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# ULTRASTRUCTURAL STUDIES ON THE PLANTHOPPER, *PEREGRINUS MAIDIS* (ASHMEAD), VECTOR OF MAIZE MOSAIC AND MAIZE STRIPE VIRUSES

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## ABSTRACT

The delphacid planthopper, *Peregrinus maidis* (Ashmead) is the only known vector of two widely distributed tropical and subtropical maize pathogens: maize mosaic virus (MMV), a rhabdovirus, and maize stripe virus (MStpV) which belongs to the new 'rice stripe' virus group. Ultrastructural studies were carried out on various organs, particularly the salivary glands of adult *P. maidis*. Striated muscle fibers were found in various acini of the principal, but not the accessory, salivary glands. This and other observations suggested that the system for transporting secretion within the principal gland is different from that within the accessory gland. In MMV-infected adult *P. maidis*, MMV particles were found in the salivary glands, brain, nerve ganglia, compound eye, leg muscle, foregut, midgut, tracheae, epidermis and fat and connective tissues. MMV particles appeared to bud on three types of membranes: (1) inner and outer nuclear membranes, (2) intracytoplasmic membranes, and (3) plasma membranes, particularly in the salivary glands. In most tissues, MMV particles accumulated mainly within cisternae connected to the perinuclear space. However, in the principal salivary gland, virus particles accumulated mainly in intercellular and extracellular spaces of various acini. These spaces are apparently connected with extracellular canaliculi, which lead to the salivary ductules and ducts. In MStpV-infective adults, no structures associated with MStpV were found. However, several structures unrelated to either MMV or MStpV were found in *P. maidis*: these included paramyxovirus- and rickettsia-like structures found in various organs, in addition to yeast-like symbiotes found in adult fat tissue and embryonic mycetome.

## INTRODUCTION

In a recent review (Ammar 1985), the paucity of knowledge on ultrastructural studies of leafhoppers and planthoppers was indicated. However, these studies are of prime importance for understanding the physiology of these insects, their disease-vector relationships, and for studying disease agents *in situ* inside their vectors. The present work illustrates this point, with particular reference to the delphacid planthopper *Peregrinus maidis* (Ashmead), the only known vector of maize mosaic (MMV) and maize stripe (MStpV) viruses. Diseases caused by these viruses are widely distributed on maize plants in some parts of Africa, Asia and North, Central and South America (Damsteegt 1981). More than twenty years ago, rhabdovirus particles of MMV were found by electron microscopy in the salivary glands and midgut cells of *P. maidis* in Venezuela (Herold & Munz 1965). However, only recently when an

ultrastructural study of the salivary glands and other organs of this insect was conducted (Ammar 1985, 1986), have the assembly and accumulation sites of MMV in its vector been identified (Ammar & Nault 1985). During these studies, several other virus or microorganism-like structures were encountered in various tissues of *P. maidis* (Ammar *et al.*, 1986). Following is a brief review of the above mentioned studies, in addition to some electron micrographs that show assembly and accumulation sites of MMV in *P. maidis*, and possible routes for transportation of assembled virus particles in the salivary glands of this vector.

## ULTRASTRUCTURE OF THE SALIVARY GLANDS OF *P. MAIDIS*

The paired salivary glands in *P. maidis* are similar in gross morphology to those described for other delphacids by Sogawa (1965). Ultrastructurally, the principal and accessory salivary glands, respectively, are composed of six and one acinar types of secretory cells, in addition to duct cells in both glands. Each of these acinar types contains secretion vesicles which are different in electron opacity, internal structure, shape and size from those of other acinar types (Ammar 1986). In the principal salivary gland, extensive basal infoldings of the plasma membrane in secretory cells (Fig. 1), and apical infoldings of the plasma membrane in duct cells are observed. Secretory cells contain elongated vacuoles partly lined by microvilli and by microtubule bundles (Fig. 2). These vacuoles are apparently connected with extracellular canaliculi deeply invaginated into secretory cells. Canaliculi of each acinus lead to the ductule lumen, which is lined with spiral cuticular intima, surrounded by duct cells. Striated muscle fibers, supplied with small nerve axons, and tracheoles, are found in various acini of the principal gland, usually around secretory and duct cells. A single myoepithelioid cell has been reported in the principal salivary gland of aphids (Ponsen 1972), but muscle fibers in insect salivary glands apparently have been reported only in leafhoppers and planthoppers (Ammar 1984).

In the accessory salivary gland of *P. maidis*, the two large secretory cells contain no elongated vacuoles or canaliculi invaginations. However, in their central region, apically, these cells border a large microvilli-lined canal with its own canal cells. This canal is apparently connected with the cuticle-lined accessory duct, formed by duct cells. Nerve axons, but no muscle fibers, are found in the accessory gland and its duct. Based on the above mentioned differences between the principal and accessory salivary glands of *P. maidis*, it was suggested that the system for transporting secretion within the principal gland, is basically different from that within the accessory gland (Ammar 1986).

## ASSEMBLY AND ACCUMULATION SITES OF MMV IN ITS VECTOR

In MMV-infected adults of *P. maidis*, bullet shaped (234 x 60 nm) or bacilliform (247 x 60 nm) virus particles of MMV were found in the following organs and tissues: principal and accessory salivary glands (Figs. 1&2), foregut, midgut, nerve ganglia, brain, (Figs. 3&4) epidermis, tracheal epithelium (Fig. 5), leg muscles , fat and connective tissues, retinula cells of the compound eyes, (Fig. 6), epithelium of the male ejaculatory duct and in follicular cells of the ovaries (Ammar and Nault, 1985). Particles longer than normal, up to four times the normal length, were found, particularly in vacuoles of the salivary gland secretory cells (Figs. 2&8). Accumulation of MMV particles in various tissues of the vector was much less massive than accumulation of these particles in cells of diseased maize plants (McDaniel *et al.* 1985). Also, a higher proportion of budding particles with intermediate

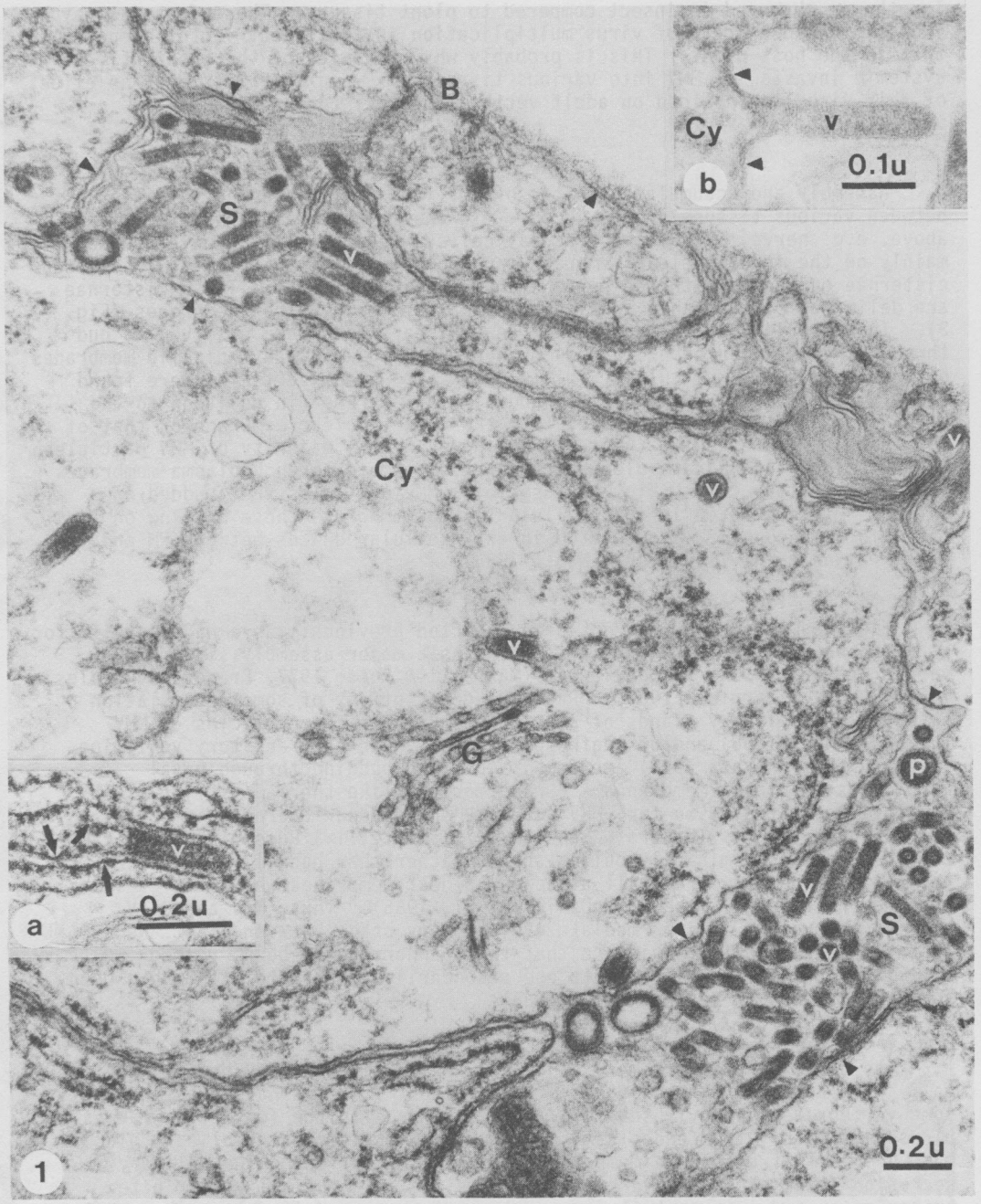


Fig. 1. Maize mosaic virus (MMV) particles (v) accumulated in intercellular and extracellular spaces (S) or scattered in the cytoplasm (Cy) of a salivary gland secretory cell in *P. maidis*. Arrowheads indicate plasma membranes. Insets: MMV particles budding through membranes of the endoplasmic reticulum (inset a) or the plasma membrane (inset b). B = basal lamina, G = golgy body, p = paramyxovirus-like particle.

length was observed in insect compared to plant tissues. These features may indicate a slower rate of virus multiplication in the insect vector compared to that in the host plant. This is probably why, in spite of the apparently systemic invasion of MMV into various tissues of *P. maidis*, little or no effect of this virus was noticed on adult vector longevity (Ammar & Nault unpublished).

Assembly and accumulation sites of MMV in *P. maidis* seemed to differ between various tissues of the vector. In the majority of tissues mentioned above, e.g. nerve, midgut and tracheal cells, MMV particles appeared to bud mainly on the inner nuclear membrane, accumulating in intracytoplasmic dilated cisternae connected with the perinuclear space (Figs. 3-5). These cisternae are delimited by membranes continuous with the outer nuclear membranes (Fig. 3). In the principal salivary gland few MMV particles were observed to bud through inner and outer nuclear membranes or through intracytoplasmic membranes of the endoplasmic reticulum (Fig. 1, inset a). These particles were found usually singly or in twos, tightly bound by a membrane (in addition to the virus envelope), probably derived from the outer nuclear membrane or that of the endoplasmic reticulum (Figs. 1&7). However, the majority of MMV particles in the principal salivary gland were observed to bud through plasma membranes (PM) of secretory cells (Fig. 1, inset b). MMV particles that budded on lateral PM accumulated in intercellular spaces, whereas those budding on the basal infoldings of PM accumulated in extracellular spaces between PM and the basal lamina (Fig. 1) (Ammar & Nault 1985).

The plasma membrane had not been reported previously as a budding site for plant rhabdoviruses, although it is known as a major assembly site for vertebrate and insect rhabdoviruses (Martelli & Russo 1977, Francki & Randles 1980, Murphy & Harrison 1980). Previous descriptions of some accumulation sites in the salivary glands of planthopper vectors for particles of MMV (Herold & Munz 1965) and of leafhopper vectors of rice transitory yellowing virus (RTYV) (Shikata 1979) are consistent with budding through basal infoldings of plasma membranes. Furthermore, unlike the majority of plant rhabdoviruses studied so far, MMV and RTYV share other characteristics with animal rhabdoviruses (Peters & Schultz 1975, Martelli & Russo 1975): 1. their particles are predominantly bullet-shaped rather than bacilliform; and 2. MMV particles frequently are still attached to host membranes when particles have reached their normal length (McDaniel et al. 1985). Thus, MMV and RTYV possibly represent an intermediate evolutionary stage between plant and animal rhabdoviruses. Further evidence that the Auchnorrhyncha-borne rhabdoviruses most likely originated in insects and secondarily adapted to plants has been forwarded and discussed by Nault (1986).

The difference in assembly and accumulation sites of MMV between the salivary glands and other tissues of the vector *P. maidis* has an analogy in some vertebrate rhabdoviruses, e.g. rabies virus. In the fox brain, rabies virus buds mainly from endoplasmic reticulum, whereas in the salivary glands it buds primarily from plasma membranes of mucous cells facing the salivary secretion space; this delivers infectious virus into the saliva for bite transmission and is essential for survival of the virus in nature (Murphy & Harrison 1980). Bearing in mind the ultrastructure of the salivary glands of *P. maidis* (Ammar 1986), it is possible to suggest the routes for transportation of MMV particles assembled in the salivary glands of the vector, until they are finally expelled with salivary secretions into the host plant during insect feeding and salivation. Since most MMV particles in the salivary glands bud on plasma membranes and accumulate in intercellular or extracellular spaces, it is likely that these particles are discharged directly into the extracellular

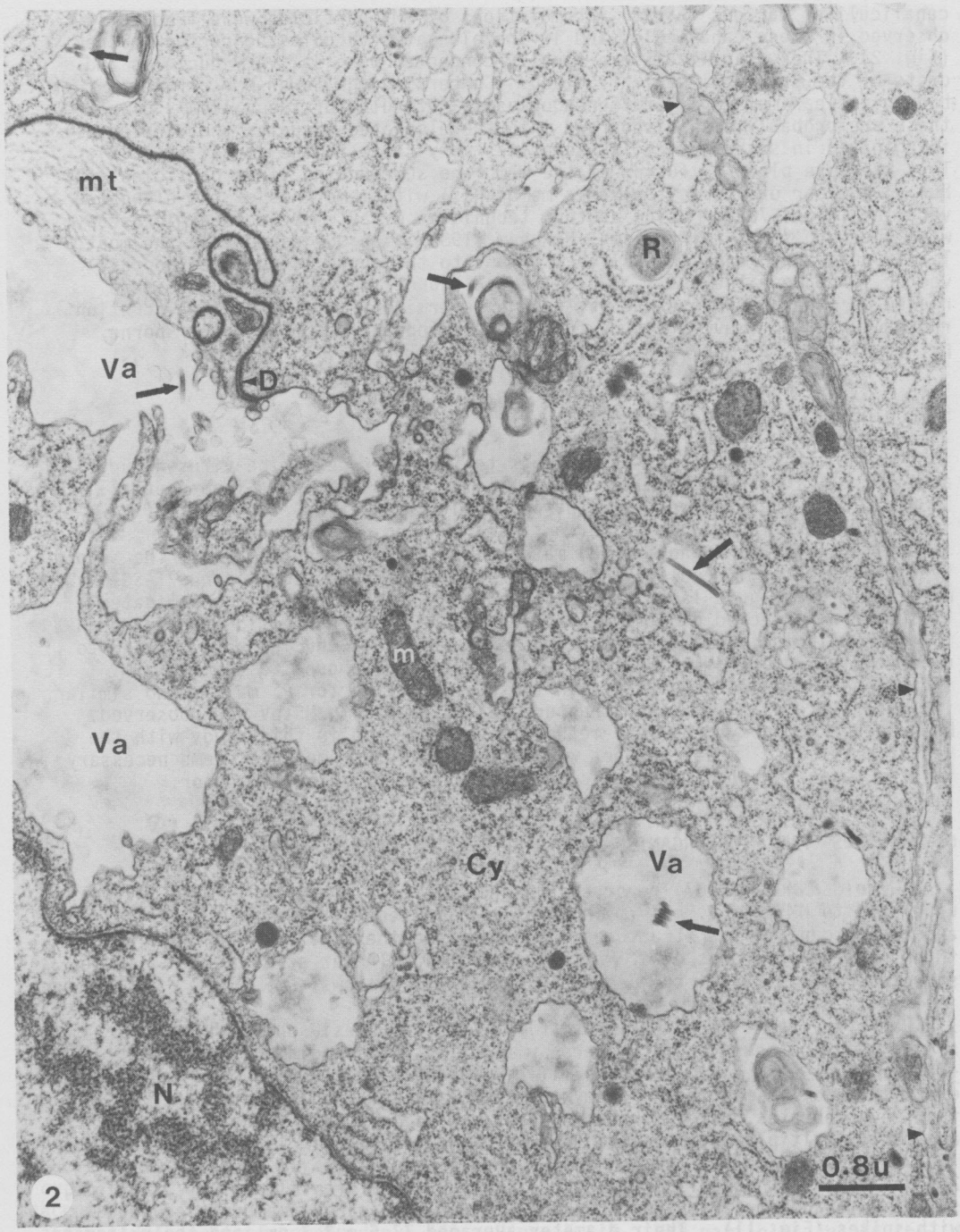


Fig. 2. Maize mosaic virus particles (arrows) in irregularly shaped or elongated vacuoles (Va), which are deeply invaginated into the cytoplasm (Cy) of a salivary gland secretory cell. Arrowheads indicate plasma membranes. D = desmosome, m = mitochondrion, mt = microtubules, N = nucleus, R = rickettsia.

canaliculi of various acini. Accumulations of MMV particles were frequently observed in these canaliculi, and in the elongated vacuoles connected to them (Fig. 2). These canaliculi are finally connected with salivary ductules and ducts of the principal salivary gland. Concerning the fewer MMV particles that bud on nuclear membranes or the endoplasmic reticulum of the salivary gland, these are probably also discharged into the elongated vacuoles that are deeply invaginated in secretory cells, sometimes very close to the nuclei (Fig. 2). This may take place by fusion of the membrane surrounding virus particles (that bud on nuclear or intracytoplasmic membranes) with membranes of the elongated vacuoles (Figs. 7&8). Using another technique, e.g., fluorescence microscopy, with the corn stunt spiroplasma, Markham (1983) also suggested that the most likely pathway through the salivary glands to the ducts of the (leafhopper) vector is between the cells, along the cell junction. Similar studies are needed on other pathogens and vectors to determine if the 'pathogen-secretion' routes suggested above are true for other leafhopper- or planthopper-borne pathogens.

## SEARCHING FOR MSTPV IN THE VECTOR

Maize stripe virus (MStpV) belongs to the new rice stripe virus group, associated with fine (ca. 3 nm diam.) filamentous particles (Gingery *et al.* 1983). In several tissues of MStpV-infected maize leaves, two types of inclusions were found: one type consisted of long narrow bundles of filamentous electron-opaque (FEO) material, with 3.5 nm periodicity; the second type consisted of irregularly shaped masses of amorphous semi-opaque (ASO) material. Using gold-labelled antibodies on thin sections of non-osmicated tissues from infected plants, antibodies to the MStpV noncapsid protein bound to the FEO inclusions, whereas antibodies to the MStpV nucleoprotein bound to unidentified cytoplasmic areas, but neither antibody bound to the ASO inclusions (Ammar *et al.* 1985). In tissues of the vector *P. maidis*, no similar inclusions or any virus-like structures associated with MStpV were observed. However, no immunocytochemical methods have been used in this study with the vector's tissues. Thus, further work using the latter methods seems necessary for the detection of MStpV particles in tissues of the insect vector.

## OTHER VIRUS- AND MICROORGANISM-LIKE STRUCTURES FOUND IN *P. MAIDIS*

Herold & Munz (1967) reported the presence of a spherical 'latent' virus, unrelated to MMV, in *P. maidis* in Venezuela. Recently, several other virus- and microorganism-like structures unrelated to either MMV or MStpV have been found in several organs of *P. maidis* from Florida (Ammar *et al.* 1986). These structures (Figs. 9-12) are described briefly as follows:

1. Paramyxovirus-like particles (PLP). These are quasi-spherical, single-membrane bound bodies covered with spikes; their diameter averaged 134.4 ± 2 nm. They were found in the salivary glands (Figs. 1&10), nerve ganglia, ovarioles and leg muscles. Paramyxoviruses have not been reported previously from invertebrates, but an orthomyxovirus, swine influenza virus, can apparently multiply in the swine lung worm (Bellett *et al.* 1973).

2. Filamentous rhabdo-like structures (FRS). These are filamentous, flexible, sometimes branched structures apparently bound by a single membrane covered with spikes (Fig. 11). Their diameter averaged 77.6 ± 3.7 nm, and their length reached 800 nm. It is suggested that the FRS are probably the filamentous forms of the PLP described above.

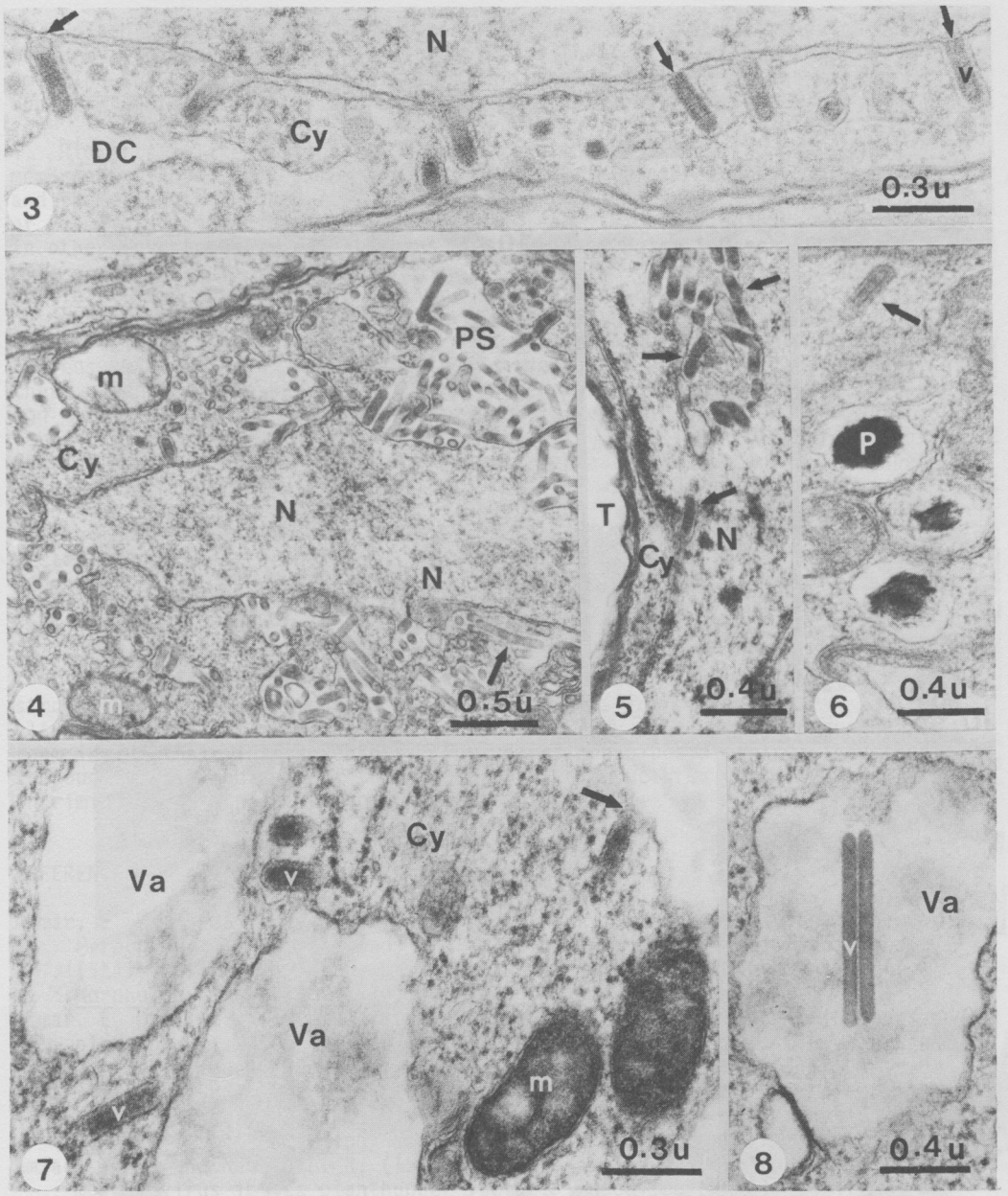


Fig. 3. Maize mosaic virus (MMV) particles (v) budding through the inner nuclear membrane (at arrows), into dilated cisternae (DC) branched in the cytoplasm (Cy) of a brain cell in *P. maidis*. Figs. 4-6. MMV particles accumulated in perinuclear space (PS) of a brain cell (4), tracheal cell (5) and retinula cell of compound eye (6). Figs. 7 & 8. MMV particles (v) in the cytoplasm (Cy) or vacuoles (Va) of salivary gland secretory cells; arrow indicates particle apparently entering a vacuole. m = mitochondrion, N = nucleus, P = pigment, T = tracheal lumen.

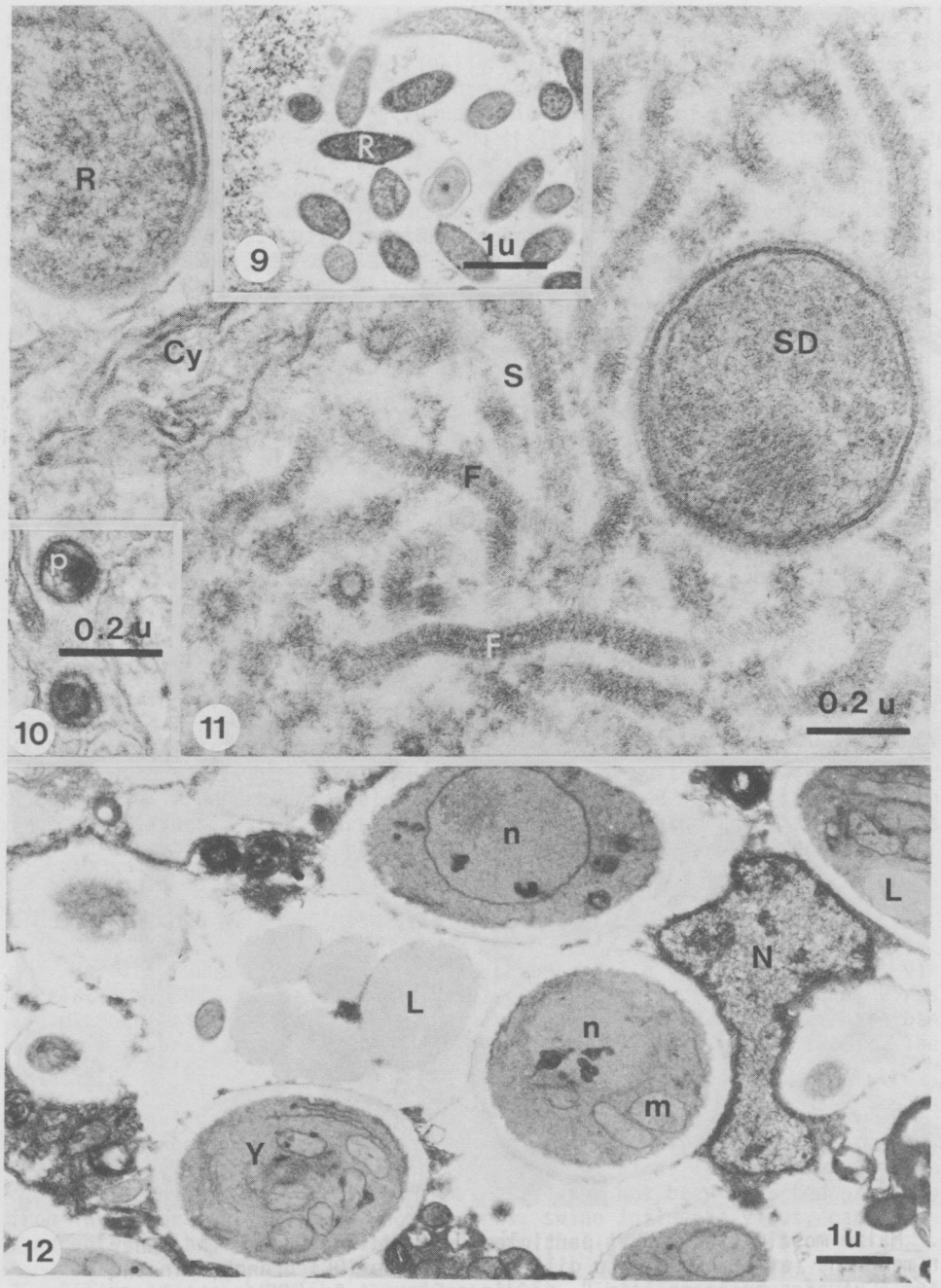


Fig. 9. Rickettsia-like structures (R) in the nucleus of a midgut cell in *P. maidis*. Figs. 10 & 11. Paramyxovirus-like (p), filamentous rhabdo-like (F) and spiked-double-membrane (SD) structures in extracellular spaces (S) of salivary gland cells. Cy = cytoplasm, R = rickettsia. Fig. 12. Cell-walled yeast-like structures (Y) in the embryonic mycetome of *P. maidis*. L = lipid, m = mitochondrion, n = yeast nucleus, N = mycetocyte nucleus.

3. Spiked double-membrane structures (SDS). These are quasi-spherical bodies, bound by two membranes covered with spikes. Their diameter averaged 394 ± 16 nm. The SDS were always associated with the FRS; frequently, the latter appeared contiguous with, and extended from, envelopes of the SDS (Fig. 11).

4. Rickettsia-like structures. These are elongated bodies, bound by two membranes with no spikes (Figs. 2,9&11). Their diameter averaged 457 ± 15.6 nm and their length reached 1.7 um. They were found in several organs and tissues of *P. maidis*, intracytoplasmic or intranuclear. Rickettsiae have been reported in other invertebrates as pathogens or symbiotes (Weiss & Dasch 1981).

5. Yeast-like symbiotes. These were abundant in the egg (embryonic) mycetome but observed less frequently in some cells of fat tissues in the head of *P. maidis* (Ammar 1985). These structures possessed a cell wall, a membrane-bound nucleus, mitochondria, lipid and electron dense inclusions (Fig. 12). Some of these organisms appeared to be in the process of budding or cleavage. In the egg mycetome and adult fat tissue, mycetocytes contained lipid material; their large nuclei were irregularly shaped, protruding sometimes to engulf one or more of the yeast-like symbiotes (Fig. 12). Yeast-like symbiotes have been reported from several other delphacids (Nasu *et al.* 1981). Other types of symbiotes have been reported in leafhoppers. A significant role for a symbiote in the mechanism of transovarial transmission of rice dwarf virus in its vector, *Nephotettix cincticeps*, was suggested (Nasu 1965). No similar studies have been reported on other Auchenorrhyncha-borne viruses to examine the possible role of symbiotes in transovarial transmission of these viruses.

The presence of the above described virus- and microorganism-like structures in various tissues of *P. maidis*, calls for caution in ultrastructural (*in situ*) studies of plant pathogens of unknown or uncertain form inside their insect vectors. For unless non-exposed control insects are thoroughly examined, any of the above or other structures could be wrongly incriminated as a plant pathogen.

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