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Additional data on the ultrastructure of inclusion bodies evoked by sharka (plum pox) virus

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Abstract

Electron microscopy of ultrathin sections of leaves of *Nicotiana clevelandii* infected with sharka virus revealed several types of cytoplasmic inclusions. Pinwheels and lamellar aggregates were frequent. Pinwheels showed a central core with a threadlike structure in the middle. Lamellar aggregates showed a striation with a periodicity of 55 Å on their surface and they were associated with the endoplasmic reticulum. Irregular crystalline structures were found less frequently. Microbodies were common in the cytoplasm of sharka virus infected *N. clevelandii* plants, but they also abounded in healthy controls.

Nuclear inclusions were present only for short periods after infection.

In leaf extracts irregularly shaped inclusions were found. They had a regular striation of 55 Å. Sometimes nearly parallel stripes were seen on their surface, always at an angle of 80 degrees with the striation.

Introduction

Cytoplasmic and nuclear inclusions in cells of plants infected by viruses of the potato virus Y group are well known. Sharka (plum pox) virus (x/x; x/x; E/E; S/Ap), belonging to this group, is also known to evoke inclusions as was demonstrated by light microscopy (Pleše et al., 1969; van Oosten and van Bakel, 1970) and by electron microscopy (Pleše et al., 1969; Bovey, 1971; Macovei, 1971). In our light microscopical study the nuclear needles seemed of similar shape and size to the cytoplasmic needles. This similarity was interesting and therefore the inclusions were compared for their ultrastructure. Another point of interest was to determine whether the inclusions could be recognized in leaf extracts, as recently found by Purcifull et al. (1970) for tobacco etch virus and potato virus Y.

Materials and methods

Virus isolates and host plant. The sharka virus isolate was the same as that used in previous experiments (van Oosten and van Bakel, 1970). Tobacco etch virus, used for comparison, was kindly provided by Dr D. E. Purcifull (University of Florida, USA). Both virus isolates were maintained in *Nicotiana clevelandii.*

Light microscopy. Methods used for light microscopy were as reported earlier (van Oosten and van Bakel, 1970).

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Electron microscopy. Samples of 1–4 mm^2 were taken from systemically infected leaves of *N. clevelandii*. These samples were fixed for 4 h in 6.5% glutaraldehyde in 0.1 *M* phosphate buffer, pH 6.8, containing 0.1 *M* sucrose. Afterwards the tissues were washed twice in phosphate buffer 0.1 *M*, pH 6.8, containing 0.1 *M* sucrose, then fixed for 1–2 h in 1% osmium tetroxide in water, washed three times in water and dehydrated in ethanol series. Each wash took 10 minutes. Dehydrated tissue was embedded in a prepolymerized methacrylate mixture (butyl: methyl ester = 4:1) and incubated at 55°C for 48 h. For sectioning a LKB ultratome III was used. Sections were stained with uranyl acetate and lead citrate.

In order to study the occurrence of inclusions in leaf extracts leaves of *N. clevelandii* were carefully homogenized in a mortar. A droplet of the extract was transferred to formvar coated grids and mixed with 2% potassium phosphotungstate, pH 6.5.

Sections and extracts were examined with a Siemens Elmiskop I electron microscope.

Results and discussion

Light microscopy. The observations showed a much slower development of the inclusion bodies in autumn than in spring. In autumn the first nuclear inclusions were usually seen 14 days later than reported by van Oosten and van Bakel (1970) for spring. In autumn the inclusions, especially those in the nucleus, were notably smaller. It appeared that the rate of formation of the inclusions and their size depended on the season and corresponded closely with the rate of growth of the host plant.

In a thorough study of the cytoplasm of *N. clevelandii* leaves infected with sharka virus we could observe pinwheels even with the light microscope. So far, this has been reported only for pumpkin leaves infected with watermelon mosaic virus (Edwardson et al., 1968).

Electron microscopy. Inclusions were easily found in leaf extracts of *N. clevelandii* infected with sharka virus and with tobacco etch virus. They showed a regular, exactly parallel striation with a periodicity of 55 ± 5 Å (Fig. 1). A similar periodicity was reported by Edwardson et al. (1968) for tobacco etch virus and watermelon mosaic virus inclusions. In leaf extracts Purcifull et al. (1970) observed a morphological difference between inclusions evoked by tobacco etch virus and those evoked by potato virus Y. In leaf extracts of *N. clevelandii* plants infected with tobacco etch virus we also found typical triangular inclusions, whereas in plants infected with sharka virus only irregular inclusions were observed on which irregular stripes sometimes occurred. These stripes were nearly parallel, but the distance between them slightly varied. They always formed an angle of about 80 degrees with the striation of the inclusion. In our opinion these stripes were not virus particles since their width was about 95 Å, while that of virus particles was about 160 Å (Fig. 1). We were unable to find these stripes on inclusions evoked by tobacco etch virus, but they were too rarely found on sharka virus inclusions to consider them typical.

In ultrathin sections pinwheels and lamellar aggregates were abundant. Serial sections showed that pinwheels have a central core with a thread-like structure in the middle (Fig. 2). Lamellar aggregates consisted of 1–6 lamellae. Each lamella was 55 ±

5 Å thick. When sectioned parallel to the lamellae a striation with a periodicity of 55 ± 5 Å was observed, identical with the striation of inclusions in leaf extracts. The lamellar aggregates were often connected with the endoplasmic reticulum. In some cases the endoplasmic reticulum was parallel to one side of the lamellar aggregate, while in other cases it was perpendicular to the ends of some lamellar aggregates (Fig. 3). Similar relations have been reported for other viruses (Arnott and Smith, 1967; Kim and Fulton, 1969; Hoefert, 1969).

In a few sections groups of crystalline structures were found in the cytoplasm. When cross-sectioned, periodicities ranging from 110–130 Å were found in two perpendicular directions (Fig. 4). Similar structures were recently reported by Harrison and Roberts (1971) for *Atropa* mild mosaic virus and by Bovey (1971) for sharka virus.

Microbodies were commonly observed in the cytoplasm of cells of *N. clevelandii* plants. The microbodies contained a crystal with a striation-periodicity of 85 ± 5 Å and were easy to distinguish from other inclusions since they were embedded in a medium quite different from the cytoplasm and because they were surrounded by a single membrane (Fig. 5). These microbodies, however, are not characteristic for a virus infection since they were also found in healthy *N. clevelandii* plants. Their occurrence in healthy tobacco was also reported by Frederick and Newcomb (1969).

Nuclear inclusions were observed in ultrathin sections of leaf samples only when their occurrence had been previously checked by light microscopy (Fig. 6). This is in agreement with the transient character of the nuclear inclusions as reported by van Oosten and van Bakel (1970). Nuclear needles were observed by light microscopy in inoculated leaves of *N. clevelandii* about 2 weeks after infection with sharka virus and they disappeared within 10 days. The reason for this transiency, however, is unknown.

The nuclear inclusions were clearly striated in the direction of their length (Fig. 7). The stripes were parallel, but their periodicity was hard to estimate reliably owing to the fact that the inclusions were very small (due to the winter season). For this reason only some cross-sections were seen suggesting striations in two perpendicular directions. If true, there is evidently a resemblance between the nuclear inclusions and the cytoplasmic crystalline structures. This fact has been noted by Bovey (1971). However, this author gave no photographic information on the presence and structure of nuclear inclusions. The resemblance mentioned gave rise to the idea that cytoplasmic crystalline structures and nuclear inclusions may also have an identical appearance under the light microscope. Similar needle-shaped inclusions have indeed been observed with the light microscope in the cytoplasm and the nucleus. They were both stainable in the same way with a mixture of brilliant green and calcomine orange (van Oosten and van Bakel, 1970). Under the light microscope the cytoplasmic needles were quite common, whereas in ultrathin sections the pinwheels and the lamellar aggregates were abundant and the crystalline structures only rarely observed. Based on their general occurrence, the identity of cytoplasmic needles in epidermal strips and pinwheels and lamellar aggregates in ultrathin sections may be expected. However, the resemblance between cytoplasmic and nuclear needles in epidermal strips and between crystalline cytoplasmic structures and nuclear inclusions in ultrathin sections is striking. Therefore, it can not be excluded that the cytoplasmic needles observed under the light microscope and the cytoplasmic crystalline structures seen under the electron microscope are identical.

Fig. 1. Part of an inclusion in a negatively stained extract of *N. clevelandii* leaves infected with sharka virus. Note the angle of 80 degrees between an exactly parallel striation and nearly parallel stripes. Some virus particles (VP) are present.

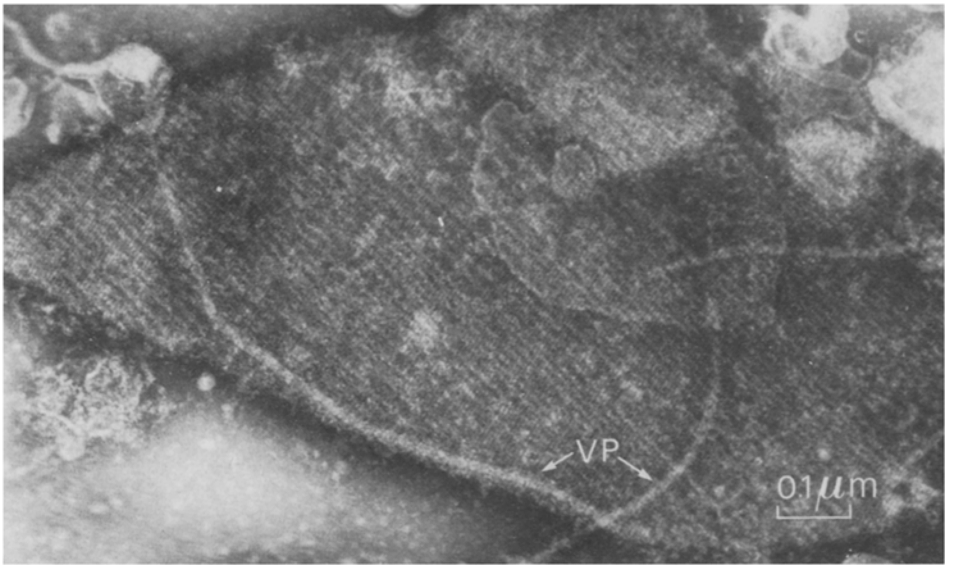

Fig. 1. Een gedeelte van een insluitsel in een negatief gekleurd extract van bladeren van N. clevelandii, geïnfecteerd met het sharkavirus. Let op de hoek van 80 graden tussen de precies evenwijdige, fijne streping en de wat onregelmatige, grovere streping. Een aantal virusdeeltjes (VP) is aanwezig.

Fig. 2. A cross-section through a pinwheel. LA: lamellar aggregates. CC: central core of the pinwheel with a dark point in the middle.

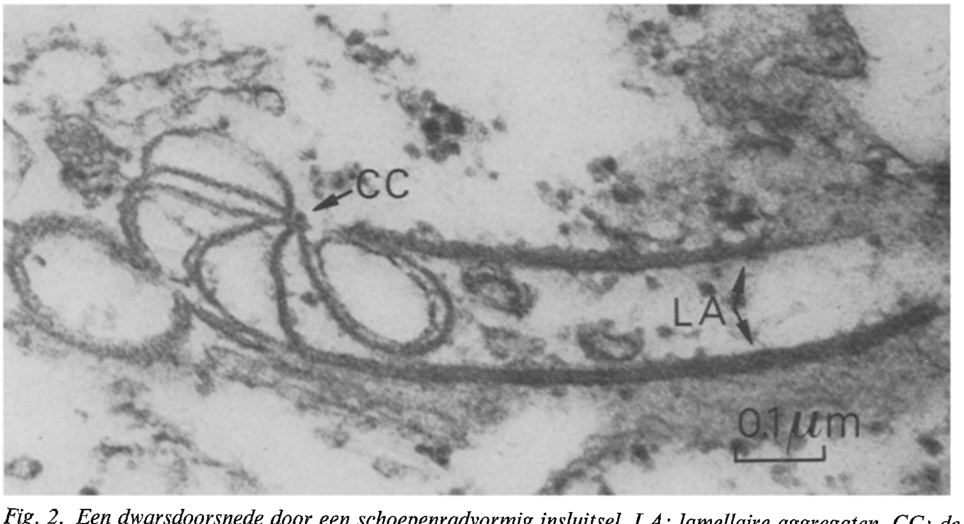

Fig. 2. Een dwarsdoorsnede door een schoepenradvormig insluitsel. LA: lamellaire aggregaten. CC: de centrale holte van het schoepenradvormig insluitsel met een donkere stip in het midden.

Fig. 3. Lamellar aggregates (LA) associated with the endoplasmic reticulum (ER) in the cytoplasm of a cell of a sharka virus infected *N. clevelandii* plant. M: a microbody.

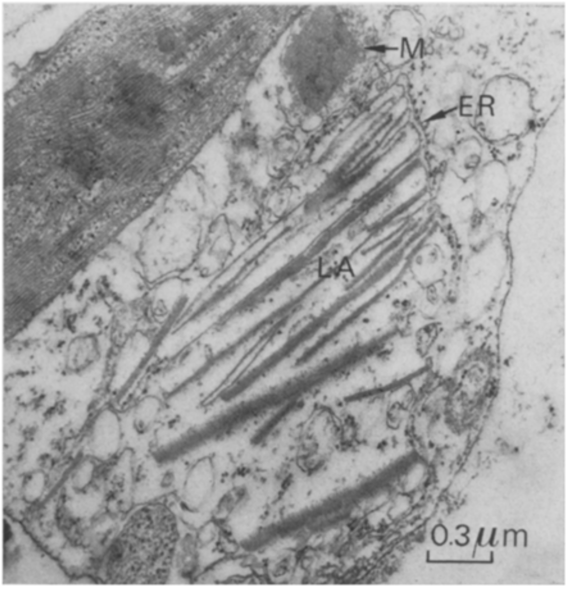

Fig. 3. Lamellaire aggregaten (LA) geassocieerd met het endoplasmatisch reticulum (ER) in het cytoplasma van een cel van een met het sharkavirus geïnfecteerde plant van N. clevelandii. M: een 'microbody'.

Samenvatting

Aanvullende gegevens over de ultrastructuur van insluitsels veroorzaakt door het (pruime-) sharkavirus

In ultradunne coupes van bladeren van *Nicotiana clevelandii*, geïnfecteerd met het sharkavirus bleken schoepenradvormige insluitsels ('pinwheels') en lamellaire aggregaten algemeen voor te komen. De schoepenradvormige insluitsels vertoonden een centrale holte met daarin een draadvormige structuur (Fig. 2). De lamellaire aggregaten vertoonden een streping met een periodiciteit van 55 $\pm$ 5 Å, zowel in ultradunne coupes als in bladextracten (Fig. 1). De lamellaire aggregaten bleken vaak geassociëerd met het endoplasmatisch reticulum (Fig. 3). Kristallijne structuren (Fig. 4) werden slechts incidenteel waargenomen. 'Microbodies' waren wel algemeen, maar zij kwamen ook voor in gezonde controleplanten (Fig. 5).

Kerninsluitsels werden met de elektronenmicroscoop aangetoond gedurende de periode, dat zij ook lichtmicroscopisch zichtbaar zijn (Fig. 6). Zij bezaten een duidelijke structuur (Fig. 7).

Gewezen wordt op een mogelijke relatie tussen de kerninsluitsels en de kristallijne structuren in het cytoplasma. Wellicht zijn de naaldvormige insluitsels, die met de lichtmicroscoop in de kern en het cytoplasma kunnen worden waargenomen, identiek aan de elektronenmicroscopisch waargenomen kerninsluitsels en kristallijne struc-

Fig. 4. Crystalline structures in the cytoplasm of a cell of a *N. clevelandii* leaf infected with sharka virus.

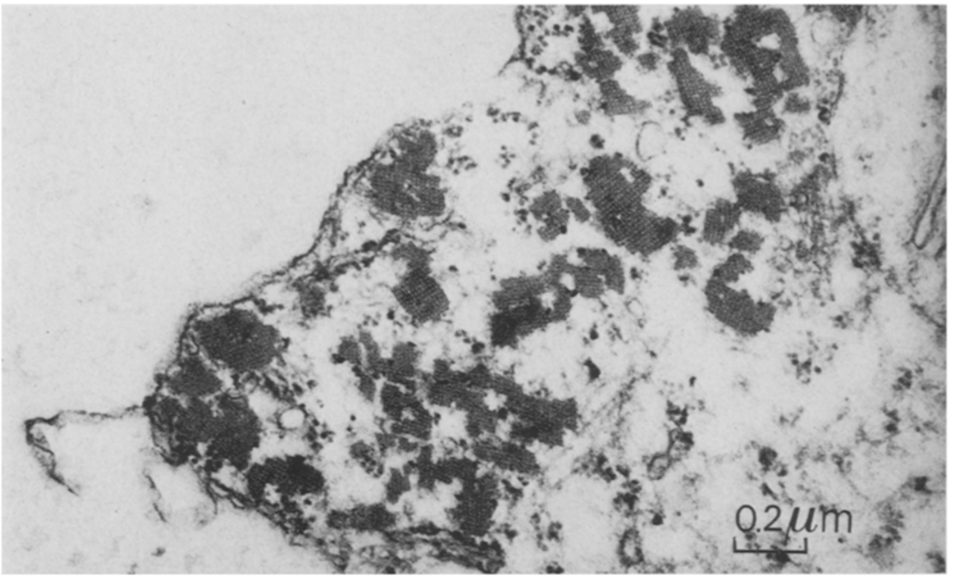

Fig. 4. Kristallijne insluitsels in het cytoplasma van een cel van een met het sharkavirus geïnfecteerde plant van N. clevelandii.

Fig. 5. A microbody (M): a single membrane surrounds a crystal lying in a medium quite different from the cytoplasm. LA: lamellar aggregate.

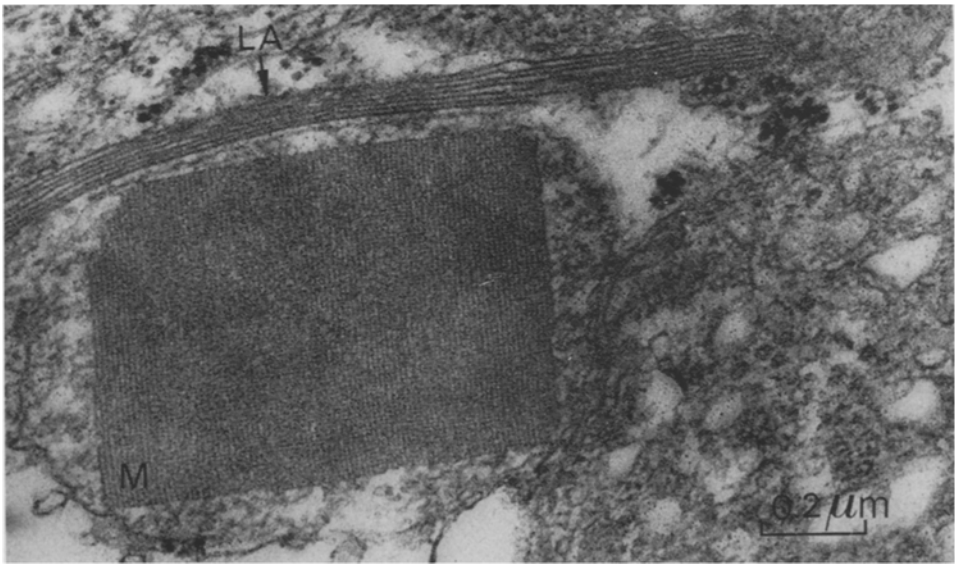

Fig. 5. Een 'microbody' (M): een enkelvoudig membraan omgeeft een kristal, gelegen in een van het cytoplasma afwijkend medium. LA: lamellair aggregaat.

Fig. 6. Intranuclear needle-like inclusions evoked by sharka virus. Nu: nucleolus.

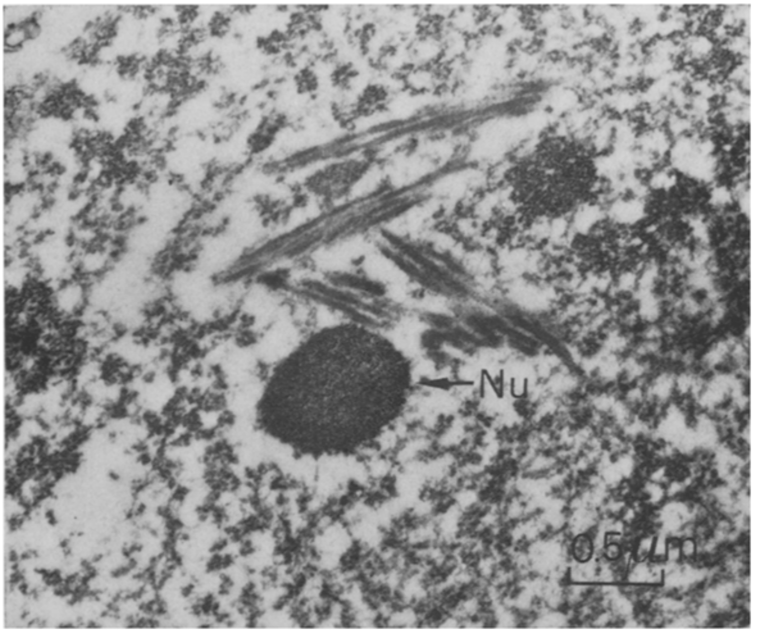

Fig. 6. Intranucleaire naaldvormige insluitsels in een met het sharkavirus geïnfecteerde cel van N. cleve-andii. Nu: nucleolus.

Fig. 7. Detail of some intranuclear inclusions showing a clear striation in their length direction. Arrows indicate vague cross-sections of nuclear needles.

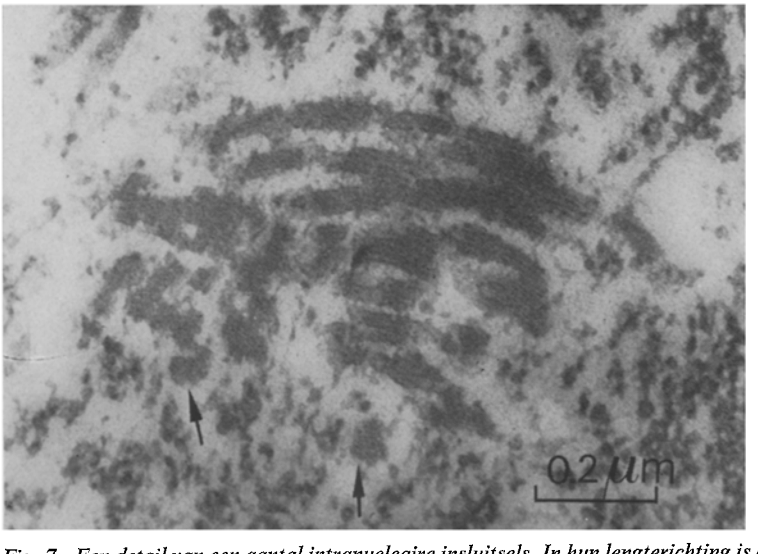

Fig. 7. Een detail van een aantal intranucleaire insluitsels. In hun lengterichting is een duidelijke streping te zien. De pijltjes wijzen op vage dwarsdoorsneden van in de kern gelegen naalden.

turen in het cytoplasma.

In bladextracten van planten van *Nicotiana clevelandii*, geïnfecteerd met het sharka-virus, werden onregelmatig gevormde insluitsels gevonden. Ze vertoonden een fijne, precies evenwijdige streping met een periodiciteit van 55 ± 5 Å. Soms bevonden zich op het oppervlak van het insluitsel ook nog wat onregelmatige, grovere strepen. Beide strepingen vormden steeds een hoek van 80 graden (Fig. 1).

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Addendum

To the paper 'Diagnosis of sharka (plum pox) by internal and external fruit symptoms' by H. J. van Oosten in No 3 on page 102, in Table 2 in *varieties with green or yellow fruits*, add 'Reine-Claude verte' for which the occurrence of symptoms was: + + — — —