# Deposition of the Eggshell Layers in the Sugar Cane Borer (Lepidoptera: Pyralidae): Ultrastructural Aspects

***Daniela Carvalho dos Santos and Elisa Aparecida Gregório***
*Centro de Microscopia Eletrônica – Instituto de Biociências de Botucatu – UNESP –Campus de Botucatu, CEP:18618000, Rubião Júnior, Botucatu, SP, Brasil*

## Abstract

The deposition of the eggshell layers in *Diatraea saccharalis* (Lepidoptera: Pyralidae) ovarian follicles from adult insects were studied by transmission electron microscopy. The eggshell is composed by the vitelline membrane, the wax layer and the chorion, which is formed by different layers, recognized as trabecular, lamellate and outer osmiophilic layers. The deposition of the eggshell started in ovarian follicles at the stage 6 of the vitellogenesis; the first layer to be secreted was the vitelline membrane, being composed by uniformly flocculated material. The thin wax layer is visualized from the stage 7 on. The three chorion layers were consecutively secreted up to stage 9 and presented morphological features that allowed their recognition at the ultrastructural level. No exochorion was visualized in the oocyte envelope. The general features of the vitelline membrane, the wax layer and the three chorion layers were similar to the ones described for other Lepidoptera species. The follicle cells were responsible for the different eggshell layers secretion; they assumed different ultrastructural aspects along the eggshell deposition, specially concerning the morphology of the apical granules. Concomitant with the secretion of the outer osmiophilic layer, the follicle cells start to degenerate by autophagic process.

**Keywords**: Eggshell, ovarian follicle, ultrastructure, Lepidoptera, transmission electron microscopy.

## Introduction

The insect egg has to be housed in such an entity to permit the embryonic process to proceed safely. Besides the architecture and composition of the oocyte itself, a major role in that direction is played by the oocyte coverings which are commonly referred as eggshell (vitelline membrane, wax layer and chorion). The eggshell played several functions: to allow and facilitate the sperm entry; to provide elasticity for easy oviposition; to protect the embryo from environmental hazards such as temperature fluctuations, humidity, dryness and bacterial attack; to insure an adequate oxygen supply for all biochemical reactions occurring within the developing embryo and, at the same time, getting rid of carbon dioxide (13).

The eggshell, in most insects, is mostly composed by protein, lipid and carbohydrates (2, 7, 13, 19, 20). In Lepidoptera the eggshell is formed by the vitelline membrane, the wax layer and the chorion (trabecular layer, lamellate layer and an outer osmiophilic layer); these layers differ in their morphology and chemical properties, according the insect species (1, 6, 13, 14, 17). The eggshell is known to be produced by the follicular epithelial cells, which surround the follicle (5, 9, 10, 11, 13, 15, 18).

There are no ultrastructural studies on the eggshell organization in *Diatraea saccharalis* (Lepidoptera: Pyralidae), although this insect has long been considered the most destructive pest attacking sugarcane in several Latin American countries. This work aims to describe the ultrastructural features of the eggshell layers deposition in D. saccharalis follicles related with the vitellogenic stages previously established (21).

## Materials and Methods

The larvae of *D. saccharalis* were reared on artificial diet (10), and the pupae were maintained in plastic recipient without the artificial diet, until the emergence of the adults. Both larvae and pupae were maintained under controlled temperature (25-27°C) and humidity (70%).

Ovaries removed from adult insects were conventionally prepared for transmission electron microscopy. The ovaries were fixed in 2% glutaraldehyde – 4% paraformaldehyde solution in 0.1M phosphate buffer (pH 7.3) for 24h, post-fixed in 1% osmiun tetroxide in the same buffer for 2h, dehydrated through a graded series of acetone and embedded in Araldite resin.

Ultrathin sections were double stained with uranyl acetate and lead citrate and examined under a Philips CM100 transmission electron microscope.

## Results and Discussion

The deposition of the different eggshell layers in *D. saccharalis* occurs concomitant with the development of the ovarian follicles along the vitellogenesis, as described for other Lepidoptera species (4, 5, 6, 11, 23, 24).

In *D. saccharalis* the deposition of the eggshell layers begins in the stage 6 of the vitellogenesis, with the vitelline membrane being secreted uniformly (Fig. 1). At these stage of the vitellogenesis the *D. saccharalis* oocyte is still engaged in the extra-ovarian protein uptake (21).

The beginning of the vitelline membrane deposition along the vitellogenic process is quite variable among the Lepidoptera species: in *Anagasta kuhniella* it occurs at the stage 7 (4, 23) and in both *Hyalophora cecropia* (11) and *Bombyx mori* (24) it happens at the stage 9.

The vitelline membrane is composed by flocculent material that is deposited into the space between the oocyte surface and the surrounding follicle cells (Figs. 1, 2). There are images showing similar flocculent material being released by the follicle cells (Fig. 1), that exhibit extensive amount of rough endoplasmic reticulum (Fig. 1). Ultrastructural evidence indicates that the vitelline membrane is produced by the follicle cells in several insects (5, 6, 11, 12, 13, 19), as observed in *D. saccharalis* ovarian follicles.

In stage 7 the wax layer is secreted; it is composed by delicate and spaced filamentous structures covering the vitelline membrane (Figs. 2, 3, 4, 5). This layer was not well preserved in our preparation (Figs. 4, 5), as the lipidic material dissolves during dehydration by conventional electron microscopy method, as observed in *Drosophila melanogaster* (19). Wax layer has been identified in several orders of insects, like Diptera, Hymenoptera, Lepidoptera and Orthoptera (13). The wax layer is composed mostly by lipid, been related with the humidity of the environment where the insect egg is deposited (19).

The wax, like all the chorion layers, seals the entire oocyte, making this cell impermeable to water but only after maturation of the egg (19). It is also known that the wax layer, as the other eggshell compartments, is produced by the follicle cells, following the vitelline membrane deposition (3, 6, 16, 18).

The chorion formation starts at the stage 8, concomitant with the nurse cells degeneration (21). The apical cytoplasm of the follicle cell contains many small, electron dense vesicles (Figs. 2-4) which are secreted into the space above the wax layer, as electron dense bodies (Figs. 2-5) to form the trabecular eggshell compartment. These bodies coalesce into a layer (Fig. 4) and latter on are recognized as a thin layer of larger dense droplets irregularly spaced (Fig. 5), giving rise to the peculiar trabecular aspect of this chorion compartment. The trabecular layer of the chorion is not well developed in Lepidoptera (6, 13, 15, 17, 20), as it was described for many Diptera (3, 12, 13, 14, 19).

Along the stage 9 the trabecular layer is covered by the lamellate and the outer osmiophilic layers (Figs. 5-9); the adjacent follicle cells exhibit vesicular rough endoplasmic reticulum, apical electron lucent vesicles, vesicular Golgi apparatus and some autophagic vacuoles (Figs. 6, 7, 9). The thick lamellate layer is composed by several layers of helicoidally arranged stacks of fibrils (Figs. 5-9), like in other Lepidoptera (13, 15, 22). The outer osmiophilic layer is a thin electron-dense layer, with small undulations toward the follicle cells (Figs. 7-9); it is the last chorion layer to be deposited. No exochorion is observed in *D. saccharalis*, as in other Lepidoptera species (13, 15, 20), while in Diptera species this is a well-developed layer in the eggshell (3, 12, 13, 14).

At the end of the eggshell deposition the follicle cells become thinner and exhibit many autophagic vacuoles (Figs. 7, 9), indicating the beginning of the degenerative process of these cells that occurs in the mature egg (21).

The eggshell layers in *D. saccharalis* ovarian follicles show the general ultrastructural features similar to the ones described for other Lepidoptera species. Ultrastructural evidences has been presented which indicate that the vitelline membrane, the wax layer and the different chorion layers are subsequently produced by the follicle cells along the ovarian follicle development.

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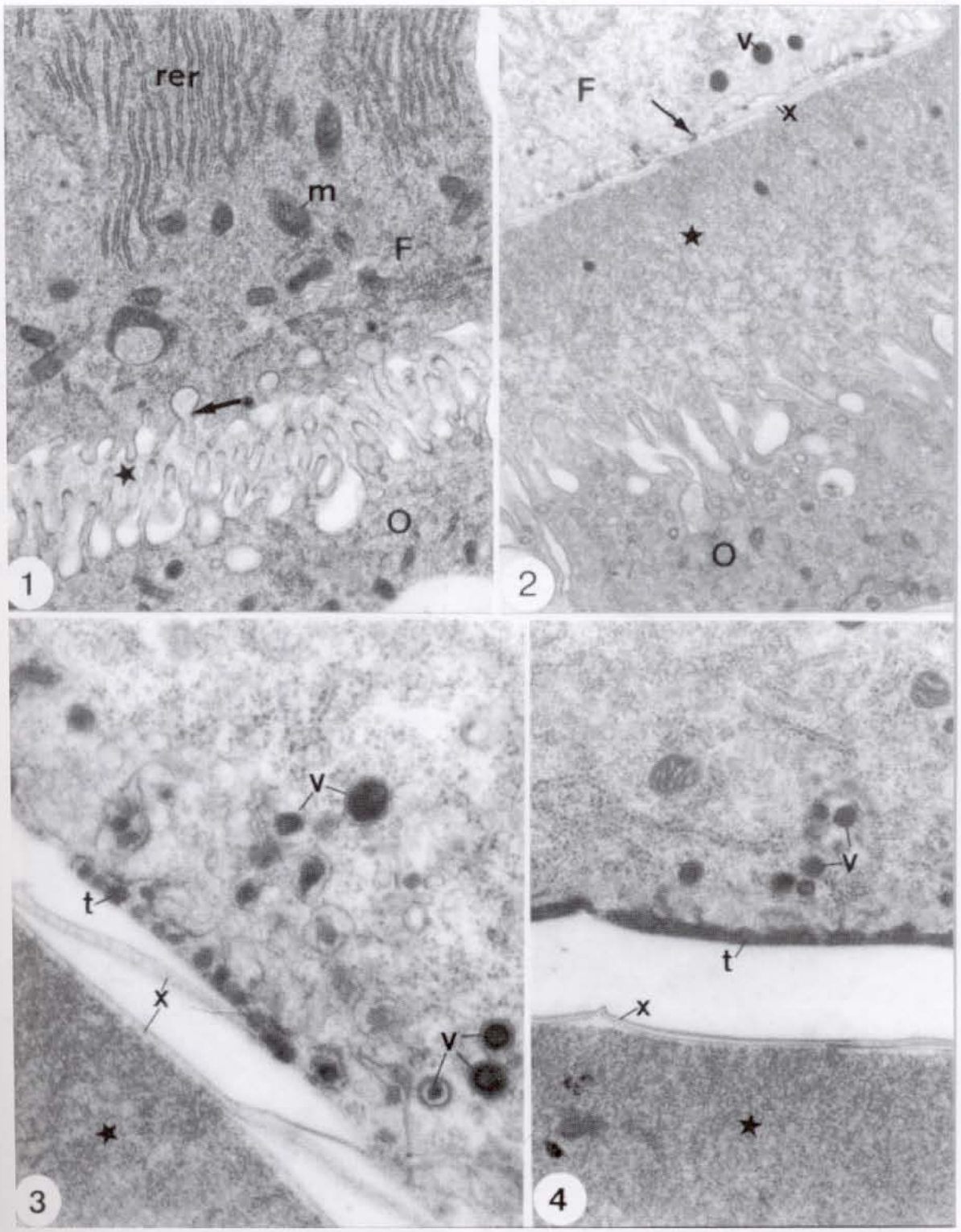


Figure 1- Vitelline membrane (★) between oocyte (O) and follicle cell (F). Flocculent material (arrow) being released by follicle cell; rough endoplasmic reticulum (rer), mitochondria (m). 21.000X. Figure 2- Wax layer (x) over the vitelline membrane (★) adjacent to the oocyte (O). Small electron dense bodies (arrow) of the trabecular layer, deposited into the space between the wax layer and the follicle cell (F), that contains electron dense vesicles (v) in the apical cytoplasm. 21.000X. Figure 3- Incomplete trabecular layer (t) formed by small dense bodies; wax layer (x) and vitelline membrane (★). Follicle cell with many electron dense vesicles (v). 50.000X. Figure 4- Trabecular layer (t) as coalescent dense bodies over the wax layer (x). Apical electron dense vesicles (v) in follicle cell cytoplasm; vitelline membrane (★). 36.000X

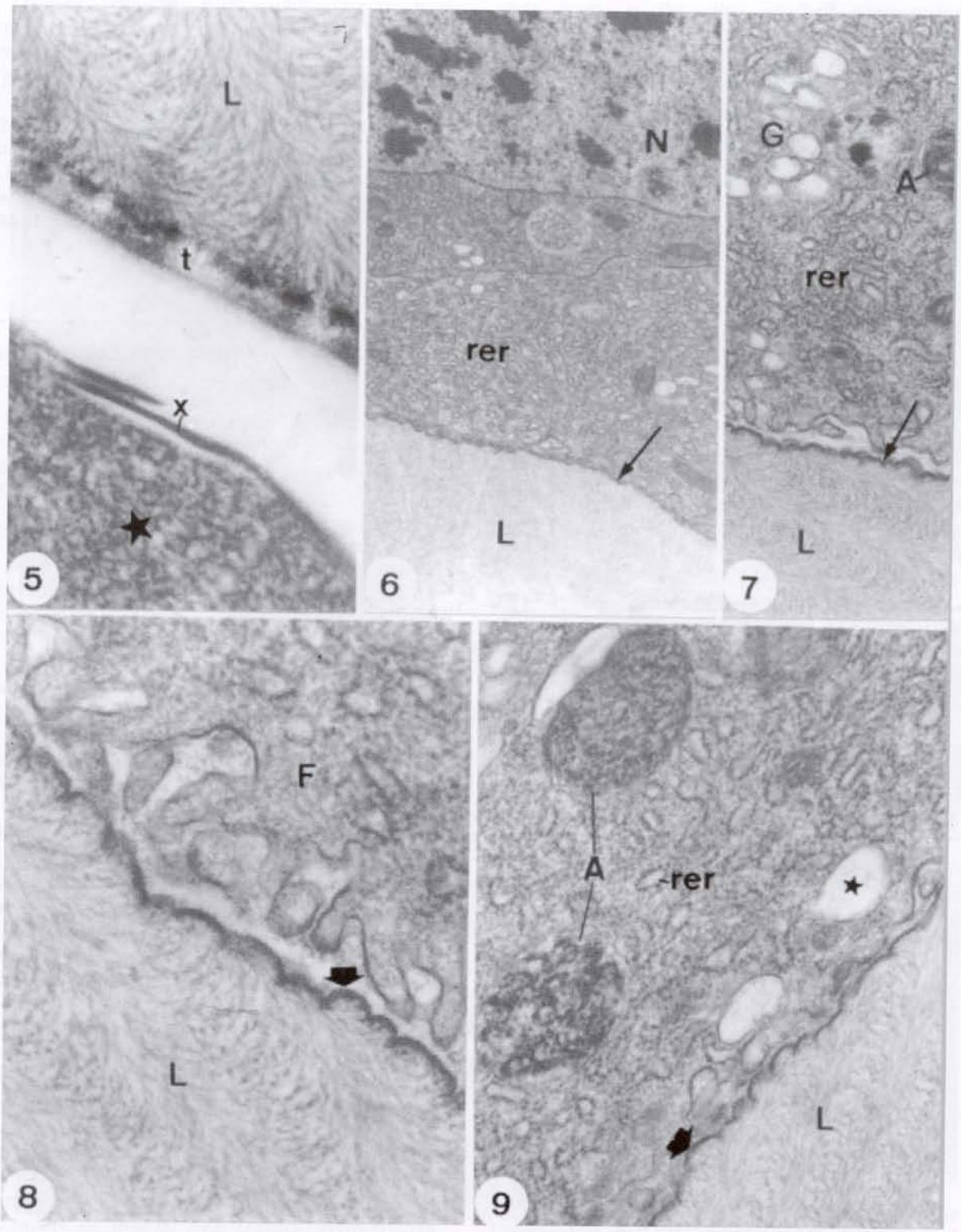


Figure 5- Complete trabecular layer (t) formed by regular spaced dense bodies. Lamellate layer (L), wax layer (x) and vitelline membrane (★). 77.500X. Figure 6- Follicle cell with vesicular rough endoplasmic reticulum (rer); nucleus (N). Lamellate layer (L) and outer osmiophilic layer (arrow). 13.250X. Figure 7- Detail of follicle cell cytoplasm exhibiting vesicular rough endoplasmic reticulum (rer), Golgi complex (G) and autophagic vacuole (A). Lamellate layer (L) and outer osmiophilic layer (arrow). 31.500X. Figure 8- Undulated outer osmiophilic layer (arrow) over the lamellate layer (L) of the eggshell. Follicle cell (F). 77.500X.Figure 9- Follicle cell cytoplasm with rough endoplasmic reticulum (rer), apical electron lucent vesicles (★) and autophagic vacuoles (A). Lamellate layer (L) and outer osmiophilic layer (arrow). 42.000X

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