

NEW EXPERIMENTAL HOSTS OF BELLADONNA MOTTLE VIRUS (BMV)*

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Introduction

In the course of several years past investigations have been carried out on belladonna mottle virus (BMV, Tollkirschenscheckungs-Virus) which made the characterization of the virus possible (cryptogram: R/1 : 2.0/37 : S/S : S/Cl). According to the chemical composition of virus particles, especially of RNA, it seems that BMV can be considered intermediate between the two subgroups of tymoviruses, i. e. turnip yellow mosaic virus (TYMV) and Andean potato latent virus (APLV) subgroups (cf. Jankulowa et al. 1968). Serologically the virus is more closely related to the members of APLV subgroup, notably to dulcamara mottle and eggplant mosaic (also APLV strain), than to other tymoviruses (Koenig and Givord 1974; cf. Paul et al. 1968, Bercks and Querfurth 1971, Paul 1971, Harrison et al. 1971).

BMV is a fairly widespread virus in Europe and apparently also in North America. So far it has been isolated only from solanaceous plants as natural hosts. Hereat it is interesting that all the known BMV isolates from Europe were recovered from *Atropa bella-donna* L. plants (Paul et al. 1968, Štefanac 1974, Horváth et al. 1976), while the American isolates from *Physalis heterophylla* L. (Moline and Fries 1972, 1974), *P. subglabrata* Mack. & Bush (Peters and Derks 1974) and *Capsicum frutescens* L. (Lee et al. 1975). It is worth-while to note that the European isolates (from Bulgaria, Hungary and Yugoslavia) are more closely serologically related to the German (type) BMV isolate of Paul et al. (1968), (Jankulowa et al. 1968, Štefanac 1974, Horváth et al. 1976) than the three mentioned American isolates.

* Dedicated to the memory of Dr. K. Schmelzer.

The experimental host range of BMV comprises mainly solanaceous plants. It seems that as yet only four species other than *Solanaceae* could be infected artificially with BMV, i. e. *Chenopodium quinoa* Willd. (Paul et al. 1968, Moline and Fries 1974), *C. foetidum* Schrad., *Sonchus oleraceus* L. (Peters and Derks 1974) and *Hibiscus manihot* L. (Horváth et al. 1976).

An attempt was made in the course of the present work to transmit a Yugoslav BMV isolate to several plants from the family *Schrophulariaceae*, to some additional *Solanaceae*, and to a few plants from 6 other families. The results are reported below.

Material and Methods

The virus isolate BMV-M used in the present investigations was the one recently found in Yugoslavia (Štefanac 1974). For transmission tests it was maintained in *Nicotiana glutinosa* L.

Transmission of the virus (mechanical inoculation) and serological reactions (gel double diffusion method) were performed in the usual manner. The medium for serological tests contained 0,9% Difco bacto agar and 0,02% NaN₃ in saline.

An antiserum was prepared against the isolate BMV-M for purpose of checking the presence of the virus in the plants examined. After the rabbit was given 5 intravenous injections within 12 days (total amount of partially purified virus c. 15 mg), the antiserum reached a titre of c. 1/256 fifteen days after the last injection. The virus was purified (Fig. 1G-I) from *N. glutinosa* and *N. megalosiphon* according to the procedure used by Paul et al. (1968).

Results

Host range of BMV-M isolate

Plants belonging to several families, predominantly to *Scrophulariaceae* and *Solanaceae*, were inoculated with the BMV-M isolate. Species which developed symptoms on their leaves are listed in Table 1. (The symbols for symptoms in the Table have the following meanings: C = chlorosis, D = leaf deformation, L = symptoms on inoculated leaves, M = mosaic, N = necrotic patches, Nl = necrotic lesions, S = symptoms of systemic infection, V = variegation, Vb = vein-banding, L*, S* = infection of inoculated and/or younger leaves was proved serologically; cf. Fig. 1F).

Lesions on inoculated leaves of *Digitalis* and *Pentstemon* spp. were often surrounded by a red-violet halo.

Symptoms on inoculated and younger leaves (systemic infection) of *Scrophulariaceae* appeared after a rather long period, in case of the latter mentioned leaves after about 30 to 60 days following the inoculation.

Necrotic spots of a weaker expression than lesions in *Scrophulariaceae* from Table 1 developed on inoculated leaves of the following plants from that family: *Digitalis ferruginea* L., *D. purpurea* L. and *Kickxia elatine* (L.) Dum. These lesion-like spots were at present not proved to be caused by BMV-M.

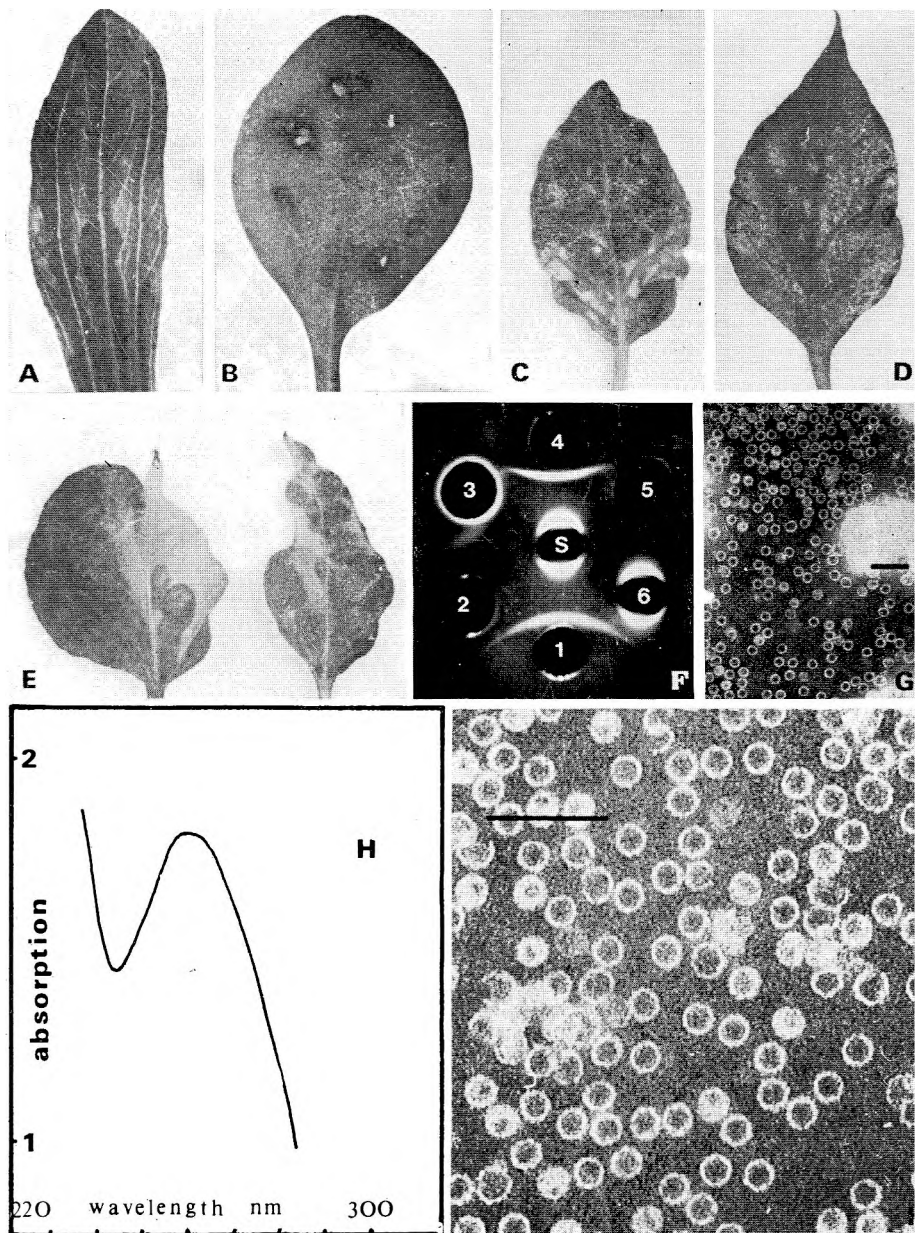


Fig. 1. — *Sl. 1*.

Fig. 1. Symptoms of isolate BMV-M in several hosts (A-E). A Vein-banding on systemically infected leaf of *Digitalis lutea*. B Necrotic lesions on inoculated leaf of *Pentstemon laevigatus* var. *digitalis* (enlarged c. 3 times). C Systemically infected leaf of *Nicotiana rustica* showing mosaic, necrosis, and slight blistering of blade. D and E Systemically infected leaves of hybrid *Nicotiana clevelandii* x *N. glutinosa*; chlorosis, necrosis, (D), variegation, mosaic, blistering, and reduction of blade (E) are present. F Serological reaction between leaf extracts of plants infected with BMV-M and of healthy plants (1-6), and antiserum to isolate BMV-M (s). Systemically infected leaves of *Digitalis laevigata* (1), leaves of healthy *D. lutea* (2), inoculated leaves of *Pentstemon angustifolius* (3), systemically infected leaves of *D. lutea* (4), systemically infected leaves of *P. angustifolius* (5), leaves of healthy *Nicotiana megalosiphon* (6). Leaf extract (sap) in 3 and 5 was diluted with distilled water. A very weak reaction was present in 5. H Ultraviolet light absorption of partially purified BMV-M. G and I Electron micrographs of partially purified BMV-M (negative staining with 4% phosphotungstate). Bar in G and I equals 100 nm.

Sl. 1. Simptomi izolata BMV-M na nekoliko domaćina (A-E). A Vrpčasto obojenje uz žile na sistematično inficiranom listu od *Digitalis lutea*. B *Pentstemon laevigatus* var. *digitalis*, nekrotične lezije na inokuliranom listu (list je povećan c. 3 puta). C Mozaik, nekroza i blaga mjehurasta ispupčenost plojke na sistematično inficiranom listu od *Nicotiana rustica*. D i E Sistematično inficirani listovi hibrida *Nicotiana clevelandii* x *N. glutinosa*; vide se kloroza, nekroza (D), šarenilo, mozaik, mjehurasta ispupčenost i redukcija plojke (E). F Serološka reakcija između ekstrakta listova od biljaka koje su inficirane izolatom BMV-M i od zdravih biljaka (1-6) i antiseruma od izolata BMV-M (s). Sistematično inficirani listovi od *Digitalis laevigata* (1), listovi zdrave biljke *D. lutea* (2), inokulirani listovi od *Pentstemon angustifolius* (3), sistematično inficirani listovi od *D. lutea* (4), sistematično inficirani listovi od *P. angustifolius* (5), listovi zdrave biljke *Nicotiana megalosiphon* (6). Ekstrakt (sok) listova u 3 i 5 razrijeđen je s destiliranom vodom. U 5 je neznatna reakcija. H. Apsorpcija ultravioletne svjetlosti pri prolazu kroz djelomično purificiranu suspenziju BMV-M. G i I Elektronskomikroskopske snimke djelomično purificiranog izolata BMV-M (negativno kontrastirano s 4% fosfovolframskom kiselinom). Linija u G i I predstavlja 100 nm.

Table 1. List of plants which reacted with symptoms after inoculation with isolate BMV-M

Tabela 1. Popis biljaka na kojima su se razvili simptomi infekcije nakon inokulacije izolatom BMV-M

Family	Species	Symptoms
SCROPHU- LARIACEAE	<i>Digitalis laevigata</i> W. & K.	L: NI; S ^x : M, N, D
	<i>D. lutea</i> L.	L: (NI); S ^x : M, Vb, C, D; Fig. 1A
	<i>Pentstemon angustifolius</i> Pursh	L ^x : NI; S ^x : N, D
	<i>P. laevigatus</i> Soland. var. <i>digitalis</i> Gray	L ^x : NI; S ^x : V; Fig. 1B
SOLANACEAE	<i>Nicotiana clevelandii</i> Gray × <i>N. glutinosa</i> L.	L: (NI); S: M, V, Vb, N, D; Fig. 1D, E
	<i>Physalis franchetii</i> Mast.	L: (NI); S ^x : M, Vb, D
	<i>Solanum luteum</i> Mill.	L: (NI); S ^x : M, N, D

Some more species were inoculated with BMV-M but they displayed no symptoms of infection. Those species apparently not infected were: *Cleome spinosa* Jacq. (*Capparidaceae*), *Chenopodium amaranticolor* Coste & Reyn., *C. murale* L. (*Chenopodiaceae*), *Tithonia speciosa* Hook. (*Compositae*), *Brassica chinensis* L., *B. rapa* L. var. *rapa* (*Cruciferae*), *Ricinus communis* L. (*Euphorbiaceae*), *Plantago media* L. (*Plantaginaceae*), *Veronica incana* L. (*Scrophulariaceae*), *Physalis alkekengi* L., *Solanum dulcamara* L. and *S. tuberosum* L. (*Solanaceae*).

Discussion

The evidence is pointed out in this paper of susceptibility of several species from families *Scrophulariaceae* and *Solanaceae* to infection with BMV under experimental conditions. Among the plants which have been found to be susceptible to infection with BMV-M members of *Scrophulariaceae* attract an attention, for species from that family were, apparently, not previously known as hosts to BMV (cf. Introduction). In the experiments presented BMV-M failed to infect certain solanaceous plants, although it appears that species from that family are the most susceptible plants to BMV. However, Paul et al. (1968), Moline and Fries (1974) and Peters and Derks (1974) also found several *Solanaceae* — among them *Solanum tuberosum* is of special interest — which were insusceptible to infection with BMV isolates.

In the course of the experiments the environmental conditions for plant growth in glasshouse were not suitable enough (winter period). It is possible that the rather long incubation period with *Scrophulariaceae* and a comparatively weak symptom expression on these plants were due

to that circumstance. Therefore, trials will be repeated to infect with BMV-M *Digitalis ferruginea*, *D. purpurea*, *Kickxia elatine* and some more plants. *D. purpurea* could not be infected with BMV in experiments of Paul et al. (1968), too.

Trials were made by several authors to transmit BMV isolates to plants from more than 30 families but only a few species outside *Solanaceae* could be infected as stated in the Introduction. According to the results reported here it seems possible that next to *Solanaceae*, *Scrophulariaceae* are the most susceptible plants to BMV.

Summary

An account is given of the results of transmission experiments with belladonna mottle virus (BMV) to plants from families *Scrophulariaceae* and *Solanaceae* and to a few plants from six other families. It was shown that the Yugoslav isolate BMV-M caused both symptoms on inoculated leaves and systemic infection in *Digitalis laevigata*, *D. lutea*, *Pentstemon angustifolius*, *P. laevigatus* var. *digitalis* (*Scrophulariaceae*), hybrid *N. clevelandii* x *N. glutinosa*, *Physalis franchetii* and *Solanum luteum* (*Solanaceae*). *Pentstemon* spp. displayed more expressed lesions than the other plants examined.

Consequently it seems that the paper brings out the first data on species belonging to *Scrophulariaceae* as experimental hosts to BMV. The same can, apparently, be stated for the three solanaceous plants mentioned.

In the course of the present work an antiserum to BMV-M with a titre of 1/256 was prepared.

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S A D R Ź A J

NEKI NOVI EKSPERIMENTALNI DOMAĆINI VIRUSA IŠARANOSTI VELEBILJA (BMV)

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U radu su izneseni rezultati pokusa prenošenja jugoslavenskog izolata (BMV-M) virusa išaranosti velebilja na biljke iz 8 porodica. U nešto većoj mjeri bile su zastupljene vrste iz porodica *Scrophulariaceae* i *Solanaceae*.

Izolat BMV-M uzrokovao je pojavu sistemične infekcije na vrstama *Digitalis laevigata*, *D. lutea*, *Pentstemon angustifolius*, *P. laevigatus* var. *digitalis* (*Scrophulariaceae*), hibrid *Nicotiana clevelandii* x *N. glutinosa*, *Physalis franchetii* i *Solanum luteum* (*Solanaceae*). Na inokuliranim listovima tih vrsta nastajale su i nekrotične lokalne lezije.

Dvanaest vrsta iz šest drugih porodica, uključujući i *Solanaceae*, nisu se pokazale prijemljivim za infekciju s BMV-M.

Kako se čini, rad donosi prve podatke o uspješnom prijenosu virusa išaranosti velebilja na pripadnike porodice *Scrophulariaceae*. Spominju se i tri *Solanaceae* kao novi pokusni domaćini BMV.

U toku rada priređen je imuni serum od izolata BMV-M s titrom oko 1/256.

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