

The Properties of a Few Isolates of the Sour Cherry Necrotic Ringspot Virus

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Abstract. Results of the investigation of four isolates of the sour cherry necrotic ringspot virus are presented in this paper. The isolates used caused characteristic symptoms on woody indicators "Bing", "Montmorency", F 12/1, and on peach seedlings. The virus was transmitted mechanically to some herbaceous species: *Antirrhinum majus*, *Cucumis sativus*, *Cucurbita maxima*, *Chenopodium quinoa*, *Crotalaria juncea*, *Momordica balsamina*, *Petunia hybrida* and *Leonorus sibiricus*. The attempts to transmit the virus mechanically to further 23 herbaceous species were unsuccessful. The thermal inactivation point of the virus lies between 46 and 58 °C and the dilution end-point between 10^{-1} and 10^{-2} . The virus is stable in vitro at room temperature for more than one day. Individual virus isolates gave a positive immunological reaction with the Fulton's "G" antiserum.

The investigation of the ringspot disease of cherries started when KEITT and CLAYTON (1939) and WILLISON *et al.* (1948) confirmed in the U.S.A. the viral nature of yellows and of necrotic ringspot disease of sour cherry. From this time on numerous papers dealing with the investigation of this problem were published. Mechanical transmission of the ringspot virus from sour cherries to cucumber (*Cucumis sativus*) accomplished by MOORE *et al.* (1948) was one of the important achievements in the study of these viruses which opened new possibilities of a further and nearer study of their properties. FULTON (1957a, b, 1958, 1959) and WATERWORTH and FULTON (1964) divided ringspot viruses into two basic groups: the sour cherry necrotic ringspot virus (NRSV), strains A, F and G, and the prune dwarf virus (PDV), isolate B. In our previous papers we already described some results of the investigations on ringspot viruses in our laboratory (PAULECHOVÁ-KRÁLIKOVÁ and KEGLER 1967, PAULECHOVÁ-KRÁLIKOVÁ 1969). In this paper we present further results on the sour cherry necrotic ringspot virus.

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Material and Methods

In preliminary experiments, many cherry, sour cherry, prune, apricot, peach, and almond trees were tested to ascertain the occurrence of some viruses belonging to the group of ringspots. A few isolates were chosen after greenhouse tests which showed typical symptoms of the sour cherry necrotic ringspot virus and which did not show the symptoms of mixed infection. Altogether four isolates were used in further experiments, three of them from sour cherry cultivars "Kö-rösská" (isolate A), "Vackova" (isolate B), "Ostheimská" (isolate C), and one from apricot cv. "Kocmankova" (isolate D).

All experiments on both woody and herbaceous indicators were performed in glasshouse conditions, from January to April.

Cherry (*Prunus avium* L.) "F 12/1" and cv. "Bing", sour cherry (*Prunus cerasus* L.) cv. "Montmorency" and peach seedlings (*Prunus persica* (L.) BATSCH) were used as woody indicators. Cherry and sour cherry indicators were grafted one month before the inoculation on cherry seedlings. The indicators were infected using the method of chip budding.

Among herbaceous plants such indicators were chosen for mechanical transmission in which the virus can easily and quickly reproduce, or such which are of importance for differential diagnostics.

When transmitting the viruses to herbaceous hosts the individual virus isolates were first transmitted from sprouted grafts of sour cherries and apricots to cucumber (*Cucumis sativus* L.) cv. "Delikates" plants and from cucumber plants to other herbaceous species. Young leaves taken from the grafts were homogenized with the same amount of pH 7.5 phosphate buffer containing 0.015 M sodium diethyldithiocarbamate, 0.015 M N,N-diphenylthiourea and 0.03 M caffeine. 1/15 M phosphate buffer pH 7.5 was used for the preparation of the inoculum from peach leaves or from infected cucumber cotyledons (4 to 5 days after the inoculation).

Crude extracts from infected cucumber cotyledons obtained after the maceration with phosphate buffer pH 8.0 (1 : 1) were used for the determination of the physical properties of the virus. The sap obtained was divided into 1 ml samples which were used for the determination of the thermal inactivation point, for the determination of the dilution end-point and for the determination of the *in vitro* stability. 18 cucumber plants were used for each test. The trials were replicated 2 to 4 times.

Immunological tests were performed with B and G antisera supplied by prof. Fulton, using the method of double-diffusion agar test. The sap used for immunological tests was prepared 4 to 5 days after the inoculation from infected cucumber cotyledons as well as directly from diseased trees.

Results

The following symptoms of the disease could be observed on the indicators used after early spring infections with the individual isolates:

Prunus persica (L.) BATSCH.: Small chlorotic spots or sometimes rings (Fig. 1A) appear after the infection on peach leaves, the symptoms later disappear or are camouflaged. Shock reactions were not observed.

"Montmorency" (*Prunus cerasus* L.): Necrotic spots, rings on older leaves (Fig. 1B), tatter leaf, enations on the lower side of the leaves in a few cases during the first year after the infection (B-enations) (Fig. 1C) and shock reactions residing in quick dying of the shoots.

"Bing" (*Prunus avium* L.): Sporadic necrotic spots on the leaves (Fig. 1D) and sporadic enations (B-enations) on the lower side of the leaves already during the first year after the infection (Fig. 1E).

"F 12/1" (*Prunus avium* L.): Sporadic necrotic spots on older leaves (Fig. 1F).

In addition to woody indicators the sour cherry necrotic ringspot virus was also tested on herbaceous indicators. The virus could be positively transmitted by means of mechanical inoculation to nine herbaceous species mentioned below:

TABLE I
PHYSICAL PROPERTIES OF THE SOUR CHERRY NECROTIC RINGSPOT VIRUS

| Virus isolate | Replicate N ^o | Control | Infectivity of raw sep* | | | | | | | | | | when exposed to room temp. days | | | | | |
|---------------|--------------------------|---------|------------------------------|----|----|----|----|-----------------|----|----|----|------------------|---------------------------------|----------------------|------------------|---|---|---|
| | | | when heated to °C for 10 min | | | | | when diluted to | | | | | 1 | 2 | 3 | | | |
| | | | 42 | 44 | 46 | 48 | 50 | 52 | 54 | 56 | 58 | 10 ⁻¹ | 2 × 10 ⁻¹ | 4 × 10 ⁻¹ | 10 ⁻² | 1 | 2 | 3 |
| A | 1 | 8 | 1 | 0 | 0 | 0 | 0 | — | — | — | — | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| | 2 | 10 | 1 | 1 | 0 | 0 | 0 | — | — | — | — | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| | 3 | 12 | 1 | 1 | 0 | 0 | 0 | — | — | — | — | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| B | 1 | 4 | 3 | 3 | 2 | 2 | 0 | 0 | — | — | — | 1 | 0 | 0 | 0 | 1 | 0 | 0 |
| | 2 | 2 | 2 | 1 | 1 | 1 | 0 | 0 | — | — | — | 1 | 0 | 0 | 0 | 1 | 0 | 0 |
| | 3 | 4 | 1 | 2 | 1 | 1 | 1 | 0 | — | — | — | 0 | 0 | 0 | 0 | 2 | 0 | 0 |
| C | 1 | 6 | — | 5 | 5 | 3 | 0 | 1 | 0 | — | — | 0 | — | — | 0 | 3 | 0 | 0 |
| | 2 | 15 | — | 15 | 14 | 14 | 12 | 9 | 2 | — | — | 1 | — | — | 0 | 5 | 0 | 0 |
| | 3 | 15 | — | — | — | 15 | 9 | 6 | 6 | 1 | 0 | 11 | — | — | 4 | 2 | 0 | 0 |
| | 4 | 8 | — | — | — | 5 | 0 | 0 | 0 | 0 | 0 | 0 | — | — | 0 | — | — | — |
| D | 1 | 11 | — | 9 | 4 | 3 | 2 | 2 | 1 | 0 | — | 6 | — | — | 0 | — | — | — |
| | 2 | 5 | — | 4 | 3 | 1 | 1 | 1 | 0 | 0 | — | 0 | — | — | 0 | — | — | — |

*) Individual numbers represent the total number of the infected plants from 18 inoculated cucumber plants for each of the replicates; — = the experiments were not performed

Antirrhinum majus L.: Necrotic zonal rings on the inoculated leaves.

Cucumis sativus L. (cv. Delikates): Light green lesions on the cotyledons 4 to 6 days after the inoculation (Fig. 2A), later necrosis and dying of the rosette leaves (Fig. 2B), terminal rosettes, mosaic of the leaves and perishing of entire plants.

Cucurbita maxima DUCH.: Chlorotic lesions on inoculated cotyledons 6 days after the inoculation, later green rings, strips on yellow underground. The symptoms can be observed only after early spring infections.

Chenopodium quinoa WILLD.: Systemic infection, chlorotic spots, chlorotic to necrotic rings on further leaves (Fig. 2C).

Momordica balsamina L.: Round necrotic lesions on inoculated leaves (5 to 30 lesions on one leaf which depended on the isolate) four days after the inoculation, further leaves healthy (Fig. 2D).

Crotalaria juncea L.: Brown-violet sporadic lesions on the inoculated leaves after five days (Fig. 2E).

Tithonia speciosa HOOK.: Unspecific symptoms, small necrotic lesions on the inoculated leaves after spring inoculations.

Petunia hybrida HORT ex VILM.: Zonal grey concentric ring patterns on the inoculated leaves (Fig. 2F).

Leonorus sibiricus L.: Yellowing of the nervature, eventually also yellowing of the area between the nervature (Fig. 2G).

The transmissions were not established to the following species: *Amaranthus caudatus* L., *Ammi majus* L., *Aquilegia vulgaris* L., *Crotalaria spectabilis* ROTH., *Datura chloranantha* HOOK., *Digitalis purpurea* L., *Emilia sagitata* DC. *Gomphrena globosa* L., *Chenopodium amaranticolor* COSTE et REYN., *Ch. foetidum* SCHRAD., *Ch. foliosum* (MOENCH.) ASCHERS., *Ch. murale* L., *Nicandra physaloides* (L.) GÜRTNER f. *violacea* BITTER, *Nicotiana glutinosa* L. (cv. Żukowski), *N. langsdorfii* L., *N. tabacum* L. (cv. "Samsun", "White Burley"), *Physalis angulata* L., *Ph. peruviana* L., *Sesbania exaltata* (RAF.) CORY, *Solanum nigrum* L., *S. sisymbriifolium* LAM., *Vigna sinensis* (L.) ENDLICHER, and *Zinnia elegans* JACQ.

The thermal inactivation point of the individual isolates of the virus lies between 46 and 58 °C and the dilution end-point between 10⁻¹ and 10⁻². The virus is stable *in vitro* at room temperature for more than one day (Table 1).

Immunological tests performed with the antisera "G" and "B", by means of which the relation of the sour cherry necrotic ringspot virus to the other viruses of the cherry ringspot disease was investigated using the double diffusion agar test, have shown that the isolates used in our experiments give positive immunological reactions with the antiserum "G".

Discussion

The sour cherry necrotic ringspot virus belongs to the group of ringspot disease viruses the common characteristic of which is the possibility of the transmission through seeds and pollen (CATION 1949, GEORGE nad DAVIDSON 1963). FULTON (1968) has specified this group of viruses as ILAR-viruses (Isometric Labile Ringspot Viruses). These viruses, especially the sour cherry necrotic ringspot virus, are known to be widely distributed to indi-

vidual species of the genus *Prunus* (COCHRAN 1946). They occur mainly in latent form, typical symptoms appear only for a short time after the infection, or after the transmission to susceptible varieties or species.

In our experiments we tried to characterize more closely a few isolates of the necrotic ringspot virus under our conditions and to compare our data with those given in the literature. All the investigated isolates gave positive immunological reactions with Fulton's "G" antiserum and could be easily transmitted mechanically to some selected herbaceous species, such as *Chenopodium quinoa* and *Momordica balsamina*. When transmitting the virus to *Chenopodium quinoa* we could not find — with the exception of one isolate from the apricot — any leaf necrosis or leaf malformations which had been found with this virus by other authors (KEGLER 1965, NÉMETH 1965), but only systemic infection residing in the formation of mosaic. Characteristic local necrotic lesions described by FULTON (1957a) on *Momordica balsamina* occurred with all the investigated virus isolates. Individual cases differed only in the number of the lesions. Transmissions to *Sesbania exaltata*, to which other ringspot viruses are well transmissible, were in all cases negative. All the typical symptoms of this virus were found on woody indicators "Bing", "F 12/1", and "Montmorency". Shock reactions found by some authors (KEGLER 1965, NÉMETH 1965) after the infection with the sour cherry necrotic ringspot virus on peach seedlings were not observed in our experiments at all. Some differences were found in some of the properties of different isolates of the sour cherry necrotic ringspot virus. The thermal inactivation point lies between 46 and 58 °C. Only two isolates had their thermal points between 54 and 58 °C, which corresponds to the lower limit of the thermal inactivation point (55 to 62 °C) determined for their isolates of the necrotic ringspot virus by WATERWORTH and FULTON (1964). The virus isolates studied in our experiments also show lower thermal inactivation points than the isolates of NRV/STV prepared by KEGLER (1965) for which the thermal points were determined between 58 and 60 °C. Most of the data obtained indicate that the virus isolates described in this paper belong to the sour cherry necrotic ringspot virus. Lower values of the thermal inactivation point obtained with the investigated isolates could be connected with the virulence of the used virus sources.

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K. PAULECHOVÁ, H. BAUMGARTNEROVÁ, Ústav experimentálnej fytopatológie a entomológie SAV, Ivanka pri Dunaji: **Vlastnosti niekoľkých izolátov vírusu nekrotickej krúžkovitosti višne.** — *Biol. Plant.* **16** : 444—449, 1974.

V práci sa podávajú výsledky štúdia 4 izolátov vírusu nekrotickej krúžkovitosti višne. Použité izoláty vírusu vyvolávali charakteristické symptómy na drevinných indikátoroch "Bing", "Montmorency", "F 12/1" a na semenáčoch broskýň. Vírus bol mechanicky prenesený na niektoré bylinné druhy a to *Antirrhinum majus*, *Cucumis sativus*, *Cucurbita maxima*, *Chenopodium quinoa*, *Crotalaria juncea*, *Momordica balsamina*, *Petunia hybrida* a *Leonurus sibiricus*. Pokusy o mechanicky prenos na ďalších 23 bylinných druhov boli negatívne. Termálny bod inaktivácie vírusu leží v závislosti od izolátu medzi 46—58 °C a konečný bod zriedenia medzi 10^{-1} — 10^{-2} . Vírus vydrží in vitro pri izbovej teplote viac než 1 deň. Jednotlivé izoláty vírusu dávali pozitívne serologické reakcie s Fultonovým "G" antisérom.

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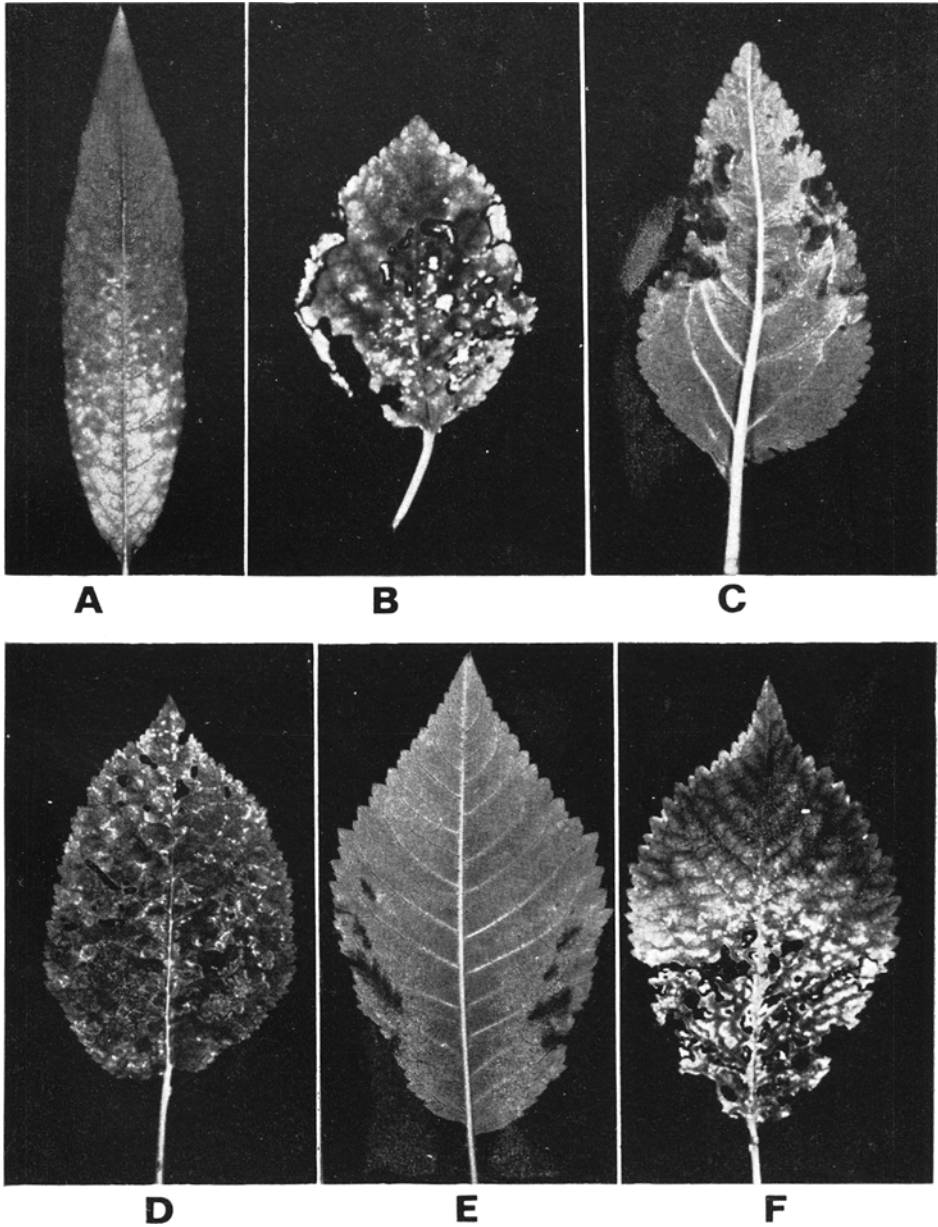


Fig. 1. Mosaic spots on peach leaves (A), necrotic lesions (B) and enations on the lower side of sour cherry leaves (C) cv. "Montmorency", individual light yellow mosaic, necrotic lesions (D) and enations (E) on cherry leaves, cv. "Bing" and necrotic lesions on cherry leaves "F 12/1" (F) after the infection with the sour cherry necrotic ringspot virus.

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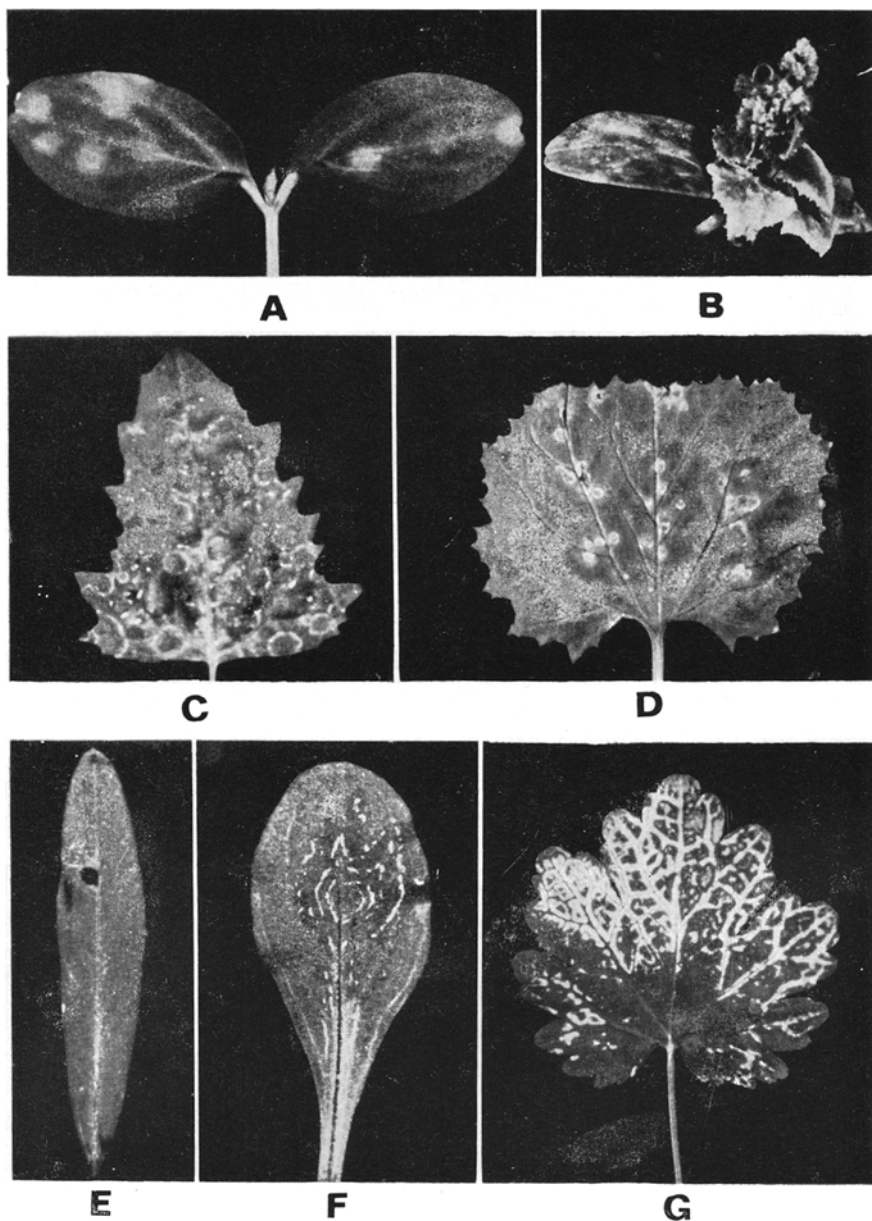


Fig. 2. Chlorotic lesions on cucumber cotyledons (A) and terminal rosettes on cucumber plants in an advanced stage of infection (B), chlorotic-necrotic spots and rings on *Chenopodium quinoa* (C), necrotic lesions on *Momordica balsamina* (D), brown-violet lesions on *Crotalaria juncea* (E), grey zonal rings on inoculated leaves of *Petunia hybrida* (F) and yellow necrotic mosaic on *Leonorus sibiricus* (G) after the infection with the sour cherry necrotic ringspot virus.