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LABURNUM ANAGYROIDES MED. —NATURAL HOST OF POTATO VIRUS X

NADA PLEŠE and MERCEDES WRISCHER

(Department of Botany, Faculty of Science, University of Zagreb and Ruđer Bošković Institute, Zagreb)

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Potato virus X has been isolated from the ornamental shrub *Laburnum anagyroides* Med. with mosaic symptoms of the leaves, growing in an uncultivated park in Zagreb. The virus has been identified on the basis of host range reactions, appearance of characteristic ultrastructural changes in cells of infected test plants and positive serological test. This is, to the best of our knowledge, the second report on potato virus X infection of a woody plant.

Introduction

In previous years arabis mosaic virus (Schmelzer 1962/63, Cooper and Sweet 1976) and tomato black ring virus (Schmelzer 1967, 1977) were isolated from various *Laburnum* species showing yellowing and leaf chlorosis. Brierley and Smith (1954) found *L. alpinum* (Mill.) Bercht. et Presl. with vein mosaic symptoms to be infected with tobacco ringspot virus. Schultz and Harrap (1975) and Pleše (1979) ascertained association of bacilliform virus particles with vein yellowing symptoms of *L. anagyroides*. From the same laburnum species with mosaic symptoms we have recently isolated potato virus X (PVX), and the present paper describes some properties and identification of the virus.

Material and Methods

Virus isolation. PVX was isolated from very young leaves of *L. anagyroides* branchlets forced for budding at room temperature. The leaf material was ground with cooled 0.1 M phosphate buffer pH 7.2 containing 1% (w/v) sodium ascorbate and the inoculum was rubbed on leaves of five specimens each of *Chenopodium quinoa, Nicotiana glutinosa* and *N. megalosiphon* plants. To provide susceptibility for infection the plants were kept in darkness 24 hours before inoculation. Inoculation was carried out in a cold room.

Biological tests. The virus was propagated in *N. megalosiphon* to provide inoculum for host range tests and the source of virus for serological test. *Gomphrena globosa* was used as assay species for back inoculations. Inocula for host range tests were prepared by grinding infected leaf tissue in the buffer used for virus isolation.

Light microscopy. Epidermal strips with hair cells taken from systemically infected leaves of *Nicotiana clevelandii* were mounted in water and in living stage examined at magnification × 450.

Electron microscopy. For cell ultrastructure analysis pieces of chlorotic foliar tissue of systemically infected *N. glutinosa* and of symptoms bearing *L. anagyroides* were fixed for 60 min in 1% (v/v) glutaraldehyde in cacodylate buffer, and after washing in the buffer postfixed in 1% (w/v) OsO_4 during 2 h. Samples of tissue were then dehydrated in ethanol series and embedded in Araldite resin. Ultrathin sections were poststained in 2% uranyl acetate and lead citrate before examination in a Siemens Elmiskop I.

To ascertain the morphology of virus particles crude sap from chlorotic lesions of infected *C. quinoa* was negatively-stained with 2% (w/v) sodium phosphotungstate pH 7.

Serological test. Slide microprecipitin test was performed for serological identification of the virus. The antigen was added as crude leaf sap of systemically infected *N. megalosiphon.* The sap of the same healthy plant served as control. Before assayng, the sap was subjected to low speed centrifugation (2500 g/20 min). Antiserum to PVX (originating from U.S.S.R.) assigned for routine testing was available for serological test. It was used in a twofold dilution series prepared with saline. The slides were incubated for 40 min at 27°C in a humid condition.

Results

Naturally Infected Host

The shrub of *L. anagyroides* from which PVX was isolated originated from an uncultivated park in Zagreb. It showed conspicuous mosaic or mosaic mottling on the leaves of most branches (Fig. 1 A), and vein yellowing symptoms on the leaves of only few separate branches. PVX was isolated from young leaves of mosaic bearing branches. From all of the test plants included in the experiment of virus isolation, two *N. glutinosa* and one *N. megalosiphon* specimens became infected in the first transmission experiment and only one specimen of *N. glutinosa* in the second.

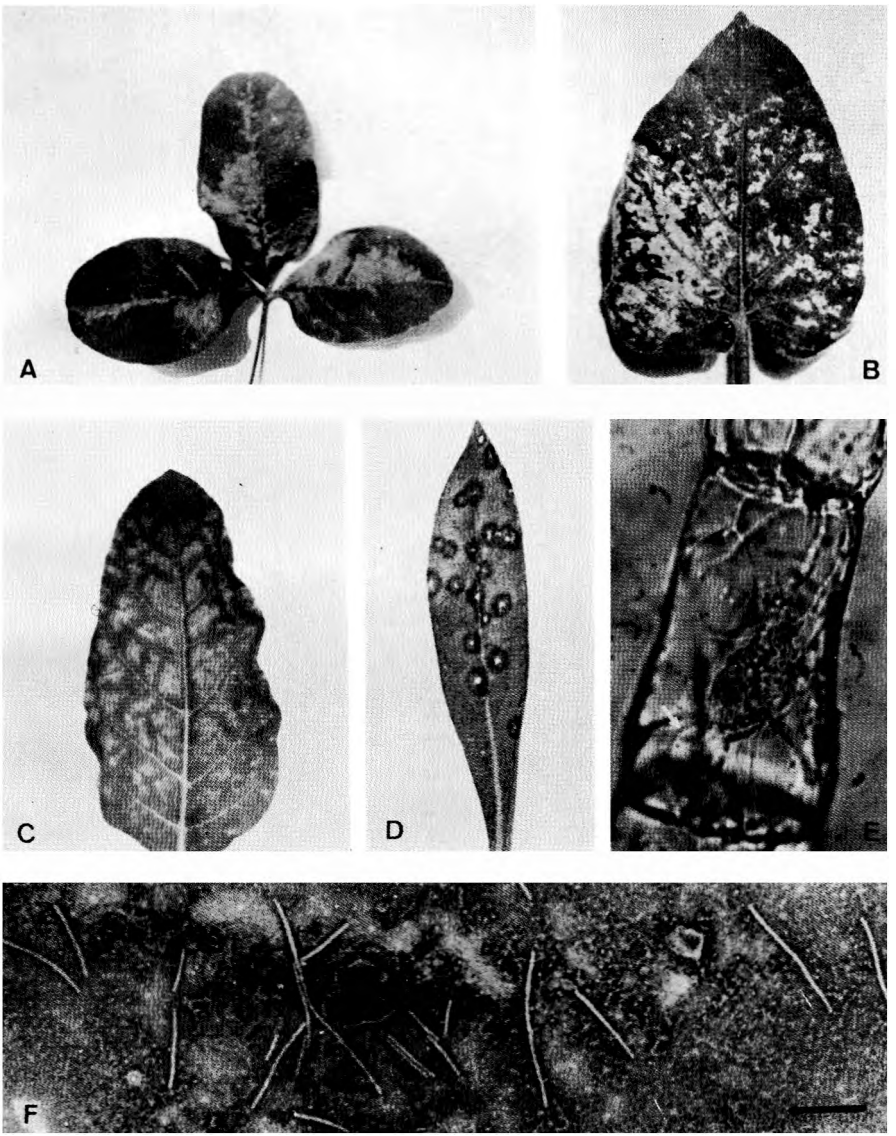

Fig. 1. **A** Mosaic leaf symptoms in *Laburnum anagyroides* from which PVX was isolated. Effect of PVX on test plants: **B** Mosaic mottling becoming necrotic in *Nicotiana glutinosa*. **C** Mosaic mottling in *N. megalosiphon*. **D** Local necrotic dots with red-rimmed ring in *Gomphrena globosa*. **E** Granular inclusion body (black arrow) near the nucleus (white arrow) in leaf hair cell of *N. clevelandii* infected with PVX. **F** Broken particles of PVX in leaf dip preparation of infected *Chenopodium quinoa*. Bar = 250 nm.

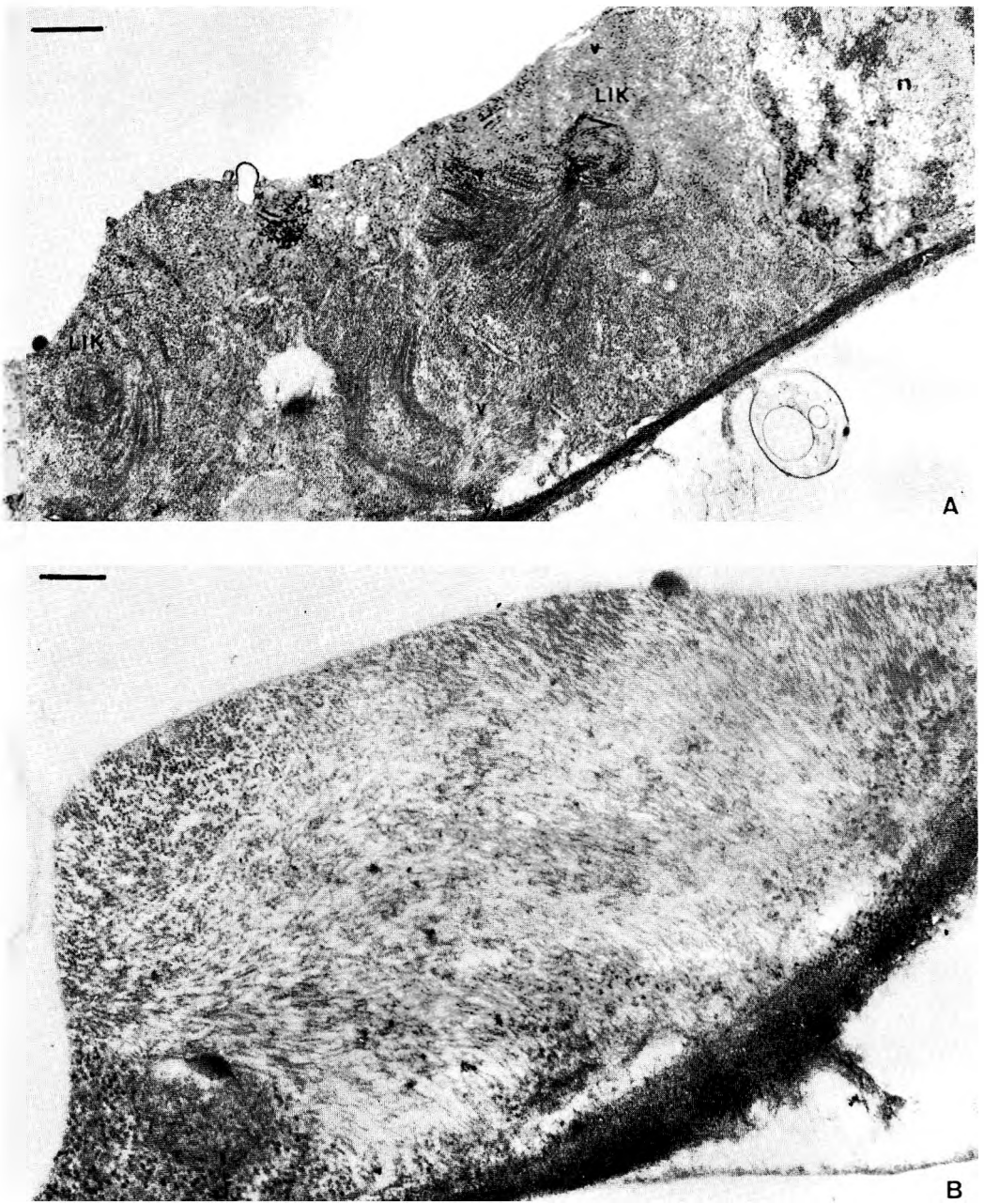

Fig. 2. Thin sections trough leaf cells of PVX infected *Nicotiana glutinosa:* **A** Laminate inclusion components (LIC) and aggregates of virus particles (v) inside the inclusion body, n nucleus; **B** Inclusion body with dense masses of virus particles aggregated in a roughly parallel arrangement. Bar = 500 nm (A) and 200 nm (B).

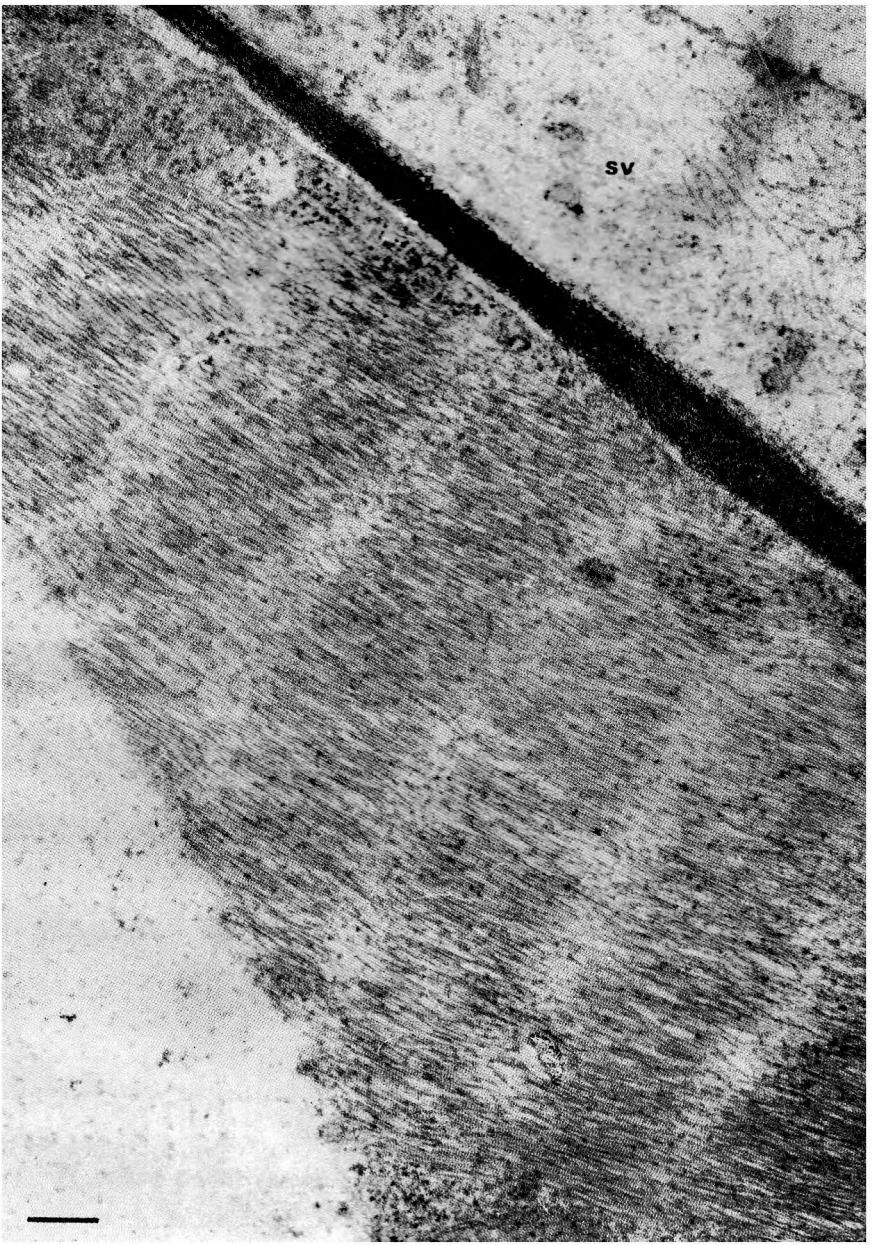

Fig. 3. Banded cytoplasmic inclusion body consisting of tiers of virions, and virions loosely scattered in the cytoplasm (sv) in electron micrograph of leaf cells of PVX infected *Nicotiani glutinosa*. Bar = 200 nm.

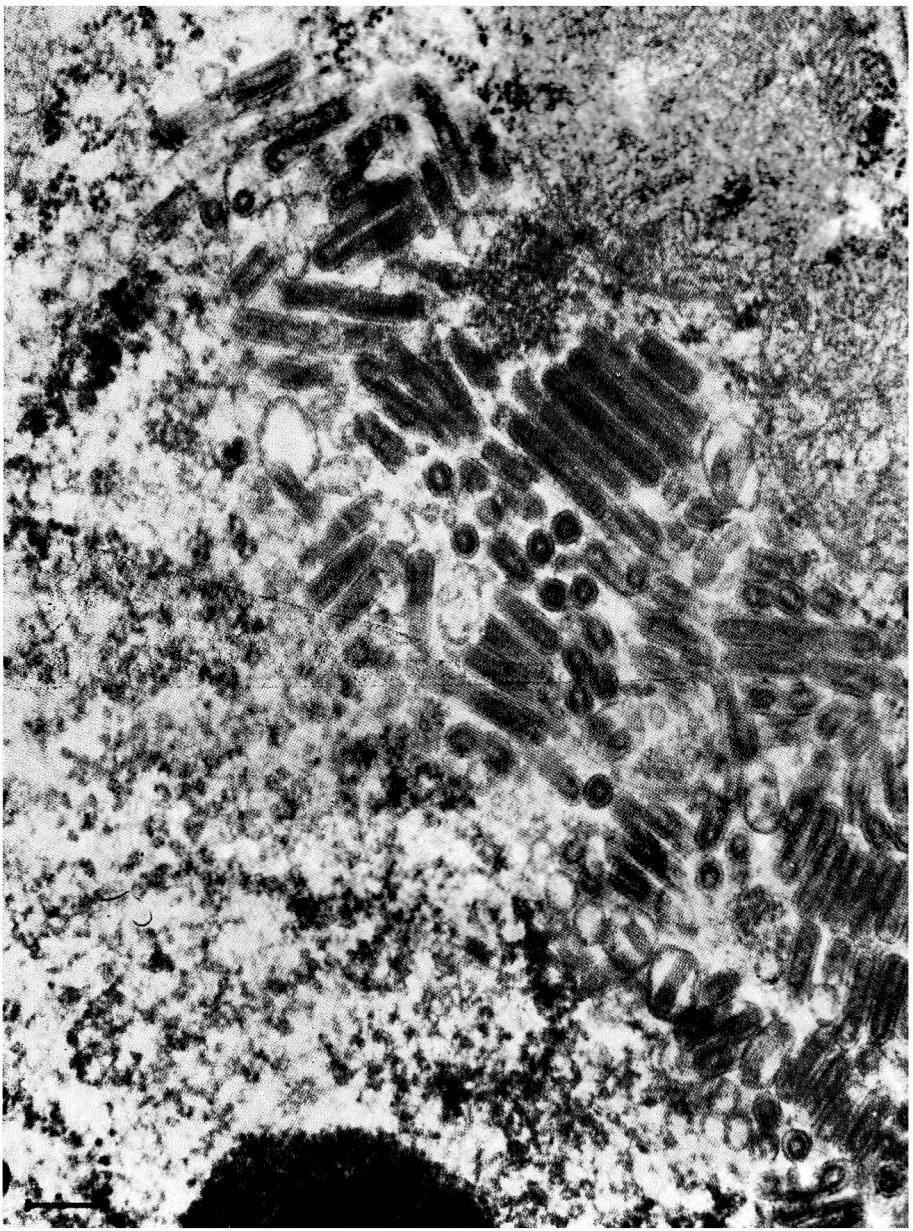

Fig. 4. Ultrathin section through cell of vein yellowing leaf tissue of *Laburnum anagyroides:* masses of rhabdovirus particles are present in perinuclear space. Bar = 200 nm.

Identification of the Virus

Test plants reactions. In conventional test plants the isolated virus induced the symptoms common to PVX infection: *C. quinoa* and *C. amaranticolor* reacted with chlorotic local lesions; in the latter one few lesions were observed on the leaves above the incolated ones. In *G. globosa* the virus induced necrotic local lesions with a red-rimmed ring (Fig. 1 D). *N. glutinosa* and *N. megalosiphon* showed pronounced necrotic dots and rings followed by systematic vein clearing and conspicuous mosaic mottling (Fig. 1 C) which later became necrotic (Fig. 1 B). *Datura stramonium* and *N. tabacum* »White Burley« developed systemic mild mosaic mottling, whereas *N. clevelandii* showed systemic mottling with necrotic dots. In *Vigna sinensis* the infection was local and latent.

Light and electron microscopy. In living cells of infected *N. clevelandii* leaves amorphous cytoplasmic inclusion bodies were observed usually close to the nuclei (Fig. 1 E). Their appearance was more or less granular or homogenous, depending on their submicroscopic composition.

The ultrastructural analysis of leaf cells of infected *N. glutinosa* showed that the inclusion bodies contained, in addition to the normal cytoplasmic constituents, a virus material which mostly occupied the greatest part or even made the complete matter of the inclusion. Dense masses of virus particles inside the inclusions were usually in a roughly parallel arrangement (Fig. 2 B). In some inclusions virions were arrayed side by side with ends more or less in one plane forming superimposed aggregates which appeared as tiers or bands in sections (Fig. 3). Such submicroscopic virus structures are characteritic of banded body inclusions and are considered together with dense masses of virions as a very probable indication of a potexvirus infection (Purcifull et al. 1966, Amelunxen and Thaler 1967, Štefanac and Ljubešić 1974, Christie and Edwardson 1977, Hiruki et al. 1980, Purcifull and Edwardson 1981, Pisi et al. 1982 and others). In addition to virus particle aggregates, we found conspicuous laminate inclusion components (LIC) established to be specific of infection with PVX (Shalla and Shepard 1972, Doraiswamy and Lesemann 1974, Christie and Edwardson 1977 and others). The LIC were composed of colateral bundles of sheets which were densely rolled into a scroll at one end and only slightly curved and loosely packed at the other giving the inclusion a broom-like appearance (Fig. 2 A).

A rather large number of rod-shaped virus particles present in dip preparations of infected *C. quinoa* was broken too much to determine their normal length (Fig. 1 F). However, the width of the tiers of banded inclusions was about 500 nm, i.e. nearly equal to the particle length of PVX (*Bercks* 1970) indicating their composition from only one particle array.

No virus particles were detected in thin sections of *L. anagyroides* leaf tissue with mosaic symptoms. However, bacilliform particles of a rhabdovirus were observed again in sections of vein-yellowing tissue (see Plešе 1979) of the leaves with the same symptoms. The particles were situated mostly in perinuclear spaces (Fig. 4).

Serology. In slide precipitin test, the antigen reacted with the serum against PVX giving floculent precipitate to the dilution of the serum 1/64. No reaction was observed in control test, i.e. with the healthy leaf sap of *N. megalosiphon.* Thus, the serological test confirmed that the isolated virus belongs to PVX.

Discussion

The symptoms on test plants and the ultrastructural characteristics of the isolated virus are in agreement with those already described for isolates of PVX (Bercks 1970, Shalla and Shepard 1972, Doraiswamy and Lesemann 1974, Christie and Edwardson 1977, Purcifull and Edwardson 1981). The finding of LIC inclusions, unique for PVX infection, and the result of the serological test excluded all the posibilities of doubt. Banded arrangement of virus particles in ultrathin sections was not rare, yet we have never observed banded bodies in living cells under a light microscope. This fact also substantiates the great susceptibility of banded structures to destruction before appropriate fixation (Christie and Edwardson 1977).

Since PVX was isolated from *L. anagyroides* in repeated experiments, the lack of virus particles in mosaic leaf tissue is most probably a consequence of low concentration of the virus in woody plant and of insufficient search with electron microscope. In addition, rhabdovirus particles were always associated only with vein yellowing symptoms of *L. anagyroides* (cf. Schultz and Harrap 1975, Pleše 1979).

According to the available literature data, the natural host range of PVX was ascertained to be rather narrow (Klinkowski 1977, Purcifull and Edwardson 1981). Nearly all of the hosts are herbaceous species, mostly from the family *Solanaceae*. Only few hosts belong to leguminous plants *(Fabaceae)*. As far as we know, among the woody plants only *Vitis vinifera* was reported as a natural host of this virus (Giunchedi 1973, cit. by Purcifull and Edwardson 1981). The present paper is the second report of a woody plant as natural host of PVX.

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SAŽETAK

LABURNUM ANAGYROIDES MED. — PRIRODNI DOMAĆIN X VIRUSA KRUMPIRA

Nada Pleše i Mercedes Wrischer

(Botanički zavod, Prirodoslovno-matematički fakultet Sveučilišta u Zagrebu i Institut »Ruđer Bošković«, Zagreb)

Iz ukrasnog grma *Laburnum anagyroides* s mozaičnim simptomima na listovima izdvojen je X virus krumpira (potato virus X). Grm je potjecao iz jednog neuređenog parka na području Zagreba. Virus je identificiran na osnovi simptoma na pokusnim biljkama, pojavi karakterističnih ultrastrukturnih promjena u stanicama pokusnih domaćina i pozitivnog serološkog testa. Koliko nam je poznato, taj rad predstavlja drugi izvještaj o nalazu X virusa krumpira u drvenastom domaćinu.

Doc. dr. Nada Pleše
Prirodnoslovno-matematički fakultet
Marulićev trg 20/II
YU-41000 Zagreb (Jugoslavija)

Dr. Mercedes Wrischer
Institut »Ruđer Bošković«
Bijenička 54
YU-41000 Zagreb (Jugoslavija)