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SPONTANEOUS INFECTION OF SOME LABIATES WITH ALFALFA MOSAIC VIRUS

DAVOR MILIČIĆ and MUJË PLAKOLLI

(Botanical Institute, University of Zagreb, and Faculty of Sciences, University of Priština)

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Introduction

Alfalfa mosaic virus (AMV) is widely spread in nature, and it does not attack only alfalfa but also many other cultivated and wild grown plants (Hull 1969, Musil and Leškovà 1969, Bos and Jaspars 1971, Beczner et al. 1972). This virus was sometimes found in herbaceous and woody plants in Yugoslavia (Babović 1968, Pleše and Miličić 1971). According to the data of Lovisolo and Luisoni (1963), Richter (1966), Schmelzer (1963) and Schmelzer et al. (1973) some labiates can be infected with AMV too. Among the labiates there were *Ballota nigra, Origanum vulgare* and *Ocimum basilicum* for which natural infection with AMV was established in various countries (Lovisolo 1961, Neubauer 1963).

In this paper natural infection of the three mentioned labiates with AMV is for the first time reported for Yugoslavia. It is pointed out that *B. nigra* can be a very frequent natural host to AMV.

Material and Method

During these investigations three viruses B, Or and Oc were isolated from *B. nigra, Or. vulgare* and *Oc. basilicum,* respectively. The Sa isolate was used for comparing purposes; it was found in Solin near Split and was identified as AMV on the basis of comprehensive host plants, serological and electron microscopical investigations (Grbelja 1974). This AMV isolate was kindly put at our disposal by Mrs. J. Grbelja (Sarajevo).

A serum against AMV used for identification purposes was kindly supplied by Dr. M. Babović (Belgrade). Serological investigation was performed by double diffusion test in agar-gel.

Results

Localities and virus isolation

In the spring of 1973 six localities of *B. nigra* plants with yellow leaf margins were found in the environments of Zagreb without special searching (Fig. 1 A). The symptoms were very similar to those previously described by Hein (1957, 1959) as characteristic of *Ballota* yellow mosaic disease. At the end of spring 1973 specimens of *Ballota* with yellow leaf margins were also collected in Priština. Since many specimens of *B. nigra* with this symptom have been found till now in Yugoslavia, it seems that such infected plants are frequent in this country.

An interesting locality of diseased *Ballota* plants was the Botanical Garden in Zagreb where a great number of various plants were cultivated in a special place for teaching purposes. The plants were arranged systematically, after their natural relationship, so that many labiates were placed near one another because of their close relationship. They grew on separate beds, and every plant had at its disposal an area of 1 m^2 approximately. Tab. 1 shows the space arrangement of our three labiates among other plants belonging to the same family. These plants were represented by several tens of specimens on each area. We were especially interested in *B. nigra*, *Or. vulgare* and *Oc. basilicum* because they showed characteristic virus symptoms on leaves.

Table 1. Space arrangement of investigated labiates growing on neighbouring beds in the Botanical Garden

Scutellaria galericulata	**Origanum vulgare**	*Plectranthus glaucocalyx*
Ocimum basilicum	*Dracocephalum ruyschiana*	*Stachys officinalis*
Teucrium chamaedrys	*Glechoma hederacea*	**Ballota nigra**

On the area where *B. nigra* grew, nearly one half of the plants had the yellow margin symptoms (Fig. 1 A). In the neighbouring area where *Or. vulgare* grew, several of plants showed intensive yellow variegation on leaves (Fig. 2 A, B). *Oc. basilicum* was planted in the third area; one half of these plants had intensively yellow variegated leaves (Fig. 1 C, D; 2 C).

Several attempts were made to isolate the virus from diseased *Ballota* plants during spring, summer and autumn but the isolation was successful only early in spring. On this occasion the B isolate was obtained from infected plants collected near Youth Bridge in Zagreb. This virus was mechanically transmitted to various herbaceous plants (Table 2). The isolation of the virus from *Ballota* plants growing in the Botanical Garden was attempted first in the summer of 1973 but without success.

From the variegated specimens of *Or. vulgare* the virus was isolated twice in spring. No attempts were made during the summer and autumn so that we do not know whether it is possible to isolate the virus in these seasons.

AMV was isolated from *Oc. basilicum* in the autumn, that is in the season which is considered as unfavourable for isolation of viruses from plants containing inhibitors. As this species is generally used as a test plant it seems that it is not difficult to isolate viruses from it.

A specimen of *B. nigra* found in Zagreb showed the yellow variegation not only on the leaf margins but also on the greater part of the leaf surface (Fig. 1 B). Early in the summer we tried to obtain the virus from it but without success.

Analysis of isolates on herbaceous plants

The B, Or and Oc isolates were mechanically transmitted to many plants which reacted with characteristic symptoms (Table 2). In order to facilitate the virus transmission from *B. nigra* and *Or. vulgare* a phosphate buffer of pH 7 and the stabilizing mixture of Opel and Kegler (1969) were used.

Table 2. Reactions of herbaceous plants after infection with B, Or and Oc isolates of alfalfa mosaic virus

Local symptoms on rubbed leaves are marked with sign I placed before the description of symptoms; systemic symptoms on younger leaves are marked II. Plants, whose symptoms are marked only I, are locally infected.

CHENOPODIACEAE

Chenopodium amaranticolor (L.) Coste et Reyn. I local lesions, II vein yellowing, mosaic, epinastic curving of leaves.

Ch. quinoa Willd. I chlorotic and necrotic lesions, II vein yellowing first on basal parts of leaves, mosaic spreading from the veins.

CUCURBITACEAE

Cucumis sativus L. »Delicatesse« I very small necrotic lesions on cotyledons, II white mosaic attacking particularly the veins.

LABIATAE

Ocimum basilicum L. II a fortnight after inoculation yellow spots on upper leaves, a few days later yellow variegation which is spread over most of the leaf surface (Fig. 1 C, D; 2 C).

PAPILIONACEAE

Phaseolus vulgaris L. I a great number of brown necrotic lesions, occasional necrotic lines and spots on petiole, II mosaic, brown lesions (Fig. 2 D).

Vicia faba L. I small brown-red local lesions followed by leaf necrosis; II brown lines and necrosis on stem.

Vigna sinensis Savi ex Hassk. I small brown or violet local lesions, II yellow spots.

SOLANACEAE

Lycopersicon esculentum Mill. I brown necrotic lesions, II brown necrotic spots, deformation of leaves.

Nicotiana glutinosa L. I necrotic lesions (B, Or), yellowish spots (Oc); II large necrotic spots, chlorotic lines, leaf deformations, later recovery of plants (B, Or); numerous small chlorotic yellowish or white spots, recovery (Oc).

N. tabacum L. I necrotic and chlorotic lesions, II a small number of chlorotic lines and spots, recovery.

Systemic infection of *Chenopodium* species and vein yellowing of upper leaves of *Ch. quinoa* were characteristic symptoms for the presence of AMV. Brown lessions on *Phaseolus vulgaris* and violet ones on *Vigna sinensis* also pointed out that the infection was caused by AMV. This opinion was especially supported by intensive yellow spots and yellow variegation which appeared on the leaves of *Oc. basilicum.* Similar yellow spots and patches sometimes arose on the leaves of *Vigna sinensis.*

Other identification experiments and observations

An attempt was made to transmit the B isolate by *Myzus persicae* from infected *Ch. quinoa* to healthy plants of the same species. During these experiments we succeeded in transmitting the virus in the non-persistent manner. As AMV can be transmitted by insects this experiment also indicates that AMV is the cause of this disease.

Double diffusion tests in agar-gel were also made in order to establish with certainty that the virus isolates from labiates belong to AMV. On this occasion the serum against AMV was used. The homologous titre of the antiserum was 1 : 256, and it was employed for testing Or isolate at a dilution of 1 : 8. The antiserum was placed in a centre well, and the infective and healthy plant sap at the dilution of 1 : 1, 1 : 2 and 1 : 4 in peripheral wells. After one day the precipitation lines appeared only between wells which contained the antiserum and the infective sap. These lines proved that the isolate Oc belonged to the AMV. Since the Or and B isolates gave the same symptoms on herbaceous plants as the Or, we abstained from doing serological tests with them.

There was yet another indication suggesting that *Ballota* yellow mosaic is caused by AMV. Three species, i. e. *B. nigra, Or. vulgare* and *Oc. basilicum* belonging to the family *Labiatae,* grew in the Botanical Garden in Zagreb on closely situated beds (Tab. 1). They showed a strong yellow variegation of leaves characteristic of calico strain of AMV. From *Or. vulgare* and *Oc. basilicum* growing on the beds, viruses Or and Oc were isolated, and were identified as AMV. We did not do isolation experiments from this *B. nigra* in spring and we did them in

Fig. 1. A and B *Ballota nigra,* C and D *Ocimum basilicum.* A yellowing of leaf margins; B yellow rings and lines on leaves; C only the basal part of leaves is green, the other is yellow; D some upper leaves are completely yellow.

Sl. 1. A i B *Ballota nigra,* C i D *Ocimum basilicum.* A žutilo na rubovima listova; B žuti prstenovi i linije na listovima; C samo bazalni dio listova je zelen, vršni je žut; D neki gornji listovi posve su žuti.

Fig. 2. Symptoms of infection with alfalfa mosaic virus. A and B *Origanum vulgare* with line pattern; C *Ocimum basilicum* with yellow variegated leaves; D *Phaseolus vulgaris,* a leaf with brown spots.

Sl. 2. Simptomi infekcije s virusom mozaika lucerne. A i B *Origanum vulgare* sa žutim vrpčastim šarama; C *Ocimum basilicum* sa žutim šarenilom listova; D *Phaseolus vulgaris,* list sa smeđim pjegama.

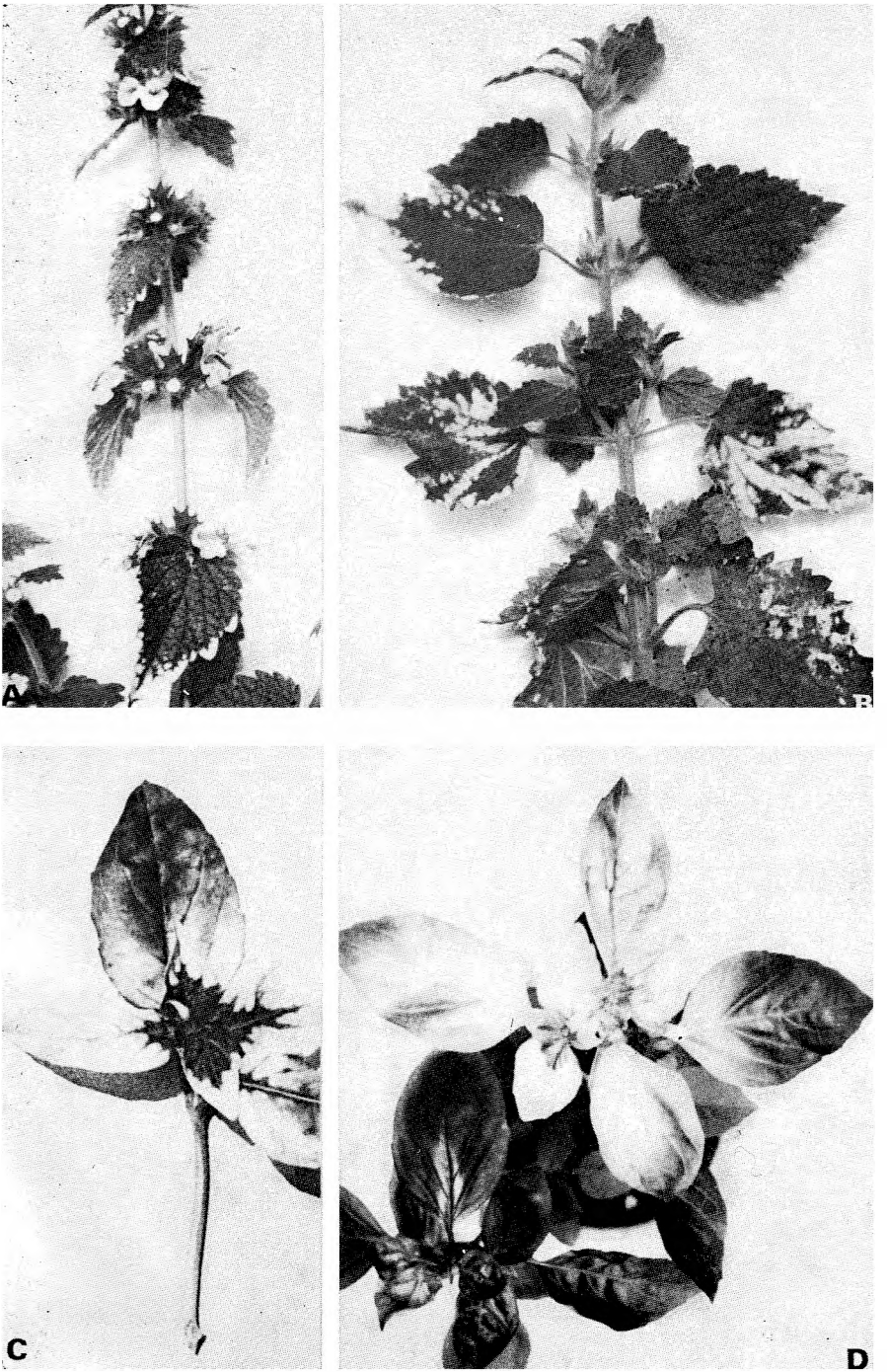

Fig. 1. — Sl. 1.

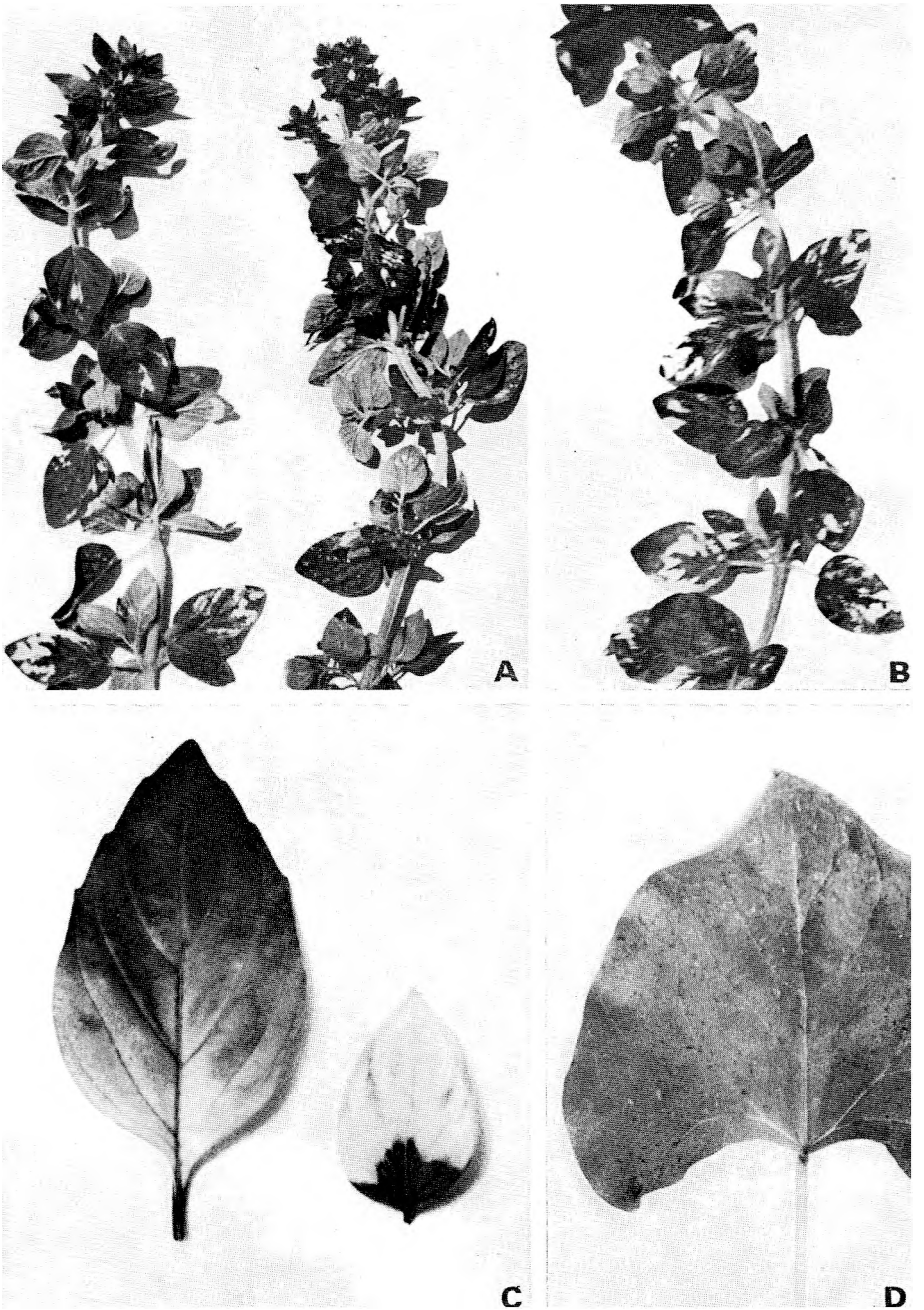

Fig. 2. — Sl. 2.

summer but without success probably because of the presence of virus inhibitors in plants during this season. Nevertheless, we think that *B. nigra* was also infected with AMV as were the two other labiates growing close to it because of the presence of characteristic calico symptoms.

Back transmission of virus

At the end of the experiments we tried to return our isolates back to *B. nigra, Or. vulgare* and *Oc. basilicum. Ballota* plants grown from seeds were inoculated with Oc and Sa isolates of AMV in October. Only two plants of the 20 inoculated developed obvious symptoms. On these plants light green and green-yellow mosaic symptoms arose on young leaves twenty days after inoculation. The colour of nearly the whole leaf surface was changed under the influence of the virus. The most prominent shade of colour was green as was the case in glasshouse experiments by Hein (1957). On the contrary, the leaves of spontaneously infected plants in nature had regularly intensive yellow margins of leaves. Accordingly, we were not able to reproduce either the intensive yellowing or the characteristic marginal location of this colour in *B. nigra.* It is possible that the plants need more light for the development of these symptoms than they had at their disposal in the glasshouse in the late autumn when these experiments were performed. Certainly, it would be interesting to know which factors are necessary for the development of marginal yellowing.

Twenty specimens of the second labiate, *Or. vulgare,* were also inoculated twice in October. By the end of December none of them had shown any symptoms of infection. As the AMV is hard to transmit in *B. nigra* and *Or. vulgare* it seems that these two species cannot be employed as good test plants for identification of AMV although they often show very intensive yellow symptoms after infection.

In contrast to *B. nigra* and *Or. vulgare, Oc. basilicum* could accept the virus more readily so that nearly all inoculated plants became infected and showed intensive yellow symptoms in the glasshouse in spring, summer and autumn. Because of this property this species is used as a good test plant for the identification of AMV (Klinkowski 1968).

Discussion

Among the diseases on labiates mentioned here our attention was especially drawn by the yellow mosaic of *Ballota* because it is widely spread in Yugoslavia. This disease is characterized by yellowing on the leaf margins. It corresponds symptomatologically well to the yellow mosaic of *Ballota* which was described by Hein (1957). However, in contrast to Hein we succeeded in isolating this virus mechanically.

Isolation of AMV from *B. nigra* was possible only in April. Many trials to isolate the virus from this plants in the late spring, summer and autumn finished without any success. The inhibitors which are present in some labiates may be the cause of this failure.

Lovisolo and Luisoni (1963) established that the plant sap of *Mentha piperita* contains inhibitors which hinder the mechanical virus transmission. Neubauer (1963) too was unable to isolate AMV

mechanically from *Mentha piperita*. However, Neubauer (1963) and Lovisolo and Luisoni (1963) transmitted a virus by grafting from this plant. *Mentha piperita* is an interesting plant because it shows, after infection with AMV, a strong yellow variegation which is very similar to the yellow symptoms produced in *B. nigra, Or. vulgare* and *Oc. basilicum* under the influence of the same virus.

Unlike these authors Richter (1966) has recently established that it is possible to isolate AMV mechanically from *Mentha piperita*. This different result can be explained by the fact that viruses are transmitted mechanically from many plants containing inhibitors only in early spring. In this season the concentration of inhibitors in plants is often very low. These conditions are especially widely spread in fruit trees from which it is very difficult to isolate viruses in other seasons (Fulton 1966, p. 88).

Inhibitors also existed in the leaves of *B. nigra* after experiments by Hein (1957). Therefore, it is possible that the absence of inhibitors in the early spring made it possible for us to transmit AMV from *Ballota* to herbaceous plants, and the presence of inhibitors in other seasons hindered the transmission. If further investigations should show the correctness of this hypothesis, this would also prove that the yellow mosaic of *Ballota* is caused by AMV.

Summary

Alfalfa mosaic virus (AMV) was mechanically isolated from *Ballota nigra* showing intensive yellow symptoms on the margins of leaves. The isolation of virus from diseased plants was possible only early in the spring but not in the summer and autumn. The specimens of *B. nigra* with yellow leaf margins are widely spread in Yugoslavia. This species could be an important reservoir of AMV in nature if this symptom is regularly provoked by AMV.

Naturally infected *Origanum vulgare* and *Ocimum basilicum* with AMV were found in the Botanical Garden in Zagreb.

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SADRŽAJ

SPONTANA ZARAZA NEKIH USNAČA VIRUSOM MOZAIKA LUCERNE

Davor Miličić i Mujë Plakolli

(Institut za botaniku Sveučilišta u Zagrebu i Prirodno-matematički fakultet Univerziteta u Prištini)

Virus mozaika lucerne (VML) izolirali smo mehanički iz vrste *Ballota nigra* koja je imala intenzivno žute rubove listova. Izolaciju smo izvršili u rano proljeće; međutim, virus nismo mogli izolirati u kasno proljeće te tijekom ljeta i jeseni. Postojanje velikog broja nalažišta bolesnih primjeraka *B. nigra* u okolici Zagreba i nekoliko nalazišta u udaljenoj Prištini pokazuje da je ta bolest raširena u Jugoslaviji. Ako samo VML uzrokuje žutilo rubova lista vrste *B. nigra*, a u tome ne sudjeluju drugi čimbenici, ta bi vrsta mogla biti važan rezervoar VML u prirodi.

Osim toga pronašli smo u Botaničkom vrtu u Zagrebu primjerke vrsta *Origanum vulgare* i *Ocimum basilicum*, koji su bili prirodno okuženi s VML.

Prof. dr Davor Miličić
Institut za botaniku
Sveučilišta u Zagrebu
Maruličev trg 20/II
41000 Zagreb (Jugoslavija)

Mujë Plakolli
Katedra za botaniku
Prirodno-matematički fakultet
Ul. Maršala Tita 22
38000 Priština (Jugoslavija)