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CONTENTS

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Infection Studies on Some Tomato Cultivars by **Colletotrichum coccodes** (Wallr.) Hughes Isolated from Potatoes

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ABSTRACT

Colletotrichum coccodes (Wallr.) Hughes isolated from the roots of potato plants grown in Erzurum Region caused infection both on underground parts and fruits of tomato plants. The disease severity on the underground parts of the cultivars varied in which Amerikan uçsuz and New Yorker 870 were being less affected while Yeşilköy 72 Yalova and Red Claud seemed to be more susceptible. The disease appeared as slight wilting of leaves towards the end of vegetation period only on the most affected cultivars.

Infection occured only in wound inoculated tomato fruits. In green fruits the fungus remained latent until the begining of ripening. In Erzurum under field conditions the fruits which are touching to soil surface and wounded were subjected to infection. The infection of ripe fruits progressed more slowly on Yuvarlak erkenci and Viktor than others. But even in these cultivars the fruits became completely rotten.

INTRODUCTION

Colletotrichum coccodes (Wallr.) Hughes (Syn. **Colletotrichum atramentarium** (Berk and Br.) Taubenh.) is a major cause of disease in potato and tomato plants in many parts of the world. The disease known as black dot of potatoes and anthracnose or brown root rot of tomatoes has wide distribution in potato growing regions of Erzurum (Karaca, 1962; Döken, 1977). From the stand point of tomato growing the climatic conditions have been always a limiting factor in enlarging tomato growing areas in the region. Unless in the recent years various adaptation studies have been conducting for introducing new, especially early cultivars which will promote tomato growing in the region. Since **C. coccodes** is known to be pathogenic both on potato

and tomato plants (Chesters and Hornby, 1965; Thirumalachar, 1967; Griffiths and Campbell, 1972) and considered to be the most serious disease of ripe tomato fruits (Barksdale, 1971), it will be worthwile to examine the pathogenicity of Erzurum potato isolate of **C. coccodes** on various tomato cultivars used in the adaptation studies.

MATERIALS AND METHODS

The present investigation was carried out by using a monoconidial isolate of a sclerotium type of **C. coccodes** which is widely distributed in the region and pathogenic on potatoes (Döken, 1977; Döken, 1982). For single spore isolation conidia were obtained from the cultures made by transferring a single sclerotium from the roots of infected potato plants. The isolate was maintained on potato agar 25 $\overline{+}$ 2° under continuous 2 x 40 watt white flurescent light for favoring sporulation (Barksdale, 1967; Döken, 1977). The inoculum was prepared from 14-21 days old cultures. The fungus colony was scraped by adding 10 ml. distilled water to each culture in petri dish. The whole inoculum was mixed throughly before inoculation.

During the studies Libby-C-52, Red Claud, Süper marmande, Valiant, Amerikan uçsuz (American topless), Fireball, New Yorker 870, Gardener, Morden 62 C 37'1, Yeşilköy 72 Yalova, Yuvarlak erkenci (Round early) and Viktor tomato cultivars were used. Their seedlings were obtained from the Horticulture Department of Faculty of Agriculture, Atatürk University, Erzurum-Turkey. The first four of the cultivars are early and from this feature they are recomended to the region. The following three cultivars are moderate and the last five are late (R. Alan Personal Communication).

The researches were conducted both in field and in growth cabinet. The temperature in growth cabinet was 24 $\overline{+}$ 2° C at 16 hours light period and 16 $\overline{+}$ 2° C at dark period. The relative humudity was being around 70 %. The seedlings grown in greenhouse were transplanted when they are eight weeks old to field and singly to each 10 cm diameter pots in four replicates. Each replicate contained six plants in field and four plants in pots. During the transplanting 10 ml. inoculum which contained mycelium, conidia and sclerotia was poured around the root and collar region of each plant. Then the inoculated portion was covered with soil and the plants were kept under continuous observation to note any wilting and yellowing symptoms. Root disease assesments were made at the end of vegetation period. The plants were lifted, the root systems were washed free from soil and visual disease estimations were made as percentage of root system covered by sclerotia. The results were log. transferred and analyses of va-

riance were made. The means of the results were compared by Duncan's Multiple Range test.

For tomato fruit inoculations mature and green tomato fruits about the same size were harvested from each cultivar. The fruits were washed and allowed to dry. For wound inoculations a glass tube at 5 mm. diameter was slightly pierced into tomato fruit and the skin inside the punctured area was peeled carefully. Four wounds were made on every fruit. A drop of inoculum about 0.05 ml. was placed on each wound and covered by the skin. For control, one wound on each fruit was not inoculated. Another group of fruits which are not injured were also inoculated by placing same amount of inoculum into the surface of the fruits. Ten green and ten ripe fruits were used from every cultivar for each type of inoculation. The inoculated fruits were placed in moist chambers on paper towels at room remperature ($20 \pm 2°$ C). The disease was measured by lesion diameter 15 days after inoculation. Analyses of variance was made and mean results were ranked by Duncan's Multiple Range test.

RESULTS AND DISCUSSION

No distinct symptoms were observed on the green parts of tomato cultivars at any growth stages. Only two cultivars Yeşilköy 72 Yalova and Red Claud exhibited slight wilting towards the end of vegetation period, while no yellowing was detected except the normal senesence of the lower leaves due to aging of the plants. There was no indication of fungal growth on leaves, although it was reported that under field conditions the fungus can grow on leaves (Pantidou and Schroeder, 1955).

The examination of the roots and the collar of tomato cultivars revealed the presence of tiny black sclerotia embedded in the surface of cortex (Fig. 1). Although disease appeared to be common on the underground parts of all cultivars, some differences in susceptibilty were recorded (Table 1). According to the analyses of variance Amerikan uçsuz and New Yorker 870 were significantly less affected. Unless the both were not among the recomended cultivars. On the other hand Yeşilköy 72 Yalova and Red Claud which is one of the recomended early cultivars was found to be significantly more susceptible than other cultivars under the field conditions, but in the growth cabinet the difference was not statistically significant. On the most affected plants the cortex of roots and collar loosened and easily detached and lost leaving the central cylinder exposed. But no cankers were observed on any of the tomato cultivars while a limited canker development on the basal stem was reported by Manning (1979).

The disease severity on the underground parts of the tomato plants grown in pots in growth ca-

binet was seemed to be little higher than tomatoes in field. It was ascribed to the decreasment of the inoculum density in contact with roots in field due to the washing away by irrigation water.

Table 1. Mean disease on the underground parts of different tomato cultivars grown under field and growth cabinet conditions

FIELD

Tomato cultivar	% Infection	% 5	% 1
Yeşilköy 72 Yalova	60.0	a	a
Red Claud	56.7	a	ab
Süper marmande	41.7	b	abc
Fireball	40.0	b	abc
Viktor	38.3	b	abc
Gardener	36.7	b	bc
Valiant	36.7	b	bc
Morden 62 C 37'1	35.0	b	bc
Libby-C-52	33.4	b	c
Yuvarlak erkenci	30.0	b	c
Amerikan uçsuz	6.6	c	d
New Yorker 870	5.8	c	d

GROWTH CABINET

Tomato cultivar	% Infection	% 5	% 1
Yevilköy 72 Yalova	66.7	a	a
Red Claud	63.3	ab	a
Fireball	48.3	ab	a
Süper marmande	46.7	ab	a
Gardener	46.7	ab	a
Viktor	45.0	ab	a
Libby-C-52	41.7	ab	a
Valiant	40.0	ab	a
Morden 62 C 37'1	39.2	ab	a
Yuvarlak erkenci	38.3	b	a
Amerikan uçsuz	12.5	c	b
New Yorker 870	10.8	c	b

Within each column means not followed by the same letter differ according to Duncan's Multiple Range test

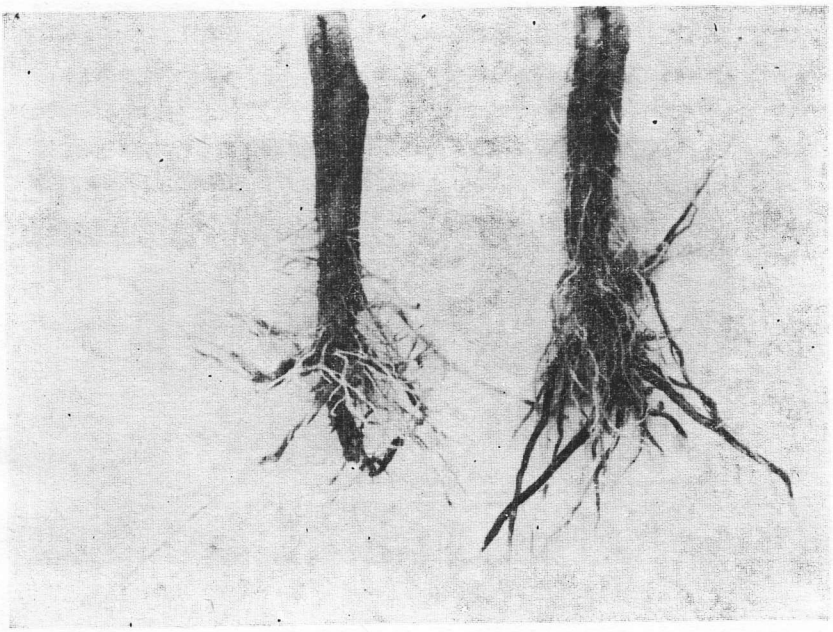

Fig. 1. Disease incidence on the roots and the collar of tomato plants. Tissues which appear white are healty, dark are covered by sclerotia

The fruit inoculations showed that **C. coccodes** is able to cause infections on wound inoculated tomato fruits. The symptoms which were as small circular depressed spots at the begining enlarged and became dark by the time (Fig. 2). On ripe fruits the disease began to appear about one week after inoculation. But on green fruits it delayed up to the begining of maturity. It seemed that in green fruits the fungus remains latent until the beginning of ripening. This could be due to the presence of some substances affecting to the development of **C. coccodes**. As a matter of fact Allison (1955), pointed out the inhibitory effect of solanine on the growth of **C. coccodes** and also Brown Adıkaram (1983), attributed it to the accumulation of phytoalexins in green tomato fruits. In our studies **C. coccodes** failed to cause infection on uninjured green and ripe fruits, although Barksdale (1967) obtained infections on uninjured tomato fruits. According to Brown and Adıkaram (1983), **C. coccodes** does not develop on unwounded green fruits, but makes slow development on unwounded ripe fruits. These discrepancies might be owing to the differences between the tomato cultivars as well as the virulance of the pathogen isolate used by different researchers.

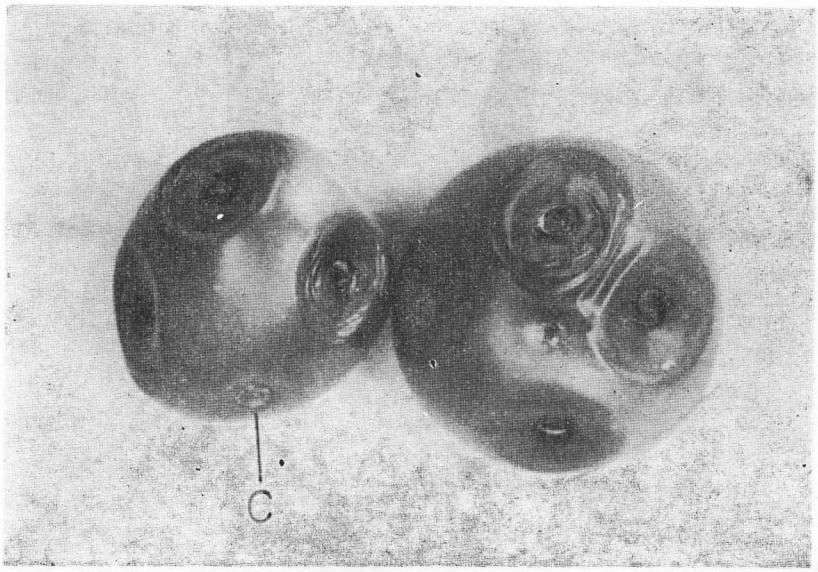

Fig. 2. Disease symptoms on wound inoculated tomato fruits.
C.Control (Not inoculated)

In field natural infections of fruits touching to the surface of soil were infected. The climatic conditions in Erzurum appeared almost preventing the aerial contamination of tomato fruits by **C. coccodes** since high relative humudity is necessary for conidial production and dissemination (Doolittle, 1953). Therefore it is believed that under the prevailing conditions of Erzurum, generally the fungus present in soil cause infection through the wounds of tomato fruits developing only on or near the soil surface.

The pathogen caused infections on the fruits of all cultivars with varying degrees of progress on the development of infection. The disease incidence was measured only on ripe fruits. Because in green fruits **C. coccodes** began to develop with the commencement of maturity which was not about the same between the fruits. So that the variation in infection rate could not be attributed to the differences in varietel reactions. Disease assesments showed that the infection progreses significantly more slowly on the fruits of Yuvarlak erkenci and Viktor (Table 2). Unless even in these cultivars the fruits were consequently rotten.

Table 2. Mean disease on the fruits of different tomato cultivars

Tomato cultivar	Lesion diameter (mm)	
Libby-C-52	43.7	a
Gardener	42 5	a
Red Claud	41.7	a
Amerikan uçsuz	41.0	a
Fireball	40.2	a
New Yorker 870	38.7	a
Valiant	38.1	a
Morden 62 C 37'1	38.0	a
Yeşilköy 72 Yalova	37.8	a
Süper marmande	37.7	a
Yuvarlak erkenci	8.2	b
Viktor	7.3	b

Within the column means not followed by the same letter differ at 0.01 and 0.05 level according to Duncan's Multiple Range test

When disease incidences on fruits and on underground parts are compared between the cultivars, it seems that there is unlikely to be a relationship between the reactions of fruits and underground parts of tomato cultivars to **C. coccodes.**

In our studies apart from the pathogen's direct effect on yield by rotting tomato fruits, the disease damage on roots and collar of the cultivars was seemed to be insufficient to cause considerable wilting even on the most affected plants. The damage which was reflected as slight wilting towards the end of growing period only on these cultivars should not be regarded as important enough to warrant consideration of the isolate of **C. coccodes** as a notable tomato wilt pathogen. However under unfavourable conditions and with other pathogens it may be an important contributor of tomato wilt. On the other hand in the view of economic consideration further investigations are necessary especially to understand the relation between disease incidence on underground parts of tomato plants and yield losses.

Ö Z E T

BAZI DOMATES ÇEŞİTLERİNDE PATATESDEN İZOLE EDİLEN **Colletotrichum coccodes** (Wallr.) Hughes İLE ENFEKSİYON ÇALIŞMALARI

Erzurum yöresinde patates köklerinden izole edilen **Colletotrichum coccodes** (Wallr.) Hughes'in farklı domates çeşitlerinde patojenite du-

rumu bitki büyütme kabini ve tarla koşullarında incelenmiştir. Çeşitlerin toprak altı aksamlarında oluşan hastalık en düşük Amerikan uçsuz ve New Yorker 870 çeşitlerinde belirlenmiştir. En hassas olarak Yeşilköy 72 Yalova ve Red Claud çeşitleri görülmekte ve solgunluk hafif olarak sadece bu çeşitlerin yapraklarında vejetasyon dönemi sonuna doğru ortaya çıkmaktadır.

Domates meyvelerinde enfeksiyon sadece yaralardan yapılan inokulasyonlar sonucu gerçekleşmektedir. Fungus yeşil meyvelerde latent olarak kalmakta ve ancak meyvenin olgunlaşmaya geçmesi ile birlikte enfeksiyon başlamaktadır. Erzurum koşullarında sadece toprağa temas eden yaralı meyvelerde hastalık oluşmaktadır. Enfeksiyon Yuvarlak erkenci ve Viktor çeşitlerinin meyvelerinde daha yavaş olarak ilerlemekte, ancak bu çeşitlerde bile meyveler sonunda tamamen çürümektedir.

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Investigations on the Identification, Seed Transmission and Host Range of Viruses Infecting The Culture Plants in the Cucurbitaceae in Marmara Region

1— The Identification of Viruses Infecting Cucurbits in Marmara Region

Abdullah NOGAY[1] and Ülkü YORGANCI[2]

ABSTRACT

Viruses obtained in surveys of various cucurbits in Marmara region in 1979-1980 were isolated and identified by use of differential hosts, their some physical properties, serology, and electron microscopy. During two tours surveys in cucurbit growing areas in 1979, macroscopically the average virus diseases rates for region was found 17.3, 24.2, 13.4 and 0.83 % on cucumber, squash, melon and watermelon respectively. In surveys of melon and watermelon plantings in Thrace in August and September 1980, the average virus diseases rates was 14.6 and 2.18 % on melon and watermelon. 269 cucurbit samples with virus like symptoms collected in 1979 and 1980 were found infected by viruses using differential hosts. Cucumber mosaic virus (CMV) was present in 142 of 269 samples (52.79 %), Watermelon mosaic virus-2 (WMV-2) was found in 118 samples (43.86 %). 9 samples (3.34 %) were infected by both CMV and WMV-2. CMV was prevalent in the provinces of Anatolian side. In Thrace the distribution pattern was different in that WMV-2 was more prevalent than CMV. Mixtures of CMV and WMV-2 in field samples were separated by the use of **Nicotiana tabacum, C. quinoa, Citrullus vulgaris** and **Hibiscus esculentus.**

Because of the some differences of their symptoms in test plants, investigations were done on 5 CMV and 7 WMV-2 isolates. The thermal inactivation points of CMV and WMV-2 isolates were between 65-80 °C and 50-75 °C, dilution end points were 10^{-4} - 10^{-6} and 10^{-3} - 10^{-6} respectively. CMV and WMV-2 isolates were tested with antisera of CMV, WMV-1 and WMV-2. CMV isolates produced reactions with CMV antisera, WMV-2 isolates reacted with WMV-2 antiserum. The isolates were

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examined in the EM and micrographs taken by R.D. WOODS at Rothamsted Experimental station (England). The electron micrographs revealed the particles of CMV to be isometric about 30 nm diameter. WMV-2 particles were long flexuous rods about 800 nm long.

INTRODUCTION

According to the statistics, cucurbits cover 30 % of the acreage devoted to the vegetable crops that are grown in 600.000 hectars of Turkey. With an area of about 37.400 ha and the production of 750.000 tons, cucurbits (cucumber, squash, pumpkin, melon and watermelon) are important vegetable crops grown in 9 provinces (Bilecik, Bolu, Edirne, Kırklareli, Kocaeli, İstanbul, Sakarya, Tekirdağ) of Marmara region (4).

In 1955, CMV were seen epidemically in vegetable areas of İstanbul and disease rate was determined 100,90 % on cucumber and squash respectively (29). İŞMEN (15) reported that the epiphytotic of CMV caused 70-80 % yield reduction in cucumber in Marmara region in 1962. In these studies, symptoms of infected plants were depended upon for the virus identification and CMV was supposed to be agent of mosaic.

During recent years, cucurbit crops (particularly cucumber and squash) grown in Marmara region have been severely affected by mosaic virus diseases. In various localitles, practically 100 % of the plants have become infected. Symptoms were mosaic, mild or severe mottling, blistering of leaves and stunted growth. On cucumber and melon wilting and drying occasionally occurred. Reduction in fruit size, abnormal shapes, colors, and patterns that make them non-marketable were fruit symptoms.

In the present study, surveys were conducted in cucurbit plantings cultivated throughout the region of Marmara during the 1979-1980 growing season and prevalance of virus diseases was determined. CMV and WMV-2 were isolated from samples with virus like symptoms collected in surveys.

This is apparently the first report of the occurrence of CMV and WMV-2 in cucurbits in Turkey.

MATERIALS and METHODS

1. Field collections and inoculations

In 1979-1980 samples of virus-infected cucurbit hosts like **Cucumis sativus** (cucumber), **Cucurbita pepo** "Sakız" (squash), **Cucurbita maxima** (pumpkin), **Cucumis melo** (melon, cantaloupe), **Citrullus vulgaris** (watermelon) were collected from the 9 provinces of Marmara region. Samples were ground with mortar and pestle in phosphate buf-

fer (0.05 M, pH 7-7,6) (11, 26, 39, 40) or in borate buffer (0.005 M, pH 9.0) (10) and the extracts were inoculated to test plants (presented in Table 1) dusted lightly with carberandum powder (500 mesh) in a greenhouse. After inoculations, the leaves were rinsed with tap water and plants were kept in a moist chamber overnight. The cucurbit hosts were inoculated in the cotyledon stage and other hosts in the 2-4 true-leafless stage. Temperatures in greenhouse ranged from 15 °C at night to 30 °C during the day. The test plants were observed everyday after inoculation for symptom development.

2. Tests of some physical properties

The thermal inactivation and dilution end points of virus isolates were determined by methods described by BOS et al. (6). In these studies, **N. tabacum** "Xanthi" and **Cucurbita pepo** "Sakız" were used as the source plants for CMV and WMV-2 isolates respectively. **C. amaranticolor** was utilized as assay plant for both viruses.

3. Purification

CMV isolates for purification were propagated in **N. tabacum** "Xanthi" and purified by the methods of LOT et al. (22) and TOMLINSON et al. (35). Two WMV-2 isolates were multiplied in **C. pepo** "Sakız" by inoculating them in their cotyledon stage and partially purified following method of BHARGAVA (5). During the purification studies Christ low-speed centrifuge and Beckman L5 50 ultracentrifuge were used.

4. The ultraviolet absorbtion spectra

The ultraviolet absorbtion spectra of the purified virus preparations between 250-290 nm were determined with a DB spectrophotometer. Virus yields of purified preparations were calculated making use of relation that absorbance at 260 nm (1 mg/ml, 1 cm light path) was 5 (13) for CMV and 3 for WMV-2 being a member of PVY group.

5. Serology

Purified CMV isolates were tested with antisera of CMV using the method of Ouchterlony agar double diffusion. CMV antisera were supplied from Dr. D.A. GOVIER and Dr. J.A. TOMLINSON.

Agar gel diffusion tests were done on 5 x5 cm glass slides containing 5 ml of 0.75 % oxoid purified agar Code L 78 in buffer (0.005 M BO_3, 0.005 M EDTA, pH 8.2 or 0.05 M KH2PO4, pH 8). Sodium azide at 0.02 % was added to the melted agar as a preservative (17, 35). Test patterns in the agar comprised 6 peripheral wells (5 mm diam) around a central well (5 mm diam) with 8 mm between the centres of the neighbour wells. Wells were fil-

led with the purified preparations of CMV and antisera at vorious combinations. Each agar slides was incubated at room temperature in a closed humid petri dish. The development of precipitin patterns was recorded over a period of 1-5 days.

WMV-2 isolates were tested with antisera of WMV-1 and WMV-2 (Supplied by A. SCOTT) using methods of SDS (sodium dodecyl sulfate) immunodiffusion (30) and microprecipitin (25).

For immunodiffusion tests with SDS treated antigens, the immunodiffusion medium of 0.8 % Noble agar, 0.5 % SDS, and 1.0 % NaN_3 (all w/v) in distilled water. 12 ml of medium were poured into each plastic petri dish. Antigen were prepared from virus-infected and healthy leaves by grinding tissue in H2O (1 ml per g of tissue). One ml of 3 % SDS was than added per g of tissue, and the mixture was expressed through cheesecloth. The extracts were used within 1-2 hrs after preparation. Purified antigens were untreated with SDS prior to use. A gel pattern consisting of 6 peripheral wells surrounding a central well (each well 5 mm in diam) was used. The edge of the center well was 3 mm from the edges of the peripheral wells. After addition of reactants at various combinations, the plates were incubated at room temperature and results were recorded after 24-48 hrs.

Microprecipitin tests were done in plastic petri dishes under liquid vaseline. Antiserum, virus, and healthy plant sap dilutions were made in 0.85 % saline in 0.01 M Tris-HCl, pH 7.5. Tests were incubated at room remperature, and results were recorded after 2 hours.

6. Electron microscopy

The isolates were examined in the EM and micrographs were taken by R.D. WOODS (Rothamsted Experimental Station. Horpenden Herts, England).

RESULTS

1. Host reactions

The reactions of test plants inoculated with CMV and WMV-2 isolates are summarized in table 1.

Table 1. Reactions of test plant useful for identifying CMV and WMV isolates

Test plant	CMV isolates					WMV-2 isolates						
	CC_1	CM_2	CM_3	CS_4	CS_5	WM_6	WM_7	WS_8	WS_9	WS_{10}	WW_{11}	WW_{12}
C. amaranticolor	L	L	L	L	L	L	L	L	LS[1]	LS	L	L
C. quinoa	L	L	L	L	L	LS	—	—	—	—	—	—
Vigna sinensis	L	L	L	L	L[2]	—	L	L	L	L	L	L
P. vulgaris "Pinto", "Prince"	L	L	L	L	L	—	—	—	—	—	—	—
D. stramonium	LS	L	L	LS	LS	—	—	—	—	—	—	—
Zinnia elegans	S	S	S	S	S	—	—	—	—	—	—	—
Gomphrena globosa	L	L	L	L	L	—	—	L	L	L	—	—
Hibiscus esculentus	S	S	S	S	S	LS	LS	LS	LS	LS	LS	LS
Lycopersicon esculentum	S	S	S	S	S	—	—	—	—	—	—	—
Capsicum annuum	S	S	S	S	S	—	—	—	—	—	—	—
N. tabacum "Xanthi", "Samsun"	S	S	S	S	S	—	—	—	—	—	—	—
N. glutinosa	L[3]S	LS	LS	LS	L[3]S	—	—	—	—	—	—	—
Vicia faba	—	—	—	—	—	—	—	—	—	—	—	—

(continued on next page)

Table 1. (continued)

Test plant	CMV isolates					WMV-2 isolates						
	CC_1	CM_2	CM_3	CS_4	CS_5	WM_6	WM_7	WS_8	WS_9	WS_{10}	WW_{11}	WW_{12}
Cucumis sativus	LS	LS	LS	LS	LS	S	S	S	S[4]	S[4]	S	S
C. melo "B 633-3"	LS	LS	LS	LS	LS	S	S	S	S	S	S	S
C. melo "34340"	—	—	—	LS[5]	—	S	S	S	S	S	S	S
Cucurbita pepo	LS	LS	LS	LS	LS	S	S	S	S	S	S	S
Citrullus vulgaris	L	LS[6]	L	L	L	S	S	S	S	S	S	S
Luffa acutangula	S	S	S	S	S	—	—	—	—	—	—	—

L = Chlorotic and/or necrotic local lesions

S = Systemic infection

— = No reaction

1 = Systemic infection not consistently observed

2 = Very few lesions occasionally seen

3 = Local lesions not clearly visible

4 = More severe symptoms than obtained with other WMV-2 isolates

5 = Infection occurred on 50 % of inoculated plants

6 = Systemic infection occurred on 20 % of inoculated "Sugar Baby" variety.

Viruses were isolated from **C. sativus** «Çengelköy» (CC_1), **C. melo** «Hasanbey» (CM_2), **C. melo** «Pamukova» (WM_3), **C. pepo** «Sakız» (CS_4), **C. maxima** (CS_5), **C. melo** «Kırkağaç» (WM_6), **C. melo** «Topatan» (WM_7), **C. pepo** «Sakız» (WS_8), **C. pepo** «Sakız» (WS_9), **C. maxima** (WS_{10}), **C. vulgaris** «Yeni Dünya (WW_{11}) and a fruit of **C. vulgaris** «Washington» (WW_{12}) in Bursa, Bilecik, Geyve, İnegöl, Akyazı, Uzunköprü, Silivri, Kartal, Yalova, Akyazı, Kırklareli and İpsala respectively.

2. Mixed infections

Mixtures of CMV and WMV-2 in field samples (3 squash and 6 melon) were separated by the use of following differential hosts; **Nicotiana tabacum, Chenopodium quinoa, Citrullus vulgaris** and **Hibiscus esculentus. N. Tabacum** was susceptible to CMV but immune to WMV-2. **Citrullus vulgaris** and **C. quinoa** were susceptible to systemic invasion by WMV-2 isolates but very highly resistant or immune to isolates of CMV. WMV-2 induced local lesions and systemic mottle in **Hibiscus esculentus**, whereas CMV produced chlorosis and stunting on same plant. Thede differential reactions permitted separation of WMV-2 from CMV.

3. Physical properties

The results obtained in these tests were summarized in Table 2.

Table 2. The some physical properties of CMV and WMV-2 isolates from cucurbits

Isolate	Dilution end point	Thermal inactivation point (°C)
CMV		
CC_1	10^{-4} - 5 x 10^{-4}	65 - 70
CM_2	10^{-5} - 10^{-6}	70 - 75
CM_3	10^{-5} - 10^{-6}	65 - 70
CS_4	10^{-5} - 10^{-6}	75 - 80
CS_5	5 x 10^{-4} - 10^{-5}	75 - 80
WMV-2		
WM_6	5 x 10^{-4} - 10^{-5}	60 - 65
WM_7	10^{-4} - 5 x 10^{-4}	50 - 55
WS_8	10^{-5} - 10^{-6}	65 - 70
WS_9	10^{-5} - 10^{-6}	70 - 75
WS_{10}	10^{-4} - 5 x 10^{-4}	60 - 65
WW_{11}	10^{-4} - 5 x 10^{-4}	50 - 55
WW_{12}	10^{-3} - 10^{-4}	55 - 60

4. Purified preparations

Five CMV and two WMV-2 isolates were purified. Properties of these preparations are summarized in Table 3.

Table 3. Yields and absorbances at 260/280 nm absorption of purified virus isolates

Isolate	Yield (mg/ml)	A260/280 wavelength (nm)	Suspansion medium of Preparation
CMV			
CC_1	1.05	1.54	0.005 M $Na_2B_4O_7$ + 0.005 M EDTA, pH9
CM_2	0.25	1.75	sterile destilled water
CM_3	0.77	1.26	0.005 M $Na_2B_4O_7$ + 0.005 M EDTA, pH9
CS_4	1.21	1.28	sterile destilled water
CS_5	1.11	1.38	sterile destilled water
WMV-2			
WS_9	2.35	1.40	1 M phosphat pH7
WW_{12}	1.08	1.25	1 M phosphat pH7

5. Serological tests

All CMV isolates (purified) reacted with the antisera of CMV - GOVIER and CMV-TOML in Ouchterlony agargel double diffusion tests (Table 4, Fig. 1, 2).

Table 4. Reactions of purified CMV isolates with CMV-GOVIER antiserum

Antigen	mg virus/ml	Dilutions of antiserum 1/1	1/2	1/4	1/8	1/16	1/32
CC_1	1.05	+ + +	-+- -+-	+ +	—	—	—
CM_2	0.25	+ +	+ +	+ +	+	+	—
CM_3	0.77	+ + +	-+- -+-	+ +	+	—	—
CS_4	1.21	+ + +	+ -+- -+-	+ +	+	+	-+-
CS_5	1.11	+ + +	-+- -+-	+ +	+ +	-+-	-+-

Precipitation line intensity (after 48 h) is indicated by the number of + signs ; — indicates no precipitation line.

The isolates of WMV-2 reacted in SDS-immunodiffusion and micro-precipitin tests with the WMV-2 SCOTT antiserum (Table 5, Fig. 3). No reaction occurred with the sera to WMV-1 (supplied A. SCOTT) and CMV.

Table 5. Reactions of WMV-2 isolates with WMV-2 SCOTT antiserum in SDS-immunodiffusion tests

Antigen (Clarified extract of infected C. pepo «Sakız»)	Antiserum dilutions 1/1	1/2	1/4	1/8	1/16	1/32
WM_6	+++	+	—	—	—	—
WM_7	++	±+	+	—	—	—
WS_8	+++	+±±	++	+	—	—
WS_9	+++	+±+	++	++	±	—
WS_{10}	+++	±±	—	—	—	—
WW_{11}	+++	±±	+	—	—	—
WW_{12}	+++	±+	++	+	—	—
Control (Healthy plant extract)	—	—	—	—	—	—

Precipitation line intensity (after 48 h) is indicated by the number of + signs ; — indicates no precipitation line.

6. Electron microscopy

The isolates identified by use of test plants were examined in the EM and micrographs were taken by R.D. WOODS. The electron micrographs revealed the particles of CMV to be isometric about 30 nm diameter (Fig. 4). WMV-2 particles were long flexuous rods about 800 nm long (Fig. 5). Mixtures of CMV + WMV-2 in field samples are seen in Figure 6.

7. Distribution of viruses

269 cucurbit samples with virus like symptoms collected in surveys in 1979 and 1980 were Marmara region and in cucurbits cultivated were presented Tables 6, 7.

Table 6. Distribution of viruses identified from field collections (1979-1980) in Marmara region

Collection sites	Cucumber		Squash			Melon			Watermelon		Total
	CMV	WMV-2	CMV	WMV-2	CMV+WMV-2	CMV	WMV-2	CMV+WMV-2	CMV	WMV-2	
Bilecik						4					4
Düzce			4	1							5
Bursa	10	2			1			3	2		18
İnegöl			6								6
İznik	4										4
Karacabey						2					2
M.Kemalpaşa						3	1				4
Orhangazi	3										3
Yenişehir	2									1	3
Akyazı			12	4							16
Geyve	2					18					20
Çatalca	2	2	5	3		2	2			1	17
Kartal	8	2	7	9	2						28
Silivri						2	4	1			7
Yalova			4	6							10
Edirne						1	3			2	6
Havsa							2			2	4
İpsala						10	12			4	26
Meriç						3	3			1	7
Uzunköprü						2	18			3	23
Kırklareli							2			3	5
Çorlu						1	5			2	8
Hayrabolu						2	2			1	5
Others	5	4	11	4		5	7	7			38
Total	36		49			55			2		269
		10		27			61			20	
					3			6			

Table 7. Distribution of viruses in cucurbits cultivated

Viruses	Cucumber		Squash		Melon		Watermelon	
	n.col.	%	n.col	%	n.col.	%	n.col.	%
CMV strains	36	78.26	49	62.02	55	45.08	2	0.09
WMV-2 strains	10	21.74	27	34.18	61	50.00	20	90.91
CMV + WMV-2	0	0	3	3.80	6	4.42	0	0
Total	46	100.00	79	100.00	122	100.00	22	100.00

n.col. = number of collections

DISCUSSION

All CMV isolates induced local lesions on **C. amaranticolor, C. quinoa, Vigna sinensis, Phaseolus vulgaris** (2-4 days), **Datura stramonium** (6-7 days), **Gomphrena globosa** (10-12 days) and systemic symptoms (mosaic mottle, narrowing of the leaves, severe malformations leading to filiformity and shoe-string formation) on **Nicotiona tabacum, N. glutinosa, Lycopersicon esculentum** and **Capsicum annuum.** The symptoms produced by our CMV isolates in tests plants were similar to those described by other authors (7, 8, 13, 14, 19, 26, 27, 28, 32, 39). CMV isolates locally and systemically infected cucumber squash and melon. Only one of them (CM_2) systemically infected Sugar baby variety of watermelon with 20 % infection ratio and it was characterized by being more prevalent than the others. Producing systemic symptoms of CMV on watermelon depends on strain and usually is not common (7, 27, 39).

The thermal inactivations and dilution end points of CMV isolates were similar to those reported by some authors but showed slight differences to others (12, 16, 17, 19 20, 23). The differences could have depended on the use of other strains or the different environmental conditions. CMV particles were measured 28-42 nm by previous workers. However, our isolates were 30 nm diam as average length of particles reported by GIBBS and HARRISON (13).

WEBB and SCOTT (40) divided Watermelon mosaic virus (VMV) isolates from the USA into two distinct groups, distinguished by host range and serologycal properties. Those isolates that failed to infect noncucurbitaceous plants were designated as WMV-1, whereas isolates that infected plants outside the **Cucurbitaceae** were designated as WMV-2. Also they had reported that WMV-1 induced pin poin local lesions on inoculated cotyledons and leaves of selection B633-3 of muskmelon PI 180280, whereas isolates of WMV-2 produced only a systemic mottle. WEBB (38) reported

Luffa acutangula to be susceptible to WMV-1 but immune to WMV-2 isolates. Our WMV isolates induced local lesions (later with red border) on **C. amaranticolor** in 519 days, failed to infect **Luffa acutangula** and **Nicotiana** sp tested, caused only systemic infection on **C. melo** «B 633-3» and produced local lesions and systemic symptoms on **Hibiscus esculentum.** Chlorotic local lesions and later systemic symptoms developed when **C. quinoa** was inoculated with several isolates (designated as WM6, WS9, WS10), this fact in egreement with the results of other workers on WMV (21, 23) though MILNE et al. (26) reported that none of the WMV isolates tested at Davis produced systemic infection of this plant. On cucurbits all the isolates induced typical WMV symptoms like those described by VAN REGENMORTEL (36). But WS_8 and WS_9 isolates induced more severe symptoms on cucumber and squash than the other WMV isolates. Also the physical properties (thermal inactivation and dilution end points) of these two isolates were found than those recorded for WMV-2 (2, 3, 5, 24, 37).

The results of our serological tests support the observations of some workers (2, 21, 31, 40), who reported evidence that WMV-1 and WMV-2 were serologically different, but on contrary, MILNE and GROGAN (25), who reported that a very close serological relationship existed between WMV-1 and WMV-2.

The particle lengths of WMV reported by earlier workers (2, 3, 5, 9, 19, 31, 33, 37) varies from 675 to 900 nm, but the normal length has been given as 750 nm (36). Our WMV particles were measured 800 nm long. The reason of that 50 nm difference between our isolates and the average length of WMV may be differences of the strains.

We belive, therefore, as a result of the accumulation of our data, that all characteristics of our WMV isolates are those of WMV-2. They show minor differences amongthemselves in the symptoms induced on test plants.

9 field samples (3.34 %) were found infected by both CMV and WMV-2 in this study as reported earlied (1, 18, 24).

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ÖZET

MARMARA BÖLGESİNDE CUCURBİTACEAE FAMİLYASI KÜLTÜR BİTKİLERİNDE GÖRÜLEN VİRUS HASTALIKLARININ TANILANMASI, TOHUMLA GEÇİŞ DURUMLARININ VE KONUKÇU DİZİLERİNİN SAPTANMASI ÜZERİNDE ARAŞTIRMALAR

1. Marmara Bölgesinde Cucurbitaceae Familyası Külür Bitkilerinde Görülen Virusların Tanılanması

Marmara bölgesinde kabakgil kültür bitkilerinde görülen virusler test bitkilerinde oluşturdukları reaksiyonlar, bazı fiziksel özellikleri, serolojik testler ve elektron mikroskop gözlemleri yoluyla tanılanmışlardır. 1979 yılında kabakgil alanlarında 2 tur olarak düzenlenen surveylerde bölge virus hastalık oranı ortalaması hıyar, kabak, kavun ve karpuzlarda sırasıyla % 17.3, 24.2, 13.4 ve 0.83 olarak bulundu. 1980 yılında Ağustos ve Eylül aylarında Trakya'da kavun ve karpuz alanlarında yapılan surveylerde virus hastalık oranı ortalaması kavunda % 14.6, karpuzda 2.18 idi. 1979 ve 1980 yıllarında toplanan 269 adet virus benzeri belirti gösteren örneğin test bitkilerinde yapılan mekanik inokulasyonlar sonucu virusla bulaşık bulundu. Bunlardan 142'sinin (% 52.79) Hıyar mozayık virusu (CMV), 118'inin (% 43.86) Karpuz mozayık virusu-2 (WMV-2) ve 9'unun (% 3.34) CMV + WMV-2 ile enfekteli oldukları saptandı. Anadolu yakası ilerinde CMV, Trakya'da WMV-2 hakim durumdadır. **Nicotiana tabacum, C. quinoa, Citrullus vulgaris** ve **Hibiscus esculentus** test bitkileri kullanılarak karışık enfekteli tarla örneklerinde CMV ve WMV-2 ayrıldılar.

Test bitkilerinde belirtileri yönünden bazı farklılıkları nedeniyle araştırmalar 5 CMV ve 7 WMV-2 isolatı üzerinde yapıldı. CMV ve WMV-2 izolatlarının sıcaklıkla inaktifleşme ve son seyreltme noktalaI rı sırasıyla 65-80 °C ve 10^{-4} - 10^{-6}, 10^{-3} - 10^{-6} dır.

Serolojik testler CMV, WMV-1 ve WMV-2 antiserumları ile yapıldı. CMV izolatları CMV antiserumları ile, WMV-2 izolatları WMV-2 antiserumları ile reaksiyon verdiler. İzolatların EM gözlemleri Rothamsted Experimental Station'da sayın R.D. WOODS tarafından yapılmış ve resimleri çekilmiştir. Resimlerden CMV partiküllerinin küresel ve takriben 30 nm çapında, WMV-2 partiküllerinin çubuk şeklinde ve 800 nm uzunluğunda olduğu anlaşılmıştır.

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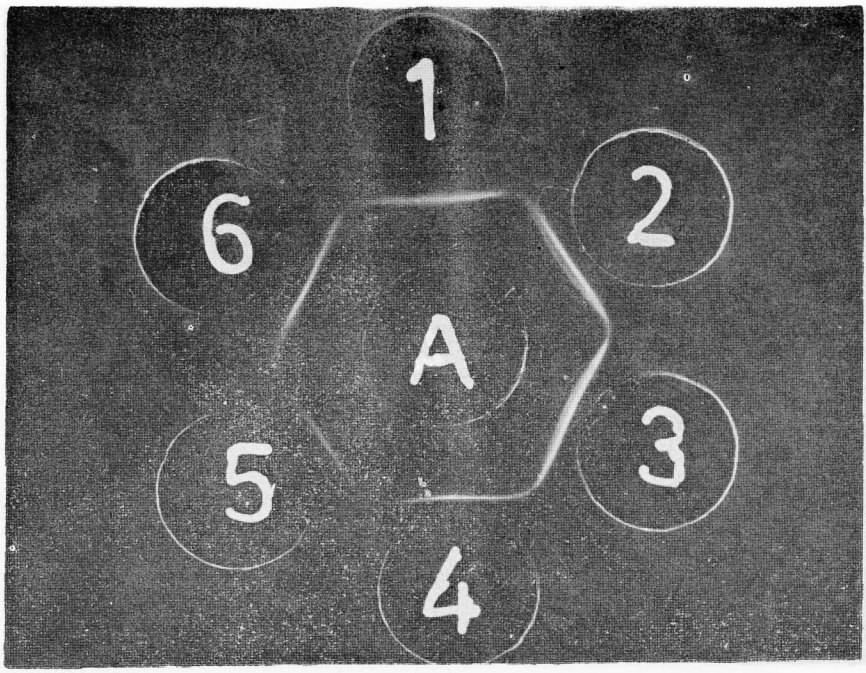

Fig.1. Serological reactions in gel diffusion tests. A=CMV TOML. antiserum (1/1). Peripheral wells contain purified CMV isolates; 1=CC_1 (1.05 mg/ml), 2=CS_4 (1.21 mg/ml), 3=CS_5 (1.11 mg/ml), 4=CM_3 (0.77 mg/ml), 5-6=CM_2 (0.25 mg/ml).

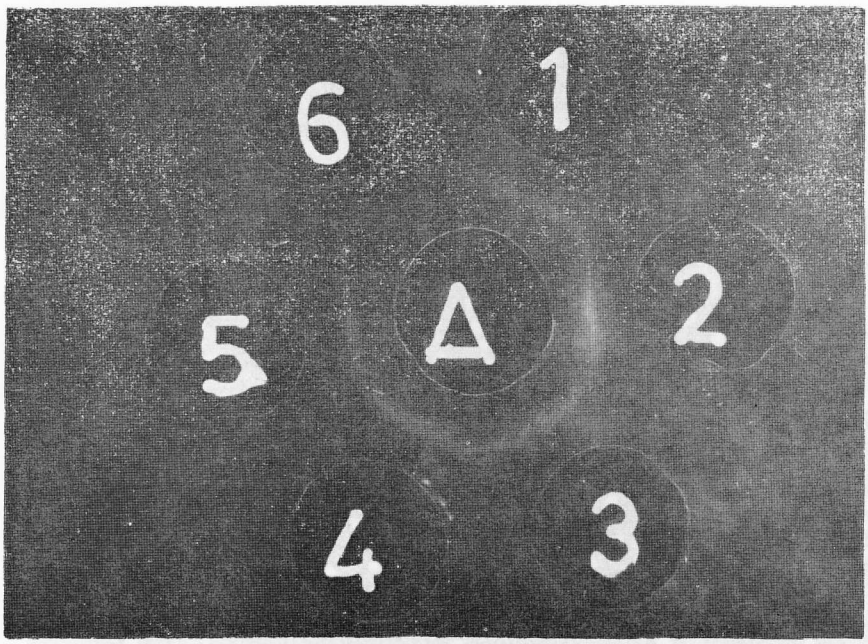

Fig.2. Serological reactions in agar gel diffusion tests A=CS_5 isolate of CMV (1.11 mg/ml). Peripheral wells contain. CMV-Govier antiserum (1=1/1, 2=1/2, 3=1/4, 4=1/8, 5=1/16, 6=1/32).

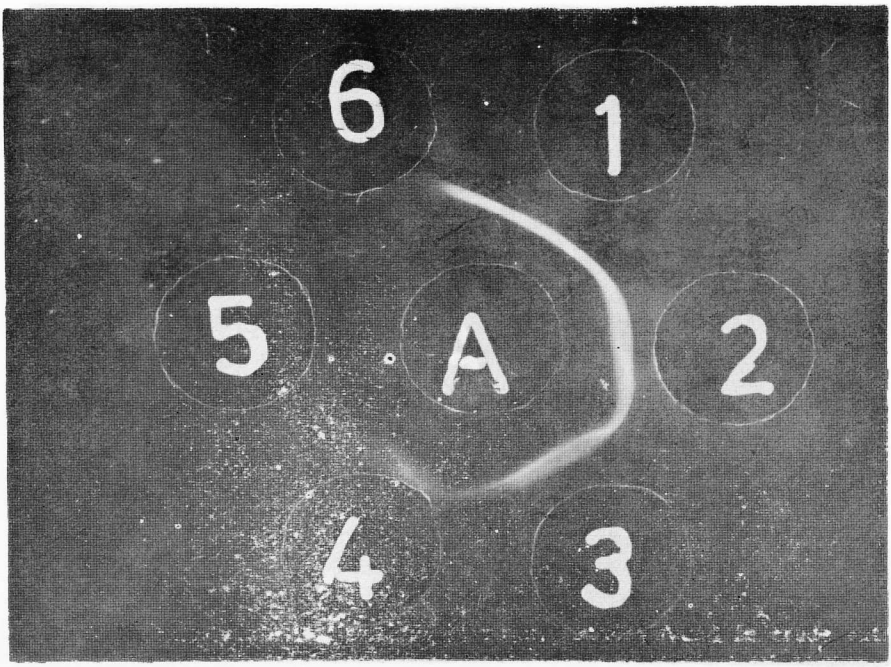

Fig.3. Agar-gel diffusion tests with SDS-treated isolates of WMV-2 and healthy sap. A=WMV-2 Scott antiserum. The outer wells contain SDS-treated antigens (1=WS_8, 2=WS_9, 3=WS_{10}, 4=WW_{11}, 5=WW_{12} multiplied in **C. pepo** «Sakız», 6=healthy sap of **C. pepo** «Sakız»)

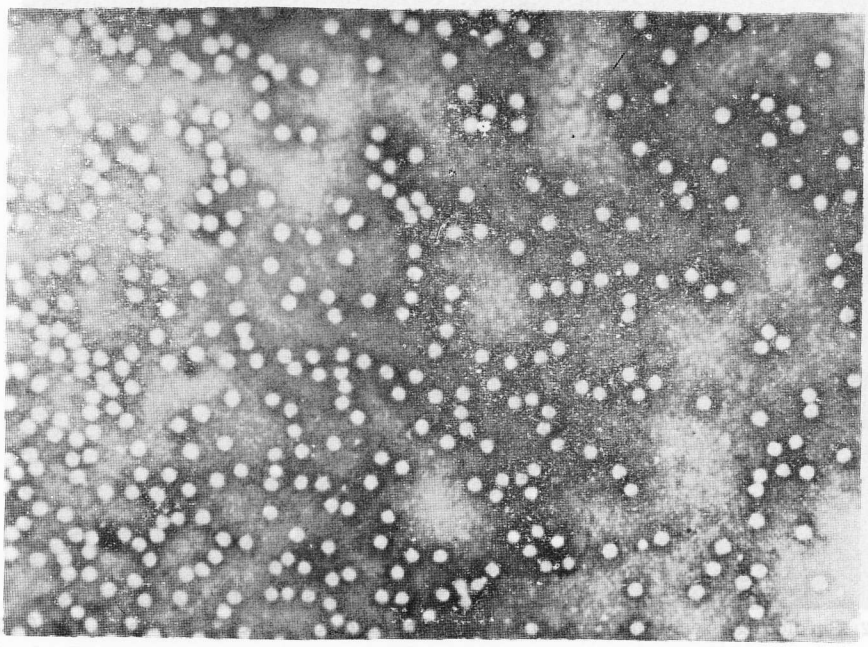

Fig.4. Electron micrograph of purified preparation of CMV isolate CC_1 (X 70.000)

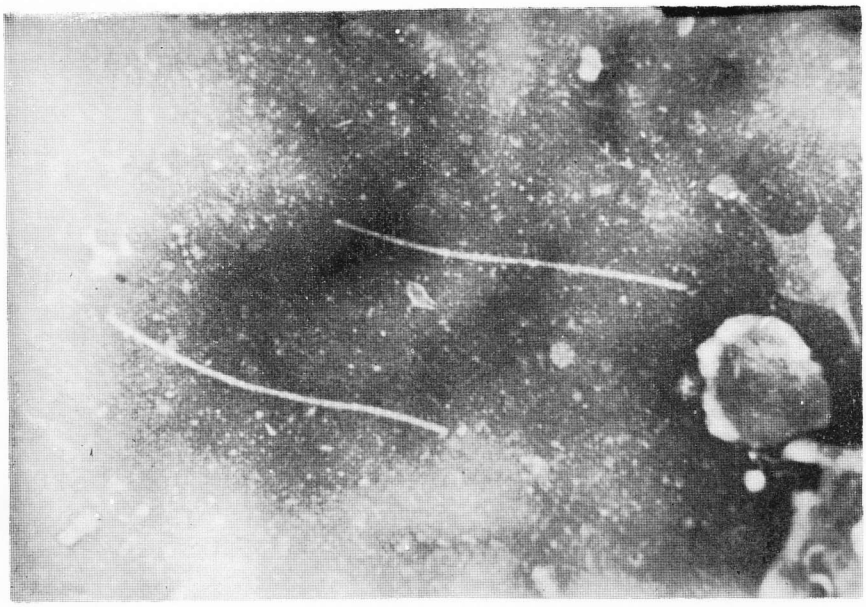

Fig.5. Negatively stained, rod-shaped virus particles found in crude extract from **C. pepo** «Sakız» infected with WM_6 isolate of WMV-2 (X 64.000)

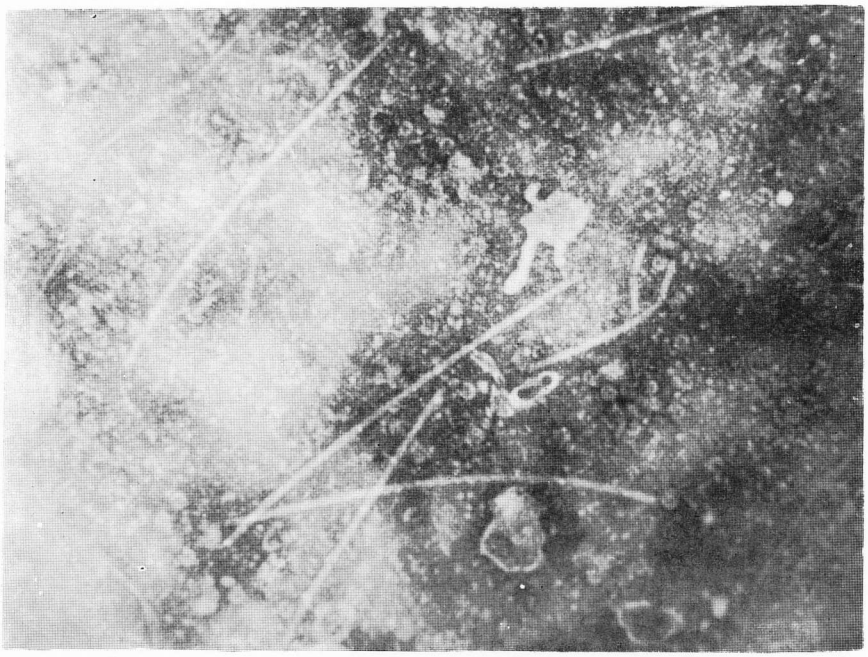

Fig.6. Electron micrograph of crude extract from **C. melo** «Hasanbey» mixed infected with CMV and WMV-2 (X 75.000)

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Purification and Particle Morphology of TMV, CMV and ZYMV Isolated from Various Cultivated Crops grown Along the Mediterranean Coast of Turkey[1]

Mehmet Asil YILMAZ[2] R.F. DAVIS[3]

ABSTRACT

CMV, ZYMV and TMV isolates were easily purified by Density Gradient Centrifugation. Ultraviolet absorbance spectrum of the purified virus preparation from the gradient layer has a typical nucleoprotein peak. The absorbance ratio of 260/280 nm was 1.74-1.79; 1.45 and 1.02-1.24 for CMV, ZYMV and TMV isolates, respectively. The purified virus yield of CMV, ZYMV and TMV isolates amounted to 0.05-0.14 mg, 0.014 mg, and 7.6 mg, per 1 g infected plant tissue, respectively after density gradient centrifugation. Electron microscopy of purified virus revealed-rigid rod, isometric and flexous rod particles typical of TMV, CMV and ZYMV particles respectively.

INTRODUCTION

The production of vegetable crops is limited due to virus diseases. During the survey along the Mediterranean sea coast it was observed that virus-like symptoms were common on the cultivated crops in each glasshouse. The infected plants show stunting, mild to severe mosaic, vein clearing and vein yellowing, blistering and deformation of leaves up and downward, leaf curling, and necrosis on the stem and the leaves. Biological assay, serological tests and leaf dip electron microscopy have shown that (Tobacco Mosaic Virus) TMV, (Cucumber Mosaic Virus) CMV and (Zucchini Yellow Mosaic Virus) ZYMV are the major viruses along the coast (8). The purpose of this study was to purify and determine particle morphology of these viruses.

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MATERIALS AND METHODS

Collection of Samples : Collection of samples at the different location along the coast and identification of viruses from the samples have been described (8).

Purification and Electron Microscopy : TMV isolates were purified from systemically infected **Nisoliona tabacum** «Samsun type» by the method of Gooding and Hebert (1966) with sliht modification (Table 1). In order to make comparision of density slight absorbance profile U.S., European and Turkish isolates of TMV were also used in this research. TMV-Alexandar + TMV-Aucuba provided by Dr. K. Corbett, University of Maryland; TMV-GPSa, TMV-SPS provided by Dr. A. Th. B. Rast, Research Institute for Plant Protection, Wageningen, The Netherlands; Tomato isolate NJ-81-1 and N-81-20 provided by Dr. R.F. Davis and Turkish tomato isolate AY isolate 1, and Turkish AY isolate 4. Isolated by Dr. M.A. Yılmaz.

CMV was purified from systemically infected **Cucurbita pepo** «Early Prolific» leaves 10-14 days after inoculation by the method of Lot et al. (1974) as modified by (2), (Table 2).

ZYMV was purified from systemically infected «Early Prolific» squash leaves 10-14 days after inoculation by the method of Sako et al. (1980) (Table 3).

Purified viruses were examined by leaf-dip electron microscopy (1).

RESULTS AND DISCUSSION

A. Purification of TMV Isolates. TMV isolates were purified easily and aggregation was reduced by using EDTA buffer as a chelating agent in the purification. Two isolates of TMV from Turkey, two from the Netherlands, and four from the U.S.A. were purified. Purification of the TMV-GPga isolate was unsuccessful and has not been repeated. Sucrose density gradient profiles of the other seven isolates are shown in Figure (1-7). Each isolate produced two major peaks, the first representing monomers of the virions, the second representing aggregated polymers. Notice that the Turkish isolates were not as severely aggregated after purification, as shown by the greater relative height of the monomer peak. This observation has not been confirmed by additional testing.

The absorbance ratio of 260/280 nm ranged from 1.02 to 1.24, whereas the accepted value for purified TMV is 1.19. The yield was determined using an extinction coefficient of $E^{0.1\ \%}_{260\ nm} = 3$, to be 7.6 mg virus/g infected tissue after density gradient centrifugation. Studies of purified virus particles under E.M. (Fig 10) showed typical TMV rod (Shikata, 1977).

Table 1. Purification of TMV Isolates.

Discard material	—	1. Homogenize the infected tobacco leaves («Samsun» type) in 0.5 M Na_2 HPO_4-KH_2 PO_4 buffer at pH 7.2+1 % 2-mercaptoethanol, 1 g of tissue/ml of buffer
		2. Squeeze through cheesecloth
		3. Add 8 ml n-butanol per 100 ml extract Stir 15 min
		4. 10000 g, 30 min
Sediment	—	5. Add 4 g PEG (mol wt 6000) per 100 ml and dissolve
		6. 10000 g, 15 min
Supernatant	—	7. Suspend pellets in 0.01 M EDTA phosphaite buffer pH 7.2, 20 ml/100 ml initial extract
		8. 10000 g, 15 min
Pellet	—	9. Add to the supernatant 0.4 g of NaCl and 0.4 g PEG per 10 ml. Add while stirring and dissolve
		10. 1000 g, 15 min
Supernatant	—	11. Dissolve the pellets in 2 ml 0.01 M EDTA buffer for each 100 ml of the initial extract and centrifuge at 1000 g for 5 min
Pellet	—	12. Save supernatant and layer the virus on 10-40 % sucrose gradient in 0.01 M EDTA pH 7.2 buffer solution, and centrifuge at 24.000 RPM for 2.5 hours in a Beckman SW 25.1 rotor
		13. Fractionate the light scattering band in sucrose gradient tubes
Supernatant	—	14. Centrifuge the fraction at 78.000 g for 60 min and suspend the pellet in 0.01 M EDTA pH 7.2 buffer

Table 2. Purification of cucumber mosaic virus[a].

DISCARD	RETAIN
	1) Homogenize tissue in 2 volumes each of 0.5 M citrate pH 6.5 with 0.1 % sodium thioglycollate and chloroform
	2) Low speed 10 min at 3000-4000 g
Chloroform, tissue	3) Remove supernatant, add PEG 6000 to final concentration of 10 % (w/v)
	4) Stir 30-45 min, low speed 10 min at 12.000-16.000 g
Supernatant	5) Resuspend pellet in 0.05 M citrate pH 7.0 with 0.2 % Triton X-100, stir 30-45 min
	6) High-speed 2 hr at 78.000 g or 1.5 hr at 105.000 g
Supernatant	7) Grind pellet in mortar and pestle with 0.02 M EDTA pH 7.0
	8) Low-speed 15 min at 12.000 g
	9) Remove supernatant (SI), low-speed pellet again with adtitional EDTA buffer
Pellet	10) Remove supernatant (S2), combine S1 and S2, high-speed 1.5 hr at 105.000 g
Supernatant	11) Resuspend pellet overnight in EDTA buffer
	12) Layer 0.5-1.0 ml virus solution onto fresh or thawed 10-40 % linear sucrose gradients prepared in EDTA buffer, high-speed 2 hr at 24.000 rpm in Beckman SW 27 rotor
	13) Collect band manually with syringe and bent needle, dilute solution in EDTA, high-speed 1.5 hr at 105.000 g
Supernatant	14) Resuspend pellet in EDTA buffer

[a]All reagents and glassware are kept at 0.4 C

Table 3. Purification of ZYMV

DISCARD	RETAIN
	1) Homogenize the leaves with 0.3 M potassium phosphate buffer pH 8.8 (1:2) containing 0.01 M DIECA, 0.1 %.
	2) Mercaptoethanol with 15 % carbon tetrachloride.
Sediment	3) Low speed 15 min. 8.500 g.
	4) Add 2 % triton X-100 and stir for 20 min.
	5) Layer the mixture over an 8 ml pad of 20 % sucrose in 0.02 M potassium phosphate buffer pH 8.5 and centrifuge 29.000 rpm for 90 min.
Supernatant	6) Resuspend pellet with 0.02 M potassium phosphate buffer pH 8.5
Pellet	7) Low speed 15 min. 8.500 g
	8) Repeat steps 5-7, omitting sucrose pad ni step 5.
Pellet	9) Layer 0.5-1.0 M 1 virus onto fresh or thawed 10-40 % sucrose linear gradient prepared in phosphate buffer high speed 4 hour at 24.000 rpm
	10) Fractionate the light scattering band in sucrose gradient tubes.
Supernatant	11) Centrfifuge the fractionate at 24.000 rpm for 1 hour and resuspend the pellet with 0.02 M phosphate buffer pH 8.5

B. Purification of ZYMV. Squash isolate of ZYMV was also purified by density gradient centrifugation. One peak of absorbance profile in 10-40 % sucrose gradient was observed after 4 hours centrifugation at 24.000 rpm (Figure 8). Flexuous virus particles (672 nm) in length were observed in electron micrograph (Figure 11). The purified virus yield was 14 mg/kg of squash leaves. The 260/280 ratio was 1.45. Virus particles are typical of flexucus rod particles of ZYMV (4).

C. CMV Purification. The watermelon isolate of CMV was purified by density gradient centrifugation. There was only one peak of absorbance profile in the 10-40 % sucrose density gradients after 2.5 hours centrifugation at 24000 rpm (Figure 9). Ultraviolet absorbance spectrum of the purified virus preparation from the gradient layer had a typical nucleoprotein peak at 260 nm The A260/A280 nm ratio was 1.74-1.79, very close to the typical

value of $E^{0.1\ \%}_{260\ nm} = 5$, to be .05 to .14 virus/g infected tissue after density gradient centrifugation. Examination of leaf dip preparation of purified virus showed purified virus particles are typical of CMV virus particles (7).

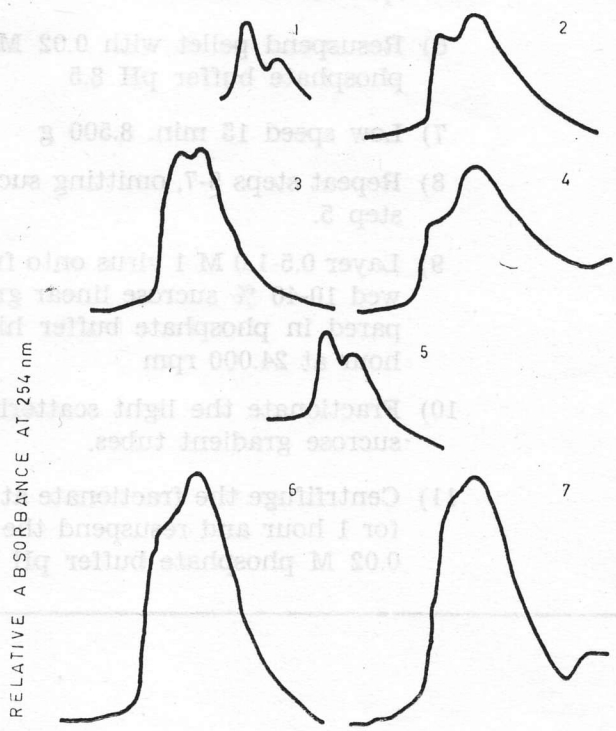

Figure 1-7. Absorbance (254 nm) of tomato isolates of TMV in the 10-40% sucrose density gradient at 24.000 rpm for 2.5 hours.
1. Turkish AY isolate 1:2. TMV Alexandar: 3. TMV NJ 81-1:4 TMV Aucuba: 5. Turkish AY isolate 4:6 TMV SPS:7. NJ 81-20.

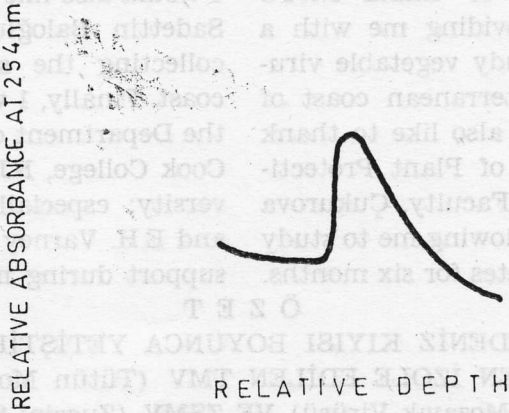

Figure 8. Absorbance of squash isolate of ZYMV in 10-40 % sucrose density gradient at 24.000 rpm in a Beckman SW 25.1 rotor for 4 hours.

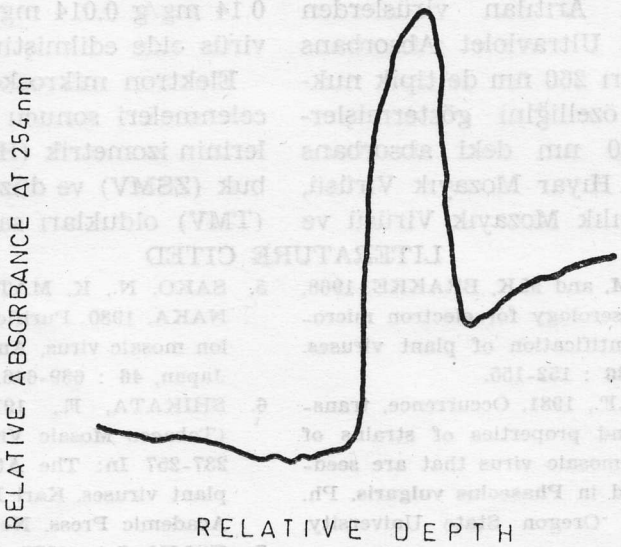

Figure 9. Absorbance of watermelon isolates of CMV in 10-40 % sucrose densty gradient at 24.000 rpm in a Beckman SW 25.- rotor for 2.5 hours.

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I would like to thank NATO (CCMS) for providing me with a followship to study vegetable viruses in the Mediterranean coast of Turkey. 1 would also like to thank the Department of Plant Protection, Agricultural Faculty, Çukurova University, for allowing me to study in the United States for six months. 1 would also like to thank Assistant Sadettin Baloğlu for his help in collecting the samples along the coast. Finally, 1 would like to thank the Department of Plant Pathology, Cook College, NJAES, Rutgers University; especially Drs. R.F. Davis and E.H. Varney for their help and support during my visit.

Ö Z E T

TÜRKİYE AKDENİZ KIYISI BOYUNCA YETİŞTİRİLEN ÇEŞİTLİ ÜRÜNLERDEN İZOLE EDİLEN TMV (Tütün Mozayık Virüsü), HMV (Hıyar Mozayık Virüsü) VE ZSMV (Zuccini Sarılık Mozayık Virüsü) ARITILMASI VE PARTİKÜL MORFOLOJİSİ

Density Gradient Santrifuj yöntemiyle HMV (Hıyar Mozayık Virüsü), ZSMV (Zuccini Sarılık Mozayık Virüsü) ve TMV (Tütün Mozayık Virüsü) izolatları kolaylıkla arıtılmışlardır. Arıtılan virüslerden elde edilen Ultraviolet Absorbans Spektrumları 260 nm de tipik nukleoprotein özelliğini göstermişlerdir. 260/280 nm deki absorbans oranlarının Hıyar Mozayık Virüsü, Zuccini Sarılık Mozayık Virüsü ve Tütün Mozayık Virüsleri için sırasıyla 174-179, 145 ve 1.02-1.24 olduğu saptanmıştır. Arıtma sonucu HMV, ZSMV ve TMV ile bulaşık bitki dokularından sırasıyla 0.05-0.14 mg/g 0.014 mg/g ve 7.6 mg/g virüs elde edilmiştir.

Elektron mikroskobu altında incelenmeleri sonucu virus partiküllerinin izometrik (HMV), ipliksi çubuk (ZSMV) ve düz çubuk şeklinde (TMV) oldukları saptanmıştır.

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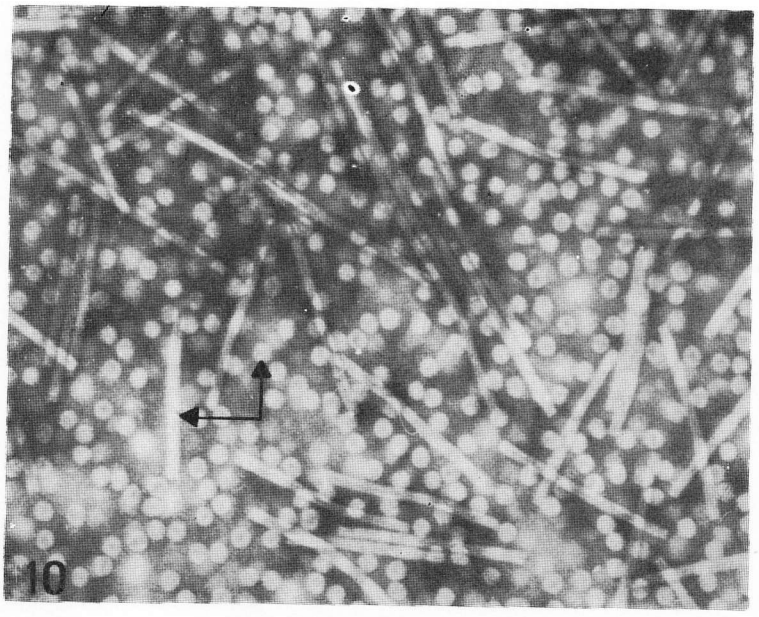

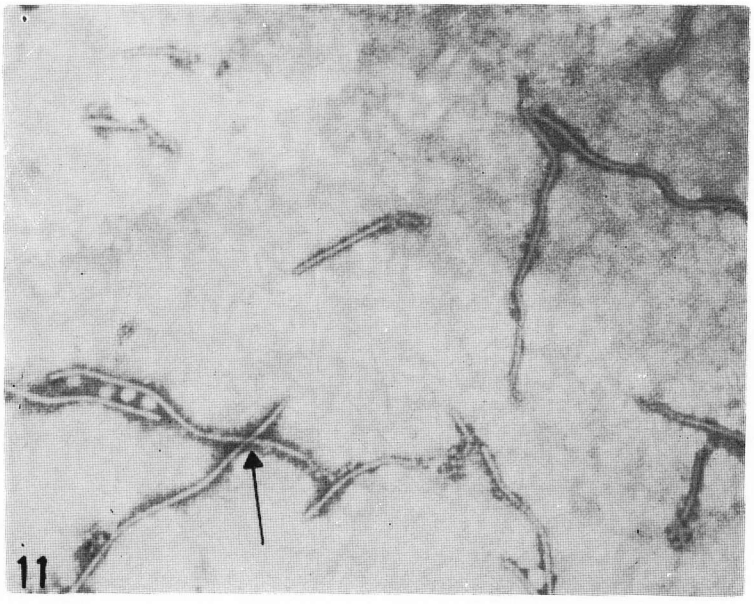

Figures 10-11. Types of virus particles (arrows) found in negatively stained leaf dip preparation from **Nicotiona tabacum** «Samsun type» and **Cucurbito pepo** «Early prolific». Fig 10. Purified TMV and CMV (× 100.650). Fig 11. Purified ZYMV (× 68.200).

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Investigations for Determination of The Hosts of The Causal Agent of Rice Blast Disease (**Pyricularia oryzae** Bri. et Cav.) in The Southern Anatolian Region

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ABSTRACT

The causal agent of rice blast disease (**P. oryzae**) was determined on **Echinochloa crus-galli, E. colonum, Cyperus longus,** and **Setaria viridis** plants in the Southern Anatolian Region.

Conidia dimensions measured in the preparates made from the leaf spots on rice, **E. colonum** and **S. viridis** plants were the same. But conidi dimensions determined for **C. longus** by using same method were different from the others.

INTRODUCTION

In the studies on rice diseases made previously in the Southern Anatolian Region, **Pyricularia oryzae** Bri. et Cav., the causal agent of rice blast disease, had never been determined on any host besides rice.

ORAN (1975), could not determine this fungus on gramineous plants which are known as hosts of **P. oryzae,** in the studies which he made in the Southern Anatolian Region. But he reported that he determined **P. oryzae** fungus on **Echinochloa crus-galli** (L.) Pal. Beauv., **Scirpus mucronatus** All., **Cyperus fuscus** L. and **Phragmites communis** Thrin. in some places of the regions of Central and Northern Anatolia. In addition, the author reported that the isolates obtained from these plants could infect the Karacadağ rice variety.

DICKSON (1974) reported that **P. oryzae** was observed on **Digitaria sanguinalis** (L.) Scop.

MC RAE (1922) reported that **P. oryzae** was observed on **Eleusine coracana** Grtn., **Panicum repens** L., **P. ramosum, Setaria italica** (L.) P.B., **Paspalum sanguinale** and **Triticum vulgare.**

NISHIKADO (1927) reported that **P. oryzae** specialized to gramineous plants and was recognized as special species for gramineous plants.

FULTON (1908), reported that the **P. oryzae** culture which he obtained from **Digitaria sanguinalis,**

and the **Pyricularia grisea** culture were the same, and **P. grisea** could infect the rice.

ROGER (1950), according to Ramakrishnan, reported that although **Pyricularia** isolates obtained from other gramineae could infect the rice, those obtained from the rice could not infect other gramineae. But the same author, according to Nishikado, reported that although the **Pyricularia** of rice could infect other gramineae, that of other gramineae could not infect rice.

There are many wheat grass species and climatic conditions are proper to **P. oryzae** to grow all seasons in our region. Therefore it was possible that there were **P. oryzae** hosts besides the rice, and **P. oryzae** could survive on these hosts during winer. This study was conducted to clarify these subjects.

MATERIALS AND METHODS

Wild gramineae on which leaf spots were found during survey studies made in rice, corn and vegetable fields were collected. The preparates made from the leaf spots on these plants directly and after incubating in moist chambers at room temperature for 72 hours were examined. In addition, the preparates made from fresh spots on the leaves of **Oryzae sativa** L., **Echinochloa** (L.) Link., **Echinochloa crus-galli** (L.) Pal. Beauv., **Cyperus longus** L. and **Setaria viridis** (L.) P.B. plants were examined. Conidia dimensions were determined during these examinations by measuring the dimensions of 100 conidia for each plant.

RESULTS

P. oryzae fungus was determined on spotted **E. colonum** (Fig 1), and **E. crus-galli** (Fig 2) plants taken from rice fields in Central, Karataş, Kozan and Kadirli counties of Adana, Tarsus district of İçel, and Andırın district of Kahraman Maraş. **P. oryzae** fungus was also determined on spotted **E. colonum**, **C. longus** (Fig 3) and **S. viridis** (Fig 4) plants taken from vegetable fields in Central district of Adana, and Tarsus district of İçel. In addition, **P. oryzae** was determined on spotted **E. colonum** plants taken from a Corn field in Karataş district. Conidia dimensions could not be measured because **P. oryzae** leaf spots were rare on **E. crus-galli** plants.

Conidia dimensions as measured on fresh leaf spots of rice (Fig 5) **E. colonum**, **S. viridis** and **C. longus** plants are given in Table 1.

Table 1. Conidia dimensions of **P. oryzae** determined in measures made on various host plants.

Host Plant	Conidia dimensions (micron)	Mean (micron)
Rice (Gritna cv.)	17.1-27.9 × 8.1-10.8	22.10 × 9.16
E. colonum	17.1-27.9 × 8.1-10.8	22.11 × 8.96
S. viridis	18.0-30.6 × 8.1-10.8	22.48 × 9.70
C. longus	23.4-31.5 × 5.4- 9.9	29.72 × 6.56

DISCUSSION

P. oryzae fungus on **E. crus-galli, E. colonum, S. viridis** and **C. longus** plants was firstly determined in Southern Anatolian Region. Determined in Southern Anatolian Region. Determination of **P. oryzae** fungus on **E. colonum, S. viridis** and **C. longus** plants is first for Turkey.

It was determined that conidia dimensions measured from rice and **E. colonum** were the same, and means of conidia dimensione were very close. Conidi dimensions measured from **S. viridis** were slightly different from those measured from rice and **E. colonum,** but the means of dimensions were very close. Conidia dimensions measured from **C. longus** plants were greatly different from those measured from other plants.

The conidia dimensions and means determined in this study for rice, **E. colonum** and **S. viridis** were very close to the dimensions (17-28 × 8.5-12 microns and mean (22.5 × 10.2 microns reported by SAWADA (1917). The conidia dimensions determined in our study were also very close to the dimensions (16-32 × 7-11 microns) reported for rice by ASUYAMA (1965). In addition, the dimensions and mean obtained in our study were the same of those (18-28.8 × 8.0-11.3 microns) reported by ASUYAMA (1965). according to YAMANAKA. As a result, it was concluded that the **P. oryzae** fungus determined on **E. colonum** and **S. viridis** and that determined on Gritna rice varisty were the identical. It was also concluded that the fungus determined on rice, and the conidia dimensions like these were not found in literature.

The fact that **P. oryzae** was observed on **E. colonum** and **S. viridis** taken from a corn field in Karataş and from a vegetable field in Tarsus, and from the institute experimental field in Adana, showed that **P. oryzae** could survive on these plants when rice fields were absent in the region.

Ö Z E T

GÜNEY ANADOLU BÖLGESİNDE ÇELTİK YANIKLIĞI HASTALIĞI ETMENİ (Pyricularia oryzae Bri. et Cav.)'NİN KONUKÇULARININ SAPTANMASI ÜZERİNDE ARAŞTIRMALAR

Güney Anadolu Bölgesinde **Echinochloa crus-galli, E. colonum, Cyperus longus** ve **Setaria viridis** bitkileri üzerinde Çeltik Yanıklığı hastalığı etmeni **Pyricularia oryzae** saptanmıştır.

Çeltik, **E. colonum** ve **S. viridis** bitkileri üzerindeki yaprak lekelerinden hazırlanan preparatlarda ölçülen konidi boyutları aynı bulunmuştur. Ancak, aynı metod ile **C. longus** için belirlenen konidi boyutları diğerlerinden farklı bulunmuştur.

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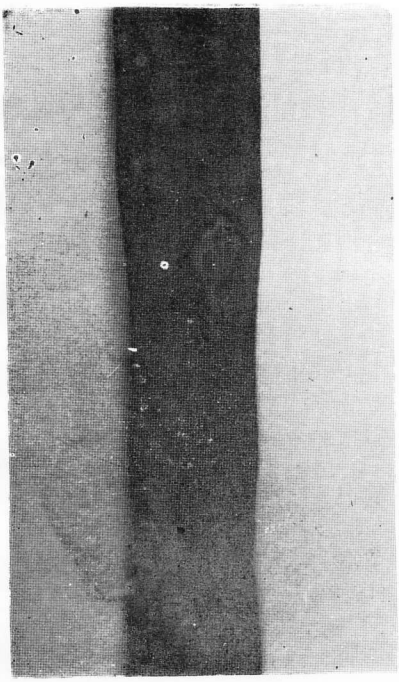

Fig 1. **P. oryzae** spots on **E. colonum** leaf.

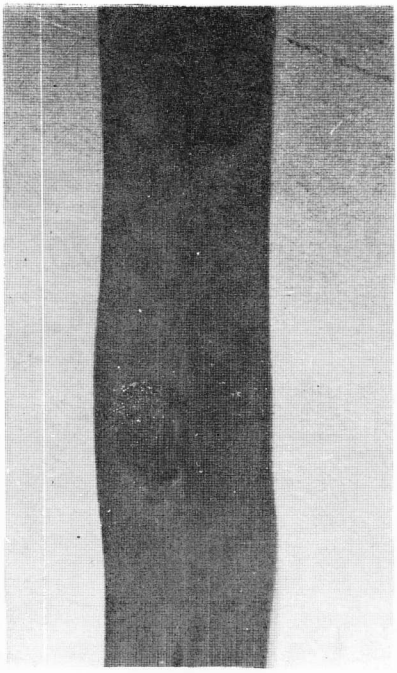

Fig 2. **P. oryzae** spots on **E. crus-galli** leaf.

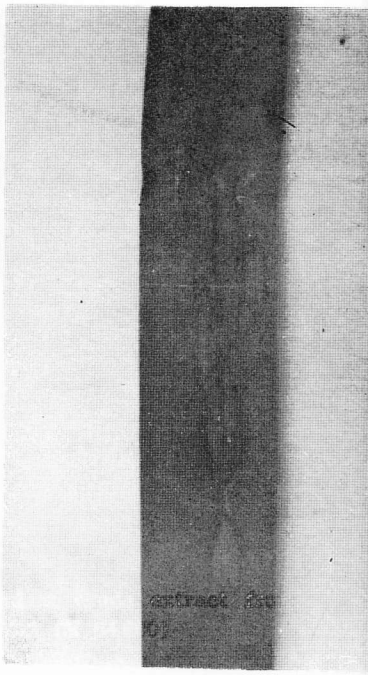

Fig 3. **P. oryzae** spots on **C. longus** leaf.

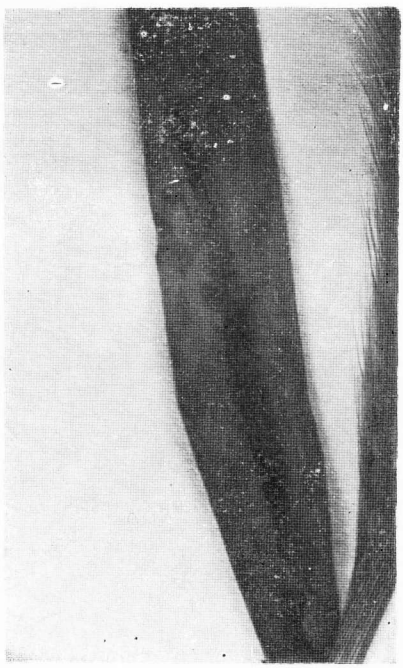

Fig 4. **P. oryzae** spots on **S. viridis** leaf.

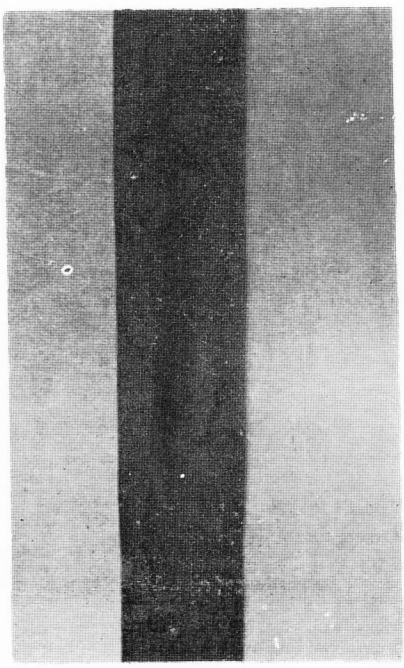

Fig 5. **P. oryzae** spots on the leaf of rice.

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NEW RECORDS :

Meloidogyne spp. infestation in the leaves of **Chlorophytum comosum** in Turkey

by

Seval TOROS[1] J.J. S'JACOB[2] Sabahat ENNELİ[3]

Meloidogyne infestations often result in knotted, swollen and distorted root systems. Hence the common name «root-knot nematodes», although normally found in the roots of the host, they have also been found in stems and leaves. Steiner (1940) first drew attention to this by reporting severe injury to the cotyledons, stems and leaves of bean plants which had germinated in a soil heavily infested with **Meloidogyne** larvae. Linford (1941) and Powell and Moore (1961) succeeded in infecting cowpea, bean, tobacco and tomato plants with root-knot larvae. Golden (1953) found the crowns, petioles and leaves as well as the roots of the African violet attacked by the **Meloidogyne arenaria** group. Numerous galls of **Meloidogyne incognita incognita** were found in the leaves of **Siderasis fuscata** (Miller and DiEdvardo, 1962).

The first author noticed unidentified galls on the leaves of the ornamental plant, **Chlorophytum comosum**. Examination of the galls under a dissecting microscope showed the presence of **Meloidogyne** females and larval stages in leaf galls. The perineal patterns were mounted in dehydrated glycerol after having been stained in lactophenol cotton blue 0.03 %. (s.Jacob and van Bezooijen, 1983) and sent for identification to J.J. s'Jacob (Nematology Department, Agricultural University, Wageningen, the Netherlands).

Almost all the specimens were identified as **Meloidogyne incognita** except one which was probably **M. arenaria**. Since the morphological differences between the two are small and there is considerable variation within each species they will be referred to as **Meloidogyne** spp.

Galls were found on both the midribs and veins. On the upper side of the leaf they were circular in outline (Fig. 1A) with a conspicu-

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ous enation when viewed laterally (Fig. 1B). On the lower side they appeared as circular depressions. Older galls were usually necrotic with a reddish brown tip. After hatching in the leaf galls the larvae have the ability to migrate short distances in the leaf to form new galls. This is shown in Fig. 1A, where relatively small galls were found near larger older ones.

According to Thorne (1961) foliar and stem inoculation with root-knot nematodes could occur with exceedingly heavy infestation and high humidity.

The roots of the affected **C. comosum** plants were heavily infested with root-knot nematodes. Apart from root infection, it has been concluded that adverse conditions in the roots caused by desiccation and irradiation (Linford, 1941) as well as unfavourable temperatures (Wong and Willetts, 1969) result in aerial infections. It is known that root-knot nematodes have a wide host range this is the first report of infestation of aerial plant parts by **Meloidogyne** spp. in Turkey.

Ö Z E T

TÜRKİYE'DE Meloidogyne TÜRLERİNİN Chlorophytum comosum'DA MEYDANA GETİRDİĞİ YAPRAK ENFEKSİYONU

Meloidogyne türleri, köklerde meydana getirdikleri urlarla bitkilerde zararlı olmakta ve bu nedenle de «kök-ur nematodları» olarak isimlendirilmektedir. Ancak, belirli koşullarda —nadir de olsa— yapraklarda da galler oluşturabildikleri bilinmektedir. Nitekim, süs bitkilerinin zararlılarının saptanması ile ilgili çalışmalar sırasında **Chlorophytum comosum**'un yaprakları üzerinde rastlanılan gallerin, yapılan mikroskobik inceleme ve etmenin Hollanda'da konunun uzmanı yardımıyla tanısı sonucu **Meloidogyne incognita** ve **M. arenaria** tarafından meydana getirildiği saptanmıştır. Etmenlerden **M. incognita** çoğunlukta olmasına rağmen yazarlar, her iki türü ayrı ayrı belirlemek yerine **Meloidogyne** spp. ifadesini uygun bulmuşlardır.

Galler, yaprakların ana ve yan damarları üzerinde yuvarlak görünümde olup lateral yönde enasyonlar şeklinde belirmektedir. Yaşlı galler kırmızımsı kahverengindedir.

Bu bulgu, Türkiye koşullarında, **Meloidogyne** türlerinin, bitkinin toprak üstü organlarında meydana getirdiği zararla ilgili ilk bulgudur.

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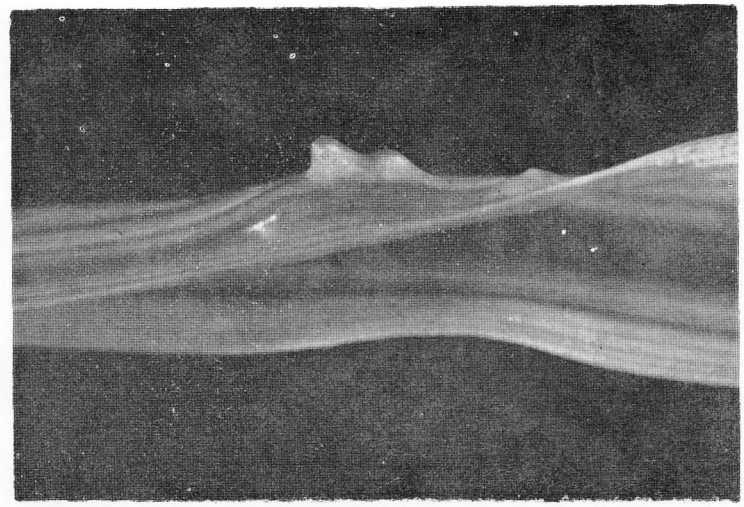

Fig.2. **Meloidogyne spp. galls on** leaf as conspicuous enation at lateral view
B— Necrotic older galls

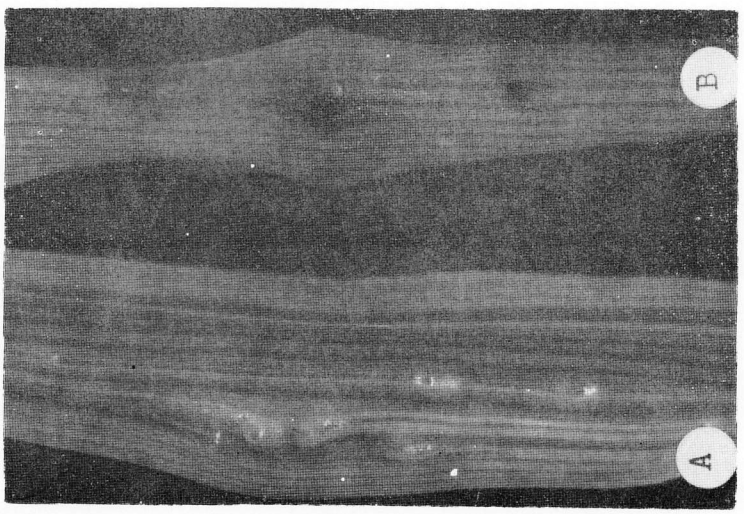

Fig.1. Root-knot nematode galls on the leaf of **C. comosum**
A— Galls as circular at top view

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BOOKS :

J. PALTI, **Cultural Practices and Infectious Crop Diseases.** 1981. Springer-Verlag, 43 figures, 51 Tables, XVI, 243 Pages. ISBN 3-540-11047-X Price : DM 98.

The book includes three main parts :

1) Climate, Cropping and Crop Disease

2) Major cultural practices and their effects on crop disease

3) Interaction between cultural practices, resistance breeding and application of chemicals = Integrated Control.

Inthe first part, after a general in formation is given on agro-ecosystems, microclimate, crop climate, the effects of soil and micro-biota on soil-borne diseases and soil resistance are outlined and examples are given. Strains of pathogens which are formed by different degrees of temperature and water (stress) and the reflections of these stresses on crop (Predisposition) are surveyed with special referrence to **Macrophomina phaseolina**, which is considered as a prototype.

After the sub-division of 1.6 where the effect of physiological and structural properties of different stages of crop development on diseases, takes place, the importance of weeds in the view point of diseases and the effects of cultural practices on weeds are discussed.

In the second main part, the writer is explaining the effects of major cultural practices on crop diseases comprehensively and in details. According to the order in the book these cultural practices are as follows :

1) Sanitation,

2) Crop sequence,

3) Soil amendments and mulches,

4) Tillage,

5) Crop nutrition,

6) Moisture management in non-irrigated crop,

7) İrrigation,

8) Rate of sowing and planting and density of stand,

9) Sowing and planting dates and manuplation,

10) Harvesting dates and practices,

11) Planning to minimize influx of air-or vector-borne inoculum to neighbouring crops,

12) Prunning and grafting,

13) Effect of physical barriers on crop infection and of optical means on virus vector control.

In the third part, the cultural practices are subjected to evaluation as one of the components of integrated control.

There is a referrence list of approximately 600 publications at the end ot the book. The pages of 231-243 include the indexes of pathogens and subjects.

Dr. PALTI, is one of the masters of phytopathology. Reading this book, one gets feeling of a very rich accumulation of the knowledges.

The book includes all the data about cultural practices with special referrence to integrated disease control, which is the subject, takes place in the first order of the agenda in the last years.

The valuable synthesis of Dr. PALTI leads us to gain new ideas in this field.

Dr. T. BORA

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