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Inclusion bodies in plants infected with sharka (plum pox) virus

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Abstract

Sharka virus was found to give rise to the formation of inclusion bodies in nucleus and cytoplasm of host cells, as is known for several other viruses of the potato virus Y group. In inoculated *Nicotiana clevelandii* needle-shaped inclusion bodies were found loosely distributed in the nucleus 10 days after the first external symptoms appeared. In the cytoplasm, bundles of needles and granular inclusions arose 14 and 18 days, respectively, after external symptoms became visible. The intranuclear needles disappeared shortly before or after the first appearance of granular cytoplasmic inclusions.

Inclusion bodies abound in parenchyma cells of fruits from sharka-diseased plum trees, but they did not occur in fruits from sharka-free trees, with or without pseudo-pox symptoms. Thus, inclusion bodies can be of value in the diagnosis of sharka and be of great help in differentiating between plum pox and pseudo-pox.

Introduction

Many plant viruses cause inclusion bodies in the cytoplasm of their hosts. The inclusions can be of a specific form and size and are therefore valuable aids in the diagnosis of various virus diseases (McWhorter, 1965). Sharka virus (*/*; */*; E/*; S/Ap) of plum (*Prunus domestica*), transmitted by aphids and consisting of flexuous rod-shaped particles with a length between 600 and 800 nm, has been shown to belong to the potato virus Y (PVY) group (Kegler et al., 1964; Kassanis and Šutić, 1965; van Oosten, 1970b). Many members of this group initiate the formation of inclusion bodies in the cytoplasm and in the nucleus of their host cells (Bos, 1969, 1970). Those in the nuclei seem to be especially specific for these viruses. Pleše et al. (1969) first described cytoplasmic inclusions in the leaves of *Nicotiana clevelandii* infected with sharka virus. The present investigation was carried out without knowing of the work of these authors. Several hosts of sharka virus described by van Oosten (1970b, 1971) as well as plum fruits were examined for inclusion bodies. It was felt that the inclusions in plum might be of great help in the diagnosis of sharka virus.

Material and methods

Virus isolates used

One Dutch isolate and 3 Yugoslavian isolates of sharka virus were cultured for more than a year in *N. clevelandii*. The isolation and identification of the Dutch isolate was reported earlier (van Oosten, 1970a). The Yugoslavian isolates were from leaves of *Prunus domestica* 'Pozegaca', *P. armeniaca* 'Kajsije' and *P. cerasifera* 'Dzenarike', and were kindly provided by Mrs. M. Jordović and M. Ranković, Institut za Voćarst-

vo, Čačak, Yugoslavia. Most experiments were done with the Dutch isolate.

Apparatus and techniques used

Observations were made with a Wild light microscope. Preparations of epidermal strips from leaves, flower stalks and petals of several hosts as well as sections of plum fruits were examined for inclusion bodies. Strips from the midribs of the leaves were preferred to those from the mesophyll because stripping was easier and cells were larger. Epidermis cells of plum petals and fruits were very small, while parenchyma cells of the fruits were very large. Therefore the latter were very useful for observations with a light microscope.

Preparations were stained either with 1 % phloxine in water for 15 minutes (Rubio-Huertos, 1950, cited by Bos, 1969) or for 5 minutes in a mixture of 1 % calcomine orange 2RS and 1 % 'Luxol' brilliant green (1:4) in a special staining solvent. This solvent contained cellosolve (ethylene glycol monomethyl ether), 95 % ethyl alcohol and aqua dest (2:1:1) (Christie, 1967). In the latter case excessive stain was washed out by rapidly dipping the preparations into 90 % alcohol and then keeping them in water for at least 15 minutes. Sections stained with phloxine were only washed with water. Both staining procedures greatly enhanced contrast but in some instances the mixture of calcomine orange and brilliant green gave better results and was therefore used in most experiments.

Occasionally unstained petals of *N. clevelandii* were examined after removal of intercellular air by vacuum pumping (McWhorter, 1951).

Fruits of several plum varieties were collected during the summer of 1969. Parts of the fruits were fixed in a mixture of formalin, glacial acetic acid and 50 % ethyl alcohol (5:5:90) (Johansen, 1940), and used later for sectioning.

Determination of virus concentration and occurrence of inclusion bodies in N. clevelandii

Every 3 days the relative virus concentration was determined in inoculated and systemically infected leaves of *N. clevelandii* according to the method described by Bos et al. (1960). *Chenopodium foetidum* was used as local lesion host. For systemic infections in *N. clevelandii* only the rosette leaves were used. The occurrence of inclusion bodies was traced in inoculated and in systemically infected leaves every day after inoculation for at least 4 weeks. For another 4 weeks determinations were done weekly.

Plants were inoculated in the 4–6 leaf stage and grown in Sherer climate chambers with a light period of 16 hours and with ligth intensity of 38 W/m^2. The dark period was 8 hours. The influence of temperature on inclusion formation was examined by growing the plants at temperatures of either 20° or 25° C during day and night or at temperatures of 20° C and 25° C during night and day, respectively. Other herbaceous hosts were grown in a glasshouse at a temperature of approximately 22° C.

Determinations of virus content in examined plum trees

The presence of sharka virus in plum fruits was shown by mechanical inoculations to *C. foetidum* according to the method described by van Oosten (1970a).

Most plum trees from which fruits were investigated were tested by chip-budding on peach seedlings (*Prunus persica*) to determine whether other viruses than sharka occurred in these trees. Symptoms were assessed by weekly observations. About 4 weeks after budding the peach seedlings were cut back and observed for another 4

weeks. Usually symptoms were more severe and earlier on pruned trees than on trees which were not pruned (Kegler, 1965; Baumann, 1967). The peach seedlings were grown in a glasshouse.

Results

In the nucleus and in the cytoplasm of cells of *N. clevelandii* infected with sharka virus different types of inclusions were found, viz. needle-shaped inclusion bodies in the nucleus as well as in the cytoplasm and amorphous masses in the cytoplasm (Fig. 1). Within the nucleus up to 15 loosely distributed needles could be found. These meas-

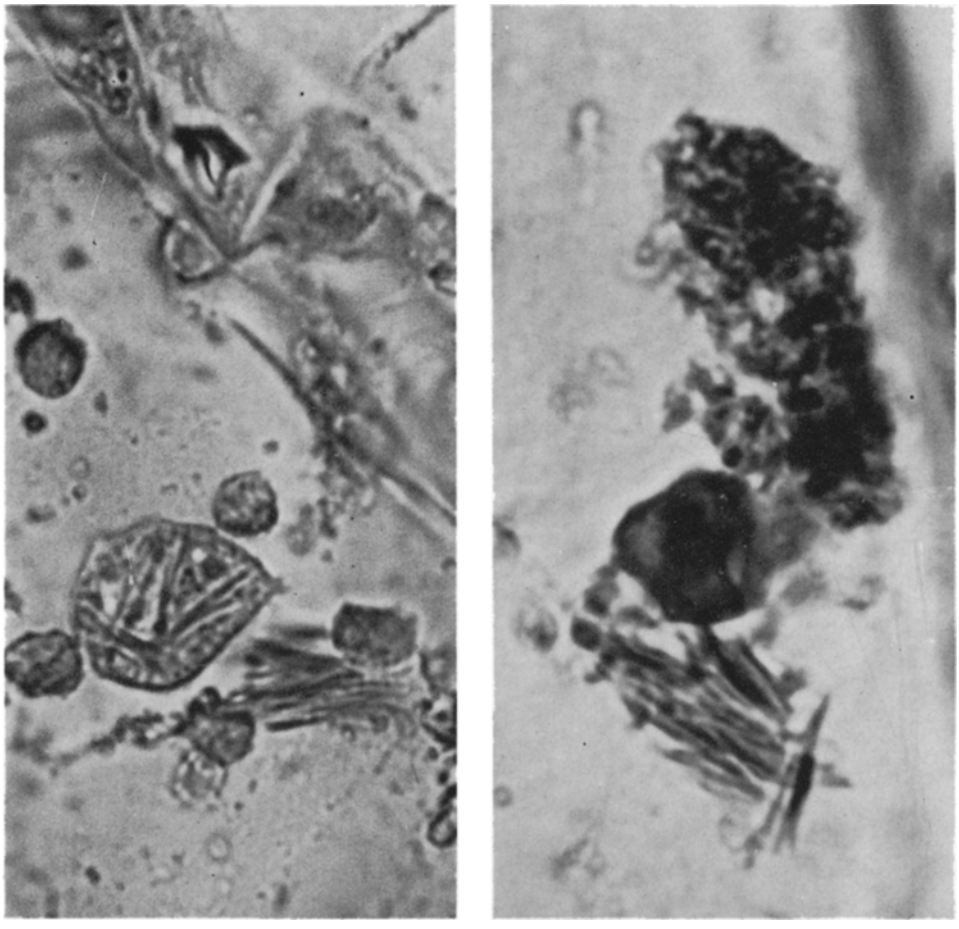

Fig. 1. Needle-shaped inclusions distributed in the nucleus (A), and in bundles in the cytoplasm (A and B) and granular inclusions in the cytoplasm (B) of leaf epidermis cells of *Nicotiana clevelandii* sap-inoculated with sharka virus. Magnification A and B: × 2500. (B-photograph made by Mr. R. E. Labruyère, IPO, Wageningen).

Fig. 1. Naaldvormige insluitsels verspreid door de kern (A) en gebundeld in het cytoplasma (A en B) en een granulair insluitsel in het cytoplasma (B) van epidermiscellen van bladeren van Nicotiana clevelandii geïnoculeerd met sharka virus. Vergroting van A en B: 2500 ×. *(Foto B gemaakt door Ir. R. E. Labruyère, IPO, Wageningen).*

ured up to 10 μm. Bundles of needles in the nucleus were rare. The cytoplasmic bundles of needles differed in form and size. Usually the bundles were oblong, sometimes compact or very loosely aggregated. Determinations of the exact number of needles in the bundles were impossible but the numbers varied greatly. Both ends of the bundles were irregular due to varying needle lengths. The bundles were between 6 and 24 μm and 1 to 6 μm thick. Usually every cell contained several bundles. The bundles were found either close to the nucleus or throughout the cytoplasm. The amorphous masses were granular. They were either round, oval or irregularly elongated. In the latter case they often contained needles or bundles of needles. The granular inclusions were 11 to 43 μm long and 10 to 24 μm thick.

Similar inclusions were found in epidermal strips from leaves of inoculated *Nicotiana exigua*, *N. megalosiphon*, *Ranunculus arvensis*, *Vicia sativa* ssp. *angustifolia*, *Lamium amplexicaule* and *Zinnia elegans*. In the last 3 species only granular inclusions were found. Other tissues also often contained inclusion bodies. Nuclear and cytoplasmic needles could easily be seen in epidermal strips from flower stalks and in unstained vacuum-treated preparations of petals of *N. clevelandii*.

Bundles of needles in the cytoplasm were occasionally found in epidermal strips from the petals of plum. They were abundant (sometimes up to 20 bundles per cell) in parenchyma cells of ripe plum fruits from infected trees of several varieties. According to the variety fruits showed either bandlike discoloration or irregular pits and grooves or both. Fruits which were not quite ripe but already showed bandlike discoloration often contained nuclear needles only. Inclusions were not found in unripe fruits. Ripe fruits from sharka-free trees did not contain inclusion bodies. Usually these fruits were symptomless, but fruits from some sharka-free trees of 'Warwickshire Drooper', 'Reine Claude d'Althan' and the damson plum showed deep irregular grooves reminiscent of those caused by sharka virus. However, the sharka virus could not be isolated from these fruits by sap inoculation on to *C. foetidum*. After chip-budding on peach seedlings the ringspot virus, line pattern virus or dark green mottle virus could be demonstrated, either singly or together. Usually these viruses were also commonly found in plum trees infected with sharka virus.

Intranuclear inclusions in leaves of *N. clevelandii* were first observed about 14 days after inoculation, 10 days after external symptoms appeared, and 3–4 days after the virus reached its highest concentration (10^{-4} to 10^{-5}) (Fig. 2). About 4–5 days after the appearance of the needle-shaped inclusion bodies in the nucleus the bundles of needles were seen in the cytoplasm. After another 4–5 days granular inclusions appeared in the cytoplasm. Shortly before the appearance of the granular inclusions the nuclear needles disappeared.

In systemically infected leaves of *N. clevelandii* inclusion bodies developed in the same sequence, but somewhat later after inoculation. The development was somewhat faster. Formation of nuclear needles started about 10 days after inoculation, 10 days after symptoms became visible or 3–4 days after the virus reached its highest concentration. Here, the nuclear needles were also transient only. Therefore the 3 types of inclusion bodies could be observed simultaneously in one cell for a short time only (Fig. 2).

When infected *N. clevelandii* plants were grown at a constant temperature of 20° or 25° C, growth and inclusion development was slower than in plants grown at varying night and day temperature (20° and 25° C respectively). No other differences were found.

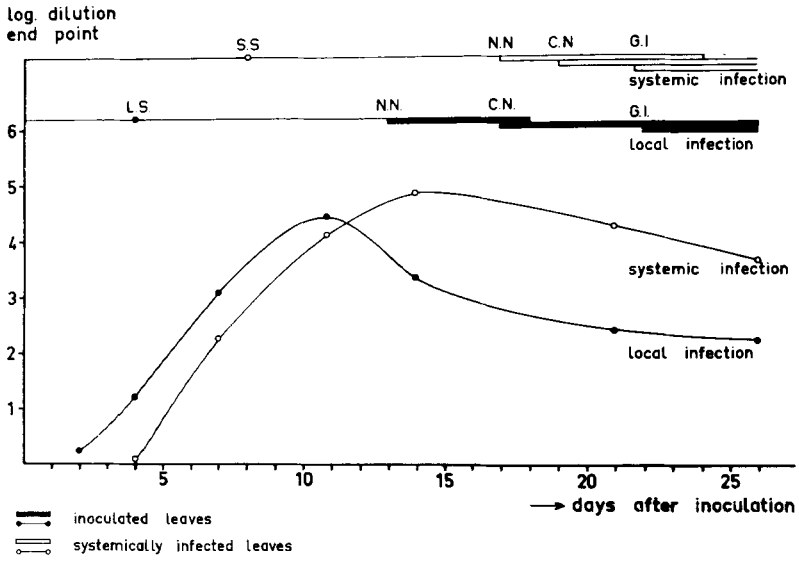

Fig. 2. The formation of inclusion bodies in the leaves of *Nicotiana clevelandii* inoculated with sharka virus, related to the appearance of symptoms and the virus concentration.
L.S.= local symptoms; S.S. =systemic syptoms; N.N.= intranuclear needles; C.N.= intracytoplasmic bundles of needles; G.I.= granular inclusions.
Fig. 2. De vorming van insluitsels in de bladeren van Nicotiana clevelandii geïnoculeerd met sharka virus, in relatie tot het verschijnen der symptomen en de concentratie van het virus.
L.S.=lokale symptomen; S.S.= systemische symptomen; N.N.= naalden in de kern; C.N.= bundels naalden in het cytoplasma; G.I.= granulaire insluitsels.

Results obtained in a few experiments with the 3 Yugoslavian isolates did not differ from those described for the Dutch one.

Discussion

The results obtained here agree with those relating to several other viruses of the PVY group. Most reports on inclusions caused by members of this group have concerned granular inclusions (Bos, 1969). Cytoplasmic bundles of needles provoked by watermelon mosaic virus had been photographed both by Schmelzer and Miličić (1966) and Edwardson et al. (1968). Purcifull and Shepard (1967) reported needle-shaped cytoplasmic inclusions in plants with western celery mosaic virus. Pleše et al. (1969) reported bundles of needles in the cytoplasm and amorphous masses for sharka virus. For a long time the only known viruses of the PVY group causing intranuclear inclusions visible with a light microscope were tobacco etch virus (Kassanis, 1939) and bean yellow mosaic virus (McWhorter, 1941). Recently Bos (1969, 1970) demonstrated that several other members of this group could cause inclusions or deviations within the nucleus. Bos (1969) and Bos and Rubio-Huertos (1969) reported the occurrence of radiating intranuclear needles in epidermis cells of *Pisum sativum* after infection with pea necrosis virus. The intranuclear needles in sharka-infected plants showed no orientation and were loosely distributed throughout the nucleus. This behaviour of the intranuclear needles seems therefore typical for sharka virus.

However, intranuclear inclusions were not provoked only by viruses of the PVY

group, as suggested by Bos (1969). There are some reports about intranuclear inclusions visible with the light microscope and caused by viruses of other groups, viz a strain of tobacco mosaic virus (Woods and Eck, 1948), cotton leaf crumple virus (Tsao, 1963) and dahlia mosaic virus (Robb, 1964). Esau (1968) reviewed some reports on inclusions within the nucleus which were seen with an electron microscope.

The transient character of the intranuclear needles in sharka virus infected plants is remarkable. The intranuclear inclusions provoked by dahlia mosaic virus were also found to be transient by Robb (1964), who suggested that this phenomenon might be due to extrusion of the inclusions from the nucleus into the cytoplasm.

So far there have been no reports on members of the PVY group which could infect plum or on plum infecting viruses which initiate inclusion formation (Sommereijns, 1967). Inclusions as described in the present paper were not found in fruits of sharka-free trees which were infected with other common plum viruses. Fruits of such trees were usually symptomless but occasionally some varieties showed irregular pits and grooves on the fruit surface. These abnormalities resembled those described by Schuch (1961), Kegler et al. (1964) and Posnette and Ellenberger (1963). The last named authors suggested the name pseudo-pox for this phenomenon. Fruits with pseudo-pox symptoms did not contain inclusion bodies.

Thus, cytoplasmic and intranuclear inclusions can be used in the diagnosis of sharka, especially in plum, one of the most important hosts of sharka virus. The inclusions of sharka virus might also be of great help in differentiating plum pox and pseudo-pox.

Samenvatting

Celinsluitsels in met sharka-(plum pox) virus geïnfecteerde planten

Sharka-virus doet insluitsels in de kern en in het cytoplasma van cellen van diverse waardplanten ontstaan. Dit is een eigenschap van verscheidene virussen, die tot de aardappel-Y-virusgroep behoren. In de kern van epidermiscellen van bladeren van met het sharka-virus geïnoculeerde planten van *Nicotiana clevelandii* werden 10 dagen na het verschijnen van de uitwendige symptomen verspreid liggende naaldvormige insluitsels gevonden. In het cytoplasma verschenen bundels naalden en granulaire massa's, respectievelijk 14 en 18 dagen na het verschijnen van uitwendige symptomen. De naaldvormige kerninsluitsels waren slechts tijdelijk zichtbaar. Zij verdwenen omstreeks het moment waarop de granulaire insluitsels verschenen.

Insluitsels bleken overvloedig voor te komen in parenchymcellen van pruimevruchten van met sharka besmette bomen. Zij ontbraken in parenchymcellen van vruchten van bomen, die vrij waren van sharka-virus. De insluitsels zijn daarom van betekenis voor de diagnose van sharka en zij kunnen tevens van doorslaggevende betekenis zijn bij het onderscheiden van vruchtsymptomen van 'plum pox' en 'pseudo-pox'.

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