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The chorionic architecture and shell structure of *Diaphania pulverulentalis* (Hampson) (Lepidoptera: Pyralidae) eggs

Архитектура хориона и строение оболочки яйца огнёвки *Diaphania pulverulentalis* (Hampson) (Lepidoptera: Pyralidae)

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КЛЮЧЕВЫЕ СЛОВА: архитектура хориона, структура оболочки, оболочка яйца, *Diaphania pulverulentalis,* СЭМ.

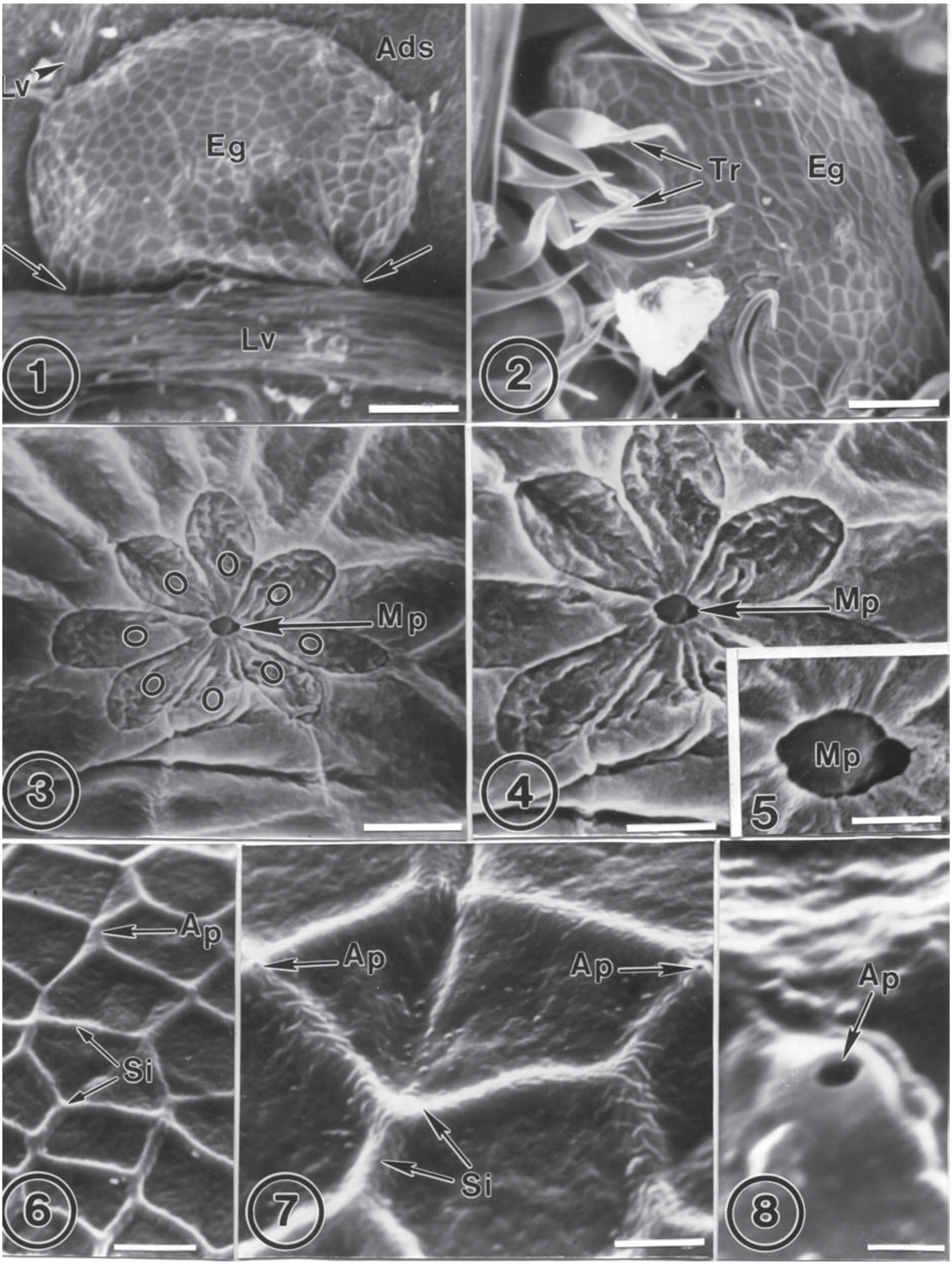
ABSTRACT: The eggshell of *Diaphania pulverulentalis* (Hampson) was investigated by scanning electron microscopy. Surface of the pyralid egg shows structural elements, viz., aeropyles and micropylar rosette around the micropyles. Eggs are longish-oval, 332.80±4.32 µm long, and 218.60±4.71 µm wide, marked by a reticulate pattern of rectangular and pentangular cells. Micropylar structures are situated at the anterior pole of the egg opposite to its point of attachment to the host plant leaf. Micropylar area has eight unequal petal-shaped cells occupying total length of 27.76±0.10 µm. Micropylar pit having 2.34±0.78 µm in diameter is at the centre of an asymmetrical rosette at the anterior pole. Aeropyles are annular, slightly raised above the chorionic surface. They have 0.32±0.005 µm in diameter, being surrounded by cell ridges. Total number of aeropyles varies from 15 to 18 per egg.

РЕЗЮМЕ: Оболочка яйца *Diaphania pulverulentalis* (Hampson) исследована при помощи сканирующей электронной микроскопии. Поверхность яиц огнёвок имеет структурные элементы, такие как аэропиле и микропилярную розетку вокруг микропиле. Яйца удлинённо-овальные, 332,80±4,32 мкм длиной и 218,60±4,71 мкм шириной, с сетчатым рисунком из прямоугольных и пятиугольных ячеек. Микропилярные структуры расположены на переднем полюсе яйца напротив точки присоединения яйца к листу растения-хозяина. Микропилярная область имеет восемь неравных лепестковидных ячеек, занимающих общую площадь 27,76±0,10 мкм. Микропилярная ямка диаметром 2,34±0,78 мкм расположена в центре асимметричной розетки заднего полюса. Аэропиле кольцевые, слегка приподнятые кад поверхностью хориона, 0,32±0,005 мкм в диаметре, окружены гребнями ячеек. Общее число аэропиле варьирует от 15 до 18 на яйцо.

Introduction

First studies of the structure and function of insect eggshell have been done by Leuckart [1855] and Korschelt [1887]. Light microscopic (LM) and physiological studies of gas exchange and permeability were followed by research [Beament, 1948; Wigglesworth and Beament, 1950] using transmission and scanning electron microscopy (TEM and SEM). Since that time, fine structure of a number of lepidopteran eggs has been described, with a particular emphasis on surface sculpture [Matheny & Heinrichs, 1972; Mazzini, 1974; Downey & Allyn, 1980; Hill, 1982; Salkeld, 1983, 1984; Kumar *et al*., 1999]. A taxonomic key of the family Pyralidae based on characters of egg chorion and its architecture in 15 species of sod worm moths has been created [Matheny & Heinrichs, 1972]. Due to small eggs size in the subfamily Crambinae it is difficult to employ conventional techniques for preparing slides and studying chorion structure. High resolution of SEM studies makes them an invaluable tool for examining diagnostic characters in insects. Hinton [1969] used scanning electron micrographs to illustrate respiratory systems of various insect eggshells.

Mulberry (*Morus* spp., family Moraceae), the sole food plant of silkworm, *Bombyx mori* L., an economically important insect used in sericulture, is also prone to attack by various insect pests in different seasons of the year. Recently, a new pest, leaf roller *Diaphania pulverulentalis* (Hampson), becomes a growing threat to mulberry in the southern sericultural states of India viz., Karnataka, Andhra Pradesh and Tamil Nadu with an average occurence of 21.77% [Rajadurai *et al*., 1999]. *D. pulverulentalis* feeds on young leaves of mulberry (Chawki leaves), thus being a serious pest and causing a quantitative crop loss. Consequently, it has a consider-



Figs. 1–8. Scanning electron micrographs of eggshell micropyle and aeropyles of *Diaphania pulverulentalis*: 1 — single egg (Eg) attached (arrow) between the leaf veins (Lv) on the adaxial surface (Ads) of mulberry leaf; 2 — lateral side of the egg (Eg) covered by leaf trichomes (Tr) of the host plant leaf; 3–4 — top view of egg pole showing micropylar region (Mp) surrounded by eight petal-shaped cells (O); 5 — magnified view of the micropyle (Mp); 6–7 — egg chorion with shell imprints (Si) and aeropyles; 8 — a single protruded aeropyle (Ap) on the shell imprints. Scale bars: 0.5 μm (8), 1.2 μm (5), 2.75 μm (7), 3 μm (3), 4 μm (4), 10 μm (6), 40 μm (2), 50 μm (1).

able impact on Indian sericulture. To our knowledge, little research of various stages of *D. pulverulentalis* has been carried out. The present study of the chorionic architecture and eggshell structure of this pest may help in establishing its effective control.

Materials and Methods

Laboratory culture of *D. pulverulentalis* has been established using freshly emerged adults. They were released in an insect cage (28 x 18 cm) and provided with 10% honey solution for feeding and fresh mulberry branches for egg-laying. After mating, females laid eggs on the abaxial leaf surface. Eggs were then gently removed with a fine paintbrush. For a SEM study, eggs were fixed for two hours at room temperature in 2.5% glutaraldehyde solution in 0.2M cacodylate buffer (pH 7.2), then dehydrated in a graded alcohol-acetone series, and dried in a critical point dryer (EMS – 850) using CO_2 as a transition fluid. Dried eggs were mounted onto copper stubs in different positions. Mounted stubs were gold coated (20 nm thick) in a sputter coater (EMS – 550) and examined using a JEOL 100 CX II ASID 4D scanning electron microscope at 20 kV.

Results and Discussion

Mated females of *D. pulverulentalis* laid eggs on the lower surface of mulberry leaves between the midribs and sub-veins (Figs. 1-2). A number of authors also describe that most of the moths lay their eggs on the lower surface of host plant leaves [Hinton, 1981, Goel & Kumar, 1987; Kumar *et al*., 1999]. Fresh eggs of *D. pulverulentalis* are creamy white, getting a pink shade before eclosion. Matheny and Heinrichs [1972] described chorion features of 15 pyralid species. Most of the eggs are creamy white or light yellow, turning yellow orange or deeper yellow before eclosion. Females of *D. pulverulentalis* laid single eggs amidst leaf trichomes to protect the developing embryo (Fig. 2). Eggs are attached to the leaf on their flat side and adhere to the substrate by a glue-like secretion. Hinton (1981) wrote that flat eggs are characteristic of families of the former Microlepidoptera — Tineidae, Gelechiidae, Gracillariidae, Pterophoridae, Pyralidae etc. This author also pointed out that size and shape of the eggs of the tortricid *Rhyacionia duplana* slightly varied according to the pine species on which they were laid. Eggs of *D. pulverulentalis* are longish-oval, 332.80±4.32 µm long and 218.60±4.71 µm at their maximum width, bluntly rounded at both ends (Figs. 1–2). Matheny and Heinrichs [1972] described longish-oval eggs in other pyralids, *Pediasia luteolella* and *Crambus decorellus*. Those eggs were 419±0.008 µm long, 295±0.004 µm wide and 582±0.012 µm long, 413±0.014 µm wide respectively. Eggs of the minimum size 396±0.006 µm long and 315±0.006 µm wide were observed in *Crambus alboclavellus*. Those dimensions are very close to the egg size of *D. pulverulentalis*. Arbogast *et al*. [1989] studied eggs of three moths under scanning electron microscope and found that eggs of *Niditinea fuscella*, *Tinea pallescentella* and *Tinea occidentella* were subcylindrical, having been 450, 640 and 620 µm long and 280, 330 and 360 µm wide respectively. The micropylar area is slightly raised at the anterior pole of the egg, and contains eight unequal petal-shaped primary cells (Figs. 3–4). Hinton [1981] found that micropyles are always situated at the anterior pole of lepidopteran eggs. However, unusual position of the micropylar structure on the lower surface of the egg of *Cydia pomonella* was observed [Fehrenbach *et al*., 1987]. The micropylar area with eight unequal petal-shaped cells is 27.76±0.10 µm wide. It forms an asymmetrical rosette at the anterior pole of the egg of *D. pulverulentalis* with a central micropylar pit (2.34±0.78 µm in diameter) (Figs. 3–4). Similar rosettes of unequal petal-shaped cells were studied in some other lepidopteran species, *Plusia orichalcea* [Goel & Kumar, 1987]; *T. pallescentella*, *T. occidentella*, *N. fuscella* [Arbogast *et al*., 1989] and *Spilarctia obliqua* [Kumar *et al*., 1999]. There are seven primary cells around the micropylar pit in *P. orichalcea*, 5–8 in *T. occidentella*, 6–9 in *N. fuscella* and 10–14 in *S. obliqua.* However, primary cells were of subequal lengths in some eggs of *N. fuscella*, thus forming symmetrical rosettes. Fehrenbach *et al*. [1987] examined fine structure of eggs of three lepidopteran species. They found that the micropylar rosette was composed of a rosette of 13–15 petal-shaped cells in *Heliothis virescens*, whereas in *Spodoptera littoris* the number of rosette petals varied from 6 to 11, with 7 and 8 having been the most frequent. In *Cydia pomonella* the micropylar rosette is composed of 8 or 9 rosette petals. Matheny and Heinrichs [1972] have used chorion characteristics of 15 species to prepare a taxonomic key of pyralid eggs of the subfamily Crambinae. They observed that the immediate micropylar region is surrounded by 5–8 cells in *Chrysoteuchia topiaria*, 5–9 cells in *Pediasia trisecta* and by a minimum of 4–6 cells in *Pediasia mutabilis*, *Crambus alboclavellus*, *Pediasia luteotella* and *Crambus decorellus*.

In *D. pulverulentalis,* the rosette of a single row of eight petal-shaped cells at the anterior pole of the egg is followed by densely ridged shell imprints, covering the rest of egg surface (Figs. 1, 2, 6, 7). These imprints are represented by rectangular and pentangular cells of variable size, 13.56±0.51 µm long and 8.61±0.22 µm

Рис. 1–8. СЭМ-фотографии микропиле и аэропиле оболочки яйца *Diaphania pulverulentalis*: 1 — одиночное яйцо (Eg), прикреплённое (стрелка) между жилками (Lv) на адаксиальной поверхности (Ads) листа шелковицы; 2 — боковая сторона яйца (Eg), покрытая волосками (Tr) листа растения-хозяина; 3–4 — полюс яйца с микропилярной областью (Mp), окружённой восемью лепестковидными ячейками (O), вид сверху; 5 — микропиле (Mp), увеличено; 6–7 — хорион яйца с отпечатками оболочки (Si) и аэропиле; 8 — одиночное аэропиле (Ap) на отпечатке оболочки. Масштаб: 0,5 мкм (8), 1,2 мкм (5), 2,75 мкм (7), 3 мкм (3), 4 мкм (4), 10 мкм (6), 40 мкм (2), 50 мкм (1).

wide (Figs. 7, 8). Fehrenbach *et al*. [1987] studied fine structure of eggshells of lepidopterous pests. They found that the network of the flanks usually consisted of rectangular plates ca. 35 µm long and ca. 25 µm wide which is about three times more than the corresponding panels of the eggs of *D. pulverulentalis*. Arbogast *et al.* [1989] examined external morphology of the eggs of three lepidopteran species using SEM. They found that egg chorion was marked by a reticulate pattern of polygonal (mostly hexagonal) cells in *T. occidentella* whereas in *N. fuscella* it was marked by bold pattern of strongly depressed polygonal (mostly hexagonal) cells outlined by heavy ridges. Recently, Kumar *et al*. [1999] wrote that the cell imprints of the eggs of *S. obliqua* were mostly pentagonal or hexagonal and the aeropyles were situated on their ridges. Similarly to results obtained by Fehrenbach *et al*. (1987), and Kumar *et al*., [1999], aeropyles were not observed on the first few rows of cells at the micropylar zone of the eggs of *D. pulverulentalis.* Aeropyles of the eggs of *D. pulverulentalis* are annular, slightly raised above the chorionic surface, having 0.32±0.005 µm in diameter. An average distance between the two aeropyles is 22.74±0.19 µm. Matheny and Heinrichs [1972] also found annular and slightly protruded aeropyles on the egg surface of *C. topiarie*s, *M. elegans* and *C. pascuellus floridus*. However, diameters of the aeropyles in these species, 3.33±0.35 µm, 2.56±0.17 µm and 2.14±0.15 µm were much larger than those structures observed in *D. pulverulentalis*. Arbogast *et al*. [1989] found that the diameter of aeropyle opening was in *T. occidentella* 0.55 to 0.93 µm (0.77±0.15), in *T. pallescentella* — 0.667 to 1.20 µm (0.94±0.15), and in *N. fuscella* — 0.65 to 1.50 µm (1.10±0.36) for anterior aeropyles and 0.55 to 0.75 µm (0.65±0.07) for posterior ones. Moreover, Fehrenbach *et al.* [1987] wrote that the approximate diameter of aeropylar openings in *H. virescens* was 1.9 µm and in *C. pomonella* — 0.6 µm. The number of aeropyles in *D. pulverulentalis* was substanially smaller, i.e. from 15 to 18 per egg, those structures having been found only at the anterior pole. Fehrenbach *et al*. [1987] reported that there were only 50 aeropyles restricted to the upper region of the egg of *H. virescens*, whilst there were about 400 apertures per egg in *S. littoralis* and about 140 ones — in *C. pomonella*. Recently, Kumar *et al.* [1999] found that the number of aeropyles in *S. obliqua* varied from 18 to 47 per shell imprint. These authors also wrote that the number of aeropyles for the whole egg was too high to be counted. Similarly to observations by Kumar *et al.* [1999] each aeropyle is surrounded by a well-defined collar in *D. pulverulentalis*. Aeropyles were also surrounded by collars in *T. occidentella* and *N. fuscella* [Arbogast *et al*., 1989].

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