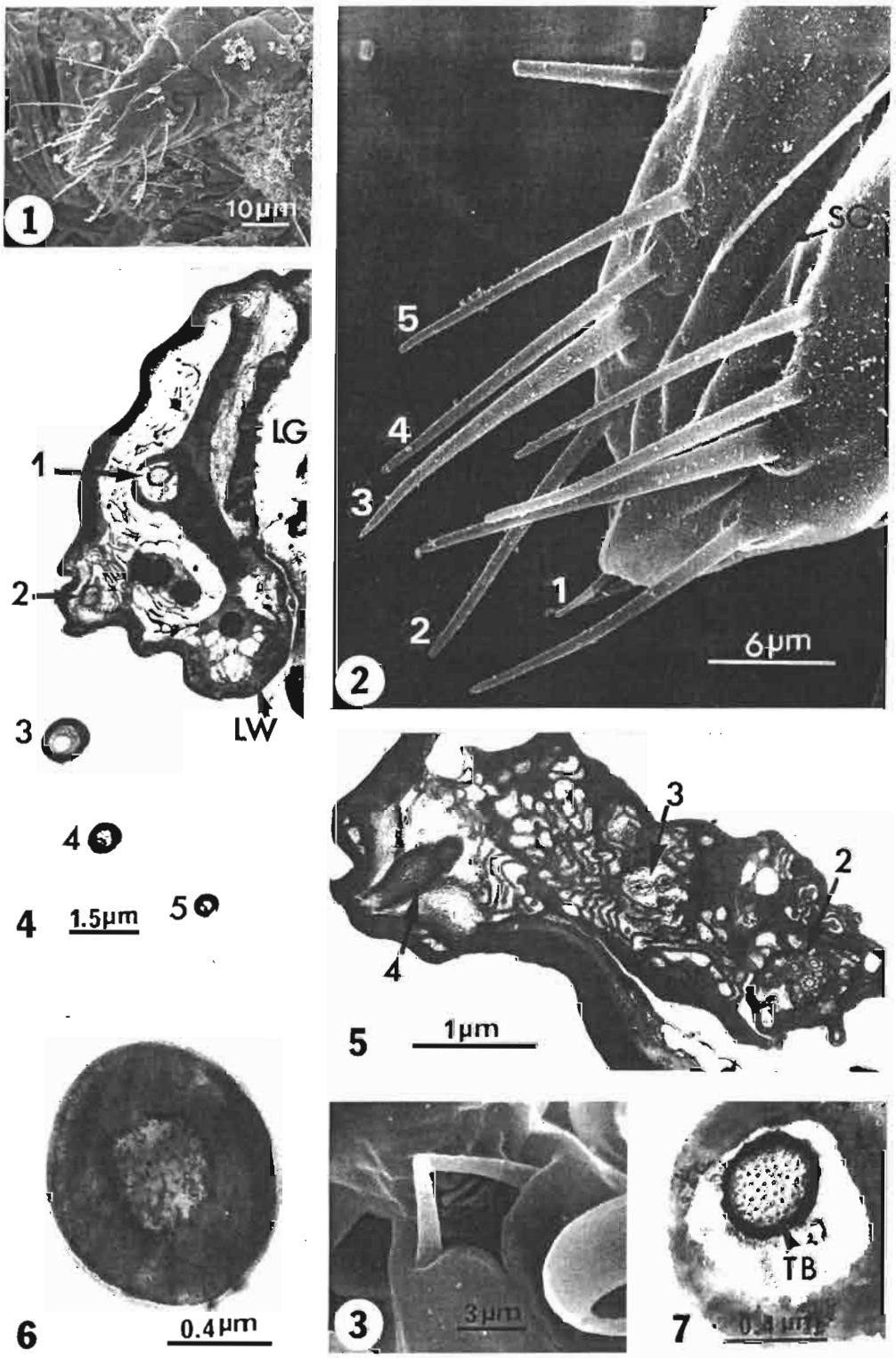
1. smooth short pegs = pegs number 1 (Figs. 2, 3)
2. smooth long pegs = pegs number 2, 4 and 5 (Figs. 2, 8)
3. grooved pegs = pegs number 3 (Figs. 2, 13)

All pegs are located on the tip of the labium (4th and 5th segments joined) in two sensory areas symmetrically placed on either side of the stylet groove (Figs. 1, 2).

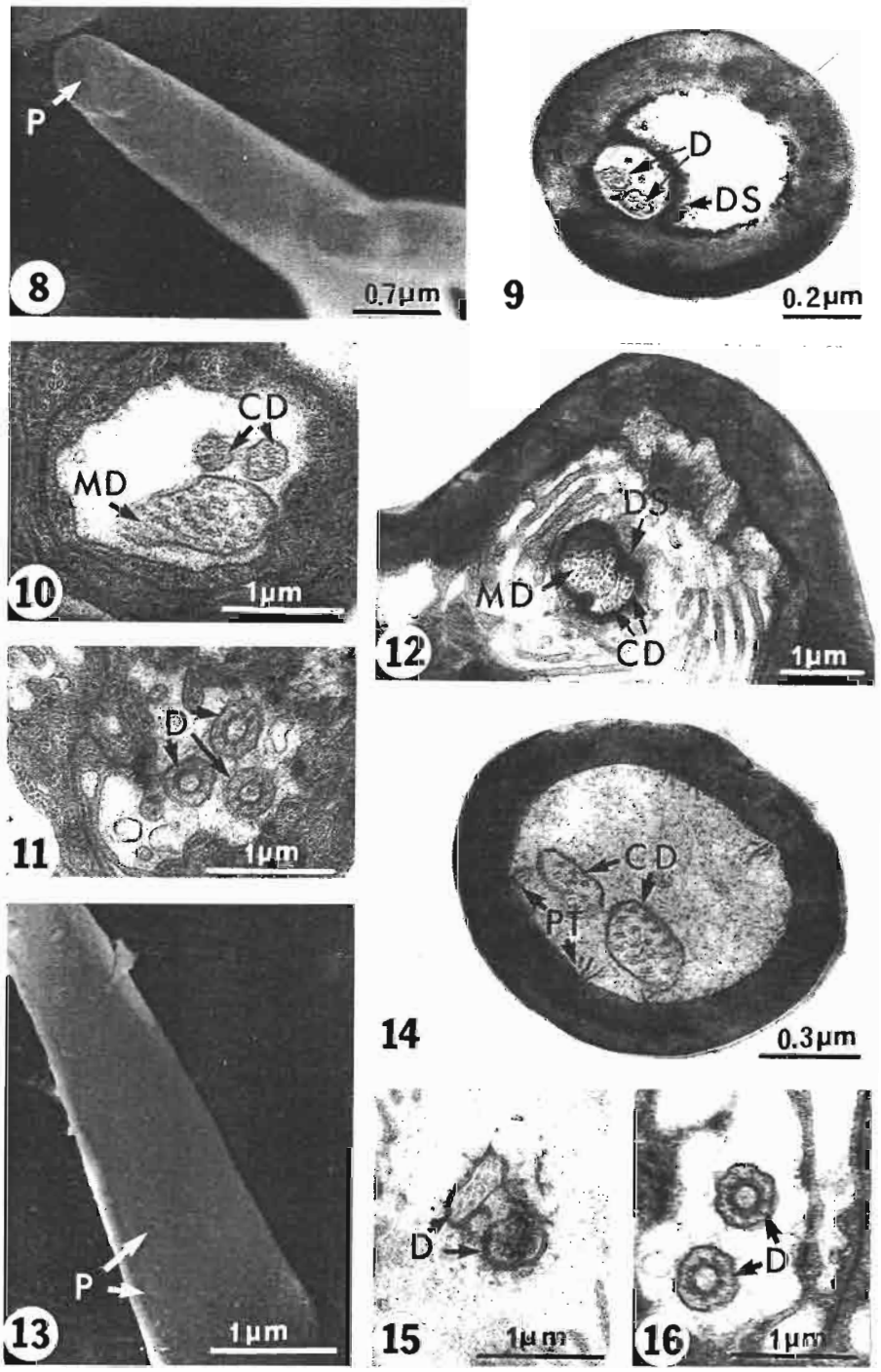
Trichoid hairs. At least twenty hairs are found on all segments of the labium (Fig. 1). They are large, 22 μm in length and 1 μm wide at the base and have a pointed tip. Their cuticle is smooth and the base in inserted into a well-defined socket on a raised rim of the cuticle. The hair has no pores. These sensilla are placed obliquely with respect to the longitudinal axis of the labium. The wall is very thick (300 nm) and the lumen of the hair does not contain a dendrite (Fig. 6). Each hair is innervated by a single dendrite which terminates in a tubular body at the base of the hair. The morphological and ultrastructural characteristics of trichoid hairs suggest that they function as simple mechanoreceptors.

Smooth short pegs. A pair of smooth short pegs is located on the distal end of the labial groove and comes into direct contact with the stylet when it protrudes past the groove (Fig. 3). Pegs insert into a socket and measure about 5 μm in length; their diameter at the base is 1 μm and get progressively thinner to a blunt tip of 0.3 μm diameter. Their cuticle also lacks pores. No section of the peg was seen by TEM. Numerous sections made just below the base of the peg, show the presence of a tubular body of a single dendrite (Fig. 4, 7). The presence of a single dendrite in cross sections made at a deeper level in the sensillum confirms the single innervation of these sensilla. The ultrastructure of smooth short pegs suggests that they are simple mechanoreceptors.

Smooth long pegs. A total of six smooth long pegs are present on the labium (Fig. 2). They are approximately 14 μm in length with a diameter of 1 μm at the base; their diameter decreases progressively to a digitated tip which contains an apical pore (Fig. 8). The base is inserted in a socket. Cross sections of the peg show a thick wall (250 nm) lacking pores; in the lumen of the sensillum there are two dendrites contained within a sheath (Fig. 4, 9). A third dendrite (Figs. 10, 11) terminates in a tubular body at the base of the peg (Figs. 4, 5, 12). The single apical pore, the presence of



Figs. 1–7. (1) Labium of 4th instar *P. manihoti* to show the position of the sensilla trichodea (ST) and sensilla chaetica or pegs (SC); (2) Labial tip showing the sensory fields on either side of the stylet groove (SG). Note the smooth short pegs, number 1, the smooth long pegs, numbers 2, 4 and 5 and the grooved pegs, number 3; (3) Smooth short pegs, number 1, to show their insertion at the distal extremity of the labial tip; (4) Right side of the labial tip showing two pairs of dendritic bundles, numbered according to the peg that they innervate and of the pegs numbers 3, 4 and 5; (5) Left side of the labial tip, more proximal than Fig. 4, showing three pairs of dendritic bundles, numbered according to the peg that they innervate; (6) Sensillum trichodeum (cuticular part) showing no dendrite in its lumen; (7) Base of a smooth short peg, number 1 (cellular parts), showing only a tubular body. D: Dendrite; LG: Labial Groove; LW: Labial Wall; TB: Tubular Body. *Figs. 1–3.* Scanning electron micrographs; *Figs. 4–7.* Transmission electron micrographs of cross section of the apical portion of the labium of *P. manihoti*.



Figs. 8–15. (8) Two smooth long pegs, showing an apical pore; (9) Hair of a smooth long peg showing two dendrites within the dendritic sheath and the thick wall; (10–11) At the base of the peg showing one mechanoreceptive dendrite (larger) and two chemoreceptive dendrites (smaller) within a dendritic sheath and, more proximally near the apex of the ciliary sinus respectively; (12) Near the base of the ciliary segments of the three dendrites; (13) A grooved peg, number 3, showing numerous pores on the cuticle; (14) Grooved peg showing two dendrites without a dendritic sheath, the thin wall, and pore tubules; (15) At the base of the peg showing two dendrites within a dendritic sheath; (16) Near the base of the ciliary segments of the two dendrites. CD: Chemoreceptive Dendrite; D: Dendrite; DS: Dendritic Sheat; MD: Mechanoreceptive Dendrite; P: Pore; PT: Pore Tubule. *Figs. 8* and *13*, scanning electron micrographs; *Figs. 9–12* and *14–16*, transmission electron micrographs of cross sections.

two dendrites within the peg and a tubular body at the base of the peg, suggest that these sensilla could have a dual role as a contact chemosensory organ and as a mechanoreceptor.

Grooved pegs. The labial tip bears two grooved pegs (Fig. 2) each about 18 μm in length and 1.5 μm in diameter at the base; the tip is slightly expanded and measures 0.3 μm in diameter. The surface of the cuticle is slightly fluted and bears numerous pores of about 10 nm in diameter, regularly distributed over the entire surface of the sensillum (20–30/μm^2) (Fig. 13). The wall of the peg is thin (150 nm) and the lumen contains two dendrites which do not branch and are not enclosed within a sheath (Fig. 14). Pores of the wall (without pore kettles) have pore tubules, ca. 10 nm in diameter (Fig. 14). Deeper sections show that only two dendrites innervate these pegs (Figs. 4, 5, 15, 16). A tubular body is absent. The ultrastructure of grooved pegs suggests that they may have an olfactory function.

Discussion and conclusion

This study shows the presence of at least 15 pairs of sensilla distributed symmetrically on either side of the labial groove of *P. manihoti*; at least 10 pairs of sensilla trichodea (hairs) are evenly distributed on all segments of the labium and 5 pairs of sensilla chaetica (pegs) are located on the labial tip.

The 10 pairs of sensilla trichodea are innervated by a single dendrite ending at the base of the hair, and their morphology and ultrastructure are typical of a mechanoreceptor (McIver, 1975). These mechanoreceptive hairs are few in number, as in most Hemiptera studied (Backus, 1988); they probably detect the depth and angle of the labium when inserted during feeding.

Sensilla chaetica of type 1 also have ultrastructural features suggesting a mechanoreceptive role. The presence of such mechanoreceptive sensilla of similar structure on the tip of the labium in Homoptera Sternorrhyncha has already been observed in Aphididae (Wensler, 1977; Tjallingii, 1978) and in Aleyrodidae (Walker & Gordh, 1989). Their position, on the end of the groove, on either side of the stylet and in direct contact with it, suggests that they could be involved in the identification of the physical characteristics of the plant surface while selecting the site of probe, and perhaps in the orientation of the stylet during its penetration into the plant.

Sensilla chaetica of type 2 are the most numerous (6 out of 10) and look like thick walled sensilla (Slifer, 1970) or like uniporous chemosensilla (Zacharuk, 1980) which would probably have a gustatory role. The two chemoreceptive dendrites extend into the peg which has only one apical pore. These peg receptors have also a tactile mechanoreceptive function. The two sensilla chaetica of type 3 are innervated by two dendrites extending into the peg with a cuticle bearing numerous pores. These sensilla have the typical structure of multiporous chemosensilla (Zacharuk, 1980) or single walled sensilla described by Altner & Prillinger (1980) which are known to have an olfactory role. To our knowledge, the presence of chemoreceptors on the labium of Sternorrhyncha is reported here for the second time, Walker & Gordh (1989) having described gustatory sensilla in Aleyrodidae similar to those we have observed, but this is the first report of olfactory chemoreceptors; the presence of such receptors in Homoptera has been reported only in *Nilaparvata lugens* Stal (Auchenorrhyncha, Delphacidae) by Foster *et al.* (1983).

As in most Hemiptera studied up to now (Backus, 1988), the tip of the labium of *P. manihoti* is equipped with a small number of sensilla, characteristic of a high specificity in feeding (Chapman, 1982). The sensory system appears nevertheless well diversified with three different types of sensilla. Mechanoreceptive and chemoreceptive functions are allocated an identical number of sensilla, eight of each, suggesting an equal importance of the two functions. Among chemoreceptors, the gustatory function seems to predominate over the olfactory function, with six sensilla innervated with 12 chemosensitive dendrites and two sensilla innervated with four chemosensitive dendrites, respectively. Our results indicate that the cassava mealybug has a sensory system on the tip of the labium which allows it to detect chemical substances present on the plant surface by olfaction and by contact. The leaf surface chemicals which are detected and elicit a response remain to be identified. However, the preference of *P. manihoti* for the genus *Manihot* exclusively suggests that host-specific chemicals could be involved, such as secondary substances that convey the main chemical information about plant quality (Städler, 1984).

Results also suggest that sensory stimuli involved in host-plant selection and detected through the tip of the labium when it comes in contact with the plant surface, are very different in *P. manihoti*, *P. myricae* and *B. brassicae*. Thus the tip of the labium would perceive mechanical, gustatory and olfactory stimuli in mealy-

bugs, mechanical and gustatory stimuli in whiteflies and only mechanical stimuli in aphids. It is surprising to find that the great variability of the sensory system on the tip of the labium does not correspond to a variety of behaviour patterns during the identification of the plant surface by the insects. If we compare the results of Ibbotson & Kennedy (1959) and those of Klingauf (1970) on aphids, Walker's work (1987) on whiteflies and our own observations (Renard, 1993) on mealybugs, the host identification behaviour appears to be highly sterotyped in all three families. The present work also confirms the unique case of aphids reported by Backus (1988). They are the only Hemiptera known to lack chemoreceptors on the labial tip. It must be emphasized, however, that a comparison between the sensory systems on the labium of different families of Sternorrhyncha remains speculative because of the small number of species studied (one species in each of the families mentioned above).

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