Acta Bot. Croat. 43, 7—12, 1984.

CODEN: ABCRA2
YU ISSN 0365—0588

UDC 576.858.8:582.669.2(497.1)=20

OCCURRENCE OF CARNATION NECROTIC FLECK VIRUS IN YUGOSLAVIA

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Received February 1, 1984

Infection with carnation necrotic fleck virus (CNFV) was established in cultivations of carnation (*Dianthus caryophyllus* L.) in Zagreb and Split. The disease was detected on the basis of characteristic symptoms which appeared as yellow and reddish necrotic streaks and spots on the leaves. From carnation the virus was transmitted by *Myzus persicae* into *D. barbatus* L. In this species alterations appeared after 2 to 3 weeks in one or two pairs of adjacent decussated leaves in the form of discoloration of leaf veins and wilting of the leaves. The virus was proved by the appearance of characteristic aggregates of filamentous virus particles c. 1380 nm long in thin sections, and by positive reaction with antiserum to CNFV.

CNFV is the fifth virus detected on carnations in Yugoslavia.

Introduction

The first survey of virus diseases present on carnation *(Dianthus caryophyllus)* in Yugoslavia was performed by Šarić et al. (1972) who established that carnation mottle virus (CarMV) is widespread in the glasshouses. They registered the finding of carnation ringspot virus (CarRSV). Recently Bezić et al. (1983) detected carnation vein mottle virus (CarVMV) in plants grown under the glass and in the open, and isolated cucumber mosaic virus from specimens which grew in the open. According to the data given by Lawson (1981) at the present time there are six harmful viruses spread in the glasshouse cultures of carnation. Consequently, we expected that in addition to CarMV, CarRSV and CarVMV some other dangerous carnation viruses might be present in this country.

In this report we give the data on detection of a closterovirus, the carnation necrotic fleck (CNFV) in Yugoslavia. The virus has been described in detail by Inouye and Mitsuhata (1973) and by Inouye (1974).

Material and Methods

Material

The symptoms of CNFV were detected on *D. caryophyllus* plants grown in the glasshouses of the firm »Žitnjak« situated in Zagreb and Ivanić-Grad. The same symptoms were noticed on carnations in a glasshouse of a private grower in Split. Diseased specimens displayed yellow or reddish, partially necrotic, streaks and spots on the leaves (Fig. 1. A). The number of plants with these symptoms was fairly large, and diseased specimens were usually situated near the entrance doors of the glasshouses. It is very probable that such distribution of infected carnations was connected with the manner of transmission of CNFV by aphids, which were also more abundant near the entrances.

Only plants with strong symptoms of CNFV, in which CarMV could not be detected serologically, served for analysis.

Methods

Transmission by aphids. Since CNFV is transmitted semi-persistently by a green peach aphid *(Myzus persicae)*, we used apterous forms of this particular aphid species for isolation of the virus. For transmission, the aphids were allowed to starve for a period of 1 h and then to feed for 24 h on infected leaves. Thereafter they were transmitted in groups of 12 to virus-free plants of *D. barbatus* which were treated with an insecticide the next day.

Electron microscopy. The leaf dip technique was used to detect individual virus particles and to determine their size. The cut surfaces of *D. barbatus* leaf infected experimentally by *M. persicae*, and of naturally infected *D. caryophyllus* leaves were dipped for contrasting into potassium phosphotungstate. The measurement of the lenght of particles was done from electron micrographs by a flexible and neatly fitted small metal chain.

Thin sections were prepared from leaves showing severe symptoms. Small pieces of tissue were fixed for 30 min in 1% (v/v) glutaraldehyde in cacodylate buffer and postfixed in 1% (w/v) OsO_4 for 2 h. After dehydration through graded ethanol, the pieces were embedded in Araldite resin. Thin sections were stained with uranyl acetate and lead citrate before examination in a Siemens Elmiskop I.

Serology. For serological detection of CNFV the antiserum kindly supplied by Dr G. L. Rana (Bari, Italy) was used. Antiserum had a titre of 1/32 and could be applied in double diffusion test. To make possible virus diffusion through agar, the virus sap was treated with 0.3 M ethanolamine at pH 10.5 according to the instructions given by Rana et al. (1977, cf. Shephard and Grogan 1967).

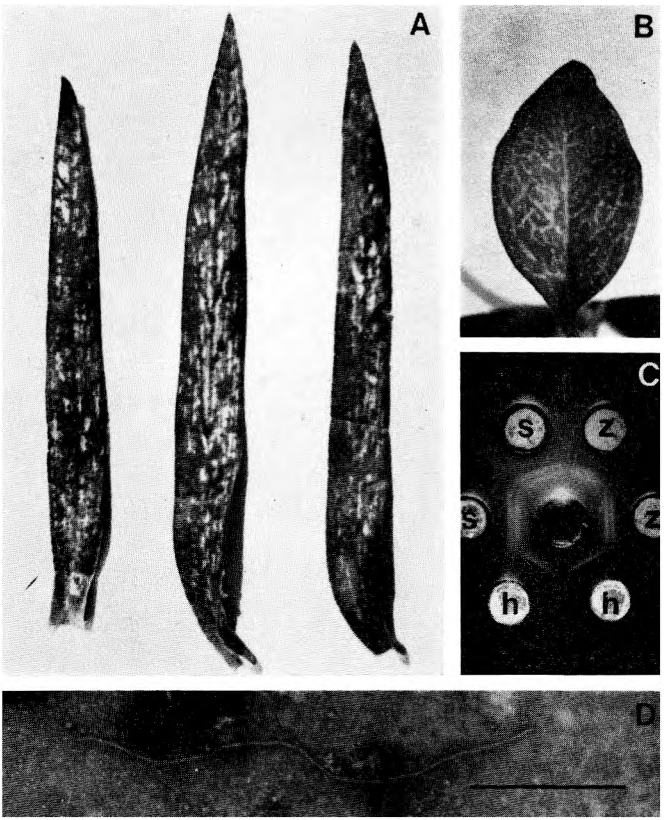

Fig. 1. A. Necrotic flecks and spots in naturally infected leaves of *Dianthus caryophyllus*. B. Pronounced whitish veinal necrosis in leaf of *D. barbatus* plant infected by aphids. C. Gel-diffusion serological test (plant extracts treated with ethanolamine); central well — antiserum to CNFV, peripheral wells — extracts from virus infected *D. caryophyllus* plants of Zagreb (z) and Split (s) origin and from healthy plant (h). The lines nearer to the antigen wells represent the reaction of CNFV, those nearer to the antiserum well the reaction of normal plant proteins. D. Virus particles in a leaf dip preparation of *D. barbatus*. Bar represents 500 nm.

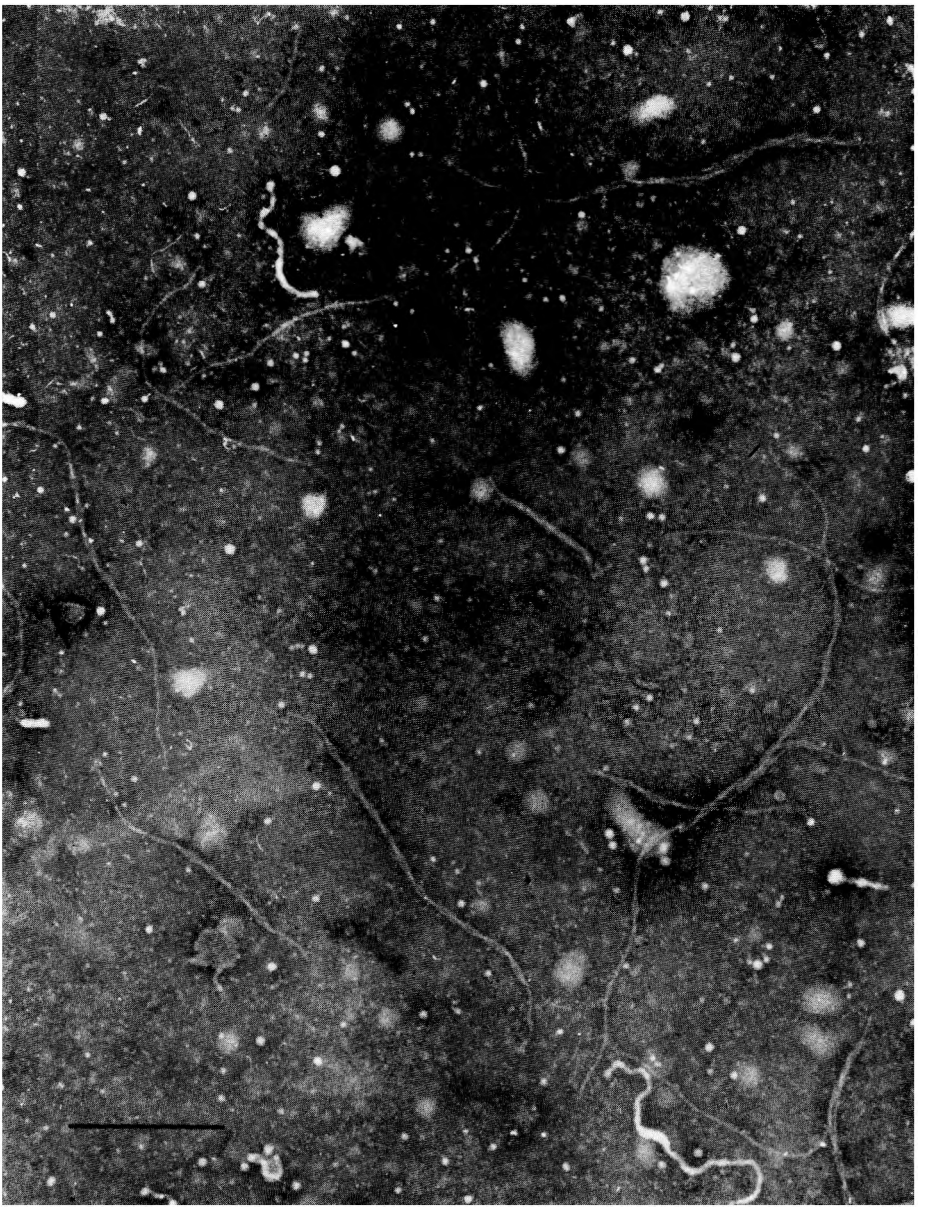

Fig. 2. Virus particle in a leaf dip preparation of *Dianthus caryophyllus*. Bar represents 500 nm.

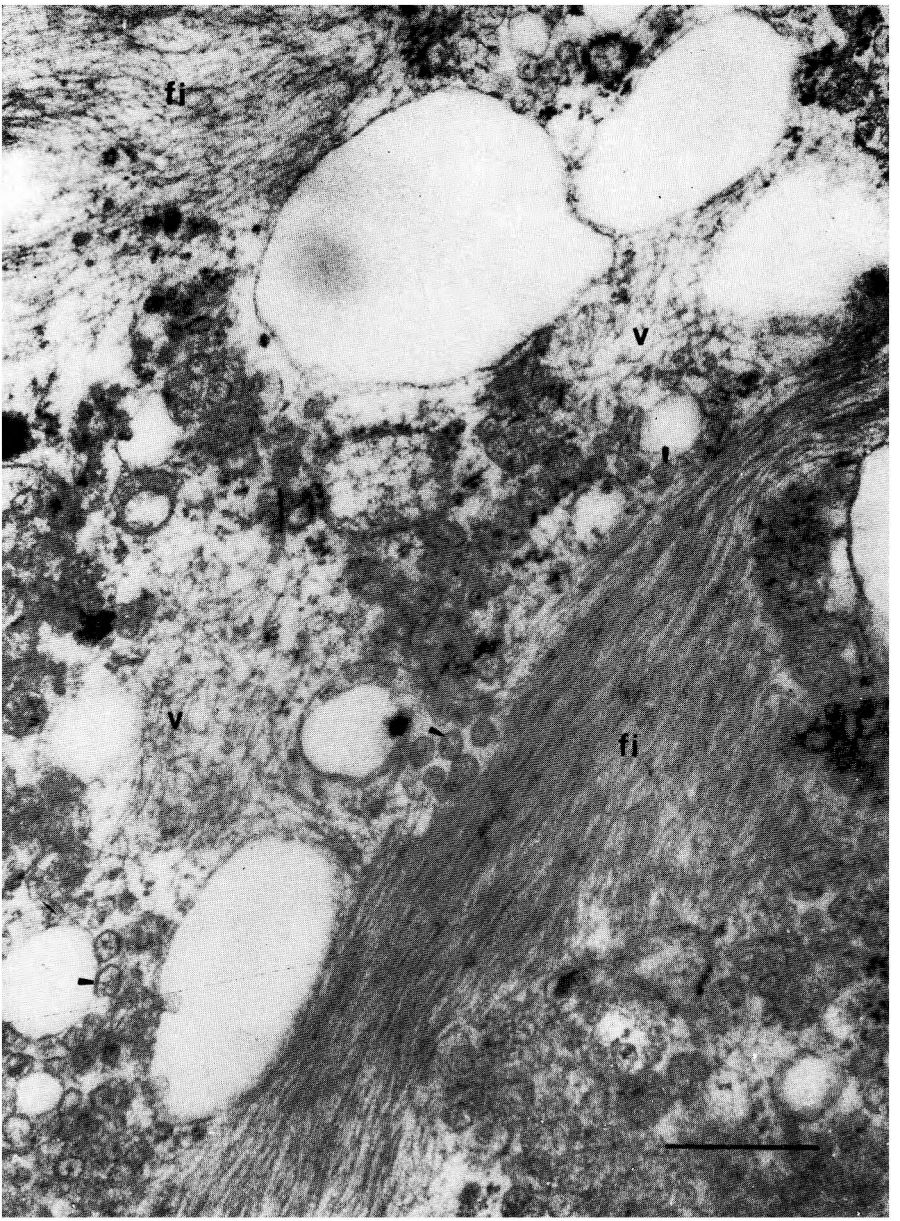

Fig. 3. Thin section of infected *Dianthus caryophyllus* leaf cell showing fibrous (fi) and orderless (v) masses of virus particles, and numerous vesicular strutures (arrow-heads). Bar represents 500 nm.

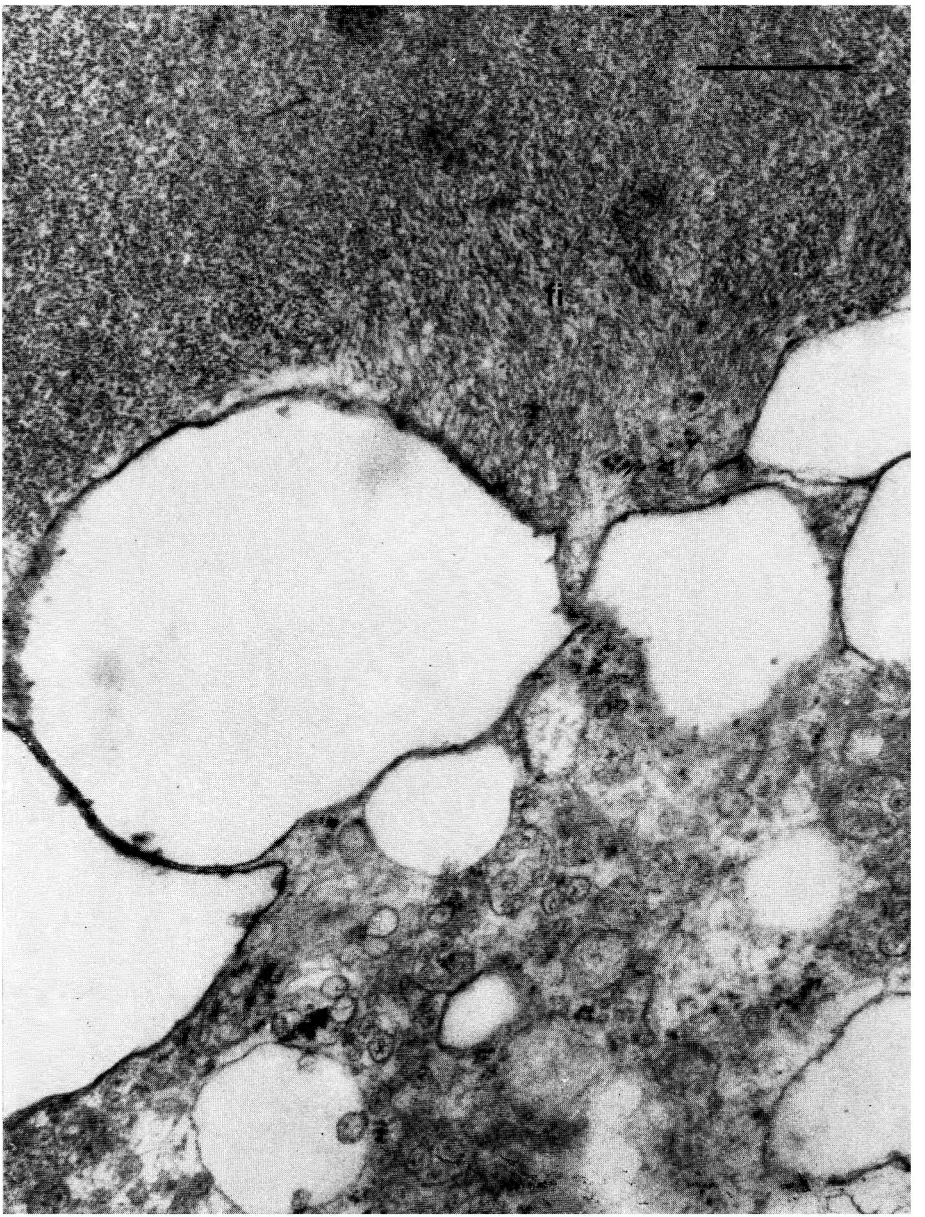

Fig. 4. Thin section of infected *Dianthus caryophyllus* leaf cell showing the virus aggregates (fi) in cross section, and numerous vesicular structures. Bar represents 500 nm.

Results

Transmission to and symptoms in D. barbatus

On the basis of characteristic symptoms on *D. caryophyllus* (see Material and Methods) we suspected that CNFV was quite widespread in the glasshouses and in the open fields in Yugoslavia. By means of *M. persicae* the virus was transmitted from a naturally infected *D. caryophyllus* to 5 out of 14 specimens of *D. barbatus* used in the experiment. The changes characteristic of CNFV appeared 3—4 weeks after infective feed. They consisted of discoloration of leaf veins which lost their green colour and became whitish or yellowish (Fig. 1. B). Discoloration was most frequently present only on two pairs of adjacent decussated leaves which soon died and dessicated. Regularly, the symptoms were not manifested on the upper leaves which retained their green colour for a long time. Alterations ordinarily showed only in one shoot of a particular plant while other branches were developed quite normally.

Attempts to transmit the virus to *D. barbatus* mechanically were not successful. Although *D. barbatus* following sap inoculation occasionally responds by appearance of necrotic local lesions, mechanical transmission seems extremely difficult (Smookler and Loebenstein 1974).

Particle size

A relatively small number of long and filamentous virus particles were present in leaf dip preparations from altered parts of infected *D. barbatus*. Their length measured c. 1380 nm, and the width c. 12 nm (Fig. 1. D). In preparations obtained from naturally infected *D. caryophyllus* virus particles were more abundant (cf. Fig. 2). The normal length of 20 measured particles also amounted to c. 1380 nm.

This characteristics correspond to the morphology and size of CNFV particles.

Ultrastructural observations

In thin sections of altered *D. barbatus* leaves with symptoms of whitish veins, the protoplasts of the cells appeared seriously damaged. Numerous loosely arranged virus particles were present in most of the cytoplasm.

To complete the information on ultrastructural changes, thin sectioning was done through naturally infected *D. caryophyllus* leaves. The cytoplasm of infected cells showed a large amount of parallelly aggregated virus particles which formed so-called fibrous inclusions (cf. Lister and Bar-Joseph 1981) (Fig. 3). Orderless masses of virus particles were also present. In some places the fibrous aggregates were cut transversally and virus particles appeared as densely arranged dots (Fig. 4). From cross sections of virus particles, their diameter was determined to amount to c. 12 nm. Membranous vesicles commonly containing a net

of fibrils occurred in the vicinity of virus aggregates (Figs. 3 and 4). In certain instances a group of vesicles was found surrounded by a common membrane. On the basis of measurement of 50 vesicles, their diameter was established to vary from 75 to 110 nm. The described vesicular structures as well as dense virus aggregates are known to be associated with infections by a number of closteroviruses including CNFV (cf. Lister and Bar-Joseph 1981, Bar-Joseph and Murant 1982). The role of the vesicles is not known, but there are opinions that they might be considered as deposits of viral nucleic acid.

Serological tests

In serological tests the peripheral wells were filled with plant sap and the central well with the serum to CNFV. To compare the sap from infected carnations from Zagreb with that obtained from diseased plants originating from Split, they were placed in adjacent wells. Several hours after the placement of reactants a positive reaction appeared (Fig. 1. C). This proved as conclusive evidence of the presence of CNFV in carnations. The virus was serologically detected in many samples of carnation plants tested.

Discussion

CNFV was found in several localities in Yugoslavia and undoubtedly is also widespread in other plantatons of *D. caryophyllus* in the country. The virus was investigated in particular by Inouye and Mitsuhata (1973) in Japan. Later it was described by Smookler and Loebenstein (1974) in Israel. These works initiated detection of the virus in other Mediterranean countries, so it was detected by Poupet et al. (1975) in France and by Rana et al. (1977) in Italy. This paper is a further contribution to the knowledge of the distribution of CNFV.

Regarding the particle length CNFV belongs to the second subgroup of closteroviruses (Bar Joseph and Murant 1982). In addition to CNFV, a closterovirus beet yellows was earlier established in Yugoslavia (Panjan 1951, Nikolić 1951). The members of the second subgroup of closteroviruses are transmitted by aphids and cause intracellular inclusions including the vesicles mentioned. The cytoplasmic membranous vesicles of CNFV (Inouye and Mitsuhata 1973) are particularly similar to those of above mentioned beet yellows (Esau and Hoefert 1971) and beet yellow stunt viruses. The similarity among the three viruses is also manifested in relation to their particle length, their serological properties and amino acid composition (Lister and Bar-Joseph 1981).

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SAŽETAK

NALAZ VIRUSA NEKROTIČNE PJEGAVOSTI KARANFILA U JUGOSLAVIJI

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U nasadima karanfila (*Dianthus caryophyllus* L.) u Zagrebu i Splitu utvrđena je infekcija virusom nekrotične pjegavosti karanfila (carnation necrotic fleck virus, CNFV). Infekcija je ustanovljena najprije na osnovi karakterističnih simptoma u obliku žutih i crvenkastih nekrotičnih pjega na listovima. Iz karanfila virus je prenesen lisnom uši *Myzus persicae* na biljku *Dianthus barbatus* L. Na toj je vrsti nakon 2 do 3 tjedna došlo do alteracije nekoliko susjednih listova koja se očitovala u dekoloraciji žila i uginuću listova. Infekcija je dokazana i na osnovi prisustva karak-

teristićnih agregata nitastih virusnih čestica dužine oko 1380 nm u tkivu zaraženih biljaka, te reakcijom u gelu agara između imunog seruma protiv CNFV i samog virusa.

Ovo je peti virus koji je utvrđen na karanfilima u Jugoslaviji.

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