SALIVARY GLAND HISTOLOGY OF THE LARVA RED PALM WEEVIL, *Rhynchophorus ferrugineus* (COLEOPTERA: DRYOPHTHORIDAE)

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ABSTRACT

Red Palm Weevil (RPW), *Rhynchophorus ferrugineus* (Olivier 1790) is a very destructive phytophagous insect pest to plants under family Arecaceae. The remarkable abilities of RPW to destroy the host plant have been attributed to its feeding behaviors and the functions of the salivary gland. The study describes the salivary gland histology of the larva RPW to assist in understanding the feeding habits of the pest. The observation was done by using light microscopy of Zeiss Image Analyzer with Axio Vision and Zeiss Axioscope with iSolutionlite softwares. RPW salivary gland tissues were stained with haematoxylin and eosin stains. Our study revealed, a unique ring-shaped acinar gland that encircled the crop of the larva. The two halves of the gland are long, wide and flatten leaf-like structures. The acini found are of different sizes and consist of two cell types, the zymogen and parietal cell. The cellular features observed suggest that the zymogen cells and parietal cells play different roles in salivary gland regulations. The results also revealed that the gland is heavily tracheated which might support the active feeding of the pest. The findings of the study could aid on preliminary understanding on the vigorous feeding of RPW larva that silently killing the host plant inside out.

Keywords: Red Palm Weevils, pest, feeding

ABSTRAK

Kumbang Merah Palma (RPW), *Rhynchophorus ferrugineus* (Olivier 1790) merupakan serangga perosak fitofagus terhadap tumbuhan di bawah famili Arecaceae. Kemampuan luar biasa RPW dalam memusnahkan tumbuhan perumah berkait rapat dengan tingkah laku pemakanan dan fungsi kelenjar liur. Kajian ini memperincikan histologi kelenjar liur larva RPW untuk membantu memahami kelakuan pemakanan serangga perosak ini. Pemerhatian dijalankan dengan menggunakan mikroskop cahaya Penganalisis Imej Zeiss dengan perisian Axio Vision dan Zeiss Axioscope dengan perisian iSolutionlite. Tisu kelenjar liur RPW telah diwarnakan dengan pewarnaan hematoksilin dan eosin. Kajian mendedahkan, kelenjar acinar unik yang membentuk gelung mengelilingi tembolok pada saluran penghadaman larva tersebut. Kedua-dua belah kelenjar tersebut adalah panjang, lebar dan leper seperti struktur daun. Asinus yang ditemui mempunyai saiz yang berbeza dan terdiri daripada dua jenis sel iaitu sel zimogen dan sel parietal. Ciri-ciri sel yang diperhatikan menunjukkan bahawa sel zimogen dan sel parietal memainkan peranan yang berbeza dalam pengawalaturan kelenjar liur larva RPW. Hasil kajian juga mendedahkan bahawa kelenjar tersebut dilitupi dengan trakea

yang mungkin menyokong pemakanan aktif larva RPW. Penemuan kajian ini dapat membantu pemahaman awal tentang pemakanan aktif larva RPW yang secara senyap membunuh pokok perumahnya dari dalam.

Kata kunci: Kumbang merah palma, perosak, pemakanan

INTRODUCTION

Red Palm Weevil (RPW), *Rhynchophorus ferrugineus* (Olivier 1790), is an economically important well-known insect pest of palm worldwide. The pest has been reported to invade all continents (Ju et al. 2010; NAPPO 2010; Kaakeh 2005) and distributed most widely within tropical Asia that extends from Pakistan, through Southeast Asia to Melanesia (Faleiro 2006; Murphy & Briscoe 1999). The range expansion of this insect was then further facilitated through the growing of commercialization of coconut, oil palm and date palm within tropical Asia (Idris et al. 2014; Nirula et al. 1953).

RPW is a major pest of plants under family Arecaceae, such as dates palms, coconut, sago palm as well as oil palm. Besides, it has also been recorded on Agavaceae (century plant), and Poaceae (sugar cane) (Malumphy & Moran 2007). RPW is a threat to date industries (Abraham et al. 1998; Al-Ajlan 2008; Kaakeh 2006; Salama et al. 2009) coconut industries (Ferry & Gomez 2002; Murphy & Briscoe 1999; Sivapragasam et al. 1990) and potentially possess a threat to oil palm industries (Idris et al. 2014). Keller et al. (2011) mentioned that an invasive species like RPW could adapt to new hosts and caused serious damage. Control measures is very essential to be taken now to avoid the potential economic damages.

Larva of RPW is the main perpetrator of all the damages to the host (Wahizatul et al. 2013). The larvae live in tunnels and feed on the trunk tissues, killing the host slowly from inside without any symptoms in the early stage of the infestation (Al-Ajlan 2008; Salama et al. 2009). One of the most important organs that involves in the feeding of this pest is the salivary gland. Salivary glands are the largest exocrine organs in insect and play major role for successful feeding (Ribeiro & Francischetti 2003). The glands are associated with the mouth or oral cavity and produce secretions known as saliva that are mixed with the food during feeding and are ingested along with the food.

The biology, ecology and systematics of RPW are actively or have been well studied by other previous researcher such as Hallett et al. (2004), Malumphy & Moran (2007), (Kaakeh et al. 2001), (Rugman-Jones et al. 2013)and many others. However, there is a gap in research on the physiological and morphological studies of the pest especially on the feeding organ that mainly contributes to the feeding of the pest. Therefore, the study was done to describe the salivary glands morphology and histology of RPW larva; the main culprit that silently destroys the host plant from inside. It is expected that the study will assist in the better understanding of the insect pest active feeding behaviour.

MATERIALS AND METHODS

Insect Sampling and Larval Instar

Samples of the adult male and female weevils of RPW were collected from infested areas in Kuala Terengganu Malaysia (Table 1), through passive sampling by using pheromone traps. The collected samples were cultured in laboratory on sugarcane adopting the method of Norzainih et al (2015). The larvae were let to grow until they reached the sixth instar stage. At

this stage, the larvae were actively feeding and large enough to be dissected. The instar stage was determined by the head measurement of the larva. The mean size of the sixth larval instar's head length and width are 6.04±0.22 mm and 4.32±0.18 mm approximately (Norzainih et al. 2015).

Table 1: Coordinates of the 10 sampling sites in Kuala Terengganu

Sampling sites	Coordinates
Site 1	5°24'03.5" N 103°06'01.3" E
Site 2	5°23'37.3" N 103°06'23.1" E
Site 3	5°22'10.0" N 103°06'47.5" E
Site 4	5°21'52.4" N 103°06'54.2" E
Site 5	5°21'30.8" N 103°07'08.6" E
Site 6	5°21'05.2" N 103°06'59.6" E
Site 7	5°20'31.9" N 103°07'04.1" E
Site 8	5°20'09.4" N 103°06'25.9" E
Site 9	5°20'02.2" N 103°06'33.1" E
Site 10	5°21'00.7" N 103°06'57.8" E

Dissection

The salivary gland of 15 individuals of RPW larva was removed through dissection in phosphate buffer (PBS). The larvae were immobilized at 4℃ for five minutes prior to dissection. A medial cut was made at the dorsal of the body from the posterior segment to the anterior head. The salivary gland was removed and taken out from the body. The dissection process was done under surgical microscope Stemi 2000-C Carl Zeiss. Microphotograph of the salivary gland was taken by using Zeiss Image Analyzer with Axio Vision software. For storage purposes, the salivary gland was fixed in 10% formalin solution.

Histology

Samples for morphological and histological studies were fixed in 10% formalin solution. The tissues were washed with 70% ethanol to remove the excess fixative and put through a series of ethanol solutions (70-100%) for dehydration followed by xylene substitute. Then the tissues were embedded in wax and labelled. Blocks of waxed tissues were then sectioned (4μm) using Leica RM2245 microtome. Four to five sections were placed on a slide and dried overnight. Sectioned tissues were stained according to haematoxylin and eosin staining method. The first step of haematoxylin and eosin staining method is to put the slides with the tissue into xylene substitute solution for 15 minutes. Then the slides were put in a series of alcohol: 100%, 95%, 80% and 70%, with three minutes for each solution. Then the slides were rinsed in distilled water before being put into haematoxylin solution for five minutes. Next, the slides were rinsed in running tap water and placed in eosin solution for three minutes and dipped in 95% alcohol four times before being put in 100% alcohol solution for two minutes. The final step in staining was to put the slides in xylene substitute solution. Lastly, the slides were mounted and microphotography was observed by light microscopy using Zeiss Axioscope with iSolutionlite software.

RESULTS

The salivary complex of the RPW larva consists of a unique ring-shaped of an acinar gland. The right and left halves of the gland appear as long and wide and flatten leaf-like structures

that form a loop around the anterior part of the crop (Figure 1). The two halves connected to a main duct at the middle of the loop. The loop of the acinar gland is about 10.14 ± 0.94 mm in circumference and 0.32 ± 0.07 mm to 1.03 ± 0.14 mm width (wider at the proximal region) and the mass is about 0.48 ± 0.88 mg (n = 10). The observation on the morphology of the salivary gland revealed that the gland is heavily tracheated (Figure 1).

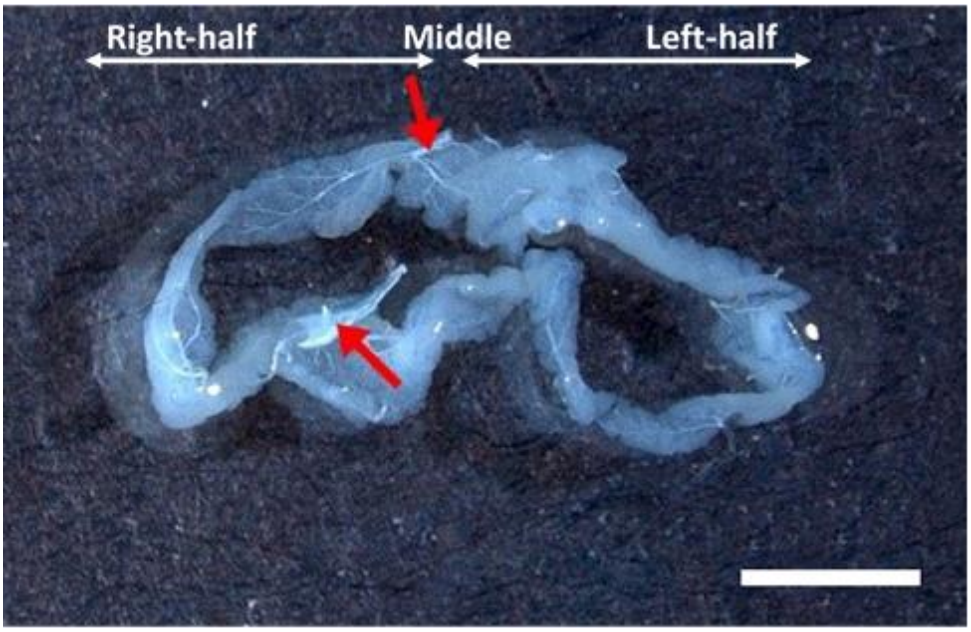

Figure 1 the salivary gland of the larva of RPW. Red arrows show the tracheal system. Scale bar = 1.0 mm

Based on the histology results, the acini of the gland are of different sizes (Figure 2a and 2b). They were connected via nerve-like salivary duct that joint to the main duct. The main duct open in the mouth cavity to secrete all the secretory materials. The lumen is lined by a thin cuticle and the entire gland is covered by basal lamina or the outer extracellular thin sheath (Figure 2d). There are two types of cell found in the acinar gland of the larva; the zymogen and parietal cells. The zymogen cells are cells with the large central nucleus that typically found in the central part of the acini (Figure 2b). They are more compact but less symmetrical in shape and stains more intensely compared to the parietal cells. The nucleus of the zymogen cells observed is spherical or oval and predominance with condensed chromatin and the cytoplasm contain numerous granular structures (Figure 2c). The parietal cells on the other hand, are typically found on the peripheral part of the acini. Some parietal cells observed were binucleated with the nuclei usually found at the basal part of the cell (Figure 2c). The parietal cells appear to be smaller and rounder or oval. The chromatin in the nucleus of the parietal cells are less condensed compared to the zymogen cells.

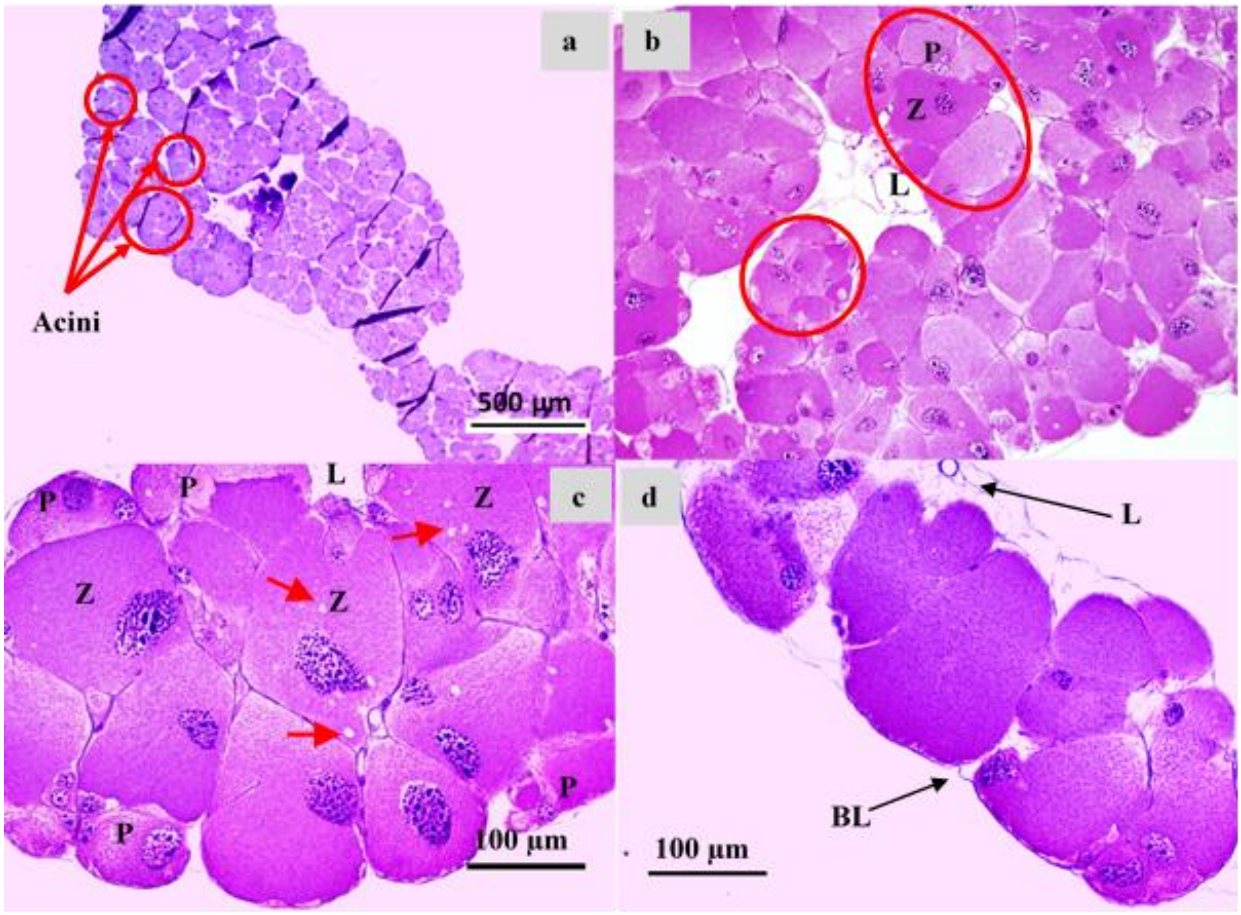

Figure 2 Tissue sections of the salivary gland of RPW larva. **(a)** the acini of the gland are of different size. **(b)** the zymogen cell (Z) found in the central of the acinus (red circle) near to the lumen (L) while the parietal cell (P) located in the peripheral. **(c)** zymogen and parietal cells with the condensed chromatin in the nuclei and granules in the cytoplasm (red arrow). **(d)** the gland was covered by a membrane or basal lamina (BL).

DISCUSSION

The ring-shaped acinar gland structure of the RPW larva was the first to be found in insects. The morphology of the acinar gland of the phytophagous larva is different from the other phytophagous insects. The acinar gland of insects usually found as a pair gland that branched from the common duct with the acini structures resemble the appearance of a bunch of grapes. That kind of structures were found in yellow winged grasshopper *Gastrimargus musicus* (Wahida & Cooper 2014) and cockroach *Periplaneta americana* (Baumann et al. 2004; Juz and Walz 1996;). The salivary gland of the RPW larva however, lack the bunch-grapes acini appearance. Instead, the acini were attached to one another to form a wide flatten leaf-like structures that encircled the anterior crop of the digestive tract.

Besides that, the acinar gland of the RPW larva was also different from the salivary gland of the adult weevil. The adult RPW possesses a tubular salivary gland (Norzainih et al. 2016; Nurul Hidayah et al. 2013) with one cell thick layer of epithelium. The tubular gland of the adult is much simpler compared to the complex acinar gland of the larva. Moreover, the gland of the larva is heavily tracheated suggesting that the gland is in need of direct oxygen

supply. This helps to explain the vigorous feeding of the larva which the most destructive stage throughout the RPW developmental growth.

Based on the gland cellular morphology, the gland of the larva is not much different from those acinar glands found in other insects. The acini were made up from two types of cells; the zymogen cell and parietal cell. The similar type of cells could be found on the acini of the cockroach *Periplaneta americana* (Just & Walz 1996) and the yellow winged grasshopper *Gastrimargus musicus* (Wahida & Cooper 2014).

The zymogen cells in the acini of the gland were found to be predominant with large nucleus and condensed chromatin, suggesting the occurrence of high metabolic activity of the cells. Li et al. (2007) stated that the nuclear mass increased in relation to the increment of the metabolic activity in the cytoplasm. The high metabolic activities observed may related to the salivary enzymes production. Numerous secretory granules were found in the cytoplasm of the zymogen cells, further suggest that the cells may involve in the protein-production activities. Just & Walz (1996) in their research on cockroaches, reported that the central cells are densely packed with secretory granules and produce protein-rich material for the gland.

Contrary to the zymogen cells, the parietal cells are smaller in size with less condensed chromatin in the nucleus and less granular structures in the cytoplasm. This indicates the occurrence of low metabolic activity in the parietal cells. Baumann et al. (2004) reported that the parietal cells of *Periplaneta americana* responsible for water and electrolyte transport which has totally different roles with zymogenic cells that secretes salivary enzymes.

CONCLUSION

The morphology of the salivary gland of RPW larva is different from the adult weevils. Larva of RPW possess an acinar gland with a ring-shaped morphology that encircles the anterior crop of the alimentary tract. The heavily tracheated gland shows that it supports the active feeding of RPW during larval stages. Two types of cells, zymogen and parietal cells described in this study could probably play different roles in salivary gland regulations based on the location, size, features and structure of the cells. However, further studies need to be done to confirm the role of these cells to RPW larva salivary gland.

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