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## Detection of Tomato Mosaic Tobamovirus (ToMV) on the Local and Imported Tomato Seed by DAS-ELISA, Molecular Techniques and Electron Microscopy<sup>#</sup>

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### ABSTRACT

The applicability of ELISA in combination with dsRNA analysis and RT-PCR were used to determine ToMV in the seeds and cotyledone leaves of seedlings. The virus was not found on the seed coats by ELISA, but detected on the cotyledone leaves of seedlings by ELISA, dsRNA analysis and RT-PCR.

**Key words:** Tomato Mosaic Virus, ELISA, Double-Stranded RNA (dsRNA), Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)

### INTRODUCTION

Because of it's suitable ecological condition and irrigation facilities, the Eastern Mediterranean region of Turkey is known as the most productive area for the early and late vegetable crops production in which tomato production takes an important place. The tomato production however is not correlated with the cultivated area, since the plants are affected by several mechanically, vector and seed transmitted viruses.

Rate of seed transmission is related to the time of infection, growth stage of plant, infected plant species and environmental condition. It was observed that plant grown from infected seeds produce higher percent of infection.

The most common virus diseases infecting tomato crops are Tomato Mosaic Tobamovirus (ToMV), Tomato Yellow Leaf Curl Geminivirus (TYLCV), Cucumber Mosaic Cucumovirus (CMV) and Potato Y Potyvirus (PVY)'es in Turkey (Güldür and Yılmaz, 1994).

<sup>#</sup> The Grants were provided by The Turkish Scientific and Technical Research Council (TÜBİTAK, TARP-2431) and The Research Funds of Çukurova University.

## DETECTION OF TOMATO MOSAIC TOBAMOVIRUS (TOMV) ON THE LOCAL AND IMPORTED TOMATO SEED BY DAS-ELISA, MOLECULAR TECHNIQUES AND ELECTRON MICROSCOPY

The seeds used in plantation are mostly imported by firms, but quarantine regulations are not sufficient for detection of viral pathogen in the seeds which results in dissemination of the diseases in the field. Therefore the seeds should be tested before the plantation in a given area by serological and the molecular techniques.

The objective of this research is to detect viruses on the tomato seeds by utilizing of DAS-ELISA test, RNA extraction, dsRNA analysis and Polymerase Chain Reaction (PCR).

### MATERIALS and METHODS

The tomato seeds from different farmers and firms (Razan (Petoseed); Roquetero FA 180 (Hazera); Fantastik (Hazera); Tomato F1 Belmonty (Enza Zaden); Falkon (MAY); Domato Mandur (Agromar); Tomato M 74 F1 (Agrotek); 73-33 RZ (RIJK ZWAAN)) were obtained. After germination of seeds in the greenhouse, young cotyledon leaves of seedlings were harvested; 1/3 of the leaf samples were assessed serological based on ELISA against to the antisera of Cucumber Mosaic Cucumovirus (CMV), Tomato Spotted Wilt Tospovirus (TSWV), Pepper Mild Mottle Tobamovirus (PMMV), the other 1/3 of the seedlings were proceeded for the total RNA extraction of ToMV and the RT-PCR tests.

The other part of the samples was mechanically inoculated to *Chenopodium quinoa* Wild, *C. Amaranthicolor* Costa & Reyn, *Datura stromarium* L., *Nicotiana glutinosa* L., *N. tabacum* L. "Samsun", *Nicotiana tabacum* L. "Samsun nn", *N. tabacum* L. "Rustica", *Nicotiana tabacum* L. "Xanthi", *Nicotiana tabacum* L. "Xanthi-nc" and *Gomphrena globosa* L.

The dsRNA analysis was made from the infected *N. tabacum* "Samsun" plants. The procedure was applied one by one to each of seed lots.

#### Virus Purification, SDS-PAGE Electrophoresis, Electron Microscopy and Serology

The young leaves of infected *N. tabacum* "Samsun" were used in purification of Tomato Mosaic Virus. The purification was made by Gooding and Hebert, (1967). Molecular weight of ToMV protein was determined with the method described by Laemelli, (1970). The purified material was examined by Elektron Microscopy (Milne, 1993). The infectivity rate of seed transmission was determined by DAS-ELISA on the seeds after germination.

In each lot 25 seeds were used for the testing. DAS-ELISA was performed according to the method of Clark and Adams (1977).

### **Total RNA Extraction, Double-Stranded RNA (dsRNA) Analysis and Electrophoresis**

The young leaves of ToMV infected *N. tabacum* "Samsun" plants and young cotyledon leaves of germinated seeds were used in total RNA extraction (Astruc et al., 1996). In the dsRNA analysis ToMV infected Samsun leaves were used (Valverde et al., 1990). Electrophoresis was performed in 1.0% agarose gel (Galitelli and Minafra, 1994). The size of amplified fragments was determined by DNA molecular weight markers (SIGMA P9577, PCR Marker, 50-2.000 bp).

### **Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)**

The purified nucleic acid of ToMV was amplified for detection of causal organism in the tomato seedlings (Castello et al., 1995). RT-PCR was accomplished in a Techne-Genius thermal cycler (model FGEN02TD).

The sequence of complete genome was used for designing the specific primers (HPLC purified Iyontec) to ToMV (Genbank in [www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)). To detect ToMV, the primers (PrForward 5'-GAAGAGCTAATGCGTCGGGC identical to bases 2671-2690, PrReverse 5'-ATCTGCGTAGCCCCTTGAGA complementary to bases 3157-3176) of ToMV complete genome from Genbank AF155507 were employed in RT-PCR.

Amplification of complementary DNA (cDNA) from the viral RNA, a 25 µl reaction mix containing AMV-Reverse Transcriptase (RT) and RT-buffer (Promega), RNasin (Promega), dATP, dGTP, dCTP, dTTP (Promega) and primer reverse (PrR) in the RT step, for the amplification of cDNA in PCR step the 25 µl of mixture containing MgCl<sub>2</sub> and MgCl<sub>2</sub>-Buffer (Promega), dATP, dGTP, dCTP, dTTP (Promega), Taq polymerase (Promega) and primers reverse and primers forward were used.

RT reaction at 37 C° for 1 hour, PCR reaction at 94 C° for 30 second, 60 C° for 1 minute and 72 C° for 1 minute, for 35 cycle with a final extension at 72 C° for 10 minute for one cycle were carried out in the thermocycler.

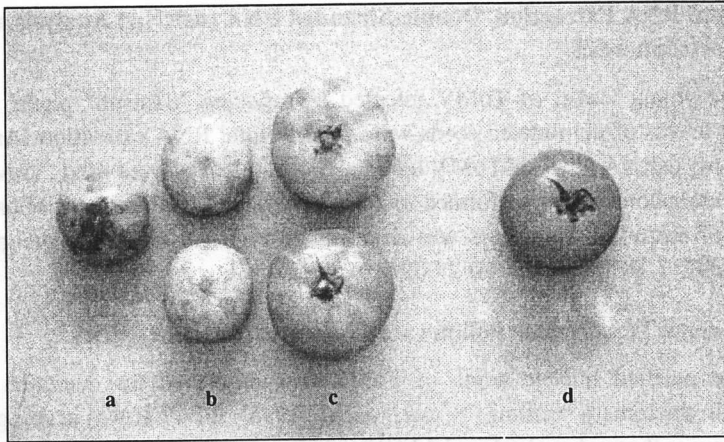
## **RESULTS and DISCUSSION**

The molecular techniques were employed to detect ToMV on the tomato seeds and seedlings that were provided by different farms and farmers.

After germination of ToMV infected tomato seeds the leaf deformation, and mosaic on the leaves were observed. At the time of harvesting, uneven ripening and discoloration, and necrosis type of symptoms developed on the fruits. The fruits were also reduced in size comparing to that of control (Figure 1). The similar type of symptoms were reported on the fruits and leaves by Jones et al., (1991); Mayhew et al., (1984); Lanter et al., (1982).

The typical symptoms of ToMV were expressed on the indicator plants (Yorgancı, 1975; Andrade ve ark., 1981; Güldür and Yılmaz, 1994).

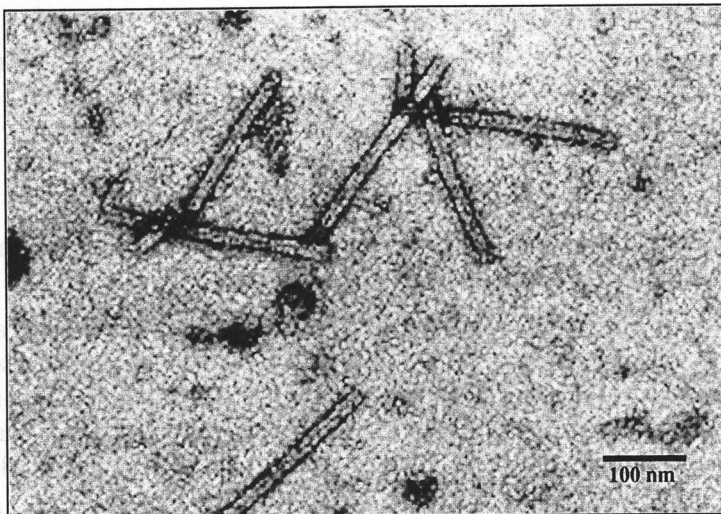
**DETECTION OF TOMATO MOSAIC TOBAMOVIRUS (TOMV) ON THE LOCAL AND IMPORTED TOMATO SEED BY DAS-ELISA, MOLECULAR TECHNIQUES AND ELECTRON MICROSCOPY**



**Figure 1.** Infected (a,b,c); healthy (d) Tomato Fruits.

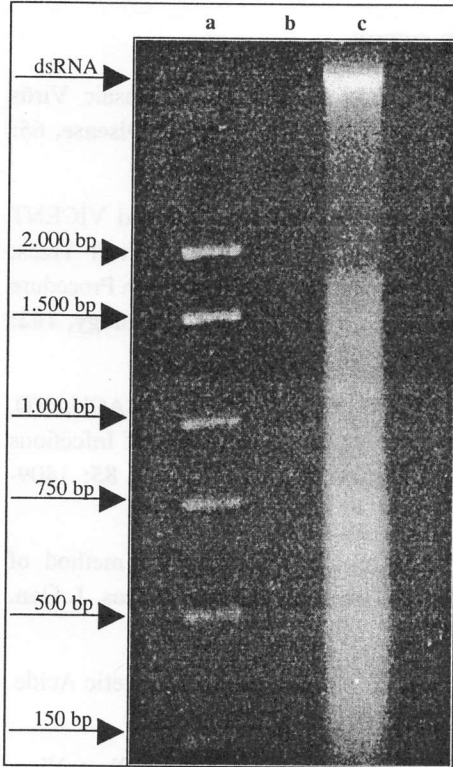
ToMV was not directly found on the tomato seed, but easily found on the cotyledone leaves of seedling by ELISA. CMV, TSWV and PMMV were not found out on the same samples.

The straight ToMV particules (300x18 nm) were observed on the purified material under electron microscopy (Figure 2). The molecular weight of the coat protein was determined as 17.6 kDa by SDS-PAGE. The other workers on the morphology of ToMV particles and the molecular weight of coat protein have reported similar findings (Mayhew et. al., 1984; Fraenkel- Conrat, 1957).

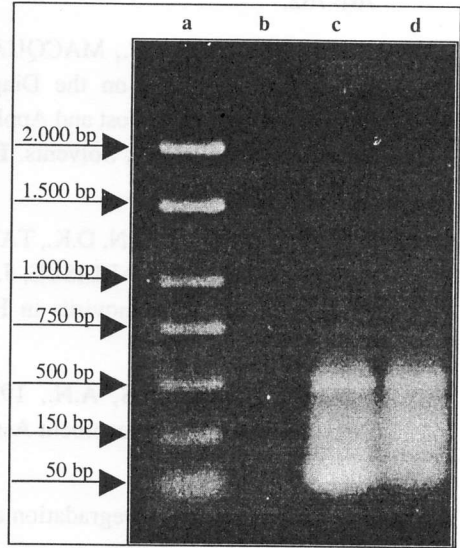


**Figure 2.** Tomato Mosaic Tobamovirus (ToMV) Particles.

ToMV was diagnosed by dsRNA analysis in the young leaves of tomato seedling (Figure 3). The extract of RNA of ToMV from infected samples were amplified using primers specific to ToMV. The fragment with size expected (505 bp between Pr forward and Pr reverse of ToMV) for amplification on ToMV RNA was observed by agarose gel electrophoresis analysis (Figure 4).



**Figure 3.** dsRNA analysis; marker (a), healthy (b), and infected tomato (c).



**Figure 4.** Agarose gel electrophoresis of RT-PCR products (500bp) by specific primers to ToMV. Marker (a), healthy (b), infected (b,c) tomato.

## ÖZET

### MOLEKÜLER TEKNİKLERLE YERLİ VE YURT DIŞINDAN GETİRİLEN DOMATES TOHUMLARINDA DOMATES MOZAYİK VIRÜSÜ (TOMV)' NÜN SAPTANMASI

Domates tohumlarında ve domates fidelerinin kotiledon yapraklarında ToMV nün varlığını saptamak için ELISA, dsRNA analizi ve RT-PCR yöntemleri kullanılmıştır. ELISA testi ile yapılan çalışmalarda domates tohumlarından hazırlanan ekstraktlarda ToMV saptanmamıştır. Buna karşılık çimlendirilen domates fidelerinin kotiledon yapraklarında



DETECTION OF TOMATO MOSAIC TOBAMOVIRUS (TOMV) ON THE LOCAL AND IMPORTED TOMATO SEED BY DAS-ELISA, MOLECULAR TECHNIQUES AND ELECTRON MICROSCOPY

ELISA testi dahil olmak üzere kullanılan dsRNA analiz ve RT-PCR teknikleri ile ToMV kolaylıkla saptanmış bulunmaktadır.

**Anahtar Kelimeler:** Domates Mozayik Virüsü, ELISA, Çift İplikli RNA RNA (dsRNA), Reverse Trascriptase Polimeraz Zincir Reaksiyonu (RT-PCR)

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## Detection of Incidence and Period of Natural Transmission of Citrus Stubborn Disease in Young Citrus Orchards in Hatay Province in Turkey

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### ABSTRACT

Surveys were carried out for determination of incidence of Citrus stubborn disease in young citrus trees (5 to 10 years old) consisting of sweet oranges, Minneola tangelo, mandarins and grapefruits which are dominant varieties grown in Erzin, Dörtüyl, İskenderun and Samandağ districts of Hatay Province in Turkey during the September to November in 2000 and 2001. For determination of natural infection rates of *S. citri*, periwinkle (*Catharanthus roseus* (L.) Don. G.) plants exposed in five young citrus orchards. For determination of periods of natural transmission of *S. citri*, healthy periwinkles were monthly placed in a W. Navel orange orchard (six years old) established by healthy (virus-free) seedlings. All test plants taken from orchards at the end of the exposes periods were kept at  $30\pm 2$  °C for 10 weeks and observed for developing symptoms and tested by double antibody sandwich immunosorbent assay (DAS-ELISA) for presence of *S. citri*. Generally, the highest infection rate of *S. citri* in the test plants was obtained in W. Navel orchard. The transmission rate of *S. citri* in periwinkle was found higher in the period of August-October. The infection rates of *S. citri* in young orchards were found between 1.6 to 12.0% and showed variation depending on districts and citrus species. The higher rates of *S. citri* were obtained in Erzin, Dörtüyl, İskenderun and Samandağ respectively. According to species, the higher rates of CSD-infected trees were obtained in sweet oranges, tangelo, grapefruits and mandarins respectively.

**Key words:** *Circulifer haematoceps*, Citrus, ELISA, insect vector, Periwinkle, *Spiroplasma citri*, stubborn

### INTRODUCTION

The causal agent of Citrus stubborn disease (CSL) was named as *Spiroplasma citri* by Saglio et al. (1973). *Spiroplasma citri* Saglio et al. (Mycoplasmatales: Spiroplasmataceae) is described and illustrated in detail by Bové (1980). Transmission of *S. citri* in California is primarily by beet leafhopper, *Circulifer tenellus*, but also *Scaphytopius nitridus* (Kaloostian et al., 1975, 1976). CSD is one of the most important diseases of citrus and its causal agent, *S. citri* is transmitted by the insect vector, *Circulifer haematoceps* (M.-R.)

## DETECTION OF INCIDENCE AND PERIOD OF NATURAL TRANSMISSION OF CITRUS STUBBORN DISEASE IN YOUNG CITRUS ORCHARDS IN HATAY PROVINCE IN TURKEY

(Hom.: Cicadellidae) in the Eastern Mediterranean Region of Turkey (Çınar et al., 1993). *S. citri* is not only an important pathogen of citrus (Calavan and Oldfield, 1979) but also of some other plants (Fletcher et al., 1981; Raju et al., 1981; Fletcher, 1983; Kersting et al., 1992; 1993). *S. citri* was shown to be spread from weed or vegetable hosts to a wide variety of weeds or vegetables by leafhoppers (Oldfield and Calavan, 1980). Sesame and some herbaceous plants are known as hosts of *S. citri* and its insect vector, *C. haematoceps*, in Turkey (Bové et al., 1979; Başpınar et al., 1993; Kersting et al., 1993; Şaş-Sertkaya et al., 1997). Periwinkle (*Catharanthus roseus* (L.) Don. G.) plants grown near a stubborn affected sweet orange citrus orchard became naturally infected by *S. citri* in Morocco (Bové et al., 1979), Iraq and Syria (Bové et al., 1984) and in U.S.A (Allen, 1975; Granet et al., 1976). Periwinkle is used in many studies on spiroplasmas as experimental plant or indicator plant of natural transmission (Bové et al., 1979; Çağlayan, 1987; Davis et al., 1990; Kersting, 1990; Şaş-Sertkaya et al., 1997; Maccherroni et al., 2001). Young citrus plants are more susceptible than older trees to the infection (Roistacher, 1991). If citrus tree is younger than 4 years old, the typical symptoms of the CSD were not observed and *S. citri* was not able to detect by culture assay successfully (Çağlayan, 1987). So, in this study the rates and periods of natural transmission of *S. citri* were detected and the incidence of citrus stubborn diseases was investigated in young citrus orchards (5 to 10 years old) in Hatay Province in Turkey.

### MATERIALS and METHODS

Natural infection rates of *S. citri* were determined by using periwinkle plants (*Catharanthus roseus* (L.) Don. G.) in five different young citrus orchards established with W. Navel orange (*Citrus sinensis* (L.) Osb.); Minneola tangelo (*C. reticulata* Bl. X *C. paradisi* Macf.); Satsuma mandarin (*C. unshiu* (Mak.) Marc.) and Star Ruby grapefruit (*C. paradisi* Macf.) in 2000 and 2001. The ages of the young orchards used for these experiments were ranged from five to seven years old.

Healthy 50 periwinkle plants (six to eight weeks old) were monthly placed in the each orchard from April to November for two years.

For determination of periods of natural transmission of *S. citri*, healthy periwinkles were placed in a W. Navel orange orchard (six years old) established by healthy (virus-free) seedlings in Erzin where is the main citrus growing area of Hatay. Every four weeks, 25 periwinkles were transferred to field and changed with a new healthy group at the end of that period. All test plants taken from orchards at the end of the exposes periods were kept at  $30 \pm 2$  °C in growth room for 10 weeks and observed for developing symptoms. All plants were tested by DAS-ELISA for presence of *S. citri* (Saillard and Bové, 1983). (ELISA kits got from Sanofi Diagnostics Pasteur-France). Absorbance at 405 nm ( $A_{405}$ ) was measured on a microtiter plate reader (Sirio S, Radim Group- Italy). If the absorbance value of the sample is 2.5 times higher than of the healthy control, the

samples were accepted positive for *S. citri* (Kersting, 1990). The insect vector, *C. haematoceps* was also sampled by using sweep net from April to November. Every two weeks 25 collections were made on weeds in the orchard.

Surveys were carried out for determination of incidence of Citrus stubborn disease in young citrus orchards (5 to 10 years old) including sweet oranges (W. Navel, Valencia and Shamouti), tangelo (Minneola), mandarins (Satsuma, Okitsu, Fremont and Clemantine) and grapefruits (Star Ruby, Henderson and Marshseedless) which are more growing varieties in Erzin, Dörtüyl, İskenderun and Samandağ districts (Figure 1) between October to November which is the most suitable period for development of symptoms and collection of plant material for DAS-ELISA test (Calavan, 1968; Bové et al., 1984; Çağlayan, 1987; Bové 1995) in 2001.

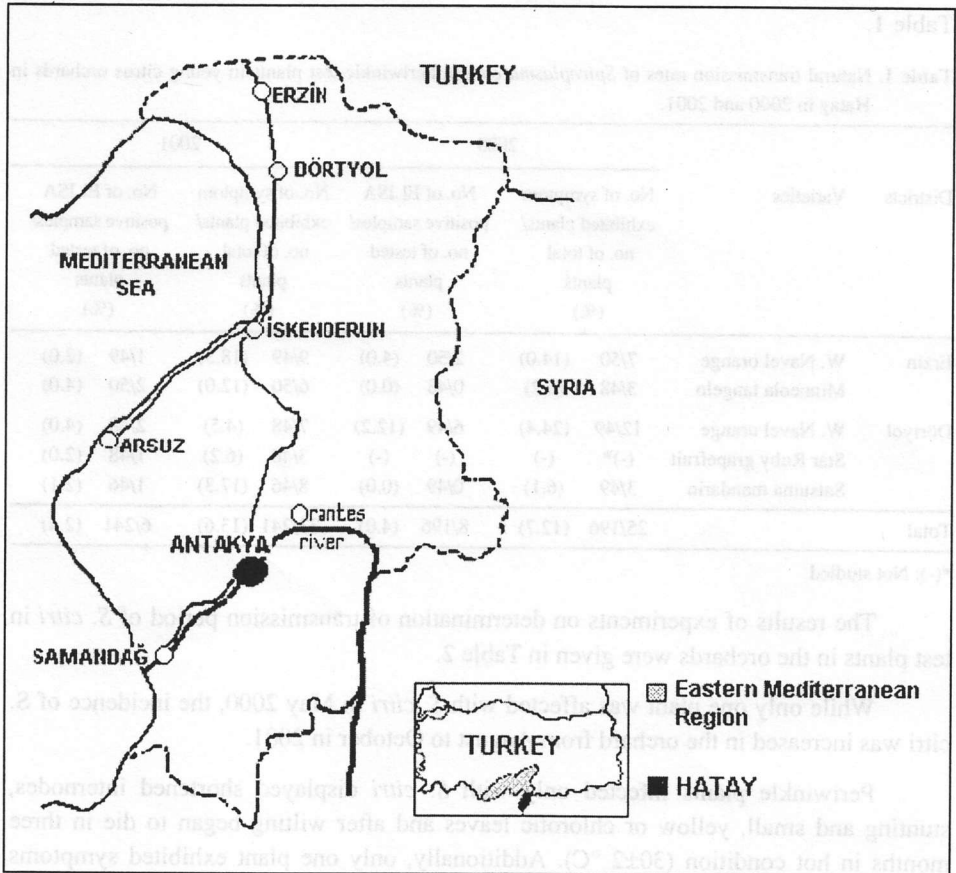


Figure 1. Citrus growing areas where surveyed for Citrus stubborn disease in Hatay in 2001-2002.

DETECTION OF INCIDENCE AND PERIOD OF NATURAL TRANSMISSION OF CITRUS  
STUBBORN DISEASE IN YOUNG CITRUS ORCHARDS IN HATAY PROVINCE IN  
TURKEY

For each species about 5-10% of all trees were surveyed for occurrence and incidence of CSD and shoot samples were randomly taken for ELISA from observed trees in the rate of 1% for each province. The symptoms of main citrus virus and virus like diseases were also observed on the trees in autumn 2001 and in early spring of 2002.

### RESULTS and DISCUSSION

According to the results of ELISA, the highest infection rate of *S. citri* was obtained in W. Navel orange orchard in Dörtüyl (12.2 %) in 2000. But the transmission rate was decreased in the next year in the same orchard. While natural infection was not occurred in Minneola tangelo in Erzin and in Satsuma mandarin in Dörtüyl in 2000, infection rates were found as 4.0% and 2.1% in 2001, respectively.

The data of natural infection rates of *S. citri* in test plants in Hatay was given in Table 1.

**Table 1.** Natural transmission rates of *Spiroplasma citri* in Periwinkle test plants in young citrus orchards in Hatay in 2000 and 2001.

Districts	Varieties	2000		2001	
		No. of symptom exhibited plants/ no. of total plants (%)	No. of ELISA positive samples/ no. of tested plants (%)	No. of symptom exhibited plants/ no. of total plants (%)	No. of ELISA positive samples/ no. of tested plants (%)
Erzin	W. Navel orange	7/50 (14.0)	2/50 (4.0)	9/49 (18.3)	1/49 (2.0)
	Minneola tangelo	3/48 (6.2)	0/48 (0.0)	6/50 (12.0)	2/50 (4.0)
Dörtüyl	W. Navel orange	12/49 (24.4)	6/49 (12.2)	7/48 (4.5)	2/48 (4.0)
	Star Ruby grapefruit	(-)* (-)	(-) (-)	3/48 (6.2)	1/48 (2.0)
	Satsuma mandarin	3/49 (6.1)	0/49 (0.0)	8/46 (17.3)	1/46 (2.1)
Total		25/196 (12.7)	8/196 (4.0)	33/241 (13.6)	6/241 (2.4)

\*(-): Not studied

The results of experiments on determination of transmission period of *S. citri* in test plants in the orchards were given in Table 2.

While only one plant was affected with *S. citri* in May 2000, the incidence of *S. citri* was increased in the orchard from August to October in 2001.

Periwinkle plants infected only with *S. citri* displayed shortened internodes, stunting and small, yellow or chlorotic leaves and after wilting began to die in three months in hot condition (30±2 °C). Additionally, only one plant exhibited symptoms consisted of phyllody, virescence, and shortened internodes in the orchard in September in 2001.

**Table 2.** Periods of natural transmission of *Spiroplasma citri* in Periwinkle test plants in a young W. Navel orange orchard in Erzin- Hatay in 2000 and 2001.

Period*	2000				2001			
	No. of simptom exhibited plants/ total no. of plants (%)		No. of ELISA positive samples/ no. of tested plants (%)		No. of simptom exhibited plants/ total no. of plants (%)		No. of ELISA positive samples/ No. of tested plants (%)	
April	2/22	(9.0)	0/22		1/25	(4.0)	0/25	
May	3/25	(12.0)	1/25	(4.0)	3/25	(12.0)	0/25	
June	2/24	(8.3)	0/25		2/25	(8.0)	0/25	
July	1/24	(4.1)	0/24		1/23	(4.3)	0/23	
August	1/24	(4.1)	0/25		5/25	(20.0)	1/25	(4.0)
September	2/25	(8.0)	2/25	(8.0)	4/24**	(16.6)	1/24	(4.1)
October	4/25	(16.0)	0/25		3/25	(12.0)	1/25	(4.0)
November	2/25	(4.0)	0/25		2/25	(8.0)	0/25	
Total	16/194	(8.2)	3/194	(1.5)	21/197	(10.6)	3/197	(1.5)

\* Periods were for four weeks and begun in the first week of each month

\*\* One plant additionally exhibited phyllody symptom

The results of survey for determination of incidence of CSD in young citrus orchards 5 to 10 years old in the districts where are citrus growing is concentrated in Hatay in 2001 were given in Table 3.

According to symptoms, the rates of CSD were showed variation between 1.55% to 40.75% depending on species and districts. When compared with ELISA results, higher rates of *S. citri*-infected trees were found by visually inspection in the orchards. Incidence of infected plants with *S. citri* varied between 1.6 to 12.0% by ELISA (Table 3).

During the surveys, 4.8% of young orchards were found to be established by virus-free seedlings in Erzin and Dörtüol.

The natural transmission rates of *S. citri* were not only varied between 0.0 and 12.2% in the orchards (5-10 years old) depending on cultivars, but also from year by year in the same orchard. The highest infection rates of *S. citri* were obtained in oranges and tangelos, and the lowest rates were found in mandarins. Şen et al. (1995) reported that 4 of 11 symptomatic samples taken from Robinson mandarin trees which were previously introduced and distributed to Eastern Mediterranean Region of Turkey were found to be infected with *S. citri* by ELISA. Highest transmission rates of *S. citri* in periwinkle test plants placed in 2-4 years old virus-free orchards were obtained in Dörtüol (13.3%), Erzin (12.5%) in 1994 (Şaş-Serkaya et al., 1995).

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**Table 3.** Incidence of Citrus stubborn disease in young citrus orchards (5 to 10 years old) in Hatay in 2001.

Districts	Species	No. of simptom exhibited plants/		No. of ELISA positive samples/		Total no. of young trees in Hatay**
		Total no. of observed plants (%)		No. of tested plants (%)		
Erzin	Sweet oranges	1150/3500	(32.85)	14/180	(8.00)	350.000
	Mandarins	70/4500	(1.55)	1/60	(1.66)	134.400
	Tangelos	1260/5000	(25.20)	11/120	(12.00)	
	Grapefruits	240/1000	(24.00)	9/90	(6.00)	16.500
Dörtyol	Sweet oranges	1370/7000	(19.57)	15/240	(6.00)	167.400
	Mandarins	115/5500	(2.09)	0/30	(0.00)	105.000
	Tangelos	254/1500	(16.93)	10/90	(9.00)	
	Grapefruits	84/1000	(8.40)	2/30	(3.00)	14.000
İskenderun	Sweet oranges	1470/6800	(21.61)	9/180	(4.00)	61.000
	Mandarins	176/2500	(7.04)	1/54	(1.85)	31.000
	Tangelos	163/400	(40.75)	5/60	(3.00)	
	Grapefruits	214/950	(22.52)	2/60	(2.00)	6.500
Samandağ	Sweet oranges	445/2000	(22.25)	4/90	(2.00)	20.000
	Mandarins	47/4000	(1.17)	0/30	(0.00)	60.000
	Tangelos	(-)*	(-)	(-)	(-)	(-)
	Grapefruits	(-)	(-)	(-)	(-)	(-)
Total	7058/45650	(15.46)	86/1300	(4.03)	965.800	

\* (-): Not detected

\*\* : No. of young Tangelo + mandarin trees (Anonymous, 2001).

The incidence of *S. citri* was found higher in period of August to October. This result was showed that the natural transmission of *S. citri* in citrus orchards was increased in end of the summer period. It was suggested that the insect vectors removed onto new young host plants as periwinkle at the end of the season. Bové (1986) was reported that when the weeds dried up under warm conditions the vectors containing *S. citri* moved from the weed hosts to citrus. It was reported that insect vector population has been increased in vegetal flora in September to November (Kersting, 1990). *Circulifer opacipennis* has been found in citrus orchards in Eastern Mediterranean Region of Turkey in April to December with in variable amount (Başpınar, 1990).

A Periwinkle plant exhibited phyllody symptoms in W. Navel orange orchard in September in 2001. But the symptoms as phyllody associated with a phytoplasma disease masked in new flush in 6-8 weeks and the symptoms of *S. citri* appeared dominantly after transferred into hot condition (30±2 °C). After observation period in controlled condition, this plant gave positive reaction for *S. citri* by DAS-ELISA. The plant infected with a concurrent infection of a phytoplasma and *S. citri* were survived longer than the plants infected with *S. citri* infection only. When the dually infected plants tissues



grafted on the young healthy periwinkles in flowering period (8-10 weeks old) were exhibited the symptoms related with the phyllody agent on leaves and flowers in 6 weeks after grafting.

Concurrent infections were found in lower rates than single infection of *S. citri*. Both pathogens *S. citri* and a phyllody phytoplasma are able to vectored by *C. haematoceps* and dually infection were reported in periwinkle and sesame in Eastern Mediterranean Region of Turkey (Kersting, 1990; Kersting et al., 1992; Kersting et al., 1993). Dually infections were also reported in first and second crop sesame as 0.0 to 0.1% and 0.2 to 0.6% in 1995 and 1996, respectively. If donor plant was concurrent infected with *S. citri* and a phytoplasma, both of the pathogens were able to transmit from sesame to periwinkle in 2.7% (Şaş-Sertkaya et al., 1997).

The rates of CSD showed variation depending on districts and citrus species. The higher rates of CSD were obtained in Erzin, Dörtiyol, İskenderun and Samandağ respectively. According to species, the higher rates of CSD were obtained in sweet oranges, tangelo, grapefruits and mandarins respectively.

Although the citrus seedlings in all commercial varieties have been producing in private nurseries (Sertkaya, 2001), climatic conditions are unsuitable for growing some cultivars as grapefruit, lemon and tangelo in Samandağ district. So, only Satsuma mandarin, Valencia and Shamouti oranges which are mostly grown varieties in the province were able to detect for CSD in Samandağ.

According to a survey, 21.6% of 10 to 15 years old W. Navel orange trees were found to be infected with *S. citri* in the Eastern Mediterranean Region of Turkey (Güllü, 1989). The infection rate of *S. citri* in mature grapefruit trees (14 years old) were reported as 22.0% in the same Region (Ertuğrul and Çınar, 1995). It was reported that the diagnostic symptoms of CSD were able to observe more intensive in young trees and after 4 to 5 years old, the trees have been exhibited all characteristic symptoms of the disease. The variability of the symptoms with season has also been reported in the East Mediterranean Region of Turkey (Çağlayan, 1997).

Because of severe symptoms (stem pittings on main trunk of young Minneola tangelo trees etc.) related to different virus diseases of citrus were observed on trees, it was suggested that, Although, W. Navel orange, Minneola tangelo and grapefruit trees were exhibited symptoms of CSD, these young trees have been affected concurrently with important vector and graft-transmissible citrus disease in Hatay where has been private citrus nurseries under outdoor (uncovered) conditions (Sertkaya, 2001). When compared oranges and tangelos with mandarins and grapefruits, the diagnostic symptoms of CSD were not observed on young Satsuma trees clearly. Young grapefruit trees were also exhibited leaf and shoot symptoms rather than flower and fruit symptoms.

In the last years, some citrus growers began to change the Minneola tangelo orchards, 8 to 10 years old, with a new variety or uproot the unfruitful trees in W. Navel

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orange orchards. Many W. Navel orchards 4 to 5 years old were being removed due to the prevalence of CSD in the Region in the mid of 1980's were also reported (Çağlayan, 1987).

During field studies, any symptom related CSD was observed in citrus orchards established with virus-free seedlings. Bozan (1995) reported that virus-free W. Navel orange seedlings were not infected with *S. citri* at the end of the 3rd year in the orchards in the Mediterranean Region of Turkey. Healthy Madam Vinous (*Citrus sinensis* (L.) Osb.) seedlings planted in 2-4 years old virus-free orchards from April to November in 1994 were not infected with *S. citri* (Şaş-Sertkaya et al., 1995). Generally, citrus seedlings have been produced by using bud-woods obtained from unknown or randomly selected source trees, and grown under outdoor conditions especially in Samandağ district in Hatay. Unfortunately, growers prefer to buy these citrus plants, because of different reasons as price of seedlings and cost of transport of plants from local nurseries are cheaper than virus-free seedlings etc. Because of the necessity of using healthy plants in a long period, citrus growers in Hatay must be encouraged to use virus-free seedlings to establish new plantations.

ÖZET

HATAY İLİNDE GENÇ TURUNÇGİL BAHÇELERİNDE PALAMUTLAŞMA  
(STUBBORN) HASTALIĞININ YOĞUNLUĞUNUN VE DOĞAL TAŞINMA  
DÖNEMLERİNİN ARAŞTIRILMASI

Hatay ilinde turunçgil yetiştiriciliğinin en fazla yapıldığı Erzin, Dörtiyol, İskenderun ve Samandağ ilçelerinde portakal, Minneola tangelo, mandarin ve Klemantin) ve altıntop çeşitlerinden oluşan 5 ile 10 yaş arasındaki genç turunçgil bahçelerinde hastalık yoğunluğunu belirlemek üzere 2000 ve 2001 yıllarında survey çalışmaları yapılmıştır. Hastalığın doğal taşınma oranlarını belirlemek için farklı çeşitlerden oluşan 5 bahçeye test bitkisi olarak Cezayir menekşesi (*Catharanthus roseus* (L.) Don. G.) yerleştirilmiştir. Hastalığın doğal taşınma dönemlerini belirlemek için virustan arındırılmış fidanlar ile kurulmuş olan 6 yaşındaki bir W. Navel portakal bahçesine aylık olarak yenileri ile değiştirilen sağlıklı *C. roseus* bitkileri yerleştirilmiştir. Dönem sonlarında bahçelerden alınan bütün test bitkileri  $30\pm 2$  °C'de 10 hafta süre ile tutularak simptom gelişimi gözlenmiş ve DAS-ELISA ile *S. citri*'nin varlığı araştırılmıştır. Genel olarak, en yüksek *S. citri* enfeksiyon oranı W. Navel portakal bahçelerine yerleştirilen test bitkilerinde elde edilmiştir. *S. citri*'nin *C. roseus*'a taşınma oranı en fazla Ağustos-Ekim döneminde belirlenmiştir. Turunçgil çeşidi ve ilçelere göre farklı olmakla birlikte genç turunçgil bahçelerinde Palamutlaşma hastalığı ile enfekteli ağaç oranları %1.6 ile %2.0 arasında değişkenlik göstermiştir. Turunçgil palamutlaşma hastalığı ile enfekteli ağaç oranları sırası ile en fazla Erzin, Dörtiyol, İskenderun ve Samandağ ilçelerinde belirlenmiştir.

Türlere göre Turunçgil palamutlaşma hastalığı ile en fazla enfekteli ağaçlar sırası ile portakal, tangelo, altıntop ve mandarinlerde belirlenmiştir.

**Anahtar Kelimeler:** Böcek vektör, Cezayir menekşesi, *Circulifer haematoceps*, ELISA, *Spiroplasma citri*, turunçgil, palamutlaşma

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## Development of cRNA Probes for Detection of Prunus Necrotic Ringspot Virus and Prune Dwarf Virus

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### ABSTRACT

cRNA probes for the detection of the ilarviruses, *Prunus necrotic ringspot virus* (PNRSV) and *Prune dwarf virus* (PDV) were developed. The probes were labeled with psoralen-biotin and used in a non-radioactive system for the detection of viral genomic RNAs in infected peach trees. Northern Analysis of total RNA extracted from young peach trees infected with either PNRSV or PDV alone or a combination of both viruses was done. The PNRSV-specific cRNA probe easily detected as little as 4 ng of purified viral RNA in a Northern assay. Both PNRSV and PDV probes were sensitive, specific, and did not cross-react in Northern assays.

**Key words:** cRNA probes, PNRSV, PDV, Non-radioactive labeling

### INTRODUCTION

Prunus necrotic ringspot virus (PNRSV) and Prune dwarf virus (PDV) are two economically important *Ilarviruses* that occur worldwide in the temperate regions where stone fruits are grown (Németh, 1986, Ulu et al.). Other than stone fruits, PNRSV can also infect rose (*Rosa* spp.) (Wong et al., 1988), and hops (*Humulus* spp.) (Smith & Skotland, 1986). Both viruses, alone or together with, can cause severe losses in fruit yield (Pine, 1964; Saunier, 1972; Schmitt et al., 1976; Ramsdell et al., 1992; Uyemoto et al., 1992), reductions in tree growth (Saunier, 1972), negative effects on the fruit set, size and color (Way & Gilmer, 1963; Topchiiska, 1982, Llacer et al., 1986), delayed fruit maturity (Saunier, 1972), reduced bud take, and reduced compatibility at the graft-union on susceptible cultivars of all their hosts (Gilmer et al., 1976). These viruses are transmitted by seed (Cation, 1949; Barba et al., 1986; Schmid, 1986; Mink, 1993), pollen (Ehlers & Moore, 1957; Howell & Mink, 1988; Uyemoto et al. (1992)), propagation materials, mechanical inoculation with sap, plant contact (Gilmer et al., 1976; Németh, 1986), and thrips (Greber et al., 1992).

PNRSV and PDV can be detected by biological indexing (Fridlund 1966), enzyme-linked immunosorbent assay (ELISA) (Barbara et al. 1978) and polymerase chain reaction (PCR) (Cambra et al. 1998). However, these methods have some limitations. Biological indexing requires long time and depends on environmental conditions (Scott, et

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al, 1986). ELISA requires specific antisera and may not detect low concentration of virus (Van Regenmortel, 1982, Scott et al, 1992). PCR is not practical for field surveys and a large number of samples (Hadidi et al, 1995). Therefore, some new technologies need to be developed for detecting PNRSV and PDV. The purpose of this study is to develop a non-radioactive and highly sensitive cRNA probes for detection of PNRSV and PDV.

### MATERIALS and METHODS

#### Viruses and Inoculation

The virus isolates (PNRSV-CA and PDV-CA) used in this experiment were the kind gift of Dr. J. K. Uyemoto, USDA/ARS, University of California, Davis. Plants were inoculated with viruses by chip-budding using buds from the stock seedlings containing PNRSV-CA and PDV-CA. The inoculum buds were placed 2 to 3 cm below the scion bud and the seedlings (Lovell) were kept in the greenhouse 2 months to allow bud union. Three replicates of each of the following treatments: inoculation with PNRSV, inoculation with PDV, inoculation with PDV + PNRSV, and a non-inoculated control were established for each treatment. The trees were transferred to a cold room (4°C) for 2 months to fulfill chilling requirements. In March the trees were transferred back to the greenhouse so growth could continue. When leaves of plants were 4-5 leaf stages total RNA was extracted.

#### Total RNA Extraction

Total RNA was extracted from leaves using a modified procedure of Hughes and Galau (1988). Fresh peach leaf tissue (100 mg) was homogenized in 1 ml of extraction buffer (200 mM Tris-HCl, pH 8.5, containing 300 mM LiCl, 10 mM EDTA, 1.5% sodium desoxycholate, 1% Nonidet P-40, 1.5% SDS and 0.5%  $\beta$ -mercaptoethanol) in a pestle and mortar. The extract was collected in a 1.5 ml microcentrifuge tube and centrifuged (8,163g) for 10 min at 4 °C. The supernatant was transferred to a clean 1.5 ml microcentrifuge tube and an equal volume of 3 M potassium acetate, pH 6.5 was added. The mixture was kept in ice for 5 to 10 m and centrifuged (11,750g) for 15 m at 4 °C. The supernatant was mixed with an equal volume of isopropanol and kept for at least 3 h at -15 °C to allow a precipitate to form. The nucleic acid precipitate was collected by centrifugation (16,000g) for 15 m, washed once with cold 70% ethanol, and allowed to air dry at room temperature. The pellet was resuspended in 20  $\mu$ l of RNase-free water and stored at -80 °C. The RNA samples were later used in PCR detection and Northern blot analysis.

#### Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) for Detection of Viruses

PCR analysis was done to verify virus infection according to Hadidi et al., (1995). PNRSV primers were designed from the sequence of isolate 30/4 (GenBank accession U57046 - Scott et al., 1998). The upstream primer (5' GTGACTATGTCACGAGCG 3')



corresponded to nucleotides 1034–1051 of the sequence and the downstream primer (5' CGGAGAAATTCGAGTGTGC 3') was complementary to nucleotides 1800–1818 of the sequence. The primers produced an amplification product of 785 bp. Primers for PDV were selected from the sequence of Genbank accession L28145 (Bachman et al., 1994). The upstream primer (5' ATGGATGCGATGGATAAAAATAGT 3') corresponded to nucleotides 1838–1860 of the sequence and the downstream primer (5' TAGTGCAGG TTAACCAAAAAGGAT 3') was complementary to nucleotides 1988–2010 of the sequence. The primers amplified a region of 172 bp. PCR products of the anticipated size were cloned and sequenced to confirm their identity.

#### **Production of PCR clones for PNRSV and PDV**

Clones for PNRSV and PDV were produced using pCR<sup>®</sup> II TOPO<sup>™</sup> cloning vector (Invitrogen, San Diego, CA).

#### **Sequencing of Plasmids**

Plasmids were sequenced using an ABI PRISM<sup>™</sup> Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin Elmer).

#### **Synthesis and Non-Radioactive Labeling of cRNA Probes**

Antisense probes that hybridize to the RNA 3 were generated for both PNRSV and PDV. The probe for PNRSV produced using a plasmid (p2-2), containing a 270 bp fragment of the viral coat protein gene in pBluescript, SK<sup>2</sup> (Staratagene, La Jolla, CA) and the probe for PDV was produced using a plasmid containing the 321 bp PCR clone of PDV prepared as described previously.

Plasmids containing the PNRSV and the PDV inserts were linearized with *EcoR* I (Promega, Madison, WI) and *Hind* III (Amersham Pharmacia Biotech, Piscataway, NJ) restriction enzymes, respectively, according to manufacturer instructions. The linearized plasmid was extracted with phenol/chloroform and precipitated with 0.1 vol. 3 M sodium acetate, 3.3 vol. cold ethanol, and incubated at –20 °C for 3 h to allow a precipitate to form. cRNA probes were synthesized using StripAble<sup>™</sup> RNA Probe Synthesis & Removal Kits (Ambion Inc., Austin, TX).

#### **Northern Blot Analysis and Non-Radioactive Detection**

Northern blot analysis was performed according to the protocol of the NorthernMax<sup>™</sup> Complete Northern Blotting kit (Ambion Inc., Austin, TX). PNRSV and PDV were detected in Northern analysis using non-radioactively labelled cRNA probes as described Figure 1.

#### **Densitometry**

Data for the relative concentration of PNRSV and PDV genomic RNAs were determined from the autoradiograph films of Northern blots using a GS-710 Calibrated Imaging Densitometer (BIORAD).

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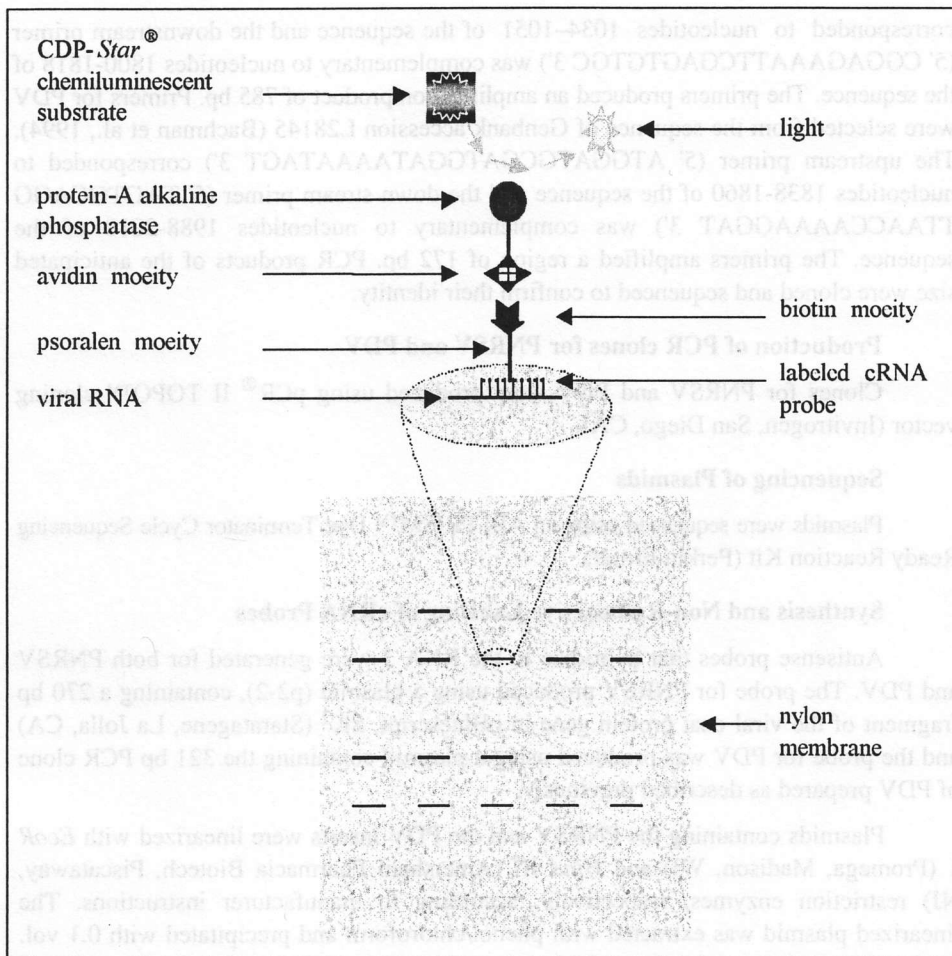
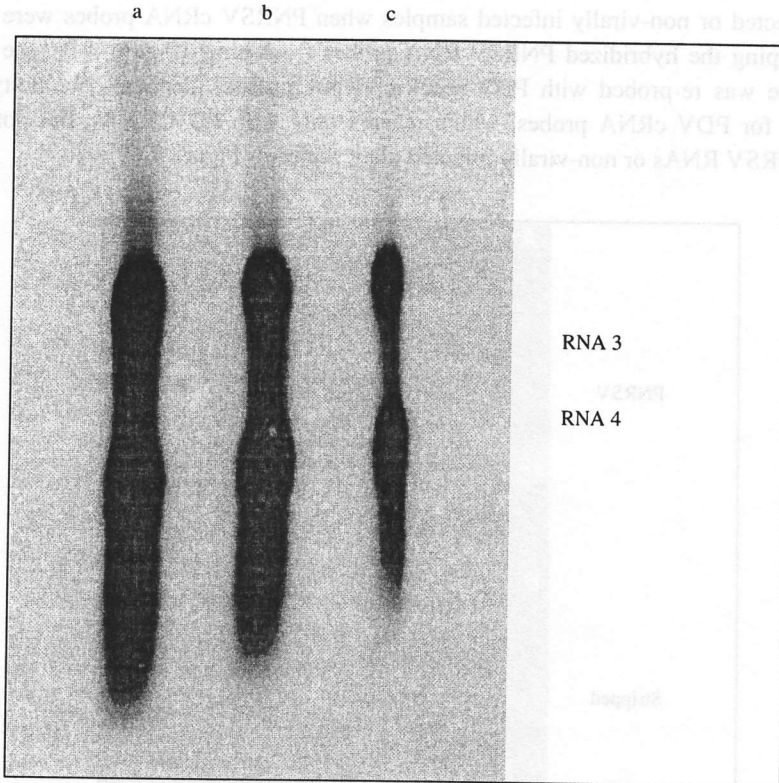


Figure 1. Graphical representation of the non-radioactive chemiluminescent detection system showing the major components involved in the attachment.

## RESULTS and DISCUSSION

Antisense PNRSV (270 bp) and PDV (321 bp) probes that hybridize to the RNA 3 were successfully transcribed and labeled with the BrightStar<sup>™</sup> Psoralen-Biotin Nonisotopic Labeling System. The cRNA probes were sensitive. The PNRSV-specific cRNA probe easily detected as little as 4 ng of purified viral RNA in a Northern assay (Figure 2).

cRNA probes have already been used for the detection of PNRSV (Crosslin et al., 1992) but there are no reports of the use of such probes for the detection of PDV.



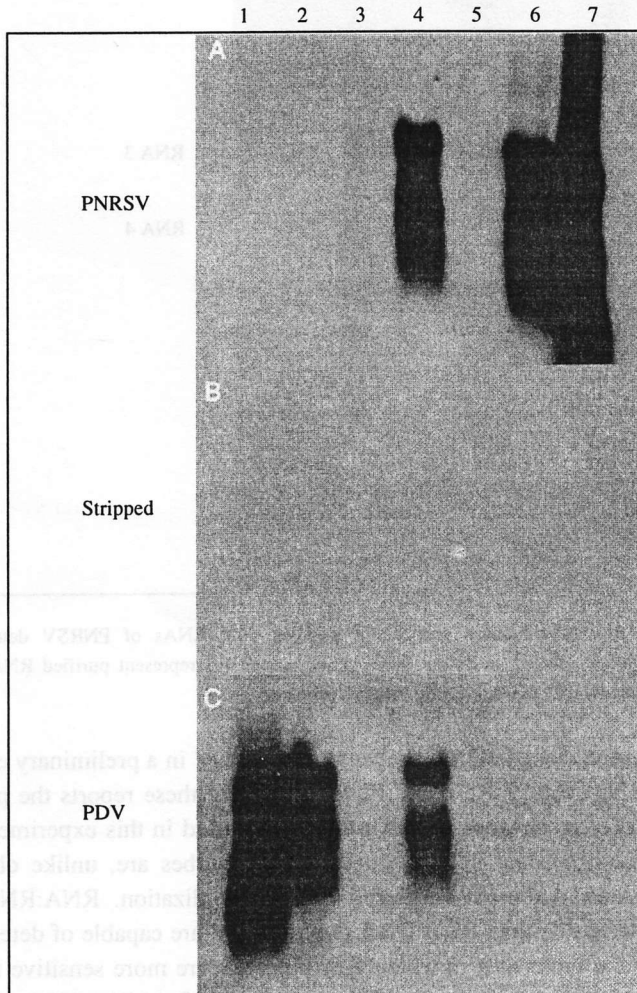
**Figure 2.** Northern blot hybridization analysis of purified viral RNAs of PNRSV detected using a psoralen-biotin labeled antisense cRNA probe. Lanes 1-3 represent purified RNAs of PNRSV used in the amounts of 40, 10, 4 ng, respectively.

Bachman (1994) used cloned cDNA probes to detect PDV in a preliminary examination of the synergism between PNRSV and PDV. In both of these reports the probes were labeled with radioactive isotopes. cRNA probes were used in this experiment, because they offered advantages over cDNA probes. cRNA probes are, unlike cloned DNA probes, single-stranded and are not depleted by self-hybridization. RNA:RNA duplexes are inherently more stable than RNA:DNA duplexes and are capable of detecting lower concentrations of the molecules to which they bind, i.e. are more sensitive than cDNA probes (Roy et al., 1989; Rao et al., 1990; Winkler & Goldrick, 1997). It has been reported that cRNA can detect picogram or even femtogram levels of viral RNA in dot-blot or Northern assays (Ambion, 1997).

The probes were specific. PNRSV-specific cRNA probes hybridized only to purified PNRSV viral RNAs and to total nucleic acids extracted from PNRSV-infected peach trees (Figure 3-A). Furthermore, no hybridization signals were observed for either

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PDV infected or non-virally infected samples when PNRSV cRNA probes were used. After stripping the hybridized PNRSV RNA probes from blots (Figure 3-B), the same membrane was re-probed with PDV-specific cRNA probes. Identical specificity was observed for PDV cRNA probes, which reacted only with PDV RNAs, but not with either PNRSV RNAs or non-virally infected plant samples (Figure 3-C).



**Figure 3.** Northern blot hybridization analysis of purified viral RNAs from PNRSV and PDV plus total RNAs from peach, detected using psoralen-biotin labeled antisense cRNA probes. The nylon membrane was first subjected to hybridization of PNRSV antisense cRNA probe (blot A), stripped (blot B), and reprobed with PDV antisense cRNA probe (blot C). The RNA samples were from: purified PDV, lane 1; total nucleic acids from PDV infected plants, lane 2; healthy peach tissues, lanes 3, 5; total nucleic acids from PDV + PNRSV-infected peach plants, lane 4; total nucleic acids from PNRSV-infected peach plants, lane 6; purified PNRSV, lane 7.

Following the development of the cRNA probes for both PNRSV and PDV and their obvious sensitivity in Northern assays, it now becomes of interest to make them applicable to the detection of these viruses in a simpler procedure that would allow for the testing of numerous samples. Currently, the simplest procedure for the detection of viruses in plant tissues is a blot in which tissues from the tree under test are pressed onto a nylon membrane and the 'print' is probed using a cRNA probe. This has proved extremely sensitive and allowed the testing of many samples for the presence of PLMVd (Skrzeczkowski et al., 1996) and has been used for the detection of PNRSV (Mas & Pallas, 1995). Adoption of such a procedure for the detection of both PNRSV and PDV would be attractive in situations in which large numbers of samples are to be processed. However, the probes used in this work are typically radioactive probes (Skrzeczkowski et al., 1996; Crosslin et al., 1992). Thus, processing large numbers of samples raises safety problems due to the handling of radioactivity and disposal of radioactive waste. Clearly non-radioactive probes that would eliminate these concerns over safety are desirable. Furthermore, non-radioactive probes do not suffer the other problems associated with the use of radioactive isotopes such as short life, requirement for long film exposure, and the need for specialized equipment not available in many diagnostic laboratories (Dietzgen et al., 1994; Nikolaeva, 1995). The work by Mas & Pallas (1995) used non-radioactive probes that were linked to digoxigenin, and they reported that tissue prints were a feasible detection system for PNRSV. However, no reports of the extensive use of this procedure have yet appeared and this may be due to the high non-specific reactions reported with some non-radioactive systems. The Brightstar system (Ambion, 1997), claims high specificity, high signal to noise ratio, and a lack of non-specific reactions. All of these claims have been met in the Northern blots that we have used in our examination of the concentrations of RNA 3 and 4 in trees infected with either PDV or PNRSV alone or with both viruses together. However, the Brightstar system involves the use of biotin, a molecule that is also present in plant tissues (a member of the B vitamin complex). In Northern blots much of the biotin present in plant tissues is eliminated from the samples during preparation and any remaining biotin is eliminated when the samples are exposed to formaldehyde during the electrophoresis that precedes blotting and the formaldehyde that is used in the pre-hybridization and hybridization buffers. In tissue prints, there is the possibility of residual biotin causing non-specific reactions with the cRNA probe and this must be overcome before tissue printing with non-radioactive cRNA probes that involve biotin can become a widely used procedure.

Further research is required to adapt this technology into "tissue blot" procedure that will enable to process quickly large number of plant samples in a short time.

#### ÖZET

#### KIRAZ HALKALI LEKE VİRUSU VE SERT ÇEKİRDEKLİLERDE CÜCELİK VİRUSUNUN TESPİTİ İÇİN cRNA PROPLARININ GELİŞTİRİLMESİ

İlaviruslar olan, kiraz halkalı leke (PNRSV) ve sert çekirdeklilerde cücelik viruslarının (PDV) tespitleri için cRNA propları geliştirildi. Bu viruslar ile hastalandırılmış

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şeftali ağaçlarında, viral genomik RNA'lar psoralin-biotin ile işaretlenmiş ve radioaktif olmayan proplar kullanılarak tespit edildi. Tek başına PNRSV veya PDV, veya her iki virus ile de hastalandırılmış genç şeftali ağaçlarından ekstrakte edilen tüm RNA'lar Northern Blot analizine tabii tutuldu. PNRSV virüsüne spesifik olan cRNA probu Northern Blot analizinde 4 ng kadar saflaştırılmış viral RNA'yı çok kolaylıkla tespit etti. Northern Blot analizlerinde her iki PNRSV ve PDV propları virüsleri tespit etmede hassas ve spesifik olması yanında, birbirleriyle karşı reaksiyona da girmedi.

**Anahtar Kelimeler:** cRNA propları, PNRSV, PDV, Radio-aktif olmayan işaretleme

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## Present Status of Verticillium Wilt of Olive in Western Anatolia and Some Factors Affecting the Disease Prevalence

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### ABSTRACT

A two-year survey was conducted in Aydın, Balıkesir, Çanakkale, İzmir, Manisa and Muğla provinces of Turkey, in order to assess the prevalence of verticillium wilt of olive in the region, the incidence and the intensity of disease in olive groves and to bring out the possible causative factors of disease prevalence through a farmers' pool.

In surveyed area the prevalence of disease was found as 49% and 60% in 1998 and 1999 respectively. Disease incidence was 0.8% in 1998 and 1.0% in 1999 in the olive groves where disease symptoms were observed. The mean disease index was 1.4 in both years in infected trees.

Verticillium wilt was most prevalent (84%) in young olive groves which had been established by grafted nursery trees. The factors such as, previous crop and intercropping also seem to be responsible factors for the prevalence of the disease as indicated by the farmers' pool.

**Key words:** *Olea europaea*, olive, verticillium wilt of olive, *Verticillium dahliae*

### INTRODUCTION

Olive is one of the most important and traditional crop in Western Anatolia. Nearly, 74% of the olive trees of Turkey grow in this region, which represent about 75% of the total national olive production (Canözer, 1991). Verticillium wilt of olive, caused by *Verticillium dahliae* Kleb., was first observed in Turkey in 1972 (Saydam and Copçu, 1972). During the last ten years the disease has become prominent in this region, especially in young, well-tended, regularly irrigated groves which had been planted with cotton previously.

The objective of this study was to assess the prevalence of verticillium wilt of olive in the region, the incidence and the intensity of disease in olive groves; to determine the causal agent/s and additionally, to bring out the possible causative factors of disease incidence through a farmers' pool.

# PRESENT STATUS OF VERTICILLIUM WILT OF OLIVE IN WESTERN ANATOLIA AND SOME FACTORS AFFECTING THE DISEASE PREVALENCE

## MATERIALS and METHODS

Systematic fields surveys were conducted in the 6 provinces in the western Turkey namely Aydın, Balıkesir, Çanakkale, İzmir, Manisa and Muğla comprising 124 olive groves which had been established on flat lands, between July and September in 1998 and between May and July in 1999. The number of groves visited in each province were determined according to its tree possession. In order to asses disease incidence, totally 500 trees were examined symptomatically in every groves surveyed. The following scale was used to determine disease severity, where the healthy trees were not considered:

- 1- Wilt symptoms on one main branch
- 2- Wilting affected half of the tree canopy
- 3- Total wilting, death of the tree

In most cases, two years old affected twig samples, 2 pieces each time, were randomly obtained from each wilted tree. Samples from older branches were rarely taken. The samples were divided transversely into 6 small sections, surface- disinfested in 0.5% sodium hypochloride for one minute and placed onto two different culture media. Ethyl alcohol- water agar was used for selective isolation of *Verticillium dahliae* (Nadakavukaren and Horner, 1959) and potato- dextrose agar (PDA) to isolate the other possible pathogens. Six sections were plated on each medium and plates were kept at 24 °C in the dark for 7- 10 days. Since the ethyl alcohol - water agar medium didn't create any important quantitative difference at the isolation frequency of *V. dahliae* in 1998, PDA was adopted as the standart isolation medium in 1999.

The identification of the pathogen and the other fungi isolated was realized according to Domsch et al., (1980), Sutton (1980) and Ellis (1993 a, b).

A farmers' pool containing 37 questions arranged under 5 main headlines such as plantation characteristics, land form, disease progress, cultural managements and farmers' opinions about the disease, were answered by the farmers whose groves were visited during the survey.

## RESULTS and DISCUSSION

The prevalence, the incidence and the severity of verticillium wilt of olive and also the rate of positive isolation of the *V. dahliae* from diseased trees in surveyed area in 1998 and 1999 are presented in Table 1.

The data presented in Table 1 demonstrate that the disease prevalence was 49% and 60% in 1998 and 1999 respectively in the surveyed area. Disease incidence was the highest in both neighbour provinces Manisa (1.9%) and Aydın (1.7%) where young and well managed olive groves were abundant. The mean disease index was found as 1.4 in infected trees as average of two years in the survey area. This means that the disease severity has not reached a high level of infection in the region yet.

**Table 1.** Prevalence and incidence of verticillium wilt, disease index and isolation rate of the pathogen in the survey area, 1998 and 1999.

Provinces	Number of groves examined	Number of groves with verticillium wilt		Disease prevalence (%)		Number of infected trees*		Disease incidence in affected groves (%)		Mean disease index		Positive isolations from infected trees (%)	
		1998	1999	1998	1999	1998	1999	1998	1999	1998	1999	1998	1999
Aydın	14	11	14	79	100	61	118	1.1	1.7	1.4	1.7	16.7	31.5
Balıkesir	44	18	25	41	57	63	110	0.7	0.9	1.4	?	3.2	8.2
Çanakkale	14	4	4	29	29	10	9	0.5	0.4	1.0	1.1	0.0	11.1
İzmir	14	10	12	71	86	51	35	1.0	0.6	1.3	1.5	3.9	37.4
Manisa	12	6	6	50	50	21	58	0.7	1.9	1.9	1.6	33.3	43.1
Muğla	26	12	14	46	54	39	37	0.6	1.2	1.5	1.2	0.0	27.0
Total/Average	124	61	75	49	60	245	367	0.8	1.0	1.4	?	7.7	25.9

\* 500 trees were examined in every orchard surveyed.

? Inadequate information

From the samples collected between late spring and early summer in 1999, the rate of positive isolation of the pathogen from diseased trees was three times higher than those of 1998, collected from late summer to fall. As already indicated by other authors, although the symptom expression is severe, living fungus in the tree tends to disappear during the summer and fall, and therefore *V. dahliae* seems to be most easily isolated in spring and early summer (Wilhelm and Taylor, 1965; Tosi and Zizzerini, 1998).

Verticillium wilt of olive, although not a new disease in Turkey, has been recorded with increasing frequency in recent years in Western Anatolia. A questionnaire answered by the farmers whose groves were visited during the survey was used to get an idea about possible ways or factors which may be responsible for the enhanced occurrence of the disease. The results are given below:

#### Relation of establishment technique and plant age to disease prevalence

The number of new, modern, intensive orchards established with grafted nursery trees have been increased in Western Anatolia during the recent years. But the dominant establishment method is still the use of wild plants uprooted from mountains as the rootstock which are grafted thereafter (Table 2). One of the most important data obtained from the farmers' pool is that the disease is most prevalent (75%) in olive groves which have been established by grafted nursery trees. But, since the percentage of prevalence in orchards established by using uprooted plants as the rootstock was found to be higher (69%) than the mean disease prevalence in 1999 (60%), so, it is not possible to consider that the infected nursery trees are the only way of disease dissemination in the region .

PRESENT STATUS OF VERTICILLIUM WILT OF OLIVE IN WESTERN ANATOLIA AND SOME FACTORS AFFECTING THE DISEASE PREVALENCE

Table 2. Relation of establishment technique and plant age to disease prevalence.

Plant age (Year)	Establishment Technique					
	Grafting wild trees in situ		Grafting uprooted plants		Grafted nursery trees	
	Number of affected groves	Number of non-affected groves	Number of affected groves	Number of non-affected groves	Number of affected groves	Number of non-affected groves
0 - 15	0	0	1	0	15	3
16 - 30	2	0	6	5	6	4
31 - 50	0	1	20	9	0	0
> 50	6	22	18	6	0	0
Total	8	23	45	20	21	7

In western Anatolia, 0-15 years old, young groves seem to be highly affected by verticillium wilt. But nearly half of the old groves which are more than 50 years old was also found to be infected in surveyed area (Fig. 1). As already indicated by various authors, although the young olive trees appear to be more susceptible to the disease, trees over 50 years old can also be affected by the disease (Balanco-Lopez et al., 1984; Al Ahmad and Mosli, 1993; Serrhini and Zeroual, 1995).

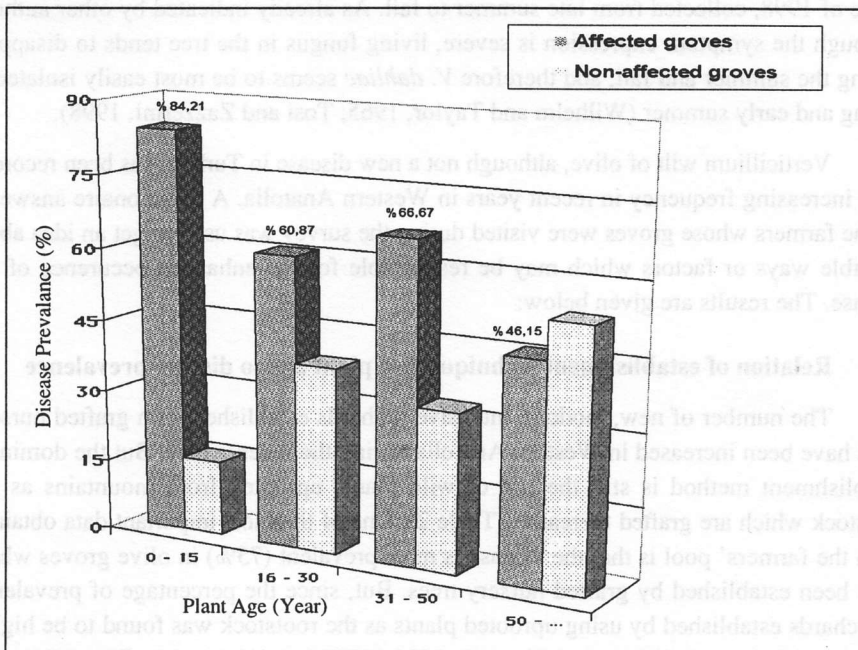
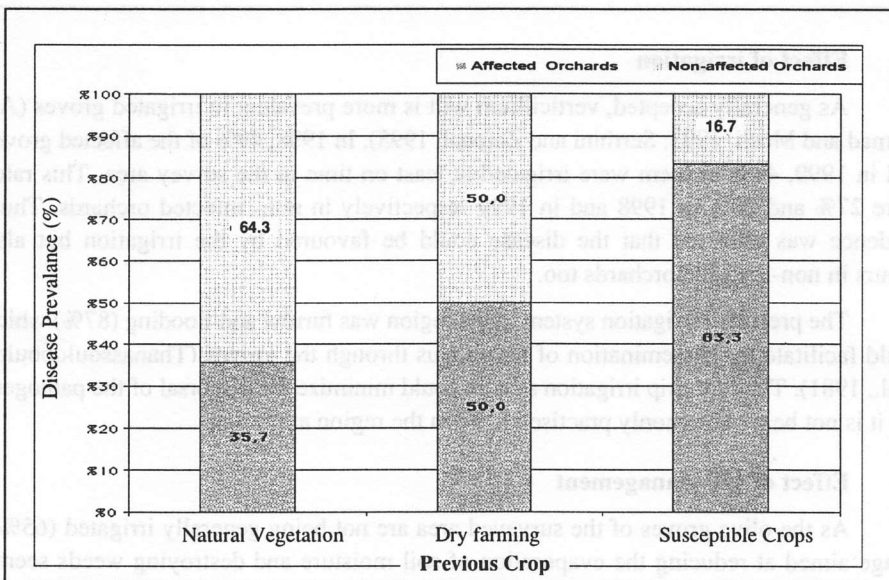


Figure 1. Relationship between plant age and disease prevalence.

**Effect of previous crop and intercropping on the disease occurrence**

High incidence of verticillium wilt may often occur in olive groves established in soils with a history of susceptible crops such as cotton or vegetables, as has already been pointed out by various authors (Wilhelm and Taylor, 1965; Caballero et al., 1980; Serrhini and Zeroual, 1995). In visited 124 orchards, 61% of them were established on lands which had never been cultivated before (scrubs, brushwoods, woods and pastures) or in fields managed mainly for dry-farming whereas only a small part (21%) of the groves were established on the soils which used previously for the cultivation of a susceptible crop, e.g., cotton, vegetables, grapevine, fig and other fruit trees. Although a high disease prevalence (%83%) was determined in groves which are estimated to be infested with the pathogen through susceptible previous crops, the percentage of disease was also found in remarkable rate in groves where such crops had never been cultivated before (Figure 2). This result are analogous with those reported by several authors (Thanassouloupoulos et al., 1979; Al- Ahmad and Mosli, 1993; Rodriguez- Jurado et al., 1993; Thanassouloupoulos, 1993).

Intercropping with *V. dahliae*- susceptible host can create serious problems in olive groves through the increase of inoculum (Thanassouloupoulos et al., 1979; Tjamos, 1993; Serrhini and Zeroual, 1995;). In our study, disease prevalence was found as 80% in intercropped 19 olive orchards, although this cultivation system has a restricted use in the survey area.



**Figure 2.** Effect of previous crop on disease prevalence.

## PRESENT STATUS OF VERTICILLIUM WILT OF OLIVE IN WESTERN ANATOLIA AND SOME FACTORS AFFECTING THE DISEASE PREVALENCE

### Tolerance of local olive cultivars against verticillium wilt

Although several studies carried out in Mediterranean countries have evaluated the reaction to verticillium wilt of well known olive cultivars (Pennisi et al, 1993; Rodriguez-Jurado et al., 1993; Tjamos, 1993; Jimenez- Diaz et al., 1998), the difference in *V. dahliae* -tolerance among Turkish olive cultivars has not been investigated yet. To form an opinion about the behaviour of local cultivars against pathogen, the cultivars of surveyed olive groves has been determined. The data presented in Table 3 demonstrate that the most dominantly cultivated ones are Edremit (Ayvalık) and Memecik (totally 72%) in surveyed area and so it can be noted that the disease was observed more often on these varieties (62%). The other cultivars seem to be also susceptible.

**Table 3.** Prevalance of verticillium wilt on different olive cultivars in the survey area.

Cultivars	Number of groves examined	Number of affected groves
Edremit(Ayvalık)	53	19
Memecik	36	19
Gemlik	13	7
Manzanilla	1	1
Domat	1	1
Çilli	1	1
Mixed	19	13
Total	124	61

### Effect of irrigation

As generally accepted, verticillium wilt is more prevalent in irrigated groves (Al-Ahmad and Mosli, 1993; Serrhini and Zeroual, 1995). In 1998, 49% of the affected groves and in 1999, 41% of them were irrigated at least on time in the survey area. This rates were 27% and 20% in 1998 and in 1999 respectively in non- infected orchards. Thus, evidence was obtained that the disease could be favoured by the irrigation but also occurs in non-irrigated orchards too.

The prevailing irrigation system in the region was furrow and flooding (87%) which could facilitate the dissemination of the fungus through the groves (Thanassouloupoulos et al., 1981). The root-drip irrigation system could minimize the dispersal of the pathogen, but it is not being commonly practised (2%) in the region at present.

### Effect of soil management

As the olive groves of the surveyed area are not being generally irrigated (65%), tillage aimed at reducing the evaporation of soil moisture and destroying weeds seems to be a very frequent local habit of olive farming, since 90% of the olive orchards were soil cultivated in 1998 (Table 4).

Soil cultivation might increase the disease incidence by causing damage to the superficial root system of olive tree and favours the entrance of the pathogen through these way (Tjamos, 1993). However, there was not a clear relation between soil management and the disease prevalence in surveyed area as indicated in Table 4.

**Table 4.** Effect of soil cultivation on disease prevalence.

	Number of orchards surveyed	Number of affected orchards	Disease prevalence (%)
Orchards with soil cultivation	111	59	53
Orchards without soil cultivation	13	2	15
Total	124	61	49

#### **Farmers' observations and opinion about the disease occurrence and development**

In 1998, 79% of the olive growers whose orchards were affected by the wilt, indicated that they had become familiar with such symptoms for only 1 to 3 years. Considering that the disease has been reported from western Anatolia in the early seventies, it seems that the increase of the pathogen attacks in olive groves could be very recent. Thus, all the adequate preventive measures should be taken as soon as possible to stop the disease from spreading.

Furthermore, during the survey, 75% of the farmers could not state any opinion about the source of the disease and only 3% of them thought that the causal agent might be a fungal pathogen.

Although the growers were ignorant of disease origine, they were carrying out an effective disease control by pruning the infected branches regularly. On the other hand, it seems possible that the real rate of disease prevalence or incidence in the region could be hidden by this pruning habit or in other words as a result of removing the infected branches from trees regularly. Thus, it should take into consideration that the disease could be detected less than it exists during the survey.

In conclusion, verticillium wilt tends to become one of the main phytopathological problems threatening the olive trees in Western Anatolia. Since the causal agent, *V. dahliae* is a hardly controlled pathogen, it is required that both olive growers and agricultural organization must pay ultimate attention to the disease. Olive growers who have insufficient information on the disease source, risk particularly spreading the disease over the uninfected areas. Taking in to consideration that any fungicides are unable to prevent or control verticillium wilt of olive yet (Biris and Thanasouloupoulos, 1980; Petsikos- Panayotarou, 1980; Tjamos, 1993) the growers must be informed about both the alternative strategies in confronting the disease and the cultural practices which favour it.

## ÖZET

### ZEYTİN VERTICILLIUM SOLGUNLUĞUNUN BATI ANADOLU'DAKİ DURUMU ve HASTALIĞIN YAYGINLIĞINI ETKİLEYEN BAZI FAKTÖRLER

Bu çalışmada, Aydın, Balıkesir, Çanakkale, İzmir, Manisa ve Muğla'da yer alan zeytinliklerde *Verticillium solgunluğunun* yaygınlık oranı, zeytinliklerde ortalama yakalanma oranı ve hastalık şiddetinin belirlenmesi, bu belirtilere yol açan etmen veya etmenlerin saptanması ve ayrıca bir anket yardımıyla hastalık çıkışında etkili olabilecek bazı faktörlerin ortaya konması amaçlandı.

Survey alanında 1998 ve 1999 yıllarında hastalığın yaygınlık oranı sırasıyla ortalama %49 ve %60 olarak saptandı. Hastalık görülen zeytinliklerde hastalığa yakalanma oranları 1998'de % 0.8, 1999'da ise %1 olarak belirlendi ve ortalama hastalık indeksi yine her iki yıl da 1.4 olarak bulundu.

*Verticillium solgunluğu* en sık olarak (%84) aşılı fidanla tesis edilmiş genç zeytin bahçelerinde görüldü. Anket sonuçlarına göre ön bitki ve ara tarım da hastalığın yaygınlığını arttıran faktörler olarak ortaya çıkmaktadır.

**Anahtar Kelimeler:** *Olea europaea*, zeytin, *Verticillium solgunluğu*,  
*Verticillium dahlia*

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First report for Aydın, Turkey: *Cryphonectria parasitica* (Murrill.) Barr.  
Threatens the Chestnut Orchards

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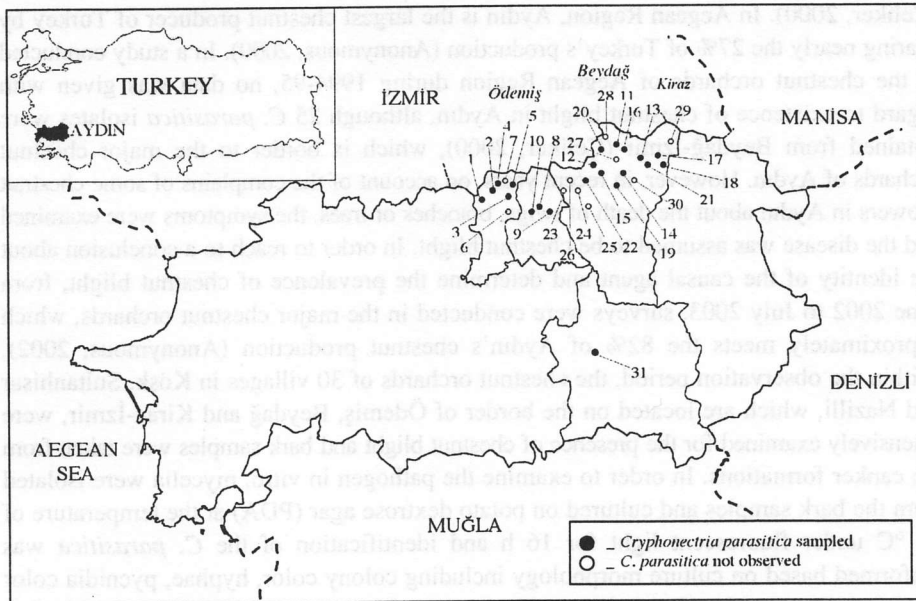
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Chestnut blight caused by *Cryphonectria parasitica* (Murrill.) Barr is one of the most significant diseases of chestnut (*Castanea* spp.) worldwide. The disease, which destroys the bark and the cambium thus inducing wilt and eventually death of distal tree parts, was first recorded in Turkey (Marmara Region) in 1967 (Akdoğan and Erkman, 1968). Later on the disease was reported from Blacksea Region (Delen, 1975; Coşkun and Kural, 1994; Coşkun et al., 1998) and Aegean Region (Balıkesir, İzmir and Manisa) (Çeliker, 2000). In Aegean Region, Aydın is the largest chestnut producer of Turkey by sharing nearly the 27% of Turkey's production (Anonymous, 2000). In a study conducted in the chestnut orchards of Aegean Region during 1994-95, no data was given with regard to existence of chestnut blight in Aydın, although 25 *C. parasitica* isolates were obtained from Beydağ-İzmir (Çeliker, 2000), which is border to the major chestnut orchards of Aydın. However, in recent years, on account of the complains of some chestnut growers in Aydın about the death of twigs, branches or trees, the symptoms were examined and the disease was assumed to be chestnut blight. In order to reach to a conclusion about the identity of the causal agent and determine the prevalence of chestnut blight, from June 2002 to July 2003, surveys were conducted in the major chestnut orchards, which approximately meets the 82% of Aydın's chestnut production (Anonymous, 2002). Within the observation period, the chestnut orchards of 30 villages in Köşk, Sultanhisar and Nazilli, which are located on the border of Ödemiş, Beydağ and Kiraz-İzmir, were extensively examined for the presence of chestnut blight and bark samples were taken from the canker formations. In order to examine the pathogen in vitro, mycelia were isolated from the bark samples and cultured on potato dextrose agar (PDA) at the temperature of 26 °C under fluorescent light for 16 h and identification of the *C. parasitica* was performed based on culture morphology including colony color, hyphae, pycnidia color and size, conidia shape and size (Anderson, 1914; Anagnostakis, 1987). Beside that each isolate was compared in morphology to a known *C. parasitica* isolate obtained from Sandra L. Anagnostakis, The Connecticut Agricultural Experiment Station, CT, U.S.A. Besides the survey areas mentioned above, chestnut orchards of Bozdoğan located at the border of Muğla were also examined to determine whether the disease has been introduced to the south parts of Aydın, although chestnut production is not high in these sites (Anonymous, 2002).

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During the surveys, in most of the orchards, similar lethal cankers were detected. The cankers appear as sunken necrotic lesions later turn to cracks that penetrate to the bark. Epicormic shoots were frequently found below the canker. Totally 84 isolates of *C. parasitica* were recovered from the bark samples collected from different chestnut orchards. According to this data, *C. parasitica* was first detected to enter the Aydın's chestnut orchards. The disease was not observed in the orchards of ten villages (Figure 1). The three isolates from the orchards of Bozdoğan indicated that the disease had been also disseminated to border of Muğla in the South. The intensities of the blighted trees were usually low in most orchards except in the ones (Akçaköy, Gökkişi, Malgaçmustafa, Appaklar, Bağcılı) located near to the border of İzmir (Ödemiş, Beydağ and Kiraz), which was previously reported to be contaminated (Figure 1). Therefore, it is suggested that the fungus was introduced to Aydın from the orchards of Ödemiş, Beydağ and Kiraz. According to some chestnut growers of Aydın, the disease has been present for approximately ten years, but the incidence has been increasing in recent years. These results may lead to conclusion that chestnut blight evolved to be a serious threat for the chestnut orchards of Aydın.



**Figure 1.** Map of the major chestnut producer villages were surveyed in Aydın (isolate number in the paranthesis). **Köşk:** 1. Gökkişi (14); 2. Akçaköy (9); 3. Cumayanı (5); 4. Kıran (1); 5. Sarıçam (1); 6. Ahatlar (0); 7. Kızılçaköy (0); **Sultanhisar:** 8. Malgaçmustafa (9); 9. Uzunlar (2); 10. Malgaçemir (1); **Nazilli:** 11. Derebaşı (8); 12. Appaklar (5); 13. Bağcılı (5); 14. Hisarcık (4); 15. Çatak (4); 16. Karahallı (3); 17. Kahveresi (3); 18. Kuşçular (3); 19. Ketendere (1); 20. Aksu (1); 21. Ovacık (1); 22. Semaili (1); 23. Çobanlar (0); 24. Kavacık (0); 25. Hasköy (0); 26. Sinekçiler (0); 27. İşıklar (0); 28. Esentepe (0); 29. Aşağıyakacık (0); 30. Bekirler (0); 31. **Bozdoğan** (3).

## ÖZET

### AYDIN-TURKEY İÇİN İLK RAPOR: KESTANE ÜRETİM ALANLARI *CRYPHONECTRIA PARASITICA* (MURILL.) BARR.'IN TEHDİTİ ALTINDA

*Cryphonectria parasitica* (Murill.) Barr.'nın oluşturduğu kanser kestanenin (*Castanea* spp.) en önemli hastalıklarından biridir. Ağacın kabuk ve kambium dokusunu tahrip ederek bu noktanın üzerinde kalan kısmın ölümüne neden olan hastalık Türkiye'de ilk kez 1967 yılında Marmara Bölgesinde saptanmıştır (Akdoğan ve Erkman, 1968). Daha sonra Karadeniz Bölgesi (Delen, 1975; Coşkun ve Kural, 1994; Coşkun et al., 1998) ve Ege Bölgesi'nin Balıkesir, İzmir ve Manisa illeri kestaneliklerinde (Çeliker, 2000) bulunduğu bildirilmiştir. Ege Bölgesinde yer alan ve ülkemiz yıllık kestanе üretimini yaklaşık %27'sini sağlayan Aydın'ı (Anonymous, 2000) da kapsayan bir çalışmada, bu İl'e sınır olan İzmir'in Beydağ İlçesi kestanе üretim alanlarından 25 adet *C. parasitica* izolatu elde edilmiş olmasına karşın Aydın kestanе üretim alanlarında bu hastalığın bulunduğu dair bir bilgi verilmemiştir (Çeliker, 2000). Son yıllarda kestanе ağaçlarında kurumaların olduğuna dair yakınmalar üzerine yapılan simptomatolojik gözlemlere dayanarak, hastalığın kestanе kanseri olabileceği kanısına varılmıştır. Ancak kesin tanıya gidebilmek ve bu hastalığın varlığı ve yaygınlığını belirlemek amacıyla, Aydın'da kestanе üretimini yaklaşık %82'sini karşılayan önemli üretim alanlarına (Anonymous, 2002) Haziran 2002 ve Temmuz 2003 tarihleri arasında surveyler düzenlenmiştir. Bu süre içinde İzmir'in Ödemiş, Beydağ ve Kiraz ilçelerine sınır olan Köşk, Sultanhisar ve Nazilli ilçelerinde üretimin yüksek olduğu 30 köyün kestanelikleri (Anonymous, 2002) geniş bir şekilde taranmış ve kanserli yapılardan kabuk örnekleri alınmıştır. Patojenin kesin tanısı için in vitro da her bir kabuk örneğinden izole edilen miselyum PDA ortamında 26 °C sıcaklıkta 16 saat süreyle floresan ışık altında kültüre alınmıştır ve *C. parasitica*'nın teşhisi kültürün morfolojik özellikleri (koloni rengi, hif yapısı, piknit rengi ve boyutu, konidi şekli ve boyutu) incelenerek yapılmıştır (Anderson, 1914; Anagnostakis 1987). Buna ek olarak her bir izolat A.B.D.'den Sandra L. Anagnostakis aracılığıyla elde edilmiş, bilinen bir *C. parasitica* izolatu ile morfolojik özellikleri yönünden karşılaştırılmıştır. Ayrıca yukarıda belirtilen survey alanlarının dışında, bu hastalığın Aydın'ın güneyinde Muğla sınırında kestanе üretimi düşük olan ilçelerinde de olup olmadığını belirlemek amacıyla Bozdoğan İlçesi kestanelikleri de incelenmiştir.

Surveyler sırasında, incelenen kestaneliklerin çoğunda benzer yapıda ölümcül kanserler belirlenmiştir. Önce kabukta çökük nekrotik lezyonlar olarak beliren ve daha sonra derin çatlaklar oluşturarak gelişen bu kanserlerin hemen alt kısmında çok sayıda sürgün gelişmektedir. Çeşitli kestaneliklerde farklı ağaçlarda bu tip kanserlerden alınan kabuk örneklerinden toplam 84 adet *C. parasitica* izolatu elde edilmiştir. Bu verilere göre, son derece tehlikeli bir hastalık olan kestanе kanserinin Aydın İli kestaneliklerine girdiği ilk kez tespit edilmiştir. Survey kapsamındaki köylerden sadece 10 köyün kestaneliklerinde bu hastalığa rastlanmamıştır. Bozdoğan İlçesi kestaneliklerinden üç adet izolatu elde edilmesi hastalığın güneyde Muğla sınırına kadar yayıldığını göster-

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mektedir. Hastalığın bulunduğu kestaneliklerde genelde hastalık oranı düşük olmasına karşın İzmir'in Ödemiş, Beydağ ve Kiraz ilçeleri kestane alanlarına komşu olan Akçaköy, Gökkişiş, Malgaçmustafa, Appaklar ve Bağcılı'da yüksek oluşu hastalığın Aydın'a bu yönden bulaştığı kanısını vermektedir. Bu köylerde bazı üreticilere göre, bu hastalık yaklaşık 10 yıldan beri görülmekte ancak son yıllarda bir artış göstermektedir. Bu verilere göre, kestane kanserinin Aydın kestanelikleri için önemli bir tehdit olabileceği sonucuna ulaşılmaktadır.

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## Effects of Soil Properties on the Occurrence of Rhizomania Disease in Sugar Beet Cultivars

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### ABSTRACT

This study was conducted to determine the relationships between physical and chemical properties of soils and the appearance of on rhizomania disease in sugar beet cultivars. Sugar beet production areas of Kastamonu sugar factory were surveyed and plant samples were taken from 68 fields that represent 2241 ha of acreage. Sixteen regions were selected based on the results of DAS-ELISA using sugar beet root samples. Physical and chemical properties of soils were determined. Sugar beet plants from three tolerant cultivars (Gina, Gabriela and Rizor) and three susceptible cultivars (Evita, Fiona and Sonja) have been grown as bait plants in room temperature for six weeks. DAS-ELISA was applied to root samples of bait plants to find out the virus content. Based on the calculated logistic regression equation, rhizomania disease was promoted as pH, phosphorus and potassium contents increased, as lime and loam contents decreased.

**Key words:** Beet necrotic yellow vein virus, rhizomania, sugar beet, soil properties, logistic regression

### INTRODUCTION

Rhizomania disease caused by Beet necrotic yellow vein virus (BNYVV) was reported in many parts of the world (Falk and Duffus, 1977; Heijbroek, 1984; Asher, 1987; Özgür, 1995). Rhizomania is a soil-borne disease and it infects sugar beet roots only via a vector fungus, *Polymyxa betae* Keskin (Asher and Thompson, 1987). Virus can survive in soil for at least 15 years inside the thick-walled resting spore of the fungus. After taking up once, fungus has carried the virus for a long time (Rush and Heidel, 1995). Rhizomania is common in poor drained soils such as kepir and vertisol which have high clay contents as well as in soils with high ground water levels. Soil is main factor in spreading of the disease. Factors that affect the infection are inoculum level of *P. betae*, soil temperature, soil moisture (Asher and Blunt, 1987) and pH (Abe,

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1987). In the present study, the relationships between the occurrence of rhizomania and the physical and chemical properties of soils were investigated.

### MATERIALS and METHODS

**Virus infected plant material:** Districts and villages in Kastamonu sugar factory production areas were surveyed in August-September periods in 1994 thru 1996. Considering virus symptoms in the plants, root samples were taken from 68 fields that represent 2241 ha of acreage.

**Material used in serological studies:** Root tips and hairy roots from selected sugar beets having virus symptoms were used in serological studies. Reagents used in DAS-ELISA were obtained from Sanofi (France). Polystyrene plate (Nunc-Denmark) were obtained from Bio-RAD company. Sugar beet extracts were prepared using porcelen mortar and pestle. Absorbance values were evaluated in a spectrophotometer Novapath™ Model 3550Bio-RAD.

**Survey method:** Surveys were conducted in Factory vicinity, Merkez, Araç, Daday, Devrekani, Taşköprü and Tosya districts in Kastamonu province; Ilgaz district in Çankırı province; and Boyabat district in Sinop province between 1994 and 1996, throughout August and September according to the methods described by Putz et al. (1990).

**Soil sampling:** Sixteen areas were selected based on the results of ELISA tests using root samples taken during surveys in 1994 thru 1996. Soil samples were taken from these regions in April 1996 and used as experimental material. At least one kilogram of soil was taken from 0-20 cm depth (Bürcky, 1994) and taken to laboratory in labeled polyethylene bags. Total salt (Richard, 1954), pH and lime (Black, 1965), organic matter (Walkey, 1947), phosphorus (Olsen et al., 1954) and potassium contents (Richard, 1954), and soil texture properties (Bouyoucos, 1952) were determined.

**Bait plant tests:** Soil samples taken from 16 selected areas were used in bait plant tests. 20 sugar beet seeds from each of the six cultivars [Gina, Gabriela, Rizor (tolerant cultivars), Evita, Fiona and Sonja (susceptible cultivars)] were planted in 500 ml of pots containing saturated soil. Each treatment was replicated three times. After the growing period of six weeks under room temperature, each pot was harvested separately and total root weight of each pot was determined. Root samples were homogenized using sterile mortars and pestles by addition of buffer solution (Anonymous, 1998). Root extracts were placed in tubes (12 ml) and centrifuged at 2500 rpm for two minutes. Supernatant was collected in eppendorf tubes and diluted with beet tip solution (1:10). These solutions were frozen at -27°C and used in ELISA tests (Anonymous, 1998).

**Serological tests:** Root tips and hairy roots selected from sugar beets with virus symptom were used in serological tests. DAS-ELISA method was performed according to Casper and Meyer (1981).  $\bar{x}+3.25s$  formula was used to differentiate healthy and contaminated samples. ( $\bar{x}$ : average absorbance value of 8-10 healthy samples;  $s$ : standard deviation of readings, Clark, 1981).

**Statistical analysis:** In order to detect the soil properties that can affect disease occurrence, Logistic Regression analysis method was applied using SPSS for Windows 6.0 software.

The logistic regression equation was calculated based on the following formula.

$$P_{(y)} = \frac{1}{1 + e^{-z}}$$

Soil properties that have significant effects on disease occurrence were detected using forward selection option (Özdamar, 1997).

## RESULTS and DISCUSSION

**Soil Properties of Rhizomania Infested Areas:** The results from soil analysis showed that the texture of the soils were generally alkaline and rich in clay. Lime contents of the soils were generally moderate while organic matter contents were low (Table 1).

**Table 1.** Soil analysis results of selected fields in the sugar beet production areas of Kastamonu sugar factory.

Fields	Area No	Salt %	pH	% Lime	% Organic Matter	P <sub>2</sub> O <sub>5</sub> (kg/da)	K <sub>2</sub> O (kg/da)	Texture component			Texture class (*)
								Sand	Clay	Silt	
Y. Ayvalı	1	0.160	7.85	16.6	2.20	6.64	126.4	24.15	46.80	29.05	C
A. Ayvalı	2	0.111	7.77	12.7	2.36	9.16	183.7	37.93	42.07	20.00	C
Kuşkara	3	0.170	7.65	17.0	2.93	13.51	92.4	22.07	48.88	29.05	C
Çavundur	4	0.105	7.92	14.6	2.28	6.64	93.6	20.00	48.88	31.12	C
Çaycevher	5	0.079	7.71	11.2	1.66	5.26	44.5	42.82	30.21	26.97	C-L
Batak	6	0.145	7.99	27.7	2.38	9.84	104.1	20.00	46.80	33.20	C
Bükkarşı	7	0.074	7.69	7.7	1.96	3.43	28.1	34.52	23.98	41.50	L
Çöroğlu	8	0.093	7.97	10.4	2.42	2.74	58.5	26.22	42.66	31.12	C
Dereköy	9	0.211	7.48	16.6	1.28	2.74	28.7	26.22	42.66	31.12	C
Ilıca	10	0.134	7.64	14.2	2.90	20.38	216.5	25.48	42.07	32.45	C
Cuma köy	11	0.170	7.70	9.6	1.83	3.43	68.9	20.00	48.88	31.12	C
Hacı Hasan	12	0.213	7.85	10.0	1.79	1.14	86.6	26.12	30.21	43.57	C-L
Akbük	13	0.086	7.76	12.0	1.53	2.29	156.8	24.15	32.28	43.57	C-L
Sofular	14	0.105	7.61	12.3	1.74	8.47	100.6	20.00	44.73	35.27	C
Kadıköy	15	0.142	7.16	3.8	1.40	17.86	79.6	26.22	46.80	26.98	C
Ethem Mh.	16	0.140	7.97	12.3	1.90	17.86	86.6	30.37	42.6	26.97	C

\* C: Clay; CL: Clay-Loam; L: Loam



## EFFECTS OF SOIL PROPERTIES ON THE OCCURRENCE OF RHIZOMANIA DISEASE IN SUGAR BEET CULTIVARS

The Effect of Soil Properties on Disease Occurrence: Different sugar beet cultivars were grown on soils from 16 selected areas. The values of DAS-ELISA obtained from plants grown on those soils are given in Table 2. Analysis of  $\chi^2$  were performed using SPSS for Windows 6.0 software between cultivars and areas that were hypothesized to affect disease occurrence (Özdamar, 1997). Differences among cultivars were not statistically significant ( $P>0.05$ ,  $\chi^2 = 4.63$ ) while differences among areas were statistically significant ( $P>0.01$ ,  $\chi^2 = 99.72$ ). ELISA absorbance values which were greater than 0.1 or equivalent to this value were accepted positive. Areas 1, 2, 4, 7, 8, 10, 15 and 16 were heavily infected. These differences could be a result of differences in soil properties.

**Table 2.** The contents of rhizomania on sugar beet plants from different cultivars grown on soils from 16 selected areas.

Area No	Areas	Sugar Beet Varieties (*)					
		Gina	Gabriela	Rizor	Sonja	Fiona	Evita
1	Y. Ayvalı	+++	+	+	+	+++	+++
2	A. Ayvalı	+++	+++	+	+++	+++	+++
3	Kuşkara	-	-	-	-	+	-
4	Çavundur	++	-	++	+	+	+
5	Çaycevher	+	-	-	-	-	-
6	Batak	-	-	-	+	-	-
7	Bükkarşı	-	-	-	+++	-	+
8	Çöroğlu	+++	++	+	+++	+	++
9	Dereköy	-	-	-	-	-	-
10	Ilica	++	-	+	+++	++	++
11	Cumaköy	-	-	-	-	+	-
12	Hacı Hasan	-	-	-	-	-	-
13	Akbük	+	+	-	-	-	-
14	Sofular	-	-	-	-	-	-
15	Kadıköy	+	+	+	-	++	+++
16	Ethem Mh.	+	+++	++	+	+++	+

(\*) Absorbance Values; -:  $>0.100$  (Clean); +:  $0.101-0.200$  (Slightly Infected); ++:  $0.201-0.500$  (Moderately Infected); +++:  $0.500 <$  (Heavily Infected)

In order to prove that the soil properties may affect the disease occurrence, logistic regression analysis was applied using SPSS for Windows 6.0 software with forward selection option (Özdamar, 1997). A total of 288 observations (16 areas x 6 cultivars x 3 replications) were used. Soil characteristics such as pH, salt, lime, organic matter, phosphorus and potassium contents and soil texture (i.e. sand, silt and clay contents) were taken as independent variables, and clean ( $ELISA < 0.1$ ) and infected ( $ELISA \geq 0.1$ ) were taken as the two possible causes of the dependent variable in the logistic regression equation. A significant relationship between independent and dependent variables were sought.

Healthy and infected samples could be predicted from the soil characteristic at a success rate of 77.08%.

The logistic regression equation was calculated based on the formula.

$$Z = -37.4171 + 5.2947 (\text{pH}) - 0.1533 (\text{lime } \%) + 0.0745 (\text{P}_2\text{O}_5 \%) + 0.0067 (\text{K}_2\text{O}) - 0.0963 (\text{silt } \%)$$

Based on this equation, disease occurrence increases as pH, P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O contents increase and silt and lime contents decrease.

Soils from 16 selected areas were generally rich in clay although some areas had soils in the textures of clayed-loam or loam. Kastir and Widera (1988) reported that *P. betae* was most common in loamy soils.

This study, in which relations between the occurrence of rhizomania disease and soil properties were investigated using a logistic regression approach, showed that higher pH, phosphorus and potassium contents and lower lime and silt contents promoted disease appearance. These factors may act separately and together as shown by the logistic regression equation.

Gencer (1989) reported that sugar beet cultivation is optimum at pH values around 7.2. pH values of soils from 16 selected areas in our study were between 7.16 and 7.97. Soil reaction was reported to be an important factor for infection of sugar beet roots by vector. Heavily infected soils have soil reactions of neutral to alkaline (Abe, 1987; Asher, 1988). Thus, pH of selected areas are close to the pH values in which *P. betae* is active and these areas were found to heavily infected. Relatively few research was conducted on the effects of soil properties and especially of plant nutrition on viral diseases. Some researchers explain this reality considering the fact that viruses are obligate parasites and have nucleo-protein structures (Erkan et al., 1993). Since the soils of Turkey are generally poor in organic matter and rich in clay and lime and have alkaline soil reactions, nutrient uptake by plants is generally low (Aksoy, 1986). Fertilization affects response of plants to certain diseases and pests through changes in plant growth, morphology, anatomy and especially chemical composition. These changes may improve, or deteriorate, plant tolerance against the disease (Marschner, 1986).

Soils from the 16 infected areas had 11.4-203.8 kg/ha phosphorus, 281-2165 kg/ha potassium and 3.8-27.7% lime. High phosphorus content promoted disease occurrence while increased vector activity. Phosphorus fertilization affects viral infections and virus biosynthesis in a way similar to nitrogen fertilization. Both make plants more susceptible and promote virus multiplication. However, it has been pointed out that there could be an interaction between different plant nutrients. For example, high nitrogen levels increase susceptibility of tobacco plants to Tobacco mosaic virus only in the availability of enough phosphorus (Foster, 1967).

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Effect of potassium applications on viral diseases can vary with the host-plant and virus combinations. Chant and Gbaja (1985) reported that higher levels of K increased the concentrations of Tobacco Mosaic Virus and Tomato Mosaic Virus in tobacco and tomato, respectively. Although a near-maximum growth is achieved by high potassium fertilization, the highest virus infection also occurred in high potassium levels (Perrenoud, 1990).

Our data showed that decreased calcium content in the soil promoted the disease. It was reported that  $\text{CaCO}_3$  application in soils with pH: 5.1 increased the severity of BNYVV infection caused by *P. betae*. On the other hand, calcium applied in the form of  $\text{CaSO}_4$  did not promote the disease since pH did not increase with this application (Abe, 1987). When the lime content was low, the vector activity was high. Lime application to soils has been a technique used for about 200 years to control club-root (Campbell and Greathead, 1989) and has been reported to be an application that suppress BNYVV vector. Silt has the ability of cation absorption (Ergene, 1987). It absorbs the cations necessary for plant nutrition and could suppress plant growth as well as vector activity. Our findings showed that BNYVV infections were promoted in soils with low silt contents.

In the light of the findings in our study, we conclude that excess phosphorus and potassium fertilizations should be avoided in rhizomania infested sugar beet production areas. Soil properties that affect the occurrence of the disease should be studied in depth. Further studies are need to show the effect of soil properties on disease occurrence.

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### ÖZET

#### ŞEKER PANCARI ÇEŞİTLERİNDE RHİZOMANIA HASTALIĞININ OLUŞUMU ÜZERİNE TOPRAK ÖZELLİKLERİNİN ETKİSİ

Şeker pancarı çeşitlerinde rhizomania hastalığının oluşumu ile toprakların fiziksel ve kimyasal özellikleri arasındaki ilişkileri belirlemek amacıyla bu çalışma yürütülmüştür. Kastamonu Şeker Fabrikası şeker pancarı üretim alanlarında yapılan survey çalışmaları sonucu 2241 ha alanda 68 adet bitki örneği toplanmıştır. Bu kök örneklerine uygulanan DAS-ELISA testine göre hastalıkla bulaşık 16 bölge seçilmiştir. Bölgelere ait toprak örneklerinin fiziksel ve kimyasal özellikleri belirlenerek tuzak bitki testi yöntemine göre 3 adet toleranslı (Gina, Gabriela, Rizor) ve 3 adet duyarlı (Evita, Fiona, Sonja) şeker pancarı bitkileri 6 hafta süre ile oda sıcaklığında yetiştirilmiştir. Deneme sonucunda bitkilerden alınan kök örneklerine DAS-ELISA testi uygulanmıştır. Elde edilen logistik regresyon formülüne göre toprak özelliklerinden pH, fosfor ve potasyum miktarlarının arttığı, kireç ve siltin azaldığı durumlarda rhizomania hastalığının teşvik edildiği sonucuna varılmıştır.

**Anahtar Kelimeler:** Beet necrotic yellow vein virus, rhizomania, şeker pancarı, toprak özellikleri, logistik regresyon

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## First Report of Yellow Vein Clearing of Lemons in Turkey

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Lemon (*Citrus limon* (L.) Burm.f.) production is an ancient and important sector in Turkey. There were 5180000 lemon trees found in this country and % 18.78 of lemon production was held in Çukurova region (Anonymous, 1999). Interdonato and Kutdiken lemon varieties on sour orange (*C. aurantium* L.) rootstock were shown yellow vein clearing (YVC) symptoms in this region. YVC symptoms was first reported in Pakistan (Catara et al., 1993), and then filamentous particles associated with YVC announced by Grimaldi and Catara (1996).

The leaves of YVC infected lemon trees in Çukurova region showed vein clearing which appears with yellow flecks at varying length on laterally veins. This symptom is combined with leaf crinkling and warped symptoms on young leaves. Field symptoms are excellent during spring and autumn flush, also visible during the flushing periods in summer.

Graft transmissible studies showed that YVC was transmissible to sour orange, Interdonato, Kutdiken, Italian and Lamas lemon varieties (Fig.1). However, Madam Vinous, Pineapple, Navelina, W. navel, Valencia (*C. sinensis* (L.) oranges, Satsuma, Klemantin Fremont, Nova, Parson's Special, Kara mandarin (*C. reticulata* Blanco) varieties, Star Ruby, Rio Red, Marsh Seedles, Duncan (*C. paradisi* Macf.) grapefruits, Mexican lime (*C. latifolia* Risso) and rough lemon (*C. jambhiri* Lush) were not shown any YVC symptoms. The YVC was compared with Citrus Psorosis Virus (CPsV), Citrus Variegation Virus (CVV) and Citrus Chlorotic Dwarf Virus (CCDV). It was found that CPsV showed symptoms on sour orange, lemon, mandarin, orange and grapefruit varieties, but YVC showed symptom only on sour orange and lemon varieties. The DAS-ELISA results for CVV by using YVC infected lemon and sour orange varieties were negative. The symptoms of YVC are very similar to CCDV which is transmitted with whitefly, *Parabemisia myricae* (Kuwana) (Korkmaz et al., 1994). However, CCDV infected mandarin, orange and grapefruit seedlings showed symptoms on leaves, but YVC was not. Up to now there is not any symptom of YVC on mechanically transmitted and further investigations were necessary about insect vector transmission. This report represents the first record of YVC in Turkey and YVC symptoms were different from CPsV, CVV and CCDV.



Fig. 1. Symptoms of yellow vein clearing on Küt diken lemon leaves. Yellow flecks combined with leaf crinkling of yellow vein clearing.

## ÖZET

### TÜRKİYE'DE LİMONLARDA SARI DAMAR AÇILMASI İLE İLGİLİ İLK RAPOR

Türkiye'de limon (*Citrus limon* (L.) Burm.f.) üretimi eski ve önemli bir sektördür. Ülkemizde 5180000 adet limon ağacı bulunmakta ve limon üretiminin % 18.78'i Çukurova bölgesinde yapılmaktadır (Anonim, 1999). Bu bölgede turuncu üzerine aşılı Enter ve Küt diken limon varyeteleri sarı damar açılması (Yellow Vein Clearing (YVC)) semptomu sergilemektedir. YVC semptomlarının ilk olarak Pakistan'da (Catara et al., 1993) bildirilmesinden sonra Grimaldi ve Catara (1996), YVC ile ilgili olan ipliksi partiküllerin varlığını bildirmişlerdir.

Çukurova Bölgesi'nde YVC ile infekteli limon ağaçlarında, sarı damar açılması semptomu yapraklarda, yan damarlar üzerinde farklı boyutlarda beneklenmeler şeklinde görülmüştür. Bu semptom genç yapraklarda, yaprak kıvrılmaları ve bükülmeleri ile birlikte gelişmektedir (Fig. 1). Arazi semptomları ilkbahar ve sonbahar sürgün gelişme dönemlerinde oldukça belirgin olup yaz gelişme döneminde de gözlenebilmektedir. Aşılı taşıma çalışmaları YVC'in turuncu (*C. aurantium* L.), Enter, Küt diken, İtalyan ve Lamas limon çeşitlerine taşındığını göstermiştir. Bununla beraber, Madam Vinous, Pineapple, Navelina, W. navel, Valencia (*C. sinensis* (L.)) portakal çeşitleri, Satsuma, Klemantin, Fremont, Nova, Parson's Special, Kara mandarin (*C. reticulata* Blanco) çeşitleri, Star Ruby, Rio Red, Marsh Seedles, Duncan (*C. paradisi* Macf.) altıntopları, Meksika laymı

(*C. latifolia* Risso) ve kaba limon (*C. jambhiri* Lush) YVC ait herhangi bir simptom geliştirmemiştir. YVC, Turunçgil Psorosis Virüs'ü (CPsV), Turunçgil Varigation Virüs'ü (CVV) ve Turunçgil Klorotik Cüceleşme Virüs'ü (CCDV) ile karşılaştırılmıştır. CPsV'ünün turunç, limon, mandarin ve altıntop çeşitlerinde simptomlar geliştirdiği, YVC ise sadece turunç ve limon çeşitlerinde simptom oluşturduğu belirlenmiştir. YVC ile infekteli turunç ve limon çeşitleri DAS-ELISA sonucunda CVV'ne karşı negatif sonuç oluşturmuştur. YVC simptomları beyaz sinek, *Parabemisia myricae* (Kuwana) (Korkmaz et al., 1994) ile taşınan CCDV'ünün oluşturduğu simptomlara benzerdir. Ancak CCDV ile infekteli mandarin, portakal ve altıntop fidanları yapraklarında simptom geliştirirken, YVC geliştirmemektedir. YVC ile mekanik taşıma çalışması yapılan bitkilerde şimdiye kadar herhangi bir simptom gelişimi gözlenmemiştir ve ileri dönemlerde vektör böcek taşınmaları ile ilgili araştırmalara gereksinim vardır. Bu rapor YVC'in Türkiye'de varlığını ve YVC simptomlarının CPsV, CVV ve CCDV'den farklı olduğunu bildirir ilk rapordur.

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