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Chemoreceptors on the proboscis of the female eastern spruce budworm[1] $\sqrt[3]{}$

I MORPHOLOGICAL AND HISTOLOGICAL STUDY

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The distribution, morphology and innervation of the various sensillae on the proboscis of the female spruce budworm *(Choristoneura fumiferana* CLEM.*)* are described. The sensillae chaetica on the proximal part must be mechanoreceptors. Two types of sensillae styloconica and papillae on the exterior surface and in the food channel are believed to be chemoreceptors innervated predominately by two sensory nerve cells.

Verteilung, Morphologie und Innervation der verschiedenen Sensillen auf dem Rostrum des Weibchens des Spruce Budworm (*Choristoneura fumiferana* CLEM.) werden beschrieben. Die Sensillae chaetica auf dem proximalen Teil müssen Mechanorezeptoren sein. Zwei Typen von Sensillae styloconica und Papillen auf der äusseren Oberfläche und dem Nahrungskanal scheinen Chemorezeptoren zu sein, die zur Hauptsache durch zwei Sinneszellen innerviert werden.

INTRODUCTION

The external morphology of the proboscis and its sense organs has been described for various *Lepidoptera* by GUYENOT (1912), FRINGS & FRINGS (1949), EASTHAM & ESSA (1955) and HEPBURN (1917). The only histological data available to us has been given by GUYENOT (1912) for Vanessa. Recently GOLDWARE & BARNES (1973) published pictures of the proboscis' sense organs in the codling moth taken with a scanning electromicroscope.

The function of these receptors has been studied for some species by FRINGS & FRINGS (1949, 1956). The authors concluded from their behavior observations that butterflies and moths can taste sucrose probably with the sensillae styloconica on the terminal part of the proboscis. Based on their morphological data GOLDWARE & BARNES (1973) suggested that the proboscis' chemoreceptors could be involved in host selection by perception of plant chemicals. However, our behavior experiments in the spruce budworm (STÄDLER 1974) using amputation techniques gave us no evidences for chemoreceptors on the proboscis which influence oviposition.

As ablation experiments can lead to erroneous conclusions, an electrophysiological investigation of the sensillae styloconica was initiated to supply more information (STÄDLER & SEABROOK, in preparation). The present study was designed to provide the morphological and histological data necessary for physiological experiments in the future.

MATERIALS AND METHODS

– *Insects:* The same rearing procedure as in our earlier work was used (STÄDLER 1974).

– *Lightmicroscopy:* Whole mounts of the proboscis were stained with the vital Methylene blue technique modified and described by ZACHARUK (1962).

The proboscis used for sectioning were obtained from animals fixed in Bouin's fluid (GRAY 1964). Isolated pieces were embedded in parafin using

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Péterfi's method of double embedding with Celloidin. Sections were obtained at a thickness of 4–6μm. They were successfully stained with Silver (ROMANES 1950), Harris's Haemotoxylin-Eosin and Azan (HUBSCHMAN 1962).

– *Scanning "electron"microscopy:* Material for use in the S.E.M. was air dried, and mounted using conductive silver paint. It was sonicated, using an ultrasonic cleaning apparatus, in detergent, absolute alcohol, and acetone for about one minute each. The specimens were coated with gold.

RESULTS AND DISCUSSION

Morphology

The following sensillae have been observed on each of the two identical galea which form the proboscis: 1) sensillae chaetica mainly on the proximal $^1/_3$ of the proboscis. 2) one lateral row of long sensillae styloconica (40 μm long, Tab. 1, Fig. 1), each with 6–9 longitudinal ridges and a central tip papillum. 3) one dorsal row of medium sized sensillae styloconica (20 μm long, Tab. 1, Fig. 1, 2) of the same appearance as the long sensillae. 4) Between the medium sized receptors usually small sensillae are found which look similar to the central papillae of the tip of the sensillae styloconica (5 μm long, Tab. 1, Fig. 1, 3). 5) A similar type of receptor may be found in the proximal part of the food channel.

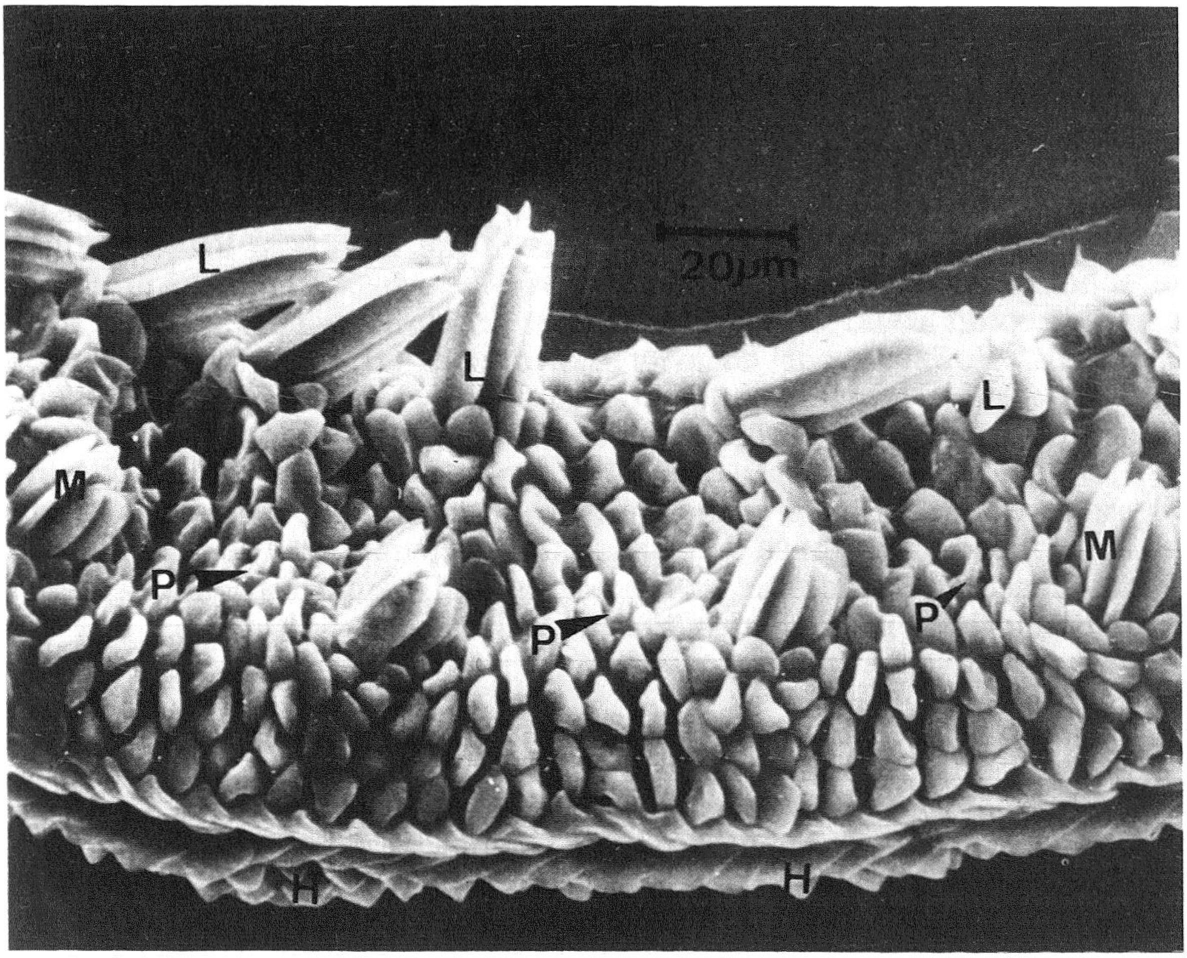


Fig. 1 Portion of one galea: SEM.

Table 1 Number, size and innervation of sensillae styloconica on the galea.

Type of sensillum	Number of sensillae per galea range	average	Average size in µm length	diameter	Average number of sensory cells per sensillum
long	25 - 42	34	40	10	1.46 (26)
medium	20 - 41	34	20	10	1.98 (64)
small	28 - 48	40	5	2	2.01 (105)
small in food channel	9 - 18	14	5	2	2.45 (22)

( ) Number of sensillae

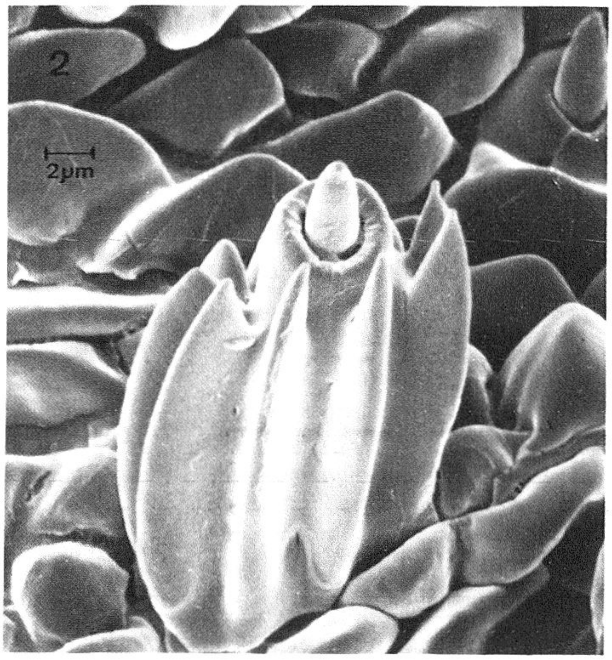


Fig. 2 Medium sized sensillum styloconicum: SEM.

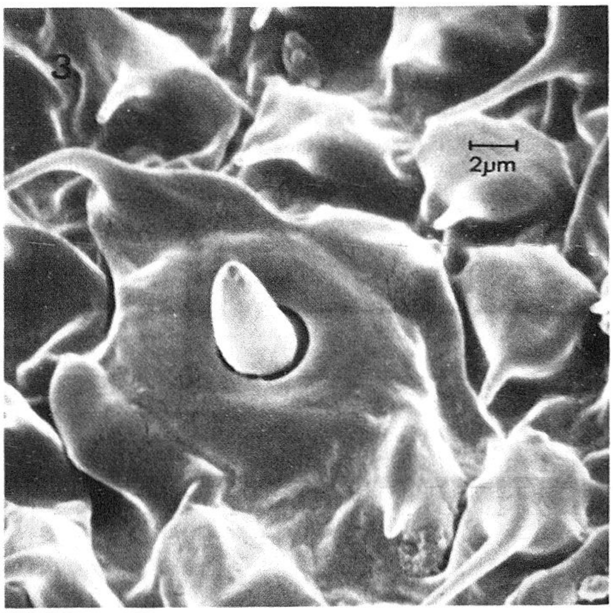


Fig. 3 Papillum: SEM.

The length of the long and the medium sized sensillae is variable. The first few receptors proximal are significantly smaller. Both types of receptors, but mainly the longer lateral ones, diminish in size apically and are practically of the same appearance near the tip of the proboscis. The number of sensillae styloconica is not constant (Tab. 1) but obviously correlated to the size of the animal and the length of the proboscis. The observed variation was probably due to the rearing conditions.

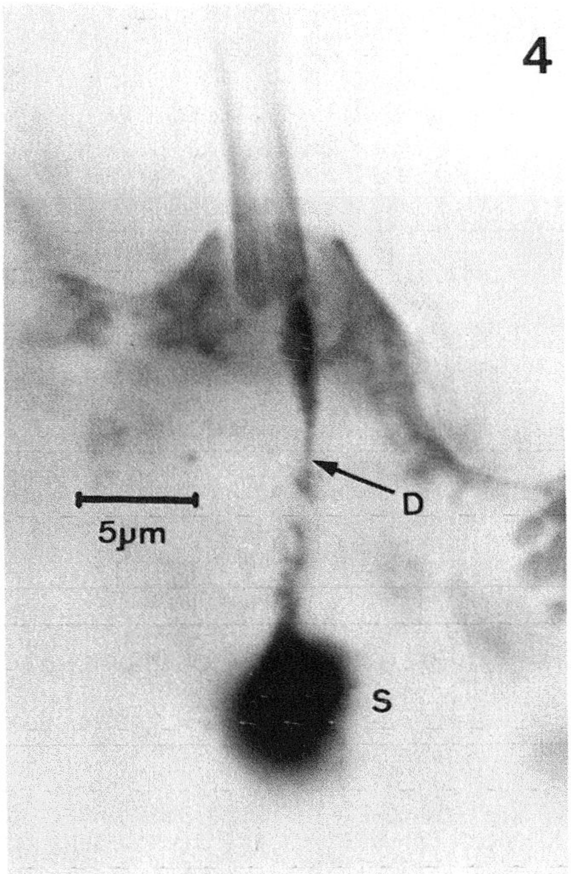
The morphology of the proboscis of the spruce budworm seems to be similar to those of other *Tortricidae* (GUYENOT 1912) and that of the closely related codling moth (GOLDWARE & BARNES 1973). We agree with GUYENOT that it is very unlikely that the proboscis can be used to rupture the plant cuticle. The distinct longitudinal wall-ridges prevent the bending of the receptor (the socket is flexible only) and enable a quick erection after deformation and wetting during feeding.

Except for the sensillae chaetica (hairs) all the receptors have a depression on the tip of their papillae looking like a pore through which the dendrites seem to be in contact with the outside. In several receptors we observed also a droplet of fluid on the tip of the papillae exuded probably from inside the receptor before or during fixation. This shows that the papillae must have a pore because no droplets have been seen on the tips of the ridges. The occurrence of such a viscous substance has also been observed by STÜRCKOW (1967) on the tip of labellar chemoreceptive hairs of the blow fly.

Histology

The whole mount preparations stained with Methylene blue show the innervation of the different receptors (Fig. 4, 5, 9). The sensillae chaetica on the proximal part of the proboscis are always innervated by one dendrite of a sensory nerve cell (Fig. 4). The dendrite ends at the socket of the base thus characterizing these hairs as mechanoreceptors.

All the other receptors have dendrites going up to the apex of the sensory papillae where we assume to be a pore providing contact with the outside environment (Fig. 5–9). Unfortunately we never succeeded in staining the whole length of a galea with Methylene blue. However, in 12 galeae we could count the sensory cells of the proximal fourth to half. As no trend of the counts



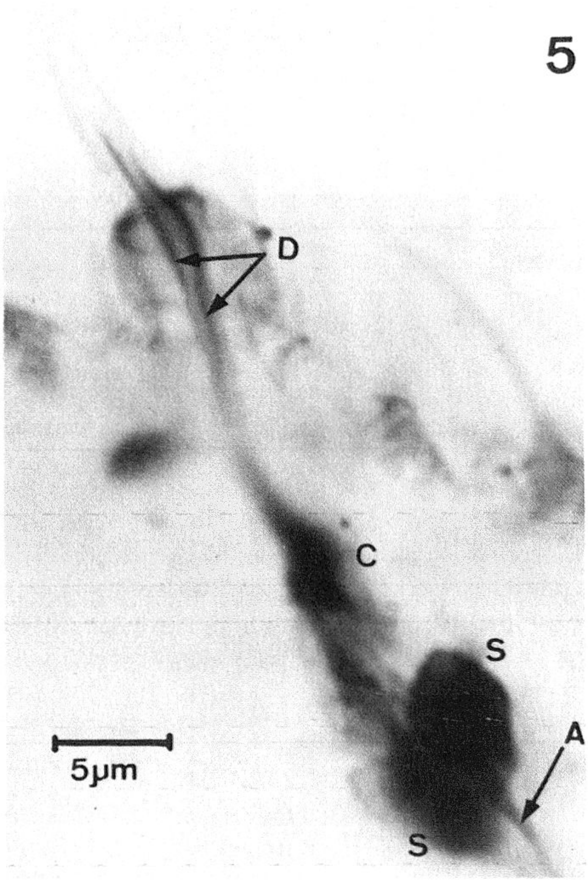
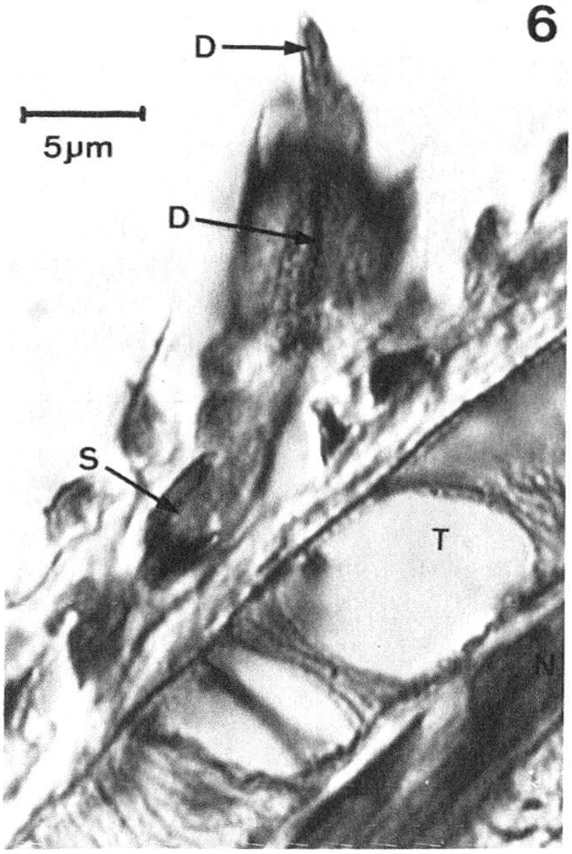
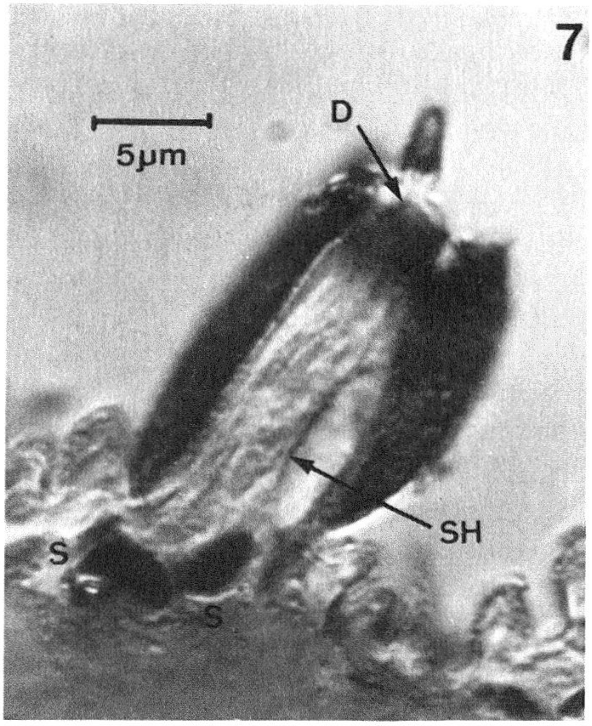
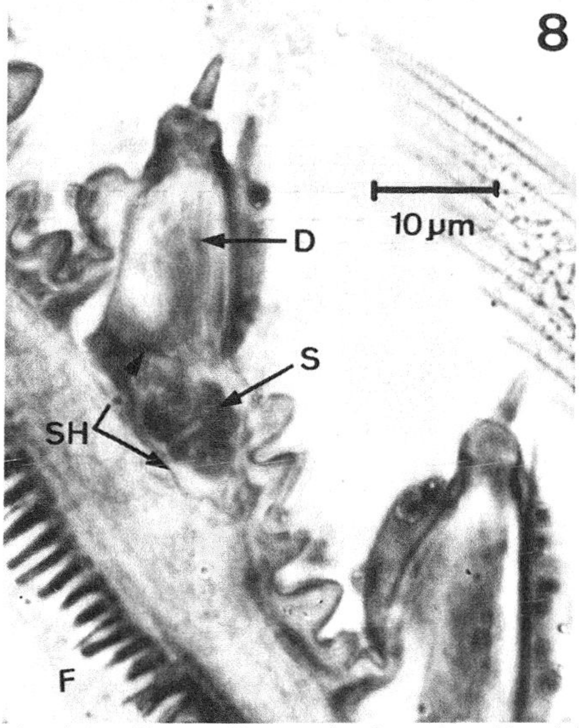


Fig. 4 Sensillum chaeticum: Methylene blue whole mount. Dendrite (D) ending in hair socket.
Fig. 5 Papillum: Methylene blue whole mount. Two dendrites entering papillum.







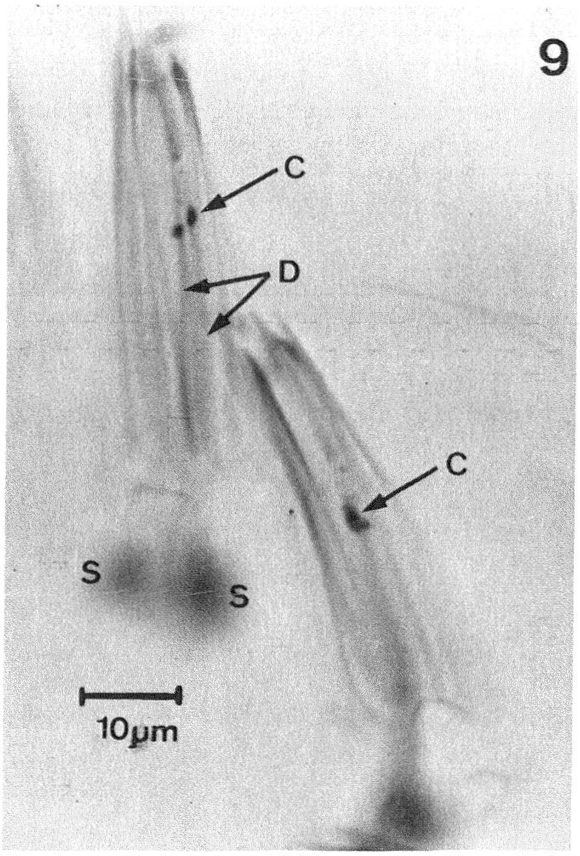


Fig. 6 Papillum: Silver stained section. Dendrite in focus in contact with pore opening of tip papillum.
Fig. 7 Medium sized sensillum styloconicum: Silver stained section.
Fig. 8 Medium sized sensillum styloconicum: Azan stained section.
Fig. 9 Long sensillum strloconicum: Methylene blue whole mount.
Abbreviations: A Axon; C Ciliary body?; D Dendrite; H Dorsal (medial) hocks which link the two galea together; L Long s. styloconicum; M Medium sized s. styloconicum; N Nerv; P Papillum; S Sensory cell body; SH Sheath; T Tracheal branch.

along the proboscis was obvious we assume that the number of cells per receptor will not be significantly different in the apical part. The results in Tab. 1 show that there is no marked difference in the innervation of the four types of sensilla styloconica. Of 215 receptors the majority (119) was innervated by 2 cells. However, the statistical analysis of the cell counts showed that the long receptors in the average have fewer, and the papillae of the food channel have more cells than the medium and the small sized sensillae.

In most well stained receptors the dendrites show in the medium part a typical swelling (Fig. 5, 9). It could represent the ciliary body even though this region of the dendrite has been found closer to the cell body in the chemoreceptors of other insects (SLIFER 1970).

Longitudinale sections stained with silver confirm that at least one dendrite is in contact with the pore opening (Fig. 6). Some sections show that the sensory cells are surrounded by a sheath which seems to extend into the sensillum enclosing the dendrites. The counts of the sensory cells in the sections support the earlier results that 2–3 cells innervate the sensillae styloconica.

Our results confirm the observation of GUYENOT that the s. styloconica are innervated. They indirectly also support the conclusion of FINCH & FINCH (1956) and KUSANO & ADACHI (1969) that these receptors could be taste receptors. Further evidences from our electrophysiological study (STÄDLER & SEABROOK, in preparation) show that the sensilla styloconica on the proboscis of the spruce budworm are indeed chemoreceptors.

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