Chemoreceptors of adult Mexican bean beetles: External morphology and role in food preference

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Abstract

The Mexican bean beetle, *Epilachna varivestis* Mulsant (Coleoptera; Coccinellidae), is an oligophagous insect that accepts leaves of soybean, *Glycine max* (L.) Merrill, as food but exhibits distinct preferences among varieties. It is believed that the basis for discrimination between the resistant PI 229358 and the susceptible cv. 'Williams' is chemical. By the ablation of chemosensilla on antennae, maxillary palpi, labial palpi, and foretarsi, followed with leaf disc preference tests, we found antennal and maxillary palp chemosensilla to be almost entirely responsible for Mexican bean beetles' discrimination between foliage of the resistant and susceptible genotypes. All appendages, and the epipharynx, were observed by scanning electron microscopy and, except for foretarsi, were found to bear several types of presumably olfactory and gustatory sensilla, which are figured and described. Since behavioral observations show that olfaction is usually sufficient to cause rejection of PI 229358, we conclude that olfactory sensilla mainly on antennae and maxillary palpi are critical in food discrimination in this system.

Introduction

The Mexican bean beetle (MBB), *Epilachna varivestis* Mulsant, is one of the few truly oligophagous Nearctic herbivores adapted to soybean, *Glycine max* (L.) Merrill, an introduced plant in the United States. The spatial patterns of soybean and the MBB in the United States are perfectly coincidental in the east but, the pattern of soybean exploitation by the MBB is disjunct (Kogan, 1980). Although capable of exploiting soybean resources in some areas, the MBB still prefers feeding on its native hosts, *Phaseolus* spp.

The MBB displays a remarkable ability to discriminate among its potential leguminous hosts. It was used to screen, from among hundreds of genotypes of soybean, a few with high levels of resistance to foliage-feeding arthropods (Van Duyn et al., 1971). These lines - plant introductions (PI's) 171451, 227687 and 229358, have been investigated with regard to possible physiological processes associated with resistance (Van Duyn et al., 1972; Kogan, 1972; Tester, 1977; Dreyer et al., 1979; Smith & Brim, 1979; Grunwald & Kogan, 1981; Hart et al., 1983; Binder & Waiss, 1984). Despite these researchers' efforts to elucidate mechanisms of soybean resistance to insects, the question is still unresolved. We believe that the answer lies in a better understanding of the fundamental host-selection mechanisms in the MBB. Therefore, we redirected our research to improve our understanding of those mechanisms at the sensory, behavioral, physiological, and broad ecological levels.

By gross examination of antennae, maxillary and labial palps, epipharynx, and tarsi we located sensory areas likely to be involved in host-selection processes. We employed scanning electron microscopy (SEM) of those sensory areas. In a preliminary attempt to connect structure and function, we systematically amputated adult beetle ap-
Sensory structures

Antenna (Figs. 1–11)

The MBB antenna consists of 12 segments, including scape and pedicel, and is very small (ca. 2 mm long) relative to the size of the beetle (Fig. 1). Antennae might be expected to be densely covered with sensilla, since surface area is small. However, sensory hairs are rather widely spaced, and only the three terminal segments bear chemosensilla (Fig. 2). These types of sensilla were identified.

Sensilla trichodea Types I & II (Figs. 1–2). Pedicel has a cluster of 40–100 μ trichoid hairs (Type I) with straight or strongly curved, finely striated shaft, without openings or pores, and with blunt tips (Fig. 1). Remaining segments are sparsely covered with sensilla trichodea closely appressed to the integument of the antenna (Type II) (Fig. 2).
These hairs do not show openings or pits, the tip is blunt, and the shaft is also finely striated. Both types appear to be mechanoreceptors (Slifer, 1970; Zacharuk, 1980).

There appear to be five types of sensilla on the three terminal segments. They can be distinguished as:

**Sensilla trichodea Type III** (Figs. 2–4). A third type of trichoid sensillum extends outward, at approximately 45° from the three apical segments (Figs. 2, 4). These are 50–80 μ long and 3 μ in diameter. Strong grooves begin at the base and spiral toward the tip, where there is an opening about 0.2 μ wide (Fig. 3). There is no evidence of pores on the side-walls of these hairs, and they are probably gustatory. Similar trichoid sensilla were observed on the antennae of *Dendroctonus ponderosae* Hopkins and *Apis mellifera* L. and were considered gustatory (Whitehead & Larsen, 1976; Whitehead, 1981).

**Sensilla chaetica** Types I & II (Figs. 4–7). Each of the three terminal segments also bears a small field of sensilla chaetica (Fig. 2). Type I is 10–15 μ long, slightly tapered distad, 2 μ at tip and produced from a bulbous base. The tip is blunt, and the shaft is apparently multiporous (Fig. 6). Type II is identical to Type I but ranges from 22–28 μ in length and appears only on the terminal segment (Fig. 7). Examination of the interior of broken s. chaetica reveals very thin walls. A similar s. chaeticum on the antenna of *D. ponderosae* is multiporous and filled with profusely branched dendrites, indicating an olfactory function (Whitehead, 1981). Multiporous external surfaces and highly branched dendrites seem to typify olfactory sensilla in antennae of insects (Slifer, 1970; Mustaparta, 1973; Hatfield et al., 1976; Dickens, 1979; Zacharuk, 1980; Bland, 1981; Rojas-Rousse & Palevody, 1983). In the MBB the size of these fields increases toward the antennal tip, with the antepenultimate segment bearing approximately 30 s. chaetica and five s. trichodea (Type III), the penultimate bearing 50–55 s. chaetica and 8–10 s. trichodea, and the ultimate segment bearing approximately 100 s. chaetica and 8–10 s. trichodea.

**Sensilla chaetica Type III** (Figs. 8–9). This type is about the same length as s. chaetica Type I (10–15 μ) and also arises from a bulbous base, but Type III is needlelike, tapering sharply from 3 μ diam to 0.5 μ through half its length and remaining at 0.5 μ diam through the tip (Fig. 8). Its surface is deeply and irregularly corrugated, and no apical pore is evident (Fig. 9). Type III sensilla are present only on the dorsal side of the terminal segment; only five were detected (Fig. 7). Honda *et al.* (1984) reported a very similar needlelike sensillum on the antenna of *Delia antiqua* (Meigen), the onion maggot, and showed by TEM examination of cross sections that it has a multiporous olfactory structure.

**Sensilla basiconica Type I** (Fig. 10). This peglike sensillum is 8 μ long on a bulbous base, 2.5 μ in diam at base, with tip grooved, clavate, and 1.0 μ in diam (Fig. 10). Eleven such sensilla are found on the dorsal surface of the terminal antennal segment and three on the penultimate segment just below the base of the terminal segment. This type of sensillum has been found on antennae of several insect species, usually as shorter pegs (Harbach & Larsen, 1977b; Hatfield *et al*., 1976; Honda *et al*., 1984). In cross section, multiple pores are found in the base of the longitudinal grooves. Harbach & Larsen (1977b) calculated the presence of approximately 214 pores in each groove of such sensilla on the antenna of *Tenebrio molitor* L. S. basiconicum Type I is very similar to the s. styloconicum of unknown function reported by Hatfield *et al.* (1976) on the antenna of *Hypera meles* (F.), the clover head weevil.

**Sensilla basiconica Type II** (Fig. 11). This stout, smooth-walled peg is 8–10 μ long, and 5 μ in diam at base, pointed at tip, and with no visible apical pore. Four s. basiconica Type II are seen on the dorsal surface of the terminal segment (Fig. 7). Smooth-walled pegs of this type are often thermoreceptors or hygroreceptors (Harbach & Larsen, 1977a; Altner & Prillinger, 1980).

**Labial Palp** (Figs. 12–14)

The three-segmented labial palp bears s. trichodea similar to those found along the shaft of the antenna (Type II). However the distal tip of the terminal segment bears a field of s. basiconica, the number varies from specimen to specimen but is about 20. All but one have a single terminal pore surrounded by several tubercles and a sidewall that appears to be multiporous (Fig. 14). The single ex-
ception is a central blunt sensillum also with pitted walls but no terminal pore (Fig. 13 arrow). The same arrangement was described by Mitchell & Schoonhoven (1974) for larvae of *Leptinotarsa decemlineata* (Say), the Colorado potato beetle, except that the sides of the pegs bearing an apical pore did not appear multiporous. In the Colorado potato beetle these sensilla are sensitive to salt, sucrose, chlorogenic acid, and some amino acids.

**Maxillary palp** (Figs. 18–23)

The three-segmented maxillary palps are also sparsely covered with *s. trichodea* that are presumed to be mechanoreceptive (Type II) (Fig. 18), but the subtriangular distal segment bears on its mesal face an oblong field with about 420 *s. basiconica* of two distinct types surrounded by ranks of appressed setae (Figs. 18–19). A similar morphology is found on the maxillary palpi of several other species of Coleoptera (Honomichl, 1980; Honomichl & Guse, 1981). Altner (1975) described a very similar field of chemosensilla on the maxillary palp of *Periplaneta americana* L. In *P. americana*, as in the MBB, the floor of this field is pliable and assumes a convex shape by hystostatic pressure. This anatomical structure allows the sensilla to maintain close contact with surfaces while protecting the sensilla from injury.

**Sensilla basiconica Type I** (Figs. 20–21). This sensillum is a grooved peg, 4–6 μ long, 2 μ in diam at base, and with a single terminal pore surrounded by several tubercles (Fig. 21). Some pegs are extended and appear to have no distinct base; others appear slightly withdrawn and are surrounded by a low rimmed base. This basiconic peg is a typical gustatory sensillum (Zacharuk, 1980).

**Sensilla basiconica Type II** (Figs. 22–23). This type is blunt, with pitted sidewalls and a tip composed of approximately 75 tightly packed tubercles (Fig. 23). It is 4–6 μ long and 2 μ in diam. No apical pores are evident, but the sidewall is highly pitted and apparently multiporous (Fig. 22). Each Type II peg is produced from a cylindrical base with a strongly crenulated rim. The function of these sensilla is difficult to assess. The clearly pitted sidewalls suggest the likelihood of a porous wall, but the morphology of the tip seems to be unique. Histological and physiological investigations will be required to determine its function. A third type of basiconic peg may be present. One area in the proximal third of the sensory field is composed of only one type of pegs (Fig. 16b). These are very similar to Type I but have more longitudinal grooves, with terminal tubercles arising from the areas between grooves. These pegs have many more protuberances and these appear longer and not as tightly grouped as in Type I pegs. The tubercles surround an apical pore.

**Epipharynx** (Figs. 15–17)

The epipharynx is densely covered with setae except for a small bare triangular area in its center (Fig. 15 arrow). In this area are found a variable number (6–8 in our sample) of sensilla basiconica projecting from circular pits. Each is 1 μ in diam and 2 μ high and appears smooth walled and without an apical pore. Some of these pegs are also found among the dense setae toward the posterior of the epipharynx. The number of these has not been accurately determined, but it is about five to ten.

**Foretarsus**

Examination of the foretarsus showed a very dense covering of *s. trichodea*, apparently mechanoreceptive. No chemosensilla were discovered, though examination by transmission electron microscopy for nerve fibers will be necessary for a
Figs. 18–23. Maxillary palp sensilla. 18) Entire maxillary palp; 19) Sensory field on terminal segment (a=chemosensilla mixed type I and II; b=circular area of only type I; c=rows of setae surrounding chemosensilla; 20) Both types of max. palp s. basiconicum (a=type I; b=type II); 21) S.b. type I (arrow=apical pore); 22) S.b. type II (arrow=apparent sidewall pores); 23) Apex of s.b. type II.
conclusive statement. However, preference test results indicate that tarsi apparently are not used in discriminating among soybean varieties.

Preference tests with MBB

A preference index ($C$) was computed as:

$$C = \frac{2A}{M + A}$$

where $A =$ leaf area eaten of the normally not preferred PI 229358 and $M =$ leaf area eaten of cv. Williams, a susceptible type. $C$ values fall between 0 and 2.0, and a value of 1.0 indicates no preference for either test plant (Kogan, 1972). Figure 24 diagrams treatments applied and average $C$ values resulting from each. Normal MBB show a strong preference for Williams leaf discs ($C = 0.35 \pm S.D. 0.22$). Treatment wherein one antenna, one maxillary palp, one labial palp, and one foretarsus were removed slightly reduced discrimination, but differences from unablated controls are not significant. Loss of chemoreceptors on antennae and maxillary palps had the most marked effect on food choice. Ablation of maxillary palps (MP) produced the greatest single effect ($C = 0.85 \pm 0.27$), with ablation of antennae (ANT) alone somewhat less effective ($C = 0.75 \pm 0.28$). When both antennae and maxillary palpi (ANT+MP) were excised, there was complete loss of discrimination between the

Fig. 24. Results of preference tests following ablation treatments. The shaded area represents a no preference zone bounded by the mean $C$ values when all discs in the preference test are Williams. Vertical lines: + and - one s.d.
two soybean types (C = 1.01 ± 0.17). When maxillary palpi alone were excised, no feeding occurred for approximately 24 h, and results were taken after 48 h. Ablation of labial palps (LP) produced a partial loss of discrimination (C = 0.65 ± 0.41) but only slightly less than in controls in which only one of each pair of appendages tested was excised (C = 0.52 ± 0.38). Removal of foretarsi (FT) had almost no effect on food choice (C = 0.38 ± 0.28). In tests where all appendages were ablated (antennae, maxillary palpi, labial palpi, and foretarsi), results were erratic, in some cases indicating strong preference and in other cases indicating no preference. This result was due to the almost complete and permanent cessation of feeding, with occasional very slight random feeding by one beetle on one disc. In total ablation, no feeding was ever observed in the first 24 h after treatment, and most beetles never resumed normal feeding.

Figure 24 illustrates the intensity of ablation effects for each treatment as measured by the degree of lost discrimination between 'susceptible' and 'resistant' plants: 2ANT + MP > 2MP > 2ANT + 2MP + 2LP + 2FT > 2ANT > 2LP > 1ANT + 1MP + 1LP + 1FT > 2FT > CONTROL. These results suggest that the sensilla involved in discrimination between these soybean types reside primarily on the antennae and maxillary palps.

Visual observation of MBB food selection suggests that olfaction at close range may produce the stimulus that results in the rejection of 229358 in favor of Williams. When unablated beetles are placed in a preference arena and provided with both types of leaves, they consistently wander over the discs and filter paper without feeding for several minutes, antennae and maxillary palps waving in the air. Some MMBs palpate discs with maxillary palps and eat 'preferred' discs without ever biting the 'nonpreferred' discs. On other occasions they do not seem to make physical contact with the leaf surface except with the tarsi. However, no obvious chemosensilla were observed on the tarsi. Since there are no apparent physical differences in the leaf surfaces of the soybean types, it is difficult to assume a physical basis for discrimination. Indications are that the discriminatory stimulus for initiation of feeding is primarily olfactory.

Our observations indicate that there is an olfactory signal that triggers a rejection or an acceptance response. In the former case the presence of volatile allomones in 229358 would account for the resistance; in the latter, the absence of a required kairomone would be the main resistance factor. Experiments with very low molecular weight volatiles emitted by Williams, 229358, and common beans, suggest that volatile allelochemics may be involved in the rejection process (Wei, Fischer, & Kogan unpublished results).

The apparent olfactory capabilities of the labial palps and maxillary palps also support the assumption of a chemosensory basis for discrimination. Sometimes, before rejection, biting and slight feeding occurs on 229358 leaf, indicating a secondary gustatory component in the discrimination process.

Ablation results clearly demonstrate the importance of the maxillary palps and the antennae in food choice among soybean genotypes. Amputation of the maxillary palps alone is nearly enough to destroy the ability to distinguish between Williams and 229358. In combination with antennal amputation, discrimination is virtually lost. The large number of sensilla on the maxillary palps (> 840) and the high concentration and anatomical location of the sensory field (Figs. 15 and 16) are other signs of their importance in food selection.

We are in the process of investigating the internal structure of sensilla on antenna, maxillary palp, and labial palp to confirm function. However, external structures have been shown to correlate well with function. S. basiconica Type I on the maxillary palp are typical of many insect gustatory organs. Their smooth, grooved walls and apical pore strongly suggest a gustatory function. The pitted nature of the wall and lack of an apical pore indicate an olfactory function for Type II s. basiconica. The tight group of tubercles at its tip is not similar to any structure previously described. Thus, on the maxillary palp, s. basiconicum Type I is the likely olfactory structure with food selection function.

The MBB antenna is quite small by comparison with the antennae of other phytophagous Coleoptera of similar size (e.g., Diabrotica spp.), and it is often positioned near the mouthparts rather than extended forward. Since each bear about 30 gustatory and about 150 olfactory hairs, it appears that a significant part of the antennal function is close-range chemoreception. We are confident that the maxillary palps and antennae contain the main chemoreceptors involved in the detection of the plant stimuli that permit the MBB to discriminate
between the two genotypes. Those plant stimuli are not currently known, but they could be (a) secondary metabolites peculiar to each genotype or (b) low molecular weight plant volatiles, in unique combinations and concentrations, that provide a 'fingerprint' pattern, as proposed by Visser (1983) for plants eaten by the Colorado potato beetle.

Analysis of soybean leaf volatiles for differences among acceptable and nonacceptable leaf types is being performed in our laboratory. The activity of these compounds is being measured with standard behavioral bioassays (preference tests, olfactometry) as well as with electrophysiological techniques applied to antennal and maxillary palp chemoreceptors.

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Résumé

Chimiorecepteurs d' Epilachna varivestis adultes: Structures et importance dans la selection des aliments

E. varivestis Mulsant, insecte oligophage, accepte les feuilles de Glycine max (L.) Merrill (soja) comme aliment, mais presente des preferences marquees parmi les varietes. On considere que l'origine de la discrimination entre le cultivar sensible 'Williams' et PI 229358 resistant, est chimique. Des experiences de choix avec des rondelles de feuilles, apres ablation des sensilles chimiques des feuilles et des palpes maxillaires et labiaux, et des tarses anterieurs, ont montre que les sensilles chimiques etaient presque entierement responsables de la discrimination entre les feuillages de genotypes resistant et sensible. Tous les appendices ont ete observes au microscope a balayage, et, a l'exception des tarses anterieurs, ils portaient plusieurs types de sensilles gustatives et olfactives, qui ont ete representing et decrites. Puisque les observations comportementales montrent que l'olfaction est normalement suffisante pour provoquer le rejct de PI 229358, nous concluons que les sensilles olfactives des antennes et des palpes maxillaires jouent un role crucial dans le choix de l'aliment.

References


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