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Zdenko Polák and Jaroslav Brčák:

Identification of the mosaic of *Arctium lappa* L. caused by the common cucumber mosaic virus

Introduction

Identification of a virus disease of *Arctium lappa* L. occurring to a great extent in ruderal associations of Greater Prague's territory is presented. In world literature there is only a limited number of references dealing mostly with symptoms of diseased *Arctium*. No exact determination of the disease was made. Only WILKINSON (1952) demonstrated the tomato ringspot virus as the causal agent of diseased *A. lappa* in the U.S.A. An unidentified disease probably of virus origin was described by SHAPIRO (1934) (cf. KÖHLER, KLINKOWSKI 1954). A virus disease of *Arctium* was mentioned also by MACCLEMENT & RICHARDS (1956) and PROCENKO (1957). MURAVIEV (1930) and BORISEVITSCH (1930) suggested that the disease of *A. lappa* observed in the Ukraine was caused by the sugar beet mosaic virus. First attempts to transmit an undetermined virus disease of *A. tomentosum* Mill. in this country were carried out by BLATTNÝ jun. (1955). He described the symptoms of the disease in *A. tomentosum* as large diffuse mosaic spots in middle-aged leaves (the symptoms described are similar to those in plants used for our experiments). Blattný succeeded to transmit the disease to sugar beet seedlings both by means of mechanical inoculation and aphid transmission (*Aphis fabae* SCOP. was used in the experiments); symptoms in sugar beet leaves developed 53 days after mechanical inoculation and 61 days after transmission by aphids. Common cucumber mosaic virus was transmitted to *A. lappa* seedlings by SCHWARZ (1959). The author suggested, however, that this plant was not a suitable host for wintering of the virus. He failed to transmit it to healthy *Arctium* seedlings by aphids. At last BAUDYŠ (1949) described a plant of *Arctium* sp. having expressive mosaic symptoms in the form of large yellow spots in the leaves. These symptoms also agree with those in our starting plants.

It follows from the survey of literary references cited in the present paper that up to this time it has not been possible to preconcieve the significance of the disease and draw any conclusions. Therefore we have tried to achieve an exact determination of the disease. We have demonstrated that the causal agent is the widespread cucumber mosaic virus (CMV) known as infectious for many species of cultural plants. In the case of *Arctium lappa*, which is a biennial plant, there is good reason to suppose that it is a suitable host plant for virus wintering being a frequent source of infection in nature.

Material

Two diseased plants of *A. lappa* (representing two observed types of the disease), rather distinct in symptoms, were used for our identification experiments. The causal virus induced symptoms especially in leaves. The main

symptom of first type diseased *Arctium* (isolate A_1) was severe yellow mosaic. Spots having bright yellow centres, sometimes becoming necrotic, were surrounded by diffuse halos (Fig. 1). Neither abbreviation of leaf area nor stunting of plants were observed.

The symptoms of the second type of the disease (isolate A_2) can be characterized as follows: expressive pale green or yellowish green mosaic mottling accompanied with distinct abbreviation of leaf area. The leaves were perforated, usually with dark brown necrotic spots 2 mm in diameter (Fig. 2). Affected plants were strikingly stunted.

Methods

Leaf tissue of infected plants was homogenized and crude untreated homogenate was used as inoculum. Before rubbing, the leaves were dusted with 600-mesh carborundum powder. In some cases the stabilization of infectivity of tissue homogenate was made by adding several drops of 0,02 M cysteine hydrochloride. Superinfection in cross-protection tests was carried out by inoculation of a yellow CMV strain (Fig. 3), kindly supplied by Dr. Bos from Holland.

Inoculated plants were incubated for suitable periods under registered conditions of air temperature and humidity. (Averages of these values are quoted for every experiment.) Test plants were grown in a virus-free greenhouse which was kept free of insects.

Identification of the two virus isolates was made according to systemic or local symptoms developed in the following differential host species: *Nicotiana tabacum* L. var. *Samsun* 656sBs, *N. tabacum* L. var. *Xanthi-nc* 593BsCs, *N. glutinosa* L., *Cucumis sativus* L. var. *Delikates*, *Chenopodium Quinoa* WILLD., *Ch. giganteum* DON., *Amaranthus caudatus* L.

Results

I. Identification of the isolate A_1 and symptoms in plants tested

1. a) The transmission to *Nicotiana tabacum* L. var. *Samsun*. Nine plants were inoculated towards the end of June. Air temperature was between 11° and 30° C, relative air humidity between 97 and 48%. 13 days after inoculation the treated leaves showed distinct primary reaction (small necrotic spots formed irregular patterns). This stage was immediately followed by light intervenial mosaic systemically spread especially in the apical parts of leaves (Fig. 5).

b) Sixteen plants were inoculated towards the end of July. (Air temperature 12.5°—33.0° C, relative air humidity 92—36%). Symptoms which developed 7—13 days after inoculation were corresponding to those in the experiment 1 a).

c) Twelve plants were inoculated towards the end of July. (Air temperature 12.7°—33.6° C, relative air humidity 93—35%.) 4—10 days after inoculation the same result was obtained.

2. The transmission to *Nicotiana glutinosa* L. Nine plants were inoculated towards the end of July. (Air temperature 12.0—32.4° C, relative air humidity 93—40%.) 12—15 days after inoculation distinct symptoms of systemic infection developed: slight mosaic in youngest leaves and necrotic patterns in middle-aged leaves, deformation of the leaves and minute necrotic areas on the edges of young leaves (Fig. 4).

3.—4. The transmission to *Chenopodium Quinoa* WILLD. and *Ch. giganteum* DON. Twelve leaves of each species were inoculated towards the end of July. (Air temperature 10.4°—28.0° C, relative air humidity 99—54%). 6 days after inoculation expressive symptoms of local infection developed (chlorotic local lesions with necrotic centres). No systemic spread of the infection was observed.

5. a) The transmission to *Cucumis sativus* L. Ten plants were inoculated towards the end of June. (Air temperature 12°—30° C, relative air humidity 95—47%.) Symptoms of systemic infection and stunting developed 27 days after inoculation. The evidence of the transmission was given by further transmission to *N. tabacum* var. *Samsun* which was carried out 28 days after inoculation of cucumber plants.

5. b) Eight plants were inoculated towards the end of July. (Air temperature 12.4°—32.0° C, relative air humidity 93—41%.) Five days after inoculation primary symptoms in inoculated leaves developed (chlorotic spots, sometimes ring-shaped, 2—3 mm in diameter). During 17 days systemic ring mosaic was observed.

6. Determination of the isolate A_1 by means of cross-protection test. The test was carried out at the beginning of September. (Air temperature 12.6° to 34.0° C, relative air humidity 94—43%.) 28 plants of *N. tabacum* var. *Samsun* were inoculated with the isolate A_1. 10—13 days after the first inoculation they were superinoculated with the yellow CMV strain. At the same time 20 check plants were inoculated with the yellow strain. In 28 plants first infected with the isolate A_1 no symptoms of challenge inoculated yellow strain developed. In control series, however, there were 14 plants infected with yellow CMV out of the 20 inoculated. This test was watched 27 days after the second inoculation. In this way the praemunity of the isolate A_1 against the yellow strain was demonstrated.

II. Identification of the isolate A_2; symptoms in plants tested

1. a) The transmission to *Nicotiana tabacum* L. var. *Samsun*. (Air temperature 13—33° C, relative air humidity 95—46%.) The test was carried out at the beginning of June. 13 days after inoculation characteristic symptoms of systemic infection developed.

1. b) The transmission to *Nicotiana tabacum* L. var. *Xanthi-nc*. The same symptoms as in *N. tabacum* var. *Samsun* were observed.

2. The transmission to *Nicotiana glutinosa* L. The results were identical with those obtained with the isolate A_1.

3.—4. The transmission to *Chenopodium Quinoa* WILLD. and *Ch. giganteum* DON. (Air temperature 5.2°—40.0° C, relative air humidity 98—32%.) The test was carried out at the beginning of May. 7 days after inoculation yellow lesions becoming slowly necrotic developed. No systemic spread of the infection was observed.

5. The transmission to *Cucumis sativus* L. At the beginning of May and at the end of June the attempts to transmit the virus to cucumber plants failed. The successful transmission was made at the beginning of July (air temperature 13°—31° C, relative air humidity 95—44%). Sixteen plants were inoculated on cotyledons. 9—10 days after inoculation systemic distinct light rings 2 mm in diameter developed in young leaves as well as in the inoculated leaves (Fig. 6).

6. The transmission to *Amaranthus caudatus* L. (Air temperature 12.5° to 30.0° C, relative air humidity 96—51%.) After 6 days primary rusty brown ring-shaped lesions appeared.

7. Determination of the isolate A_2 by means of cross-protection test. Transmission of the challenging yellow CMV strain to *N. tabacum* var. *Samsun* first

infected by the isolate A_2 was not successful. Thus praemunity of the isolate A_2 against the yellow strain was proved.

Symptoms in differential host plants used in the above-mentioned experiments corresponded with those described for the common cucumber mosaic virus (CMV) in numerous recent papers.(For instance symptoms in *Nicotiana tabacum* (FULTON 1950, ROLAND 1955, SMITH 1957, GOVIER 1957, SIMONS 1957, WILLISON and WEINTRAUB 1957, SOLYMOSY 1960); symptoms in *Nicotiana glutinosa* (GOVIER 1957, SMITH 1957, SOLYMOSY 1960, WILLISON and WEINTRAUB 1957); symptoms in *Cucumis sativus* (SIMONS 1957, SMITH 1957, SOLYMOSY 1960, WILLISON and WEINTRAUB 1957); in *Chenopodium Quinoa* (USCHDRAWEIT 1955, SOLYMOSY 1960); in *Chenopodium giganteum* (HARRISON 1958), and in *Amaranthus caudatus* (FULTON 1950, SOLYMOSY 1960).

The phenomenon of praemunity in both A_1 and A_2 isolates served as the principal proof for the mosaic of *Arctium lappa* to be caused by CMV. On the basis of these facts the authors consider the identity of the causal virus and CMV to be wholly demonstrated.

Discussion

The authors have proved experimentally that the widespread mosaic disease of *Arctium lappa* is caused by CMV. During the experimental work with two isolates of the virus it has been ascertained that mechanical transmission of the virus from *Arctium* to some differential hosts is not always easy. For that very reason drops of cysteine hydrochloride solution were added to crude inoculum, besides carborundum powder with which the leaves were dusted. Infectivity of both isolates, however, was not the same; the transmissibility of the isolate A_2 was more difficult than that of A_1 even though both isolates had been prepared from the same species of *Arctium* and transmitted to the same differential host species in the same way. On the basis of our experiments we can not conclude whether these isolates are adapted strains of CMV to which other plant species are less susceptible. In no case it is possible to agree with the statement of BAUDYŠ (1949) — even if we gather from the symptoms described that the same virus is concerned — and that it might be a "useful disease" as the diseased *Arctium* gets stunted and wilts. Rather we must allow for the possibility that the virus is transferred from *Arctium* to cultural plants analogically as in the case of natural sources of infection. A strong infestation of *Arctium* by *Aphis fabae* SCOP., the vector of CMV, clearly points out such a danger. According to our findings SCHWARZ's (1959) opinion that *Arctium* is no suitable host for wintering of CMV is incorrect.

Summary

The authors have demonstrated that the virus disease of *Arctium lappa* L. manifested in natural conditions by green or yellow mosaic sometimes accompanied with necrotic spots, perforations of leaves and stunting of plants is caused by strains of common cucumber mosaic virus. The virus was identified by means of artificial transmission to *Nicotiana tabacum* L., *N. glutinosa* L., *Cucumis sativus* L., *Chenopodium Quinoa* WILLD., *Ch. giganteum* DON. and *Amaranthus caudatus* L. and cross-protection tests with a yellow CMV strain. The mosaic of *Arctium* is considered to be a dangerous source of CMV for cultural plants.

Souhrn

Identifikace mozaiky *Arctium lappa* L. působené virem mozaiky okurky. Autoři dokázali, že virová choroba lopuchu většího *Arctium lappa* L., projevující se zelenou nebo žlutou mozaikou provázenou někdy nekrotickými skvrnami, proděravěním listů a zakrsáním rostlin, je působena

virem mozaiky okurky. Determinace choroby (u dvou isolátů viru) byla provedena jednak umělými přenosy na diferenční hostitele *Nicotiana tabacum* L., *N. glutinosa* L., *Cucumis sativus* L., *Chenopodium Quinoa* Willd., *Ch. giganteum* Don. a *Amaranthus caudatus* L., jednak pomocí křížových testů se žlutým kmenem viru mozaiky okurky. Autoři považují mozaiku lopuchu za nebezpečný přirozený zdroj nákazy, z něhož může virus mozaiky okurky přecházet na kulturní rostliny.

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Explanation of Plates XXVI—XXIX

Fig. 1. Symptoms of the mosaic of *Arctium lappa* (isolate A_1). Photograph by J. Brčák.

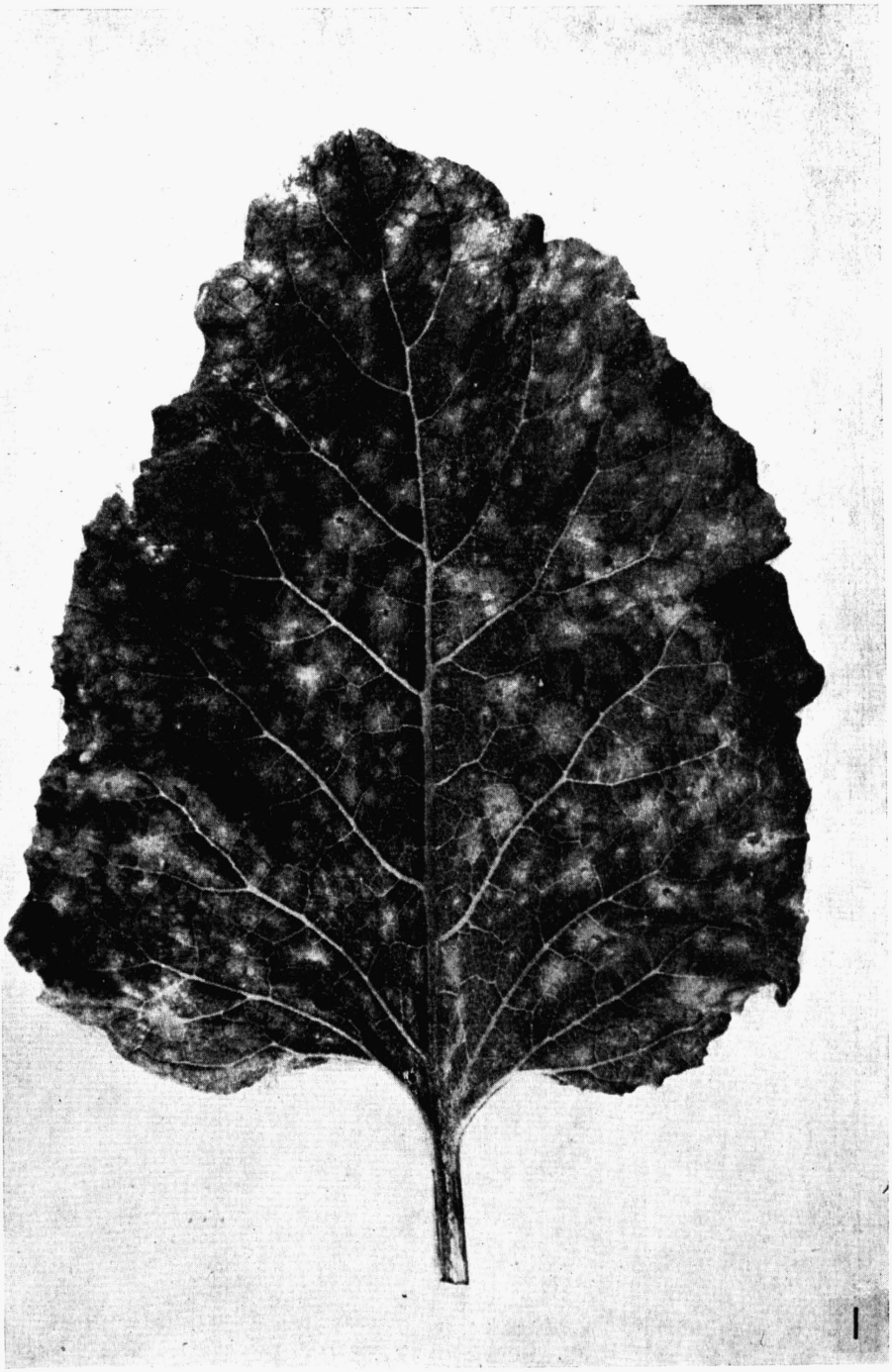
Fig. 2. Symptoms of the mosaic of *Arctium lappa* (isolate A_2). Photograph by J. Brčák.

Fig. 3. Symptom expression in the leaves of *Nicotiana tabacum* var. *Samsun* by the yellow strain of CMV used for cross-protection tests. Photograph by J. Brčák.

Fig. 4. Symptoms of systemic infection caused by CMV (isolate A_1) in *Nicotiana glutinosa*, 17 days after inoculation. Photograph by J. Brčák.

Fig. 5. Symptoms of systemic infection caused by CMV (isolate A_1) in *Nicotiana tabacum* var. *Samsun*, 14 days after inoculation. Photograph by J. Brčák.

Fig. 6. Primary symptoms caused by CMV (isolate A_2) in the rubbed leaf of *Cucumis sativus* var. *Delikates*, 10 days after inoculation. Photograph by J. Brčák.



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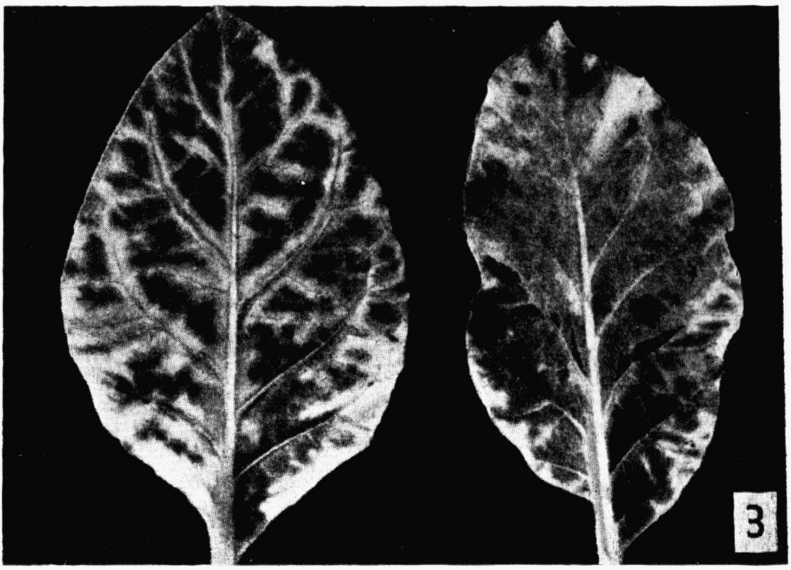

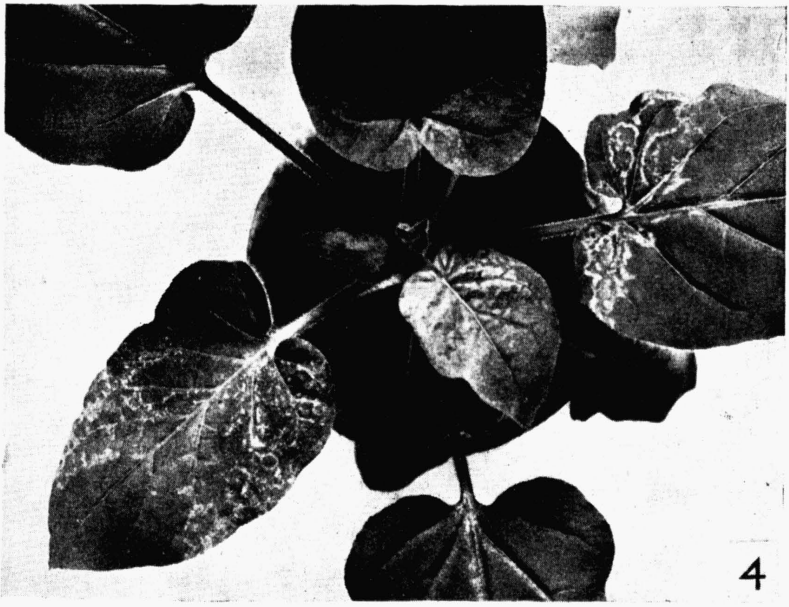

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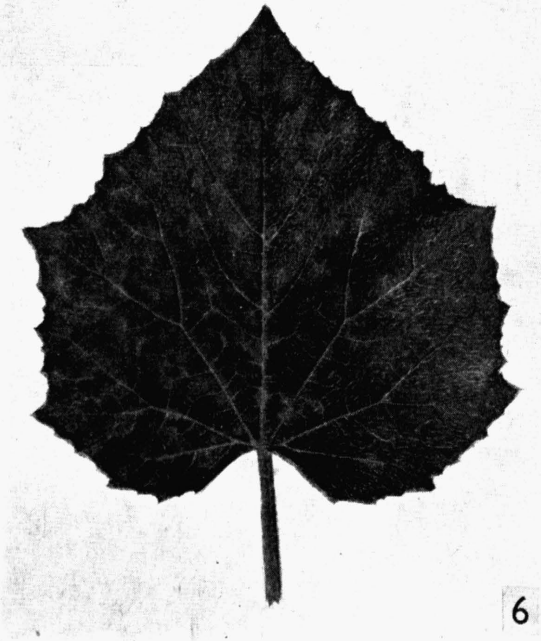

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