SYSTEMATICS, MORPHOLOGY AND PHYSIOLOGY

Histochemical and Ultrastructural Evidence of Lipid Secretion by the Silk Gland of the Sugarcane Borer *Diatraea saccharalis* (Fabricius) (Lepidoptera: Crambidae)

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Neotropical Entomology 36(5):707-711 (2007)

Evidências Histoquímicas e Ultra-Estruturais de Secreção Lipídica pela Glândula da Seda da Broca da Cana-de-Açúcar, *Diatraea saccharalis* (Fabricius) (Lepidoptera: Crambidae)

RESUMO - A glândula da seda de larvas de Lepidoptera é responsável pela produção da seda usada na construção do casulo ou do abrigo. A secreção de fibroína e sericina pelas diferentes regiões da glândula da seda está bem estabelecida. Existem poucos trabalhos tentando identificar componentes lipídicos na secreção de seda de insetos, embora a presença desse componente contribua para a resistência da seda a ambiente úmidos. Este estudo teve como objetivo identificar a presença de componente lipídico na secreção da glândula da seda de larvas de *Diatraea saccharalis* (Fabricius), bem como caracterizar a região glandular responsável pela secreção. A glândula da seda foi submetida a procedimentos histoquímicos para detecção de lipídeos e convencionalmente preparada para análise ultra-estrutural. Gotas lipídicas foram histoquimicamente detectadas no citoplasma apical de células da região anterior e no lúmen, entre os microvilos. A análise ultra-estrutural da região anterior mostrou material lipídico, visualizado como figuras do tipo mielina dentro do complexo de Golgi vesicular e nos glóbulos secretores apicais, misturados com sericina; material semelhante foi observado dentro do lúmen, adjacente aos microvilos. Nenhum componente secretor lipídico foi detectado nas células ou no lúmen da região posterior. Os resultados sugerem que a seda produzida pela *D. saccharalis* tem, pelo menos, um discreto componente lipídico, que é secretado pela região anterior, junto com a sericina.

PALAVRAS-CHAVE: Célula secretora, inseto, larva, lipídio

ABSTRACT - The silk gland in Lepidoptera larvae is responsible for the silk production used for shelter or cocoon construction. The secretion of fibroin and sericin by the different silk gland regions are well established. There are few attempts to detect lipid components in the insect silk secretion, although the presence of such element may contribute to the resistance of the shelter to wet environment. This study characterizes the glandular region and detects the presence of lipid components in the secretion of the silk gland of *Diatraea saccharalis* (Fabricius). The silk gland was submitted to histochemical procedure for lipid detection or conventionally prepared for ultrastructural analyses. Lipid droplets were histochemically detected in both the apical cytoplasm of cell of the anterior region and in the lumen among the microvilli. Ultrastructural analyses of the anterior region showed lipid material, visualized as myelin-like structures within the vesicular Golgi complex and in the apical secretory globules, mixed up with the sericin; similar material was observed into the lumen, adjacent to the microvilli. Lipids were not detected in the cells neither in the lumen of the posterior region. Our results suggest that the silk produced by *D. saccharalis* has a minor lipid content that is secreted by the anterior region together with the sericin.

KEY WORDS: Secretory cell, insect, larva

The silk gland of Lepidoptera larvae is responsible for silk secretion used for shelter or cocoon construction (Wigglesworth 1972, Sehnal & Akai 1990). The silk is mainly constituted by fibrous proteins and glycoproteins, which are variable in the composition and molecular array depending on the insect species (Lucas & Rudall 1968, Rudall & Kenchington 1971, Richards & Davies 1977). The silk produced by these glands in Lepidoptera larvae has two main components, the highly elastic friboin and the gelatinous sericin (Akai 1984).

Although the morphology of the silk gland of *Bombyx mori* (L.) (Lepidoptera: Bombycidae) larvae has been exhaustively studied as its secretion has a great economic value (see Sehnal

& Akai 1990), the gland morphology of several other insect species have also been investigated. However, most of the papers emphasize the morphology of the secretory cells and their relationship with the synthesis, intracellular transport and secretion of the different silk elements, mainly the fibroin and sericin (Wiley & Lai-Fook 1974, Engster 1976, Silva-Zacarin *et al.* 2003).

Morphological differences along this gland allowed the identification of species-specific secretory regions (Wiley & Lai-Fook 1974, Silva-Zacarin *et al.* 2003). Although it is well established that in the Lepidoptera silk gland the fibroin is secreted by the posterior glandular region while the sericins are produced by the anterior one (Shibukawa 1959, Sehnal & Akai 1990), there are few histochemical studies identifying the elements of the silk in insect gland, either into the lumen or in the secretory cells (Machida 1926, Shibukawa 1959, Wiley & Lai-Fook 1974, Engster 1976). The presence of lipid in the silk secretion in insects is controversial, although it would make the cocoon or shelter waterproof. There are few works concerning the histochemical detection of lipids in the secretion of the Lepidoptera silk gland cells (Shibukawa 1959, Engster 1976).

The sugarcane borer, *Diatraea saccharalis* (Fabricius), is an important pest of sugarcane and many others crops (Long & Hensley 1972). The silk gland of last instar *D. saccharalis* produces large amount of silk to protect the insect. The silk gland ultrastructure of *D. saccharalis* has been previously described (Victoriano & Gregório 2002, Victoriano *et al*. 2003), but there are no reports on the characterization of the silk secretion in this species. Therefore, this work aims to verify the occurrence of lipid components in the secretion of *D. saccharalis* silk gland, as well as to determine the gland region responsible for this secretion.

Material and Methods

D. saccharalis larvae were reread on artificial diet (Hensley & Hammond 1968) and maintained in laboratory under controlled conditions (temperature: 26 ± 1ºC; humidity: 70 ± 5% and photophase: 14h).

Silk glands obtained from last-instar larvae (three days after the ecdysis) were prepared for histochemistry as described by Hernández-Blazquez *et al.* (1989). Briefly, the silk glands were fixed in 4% paraformaldehyde, 1% calcium chloride, 1% cadmium chloride for 24h, post-fixed in 1% osmium tetroxide in 0,1M phosphate buffer (pH 7.2) for 3h, and embedded in Historesin (JB4-Polysciences). Histological sections were treated with hydrogen peroxide, stained for 15 min with 1% -Sudan Black B and observed under light microscope. Detailed ultrastructure analyses were done in silk glands fixed in 2% glutaraldehyde–4% paraformaldehyde solution in 0.1 M phosphate buffer (pH 7.3) for 24h, post-fixed in 1% osmium tetroxide in the same buffer for 2h, and embedded in Araldite resin. Ultra thin sections were double stained with uranyl acetate and lead citrate and examined under a Philips CM100 transmission electron microscope.

Results

The histochemical analysis showed positive reaction for lipids at the anterior glandular region, mainly adjacent to the excretory duct at the apical cytoplasm and in the lumen, among the apical microvilli, but not in the lumen content (Fig. 1A). The ultrastructural analysis showed that the cells of this region present lipid components, morphologically similar to myelin-like structures; these elements were found in both the apical secretory vesicles (Fig. 2A) and vacuoles of Golgi complex (Fig. 2C), mixed up with fibrilar electron-lucent material reported as sericin secretion (Akai 1984). Myelin-like structures were also observed being released into the lumen (Fig. 2E) and adjacent to the microvilli (Figs. 2A and D).

The histochemical analysis of the posterior glandular region showed small droplets positive for lipid reaction concentrated in the basal and perinuclear cytoplasm (Fig. 1B); we did not observe positive reaction for lipid in the

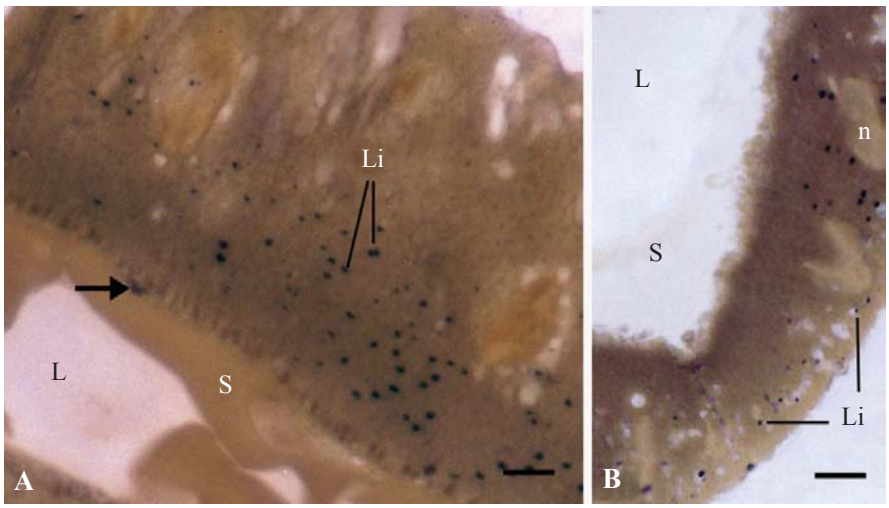

Fig. 1. The silk gland of *D. saccharalis* larvae stained with Sudan Black B for lipid detection A) Anterior secretory region. B) Posterior secretory region. L = lumen; Li = lipid droplets; S = silk secretion; arrow = lipid among the apical microvilli; n = nucleus. Bar = 10 µm.

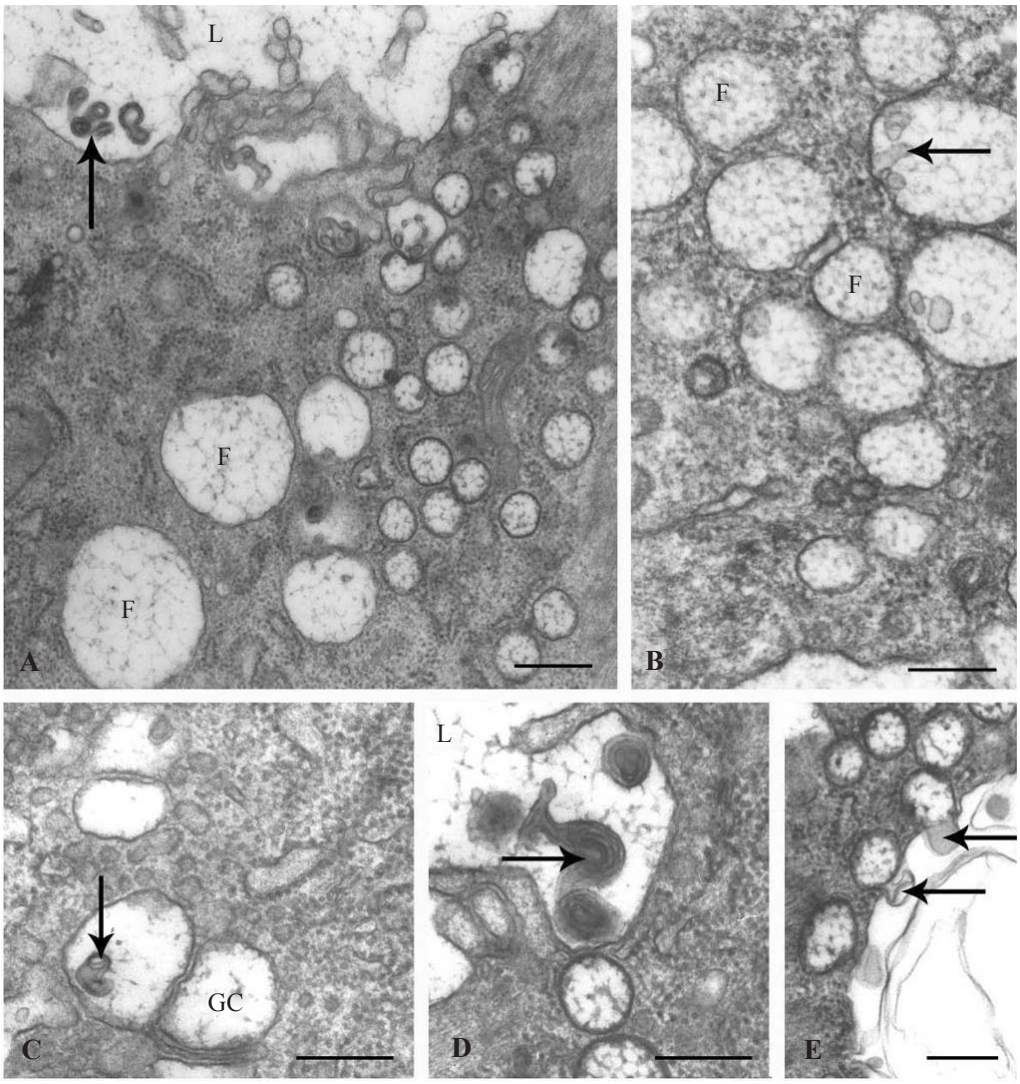

Fig. 2. Ultrastructure of the anterior region in the *D. saccharalis* silk gland A) Apical cytoplasm with myelin-like material (arrow) in the secretory vesicles containing fibrilar material (F) of sericin, and in the lumen (L). Bar = 0.5 μm. B) Detail of the secretory vesicles containing fibrilar material (F) and myelin-like material (arrow). Bar = 0.25 μm. C) Detail of the Golgi complex vacuoles containing myelin-like material (arrow) together with the fibrilar secretion of sericin; Golgi complex cisternae (GC). Bar = 0.25 μm. D) Myelin-like material (arrow) in the lumen (L), among the microvilli. Bar = 0.25 μm. E) - Exocytosis of the secretory vesicles releasing myelin-like material secretory vesicles (arrows) into the lumen. Bar = 0.25 μm.

lumen (Fig. 1B). The ultrastructural analysis of this region showed sparse lipid droplets in the basal cytoplasm (Figs. 3A and B), visualized as homogeneous material not bounded by membrane. The content of the apical secretory granules (Fig. 3C) and the vesicular Golgi complex (Fig. 3D) were represented only by filamentous material.

Discussion

There are few reports presenting the ultrastructural features of the different silk gland regions correlated with the histochemical analyses of their cells in insects; however, they emphasize the detection of fibroin and sericin, the major components of the silk gland secretion (Shibukawa 1959, Wiley & Lai-Fook 1974, Engster 1976).

The morphological evidence of lipid elements in the insect silk is not well determined. Although there was positive reaction for lipid in the internal and external layers of the luminal silk in *B. mori* larvae, no reactive product was visualized into the secretory cells (Shibukawa 1959). On the other hand, the silk of *D. saccharalis* tested negative for lipid into the lumen was negative for lipid staining, but lipid components were detected among the microvilli of the apical cell region and in the apical cytoplasm. Although we could not detect lipids in the lumen, we believe that the lipid

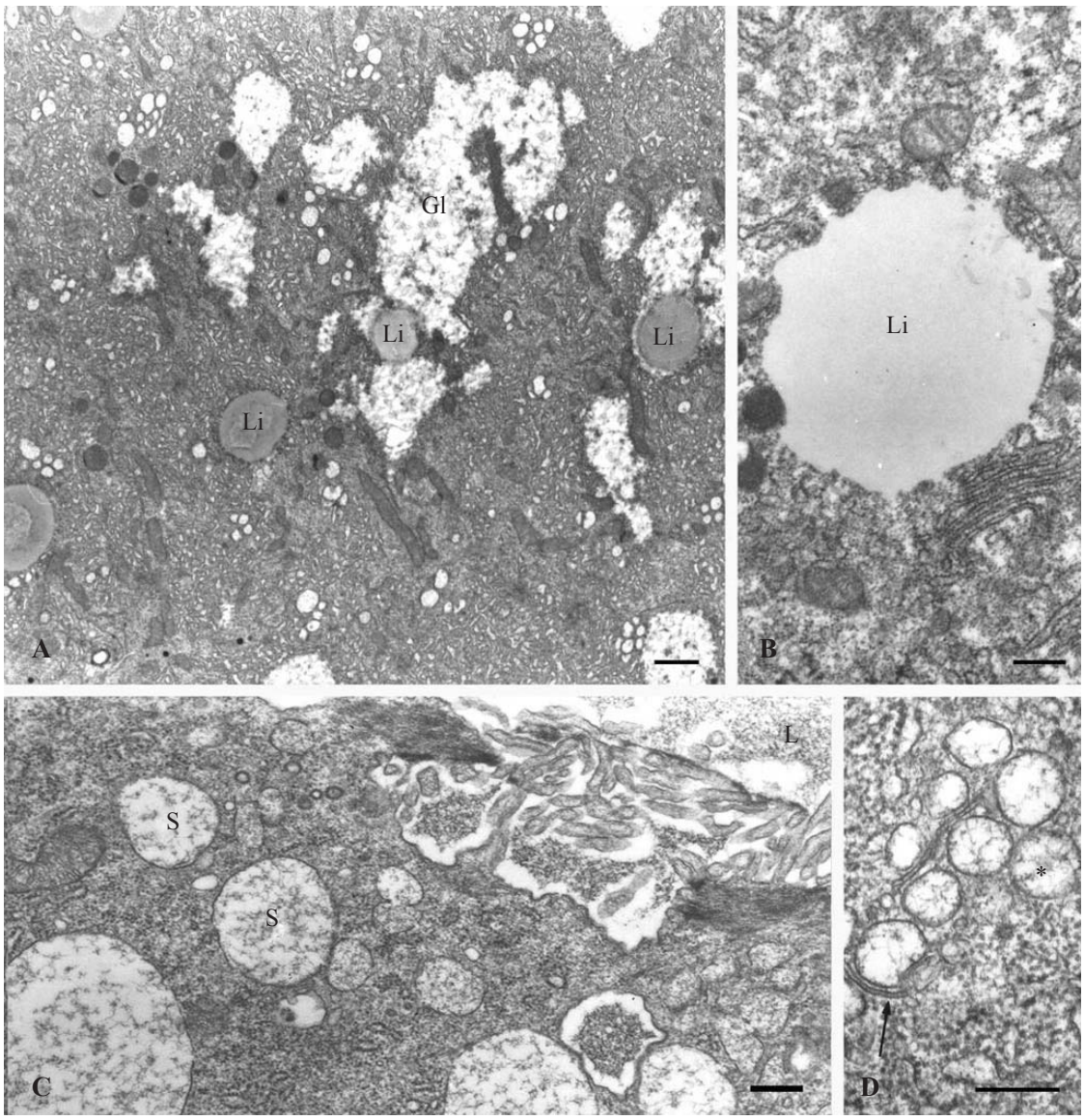

Fig. 3. Ultrastructure of the posterior region in the *D. saccharalis* silk gland A) Basal cytoplasm with lipid droplets (Li) and glycogen deposit (Gl). Bar = 1 μm. B) Detail of lipid droplet (Li), not surrounded by membrane. Bar = 0.5 μm. C) Apical cytoplasm with secretory vesicles (S) containing only filamentous material of fibroin, present also in the lumen (L). Bar = 0.5 μm. D) Vesicular Golgi complex containing only filamentous material of fibroin (*); Golgi complex cisternae (arrow). Bar = 0.25 μm.

component secreted in small amount by the glandular cells is incorporated in the luminal silk, being mixed with the silk major proteins, sericin and fibroin.

The secretory cells of the anterior region tested positive for lipids, indicating these cells to be the active site for lipid secretion. In *B. mori*, Komatsu (1975) also determined the preferential localization of insoluble material, constituting the wax of the cocoon, in the silk gland region adjacent to the excretory duct. These findings suggest that the lipid component of the silk is deposited at the external surface of the silk strand, where it could contribute for the waterproof role played by the silk and, consequently, by the cocoon.

The ultrastructural analysis of the different silk gland regions confirmed that the anterior glandular region secrets the lipid element, visualized as dense material forming myelin-like structures in the secretory cells and among their microvilli. This lipid secretion was detected both in vacuoles of Golgi complex vacuoles and secretory vesicles, together with the sericin secretion. There is no description of such material in the secretory vesicles of Lepidoptera silk gland (Sehnal & Akai 1990, Akai *et al*. 1993).

The ultrastructure of the posterior gland region showed the presence of lipid droplets in the basal and supranuclear cytoplasm, corresponding to the positive to histochemically

reactive for lipid droplets detected under light microscope. These droplets were not bounded by membrane and correspond to the lipid storied as reserve in animal and plant cells. There was no sign of myelin-like figures in the secretory vesicles at the apical cytoplasm, these secretory vesicles contained only fibrous elements interpreted as fibroin (Victoriano *et al*. 2003), similar to those described for other Lepidoptera (Willey & Lai-Fook 1974, Akai 1984, Sehnal & Akai 1990).

Our results suggest that the silk produced by *D. saccharalis* larvae silk gland presents a discrete amount of lipid content, which are secreted by cells of the anterior glandular region together with the sericin, in the same secretory vesicle.

Acknowledgments

We gratefully acknowledge the Entomological Laboratory of Usina Barra Grande, Lençóis Paulista, state of São Paulo, for rearing the insects. This work was partially supported by FAPESP (Proc. 98/03020-0).

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Received 23/VI/06. Accepted 21/IV/07.