ISSN 0378-8024

THE JOURNAL OF TURKISH PHYTOPATHOLOGY

PUBLISHED BY THE TURKISH PHYTOPATHOLOGICAL SOCIETY

Volume : 23 Number : 3 Sept : 1994

The Journal of Turkish Phytopathology is published by Turkish Phytopathological Society and issued twice or three times a year to from volume. The subscripton rate per volume is $ 21.00

Doğruluk Matbaacılık San. ve Tic. Ltd. Şti. 1310 Sokak No: 35
Çankaya - İZMİR Tel: 483 69 56 Fax: 484 31 30

THE JOURNAL OF TURKISH PHYTOPATHOLOGY

TURKISH PHYTOPATHOLOGICAL SOCIETY

VOL. 23 SEPT. NO. 3

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J. Turk. Phytopath., Vol. 23, No.3, 105-111, 1994 ISSN 0378 - 8024

Investigations on the Comparison of **In-Vitro** and **In-vivo** Reaction Tests for the Determination of Susceptibility of Some Cucurbits to **Pseudomonas syringae** pv. **lachrymans**

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ABSTRACT

*In order to determine the susceptibility of some cucurbit plants to **P.s. pv. lachrymans**, causal agent of Angular Leafspot disease, the results of **in-vitro** and **in-vivo** reaction tests were compared. The results obtained from **in-vitro** and **in-vivo** reaction tests were extremely similar. On the other hand, **in-vitro** cotyledone reaction test resulted more quicikly than **in-vivo** test. At the end of these tests, it was observed that melon, hairy cucumber and pumpkin were highly susceptible to **P.s. pv. lachrymans**; where as zucchini squash, and the cultivars of cucumber and watermelon were moderately or weakly susceptible to **P.s. pv. lachrymans**, isolated from melon.*

INTRODUCTION

Angular Leafspot, which is incited by the bacterium **Pseudomonas syringae** pv. **lachrymans** (Smith and Bryan) Young **et al.) (Ps1)** is a serious problem of cucurbit plants grown in humid areas. The disease was firstly described symptomatologically in 1952 (SÖKMEN, 1952) and the causal agent was identified in 1974 in Türkiye (ÇINAR, 1974). The infested seeds are primary inoculum source of the causal agent (WILES and WALKER, 1951; VAN GUNDY and WALKER, 1957; LEBEN, 1979), and the disease is characterized by angular local lesions primarily on the leaves. The property of systemic invasion of the pathogen was also reported by several researchers (POHRONEZNY **et al.**, 1977; LEBEN, 1979; LEBEN, 1986).

Various plant species in **Cucurbitaceae** family such as cucumber, melon, watermelon, zucchini squash and pumpkin are involved in the host range of Angular Leafspot disease (VAN GUNDY and WALKER, 1957; HOPKINS and SCHENCK, 1972; CORTESMONLLOR and RODRIGOEZ-MARCANO 1992; SCORTICHINI and TROPIANO, 1993). It has been observed that the pathogen of Angular leaf spot attacks the leaves of hairy cucumber in Ege Region (ÖZAKTAN and BORA, 1994).

It is important to identify the bacterium on the contaminated seed within a short time since the primary inoculum source of **Ps1** is infested seeds. In order to identify the causal agent of Angular Leafspot, the methods such as isolation onto semi-selective media, and the application of some biochemical, physiological and pathogenicity tests are

used, respectively (LEBEN, 1972; ÇINAR, 1974; BRADBURY, 1986; KAGIWATA, 1992). But, these tests are rather time consuming. It may be concluded that the method used and described in detail by STANKIEWICZ **et al.** (1989) is also a rapid technique for detection of the disease development in compare with the others. Therefore, it was aimed in this study to compare the results of the tests of **in vitro** cotyledon reaction with **in vivo** cotyledon and true leaf reactions in respect of time consumption and the incidence of disease, and to describe the appearence of disease on various plant species in **cucurbitaceae** family by using the tests of cotyledon and true leaf reaction.

MATERIALS and METHODS

Bacterial Inoculum

The strain of **Ps1** used in this study was isolated from melon leaves in Ödemiş. The pathogen was cultivated on King Medium B for 48 h. Then the cells were removed from agar with sterile distiled water.

Test Plants

Various plant species in Cucurbitaceae family shown on Table 1 were tested for their reaction to **Ps1** in this study.

Table 1. The Plant Species in Cucubitaceae family tested for their reaction to **Ps1**.

PLANT SPECIES	CULTIVAR
CUCUMBER (**Cucumis sativus,** L.)	Fancypak Vicky Conquest Çengelköy
MELON (**Cucumis melo.,** L.)	Altınbaş Kırkağaç
WATERMELON (**Citrillus vulgaris,** Schrad.)	Washington Sugarbaby
ZUCCHINI SQUASH (**Cucurbita pepo.,** L.)	UK*
PUMPKIN (**Cucurbita moschatar,** Poir)	UK
HAIRY CUCUMBER (**Cucumis melo L.** var. **chate,** Naud.)	Gaziantep

* Unknown

In-vitro Test

Surface sterilized cucurbit seeds were placed into plates with wet sterile filter paper. Each cultivar was placed as five seeds/plate with ten replications. Plates were incubated under high relative humidity at 24°C in darkness for 48 hrs. After germination, plates were opened. The cotyledons were sprayed by **Ps1** suspension (~10^9 cfu/ml). They were kept in a growth chamber (in high relative humidity and at 24°C). Four days later, cotyledons were examined visually and the percentage of the infection was estimated for each plate (STANKIEWICZ **et al.**, 1989).

In-vivo Tests

Ten seeds were sown in each pot with 12 cm diameter. The pots were kept at 24°C in a 12 h dark/light cycle. After appearance of the cotyledons, the plants were sprayed by **Ps1** suspension (~10^9 cfu/ml) and kept in high relative humidity. Four days later, the cotyledons were examined visually and the percentage of the infection was estimated for each pot.

The plants reaching 1-2 leaf stage were sprayed by **Ps1** suspension ((~10^9 cfu/ml) and kept in high relative humidity. Five days later, the disease symptoms on the true leaves were evaluated by 0-4 scale (0 = Not infected, 1 = one or two chlorotic or necrotic lesions on the true leaf, 2 = Showing necrotic lesions up to 25% of true leaf surface, 3 = Showing necrotic lesions 25-70% of true leaf surface, 4 = Leaves completely dried).

RESULTS and DISCUSSION

The percentages of infection obtained by **in-vitro** cotyledon test are seen on Table 2. Total period required for **in-vitro** cotyledon reaction test was seven days. Symptoms on cucurbit cotyledons produced by **Ps1** were seen as necrosis of the edges of cotyledon, and were not localized.

According to **in-vitro** cotyledone reaction test, melon, zucchini squash, pumpkin and hairy cucumber were highly susceptible to **Ps1.** Although the disease symptoms appear on cv Sugarbaby and cv. Washington of watermelon, the percentages of infection were rather low as 12.41% and 22.08% respectively. Tested cultivars of cucumber were moderately or weakly susceptible to **Ps1** (Table 2).

The percentage of infection determined by **in-vivo** cotyledon reaction test with **Ps1** on the cotyledons of the cucurbits are seen on Table 3.

Table 2. Percentage of the Infection Determined on the Cucurbit Plants after Inoculation of Cotyledon with **Ps1 in-vitro**

TESTED CUCURBIT PLANTS	PERCENTAGE OF INFECTED COTYLEDONS*					
MELON (cv. Kırkağaç)	65.99**	a				***
MELON (cv. Altınbaş)	61.58	a	b			
HAIRY CUCUMBER	48.83	a	b			
ZUCCHINI SQUASH	53.00	a	b			
PUMPKIN	45.08		b	c		
CUCUMBER (cv. Conquest)	25.00			c	d	
CUCUMBER (cv. Fancypak)	25.75			c	d	
CUCUMBER (cv. Çengelköy)	22.50			c	d	
WATERMELON (cv. Washington)	22.08			c	d	
CUCUMBER (cv. Vicky)	21.25			c	d	
WATERMELON (cv. Sugarbaby)	12.41				d	

* The percentage of infection was estimated according to the existence of disease symptoms on the cotyledons.

** Each cultivar was placed as five seed/plate with ten replications.

*** Means followed by the same letter are not significantly different (P = 0.05) according to Duncan's test.

Table 3. Percentage of the Infection Determined by **In-vivo** cotyledon reaction test with **Ps1** on the cotyledons of the cucurbits

TESTED CUCURBIT PLANTS	PERCENTAGE OF INFECTED COTYLEDONS*						
MELON (cv. Kırkağaç)	69.64**	a					***
MELON (cv. Altınbaş)	48.80	a	b				
PUMPKIN	55.35	a	b	c			
HAIRY CUCUMBER	33.92		b	c	d		
CUCUMBER (cv. Çengelköy)	32.14		b	c	d	e	
CUCUMBER (cv. Conquest)	30.35			c	d	e	
ZUCCHINI SQUASH	28.80				d	e	
WATERMELON (cv. Washington)	22.50				d	e	
WATERMELON (cv. Sugarbaby)	12.50				d	e	
CUCUMBER (cv. Vicky)	25.00				d	e	
CUCUMBER (cv. Fancypak)	7.14					e	

* The percentage of infection was estimated according to the existence of disease symptoms on the cotyledons.

** Each treatment was replicated seven times. There were ten plants in each pot.

*** Means followed by the same letter are not significantly different (P = 0.05) according to Duncan's test.

Total period required for **in-vivo** cotyledon reaction test was eleven days. According to the results of **in-vivo** cotyledon reaction test (Table 3) melon, hairy cucumber and pumpkin were highly susceptible to **Ps1** whereas the cultivars of cucumber were moderately or weakly susceptible. Also the cultivars of watermelon were weakly susceptible to **Ps1**.

As shown on Table 2 and 3, these test results are extremely similar. As the result of the calculation, the correlation between **in-vitro** and **in-vivo** cotyledone reaction tests was found to be significant (r = 0.789).

The results of the reaction of cucurbit plants to **Ps1** obtained by trueleaf inoculation are seen on Table 4. The total period required for true leaf inoculation test was twenty days.

According to the results of true-leaf inoculation test, hairy cucumber, melon (except cv. Altınbaş) and pumpkin were highly susceptible to **Ps1**. The cv. Conquest and cv. Vicky of cucumber were moderately susceptible to **Ps1** but cv. Fancypak was weakly susceptible to **Ps1**. It is seen on Table 4 that percentage of the infection on the cv. Washington of watermelon is extremely low as 0.78%. However cv. Sugarbaby of watermelon was more susceptible to **Ps1** than cv. Washington in true-leaf stage.

Table 4. Percentage of the Infection Determined on Cucurbit Plants, after **In-vivo** True-Leaf Inoculation with **Ps1.**

TESTED CUCURBIT PLANTS	PERCENTAGE OF THE INFECTION*				
HAIRY CUCUMBER	24.29**	a			***
MELON (cv. Kırkağaç)	19.20	a			
CUCUMBER (cv. Çengelköy)	18.97	a			
PUMPKIN	18.23	a			
WATERMELON (cv. Sugarbaby)	16.83	a	b		
CUCUMBER (cv. Conquest)	13.69	a	b	c	
CUCUMBER (cv. Vicky)	11.28	a	b	c	
MELON (cv. Altınbaş)	12.15	a	b	c	
ZUCCHINI SQUASH	6.76		b	c	
CUCUMBER (cv. Fancypak)	6.43		b	c	
WATERMELON (cv. Washington)	0.78			c	

* The disease symptoms on the true leaves were evaluated by a 0-4 scale; 0 = No disease; and 4 = necrotic and chlorotic lesions on all of the leaf surface

** Each treatment was replicated four times. There were four plants in each pot.

*** Means followed by the same letter are not significantly different (P = 0.05) according to Duncan's test.

In comparison to the results from Table 2 and 4, it may be concluded that there is a similarity between the results of **in-vitro** and **in-vivo** reaction tests, except some cultivars (i.c., cv. Çengelköy, cv. Altınbaş, cv. Sugarbaby and Zucchini Squash). But, the cotiledonary stage of Cucurbits was more susceptible to Angular Leafspot disease than true-leaf stage. Moreover, the results of the **in-vitro** cotyledon reaction test could be obtained more quickly than the results of the **in-vivo** tests (STANKIEWICZ **et al.**, 1989). These findings show that the **in-vitro** cotyledon test are able to ensure the identification of **Ps1** on the seeds or other organs of the cucurbit plants in a short time. In addition, usage of the **in-vitro** cotyledon reaction test as first screening method on the selection of effective biocontrol agents may shorten the time required for the procedure (STANKIEWICZ **et al.**, 1989; ÖZAKTAN and BORA, 1994).

Our studies confirmed that a strain of **Ps1**, isolated from melon, was pathogenic on several species in Cucurbitacea such as cucumber, melon, zucchini squash, pumpkin, watermelon and hairy cucumber (JINDAL and BHARDWAJ, 1993; SCORTICHINI and TROPIANO, 1993; CORTES-MONLLOR and RODRIGUEZ-MARCANO, 1992; GROGAN **et al.**, 1971; YELBOĞA and BAYKAL, 1980; HOPKINS and SCHENK, 1972). The pathogenicity of **Ps1** on hairy cucumber may be the first record in the world.

ÖZET

BAZI KABAKGİL BİTKİLERİNİN Pseudomonas syringae pv. lachrymans'a DUYARLILIKLARININ SAPTANMASINDA In-vitro ve In-vivo REAKSİYON TESTLERİNİN KARŞILAŞTIRILMASI ÜZERİNDE ARAŞTIRMALAR

Bu araştırmada bazı kabakgil bitkilerinin Hıyar Köşeli Leke Hastalığı etmeni **P.s.** pv. **lachrymans**'a duyarlılıklarının saptanmasında **in-vitro** kotiledon ve **in vivo** saksı testleri karşılaştırılmıştır. Her iki test sonucu arasında infeksiyon başarısı bakımından güçlü bir korelasyon olduğu saptanmıştır. Ayrıca, **in-vitro** kotiledon inokulasyon testi saksı testlerine oranla daha kısa sürede sonuç vermiştir. Bu testler sonucunda; genel olarak, kabakgil bitkilerinden kavun, acur ve tatlı kabağın kavundan elde edilen bir **Ps1** izolatına karşı yüksek oranda duyarlı olmasına karşın; sakız kabağı, hıyar ve karpuz çeşitlerinin orta veya düşük duyarlılıkta olduğu gözlenmiştir.

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J. Turk. Phytopath., Vol. 23, No.3, 113-117, 1994 ISSN 0378 - 8024

Diagnosis of Citrus Chlorotic Dwarf Virus by Biological Indexing

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ABSTRACT

*In this paper we describe biological indexing techniques of citrus chlorotic dwarf virus (CCDV), a new and serious disease of citrus in the Eastern Mediterranean region of Turkey. Rough lemon (**Citrus jambhiri** Lush.) was found the superior indexing plant, producing many new flushes and distinct symptoms in a very short time. CCDV expressed leaf symptoms under warm and cool conditions in greenhouse within six to twelve weeks. This disease resembles to citrus infectious variegation (CIVV), citrus leaf rugose (CLRV), and Satsuma dwarf (SDV) that may lead to considerable confusion. However, serological methods, mechanical inoculation and the correct selection of citrus indexing plants will specifically diagnose CCDV.*

INTRODUCTION

The citrus production in Turkey is limited by many virus and virus-like diseases that reduce the vigor of trees, produce losses in yield and fruit quality and shorten the lifetime of trees (Çınar et al., 1993). There is increasing risk in importing severe citrus diseases due to the worldwide exchange of citrus tissue (Roistacher, 1993) and the illegal import of budsticks into Turkey. These introduced diseases once established may have the capability to destroy a citrus industry. Furthermore, almost every year new diseases and more severe strains of already existing pathogens are recognized in many citrus production areas all over the world (Roistacher, 1993).

The authors observed a new graft-transmissible citrus disease of probable viral etiology in many orchards in the East Mediterranean region in 1990 that spread fast in the whole citrus growing area. A survey conducted in 1993/94 showed that about 20% of all citrus trees in the Çukurova region were infected by this disease. However, most affected were citrus trees in İçel where on average 49% of all trees showed distinct symptoms (Korkmaz et al., 1994a). A proposed name of this new disorder is **citrus chlorotic dwarf virus** (CCDV) (Çınar et al., 1995). Korkmaz et al. (1994b) reported that most citrus varieties tested were susceptible to a CCDV infection.

Because this disease was recognized just recently, the causal agent is not isolated and characterized yet. Thus, no detection tools for CCDV are available that might be used in surveys or in sanitation programs. The diagnosis of CCDV therefore relies only on biological indexing. In the present paper we report on biological indexing techniques

for CCDV on citrus indicator plants as they were established at the Subtropical Fruits Research and Experimental Centre in Adana in the last two years. In addition, some information on symptomatologically characteristics and on dissemination of CCDV in field are given.

MATERIALS OF DETECTION

In this paper we followed the technical scheme set up by Roistacher (1991) in his "Handbook for detection and diagnosis of graft-transmissible diseases" since a quick and easy comparison with biological indexing of symptomatologically similar diseases was anticipated.

Field diagnosis: The primary symptoms of CCDV in field trees of lemon, sweet orange, mandarin or grapefruit are seen in young leaves, which show strong chlorotic flecking along the leaf margins. Sometimes the chlorotic flecking is reduced to diffuse flecking similar to a psorosis infection. Young leaves are distorted and start to crinkle, especially in lemon, some mandarin varieties (e.g., Fremont) and Minneola tangelo. Leaf size in lemon varieties and Fremont mandarin is reduced.

Elder leaves are smaller and boat -or spoon- shaped, similar to Satsuma dwarf virus (SDV) in susceptible varieties. In grapefruit and sweet orange varieties elder leaves recover from almost all symptoms, but sometimes slight to moderate crinkle can be observed.

Field symptoms are excellent during spring flush, however, are readily visible during all flushing periods in summer and autumn. Sometimes the symptoms may cause considerable confusion with citrus infection variegation (CIVV) and citrus leaf rugose (CLRV).

Graft transmission to citrus indicator plants: In spite of that CCDV can be diagnosed specifically by field symptoms in lemon varieties and some mandarin varieties, this disease is symptomless or field symptoms are not specific in most sweet orange and grapefruit varieties. Selected trees should be therefore always biological indexed to detect CCDV.

Collection of budwood

Inoculation: There is evidence that CCDV is a phloem restricted pathogen and not equal distributed in trees. For this reasons at least two buds should be grafted to each indicator seedling. We achieved excellent results by grafting two chip buds and one blind bud per indicator plant. It is important that inoculum tissue contain phloem and that cut surfaces of phloem tissue of donor and receptor plants are in good contact. Seedlings can be cut back at the time of inoculation, or two to three weeks after inoculation when wrapping tapes are cut or removed.

Indicator plants: We tested more than 40 citrus varieties as indicator plants for CCDV. Excellent symptoms were produced in all lemon varieties (e.g. Kütdiken, La-

mas), some mandarins (Bover, Fremont), as well as Mexican lime, sour orange, rough lemon and etrog citron. The superior indicator was rough lemon since it produced many flushes and did not interfere with exocortis infection. Although we used only one indicator plant per container, there is any reason for not using two inoculated plants and one non-inoculated control plant per container.

Controls: Besides negative controls a positive control should be included to each index test. Small amounts of CCDV infected budsticks can be obtained from the authors. In addition, it is useful to add a severe strain of CIVV in the indexing scheme.

Inoculum survival: After two to three weeks the wrapping tapes should be cut and removed and the survival of buds should be recorded. Although mechanical transmission of CCDV by knife blades is quite difficult, it is important that the knife or razor-blade used to cut the tapes are disinfected in sodium hypochlorite solution between plants. This is especially the case if CIVV is added in the indexing scheme, because transmission via infected tools is a primary mean of spread of this virus.

Post-inoculum care: After the initial cut-back at time of inoculation or after the wrapping tapes were removed, new growth should be allowed to grow without trimming. The application of pesticides should be avoided during this stage, since they often resulted in leaf damage that may confuse with symptoms. Instead of pesticides plants should be regularly sprayed with water to wash down initial infestations. Since rough lemon is sensitive to a **Phytophtora** infection causing foliage yellowing and plant death, plants should be maintained without any contamination, e.g., through irrigation water.

Temperature requirement: We kept our plants usually in cool temperature greenhouses during indexing, maximum 26 °C at day and minimum 20 °C at night, without any additional illumination. However, the diagnosis of CCDV was independent from temperature since excellent symptoms were also reproduced under warm conditions (30/36 °C n/d).

Time for appearance of first symptoms: Symptoms of CCDV should appear in the first flush within six weeks after the initial cut-back. Definitely the pathogen can be diagnosed in the second flush after the plant was cut-back again following within eight to twelve weeks. The most distinct symptoms appear always in the very young leaves.

Symptoms: Symptoms induced by CCDV in rough lemon include a V-shape chlorotic inlet that starts at one leaf margin and pronounced chlorotic flecking on the rest of the leaf. Young rough lemon leaves are reduced in size and extremely boat-shaped to hook-shaped. In mature leaves symptoms become quite variable, especially recover from the V-shape chlorotic inlet. They show leaf distortion, pronounced chlorotic flecking and if the leaf hardened typically puckered elevated segments may remain. The symptoms in mature leaves are similar to those produced by severe CIVV isolates, but in general much stronger.

Termination: If positive CCDV were used the indexing can be terminated after

12 weeks when definitive symptoms are observed in new flushes of the positive control. If no positive control is available, it is useful to cut-back the indicator plants a third time when no symptoms developed within the first twelve weeks. A severe CIVV control that developed excellent symptoms clearly demonstrates the differences in symptoms expressions between CCDV and CIVV.

Confusion with other graft-transmissible citrus diseases: CCDV produced some symptoms already described for several other graft-transmissible citrus diseases. Boat-or spoon-shaped leaves are characteristic for Satsuma dwarf (SDV), but usually only observed in spring flushes and not in other flushes of the year. Leaf distortion, crinkle, pronounced chlorotic flecking and puckered segments are typical symptoms of citrus infectious variegation (CIVV) and citrus leaf rugose (CLRV) on rough lemon and citron. As for SDV, these symptoms are usually expressed in spring flushes or under cool temperature in greenhouse. The symptoms of SDV, CIVV and CRLV are masked under warm conditions. Mandarins of the Clementine group, e.g., Marisol, can be used to separate CCDV from CIVV. On Marisol, CCDV produced no or only very mild symptoms, while in opposite CIVV induced strong crinkled leaves. Mechanical inoculation of suspected citrus tissue to white sesame will separate SDV from CCDV. In addition, ELISA can diagnose CIVV and SDV, since CCDV did not react with either of both antigens.

CONCLUSION

Citrus chlorotic dwarf virus is reliable diagnosed by biological indexing on rough lemon and other citrus indicator plants. Since this disease is a threat for every citrus industry, routine indexing for CCDV should be implemented in citrus butwood improvement programs. As this disease is vectored by the whitefly **Parabemisia myricae** (Kuwana), some serious problems arise. A whitefly infestation is difficult to control even in greenhouses. Thus, there is a risk of an accidental infection of indicator and mother plants. Furthermore, since CCDV displayed strong leaf symptoms on almost all citrus indicator plants, biological indexing of other diseases like psorosis complex, citrus tristeza, and mild exocortis strains is not possible in concurrent infected plants. On the other hand, CCDV can be eliminated from infected plants by shoot-tip grafting *in vitro* (Şaş and Çınar, 1994).

The rapid spread of CCDV in the Eastern Mediterranean region of Turkey was possible since no mandatory citrus certification program prevents the dissemination of this disease. Before this new disease was regocnized, it spread already to several citrus growing areas in the Çukurova most likely not by insect vectors but by infected seedlings (Korkmaz et al., 1994a). We have currently deep concern that CCDV will spread to other citrus growing areas in Turkey and to other countries of the Eastern Mediterrane-

an, if no serious and immediate attempts are undertaken to control the further spread of CCDV.

ÖZET

BİYOLOJİK İNDEKSLEME YÖNTEMİ İLE CİTRUS CHLOROTIC DWARF VİRÜSÜNÜN TEŞHİSİ

Bu çalışmada, Türkiye'nin Doğu Akdeniz Bölgesi'nde bulunan yeni ve önemli bir turunçgil hastalığı olan citrus chlorotic dwarf virüs (CCDV) (Beya sinekle taşınan virüs hastalığı) hastalığının biyolojik indeksleme teknikleri ortaya konmuştur. Kaba limon (**Citrus jambhiri** Lush.) kısa sürede çok sayıda yeni sürgün ve belirgin simptomlar oluşturması bakımında fevkalade bir indeks bitkisi olarak bulunmuştur. CCDV sıcak ve soğuk koşullarda oniki hafta içinde yaprak simptomları oluşturmaktadır. Bu hastalık citrus infectious variegation (CIVV), citrus leaf rugose (CLRV) ve Satsuma dwarf (SDV) hastalıklarına benzer simptomlar oluşturdukları için önemli yanılgılara yol açabilmektedir. Buna karşın serolojik metotlar, mekanik inokulasyon ve turunçgil indeks bitkilerinin doğru seçimi CCDV'nin spesifik teşhisine imkan sağlayacaktır.

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J. Turk. Phytopath., Vol. 23, No.3, 119-126, 1994 ISSN 0378 - 8024

Plant Viruses in Ankara Rivers and Lakes

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ABSTRACT

Water samples were collected from nine different water sources in district of Ankara. These water samples were filtered and santrifuged, then inoculated to herbaceous plants. Infected plants were tested serologically with agar gel and microprecipitin tests.

As a result, the existence of alfalfa mosaic virus (ALMV), cucumber mosaic virus (CMV), tomato black ring virus (TBRV), watermelon mosaic virus-2 (WMV-2), zucchini yellow mosaic virus (ZYMV) were found out.

INTRODUCTION

The pollution of environmental waters of ground and tap water with human and animal viruses has received considerable attention for studies on the epidemiological ecology and for considerations of public health (Berg, 1983). Recently, it was shown that in different areas of the world environmental waters also contain considerable amounts of plant viruses (Gerba, 1983; Gerik **et al.,** 1990; Jacobi and Castello, 1990; Koenig and Lesemann, 1984, 1985; Koenig **et al.,** 1984; Koenig, 1986; Piazzolla **et al.,** 1986; Smith **et al.,** 1969; Stage-Smith, 1989; Tomlinson **et al.,** 1983a; Tomlinson **et al.,** 1983b; Tomlinson and Faitful, 1984; Tosic and Tosic, 1984). Plant viruses from soil or river water could be an important means of their transmission. It was demonstrated that some viruses were soil transmitted and suggested that the fungus vector might be involved (Broadbent and Fletcher, 1966; Hollings **et al.,** 1977; Lavisolo **et al.,** 1965; Van Dorst, 1969). It was reported that 475-495 million tones of agricultural soil swept to the sea by erosion every year in Türkiye (Haktanır, 1989). This prompted us to check the presences of plant viruses in some rivers, pools and lakes in Ankara which their water is normally used for irrigation.

MATERIALS and METHODS

Water samples were collected from Ankara, Çubuk and İncesu streams; irrigation water in Kazan; Sugar Factory's waste water which flows to Ankara stream; Kızılırmak and Asartepe and İkizce ponds and Mogan lake. Water samples were collected from April through the end of October in 1992-1993. Samples were stored at 4 °C until processing. Samples were filtered through Schleicher & Schull 100 SxRundfilter first and then GMBH Sartorius membrane filter (pore size 0.45 and 0.2Um) and santri-

fuged at 5000 rpm for 15 min. Supernatants were incoulated to 16 different herbaceous plants and the plants were incubated at 20-28 °C for 4 weeks. Tap water was treated as control. Infected plants were tested serologically with agar gel and microprecipitin tests.

RESULTS and DISCUSSION

The samples collected from enviromental waters analized with the same method, revealed the presence of alfalfa mosaic, cucumber mosaic,tomato black ring and water-melon mosaic, zucchini yellow mosaic virus. Preliminary results indicated that it was possible to infect herbaceous test plants simply by rubbing on to them supernatants after a low speed centrifugation and filtration of water samples. Thirteen of sixteen species showed symptoms (Table 1). Serological tests also indicated that presence of same viruseas (Table 2).

Table 1: Symptoms observed on the test plants infected with water samples

TEST PLANTS	SYMPTOMS
Beta vulgaris L.	No symptoms
Capsicum annuum L.	YM, SBS, SBF, VC
Chenopodium amaranticolor	CLL, FNLL
C. murale L.	CLL, FNLL, Ep, ApLD
C.quinoa Wild.	VC, CLL
Citrillus vulgaris	CLL
Cucumis sativus L.	No symptoms
C. melo L.	VC, Ch, CLL
Datura stramonium L.	VC, SY, CLL, ApLD
Lycopersicum esculentum	BNS, FNLL, BNLL
Nicotiana clevelandii Gray.	CLL, SY
N. glutinosa L.	VC, YM, GM
N. tabacum L. "Maden"	GrLL, FNLL, RS
N. tabacum L. "Muş"	No Symptoms
N. tabacum L. "Tömbeki"	No Symptoms
N. tabacum L. "Xanthii"	No Symptoms
Phaseolus vulgaris L.	FNLL, BLNLL, M
Tetragonia expansa	SY
Vigna sinensis L.	FNLL, BNLL, RLL, M

ApLD: Apical Leaf Distortion; BNS: Brown Necrotic Streaks; BNLL: Brown Necrotic Local Lesions; Ch: Chlorosis; CLL: Chlorotic Local Lesions; Ep: Epinasty; FNLL: Fawn Necrotic Local Lesions; GM: Green Mosaic; GrLL: Green Local Lesions; M: Mosaic; RLL: Reddish Local Lesions; RS: Ring Spot; SBF: Systemic Brown Fleck; SBS: Systemic Brown Streak; SY: Systemic Yellowing; VC: Vein Clearing; YM: Yellow Mosaic

Table 2. Detected virus diseases in different water samples in Ankara province.

Water Samples	AIMV	BCMV	BNYVV	BYMV	CMV	CGMV	PVM	PVY	TBRV	TMV	WMV-2	ZYMV
STREAMS												
Ankara	+				+				+		+	+
Çubuk					+						+	
Incesu												
Kazan												
Sugar Fac.					+							
Kızılırmak									+			
PONDS												
Asartepe									+			
İkizce	+				+				+			
LAKE												
Mogan									+		+	

It is important that the healty plants may become infected via roots with viruses present in the surrounding medium. From the epidemiological point of view this transmission may occur without vector (Gerharson and Insunza, 1979; Hollings **et al.,** 1977; Kegler and Kegler, 1981; Kegler **et al.,** 1983), may be aided for instance by zoospors of **Olpidium brassicae** and **Polymyxa betae** which acquire the virus outside the host plant (Gerharson and Insunza, 1979; Smith **et al.,** 1969). Irrigation water from rivers and lakes may therefore be an important factor in the dissemination of plant virus disease.

Sewage may be a source of infectious viruses in river. Tomato bushy stunt virus can survive a passage through the human alimentary tract in an infectious state (Tomlinson **et al.**, 1983b; Tomlinson and Faitful, 1984). Dump material of vegetables and ornemental and compost may be the other sources of viruses in rivers, pools and lakes. Infectious particles of alfalfa mosaic, barley stripe mosaic, brome mosaic, cucumber green mottle mosaic, tomato bushy stunt, tobacco mosaic and tobacco rattle viruses in the feaces of mice and rabbits which had been fed with these viruses have been detected.

It has been demonstrated that a number of viruses can be released from uninjured roots into the soil and drainage water and that they can survive for long periods of time. Such observations have been made for petunia asteroid mosaic virus (Lavisolo **et al.,** 1965), tobacco necrosis, cucumber mosaic, petunia asteroid mosaic,tomato bushy stunt, tobacco mosaic and southern bean mosaic viruses (Smith **et al.,** 1969), cucumber green

mottle and tomato mosaic viruses (Van Dorst, 1969), cymbidium ring spot virus (Hollings **et al.,** 1977), tomato bushy stunt and carnation ring spot viruses (Kegler and Kegler, 1981), tomato mosaic virus (Kegler **et al.,** 1983) and tobacco mosaic virus (Barchend **et al.,** 1984).

It is believed that most human and animal viruses do not occur free in the water (Block, 1983). The adsorption of plant viruses to and protection by particulate matter has been described by several autors (Koenig, 1986). Adsorption of tomato bushy stunt virus to clay particles was demonstrated (Kegler and Kegler,1981). Virus adsorbed to clay containing soil was more resistant to extreme temperatures (autoclaving) than non-adsorbed virus or virus in sand which was free of colloids (Koenig, 1986; Lavisolo **et al.,** 1965). Petunia asteroid mosaic virus could survive more than twice as long in soil than in sap, provided that the soil contained clay. It was postulated that clay protects the virus from inactivation. The majority of viruses which have been isolated from enviromental waters are stable viruses, but cucumber mosaic virus is rather unstable. Pizzolla **et al.,** (1986) explained the recovery of the rather labile cucumber mosaic virus from river water samples by the fact that the virus was "sediment protected".

The strength of the adsorption of plant viruses to organic or inorganic matter may vary with different viruses, different adsorbing materials and environmental conditions, such as pH, salts and the presence of other materials. Adsorpotion, desorption and readsorption seem to occur continously under natural conditions (Block, 1983).

ÖZET

ANKARA'NIN AKARSU VE GÖLLERİNDEKİ BİTKİ VİRUSLARI

Ankara ilinde bulunan 9 farklı su kaynağından su örnekleri toplanmıştır. Bu su örnekleri filitre ve santrifüj edildikten sonra otsu bitkilere inokule edilmiştir. Enfekteli bitkiler, agar jel ve mikropresipitasyon testleri ile serolojik olarak test edilmiştir.

Bu testler sonucunda yonca mozaik virusu (AIMV), hıyar mozaik virusu (CMV), domates halkalı leke virusu (TBRV), karpuz mozaik virus-2 (WMV-2) ve kabak sarı mozaik virusunun (ZYMV) varlığı saptanmıştır.

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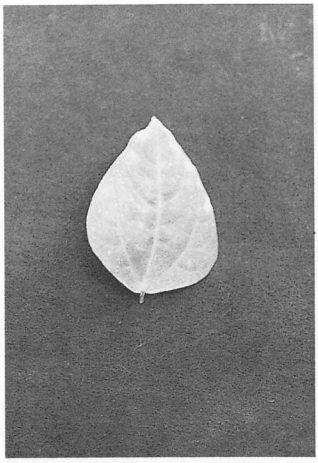

Fig.1. Reddish necrotic local lesions on the leaf of **V. sinensis** caused by CMV

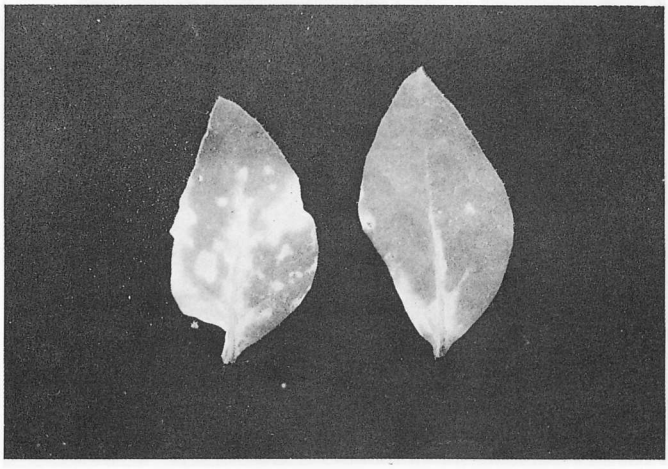

Fig.2. Chlorotic local lesions on the leaves of **N. clevelandii** caused by WMV-2

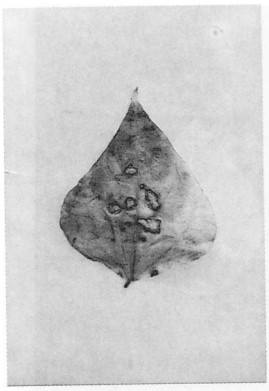

Fig.3. Necrotic local lesions on the leaf of **P. vulgaris** caused by AIMV

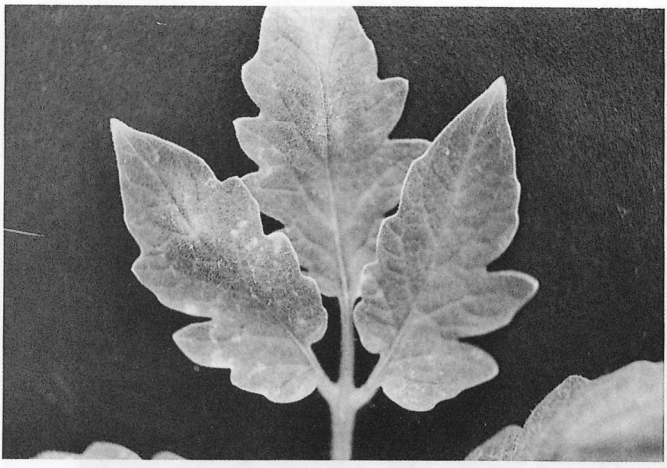

Fig.4. Brown necrotic local lesions on the leaves of **L. esculentum** caused by TBRV

J. Turk. Phytopath., Vol. 23, No.3, 127-132, 1994 ISSN 0378 - 8024

Indexing of Apple Trees for Apple Mosaic Virus, Apple Chlorotic Leaf Spot Virus and Apple Stem Grooving Virus by Elisa

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ABSTRACT

In this study, leaf samples were collected from apple trees growing areas in Aegean Region. Enzyme linked immunosorbent assay (Elisa) was used to detect apple mosaic virus (AMV), apple chlorotic leaf spot virus (ACLSV) and apple stem grooving virus (ASGV) in apple leaf extracts. The results of Elisa tests revealed that of the 450 samples tested in 1992, 4 ones displayed apple mosaic virus. Of the 220 samples in 1993, 63 ones (28,6%) exhibited chlorotic leaf spot virus and 52 samples (23,6%) indicated apple stem grooving virus. Apple trees which gave the positive results by Elisa have also been tested on herbaceous test plants.

INTRODUCTION

Apple is grown extensively in Balıkesir, Çanakkale, Denizli, İzmir and Uşak. Total 39.780.00 apple trees have been planted in the Aegean Region and the production is 1.950.00 tons per year (Anonymous,1990)

It is certain that various pests and diseases attack this crop of economic importance. Most virus diseases affect the growth and productivity of the apple trees.Through the various researches, it is known that there are abaut 40 viruses and virus-like diseases on apple trees, besides genetic and physiological disorders cause deformations on fruits and leaves of apple (Klınkowski,1958; Mc Grum et al, 1960; Posnette, 1963; Canova et al, 1964; Nemeth,1986). In England apple mosaic virus reduced the fruit production 30 percent (Posnette, 1963).

The studies about virus diseases of apples in Türkiye was made by Özkan and Kurçman (1976), and Çalı (1992).

This study was carried out to determine the presence of apple mosaic, apple chlorotic leaf spot and apple stem grooving virus in Aegean Region of Türkiye.

MATERIALS and METHODS

The surveys made between 1992 and 1993 for detection of the virus diseases on apple orchards in Balıkesir, Çanakkale, Denizli, İzmir and Uşak. Leaves were collected

from 670 trees in the five provinces of Aegean Region. The number of the apple trees in the region and the number of sampled trees were given in Table 1.

Table 1. The number of apple trees in Balıkesir, Çanakkale, Denizli, İzmir and Uşak, and number of tested apple trees.

County name	The number of apple trees	Number of tested apple trees	
		1992	1993
Balıkesir	447.587	56	40
Çanakkale	722.840	157	79
Denizli	766.439	126	91
İzmir	319.876	77	-
Uşak	239.545	34	20
Total	2.496.787	450	220

Young shoot samples for sap inoculation test and Elisa were collected in spring (in April and May) during the survey. Young leaf samples from 670 trees were collected for testing.

The Elisa tests were carried out in polystyrene microtitre plates in accordance with the procedure outlined by Clark (1981), and absorbance values over 0,1 were considered positive.

Reagents

Coating reagent : Dilution of the anti AMV,ACLSV, ASGV-Ig G with coating buffer 1/200 (v/v)

Antibody-AP-conjugate reagent: Dilution of the antibody-AP-conjugate with sample buffer 1/200 (v/v)

Substrate reagent : Dilution of 4- nitrophenylphosphate with sample buffer1/1000 (w/v)

Indexing

Leaf samples were tested with three virus antiserum by Elisa method. Then mechanical inoculations were done with leaf samples giving positive results in Elisa. Infected leaf samples were triturated in Phosphate buffer 0,05 M. 7.00 PH +%2 Polivinil prolidon. The prepared inoculum adding the carborandum duster (500 mesh) were rubbed on the leaves of the **Chenopodium quinoa, C. amaranticolor, Cucumis sativus and**

Table 2: Elisa detections of AMV, ACLSV and ASGV in the leaf sample extracts collected from the apple trees the Aegean Region (405 nm)

Apple mosaic virus				Apple chlorotic leaf spot virus				Apple stem grooving virus							
Plate No	Tree No	Ab.Va	Cont.Ab.Va	Plate No	Tree No	Ab.Va	Cont.Ab.Va	Plate No	Tree No	Ab.Va	Cont.Ab.Va	Plate No	Tree No	Ab.Va	Cont.Ab.Va
1	8/1 S	0,135	0.000	1	2/4 S	0,123	0.010	1	2/1 G	0,132	0.018	2	3/4 S	0,322	0.018
	15/2 S	0,280	0.006		5/4 S	0,117	0.030		2/2 S	0,199	0.019		6/3 S	0.143	0.005
	26/1 S	0,158	0.005		9/5 S	0.311	0.032		2/3 G	0.155	0.018		6/4 G	0.147	0.003
	30/6 G	0,344	0.007		10/1 S	0.448	0.031		2/5 G	0.103	0.018		28/4 G	0.197	0.006
					12/2 S	0.114			4/1 S	0.227					
					15/2 S	0.265			4/2 S	0.131		3	11/4 S	0.150	0.019
					15/3 S	0.189			5/3 G	0.154			21/2 S	0.150	0.001
					18/1 S	0.223			8/1 S	0.133			22/1 S	0.251	0.026
									8/2 S	0.136			30/4 S	0.174	0.036
				2	1/1 G	0.115	0.056		8/5 S	0.149			46/4 S	0.313	
					1/2 S	0.257	0.025		9/1 G	0.132			48/1 G	0.111	
					1/3 S	0.158	0.015		9/3 G	0.422					
					3/1 S	0.205	0.018		9/5 S	0.432		5	30/3 S	0.209	0.048
					3/4 S	0.236			10/3 S	0.213			31/1 G	0.170	0.055
					6/2 S	0.140			12/4 S	0.169			31/2 S	0.219	0.024
					6/3 S	0.319			12/5 S	0.112			31/3 G	0.177	0.067
					8/3 G	0.104			15/1 S	0.111			32/4 S	0.117	
					12/1 S	0.261			15/3 S	0.167			38/4 G	0.119	
					14/5 G	0.247			15/4 S	0.109			40/2 G	0.100	
					23/1 S	0.110			16/3 S	0.124			41/2 S	0.179	
					23/2 S	0.351			17/1 S	0.158			51/3 G	0.413	
					23/3 S	0.165			18/2 S	0.101					
					28/4 G	0.157			18/3 S	0.104					
									18/4 S	0.102					
				3	7/4 S	0.238	0.003		19/1 S	0.145					
					11/2 S	0.102	0.003		19/2 G	0.210					
					11/4 S	0.168	0.039		19/3 S	0.224					
					13/4 G	0.136	0.035		19/4 S	0.184					
					14/2 S	0.162			26/1 S	0.169					
					21/2 S	0.168			26/2 S	0.191					
					28/2 G	0.175			26/3 S	0.157					
					28/5 G	0.144			26/4 S	0.117					
					30/4 S	0.113			26/5 S	0.198					
					33/1 S	0.457									
					43/4 S	0.185									
					45/3 G	0.152									
					46/3 S	0.386									
					46/4 S	0.264									
				4	34/4 S	0,283	0.035								
					40/1 G	0.104	0.015								
					42/2 S	0.467	0.040								
					43/3 S	0.241	0.003								
					50/2 S	0.190									
				5	24/1 S	0.142	0.004								
							0.012								
							0.011								
							0.019								

Nicotiana glutinosa plants. Inoculated plants were kept at 25-28°C, and 3-30 days after the inoculations the symptoms of the virus disease was observed (Fulton ,1972; Smith, 1957; Posnette,1963; Lister, 1970 a; Lister, 1970 b).

RESULTS and DISCUSSION

The results of Elisa tests revealed that of the 450 samples tested in 1992, 4 ones displayed apple mosaic virus of the 220 samples in 1993, 63 ones (28,6%) exhibited apple chlorotic leaf spot virus and 52 samples (23,6 %) indicated apple stem grooving virus. Positive readings were obtained belong to the apple trees in survey districts can be seen in Table 2.

Table 3. Reactions of test plants used for identification the AMV, ACLSV and ASGV on apple

Test plants	Virus		
	AMV	ACLSV	ASGV
C.amaranticolar	NLL	-	-
C.quinoa	-	NLL	-
N.glutinosa	-	-	LP,Y
C.sativus	CLL	-	-

NL = Necrotic local lesion
CLL = Chlorotic local lesion
LP = Line patterns
Y = Yellowing

Mechanical inoculation tests have been applied to **C. sativus , N . glutinosa** and **C. amaranticolor** indicator plants for identification of AMV. Most of the inoculations on the **C. amaranticolor** have been produced necrotic spotting (0,1 mm calibered) on the inoculated leaves (Fig1) and caused chlorotic local lesion on **C. sativus** cotyledon leaves but no symptoms in **N. glutinosa** in 1992. These results were similar to those in literatures (Fulton 1972) (Table 3).

As a result of mechanical inoculations on 63 samples determined ACLSV in Elisa test 1993; necrotic spots produced on **C. quinoa** are similar to symptoms reported by Lister (1970 a) (Table 3).

In the results of tests done from the samples found ASGV on apple by Elisa line patterns and yellowing on **N. glutinosa** (Fig 2) were seen as reported by Lister (1979 b). (Table 3)

Consequently, apple mocaic virus, apple chlorotic leaf spot virus and apple stem grooving virus were first determined on apples in Türkiye.

Fig.1. Symptoms by AMV on **C. amaraticolor**

Fig.2. Symptoms caused by ASGV on **N. glutinosa**

ÖZET

ELMA AĞAÇLARINDAKİ APPLE MOSAIC VIRUS, APPLE CHLOROTIC LEAF SPOT VIRUS VE APPLE STEM GROOVING VIRUS'UN ELISA TESTİ İLE SAPTANMASI

Balıkesir, Çanakkale, Denizli, İzmir (1992) ve Uşak illerindeki elma bahçelerinden toplanan örneklere Elisa testi uygulanmıştır. 1992 yılında 450 örnek toplanmış ve 4 örnekte apple mosaic virus (elma mozayik virusu) saptanmıştır. 1993 yılında 220 örnek toplanmış ve 63 örnekte (% 28,6) apple chlorotic leaf spot virus (elma renkli yaprak leke virusu) ve 52 örnekte (% 23,6) ise apple stem grooving virus (elma gövde oluklaşması virusu)bulunmuştur. Elisa testinde pozitif sonuç veren örneklerin test bitkilerinde mekanik inokulasyonları yapılmış ve oluşan belirtiler değerlendirilmiştir.

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J. Turk. Phytopath., Vol. 23, No.3, 133-142, 1994 ISSN 0378 - 8024

Morphology of **Olpidium brassicae** (Wor.) Dang. and its Transmission of Big-vein Virus to Lettuce*

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ABSTRACT

*Big-vein disease of lettuce was determined to present on lettuces in Erzurum-Türkiye. The virus was successfully transferred to healthy lettuce cultivar Yedikule with its vector **Olpidium brassicae** (Wor.) Dang. obtained from the root washings of infected lettuces. The occurrence of transmission was higher in lettuces inoculated by immersing the roots of the seedlings into zoospore inoculum than adding the inoculum to root zones or infesting the soil with infested root debris.*

*Histological studies on the morphological features of **O. brassicae** in root tissues showed that mostly spherical sporangia really bearing single exit tubes and besides thick walled usually spherical stellate type resting spores were present. The zoospores which released from the sporangia in root epidermis had a round body possessing a single long posterior flagellum.*

*It was determined that lettuce big-vein virus (LBVV) together with in the resting spores of **O. brassicae** in root debris was overwintered in soil under severe winter conditions of Erzurum Region. Survival of **O. brassicae** during severe winters is new record that has not been reported previously.*

INTRODUCTION

Lettuce big-vein virus (LBVV) which is considered as a serious problem of lettuce growing is prevalent in many countries including Türkiye. The disease appears as slight clearing around the leaf veins of seedlings later develops into distinctive typical vein banding rather than enlargement of veins as stated by its known name " big-vein ". The disease caused by an infectious graft transmissible virus (Campbell **et al.,** 1961) is now known to be transmitted by the soil-inhabitating holocarpic chytrid fungus **Olpidium brassicae** (Wor.) Dang. in a persistent manner (Fry, 1958; Campbell and Grogan, 1963; Tomlinson and Garrett, 1964; Hiruki, 1965).

* These studies are supported by Turkish Scientific and Technical Research Council (TÜBİTAK, TBAG-1120) Ankara-TÜRKİYE

In the etiology of the disease the functions of the **Olpidium** was gathered in two distinctive parts (Campbell and Fry, 1966). Firstly, the zoospores which liberated through the exit tubes of sporangia enveloped in cells close to the root surface carry the virus internally and inoculate into host cells. Secondly thick walled resting spores surviving in adverse conditions in the absence of the host also carry the pathogen internally and enable its survival in soil from crop to crop. The period of their survival was determined as at least eight years by Pryor (1946). However according to Campbell (1985) the longevity end point of **O. brassicae** was between 20.8 and 22.5 years while the persistence end point of LBVV was between 18.7 and 20.8 years.

In Erzurum-Türkiye where the studies were conducted LBVV was first noticed on various lettuce cultivars in 1991 (Döken **et al.,** 1993) although it was probably present long before then. The disease was previously determined and defined according to symptom expression in İzmir-Türkiye by Fidan and Türkoğlu (1988). However their attempts to transmit the virus were not successful. The objectives of researches reported in this paper are to study the transmission of the virus with regard to behavior of **O. brassicae** in root tissues including its morphology besides to determine whether the virus survives and translocated in the debris of infected plants under the severe winter conditions of Erzurum.

MATERIALS and METHODS

Isolation and inoculum preparation

In the lettuce growing areas of Erzurum plants exhibiting typical vein banding symptoms were carefully removed from the soil and brought to laboratory. The roots were washed and zoospores were obtained by immersing in tap water equivalent 5-10 times of the amount of roots (v/w) for about 15 minutes (Bos and Huijberts,1990). The suspension was filtered through a double-layer cheesecloth to remove plant residues and soil particles. Then the concentration of zoospore inoculum was determined with a hemocytometer and adjusted to 10^6 zoospore per ml (Huijberts **et al.,** 1990).

Inoculation and transmission assay

The inoculum was applied to healthy Yedikule lettuce cultivars which raised from seeds sown seven days earlier in sterilized sand + soil + manure (1:1:1) mixture by pipetting 1 ml of inoculum on to sand surrounding the roots of each seedling after transplanting (Huijberts **et al.,** 1990). On the other hand the roots of seedlings were immersed into the inoculum for 20 minutes just before transplanting them singly to pots filled with sterilized sand. As a third method the root residues of infected lettuces were placed around the roots of seedlings during transplanting. In each method 80 seedlings were inoculated. All the transplants were kept in growth cabinets at 16 ± 2 °C (Westerlund **et al.,** 1978 a) and providing 16 hours photoperiod by illuminating with daylight fluorescent lamps. To detect transmission the plants were examined daily whether to

show typical vein-banding symptoms. After 6-8 weeks, the symtomless plants were removed and placed in tap-water for 10-15 minutes. This fluid examined for motile zoospores of **O. brassicae.** If no zoospores were detected epidermal root cells were examined with microscope for the presence of sporangia and resting spores (Westerlund **et al.**, 1978 b).

Propagation and maintenance

Throughout these studies the LBVV and its vector **O. brassicae** were maintained and propagated in lettuce cultivar Yedikule by regular transfers done by immersing the roots of healthy seedlings into inoculum before transplanting. For a long term storage of the inoculum roots and soils of infected lettuces were air dried at room temperature at about % 40-50 relative humidity since both **O. brassicae** and the virus could survived together in dry roots and soil for many years, as providing long term inoculum (Campbell,1985).

Study of O. brassicae in root tissues

To observe the development of **O. brassicae** in root tissues of lettuces, young roots were excised at time intervals from seedlings being inoculated as described before. Then epidermal layer of the roots were carefully removed, stained by gentle heating in lactophenol-analin blue for about 1-2 minutes and rinsed in clear lactophenol to remove excess stain. They were mounted in clear lactophenol on slides, covered by coverslips. The edges of the coverslips were sealed with a band of nail-varnish to make them semipermanent. All slides were observed directly by light microscope and microphotographs were taken.

In studying zoospores of **O. brassicae** the modified method of Alderson and Hiruki (1977) were applied in preparation of zoospore slides for microscopic examination as follows. Initially the zoospore suspension prepared by soaking the roots of infected seedlings into sterile water (15 min) was filtered through Wathman No: 40 filter paper to remove the particles other than zoospores. Then a drop from this suspension was poured on a microscope slide and an equal amount of osmium tetroxide was added as fixative. A cover slip was gently placed on it and surrounded with a band of nail varnish. The slides were examined and photographs were taken by phase-contrast microscope.

During the microscopic observations the measurements of **O. brassicae** were taken by eye-piece micrometer on 100 specimens.

Overwintering in soil

Overwintering of LBVV and its vector **O. brassicae** in plant debris left in soil was studied under the ecological conditions of Erzurum in 1992-93, 1993-94. In the beginning of November a piece of an uncultivated area before was uniformly infested to the dept of 30 cm by spreading the roots of infected lettuces and equal portion of an uninfested land was used as control. During the following vegetation period of July the seedlings of lettuce cultivar Yedikule raised in sterile sand + soil manure mixture were

transplanted to this area in the first week of July. The incidence of overwintering was rated positive if symptoms of LBVV was visible on leaves of the plants. If not the presence of zoospores in root washings and sporangia, resting spores in epidermal tissues were observed as a further step by following previously described methods.

RESULTS and DISCUSSION

The lettuce cultivar Yedikule inoculated with **O. brassicae** began to exhibit viral symptoms on leaves 20-21 days after inoculation. The period required for symptom development was same with the minimum time determined by Campbell and Grogan (1963) under controlled environmental conditions. The symptoms initially noticeable as slight clearing of veins developed into distinct yellow banding by clearing of the chlorophyll adjacent to main veins (Fig.1) same as described by Westerlund **et al.**, (1978 a). The appearance of these characteristic symptoms evidenced that the transmission of the virus by zoospores applied to lettuce seedlings either by immersing the roots into zoospore suspension or adding it to root zones and by infesting their soil with root debris from infected lettuces. Among them the inoculation done by immersing the roots of lettuce seedlings into zoospore suspension resulted in more plants carrying typical symptoms than others. This might be due to the rapid and easy movement of zoospores towards to roots in complete water than soil thus providing higher number of contacts

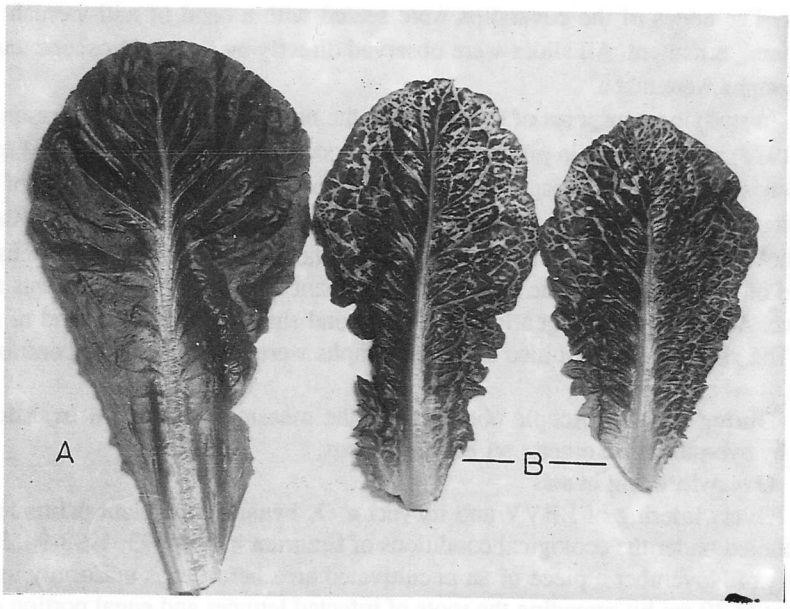

Fig.1. Comparison of leaves from healthy (A) and infected lettuce leaves with LBVV (B).

with roots in a short limited time and in such cases more entry might occur giving higher disease incidences. Since the microscopic observations on motility of zoospores in suspension showed that the movement began to cease in about 20-30 minutes after being collected in tab water.

In the assays to determine the transmission, some seedlings inoculated with **O. brassicae** did not showed any big-vein symptoms. However in microscopic examinations of root washings and root epidermal cells, zoospores and sporangia were observed respectively in all plants as well as in symptomless lettuces. The reason for the failure to develop big-vein symptoms probably related to the lower level of zoospores attached to roots. As a matter of fact on symptom development besides temperature, the influence of inoculum potential was also determined (Campbell and Grogan, 1963) and noted by these researchers the increase in temperature above 16-18 °C which was optimum for symptom development (Westerlund **et al.**, 1978 a) decreased their severity or prevented new symptom development. But return back into optimum range induced typical symptoms of LBVV. The preventation of symptom development at higher temperatures (above 16-18 °C) was attributed to the slower synthesis of LBVV (Westerlund **et al.**, 1978 a).

In histological studies of sporangia and resting spores in varying dimensions were observed to be present singly or more in each cell of root epidermis and occasionally large sporangium completely filled an epidermal cell. In tissues they were not uniformly distributed but usually formed in groups. Sporangia were mainly spherical in diameter ranging from 17.5 to 56.3 (30.1) µm (Fig.2a). Rarely sporangium were elongated in a cylindrical shape with measurements of 25 - 47.5 (31.8) x 36.3 - 76.3 (55.3) µm (Fig.2b). Mature sporangia were observed to be filled with zoospores (Fig.2c). Less frequently sporangia possesing single exit tubes were detected (Fig.2d). The length of exit tubes varied 7.5 - 11.3 (9.1) µm. Exit pores which classified as exit structures less than 1 µm length (Campbell and Lin, 1976) were not observed probably because of their small indistinctable appearance. After the release of zoospores empty sporangia left behind within the cells (Fig.2e). Usually, resting spores with stellate bodies and thick folded walls were spherical (Fig.3a) besides the rare occurrence of cylindrical ones (Fig.3b). In spherical resting spores the diameter was measured as 12.5 - 30 (20.4) µm and in cylindrical forms the lengths were 13.8 - 15 (14.4) x 22.5 - 33.8 (28.8) µm. The free zoospores which liberated into surrounding water from sporangia had a round body 4 - 5 (4.3) µm in diameter and a single whiplash flagellum 19 - 20 (19.5) µm in length (Fig.4).

If slight differences ignored the dimensions indicated above were mostly within the limits of the measurements done by Campbell and Lin (1976) on **O. brassicae** isolated from lettuces. Only sporangia were bigger than others. On the other hand morpho-

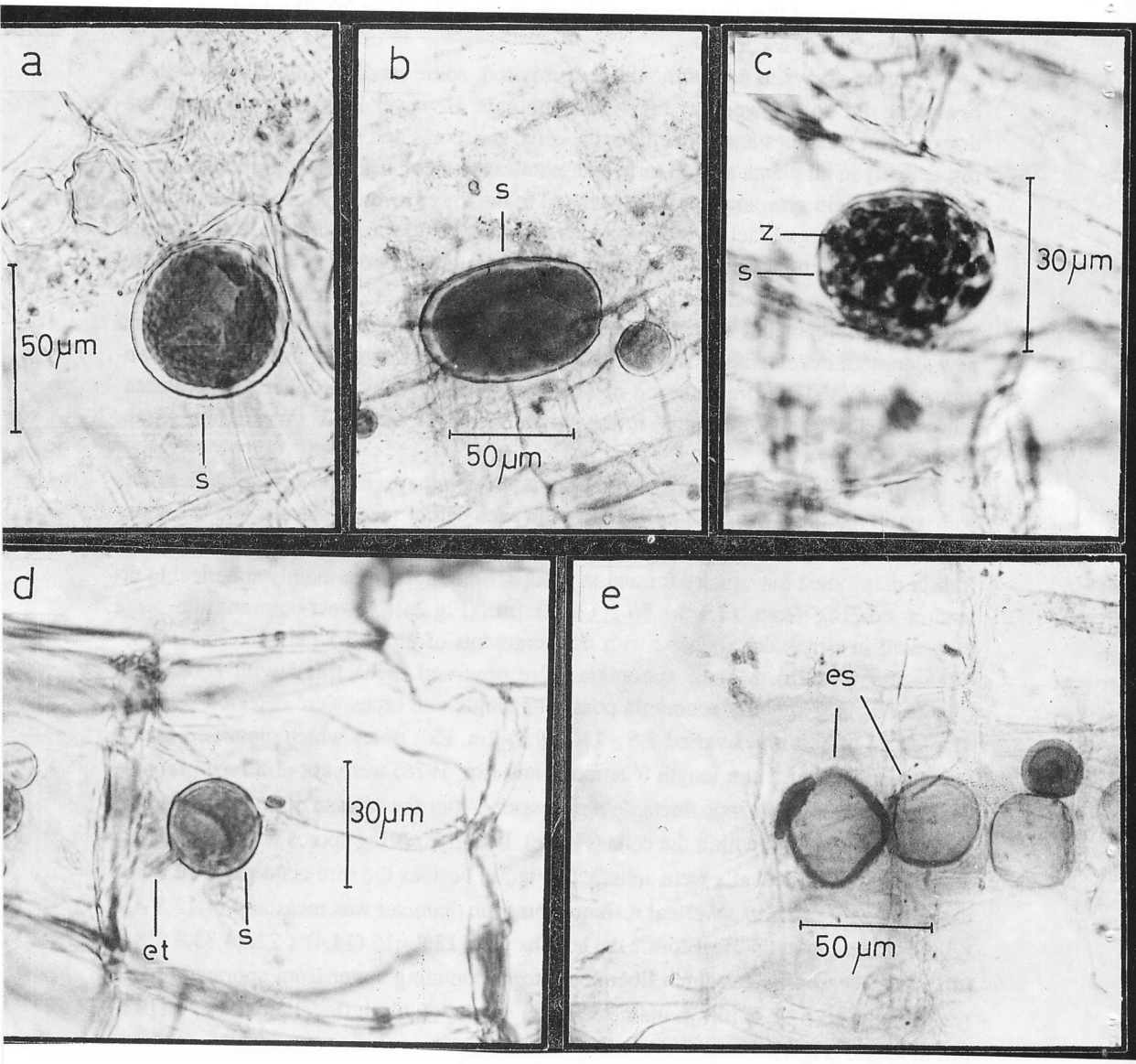

Fig. 2. Sporangia of **Olpidium brassicae**. a, Spherical sporangium; b, cylindrical sporangium; c, mature sporangium just before the release of zoospores; d, sporangium with single exit tube; e, empty sporangia. s: sporangium, z: zoospore, et: Exit tube, es: empty sporangium.

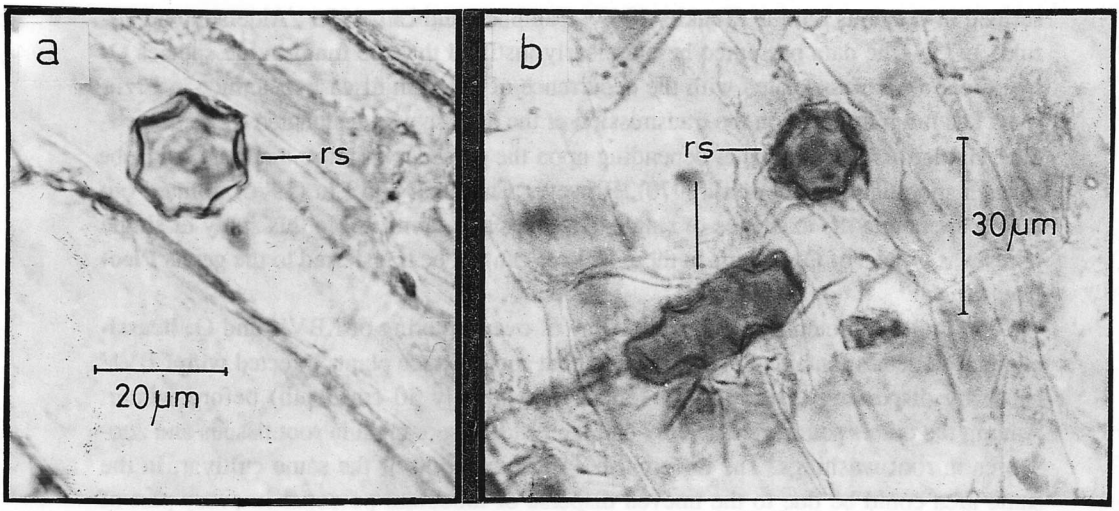

Fig. 3. Resting spores of **Olpidium brassicae.** a, Spherical resting spore; b, cylindrical and spherical resting spores. rs: resting spore.

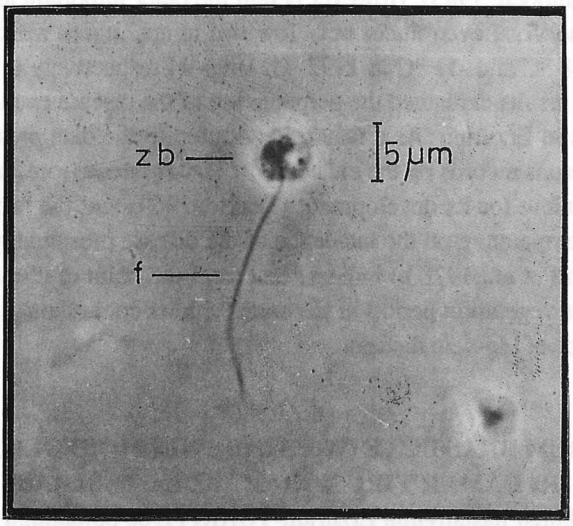

Fig. 4. Zoospore of **Olpidium brassicae.**

logical appearance of sporangia, resting spores and zoospores were similar to those described in previous reports (Teakle, 1969 ; Campbell and Lin, 1976 ; Alderson and Hiruki, 1977). The data presented herein clearly justified that the fungi in the species **O. brassicae** which associated with the occurrence of big-vein disease of lettuce in Erzurum. The fungi involved in the transmission of the disease was designated as **Pleotrachelus virulentus** by Sahtiyancı depending upon the presence of more than one exit tube in each sporangium (Lin **et al.**,1970). However Campbell and Lin (1976) pointed out that in sporangia the existence of single exit tubes as determined in this study or single pores was typical of **Olpidium** so these isolates can not be transferred to the genus **Pleotrachelus**.

Studies to determine the natural way of overwintering of LBVV and **O. brassicae** in root debris remained in soil resulted that some lettuce plants infected with LBVV cause by dispersing their roots in soil (approximately 30 cm depth) before winter. Among the others some were healthy and the rest had sporangia in root tissues and zoospores in root washings. The differences in the infection of the same cultivar, in the same area could be due to the uneven disperse of inoculum potential in every part of soil. In uninfested control plots all the plants were found to be healthy. So it can be stated that LBVV together with its vector **O. brassicae** survive in soil at least to reach the vegetation period of the coming year which covers about nine months period in Erzurum. In earlier reports the persistence of LBVV in soil within the resting spores of **O. brassicae** for many years was determined (Campbell and Grogan, 1963; Tomlinson and Garrett, 1964; Campbell, 1985). However herein the other important aspect was the occurrence of this survival even under very low soil temperatures which recorded to be dropped up to - 18 °C and -11 °C in 1992-93, 1993-94 respectively in the upper 30 cm of the soil. These results explained the perpetuation of the disease even under the severe winter conditions in Erzurum. As a result, in addition to the data presented above, the presence of numerous records on the existence of **O. brassicae** from Europe where cool conditions convenient for its development (Teakle, 1969) and the promoting effect of low air and soil temperature on the incidence of the disease presumably by favoring the vector (Westerlund **et al.**, 1978 b) indicate that from the point of climatic factors especially in course of vegetation period in Erzurum with its cool climate seems to be suitable for occurrence of big-vein disease.

ÖZET

OLPIDIUM BRASSICAE (Wor.) Dang.'NIN MORFOLOJİSİ VE İRİ DAMAR VİRUSUNU MARULA TAŞIMASI

Marul iri damar hastalığının Erzurum-TÜRKİYE'de bulunduğu saptanmıştır. Virus, enfekteli marul köklerinin yıkanmasıyla elde edilen vektör **Olpidium brassicae** (Wor.) Dang.'nın zoosporları ile sağlıklı Yedikule marul çeşidine taşınmıştır. Marul fi-

delerinin zoospor inokulumuna daldırılması ile yapılan inokulasyonlar fidelerin kök bölgesine zoospor inokulumunun veya enfekteli kök artıkları içeren toprak inokulumunun eklenmesine göre daha başarılı olmuştur.

Kök dokusunda **O. brassicae**'nın morfolojik özellikleri üzerinde yapılan histolojik çalışmalar ile nadiren tek çıkış tüpü bulunan çoğunlukla yuvarlak sporangiumların ve genellikle kalın duvarlı yuvarlak yıldız şekilli dinlenme sporlarının bulunduğu saptanmıştır. Kök epidermisinde bulunan sporangiumlardan çıkan zoosporlar yuvarlak vucutlu olup posterior olarak tek kamçıya sahiptir.

Erzurum bölgesinde şiddetli kış şartlarında marul iri damar virusunun topraktaki kök artıklarında **O. brassicae**'nın dinlenme sporları içerisinde kışladığı belirlenmiştir. **O. brassicae**'nın şiddetli kış şartlarında yaşaması daha önce rapor edilmemiş yeni bir bulgudur.

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J. Turk. Phytopath., Vol. 23, No.3, 143-152, 1994 ISSN 0378 - 8024

Post-harvest Disease Control of Apple, Quince, Onion and Peach, With Radiation Treatment*

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ABSTRACT

The gamma irradiation doses which delays rottenness were determined in apple, quince, onion and peach inoculated with ***Penicillium expansum*** *Link.ex S.F. Gray,* ***Monilinia fructigena*** *(Aderh. ex Ruhl.) Honey,* ***Botrytis aclada*** *Fresen. and* ***Rhizopus stolonifer*** *(Ehrenb. ex Fr.) Lind, respectively. Doses of 1, 2, 3, and 3,5 kGy did not inhibit rotenness on fruit, but infection was delayed for a certain period.*

And also effect of irradiation on pathogenicity, cultural characteristics, and sporulation of fungi have been investigated by reisolation. It was found that there is no differences at these properties of fungi between irradiated and unirradiated samples.

INTRODUCTION

Penicillium expansum Link.ex S.F. Gray on apple, **Monilinia fructigena** (Aderh. ex Ruhl.) Honey on quince, **Botrytis aclada**-Fresen (**B. cinerea**) on onion bulbs and **Rhizopus stolonifer** (Ehrenb. ex Fr.) Lind on peach are the main causes of storage rot in Türkiye (Gürer and Tiryaki, 1991).

A series of experiments were undertaken to determine the effect of irradiation with 60 Co gamma rays. The results indicate that, although the development of the fungi was not absolutely controlled, their progress in the fruit was inhibited in various degrees. In other words the irradiation dose which killed the fungi occures unacceptable fruit damage (Matthee and Potgieter, 1965; Beraha **et al.**, 1961; Roy, 1975).

The effect of irradiation is more promising when applied in combination with hot water treatment, chemicals such as SO_2 fumigation, and cold storage treatment. It is possible to obtain more hopeful results with decreasing the time between irradiation and harvest. Less decay observes when fruits were irradiated soon after picking than after a storage period (Matthee and Potgieter, 1965; Beraha **et al,** 1961; Roy, 1975; Tiryaki, 1990; De Kock and Holz, 1991; Thord-Gray **et al**, 1985).

* Recent advances in Botrytis research. Proceedings of the 10th International Botrytis Symposium Heraklion, Crete, Greece 5-10/12/1992.

A Joint Expert Committee on the Wholesomeness of Irradiated Foods (JECFI) convened by the WHO, FAO, IAEA concluded in 1980 that foods irradiated a dose of up to 10 kGy are wholesome for human consumption (Anonymous, 1987).

The main scope of this investigation was inhibiting the growth of the fungi causing storage rots on apples, peaches, quinces and onions which are stored economically by irradiating + cooling.

MATERIALS and METHODS

a) Effect of Irradiation on rot of fruit

For in-vivo studies fruits were surface- sterilised with 0.5 % NaOCl for 2 to 3 min. Wound inoculations of apple, peach, quince and onion were made by removing a 6 mm diameter disc of tissue with a corkborer, placing an agar-grown (PDA) culture disc of the **Penicillium expansum, Monilinia fructigena, Botrytis aclada** and **Rhizopus stolonifer** in the cavity and replacing the fruit disc respectively. After incubation at 23°C for 21 h fruits were exposed to 1, 2, and 3 kGy (additional dose 3.5 kGy for **R.stolonifer**) and stored at 0°C and 85-90 % relative humidity (onion stored at 10-15 °C and 60-70 % relative humidity). Ten inoculums were filled the holes for each dose and each pathogen. The diameter of rot was measured 10 days intervals for apple, quince and onion, and 5 days intervals for peach (Tiryaki and Maden 1991, Gürer and Tiryaki 1992).

b) Long-term effects of irradiation on pathogenicity and change on cultural characteristics of fungi

Reisolation were made at irradiated and non-irradiated fruits for each fungi and these fungi were cultured on potato dextrose agar (PDA, Difco).

For the pathogenicity test, two disks with a diameter of 6 mm were removed from either side of each fruit as mentioned before. The holes of fruits were filled with disks of re-isolated cultures, and incubated at 23°C. And the diameter of rots was measured as mm for each pathogen and each dose, one day interval.

For investigation of the sporulation of fungi, 6 mm agar disks removed from re-isolated cultures and transferred to fresh PDA and incubated at 23°C. And the diameter of colonies was measured as mm for each pathogen and each irradiation dose.

RESULTS

a) Effect of Irradiation on rot of fruit

Penicillium expansum

The diameter of rot on apples which were wound-inoculated with **P. expansum** at each dose level are shown in Table 1 and Fig. 1. The most inhibitory irradiation dose for apple rot after 62 days was 3 kGy. This effect was also statisticaly significant at $P < 0.05$.

Monilinia fructigena

The diameter of rot on quince which were wound-inoculated with **M. fructigena** at each dose level are shown in Table 2 and Fig. 2. Although the differences between 2 and 3 kGy was not statisticaly significant at $P<0.05$, the most inhibitory irradiation dose for quince rot after 45 days was 2 kGy.

Botrytis aclada

The diameter of rot on onion which were wound-inoculated with **B.aclada** at each dose level are shown in Table 3 and Fig. 3.

Table 3. Development of rot on onion bulbs wound-inoculated with **B.aclada**, exposed to irradiation doses and warm stored (at 10-15 °C and 60-70 % relative humidity).

Irradiation Dose (kGy)	Rot diameter (mm) Days After Irradiation			
	7	17	24	32
0	17.45 A	59.05 A	72.75 A	86.50 A*
1	11.30 B	37.90 B	58.10 B	68.60 B
2	9.25 B	27.20 B	47.00 B	75.60 AB
3	8.80 B	29.25 B	49.50 B	73.90 AB

* figures followed by different capital letters differ significantly at $P<0.05$.

Rhizopus stolonifer

The diameter of rot on peach which were wound-inoculated with **R.stolonifer** at each dose level are shown in Table 4 and Fig. 4. The most inhibitory irradiation dose for peach rot after 25 days was 3.5 kGy.

b) Long-term effects of irradiation on pathogenicity and change on cultural characteristics of fungi

The diameter of rot on apples, quince, onion and peach at each dose level are shown in Table 5, Table 6, Table 7, and Table 8, respectively. There is no differences between the irradiation doses either at $P<0.05$ for each pathogen related to rot diameter. And also there is no differences between the irradiation doses for each pathogen related to cultural charecteristics, colony diameter, and sporulation.

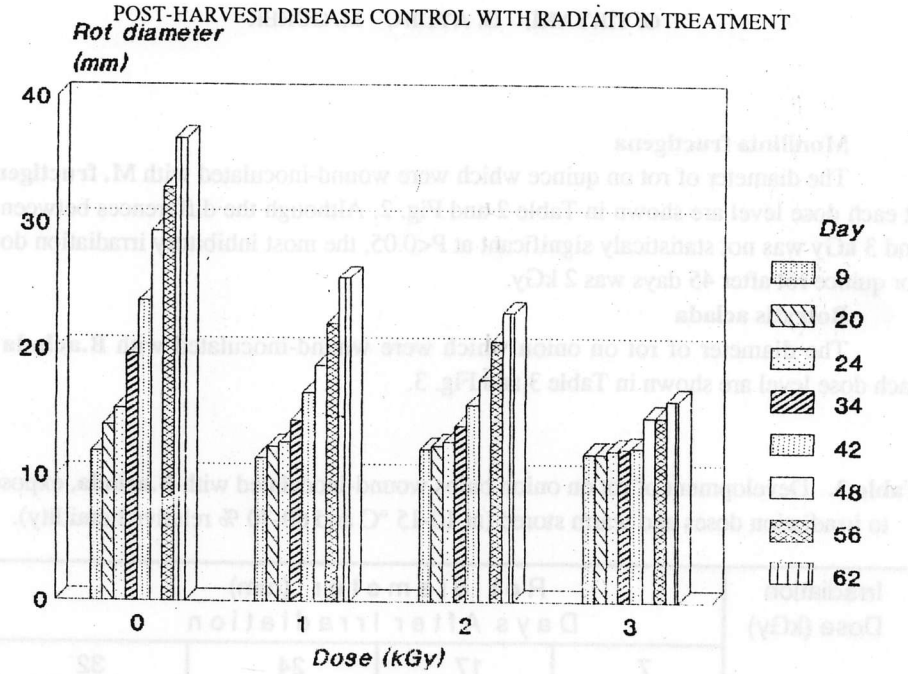

Figure 1. Development of rot on apples wound-inoculated with P. expansum, after 62 days irradiation at 0°C and 85-90 % relative humidity

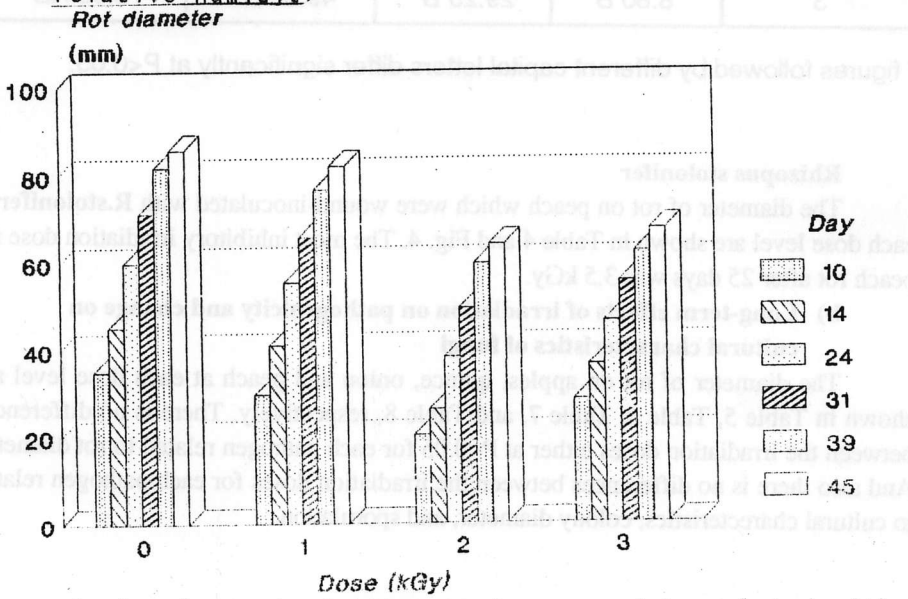

Figure 2. Development of rot on quince wound-inoculated with M. fructigena, after 45 days irradiation at 0°C and 85-90 % relative humidity.

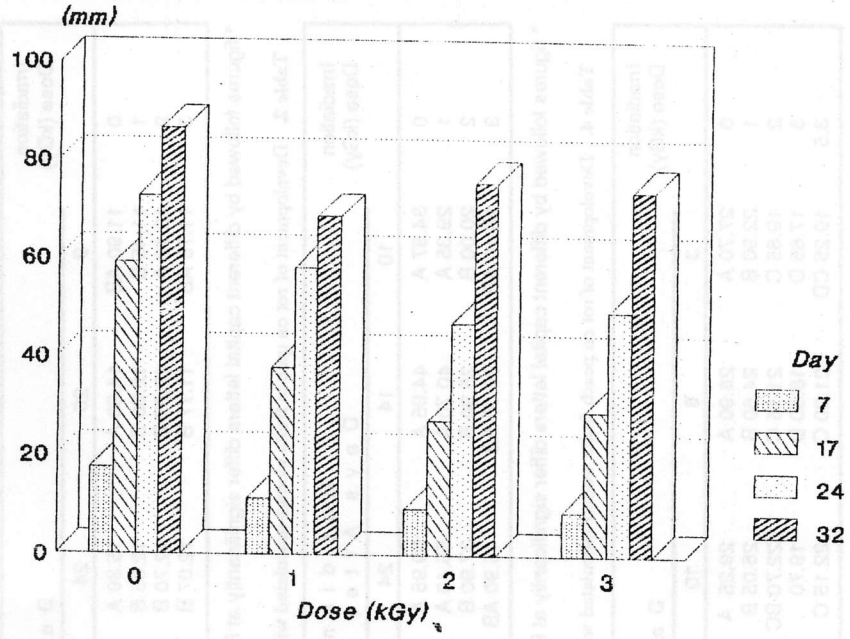

Figure 3. Development of rot on onion wound-inoculated with B. aclada, after 24 days irradiation (at 10-15°C and 60-70 % relative humidity).

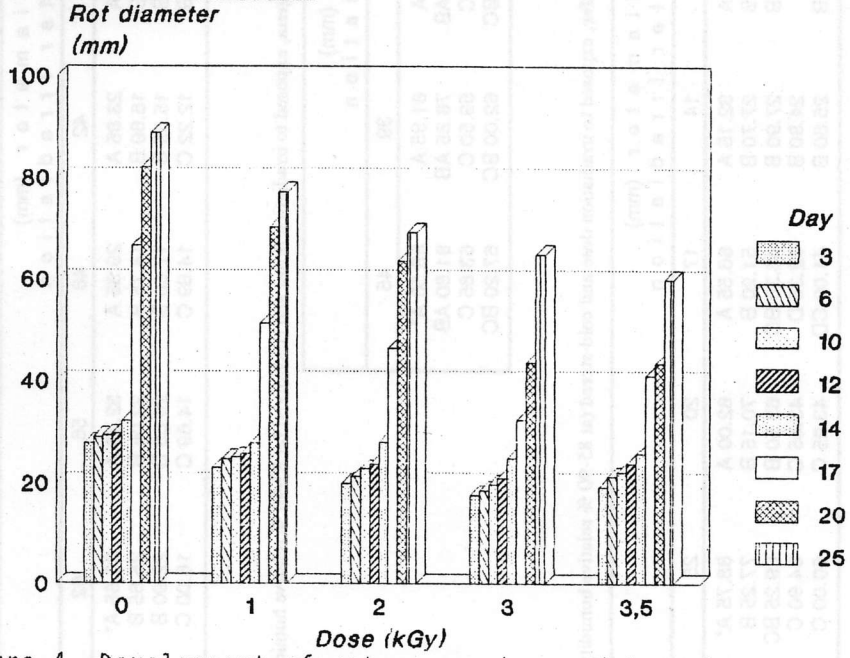

Figure 4. Development of rot on peach wound-inoculated with R. stolonifer, after 25 days irradiation (at 0°C and 85-90 % relative humidity)

Table 1. Development of rot on apple fruits wound-inoculated with **P.expansum,** exposed to irradiation doses and cold-stored (at 85-90 % relative humidity)

Irradiation Dose (kGy)	Rot diameter (mm) Days After Irradiation							
	9	20	24	34	42	48	56	62
0	11.90 AB	14.00 A	15.30 A	19.65 A	23.85 A	29.35 A	32.75 A	36.65 A*
1	11.45 B	12.30 B	12.65 B	14.40 B	16.60 B	18.75 B	22.05 B	25.65 B
2	12.12 A	12.37 B	12.70 B	14.00 B	15.65 B	17.60 B	20.65 B	22.90 B
3	11.75 AB	11.77 B	12.07 B	12.17 B	12.22 C	14.69 C	14.69 C	16.00 C

* figures followed by different capital letters differ significantly at P<0.05.

Table 2. Development of rot on quince fruits wound-inoculated with **M. fructigena,** exposed to irradiation doses and cold-stored (at 85-90 % relative humidity)

Irradiation Dose (kGy)	Rot diameter (mm) Days After Irradiation					
	10	14	24	31	39	45
0	34.87 A	44.95 A	59.95 A	71.15 A	81.55 A	88.50 A*
1	29.35 A	40.70 A	55.40 A	65.70 AB	76.35 AB	81.80 AB
2	20.00 B	25.90 B	37.90 B	49.15 C	59.50 C	63.85 C
3	28.10 AB	36.00 AB	45.90 AB	55.30 BC	62.00 BC	67.20 BC

* figures followed by different capital letters differ significantly at P<0.05.

Table 4. Development of rot on peach fruits wound-inoculated with **R.stolonifer,** exposed to irradiation dose and cold-stored (at 85-90 % relative humidity).

Irradiation Dose (kGy)	Rot diameter (mm) Days After Irradiation							
	3	6	10	12	14	17	20	25
0	27.70 A	28.90 A	29.25 A	29.65 A	32.15 A	66.55 A	82.00 A	88.75 A*
1	22.90 B	24.60 B	25.05 B	25.70 B	27.70 B	51.20 B	70.15 B	77.25 B
2	19.85 C	21.15 C	22.70 BC	23.60 B	27.90 B	46.30 BC	63.50 B	69.25 BC
3	17.65 D	18.50 D	19.70	20.95	24.80 B	32.35 D	43.55 C	64.90 C
3.5	19.25 CD	21.20 C	22.15 C	23.85 B	25.60 B	41.00 CD	43.25 C	60.00 C

* figures followed by different capital letters differ significantly at P<0.05.

Table 5. Development of rot on apple fruits which were wound-inoculated with re-isolated irradiated and non irradiated **P.expansum** cultures (at 23 °C)

Irradiation Dose (kGy)	Rot diameter (mm) Days After Irradiation			
	6	7	8	9
0	27.40 A	31.65 A	37.10 A	42.00 A*
1	27.55 A	32.00 A	37.35 A	43.10 A
2	27.70 A	31.85 A	36.75 A	42.40 A
3	27.55 A	31.75 A	37.45 A	41.80 A

* figures followed by different capital letters differ significantly at P<0.05.

Table 6. Development of rot on quince fruits which were wound-inoculated with re-isolated irradiated and non -irradiated **M.fructigena** cultures (at 23°C)

Irradiation Dose (kGy)	Rot diameter (mm) Days After Irradiation			
	3	6	7	10
0	33.85 B	66.95 A	74.85 A	90.95 A*
1	38.40 A	69.95 A	78.75 A	95.55 A
2	36.25 AB	72.00 A	84.90 A	101.55 A
3	34.70 AB	66.00 A	75.05 A	92.65 A

* figures followed by different capital letters differ significantly at P<0.05.

Table 7. Development of rot on onion bulbs which were wound-inoculated with re-isolated irradiated and non -irradiated **B.aclada** cultures (at 23°C)

Irradiation Dose (kGy)	Rot diameter (mm) Days After Irradiation			
	3	6	7	10
0	11.70 B	19.50 A	36.30 A	45.40 A*
1	11.00 B	21.65 A	39.60 A	46.40 A
2	13.40 A	23.25 A	40.70 A	49.15 A
3	13.40 A	22.40 A	46.45 A	53.65 A

* figures followed by different capital letters differ significantly at P<0.05.

Table 8. Development of rot on peach which were wound-inoculated with re-isolated irradiated and non-irradiated **R.stolonifer** cultures (at 23 °C)

Irradiation Dose (kGy)	Rot diameter (mm) Days After Irradiation	
	2	5
0	44.70 A	91.00 B*
1	35.65 BC	92.75 AB
2	36.30 BC	95.00 AB
3	35.15 C	97.25 A
3.5	41.60 AB	90.25 B

* figures followed by different capital letters differ significantly at $P<0.05$.

DISCUSSION

According to the our data, the most inhibitory irradiation dose for apple wound-inoculated with **P.expansum** was 3 kGy at 0°C. Beraha **et al** (1961) reported that the fungal development of **P.expansum** on apple was decreased at $200x10^3$ rep (1.86 kGy). And Beraha et al (1960) reported that the lethal gamma radiation dose was 1.82-2.74 x (100.000 rad) for **P.expansum** on apple fruits.

And the most inhibitory irradiation dose for quince wound -inoculated with **M.fructigena** was 2 kGy at 0°C. Paralelling these findings, it was found 1.37-1.82 x (100.000 rad) as lethal gamma radiation dose **M.fructicola** on peach by Beraha **et al** (1960).

For onion which were inoculated with **B.aclada** there is no differences between irradiation doses at 10-15 °C. Where as Beraha **et al** (1960) reported that the lethal gamma irradiation dose was 2.34-3.65 x (100.000 rad) for **B.allii** on onion bulbs.

And for peach which were inoculated with **R.stolonifer,** the most inhibitory dose was 3.5 kGy. Our data result is different to beraha's (1960) findings. These researcher has been declared that lethal gamma irradiation dose was 1.82-2.28 x (100.000 rad) for **R.stolonifer** on peach fruits.

It was determined with the investigatious carried out up to now that, irradiation is much more effective against the pathogens than the chemicals, because of deep penetration. The effect of the irradiation is not as the complete inhibition of the fungus growth, but it is as delaying the growth with different degrees. On the other hand, the doses necessary to eradicate the fungi, causes irreversible damages on the product. In these kinds of studies, not only the given total dose but also, the dose ratio and the power of the radiation source is important. Storage under low temperatures or chemical surface disinfectants control only the partial rottings. This is an important subject where

the advase effects of the chemical preparations especially when used as high doses and frequently are being discussed. The expected result is always better when the irradiation is combined with the low temperature, hot water treatment and the low doses of the chemicals (Beraha 1957, Beraha **et al** 1961, Matthee and Potgieter 1965, Sommer **et al** 1967).

In our investigation, infection increases with all the doses, as the period after the irradiation elongates, in other words, the effect of the irradiation decreases. These results are similar with the results of the researchers, who explains the effect of the irradiation not as fungisit as but fungistatic, it means delaying the infection for a while (Beraha **et al** 1961, Cappelini and Strecth 1962).

The effect of irradiation is not as inhibiting the fungal growth completely, but delaying its growth to different degrees (fungustatic effect). But the doses necessary to inhibit the fungal growth completely, sometimes causes some irreversible damages on the product (Anonymous 1987).

ÖZET

ELMA, AYVA, SOĞAN VE ŞEFTALİNİN HASAT SONRASI ÇÜRÜKLÜKLERİNİN RADYASYON İLE ENGELLENMESİ

Bu çalışmada **Penicillium expansum**'un elmada, **Monilinia fructigena**'nın ayvada, **Botrytis aclada**'nın, soğanda ve **Rhizopus stolonifer**'in şeftalide, oluşturduğu çürüklükleri erteleyen gamma radyasyonu dozları araştırılmıştır.

Uygulanan hiçbir gamma radyasyonu dozu çürümeyi tamamen engellememiş, sadece herbir patojen için çeşitli sürelerde gelişmeleri geciktirilmiştir. Işınlamadan sonra fungusların hastalandırma yeteneklerinde ve kültürel özellikleri ve sporulasyonlarında önemli bir değişiklik olmamıştır.

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CORRIGENDUM

In the article in J. Turkish Phytopath. Vol. 22, No: 2-3, 65-74, 1993, named "Occurrence and Detection of Citrus Tristeza Virus Decline on Satsuma Mandarins Buded on Trifoliata Orange in İzmir Province" by Turhan AZERİ, in page 66 and 77, **Toxoptera citricidus** Kirkaldy tropical vector of Tristeza was printed instead of **A. spiraecola** by mistake. The author has previously reported that the most efficient tropical vector of Tristeza, "**T. citricidus** Kirk., is not present in the Mediteranean Countries and Türkiye (In article No: 1 "Investigation on the tristeza virus in the Satsuma Mandarins, J. Turkish Phytopath. Vol: 7, Num., 2-3, 51-68, 1978; "Decline of Satsuma Mandarin Orange in Turkey, J. Turk., Phytopath., Vol: 10, Num. 1, 37-44") In the same articles only **A. gossypii** Glov. and **A. spiraecola** were reported as tristeza vector in Türkiye.

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2. Manuscripts must be written in English, German or French.

3. Papers accepted for the Journal of Turkish Phytopathology may not be published elsewhere, in any form or language.

4. In addition to ressearch papers, the journal publishes letters the editor, book reviews and short communications that the author does not intend to publish in more detail at a later date.

5. Papers must have a short abstract which will be printed in the beginning, introduction, materials and methods, results, discussion, summary, acknowledgment (if necessary) and literature cited.

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