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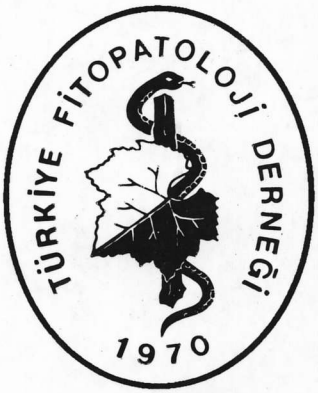

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Symptomatology, Etiology and Transmission of Sesame Phyllody in Turkey

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ABSTRACT

The occurrence, distribution, etiology, and leafhopper vectors of sesame phyllody were studied in Turkey in 1990 and 1992. This disease was common on all sesame fields along the South Mediterranean coast and in Southeast Turkey. The main symptoms observed in the field consisted of phyllody, virescence and witches' broom. In some sesame plants, capsule gigantism occurred. Mycoplasma-like bodies were observed in the sieve elements of infected sesame plants, while sieve cells of healthy plants revealed no mycoplasma-like bodies. Among various leafhoppers encountered on sesame, only ***Circulifer haematoceps*** *(M. &R.) transmitted the disease from sesame to periwinkle (****Catharanthus roseus*** *L.). The rate of transmission to periwinkle using field collected leafhoppers was about 22%. According to vector specificity, the sesame phyllody MLO in Turkey appeared different from that reported from India and Burkina Faso, but similar to that found in Iran.*

INTRODUCTION

In a survey on pests and diseases of sesame (**Sesamum indicum** L.) in the South Mediterranean region and in Southeast of Turkey many sesame plants were observed, showing virescence, phyllody and witches' broom. Similar symptoms have been described on sesame in India, Iran, Israel, Thailand and Burkina Faso and found associated with MLOs (Vasudiva and Sahambi, 1959; Cousin *et al.*, 1970; Choopanya, 1973; Klein, 1977; Salehi and Izadpanah, 1992).

Phyllody is a serious and destructive disease of sesame, since infected plants set generally no fertile flowers and capsules. Although the appearance of sesame phyllody in Turkey is known since several years, the etiology and the insect vectors of this disease are still unknown. The objective of the presented paper is to report on the sym-

ptomatology, the distribution, the etiology and the leafhopper vectors of sesame phyllody in Turkey.

MATERIALS AND METHODS

Fields surveys on the distribution, symptoms and leafhopper vectors of sesame phyllody were conducted in July and August 1990 as well as from June to October in 1992. The area studied, consisted of the provinces Antalya, Silifke, Adana and Hatay along the Mediterranean coast and Gaziantep and Urfa in Southeast region of Turkey.

The etiology of sesame phyllody in Turkey was determined by collecting leaves from sesame plants with exhibited symptoms. Such leaves were cut in 1 x 1 mm sections and immediately fixed in Karnowsky buffer (Karnowsky, 1965). The fixation of plant material was done in field and such material was brought to the laboratory in an ice-chest and stored at 4°C. The samples were later embedded for electron microscopy according to Spurr (1969) in the Institute of Phytopathology in Bonn, FRG.

To find vectors of the sesame phyllody MLO in Turkey, leafhoppers were collected using a mechanical insect collector (D-VAC) at various sesame growing locations, covering the entire survey-area. Field identification of the leafhoppers was confirmed by microscopic examination of dissected male genitalia in the laboratory. The leafhoppers were separated into species and batches between 10 and 20 individuals of the most important species were caged on young, healthy **Catharanthus roseus** L. held at 32±1°C, 75±5% RH and 16 hrs of artificial light in a climate chamber. After an inoculation period of 72 hrs the plants were sprayed by Supracide and kept in a greenhouse at 30±2 °C for at least 12 weeks and observed for MLO symptoms. Phloem tissue of some leafhopper inoculated **C. roseus** were examined under electron microscope for the presence of MLOs as described above.

RESULTS AND DISCUSSION

Sesame phyllody infected plants were commonly found along the South Mediterranean coast from Antalya in the West to Samandağ in the East part in 1990 and 1992. In addition, this disease was also noticed in the Southeast, namely in the provinces Gaziantep and Urfa in 1992. The appearance of sesame phyllody infected plants was previously reported from the Aegean coast (Türkoğlu and Fidan, 1985). According to the field observations, the infestation rate was higher in Antalya and Silifke than in Adana, Hatay, Urfa and Gaziantep, especially in 1990, when only a few sesame phyllody infected plants were observed in the Eastern Mediterranean.

Field symptoms consisted of phyllody, virescence and withches' broom, the latter as a result of flower proliferation. Occasionally, sesame plants showing capsule gigant-

ism were observed. Such capsules contained hundreds of small shoots. On the base of symptomatology, sesame phyllody in Turkey seems not to be different from that found in India, Israel and Burkina Faso (Vasudiva and Sahambi, 1959; Cousin **et al.** 1970; Choopanya, 1973; Klein, 1977). However, in Iran this disease appeared different from that described elsewhere, since sesame phyllody was usually accompanied by foliar yellowing, cracking of seed capsules, germination of seeds in the capsule and formation of dark exudates on the foliage (Salehi and Izadpanah, 1992). The authors reported that some of these symptoms might be due to a mix-infection of sesame by the phyllody agent and sugar beet curly top virus. On the other hand, foliar yellowing of sesame is often caused by a concurrent infection with the sesame phyllody agent plus **Spiroplasma citri** Saglio **et al.** (Entomoplasmatales: Spiroplasmataceae) as frequently noticed in the Çukurova in 1991 (Başpınar **et al.,** 1993).

Mycoplasma-like bodies were observed in the ultrathin sections of the sieve elements of phyllody infected sesame plants under electron microscope, but not in any of the apparently healthy ones. MLO cells were generally rounded, but sometimes elongated, especially when concentrated at the sieve plate.

Over all, fifteen different leafhoppers species were determined on sesame, the most abundant being **Circulifer haematoceps** (M. & R.), followed by **Orosius orientalis** (Matsumura) as well as **Asymmetrasca decedens** (Paoli) and **Empoasca decipiens** Paoli, treated as likes (Kersting **et al.,** 1992; Başpınar **et al.,** 1993). Only **C. haematoceps** transmitted the sesame phyllody agent from sesame to **C. roseus** (Table 1). This was true for leafhoppers collected in all provinces in 1990 and 1992. Like in Turkey, **C. haematoceps** was proved to be the vector of the sesame phyllody MLO in Iran, transmitting the pathogen from sesame to sesame and to various other herbaceous hosts (Salehi and Izadpanah, 1992).

The MLO infected **C. roseus** developed virescence sympotms within 14 days. Later the petals altered to normal leaf structures (phyllody) and after several weeks chlorosis and slight stunting were observed. The electron microscope studies revealed MLOs in the phloem of the infected **C. roseus** plants, but not in any of the symptomless plant (Fig. 1). The rate of transmission of field collected leafhoppers were 22.2% in 1990 and 22.5% in 1992 (Table 1). These data are in coincidence with the findings of Salehi and Izadpanah (1992), who mentioned transmission rates of 21.4% when field collected **C. haematoceps** were used, but transmission rates as high as 83% when sesame plants were inoculated by individuals that had been reared on infected sesame.

C. haematoceps was recently found to be the most important vector of the citrus stubborn disease pathogen (CSD), the mollicute **S. citri,** in the Çukurova region, causing heavy losses in yield and fruit quality in citrus each year (Kersting and Şengonca, 1992, Kersting **et al.,** 1992). Moreover, sesame was found as an excellent natural host both, for the leafhopper **C. opacipennis** and the pathogen **S. citri** (Kersting **et al.,** 1992).

Table 1: Rate of transmission of the sesame phyllody MLO to **Catharanthus roseus** by leafhoppers collected from sesame fields in South and Southeast Turkey in 1990 and 1992.

Leafhopper species	No. of infected plants/ No. of exposed plants 1990		No. of infected plants/ No. of exposed plants 1992	
Circulifer haematoceps	2/9	22.2 %	11/49	22.5 %
Asymmetrasca decedens+ Empoasca decipiens	0/6	0.0 %	-	-
Orosius orientalis	0/8	0.0 %	0/18	0.0 %

It is reported that **C. opacipennis** and **S. citri** constitu a well-adapted vector-pathogen system (Şengonca **et al.,** 1991). The appearance of concurrent infected sesame (**S. citri** plus sesame phyllody MLO), both transmitted by the same vector **C. haematoceps,** does not only cause considerable confusion in diagnosis and in studies of etiology, but in the epidemiology of both pathogens. Herbaceous plants infected with **S. citri** die within twelve weeks when kept at 32 °C. It is known, however, that herbaceous plants inoculated with **S. citri** and MLO will survive and retain **S. citri** for more than two years (Oldfield, 1982; Saillard **et al.,** 1984). This influences the field ecology of both pathogens, by limiting the titer of **S. citri** in plants, by limiting the pathogenic effect to plants and by greatly extending the period during which vectors may acquire **S. citri** from herbaceous plants.

Furthermore it is assumed that the titer of **S. citri** in leafhopper vectors will be limited by coinfection with other MLOs (Calavan and Bové, 1989). It seems that the MLO multiplication somehow interferes with the multiplication of **S. citri.** Hsu and Banttari (1979) reported that the transmission of oat blue dwarf virus and Aster yellows (AY-MLO) by the leafhopper **Macrosteles fascifrons** (Stal) was reduced substantially in dually infected leafhoppers compared to leafhoppers that were only infected with a single pathogen type. The coinfection of **C. haematoceps** with sesame phyllody MLO and **S. citri** may also explain why the leafhopper-borne CSD pathogen is very variable both in its annual and local spread (Calavan **et al.,** 1977, Kersting **et al.,** 1991).

Interestingly, field collected **O. orientalis** did not transmit the sesame phyllody MLO to **C. roseus,** although **O. orientalis** (syn.: **O. albicinctus** Distant) and the related species **Orosius cellulosus** (Lindberg) are vectors of a sesame phyllody agent in India and Burkina Faso, and cotton virescence in Burkina Faso (Laboucheix **et al.,** 1972; Ishihara, 1982).

This unexpected result may be due to two reasons. The limited transmission experiments using field collected leafhoppers are inconclusive. Factors like target plant, strain of pathogen and biotype of the vector strongly affect the transmission rate (Chiykowski, 1981). The obtained results, however, show that **C. haematoceps** is an efficient vector of the sesame phyllody MLO in Turkey, which remains to be proven for **O. orientalis.** More important, the provided data did not analyze the taxonomic relationship between the MLO observed on sesame in the Near East, India or Africa and that found in Turkey. Because plants infected with different MLOs show similar symptoms, the vector relation in MLO taxonomy is more important (McCoy **et al.,** 1989). This was illustrated for the beet leafhopper-transmitted virescence MLO (BLTV), which was first distinguished from Aster Yellow (AY-MLO) only by its unique vector specifity, although in many hosts the symptoms are similar to each other (Oldfield **et al.,** 1977; Golino **et al.,** 1989). Thus it might be possible that the sesame phyllody MLO found in Turkey is not related to those found in India and Burkina Faso. The transmission test, however, provided evidence that sesame phyllody in Turkey is related to that observed in Iran, since both are transmitted by **C. haematoceps.**

The results obtained in this study provide strong evidence that **C. haematoceps** is an important vector of the sesame phyllody MLO in the Çukurova region of Turkey. However, the failure in transmitting this disease using field collected **O. orientalis,** requires further studies in the vector-pathogen relationship and the taxonomy of MLO by using DNA-hybridization.

ÖZET

Türkiye'de Susam 'Phyllody' Hastalığının Belirtileri, Nedeni ve Taşınması

Susam 'phyllody' hastalığının ortaya çıkışı yayılışı, nedeni ve vektörü olan Cüce Ağustosböcekleri ile ilişkileri 1990 ve 1992 yıllarında araştırılmıştır. Bu hastalığın Güneydoğu Anadolu ve Akdeniz bölgelerinin susam yetiştirilen tüm alanlarında yaygın olduğu belirlenmiştir. Hastalığın tarladaki esas belirtileri phyllody, aşırı yeşillenme (virescence) ve cadı süpürgesi oluşumu şeklindedir. Bazı susam bitkilerinin kapsüllerinde aşırı büyümeler gözlenmiştir. Sağlıklı bitkilerin iletim demeti hücrelerinde Mikoplazma Benzeri Organizmalara (MBO) rastlanılmamasına karşın, hastalıklı susam bitkilerinin iletim demetlerinde MBO'lar saptanmıştır. Susam üzerinden toplanan değişik Cüce Ağustosböceklerinden sadece **Circulifer haematoceps** (M. & R.) bu hastalığı susam bitkisinden Cezayir menekşesine (**Catharanthus roseus** L.) taşımıştır. Doğadan toplanan böceklerin hastalığı bu bitkiye taşıma oranı ise % 20'dir. Vektöre özelleşmesi bakımından Turkey'deki susam phyllody hastalığının Hindistan ve Burkina Faso'da saptanandan farklı, buna karşın İran'da bulunanla aynı olduğu görülmektedir.

ACKNOWLEDGEMENTS

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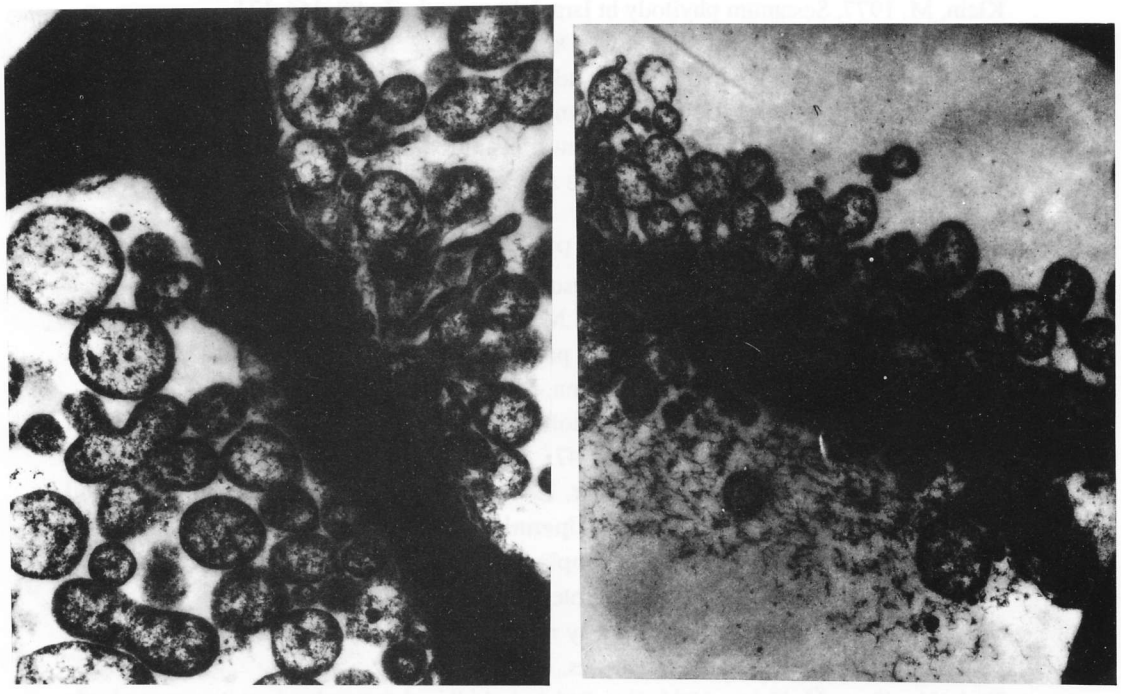

Fig. 1: Thin sections of **Catharanthus roseus** infected phloem tissue embedded in Spurr's resin. MLO's are concentrated at the plate of the sieve cell.

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Researches on Grapevine Virus Diseases and Determination of Their Incidences in Ankara, Türkiye

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ABSTRACT

As a result of surveys made in districts of Ankara, alfalfa mosaic virus (AIMV), arabis mosaic virus (ArMV), grapevine fanleaf virus (GFLV), strawberry latent ring-spot virus (SLRV), tomato black ring virus (TBRV) and grapevine leafroll disase were found out.

The existence of these viruses were proved to be based on its spesific symptoms, inoculation tests on herbaceous and woody indicator plants and positive reactions with agar gel, microprecipitation and ELISA tests.

INTRADUCTION

Grape is a very useful fruit as human food and healthy thanks to its sugar, nitrogen, mineral substance and vitamin contents. Grape is exhausted both fresh and various products in Türkiye (Ağaoğlu, 1986).

According to the statistical data of the State Institute of Statics of Türkiye in 1989 grapevine area and production of Türkiye were 597.000 hectares and 3.430.000 tons respectively (Anonymous, 1989), in 1988 grapevine area and production of Ankara were 6.125 hectares and 13.576 tons respectively (Anonymous, 1988).

Türkiye is at the top in the world with raisin producting approximately 250-300 thousand tons every year. Approximately, half of the production is exported and many million dolars of currency is provided every year (Ağaoğlu, 1986).

Virus diseases are one of the most important factors, which affect output and quality in the grapevines. Actual studies about virus diseases have begun in 1940s. Two virus were isolated in the grapevines in 1962. These viruses were GFLV and TBRV (Hewitt, 1987). Now it is confirmed that 29 viruses (Martelli, 1979, 1982) 3 viroids (Goheen, 1988) and 21 virus-like diseases (Hewitt, 1987) induce economic problems.

Özaslan **et al.** (1991) expressed that grapevine virus diseases caused reduction of yield about 45.4 %.

Nevertheles it is known that virus diseases cause yield losses about 80 % rate by

reducing yield quality, causing shorter generative period, reducing grafting and rooting of generative material, and also loosing sugar contents of yield and market value.

MATERIALS AND METHODS

White and red **Vitis vinifera** L. varieties (Alphones, Çavuş, Gül Üzümü, Hafız Ali, Hamburg Misketi, Hasan Dede, Hoca Üzümü, Kadın Parmağı, Kalecik Karası, Razakı, Sultani, Tilki Kuyruğu, Tokat Üzümü) were observed from 1990 to 1992. The survey area of this research was selected as Ayaş, Beypazarı, Güdül, Kalecik, Kızılcahamam, Şereflikoçhisar and Centrum of Ankara, which were main grapevine areas in the province. Approximately 3500 grapevines in 57 vineyards or gardens were investigated during the vegetation period of grapevines.

Barnett's "c" pattern was used as sampling patterns (Barnett, 1986).

Leaves of grapevine showed virus symptoms were homogenized in a mortar with 0.01 M phosphate buffer (pH : 7.2) which contains 2.5 % nicotine. Extracting sap was inoculated on 11 different herbaceous test plants. Each 5 plants were inoculated and 3 plants were seperated as control. Inoculated test plants were placed in the climate chamber which conditioned to 25°C and 16 hours of illumination (4000 lux) or 20°C at dark and 70-80 % humidity.

For the grafting to the woody test plants (**V. rupestris** st George and Pinot Noir), collected shoots which show nepovirus and leafroll symptoms in the middle of August were kept at 4°C. Then shoots buds were grafted by chip budding. Grafting were made under the greenhouse conditions.

Shoots which showed degeneration symptoms were used as material for detection cordon and thyllosis in the endovascular system, which are the microscopic symptoms of the degeneration disease. These shoots were fixed according to Sass (1958) in the complex of ethyl alcohol (96 %), glacial acetic acid, formaldehyde (37-40 %) and distilled water (50, 5, 10, 35), then sections which had a thickness of 30 µm were taken between 3 th. and 4 th. node acording to Kepsutlu and Saydam (1972), then these sections were examined under the light microscope. As for detection of inclusion body. **Chenopodium amaranticolor, Petunia hybrida** and **Phaseolus vulgaris** leaves epidermis tissues were stained by a complex of calcomine orange (0.2 %) luxol brilliant (1 %) (1;5) about 20-30 minutes according to Christie (1967). Methyl cellosolve (ethylene glycol monomethyl ether), ethyl alcohol and distilled water (2;1;1) were used as solvent.

Agar gel, microprecipitation and ELISA serological test techniques were applied. Agar gel tests were used to the virus infected leaf samples of grapevines and leaves of **C. amaranticolor, C. quinoa** and **P. vulgaris.** Agar gel test was performed according to the method described by Erdiller (1982). Microprecipitation tests were used to leaves of

C. amaranticolor, C. quinoa, P. pybrida and **P. vulgaris.** Microprecipitation test was performed according to the method of Noordam (1973).

DAS-ELISA serological test technique was used. The test was based on Clark and Adams (1977).

RESULTS AND DISCUSSION

Virus symptoms were observed on the majority of grapevine varieties in the surveyed areas (Table 1). The symptoms of leafroll disease were observed only vineyards in Ayaş, Beypazarı, Güdül, Kalecik districts and vineyard of Agricultural Faculty of Ankara University.

Results of mechanical inoculation to 11 apart test plants were summarized in Table 2. In this research, identification of the causative agents of symptoms occured herbaceous indicator plants was made according to Hewitt **et al.**, 1970; Erdiller, 1982; Martelli and Quacquarelli, 1972; Hiruki and Miczynski, 1987; Murant 1970 a;b, 1974.

Nepovirus and leafroll diseases symptoms have been determined after grafting 2 apart woody test plants by chip budding. Chlorotic lesions and then shock symptoms on **V. rupestris** st. George were similar to Martelli (1986)'s reports. Symptoms on Pinot Noir changed leaves colour from green to reddish, but veins kept their green colour and then leaves rolled down. These symptoms observed on Pinot Noir test plants were similar to Azeri (1990)'s reports.

In order to detect microscobic symptoms, shoot showed zigzag form and short noding interval symptoms were used and examined under the light microscope. Thyllosis and cordon forming was observed in the endovacular systems.

In order to detect inclusion body. **C. amaranticolor, P. hybrida** and **P. vulgaris** epidermis tissues were examined under the light microscope. The cell nuclei were stained to orange colour, plastids were stained to yellow. But only in the epidermis cells of **P. vulgaris,** greenish brown and green granular bodies were searched. These bodies were similar to granular and amorph bodies of AIMV according to Desjardins (1966)'s reports (Bos and Jaspars, 1971).

In the results of serological tests, ArMV found out as the most widespread virus disease in Ankara province. GFLV, TBRV, SLRV and AIMV have been deteced as the other important viruses respectively (Table 3). Spreading of these viruses on the grapevine varieties was summarized in Table 4.

One line precipitate in gel-diffusion tests and small granular precipitates in microprecipitation test were got according to AIMV, ArMV, GFLV, SLRV and TBRV antisera.

Two different substrats were used in ELISA tests depending on enzyme. 5-amino-salicylic acid (5AS) were used as substrat for linked IgG of TBRV by horseradish

Table 1. Observed symptoms on surveyed grapevine varieties in Ankara Province.

Grapevine Varieties	Observed Symptoms *		
	Leaves	Shoots	Clusters
ALPHONSE	**Df**	ShN; ZZD; WD	SmCF; DCF
ÇAVUŞ	**Df, Y, CLL; Wr**	ShN; ZZD; WD; DNF	DCF
GÜL ÜZÜMÜ	**Df, CLL; FL**	ShN; ZZD; WD	SmCF; DCF
HAFIZ ALİ	**Df**	ShN; WD	SmCF; DCF
HAMBURG MİSKETİ	**Df; Y; WR; FL**	ShN; WD	SmCF; DCF; RBN
HASAN DEDE	**Df**	ShN; WD; DNF	SmCF; DCF
HOCA ÜZÜMÜ	**Df; Wr; FL**	ShN; WD	SmCF; DCF
KADIN PARMAĞI	**Df; Wr; FL**	ShN; ZZD	SmCF; DCF
KALECİK KARASI	**Df; Y; CLL; Wr**	ShN; ZZD	SmCF; DCF
RAZAKI	**Df**	ShN; WD	SmCF; DCF
SULTANİ	**Df;Y;Wr;FL;VB**	ShN; ZZD; DNF; NS	SmCF; DCF; ACF
TİLKİ KUYRUĞU	**Df; CLL**	ShN; ZZD	SmCF; DCF
TOKAT ÜZÜMÜ	**Df; CLL**	ShN; ZZD	SmCF; DCF

* Df ; Deformation
Y ; Yellowing
CLL ; Chlorotic local lesion
Wr ; Wrinkle
FL ; Fan leaf
VB ; Vein banding
ShN ; Shortened internal nodes
ZZG ; Zigzag development
WD ; Weakly development
DNF ; Double node form
NS ; Node swelling
SmCF ; Small cluster form
DCF ; Delay cluster form
RBN ; Reducing berry number
ACF ; Anormal cluster form

Table 2. Symptoms observed on the test plants infected by grapevine viruses

Test Plants	Symptoms *
Chenopodium amaranticolor Costa et Reyn	CLL; VC
C. foetidum	SMot
C. murale L.	NLL; Nec; E
C. quinoa Willd.	CLL; NLL; VC; Nec
Cucumis sativus L.	CLL; CRS; NRS; Cl; VC
Datura stramonium L.	CLL; NLL
Gomphrena globosa L.	RLL; BLL; SM
Petunia hybrida Hort	CLL; VC; BLL
Phaseolus vulgaris L. Yalova 5	CLL; NLL; BLL; Cl; VNec
Nicotiana clevelandii Gray	SCL
N. tabacum L. "Xanthii"	NLL

* CLL ; Chlorotic local lesion
Cl ; Chlorosis
NLL ; Necrotic local lesion
VNec ; Vein necrosis
CRS ; Chlorotic ring spot
NRS ; Necrotic ring spot
SMot ; Systemic mottle
BLL ; Brownish local lesion
E ; Epinasty
SM ; Systemic mosaic
VC ; Vein clearing
RLL ; Reddish local lesion
Nec ; Necrosis
SCL ; Systemic chlorotic lesion

Table 3. Detected number of grapevine viruses (AIMV, ArMV, GFLV, SLRV, TBRV) infections using serological tests methods in Ankara province.

Survey Area	Number of Sample	Number of Detected Virus Infections Grapevine					Total Number of Infectious Plant
		AIMV	ArMV	GFLV	SLRV	TBRV	
Altındağ	8	0	5	3	0	3	8
Ayaş	18	0	12	6	0	6	12
Beypazarı	24	0	16	8	0	8	20
Çankaya	2	0	2	1	0	0	2
Güdül	48	0	17	16	15	13	42
Kalecik	24	1	10	3	0	8	13
Keçiören	72	25	31	34	8	17	66
Kızılcahamam	8	0	6	4	0	1	8
Şereflikoçhisar	21	0	12	21	12	18	21
TOTAL	225	26	111	96	35	74	192

Table 4. Detected virus diseases on different grapevine varieties in Ankara province.

Grapevine Varieties	Detected Virus Diseases				
	AIMV	ArMV	GFLV	SLRV	TBRV
Alphonse	–	+	+	–	+
Çavuş	+	+	+	–	+
Gül Üzümü	–	+	+	+	+
Hafız Ali	–	+	+	+	+
Hamburg Misketi	+	+	+	–	+
Hasan Dede	–	–	+	–	–
Hoca Üzümü	–	+	+	+	+
Kadın Parmağı	–	+	+	+	+
Kalecik Karası	+	+	+	+	+
Razakı	–	+	+	+	+
Sultani	+	+	+	+	+
Tilki Kuyruğu	–	–	+	–	–
Tokat Üzümü	–	+	+	+	+

+ : Presence of detected grapevine virus disease
– : Healty

peroxidase enzyme, and a light brown colour reactions was got, absorbans values were read in ELISA reader at 450 nm. Paranitro phenyl phosphate were used as substrat for linked IgG of ArMV and GFLV by alkaline phosphatase enzyme, and getting yellow colour reaction were read in ELISA reader at 405 nm.

ÖZET

Ankara'nın 8 ilçesindeki bağ alanlarında yapılan surveyler sonucu asma yelpaze yaprak virusu (GFLV), kazotu mozaik virusu (ArMV), domates siyah halka virusu (TBRV), çilek latent halkalı leke virusu (SLRV), yonca mozaik virusu (AIMV) ve asma yaprak kıvrılma hastalığına rastlanmıştır.

Bu virus hastalıklarının mevcudiyeti gösterdikleri belirtiler, otsu ve odunsu indikatör test bitkilerinin kullanımı ve serolojik test yöntemlerinden agar jel, mikropresipitasyon ve ELISA test yöntemleri uygulanarak ortaya çıkarılmıştır.

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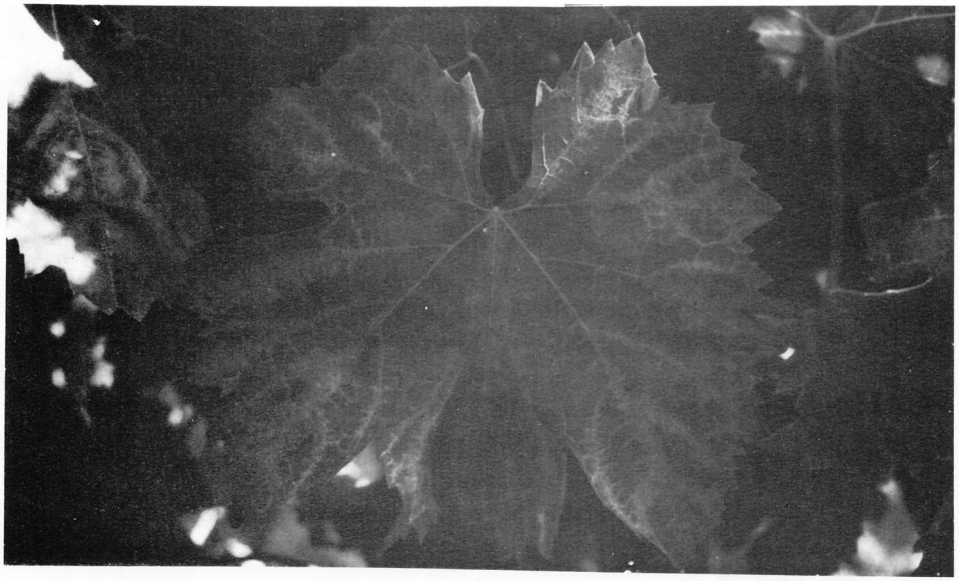

Fig 1. Vein banding symptoms on leaves of Sultani vine cultivar.

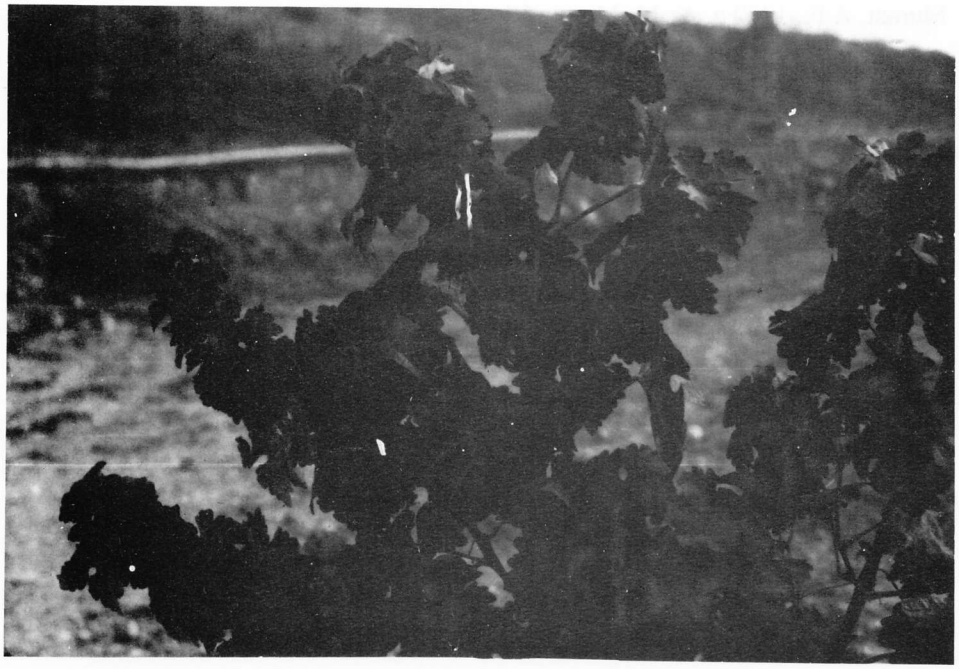

Fig 2. Symptoms caused by Grapevine leafroll disease on Kalecik Karası vine cultivar.

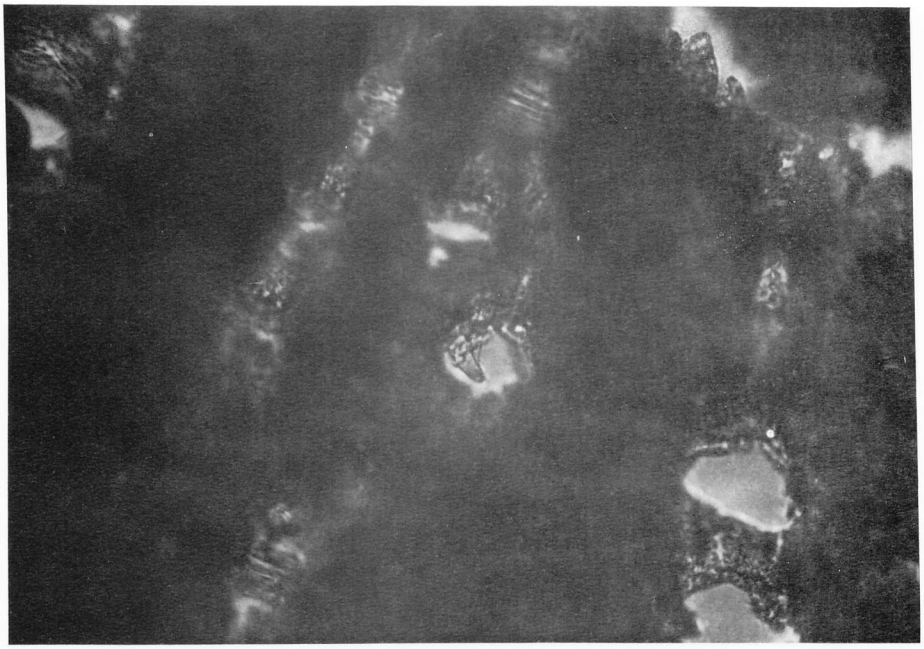

Fig 3. Cordon formation in the endovascular system

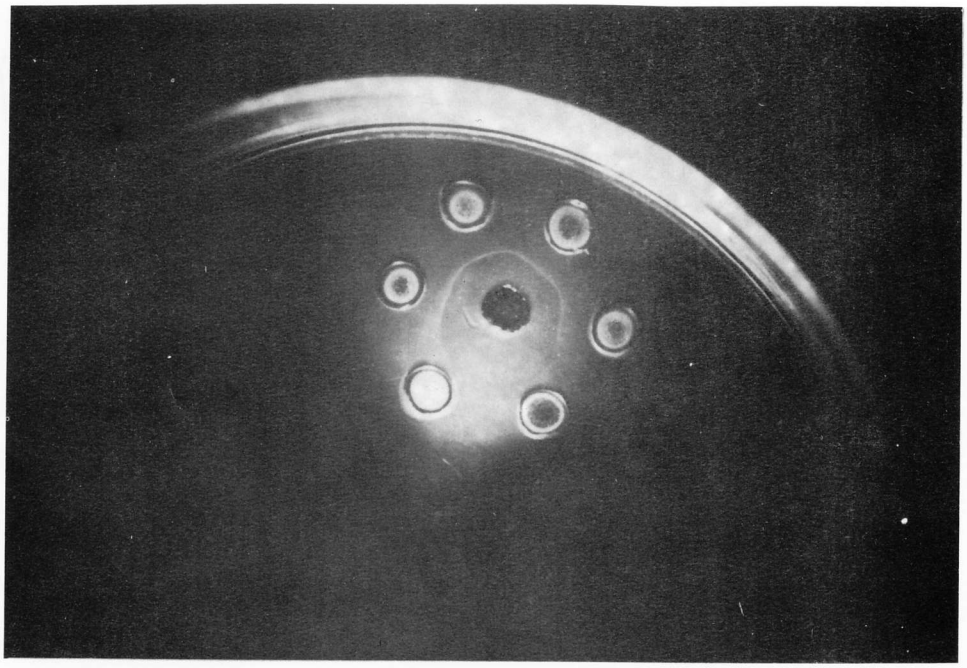

Fig 4. One line precipitates of extracts against to ArMV antiserum which is placed in the centrum of wells in gel diffusion tests.

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Occurrence And Detection of Citrus Tristeza Virus (CTV) Decline on Satsuma Mandarins Buded On Trifoliata Orange In Izmir Province

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ABSTRACT

*In this study, the relation of the present severe isolate of CTV and its decline symptoms on Satsuma mandarins (**Citrus unshiu** March.) buded on trifoliata rootstock were detected by biological indexing and ELISA test. Leaf insert graft inoculated on Mexican lime (**C. aurantifolia** (L.) Swingle) plants developed characteristic severe CTV symptoms of veinlet-netting, small and upright cupped yellow leaves, vein-corking and stem pitting 6 or 8 weeks after the inoculations. Leaf samples collected from the declined Satsuma trees gave the positive (+) results in ELISA test. It was most evident that, severe CTV isolate was responsible for declining symptoms of Satsuma mandarin on the trifoliata orange rootstock in İzmir Province.*

INTRODUCTION

Citrus tristeza virus (CTV), also quick decline or bud-union decline, has existed for many years in the tropical citrus growing countries. Tristeza caused extensive losses between 1930 and 1940 in Argentina, Brazil and California. More than 20 million citrus trees on sour orange (**C. aurantium** L.) rootstocks were completely destroyed in Argentina, Brazil and other South American countries, and about 3 million in Southern Calif. as reported by Wallace (1957). Klotz **et al.**, (1972). CTV is present world wide, however, mostly on Mayer lemons, Satsuma mandarins (**C. unshiu** Marc.) and in certain tangerin selections (Olson and Mcdonald, 1955). It has been reported that, Satsuma mandarins were infected with tristeza in Italy and in the other parts of the Satsuma growing Mediterranean area particularly in North Africa. Because of CTV infections, Satsuma trees in N. Africa have been severely dwarfed as reported by Reichart (1959), Reichert and Bental (1960). CTV was reported also in Cyprus, Southern France, Greece, Israel by the same authors and in Spain (Reichert and Bental, 1960). It caused epidemic decline in Satsuma mandarins in Valancia district in Spain (Anonymous 1986). Different strains and decline symptoms of CTV that varied in severity citrus hosts have been described world wide.

The presence of 5 different strains of CTV (mild, moderate and severe strains) in Satsuma mandarins have been previously reported by Azeri and Karaca (1978). Mild and moderate strains of CTV were found more distributed than the severe strain in İzmir region. Severe strain of CTV that caused severe stunting, declining, stem pitting (SP) and seedling yellows (YS) has also been reported by the same authors and Azeri (1981). The two efficient vectors of CTV; The melon aphid, **Aphis gossypii** Glover and **Toxoptera citricidus** Kirkaldy have been found endemic in most Citrus growing areas in İzmir (Lodos, 1981; Azeri, 1981). A high rate of CTV decline in Satsuma mandarins on trifoliata orange rootstocks (**Poncirus trifoliata** L. Raf.) was reported by Azeri and Karaca (1978) and Azeri (1981).

During the last year, some Satsuma mandarins on **P. trifoliata** rootstocks were showing severe decline and SP symp. on the Satsuma scions. These symptoms led us to investigate whether the severe strain of CTV caused or not, and the distribution of decline in Satsuma orchards in İzmir province.

MATERIALS AND METHODS

Survey : The survey was made in 1990 in the Satsuma producing provinces, namely (Gümüşsu, Özdere, Güzelbahçe, Ürkmez), Seferihisar and Urla. The total number of 4797 Satsuma trees older then 8 years old and buded on **P. trifoliata** rootstocks in the 57 different orchards were examined for CTV-SP symptoms in the survey as described by Bitters and Parker (1952), Olson (1956), Reichert and Ginsburg (1960), Harpaz (1964), Yamada and Tanaka (1969), Pujol **et al.**, 1972.

indexing: During the survey, leaf samples from the declined Satsuma trees with SP symptoms were collected for leaf insert graft inoculation test on Mexican lime (ML) (**Citrus aurantifolia** (Christm.) Swingle) for the identification of CTV strains that caused severe decline and SP symptoms.

Leaf insert grafting was used in the tests as described by Gohen (1972), Azeri and Karaca (1978). Pencil-size of ML seedlings were used in the tests. Four or 5 leaf stage graft were inserted in each ML. The temperature varied in between 20°C - 27°C during the indexing period in the laboratory. Inoculated ML seedlings were checked weekly for the presence of the symptoms as described by McClean (1974), Wallace (1968), Azeri and Karaca (1978). A scale illustrated by McClean (1963) was used to determine CTV strains.

Staining Pathological Phloem: During the survey and examination of the declined Satsuma trees, a piece of bark sample, approximately 5 mm x 30 mm were cut out from just above the bud-union of the examined and declined (with SP) Satsuma trees. Sectioning and staining of the pathological phloem was done by using the technique as previously described by Schneider (1954, 1959).

ELISA: ELISA tests were carried out for detection of the spreading of CTV at the declined Satsuma orchards. The young leaves and the shoot samples were collected in Spring (in March and April, 1990) from the several Satsuma orchards given in table 2. Totally 137 samples were collected from the several Satsuma trees in the 57 Satsuma orchards (Leaves and the shoot samples taken from 4 or 5 trees were collected together in the same plastic bag and accepted as one sample).

In ELISA test, leaf samples were homogenized in PBS-Tween-extraction buffer at a dilution of 1/10 (W/V), pH 7,4. The buffer was freshly prepared and fresh egg white was added in it just before used (Bar-joseph **et al.**, 1980). CTV infection on declined Satsuma trees was verified using double antibody sandwich enzymelinked immunosorbent assey (ELISA). The monoclonal antibodies and conjugated IgG specified and produced against the very severe CTV isolate "T-308" isolated from Spain (Which enable the detection of severe decline, SY and SP isolates of CTV from different parts of the world) were used in ELISA as described by Bioreba AG. Test procedure was carried out in polystyrene microtitre plates according to ELISA procedure outlined by Clark and Adams (1977). Optical density (extinction values) was measured at 405 nm in a Multiskan plus MK II ELISA reader. Two healty ML seedlings Och. No: 1/1, 1/2 and 16 Sampl. buff. Control were used as (–) controls. "X" value and "X + 3 S" value of healthy control samples as given in fig. 1 was calculated for setting histogram of ELISA results. Extinction values over ≥ 0.1 were considered positive.

RESULTS AND DISCUSSION

Symptomatology of CTV: The obvious external symptoms on the severely CTV affected Satsuma trees on **P. trifoliata** rootstocks were: severe defoliations, reduceing of the foliage, chlorosis, very small cupped leaf showing as zinc deficiency, shoot dieback, gradual decline and death of the trees in the latter stages. Most of the severly CTV affected Satsuma trees also displayed severe SP and pegs on the woody cylinder of CTV affected Satsuma scion over the **P. trifoliata** rootstocks as seen in fig 2. The ave-rage rates of incidence of CTV decline were 17.65 % for İzmir Central County; 13,66 % for Seferihisar; 1.82 % for Urla (Central) provinces. The general average rate of CTV decline for İzmir Province was 16.06 % as showen in table 1.

Indexing Result :

Since 1990, more than 50 declined CTV affected Satsuma mandarins on **P. trifoliata** have been indexed for strain determination of CTV. Leaf insert graft inoculated ML seedlings developed severe vein-clearing (Fig 3) very small and upright cupped leaves (Fig. 4) vein corking in the advanced stage. The indexing test revealed that, the severe strain of CTV was the causal of Satsuma decline.

Table 1. The rate of incidence of CTV decline and SP on Satsuma trees in İzmir Central County, Seferihisar and Urla Counties in 1990.

Survey Place	Number of Examined Orchards	Total Number of the trees in the Examined Orchards	The number of the declined Satsuma trees with SP symptom	The rates Incidance of CTV decline (%)
(Central County)				
Gümüşsu	21	29.210	3040	19.06
Özdere	3	1800	17	9.0
Güzelbahçe	6	2625	350	7.9
			Average	17.65
Seferihisar				
Ürkmez	4	2200	220	15.9
Merkez	14	5455	740	12.74
			Average	13.66
Urla (Central)	9	2490	430	1.82
Total	57	4797		
General Average Rate of CTV decline for İzmir Province.				16.06

The microscopic examination of the stained pathological phloem revealed that, the cytoplasm of the paranchymatic cells showed darkly staining material (chromatic cells) in the CTV infected Satsuma scion and **P. trifoliata** bark phloem. Sieve tube necrosis in the bark phloem of Satsuma scions were observed in the staining phloem. Callus depositions were also observed on the sieve plates near the cambium of the Satsuma and **P. trifoliata** phloem tissue as reported by Azeri (1984). This internal necrosis or nonfunctioning sieve tubes symptom was known the characteristic phloem effect of the severe CTV strain which causes decline of the citrus trees as reported by Schneider (1954) and Batzer and Schneider (1960).

ELISA: The results of ELISA tests revealed that, leaf extracts from the declined trees gave the positive reactions as showen in Table 2. CTV infected Satsuma tree were showing severe SP and decline symptoms on Satsuma scion below the bud-union. Some

Table 2. ELISA detection of CTV in the leaf sample extracts collected from the Satsuma trees in the several locations of İzmir.

Orch./Tree No (localities)	Ab. Va (at 405 nm) ELISA Plate No:1	Orch./Tree No (Loc.)	Ab. Va (at 405 nm) ELISA Plate No:1	Orch./Tree No (Loc.)	Ab. Va (at 405 nm) ELISA Plate No:1	Orch./Tree No (Loc.)	Ab. Va (at 405 nm) ELISA Plate No:1
1/1	0.017 (a)	14/1 G	0.014	20/3 İn.	0.019	21/1 Ü	0.058
1/2	0.012 (a)	14/2 G	0.203 +	20/4 İn.	0.021	21/2 Ü	0.799 +++
3/1 G	0.079	14/3 G	0.029	20/5 İn.	0.020	21/3 Ü	0.078
3/2 G	0.009	14/4 G	0.023	20/6 İn.	0.025	21/4 Ü	0.110 (L.) +
3/3 G	0.039	14/5 G	0.240 +	E.Plate No:2)		22/1 Ü	0.130 +
3/4 G	0.544 +	14/6 G	0.007	20/7 İn.	0.047	22/2 Ü	0.082
3/5 G	0.019	14/7 G	0.030	20/8 İn.	0.079	22/3 Ü	0.133 +
3/6 G	0.023	14/8 G	0.060	20/9 İn.	0.083	22/4 Ü	0.085
3/7 G	0.018	14/9 G	0.063	20/10 İn.	0.024	24/1 Ü	0.075
3/8 G	0.013	15/1 G	0.064	20/11 İn.	0.347 ++	24/2 Ü	0.090
3/9 G	0.013	15/2 G	0.012	20/12 İn.	0.075	24/3 Ü	0.012
4/1 G	0.694 (b)++	15/3 G	0.064	20/13 İn.	0.088		(X values)
4/2 G	0.116 (b)+	15/4 G	0.064	20/14 İn.	0.078		0.047
4/3 G	0.114 (b)+	16/1 İn.	0.022	20/15 İn.	0.081		0.000
5/1 G	0.229 +	16/2 İn.	0.064	20/16 İn.	0.071		0.099
6/1 G	0.067	16/3 İn.	0.239 +	20/17 İn.	0.038		0.001
7/1 G	0.013	16/4 İn.	0.102 +	20/18 İn.	1.310 +++		0.035
8/1 S	0.013	16/5 İn.	0.288 +	20/19 İn.	0.089		0.005
8/2 S	0.109 +	16/6 İn.	0.008	20/20 İn.	0.079		0.051
9/1 S	0.041	16/7 İn.	0.117 +	20/21 İn.	0.913 +++		0.073
9/2 S	0.445 +	16/8 İn.	0.053	26/1 İn.	0.072		0.062
10/1 S	0.056	16/9 İn.	0.073	27/1 İn.	1.268 +++		0.000
10/2 S	0.029	17/1 İn.	0.195 +	28/1 İn.	0.085		0.055
10/3 S	0.026	17/2 İn.	0.004	28/2 İn.	0.075		0.096
11/1 S	0.012	17/3 İn.	0.010	28/3 İn.	0.169 +		0.099
12/1 G	0.407 (b)++	17/4 İn.	0.004	28/4 İn.	0.550 ++		0.099
12/2 G	0.861 (b)+++	17/5 İn.	0.010	28/5 İn.	0.096		0.017
12/3 G	0.518 ++	18/1 İn.	0.017	28/5 İn.	0.078		0.012
12/4 G	0.147 +	18/2 İn.	0.334 +	28/6 İn.	0.087		
12/5 G	0.196 +	18/3 İn.	0.011	28/7 İn.	0.084		
12/6 G	0.027	18/4 İn.	0.015	29/1 İn.	0.090		
13/1 G	0.022	18/5 İn.	0.034	29/2 İn.	0.081		
13/2 G	0.647 ++	18/6 İn.	0.200 +	30/1 İn.	0.272 +		
13/3 G	0.248 +	18/7 İn.	0.005	31/1 İn.	0.596 ++		
13/4 G	0.313 +	18/8 İn.	0.128 +	32/1 İn.	0.004		
13/5 G	0.179 +	18/9 İn.	0.593 ++	33/1 G	0.330 +		
13/6 G	0.217 +	18/10 İn.	0.348 ++	33/2 G	0.444 ++ (b)		
13/7 G	0.529 +++	18/11 İn.	0.004	33/3 G	0.070		
13/8 G	0.031	18/12 İn.	0.014	33/4 G	0.249 + (b)		
13/9 G	0.465 ++	19/1 İn.	0.456 ++	33/5 G	0.161 + (b)		
13/10 G	0.021	19/2 İn.	0.157 +	33/6 G	0.303 ++ (b)		
13/11 G	0.469 ++	20/1 İn.	0.022				
13/12 G	0.012	20/2 İn.	0.025				

(a) : Helthy ML. control; (x) Extraction buf. Controls; (b) Leaf samples from severe CTV SP affected trees; Localities : G = Gümüşsu, In = İnciraltı, Ü: Ürkmez, S = Seferihisar; Ab. Va = Absorbance; (L) Lemon; E. Plate No: 1 = ELISA Plate No: 1.

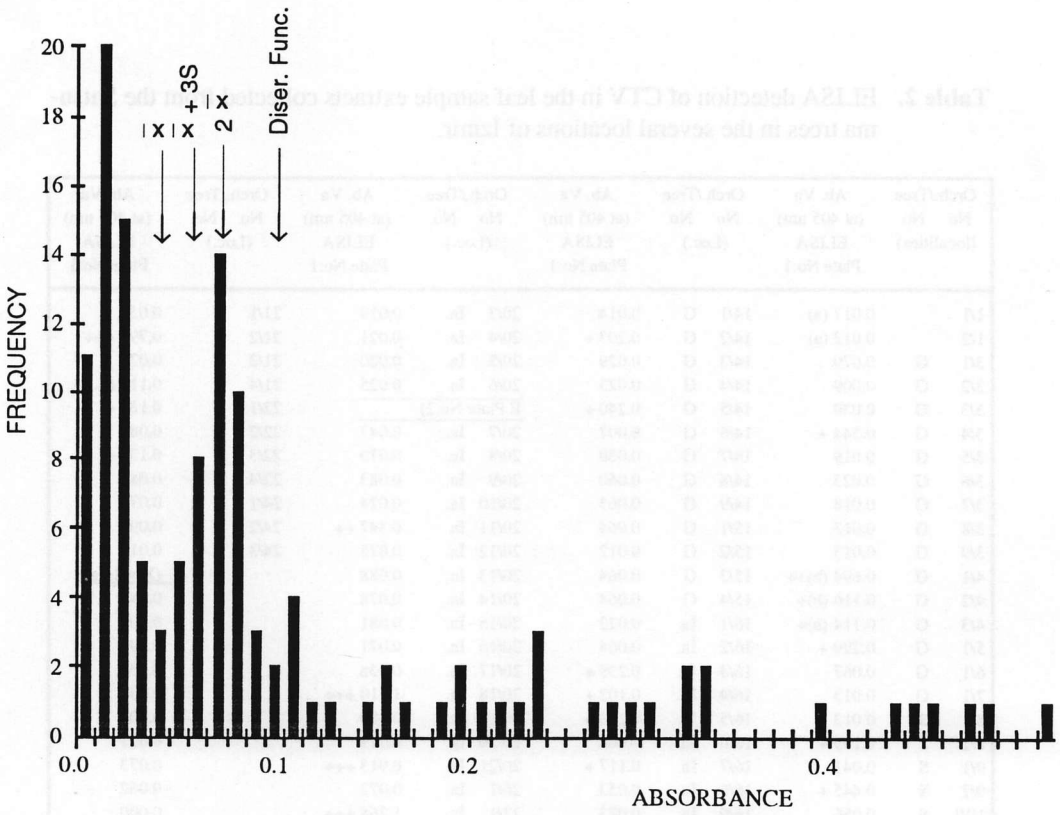

Fig. 1. Histogram of ELISA results (belong to table 2) for CTV in 57 Satsuma orchards. (Two negative 16 sample buffer control were used for healty mean value.)
X = 0.043 (Average value of negative control samples) ; (X + 3x S) = 0.048; S (Standart deviatio trol samples) = 0.0017; Discr. Func. = 0.1 nm, (Discriminate Function)

Satsuma trees were still free of CTV or infected by mild strain of the virus as shown in Tab. 2. A histogram prepared from the ELISA results given in Table 2 was clearly setting positive-negative thresholds as shown in Fig. 1. Some Satsuma trees were still free of CTV or infected by the mild CTV strains. It was proviously reported, 5 different CTV isolates (severe, mild and moderate strains) have been present in Satsuma mandarin trees in the region (Azeri and Karaca, 1978; Azeri 1981). It is clear that, CTV isolates already constitute a damage with endemic efficient vectors, melon aphid. **A. gossypii** Glover and aphid **T. citrucidus** Kirkaldy in the Citrus growing areas (Azeri and Karaca, 1978). Eradication of these CTV declined Satsuma trees and replanting with the virus free registrated healthy plants is necessary in the short time for protection of the virus free Citrus trees from field spreading of severe CTV isolates in the Citrus orchards.

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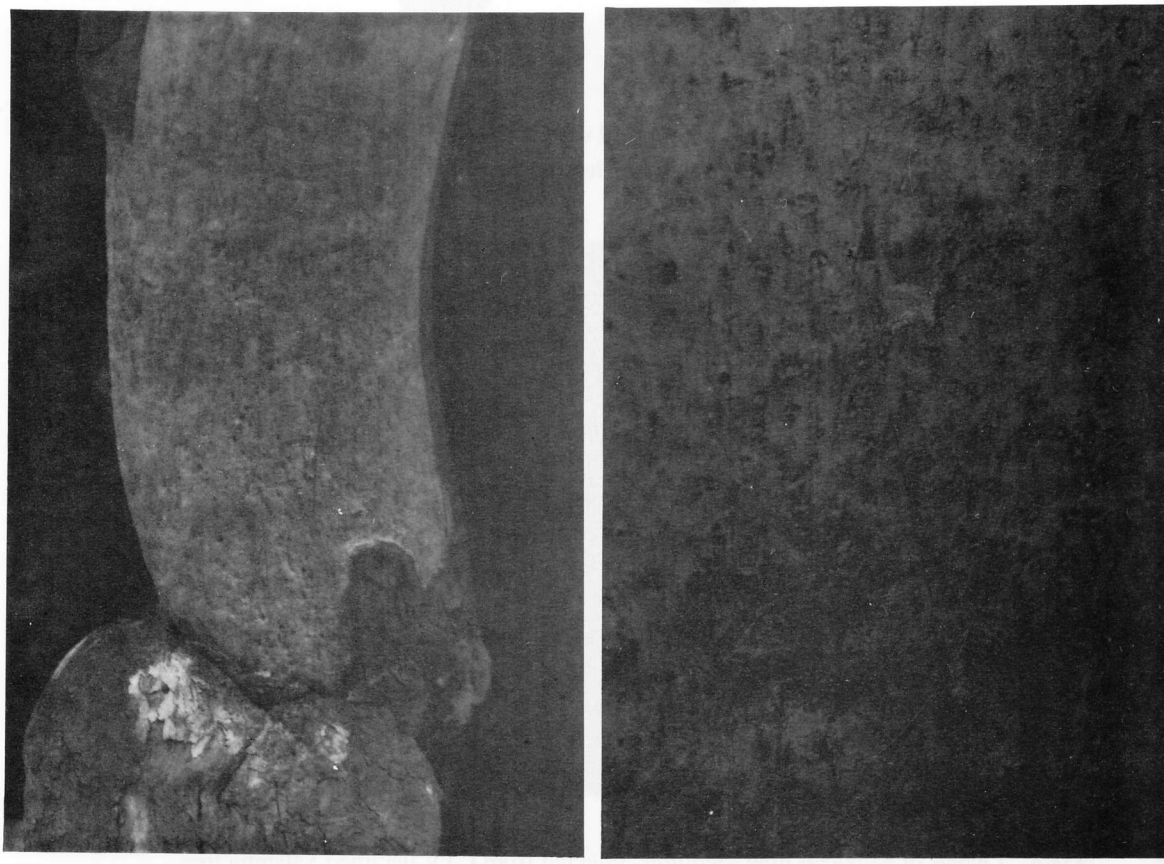

Fig 2. A-B : Severe invers-pittings and pegs on the woody sylinder of the Satsuma scion affected by CTV isolate.

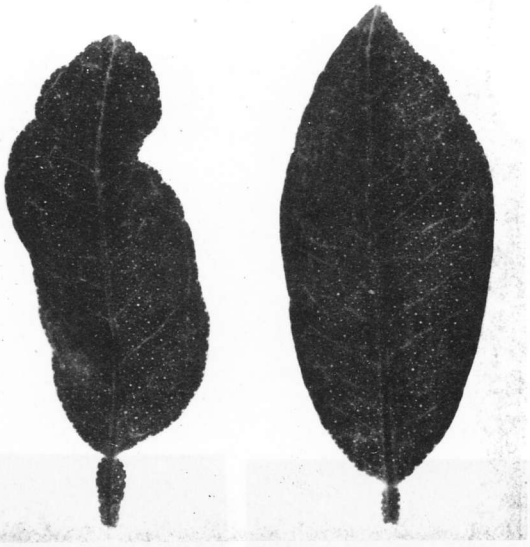

Fig 3 : The leaves of Mexican lime, infected with the severe strain of CTV showing severe vein-clearing.

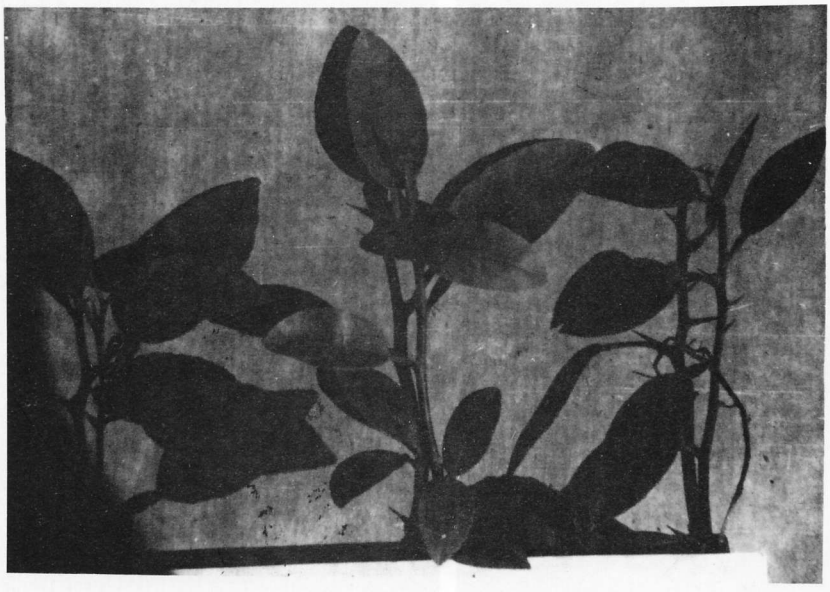

Fig 4. Leaves of Mexican lime seedlings, infected from CTV declined Satsuma tree showing severe vein-clearing, very small and upright cupped leaves (typical leaf symptom of severe CTV + Seedling Yellows (SY) strains)

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Evaluation of Two Serological Methods For The Identification of Halo Blight Pathogen (**Pseudomonas syringae pv. phaseolicola**) of Beans

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ABSTRACT

3 antisera were produced in rabbits against heat-treated and glutaraldehyde fixed cells of ***Pseudomonas syringae pv. phaseolicola*** *with two different injection schedules.* ***P. s. pv phaseolicola*** *strains having different origins, some fluorescent* ***Pseudomonas*** *isolated from dry beans and some other plant pathogenic bacteria were tested with two serological methods; Indirect fluorescent antibody staining (IFAS) and ELISA. The detection limit of these two assays and cross-reactions with bacterial strains under test were evaluated. Out of 3 different antisera, one, produced against glutaraldehyde fixed cells of* ***P.s. pv. phaseolicola*** *was more specific, and IFAS was proved to be more sensitive than ELISA for the detection of halo blight disease agent.*

INTRODUCTION

Pseudomonas syringae pv. **phaseolicola** the causal agent of halo blight, is a serious seedborne pathogens of beans worldwide (Zaumeyer and Thomas, 1957). Karaca (1977) first reported that halo blight was seen in Black Sea region and Bursa province of Türkiye. Detection of this pathogen in seed is essential for effective disease control. Laboratory and field tests for seed certification requires the rapid and accurate methods for the identification of **P.s. pv. phaseolicola.** Serological tests have been used for this purpose by Guthri **et al.** (1965), Taylor (1970). Trigalet and Bidaud (1978) used immunofluorescence microscopy (IF) for the direct identification of **P.s. pv. phaseolicola** cells in seed extracts. Recent investigations have demonstrated the potential use of enzyme linked immunosorbent assay (ELISA) for the detection of **P.s. pv. phaseolicola** (Van Vuurde & Van den Bovenkamp, 1981; Barzic & Trigalet, 1982; Van Vuurde **et al.**, 1983; Wyatt **et al.**, 1989).

The main objective of this study was to evaluate two serological methods-IFAS and ELISA for the specific detection of halo blight disease agent of beans by producing antisera against glutaraldehyde-fixed and heat treated cells of **P.s. pv. phaseolicola.**

MATERIALS AND METHODS

Bacterial cultures

A strain (NCPPB-52) of **Ps. pv. phaseolicola** was used for antiserum preparation. Sources of **Ps. pv. phaseolicola** and other species used for testing antiserum specificity are listed in the result section. All bacteria were grown on King's Medium (Kings et al., 1954) and suspended in sterile distilled water, unless otherwise stated.

Antisera production

For all preparations, bacterial cells were grown in Nutrient Broth (Difco) and shaker incubated at 27 °C for 24 h. Bacteria were washed three times in PBS [Phosphate buffer saline = 0.01 M potassium phosphate buffer (pH = 7.2) and 0.15 M Na Cl] and centrifuged at 12.000 x g for 20 min. The washed bacteria were resuspended in PBS, and the density adjusted OD 630 = 1.2 (Bio-Tek micro plate reader) (~10^9 - 10^{10} cells per ml). The cell suspensions used as antigen were; cells fixed in glutaraldehyde by the method of Allan & Kelman (1977), and cells heat-treated for 1 h at 60 °C.

Two antisera were produced against whole heat-treated, and fixed cells in the following injection schedule. For the initial injection, 1 ml of cell suspension was emulsified with and equal volume of Freund's Incomplete adjuvant and injected in the hind leg of each of two New Zealand white test rabbits from which normal sera were obtained prior to immunization. Then four intramuscular injections of 2 ml of each antigen preparation without adjuvant were given at weekly intervals. The third antiserum was produced against whole fixed cells and the same injection schedule was followed except three weeks after the initial injection, a serious of intravenous injections of 0.3, 0.6, 1.2 and 2.4 ml of cell suspension without adjuvant were given at 3-to 4-day intervals. One week after the last injections, rabbits were test bleed. Blood was collected into hearinized tubes, the plasma removed and sera preserved by mixing with an equal volume of glycerol and stored at -20 °C.

Agglutination titers were determined by the slide agglutination and the microagglutinin test described previously (Ball, 1974). Immunofluorescent titers were determined on suspensions (OD 630 mm = 0.1 ~10^6 cell/ml) of washed homologous bacteria, using antiserum dilutions ranging from 1/10 to 1/10.000 in PBS. The titer was taken as the greatest dilutions at which cells were even faintly visible in the stained preparations.

Immunofluorescence staining

The indirect fluorescent antibody staining (IFAS) procedure as described by Van Vuurde et al. (1983) were followed. Cultures were suspended in sterile distilled water to OD 630 mm = 0.1. The cells in single drops (0.025 ml) were air dried and ethanol (96 %) fixed to the windows of microtiter slides. To each preparation a drop of antiserum

(0.025 ml) at dilutions of 10, 100, 500, 1000, 2500, 5000 in PBS was applied. Slides were incubated in a moist chamber at 37 °C for 30 min., washed with PBS diluted 1/10, rinsed with distilled water, and blotted dry. 0.025 ml of the antirabbit Ig G antiserum conjugated with fluorescein isothiocynate (Dakopatts, F-205) diluted 1/40 with PBS was then applied to each preparation. Slides were incubated at 37 °C another 30 min. in a dark moist chamber, and washed as before. Coverslips (No = 1 size) were mounted with Glycerol -PBS (containing per 100 ml: Na_2HPO_4. $12H_2O$ 3.2 g, NaH_2PO_4. $2H_2O$ 0.15 g, glycerol 50 ml, pH = 7.6) and preparations were observed with Zeiss photomicroscope equipped with an oil-immersion objective (x 100), a 48 79 09 filter set, and HBO 50 light source.

ELISA

The assay was carried out in 96-well polystyrene plates (NUNC) according to Indirect ELISA procedures (Şahingöz **et al.**, 1991). Shortly, the wells of microtiter plates were coated by incubation with a suspension of bacterial cultures (0.1 ml) at a concentration of ca 10^8 cell/ml (OD 630 nm = 0.5) overnight at 4 °C. Subsequently, the wells were treated with 0.2 % glutaraldehyde solution for 10 min. After excess antigen was washed three times with PBST (PBS plus 0.025 % Tween 20) from plates, nonspecific protein binding sites were blocked by incubation with 1 % Bovine serum albumin in PBS for 1 h, and washed as before. The wells were treated with 0.1 ml of antiserum diluted 1/1.000, 1/5000, 1/10.000 with PBS and incubated for 2 h at 37 °C. After washing with PBST, Alkaline phosphatase conjugated antirabbit Ig G (Sigma A-8025) at recommended dilution was incubated in the plates for 1 h at 37 °C. The plates were again washed five times with PBST before adding p-nitrophenyl phosphate substrate. After 30 min., substrate conversion was quantified by measuring absorbance at 405 nm with a Bio-Tek EL-311 plate reader.

Sensitivity of IFAS and ELISA

To evaluate the sensitivity of two serological tests, 5 ten fold dilutions of a known concentration of **P.s. pv. phaseolicola** (NCPPB-52) ranging from 8 x 10^3 to 8 x 10^7 cfu/ml were prepared and each dilution series was tested with both IFAS and ELISA. The number of colony forming cells in starting suspension was determined by the dilution plate technique. Counts of bacteria were made on triplicated 50 µl droplets on multiwindow slides in IFAS. The number of fluorescent cells per ml of each dilution series was calculated by counting the average number of fluorescing cells in ~20 microscope fields for each window. The absorbance values at 405 nm in triplicated microplate wells were measured for each dilution series in ELISA.

RESULTS

Antisera were intially assessed by determining the agglutination titers (Table 1). The titers for antiserum II and III, prepared against glutaraldehyde-fixed cells were higher than for antiserum I, which is prepared against heat-treated whole cells. However, the highest titer was achieved with glutaraldehyde-fixed cells by combining with intramuscular and intravenous injections. The IFAS titers of each antiserum (I, II, III) were significantly higher than their agglutination titers. Antiserum III, prepared against glutaraldehyde-fixed cells had the highest IFAS titer and was used for all tests in this study. No reaction was determined with normal sera.

Table 1: Agglutination and indirect fluorescent antibody staining titers of three antisera produced against **Pseudomonas syringae pv. phaseolicola**

Antiserum number*	Antigen	Reciprocal of agg. titer slide agg.	Micro agg.	Reciprocal of IFAS titer
I	Heat-treated	100	250	1.000
II	Glutaraldehyde-fixed	250	1.000	5.000
III	Glutaraldehyde-fixed	250	2.500	10.000

(*) Rabbits for antisera I and II received five intramuscular injections; for antisera III one intramuscular followed by four intravenous injections.

In IFAS tests, at the dilution of 1/2.500 and 1/5.000 antiserum III stained all **P.s. pv. phaseolicola** strains tested, but other species were not stained. Less diluted antiserum crossreacted with some other **P. syringae** pathovars, unidentified fluorescent pseudomonads isolated from bean seeds, and **X. campestris pv. phaseoli** from other genera. However, only **P.s. pv. pisi, P.s. pv. glycinea** and **P.s. pv. lachrymans** gave weak fluorescence with antiserum diluted 1/1.000 while the other cross reacted bacteria were not stained at this dilution (Table 2).

Similar results were obtained in ELISA test. The less diluted antiserum (1/1000) cross-reacted with all **P. syringae** pathovars under test including **P. cichorii** and **X. campestris pv. phaseoli.** At the dilution of 1/5000, absorbance values of cross-reacted strains were quite smaller than those of all **P.s.pv phaseolicola** strains. However, cross reactions were nearly eliminated when using antisera diluted 1/10.000 (Table 3).

Table 2: Reaction of various bacterial species in indirect fluorescent antibody staining tests with **Pseudomonas syringae pv. phaseolicola** antiserum at six dilutions

Bacterial species and origins*	Antiserum dilutions					
	1/10	1/100	1/500	1/1000	1/2500	1/5000
P.s.pv. phaseolicola (NCPPB-52) race I	+	+	+	+	+	+
P.s.p. (1281A j.D. Taylor, U.K.) race I	+	+	+	+	+	+
P.s.p. (882 j.D. Taylor, USA) race II	+	+	+	+	+	+
P.s.p. (1299A j.D. Taylor, Tanzania) race II	+	+	+	+	+	+
P.s.p. (1776A j.D. Taylor, Bulgaria) race II	+	+	+	+	+	+
P.s.p. (1301A j.D. Taylor, Tanzania) race III	+	+	+	+	+	+
P.s.p. (1302A j.D. Taylor, Rwanda) race III	+	+	+	+	+	+
P.s.p. - 30 (Samsun-Türkiye)	+	+	+	+	+	+
P.s.p. - 2 (Niğde-Türkiye)	+	+	+	+	+	+
P.s.p. - 1 (Çubuk-Türkiye)	+	+	+	+	+	+
P.s.p. - 4 (Çankırı-Türkiye)	+	+	+	+	+	+
P.s.p. - 14 (Konya-Türkiye)	+	+	+	+	+	+
P.s.p. - 129 (Balıkesir-Türkiye)	+	+	+	+	+	+
P.s.pv. glycinea (NCPPB-2411)	+	+	+	±	-	-
P.s.pv. lachrymans (E.Kahveci, Türkiye)	+	+	+	±	-	-
P.s.pv. pisi (NCPPB-2585)	+	+	+	±	-	-
P.s.pv. syringae (NCPPB-1093)	+	+	±	-	-	-
P.s.pv. syringae (Ankara - Türkiye)	+	+	±	-	-	-
P.s.pv. tomato (Antalya - Türkiye)	+	+	±	-	-	-
Pseudomonas cichorii (E.Kahveci, Türkiye)	+	+	±	-	-	-
Pseudomonas marginalis (E.Kahveci, Türkiye)	+	±	-	-	-	-
Pseudomonas fluorescens (Ankara - Türkiye)	+	+	-	-	-	-
Pseudomonas tolaasii (T.F.Preece, UK)	-	-	-	-	-	-
Unidentified fleuroscent pseudomonades**						
1. Nevşehir	+	±	-	-	-	-
2. Samsun	+	±	-	-	-	-
3. Çorum	+	+	±	-	-	-
4. Kayseri	+	+	±	-	-	-
Agrobacterium tumefaciens (NCPPB-1651)	-	-	-	-	-	-
C.michiganense ssp. flaccumfaciens (NCPPB-1446)	-	-	-	-	-	-
C.michiganense ssp. michiganense (Ankara)	-	-	-	-	-	-
Erwinia amylovora (NCPPB-595)	-	-	-	-	-	-
E.carotovora ssp. atroseptica (SCRI-1039)	-	-	-	-	-	-
X. campestris pv. phaseoli (NCPPB-3035)	+	±	-	-	-	-
X.c. pv. phaseoli var. fuscans (Sinop-Türkiye)	+	±	-	-	-	-

(+) Positive (-) Negative (±) Weak fluorescence
SCRI-Scottish Crop Research Institute UK
NCPPB-National Collection of Plant Pathogenic Bacteria UK

* Bacterial species originated from Türkiye were isolated by the authors

** Fluorescent pseudomonads isolated from differently originated bean seeds

Table 3: ELISA reactions (at 405 nm) of **Pseudomonas syringae pv. phaseolicola** antiserum at three dilutions with representative strains of various bacteria

Bacterial species and origins*	Antiserum dilutions		
	1/1000	1/5000	1/10.000
P.s.pv. phaseolicola (NCPPB-52) race I	2.21**	1.97	1.82
P.s.p. (1281A j.D. Taylor, U.K.) race I	2.01	1.57	1.41
P.s.p. (882 j.D. Taylor, USA) race II	1.72	1.52	1.32
P.s.p. (1299A j.D. Taylor, Tanzania) race II	1.65	1.36	1.28
P.s.p. (1776A j.D. Taylor, Bulgaria) race II	2.05	1.86	1.56
P.s.p. (1301A j.D. Taylor, Tanzania) race III	1.82	1.65	1.46
P.s.p. (1302A j.D. Taylor, Rwanda) race III	2.08	1.73	1.55
P.s.p. - 30 (Samsun-Türkiye)	1.79	1.58	1.34
P.s.p. - 2 (Niğde-Türkiye)	1.55	1.45	1.28
P.s.p. - 1 (Çubuk-Türkiye)	1.79	1.58	1.48
P.s.p. - 4 (Çankırı-Türkiye)	2.16	1.96	1.74
P.s.p. - 14 (Konya-Türkiye)	1.78	1.48	1.34
P.s.p. - 129 (Balıkesir-Türkiye)	1.86	1.64	1.44
P.s.pv. glycinea (NCPPB-2411)	1.54	0.94	0.67
P.s.pv. lachrymans (E.Kahveci, Türkiye)	1.31	0.67	0.46
P.s.pv. pisi (NCPPB-2585)	1.17	0.55	0.39
P.s.pv. syringae (NCPPB-1093)	1.36	0.85	0.45
P.s.pv. syringae (Ankara - Türkiye)	1.28	0.72	0.36
P.s.pv. tomato (Antalya - Türkiye)	1.04	0.52	0.34
Pseudomonas cichorii (E.Kahveci, Türkiye)	0.73	0.37	0.33
Pseudomonas marginalis (E.Kahveci, Türkiye)	0.60	0.28	0.16
Pseudomonas fluorescens (Ankara - Türkiye)	0.46	0.34	0.27
Pseudomonas tolaasii (T.F.Preece, UK)	0.20	0.12	0.06
Unidentified fleuroscent pseudomonades***			
1. Nevşehir	0.12	0.04	0.03
2. Samsun	0.58	0.26	0.20
3. Çorum	0.79	0.38	0.21
4. Kayseri	0.66	0.42	0.34
Agrobacterium tumefaciens (NCPPB-1651)	0.22	0.12	0.06
C.michiganense ssp. flaccumfaciens (NCPPB-1446)	0.30	0.16	0.15
C.michiganense ssp. michiganense (Ankara)	0.10	0.08	0.06
Erwinia amylovora (NCPPB-595)	0.16	0.10	0.08
E.carotovora ssp. atroseptica (SCRI-1039)	0.41	0.25	0.20
X. campestris pv. phaseoli (NCPPB-3035)	1.09	0.65	0.36
X.c. pv. phaseoli var. fuscans (Sinop-Türkiye)	0.86	0.36	0.24

SCRI-Scottish Crop Research Institute UK
NCPPB-National Collection of Plant Pathogenic Bacteria UK

* Bacterial species originated from Türkiye were isolated by the authors

** Mean value of three wells

*** Fluorescent pseudomonads isolated from differently originated bean seeds

The results of the sensitivity tests with IFAS and ELISA has been shown on table 4.

Table 4: Sensitivity threshold of IFAS and ELISA

	IFAS			ELISA	
No. of bacteria in starting susp. cfu/ml*	No. of cell added to each well	No. of cell detected cell/ml*	% Recovery	No. of cell added to each well	Absorbance values at 405 nm*
8×10^3	4×10^2	0.9×10^2	22.5	8×10^2	0.16
8×10^4	4×10^3	1.3×10^3	32.5	8×10^3	0.17
8×10^5	4×10^4	0.8×10^4	20.0	8×10^4	0.42
8×10^6	4×10^5	1.2×10^5	30.0	8×10^5	0.78
8×10^7	4×10^6	**		8×10^6	1.20
		Mean 26.2			

* Average of three replicates

** Bacterial number detected were too high to be counted

DISCUSSION

A highly specific and high titre antiserum was produced against glutaraldehyde fixed cells of **P.s. pv. phaseolicola** by an immunazation procedure in combination with one intramuscular and 4 successive intravenous injections. Positive reactions were obtained by IFAS and ELISA with the all known and differently originated strains of **P.s.pv. phaseolicola** (Table 2, 3). No considerable cross-reactions were observed when using 1/2500 and 1/5000 antiserum dilution for IFAS and 1/10.000 for ELISA. Nevertheless, at the lower dilutions, few unidentified fluorescent pseudomonads isolated from dry beans, soma pathovars of **P.syringae (P.s.pv tomato, P.s.pv pisi, P.s. pv glycinea, P.s.pv syringae, P.s.pv lachrymans)** and **P.cichorii** cross reacted with the antisera in both tests. From the other genera under test, only **X.c.pv phaseoli** gave cross reaction with less dilluted antisera.

These cros reactions indicate that other organisms may have antigens similar to those on the **P.s.pv phaseolicola** cell wall. Lucas & Grogan (1969) reported that the antigens were common between isolates of **P.s.pv phaseolicola, P.s.pv mori, P.s.pv tomato** and **P.s.pv lachrymans.** Taylor (1970) also reported that antisera against **P.s.pv phaseolicola** gave cross reactions with whole non-autoclaved strains of **P.morsprunorum, P.antirrhini, P.maculicola, P.lachrymans, P.tomato, P.primulae,**

P.delphini, P.viridiflava and **P.cichorii.** However, these researchers also stated that the common antigen bands were particularly eliminated by heating antigen preparations. The specificity in our work may have ensued from an antigen preparation with glutaraldehyde fixation and serologic procedure. Likewise, Wyatt **et al.** (1989) showed typical cross reactions of some pathovars of **P.syringae (P.s.pv syringae, P.s.pv maculicola, P.s.pv lachrymans)** with competitive ELISA, and also emphasized that heating cells was not necessary to eliminate non specific reactions.

In our sensitivity assays detecting of **P.s.pv phaseolicola** cells in a suspension, IFAS turned out to be more sensitive than ELISA. Because, it was possible to detect 8 x 10^3 cell/ml with IFAS while 8 x 10^6 cell/ml were required obtaining positive results (absorbance values ≥ 1.0 at 405 nm) by ELISA (Table 4). However, percentage recovery of cells in IFAS was rather low with an average of 26.2 %. Wyatt **et al.** (1989) found the sensitivity level with fluorescence microscopy as 2.5 x 10^6 on slide, 2.5 x 10^5 on membrane, but also reported that many cells must had been lost during preparation or remained unstained. Nevertheless, the limit of detection in our ELISA was quite low compared to that of Wyatt **et al.** (1989). This is probably due to the coating plates with whole cells rather than exopolysaccharide. Much as ELISA had the advantage of applicability to large amounts, Barzic and Trigalet (1982), and Van Vuurde **et al.** (1983) demonstrated that ELISA in its present stage for development was, for the identification of **P.s.pv phaseolicola** in seed extracts, less sensitive than immunofluorescent (IF). Further work is necessary to improve the sensitivity of ELISA.

The work reported here showed that a rather specific and high titre polyclonal antisera against **P.s.pv phaseolicola** could be produced, and serological techniques such as ELISA, particularly IFAS could be used to complement current diagnostic procedures in seed certification and quarantine in Türkiye

ÖZET

Fasulyelerde Hale Yanıklığı Etmeni (Pseudomonas syringae pv phaseolicola)'nin Tanılanmasında iki Serolojik Yöntemin Değerlendirilmesi

P.s.pv phaseolicola'nın ısı uygulanmış ve glutaraldehitle fikse edilmiş hücrelerine karşı 2 farklı injeksiyon yöntemiyle tavşanlarda 3 antiserum üretildi. Farklı kaynaklardan **P.s.pv phaseolicola** bakterileri, kuru fasulye tohumlarından izole edilen fluoresan pseudomonaslar ve diğer bitki patojeni bakteriler 2 serolojik yöntemle; İndirekt Fluoresan Antikor Boyama (IFAS) ve ELISA ile testlendi. Bu iki testin duyarlılık sınırı ve test edilen bakteri türleri ile çapraz reaksiyonlar değerlendirildi. Üretilen 3 farklı antiserumdan, **P.s.pv phaseolicola** nın glutaraldehidle fikse edilen

hücrelerine karşı üretileni diğerlerinden daha spesifik bulundu. Hale lekesi hastalığı etmeni **P.s.pv phaseolicola**'nın tesbitinde IFAS'ın ELISA dan daha duyarlı olduğu saptandı.

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Effect of Soil Solarization on the Viability of **Verticillium dahliae** Kleb. Microsclerotia in Aegean Region of Türkiye

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ABSTRACT

Mulching with polyethylene sheets increased soil temperature. Temperatures achieved at the upper soil layers were in the range of those found to be lethal to the pathogens. The viability of ***Verticillium dahliae*** *microsclerotia either dissappeared completely or decreased greatly and remained very low at the end of 30 days in solarized plots.*

INTRODUCTION

Verticillium wilt, caused by **Verticillium dahliae** Kleb., is perhaps the most economically important disease in Aegean Region. Once the pathogen has been introduced into a field, it may survive for years in the soil. When wilt-susceptible cultivars are grown, losses can exceed about 75 % of the potential crop. In many instances, **Verticillium** wilt is a limiting factor in production. In the region, it is seen on cotton, vegetables, various fruit trees, vineyard and ornamental plants (Kamal and Saydam, 1970; Saydam and Kamal, 1970; Karaca **et al.,** 1971; Saydam **et al.**, 1971; Esentepe **et al.**, 1972; Saydam and Copçu, 1972; Saydam **et al**., 1973; Kocatürk and Karcılıoğlu, 1980; Kapkın and Arı, 1982; Sarıbay and Demir, 1984; Sezgin **et al.,** 1985).

Chemical control of **Verticillium** wilt is erratic and expensive, thus control has generally been restricted to the use of wilt-resistant cultivars, long-term rotations and virgin-land plantings. Although sometimes successful, these control methods are not always acceptable or practical to the grower. In many instances, the widely used commercial cultivars are higly susceptible to **Verticillium** wilt and longterm rotations or fallow periods are not economically feasible. Likewise the use of virgin land is becoming increasingly more difficult and costly.

Soil solarization (solar heating) is a significant departure from the old disinfestation procuderes used against soil-borne pathogens (Katan, 1981). This technique involves the use of a clear, polyethylene tarp placed over moist soil during a summer fallow period to trap radiation and build up enough heat to reduce populations of soilborne

pests (Katan, 1980). Numerous field experiments conducted around the world during the last 15 years have clearly shown that solar heating has potential for replacing traditional soil disinfestation methods in numerous crops (Stapleton and DeVay, 1986), provided that certain requirements, such as suitable climatic conditions and soil types, are met. Solarization has been shown to be effective on control of a number of diseases caused by soilborne pathogens, including **Fusarium** wilt of tomato (Katan, 1980; Tjamos and Faridis, 1980), damping-off caused by **Rhizoctonia solani** (Elad **et al.**, 1980; Katan, 1980; Pullman **et al.**, 1981b) and **Pythium** spp. (Pullman **et al.**, 1981 b), **Verticillium** wilt (Pullman **et al.**, 1981 a, 1981 b; Tjamos and Paplomatas, 1988).

The main objective of this study was to investigate the effectiveness of soil solarization on the viability of **Verticillium dahliae** microsclerotia under the Aegean Region conditions.

MATERIALS AND METHODS

Production of microsclerotia

Microsclerotia of **V.dahliae** were produced on wheat straw according to Katan et al. (1976). For this, autoclaved wheat straw pieces 5 mm long were infested with conidia of the pathogen and incubated at 24 °C. After 3 weeks they were densely colonized with microsclerotia and conidia. The conidia were removed by washing the straw pieces with running water. The straw pieces were then dried, ground and sieved to 0.97 mm.

Artificial infestation of soil with microsclerotia

Soil from Institute experimental field was air dried and passed through 2 mm sieve before use. Microsclerotia were added to soil at the rate of 3 % (w/w). Thirty g soil samples were distributed in 15 x 15 cm nylon cloth pieces and tied with nylon strings into small bags. These bags were uniformly buried in experimental plots at 5, 10 and 20 cm depth.

Application of solarization

Soil solarization was conducted on 18 July 1991 in the Institute experimental field. The soil was divided into 12 plots (1 x 3 m): a) three days after irrigation, three plots were tightly covered with transparent polyethylene sheets (100 μm thick); b) immediately after irrigation, similarly, three plots were tarped; c) three plots were tarped without irrigation for simultaneous solarization; d) three plots were left untarped and used as untreated controls.

The polyethylene was left in place for 30 days and the maximum values of temperatures of the solarized and non-solarized soil were measured by inserting thermometers at soil depths of 5, 10 and 20 cm. Readings were recorded from 2 p.m. until 5 p.m. The polyethylene was removed on 16 August.

Quantitative assessment of the viable Verticillium dahliae sclerotia

Microsclerotia of **V. dahliae** were quantified from 3 bags of each treatment containing microsclerotia recovered from 5, 10 and 20 cm depths. Bags were collected at 0 time and 3, 6, 12, 18 and 30 days interval. Triplicate 15 g samples of air-dried soil were treated by wet sieving as described by Huisman and Ashworth (1974) with the following modifications: the soil was washed through sieves of mesh 150 and 42 µm; the residue retained by the 42 µm sieve was washed for 30 s with 40 g/l NaOCl, transferred to 50 ml tubes and made up to 30 ml with sterile distilled water. The soil particles were left to precipitate for 30 min and excess water was removed by aspiration. The final 10 ml residue was spread uniformly over 10 agar plates containing a verticillium-selective growth medium (Ausher **et al.**, 1975) and incubated for 21 days at 18 C. **Verticillium dahliae** colonies were counted.

Percentage of pathogen control due to solarization was calculated as (1- B/A x 100) where A: number of microsclerotia per 15 g soil in the non mulched soil; and B: the corresponding data in the mulched soil.

RESULTS

Effect of solarization on soil temperatures

The maximum temperatures recorded in solarized soil were about 10 °C higher than in the uncovered plots (Table 1). Maximum temperatures in covered plots reached 55 and 48.0 °C at depths of 5 and 10 cm respectively, as compared to 48 and 39 °C in the control plots (5 and 10 cm). Maximum temperature recorded during one month period is presented in Fig 1.

Table 1. Maximum soil temperatures recorded between 18 July and 16 August 1991, in the Institute experimental field, Bornova

Soil treatment	Maximum soil temperature in °C at depths of					
	5 cm		10 cm		20 cm	
	mini-mal	maxi-mal	mini-mal	maxi-mal	mini-mal	maxi-mal
Solarized:*						
- Three days after irrigation	51	55	45	48	37	39
- Immediately after irrigation	52	56	46	49	37	39
- without irrigation	49	53	42	45	33	35
Control	44	48	36	39	32	34

* Transparent 100 µm polyethylene

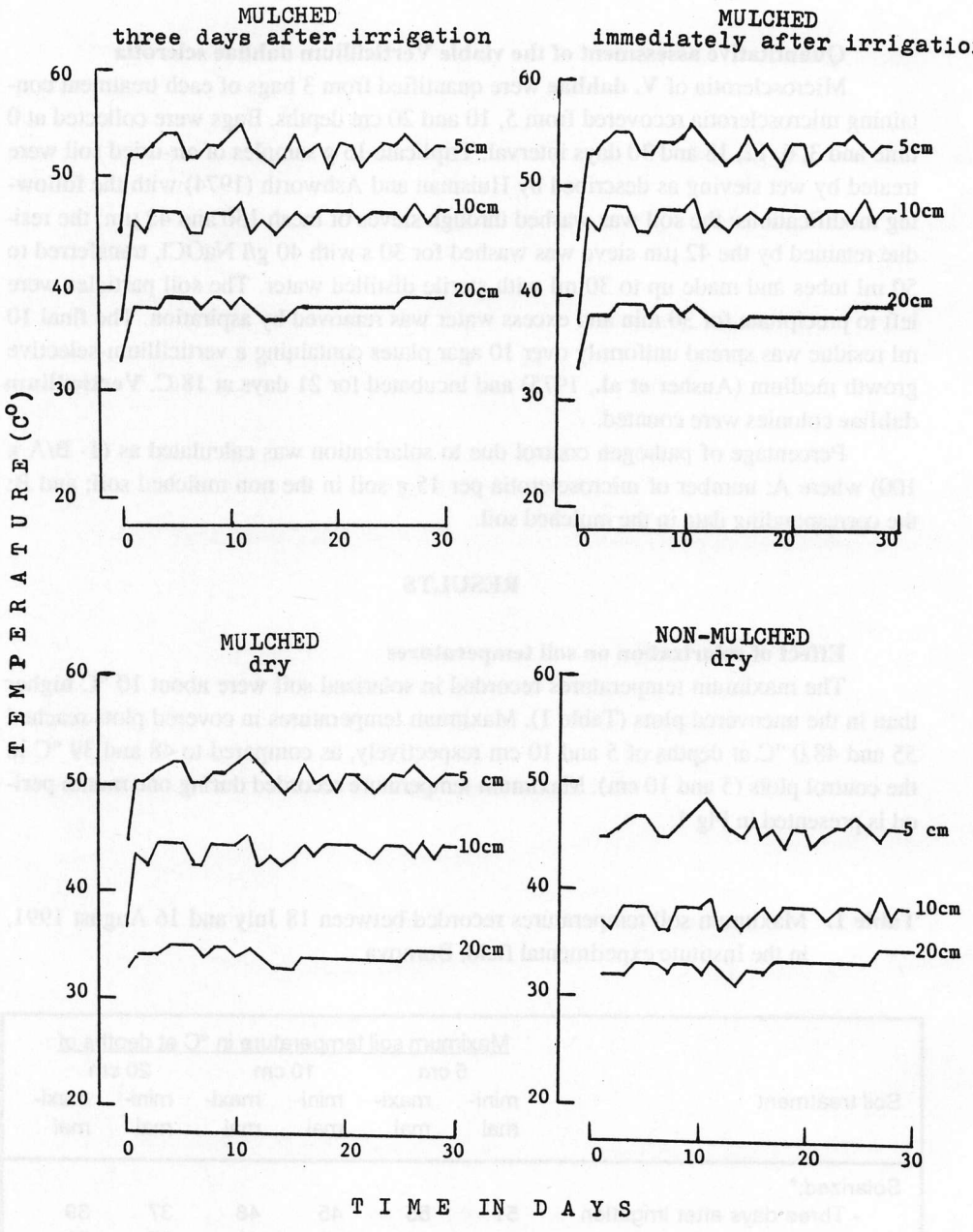

Fig. 1. Effect of soil mulching with transparent polyethylene sheets on the increase in soil temperature under wet and dry conditions (Daily maximum temperature of soil between 18 july - 16 August, Bornova)

Effect of solarization on the viable V.dahliae microsclerotia

Populations of **V.dahliae** microsclerotia were either eradicated or greatly reduced and remained very low at the end of 30 days in solarized plots. Population reductions (%) at depths of 5, 10 and 20 cm are seen in Table 2. The decrease in viability of microsclerotia of **V. dahliae** in soil under wet and dry conditions is presented in Fig. 2.

Table 2. Effect of 3, 6, 12, 18 and 30 days of soil mulching with polyethylene sheets on the population of **V. dahliae** microsclerotia

Treatments	Population reduction* (%) at dephts of 5 cm	10 cm	20 cm
3 days			
Solarized:			
- three days after irrigation	24,0 a**	13,0 b	1,0 ef
- immediately after irrigation	26,0 a	15,0 b	1,1 ef
- without irrigation	7,0 c	2,9 de	0,4 ef
Control	5,9 cd	0,9 ef	0,3 ef
6 days			
Solarized:			
- three days after irrigation	58,0 a	43,0 b	9,0 d
- immediately after irrigation	60,0 a	42,3 b	10,3 d
- without irrigation	45,0 b	31,0 c	2,9 e
Control	9,0 d	2,0 ef	1,1 f
12 days			
Solarized:			
- three days after irrigation	75,0 a	60,0 b	52,9 c
- immediately after irrigation	78,0 a	63,0 b	57,0 bc
- without irrigation	61,9 b	51,9 c	6,0 e
Control	25,0 d	9,0 e	2,0 f
18 days			
Solarized:			
- three days after irrigation	84,9 a	72,9 bc	61,9 e
- immediately after irrigation	89,0 a	75,9 b	69,9 cd
- without irrigation	72,9 bc	63,9 de	16,9 g
Control	36,0 f	12,0 g	3,9 h
30 days			
Solarized:			
- three days after irrigation	97,9 b	93,9 c	86,9 d
- immediately after irrigation	100,0 a	97,0 b	90,0 c
- without irrigation	92,9 c	71,9 e	20,9 g
Control	38,0 f	17,9 g	5,0 h

* Values are the means of three replicates

** Within a column, values followed by the same letter are not significantly different at P = 0,01 according to Duncan's Multiple Range Test.

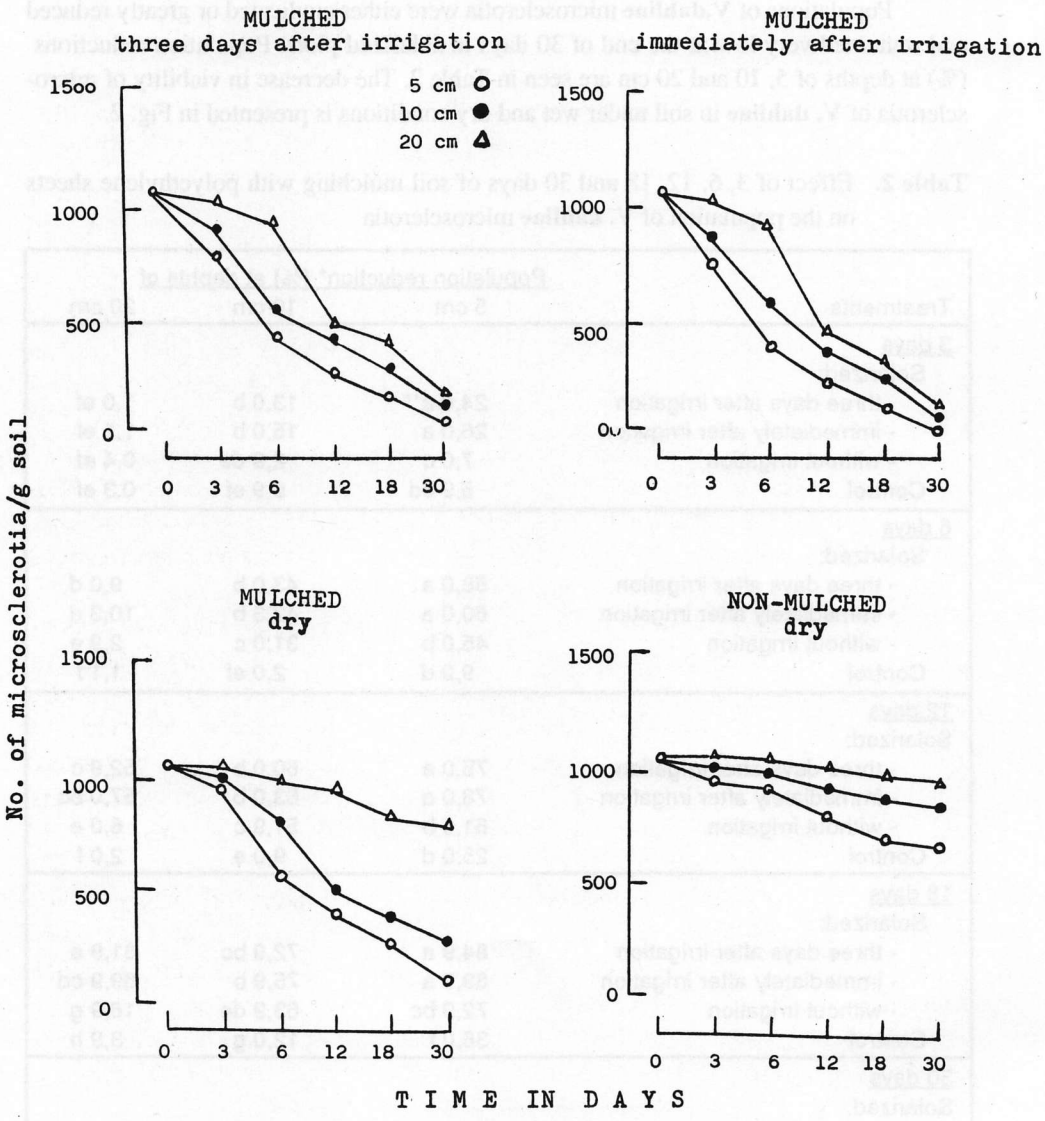

Fig. 2. Effect of soil mulching with transparent polyethylene sheets on the decrease in viability of microsclerotia of **Verticillium dahliae** in soil under wet and dry conditions (18 july - 16 August, 1991, Institute Experimental plots, Bornova)

DISCUSSION

Soil solarization has been shown to have a detrimental effect on several soil-borne pathogens (Katan, 1981). **V. dahliae** causing **Verticillium** wilt of several annual crops could be successfully controlled by this technique (Katan **et al.**,1976; Pullman **et al.**, 1981 b). Data obtained in Aegean Region of Türkiye demonstrate that mulcing with polyethylene sheets increased soil temperature and resulted in pronounced reduction in the populations of **V.dahliae** microsclerotia.

From this study, it is seen that there was no difference among the population of **V.dahliae** microsclerotia at depths of 5, 10 and 20 cm in soil solarized, three days after irrigation and immediately after irrigation, on 3rd, 6th, and 12th day. Difference among the population reductions occured at depth of 20 cm on 18th day. The population reduction in soil solarized immediately after irrigation was higher than in soil solarized three days after irrigation. Similarly, these differences were pronounced at depths of 5, 10 and 20 cm on 30th day (Table 2). The differences in the population reduction in solarized soil may be due to different moisture ratio in soil. Moist soil, either irrigated before mulching or irrigated under the plastic film, increases the thermal sensitivity of soil-borne pathogens as well as heat transfer or conduction in the soil, and it is known that saturated soils are optimal (Stapleton and DeVay, 1986).

From the study it follows that temperatures achieved at the upper soil layers by mulching in Aegean Region are in the range of those found to be lethal to the pathogens (Nelson and Wilhelm, 1958). Thermal death studies of various micro-organisms in vitro have shown that at or above 50 °C, survival is limited to a maximum of a few hours. At temperatures of 37-50 °C eradication or marked reductions in viable populations occur within 2-5 weeks (Pullman **et al.**, 1981 a and b). At lower soil layers, where the temperatures were lower, the populations were also reduced. This may be due to a direct cumulative effect of sublethal temperature on the microsclerotia.

Consequently, our results demonstrate that soil solarization for 30 days may be effective on **Verticillium** wilt under the Aegean Region conditions. But these results were obtained in the Institute experimental field. Mulching for disease control might be effective in the Aegean Region if its effectiveness is increased, e.g. by mulching the whole field, by extending the mulching period. In glasshouses, this method might be especially easy to handle. It will be interesting to examine this method in the fields and glasshouses naturally infested with **V.dahliae.**

ÖZET

Ege Bölgesinde Toprak Solarizasyonunun **Verticillium dahliae** Kleb. da Mikrosklerot Canlılığı Üzerine Etkisi

Polyethylene örtülerle toprağı örtme işlemi, toprak sıcaklığını arttırmıştır. Üst toprak tabakalarında ulaşılan sıcaklık, patojenlere letal olarak saptanan sıcaklık sınırları içerisinde oluşmuştur. 30 günlük toprak solarizasyonunun sonunda **V.dahliae**'nin canlı kalan mikrosklerot sayısı ya büyük oranda azalmış ya da canlılık tamamen yok olmuştur.

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Anastomosis Groups and Pathogenicity of **Rhizoctonia solani** Kühn Isolates From Potatoes in Erzurum - TÜRKİYE

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ABSTRACT

*184 isolates of **Rhizoctonia solani** Kühn from sclerotia on tubers and from lesions and mycelia on stems were obtained from potatoes grown in Erzurum-Türkiye. Most of the isolates (88.04 %) recovered belonged to AG-3. The rest were members of AG-2 type 1, AG-2 type 2, AG-4 and AG-5 which accounted for 1.09 %, 1.63 %, 5.44 % and 3.80 respectively.*

In pathogenicity tests, isolates of AG-3 were found to be the most virulent on potato cultivar Resy. On the other hand the infections caused by the isolates of sclerotia and lesions were somewhat more severe than mycelial ones.

INTRODUCTION

Rhizoctonia solani Kühn, (teleomorph **Thanatephorus cucumeris** (Frank) Donk) the causal agent of stem canker and black scurf of potatoes (**Solanum tuberosum L.**), occurs in all potato growing areas of the world (Frank, 1986) and has a wide host range (Mordue, 1974). The pathogen is also found to be widely distributed in the potato fields of Erzurum-Türkiye (Demirci and Döken, 1989).

R.solani is divided into anastomosis groups (AGs) based on hyphal anastomosis. Hyphal anastomosis occurs as perfect fusion, imperfect fusion and contact fusion (Parmeter **et al.**, 1969; Ogoshi, 1976). So far 11 anastomosis groups (AG-1, AG-2, AG-3, AG-4, AG-5, AG-6, AG-7, AG-8, AG-9, AG-10 and AG-BI) have been determined (Parmeter **et al.**, 1969; Ogoshi, 1975; Kuninaga **et al.**, 1978; Homma **et al.**, 1983; Neate and Warcup, 1985; Carling **et al.**, 1987; Ogoshi **et al.**, 1990) Among them AG-2 was further divided into two sub groups (AG-2 type 1 and AG-2 type 2) by the relative frequency of hyphal fusion between the isolates (Ogoshi, 1975).

The isolates collected from potatoes in various parts of the world fall into AG-1, AG-2 type 1, AG-2 type 2, AG-3, AG-4, AG-5 and AG-9; among them AG-3 is most frequently obtained (Abe and Tsuboki, 1978; Chand and Logan, 1983; Bolkan and

Rıbeiro, 1985; Carling and Leiner, 1986; Carling **et al.**, 1987; Bandy **et al.**, 1988; Anguiz and Martin, 1989). As a matter of fact AG-3 is also the only anastomosis group isolated from stems, hypocotyls and tubers of potatoes in the Central Anatolia Region of Türkiye (Tuncer and Erdiller, 1990).

The objective of this study is to determine which anastomosis groups are the cause of **Rhizoctonia** disease of potatoes in Erzurum-Türkiye and to find out and compare the pathogenicity of these anastomosis groups.

MATERIALS AND METHODS

Collection, isolation and identification

Plants exhibiting symptoms of **Rhizoctonia** disease and sclerotia bearing tubers were randomly taken from potato fields and storages from Erzurum province respectively. Initially stems bearing mycelia or lesions and tubers having sclerotia were washed from soil under tap water. Then 0.5 x 1 cm^2 tissue pieces taken from the samples were surface-disinfected in 0.5 % sodium hypochlorite for 1 min. and placed on 1.5 % water agar containing 50 mg/l streptomycin sulfate. After 48-72 hours of incubation at room temperature the discs taken from the margins of actively growing colonies containing hyphal tips were transfered on to water agar slants. Pure **R. solani** isolates obtained in this way were transfered to potato dextrose agar (PDA) in test tubes and kept at 10 °C to be used in these studies.

The identification of **Rhizoctonia** isolates as **R. solani** were done by determining the presence of multinucleated cells having dolipore septum in the hyphae (Ogoshi, 1987) taken from 2-3 day old cultures grown on PDA and stained by Safranin -0 method as described by Bandoni (1979) and by considering the hyphal fusion of multinucleated isolates with tester isolates.

Anastomosis group typing

The isolates obtained from stems and tubers of potatoes were paired with tester isolates (AG-1, AG-2 type 1, AG-2 type 2, AG-3, AG-4, AG-5, AG-6, AG-7, AG-8, AG-9, AG-10 and AG-BI) supplied from various sources. Mycelial discs (5 mm in diameter) taken from either actively growing tester isolates and the isolates recovered from stems and tubers were placed 2-4 cm apart on 2 % water agar in 9 cm diameter petri dishes (Parmeter **et al.**, 1969). They were incubated usually for 48-72 hours at room temperature until advancing hyphae made contact and slightly overlapped. A 4-5 cm^2 portion in the area of contact was then removed, placed on a slide, stained with 0.05 % trypan blue in lactophenol and examined with light microscope (Carling and Leiner, 1986) or with phase-contrast microscope without staining. This portion was observed

from the point of perfect, imperfect and contact fusions (Parmeter **et al.**, 1969; Ogoshi, 1976).

All isolates either from stem and tuber were paired with a tester isolate AG-3. The isolates failing to anastomose with AG-3 were subjected to hyphal anastomosis with other tester isolates until anastomosis affiliation had been established.

Pathogenicity testing

Pathogenicity of AG-3 isolates selected according to their source of isolation and all the isolates of other anastomosis groups were determined on potato sprouts. Single eye seed pieces cut from the tubers (potato cultivar Resy susceptible to **R. solani)** which had been surface-disinfected in 2 % (v/v) formaldehyde for 5 min. were kept in moist chamber at room temperature for two weeks before planting for formation of suberine layer (Bandy **et al.**, 1984). In the preparation of inoculum for infestation of soil, 98 % sand + 2 % maize meal mixture moisturized at 20 % (w/v) level and autocloved twice for 1 hour at 24 hour intervals (Papavizas and Ayers, 1965). Then four pieces of agar discs (5 mm in diameter) containing mycelia cut from 15 days old cultures were added to every 30 g sand + maize meal mixture and incubated at 25 °C for 18 days. After sprouting was initiated seed pieces were placed on one cm thick layer of sand + soil mixture (2:1) at the bottom of 10 cm diameter plastic pots and covered with 5 cm of sand + soil mixture. Then 7.5 g of inoculum for each pot was dispersed on to surface and covered with sand + soil mixture. Four pots were inoculated with each isolate. Plants were grown in growth cabinets under alternative 12 hours fluorescent light and 12 hours dark at 18-24 °C. Two monhts after inoculation plants were removed, washed free of soil, and damage on plants were assessed according to disease index indicated by Carling and Leiner (1986). The isolates collected from those plants were paired with tester isolates from the appropriate group to confirm anastomosis group identity.

RESULTS AND DISCUSSION

Anastomosis groups.

A total of 192 **Rhizoctonia** isolates were recovered from lesions and mycelia on stems and from sclerotia on tubers. 184 isolates possessed multinucleate cells with dolipore septa were identified as **R. solani.** The remaining 8 isolates were binucleate **Rhizoctonia**-like fungi. As a result of pairing **R. solani** isolates with tester isolates five anastomosis groups belonging to AG-2 type 1, AG-2 type 2, AG-3, AG-4 and AG-5 were determined. As seen in the distribution of them based upon their isolation source (Table 1) most of the isolates were members of AG-3.

Table 1. Anastomosis group typing of **Rhizoctonia solani** isolates collected from potatoes in Erzurum, Türkiye

Source of isolate	Number of isolates	*R. solani* AG (Anastomosis groups)				
		AG-2 type 1	AG-2 type 2	AG-3	AG-4	AG-5
Mycelium (Stem)	34	2	2	22	5	3
Lesion (Stem)	68	-	-	61	4	3
Sclerotium (Tuber)	82	-	1	79	1	1
Total	184	2	3	162	10	7
% of total		1.09	1.63	88.04	5.44	3.80

Among the 102 **R. solani** isolates obtained from mycelia and lesions on stems 81.37 %, 1.96 %, 1.96 %, 8.83 % and 5.88 % were members of AG-3, AG-2 type 1, AG-2 type 2, AG-4 and AG-5 respectively. From the out of 82 tuberborne sclerotial isolates 96.34 % belonged to AG-3 and remainder to AG-2 type 2, AG-4 and AG-5 with somewhat equal rates. In accordance with the similar studies about the anastomosis groups and their distribution according to different sources of isolation (Abe and Tsuboki, 1978; Davis, 1978; Chand and Logan, 1983; Carling and Leiner, 1986; Bandy et al., 1988; Tuncer and Erdiller, 1990). AG-3 isolates were also obtained more commonly from plants and tubers of potatoes in our studies. However Bolkan and Ribeiro (1985) isolated only AG-4 from stems and AG-3 from tubers of potatoes. In the studies of Anguiz and Martin (1989) AG-4 was recovered more frequently than AG-3 on roots and stems, whereas only isolates of AG-3 were isolated from tubers. The isolates of AG-2 type 1 were obtained only from stems although they were also isolated from tubers by Chand and Logan (1983) and both from stems and tubers by Carling and Leiner (1986). On the other hand AG-1 obtained both from tubers and plants (Abe and Tsuboki, 1978; Chang and Tu, 1980; Bandy **et al.**, 1988) and AG-9 only from plants (Carling **et al.**, 1987) were not encountered in our isolations. AG-2 type 2 which was only recovered from tubers by Abe and Tsuboki (1978) were present both on stems and tubers in this work. AG-4 and AG-5 were obtained from stems and tubers as in the studies of Abe and Tsuboki (1978), Chang and Tu (1980), Bolkan and Ribeiro (1985), Bandy **et al.**, (1988), Anguiz and Martin (1989).

Pathogenicity tests.

The pathogenicity tests carried on Resy potato cultivar showed that the isolates belonging to five anastomosis groups recovered from the stems and tubers of potatoes

grow in Erzurum Region (Table 2) were capable of causing infection, however isolates of AG-3 were appearently more virulent than others. Thus AG-3 was observed to induce sunken big brown necrotic lesions on the subterranean portions of potato plants in contrast to small superficial lesions associated with isolates of other anastomosis groups.

Table 2. Disease reactions on potato sprouts infected by the isolates of **Rhizoctonia solani** anastomosis groups obtained from stems and tubers of potatoes from Erzurum, Türkiye.

Anastomosis groups of isolates	Origin of isolates	Number of isolates	Disease index* mean
AG-2 Type 1	Mycelium	2	1.19
AG-2 type 2	Mycelium	2	0.75
	Sclerotium	1	1.75
AG-3	Mycelium	10	2.60
	Lesion	10	3.05
	Sclerotium	10	3.07
AG-4	Mycelium	5	0.65
	Lesion	4	0.88
	Sclerotium	1	1.75
AG-5	Mycelium	3	0.21
	Lesion	3	1.04
	Sclerotium	1	1.07

* Disease index: 0 = no lesions, 1 = one to several lesions less than 1 mm in diameter, 2 = several lesions 1-3 mm in diameter, 3 = lesions larger than 3 mm in diameter and sprout girdling and 4 = sprout girdling and sprout death (Carling and Leiner, 1986).

The evidences about the extensive occurrence and more virulence of AG-3 isolates in Erzurum (one of the major potato growing region of Türkiye) imply that members of, AG-3 which is reported to be the principal cause of *Rhizoctonia* diseases in various parts of the world (Tsuboki **et al.**, 1977; Abe and Tsuboki, 1978; Davis, 1978; Chand and Logan, 1983; Carling and Leiner, 1986; Carling **et al.**, 1986; Bandy **et al.**, 1988) are also potentially responsible for stem canker and black scurf of potato in Erzurum-Türkiye. Anguiz and Martin (1989) indicated that the isolates of AG-3 which are more virulent in cool environments at high elevations were lower in agressiveness than AG-4 in warm conditions at low altitudes. From this point of view the dominance of AG-3 isolates can be expected in Erzurum Region since it is located at high altitudes (1800-2000 m high from sea level) where cool environmental conditions prevail. The results of artificial inoculations done by using the members of various anastomosis

groups obtained from different isolation sources (Table 2) showed that usually isolates from lesions and sclerotia appeared to be somewhat more virulent than mycelial isolates. However in a similar study of Carling and Leiner (1986) with isolates of AG-2 type 1 and AG-3, it was found that sclerotial isolates cause less infection than isolates from lesions and mycelia. Differences between these two studies about the virulence of a given anastomosis group from the similar sources might partly arise from the different virulence of the isolates obtained from different locations.

ÖZET

Erzurum İlinde Patateslerden Elde Edilen **Rhizoctonia solani** Kühn İzolatlarının Anastomosis Grupları ve Patojenisiteleri

Erzurum ilinde patates yetiştirilen alanlardan toplanan yumrulardaki sklerotium'lar ile gövdelerdeki lezyon ve miselyumlardan 184 **Rhizoctonia solani** Kühn izolatı elde edilmiştir. İzotların çoğunluğunun (% 88.04) AG-3'e ait olduğu saptanmıştır. Geri kalanların % 1.09'unun AG-2 tip 1, % 1.63'ünün AG-2 tip 2, % 5.44'ünün AG-4 ve % 3.80'inin AG-5 olduğu belirlenmiştir.

Resy patates çeşidi ile yapılan patojenisite testlerinde AG-3 izolatlarının en virulant olduğu gözlenmiştir. Diğer taraftan sklerotial ve lezyon izolatları miselyum izolatlarına göre daha yüksek enfeksiyona neden olmuştur.

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A New Carnation Disease For Türkiye; **Cladosporium** State of **Mycosphaerella dianthi**

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Cladosporium state of **Mycosphaerella dianthi (C. echinulatum)** was detected on diseased carnation plants sent to our department of Plant Protection for identification from Antalya. It has also been reported to be widespread in that province.

The disease caused spots specially on the leaves and calyx. Intensive leaf spots gave the plants a blighted appearence (Figure 1a) and the spots were round and tan in colour. They appeared in concentric zones due to sporulation of pathogen, which was light brown (Figure 1b and c). The pathogen produced pale or mid pale brown conidia of 1-4 septate on flexious, often geniculate conidiophores. They were straight or slightly curved, oblong or cylindrical and rounded at the ends (Figure 1d, e, f). Colonies of the pathogen were effuse and olivaceous gray in both PDA ve MEA (Figure 1g and h) and their mean diameters were 1.6 and 1.5 cm respectively in 8 days.

Since there is only a **Cladosporium** sp. reported on carnation, that is **Cladosporium echinulatum,** and its description is in accordance with the literature (Ellis, 1983). Identification of the pathogen did not create any difficulty.

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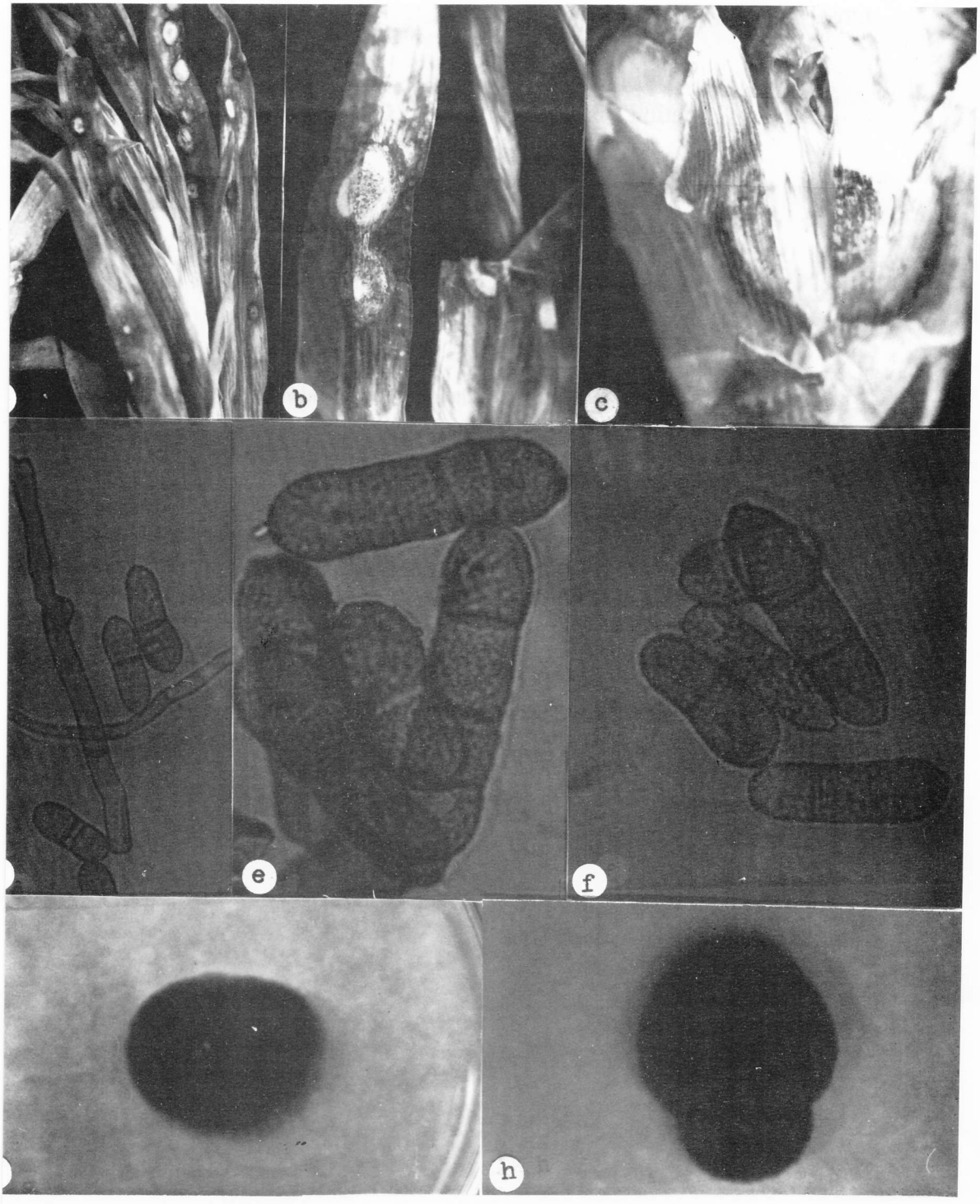

Figure 1: Various aspects of **Cladosporium echinulatim.** a,b) Symptoms on the leaves, c) Symptoms on the calyx, d) Geniculate conidiophore, e, f) 2-4 celled conidia, g) A colony in PDA, h) A colony in MEA

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