Poplar mosaic virus detected in Lithuania

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Eastern poplar trees (*Populus deltoides*) with slight leaf mosaic symptoms were observed in Vilnius environs. Virus infection from such trees was mechanically transmitted to herbaceous plants. Using diagnostic species of plants it was determined that the host range and symptoms are characteristic of poplar mosaic virus from Carlavirus group. Virion morphology (668 nm) and cytopathological changes in infected tissues of *Nicotiana glutinosa* and *N. megalosiphon* plants confirmed this finding. It is the first detection of *Poplar mosaic carlavirus* in Lithuania.

Key words: *Populus*, carlavirus, poplar mosaic virus, identification, electron microscopy

INTRODUCTION

Poplar trees in Vilnius and other regions of Lithuania were observed to exhibit symptoms of mild mosaic or light yellow mottling in completely expanded leaves, especially in early summer. Poplar mosaic virus (PMV) naturally infects species and hybrids within the genus *Populus* L. (*Salicaceae*) [1]. Poplar is the only natural host of PMV. The virus has been known for a long time since its description in Bulgaria in 1935 [2]. PMV is distributed and described in many European countries and in other continents [1]. From Lithuania neighbouring countries, PMV is known in Poland. The worldwide distribution of poplar with mosaic symptoms is probably due to dissemination of the virus by infected cuttings. PMV is transmitted mechanically, no vector is known [3]. PMV-affected poplar trees usually show mosaic or diffuse yellow spotting symptoms on leaves. According to some investigations, very sensitive cultivars of poplar can develop necrosis of bark, petioles and leaf veins, and growth of trees may be severely decreased. In such cases possibly more than one virus may be involved [1].

A preliminary electron microscopic investigation of crude extracts from mosaic-affected leaves (Fig. 1) of eastern poplar (*Populus deltoides* W.Bartram ex Marshall) from Vilnius region revealed the presence of elongated carlavirus-like particles. The subject was identification of causal agent of mosaic disease of poplar population grown in Vilnius environs, considering that poplar could be affected by other viruses as well.

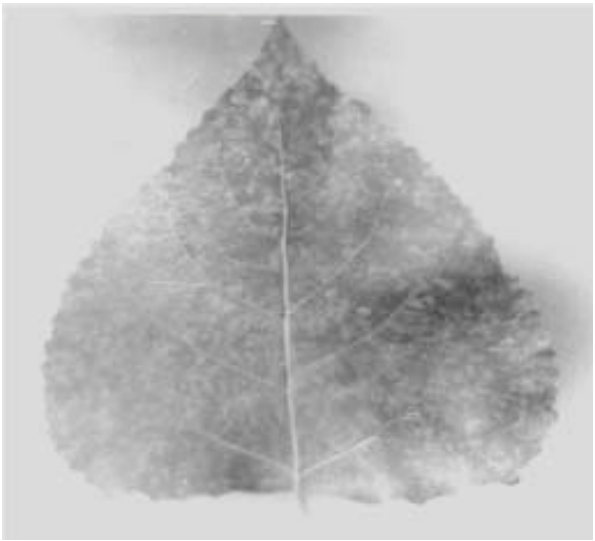

Fig. 1. Mosaic symptoms on poplar leaf

MATERIALS AND METHODS

Attempts to transmit mechanically possible viruses from poplar leaves with mosaic symptoms to herbaceous plants were made by using phosphate buffer solution supplemented with some reducing or chelating agents. Test plants were grown and inoculated in greenhouse conditions. In the host range studies, species with a characteristic reaction to PMV were included. Presence of virus particles was observed in the dip preparations, using a JEM-100S electron microscope after negative staining with 2% uranyl acetate (UA) solution. For cytopathological investigation of ultrathin sections, *Nicotiana glutinosa* and *N. clevelandii* plants infected with the virus from mosaic-diseased poplar were used. The tissue samples were fixed in 4% glutaraldehyde-paraformaldehyde solution in 0.1 M cacodylate buffer pH 7.2 over-

night at 4 °C and postfixed with 2% osmium tetroxide solution in the same buffer for 3 h at 4 °C. The samples were dehydrated in a graded ethanol series with *en block* staining with 3% UA during dehydration in 70% ethanol. After final dehydration in absolute acetone, tissue samples were embedded in Epon 812. Ultrathin sections were cut with LKB Ultratome V, using glass knives stained with UA and lead salts according to Sato [4] and observed with a transmission electron microscope at an accelerating voltage of 60 kV.

RESULTS AND DISCUSSION

Tests of mechanical virus transmission from mosaic-affected poplar leaves to limited herbaceous hosts were successful only in case when 0.05 M phosphate buffer solution pH 7.2 contained 1% nicotine, 0.01 M Na DIECA and 1% polyvinylpyrrolidon (PVP). Inoculations failed when only PVP, Na DIECA, nicotine, thioglycolic acid or caffeine were used as single additives. The plant species used for the host range studies of virus isolate from poplar and the results obtained are presented in Table.

Susceptibility of inoculated species and symptoms caused by the virus isolated from poplar in *Cucumis sativus*, *Datura stramonium*, *Nicotiana glutinosa*, *N. clevelandii*, *N. megalosiphon* and *Vigna sinensis* (Fig. 2) are in accordance with the description of PMV [1, 5]. Observation and measurement of virus particles in crude sap preparations of infected plants indicated the presence of filamentous rather rigid or slightly curved particles about 668 nm long (Fig. 3). This length is within the limits for representatives of *Carlavirus* group (610–700 nm) [4, 6], (620–680 nm) [7]. The modal length of PMV investigated in Czechoslovakia was estimated to be 626 nm [8], in Germany 670–685 nm [9]. In the descrip-

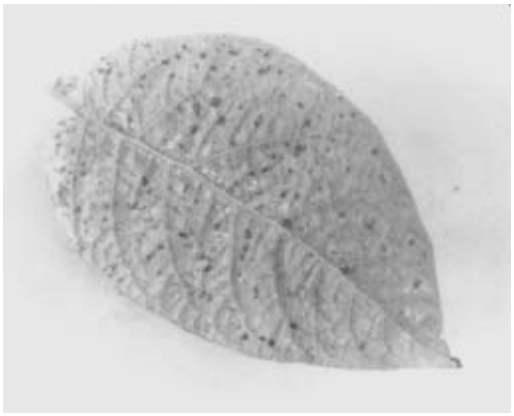

Fig. 2. Local lesions in *Vigna sinensis* inoculated leaf

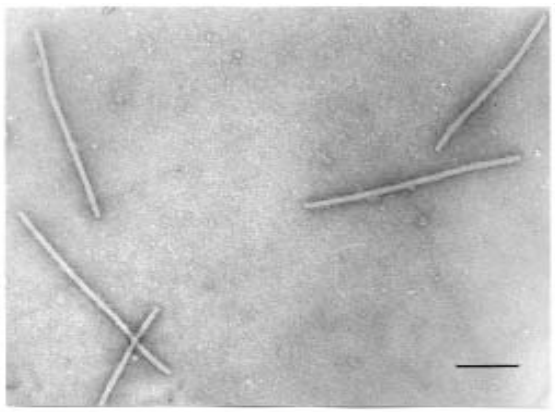

Fig. 3. Filamentous virus particles in crude sap of infected *Nicotiana clevelandii*. Bar represents 200 nm

Table. **Host range and symptoms caused by the virus isolated from mosaic affected poplar**

Plant species	Symptoms (local/systemic)	Back inoculation
Amaranthus caudatus L.	0/0	–
Celosia cristata Kuntze	0/0	–
Chenopodium amaranticolor Coste et Reyn.	0/0	–
C. foetidum Schrad.	0/Mo, Ma	+
C. murale L.	0/0	–
C. quinoa Willd.	0/0	–
Cucumis sativus L., cv. 'Polan'	0/VC, (Mo)	+
Datura stramonium L.	0/Mo, Ma	+
Nicandra physalodes (L.) Pers.	0/0	–
Nicotiana clevelandii Gray	LL/Mo, Ma	+
N. glutinosa L.	0/VC, Mo	+
N. megalosiphon Heurck et Muell.	LL/VC, Mo, Ma, Stunt	+
N. rustica L.	0/VC	+
N. tabacum L., cv. 'Samsun'	0/0	–
Tetragonia expansa Murr.	0/0	–
Vigna sinensis Endl.	LL/0	+

Note: 0/0 indicates absence of local and systemic symptoms; LL – local lesions; Mo – systemic mottle; M – mosaic; VC – vein clearing.

tion of the virus, the modal length (675 nm) is indicated [5].

The majority of cytologically investigated carlaviruses in infected tissues are forming aggregated virus particles. Aggregates of particles not organized into banded bodies are also reported in cells infected with poplar mosaic virus [10]. Quite similar loose bundles of virus particles we observed in cytoplasm of mesophyll cells of *Nicotiana clevelandii* infected with the virus isolate under investigation (Fig. 4). Moreover, in the cytoplasm of this species and infected *Nicotiana glutinosa,* paracrystalline inclusions were observed (Figs. 5, 6). Similar cytoplasmic crystalline inclusions have been described in some carlavirus infections [11]. Pinwheel inclusion bodies characteristic of potyviruses were not present in infected tissues of the species studied.

Thus, according to the morphology of virus particles, host range and induced symptoms in susceptable plants, as well as cytopathology of infected tissues it can be concluded that the virus isolated from poplar (*Populus deltoides)* with leaf mosaic symptoms is identical with *poplar mosaic carlavirus* from the genus *Carlavirus*.

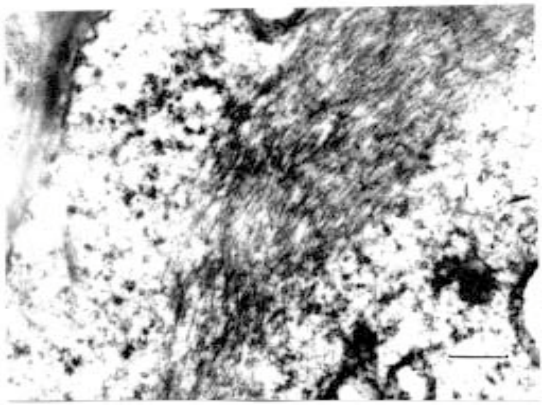

Fig. 4. Loose virus particles in cytoplasm of mesophyll cells of *Nicotiana clevelandii*. Bar represents 500 nm

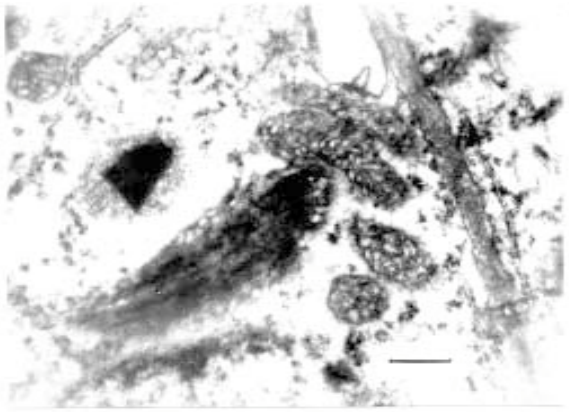

Fig. 5. Inclusion body in cytoplasm of mesophyll cell of infected *Nicotiana clevelandii*. Bar represents 500 nm

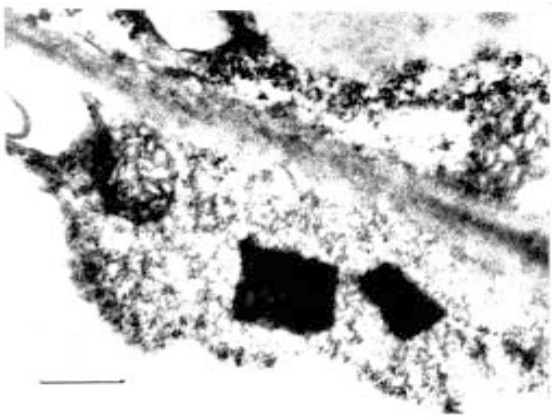

Fig. 6. Inclusion body in cytoplasm of mesophyll cell of infected *Nicotiana glutinosa*. Bar represents 500 nm

References

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TUOPŲ MOZAIKOS VIRUSAS LIETUVOJE

S a n t r a u k a

Tuopos (*Populus* L.) dažnai pastebimos su šviesiai margais, silpnai mozaikiškais lapais. To priežastis, literatūriniais duomenimis, yra virusinė infekcija. Siekiant išsiaiškinti Vilniaus apylinkėse ir Vilniaus mieste augančių didžiųjų tuopų (*Populus deltoides* W. Bartram ex Marshall) lapų margligės priežastį, buvo tyrinėjama virusinė ligos etiologija. Yra žinoma, kad karlavirusų grupei priklausantis tuopų mozaikos virusas (*Poplar mosaic virus*) pažeidžia tik tuopas, nors jas gali pažeisti ir kiti virusai. Naudojant buferius su antioksidantais, virusinė infekcija iš tuopų buvo pernešta į žolinius augalus indikatorius, nustatyta aptinkamų virusinių dalelių morfologija (apie 670 nm). Pagal infekcijos pernešimo būdą, pažeidžiamų augalų spektrą ir sukeliamus simptomus, virionų morfologiją bei ląstelių citoplazmoje aptinkamus virusinius intarpus tuopų lapų margligės sukėlėjas yra identifikuotas kaip tuopų mozaikos karlavirusas (*poplar mosaic carlavirus*).