Acta virol. 32 : 50—54, 1988

ELECTRON MICROSCOPIC STUDY OF DEVELOPMENTAL STAGES OF *RICKETTSIELLA PHYTOSEIULI* IN *PHYTOSEIULUS PERSIMILIS* ATHIAS-HENRIOT (GAMASOIDEA: PHYTOSEIIDAE) MITES.

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Received January 23, 1987; April 21, 1987

Summary. — *Rickettsiella phytoseiuli* was found in great amounts in all tissues except of the nervous system of adult *Phytoseiulus persimilis* mites. Six morphologically different stages (dense, intermediate, bacterial, giant, crystal-forming and small dark particles) of *R. phytoseiuli* were detected. No rickettsiae were seen in the larvae and in phase 1 and 2 nymphae of these mites.

Key words: Phytoseiulus persimilis; Rickettsiella phytoseiuli; electron microscopy

Introduction

Rickettsiella phytoseiuli was discovered in the mite *P. persimilis* (Šuťáková, 1977 *a*, *b*). It was seen accidentally when looking at the ultrastructure of the gastrointestinal tract of these mites. Up to now rickettsia-like microorganisms were observed in mites *Metaseiulus* (=*Typhlodromus*) *occidentalis* (Hess and Hoy, 1982). In *P. persimilis* closer unidentified bacteria were described in the lumen of the alimentary tract (Arutunjan, 1985) and double infection with virus-like particles and *R. phytoseiuli*, was also reported (Šuťáková and Rüttgen, 1978).

The difference between the presence of *R. phytoseiuli* in mites and the described rickettsiella in insects and spiders is based on the fact that the mites *P. persimilis* remained alive even at massive infestation while the infection in insects and spiders was lethal. In insects and spiders mainly the fat body was infected. In addition haemocytes were rarely involved. The infected individuals died mainly in the larval stadium. Several observations testify against the possible symbiotic relationship between rickettsiae and *P. persimilis* such as: spread of infection, the presence of rickettsiae in the majority of organs and also the fact that *P. persimilis* mites studied by Arutunjan (1985) had not been infested with rickettsiae.

Up to now no attention was paid to the developmental stages of *P. persimilis*. All these data as well as the increasing practical application of *P. persimilis* for biological control against *Tetranychus urticae* mites in

glasshouse farming prompted us in 1986 to return again to the problem of the relationship between *R. phytoseiuli* and *P. persimilis*.

The present paper deals with the ultrastructure of the developmental stages and adults of *P. persimilis* mites owing to the occurrence of rickettsiae.

Materials and Methods

P. persimilis mites were obtained from the laboratory breed of the Institute of Experimental Phytopathology and Entomology (Jedličková, 1983; 1985). A sample of mites originated from the Institute while others were obtained after their introduction into the glasshouse of the Cooperative farm Kvetoslavov. In both cases the predators were fed by *T. urticae* KOCH mites. In the laboratory breed *T. urticae* were kept on bean plants and in the glasshouse on cucumbers.

For electron microscopy the larvae, nymphae of the first and second stage as well as adult individuals, males and females, were used from the laboratory breed. The mites were not autopsied. In order to obtain maximum penetration of the fixative, the heads, legs or end parts of the body of mites were cut off, and apertures in the cuticle were made. The mites were fixed for 6 hr in cold 4% glutaraldehyde in 0.2 mol/l cacodylate buffer, pH 7.4, and post-fixed for 60 hr at room temperature in the same buffered 2% OsO_4. Tissues were dehydrated in ethanol and embedded in Spurr's low-viscosity medium (Spurr, 1969). Ultrathin sections were prepared on ultramicrotome LKB III and REICHERT III and were contrasted with uranyl-formol (Mazza and Casale, 1979; Mazza *et al.*, 1981). The ultraphin sections were examined in a TESLA electron microscope BS 500 and photographed on glass plates ORWO EU 2.

Results

Ultrathin sections (transversal, longitudinal, and oblique) were prepared from whole larvae and nymphae of the first and second stage; from each group five individuals were evaluated. So far no rickettsiae could be seen in any of the given groups (Figs 1—3).

Similarly to the larvae and nymphae, the adult individuals were not autopsied, but were processed as whole regardless whether coming from the breed or from a given locality. No difference was observed between these two groups of mites. All the mites, i.e. 10 individuals (5 from each group) were considerably infested with the rickettsiae. The rickettsiae were present in the whole body of the mite (Fig. 4) except of nervous tissue; a less numerous occurrence was examined in muscles. The infestation of fat tissue was the most extensive (Fig. 5). A great number of rickettsiae was observed in the lumen of all parts of the gut, gut diverticula (Fig. 6) as well as in the lumen Malpighian tubules (Fig. 7).

Rickettsiae were rarely found individually, they were mostly in clusters — accumulated in groups or in vacuoles. The size of vacuoles considerably varied from the smallest ones filled with a few rickettsiae to the largest ones occupying a considerable part of the cell. Some of the vacuoles had exclusively one type of rickettsiae while in other vacuoles mixed stages of rickettsial cells were found.

In all samples of the investigated adult individuals the following morphologically different rickettsiae were observed: dense, intermediate, bacterial, giant, crystal-forming and small dark particles (rickettsiae) within the latter (Fig. 8). Binary fission of bacterial forms of rickettsiae, particularly in the fat tissue occurred occasionally (Fig. 9).

Discussion

Adult individuals of the mite *P. persimilis* either obtained from the breed of the institute and or those after introduction into glasshouses contained a new species of rickettsiae designated *R. phytoseiuli* (Šuťáková, 1977*a*, *b*). In contrast to preceding results, the occurrence of these microorganisms was detected also in salivary glands, Malpighian tubules and gut diverticula. The rickettsiae did not occur in the nervous tissue.

In all so far studied individuals we found again 6 developmental stages of rickettsia *P. persimilis*, i.e. dense rickettsia, intermediate, bacterial (among them binary fission of cells was seen), giant, and crystal-forming in which small dark particles had developed. We consider these small dark particles for the first developmental phase. When the small dark particles are released from the crystal-forming rickettsiae, they grow, gain their membrane complex and change in this way to the dense type of rickettsiae. These are transformed to intermediate and later to bacterial types of rickettsiae. The bacterial types of rickettsiae can multiply by binary fission. Some bacterial rickettsiae increase largely and form the giant type rickettsiae. These form a crystal inside and so they are converted into crystal-forming types of rickettsiae in which the above mentioned small dark particles had already developed. For the genus *Rickettsiella* no such reproductive cycle has been reported. The significance of the crystal in the developmental cycle is unknown, although noticed by many authors (Krieg, 1960; Huger and Krieg, 1967; Weiser and Žižka, 1968; Götz, 1972; Federici, 1980). We think that the small dark particles can be liberated into the surrounding after breaking the cell wall, and after undulation and enlargment of cell wall of crystal--forming rickettsiae a new vacuole arises in which small dark particles remain and continue the next reproduction cycle. Similar formation of new vacuoles was assumed by Huger and Krieg (1967). Concerning other members of genus *Rickettsiella* about 3 to 5 developmental stages were observed. From the species having 5 developmental stages, *R. phytoseiuli* differ by occurrence of small dark particles inside crystal-forming cells. In the same way this rickettsia multiplied in haematophagous ticks *Dermacentor reticulatus*, in which *R. phytoseiuli* were cultivated after their previous isolation from mites *P. persimilis* (Šuťáková and Řeháček, 1988).

Owing to a considerable infection of adult individuals of *P. persimilis* the occurrence of rickettsiae was revealed both in larvae and nymphae of this mite. Up to now we did not succeed in detection of rickettsiae in any sample of larvae or nymphae of this mite. In the literature we found microorganisms in eggs and in other developmental stages as well as in audult individuals of *M. occidentalis* (Hess and Hoy, 1982) . The above mentioned authors have described two types of microorganisms in healthy as well as in unhealthy mites (according to external symptoms). Type A occurred only intracellullarly, type B — intra — and extracellularly. These two cell types differed morphologically and it may be presumed that in the first case we have to do with symbionts and in the second one with pathogens. In addition closer unidentified bacteria were seen, in the lumen of the alimentary

tract of *P. persimilis* when in some cases they were attached to microprojections in the gut diverticula but they were not found within epithelial cells (Arutunjan, 1985). These bacteria morphologically differed from the rickettsiae. The results obtained so do not allow to distinguish whether the rickettsiae are or are not pathogenic for their hosts. This will be the aim of our future work. We also wish focus our attention on the source of rickettsiae infection of *P. persimilis*. The extension of infection (both in the years 1974—75 and in the years 1984—86) does not indicate an occasional event. An accidental and not very massive viral infection was recorded for *P. persimilis* in a part of the material used for the study of the ultrastructure of *P. persimilis* (Šuťáková and Rüttgen, 1978).

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Explanation of Electron Micrographs (Plates II—VII):

Fig. 1. Part of oblique section of the larva of mite *Phytoseiulus parsimilis*. FB — fat tissue, He — haemocoel, NC — new cuticle, OC — old cuticle, SG — salivary glands. × 1,840.

Fig. 2. Part of oblique section of the protonymph of mite *P. persimilis*. D — diverticulum, EC — epithelial cell, L — lumen of midgut, H — hypodermis, NC — new cuticle. × 2,900.

Fig. 3. Part of longitudinal section of the end part of deutonymph of mite *P. persimilis*. H — hypodermis, NC — new cuticle, OC — old cuticle, O — ovary. × 9,850.

Fig. 4. Part of longitudinal section of the adult mite *P. persimilis*. C — cuticle, FB — fat tissue, H — hypodermis, O — ovary. × 1,600.

Fig. 5. Transversal section of the fat tissue of mite *P. persimilis* with a massive rickettsial infection. BR — bacterial rickettsiae, CR — crystal-forming rickettsiae, DR — dense rickettsiae, N — nucleus. × 4,250.

Fig. 6. Rickettsiae in the lumen and epithelial cell of the midgut of *P. persimilis*. BR — bacterial rickettsiae, DR — dense rickettsiae, EC — epithelial cell, L — lumen. × 7,050. (inset × × 22,000).

Fig. 7. Rickettsiae in the lumen of Malpighian tubule of *P. persimilis*. Cu — cuticle, ML — lumen of Malpighian tubule. × 4,600.

Fig. 8. Different variations of crystal-forming rickettsiae the mite *P. persimilis*. CR — crystal-forming rickettsiae. SP — small dark particles. × 14,300.

Fig. 9. Dense, intermediate, bacterial and giant rickettsiae of mite *P. persimilis*. BR — bacterial rickettsiae, DR — dense rickettsiae, IR — intermediate rickettsia, GR — giant rickettsia. × 57,850.