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Cucumber mosaic virus isolated from Yucca flaccida

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Abstract

Cucumber mosaic virus (CMV) was isolated from *Yucca flaccida*. The isolate was identified as CMV by host range, ways of transmission, physical properties and serology. No symptoms appeared on healthy *Yucca* seedlings inoculated with purified virus or virus-containing sap, neither could the virus be recovered from these plants.

Introduction

Some years ago the Plant Protection Service (PD) at Wageningen received plants of *Yucca flaccida* Haw. (Agavaceae) with yellow oval ringspots on the leaves from a nursery at Hillegom, the Netherlands. Routine tests carried out at the above institution did not reveal the presence of any pathogen. The diseased plants, kept in the experimental garden of the PD, exhibited symptoms throughout the years. The symptoms were most prominent in early spring, consisting of bright yellow ringspots (Fig. 1A). Later in the season the younger leaves showed a more diffuse chlorotic streaking (Fig. 1B).

In two inoculation experiments carried out in the Laboratory of Virology a few test plants (nine different species and cultivars) were inoculated with crude sap from *Yucca* leaves showing ringspots. In one experiment only plants of *Nicotiana tabacum* 'White Burley' developed symptoms (chlorotic mottling followed by fine etching), whereas in the other only plants of *Gomphrena globosa* and *N. tabacum* 'Samsun NN' reacted by showing local necrotic lesions, and etched rings and line pattern, respectively.

Although no virus disease of *Yucca* has been reported so far, the results from the above-mentioned preliminary experiments indicated the presence of a virus. We, therefore, further investigated the disease. The present paper describes the virus isolated from diseased *Yucca* plants.

Materials and methods

All plants were grown in the glasshouse at temperatures ranging from 20°C–25°C.

Yucca seedlings were raised from commercially obtained seed of *Y. filamentosa*, as seed of *Y. flaccida* was not available. However, according to Bailey (1966), *Y. filamentosa* Auth. non L. is a synonym of *Y. smalliana* Fern. and some material cultivated as *Y. filamentosa* belongs to *Y. flaccida* Haw.

When the virus was mechanically transmitted from diseased *Yucca* plants to

Fig. 1. Yellow ringspots (A) and chlorotic streak (B) in leaves of *Yucca flaccida*.

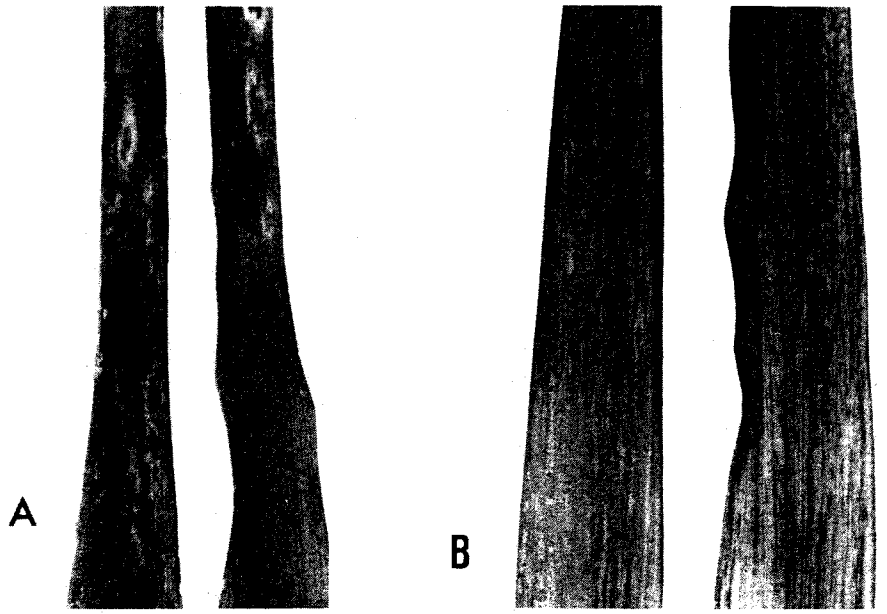

Fig. 1. Gele kringvlekken (A) en chlorotische streping (B) op bladeren van Yucca flaccida.

'White Burley' tobacco plants, it invariably evoked the same symptoms on the latter. Therefore, infected 'White Burley' plants served as virus source in most of the experiments.

In host range tests, plants of 26 species and cultivars were inoculated manually after dusting the leaves with carborundum (600 mesh) with water-diluted crude sap of symptom-showing leaves of *N. tabacum* 'White Burley'. After 1 to 2 weeks non-inoculated, symptomless leaves and inoculated leaves of symptomless plants were tested for virus by return inoculations to 'Samsun NN' and 'White Burley' tobacco plants. *Chenopodium amaranticolor* was used as assay plant in experiments on properties of the virus in crude sap.

Transmission experiments with *Myzus persicae* were carried out as follows. After a starvation period of 30 min apterous aphids were allowed an acquisition access period of 10 min on symptom-showing plants of either *Y. flaccida* (clones of the original diseased plants from the PD) or 'White Burley' tobacco, whereafter they were transferred to young seedlings of *Y. filamentosa* and 'White Burley' tobacco for inoculation feeding. As the aphids did not seem to be eager to probe into the *Yucca* leaves we extended the starvation period to 18 h in later experiments.

In dodder transmission experiments shoots of *Cuscuta subinclusa* established on symptom-showing plants of *Y. flaccida* and 'White Burley' tobacco, respectively, were trained on to both *Y. filamentosa* and 'White Burley' tobacco plants.

The virus was purified from infected 'White Burley' tobacco plants by the method used by Murant (1965) for cucumber mosaic virus (CMV).

The optical density of purified virus preparations was measured in a Zeiss spectro-

photometer. Virus concentrations were estimated using an extinction coefficient $E_{260}^{0.1\%}_{1\ cm}$ of 5.0, the value given for CMV (Gibbs and Harrison, 1970).

For electron microscopy purified virus was negatively stained with 2% potassium phosphotungstate (pH 6.5) or 2% ammoniummolybdate (pH 5.0). The mixture was mounted on carbon-reinforced formvar-coated copper grids and examined with a Siemens Elmiskop I electron microscope. Particles of tobacco mosaic virus were used as an internal size standard as described by Bos (1975).

Antiserum was produced in a 13-month-old rabbit, injected intravenously with 2 mg of purified virus in 0.06 M phosphate buffer (pH 7.0) on the 1st, 6th and 16th day. Thereafter, the rabbit was injected intramuscularly on the 27th, 38th and 48th day with 5 mg, 10.8 mg and 11.4 mg of virus, respectively, emulsified with Freund's incomplete adjuvant. On the 61st day blood was collected. Microprecipitin method according to Noordam (1973) and conventional agar gel double-diffusion method were used for serological tests. Antisera to the following strains and isolates of CMV were kindly provided by Ing. D. Z. Maat, Research Institute for Plant Protection at Wageningen: a strain of CMV, seed-transmitted in bean (Bos and Maat, 1974; CMV-B 32), CMV-related chrysanthemum virus (Noordam, 1952; CV-Noordam), CMV, Lisse (CMV-Lisse), CMV from *Nerine* (CMV-*Nerine*) and CMV, Y-strain (Scott, 1963; CMV-Y).

Fig. 2. Leaves of *Datura stramonium* with chlorotic (ring)spots (A) and necrotic lesions (B) 6 and 32 days, respectively, after inoculation with cucumber mosaic virus.

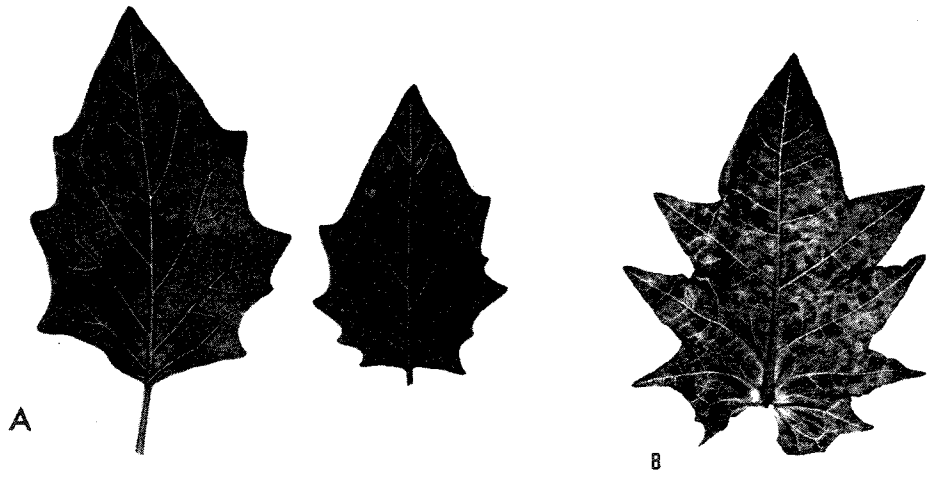

Fig. 2. Bladeren van Datura stramonium met chlorotische (kring)vlekken (A) en necrotische lesies (B), respectievelijk 6 en 32 dagen na inoculatie met komkommermozaïekvirus.

Fig. 3. Leaves of *Hyoscyamus niger* with pin-point necrotic local lesions (A) and systemic mosaic (B) 14 and 11 days, respectively, after inoculation with cucumber mosaic virus.

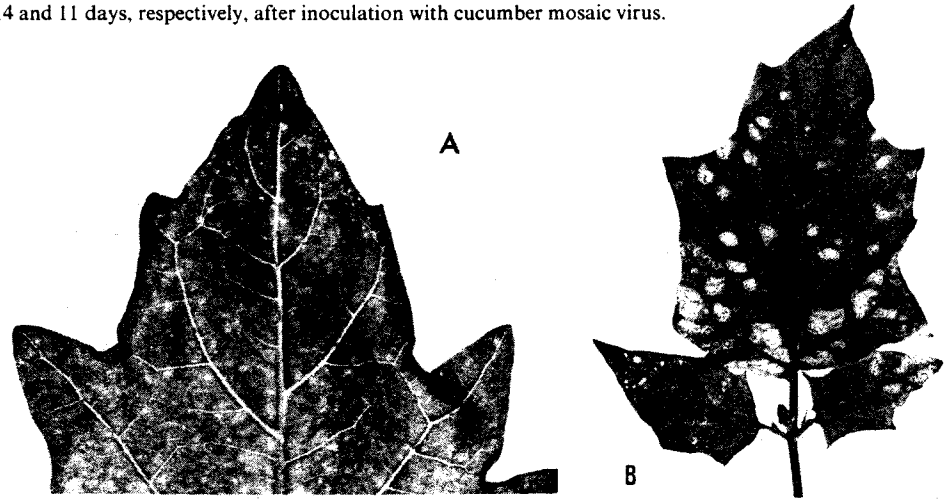

Fig. 3. Bladeren van Hyoscyamus niger met speldeknopgrote, necrotische lokale lesies (A) en systemisch mozaïek (B), respectievelijk 14 en 11 dagen na inoculatie met komkommermozaïekvirus.

Results

Host range tests. The plants used in these tests reacted as follows:
Beta vulgaris 'Groeningia'. Many small local chlorotic spots; systemic symptomless infection.
Browallia speciosa 'Blue bell'. Faint local mottle; systemic chlorotic areas.
Chenopodium amaranticolor. Two days after inoculation numerous local necrotic pin-point lesions with a narrow chlorotic halo; no systemic infection.
Chenopodium quinoa. Two days after inoculation many necrotic local lesions with a yellow halo; no systemic infection.
Cucumis sativus 'Lange gele tros'. Local chlorotic lesions on the cotyledons; systemic yellow mottle beginning at the base of the leaf followed by wrinkling and distortion of the leaves, and stunting of the plant. Occasionally recovery after the third leaf had developed.
Datura stramonium. Local chlorotic (ring)spots, beginning at the tip of the leaves, and faint mottle; systemic chlorotic (ring)spots (Fig. 2A), occasionally followed by necrotic brown dots arranged in rings (Fig. 2B).
Gomphrena globosa. Many diffuse, reddish brown local lesions with a white centre; systemic reddish brown lesions and yellow spots in the youngest leaves.
Hyoscyamus niger 'Pallidus'. Local necrotic pin-point lesions appearing after the systemic symptoms (Fig. 3A); systemic mosaic in the upper-most fully-developed leaves (Fig. 3B).
Lycopersicon esculentum 'Moneymaker'. Three days after inoculation some brown necrotic local spots; faint mottle or symptomless systemic infection.
Nicotiana clevelandii. Faint local mottle, followed by yellow-orange flecks; systemic mottle and yellow mosaic, narrowing of the leaves and stunting of the plant.
Nicotiana glutinosa. Diffuse local mottle, occasionally vein-clearing beginning at the

Fig. 4. Leavus of *Solanum capsicastrum* showing necrotic flecks with red-brown dots, and faint mottle 14 days after inoculation with cucumber mosaic virus.

Fig. 4. Bladeren van Solanum capsicastrum die 14 dagen na inoculatie met komkommermozaïekvirus chlorotische vlekken met roodbruine stipjes, alsook vage vlekkerigheid vertoonden.

base of the leaf; systemic green mottle and curling of the leaves, stunting of the plant.
Nicotiana rustica. Faint local mottle; systemic yellow mosaic and mottle, occasionally dark-green areas in some of the top leaves, followed by recovery.
Nicotiana tabacum 'Samsun NN'. Local chlorotic and slightly necrotic (ring)spots, and fine necrotic etching with concentric rings; systemic chlorotic (ring)flecks, followed by recovery.
Nicotiana tabacum 'White Burley'. Pale chlorotic (ring)spots, beginning at the base and faint mottle in the older inoculated leaves, occasionally followed by faint etching in the latter leaves; systemic vein yellowing and chlorotic ringspots, sometimes accompanied with fine necrotic etching. Usually recovery four weeks after inoculation.
Petunia hybrida. Inoculated leaves with black veins and large, irregularly shaped brown or white necrotic lesions; systemic faint mottle or symptomless infection.
Phaseolus vulgaris 'Noordhollandse bruine'. No infection.
Solanum capsicastrum. No local symptoms; occasionally a fortnight after inoculation chlorotic lesions with reddish brown dots in the centre and faint mottle (Fig. 4), followed by recovery.
Solanum melongena 'Radja RZ'. Local chlorotic (ring)spots; faint systemic mottle and occasionally bright yellow line pattern in some leaves.
Tetragonia expansa. Faint local mottle or symptomless; systemic chlorotic (ring)-spots and chlorotic bands.
Trifolium incarnatum. On some leaves local chlorotic spots; systemic mottle, bright

yellow mosaic, vein yellowing, reddish brown discoloration, and malformation of the younger leaves.
Vicia faba 'Driemaal wit'. Few reddish brown lesions; no systemic infection.
Vigna unguiculata 'Blackeye Early Ramshorn'. Three days after inoculation reddish brown local lesions on primary leaves; no systemic infection.
Vinca rosea. More than two weeks after inoculation yellow flecks and patches on some of the inoculated and non-inoculated leaves.
Zinnia elegans 'Californische reuzen'. Reddish brown discoloration of the inoculated leaves accompanied with chlorosis and mottle; faint systemic mottle.

Mechanical inoculation of Yucca. Eighty three seedlings of *Y. filamentosa*, 2—4 months of age, were inoculated with either purified preparations of the virus (69 seedlings, in November 1977) or crude sap of symptom-showing leaves of 'White Burley' tobacco (14 seedlings, in February 1978). Similar numbers of seedlings were not inoculated and served as controls. No symptoms appeared, neighter could virus be recovered from plants in the two series at the time of assessment (May 1978).

Aphid transmission. The virus was transmitted from diseased 'White Burley' plants to healthy plants of the same cultivar by *M. persicae*; about eight days after the aphids had been transferred from the diseased plants to the healthy ones symptoms appeared

Fig. 5. Electron micrograph showing particles of cucumber mosaic virus mounted in 2% ammonium molybdate. Bar represents 100 nm.

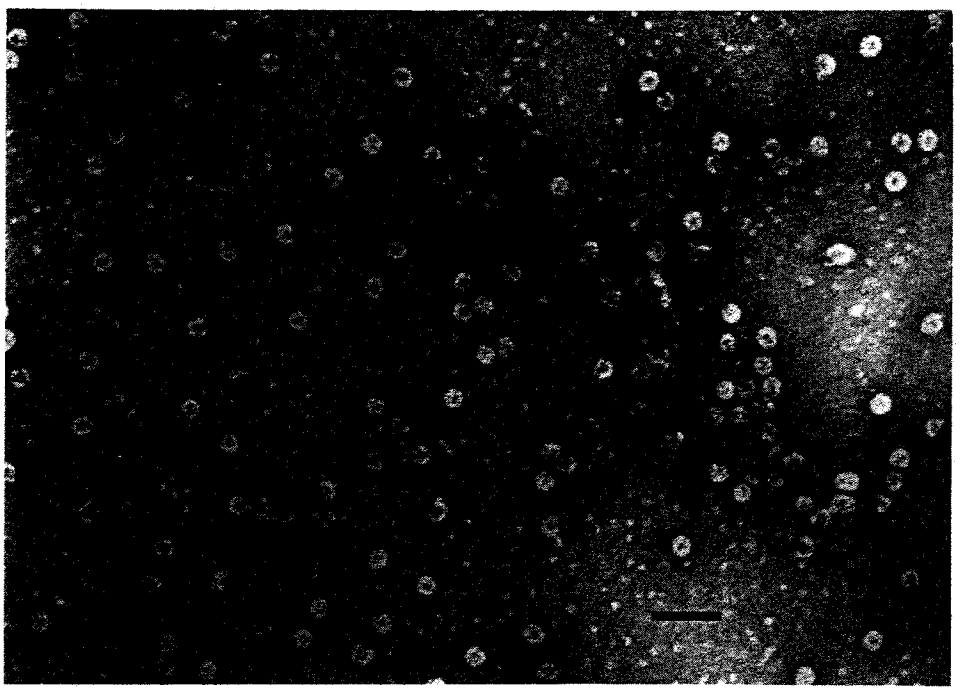

Fig. 5. Elektronenmicroscopische opname van komkommermozaïekvirus dat met 22% ammoniummolybdaat werd gecontrasteerd. De vergrotingsstreep geeft 100 nm weer.

on the latter. The virus was neither transmitted from diseased *Yucca* plants to either *Yucca* or 'White Burley' tobacco plants, nor from diseased 'White Burley' tobacco to healthy *Yucca* plants. During the acquisition access period on *Yucca* many aphids were not found probing and during the inoculation feeding period on *Yucca* some of the aphids disappeared from the leaves.

Dodder transmission. The virus was readily transmitted from diseased 'White Burley' tobacco plants to healthy plants of the same cultivar by *C. subinclusa*; about 14 days after shoots of dodder on diseased plants had established on healthy plants symptoms appeared on the latter. A healthy *Y. filamentosa* seedling which had been in contact with a diseased plant of *Y. flaccida* through dodder showed characteristic symptoms (diffuse chlorotic streaking) about five months after the beginning of the experiment, but we were unable to recover the virus from this plant by mechanical inoculation. No transmission was obtained from diseased *Yucca* plants to healthy 'White Burley' tobacco and from diseased 'White Burley' tobacco to healthy *Yucca* plants.

Properties in crude sap. Crude sap from symptom-showing leaves of 'White Burley' tobacco was infective after dilution to 10^{-5} but not 10^{-6}. The thermal inactivation point was 65 °C–70 °C, and at 22 °C infectivity was retained for 9 days but not for 10 days.

Properties of purified preparations. – Ultraviolet absorption spectrum: Purified preparations had an absorption maximum at 258 nm and minimum at 242 nm. The 260: 280 nm ratio varied between 1.64 to 1.71 with a mean ratio of 1.68. The average yield was 450 mg virus per 1000 g leaf material.
Electron microscopy. When purified preparations were examined in the electron microscope in 2% potassium phosphotungstate (pH 6.5) almost all virus particles were disrupted. Preparations mounted in 2% ammoniummolybdate (pH 5.0), on the other hand, contained many intact isometric virus particles ca 30 nm in diameter (Fig. 5).
Serology. The antiserum to the purified virus had a homologous titre of about 2048 in microprecipitin tests. Purified preparations of the virus reacted in gel diffusion tests with antisera to the following isolates and strains of CMV (homologous titres in parentheses): CMV-B 32 (64), CV-Noordam (2048), CMV-Lisse (256), CMV-*Nerine* (64) and CMV-Y (128). The reaction was weak in case of CMV-B 32 and CMV-Y. No reaction was obtained with sap from healthy tobacco plants.

Discussion

The host reactions resembled those caused by CMV. On the basis of the symptoms the isolate from *Yucca* falls into group II of 'Typical field isolates in Britain', a CMV classification proposed by Hollings et al. (1968); all the CMV isolates described by Lovisolo and Conti (1969) also belong to this group. In the classification of Marrou et al. (1975) the virus from *Yucca* belongs to their group B characterized by ring-spotting, etching and eventually recovery on tobacco. According to the latter authors, this group B compares well with group II of Hollings et al. (1968) and with serotype group ToRS of Devergne and Cardin (1970, 1975). However, there were a few exam-

ples of host reactions which resembled more those from Price's yellow strain of CMV (CMV-PY; group III of Hollings et al., 1968) than those from strains of group II, viz. chlorotic ringspot in *D. stramonium*, local necrotic lesions on *H. niger*, systemic mottle and yellow mosaic in *N. clevelandii* and bright systemic yellow mosaic in *T. incarnatum*.

The transmission characteristics were in agreement with those of CMV. The failure to transmit the virus from *Yucca* to tobacco vice versa by *M. persicae* may be due to the fact that the aphids scarcely probed into the *Yucca* plants. Our inability to transmit the virus mechanically to *Yucca* may be explained by a possible genetical heterogeneity of the *Yucca* seedlings and a very slow multiplication of the virus in this plant. In our dodder transmission experiments one *Yucca* seedling showed symptoms only five months after the beginning of the experiment, but even from this plant we were unable to recover the virus, possibly due to low virus concentration and presence of inhibitors of infectivity in expressed *Yucca* sap.

Thermal inactivation point and dilution end-point fell within the range of CMV strains (Gibbs and Harrison, 1970; Smith, 1972), but the longevity in vitro was much longer (9 days) than that reported in the literature (Gibbs and Harrison, 1970, 3–6 days; Smith, 1972, 3–4 days).

The 260/280 absorbance ratio and the particle structure were comparable to those of CMV (Gibbs and Harrison, 1970).

The serological reaction confirmed that the isolate from *Yucca* was related to CMV. It is known that with broad-spectrum antiserum CMV strains belonging to group II of Hollings et al. (1968) can be shown to be serologically related to virus strains of group III (CMV-PY and CMV-Y; Hollings et al., 1968). This may explain the weak serological reaction between the virus from *Yucca* and antiserum to CMV-Y.

Samenvatting

Komkommermozaïekvirus, geïsoleerd uit Yucca flaccida

Enkele jaren geleden ontving de Plantenziektenkundige Dienst (PD) te Wageningen van een kwekerij in Hillegom enkele exemplaren van *Yucca flaccida* Haw. (Agavaceae) met gele, ovale kringvlekken op de bladeren. In routinetoetsingen, uitgevoerd op de PD, kon in het zieke materiaal geen pathogeen worden aangetoond. De zieke planten, overgebracht naar de proeftuin van de PD, vertoonden in het vroege voorjaar heldergele kringvlekken (Fig. 1A) en in de rest van het jaar diffuse, chlorotische strepen op de nieuw-gevormde bladeren (Fig. 1B). In twee inoculatieproeven, uitgevoerd op het Laboratorium voor Virologie, kon een virus uit het zieke *Yucca*-materiaal worden overgebracht naar planten van *Gomphrena globosa*, *Nicotiana tabacum* 'Samsun NN' en *N. tabacum* 'White Burley'. Reacties op een uitgebreidere serie toetsplanten, geïnoculeerd met sap van een zieke 'White Burley'-tabaksplant, deden denken aan die welke veroorzaakt worden door komkommermozaïekvirus (KMV), en wel door die groep van KMV-isolaten die in de indeling van Hollings e.a. (1968) aangeduid wordt als groep II 'Typical CMV field isolates in Britain'. Figuur 2 geeft een karakteristiek beeld van infectie van *Solanum capsicastrum* door het virus uit *Yucca*. Slechts enkele toetsplanten vertoonden symptomen die kenmerkend zijn voor de gele stam van KMV (CMV-PY), die in het systeem van Hollings e.a. (1968) be-

hoort tot groep III (Fig. 3 en 4).

Het is niet gelukt gezonde *Yucca* zaailingen te infecteren met hetzij een gezuiverde virussuspensie, hetzij ruw sap van zieke 'White Burley'-planten.

Het virus kon door *Myzus persicae* in korte zuigtijden worden overgebracht van zieke 'White Burley'-tabak naar gezonde planten van dezelfde cultivar, doch niet uit zieke *Yucca* naar gezonde tabak of uit zieke tabak naar gezonde *Yucca*. In overdrachtsproeven met warkruid *(Cuscuta subinclusa)* kon het virus van zieke 'White Burley' naar gezonde 'White Burley' worden overgebracht en in één geval, te oordelen naar de symptomen, ook van zieke *Yucca* naar gezonde *Yucca*, naar niet van zieke *Yucca* naar gezonde tabak of van zieke tabak naar gezonde *Yucca*.

In ruw sap behield het virus zijn infectievermogen bij een verdunning van 10^{-5}, een temperatuur van ongeveer 70 °C en een bewaarperiode bij 22 °C van 9 dagen. De twee eerstgenoemde waarden komen overeen met, de laatste is daarentegen hoger dan, die welke in de literatuur worden vermeld voor KMV.

De 260/280 absorptieverhouding en de structuur van de virusdeeltjes (Fig. 5) kwamen overeen met die van KMV. Serologische reacties uitgevoerd met antiserum tegen verschillende stammen en isolaten van KMV bevestigen de verwantschap tussen het virus uit *Yucca* en KMV

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