VOLUME: 16 NUMBER: 1 JAN. 1987

THE JOURNAL OF TURKISH

PHYTOPATHOLOGY

Published by the Turkish Phytopathological Society

The Journal of Turkish Phytopathology is published by Turkish Phytopathological Society and issued twice or three times a year to form a volume. The subscription rate per volume is $ ~~13.00~~ 21.00

CONTENTS

DOĞRULUK Matbaacılık ve Tic. Koll. Şti. — 1987

J. Turk. Phytopathol., Vol. 16, No. 1, 1-8 ISNN 0378-8024

Seed-borne Fungal Diseases of Chick-pea in Turkey

Salih MADEN

Ankara University, Agricultural Faculty, Department of Plant Protection

ABSTRACT

As the result of the examination of 140 chickpea seed samples brought from the important chickpea producing areas of Turkey, the following fungal agent were determined.

Ascochyta rabiei, Botrytis cinerea, Fusarium equiseti, F. moniliforme, F. oxysporum, F. sambucinum, F. solani, Macrophomina phaseolina, Rhizoctonia solani, Stemphylium spp., **Verticillium dahliae** and a bacterial pathogen **Bacillus subtilis.** Along with the pathogenic fungi, **Acromoniella** sp., **Alternaria alternata, A. tenuissima, Aspergillus** spp., **Chaetomium** sp., **Cladosporium** spp., **Epicoccum purpurascens, Gonatobotrys** sp., **Mucor** sp , **Myrothecium** spp., **Penicillium** spp., **Rhizopus** spp., **Trichoderma pseudokoningii, Trichothecium roseum, Ulocladium** sp. were also recorvered on the seeds.

INTRODUCTION

Chickpea is the second crop among legumes after lentils from the point of both yield and acreage in our country. In 1983, it was sown in 334.000 ha and 290.000 tons harvested.

One of the most important problem of chickpea productuon is diseases. So for, the most widespread disease of this crop has been chickpea blight known as antrachnose in Turkey. This disease, caused by **Ascochyta rabiei** (Pass.) Labr. is also known in the other chickpea producing countries (Karahan 1968, Maden et al. 1975, Nene 1981).

Along with the Chickpea Blight, frequently root-rot and wilt diseases caused by various fungi also have become serious on this crop. Soran (1977) found out that **Fusarium acuminatum** Ell. and **F. oxysporum** Schlecht. en S. and H. were the most important root-rot agents under field conditions. **Fusarium** spp. were also reported as the causal agents of root-rot of chickpea in the other countries too. Specially, **Fusarium oxysporum** f. sp. **ciceri** Padwick were reported in many countries as the agent of wilt (Kotasthane et al. 1979, Kaiser and

Gupta 1980, Nene et al. 1981). Besides **F. oxysporum** f. sp. **ciceri**; **F. moniliforme** Sheld em S. and H. (Gurha and Misra 1980) and **F. solani** (Mart.) Sacc. (Shukla and Bhargava 1977) were also reported as wilt pathogens.

Basides the above mentioned pathogens, **Botrytis cinerea** Pers. ExFr, **Opercullella padwickii, Macrophomina phaseolina** (Maubl.) Ashby, **Rhizoctonia solani** Kühn, **Sclerotium rolfsii** Saw. and **Sclerotinia sclerotiorum** (Lib.) de Bary were isolated from the wilted chickpea plants (Cother 1977 a, Kotasthane et al. 1979, Maden 1983).

Seed-borne nature of chickpea diseases have not been investigated both in the other countries and Turkey extensively. In his review, Richardson (1979) cited the following fungi as seed borne in chickpea; **Ascochyta rabiei, Fusarium moniliforme, F. semitectum** Berk and Rav., **F. solani** f. sp. **pisi, F. lateritium** Nees ex. Fr. emend Snyder and Hansen **Pleospora herbarum** (Pers. ex Fr.) Rabenh. **Stemphylium sarcinaeforme** (Cav.) Wilts. Later on Cother (1977 b) isolated **Botrytis cinerea and Sclerotinia** sp. from the seed samples and proved their pathogenicity on this plant. Mengistu and Sinclair (1972), from 17000 seeds of chickpea, recovered **Fusarium equiseti** (Corda) Sacc., **Phoma exigua** Desm., **P. insidiosa** Desm. and **Bacillus subtilis** Cohn emend Prazmowski along with a lot of saprophytic fungi and they found out that **B. subtilis** affected emergence the most. However these researchers determined the seed-borne fungi by applying surface disinfection and plating the seeds on agar media. Again, by using the same method D'Ercole and Sportelli (1982) investigated the mycoflora of chickpea seeds. They also, together with various saprophytic fungi, isolated **Alternaria solani** Sorauer, **Fusarium moniliforme, F. oxysporum, F. roseum** Schwabe very often while **Botrytis cinerea, Mycosphaerella** spp. and **Ascochyta** spp. more rarely.

In Turkey, only Maden et al. (1975) investigated seed borne nature of **Ascochyta rabiei.** The other seed-borne root-rot agents have not been studied so far.

In this study, seed-borne fungi were determined by using 140 seed samples brought important chickpea producing areas. Along with the fungal pathogens, saprophytic fungi and since its frequent occurrence **Bacillus subtilis** were taken into account. This work was carried out in the years 1982-1984.

MATERIALS AND METHODS

In this work, 140 seed samples, representing 1 % of the total acreage and brought from various provinces (Table 1) were examined.

Table 1. Places of samples and their numbers

Provinces	Numbers of the Samples	Provinces	Numbers of the Samples	Provinces	Numbers of the Samples
Adana	1	Denizli	40	Manisa	5
Adıyaman	2	Diyarbakır	3	Mardin	5
Afyon	2	Gaziantep	1	Malatya	5
Amasya	2	Isparta	3	Nevşehir	3
Balıkesir	8	İzmir	1	Samsun	1
Burdur	5	Kahramanmaraş	24	Tokat	3
Çanakkale	2	Kayseri	4	Urfa	2
Çorum	3	Konya	7	Uşak	2
				Yozgat	3

A hundred seeds from each sample were incubated on moistened blotter papers in 9 cm diameter glass petri plates at 22 ∓ 2°C and 12 hours on/off cycles of Near Ultra Violet Light (NUV) for 7 days. On the 8. day, seeds were examined under a zoom-stereo microscope and a compound microscope in case of need.

Various fungi, growed on different seed samples, were isolated and their cultures were stored for testing their pathogenicity.

The fungi of which pathogenicity have been proved so far did not tested for their virulence. However some **Fusarium** spp. and others about which contraversial results have been given were tested by inoculating seeds with dense spore suspensions and sowing them in sterile soils in pots.

RESULTS

With the examination of fourteen thousend seeds of chickpea, 11 fungus species and **Bacillus subtilis** were detected at different percentages (Table 2). As seen in table 2, **Ascochyta rabiei** was recorded at the highest frequency and intensity. This fungus was observed at 56,42 % of the seed samples. Two **Fusarium** species, **F. oxysporum** and **F. equiseti** which were seen at 50 % and 45,71 % of the seed samples, followed the causal agent of chickpea blight, **Ascochyta rabiei.** Five of the other agents which were **Fusarium moniliforme, F. solani, Macrophomina phaseolina, Stemphylium botryosum** and **Bacillus subtilis** were present in more than 10 % of the samples. Four fungi were recovered in less than 10 % of the samples. Among them, **Fusarium sam-**

bucinum was found in 7,14 % of the samples, while the other three were present in 5 % and less than that.

The distribution of the percent seed infection in various seed samples was also similar to the percent infected samples. 43,57 % of the samples which were infected by **Ascochyta rabiei** had 1-5 % infected seeds with this pathogen, 7,14 % of the samples had 6-10 % infected seeds and 3,57 % and 2,14 % of the samples had 11-20 % and 21-50 % infected seeds respectivelly. **Fusarium oxysporum** also showed the same trend.

Along with the above mentioned pathogenic fungi, the following ones were also isolated from chickpea seeds in various rates. These were:

Acremoniella sp., **Alternaria alternata, Alternaria** sp., **Aspergillus** spp., **Chaetomium** sp., **Cladosporium** spp., **Curvularia inaequalis, Drechslera spicifer, Epicoccum purpurascens, Gonatobotrys** sp., **Mucor** sp , **Myrothecium** spp., **Penicillium** spp., **Rhizopus** sp., **Septonema** sp., **Trichoderma pseudokoningii, Trichothecium roseum** and **Uloclodium** sp.

In the pathogenicity trials made by inoculating seeds with a dense spore suspension and sowing them in sterile pots after 8 hours incubation on blotters and evaluating on the 25. day, 2 **Fusarium equiseti** isolate yielded 13,5-46,6 % mortality on young plants, while 1 **F. moniliforme** isolate gave 83,3 % mortality, 10 **F. oxysporum** isolates gave 40-100 % and 4 **F. solani** 76-96 % and 2 **Macrophomina phaseolina** 33-56 % mortalities.

DISCUSSION

As the result of this work, many of the causal agents of diseases of chickpea were found to be seed-borne. Many of them have been detected on seeds so far (Maden et al. 1975, Mengistu and Sinclair 1979, D'Ercole and Sportelli 1982). Only three fungi, determined in this work, **Fusarium sambucinum, Rhizoctonia solani** and **Verticillium dahliae** have not been recorded up to date on seeds so far. On the other hand, it has not been come across to **Sclerotinia** sp., as stated by Cother (1977 b).

Some fungi, such as **Botrytis cinerea, Fusarium equiseti, F. sambucinum, F. solani, Macrophomina phaseolina, Rhizoctonia solani, Stemphylium** spp., **Verticillium dahliae** were recorded the first time on the seeds of this crop and they certainly present and cause disease on this plant, in Turkey; Even though they have not been reported.

Table 2. Seed-borne fungi, determined on 140 seed samples, percentages of the diseased samples of every fungus and their distribution in various percentages of diseases.

Fungi	Percent infected samples	Percentages of samples in various categories of percent seed infections				
		1-5 %	6-10 %	11-20 %	21-50 %	50-100 %
Ascochyta rabiei	56,42	43,37	7,14	3,57	2,14	—
Botrytis cinerea	2,85	2,85	—	—	—	—
Fusarium equiseti	45,71	42,14	2,85	0,71	—	—
Fusarium moniliforme	15,71	13,57	2,14	—	—	—
Fusarium oxysporum	50,00	43,57	2,85	2,85	0,71	—
Fusarium sambucinum	7,14	7,14	—	—	—	—
Fusarium solani	14,28	14,28	—	—	—	—
Macrophomina phaseolina	11,42	11,42	—	—	—	—
Rhizoctonia solani	5,00	5,00	—	—	—	—
Stemphylium spp.	11,42	11,42	—	—	—	—
Verticillium dahliae	2,14	2,14	—	—	—	—
Bacillus subtilis	15,71	15,71	—	—	—	—

The most frequently recorded fungi were **Ascochyta rabiei, Fusarium oxysporum** and **F. equiseti.**

The fungus, named as **Ascochyta rabiei**, did not show a typical characteristics of the genus **Ascochyta**. For this reason, Sutton (1980) described, this fungus as a synonym of **Phoma medicaginis.** In my opinion too, this is the correct nomenclature of the fungus and if there is a host speciation it can be named as **P. medicaginis** f. sp. **ciceri.** There were also cultural differences of the various isolates of this fungus. By examining so many isolates the exact situation of this fungus should be clarified. Because some authors reported diseases on chickpea caused by some **Phoma** species. For example, Haware and Nene (1981) reported **Phoma medicaginis** on chickpea and mentioned that this agent was different than **A. rabiei.** On the other hand, Mengistu and Sinclair (1979) revovered **Phoma exigua** and **P. insidiosa** from chickpea seeds and mentioned that their role on germination was not important. In a work like this where identifications largly based on the examination under a stereomicroscope misunderstanding might be possible and an extensive taxanomic study in needed.

The second frequent fungus on the chickpea seeds, **Fusarium exysporum,** was mainly carried superficially. The reason of the low percent recovery of this fungus by the other authors (Mengistu and Sinclair 1979, D'Ercole and Sportelli 1982) might be incubation of seeds after surface disinfection. This, surface contamination of the fungus may be eliminated. For this reason, the importance of the Blotter Method should be stressed here. In addition, as the result of the pathogenicity trials, done by soaking the seeds in a dense spore suspension and sowing them in sterilized pots, 40-100 % mortality was obtained. This also shows the importance of the pathogen. Again by the above mentioned method, two isolates of **Fusarium equiseti,** also a very common fungus, gave 13.5 and 46.6 % mortalities. It was not anticipated that **F. equiseti** might cause severe disease as **F. oxysporum.** Among the other **Fusarium** species, **F. moniliforme** and **F. solani** can be serious on root rot and wilt diseases.

The other seed borne fungal pathogens are not expected to be serious under our conditions .

ÖZET

TÜRKİYE'DE NOHUTTA TOHUMLA TAŞINAN FUNGAL HASTALIK ETMENLERİ

Türkiye'nin önemli nohut yetiştirme alanlarından getirtilen 140 tohum örneğinin incelenmesi sonucunda tohumlarda değişik oranlarda **Ascochyta rabiei, Botrytis cinerea, Fusarium equiseti, F. moniliforme, F. oxysporum, F. sambucinum, F. solani, Macrophomina phaseolina, Rhizoctonia solani, Stemphylium** spp., **Verticillium dahliae** ve **Bacillus subtilis** etmenleri saptanmıştır. Patojenik funguslar yanında **Acremoniella** sp., **Alternaria alternata, A.** sp., **Aspergillus** spp., **Chaetomium** sp., **Cladosporium** sp., **Curvularia inaequalis, Drechslera spicifer, Epicoccom purpurascens, Gonatobotrys** sp., **Mucor** sp., **Myrothecium** spp., **Penicillium** spp., **Rhizopus** spp., **Trichoderma** sp., **Trichothecium roseum, Ulocladium** sp. fungusları da tohumlarda gelişmişlerdir.

LITERATURE CITED

Cother, E.J., 1977 a. Identification and control of root-rot fungi in **Cicer arietinum** (Chickpea). Plant Dis. Reptr., **61**, 736-740.

————, 1977 b. Isolation of important pathogenic fungi from seeds of **Cicer arietinum.** Seed Science and Technol., **5**, 593-597.

D'Ercole, N. et M. Sportelli, 1982. (Mycoflora of **Cicer arietinum** seeds). Informatora Fitopathologica, 32, 51-54 (Rev. Plant Pathol., **61**, 572).

Gurha, S.N. and D.P. Misra, 1980. A new wilt disease of gram caused by **Fusarium moniliforme** Sheld., Current Science, 49, 402 (Rev. Plant Path., 1981, **60**, 572).

Haware, M.P. and Y.L. Nene, 1981. Phoma blight, a new disease of chickpea. Plant Disease, **65**, 282.

Kaiser, S.A.K.M. and P.K.S. Gupta, 1980. Reduction of **Fusarium** wilt of gram by formae speciales of **Fusarium oxysporum** non pathogenic to gram. Indian Journal of Microbiology, 20, 239-241 (Rev. Plant Path. **61**, 516).

Karahan, O., 1968. Nohut antraknozunun (**Ascochyta rabiei** (Pass.) Labr.) mücadele metodunun tesbiti üzerinde araştırmalar. Bitki Koruma Bülteni, **8**, 77-109.

Kotasthane, S.R., P.S. Agrawal, L.K. Soshi and L. Singh, 1979. Studies on wilt complex in Bengal gram (**Cicer arietinum** L.). J.N.K.V.V. Research Journal, **1**, 257-258.

Maden, S., D. Singh, S.S. Mathur and P. Neeargaard, 1975. Detection and location of seed-borne inoclum and its transmission in chickpea (**Cicer arietinum**). Seed Science and Technol., **3**, 667-681.

Maden, S., 1983. Transmission of seed-borne infections of **Ascochyta rabiei** (Pass.) Labr. to seedlings and its control. J. Turkish Phytopath., **12**, 77-82.

Mengistu, A., J.B. Sinclair, 1979. Seed-borne microorganisms of Ethiopian-grown soybean and chickpea seeds. Plant Dis. Reptr. **63**, 616-619.

Nene, Y.L., 1982. A review of Ascochyta bilght of chickpea. Tropical Pest Management, **28**, 61-70.

————, M.P. Haware, M.V.I. Reeddy, R.P.S. Pundir, 1981. Sources of resistance to selected chickpea diseases. Pulse Pathology Progress Report No. 15 (Rev. Plant Pathol., 61, 3791).

Richardson, M.J., 1979. An annotated list of seed-borne diseases. Commonwealth Mycological Institute, Kew, Surrey, England, 191 s.

Shukla, D.N. and S.N. Bhargava, 1977. Some studies on **Fusarium solani** (Mart.) Sacc. isolated from different seeds and oil crops. Procedings of National Academy of Sciences, India, B 47, 199-203 (Rev. Plant Pathol., 1981, **60**, 396).

Soran, H., 1977. The fungus disease situation of edible legumes in Turkey. J. Turkish Phytopath., **6**, 1-7.

Sutton, B.C., 1980. The coelomycetes. Commonwealth Mycological Institute, Kew, Surrey, England.

J. Turk. Phytopathol.. Vol. 16, No. 1, 9-15 ISNN 0378-8024

Resistant Source Indication Against Ascochyta Blight of Chickpea in Central Anatolian Region

Engin KINACI and Hatice DALKIRAN

Central Anatolian Regional Agricultural Res. Inst.
P.O. Box 226, Ankara, Turkey

ABSTRACT

Chickpea has been important to meet food demand, mainly in Asia, Nort Africa and Middle East. Turkey is one of the major producer and exporter. Ascochyta Blight is a serious limiting factor of production in Turkey, like in many other producers. Reducing of yield losses can be manage by using entegrated control methods that includes growing resistant or at least tolerant varieties. Such varieties can be obtain if resistant sources are available.

In this study 1100 samples of chickpea were tested against Ascochyta Blight under both, natural and artificial epiphtotic condition in two locations. As a result, 6 highly resistant and 18 resistant entries were identified in Ankara. In Çankırı location, number of highly resistant entries were 4, number of resistant entries were as 6. The entries, ILC 183 and 82-II were found as resistant in both locations.

INTRODUCTION

Although, the total food production of world and production per se in developing countries has been increased for the last three decades, still there is an increasing food deficit. Beside cereals, food legumes were important to meet food demand as one of the main nutrients of people in Asia, North Africa and Middle East.

One of this, chickpea, has been counted as fifth important legume crop (1). This not only due to its validity as food crop but also its adaptability to wide range of agro-ecological zones and ability to grow under rainfed conditions (2).

Turkey is one of the larger producer and frequent exporter of chickpea (Table1) (3).

One of the most important limiting factor to meet increasing internal and external demand of chickpea is Ascochyta blight (so called Antrachnose) caused by. **Ascochyta rabiei.** Researchers from various countries have been given reports on the effects, prevalence and economic damage caused by Antrachnose since 1930's (2, 4, 5, 6, 7, 8).

Table 1. 1973-1983 Area Sown, Production and Yield of Chickpea in Turkey.

Year	Area Sown (Ha)	Production (Tons)	Yield (kg/ha)
1973	186.000	185.000	995
1974	175.000	195.000	1114
1975	140.000	172.000	1229
1976	138.000	170.000	1232
1977	138.000	180.000	1304
1978	168.000	205.000	1220
1979	200.000	225.000	1125
1980	240.000	275.000	1145
1981	200.000	235.000	1175
1982	245.000	280.000	1143
1983	334.000	290.000	867

Nene (2), reported that for chickpea, which is an important crop in Asia, North Africa, Central and South America, the most harmful disease is Ascochyta Blight. Singh, Reddy and Nene (9) also added that, Antrachnose is the main limiting factor for chickpea production in Northwest India, Pakistan and Mediterranean region. They also stated that, between 1978-81, in three years loss, due to Ascochyta Blight was 50 % in Pakistan. In 1982, 30 % production loss has experienced at Northern Syria.

According to the Bremmer (10), chickpea Antrachnose is listed as one of the main important disease of cultivated crops in Turkey. Later on, various sources (11, 12, 13, 14, 15) declerated that, Antrachnose can cause damage in all chickpea growing areas of Turkey; in some years depend on climatic condition, 100 % yield loss may occur.

Currently, Ascochyta Blight is the principal disease of chikpea in many locations of Central Anatolia. Although, localized epidemics are frequent, region-wide spectacular losses which are caused by Ascochyta Blight were rare. Latest damaging epidemic was in (1973) (18) but the last and worst has experienced in 1983.

The first study on race identification has been started by Luthra and his colleagues in 1939. From this study and the study which has been made by Arif and Jabbar in 1965, no results has been obtained (2).

The report that is published in India, in 1963, the line lost its resistance because of possible new race. Bedi and Aujla reported that, various races has been found in India (2). Kaizer (16) reported that, isolates collected from India, Iran, Pakistan and Turkey has showed

wide difference of growth rate and colony appereance. Later, in his works he also observed differences in pathogenicity on these isolates.

In her study on resistance sources against Anthracnose and inheritance of resistance, Açıkgöz (15), has figured out some isolates with different virulence pattern.

As if in all plant diseases, the easiest and economic aplication to control of Ascochyta Blight is to grow resistant culticars. Development of resistant cultivars needs indication of source material.

Research on resistance source has been started by Luthra et al, in 1938. Since than, many scientiest has been working on this subject (15).

In Turkey, the research carried on this subject is not very old. Eser (13) has declareted a resistant variety to Anthracnose, later on Eser and Soran (17) has reported existance of 4 varieties with tolerance in their works which carried on with 52 varieties. Açıkgöz (15) has indicated 36 of about 5000 chickpea samples were resistant to Ascochyta Blight in various levels. In another work, she has found that, 6 varieties were resistant to all isolates collected from Aegean Region, two were resistant to the most, and two were resistant to some of those isolates.

For indication of resistance source, this study has been carried out in two phase and it covers the 1983 and 1984.

MATERIALS and METHODS

1100 samples of chickpea which consist of Kabuli and Desi type are included for test. 50 seeds of each entyr were planted in single rows, 2 m long and 40 cm apart. A susceptible line, 19-1-5 was also planted as a spreader after every 10 entries with line, 65-C-830 which was used as a local check. 19-1-5 was also planted as borders around the nursery. Seeding of nursery in Ankara for first phase of this study in 1983 was done at the begining of March.

Material inoculated artificially by infected crop debris which are collected in the previous years from different locations of Central Anatolia. While some of that were scattering between rows, others were used to obtain spor suspansions. Spor suspansions were obtained by soaking the infected crop debris in water for two hours at room temparature. The suspansions were sprayed on material for one week at a time. Essential humidity level for disease development was obtained and maintained by sprinkler irrigations.

In the second phase of this study, in, 1984, the artificial epidemic was created in the same way at the nursery which was conducted in Ankara. The same nursery which is also conducted in Çerkeş-Çankırı is left for natural epidemic where has been selected as hot-spot (2).

In 1983, material was scored on a «0-5» scale (2) which was developed by Morral and McKenzie. In 1984, it was scored on a «1-9» scale (2) which was developed by Singh et al. Both scales are given in tables 2 and 3.

Table 2. 0-5 Scale Scores are Defined in 1983.

0	No lesions visible on any plant in the plot
1	A few scattered lesions on the plants, usually found only after careful searching
2	Lesions common and readily observed on plants, but defoliation and damage not great, or in only one or two patches in plot
3	Lesions very common and damaging, severity intermediate between 2 and 4
4	All plants in plot with extensive lesions, defoliation and dying branches, but few if any plants completely killed
5	All plants, or all but parts of a few, completely killed

Table 3. 1-9 Scale Scores are Defined in 1984.

1	No disease visible on any plant (highly resistant)
3	Lesions visible on up to 10 % of plants, no stem girdling (resistant)
5	Lesions visible on up to 25 % the plants, stem girdling on less than 10 % of plants, but little damage (tolerant)
7	Lesions present on most plants, stem girdling on less than 50 % of the plants, resulting in the dead of a few plants and causing considerable damage (susceptible)
9	Lesions profuse on all plants, stem girdling present on more than 50 % of the plants and dead of most plants (highly susceptible)

RESULTS and DISCUSSION

In both locations, in two years, susceptible line 19-1-5 was scored as highly susceptible on almost 100 % of plants and it was beleived that required disease level was created.

In the first phase of study, in 1983, the 125 samples out of 1100 were indicated as resistant under the artificial epiphytotic condition. In 1984, in Ankara, the artificial epidemic has created for 125 samples by using infected crop debris which were collected from the region where a region wide disasteraus epidemic had also been occured. The selected resistant and highly resistant lines or varieties are given in table 4.

Table 4. Resistant and Highly Resistant Lines and Varieties Selected in Ankara, in 1984.

Highly Resistant	Resistant	
ILC 182	ILC 195	ILC 2548
ILC 201	PLJC 128	77MS73022-2
ILC 192	ILC 72	NEC 308
ILC 2596	ILC 183	82-10
ILC 173	ILC 187	82-11
NEC 1894	ILC 200	82-16
	ILC 2380	93039
	ILC 2506	
	ILC 3279	
	ILC 3346	
	NEC 138-2	

In 1984, at Çerkeş-Çankırı, amongst the 125 samples that are tested under natural epidemic condition, the lines and varieties are indicated as resistant and highly resistant are given in table 5.

Table 5. Resistant and Highly Resistant Lines or varieties selected in Çerkeş-Çankırı, in 1984.

Highly Resistant	Resistant
ILC 187	ILC 183
NEC 138-2	ILC 196
NEC 1256	ILC 201
NEC 1894	ILC 2956
	ILC 173
	82-11

In 1984, NEC 1894 is recorded as highly resistant in Ankara and in Çerkeş-Çankırı. ILC 183 and 82-11, in both locations has been found as resistant.

With the consideration of this study, the existance of different races in different locations where infected crop debris were collected are found beliavable.

In 1985, in the lab study, different color, growth rate and spor size were observed in some pure isolates; so the existance of different races became more evident.

ÖZET

ORTA ANADOLU BÖLGESİNDE NOHUT ANTRAKNOZUNA DAYANIKLILIK KAYNAKLARININ SAPTANMASI

Nohut, Asya, Kuzey Afrika ve Orta Doğuda önemli bir besin maddesidir. Türkiye en büyük nohut üreticisi ve ihracatçıları arasında yer alır. Diğer önemli üretici ülkelerde olduğu gibi Türkiyedede Ascochyta yanıklığı üretimi sınırlayan ciddi bir faktördür.

Verim kaybını azaltmak, dayanıklı yada en azından toleranslı çeşitlerin yetiştirilmesinide kapsayan entegre kontrol metodlarını kullanmakla sağlanabilir. Böyle çeşitler ise dayanıklılık kaynakları bulunduğu taktirde elde edilebilir.

Bu çalışmada, 1100 nohut örneği, iki lokasyonda, doğal ve yapay epidemi koşulları altında, Ascochyta yanıklığına karşı test edildi.

Sonuçta, Ankara lokasyonunda, 6 yüksek derecede dayanıklı ve 18 dayanıklı, Çankırı'da ise 4 yüksek derecede dayanıklı ve 6 dayanıklı nohut hattı belirlendi. ILC 183 ve 82-11, her iki lokasyonda dayanıklı olarak gözlendi.

LITERATURE CITED

1. Havtin, G.C. and K.B. Singh, 1984. Prospects and Potential of Winter Sowing of Chickpeas in the Mediterranean Region. **In** Ascochyta Blight and Winter Sowing of Chickpeas, World Crops; Production, Utilization, Descripion, vol 9, Martinus Nijhoff/Dr. W. Junk Publishers for ICARDA, pp. 7-16.

2. Nene, Y.L., 1984. A review of Ascochyta Blight of chickpea. **In** Ascochyta Blight and Winter Sowing of Chickpeas. World Crops; Production Utilization, Description, vol 9, Martinus Nijhoff/Dr. W. Junk Publishers for ICARDA, pp. 17-33.

3. Anonim, 1984. İGEME haftalık enformasyon bülteni. Sayı 44.

4. Labrousse, F., 1930. Anthracnose of the Chickpea (Cicer arietinum). **In** An Annoated Bibliography of Chickpea Genetics and Breeding 1915-1983 No: 671, p. 87.

5. Luthra, J.C., O.A. Sattar and K.S. Bedi, 1941. Determination of Resistance to the Blight Disease (**Mycosporella rabiei**), Vileknin Kovacevski, **Ascochyta rabiei** (Pass) Labr in Gram Types In Indian Jour. of Agr. Sci. II: 249-264.

6. Retig, B. and Tobolsky, 1967. A Trial for the Control of Ascochyta in Chickpeas, The First Congress of Plant Path. pp. 50-51.

7. Kaiser, V.S., 1973. Factors Affecting Growth, Sporulation, Pathogenicity, and Survival of **Ascochyta rabiei, In** Mycologia vol 65, pp. 445-457.

8. Morral, R.R.A. and D.L. McKenzie, 1974. A note on the Inadvented Introduction to North America of **Ascochyta rabiei,** A Destructive Pathogen of Chickpea, **In** Plant Disease Reporter vol 58 : 4.

9. Singh, K.B., Y.L. Nene and W.V. Reddy, 1984. International Screening of Chickpea for Resistance to Ascochyta Blight. **In** Ascochyta Blight and Winter Sowing of Chickpeas, World Crops, Production, Utilization, Description vol 9, Martinus Nijhoff/Dr. W. Junk Publishers for ICARDA pp. 67-68.

10. Bremmer, 1948. Türkiye Fitapatolojisi, cilt 2, özel basım, 237 s.

11. Karahan, O., 1968. Nohut Antraknozunun (**Ascochyta rabiei** (Pass) Labr) Mücadele Metodunun Tespiti Üzerine Araştırmalar, Bitki Koruma Bülteni cilt 8 : 2.

12. Karahan, 1941. Nohut Antraknozu (**Ascochyta rabiei** (Pass) Libr.) Hastalığı, Sebze Hastalıkları ve Mücadele Usulleri, Türkiye Cumhuriyeti Tarım Bakanlığı Zirai Mücadele ve Zirai Karantina Genel Müdürlüğü, Mesleki Kitaplar Serisi, s. 116-121.

13. Eser, D., 1976. Nohut (**Cicer arietinum** L.)'ta Başlıca Bitki Özelliklerinin Kalıtım Değerleri, Bu Özellikler ile Bitki Verimi Arasındaki İlişkiler ve **Ascochyta rabiei** (Pass)'ye Dayanıklılığın Kalıtımı, Ankara Üniversitesi Ziraat Fakültesi Yayınları 620, Bilimsel Araşt. ve İncelemeler, 363, s. 8.

14. Maden, S., 1983. Transmission of Seed borne Infections of **Ascochyta rabiei** (Pass) Labr to Seedlings and Its Control, J. Turk. Phytopath. vol 12, no 2-3 : 77-82.

15. Açıkgöz, N., 1983. Nohut Antraknozu **Ascochyta rabiei** (Pass) Labr'nin Dayanıklılık Kaynakları ve Dayanıklılığın Kalıtımı Üzerine Araştırmalar, Ege Bölge Zirai Araştırma Enstitüsü Yayınları, no: 29.

16. Creval, J.S., 1984. Evidence of Physiologic Races in **Ascochyta rabiei** of Chickpea, In Ascochyta Blight and Winter Sowing of Chickpeas, World Crops; Production. Utilizatin Description, vol 9. Martinus Nijhoff/Dr. W. Junk Publishers for ICARDA pp. 55-65.

17. Eser, D. ve H. Soran, 1978. Yerli ve Yabancı Kökenli Nohut Çeşitlerinin Orta Anadolu Çevre Koşullarında Erkencilik, Verimlilik ve Hastalıklara Dayanıklılık Yönünden Mukayeseli İncelemesi, Ankara Üniversitesi Ziraat Fakültesi Yayınları 684, Ankara, 40 s.

18. Soran, H., 1975. Die Wichtigste Krankheiten der Kichererbse in Mittelanatien, J. Turk., Phytopath., Vol. 4, No .2 : 53-62.

19. Kınacı, E., H. Dalkıran, 1983. Yemeklik Dane Baklagil Hastalıkları Sürveyi, Orta Anadolu Bölge Zirai Araştırma Enstitüsü Müdürlüğü, 1983 Yılı Faaliyet Raporu, s. 256-257.

J. Turk. Phytopathol., Vol. 16, No. 1, 17-22 ISNN 0378-8024

Studies on Possibilities of Using Troleandomycin as a Seedling Treatment Chemical Against Tomato Bacterial Canker (**Corynebacterium michiganense** pv. **michiganense** 'Smith' Jensen) : I. In vitro Effectiveness and Phytotoxicity of the Antibiotic

Oya PİLAVCI **İsmail ULUKUŞ**

Biological Control Research Institute
Antalya, TURKEY

ABSTRACT

The 10 000, 1000, 100, 10 and 1 ppm doses of Troleandomycin have prevented the development of the causal agent of Tomato Bacterial Canker disease, **C.m.** pv. **michiganense**, at **in vitro** studies. On the other hand, the antibiotic have penetrated through the roots to the upper parts of the plants, but even the high doses did not cause any phytotoxicity on the tomato seedlings.

INTRODUCTION

The total vegetable production of Turkey is 11 989 770 tons. Tomato has an important place in this with 3 550 000 tons of production (Anonymous, 1982). An important disease of tomatoes is Tomato Bacterial Canker that is caused by **Corynebacterium michiganense** pv. **michiganense** 'Smith' Jensen. Up to date, the disease has been determined in Central, Southern and South-Eastern Anatolia, Aegean and Marmara regions (Karaca, 1977; Karaca and Saygılı, 1982; Ulukuş, 1982) and causes serious damages to the tomato production in Turkey.

The spread of the disease is by seeds essentially (Karaca, 1977) and seed transmission is regarded as a major means of dissemination (Strider, 1969). For this reason, the studies on the control of the disease have been directed to seed treatments and some successful control measures have been found out. However, Thyr **et al.** (1973) attributing to Blood (1937) and Ercolani (1968) stated that no wholly effective chemical treatment have been found, and add to their words that the most important problem is that the bacteria are in the inner parts of the seed. Therefore, it can be easily seen that the seed treatments need to be supported by additional measures.

The combination of seed and seedling treatments will provide high effectiveness against the disease. Seedling treatment will also

provide an important yield profit to the farmers who could not make seed treatment by various reasons but only made seedling treatment during transplanting. This study has been carried out for this purpose and Troleandomycin has given the promising results.

MATERIALS AND METHODS

The antibiotic «Tao Capsule», which belongs to Pfizer Co., has been tested against **C.m.** pv. **michiganense** isolate numbered C.1 (NCPPB 1468) that was received from Dr. Y. Emin Öktem. Each capsule of this antibiotic preparation contains Troleandomycin equal to 250 mg Oleandomycin.

The **in vitro** tests have been carried out in Petri dishes (15 cm in diam.) containing sNA (Standart 1-Nutrient Agar, Merck 7881) medium. For the tests, a dense bacteria suspension (approx. 10^{8-9} cells/ml) prepared from 2 days old cultures was added to the sterilized medium that was cooled until 48°C and then mixed thoroughly. Then poured into the Petri dishes at 5 mm thickness. Six holes that is 5 mm in diameter were opened at equal intervals on solidified agar plates with a sterilized cork-borer, and 2 drops from 10 000, 1000, 100, 10 and 1 ppm solutions of the chemical were dropped into each hole. Sterile distilled water was dropped into 6th hole as check. The result was observed after an incubation for 4 days at 26°C.

Chemical dilution series, which had been prepared beforehand for **in vitro** effectiveness test, was used for determining the phytotoxicity of the antibiotic to tomato seedlings. The seedlings with 4-5 leaves were put into each solution of this series. The roots of the seedlings were kept for 72 hours in these solutions. At the end of this period, it was determined whether or not any phytotoxic symptom can be seen.

In order to see if the antibiotic has penetrated into plant or not, the petioles of the leaves at the tops of the treated seedlings were crushed in a sterile mortar, and the obtained juice was tested against the bacterium with the method used for in vitro effectiveness test.

RESULTS AND DISCUSSION

It has been determined that the 10 000, 1000, 100, 10 and 1 ppm doses of Troleandomycin prevent the development of **C.m.** pv. **michiganense** significantly (Fig. 1). The average widths of the effect zones that were received from the measurements have been given in Table 1.

Table 1. The results of the in vitro effectiveness test of Troleandomycin against **C.m. pv. michiganense**

The Doses of the Antibiotic (ppm)	The Average Widths of the Effect zones (mm)
10 000	22.8
1000	20.1
100	16.2
10	11.0
1	7.6

As it was seen in Table 1, even a very low dose of the antibiotic, for example 1 ppm, has given a high effect on the bacterium.

At the end of the phytotoxicity test, the plants that were put for 72 hours into the 10 000, 1000, 100, 10 and 1 ppm solutions of the antibiotic did not give any obvious phytotoxic symptoms. These plants continued to grow healthily (Fig. 2).

The juices obtained from the tops of the plants that had been treated with the antibiotic inhibited the development of the bacterium at four doses. But this is very significant especially at the first two doses (Table 2).

Table 2. The effect of the juices obtained from the tops of the seedlings which treated with different doses of Troleandomycin for 24 hours

Concentrations of the antibiotic solutions into which the roots of the seedlings were dipped	The effect of the juices on the bacterium
10000 ppm	+ + +
1000 »	+ + +
100 »	+ +
10 »	+
1 »	—

+ : Approx. 5 mm effect zone width.

If the results of the study are reviewed generally it is seen that all of the doses of Troleandomycin prevent the development of **C.m.** pv. **michiganense**. Even at very low dilution the level of prevention does not differ much. On the other hand, the chemical does not cause any obvious phytotoxicity even if the plant contact with the high doses of the chemical for a long time. In addition, the inhibitive effect of the plant juices obtained from the tops of the seedlings on the bacterium show that the antibiotic can penetrate into the plant and transport in it systemically. All of these encourage that this antibiotic can be used successfully as a seedling chemical against Tomato Bacterial Canker disease which is caused by **C.m.** pv. **michiganense**.

Kruger (1962), who had made studies on the absorption and stabilities on some antibiotics that can be used against **C.m.** pv. **michiganense** on tomatoes, states that Oleandomycin becomes inactive in plant tissues after four weeks. From this it is understood that Troleandomycin, which has similar structure and effect mechanism with Oleandomycin, will lose its activity probably long ago before the harvest time, and so it will not cause a problem on human health.

ÖZET

TROLEANDOMYCİN'İN DOMATES BAKTERİYAL SOLGUNLUĞU (**Corynebacterium michiganense** pv. **michiganense** 'Smith' Jensen)'NA KARŞI FİDE İLACI OLARAK KULLANILMA OLANAKLARI ÜZERİNDE ÇALIŞMALAR: I. ANTİBİYOTİĞİN IN VITRO ETKİNLİĞİ VE FİTOTOKSİSİTESİ

Yapılan **in vitro** çalışmada Troleandomycin'in 10 000, 1000, 100, 10 ve 1 ppm'lik dozlarının hemen hepsinde Domates Bakteriyal Solgunluğu etmeni **C.m.** pv. **michiganense**'nin gelişmesini önlediği; diğer yandan antibiyotiğin köklerinden bitkilere nüfuz ederek sistemik olarak ilerlediği, buna karşılık yüksek dozlarında bile domates fidelerinde gözlenebilir bir fitotoksisiteye sebep olmadığı tesbit edilmiştir.

ACKNOWLEDGEMENT

The authors gratefully thank Dr. Y. Emin Öktem, from Regional Plant Protection Research Institute, Ankara, Turkey, who supplied the **C.m.** pv. **michiganense** culture.

LITERATURE CITED

ANONYMOUS, 1982. Tarımsal Yapı ve Üretim, 1980. Devlet İstatistik Enstitüsü yayını, No. 985, Ankara.

KARACA, İ., 1977. Fitobakteriyoloji ve Bakteriyel Hastalıklar. E.Ü. Ziraat Fak. yayınları, No. 294, Bornova, İzmir.

KARACA, İ. and H. SAYGILI, 1982. Investigations on disease rate, causal agents and symptoms of bacterial diseases of Tomatoes and sensitivity of the host varieties in some parts of Western Turkey. (Abstr.) J. Turkish Phytopath., 11 (3) : 120-121.

KRUGER, W., 1962. Das Schicksal von Antibiotika in Tomaten Pflanzen, II. Aufnahme Transport und Stabilitat von Antibiotika in Pflanzen. Phytopath. Z. 42, 1961 (Review of Appl. Mycol. 41)

STRIDER, D.L., 1969. Bacterial canker of tomato caused by **Corynebacterium michiganense.** A literature review and bibliography. North Carolina Agr. Exp. Sta. tech. bull. No. 193.

THYR, B.D., R.E. WEBB, C.A. JAWORSKI and T.J. RATCLIFFE, 1973. Tomato bacterial canker: Control by seed treatment. Plant Dis. Reptr., 57 : 974-977.

ULUKUŞ, İ., 1982. Elazığ, Diyarbakır ve Mardin illerinde Domates ve Biberlerde bakteriyel hastalıkların sürveyi, belirtileri, etmenlerinin tanısı ve önemlisine karşı korunma çareleri üzerinde araştırmalar. (Unpublished)

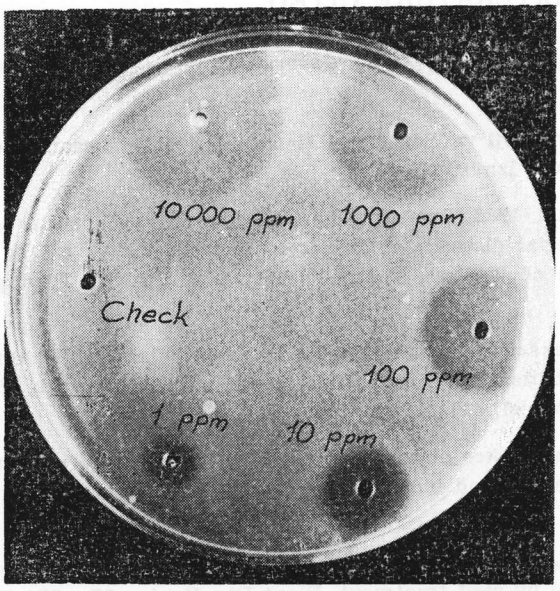

Fig. 1. The effects of different doses of Troleandomycin on agar plate against **C.m. pv. michiganense.**

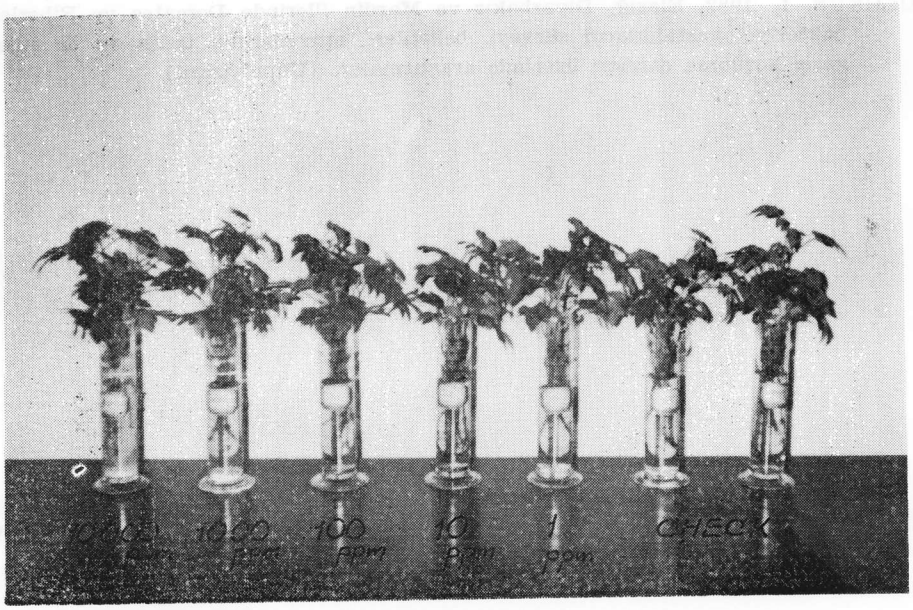

Fig. 2. The tomato seedlings that remained alive completely even at the end of a 72 hours period in different doses of Troleandomycin.

J. Turk. Phytopathol., Vol. 16, No. 1, 23-34 ISNN 0378-8024

Preliminary Investigations About Identity Of The Causal Agent Of Amasya Cherry Disease In Turkey

Ahmet ÇITIR

Faculty member of Department of Plant Protection, Faculty of Agriculture, Atatürk University, Erzurum, Turkey

ABSTRACT

A destructive sweet cherry disease in Amasya area has been prevailing since 1959. It caused the death of thousands of fruit trees in the orchards along the Valley of Yeşilırmak River. Tentative investigations have revealed that the infection causes chlorotic spots on leaves, reduction in size and quality of fruit, low yield and the death of trees in a several years. The causal agent might be described as a graft-transmissible agent. As the results of this investigations, transmission of the agent by mechanical means is possible to **Antirrihinum majus** L. and **Petunia hybrida** Vilm. with some difficulty. UV-absorbtion spectrophotometric features of partially purified preparations of infected sweet cherry sap revealed the presence of nucleoprotein molecules in the sucrose density gradient colums at the fraction numbers of 6, 21 and 24 from meniscus. Electron micrographs taken from carbon-gold covered grids indicated some true rod-shape bacteria instead of virus particles. So Amasya Cherry Disease agent needs much more detailed investigations before drawing any conclusion.

INTRODUCTION

Sweet cherry (**Prunus avium** L.) is susceptible fruit tree to a number of diseases caused by different kinds of pathogens. Among them virus and virus-like diseases which occur on **Prunus** species as well as on sweet cherry accounted in some detail by Anonymous (1951). Later on Smith (1972) compiled sufficient information about some of these sweet cherry diseases caused by Cherry Chlorotic-Necrotic Ringspot, Cherry Leaf Roll, Cherry Necrotic Rusty Mottle, Cherry Rasp Leaf, Cherry Twisted Leaf and Prunus Necrotic Ringspot viruses. He also stated that most of the cherry virus diseases caused by mixture of viruses in most cases rather than by an individual virus. Beside viruses, sweet cherry is susceptible to some infections caused by virus-like pathogens too. Such as Rickettsia-like bacteria (RLB) was reported by Wells and Weaver (1980) and Mycoplasmas-like bodies (MLB) was decribed by Florace and Cameron (1978). There are some other

diseases, however, caused by unknown agents. Due to lack of any other information about the properties of causal agents of such diseases, Schneider (1985) had named ten of them as graft-transmissible pathogens (GTP).

Amasya Cherry Disease, caused by a graft transmissible pathogen has initiated an unusual infection on sweet cherry trees in the orchards of Yeşilırmak Valley, Amasya, Turkey since 159. Studies conducted by Blodgett **et al** (1970) and Alay **et al** (1973) were revealed that at least 65,000 trees had been killed in ten years. Observations of both groups of workers indicated that causal agent of this destructive disease was neither fungus nor bacteria but a graft transmissible agent. They could not isolate any plant parasitic nematode from the soils of infected orchards either. Teherefore they suggested that it is a new kind of grafttransmissible infectious pathogenic disease and named it as Amasya Cherry Disease. Because of the effective quarantine regulations this disease is almost isolated in Amasya area.

Graft-transmissible Amasya Cherry Disease could be recognized as brick-color spots on leaves after apperance of chlorotic small lesions in early stages. Those spots are about two cm in diameter become necrotic and cover most of the leaf area, and induce early dry, up, and death of the trees. This systemic infection cause poor blossoms, small light pink colored fruits in contrast to sweet marketable size and dark colored healthy cherries. During the following seasons dieback symptoms occur on shoots, branches and limps of the infected trees and death is inevitable. Local varieties like Tabanıyarık, Erkara, Köroğlu, Türkay and Cemal Kiraz exhibit much more severe symptoms and infected trees die in several years, in contrast to those varieties of Napoleon, Bing, Vista and Lambert which are tolerant to Amasya Cherry Disase and show mild symptoms. In newly established orchards with these tolerant varieties even 15 years old trees are still productive. Neverthelless Amasya Cherry Disease is still prevailing infection and cause reduction in yield and quality. Because of the economic importance of this graft-transmissible sweet cherry disease to growers, this study was initiated in order to identify, bioassay and characterize the causal agent.

MATERIAL AND METHOD

Observations were made on Amasya Cherry Disease in different locations of Yeşilırmak Valley from 1981 to 1985. Samples were collected from the infected Tabanıyarık and Lambert varieties of sweet cherry trees in five different locations as shown in Fig. 1.

Mechanical Transmission

Samples of leaves were collected from the orchards in five locations. Samples of flowers, ripe and unripe fruits, however, were taken from both infected Tabanıyarık and Lambert trees from the Kiremitocağı orchards of Yeşilyenice with their healthy controls. All the samples were stored in deep freeze.

Young plants of 21 species and cultivars in eight families which were selected from Smith (1972) as listed in Table 1. were dusted with carborundum (grit number 500) and inoculated with sap from naturally infected sweet cherry materials, prepared as different inocula with the help of buffer solutions and chemicals as listed in Table 2. Dusted plant leaves were inoculated by sweeping with a camel hair brush wetted with an inoculum as described by Fulton (1966). Three pots of herbaceous plants and one pot of two-year old tree from sweet cherry and peach were inoculated with each inoculum. Partially purified preparations were also used as inoculum. Inoculated plants were washed with tap water and kept in greenhouse at temperature ranging from 20 to 30 C. All the inoculated plants were observed for the apperance of characteristic virus symptoms during the years of 1982 to 1985.

Purification of the Causal Agent

Naturally infected leaves of Lambert and Tabanıyarık sweet cherries and ripe fruits of infected Tabanıyarık with Amasya Cherry Disease were obtained in sufficient, quantities with their healthy analogs by harvesting them on June 21 and June 22, 1985. All the samples kept in deep freeze for one week. Both infected and healthy tissues were run thriugh all the steps of the following purification schedule which was adapted from Kira'ly **et al** (1974).

1. Homogenize plant tissue in food blender with 0.08 M phosphate buffer pH: 7.5 containing 0.1 % mixture of 0.2 M $NaSO_3$ and 0.2 M NaN_3 at the ratio of 1 gr tissue to 10 ml buffer solution.
2. Filter homogenate through cheesecloth and discard residue.
3. Centrifuge the filtrate at 26,600 g for 20 min. Discard pellet.
4. Centrifuge supernatant in a IEC Diamond ultracentrifuge at 170,000 g for 120 min. and discard the supernatant.
5. Re-suspend pellet in each tube in 1.5 ml of 0.02 M phosphate buffer at pH: 7.5 and use it as partially purified preparation.

Re-suspended pellets contained partially purified agent were inoculated mechanically to plants listed in Table 1. Partially purified agent of Amasya Cherry Disease was purified further by sucrose density

Table 1. List of Plant Species and Cultivars used for the Transmission of Amasya Cherry Disease Agent by Mechanical Means during the years of 1982 to 1985.

Family Name	Name of Species	Name of Cultivars
Amaranthaceae	**Gomphrena globosa** L.	Mixed colors
Chenopodiaceae	**Chenopodium album** L.	—
	C. amaranticolor Coste + Reyn.	—
	C. quinoa Willd.	—
Compositae	**Zinnia elegans** Jacq.	Pink
Cucurbitaceae	**Cucumis sativus** L.	Chicagos picling
	C. sativus L.	Çengelköy
	C. sativus L.	Maltepe
	C. melo L.	Kırkağaç
	Cucurbita pepo L.	Sakız
Leguminosae	**Phaseolus vulgaris** L.	Pinto III
	Vigna sinensis (Turner) Savi	Black eye
Rosaceae	**Prunus avium** L.	Vista
	P. avium L.	Lambert
	P. avium L.	Van
	P. persicae S. et Z.	Dixired
Scrophulariaceae	**Antirrhinum majus** L.	Glacier white
Solanaceae	**Nicotiana glutinosa** L.	—
	N. langsdorffii Weinm.	—
	N. tabacum L.	Samsun
	Petunia hybrida Vilm.	Mixed colors

gradient centrifugation. One ml preparation of each were layered on preformed 100-400 mg/ml sucrose gradients, keeping one gradient as control layering only 1 ml supernatant of healthy Lambert leaves. Gradients were centrifuged for 2 hours at 60,000 g in spindle-bucket ultracentrifuge rotor. By using 20 gauge infection needles, gradients were fractionated into 25 one ml fractions. All the fractions were dialized in ultrafiltered distilled water in order to separate the agents from sucrose solution (Kira'ly et al 1974). UV-absorbtion spectrophotometric values of fractions were recorded at 260 nm.

Electron microscopy

Corbett (1964)'s method was adapted for the electron microscopic studies. Copper electron microscopic grids (200 mesh/square inch)

were covered with 2.5 % Formvar. One drop of purified agent of Amasya Cherry Disease was put on each grid and let dry. Excess liquid was gently absorbed without touching the grids with filter paper. One group of grids were stained negatively by putting one drop of 0.2 % phosphothungistatic acid on each, after carbon fixation. The other group of grids were shadow cast with gold and carbon. All the grids were examined under transmission electron microscope. Characteristic bodies or molecules attributed to causal agent of Amasya Cherry Disease were recorded on electronmicrographs in the magnifications of 1800 to 95,000.

Table 2. Sources and Types of Inocula prepared from sweet cherry materials infected with Amasya Cherry Disease and the chemical additives used.

1. Crude sap from Tabanıyarık leaf tissue homogenized in 0.02 M phosphate buffer at pH: 7.5 containing 0.1 % 2-mercaptoethanol.
2. Crude sap from Tabanıyarık leaf tissue homogenized in 0.02 M phosphate buffer at pH: 7.5 containing 0.1 % mixture of 0.2 M $NaSO_3$ and 0.2 M NaN_3 in equal amount.
3. Crude sap from Lambert leaf tissue homogenized in 0.02 M phosphate buffer at pH: 7.5 containing 0.1 % 2-mercaptoethanol.
4. Crude sap from Lambert leaf tissue homogenized in 0.02 M phosphate buffer at pH: 7.5 containing 0.1 % mixture of 0.2 M $NaSO_3$ and 0.2 M NaN_3 in equal amount.
5. Crude sap from Tabanıyarık flowers homogenized in 0.02 M phosphate buffer at pH: 7.5 containing 0.1 % 2-mercaptoethanol.
6. Crude juice from unripe fruit of Tabanıyarık, homogenized in 0.02 M phosphate buffer at pH: 7.5 containing 0.1 % 2-mercaptoethanol.
7. Crude juice from ripe fruit of Tabanıyarık, homogenized in 0.02 M phosphate buffer at pH: 7.5 with 0.1 % of 2-mercaptoethanol.
8. Supernatant containing partially purified agent of Amasya Cherry Disease prepared from infected Tabanıyarık leaves.
9. Supernatant, containing partially purified agent of Amasya Cherry Disease, prepared from infected Lambert leaves.

RESULTS

Field observations made during the years from 1981 to 1985 in Yeşilırmak Valley revealed that infected sweet cherry trees were found in all the orchards. Young and old almost all the trees exhibited some degree of Amasya Cherry Disease symptoms. There were a few healthy looking trees in some orchards which were established with the varieties of Napoleon, Bing, Vista or Lambert which have tolerated much

better than those local cultivars like Tabanıyarık, Erkara, Köroğlu, Cemal kiraz and others. It is very hard to find out healthy looking and older sweet cherry trees more than ten years of age among these local cultivars. On the other hand even 15 years old trees of those imported cultivars showing mild symptoms of infection are still productive.

Mechanical Inoculation

Amasya Cherry Disease agent could not be transmitted by mechanical means to any of those tested plants. None of the tested buffer solutions and chemical additives were helpful for a succesful inoculation. Only two of those tested plants showed some symptoms to the inoculations with the partially purified preparations of both infected lambert and Tabanıyarık leaves. Symptoms of the reactive hosts are described as follows.

SOLANACEAE - **Petunia hybrida** Vilm. - Mixed colors

There was no visible symptoms on leaves of inoculated plants. After flowering stage, however, petals became ligher pink in color and developed color breaks in contrast to dark pink color of healty flowers. In addition to those flower symptoms of inoculated petunias with partially purified agent of Amasya Cherry Disease, pruduce less amount of seed and died earlier than healthy controls as indicated Figure 2.

SCROPHULARIACEAE - **Antirrhinum majus** L. - Glacier white

Inoculated plants with partially purified agent of Amasya Cherry Disease exhibited some visible chlorotic yellow spots on leaves one month after inoculation. They could not grow sufficiently and never open into blossoms in contrast to healthy controls.

Purification of causal agent of Amasya Cherry Disease

As a result of preparative low and high speed centrifugation as applied for plant viruses, Amasya Cherry Disease agent was isolated. Reactions of **P. hybrida** and **A. majus** plants to the inoculations of partially purified agent revealed that purification precedure was successful. Both of the partially purified samples of infected Lambert and Tabanıyarık leaves caused identical symptoms, as partially purified preparations of infected Tabanıyarık fruits and healthy Lambert leaves could not cause any visible symptoms on tested plants.

After further purifications by using sucrose density-gradient centrifugations of both infected Lambert and Tabanıyarık leaf preparations, two peaks of uv-absorbtion spectra were obtained. Due to lack of any reliable indicator plants, the infectivity of those peak bearing fractions

could not be tested. Both ultravioled absorbtion spectra, however, were the characteristic of virus protein and nucleic acid absorbancy at 260 nm (Figure 3). Purified preparations of infected Tabanıyarık fruit and healthy Lambert leaves did not show any peak at all.

Electron microscopy

Electron microscopic studies revealed that causal agent of Amasya Cherry Disease could not contained any virus particles or virus-like pathogens. In some grids, however, bacteria like bodies were encountered under the magnifications of 1800 and 9500.

DISCUSSION

Because of their obligat parasitic nature, it is rather difficult to investigate virus and virus-like pathogens according to all the principles of Koch Postulates. In the case of virus and virus-like diseases of woody plants it is almost impossible to do so. Bos **et al** (1960) and Ross (1964), however, suggested a step-wise characterization of viruses in a sequence of host range, symptomatology, characteristics of transmission, serological relationships, properties of virus particels in sap, and the shape and size of those virus particels. This approach of identification has been applied to most of the mechanically transmissible viruses of herbaceous plants. For the viruses and virus-like pathogens of woody plants step-wise characterization could not work so easily. In order to solve this problem of perennial plants Bawden (1964) suggested some methods of isolation of those viruses on herbaceous plants. Such as dilution of mixture of virus and inhibitor, test of seed transmission, usage of carborundum and preconditioning of the test plants may help before mechanical transmission test. Fulton (1966) expressed that without mechanical transmission of viruses of woody plants it is very difficult to expand the knowledge about them. He also suggested some techniques of investigating stone fruit viruses which were employed for the investigation of Amasya Cherry Disease agent. Beside the mechanical transmission techniques, serological test of plant viruses expanded to the viruses of woody plants by using I-ELISA and D-ELISA tests as suggested by Rowneni **et al** (1985). Even though the enzyme-linked immunosorbent assay tests has been demonstrated by Mink and Aichele (1984) for the presence and mapping Cherry Rugose Mosaic Disease (CRMV) in an orchard.

In this study plant virological experiments provided very little information about the identity of Amasya Cherry Disease agent. If Amasya Cherry Disease agent could be one of those known viruses of

cherry or **Prunus** species, then at least some of those indicator plants have to show symptoms of those viruses. Although inoculations of partially purified preparations of disease agent caused some changes of flower color and habitus of **P. hybrida** and **A. majus** as indicated in Fig. 2, their reactions could not be tested on sweet cherry trees by back inoculations. Spectrophotometric data of purified preparations of Amasya Cherry Disease agent, however, indicated some peak absorbance of certain fractions of sucrose column Fig. 3. This result could not confirmed by repeated experiments either. Electron micrographs of those grids exhibited some bacterial cells instead of virus particles. It should be remembered that Wells and Weaver (1980) reported the incidence and presence of rickettsia-like bacteria as a causal agent of some **Prunus** diseases. So Amasya Cherry Disease has to be investigated in the further studies for true identification of its causal agent.

ÖZET

TÜRKİYE'DE AMASYA KİRAZ HASTALIĞI ETMENİNİN TANISI HAKKINDA ÖN ÇALIŞMALAR

1959 yılından itibaren Amasya yöresinde oldukça tahripkâr bir kiraz hastalığı görülmeye başladı. Bundan dolayı Yeşilırmak Vadisi'ndeki bahçelerde onbinlerce kiraz ağacı kuruyup ölmüş bulunmaktadır. Yapılan gözlem ve incelemelere göre hastalık, yapraklarda hafif klorotik küçük lekelerle ortaya çıkmakta, daha sonra meyve verimi ve kalitesini düşürürken ağaçlar zayıflayıp birkaç yıl içerisinde ölmektedirler. Hastalık etmeni aşı ile taşınabilen bir patojendir. Bu çalışma sonucu kısmen arılaştırılmış inokulumlarla etmenin **Anthirrhinum majus** L. ve **Petunia hybrida** Vilm. bitkilerine mekaniksel olarak bir ölçüde güçlükle taşınabildiği saptanmıştır. Ayrıca uv-absorpsiyon spektrofotometrik ölçümler, sükroz densiti gradient santrifügasyonu sonucu menisküsten itibaren elde edilen 6, 21 ve 24 numaralı fraksiyonların nükleoprotein molekülleri içerdiklerini göstermiştir. Bu fraksiyonlardan karbon-altın kaplama ile hazırlanan gridlerden çekilen elektronmikrograflarda herhangi bir virus partikülüne rastlanmamış olup onun yerine bazı bakteri hücrelerinin bulunduğu görülmüytür. Bu durum Amasya Kiraz Hastalığı etmeni hakkında kesin bir kanıya varmadan önce konunun daha kapsamlı bir şekilde yeniden araştırılması gereğini ortaya koymuştur.

LITERATURE CITED

Alay, K., F.H. İşmen, N. Altınyay, Ö. Hancıoğlu, F. Dündar ve E.C. Blodgett, 1973. Amasya İli Kirazlarında Zarar Yapan Amasya Kiraz Hastalığı Üzerinde Araştırmalar. Bitki Koruma Bülteni 13 (3) : 163-171.

Anonymous, 1951. Virus Diseases and Other Disorders with Viruslike Symptoms of Stone Fruits in North America, Agriculture Handbook Number: 10, U.S.D.A. 276 p.

Anonymous, 1976. Virus Diseases and Noninfectious Disorders of Stone Fruits in North America. Agriculture Handbook No. 437, U.S.D.A., U.S. Govt. Printing Office, Washington, D.C. 433 p.

Bawden, F.C., 1964. Plant Viruses and Virus Diseases. 4th ed., 361 p. Ronald Press, New York.

Blodgett, E.C., F.H. İşmen, B. Gömeç, K. Alay, N. Altınyay and H.K. Wagnon, 1970. Amasya Cherry Disease in Turkey. FAO Plant Protection Bullettin 18 (3) : 49-52.

Bos, L., D.J. Hagedorn and L. Quantz, 1960. Suggested Procedures for International Identification of Legume Viruses. T. Pl. Ziekten 66 : 328-343.

Corbett, M.K., 1964. Electron microscopy of Partially Degraded Tobacco Mosaic Virus. Virology 22, 539-543.

Florance, E.R. and H.R. Cameron, 1978. Three-dimensional Structure and Morphology of Mycoplasmalike Bodies Associated with the Albino Disease of **Prunus avium**. Phytopathology 68 (1) : 75-80.

Fulton, R.W., 1966. Mechanical Transmission of Viruses of Woody Plants. Ann. Rev. Phytopathol. 4, 79-102.

Kira'ly, Z., Z. Klement, F. Solymosy and J. Vörös, 1974. Methods in Plant Pathology. Elsevier Scientific Publisning Comany. Amsterdam, London, New York, 509 p.

Minn, G.T. and M.D. Aichele, 1984. Use of Enzyme-linked Immunosorbent Assay Results in Effords to Control Orchard Spread of Cherry Rugose Mosaic Disease in Washington. Plant Disease 68 (3) : 207-210.

Ross, A.F., 1964. Identification of Plant Viruses. p. 68-92. **In** M.K. Corbett and H.D. Sisler (Ed.), Plant Virology. University of Florida Press, Gainesville, Flo., USA.

Rowhani, A., S.M. Mircetich, R J. Shepherd and J.D. Cucuzza, 1985. Serological Detection of Cherry Leafroll Virus in English Valnut Trees. Phytopathology 75 (1) : 48-52.

Schneider, R.W., 1985. Common Names of Plant Diseases Cherry (**Prunus** spp.). Plant Disease 69 (8) : 655-656.

Smith, K.M., 1972. A Textbook of Plant Virus Diseases. Academic Press, New York and London. 684 p.

Wells, J.M. and D.J. Weaver, 1980. Distribution of Rickettsia-Like Bacteria in Peach, and Occurance in Plum, Cherry and some Perennial weeds. Abstract, Phytopathology 70 (6) : 572.

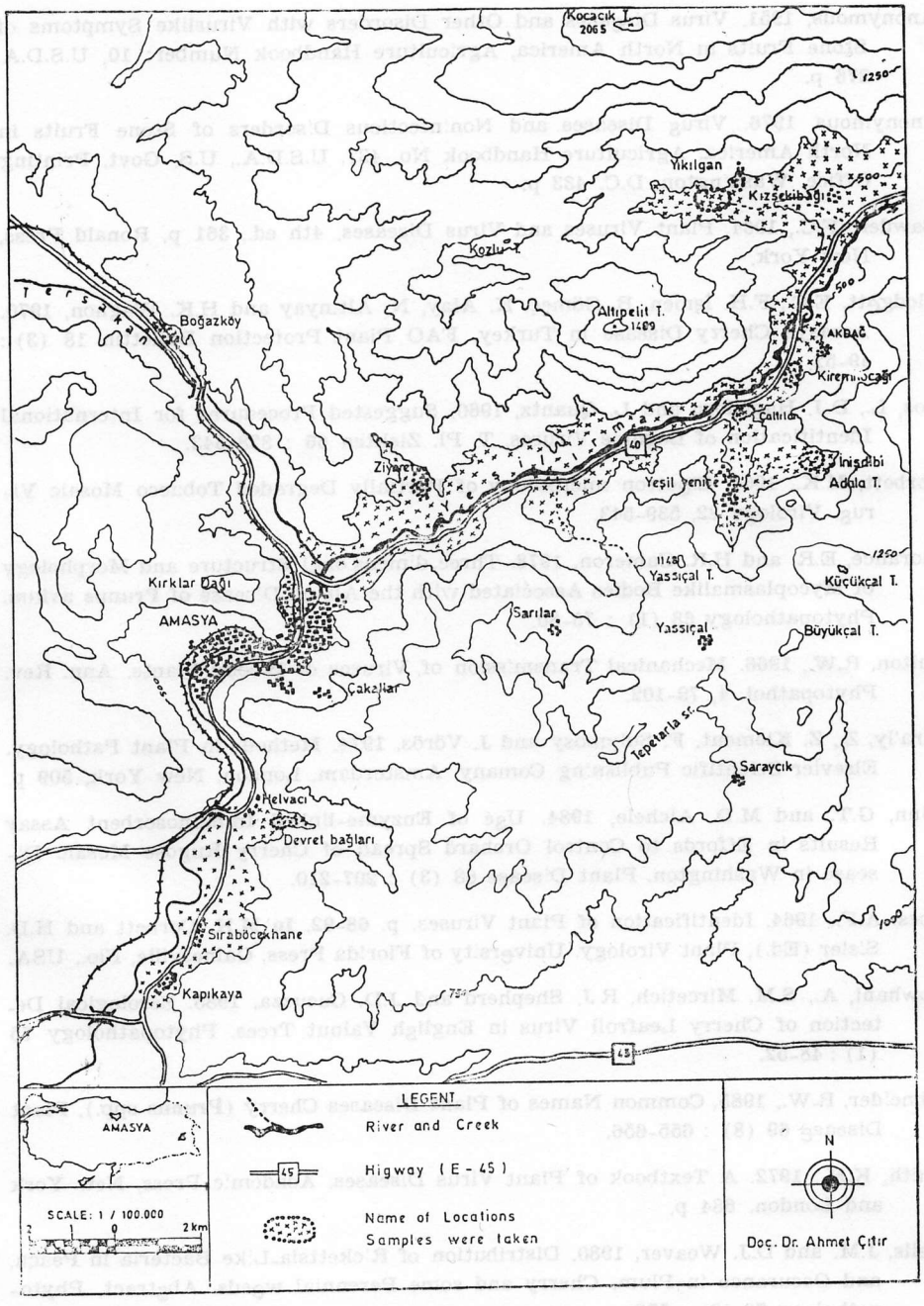

Figure 1. Map of Amasya Area showing the distribution of Sweet Cherry orchards affected by Amasya Cherry Disease, and the locations where samples were taken.

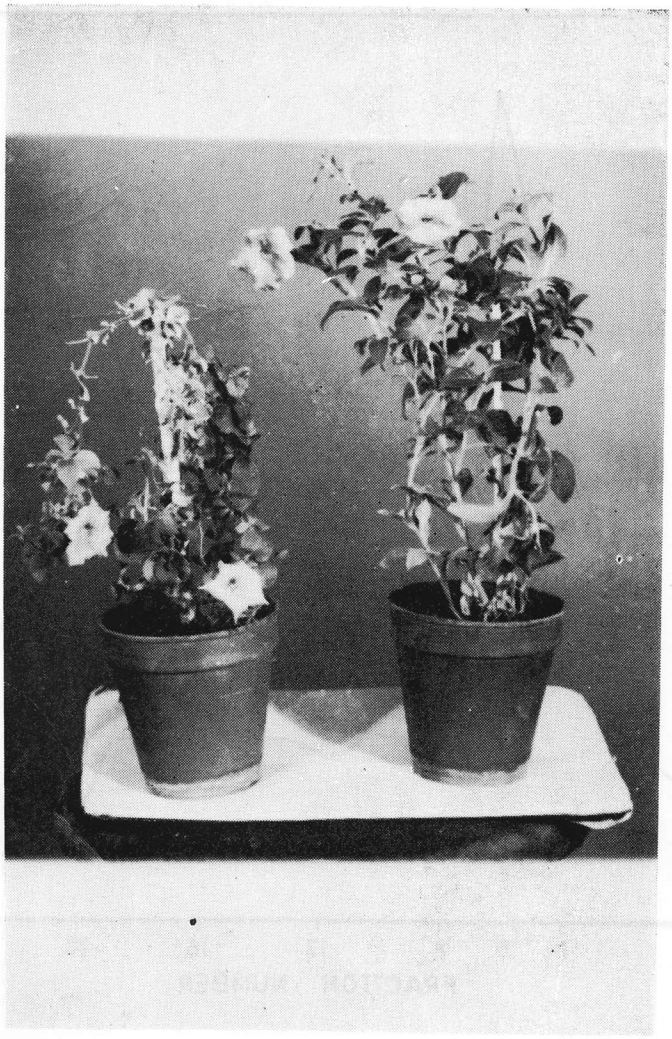

Figure 2. Flower symptoms on **Petunia hybrida** Vilm. - Mixed Colors - 3 months after inoculation with partially purified agent of Amasya Cherry Disease. Healthy plant is on right.

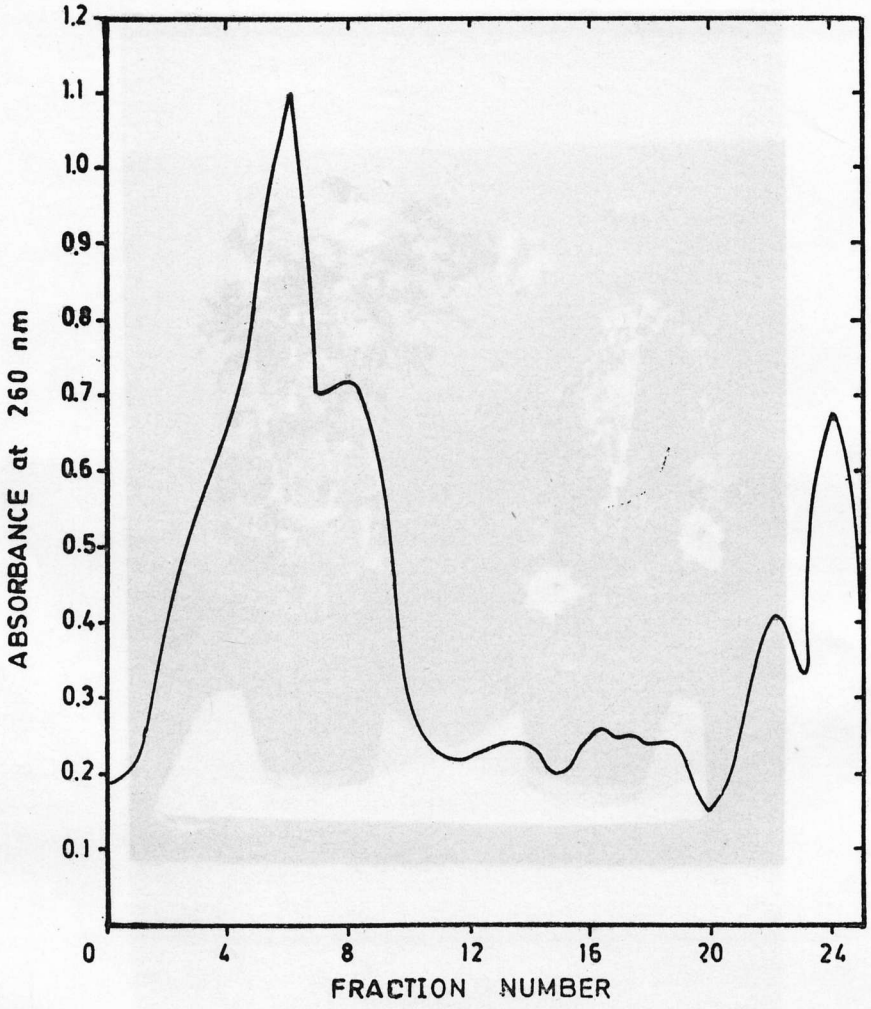

Figure 3. Absorbance at 260 nm of partially purified causal agent of Amasya Cherry Disease prepatation which centrifuged on a sucrose gradient.

J. Turk. Phytopathol., Vol. 16, No. 1, 35-39 ISSN 0378-8024

Reactions of Tomato Cultivars to Infection by Tomato Mosaic Virus

Semih ERKAN

Department of Plant Protection, Agricultural Faculty, Ege University, 35100 İzmir, Turkey

ABSTRACT

Twenty one tomato cultivars from various canning companies were tested against tomato mosaic virus and their reactions were noted. It was observed that none of these cultivars were completely resistant to infection by virus mentioned. However, some cultivars such as Cardinal, Deneb, E.Ü Z.F. 2271, H 1706, Rigel, Royal Chico, T2 Imp, Titano M and Ventura showed less susceptibility than the remaining ones. Furthermore, it was experimentally found that the virus reduced the yield and quality of fruits produced and was present in seeds from affected plants.

INTRODUCTION

Tomato is one of the most popular and profitable vegetable crops in Turkey. It is cultivated in both field and glass-or plastic houses for fresh market and processing all year round. As it is known, tomatoes have great potential for improving the income, employment and nutrition of populations (2). However, certain problems prevent tomatoes from achieving their full potential. Among them, disease problems, especially virus diseases, have more importance in comparison to others. Smith (11) recorded that there were 14 different affected tomato plants, alone or in combination. In previous studies (7, 12, 15) tomato mosaic virus (ToMV) was found to be predominant on tomato plants in Turkey, followed by potato virus X (PVX) and cucumber mosaic virus (CMV). In many instances, the only measure to eliminate or minimize virus diseases of tomato has been the use of resistant or tolerant cultivars. So, the present study was undertaken in order to determine the response of main tomato cultivars raised by the canning companies in Turkey.

MATERIALS and METHODS

The seeds of tomato cultivars (Table 1) obtained from the different canning companies in Turkey were individually sown in wooden flats with the sterilized soil mixture on July 11, 1985. The healthy

seedlings of each cultivars were transplanted into big clay pots (ca. 30 cm in diameter) on August 7 and kept under greenhouse conditions at temperatures of 15 to 28°C, as four replicates per cultivar and five plants per replicate.

In the assays the most virulent isolate «Kemalpaşa» of ToMV determined by Yorgancı (15) was used. The virus isolate was obtained from systemically infected leaves of **Nicotiana tabacum** L. cv. Maden and purified according to the earlier procedure (5).

Inoculations were made with the purified preparation of virus in question by rubbing the celite-dusted top leaves of tomato plants with four to six expanded leaves about two weeks after transplanting. The observations for symptom appearance on each of the inoculated plants were performed at the intervals of ten days during a period of approximately four months and **N. glutinosa** L. was employed in recovery tests. Moreover, first flowering and fruit setting dates of cultivars were recorded.

RESULTS

The tomato cultivars in the study and their response to infection of ToMV are presented in Table 1.

It is evident from the data in Table 1 that all the cultivars investigated generally showed susceptibility to ToMV, though little differences in their reactions. The early symptoms of virus were observed on the newly formed leaves of plants. The virus induced yellow-green mottling in certain cultivars like Chonto Mejarado, H 1706, Roma VF and Tridoro whereas the resting ones produced mild to severe mosaic on younger leaves. As the time proceeded, the main symptoms observed in cultivars under study consisted of yellowing, crinkling, narrowing and distortion on the leaves and growth retardation in plants. At the later stages, plants of affected cultivars exhibited severe stunting (Bull, Chico III, Petomech, Rigel and VF 198) and longitudinal necrotic streaks on stems (Chonto Mejarado, Harvester, Mech East 55 and Tridoro). Most of fruits from infected cultivars were small, distorted in shape and colour and had poor quality. In some cultivars (Chico III, Early Stone, Mech East 55, Petomech, Roma VF, Royal Chico and VF 198) it was seen that only one or two plants died as a results of infection by ToMV.

Recovery tests with samples of leaves and seeds from the diseased plants revealed that the virus was recoverable from the plants of all cultivars tested.

DISCUSSION

The results in Table 1 indicate that none of cultivars tested are completely resistant to infection by ToMV. However, considering our observations throughout the study we could roughly divide the cultivars in two groups on the basis of their response to infection. The least susceptible group included Cardinal, Deneb, E.Ü.Z.F. 2271, H 1706, Rigel, Royal Chico, T2 Imp, Titano M and Ventura. Deneb, H 1706, Royal Chico and Titano M were at the top of this group. The remaining cultivars comprised more susceptible group. In this group the most severely affected cultivars were Chonto Mejarado, Harvester, Mech East 55 and VF 198. On the other hand, in the studies cultivars the virus caused more or less decrease in growth, depending on cultivars, compared with their non-inoculated plants.

As it is previously reported (4, 8) resistance in tomato to viruses could well be complex and depends on a series of basic metabolic steps which are variously influenced by virus strain, the environment in which the plants are grown and the gene content of the sensitive hosts into which the resistance genes are incor porated. Furthermore, it is not forgetten that the disease symptoms as well can be greatly varied by temperature, daylength, light intensity, age of plant and cultivar of tomato as described in earlier studies (1, 3, 6, 9, 10, 13, 15). Some researcher (8, 14) demonstrated that the resistance of some tomato lines could be controlled polygenically and in most cases, virus could defeat the resistance controlled by single gene. Indeed, it was mentioned that single-gene resistance was soon overcome but three-gene resistant cultivars like «Kirford Cross» and «Pagham Cross» were still effective (4, 6).

In addition, it was determined that virus under test reduced the yield and quality of tomato fruits in all cultivars and seeds from diseased fruits were contaminated with virus.

So, from the overall results of the present study it is concluded to give special consideration the selection of cultivar(s) to be grown and to determine the reactions of selected cultivar(s) to viruses before the production season.

ÖZET

DOMATES ÇEŞİTLERİNİN DOMATES MOZAYIK VİRUSU'NA KARŞI REAKSİYONLARI

Bu çalışmada, değişik konservecilik şirketlerinden temin edilen 21 adet domates çeşidinin domates mozayık virusuna karşı ortaya koy-

dukları reaksiyonlar incelenmiş ve meydana gelen belirtiler saptanmıştır. Deneme sonuçları, çalışmadaki domates çeşitlerinden hiç birinin virusa karşı kesin bir dayanıklılığa sahip olmadığını, ancak Cardinal, Deneb, E.Ü.Z.F. 2271, H 1706, Rigel Royal Chico, T2 Imp, Titano M ve Ventura gibi bazı çeşitlerin virusa, diğer çeşitlere oranla, biraz daha az duyarlılık gösterdiklerini ortaya koymuştur. Ayrıca, virusun meyve verimi ve kalitesini azalttığı ve hastalıklı bitkilerden elde edilen tohumların virus ile bulaşık oldukları deneysel olarak bulunmuştur.

LITERATURE CITED

1. Alexander, L.J. and G.L. Oakes, 1970. Two new greenhouse tomato varieties re. sistant to all five Ohio strain of tomato mosaic virus. Phytopathology, **60** : 1281.
2. Calkings, P.H., 1979. Improving small-scale tomato production in the tropics. 1st Int. Symp. Tropical Tomato, 23-29 Oct. 1978, Taiwan, p. 22-32.
3. Crill, P., D.S. Burgis, J.P. Jones and J.W. Strobel, 1973. Effect of tobacco mosaic virus on yield of fresh market, machineharvest type tomatoes. Pl. Dis. Reptr. **57** : 78-81.
4. Dawson, J.R.O., 1967. The adaptation of tomato mosaic virus to resistant toma. to plants. Ann. appl. Biol. **60** : 209-214.
5. Gooding, G.V. and T.T. Hebert, 1967. A simple technique for purification of tobacco mosaic virus in large quantities. Phytopathology, **57** : 1285.
6. Hollings, M. and H. Huttinga, 1976. Tomato mosaic virus. C.M.I./A.A.B. Descriptionsoof Plant Viruses No. 156, 6 p.
7. Özalp, M.O., 1962. Ege Bölgesinde görülen sebze virusları. Bitki Kor. Bül. **2 (10)** : 25-30.
8. Pelham J., 1966. Resistance in tomato to tobacco mosaic virus. Euphytica **15** : 258-267.
9. Rast, A.TH.B., 1967. Yield of glasshouse tomatoes as affected by strains of tobacco mosaic virus. Neth. J. Pl. Path. **73** : 147-156.
10. ———————, 1973. Systemic infection of tomato plants with tobacco mosaic virus following inoculation of seedling roots. Neth. J. Pl. Path. **79** : 5-8.
11. Smith, K.M., 1972. A Textbook of Plant Virus Diseases, 3rd Edition, Longman Ltd. London, 167 p.
12. Tekinel, N., 1973. Adana, Antalya, Hatay ve İçel illerinde domates virus hastalıklarının yayılış alanlarının ve oranlarının tesbiti üzerinde araştırmalar. Bitki Kor. Bül. **13 (3)** : 107-142.
13. Türkoğlu, T., 1978. Effect of virus infection times on yield of five tomato varieties. J. Turkish Phytopath. **7 (1)** : 33-37.
14. Walter, J.M., 1956. Hereditary resistance to tobacco mosaic virus in tomato. Phytopathology **46** : 513-516.
15. Yorgancı, Ü. 1979. Virus diseases of tomato plantations of İzmir city, incidence and yield losses, biological and serological investigations on the viral isolates of this area. Abstr. 6th Interbalcanic Plant Protection Conf., 10-16 Oct. 1977, Izmir-Turkey, p. 226-228.

Table 1. The reactions of tomato cultivars to infection by ToMV-Kemalpaşa isolate(*)

Cultivars tested	(1) I/D	Symptoms(2) Observation dates									
		2.9.85	12.9.85	22.9.85	2.10.1985	12.10.85	22.10.85	2.11.85	2.12.85	12.12.85	22.12.85
Full	20/0	M	Y	Le Cr(3)	Le Dis			(4)	Stu		V
Cal-j	20/0	mM	(3)	sM	Le Cr	Le Dis(4)				Le Na	V
Cardinal	20/0	M	Y	sM (3)		Le Dis(4)					V
Chico III	2 /1	mM		Le Cr, Y (3)	sM	Le Dis(4)				Stu	V
Chonto ejarado	20/0	Ch Mot	Y	(3)		sM(4)	Le Cr			St N	V
Beneb.	20/0	m	Le Cr(3)	Y	sM	Le Dis	(4)				V
Early Stone	20/	m		Le Dis(3)	L			(4)			V
. . . .2271	20/1	mM	(3)	L Cr	M	(4)					V
M 1/85	0/0	Ch Mot	Le Cr	Le Dis(3)	M	(4)					V
Harvestor	20/0	M		Y (3)	Le Cr	L Dis		(4)	L Na	St N	V
Mech Last 0/	20/1	m	Le Cr	Y (3)			(4)			St N	V
Petomech	20/1	M	L Cr	Y (3)		Le Dis	Le Na (4)			St	V
Nagel	20/0	mM		Le Dis(3)		Y (4)				Stu	V
Rome VF	20/1	Ch Mot	M	(3)		Le Cr (4)	Le Dis				V
Royal Chico	20/1	M		(3)	Le Cr	Le Dis (4)					V
Super California	20/0	mM		Le Cr (3)	Le Dis		Le Na (4)				V
T2 Imp.	20/0	Y	mM	sM, LeCr	Le Dis (3)			(4)			V
Titano M	20/0	mM		(3)			(4)		Y		V
Tridoro	20/0	Ch Mot		mM (3)			(4)	Le Dis		St N	V
Ventura	20/0	mM	(3)		Y, Le Dis		(4)				V
VF 198	20/2	mM	sM, LeCr	LeDis	Y (3)			L Na	Stu (4)		V

(*) Key to abbreviations in Table 1:

(1) The number of inoculated plants/The number of died plants

(2) Symbols used for virus-induced symptoms:
Ch:chlorotic, Cr:crinkling, Dis:distortion, Le:leaf or leaves,
m: mild, M:mosaic, Mot:mottling, N:necrosis, Na:narrowing
s: severe, St:stem, Stu:stunting, V:virus recoverable, Y:yellowing

(3) first flowering date

(4) first fruit set date

J. Turk. Phytopathol., Vol. 16, No: 1, 41-45 ISNN 0378-8024

Reaction of Seedborne Bean Common Mosaic Virus And Cowpea Mosaic Virus On Differential Host Plants*

M.D. PATIL[1] and B.M. GUPTA[2]

Department of Plant Pathology Rajasthan College of Agriculture, UDAIPUR-313001, INDIA

ABSTRACT

In the present study, a differential key consisting of three differential host plants has been developed to differentiate and identify the seedborne Bean Common Mosaic Virus (BCMV) and Cowpea Mosaic Virus (CpMV). The reaction of these test plants was tested for the other characters like transmission, host range, physical properties, electron microscopy and serology. The viruses were identified on the basis of symptoms developed as under and confirmed by other characters.

A. Local lesions on **Chenopodium amaranticolor**
B. No reaction on **C. murale**
C. Systemic symptoms on **Crotalaria juncea** ..... CpMV
Cc. No reaction on **C. juncea** ..... BCMV

INTRODUCTION

The viral diseases cause enormous loss to plants annually. The seed transmission is the only possible mode of natural spread for some viruses. The seed transmission is a characteristic of the virus rather than of a plant family. Family leguminosae is prone to seed borne virus transmission and cowpea (**Vigna unguiculata** (L) Walp.), is one of them. Although cowpea is an important crop of Rajasthan and the studies on cowpea seedborne virus diseases have ben made elsewhere, yet no reports of any detailed work done on cowpea mosaic virus and its nature have been undertaken in Rajasthan. Since **Chenopodium**

* A portion from Ph. D. thesis submitted to the Sukhadia University Udaipur, Rajasthan by the Senior Author.

Present Address : 1. Department of Plant Pathology. Konkan Krishi Vidyapeeth, Dapoli 415 712, (Ratnagiri), Maharashtra.

2. Professor and Head, Department of Plant Pathology, SKN College of Agriculture, Jobner, Jaipur, Rajasthan.

species are used as indicator plants for various viruses, detailed description of the viruses under study against **Chenopodium** spp and other differential host plants are studied for developing a differential key to differentiate and identify the two viruses, which are confirmed by other characters. Taking these factors into account, the present investigations were undertaken.

MATERIALS and METHODS

Since cowpea mosaic viruses are known to be seedborne in nature, 500 seeds of each of the cowpea cultivars Jaipur Local Collection-A, Jaipur Local Collection-B, C-152, Jaipur Local Collection-D, Jaipur Local Collection-E, Jaipur Local Collection-G, Jaipur Local Collection-H, Jaipur Local Collection-10-RZ and Pusa-4 were sown in steam sterilized mixture of soil, sand and compost (2:1:1 v/v) under the glasshouse conditions. On germination, some plants of C-152 developed mosaic, vein clearing, vein banding, puckering and distortion of cotyledonary/primary leaves, 7-9 days after sowing while Pusa-4 developed dark green banding along the veins accompanied by slight crinkling of cotyledonary/primary leaves, 6-7 days after sowing. These were the two different viruses characterized and identified on the basis of other characters.

After identification of the viruses under study, 15 indicator host plant species belonging to different families were grown in the earthen pots. Standard inocula were prepared by separately macerating BCMV-infected leaves of cowpea cv. C-152 and CpMV-infected leaves of cv. Pusa-4 with the sterilized pestle and mortar in 0.1 M Boric acid buffer (ph 7.5). Five plants of each indicator host were inoculated separately with these inocula and they were observed for symptom expression up to 30 days and their reactions were recorded. The plants showing no symptoms were indexed back on healthy **Chenopodium amaranticolor** plants to test whether they acted as symptomless carriers.

RESULTS

Both the viruses were inoculated on the indicator plants and their different reactions have been presented in Table-1. Bean Common Mosaic Virus produced large chocolate brown local lesions on **Chenopodium album.** Chlorotic local lesions with white pin point centres and chlorotic local lesions were produced by BCMV and CpMV respectively, on **C. amaranticolor.** Both the viruses produced necrotic local lesions on another **Chenopodium** sp. CpMV produced systemic mosaic on **Crotalaria juncea** and **Phaseolus vulgaris** (cv. local). Systemic mosaic mottling and systemic mosaic with vein banding were produced, by

BCMV and CpMV respectively on cowpea cvs. C-152 and Pusa-4.

BCMV under study was transmitted by sap-inoculation, seed (1.2 %) and insect (**A. craccivora** and **A. gossypii**). The virus had a TIP between 75° to 80°C, DEP between 1:10,000 to 1:50,000 and LIV 4 days at room temperature (27° - 35°C). Host range was restricted to cowpea and few members of the family Chenopodiaceae. The virus particles were flexuous rods of 750-790 nm in length. Since the results are identical to that of BCMV reported by Sachchidananda **et al.** (1973), it is identified as Bean Common Mosaic Virus. CpMV also transmitted by sap inoculation, seed (11.44 %) and insects (**A. craccivora** and **A. gossypii**). The virus had a TIP between 50° - 55°C, DEP between 1:500 to 1:1,000 and LIV 7 hrs. at room temperature (27° - 35°C). Host range of the virus was confined to some species of family leguminosae and

Table 1. Reaction of BCMV and CpMV on differential host plants

Sr. No.	Name of the differential host	Symptoms on differential hosts	
		B C M V	C p M V
1.	**Cassia tora**	—	—
2.	**Chenopodium album**	Large chocolate brown L.L.	Small chocolate brown L.L.
3.	**C. amaranticolor**	Chlorotic L.L. with white pin point centres	Chlorotic L.L.
4.	**C. murale**	—	—
5.	C. sp.	Necrotic L.L.	Necrotic L.L.
6.	**Crotalaria juncea**	—	Systemic mosaic
7.	**Cucumis sativus**	—	—
8.	**Nicotiana tabacum**		
	(a) White burley	—	—
	(b) Xanthi	—	—
9.	**Ocimum basilicum**	—	—
10.	**Petunia hybrida**	—	—
11.	**Phaseolus vulgaris**		
	(a) Local	—	Systemic mosaic
	(b) Waghya	—	—
12.	**Vigna unquiculata**		
	(a) C-152	Systemic mosaic mottling.	Systemic mosaic, vein banding.
	(b) Pusa-4	Systemic mosaic mottling.	Systemic mosaic, vein banding.

L.L. = Local Lesions.

Chenopodiaceae. As the results are in agreement with that of CpMV reported by Chenulu **et al.** (1968), it is identified as Cowpea Mosaic Virus. Both the viruses reacted negatively with the standard antisera of CpMV (Sb-Isolate from surinam), Cowpea Mild Mottle and two antisera of seedborne Cowpea Mosaic Viruses indicating that they were not related to these antisera.

DISCUSSION

The reaction of tests plants was confirmed against other characters. Considering the results on host range, the transmission of by seed and insects, serological reaction and particle morphology in the present study, the viruses in question were categorized into two groups on the basis of symptoms developed as under:

A. Local lesions on **C. amaranticolor**
B. No reaction on **C. murale**
C. Systemic Symptoms on **Crotalaria juncea** ..... CpMV
Cc. No reaction on **C. juncea** ..... BCMV

Hampton **et al.** (1978) proposed the differential key to differentiate and identify the legume viruses based on symptomatology on differential indicator plants, but the test plant species were many and their reactions were not supported by other criteria like electron microscopy and serology.

On the basis of above study, the viruses were identified and confirmed as (i) Bean Common Mosaic Virus reported by Sachchidananda **et al.** (1973) and (ii) Cowpea Mosaic Virus reported by Chenule **et al.** (1968) in cowpea.

ACKNOWLEDGEMENT

The authors are grateful to Dr. R.D. Woods, Rotham Sted Experimental Station, Harpenden, Herts AL5, 2 J Q England, U.K. for completion of electron microscopy of BCMV.

ÖZET

AYIRT EDİCİ KONUKÇU BİTKİLERDE TOHUM KÖKENLİ FASULYE ADİ MOZAYIK İLE BÖRÜLCE MOZAYIK VİRUSLARININ REAKSİYONLARI

Bu çalışmada, tohum kökenli fasulye adi mozayık virusu (BCMV) ile Börülce Mozayık Virusu (CpMV)'nu ayırt etmek ve teşhis etmek için 3 test konukçusundan oluşan bir anahtar geliştirilmiştir. Bu test

bitkilerinin reaksiyonu; taşınma, konukçu dizini, fiziksel özellikler elektrojen mikroskop ve seroloji gibi diğer karakterler yönünden de denenmiştir. Söz konusu Viruslar aşağıdaki gibi saptanan belirtiler dikkate alınarak tanılanmış ve bu bulgular diğer karakterler tarafından desteklenmiştir.

A. **Chenopodium amaranticolor**'da lokal lekeler

B. **C. murole**'de belirti yok

C. **Crotalaria juncea**'da sistemik belirtiler ..... CpMV

D. **C. juncae**'da belirti yok ..... BCMV

LITERATURE CITED

* Chenulu, V.V., S.C. Sachchidananda and S.C. Mehta (1968). Studies on mosaic disease of cowpea (**Vigna sinensis**) from India. Phytopath Z. **63** : 381.387.

Hampton, R.P. (1975). The nature of bean yield reduction by bean yellow and bean common mosaic virus. **Phytopathology, 06** : 1342-1436.

* Sachchidananda, J., S. Singh, N. Prakash and V.S. Varma (1973). Bean Common Mosaic Virus on cowpea in India. **Phytopath** Z. **2** 88-91.

* Original not seen.

All Correspondance Should Be Made To
TÜRKİYE FİTOPATOLOJİ DERNEĞİ
Zirai Mücadele Araştırma Enstitüsü
Bornova, İZMİR TURKEY