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Detection of Seed-borne Viruses on Certified Seeds of Lettuce[1]

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ABSTRACT

In this study, seed specimen of lettuce obtained by the seed control and certification laboratory for the purpose of being certified were evaluated for the presence of seed-borne viruses. One of the serological method, microprecipitation test and specially selected indicator plants were used.

All the seed specimen tested were found to be infected by 66.7 %, 58.3 % and 42.3 % with Lettuce Mosaic Virus (LMV) and Cucumber Mosaic Virus (CMV) and both respectively. The results of experiments are discussed for the procedures of seed control and certification.

INTRODUCTION

LMV, a seed-borne virus, occurs in all areas of the world where lettuce (**Lactuca sativa** L.) is cultivated. The percentage of seed transmission varies 3-10 % with the cultivar and age of the plant. In some susceptible cultivars, seed transmission does not exist (hypersensitive reaction). LMV is established as embriyo-borne in lettuce, transmission occurs through the pollen and ovules of infected plants (ROHLOFF, 1967; RYDER, 1964).

In is known that LMV does not affect seed germination, but since dissemination by aphid is rapid, epidemics can quicly develop, even from a small number of infected seedlings. The virus is effiçiently spread by several aphid species, notably **Myzus persicae** Sulz., **Macrosiphum euphorbiae** Thos. and **Aphis gossypii**. Secondary spread by aphids is responsible for serious damage. A transmission percentage of 0.5 may give a total loss of crop when aphid vectors are active early in the season (GROGAN, **et al**, 1952; TOMLINSON, 1962). The earliest infected lettuce plants inoculated with LMV developed the

1) Adapted from a thesis submitted and approved for the degree of Doctor of Philiosophy of Aegean University.

highest rate of transmission, while seed from plants inoculated with the same virus after flowering were virus free (NEEGARD, 1977).

Lettuce plants infected through the seed develop mosaic followed by veinal necrosis and leaf puckering depending on the cultivars. Infected plants are stunted and frequently fail to head. Thus yield and also the quality of the crop are poor (TOMLINSON **et al**, 1970; HORVÂTH, 1980).

In the future, LMV could probably be controlled by using resistant cultivars. However, because most of the commercial cultivars in European Countries and U.S.A. are susceptible to the virus, control still largely depends upon the use of LMV-free seed stocks.

LMV is one of the most destructive virus followed by CMV in lettuces. In some areas of the world, this virus is more important than LMV. CMV is easily transmitted mechanically and through endosperm of the seed in 19 species of plants and it is the most common and destructive of the viruses affecting cucurbits. It causes mosaic and severe plant stunting. Leaves remain small, crinkled and often deformed. Fruits are also severely affected by distortion and discolaration. In lettuce, CMV causes of a yellowing and often occurs in several annual weeds common in lettuce fields like LMV (CROWLEY, 1957; TOMLINSON **et al**, 1970). CMV and LMV can be carried symptomless in **Sonchus asper**, in groundsel (**Senecio vulgaris**) and in **Chenopodium quinoa**. Seeds of these weeds may be abundantly present in soil (TOMLINSON **et al**, 1970; PHATAK, 1972).

These two viruses have been known for a long time on lettuce crop in Turkey (ÖZALP, 1964; TEKİNEL **et al**, 1969; YILMAZ, 1981). FİDAN and TÜRKOĞLU (1984) reported that lettuce crops had 5,4 infected rate with LMV in İzmir and its seed transmission in the same province by 8-9 percent based on the indicator plants and physical properties of the virus, ERKAN and SCHLÖSSER (1985) found that LMV was the most widespread virus and CMV was the second one, both transmitted by seed in the main lettuce growing locations in Turkey by using indicator plants, serology and particle morphology.

This study was undertaken to identify whether the certified seedlots of lettuce were infected with any seed-transmitted virus (es) being sent to the Seed Control and Certification Laboratory.

MATERIALS and METHODS

Lettuce seedlots were obtained from the Seed Control and Certification Laboratory. They consisted of 11.9 % (32 samples out of 269 samples as total) of the total seed being sent the laboratory for testing.

Extensive investigations were performed on suitable test plants for detection and differentiation of LMV and CMV. These include; **Chenopodium amaranticolor** Costa and Reyn, Chenopodium quinoa Wild., **Nicotiana debneyi** Domin., **Nicotiana glutinosa L.**, **Cucumis sativus** L. Seeds of indicator plants were sown periodically in trays to meet the requirement of the studies. The seedlings were then transferred into pots with the sterilized soil. Indicator test series were incubated at 3500 to 5000 lux light intensity for 16 hours a day at 18°-25° C (NOORDAM, 1973).

For mechanical inoculation, indicator plants were sprayed with 500 mesh carborandum and after inoculation were treated with water. Phosphate buffer containing 0.5 % sodium sulphite and 0.1 % tioglicolic acid were added. Then they were placed in the incubation room. In order to achieve pure isolates of the virus concerned, inoculations of single lesion were refered.

In three to four weeks, the inoculated plants were checked for the incidence of infection by serology. The sap of leaves from infected test plants showing clear syptoms were diluted with distilled water (1: 3, w/v) and centrifuged for 15 min. at 3000 rpm. Juice was cleared by sieving through fiber. The antisera to CMV and LMV (the original titres of LMV and CMV is 1/128) in lettuce were diluted as 1:4 with 0.9 % NaCl.

Performing the micro-precipitation test, a drop of inoculum (0.03 - 0.05 ml) were placed of a sterile microscope glass and diluted antisera were added onto it. Labelled glasses were then incubated at 25°C for an hour in wet petri dishes. At the end of this incubation period, the glasses were examined if any precipitation occurred under x80 to x100 magnification. The one that precipitated was recorded as +++, ++ or + depending upon the severity of precipitation.

In this paper, the term «Infected Seeds» refers to seed contaminated with virus in/on meaning that seed lots were a mixture of infected and healthy seeds.

RESULTS and DISCUSSION

32 lettuce samples received by the laboratory for being certified were tested by means of both biological and serological detection techniques. As symptoms occurred on test plants seemed to be LMV and/or CMV, after single-lesion inoculations, they were re-tested against antisera of these two viruses. According to the results, infection rate with LMV and CMV were estimated to be 66.7 % (eight

Table 1: Distribution of lettuce seed samples infected with LMV/CMV

Cultivar	No of samples		Amount (Kg) in Diff. Seed Classes						Total (Kg)	
	Total	LMV/CMV	Anaç	Cert.	Cont.	Being Cont.	Seed Ana. Rep.	Seed Rep.	Infected	Healthy
Yedikule 5701	14	4	—	—	900	700	44	—	644	1000
Şemikler	6	6	—	—	814	—	—	—	814	0
NR 7910	5	0	495	—	—	—	—	—	0	495
Paris Island	5	0	—	485	—	—	—	—	0	485
Yedikule 44	2	2	—	—	—	13	—	2.85	15.85	0
TOTAL	32	12	495	480	1714	713	44	2.85	1473.85	1975
%	100	37.5	14.35	13.92	49.70	20.67	1.28	0.08	42.73	57.27

samples) and 58.3 % (seven samples) respectively. Percentages of lettuce samples that contained both viruses was 42.73 % (five samples) (Table 1).

While **C. quinoa** showed systemic symptom by inoculation with LMV whereas **C. amaranticolor** gave local lesions only with the same virus. CMV caused local lesions on these two indicator plants. Some lettuce samples were infected with both LMV and CMV at the same time. They were distinguished each other by symptomatology and serology (MARROU und MESSIAEN, 1967).

As it is seen from the table 1, imported seeds of cvs. NR 7910 and Paris Island were both free of infection. This is an important indication that the production of virus free seed is crucial as KIMBLE **et al,** (1975) pointed out. In contrast to this, half of the seedlots received by the laboratory such as Yedikule cv. 44 and 5701, which cover most production areas in the region, were found to be totally or highly infected.

The earliest infected lettuce plants with LMV or CMV developed with the highest rate of transmission, while seed from plants inoculated with the same virus after flowering were virus free (NEEGARD, 1977)

CMV and LMV are transmitted by aphids to lettuce crop as non-persistent manner from the infectious plant in or around the field. These plants may be either lettuce plants grown by infected seed or several annual weeds common in lettuce fields. LMV can be carried symptomless in **Sonchus asper, Senecio vulgaris** and **C. quinoa**; where as CMV is seed-transmitted and often symptomless in **Senecio vulgaris, Urtica urens, Matricaria** spp. and **Capsella bursa-pastoris** (TOMLINSON, 1970; PROVVIDENTI, 1980).

Unfortunately, according to the rules. the inspecter classifies the crop by number of plants with symptoms in the field. According to the our experimental results, field controls need laboratory check of the seed samples with biological and / or serological tests regularly.

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We are grateful to Prof. Dr. H.L. PAUL, Biologische Bundesanstalt für Land and Fortstwirtschaft Institut für Viruskrankheiten der Pflanzen Baunschweig / West Germany for kindly supply on providing antisera of LMV and CMV.

Ö Z E T

SERTİFİKALI MARUL TOHUMLARINDA TOHUMLA TAŞINAN VİRUSLARIN SAPTANMASI

Bu çalışmada, Tohumluk Kontrol ve Sertifikasyon Laboratuvarına sertifikasyon amacıyla gönderilen marul tohum örneklerinde tohumla taşınan virüslerin varlığı araştırılmıştır. Sistemik ve lokal lezyon veren test bitkilerinin aşılanmasına dayanan indikatör bitki testi ve mikropresipitasyon serolojik testi, marul tohumlarıyla taşınan virüslerin araştırılmasında kullanılmıştır.

Laboratuvara gelen 32 marul tohum örneğinden, sekiz örnekte % 66.7 LMV, yedi örnekte % 58.3 CMV ve 5 örnekte de % 42.73 oranında iki virüs karışık olarak birlikte bulunmuştur. Sonuçlar tohumluk kontrol ve sertifikasyon açısından tartışılmıştır.

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Detection of Cucumber Mosaic Virus Strains by Latex Flocculation and Protein A-coated Latex-linked Antisera Tests

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ABSTRACT

Cucumber mosaic virus yellow strain (CMV-Y) and Zinnia strain (CMV-Z) were detected by latex flocculation test (LF test) in which gamma-globulin fraction of CMV-Y antiserum coated by latex particles and by protein A-coated latex-linked antisera test (PALLAS test) in which latex particles coated with protein A before sensitization with antibody were used. Both serological tests, can aesily be applied to CMV strains and can detect them in very short time. Crude saps and purified preparations of both strains were used as antigen. Better results were obtained by PALLAS tests than those of LF test.

INTRODUCTION

The mosaic type virus infections of cucurbitaceous plants cause similar symptoms which can not be differentiated visually. Therefore, the indicator plant reactions and the serological assays play important roles for detection of those virus infections. The serological assays are usually specific and sensitive enough to detect the viruses even in individual insects (Hibino and Kimura, 1982). They also provide the rapid detection of plant virus infections.

The latex flocculation (LF) test was found to be a rapid and useful procedure for detecting plant viruses (Bercks and Querfurth, 1969, 1971, Koenig et al., 1979, Omura et al., 1984, Somowiyarjo et al., 1987). Using the latex coated with antibody or its gamma-globulin fraction, much lower virus concentrations could be detected than with slide precipitin or agar gel doublediffusion test (Bercks and Querfurth, 1971). However, the test is not adaptable if the avaible antiserum has low titer or contain some inhibitory compounds (Somowiyarjo et al., 1987). These disadvantages could be successfully overcome by coating the latex particles using protein A before sensitization with antibody

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and this procedure was called latex-linked antisera (PALLAS) test (Querfurth and Paul, 1979).

This paper described a comparison of the sensitivity of LF and PALLAS tests for detecting cucumber mosaic virus yellow and Zinnia (CMV-Y and CMV-Z) strains.

CMV strains: Y and Z strains of CMV were maintained in tobacco (**Nicotinia tabacum** var. «Samsun NN) plants at Saga University, Plant Pathology Department and purified as described in Özyanar and Sako (1978) 6 days after inoculation. The tests were done with purified preparations and crude extracts of infected tobacco plants. The crude extracts used in FL test were prepared by grinding 1.0 g leaves with 1.0 ml 0.05 M Tris-HCl buffer, pH 7.2, containing 0.02 % Tween 20 and 0.02 % polyvinylpyrolidone (PVP) 40 000 (Tris-HCl buffer-TPO) in a mortar with pestle. After squeezing through two layers of gause, the sap was then centrifuged at 6 000 rpm for 15 min and was used as antigen in the tests. The procedure was also used to prepare antigen in PALLAS test, except that the extraction medium was 0.1 M glycine buffer, pH 8.2, containing 1.0 % saline, 0.02 % Tween 20, and 0.02 PVP (glycine buffer-STPO).

CMV antiserum: CMV-Y antiserum was kindly supplied by Mr. Maeda (Okayama University, Japan) and was used throughout the research Gamma-globulin from rabbit antiserum against CMV-Y was prepared by a method as described by Clark and Adams (1977).

LF test: The assay method of Bercks and Querfurth (1971) was adopted in this experiments. The tests were carried out with a suspension of Bacto latex 0.81 μm (Difco Laboratories, Detroit, Michigan) which had been sensitized with gamma-globulin of CMV antiserum. In the preliminary tests, gamma-globulin concentration of 100 μg/ml resulted in best detection of several concentrations tested.

Latex was firstly sensitized by diluting 1 part of latex suspension with 14 parts of 0.15 M NaCl. The mixture of an equal volume of gamma-globulin in 0.05 M Tris-HCl buffer, pH 7.2, and the diluted latex suspension was incubated 1 hr at 25°C with occasional stirring. It was then twice washed by a centrifugal force at 6000 rpm for 30 min, sucking the supernatant and resuspending the pellet with Tris-HCl buffer-TPO. The final pellet was resuspended in the same buffer containing 0.02 sodium azide (NaN_3) and was stored at 4°C before use.

The tests were done in polystyrene microtitre plates (Wako Pure Chem. Ind. Ltd.) The sensitized latex was used by adding 25 μl suspension to 50 μl antigen preparation. The plates were shaken at about

100 oscillations per minute for 15 min at 30°C. Detection end point was determined as the as the highest dilution of extracts from infected leaves or minimum concentration of purified virus which resulted in visible flocculation.

The reactions were investigated under stereoscobic microscope.

PALLAS test: This procedure was employed according to the method of Querfurth and Paul (1979). In this tests, latex particles were coated with protein A before sensitization with antibody. Protein A (Zymer Laboratories) was dissolved in distilled water containing 0.05 % NaN_3 at a concentration of 1.0 mg/ml. Before used, it was further diluted 200 times with 0.1 M Glycine buffer pH 8.2 containing 1.0 % NaCl. Latex particles were coated with protein A by mixing an equal volume of latex suspension diluted 1:14 with 0.15 M NaCl diluted protein A. The mixture was incubated at 20°C for 4 hr with occasional stirring.

It was then further incubated overnight at 4°C. To the final suspension, 0.5 % NaN_3 was added. The protein A-latex complex was coupled with gamma-globulin by mixing an equal volume of solution and 100 µg/ml gamma-globulin from rabbit antiserum diluted in Glycine buffer-STPO containing 0.02 % PVP, 0.02 % Tween 20 and 1.0 % NaCl. The mixture was then incubated and washed in the same way as those for coating of the latex with protein A. To the ready PALLAS complex, 0.05 NaN_3 was added.

The flocculation procedure of PALLAS test was principally identical to of LF test. In both tests, shaking time of 15 min and incubation time of 30 min at 25°C were the best conditions.

Extraction buffer and healthy tobacco sap were used as controls in both assays.

RESULTS and DISCUSSION

Results of both assays were estimated as shown in the tables 1 and 2.

Table 1. Detection of furified CMV strains by latex flocculation (LF) and protein A-coated latex linked antisera (PALLAS) tests [a]

Test	Virus strain	Visible flocculation at virus concentration (μg/ml) [b]				
		100	10	5	1	0.1
LF	CMV-Z	9/9	9/9	0/9	0/9	0/9
	CMV-Y	9/9	9/9	0/9	0/9	0/9
PALLAS	CMV-Z	9/9	9/9	9/9	0/9	0/9
	CMV-Y	9/9	9/9	9/9	0/9	0/9

a) Data of three experiments using three wells at each.

b) Numerator: Number of wells showed flocculation.
Denaminator: Number of wells used.

Table 2. Detection of CMV strains in crude extracts of infected tobacco leaves by latex flocculation (LF) and protein A-coated latex linked antisera (PALLAS) tests [a]

Test	Virus strain	Visible flocculation at dilution of [b]				
		10	100	100	10 000	100 000
LF	CMV-Y	9/9	9/9	0/9	0/9	0/9
	CMV-Z	9/9	0/9	0/9	0/9	0/9
	Healthy	0/9	0/9	0/9	0/9	0/9
PALLAS	CMV-Y	9/9	9/9	9/9	0/9	0/9
	CMV-Z	9/9	9/9	0/9	0/9	0/9
	Healthy	0/9	0/9	0/9	0/9	0/9

a) Data of three experiments using three wells at each.

b) Numerator: Number of wells showed flocculation.
Denaminator: Number of wells used.

As shown in Table 1, minimum detectable concentration of purified CMV-Y and CMV-Z strains by LF test was 10 μg/ml, for both strains compared with 5 μg/ml by PALLAS test. When the antigens in crude extracts were examined, PALLAS test proved to be more sensitive than LF test, resulting in 1000 times for CMV-Y and 100 times for CMV-Z where as these results were 100 times for CMV-Y and 10 times for CMV-Z respectively in LF test. No flocculation was observed when crude extracts of healthy tobacco extracts were tested (Table 2.)

Accoding to the data obtained, it was found that sensitivity of PALLAS test for detecting CMV strains was higher than of LF test.

In PALLAS test, protein A enables gamma-globulin to attach perfectly onto latex particles. It is also possible that if protein A becomes attached to the Fc portion of gamma-globulin, the antigenic sites of all the globulin molecules should be free to react with antigen (Somowiyarjo et al., 1987).

Since the other serological procedures were also found to be useful for detecting CMV (Özyanar and Sako, 1987), the present results will extend the possibilities to choose the best detection method for CMV that archieves the most accuracy under certain experimental condition.

Ö Z E T

HIYAR MOZAYIK VİRUS IRKLARININ LATEX ÇÖKTÜRME VE PROTEİN-A KAPLI LATEX'E BAĞLI ANTİSERUM TESTLERİ İLE TESBİTİ

Hıyar mozayık virusunun sarı (CMV-Y) ve Zinnia (CMV-Z) ırkları, latexle kaplı CMV-Y antiserumunun kullanıldığı latex çöktürme (LF testi) ve antikorlarla hassaslaştırılmadan önce latex partiküllerinin protein A ile kaplandığı antiserumun kullandığı protein A kaplı latexe bağlı antiserum testi (PALLAS testi) ile serolojik olarak tesbit edilmiştir. Her iki testte de, CMV-Y antiserumunun gamma-globulin fraksiyonu kullanılmıştır. Yapılan çalışma sonucunda her iki testinde, CMV ırklarına kolaylıkla uygulanabildiği ve kısa bir süre içinde tespit ettiği görülmüştür. Testler birbirleri ile kıyaslandığında, PALLAS testinin, LF testine oranla daha hassas olduğu görülmüştür.

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Investigations on the Detection and Seed Transmissions of the Virus Diseases Occurring on the Pulse Crops in Aegean Region

I. The identification of viruses infecting pulse crops in Aegean Region

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ABSTRACT

Leaf samples were collected from the pulse crops growing areas in Aegean Region. Mechanical inoculations were made to the certain test plants. The viruses in the samples were detected according to the symptoms on test plants. In further studies, the dilution end points and thermal inactivation points of the viruses in question were determined. The detection of the viruses was completed by serological tests.

INTRODUCTION

Pulse crops can be easily grown in various climatical conditions. They contain high percentage of protein and the **Rhizobium** bacteria in their roots that can fix free nitrogen of air and convert it into the form which plants can use. Therefore, these crops are grown by progressively increasing interest by the growers (39).

According to the statistical data, pulse crops have covered 9,7 % acreage devoted to the field crops in Turkey. With an cultivated area of about 10 and the production of 9 %, pulse crops (chickpea, broad bean, bean, lentil, cowpea, pea, soybean) are grown in 8 Provinces (Aydın, Balıkesir, Çanakkale, Denizli, İzmir, Manisa, Muğla, Uşak) of Aegean Region (4).

It is certain that various pests and diseases attack these economically important crops. Although it may change according to the variety, it is obvious that the crop losses due to the virus diseases are about 20 % (12). Another important fact is that the viruses of pulse crops are mostly seed-transmitted. Main viruses infecting broad bean, bean and Soybean in Turkey have been reported previously 2, 10, 13, 16, 32, 35, 38, 42, 44, 46, 47).

This study has been conducted from 1985 to 1988 in Aegean Region in order to determine the virus diseases affecting pulse crops. Surveys

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were carried out in 1985-1986 to collect the leaves of plants with virus-like symptoms.

MATERIAL and METHODS

Leaves of chickpea, broad bean, bean, lentil, cowpea, pea and soybean with virus-like symptoms are collected from the eight Provinces of Aegean Region. Then, these samples were homogenenized with mortar and pestle using 0,1 - 0,01 M phosphate buffer (pH. 7.0). For bean samples 0,1 % 2-Mercapto ethanol and for soybean samples citrate buffer and 0.1 % 2-Mercapto ethanol were added to this buffer. In addition, small amount of carborandum dust (500 Mesh) was added into the inoculum as an abrasive. Then, the inoculum was applied to host plants mechanically. After inoculations the leaves were rinsed with tap water. Primary leaves of leguminosae plants, cotyledon leaves of cucurbit plants and 3-4 true-leaf of other hosts were used for inoculations (Table-1). The test plants were observed after inoculation for symptom development.

The sap of systemically infected leaves of pulse crops cultivars and **N. clevelandii** were used as the source of inoculum in the test of dilution-end point and thermal inactivation point. These tests were made on **C. amaranticolor** and **C. quinoa.**

The serological techniques used were micropresipition drop tests on slides (5). Antisera were kindly supplied from L. Large (Denmark) and Dr. H.L. Paul (W Germany).

RESULTS AND DISCUSSION

Mechanical inoculations were made to the test plants and 102 of virus isolates were obtained from 225 samples. These isolates were put into groups considering that they produced some symptoms on test plants. Then, 13 isolates were selected for the detection of viruses on the wider, host range and viruses were identified by their host reactions in Table 1. The physical properties of the isolates are shown in Table 2. The isolates which showed positive serological reactions are in Table 3.

Thirteen virus isolates were identifield by host range, symptoms produced on differential and diagnostic hosts, physical properties and serology.

Two different alfalfa mosaic virus (AMV) strains (chickpea N-3 and lentil M-2 strain) were found according to the symptoms produced on test plants. Two isolates induced local lesion on **C. amaranticolor** and **C. quinoa**. N-3 isolate caused chlorotic ring spot and necrotic local

lesion on **N. tabacum** «White Burley. M-2 isolate gave rise to necrotic local lesion on same plant (Fig. 1-2). These differences may result from the use of different strains (9, 23, 24, 26, 28).

Bean yellow mosaic virus (BYMV) (F-3 and F-67 isolates from bean and Be-2 isolate from pea) produced chlorotic local lesion and mosaic on **C. amaranticolor.** Ba-2 isolate (broad bean) of the same virus had chlorotic local lesion on same plant. Ba-2, F-3, F-67 isolates induced chlorotic local lesion on same plant. Ba-2, F-3, F-67 isolates induced chlorotic ring spots and necrotic local lesions on **C. quinoa** (Fig. 5). These differences may result from the use of differant strains (6, 9, 21, 27, 31, 36, 45, 47).

Broad bean stain virus (BBSV) as a second virus was identified on broad bean. caused local lesions on **C. quinoa** and **V. unguiculata** but not symptoms on **C. amaranticolor** (Fig. 3). These symptoms confirmed the findings in previous reports (9, 15, 18, 20, 33, 39).

Bean common mosaic virus (BCMV) has widespread distribution on beans in Aegean Region. F-62 isolate (Bean) caused symptoms on test plants. These symptoms were similar to that of previous reports (2, 7, 9, 36, 38).

Cowpea aphid-borne mosaic virus (CabMV) produced local lesions on **C. amaranticolor, C. quinoa, N. tabacum** «White Burley» but no symptoms on **N. glutinosa, G. globosa, C. sativus** and **N. clevelandii** (Fig. 4). These results were similar to those in literatures (9, 14, 17, 36).

A second virus also isolated from cowpea was cucumber mosaic virus (CMV). Bö-26 isolate caused local lesions on **C. amaranticolor, C. quinoa, C. album, N. tabacum** «White Burley» and systemic mosaic on **N. clevelandii, N. gutinosa** and **C. sativus.** These results have also been confirmed by literature (19, 30, 36, 40).

Second virus was identified as pea seed borne mosaic virus (PsbMV) on pea. Be-9 isolate caused local lesions on **C. amaranticolor, C. quinoa, C. murale** but no symptoms on **C. sativus, N. tabacum** «Samsun» **V. unguiculata** and **D. stramonium.**

The results obtained from these studies have agreed with the studies made in various countries (1, 22, 34, 41).

Soybean mosaic virus (SMV) (S-1 isolate) induced local lesion on **C. amaranticolor, C. quinoa, C. album** but no symptoms in **C. globosa. C. sativus, D. stramonium.** However, some workers (8, 11, 13, 25, 43, 48) have reported positive results.

The certain physical properties of viruses under study were deter-

mined. Dilution end points for all isolates were 10^{-3} - 10^{-4} in exception of chickpea and lentil isolate of AMV which was found to be 10^{-2} - 10^{-3}.

Thermal inactivation points of the isolates were as follows: 50-55°C for BYMV F-3 isolate; 55-60°C for Ba-2, F-67, F-62, M-2, Be-2, Be-9 and S-1 isolates; 60-65°C for N-3, Ba-1, Ba-19, Bö-1 and Bö-26 isolates. The physical properties (dilution end points and thermal inactivation points) of these isolates were also confirm by the data in the previous works (6, 7, 8, 9, 18, 22).

Serological assays were performed with the antisera supplied from abroad according to the microprecipitin test. Positive reactions were obtained between N-3, M-2 isolates and AMV; Ba-1, Ba-19 isolates and BBSV; Ba-2, F-3, F-67, Be-2 isolates and BYMV; F-62 isolates and BCMV; Bö-1 isolate and Ca-bMV; S-1 isolate and SMV.

As a result of this study, broad bean stain virus and pea seed-borne mosaic virus are the first records and bean yellow mosaic virus on pea and alfalfa mosaic virus on chick pea and lentil seem also to be the first record for Turkey.

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Ö Z E T

EGE BÖLGESİNDEKİ BAKLAGİL BİTKİLERİNDE GÖRÜLEN VİRUS HASTALIKLARININ TANILANMASI VE TOHUMLA TAŞINMA DURUMLARININ BELİRLENMESİ ÜZERİNE ARAŞTIRMALAR

1. EGE BÖLGESİNDE YEMEKLİK BAKLAGİLLERDE GÖRÜLEN VİRUS HASTALIKLARININ TANILANMASI

Ege Bölgesinde yemeklik baklagillerde görülen virus hastalıkları, test bitkilerinde oluşturduğu belirtiler fiziksel özelliklerine ve serolojik testlere göre tanılanmıştır. 1985-1986 yıllarında Ege Bölgesi baklagil ekiliş alanlarında virus belirtisi gösteren 225 örnek toplanmıştır. Bu örneklerin test bitkilerine yapılan inokulasyonları sonucunda 102 virus izolatı elde edilmiştir. İzolatlar, test bitkilerinde oluşturduğu belirtilere göre gruplandırılmış ve tanılama çalışmaları 13 izolatla, konukçu dizileri genişletilerek yürütülmüştür.

Table 1. Reactions of test plant used for identifying the virus diseases on pulse

Viruses	AMV	BBSV		BYMV		
Isolates / Test Plant	N. 3	Ba-1	Ba-19	Ba-2	F.3	F.67
C. amaranticolor	NLL	—	—	KLL	KLL, Mo	KLL
C. quinoa	NLL	NLL	NLL	KLL	KLL	NLL
C. album	KLL	KLL	KLL	KLL	KLL	KLL
C. murale	KLL, Mo	NLL, Mo	KLL, Mo	NLL, Mo	Mo	—
C. hybridium	NLL	—	—	—	—	—
N. tabacum «Samsun»	—	—	—	—	—	—
N. tabacum W.B	NLL, Mo	—	—	—	—	—
N. glutinosa	NLL, Mo	—	—	—	—	—
N. clevelandii	NLL, Mo	—	—	Mo	Mo	Mo
N. rustica	NLL	—	—	NLL	—	—
G. globosa	NLL	—	—	NLL	NLL	NLL
P. hybrida	NLL, Mo	—	—	—	—	—
D. stromonium	NLL	—	—	—	—	—
T. hybridium	—	—	Mo	—	Mo	Mo
T. incarnatum	—	—	—	—	—	—
T. repens	Mo	—	—	Mo	—	Mo
M. sativa	—	—	—	—	—	Mo
M. lupiluna	—	—	—	Mo	—	Mo
M. alba	Mo	Mo	—	Mo	—	—
L. hrsitus	—	—	—	Mo	Mo	—
L. angustifolius	—	—	—	—	—	—
C. sativus	—	—	—	—	—	—
V. unguiculata «Blackeye»	NLL	NLL	NLL	—	Mo	Mo
V. faba	—	Mo	Mo	Mo	—	—
V. sativa	—	—	Mo	—	Mo	Mo
P. vulgaris «Pinto»	—	—	Mo	NLL, Mo	Mo	NLL
«Pince»	—	NLL	—	—	Mo	—
» «Red Kidney»	Mo	Mo	—	Mo	NLL, Mo	Mo
» «Black Turtle»	Mo	—	—	NLL	Mo	Mo, Ö
» «Sanilac»	NLL, Mo	—	—	Mo	—	—
» «I.59 GV»	—	—	NLL	NLL, Mo	Mo	NLL
» «Ibian»	—	—	—	—	Mo	—
P. sativum «Miva	—	Mo	Mo	Mo	—	—
G. max «Lincalin»	—	—	—	—	—	—
C. arietinum	Mo, Ö	—	—	—	—	—

Db: Vein banding Dn: Vein nekrosis KL

crops

	BCMV	AMV	CabMV	CMV	BYMV	Ps-bMV	SMV
	F.62	M.2	Bö.1	Bö.26	Be.2	Be.9	S.1
Mo	KLL	KLL	KLL	KLL	KLL, Db	KLL	KLL
Mo	KLL	NLL	NLL	NLL, Mo	NLL	NLL	KLL
	KLL	KLL	KLL	KLL	NLL	KLL, Mo	KLL
	Mo	KLL, Mo	—	KLL	KLL	KLL, Mo	—
	—	—	—	—	—	—	—
	—	—	NLL	—	—	—	—
	NLL	NLL	NLL	NLL	—	—	—
	—	NLL, Mo	—	Mo	—	—	—
	—	NLL, Mo	—	Mo	Mo	—	—
	Mo, Ö	NLL	Mo	NLL	—	—	Mo, Dn
	—	NLL	NLL	KLL	NLL	—	—
	—	Mo	—	Mo	—	—	Mo
	—	NLL	—	KLL, Mo	—	—	—
	—	Mo	—	—	Mo	Mo	—
	—	—	—	—	—	—	—
	Mo	—	—	—	—	—	—
	Mo	—	Mo	Mo	—	—	—
	—	—	—	Mo	—	—	—
	Mo	—	—	Mo	—	—	—
	Mo	—	Mo	—	—	Mo	—
	Mo	—	—	—	—	—	Mo
	—	—	—	Mo	—	—	—
	NLL, Mo	NLL	Mo, Db	Mo	—	—	Mo
	—	—	—	—	—	—	—
	—	—	—	—	—	Mo	—
Mo	NLL	NLL	—	—	—	—	—
	Mo	Mo	—	—	—	—	—
	Mo	NLL, Mo	—	—	—	—	—
	Mo	—	Mo	—	—	—	Mo, Dn
	—	—	—	—	—	—	—
	NLL, Mo	—	—	—	—	—	—
	—	—	—	—	—	—	—
	—	Mo	—	—	Mo	Mo, Db	—
	—	—	—	—	—	—	Mo, Dn
	—	—	—	—	—	—	—

: Clorotic local lesion Mo: Mosaic NLL: Necrotic local lesion

Table 2. The some physical properties of isola

Viruses	AMV	BBSV		BYMV		
Isolates	N-3	Ba-1	Ba-19	Ba-2	F-3	F-
Dilution and Point	10^{-2} - 10^{-3}	10^{-3} - 10^{-4}	10^{-3} - 10^{-4}	10^{-3} - 10^{-4}	10^{-3} - 10^{-4}	10^{-3}
Thermal inac Point (°C)	60 - 65°C	60 - 65°C	60 - 65°C	55 - 60°C	50 - 55°C	55 -

tes from pulse crops.

	BCMV	AMV	Ca-bMV	CMV	BYMV	Ps-bMV	SMV
7	F-62	M-2	Bö-1	Bö-26	Be-2	Be-9	S-1
10^{-4}	10^{-3} - 10^{-4}	10^{-2} - 10^{-3}	10^{-3} - 10^{-4}	10^{-3} - 10^{-4}	10^{-3} - 10^{-4}	10^{-3} - 10^{-4}	10^{-3} - 10^{-4}
60°C	55 - 60°C	55 - 60°C	60 - 65°C	60 - 65°C	55 - 60°C	55 - 60°C	55 - 60°C

Table 3. Serological reactions of isolates from pulse crops

Isolates	Antiserum										
	AMV	BBSV	BYMV	BCMV	BBTMV	CMMV	Ca-bMV	CpMV	CMV	Ps-bMV	SMV
N-1	++	—	—	—	—	—	—	—	—	—	—
Ba-1	+	+++	—	—	—	—	—	—	—	—	—
Ba-19	+	+++	—	—	—	—	—	—	—	—	—
Ba-2	+	—	++	—	—	—	—	—	—	—	—
F-3	—	—	++	—	—	—	—	—	—	—	—
F-67	—	—	++	—	—	—	—	—	—	—	—
F-62	—	—	—	++	—	—	—	—	—	—	—
M-2	++	—	—	—	—	—	—	—	—	—	—
Bö-1	—	—	—	—	—	—	++	—	—	—	—
Bö-26	—	—	—	—	—	—	—	—	++	—	—
Be-2	—	—	—	—	—	—	—	—	—	—	—
Be-9	—	—	—	—	—	—	—	—	—	++	—
S-1	—	—	—	—	—	—	—	—	—	—	++

Araştırma bölgesindeki nohut ve mercimeklerde Yonca Mozayık Virusu (AMV); baklalarda Bakla Benek Virusu (BBSV) ve Fasulya Sarı Mozayık Virusu (MYMV); fasulyelerde, Fasulye Adi Mozayık Virusu (BCMV) ve Fasulye Sarı Mozayık Virusu; börülcelerde Börülce Afid Kökenli Mozayık Virusu (Ca-bMV) ve Hıyar Mozayık Virusu (CMV); bezelyelerde, Fasulye Sarı Mozayık Virusu (=Bezelye Mozayık Virusu) ve Bezelye Tohum Kökenli Mozayık Virusu (Ps-bMV) ve soya fasulyelerinde Soya Fasulyesi Mozayık Virusu (SMV) saptanmıştır.

Saptanan virusların fiziksel özellikleri araştırılmış, son seyreltme noktası; AMV'nun 10^{-2} - 10^{-3} diğer izolatların ise 10^{-3} - 10^{-4} olarak saptanmıştır. Sıcaklıkla inaktifleşme noktası BYMV (F-3 izolatı) 50-55°C AMV (M-2 izolatı), BYMV (Ba-2 ve Be-2 izolatları), Ps-bMV ve SMV için 55-60°C, AMV (N-3 izolatı) BBSV, Ca-bMV, CMV için 60-65°C olarak bulunmuştur.

Serolojik testler, yurt dışında sağlanan antiserumlarla mikropresipitin yöntemiyle yapılmıştır.

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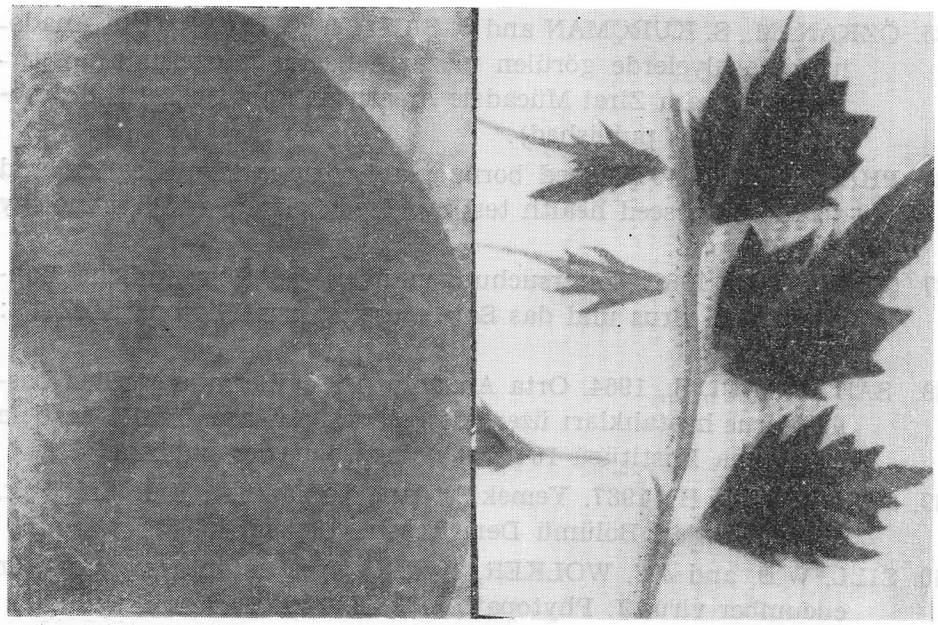
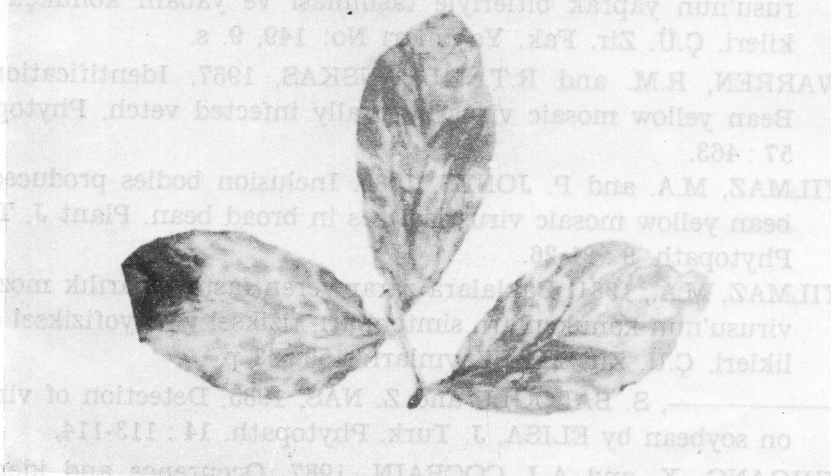
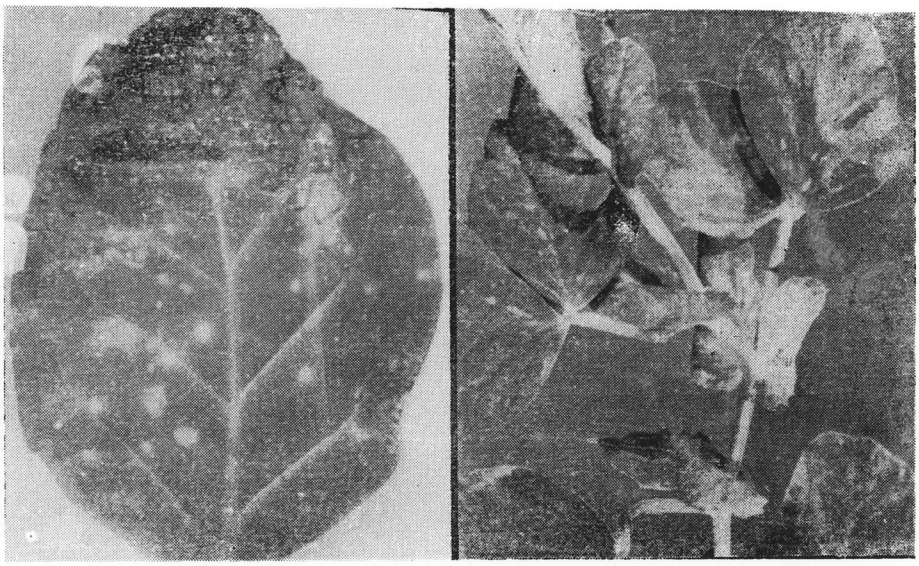


1. Leaf of N. tabacum «White Burley» showing concentric ring spots caused by AMV (Chicpea isolate).

2. Leaf of chicpea showing systemic mosaic and malformation caused by AMV.



3. Symptoms caused by broadbean stain virus on broad bean.



4. Leaf of **N. tabacum** «Samsun» showing local lesions caused by C a-b MV.

5. Symptoms caused by PMV on pea.

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Studies on Chemical Control of Sunflower Downy Mildew (**Plasmopara helianthi** Novot.) and on Resistance of the Pathogen to Metalaxyl

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ABSTRACT

The effects of Previcur N (propamocarp hydrochloride, 722 g/l, w/v), Ceresan P (quintozene, 180 g/kg, w/w) and Aprin 35 SD (metalaxyl, 350 g/kg, w/w) are determined on downy mildew of sunflower (**Plasmopara helianthi** Novot.) as seed treatment. Besides, to find whether **P. helianthi** acquires the resistance to metalaxyl, it is carried out by small field plots and under the controlled conditions.

It is seen that Aprin 35 SD is effective to downy mildew but Previcur N and Ceresan P are not. It is determined that the sensitivity of **P. helianthi** decreases to low doses of Aprin 35 SD but does not to above the dose of 200 gram and the disease occurs at the dose of 200 g as from 12th transfer.

INTRODUCTION

Plasmopara helianthi Novot., which is a soil and seed borne pathogen, causes one of the most important disease of sunflower i.e. downy mildew. Under the favourable conditions, pathogen may cause disease on average 70-80 % of the plants in the field and give rise to plant loss at the important rate (Zimmer, 1971). In Aegean Region, farmers sow over again when the great amount of seedlings are destroyed by the pathogen during the wet days after sowing.

Fungicide treatments to control the disease could not be efficient until the fungicides of phenylamide group have been thrown on the market. Investigations on this matter brought to light that the fungicides in question were highly effective to downy mildews on many plants especially (Cohen et al., 1979; Crute, 1980). It was found that there was also a similar effect on downy mildew of sunflower (Sackston, 1981; Vernescu and Iliescu, 1981; Maden, 1982; Nikolov, 1982).

In this study, it has been investigated the effects of Previcur N, Ceresan P and Aprin 35 SD to downy mildew as seed treatments. In addition, because of the capacity of the pathogen to build up resistance to metalaxyl when it causes symptoms on the leaves (Davidse

et al., 1981), it has also been studied whether the pathogen can acquire resistance to this fungicide when applicated as seed dressing.

MATERIALS and METHODS

Seeds of Vniimk 8931 which is known to be a sensitive variety to **P. helianthi** and the fungicides in Table 1 were used. Treated seeds were sown in the infested plots.

Table 1. The fungicides used to downy mildew of sunflower (**P. helianthi**) and doses

Fungicides	Firm	a.i. and percentage	Formulation	Dosage prep./100 kg seeds
Aprin 35 SD	Ciba-Geigy	metalaxyl, 350 g/kg w/w	SD	600 gram
»	»	»		500 »
»	»	»		400 »
»	»	»		300 »
»	»	»		200 »
»	»	»		100 »
Previcur N	Türk-Hoechst	propamocarp hydrochloride, 722 g/l, w/v	LC	2 liter
»	»			1 »
Ceresan P	Bayer	quintozene, 180 g/kg w/v	Dust	500 gram
»	»			400 »

Small Plot Experiments: Treatments were done as a seed-dressing before sowing. For homogeneous treatment with dust formulated fungicides, seeds were moistened before not being mixed fungicides (1 liter water/100 kg seeds at Aprin 35 SD), then fungicides were added and mixed completely. With liquid formulated fungicide, seeds and fungicide were shaken together. To facilitate the disease emergence, plots were watered every other day by beginning just after sowing. When the plants were at the stage of four leaves, diseased and healthy plants were counted and then disease ratio was determined. Efficacy of fungicides were calculated according to Abbott and the differences among the efficacies were found out by F tests. Experiments were designed in accordance with randomized blocks with three replicates.

In 1985, the characters of the experiments consisted of 400, 500, and 600 gram of Aprin 35 SD, 1 and 2 liter of Previcur N and 400 and 500 gram of Ceresan P and control.

In 1986, on the other hand, the characters were made up of 100, 200, 300, 400, 500 and 600 gram of Aprin 35 SD and control.

Laboratory Experiments: Sporangium suspension was prepared by using the leaves with sporangia of the diseased plants at the dose which the disease occured. Seeds were artifically inoculated with sporangium suspension according to Cohen and Sackston (1973). Pathogen was transferred to high doses from low ones.

The seeds treated with 100, 200, 300, 400, 500 and 600 gram/100 kg seeds doses of Aprin 35 SD were allowed to germinate on sterile blottting paper for 3 days. Germinated seeds were immersed in a suspension of 100.000 sporangia/ml in distilled water at 18°C for 4 h and then they were sown in pots.

Experiments were designed according to randomized plots with 7 characters (6 doses + 1 control) and 5 replicates in 1986 and 4 replicates in 1987. 15 seeds in 1986 and 20 seeds in 1987 were sown per pot.

The pots were placed on benches illuminated with 40 w fluorescent tubes. Light intensity was about 13.000 lux. Day lenght was 14 h and temperature was 20 ∓ 2°C.

Ten day old infected seedlings were covered with plastic bags for about 20 h in order to stimulate the sporulation on cotyledons.

Evaluations were done by counting the diseased and healthy plants per pot and the disease ratio was determined at the characters.

Above mentioned procedures were repeated 4 times in 1986 and 14 times in 1987 with the diseased plants at the dose which the disease occured.

RESULTS

Small Plot Experiments: Mean disease ratio and percentage of effects of fungicides from the experiment in 1985 are seen in Table 2.

Table 2. Mean disease ratio and percentage of effects of fungicides in the plots (1985)

Fungicides	Mean disease ratio [x]	Effect % [x]
Aprin - 400 g	0	100 a [xx]
» - 500 g	0	100 a
» - 600 g	0	100 a
Previcur - 1 l	18,65	49,46 b
» - 2 l	12,10	65,88 c
Ceresan - 400 g	31,08	18,01 d
» - 500 g	26,64	30,35 e
Control	38,28	

x : Values are the means of three replicates.

xx : Any two means designated by the same letter are not signaficantly different (Duncan's multiple range test, P=0,01).

The data from the experiment in 1986 are also given in Table 3.

Table 3. Mean disease ratio and percentage of effects of fungicides in the plots (1986)

Fungicides	Mean disease ratio [x]	Effect % [x]
Aprin - 600 g	0	100
» - 500 g	0	100
» - 400 g	0	100
» - 300 g	0	100
» - 200 g	0	100
» - 100 g	8,3	80,56
Control	45,5	

x : Values are the means of three replicates.

Laboratory Experiments: Mean disease ratio and percentage of effects of fungicides from transfers in 1986 are seen in Table 4.

Table 4. Disease ratio and percentage of effects of fungicides from transfers in 1986

Fungicides	Disease ratio x				Effect % x				
	Transfer no				Transfer no				
	1	2	3	4	1	2	3	4	mean
Aprin - 600 g	0	0	0	0	100	100	100	100	100
» - 500 g	0	0	0	0	100	100	100	100	100
» - 400 g	0	0	0	0	100	100	100	100	100
» - 300 g	0	0	0	0	100	100	100	100	100
» - 200 g	0	0	0	0	100	100	100	100	100
» - 100 g	11,9	13,3	14,6	10,6	88,1	86,7	85,4	89,4	87,4
Control	100	100	100	100					

x : Values are the means of five replicates.

The results obtained from transfers in 1987 are also given Table 5.

Table 5. Disease ratio obtained from transfers in 1987

Transfer no	Disease ratio x						
	100 g	200 g	300 g	400 g	500 g	600 g	Control
1	10,0	0	0	0	0	0	100
2	13,7	0	0	0	0	0	100
3	11,2	0	0	0	0	0	100
4	12,5	0	0	0	0	0	100
5	11,2	0	0	0	0	0	100
6	8,7	0	0	0	0	0	100
7	15,0	0	0	0	0	0	100
8	13,7	0	0	0	0	0	100
9	17,5	0	0	0	0	0	100
10	18,5	0	0	0	0	0	100
11	21,2	0	0	0	0	0	100
12	22,5	6,2	0	0	0	0	100
13	21,2	7,5	0	0	0	0	100
14	25,0	8,7	0	0	0	0	100

x : Values are the means of four replicates.

DISCUSSION

During the years when sunflower sowing time is rainy, chemical control of downy mildew is necessary owing to the fact that farmers have to sow sunflower over again.

According to the results of 1985, there are the effects 100 % at the doses of 400, 500 and 600 gram/100 kg seeds of Aprin 35 SD; 49,46 % and 65,88 % at the doses of 1 and 2 liter/100 kg seeds of Previcur N; 18,1 % and 30,35 % at the doses of 400 and 500 gram/100 kg seeds of Ceresan P respectively (Table 2). From these data, it follows that Previcur N and Ceresan P are not effective to downy mildew. However, it is seen that Aprin 35 SD is the most effective.

Metalaxyl controlled downy mildew of sunflower successfully as reported for the other downy mildews (Cohen et al., 1979; Crute, 1980). But metalaxyl is one of the active integridents having high resistance risk (Davidse et al., 1981). In addition, it was determined that there were some differences among the isolates of downy mildew of sunflower (Delen et al., 1985).

From the small plot experiments in 1986, it is seen that the pathogen is sensitive to the doses of 200, 300, 400, 500 and 600 gram/100 kg seeds of Aprin 35 SD. On the other hand, the disease ratio is 8,3 % at the dose of 100 gram (Table 3). Inoculum prepared by using the diseased plants at this dose was transferred four times to high doses from low ones. In all transfers, the disease did not occur at the dose of 200 gram and above, but the disease ratios were 11,9 %, 13,3 %, 14,6 % and 10,6 % at the dose of 100 gram (Table 4).

In 1987, the transfer procedures could be carried out 14 times and the disease did not occur at the dose of 200 gram and above until 11th transfer. In 12th, 13th and 14th transfers, the disease ratios were 6,2 %, 7,5 % and 8,7 % respectively at the dose of 200 gram. On the other hand, although the disease ratios were between 8,7 % and 15 % at the dose of 100 gram, they kept increasing from 9th transfer to 14th transfer. Although the disease ratio was 17,5 % in the 9th transfer, it rose to 25 % in 14th transfer.

The fact that the disease ratios increase gradually both at the dose of 100 gram and the dose of 200 gram as from 12 th transfer shows that the sensitivity of the pathogen decreases gradually to Aprin 35 SD.

Although under the laboratory conditions pathogen very often contacted with Aprin 35 SD, it is interesting that the disease merely

occured at the dose of 200 gram as from 12th transfer. It is not possible that pathogen frequently contacts with Aprin 35 SD as under the laboratory conditions as under the field conditions. On the other hand, the fact that metalaxyl is applied to seed may obtain an advantage for itself. So, metalaxyl, which is used as seed dressing before sowing, will contact with pathogen once a year. When the dose of 200 gram is used, thefact that disease occured in the 12th transfer may show that the resistance problem can appear in the 12th year under the field conditions. Whereas Aprin 35 SD is registered at the dose of 500 gram per 100 kg seeds. If this chemical is used as seed treatment, it seems that there is no probability for the pathogen to acquire resistance to metalaxyl under the field conditions by the proper application.

Ö Z E T

AYÇİÇEĞİ MİLDİYÖSÜ (**Plasmopara helianthi** Novot.) HASTALIĞININ KİMYASAL SAVAŞIMI VE PATOJENİN METALAXYL'E KARŞI DAYANIKLILIĞININ SAPTANMASI ÜZERİNDE ÇALIŞMALAR

Ayçiçeği mildiyösü (**Plasmopara helianthi** Novot.)ne karşı Previcur N (propamocarp hydrochloride, 722 g/l, w/v), Ceresan P (quintozene, 180 g/kg, w/w) ve Aprin 35 SD (metalaxyl, 350 g/kg, w/w)'nin tohum ilacı olarak etkililikleri belirlenmiştir. Aynı zamanda Aprin 35 SD'nin aktif maddesi olan metalaxyl'e karşı bir dayanıklılık sorunu oluşup oluşmayacağını saptamak amacıyla kontrollü koşullarda küçük parsel çalışmaları ve laboratuvar çalışmaları yapılmıştır.

Previcur N ve Ceresan P hastalığa karşı etkisiz, Aprin 35 SD ise etkili bulunmuştur. Aprin 35 SD'nin düşük dozlarına karşı etmenin duyarlılığı azalmış, 200 gramın üzerindeki dozlarda ise etmenin duyarlılığında bir değişiklik olamamıştır. 12. alıştırmadan itibaren 200 gramlık dozda hastalık görülmeye başlanmıştır. Uygun dozda kullanılırsa, etmenin bu kimyasala karşı dayanıklılık riski yoktur.

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Phytium torulosum, a New Causal Organism of Watermelon Fruit in the Field and Its Comparison with **Phytophthora capsici**

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ABSTRACT

A new fruit rot of watermelon caused by **Phythium torulosum** was isolated from rotten fruits, together with **Phytophthora capsici** in Erzincan, Turkey. This pathogen caused a faster decay than **P. capsici** but affected only the wounded fruits. However, loss of fruit rot of **P. capsici** was more common. **Pythium torulosum** grew as a fluffy, white mycelium after the diseased fruit collapsed. At the begining it was indifferent and on an even level without fungal growth. On the other hand, **P. capsici** caused a sunken decay, and later on thin cover of sporangial mass appeared.

INTRODUCTION

Fruit and stem rot of watermelon caused by **Phytophthora capsici** have been reported by the other workers so far (1, 2).

During a study of watermelon fruit rot, along with **P. capsici, Phythium torulosum** was also isolated from the diseased samples brought from Erzincan, Turkey.

This paper deals with descriptions of **P. torulosum** and its comparison with **P. capsici.**

MATERIALS AND METHODS

Diseased fruit samples were brought from Erzincan where watermelon fruit rot is widespread in the field. Isolation of the pathogens was done by the selective medium containing (mg/1): Pimaricin 10, Vancomycin 300, Quintozene 100. The basal medium was grated carrot agar (40 g grated carrots and 20 g agar per litre of medium). This medium was used both with and without antibiotics and PDA was also employed in the isolations.

Pathogenicity of the isolates was tried by placing small triangular culture pieces of the fungi on intact and punctured fruits. Fruits were

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disinfected by rubbing with a cotton pad dipped in 70 % alcohol. Culture pieces were covered with plastic tapes.

Fruits were kept 20 days for observations at the laboratory (22°C). Identification of the pathogens were made according to their descriptions in literature (3, 4).

RESULTS

On some of the rotten fruits abundant grayish-white masses of sporangia of **Phytophthora capsici** were observed. These fruits regularly yielded the pathogen on the selective medium of PVQ.

Some of the fruits were externally similar and covered by a white fluffy fungal growth. From these ones, **Phytium torulosum** was isolated on all the media.

Pythium torulosum was easily identified with its sporangia and oospores based on the descriptions (3). These are given at Figure 1 a, b and c. The pathogen caused decay only on the wounded fruits. This decay remained on an externally alike level for a period. Infections could only be differentiated with a slight colour tone of darker green (Figure 1 d). Later on white fungal growth of the pathogen appeared and this was followed by the collapse of the infected area (Figure 1 e). The rate of the decay was 3.8 cm/day in diameter.

Phytophthora capsici, on the other hand, caused rots both on intact and wounded fruits. On the latter, the decay appeared the following day after inoculation, while on the former ones it was observed two days later. All of the four inoculated fruits showed symptoms. **P. capsici** caused a characteristic decay which was circular and sunken and without fungal sporulation on the first three days (Figure 2 a and b). Later, grayish, thin and powdery masses of sporangia of the pathogen appeared on the fruits (Figure 2 c). Sporangial characteristics of the pathogen were identical with the ones described (4) and the pathogenicity of it on **Capsicum annuum** was also proved. The rate of the decay on the wounded fruits was 2.0 cm/day. Sixty percent of the samples yielded the pathogen.

From the fruits infected with both **P. capsici** and **P. torulosum,** very often, **Geotrichum** sp. was also obtained. This fungus did not cause any rot on both injured and intact fruits for the duration of 20 days. Control fruits did not show any disease during this period.

DISCUSSION

Since **Phytophthora capsici** was isolated at a higher percentage and it caused decay on intact fruits, the pathogen could be admitted

as a primary causal agent of watermelon fruit rots in Erzincan province in Turkey. **Phythium torulosum**, on the other hand, might induce extensive rot in case of the predisposing conditions were present. Although **Geotrcihum** sp. was reported as a causal agent (5) we could not prove it. We used a black skinned native cultivar for the pathogenicity of the fungus similar to the ones obtained for isolations. The other cultivars might indicate different reactions.

Ö Z E T

KARPUZLARDA YENİ BİR MEYVE ÇÜRÜKLÜĞÜ, **Pythium torulosum** VE ONUN **Phytophthora capsici** İLE KARŞILAŞTIRILMASI

Karpuz meyvelerinden **Phytopthora capsici** ile birlikte yeni bir çürüklük etmeni, **Pythium torulosum** izole edilmiştir. Bu patojen sadece yaralanmış meyvelerde çürümeye neden olmuştur, ancak bu çürüme **P. capsici**'den daha hızlı olmuştur. Erzincan'da tarlada çürümeye neden olan bu etmen **P. capsici**'den daha az sıklıkta (% 40) izole edilmiştir.

P. torulosum meyvelerde başlangıçta düzgün olan ve yalnızca çok hafif koyu bir renk ile ayrılan, daha sonra çöken ve üzerinde beyazımtırak pamuğumsu fungal gelişme olan belirtilere yol açmıştır. Halbuki **P. capsici çökük** lekeler oluşturmuş, sonra buralarda grimtırak beyaz, tozlu bir görünümde olan sporangium kümeleri meydana gelmiştir.

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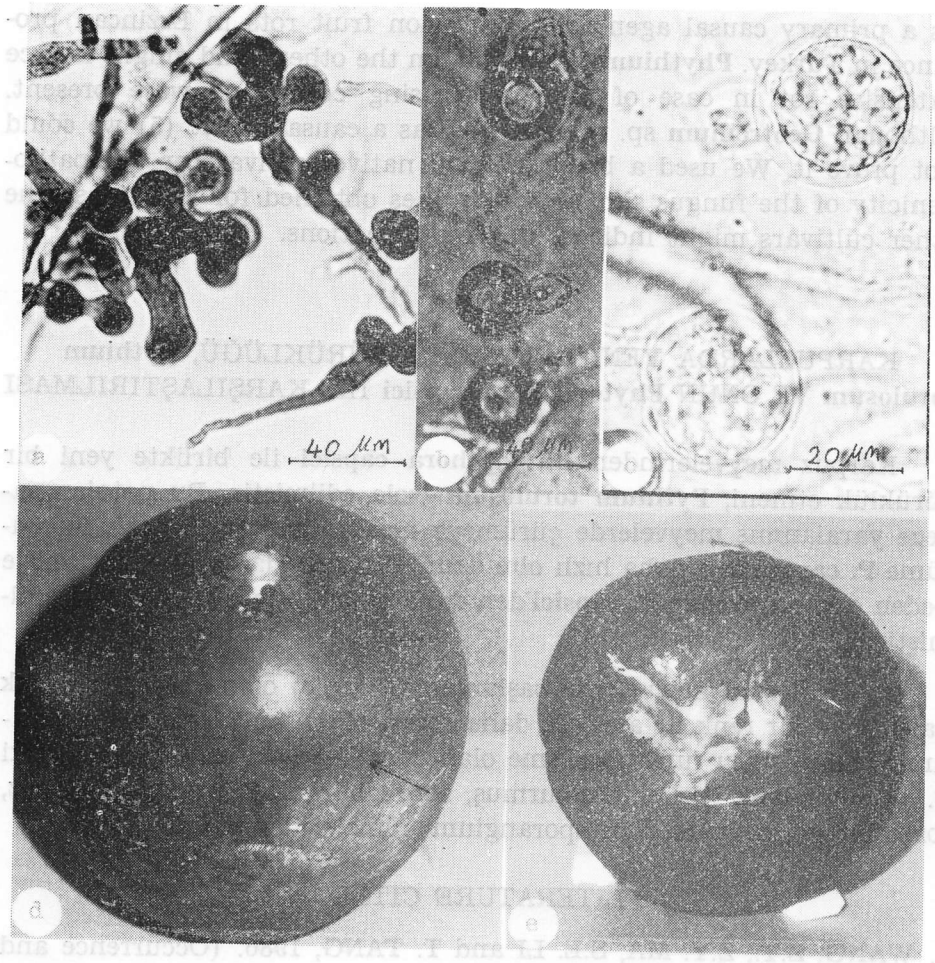

Figure 1. Varioius aspects of **Phythium torulosum.**
a) Sporangia b) and c) Oospores d) Earlier fruit decay
e) Final decay and white fungal growth on the fruit

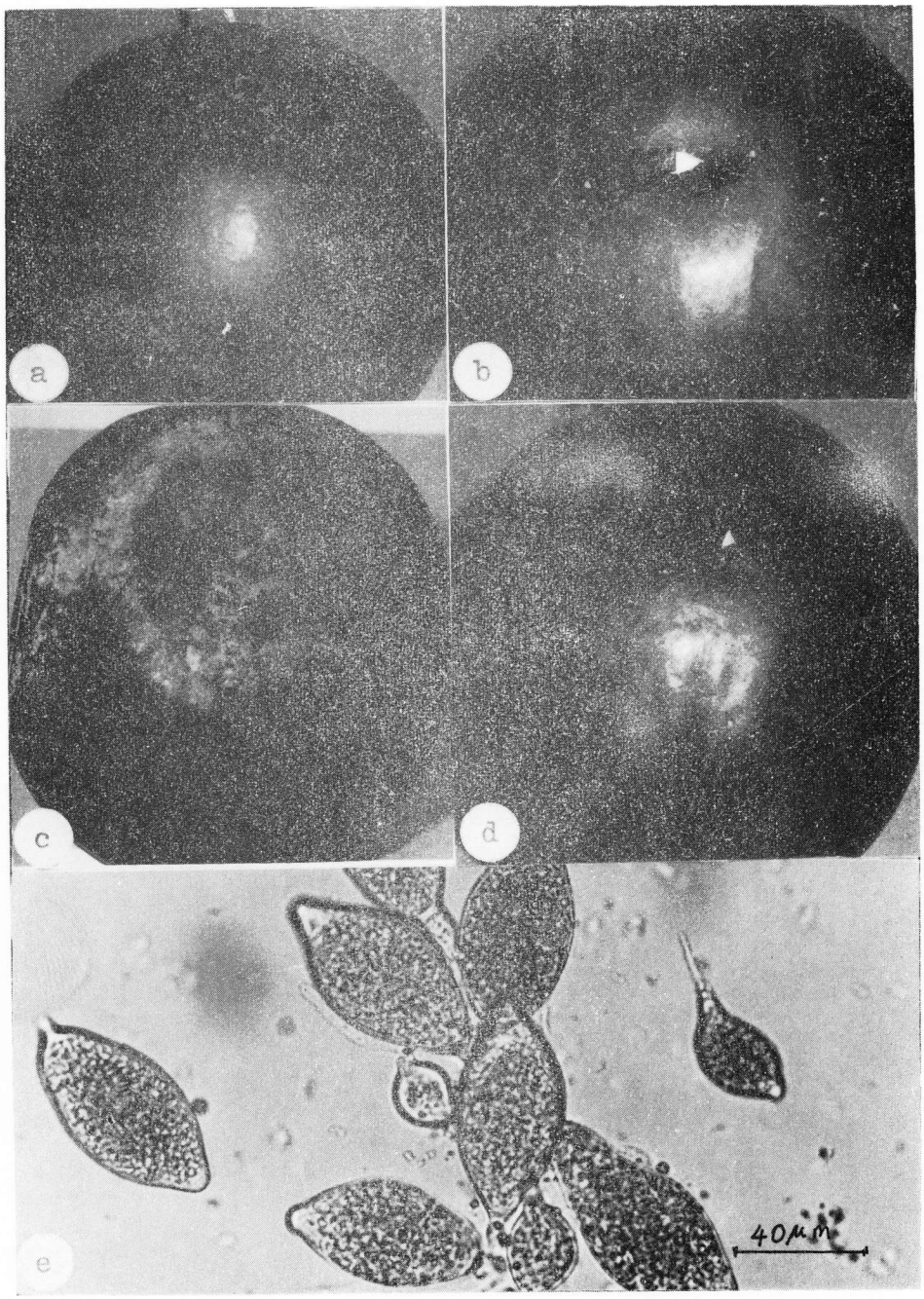

Figure 2. Various aspects of **Phytophthora capsici.**
a) and b) Earlier fruit decay c) Later fruit decay and grayish-white masses of sporangia d) Control
e) Sporangia

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