

SOME DATA CONCERNING NATURAL  
HOSTS OF CUCUMBER MOSAIC VIRUS IN  
HUNGARY AND JUGOSLAVIA

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Introduction

The present paper deals with identification of cucumber mosaic virus (CMV; cryptogram R/1 : 1/18 : S/S : S/Ap) in spontaneously infected weed plants in Hungary (*Aristolochia clematitis* L., *Aristolochia durior* Hill. (syn.: *A. siphon l'Hérit*), *Aristolochia elegans* Mast.) and in Jugoslavia (*Aristolochia macrophylla* Lam., *Aquilegia vulgaris* L., *Scopolia carniolica* Jacq.).

For several years now virus-like symptoms have been noticed on various *Aristolochia* spp. in Hungary, but the identification of those diseases was not carried out (Schmelzer et al. 1969, Horváth 1972 and 1973, unpublished data). CMV has already been found in four plant species in Jugoslavia (Juretić 1974) which grew in the same locality where the above mentioned plant specimens were collected.

Some *Aquilegia* species are known to be natural hosts or susceptible under experimental conditions to several viruses. CMV has been detected in *Aq. alpina*, *Aq. coerulea*, *Aq. canadensis*, *Aq. chrysanthra*, *Aq. flabellata*, *Aq. haylodgensis* and *Aq. vulgaris*. In addition, *Aq. alpina*, *Aq. coerulea* and *Aq. chrysanthra* can be infected with tobacco rattle virus, *Aq. coerulea* also with anemone mosaic, anemone brown ring, alfalfa mosaic and tobacco ringspot viruses, while on *A. vulgaris* infections with tomato spotted wilt virus and aster yellows mycoplasma were described (cf. Klinkowski 1968, Schmelzer and Wolf 1971).

The only known virus-infected *Aristolochia* spp. in nature are *A. durior* and *A. clematitis*. The former species has been found in Sweden as natural host of CMV (Lihnell 1951), while in Germany Schmelzer and Schmidt (1968) isolated CMV and arabis mosaic virus from that species. Schmelzer and Schmidt (1968; cf. Brückbauer 1960) and recently Juretić (1974) established spontaneous infection of *A. clematitis* with CMV in Germany and Jugoslavia, respectively.

*Scopolia* spp. have so far been noticed to be susceptible only to infection with virus-like agents (viroids) under experimental conditions. First it was found (Singh 1971) that *S. sinensis* Hemsl. reacts with local infection following inoculation with potato spindle tuber viroid. After inoculation the same viroid causes local response in *S. anomala*, *S. lurida*, *S. stramonifolia* and *S. tangutica*, as well, and symptomless infection in *S. physaloides* (Singh 1973). *S. sinensis* is capable of infection with citrus exocortis viroid locally, too (Singh and Clark 1973; cf. also Diener 1972).

### Material and Methods

The origin of virus isolates is shown in Table 1, together with some other relevant data.

Table 1. Data concerning the origin of isolates of cucumber mosaic virus discussed in this paper.

Tablica 1. Podaci o porijeklu izolata virusa mozaika krastavaca u ovom radu.

Plant	Symptoms on spontaneously infected plant	Locality	Isolate symbol
<i>Aristolochia clematitis</i>	chlorotic variegation, mosaic, leaf deformations, stunting	Hungary, Dunabogdány (weed in private garden)	Ac
<i>A. durior</i>	"	Hungary, Budapest (grown in Botanical Garden)	Ad
<i>A. elegans</i>	"	Hungary, Vácrátót (grown in Botanical Garden)	Ae
<i>A. macrophylla</i>	"	Jugoslavia, Zagreb (grown in Botanical Garden)	Am
<i>Aquilegia vulgaris</i>	"	"	Aq
<i>Scopolia carniolica</i>	chlorotic vein-banding, oak leaf pattern, mosaic, leaf deformations, stunting	"	Sc

**Transmission tests.** Isolates Ac, Ad, Ae, Am, Aq and Sc were transmitted from originally infected plants to test plants by means of mechanical inoculation, with addition of two parts of M/15, pH 7.0 phosphate buffer to ground infectious tissue.

**Plant-protection experiments.** Isolates Ac, Ad, Ae and Am were investigated for this purpose with *Nicotiana tabacum* L. cv. "Xanthi"-nc specimens as test plants and "white" strain (W) of CMV as "challenge" strain.

**Physical properties.** Conventional methods were applied (cf. Horváth 1969) in the examination of thermal inactivation point, dilution end

point and longevity in vitro of the isolates (Ac, Ad, Ae, Am). Infectious extract was obtained from *N. tabacum* cv. "Xanthi"-nc., and the test plant was *Chenopodium amaranticolor* Coste et Reyn.

*Aphid transmission.* The experiments were performed with isolates Ac, Ad, Ae and Am from *Aristolochia* spp. using *Myzus persicae* Sulz. as vector. Infected *N. tabacum* cv. "Samsun" and *N. glutinosa* plants were used as source plants and young "Samsun" and "Xanthi"-nc as test plants. Before acquisition feeding aphids starved for 3 hours. Each test plant was inhabited with 10 aphids. Aphids were destroyed with Phosdrin at the end of test feeding.

*Serology.* The experiments were performed by means of double-diffusion method in 0.9% agar-gel which contained standard additives. Crude extracts of infected and healthy leaves (*N. glutinosa*, *Cucumis sativus*) were used in the experiments. Antisera against CMV (supplied by Dr. Y. Komuro, Japan, and Dr. E. Luisoni, Italy) were at our disposal for serological experiments. Homologous antisera titres were about 1/64.

## Results

### *Symptoms in test plants*

Twelve plant species were used in inoculation tests with isolates Ac, Ad, Ae, Am, Aq and Sc. As the symptoms caused by the isolates showed similarity, the reactions of test plants are only shown in general in Table 2. Small differences in symptoms from isolate to isolate are neglected in the Table.

### *Physical properties*

The results are presented in Table 3.

### *Plant-protection tests*

After development of systemic symptoms in tobacco plants following the inoculation with single Ac, Ad, Ae or Am isolate, the subsequent inoculation of the infected plants with isolate W of CMV was done. The latter isolate produced in the reinoculated plants no visible "white" symptoms which are its characteristic.

### *Aphid transmission*

In the experiments isolates Ac, Ad, Ae and Am were readily transmitted from diseased tobacco and *N. glutinosa* plants to healthy tobacco plants through *M. persicae*. The transmission occurred in a stylet-borne manner, i. e. after an acquisition period of 8 minutes, followed by a test feeding of 24 hours.

### *Serological relationship*

Isolates Ac, Ad, Ae, Am, Aq and Sc gave positive serological reactions with the two antisera against CMV. The antisera titres with our isolates were lower by about a step of twofold antisera dilutions than the homologous antisera titres. The reactions with isolates Ac, Ad and Ae were somewhat stronger than in isolates Am, Aq and Sc.

Table 2. Symptoms in test plants of isolates Ac, Ad, Ae, Am, Aq and Sc of cucumber mosaic virus (L: Symptoms in inoculated leaves; S: Systemic symptoms; O: No symptoms.)

Tablica 2. Simptomi koje uzrokuju na pokusnim biljkama izolati Ac, Ad, Ae, Am, Aq i Sc virusa mozaika krastavca. (L.: Simptomi na inokuliranim listovima; S: Simptomi sistemične infekcije; O: Bez simptoma).

Test plant	Symptoms
<i>Chenopodium amaranthoides</i> Coste et Reyn.	L: chlorotic lesions with minute necrotic centre which enlarges later; S: O
<i>C. quinoa</i> Willd.	L: chlorotic local lesions, later becoming necrotic; S: O
<i>Cucumis sativus</i> L.	L: chlorotic lesions; S: vein-clearing, green mosaic, leaf deformation, stunting Symptoms as in <i>C. sativus</i>
<i>Cucurbita pepo</i> L. var. <i>patissonina</i> Greb. f. <i>radiata</i> Nois.	L: chlorotic lesions; S: vein-yellowing (sometimes), chlorotic spots and rings, mottling, leaf deformation
<i>Datura stramonium</i> L.	L: chlorotic lesions; S: O
<i>Gomphrena globosa</i> L.	L: sporadic chlorotic lesions; S: mosaic (sometimes blistered), necrotic patches, leaf deformation, stunting
<i>Nicotiana glutinosa</i> L.	L: sporadic chlorotic lesions and faint zonal necrotic rings; S: green mosaic (sometimes blistered), faint necrotic line pattern, leaf deformation, stunting
<i>N. tabacum</i> L. cv. "Samsun", "Xanthi"-nc	L: O; S: mosaic, leaf deformation, stunting
<i>Petunia hybrida</i> Vilm.	L: O; S: green mosaic, moderate stunting
<i>Solanum nigrum</i> L.	L: chlorotic lesions; S: O
<i>Tetragonia echinata</i> Ait.	L: chlorotic lesions; S: O
<i>T. tetragonoides</i> (Pall.) O. Ktze.	

Table 3. Physical properties of cucumber mosaic virus isolates Ac, Ad, Ae and Am from *Aristolochia* spp. TIP: thermal inactivation point, Liv: longevity in vitro ( $20^{\circ}\text{C}$ ), DEP: dilution end point.

Tablica 3. Fizička svojstva izolata Ac, Ad, Ae i Am virusa mozaika krastavca iz vrsta *Aristolochia*. TIP: temperatura inaktivacije virusa, Liv: postojanost in vitro ( $20^{\circ}\text{C}$ ), DEP: krajnja točka razrjeđenja.

Physical property	Isolate			
	Ac	Ad	Ae	Am
TIP °C	64	60—62	60—62	60—62
Liv (in days)	9	6	6	6
DEP	$10^{-4}$	$2 \times 10^{-4}$	$2 \times 10^{-4}$	$2 \times 10^{-4}$

**Fig. 1.** Symptoms of isolates Aq (A) and Am (B-D) of cucumber mosaic virus. A: *Aquilegia vulgaris* spontaneously infected with isolate Aq; bright variegation and mosaic are present on leaves. B: *Datura stramonium*, leaf systemically infected with isolate Am; moderate chlorosis and mosaic are visible. C: Leaf of spontaneously infected *A. macrophylla* displaying mosaic. D: *Nicotiana glutinosa*, systemically infected with isolate Am from *A. macrophylla*; mosaic, leaf deformations and necrotic

Sl. 1. Simptomi izolata Aq (A) i AM (B-D) virusa mozaika krastavca. A: *Aquilegia vulgaris* spontano inficirana izolatom Aq; nazočni su svijetlo šarenilo i mozaik na listovima. B: *Datura stramonium*, list sistemično inficiran izolatom Am; vide se umjerena klorozna i mozaik. C: List spontano inficirane *A. macrophylla*; na listu se opaža mozaik. D: *Nicotiana glutinosa*, sistemično inficirana izolatom Am iz vrste *A. macrophylla*; prevladavaju mozaik, nekrotična žarišta i deformacije listova.

**Fig. 2.** Symptoms of the TK isolate on a spontaneously infected *Scopolia carniolica* plant. Mosaic variegation and leaf deformations are present; The tip of the left leaf is pointed.

Sl. 2. Simptomi izolata TK na prirodno inficiranoj biljci *Scopolia carniolica*. Vide se mozaik, šarenilo i deformacije lista; vršak lijevog lista je ušiljen.

**Fig. 3.** Symptoms of isolate Sc from *S. carniolica* (A, B). A: Artificial systemic infection on leaf of *Cucumis sativus* showing strong mosaic. B: *N. glutinosa* with systemic symptoms, i. e. mosaic, necrotic patches, chlorosis and leaf malformations.

Sl. 3. Simptomi izolata Sc iz *S. carniolica* (A, B). A: Umjetna infekcija na listu krastavaca (*Cucumis sativus*); uočljiv je jaki mozaik. B: *N. glutinosa* sa simptomima sistemične infekcije: mozaik, nekrotična žarišta, klorozna i deformacije lista.

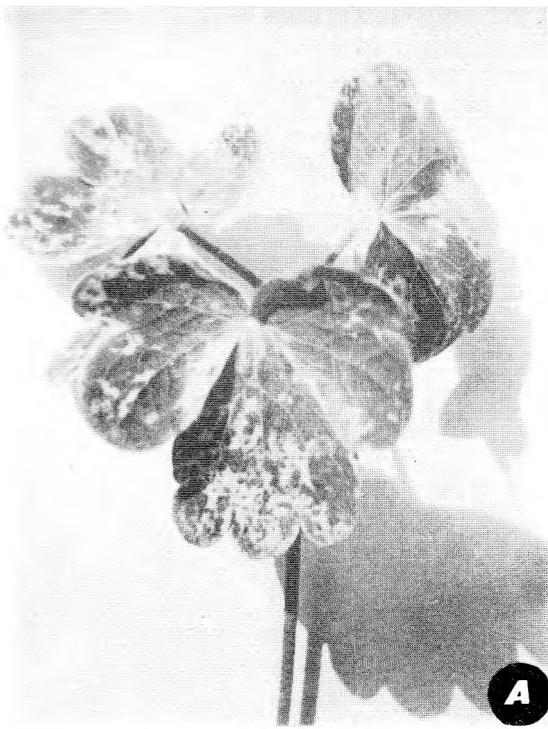
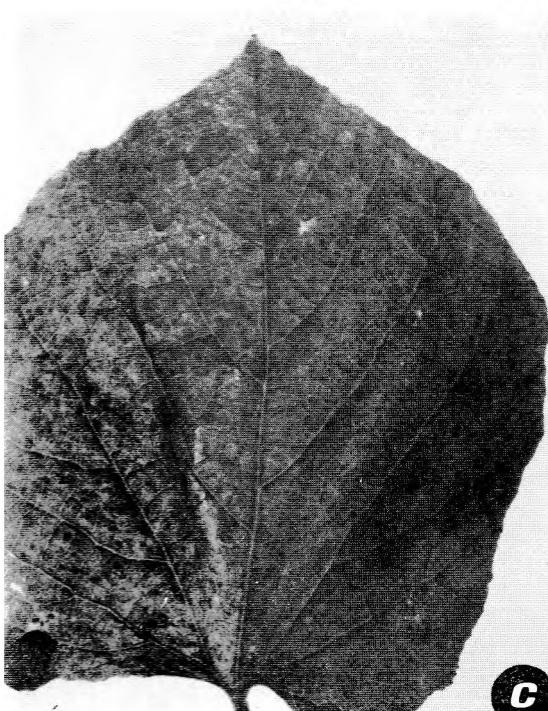
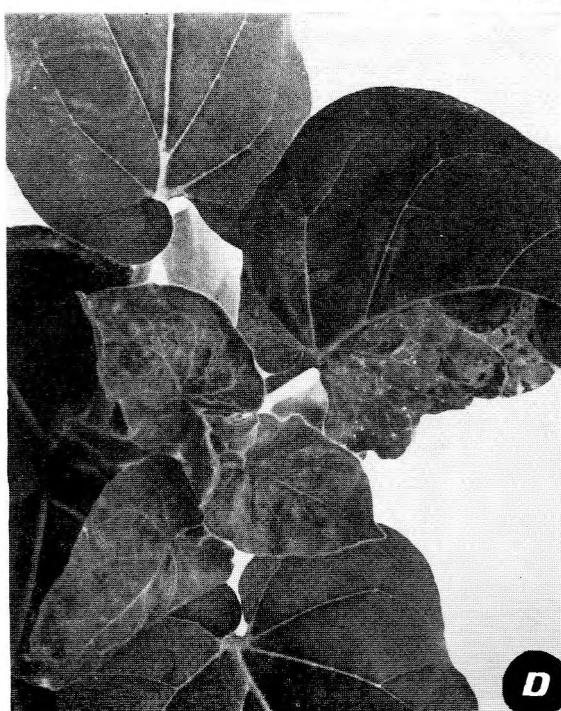
**A****B****C****D**

Fig. 1. — Sl. 1.

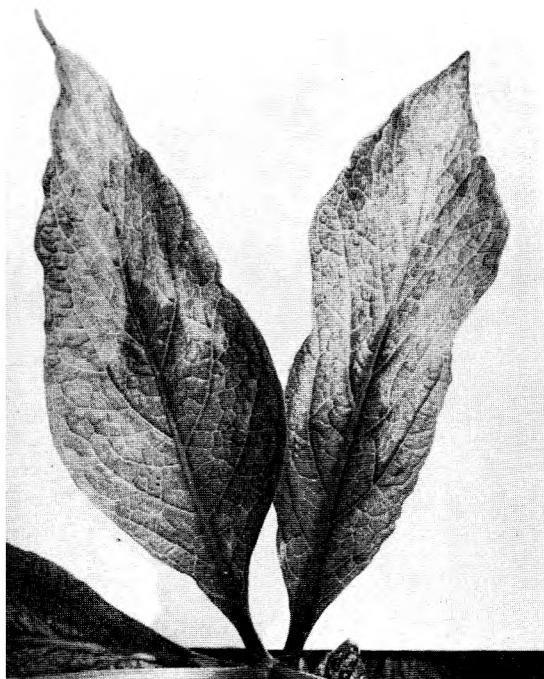


Fig. 2. — Sl. 2.

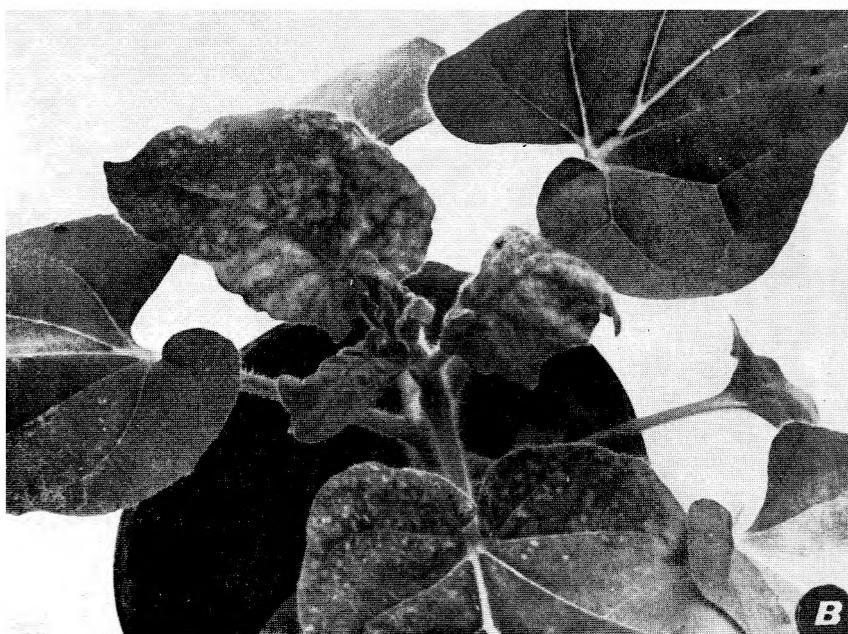
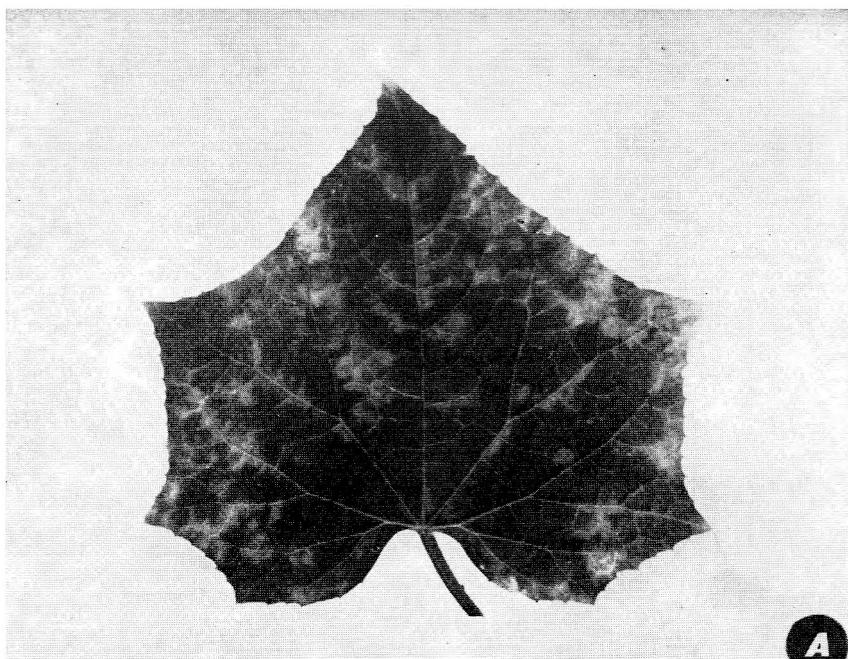


Fig. 3. — Sl. 3.

## Discussion

As known, CMV has a world-wide distribution, and under natural conditions attacks numerous weed and cultivated plants including herbaceous and woody species (cf. Gibbs and Harrison 1970, Smith 1972, Klinkowski 1968). Spontaneous infections of plants with CMV in Hungary and Jugoslavia have attracted attention of several workers. In Hungary CMV has been isolated from several plants, among others *Brassica napus* L., *Cucurbita pepo* L. var. *patissonina* Greb. f. *radiata* Nois., *Echinocystis labata* (Michx.) Torr. et Gray., *Lycopersicon esculentum* Mill., *Paulownia imperialis* Sieb. et Zucc. (Horváth 1969, 1973, Horváth and Hinfner 1969, Horváth and Szirmai 1973, Horváth and Beczner 1973, Horváth et al. 1974). In Jugoslavia the virus was found spontaneously infecting a certain number of species, e. g.: *Lycopersicon esculentum* (Panjan 1957, Juretić 1968), *Capsicum annuum* L. (Delević 1963), *Medicago sativa* L. (Babović 1968), *Maclura pomifera* (Raf.) Robinson (Pleše and Miličić 1973), *Forsythia suspensa* Vahl., *Lycium halimifolium* Mill. (Pleše and Miličić 1974), *Trifolium pratense* L., *Cucumis sativus* L. (Proc. IV Congr. Biolog. of Jugoslavia, Sarajevo 1974) and *Echinocystis lobata* (Mamula 1969, unpublished data).

This paper brings out several natural hosts of CMV unknown in the two countries so far. Results presented indicate that the *Aristolochia* spp. are an important reservoir of CMV in nature. Beside the already known *A. clematitis* and *A. durior*, two more *Aristolochia* spp., i. e. *A. elegans* and *A. macrophylla* were found to act as a source of this virus. Because of their comparatively wide distribution *Scopolia* species could also be quite important hosts with respect to spread of CMV. Concerning the question as to which of the known strains of CMV, "green", "yellow" or "white" (comp. Schmelzer 1962/63, Juretić 1968) the investigated isolates belong, the present paper cannot give a precise answer as appropriate experiments were not employed in the research. However, isolates Am, Aq and Sc could probably be assigned to the "green" strain, because such a strain was drawn from several species (*Aristolochia clematitis* L., *Hesperis dinarica* Beck, *Nicandra physaloides* Gaertn., *Sonchus oleraceus* (L.) Gou.) which grew in the same place where hosts of Am, Aq and Sc isolates were collected (Juretić 1968, 1974). Similarly, the isolates Ac, Ad and Ae displayed symptoms of the "green" type in systematically infected hosts and it was shown in plant-protection tests that isolates Ac, Ad, Ae and Am did not belong to the "white" strain of CMV.

## Summary

In the course of the present investigations virus isolates from spontaneously infected *Aristolochia clematitis* (Ac), *Aristolochia durior* (Ad), *Aristolochia elegans* (Ae) originating from Hungary, as well as an isolate from *Aristolochia macrophylla* (Am) found in Jugoslavia were identified as cucumber mosaic virus (CMV). In addition, two further isolates found in *Aquilegia vulgaris* (Aq) and *Scopolia carniolica* (Sc) in Jugoslavia also proved to belong to CMV. Identification was based on experiments with test plants, examination of serological and physical properties, plant-protection tests and experiments on transmission of the isolates by means of aphid *Myzus persicae* (stylet-borne manner). It is likely that isolates Am, Aq, Sc, and also Ac, Ad and Ae belong to the "green" strain of CMV.

Species *A. elegans*, *A. macrophylla* and *S. carniolica* are reported for the first time, at least for the European area, as being spontaneously

infected with CMV in nature. None of the six plant species under the investigation were known till now as natural hosts to CMV in the country where the specimens were collected.

### Z u s a m m e n f a s s u n g

Während der Untersuchungen der Virusisolierungen aus spontan infizierten *Aristolochia clematitis* (Ac), *A. durior* (Ad) und *A. elegans* (Ae) (aus Ungarn), sowie einer Isolierung aus *A. macrophylla* (Am) (aus Jugoslawien) wurden alle diese Isolierungen als Gurkenmosaik-Virus identifiziert. Dazu wurden weitere zwei Isolierungen aus *Aquilegia vulgaris* (Aq) bzw. *Scopolia carniolica* (Sc), beide Jugoslawischer Herkunft, auch als Isolate des CMV nachgewiesen. Die Identifizierung der Virusisolierungen wurde bekräftigt durch einige Experimente mit Testpflanzen, durch Untersuchungen der serologischen und physikalischen Eigenschaften der Isolate, sowie durch Prämunitätsteste und auch Übertragungsteste mit *Myzus persicae*. In den letztgenannten Testen wurden die Isolate auf die nichtpersistente Weise übertragen. Die Isolate Am, Aq, Sc und auch die Isolate Ac, Ad und Ae gehören wahrscheinlich zu dem "grünen" Stamm des CMV.

Unseres Wissens ist diese Arbeit die erste, die auf *A. elegans*, *A. macrophylla* und *S. carniolica* als Wirte des CMV in der Natur hingewiesen hat. Keine der untersuchten Pflanzenarten war bisher bekannt als natürlicher Wirt des CMV in dem Lande, in dem die Exemplare gesammelt wurden.

\*

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## SADRŽAJ

### NEKI PODACI O PRIRODnim DOMAĆINIMA VIRUSA MOZAIIKA KRASTAVCA U MAĐARSKOJ I JUGOSLAVIJI

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U ovom radu iznijeli smo rezultate istraživanja virusnih izolata koji su potjecali iz vrsta *Aristolochia clematitis* (Ac), *A. durior* (Ad), *A. elegans* (Ae), *A. macrophylla* (Am), *Aquilegia vulgaris* (Aq) i *Scopolia carnatica* (Sc). Osim vrste *A. clematitis* (primjerici ubrani u Mađarskoj), koja je rasla samoniklo, sve su ostale biljke bile uzgajane u botaničkim vrtovima u Mađarskoj (*A. durior*, *A. elegans*) i Jugoslaviji (*A. macrophylla*, *Aq. vulgaris*, *S. carnatica*). Na listovima inficiranih primjeraka svih spomenutih vrsta uočavali su se simptomi, kao što su: bljedo šare-

nilo, mozaik, morfološke deformacije; uz to su biljke zaostajale rastom. *S. carniolica* je na listovima imala često ispoljene klorotične vrpce uz veće žile i tzv. simptom "hrastovog lista".

Izolate smo istražili u cilju njihove identifikacije, koristeći pri tom metodu pokusnih biljaka, zatim serološke i premunitetne pokuse, određivanje fizičkih svojstava i pokuse prijenosa izolata pomoću lisnog ušenjka *Myzus persicae*. Na temelju rezultata tih istraživanja utvrdili smo da su sve istraživane vrste bile inficirane virusom mozaika krastavca. Dosta je vjerojatno da izolati Ac, Ad, Ae, Am, Aq i Sc pripadaju tzv. "zele-nom" soju tog virusa.

Čini se da ovaj rad donosi prve podatke o vrstama *A. elegans*, *A. macrophylla* i *S. carniolica* kao prirodnim domaćinima virusa mozaika krastavca. Pored toga, od šest istraženih vrsta nijedna dosad nije bila poznata kao prirodni domaćin virusa mozaika krastavca u zemlji u kojoj su inficirani primjerici biljaka bili sabrani.

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