Neth. J. Pl. Path. 79 (1973) 249–256

Tomato spotted wilt virus in the anthers of Tropaeolum majus

T. S. IE

Laboratory of Virology, State Agricultural University, Wageningen

Accepted 17 July 1973

Abstract

Electron microscopy of ultrathin sections of anthers of *Tropaeolum majus* plants infected with tomato spotted wilt virus, revealed particles of the virus only in the cells of the endothecium tissue of the anthers. In these parenchyma cells the particles were localized, sometimes in high concentrations, only in the cytoplasm, in clusters in the cisternae of the endoplasmic reticulum. They were not present in the tapetal cells, nor in the pollen grains.

Introduction

During a study on the occurrence of tomato spotted wilt virus (TSWV) (R/*:*/*: S/S:S/Th) in situ in plant cells (Ie, 1964, 1970, 1971) it was observed that sap extracted from flowers of TSWV-infected *Tropaeolum majus*, shows a high infectivity on *Petunia hybrida* 'Pink Beauty'. Besides extracts of petals, extracts of anthers gave also rise to large numbers of local lesions on *Petunia* leaves, 2–3 days after mechanical inoculation.

Therefore an electron microscope study of anther tissues and pollen grains was started to localize the distribution of TSWV in these tissues.

Material and methods

Clones of *T. majus* were maintained by cuttings and were grown in pots in a glasshouse. As some plants of *T. majus* have been found to contain solitary bodies ('S-bodies') in the cytoplasm (Ie, 1964, 1972), both clones (S-body positive and S-body negative) of *T. majus* were infected with TSWV during summer, close to the flowering period. The plants were inoculated with sap from leaves of *N. tabacum* 'Samsun NN', systemically infected with TSWV, using carborundum (500 mesh) as an abrasive. The infected plants developed a systemic mosaic on the leaves about 10–12 days after inoculation. Anthers were collected from these plants when the flowers were still closed.

Preparation for electron microscopy was as follows. Small pieces of young anthers were cut transversally and were immediately fixed in 1% OsO_4 in veronal acetate buffer, pH 7.4 for 2–4 h, chilled in ice. Dehydration was performed in ethanol. Embedding was in prepolymerized methacrylate mixture of the butyl and methyl ester in the ratio 4:1 (Pease, 1960). Ultrathin sections, cut with an LKB ultramicrotome using glass knives, were mounted on formvar-coated copper grids. These were stained with lead tartrate after Millonig (1961), or with lead citrate after Reynolds (1963).

The stained sections were examined with a Siemens Elmiskop 1 electron microscope, operating at 80 kV.

Results

Anther. A general view of a part of a loculus of the anther of *T. majus* is shown in Fig. 1. This photo-micrograph was made with a Leitz Wetzlar light microscope of a 1–2 μm thick methacrylate section, stained with methylene blue. Mostly the peripheral endothecium tissue of the anthers was used for electron microscopy. This tissue, bordering the loculus (L) which is crowded with almost mature pollen grains, is composed of one innermost layer of tapetal cells, the tapetum (T), followed by

Fig. 1. Part of an anther of *T. majus*, infected with TSWV, showing a loculus (L), containing numerous pollen grains, and the bordering tapetum layer (T) and endothecium tissue (En). Photo-micrograph of a thick section. E = exothecium, F = lamina fibrosa.

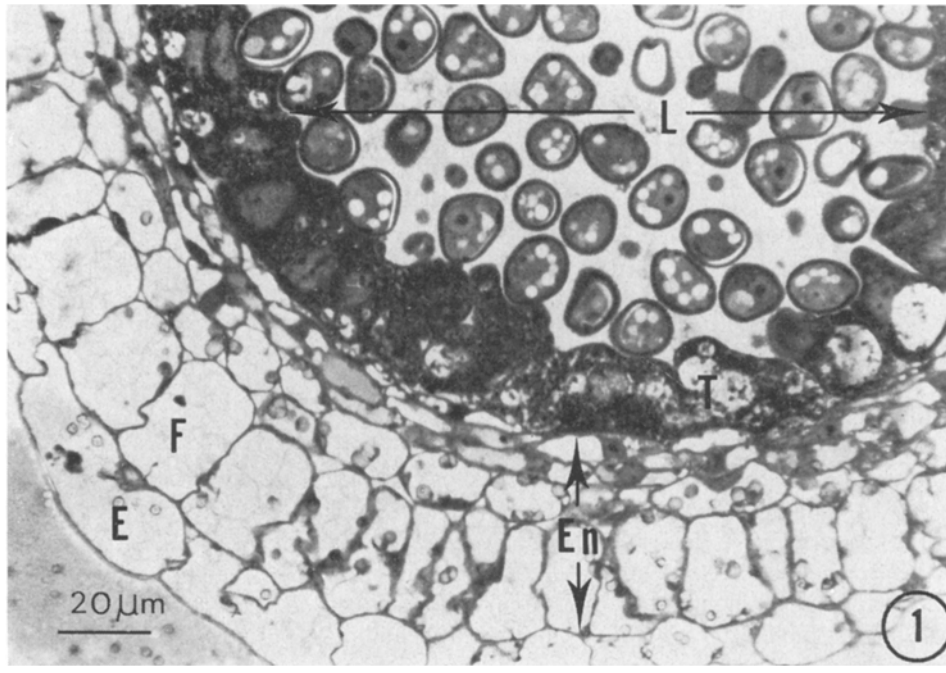
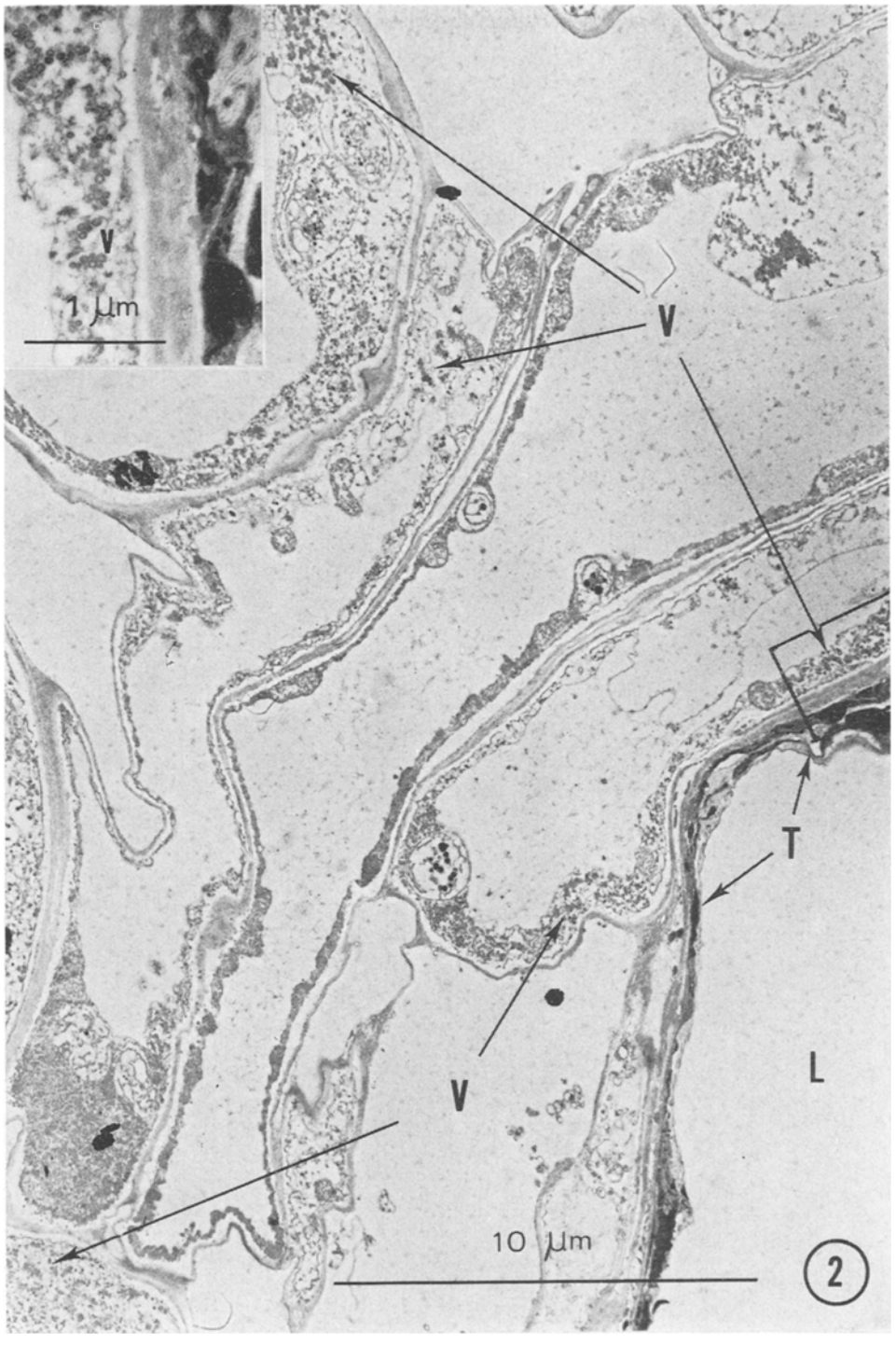


Fig. 1. Lichtmicroscopisch beeld van een gedeelte van een helmknop van T. majus, geïnfecteerd met *het TSWV. Overzicht van een stuifmeelzak (L), met talrijke stuifmeelkorrels, en zijn begrenzende tapetumlaag (T) en endotheciumweefsel (En). E = exothecium, F = lamina fibrosa.*

Fig. 2. Electron-micrograph of the endothecium tissue of TSWV infected anther of *T. majus*. Tapetum layer (T) already in degenerated (periplasmodial) stage. Clusters of virus particles (V) occur in almost every endothecium cell. L = loculus. Inset: enlargement of indicated area.
Fig. 2. Elektronenmicroscopisch beeld van het endotheciumweefsel van een met TSWV geïnfecteerde helmknop van T. majus. De tapetumlaag (T) reeds in gedegenereerd, periplasmodiumstadium. Virusdeeltjes (V) aanwezig in vrijwel elke endotheciumcel. L = loculus. Links boven: vergroting van aangegeven gebied.


V
1 µm
V
T
V
L
10 µm
2

3–4 concentric layers of endothecium cells (En) of which the outermost layer consists of large cells, the lamina fibrosa (F). Externally, there is one layer of epidermal cells, the exothecium (E).

Endothecium tissue. TSWV particles (70–90 nm in diameter) clustering in membranous sacs in the cytoplasm, were frequently present in fairly high concentrations in the endothecium cells. This general appearance of TSWV in the cytoplasm in the cisternae of the endoplasmic reticulum, fits the observation of various authors (Martin, 1964; Ie, 1964, 1970, 1971; Kitajima, 1965; Milne and De Zoeten, 1967; Milne, 1970; Francki and Grivell, 1970). The virus particles were never found in other cytoplasmic organelles, nor in the vacuoles.

They occur as well in the endothecium cells adjacent to the tapetal cells, as in the outermost large endothecium cells with characteristic secondary thickenings of the radial walls (Figs 2, 3 and 4).

Tapetum layer. The virus particles were never found in the tapetal cells, nor in the younger stages or in the ultimate plasmodial stage. The tapetum in the anther of *T. majus* does not remain as a discrete layer, but desintegrates to a plasmodial mass during development of the pollen grains in the loculus (L).

Ubisch bodies, a common 'star-shaped' structure occurring in the tapetal area

Fig. 3. Cells of the more inner layer of the endothecium tissue of TSWV infected anther of *T. majus*, containing clusters of virus particles (V) in their cytoplasm.

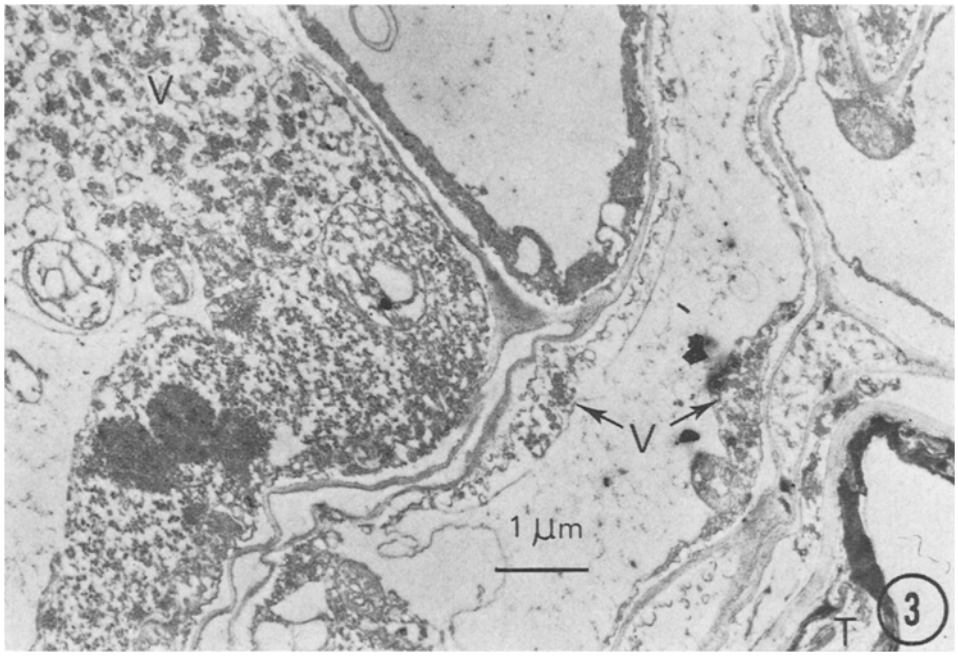


Fig. 3. Meer inwendig gelegen cellen van het endotheciumweefsel van een met TSWV geïnfecteerde helmknop van T. majus, met groepjes van virusdeeltjes (V) in het cytoplasma.

adjacent to the loculus space of anthers from many plants (Clowes and Juniper, 1968; de Vries and Ie, 1969), were not found to occur in the anthers of *T. majus*.

Very typical is the occurrence of plastid-like structures (Pl), containing high numbers of droplets of osmiophilic material and of fairly large 'sacs' (Sa) of high electron density in the cytoplasm of the tapetal cells. The occurrence of these structures seems very specific for the tapetal cells (Fig. 6).

S-bodies are frequently found in the tapetal cells of anthers from S-body positive plants (Ie, 1972).

Pollen grains. Virus particles were never found in the pollen grains of infected anthers from TSWV infected *T. majus* plants, although the parenchyma cells of the endothecium tissue do contain the virus particles in high concentrations (Fig. 5).

S-bodies, however, do occur in the pollen grains of S-body positive *T. majus* plants (Ie, 1972). They were also found in pollen grains in younger stages as well as in the more mature stage, in which the generative nucleus with some cytoplasmic material was already isolated by a callose wall. In both areas of the cytoplasm S-bodies were found, although in low concentrations.

Exothecium. Virus particles were not localized in the exothecium cells, although they have been found in the epidermal cells of leaves and petals of many TSWV infected plants.

Fig. 4. Large cells of the lamina fibrosa layer of a TSWV-infected anther of *T. majus*, with characteristic virus clusters (V) in their cytoplasm. Inset: enlargement of indicated area.

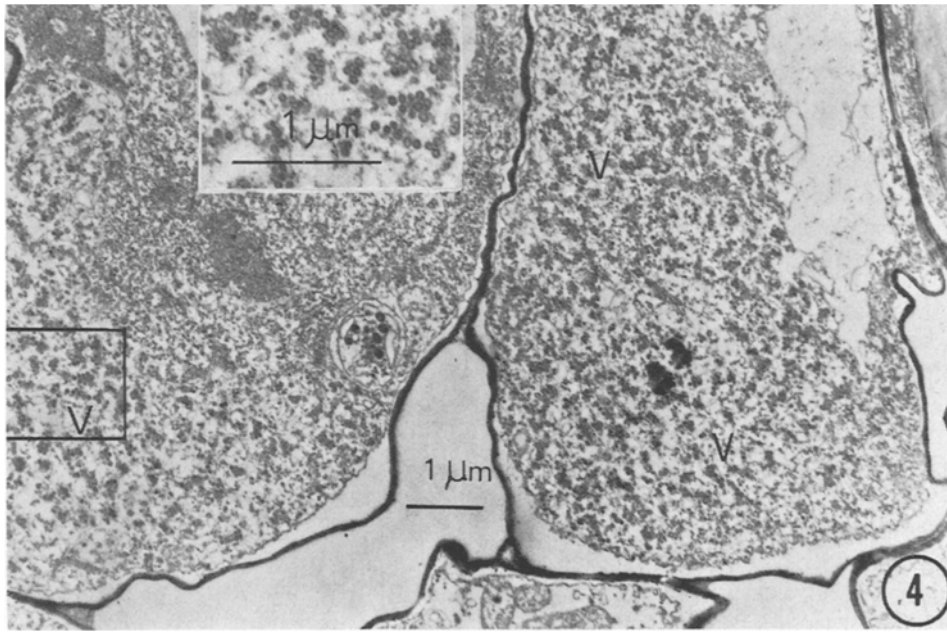


Fig. 4. Grote cellen van de lamina-fibrosa-laag van een met TSWV geïnfecteerde helmknop van T. majus, met karakteristieke virusdeeltjes (V) in het cytoplasma. Links boven: vergroting van aangegeven gebied.

Fig. 5. An ultrathin-section of a pollen grain of TSWV-infected *T. majus* of the S-body negative type. Neither virus particles, nor S-bodies occur in the cytoplasm. N = nucleus, M = mitochondrion, D = dictyosome, P = plastid, Ex = exine wall.

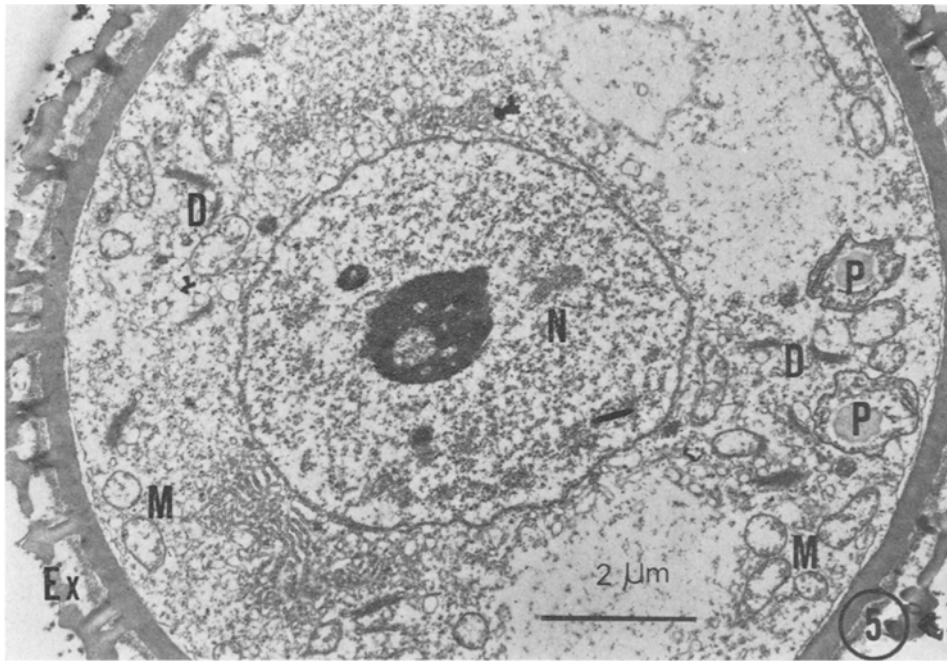


Fig. 5. Een ultracoupe van een pollen-korrel van een met TSWV geïnfecteerde plant van T. majus, van het S-body-negatieve type. Noch virusdeeltjes, noch S-bodies zijn in het cytoplasma aanwezig. N = nucleus, M = mitochondrion, D = dictyosoom, P = plastide, Ex = exine wand.

Discussion

Electron microscopy of sectioned material has enabled a precise visualization of TSWV particles in the infected anthers.

It is surprising that the tapetal cells and the developed pollen grains are virus-free, although the surrounding cells of the endothecium tissue are full of virus particles. The tapetum layer may form a barrier for the virus, but it is not known why.

However, S-bodies do occur in the tapetal cells in fairly high concentrations and also in the pollen grains, although in small numbers. Probably because these S-bodies are true cell components in the S-body positive *Tropaeolum* plants and TSWV particles are not.

Although the anthers are infected with TSWV, pollen development seems normal, but no fertility tests were made.

These observations suggest that TSWV is not pollen-transmitted in *T. majus*.

Fig. 6. Part of a not yet degenerated tapetum cell of TSWV-infected anther of *T. majus* of the S-body positive type. Only S-bodies (S), but no virus are seen in the cytoplasm. En = endothecium, Pl = plastid-like structure, L = loculus, Ex = exine wall of a pollen grain, Sa = sac-like structure.

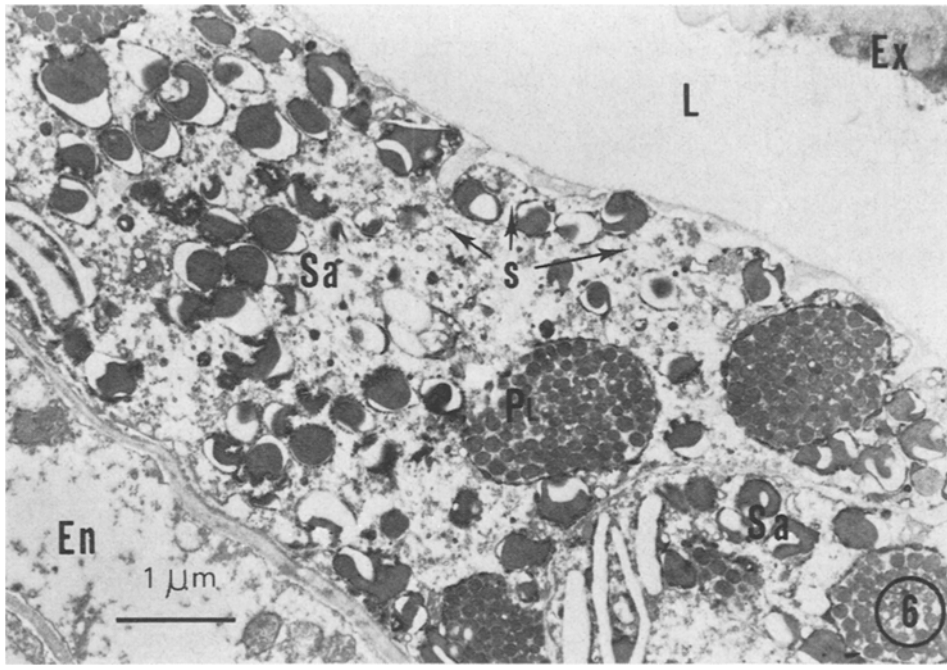


Fig. 6. Gedeelte van een nog niet gedegenereerde tapetumcel van een met TSWV geïnfecteerde helmknop van T. majus, die S-body-positief is. Slechts S-bodies (S), maar geen virusdeeltjes zijn in het cytoplasma te vinden. En = endothecium, Pl = plastide-achtige structuur, L = loculus, Ex = exinewand van een pollenkorrel, Sa = zak-achtige structuur.

Acknowledgment

I am grateful to Professor J. P. H. van der Want for his stimulating interest during the course of this work and for his suggestions during the writing of this manuscript. Corrections of the English text and critical comments by Dr G. A. de Zoeten are much appreciated. Thanks are also due to Miss M. Usmany and D. Lohuis for competent technical assistance.

Samenvatting

Tomatebronsvlekkenvirus in de helmknoppen van Tropaeolum majus

Bij elektronenmicroscopisch onderzoek van ultradunne coupes van helmknoppen van planten van *Tropaeolum majus*, geïnfecteerd met het tomatebronsvlekkenvirus (tomato spotted wilt virus TSWV), is waargenomen dat de virusdeeltjes uitsluitend voorkomen in de cellen van het endotheciumweefsel van de helmknop. In deze

parenchymcellen zijn de virusdeeltjes beperkt tot het cytoplasma, en wel in groepjes in de cisternae van het endoplasmatisch reticulum (Fig. 2, 3 en 4). De virusdeeltjes komen echter niet voor in de cellen van de tapetumlaag, evenmin in de pollenmoedercellen of in de rijpe stuifmeelkorrels (Fig. 5, 6). Solitaire deeltjes (S-bodies) daarentegen, komen wel voor in alle cellen van de helmknop, evenals in de stuifmeelkorrels van planten, die S-body-positief zijn (Fig. 6).

References

Clowes, F. A. L. & Juniper, B. E., 1968. Plant cells. Blackwell Scient. Publ., Botanical Monographs Vol. 8: 280–284.

Francki, R. I. B. & Grivell, C. J., 1970. An electron microscope study of the distribution of tomato spotted wilt virus in systemically infected *Datura stramonium* leaves. Virology 42: 969–978.

Ie, T. S., 1964. An electron microscope study of tomato spotted wilt virus in the plant cell. Neth. J. Pl. Path. 70: 114–115.

Ie, T. S., 1970. Tomato Spotted Wilt Virus. Descriptions of Plant Viruses No 39, Commonw. Mycol. Inst.–Assoc. appl. Biol.

Ie, T. S., 1971. Electron microscopy of developmental stages of tomato spotted wilt virus in plant cells. Virology 43: 468–479.

Ie, T. S., 1972. Cytoplasmic particles in *Tropaeolum majus*. Planta 106: 227–236.

Kitajima, E. W., 1965. Electron microscopy of vira-cabeça virus (Brazilian tomato spotted wilt virus (TSWV) from tomato roots. Virology 26: 89–99.

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.

Milne, R. G., 1970. An electron microscope study of tomato spotted wilt virus in sections of infected cells and in negative stain preparations. J. gen. Virol. 6: 267–276.

Milne, R. G. & Zoeten, G. A. de, 1967. A comparison of some methods of preparation of thin sections of virus-infected leaves for electron microscopy. J. Ultrastruct. Res. 19: 398–407.

Pease, D. C., 1960. Histological techniques for electron microscopy. Acad. Press, New York, London.

Reynolds, E. S., 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol. 17: 208–212.

Vries, A. Ph. de & Ie, T. S., 1970. Electron microscopy on anther tissue and pollen of male sterile and fertile wheat (*Triticum aestivum L.*). Euphytica 19: 103–120.

Address

Laboratorium voor Virologie, Binnenhaven 11, Wageningen, the Netherlands.