Neth. J. Pl. Path 77 (1971) 106–112

Electron microscopy of plant tissues infected with potato viruses A and S

J. A. DE BOKX AND H. A. J. I. WATERREUS

Institute of Phytopathological Research (IPO), Wageningen

Accepted 25 January 1971

Abstract

Electron microscopy of ultrathin sections of plant tissues infected with potato virus A, revealed pinwheel inclusions and electrondense bundles. In tissues infected with potato virus S bundles of particles or accumulations of closely packed black dots were observed.

Introduction

Potato virus A (PVA) [*/*:*/*:E/E:S/AP] and potato virus S (PVS) [*/*:*/*:E/E:S/AP] differ clearly in host range and particle length and morphology, although both are elongated. PVA, with an average length of 730 nm belongs to the group of potato virus Y, whereas PVS with an average length of 650 nm is the type virus after which an other group is named.

Tissues containing viruses of the potato virus Y group have been widely studied with the electron microscope (Matsui and Yamaguchi, 1964; Edwardson, 1966; Edwardson et al., 1968; Zettler et al., 1968; Bos and Rubio-Huertos, 1969; Herold and Munz, 1969; Edwardson and Purcifull, 1970; Purcifull et al., 1970).

The viruses concerned usually produced cytoplasmic inclusions visible with the light microscope and these always contained typical 'pinwheel' structures when studied with the electron microscope. Edwardson (1966) suggested that these structures were characteristic of viruses of that group and were of diagnostic value.

As far as we know only a few representatives of the potato virus S group have been investigated in this respect: wheat streak mosaic virus with an average length of 700 nm (Lee, 1965; Shepard and Carroll, 1967) and potato virus M (PVM) with an average length of 650 nm (Tu and Hiruki, 1970). However, in cells of wheat and barley with wheat streak mosaic virus pinwheel structures occurred, whereas in PVM infected cells they were absent and individual rods and bundles of particles were visible.

We investigated whether elongated particles of PVA and PVS were present in infected plant cells, studied the effect of PVA and PVS on the tissues of their hosts and tried te detect pinwheel structures in infected cells as reported for other representatives of the potato virus Y group.

As isolates of one virus may vary considerably with various properties, a number of isolates were used to obtain more representative results.

Materials and methods

Virus isolates and hosts used

Four isolates of PVA were obtained from the potato varieties 'Saucisse Rouge', 'Lichte Industrie' and 'Allerfrüheste Gelbe', respectively. The isolates were inoculated to 'Whit Burley' tobacco to free them from PVS. Healthy potato plants 'Eigenheimer' and plants of *Nicotiana langsdorffii* were inoculated with each of the isolates.

Five isolates of PVS were obtained from and cultured in the potato varieties 'Ysselster', 'Eersteling', 'Leona', 'Fortuna' and 'Industrie'. The plants were checked serologically and by test plants for the possible presence of unwanted viruses.

Electron microscopy

For PVA, leaves were used of potato plants with secondary infection, showing clear symptoms 5-9 weeks after planting, or leaves of *Nicotiana langsdorffii* 3-4 weeks after inoculation. PVS was investigated only in leaves of potato with secondary infection 5-9 weeks after planting. Leaves of healthy potato and tobacco of similar age were also examined.

Small pieces of infected and healthy potato and tobacco leaves were cut and fixed for 5 h with 2% glutaraldehyde in Sörensen buffer (0.1 M phosphate buffer, pH 6.8). The tissue pieces were rinsed three times in Sörensen buffer, postfixed for 2 h in 1% osmium tetroxide in 0.1 M phosphate buffer, pH 6.8, rinsed three times in Sörensen buffer and then kept in 0.5% uranyl acetate overnight, followed by rinsing in distilled water. After fixation, the tissue pieces were dehydrated in a graded series of ethanol solutions and embedded in a mixture of Epon-Araldite.

Sections were cut with an LKB ultramicrotome using a glass knife. They were

Fig. 1-2. Sections of potato leaves 'Eigenheimer', infected with potato virus A:1. isolate 'Saucisse Rouge' (× 19,000), 2. isolate 'Dora' (× 38,000).

In the cytoplasm 'rings' (R), pinwheels (PW) and electron-dense bundles (DB). CW = cell wall; V = vacuole; I = intercellular; C = chloroplast.

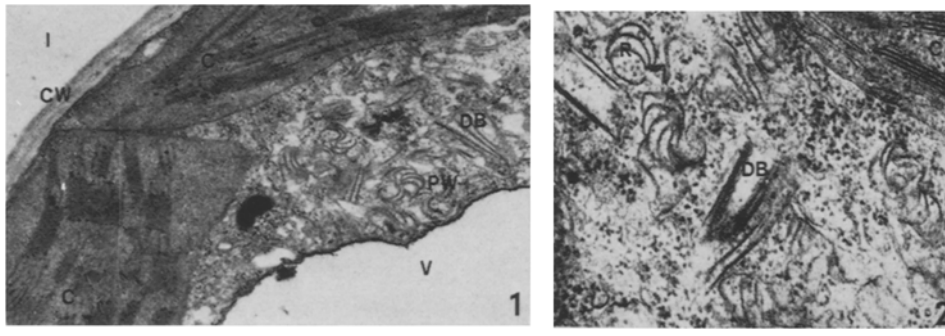
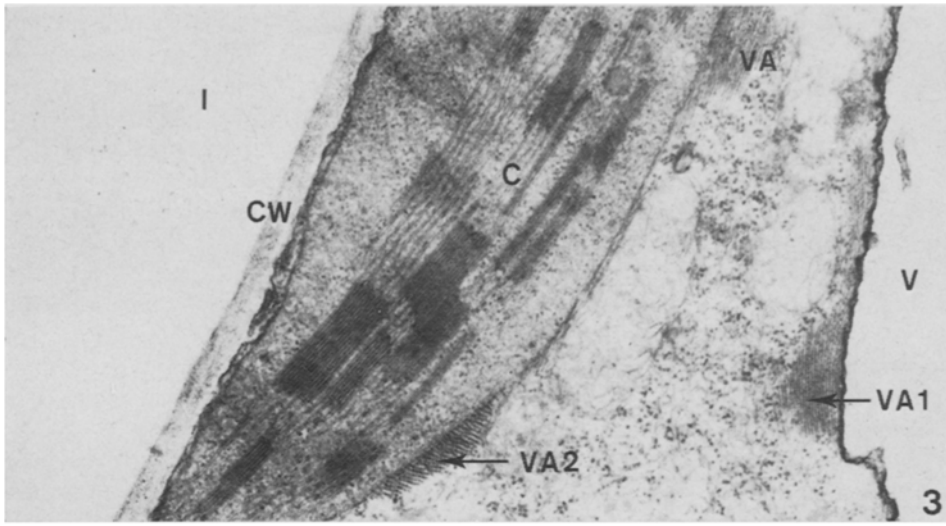
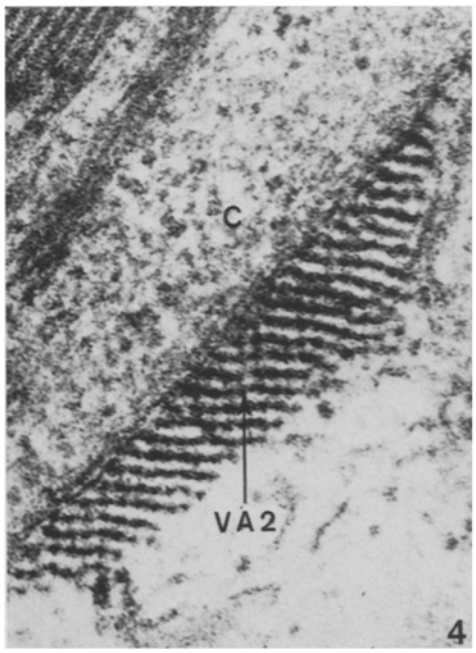


Fig. 1-2. Coupes van aardappelbladeren 'Eigenheimer', geïnfecteerd met aardappelvirus A:1. isolaat 'Saucisse Rouge' (19.000 ×), 2. isolaat 'Dora' (38.000 ×). In de cytoplasmakringen (R), 'pinwheel'-structuren (PW) en 'electron-dense' bundels (DB). CW = celwand; V = vacuole; I = intercellulair; C = chloroplast.

Fig. 3-5. Section of potato leaf 'Leona' infected with potato virus S, isolate 'Leona'. 3. Cytoplasm containing three virus aggregates (VA, VA1, VA2) × 37,000. 4. Virus aggregate (VA2) cut obliquely to the long axes of the particles × 143,500. 5. A longitudinally aligned layer (VA1) of particles × 143,500. CW = cell wall; V = vacuole; I = intercellular; C = chloroplast.





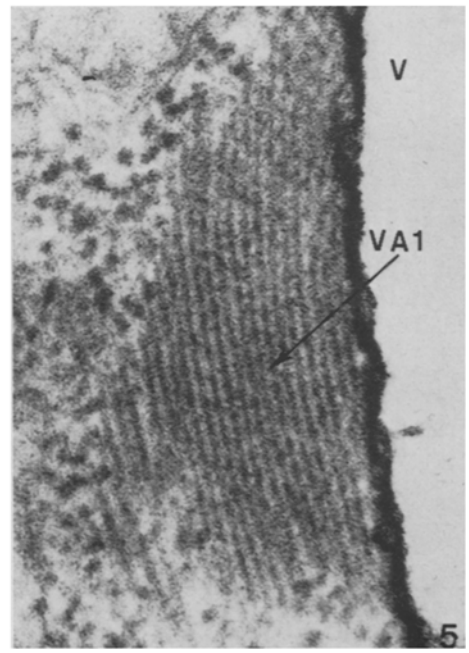


Fig. 3-5. Coupe van aardappelblad 'Leona', geïnfecteerd met aardappelvirus S, isolaat 'Leona'. 3. Cytoplasma, dat drie virusaggregaten (VA, VA1, VA2) bevat, 37.000 ×. 4. Virusaggregaat (VA2) schuingesneden op de lengteas van de virusdeeltjes, 143.500 ×. 5. Virusaggregaat (VA1) in de lengterichting gesneden, 143.500 X. CW = celwand; V = vacuole; I = intercellulair; C = chloroplast.

stained in a 2% aqueous solution of uranyl acetate for 1 h, followed by lead citrate (Reynolds, 1963) for 5 min, and then rinsed and dried. The sections of healthy and infected material were examined with a Philips EM 300 electron microscope (Technical and Physical Engineering Research Service, Wageningen).

Fig. 6-7. Sections of potato leaves 'Leona', infected with potato virus S, isolate 'Leona'. Virus aggregate (VA) cut perpendicularly to the long axes of the particles, 6. regular arrangement of the particles × 130,000, 7. less regular arrangement of the particles, presence of rings (R) × 66,600. CW = cell wall; V = vacuole; C = chloroplast; I = intercellular; M = mitochondrion.

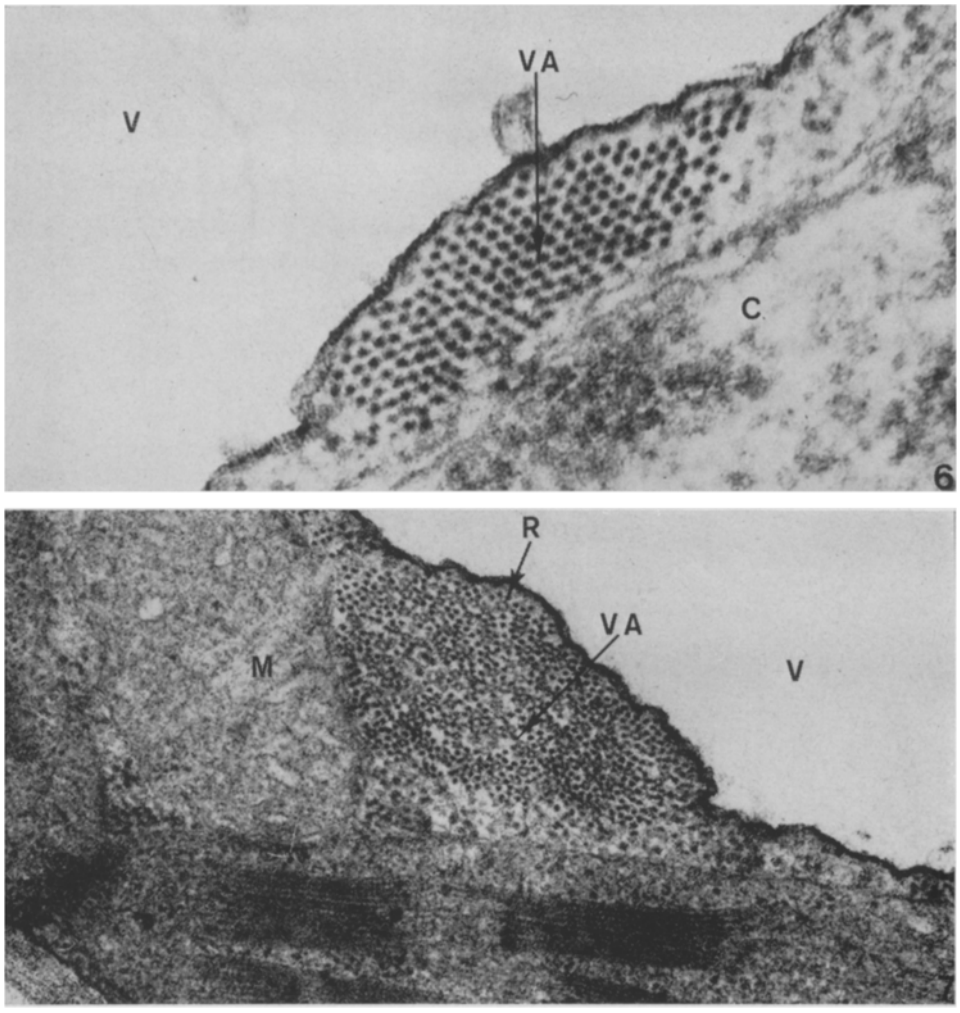


Fig. 6-7. Coupes van aardappelbladeren 'Leona', geïnfecteerd met aardappelvirus S, isolaat 'Leona'. Virusaggregaten (VA) loodrecht gesneden op de lengteas van de virusdeeltjes: 6. regelmatige rangschikking van de deeltjes 130.000 ×, 7. minder regelmatige rangschikking van de deeltjes, aanwezigheid van kringen (R), 66.600 ×. CW = celwand; V = vacuole; C = chloroplast; I = intercellulair; M = mitochondrium.

Results

Unlike the sections of tobacco, those of potato showed markedly little cytoplasm. Against the cell wall chloroplasts were observed, which were separated from the very large vacuole by small amounts of cytoplasm.

In the cytoplasm of PVA-infected potato leaves conglomerates of abnormal structures were observed. Depending on the plane of sectioning, pinwheel structures (PW), electron-dense bundles (DB) and 'rings' (R) are revealed in our electron micrographs (Fig. 1 and 2).

Single virus particles could not be observed in the cytoplasm nor in the chloroplasts. The chloroplasts in infected tissues did not differ visibly from those in uninfected cells. The inclusions did not appear different in either cells of tobacco or potato leaves infected with each of the five isolates of PVA.

In PVS-infected cells of potato plants inclusions occurred rarely. The aggregates found were rather small. They were mainly located against the tonoplast and the chloroplasts of the infected cells (Fig. 3). The aggregates consisted of particles in a parallel arrangement. When cut obliquely the aligned particles appeared as dotted lines (Fig. 5.3 and 4-VA2) and in cross section as black dots (Fig. 6). Since these particles were absent in tissues of non-infected plants and their diameter equaled that of PVS particles it is assumed that the black dots represent virus particles. In Fig. 3 and 5 (VA1) a longitudinally aligned layer of particles is shown.

Fig. 7 gives a cross section of an aggregate occurring in infected tissue.

Besides the solid black dots which presumably represent virus particles, rings (R) are visible, with a diameter somewhat larger than that of the black dots. The black dots are arranged in a more or less regular pattern but the rings are not.

In the healthy material no inclusions were detected.

Discussion

Edwardson (1966) suggested that in tissues infected with elongated viruses of the potato virus Y group inclusions occur of a complex cylindrical structure. They have been described in detail by Edwardson et al. (1968). The results obtained here with PVA completely agree with their description.

In PVS-infected tissues of potato such cylindrical inclusions were absent. Ultrathin sections of infected potato leaves revealed cytoplasmic inclusions much like the aggregates reported for clover yellow mosaic virus (Purcifull et al, 1966) and PVM (Tu and Hiruki, 1970). The latter may be understandable since PVM and PVS are related viruses. However, we did not observe individual virus rods in the cytoplasm of PVS-infected cells. In the cytoplasm (Fig. 3) membranes are visible; the black dots near the membranes represent ribosomes. Virus particles or aggregates were not observed in chloroplasts or mitochondria. The origin and the function of the rings observed in tissue of PVS-infected 'Leona' is not yet known. The presence of only a few small virus inclusions and the absence of visible individual virus rods may correspond to low virus concentration in the plant and may be connected with the difficulty of testing potato plants at a similar stage of development for PVS by serology (de Bokx, 1969).

Although Lee (1965) and Shepard and Carroll (1967) observed cylindrical in-

clusions in wheat tissue infected with wheat streak mosaic virus we conclude from our findings and from the results of the experiments of Tu and Hiruki (1970) that induction of cylindrical inclusions is not a specific property of the viruses of the potato virus S group.

It is questionable whether wheat streak mosaic virus, which is transmitted by mites, can be classified with the potato virus S group. Our findings support the idea of Gibbs (1969), that wheat streak mosaic virus probably does not belong to that group and must be regarded as a representative of a subgroup of the potato virus Y group or as one of a distinct group.

Samenvatting

Elektronenmicroscopisch onderzoek van weefsels geïnfecteerd met de aardappelvirussen A en S

Bij elektronenmicroscopisch onderzoek van ultradunne coupes van aardappel- en tabaksblad werden de uit de literatuur – van de aardappelvirus Y-groep alsmede van het tot de S-virusgroep gerekende strepenmozaïekvirus van tarwe – bekende 'pinwheel'-structuren alleen waargenomen na infectie met aardappelvirus A (Fig. 1 en 2). In cellen van aardappelblad geïnfecteerd met aardappelvirus S werd in het cytoplasma een gering aantal aggregaten waargenomen. De cellen van het aardappelblad hadden in het algemeen grote vacuolen. De aggregaten waren veelal tegen de tonoplast of chloroplasten gelegen. In dwarsdoorsneden manifesteerden zij zich als regelmatig gerangschikte zwarte punten, in lengtedoorsneden als een pakket evenwijdige deeltjes (Fig. 3, 4 en 5). Omdat deze deeltjes niet voorkwamen in niet geïnfecteerd weefsel en hun diameter ongeveer gelijk was aan die van aardappelvirus S wordt aangenomen, dat de in bundels zichtbare evenwijdig lopende deeltjes en de zwarte punten virusdeeltjes zijn.

In Fig. 7 wordt een dwarsdoorsnede van een virusaggregaat getoond, voorkomend in het cytoplasma van een aardappelblad geïnfecteerd met aardappelvirus S, isolaat 'Leona'. Hierin zijn niet alleen min of meer regelmatig gerangschikte zwarte punten zichtbaar, maar ook kringen. De diameter van deze kringen is groter dan die van de zwarte punten. De betekenis van deze kringen is onduidelijk.

De resultaten van onze proeven geven aan, dat het induceren van 'pinwheel' structuren waarschijnlijk geen eigenschap is van virussen behorend tot de aardappelvirus S-groep. Hiermee wordt de veronderstelling van Gibbs (1969) ondersteund, dat het strepenmozaïekvirus van de tarwe (700 nm), op grond van het voorkomen van 'pinwheel'-structuren in met het virus geïnfecteerde weefsels, eerder gerekend moet worden tot de groep of een subgroep van het aardappelvirus Y, of misschien tot een aparte groep, dan tot de groep van het aardappelvirus S.

References

Bokx, J. A. de, 1969. Detection of elongated potato viruses by electron microscopy. Meded. Rijksfak. LandbWet., Gent 34: 903–916.

Bos, L. & Rubio-Huertos, M., 1969. Light and electron microscopy of cytoplasmic and unusual nuclear inclusion bodies evoked by a virus from necrotic peas. Virology 37: 377–385.

Edwardson, J. R., 1966. Electron microscopy of cytoplasmic inclusions in cells infected with rod-

shaped viruses. Am. J. Bot. 53: 359–364.
Edwardson, J. R., Purcifull, D. E. & Christie, R. G., 1968. Structure of cytoplasmic inclusions in plants infected with rod-shaped viruses. Virology 34: 250–263.
Edwardson, J. R. & Purcifull, D. E., 1970. Turnip mosaic virus-induced inclusions. Phytopathology 60: 85–88.

Gibbs, A. J., 1969. Plant virus classification. Adv. Virus Res. 14: 263–328.
Herold, F. & Munz, K., 1969. Peanut mottle virus. Phytopathology 59: 663–667.
Lee, P. E., 1965. Electron microscopy of inclusions associated with wheat streak mosaic virus. J. Ultrastruct. Res. 13: 359–366.
Matsui, C. & Yamaguchi, A., 1964. Electron microscopy of host cells infected with tobacco etch virus. II Fine structures of leaf cells before and after the appearance of external symptoms. Virology 23: 346–353.
Purcifull, D. E., Edwardson, J. R. & Christie, R. G., 1966. Electron microscopy of intracellular aggregates in pea *(Pisum sativum)* infected with clover yellow mosaic virus. Virology 29: 276–284.
Purcifull, D. E., Edwardson, J. R. & Christie, S. R., 1970. A morphological comparison of inclusions induced by tobacco etch and potato viruses. Phytopathology 60: 779–782.
Reynolds, E. S., 1963. The use of lead citrate at high pH as an electronopaque stain in electron microscopy. J. Cell Biol. 17: 208–212.
Shepard, J. F. & Carroll, T. W., 1967. Electron microscopy of wheat streak mosaic virus particles in infected plant cells. J. Ultrastruct. Res. 21: 145–152.
Tu, J. C. & Hiruki, C., 1970. Ultrastructure of potato infected with potato virus M. Virology 42: 238–240.
Zettler, F. W., Edwardson, J. R. & Purcifull, D. E., 1968. Ultramicroscopic differences in inclusions of papaya mosaic virus and papaya ringspot virus correlated with differential aphid transmission. Phytapathology 58: 332–335.

Address

Instituut voor Plantenziektenkundig Onderzoek (IPO), Binnenhaven 12, Postbus 42, Wageningen, the Netherlands