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Use of 5' - Labeled ds RNAs as Probe to Detect Citrus Tristeza Virus (CTV)

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ABSTRACT

Citrus tristeza virus (CTV) nucleic acid was detected in clarified sap spotted onto the nitrocellulose membrane by hybridization with end-labeled ds RNAs probe. Specific probes were prepared from citrus tristeza virus (CTV) double stranded ds RNA in extracts of infected plants by purifying and labeled the 5' ends with radioctive nucleotides using polynucleotide kinase.

INTRODUCTION

Citrus tristeza virus (CTV), a member of closterovirus group, (Bar-Joseph *et al.,* 1979), is economically the most important viral disease of citrus and one of the major disease problems affecting citrus production worldwide. Millions of trees on sour orange rootstack have been killed by CTV infection in Argentina, Brazil, the United States, Spain and Venezuela. There are many strains of CTV which differ in their biological activity (Bar-Joseph *et al.,* 1981).

Citrus tristeza virus (CTV) has been wide spread in Florida for many years (Garnsey and Jackson, 1975), and Most of CTV strains were mild and not cause decline (Lee *et al.,* 1987). The planting of citrus trees on sour orange rootstock has been increasing in Florida throughout the last decade. With increased plantings on sour orange areas of CTV induced quick decline and severe dwarfing have develoned some parts of the Florida. Infection by severe CTV strains causes considerable economic loss due to decline of trees on sour orange rootstock (Bar-Joseph *et al.,* 1981).

Diagnosis of plant viruses can be diffucult when one is dealing with unstable viruses, unusual strains, or viruses in wody plants. The detection of virus infection in plant tissue samples has traditionally involved biological, immonological and physical techniques. Serology is the most widely used method for CTV detection because serological tests are simple and practical. However, there is need, expecially in Florida, for a rapid diagnostic test to differentiate CTV strains (Rosner *et al.,* 1986).

A rapid and sensitive dot blot hybridization technique is defined that can be ued to detect CTV in nucleic acids. Purified nucleic acids are prepared from infected plant, spotted onto a nitrocellulose membran, and hybridized with a 5' labeled ds RNA (Garger *et al.,* 1983).

This paper reports on the use of a ^{32}p labeled ds RNA probe isolated CTV infected plants for detection of CTV in plant extracts.

MATERIALS and METHODS

CTV isolates and plant material.

45 samples of CTV infected and 2-healthy samples from Beltsville collection were collected for dot blot onto nitrocellulose membran, and used in this study.

Two isolates of CTV from Florida were used for probe preparation. The T-30 isolate produces very mild symptoms and little stunting on Mexican Lime, does not produce seedling yellows symptoms on indicator plants, and does not cause decline of trees on sour orange rootstock. The T-36 isolate produces severe veinclearing, stunding and stempitting on Mexican Lime mild seedling yellows symptoms on Eureka Lemon and sour orange seedlings and quick decline of sweet orange trees on sour orange rootstock.

Virus Purification and Extraction of RNA

Purified CTV was prepared essentially as described by Lee *et al.,* (1981).

The RNA was extracted from purified virus preparetions using phenol mix (phenol/choloform/pentanol 24/24/2 v/v/v). The tubes were sealed, kept on ice, and shaken every 1-2 min over a 15 min time.

The aqueous phase was removed after centrifugation for 10 min at 10.000 g, and 12.5 ml of cold 95 % ethanol and 50 µl of 3.0 M NaAc were added to precipitate the nucleic acids. The precipitate was collected by centrifugation, at 10.000 g for min. Pellet was air dried, resuspended in 0.4 ml sterile water, and transferred to microcentrifuge tube RNA samples were precipitated by adding 0.8 ml of cold 95 % ethanol and 40 µl 3.0 M NaAc, and then stored at - 20 °C for later use.

Preparation of Nitrocellulose Membrane

Dots were prepared by applying purified nucleic acid samples (2-5 µl) onto a nitrollulose membrane presoaked in 20 x SSC buffer. After spotting, the paper was air dried, and then baked for 4 hrs at 75-80 °C.

Extraction of double-stranded RNAs for Preparation of Probe

The dsRNAs were extracted and purified from infected citrus tissue by modifying the procedure used to detect citrus viroid (Duran-Vila *et al.,* 1986).

Five grams of tender bark tissue were ground to a powder in liquid nitrogen, then transferred to a 50 ml centrifuge tube containing 10 ml of extraction buffer (0.4 M Tris, 1 % SDS, 0.005 M EDTA pH 8.9), a mixture of liquified phenol/choloform/pentanol (96:96:8 v/v/v). The mixture was shaken vigorously several times for the next 15 to 20 min, then centrifuged at 10.000 g at 4 °C for min. The aqueous phase was transferred to a sterile tube and added 1/10 volume of 3M sodium acetate, pH 5.5, 3 volumes of 95 % cold ethanol, then stored, -20 °C for min or longer. Pellet was collected by centrifugation for 10.000 g for 20 min at 4 °C and resuspended with TKM buffer (0.01 M Tris, 0.01 M KCl, 0.0001 M $MgCl_2$, pH 7.4). The suspension was transferred to boiled dialysis tubing, dialyzed overnight on a magnetic stirrer in cold room against 1 Liter of TKM buffer.

Sample was removed from dialysis tubing into centrifuge tube, added 1 volume of 4 M LiCL, mixed gently then holded at 4 °C to overnight. Pellet was collected by centrifugation at 10.000 g at 4 °C for 20 min, resuspended in 1 ml of 0.1 m NaCl plus 0.05 M $MgSO_4$ plus 0.2 M NaCl pH 5.0, added 10 µl DNase I stock solution, and then incubated for 30-40 minutes at room temparatures with slow shaking. The mixture was made to 17.5 % ethanol (v/v) by the addition of 1.225 ml of cold 95 % ethanol and added 4 ml STE buffer (0.5 M Tris, 0.01 M EDTA, 1.0 M NaCl pH 6.9). After mixing well, 1.0 g CF 11 Whatman cellulose powder was added. This suspension was poured into colomn. The cellulose precipitate was washed with 50 ml of STE buffer containing 17 % ethanol until color clears. Material bound to the cellulose were eluted by adding 6 ml STE buffer, collected into autocloved tubes, precipitated by adding 3 volumes cold 95 % ethanol and 600 µl of 3 M sodium acetate and stored overnigh at -20 °C. The precipitate was collected by centrifugation at 10.000 g for 20 min. at 4 °C. The pellet was aid dried, resuspended in 0.3 ml TKM buffer, then transferred into sterile 1.5 ml microcentrifuge tubes. Nucleic acids were precipitated in 0.9 ml of cold 95% ethanol and 30 µl of 3.0M sodium acetate. The dsRNs were stored at -20 °C.

End-Labelling of dsRNA

Labeling of the purified dsRNA with ^{32}p was carried out mainly as described by Rosner et al, (1983). The dsRNA-containing pellet obtained by contrifugation was dried and resuspensed in 9 µl of Depc Traated H_2O, and then added 1 µl of 10 x Hydrolysis buffer. Suspansion was heated in a boiling water bath for 1-2 min. After quickly cooling on liquid nitrogen, added 3 ul of ^{32}p ATP, 5 ul of H_2O, 5 µl of 5 x PNK that is prepared into siliconized tube, and 2 ul of T Polynucleatide kinase (PNK) enzyme. The mixture was incubated at 37 °C for 1 1/2 hours, and the reaction was terminated by adding 4 µl of 10 % SDS. The labeled dsRNA was separated from free ATP by G- 25 spin column. Specific activity of about 5 x 10^6 cpm per 1 µg of dsRNA was obtained.

Dot-blot Hybridization and Autoradiography

The ^{32}p dsRNA probe was denatured at 100 °C for 2 min followed by rapid cooling on liquid nitrogen. Any free sites on nitrocellulose were blocked with prehybridization solution before the labeled dsRNA probe was hybridized to the nucleic acids. Pre hybridization was carried out at 42 °C in a buffer containing 50 % formamide, 20 x SSC, 20 x Denhardt solution, 50 mM sodium phoshate buffer pH 6.5, and 500 ug/ul salmon sperm DNA (Rosner and Bar-Joseph, 1984). Hybridizations were done with labeled dsRNA probe in same prehybridization solution. The nitrocellulose membran was washed in 20 x SSC, 10 % SDS, 5 % tetra Na pyrophosphate 4 times at room temperature for 15 min each time after hybridization to remove unbound probe, and then washed again 2 time at 55-60 °C in 2 x SSC, 0.1 % SDS, 0.1 % tNaPi. After washing membran was dried on Whatmann 3 mm, covered with saran Wrap and then autoradiographed with Kodak x Omat x-ray film for an appropriate expusure time (usually 8-10 hours) at -80 °C. Thus, the amount of bound probe was determined by autoradiography.

RESULTS and DISCUSSION

Citrus tristeza virus (CTV) nucleic acid was detected in extracts from infected plants by hybridization with 5' labeled dsRNA. The (T-30 and T-36), respectively, dsRNA probes effectively hybridized with PEG-RNA extracts from infected tissues (Fig 1,2). The dsRNA probes do not hybridize with samples from healthy plants.

The spot hybridization could be used for the detection of plant pathogenic viroids which have unencapsidated RNA (Owens and Diener, 1981). This technique can also be applied to the detection of a whole range of plant viruses (Maule *et al.* 1983). The viroid detection technique of Owens and Diener (1981) utilized a DNA probe, it is also possible to use ^{32}p end - labeled RNA as a probe (Rosner *et al.,* 1983, Garger *et al.,* 1983). The main advantages of using dsRNA as a probe for hyblridization are its relatively simple isolation procedure from many infected plants (Morris and Dodds, 1979).

Garger and Turpen (1988) demostrated the detection and diagnosis of viral disease agents by nucleic acid is a valuable technique, and preferred isolating dsRNA as a probe because the RNA is easily isolated by CF-11 chromatography or LiCL fractionation, more stable and resistant to residual contaminatus ribonuclease activity than ssRNA.

Is developed for acreening of virus related RNAs in plant extracts. The method is based on the hybridization a polynucleotide kinase (Jordon and Dodds, 1983). Mediated, ^{32}p labeled, dsRNA probe with RNA or sap extracts. Sap extracts were prepared and spotted directly onto presoaked nitrocellulose membran.

The present work was intended to show the applicability of hybridization methods in which polynucleotide kinase mediated ^{32}p - labeled dsRNA probes were used for the rapid and simple screening of CTV virus RNA species. This hybridization method can be applied to diagnose CTV.

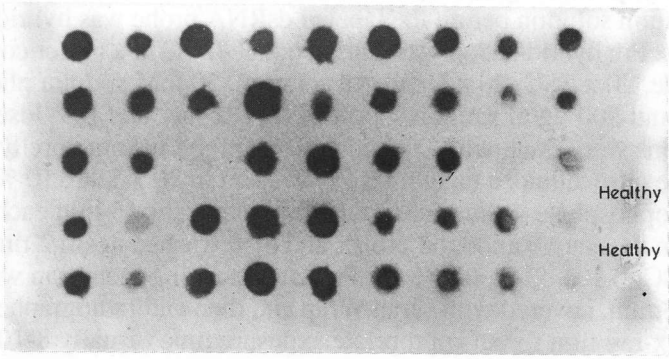

Fig. 1. Dot hybridization of 5' - labeled dsRNA (T-30) to RNA extracted from healthy and citrus tristeza virus (CTV) infected plant tissues.

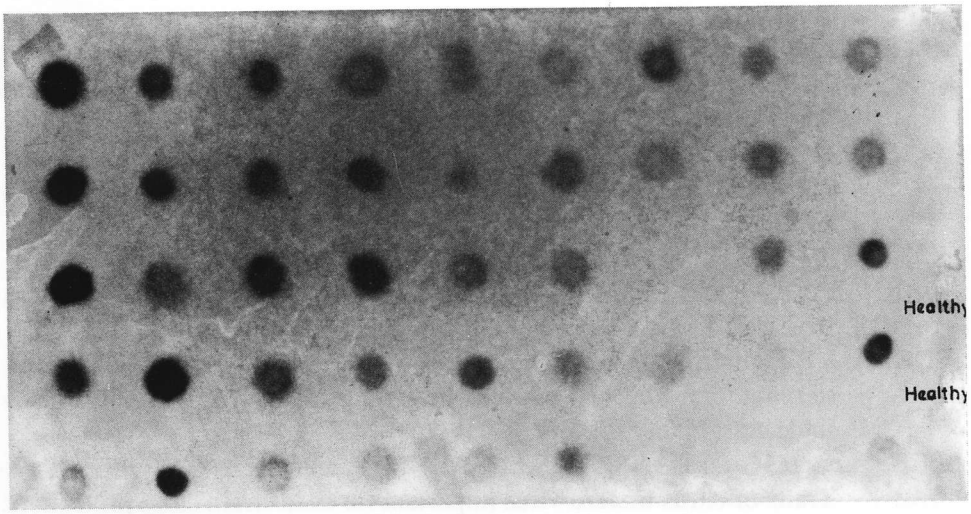

Fig. 2. Dot hybridization of 5 labeled dsRNA (T-36) to RNA extracted from healthy and citrus tristeza virus (CTV) infected plant tissues.

ÖZET

TURUNÇGİL TRİSTEZA VİRUS (CTV) TANISINDA 5' ETİKETLİ dsRNA'LERİN PROB OLARAK KULLANILMASI

Nitroseluloz membran üzerine emdirilmiş saflaştırılmış turunçgil tristeza virus (CTV) nukleik asiti, 5' etiketli dsRNA sondalar (probe) kullanılarak hibridizasyon ile teşhis edilmiştir. Spesifik sonda (probe), CTV enfekteli bitkilerden ekstrakte edilen dsRNA'lerden hazırlanmış ve polinukleotid kinase kullanılarak radyoaktif nukleotid ile etiketlenmişlerdir. CTV'nin T-36 ve T-30 izolatlarından elde edilen dsRNA sondaları pürifiye CTV RNA'leri etkili olarak hibridize etmiştir.

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Production of Antiserum Against Cucumber Mosaic Virus

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ABSTRACT

Cucumber mosaic virus Zinnia strain (CMV-Z) was isolated from an ornamental plant ***"Zinnia elegans"*** *and purified according to the method described in the text. Purified preparations were used as immunogens and injected to two New Zealand rabbit intramuscularly and intravenously. The animals were sacrified seven days after the booster, the antiserum was collected and its titre was determined as 1/512 by ring interface precipitin test and microprecipitin test against the purified preparation of CMV-Z strain.*

INTRODUCTION

Cucumber mosaic virus (CMV), among the other cucurbit virus infections, is one of the most widespread vegetable virus all over the world especially in temperate regions (Smith, 1972). It is the type member of cucumovirus group and its host range spectrum is quite wide including some of vegetables, wild plants and ornamental plants. CMV causes dwarfing and color breaking of the ornamental plants (Francki *et al.,* 1979).

Serological methods are the great tools for rapid detection of the plant viruses (Van Regenmortel, 1982). For the application of these methods the antiserum against that plant virus infection must be produced at first. Then, the identification of the viruses can be done in a simple manner by applying them to the one of the serological assays. Those tests provide the rapid detection of the viruses even if they are in very small quantities. Also, the virus epidemiology can be investigated and seed stocks can be checked for the presence of virus infections by the aid of the antisera produced (Jafarpour et al., 1979, Erkan *et al.,* 1991). It is also possible to detect the plant virus infections from their insect vectors even if from a single one by the application of DAS-ELISA or Indirect-ELISA assays (Crook and Payne, 1980; Hibino and Kimura, 1982; Omura *et al.,* 1984).

Since ornamental plant production is an important field of horticulture in Marmara, Aegean and Mediterranean regions of Turkey, an ornamental plant pathogen has been selected as research subject.

The purpose of this research was to produce the antiserum against the Zinnia strain of CMV.

MATERIALS and METHODS

Virus source : CMV-Z strain was isolated from an ornamental plant **"Zinnia elegans"** in Japan and identified as a strain of CMV (CMV-Z strain) by Prof.Dr.N.SAKO (personal communication, 1986). Virus was propagated in tobacco ("**Nicotiana tabacum** var. **Samsun** NN") plants and 6 days after the inoculation, systemically infected leaves were collected, packed separately as 150 g and stored in a deep-freezer at -25 °C.

Purification of CMV-Z strain : The virus was purified from infected leaves according to the method of Maeda et al. (1983) with slight modifications. 150 g of infected leaves was homogenized in 0.5M citrate buffer (pH: 6.5) containing 0.0025 M EDTA and 0.1 % thioglycollic acid at the rate of 1:2 (w/v). The homogenate was squeezed through two layers of gauze and CCl 4 was added to the homogenate in cold room at the rate of 1/4 of the total volume and let to stir for 10 minutes. The other steps of the purification procedure are shown below as figurative :

1) Crude sap prepared as above
2) Low speed centrifugation, 4500 rpm 15 min.

Pellet** _____ 3) 1%Triton X-100, 6% PEG 6000, 0.1M NaCl were added to supernatant and all mixed in laboratory conditions for 30 min

4) Low speed centrifugation at 6200 rpm for 20 min.

Supernatant _____ 5) Pellets were dissolved in 0.005 M borate buffer (pH: 9.0), containing 0.005 M EDTA

6) Shaked 1 h at 4 °C
7) Centrifugation at 8800 rpm for 20 min

Pellet _____ 8) Supernatants were subjected to high speed centrifugation at 28000 rpm for 2 h. (repeated twice)

9) Pellets were dissolved in EDTA-borate buffer containing 4 % formalin
10) This solution was shaken overnight
11) Low speed centrifugation at 8800 rpm for 20 min.

Pellet _____ 12) Supernatant was layered on sucrose density gradients composed of 35 %,

25 %, 15 %, 5 % sucrose solutions in borate buffer (pH : 9.0).

13) Light scattering virus bands were checked and collected to a centrifuge tube in cold room

14) Tube was filled with EDTA-borate buffer up to top

15) Centrifugation at 28000 rpm for 2 h

Supernatant _____ 16) Pellet was suspended in 0.5 ml borate buffer (pH : 9.0) and shaken for 3 h

17) Low speed centrifugations at 8800 rpm for 20 min.

18) Purified CMV preparation

** : The material on the left were discarded

EDTA : Ethylene di - amine tetra acetic acid.

Ultraviolet absorbance spectra of all purified preparations were determined by a Shimadzu UV-VIS spectrophotometer and A_{260} / A_{280} A_{max} / A_{min} values were estimated separately for each purified preparations. Virus yields were obtained by using the formulae ($E^{0.1\ \%}_{10\ mm}$) = 5.0 at 260 nm (Francki et al., 1979).

Immunization schedule : Two male New Zealand rabbits of 4 months old were selected for immunization. The first injection was done with 0.5 mg of purified CMV-Z preparation emulsified in Freunds complete adjuvant, second and third injections were performed 1 and 1.5 mg purified virus preparations emulsified in Freund's incomplete adjuvant respectively. All of the three injections were done intramuscularly with 10 days intervals and 4 th and 5 th injections were realised 3 weeks later the third one with 3 weeks intervals. Booster injection was done 10 days later the fifth injection via ear vein of the animals, using only the purified preparation of virus at the rate of 2 mg. Some blood specimen was taken out by a sterile syringe 2 days earlier than the fourth and fifth injections in order to check the antibody production. Totally 8 mg of purified virus was injected to the each of the assay animals.

Collection of antiserum : The animals were sacrifised one week after the booster injection. Bloods of the animals were collected from throat artery to the sterilized flasks (30-40 ml totally), incubated at 37°C for 1 hour and then they were put into the refrigerator and left there overnight. Antiserum was collected by using a sterile micropipet and centrifuged at 3000 rpm for 15 minutes. 0.02 % NaN_3 was added to the supernatant. The resulting antiserum was placed into the glass ampuls as 1 ml, freezedried, stored in a deepfreezer at -25°C and then carried to Turkey.

Titre determination and the control of the antiserum : Titre determination of the present antiserum was done by ring interface precipitin test and microprecepitin test as decribed by Ball (1974). Ring interface precipitin test was performed in glass tubes of 4 mm diameter. Antiserum was diluted with serum physiologic solution at the rates of 1/2 ~ 1/1024 and layered onto the purified preparation of CMV-Z at the concentration of 100 mg/ml as in the same volume of 100 µl. Tubes were incubated at 37°C for 2 hours and resulting reactions were recorded after the end of incubation period. Controls were made with healthy tobacco sap. Microprecipitin test was performed in plastic petri dishes and one drop of purified CMV-Z preparation was mixed with one drop of diluted antiserum at the same concentrations as above. The plastic petri dishes were incubated at 37°C for 2 hours and resulting reactions were observed by a stereoscobic microscope.

Cross reactions of the produced antiserum with the other strains of CMV : Cross reactions of the produced antiserum was checked by using the Y strain (Japanese) and common strain of CMV (Turkish), isolated from cucumber, by microprecipitin test and Ouchterlony agar-gel double diffusion test. 0.005 M phosphate buffer saline agar medium (containing 0.002 % NaN_3) was used as the solid state of the agar-gel double diffusion tests.

RESULTS and DISCUSSION

Although the purification procedure requires 3 days to be completed, it is rather sensitive and almost allows no host protein to be involved to the comletely purified preparation. Virus yields of the purified preparations were ranging between 0.705 mg - 2.48 mg / 150 g infected tobacco leaves. An absorbance spectrum of a purified CMV-Z strain preparation is shown at Figure 1, with absorbance values at $A_{260\ nm}$: 0.827, $A_{280\ nm}$: 0.593 A_{260} / A_{280} : 1.59 and A_{max} / A_{min} : 1.22 and the virus yield was 2.488 mg/150 g infected tobacco leaves.

The titre of the obtained antisera of both animals were 1/512 as determined by ring interface precipitin test and microprecipitin tests. It gave positive reactions with common strain (Turkish) and Y strain (Japanese) of CMV, in Ouchterlony agar-gel double diffusion test. CMV-Z antiserum, reacted with crude sap of common strain (Turkish) of CMV, up to 1/8 dilution in agar-gel double diffusion test and up to 1/16 dilution in microprecipitin test. No reaction was observed with healthy tobacco leaf sap in both tests.

Those tests proved us that the produced CMV-Z antiserum had cross reacted with the heteroloug strains of CMV, besides it homoloug strain, CMV-Z. Although Zinnia strain was rather a weak strain, comparing to yellow and common strains of CMV, the produced antiserum had enough titre for serological determination of the homoloug and heteroloug strains of the pathogen where as the antiserum produced against the common strain of CMV by Danish Government Seed

Pathology Institute for Developing Countries, Denmark had a titre of 1/128 (personal communication with Dr. Albrechtsen, 1990).

The protocol of CMV-Z antiserum is presented at page 12.

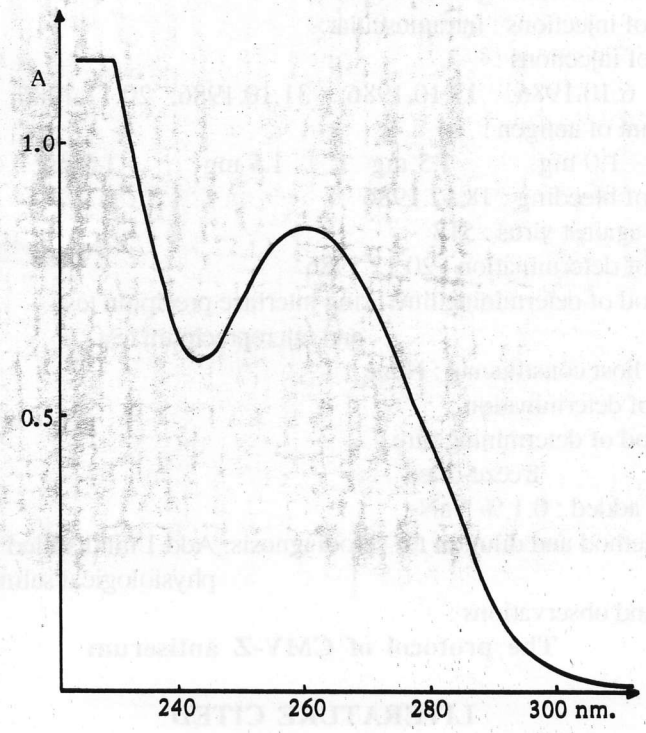

Figure 1. The ultraviolet absorbance spectrum of a CMV-Z purified preparation.

ÖZET

HIYAR MOZAYIK VİRUSUNA KARŞI ANTİSERUM ÜRETİMİ

Hıyar mozayık virusunun (Cucumber mosaic virus - CMV) Zinnia ırkı, **Zinnia elegans** bitkisinden izole edilip, tütün bitkisinde çoğaltılmıştır. Daha sonra saflaştırılarak, Yeni Zelanda türü iki erkek tavşana, belirli aralıklarla, toplam 8 mg enjekte edilmiştir. Son enjeksiyondan bir hafta sonra hayvanların kanı alınmış ve gelişen antiserumun 1/512 titreye sahip olduğu, halka fazla arası presipitasyon testi ve mikropresipitasyon testi ile tesbit edilmiştir.

Antiserum against : Cucumber mosaic virus - Z strain
(isolate from **Zinnia elegans**)
our identification number :
virus purified from : tobacco (**Nicotiana tabacum** var. Samsun NN)
number of injection : 6 times
kind of immunizing animal : rabbit
type of injections : intramuscular
date of injections :
28.9.1986, 6.10.1986, 17.10.1986, 31.10.1986, 20.11.1986, 11.12.1986
amount of antigen :
0.5 mg 1.0 mg 1.5 mg 1.5 mg 1.5 mg 2.0 mg
date of bleeding : 18.12.1986
Titre against virus : 512
date of determination : 20.12.1986
method of determining titre : ring interface precipitin test
and microprecipitin test
Titre against host constituents : None
date of determination :
method of determining titre :
Storage : freeze-dried
Preservative added : 0.1 % NaN_3
Suggested method and dilution for serodiagnosis: Add 1 ml distilled water or physiological saline before use
References and observations :
The protocol of CMV-Z antiserum

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Studies on Alfalfa Mosaic Virus of Alfalfa in Aegean Region

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ABSTRACT

Surveys were carried out in 1989-1990 to collect the leaves of alfalfa showing virus like symptoms. The viruses in the samples were detected according to the symptoms on the test plants. In further studies the dilution end points and thermal inactivation points of isolates were found. Seed transmission of virus has been studied through this study by testing the seeds obtained from local growers. Virus infections were detected on all growers seed.

INTRODUCTION

Alfalfa is a very important crop as food source for animal because of its high protein content and also because of Rhizobium bacteria in their roots that can fix free nitrogen of air and convert it into the form which plants can use. Virus disease are of a great importance for alfalfa besides insecte, weeds, fungal and bacterial diseases. It is certain that various pests and diseases also attack the alfalfa. Important fact is that the virus of alfalfa is mostly seed transmitted (Çalı and Erdiller, 1990).

The present study has been carried out in order to determine the virus diseases affecting alfalfa in Aegean Region, during the years of 1989-1991. Surveys were carried to collect the leaf samples of plants showing virus-like symptoms.

MATERIALS and METHODS

Methods of surveys and determination of disease ratio were carried out according to BORA and KARACA (1970). Leaves of alfalfa showing virus-like symptoms are collected from the six provinces of Aegean Region (Table-1). Leaves of diseased plants were homogenized in a mortar with addition of a little 0.01 M Phosphate buffer (pH 7.0); than small amount of 500 mesh carborandum powder was added to the inocula as an abrasive before inoculation of test plants. Mechanical inoculations were made on primary leaves of leguminosae plants and 3-4 true leaves of other hosts (Table-2). Inoculated leaves were rinsed with tap water. The test plants were observed after inoculation for symptom development.

The sap of systemically infected leaves of alfalfa and **N. glutinosa** plants were used as the source of inoculum in the experiments of dilution end point and thermal inactivation point. These tests were made on **C. amaranticolor.**

The serological technique used was microprecipition drop test on slides (Bercks, 1963).

Seeds collected from growers in the region were used in seed-transmission experiments. Three hundreds seeds from each sample were put in sterile pot soil in greenhouse. The leaves of these plants were used to obtain the inocula and mechanical inoculations were made on **C. amaranticolor.**

Table 1. Existence and widespread area of mosaic virus in Aegean Region

Survey Area (Country name)	Number of field examined	Total area (da)	Existence of mosaic virus (%)	Widespread area of mosaic virus (%)
AYDIN				
Central Country	2	4	–	–
Nazilli	7	49.5	–	–
Kuyucak	6	26	18.6	50
Bozdoğan	7	56	6.6	100
Total	22	135.5 (Average)	6.2	
BALIKESİR				
Gönen	12	33	–	–
Susurluk	17	118	10.88	52.9
Total	29	151 (Average)	8.50	
ÇANAKKALE				
Central Country	8	22	–	–
DENİZLİ				
Tavas	5	15	0.2	60
Çivril	30	136	32.8	76.6
Total	35	151 (Average)	29.5	
İZMİR				
Bergama	14	108	2.68	35.7
Ödemiş	11	26	0.61	27.2
Torbalı	8	35	3.28	50
Total	33	169 (Average)	2.50	
MANİSA				
Central Country	3	14	0.35	33.3
Saruhanlı	3	25	0.2	33.2
Salihli	4	23	2.82	50
Total	10	62 (Average)	1.20	

Table 2: Reactions of test plants used for identification the virus isolates on alfalfa

Test Plants	Isolates					
	Y-10	Y-14	Y-15	Y-38	Y-51	Y-53
Chenopodium amaranticolor	NLL,S	KLL	KLL	KLL	–	KLL,S
C. quinoa	NLL	NLL	–	–	–	NLL,S
Datura stromonium	–	S	S	S	–	S
Gomphrena globosa	–	–	–	–	NLL	NLL,S
Nicotiana glutinosa	NLL,KL	NLL,S	NLL,KL	NLL,KL	S	NLL,S
N.tabacum "White Burley"	–	NLL	NLL	NLL,S	NLL	–
Phaseolus vulgaris "Pinto"	NLL	S	NLL,S	NLL	DA	–
"Prince"	–	–	–	–	NLL	S

NLL : Necrotic local lesion
KLL : Clorotic local lesion
KL : Concentric lesion
Mo : Mosaic
DA : Vein clearing
S : Systemic

Table 3. Serological reactions of isolates from alfalfa

Isolates	Antiserum			
	AMV	CMV	BCMV	BYMV
Y-10	–	–	–	–
Y-14	+ + +	–	–	+
Y-15	+ +	–	–	–
Y-38	+ +	–	–	–
Y-51	+ +	–	–	–
Y-53	+ +	–	–	–

Table 4. Seed transmission by growers seed

Samples	1	3	5	7	2	4	6	6
Variety	Kayseri	Kayseri	Kayseri	Kayseri	Peru	Peru	Peru	Peru
Seed transmission %	2.4	2	4	2	10	4	4	1

RESULTS and DISCUSSION

According to the survey results, it was found that existence and widespread area of mosaic virus on alfalfa were between the rate of 1.20 % and 29.5 % and 0 % and 76.6 % respectively in Aegean region (Table 1). Mechanical inoculations were made to the test plants and 80 virus isolates were obtained from 60 samples. These isolates were divided into groups considering that they produced same symptoms on test plants. Then 6 isolates were tested for the detection of viruses on the a wider, hostrange and viruses were identified by their host reactions as shown in Table 2.

Six different alfalfa mosaic virus (AMV) isolates were found according to the symptoms producted on test plants (Fig 1, 2, 3).

Alfalfa mosaic virus (AMV) Y-14, Y-15 and Y-38 isolates produced chlorotic local lesions, Y-10 isolates induced necrotic local lesion and mosaic Y-53 isolates led to chlorotic local lesion and mosaic on **C. amaranticolor.** Y-14 and Y-53 isolates originated necrotic local lesion and mosaic on **N. glutinosa.** Y-10, Y-15 and Y-38 isolates caused necrotic local lesion and consentric ringspot on same plant. Y-51 isolate procuded mosaic on some plants. Y-51 isolate led to nectoric local lesion on **P. vulgaris** "Prince" Y-53 isolates induced mosaic on some plants. The differences might be the result using different strains (Zaumeyer, 1953, 1963; Hagedorn and Hanson, 1963; Jaspars and Boss, 1980; Boswell and Gıbbs, 1983).

The certain physical properties of viruses under study were determined. Dilution end point for all isolates were 10^{-3} - 10^{-4}. Thermal inactivation points of all isolates were 60-65°. The physical properties of these isolates were also confirmed by the data in the previous works (Boswell and Gıbbs, 1983; Jaspars and Boss, 1980).

Serological assays were performed with the antisera supplied abroad according to the microprecipitin test. Positive reactions were obtained Y-14, Y-15, Y-38, Y-51 and Y-53 isolates and AMV (Table 3).

Through this study, it is found that 8 different seed samples taken from the growers were infected with virus disease between 1 % and 10 % (Table 4). According the several records this ratio changes between 0-50 % (Boswell and Gıbbs, 1983; Jaspars and Boss, 1980).

ÖZET

EGE BÖLGESİNDEKİ YONCALARDA YONCA MOZAYIK VİRUSU ÜZERİNDE ÇALIŞMALAR

1989-1990 yıllarında Aydın, Balıkesir, Çanakkale, Denizli, İzmir ve Mani-

sa'da yapılan surveyler sonucunda yoncalarda % 1.2 - 29.5 arasında mozayık belirtileri saptanmıştır. Hastalığın yaygınlık oranı ise % 0-76.6 arasında değişmektedir. Yonca üretim alanlarından alınan örneklerle mekanik inokulasyon, fiziksel özelliklerini belirleme ve serolojik testlerle tanılama çalışmaları yapılmıştır. Yapılan testler sonucunda yoncalarda Yonca mozayık virusu'nun (AMV) varlığı saptanmıştır. 8 farklı üreticiden alınan tohum örneklerinin testlenmesi sonucunda yonca tohumlarının % 1-10 arasında değişen oranlarda virusla bulaşık olduğu saptanmıştır.

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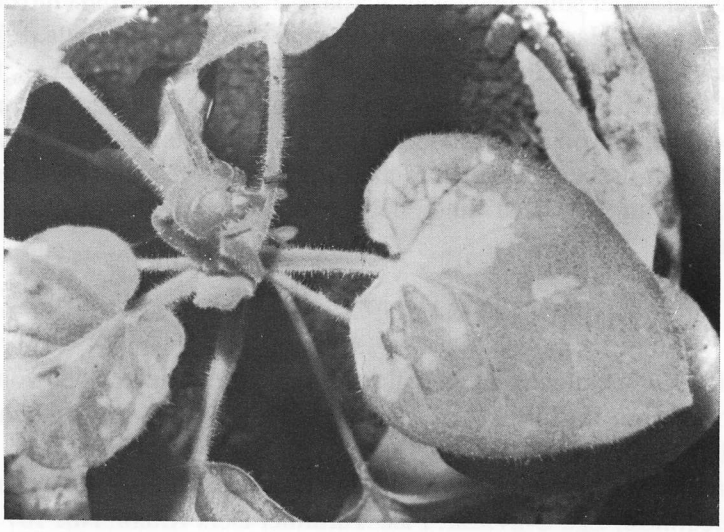

Fig. 1. Leaves of **N. glutinosa** showing mosaic and vein banding caused by AMV.

Fig 2. Symptoms caused by AMV on **D. stromonium**

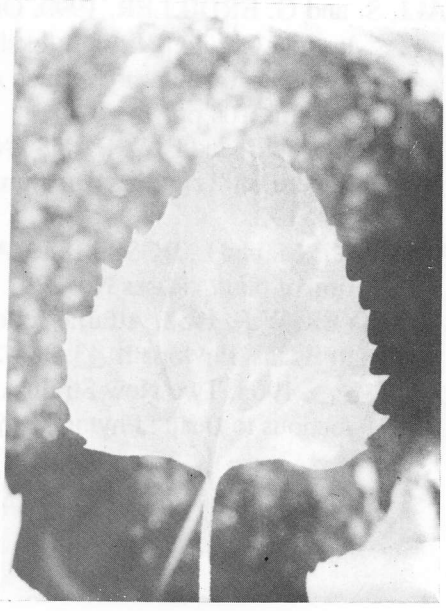

Fig 3. Symptoms caused by AMV on **C. amaranticolor**

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The Location Of Two Mosaic Viruses In Cowpea Seeds*

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SUMMARY

Cowpea Mosaic Virus (CpMV) is highly seed transmitted whereas bean Common Mosaic Virus (BCMV) is not, although both are seedborne in nature. Studies on the site of location of these two viruses revealed that CpMV is maintly in the plumular bud and cotyledons of diseased seeds while BCMV is primarily in the testa.

INTRODUCTION

The majority of viral diseases which are of commen occurence in cowpea (**Vigna unquiculata** (L.) Walp.) cultivation are known to be seed transmitted. Anderson (1955 b.) has reported seed transmission of CpMV-CMV strain in cowpea varieties from 4 to 25 percent. Chenulu ***et al.*** (1968) have reported seed transmission of cowpea mosaic virus through true seed to the extent of 5 to 16 per cent. Ekpo ***et al.*** (1974) have studied Bean Common Mosaic Virus in the cotyledons and embryo but not in seed coats developing on infected plants of monroe bean. On a symptomatological basis CpMV is characterised in cowpea plants by the presence of mosaic mottling with typical dark green vein banding, whereas BCMV is less virulent and is characterised by deformation and puckering of mosaic affected leaves, reduction in leaf lamina and poor pod formation. These viruses are distinguished on the basis of local lesions on the indicator plant **Chenopodium amaranticolor** Coste and Reyn. Local lesions produced by CpMV are chlorotic and take nearby 6-8 days while those of BCMV are chlorotic with white pin point centres and take only 3 to 5 days. However, it is not known why these two viruses differ in their transmission as far as their seedborne nature is concerned. The following studies were undertaken to find out their site of location in the seeds of cowpea.

* Part of the Ph. D. thesis submitted by the senior author to the Sukhadia University, Udaipur 313001.

MATERIALS and METHODS

In order to have a large number of virus infected seeds, they were harvested from artificially inoculated cowpea cultivars C-152 and Pusa-4 with the BCMV and CpMV, respectively.

Seeds thus collected were soaked in sterile distilled water separately for two days and then the testa, cotyledons and plumular buds of each seed were separately crushed with a sterile pastle and mortar and indexed on **C.amaranticolor.** Lesions thus obtained on indicator plants were back indexed on **C.amaranticolor** for confirmation.

RESULTS

Table-1 indicates that indexing of seed parts collected from CpMV infected plants, indicated virus location in cotyledons and plumular buds, the infection percentage being 1.94, and 16.5 respectively. On the other hand indexing of seed parts collected from BCMV infected plants indicated virus location in the testa and the infection percentage was 6.9 whereas cotyledons and plumular buds extract did not produce lesions on **C.amaranticolor** from any of the seeds indexed. The lesions pruduced by both the viruses were characteristic of virus concerned.

Table 1. Screening of seed parts on **C.amaranticolor** for detecting the site of viruses.

Number of seeds tested	Virus	Seeds with positive response	Percentage of virus in seed parts			Percentage infection		
			Testa	Coty-ledons	Plumular bud	Testa	Coty-ledons	Plumular bud
103	CpMV	19	0	2	17	0.0	1.94	16.5
58	BCMV	4	4	0	0	6.9	0.0	0.0

DISCUSSION

Location of seedborne viruses of cowpea as well as in other crops has drawn the attention of many workers (Gay, 1969; Tailor, Grogan and Kimble, 1961; Hamilton, 1965; Vashisth and Nagaich, 1965; Kennedy and Cooper, 1967; Ekpo, Ephraim and Saettler, 1974; Phatak 1974 and Agarwal, Nene and Beniwal, 1977) but they did not study the correlation between the field incidence

and the site of location of the viruses. Based on infectivity, present findings reveal that CpMV is located in cotyledons and the plumular bud but not in the testa and this probably leads to the higher percentage of seed transmission. On the other hand BCMV is located in the testa but not in the cotyledons and plumular bud, virus transmission through the seeds in less and much of the inoculum is dropped as the seedcoat is shedded off in the soil during germination. During the process of germination, the embryo infection is purely mechanical and secondery spread of the virus is by insect vectors.

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* Original not seen.

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Investigations on the Detection of Bean Diseases of Van Province

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ABSTRACT

*Diseased plant samples were collected from bean (**Phaseolus vulgaris** L.) fields of Van region in 1989. As the results of surveys, 51 fungus-infected and 4 virus-infected specimens were collected from 68 bean fields located in different locations. The pathogenic fungus isolates on beans in the region were identified as **Alternaria alternata** (35 %), **Fusarium solani** (25 %), **Macrophomina phaseoli** (17.5 %), **Drechslera sorokiniana** (10 %), **Rhizoctonia solani** (5 %), **Stemphylum** sp. (5 %) and **Botrytis cinerea** (2.5 %). Virus infected specimens were mechanically inoculated to indicator plants and according to the mechanical inoculations and serological tests, the widespread virus agent was determined as bean yellow mosaic virus in the region.*

INTRODUCTION

Van province is located at the right side of Lake Van (the biggest soda lake in Turkiye), and has its own microclimatic and soil conditions. The weather is warmer comparing the many locations in Eastern Anatolia. Although the provence is an old volcanic plain, horticulture is the major agricultural activity of towns and villages located near to the lake.

Bean (**Phaseolus vulgaris** L.) is one of the major crop of the province and commonly cultivated local bean varieties are named as Çalı and Ayşe. According to the statistical data of The National Statistical Institute of Turkiye in 1987, bean cultivation area and production of Van province were 847 hectares and 1088 tons respectively, where as the average yield was 1285 kg/ha (Anonymous, 1989).

Up to now, many researches were conducted in order to determine the diseases of bean in different parts of Turkiye (Tekinel *et al.*, 1969; Özalp, 1971; Karahan, 1971). Root-rot diseases and their distribution in bean cultivated areas of Adana and İçel were investigated by Soran (1981). The seedborne fungus diseases beans were found out and identified by Maden and İren (1984). Virus infections of beans were studied by various authors and according to their results, bean common mosaic virus (BCMV) and bean yellow mosaic virus (BYMV) were determined as the most prevalent and destructive viruses of Erzincan plain

and Western Anatolia (Açıkgöz and Çıtır, 1986; Fidan and Yorgancı, 1989). On the other hand, Erdiller (1979) has determined the effects of BCMV on yield components of different bean varieties. The diagnostic features of BCMV and BYMV were summarized by Bos (1971 and 1970) and Smith (1972).

There was no research conducted for to determine the bean diseases in Van province and therefore, the main purpose of this research was to survey and to determine the pathological problems of bean cultivation in the area.

MATERIALS and METHOD

Survey area

The survey area of this research was selected as Erçiş, Gevaş and Centrum of Van which were mainly bean cultivation areas in the province (Figure 1). Surveys were conducted at 6 villages of centrum, 6 villages of Erçiş and 5 villages of Gevaş, therefore, totally 17 villages were investigated. Four bean fields were selected in each village, so totally 68 bean fields were surveyed. The selected bean fileds were investigated as a whole. Surveys were started in July 1989 when the blossom time of beans and stopped in September 1989. The infected plants were taken out as a whole and put into polyethylene bags, labelled and brought to the laboratory immediately for microscopic and macroscopic investigations. Fungus infected plants were kept in refrigerator and virus infected one in deep- freezer at –25°C.

Identification and isolation of fungal pathogens

The fungi were isolated by plating the infected plant parts (root, stem or leaf) to the potato dextrose agar medium (PDA) in petri dishes (Anonymous, 1968). Disinfection has been done with 70 % alcohol. The dishes were incubated in incubation chamber at 25°C and then carried to Departments of Plant Protection of Aegean and Ankara Universities for examination and identification of fungal pathogens.

Detection of virus isolates:

Mechanical inoculation

The plants showing mosaic type of symptoms were mechanically inoculated to the test plants: **Chenopodium amaranticolor** Coste et Reyn., **C.quinoa** Wild, **Nicotiana rustica** L. **Medicago sativa** L. **Phaseolus vulgaris** L., **Cucumis sativus** L. according to the method of Noordam (1973). Inoculated plants were kept in the greenhouse at 20-25°C.

Serological tests

Antisera against BCMV and BYMV were kindly supplied by Institute of Seed Pathology for Developing Countries-Denmark and agglutination and micro-precipitin tests were applied to the virus infected leaf samples of been isolates. Both tests were performed according to Noordam (1973) and healthy plant sap and normal rabbit antiserum were used as control.

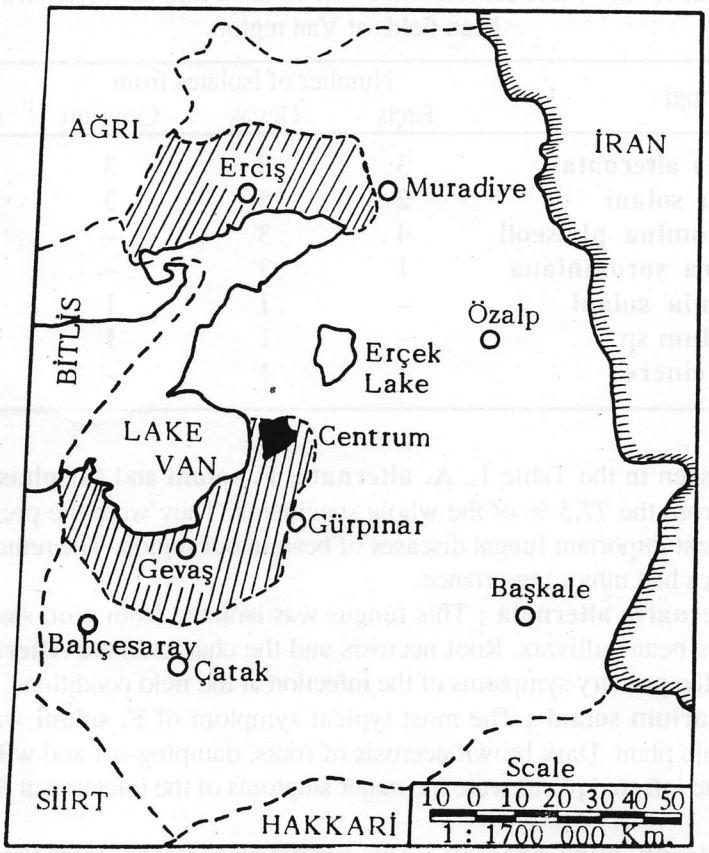

Figure 1. The map of the survey area.

RESULTS

Incidences and distribution of the isolated fungi species

During the surveys done in 1989, July-October, (from blossom to harvesting period of beans in the region), totally 51 specimens supposed to be infected by fungi were collected from the survey area. No parasitic fungus was isolated from 11 specimens but 7 different parasitic fungi were isolated from the remaining 40 specimens, at genus and species level. Those fungi were, with the rate of incidence **Alternaria alternata** (35 %), **Fusarium solani** (25 %), **Macrophomina phaseoli** (17.5 %), **Drechslera sorokiniana** (10 %), **Rhizoctonia solani** (5 %), **Stemphylum** sp. (5 %), and **Botrytis cinerea** (2.5 %) and their distribution was shown in Table 1.

Table 1. Table distribution of the isolated fungus species from bean fields at Van region.

Fungi	Number of isolates from			Total number
	Erçiş	Gevaş	Centrum	
Alternaria alternata	3	8	3	14
Fusarium solani	2	5	3	10
Macrophomina phaseoli	4	3	–	7
Drechslera sorokiniana	1	3	–	4
Rhizoctonia solani	–	1	1	2
Stemphylum sp.	–	1	1	2
Botrytis cinerea	–	1	–	1

As seen in the Table 1., **A. alternata, F. solani** and **M. phaseoli** were detected from the 77.5 % of the whole specimens. They were the prevelant and also the most important fungal diseases of bean in our region. The remaining fungus diseases had minor importance.

Alternaria alternata : This fungus was isolated from root, stem and the pods of the bean cultivars. Root necrosis and the characteristic **Alternaria** leaf spot were the primary symptoms of the infection at the field conditions.

Fusarium solani : The most typical symptom of **F. solani** was wilting of the whole plant. Dark brown necrosis of roots, damping-off and wilting at the entire of the infected plants were the major smptoms of the infection at field conditions.

Macrophomina phaseoli : This fungus was isolated from the roots and the stems of the infected bean plants. General necrosis of the roots besides the chlorosis of the foliage were the major symptoms of the infection in field conditions. During the isolation assays, this fungus was detected as mixed infection with **F. solani, A. alternata** and **D. sorokiniana** from some specimens.

Identification of the virus isolates

Virus infections weren't so prevalent in the research area. During the surveys done in 1989, only four virus infected specimens (two from Erçiş and two from Gevaş towns) were collected. Severe sistemic, chlorotic mosaic on the leaves of the infected plants was the major symptom of the infection, at field stage. According to the symptoms produced on the test plants and the serological reactions, they were all identified as bean yellow mosaic virus.

Table 2. The list of the test plants used and the symptoms produced by BYMV isolates of Van.

Test plant	Types of the symptoms
Chenopodium amaranticolor	CLL
C. quinoa	CLL
Medicago sativa	SM
Phaseolus vulgaris L.	SM
Nicotiana rustica	--
Cucumis sativus	--

CLL: chlorotic local lesion, SM: systemic mosaic infection,
NLL: necrotic local lesion, --: no reaction

All of the four isolates reacted only with BYMV antiserum in the agglutination tests, and among them, isolates no: V-102 gave the strongest reaction. Therefore, this isolate was selected for the microprecipitin test. In microprecipitin test, the precipitates were formed up to 1/128 dilution of the crude sap and up to 1/512 dilution of the antiserum.

DISCUSSION

Agriculture is performed in an traditional way, so no precaution has been taken in preventing diseases in the area.

The major pathological problem of bean cultivation of Van province was root-rot disease. Root-rot of beans had been a problem in Çukurova also, previously (Soran, 1981). All of the fungi detected in this research had been isolated from the roots and stems of the infected plants. They were soil-borne fungi and all of them were determined as seed-transmitted also by Maden and İren (1984).

Although almost all of the bean fields surveyed at Gevaş were contaminated with fungus infections, in 14 fields at Erçiş and in 16 fields at centrum, no disease symptoms were observed. No bacterial disease symptom was observed in the area. Virus diseases of bean were not prevalent in the research area and only four virus infected specimens were collected which were later identified as BYMV. BYMV is not a seed-transmitted virus infection, so, it is thought to be spreaded by insect vectors. This virus was also detected in Adana and Mersin at broad bean fields by Yılmaz (1981), at the bean fields of Erzincan plain by Açıkgöz and Çıtır (1986) and from the pulse crops cultivated in Aegean Region by Fidan and Yorgancı (1989).

The symptoms produced on the test plants were the same as reported by Bos (1970), Smith (1972), Boswell and Gibbs (1983) and Edwardson and Christie (1986). They were only reacted with BYMV antiserum.

ÖZET

VAN İLİ ÇEVRESİNDEKİ FASULYE HASTALIKLARININ TESBİTİ ÜZERİNDE ARAŞTIRMALAR

Van ili çevresindeki fasulye ekim alanlarından, 1989 yılında hastalıklı fasulye örnekleri toplanmış ve laboratuvar incelemeleri sonucunda, bölgede hakim olan fungal etmenler, **Alternaria alternata** (% 35), **Fusarium solani** (% 25), **Macrophomina phaseoli** (% 17.5), **Drechslera sorokiniana** (% 10), **Rhizoctonia solani** (% 5), **Stemphylum** sp. (% 5), **Botrytis cinerea** (% 2.5). Virus enfekteli örneklerde ise, mekanik inokulasyon denemeleri ve serolojik testler sonucunda, mevcut etmenin, fasulye sarı mozayık virusu (Bean yellow mosaic virus-BYMV) olduğu saptanmıştır.

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Studies On Bacterial Streak Disease (**Xanthomonas campestris** pv. **translucens** (Jones et al.) Dye.) Of Wheat and Other Gramineae

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ABSTRACT

Bacterial streak disease is one of serious diseases on wheat and triticale. Bacterium was isolated from diseased plants in the experiment plots of Aegean Agricultural Research Institute, and its pathogenicitiy was determined by performing artificial inoculation and it was reisolated. Bacterium is defined as ***Xanthomonas campestris*** *pv.* ***translucens*** *according to the morphological, physiological and biochemical characteristics.*

It was found that isolates from wheat caused disease on some wild Graminae spp. except barley, rye, rice and triticale. In Aegean region, some widespread durum and soft wheat varieties were determined to be susceptible to ***X.campestris*** *pv.* ***translucens.*** *Ege 88 cultivar was found to be very susceptible to the pathogen.*

INTRODUCTION

It is known that cereals are important from the standpoint of both economy of Turkey and food of society. From cereals which have an important portion in agricultural production, wheat is in the first row. Seeds used in the production are obtained from the growers, public and private organizations, and adaptation and improvement studies supported by the national and international projects.

During April-May of 1989 and 1991 it was observed that leaves of wheat had streak spots and there was leakage as small droplets on them when it was rainy, and leaf samples were taken from the diseased plants in the experiment plots of Aegean Agricultural Research Institute.

Bacterial streak or black chaff caused by **Xanthomonas campestris** pv. **translucens** is one of the most important diseases on wheat and the other some Graminae spp. and is a limiting factor on wheat production in the world (Jones *et al,* 1916; Zillinsky and Borlang, 1971; Schaad and Forster, 1985; Shane *et al.,* 1987; Alizadeh and Rahimian, 1989).

It is reported that the disease caused 40 % of yield loss in South Idoha (USA) and its control measures were not adequate (Forster, 1982). It was brought out that soft and durum wheat varieties as well as barley and triticale were severely infected among the gramineae spp. (Sands and Fourrest, 1989)

and while some isolates caused the disease on all hosts (wheat, rye, barley, and triticale) the other ones were more specific (Hagborg, 1942). It was determined that the pathogen was seed borne (Schaad and Forster, 1985; Sands and Fourrest, 1989; Sands *et al,* 1985; Duveiller, 1989) and caused 10-30 % of yields loss in Minnesota (Shone *et al.,* 1987).

In this study identification of the pathogen, its hosts, and the reaction of wheat varieties commonly produced in the Aegean Region to pathogen were investigated.

MATERIALS and METHODS

Isolation of bacteria:

Leaf samples (Fig 1) collected from experiment plots of Aegean Agricultural Research Institute were washed under tap water. Small pieces of the affected leaves were macerated in sterile distilled water and the resulting suspensions were plated on to Wilbrink's Medium (Sands and Fourrest, 1989). The medium contains 5g. Bacto peptone, 10 g. sucrose, 0.5 g. K_2HPO_4, 0.25 g. $MgSO_4.7\ H_2O$, 0.05 g. Na_2SO_4, 15g. Bacto agar, and 1 liter distilled water was adjusted if necessary to pH 6.8. To avoid fungi, we added 75 mg cycloheximide (Sigma) after cooling. The plates were incubated for 3-5 days at 25°C. Single colonies of the flat yellow bacterium were purified. Stock cultures were maintained on NA (Nifco) at +4°C.

Characterization of the bacteria

– Physiological and biochemical properties:

Mucoid growth was examined on yeast extract-dextrose-calcium carbonate agar (YDC) (Schaad, 1980). Lesithinase production was tested (Lelliott *et al,* 1966) and other physiological and biochemical properties were tested according to Schaad (1980) and Lelliott and Stead (1987).

– Xanthomonadin production: Isolates were examined for Xanthomonadin production by the spectroephotometric analysis of hot-methanol extracts of the cells (Irey and Stall, 1981).

– X-Gal test:

In Wilbrink's medium lactose instead of sucrose, the X-Gal (5-bromo-4chloro-3indolyl-B-D-galactropyranoside (Sigma) at 20 mg l^{-1} was solubilized in 1 ml N-N-dimethyl formamide (Sigma) and was added after cooling. Suspensions were inoculated on plates of above mentioned medium and plates were incubated for 4-5 days at 28°C. The plates after 5 days incubation were refrigerated for a few days.

– Ice nucleation activity (INA):

Strains of **X.c.** pv. **translucens** (isolated from wheat), a strain of **Pseudomonas syringae** pv. **syringae** (isolated from citrus) were grown on YDC (yeast extract-dextrose-calcium carbonate) and King B medium (King *et al.,*

1954) agar plates at 23-25°C Cells (approximately 10^6 - 10^9 cell/ml) were suspended in 0.1 M phosphate buffer saline, pH 7.0, and tested for INA at –9°C using the droplet freezing procedure of Lindow *et al.* (1978). Aluminum foil weighing pans were sprayed with a solution of % 1 paraffin in xylene. The pans were than placed in an oven set at 55°C to evaporate the xylene. The aluminium pans were floated in a 1-1 beaker containing ethanol-water (1:1 v/v) and cooled to –9°C by refrigerated water bath. Ten 10 µl droplets of each bacterial suspension and only PBS pH 7.0 were placed on a pan. The time required for each droplet to freeze was record in seconds.

In addition, requeous suspensions of bacterial strains (**X.c.** pv. **translucens, X.c.** pv. **vesicatoria** and **X.c.** pv. **vignicola**) were diluted to about 10^6 cell/ml. Three mililiters of each bacterial suspension were placed in each of 10 sterile test tubes and cooled to –8°C in a freezer for 3 h. A bacterial strain was determined to have ice nucleation activity if one or more tubes of bacterial suspension froze. Uninoculated tubes containing only water were used as negative control.

Pathogenicity test:

Bacterial strain were grown on Wilbrink's medium (Sands and Fourrest, 1989) 48 h at 28°C. Cells were suspended in sterile distilled water and the suspensions adjusted spectrophotometrically to contain approximately 10^7 cfu/ml. Test plants were grown in 20x20x50 cm plastic pots containing sterilized field soil. Plants, at 2-3 leaf stage were inoculated by injecting one droplet of bacterial suspensions through the tip of hypocotyls with a 1 ml syringae. In addition plants were inoculated with sterile distilled water as negatif controls. Plants were grown at 20-22°C in growth chamber. The following species were used in pathogenicitiy studies: soft wheat (cvs İzmir 88, Ata 81, Gönen, Cumhuriyet 75, and Kaklıç 88), durum wheat (cvs Ege 88 and Gediz 75), barley (cvs Kaya and Gem), rice, rye (local cultivar), **Cynodon dactylon** and **Lolium multiflorum.**

Reactions of durum and soft wheat varieties against to pathogen were recorded 10 days after inoculation using the following scale (El-Banoloy and Rudolph, 1989): (1) small yellow area; (2) Large yellow area; (3) Small water - soaked area or small translucent area; (4) large water-soaked area.

Seed detection

Ten gram seed samples were washed for 30 min in 100 ml of a sterile saline solution (NaCl 0.85 %) containing Tween 20 (0.02 %). Dilutions were made an 0.1 ml of the mixture was assayed on WBC (Wilbrink's-Boric acid - Cephalexin). The composition of the semi-selective WBC medium was: 5 g. bactopeptone; 10 g. sucrose; 0.5 g K_2HPO_4; 0.25 g. $MgSO_4.7H_2O$; 0.05 g. Na_2SO_3 (anhydrous); 15 g Agar, 850 ml distilled water; 0.75 g. boric acid was autoclaved seperately in 150 ml of distilled water and mixed. After cooling added 75 mg cy-

cloheximide in 2 ml 75 % ethanol and 10 mg cephalexin (1 ml of a 10 mg ml^{-1} stock solution in 75 % ethanol) (Duveiller, 1989).

5 seed samples taken from the plants showing-disease symptoms on the different wheat varieties during early stage were assayed.

Colonies were counted after 4-5 days incubation at 30°C. Suspected colonies were transferfed on GYCA slants to confirm identification.

RESULTS and DISCUSSION

Isolation and characterization of bacteria:

Isolation from diseased leaves of wheat plants yielded numerous flat yellow colonies on Wilbrink's medium plates after 2 days of incubation. Two isolates from wheat were used in subsequent studies. All isolates were gram (–) and oxidasenegative. The strains gave positive reactions for catalase and gelatin hydrolysis, mucoid growth on GYCA and hydrolysis of Tween 80 and a esculin. The isolates grow at 36°C, produced hydrogen sulfide from cystein and tolerated 1.5 but not 3 % NaCl. Negative results were obtained for arginine dihydrolase, urease, amilase, nitrat reduction indole production and lecithinase. The strains tolerated 0.02 % but not 0.1 % TTZ (Triphenyl Tetrazolium Chloride). Wheat strains produced acid from glucose, galactose, fructose, sucrose, mannose, threhalose, arobinose, xylose, glycerol, dextrose and lactose, but not from rhamnose raffinose, maltose, sorbitol mannitol and meso inositol. Test results were essentially the same as that reported for **X.c.** pv. **translucens** (Fang *et al.,* 1950, Batry *et al.,* 1982; Alizadeh and Rahiman, 1989).

Xanthomonadin pigment with absorption maxima at 448 nm produced by isolates. Xanthomonadin pigment with absorption maxima at 420, 444 and 467 nm was produced by Iranian isolates (Alizadeh and Rahimion, 1989).

X-Gal test: The positive reaction developed after 5 days incubation. Colonies appeared as blue-green.

It was identified as **X.cam.** pv. **translucens** according to the morphological, physiological and pathogenicity results.

Ice nucleation activity:

Strains of **X.c.** pv. **translucens** (isolated from wheat) and **P.s.** pv. **syringae** (isolated from citrus) were active as ice nuclei. The mean time droplets of cell suspensions of **X.c.** pv. **translucens** strains to freeze ranged from 27 to 31 second. Droplets of cell suspensions of **P.S.** pv. **syringae** strain required 57 second to freeze. Droplets of phosphate buffer saline without bacteria required 285 sec. to freeze.

Strains of **X.c.** pv. **translucens** were active in ice nucleation when tested by a tube-freezing method. Cell suspensions of **X.c.** pv. **translucens** strains

required 70 min. to freeze. Ice nucleation activity was not detected in **X.c.** pv. **vesicatoria,** pv. **campestris** pv., **phaseoli** and pv. **vignicola,** Kim *et al.*, 1987 have found this Xanthomonads can cause ice to form at minus 2-8°C.

Pathogenicity tests:

In pathogenicity tests, the strains from wheat infected wheat, barley, rye, rice, **C.dactylon** an **L.multiflorum.** Symptoms were more severe on wheat and barley compared to rye and **C.dactylon.** The lesions on leaves were water-soaked showing shiny exudate drops and brown (Fig 2).

On barley leaves water soaked lesions, were scarce but longer than those on wheat leaves (Fig 3).

Compared to published host-range date of **X.c.** pv. **translucens,** the wheat isolates are **X.c.** pv. **translucens.** This pathovar is known to have a relatively wide host range, infecting wheat, barley, triticale, rye, **Agropyron** spp., **Bromus** spp. (Fang *et al.,* 1950; Bradbury, 1984; Barry *et al.,* 1982, Alizadeh and Rahimian, 1989), oat (Boosalis, 1952), wild rice (Bawden and Percich, 1983), maize, sorghum, and **Echinochloa crus-galli** (Moffett and Mc carthy, 1973).

Results of the inculations which were performed on the durum and soft wheat varieties commonly cultivated in the Aegean Region were evaluated to the 1-4 scale (El-Banoby and Rudolph, 1989).

All the wheat varieties were found to be in the class 4 and susceptible while Ege 88 variety was highly susceptible having more water-soaked lesions and bacterial exudation from the lesions (Fig. 4).

Seed detection:

No **X.cam.** pv. **translucens** was isolated from five collected seeds in İzmir. This disease is undoubtly seed-borne although it wasn't isolated which can be attributed to the wheather conditions resulted in no black chaff symptoms.

This is the first record of this disease in Turkey which was isolated and identified in the Aegean Region. It is suggested that wheat growing areas must be surveyed for this disease and the seeds from the infected areas shouldn't be used in production.

ÖZET

BUĞDAY VE DİĞER GRAMİNEAE'lerde BAKTERİYEL ÇİZGİ (**Xanthomonas campestris** pv. **translucens**) HASTALIĞI ÜZERİNDE ÇALIŞMALAR

Bakteriyel çizgi hastalığı buğday ve triticale'nin en ciddi hastalıklarından biridir. Ege Tarımsal Araştırma Enstitüsü buğday deneme parsellerinde görülen hastalıklı bitkilerden bakteri izole edilmiş ve yapay inokulasyonlarla patojenliği

belirlenmiştir. İzolatlar morfolojik, fizyolojik ve biyokimyasal özelliklerine göre **Xanthomonas campestris** pv. **translucens** olarak tanılanmıştır.

Bauğday izolatlarının arpa, çavdar, çeltik ve triticale yanında bazı yabani Gramineae türlerini de hastalandırdığı belirlenmiştir. Ege Bölgesinde yaygın üretimi yapılan ekmeklik ve makarnalık buğday çeşitlerinin **X.c.** pv. **translucens**'e duyarlı olduğu bu çeşitler içinde "Ege 88" çeşidinin ise çok duyarlı olduğu saptanmıştır.

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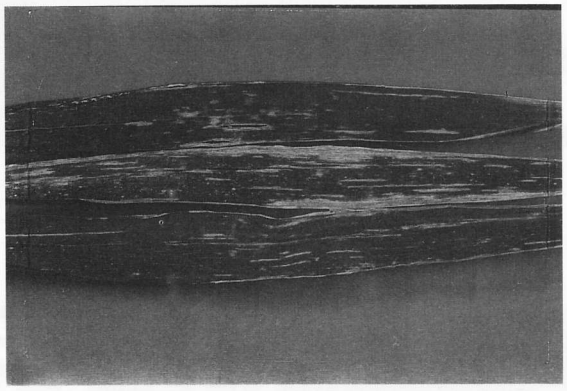

Fig 1. Symptoms of bacterial leaf streak on naturally infected wheat leaves

Fig 2. Wheat leaves showing water-soaked brown lesions.

Fig 3. Barley leaves artifically infected with **X.c.** pv. **translucens** (wheat strain)

Fig 4. More water-soaked lesions on cv. Ege 88 than the others.

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Identification of **Erwinia amylovora** and the Occurrence of Fire Blight of Pear in Western Mediterranean Region of Turkey

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ABSTRACT

*Fire blight, incited by the bacterium **Erwinia amylovora** has been one of the most serious and unpredictable disease of pomaceous fruit trees. The identification of the causal organism by a simple method on a modified MS medium and the occurence of fire blight of pear were determined in the Western Mediterranean region of Turkey. **E.amylovora** was isolated from pear twigs and from exudates of young quince twigs from orchards in Bucak and Korkuteli in 1990. Incidence of blighted trees of cultivar Santa Maria. Ankara and Williams was determined. The length of primary blossom period was 20-25 days. Some varietal difference were seen, for instance Santa Maria was more susceptible than Ankara and Williams, and Ankara was the most resistant one. Based on the behaviour of the fire blight in other countries, it is expected to become established in Turkey and constant control measures, particularly forecasting system for timing protective bactericide applications, should be practiced to minimize losses.*

INTRODUCTION

Fire blight, incited by the bacterium **Erwinia amylovora** (Burrill) Winslow et al, has been one of the most serious and unpredictable disease of pomaceous fruit trees (van der Zwet and Keil, 1978). Fire blight was detected in Turkey for the first time in 1985 on pears. The initial discovery occurred in Sultandağ, Afyon, in 1987 the disease was also detected in Isparta and Burdur (Öktem and Benlioğlu, 1988).

In 1982, a severe outbreak of fire blight was noted on pears in the Nile Delta of Egypt, since then the disease has been a severe problem (van der Zwet, 1986). Fire blight was observed in Israel for the first time in 1985 (Beer et al., 1986). Therefore, the appearance of fire blight in Turkey in 1985 was not unexpected.

Fire blight varies in severity from location to location and from year to year. Although all commercial cultivars of apple and pear are susceptible to fire blight in the sense that some infection can occur, there are large differences in degrees of susceptibility. Furthermore, susceptibility can be affected by growing conditions and cultural practices (Aldwinckle and Beer, 1978).

The present study was undertaken to identify **E.amylovora** by a simple method on a modified MS medium (Brulez and Zeller, 1981) and to determine the occurrence of fire blight of pear in Western Mediterranean Region of Turkey. Preliminary report has been published (Momol et al., 1991).

MATERIALS and METHODS

Identification: Isolation of **E. amylovora** was made for identification purposes at two locations from pear and quince (**Cydonia oblonga** Mill.) twigs.

Semiselective medium for isolation of **E. amylovora** 8 g Difco nutrient broth, 50 g sucrose, 20 g Bacto-agar, 9 ml of 0.5 % Bromthymol Blue, 2.5 ml of 0.5 % Neutral Red, 1000 ml distilled water, pH adjusted to 7.4 by 1 N NaOH, 50 mg of sterile filtrated cycloheximide (in 5 ml water) was added after autoclaving (Miller and Schroth, 1972; Brulez and Zeller, 1981). Isolation of **E. amylovora:** Twigs were superficially disinfected with 70 % ethanol or 0.5 % NaOCl. The edges of the lesions and 1-2 cm of the healthy tissue were crushed with pestle and mortar in sterile 0.01 M MgSO4. After serial dilution in 0.01 M MgSO4, 0.1 ml were streaked on petri plates with the semiselective medium and incubated at 27 C for 48-72 h (Rudolph, 1990). Green pear tests were performed with the isolated bacteria (van der Zwet, 1986).

Occurrence in 1989: Occurrences of fire blight on different cultivars of pear trees at Korkuteli, Antalya in 1989 were recorded. The disease assessments on June 16 were conducted in an orchard at Uzunoluk. A hundred pear trees per cultivar (Santa Maria 5 - year - old, Ankara 9 - year - old) were selected randomly, and number of blighted trees and number of blighted blossom clusters and shoots per tree (strikes/tree) were counted based on the characteristic symptomps of the fire blight (blighted blassoms, cane like twigs, extensions of blighted tissues). The second disease assessment was made on June 22 in a different orchard at Imrahor. In this orchard, there were three cultivars, Santa Maria, Ankara and Williams (7 - year - old). Twenty five pear trees per cultivar were selected randomly and the same type of disease assessment was made in this orchard.

Occurrence and disease progress in 1990: The assessment of fire blight in time was made in two locations (Korkuteli, Antalya and Bucak, Burdur). A susceptible cultivar (Santa Maria) to fire blight was chosen. Blighted blossom clusters and shoots per tree (strikes/tree) were pruned and recorded from 24 trees on each of three assessment dates. Disseased units were determined from visible symptoms.

RESULTS

Identification: E. amylovora was isolated from pear twigs with typical disease symptomps and from exudates of young quince twigs from orchards in Bucak and Korkuteli in 1990. The pathogen was identified by domed, orange-red collonies on MS medium. When unripe pears were inoculated with the isolated bacteria by pricking, typical white exudates (ooze) appeared on the fruitlets within 2-3 days when incubated at 27 C at high relative humidity.

Occurrence in 1989: Incidence of blighted trees of cultivar Santa Maria were 47 percent at Uzunoluk and 44 percent at Imrahor orchards, but strikes per tree were low (1.21 and 1.64 respectively) (Table 1.) (Table 2.). This indicates that infections were light. Under these conditions, pear cultivar Ankara showed no fire blight symptoms at both orchards. At Imrahor (Table 2.) most infections were recorded on cultivar Santa Maria, and almost 4 times less strikes per tree were recorded on cultivar Williams, and no infection on cultivar Ankara. It was observed that, in cultivar Ankara, blossom period was started one week earlier than cultivar Santa Maria. In 1989, the disease affected several pear and quince orchards at Korkuteli, Antalya.

Occurrence and disease progress in 1990: In 1990, blossom periods of Santa Maria pear were recorded at both locations (Table 3.). Flowering started earlier and ended earlier at Bucak. Early flowering at Bucak affected the onset of the disease progress.

Flowering occurred between 11 April an 3 May in Korkuteli, first blighted blossoms were seen on May 25. Based on the disease assessment made on June 12, the results indicate that the cumulative number of strikes per tree were 6.41 (Table 4.). The disease was progressed to a much higher level in Korkuteli than Bucak (Fig. 1.). In Bucak, trees started to bloom on about 6 April and continued until 26 April (Table 3.). In the orchard, first blight symptoms occurred on May 18, and no blight symptoms were found at Korkuteli on the same day (Table 4.). Very light infections were observed in Bucak during the disease assessment period.

DISCUSSION

In the Western Mediterranean Region of Turkey most symptoms observed on pear were those of blossom blight, all caused by blossom infections, shoot infections were rare. Ooze droplets were seen easily and bacterial strands were never observed. The length of primary blossom period was 20-25 days. Therefore, this information should be taken into account during the protective spray of blossoms for the control of fire blight on pears.

In 1989, almost no rainfall occurred during April and May in Korkuteli. In general, it was an extremely dry year in Turkey. Fire blight is most favoured by warm (18-27 C) weather plus rain and least by cool dry weather. Temperature has a very important role for the development of blight epidemic but rainfall and surface moisture should be taken into account (Billing, 1984). Therefore, light infection (1.64 strikes per tree) during 1989 in Korkuteli (Table 2.) was predictable. In 1990, more rainy days were observed in Korkuteli and this resulted in 6.41 strikes per tree (Table 4.) at the same orchard (Imrahor) on the same cultivar (Santa Maria).

In 1990, the different level of disease observed in Korkuteli and Bucak (Table 4.) could be explained by the different management system, soil type, blossom period, mean daily temperature, relative humidity and amount of rainfall.

Some varietal differences were seen, for instance Santa Maria was more susceptible than Ankara and Williams and Ankara was the most resistant one. In cultivar Ankara blossom period was one week earlier (cool weather) than cultivar Santa Marika. Besides their genetic difference, the flowering period could explain the reaction of these two cultivars to fire blight. All of the results obtained during this study were under natural infection conditions. In order to evaluate the susceptibility of pear, apple and quince cultivars grown in Turkey to fire blight, an experimental orchards should be established far away from any commercial pear, apple and quince orchard areas. Then, artificial inoculation could be exercised for selection of resistant cultivars.

Based on the blehaviour of the fire blight in other countries, it is expected to become established in Turkey and constant control measures, particularly forecasting system for timing protective bactericide applications, should be prasticed to minimize losses.

ÖZET

TÜRKİYE'NİN BATI AKDENİZ BÖLGESİNDE ARMUTLARDA ATEŞ YANIKLIĞI HASTALIĞININ BELİRLENMESİ VE TANISI

Erwinia amylovora tarafından oluşturulan ateş yanıklığı, yumuşak çekirdekli meyve ağaçlarının en ciddi ve tahmini güç hastalıklarından birisidir. Türkiye'nin Batı Akdeniz Bölgesinde armutlarda ateş yanıklığının belirlenmesi ve basit bir yöntemle modifiye edilmiş MS ortamında etmenin tanısı yapılmıştır. Bucak ve Korkuteli'nde 1990 yılında armut ve ayva sürgünlerinden **E. amylovora** izole edilmiştir. Santa Maria, Ankara ve Williams çeşitlerinde ateş yanıklığı gösteren ağaçların buluma oranı belirlenmiştir. Çiçeklenme dönemi 20-25 gün kadar sürmektedir. Çeşitler arasında bazı değişiklikler görülmüştür, örneğin Santa Maria çeşidi Ankara ve Williams tan daha hassastır, Ankara çeşidi ise içlerinde en dayanıklı olanıdır. Ateş yanıklığının diğer ülkelerdeki davranışına bakılacak olursa, hastalığın Türkiye'de de yerleşeceği beklenmektedir, bu hastalığa karşı tüm mücadele olanakları, özellikle koruyucu bakterisidlerin zamanlamasına yardımcı olacak önceden tahmin sistemleri, kayıpları en aza indirmek için uygulanmalıdır.

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Table 1: Occurence of fire blight in cultivars Santa Maria (5-year-old) and Ankara (9-year-old) pear orchard on June 16, 1989 at Uzunoluk, Korkuteli, Antalya.

Cultivar	Number of trees	Number of blighted trees	% Incidence of blighted trees	Strikes per tree
Santa Maria	100	47	47	1.21
Ankara	100	0	0	0.00

Table 2: Occurence of fire blight in cultivars (7-year-old) Santa Maria, Ankara and Williams pear orchard on June 22, 1989 at Imrahor, Korkuteli, Antalya.

Cultivar	Number of trees	Number of blighted trees	% Incidence of blighted trees	Strikes per tree
Santa Maria	25	11	44	1.64
Ankara	25	0	0	0.00
Williams	25	7	28	0.44

Table 3: Blossom development of Santa Maria pear trees at Korkuteli, Antalya and Bucak, Burdur in 1990.

Location	Blossom Development					
Korkuteli	**********					
Bucak	**********					
	5	15	25	5	15	25
	[	APRIL	]	[	MAY	]

Table 4: Occurence of fire blight based on cumulative number of strikes per tree (blighted blossom clusters and shoots per tree) on susceptible pear cultivar Santa Maria (8-year-old) in 1990.

	Disease assessment dates			
Location	18 May	25 May	7 June	12 June
Imrahor Korkuteli Antalya	0	0.08	–	6.41
Karapınar Bucak Burdur	0.04	0.25	0.41	–

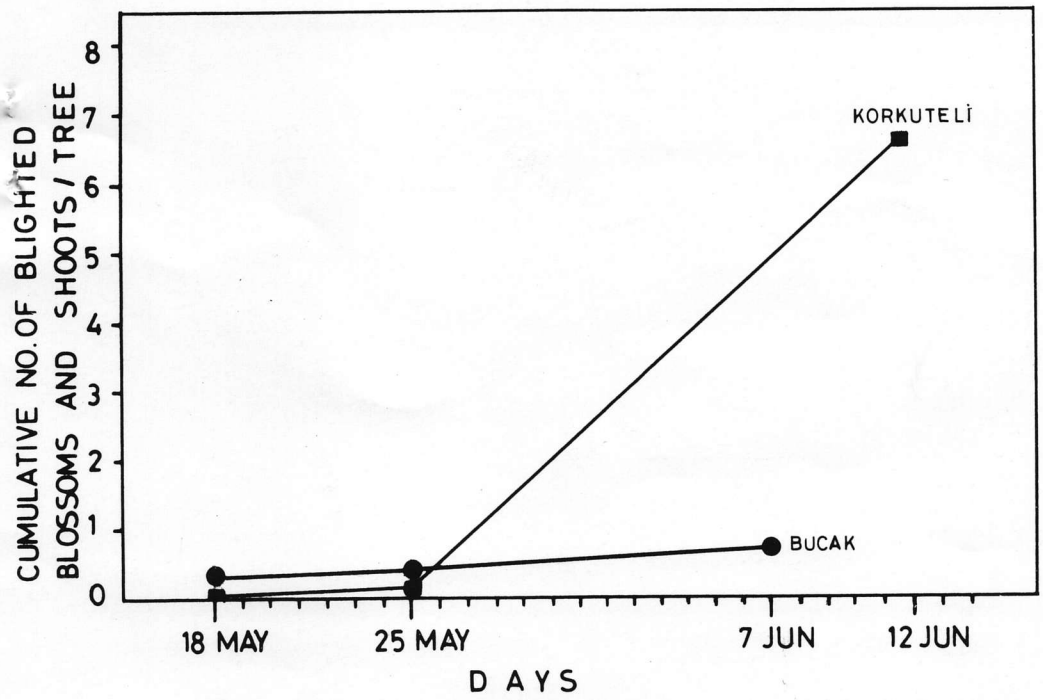

Figure 1: The progress of fire blight based on blighted blossoms and shoots per tree (strikes per tree) on Santa Maria pear orchards at Korkuteli, Antalya and Bucak, Burdur.

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Behaviour of Some Cotton Varieties to Cotton Wilt Disease Caused by **Verticillium dahliae** Kleb.

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ABSTRACT

In the present study, eighteen cotton varieties were tested for their behaviour against ***V. dahliae,*** *the caused organism of cotton wilt disease. The studies were designed as pot and field experiments. The pot experiments were in responsibility of Bornova Plant Protection Research Institute and the field experiments were carried out by Nazilli Cotton Research Institute.*

According to the results of these experiments, the varieties ST-250/1, ST-250/2 and ST-226/2 were found to be tolerant and Stoneville-506, Stoneville-825 were susceptible varieties.

INTRODUCTION

Cotton is an important income source for both countries which have grown it and industrialized ones. Cotton is a plant which provides foreign currency at most with proportion of 34,1 % at total exports for our country. Besides it is an important raw material in textiles and oil industry.

Aegean Region, which has cotton growing areas over 200.000 ha, is the second cotton production centre in Turkey. This region has 32 % of cotton growing areas and provides 41 % of Turkey's production (İncekara, 1979). In the region, one of the most important problems of cotton is wilt disease caused by **Verticillium dahliae.** The population of **V. dahliae** has gradually increased in soil and threatened the cotton growing in our region due to the fact that the sensitive cotton varieties were grown and the crop rotation and the sufficient cultural practices have not been applied.

The cotton wilt was first reported by Iyriboz (1941) from Kırkağaç in Turkey and the pathogen was determined as **Verticillium** sp. Studies, which were carried out to determine the causal agent, indicated that the pathogen was **V. dahliae** (Karaca et al., 1971). The most effective way for avoiding the disease is to grow resistant varieties, so studies have been carried out on the determination of susceptibility of various cotton varieties against cotton wilt disease. The aim of these experiments is to provide the materials to tests about breeding and crossing to be done to obtain cotton varieties which are resistant to disease, fruitful and suitable to region circumstances.

The present study was carried out with collaboration of Bornova Plant Protection Rescarch Institute and Nazilli Cotton Research Institute during 1985-1986.

MATERIALS and METHODS

The following cotton varieties were tested in the experiments.

Variety	Origin	Variety	Origin
1. Nazilli 66-100 (K)	Nazilli	10. M-433	Nazilli
2. Nazilli 66-100 (79-13)	Nazilli	11. Ege-69 (79-2)	Nazilli
3. Nazilli - 84 (K)	Nazilli	12. Ege-69 (80-9)	Nazilli
4. Del Cerro (78-11)	Nazilli	13. Glandsız-129	E.Ü.Z.F.
5. Del Cerro (80-9)	Nazilli	14. Stoneville-825	A.B.D.
6. ST-250/1	Nazilli	15. Stoneville-506	A.B.D.
7. ST-250/2	Nazilli	16. Rax-4505	A.B.D.
8. ST-266/2	Nazilli	17. Rax-4562	A.B.D.
9. M-430	Nazilli	18. Rax-4632	A.B.D.

1. Pot Experiments: Pot experiments were arranged according to the randomized plot design. Each treatment was replicated seven times and 50 cm diameter of pots were used. The inoculum prepared in accordance with Zunnunov (1962) was added into each pot and then cotton seed were sown. Five cotton-seedlings were left in each pot when they had four leaves.

The final observations were done at the end of the vegetation period according to the vascular browning of the cotton plants.

2. Field Experiments: Field experiments were set up according to the randomized block design with four replication in a naturally infested field in Cotton Research Institute in Nazilli. The plots were made by two rows and each was 8 m. long. Sowing was done by hand. At capsule stage (in September), all plants in the plots were estimated by using 0-3 scale to determine the disease severity. At the end of the vegetation (in October), plants in one row of the plots were cut individually and disease ratio was measured.

0-3 scale:

0 : no disease symptoms

1 : moderately severe symptoms; the leaves turning yellow and withering up to 50 % of plants but no drying

2 : falling of leaves, withering of plants and died plants

The pot and field experiments were repeated for two years.

RESULTS

1. Pot Experiments:

The average rates of the incidence of **V. dahliae** are shown in Table 1.

Table 1. The average rates of the incidence of **V. dahliae** for the cotton varieties tested in 1985 and 1986

Varieties	1985	1986
ST-250/1	0.0	0.0
ST-250/2	2.9	0.0
ST-266/2	5.7	8.6
Rax-4505	14.3	28.6
Rax-4632	20.0	65.7
Rax-4562	42.8	42.8
Nazilli 84	22.8	25.7
M-433	28.6	17.1
Nazilli (66-100) Control	34.3	45.7
Ege 69 (79-2)	40.0	60.0
Glandsız-129	42.8	68.6
Delcerro (78-1)	48.6	80.0
Delcerro (80-9	48.6	88.6
Nazilli 66-100 (79-13)	51.4	54.3
Stovenille 825	54.3	80.0
Ege 69 (80-9)	57.1	65.7
M-430	65.7	37.1
Stovenille-506	88.6	80.0

From the above table, it is clear that ST-250/1, ST-250/2 and ST-266/2 are the most tolerant as compared to the all eighteen cotton varieties.

2. Field Experiments :

The rates of the incidence of **V. dahliae** according to the vascular browning, the disease severity by 0-3 scale and yield per da are given in Table 2.

Table 2. The rates of the incidence of **V. dahliae,** the disease severity and yield per da of the cotton varieties

Varieties	1985			1986		
	Disease incidence	Disease severity (index)	Yield kg/da	Disease incidence	Disease severity (index)	Yield kg/da
ST-266/2	0	0	372.0	5.0	0.03	401.2
ST-250/2	2.5	0.01	400.9	2.5	0.03	498.2
ST-250/1	5.0	0.03	417.6	12.5	0.18	394.0
M-433	7.5	0.14	391.7	27.5	0.99	360.4
M-430	2.5	0.10	360.1	45.0	1.21	358.3
Nazilli 84	77.5	0.70	450.9	72.5	0.38	434.8
Rax 4632	82.5	1.18	233.3	67.5	1.01	255.6
N-66/100 (K)	85.0	0.76	348.2	77.5	0.45	326.8
Stoneville 825	85.0	0.85	377.8	97.5	1.24	393.2
Rax 4562	87.5	0.77	309.8	75.0	1.08	283.0
N-66/100 (79-13)	87.5	0.87	403.0	77.5	0.89	415.8
Ege-69 (79-2)	87.5	0.94	393.6	80.0	1.04	341.4
Rax-4505	90.0	1.30	347.0	80.0	1.07	272.9
Delcerro (78-11)	90.0	0.79	364.2	82.5	0.86	353.0
Ege-69 (80-9)	92.5	1.14	364.3	90.0	0.89	383.0
Stoneville (506)	95.0	1.60	336.9	97.5	1.43	400.6
Delcerro (80-9)	95.0	0.77	360.1	85.0	0.81	380.4
Clandsız-129	97.5	1.15	354.8	90.0	0.99	382.4

From Table 2, it follows that ST-250/1, ST-250/2 and ST-266/2 were the most tolerant varieties in the field experiments as well.

DISCUSSION

Eighteen cotton varieties were tested by pot and field experiments for determination of susceptibilities against cotton wilt disease caused by **V. dahliae** in Aegean Region.

ST-250/1, ST-250/2 and ST-266/2 were found the most tolerant in the other cotton varieties by both pot and field experiments in two successive years. ST is a crossbred of SahelxTaşkent. It was determined that Taşkent varieties were the most tolerant through the previous studies as well (Karcılıoğlu et al., 1982).

Milkovski and Bozhinov (1977) has also reported that Taşkent varieties were highly tolerant. It can be said that the tolerant of ST is due to the parents of Taşkent. This is followed by M-433 and M-430 varieties from the standpoint of tolerant.

It was found that Rax varieties were sensitive in the field experiments. Whereas they were determined to be tolerant in the pot experiments. Nazilli 84 variety was improved as tolerant to **Verticillum** wilt by Cotton Research Institute, Nazilli.

It was seen that Stoneville varieties were the most sensitive. On the other hand, the other varieties were affected by the wilt disease in the different measure.

ÖZET

BAZI PAMUK ÇEŞİTLERİNİN SOLGUNLUK (**Verticillium dahliae** Kleb.) HASTALIĞINA DUYARLILIKLARININ SAPTANMASI ÜZERİNDE ARAŞTIRMALAR

Çalışmada 18 pamuk çeşidinin pamuk solgunluk hastalığı etmeni **V. dahliae**'ya duyarlılıkları saptanmıştır. Çalışma saksı ve tarla denemeleri şeklinde yürütülmüştür. Saksı çalışmaları Bornova Zirai Mücadele Araştırma Enstitüsünce, tarla çalışmaları da Nazilli Pamuk Araştırma Enstitüsü tarafından yapılmıştır.

Denenen 18 pamuk çeşidi arasında ST-250/1, ST-250/2 ve ST-266/2 çeşitleri dayanıklı, Stoneville-506, Stoneville 825 çeşitleri de duyarlı olarak bulunmuşlardır.

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