

PARTIAL CHARACTERISATION OF CUCUMBER MOSAIC VIRUS ISOLATE INFECTING *Lonicera caprifolium* L. PLANTS

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Abstract. Plants of honeysuckle (*Lonicera caprifolium* L.) from commercial nursery, showing stunted growth and severe leaf and flower malformation were found to be naturally infected with *Cucumber mosaic virus* (CMV). The virus was identified on the basis of its host range and *in vitro* and serological properties. It was mechanically transmitted onto thirteen herbaceous test plants and induced local or local and systemic symptoms. The isolated virus had a TIP of 65–70°C, a LIV of 4–5 h and DEP of 10^4 – 10^5 . It reacted positively in DAS-ELISA with CMV-ToRS (II) commercial antibodies but not with antibodies against CMV-DTL (I). Rabbit antiserum was produced, and it showed the titre at least 128 000 in F(ab')₂-ELISA with homologous isolate, as well as with isolate CMV-M belonging to serogroup DTL.

Key words: *Lonicera*, *Cucumber mosaic virus*, identification, serology

INTRODUCTION

Cucumber mosaic virus (CMV) occurs throughout the temperate climate regions of the world and it is pathogenic to extremely wide variety (more than 1000 species) of herbaceous and woody plants [Kaper and Watherworth 1981]. In Poland, CMV has been found in several crops including vegetable and ornamental plant species [Błaszczak and Fiedorow 1969, Kamińska 1976, 1984, 1995, 1996; Korbin and Kamińska 1998].

Lonicera spp. are ornamental woody plants very popular worldwide. Disease of *L. periclymenum* L., characterised by distorted leaves with ringspot symptoms, has been reported to be associated with *Cucumber mosaic virus* infection [Lihnell 1951]. However, the reaction of inoculated plants has not been described. Sweet, according to Cooper [1979], frequently isolated CMV from *L. japonica* Thunb. cv. aureo-reticulata plants and transmitted the disease agent by grafting. In a brief statement Brunt and Thomas

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[1975] reported detection of a virus which they called “*Lonicera latent virus*”, actually known as *Honeysuckle latent virus* [van der Meer et al. 1980]. The virus, previously recorded in Europe, was reported also from Canada on *L. periclymenum* [Chiko and Godkin 1986]. However, its pathogenity was not determined. In Japan, plants of *L. japonica* with yellow vein symptoms were frequently infected with *Tobacco leaf curl virus* [Kobatake et al. 1981], while in Tunisia, from *Lonicera* spp. with similar symptoms *Eggplant mottled dwarf virus* was isolated [Martelli and Cherif 1987].

Studies on the diversity of CMV isolates led to a definition of two major serogroups or subgroups. Using immunodiffusion and ELISA, Devergne et al. [1973] determined DTL and ToRS groups. Equivalent classification based on genomic nucleic acids sequence homology (groups WT and S, or I and II) was proposed by Piazzolla et al. [1979], and Owen and Palukaitis [1988].

During inspection of commercial nurseries in Poland, *Lonicera caprifolium* plants with stunted growth, leaf lesions and severe leaf and flower malformations were found. They contained a mechanically transmissible virus, identified as CMV isolate Wic. The properties of the isolated virus are presented in this paper.

MATERIALS AND METHODS

Virus source and mechanical transmission. Naturally infected plants of *L. caprifolium* found during inspection of commercial farms in central Poland in summer 2002 served as source plants in transmission experiments.

Virus isolation. Virus was transmitted mechanically using inocula prepared by grinding infected leaves in cold 0.02 M phosphate buffer (pH 7.6) containing 1% Na₂SO₃. Plants were maintained in darkness for 12 h before inoculation. The inoculation was performed on five-seven plants of each species in spring and autumn. Test plants were observed up to four weeks after inoculation with the exception of *L. caprifolium* seedlings which were observed for two months. When symptoms were absent, inoculated and non-inoculated leaves were tested by DAS-ELISA. All plants were grown in a greenhouse at 20-25°C with supplementary lighting.

In vitro properties. Thermal inactivation point (TIP), dilution end point (DEP) and longevity *in vitro* (LIV) determinations were performed using crude sap from systemically infected *Nicotiana rustica* L. Leaves were ground in phosphate buffer (1 g/1 ml). Seedlings of *Chenopodium quinoa* Willd. and *N. tabacum* L. ‘Samsun’ were used as indicator plants in these experiments.

Virus testing. Samples of leaves of naturally infected *L. caprifolium* and experimentally inoculated herbaceous test plants were tested for the presence of CMV using DAS-ELISA. Commercial kits containing immunoglobulines and alkaline phosphatase conjugates of CMV-I (DTL) and CMV-II (ToRS) were obtained from Agdia, (Elkhart, IN, USA). ELISA reagents prepared from CMV antiserum produced in our laboratory against isolate Cas, reacting with virus isolates from serogroup DTL as well as ToRS

[Korbin and Kamińska 1998], was used in addition. The tests were carried out according to the manufacturers' instructions.

Virus purification. Systemically infected leaves of *N. rustica* L., collected 15–18 days after inoculation, were used for virus purification according to a protocol published by Tobias et al. [1982], with minor modifications. Final purification of the virus was achieved by centrifugation in 5–30% sucrose density gradient (120 minutes at 25 000 rpm in SW28 rotor, Beckman). Virus particles were centrifuged (180 minutes at 45 000 rpm in Ti 50.2 rotor, Beckman), and resuspended in 1 ml of borate buffer. CMV concentration was estimated spectrophotometrically, using an extinction coefficient $E_{260}^{0.1\%, 1\text{ cm}} = 5$ [Francki et al. 1966].

Antiserum preparation. Antiserum was produced by immunization of rabbit with CMV preparations emulsified with Freund's adjuvant (Sigma-Aldrich), complete for the first injection, incomplete for the following ones. Rabbits were injected 7 times, at approximately two weeks intervals. Blood samples were collected at intervals of two weeks, starting 5 weeks after the first injection.

Immunoglobulines were isolated from antiserum using affinity adsorption on protein-A sepharose column. Fractions containing the highest concentrations of IgG were used for preparations of F(ab')₂ fragments [Barbara and Clark 1982]. Alkaline phosphatase (ALP) – IgG conjugate was produced using one-step procedure as described by Clark and Bar-Joseph [1984].

The antiserum, immunoglobulines, F(ab')₂ fragments and ALP-IgG conjugate were tested using F(ab')₂-ELISA or DAS-ELISA.

RESULTS

Symptoms. The naturally infected honeysuckle plants, grown in commercial nursery, were less attractive than healthy plants due to severe leaf malformation (fig. 1) and discoloration. The leaves were twisted with chlorotic spots and ringspots. The growth of symptomatic plants was affected and they produced flowers with malformed petals. The most severe symptoms were observed in spring-summer months on fully developed young leaves.

Isolation of virus. The virus was transmitted by mechanical inoculation from symptomatic leaves of *L. caprifolium* to *C. quinoa* and *N. tabacum* L. 'Samsun'. Isolate, named Wic, was obtained by single local lesion passages from *C. quinoa* to *C. quinoa*. Then it was propagated in *N. rustica* plants and from these it was transmitted to the other plants. We did not isolate any virus from symptomless honeysuckle plants.

Host range and symptoms. Fifteen plant species were used to demonstrate infectivity and to identify the virus, and the virus was detected in thirteen of them. Three plant species were infected only locally, the other ones showed local and systemic symptoms.



Fig. 1. Leaf malformation of *Lonicera caprifolium* plant naturally infected with *Cucumber mosaic virus*

Rys. 1. Deformacja liści *Lonicera caprifolium* naturalnie zaatakowanych przez *Cucumber mosaic virus*

Local and systemic symptoms were observed on:

– *N. benthamiana* Domin., *N. christii* (*N. glutinosa* L. × *N. clevelandii* Gray) – Local chlorotic or grayish diffuse lesions developed in 5 days. They were followed by systemic mosaic and retarded growth visible only for few days.

– *N. glutinosa* L. – Local diffused chlorotic lesions developed in 5–7 days. Systemic mosaic, chlorosis and necrotic lesions, leaf diminishing and malformation followed in 10–12 days. Infected plants were dwarfed.

– *N. tabacum* L. ‘Samsun’ – Local faint chlorotic lesions occurred in 5–7 days, followed by very gentle systemic mosaic in 10–12 days.

– *N. rustica* L. – Local faint spots developed in 5–7 days. They were followed by gentle systemic mosaic and yellowish rings (fig. 2a) and patterns in 10–12 days.

– *Cucumis sativus* L. ‘Kronos’ (fig. 2b), *Datura stramonium* L., *D. metel* L. – local diffuse chlorotic lesions developed in 5 days. Severe systemic infection with mosaic, leaf epinasty and retarded growth followed in 10–14 days

– *Nicandra physaloides* L. (fig. 2c), *Chenopodium urbicum* L. – Local and systemic chlorotic lesions developed in 6–8 days. Systemic mosaic, top leaf distortion followed in 10–12 days. Infected plants were severely dwarfed.

The following species were infected only locally:

– *Atriplex hortense* L, *Chenopodium amaranticolor* Coste et Reyn and *C. quinoa* Willd. – local chlorotic and/or necrotic lesions were produced in 4–5 days after inoculation.

The virus failed to infect the seedlings of *Lonicera caprifolium* and *Phaseolus vulgaris* L. ‘Saxa’.

In vitro properties. The virus in a sap of systemically infected leaves of *N. rustica*, had a TIP of 65–70°C, a LIV of 4–5 hours and a DEP of 10^4 – 10^5 , as checked using *C. quinoa* and *N. tabacum* ‘Samsun’ as indicator plants.

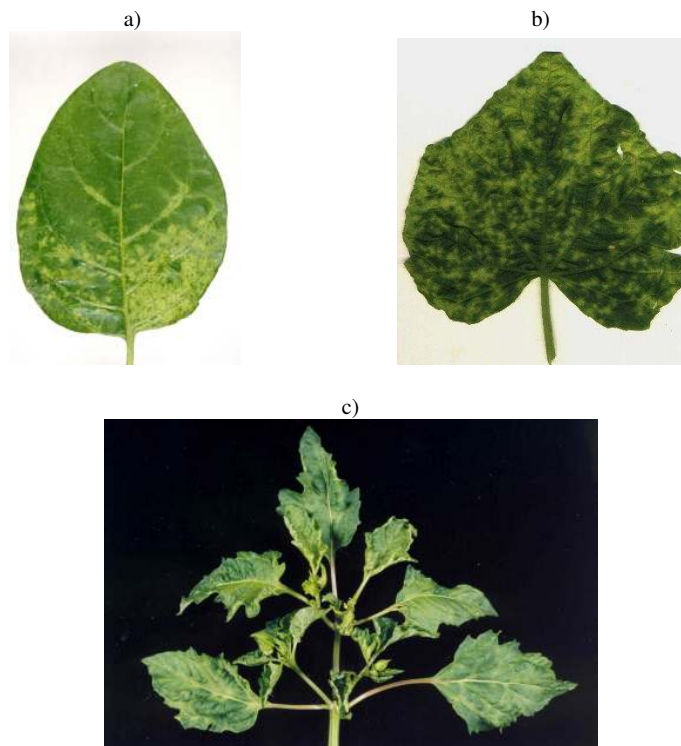


Fig. 2. Systemic symptoms induced by *Cucumber mosaic virus*; isolate Wic in leaves of experimentally inoculated plants: a) *Nicotiana rustica*, b) *Cucumis sativus*, c) *Nicandra physaloides*

Rys. 2. Objawy chorobowe powodowane przez *Cucumber mosaic virus*; izolat Wic na liściach sztucznie inokulowanych roślin: a) *Nicotiana rustica*, b) *Cucumis sativus*, c) *Nicandra physaloides*

Serological reactions. The sap from naturally infected honeysuckle or mechanically inoculated seedlings of *L. caprifolium*, tested by DAS-ELISA assay, did not show positive reaction with CMV-I, CMV-II, and CMV-Cas reagents. In contrast, the sap from the symptomatic leaves of mechanically inoculated plants of tobacco species, *D. metel* and *C. sativus* reacted strongly in ELISA with CMV-II (ToRS) and CMV-Cas antibodies. No positive reaction was obtained using DAS-ELISA reagents for CMV-I (DTL) detection.

Evaluation of prepared antisera. The titre of antisera obtained from 5 different bleedings (1, 2, 3, 4 and 7) was compared in F(ab')₂-ELISA with homologous CMV-Wic isolate and isolate CMV-M (serogroup DTL). The titre of antisera 1, 2, 3 and 4 was at least 128 000 in ELISA with both isolates. The titre of antiserum 7 was 256 000.

Optimal dilution of IgG and conjugate for use in DAS-ELISA was found in a series of experiments. It was 1:1500 and 1:2000, respectively.

DISCUSSION

Plants of *L. caprifolium* cultivated in a commercial nursery showed retarded growth and distinct leaf and flower symptoms similar to those described for *L. periclymenum* and associated with *Cucumber mosaic virus* infection reported from Sweden by Lihnell [1951].

On the basis of host range studies, *in vitro* properties and serological reaction with CMV specific antibodies, the virus isolated from diseased honeysuckle was identified as *Cucumber mosaic virus*. The host range and physical properties *in vitro* of the isolate Wic resembled those described by Kaper and Waterworth [1981] although some differences in the reaction of several herbaceous host plants were found. The properties of CMV-Wic were different also from those reported for CMV isolates from impatiens and other horticulture crops in Poland [Kamińska 1995, Korbin and Kamińska 1998]. The symptoms induced by isolate Wic were less severe than observed for CMV isolates from cucumber (J and M), and much more similar to the symptoms of CMV isolates from lilies (Cas, P26) and impatiens (Imp-1) [Korbin and Kamińska 1998].

Extract of naturally infected *L. caprifolium* showing leaf and flower symptoms did not react in ELISA specific for CMV. However, tobacco, cucumber and *D. metel* plants, mechanically inoculated with the virus isolated from diseased honeysuckle, reacted positively in DAS-ELISA with CMV-II commercial antibodies and the antibodies against CMV isolate Cas, but not with commercial antibodies against CMV-I. These results suggest that the examined isolate belongs to the ToRS serogroup. It is possible, that concentration of virus particles in naturally infected host – *L. caprifolium*, was too low for ELISA detection, or the reaction was inhibited by plant components. These will be verified in further study. Similar problems, as observed here with virus detection in woody plant by ELISA, were reported by Borodynko [2004] in the case of nepo- and cucumoviruses.

The virus isolated from diseased honeysuckle was purified, and rabbit antiserum was produced. The obtained antiserum showed the titre at least 128 000 in F(ab')₂-ELISA with homologous isolate, as well as with cucumber isolate CMV-M belonging to serogroup DTL [Korbin and Kamińska 1998].

Attempts have been made to transmit the virus isolate by mechanical inoculation from herbaceous plants to the seedlings of *L. caprifolium*, but they failed. Lack of experimental transmission of CMV into *Lonicera* spp. healthy plants was reported by Lihnell [1951] and Cooper [1979]. Therefore, we conclude that this species can not be infected with CMV by mechanical inoculation. To the best of our knowledge, this is the first report on the natural occurrence of CMV in *Lonicera caprifolium*.

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CZEŚCIOWA CHARAKTERYSTYKA IZOLATU WIRUSA MOZAIKI OGÓRKA (CMV) Z ROŚLIN *Lonicera caprifolium* L.

Streszczenie. Stwierdzono obecność wirusa mozaiki ogórka (CMV) w naturalnie porażonych roślinach wiciokrzewu (*Lonicera caprifolium* L.) wykazujących zahamowanie wzrostu oraz silną deformację liści i kwiatów. Wirusa zidentyfikowano na podstawie reakcji testowych roślin zielnych, jego właściwości w warunkach *in vitro* oraz właściwości serologicznych. Wirusa przeniesiono w sposób mechaniczny na trzynaście gatunków roślin zielnych, u których wywoływał objawy lokalne lub lokalne i systemiczne. TIP wirusa określono na 65-70° C, LIV 4-5 godzin a DEP 10⁻⁴ do 10⁻⁵. W teście DAS-ELISA wirus reagował pozytywnie z przeciwciałami na CMV-ToRS (II), a nie reagował z przeciwciałami na CMV- DTL (I). Przygotowano surowicę króliczą, której miano w teście F(ab')₂ ELISA wynosiło co najmniej 128 000, zarówno w reakcji z izolatem homologicznym jak i z izolatem M, należącym do serogrupy DTL.

Słowa kluczowe: *Lonicera*, wirus mozaiki ogórka, identyfikacja, serologia

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