



RESEARCH ARTICLE

MORPHOLOGICAL DESCRIPTION OF THE SEX PHEROMONE GLAND OF *HELICOVERPA ARMIGERA* (HÜBNER) (LEPIDOPTERA: NOCTUIDAE)

¹Juliana Souza dos Santos, ¹Miryan Denise Araujo Coracini, ¹Regina Inês Kunz, ²Samuel Roggia and ¹Rose Meire Costa Brancalhão

¹State University of Western Paraná – University street, 2069, University neighborhood, Cascavel – Paraná, Brazil

²Embrapa-CNP So of Londrina Paraná - Carlos João Strass highway, Warta district, Londrina – Paraná, Brazil

ARTICLE INFO

Article History:

Received 24th March, 2017
Received in revised form
29th April, 2017
Accepted 15th May, 2017
Published online 30th June, 2017

Key Words:

Cotton Bollworm,
Morphology,
Gland Structure,
Bouin Solution.

ABSTRACT

Helicoverpa armigera (Lepidoptera: Noctuidae) is a polyphagous pest insect of wide distribution and observed in Brazil for the first time in 2013. The population control of *H. armigera* can be affected by using sex pheromones that is a product of glandular synthesis and secretion, whose location and morphology show heterogeneity according to the group. Considering that there are few studies on the glandular structure in *H. armigera*, we have analyzed the morphology its sex pheromone gland, revealing its anatomical position. For this, the insects were obtained from the Brazilian Agricultural Research Corporation - Embrapa-CNP So and were raised until adulthood. At this stage, the ovopositor apparatus of the female moths were collected and fixed, following the histological processing. The morphological analysis of *H. armigera* ovopositor apparatus revealed that the sex pheromone gland was positioned between the 8th and 9th abdominal segments. The gland was organized into a simple, continuous epithelium of the tegument and the cell format varied from pavement to slightly cubic and, depending on proximity to villi, columnar. The pheromone gland can be bulging in the "calling" period and is covered by the cuticle and hairs that aid in the spread of the sex pheromone.

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Citation: Juliana Souza dos Santos, Miryan Denise Araujo Coracini, Regina Inês Kunz, Samuel Roggia, Rose Meire Costa Brancalhão¹, 2017. "Morphological description of the sex pheromone gland of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae)", *International Journal of Development Research*, 7, (06), 13298-13301.

INTRODUCTION

The moths of the genus *Helicoverpa* (Lepidoptera, Noctuidae) are agricultural pests of world importance, being known at the moment about 80 species (Todd, 1978). Among them, the cotton bollworm, *Helicoverpa armigera*, is a widely distributed pest insect (Common, 1953; Kriticos, 2015) first observed in Brazil in 2013 (Tay et al., 2013) and establishing itself as a plague by its high capacity of polyphagia (Suzana et al., 2016), acquiring quick resistance to the country's transgenic plants (Ávila et al., 2013). Due to the growing concern to preserve the ecosystem, researchers are increasingly seeking alternative methods aiming to reduce or even replace the use of dangerous products such as pesticides (Trapé, 2003; Rangel et al., 2011).

*Corresponding author: Juliana Souza dos Santos, State University of Western Paraná – University street, 2069, University neighborhood, Cascavel – Paraná, Brazil.

Among the various ways, behavioral control is very advantageous because it aims to limit the action of pests through resources with low environmental impact, involving adequate management of natural resources, thus enabling the satisfaction of human needs without harming the environment (Junior et al., 2000; Campanhola et al., 2003; Morandi and Bettiol, 2009). This control can be done in several ways, such as the use of pheromones in monitoring traps, or for disrupting mating or in attracticide formulations (Thomson et al., 1998; Kovaleski et al., 2002; Witt, 2016). Pheromones are chemicals used in communication between individuals of the same species (Cardé and Minks, 1996). In moths (Lepidoptera) the sex pheromones are synthesized and secreted by a gland (Lofstedt and Koslov, 1997) normally reversible (Jefferson et al., 1966), formed of epithelial cells and located between the 8th and 9th female abdominal segment (Hollander and Schwalbe, 1982).

However, within the order the sex pheromone glands presents great morphological disparity (Hajibabaei *et al.*, 2006). Therefore, not all these insects have similarity in the secretory structure of sex pheromones. In this sense, and considering the scarcity of information about this insect in Brazil, the work aimed to locate and describe the tissue morphology of the sex pheromone gland of *H. armigera* females, aiming to provide subsidies for further studies about this important pest in Brazil.

MATERIALS AND METHODS

The experimental research was developed at Laboratory of Structural and Functional Biology (LABEF) and Laboratory of Agricultural Biotectonogy, both at Parana West State University - Unioeste, Cascavel, PR. The *H. armigera* larvae were obtained at the Brazilian Agricultural Research Company - Embrapa-CNP So, located in Londrina, PR, and maintained at Laboratory of Agricultural Biotectonogy at 25±2°C, 60±10% relative humidity, and a 14L:10D hr photoperiod. The larvae were separated and placed in plastic cups (5cm diameter x 6cm height), capped with a perforated cap for the air intake, and reared on an artificial agar/white bean-based diet (Parra, 1999) until they reached the pupal stage. The insects were sexed in the pupae stage, separated and were transferred to plastic containers (15 cm diameter x 30 cm height), covered with white voil attached by an elastic band, until the moment of the butterfly emergence. The adult insects were fed with a 10% honey solution (Pratissoli *et al.*, 2004). Single pheromone glands of 3-day old calling females were extracted during the scotophase, which is the period when *H. armigera* mates (Hou and Shang, 2000). The female moths were placed in Petri dishes and subjected to a decrease in temperature in the refrigerator during?? minutes, for later decapitation. After decapitation, with the aid of tweezers, the female abdomen was pressed until the ovopositor apparatus present in the last abdominal segments was protruded, sectioning the structure at the height of the intersegmental membrane between the abdomen and the 8th segment. The material was fixed in a Bouin solution (10%) during 24 hours, and after that was kept in a glass vial containing alcohol solution (70%). The fully extended and already fixed ovopositors were dehydrated and placed in Paraplast®. Semi-serial cross-sectional and longitudinal sections of 7 µm were obtained from structures stained with hematoxylin and eosin (Junqueira and Junqueira, 1983). The slides were analyzed under light microscopy and photomicrographs.

RESULTS AND DISCUSSION

The morphological analysis of the *H. armigera* ovopositor apparatus (Figure 1A) revealed that the sex pheromone gland was located in the ovopositor apparatus (OA) between the 8th and 9th abdominal segments (Figure 1C), forming an epithelial, continuous structure to the integumentary epithelium. The glandular epithelium was coated by hairs (H), and a highly chitinized cuticle (CC). Vilosities were also visualized in some regions of the glandular epithelium (Figure 2A). The pheromone secretory epithelial cells extended along the intersegmental membrane, positioned below the intersegmental cuticle, forming a "ring" of simple epithelium that surrounds the entire structure (Figure 2A). The cell format varied from pavement, slightly cubic and, depending on proximity to villi, columnar.



Figure 1. Female of *H. armigera* (A); abdomen fixed in Bouin before the ovopositor apparatus is collected (B), focus on the ovopositor apparatus (OA) (C)

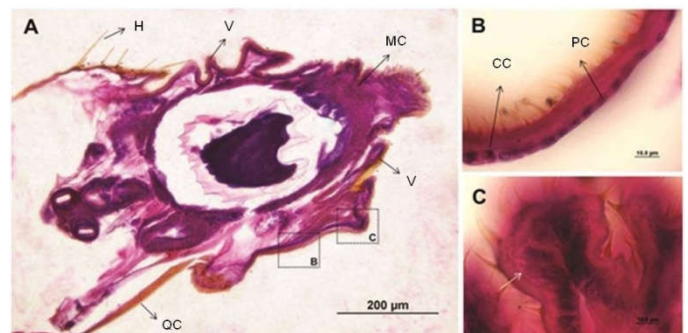


Figure 2. Longitudinal cut of female *H. armigera* ovopositor apparatus (A), where hairs (H), villi (V), cuticle (QC) and muscle cells (MC) are observed. In B, glandular epithelium showing the pavement (PC) and cuboidal (CC) epithelial cells, the cuticle and the hairs. In C, glandular epithelium in the villi, with columnar cells (white arrow). HE coloring

The morphology of the nucleus followed that of the cells, where in the pavements and cubic it was flattened (Figure 2B), and in the columnar ones the nucleus presented spherical, occupying much of the cytoplasmic volume, with more apical positioning. Internally to the epithelium there was a layer of striated muscle cells (Figure 2C). Raina *et al.* (2000) and Alstein *et al.* (2003) divide the ovopositor apparatus of *H. zea* and *H. peltigera*, in three regions: the 8th abdominal segment, the sex pheromone gland and the 9th abdominal segment. Still, Noirot and Quennedey (1974) suggest that the simpler type of gland is composed of a thin layer of epidermal cells, and that females of many Lepidoptera produce their sex pheromones by such structures and their positioning normally its in intersegmental membranes.

The same organization was found for *H. armigera* in the present study. The tissue of the glandular epithelium presented many vilosities, where the cuticle is internally perceived in the striated muscle cells suggesting that the ovopositor apparatus is retracted, having the possibility of extending when necessary. This conformation differs in other moths, such as *Cydia pomonella* (Lepidoptera: Tortricidae) where glandular cells are also continuously disposed to the epithelium and located between the 8th and 9th abdominal segments, but it is invaginated inside a body cavity, with the exterior through a duct (Barnes *et al.*, 1966). The authors verified that in this conformation of the gland, the cells of the intersegmental membrane are covered by the cuticle, which in turn is covered by hairs. The presence of the hairs on the surface acts on the release of the secretion of the adjacent columnar cells, since

the duct forms a channel of passage for the pheromone (Stobbe and Rudolf, 1911). Müller (1877) states that there is no more effective method for depositing any substance than its association with hairs pointing in all directions, thus promoting a large area of evaporation. Considering that the pheromone contains volatile compounds (De Bruyne and Baker, 2008) the presence of hairs on the cuticle probably has this function, where the insect "calling" behavior, in which the ovopositor apparatus is protracted and agitated, is related to the optimization of the pheromone emission and diffusion (Viana, 2015). Therefore, the *H. armigera* sex pheromone gland is located on the intersegmental membrane between the 8th and 9th abdominal segments and is constituted of columnar epithelial tissue. It can be bulging in the "calling" period and is covered by the cuticle and hairs that aid in the spread of the sex pheromone.

Acknowledgment

To Embrapa-CNP So-Londrina, PR for assistance affording the insects, and to Biotechnology Laboratory-Unioeste for use of rearing rooms and provide the artificial diet ingredients. This study was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

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