



E-ISSN: 2320-7078

P-ISSN: 2349-6800

JEZS 2018; 6(2): 730-734

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Received: 13-01-2018

Accepted: 14-02-2018

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## Biology and internal ultrastructure of oleander scale (*Aspidiotus nerii* Bche) at transmission electron microscope

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### Abstract

*Aspidiotus nerii* Bche. causes more damage to olive trees in Absheron. Despite its high damaging degree, this species has not widely been studied. There is no any information on the ultrastructure of the *Aspidiotus nerii* Bche. that has a great practical importance, neither in local nor in foreign publications. Therefore, taking into account the relevance of research in this direction, the ultrastructure of *Aspidiotus nerii* Bche. has fitsly been studied by Transmission Electron Microscope, diffraction pattern has been captured and depicted.

**Keywords:** coccid, *Aspidiotus nerii* Bche, olive tree, transmission electron microscope, mitochondria, basal membrane

### Introduction

Coccids (Homoptera, Diaspididae) are very dangerous pests of fruit trees and park - decorative plants. In particular, by spreading over the trunks, boughes, leaves of the plants, and even over the fruits in subtropical and hot zones, these pests can cause the drying of the boughes and the weakening of plants. Coccids damage the surface of the plants and infect especially the young boughs, and leaves. In some cases, Coccids cover the body of plants in complete colonies and cause a great damage to them. As a result of infection with the coccneas, the growth and the development of the boughes weakens, stops and the decorative beauty of green plants disappears, and the plants dry in most cases. There are many spots on the fruits that are damaged by the coccineas, the fruits are shriveled and their taste is deteriorated. Coccids winnow the juice and nutrients of plants and destroy their seeds. These pests lead to the destruction of the plants in severe infections. Their being polipacious, high egg-laying ability and their plasticity from the environmental point of view allows their more spreading <sup>[4]</sup>.

*Aspidiotus nerii* Bche. causes more damage to olive trees in Absheron. Despite its high damaging degree, this species has not widely been studied. There is no any information on the ultrastructure of the *Aspidiotus nerii* Bche. that has a great practical importance, neither in local nor in foreign publications. Therefore, taking into account the relevance of research in this direction, the ultrastructure of *Aspidiotus nerii* Bche. has fitsly been studied by Transmission Electron Microscope, diffraction pattern has been captured and depicted.

### Materials and Methods

The research was carried out in 2014-2016. The samples were collected from olive trees and oleander bushes in Absheron region <sup>[3]</sup>. It has been multiplied by a thermostat with a temperature of 25° C on the pest potato rings in laboratory conditions. Samples were taken from fully infected potato rings. The obtained samples (homopterous and a part of their localized potatoes) were prepared in 0.1M phosphate buffer (pH = 7.4), fixed in a solution of 2.5% glutaraldehyde, 2% paraformal-aldehyde, 4% surcose, 0.1% picric acid and introduced to the Electron Microscopy Laboratory of the Scientific Research Center of AMU. First of all, each of the samples were thrice washed in a 0.1 M phosphate buffer (pH = 7.4) for 15 minutes (45 minutes in total) in the laboratory. As per the common methods adopted in light and electron microscopic investigations, the slices were kept in the mixed solution of 1.5% red blood salt and 1% OsO<sub>4</sub>, 0.1M phosphate buffer for 1.5 hours in the post fixation phase. After the post fixation phase, each of the specimens were thrice washed again in 0.1 M phosphate

buffer (pH = 7.4) for 15 minutes and the slices were dehydrated at the III phase. In this case, each of the specimens is thrice passed through 50% alcohol, and then accordingly 70% and 96% alcohol, within 15 minutes (45 minutes in total). Then each of the slices has thrice been washed in the solution of 50% pure alcohol and 50% pure acetone for 15 minutes (45 min. in total) and each of them has thrice been fixed again in pure acetone for 15 minutes (45 minutes in total).

The next IV phase was the preparation of blocks. For this purpose, the aralidide epon solution consisting of 5 components (Epon-812, DDSA, Araldid M, Dibutalftalat and DMP-30) was used. Their weight ratio varies depending on the number of samples that will be taken. The block preparation phase is divided into several parts:

- 75% of the solution is pure acetone and 25% is aralidide-epon;
- 50% of the solution is pure acetone and 50% is aralidide-epon;
- 25% of the solution is pure acetone and 75% is aralidide-epon;
- The slices are kept in the solution of 100% aralidide-epon for an hour [5, 8].

After completing the last stage, the samples were placed in pre-selected and numbered ready-molds and placed in a thermostat. The slices were kept at 37° C for a day, at 45° C for a day and at 60° C for a day (total 3 days) in thermostats. The ready-made blocks have removed from the molds and the semi-slim (1 μ) cuts were prepared via Leica EM UC7 thermal ultramicrotome. Sections are stained by double-dyeing method (a solution of 0.5% methylene abundance, 0.5% azur II, 0.5% buffer and b-solution - 5% alcohol, 0.1% fuksin) [2]. Semi-slim cuts have been reviewed in the Latimet (Leitz) microscope and the images of the necessary parts have been captured by the digital photocamera systems Pixera (USA) and Canon (Japan). After the pyramids were developed from the selected areas of the necessary parts of semi-slim cuts observing by the light microscope, silver and gold ultraslim cuts were developed at 50-80 nm thick via the above mentioned ultramicrotomy and were collected into the grids. In order to observe the cuttings at the Transmission Electron Microscope, these cuttings were firstly stained at 2% uranyl acetate solution for 15 minutes, then at 0.6% pure lead-citrate prepared in 0.1 M NaOH solution for 0.5-1 minutes [5]. Ultraslim cuttings were investigated under the tension of 80-120 KV at JEM-1400 transmission electron microscope (JOEL-Japan), and electrogrammes were taken through the lower and lateral cameras (Veleta). As a result of the research, 6 blocks were developed, 64 of them were semi-slim, 30 were ultraslim cuttings. 26 photos and 123 electrogrammes were taken.

## Results and Discussion

*Aspidiotus nerii* Bche. is a tropical origin, and is now spread all over the world. It was spread in the former USSR territory on the southern shores of the Crimea, Central Asia and the Caucasus. And almost everywhere in greenhouse conditions. It is a very serious pest of various greenhouse and room plants. This scale is a polyphage.

*Aspidiotus nerii* Bche damages palm, olive, asparagus, bay, acacia, oleander and other decorative and agricultural plants in the territory of Azerbaijan. Some authors provide information on its bioecological features [1]. Z.K.Hajibeyli reported about the great damage of oleander scale to the

lemon plant [3]. A.A.Imamguliyev researched this pest at the Lankaran region of Azerbaijan and studied its biology [4]. G.A.Mustafayeva informed about the bioecological features and some local entomophages of the pest in Khachmaz and Absheron regions [6, 7].

The pest has been found to live on different organs of palm, plum, shumshad, olive, owl, asparagus, legistrum, acacia, oleander, and ordinary laurel plants. It spreads over citrus plants in Lankaran region. These coccids also harm the plants grown in greenhouse and used for decorative purposes. The decorative properties of the plants infected with the oleander scales are lost, poorly developed, the leaves are quickly poured, and sometimes the plant is dried early. Oleander scale that live on olive trees clings to olive fruits, creates sprouts, fruits do not grow normally, and poured ahead of time.

*Aspidiotus nerii* Bche. has a armor of 3-7 mm. The armor is round, thin and flat. Younger individuals are mostly white, and adults are light brown. The larval armor is yellow and located in the center. The body of the female under armor is light yellow. The body is egg-shaped, and weakly chitinized. Male's pronimpha is long in oval shape, and the division of the body into head, thorax and abdomen is felt very weak. Eyes, sacks and wings are visible very slightly. Males' nymphs are clearly divided into head, breast and abdomen. Eyes, antennas, wings and legs have been well developed. Adult males have 1 double wing, legs, and move freely. They die after living for 1-2 days.

According to our research, in Absheron conditions the youngs, adult females, I and II-instar larvae of *Aspidiotus nerii* Bche. winter. Mass going out of wintering of *Aspidiotus nerii* Bche. occurs in March-April. In the second and third decades of April, the flight of males takes place. The males diea after the mating. These individuals do not have mouthparts. They survive just for 1-2 days. The wings have developed very well, the copulativ apparatus is very long and well visible.

Females lay eggs in 12 to 15 days after mating. The period of embryonic development is short and is completed within 2-3 days. The larvae are hatched in the beginning and the second decade of May. The mass hatching of the larvae occurs in the 2nd and 3rd decades of May. Larvae move actively, have legs and the antennae. After a very short wandering period, the "crawlers" flatten against the young leaves and shoots of the plant, and begin to secrete their armor. The 1<sup>st</sup> instar larvae are transformed into the 2<sup>nd</sup> instar ones in the 2<sup>nd</sup> decade of June. In this period, males and females differ from each other. Male armor is long, white, but the female one is round, partly dark. The body under armor also differs from each other. Male's body is long and covered with red spots. Female has a round yellowish body. Males fly in the II-III decade of June. The development of the first generation spends for 55-60 days in Absheron territory.



**Pic 1:** *Aspidiotus nerii* Bche.- Oleander scales on the oleander bush



**Pic 2:** *Aspidiotus nerii* Bche. - Oleander scales on the potato rings



**Pic 3, 4:** Egg laying process of oleander scales

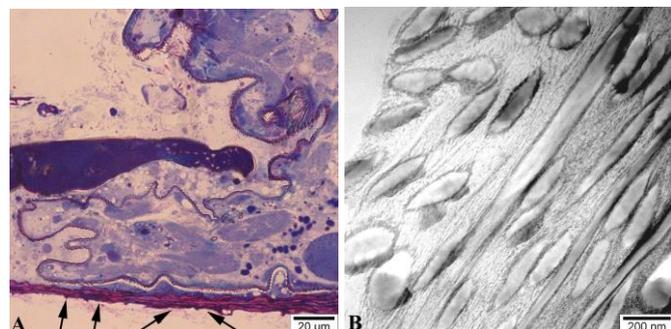
Egg - laying females of the second generation are appeared in the 1st and 2nd decades of July. The larvae hatch in the 1st and 2nd decades of July. The mass flight of the males of the II generation is observed in the second and third decades of August.

As the temperature is relatively higher than summer, full development of the II generation is completed for 50-55 days. The larvae of the III generation are hatched in the second and third decades of September. They turn into the 2<sup>nd</sup> instar larvae in the I - II decade of October. The 2<sup>nd</sup> instar larvae are turned into adult females at the end of October and beginning of November. The 1<sup>st</sup> instar males are turned into the 2<sup>nd</sup> instar ones under the nymphal armor.

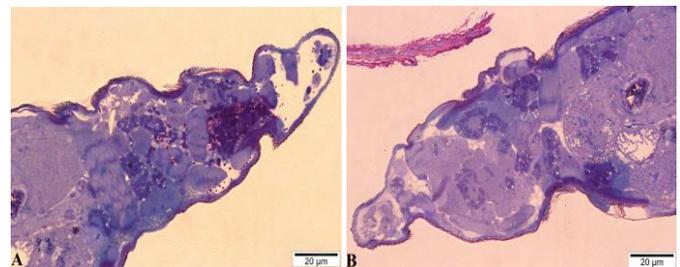
Therefore, the biology of *Aspidiotus nerii* Bche was studied on olive and oleander plants in Absheron and it was clear that it gives 3 generations for a year.

First of all, it should be noted that there is no information on the ultrastructure of *Aspidiotus nerii* Bche. having great practical value neither in local nor in foreign publications. The investigations carried out in this direction were firstly performed by us.

The pictures of the structure of armor covering the whole body surface of the pest are made through both the light (Fig. 5A) and the electron microscope (Fig. 5B).



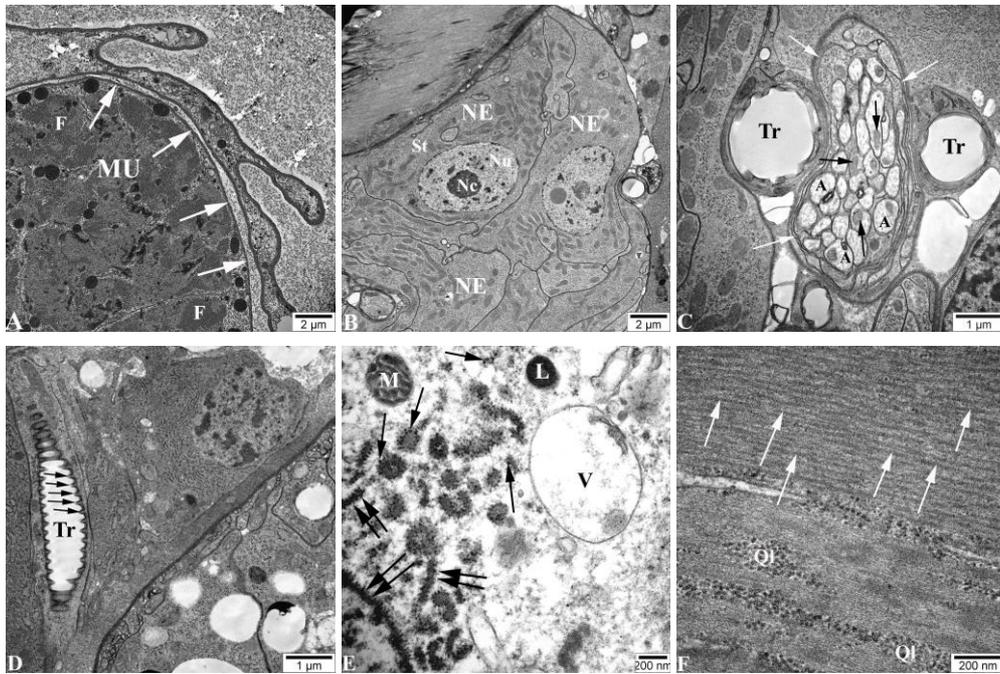
**Pic 5:** Light –(A) and electron microscopic-(B) view of ararmor of *Aspidiotus nerii* Bche. (explained in the text). By the method A- D'Amico; B- was stained with 2% uranyl-acetate and 0.6% pure lead-citrate



**Pic 6:** Internal structure of the *Aspidiotus nerii* Bche. in the light microscope (the explanation is given in the text). It was stained by A and B - D'Amico method

The armor composed of dense chitin is indicated in the light microscope by black arrows. These chitin plates (composed of Ca<sup>+</sup>) provide strength to the armor by even distribution and protect homoptera from damaging and other environmental effects. The chitin plates shown in Figure 5B are clearly visible. The armor is attached to the body's tissue (Picture 5A).

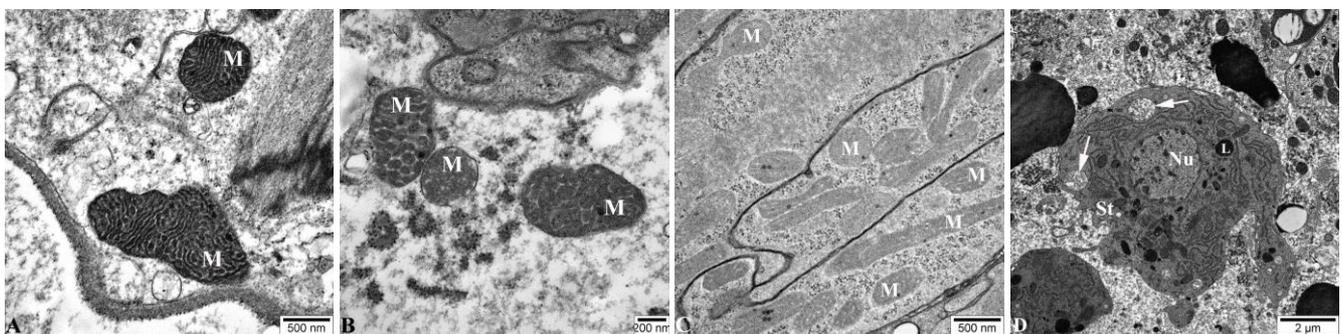
The internal structure of the pest is also studied under the light and electron microscopes. The histological structure of the scale is given in Pictures 6A and 6B. In both ways, the implicit texture, as well as the internal structure is apparent as well. Picture 7A shows transversal striated muscle layer (MU) and its basal membrane (indicated by arrow). Separately, fibrillas are also indicated inside the muscle cells (F). Pictures 7B and 7C show different parts of the scale's nerve cells. Picture 7B clearly shows the cytoplasm of the neuron (NE) cell (St), nucleus (Nu) and the nucleolus (Nc). Numerous mitochondria are observed in the neuron cytoplasm, which indicates the activity of the cells. Picture 7C shows the electrogram of the axons, which is the other part of the nerve cell. The axons are covered with thick double membrane (shown in white axes). The mitochondria are observed in the cytoplasm of the axons (indicated by the black arrow). Tracheal tube (Tr) is located on both sides of the nerve cell. Trachea's longitudinal cross-section is also provided in Picture 7D.



**Pic 7:** Ultrastructure of *Aspidiotus nerii* Bche. scale in electron microscope (explained in text). A-F – was stained with 2% uranyl-acetate and 0.6% pure lead-citrate.

Here, spiral thickening (tenidium), which forms the basis of the wall of the tracheal tube and gives it elasticity, is clearly visible (indicated by black arrows). Other organoids in the cytoplasm of the muscle cells are ribosomes that are freely available in cytoplasm - granular endoplasmic reticulum (indicated by two black arrows), ribosomes on it (shown in a

black axis), vacuole (V), lysosome (L), mitochondria (M) and ribosomes free in the cytoplasm are also reflected in Picture 7E. Fibrillars in the transversal striated muscle (indicated by white axis) and glycogen (QL) collected around them are clearly seen in the large lenses (see Picture 7F).



**Pic 8:** Different shapes of mitochondria (A, B, C) and ultrastructure of macrophage (D) in muscle cells of the *Aspidiotus nerii* Bche. scale in electron microscopy (explained in text). A-D – It was stained with 2% uranyl acetate and 0.6% pure lead-citrate

One of the key points is the observation of mitochondria (M) in different types and sizes during the study of the ultrasonography of the pest (Pictures 8A, 8B, 8C). Most of them are dark (Pictures 8A and 8B) and some are light colors (Picture 8C). Our research has revealed that the amount of mitochondria in muscle and nerve cells is higher than other organs and tissues. Its reason is that these cells constantly need energy. Dark mitochondria are believed to be more active in energy exchange than others. Picture 8D shows macrophages that act as "sanitarians" in the body and digest foreign bodies. Its cytoplasm (St), nucleus (Nu), lysosomes (L) with digestive bubbles (shown in white axes) are shown in picture.

**Conclusions**

The ultrastructure of the pest *Aspidiotus nerii* Bche. has been researched for the first time through the Transmission electron microscope. During the study of the pest's ultrastructure, mitochondria were recorded in different sizes and types, and

their amount was found to be significantly higher in muscle and nerve cells than other parts and tissues.

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