Neth. J. Pl. Path. 90 (1984) 55-69

Further characterization of melon necrotic spot virus causing severe disease in glasshouse cucumbers in the Netherlands and its control

L. BOS[1], H.J.M. VAN DORST[2], H. HUTTINGA[1] and D.Z. MAAT[1]

[1] Research Institute for Plant Protection (IPO), P. O. Box 9060, 6700 GW Wageningen, the Netherlands
[2] Glasshouse Crops Research and Experiment Station, Zuidweg 38, 2671 MN Naaldwijk, the Netherlands

Accepted 23 September 1983

Abstract

A severe leaf necrosis, observed since 1978 in glasshouse cucumbers grown on rockwool and later also in crops on soil, is described. A virus could be isolated and the disease be reproduced in cucumber and melon. The virus could be transmitted by leaf inoculation with expressed sap and by pouring rockwool leakage water onto sterilized soil containing cucumber seedlings. Infectivity steeply declined in expressed sap between dilutions 10 and 100 (dilution endpoint ca 10^6), at temperatures between 55 and 65°C (thermal inactivation point 75°C) and during storage between 1 and 1½ month at room temperature.

Out of 40 plant species tested only three species, viz. cucumber, melon and watermelon, were susceptible. All 21 cucumber cultivars and all 8 melon cultivars tested reacted severely with local lesions and some with systemic necrosis, but systemic infection and reaction were erratic under experimental conditions.

Purified virus sedimented in sucrose and CsCl gradients and during analytical ultracentrifugation in a single peak. The s_{20} was 134 *S* and buoyant density in CsCl was 1.33 g.cm^{-3}. Virus particles in crude sap and purified suspensions were spherical and ca 30 nm in diameter. They contained one type of protein with a relative molecular mass of 46 000 and one RNA species. An antiserum with a titre of 1024 did not react with cucumber and tobacco necrosis viruses, nor did their antisera react with our cucumber virus. Serologically and in physicochemical properties the virus is similar to if not identical with the melon necrotic spot virus incompletely described in Japan.

Disease control may be through improved hygiene, including steam sterilization of rockwool, soil disinfection by steam sterilization or with methyl bromide, and addition of a surfactant to nutrient solutions, and prevention may be by grafting cucumber onto *Cucurbita ficifolia* rootstocks, immune to the virus.

Additional keywords: cucumber necrosis virus, *Olpidium* sp., soil transmission, tobacco necrosis virus.

Introduction

Since 1978 severe leaf necrosis and plant death of cucumber plants *(Cucumis sativus)* caused concern to glasshouse vegetable growers in the Netherlands. The disease was first observed in cucumber crops grown on rockwool and was soon associated with the

introduction of rockwool as a substrate. Necrotic leaf flecking suggested infection by a bacterium or fungus, but initial tests at Naaldwijk and by the Plant Protection Service, Wageningen, to detect such pathogens were negative (M. de Witte, personal communication, 1980).

During 1980 our trials soon led to the isolation of a virus reminiscent of tobacco and cucumber necrosis viruses (TNV and CNV), but its host range was different. It was found to be soil-borne and to resemble the incitants of 'criblure du melon' in France and melon necrotic spot (MNSV) in Japan, incompletely described by Marrou and Risser (1967) and Kishi (1966), respectively.

This paper reports on a further characterization of the virus and presents results of initial endeavours to control the disease. Tentative results have been published elsewhere (Bos, 1981). After termination of our investigations independent publications appeared on three apparently different soil-borne viruses, viz. cucumber leaf spot virus (CLSV) from glasshouse cucumber in East Germany (Weber et al., 1982), cucumber fruit streak virus (CFSV) from cucumber grown in plastic tunnels in Crete (Greece) (Gallitelli et al., 1983) and cucumber soil-borne virus (CSbV) from cucumber seedlings used as bait plants in soil samples in Lebanon (Koenig et al., 1983). Antisera to CLSV, CFSV and CSbV were included in a serological test.

The disease. Symptoms start in still-developing leaves half-way the stem as a local vein clearing and chlorotic leaf spotting, which is particularly striking in transmitted light (Fig. 1, left). Soon thereafter these lesions develop into desiccating necrotic spots. Later entire leaves may wither with the original lesions still standing out (Fig. 1, right). In autumn affected plants may soon die (Fig. 2). Plants suffer less during spring. They may then continue to produce fruits, which are normal in colour. When the disease occurs in complex with cucumber green mottle mosaic, which is usually not associated with fruit symptoms (Van Koot and Van Dorst, 1959), the fruits have sunken chlorotic spots with dark-green water-soaked edges (Fig. 3).

Though first attracting attention in a few crops on rockwool in the province of Utrecht, the disease has later also been observed in Groningen and more recently on separate holdings throughout the glasshouse district of South Holland and in crops on soil as well. Affected plants may occur in groups but are often irregularly spread within a crop. In one block of rockwool diseased and healthy plants may alternate. In affected crops incidence usually is low, but may be as high as 45%.

Materials and methods

Virus isolates and antisera. Several glasshouse-grown cucumber plants have been tested for infection but most research at Wageningen was with isolate Cu18 from cucumber 'Corona' from a holding in Mijdrecht. Part of the host-range tests were done at Naaldwijk with an apparently identical isolate from the same holding. The isolate Cu18 was propagated in cucumber seedlings 'Gele Tros' and stored in harvested inoculated cotyledons at $-20°C$ and in such material dried and stored over $CaCl_2$ at 4°C. The same cucumber cultivar was used as an indicator host and for local-lesion assay. Often one to three plants of *Chenopodium amaranticolor* and *C. quinoa* were also inoculated to detect possible contamination with TNV or CNV. At Naaldwijk cucumber 'Sporu' was used as an indicator.

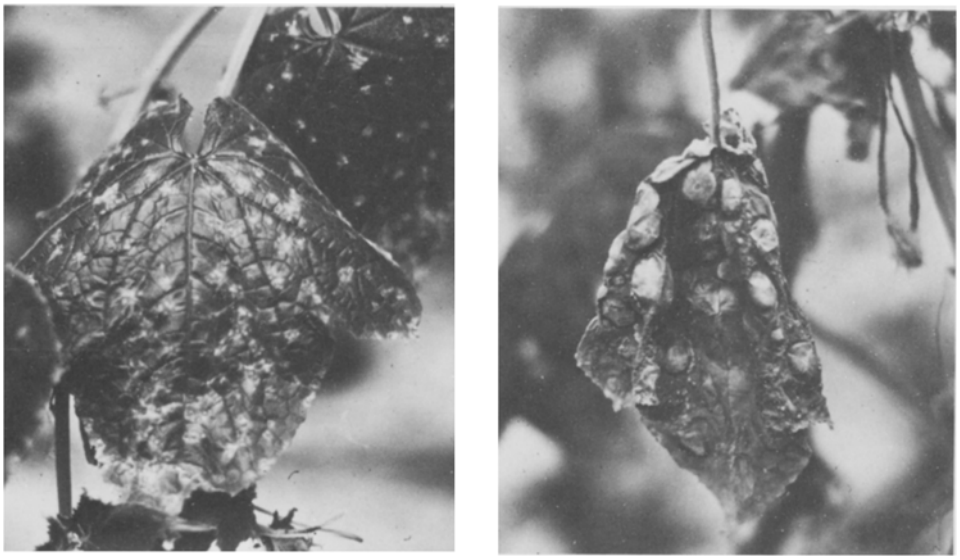

Fig. 1. Chlorotic leaf spotting (left) and later developing necrotic leaf flecking and further desiccation (right) in cucumber 'Corona' after natural infection.

Fig. 2. Overall necrosis and plant withering in affected crop of 'Corona'.

Fig. 3. Part of a cucumber fruit with severe symptoms of simultaneous infection by melon necrotic spot virus and cucumber green mottle mosaic virus.

Purified MNSV-Japan and CNV and their respective antisera were obtained from Dr Y. Saito, Japan, and Dr J.H. Tremaine, Canada, respectively. Antiserum to a virus resembling MNSV (MNSV-Cal) was obtained from Dr D.J. Gumpf, California, USA. Antisera and antigens of two serologically distinct strains of TNV from bean (TNV1) and potting soil (TNV2) and antisera to carnation mottle and ringspot viruses were from our own stock. Antisera to CLSV, CFSV and CSbV were obtained from the Institut für Phytopathologie, Aschersleben (DDR), Dr Gallitelli, Italy, and Dr R. Koenig, Federal Republic of Germany, respectively, and those to pelargonium leaf curl and tomato bushy stunt viruses had earlier been obtained from Dr M. Hollings, Great Britain.

Transmission. Mechanical transmission from plants was in the conventional way using water for grinding of leaves and diluting of plant sap, and carborundum as an abrasive. Inoculation from rockwool and via soil was by grinding rockwool or soaking rockwool for 3 or 24 h in water, filtering it through cheesecloth, and suspending cucumber seedlings with their roots for 24 h in the resulting liquid, or pouring this onto sterilized potting soil containing young cucumber seedlings. Plant infection was 5 days later tested by back inoculation from roots and first foliage leaves.

Host-range and varietal tests. For host-range tests 2 to 8 plants of each species, and for varietal tests 9 to 18 plants of each cultivar were inoculated. Later back inoculation from inoculated and non-inoculated leaves was onto 3-6 cucumber seedlings each.

Virus purification was from cucumber cotyledons infected with Cu18 and harvested 3 to 4 days after inoculation just before the local lesions became necrotic. Portions of 400 g were homogenized with a Waring blender in 1000 ml of 0.05 M sodium phosphate buffer (pH 7) containing 0.02 M sodium ascorbate. While still blending 100 ml of chloroform was added. The slurry was centrifuged for 10 min at 10 400 *g* (all *g* values will be given at R_{max} and all centrifugations were at 4°C) and the supernatant was adjusted to pH 5.3 with a 15% HCl solution. The precipitate was removed by centrifuging for 10 min at 16 000 *g*. The supernatant was decanted and centrifuged for 2½ h at 116 000 *g*. The pellets were resuspended in 70 ml of 0.017 M sodium phosphate buffer (pH 7) (phosphate buffer) and the suspension stirred for 2 h at 4°C and centrifuged for 10 min at 7700 *g*. The supernatant was then centrifuged for 2½ h at 85 600 *g* and the resulting sediments were resuspended in 6 ml of phosphate buffer and stirred for 2 h at 4°C. One-ml portions were layered onto 10-40% sucrose gradients in phosphate buffer and centrifuged for 2½ h at 104 000 *g* in a Beckman SW27 rotor. The virus zone was recovered using an Isco density-gradient fractionator. The virus fractions were diluted 1 : 1 with phosphate buffer and centrifuged for 4 h at 127 000 *g*. Pellets were resuspended in 2 ml of phosphate buffer and stirred for at least 2 h at 4°C. One-half-ml portions of the virus suspension were each mixed with 1.2 ml of water and 1.8 ml of a CsCl solution (21.76 g/25 ml) in a centrifuge tube. The tubes were filled with paraffin oil and centrifuged for 20 h at 153 000 *g* in a Beckman SW41 rotor. The virus was recovered by puncturing the bottom of the tubes and collecting appropriate fractions. CsCl was removed by dialysis overnight against phosphate buffer at 4°C.

Biophysical characterization. The sedimentation coefficient was determined by the

graphical method of Markham (1960) using a Spinco Model E analytical ultracentrifuge with schlieren optics. The buoyant density in CsCl was determined as described by Maat et al. (1978). For determination of the relative molecular mass of the coat protein, virus was dissociated and the protein and nucleic acid analysed as described by Bos et al. (1980).

Electron microscopy. Diseased tissue was chopped in 1% potassium phosphotungstate (PTA) (pH 6.5) in water and some liquid was then transferred to a carbon-coated copper grid. Purified samples were transferred to the grid and then stained with PTA. Preparations were examined in a Philips EM300.

Serology. For antiserum preparation a rabbit was injected with purified virus. Two intravenous injections, given with a three-days interval, were followed two weeks later by an intramuscular injection. For the latter the virus suspension was emulsified with an equal volume of Freund's incomplete adjuvant. Three weeks thereafter a subcutaneous injection was given. Per injection 1.5 or 2 ml of purified virus were administered.

Serological tests were by agar double-diffusion, using 1% purified agar in saline and sodium azide as an antiseptic, and purified virus suspensions.

Results

The virus, giving a characteristic reaction on cucumber cotyledons and failing to react on *C. amaranticolor* and *C. quinoa* (see below), was isolated from diseased plants from 15 holdings and one breeding station. Cucumber plants with characteristic symptoms always proved to contain the virus. In one instance six healthy plants growing in the vicinity of diseased ones were tested and five of these were found to contain a low concentration of virus. In the varietal test the virus could also easily be transmitted from symptomless fruits of inoculated cucumber plants.

On two holdings plants with leaf symptoms, characteristic of the necrotic disease described here, had fruits with sunken chlorotic spots with dark-green water-soaked edges (Fig. 3). Inoculation from such fruits onto cucumber cotyledons in both cases led to necrotic local lesions appearing in 4 days and characteristic of the new cucumber virus and 8 days later to systemic symptoms characteristic of cucumber green mottle mosaic virus.

Transmission tests. Mechanical transmission was easy in sap obtained by grinding infected tissue in water. Transmission via rockwool and soil could soon be demonstrated. From the roots of cucumber seedlings, grown in sterilized potting soil watered with leakage water from infested rockwool, the virus could readily be recovered by back inoculation (especially after watering with leakage water obtained after 3 h of soaking). Five out of 6 seedlings thus infected were found to contain *Olpidium* sp. Roots of three seedlings from non-treated soil remained free of the virus. The virus could with varying results be proved to be present in water from infested rockwool by direct inoculation onto cucumber seedlings. Water from a sample of rockwool from a trisodium phosphate-disinfected glasshouse could in this way be proved to still contain the virus (many local lesions on cucumber, whereas *C. amaran-*

ticolor and *C. quinoa* remained free). Some virus was also recovered from the roots of 7 out of 10 cucumber seedlings grown in a thin layer of sand covering a sample of infested rockwool. In some instances TNV was found as a contaminant.

Persistence of infectivity. Infectivity in plant sap steeply declined between dilutions 10 and 100 (with a few local lesions still erratically formed up to dilution 10^6), at heating for 10 min between 55 and 65°C (with all infectivity gone at temperatures above 75°C) and during storage in plant sap at room temperature between 1 and 1½ months (with very little left after 9 weeks). In cotyledons stored at −20°C the virus was still highly infectious after 4 weeks of storage, as it was in inoculated cotyledons dried and stored over $CaCl_2$ for 3 years. The virus could not be detected in naturally desiccated cucumber foliage leaves with characteristic symptoms (Fig. 1, right) from an infested crop.

Host range and symptomatology. Out of 40 plant species tested only three were susceptible: cucumber*(Cucumis sativus),* melon *(C. melo)* and watermelon *(Citrullus vulgaris).* Cucumber and melon were the only species contracting systemic infection, though erratic under experimental conditions. No symptoms were produced in, nor could virus be recovered from inoculated plants of *Ammi majus*, Apium graveolens* var. *dulce*** (celery) 'Amsterdamse Fijne Donkergroene', *A. graveolens* var. *rapaceum*** (celeriac) 'Albatros', *Beta vulgaris* (sugarbeet), *Benincasa hispida*** (white gourd), *Brassica oleracea* var. *botrytis*** (cauliflower), *Capsicum annuum*** (sweet pepper) 'Tisana' and 'Turrialba', *C. chacoense*** 'PI 260426', *C. chinense*** 'PI 281417', *Chenopodium amaranticolor*, C. bonus-henricus*, C. murale*, C. quinoa, C. urbicum*, Cucurbita ficifolia*** (figleaf gourd), *Datura stramonium*, Gerbera jamesonii*** 'Appelbloesem', *Lactuca sativa* (lettuce) 'Attractie' and 'Renate', *Lapsana communis*** (nipplewort), *Lycopersicon esculentum* (tomato) 'Ailsa Craig' and 'Moneymaker', *Nicotiana benthamiana, N. clevelandii, N. glauca**, N. glutinosa, N. megalosiphon, N. rustica, N. tabacum* (tobacco) 'White Burley', *Petunia hybrida**, Phaseolus vulgaris** (common bean) 'Bataaf', 'Brittle Wax', 'Harvester', 'Kievitsboon', 'Mont d'Or', 'Ras Verschoor', 'Rolando', 'Sanilac', 'The Prince', 'Supermètes' and 'Westlandia', *Pisum sativum** (pea) 'Koroza' and 'Rondo', *Solanum melongena*** (eggplant, aubergine) 'Mammouth', *S. ovigerum**, Tetragonia expansa, Trifolium pratense** (red clover), *T. repens* (white clover), *Vicia faba** (broad bean) 'Kompakta', *Vigna unguiculata** (cowpea) 'Bermulti' and 'Kor', and *Zinnia elegans.* Single asterisks indicate species that have not been back inoculated from non-inoculated leaves. Double asterisks indicate species that have only been back inoculated from non-inoculated leaves.

In all 21 cucumber cultivars and lines tested many small dry and larger, diffusely bordered chlorotic and often concentric local lesions were formed on inoculated cotyledons (Fig. 4, left) and foliage leaves 3 to 4 days after inoculation. The two types of lesions often occurred together, but single-lesion back inoculations from each of them did not differentiate isolates. Under experimental conditions systemic infection of cucumber, tested by back inoculation from various levels of the plant, was erratic and often symptomless. At Naaldwijk, during maintenance of the virus in cucumber 'Sporu', on average 10% of the plants produced systemic symptoms. In tip leaves of 12 cultivars systemic reaction started with chlorotic, often reticulate spotting (Fig. 4,

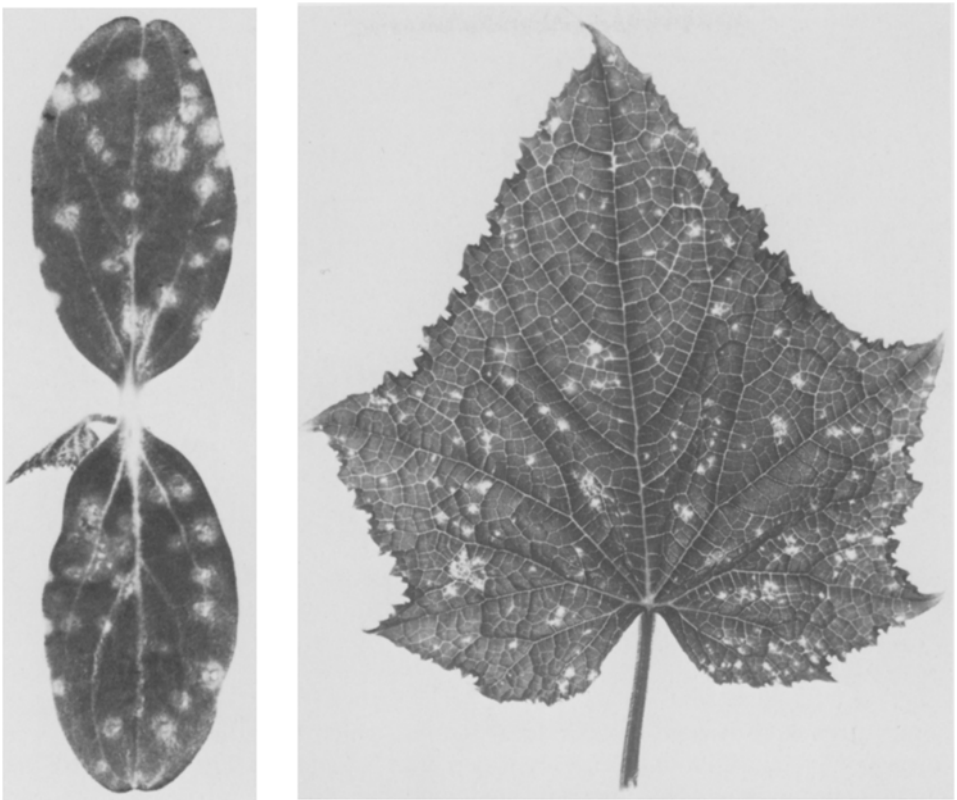

Fig. 4. Local lesions on cotyledons of cucumber 'Gele Tros' (left) and reticulate chlorotic systemic spotting in young foliage leaf of 'Formosa' (right), four days and two months after inoculation, respectively.

right). Six cultivars (indicated below with an asterisk) produced systemic leaf necrosis similar to the effect of natural infection. From 12 plants of 9 cultivars 24 (symptomless) fruits were harvested and some fruit pulp of each of them was back inoculated. Twenty-one fruits proved infected. A few seeds were obtained, but they did not germinate well, so that seed transmission could not be tested. The cultivars and lines tested were Aurora, Brilliant, Curio, DP 239, DP 369*, Famosa, Farbio*, Farbiola, Gele Tros*, Hokus, Levo, Nunhem's 32/77, Pandorex, Primio*, Sandra, Sporu*, Stereo, T 144*, Type 700, 79T62 and 1282/76.

All 8 melon cultivars tested reacted with many large brownish yellow local lesions on inoculated cotyledons (Fig. 5, left) beginning 4 days after inoculation. These lesions often coalesced, and then led to death and shriveling of inoculated cotyledons. In several plants later systemic necrosis developed similar to that of cucumber, but experimental conditions were not suitable for extended plant development. The cultivars tested were Enkele Net, Fusano, Ha'on, Ixy, Oranje Ananas, Overgen, Polydor and Witte Suiker.

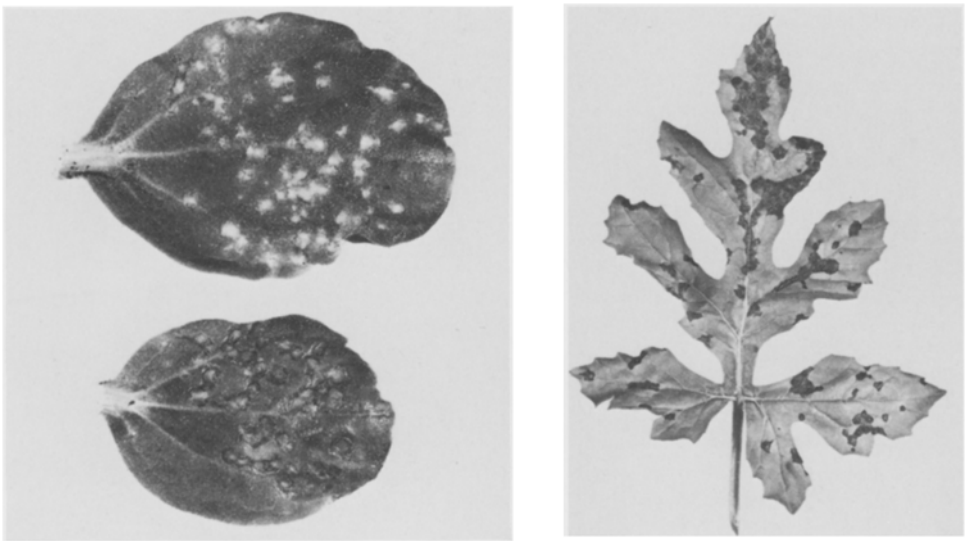

Fig. 5. Local lesions on cotyledons of melon 'Enkele Net' (left) and on foliage leaf of watermelon 'Early Canada' (right), eight and six days after inoculation, respectively.

In watermelon discrete necrotic local lesions, which sometimes coalesced, were formed on inoculated foliage leaves ca 6 days after inoculation (Fig. 5, right). Neither systemic sysmptoms were observed, nor could the virus be recovered from non-inoculated leaves.

Cucurbita ficifolia, often used as a rootstock for non-heated cultivated cucumbers to prevent wilt diseases caused by some soil-borne fungi, had been found immune to the virus if inoculated mechanically (see above). It was later also tested for resistance to infection from contaminated rockwool as a possible way to prevent the disease in cucumber crops. In practice, cucumber seedlings are approach-grafted to seedlings of *C. ficifolia.* After tissue union the cucumber plants are severed from their own roots. Sixteen plants of cv. Sporu on rootstocks did neither produce symptoms nor could the virus be isolated from them when grown on contaminated rockwool from a holding where the disease occurred. However, out of 16 identical plants on the same rockwool and still having their own roots as well as those of the rootstock, 4 contracted infection as judged by symptoms and back-inoculation. Half of the plants had been inoculated with cucumber green mottle mosaic virus and its symptoms were greatly enhanced by the soil-borne virus (Fig. 3). In another experiment with 4 plants each of 'Sporu' and *C. ficifolia* growing in one block of contaminated rockwool, roots of all 8 plants later contained resting spores and zoospores of *Olpidium* sp., though they were detected earlier in 'Sporu' than in *C. ficifolia.* In a commercial crop of 'Corona' on rootstock growing in an infested glasshouse only 10 out of 6 000 plants contracted the disease. However all diseased plants turned out to have redeveloped their own roots. Ca 10% of the 18 000 non-grafted plants of the same cultivar produced characteristic symptoms.

Virus purification and biophysical characterization. The described purification method resulted in pure virus preparations and the yield was good. The virus sedimented in a single zone in sucrose gradients and showed a single peak in the analytical ultracentrifuge. The sedimentation coefficient in 0.017 M sodium phosphate buffer (pH 7) at 20°C and at infinite dilution was 134 *S*. The virus reached equilibrium in CsCl gradient in a single zone. The buoyant density in CsCl was 1.33 $g.cm^{-3}$. The virus contained only one type of coat protein. Its relative molecular mass was 46 000. On 2.6% polyacryl amide gels only one band of RNA was found.

Electron microscopy. Spherical virus particles could readily be detected in crude sap preparations from cucumber cotyledons with local lesions (Fig. 6, left). They occurred in high concentration in purified preparations (Fig. 6, right) and had a diameter of ca 30 nm.

Serology. The homologous titre of the antiserum prepared to Cu18 was 1024. The results of comparative serological tests summarized in Table 1 show that Cu18 is closely related to MNSV-Japan. This could be confirmed in Japan with our antiserum and

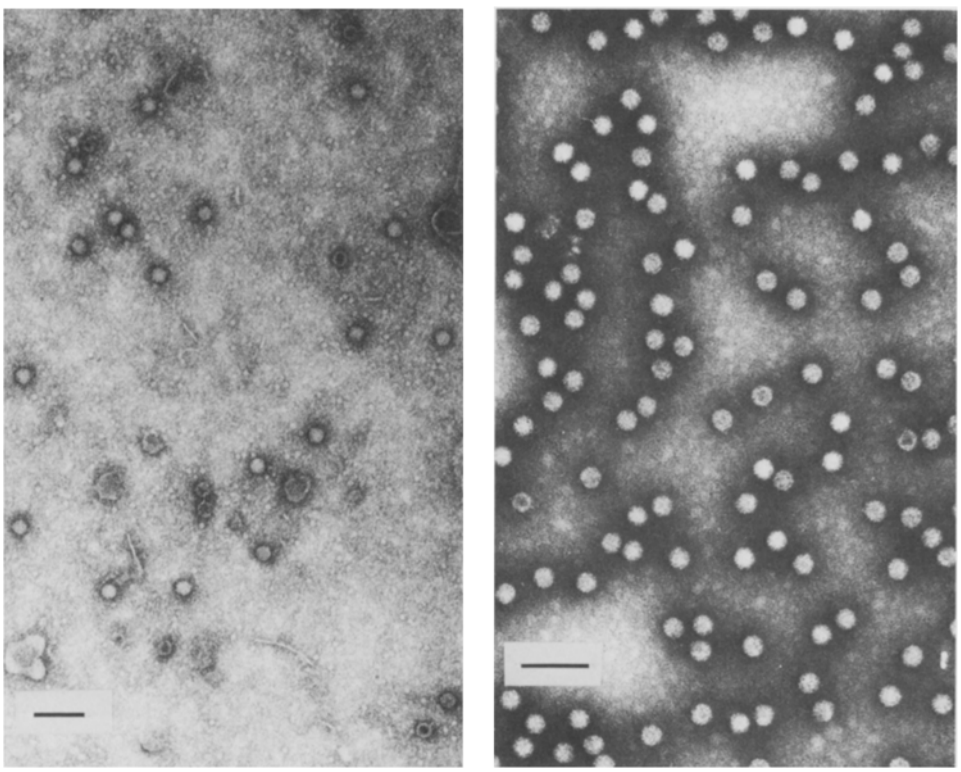

Fig. 6. Electron micrographs of the virus in crude sap (left) and purified preparation (right) after staining with PTA. Bars represent 100 nm.

Table 1. Titres in serological tests.

Antigens	Antisera					
	Cu18	MNSV-Japan	MNSV-Cal[1]	TNV1	TNV2	CNV
Cu18	1024	1024	256	—[3]	—	—
MNSV-Japan[2]	1024	1024	256	—	—	—
TNV1[2]	—	—	—	1024	64	—
TNV2[2]	—	—	—	64	1024	—
CNV	—	—	—	—	—	1024

[1] Antiserum to Californian isolate of melon necrotic spot virus; homologous titer determined in ring-interface precipitation test 1024 (Gonzales-Garza et al., 1979).

[2] MNSV-Japan = melon necrotic spot virus (purified preparation from Japan).
TNV1,2 = tobacco necrosis virus (Dutch isolates from bean and potting soil, respectively).
CNV = cucumber necrosis virus (purified preparation from Canada).

[3] — = no reaction.

the Japanese, both reacting with MNSV-Japan and producing a fused single line without spur (Y. Saito, personal communication, 1983). Table 1 also shows that the antiserum to MNSV-Cal reacted equally well with Cu18 and MNSV-Japan. No relationships were observed between these viruses and TNV or CNV. In later tests the antigens listed in Table 1 did not react with antisera to CSbV from Lebanon and to CFSV from Crete, and purified Cu18 did not react with antisera to CLSV, carnation mottle, carnation ringspot, pelargonium leafcurl and tomato bushy stunt viruses.

Discussion and conclusions

Our investigations have demonstrated the infectious and viral nature of the severe necrosis disease of glasshouse cucumbers observed in the Netherlands, its transmission via soil or rockwool and in leakage water, and the probable involvement of *Olpidium* sp. Symptoms in cucumber have been reproduced under artificial conditions.

The virus can easily be distinguished from TNV (Kassanis, 1970), inclusive its cucumber necrosis strain (Van Koot and Van Dorst, 1955, 1959) and CNV (Dias and McKeen, 1972) in being restricted to Cucurbitaceae and not producing local lesions in *Chenopodium* spp. and *Nicotiana* spp. Both *Olpidium*-transmitted viruses may also occur in roots of cucumber, and CNV and the cucumber necrosis strain of TNV in aboveground parts as well, and cause disease in the crop. They are serologically unrelated mutually and differ in particle size and sedimentation coefficient, which are 26 nm and 116 *S* for TNV and 31 nm and 133 *S* for CNV (Dias and Doane, 1968). TNV is transmitted by *O. brassicae* and CNV by *O. cucurbitacearum* (Dias, 1970a, b), now called *O. radicale* (Lange and Insunza, 1977).

Our virus resembles CNV in particle size (30 nm) and sedimentation coefficient (134 *S*), but it does not infect *Nicotiana* spp. and *Chenopodium* spp. and in our ex-

periments it did not show any serological relationship to CNV (or TNV) (Table 1). However it closely resembles or is identical with the Japanese muskmelon necrotic spot virus (MNSV), which is also restricted to Cucurbitaceae (Kishi, 1966) and has spherical particles with a diameter of 30 nm (Saito and Kishi, 1967). It occurs in the Shizuoka Prefecture since 1955 and is epidemic there since 1968 (Komuro, 1971). It has been reported to be soil transmitted (Komuro et al., 1970; Yoshida et al., 1980) by the same vector as CNV (Furuki et al., 1980). The Japanese MNSV has also been found to closely resemble CNV in sedimentation coefficient (123 and 128 *S*, respectively), buoyant density (1.34 and 1.35 $g.cm^{-3}$, respectively) but to be unrelated serologically (Hibi et al., 1980). The buoyant density of our virus in CsCl is very similar to the above values of MNSV and CNV. The *s* value of our virus (134 *S*) is close to that of CNV (136 *S*) found by Tremaine (1972), who also reported a larger diameter (35 nm) for CNV. However when we ultracentrifuged a mixture of our virus and the purified sample of MNSV, obtained from Dr Saito, only one peak was obtained. Our virus, like MNSV and CNV (Hibi et al., 1980) sediments in a single zone in sucrose density-gradients and we have also found a single peak with our virus in analytical ultracentrifugation.

Results of our serological tests (Table 1) have shown close serological affinity, if not identity, between our virus and the Japanese MNSV and have confirmed lack of serological relationships between these virus isolates and TNV or CNV when using our and the Japanese antisera. The Dutch virus is therefore now considered to be melon necrotic spot virus which differs from TNV or CNV in being restricted to Cucurbitaceae and having no serological affinity to TNV or CNV. Our paper is the first report on pathogenicity of the virus to cucumber crops grown in glasshouses.

MNSV has several features in common with the soil-borne incitant of 'criblure du melon', a severe necrotic disease of 'Charentais' melon grown in glasshouses in southern France. When tentatively describing the virus Marrou and Risser (1967) claimed it to cause local lesions on *C. amaranticolor* and *C. quinoa.* However this could not be confirmed in recent years by Dr H. Lecoq (Montfavet, personal communication, 1983). MNSV has recently also been isolated from cucumber in Britain and been identified there with help of our antiserum (Dr B.J. Thomas, Littlehampton, personal communication, 1983).

Unclear are the relationships between MNSV and the (musk)melon necrotic spot virus causing decline and premature death of glasshouse-grown melon plants in a breeding program in California. Studies of host range, symptomatology, physical properties, transmission, and serology (using the antiserum to Japanese MNSV) indicated that the Californian virus is a strain of MNSV (Gonzalez-Garza et al., 1979). Close serological relationships between the Californian virus, the Japanese MNSV and our virus was confirmed in our experiments when testing the Californian antiserum with both viruses (Table 1). Puzzling, however, are reports on seed transmission of the Californian virus (Gonzalez-Garza et al., 1979), and on its transmission by beetles *(Diabrotica* spp.) (Coudriet et al., 1979) which would be new for an *Olpidium*-transmitted virus related to TNV. Seed transmission of MNSV in melon had also been mentioned by Kishi (1966) but could not be confirmed by Yoshida et al. (1980) and by I. Furuki (Dr Y. Saito, personal communication, 1982). We have not been able to test beetle transmission because of absence of cucumber beetles in this country. We could not test seed transmission because of erratic systemic infections, insufficient

seed set in our experiments with inoculated cucumber plants, and poor germination of the few seeds obtained. Seed transmission seems unlikely in view of the wide distribution of seeds of the few cultivars grown here and limited occurrence of the disease. With *Olpidium*-transmitted viruses like TNV, contamination from soil may readily occur during experimentation because of unwanted *Olpidium* dispersal (Grogan and Campbell, 1966).

The recently reported new soil-borne CLSV from East Germany, CFSV from Greece, and CSbV from Lebanon also have some features in common with MNSV. They clearly differ from MNSV in having artificial hosts other than Cucurbitaceae including *C. amaranticolor, C. quinoa* and some *Nicotiana* spp., though most of them react locally only. The viruses have neither been tested mutually nor with MNSV for serological relationships, but like MNSV, CFSV was not related with CNV and TNV (Gallitelli et al., 1983), and CSbV not with TNV (Koenig et al., 1983). The German CLSV did not react with our MNSV (Cu18) antiserum (Weber et al., 1982) and Cu18 did not react with antiserum tot CLSV. In our comparative tests antisera to CFSV and CSbV did not react with antigens of MNSV, both from the Netherlands and Japan, or two strains of TNV and of CNV.

Like MNSV, all three new viruses contain one type of small isometric particle and one RNA species. They differ from the recently recognized dianthovirus group comprising small isometric monopartite viruses with two RNA species (Matthews, 1982). Transmission of one of its members, red clover necrotic mosaic virus, by *Olpidium brassicae* has been suggested but also questioned (Gerhardson & Insunza, 1979). In our tests MNSV did not react with antiserum to carnation ringspot virus, type member of the group. CLSV and CSbV have tentatively been assigned to a carnation mottle virus group (Hull, 1977) comprising such viruses as carnation mottle, elderberry latent, narcissus tip necrosis and pelargonium flower break viruses. Most of these have later been tentatively grouped with tombusviruses (e.g. Murant and Harrison, 1983) such as tomato bushy stunt and other possibly soil-borne viruses without known vectors. A possible tombusvirus, viz. turnip crinkle virus, has been reported to be transmitted by flee beetles (Broadbent & Heathcote, 1958). Our virus did not react with antisera to carnation mottle, pelargonium leafcurl and tomato bushy stunt viruses. The sobemoviruses are another group of closely related, highly infectious monopartite viruses with an undivided genome. Their host-ranges are narrow, some are seed-borne and they are beetle transmitted. Like those of the TNV group, sobemoviruses do not cross react serologically.

Thus taxonomically these viruses are hard to deal with. MNSV shares several properties with viruses of the TNV group, but its belonging to the carnation mottle virus group (sensu Hull, 1977) or to the tombusvirus group (sensu Hull, 1977, Matthews, 1982, or Murant and Harrison, 1983) cannot be excluded. In its erratic systemic infection of cucumber plants and symptom development MNSV behaves like CNV, with absence of symptoms during the long-day season (McKeen, 1959), and like the cucumber necrosis strain of TNV, with recovery of plants during spells of warm weather (Van Koot and Van Dorst, 1959). MNSV does not seem to easily spread aboveground.

In the Netherlands the appearance of the disease has been associated with the introduction of cucumber cultivation on rockwool and in nutrient solution. It now seems to occur in soil as well. During 1983 the number of holdings having the disease

has increased, but on holdings accustomed to the disease, incidence has decreased or been reduced to nil due to a series of hygienic measures. These are steam sterilization of soil and rockwool, if reused, or treatment of soil with methyl bromide, and the addition to nutrient solutions of the surfactant Agral known to be effective against the zoospores of *O. brassicae* transmitting lettuce big vein (Tomlinson and Faithfull, 1979). The disease can be prevented by grafting cucumber plants at the seedling stage onto immune *Cucurbita ficifolia* rootstocks.

Acknowledgments

We are greatly indebted to Mr. M. de Witte, Extension Service for Horticulture, Aalsmeer-Utrecht, for drawing attention to the problem and for field observations, to Mr A. Visser, Plant Protection Service for assisting in some of the initial experiments, to Dr D. Gallitelli, Bari (Italy) for antiserum to CFSV, to Dr D.J. Gumpf, Riverside (California, USA) for antiserum to the Californian MNSV, to Dr M. Hollings, Littlehampton (Great Britain) for antisera to pelargonium leafcurl and tomato bushy stunt viruses, to Dr. R. Koenig, Braunschweig (Federal Republic of Germany) for antiserum to CSbV, to Dr Y. Saito, Tsukuba Science City (Japan) for purified MNSV and its antiserum and for testing our antiserum, to Dr B.J. Thomas, Littlehampton (England) for testing our antiserum with a British isolate of the virus, to Dr J.H. Tremaine, Vancouver (Canada) for purified CNV and its antiserum, and to the Institut für Phytopathologie, Aschersleben (DDR) for antiserum to CLSV.

Samenvatting

Verdere beschrijving van het meloenenecrosevirus als verwekker van een ernstige ziekte van kaskomkommer in Nederland en de bestrijding ervan

Sinds 1978 komt in de op steenwol en in grond geteelde kaskomkommer een ernstige bladnecrose voor, die vooral in het najaar tot afsterving van planten kan leiden en in wel 45% van de planten van een aangetast gewas is geconstateerd. Uit zieke planten kon een virus worden geïsoleerd dat gemakkelijk overging door sap-inoculatie en in lekvocht uit besmette steenwol (waarschijnlijk door tussenkomst van een *Olpidium*-soort), nadat dit werd gegoten op gesteriliseerde grond waarin komkommerzaailingen groeiden. Met dit virus konden de symptomen van de ziekte worden gereproduceerd.

Het infectievermogen van ruw plantesap nam snel af bij verdunning tussen 10 en 100× (verdunningseindpunt ca 1 millioen) en bij warmtebehandelingen tussen 55 en 65°C (inactiveringstemperatuur 75°C) en bij bewaring bij kamertemperatuur tussen 1 en 1½ maand.

Slechts 3 van de 40 getoetste plantesoorten bleken vatbaar voor het virus, te weten komkommer, meloen en watermeloen. Alle 21 getoetste komkommercultivars en alle 8 getoetste meloenerassen reageerden hevig met lokale lesies en enkele, onder de heersende proefomstandigheden onvoorspelbaar, met systemische necrose. De wel als onderstam gebruikte *Cucurbita ficifolia* is onvatbaar.

Gezuiverd virus sedimenteerde in suiker- en CsCl-gradiënten en bij analytische ultracentrifugering in één piek. De s_{20} was 134 *S* en de zweefdichtheid in CsCl 1.33 $g.cm^{-3}$. In ruw sap en gezuiverde suspensies deden de virusdeeltjes zich voor als

bolletjes met een diameter van ongeveer 30 nm. Ze bevatten slechts één soort eiwit met een relatieve moleculaire massa van 46 000 en één RNA-soort. Een antiserum met titer 1024 werd bereid. Het reageerde niet met komkommernecrosevirus en tabaksnecrosevirus. Wel reageerde het virus met een uit Japan ontvangen antiserum tegen het daar sinds 1966 bekende 'melon necrotic spot virus', terwijl het Japanse virus reageerde met het Nederlandse antiserum. Serologisch, zowel als in biologische en fysisch-chemische eigenschappen lijken de Nederlandse en Japanse isolaten identiek. Voor het virus wordt daarom de Nederlandse naam meloenenecrosevirus voorgesteld. Het verschilt van drie andere, onlangs min of meer gelijktijdig in Oost-Duitsland, op Kreta en in Libanese grond aangetroffen, via de bodem overgaande komkommervirussen, die evenals tabaks- en komkommernecrosevirus ook andere plantesoorten dan cucurbitaceeën kunnen infecteren.

Waarschijnlijk is meloenenecrosevirus al sinds 1967 bekend in Frankrijk als verwekker van 'criblure du melon'. Het is ook nauw verwant aan de verwekker van een in een veredelingsprogramma van meloen in Californië opgedoken necrosevirus, waarvan echter wordt beweerd dat het overgaat met zaad van meloen en wordt overgebracht door bladkevertjes, *Diabrotica*-soorten. Het meloenenecrosevirus is in ons land voor het eerst geconstateerd als ziekteverwekker van kaskommer. Ook in England is het daarin onlangs aangetroffen. De ziekte kan op verschillende manieren bestreden, respectievelijk voorkomen worden. De grond dient gestoomd te worden of begast met methylbromide. Steenwolmatten kunnen bij hergebruik gestoomd worden, terwijl aan de voedingsoplossing uitvloeier (Agral) toegediend kan worden. Zowel bij grond- als steenwolteelten is de ziekte te voorkomen door komkommerplanten te enten op de onvatbare onderstam *Cucurbita ficifolia.*

References

Bos, L., 1981. Nieuwe ziekte in komkommer op steenwol veroorzaakt door het voor Nederland nieuwe meloenenecrosevirus. Gewasbescherming 12: 135-141.

Bos. L., Huttinga, H. & Maat, D.Z., 1980. Spinach latent virus, a new ilarvirus seed-borne in *Spinacia oleracea.* Neth.J.Pl.Path. 86: 79-98.

Broadbent, L. & Heathcote, G.D., 1958. Properties and host range of turnip crinkle, rosette and yellow mosaic viruses. Ann. appl. Biol. 46: 585-592.

Coudriet, D.L., Kishaba, A.N. & Carroll, J.E., 1979. Transmission of muskmelon necrotic spot virus in muskmelons by cucumber beetles. J. econ. Ent. 72: 560-561.

Dias, H.F., 1970a. Transmission of cucumber necrosis virus by *Olpidium cucurbitacearum* Barr and Dias. Virology 40: 828-839.

Dias, H.F., 1970 b. The relationship between cucumber necrosis virus and its vector, *Olpidium cucurbitacearum.* Virology 42: 204-211.

Dias, H.F. & Doane, F.W., 1968. Evidence of the lack of relationship between Canadian cucumber necrosis virus and tobacco necrosis virus. Can. J. Bot. 36: 47-50.

Dias, H.F. & McKeen, C.D., 1972. Cucumber necrosis virus. CMI/AAB Descr. Pl. Viruses 82: 4 pp.

Furuki, I., Hibi, T., Honda, Y., Saito, Y. & Komuro, Y., 1980. Relationship between cucumber necrosis virus and melon necrotic spot virus. Ann. phytopath. Soc. Japan 46: 419 (Abstr.)

Gallitelli, D., Vovlas, C. & Avgelis, A., 1983. Some properties of cucumber fruit-streak virus. Phytopath. Z. 106: 149-162.

Gerhardson, B. & Insunza, V., 1979. Soil transmission of red clover necrotic mosaic virus. Phytopath. Z. 94: 67-91.

Gonzales-Garza, R., Gumpf, D.J., Kishaba, A.N. & Bohn, G.W., 1979. Identification, seed transmission and host range pathogenicity of a California isolate of melon necrotic spot virus. Phytopathology 69: 340-345.
Grogan, R.G. & Campbell, R.N., 1966. Fungi as vectors and hosts of viruses. Ann. Rev. Phytopath. 4: 29-52.
Hibi, F., Furuki, I., Honda, Y., Saito, Y. & Komuro, Y., 1980. Relationship between cucumber necrosis virus and melon necrotic spot virus. 2. Properties of the viruses and serology. Ann. phytopath. Soc. Japan 46: 419 (Abstr.)
Hull, R., 1977. The grouping of small spherical plant viruses with single RNA components. J. gen. Virol. 36: 289-295.
Kassanis, B., 1970. Tobacco necrosis virus. CMI/AAB Descr. Pl. Viruses 14: 4 pp.
Kishi, K., 1966. Necrotic spot of melon, a new virus disease. Ann. phytopath. Soc. Japan 32: 138-144.
Koenig, R., Lesemann, D.E., Huth, W. & Makkouk, K.M., 1983. Comparison of a new soilborne virus from cucumber with tombus-, diantho-, and other similar viruses. Phytophathology 73: 515-520.
Komuro, Y., 1971. Cucumber green mottle mosaic virus on cucumber and watermelon and melon necrotic spot virus on muskmelon. Jap. agric. Res. Q. 6: 41-45.
Komuro, Y., Furuki, I., & Iizuka, K., 1970. Melon necrotic spot virus is soil-borne. Pl. Prot. 24: 399-402.
Koot, Y. van & Dorst, H.J.M. van, 1955. Een nieuwe virusziekte bij komkommers. Tijdschr. PlZiekt. 61: 163-164.
Koot, Y. van & Dorst, H.J.M. van, 1959. Virusziekten van de komkommer in Nederland. Tijdschr. PlZiekt. 65: 257-271.
Lange, L. & Insunza, V., 1977. Root-inhabiting *Olpidium* species: the *O. radicale* complex. Trans. Br. mycol. Soc. 69: 377-384.
Maat, D.Z., Huttinga, H. & Hakkaart, F.A., 1978. Nerine latent virus: some properties and serological detectability in *Nerine bowdenii.* Neth. J. Pl. Path. 84: 47-59.
Markham, R., 1960. A graphical method for the rapid determination of sedimentation coefficients. Biochem. J. 77: 516-519.
Marrou, J. & Risser, G., 1967. La criblure du melon: Etude préliminaire d'une nouvelle maladie à virus du melon en culture sous serre. Annls Epiphyt. 18 (No. hors série): 193-203.
Matthews, R.E.F., 1982. Classification and nomenclature of viruses. Fourth report of the International Committee on Taxonomy of Viruses. Intervirology 17: 1-199.
McKeen, C.D., 1959. Cucumber necrosis virus. Can. J. Bot. 37: 913-925.
Murant, A.F. & Harrison, B.D. (Eds), 1983. Description of plant viruses. CAB/AAB Farnham Royal, England, 17, sheets 261-275.
Saito, Y. & Kishi, K., 1967. Purification and morphology of melon necrotic spot virus. Ann. phytopath. Soc. Japan 33: 59-60.
Tomlinson, J.A. & Faithfull, E.M. 1979. Effects of fungicides and surfactants on the zoospores of *Olpidium brassicae.* Ann. appl. Biol. 93: 13-19.
Tremaine, J.H., 1972. Purification and properties of cucumber necrosis virus and a smaller top component. Virology 48: 582-590.
Weber, I., Proll, E., Ostermann, W.D., Leiser, R.M., Stanarius, A. & Kegler, H., 1982. Charakterisierung des Gurkenblattflecken-Virus (cucumber leaf spot virus), eines bisher nicht bekannten Virus an Gewächshausgurken *(Cucumis sativus* L.). Arch. Phytopathol. PflSch. 18: 137-154.
Yoshida, K., Goto, T., Nemato, M. & Tsuchizaki, T., 1980. Five viruses isolated from melon *(Cucumis melo* L.) in Hokkaido. Ann. phytopath. Soc. Japan. 46: 339-348.