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Additional Data on Complex Inclusions Evoked by Wisteria Vein Mosaic Virus in Pea Leaf Cells

J. Brčák and O. Králík

Institute of Experimental Botany, Czechoslovak Academy of Sciences, Praha*

Abstract. Large complex inclusions evoked by wisteria vein mosaic virus contain cylindrical inclusions, mostly pinwheels (and bundles) and rarely also scrolls and laminar inclusions. Bundles are often abutted to cell walls at plasmodesmata and show association with endoplasmic reticulum and cisternae. Virus aggregates are attached to cylindrical inclusions, various membranes and/or plasmalemma.

Cytoplasm contains more or less disintegrated chloroplasts and mitochondria, abnormal membranes, many ribosomes, bounded vesicles with fibrillar contents, numerous and large microbodies, and membranous whorls. These phenomena are not specific.

Properties of the isolate of wisteria vein mosaic virus (WVMV) used for our investigation were described in a previous paper (Brčák 1980) presenting first light micrographs of massive granular inclusions induced by WVMV in epidermal cells of pea leaflets. Some data on the ultrastructure of these complex inclusions were already reported by Brčák and Králík (1980): Cylindrical inclusions, mostly pinwheels and bundles and rarely also scrolls and laminar inclusions were stated, so that WVMV could be classified, according to Edwardson (1974), as a member of Subdivision III of the *Potyvirus* group. The paper by Brčák and Králík (1980) also contains evidence of the association of virus aggregates with pinwheels and membranes and with envelopes of disintegrated chloroplasts, and describes some cytopathological changes, *e.g.* disruption of bounding membranes and degeneration of chloroplasts and mitochondria, electron lucent regions containing fibrous material in mitochondria, and some further details concerning the association of cylindrical inclusions with rough endoplasmic reticulum *etc.* This paper presents additional data on the location of cylindrical inclusions and virus aggregates in large complex inclusions and on their cytopathological structure.

MATERIAL AND METHODS

Pea plants (*Pisum sativum* L. cv. Raman Elite) were inoculated in a greenhouse with WVMV in January. Samples of tissues taken three weeks later from leaflets showing systemic mottle were fixed in glutaraldehyde and in

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* *Address:* Na Karlovce 1a, 160 00 Praha 6, Czechoslovakia.

osmium tetroxide, dehydrated in an ethanol series, transferred to saturated solution of uranyl acetate in 70% ethanol overnight, passed through propylene oxide and embedded in Durcupan ACM or Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate (RAYNOLDS 1963) and examined with Tesla BS 500 and JEM 100B electron microscopes.

RESULTS AND DISCUSSION

Large complex inclusions evoked by WVMV show many properties which are common with other potyviruses. They contain pinwheels or bundles in longitudinal sections, which are often abutted to the cell walls at plasmodesmata (Figs. 1, 2, 12) where they originate (ANDREWS and SHALLA 1974). At a later stage they may be released into the cytoplasm and may get a more complicated structure, as represented by scrolls (Fig. 3) and/or laminar inclusions occurring inside complex inclusions in ground cytoplasm. Cylindrical inclusions are often connected to endoplasmic reticulum cisternae, as also observed by MARTELLI and RUSSO (1976). Virus aggregates may be attached not only to pinwheels but also plasmalemma or other membranes (Figs. 4, 5), or lie along plasmalemma (Fig. 12).

The cytoplasm shows strong degenerative changes, esp. various abnormal membranes and large amounts of ribosomes (Fig. 6). Vesicles bounded by a single membrane and containing fibrillar material may occur among pinwheels (Fig. 7). These vesicles differ from those of dictyosomal origin and are not specific for potyviruses (or generally for phytoviruses), because similar vesicles could be seen *e.g.* in plant cells infected with some fungi (STEINKAMP *et al.* 1979). Another non-specific phenomenon, also known with other virus or fungus infections, are numerous microbodies (Fig. 8), which may be very large (Fig. 9). Contrary to some other potyviruses and hosts, no proteinaceous crystals were seen inside microbodies. As with other host-virus combinations the ground cytoplasm often contains membranous whorls probably of endoplasmic reticulum origin (Figs. 3, 10). Further sac-like structures being simple or showing lamellar structure and resembling membranous whorls in healthy senescing cells intrude the vacuole as plasmalemmasomes (paramural bodies). They may be connected to plasmalemma with a stalk or be released into the vacuole. Plasmalemmasomes seen at the cell wall have either vesicular (Figs. 11, 13) or tubular contents (Fig. 12) and are similar to those found in healthy plants by WAHN *et al.* (1980). These formations are apparently not specific for WVMV infection.

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Figures at the end of the issue.

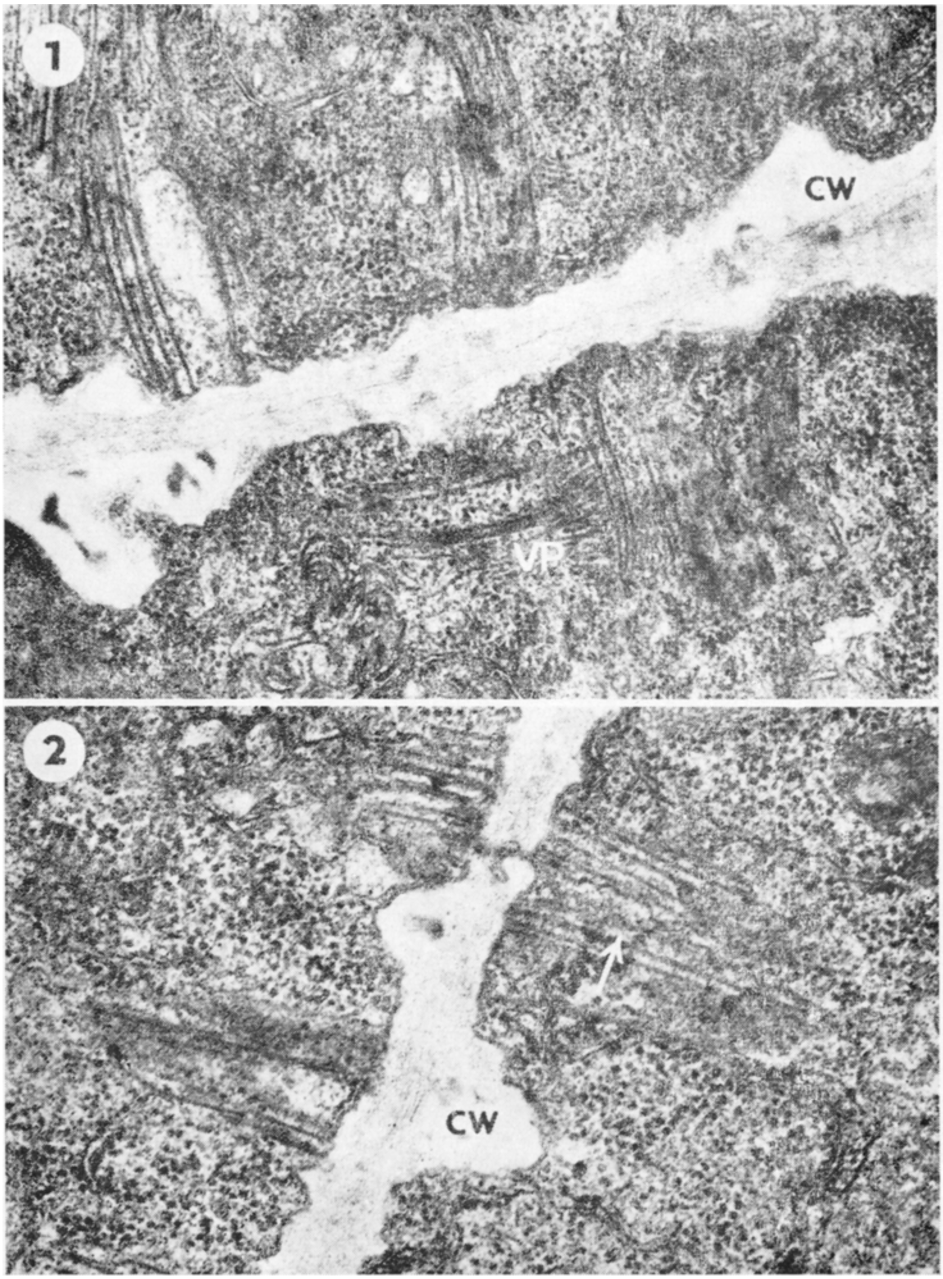
BOOK REVIEW

POTASSIUM RESEARCH — REVIEW AND TRENDS. Proceedings of the 11th Congress of the International Potash Institute 1978. — International Potash Institute, Bern 1979. Pp. 499. US$ 21.15.

This book presents the proceedings of the Congress, held on September 4—8, 1978 in Bern, on the occasion of the 25th aniversary of the Scientific Board of the International Potash Institute. Specialists from forty-eight countries of Africa, America, Asia and Europe, from FAO and five other international organizations assembled to survey the progress achieved over the past twenty-five years, to assess the gaps in present knowledge, decide on the future work and suggest how the improvements may be translated into agricultural practice. The Congress agenda was divided into six sessions, the first of which was devoted to the Jubilee of the Scientific Board of IPI. The themes of the remaining sections were organized under following headings: (2) Potassium in the soil/plant root system; (3) The role of potassium in yield formation; (4) Potassium requirements of crops; (5) Potassium fertilization in agricultural practice and (6) Translation of research into practice. In section 2 articles on "Structure and weathering of potassium containing minerals", "The physical chemistry of equilibria involving the potassium ion in soils", "Factors affecting the availability of potassium in soils" and "The evaluation of soil K status by means of soil testing" were included. Section 3 was devoted to "Potassium transport through plant cell membranes and metabolic role of potassium in plants", "The effect of potassium on growth and yield components of plants" and "Influence of K nutrition on the response to environmental stress". The most voluminous fourth section provides a comprehensive survey of the requirements of different crop types for potassium. The last but one session was dedicated to potassium effects in long term cultivation, to the concepts of potash use and interaction of potassium with other nutrients. The brief closing section summarizes the present knowledge in two chapters which also show how scientific knowledge can be better applied in modern agriculture.

There is such a wealth of information in this publication that it is beyond my scope to mention here all the items. Perhaps the only serious defect of the book is the absence of an author and especially a subject index. In every other respect, it is very interesting and should have a general appeal to all those working on this and related problems.

JARMILA SOLÁROVÁ (*Praha*)



Figs. 1, 2. Cylindrical inclusions abutted to the cell wall (CW) at plasmodesmata. Virus particles (VP) are attached to a pinwheel and a bundle (Fig. 1). Association of endoplasmic reticulum (arrow) with a bundle (Fig. 2).

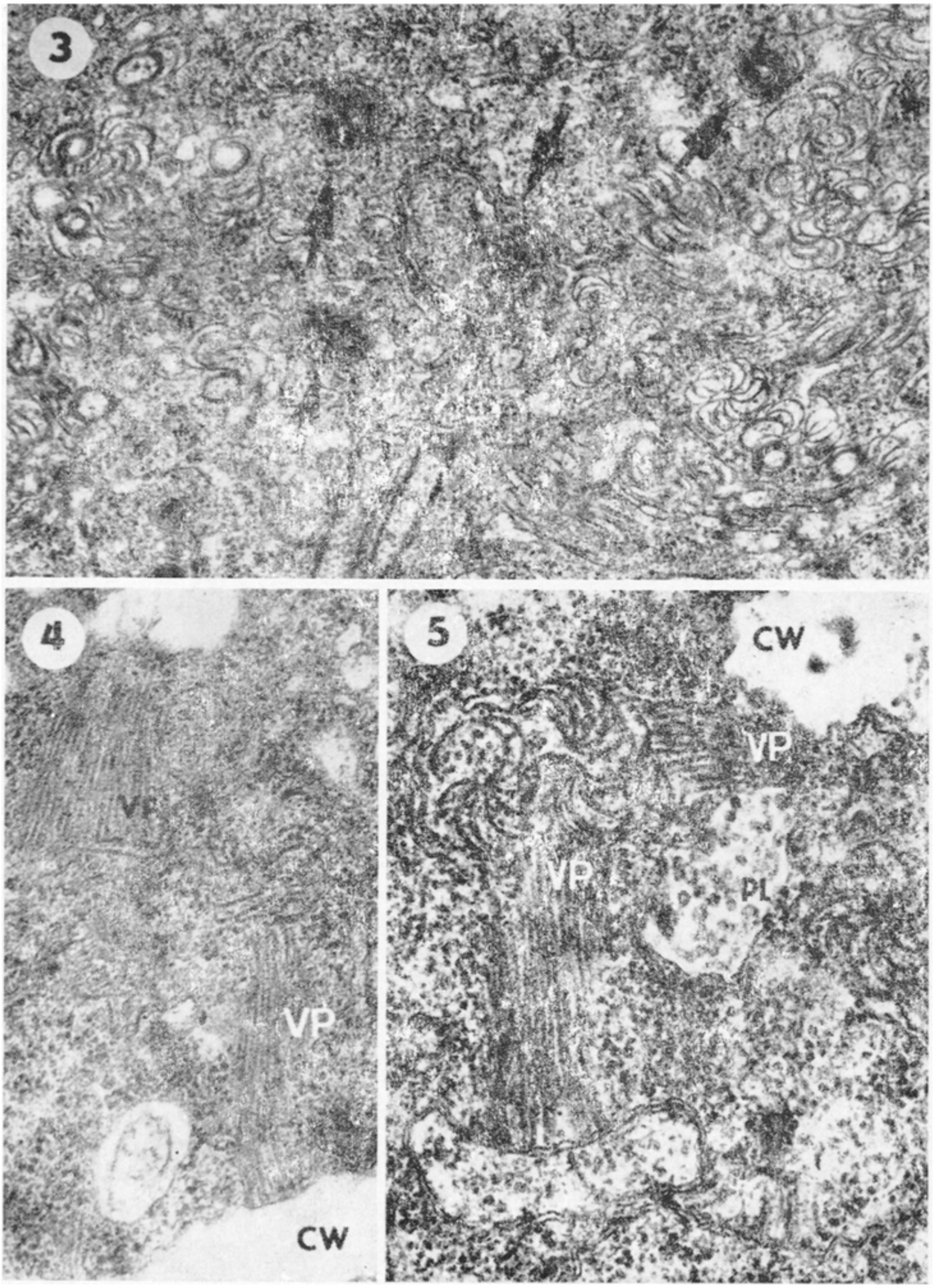


Fig. 3. Membranous whorls (arrows) in a complex inclusion among pinwheels, bundles and scrolls. Figs. 4, 5. Association of virus particles (VP) with cylindrical inclusions, plasmalemma and other membranes. CW — cell wall, PL — plasmalemmasome.

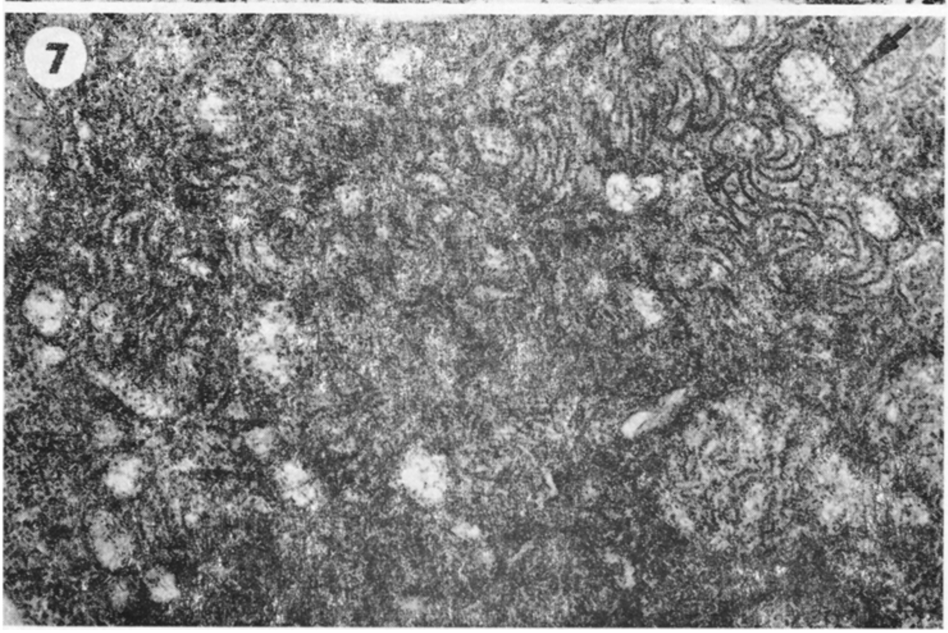


Fig. 6. Disintegrated cytoplasm of a complex inclusion containing pinwheels, bundles, vesicles, ribosomes, mitochondria (M) with partly degraded envelopes *etc.* N — nucleus.
Fig. 7. Vesicles containing fibrous material (arrows) among pinwheels.

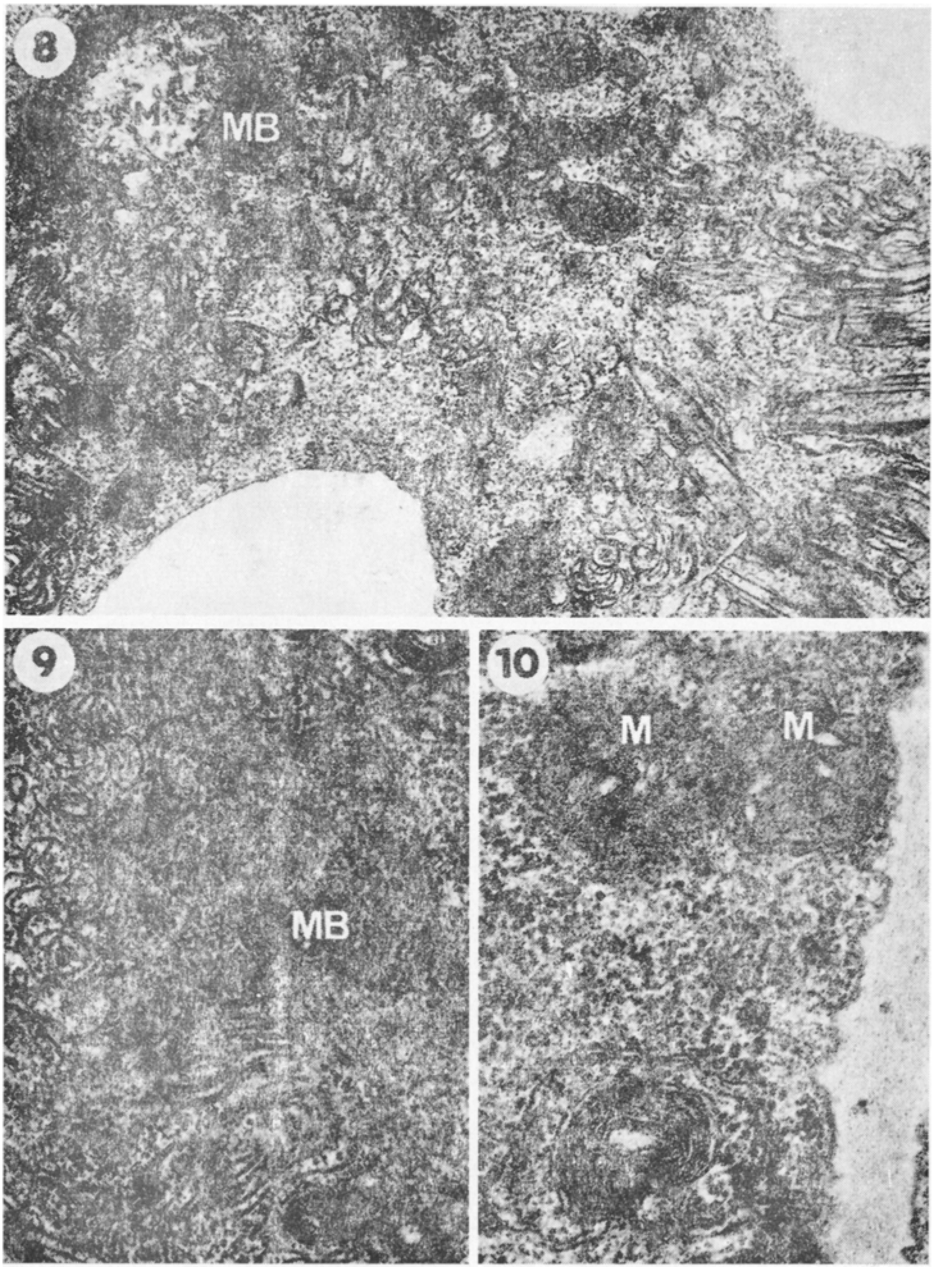
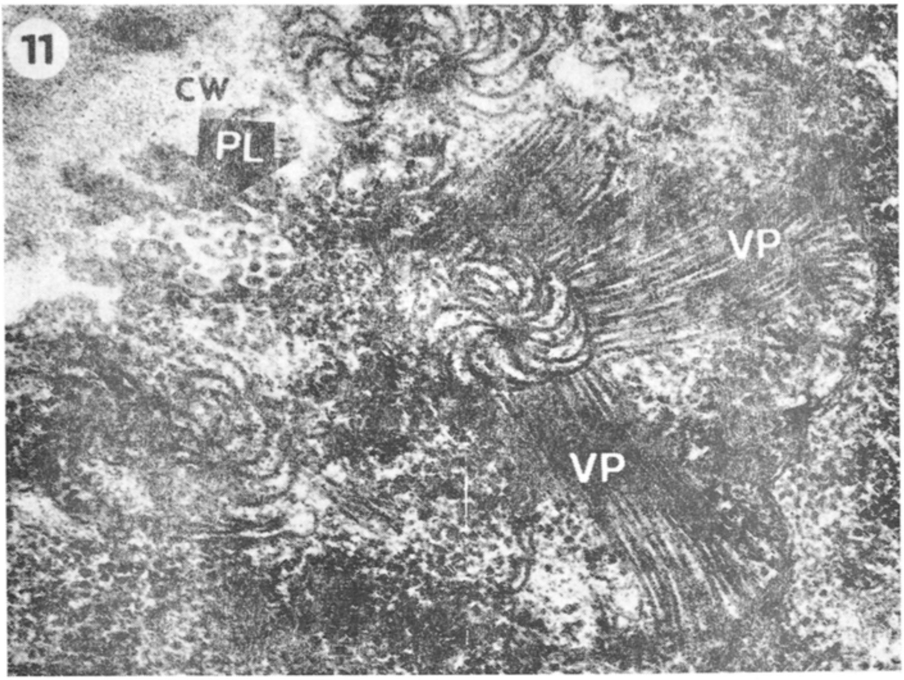
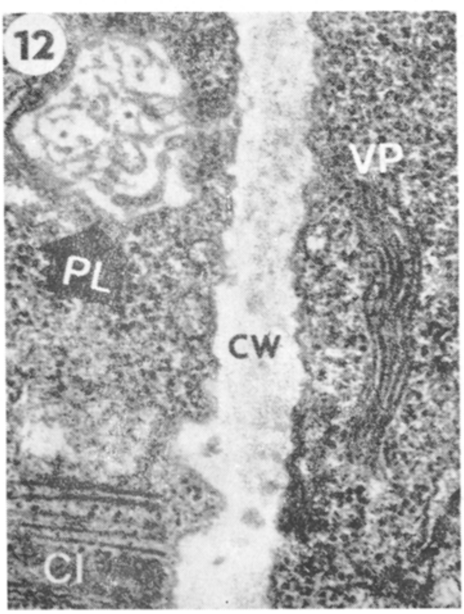


Fig. 8. Many microbodies (MB) and cylindrical inclusions in cytoplasm of a complex inclusion. M — mitochondrion.
Fig. 9. A large microbody (MB) at pinwheels and scrolls.
Fig. 10. Two mitochondria (M) undergoing degradation (to the left the mitochondrial envelope is absent) and a membranous whorl.





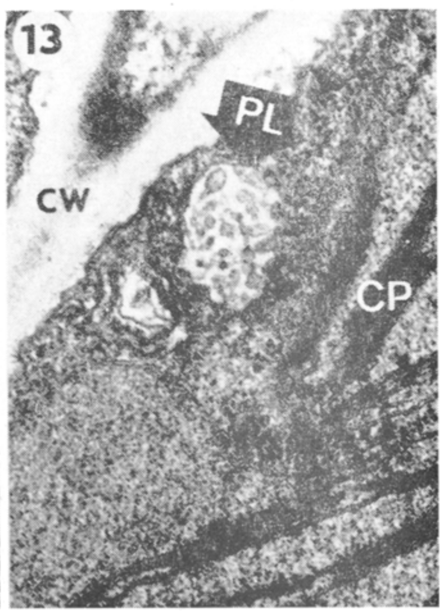


Fig. 11. Virus particles (VP) attached to a pinwheel and membranes. A plasmalemmasome (PL) containing vesicles at the cell wall (CW).
Fig. 12. Plasmalemmasome (PL) with tubular contents, cylindrical inclusions (CI) at plasmodesmata and virus particles (VP) situated along the cell wall (CW).
Fig. 13. A plasmalemmasome with vesicles (PL). CW — cell wall, CP — chloroplast.