OCCURRENCE OF COLOMBIAN DATURA VIRUS IN *BRUGMANSIA* HYBRIDS, *PHYSALIS PERUVIANA* L. AND *SOLANUM MURICATUM* AIT. IN HUNGARY

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Summary. – Colombian datura virus (CDV) has been found to infect angel trumpets (*Brugmansia* spp.) frequently and cape gooseberry (*Physalis peruviana*) and pepino (*Solanum muricatum*) sporadically in Hungary. A CDV BRG/H isolate was characterized. It had flexuous thread-like virions of about 750 x 12 nm in size. Host range and symptomathological studies revealed its great similarity to authentic CDV isolates. *Nicotiana tabacum* cultivars and lines resistant to Potato virus Y (PVY^N) either genically or transgenically proved highly susceptible to the BRG/H isolate. Tomato (*L. esculentum* cvs.) was systemically susceptible to this isolate, but some lines of *Lycopersicon hirsutum* and *L. peruvianum* turned out to be resistant. *Browallia demissa, Ipomoea purpurea, N. megalosiphon* and *S. scabrum* were demonstrated as new experimental hosts of CDV. The BRG/H isolate proved to be transmissible by the aphid *Myzus persicae* Sulz. in a non-persistent manner. Potyvirus-specific coat protein (CP) gene sequences of about 1700 bp from angel trumpet, cape gooseberry and pepino plants were amplified by RT-PCR. The cloned BRG/H CP gene showed a 99.12–99.31% identity with other CDV isolates. CDV has been found for the first time to infect naturally cape gooseberry and pepino. Since the botanical genus name of original hosts of CDV has changed from *Datura* to *Brugmansia*, we propose to change the virus name from CDV to Angel trumpet mosaic virus (ATMV).

Key words: Angel trumpet mosaic virus; coat protein; Colombian datura virus; nucleotide sequence; natural hosts; potyvirus

Introduction

Recently, a number of exotic ornamental and vegetable plants belonging to the family *Solanaceae* became known and popular in many European countries. Among them the angel trumpet (*Brugmansia* species and hybrids) is widely used as a perennial outdoor ornamental plant, while cape gooseberry (*Physalis peruviana* L.) and pepino (*Solanum muricatum* Ait.) are produced for their edible fruits. The above plants of South-American origin are known to be affected by several plant viruses in their homelands (Brunt *et al.*, 1996; Edwardson and Christie, 1997). Some of these viruses have already been introduced into Europe with propagating materials (seeds or cuttings) causing new and great risks to important crops, i.e. tomato, tobacco, pepper etc. Pepino mosaic virus (PepMV, the *Pepino mosaic virus* species, the *Potexvirus* genus), discovered some 25 years ago in Peru (Jones *et al.,* 1980) has been noticed occurring in tomato in many European countries (for review see Anonymous 2004). Furthermore, PepMV has been

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Abbreviations: ATMV = Angel trumpet mosaic virus; BRG = *Brugmansia*; CDV = Colombian datura virus; CMV = Cucumber mosaic virus; CP = coat protein; Nib = nuclear inclusion protein b; PepMV = Pepino mosaic virus; PHYS = *Physalis*; PVY^N = Potato virus Y necrotic strain; PVY^{NTN} = Potato virus Y tuber necrotic strain; 3'-UTR = 3'-untranslated region

naturalized in Spain, affecting the weeds *Amaranthus sp., Malva parviflora, N. glauca, S. nigrum* and *Sonchus sp.* (Jorda *et al.*, 2001).

Like PepMV, CDV (the *Colombian datura virus* species, the *Potyvirus* genus, the *Potyviridae* family), first described by Kahn and Bartels (1968) on angel trumpets (*Datura* spp. classified now to the genus *Brugmansia*), has also been introduced to Europe. CDV was found in Germany and the Netherlands infecting not only angel trumpets but also petunias and an exotic ornamental *Juanulloa aurantiaca* (Lesemann *et al.*, 1996; Verhoeven *et al.*, 1996; Feldhoff *et al.*, 1998). It has also caused epidemics in greenhouse tomato crop in the Netherlands, where an affected *Brugmansia* plant had been kept for wintering (Verhoeven *et al.*, 1996). It is worth noting that besides CDV two other non-identified species of potyviruses have been detected in angel trumpets in Germany (Lesemann *et al.*, 1996). Recently, CDV was reported to infect the terrestrial orchid *Spiranthes cernua* (Fry *et al.*, 2004)

As *Brugmansia* hybrids spread rapidly in home gardens all over Hungary and experiments are carried out to grow pepino and cape gooseberry, we decided to start investigations to discover the pathogens of these little known plants with special regard to viruses. The present paper gives a short account of the occurrence and symptomathology of viral diseases found in the above plants and deals with the identification of CDV as the pathogen associated with the symptoms.

Materials and Methods

Plants. Cuttings of mosaic-affected angel trumpets of yellow, pink or white flowers were collected from four distant places. They were rooted in sterile soil mixture and grown in an insect-controlled greenhouse. Leaves of cape gooseberry plants showing severe mosaic were collected from experimental fields at north-eastern and western Hungary. The infected pepino was collected from a forced culture in Gödöllö.

Virus isolation. The leaves of five mosaic-affected angel trumpets, two specimens of cape gooseberry and pepino were homogenized in 0.15 mol/l phosphate buffer pH 7.00 (1: 5 v/w), respectively. Test plants (*Capsicum annuum, Chenopodium amaranticolor, Ch. murale, Ch. quinoa, Cucumis sativus, Datura stramonium, Nicotiana* spp., and *Phaseolus vulgaris)* dusted with cellite were rubbed with different inocula. In order to assess the presence of pathologically distinct viruses or virus strains local and systemic symptoms were evaluated and cross inoculations to different test plants were made.

After preliminary studies an isolate from *Brugmansia* plants designated BRG/H was selected for detailed characterization. The BRG/H isolate was propagated from a single local lesion in *Ch. amaranticolor*. An isolate from pepino, tentatively designated CDV-SMU/H was found to contain besides CDV also Cucumber mosaic virus (CMV).

Host range studies. Three to five plants of 27 species were inoculated mechanically with the isolates BRG/H and PHYS/H, respectively. The symptoms were evaluated for 3–4 weeks and re-isolations in *Ch. amaranticolor* and *N. tabacum* cv. Xanthi-nc were made from inoculated as well as top leaves. Different plant species were inoculated with the CDV-SMU/H isolate ad re-isolations were also made with the aim to separate CDV from CMV present in this isolate.

Electron microscopy. For the visualization of the virus particles 15–20 µl of leaf extracts of the original leaf extracts of original angel trumpet, cape goosberry and pepino plants were dropped onto carbon-coated grids, washed with distilled water and stained with 1% uranyl acetate. A Zeiss EM910 electron microscope was used.

Aphid transmission was carried out using the green peach aphid (*Myzus persicae* Sulz.) reared on healthy pepper (*Capsicum annuum* L.). Adults were starved in Petri dishes and then placed onto tobacco leaves infected with the BRG/H isolate. After 5 mins (a virus acquisition period) 5 aphids were transferred to 5 young healthy *N. tabacum* cv. Xanthi-nc leaves each for 10 mins The insects were then killed by spraying the acceptor plants with Lannate.

RT-PCR. Total RNA was prepared from plants according to White and Kaper (1989). To prepare cDNA by the RT step a polyT-primer (5'-CGGGATCCGTCGACAAGCTTTTTTTTTTTTT TTTT-3', the *Bam*HI site underlined) was used (Deborré *et al*., 1995). In PCR (Deborré *et al.,* 1995), potyvirus-specific sequences were amplified using the polyT-primer mentioned above and poty-7941 primer (5'-GGAATTCCCGCGGAAAAGCCCCGTA CATTGC-3', the *Eco*RI site underlined) with some modifications (Chen *et al.,* 2001). PCR products were cleaved with *Bam*HI and *Eco*RI and separated by electrophoresis in 1% agarose gel. The fragments of interest (about 1700 bp long) were cloned into the Bluescript SK+ plasmid (Stratagene). A recombinant clone from the BRG/H containing the 3'-end of the NIb gene, CP gene and 3'-untranslated terminal region (3'-UTR) characteristic for potyviruses was sequenced and compared with the available sequence data.

Results

Natural occurrence of the diseases

Angel trumpet plants grown in home gardens were studied in different regions of Hungary. A great majority of them showed mild mottling (Fig. 1A), mosaic or severe vein banding (Fig. 1C), while chlorotic and etched necrotic rings on leaves were rare. Besides home gardens, lots of heavily infected potted plants kept for sale in greenhouses of several companies were also observed.

In the summer 2000, some cape gooseberry plants grown in experimental fields at Velence (middle western Hungary) and Berkesz (north-eastern Hungary) were found to show severe viral symptoms: strong mosaic, leaf drop, stem necrosis and, finally the death of infected plants (Fig. 1B).

Pepino plants were inspected at several places, but viral diseases affecting this species have not been observed until

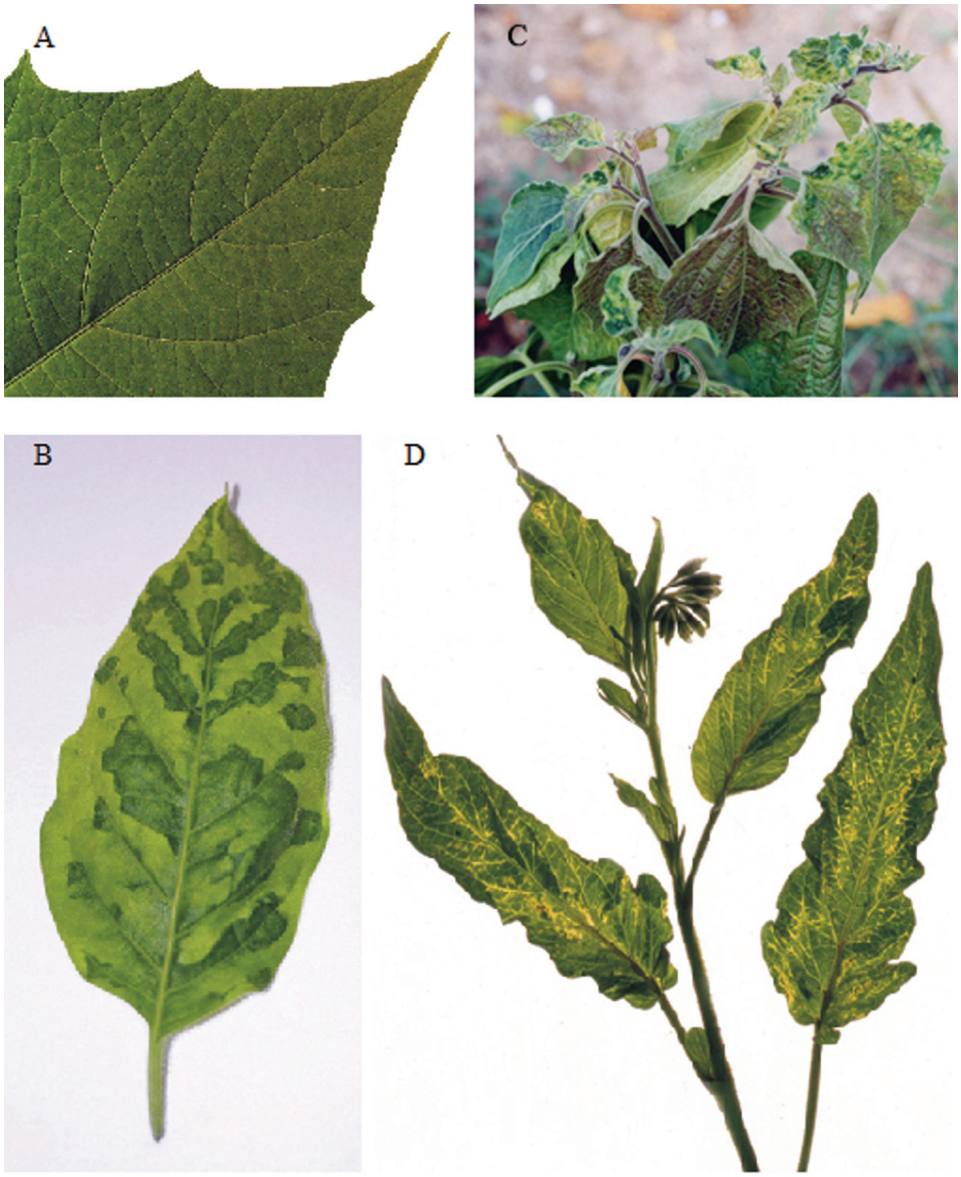

Fig. 1

Symptoms on leaves of natural hosts of CDV

Chlorotic mottle (A) and severe vein banding (B) to angel trumpet and severe leaf drop on cape gooseberry (C) plants naturally infected with CDV. Mosaic and line pattern on pepino infected with CDV and CMV (D).

plants grown in a polythene tunnel at the field of St. István University, Gödöllö were examined in 2001. Here, some plants showed mild mosaic as well as necrotic line pattern. The symptoms were conspicuous under transparent light (Fig. 1 D).

Isolation and differentiation of viruses

Test plants inoculated with leaf extracts from infected angel trumpet, cape gooseberry and pepino plants reacted with local and/or systemic symptoms characteristic of virus infections. In preliminary tests, remarkable differences between the *Brugmansia* (BRG) and *Physalis* (PHYS) isolates could not be established. These isolates caused vein clearing, mild veinal necrosis, interveinal etched necrotic spots and mosaic on *N. tabacum* cv. Xanthi-nc, total necrosis on *Datura stramonium*, *N. benthamiana* and *N. clevelandii* and local chlorotic-necrotic lesions on *Chenopodium* spp. They did not induce symptoms on *Capsicum anuum,*

Table 1. Host range and symptomathology of the-BRG/H isolate

Plant families and species	Symptoms	
	L	S
AMARANTHACEAE		
Gomphrena globosa	Cnll	Sl(-)
CHENOPODIACEAE		
Chenopodium amaranticolor	Cnll	Sl(-)
Ch. murale	Cnll	Sl(-)
Ch. quinoa	Cnll	Sl(-)
CONVULVULACEAE		
Ipomoea purpurea	Nll	Sl (-)
CUCURBITACEAE		
Cucumis sativus cv. Budai csemege	Sl (-)	Sl (-)
LEGUMINOSAE		
Phaseolus vulgaris cv. Red Kidney	Sl (-)	Sl (-)
Vigna sinensis cv. Black Eye	Sl (-)	Sl (-)
SOLANACEAE		
Browallia demissa	Cnsp	Mo, D
Capsicum annuum cv. Albaregia	Sl (-)	Sl (-)
C. frutescens cv. Tabasco	Sl (-)	Sl (-)
C. baccatum var. *pendulum*	Sl (-)	Sl (-)
Datura stramonium	Csp	Mo, W[c]
Lycopersicon hirsutum P.I. 147087	Sl (-)[d]	Sl (-)[d]
L. esculentum cv. Jubileum	Sl	Mo, Ln, D
L. peruvianum P.I. 128650	Sl (-)[d]	Sl (-)[d]
Nicotiana benthamiana	Csp	Smo, Dth
N. clevelandii	Csp	Smo, Dth
N. glutinosa	Csp	Mo, Vb, D
N. megalosiphon	Csp	Smo, Dth
N. tabacum cvs. Xanthi-nc, B21, Pallagi 3[a]	Cnsp	Vn, Nr, Stu
N. tabacum lines „G2"[b] and „G3"[b]	Cnsp	Vn, Nr, Stu
Petunia hybrida	Csp	Mo, Stu
Physalis peruviana	Csp	Mo, Stn, W
Solanum scabrum	Csp	Mo, D
S. nigrum	Csp	Mo, D
S. glaucophyllum	Sl (-)	Sl (-)
S. tuberosum cv. Kisvárdai rózsa	Sl (-)	Sl (-)[e]

[a]Resistant genically to PVY^N; [b]transgenic tobacco lines resistant to PVY^N; [c]the presence of CDV was detected also by RT-PCR; [d]CDV could not be detected by RT-PCR; [e]inoculation with the extract from pepino resulted in mosaic and CMV was re-isolated from top leaves. L = local symptoms; S = systemic symptoms; Cnll = chlorotic-necrotic local lesions; Cnsp = chlorotic-necrotic spots; Csp = chlorotic spots; D = deformations; Dth = death of plants; Ln = leaf narrowing; Mo = mosaic; Nr = necrotic rings; Sl = symptomless; Stn = stem necrosis; Stu = stunting; Vb = veinbanding; Vn = veinal necrosis; W = wilt; (-) = re-isolation to *Chenopodium amaranticolor* was negative.

Cucumis sativum and *Phaseolus vulgaris* test plants. A mixed infection was not indicated by cross inoculation tests.

Inoculation of *N. tabacum* cv. Xanthi-nc, *N. benthamiana, N. clevelandii* and *D. stramonium* plants with the pepino leaf extract resulted in symptoms very similar to those caused by the isolates BRG/H and PHYS/H, respectively, but systemic mosaic and leaf narrowing characteristic for the infection with CMV were induced in *C. anuum* and *C. sativus.* These results suggested that the pepino plant was infected by at least two pathologically distinct viruses.

Host range and symptomathology of virus isolates

Out of 27 plant species belonging to 6 families, five were infected locally with the BRG/H isolate (Table 1.). Usually, chlorotic-necrotic lesions appeared on these plants including *Gomphrena globosa* and *Chenopodium* spp., but violet-black necrotic lesions were observed on *Ipomoea purpurea*. Ten species of the families *Cucurbitaceae*, *Leguminosae* and *Solanaceae* could not be infected, while 12 species belonging to the family *Solanaceae* were susceptible both locally and systemically. The BRG/H isolate induced systemic necrosis and death of several tobacco species (*Nicotiana benthamiana, N. clevelandii* and *N. megalosiphon*) and cape goosberry. The tomato (*L. esculentum*) cultivar Jubileum (and other tomato hybrids and lines not listed in Table 1.) developed systemic mosaic and leaf deformation similar to CDV infection (Verhoeven *et al.*, 1996). In spite of repeated inoculations lines of *Lycopersicon hirsutum, L. peruvianum,* and *Capsicum* species, *S. glaucophyllum* and *S. tuberosum* could not be infected. On the basis of host range the isolates BRG/H and PHYS/H could not be distinguished from each other, but PHYS/H slightly differed from BRG/H in causing more severe symptoms in *N. tabacum* cultivars and lines.

Plants of the potato (*S. tuberosum* cv. Kisvárdai rózsa) inoculated with the extract from diseased pepino became infected systemically, showing mild mosaic and leaf narrowing. The virus transmitted from the top leaves was pathologically identical to the B pathotype of CMV (Marrou *et al.,* 1975; Salamon, 1989) and hybridized strongly with CMV-specific DNA in Northern blot analysis (data not shown).

Electron microscopy

Flexuous threads-like virions of about. 700–750 x 12 nm in size were observed in preparations from angel trumpets, cape gooseberry and pepino plants and in test plants infected with the isolates BRG/H or PHYS/H (Fig. 2).

Transmission by aphids

The isolate BRG/H was successfully transmitted by *M. persicae* Sulz. from tobacco to tobacco in non-persistent manner. Four of the five acceptor tobacco plants became infected showing vein clearing followed by vein necrosis and interveinal etched pattern symptoms identical with those caused by the original BRG/H isolate.

Molecular characterization

Using RT-PCR with potyvirus-specific primers fragments of predicted size of about 1700 bp representing CP gene and 3'-UTR were amplified from the examined plants. The nucleotide sequence of CP gene of the BRG/H isolate (Acc. No. AJ437482) showed an identity of 99.12–99.31% with the corresponding sequences of five CDV isolates available in the GenBank database (Acc. Nos: AB179622, AJ237921, AJ237923, AJ237922, and AF030689).

Discussion

The *Brugmansia* hybrids *Physalis peruviana* and *S. muricatum* represent new cultivated plants the virus diseases of which have not been so far known to have occurred in Hungary. Symptomathological surveys have proved, that like other economically important crops of the family *Solanaceae* these plants are often affected by viral symptoms. The high frequency of infection of *Brugmansia* plants apparently arise from the propagation of infected mother plants, while infection of the annual cape gooseberry grown from healthy seedlings can be obviously traced back to primary infections mediated by aphids. As the pepinos are propagated by stem cuttings, virus infections in this species are of special importance.

On the basis of morphology and size of viral particles, the virus isolates BRG/H and PHYS/H should be classified as potyviruses (viruses of the *Potyvirus* genus).

Host range and symptomathological studies revealed that both isolates represent the same virus, which could be differentiated from other potyvirus species (*Potato virus Y, Potato virus A* and *Henbane mosaic virus*) known to infect *Solanaceous* plants in Hungary. However, apart from the reaction of some test plants they showed marked pathological similarities to CDV. Indeed, potyvirus-specific nucleotide sequences were demonstrated by RT-PCR either in original hosts or test plants. The identity of the BRG/H isolate with CDV was unambiguously documented by nearly100% nucleotide sequence identity of their CP genes.

The pathological investigations suggested that the Hungarian isolates of CDV differed from others (Kahn and Bartels, 1968; Verhoeven *et al.*, 1996) in causing systemic infection in *D. stramonium* and much more severe symptoms in some *Nicotiana* species. The susceptibility of *Browallia demissa, Ipomoea purpurea, N. megalosiphon* and *S. scabrum* to CDV has not been so far reported, while *S. glaucophyllum* turned out to be a resistant plant.

As tomato proved highly susceptible to CDV, the extreme resistance of some *L. hirsutum* and *L. peruvianum* lines is of special interest. These *Lycopersicon* lines showed extreme resistance also to the tuber necrotic ring spot strain of *Potato virus Y* (PVY^{NTN}, Salamon *et al*., 2003). The genetics of the resistance of *Lycopersicon* species to CDV is completely unknown. Further on, we could establish that the *N. tabacum* tobacco cultivars and lines resistant to PVY^{N} either genically or transgenically were equally susceptible to CDV and reacted with strong symptoms.

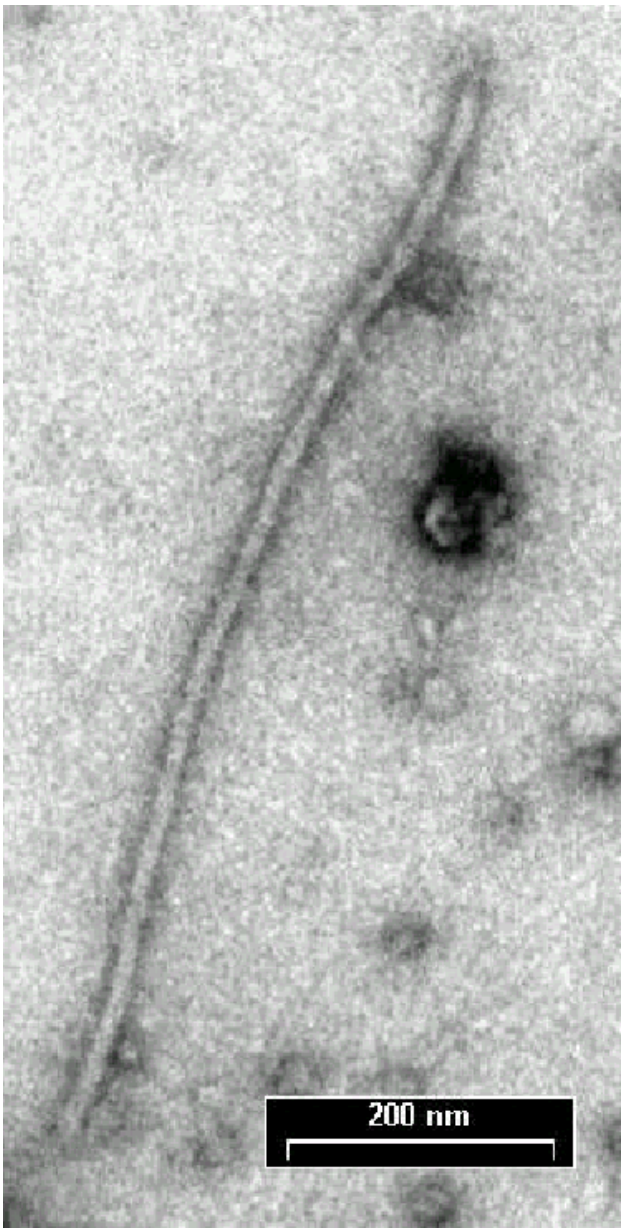

Fig. 2

Electron micrograph of a particle of the BRG/H isolate

Because of the country-wide distribution of the aphid-transmitted CDV in *Brugmansia* plants it was not a surprise to detect it in other susceptible plants, namely in cape gooseberry and pepino. To our knowledge these plants are new natural hosts of the virus.

CDV was first described as a new plant virus infecting *Datura* species some 35 years ago and re-discovered about 30 years later in the same and closely related host plants the genus name of which has changed in the meantime to *Brugmansia.* Considering that CDV has not yet been described naturally infecting any of *Datura* species it is a matter of speculation whether the name Colombian datura virus should be used in the future or it would be topical and appropriate to change the virus name as well. To our opinion the new name ATMV seems to be suitable and stable because the common English name of *Brugmansia* plants will presumably not be changed in the future.

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