# Viral diseases of flower plants 15. Identification of viruses affecting gladiolus (*Gladiolus* L.)

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Three viruses were isolated and identified from gladiolus (*Gladiolus* L.) in Lithuania: *Bean yellow mosaic potyvirus* (BYMV), *Cucumber mosaic cucumovirus* (CMV), and *Tobacco rattle tobravirus* (TRV). The viruses were identified by the methods of test-plants and electron microscopy. The optimum technique for obtaining the purified preparation of BYMV was selected and the purified preparation was obtained.

**Key words:** gladiolus, *bean yellow mosaic potyvirus, cucumber mosaic cucumovirus, tobacco rattle tobravirus*

## INTRODUCTION

The genus *Gladiolus* belongs to the family *Iridaceae* Juss. It includes more than 150 species, most of them native to Africa and a few originated from the Mediterranean area, Asia and South Europe. Cultivars of gladiolus exhibit a great diversity of colour, size, shape, flowering time, and bulbing and dormancy behavior. This variability arose from a complex of crosses among several diploid and polyploid botanical species.

Gladiolus is a popular garden plant and commercial flower crop which has been grown in Lithuania for many years. Local breeders have created a great number Lithuanian gladiolus cultivars and hybrids.

Due to large commercial worldwide trade and vegetative propagation by corms and cormels gladioli are affected by a large number of viruses. Gladiolus is a highly susceptible crop that may suffer considerable losses if control measures are not taken. The main viruses reported from gladioli in different countries are: *tomato ringspot nepovirus* [1], *tomato black ring nepovirus* [2], *tomato spotted wilt tospovirus* [3], *tobacco mosaic tobamovirus* and *tobacco ringspot nepovirus* [4], *arabis mosaic nepovirus* and *strawberry latent ringspot nepovirus* [5], *tobacco streak ilarvirus* [6].

None of the viruses found on gladioli was specific to the crop species or to the *Iridaceae.* All of them have a wide host range as well as geographic distribution and can be transmitted by mobile vectors. In several cases viruses specific for gladiolus strains were isolated and characterized. Different strains of viruses are known to occur on gladiolus, and probably they differ with the region.

The aim of the present work was to isolate, investigate and identify the main viruses affecting gladioli in Lithuania.

## MATERIALS AND METHODS

The material for investigation was collected in different floriculture farms in Lithuania: Botanical Gardens' of Vilnius University and Vytautas Magnus University, flower collections of Lithuanian Institutes of Agriculture and Horticulture, Experimental Station of Field Floriculture and private gardens of flower growers. The samples were collected from gladioli expressing viral symtoms on leaves and flowers. The experimental work was carried out at the greenhouse and at the Laboratory of Plant Viruses of Institute of Botany. Viruses were identified by test-plant reaction and virus particle morphology. Virus particles were visualized in negatively stained dip preparations [7, 8], using a JEM-100S electron microscope. For mechanical sap inoculation the following test-plants were used: *Amaranthus caudatus* L., *A. paniculatus* L., *Atriplex hortensis* L., *Chenopodium amaranticolor* Coste et Reyn, *C. ambrosioides* L., *C. murale* L., *C. urbicum* L., *C. quinoa* Willd., *Cucumis sativus* L. 'Delikates', 'Libelle', 'Niežinskij miestnyj', *Datura stramonium* L., *Gomphrena globosa* L., *Nicandra physalodes* L., *Nicotiana glutinosa* L.,

*N. tabacum* L. 'Samsun', 'Xanthi', *N. rustica* L., *Petunia hybrida* Vilm., *Phaseolus vulgaris* L. 'Baltija', 'Bataaf', 'Early Refuge', 'Saksa', *Pisum sativum* L. 'Greitukai', 'Žalsviai', *Tetragonia expansa* Murr., *Vicia faba* L. 'Aušra'. The inoculum for mechanical inoculation was prepared by homogenizing infected leaves with 0.1 M phosphate buffer (pH 7.0), containing stabilizing agents such as 0.2% mercaptoethanol, 0.1% thioglycolic acid or 0.01 M sodium diethyldithiocarbamate (DIECA).

The BYMV was purified by a modified method presented in [9]. Infected leaves were grinded in 0.18 M phosphate-citric acid buffer at pH 7.0 containing 0.01 M DIECA, 0.2% thioglycolic acid, and 0.5 M urea. The extract was clarified by blending with 6% chloroform and 5% n-butanol. Virus particles were precipitated with 4% w/v polyethylene glycol MW 6000 and 2% NaCl. Pellets were resuspended in 0.18 M phosphate-citric acid buffer (pH 7.0). The virus was purified by two cycles of differential centrifugation. Centrifugation at low speed was at 8000 rpm during 10 min in a K-24 centrifuge. To sediment virus preparations they were centrifuged in VAC-601 at 28000 rpm for 2.5 h. Final purification was accomplished by sedimentation through a 20% sucrose cushion. The concentration and purity of virus preparation was estimated by electron microscopy and spectrophotometrically.

## RESULTS AND DISCUSSION

### Bean yellow mosaic potyvirus (BYMV)

Leaves of naturally infected gladiolus plants show chlorotic spots and streaks of irregular shape (Fig. 1a). Corms of infected plants are distorted, unnormally elongated, sometimes have outgrowths. A coarse breaking pattern, expressed by intensification or dissapearence of pigment in flowers occurs in some cultivars (Fig. 1b). The mixed viral infection is common in gladiolus, so the symptom expression is very variable. The great variety of cultivars of gladiolus also contributes to variability of symptoms. Infected plants reduce in growth and development year after year, their corms lose their vital capacity and during winter storage can be lost.

BYMV on gladiolus in Lithuania for the first time was reported by J. Staniulis in 1967 [10]. Later BYMV has been isolated and identified in a great number of gladiolus cultivars.

For virus identification the test-plants were inoculated by mechanical sap inoculation. They produced following symptoms:

*Chenopodium amaranticolor, C. quinoa* and *Gomphrena globosa* developed chlorotic local lesions,

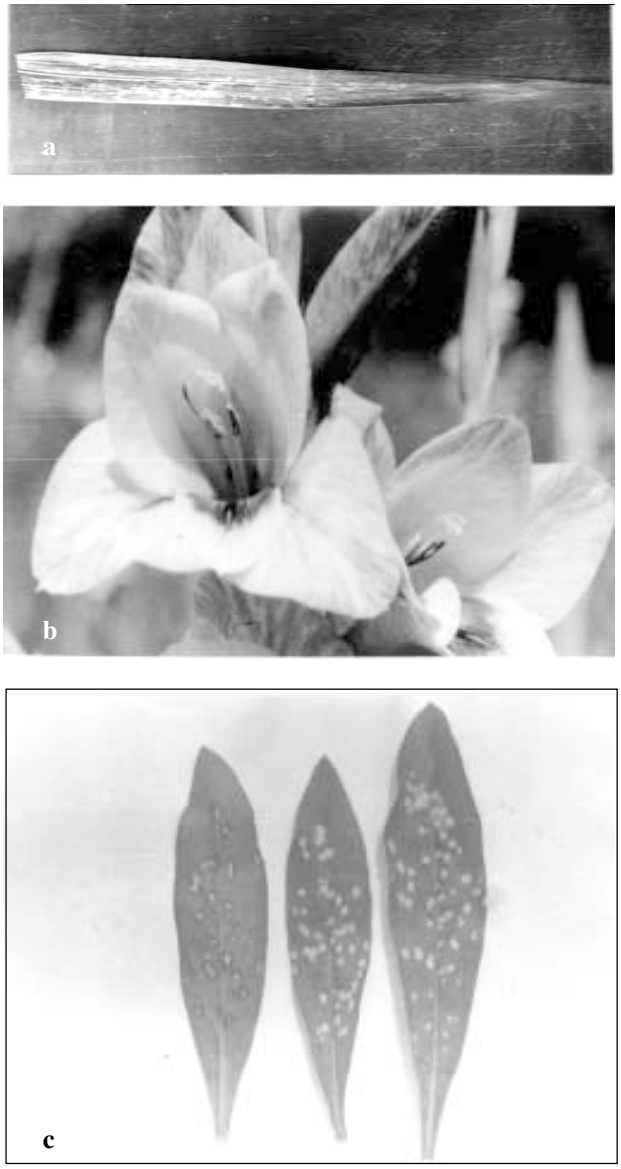


Fig. 1. Bean yellow mosaic potyvirus (BYMV) symptoms on naturally infected gladiolus leaf (*a*) and flowers (*b*); *c* – local lesions on *Gomphrena globosa* leaves caused by BYMV

which appeared on the inoculated leaves 6–7 days after inoculation (Fig. 1c).

Quite susceptible *Phaseolus vulgaris* cultivars were 'Baltija', 'Bataaf', 'Early Refuge'. They reacted to BYMV infection systemically in 10–12 days. Later grown apical leaves showed chlorotic mottling followed by necrosis of main veins and leaf curling. Later grown leaves were smaller than normal, blistered and reduced in development.

*Phaseolus vulgaris* 'Saksa' sometimes showed chlorotic spotting, but not always. A deviation in symptom expression severity depended on the temperature and other environmental conditions in a greenhouse.

Inoculated *Vicia faba* 'Aušra', *Pisum sativum* 'Greitukai' and 'Žalsviai' developed a systemic reac-

tion. Greenish mosaic appeared on *Vicia faba* apical leaves and remained for the whole vegetative season. Later grown *Pisum sativum* leaves showed mild chlorotic mottling, the development of plants was reduced.

Other inoculated test-plants showed no reaction to BYMV infection.

Electron microscopy investigation of negatively stained preparations from leaves of naturally infected gladioli and inoculated test-plants revealed slightly flexuous filamentous particles with normal length about 720 nm. Virions were found in a low concentration. Virus in higher concentrations was found in *Vicia faba* plants.

On the basis of test-plant reaction data, particle morphology and according to literature data [11–13] the virus was identified as BYMV.

For purification BYMV was propagated in *Vicia faba.* Purification was carried out from fresh leaves, according to the method [9]. The purified BYMV preparation had $A_{max}$ at 260 nm and $A_{min}$ at 240 nm, the $A_{260/280}$ ratio being 1.23. The yield of purified virus, taking into account specific absorbance $A^{0,1\%,\ 260\ nm}$ being 3.0 [9], was calculated to be 4.7 mg from 1 kg of infected plant tissue.

BYMV is readily transmitted mechanically by numerous aphid species in nonpersistent manner, mechanical sap inoculation. The virus survives in infected corms and spreads due to vegetative propagation by corms and cormlets. BYMV is widespread in Lithuania and affects almost all gladiolus cultivars grown in our country. Usually it is found in mixed infections with other viruses affecting gladiolus [14].

**Cucumber mosaic cucumovirus (CMV)**

Severe colour break and deformation of flowers are the most common symptoms associated with CMV infection in gladiolus. However, symptoms vary from a few streaks on coloured petals to various degrees and forms of distortion of flowers and whole plants. Chlorophyll break or mosaic is sometimes observed on leaves of young plants, but only in a few very susceptible cultivars. In general most infections are symptomless until start of flowering. Pitting and discoloration of corms occur in some cultivars.

CMV on gladiolus in Lithuania for the first time was reported in 1969 [14].

The following reactions were recorded on the test-plants inoculated with CMV:

*Atriplex hortensis* – local chlorotic lesions approximately 8–10 days after inoculation;

*Chenopodium quinoa* – local chlorotic lesions on inoculated leaves in 5–6 days (Fig. 2b);

*Cucumis sativus* 'Delikates' – light green diffuse spots on inoculated leaves 5 days after inoculation, followed by systemic light green mottling in the next 5 days (Fig. 2a);

*Gomphrena globosa* – local necrotic lesions on inoculated leaves in 10 days;

*Nicotiana glutinosa* – systemic mild chlorosis and leaf lamina narrowing in 15 days (Fig. 2c);

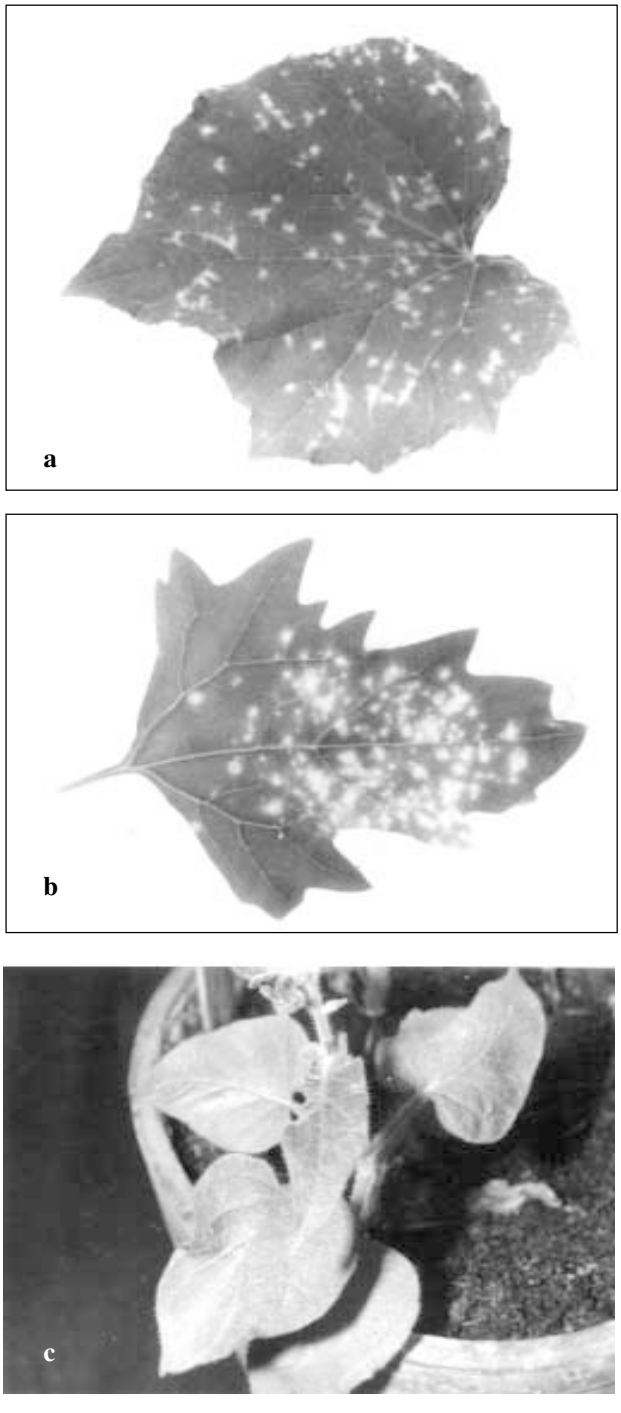


Fig. 2. Symptoms on test-plants caused by cucumber mosaic cucumovirus: *a* – systemic green mottling on *Cucumis sativus* leaf; *b* – local chlorotic lesions on *Chenopodium quinoa* leaf; *c* – systemic mild chlorosis and leaf lamina narrowing in *Nicotiana glutinosa*

*N. rustica* – systemic mild mosaic and slight leaf deformation in 15 days;

*N. tabacum* 'White Burley' – systemic light green mottling on young leaves in 15 days;

*Petunia hybrida* – chlorotic spots on inoculated leaves 10–12 days followed by systemic mottling and mosaic on young leaves in the next 5 days;

*Tetragonia expansa* – local necrotic ringspot which occurred in 8 days.

Electron microscopic examination of negatively stained dip preparations made from infected test-plants revealed isometric particles about 30 nm in diameter.

According to the test-plant reaction, electron microscopy, and literature data [12, 15], the isolated virus was identified as CMV.

CMV is mechanically transmitted by sap inoculation and by several species of aphid in non-persistent manner, spreads by vegetative propagation in corms. CMV has been recognized as the major disease of gladiolus cultivars and presents a serious problem in the production of cut flowers and corms. CMV in gladiolus often occurs in mixed infections with other viruses affecting this crop [14].

**Tobacco rattle tobravirus (TRV)**

Affected plants are usually stunted. Leaves develop yellowish brown streaks along veins, followed by leave's tearing and notching. Severely affected plants form distorted flower stalks with malformated flowers or do not form flowers at all. Roots are developed poorly and have abnormaly ramified rootlets.

TRV on gladiolus in Lithuania for the first time was reported in 1973 [16].

For virus identification test-plants were inoculated and expressed the following reaction to:

*Amaranthus paniculatus* – local light brown lesions in 7 days (Fig. 3b), which became dark-brown later.

*Atriplex hortensis* – local lesions on inoculated leaves 5–7 days after inoculation;

*Chenopodium amaranticolor* – local lesions in 5 days on inoculated leaves;

*C. ambrosioides* – local lesions with a necrotic center and red margins in 5 days (Fig. 3c);

*C. quinoa* – local chlorotic and necrotic spots on inoculated leaves in 5–6 days;

*Gomphrena globosa* – local necrotic spots in 9 days;

*Nicandra physalodes* – chlorotic and necrotic spots, vein necrosis, leaf distortion appeared in 8 days (Fig. 3d).

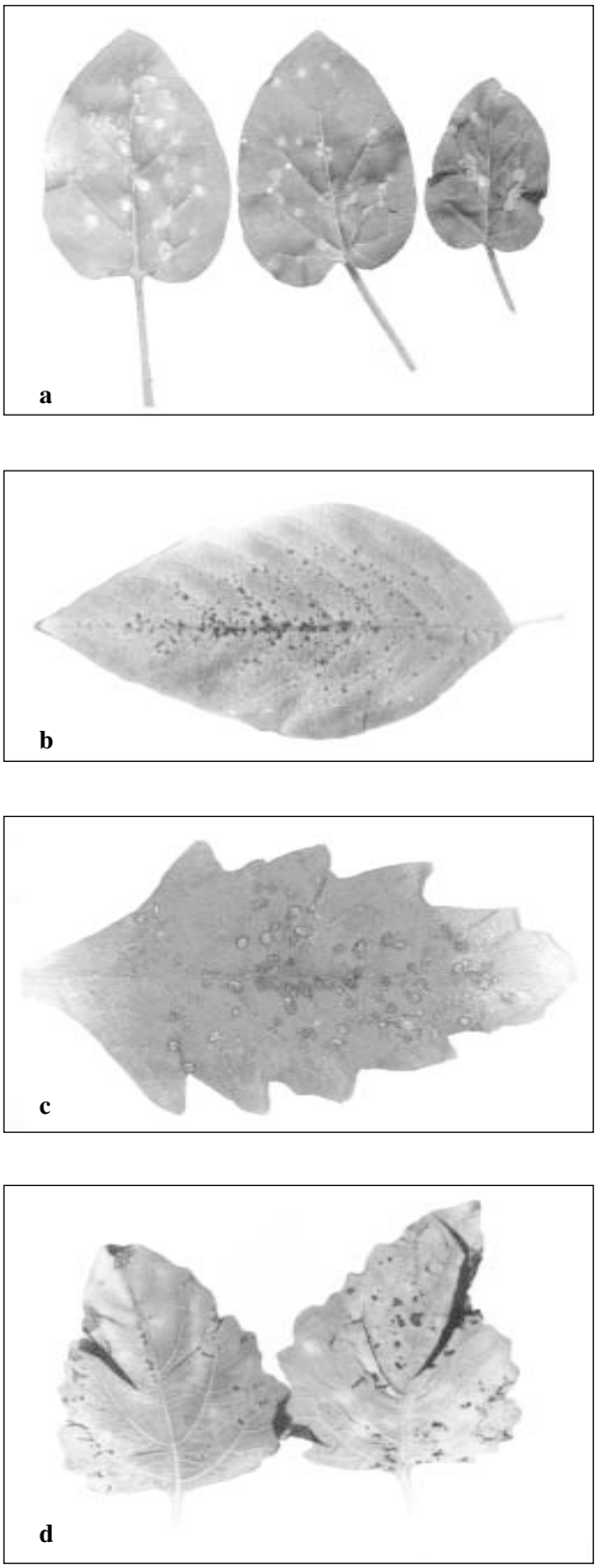


Fig. 3. Symptoms on test-plants caused by tobacco rattle tobravirus: *a* – local necrotic lesions on *Nicotiana glutinosa* leaves ; *b* – local light brown lesions on *Amaranthus paniculatus* leaf ; *c* – local lesions with necrotic center and red margins on *Chenopodium ambrosioides* leaf; *d* – chlorotic and necrotic spots, leaf distortion on *Nicandra physalodes* leaves

*Nicotiana glutinosa* – severe necrotic lesions appeared on inoculated leaves in 6 days (Fig. 3a);

*N. tabacum* 'Samsun' – local various shape lesions appeared in 7 days on inoculated leaves, followed by systemic reactions as leaf etching and necrotic vawy lines.

Electron microscopic examination of negatively stained dip preparations from naturally infected plants and inoculated test-plants revealed tubular particles of two lengths, 190 and 45–115 nm.

On the basis of test-plant and electron microscopy data and according to the literature the virus was identified as TRV [17].

TRV is transmitted by mechanical sap inoculation and by nematodes of the family *Trichodoridae* family. The virus survives in gladiolus corms and cormels during winter storage and spreads due to vegetative propagation.

During many-year investigations of gladiolus viral diseases we often observed plants showing different kinds of infection named grassy top disease symptoms. Gladiolus grassy top disease is expressed by a number of symptoms. Their intensity and frequency depend on climatic conditions and susceptibility of individual cultivars. Characteristic symptoms are flavescence proceeding from tips of the undermost leaves. Severely affected plants develop very dwarfed leaves in dense clusters sprouting from corms and mostly stop their growth within a short time. Corms do not root or roots are very poor. A diminishing and differently expressive flower malformation appeared in some plants, while in others a partial or entire greening of iniciated flowers appeared and the flower buds never came to bloom. A full development and elongation of flower stalk did not occur at all. Infected gladiolus corms are small and shrunk, they survive badly during the storage in winter period. Primary late infection in the period of flowering may remain symptomless for one season, and the symptoms occur next year when infected corms are planted. The agent of this disease is phytoplasma transmitted by the leafhopper *Macrosteles sexnotatus.*

Epidemic spread of phytoplasmic diseases often occurs the next season after a warm summer due to optimal conditions for leafhopper to develop, and can be controlled by eradicating the vector.

Methods of controlling viral diseases consist in growing and propagating healthy planting material tested for the presence of virus, inspection of plants during vegetation for presence of symptoms and elimination of affected plants. Virus-free valuable cultivars can be produced by heat treatment and meristemic culture. The search for genetic resistance to the major viruses of gladioli is a long-term approach to the problem.

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**GĖLIŲ VIRUSINĖS LIGOS. 15. VIRUSŲ, PAŽEIDŽIANČIŲ KARDELIUS (*GLADIOLUS* L.), IDENTIFIKACIJA**

S a n t r a u k a

Iš kardelių (*Gladiolus* L.) išskirti ir identifikuoti trys virusai: pupelių geltonosios mozaikos (*Bean yellow mosaic potyvirus*), agurkų mozaikos (*Cucumber mosaic cucumovirus*) ir tabako garbanotosios dryžligės (*Tobacco rattle tobravirus*). Virusai identifikuoti augalų-indikatorių ir elektroninės mikroskopijos metodais. Parinkta optimali metodika pupelių geltonosios mozaikos potyvirusui gryninti ir paruoštas išgrynintas šio viruso praparatas.