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Identification of Genes Which are Specifically Expressed in the Compatible Interaction Between Lettuce and **Bremia lactucae**

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ABSTRACT

*cDNA clones representing genes that are expressed during compatible interaction between lettuce and downy mildew pathogen **B. lactucae** were identified by differential screening of a cDNA library made from RNA isolated from cotyledons infected with **B. lactucae.** The cDNA library was screened against mRNA from the infected cotyledons and mRNA from control uninoculated cotyledons. The expression of these genes in compatible pathogen-plant interaction was examined by Northern blot analysis and several different patterns of expression were observed. Some of them showed the same expression level in both infected and uninfected RNA, while others were expressed strongly in infected cotyledons but showed a low level of expression in the uninfected control.*

INTRODUCTION

Gene expression, in most cases, is regulated by DNA sequences, 5' upstream of the coding region called the promoter. This expression can be in all cells of the plant or can be tissue specific. Promoters also determine the expression of genes specifically induced after heat, frost, wounding, UV, pathogen attack, etc. Isolation of promoters and analysis of their properties make possible the expression of foreign genes in certain tissues or under certain environmental conditions, such as when the tissue is challenged by a pathogen. Therefore, much effort has been put into the isolation of DNA sequences involved in the regulation of gene expression. For example, the proteinase inhibitor II gene promoter is inducible by wounding and has been cloned from potato. This promoter is suitable to drive the expression of genes coding proteins toxic to plant pathogens which can infect the plant through wound tissue. Other promoters are induced by heat shock and light. Organ specific gene expression has also been show for a number of genes such as the phaseolin gene that is expressed only in seeds of **Phaseoulus vulgaris.** There are some studies on resistance response genes and their promoters (Raghothoma et al., 1991). However, there are no reported studies concerning plant genes that

are switched on or off when challenged by a pathogen in a compatible host-pathogen interaction. In this case it is the temporal pattern of expression that is important and not the product produced by the gene. Therefore, the isolation of a cDNA (complementary DNA) clone from mRNA specifically expressed on pathogen attack was attempted. The use of cDNAs allows for the selection of gene sequences that are only epressed order certain circumstances. Appropriate clones can then be used to obtain the genomic copy of the gene with the promoter attached.

In this tudy, our aim was to see if it was possible to identify plant genes which are expressed during the susceptible plant-pathogen interaction. If such genes could be identified, the promoter could then be isolated making it possible to express the gene or genes of interest in the susceptible host. These genes would , therefore, be produced only after the plant was challenged by the pathogen.

MATERIALS and METHODS

Plants Material

Lettuce plants (**Lactucae sativa**) cultivar (cv) Cobham Green with no **Dm** gene determined for resistance to downy mildew disease (Crute and Johnson, 1976; Woods et al., 1988; Paran et al., 1991) were used for the experiments. Seedlings for RNA isolation were grown on 3MM paper moistened with fungicide, Rovral (Rhone Poulenc) solution (20 mg/L) in clear perspex boxes in a growth room at 21 °C under 16 hours photo-period for 10-15 days. Preparation of spore suspension was based on the method of Maclean and Tommerup (1979). Spore concentration of **Bremia lactucae** isolate CL9W was adjusted to 10^5 spores/l by counting in a haemocytometer. The seedlings were sprayed with a spore suspension and incubated in a illuminated incubator at 15 °C with a 12 hour photoperiod. Control seedlings were sprayed with sterile distilled water.

RNA Isolation

Total RNA was isolatad from cotyledons of lettuce ev Cobham Green 4 days after inoculation with **B. lacucae** isolate CL9W and sterile distilled water as a control.

The RNA isoation method was similar to that described by Ainsworth et al. (1993). Frozen material was ground to a fine powder in liquid nitrogen with a pre-cooled mortar and pestle. The frozen powder was transferred to a 50 ml disposable tube containing 5 ml/g plant tissue of extraction buffer (100mM Tris-HCI pH 9.0, 200 mM NaCI, 1% Sarcosyl, 20mM EDTA). Then 1 ml phenol was added and the tissue was fragmented using a polytron for 1 min. After centrifugation to remove insoluble material, the aqueous phase was once with phenol/chloroform (50:50) and twice with chloroform. The solution containing RNA was adjusted to 2M with 8M LiCl solution and incubated overnight at 4°C, to precipitate the RNA. The RNA was pelleted by centrifugation (11 900 g for 10 min at 4°C), washed twice in 2M LiCl by resuspension and centrifugation (11 900 g 10 min) and dissolved in TE. After centrifugation to remove any insoluble material, the RNA was ethanol precipitated and dissolved in sterile distilled water.

mRNA isolation and cDNA library construction

Polyadenylated mRNAs were isolated by the Promega PolyA Tract™ system (Z5310), which employs paramagnetic particles and a biotinylated oligo (dT) primer, according to manufacturers instructions. 5 µg of polyA RNA was used as template for cDNA synthesis using a kit (Pharmacia) and processed according to manufacturers instructions. cDNA was cloned into λZAPII (Stratagene). Phage DNA was packaged using Gigapack Plus (Stratagene) and plated using XL1 Blue (Stratagene) as plating bacterial strain.

Differential screening

For this screening, 10 000 - 20 000 pfu (plaque forming unit) were plated, with XL1 Blue, on to 20 x 20 cm plates. The plaques were lifted in duplicate on to Hybond N+membranes (Amersham), denatured on a 0.5M NaOH/0.5M NaCl saked pad, neutralised with 1.5M NaCl/0.5M Tris-Cl pH 7.2, rinsed in 2xSSC, dried and fixed by UV irradiation. Filters were prehybridised in 0.6M NaCl, 20mM Pipes pH 6.8, 4mM EDTA, 0.2% gelatin, 0.2% PVP, 0.2 % Ficol 400, 1 % SDS, containing 500 µg/ml denatured (autoclaved) salmon sperm DNA for 4 hours at 65°C. Differential probes were made using 2 µg of mRNA purified from total RNA isolated from the infected cotyledons or uninoculated control cotyledons. Labelled cDNA was synthesised from this mRNA using random primers in a DNA oligolabelling kit (Pharmacia). In a 50 µl, 2 µg of mRNA (denatured at 65°C for 5 min) was mixed with 10 µl 5X random primer buffer, 10 unit reverse transcriptase, 1 unit RNAsin (Promega), KCl to give a final concentration of 50 mM and 50 µCi ^{32}P dCTP and this was incubated at 37°C for 3-4 hours. Unincorporated nucleotides were hybridised with the denatured labelled probes in the buffer described above, for 16 hours at 65°C. Hybridised filters were washed twice in 2xSSC, 0.5% SDS (15 min) and once in 0.2xSSC, 0.5 % SDS and exposed to X-ray film.

Plasmid DNA isolation

Bulescript clones carrying cDNA insert were excised from the selected λZAP clones using helper phage as described in the manufacturers instructions and selected on ampicillin. Plasmid DNA was purified by a miniprep method (Sambrook et al., 1989). The cDNA fragments were gel purified and extracted from agarose using an oligo-labelling kit (Pharmacia) and used in hybridisation with Northern blots.

Northern blotting and hybridisation

Total RNA (10 µg per track) was separated on 1.2 % agarose/formaldehyde gels and blotted to Hybond N$^+$ membrane (Amersham) using 0.05 M NaOH as the transfer buffer for 6 to 9 hours. After transfer had been completed, the membrane was removed with forceps and rinsed in 2xSSC and then air dried on a piece of 3MM paper for 30 min. Blots were hybridised to labelled probes using prehybridisation and hybridisation conditions as described above for plaque screening.

RESULTS

Library construction and screening

Cultivar Cobham Green which is known to be a universally susceptible lettuce cultivar to **B. lactucae** isolates (Farrara et al., 1987) was chosen for this study. Total RNA was isolated from 2 week old seedlings that were infected with the CL9/W isolate of **B. lactucae** 4 days previously. The 4 days after inoculation time point was chosen as it allows the fungus to produce primary vesicles, secondary vesicles as well as intercellular hyphae and haustoria (Maclean and Tommerup, 1979), so that the maximum number of cells had been challenged by the pathogen. cDNA was synthesised using mRNA purified from the total RNA of infected cotyledons. cDNA was cloned into EcoRI site of λZAPII after addition of EcoRI/NotI adaptors. Following cDNA synthesis ligation into λZAPII was carried out at three different fragment: vector ratios and then packaged into phage lambda. The most successful combination was determined by plating dilutions from 10^{-1} to 10^{-4} and the number of plaque forming units (pfu) per ml of the original packaged ligation was calculated from each dilution series of each mixture. As can be seen from Table 1, a range pfu was obtained with ligation mixture 2 and 3 containing higher numbers than ligation mixture 1.

Table 1. Number of plaque forming units of each dilution of each ligation mixture.

dilutions	10^{-2}	10^{-3}	10^{-4}
ligation mixture 1 (pfu/m)	$0.4x10^5$	$0.8x10^5$	$1x10^5$
ligation mixture 2 (pfu/ml)	$1.07x10^5$	$1.9x10^5$	$1.6x10^5$
ligation mixture 3 (pfu/ml)	$1.2x10^5$	$1.7x10^5$	$2x10^5$

Ligation mixture 3 was used for construction of the library because pfu was high and also gave the highest ratio of white plaques/blue plaques.

Phage were plated on 20x20 cm plates (2 plates) containing 250 ml NZYM medium at the density of 12,100-20,000 pfu per plate and duplicate filters were lifted from each plate. mRNAs were extracted from infected and uninfected (sterile distilled water treated) cotyledons for the preparation of probes. First strand cDNA was synthesised in a reaction mix containing ^{32}P-labelled dCTP from these mRNAs using reverse transcriptase to produce labelled copies of mRNA. One membrane was hybridised with ^{32}P-labelled first strand cDNA from the same mRNA as was used for library construction (infected), and the other one was hybridised with the probe prepared from mRNA from water treated (uninfected) cotyledons. Hence, about 40 000 clones were differentially screened to identify those that showed increasing expression in the plant after infection. As can be seen from Figure 1 some clones hybridised to the probe

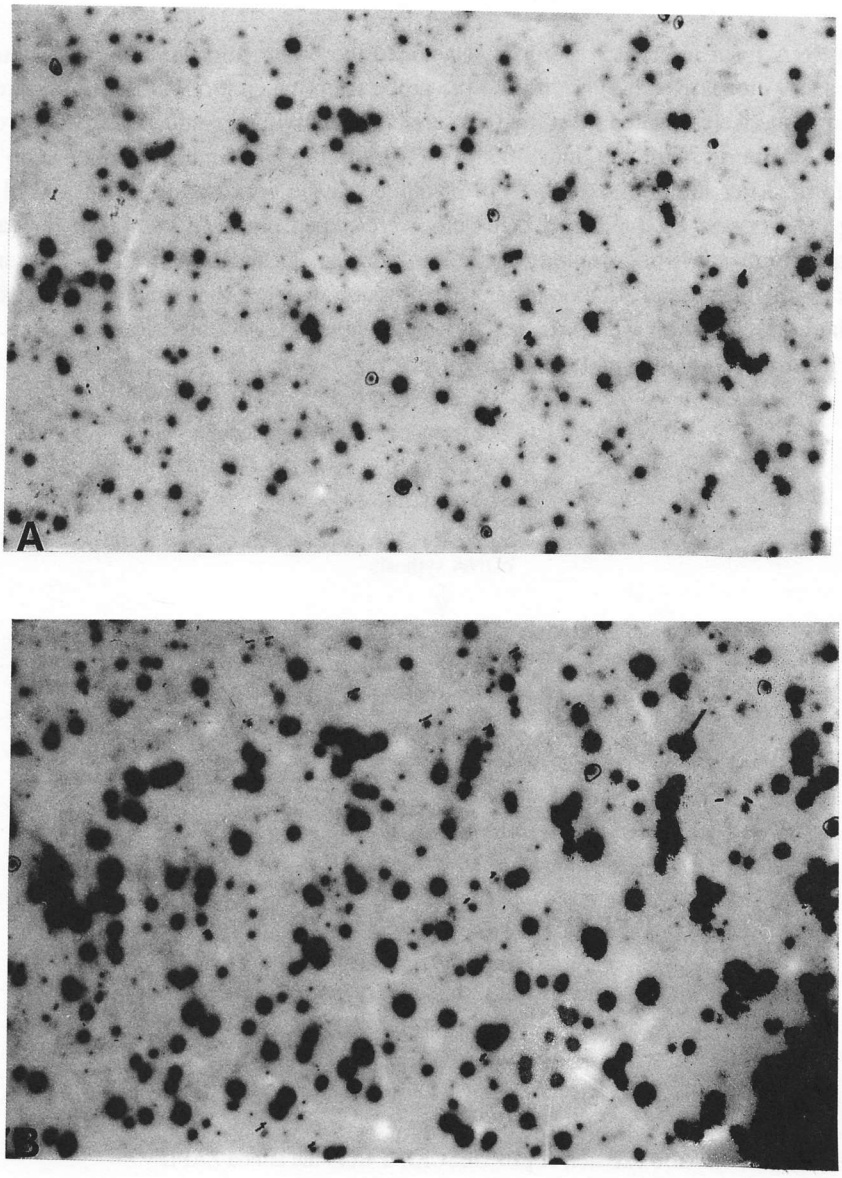

Figure 1. Differential screening of the cDNA library prepared from lettuce cultivar Cobham Green infected with CL9/W isolate of **B. lactucae.** Duplicate filters from each plate were lifted and hybridized. One of each duplicate (**A**) were probed with ^{32}P-labelled cDNA from mRNA from uninfected cotyledons. The other filter (**B**) were hybridized with the probe prepared from the same mRNA as was used for library construction (infected). The clones hybrized to one probe but not the other were picked for further analysis. Clones of interest are circled.

from mRNA from infected cotyledons but not to the probe from the control. 63 such clones and 9 clones which hybridised to the probe from the control but not to that from infected tissue were isolated from the first screening. Individual plaques picked up from the first screening were put into 500 µl of SM buffer containing a small drop of chloroform. After dilution to 10^{-2}, 10 µl of diluted phage was used to infect 200 µl of plating cells (strain XL-blue). Infected cells were plated out on a petri dish for each clone and duplicate lifts on nylon membranes were taken and hybridised in the same way as for the first round. 48 clones whose expression was switched on or off as a result of **B. lactucae** infection were selected for further analysis. A summary of all steps of cDNA library construction and secreening is given in Figure 2.

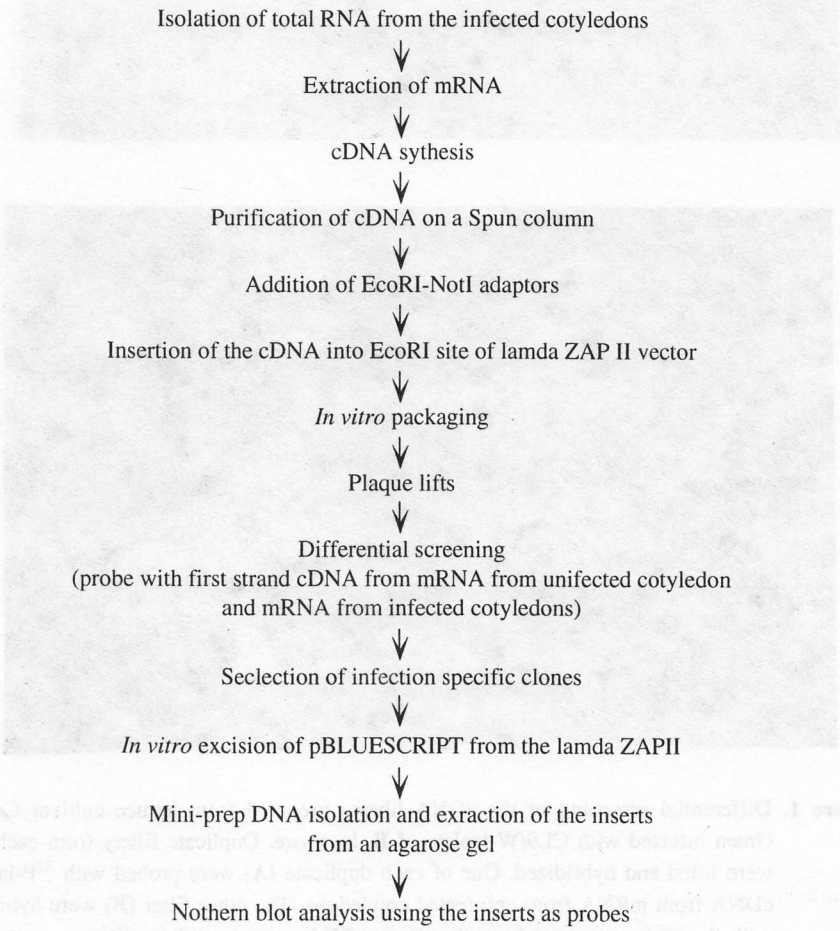

Figure 2. A flow diagram of cDNA library construction and secreening

Identification of cDNA clones expressed specifically in the compatible interaction

Bluescript derivates of the cDNA were excised from each λZAP clone that had been selected after secondary screening. XL1-Blue hostcells carrying the Bluescript derivates were grown at 37°C overnight, and 3-4 individual colonies from each plate were grown in a selective liquid medium (LB) and mini prep DNA isolated. These clones were checked for insert DNA; 72 individual colonies had inserts of different size ranging from 500 bp to 2.8 kb, and these inserts were isolated from agarose gels. The inserts purified from the clones were used as probes for to Nothern blots containing from total RNA from infected and uninfected cotyledons. cDNA clones of genes showing different levels and patterns of expression were seen (Figure 3). Some of them showed the same expression level in both infected and uninfected RNA, while others were expressed strongly in infected cotyledons but also showed a low level of expression in the uninfected control.

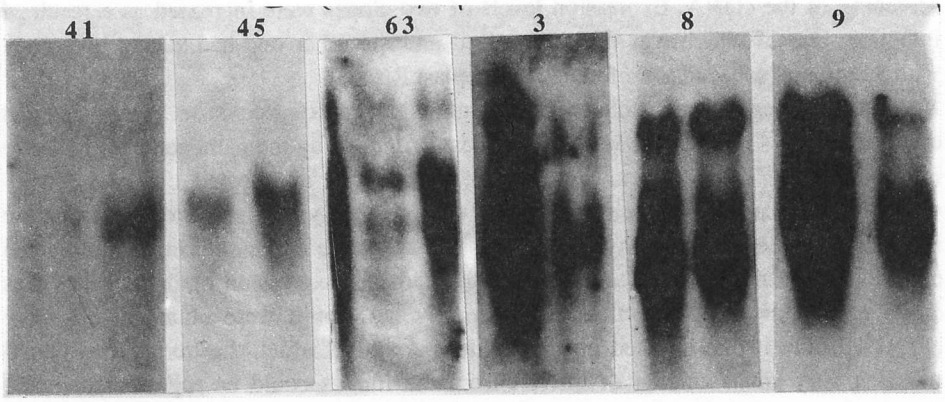

Figure 3. mRNA expression during infection of lettuce by **B. lactucae** isolate CL9W. Northern blots were prepared from the RNA isolated from uninfected cotyledons (first track on each filter) and infected cotyledons (second track). Each Northern blot was hybridised with a different cDNA. Clones 41, 45 and 63 were more strongly expressed in infected tissues than in the uninfected controls. Clones 3, 8 and 9 showed relatively higher expression in the uninfected control than in the infected cotyledons.

DISCUSSION

Plants react to challenge by phytopathogens, such as viruses, fungi or bacteria, in a number of different ways to protect themselves by eliminating or restricting the pathogen and limiting the damage that the pathogen causes. In some cases, defence attempts are unsuccessful and the pathogen grows and spreads through the plant usually causing disease or even death of the host (Malamy and Klessig, 1992). One possibility

for this is that pathogen is recognised but the defence responses are not initiated quickly enough. Several studies have been carried out to analyse the defence mechanisms of plants. These studies showed some responses such as accumulation of hydrolytic enzymes, are similar in the incompatible and compatible plant-pathogen interactions. Most studies have concentrated on genes expressed in the resistance reaction.

There are two studies on the expression of susceptible specific genes in compatible plant-pathogen interaction. Gurr et al., (1991) carried out work on potato cyst nematode infection of potato plants where their goal was to identify syncytium-specific expressed genes (syncytia; group of enlarged cells caused by nematode invasion). They reported that expression of one cDNA clone was particularly infection specific. Comparison of partial sequence information with databases indicated no homology with known genes.

Saunders et al., (1989) have studied the gene expression of turnip plants in response to cauliflower mosaic virus (CaMV) infection. Three clones representing turnip leaf RNAs whose levels changed during infection were isolated by differential screening of the cDNA. Expression levels of two clones were increased as a result of infection, one of which was uncharacterized and the other one had strong homology with a region of tobacco and of maize chloroplast RNA.

Eight clones were isolated representing lettuce RNAs whose levels changed during the first 4 days of the infection process of lettuce by the pathogenic **B. lattucae** isolate CL9/W. Two of them were strongly expressed in infected tissues but very weakly in the control and four other clones showed relatively higher expression on the infected cotyledons. In contrast, two clones showed more expression in the uninfected control than in the infected cotyledons suggested that expression of these genes was down regulated during infection. Unfortunately, no clones were obtained that were expressed only in the infected plant. Due to the way in which the library was constructed a very small amount of mRNA from the fungus could be represented in the library. If an appropriate clone had been identified, it would have been necessary to check whether this clone represented mRNA from the fungus or the plant. Hybridisation of the clone to total plant genomic DNA would have confirmed the clone as being of plant origin.

The clones that were isolated may not be sufficiently tightly regulated to be of use in this study. Ideally the expression of the gene would be high in the infected but absent in uninfected tissue. However, a low level of expression in uninfected tissue may not be developmental to the plant and, hence, clones such as number 41 may be useful. These clones were not analysed further and, hence, no information on their primary structure is available. These clones could be sequenced and compared with the available databases to finid out if they have homology with known sequences. The next step would be to isolate the promoter of the gene of interest by using the cDNA as a probe to a genomic library from lettuce. Such promoters would be of use in the expression of gene in plants that enhance disease resistance.

ÖZET

MARULDA **Bremia lactucae** İNFEKSİYONU
SPESİFİK ÇALIŞAN GENLERİN TEŞHİSİ

Marulda **B. lactucae** infeksiyonuna karşı spesifik çalışan genleri temsil eden cDNA klonları **B. lactucae** ile infekteli kotiledonlardan izole edilmiş mRNA kullanılarak yapılan cDNA arşivi testlenerek teşhis edilmiştir. cDNA kütüphanesi infekteli kotiledonlardan izole edilen mRNA ile testlenmiştir. Patojen-bitki interaksiyonuna spesifik olarak bu genlerin çalışması Northern hibridizasyon analizi ile kontrol edilmiştir. Northern hibridizasyon analizinden birçok farklı çalışma (expression) seviyelerini ifade eden gen profilleri gözlenmiştir. Bunlardan bazıları infekteli bitkide kontrole oranla daha yüksek seviyede çalıştığı saptanmıştır ve bunların infeksiyona spesifik olduğu kanısına varılmıştır.

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Ultrastructural Changes in Crowns of Peppers Resistant And Susceptible To **Phytpohthora capsici**

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ABSTRACT

The ultrastructural examination of resistant and susceptible host-pathogen interactions was conducted in the pepper cultivars Ince Sivri-35, PM 217, and PM 702 = CM 334 following inoculation with ***Phytophthora capsici*** *Leonian. Responses were characterized and compared with healthy non-inoculated controls at 2, 4 and 6 days after inoculation. After 2 days in susceptible interactions, the pathogen grew intercellularly in crowns, whereas in resistant interactions only a few intercellular hyphae were observed penetrating the host cells and forming haustoria. The consistent features of ultrastructural changes in susceptible interactions at 2, 4 and 6 days after inoculation were degeneration of cell organelles and dissolution of host cell walls. The host cell walls were poorly stained and swollen, possibly due to alteration by fungal macerating enzymes. In the resistant cultivar, PM 217, at 2 and 4 days after inoculation, the organelles appeared healthy, but at 6 days after inoculation organelles had degenerated. In another resistant cultivar, PM 702, at 2, 4 and 6 days after inoculation fungal hyphae grew usually intercellularly, and mitochondria and chloroplasts, but not microbodies, were degenerated and papilla had formed. In general, reactions of the resistant cultivars to* ***P. capsici*** *were intercellular, rather than intracellular fungal growth, papilla (callose) formation, resistance of the cell wall to dissolution and microbodies with normal appearing inclusions.*

INTRODUCTION

The infection by **Phytophthora capsici** Leonian causes foliar blight and crown and root rot of pepper plants (Leonian, 1922; Barsdale et al., 1984)). A brown girdling rot may extend up the stem before the plant finally wilts and dies. Numerous ultrastructural studies have been made of the interaction of various host plants with **Phytophthora** spp. (Ehrlich and Ehrlich, 1966; Jones et al., 1974; Hohl et al., 1976; Hohl and Suter, 1976; Allen and Friend, 1983; Hohl, 1984; Hwang et al., 1989). In an earlier study of **P. capsici** in pepper, Jones et al., (1974) examined the compatible interaction only in fruit cells injected with a zoospore suspension in an attempt to demonstrate ultrastructural events during pathogenesis. Recently Hwang et al., (1989) examined ultra-

structural changes, at 2 days after inoculation, in the infected root and stem of pepper (**Capsicum annuum** L.) cultivar "Hanbyul" susceptible to **P. capsici.**

It is important to examine the ultrastructural changes in infected tissues of resistant and susceptible pepper cultivars to **P. capsici** Leon. No ultrastructural studies comparing the resistant and susceptible reactions of pepper cultivars to **P. capsici** have been reported. Information reported here characterizes the processes of pathogen containment in resistant interactions and compares these with the processes occuring in susceptible interactions.

MATERIALS and METHODS

Pepper plants and fungus. Three cultivars of **Capsicum annuum** L. were used in this study: one susceptible (Ince Sivri-35) and two resistant (PM 217 and PM 702 = CM 334) to **Phytophthora capsici** Leonian. Seeds of resistant pepper cultivars were obtained from Prof. Dr. Mas (Station d'Amelioration des Plantes Maraicheres Domanie St-Maurice, Monfavet-France). Seeds of susceptible pepper were obtained from Antalya, Vegetable Research Institute, Turkey.

Plants were grown in a plastic pots (75x15x10 cm) containing a mixture of steam-sterilized loam soil, sand and peat (1:1:1, v/v/v). Plants were maintained in a glasshouse at 25±2°C during a 16 hr light period (about 5000 lux) and at 16±2°C during darkness. Humidity levels were 65 %.

An isolate of **P. capsici** Antalya was obtained from Prof. Dr. Maden (Department of Plant Protection, Agricultural Faculty of Ankara University, Turkey) and was used for inoculation. The fungus was grown in V8 juice agar plates at 25±2°C for 7-10 days. Then zoospore suspension was prepared as previously described (Üstün, 1993). Zoospores were separated from the empty sporangia by decanting through cheesecloth. Zoospores in suspension were counted with a haemocytometer and adjusted to the required concentration ($1x10^4$ zoospores/ml) with sterile distilled water.

Inoculation and sampling. At the 7-8 leaves stages, the seedlings were uprooted ant their roots were washed in tap water and plants were transfered to Hoagland Liquid medium culture. Five days later, when plants had recovered from the stress of being uprooted and roots and crowns were soaked for one hour in a suspension of motile zoospores of **P. capsici.** After inoculation plants were then replaced in liquid culture. Samples of crowns were taken 2, 4 and 6 days after inoculation.

Electron microscopy. One-mm segments of controls and rotted crowns infected with P. capsici were excised in 0.1 M phosphate buffer at pH 7.4. These segments were immersed in a fixative containing 3 % glutaraldehyde in 0.1 M phosphate buffer at pH 7.4, under vacuum for 4-6 h at room temperature to facilitate penetration of the fixative. Following initial fixation tissues were washed (overnight) in three changes of phosphate buffer. The tissues were then post-fixed in 1 % OsO_4 in the same buffer for 2 h at room temperature, washed in buffer, dehydrated in a graded series of ethanol up to 100 %, and

transferred to a prophylenoxite+Epon (2/1, 1/1, 1/2, v/v) series for 1 h each. The segments were saturated overnight on a rotary shaker with Epon mixture and then embedded in Epon 812. Polymerization was done at 60°C for 24 h. Thin sections were cut on a Reichert OMU_4 ultramicrotome and then mounted on coated 75-100 mesh copper grids. Sections were stained with uranyl acetate, and poststained with lead citrate (Sato, 1967). Observations were made with a Jeol-100CXII transmission electron microscope operated at 80 kV.

RESULTS

Transverse sections of susceptible Ince Sivri-35 crowns infected with zoospore suspension of **P. capsici** were observed by electron microscopy at 2 days after inoculation (Fig. 1). Fungal hyphae had invaded regions of the crown tissue including xylem, phloem and parenchyma cells. The fungus grew intercellularly and in the lumen of the xylem vessels (Fig. 1A). Ultrastructural changes in infected parenchyma cells were more severe than those of vascular tissues. Swollen mitochondria and degenerated dictyosomes and microbodies including crystals devoid of their mebrane and granular matrix were observed within plasmolyzed plasma membranes. In the resistant cultivar PM 217, fungal hyphae were rarely observed intracellularly. In the other resistant cultivar PM 702, internal organization was normal even though the fungal hyphae were present intra- and intercellularly within most of parenchyma cells. The route of hyphal growth was invariably though the middle lamella in parenchyma cells (Fig. 1B) and the infected host wall was swollen. Host cells which were in contact with fungal cells exhibited degenerated mitochondria and chloroplasts containing disorganized lamellae (Fig. 1C). Small aggregations of vesicles were observed occasionally in the separation zone between the fungal plasmalemma and fungal cell wall of the fungal hyphae penetrating parenchyma cells (Fig. 1D). Most of the fungal hyphae penetrating parenchyma cells had numerous vacuoles and numerous mitochondria, ribosomes, and smooth endoplasmic reticulum (ER) (Figs. 1A, B).

Four days after inoculation, severe lesions appeared on the susceptible Ince Sivri-35 crowns. The cortex region developed brown lesions and began to separate from the cambium. Cell walls were destroyed and the cells became plasmolysed (Fig. 2A). Fungal hyphae had invaded intra- and intercellularly. Most fungal hyphae in infected phloem grew intercellularly in the middle lamella, resulting in the separation of the host cell wall into two parts (Fig. 2D). Penetrating hyphae were narrowed where they perforated the host cell wall and electron-dense staining material between host cell wall and fungal cell wall accumulated around the neck of the fungus (Fig. 2B). Small aggregations of vesicles were observed occasionally between fungal plasmalemma and fungal wall (Fig. 2B). The plasma membranes and cytoplasmic organelles (swollen, distorted chloroplasts) and walls of host cells were dissolved by the penetrating hyphae (Figs. 2C, D). In resistant PM 217 cultivar, generally, cytoplasmic organelles appeared healthy

except in a few of the infected cells. In the ray parenchyma cells with adjacent xylem cells, chloroplasts were observed containing large and numerous starch granules. Fungal hyphae were rarely found in resistant PM 217 cultivars cells. In another resistant combination (PM 702) fungal hyphae were observed inter- and intracellularly, and cytoplasmic organelles had degenerated more than in the other resistant combination (PM 217) (Fig. 2E).

Six days after inoculation, in susceptible cultivar (Ince Sivri-35), the cytoplasm of parenchyma cells below the epidermis appeared amoeboid in shape vacuolate and the host cell wall had degenerated directly under the epidermis (Fig. 3A). Epidermal, parenchyma and phloem cells were intra-and inter-cellularly colonized by zoospores (arrows) and fungal hyphac had undergone degradation (Fig. 3B). Most of the fungal hyphae penetrating parenchyma cells were vacuolated or completely degenerated (Fig. 3C). Swollen and sac-like chloroplasts containing disorganized lamellae, degenerating mitochondria, microbodies with crystalline inclusion devoid of granular matrix were observed (Figs. 3B, C). Generally, zoospores were found in the intercellular spaces (Fig. 3D). In the resistant PM 217 cultivar, cortical parenchyma cells, although themselves appearing normal, had irregularly-shaped, degenerated sac-like chloroplasts which contained disorganized and disintegrating lamellae (Fig. 3E). Some non-infected cortical and phloem cells had normal internal organization (Figs. 3F, G, H). Some of the phloem cells contained normal plastids but had mitochondria with bulging outer membranes and which were vacuolated (Fig. 3J). Very few fungal hyphae were observed intercellularly. Papillac (callose deposits) were formed at the region of contact between the plasma membrane and the cell wall of the host cell (Fig. 3I). In the other resistant cultivar PM 702, fungal cells had cytoplasm with dense ribosomes, ER, numerous mitochondria and vacuoles (Fig. 3K). Fungal hyphae had invaded inter- and intracellularly in epidermis, cortex parenchyma and xylem. Distorted chloroplast, normal microbodies containing crystalline inclusions and disintegrated nuclei were observed in parenchyma cells. Fungal lomasomes were usually associated with the fungal wall at the interface (Fig. 3K).

DISCUSSION

The infected cells of the susceptible cultivar (Ince Sivri-35) exhibited disruption of chloroplasts, mitochondria and microbodies, possibly as a result of damage to cellular membranes. The possibility of damage to the cellular membranes was evident by the appearance of incomplete cell walls and distinctive plasmolysis. The middle lamella appeared to have pulled away from the cell wall and an increase in the number of damaged cells was evident. Infecting hyphae of **P. capsici** grew intercellularly in infected tissues of pepper crowns. The common route of hyphal growth was through the middle lamella of the host wall. This is characteristic of **Phytophthora** species on numerous hosts (Hanchey and Wheeler, 1971; Tippett et al., 1977; Stoessl et al., 1981;

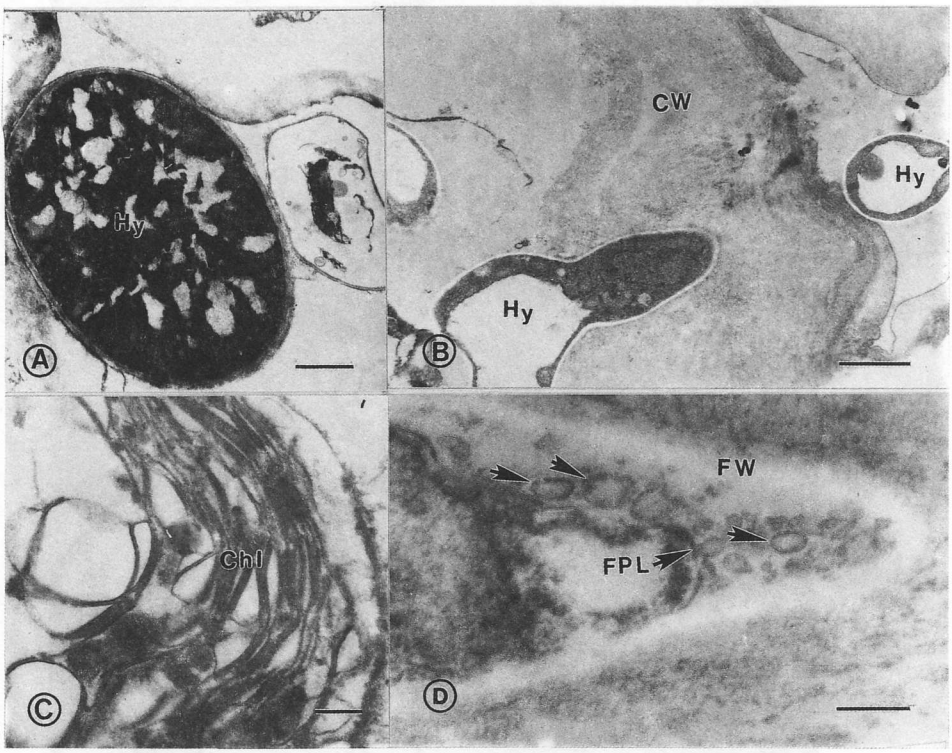

Figure 1. Electron micrographs of the reactions of susceptible Ince-Sivri 35 (A) and resistant PM 702 pepper cultivar (B, C, and D) two days after inoculation with **P. capsici.** **A)** Intercellular hyphae (Hy). Bar = 1.5 μm. **B)** Hyphae (Hy) penetrating the parenchyma wall (W). Note the swollen and dissolved host walls (W) and middle lamella. Bar = 2 μm. **C)** Swollen, sac-like chloroplast (Chl) containing disorganized lamellae in the parenchyma cell. Bar = 0.2 μm. **D)** Vesicles (arrows) are aggregated in the hyphal tip between fungal plasmalemma (FPL) and the fungal cell wall (FW). Bar = 0.2 μm.

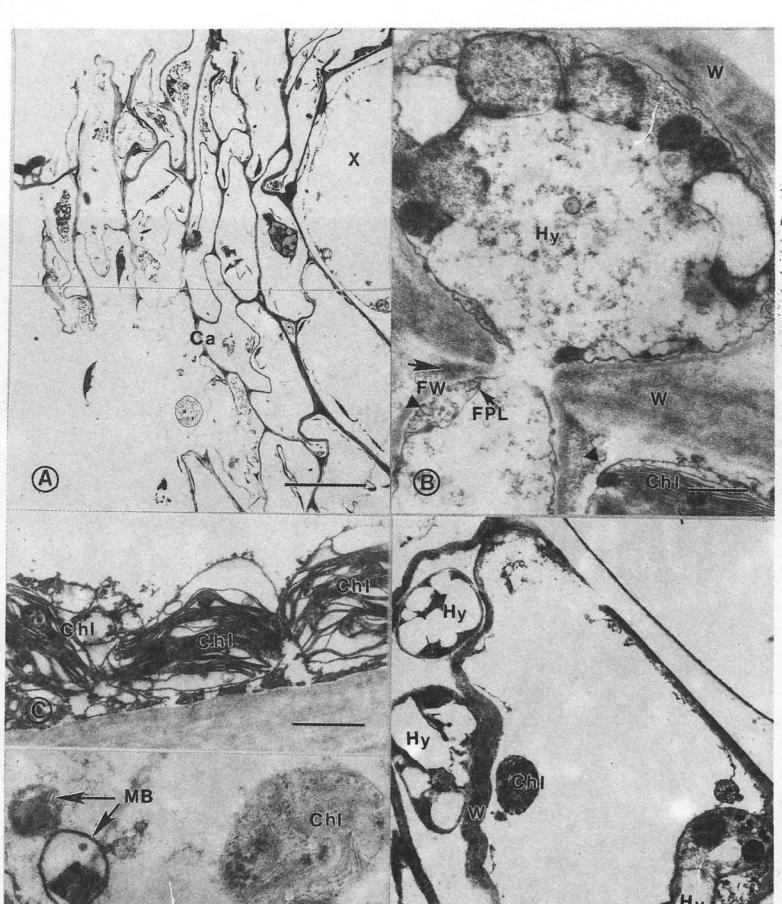

Figure 2. Electron micrographs of the reactions of susceptible Ince Sivri 35 (A-D) and resistant PM 702 (E) pepper cvs. to **P. capsici**, four days after inoculation. **A)** Infected cambium (Ca) and xylem (X) cells, which appear to be plasmolyzed. Bar = 1.5 μm. **B)** The hyphae at the penetration (big arrow) site of host wall (W) is narrowed and the densely stained wall material is apposed at the neek of fungus. The vesicles (small arrows) are aggregated between fungal plasmalemma (FPL) and the fungal cell wall (FW) at the neek of fungus. Bar = 0.5 μm. **C)** Electron micrographs of the reaction of resistant pepper cultivar 4 days after inoculation with **P. capsici.** Swollen, sac-like chloroplast (Chl) containing disorganized lamellae. Bar = 1.5 μm. **D)** Fungal hyphae (Hy) which have grown intercellularly, within the host middle lamella adjacent to the cell wall (W) as well as intracellularly in a phloem cell. Bar = 2 μm **E)** Degenerated cytoplasmic organelles [microbody (MB), chloroplast (Ch)] in the resistant PM 702 cultivar. Bar = 0.5 μm.

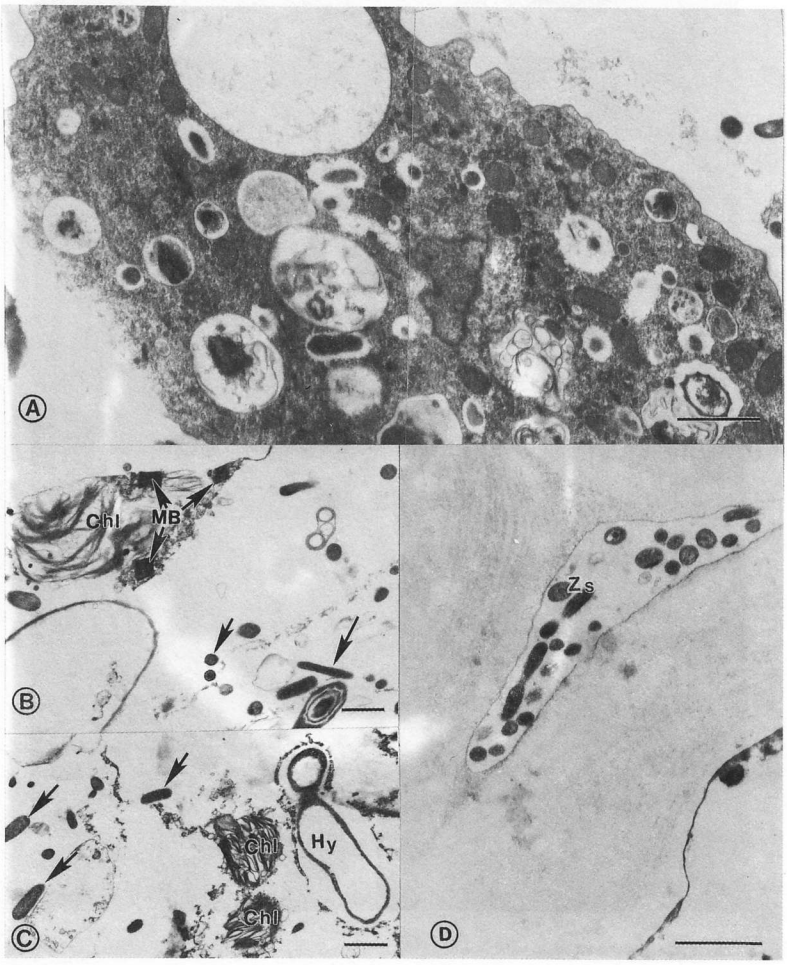

Figure 3. Electron micrographs of the reactions of susceptible İnce Sivri 35 (A-D) and resistant PM 217 (E-J), PM 702 (K) pepper cvs. to **P. capsici** six days after inoculation. **A)** Amoeboid shaped cytoplasm and dissolved host cell wall. Bar = 2 µm. **B)** Zoospores ((arrows) and degenerated chloroplast (Chl) and microbody (MB) with crystalline inclusion devoid of granular matrix. Bar = 1.5 µm. **C)** Zoospores (arrows), empty hphae (Hy), disintegrated host wall, and degenerated chroplast (Chl). Bar = 1.5 µm. **D)** Zoospores (Zs) within an intercellular space are seen. Bar = 2 µm. Electron micrographs of the reactions of resistant pepper cultivar 6 days after inoculation.

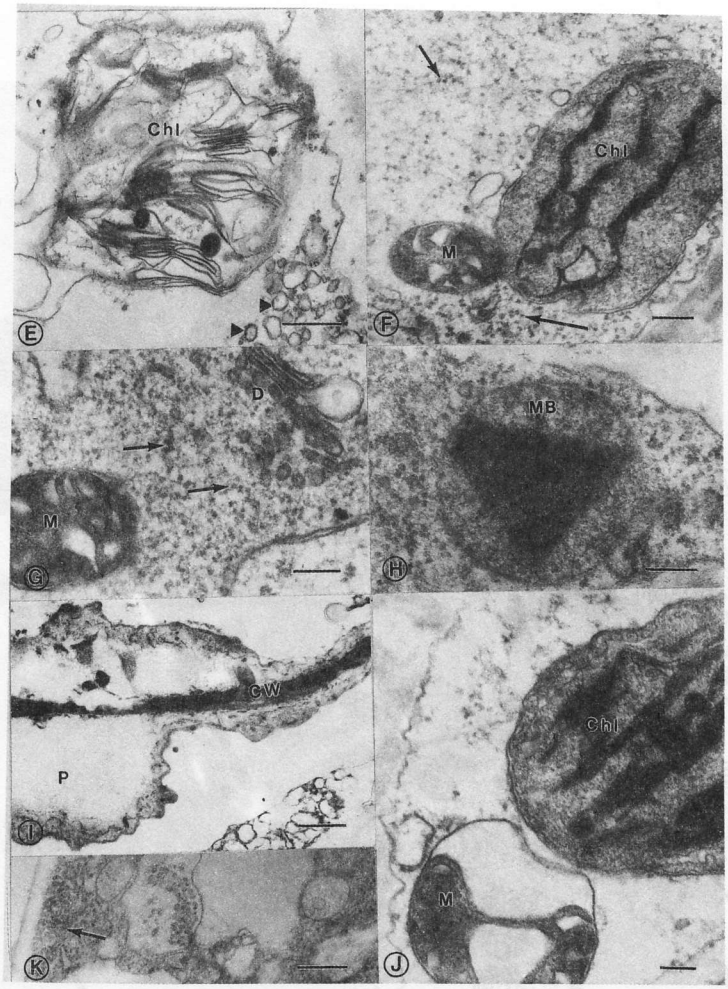

Figure 3. **E)** Degenerated chloroplast (Chl) which contains disorganized and disintegrating lamellae and vesicles (arrows) are seen. Bar = 0.5 μm. **F)** Normal chloroplast (Chl), mitochondrion (M), and ribosomes (arrows) in phloem cell. Bar = 0.2 μm. **G)** Normal dictyosome (D) and mitochondrion (M) and ribosomes (arrows). Bar = 0.2 μm. **H)** Normal microbody (MB) with crystalline inclusion. Bar = 0.2 μm. **I)** Papillae (P) (callose deposits) formation between plasma membrane and cell wall (CW) of the host cell. Bar = 1 μm. **J)** Normal chloroplast (Chl) and vacuolated mitochondrion (M) with bulging outer membrane. Bar = 0.2 μm. **K)** Fungal cell (arrow) in cortical parenchyma cell. Bar = 0.3 μm.

Hinch et al., 1985; Hwang et al., 1989). Passage through the middle lamella may be due to physical weakness of their material compared with cell walls (Hinch et al., 1985; Hwang et al., 1989). The host cell walls were poorly stained and swollen, possibly due to biochemical alteration by the action of fungal macerating enzymes. Our ultrastructural observations of the susceptible cultivars (intra- and intercellular fungal hyphae growth) supported Hwang's results (Hwang et al., 1989). But in the two resistant cultivars (PM 217, PM 702) fungal hyphae usually grew intercellularly and therefore host cell walls not severely damaged. This could be either the cause or the result of the resistance in these cultivars to the fungus. In addition, much less intercellular fungal growth was seen in PM 217 than PM 702. This latter observation correlates with the relative levels of resistance between the two cultivars.

Infected parenchyma cells of susceptible cultivars were completely macerated whereas lignified walls of the xylem vessels were not severely disrupted, even though the vessels were filled with fungal hyphae. These observations support those of Hwang et al., (1989). The observations of lignified xylem walls in cells of the resistant cultivars are supported by the histopathological results from light microscopy of the same pepper tissues previously reported (İlarslan et al., 1994). The membrane and granular matrix were intact in microbodies in the infected resistant cultivars but disappeared in susceptible cultivars. Hwang et al., (1989) observed microbodies including crystals devoid of their membrane and granular matrix. In contrast, Jones et al., (1974) observed normal microbodies containing crystalline inclusions in infected and uninfected tissues. The observation of normal microbodies in the resistant cultivars 6 days after the inoculation might be a resistance response.

As is well known, microbodies play important roles in the peroxidase activity (Hammerschmidt et al., 1982). Undistorted microbodies may be a requisite for normal peroxidase activity. In order to understand the mechanisms behind the resistance it may be necessary to investigate peroxidase activity and isozymes. Several investigations (Hammerschmidt et al., 1982; Kollattukudy et al., 1992) indicate that peroxidase may affect resistance through cell wall lignification. The degeneration of cytoplasmic organelles may result from action of fungal toxins. Separation of the cytoplasm from cell walls and degeneration of cell organelles prior to penetration has been postulated to be the result of the effects of fungal metabolites (Jones et al.., 1974). In the resistant cultivars the fact that this degeneration occurs late and to a lesser degree might be the result of the very low number of fungal hyphae that can penetrate into the cells and thus, due to a reduced amount of these fungal toxins (metabolites). In resistant result of the very low number of fungal hyphae that can penetrate into the cells and thus, due to a reduced amount of these fungal toxins (metabolites). In resistant genotypes no fungal hyphae were observed in the xylem lumens of the roots and may be an indication that the vascular conduction is not inhibited.

We observed that hyphae of **P. capsici** rapidly grew intra- and intercellularly in the susceptible cultivar, and caused death of the plant after severe cell deformation. But in the resistant genotypes (PM 217 and PM 702), the fungus usually was found only in the intercellular regions and therefore host cells were not severely damaged. Callose formation (papillae) was observed in the resistant cultivars. According to other researchers, callose formation plays a possible role in the arrest of hyphal growth and protection of host cells in contact with hyphae (Hinch and et al., 1985; Wetherbee and et al., 1985). Our observations indicate that the occurence of host responses such as intercellular rather than intracellular fungal growth, papillae formation, resistance of the cell wall to dissolution, and normal microbody inclusions may be resistance responses. The differences in resistance responses of the two resistant cultivars is likely related to the genetic backround of these genotypes.

ÖZET

Phytophthora capsici'YE DAYANIKLI VE DUYARLI BİBERLERİN KÖKBOĞAZLARINDA MEYDANA GELEN ULTRASTRÜKTÜREL DEĞİŞİMLER

Phytophthora capsici Leonian inokule edilmiş dayanıklı ve duyarlı İnce-Sivri 35, PM 217 ve PM 702 = CM 334 biber çeşitlerinde konukçu-patojen ilişkisi ultrastrüktürel olarak incelenmiştir. İnokulasyondan 2, 4 ve 6 gün sonra, inokule edilmemiş kontrol bitkiler ile karşılaştırılarak dayanıklılık özellikleri ortaya konmuştur. Patojen, inokulasyondan 2 gün sonra duyarlı çeşidin kökboğazlarında intersellüler olarak büyümesine karşılık, dayanıklı çeşitlerde konukçu hücrelere diffüz eden ve haustoria formunu oluşturan az miktarda intersellüler hifler gözlenmiştir. Duyarlı çeşitde inokulasyondan 2, 4 ve 6 gün sonra gözlenen ultrastrüktürel değişimler, hücre organellerinin dejenerasyonu ve konukçu hücre duvarlarının erimesidir. Konukçu hücre duvarları az boyanır ve şişer, muhtemelen bunun sebebi fungusun maserasyon enzimleridir. Dayanıklı çeşit olan PM 217 de inokulasyondan 2 ve 4 gün sonra, organeller normal görünür fakat inokulasyondan 6 gün sonra, organeller dejenere olurlar. Diğer dayanıklı çeşit PM 702 de inokulasyondan 2, 4 ve 6 gün sonra fungal hif genellikle intersellüler olarak büyür, mitokondri ve kloroplastlarda dejenerasyon görülmesine karşılık, mikrocisimlerde herhangi bir dejenerasyon gözlenmez ve papilla oluşur. Genel olarak, dayanıklı çeşitlerin **P. capsici**'ye karşı oluşturduğu dayanıklılık reaksiyonları, fungusun intrasellüler olarak büyümesinden ziyade intersellüler olarak büyümesi, papilla (kallos) formasyonu, hücre çeperinin dayanıklılığı ve normal iç yapıya sahip mikrocisimlerin bulunması şeklindedir.

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Studies on the Sporulation of the Early Blight Agent [**Alternaria solani** (Ell. and Mart.) Jones and Grout] of Tomatoes

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ABSTRACT

*It is rather difficult to induce the sporulation of **Alternaria solani** (Ell. and Mart.) Jones and Grout and some **Alternaria** spp. on artificial media by the conventional methods. Nine different artificial media in combination with 4 differenet light and temperature conditions, and the other several media were tested for inducing the sporulation of **A. solani** in-vitro. As a result, T media containing tomato juice was found to be the most suitable media with 6 days dark at 23°C - 12 h light at 26°C - 12 h dark at 18°C incubation for the best sporulation of different **A. solani** isolates.*

INTRODUCTION

Early blight caused by **Alternaria solani** (Ellis and Martin) Jones and Grout has recently been one of the most important annually-occurring foliage diseases of tomatoes. It is very difficult to induce the sporulation of **A. solani** and some **Alternaria** spp. on the artificial media by the conventional methods, and many researchers such as, Lukens, 1960; Miller, P.M., 1965; Kilpatrick, 1966; Rotem and Bashi, 1969; Prasad, Dutt and Nagaich, 1973; Prasad and Dutt, 1974; Shahin and Shepard, 1979 worked on the inducing sporulation of **A. solani** and tried to use different methods. However, our preliminary experiments related to inducing sporulation of **A. solani** didn't confirm the above mentioned methods, at all. As it is known, obtaining abundant spore in-vitro, forms the basis of studies related to taxonomy host and fungicide resistance.

In this report, we describe the development of processes of sporulation in **A. solani.**

MATERIALS and METHODS

In this study, we used three isolates which had the highest virulence. The following methods were carried out to induce sporulation of **A. solani.**

1. Mycelial mat of **A. solani,** grown in Potato Dextrose Broth (potato 200 g, dextrose 20 g, distilled water 1l) in a shaker at 23±2°C for 7 days was

disintegrated with waring blender, and centrifuged at 6000 gr for 10 min. The pellet was suspended in 0.02 μ phosphate buffer (pH = 6.4) and filtered by a filter paper. 2 ml of the filtrate was spread on PDA plates and incubated at 23°C for 3 days (Lukens, 1960).

2. Double plate method: Filter paper (non-sterile) saturated with distilled water were placed in a petri dish. Small agar plugs, containing hyphae of **A. solani** were transferred to the filter paper, and incubated them at room temperatures (23°C) in 3-10 days when necessary, the filter paper was moistened at frequent intervals without disturbing growth of the fungi (Kilpatrick, 1966).
3. Water treatment: **A. solani** isolate was grown on PDA plates at 26°C for about 9 days. The cultures were dipped first in cold (4°C) and then hot (58°C) water 4 minutes by opening the lids. Thereafter, the treated cultures were incubated at room temperature (23°C) in diffused sunlight. A similar set of water treated cultures were placed in complete darkness at 26°C. In the second method, the cultures were sprayed with 2 ml of cold (4°C) and 2 ml of host (58°C) water. One part of them was incubated at room temperature (23°C) in diffused sunlight. Other part of them were placed in darkness at 26°C in incubator (Prasad et al., 1973).
4. Exposition to sunlight: Sporulation was tried to obtain 6 days old partially grown cultures by inducing the formation of sporulating zone which appeared in 24 hours after every exposure of 60 minutes to sunlight (Prasad and Dutt, 1974).
5. **A. solani** cultures were incubated on PDA plates containing 14 ml PDA in 9 cm diameter petri dishes at 25°C. After 48-72 h incubation, agar plaques containing mycelium was cut into small blocks and then the half of the grown colony transferred to the S-medium (20 g sucrose, 30 g $CaCO_3$, 20 g agar, 11 distilled water, pH = 7.4). Petri dish was flooded with 2 ml of sterile distilled water and incubated 48-72 h at 18°C (Shahin and Shepard, 1979).
6. Nine different media were tried under 4 different light-temperature conditions. These media:

a. PDA (Potato Dextrose Agar): Potato 200 g, dextrose 20 g, agar 20 g, distilled water 11.
b. Tap Water Agar (TW): Agar 15 g, tap water 11.
c. Corn Meal Agar (CMA, Oxoid)
d. Malt Extract Agar (MEA): Malt extract 20 g, agar 20 g, distilled water 11.
e. Czapek (Dox) Agar (Cz) (Anonymous, 1983): $NaNO_3$ 2 g, KH_2PO4 1 g, Mg SO_4. $7H_2O$ 0.5 g, KCl 0.5 g, FeSO4, 7 H_2O 0.01 g, sucrose 30 g, agar 20 g, distilled water 11.
f. S-medium (Shahin and Shepard, 1979); Sucrose 20 g, $CaCO_3$ 30 g, agar 20 g, distilled water (pH=7.4) 11.

g. Tomato juice agar (T, prepared by us): Tomato juice 200 ml, agar 20 g, distilled water 800 ml.

h. V8-Agar (Anonymous, 1983): V8 vegetable juice 200 ml, agar 20 g, distilled water 800 ml.

ı. V88-Agar (prepared by us): Tomato 400 g, carrot 100 g, beet root 60 g, lettuce 20 g, celery 10 g, parsley 10 g, cress 10 g, spinach 10 g, distilled water 11.

The light-temperature conditions:

a. 26°C continuous dark (D)

b. 26°C continuous light (L)

c. 12 h dark in 23°C - 12 h light in 26°C (D-L)

d. 6 days dark in 23°C - 12 h light in 26°C-at least 12 h dark in 18°C (D-L-D) (Hainaut, 1978).

At the end of preliminary experiments, the most suitable light and temperature conditions and the best media in order to produce the abundant sporulation of **A. solani** were determined.

Three highly virulent **A. solani** isolates were tested by using three different media causing the best sporulation and by applying the determined light-temperature conditions in an experiment designed by 3x3 factorial desing.

In all sporulation experiments, the spore suspension of **A. solani** was prepared by agitating the spores with jets of sterile distilled water containing 0.1 % Tween 80 on each plate. The spore suspension was centrifuged at 4000 g for 15 min., the supernatant was removed and then pellet was suspended in 1 ml sterile distilled water. 5 µl of spore suspension were pipetted and number of spores per ml were determined under the light microscope.

RESULTS

Few **A. solani** spores were obtained by the methods of Lukens (1960), Kilpatrick (1966), Prasad et al. (1973), Prasad and Dutt (1974), Shahin and Shepard (1979). No sporulation was seen under continuous dark or light conditions at 26°C in the preliminary experiments aiming to determine the most suitable media and light-temperature combinations. But, 12 h dark - 12 h light conditions caused rather good sporulation (<100 spores per 5 µl) on V8, V88 and T media (Table 1). The best result was determined in 6 days dark in 23°C - 12 h light in 26°C - at least 12 h dark in 18°C, and it was obtained more than 100 spores of **A. solani** per 5 µl on each V8, V88 and T media. The same results were also confirmed with 3 differenet **A. solani** isolates by using the same media and dark and light combinations. The data determined are presented in Table 2.

According to the statistical analysis, no interaction was seen between **A. solani** isolates and media.

Table 1. The sporulation of **A. solation** in different media under altered growth conditions

Media	Conditions			
	Dark	Light	Dark-Light	Dark-Light-Dark
V8	-	-	+	++
V88	-	-	+	++
T	-	-	+	++
MEA	-	-	-	-
CMA	-	-	-	-
Cz	-	-	-	-
S	-	-	-	-
PDA	-	-	-	-
TW	-	-	-	-

(+) < 100 spores per 5 µl

(++) > 100 spores per 5 µl

Table 2. The sporulation of 3 **A. solani** isolates in V8, V88 and T media at darklight-dark period

Isolates	Replications	Media			
		V8	V88	T	Average
20/2	3	513.7	323.3	382.3	406.4 a
18/7	3	510.3	304.3	510.3	441.6 a
8/4	3	208.7	244.7	143.7	199 a
Average		410.9 b	290.8 b	345.4 b	349

(P<0.01)

DISCUSSION

No spore was obtained from all media incubated under the continuous dark or light conditions at 26°C. We determined only a few **A. solani** spores in V8, V88 and T media under 12 h dark - 12 h light combination. Probably, the fungus has exposed to light period before it could complete its vegetative growth. Rath and Padhi (1973) reported that the young cultures more than 3 days old never gave good sporulation even any inducible sporulation methods. The most suitable result was obtained from the combination with 6 day dark at 23°C - 12 h light at 26°C - at least 12 h dark at 18°C conditions from V8, V88 and T media. **A solani** completed its vegetatif growth in this combination for 6 days in 23°C. Many researchers (Klaus, 1940; Neergaard, 1945; Kasarani, 1957;

Aragaki, 1961, 1964; Rath and Padhi, 1973) claimed that the temperature between 20-26°C helped to increase the sporulation of **A. solani.** The light induce to produce conidiophore in this combination (Waggoner and Horsfall., 1969). Lukens (1963) reported that the light inhibited spore production in many conidiophores which were stimulated with light and it was necessary to incubate plates approximately 10 h dark in order to with light and it was necessary to incubate plates approximately 10 h dark in order to 50 %. Therefore, the 12 h dark period at 18°C in 4th combination of our experiment may be one of the reasons for the abundant sporulation.

We can conclude that the most suitable method for inducing sporulation of **A. solani**, incubating V8, V88 or T media inoculated with **A. solani** isolate under dark-light-dark period. V8 juice can be commercially available in European country but not in Turkey, the preparation of V88 media could be difficult at all times due to the difficulties supplying 8 different vegetables. The best and simple medium is T medium containing only tomato juice.

ÖZET

DOMATESLERDE ERKEN YANIKLIK ETMENİ (**Alternaria solani**)'NİN SPORULASYONU ÜZERİNE ARAŞTIRMALAR

Alternaria solani (Ell. and Mart.) Jones and Grout'nin ve bazı **Alternaria** türlerinin kültür koşullarında bilinen yöntemlerle sporulasyonu oldukça zordur. **A. solani**'nin in-vitro'da sporulasyonunu teşvik etmek amacıyla birçok metod ve ışık-sıcaklık kombinasyonunun kapsayan 4 farklı kombinasyon 9 ayrı besiyerinde denenmiştir. Sonuç olarak **A. solani**, domates suyu içeren T besiyerinde, 23°C'de 6 gün karanlık - 26°C'de 12 saat aydınlık - 18°C'de 12 saat karanlık ile kombinasyonunda en iyi sporulasyon vermiştir.

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Sensitivity in **Alternaria solani** Isolates to Flusilazole

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ABSTRACT

Alternaria solani *is a very important pathogen of tomato. While the pathogen could be controlled successfully by multiside fungicides and iprodine previously, in present sensitivity of the pathogen to these fungicides decreased. For this reason, growers have become using flusilazole from triazole group intensively.*

Laboratory tests showed that sensitivity of ***A. solani*** *isolates to flusilazole have become reducing in time. Moreover, flusilazole was found to be less effective to the isolates with reduced sensitivities than sensitive ones during pot conditions. However, it was observed that the flusilazole has still had its high efficacy to* ***A. solani*** *during field trials conducted in the same area for two years one after another. Despite this high effectiveness, the results obtained from laboratory and pot conditions revail some concern for the future of this group fungicides in Türkiye.*

INTRODUCTION

Alternaria solani is one of the very important pathogens of tomato, grown under the greenhouse and field conditions in Türkiye. Because of high humidity in the greenhouse or under humid conditions in the fields pathogen can cause economical losses. For this reason, amongst control programs of the pathogen, chemical method has a very significant place (Benlioğlu and Delen, 1991; Delen and Özbek, 1994).

In the past, the pathogen could be controlled by some multiside inhibitors and iprodione. But now, sensitivity of the pathogen to these chemicals decreased as summarized in Table 1 (Benlioğlu, 1991; Delen and Özbek, 1992).

As a result of situation in Table 1, it shows that chemical control of pathogen in tomato may become more difficult day by day (Benlioğlu, 1991; Delen and Özbek, 1992). Therefore, although the chemicals from DMI's groups have not been suggested offically (Yücer, 1993), growers tend to use especially flusilazole from triazoles intensively in greenhouses according to our observations in Muğla-Fethiye and Antalya for controlling **A. solani.**

Table 1. Sensitivity of **A. solani** isolates to some fungicides in 1986 and 1992.

Year	Fungicide	No. of the tested isolates	Sensivity of the isolates as to their ED_{50} values (µg/ml) (%)					Reference
			< 3	3-10	10-30	30-100	> 100	
1986	Mancozeb	33	18.18	36.36	21.21	21.21	3.03	Benlioğlu, 1991
	Chlorothalonil	33	33.33	6.06	0.00	3.03	57.57	
	Dichlofluanid	25	52.00	32.00	16.00	0.00	0.00	
	Iprodine	32	81.25	15.62	3.12	0.00	0.00	
1992	Mancozeb	20	0.00	0.00	50.00	45.00	5.00	Delen and Özbek, 1992
	Chlorothalonil	20	0.00	0.00	0.00	15.30	85.00	
	Dichlofluanid	20	5.00	0.00	70.00	20.00	5.00	
	Iprodine	20	55.00	0.00	0.00	0.00	45.00	

As it has been known, different groups as piperazines, pyridines, pyrimidines, imidazoles, and triazoles are DMI's. Expect for morpholines, the others are the inhibitor of demethylation at position 14 of lanosterol or 24-methylene dihydrolanosterol (Kaypteyn, 1993; Scheinpflug, 1988). Although these group of fungicides have low or very low resistance risks (Dekker, 1982; Fuchs and de Waard, 1982) after intensive uses some pathogenic isolates can become resistant to DMI's (Kaypteyn, 1993; Ohtsuka et al., 1988; Scheinpflug, 1988; Sholberg and Haag, 1993). Acoording to these data, our main objective is to obtain the first information on the resistance risks of DMI's in Türkiye. For this reason sensitivity in **A. solani** isolates especially to flusilazole was studied in this paper.

MATERIALS and METHODS

In this study, flusilazole (Punch 40 EC, Du Pont) was the mainly used fungicides. Imazalil (Magnet 80 %, Maktheshim, Agan), myclobutanil (Systhane 12E, AgrEvo), and tebuconazole (Folicur 25 %, Bayer) were also used for testing cross-resistance relations between the flusilazole and the others DMI's.

A. solani isolates were collected from tomato plants grown in the greenhouses in Antalya-Kumluca and in Muğla-Fethiye or grown on the fields in Bursa Mustafakemalpaşa and Karacabey in 1991, 1992 and 1993.

The sensitivity tests of the isolates to the fungicides were conducted on fungicide amended PDA medium (Benlioğlu, 1991; Benlioğlu and Delen, 1991). For obtaining the relation between the sensitivity and virulence of the isolates, two tests were done under the pot conditions. In the first test, two less sensitive and one sensitive isolates to flusilazole were used. No fungicide was applied to the tomato plants (cv. Rio Grande) grown

in the pots. In the second test, flusilazole applied and nonapplied (control) tomato plants (cv. Rio Grande) were inoculated by six isolates individually. After the first inoculation, every isolate was inoculated to the tomato plants, containing increasing concentration of flusilazole. In the first application, flusilazole was applied at sublethal concentration as suggested by SCHEINPFLUG (1988) and then concentrations were increased slowly. Every application was applied seven days intervals. After the first and the last (fourth) inoculations, virulence of the isolates on the controls and on the applied plants were estimated (Benlioğlu, 1991; Benlioğlu and Delen, 1991). Disease severities were evaluated according to the 0 (no infection) - 5 (100 % infected) grading system seven days after inoculation (Horsfall and Barratt, 1945; Kirpal and Grover, 1969). For inoculation of the tomato plants, cultur (mycelium) suspension of the pathogen were sprayed by the hand pulverizator. Half petri dishes of cultur were used as the inoculum for each pot have four plants. After inoculation pots were covered by the plastic bags.

For obtaining the effectiveness of flusilazole and imazalil to a sensitive and a less sensitive isolates, a test were done under the pot conditions (cv. Rio Grande). In additions, flusilazole applications were also done in the field to find out the effectiveness of the chemical in practice. These studies were also done and estimated according to the methods summarized above. In the field studies applications were done four times in 10 day-intervals. Ten days after the last application, calculations were done according to the 0-5 scale.

All the tests were done according to the randomized plot design with three replications.

RESULTS

The sensitivity of the collected **A. solani** isolates to flusilazole was given in Table 2.

Tablo 2. Sensitivity **A. solani** isolates to flusilazole

Year	No. of the tested isolates	Distribution of ED_{50} values (µg/ml) in isolated population (%)						
		< 0.1	0.1-0.3	0.3-1	1-3	3 - 10	10 - 30	> 30
1991	10	30.00	50.00	10.00	10.00	0.00	0.00	0.00
1992	10	30.00	10.00	40.00	10.00	0.00	0.00	0.00
1993	13	38.46	30.76	7.96	0.00	0.00	15.38	7.96

The data in Table 2 shows that especially less sensitive isolates were emerged in 1993 although most of the isolates were very sensitive to flusilazole.

For obtaining the relation between the sensitivity and virulence of the isolates, no fungicide applied tomato plants were inoculated by two less sensitive and one sensitive isolates to flusilazole (Table 3).

Table 3. Virulences of flusilazole sensitive and less sensitive isolates to flusilazole to **A. solani** on tomato.

Isolates	ED_{50} Values (µg/ml)	Disease severity (%)	
		1 week after inoculation	2 week after inoculation
S-92-5/1K	0.70	27.60	56.25
T-93/6 K	23.50	81.25	93.75
T-5/F	> 30.00	38.44	62.50

According to the data in Table 3, less sensitive isolates (T.93/6 K and T5/F) to flusilazole were found as virulent as the sensitive (S.92-5/1 K) one.

For showing the iportance of continuos flusilazole applications to **A. solani** isolates, flusilazole applied tomato plants with increasing concentrations and non applied plants were inoculated by six isolates of the pathogen continuously. Disease severity and flusilazole sensitivity of the isolates before and after flusilazole applications were given in Table 4.

Table 4. Disease severity and flusilazole sensitivity of 6 **A. solani** isoletes on the tomato plants before (original isolates) and after the increasing concentrations of 4 flusilazole applications.

Isolate	Orginal isolates's		After 4 application		
	Highest concentration can infect (µg/ml)	Disease severity on the control plants (%)	Highest concentration can infect (µg/ml)	Disease severity on the highest concentration (%)	Disease severity on control plants (%)
T-92/6-K	50.00	20.00	60.00	12.50	18.75
T-92/3K	87.50	40.00	20.00	12.50	12.50
S-92-3/51	37.50	20.00	40.00	12.50	18.75
T-93/5-K	75.00	40.00	80.00	31.25	87.50
T-93/6-K	87.50	80.00	100.00	37.50	100.00
T-93/2-K	37.50	40.00	80.00	62.50	87.50

According to the results in Table 4, less sensitivity of five isolates increased after the applications, and moreover, virulence of the last three isolates did not decrease.

Cross-resistance between the flusilazole and other 3 DMI's were also studied. For this reason, 4 isolates were tested. ED_{50} values of these isolates were given in Table 5.

Table 5. Sensitivity of 4 selected **A. solani** isolates to flusilazole and other 3 DMI's.

Fungicides	Isolates and ED_{50} values (µg/ml)			
	T-5/F	T-93/14-K	T-92-5/1-K	T-93/6-K
Flusilazole	> 30.00	10.50	0.70	23.50
Myclobutanil	> 30.00	16.00	4.40	11.00
Imazalil	13.00	0.86	3.00	1.50
Tebuconazole	2.50	3.00	4.50	2.60

The data in Table 5 indicate that there are close relation between the ED_{50} values of the isolates for flusilazole and myclobutanil.

For obtaining the effectieveness of flusilazole and imazalil to sensitive and less sensitive isolates, a test were done under the pot conditions (Table 6).

Table 6. Effectivenes of flusilazole and imazalil to sensitive and less sensitive **A. solani** isolates.

Isolate and ED_{50} value (µg/ml)		Fungicide and concentration (µg/ml)		Disease severity (%)	Effectiveness (%)
S-91-2/1-I	1.0	Flusilazole	0.0	70.83	-
			6.0	8.33	88.23
			12.0	6.25	91.17
			24.0	0.00	100.0
T-5/F	> 30.0	Flusilazole	0.0	38.54	-
			6.0	15.62	59.47
			12.0	12.50	67.56
			24.0	10.93	71.63
S-91-2/1-I	1.0	Imazalil	0.0	70.83	-
			74.9	4.16	94.12
			149.8	2.08	97.06
T-5/F	13.0	Imazalil	0.0	38.54	-
			74.9	12.50	67.56
			149.8	14.06	63.51

According to Table 6, it can be said that flusilazole and imazalil could not control the less sensitive isolates as effective as the sensitive ones.

In addition of these studies, flusilazole applications were done under the field conditions at the same arrea in 1992 and 1994 to find out the effectiveness of the chemical in practice.

Table 7. Effectiveness of flusilazole to **A. solani** under the field conditions in 1992 and 1994.

Flusilazole doses (µg/ml)	Diseases severity (%)	Effectiveness (%)	Yer
0.0	33.54	-	1992
20.0	11.60	65.41	
30.0	8.11	75.81	
40.0	4.27	87.26	
0.0	37.93	-	1994
20.0	5.93	84.36	
30.0	4.73	87.52	
40.0	4.40	88.39	

The values in Table 7 shows that under the field conditions flusilazole controlled the pathogen effectively in both years.

DISCUSSION

First member of DMI's (triadimefon) was registered in Türkiye in 1980. After triadimefon, the other members from this group have been registered. Flusilazole was registered in 1988 (Yücer, 1993). According to our observations the most intensive usage of the fungicides from this group started after 1990. For this reason, in 1992 and especially in 1993 less sensitive **A. solani** isolates to flusilazole started to be obained from the tomato growing areas. Although this group fungicides have a low resistance risk (Dekker, 1982; Delen and Özbek, 1994; Delen and Özbek, 1992; Guan, 1992; Scheinpflug, 1988), development of resistance in fungi to DMI's in the field has been reported for a long time ago (Kaypteyn, 1993; Köller, 1988; Ohtsuka, et al., 1988; Sholberg and Haag, 1993) moreover, increased resistance to DMI's is linked to decreased fitness (Guan, 1992; Kayptyn, 1993). Howewer, in this study it was found that virulence of some less sensitive isolates to flusilazole was not affected by the decreaed sensitivity, and less sensitive isolates could not be controlled as effective as the sensitive isolates under the pot conditions. On the other hand, as reported by PEEVER and MILGROOM (1994), fitness are not generaly associated with resistance to DMI's in field collected

isolates and management strategies to EBI- resistance cannot depend upon the existence of fitness. Parallel to the statements of KÖLLER (1988), some relations weree also obtained between the ED50 values of tested **A. solani** isolates for flusilazole and the other chemicals from the groups DMI's. Although the high effectiveness of flusilazole to the pathogen under the field conditions, in the light of the results from the laboratory and pot studies may igve us some serious signals for the future of DMI's in Türkiye.

ÖZET

Alternaria solani İZOLATLARININ FLUSİLAZOLE'E DUYARLILIKLARI

Alternaria solani domateslerin önemli hastalıklarından biridir. Özellikle seralarda büyük zararlara neden olabilmektedir. Önceleri, mancozeb, chlorothalonil, dichlofluanid gibi çok yer engelleyici (klasik) fungusidlerin yanısıra tek yer engelleyici fungusidler olan iprodione kullanılarak başarıyla önlenebilen patojen, şimdi bu fungisidlere daha az duyarlı hale gelmeye başlamıştır. Bu durumun bir sonucu olarak domates seralarında triazole grubundan flusilazole yoğun olarak uygulanmaktadır.

1991, 1992 ve 1993 yıllarında toplanan **A. solani** izolatları ile yapılan laboratuvar çalışmalarında yıldan yıla izolatların flusilazole'e duyarlılıklarının azalmakta olduğu ve saksı denemelerine göre ise duyarlılığı azalmış izolatların duyarlılardan virulent olabildikleri ortaya çıkmıştır. Yine saksı denemeleri sonucu sürekli flusilazole uygulamalarının izolatların flusilazole'e duyarlılığını azaltıcı etki gösterebildiği saptanmıştır.

İki yıl üst üste aynı yörede yapılan tarla denemeleri ile ise flusilazole'ün **A. solani**'ye yüksek etkinliğini sürdürdüğü sonucuna varılmıştır. Ancak bu etkinliğe karşın laboratuvar ve saksı koşullarında elde edilen sonuçlar giderek kullanımı yoğunlaşan bu grup fungisidlerin Türkiye'deki geleceği konusunda bazı kuşkuları gündeme getirmektedir.

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Influence of the Gibberellic Acid (GA_3) Applications on the Powdery Mildew and Grey Mould on the Grape Variety Sultana in the Aegean Region

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ABSTRACT

*The effects of GA_3 applications on grey mould and powdery mildew have been studied in 1990 and 1992. The applications have been conducted on **Vitis vinifera** L. spp. **sativa** var. Sultana, at the dosages 10 ppm when the inflorescense 2-4 cm. long, 15 ppm when the blossoming 60-80 %, end 20 ppm when the berries 3-5 mm diameter.*

GA_3 applications have been found to be effective on disease severity reduction of grey mould while uneffective on powdery mildew.

INTRODUCTION

Plant growth regulators are used along with the other cultural applications such as tillage, irrigation, prunning, fertilization and plant protection.

The most widely applied plant growth regulator is gibberellic acid on the vineyards where the Sultana variety has been prevailing which have been known for its role in shoot growth and berry enlargement by inducing hyperplasia and cell elongation (Kısmalı, 1974).

The proper application times has been determined as at 60-80 % blossoming at 15 ppm rate, when the berries 4-5 mm diameter at 30 ppm for table grapes and as 15 ppm at 60-80 % blossoming for table grapes and raisins and 30 ppm when the berries 4-5 mm diameter for only table grapes at which GA_3 residues have been under the tolerance levels (Gökçay et al., 1990 and 1992).

It has been reported that GA_3 applications on Sultana grapes 5-10 ppm rate at the end of flowering have caused 35-90 % increase in berry size and 35-100 % increase on fresh weight, and 5 ppm application have enhanced raisin quality (Onaran, 1964).

Consequently GA_3 has become the most widely applied PGR in vineyards and it has been necessary to study its effect on powdery mildew and grey mould. The experiments were conducted in 1990-1992 in Bornova (İzmir) and Alaşehir, Sarıgöl (Manisa).

MATERIALS and METHODS

In 1990, the experiment was laid out according to the randomized block design with 3 characters (GA_3 application on table grapes, on raisins and control) and 6 replications, in the Institute vineyard in Bornova on Sultana variety. In 1991 the experiment was carried out according to the same design with 4 characters (two different GA_3 application on table grapes, one applications on raisins, and control) and 5 replications in the same vineyard (Table 1). These tests were conducted to determine the effect of GA_3 on **U. necator**.

Table 1. GA_3 concentrations and application times used in 1990 and 1991 to determine its effect on ***Uncinula necator.***

Year	Chemical company and trade name	Active ingredient (%) and formulation	Application time	Concentration (ppm)
1990	Gibbex ICI, Turkey	"GA_3" 1,66 % Em.	**On table grapes**	
			1) at 60-80 % flowering 21.5.1990	15
			2) Berries 3-5 mm. Ø 29.5.1990	20
			On raisins Berries 3-5 mm. Ø 29.5.1990	15
1991	Gibbex ICI, Turkey	"GA_3" 1,66 % Em.	**On table grapes**	
			1) When inflorescence 2-4 cm length 12.4.1991	10
			2) at 60-80 % flowering 29.5.1991	15
			3) Berries 3-5 mm Ø 11.6.1991	20
			On table grapes	
			1) At 60-80 % flowering 29.5.1991	15
			2) Berries 3-5 mm Ø 11.6.1991	20
			On raisins At 60-80 % flowering 29.5.1991	15

In 1992, the experiment was carried on according to the randomized block design with 4 characters and 5 replications to find out the effects of GA_3 application on **B. cinerea** infection on the grapes in two locations, Alaşehir and Sarıgöl, both in Manisa region (Table 2). No chemical was used against grey mould and **Lobesia botrana** Den. et. Schiff.

Table 2. The concentration and application time of GA_3 in 1992 in Alaşehir and Sarıgöl

Chemical company and trade name	Active ingredient (%) and formulation	Application time	Concentration (ppm)
Gibbex	"GA_3" 1.66 %	**Table grapes**	
ICI Turkey	Em.	1) When inflorescence	
		2-4 cm length	10
		20.4.1992	
		2) At 60-80 %	
		flowering	15
		4.6.1992	
		3) Berries 3-5 mm Ø	
		22.6.1992	20
		Table grapes	
		1) At 60-80 %	
		flowering	15
		4.6.1992	
		2) Berries 3-5 mm Ø	20
		22.6.1992	
		Raisins	
		Berries 3-5 mm Ø	20
		22.6.1992	

In all treatments GA_3 was applied by knapsack pulverizator and a surfactant was added to the solution in 0.02 %.

A plot was composed of 6 vine and 4 of them were subjected to counts.To evalute powdery mildew infection totally 20 bunches of which 5 was selected from each vine vere counted as diseased and healthy berries and disease ratio was determined. Counts were made on 21.7.1990 and 30.7.1991 in Bornova.

Grey mould evaluation was made on totally 20 bunches as described above according to the 0-4 scale when the disease had appeared in the control plots on 1.9. 1992 in Alaşehir and on 15.9.1992 in Sarıgöl. The data were subjected to Towsend-

Heuberger formula and the disease ratio was determined. All data were subjected to analysis of variance.

0-4 scale

0 No infection	:	No symptom on berries
1 Weak infection	:	At most 5 diseased berries on cluster
2 Medium infection	:	1/5 of the cluster with disease symptoms
3 Considerable infection	:	2/5 of the cluster with disease symptoms
4 Heavy infection	:	3/5 of the cluster with disease symptoms

RESULTS and DISCUSSION

Disease ratios of powdery mildew (Table 3) and grey mould (Table 4) are given below.

Table 3. Powdery mildew ratio (%) on the bunches applied GA_3 in Bornova in 1990 and 1991.

		Replications						
Year	Characters	I	I	III	IV	V	VI	Average
1990	Table grapes	61.5	54.5	29.2	59.0	65.0	55.5	54.1
	Raisins	33.5	66.0	49.5	50.5	51.5	61.5	52.0
	Control	67.5	77.5	69.5	60.0	69.0	63.3	67.8
1991	Table grapes (3 applications)	75.0	74.5	80.0	80.5	78.5	-	77.7
	Table grapes (2 applications)	77.0	76.0	80.5	79.5	79.5	-	78.5
	Raisins	75.5	76.5	75.5	79.0	80.5	-	77.4
	Control	76.5	78.5	79.0	80.0	79.0	-	78.7

Disease rates were almost the same as in the control plots in both years showing 50-60 % in 1990 and 70 % in 1991. There was no statistically difference between the characters; meaning no effects of GA_3 applications by reducing or inducing powdery mildew infection. There is little information about the effects of PGR's applications on the pathogen, especially no studies have been done on **U. necator** yet.

Disease ratios were 26.75 % on the table grapes with three GA_3 applications, 45.50 % on the table grapes with two applications and 31,50 % on the raisins while 67,75 % in controls in Alaşehir. In Sarıgöl disease ratios were 17,5 %, 35,25 %, 23,05 % and 44,75 %, respectively. There was a significant difference between the characters. GA_3 applications resulted in reducing grey mould infection on both table grapes and raisins.

Tablo 4. Grey mould ratio (%) on the bunches applied GA_3 in Alaşehir and Sarıgöl in 1992.

Districts	Characters	Replications					
		I	II	III	IV	V	Average
Alaşehir	Table grapes (3 appl.)	27.5	25	21.25	28.75	31.25	26.75
	Table grapes (2 appl.)	40	46.25	46.25	51.28	43.75	45.50
	Raisins	30	23.75	36.25	40	27.5	31.50
	Control	73.75	72.5	70	58.75	63.75	67.75
Sarıgöl	Table grapes (3 appl.)	16.25	17.5	20.0	20.0	13.75	17.5
	Table grapes (2 appl.)	42.5	32.5	27.5	40.0	33.75	35.25
	Raisins	21.25	26.25	25.0	20.0	22.5	23.05
	Control	53.75	46.25	42.5	37.5	43.75	44.75

It was reported that preflowering application of 15 ppm GA_3 caused bunch elongation and reduced bunch compaction and consequently decreased Botrytis infection in Italy. (Ronisondo et al., 1972). It was also stated that pre-flowering and during flowering application of GA_3 at 1-5 ppm rates in California resulted in significant Botrytis reduction (Weaver et al., 1962). Similar results were obtained from some researches conducted in New Zealand (Hopping, 1976).

It was concluded that reduced Botryis infection was due to changes occured on the bunches applied with GA_3, not because of any fungitoxic influence.

It has been known that pre-flowering GA_3 application when the inflorescens 2-4 cm length stimulates the axis elongation; application at 60-80 % flowering causes berry dropping resulting in reduced bunch compaction; application after the berry formation enhances berry enlargement (İlter, 1984). Consequently, bunch aeration improves and unfavorable conditions for disease development exist.

It has been declared that bunch shape and structure influence the Botrytis infection and disease development and microclima in the bunch. It has also been emphasized that in the compact bunches the berries touch each other giving rise to lowering cuticular secretions makes them more susceptible to **B. cinerea** infection (Vail and Marois, 1991).

It has been found that single pre-flowering GA_3 application when the inflorescens 2-5 cm length at 10 ppm rate excites bunch length creating good aeration and consequently grey mould infection rate significantly reduces (Rivera and Mavrich, 1978).

In conclusion, it has been shown that pre-flowering and during flowering GA_3 applications create unfavorable conditions on the bunches for **B. cinerea** infections.

ÖZET

EGE BÖLGESİ'NDE ÇEKİRDEKSİZ ÜZÜMLERDE GİBBERELLİK ASİT (GA_3) UYGULAMALARININ BAĞ KÜLLEMESİ VE KURŞUNİ KÜF HASTALIĞINA ETKİSİ ÜZERİNE ARAŞTIRMALAR

Bağlarda kullanılan gibberellik asit (GA_3)'in Külleme (**Uncinula necator** (Schw) Burr.) ve Kurşuni Küf (**Botrytis cinerea** Pers.) hastalıklarına etkisi 1990-1992 yıllarında araştırılmıştır.

Denemeler çekirdeksiz üzüm çeşidinin (**Vitis vinifera** L. spp. **sativa** var. **Sultana**) hakim olduğu bağlarda açılmıştır.

GA_3 sofralık ve kurutmalık olmak üzere iki amaçlı kullanılmıştır. Bu amaçlar doğrultusunda, salkımlar 2-4 cm iken 10 ppm; çiçekler % 60-80 açtığında 15 ppm; taneler 3-5 mm çapında olunca 20 ppm uygulanmıştır.

GA_3 uygulamaları, Bağ Küllemesi'nde hastalık şiddetini azaltıcı ve arttırıcı yönde etkili olmazken, Kurşuni Küf'de kontrole göre hastalık şiddetinin azalmasına yol açmıştır.

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A New Bacterial Disease of Watermelon in Türkiye: Bacterial Fruit Blotch of Watermelon (**Acidovorax avenae** subsp. **citrulli** (Schaad et al.) Willems et al.))

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ABSTRACT

*A bacterial disease significantly reduced production of watermelon (**Citrullus lanatus**) in commercial watermelon fields of Sultaniçe village (Enez-Edirne) in the summer of 1995. The disease was characterized by necrosis on the true leaves, extensive stem necrosis and fruit blotch.*

*A nonfluorescent, gram (-), oxidase (+) bacterium was isolated from the margins of fruit, leaf, petiole; branch and stem lesions. Our strain induced hypersensitivity in tobacco (cv. White Burley). Pathogenicity were completed on both watermelon fruit and cotyledons by stabinoculation and injection using a 10^8 cfu/ml suspension. Natural seed-borne inoculum of bacterium caused symptoms on seedlings of 44 out of 260 seeds removed from infected watermelon fruit. Morphological, physiological and biochemical properties of isolated bacterium were similar to reported properties of **Acidovorax avenae** subsp **citrulli** and was identified as **A. avenae** subsp. **citrulli.** This is the first report of bacterial fruit blotch in Türkiye.*

INTRODUCTION

In the crop season of 1995 some watermelon cultivars grown in several fields of Sultaniçe village (Enez-Edirne) were infected with a severe fruit blotch. The disease was characterized by necrosis on the true leaves, extensive stem necrosis and blighting of young shoots. Just before harvest, small dark green blotches appeared on the portion of the fruit. These blotches expanded rapidly to cover the entire upper surface within a week. Young unmaturated fruits were blighted. As fruit maturated the rind became brown and cracked, followed by decay (Fig 1).

Webb and Goth (1965) reported a nonfluorescent, pathogenic bacterium associated with seed of two watermelon (**Citrullus lanatus**) plant introductions, and later demonstrated the seed transmissibility of the bacterium in watermelon fruits inoculated in the field (Goth and Webb, 1975). Schaad et al. (1978) named the pathogen **Pseudomonas pseudoalcaligenes** subsp. **citrulli** subsp. nov. but the bacterium subsequently placed in the genus **Acidovorax** by Willems et al. (1992) and named as **P. avenae**

subsp. **citrulli.** Sovell and Schaad (1979) reported natural seedborne inoculum of **P.p.** subsp. **citrulli** caused watermelon tested. Latin and Rane (1990) reported more than 500 ha of watermelons in south western Indiana were affected by bacterial fruit blotch with nearly 100 ha sustaining losses approaching 90 %. Apart from these reports, several researches were done about resistance of commercial watermelon cultivars against bacterial fruit blotch (Sowell and Schaad, 1979; Goth and Webb, 1981; Hopkins et al., 1993; Rhodes et al., 1993).

The purposes of this investigation was to determine the causal agent of fruit blotch disease of watermelon.

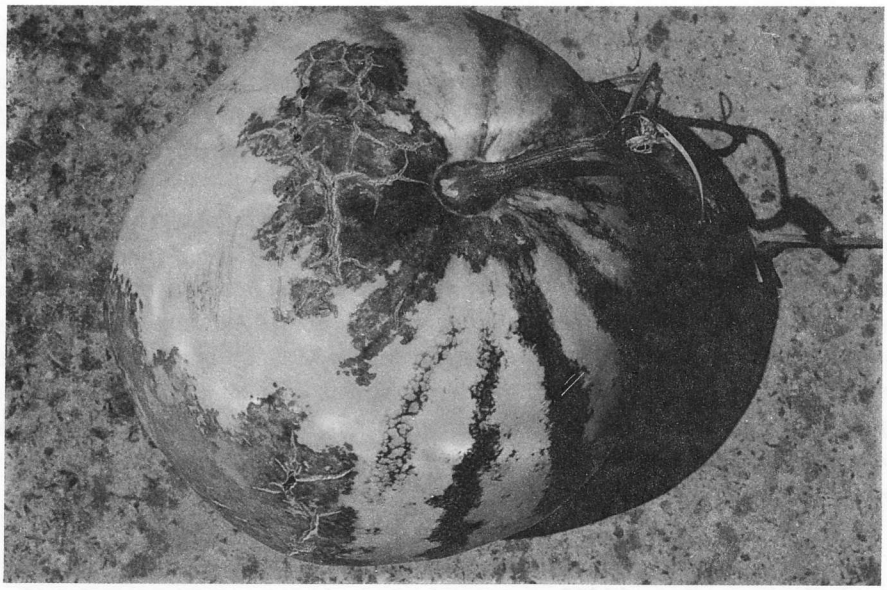

Figure 1. Symptoms of natural infected watermelon fruit.

MATERIALS and METHODS

Isolation of bacterium

Diseased watermelon plants and fruits (fig 1) were supplemented from the fields of cultivated watermelon in Sultanişe by Agricultural Directorate of Edirne province. Small pieces of infected leaves, petioles, stems and fruit rind were surface sterilized with 70 % ethanol and washed in sterile water. These infected parts macerated in 5 ml. sterile water and suspensions were streaked on plates of King's medium B (KB) (King et al., 1954) and Sucrose Nutrient Agar (SNA). Plates were incubated at 27°C for 72 hr. Isolates were maintained on Nutrient Agar (NA) (Difco) at 4°C.

Seed detection

Twenty watermelon seeds from infected fruit were washed for 30 min in 100 ml of a sterile saline solution (NaCl 0.85 %). Dulition were made an 0.1 ml. of the mixture was asseyed on KB and SNA. Colonies were counted after 2-3 days incubation at 27°C. Suspected colonies were transferred on NA slants to confirm identification.

Seed transmission

Two hundred-sixty seeds removed from natural infected watermelon fruit were planted in a perlite growing medium. We recorded seedling emergence and percent infection 11-13 days after planting.

Pathogenicity tests

Isolates were grown for three days on KB at 27°C. Cells were suspended in sterile distilled water and the suspensions adjusted spectrophotometrically to contain approximately 10^8 cfu/ml. Pathogenicity of the single colony isolates were tested on one-week-old seedlings of watermelon and mature fruits. Bacterial suspensions were applied to the cotyledons with a hair brush. Inoculated plants were incubated in moist chamber for 48 hr at 25±2°C. The plants were examined for the typical dark green water-soaked lesions about 1 week later. The pathogenicity of representative isolates were comfirmed by injecting 0.2 ml of a suspension of 10^8 cfu/ml into the mature, harvested fruits. Sterile water was applied as a negative control.

Characterization of the bacteria

Colony characteristics were determined after 3 days incubation at 27°C on SNA and KB medium. Gram-stain was determined using 48-hr NA cultures.

Growth at 4 and 41°C was determined after 72 hr in nutrient broth (NB, Difco) in a water-bath. For oxidase activity, the Kovacs (1956) method's was applied. 3-keto gluconate production, gelatin hdyrolysis, nitrate reduction, catalase, anaerobic break-down of L (+) arginine dehydrolysis were tested according to Lelliott and Stead (1987) and Klement et al. (1990) Hypersensitivity reaction was determined in tobacco leaves (cv. White Burley) with a suspension of 10^8 cell ml^{-1} in sterile water from 48-h NA culture (Klement, 1963).

Growth on carbon sources was recorded on the minimal medium (MM) of Ayers et al (1919). Sugars and other substances likely to be decomposed by autoclaving were filter sterilized (0,2 µm Millipore) and included in MM (1 % w/v) In addition alkaline from L (+) tartrate and propionate were tested on the Hayward's medium (Hayward, 1964.

RESULTS and DISCUSSION

Isolation and characterization of the pathogen

Non fluorescent bacteria that produced rounded, slightly irregular, cream-yellow colonies were grown on KB medium for 72 hr were consistently isolated from lesions of leaf, branch, stem and fruit.

Our strains were gram-negative and induced hypersentivity in tabocco within 18 hr. Colonies were non-fluorescent on KB and grew at 41°C but not at 4°C. Oxidase, catalase and 3 ketogluconate reaction were positive. Isolates utilized ethanol D-fructose, sucrose, glucose, propionate, DL-tartrate but D-mannitol, D-sorbitol, meso-erythritol, D-mannose, maltose, dulcitol are not used. Gelatin hydrolsed weakly, produced nitrate reductase, but not arginine dihydrolase.

The results of physiological and biochemical comparison tests were mostly similar to the reported characteristics of **A. avenae** subsp. **citrulli** (IMI Descriptions of Fungi and Bacteria No. 1213) with the exception of growing on sucrose, the characteristics of the bacterium were consistent with those desribed for **A. avenae** subsp. **citrulli** (Schaad et al., 1978). Williems et al. 1992. Our strains grew on sucrose whereas the type strain of **A.a** subsp. **citrulli** was reported did not grow on sucrose. In the original description (Schaad et al. 1978) nitrate is not reduced and there is no hypersensitive response on tobacco, which is odds with the recent work by Hu et al. (1991) in which same strain, the type ATCC 29625, reduced nitrate and was tobacco HR positive. Latin and Rane (1990) and Somodi et al. (1991) also reported that some strains were tobacco HR positive and our results were confirmed. In the original description glucose are not use as a carbon source in contrast to work by Willems et al. (1992) in which the same strain produced growth on this substrate.

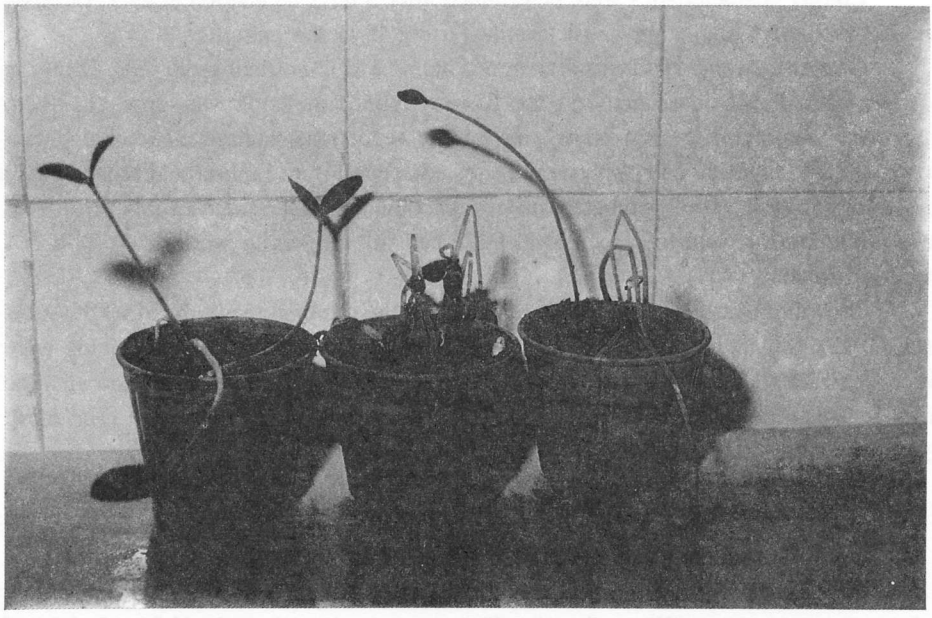

Figure 2. Pathogenicity tests on watermelon seedlings (left: negative control)

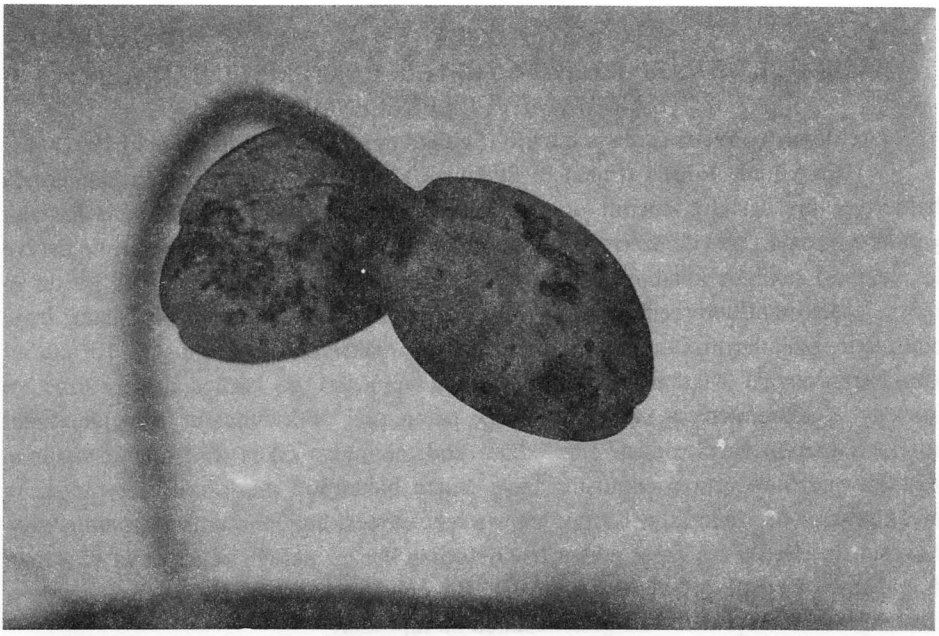

Figure 3. Water-soaked lesions on cotyledons in pathogenicity tests

Pathogenicity

In pathogenicity tests of the bacteria isolated from infected leaf, stem, petiole, branch and fruit were pathogenic on watermelon seedlings. Most of the inoculated seedlings died three days after inoculation (Fig. 2). Small water-soaked lesions appeared on cotyledons of watermelon seedlings five days after inoculation (Fig. 3). Inoculated fruits showed discoloured areas below all of 10 points at which the pathogen was infected. No symptoms was observed at the 10 points where sterile water was injected. At the end of the inoculation pulp of fruits were destroyed. The bacterium was also isolated from infested seeds. Natural seed-borne inoculum of bacterium caused symptoms on seedlings of 44 out of 260 seeds removed from infected watermelon fruit. Sowell and Schaad (1979) reported the bacterium produced a firm necrosis when injected into the interior of mature fruits and the seed from watermelon harvested from plants produced from infested seed produced infected seedlings.

Consequently, it was determined that disease of watermelon was caused by **A.a.** subsp. **citrulli.** Since the disease was transmitted by infested seed, it will be appropriate that pathogen free seed was introduced and included to the seed certification programme quarantine lists.

ÖZET

TÜRKİYE'DE KARPUZLARDA YENİ BİR BAKTERİYEL HASTALIK: KARPUZ MEYVE LEKELENMESİ

(Acidovorax avenae subsp. citrulli (Schaad et al. 1978) Willems et al 1992)

1995 yılında Edirne (Enez) ili Sultaniçe köyü ticari karpuz üretim alanlarında bakteriyel bir hastalık önemli oranda ürün azalışına neden olmuştur. Bu hastalık yaprak nekrozları, gövde üzerinde yaygın nekrozlar, genç sürgün yanıklıkları ve meyve lekelenmesi şeklinde kendini göstermiştir. İnfekteli yaprak, yaprak sapı, dal, gövde ve meyvelerden nonfluorescent, gram (-) ve oksidaz (+) özellik gösteren bir bakteri izole edilmiştir. İzolatlarımız tütünde hypersensitif bir reaksiyon oluşturmuştur. 10^8 cfu/ml konsantrasyondaki bakteri inokulumu karpuz meyveleri ve kotiledonlara sürme ve injeksiyon yöntemleriyle inokule edilerek patojenliği belirlenmiştir. Etmenle doğal enfekteli karpuz meyvesinden elde edilen 260 tohumdan çıkan 237 fidenin 44'ünde hastalık belirtileri ortaya çıkmıştır. İzole edilen bakterinin morfolojik, fizyolojik ve biyokimyasal özelliklerinin; Karpuz meyve lekelenmesi etmeni olarak tanılanmış olan **A.a.** subsp. **citrulli**'nin rapor edilen özellikleriyle benzer olduğu belirlenmiş ve etmen **A.a.** subsp. **citrulli** olarak tanılanmıştır. Bu çalışma, Karpuz Meyve Lekelenmesi Hastalığının Türkiye'deki varlığı konusunda ilk rapordur.

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Citrus Viroids Inducing Dwarfing on Meyer Lemon Grafted on Sour Orange in the East Mediterranean Region in Turkey

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ABSTRACT

*Viroids are the smallest known pathogen group and used as graft-transmissible dwarfing factor for controlling citrus tree size. In this study the viroids of dwarfed and normal sized Meyer lemon (**Citrus lemon** (L.) Burm. f.) grafted on sour orange (**C. aurantium** L.) were analyzed. CVd-lb, CVd-II, -III Group and CVd-IV viroids were identified to induce dwarfing as graft transmissible complex. No viroids were detected in the normal sized Meyer lemon.*

INTRODUCTION

One of the crucial issues in contemporary citriculture is to obtain a high yield. One possibility to improve yield is increasing of planting density by tree size control (use of dwarfed trees). The use of dwarfed trees at high densities not only increase the amount of production (per area) but also simplifies orchard practices (convenience of tree-care, plant-protection operations and harvesting) (BROADBENT et al., 1986; 1993). Various techniques have been employed to produce dwarfed citrus trees as the use of rootstocks with a dwarfing effect, angle planting, specific cultivation practices, growth inhibiting chemicals and citrus viroids (GOLOMB, 1988).

Naturally dwarfed citrus trees are often used to study the cause of this dwarfing and as source of graft-transmissible agents.

In this study, the dwarfing observed in Meyer lemon variety grafted on sour orange in the East Mediterranean region was examined in terms of citrus viroids and the possible utilization of these viroids as graft-transmissible dwarfing factor is discussed.

MATERIALS and METHODS

Field observations and plant material. This study was carried out on naturally dwarfed. Meyer lemon trees in the East Mediterranean region of Turkey. The dwarfed trees have had about 50 % smaller canopies that normal sized Meyer lemon of the same age.

For laboratory experiments two field sources, dwarfed and normal sized are selected. Both source trees were 4 years old and grafted on sour orange. Buds and tissues were collected from these trees and grafted on Etrog citron (**C. media** L.) 861-S-1 to amplify the titer of viroids.

Nucleic acid extraction and viroid purification. Viroid RNA's were extracted according to SEMANCIK et al., (1988) using 3 to 5 weeks old leaves. Preparations of 2M LiCl soluble nucleic acids were applied to CF-II cellulose columns. Viroid RNA's were detected and isolated by sequential polyacrylamide gel electrophoresis (sPAGE) (RIVERA-BUSTAMANTE et al., 1986) followed by staining with silver for greater sensitivity in viroid detection (IGLOI, 1983).

RESULTS and DISCUSSION

In the East Mediterranean region dwarfed and normal sized Meyer lemon trees were observed showing no differences in fruit quality. Both Meyer lemons originated from California, USA but were introduced by two different growers and propagated in Turkey.

The dwarfed Meyer lemon was associated with several citrus viroids while in contrast no citrus viroid was detected in the normal sized tree. Viroid analysis revealed four viroid groups: CVd-Ib, CVd-II, -III Group and CVd-IV (Fig. 1).

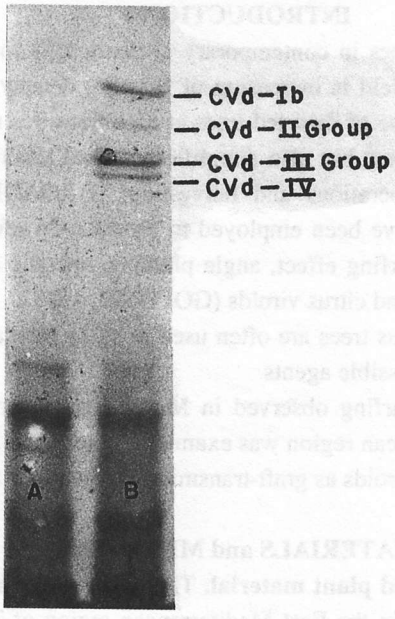

Figure 1. Polyacrylamide (% 5) gel containing 8M urea and stained with silver after processing by sequential PAGE. A) Normal sized Meyer B) Dwarfed Meyer lemon.

CVd-II and III groups are known to induce dwarfing in Washington navel (**C. sinensis** (L) Obs.) grafted on trifoliate orange (**Poncirus trifoliata** (L.) Raf.) and its hybirds (BROADBENT et al., 1993; ROISTACHER et al., 1991). Experiments in Australia showed that citrus viroids (CEVd (Citrus exocortis viroid), CVd-IIa, -IIc, -IIIa) induced dwarfing did not have any adverse effect on trees and fruit quality for over 35 years (BROADBENT et al., 1993).

BEN-SHAUL et al., (1995) reported that five different citrus sources used and graft-transmissible dwarfing complex in commercial orchard in Israel contained CEVd, CVd-Ib; additionally, some of these sources carried CVd-IIb and CVd-IIIb.

Thus all viroids known to induce dwarfing were detected in the Turkish Meyer lemon indicating that these viroids are the cause of dwarfing observed on sour orange.

Trifoliate and its hybrids are slow growing rootstocks and this effect is enhanced by several viroids. In opposite sour orange is a vigorous rootstock producing larger canopies. This rootstock is preferred in the Mediterranean region because

i) they produce vigorous and highly uniform seedlings easy to graft
ii) this rootstock develops productive trees with a long life span of 50 years and induce a good quality of fruits
iii) it is tolerant to **Phytophthora** and viroids (Exocortis)
iv) it is tolerant to high lime content in the soil and high salinity in the irrigation waters, these latter characteristics are highly appreciated by Mediterranean farmers.

For these reasons it would be advantageous to select viroids inducing dwarfing in scions grafted on sour orange. The viroids detected in Meyer lemon in East Mediterranean region are promising agents which could be further studied on different scion combination.

No CEVd was detected in any of the source trees although mild strains of CEVd are a common component in dwarfing factors in Israel (BEN-SHAUL et al., 1995). Since CEVd cause a severe disease on trifoliate and its hybrids, the use of CEVd is always risky. The Meyer lemon dwarfing factor, however could be used even on CEV-sensitive rootstocks as trifoliate.

ÖZET

DOĞU AKDENİZ BÖLGESİNDE TURUNÇ ÜZERİNE AŞILI MEYER LİMON AĞAÇLARINDA BODURLUK OLUŞTURAN TURUNÇGİL VİROİDLERİ

Bilinen en küçük patojen grubunu oluşturan viroidler, günümüzde ağaç büyüklüğünün kontrolu amacıyla, aşı ile taşınabilir bodurluk faktörü olarak kullanılmaktadır. Bu çalışmada Doğu Akdeniz bölgesinde yetiştirilen turunç anacına aşılı normal ve bodur Meyer limon ağaçları içerdiği viroidler açısından incelenmiştir. Aşı ile taşınabilen bu bodurluğa turunçgil viroidlerinden CVd-Ib, CVd-II, -III Group ve CVd-IV oluşan bir kompleksin neden olduğu belirlenmiştir. Normal Meyer limon ağacında ise hiçbir viroide rastlanmamıştır.

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Researches on the Detection of Virus Diseases of Sugar Beet in Ankara

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ABSTRACT

Diseased leaf samples were collected from sugar beet fields of Ankara region in 1992. During the surveys, 94 specimens with virus like symptoms were taken from 150 sugar beet fields located in 14 towns and 1 location of Ankara. They were mechanically inoculated to indicator test plants and diagnosed according to the symptoms produced, serological test results and electron microscopy. The specimens suspected as Rhizomania infection were applied to Indirect ELISA test against Beet necrotic yellow vein virus (BNYVV) IgG. According to the results of mechanical inoculation and serological test results, 41 specimens were identified as beet mosaic virus (BMV) and 5 specimens as beet yellows virus (BYV). Rhizomania was not existing in the research area. No results was obtained with the other specimens in the tests, so they were identified as physical disorders or non-mechanical insect transmitted virus infections of sugar beet.

INTRODUCTION

Sugar beet (**Beta vulgaris** subsp. **saccharifera**) is the only raw material of the sugar industry in Türkiye. It is also the most important industrial of Ankara region. According to data in 1991, the industrial plants sown total area of Ankara was 18436 ha and total production was 660743 tons where as sugar beet sown area was 15211 ha (82.5 % of total area) and total production was 659001 tons. Yield of sugar beet in Ankara is over the Türkiye's average. It was 38558 kg/ha as Türkiye's average and 43324 kg/ha as Ankara's average in 1991 (Anonymous, 1994).

The most important virus infections of sugar beet were Beet yellows virus (BYV), Beet mosaic virus (BMV), Beet yellow stunt virus (BYSV), Beet western yellows virus (BWYV), Lettuce infectious yellows virus (LIYV), Beet yellow vein virus (BYVV), Beet yellow net virus (BYNV), Rhizomania (Beet necrotic yellow vein virus (BNYVV), Cucumber mosaic virus (CMV), Beet leaf curl virus (BLCV) and Beet curly top virus (BCTV) (Smith, 1972; Whitney and Duffus, 1991). Among them, BYV, BWYV, LIYV, BYNV, BLCV, BCTV are all insect-transmitted virus infections, only BYV can hardly be mechanically transmitted to limited number of host plants. BNYVV is the only fungus-transmitted virus infection of sugar beet and transmitted by a

plasmodiophoraceous fungus **Polymyxa betae** (Keskin). Diagnostic features of BYV and BMV were summarized by Russel (1970 and 1971), BWYV by Duffus (1972) and Hoefert (1984), BNYVV by Tamada (1975) and Putz et al., (1990), CMV by Francki et al. (1979). They were all reviewed by Smith (1972) and Whitney and Duffus (1991).

First reports on the sugar beet virus diseases in Türkiye belongs to Tanrısever (1961). In his book named "Sugar beet diseases and pests in Türkiye", he mentioned that BMV and BYV were prevalent at different locations of Türkiye. He also reported that BCTV was also present and its vectors were identified as **Psyllopsis froxinicola** (Foerst) and **Macropsis cerae** (Germar).

Rhizomania (BNYVV) has been detected as the most destructive and wide spread virus infection of sugar beet cultivation recently (Erdiller and Özgür, 1994 b). There are several reports on the distribution and prevalence of the pathogen in Türkiye (Vardar and Erkan, 1992; Erdiller and Özgür, 1994 a).

This research was conducted in order to determine the virus infections of sugar beet in Ankara. This is the first report on this subject.

MATERIALS and METHODS

Survey area

The survey area of this research has been selected as 14 towns and 1 location of Ankara (Figure 1). Surveys were conducted to the mainly sugar beet cultivation areas and totally 150 sugar beet fields were investigated in 1992. Fields were examined

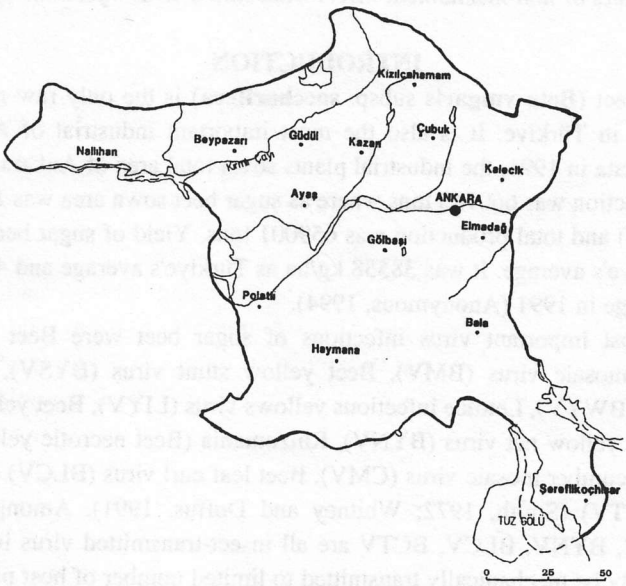

Figure 1. The map of survey area of the research

according to Barnet (1986)'s "W type" survey method. Virus like symptoms showing plants were collected as leaf and root samples and brought to the laboratory in poly-ethylene bags. Specimens were kept in deep-freezer at -25°C.

Detection of virus isolates:

Mechanical inoculation

The plants showing virus like symptoms were mechanically inoculated to the test plants: **Chenopodium quinoa** Wild., **C. amaranticolor** Costa et Reyn., **Tetragonia expansa** Murr., **Amaranthus caudatus, A. retroflexus., Claytonia perfoliata** Donn., **Beta vulgaris** c. v. Eva, c. v. Perla and c. v. Kawepura, **Nicotiana tabacum** "Maden" according to the method of Abe and Tamada (1986). Inoculated plants were kept in greenhouse at 20±5°C. **T. expansa** and **C. perfoliata** were kindly supplied by International Horticultural Research (United Kingdom), **Chenopodium** species by Ministere de Agriculture INRA-(France), **Beta vulgaris** species by Sugar Research Institute (Ankara).

Serological Tests

Ouchterlony agar-gel double diffusion test has been applied to the all of the specimens by using two different agar medium prepared according to Purcifull and Hiebert (1979) and Erdiller (1982). Antisera used were: BNYVV antiserum (MAFF, Virology Laboratory, Harpenden, UK), BWYV and BMV (Biologische Bundesatalt, Institute for Biochemistry and Plant Virology, Braunschweig, Germany) CMV (Arkansas Univerity, USA). BYV antiserum was kindly supplied by Prof. Dr. G. erdiller (Ankara Universtiy). Indirect ELISA test was applied to the specimens suspected as BNYVV infection according to Brewer (1993) by using BNYVV-H3 polyclonal IgG and BNYVVMAFF 9 IgG (MAFF Virology Laboratory, Harpenden, UK) in same assay. Microprecipitation test has been applied according to Noordam (1973).

Deterination of Physical Properties of Sugar beet Viruses in sap:

Thermal inactivation point, longevity in vitro and dilution and points were determined according to Noordam (1973). **Chenopodium quinoa** Wild. plants were used as local lesion host for BMV and **Claytonia perfoliata** for BYV, but **C. Perfoliata** seeds couldn't emerged, therefore, it was not possible to detect the physical properties of BYV in sap.

Electron microscopy:

Infected plant sap were examinid using 2% PTA, after a low speed centrifugation at 5000 rpm. Zeiss M-9 S-2 model electron microscope (U.A. Faculty of Agriculture) was used for investigation.

RESULTS

Survey results:

Surveys were started in June and contiuned to October 1992. During the surveys 150 sugar beet fields were observed as completely free of virus infections in the research area. The major symptoms of virus infections were severe mosaic on young leaves or severe chlorosis on old leaves at field conditions.

According to the results of mechanical inoculation test 41 specimens were infected by BMV and 5 were by BYV. 5 specimens were suspected as Rhizomania infection but no symptom development had occured on the test plants. As seen from the Table 1, BMV was prevalent but BYV was present only in Haymana and Ayaş. Rhizomania suspected specimens were collected only from Nallıhan which is near to Bolu and has its own microclimatic conditions. Typical symptoms of BMV and BYV are shown in Figures 2 and 3.

Table 1. The results of mechanical inoculation tests.

Specimen collected Towns	Total speciman	Mechanical inoculation results			Non-mechanical transmitted
		BMV	BYV	BNYVV suspected	
Polatlı	21	19	0	0	2
Kızılcahamam	1	0	0	0	1
Şereflikoçhisar	4	3	0	0	1
Haymana	7	4	3	0	0
Kalecik	12	4	0	0	8
Çubuk	6	2	0	0	4
Sincan	6	0	0	0	6
Bala	6	0	0	0	6
Ayaş	6	2	2	0	2
Beypazarı	4	3	0	0	1
Gölbaşı	4	0	0	0	4
Güdül	4	2	0	0	2
Kazan	4	1	0	0	3
Nallıhan	9	1	0	5	3
Total	94	41	5	5	43

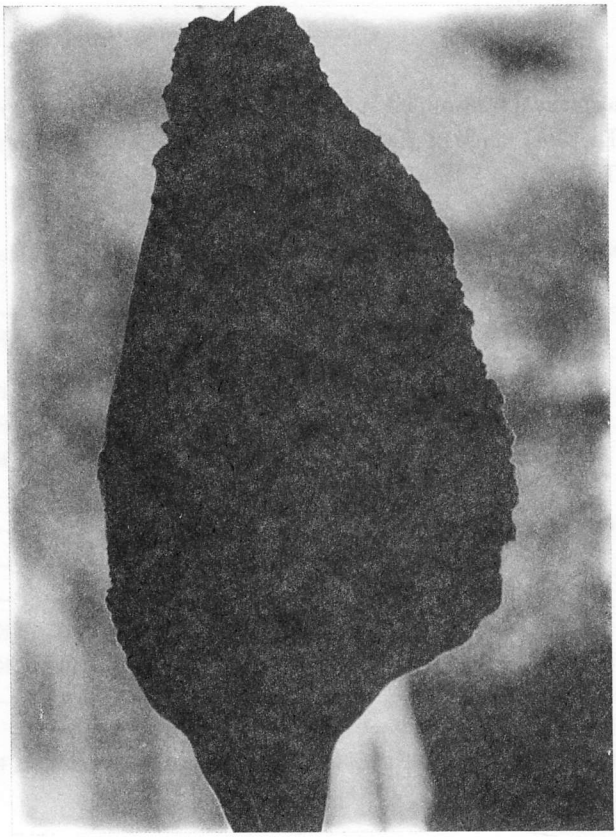

Figure 2. Symptoms of BMV on naturally infected sugarbeet leaf.

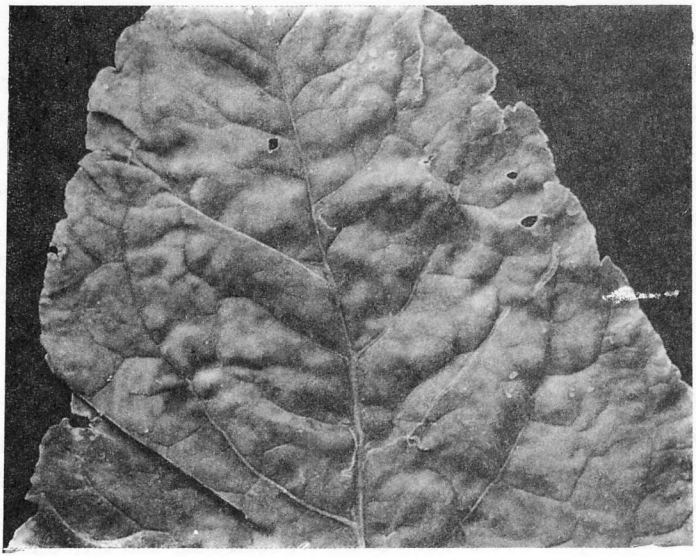

Figure 3. Symptoms of BYV on naturally infected sugarbeet leaf.

Other mechanical transmitted sugar beet virus infections were not present in the research area. The host range of BMV and BYV isolates and the symptoms produced were shown in Table 2.

Table 2. Symptoms produced on the test plants by BMV and BYV isolates.

Test plant	BMV	BYV
B. vulgaris c.v. Eva	SM	K
B. vulgaris c.v. Perla	SM	K
B. vulgaris c.v. Kawepura	SM	K
C. quinoa	KLL, NLL	--
C. amaranticolor	KLL	--
A. caudatus	KLL	--
A. retroflexus	NLL	--
S. oleracea	KLL	K
T. expansa	--	K
N. tabacum var. Maden	--	--

SM: Systemic mosaic, KLL: Chlorotic local lesion, NLL: Necrotic local lesion, K: Chlorose

Serological test results:

Ouchterlony agar gel double diffusion test results:

BMV isolates showed weak precipitation when applied to the agar medium prepared according to Erdiller (1982) but when same isolates were applied to the medium of Purcifull and Hiebert (1979) with application of 3 % SDS to the plant sap at 1:1 ratio, the occurred precipitates were stronger. The strongest reaction was obtained by isolate obtained from A.U. A.F. Haymana Kenan Evren Research Farm. All of the BMV isolates reacted against only BMV antiserum. No reaction was observed by BYV isolates and BNYVV suspected isolates against BYV and BNYVV antisera respectively and the other antisera tested in both applications.

Microprecipitation test results:

BMV isolates reacted with BMV antiserum up to 1/16 dilution of sap and up to 1/16 dilution of antiserum in the test. Although there was no reaction in agglutination test with BYV isolates against BYV antiserum, in microprecipitation test, defined precipitates occured up to 1/32 dilution of BYV infected sap and 1/16 dilution of BYV antiserum.

Indirect ELISA test results:

No pozitif reaction was obtained with BNYVV suspected isolates against BNYVV IgGs.

Physical properties in sap:

Physical properties of BMV in sap were as: thermal inactivation point 50-55°C, dilution end point 10^{-2}-10^{-3}, longevity in-vitro 48-56 hours.

Electron microscopy:

Both viruses were detected as tiny flexible rods on the electromicrographs.

DISCUSSION

Sugar beet is the major industrial plant cultivated in Ankara. During the surveys done in 1992, the major symptoms observed on naturally infected plants were systemic mosaic on young leaves or severe chlorosis on old leaves at field conditions, therefore the collected isolates were differentiated into two groups as mosaic-type and chlorosis-type isolates.

The mosaic-type infections of sugar beet are CMV, BMV and it very difficult to differentiate them at field conditions (Whitney and Duffus, 1991). The symptoms produced on test plants by mosaic type isolates of Ankara province were similar to the ones produced by BMV (Russel, 1971; Smith, 1972; Whitney and Duffus, 1991). They all reacted against BMV antiserum in Ouchterlony agar-gel double diffusion test, especially, the reactions were stronger when they applied to the method of Purcifull and Hiebert (1979). There was no reaction against CMV antiserum and the other antisera tested.

The strongest reaction against BMV antiserum in agar-gel double diffusion test was observed with the isolate obtained from A.U. Kenan Evren Reseach Farm (Haymana), so this isolate was used for microprecipitation test, determination of physical properties and electron microscopic observations. In microprecipitation test, the isolate gave reactions up to 1/16 dilution of plant sap and 1/16 dilution of BMV antiserum. As a result of this test, minimum detection dilution of BMV infected sap was determined as 1/16. Physical properties in sap of this isolate were in the range reported by Russel (1971) and Smith (1972) for BMV. In electron microscobic observation, the particles of this isolate were observed as long flexible rods. Therefore, all of our mosaic-type isolates were identified as BMV infection.

Chlorosis-type isolates were all indexed on test plants but only 5 of them gave reactions similar to BYV (Russel, 1970 and Smith, 1972). BYV is a member of Closterovirus group, it is an insect-transmitted virus infection in persistent manner by aphid vectors and also mechanically transmitted to a very narrow host range. They all didn't react in agglutination test against the antisera tested but produced defined

precipitates in microprecipitation test against BYV antiserum. We failed to detect the physical properties in sap of BYV isolates because **C. perfoliata** is the only local lesion host but the seeds we had didn't emerge at in laboratory and also greenhouse conditions, although different applications (such as cracking of seed coat, application of gibberellic acid) had been done. Their particles were observed as long flexible rods but longer than BMV in electronmicroscopic observations.

The other chlorosis type infected specimens didn't produce any reactions on the test plants and also Ouchterlony agar-gel double diffusion test against the antisera tested. Since they were not mechanically transmitted, they were taken out of the research and were considered as physiological disorders or insect-tranmitted virus infections. There was no symptom development with the isolates suspected as BNYVV infection on the test plants. They didn't react with BNYVV antiserum and BNYVV IgGs in the serological tests.

According to the results of this investigation, BMV is the most prevalent virus infection of sugar beet, followed by BYV, in the research area.

ÖZET

ANKARA İLİNDE ŞEKERPANCARI VİRÜSLERİNİN TESBİTİ ÜZERİNDE ARAŞTIRMALAR

Ankara ilindeki şekerpancarı tarlalarından 1992 yılında hastalıklı yaprak örnekleri toplanmıştır. Ankara'nın 14 ilçe ve 1 beldesinde bulunan 150 tarladan 94 adet örnek alınmıştır. Bunlar mekaniksel olarak indikatör test bitkilerine aşılanmış, bunlar üzerinde gelişen simptomlar, serolojik test sonuçları ve electron mikroskop gözlemleri ile teşhis edilmişlerdir. Rhizomania enfeksiyonundan şüphelenilen örnekler, Beet necrotic yellow vein virus (BNYVV) anti-serumlarına karşı İndirekt-ELISA testine tabi tutulmuşlardır. Mekaniksel inokulasyon testi sonucuna göre, 41 izolat Beet mosaic virus (BMV-Pancar mozayık virusu), 5 isolat Beet yellows virus (BYV= Pancar sarılık virusu olarak belirlenmiştir. Araştırma bölgesinde Rhizomania'nın bulunmadığı saptanmıştır. Diğer örneklerde ise, mekanik inokulasyon ve serolojik testlerde hiç bir sonuç alınmamış ve bu nedenle bunlar fizyolojik bozukluklar veya mekaniksel olarak taşınmayıp sadece böceklerle taşınan viruslar olarak nitelendirilmişlerdir.

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Virus Diseases of Peach Trees in Hatay Province

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ABSTRACT

A survey for virus diseases prevailing in peach was carried out in Hatay province of Turkey. Virus detection was made by using enzyme linked immunosorbent assay (Das-Elisa) technique. The suspected plants were checked individually for the presence of plum pox potyvirus (PPV), prunus necrotic ringspot (PNRV), prune dwarf (PDV) and apple mosaic (ApMV) ilar viruses and apple chlorotic leaf spot closterovirus (ACLSV).

A total of 58 suspected plants were tested belonging to different cultivars.

According to the Elisa results about 15.51 % of the plants were shown infected by PNRV and 5.17 % had mixinfection by PNRV + PDV. Only 3.44 % trees were found to be infected by ApMV. The all tested plants were free of PPV and ACLSV.

INTRODUCTION

Peach production in Hatay province is getting more popular and the number of peach trees was reached to 70120 and production was 2660 tons (Anonymous, 1993).

It is obsivious that many virus diseases are a serious danger for the industrial growing of peaches. Studies on the harmful effect of the representatives of different virus, viroid and mycoplasma like organisms on peach have been published by a number of authors (Topchiiska, 1982., Morran and Cameron, 1983., Flores et al., 1990, Varveri, 1992., Uyemoto and Scott, 1992).

Viruses present in peaches belong to the Ilarvirus, Nepovirus, Closterovirus and Potyvirus groups. Main virus diseases in peach tree are prunus necrotic ringspot (PNRV), prune dwarf (PDV), apple mosaic virus (ApMV) apple chlorotic leaf spot (ACLSV), tomato ring spot (TomRSV) and plum pox virus (PPV) (Dunez, 1988, Dunez and Sutic, 1988). They are the major cause of crop losses despite often being carried latently (Lansac et al., 1980). In California for the trees infected with PNRV and PDV, fruit production, trunk diameter and tree height were reduced by an average of 30,23 and 12 %, respectively over 3 years (Uyemoto et al., 1992).

The studies about virus and virus-like diseases of peach trees in Turkey is limited. One of the most studied virus disease in sharka. A survey were done in Marmara region between 1976-1982 in order to determine the incidence of sharka disease on

apricot, plum and peach trees. Peach trees were found to be infected by sharka, some ilar viruses and apple chlorotic leaf spot according to indexing results on GF 305 peach variety (Yürektürk, 1984). Elibüyük and Erdiller (1991) found that apricots and plums in Ankara province were infected by sharka but they could not find any infected peach trees. According to Baloğlu et al. (1995), plum and peach trees in Adana and Tarsus provinces were not infected by Sharka when the samples tested by Elisa. This study was carried out to determine virus diseases of peach trees growing in Hatay province.

MATERIALS and METHODS

Field inspections were carried out from mid-May onwards between 1994 and 1995. Plants that showed no symptoms on the foliage were re-inspected at fruit ripening. The leaf samples were mainly collected from the governmental nurseries and mother plant stocks and also from the private nurseries and orchards.

Das-Elisa was made according to Clark and Adams (1977). Antisera to the following viruses were utilized: Plum pox poty virus (PPV), apple chlorotic leaf spot closterovirus (ACLSV) and apple mosaic virus (ApMV), prunus necrotic ringspot (PNRV) and prune dwarf (PDV) ilarviruses. Commercial kits (Sanofi Sante' Animale) and also IgG+Conjugates produced by Prof. Fuchs-Halle University, GERMANY were used for Elisa tests. Commercial reagents were used as suggested. However a serial of dilution tests were applied for the reagents from Prof. Fuchs. We found that optimal IgG dilution was 1/100 whereas conjugate was 1/400.

RESULTS and DISCUSSION

During the survey work it was observed that some peach trees had very typical symptoms of ApMV and PNRV. Symptoms caused by ApMV on peach consisted of various types of mottle with most commonly small, irregularly shaped cream-to yellow spots (Fig. 1). Infected leaves eventually became chlorotic. In some cases chlorotic vein-banding observed and no fruit symptoms were recorded. The initial symptoms of PNRV were chlorotic spots and rings that turned necrotic on recently developed leaves. Later this necrotic tissues fallen out and left a shot-hole appearance of the leaves (Fig. 2). No obivious symptoms of PDV were on the trees which were mixinfected by PNRV and PDV. The symptoms were very similar to the ones described before (Di Terlizzi et al. 1992, Nemeth, 1986). Those trees were also found infected when tested by Elisa.

According to the Elisa results from 58 tested trees 9 were infected by PNRV (15.51 %), 3 were infected by PNRV+PDV (5.17 %) and 2 were infected by ApMV (3.44 %). The all tested plants were free from PPV and ACLSV. Das-Elisa is one of the most reliable method to determine stone fruit viruses for field surveys (Morran and Cameron, 1983, Varveri, 1992). This is the first study for virus definition of peach trees in Hatay province. Although some peach trees in this area were infected by ApMV, PNRV and PDV and PDV they were free of the most dangerous sharka disease. However Azeri (1994) found that peach trees in Aegean region is severely infected with PPV and also ACLS according to field symptoms and Das-Elisa results.

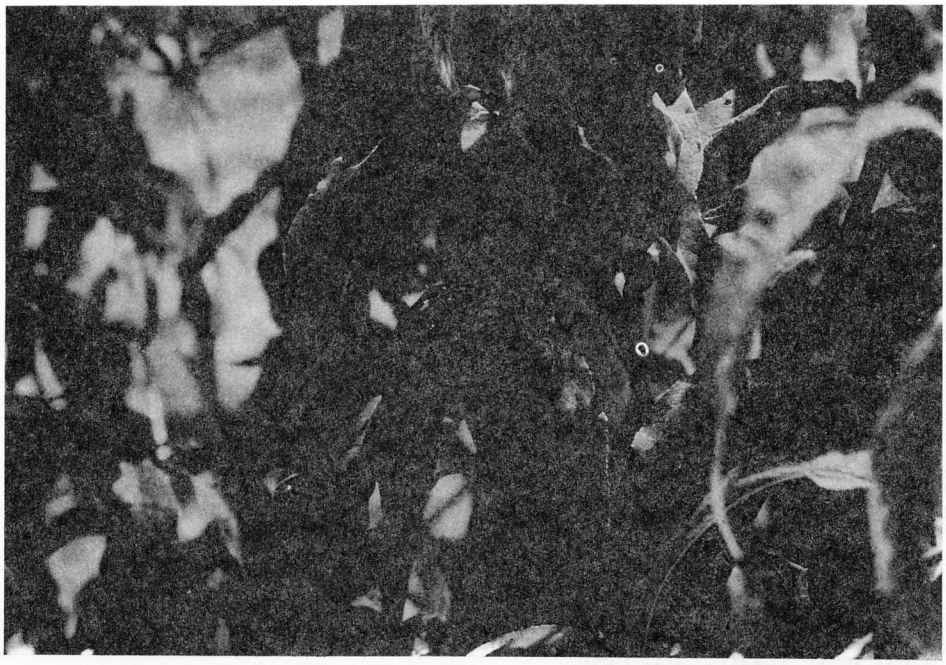

Figure 1. Typical mosaic symptoms of Apple Mosaic Virus (ApMV) on peach leaves.

Figure 2. Shoot-holing and yellowing symptoms of Prunus necrotic ringspot (PNVR) on peach leaves.

ÖZET

HATAY İLİNDEKİ ŞEFTALİ AĞAÇLARINDA GÖZLENEN VİRUS HASTALIKLARI

Antakya yöresinde yetişen şeftali ağaçlarında virus hastalıklarını saptamak amacıyla bir survey yapılmıştır. Viruslerin saptanmasında Das-Elisa yöntemi kullanılmıştır. Şüpheli bitkiler erik kanseri virusu (sharka=plum pox-PPV), nekrotik halka leke virusu (prunus necrotic ring spot-PNRV), cüceleşme virusu (prunus dwarf-PDV), elma mozaik virusu (apple mosaic-ApMV) ve elma klorotik yaprak leke virusleri (apple chlorotic leaf spot-ACLSV) açısından testlenmiştir.

Farklı çeşitlere ait şüpheli bitkilerden 58 adedi Elisa ile testlendiğinde bitkilerin % 15.51'i PNRV, % 5.17'si PNRV+PDV ile karışık olarak infekteli bulunmuştur. Bitkiler sadece % 3.44 oranında ApMV ile infekteli bulunurken testlenen bütün bitkiler PPV ve ACLSV'den ari olarak saptanmıştır.

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Transmission and Epidemiology of Citrus Chlorotic Dwarf (CCD) Disease in the Eastern Mediterranean Region of Türkiye

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ABSTRACT

A new virus-like disease of citrus was observed in the eastern Mediterranean region of Türkiye in late 1980s. Field symptoms consist of a V-shaped notch and chlorotic flecking on young leaves, warping, crinkling, inverted cupping and variegation on mature leaves. This disease has been named "citrus chlorotic dwarf (CCD)". In the laboratory, the pathogen was frequently transmitted from citrus to citrus by the Japanese bayberry whitefly, ***Parabemisia myricae*** *(Kuwana) (Hom.: Aleyrodidae). The transmission rate was increased from 18 % to 46 % by increasing the inoculation access period from 24 to 48 h. The pathogen was transmitted to rough lemon by stem-slash inoculation in a rate of 5 % (5 cuts) to 72 % (100 cuts). It was not possible to transmit the causal agent mechanically to citrus seedlings or herbaceous plants neither by leaf-inoculation nor by knife, simulating pruning. According to the results, vector transmission seems to be the primary mean of spread of CCD.*

INTRODUCTION

During general citrus orchard inspections a new virus-like disorder was observed in the eastern Mediterranean region of Türkiye. The disease spreads rapidly along the south coast and reached epidemic levels at the beginning of the 1990s. Currently this disorder is considered the most serious citrus disease occuring in the eastern Mediterranean region of Türkiye (Çınar et al., 1993; 1994).

Field symptoms consist of a V-shaped notch and chlorotic flecking on young leaves, warping, crinkling, inverted cupping and variegation on mature leaves. Sweet orange was much less affected than lemon, grapefruit or mandarin, not only in the number of diseased trees, but also according to the severity of symptoms (Korkmaz et al. 1994a). Based on field symptoms, this disease was at first believed to be caused by a form of citrus infections variegation virus (CIVV) (Çınar et al., 1993). Subsequent studies in the greenhouse, however, revealed important differences from CIVV, particularly strong chlorosis, reduced leaf size, and in some varieties severe leaf dropping. As such, the name "citrus chlorotic dwarf (CCD)" was proposed for this new disease

(Kor kmaz et al, 1995). The causal agent was graft-transmitted to 20 out of 36 citrus varieties inoculated. The best symptoms developed on rough lemon (**Citrus jambhiri** Lush.) five to eight weeks after inoculation. Symptoms occurred at 20-25°C, but even better symptoms were observed at 30-35°C (Kersting et al., 1994; Korkmaz et al., 1994b).

Because of the intense and rapid outbreak of CCD, vector transmission was suspected. This suspicion was further strengthened when CCD was found on rough lemon seedlings used as host plants for a **Parabemisia myricae** (Kuwana) (Hom.: Aleyrodidae) mass culture maintained at the Department of Plant Protection in Adana. In primary laboratory experiments, the putative pathogen was transmitted by **P. myricae** from infected rough lemon to half of the 20 exposed rough lemon seedlings (Çınar et al., 1993).

MATERIALS and METHODS

Plant material. Rough lemon plants were grown from seeds in sterilized potting mixture and kept in a partially shaded greenhouse cooled by evaporative coolers. The temperature ranged from 20 to 25°C and the relative humidity was about 65 ± 5 %. Rough lemon was chosen as test plant because it produced many flushes in a short time and is an excellent indicator plant of CCD (Kersting et al., 1994).

The source of CCD used in all experiments was originally obtained from a rough lemon seedling inoculated with CCD by **P. myricae** and proved to be free from any other detectable citrus virus or virus-like diseases as determined by biological indexing. Other virus sources used for comparison were two strains of CIVV supplied by Prof. C.N. Roistacher, University of Riverside, California (Strain 401 A), citrus leaf rugose virus (CLRV) was obtained from the University of Catania, Italy, and Satsuma dwarf virus (SDV 58) provided by Dr. T. Iwanami, Okitsu Research Institute, Japan. All virus sources were maintained on rough lemon seedlings in the greenhouse.

Transmission test. P. myricae were reared on rough lemon seedlings in a climate room as described previously (Uygun et al., 1993). For each experiment about 500 adult whiteflies were transferred onto 1 yr old CCD infected rough lemon seedlings for acquisition access period (APP) of 24 h. Without any latent period, groups of 15 to 20 **P. myricae** were caged with six month old rough lemon seedlings having many young flushes for two different inoculation access periods (IAP) of 24 and 48 h, respectively. After inoculation the plants were sprayed twice insecticides and were cut back after one month.

Mechanical inoculations to red kidney bean, cowpea (local Turkish variety), white sesame were made by conventional leaf-inoculation procedures. Inocula were prepared from young, symptomatic citrus leaves in cold 0.05 M neutral potassium phosphate buffer, and applied with sterile cotton swabs to leaves dusted with 500-mesh Carborundum (Garnsey et al., 1977). A minimum of five plants was used for each inoculation experiment. In a second experiment, it was attempt to transmit the causel

agent from rough lemon to rough lemon by stem-slash inoculation. For simulation of pruning in field, CCD-infected plants were slashed by a sterilized knife and one to three healthy plants were inoculated with one cut each. This experiment was repeated 20 times. In addition, the causel agent of CCD was inoculated by a stem-slash inoculation technique to six months old rough lemon seedlings as described by Müller and Garnsey (1984). Inoculum was prepared in the same way as for leaf-inoculation without further concentration of extracts. Test plants were inoculated by a razor blade to which single drop of inoculum was applied for three to five cuts. Five treatment from five to 100 cuts per plant were tested. About ten days after inoculation all leaves were removed and the plants were cut back above the inoculation site.

All inoculated plants (insect and mechanical transmission tests) were kept at 20-25°C in the greenhouse and periodically examined for CCD symptoms for at least six months. Usually the plants were cut back after each flush (every second month).

RESULTS

Vector transmission. P. myricae transmitted the putative agent of CCD from rough lemon to rough lemon. Symptoms occurred in general in the first flush as quick as 14 days after inoculation, and they were much stronger than those observed after graft-transmitting of the causal agent. In a few plants, however, symptoms were obtained after 3 or even 6 months. With increasing inoculation access periods, an increase in transmission rate was observed from 18 % (IAP: 24 h) to 46 % (IAP: 48 h). These findings indicate that CCD is probably transmitted in a semi-persistent or persistent way where the virus is retained for several days or sometimes along the life span than in non-persistent manner where the virus is retained for not more than some hours (Duffus, 1987).

Mechanical transmission. It was not possible to transmit CCD mechanically to red kidney bean, cowpea or sesame by leaf-inoculation, while CIVV and SDV were readily transmitted in the same tests. All attempts failed to transmit CCD from citrus to citrus by simulated pruning.

In contrast, CCD was transmitted by stem-slash inoculation from rough lemon to rough lemon at a rate of 5 % for five to ten cuts and 72 % for 100 cuts (Table 1).

Only few plants developed symptoms in the first flush; most of the symptomatic plants were observed in the second flush after three to four months. The transmission efficiency of CCD by stem-slash inoculation was comparable to that obtained in similar transmission studies for citrus tristeza virus (CTV) using concentrated bark extracts (Müller and Garnsey, 1984). CTV is a phloem restricted, semi-persistent transmitted virus and not transmissible to citrus and herbaceous plants by conventional leaf-inoculation (Müller and Garnsey, 1984; Garnsey and Müller, 1988). Because of these similarities, our results suggest that CCD might be a phloem-restricted pathogen.

Table 1. Transmission of citrus chlorotic dwarf (CCD) disease by stem-slash inoculation from rough to rough lemon seedlings

Treatment	Number of cuts	Transmission rate (%)	
1	5	5	1/20*
2	10	5	1/20
3	25	10	2/20
4	50	30	6/20
5	100	72	36/50

* Number of plants infected/number of plants inoculated

DISCUSSION

CCD spread over the whole citrus-growing area in the province of İçel within only three years, resulting in a disease incidence of about 50 % (Korkmaz et al., 1994a). CCD has now to be considered as a most serious citrus disease in the eastren Mediterranean. The origin and the causal agent of this disease is not known, and furthermore the rapid spread of CCD is yet not fully understood.

The causal agent of CCD is graft-transmissible (Korkmaz et al., 1994b) and as shown in the results transmitted by stem-slash inoculation and by the whitefly **P. myricae.** In contrast, it was not possible to transmit CCD by conventional leaf-inoculation and knife-inoculation, simulating prunning. These results clearly demonstrate that CCD is, if at all, spread in a negligible rate by prunning, a common horticultural practice on lemon in the province of İçel. The presence of CCD-infected root stocks in nurseries and a high disease incidence in elder trees suggest that insect vector transmission is the primary mean of spread.

The role of **P. myricae** in the spread of CCD in field is difficult to judge. According to our field observations, and statements of local citrus growers, CCD outbreaks occurred after a heavy infestation of citrus by the Japanese bayberry whitefly (Çınar et al., 1994). However, since 1990, the abundance of **P. myricae** in field has been very low (<0.1 stages/leaf). A program for biological control by releasing the aphelinid wasp **Eretmocerus debachi** Rose and Rosen (Hym.: Aphelinidae) was launched in 1988 and this had proved to be extremely successful (Uygun et al., 1990; Şengonca et al., 1993; 1995). However, natural transmission of CCD in field still occurs (Korkmaz et al., 1994a). One possible explanation for this situation might be that the remaining **P. myricae**-populations in combination with the high transmission efficiency is responsible for the spread of this disease in field. Less than 0.1 **P. myricae**-stages/leaf add up to several hundreds or thousands of individuals per tree, a population most likely large enough for the dissemination of CCD. However, it might be also possible that other whitefly species are vectoring CCD. Due to effective control of **P. myricae**, the

citrus whitefly, **Dialeurodes citri** (Ashmead), is now the dominant species on citrus in the eastern Mediterranean region (Uygun et al., 1994). Whether **D. citri** is a also vector of CCD and should be implicated in the rapid spread of this disease in the eastern Mediterranean region is yet not known.

ÖZET

TÜRKİYE'NİN DOĞU KARADENİZ BÖLGESİ'NDE TURUNÇGİL KLOROTİK CÜCELEŞME (**Citrus Chlorotic Dwarf-CCD**) HASTALIĞININ TAŞINMASI VE EPİDEMİYOLOJİSİ

Türkiye'nin Doğu Akdeniz Bölgesi'nde 1980'li yılların sonlarında turunçgillerde yeni bir virüs benzeri hastalık gözlenmiştir. Hastalık simptomları genç yapraklarda V şeklinde girinti, olgun yapraklarda ise buruşukluk kıvırcıklık, kayık şeklinde oluşum ve renk açılmasından ibarettir. Hastalık "Turunçgil Klorotik Cüceleşme" (Citrus chlorotic dwarf) olarak adlandırılmıştır. Laboratuvar koşullarında etmen Japon defne beyazsineği, **Parabemisia myricae** (Kuwana) (Hom.: Aleyrodidae), ile turunçgilden turunçgile taşınmıştır. Taşınma oranı 24 saatlik bir beslenme periyodundan sonra % 18 olarak gerçekleşirken 48 saatlik bir beslenme periyodunun sonunda % 46'ya ulaşmıştır. Etmen aynı zamanda gövdeye kesik atılarak yapılan inokulasyon yolu ile kaba limona % 5 (5 kesik) ile % 72 (100 kesik) arasında değişen bir oranda taşınmıştır. Etmen mekanik olarak yaprak özsu inokulasyonu veya bıçakla turunçgile yada yabancı otlara taşınamamıştır. Bu bulgular sonucunda vektörle taşınma hastalığın yayılmasında birincil derecede önemli olarak bulunmuştur.

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The Species of Tylenchida (Nematoda), Found in The Onion Growing Areas of Central Anatolia

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ABSTRACT

This paper, comprises studies on plant parasitic Tylenchids, carried out in the most economic onion growing areas of Konya, Karaman and Nevşehir provinces in Central Anatolia in 1984-1989. Soil and onion samples were taken from the mentioned provinces, especially in summer months when the adults were found abundantly.

As a result of this study, totally 19 species, 17 of these belonging to 5 families, 8 subfamilies and 12 genera of the Tylenchina Suborder and Tylenchoidea, Dolichodoroidea and Hoplolaimoidea superfamilies of the order Tylenchida; and 2 species of the ***Ditylenchus*** *genus belonging to the Anguinidae family of the Anguinidea superfamily of the suborder Hexatylina, were determined. According to the literature reviewed by the authors, 5 species have been recorded for the first time in Turkiye.*

INTRODUCTION

The order Tylencida is the most important plant parasitic nematode group since it covers the majority of harmful and econoic important species.

Literature search related with the Tylenchida species exist in the adjacent soil of the onion bulbs and roots has shown that the bulb and stem nematode of onion (**Ditylenchus dipsaci** (Kühn)) is the species that received the highest number of studies. There exists so many studies related with the hosts, damage types, distribution (Thorne, 1961; Bovey et al., 1967; Jensen, 1972), and strain discrimination (Gibbins and Grandison, 1968; Erikkson, 1974) of this species. Other species of the Tylencida order like Root-knot nematode species (**Meloidogyne** spp.), spiral nematode species (**Helicotylenchus** spp.) and potato rotting nematode (**Ditylenchus destructor** Thorne) were also found on onion in Asia and Europe (Jensen, 1972).

The highest number of studies related with the onion damaging nematodes in Türkiye have been carried on onion bulb and stem nematode. This nematode has been

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determined on many crops including onion in all regions of Türkiye (Yüksel, 1958; Ağdacı et al., 1979; Ağdacı, 1980; Hekimoğlu, 1980; Borazancı et al., 1985; attributing to Borazancı, 1977). In some of these studies, the other plant parasitic Tylenchida species in soil were also determined in genus (Ağdacı et al., 1979; Ağdacı, 1980) and species (Hekimoğlu, 1980) basis.

This study was done to determined the Tylenchida species in onion grown areas of Central Anatolia Region and the study was carried out in three provinces of the region.

MATERIALS and METHODS

The main materials of the study were soil and onions collected from onion growing areas of the three province; Nevşehir, Konya and Karaman in the summer months.

Soil samples were collected according to the standard methods of nematology literature from 114 fields equal to 1 % of the total cultivation area of the provinces. Fresh onion samples were collected and in addition shallots from the 14 grower stores in Nevşehir province.

In laboratory studies, sieve and funnel methods were used to obtaining active nematodes from soil (Christie and Perry, 1951), and incubation method (Young, 1954) and Cobb sieves in obtaining nematodes from onion samples. For identification, nematodes were fixed and permanently prepared according to De Grisse (1969). Measurements were done according to the formula cited by Siddiqi (1986).

Genera were identified according to Thorne (1961), Goodey (1963) and Siddiqi (1986) and species according to various literature and comparing with identified samples.

RESULTS and DISCUSSION

In studies carried out in Nevşehir, Konya and Karaman provinces during 1984-1989, 19 species were determined belonging to the order Tylenchida. Distribution and occurence number of these species in samples are given in Table 1.

The species **Filenchus filiformis, Discotylenchus discretus, Psilenchus clavicaudatus, Pratylenchus zeae** and **Ditylenchus obesus** were determined for the first time in Türkiye. **F. filiformis, D. dipsaci, B. goffarti, H. digonicus, B. thylactus, M. brevidens, P. zeae, P. alkani, P. conincki** and **P. penetrans** were most common species in soil samples.

F. filiformis, D. dipsaci and **H. digonicus** also known widely distributed throughout in the world (Thorne, 1961; Jensen, 1972; Siddiqi, 1986; Raski and Geraert, 1986). **Paratrophurus loofi** was found in only one sample but in high number.

Either in onion storages and soil samples or in onion samples collected from sample fields, the most common Tylenchida species was the endoparasit nematode **D. dipsaci.** This species was recovered in 11 of the 14 (78.5 %) investigated onion stores in

Table 1. Distribution of the Tylenchida Species in Sample and Province Basis According to 114 Soil Samples Examined

Species	Provincies and Counties	Numbers of the Sample Found	Percent in the Whole Sample (%)
Tylenchina			
Tylenchinae (Tylenchidae)			
Filenchus filiformis (Butschli, 1873)	Karaman (Merkez, Ermenek)	76	66.7
	Konya (Merkez, Cumra), Nevşehir (Merkez, Derinkuyu, Ürgüp, Gülşehir) Karaman (Ermenek)	1	0.9
Discotyenchus discretus Siddiqi, 1980			
Boleodorinae (Tylenchidae)			
Boleodorus thylactus Thorne, 1941	Konya (Merkez), Karaman (Merkez, Ermenek), Nevşehir (Merkez, Ürgüp)	14	12.3
Psilencinae (Psilenchidae)			
Psilenchus clavicaudatus (Micoletzky, 1922)	Karaman (Ermenek)	1	0.9
Tylenchorhynchinae (Dolichodoridae)			
Quinisulcius capitatus (Allen, 1955)	Konya (Merkez)		
Bitylenchus dubius (Butschli, 1873)	Konya (Cumra)	1	0.9
Bitylenchus goffarti (Sturhan, 1966)	Konya (Merkez), Karaman (Merkez, Ermenek)	1	0.9
	Nevşehir (Merkez, Derinkuyu, Ürgüp, Gülşehir) Karaman (Ermenek)	22	19.3
Paratrophurus loofi Arias, 1970		1	0.9
Merliniinae (Dolichodoridae)			
Merlinius brevidens (Allen, 1955)	Konya (Merkez), Karaman (Merkez, Ermenek), Nevşehir (Merkez, Derinkuyu)	12	10.5
Merlinius bseudobavaricus Saltukoğlu, Geraert and Coomans, 1976)	Karaman (Ermenek)	1	0.9
Amplimerlinius dubius (Steiner, 1914)	Karaman (Ermenek)	1	0.9
Amplimerlinius viciae (Saltukoğlu, 1973)	Karaman (Ermenek)	1	0.9
Radopholinae (Pratylenchidae)			
Pratylenchoides alkani Yuksel, 1977	Karaman (Ermenek)	8	7.0
Pratylenchoides conincki Ökten, 1989	Karaman (Merkez, Ermenek), Konya (Merkez), Nevşehir (Merkez, Avanos)	8	7.0
Pratilenchinae (Pratilenchidae)			
Pratylenchus penetrans (Cobb, 1917)	Karaman (Ermenek), Nevşehir (Ürgüp)	5	4.4
	Konya (Merkez, Cumra), Karaman (Merkez, Ermenek), Nevşehir (Merkez, Ürgüp)	10	8.8
Paratylenchus zeae Graham, 1951	Konya (Merkez, Cumra), Karaman (Merkez, Ermenek), Nevşehir (Merkez, Derinkuyu, Ürgüp, Avanos, Gülşehir, Hacıbektaş)	17	14.9
Helicotylenchus digonicus Perry 1959			
Hexatylina			
Anguininae (Angunidae)			
Ditylenchus dipsaci (Kuhn, 1857)	Konya (Merkez, Cumra) Karaman (Merkez, Ermenek), Nevşehir (Merkez, Ürgüp, Derinkuyu, Gülşehir)	27	23.7
Ditylenchus obesus Thorne and Malek, 1968	Karaman (Ermenek), Konya (Merkez)	2	1.8

Nevşehir. It feeds on the stem and leaves of the onion, being rarely on roots. 3 weeks after infection, onions being rotting from the bottom side; very apparent symptom which enables it to be determined easily. Yüksel (1958) and Diker (1959) informed that this species damages the consumed onions in Nevşehir.

Pratylenchus zeae, a member of genus **Pratylenchus** known as active endoparasitic in roots and inserts its body in tissues only during feeding, was also found as a female individual.

B. thylactus, B. dubius, B. goffarti were determined the first time in onion growing areas. Jensen (1972) repoted that some species of **Pratylenchus** and **Helicotylenchus** exist on the circumstances of some vegetable roots and feed there. The species determined in soil around the onion bulbs or onion roots, those belong to Pratylenchidae and Dolichodoridae feed on the plant with their strong stylets, cause reduction in plant development and open entrances for some microorganism (Thorne 1961; Webster, 1972; Siddiqi, 1986.

ÖZET

İÇ ANADOLU BÖLGESİ SOĞAN EKİLİŞ ALANLARINDA BULUNAN TYLENCHİDA (NEMATODA) TÜRLERİ

İç Anadolu Bölgesinin önemli soğan ekiliş alanlarına sahip olan Konya, Karaman ve Nevşehir illerinde 1984-1989 yılları arasında yürütülen çalışmada; özellikle ergin Tylenchida türlerinin bol bulunduğu yaz aylarında sözkonusu alanlardan toprak ve soğan örnekleri alınarak bitki paraziti Tylenchida türleri saptanmaya çalışılmıştır. Örneklerin incelenmesi sonucunda Tylenchida takımının Tylenchina alttakımından ve Tylenchoidea dolichodoroidea, Hoplolaimodea üst familyalarından 5 familya, 8 alt familya, 12 cinse bağlı 17 tür ile; Hexatylina alt takımından ve Anguinoidea üst familyasından Anguinidae familyasına bağlı **Ditylenchus** cinsinden 2 tür olmak üzere toplam 19 tür saptanmıştır. Bunlardan 5 türün Türkiye faunası için yeni kayıt niteliğinde olduğu belirlenmiştir.

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Effects of Some Sulfonylurea Group Herbicides on Growth of Melon, Water Melon, Cucumber, Tomato and Pepper

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ABSTRACT

Some sulfonylurea herbicides caused various type of phytotoxicity on melon, water melon, cucumber, tomato and peppers when they have applied as soil incorporation at field application rates. Of these herbicides, Chlorsulfuron reduced emergence of water melon and pepper and caused post-emergence death on these plants, in addition to this it delayed the growth of all the plants.

Tribenuron-methyl induced death and retardation in growth in all the plants. Similar effects were observed by Tribenuron-methyl+Thifensulfuron-methyl. Metsulfuron-methyl impeded germination of tomato and pepper and hampered the growth of all plants tested by causing post-emergence death and growth retardation Terbutryn+Triasulfuron prevented germination of water melon and pepper and killed all the test plants after emergence.

INTRODUCTION

The increase in use of sulfonylurea group of herbicides on broad-leaved weeds in cereal fields and long-lasting residual effects of some of these herbicides in soil have created some restrictions in the selection of rotational plants after cereals. NILSSON (1991) reported that Triasulfuron applied at the rate of 0.04-0.8 g/da in the spring maintained its residual effect in autumn; on the other hand Tribenuron-methyl at the same rate and Thifensulfuron-methyl at 0.75-1.50 g/da showed residual effect for two months after application in the spring. RAHMAN et al. (1991) detected residual effects of Metsulfuron-methyl after 1-9 weeks of application at the rates of 0.6, 1.2, 1.8 g/da. VICARI et al. (1991) determined the effects of Chlorsulfuron at low rates on a lot of culture plants and found out that pre-emergence application of this herbicide at 0.05-0.072 g/da did not affect tomato much. It was also reported that the same herbicide did not induce phytotoxicity on Cantoloup melon cultivars at 0.82-3.3 g/da (BARSTOW and CHERNICKY, 1988). PAVLOVA et al. (1992) found that cucumber stems were more susceptible to this herbicide than the leaves.

In Türkiye; melon, water melon, cucumber, tomato and pepper have been grown as rotation plants in cereal growing areas and effects of sulfonylurea group of herbicides on these crops have not been investigated so far. Sulfonylurea herbicides are widely used in cereal fields and in this research effects of them on germination, post-emergence death and growth of these rotational crops at field rates at pre-plant incorporation are taken into consideration.

MATERIALS and METHODS

Trials were set up in greenhouse conditions at plastic post of 16x15 cm with four replicates. The post were filled with 2 kg sand/perlite/soil mixture (1/1/2). Herbicides in the calculated amount per pot, were diluted in 50 ml water and mixed with the upper half of the soil mixture of the pots. Seeds of various crops, 10 seeds in a pot, were sown after 2 days of the treatment. The herbicides and their rates are shown in Table 1.

Table 1. Trade marks, common names and percent of active ingredients and rate of the herbicides used.

Herbicides	Active ingredient and percentage		Application rate (g(a.i)/da)
Glean	Chlorsulfuron	75	0.75
Granstar	Tribenuron-methyl	75	7.5
Harmony-extra	Tribenuron-methyl + Thifensulfuron-methyl	25+50	0.5+1
Ally	Metsulfuron-methyl	20	0.4
Logran extra 64WG	Terbutryn+Triasulfuron	60+4	15+2.4

Herbicidal influence was determined by counting germinated seeds and making observations on the post-emergence growth of seedlings. Post-emergence growth was evaluated by seedling death and phytotoxicity (chlorosis, dwarfing, leaf mottling, weakness etc.) on the seedlings. Effects of herbicides on germination was assessed by counting germinated seedlings after completition of germination in the check plots. Statistical analysis was employed on each crop with their controls.

RESULTS

Sulfonylurea herbicides had a varying impact on germination of some crops (Table 2).

As seen in Table 2, germination of cucumber was not affected by the herbicides but water melon and pepper by all of them. Melon was affected by Tribenuron-methyl and Tribenuron-methyl+Thifensulfuron-methyl while tomato affected by Tribenuron-methyl and Metsulfuron-methyl.

Table 2. Effects of some sulfonylurea herbicides on germination of cucumber, melon, pepper, tomato and water melon

Herbicides	Plants and mean germination percentages				
	Cucumber	Melon	Pepper	Tomato	Water melon
Chlorsulfuron	92.5 a	90 ab	27.5 def	87.5 abc	70 bcdef*
Tribenuron-methyl	85 a	57.5 c	27.5 def	80 bc	62.5 def
Tribenuron-methyl + Thifensulfuron-methyl	75 a	72.5 bc	20 f	85 abc	60 ef
Metsulfuron-methyl	75 a	82.5 abc	30 bcdef	75 c	57.5 f
Terbutryn+Triasulfuron	87.5 a	87.5 ab	27.5 ef	90 ab	67.5 cdef
Control	92.5 a	95 a	55 a	92.5 a	92.5 a

* Means followed by different letters are significantly different at P=0.05 according to Duncan's Multiple Range Test.

Post-emergence growth of the rotational crops was observed for 60 days and percentage of plants in this period was calculated and presented in Table 3.

Table 3. Percentages of death plants after 60 days from emergence

Herbicides	Plants and mean germination percentages				
	Cucumber	Melon	Pepper	Tomato	Water melon
Chlorsulfuron	8.1 f	2.7 ef	81.8 ab	0 d	57.1 bc*
Tribenuron-methyl	44.1 d	43.4 de	45.4 bc	87.5 b	88 ab
Tribenuron-methyl + Thifensulfuron-methyl	80 bcd	55.1 bcd	87.5 abc	100 ab	50 c
Metsulfuron-methyl	63.3 cd	39.3 cd	16.6 c	100 ab	95.6 ab
Terbutryn+Triasulfuron	100 a	100 a	100 a	100 a	100 a
Control	10.8 ef	0 f	54.5 a	8.1 cd	0 d

* Means followed by different letters are significantly different at P=0.05 according to Duncan's Multiple Range Test.

All the herbicides except Chlorsulfuron caused post-emergence death on cucumber, melon and tomato. On the other hand on pepper Tribenuron-methyl and Metsulfuron-methyl showed less death than the other herbicides and the control in

which some other factors (as pathogens) might have caused the death. Water melon was affected by all the herbicides.

The most prominent impact of the herbicides was on the post-emergence growth of the crops. All the hercibides significantly reduced plant height (Table 4). Besides this dwarfing effect they also produced various symptoms (Table 5).

Tablo 4. Effects of the herbicides on plant height of five culture plants

Herbicides	Plants and mean germination percentages				
	Cucumber	Melon	Pepper	Tomato	Water melon
Chlorsulfuron	17.75 b	6.12 b	1.5 def	6.87 b	5.5 b*
Tribenuron-methyl	2.37 cde	2.37 cde	2.5 bcd	1.12 cdef	1.75 def
Tribenuron-methyl + Thifensulfuron-methyl	1.62 ef	2.0 e	0.5 ef	0 ef	1.87 cde
Metsulfuron-methyl	2.37 de	2.12 de	2.38 cd	0 ef	2.12 de
Terbutryn+Triasulfuron	0 f	0 f	0 f	0 f	0 f
Control	55 a	27.5 a	6.75 a	13.37 a	17.50

* Means followed by different letters are significantly different at P=0.05 according to Duncan's Multiple Range Test.

Table 5. Other phytotoxic effects of the herbicides on the rotational crops

Herbicides	Plants and mean germination percentages				
	Cucumber	Melon	Pepper	Tomato	Water melon
Chlorsulfuron	c	a	a, b, d	g	a, d
Tribenuron-methyl	a, d	a, d	a, b, d	a, b, d	d
Tribenuron-methyl + Thifensulfuron-methyl	a, b, d	a, b, d	a, b, d	f	a, d
Metsulfuron-methyl	a, b	a, d, e	a, b, d	f	c
Terbutryn+Triasulfuron	f	f	f	f	f
Control	g	g	g	g	g

a chlorosis
b poor growth
c small leafness
d fail in emergence of true leaves
e leaf spotting
f total plant death
g no fitotoxicity

DISCUSSION

Sulfonylurea herbicides are normally used on cereals against mainly some broad-leaved weeds as post-emergence application. Their residual effects are important for the rotational crops of cereals. For this season influence of some sulfonylurea group of herbicides at field rates was taken into consideration.

The germination of the plants were influenced at varying degrees. For instance germination of cucumber did not show a significant difference while water melon and pepper showed a decrease. Germination of melon and tomato almost behaved similarly. When the whole ill effects of the herbicides were evaluated, their results on germination might not be important but the growth of almost all the crops were affected by them at field rates to some extent.

Tomato seemed to tolerate only to Chlorsulfuron and this result was also confirmed by VICCARI et al. (1991) who found out that 1/10 rate of it slightly affected the growth of tomato. Melon also tolerated to Chlorsulfuron, which yielded only weak, chlorosis, at field rates and these effects might be more strong at low dosages. This was supported by BARSTOW and CHERNICKY (1988). Cucumber too might be tolerable to low dosages of Chlorsulfuron, which could be used in the preference of rotational crops. Since Metsulfuron-methyl, Tribenuron-methyl and Thifensulfuron-methyl were strongly phytotoxic on all the plants tested their persistence in soils should be found out, because there are reports relating their persistence in the soil (NILSSON, 1991; RAHMAN et al. 1991).

Although Terbutryn+Triasulfuron killed all the crops, this effect might have been caused by Terbutryn, in the mixture. Any conclusion can not be drawn about Triasulfuron since we could not test its effects directly.

ÖZET

TAHILLARDA KULLANILAN BAZI SULFONYLURE GRUBU HERBİSİTLERİN KAVUN, KARPUZ, HIYAR, DOMATES VE BİBERE ETKİLERİ

Bazı sulfonylüre grubu herbisitler tarla kullanım dozlarında ekim öncesi toprağa karıştırılarak (PPI) uygulandıklarında kavun, karpuz, hıyar, domates ve biberde değişik tiplerde fitotoksisiteye neden olmuşlardır. Bu herbisitlerden Chlorsulfuron, karpuz ve biberin çıkışını önemli derecede azaltmış ve çıkış sonrası ölüme, tüm bitkilerde de gelişme geriliğine yolaçmıştır. Tribenuron-methyl tüm bitkilerde ölüme ve gelişme geriliğine neden olmuştur. Aynı etkiler Tribenuron-methyl+Thifensulfuron-methyl'de de görülmüştür. Metsulfuron-methyl ise, kavun ve karpuzun çıkışını fazla etkilememiş, ancak hıyar ve domateste çıkış sonrası ölüm ve gelişme geriliği meydana getirmiştir. Terbutryn+Triasulfuron, karpuz ve biberde çıkışı engellemiş fakat çıkış sonrasında tüm bitkileri öldürmüştür.

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