Neth. J. Plant. Path. 72: 114–115

SHORT COMMUNICATION
AN ELECTRON MICROSCOPE STUDY OF TOMATO SPOTTED WILT VIRUS IN THE PLANT CELL[1]

T. S. IE
Department of Virology, State Agricultural University, Wageningen

Characteristic particles of about 70 mμ diameter were found in tomato spotted wilt virus (TSWV) infected cells of roots, stems, leaves and petals of several plant species. These particles were similar in size and form to those found in the pellets of partially purified infective TSWV.

Electron microscope studies of purified tomato spotted wilt virus (TSWV) by BLACK, BRAKKE & VATTER (1963) and by BLACK (1955) revealed fairly large-sized particles (about 85 mμ across) by the shadow-casting method. This fact offers a promising possibility for a study of TSWV particles *in situ* (MARTIN, 1964).

Ultramicrotome sections were made from parts of pellets of partially purified TSWV from infected tomato plants, and from parts of leaves, roots and petals of several infected host plants viz. *Lycopersicum esculentum* Mill. cv. 'Moneymaker', *Nicotiana tabacum* L. cv. 'White Burley' and 'Samsun NN', *N. rustica* L., *Tropaeolum majus* L., *Zinnia elegans* Jacq. and *Senecio cruentus* DC.

The pellets were obtained from purified extracts of roots of tomato plants which were systemically infected twelve days after inoculation of the leaves. These extracts were purified by means of differential and sucrose gradient centrifugation by Mr. M. M. MARTIN (University of Natal, Pietermaritzburg, South Africa) who worked as a guest in our laboratory. The small pellets in the bottom of the tubes were immediately fixed by OsO_4 and finally divided in small pieces of about $1 \times 1 \times 1$ mm. Small pieces of about 1 mm from various organs of the above-mentioned species were fixed in 1 % OsO_4 in veronal acetate buffer pH 7.4 (PALADE, 1952) for 2–4 hours at 4° C.

The embedding was done in pre-polymerized methacrylate mixture of n-butyl and methylmethacrylate (4 : 1), which was polymerized in gelatin capsules at 60° C for 24 hours (PEASE, 1960). The ultrathin sections were cut with a Porter-Blum ultramicrotome, stained with lead-hydroxide (MILLONIG, 1961) and examined in the Siemens Elmiskop I electron microscope.

Ultramicrotome sections of pellets from partially purified TSWV from tomato plants revealed characteristic, more or less spherical particles among the mass of unidentified material (Fig. 1). The infectivity of these TSWV pellets was proved by a local lesion test on detached leaves of *Petunia hybrida* Vilm. before fixing and embedding.

Ultramicrotome sections of infected tomato leaf and root cells, about 14 days after inoculation, invariably showed the characteristic particles of about 70 mμ diameter in the cytoplasm of the cells. These particles were present in clusters surrounded by enveloping membranes, which may be the cisternae of the endoplasmic reticulum (Fig. 3). They were present only in the cytoplasm

[1] Accepted for publication 21 May, 1964.

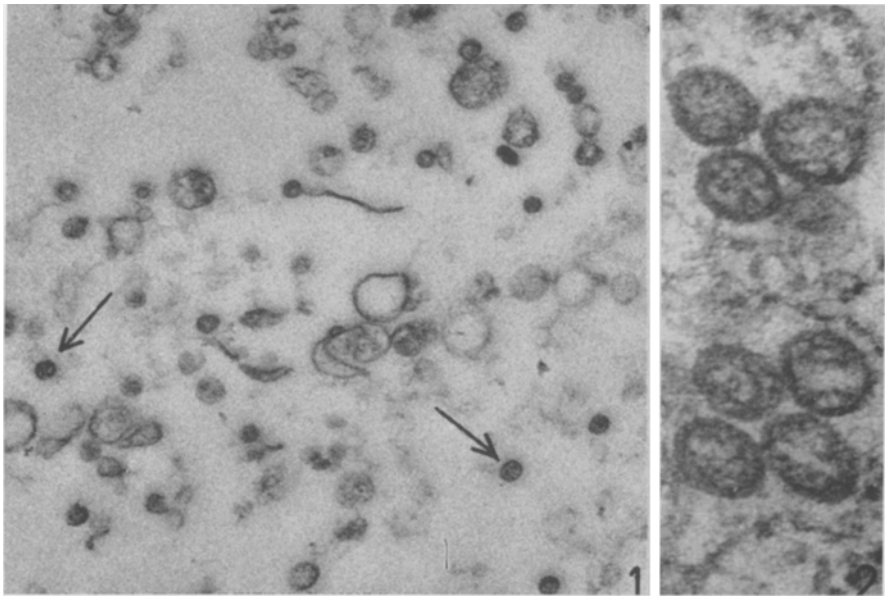

FIG. 1. Section of a pellet of partially purified TSWV. The dense virus particles are clearly seen among the cell debris. Some are indicated by arrows. Magnification: × 40,000.
FIG. 2. Detailed structure of TSWV particles present in a section of a tomato leaf cell. Magnification: × 160,000.

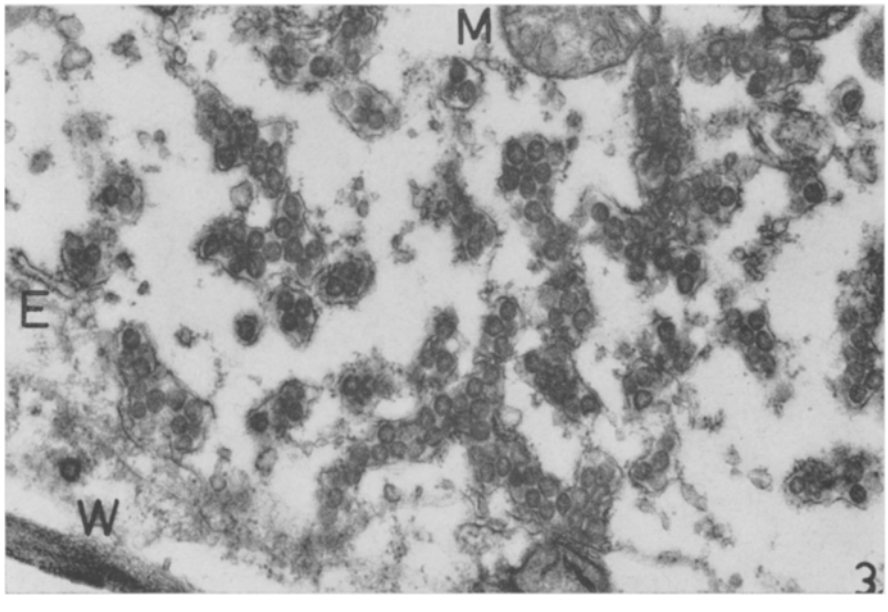

FIG. 3. Section of a tomato root cell infected with TSWV. Clusters of virus particles surrounded by a membrane are seen in the cytoplasm. Magnification: × 40,000. M = mitochondrion; E = endoplasmic reticulum; W = cell wall.

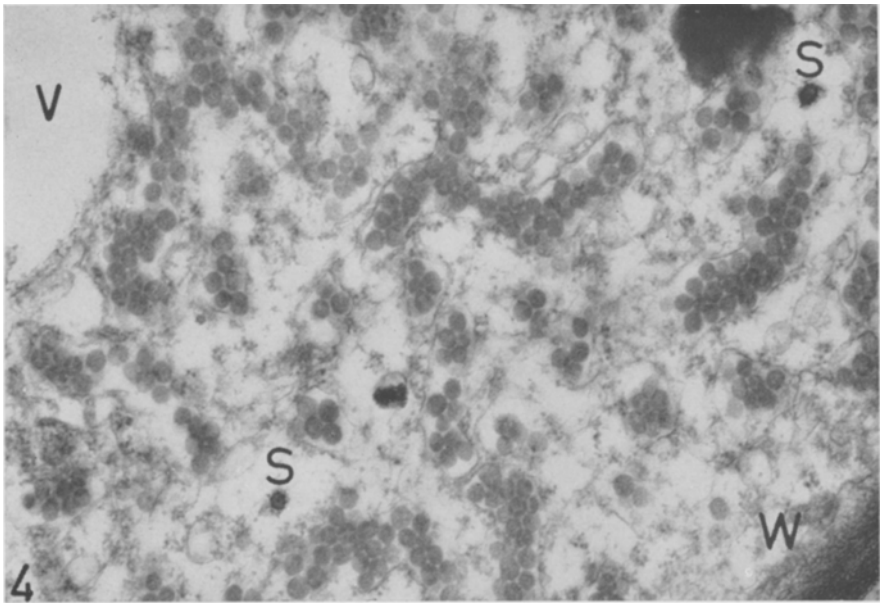

FIG. 4. Section of a TSWV infected leaf cell of *Tropaeolum majus*, showing two particles of the solitary type (S) in the cytoplasm besides the usual particle clusters. Magnification: × 40,000. V = vacuole; W = cell wall.

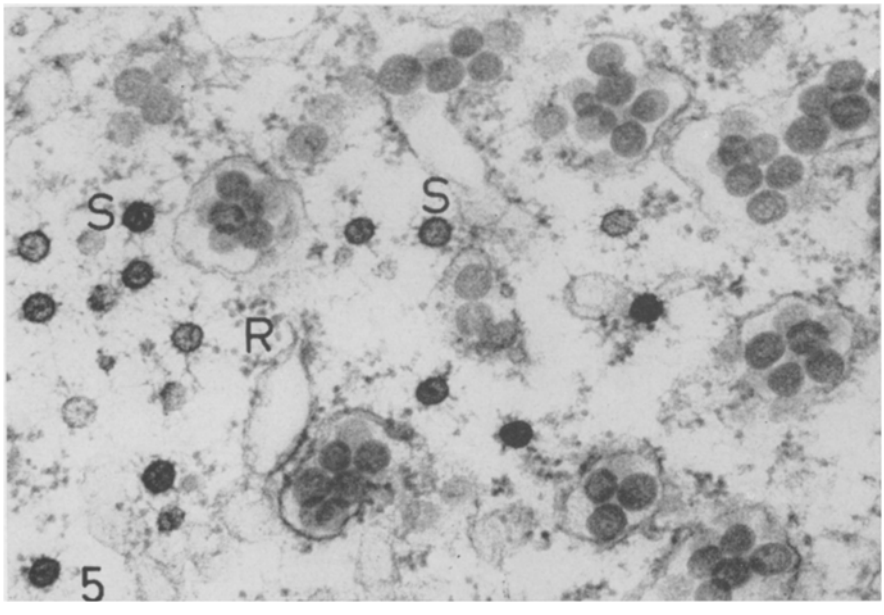

FIG. 5. Section of a TSWV infected leaf cell of *Tropaeolum majus*, showing a more frequent occurrence of the solitary type (S) among the particle clusters. Magnification: × 80,000. R = ribosomes.

Photos Service Institute for Technical Physics in Agriculture, Wageningen.

and could not be found in the nuclei, chloroplasts, or mitochondria. The individual particles seem to possess a membrane (Fig. 2). No comparable particles were found in the cells of roots or leaves from healthy tomato plants.

The other above-mentioned host species were also checked for the presence of these particles. The characteristic particles of about 70 mμ diameter were invariably found in the various parts of all these plant species.

The 70 mμ particles are presumed to be the TSWV particles for the following reasons: 1. their great regularity in shape and size; 2. their occurrence only in the cells of TSWV infected plants belonging to different species, and their absence in all the healthy plants; 3. a similarity with particles found in the partially purified TSWV pellets, which were infective; 4. no such particles have yet been reported as normal constituents of plant cells.

The particles reported by BLACK *et al.* (1963) are somewhat larger than those found in the sections in the present work. This may be due to flattening caused by air-drying employed in the shadow-casting method.

Besides these characteristic particles, a different type of particle was also found in the infected leaf cells of *T. majus*. These particles were solitary and did not occur in clusters in the cytoplasm. They were more electron dense and the inner-structure appeared different from the characteristic type (Figs. 4 and 5). The relationship of these two types of particles is not clear at the moment.

Further studies on the developmental stages of these particles in plant tissue and on their possible occurrence in the body of the insect vector (*Thrips tabaci* Lind.) are in progress.

ACKNOWLEDGEMENTS

The author expresses his sincere thanks to Prof. Dr. J. P. H. VAN DER WANT for the valuable advice and criticism. Many thanks are due to Miss Drs. C. VAN DER SCHEER for valuable discussions, to Mr. S. HENSTRA for making the electron micrographs and to Mr. A. MASTENBROEK for making the prints, all of the Electron microscopy section of the Service Institute for Technical Physics in Agriculture at Wageningen.

The author is also greatly indebted to Dr. H. AGRAWAL for correcting the English text.

REFERENCES

BLACK, L. M., – 1955. Concepts and problems concerning purification of labile insect-transmitted plant viruses. Phytopathology 45: 208–216.

BLACK, L. M., M. K. BRAKKE & A. E. VATTER, – 1963. Purification and electron microscopy of tomato spotted wilt virus. Virology 20: 120–130.

MARTIN, M. M., – 1964. Purification and electron microscope studies of tomato spotted wilt virus (TSWV) from tomato roots. Virology 22: 645–649.

MILLONIG, G., – 1961. A modified procedure for lead staining of thin sections. J. biophys. biochem. Cytol. 11: 736–739.

PALADE, G. E., – 1952. A study of fixation for electron microscopy. J. exp. Med. 95: 285–298.

PEASE, D. C., – 1960. Histological techniques for electron microscopy. Academic Press, New York, London.