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Scanning Electron Microscopic Observations on the Hatching of Egg of Citrus Red Mite, *Panonychus citri* (McGregor) (Acari: Tetranychidae)

Abdul Razaq, Masaya Shiraishi[1], Pear Mohammad, Ryoko Tsutsumi[1] and Junko Toda
United Graduate School of Agricultural Sciences,
[1]Faculty of Agriculture, Ehime University, 3-5-7 Tarumi, Matsuyama 790'8566, Japan

Abstract: The changes in egg of citrus red mite, *Panonychus citri* (McGregor) Mari: Tetranychidae) during hatching were observed under scanning electron microscope. The egg was jewel-shaped having a diameter of 120-145 pm. The guy ropes and stalk broken down after two weeks of laying eggs. The characteristics of the shell surface gradually changed before hatching. The vertically patterned ridges appeared from the stalk base toward the middle portion of the egg. With the passage of time, the egg surface converted into deep patchy form. The embryo developed and the larva made a pair of hales in the middle region of egg using claws. The inside surface of the shell was smooth. The internal surface was spongy and external surface was patchy type having a thickness of 1 pm. The cleavage appeared on the equatorial portion of the egg and the larva came out. The deposited eggs had sticky wax materials on its ventral side for attachment on the leaf.

Key words: Egg, holes, larva, shell, stalk

Introduction

Citrus red mite, *Panonychus citri* (McGregor) is exclusively a citrus pest. It is considered as the most serious mite in Japan as well as in many other countries. Their eggs are seen on twigs leaves and fruits (Jeppson *et al.*, 1975). The egg differs from that of the other species on citrus by its red color and presence of vertical stalk (Jeppson, 1989).

During oviposition, the tube-like ovipositor is formed as a continuation of the posteroventral body surface and consists of the flap, folded integument of the genital region and copulatory pore (Crooker, 1985). The oviposition period varies widely on species and environmental conditions but 9-41 days are required for hatching depending on the temperature and humidity (Sao *et al.,* 1988).

Protection of eggs against water loss is necessary because of their relatively large surface/volume ratio. Some eggs must survive under adverse environmental conditions for long period. The overwintering of eggs of *Punonychus ulrni* withstands low humidity and temperatures throughout 5-9 months of diapause. The summer diapause of eggs of *Panonychus* latens must whitstand hot and desiccating conditions (Crooker, 1985). The resistance of *P. ulmi* eggs to the water loss was due primarily to the secretion of a very thin wax layer around the inner surface of the shell (Beament, 1951; Hopp, 1954). Dittrich and Streibert (1969) suggested that this layer might arise from clear yolk. The eggshell of *Tatranychus cinnabarinus* (Boisd) was not totally impermeable to gas exchange and that metabolism increased during incubation (Thurling, 1980). In *Tatranychus uticae* long conic protrusions for along the zones of the intermediate lamella after egg deposition (Dittrich, 1971).

Embryonic development takes place in the eggs deposited on the substrate by the primitive ovipositor of the adult female (Lees, 1961; Barend van de Lustgraaf, 1977). The breeding in *Panonychus* species has been multi instared and rapid. The population of mite reached *their* maximum density in late May and mid June (Gotoh and Kubuta, 1997).

There are few reports on the internal development and chemical changes of hatching eggs (Beament, 1951; Hopp, 1954). The observations on the structural variations in the external surface of hatching egg and its characteristics have riot been reported. The present study was conducted to observe the structural changes in egg during embryonic development and hatching under scanning electron microscope.

Materials and Methods

The experiment was conducted in the Citriculture Laboratory, Faculty of Agriculture, Ehime University, Japan. The leaves of citrus plants infested by several citrus red mites were collected. The mites were transferred to the healthy leaves for oviposition under laboratory condition, Freshly laid and matured eggs were isolated with 3 mm of leaf area by cutting with a blade. Samples were pre-fixed in 4% glutaraldehyde in 0.1 M phosphate buffer in pH 7.2 for 48 hours, followed by rising in the same buffer and were post-fixed in 1% osmium tetroxide for 1 hour. Dehydration was carried out with a graded ethanol series and were dried in a Vacuum Device Inc., VOF-20 freeze dryer. Dried samples were mounted on the specimen stubs. Coated with gold using Eike Engineering Co. Ltd., 18-2 ion sputter and finally were viewed under hitachi 52250N Scanning electron Microscope at 20KV and photographed.

Results

The egg of *P. citei* was jewel-shaped having a diameter of 120145 pm. The embryo developed after two weeks of oviposition and started hatching of eggs. The guy ropes broken down, their net pattern was disturbed and the stalk base was cracked (Fig. 1). The base of the egg stalk was roughly surfaced having vertical lines. The shell had a sandy surface from the top up to the middle portion of the egg (Fig. 2). Matured egg developed ridges which originated from the dorsal area and extended towards the middle portion of the egg. The ridges were irregularly arranged and had various lengths and depths (Fig. 3). There middle part was converted into patchy surface, which revealed oval and irregular shapes. These patches were engraved and extended towards the same direction on the egg (Fig. 4). Two holes vertically parallel to each other were made on the maturation of egg. The shell was punctured by applying internal force and the broken pieces were pushed outward. The lower hole was not completely developed but the cracks appeared externally. Both holes were in similar shape and pattern (Fig. 5). Magnified view of the punctured area revealed

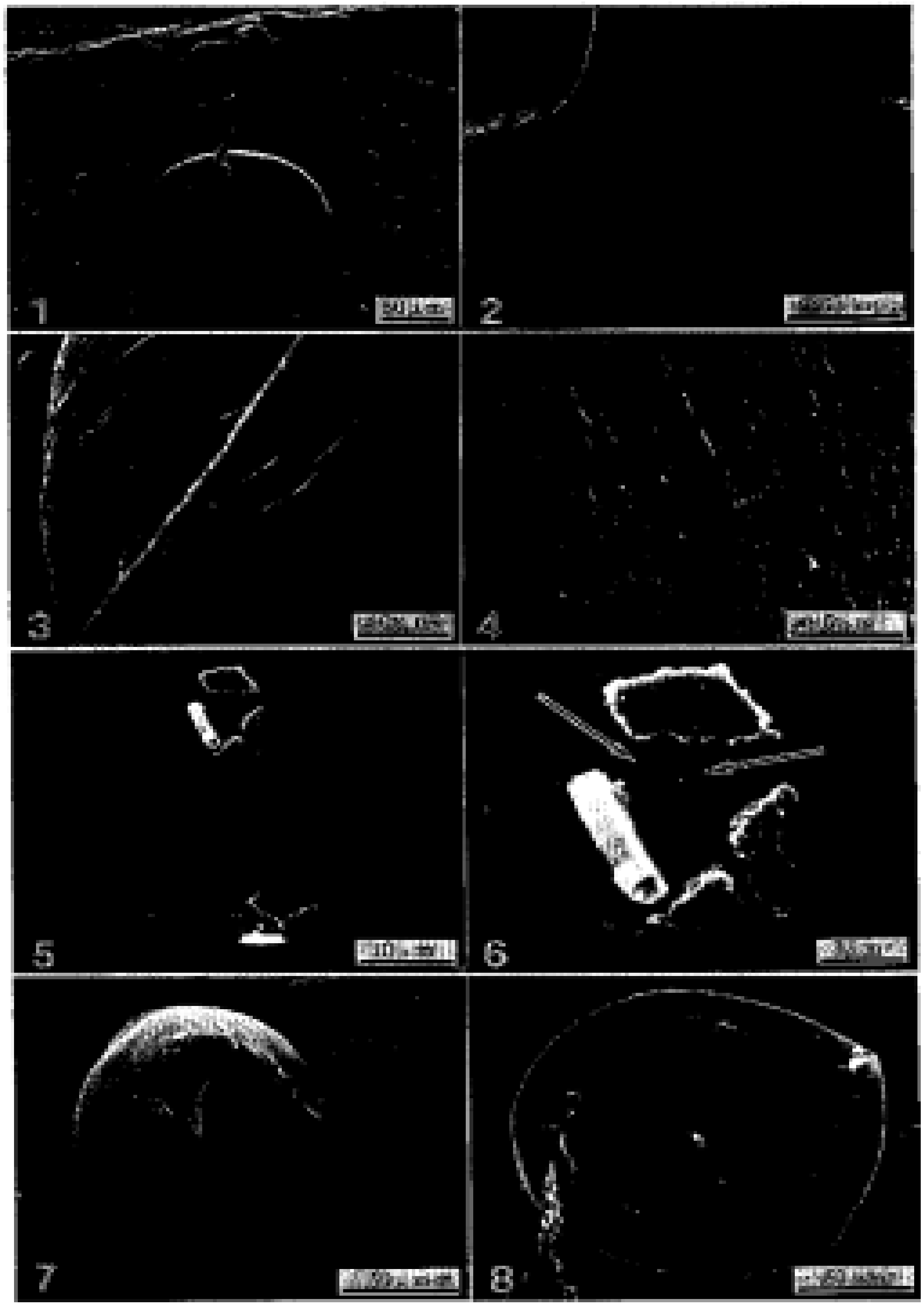

Fig. 1-8: SEM micrographs on the strucutral changes of eggshell and hatching of egg in *Panonychus cirir* (McGregor). 1: Mature jewel-shaped egg having broken stalk close to its base, 2: Roughly surfaced stalk base and eggshell, 3: Deep vertical ridges on the egg surface extended from top to the middle portion of egg, 4: Net patterned patches on the equator region of the egg, 5: A pair of holes made on the middle portion of egg by developing embryo, 6: Magnified view of the punctured wall of the shell having smooth inside, spongy internal and patchy external structures, 7: Matured larva coming out of egg by tearing the shell from the middle of egg and extended leg out and 8: The ventral side of egg possessing a thick layer of sticky materiel

the structure of shell. The internal wall of the shell possessed spongy surface. The inner layer was smoothly surfaced and the external layer was densely patched. A portion of the palpal claw of larva was projected from the upper portion of this cavity (Fig. 6). The eggshell was broken from the middle and was divided into two parts. The upper side was completely separated from the lower one of the broken shell. The ventral portion of egg was stick with the leaf. The larva extended leg to come out from the egg (Fig. 7). When egg was pulled up from the leaf surface, a thick layer of sticky material was found, which was fixed with the bottom side. The ventral surface was smooth and formed a rounded flat shape (Fig. 8).

Discussion

Tetranychid eggs varies from round to onion-like and its diameter in spider mite varies from 110-150 gm depending on the species (Dittrich and Streibert, 1969). The egg surface may he smooth, light sculptured, straited or ribbed as a result of contact with the averted genital canal (Dittrich and Streibert, 1969). We found that the egg was jewel-shaped and had a stalk on the top portion while the diameter of the egg was 1.20-145 pm in *P. cirri.* The various structures of the egg surface during the developmental stages may be due to the external and internal effects of temperature and oxidation of the egg components. The ropes were bro-ken down and the stalk cracked near the base. This indicated the beginning of hatching process of the embryo.

The egg of *P. loirni* consisted of an outer thick layer of oil and protein that attached the wax to an under laying inner shell layer. The cement and wax layers contacted the substrate during oviposition (Beament, 1951). Where as the egg of the *Petrobia latens* has and external coating of hard wax. Differences in the amount of this material and the ovipositional behavior of the female are responsible for the dissimilar appearance of the diapause and non-diapause eggs (Lees, 1961). We observed that the eggs upper layer of eggs was rough-surfaced, which favoured the deposition of wax and cement layer. By the passage of time, the erosion of egg surface layer started and deep patches appeared. This phenomenon leads to the exposure of second layer and the weakening of eggshell. It might help the breaking of shell by the larva.

The shell consisted of an outer granular layer and an inner electron transparent layer in case of *T. urticae.* The chitin of the shell did not appear to be deposited in the form of chitin microfibrils (Mothes and Seitz, 1981). In our experiment we observed the broken shell pieces projected outward which had smooth inside wall and spongy internal structure. The outer surface was also densely patchy around the hole. The shell lied a thickness of 1 pm.

The developing embryo must be protected from desiccation, but respiration must also be possible. During early embryonic stage, gas exchange is possible through the water-resistant eggshell (Thurling, 1980). Our experiment showed that there were always two holes at the middle of the egg surface. During the process of embryo development it might be that the oxidation process increased. Before rnaturtion and hatching, the larva made these holes to comply with the sufficient amount of oxygen. When the larva was sufficiently hatched, the shell was torn from the middle portion of the egg and pushed its leg out of the shell.

There was no wax at the base of the egg where it was in contact with the substrate (Beament, 1951; Hopp, 1954). Cement and wax layers were also not observed in T. *erricae* by Dittrich (1971). In contrast, we found that the ventral portion of the eggshell had a thick layer which attached it with the surface of the leaf. This layer showed that it was delivered prior to the oviposition and contained a sticky material.

In conclusion the results of this study revealed that the egg surface was rough because of the cement and wax which slowly eroded and the second layer of egg was exposed. Consequently, the eggshell weakened and it became possible for the larva to have sufficient supply of oxygen by making two holes. Another advantage of this development was to puncture the egg by the larva for coming out. The ventral side of the egg was provided with a thick layer of sticky material which fix the egg on the leaf surface.

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