

Research Article

Histology and histochemistry of *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) fat body during the post embryonic development

Histología e histoquímica del cuerpo graso de *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) durante el desarrollo postembrionario

Gloria Valeria Vaca^{*}, Adriana Azucena Michel¹ and Franco José Pucci Alcaide¹

¹Instituto de Morfología Animal, Dirección Zoológica, Fundación Miguel Lillo. Miguel Lillo 251, (4000) San Miguel de Tucumán. Tucumán, Argentina. E-mail: gvvaca@lillo.org.ar

ZooBank: [urn:lsid:zoobank.org:pub:7A7FB77D-D468-41F2-B8BC-A334D85A895E](https://doi.org/10.35249/rche.45.4.19.03)
<https://doi.org/10.35249/rche.45.4.19.03>

Abstract. *Phyllocnistis citrella* (Stainton), is one of the most worrying pests in the citrus growing sector. Insects' fat body is an important tissue where numerous metabolic processes take place; its significance can be clearly seen in its main functions, i.e., assimilation and oxidation of substances so as to obtain energy and generate tissue; beside, it is the main storage source of fats and carbohydrates. The objective of the present work is to analyse its histological and histochemical changes during the post embryonic development of this species. The study material was collected in a lemon growing area free from pesticides. This material was fixed in specific solutions so as to keep proteins, lipids and carbohydrates and was stained with Hematoxylin-Eosin, Schiff Periodic Acid, Nile Blue, Sudan III and Sudan Black. During larval development, the trophocyte cytoplasmatic drops have a lipidic nature. In the prepupa stage, a decrease of lipidic drops and an increase of basophilic granulations and mild PAS positivity in the trophocyte cytoplasm were observed, indicating the beginning of the synthesis and storage of proteins and carbohydrates. Pupa's fat body accumulates protein granules with low content of glycogen. The lipid content decreases with a predominance of acid lipids. Changes in the fat body chemical composition during the post-embryonic development of *Phyllocnistis citrella* are related to lysis of larval tissues and the formation of adult structures during metamorphosis.

Key words: Citrus miner, larval development, metamorphosis, morphology, trophocytes.

Resumen. *Phyllocnistis citrella* (Stainton) es una de las plagas que más ha preocupado al sector citrícola. El cuerpo graso de los insectos es un importante tejido en el cual ocurren procesos metabólicos, como la utilización de sustancias absorbidas por el intestino, su asimilación y oxidación para la obtención de energía y génesis de tejidos, siendo también la principal fuente de reserva de grasas y carbohidratos. El objetivo del presente trabajo es analizar los cambios histomorfológicos e histoquímicos durante el desarrollo postembrionario de la especie. Las muestras obtenidas de quintas sin aplicación de insecticidas se fijaron en soluciones específicas para la conservación de proteínas, carbohidratos y lípidos. Se coloreó con Hematoxilina-Eosina, Ácido Peryódico Schiff, Azul de Nilo, Sudan III y Sudan Black. Durante el desarrollo larval se identifican trofocitos de aspecto vacuolar con contenido lipídico. En prepupa se observa disminución de vacuolas lipídicas, aumento de granulaciones basófilas y leve PAS positividad en el citoplasma de los trofocitos, confirmando síntesis y almacenamiento de proteínas y carbohidratos. En la pupa el cuerpo graso acumula gránulos proteicos con un escaso

Received 30 August 2019 / Accepted 2 October 2019 / Published online 16 October 2019
Responsible Editor: José Mondaca E.

contenido de glucógeno. El contenido lipídico disminuye predominando lípidos ácidos. Los cambios en la composición química del cuerpo graso durante el desarrollo posembrionario de *Phyllocnistis citrella* están relacionados con la lisis de tejidos larvales y la formación de estructuras del adulto durante la metamorfosis.

Palabras claves: Minador de los cítricos, desarrollo larval, metamorfosis, morfología, trofocito.

Introduction

Phyllonictis citrella (H.T. Stainton), the “citrus leaf miner”, is one of the most worrying pests in the citrus growing sector. Damage caused by this species during its larval development entail cuticle tearing, and both leaf and sprout furling, which, in turn, lead to drying, necrosis and fall of leaves, with the ensuing loss of its photosynthetic capacity. In addition, the miner acts as cancrrosis amplifier, augmenting disease incidence and severity (Heppner 1993; Garrido Vivas and Gascón López 1995; Urbaneja *et al.* 1998; Goane *et al.* 2007; Furman 2013).

The citrus miner oviposits preferably on young growing leaves. When feeding on cell juices, larvae separate epidermis from parenchyma, and as *P. citrella* three larval stages take place, the winding galleries become more visible. Toward the end of its development, the larva goes toward the edge of the leaf and reaches the stage of prepupa. During this post embryonic development stage, the insect does not feed but builds a chamber; it covers the inner walls of the mine with silk threads, which shrink while drying, causing the edge of the leaf to furl. Thus, a protection structure, or pupal chamber, is built for the insect to go through the pupa stage, during which the processes of metamorphosis take place (Urbaneja *et al.* 1998; Asplanato 2009; Vaca and Michel 2016).

Insects’ fat body is an important tissue where numerous metabolic processes take place. Its significance can be clearly seen in its main functions, i.e., assimilation and oxidation of substances so as to obtain energy; besides, it is the main storage source of fats and carbohydrates (Chapman 1969).

The main cells of the fat body in insects are the trophocytes. However, other cell types, such as urate cells or urocytes, mycetocytes and the chromatocytes, can be associated with them. This tissue is distributed all over the body of the insect. Although it is frequently found scattered over the abdomen, most authors identify the parietal fat body between the tegument and the inter-segmentary muscles and the perivisceral fat body in contact with the midgut and the ovaries (Chapman 1998; Paes de Oliveira and Cruz-Landim 2003; Roma *et al.* 2010).

Holometabolous insects have an intense feeding period in their immature or larval state, a period in the post embryonic development during which the nutrients are consumed particularly to meet the insect’s needs, while the rest is stored in the fat body. This organ is mainly responsible for metabolite exchange and nutrient storage, with cells having an intense bio synthetic activity producing lipids and carbohydrates, as well as proteins liable to be stored or released to haemolymph (Marques-Silva *et al.* 2003).

Moreover, histological changes take place in holometabolous insects in the metamorphosis during the pupa stage. These changes involve both histolysis and histogenesis of various tissues, for instance, the histolysis process is partial in various Coleoptera, whereas trophocytes experience total histolysis in some superior Hymenoptera and Diptera. In this case, the adult’s fat body is formed by cell differentiation of imaginal discs. In Lepidoptera, the parietal fat body, which synthesizes proteins during the larval stage, goes through histolysis, whereas the cells in the perivisceral fat body, which stores proteins, persist in the adult’s fat body (Snodgrass 1935; Cruz-Landim 1983; Wigglesworth 1984; Gillot 1995; Chapman 1998).

Considering the importance the fat body has in the development of insects, especially in holometabolous insects, it is the aim of the present study to analyse the histological changes this tissue goes through during *Phyllocnistis citrella* post embryonic development. Through a histochemical analysis, the present study seeks to determine the nature of the molecules synthesized and stored by trophocytes; showing, the direct incidence of the fat body on the development of this species, has been determined.

Materials and Methods

The material for the present study was obtained from leaves collected at a lemon field, *Citrus limon* (L.), during the months of March-May. During this period, the temperature values favourable for the oviposition and development of the different cycle stages of the species were registered. The growing field was under trimming and path-cleaning conditions at all times, and no insecticides were applied in order to control the miner.

Sampling was performed every ten days and was done as follows: ten trees were selected randomly in different parts of the sampling area, both in the centre and in the boundaries of the field. A tender shoot was taken from each tree, put inside a plastic bag each and taken to the lab. Once there, identification and classification of specimens in different stages during the post embryonic development was performed to process them with routine histological techniques.

The larvae, prepupae and pupae used in the histo-morphological study were fixed in Bouïn (saturated aqua solution of picric acid, pure formalin and acetic acid 70: 25: 5) for 24 hours. They were dehydrated in an ethanol ascending battery and kept in n-butylic alcohol. Due to the small size of the samples, they were soaked in agar-paraplast twice. A series of 6 μm -thick cuts were made both frontally and sagittally by using a Zeiss HM325 manual rotary microtome and stained with Hematoxiline (Erich)-Eosine (HE).

The specimens to be used in the histochemical study were soaked in specific fixing solutions so as to keep proteins, lipids and carbohydrates. The material used for protein and carbohydrate identification was fixed in Bouïn, kept in n-butylic alcohol and stained with Hematoxiline (Erich)-Eosine (HE), Periodic Acid Schiff (PAS) and PAS-Hematoxiline (PAS-H). For lipid identification, Formol-Calcium (Baker) was used. 4 o 5 μm -thick frozen section were made by using a Carl Zeiss Criostate (cryostat) Model Hyrax C25, and stained with Sudan Black (SB), Sudan III (SIII) and Nile Blue (NB).

The identification of specimens in different stages was performed by using a Leica EZ4 simple stereoscopic microscope. The cell-tissue observations were made on a Leica DM2000 high-resolution photonic-trinocular microscope and the results obtained were illustrated with microphotos taken with a digital camera added to the Leica ICC 5 HD microscope, software Leica LAZ V4, 12.

Results and Discussion

Larval stages. *Phyllocnistis citrella* is an insect with an endophytic habit, which lives and feeds on the leaves of citrus tender shoots (Vargas *et al.* 1998; Valarezo *et al.* 2004). According to Snodgrass (1935), the fat body synthesizes and stores different types of energy substances, by using the nutritional supplies stored during the larval development.

Although the fat body has been considered a homogeneous tissue, various types of fat bodies have been identified in different species of insects. In the studies on this tissue made in larvae belonging to several Lepidoptera species, two types of fat body for this order have been mentioned, both defined according to different criteria. Thus, *Plodia interpunctella* Hübner (Shirk and Malone 1989) shows one dark-colored fat body located in the posterior part of the abdomen, and another whitish one in its anterior part; whereas

Helicoverpa zea (Boddie) (Haunerland and Shirk 1995) presents a parietal fat body in the anterior part of the abdomen, its color being lighter than that of the previsceral when treated with hematoxiline-eosine. In *Anticarsia gemmatalis* Hübner (Carvalho *et al.* 2013), however, two fat bodies, one parietal and one perivisceral, can be observed. The former is underneath the tegument and beside the digestive tube, with an irregular distribution between the thorax-abdomen regions, and the latter is beside the digestive tube. During the larval development of *Phyllocnistis citrella* (Vaca and Michel 2016) two types of fat bodies are clearly differentiated: 1) a parietal fat body, clearly visible since the first larval stage, with a well-defined location between the epidermis and the abdominal muscular fibers; and 2) a visceral fat body which starts to be differentiated in the larval third-instar as small trophocyte masses surrounding the digestive tube scattered randomly.

For the present study, the observations were made by means of histomorphological techniques; they showed that in *Phyllocnistis citrella*'s first-instar larvae, the trophocytes present a rounded nucleus in central position and a uniform basophilic cytoplasm; in the second and third instar the nucleus of these cells has an irregular shape and the vacuolated cytoplasm (Figs. 1-4).

Moreover, the analysis performed based on histomorphological techniques on samples chosen throughout *P. citrella* second and third instar larval shows that the content of the trophocyte cytoplasm, which are not stained when treated with hematoxiline-eosine, has a lipidic nature.

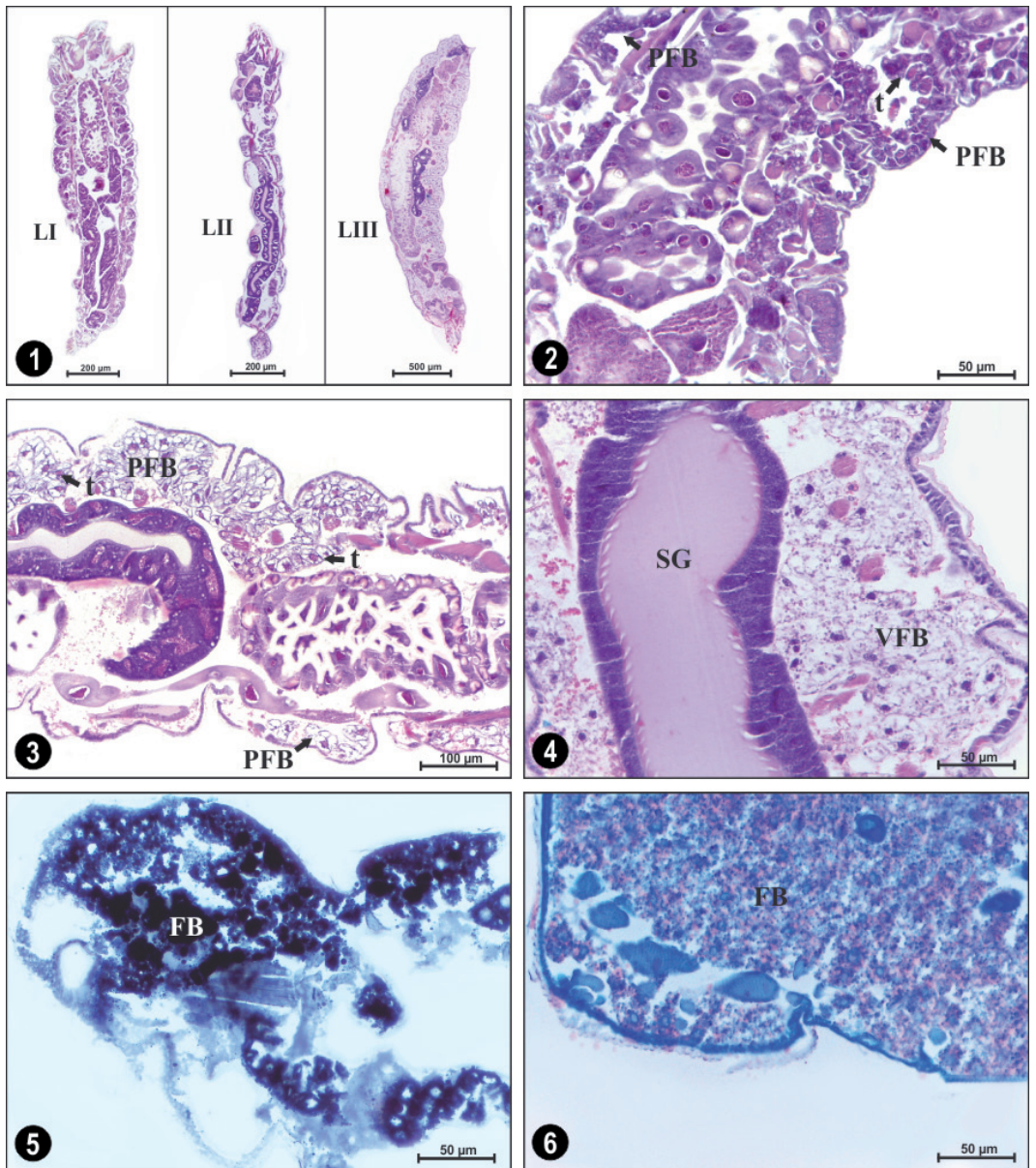
The presence of big amounts of lipidic drops in the trophocyte cytoplasm in the larval third instar is accounted for through the Black Sudan technique (Fig. 5). Moreover, the positive reaction to Nile Blue reveals the presence of both neutral and acid lipids in the fat body. The neutral lipids with a higher concentration are stored in small drops surrounded by acid lipid granules of different sizes (Fig. 6).

This predominance of neutral lipids at the end of the larval development in *Phyllocnistis citrella* indicates a higher synthesis and storing of triglycerides which are the main source of energy during the nonactive period of metamorphosis. Gejage and Awate Manisha (2009), in their study on lipase activity in the *Leucinodes orbonalis* Guenée larva fat body, suggest that, from 6-8-day larval age, a gradual increase in the fat body lipase activity is observed. This indicates that this period of larval development is most active and requires maximum energy, which is supplied through triglycerides catabolism for its active life.

Various authors have observed significant differences in lipid accumulation of the fat body in relation to sex; Yurkiewicz (1969) observed in his study that *Plodia interpunctella* male larvae have a slightly higher neutral lipid content than females. These results would indicate a sexual lipidic dimorphism in this species, whereas Subramanyam and Cutkomp (1987) have determined that *P. interpunctella* male larvae have a significantly higher amount of lipids than females, while no differences between the sexes of *Cadra cautella* Walker larvae were observed. This author suggests that the differences in the total amount of lipidic content in the body between the sexes and species are related to both physiological and metabolic differences between the species. In the present study, the results show that there were no significant differences in the fat body lipidic components during the larval development of the male and female *Phyllocnistis citrella*.

Regarding the substance stored in the fat body during the larval development, it must be said that various authors have identified a significant content of carbohydrates in the trophocyte cytoplasm during the larval development of other insect species, as the studies by Cruz-Landim (1983) have demonstrated. This author found glycogen in trophocyte cytoplasm in *Melipona quadrifasciata anthidioides* Lepelletier second instar larvae. While analysing the fat body in *Thyrinteina arnobia arnobia* (Stoll) larvae, the Marques-Silva *et al.* (2003) team identified carbohydrates in trophocytes of third instar larvae, fed with

Eucalyptus cloeziana F. Muell., as well as in sixth instar larvae fed with *Psidium guajava* L.; Zara and Caetano (2004) perceived a strongly positive reaction to PAS in the cytoplasm of *Pachycondyla villosa* (F.) larva trophocytes. Conversely, during *Phyllocnistis citrella* larval development, a positive response to PAS was not observed in the fat body.



Figures 1-6. *Phyllocnistis citrella* larvae. 1. Sagittal and frontal section larval instar I, II, III. Hematoxylin-Eosin technique (HE). 2. Larva I: Parietal fat body (PFB) with trophocytes (t) showing irregular shape and basophilic cytoplasm with few droplets. (HE). 3. Larva II: Parietal fat body (PFB) made up of masses of trophocytes (t) with abundant cytoplasmic droplets. (HE). 4. Larva III: Spongy visceral fat body (VFB) in contact with the silk glands (SG). (HE). 5. Larva III: Fat body (FB) with big cumuli of Sudan Black positive lipidic droplets. Sudan Black technique (SB). 6. Larva III: Fat body (FB) with high concentration of neutral lipidic droplets surrounded by acid lipid granules of different sizes. Nile Blue technique (NB).

Prepupa. Histomorphological techniques demonstrated that at the beginning of *Phyllocnistis citrella* prepupa stage, the boundaries between parietal and visceral fat body cannot be clearly differentiated in the abdominal region due to the significant volume of trophocytes (Fig. 7). These boundaries are easily identified during the larval development. In this former stage trophocytes show basophilous cytoplasm with droplets of different sizes that respond positively to the histochemical technique used for the identification of lipids.

The Sudan Black technique used for the lipid identification in *Phyllocnistis citrella* prepupa indicates that the lipid content in the fat body decreases slightly in comparison with that of the larval third instar (Fig. 8). The Blue Nile technique shows that the abdominal fat body has a homogeneous appearance where the acid lipid concentration is significantly higher than the neutral lipid content. The observation suggests predominance in the synthesis and storage of the phospholipids which will constitute the cell membrane of the histogenetic structures (Fig. 9). Regarding this issue, various authors such as Gilbert and Chino (1974), Ünler (1988), Arrese and Soullage (2010) and Zhang and Xi (2014) suggest that neutral lipids, mainly triglycerides, constitute 90% of the fat body lipidic droplets; they are also the main source of energy, a function that is essential for holometabolous, which must keep a minimal amount of nutrients during larval stage to survive during both their starvation period during metamorphosis.

In a further phase during the prepupa stage, *Phyllocnistis citrella* fat body appears as isolated masses, made up of three or four trophocytes, whose cytoplasm shows small basophilous granules and few lipid droplets (Fig. 10). The striking feature in these cells is the strongly basophilous, irregularly-shaped, granular-looking nucleus with chromatin in different degrees of condensation and acidophilic nucleoli. Both the presence of these granules in the cytoplasm and the features of the nucleus would point toward a greater development in the rugged endoplasmic reticulum at this stage of the prepupa. This would indicate the beginning of synthesis of both the proteins and membrane materials of secreting vesicles in the trophocytes.

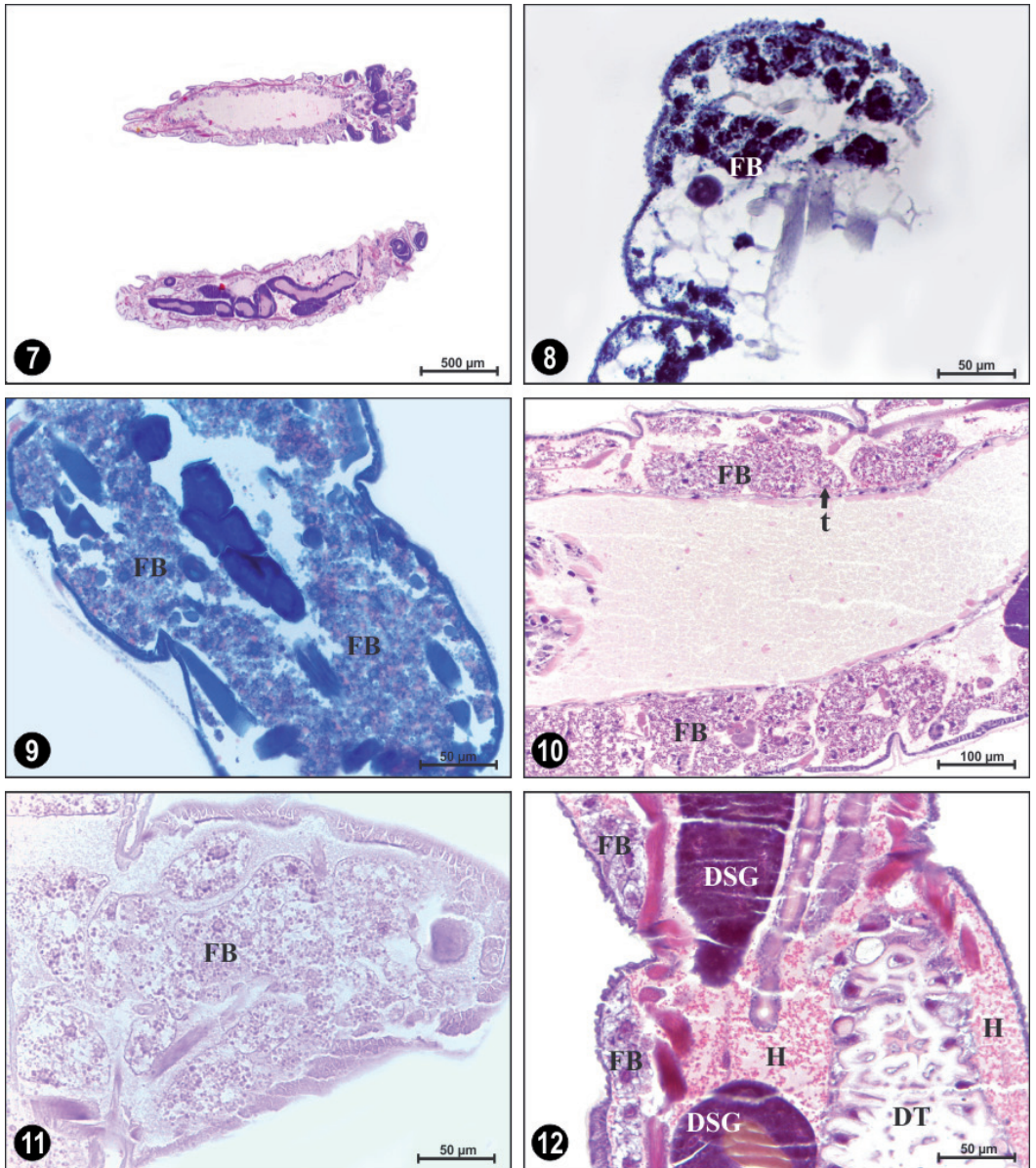
The decrease in the number of lipidic droplets in the trophocyte cytoplasm would imply that the drops that were stored during the larval development are being used in the morphogenesis processes which started in the prepupa stage to build new structures during metamorphosis of this species. Gakhar and Maleyvar (1985); in their study on content variation in carbohydrates, lipids and proteins during *Trabala vishnou* (Lefèbvre) ontogeny, they determined that the lipidic content diminishes during the prepupa stage. Basing their observations on those made by Fast (1964), the former suggest that this phenomenon is a trait in holometabolous insects, where the energy necessary for pupa transformation derives from lipids accumulated during the larval stages.

As was mentioned by Wigglesworth (1972), Gakhar and Maleyvar (1985), Chapman (1998), Paes de Oliveira and Cruz-Landim (2003), Roma *et al.* (2010), apart from lipids and proteins, the fat body also stores carbohydrates, mainly in the form of glycogen, this being a significant source of energy for insects and other animals. In several insects, carbohydrates appear during feeding periods, and are then used in the chitinic synthesis of the new cuticle or are turned into storing carbohydrates such as trehalose and glycogen. A light PAS positive response in the trophocyte cytoplasm located next to the digestive tube is observed in *Phyllocnistis citrella* during the prepupa stage. This would indicate the beginning of synthesis and the ensuing carbohydrate storage (Fig. 11).

At the end of the prepupal stage, very few small acidophilic granules appear dispersed in the trophocyte cytoplasm for the first time during *P. citrella* post embryonic development. The results would indicate the beginning of the protein synthesis and storage in the fat body (Fig. 10).

Vaca and Michel (2016) in their study on cellular-tissue variations during *Phyllocnistis citrella* third instar larvae showed that the haemolymph in the abdominal cavity presented numerous intensely acidophilic granules. For the present study, an increase both in the number and size of acidophilic granules of the haemolymph can be observed in specimens fixed at end of prepupal stage. These granules surround the fat body, the digestive tube and the distal silk gland in the

haemocoel (Fig. 12). This increase of acidophilic granules in the haemolymph would indicate an increase of precursors for the synthesis of the fat body proteins, and would coincide with the start of protein synthesis and storage in the trophocyte cytoplasm during *Phyllocnistis citrella* prepupa stage.



Figures 7-12. *Phyllocnistis citrella* prepupae. 7. Sagittal and frontal section prepupae. Hematoxylin-Eosin technique (HE). 8. Abdominal fat body (FB) with scarce Sudan Black positive lipid droplets in the trophocyte cytoplasm. (SB). 9. Abdominal fat body (FB) with a significantly high acid lipidic concentration. (NB). 10. Abdominal fat body (FB) showing masses of trophocytes (t) with numerous basophilous granules and few lipidic droplets. (HE). 11. Fat body (FB). Posterior region showing trophocytes with numerous slightly PAS-positive granules. Periodic Acid-Schiff technique (PAS). 12. Haemolymph (H) with acidophilic granules surrounding the fat body (FB), digestive tube (DT) and distal silk gland (DSG). (HE).

Pupa. Metamorphosis of holometabolous insects involves the breakdown of larval tissues and the histogenesis of adult structures. During this stage, the insect development depends on what was accumulated during larval growth for both energy and the provision of anabolic precursors. Lipids and carbohydrates may contribute to the energy expenditure of pupa, depending on the species and sex of the insect under study. Besides, the lipidic composition of the fat body also changes, especially free fatty acids and cholesterol. Some authors suggest that the fatty acids stored in the fat body, produced by the triglyceride hydrolysis, serve a number of purposes, including the provision of energy for flight muscles, for ovary lipids, and the overall maintenance of the metabolic activity of other tissues (Downer and Mattehews 1976; Arrese and Soulage 2010; Cerkowniak *et al.* 2015).

In specimens fixed at the beginning of the *Phyllocnistis citrella* pupa stage, an increase in the number of small acidophilic granules, as well as an increase in the size of the trophocytes of the entire abdominal cavity, can be observed (Fig. 13). In further phases during the pupa stage, the trophocytes of the anterior abdominal region present clear cellular boundaries. In addition to that, their cytoplasm shows protein granules equal in quantity and distribution to those observed in the initial phase of this stage (Fig. 14); in the middle and posterior region of the abdomen, however, the fat body lacks clear cellular boundaries, and its cytoplasm is filled with intensely acidophilic numerous protein granules of different sizes. The large granules in these trophocytes, which probably stem from a coalescence of small granules, show a central strongly acidophilic region (Fig. 15).

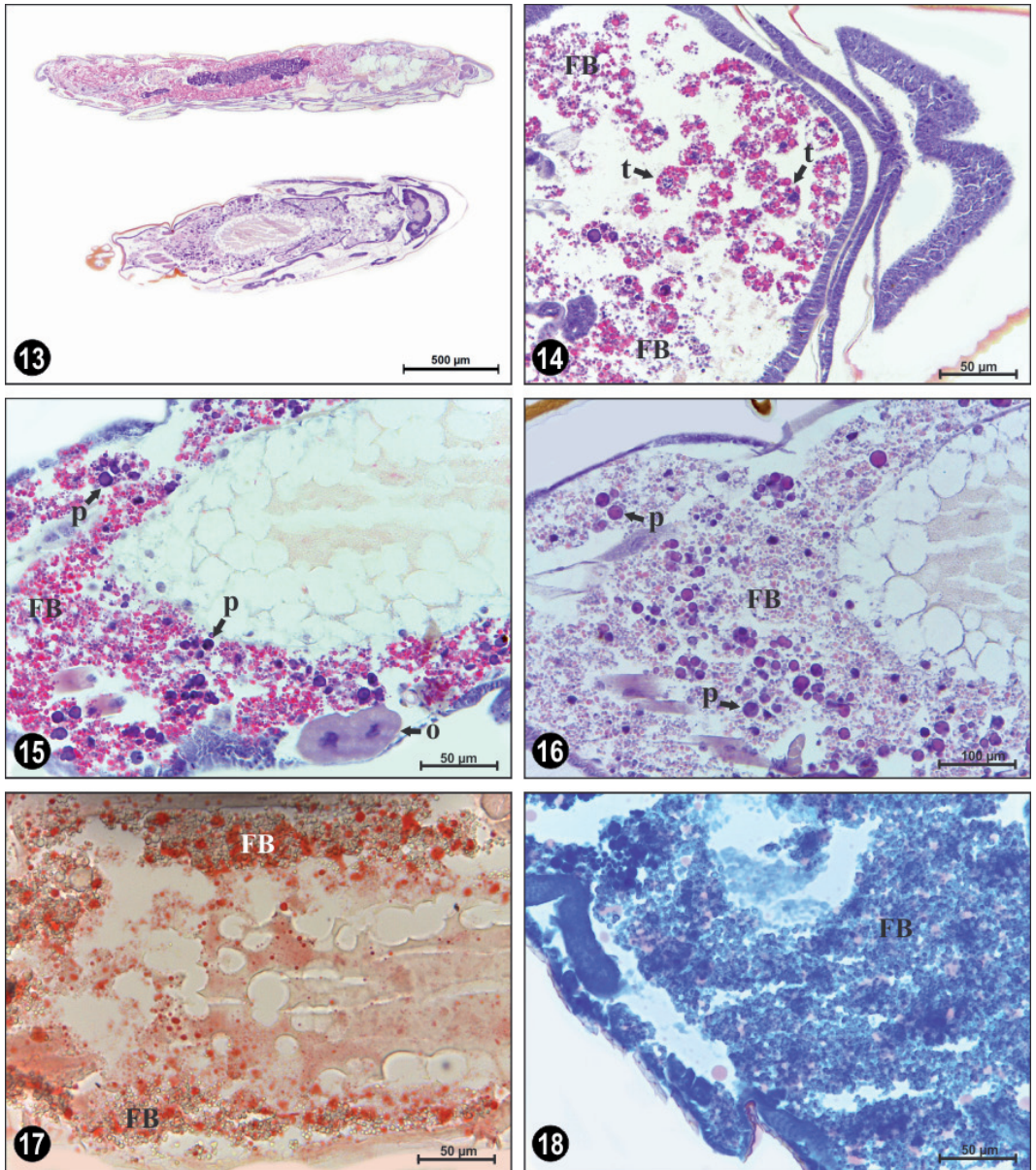
This progressive increase of the protein granules in the trophocytes during of pupa stage would indicate that at this stage of post-embryonic development, an active synthesis and storage of the cytoplasmic proteins take place in these cells.

Locke and Collins (1965) identified two different sorts of protein granules in the fat body of *Calpodes ethlius* (Stoll) pupae, one containing only protein and one containing proteins and RNA. According to these authors, the first appearance of the protein granules coincides exactly with the time when the cuticle is digested by the fluids segregated by the epidermis during molting. This suggests that the protein granules in the fat body derive from the protein of the cuticle. They also put forward that the time when the protein-RNA granules first appear coincides with the formation of pupal epicuticle. The observations made by Locke and Collins (1965) and the results obtained with the histomorphological and histochemical techniques used in the present work show that during the *Phyllocnistis citrella's* pupa stage, the fat body stores protein granules with a low content of glycogen. In this species, the protein granules are synthesized from precursors stored in the haemolymph during the prepupa stage and are used for the various processes during the metamorphosis.

Cruz-Landim (1983) mentioned that, at the beginning of the prepupa stage in *Melipona quadrifasciata anthidioides*, the trophocytes lack clear boundaries and Wang and Haunerland (1992) remarked that at the end of the larval stage of *Helicoverpa zea*, the plasmic membrane is partially destroyed. The present work deals with *Phyllocnistis citrella*, whose trophocytes lack the plasmic membrane boundaries at the end of the pupa stage. Wang and Haunerland (1992), as well as Paes de Oliveira and Cruz-Landim (2003), suggest that in the pupa stage, the lysosomal activity in the fat body, releases the supplies accumulated during the larval phase in order to keep the provision of energy for this transformation phase.

Among the cell-tissue changes in *Phyllocnistis citrella* fat body towards the end of the pupa stage, some small cells become visible scattered among the fat body granules in the abdominal cavity; their shape is varied, with some strongly basophilous cytoplasmic areas and ovoid nucleus slightly basophilous (Fig. 15). These cells are easily identified through the hematoxilline-eosine technique. According to Snodgrass (1935), Wigglesworth (1955, 1956) and Chapman (1998), these cells would belong to a type of haemocytes called plasmatocytes o amoebocytes, which are frequently found in the holometabolous insect haemolymph, mainly during metamorphosis, having a phagocytary function. By using the PAS technique, some

cytoplasmic areas appear as slightly PAS positive, which would indicate a low carbohydrate content of these cells (Fig. 16). According to the results obtained in the present study, the plasmatocytes present in the haemolymph at the end of the *P. citrella* pupa stage would be responsible for the trophocytes plasmatic membrane phagocytosis.



Figures 13-18. *Phyllocnistis citrella* pupae. 13. Sagittal and frontal section pupae. Hematoxylin-Eosin technique (HE). 14. Fat body (FB). Anterior abdominal region with spherical trophocytes (t) and cytoplasm filled with intensely acidophilic granules. (HE). 15. Fat body (FB). Median region with numerous intensely acidophilic granules and numerous strongly basophilous plasmatocytes (p). Oenocytes (o). (HE). 16. Fat body (FB). Median region with PAS-positive granules and big intensely basophilous plasmatocytes (p). Periodic Acid-Schiff technique-Hematoxylin (PAS-H). 17. Fat body (FB). Median region with Sudan III-positive lipidic droplets. Sudan III technique (SIII). 18. Fat body (FB). Median region with increase in the lipidic acid concentration related to neutral lipids. (NB).

The histochemical technique used for the carbohydrate identification in *Phyllocnistis citrella* pupae indicates a slight reaction to PAS in both small and medium protein granules; however, a stronger positive reaction to PAS, even more remarkable in the granule central part, is seen in large protein granules. The results in these trophocytes cytoplasmic granules would indicate that they contain carbohydrates besides proteins (Fig. 16). The results obtained through the Sudan III technique, indicate that the lipidic content in *P. citrella* fat body during the pupa stage decreases considerably regarding the larval and prepupal stages (Fig. 17). In addition, the results obtained through the Nile Blue technique suggest that an increase in the acid lipid concentration related to neutral lipids takes place during the pupa stage (Fig. 18).

Conclusions

The study performed on *Phyllocnistis citrella*, it can be confirmed that, in this species, the larval fat body's function is mainly focused on both the synthesis and storage of lipids. In the prepupa stage, the lipid content decreases and protein synthesis and storage in the trophocyte cytoplasm begins. While, the fat body of pupa shows few lipids with a predominance of acidic lipids and abundant granules of proteins with low glycogen. The results obtained through the histomorphological and histochemical techniques show that changes in cell tissue in the fat body during the *Phyllocnistis citrella* post embryonic development are closely related to larval histolysis and tissue reconstruction during metamorphosis.

Acknowledgments

We would like to thank Mr. Jose Rodriguez for allowing us to carry out the sampling in his lemons field at Tafi Viejo, Tucumán. Luisa Montivero helped with the English text. The present study was funded entirely by Foundation Miguel Lillo (Tucumán, Argentina).

Literature Cited

- Asplanato, G. (2009)** Reconocimiento, daño e importancia económica. In: Asplanato G, Amuedo S, Bao L, Buenahora J, rubio L. El minador de la hoja de los cítricos, *Phyllocnistis citrella* (Lepidoptera: Gracillariidae): Bioecología y control biológico. Montevideo, Uruguay: Asplanato G. *Serie FPTA-INIA*, 24: 13-22.
- Arrese, E.L. and Soulages, J.L. (2010)** Insect fat body: energy, metabolism, and regulation. *Annual Review of Entomology*, 55: 207-255.
- Carvalho, R.R.B., Goulart de Andrade, F., Levy S.M., Moscardi, F. and Ferreira Falleiros, Â.M. (2013)** Histology and ultrastructure of the fat body of *Anticarsia gemmatalis* (Hübner, 1818) (Lepidoptera: Noctuidae). *Brazilian Archives Biology and Technology*, 56(2): 303-310.
- Cerkowniak, M., Ostachowska, A., Slocinska, M., Rosinki, G., Stepnowski, P. and Golebiowski, M. (2015)** The influence of hormones on the lipid profile in the fat body of insects. *Investment Survey Journal*, 12: 225-232.
- Chapman, R.F. (1998)** The Insects, Structure and Function. Cambridge University Press, Cambridge, 770 pp.
- Chapman, R.F. (1969)** The Insects, Structure and Function. Elsevier, New York, 891 pp. 16: 733-745.
- Cruz-Landim, C. (1983)** O corpo gorduroso da larva de *Melipona quadrifasciata anthidioides* Lep. (Apidae: Meliponinae). *Naturalia*, 8: 7-23.
- Downer, R.G.H. and Matthews, J.R. (1976)** Patterns of lipid distribution and utilization in insects. *American Zoologist*, 16: 733-745.

- Fast, P.G. (1964)** Insect lipids. A review. *Memoirs of the Entomological Society of Canada*, 37: 1-50.
- Furman, N. (2013)** Desarrollo de alternativas biotecnológicas para la obtención de plantas de *citrus sinensis* (L) Osbct resistentes a la enfermedad de la cancrrosis de los cítricos. Tesis Doctoral. Buenos Aires: UBA Fac.de Cs Exac. y Nat. https://digital.bl.fcen.uba.ar/download/tesis/tesis_n5346_Furman.pdf
- Gakhar, S.K. and Maleyvar, R.P. (1985)** Ontogenetic variations in carbohydrates, lipid and protein contents in *Trabala vishnou* Lef. (Lepidoptera: Insecta). *Proceedings of the Indian National Science Academy*, B51: 461-467.
- Garrido Vivas, A. and Gascón López, I. (1995)** Distribución de fases inmaduras de *Phyllocnistis citrella* Stainton, según el tamaño de la hoja. *Boletín de sanidad vegetal. Plagas*, 21: 559-571.
- Gejage, R.M. and Awate Manisha, R.A. (2009)** Lipase activity in the fat body of developing larva of *Leucinodes orbonalis* (Guenee). *Journal of Cell and Tissue Research*, 9(2): 1849-1850.
- Gilbert, L.I. and Chino, H. (1974)** Transport of lipids in Insects. *Journal of Lipid Research*, 5: 439-456.
- Gillot, C. (1995)** Entomology. 2nd edn. Plenum Press, New York, 798 pp.
- Goane, L., Salas, H., Casmuz, A.S., Lazcano, J.M., Zapatiel, S.A. and Willkin, E. (2007)** Fluctuación poblacional del minador de la hoja de los cítricos y su parasitoides exótico *Ageniaspis citricola* en la provincia de Tucumán, Argentina. *Revista Industrial y Agrícola de Tucumán*, 84(2): 9-18.
- Hauerland, N.H. and Shirk, P.D. (1995)** Regional and functional differentiation in the insect fat body. *Annual Review of Entomology*, 40: 121-145.
- Heppner, J.B. (1993)** Citrus leafminer, *Phyllocnistis citrella* in Florida (Lepidoptera: Gracillariidae: Phyllocnistinae). *Tropical Lepidoptera*, 4(1): 49-64.
- Locke, M. and Collins, J.V. (1965)** The structure and formation of protein granules in the fat body of an insect. *Journal of Cell Biology*, 26: 857-884.
- Marques-Silva, S., Gomes de Oliveira, H., Holtz, A.M., De Almeida Sarmiento, R. and Serrao, J.E. (2003)** Fat body morphology of *Thyrintina arnobia arnobia* (Lepidoptera: Geometridae) larvae in function of two alimentary sources. *Revista Chilena de Entomología*, 29: 105-110.
- Paes de Oliveira, V.T. and Cruz-Landim, C. (2003)** Morphology and function of insect fat body cell: a review. *Biociências*, 11(2): 195-205.
- Roma, G.C., Bueno, O.C. and Camargo-Mathias, M.I. (2010)** Morpho-physiological analysis of the insect fat body: a review. *Micron*, 41: 395-401.
- Shirk, P.D. and Malone, C.C. (1989)** Regional differentiation of fat bodies in larvae of the indianmeal moth, *Plodia interpunctella*. *Archives of Insect Biochemistry and Physiology*, 12: 187-199.
- Snodgrass, R.E. (1935)** Principles of Insects Morphology. Ed. McGraw-Hill, New York, 667 pp.
- Subramanyam, B.H. and Cutkomp, L.K. (1987)** Total lipid and fatty acid composition in male and female larvae of indian-meal moth and almond moth (Lepidoptera: Pyralidae). *The Great Lakes Entomologist*, 20(2): 99-102.
- Üner, N. (1988)** Lipid composition of the fat body, haemolymph and muscle in *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) larvae. *Communications Faculty of Sciences University of Ankara, Serie C*. 6: 147-157.
- Urbaneja, A., Llacer, E., Hinarejos, R., Jacas, J. and Garrido, A. (1998)** Sistema de cría del minador de las hojas de los cítricos, *Phyllocnistis citrella* Station y sus parasitoides *Cirrospilus* próximo a *Lyncus* y *Quadrastichus* sp. *Boletín Sanidad Vegetal. Plagas*, 24: 787-796.
- Vaca, G.V. and Michel, A.A. (2016)** Morfología del desarrollo larval de *Phyllocnistis citrella* (Lepidoptera: Gracillariidae) en cultivos de citrus de Tucumán. *Acta Zoológica Lilloana*, 60(2): 148-169.

- Valarezo, O., Cañarte, E. y Navarrete, B. (2004)** Distribución, bioecología y manejo de *Phyllocnistis citrella* Stainton en Ecuador. INIAP-PROMSA. Estación Experimental Portoviejo, Manual N.º 62, 50 pp.
- Vargas, H., Bobadilla, D., Jiménez, M. and Vargas, H. (1998)** Algunas características biológicas del minador de los cítricos, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae: Phyllocnistinae) observadas en el valle de Azapa, I region, Chile. *IDESIA* (Chile), 15: 65-75.
- Wang, Z. and Haunerland, N.H. (1992)** Fate of differentiated fat body tissues during metamorphosis of *Helicoverpa zea*. *Journal of Insect Physiology*, 38(3): 199-213.
- Wigglesworth, V.B. (1955)** The role of the haemocytes in the growth and moulting of an insect, *Rhodnius prolixus* (Hemiptera). *Journal of Experimental Biology*, 32: 649-63.
- Wigglesworth, V.B. (1956)** The haemocytes and connective tissue formation in an insect, *Rhodnius prolixus* (Hemiptera). *Quarterly Journal of Microscopical Science*, 97: 89-98.
- Wigglesworth, V.B. (1972)** The principles of Insect Physiology- 7th Ed. London, Chapman and Hall, 827 pp.
- Wigglesworth, V.B. (1984)** The principles of insect Physiology, 8 th Ed London: Chapman and Hall, London, 191 pp.
- Yurkiewicz, W.J. (1969)** Sexual dimorphismo in neutral lipid metabolism in the indian-meal moth, *Plodia interpunctella* (Hubner). *Ohio Journal of Science*, 69(2): 70-73.
- Zara, F.J. and Caetano, F.H. (2004)** Ultramorphology and histochemistry of fat body cells from last instar larval of the *Pachycondyla* (=Neoponera) *villosa* (Fabricius) (Formicidae: Ponerinae). *Brazilian Journal Biology*, 64(3B): 725-735.
- Zhang, Y. and Xi, Y. (2014)** Fat body development and its function in energy storage and nutrient sensing in *Drosophila melanogaster*. *Journal of Tissue Science & Engineering*, 6: 141. Doi:10.4172/2157-7552.1000141.