

Influence of the development and host-plant on the differential haemocyte counts in the cotton whitefly, *Bemisia tabaci* Genn. (Hom.:Aleyrodidae).

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ABSTRACT

Haemocytes of different developmental stages of *Bemisia tabaci* Genn. were examined. In all stages, six types of haemocytes were recognized and found : prohaemocytes (Pr.), granulocytes (Gr.), non-granulocytes (nGr.), oenocytes (Oen.) and spherule cells (Sph.). Changes occurring in the differential haemocyte count (DHC) and in surface area were investigated during the different metamorphic stages of the subject insect. The regression analysis of the obtained results showed a positive regression between the age and DHC in each of Pr., Gr., Oen. and Sph.. In the contrary, negative regression was recorded between the age and counts of Pl. and nGr. In addition, the data showed a positive relationship between the age and surface area of all haemocyte types. The changes in haemocytes picture were examined in both male and female of *B. tabaci* adults reared on different host-plants (tobacco, tomato and malabar nut). The results proved the existence of host-correlated variations phenomena in DHC of adults. The DHC were highly significantly affected by host-plant, and significantly affected by adult-sex. In addition, the dual interacting effect of the studied factors (DHC and host-plant; sex and host-plant) proved to be highly significant. While, the dual interacting effect between DHC and sex proved to be significantly different. Also, the interacting effect of the three studied factors (DHC, sex and host-plant), all together, proved to have highly significant influence. Tobacco plants had a positive superior effect on the DHC of Pl., Gr. and nGr. However, malabar nut plants showed a supreme effect on Oen. count. On the other hand, no significant differences were recorded in Sph. counts among tested host-plants. Concerning the sex effect on DHC, the study proved that Pr. counts always exceed in females than that in males within the same host-plant. However, irrespective of Pl. counts in malabar nut culture, Oen. and Sph. in tobacco culture, the DHC in males always exceed than those in females.

Keywords: Cotton whitefly, *Bemisia tabaci*, Haemocytes picture, Developmental stages, Host-plant effect.

INTRODUCTION

The sweet potato or cotton whitefly, *Bemisia tabaci* Gennadius (Homoptera : Aleyrodidae) is a key insect pest of vegetables, field crops and ornamentals in both greenhouses and field cultures (Brown and Bird, 1992). It is globally distributed, being found on all continents except Antarctica (De Barro and Hart, 2000). The reported host range of *B. tabaci* includes over 500 plant species (Azab *et al.*, 1971; Gameel, 1972 and Greathead, 1986). It is considered as a most important vector of plant viruses that drew back the agricultural production (Costa, 1976 and Cock, 1986). Larval instars and adults of *B. tabaci* directly damage plant leaves by de-sapping them and injecting their saliva therein. Excretion of honeydew by all juvenile stages and adults serves as a medium for sooty moulds, which severely reduces the productivity of plants by interfering with photosynthesis (Perkins, 1983). Cotton fiber stickiness due to honeydew is a severe problem in many cotton producing countries as this leads to significant loss of cotton quality and marketability (Ayars *et al.*, 1986 and Butler and Henneberry, 1986).

Investigation of whiteflies haemocytes or *B. tabaci* haemocytes in particular had no attention till now. Therefore, the present study was carried out to add fundamental knowledge about the changes in the differential haemocyte counts and variations in haemocytes surface area in different *B. tabaci* developmental stages. Besides, the present study was conducted in order to explore any possible effects of host-plant on the haemocytes picture of *B. tabaci* male and female adults, taking into consideration the previously recorded phenomena about host-correlated variations in whiteflies. These phenomena have been documented in the morphology of whitefly pupae since 1957 (Russell, 1957). In addition, these phenomena have been investigated and recorded in *B. tabaci*, in particular, at different physiological aspects such as in susceptibility to insecticides (i.e. El-Helaly, 1973; El-Meniawi, 1992 and Anthony *et al.*, 1998), in kinetics and electrophoretic profiles of enzymes (i.e. Wool *et al.*, 1993; Brown *et al.*, 1995 and Rawash *et al.*, 2005); and in olfaction response (i.e. Heinz *et al.*, 1993 and El-Meniawi *et al.*, 2005).

Eventually, the present study intended to serve as a basis for investigations of the physiology of *B. tabaci* haemocytes which thought to be necessary for controlling whiteflies through the IPMs strategies.

MATERIALS AND METHODS

I- Whitefly populations:-

A- The whitefly *Bemisia tabaci* tobacco culture: -

A susceptible population of the whitefly, *Bemisia tabaci* Genn., was taken from the whitefly laboratory culture of the Faculty of Agriculture, Alexandria University. This culture was firstly established by El-Helaly and has been reared on tobacco plants *Nicotiana tabacum* (Solanaceae) in greenhouses at 25 ± 7 °C, 65 ± 5 % RH and under natural light conditions since 1966.

B- The whitefly tomato culture: -

B. tabaci adults from the laboratory tobacco culture have been reared on tomato plants *Lycopersicon esculentum* (Solanaceae) since 1999, and maintained under the aforementioned greenhouse conditions without exposure to insecticides.

C- The whitefly malabar-nut culture: -

B. tabaci adults from the laboratory tobacco culture have been reared on malabar nut plants *Adhatoda vasica* (Acanthaceae) since 2001. This population maintained under the same aforementioned greenhouse conditions.

II- Changes in blood picture during *B. tabaci* developmental stages:

Differential haemocyte counts (DHC) were determined in different developmental stages of the subject insect reared on tobacco plants. Blood samples obtained from 25 individuals of each of the three larval instars, pupae and newly emerged male and female adults were prepared for DHC. Each blood sample was replicated seven times.

In addition, the surface areas of each haemocyte type were measured by micrometric slide in all the abovementioned developmental stages of the subject insect. All the tested criteria were statistically analysed and compared using "F" test and Least Significant Differences (L.S.D.) at 0.01 probability level. Also, the regression analysis (Little and Hill, 1977) was adopted to determine the relationship between the time (age) and each of DHC and haemocyte surface areas.

III- Host-plant effect on *B. tabaci* haemocyte counts:-

To study the effects of three different host-plants (tobacco, tomato and maiabar nut) on DHC, blood samples obtained from 25 newly emerged male and female *B. tabaci* adults were used in order to investigate any probable effect of the tested host-plants on the blood picture. Each blood sample was replicated ten times.

Data and all the probable comparison combinations were analysed in factorial design by using SAS procedures (1986) at probability level of 0.01.

IV- Haemocytes staining and counting:-

The appropriate number of each of the three larval instars, pupae and both adult sexes were separately smeared to a thin film between two glass slides. The blood smears were air-dried, and stained with Wright's blood stain (Conn, 1948 and Martha and Hachiro, 1971) for 1 min. and destained for 2 min. with 70 % ethyl alcohol.

The blood cell types were examined and identified under oil immersion (100 X) using a stereomicroscope. DHC were carried out in random scan of blood films (100 haemocytes from each film). The identification of haemocyte types were performed according to Arnold (1974).

RESULTS AND DISCUSSION

I - Differential haemocyte counts in different *B. tabaci* developmental stages :

Examination of stained blood smears from different developmental stages (1st, 2nd, 3rd larval instars, pupae and newly emerged male and female adults) of *B. tabaci* tobacco culture revealed six types of haemocytes: prohaemocytes (Pr.), plasmatocytes (Pl.), granulocytes (Gr.), non-granulocytes (nGr), spherule cells (Sph.) and oenocytes (Oen.) Fig. (1).

Table (1) presents mean values of DHC among different developmental stages of *B. tabaci*. These data show that the DHC varied throughout the development. Prohaemocytes were gradually increased throughout the development from the 1st larval instar to the adult stage. The maximum count of prohaemocytes was recorded in each of pupal and adult stages. A significant difference was recorded in Pr. count between the 1st larval instar and all other developmental stages.

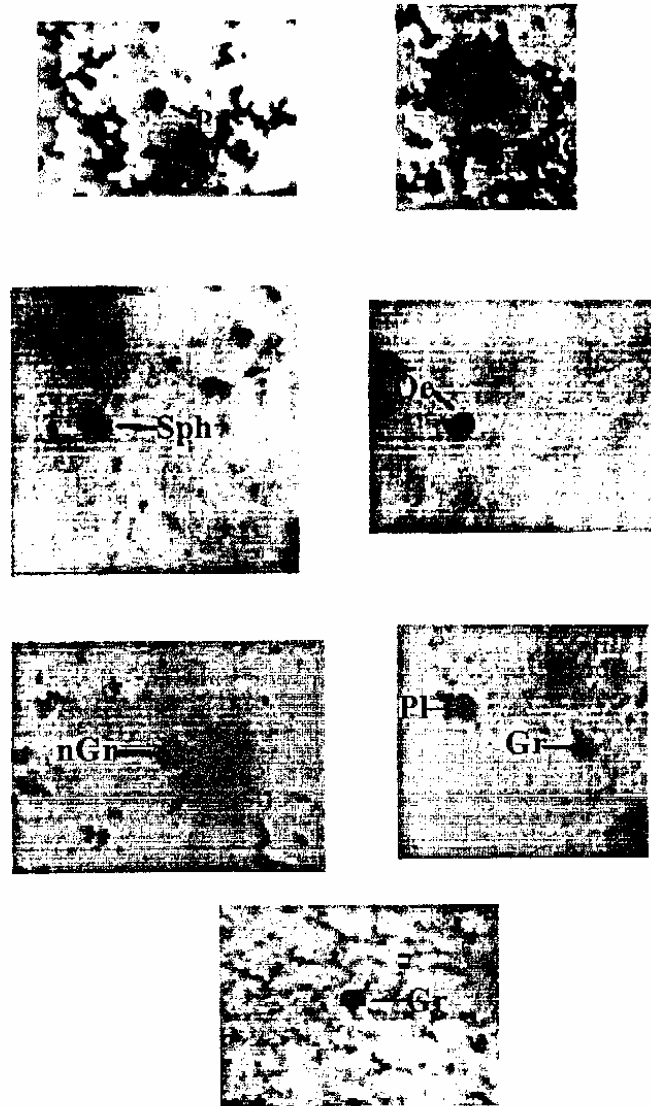


Fig. (1): Fresh haemolymph samples taken from different developmental stages of *B. tabaci*, all phase contrast micrographs. Smears stained with Wright's stain. Prohaemocyte (Pr.), plasmatocyte (Pl.), granulocyte (Gr.), non-granulocyte (nGr.), oenocyte (Oe.), spherule cell (Sph.).

No significant differences were recorded in the cases of Gr., Pl. and Sph. among all developmental stages. The maximum count of Gr., was recorded for the 3rd larval instar (37.4 ± 2.0 cell / mm³) and declined thereafter in the

show highly significant differences between pupal stage and each of the three larval instars.

It is clear from the obtained results shown in Table (1) that adult sex revealed no significant effect on DHC. However, in the case of Oen. count, a significant difference was recorded between male and female adults.

The regression analysis of the obtained data showed a positive regression between the age (time) and DHC in each of Pr., Gr. and Sph. cells. In the contrary, negative regression was recorded between the age and counts of Pl. and nGr.

The present results also showed that during *B. tabaci* development, the two immunocytes (Pl. and Gr.) represented the majority of total haemocytes (about 60 %). Similar observation was noted in *Philosamia ricini* (Essawy, 1999).

It has been frequently demonstrated that the DHC changes markedly during the development stages of many kinds of insects (Hoffmann, 1970; Bahadur and Pathak, 1971; Essawy, 1985; Essawy and Idriss, 1990; Essawy, 1991 and Slovak *et al.*, 1991).

II-Changes in the haemocyte surface areas in different *B. tabaci* developmental stages:

Figure (2) shows variations in the surface area of each haemocyte type during the development of *B. tabaci*. The obtained results proved that haemocyte surface areas were changed with varying degrees throughout *B. tabaci* development.

The data revealed no significant differences in the surface area of Pr. among all developmental stages of *B. tabaci*. The highest value of Pr. surface area was recorded for pupae ($33.5 \pm 0.2 \mu\text{m}^2$), followed in a descending order by those of the 3rd larval instar and male adults.

The changes in the surface area of Pl. showed the same trend of that of Pr., as the maximum value was recorded in the pupal stage. Besides, there were no significant differences in Pl. surface area among all developmental stages of *B. tabaci*.

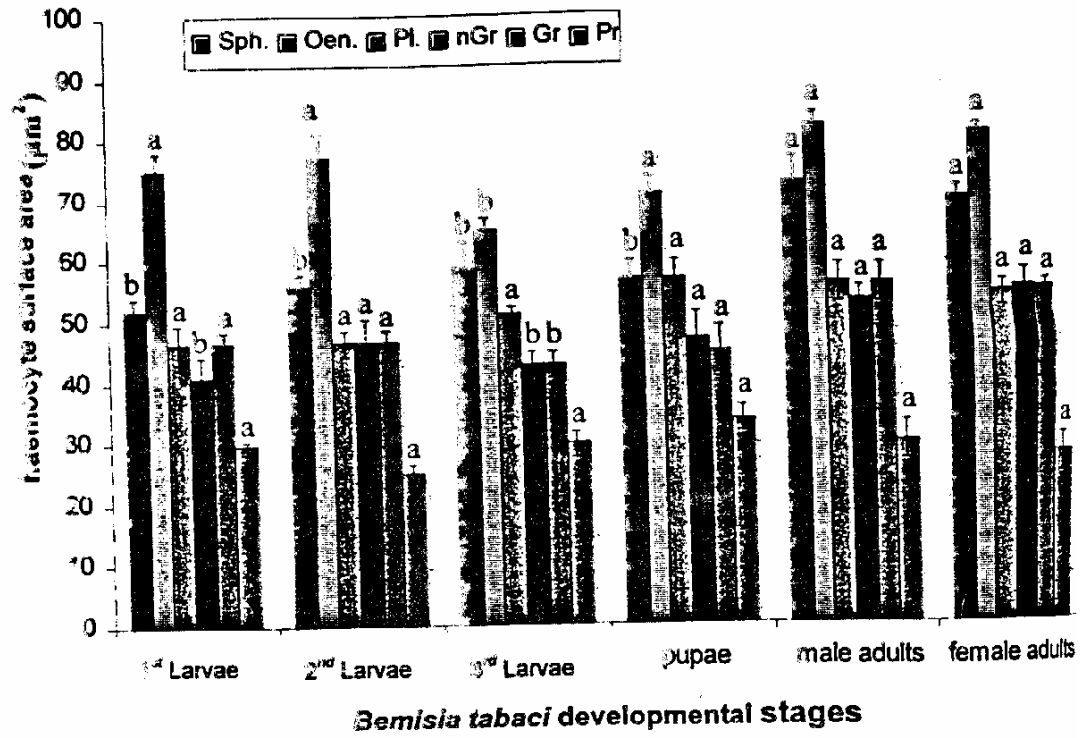


Fig. 2: The surface area of each haemocyte type in different developmental stages of *Bemisia tabaci*.

The surface area of the Gr. decreased significantly to reach the minimum value at the 3rd larval instar ($42.8 \pm 0.2 \mu\text{m}^2$) and increased significantly thereafter to reach the maximum value at male and female adult stage (55.8 and $54.72 \mu\text{m}^2$, respectively).

Similar to Gr., the minimum value of the Oen. surface area was detected in the 3rd larval instar. Also, significant difference was recorded in Oen. surface area between the 3rd larval instar and each of other developmental stages. The maximum value of Oen. surface area was recorded in the case of male adults followed by that of female adults, with no significant effect between the two sexes.

Concerning the surface area of nGr., the results showed that it increased significantly from the 1st larval instar to the 2nd larval instar. After that, the surface area of nGr. decreased significantly at the 3rd larval instar, and then increased significantly thereafter to reach the maximum in the adult stage.

In regard to spherule cells, the surface area significantly increased throughout the three larval instars, and insignificantly decreased thereafter in the pupal stage. The maximum value of the Sph. surface area was obtained in the case of male adult ($72.5 + 0.4 \mu\text{m}^2$), followed by that of female adults ($69.9 + 0.2 \mu\text{m}^2$). The changes in Sph. surface area were significant between the adult stage and each of larval and pupal stage.

The present findings agreed with those of Essawy and Idriss (1990) which proved that the counting percentage of Sph. and Oen. increased significantly at the 5th day of the last larval instar of *Ph. ricini*.

It is clear that, irrespective of nGr., the haemocyte surface areas in male adults always exceeded those of females, with no significant differences between two sexes.

The regression analysis of the obtained results showed positive relationship between the age (time) and surface area of all haemocyte types. Therefore, it could be concluded that the surface areas of haemocyte increased with the development of *B. tabaci*.

III- Host plant effect on DHC in *B. tabaci* male and female adults:

The factorial statistical analysis of the obtained data proved highly significant differences among all means of DHC of *B. tabaci* male and female adults reared on three different host plants (tobacco, tomato and malabar nut). Analysis of variance presented in Table (2) reveal that DHC was highly significantly affected by the host plant, and significantly affected by the adult sex. In addition, the interacting effects of the studied factors (DHC and host-plant; sex and host-plant) also proved to be highly significant different on the bases of their dual interaction effect. Furthermore, the dual interacting effect between DHC and sex proved to be significantly different. Moreover, the interacting effect of the same three studied factors (DHC, sex and host-plant), all together, proved to have highly significant influence.

Table (2): Analysis of variance for mean values of differential haemocyte counts as affected by *B. tabaci* sex and host-plant.

S.O.V.	d.f	Mean square
A	5	1148.83**
B	1	4.358*
C	2	1117.24**
A*B	5	5.668*
A*C	10	5531.272**
B*C	2	10886.15**
A*B*C	10	292.224**
Error	109	15.06
Total	179	-----

d.f = degrees of freedom.

A = differential haemocyte counts, B = *B. tabaci* sex, C = host-plant.

** = Highly significant at 0.01 level of probability.

Table (3) summarizes the variations in DHC of *B. tabaci* male and female adults reared on different host-plants. Concerning the host-plant effect, it was found that tobacco plants, when compared with tomato and malabar nut plants, had a positive superior effect on the haemocyte counts of *B. tabaci* in the cases of Pl., Gr. and nGr. However, the malabar nut plants showed a supreme effect on Oen. count. On the other hand, no significant differences were recorded in Sph. counts among all the three tested host-plants (Table 3). In general, it is clear that there were no

significant differences in counts of Pr., Gr. and nGr. between tomato and malabar nut *B. tabaci* cultures.

Table (3): Variations in the differential haemocyte counts of *B. tabaci* male and female adults reared on three different host-plants.

	Tobacco culture		Tomato culture		Malabar nut culture	
	males	females	males	females	males	females
Pr.	7.2±0.4 b	8.8± 0.5 a	8.0±0.5 a	9.4± 0.5 a	7.6±0.6 a	8.0± 0.3 a
Gr.	37±0.7 a	34.4±0.6 b	12.2±0.6 c	11.8± 0.6 c	12.0±0.6 c	11.2± 0.5 c
NGr.	14.8±0.4 a	14.0±0.7 a	11.4±0.7 b	10.4±0.2 b	9.8±0.8 b	9.6±0.8 b
Pl.	30.2±0.6 a	28.4±0.6 a	11.6±0.7 b	9.6± 0.5 c	10.8±0.2 b	11.8±0.5 b
Ocn.	4.6±0.5 c	6.0±0.5 c	5.6±0.4 c	5.2± 0.4 c	11.8±0.6 b	13.2± 0.3 a
Sph.	3.8±0.3 a	4.0±0.3 a	3.0±0.3 a	2.6± 0.5 a	3.0±0.2 a	2.6± 0.2 a

Means in same row followed by the same letter(s) are not significantly different at 0.01 probability level.

In regard to the sex effect on the DHC, the results proved that Pr. count always exceeded in females than that of males within the same host-plant. However, irrespective of counts of Pl. in malabar nut culture, Ocn. and Sph. in tobacco culture, the haemocyte counts in males always exceeded those of females. The significant differences in DHC between male and female adults were recorded for Pr., Gr. of tobacco culture, Pl. in tomato culture and Ocn. in malabar nut culture.

In the light of the aforementioned interpreted results, the obtained findings proved the existence of host-correlated variations phenomena in the DHC of the subject *B. tabaci* adults but with varying degrees of effect. Taking into consideration the probability of the presence of certain allelochemicals in each tested host-plant.

These phenomena referred to the abruptive physiology of the whiteflies, and in return their promising highly important role in enriching our knowledge of insect genetics and physiology.

Unfortunately, no available references on host-plant effect in haemocyte picture of whitefly were found to include them in the discussion of the present study.

Finally, the present study revealed the existence of six haemocyte types in the subject Aleyrodid, *B. tabaci*. Besides, the obtained results demonstrated the changes in the differential haemocyte counts during the development of *B. tabaci*. Also, the present study recorded variations in surface area of each haemocyte in different developmental stages. Moreover, the present study proved the existence of host-correlated variation phenomena in differential haemocyte counts in both male and female adults.

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تأثير النمو والنبات العائل على أعداد الأنواع المختلفة لخلايا الدم في ذبابة القطن البيضاء *Bemisia tabaci* Genn.

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درست خلايا الدم في مراحل النمو المختلفة لذبابة القطن البيضاء وذلك بداية من العمر اليرقي الأول وحتى طور الحشرة الكاملة. ولقد تم تمييز ستة من أنواع خلايا الدم وجدت في جميع الأطوار النموية للحشرة وهذه الخلايا هي : خلايا الدم الأولية prohaemocytes، وخلايا الدم الحبيبية granulocytes، وخلايا الدم الغير حبيبية non-granulocytes، وخلايا الدم النبيذية oenocytes، وخلايا الدم الكروية spheriole cells. كذلك فقد درست التغيرات في أعداد كل نوع من أنواع خلايا الدم الستة ، وفي مساحة السطح لهذه الخلايا أثناء تطور الحشرة . ولقد أوضح التحليل الإحصائي للنتائج المتحصل عليها أن أعداد خلايا الدم الأولية والحبيبية والنبيذية والكروية تتناسب طرديا مع نمو وتطور الحشرة ، وعلى العكس من ذلك فإن أعداد خلايا الدم البلازمية والغير حبيبية تقل بزيادة نمو وتطور الحشرة . وبالإضافة إلى ذلك ، أوضحت النتائج أن مساحة سطح الأنواع المختلفة لخلايا الدم تزيد بزيادة نمو وتطور الحشرة

كذلك فقد تم دراسة تأثير إختلاف العائل النباتي المرباه عليه الحشرة على صورة الدم في كل من الذكور والإناث وذلك بتربية الحشرة على ثلاث عوائل نباتية مختلفة هي : الدخان والطماطم والبوستاشيا البيضاء . وأثبتت النتائج وجود ظاهرة الإختلافات المرتبطة بالعائل host-correlated variations phenomena في أعداد كل نوع من أنواع خلايا الدم للحشرات الكاملة لذبابة القطن البيضاء . ولقد كان تأثير إختلاف العائل النباتي على المعنوية بينما كان تأثير جنس الحشرة على أعداد الأنواع المختلفة لخلايا الدم معنوى فقط . كذلك أثبت التحليل الإحصائي للنتائج أن تأثير التداخل بين العوامل الثلاثة موضوع الدراسة ، وهي أعداد كل نوع من أنواع خلايا الدم والنبات العائل وجنس الحشرة ، كان عالى المعنوية . ولقد أظهر نبات الدخان تأثير إيجابى فائق على أعداد خلايا الدم البلازمية والحبيبية والغير حبيبية . بينما أظهر نبات البوستاشيا البيضاء تأثير فائق على أعداد خلايا الدم النبيذية . ومن ناحية أخرى ، لم تسجل أى فروق معنوية في أعداد خلايا الدم الكروية نتيجة تربية الحشرة على العوائل النباتية المختلفة .

أما بالنسبة لتأثير جنس الحشرة على أعداد كل نوع من أنواع خلايا الدم ، فقد أثبتت النتائج أن أعداد الخلايا الأولية كانت دائما في الإناث تفوق مثلتها في الذكور وذلك في الحشرات المرباة على نفس العائل النباتي. بينما ، باستثناء أعداد الخلايا البلازمية في الحشرات المرباه على نبات البوستاشيا البيضاء والخلايا النبيذية والكروية في الحشرات المرباه على نبات الدخان ، فإن أعداد كل نوع من خلايا الدم في الذكور كانت دائما تزيد عن مثلتها في الإناث.