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Intracellular accumulation of Passiflora latent virus in Chenopodium quinoa

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Abstract

In ultrathin sections of *Chenopodium quinoa* plants, with systemic symptoms after inoculation with *Passiflora* latent virus, high concentrations of virus particles could be easily observed.

The virus particles occurred in bundles or extensive, slightly contorted plates in the cytoplasm, in bundles externally attached to the membranes of mitochondria and chloroplasts or in bundles of regular size attached to tonoplasm in protoplasmic strands. The excessive production of such abnormal protoplasmic strands with rather regularly distributed bundles of virus particles has not been reported before.

No pinwheels or other structures characteristic of infection with representatives of the potato virus Y group have been observed. The accumulations of particles attached to chloroplasts resemble those known of potato viruses S and M.

Introduction

The intracellular localization of plant viruses and their corpuscular participation in the formation of inclusion bodies have received rapidly increasing interest during the last few years. Representatives of the potato virus Y group (730 to over 800 nm long) have been most intensively studied (e.g. Rubio-Huertos and Hidalgo, 1964; Edwardson *et al.*, 1968).

Only since very recently, attention is also turning to the potato virus S group (650–680 nm long): Tu and Hiruki (1970) studied potato virus M and de Bokx and Waterreus (1971) potato virus S. Earlier investigations on the 700 nm long wheat streak mosaic virus by Lee (1965) and Shepard and Carroll (1967) have shown that this virus may belong to the Y group rather than to the S group on account of the pinwheel structures produced.

A few photographs on *Passiflora* latent virus ($^{*}/_{*}$ $^{*}/_{*}$ $^{E}/_{E}$ $^{S}/_{*}$) by Schnepf and Brandes (1961) are among the first published on the intracellular localization of plant viruses in their hosts. Schnepf's work on the ultrastructure of extrafloral nectaries of *Passiflora caerulea* revealed abnormal structures in ultrathin sections, with help of Brandes characterized as accumulations of slightly curved particles of 15 × 650 nm. It led to the discovery of this virus, later described in more detail by Brandes and Wetter (1963).

When working on some other members of the potato virus S group isolated from legume crops, we included the *Passiflora* latent virus and have further studied its intracellular distribution.

Materials and methods

The virus was isolated from *P. caerulea* grown in a private garden. It was transmitted with sap to *Chenopodium quinoa* and easily maintained in this test plant. It was identified as *Passiflora* latent virus on the basis of its very limited host range outside the genus *Passiflora*, the very characteristic systemic symptoms in *C. quinoa*, and the particle length of about 650 nm (determined for 61 particles as compared with 23 TMV particles used as internal standard).

For light microscopy, epidermal strips of petioles, main veins and young stems of *C. quinoa*, were stained with 1 % phloxine and 1 % methylene blue in Christie's solution (Bos, 1969).

For electron microscopy, pieces of about 1 mm^2 of infected leaves of *C. quinoa* with bright vein chlorosis were fixed for 2h at 4 °C with a 2 % osmium tetroxide solution in Veronal acetate buffer pH 6.8. The samples were dehydrated in a graded series of acetone concentrations, embedded in Durcupan, and sectioned with a glass knife in an LKB Ultrotome ultramicrotome. The sections were expanded in chloroform, mounted on Formvar-coated grids, and stained with lead citrate according to Reynold's method. The preparations were examined in a Siemens Elmiskop I electron microscope.

Results

Light microscopy of epidermal strips stained with phloxine and methylene blue did not reveal any inclusion bodies.

With the electron microscope in ultrathin sections extensive accumulations of elongated particles could easily be found. They were usually aggregated in more or less regular parallel array.

In longitudinal section the particles often occurred in single bundles or in plates obviously one particle long or high (Fig. 1). Evidently particles in the upper left of Fig. 1 have been cut obliquely because of a contortion of the plate. This arrangement in slightly contorted plates or groups of plates is further demonstrated in Fig. 2. Here a massive amount of particles is shown, most of them cut transversely, part more or less obliquely, thus giving the impression of a whirlpool.

Sometimes particles overlap forming elongate bundles as in some protoplasmic strands (Fig. 3).

The most characteristic feature of the ultrastructure of the infected plants is the production of protoplasmic strands in which the particles occur transversely to the longitudinal axis of the cytoplasmic strand producing a cross-banding effect (Fig. 4). These particles are connected with the tonoplast usually at both ends. The inset of Fig. 4 obviously represents a tangential section of one bundle protruding from such a protoplasmic strand. A strand with many bundles sectioned more or less obliquely is shown in Fig. 5. In Fig. 6 these bundles have been cut almost and sometimes completely transversely. This photograph suggests the bundles to be within or associated with cytoplasmic membranes, possibly modified endoplasmic reticulum.

In Fig. 4 the upper right shows an accumulation of particles close to or attached to the chloroplast membrane, and in the middle right attached to a mitochondrion.

Fig. 1. Part of an infected cell with bundles and plates of virus particles in parallel array; × 34,500; *w* cell wall.

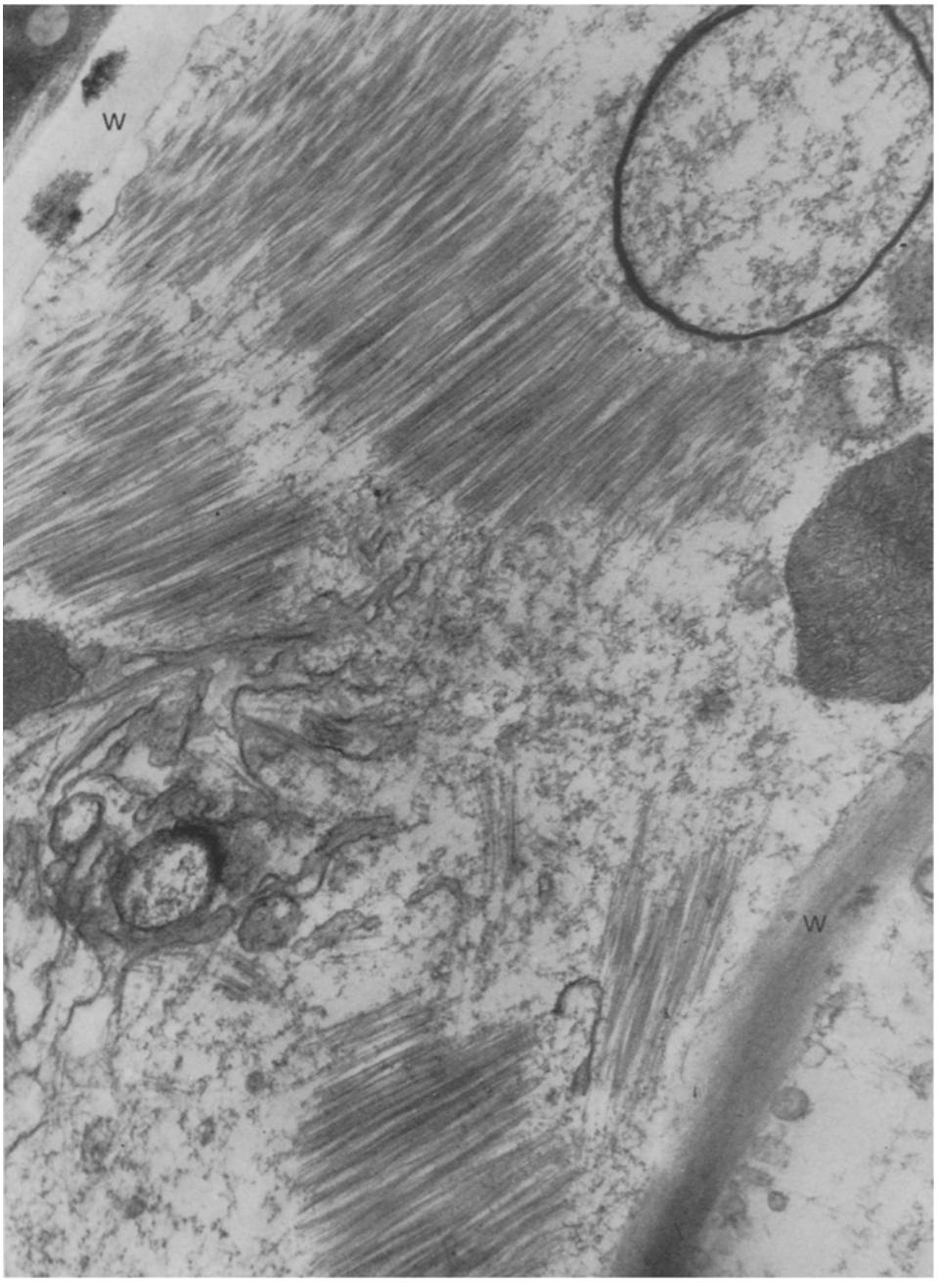

Fig. 1 Deel van een geïnfecteerde cel met bundels en platen van evenwijdig gerangschikte virusdeeltjes; vergr. 34.500 ×; w celwand.

Fig. 2. Part of an infected cell with extensive slightly contorted plates of particles cut transversely or obliquely; × 52,900; *c* chloroplast, *n* nucleus.

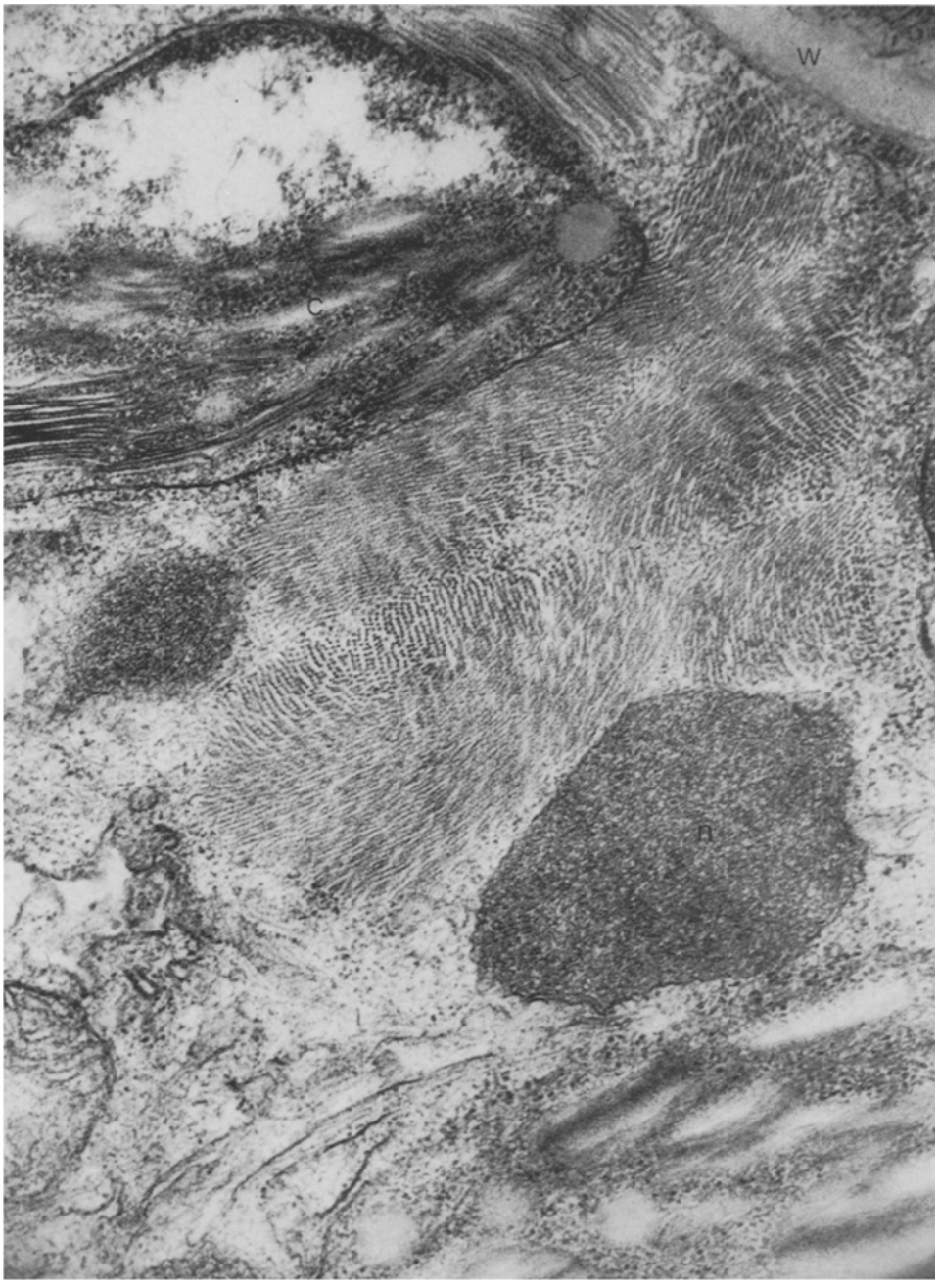

Fig. 2. Deel van een geïnfecteerde cel met omvangrijke, iets verwrongen platen, bestaande uit dwars- of schuingesneden virusdeeltjes; vergr. 52.900 ×; c chloroplast, n kern.

Fig. 3. Strand of protoplasm with virus particles attached to degenerated chloroplast (*c*) and overlapping lengthwise; × 34,000.

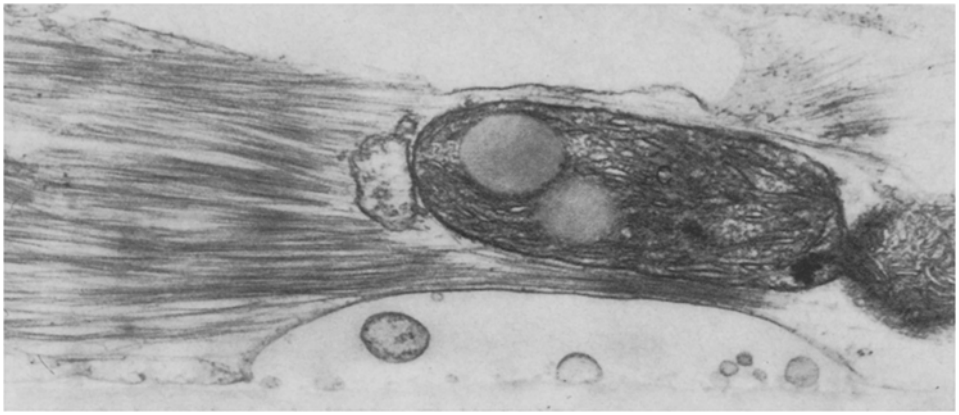

Fig. 3. Streng protoplasma met virusdeeltjes gehecht aan een gedegenereerde chloroplast (c) *en elkaar in de lengte overlapppend tot een langgerekte bundel; vergr. 34.000* ×.

Fig. 4. Part of a cell with virus particles attached to chloroplast (*c*) and mitochondrion (*m*) and with regular bundles of virus particles in protoplasmic strand; × 46,000. Inset, tangential section of a bundle of virus particles protruding from a protoplasmic strand; × 30,000.

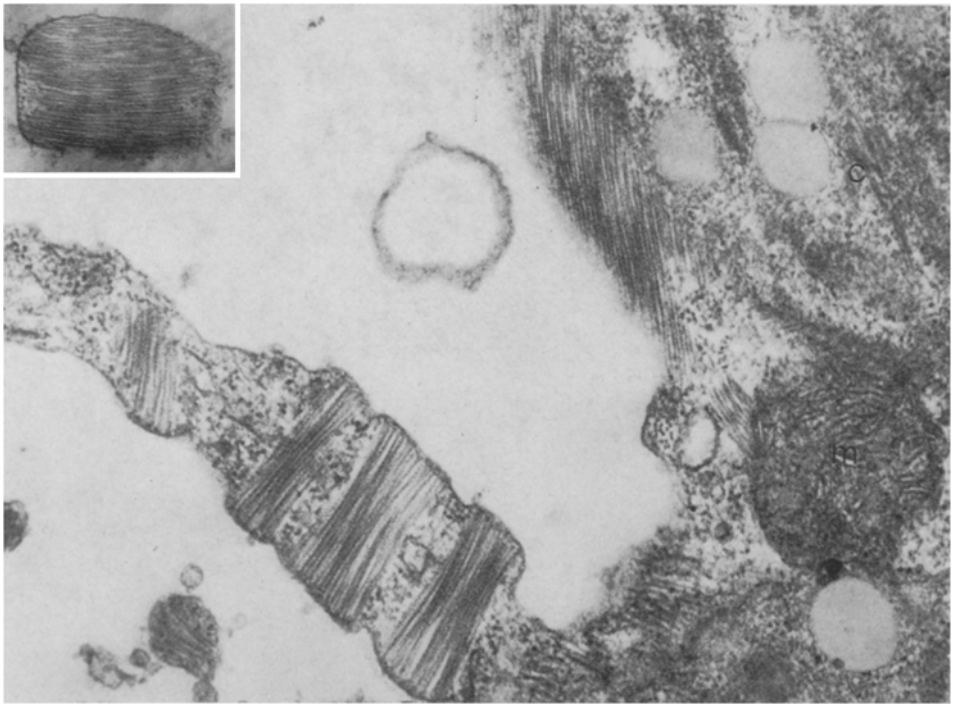

Fig. 4. Deel van een cel met virusdeeltjes gehecht aan chloroplast (c) *en mitochondrion* (m) *en met regelmatige bundels van deeltjes in protoplasmastreng; vergr. 46.000* ×. *Inzet, overlangs gesneden bundel virusdeeltjes, uitstekend uit een protoplasmastreng; vergr. 30.000* ×.

Fig. 5. Section of a protoplasmic strand with regularly distributed bundles of virus particles cut tangentially or obliquely; × 50,000.

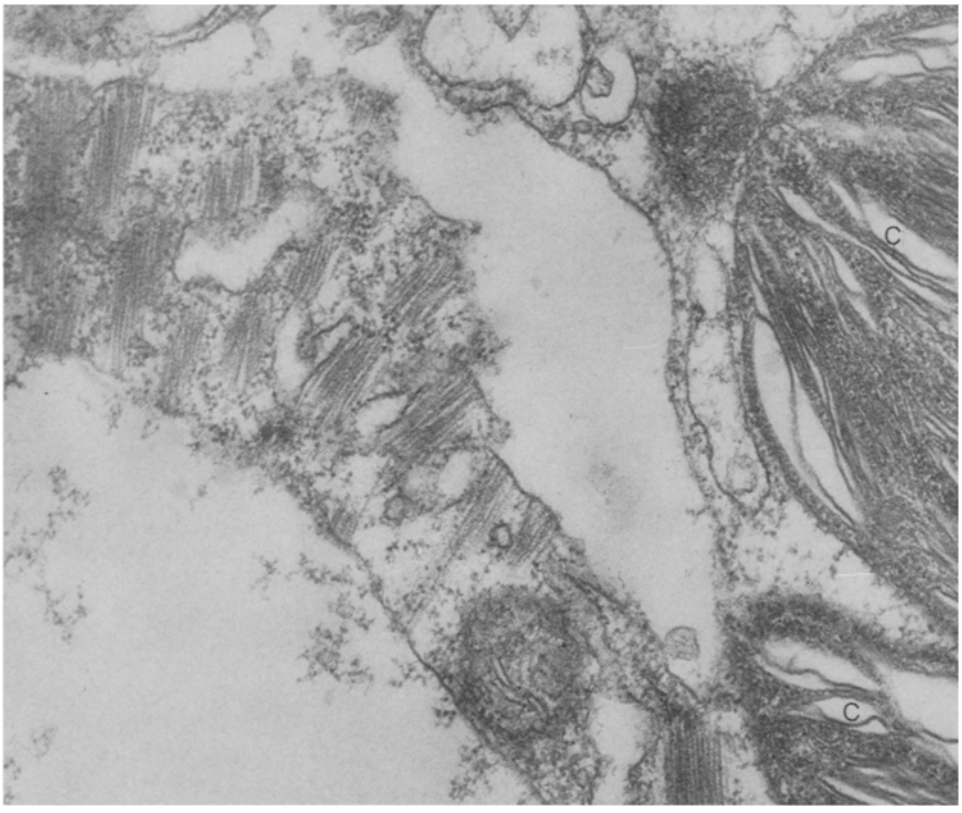

Fig. 5. Coupe van een protoplasmastreng met regelmatig gespreide bundels virusdeeltjes, in de lengte of schuin gesneden; vergr. 50.000 ×.

Discussion

Although it is hard to accurately determine the length of the elongated particles occurring in extensive accumulations in infected tissue, they undoubtedly represent the *Passiflora* latent virus because of comparable length and diameter. The accumulations of Fig. 2 are comparable to those found by Schnepf and Brandes (1961) in *Passiflora caerulea*, but the meanwhile improved techniques allow a much better resolution. The formation of bundles in protoplasmic strands and the attachment of particles of the virus to membranes has not been reported before.

Despite the tendency towards a rather orderly array, as illustrated in Fig. 1 and 2, the accumulation of particles is not sufficiently regular to form crystals easily visible with the light microscope, as with the related red clover vein mosaic virus (publ. in prep.). Although *Passiflora* latent virus clearly tends to form plates (Fig. 1), the particles do not form stacks of more or less regular plates, as is the case with the much longer beet yellows virus (Esau *et al.*, 1966) and the related beet yellow stunt virus (Hoefert *et al.*,

1970). This may be due to the fact that the virus particles are not completely rigid and are more inclined to aggregate lengthwise rather than regularly side by side. Similar structures occur even with tobacco mosaic virus when it forms bundles, rods, needles and fibres.

The frequent appearance of virus particles with their ends stuck to tonoplasm and chloroplasts has also been reported by de Bokx and Waterreus (1971) for potato virus S, whereas Tu and Hiruki (1970) had pointed to the fact that particles of potato virus M were closely associated with or attached to the membrane of chloroplasts, mitochondria and nuclei.

The formation of bundles in more or less parallel orientation, attached with their ends to tonoplast inside abnormal numbers of protoplasmic strands, is a typical feature of infected cells, not reported before. In our electron microscope studies we never observed such an abnormal amount of cytoplasmic strands in *Chenopodium* plants, healthy or infected with other viruses. Their excessive production may very well be caused by a strong affinity of the virus particles to associate with tonoplasm. It

Fig. 6. Section of a flat protoplasmic strand with regularly distributed bundles of virus particles cut obliquely or transversely; × 48,000.

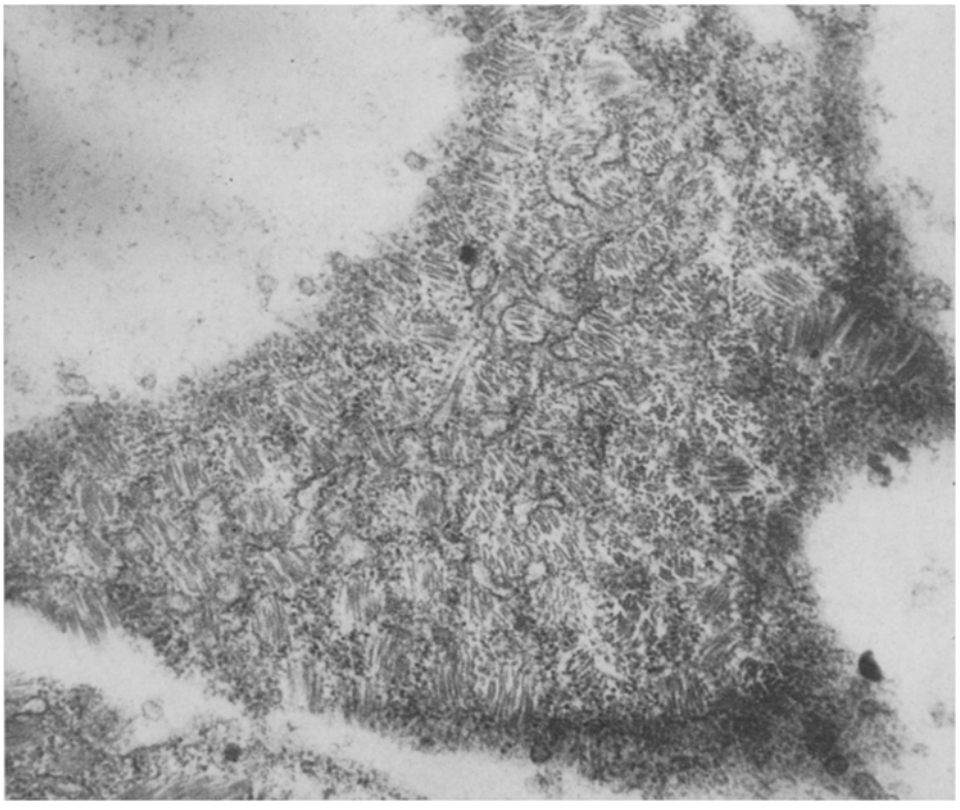

Fig. 6. Coupe van een protoplasmastreng met regelmatig gespreide bundels virusdeeltjes in schuine of dwarse doorsnede; vergr. 48.000 ×.

may also explain the flat shape of the 'strand' of Fig. 6 cut parallel to the flat side of the strand and across the virus bundles.

Evidently, the passion-flower virus has a much stronger tendency to aggregate *in vivo* than potato viruses M and S do. Their accumulations found by Tu and Hiruki (1970) and by de Bokx and Waterreus (1971), respectively, are of very limited size. The first authors easily found individual particles distributed in the cytoplasm. Both potato viruses are known to occur in high concentration, as they can readily be detected by electron microscopy of crude dip preparations (de Bokx, 1969).

With *Passiflora* latent virus no multimembrane bodies were found as reported for potato virus M by Tu and Hiruki (1970). These bodies may not be typical of the potato virus S group. They can be found with different viruses and in healthy cells and may be a symptom of cell degeneration or fixation artifacts (e.g. myeline figures of Coulomb, 1968).

Neither pinwheels, nor electron-dense bands, typical of the potato virus Y group, were observed with our virus. This agrees with the results published for the potato viruses M and S. Because of the presence of these structures in tissues with the slightly longer (700 nm) and mite-transmitted wheat streak mosaic virus (Lee, 1965; Shepard and Carroll, 1967), Gibbs (1969) suggested that this virus and the related *Agropyron* mosaic and ryegrass mosaic viruses might be regarded as a distinct group or perhaps as a subgroup of the potato virus Y group. The loose or more definite parallel arrangement of wheat streak mosaic virus particles in small groups in plant tissue (Shepard and Carroll, 1967) resembles that of the potato viruses M and S. When aggregated into bundles and plates and even in densely packed irregularly formed streamlike patterns in the posterior part of the midgut and especially the hindgut of viruliferous mites (*Aceria tulipae*) (Paliwal and Slykhuis, 1967), it somewhat resembles the *Passiflora* virus. With members of the potato virus Y group such accumulations of virus particles have never been observed. Thus in various respects the viruses related to wheat streak mosaic virus may form some sort of intermediate group, rather than a subgroup of the potato virus Y group.

Our results with *Passiflora* latent virus confirm and further extend the early data by Schnepf and Brandes (1961).

Samenvatting

Ophoping van latent Passiflora-virus in de cellen van Chenopodium quinoa

In ultradunne coupes van stukjes blad van *Chenopodium quinoa*, met systemische symptomen na inoculatie met het latente *Passiflora*-virus, konden opeenhopingen van grote hoeveelheden draadvormige deeltjes worden waargenomen. Van deze deeltjes wordt op grond van rangschikking, afmetingen en vorm aangenomen dat ze virusdeeltjes zijn.

De deeltjes kwamen vaak vrij in het protoplasma voor, in bundels van verschillende afmetingen of in licht gebogen of verwrongen platen. Daarin lagen de deeltjes stijf naast elkaar (Fig. 1 en 2). Door het niet geheel vlak liggen der platen gaf een doorsnede dwars op de lengterichting van de meeste deeltjes een draaikolkbeeld (Fig. 2). Soms vormden de virusdraden door overlapping meer langgerekte bundels (Fig. 3). Ze werden ook vaak waargenomen in groepen en met hun ene uiteinde liggend tegen een

chloroplast of mitochondrion (Fig. 4, rechts, en 3). Daarnaast bleken de virusdeeltjes veel voor te komen in protoplasmastrengen en wel in overdwars liggende bundels van vrij regelmatige afmetingen (Fig. 4 tot 6). Waarschijnlijk werkt de affiniteit van de deeltjes tot de tonoplastmembraan de vorming van deze strengen in de hand. Misschien is de betrokken kracht ook verantwoordelijk voor het vaak afgeplat zijn van de protoplasma 'strengen', wat blijkt uit de scheef of zelfs nagenoeg vlak gesneden strengen van respectievelijk Fig. 5 en 6. In de laatste zijn de virusbundels vrijwel dwars op de lengterichting der deeltjes getroffen.

In geïnfecteerde planten werden geen 'pinwheel' structuren, zoals bekend van de aardappelvirus-Y-groep, gevonden. In neiging tot aggregatie met chloroplasten en mitochondria vertoont het latente *Passiflora*-virus overeenkomst met de aardappelvirussen S en M uit de aardappelvirus-S-groep. Het virus uit passiebloem komt echter in veel grotere opeenhopingen voor. Toch vormt het in *Chenopodium quinoa* geen lichtmicroscopisch zichtbare celinsluitsels, zoals het verwante nerfmozaïekvirus van rode klaver doet. De bij het latente *Passiflora*-virus aangetroffen protoplasmastrengen met overdwars liggende virusbundels zijn tot dusver uniek.

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