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OCCURRENCE OF CARNATION VEIN MOTTLE
AND CUCUMBER MOSAIC VIRUSES ON
CARNATIONS IN YUGOSLAVIA

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Carnation vein mottle virus (CarVMV) was isolated from glasshouse-grown *Dianthus caryophyllus* L. originating from Split. The same virus was obtained also from *D. caryophyllus*, *D. carthusianorum* L. and *D. barbatus* L. which were grown in the open in Zagreb and the surroundings. The virus was identified on the basis of host plants, non-persistent mode of transmission by *Myzus persicae* Sulz. and by analysis of ultrastructural changes in host cells. Light microscopic inclusions of CarVMV in the epidermal cells of *Chenopodium quinoa* Willd. and *C. amaranticolor* Coste et Reyn. leaves are described. Three forms of these inclusions are illustrated: granular bodies, bodies showing strong light diffraction and containing vacuoles, and aggregates of cylindrical cytoplasmic inclusions in the form of clusters of needles.

Cucumber mosaic virus was isolated from *D. caryophyllus* which was grown in the open in Zagreb.

This is the first finding of the two viruses on carnations in Yugoslavia.

Introduction

The first data concerning the occurrence of carnation viruses in Yugoslavia are given by Šarić et al. (1972). These authors established the presence of carnation mottle virus (CarMV) and carnation ringspot virus (CarRSV) using test plants and serological tests for identification of CarMV and test plants reactions for identification of CarRSV. Šarić et al. (1972) found that a large number of carnation cultivars grown under the glass in the coastal area and the inland parts of the country were

infected with CarMV. Not long ago Kačić (1980) also investigated glasshouse-grown carnations and ascertained that 14 cvs out of 15 were infected with CarMV.

In this paper we bring out the data which show that in addition to the viruses mentioned two other viruses are present on carnations in Yugoslavia: carnation vein mottle virus (CarVMV) and cucumber mosaic virus (CMV). We also describe light microscopic inclusions in cells infected with CarVMV.

Material and Methods

Material

We used several sources for the isolation of viruses from carnations. The first experiments were performed on six cvs of *Dianthus caryophyllus* L. with double flowers which were purchased in a flower shop in Split. In addition, *D. caryophyllus* and *D. carthusianorum* L. were used which grew in the University Botanic Garden, Zagreb. These plants had simple flowers and were cultivated outdoors. The specimens of *D. barbatus* L. were collected at the village of Odranski Obrež near Zagreb, where they were cultivated outdoors in a small backyard garden.

Methods

Virus transmission by aphids. Specimens of aphid *Myzus persicae* Sulz. were used for separation of CarVMV from a mixture with CarMV. Transmission was performed in the non-persistent manner. After being starved for two hours the aphids fed for 2 to 3 min on diseased plants of *D. barbatus* and were then transferred in 4 groups of 15 aphids to four young plants of the same species. After two days the aphids were destroyed with insecticide and the plants were placed in an insect-proof glasshouse. The other transmissions with *M. persicae* were also performed in the non-persistent manner.

Light microscopy. Thin sections containing the intact upper epidermis from the region of the chlorotic spots were prepared in order to investigate the cells of infected tissue by light microscopy. Sections were placed in the tap water and were examined in living state, or were stained with Lugol's solution [0.7% (w/v) aqueous solution of potassium iodine containing 0.3% (w/v) of iodine].

Electron microscopy. Leaves of *Chenopodium quinoa* Willd. and afterwards again fixed in 1% OsO₄ during 2 h. Fixed pieces of fixed in 1% (v/v) glutaraldehyde in cacodylate buffer during 30 min and afterwards again fixed in 1% OsO₄ during 2 h. Fixed pieces of tissue were dehydrated in ethanol and then embedded in Araldite resin. Ultrathin sections were stained with uranyl acetate and lead citrate. Finally, the sections were investigated with electron microscope.

Serological experiments. Serological experiments with CMV were done by double diffusion method in 0.7% agarose with 0.05% NaN₃ which was prepared in distilled water. The inoculated *C. quinoa* leaves or systemically infected shoots of *Silene armeria* L. were the source

of viral sap. The serum used against Car strain of CMV from carnation (L o v i s o l o et al. 1968) contained besides antibodies against the whole virus particles (titre 1:128) also antibodies against decomposed (soluble) protein, i. e. protein subunits (titre 1:64). It was supplied earlier by Dr E. L u s i o n i, Torino.

Serological tests with CarMV were performed in gels of agar prepared with physiological solution and by means of a serum made in our laboratory earlier.

Results

Most of carnation plants with double flowers were infected with CarMV. This was established by serological tests in which crude carnation sap was used.

Finding of carnation vein mottle virus

The first isolate of CarVMV derived from a carnation plant with double flowers of a dark violet colour originating from Split. The plant contained CarVMV in a mixture with CarMV. The mixture of viruses was first transmitted mechanically to *D. barbatus* from which CarVMV was isolated by *Myzus persicae* to healthy specimens of *D. barbatus*. By means of the serum against CarMV it was repeatedly established that CarMV was eliminated. After a month chlorotic changes appeared on the young plants infected by aphids (Fig. 1 C). During aging of the leaves the chlorosis gradually disappeared so that the older leaves were normally green. Similar symptoms on *D. barbatus* are cited in the literature for other isolates of CarVMV (K a s s a n i s 1955). CarVMV freed of CarMV was then transmitted to test plants *C. quinoa* and *C. amaranticolor* Coste et Reyn. The former species reacted with local and systemic symptoms (Fig. 1 B), and the latter only with local lesions on inoculated leaves (Fig. 1 A). The morphology of the symptoms corresponded also to the changes described by other authors for CarVMV (H o l l i n g s 1956, H o l l i n g s and S t o n e 1971). Since *C. amaranticolor* did not react systemically, it was clear that the species was not infected with a mixture which contained also carnation latent virus, the virus also transmissible by aphids in a non-persistent manner (W e t t e r 1971). The symptoms on *Silene armeria* were a chlorotic mosaic or small chlorotic spots on young apical leaves, and large chlorotic spots with brown necrotic margins and a general chlorosis on old leaves (Fig. 1D, E). In some specimens the intercostal yellowing was obvious.

Infected tissue of *C. quinoa* and *C. amaranticolor* was examined by light microscopy. Virus inclusions were found in the epidermal strips taken from old chlorotic lesions, but only from the plants cultivated at low temperature. The inclusions were often beside the nucleus and had the form of granular bodies (Fig. 2A, B) or they were more compact bodies exhibiting high light diffraction and usually containing vacuoles (Fig. 2C, D). Some inclusions appeared as clusters of needles which represented the aggregates of cylindrical cytoplasmic inclusion bodies (Fig. 2E). According to their appearance, the inclusion bodies were similar to the inclusions which are often seen with a light microscope in cells infected by a large number of different potyviruses (cf. E d w a r d s o n 1974). Under the

influence of Lugol's solution the first two types of inclusions were stained more intensely than the nuclei and were more conspicuous than those of unstained living cells.

For reliable identification of the virus, the analysis of infected tissues was performed with electron microscope. In the ultrathin sections of infected cells, cylindrical cytoplasmic inclusions in the form of pin-wheels, loops, scrolls, tubes, and laminated aggregates without expressed striation were present (Figs. 3, 4A, 5A, B). Cylindrical inclusions resembled those of the viruses belonging to the third subdivision of potyviruses (Edwards 1974) and their configuration corresponded to the one described for CarVMV previously (Weintraub and Ragetli 1970, Edwards 1974). In addition to that, a group of parallelly arranged virus particles forming a sheet near the periphery of the cytoplasm was observed (Fig. 5B). Such ultrastructural particularities of CarVMV have been described and studied by Weintraub and Ragetli (1970). Special vesicles were often present in places where cylindrical inclusions were developed (Fig. 4A). Most of the vesicles were surrounded by two or more concentric envelopes resembling myeline figures. Sometimes a very fine net of fibrils was visible in the vesicles. These structures are also present on micrographs of CarVMV infected cells published by Weintraub and Ragetli (1970).

Besides the above described isolate of CarVMV, we obtained three more isolates of the same virus. One of them derived from *D. barbatus*, one from *D. carthusianorum* and one was isolated together with CMV from *D. caryophyllus*. All three isolates were analysed by means of test plants and by transmission with aphids, and the intracellular changes they provoked in infected cells were studied. These investigations did not show considerable differences among the isolates.

Twelve virus particles of the isolate from *D. carthusianorum* were micrographed in preparations made by dipping method. The particles were in the form of flexuous rods (Fig. 4B), and their normal length was 770 nm.

Finding of cucumber mosaic virus

Four specimens of *D. caryophyllus* were gathered in the Botanic Garden, and two of them were infected with CMV. The virus was isolated by mechanical transmission to *C. quinoa*. From one plant it was obtained together with CarVMV from which it was separated by passage through cucumber.

CMV was mechanically transmitted to 15 host plants. *Chenopodium amaranticolor*, *C. murale* L., *C. quinoa*, *Phaseolus vulgaris* L. and *Vicia faba* L. reacted locally, i. e. without systemic infection. However, *Cucumis sativus* L. cv Delicatess, *Cucurbita pepo* L., *Datura stramonium* L., *Nicotiana clevelandii* Gray, *N. glutinosa* L. cv Corvallis strain, *N. megalosiphon* Heurck et Muell., *N. tabacum* L. cv Samsun, *Silene armeria* and *Spinacia oleracea* L. cv Matador reacted systemically. Symptoms which developed on test plants were characteristic of many CMV isolates and they mainly corresponded to the symptoms described for Car strain of CMV (cf. Lovisolo et al. 1968).

Crude sap obtained from leaves infected with our isolate reacted with the serum against Car strain of CMV with the apparition of a right precipitation line (Fig. 6A) specific for a decomposed virus protein

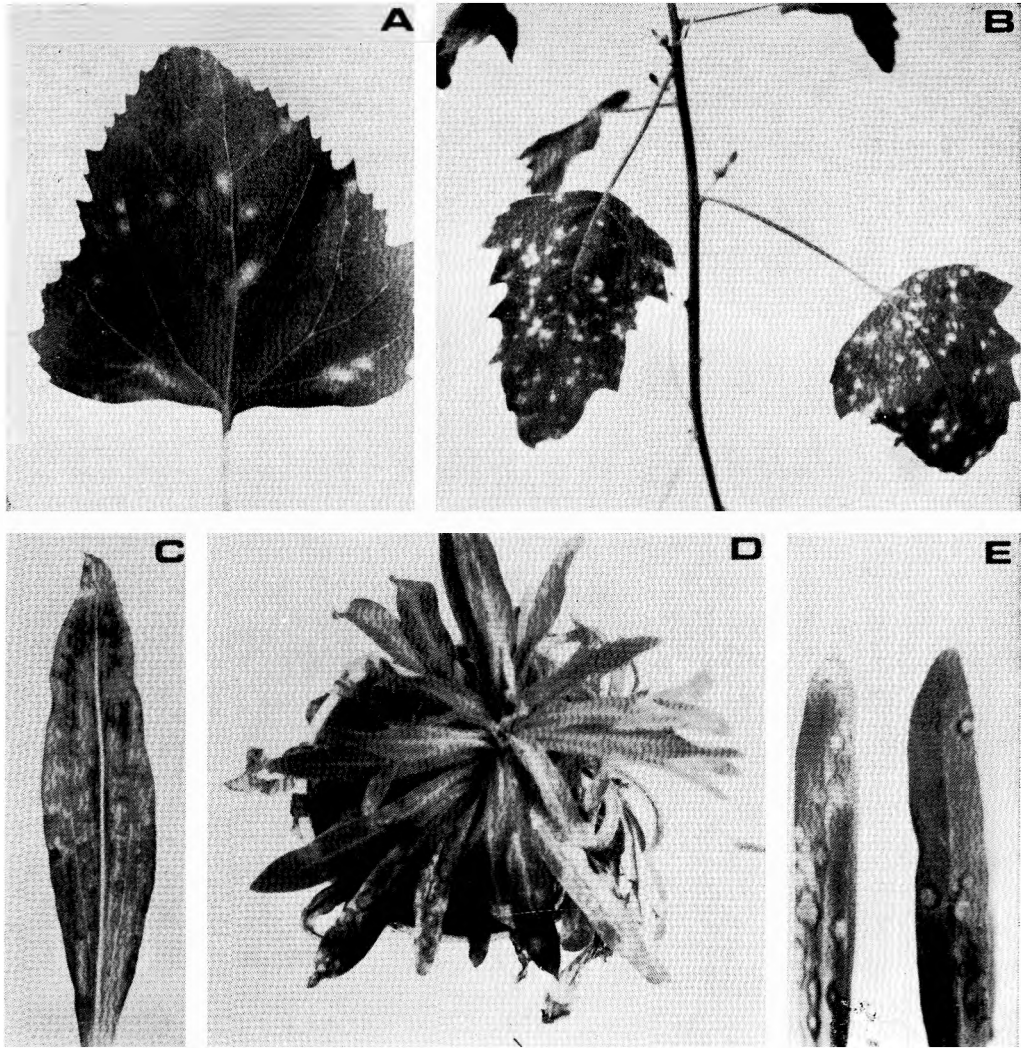


Fig. 1. Effect of CarVMV on test plants. **A.** Chlorotic lesions in inoculated leaf of *Chenopodium amaranticolor*. **B.** Severe yellowish flecks in systemically infected leaves of *C. quinoa*. **C.** Yellow green mottling in systemically infected leaf of *Dianthus barbatus*. **D.** Systemic symptoms induced in *Silene armeria*. **E.** Old leaves of *S. armeria* showing symptoms similar to those in **D.**

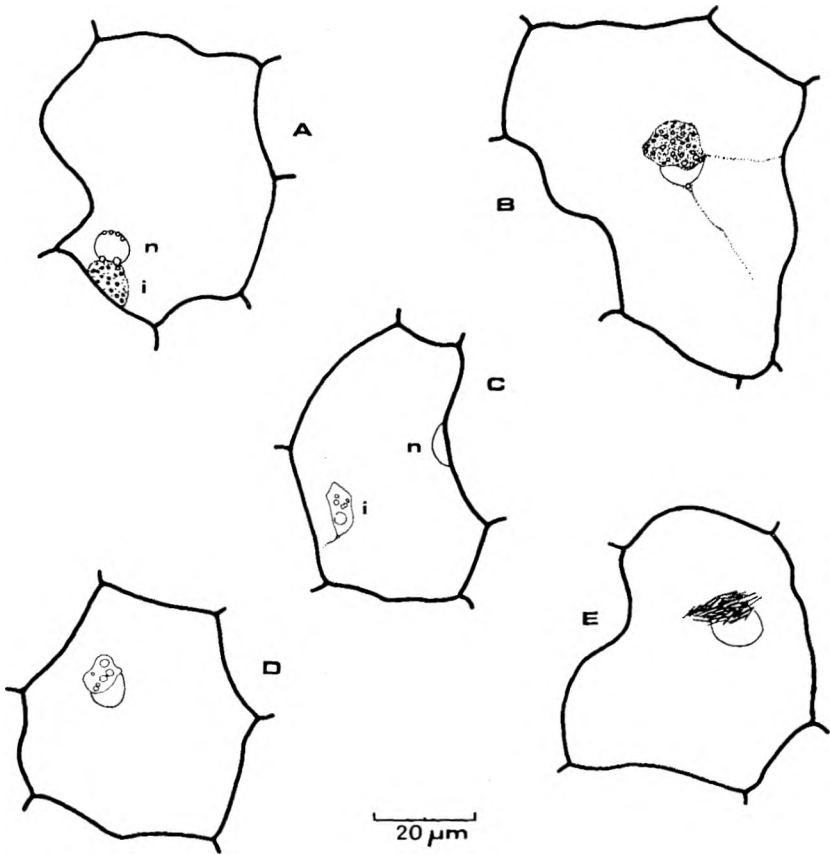


Fig. 2. Drawings of inclusion bodies induced by CarVMV in epidermal cells of *Chenopodium quinoa*. **A, B.** Granular bodies near the nuclei. **C, D.** More compact vacuolate inclusions. **E.** Cluster of needles (aggregate of cylindrical cytoplasmic inclusions, side view).



Fig. 3. Electron micrograph showing various aspects of cylindrical cytoplasmic inclusions in ultrathin sections of *Dianthus barbatus* leaf cell infected with CarVMV. Pin-wheel (pw), loops (l), laminated aggregate (la), tube (t).

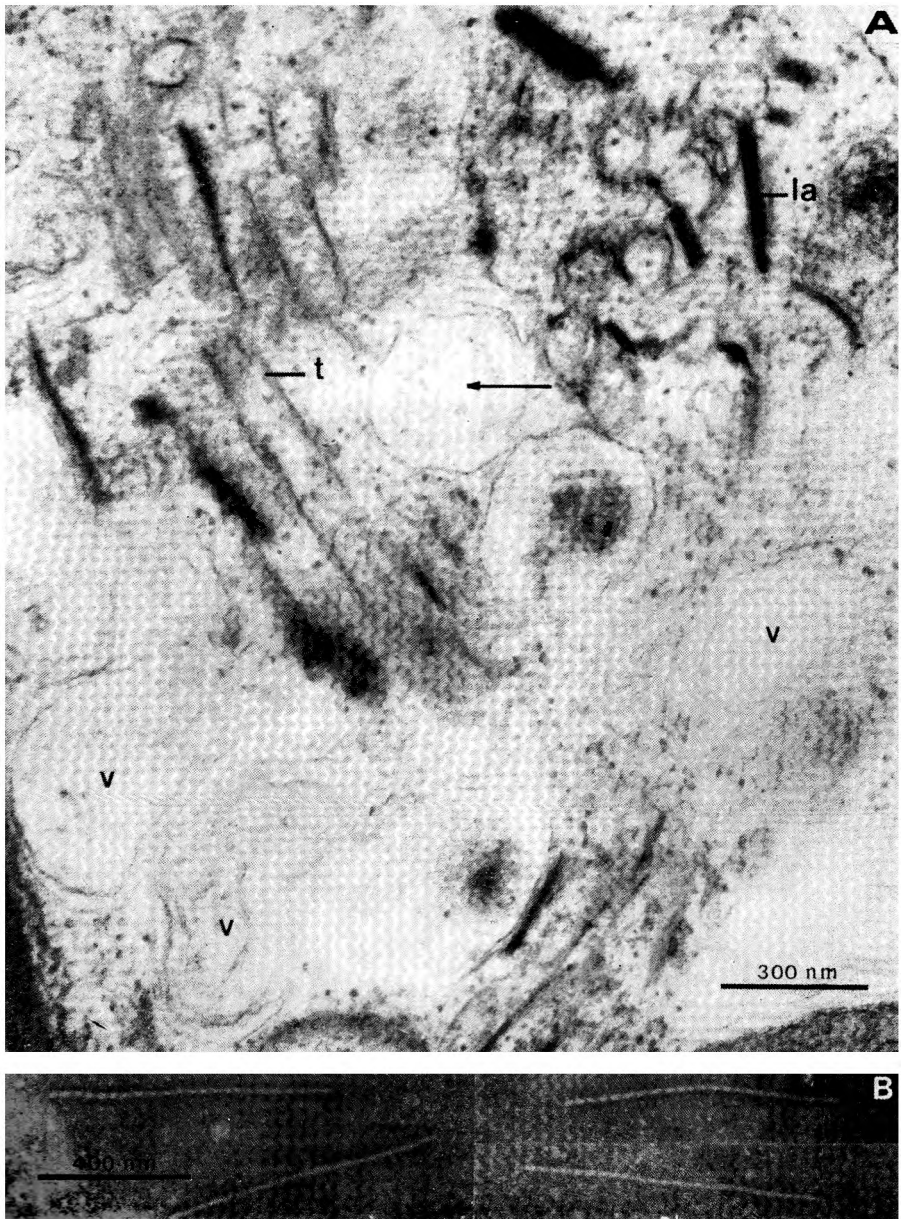


Fig. 4. **A.** As in Fig. 3. showing numerous vesicles with concentric envelopes (v) close to the cylindrical inclusions. Vesicle containing a net of fibrils reminiscent of nucleic acid (arrow), laminated aggregate (la), tube (t). **B.** Electron micrograph of CarVMV particles in crude sap from infected *C. quinoa* stained with phosphotungstate.

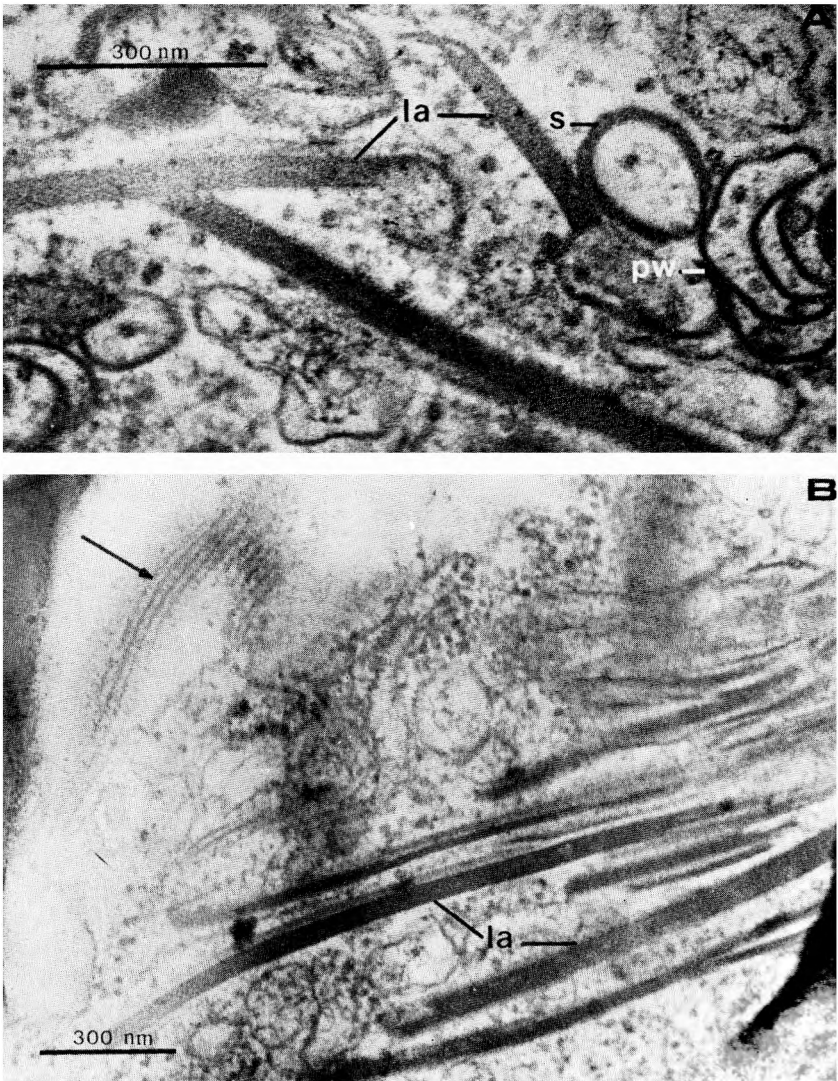


Fig. 5. Pin-wheels (pw), laminated aggregates (la) and scrolls (s) induced by CarVMV in *C. quinoa*. Note the aggregate of virus particles (arrow) (micrographed by Z. Erić, Faculty of Science, Sarajevo).

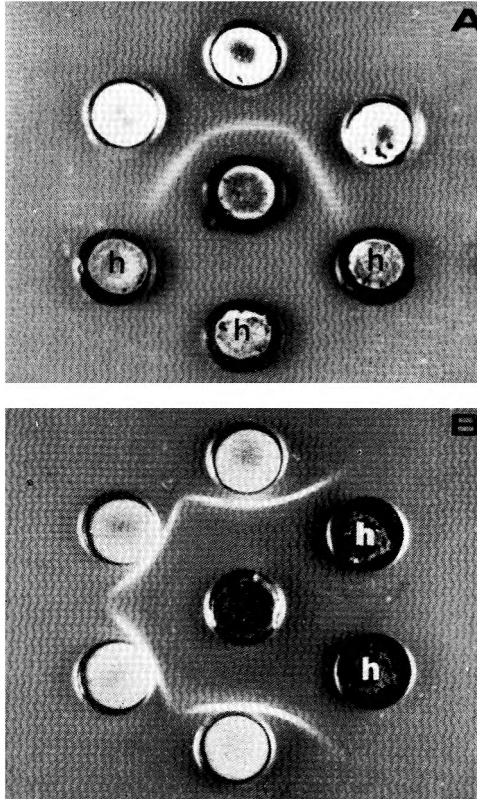


Fig. 6. Immunodiffusion reactions obtained with Yugoslav isolate of CMV from carnation (outer wells) and antiserum against CMV-Car (centre wells). In **A** the peripheral wells contain the isolate in raw sap of *S. armeria*, in **B** in raw sap extracted by stabilizing buffer. Sap from healthy *S. armeria* (h).

(Scott 1968). Virus stabilization was achieved by means of 0.1 M citrate buffer pH 6.5 with 20 mM ethylene diamine tetra-acetate and 0.1% thyo glycollic acid (Devergne et al. 1972, Štefanac et al. 1981) which was added to the leaves before squeezing the sap in proportion 1 ml/l g. In this case a specific virus line appeared next to the virus well together or without a non specific line of soluble virus protein (Fig. 6B). The serum titre with the whole virus was 1 : 128. i. e. it was equal to the homologous virus titre. During the winter *S. armeria* was a better source of the virus than *C. quinoa* which at a low temperature and shorter day was very sensitive and reacted usually with necrosis.

Discussion

No doubt CarMV is the most dangerous virus for carnation cultures in Yugoslavia. During our investigation it was present in nearly all commercial carnation plants. In order to reduce the number of infected carnations Institute for Adriatic Plant Cultures and Melioration of Karst in Split has been making efforts for several years to eliminate CarMV from commercial carnation cultivars and to breed healthy stocks by means of the tip meristem culture (Kačić et al. 1982).

The other virus which was found earlier on carnations in Yugoslavia is CarRSV (Šarić et al. 1972). This virus is also very infectious and resistant. It is readily transmissible from plant to plant during handling with infected carnations. Infected plants can easily be recognized by obvious symptoms and therefore this virus has practically been eradicated from all plantations. We were not able to find CarRSV during this investigation. In a detailed review concerning virus control of ornamental plants Lawson (1981) has recently enumerated six viruses which present a great problem in the culture of carnations. CarRSV is the last in this list.

Of greater importance for the cultivation of carnations is CarVMV which is the second in Lawson's list, immediately after CarMV. CarVMV is spread in all places where carnations are cultivated, especially in the Mediterranean region. Therefore, it is not surprising that during the search for carnation viruses we immediately obtained four isolates of CarVMV.

The fourth virus concerned is CMV. The reaction of test plants and serological experiments show that our isolate is similar to the carnation isolate of CMV which has been studied by Lovisolo et al. (1968). Regarding the significance of CMV for the culture of carnations, it seems that it is not great. This virus is not quoted in Lawson's list which contains six most important carnation viruses.

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S A Ž E T A K

NALAZ VIRUSA IŠARANOSTI ŽILA KARANFILA I VIRUSA MOZAIKA KRASTAVCA
NA KARANFILIMA U JUGOSLAVIJI

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Iz karanfila (*Dianthus caryophyllus* L.) s punim cvjetovima uzgojenog u Splitu te iz vrsta *D. caryophyllus*, *D. carthusianorum* L. te *D. barbatus* L., koje su rasle na otvorenom u Zagrebu i okolici, izoliran je virus išaranosti žila karanfila (CarVMV). Virus je identificiran na osnovi reakcije domaćina, neperzistentnog prenošenja s pomoću *Myzus persicae* Sulz. te analize ultrastrukturnih promjena. U stanicama epiderme lista zaraženih primjeraka *Chenopodium quinoa* Willd. i *C. amaranticolor* Coste et Reyn. opisane su svjetlosnomikroskopske inkluzije od CarVMV, i to tri tipa: zrnata tijela, tijela koja jače lome svjetlo i sadržavaju vakuole, te agregati cilindričnih citoplazmatskih uklopina koji nalikuju nakupinama iglica.

Iz karanfila *D. caryophyllus*, koji je rastao na otvorenom u Zagrebu, izoliran je virus mozaika krastavca (CMV).

Ovo je prvi nalaz CarVMV i CMV na karanfilima u Jugoslaviji.

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