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LIGHT AND ELECTRON MICROSCOPY OF CELLS INFECTED WITH MACLURA MOSAIC VIRUS

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Introduction

Maclura pomifera (Raf.) Robinson (osage orange) is a North American woody plant belonging to the family *Moraceae*. Its orange like fruits are very attractive. In Europe this plant can be found as an ornamental tree grown in the parks as well as a hedge plant because of its strong thorns.

An elongated virus has been isolated from hedge grown *Maclura* plants with obvious mosaic symptoms (Pleše and Miličić 1973) (Fig. 1. A). Its host range, transmissibility, properties in vitro, particle size and cell inclusions were investigated in detail, and the virus was named maclura mosaic virus (MacMV) (Pleše and Štefanac 1976).

In the host cells MacMV induced lamellar inclusions, a characteristic feature of the potyvirus group. However, it has a considerably shorter virus particle than potyviruses. In addition, this virus differs from potyviruses also in some other properties. Since the results of light microscopic and submicroscopic investigations are mentioned only partially in the above paper, detailed data of these investigations are completely presented here.

Materials and Methods

Light microscope investigations were carried out on infected leaf tissue of *Tetragonia expansa* Thunb. which was mechanically inoculated with MacMV. MacMV causes diffuse chlorotic local lesions (Fig. 1. B) on this plant. Epidermal strips of living tissue were taken from the local lesion area and examined.

Electron microscope examinations were done by using infected tissue of *T. expansa* and *Maclura pomifera*. The leaf tissue was sectioned in the region of local lesions of *Tetragonia* plant, and in the region of mosaic alterations of *Maclura* plant. Pieces of leaf tissue were fixed for 30 min in 1% (v/v) glutaraldehyde in cacodylate buffer pH 7.2 and after appropriate washing in buffer they were postfixed for 2 hr in 1% (w/v) osmium tetroxide. After fixation samples of tissue were dehydrated in ethanol series and embedded in Araldite resin. The ultrathin sections were stained with uranyl acetate and lead citrate, and analysed in a Siemens Elmiskop I.

Results and Discussion

Light microscopy

Amorphous inclusion bodies could be easily found in the region of local lesions of *T. expansa*. The amorphous inclusions show a granular structure. They were commonly placed near the nucleus and mostly were larger than the nucleus (Fig. 1. C). Sometimes single minute needle-shaped crystals were seen within the amorphous inclusion body. How-

Fig. 1. Symptoms and inclusion bodies induced by MacMV: *A* — branchlet of mother plant *Maclura pomifera* with mosaic symptoms on the leaves, *B* — diffuse local lesions on *Tetragonia expansa*, *C* — amorphous granular inclusion body (ai) near the nucleus (n) in the large leaf epidermal cell of infected *T. expansa*.

Sl. 1. Simptomi i virusne stanične uklopine izazvani MacMV: *A* — grančica izvorne biljke *Maclura pomifera* s mozaičnim simptomima na listovima, *B* — difuzne lokalne lezije na vrsti *Tetragonia expansa*, *C* — amorfna zrnata uklopina (ai) uz jezgru (n) u velikoj epidermskoj stanici lista zaražene biljke *T. expansa*.

Fig. 2. Submicroscopical view of leaf cells infected with MacMV: *A* — ultrathin section of the mesophyll cell of mother plant *Maclura pomifera;* pw pinwheels, l laminated aggregates, n nucleus, v vacuole. *B* — ultrathin section of amorphous inclusion body in epidermal cell of *Tetragonia expansa;* amorphous inclusion body contains pinwheels (pw), bundles (b) and laminated aggregates (l); cm crystal containing microbody, m mitochondrion, mb myelinic body, v vacuole, w cell wall.

Sl. 2. Submikroskopske promjene u stanicama lista zaraženim MacMV: *A* — ultratanki presjek kroz stanicu mezofila matične biljke *Maclura pomifera;* pw strukture pinwheel, l laminarni agregati, n jezgra, v vakuola. *B* — ultratanki presjek kroz amorfnu uklopinu u epidermskoj stanici biljke *Tetragonia expansa;* amorfna uklopina sadrži strukture pinwheel (pw), svežnjiće (b) i laminarne agregate (l); cm mikrotijelo (peroksisom) s kristalom, m mitohondrij, mb mijelinska figura, v vakuola, w stanična stijenka.

Fig. 3. Ultrathin section of leaf tissue of *Tetragonia expansa* infected with MacMV. Extraprotoplasmic membrane-bound aggregates of virus particles (mvp) situated between the plasmalemma (p) and the cell wall (w); b bundle, c callose, pd plasmodesma, v vacuole.

Sl. 3. Ultratanki presjek kroz lisno tkivo vrste *Tetragonia expansa* zaražene MacMV. Ekstraprotoplazmatski membranom omeđeni agregati virusnih čestica (mvp) smješteni između plazmaleme (p) i stanične stijenke (w); b svežnjić, c kaloza, pd plazmodezma, v vakuola.

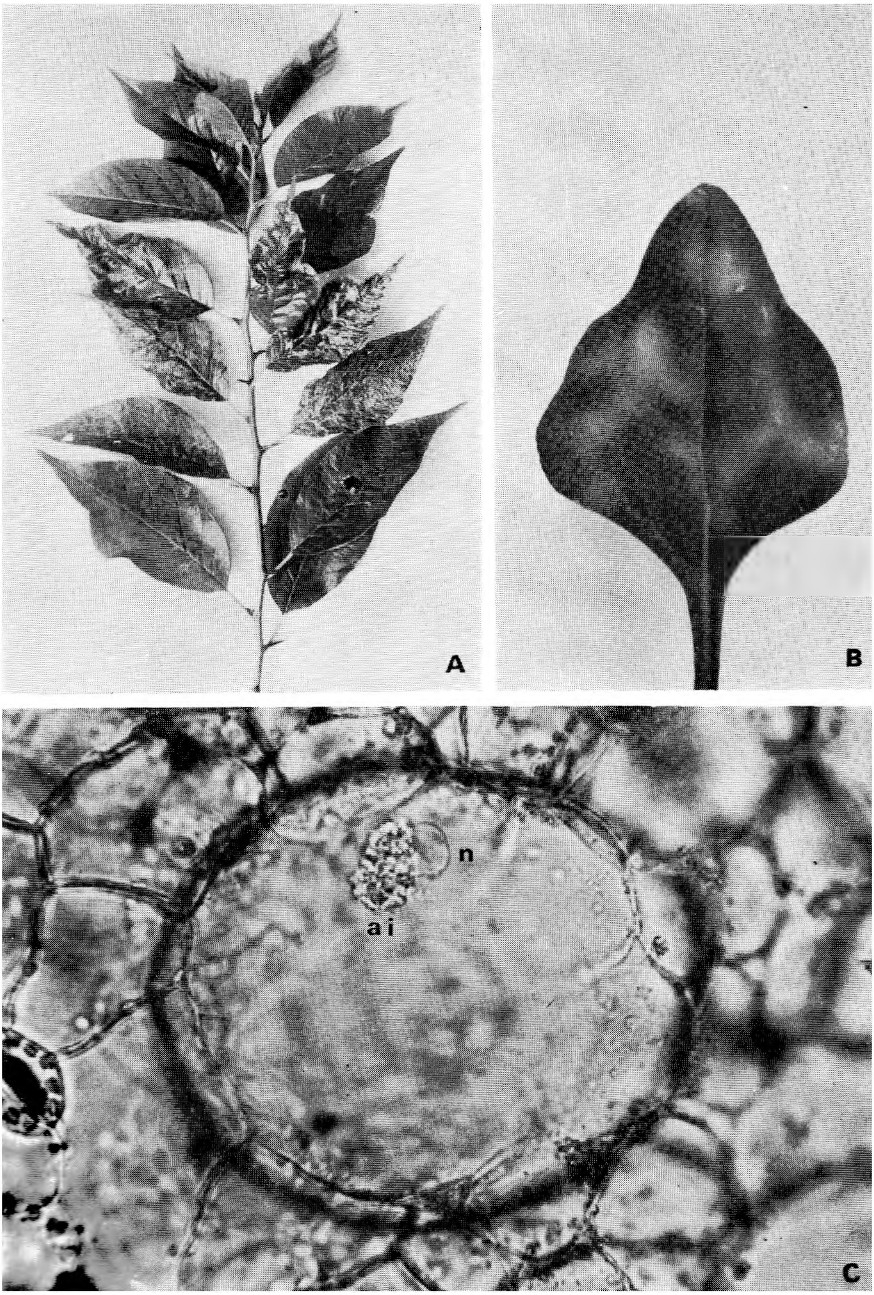

Fig. 1. — Sl. 1.

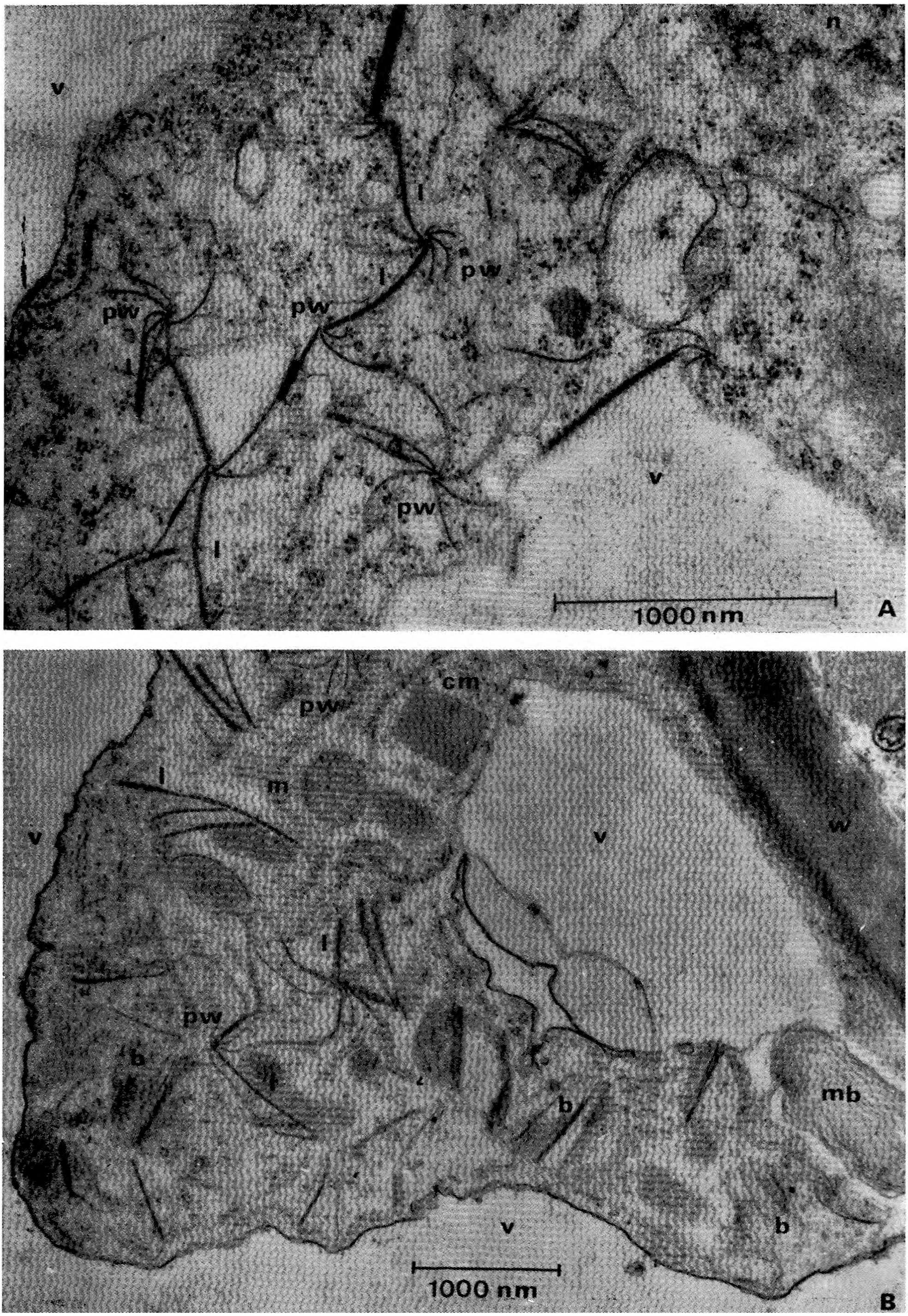

Fig. 2. — Sl. 2.

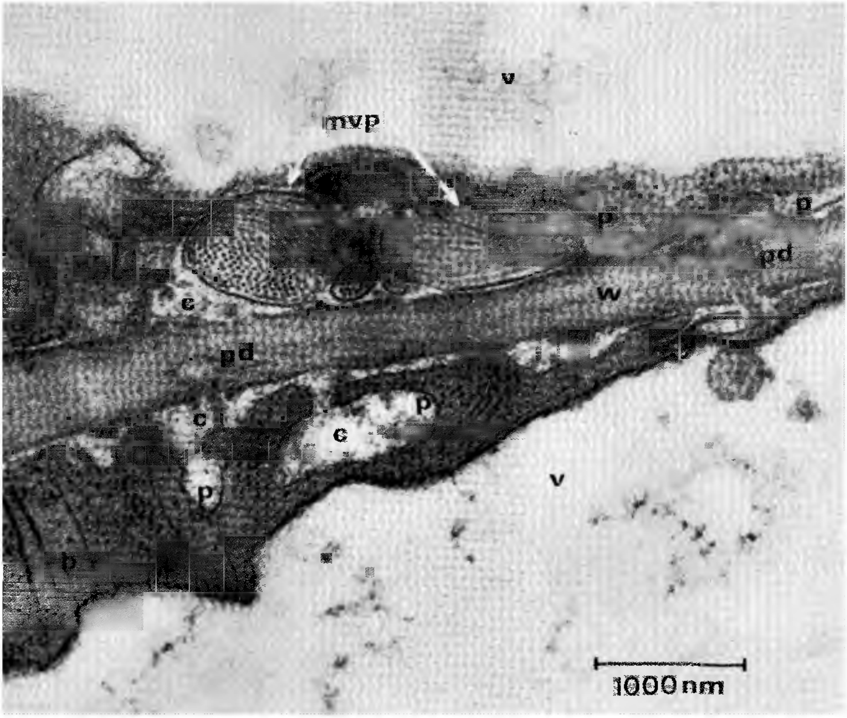

Fig. 3. — Sl. 3.

ever, inclusion bodies built of many needle-shaped crystals which are a characteristic of some potyviruses (Schmelzer and Miličić 1965, Štefanac and Miličić 1965, Pleše et al. 1969, van Oosten and van Bakel 1970, Christie and Edwardson 1977) were never observed.

Electron microscopy

The electron microscope analysis of ultrathin sections of *T. expansa* as well as *M. pomifera* revealed the presence of lamellar inclusions (Fig. 2. A, B) whose appearance is, otherwise, typical of infection with potyviruses (Edwardson 1974). In the cytoplasm, especially in the areas of amorphous bodies, many cylindrical inclusions were present. These inclusions appeared either as pinwheels or as bundles depending on its orientation. Arm plates of pinwheels were straight or slightly curved. The pinwheel structures were very frequently connected with each other by means of thicker or thinner laminated aggregates (Fig. 2. A). Potyviruses which form pinwheels and laminated aggregates belong to the subdivision II of the group (Edwardson 1974). However, the particle length (672 nm) and the particle structure as well as coat protein molecular weight (45,000 to 48,000 d) of MacMV differ significantly from those of potyviruses (Pleše et al. 1978). Therefore, it cannot be decided whether MacMV represents a potyvirus of unusual properties or the first known member of some new virus group.

Besides lamellar inclusions crystal-containing microbodies (Edwardson 1974) were also observed in the cytoplasm of *T. expansa* cells (Fig. 2. B).

In an ultrathin section through leaf tissue of *Tetragonia* infected for a long time membrane-bound aggregates of filamentous particles in the longitudinal and cross-sectional view were seen in extraprotoplasmic space (Fig. 3.). Consequently, these aggregates were situated between the plasmalemma and the cell wall. On the basis of the appearance and size of enveloped filamentous particles (c. 650 nm long and 12 to 15 nm wide) it can be concluded that they represent the aggregates or bundles of virus particles, which are excluded from cytoplasm and sealed off by single unit mebrane as "sacs of virus". This conclusion is supported by similar notices of some other investigators. In fact, such extraprotoplasmic sacs of elongated virus particles, often called micro--inclusion bodies, have been described in several papers (Krass and Ford 1969, Russo and Martelli 1969, Barnett et al. 1971, Macovei 1971, Murant and Roberts 1971, Allison and Shalla 1974, Horvat and Verhoyen 1975). Most reports deal with the finding of these structures in host cells infected by potyviruses. Only Allison and Shalla (1974) have observed such structures in host cells affected by potato virus X.

Extraprotoplasmic membrane-bound aggregates or sacs of virus were mostly located near the plasmodesmata. Allison and Shalla (1974) believe that the formation of these extraprotoplasmic sacs of virus was probably due to excessive callose deposition along the cell wall of infected cells. They have proved that the sacs of virus particles are embedded in the callose present in such cells in a large amount at the cell wall. With the exception of Allison and Shalla (1974), other previously mentioned authors have not investigated the deposition of callose up to the cell wall. In agreement with the findings of Allison and Shalla (1974) it can be concluded that the protrusions of plasmodesmal canals from the cell wall in Fig. 3. are a consequence of heavy callose deposition around plasmodesma and along the wall.

It is difficult to decide whether these extraprotoplasmic membrane-bound aggregates of virus reflect a manner in which the intracellular and cell-to-cell movement of virus is prevented, or they are incidental manifestation of excess callose deposition. Nevertheless, it should be pointed out that such virus structures have been found in local lesion hosts as well as in systemic hosts in which the spreading of virus is comparatively unrestricted.

Summary

Leaf tissue of *Tetragonia expansa* Thunb. and of *Maclura pomifera* (Raf.) Robinson plants infected with maclura mosaic virus (MacMV) was studied by light and electron microscopy.

In the cytoplasm of the epidermal cells of *T. expansa* amorphous granular inclusion bodies were commonly observed with the light microscope.

Submicroscopic cytoplasmic lamellar inclusions in infected *T. expansa* and *M. pomifera* cells included the same forms (pinwheels and laminated aggregates) as those observed in plants infected with viruses belonging to subdivision II of potyviruses (Edwardson 1974).

In *T. expansa* another type of submicroscopis virus inclusions was also established. These are the extraprotoplasmic membrane-bound aggregates of virus particles or the so-called micro-inclusion bodies (Krass and Ford 1969) situated outside the cytoplasm, i. e. between the plasmalemma and the cell wall. A possible role of these structures as the mechanism for localizing a virus infection is discussed.

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SADRŽAJ

SVJETLOSNA I ELEKTRONSKA MIKROSKOPIJA STANICA ZARAŽENIH VIRUSOM MOZAIKA MAKLURE

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Lisno tkivo izvorne biljke *Macura pomifera* (Raf.) Robinson i pokusne biljke *Tetragonia expansa* Thunb., zaraženih virusom mozaika maklure, istraživano je svjetlosnim i elektronskim mikroskopom.

U svjetlosnom mikroskopu zapažene su u citoplazmi stanica pokusne biljke *T. expansa* virusne uklopine koje su imale izgled povećih amorfnih zrnatih tijela.

Submikroskopske citoplazmatske lamelarne inkluzije u stanicama vrste *M. pomifera* i *T. expansa* imale su oblike koji su karakteristični za II podskupinu potivirusa (Edwardson 1974), tj. sadržavale su strukture pinwheel i laminarne agregate.

U vrste *T. expansa* utvrđen je također još jedan drugi tip submikroskopskih virusnih uklopina. To su membranom omeđeni ekstraprotoplazmatski agregati virusnih čestica ili tzv. »micro-inclusion bodies« (Krass i Ford 1969), koji su smješteni izvan citoplazme, tj. između plazmaleme i stanične stijenke. Diskutirano je o mogućoj ulozi ovih struktura kao mehanizma za lokaliziranje virusne infekcije.

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