Ultrastructure of Pea Leaf Cells Infected with Three Strains of Red Clover Mottle Virus

(Accepted 4 March 1982)

SUMMARY

Electron microscopy of pea (Pisum sativum L. cv. English Sabre) leaf cells, infected with three strains (N, S and O) of red clover mottle virus, revealed membranous inclusions characteristic of many comoviruses. The inclusions contained membranous vesicles with fine fibrils, osmiophilic globules and microbodies. The inclusions were not present in dark-green regions of systemically infected leaves (strains N and S). Membranous structures were also seen inside a few nuclei. In the chloroplasts of chlorotic regions of systemically infected leaves (strains N and S) phytoferritin was frequently observed. Chloroplasts in cells infected with strain O had no phytoferritin crystals, but were deformed by very large starch grains. Cell wall modifications were common in mesophyll regions infected with all three strains. Virus-like particles were found in all cell types, scattered or in crystals but not in the aggregated form in dark-green regions of systemically infected leaves (strains N and S).

Membranous inclusions are present in cells infected with cowpea mosaic virus (CPMV) and they have also been found in cells infected with other comoviruses (Martelli & Russo, 1977). The biological and physicochemical characteristics of three strains of red clover mottle virus (RCMV), another comovirus, have been described previously (Oxelfelt, 1976). The present investigation was carried out primarily to search for similar structures in RCMV-infected pea leaf cells and to compare the cytopathology of the three strains, designated N, S and O.

Five-day-old plants of pea, Pisum sativum L. cv. English Sabre, were inoculated with the three RCMV strains, N, S and O. When typical symptoms had developed 13 days later, tissue pieces, 0·5 to 1 mm², were cut as follows. Dark-green and chlorotic areas were cut out separately from N- and S-infected young leaves with mosaic. Samples were also taken from the older inoculated leaves with mottle symptoms. Inoculated leaves had no distinct chlorotic or dark-green areas. Tissue pieces were taken only from inoculated leaves of plants infected with strain O which showed top necrosis. Tissue pieces were also cut from corresponding young and old leaves of healthy plants as controls. After fixation and embedding in Spurr's epoxy resin the sections were examined in a Philips EM 200.

Membranous inclusions were observed in mesophyll cells infected with all three virus strains. The inclusions, usually adjacent to the nuclei, were made up of masses of membranous vesicles together with fine fibrils, osmiophilic globules and microbodies. Ribosomes and/or virus-like particles were scattered throughout the inclusions, and many mitochondria were present at their periphery (Fig. 1a). Membranous inclusions were not seen in sieve tubes, xylem tracheal elements, or epidermal cells from chlorotic regions. In dark-green regions of systemically infected leaves (strains N and S) the inclusions were absent.

The origin of the inclusions is not known, but it has been proposed that the vesicles in the similar inclusions induced by two other comoviruses, radish mosaic virus and CPMV, may have some connection with or emanate from dictyosomes (Stefanac & Ljubesic, 1971; van...
Membrane synthesis is obviously stimulated during early stages of infection with RCMV but no association between dictyosomes and membranes of the vesicles was observed in our material; however, this was sampled at a late stage of infection.

The replication of CPMV nucleic acid was demonstrated by de Zoeten et al. (1974) to be associated with the vesicles in the inclusions; the fibrils in the vesicles of the inclusions induced by Echtes Ackerbohnenmosaikvirus have been identified as double-stranded RNA (Hatta & Francki, 1978). The site of assembly of CPMV particles is not known. Small electron-dense bodies were scattered in the RCMV-induced inclusions and occasionally associated with its membranes, but it could not be ascertained whether these bodies were ribosomes or virus particles. In contrast to some other comoviruses (Stefanac & Ljubesic, 1971; Kitajima et al., 1974; Bowyer et al., 1980), RCMV does not develop large aggregates of virus particles among the membranes of the inclusions or in their proximity. No virus production seems to take place in dark-green regions (strains N and S); there are no inclusions or accumulation of virus-like particles in vacuoles of mesophyll cells or in any other cell type in these regions.

Nuclei in inoculated leaves (all three strains) had a normal appearance when compared to controls. In a sample from a chlorotic region (strain N) membranous structures were found inside some nuclei (Fig. 1 b). Intranuclear membranous structures, very similar to those observed in RCMV-infected tissue, have been found in nuclei in cells infected with CPMV (van der Scheer & Groenewegen, 1971; Langenberg & Schroeder, 1975). Interactions between the membranes of the nuclear inclusion and the nuclear envelope such as were found in CPMV-infected cells (Langenberg & Schroeder, 1975) were not seen in RCMV-infected cells.

Chloroplast structure was affected in the chlorotic regions of systemically infected young leaves (strains N and S). In the stroma, crystals of phytoferritin were frequently found. Chloroplast inclusions were not observed in leaves infected with strain O and were rare in dark-green regions (strains N and S) and in the older inoculated leaves (strains N and S). In cells infected with strain O, chloroplasts had numerous and very large starch grains and were much deformed (Fig. 2 a).

Aggregates or crystals of virus-like particles were numerous in all cell types in inoculated leaves and in chlorotic areas of systemically infected leaves but were absent in the dark-green regions. Virus particles could not be distinguished from ribosomes when they were scattered in the cytoplasm. Virus-like particles were found scattered or in aggregates in the vacuoles of mesophyll, epidermal and guard cells; in the aggregates the particles were sometimes in a crystalline array. In sieve tubes, particles were loosely scattered or aggregated into large crystals, which were always partly or completely enveloped (Fig. 2 b). In some tracheal elements of leaves infected with strains N and O, virus-like particles were observed either in crystalline aggregates or scattered and located close to the cell walls (Fig. 2 c). Enveloped crystals were not seen in tracheal elements.

Modifications of cell walls were seen in mesophyll cells of older inoculated leaves infected with all three virus strains. These modifications consisted of paramural bodies associated with plasmodesmata containing virus-like particles. Sometimes, adjacent cell walls showed slight localized protrusions (Fig. 2 d). Virus-like particles were also present in plasmodesmata without plasmalemmosomes (Fig. 2 e). In dark-green regions (strains N and S) and in healthy tissue virus-like particles were not seen in plasmodesmata; however, paramural bodies were present.

Cell wall changes are frequently reported as an effect of infection by members of many virus groups including comoviruses (Martelli & Russo, 1977). Extensive cell wall changes including tubules filled with virus-like particles either embedded in the cell walls or in
protrusions of the cell walls have been described in plants infected with CPMV (van der Scheer & Groenewegen, 1971) and with bean pod mottle virus (Kim & Fulton, 1971); however, such structures were not seen in RCMV-infected cells.
Fig. 2. Ultrastructure of pea leaf cells infected with three strains of red clover mottle virus. (a) Chloroplasts deformed by large starch grains characteristic of cells infected with strain O. Membranous inclusion (arrow) is also present. Bar marker represents 5 μm. (b) Densely packed and partly enveloped virus crystal in a sieve tube. Bar marker represents 250 nm. (c) Virus-like particles in a xylem tracheal element. Scattered particles are associated with the cell wall. Bar marker represents 250 nm. (d) Membranes and small vesicles between plasmalemma and cell wall associated with a plasmodesma filled with virus-like particles. Bar marker represents 250 nm. (e) Plasmodesma with virus-like particles. Bar marker represents 250 nm.

The most important ultrastructural difference between the three strains of RCMV seems to be restricted to the appearance of the chloroplasts. Nuclear membranous inclusions were observed only in a few cells infected with strain N, but nuclear inclusions may occur only at
early stages of infection. The ultrastructural modifications induced by RCMV in pea are similar to those reported for other members of the comovirus group.

We thank Mrs Birgit Wallentinsson for photographic work.

Swedish University of Agricultural Sciences
Department of Plant and Forest Protection
P.O. Box 7044, S-750 07 Uppsala, Sweden

KARIN TOMENIUS*
PER OXELFELT

REFERENCES


*(Received 27 November 1981)*