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ABSTRACT

The relative weight of haemolymph was determined in the full grown larvae of *Alabama argillacea*. The haemocytes were classified and differentially counted. They fall in 9 types: Proleucocytoids, plasmatocytes, adipocytes, oenocytoids, cystocytes, podocytes, spherocytes, rhegmatocytes, and degenerating cells.

The interrelationships between these types were discussed.

INTRODUCTION

Haemolymph is the only tissue in the body cavity of insects which exists in a liquid form. The appearance, structure and composition of this tissue vary greatly from one species to another and also, within the same species according to habitat and season (BABERS, 1938).

The haemolymph has many functions such as phagocytosis, immunity, protection from metazoan parasites, connective tissue formation and blood coagulation, and plays an important role in intermediary metabolism (WIGGLESWORTH, 1959; RIZKI, 1953; TAUBER, 1940; MULDREW, 1953; SALT, 1956; JONES, 1954 and YEAGER & MUNSON, 1942).

The classification of the different types of haemocytes is rendered difficult by the diversity of forms encountered in different insect species, the variation in the histological features of the blood cells within the same insect and by the varying techniques used in fixation and staining (WIGGLESWORTH, 1959 and YEAGER, 1945). YEAGER (1945) recognized 10 classes of haemocytes containing 32 distinct morphological cell types in the larva of *Prodenia eridania*. On the other hand, JONES (1954), ARNOLD (1952), DENNELL (1947), SOLIMAN & SOLIMAN (1958), WIGGLESWORTH (1959) and EL-MINSHAWY et alii (1973) recognized only 3 or 4 classes which do not necessarily correspond from one author to another.

JONES & TAUBER (1951) using heat-fixed *Tenebrio* larvae (55°C. 2 min.) obtained an average count of 48,000 cells per mm³ of haemolymph with a standard deviation of 17,000. In unfixed larvae, the values were significantly less.

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Recently, there is a tendency to utilize the haemocyte complex in the classification of insects (ARNOLD, 1972a and 1972b).

The present paper reports on the relative weight of haemolymph and the haemocyte count, as well as the description of nine types of blood cells (haemocytes) and their relative proportions in the full grown larva of *Alabama argillacea*, one of the most important pests of cotton plants.

MATERIALS AND METHODS

The larvae used in this study were reared at room temperature and relative humidity and were fed fresh tender leaves of cotton plants. Adult moths were supplied with 20% sugar solution.

To determine the relative weight of haemolymph, 50 larvae were etherized and externally dried with filter paper before weighing. The weighed larvae opened along a mid-ventral line, haemolymph was removed from the body cavity by absorbing with filter paper and then the larvae were reweighed. The difference between the original weight and the weight after absorption of haemolymph corresponds to the approximate weight of the latter.

The Neubauer slide technique was used to determine the haemocyte count (cells per mm³ of haemolymph) utilizing 35 larvae.

For classification and determination of the different proportions of all types of haemocytes, 70 larvae were heat-fixed by immersion in water at 60°C. for minutes. The fixed blood was obtained by piercing the base of the third thoracic leg and smearing the blood on a slide. After air drying, the cells were fixed with methyl alcohol for 2 minutes and stained with Giemsa, for 5 minutes.

RESULTS AND DISCUSSION

A. Relative weight of haemolymph:

The mean percent contribution of haemolymph in last instar larvae of *Alabama argillacea* was found to be 12.3% of the body weight. This corresponds to a total haemolymph weight of 0.424 g. Maximum of 17.7% and minimum of 8.3% were registered, as shown in Table 1.

TABLE 1 - Relative weight of haemolymph in the full grown larvae of *Alabama argillacea*.

Weight	Entire larva (g.)	Weight of Haemolymph (g.)	% of haemolymph by weight
Maximum	0.521	0.092	17.7 %
Minimum	0.433	0.039	8.3 %
Average	0.482 ± 0.0017	0.057 ± 0.0016	12.3 ± 0.07 %

B. Haemocyte count:

In heat-fixed larvae, an average count of $21,723 \pm 944$ cells per mm^3 of haemolymph was obtained, with a maximum of 40,010 and a minimum of 12,750 cells per mm^3 .

According to BURSELL (1970), the total number of haemocytes in insects, principally during moulting, may reach levels higher than 50,000 cells per mm^3 . CORNWELL (1968) reported that the number of haemocytes in *Periplaneta fuliginosa* varied from 15,000 to 60,000 cells per mm^3 with an average of 30,000; while in *Tenebrio* larvae the average was $48,000 \pm 17,000$ (JONES & TAUBER, 1951).

C. Types of haemocytes and their proportions:

In this study, nine types of haemocytes were identified in the last larval instar of *Alabama argillacea* (Figure 1). The major characteristics of each type are given here:

1. Proleucocytoids:

HOLLANDE (1911) and WIGGLESWORTH (1967) assigned the term "Proleucocytes" to this type of haemocytes, while YEAGER (1945) used the term "Proleucocytoids" and subdivided this category according to size into minute microcytes and larger proleucocytes.

These are small cells ranging in shape from round to oval. The cytoplasm is always basophilic with fine colourless vacuolization. They have relatively large round nuclei with a small amount of cytoplasm. Mitotic forms were observed in this type. This type, according to WIGGLESWORTH (1967) and ROMOSER (1973), may give rise to all the other types of haemocytes.

It was found that 31.49% of the total number of haemocytes were proleucocytoids in the last larval instar of *A. argillacea*.

2. Plasmatocytes:

The same term was used by HOLLANDE (1911), YEAGER (1945) and WIGGLESWORTH (1967), while GUPTA (1969) grouped the proleucocytoids with the plasmatocytes.

The cells of this type were found to be ovoid, round or elongated, with an eosinophilic nucleus round or ovoid. These cells were characterized by the presence of cytoplasmic inclusions as well as variable proportions of vacuolized basophilic cytoplasm. According to YEAGER (1945), who divided this group into 4 divisions, the cytoplasmic inclusions are mucopolysaccharide in nature. Mitotic forms were encountered in this type.

These plasmatocytes, in larvae of *A. argillacea*, represented 18.84% of the total number of haemocytes.

3. Adipocytes:

These cells range in shape from round to ovoid. They also have fine droplets of lipids and granular inclusions in the cytoplasm. These cells showed a weak basophilic reaction.

About 22.17% of the total number of haemocytes fell into this type.

4. Oenocytoids:

HOLLANDE (1911), WIGGLESWORTH (1967) and ROMOSER (1973) applied the same term, while YEAGER (1945) used the term "Oenocyte-like cells" for this cell type.

The cells of this type range from medium to large in size with relatively small round nuclei. They are characterized also by the greater amount of homogenous cytoplasm, which varies from basophilic to slightly eosinophilic.

It was found that 7.89% of the total number of haemocytes were oenocytoids.

5. Spherocytes:

This type was identified by HOLLANDE (1911) as "Granular leucocytes", by WIGGLESWORTH (1967) as "Spherule cells" and by YEAGER (1945) as "Spheroidocytes", subdividing it into 4 divisions.

In the last larval instar of *A. argillacea* this type of cells was characterized by a round or ovoid form with a great quantity of basophilic cytoplasm. The cytoplasm contained fine colourless vacuolization and eosinophilic inclusions. Some mitotic forms were observed in this type.

This type of blood cells represented only 3.41% of the total haemocyte number.

6. Podocytes:

ROMOSER (1973) applied the same name, while YEAGER (1945) subdivided it in two divisions.

These cells, which represented only 1.89%, were thin, flat and elongated with cytoplasmic extensions. The cytoplasm showed a weak basophilic reaction and a colourless vacuolization, while the nucleus was relatively small and acidophilic.

7. Cystocytes:

YEAGER (1945) applied the same term, subdividing this group into 4 divisions.

These cells are round or ovoid with a great quantity of a weak basophilic cytoplasm, containing a variable number of eosinophilic inclusions (Cysts, according to YEAGER, 1945). The nucleus is relatively small, and eosinophilic. Some mitotic forms were observed in this type also.

About 5.52% of the total haemocyte number were found to be cystocytes.

8. Rhegmatocytes:

YEAGER (1945) considered this category as a subdivision of "Eruptive cells".

These cells, which represented only by 4.81%, were found to be polymorphic in form. The cytoplasmic eosinophilia was weak. The cytoplasm showed a very fine vacuolization. The nucleus was eosinophilic, and the cell walls were sometimes indistinct. Mitotic forms were observed in this type.

9. Degenerating cells:

This type was found to compose 3.67% of the total number of haemocytes in the last larval instar of *A. argillacea*. It was difficult to describe accurately the features of these cells because of their nature as degenerative parts of all the previous types of cells. This fact was also reported by YEAGER (1945).

According to YEAGER (1945), the interrelationships of the different types of haemocytes indicated that the spherocytes, plasmatocytes and oenocytoids are derivable from proleucocytoids, while podocytes and cystocytes are derivable from plasmatocytes.

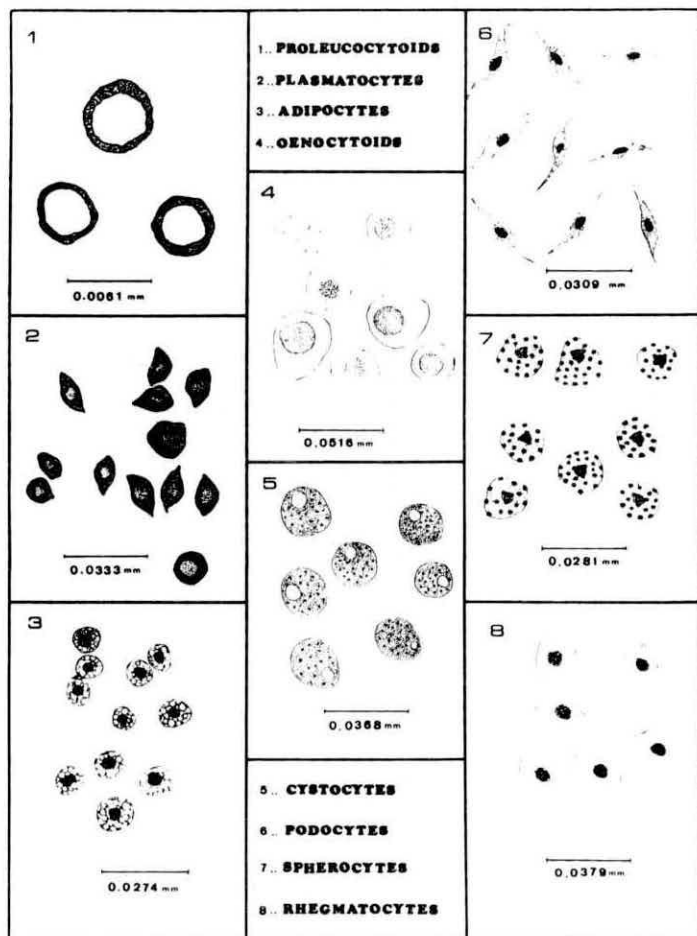


FIGURE 1 - Different types of haemocytes in the last larval instar of *Alabama argillacea*.

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RESUMO

Hemolinfa e hemócitos do último estágio larval de *Alabama argillacea* (Hubner, 1818) (Lepidoptera, Noctuidae)

O trabalho trata de estudos qualitativos e quantitativos da hemolinfa e dos hemócitos de *Alabama argillacea*, uma das importantes pragas do algodoeiro.

Foi determinado o peso relativo da hemolinfa em larvas de último estágio. Foi calculado o número total dos hemócitos, e estes foram classificados e avaliados quanto à proporção numérica em cada categoria.

Determinou-se 9 categorias:

Proleucocitoides (31,49%), Plasmatócitos (18,84%), Adipócitos (22,17%), Oenocitoides (7,89%), Cistócitos (5,52%), Podócitos (1,89%), Esferócitos (3,41%), Regmatócitos (4,81%) e Células Degenerativas (3,67%).