

MORPHOLOGICAL EVIDENCE FOR POSSIBLE SITES OF
PRODUCTION OF THE FEMALE SEX PHEROMONE OF
COPITARSIA CONSUETA (LEPIDOPTERA: NOCTUIDAE)

JULIO C. ROJAS¹, JORGE VALDEZ-CARRASCO², AND JUAN CIBRIAN-TOVAR²

¹Centro de Investigaciones Ecológicas del Sureste. Apdo Postal 36,
30700 Tapachula, Chiapas, México.

²Centro de Entomología y Acarología, Colegio de Postgraduados,
56230 Chapingo, Edo. de México, México.

Copitarsia consueta (Walker) is a polyphagous insect that could be a serious pest of several cultivated plants in Mexico (Gutierrez & MacGregor 1983). Recently, some aspects of reproductive behavior were studied (Rojas & Cibrian 1994). Crude extracts of the female terminal abdominal segments tested in a wind tunnel evoked in males the following behavioral acts: oriented flight, landing, wing-fanning, spreading genitalia and attempted copulation (Rojas et al. 1993). Until now, the exact location of the sex pheromone gland remained unknown. This paper provides morphological and histological evidence of putative cells involved in the production of sex pheromone of *C. consueta*.

The insects used in this study were reared at $25 \pm 2^\circ\text{C}$, $65 \pm 5\%$ RH, and 14:10 (L:D) photoperiod regimen on an artificial diet (Rojas et al. 1993). Virgin females 4-6 days old were anesthetized with CO_2 , the terminal portions of their abdomen extruded to a normal degree by gentle squeezing, and the abdomen ligated to keep the last segments extruded. The abdominal tips were cut off anterior to the ligation and fixed in Duboscq-Brasil (Pantin 1946) for 72 h. For scanning electron microscopy, the material was dehydrated in ethanol and critical-point dried. The tissue was mounted on metallic cylindrical stages, coated with gold and examined with a JEOL 35-C scanning electron microscope, operated at 10 Kv. For histological observations, the tissue was dehydrated in ethanol and embedded in paraffin. Serial, longitudinal and transverse sections were made at $10\ \mu\text{m}$, stained with hematoxylin-erythrosin and mounted in Canada balsam. Photomicrographs were taken with a Zeiss photomicroscope.

Analysis of the integument of the last abdominal segments suggests that two types of structures are involved in the production and release of pheromonal components. The first involves intersegmental membranes VII-VIII and VIII-IX (Fig. 1, No. 1,2) which reveal a characteristic histological structure on which the external surfaces are covered with a multitude of sharp epicuticular projections (Fig. 1, No. 4). These epicuticular projections rest over a stratum of flexible procuticle. Possible glandular cells present on this integument are similar to those found in other noctuids (Percy-Cunningham & MacDonald 1987). The pheromone producing glands in other Noctuidae are located in intersegmental membrane VIII-IX (Percy-Cunningham & MacDonald 1987). No prior study has found pheromone-producing cells in intersegmental membrane VII-VIII in this family. However, in the butterfly *Argynnis adippe*, the gland is located over the intersegmental membrane of abdominal segments VII-VIII (Percy & Weatherston 1971).

The second structures involved in pheromone production are the two lateral cuticular sacs situated in intersegmental membrane VII-VIII (Fig. 1, No. 3). At this place the integument is invaginated at each side to form an almost spherical cavity of extremely thick cuticle. It is formed by a thick layer of lamellated procuticle and a thin layer of superficial epicuticle (Fig. 1, No. 5,6). The hypodermis of this integument is

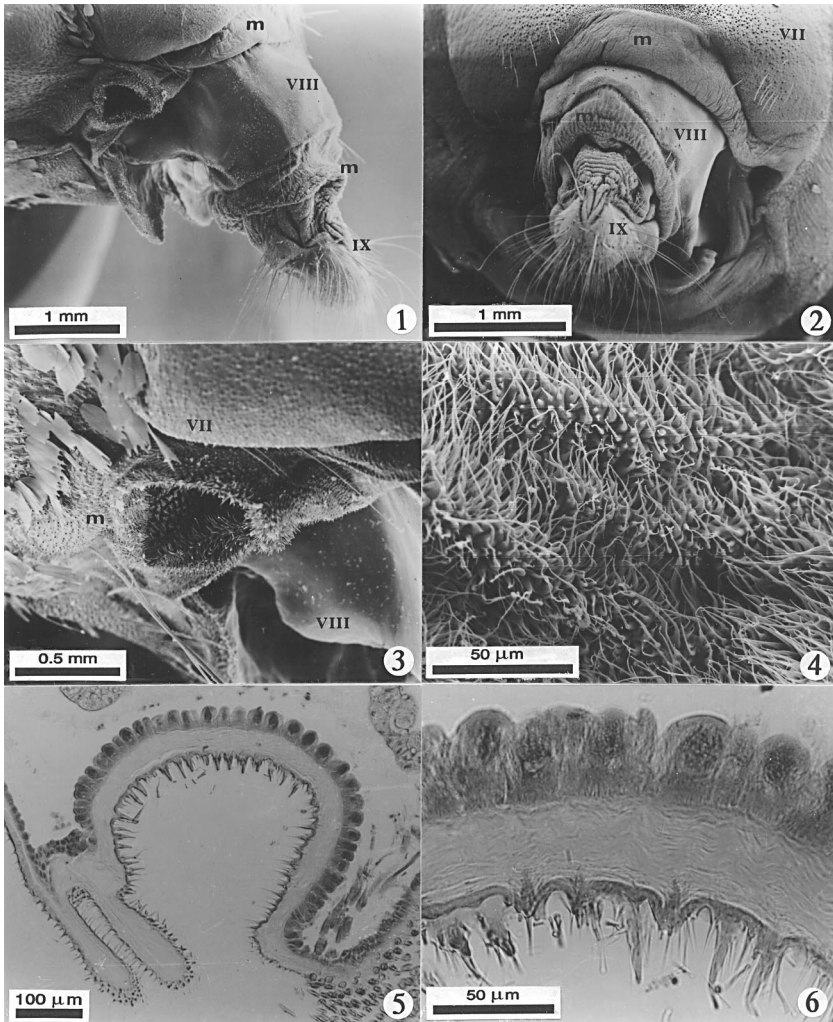


Fig. 1. Electron micrograph of possible site of production of female sex pheromone of *Copitarsia consueta*. **No. 1**, left lateral view of the abdominal segments everted during the calling behavior; m, intersegmental membrane; VIII, eighth abdominal segment; IX, ninth abdominal segment. **No. 2**, Dorsal view of the female abdominal terminalia; m, intersegmental membrane; VII, seventh abdominal segment; VIII, eighth abdominal segment; IX, ninth abdominal segment. **No. 3**, Aperture of the lateral glandular sacs in the intersegmental membrane VII-VIII; m, intersegmental membrane; VII, seventh abdominal segment; VIII, eighth abdominal segment. **No. 4**, Microtrichia covering the membranes VII-VIII and VIII-IX involved in the release of pheromonal components. **No. 5**, Longitudinal section of the tegumentary glandular sacs. **No. 6**, Section of the cuticle and hypodermis of the lateral tegumentary sacs.

formed by globe-shaped cells with nuclei that occupy approximately 70% of their volume (Fig. 1, No. 6). These cells exhibit typical characteristics of glandular epithelium, such as cellular membrane infolding along the whole line between cell and cuticle. In a light microscope, the cytoplasm has a fibrous appearance because it is always observed in cells with deep basal folds of the cellular membrane and in cells loaded with endoplasmic reticulum. These characteristics are typical of cells with intense synthesis activity. The nuclei are granulate and show a clear nucleolus. The cuticular structures continue beyond the borders of the orifices (Fig. 1, No. 3) with structures of the intersegmental membrane already described. The histological structure of the cuticular sacs suggests that these semispherical cells could synthesize the sex pheromone of this species, which would then have to diffuse through the cuticle to reach the cavity of the organ. This type of cell could be classified as class 1 (Noirot & Quennevey 1974). The females of many Lepidoptera produce their sex pheromone from glands of this type (Noirot & Quennevey 1974). The lateral cuticular sacs located in intersegmental membrane VII-VIII have not been reported in any species of any family. In *Bombyx mori*, the gland is represented by ventrolateral sacs in membrane VII-VIII (Hecker & Butenandt 1984).

The fact that in *C. consueta* we found different regions involved in the possible biosynthesis of pheromones is not new in the literature, since the same phenomenon is found in other species (Chow et al. 1976; Teal et al. 1983). In *Heliothis virescens*, it is suggested that different areas could produce or release mixtures of several pheromonal components (Teal et al. 1983).

The authors thank Florencio Navarrete Medina for technical assistance. JCR was sponsored by a CONACyT graduate scholarship (62158-). This research was supported by CONACyT (0691-N9111).

SUMMARY

This paper provides morphological and histological evidence of the putative cells involved in production of the sex pheromone of *Copitarsia consueta* (Lepidoptera: Noctuidae). The analysis of the integument of the last abdominal segments suggests two types of structures may be involved: (1) the intersegmental membranes VII-VIII and VIII-IX, and (2) the two lateral cuticular sacs situated in intersegmental membrane VII-VIII.

REFERENCES CITED

- CHOW, Y. S., J. CHEN, AND S. H. LIN-CHOW. 1976. Anatomy of the female sex pheromone gland of the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae). Int. J. Insect Morphol. and Embryol. 5: 195-203.
- GUTIERREZ, O., AND R. MACGREGOR. 1983. Guía de insectos nocivos para la agricultura en México. Alhambra Mexicana, S.A., México, D.F.
- HECKER, E., AND A. BUTENANDT. 1984. Bombykol revisited. Reflections on a pioneering period and on some of its consequences, pp. 1-44 in Hummel, H. E. and T. A. Miller [eds.], Techniques in Pheromone Research. Springer-Verlag, New York.
- NOIROT, C., AND A. QUENNEVEY. 1974. Fine structure of insect epidermal glands. Ann. Rev. Entomol. 19: 61-80.
- PANTIN, C. F. A. 1946. Notes on microscopical technique for zoologist. The Sindics of the Cambridge University Press, London.
- PERCY, J. E., AND J. WEATHERSTON. 1971. Studies of physiologically active arthropod secretions. X. Morphology and histology of the pheromone-producing glands of some female Lepidoptera. Canadian Entomol 103: 1733-1739.

- PERCY-CUNNINGHAM, J. E., AND J. A. MACDONALD. 1987. Biology and ultrastructure of sex pheromone-producing glands, pp. 27-75 in Prestwich, G. D. and G. J. Blomquist [eds.], Pheromone Biochemistry. Academic Press, Inc. New York.
- ROJAS, J. C., AND J. CIBRIAN-TOVAR. 1994. Reproductive behavior of *Copitarsia consueta* (Lepidoptera: Noctuidae): Mating frequency, effect of age on mating, and influence of delayed mating on fecundity and egg fertility. Pan-Pacific Entomol. In press.
- ROJAS, J. C., J. CIBRIAN-TOVAR, J. VALDEZ-CARRASCO, AND R. NIETO-HERNANDEZ. 1993. Analisis de la conducta de cortejo de *Copitarsia consueta* (Lepidoptera: Noctuidae) y aislamiento de la feromona sexual. Agrociencia 4: 23-39.
- TEAL, P. E. A., T. C. CARYSLE, AND J. H. TUMLINSON. 1983. Epidermal glands in terminal abdominal segments of female *Heliothis virescens* (F.) (Lepidoptera: Noctuidae). Ann. Entomol. Soc. America 76: 242-247.