SURVEY AND DIFFERENTIATION OF CUCUMBER MOSAIC VIRUS STRAINS IN LEBANON

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CUCUMBER MOSAIC VIRUS

IBRAHIM

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AN ABSTRACT OF THE THESIS OF

Muhammad Ibrahim for M.S. in Plant Pathology

Title: Survey and differentiation of cucumber mosaic virus strains in Lebanon.

A study was conducted in 1965-66 to survey and identify cucumber mosaic virus (CMV) strains in Lebanon. It was observed that 15 different species of host plants of five different plant families at 16 different localities in Lebanon were naturally infected with the CMV. The identification and differentiation of CMV strains was done through bio-assay and physical property studies of the virus. There seems to be one main strain of CMV in Lebanon. Two isolations from pepper and pumpkin from Beirut area reacted slightly different from the main strain and are to be considered as different strains of the main CMV strain. The closely related viruses to CMV, i.e., squash mosaic virus and watermelon mosaic virus, were not found in Lebanon. Investigations on the possibility of CMV transmission, through locally planted cucurbitaceous seeds, demonstrated that a certain percentage of local cucumber and squash seeds transmit CMV while the watermelon seeds were entirely free of infection.

Suggestions on how to control and reduce diseases due to CMV infections are discussed.

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I. INTRODUCTION

Mosaic diseases are commonly observed on many cultivated plants in Lebanon, particularly in the littoral region where different vegetables and other crops are interplanted and grown close to each other. Cucumber mosaic virus (CMV) is one of the most common mosaic viruses infecting horticultural crops in many parts of the world. In general, the symptoms of CMV consist of mosaic, mottling, distortion of leaves and stunting of the affected plants. Malformation and mottling of fruits may also occur. The losses caused by this virus are due to gradual decline and ultimate death of the infected plants. The damage is also done through degradation of the quality of the produce.

CMV has a wide host range and attacks a great variety of field crops, vegetable crops, ornamentals and weeds. The virus and its strains can attack, for instance, different species of Chenopodiaceae, Cucurbitaceae, Solanaceae, and Papilionaceae, and banana plants. These crops are also attacked by other viruses like tobacco mosaic virus, alfalfa mosaic virus, potato viruses, and especially the cucurbits by squash mosaic and watermelon mosaic viruses.

In Lebanon, no systematic efforts have been made, so far, to survey and record the incidence of CMV on different

host plants. Taking into consideration the wide host range and importance of CMV this investigation was conducted to find out (1) to what extent the CMV may be involved in causing mosaic diseases on plants in Lebanon; (2) to identify the various strains of the virus occurring on different host plants from different areas of this country; (3) to check the possibility of transmission of CMV through seeds of cucumber, squash and watermelon. The research work carried out in this connection is presented in this treatise.

II. REVIEW OF LITERATURE

In accordance with the different parts of this study, the available literature will be reported on the following three aspects: (1) Host range and symptomatology of CMV; (2) Identification and differentiation of the CMV strains; and (3) Transmission of CMV through seeds.

Host Range and Symptomatology of CMV

Host range: The host range of CMV among the economic plants, ornamentals and weeds, as reported by a number of investigators is summarised below:

Doolittle (1916) reported the attack of CMV on cucurbitaceous plants. In 1921 the same author described the transmission of this virus to plants outside the Cucurbitaceae family, such as milkweed (Asclepsis syriaca), pepper (Capsicum annum), pigweed (Amaranthus retroflexus) and Martynia lousiana. Jagger (1916) also reported that CMV, which he called "white pickle disease", was infectious to all cucurbits except watermelon, (Citrullus vulgaris). Doolittle and Walker (1923) confirmed Jagger's findings and observed that CMV was readily transmissible to pokeweed (Phytolaca sp.), potato plants, potato tubers and back to the cucumber plants. Johnson (1933) in Wisconsin observed the attack of CMV on tobacco plants.

Wellman (1935), while studying the host range of the southern celery mosaic virus on celery and parsley plants, described it as a strain of CMV. Smith (1937, pp. 52-86), reviewed a very comprehensive record of the host range of CMV and reported that 100 species of plants in 32 families were being attacked by CMV. Price (1940, pp. 530-541), in the course of his studies on the comparative host ranges of six viruses, namely, tobacco-necrosis virus, cucurbit mosaic virus, alfalfa mosaic virus, tobacco ringspot virus, tomato ringspot virus and cucumber mosaic virus, observed that CMV was infective to 191 species in 12 different families. Pound and Walker (1948) reported the CMV infection on dam's voilet (Hesperis matronalis), an ornamental crucifer, in the area of Wisconsin. Magee (1940) in his research findings revealed that CMV from cucurbitaceous plants was transmissible to banana plants and vice versa.

Symptomatology: The different types of symptoms produced by CMV on different host plants are presented briefly in the following paragraphs:

Doolittle (1916) described the most characteristic symptoms of CMV on cucurbitaceous plants. He observed mosaic, mottling and distortion of leaves and shortening of stems and petioles. The affected fruits were slightly pale-green to dark-green and had wartlike projections, giving a rough appearance. Similar symptoms on the

cucurbitaceous plants have been reviewed by Jagger (1916), Smith (1937, pp. 52-86), and Sherf (1965).

The most conspicuous symptoms on Chenopodiaceae plants were reviewed by Doolittle (1921), Smith (1937, pp. 52-86) and Sherf (1965). They described them as slight yellowing of the younger leaves of infected plants and later malformation of the leaves which resulted in wrinkling, narrowing and mottling of leaves and stunting of the plant.

Most spectacular symptoms of CMV on solanaceous plants consisted of mosaic, mottling and distortion of leaves and stunting of the plants. Fern leaves and shoe stringing were the obvious characteristic symptoms on tomatoes and sometimes on pepper and tobacco too. Malformation and mottling of the fruit might occur as reported by Doolittle (1921), Johnson (1933), Smith (1937, pp. 52-86), Paulus et al. (1962) and Sherf (1965).

Wellman (1935), Smith (1937, pp. 52-86) and Sherf (1965) reported CMV on celery and parsley and described early symptoms of the mosaic resulting in pronounced outward and downward curling of leaves which depicted the characteristic opening of the plant. Plants tended to be dwarfed and petioles badly shrivelled and brownish in colour.

Smith (1937, pp. 52-86) and Magee (1940) described the chlorotic streaking of leaves accompanied by drooping

and brittleness as the major symptoms of CMV on banana plants. Petioles were reduced in size and showed chlorotic spotting. Sometimes this chlorotic stage coincided with rotting of the heart-leaf and central cylinder. The bunches formed on affected plants were often small.

In general these reports on host range and symptomatology revealed that CMV has a wide host range and can infect many different vegetables, ornamentals, weeds and banana plants. The main symptoms consist of mosaic and mottling of leaves, stunting and dwarfing of the whole plants and mottling of fruits. The symptom expressions differ to a certain degree with different host plants.

Identification and Differentiation of the CMV Strains

Johnson and Hoggan (1931) described four chief types of differential or diagnostic features of plant viruses as (i) symptom expression; (ii) physical properties of the virus; (iii) modes of transmission; and (iv) cytological features of infected host. Again in 1935, they elaborated on the diagnostic features of plant viruses and described detailed procedures for identification of viruses and for differentiation of their strains.

All or some of the above said diagnostic features were employed by various workers as the basis for identification and differentiation of various strains of different viruses including CMV and its strains.

Walker (1926) studied the infective principle in the expressed juices from three host plants viz, cucumber, tomato and Physalis species. He found that the CMV from the above mentioned hosts manifested characteristics similar to those of the CMV I type virus, such as aging 24 to 48 hours, heating 70°C, diluting 1:1,000, and negative treatment with alcohol. Smith (1937, pp. 52-86) described the physical properties of different strains of CMV which ranged as follows: heat inactivation point from 60-90°C and longevity in vitro from 72 hours to one year. He also identified two species of viruses as CMV 1 and CMV 2 both having 12 and two strains, respectively.

Whipple and Walker (1941) investigated some mosaic viruses on peas and beans. He found that they were strains of CMV and called them strain 14 and 17. They remained infectious in vitro for 7 days at 20 to 22°C. The thermal inactivation point for strain 14 was about 65°C and for strain 17 between 65 to 70°C. Pound and Walker (1948), working on a mosaic disease of the ornamental crucifer dam's voilet, observed that the physical properties of the virus agreed very closely with those of CMV: aging in vitro 4 to 5 days at 20°C and thermal inactivation at 70°C.

Anderson (1954), Grogan et al. (1959) and Lindberg et al. (1956) used host range, symptomatology, physical properties and insect transmission as criteria for the

identification of CMV, watermelon mosaic virus and squash mosaic virus. Anderson (1954) found the thermal inactivation point of two watermelon mosaic virus strains between 55 and 60°C and the longevity *in vitro* from 4½ hours to 6 hours. Lindberg *et al*. (1956) observed that the physical properties of squash mosaic virus and melon mosaic virus were the same but the squash mosaic virus could only be transmitted through aphids.

Nitzany and Wilkinson (1960-62) and Cohen and Nitzany (1963) in Israel described methods for the separation and identification of five viruses, infectious to cucurbits, viz, CMV, melon mosaic virus, squirting cucumber mosaic virus, bottle gourd mosaic virus and squash mosaic virus, and gave their different host ranges and physical properties such as thermal inactivation point between 45 and 70°C, dilution end point 10^{-3} to 10^{-6} and longevity *in vitro* from one day to 22 days.

These results reported by various workers make it clear that the differentiation of various strains of CMV was based on the differences in symptom expressions on different host plants, thermal inactivation point, longevity *in vitro*, dilution end point and in the ways they were transmitted. According to these reports there are two species of the virus, namely, CMV 1 and CMV 2, and these two species have further 12 and two strains, respectively.

Transmission of CMV Through Seed

Doolittle and Gilbert (1919) established the possibility of CMV spread through infected seed in wild cucumber plants. Kendrick (1934) studied the transmission of CMV through the muskmelon seeds and found that it occurred in a small percentage of seeds.

Middleton (1944) reported that the percentage of virus transmission through seed was more in light, poorly filled and deformed seed than in heavy, well filled seed of squash.

Webb and Morton (1963) described their study of the seed transmission of watermelon mosaic but they could not get infection in any seed out of several hundred tested for this purpose.

The foregoing reports show that while there was a low percentage of transmission of CMV through the seeds of cucumber, muskmelon and squash there was no transmission at all through the seeds of watermelon.

III. MATERIALS AND METHODS

In order to find out the host range of cucumber mosaic virus (CMV) and to study its prevalent strains in Lebanon, an extensive field survey was initiated especially in the littoral zone and the Beqa'a Plain in March, 1965. Diseased specimens apparently suffering from mosaic diseases were collected from different field crops, ornamentals and banana plants throughout the year until spring 1966.

On specific plant species different viruses cause different symptoms which are distinct enough to be used as basis for identification of these viruses.

Inoculum was obtained from diseased plant specimens and inoculated on indicator plants. The following test plants were grown in a room supplied with artificial light:

1. Chenopodium amaranticolor (pigweed species)
2. Citrullus vulgaris (watermelon)
3. Cucurbita pepo (squash)
4. Cucurbita moschata (pumpkin)
5. Cucumis sativus (cucumber)
6. Datura stramonium (jimsin-weed)
7. Gomphrena globosa (globe-amaranth)

8. Nicotiana glutinosa (Bolivian tobacco)

9. Nicotiana tabacum var. Samsun (Turkish tobacco)

10. Nicotiana tabacum var. Xanthi (Tobacco hybrid of N. glutinosa and N. tabacum)

The above mentioned test plants were seeded in sterilized pots or flats containing sterile soil. After 8 to 12 days Chenopodium amaranticolor, Datura stramonium, Gomphrena globosa and Nicotiana species were transplanted to other flats and finally potted in sterilized soil. Liquid fertilizers[1] were frequently added to the test plants to induce vigorous growth. Weekly sprayings with systemic insecticides[2] were done to protect the indicator plants from virus infection being transmitted by insects from other stock plants present in the laboratory. At the time of inoculation the cucurbits were 6 to 12 days old (cotyledonal stage), Chenopodium amaranticolor and Nicotiana tabacum 8 to 12 weeks old, and Gomphrena globosa and Nicotiana glutinosa 12 to 14 weeks old. The inoculum was prepared by grinding the leaves of diseased plants with a small amount of water in sterilized mortars. Inoculations were made using 400 mesh carborundum as an abrasive. Sterilized cotton swabs or pestles were used for

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1. $Ca(NO_3)_2 \cdot 4H_2O$ = 2.5 gms/litre; KH_2PO_4 = 0.6 gms/litre; and $MgSO_4 \cdot 7H_2O$ = 0.5 gms/litre of water.
2. Demicron 50 (phosphamidon) applied at the rate of 0.05% active ingredient.

inoculation purposes. To stimulate the wound healing process of inoculated plants, the treated leaves were washed with water soon after they had been inoculated, removing carborundum and excessive inoculum. After inoculations the plants were kept in the laboratory where the temperature ranged between 23°C and 27°C (temperatures higher than 27°C are unfavorable for virus infection and symptom expression). After 14 to 21 days of inoculation, observations were recorded for the symptom expressions on the various indicator plants.

Stock cultures of various virus isolations from different host plants were maintained in Nicotiana glutinosa to ensure freedom from any contamination with tobacco mosaic virus and, alternately, in Cucumis sativus to safeguard from certain other viruses. Accordingly, alternative passages between these two plant species ensured elimination of other viruses, if present.

In order to confirm the findings that the mosaic diseases found were caused by CMV and to differentiate the particular strains, the thermal inactivation point method was also used, because different viruses and, sometimes, different strains of the same virus, can be differentiated by their reaction to a short heat treatment in vitro. To facilitate these investigations, six grams of Nicotiana glutinosa leaves, affected with CMV were macerated in sterilized mortars. The grinded material was squeezed

through sterilized 2 X 2 inches cheesecloth which gave about 3 ml of the expressed juice. Then the volume of the sap was made upto 7 ml by adding water. In this way the requisite quantity of sap for seven different tests of thermal inactivation point was obtained. Two ml of such sap was filled in each of six screw-cap test tubes which were divided into 6 batches. The tubes were immersed in a hot waterbath (Hoeppler thermostat) for 10 minutes at 40, 50, 60, 70, 80, and 90°C, respectively, for each batch. Immediately after treatment the test tubes were cooled under running cold tap water in order to cool down the plant extract quickly. The cooled sap was used for inoculations on Nicotiana glutinosa and Nicotiana tabacum var. Xanthi. Unheated controls were used in all tests. The incidence of infection was recorded after 14 to 30 days. A second investigation was performed by narrowing down the temperature range from 10°C to 2°C intervals within a 15°C range from 55 to 69°C. The six batches of saps were heated up to 55, 57, 59, 61, 63, 65, 67, and 69°C, respectively, and cooled down as before. Then the indicator plants were inoculated and symptom expressions were recorded after 30 days.

Repeating these experiments the cooled extracted juice was diluted 1:10 with water after heat treatment and used as inoculum according to the above mentioned method.

As a third method of differentiation of the isolated

viruses, the longevity *in vitro* was employed. Viruses in plant extractions are able to stay active for certain periods ranging from few hours up to several months depending upon the virus species. In this method the extraction of juice was made in a similar way as already described. Five ml of expressed sap was taken in screw cap test tubes to which a small quantity of streptomycin (10 mg/ml of streptomycin sulphate, potency 745 units/mg) was added as an antibiotic to suppress the bacterial growth in the sap. These samples were kept in the laboratory at 22 to 24°C and inoculations of the juice were made after 24, 48, 72, and 120 hours on *Nicotiana glutinosa* and *Nicotiana tabacum* var. *Xanthi*. Fresh virus extract was used as a control in each trial. The incidence of the disease was recorded after 8 to 21 days.

For testing the transmission of CMV through seeds, samples of seeds of cucumber, squash and watermelon were collected from the Agricultural Research and Education Centre of the American University of Beirut (Certified U.S. Seed) and local seeds from shops in Beirut, Saida and Zahlé. From each location 100 seeds of each crop were taken for trial purposes and treated with Arasan to prevent seedling infection by seed-borne and soil-borne fungal and bacterial pathogens. The treated seeds were sown in sterilized wooden flats containing sterilized soil at two different dates during winter and spring. The incidence of CMV infection in each crop was recorded after one month.

IV. RESULTS AND DISCUSSION

The results of the study conducted to survey; (1) the host range of CMV in Lebanon; (2) the different strains of CMV found on different host plants; and (3) the transmissibility of CMV through seeds, are reported in sections as follows:

Host Range

Samples of different host plants collected were tested with the indicator plant method as described under materials and methods, to identify the virus causing the mosaic diseases. The characteristic symptoms of CMV on these indicator plants were:

Mosaic symptoms on Cucumis sativus (see Fig.2), Cucurbita moschata and C. pepo, mosaic and malformation of leaves of Nicotiana glutinosa, N. tabacum var. Samsun and N. tabacum var. Xanthi, (see Fig. 15, 16, and 19), on Chenopodium amaranticolor local lesions which were yellowish in the centre and reddish brown at the periphery (see Fig.13), and faint mosaic and mosaic symptoms on the leaves of Datura stramonium and Gomphrena globosa (see Fig.14 and 20), respectively. The following 15 host plants at 16 different locations in Lebanon were demonstrated to be naturally infected with the CMV:

Chenopodiaceae:	
Spinach,	Spinacea oleracea
Swiss-chard,	Beta vulgaris var. Cicla
Cucurbitaceae:	
Cucumber,	Cucumis sativus
Snake cucumber,	Cucumis chate
Pumpkin,	Cucurbita pepo
Squash,	Cucurbita moschata
Muskmelon,	Cucurbita melo
Solanaceae:	
Eggplant,	Solanum melongena
Pepper,	Capsicum annuum
Potato,	Solanum tuberosum
Tobacco,	Nicotiana tabacum
Tomato,	Lycopersicon esculentum
Musaceae:	
Banana,	Musa cavendishii
Umbelliferae:	
Celery,	Apium graveolens
Parsley,	Petroselinum hortense

More details of the above are given in Table 1 and Figure 1.

Table 1. Cucumber mosaic virus occurring on different host plants at different locations as surveyed in Lebanon during 1965-66.

M = mosaic, Mf = malformation)

Time of sample collection	No. of samples	Location	Symptoms	Virus identification
	Host: Spinach (Chenopodiaceae)			
March, 1965	1	Saida	M	CMV
	Host: Swiss-Chard			
March, 1965	1	Abdeh	M	CMV
March, 1965) April, 1966)	5	Beirut	M + Mf	CMV
Jan., 1966	3	Tyre	M + Mf	CMV
	Host: Cucumber (Cucurbitaceae)			
May, 1965	1	Abdeh	M + Mf	CMV
June, 1965	1	Beirut	M + Mf	CMV
Oct., Nov., 1965	3	Eljieh	Mottled fruits only	CMV
March, May, 1965	2	Saida	M + Mf	CMV
	Host: Snake Cucumber			
Aug., 1965	1	AREC[1]	M	CMV
	Host: Muskmelon			
Aug., 1965	1	AREC	M	CMV
	Host: Pumpkin			
Aug., 1965	1	Adloun	M	CMV
Aug., 1965	1	AREC	M	CMV
Sept., 1965	1	Beirut	M	CMV
Oct., 1965	1	Eljieh	M	CMV

(Table 1 continued)

	Host:	Squash		
Aug., 1965	1	AREC	M + Mf	CMV
June, 1965 May, 1966	2	Beirut	M + Mf	CMV
March, Oct., 1965 April, 1966	4	Eljieh	M + Mf	CMV
May, 1966	2	Halba	M + Mf	CMV
July, 1965	1	Nahr-Ibrahim	M + Mf	CMV
June, July, 1965	3	Saida	M + Mf	CMV
Nov., 1965	2	Tabarja	M + Mf	CMV
	Host:	Eggplant	(Solanceae)	
March, 1965	1	Adloun	M	CMV
March, 1965	1	Saida	M	CMV
	Host:	Pepper		
Nov., 1965	1	Adloun	M	CMV
May, Nov., 1965	2	Beirut	M + Mf	CMV
March, 1966	1	Bwar	M + Mf	CMV
July, 1965	1	Nahr-Ibrahim	M	CMV
April, 1966	1	Saadiat	M	CMV
	Host:	Petunia		
March, 1965	1	Tripoli	M	CMV
	Host:	Tobacco		
July, 1965	1	Abdeh	M	CMV
Oct., 1965	1	Beirut	M	CMV
July, 1965	1	Chekka	M	CMV
Oct., 1965	2	Kfarhata	M	CMV
July, 1965	1	Tripoli	M	CMV
	Host:	Tomato		
March, 1965	1	Abdeh	M	CMV
July, 1965	1	AREC	M	CMV

(Table 1 continued)

June, 1965	1	Beirut	Fern leaf	CMV
Aug., 1965	1	Chekka	M + Mf	CMV
March, 1965	2	Eljieh	M + Mf	CMV
May, 1965	2	Saida	M + Mf	CMV
	Host:	Banana	(Musaceae)	
June, 1966	1	Adloun	M	CMV
March, 1966	2	Bwar	M	CMV
Nov., 1966	1	Saida	M	CMV
Nov., 1965	1	Tabarja	M	CMV
March, 1965	1	Tyre	M	CMV
	Host:	Celery	(Umbelliferae)	
March, 1965	1	Beirut	M + Mf	CMV
April, 1966	1	Eljieh	M + Mf	CMV
	Host:	Parsley		
April, 1966	1	Tyre	M	CMV

The watermelon is conspicuously absent from the above list as throughout the survey it was found to be free of any mosaic disease. The list of host plants of CMV as studied in this survey is by no means exhaustive. There could be many other potential hosts among the cultivated plants and weeds in Lebanon and it is probable that the recognised host range of this disease will become much more extensive in the future.

1. AREC = Agricultural Research and Education Centre.

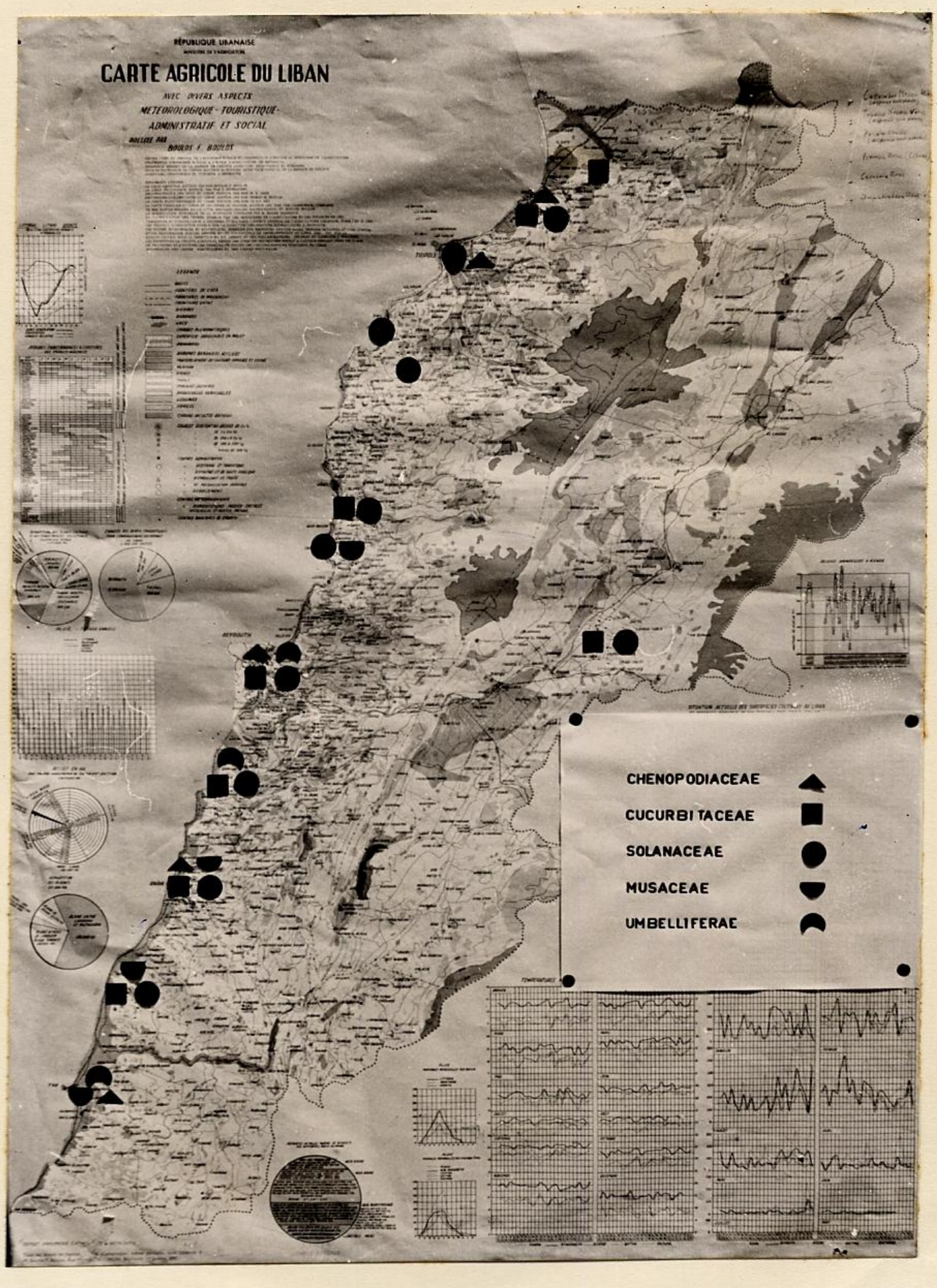

Figure 1. Survey of cucumber mosaic virus in Lebanon.

Symptomatology

CMV causes a wide variety of reactions in leaves, stems, flowers and fruits of infected plants. The host plants in various locations at different times of the year developed somewhat different symptoms when infected with CMV as may be seen in the summary of observations in Table 1.

Symptoms on cucumber: Cucumber plants can become infected at any stage of their growth but the symptoms were more pronounced and severe on plants attacked at early stages of growth. The first symptoms of the disease were yellowing and wilting of the cotyledonal leaves and a faint mottling of the young leaves. After few days of the appearance of first symptoms, the young developing leaves became mottled, distorted, and wrinkled, with their edges curled downward. Growth of leaves was checked, and the later formed leaves remained small in size with a distinct mottle of yellowish green called mosaic, (see Fig.2). The plants often became stunted and were short lived. Symptoms generally appeared on the fruits too, and the affected fruits became pale to dark green, with rough surface and wartlike projections (see Fig. 3).

Symptoms on muskmelon: The symptoms on muskmelon plants were generally similar to those on cucumber plants (see Fig. 4). The young fruits showed mottling in the early

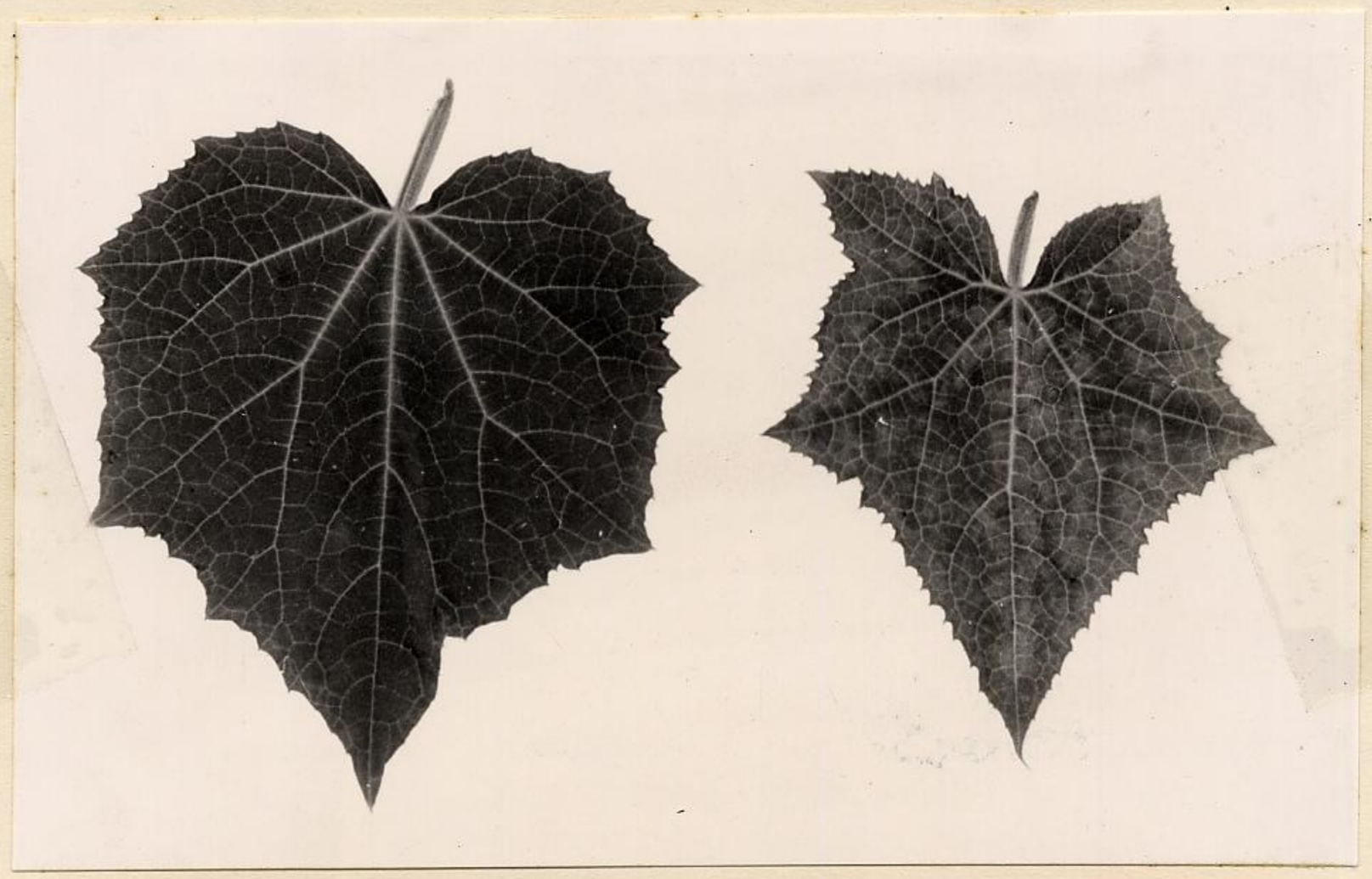

Figure 2. Mosaic symptoms on cucumber leaf, right diseased, left healthy leaf.

Figure 3. Mosaic symptoms on cucumber fruit.

stages, but later on the symptoms on mature fruits seemed to become masked.

Symptoms on squash: The young leaves showed rough, dark raised areas, and yellow-green blotches (see Fig.5). The affected leaves remained smaller in size than the normal healthy leaves. The symptoms on fruits were mottling and bright yellow or orange warts.

Symptoms on pumpkin: The pumpkin leaves depicted symptoms identical with those of squash. The young leaves were mottled and wrinkled while the older leaves often turned yellow and wilted rapidly. The affected shoots had the tendency to branch and the plants sometimes remained stunted. Young fruits were mottled and mature fruits were generally irregular in form and warty.

On cucurbits, in general, the yields are reduced by the virus infection to such a great extent that in certain areas of the littoral zone of Lebanon the farmers are intending to give up the growing of cucurbitaceous plants.

Symptoms on eggplant: The symptoms on leaves were mosaic, mottling and distortion. Malformation and mottling of the fruits was not observed in this survey although the occurrence of these symptoms has been reported in literature.

Symptoms on pepper: The infected plants were often stunted by shortening of the internodes and petioles. The young leaves showed mosaic symptoms and were mostly mottled and

Figure 4. Cucumber mosaic virus symptoms on muskmelon plants.

Figure 5. Cucumber mosaic virus symptoms on a squash plant.

sometimes curled downwards (see Fig.6). The older leaves were abnormally narrowed. The few fruits produced were smaller than normal.

Symptoms on tobacco: The infections of the tobacco plants were systemic. The leaves at first showed vein-clearing followed by general mosaic symptoms. Sometimes the leaves were distorted, and malformed (see Fig.11). Under hot conditions in summer symptoms were often masked but the virus was found to stay highly concentrated in the latent carriers.

Symptoms on tomato: Tomato could be infected at any stage of growth, 6 to 8 weeks old plants already showed pronounced symptoms. Mosaic symptoms, sometimes combined with necrotic spots and streaks, and slight malformation were the most common symptoms of many varieties. The distortion or narrowing of the leaf blades like a string, called fern leaf and shoe-stringing, which is mentioned in literature as a diagnostic evidence of the presence of CMV, was found very often not to be a characteristic symptom of CMV infection, because it was also observed on tomatoes infected only by tobacco mosaic virus (see Fig.7). The fruits which they set did not show pronounced symptoms due to CMV.

Symptoms on spinach: The first sign of infection was yellowing of the younger leaves, which gradually spread to the older leaves. In later stages the foliage curled and wrinkled. The dwarfing, and yellowing was not conspicuous

Figure 6. Cucumber mosaic virus symptoms on a pepper plant.

Figure 7. Symptoms of malformation on leaves of tomato, right healthy leaf, left shoe stringing.

enough to be of diagnostic value.

Symptoms on swiss-chard: The symptoms on leaves appeared as severe mosaic and malformation (see Fig. 8, and 9). In one location pronounced yellow spotting was also observed. In another location severe distortion and curling occurred but it was found to be caused by curly top virus of beets.

Symptoms on banana: In the banana plants, mainly the young suckers were stunted in growth. Leaves showed chlorotic streaking (see Fig. 10). Sometimes this chlorotic stage was accompanied by rotting of the heart leaf and the central cylinder of the plant but according to unpublished data in the Department of Plant Pathology of the American University of Beirut these latter symptoms were not due to CMV infection but were caused by certain fungi. The bunches formed on CMV infected plants were often small and unmarketable.

Symptoms on celery: The symptoms on leaves were mosaic and malformation (see Fig.12). The plants were stunted and showed flattened and open appearence due to outward and downward curling of petioles.

Symptoms on parsley: The leaves of the parsley depicted mosaic symptoms and curling. The plants remained stunted in growth.

Differentiation of CMV Strains

Differential host investigations: The symptoms expressed on the various differential hosts inoculated by ten

Figure 8. Symptoms of mosaic and malformation on leaves of swiss-chard.

Figure 9. Heavy malformation of young leaves of swiss-chard.

Figure 10. Leaf streaking on a leaf of banana plant.

Figure 11. Symptoms of mosaic and malformation on Nicotiana tabacum var. Samsun.

Figure 12. Mosaic symptoms on leaves of celery.

representative isolations previously identified as CMV are presented in Table 2.

It is evident from Table 2 that nine out of the ten different isolations from different areas have given the same reaction on all the indicator plants. Isolation No. VII from Beirut area on pumpkin plant showed reactions similar to above isolations on six hosts, but different symptom expressions on the three other test plants. On Gomphrena globosa isolation No. VII exhibited faint local lesions, on Datura stramonium faint mosaic (see Fig.20), and on Nicotiana glutinosa mottling and spotting (see Fig.18) in comparison to the other isolations which have given mosaic symptoms on Gomphrena globosa (see Fig.14), mottling on Datura stramonium and mosaic and heavy malformation on Nicotiana glutinosa (see Fig. 17).

It is noteworthy that no isolation could be transmitted to watermelon plants. These results are in accordance with field observations where no watermelon plants showed any mosaic symptoms.

The symptoms observed on the different test plants and on the host range of these isolations corresponded in general with those caused by CMV as described by Doolittle (1916); Severin (1948); Severin and Freitag (1948); Smith (1937, pp. 52-86), and Wellman (1935).

The results of this research indicate the absence from Lebanon of watermelon mosaic virus described by

Table 2. The reactions of indicator plants used for identification of cucumber mosaic virus isolations from different hosts and different locations in Lebanon.

S.No.	Host plant (isolated)	location	Test plants								
			Citrullus vulgaris	Chenoposium amaranticolor	Cucumis sativus	Cucurbita pepo	Datura stramonium	Gomphrena globosa	Nicotiana glutinosa	Nicotiana tabacum var. Samsun	Nicotiana tabacum var. Xanthi
I	Banana	Tyre	-	L.L.	M	M	M	FM	Mf	M	M
II	Cucumber	Beirut	-	L.L.	M	M	M	FM	Mf	M	M
III	Cucumber	Saida	-	L.L.	M	M	M	FM	Mf	M	M
IV	Cucumber	Tripoli	-	L.L.	M	M	M	FM	Mf	M	M
V	Pepper	Beirut	-	L.L.	M	M	M	FM	Mf	M	M
VI	Petunia	Tripoli	-	L.L.	M	M	M	FM	Mf	M	M
VII	Pumpkin	Beirut	-	L.L.	M	M	FM	L.L.	Mo	M	M
VIII	Tomato	Beirut	-	L.L.	M	M	M	FM	Mf	M	M
IX	Tomato	Tripoli	-	L.L.	M	M	M	FM	Mf	M	M
X	Swiss-chard	Tripoli	-	L.L.	M	M	M	FM	Mf	M	M

M = mosaic; FM = faint mosaic; Mf = malformation;
L.L. = local lesions; Mo = mottling and spotting

Figure 13. Local lesions on leaves of Chenopodium amaranticolor.

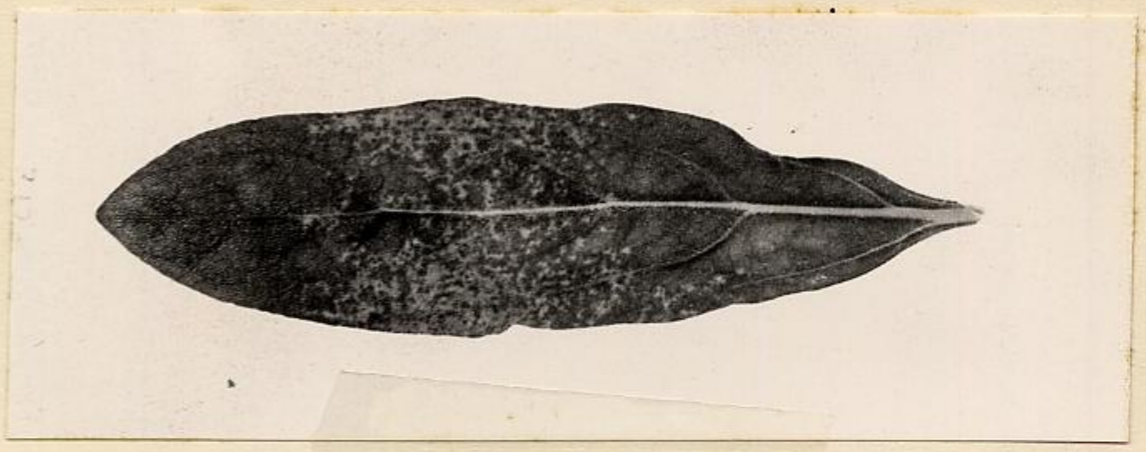

Figure 14. Mosaic symptoms on Gomphrena globosa.

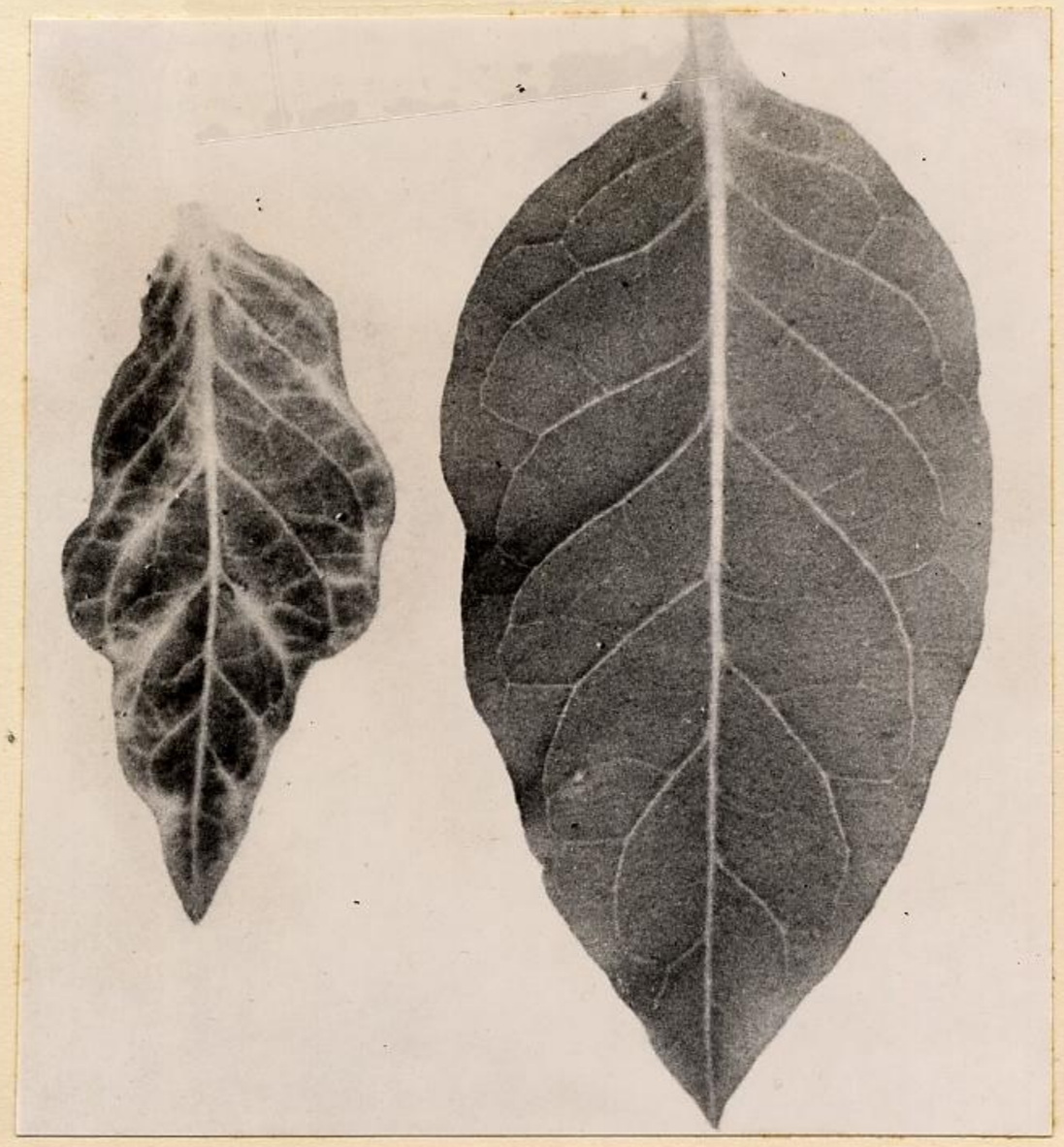

Figure 15. Mosaic symptoms on Nicotiana tabacum var. Xanthi on the left, healthy leaf on right.

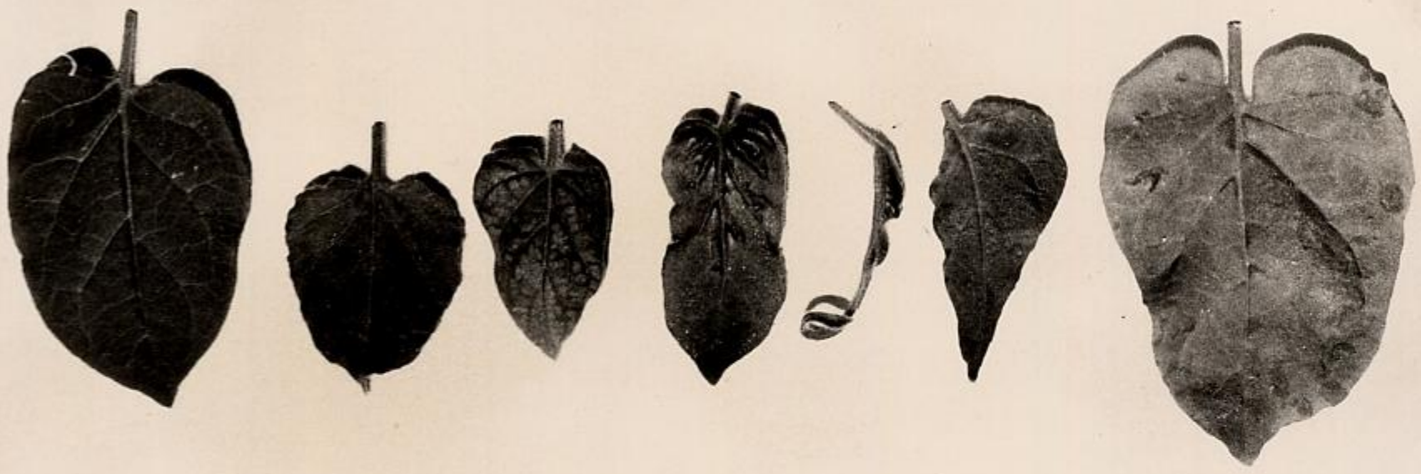

Figure 16. Symptoms of mosaic and malformation on leaves of Nicotiana glutinosa, left healthy leaf.

Figure 17. Mosaic symptoms on leaves of Nicotiana glutinosa due to the main strain of CMV.

Figure 18. Mottling of leaves of Nicotiana glutinosa due to the pumpkin strain of CMV.

Figure 19. Mosaic symptoms, on Nicotiana tabacum var. Xanthi on left, and on N.tabacum var. Samsun on right.

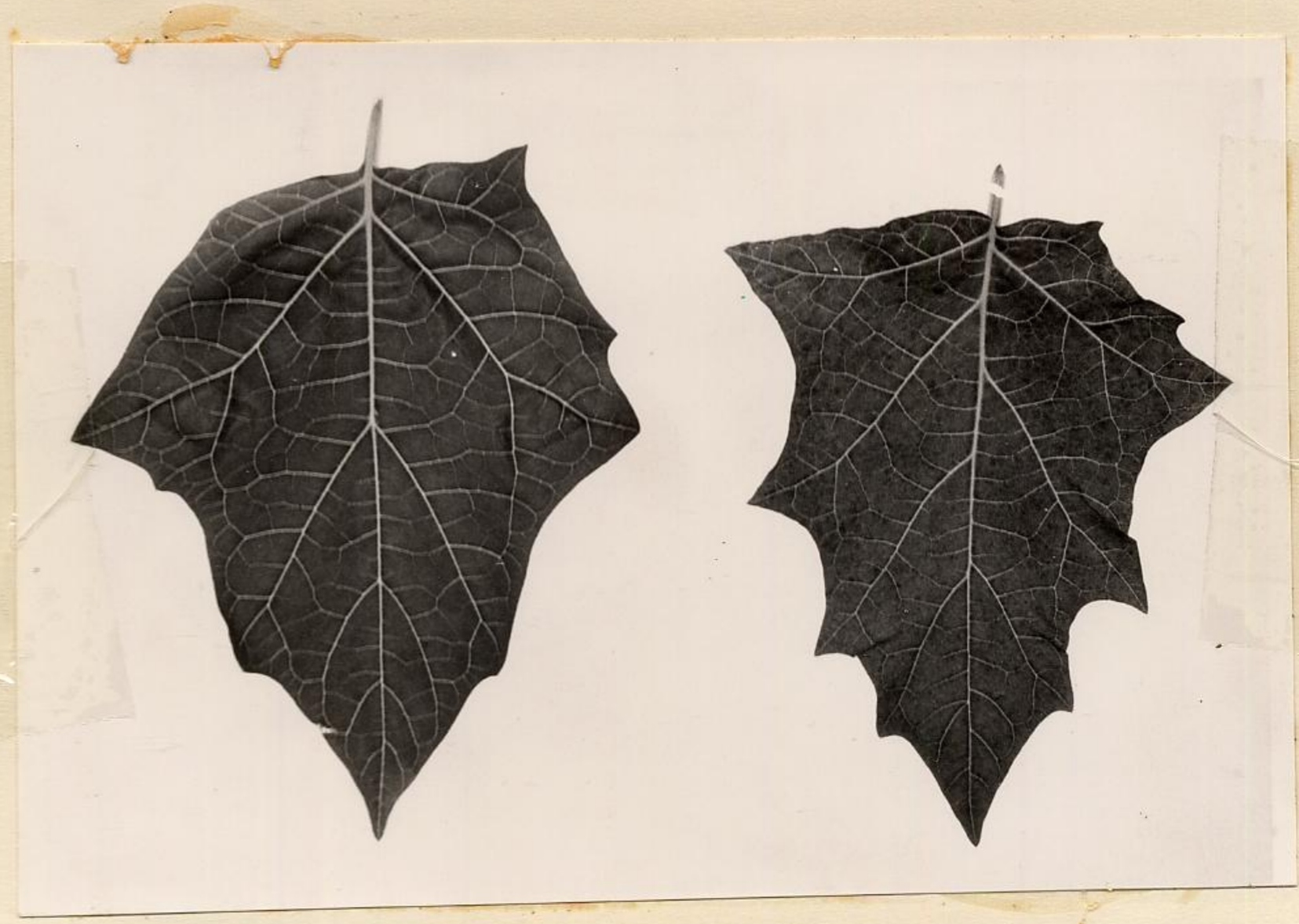

Figure 20. Faint mottling on a leaf of Datura stramonium on right, healthy leaf on left.

Anderson (1954); Cohen and Nitzany (1963), and Lindberg et al. (1956) in other countries. The symptom expressions as observed in this study were different from those reported by Cohen and Nitzany (1963) and Freitag (1956), for squash mosaic virus. This gives an indication that the squash mosaic virus could also be absent from Lebanon.

Physical properties of the virus:

Thermal inactivation point (T.I.P.): The T.I.P. of the same ten isolations as used in differential host studies, was tested at 10°C intervals, the results of which are presented in Table 3.

Table 3. Thermal inactivation point of 10 different isolations of CMV at 10°C intervals.

Isolation No.	Control	40°C	50°C	60°C	70°C	80°C	90°C
I	+	+	+	+	-	-	-
II	+	+	+	+	-	-	-
III	+	+	+	+	-	-	-
IV	+	+	+	+	-	-	-
V	+	+	+	+	-	-	-
VI	+	+	+	+	-	-	-
VII	+	+	+	+	-	-	-
VIII	+	+	+	+	-	-	-
IX	+	+	+	+	-	-	-
X	+	+	+	+	-	-	-

+ = infection ; - = no infection.

It may be seen from Table 3 that all the isolations lost their infectivity below 70°C, so it can be concluded that T.I.P. of all the isolations is between 60°C and 70°C.

To check closer ranges of temperature of the T.I.P. of the ten isolations, the temperature intervals were narrowed from 10°C to 2°C. The observations thus obtained are shown in Table 4.

Table 4. Thermal inactivation point of 10 isolations of CMV at 2°C intervals.

Isolation No.	Control	55°C	57°C	59°C	61°C	63°C	65°C	67°C	69°C
I	+	+	+	+	+	+	-	-	-
II	+	+	+	+	+	+	-	-	-
III	+	+	+	+	+	+	-	-	-
IV	+	+	+	+	+	+	-	-	-
V	+	+	+	+	+	+	+	-	-
VI	+	+	+	+	+	+	-	-	-
VII	+	+	+	+	+	+	+	+	-
VIII	+	+	+	+	+	+	-	-	-
IX	+	+	+	+	+	+	-	-	-
X	+	+	+	+	+	+	-	-	-

+ = infection; - = no infection.

It is evident from Table 4 that all the isolations were infective at 63°C but at 65°C only isolation No. V and VII were still infective and at 67°C only isolation No. VII caused infection while all others were inactive.

In the two T.I.P. experiments presented above, the inoculations were made with undiluted sap. In order to see the effect of diluting of the sap after heating, another experiment was done in which the sap was mixed with water in the ratio of 1:10 before making inoculations on the test plants. The results are shown in Table 5.

Table 5. Thermal inactivation point of 10 isolations of CMV at 2°C intervals after 1:10 dilution of the sap.

Isolation No.	Control	55°C	57°C	59°C	61°C	63°C	65°C	67°C	69°C
I	+	+	+	+	+	-	-	-	-
II	+	+	+	+	+	-	-	-	-
III	+	+	+	+	+	-	-	-	-
IV	+	+	+	+	+	-	-	-	-
V	+	+	+	+	+	+	-	-	-
VI	+	+	+	+	+	-	-	-	-
VII	+	+	+	+	+	+	+	-	-
VIII	+	+	+	+	+	-	-	-	-
IX	+	+	+	+	+	-	-	-	-
X	+	+	+	+	+	-	-	-	-

+ = infection; - = no infection.

The results in Table 5 indicate that when the sap solution was diluted after heating, the T.I.P. of all the isolations decreased by 2°C from what was shown in Table 4. With dilution the T.I.P. for all the isolations was between 61°C and 63°C except for isolation No. V and VII in which it was 2°C and 4°C, respectively, higher than that of other isolations as previously observed.

The results of T.I.P. experiments suggest that the two isolations No. V and VII are different from others in so far as their T.I.P. is concerned.

The T.I.P. observations determined for these isolates are within the limits of variability known for the CMV as described by Doolittle (1921); Cohen and Nitzany (1960-62) and Smith (1937, pp. 52-86).

The T.I.P. results also confirmed the earlier conclusion based on symptom expression on differential hosts that no squash mosaic virus could be isolated in Lebanon. The T.I.P. ranges for various isolates have not been in agreement with those recorded for squash mosaic virus by Freitag (1956) and Cohen and Nitzany (1963) who reported that the T.I.P. for squash mosaic virus was 70-75°C and 65°C, respectively. The T.I.P. of 65°C to 67°C (undiluted sap) as observed in this study for isolates V and VII was close to the T.I.P. of squash mosaic virus as mentioned above but the symptom expressions on indicator plants were different from those reported by the same authors.

Longevity in vitro: Three isolations were checked for their longevity in vitro. The results are shown in Table 6.

Table 6. The longevity in vitro of three different isolations of CMV.

Isolation No.	Longevity in vitro (hours)					
	0	24	48	72	96	120
V	+	+	+	+	-	-
VII	+	+	+	+	+	-
X	+	+	+	-	-	-

+ = infection: - = no infection.

It may be seen from Table 6 that isolation No. X, also used as a representative of the remaining 7, was not infective at 72 hours when the other two were still infective. Isolation No. V was not infective at 96 hours while, isolation No. VII remained infective even at 96 hours.

The longevity in vitro of CMV reported by various workers is different. Fulton (1950) reported a period of 10 days; Cohen and Nitzany (1960-62) noted 18 to 22 days; Smith (1937, pp. 52-86) described a period of 72 to 96 hours

at room temperature while Whipple and Walker (1941) recorded 7 days at 20 to 22°C. The isolates in the present study fall within the range of the one described by Smith (1937, pp. 52-86).

The foregoing results of host range, symptomatology of indicator plants and physical properties of the virus are in accordance with those reported by Smith (1937, pp. 52-86) for the CMV I type of virus, (Doolittle).

In conclusion it can be summarised that there seems to be only one main strain of CMV in Lebanon and that the isolation No. V and VII from pepper and pumpkin hosts from Beirut area are special strains of CMV I type virus. In Lebanon, the present studies revealed no other strain of CMV that could fit those in other countries reported by Anderson (1954); Grogan et al. (1959); Lindberg et al. (1956); Rader et al. (1947), and Smith (1937, pp. 52-86). The host range described by these authors was very limited as compared to the wide host range observed in the present study.

Transmission of CMV Through Seed

An experiment was laid out to test the degree of CMV transmission through certified U.S. seed of cucumber, as well as local seeds of cucumber, squash and watermelon from three different areas viz, Beirut, Saida and Zahlé. The findings of this experiment are tabulated in Table 7.

Table 7. Transmission of CMV through seeds of cucumber, squash and watermelon.

Location	October, 1965			March, 1966		
	Total plants	Diseased plant	% infection	Total plant	Diseased plant	% infection
		A = Cucumber seeds				
Beirut	80	8	10	50	2	4
Saida	92	9	11	45	2	3
Zahlé	85	9	10	40	1	4
U.S. certified seed	-	-	-	94	-	-
		B = Squash seeds				
Beirut	85	2	2	60	1	2
Saida	78	1	1	47	-	-
Zahlé	88	1	1	45	-	-
		C = Watermelon seeds				
Beirut	85	-	-	55	-	-
Saida	83	-	-	42	-	-
Zahlé	87	-	-	50	-	-

The above tests indicate that CMV was transmitted through seeds of cucumber and squash to a certain extent. It is further confirmed that watermelon seeds in Lebanon are free of CMV and there is no incidence of seed-borne watermelon mosaic virus.

According to Table 7, seeds under room temperature seem to loose virus activity during prolonged storage. The relatively low percentage of infected plants arising from infected seed is in harmony with the low amount of the disease usually present in early field plantings, but the presence of these infected seedlings provides an excellent and immediate reservoir of the virus which then can be readily and rapidly spread throughout the fields, either by mechanical means or by insect vectors. In the same way, apparently, the virus is spread from field to field.

V. SUMMARY AND CONCLUSIONS

An extensive survey on cucumber mosaic virus (CMV) was done by collecting disease specimens of a wide variety of host plants from 16 different areas in Lebanon. The results of the survey indicated that CMV has a wide host range including many vegetable crops of Chenopodiaceae, Cucurbitaceae, Solanaceae, Umbelliferae, ornamentals and banana plants.

Under the environmental condition of Lebanon, the CMV symptoms are characterised by mosaic, mottling and distortion of leaves, dwarfing and stunting of plants. Fruits of some crops may be mottled or altered in shape and size, in addition to impaired quality and flavor.

The identification of CMV and its strains was done through bio-assay on different indicator plants and by the determination of physical properties. The thermal inactivation point was in the limit of 63 to 65^{o}C (undiluted sap) for eight isolations but for the two isolations from pepper and pumpkin host plants it was 65^{o}C and 67^{o}C, respectively. The sap of a representative of the 8 isolations stored at room temperature lost infectivity between 48 to 72 hours, while in pepper and pumpkin isolations it ranged between 72 to 96 hours and 96 to 120 hours, respectively.

According to the results there is only one main strain of CMV in Lebanon. The pepper and pumpkin isolations can be considered as special strains of the virus not very much different from the main one. The watermelon mosaic virus and squash mosaic virus could not be obtained during the course of the survey program.

An experiment to check the percentage of CMV infection in local cucumber, squash and watermelon seeds was conducted. CMV was not detected in watermelon seed while a low percentage of cucumber and squash seeds were infected with the virus. Even this partial transmission presents a potential danger of the spread of the virus from one area to another through seed movements as the virus can easily spread within the fields from a few infected plants to all healthy plants either through mechanical contamination or by insects.

A number of methods could be employed to prevent the disease. It could be suggested that even though a very small percentage of cucumber and squash seed may harbor CMV the first prerequisite to adequate alleviation of the disease condition is securing and planting of disease-free seed. The perennials should not be grown in the same field with cucumber, tomatoes, and potatoes. Also the interplanting of cucurbitaceous and solanaceous plants with banana plants should be avoided. Infected banana plants should be removed and destroyed as soon as

they show symptoms and suckers from infected plants should not be used for new plantations. Vegetable plants left in the field after the last harvest should be removed because they can be a dangerous source of inoculum for the new crop. Weeds should be kept down since they may be a source of virus inoculum and also harbor insect vectors. No crop rotation is needed since CMV does not stay infective longer than a few days in dead tissue and soil.

Disease resistance is, of course, the best means to combat parasitic diseases of plants. It is suggested that efforts should be made to introduce or evolve varieties resistant to the main strain of CMV found in Lebanon.

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