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INVESTIGATION OF VIRUSES AND VIRUS DISEASES OF SPINACH IN CROATIA

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Introduction

Spinach (*Spinacia oleracea* L.) is known as a species particularly susceptible to infections by different viruses. Hitherto, a large number of viruses have been transmitted to spinach experimentally but considerably less have been isolated from naturally infected plants. Numerous data concerning viruses infecting spinach can be found in texbooks by Klinkowski (1968) and Smith (1972), and in C.M.I./A.A.B. Descriptions of Plant Viruses.

One of the most important and most disseminated viruses on spinach certainly is the cucumber mosaic virus (CMV; R/1: $\frac{1.1}{18} + \frac{1.0}{18} + \frac{0.7 + 0.3}{18}$: S/S: S/C, Ve/Ap). This virus exists in spinach in a number of different strains causing a severe disease known as 'spinach blight'. A few years ago CMV was recorded for the first time on spinach in Yugoslavia in some localities in Bosnia and Herzegovina were it caused remarkable deterioration of plants and considerable losses in yield (Buturović 1974). Judging by the number of relatively recent papers and information on the presence of broad bean wilt virus (BBWV; R/1: */33 : S/S : S/Ap) on spinach, which first came from the USA (Schroeder and Provvidenti 1970) and then from Japan (J. Komuro, cited by Taylor and Stubbs 1972) and some European countries (Bailis, Brunt and Dale 1975, Schmelzer 1975, Weidemann et. al. 1975), it seems that spinach too can often be infected with BBWV. Since BBWV causes severe symptoms in spinach which may be confused with those of CMV (Schroeder and Provvidenti 1970), accurate identification has to be done to recognize and distinguish these two viruses. The other viruses deseribed to cause considerable losses in spin-

ach are spinach yellow dwarf virus, which was found widespread in commercial fields in California and Texas (Thomas, Halliwel and Webb 1973), and lettuce mosaic virus described on spinach in New York (Provvidenti and Schroeder 1972).

In order to determine viruses present on spinach in the vicinity of Zagreb, initial work was started at the beginning of 1977. So far altogether four viruses were found: CMV, turnip mosaic virus (TuMV; R/1 : 3.1/5 : E/E : S/Ap), tomato bushy stunt virus (TBSV; R/1 : 1.5/17 : S/S : S/*) and an unidentified virus tentatively named spinach latent virus (SLV). In this paper some results concerning the identification of cited viruses are presented, their influence on spinach and some other characteristics.

Materials and Methods

The viruses were isolated from spinach of the cultivar Matador by macerating the tissue 1 : 10 (w/v) in 0.06 M phosphate buffer ph 7.6 containing 0.1% thioglycolic acid and rubbing the extracts on leaves of the following test plants: *Chenopodium amaranticolor* Coste et Reyn., *Ch. quinoa* Willd. and *Nicotiana glutinosa* L. Except in the case of CMV, a single isolate of each virus was used in all experimental inoculations. In host range tests, return inoculations were always made from inoculated and from uninoculated leaves.

For transmission tests with aphids, apterous *Myzus persicae* (Sulz.), after 3 to 4 hours of starvation, were placed on infected leaves for 1—2 min and then in groups of 6 transferred to each test plant for 24 hours.

Seed-transmission of SLV was studied in spinach. The analysis of the seed samples was performed by testing seedlings developed from particular seed lots. The seedlings in the stage of first true leaves were indexed for virus, singly or in groups, by inoculating sap to *Ch. quinoa*. Before inoculation with respective virus, all spinach seedlings (cv. Matador) were also tested in the same way for the presence of SLV or may be some ohter viruses transmitted by spinach seed.

All experiments with test plants were done in insect-proof glasshouse.

For electron microscopy, the leaf pieces were fixed, embedded and sectioned as described previously (Štefanac and Ljubešić 1971).

Serological microprecipitin tests were conducted on slides by mixing a drop of crude virus sap with a drop of appropriately diluted antiserum. Agar gel diffusion tests were also made with antigens using crude leaf extracts.

The following sera were used to check the identification of CMV and TuMV: the serum against CMV (titre 1/16, no antibodies against normal plant components) provided by Dr Renate Koenig, BBA Institute für Virusserologie, Braunschweig, and serum against *Alliaria* strain of TuMV (Lisa and Lovisolo 1976) (titre 1/1024) provided by Dr Vittoria Lisa, Laboratorio di Fitovirologia applicata del CNR, Torino. The sera against raspberry ringspot virus (Scottish strain from Lloyd George raspberry, titre 1/256), tomato black ring virus (German Kartoffel-Buckett strain, titre 1/256), arabis mosaic virus (titre 1/256) and strawberry latent ringspot virus (titre 1/2048) were supplied by Dr A. F. Murant, S.H.R.I., Invergowrie, Dundee.

Results

Disease symptoms

In March 1977 virus-like symptoms were noticed in quite a number of plants on a 5-acre field of autumn-sawn spinach in the locality of Brezovica near Zagreb. Some affected plants were more or less stunted and rosetted with somewhat chlorotic and wrinkled leaves (Fig. 1). Some plants were extremely rosetted and exhibited slight vein clearing on the leaves. Some others were badly stunted, chlorotic and somewhat wilted, with conspicuously malformed and reduced leaves, generally looking as if infected with CMV (cp. S m i t h 1972, Fig. 37).

In January 1978 another large field of autumn-sawn spinach was inspected in the same area, which was sawn approximately at the same time as the one visited in 1977. Again, a rather large number of infected plants was observed. On this occasion the most frequent symptoms were a slight yellowing of the younger leaves and some stuntng of the plants, the first evidence of the infection with CMV (cp. S m i t h 1972).

Fifteen spinach plants from each of the two places were collected at random and tested individually for the presence of viruses. All were shown to be infected at least with one virus. CMV was the most frequent virus and was isolated from 26 plants, both TuMV and TBSV were isolated each from two plants, and SLV occurred in 3 plants.

Cucumber mosaic virus

The isolates of CMV were recognized already according to the symptoms they caused when inoculated mechanically from field grown spinach to *Chenopodium quinoa, Ch. amaranticolor* and *Nicotiana glutinosa*. Two *Chenopodium* species always developed characteristic local lesions without systemic infection and *N. glutinosa* showed systemic mosaic or faint chlorotic spots followed by general chlorosis of the plants, sometimes accompanied by necrosis and frequently by a distortion of leaves which became more or less filamentous. As mentioned, the symptoms in *N. glutinosa* were not exactly the same with all isolates but further tests were not carried out to establish whether they represented different strains or not. Using the sap from locally infected *Ch. quinoa* leaves, a few isolates were transmitted to spinach. The symptoms which developed in glasshouse grown plants were chlorotic local lesions followed by systemic yellowing, malformation of leaves and stunting (Fig. 2) and corresponded to those seen in the field.

The virus was transferred from field infected spinach to healthy spinach seedlings by *M. persicae* after acquisition feed of 1—2 min. The infection occurred in all 4 plants which were used in this experiment and the symptoms were typical.

Light microscope examination of various infected plants did not show virus inclusion bodies.

The identity of all 26 isolates of CMV was comfirmed serologically by gel diffusion serological tests in which saps from systemically infected *N. glutinosa* reacted with the serum against CMV. Healthy controls did not react.

Turnip mosaic virus

TuMV was isolated in 1977 from two rosetted plants with wrinkled leaves exhibiting yellow vein clearing and lying on the soil surface. The

symptoms produced in spinach seedlings initially were the same as in the originally infected plants. Later their leaves became differently malformed and mottled (Fig. 3) and plants badly stunted. The virus caused symptoms typical of TuMV (Tomlinson 1970) also in *Brassica chinensis* L. cv. Michihli, *B. nigra* (L.) Koch, *B. rapa* L. var. *rapa*, *Gomphrena globosa* L. (Fig. 4) and in *Impatiens balsamina* L. (Fig. 6). In *B. oleracea* L. cvs. it provoked mostly mild systemic symptoms. In *Ch. amaranticolor* and in *Ch. quinoa*, in addition to characteristic lesions in the inoculated leaves systemic flecks and spots developed (Fig. 5), especially pronounced from March to June. The isolate did not produce symptoms in *Petunia hybrida* Vilm. and could be recovered from inoculated leaves only.

The isolate of TuMV was readily transmitted from mechanically inoculated turnip to spinach seedlings by *M. persicae* in a non-persistent manner.

The cells of infected spinach and most other experimental hosts contained large and complex cytoplasmic inclusion bodies visible by light microscope which were characteristic of TuMV (cp. Stefanac and Miličić 1965, Christie and Edwardson 1977). In ultrathin sections of turnip leaf tissue cylindrical inclusions appeared as scrolls and as long and flat laminated aggregates (Fig. 7). In their morphology these cylindrical inclusions were similar to those reported for most isolates of TuMV (cp. McDonald and Hiebert 1975).

The identity of spinach isolate was further confirmed serologically. In the slide microprecipitin test, the isolate reacted with the serum against *Alliaria* strain of TuMV to the serum dilution 1/64.

Tomato bushy stunt virus

TBSV was isolated from two spinach plants collected in 1977 which were reduced in growth and with somewhat chlorotic and wrinkled leaves (cp. Fig. 1). The isolates could be distinguished from other spinach isolates by causing brown necrotic lesions in inoculated leaves of *N. glutinosa* (Fig. 10) without systemic infection. The two isolates were indistinguishable from each other and are treated as one strain.

Under experimental conditions, the virus caused in spinach severe systemic infection. The symptoms in inoculated leaves consisted of numerous lesions and in top leaves of curling, shortening of the main veins, mottle, vein banding and necrosis. The experiments were done during the winter and the plants quickly died. The isolate produced also systemic symptoms in pepper (*Capsicum annuum* L.) in the form of chlorotic spots, mottling and scattered small brown necrotic lesions followed by zonated ring spots (Figs. 8 and 9) and stronger mottling.

The other characteristics and serological relationship of spinach isolate to some known strains of TBSV will be discussed further elsewhere.

An unidentified virus

This virus, provisionally marked as SLV, was first detected in 3 spinach plants from which CMV was also isolated. The virus differed from CMV by the ability to infect *Ch. quinoa* systemically. Afterwards, this virus was found in spinach seed which were used for sowing the field visited in 1977. From 145 seedlings which germinated from the mentioned seed and were tested individual for the presence of SLV, 17 con-

tained this virus, i. e. about 11%. Plants germinated from infected seed did not show any symptoms at all and grew with equal vigour as the healthy ones.

Mechanical inoculation with SLV caused in spinach faint mosaic and vein clearing in top leaves after about 6 days. These symptoms soon disappeared and the plants could not be distinguished from healthy controls, but still carried the virus. Transmission experiments to a few test plants gave the following results:

In *Ch. quinoa,* diffuse chlorotic local lesions were produced 3—5 days after inoculation followed by chlorosis or chlorotic lesions at the basis of top leaves and by top necrosis. In *Ch. amaranticolor,* the virus was restricted to the inoculated leaves where after 3—5 days produced numerous small chlorotic lesions which did not enlarge with time. The virus infected *Nicotiana glutinosa, N. megalosiphon* Heurek et Muell, and *N. tabacum* L. ev. Samsun only during the winter and early in the spring where produced systemic symptoms.

To determine the percentage of transmission through spinach seed, seeds collected from infected plants which were exposed concurrently to the pollination from healthy and infected plants were tested. SLV was found in 103 out of 170 seedlings tested, that is in 60% of progeny. SLV was detected also in a big proportion of seeds which were collected from healthy spinach plants also exposed concurrently to the pollination from healthy and infected sources. In this case, the virus was found in 35 out of 66 seedlings tested, that is in 56%.

The symptoms in test plants and especially the high level of transmission by seed and by pollen suggested that virus latent in spinach could perhaps be a nepovirus. In the preliminary gel diffusion tests, the sap from diseased *Ch. quinoa* plants containing virus in adequate concentration did not react with sera against the following nepoviruses: raspberry ringspot virus, tomato black ring virus, arabis mosaic virus and strawberry latent ringspot virus.

Discussion

From four viruses, i. e. CMV, TuMV, TBSV and the virus tentatively named SLV, which were found in spinach during this initial work, to the author's best knowledge only CMV has been reported previously from spinach. The others have been detected in spinach apparently for the first time.

Under experimental conditions TuMV and TBSV caused acute systemic symptoms in spinach seedings, which showed that infection with each of these viruses alone could be very important. Each of the two viruses was isolated only from two plants but considering the relatively small number of plants tested both viruses were certainly present in many more plants. TuMV unlike TBSV (Martelli, Quacquarelli and Russo 1971) systemically invades a wide range of natural hosts. Therefore TuMV infected spinach can be an important source of infection for other susceptible crops or the other way round. Nevertheless, one should not forget that the isolate of TBSV caused intense systemic symptoms not only in spinach but also in pepper and consequently it could be dangerous both for spinach and for pepper. Actually, according to the reaction of these two species, the variant of TBSV from spin-

ach shows the similarity with the variant of the same virus recently reported from pepper from Morocco (Fischer and Lockhart 1977).

SLV shares some properties with nepoviruses and seems quite distinct from other viruses previously reported from spinach. SLV seemingly does not cause any damage to spinach but it will certainly make some problems if present in spinach seeds used in experimental work with ohter viruses. I suppose that the number of viruses transmitted by spinach seed is larger than it appears on the basis of recent data. Phatak (1974) lists a little over two hundred combinations of viruses and host plants in which the virus is seed-transmitted but in the list there are no data on spinach.

BBWV was not detected during this survey but it certainly causes periodical losses in spinach in Yugoslavia. So far this virus has been found on a few occasions in different plant species in this country (Miličić et al. 1976).

Fig. 1. Right, distinct symptoms in naturally infected spinach *(Spinacia oleracea)* cv. Matador plant; left, healthy plant.

Sl. 1. Desno, izraziti simptomi na prirodno zaraženom špinatu *(Spinacia oleracea)* cv. Matador; lijevo, zdrava biljka.

Fig. 2. Right, severe spinach blight symptoms in plant experimentally infected with CMV; plant on the left is control.

Sl. 2. Desno, jaki znakovi propadanja špinata na primjerku pokusno zaraženom s VMK; lijevo, kontrolna biljka.

Figs. 3—6. Symptoms in plants induced by spinach isolate of TuMV. Fig. 3. Spinach plant about 6 weeks after infection showing systemic chlorotic mottle and leaf distortion. Fig. 4. Local lesions in *Gomphrena globosa*. Fig. 5. Systemically infected *Ch. quinoa* with star-like spots and leaf distortion. Fig. 6. Dark brown stem necrosis and leaf distortion in *Impatiens balsamina*.

Sl. 3—6. Simptomi na biljkama koje uzrokuje izolat VMPR iz špinata. Sl. 3. Špinat, otprilike 6 tjedana poslije zaraze, s klorotičnim šarenilom i iskrivljenim listovima. Sl. 4. Lokalne lezije na vrsti *Gomphrena globosa*. Sl. 5. Sistemično zaraženi primjerak *Ch. quinoa* sa zvjezdolikim pjegicama i iskrivljenim listovima. Sl. 6. Tamnosmeđe nekroze stabljike te deformacije lista na vrsti *Impatiens balsamina*.

Fig. 7. Electron micrograph of cylindrical inclusions in turnip *(Brassica rapa)* cell infected with spinach isolate of TuMV. Note scrolls in cross and oblique sections (S) and long laminated aggregates (LA). Small bundles composed of loosely packed and curved virus like particles (V) are scattered in the cytoplasm of this area.

Sl. 7. Elektronsko mikroskopska snimka cilindričnih uklopina u stanici postrne repe *(Brassica rapa)* zaražene izolatom VMPR iz špinata. Smoci u poprečnom i kosom presjeku (S), dugi lamelarni agregati (LA). U citoplazmi ovog područja prisutni su snopići labavo povezanih i zakrivljenih čestica koje nalikuju česticama virusa (V).

Figs. 8—10. Symptoms in test plants induced by spinach isolate of TBSV. Figs. 8 and 9. Systemic symptoms in pepper *(Capsicum annuum)*. Fig. 10. Typical local lesions in *Nicotiana glutinosa*.

Sl. 8—10. Simptomi na pokusnim biljkama koje uzrokuje izolat VGKR iz špinata. Sl. 8 i 9. Sistemični simptomi na paprici *(Capsicum annuum)*. Sl. 10. Tipične lokalne lezije na vrsti *Nicotiana glutinosa*.

Fig. 1. — Sl. 1.

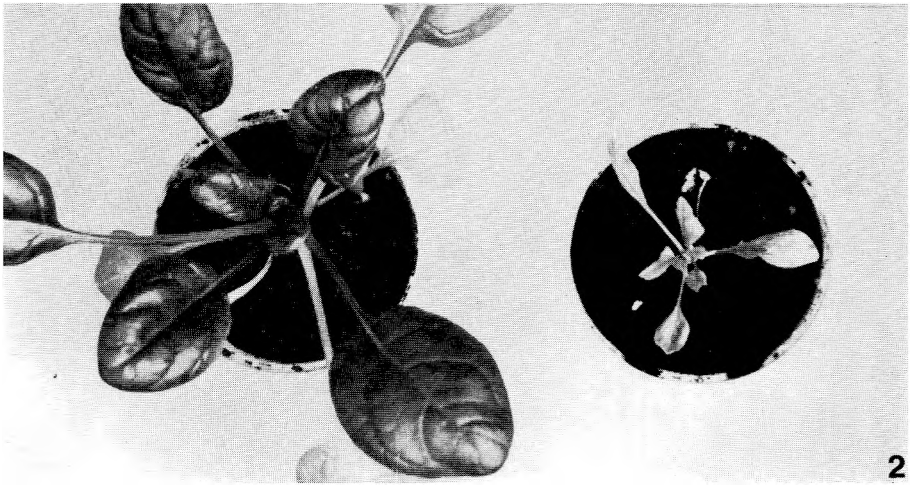
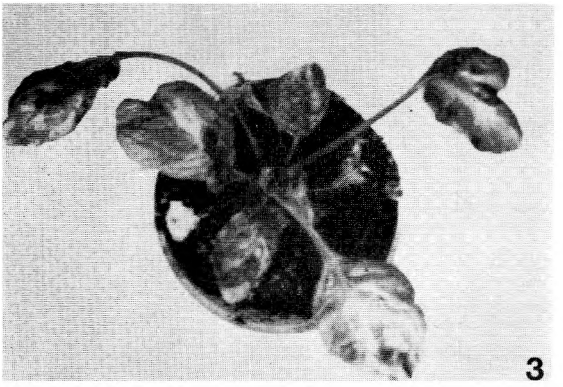
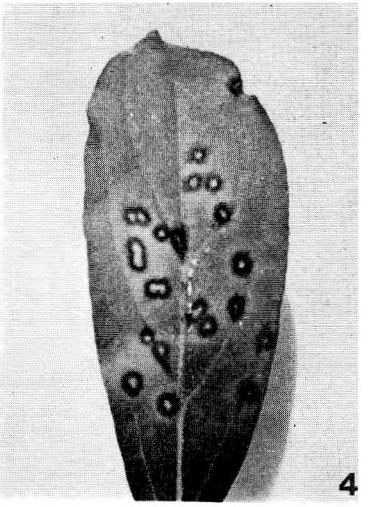
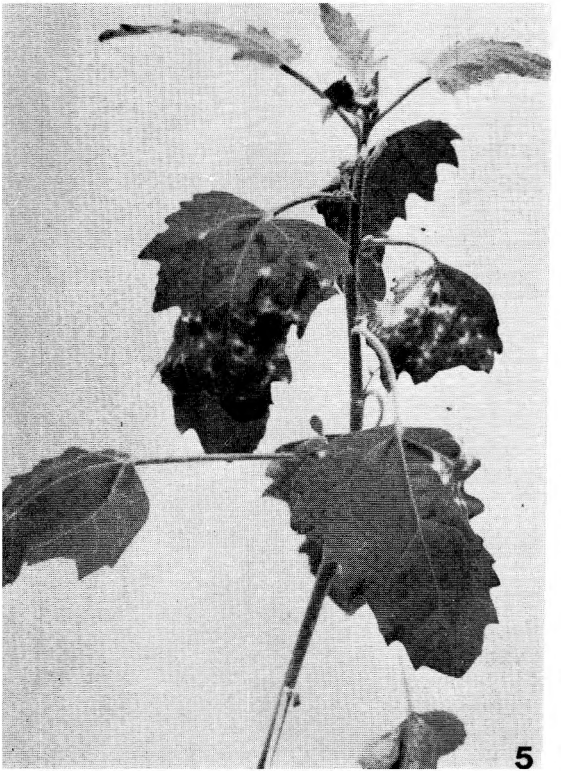
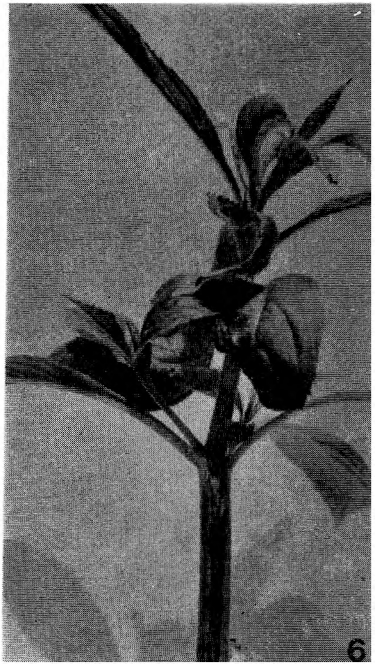


Fig. 2. — Sl. 2.









Figs. 3—6. — Sl. 3—6.

Fig. 7. — Sl. 7.

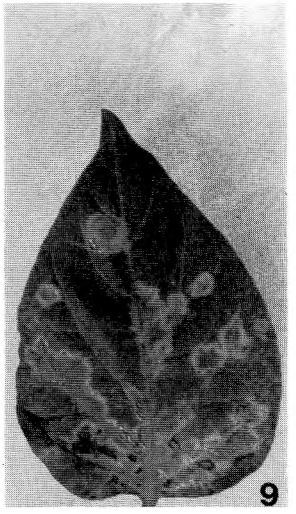


Fig. 8—10. — Sl. 8—10.

Summary

Affected spinach (*Spinacia oleracea*) plants cv. Matador with various symptoms of virus infection were collected from two places in the vicinity of Zagreb and four viruses were isolated. Out of 30 plants tested, 26 plants yielded cucumber mosaic (CMV), 2 turnip mosaic (TuMV) and 2 tomato bushy stunt (TBSV) viruses: each of these viruses was re-transmitted to spinach where it induced strong disease symptoms. In addition, from 3 plants infected with CMV one not fully identified virus was isolated which caused mainly symptomless infection in spinach and was efficiently transmitted by spinach seed and pollen.

Among for isolated viruses only CMV has been reported previously from naturally infected spinach. Others have apparently been detected in this species for the first time. Together, this is the first detection of TBSV in Yugoslavia.

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SADRŽAJ

ISTRAŽIVANJE VIRUSA I VIRUSNIH BOLESTI NA ŠPINATU U HRVATSKOJ

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U toku 1977. i početkom 1978. sabrani su s dviju ploha ozimog špinata u okolici Zagreba primjerci špinata (*Spinacia oleracea* L.) cv. Matador s različitim znakovima virusne zaraze iz kojih su izdvojena ukupno četiri različita virusa. Od 30 istraženih primjeraka špinata, 26 primjeraka je sadržavalo virus mozaika krastavca (VMK), 2 primjerka virus mozaika postrne repe (VMPR) a 2 virus grmolike kržljavosti rajčice (VGKR). Svaki od navedenih virusa uzrokovao je na pokusno zaraženom špinatu veoma jake degenerativne promjene. Iz manjeg broja biljaka u kojima je bio utvrđen VMK izdvojen je također još jedan virus koji u toku ovog rada nisam u cijelosti identificirala, ali sam ustanovila da zaražava špinat latentno i da se prenosi u visokom postotku sjemenom i polenom špinata.

Od četiri izdvojena virusa samo je VMK bio ranije utvrđen na špinatu. Taj virus ujedno je jedan od najraširenijih virusa na navedenoj kulturi kod koje znatno smanjuje prinos. Ostala tri virusa, čini se prvi put su utvrđena na prirodno zaraženom špinatu. To je ujedno prvi nalaz VGKR na području Jugoslavije.

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