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Jaroslav Brčák and Zdenko Polák:

Identification of the Viruses Responsible for the Mosaic Disease of *Alliaria officinalis* ANDR. in Central Bohemia

Introduction

The mosaic disease of *Alliaria officinalis* ANDR.[1]) has been observed to a great extent in ruderal associations of Greater Prague's territory and its wide surroundings. *A. officinalis* being a two-year or persistent wild plant of frequent occurrence, determination of the causal agent inducing its mosaic disease was supposed to be of great importance. We assumed that it might be a question of the virus easily transmissible to some other cultural plants. *Alliaria* plants would serve as the reservoir plants for wintering of the virus.

The determination of the mosaic disease of *A. officinalis* was started with more virus isolates. Three of them (i.e. A_{59}, A_{60} and A_{61}) described in the present paper have been identified. These isolates issued from the locality in Prague 6 - Dejvice. Our experiments lasted from 1955 since 1960. In Czechoslovak literature there were no communications dealing with virus diseases of the genus *Alliaria*. For that very reason we presupposed the identity of this disease with that recorded in the meantime from Germany.

The first isolation of the cucumber mosaic virus [*Cucumis* virus 1, *Marmor cucumeris* (CMV)] from *A. officinalis* was made by USCHDRAWEIT and VALENTIN (1956), later by HEROLD and BREMER (1958). USCHDRAWEIT and VALENTIN (1957) also described the natural infection of *A. officinalis* by a special strain of the cabbage black ringspot virus (*Brassica* virus 1, *Marmor brassicae*) (CBRV) occuring especially in Berlin's parks. A variant of the same virus in *A. officinalis* was suggested by SCHWARZ (1959). BODE and BRANDES (1958) worked with a strain of the cabbage black ringspot virus isolated from *A. officinalis*. At last MILIČIĆ et al. (1958) described a virus disease of *A. officinalis* but they did not determine it. All the papers mentioned above dealt either with virus isolates the properties of which were not described or with strains of the cabbage black ringspot virus differing from common ones isolated from cultural species of the genus *Brassica*.

We have isolated four strains of viruses from *A. officinalis*: the strain of the cucumber mosaic virus (the isolate A_{59}), the strain of the cabbage black ringspot virus (the isolate A_{61}), and the mixture of the two viruses mentioned (the isolate A_{60}). Properties of all these strains are presented in the experimental part of the paper.

Material and Methods

Isolation of the viruses was made from three *Alliaria* plants having similar symptoms. The leaves had mosaic symptoms in the form of dark-green mottle accompanied by deformation and curling, sometimes with necrotic changes. In some cases the dark-green areas spread along the veins. (Fig. 1.) The invaded leaves were usually shortened and diseased plants were stunted. The symptoms described above appeared in plants from the beginning of their vegetation period.

[1]) The valide name *Alliaria petiolata* (BIEB.) CAVARA et GRANDE.

Mechanical inoculation of various host plants by means of 600-mesh carborundum powder was used for identification. Glasshouse conditions are quoted for every experiment; average values of minimum and maximum temperature and relative air humidity are presented in brackets in the above mentioned order.

The identification of the cucumber mosaic virus was completed by cross-protection tests with a yellow strain of the same virus kindly supplied by Dr. Bos from Holland. The identification experiments with the cabbage black ringspot virus were completed by comparing with the isolate from *Matthiola incana* (L.) R. Br. and by electron microscopic examination. The size of flexible particles was compared with electron micrographs of the tobacco mosaic virus prepared at the same time.

Results

I. Identification of the Isolate A_{59}

(1) Mechanical transmission to *Nicotiana glutinosa* L. 6 days after inoculation severe systemic infection appeared: mosaic in the youngest leaves (Fig. 2) and necrotic patterns in middle-aged ones.

(2) Transmission by means of aphids *Myzus persicae* Sulz. to *N. glutinosa* (12.3°—29.6° C; 94—42% r.h.). Aphids after starvation lasting three hours were transferred to the infectious source. Immediately after 15 min. acquisition threshold they were placed on the leaves of healthy plants kept in silon isolators (cf. Brčák 1959). The aphids were sprayed with Ekatine two days later. Characteristic symptoms of systemic infection developed after five days.

(3a) *Nicotiana tabacum* L. var. Samsun $T_{656s}Bs$ (12.8°—34.0° C; 95—50% r.h.). Systemic symptoms characteristic for CMV infection resulted in 13 days.

(3b) The same experiment was repeated (12.5°—29.8° C; 95—51% r.h.). The symptoms mentioned above appeared 6 days after inoculation.

(4) *Nicotiana glauca* Grah. (15.1°—30.6° C; 95—57% r. h.). The infection resulted in dark-green mosaic with not sharply defined spots which had been practically identical with the symptoms in plants infected by the check CMV strain.

(5) *Datura innoxia* Mill. (same conditions as in 4th experiment). 13 days after inoculation systemic mosaic symptoms developed: dark green spots with diffuse edges; triple parallel strips and slight rings were observed in the basal parts of some leaves. These symptoms were also similar to those caused by the CMV strain compared.

(6) *Physalis floridana* Rydb. (12.6°—34.1° C; 99—44% r.h.). Systemic symptoms identical with those of the check CMV strain appeared 9 days after inoculation.

(7) *Petunia hybrida* Vilm. (same conditions as in 4th experiment). Dark green diffuse mosaic of the youngest leaves accompanied with vein-clearing of the older ones (Fig. 3) resulted in 13 days. Afterwards the symptoms grew mild.

(8) *Amaranthus caudatus* L. (same conditions as in 6th experiment). Brown primary lesions 1 mm in diameter developed 7 days after inoculation.

(9) *Chenopodium giganteum* Don. (5.1°—39.7° C; 98—32% r.h.). Only primary infection developed: 7 days after inoculation chlorotic lesions 0.5 mm in diameter, with centres becaming necrotic, were observed.

(10) *Ch. Quinoa* Willd. (same conditions as in 9th experiment) 7 days after inoculation orange-coloured lesions 1 mm in diameter appeared. Systemic spread was not observed.

(11) *Vigna sinensis* (L.) Endlicher var. Black (11.9°—31.7° C; 97—47% r.h.) 10 days after infection both primary symptoms (chlorotic lesions 1 mm in diameter) in inoculated cotyledons (Fig. 4) and systemic symptoms (mosaic in the leaves) appeared.

(12) *Cucumis sativus* L. var. Delikates (11.5°—31.2° C; 99—49% r.h.). Systemic symptoms characteristic for CMV infection developed.

(13) Cross-protection test (12.1°—31.5° C; 94—44% r.h.). Tobacco plants (*Nicotiana tabacum* var. Samsun $T_{656s}Bs$) having distinct symptoms of systemic infection were superinoculated with the yellow CMV strain 11 days after inoculation with the isolate A_{59}. No symptoms of the yellow strain were observed during 44 days. By this test the mutual antagonism of the isolate A_{59} against the yellow CMV strain was clearly proved.

On the basis of the above mentioned experiments it was ascertained that the causal virus had been mechanically transmissible and nonpersistent causing in differential hosts symptoms identical with those caused by other strains of CMV. Positive cross-protection with the yellow CMV strain was stated.

Literary references dealing with *N. tabacum*, *N. glutinosa*, *A. caudatus*, *Ch. giganteum*, *Ch. Quinoa*, and *C. sativus* as differential hosts for CMV were presented in a previous paper (POLÁK and BRČÁK, 1961). For that very reason there are not included here. The similar or identical symptoms in additional differential hosts used in our experiments were described by: HEIN (1957), KOVAČEVSKI (1960), HARRISON (1958), WILLISON and WEINTRAUB (1957) for *Petunia hybrida* and by WILLISON and WEINTRAUB (1957) for *Vigna sinensis*. The symptoms of systemic infection caused by the isolate A_{59} in *N. glauca*, *D. innoxia* and *P. floridana* corresponded always with those induced by the check CMV strain isolated from glasshouse grown cucumber plants. Thus the identity of the isolate A_{59} with the common CMV strain was unambiguously demonstrated.

II. Identification of the Isolate A_{60}

Transmission to *Nicotiana glutinosa* L. (11.2°—36.1° C; 97—43% r.h.). The infection resulted in veinclearing of young leaves after 5 days; after 16 days severe mosaic symptoms appeared. Sap extracted from systemically infected leaves was inoculated on tobacco leaves (*N. tabacum* var. Samsun). Brown primary lesions and systemic infection of CMV type appeared.

Transmission to *Nicotiana tabacum* L. var. Samsun (14.0°—41.1° C; 97—34% r.h.). Primary infection developed 5 days after inoculation: brown necrotic lesions in the inoculated leaves; systemic infection developed after 6 days: clearing of veins and convexities of intervenial areas (Fig. 5).

These symptoms suggested that it was a question of a mixed infection. Therefore a further passage was made from the leaves with primary infection to healthy tobacco plants. The tobacco plants developed both brown primary necrotic lesions and faint grey necrotic paintings with rings 3—4 mm in diameter 4 days after inoculation (Fig. 6). Systemic infection appeared simultaneously: chlorosis and faint necrotic vein banding (Fig. 7) or faint necrotic rings (Fig. 8), (13.2°—34.5° C; 95—46% r.h.).

For the second passage (11.9°—31.7° C; 97—47% r.h.) inoculum from primary infected leaves and inoculum from systemically infected ones were prepared separately (the plants used for this purpose were infected 14 days before). The inoculation with an extract prepared from the leaves with primary necrotic symptoms resulted again in formation of brown primary necrotic lesions; 10 days after infection systemic symptoms were observed, too. The tobacco plants infected with the material obtained from leaves systemically invaded developed symptoms of systemic infection only (no brown necrotic lesions were observed).

In this way the virus causing systemic infection in tobacco, being identical with that of CMV, was separated from the virus complex. For determination if this component both differential hosts and cross-protection tests with yellow CMV strain were used as follows:

(1) *Cucumis sativus* L. (12.5—34.2° C; 98—44% r.h.) — diffuse chlorotic lesions appeared in inoculated cotyledons during 5 days; 2 days later systemic mosaic with ringlike chlorotic spots developed (Fig. 9).

(2) *Physalis floridana* RYDB. (the same experimental conditions). The infection resulted in systemic mosaic of CMV type after 7 days.

(3) *Amaranthus caudatus* L. (11.2—32.2° C; 98—45% r.h.) — characteristic symptoms for CMV infection (primary brown local lesions) appeared (Fig. 10).

(4) *Gomphrena globosa* L. (12.6—34.1° C; 99—44% r.h.) — symptomless.

(5) *N. tabacum* var. Samsun (13.0—34.3° C; 99—45% r.h.) primary systemic infection accompanied by grey stripe necrosis of CMV type appeared.

(6) *Chenopodium Quinoa* Willd. (Fig. 11) and *Ch. giganteum* Don. (12.5—34.2° C; 99—44% r.h.). Primary lesions developed during 2 days.

(7) Cross-protection tests.

For the first cross-protection test tobacco plants infected 11 days before with the component preliminarily considered for CMV strain were used (12.1°—31.5° C; 94—44% r.h.). After inoculation with the yellow CMV strain no symptoms of infection by this strain were observed during 44 days.

For the second cross-protection test twelve tobacco plants infected 7 days before with the complex of both viruses tested were used (these were the plants originally infected from *Alliaria*). Also in this series no symptoms of the yellow strain of CMV after reinoculation were observed. The experiment was watched for 27 days. In the check series inoculated only by the yellow CMV strain (simultaneously) there were thirteen infected plants out of fourteen inoculated ones.

The results of the experiments mentioned have demonstrated the identity of the isolated component (causing systemic infection after transmission from *Alliaria*) with the CMV.

The second component of the complex inducing only primary symptoms in the inoculated leaves of tobacco (the systemic infection is not induced) could not be separated from the complex with CMV by mechanical inoculation. For that very reason the characterisation of this component was made only by comparing the material obtained from tobacco leaves with symptoms of primary infection by both viruses with that of tobacco leaves having systemic symptoms only identified above as caused by CMV.

(1) In *N. tabacum* var. Samsun as distinct from the symptoms of CMV always only brown necrotic lesions were formed during 7 days. (13.0—34.3° C; 99—45% r.h.).

(2) *Gomphrena globosa* L. (12.6—34.1° C; 99—44% r.h.) reacted by formation of lesions 9 days after inoculation as distinct from the symptomless reaction to infection by the CMV component.

(3) *Chenopodium giganteum* Don. (12.5—34.2° C; 99—45% r.h.). In the leaves inoculated by the homogenate of the tobacco leaves having symptoms of primary infection by both viruses, two kinds of lesions developed: light-coloured small lesions 0.5—1.0 mm. in diameter with a nearly white necrotic spot in the centre surrounded by light-brown halo (these lesions were formed also after infection by CMV component — Fig. 12 — the two lower leaves) and large lesions 1.5—2.0 mm. in diameter with red centres, which had never been induced by inoculation with CMV (Fig. 12 — the two upper leaves).

All these experiments indicated that the second component of the mixture might be identical with the cabbage black ring virus (CBRV) (*Brassica* virus 1, *Marmor brassicae*).

The fact that the two types of lesions occurring in *Ch. giganteum* leaves are due to the two components of the mixture was proved by the passage of the separated lesions to *Nicotiana tabacum* var. Samsun leaves: after inoculation with the homogenate prepared from cut out large lesions brown necrotic lesions in tobacco leaves developed. The homogenate of small lesions being used as inoculum the symptoms mentioned above did not appear.

For the identification of the second component of the virus mixture the isolate of the virus prepared from petals of *Matthiola incana* (L.) R. Br. was used as comparative material. After transmission to tobacco plants this isolate induced the characteristic brown primary lesions but never systemic infection. (Negative evidence of systemic infection was given by inoculation with the sap extracted from top leaves of infected tobacco plants.) The virus isolate from *Matthiola* was identical with that described by Uschdraweit and Valentin (1957) and Herold (1957).

The identification of the second virus component by means of differential host reactions is considered to be wholly sufficient. The symptoms correspond to those quoted in literature: the first description of this virus by Smith (1935) and the results of other authors indicated that the viruses of *Brassica* virus 1 group were characterized by the primary necrotic reaction of tobacco leaves without any systemic infection. *N. glutinosa* reacts systemically (converted relation between CBRV and TMV). The component of the isolate A_{60} reacted in the same way. The reaction of *Chenopodium giganteum* (= *amaranticolor*) leaves also corresponds to the data of Smith (1957) and Uschdraweit and

VALENTIN (1957), and the same reaction of *Gomphrena globosa* was described by HEROLD (1957).

Additional evidence that this component belonged to the *Brassica* virus 1 group were given by experiments with the third isolate (A_{61}).

III. Identification of the Isolate A_{61}

(1) *Nicotiana tabacum* var. Samsun (11.7—33.5° C; 97—49% r.h.) only brown primary necrotic lesions developed; no systemic infection occurred. There was evidence that there was no systemic infection: The leaves of healthy plants were inoculated with the sap from the top leaves (non inoculated); no infection resulted. In a further experiment cut out lesions and cut out areas of leaf edges between lesions were inoculated separately on the leaves of two tobacco series. The homogenate prepared from lesions resulted in lesions formation again, the other homogenate was noninfectious (no symptoms developed).

(2) *Nicotiana glutinosa* L. (12.7—32.4° C; 94—44% r.h.). The inoculation by the homogenate prepared from lesions in tobaco leaves (plants from the previous experiment were used) resulted in systemic infection: large chlorotic spots with faint necrosis in the centre occured (Fig. 13). Two months later necrotis rings were formed around the chlorotic spots. The spots were 8—10 mm. in diameter. Sometimes necrosis spread on into the spots. After backward transmission of the sap of these leaves to *Nicotiana tabacum* var. Samsun characteristic necrotic lesions developed.

(3) *Chenopodium giganteum* Don. (12.5—31.6° C; 97—42% r. h.). Yellowish-green lesions with necrotic centres occurred 8 days after inoculation of the homogenate from lesions formed in tobacco leaves. After 20 days the lesions were crimson-coloured, surrounded by a chlorotic halo, with a white necrosis in the centre; they had 3 mm in diameter (Fig. 14).

(4) The transmission to seedlings of *Brassica oleracea* L. var. *capitata* L. (Dobrovodské pozdní), repeated twice, was unsuccessful.

(5) The electron microscopic examination. Preparations for electron microscopic examination were made by grinding individual local lesions from *Nicotiana tabacum* var. Samsun (approximately 1 cm^2 of leaf area containing lesions was homogenized in 10 ml. of sterile redistilled water). The suspension was spread on specimen grids covered with collodion membrane.

Shading was carried out with platinum and carbon. For the examination and photography the table electron microscope Tesla BS 242 was used. Flexible thread-like particles were observed on electron micrographs (Fig. 15). The size of these particles was determined by comparison with the known size of tobacco mosaic virus rod-like particles prepared in the same way. The normal length of thread-like particles was 759 mμ. BODE and BRANDES (1958) indicated that the normal length of CBRV particles was 754 mμ and the diameter of about 12—13 mμ. Our results fully correspond to those of BODE and BRANDES. USCHDRAWEIT and VALENTIN (1957) described identical particles of the same virus which had been isolated from *Matthiola incana* and *Brassica pekinensis* RUPR.; an average length of 756 mμ was given.

In the case of the isolate A_{61} CBRV was the causal agent as indicated above.

Discussion

The mosaic disease of *Alliaria officinalis* was caused either by CMV infection (the isolate A_{59}) or by CBRV (the isolate A_{61}) or by the complex of both viruses (the isolate A_{60}). According to the symptoms in *Alliaria officinalis* it seems to be impossible to distinguish the three types mentioned. All these types were collected in one locality and so it is not possible to say which of the agents prevails. Both viruses are of a great epidemiological and therefore economical importance. Both winter in the underground parts of *Alliaria officinalis*. It will be necessary to carry out a detailed analysis of most infested rudderal associations. CBRV seems to have a wide natural host range as in the case of CMV; additional new hosts are still described (e.g. LOVISOLO and BENETTI, 1960). In accordance with publications by German authors we suggest that CBRV occurs mostly in large European areas in *Alliaria officinalis*. The communication of MILIČIĆ et al. (1958) is in agreement with our suggestion. These authors did not determine the causal virus; we suggest

however on the basis of symptom expression in *N. glutinosa* and *Hesperis matronalis* L. that their isolate belongs to the group of CBRV.

It is more suitable to speak about the viruses of the CBRV group than about one virus. A lot of experimental results suggest that CBRV has many strains which differ substantially namely in reactions on various hosts. The infection of *Brassica oleracea* var. *capitata* by the isolate A_{61} failed although this isolate belongs to the CBRV group undoubtedly; HEYLAND-STEINHILBER (1958) failed to transmit a strain of the same virus to *B. oleracea* var. *capitata* as well as to some other varieties. On the other hand USCHDRAWEIT and VALENTIN (1957) got distinct symptoms in some forms of *B. oleracea* var. *capitata* by their strain of CBRV, but in one form symptomless infection only. These authors failed to infect *Nicotiana glutinosa* and *Gomphrena globosa* by their CBRV strain which should be very interesting. All the results mentioned above proved the wide breaking up of the CBRV group.

In view of a high economic importance of both viruses [HEROLD (1957) indicated in the case of CBRV crop loses of about 20—45%], we find it necessary to study further ecological relations which could be very important for destroying dangerous sources of viruses transmissible to cultivated plants.

Summary

The mosaic disease of *Alliaria officinalis* characterized by dark green areas and deformations of the leaves, sometimes by their necrosis, and by dwarfing of the whole plant was identified. The following differential hosts were used for this purpose: *Nicotiana tabacum* var. Samsun, *N. glutinosa*, *N. glauca*, *Physalis floridana*, *Datura innoxia*, *Petunia hybrida*, *Vigna sinensis* var. black, *Amaranthus caudatus*, *Chenopodium giganteum*, *Ch. Quinoa*, *Cucumis sativus*, and *Gomphrena globosa*. Artificial transmission by *Myzus persicae* SULZ., cross-protection tests and electron microscopic examinations were used for further identification experiments. The following causal viruses were described: the common cucumber mosaic virus (CMV), and a special strain of the cabbage black ringspot virus (CBRV); the mosaic of *A. officinalis* can be induced either by a single virus (by CMV or by CBRV) or by a double infection of the two viruses. CMV can be separated from CBRV in a double-infected plant after the transmission to *N. tabacum*. It is also possible to distinguish the double infection (CMV with CBRV) in *Ch. giganteum*. Small lesions are formed on leaves of this plant by CMV and larger lesions are formed by CBRV when inoculated simultaneously. Attempts to transmit the strain of CBRV to *Brassica oleracea* var. *capitata* failed. The properties of the CBRV strain were compared with those of another strain of CBRV obtained from petals of *Matthiola incana*.

Thread-like particles, having normal length of 759 mμ, were observed on electron micrographs in the case of CBRV.

A. officinalis is considered to be an important source of CMV and CBRV infection representing danger for cultural plants. Both viruses winter in the underground parts of *A. officinalis*.

References

BODE, O. und BRANDES, J. (1958): Elektronenmikroskopische Untersuchung des Kohlrübenmosaik-Virus (turnip mosaic virus). — Phytopath. Z. 34 (1) : 103—106.

Brčák, J. (1959): Transmission of beet mosaic virus by the green peach aphid starved before infection feeding under different conditions of air humidity. — Biol. plant. 1 (4) : 330—332.
Harrison, B. D. (1958): Cucumber mosaic virus in raspberry. — Plant Path. 7 (3) : 109—111.
Hein, A. (1957): Beiträge zur Kenntnis der Viruskrankheiten an Unkräutern. III. Das Gurkenmosaikvirus. — Phytopath. Z. 29 (2) : 204—229.
Herold, F. (1957): Zur Symptomatik und Schadwirkung des Kohlschwarzringfleckenvirus. — Phytopath. Z. 31 (2) : 149—157.
Herold, F. und Bremer, H. (1958): Untersuchungen zur Epidemiologie, Ökologie und Bekämpfung des Gurkenmosaikvirus. — Gartenbauwissenschaft 23 : 254—274.
Heyland-Steinhilber, H. (1958): Untersuchungen über ein in Württemberg an Kohlrüben (*Brassica napus rapifera*) vorkommendes Virus. — Phytopath. Z. 32 (2) : 181—206.
Kovačevski, I. (1960): Изследвания върху жиловата некроза по домата. (Untersuchungen über die Andernnekrose der Tomaten.) — Research in memoriam Dontcho Kostoff, Published by the Bulgarian Acad. Sci., Sofia, 1960 : 143—172.
Kvíčala, B. A. (1949): Některé vztahy mezi virem mosaiky zelí a mšicí broskvoňovou (*Myzodes persicae*) pokusně zjišťované na tabáku. — Sb. ČAZ, 22 (1) : 121—138.
Lovisolo, O. et Benetti, M. P. (1960): Virus e piante spontanee. II. Nuovi ospiti del virus della muculatura anulare nera del cavolo. — Boll. Staz. Patol. Veg. 17 (1) : 61—70.
Miličić, D., Panjan, M., Bilanović, D. und Katić, B. (1958): Viruskrankheit von *Alliaria officinalis*. — Acta botan. Croatica 17 : 159—176.
Polák, Z. and Brčák, J. (1961): Identification of the mosaic of *Actium lappa* L. caused by the common cucumber mosaic virus. — Preslia 33 : 357—362.
Schwarz, R. (1959): Epidemiologische Untersuchungen über einige Viren der Unkraut- und Ruderalflora Berlins. — Phytopath. Z. 35 (3) : 238—270.
Smith, K. M. (1935): A virus disease of cultivated crucifers. — Ann. appl. Biol. 22 (2) : 239—242.
Smith, K. M. (1957): A textbook of plant virus diseases. — London, 1957.
Ullrich, J. (1955): Schwarzringfleckigkeit des Kohls in Deutschland. — Nachrichtenblatt d. Deutsch. Pflanzenschutzd. 7 (10) : 2 pp. sep.
Uschdraweit, H. A. und Valentin, H. (1956): Winterwirte des Gurgenmosaiks. — Angew. Bot. 30 (3) : 73—79.
Uschdraweit, H. A. und Valentin, H. (1957): Untersuchungen über ein Kruziferen-Virus. — Phytopath. Z. 31 (2) : 139—148.
Willison, R. S. and Weintraub, M. (1957): Properties of a strain of cucumber-mosaic virus isolated from *Prunus* hosts. — Canad. J. Bot. 35 (5) : 763—771.

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Explanations of the photographs on the plates

(All photographs by Dr. J. Brčák)

Plate XII. Fig. 1. Symptoms of systemic infection on the leaves of *Alliaria officinalis*: dark-green areas along the veins.
Fig. 2. Systemic infection of *Nicotiana glutinosa* caused by a strain of the cucumber mosaic virus isolated from *A. officinalis* (the isolate A_{59}).

Plate XIII. Fig. 3. Systemic veinclearing of the *Petunia* leaf caused by the isolate A_{59}.
Fig. 4. Chlorotic primary symptoms in the inoculated cotyledon of *Vigna sinensis* var. Black, 15 days after rubbing with the isolate A_{59}.
Fig. 5. Leaves of *Nicotiana tabacum* showing symptoms of infection by the isolate A_{60} six days after inoculation. To the left: primary necrotic lesions on the rubbed leaf caused by a strain of the cabbage black ringspot virus (CBRV). To the right: systemic infection of a young leaf (convexities of intervenial areas) caused by the common cucumber mosaic virus (CMV).
Fig. 6. Necrotic symptoms in the inoculated tobacco leaf caused by the mixed infection of CBRV and CMV (the isolate A_{60}) ten days after rubbing: large faint grey necrotic rings and paintings were caused by CMV, brown necrotic local lesions by CBRV.

Plate XIV. Fig. 7. Chlorosis and necrotic veinbanding in tobacco leaf caused by systemic infection with the CMV component of the isolate A_{60} 15 days after infection.

Fig. 8. Faint necrotic rings in *N. tabacum* var. Samsun induced by systemic infection of the CMV component of the isolate A_{60} six days after infection.
Fig. 9. Chlorotic symptoms of systemic infection by the CMV component of the isolate A_{60} in a cucumber leaf after eight days.
Fig. 10. Brown local lesions on *Amaranthus caudatus* caused by the CMV component of the isolate A_{60} four days after rubbing.
Fig. 11. The leaf of *Chenopodium Quinoa* showing necrotic local lesions by CMV (the isolate A_{60}) four days after rubbing.
Fig. 12. Leaves of *Chenopodium giganteum* with local lesions (after nine days) caused by the two components of the isolate A_{60} which were separated in *N. tabacum* var. Samsun: The two upper leaves were rubbed with crude sap taken from primary infected leaves of *N. tabacum* by the isolate A_{60} and showed two kinds of lesions caused either by CBRV (larger lesions) or by CMV (smaller lesions) respectively. The two lower leaves were rubbed with sap taken from systemic infected leaves of *N. tabacum* by the isolate A_{60}; smaller lesions caused by the CMV component appeared on inoculated leaves only.
Fig. 14. Crimson — coloured necrotic lesions in *Ch. giganteum* formed after rubbing with the isolate A_{61} (= CBRV) twenty days after developing.

Plate XV. Fig. 13. Symptoms of systemic infection of *Nicotiana glutinosa* caused by the isolate A_{61} (= CBRV): chlorotic spots with faint necrosis 24 days after inoculation.
Fig. 15. Electron micrographs of flexible CBRV particles (the isolate A_{61}). Shaded with Pt + C, magnified by 31,000.

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Jaroslav Brčák a Zdenko Polák:

Identifikace virů způsobujících mozaiku česnáčku lékařského (*Alliaria officinalis* Andr.) ve středních Čechách

Byli vyšetřeni původci mozaiky česnáčku, která se projevuje nejčastěji tmavými skvrnami a deformacemi listů, později též jejich nekrózou a zakrslostí celé rostliny. Pomocí přenosů na diferenční hostitele (*Nicotiana tabacum* var. Samsun, *N. glutinosa*, *N. glauca*, *Physalis floridana*, *Datura innoxia*, *Petunia hybrida*, *Vigna sinensis*, *Amaranthus caudatus*, *Chenopodium giganteum*, *Ch. Quinoa*, *Cucumis sativus*, *Gomphrena globosa*), pomocí přenosu *Myzus persicae*, pomocí křížových testů a elektronovým mikroskopem bylo zjištěno, že mozaika česnáčku může být vyvolávána buď virem mozaiky okurky, nebo kmenem ze skupiny viru černé kroužkovitosti zelí, nebo směsí obou těchto virů. Virus mozaiky okurky byl z komplexu oddělen od viru černé kroužkovitosti zelí pasáží přes *N. tabacum*. *Ch. giganteum* podle typu lézí rovněž rozlišilo oba viry. Isolovaný kmen viru černé kroužkovitosti zelí se nepodařilo přenést na *Brassica oleracea* var. *capitata*; tento virus byl srovnáván s jiným kmenem téhož viru získaným z korunních plátků *Matthiola incana*. U viru černé kroužkovitosti zelí byly zjištěny elektronkovým mikroskopem ohebné částice uniformní délky 759 mμ. Autoři považují česnáček za významný zdroj infekce oběma viry pro kulturní rostliny, protože v česnáčku tyto viry přezimují.