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CHERRY LEAFROLL VIRUS AND ELDERBERRY CARLAVIRUS ON *SAMBUCUS NIGRA* L. IN SOUTH-EAST EUROPE

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The virus symptoms of yellow net on *Sambucus nigra* leaves were observed in two localities for the first time in Yugoslavia. A detailed analysis of virus by means of test plants showed that the causal agent of yellow nets in both localities was cherry leafroll virus (CLRV). The yellow nets were investigated with two sera against CLRV, both of which reacted positively.

During these investigations, in many specimens of elderberries with yellow net symptoms, a large number of elongated virus particles about 680 nm long and 12 nm wide were found. The particles were observed by means of ultrathin sections and by means of dip preparations. This elongated virus is elderberry carlavirus (ECV), which was on this occasion found in Yugoslavia for the first time.

According to some data in the literature ECV is very probably present in Hungary and was found also in Tschechoslovakia many years ago. Therefore it can be claimed that ECV is spread in south-eastern Europe.

Introduction

The first find of a virus disease on elderberry shrubs in Yugoslavia was reported by Plavšić-Banjac and Miličić (1968) who found many infected shrubs in Sarajevo. This virus was later identified as CLRV by Štefanac (1969). The same author prepared an antiserum against CLRV and established that this serum was able to make spurs with CLRV from rhubarb and from cherry in agargel tests.

Later Grbelja (1972) investigated the spreading of this elderberry virus in Yugoslavia with a serum against CLRV from *Sambucus nigra*. She found this virus in various parts of Slovenia, Croatia and Bosnia. The other parts of Yugoslavia have not been investigated till now. The same author has found that on the mountain Medvednica near Zagreb many wild red-berried elders (*Sambucus racemosa* L.) are infected with CLRV. By means of serological tests in agargel Grbelja established that the virus of *S. racemosa* was serologically identical with the virus from *S. nigra* because the precipitation lines of saps of CLRV from *S. nigra* and *S. racemosa* coalesced without the formation of spur. CLRV from *S. racemosa* is important for forestry because it is transmissible by seeds (Schimanski and Albrecht 1980) in a high percentage of 24.7%.

By means of a serum against CLRV Mamula and Miličić (1975) established the presence of this virus in plants of *Sambucus ebulus* L. which are widely spread in the country. Afterwards, Jones (1976) confirmed that various CLRV isolates of *S. nigra, S. racemosa* and *S. canadensis* were serologicaly very similar, but he also established that CLRV infecting *S. ebulus* was serologically distinguishable.

In the time when the papers by Plavšić-Banjac and Miličić (1968), Štefanac (1969) and Grbelja (1972) were published, we did not know any finding place of yellow net on elderberry in Yugoslavia. About the year 1980 we found about five shrubs of *S. nigra* with yellow net in the village of Šestine north of Zagreb. Two years ago we found the second finding place of elderberry with yellow net symptoms in southern part of Zagreb.

Yellow or white nets consisted of yellow or white lines stretching along the leaf nerves and were especially well expressed on young leaves. The appearance of yellow net on elderberry is very conspicuous so that it cannot be easily overlooked (Fig. 1 A). According to the data in the literature (Schmelzer 1966, Murant 1980) three nepoviruses can cause yellow net symptoms on elderberry: CLRV, tomato black ring virus (TBRV) and arabis mosaic virus (AMV).

Material and Methods

Material

Sambucus nigra plants with obvious symptoms of yellow net were used for this study. They were found in two localities in the environs of Zagreb. The first group of plants consisted of seven elderberries growing in the southern outskirts of Zagreb. These plants were conspicuous because they had well developed symptoms of yellow net in the apical parts. On these parts along the leaf nerves, yellow or white lines were present (Fig. 1 A). Elderberry plants in this locality had almost exclusively the symptoms of yellow net during the spring. One plant of this group designated Zg served for most of the experiments described here.

Methods

Leaves of naturally infected *S. nigra* Zg with yellow net symptoms were taken for electron microscopy. Small pieces of leaves were fixed in 1% (v/v) glutaraldehyde in cacodylate buffer for 30 min and then

postfixed in OsO_4 for 2 h. The samples were dehydrated in alcohol series and embedded in Epon 812. The material was subsequently cut with a diamond knife. Ultrathin sections were stained with uranyl magnesium acetate (K i m u r a et al. 1975) and lead citrate. Finally, the sections were examined in a JEM 100 B electron microscope.

The serological tests were made by double diffusion method in agar gel. In these tests we used two sera against CLRV marked 28 and 50, which were prepared by G r b e l j a (1972) and had a titre 128. The sera were used undiluted and diluted 1 : 15. Healthy sap of *Chenopodium quinoa* was taken as control.

The plant material for serological tests was homogenized in a mortar by means of carborundum powder and 0.15 M phosphate buffer of pH 7. The mixture was filtered through a cheese-cloth and used for serological tests. The tests were performed by double diffusion method in 0.9% Bacto-agar gel containing 0.05% NaN_3.

The presence of elongated ECV in the leaves of elderberry shrubs was established by using the dip method and the negative staining with 2% phosphotungstic acid.

Results

Finding places and symptoms

Elderberry shrubs in the above mentioned locality in the s o u t h e r n p a r t of Zagreb had symptoms of yellow net in early spring almost exclusively. Only on one shrub the symptoms of yellow net were mixed partly with round chlorotic flecks. However, in the late spring, part of these shrubs changed the appearance and showed symptoms of the so called green net which was rich in chlorophyll. This symptom was characterized by the green colour of main nerves, but the interspaces (cell lumina were chlorotic and yellowish (Fig. 1 B).

The second finding place of elderberry shrubs with yellow net symptoms was situated in the n o r t h e r n s u b u r b s of Zagreb in the village of Šestine. In this place the symptoms of diseased elderberries were weak. Only some apical branches had yellow net symptoms. In the spring the symptoms of green net appeared with characteristic yellowish interspaces (Fig. 1B). The third kind of symptoms consisted of small round chlorotic flecks on leaflets. Somewhere the chlorotic spots coalesced formig a continuous chlorotic surface.

The symptoms of green net was observed earlier on young elderberries studied by P l a v š i ć-B a n j a c and M i l i č i ć (1968), Š t e f a n a c (1969, Pl. 1A) and by G r b e l j a (1972).

Host plants of the Zg isolate of CLRV

The first isolation of virus was performed from infected elderberry trees growing in the southern suburbs of Zagreb, which showed symptoms of well developed yellow net. This material was homogenized in a mortar by means of 0.15 M phosphate buffer of pH 7 in addition to 0.01% thioglycollic acid. The slurry was inoculated to carborundum dusted leaves of herbaceous hosts belonging to the plant families *Amaranthaceae, Chenopodiaceae, Fabaceae* and *Solanaceae*. During this activity several plant species were infected. The symptomatical response of these plants to infection is listed in Table 1.

Table 1. Host plants of the Zg isolate of cherry leafroll virus

Host plants	Symptoms (I: symptoms on inoculated leaves, II: symptoms on upper leaves)
AMARANTHACEAE	
Celosia cristata	I: necrotic spots II: yellow flecks
CHENOPODIACEAE	
Chenopodium amaranticolor Coste et Reyn.	I: chlorotic flecks and rings II: sometimes necrosis of plant (Fig. 2 A)
C. murale L.	I: necrotic flecks, leaves fallingoff II. wilting and necrosis of plant top (Fig. 2 B)
C. quinoa Willd.	I: chlorotic flecks and necrotic spots II: strong chlorosis
FABACEAE	
Phaseolus vulgaris L. cv. Pinto	I: chlorotic spots and flecks II: chlorotic flecks
SOLANACEAE	
Nicotiana clevelandii Gray	I: necrotic ringspots II: necrotic points and rings
N. glutinosa L.	without symptoms
N. tabacum L. cv. White Burley	I: necrotic rings and lines II: without symptoms
N. tabacum L. cv. Samsun	I: necrotic rings and recovery in upper leaves (Fig. 1 C and D)
N. megalosiphon Heurck et Muell.	I: necrotic rings and lines II: necrotic rings and lines

Fig. 1. Symptoms on plants infected with elderberry strain of CLRV. **A.** Young leaf of *Sambucus nigra* with yellow net symptoms. **B.** Develocotiana tabacum var. Samsun with necrotic spots, lines and rings. **D.** Upper leaf of N. tabacum of the same variety with necrotic lines and rings.

Fig. 2. **A.** *Chenopodium amaranticolor* with deformations, chlorosis and necrotic spots on leaves. **B.** *Chenopodium murale*, necrosis of plant. **C.** Serological test. Squeezed sap, derived from upper leaves of *Chenopodium quinoa* with intensive symptoms of infection with Zg isolate of elderberry strain of CLRV, was put in the left well. Infectious plant material from crushed leaves of young elderberry with obvious symptoms of yellow net (YN) was placed in the right well. In the lower well the serum against elderberry strain of CLRV was set. The appearance of the white precipitation line shows that the reaction was positive.

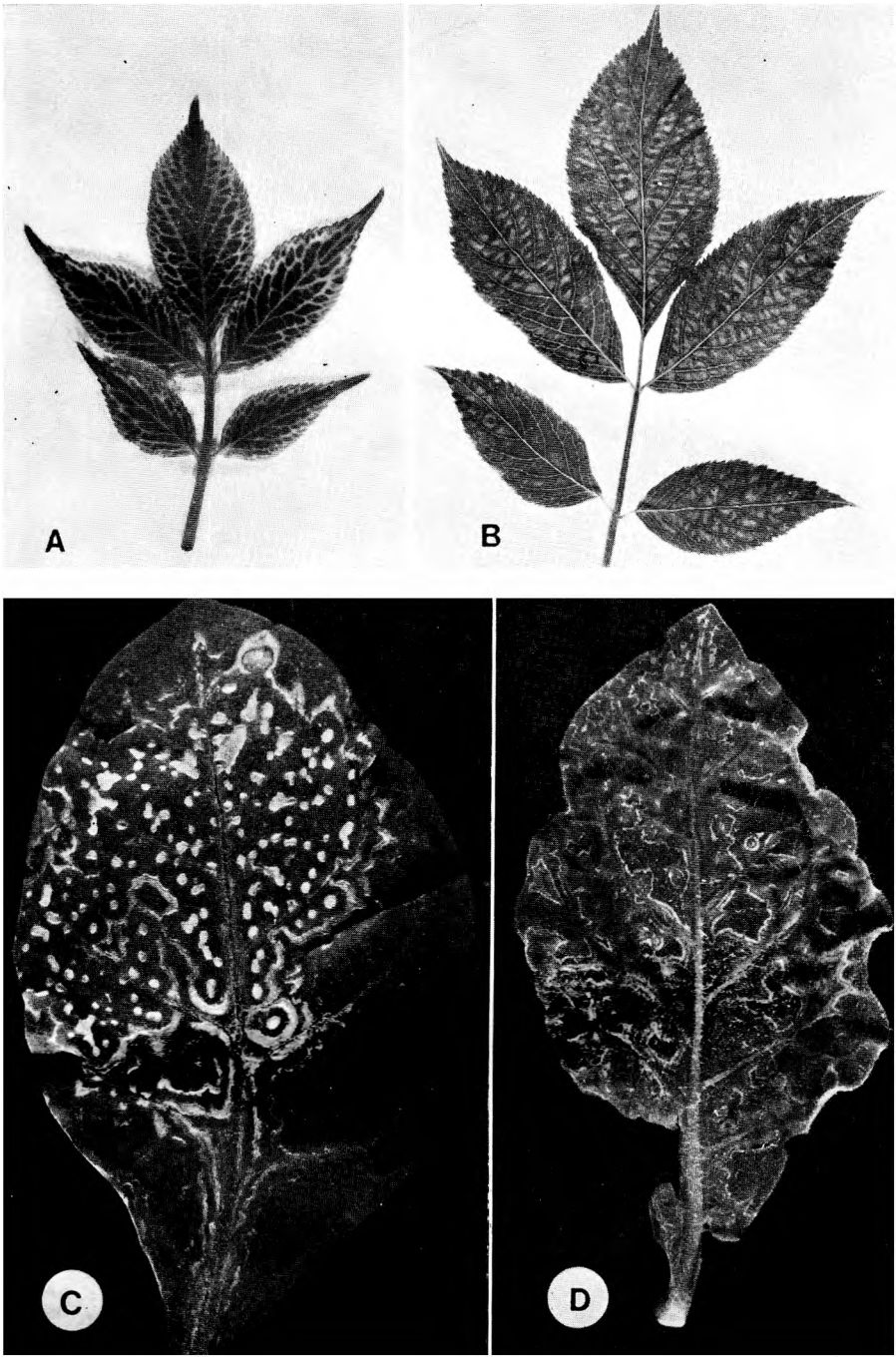

Fig. 1.

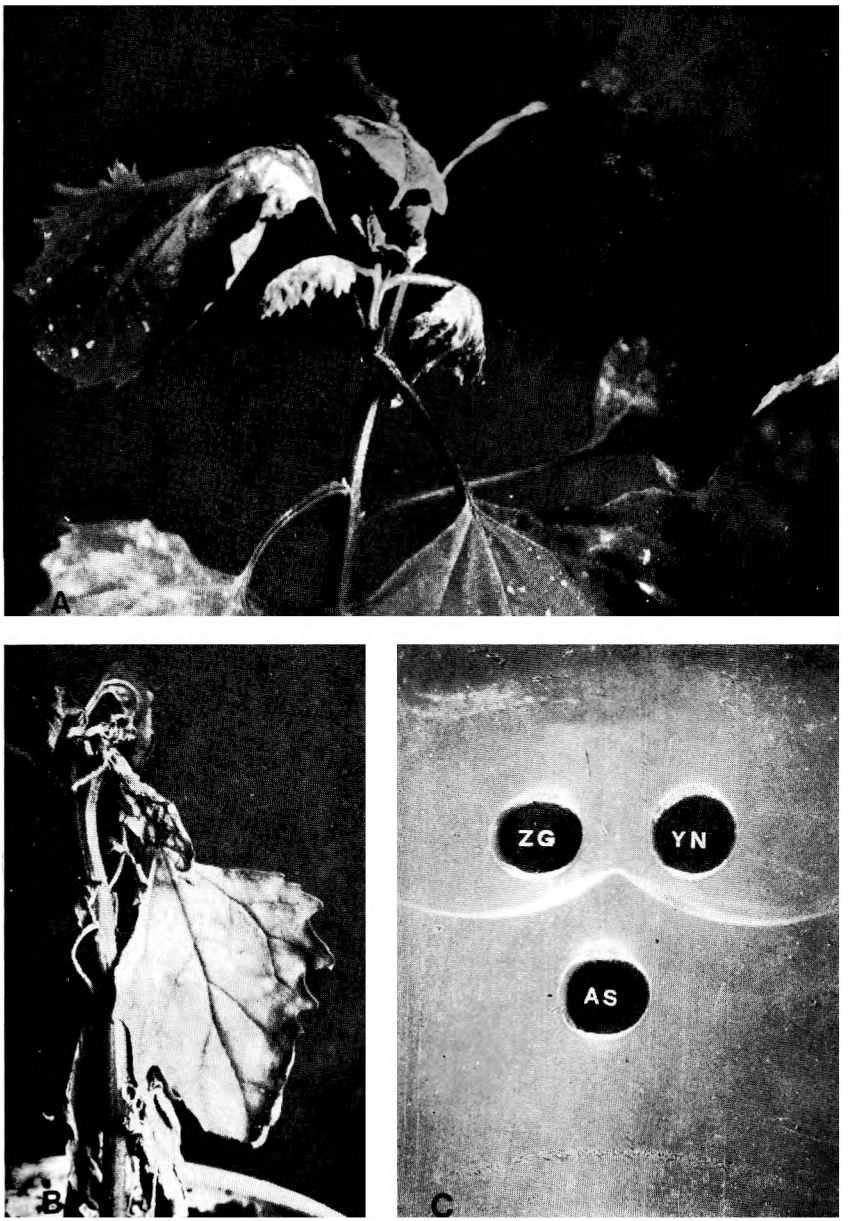

Fig. 2.

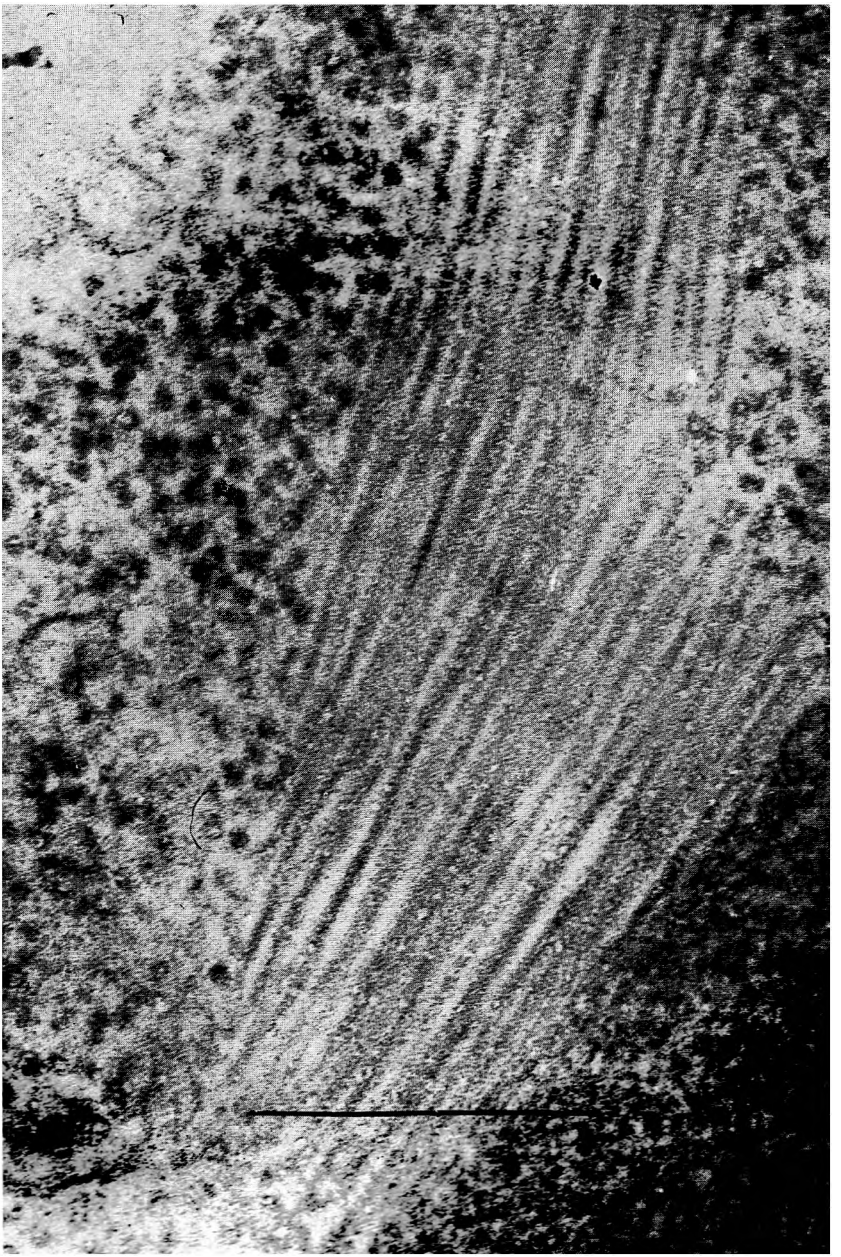

Fig. 3. Ultrathin section of a *S. nigra* leaf showing yellow net symptoms. A large number of elongated virus particles of elderberry carlavirus are parallelly arranged. Bar is 500 nm.

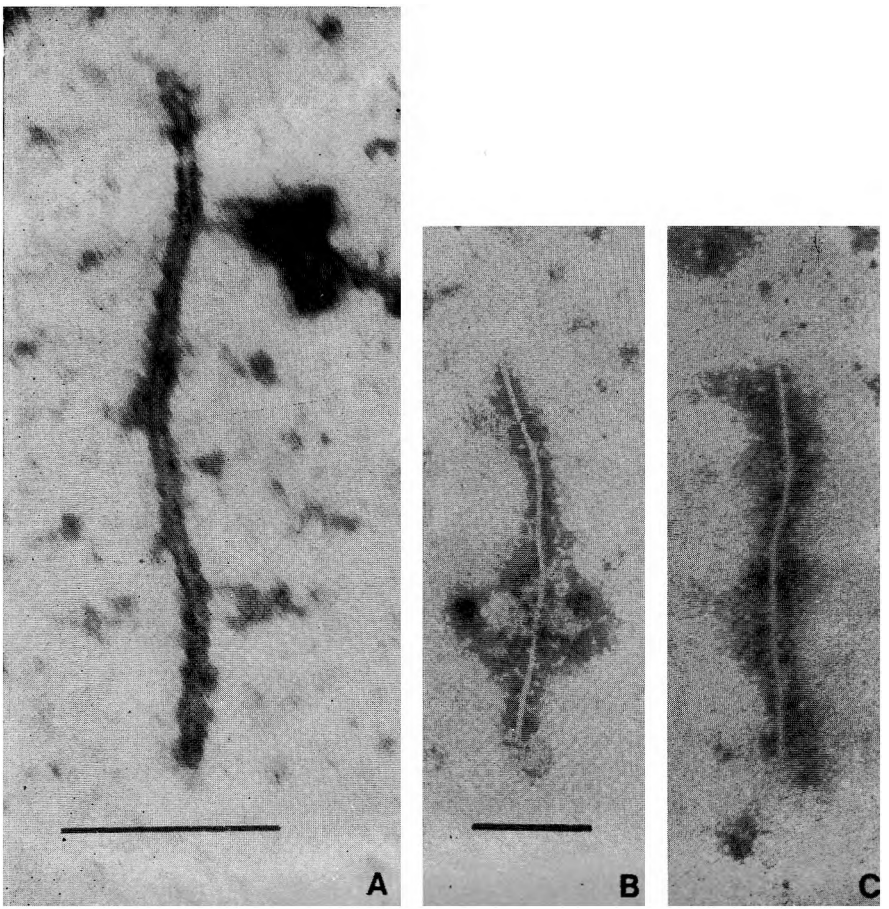

Fig. 4. Some particles of elderberry carlavirus isolated by means of the dip method. Bars are 200 nm.

Serological evidence of the presence of CLRV in elderberry

Since examinations of test plants pointed out that the yellow net on *S. nigra* from southern suburbs of Zagreb was caused by CLRV, we performed serological investigations in order to establish whether this virus was present in the shrubs. Fortunately we had two sera against CLRV in our collection under the number 28 and 50. The sera were prepared by Grbelja (1972) during her stay in Zagreb.

For serological investigations we first used the squeezed sap of upper leaves of *Ch. quinoa* which had strong symptoms of virus infection. This virus was marked Zg and was put in the left well (Fig. 2C). Then we homogenized the upper leaves of *S. nigra* which had very strong symptoms of yellow net. The right well was filled up with this sap. The third lower well was assigned to antisera 28 and 50. After some hours the reaction appeared; a white precipitation line appeared (Fig. 2C) between the upper wells and the lower well, which showed that the viruses from *C. quinoa* and *S. nigra* belonged to CLRV.

Another serological test showed directly that the sap from elderberry leaves with yellow net symptoms from the southern locality also contained CLRV.

Finding of elderberry carlavirus (ECV) in Yugoslavia

In order to thoroughly investigate the elderberry leaves with yellow net symptoms from southern locality in Zagreb, the second author of this paper B. Plavšić performed electron microscopic investigation. She found a large number of elongated parallelly arranged virus particles about 680 nm long in the leaf parenchyma cells (Fig. 3).

This Figure is — with regard to the parallel arrangement of elongated virus particles and their abundance in the parenchyma — very similar to Fig. 9 of the paper by Van Lent et al. (1980) and to Fig 4 by Dijkstra and Van Lent (1983). Our isolate Zg was studied only on ten isolated particles and it was found that its length was about 680 nm, i. e. it was similar to the virus from the Netherlands. Similar virus was found in several *Sambucus* species many times.

A similar virus was for the first time isolated by Brčák (1964) and Brčák and Polák (1966) in *S. nigra* and it was fairly well established that the dimensions of virus particles were 647 × 11-12 nm. Later, this virus was found by Uyemoto and Gilmer (1971) and Uyemoto et al. (1971) in *Sambucus canadensis* and it was established that the virus was a member of the group of carlaviruses. A similar virus was found again in *S. canadensis* by Jones (1972). This virus has recently been thoroughly studied by Van Lent et al. (1980) who named it elderberry carlavirus (ECV) and found it in *S. racemosa*. They also established that ECV was 12 × 678 nm large. Later Jeanne Dijkstra and Van Lent (1983) described this virus in CMI/AAB Descriptions of Plant Viruses No. 263. Consequently, ECV is found in *S. nigra, S. canadensis* and *S. racemosa*. On the basis of the investigations by Jones (1976) these *Sambucus* species have many antigenic determinants in common. It would be interesting to know whether *Sambucus ebulus* which has different antigenic determinants can also be a host to ECV.

Besides the method of cutting ultrathin sections with ultramicrotom, we also used the dip method for studying ECV. With this method it was

easy to find elongated virus particles (Fig. 4). It seems that this carlavirus is abundantly present in diseased tissue.

Discussion

Green and yellow net

Already at the beginning of investigations into elderberry virus diseases in Yugoslavia we described the symptoms of green net on *S. nigra* leaves (Plavšić-Banjac and Miličić 1968). The symptoms consisted of dark green colour of thicker leaf veins and of yellowing of interstitial leaf parts which became chlorotic and yellowish. This symptom was later observed often and was illustrated (Fig. 1B). The green net symptom is only reliable in young shrubs in spring when they are not yet altered by injuries and pests.

In this paper we have also described the yellow net symptoms on elder shrubs which were observed in Yugoslavia for the first time. This symptom is very conspicuous in the crown of elderberry shrubs, especially in spring (Fig. 1A).

Elderberry carlavirus

In our elderberry shrubs with yellow net not only CLRV was present but also another elongated virus named elderberry carlavirus (ECV, Figs. 3 and 4). This virus is a typical representative of the group of carlaviruses. It is latent in most of its hosts and has a narrow host range (Van Lent et al. 1980). Two other carlaviruses are similar to this virus. They were found in Great Britain by Jones (1970, 1972) and by Brunt and Stace-Smith (1972), but these two viruses have not been so intensively investigated as ECV.

Synergistic activity

Van Lent et al. (1980) have isolated ECV many times from elderberry plants growing in parks and showing obvious chlorotic symptoms. These intensive symptoms could not be provoked only by filamentous ECV because this virus does not cause symptoms, or the symptoms are very weak. In experiments of Van Lent et al. (1980) *Sambucus pubescens* Michx. plants inoculated only with the filamentous virus usually remained symptomless.

When elderberry plants carrying only ECV were also inoculated with CLRV, then intensive symptoms appeared in the form of concentric rings, ringspots, line pattern or mosaic (Van Lent et al. 1980). Consequently, these authors experimentally showed that ECV, through the agency of CLRV, can cause intensive symptoms. Therefore these authors consider that ECV and CLRV work synergistically because the symptoms on double infected *S. pubens* (that is both with ECV and CLRV) are much more severe than with single infection with ECV.

Van Lent et al. (1980) admit that besides CLRV other nepoviruses such as AMV, TBRV or TobRV (tobacco ringspot virus; cf. p. 131) can, instead of CLRV, work synergistically in this system and cause intensive symptoms.

Again about yellow net

An interesting phenomenon on elderberry was studied by Horváth et al. (1974). These scientists observed in Hungary two *S. nigra* shrubs, one of which showed well expressed yellow net and the other intensive symptoms in the form, of chlorotic rings. In order to explain this difference a detailed serological analysis of viruses was performed. It was established that both shrubs were infected with CLRV and belonged to the same serotype of this virus (Horváth et al., 1974). As both symptoms of yellow net and chlorotic spots were very strong ECV might have been present in the altered leaves.

An important finding is the first report of ECV in Yugoslavia. This virus was found in *S. nigra* leaves which had very pronounced symptoms of yellow net (Fig. 1A). As in these leaves both elongated ECV and CLRV were present, conditions were available for synergistic influence. As a consequence of this influence an intensive yellow net appeared in concordance with the synergistic theory of Van Lent et al. (1980). It would be interesting to establish whether other yellow nets also have ECV particles in their cells.

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SAŽETAK

VIRUS UVIJENOSTI LISTA TREŠNJE I KARLAVIRUS BAZGE NA VRSTI *SAMBUCUS NIGRA* U JUGOISTOČNOJ EVROPI

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Zapaženi su simptomi žute mreže na listovima crne bazge na dvama lokalitetima prvi put u Jugoslaviji. Podrobna analiza s pomoću pokusnih biljaka pokazala je da je uzročnik žute mreže na obim lokalitetima virus uvijenosti lista trešnje (VULT). Žute mreže istražene su s pomoću dva seruma protiv VULT-a koji su reagirali pozitivno.

Tijekom istraživanja u primjercima bazgâ sa simptomima žute mreže nađen je velik broj produženih čestica koje su bile oko 680 nm duge i 12 nm široke. Čestice su nađene prilikom priređivanja ultratankih presjeka i s pomoću metode uranjanja. Taj produženi virus je karlavirus bazge koji je tom prilikom prvi put nađen u Jugoslaviji.

Na osnovi podataka iz literature karlavirus bazge vjerojatno je prisutan i u Madžarskoj, a nađen je prije mnogo godina i u Čehoslovačkoj. Stoga se može kazati da je karlavirus bazge rasprostranjen u jugoistočnoj Evropi.

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