ULTRASTRUCTURAL AND CYTOCHEMICAL CHARACTERIZATION OF THE OENOCYTOID IN LARVAE OF *DIATRAEA SACCHARALIS* (LEPIDOPTERA: PYRALIDAE) PARASITIZED BY THE WASP *COTESIA FLAVIPES* (HYMENOPTERA: BRACONIDAE)

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ABSTRACT

The hemocytes participate in the cellular encapsulation around foreign material implanted into the insect hemocoel. However, the suppression of this reaction by entomopathogens remains largely misunderstood. This study describes the ultrastructural modifications in one type of hemocyte of *Diatraea saccharalis* larvae, the oenocytoid (OE), along the parasitism by the wasp *Cotesia flavipes*, using conventional and cytochemical techniques. The OE of parasitized *D. saccharalis* larvae exhibited areas of cytoplasm rarefaction with intensity proportional to the time of parasitism. There was cell lyse at long-term parasitism, although we did not observe melanization either into the host haemocel or around of parasitoid. The OE of either control or parasitized larvae did not show phenoloxidase activity in the ultrastructural preparations; these absence was related with the morphological modifications caused by the parasitism. The trimetaphosphatase and acid phosphatase activities were not detected in OE of control larvae, but were visualized along the parasitism, reinforcing the role played by the OE in the defense reactions.

Key words: Ultrasctructural, oenocytoid, *Diatraea saccharalis*

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INTRODUCTION

The processes of phagocytosis and nodule-capsule formation are efficient mechanisms against invaders into the insect hemocoel, with the direct participation of the hemocytes in these reactions [12, 20, 27, 32, 34, 35, 36, 37, 42]. Parasites and pathogens, however, can develop into their host insects without an efficient defense reaction [14, 36, 37, 39]. The ability of same parasitoids to escape of the host insect immune reaction has been used in the biological control of insects that affect the agricultural production [3, 7, 36, 37, 47]. The strategies utilized by the parasites to avoid the host response have been studied and revised by many authors [2, 3, 4, 36, 37, 39]. However, there are few investigations correlating the immune response and morphology of the host insect hemocytes along the parasitoid development [15, 45, 46, 47].

The *Diatraea spp* is the major pest of the sugarcane crop; this insect may be controlled by the parasitoid wasp *Cotesia flavipes* [29, 38,48]. Six hemocytes types has been morphologically described in the hemolymph of non-parasitized *D. saccharalis* larvae: prohemocyte (PR), plasmatocyte (PL), granulocyte (GR), oenocytoid (OE), spherule cell (SP) and vermicyte (VE) [11, 17, 18, 19].

The PL and GR are the two hemocyte types that are frequently associated with phagocytosis or encapsulation of the foreign material in many insect species [14, 15, 17, 19, 20, 24]. However, very little is known about the role played by the OE in the defense reactions [9, 20, 24, 25, 35].

The prophelоloxidase (proPO) system is one of the most important component of the defense mechanisms in insects [5, 6, 22]. The phenoloxidase (PO) is the main produced enzyme, derived from the proPO by the activation of the system that involves enzymatic reaction in cascade [6]. The PO catalyses the initial stage of the melanization process observed into the hemocoel of the insects in response to the presence of strange material or injury. The OE of several Lepidoptera species contains the PO, as detected in *Galleria mellonella* [12, 13], *Bombyx mori* [1, 6, 22] and *Spodoptera litura* [23, 24]. Falleiros [17] showed positive reaction for PO in the OE of *D. saccharalis* larvae, using light microscopic preparations.

This work aims to describe the ultrastructural modifications in the OE of the *D. saccharalis* larvae along the reaction against the parasitoid *C. flavipes*, using conventional and cytochemical techniques.

MATERIAL AND METHODS

The insects *Diatraea saccharalis* and *Cotesia flavipes* were provided by the Laboratório Entomológico, Usina Barra Grande, Lençóis Paulista, S.P., Brazil. The *D. saccharalis* larvae were maintained with artificial diet [21] under controlled temperature (25-27°C) and photoperiod (14h light/10h dark), and 80% relative humidity [28].

Twelve days old *D. saccharalis* larvae were naturally infected by the wasp *C. flavipes*. After different times of parasitism (6, 72 and 144h), the hemocyte pellets were obtained by centrifugation of the hemolymph.

For conventional preparations, the pellets were fixed for 18 h in 2.5% glutaraldehyde in 0.1M phosphate buffer pH 7.3, post-fixed for 2h in 1% osmium tetroxide using the same buffer, dehydrated through a graded series of acetone and embedded in Araldite resin. Ultrathin sections were double stained with uranyl acetate and lead citrate.

For cytochemistry preparations, the hemolymph was dropped in insect anticoagulant solution (62 mM NaCl; 100 mM glucose; 10 mM EDTA; 30 mM trisodium citrate; 26 mM citric acid) [30]. The hemocyte pellet was obtained by centrifugation and was immediately fixed for 20min in 2% glutaraldehyde in 0.1 M sodium cacodylate buffer pH 7.2 (GA) at 4ºC. The fixed pellets were embedded in 2.5% Bacto-agar (DIFCO) prepared in the same buffer, cooled in ice and sliced into small fragments with a razor blade. The fragments were incubated in different solutions at 25ºC for detection of the following enzymatic activities: a)- acid trimetaphosphatase (TMPase): incubated for 10-60min in solution containing sodium trimetaphosphate, according to Berg [10]; b)- other acid phosphatases (AcPase): incubated for 60min in solution containing citydine-5-monophosphate, according to Pino *et al.* [31]; c)- phenoloxidase (PO): pre-incubated with 1% trypsin in sodium cacodylate buffer solution for 30-60min and post-incubated with L-DOPA saturated solution in sodiun cacodylate buffer for 30min-3h, according to Söderhäll & Smith [43]. Independent of the incubated solution, the materials were re-fixed in 2.5% GA for 40min, post fixed for 2h in 1% osmium tetroxide in cacodylate buffer and embedded in Araldite resin. Stained and non-stained ultrathin sections were examined under transmission electron microscope.

RESULTS

The OE of non-parasitized *D. saccharalis* larvae is a rounded cell, larger than all the other hemocyte types; scarce vesicles are observed in close association with the regular plasma membrane (Figs. 1 A-B). The nucleus is small and eccentric and shows a distinct chromatin distribution pattern, alternating loose and dense chromatin packages attached to the nuclear envelope (Figs. 1 A-B). The cytoplasm is homogenous and electron dense with few organelles, presenting abundant free ribosome (Figs 1 A-B). Mitochondria, many of them ring-shaped, are observed (Figs. 1 C). The reaction for TMPase, AcPase and PO activities were not detected in our control preparations.

The OE of *D. saccharalis* larvae parasitized by the wasp *C. flavipes* exhibited cytoplasmic areas of low electron density with intensity proportional to the time of parasitism (Figs. 1 D-E, 2 A-B); there are plasma membrane disruption and cell lyses at long-term parasitism, mainly after 144 h of parasitism (Figs 2 C-D). With 6 h of parasitism the OE presented an increase in both the number and volume of the cortical vacuoles (Figs. 1 D-E), and moderate reaction for AcPase was detected in the cytoplasmic vacuoles. With 72 h of parasitism the OE still showed the increase of the vacuoles (Fig 2 A) and heterogeneous electron-dense bodies (Fig 2 B) were observed. With 144 h of parasitism the few better-preserved OE showed moderate reaction for TMPase in the heterogeneous bodies (Fig. 2 D-detail). No PO activity was detected in the OE of *D. saccharalis* larvae along the

parasitism. Concomitant macroscopic observations of the dissected insects did not show melanization either into the host hemocoel or around the parasitoid at any time o hemocoel or around the parasitoid at any time of parasitism.

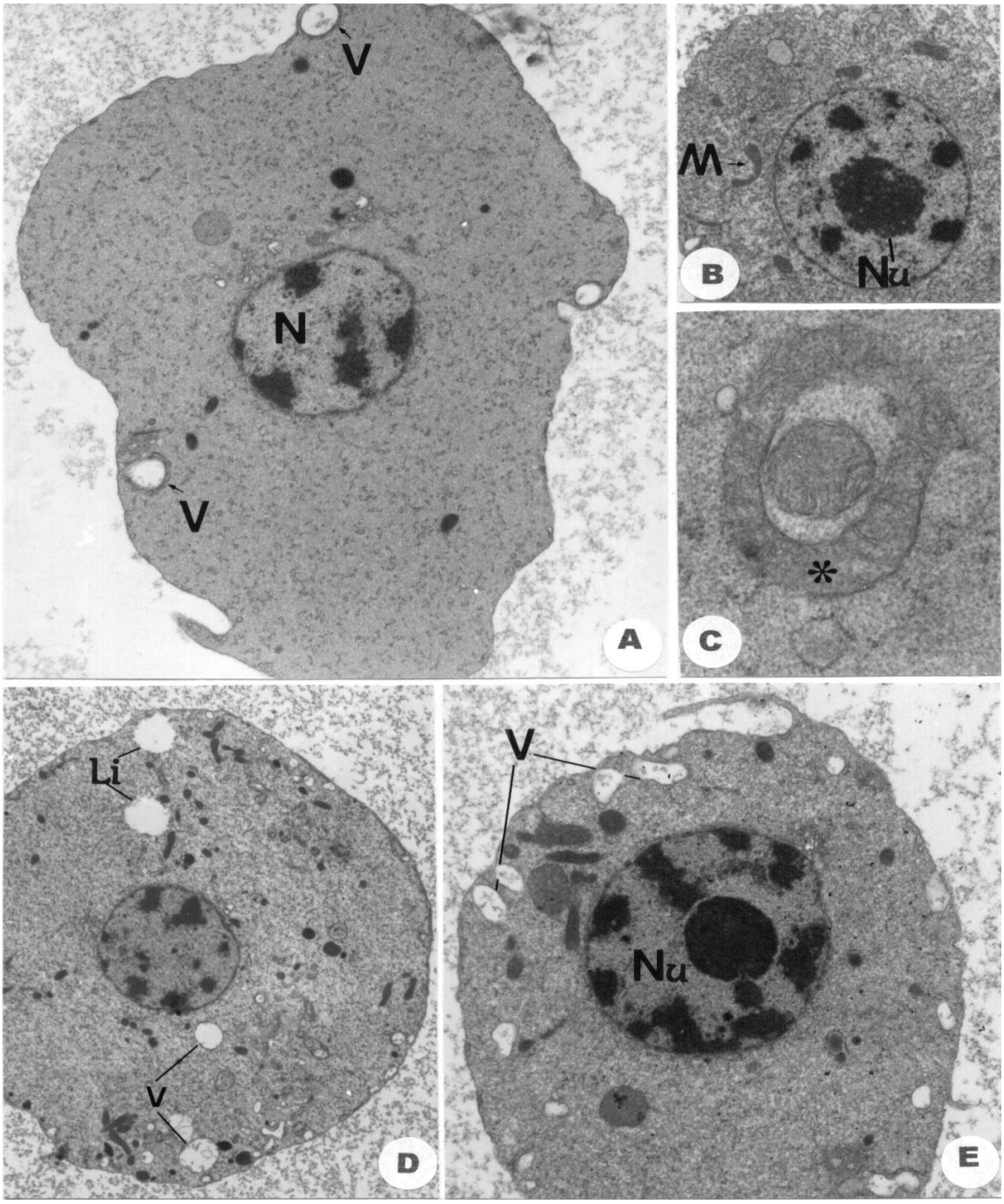

Figure 1- **A-C.** OE of non-parasitized *D. saccharalis* larvae. **D-E.** OE of *D. saccharalis* larvae after 6 h of parasitism by the wasp *C. flavipes.* A= 13.000x; B= 10.000x; C= 41.000x; D= 6.500x; E= 13.000x. Nucleus (N); vacuoles (V); nucleolus (Nu); mitochondria (M); ring-shaped mitochondria (*); lipid droplets (Li).

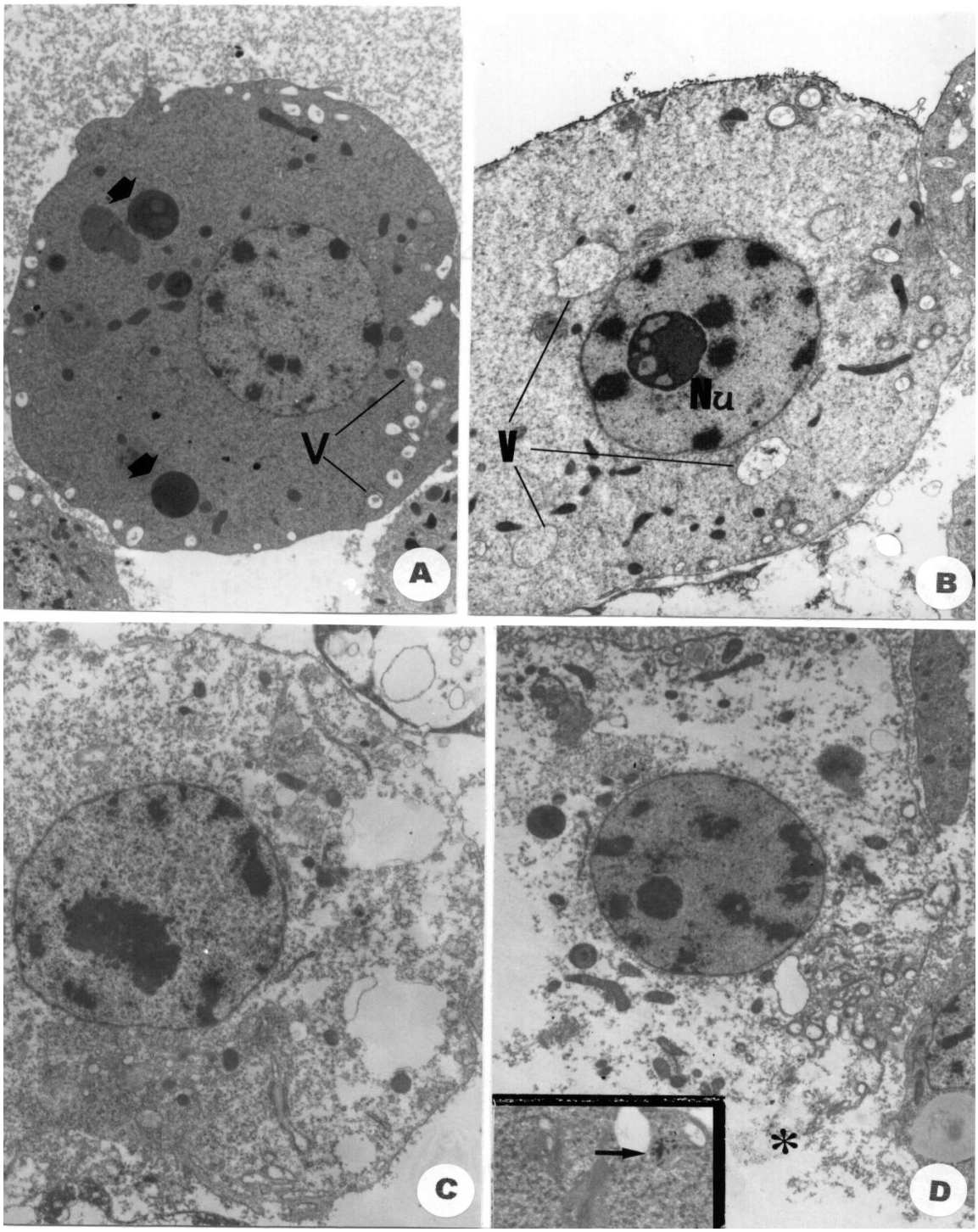

Figure 2- OE of *D. saccharalis* larvae parasitized by the wasp *C. flavipes*. **A** to **B-** 72 h of parasitism. **C** to **D-** 144 h of parasitism. A= 8.000x; B= 10.000x; C= 10.250x; D= 10.250x. Vacuoles (V); heterogeneous dense bodies (large arrow); nucleolus (Nu); plasma membrane disruption (*). Detail: positive reaction for TMPase (arrow). 14.500x.

DISCUSSION

The general ultrastructural aspects of the OE in non-parasitized *D. saccharalis* larvae are in agreement with the previous description for the same insect [11, 17, 18] as well as for many other Lepidoptera species [1, 8, 9, 16, 26, 33, 35, 41, 44].

The lyses of OE is often described in insects after different injury. Kurihara *et al* [24] observed the same phenomenon in OE of *Spodoptera litura* during an *in vivo* study of either India ink phagocytosis or latex particles encapsulation. The host *Heliothis virescens* parasitized by *Campoletis sonorensis* also exhibited lyses of OE, as described by Davies *et al* [14]. Ribeiro *et al* [35] describe the electron lucent OE as ghost hemocyte in *Mythimna unipuncta* (Lepidoptera) after injection of iron saccharate or pig red blood cells. Our results also showed lyses of OE at long-term of parasitism; however, our data were not enough to determine whether the OE disrupted to release the cytoplasm content, including the PO or if the hemocyte is affected to prevent the release of the activated enzyme. There was no activation of proPO system in parasitized *D. saccharalis* larvae as the parasitoid *C. flavipes* did not exhibit any sign of melanization inside the host hemocoel.

The proPO in insects is activated through the action of a protease cascade triggered by minutes amounts of foreign components. In most of the insects, the enzyme was found in the inactive form (proPO), in the plasma [40] or into the hemocytes [12, 28]. Falleiros [17] showed positive reaction for PO into the OE of *D. saccharalis*, under light microscopy; the reaction was detected when the hemocytes were pre-incubated with trypsin, indicating the presence of the enzyme into this cell type in the proPO form. The reaction was visualised in a diffuse way all over the cytoplasm, not being apparently confined to any cytoplasmic compartments. In our ultrastructural preparations the detection of the proPO (in the trypsin pre-treated hemocytes) or even of the PO was not successfully. The discrepancy of these results with the one for light microscope observation showed by Falleiros [17] can be credited to the difficulty for visualising a soluble enzyme in the naturally electron-dense citosol, as observed in our OE ultrastructural images.

The detection of lysosomal enzymatic activity (TMPase and AcPase) into the heterogeneous vacuoles observed in the OE of infected larvae reinforce the role played by this cell type in the defense of the insect. Falleiros & Gregório [19] showed that the PL, GR and occasionally ES and OE of *D. saccharalis* were involved in the phagocytic process of microorganisms. Although the PL and GR cells are the two types of hemocytes most often related with the defense reactions in Lepidoptera [14, 15, 17, 19], the OE was also associated with phagocytosis, showing strong lysosomal enzyme activity (AcPase and unspecific esterase) during the process [13]. Works concerning the participation of the OE in the reaction against invaders are

scarce, but these results indicate that the involvement of this hemocyte type in the immune reactions of Lepidoptera species should be more deeply investigate.

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