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Determination Of Resistance Of Chickpea Cultivars To *Ascochyta rabiei* (Pass.) Labr. in Türkiye.

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ABSTRACT

Reactions of seven chickpea cultivars against three races (race 1, 4 and 6) of Ascochyta rabiei, known to be existent in Türkiye, were determined. ILC 195 was found highly resistant to all three races, whereas ILC 629, 19/1-5 and one cultivar from farmer populations, named Spanish were susceptible to all three races. ILC 482 and 65C830 were revealed as resistant to race 1 and 4 but susceptible to race 6. The hybrid cultivar 65C830xICP114 was resistant to race 1 and susceptible to the other two races.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is an important legume crop in several parts of the world, such as West Asia, North Africa, Central and South America (Nene, 1982; Singh and Reddy, 1990). However chickpea is the second in production and acreage among the food legumes in Türkiye after lentil (Eser et al., 1991). Of a variety of diseases that affect the crop, the most important one is *Ascochyta* blight caused by *Ascochyta rabiei* (Pass.) Labr. This fungus, which is known to be seedborne, causes brownish lesions on the stems, leaves and pods of the host, and severe infections can kill the plant (Maden et al., 1975; Morrall and McKenzie, 1974). Singh and Reddy (1990) have demonstrated, that *A. rabiei* can cause 100 % crop loss when conditions are favourable to the development of the disease. In Türkiye, there are not any information about crop losses caused by chickpea blight.

Late- sown chickpeas can escape the disease but the drop in yield due to late sowing does not make this procedure very acceptable. The most effective ways to control this disease is through the use of resistant cultivars and certificated seeds. But breeding resistant varieties have become more difficult because there are many physiological races of *A. rabiei* (Katiyar and Sood, 1985; Singh et al., 1981; Singh and Reddy, 1989). Bedi and Aujla (1969) reported eleven races, whereas Vir and Grewal (1974) reported two races (race 1 and 2) and one biotype of race 2. Recently six physiological races of *A.rabiei* have been reported by Reddy and Kabbabeh (1985).

The purpose of this study was to determine the resistance of some of chickpea cultivars to three races (race 1, 4 and 6) of *Ascochyta rabiei* in Türkiye. So far, some workers have tried to determine resistant varieties to Ascochyta blight in Türkiye, but none of them have taken into consideration of races of *A.rabiei* in their studies. In our study, for the first time, the races of *A.rabiei* were used for the first time, the races of *A.rabiei* were used for determination of resistant cultivars to Ascochyta blight in Türkiye.

MATERIALS and METHODS

1. Plant Material:

Seven chickpea cultivars (65C830, 19/1-5, ILC 482, ILC 629, ILC 195, 65C830XICP114 and one cultivar from farmer populations, named as Spanish) were used for determination of resistance to 3 races of *Ascochyta rabiei*. Chickpea cultivars were obtained from different Research Centers in Türkiye; two registered chickpea cultivars (65C830 and 19/1-5) from Central Anatolia Research Institute, other two cultivars (ILC 629, 65C830XICP114) from Eskişehir Agricultural Res. Ins. and cultivar ILC 195 (proregistered) from Menemen Agricultural Res. Ins. and cultivar ILC 482 from Diyarbakır Agricultural Res. Ins. In addition, one cultivar, a large seeded that originated from Spain was collected from farmer populations.

Seeds of each cultivar were surface - sterilized with sodium hypochloride (1%) for 5 min. and washed 3 times with sterile distilled water. Eight seeds were sown in 15 cm earthen pots containing sterilized Pro-Mix. Bx. After germination, five plants per pot were allowed to grow for 15 days. Plants were grown in growth chambers regulated at $22\pm 1^{\circ}\text{C}$ and relative humidity of 25-50 %. Plant were illuminated for 12 hour per day with a white fluorescent light (light intensity of 14 850 lux.).

2. Fungal Material:

Utilized in this study, the three races (1, 4 and 6) of *A.rabiei* were determined from different isolates of *A.rabiei* collected from different locations in Türkiye (Dolar, unpublished). Origins of isolates of *A. rabiei* used in this experiment are given in Table 1. Cultures of the pathogen were maintained on CSMDA (Chickpea- Seed Meal Dextrose Agar). Petri plates were incubated for 14 days at $20\pm 1^{\circ}\text{C}$ with an illumination of 12 hours per day. Spore suspensions of 1.2×10^6 spores per ml were prepared using sterile distilled water.

Table 1. Origins of race 1, 4 and 6 of *Ascochyta rabiei*.

Race	Isolate Name	Locality	Country
1	Çr. 1	Çorum	Türkiye
4	Nv. 1	Nevşehir	Türkiye
6	Ank. 4	Ankara	Türkiye

3. Inoculation of Plants:

Fifteen days old plants for each cultivar were inoculated with the three races of *A.rabiei* by employing five pots of five plants for each race. Spore suspensions were prepared as above and sprayed onto the plants (to run-off) using a pressure sprayer. After spraying, the plants were covered with transparent polyethylene bags for six days to maintain leaf wetness and incubated in a growth chamber a 12 hour photoperiod (light intensity of 14 850 lux.) and day and night temperatures of approximately 20°C and 18°C respectively.

4. Disease Assessment:

Twenty one day after inoculation, plants were rated on a scale of 1 to 9 where 1: no visible lesions on any plants; 3: lesions visible on less than 10 % of the plants, no stem girdling; 5: lesions visible on up to 25 % of the plants, stem girdling on less than 10 % of the plants but little damage; 7: lesions on most plants, stem girdling on less than 50 % of the plants resulting in the death of a few plants; 9: lesions profuse on all plants, stem girdling on more than 50 % of the plants and death of most plants (Singh et al., 1981). Those plants rated from 1 to 5 were considered resistant and those rated from 5.1 to 9.0 were considered susceptible.

RESULTS and DISCUSSION

Use of resistant cultivars have been recommended for control of this disease in all of the world because other control methods, such as fungicide sprays, disease-free seed, mixed cropping and destruction of infected plant debris have proven to be unreliable and uneconomical (Singh et al., 1981; Bashir et al., 1986).

As results of this research, the cultivar ILC 629, 19/1-5 and Spanish as a representative cultivar of farmer populations were found susceptible to all three races tested. On the other hand, ILC 195 was resistant to the three races. The cultivar 65C830xICP114 was resistant to race 1 but susceptible to races 4 and 6. Two cultivars, namely 65C830 and ILC 482 were resistant to races 1 and 4, but susceptible to race 6

RESISTANCE OF CHICKPEA CULTIVARS TO ASCOCHYTA BLIGHT

(Table 2). The cultivar ILC 195 could be recommended to farmers for control of chickpea blight because of its resistance to all three races of *A.rabiei*. But farmers might not prefer the this cultivar because of its small seeds.

Singh and Reddy (1990) determined that the cultivar ILC 482 was resistant to race 1 but susceptible to races 4 and 6. Whereas ILC 482 was resistant to races 1 and 4 in our study. The reason of this difference between the two studies could be the repeated inoculations at two stages (vegetative and podding stage) by Singh and Reddy.

Table 2. Reaction of seven chickpea cultivars to three races of *Ascochyta rabiei* three weeks after inoculation

Disease Rating ¹							
Cultivars							
Races	ILC 629	19/1-5	Spanish	65C830	65C830	ILC 482	ILC 195
x							
ICP114							
1	7.0	6.5	7.0	4.0	3.5	3.0	3.0
4	7.5	8.5	8.0	6.0	3.0	3.5	3.5
6	9.0	8.5	8.5	8.0	6.5	5.5	4.5
1	0 - 5.0 = Resistant		5.1 - 9.0 = Susceptible				

Singh et al. (1984), reported that the cultivar ILC 195 had showed resistant reaction in six of eleven countries (Algeria, India, Greece, Jordan, Lebanon, Morocco, Pakistan, Spain, Syria, Tunisia and Türkiye). However Açıkgöz and Demir (1988) reported that ILC 195 had been resistant to 25 isolates of *A.rabiei* in their experiment. Furthermore, ILC 195 was also found to be resistant by some workers while 65C830 was tolerant cultivar and 19/1-5 was susceptible cultivar to *A.rabiei* (Dalkıran et al., 1988). All the above mentioned researchers utilized a population of *A.rabiei* for determination of resistant cultivars and, in their studies, it is not clear which races of *A. rabiei* they used.

ÖZET

TÜRKİYE'DE *Ascochyta rabiei* (Pass.) Labr.'YE DAYANIKLI NOHUT ÇEŞİTLERİNİN TESBİTİ

A.rabiei 'nin Türkiye'de var olduğu bilinen üç ırkına (ırk 1, 4 ve 6) karşı yedi nohut çeşidinin dayanıklılık durumu bu çalışmada saptanmıştır. Bu denemenin sonucunda ILC 195 üç ırka da dayanıklı bulunurken ILC 629, 19/1-5 ve yetiştirici popülasyonunu temsil eden ve İspanyol olarak adlandırılan çeşitin üç ırka da duyarlı olduğu tespit edilmiştir. ILC 482 ve 65C830 ırk 1 ve 4'e dayanıklı, ırk 6'ya duyarlı, 65C830xICP114 ise ırk 1'e dayanıklı diğer iki ırka karşı duyarlı reaksiyon göstermiştir.

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Pathogenic Variability and Race Appearance of *Ascochyta rabiei* (Pass.) Labr. in Türkiye

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ABSTRACT

In this study, twenty isolates of Ascochyta rabiei (Pass.) Labr. were isolated from disease leaves, stems and pods of chickpea collected from 11 different locations in Türkiye. Morphological and cultural characters of the isolates were studied on CSM DA. On the basis of growth rate, colony color, sporulation, size of pycnidia and pycnidiospores, the twenty isolates were divided into 8 groups. One isolate was selected as representative for each group for race determination. A set of six different chickpea cultivars (ILC 1929, F-8, ICC 1903, ILC 249, ILC 3279, ICC 3996) was used to identify pathogenic variability in A. rabiei. According to this experiment races of A. rabiei 1, 4 and 6 were identified in Türkiye.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is an important legume crop in Türkiye. Of the several diseases recorded on chickpea, ascochyta blight caused by *Ascochyta rabiei* (Pass.) Labr. (telioform *Mycosphaerella rabiei* Kov.) is the most damaging disease of chickpea. In the favourable environment, yield losses due to the disease can reach 100 %.

Presently, the best method of controlling the disease is through the use of resistant cultivars. Because other control methods have proven to be unreliable and uneconomical (Singh et. al., 1981; Singh and Reddy, 1989). The production of resistant cultivars have become more difficult because of resistant cultivars have become susceptible by the time. Loss of resistance or tolerance to the pathogen may be due to differences in the pathogenic behaviour of *Ascochyta rabiei* isolates, as reported by several workers (Kaiser, 1973; Qureshi and Alam, 1984). Several workers reported many physiological races of *Ascochyta rabiei* using local chickpea cultivars (Bedi and Aujla, 1969; Vir and Grewal, 1974; Reddy and Kabbabeh, 1985). This research project on existing pathogenic variability in *A. rabiei* in Türkiye.

MATERIALS and METHODS

Twenty isolates of *A. rabiei* isolated from disease leaves, stems and pods of chickpea collected from 11 different locations (Ankara, Afyon, Uşak, Tokat, Kahramanmaraş, Nevşehir, Mardin, Malatya, Diyarbakır, Yozgat and Çorum) in Türkiye were used in this study. Single spore isolations of the cultures were obtained and maintained on slants of PDA. Morphological and cultural characters of the isolates were studied on CSM DA (Chickpea Seed- Meal Dextrose Agar). Plates were incubated at $20 \pm 2^\circ\text{C}$ under 12 hours dark and 12 hours UV light. To measure growth rate, 5 mm discs from the peripheries of 10 days old cultures were transferred to CSM DA plates (3 plates per isolate) and colony diameter measured at weekly intervals for three weeks durations. One hundred fifty pycnidia and pycnidiospores were measured for each isolates. On the basis of growth rate, colony color, sporulation, size of pycnidia and pycnidiospores, the twenty isolates were divided into 8 groups. The data were subjected to statistical analysis using Duncan Multiple test.

One isolate was selected as representative from each group for race determination. A set of six different chickpea cultivars (ILC 1929, F- 8, ICC 1903, ILC 249, ILC 3279, ICC 3996) was used to identify pathogenic variability in *A. rabiei* (Reddy and Kabbabeh, 1985). Utilized in this study, chickpea cultivars obtained from the germplasm collection of the ICRISAT and ICARDA. Seeds of each cultivar were treated with a mix of Benomyl and Thiram (2.5 and 2 g respectively per kg of seed). Chickpea plants of 6 cultivars were grown in 15 cm earthen pots filled with sterilized Pro- Mix Bx. After germination, five plants per pot were allowed to grow for 15 days. The plants were watered daily and fertilized twice a week with a dilute solution of 20- 20- 20 (N- P- K). Plants were grown in growth chambers regulated at $22 \pm 1^\circ\text{C}$ and relative humidity of 25- 50 %. Plants were illuminated for 12 hours per day with a white fluorescent light (light intensity of 14 850 lux). Fifteen days old seedlings for each cultivar were inoculated with the 8 isolates of *A. rabiei*, with 5 replications. Seedling were sprayed until run off with spore suspensions (1.2×10^5 spores ml^{-1}) from 14 days- old cultures propagated on CSM DA (Reddy and Kabbabeh 1985). After spraying, seedlings were covered with transparent polyethylene bags for six days to maintain leaf wetness and incubated in growth chamber with a 12 hour photoperiod (light intensity of 14 850 lux) and day and night temperatures of approximately 20°C and 18°C respectively. Twenty- one days after the inoculation, disease severity was recorded on a 1- 9 rating scale (Singh et al., 1981). Cultivars rated 1 to 5 were considered resistant and those rated 5.1 to 9.0 were considered susceptible.

1- 9 Rating scale (Singh et al., 1981)

1= No visible lesions on any plants;

- 3= Lesions visible on less than 10% of the plants, no stem girdling;
 5= Lesion visible on up to 25% of the plants, stem girdling on less than 10% of the plants but little damage;
 7= Lesions on most plants, stem girdling on less than 50% of the plants resulting in the death of a few plants;
 9= Lesions profuse on all plants, stem girdling on more than 50% of the plants and death of most plants;

RESULTS and DISCUSSION

On the basis of cultural and morphological characters 20 isolates were categorised into eight groups. Observation on cultural and morphological characters of 8 groups are presented in Table 1. Each group showed some differences in colony color, rate of growth, sporulation, size of pycnidia and pycnidiospores. Group 3 was the fastest growing, group 2 had the most pycnidial formation. Colony color on CSMDA was changing between pink- orange and brown. But group 2 had green- brown color that was different than other group. Several workers (Kaiser, 1973; Vir and Grewal, 1974; Grewal, 1981; Qureshi and Alam, 1984) have reported differences in the cultural and morphological characters of *A.rabiei*.

Table 1. *Ascochyta rabiei* isolates grouped on the basis of morphological and cultural characters^c

Group	Colony diameter (mm)	Colony ^a color	Pycnidial ^b formation	Sizes of pycnidia (μ)	Sizes of pycnidiospores (μ)	No. of Isolates
1	b 85±0.40	P113- F6	c ++	a 227+2.0x233+2.8	a 5.0+0.5x11.7+1.5	2
2	b 86±0.40	P115- C5	a ++++	b 202+2.7x210+3.9	b 4.6+0.4x10.4+0.8	3
3	a 88±0.26	P113- F7	c ++	b 203+1.6x214+2.6	a 4.7+0.5x11.9+0.7	2
4	d 75±0.50	P113- F6	c ++	a 225+6.4x242+4.3	a 5.5+0.4x11.8+0.3	3
5	d 75±1.0	P113- F7	c ++	a 231+5.5x255+5.8	b 4.1+0.2x10.9+1.1	2
6	c 80±1.0	P113- F7	b +++	a 222+2.3x241+2.2	b 4.5+0.2x11.0+0.7	4
7	d 75±0.98	P113- F6	b +++	a 210+6.5x220+7.4	a 5.5+0.4x12.0+0.7	1
8	d 74±1.41	P113- F7	b +++	b 200+5.1x211+6.0	a 4.8+0.3x11.7+0.3	3

a Colony colors were determined according to "Dictionary of Color Card"
 b ++ = Fair, +++ = Good, ++++ = Abundant
 c Means within each column value followed by the same letter are not significantly different (p= 0.01) according to Duncan multiple test.

PATHOGENIC VARIABILITY AND RACE APPEARANCE OF ASCOCHYTA BLIGHT

The cultivars of chickpea released earlier as resistant to blight became susceptible in subsequent years probably due to the existence of *A. rabiei* races (Qureshi and Alam, 1984; Bashir and Haware, 1986). In this study one isolate was selected as representative from each group for race determination. Based on disease reaction of six differentials, the 8 groups were classified into three races (Table 2). The results of this experiment showed that three races of *A. rabiei*, 1, 4 and 6 were identified first time in Türkiye but other three races (race 2, 3 and 5) were not found. Race 4 and 6 were determined more common than race 1 in Türkiye (Table 2). Bedi and Aujla (1969) reported eleven races whereas Vir and Grewal (1974) and Grewal (1981) found two races (race 1 and 2) and 1 biotype of race 2. Six physiological races of *A. rabiei* has been reported by Reddy and Kabbabeh (1985). However Singh et al. (1988) have asserted twelve physiological races of *A. rabiei*. This study showed that existence of pathogenic variability in *A. rabiei* in Türkiye.

Table 2. Pathogenic variability in *Ascochyta rabiei* in Türkiye.

Race	Reactions of chickpea cultivars						No. of isolates representing the race
	ILC 1929	F8	ICC 1903	ILC 249	ILC 3279	ICC 3996	
1	S	R	R	R	R	R	4
2	S	S	R	R	R	R	not found
3	S	S	S	R	R	R	not found
4	S	S	S	S	R	R	9
5	S	S	S	S	S	R	not found
6	S	S	S	S	S	S	7

S= Susceptible
R= Resistant

The existence of physiologic races emphasizes the need for further research on sources and inheritance of resistance and breeding strategies for the development of cultivars with stable resistance.

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ÖZET

TÜRKİYE'DE *Ascochyta rabiei* (Pass.) Labr. 'İN PATOJENİK DEĞİŞKENLİĞİ VE IRKLARI

Türkiye'de 11 farklı ilden toplanan hastalıklı nohut yaprak, gövde ve kapsüllerinden 20 *Ascochyta rabiei* (Pass.) Labr. izolatu elde edilmiştir. İzolatların morfolojik ve kültürel karakterlerini tespit amacıyla CSMDA ortamı kullanılmıştır. Morfolojik ve kültürel karakterlerinden gelişme hızı, koloni rengi, sporulasyon, piknit ve pikniospor büyüklükleri esas alınarak 20 izolat 8 grupta toplanmıştır. Irk tespit çalışması için her bir gruptan 1 izolat tesadüfi olarak seçilmiştir. İzolatlar 6 nohut hattı (ILC 1929, F-8, ILC 249, ICC 1903, ILC 3279 ve ICC 3996) ile reaksiyon denemesine alınarak ırk tespit çalışması yapılmıştır. Bu çalışmanın sonucunda Türkiye'de *A. rabiei*'nin 1, 4 ve 6 nolu ırklarının mevcut olduğu saptanmıştır.

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The Determination of Weeds in The Cereal Fields Around Çıldır Lake in Türkiye

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ABSTRACT

In this study, weed species were determined in the cereal fields. Sinapis arvensis L. was densely and widespread in the fields. The conversion of the pastures into the fields, plowing the fields improperly and no application of any herbicide against the weeds are the reasons for the dense weed growth. It is necessary to modernize the agricultural techniques and to prevent the plowing of the pastures for good production and stock- breeding in this area.

INTRODUCTION

The elevation of Çıldır Lake is rather high, over 1950 m. Surroundings of Çıldır lake is suitable for pastures. However, the plowing of the pastures and the using of these lands as cereal fields in the last years caused to decline of stock- breeding and decrease of the cereal yields. Essentially, the area is not suitable for crop production because of very cool and short summer season. Generally, the pastures have been changed into barley and wheat fields. But, the fields have not been plowed sufficiently and the herbicides have not been applied against the weeds in this area. For this reason, the yield decreases and the weeds increase more in fields. We have not encountered any published research work on this topic in our literature survey. The problem of the weed invasion had not been known in the area. This study was carried out to establish the weeds and their density in the fields in this area.

MATERIALS and METHODS

The area was visited for the purpose of examination twice in 1988, namely during June 24- 30 th and September 17- 23 rd; and twice in 1989, that is during July 8- 14 th and October 1- 6 th. 3 wheat and 2 barley fields were chosen from every village around the lake as being representative of these villages. During surveys, the wheat and

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barley plants were either at tillering and stem extension, or ripening stages. The weed samples were taken from these fields and brought to the laboratory. Meanwhile, we tried to establish the species of weeds which seem widespread and densely. They were identified according to Davis (1965- 1985).

RESULTS

In this study, 53 species of weed were established in the wheat and barley fields in this area. These species have been shown in Table 1. These weeds are seen almost in every field. Especially, *Sinapis arvensis* L. was found every field densely and cause great yield losses.

Table 1. The weed species which were established in the wheat and barley fields around Çıldır Lake.

Familia	Species
Apiacea	<i>Chaerophyllum bulbosum</i> L.
	<i>Falcaria falcarioides</i> (Bornm. et Wolff) Wolff
	<i>Ferula orientalis</i> L.
Asteraceae	<i>Achillea millefolium</i> L. subsp. <i>millefolium</i>
	<i>Bidens tripartia</i> L.
	<i>Centaurea triumfettii</i> All.
	<i>Cirsium arvense</i> (L.) Scop. subsp. <i>vestitum</i> (Wimmer et Grab) Petrak
	<i>Crepis foetida</i> L. subsp. <i>commutata</i> (Spreng.) Babcock
	<i>Inula oculus - christi</i> L.
	<i>Lactuca serriola</i> L.
	<i>Scozonera cana</i> (C.A. Meyer) Hoffm.
	<i>Taraxacum bellidiorme</i> Van Soets
	<i>Tragopogon aureus</i> Boiss.
	<i>Tripleurospermum callosum</i> (Boiss. et Heldr.) E.Hossain
	<i>Tripleurospermum oreades</i> (Boiss.) Rech fil.
<i>Tripleurospermum caucasicum</i> (Willd.) Hayek	
<i>Arctium minus</i> (Hill.) Bernh.	
Boraginaceae	<i>Anchusa azurea</i> Miller
	<i>Brunnera orientalis</i> (Schenk) Johnston
	<i>Echium vulgare</i> L.
Brassicaceae	<i>Descurainia sophia</i> (L.) Webb ex Prantl.
	<i>Hesperis matronalis</i> L.

- Thlaspi huetii** Boiss.
Sinapis arvensis L.
- Campanulaceae **Asyneuma limonifolium** (L.) Janchen
Caryophyllaceae **Gypsophila elagans** Bieb.
Silene sp.
Silene dichotoma Ehrh.
- Chenopodiaceae **Chenopodium album** L.
- Convolvulaceae **Convolvulus arvensis** L.
- Fabaceae **Lathyrus tuberosus** L.
Trifolium ambiguum Bieb.
Trifolium pratense L.
Trigonella sp.
Vicia cracca L. subsp. **tenuifolia** (Roth) Gaudin
- Iridaceae **Gladiolus atrovioleaceus** Boiss.
- Lamiaceae **Lallemontia peltata** (L.) Fish et Mey.
Leonurus glaucescens Bunge
Salvia verticillata L. subsp. **amasiaca** (Frey et Bomm.) Bornm
Stachys cretica L.
- Plantaginiaceae **Plantago major** L.
- Poaceae **Avena sativa** L.
Bromus japonicus Thunb.
Cynodon dactylon (L.) Pers.
Lolium persicum Boiss. et Hohen. ex Boiss.
Lolium rigidum Gaudin
Phleum pratense L.
- Polygonaceae **Polygonum alpinum** All.
Polygonum lapathifolium L.
Rumex angustifolius Campd.
- Scrophulariaceae **Rhinanthus angustifolius** C. Emelin subsp. **grandiforus** (Wallr.) D.A. Webb
Linaria kurdica Boiss. et Hohen subsp. **kurdica**

DISCUSSION

The conversion of the pastures into the wheat and barley fields, plowing the fields improperly and no application of any herbicide against the weeds are the reasons for the fields having so much weeds. In addition, the cereal production and stock - breeding decreases. In this respect, the conversion of the pastures must be forbidden, the fields should be plowed using modern techniques and the herbicides should be applied against the weeds in the wheat and barley fields.

ÖZET

ÇILDIR GÖLÜ ETRAFINDAKİ TAHİL TARLALARINDA GÖRÜLEN YABANCI OTLARIN TESPİTİ

Bu çalışmada yörede 53 yabancı ot türü bulunmuştur. Bunlardan en yaygın ve yoğun bulunanı *Sinapis arvensis* L.dir. Meraların sökülerek tarla haline çevrilmesi, tarlaların tekniğe uygun olarak işlenmemesi ve yabancı otlara karşı tarımsal savaşın uygulanmaması yabancı ot türlerinin çok ve yoğun olmasına neden olmuştur. Bölgede daha iyi hayvancılık ve tahıl üretimi için meraların sökümünü önlemek ve tekniğine uygun tarım yapmak gereklidir.

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The First Studies On The Detection Of Beet Necrotic Yellow Vein Virus In Sugar Beet In Türkiye

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ABSTRACT

In the present study performed in Inst. f. Phytopathologie (Giessen- Germany) and our laboratory the presence of BNYVV (beet necrotic yellow vein) in sugar beet samples from Türkiye and some countries was investigated with Elisa. The findings showed that the commercial detection kits can be used for determining the virus in routine testing and Elisa test method was well suited to the situations where many plant samples had to be processed rapidly. The data from assays showed that some of the samples from Türkiye and France was infected by the virus and there was no virus in the most of the samples from Germany. Moreover, it is necessary to perform the detailed studies in the samples to be collected from the major sugar beet plantations in order to bring up definitely the state of mentioned virus in Türkiye.

INTRODUCTION

Rhizomania (or root madness) is an important disease of sugar beet caused by beet necrotic yellow vein virus (BNYVV). It is reported that yield losses can be considerable levels, up to 80% in severe cases (7,10). Since it was first detected in Italy in the mid of 1950s (Canova) the disease has been described in most sugar beet growing areas of Europe, Asia and the USA (6, 9, 10, 11), Rhizomania of sugar beet appears now to be distributed in many countries such as Greece, France, Germany, Yugoslavia, Austria, Romania, Czechoslovakia, Switzerland, Netherlands, Bulgaria, Sweden, England, Poland in Europe, Japan, The People's Republic of China and the U.S.S.R. in Asia and various locations in the U.S.A. (7,9).

In the course of the surveys in the growing seasons 1990 and 1991 it was observed that a causal like virus agent became a serious problem in some sugar beet producing areas, in our country, too. The agent caused the symptoms of wilting and yellowing areas along the veins. However, it was seen that these symptoms appeared only occasionally in infected plants. But, since these kinds of symptoms cannot be considered as a general diagnostic tool and they are often attributed to other causes it is necessary to use the reliable and sensitive methods to detect this agent. So, we tried to detect the

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agent involved in the beet samples by Elisa which has been presently the best method used for routine diagnosis of plant viruses in this study.

MATERIALS and METHODS

In the present study conducted in Institut für Phytopathologie und Angew., Zoologie (Giessen- Germany) and our laboratory beet rootlet samples coming from various locations of Türkiye and some countries (France and Germany) were tested for the presence of Rhizomania.

First, rootlets from each sample were washed to remove soil debris and dried shortly in air. Then 0,02 g. pieces of air dried rootlets were homogenized in 2 ml. sample- buffer (w/v: 1/10) by Homex 5 extractor and the obtained sap was filtered through a textile tissue. In the assays the test kit (globulin and conjugate) from Boehringer company (Germany) was used. The assays were performed according to the methods used previously by certain researchers (1, 2, 3, 4, 5, 6).

Buffers

- a) Coating buffer: 1,59 g Na_2CO_3 , 2,93 g NaHCO_3 , 0,20 g NaN_3 , filled up with distilled, water to 1 lt (pH = 9,6)
- b) Washing buffer: 8,0 g NaCl , 2,9 g $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, 0,2 g KCl , 0,2 g NaN_3 , 0,5 ml Tween 20, filled up with distilled, water to 1 lt (pH = 7, 2-7, 4)
- c) Sample buffer: 20 g Polyvinylpyrrolidon, 2 g Bovine serum albumin, 1 lt washing buffer
- d) Substrate buffer: 97,0ml Diethanolamin, 0,2 g $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 0,2 g NaN_3 , filled up with distilled, water to 1 lt (pH= 9,8)

Reagents

Coating reagent: Dilution of the anti BNYVV - IgG with coating buffer 1/100 (v/v)

Antibody- AP- conjugate reagent: Dilution of the antibody- AP- conjugate with sample buffer 1/1000 (v/v)

Substrate reagent: Dilution of 4- nitrophenylphosphate with sample buffer 1/1000 (w/v)

Test Procedure:

1. Coating of the plate (wells), Incubation at 37 °C for 4 h, Washing
2. Addition of test sample, Incubation at 4- 10 °C for 10- 20 h, Washing
3. Addition of the antibody-AP-conjugate, Incubation at 37°C for 4h, Washing
4. Addition of substrate Incubation at room temperature for 1- 3 h, Stopping of reaction with 3 M NaOH

During the application of assays the plates were washed three times with a bottle and removed washing buffer on dry cloth and after the introduction of substrate reagent the extinction values of samples in the plates were recorded with a Titertek Multiskan MKII photometer (Flow) at 405 nm.

RESULTS and DISCUSSION

As the result of the assays it was observed that the test kit can be used for the detection of the virus in sugar beet samples. The suggested dilution ratios of reagents and the incubation periods were found to be convenient for the diagnose of the virus in samples. So, some of researchers (4, 6, 7, 10) stated that the detection of this virus in samples with Elisa kit was suitable. Because the mentioned virus did not exhibited clear and easily- defined symptoms and can be mechanically inoculated to only a few of the test plants (6,7).

The existence of the virus in sugar beet samples in our work was given in Table 1.
Table 1. Mean Absorbance Values Recorded in Sugar Beet Samples Checked for the Presence of BNYVV with Elisa

Sample Nos.	Collecting Area	Infectivity Level (x)
1	Türkiye	+ + +
2	"	+ + +
3	"	+ + +
4	"	0
5	"	+ +
6	"	0
7	"	0
8	"	0
9	"	0
10	"	0
11	"	+ + +
12	"	+ + +
13	"	+ + +
14	"	+ + +
15	"	+ + +

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(Continuous of Table 1.)

Sample Nos.	Collecting Area	Infectivity Level (x)
16	Germany	0
17	"	+
18	"	0
19	"	0
20	"	+ +
21	"	+
22	"	0
23	"	+
24	"	0
25	"	+
26	"	0
27	"	+
28	"	0
29	"	0
30	"	+
31	"	0
32	"	0
33	France	+ +
34	"	+ +
35	"	+ +
36	"	+ +
37	"	+ +
38	"	+ +
39	"	+ +
Healty: 0,038	(+): 0.980 - 1.100	(-): 0.021- 0.037

(x)	0,001 - 0,100	:	0
	0,101 - 0,200	:	+
	0,201 - 1,500	:	+ +
	> 1,501	:	+ + +

As seen in Table 1, it can be observed that some of the samples obtained from Türkiye was infected by the mentioned virus. It is possible to say the same situation also for the presence of virus in the samples from France. In contrast to this, it has been seen that most of the samples from Germany were not infected by this virus.

The data from the experiment shows that Rhizomania can be existed in our country, too. However, in order to bring up this situation definitely more detailed works

should be conducted by more samples getting from the major sugar beet plantations of our country. On the other hand, in our opinion it is necessary to perform the further experiments about the concentration of the virus in host plants, the host range and the vector of the virus which is a Plasmodiophoromycetes fungus; *Polymyxa betae*. In previous researches (10), it has been pointed out that the virus has the ability of infection inside the fungus *Polymyxa betae* and air-dried soil for about 15 years and can also be transmitted by the zoospores of the fungus (2, 6, 10).

Recent studies are focused on the matters of the temperature and certain properties of soil, the level of inoculum and the control of the disease. Control of Rhizomania disease is now based on the host-plant resistance because chemical control seems to be very expensive (7,8).

As it has been observed in this study, in both determining the lines resistant to the disease and testing for the presence of virus in soil, sensitive and rapid detection methods like Elisa could be suggested to use. Since BNYSV is now investigated in many countries in details, the necessary knowledge on its viral replication, inheritance and plant resistance and the control of its fungal vector will rapidly increase in the shortest time.

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TÜRKİYE'DE ŞEKER PANCARI BİTKİLERİNDE ŞEKER PANCARI NEKROTİK SARI DAMAR VİRUSUNUN TANILANMASI KONUSUNDA ÖN ÇALIŞMALAR

Bu çalışmada Türkiye ve diğer bazı ülkelerden elde edilen şeker pancarı örneklerinde şeker pancarı nekrotik sarı damar virusunun bulunma durumu Elisa yöntemi aracılığı ile araştırılmıştır. Denemelerden elde edilen bulgular, bu virusa ait ticari kitin rutin testlemelerde güvenle kullanılabilceğini ve çok sayıda bitki örneği ile çalışılması halinde virusun teşhisinde Elisa yönteminden yararlanılabileceğini ortaya koymuştur. Deneme sonuçları Türkiye'den ve Fransa'dan sağlanan örneklerin bir kısmında bu virusun bulunduğunu göstermiştir. Buna karşın Almanya'dan alınan örneklerin çoğunda virusun olmadığı gözlenmiştir. Ancak bu virusun ülkemizdeki durumunu daha net olarak ortaya koyabilmek için şeker pancarı üretiminin yoğun olarak yapıldığı üretim alanlarından daha fazla sayıda örnek toplanması ve virus teşhisi, virus-vektor ilişkileri, virus konukçuları ve bunun gibi konularda detaylı çalışmaların yapılması gerekmektedir.

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Detection of Natural Transmission of **Spiroplasma citri** by ELISA in İzmir.

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ABSTRACT

In this study, biological indexing and serological test (ELISA) have been used for detection of the natural field transmission and spreading of S. citri Saglio et al. Which has become the second serious disease of Citrus after tristeza virus in İzmir province. As a natural herbaceous host, periwinkle plants (Vinca rosea L.) were used in the natural transmission test displayed chlorotic leaves wilting, severe water stress and stunting. Wilted plants have borne few irregularly shaped flowers of smaller size. Citrus indicator seedlings of Madam vinous sw. or., pineapple Sw. Or., Duncan and March grapefruit developed leaf mottling, tip yellowing, small cupped and yellow mottled leaves after the natural field infections by leafhoppers. The ELISA results indicated that S.citri is naturally widespread in the most Citrus orchards in İzmir.

INTRODUCTION

Citrus Stubborn disease has caused widespread damage to orange, grapefruit, mandarin and tangelo trees in arid Citrus growing areas of the world. It has been present in California since 1915. It was first thought that, Stubborn was caused by a virus. Close relation of mycoplasma like bodies and Stubborn was first described by Igwegbe and Calavan in 1970. Then, Saglio et al. (1973) was characterized and named the disease as **Spiroplasma citri**. Natural transmission of **S.citri** was first described by Calavan and Carpenter (1965) in California. Also, the same authors have reported that, more then 50 percent of the trees in many plantings were affected and worthless because of **S. citri**. It has been reported to be transmitted by leafhopper vectors to Citrus trees and wide range of herbaceous plants by several researchers (Calavan, 1968; Oldfield et al. 1976; Rana et. al. (1975); Anonymous, 1985; Bové 1986; Azeri, 1986; Kersting et al. (1991). Natural incidence of infection by **S. citri** in sweet orange seedlings was reported 90 percent in Morena location of California containing several hundred naturally infected trees that are surrounded the young planted citrus seedlings (Calavan, 1976). Also, the widespread occurrence of **S. citri** has been reported in Israel by Klein et al., In Syria and Jordan (Anonymous, 1984). It has previously been reported that (Azeri, 1986), the efficient vector of **S. citri**, **Neolaliturus haematoceps** is a wide spreaded species on the numerous host plants in Türkiye, and according to the indexing

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tests Satsuma trees surrounded the infected sweet orange trees were also found affected by *S. citri*.

MATERIAL AND METHODS

The Citrus indicator plants Madam vinous, Pineapple sweet orange (*Citrus sinensis* L. Osb.) and Duncan. March grapefruit (*Citrus paradisi* Macf.) seedlings and *Vinca rosea* L., periwinkle plants were used as indicators in natural leafhopper transmission experiments in Satsuma plantations.

Natural transmission trial:

Natural field transmission tests have been carried out between 1986- 1991 in 5 Satsuma orchards located in different areas and contain *S. citri* infected Satsuma, clemantine, sweet orange and lemon trees. Periwinkle plants, "Duncan" and "Marsh" grapefruit, Madam vinous and Pineapple Sw. Or. seedlings were grown in 30 x 30 cm. clay pots and plastic bags in the greenhouse. Totaly 25 *S. citri* infected citrus trees in 5 Satsuma orchards were chosen as *S. citri* infection sources for natural transmission by leafhopper vectors. 3 plants from each indicators were used in each test. Indicator plants were kept under the crown branches of the infected trees from April till late November. The typical symptoms of *S. citri* described by the several researchers (Calavan and Carpenter 1964; Calavan, 1968; Calavan et al. 1976; Granett et al, 1976) have been periodically observed on the indicator plans.

ELISA: ELISA tests were carried out for detection of the natural transmission and spreading of *S. citri* in the Satsuma orchards. The young leaves and the shoot samples were collected in spring (in March and April, 1992) from the several Citrus orchards and *S. citri* affected indicator plants. Leaves and shoot samples taken from 4 or 5 trees were collected together in the same plastic bag and accepted one sample. Totally 137 samples were collected from 548 Citrus trees in the 32 Satsuma orchards and 31 samples from the Citrus indicators as shown in table 1 and table 2. The leaf samples were homogenized in PBS- Tween- PVP- extraction buffer at a dilution of 1/10 (W/V) pH 7,4. The buffer was freshly prepared and fresh egg white was added in it just before used. I ml of freeze- dried antibodies and alkaline phosphatase conjugated antibodies (Produced by Sanofi- phyto- Diagnostigs- France, and provided from Antalya Citrus Res. Institute) were reconstituted by adding 1 ml of distilled water before used.

ELISA test procedure was carried out with polystyrene microtitre plates in accordance with the ELISA procedure outlined by Clark and Adams (1977), and recommendation by Sanofi phyto- Diagnosties (France). Optical density (A 405 nm) was measured in an Multiskan plus MK II. ELISA reader. Positive control and extraction buffer controls used in the tests were as shown in table 1 and 2. Absorbance values in the table 1 and 2 (at a wavelength of 405 nm) over 0,1 were considered positive.

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Table 1. ELISA detection of Spiroplasma citri in the leaf samples collected from the Citrus trees in the several locations of İzmir.

Orchard dNo:	Tree No:	Ab. Va (A 405)	Orchard No:	Tree No:	Ab. Va	Orchard No:	Tree No:	Ab. Va	Orchard No:	Tree No:	Ab. Va	Orchard No:	Tree No:	Ab. Va.
18/1	A - In.	0.635 (a) Sw.	12/1	A - G	0.462	33/4	A - G	0.405	20/1	A - In.	0.189 Sw.	28/6	B - In	0.513
18/2	A - In.	Or.	12/2	A - G	0.472	33/5	A - G	0.418	20/2	A - In.	Or.	28/7	B - In	0.568
-	-	0.679 (a)	12/3	A - G	0.607	33/6	A - G	0.317	20/3	A - In.	0.457 Sw.	28/8	B - In	0.313
-	-	0.036 (b)	12/4	A - G	0.518	33/7	A - G	0.360	20/4	A - In.	Or.	29/1	B - In	0.627
-	-	0.011 (b)	12/5	A - G	0.697	16/1	A - In	0.578	20/5	A - In.	0.364	29/2	"	0.540
1/1	A - B	0.024 (b)	12/6	A - G	0.622	16/2	A - In	0.407	20/6	A - In.	0.418	30/1	"	0.527
1/2	A - B	0.567 M.L.	13/1	A - G	0.588	16/3	A - In	0.356	20/7	B - In.	0.364	31/1	"	0.327
3/1	A - G	0.455 M.L.	13/2	A - G	0.552	16/4	A - In	0.514	20/8	B - In.	0.256	32/1	"	0.550
3/2	A - G	0.602	13/3	A - G	0.454	16/5	A - In	0.592	20/9	B - In.	0.506	21/1	B - Ü	0.303
3/3	A - G	0.475	13/4	A - G	0.369	16/6	A - In	0.511	20/10	B - In.	0.307	22/2	B - Ü	0.517
3/4	A - G	0.443	13/5	A - G	0.506	16/7	A - In	0.534	20/11	B - In.	0.697 (a)	22/3	B - Ü	0.405
3/5	A - G	0.667	13/6	A - G	0.478	16/8	A - In	0.360	20/12	B - In.	0.843 (a)	22/4	B - Ü	0.305 Le.
3/6	A - G	0.376	13/7	A - G	0.565	17/1	A - In	0.598 Sw.	20/13	B - In.	0.809	22/5	B - Ü	0.352
3/7	A - G	0.577	13/8	A - G	0.624	17/2	A - In	Or.	20/14	B - In.	0.406	22/6	B - Ü	0.463
3/8	A - G	0.584	13/9	A - G	0.798	17/3	A - In	0.468 Sw.	20/15	B - In.	0.378 Sw.	22/7	B - Ü	0.323
3/9	A - G	0.573	13/10	A - G	0.595	17/4	A - In	Or.	20/16	B - In.	Or.	22/8	B - Ü	0.310
4/1	A - G	0.587	13/11	A - G	0.463	17/5	A - In	0.606 Sw.	20/17	B - In.	0.390	24/1	B - Ü	0.371
4/2	A - G	0.609	13/12	A - G	0.527	18/3	A - In	Or.	20/18	B - In.	0.693	24/2	B - Ü	0.446
5/1	A - G	0.889	14/1	A - G	0.429	18/4	A - In	0.547	20/19	B - In.	0.459	24/3	B - Ü	0.256
6/1	A - G	1.030	14/2	A - G	0.668	18/5	A - In	0.652	20/20	B - In.	0.394	-	-	0.004 (b)
7/1	A - G	0.495	14/3	A - G	0.527	18/6	A - In	0.550	20/21	B - In.	0.612	-	-	0.030 (b)
8/1	A - G	0.587	14/4	A - G	0.560	18/6	A - In	0.531	26/1	B - In.	0.434 Sw.	-	-	0.020 (b)
8/2	A - G	0.414	14/5	A - G	0.380	18/7	A - In	0.359	27/1	B - In.	Or.	-	-	0.074 (b)
9/1	A - G	0.433	14/6	A - G	0.423	18/8	A - In	0.461	28/1	B - In.	0.605	-	-	-
9/2	A - G	0.972	14/7	A - G	0.530	18/9	A - In	0.205 W.H.	28/2	"	0.278	-	-	-
10/1	A - G	0.725	14/8	A - G	0.455	18/10	A - In	0.530	28/3	"	0.567	-	-	-
10/2	A - G	0.588	14/9	A - G	0.411	18/11	A - In	0.652	28/4	"	0.671	-	-	-
10/3	A - G	0.785	15/1	A - G	0.767	19/1	A - In	0.550	28/5	"	0.535	-	-	-
11/1	A - G	0.730	15/2	A - G	0.581	19/2	A - In	0.695 Sw.	-	-	0.468	-	-	-
-	-	0.596	15/3	A - G	0.920	-	-	Or.	-	-	0.464	-	-	-
-	-	-	15/4	A - G	0.549	-	-	0.304	-	-	0.346	-	-	-

(a): S. citri affected positive control trees; (b): Extraction buf. Control; A: Test plate number 1; B: test plate number 2; Localities: G: Gümüşsu; İn.: İnciraltı; Ü: Ürkmez; Ab. Va: Absorbance; Sw. Or.: Sweet Orange; Le: lemon;

B: Bornova

W.H.= Weed host: Malva silvestris L.; M.L.= Mexica lime

Table 2. ELISA test results of Citrus indicators, *S. citri* transmitted by leafhopper vectors in the Citrus Orchards.

(a) Indicators Name	No.	Symptoms of <i>S.citri</i> on the indicators ^(b)	Infectivity Level ^(c)
Ma. Gr.	30	-	0 (d)
Dun. Gr.	31	-	o (d)
Ma. Gr.	1	In. V. Mot+ Tip. Yel. + S. Cap. L.	+++
M. V. Sw. Or.	2	In. V. Mot+ Tip. Yel. + S. Cap. L.	+
Dun. Gr.	3	In. V. Mot+ Tip. Yel. + S. Cap. L.	+
M.V. Sw. Or.	4	In. V. Mot+ Tip. Yel. + S. Cap. L.	++
Dun. Gr.	5	In. V. Mot+ Tip. Yel. + S. Cap. L.	+++
Dun. Gr.	6	In. V. Mot+ Tip. Yel. + S. Cap. L.	++
Dun. Gr.	7	In. V. Mot+ Tip. Yel. + S. Cap. L.	++
Ma. Gr.	8	In. V. Mot+ Tip. Yel. + S. Cap. L.	++
Ma. Gr.	9	In. V. Mot+ Tip. Yel. + S. Cap. L.	+
P. Sw. Or.	10	In. V. Mot+ Tip. Yel.	+++
P. Sw. Or.	11	In. V. Mot+ Tip. Yel.	++
P. Sw. Or.	12	In. V. Mot+ Tip. Yel.	+
Ma. Gr.	13	-	0 (0.098)
Dun. Gr.	15	In. V. Mot+ Tip. Yel. + S. Cap. L.	+
Ma. Gr.	17	In. V. Mot+ Tip. Yel.	++
Ma. Gr.	18	In. V. Mot+ Tip. Yel. + S. Cap. L.	+++
Dun. gr.	19	In. V. Mot+ Tip. Yel. + S. Cap. L.	++
P. Sw. or.	21	In. V. Mot+ Tip. Yel. + S. Cap. L.	++
Dun. Gr.	22	In. V. Mot+ Tip. Yel. + S. Cap. L.	++
P. Sw. Or.	23	In. V. Mot+ Tip. Yel. + S. Cap. L.	++
Dun. Gr.	26	In. V. Mot+ Tip. Yel. + S. Cap. L.	+++
Pin. Sw. Or.	27	In. V. Mot+ Tip. Yel. + S. Cap. L.	+++
Dun. Gr.	28	In. V. Mot+ Tip. Yel. + S. Cap. L.	+++
P. Sw. Or.	29	In. V. Mot+ Tip. Yel. + S. Cap. L.	++
(e) Ex. buf.	Control	In. V. Mot+ Tip. Yel. + S. Cap. L.	0

(a): Ma. Gr.= March grapefruit; Dun. Gr.= Duncan grapefruit;

M.V. Sw. Or.= Madam Vinous sweet Orange; P. Sw. Or.=Pineapple Sweet Orange.

(b): In. V. Mot= Interveneal mottle; Tip. Yel.= Tip yellowing;

S. Cap. L.= Small Capped leaf and yellow mottling.

(c): 0.001 - 0.100 = 0

(d): Healty control (0.002 - 0.004)

0.100 - 0.200 = +

(e): Extration buf. Control (0.004 - 0.030)

0.200 - 0.300 = ++

0.300 - 0.600 = +++



Fig.1. Typical leaf symptoms of *Spiroplasma citri* on periwinkle (*Vinca rosea* (L.)) plants infected by leafhopper vector in the field.

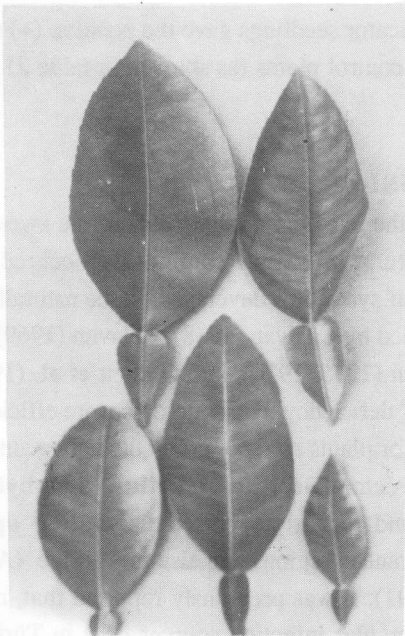


Fig. 2. Leaf symptoms of *S. citri* on grapefruit leaves, naturally infected by leaf hoppers in the field. Leaf at upper Left belongs to healthy seedling.

RESULTS

Natural transmission tests:

Periwinkle plants used in the natural transmission tests showed yellowing and chlorosis that was appeared and progressed from leaf margins toward the midribs. The leaf midribs of the yellow leaves were generally green as shown in fig. 1. Some severely affected plants developed brown lesions on the stem and at the margins of some leaves. 2 or 3 months after the infections new bloomed flowers were much smaller, broken and irregular in color. In the latter stages, infected periwinkle plants showed some slow dieback, stunting, severe chlorosis yellowing and bunching in appearance.

Citrus indicator plants Madam Vinous, Pineapple Sw. Or., Duncan and Marsh grapefruit seedlings showed typical leaf symptoms of *S. citri* 5 or 6 months after the field natural infections. These indicators showed tip yellowing, interveinal mottle on the new developed leaves. 6 months later from the first field infection by leafhoppers, many Citrus indicator plants showed small cupped leaf and yellow mottling symptoms as shown in fig. 2. Control indicator plants grown in the greenhouse conditions were in the healthy appearance.

Serological test (ELISA):

The results of ELISA detection concerning with the Satsuma and some other Citrus trees in the orchards were given in Table 1. All samples gave the positive reaction only one exception test no. 28/5 (0,077).

Samples collected from the Citrus indicator seedlings gave the positive (+) reactions only 2 exceptions belong to the healthy control plants (as shown in table 2) gave the negative (-) results.

DISCUSSION

According to the obtained results, in the Satsuma orchards where the sweet orange trees present as the *S. citri* source, natural infections continuously occurred and were seriously overspread in the places, Leaf symptoms developed on the naturally infected indicator plants were similar as described by Calavan (1968), Calavan (1969), Olson (1969), Calavan et al. (1976). Kaloostian (1975, 1976), and Granett et al. (1976). Mottled- leaf, yellowing cupped leaf and "Zn" deficiency like symptoms were efficiently characteristic symptoms on the Citrus indicator plants as reported by the above authors. It is known that, the efficient leafhopper vector *Circulifer tenellus*, *Scaphytopius nitridus*, *Neotalitrus haematoceps* and recently reported *Circulifer opacipennis* (Iethierry) are present in The Mediterranean regions and also in Türkiye (Azeri, 1986; Anonymous, 1990; Kersting et al. 1991). It was previously reported that, many weed species were the host of *S. citri* and regular infection sources of it in Türkiye

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(Uygun et al. 1991). During the survey and the observations in the Satsuma orchards, we noticed that, many weed host plants including *Malva silvestris* L. were showing chlorosis, yellowing, yellow mosaic like symptoms suspected by *S. citri*. Leafhoppers were perpetually present.

As shown in table 1., samples collected from the weed host *Malva silvestris* L. which were showing vein mosaic and yellowing symptoms gave positive ELISA result (test No: 28/5). ELISA results belong to the Satsuma and the other Citrus were positive as shown in table 1. These results indicated that, natural transmission of *S. citri* has been occurred in many Satsuma orchards. *S. citri* affected Satsuma trees were found seriously damaged in İzmir as previously reported by Azeri (1986). Since then, natural transmission and disease damages have been increased in many orchards.

ÖZET

Spiroplasma citri'NİN İZMİR'DEKİ YAYILIŞININ ELISA TESTİ İLE SAPTANMASI

Bu çalışmada, İzmir ilindeki Satsuma mandarini plantasyonlarında *S. citri* 'nin yaprak piresi vektörleri ile doğal yayılışı belirlenmiştir. Doğal taşınma testlerinde endikatör bitki olarak Cezayir menekşesi (*Vinca rosea* L.) bitkisi ile Turunçgil cinsi Madam vinous ve Pineapple portakalı ile "Duncan" ve "Marsh" altıntop çöğürleri kullanılmıştır. 1986- 1991 yılları Nisan- Kasım ayları arasında Satsuma bahçelerinde periyodik olarak bırakılan endikatörler üzerinde *S. citri*'nin tipik belirtileri oluşmuştur. İnfekteli endikatörlerin sürgünleri ile, bölgedeki 31 Satsuma mandarini bahçesindeki toplam 546 ağacın sürgünleri ile Enstitü bahçesindeki 2 Mexican, Lime çöğüründen alınan yaprak örnekleri ile ELISA testi uygulanmıştır. Uygulanan her iki test sonucu, *S. citri*'nin İzmir ili Satsuma bahçelerinde tehlikeli boyutlarda doğal bulaşmaların olduğu saptanmıştır.

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Do Viruses and Mycoplasmas Cause to Small Sized Apple Fruit in Isparta?

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ABSTRACT

The trouble of small sized apple fruit was investigated in regard to infection of virus (green crinkle, stem pitting) and mycoplasma (Chat fruit, proliferation) by using single and double budding with apple cultivars of Lord Lambourne, Golden Delicious, Virginia Crab and Spy 227 as indicators through field indexing between 1987 and 1992. Visual observations and fruit sizes in different combinations made with indicators and ultrathin sectioning and EM examining revealed that virus and mycoplasma do not cause to small sized fruit in apple trees showing this symptom and the possible agent is not bud (graft) transmissible.

INTRODUCTION

Türkiye is a very well known country with its production of different crops. Among these crops, apple is one of the major fruits with more than 30 million trees yielding 1.900.000 ton and Isparta province apple production is about 1/8 of the total amount (Anonymous, 1992).

In recent years, some Starkrimson apple trees have been producing small fruits in Isparta region and the farmers claimed to take precautions against this trouble. Observations done by some experts have reported that the possible reasons could be viruses or false growing applications by farmers, and suggested that the trouble be investigated in regard to virus diseases.

In early reports given by Smith (1957), Klinkowski (1958), Mc Grum et al. (1960), Canova et al. (1964), and Posnette (1963) apple virus and virus-like diseases were causing to crop loss in apple orchards. Nemeth (1986) pointed out 43 virus and virus-like diseases of apples and discussed in details.

Some viruse (green crinkle, stem pitting) and mycoplasma (chat fruit, proliferation) diseases showing symtoms of small fruits and bushy shoots in apples (Cenova, 1964; Nicolas and Wagnon, 1962; Posnette et al., 1965; Cropley et al., 1968; Anonymous, 1981; Nemeth, 1986) similar to the symptoms in Isparta apple orchards. Apple mosaic virus caused 55% crop loss (Mc Crum et al., 1960) while green crinckle caused 22% small sized fruit (Fridlund and Drake, 1987) and apple rubbery wood 3% crop loss in apple orchards (Zawadska, 1985).

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The only study about virus diseases of apples in Türkiye was made by Kurçman and Özkan (1976). In this article, it was reported that the symptoms of apple green crinkle and proliferation have been observed, but not small sized fruits, in some provinces of Central Anatolia.

MATERIALS and METHODS

Normal and small- sized fruit producing Starkrimson apple trees in Eğirdir (Isparta) were used as inoculum sources. The scionwoods with buds of Lord Lambourne (LL), Golden Delicious (GD), Spy 227 (Spy) and Virginia Crab (VC) kept in screenhouse at Atatürk Horticultural Research Institute (Yalova) were used as indicators and the nursery plants of GD, Starking (ST) and the seedlings grown in Atatürk Forest Farm (Ankara) were used as rootstocks.

GD, St and apple seedlings (totaly 135 nursery plants) obtained from AFF were planted in the nursery plants of Eğirdir Nursery Station in April, 1987. The buds belonging to the inoculum sources were inserted in the rootstocks 10-20 cm above the ground level and indicator buds were inserted 10- 20 cm above the former buddings in what is known as double budding in August, 1988. In order to make controls, some of the buds of inoculum sources and indicators were left with single budding and some of the rootstocks were not budded.

The buds of inoculum were dulled in March, 1989 and all the test plants pruned to induce early fruiting in the following years. The test plants were grown in usual ways and inspected for any virus and virus- like disease symptoms during each growing season. By 1990, some of the test plants were bearing several fruits and in 1991 and in 1992 mean fruit size (MFS: width (diameter) measured from the equatorial region/ height measured from sepal end to pedicel end) was measured by compass for at least 25 fruits per tree. MFS of the trees used as inoculum sources (Normal sized fruit producing tree (NSFPT) and small sized fruit producing tree (SSFPT) was also calculated for 25 fruits of the each three tree. Knives and other tools used for budding, pruning etc. were disinfected with 10% hypochloride solution just before use.

Leaf samples taken from NSFPT and SSFPT were cut into small pieces (1-2 mm long), and put in plastic tubes containing 4% glutaraldehyde solution and sent Prof. G.P. Martelli (Bari Univ., Italy) to examine by ultra thin sectioning and electron microscope.

RESULTS

Visual observations made at least twice a year showed that virus and virus-like disease symptoms did not occur in any part of the trees (twigs, branches, leaves, fruit). Combinations done with seedlings, GD, St and indicators' buds grew well but 10 seedlings (left as controls) and 26 budded seedlings did not produce fruit by 23 Sept., 1992.

Although several nursery plants of GD and St beared some fruits in 1990, it was disregarded because of less amount of fruit. The number of the nursery plants (trees), amount and kind of fruits and mean fruit sizes in 1991 and 1992 were given in Table 1 and classification of the trees in Table 2. The number of trees beared fruits of St, GD, LL, VC and Spy were 10; 10; 6; 4 and 1, respectively. The lowest and the highest MFSs (mm) were St: 54/52 and 75/67, GD: 60/55 and 70/63, LL: 56/54 and 77/63, VC: 36/30 and 43/33, Spy: 24/22 and 24/22 (Table 2).

MFSs of NSFPT and SSFPT were 68/62 and 35/29 mm, respectively.

Ultra thin sectioning and electron microscope examining of the leaf samples taken from NSFPT and SSFPT were reported as free of virus and virus-like agents (mycoplasma) by Prof. G.P. Martelli (Bari Univ. Italy).

Figures 1, 2, 3, 4, 5, and 6 show the SSFPT and the fruits of the budding combinations of S_2 /NSF, LL, VC, Spy, S_1 /LL+SSF, S_5 /VC, S_1 /Spy+SSF, respectively.

DISCUSSION

Apple fruit smalling in Isparta region was first observed by the farmers, and experts in Plant Protection Department of Isparta surveyed the apple orchards and found that 7- 66% of the Starkrimson, Starking, but not Golden Delicious trees, produced small sized fruits in 1984. In order to, objectively, compare the sizes of the fruits whether small or normal, width and height of the fruits were measured instead of weighing, while Posnette and Cropley (1965) and Cropley and Posnette (1968) have preferred weighing.

The MFSs of St fruit producing nursery plants (Table 2) did not show any significant differences between the combinations made with the NSFPT (the smallest MFS 59/53 mm for St_1 /NSF and the highest MFS 67/58 mm for St_2 /NSF) and SSFPT (the smallest MFS 64/59 mm for St_1 /SSF and St_2 /SSF, and the highest MFS 69/58 mm for St_2 /SSF) while the MFS of NSFPT and SSFPT were 68/62 mm and 35/29 mm. This situation indicates that the possible causing agent was not transmitted to St nursery plants or did not cause to make small fruits since the fruits beared in St /SSF and St /NSF were nearly twice bigger than the fruits beared in SSFPTs. On the other hand, MFS of fruits belonging to S_1 /Spy+NSF, S_2 /NSF, S_2 /LL+SSF, S_3 /SSF+NSF, GD_1 /SSF and GD_1 /NSF changed from 54/52 to 75/67 mm which became in normal sizes comparing to MFSs of SSFPTs (35/29 mm).

DO VIRUSES AND MYCOPLASMAS CAUSE TO SMALL SIZED APPLE FRUIT IN ISPARTA?

Table 1. Mean fruit sizes (MFS: length/height), amount and kinds of fruits of the nursery plants (NP) in the field - indexing plots in 1991 and 1992

(1) NP _s	(2) Kind of fruit	amount		MFS (mm)		NP _s	Kind of fruit	amount		MFS (mm)		NP _s	Kind of fruit	amount		MFS (mm)	
		24.9.91	23.9.92	24.9.91	23.9.92			24.9.91	23.9.92	24.9.91	23.9.92			24.9.91	23.9.92	24.9.91	23.9.92
S ₁ / Spy + SSF	Spy	22	25 <	24/22	24/21	S ₁ / SSF	St	-	1	-	63/60	S ₂ / NSF	D	-	-	-	-
S ₁ / Spy + SSF	Spy	5	25 <	25/22	26/22	S ₁ / SSF	St	5	-	70/63	-	S ₂ / NSF	St	7	1	67/60	48/47
S ₁ / Spy + SSF	Spy	25 <	25 <	25/22	25/22	S ₁ / SSF	St	8	-	64/62	-	S ₂ / NSF	St	14	2	57/50	40/40
S ₁ / Spy + SSF	Spy	12	25 <	20/22	24/22	S ₁ / NSF	St	-	3	-	65/58	S ₂ / NSF	St	25 <	15	62/57	64/61
S ₁ / Spy + SSF	Spy	-	25 <	-	23/21	S ₁ / NSF	St	3	-	60/54	-	S ₂ / NSF	St	25 <	11	62/57	62/61
S ₁ / Spy + NSF	D	-	-	-	-	S ₁ / NSF	St	1	-	65/56	-	S ₂ / LL + SSF	St	5	19	75/67	64/61
S ₁ / Spy + NSF	St	-	3	-	62/60	S ₁ / SSF	St	-	3	-	67/65	S ₂ / LL + SSF	IL	8	22	70/55	69/56
S ₁ / LL + SSF	IL	9	19	73/57	74/60	S ₁ / SSF	St	-	22	-	62/59	S ₂ / LL + SSF	D	-	-	-	-
S ₁ / LL + SSF	IL	13	25 <	70/55	69/55	S ₁ / SSF	St	-	1	-	64/53	S ₂ / LL + SSF	D	-	-	-	-
S ₁ / LL + SSF	IL	6	24	74/55	73/57	S ₁ / NSF	St	1	-	62/57	-	S ₂ / LL + SSF	IL	4	13	70/52	73/63
S ₁ / LL + SSF	IL	-	22	-	76/63	S ₁ / NSF	St	-	14	-	64/61	S ₂ / LL + SSF	S	-	-	-	-
S ₁ / LL	IL	11	25 <	72/55	72/58	S ₁ / NSF	St	-	1	-	48/41	S ₂ / LL + NSF	IL	16	25	73/58	70/56
S ₁ / LL	S	-	-	-	-	S ₁	St	-	-	-	-	S ₂ / LL + NSF	S	-	-	-	-
S ₁ / LL + NSF	IL	3	17	74/62	78/63	S ₁	St	-	-	-	-	S ₂ / LL + NSF	IL	3	25 <	79/63	77/63
S ₁ / LL + SSF	IL	7	25 <	75/61	76/62	S ₁	St	-	-	-	-	S ₂ / LL + NSF	D	-	-	-	-

(1) The first initials indicate the rootstocks, second and thirds indicate the buds taken from inoculum sources or indicators. NSF: Normal sized fruit producing tree, SSF: Small sized fruit producing tree., S: Seeding, St: Starking (Starkrimson), GD: Golden Delicious, Spy: Spy 227, VC: Virginia crab, LL: Lord Lambourne

Con't (Table 1.)

NP _s	Kind of fruit	amount		MFS (mm)		NP _s	Kind of fruit	amount		MFS (mm)		NP _s	Kind of fruit	amount		MFS (mm)	
		24.9.91	24.9.92	24.9.91	23.9.92			24.9.91	23.9.92	24.9.91	23.9.92			24.9.91	23.9.92	24.9.91	23.9.92
St ₂ /SSF	St	5	-	60/52	-	S ₃ /SSF+NSF	D	-	-	-	-	GD ₁ /SSF	St	14	3	65/60	65/59
St ₂ /SSF	St	3	-	67/56	-	S ₃ /SSF+NSF	St	6	4	69/65	64/56	GD ₁ /SSF	St	18	4	62/57	69/62
St ₂ /SSF	St	1	-	78/66	-	S ₃ /SSF+NSF	St	25 <	11	84/57	62/57	GD ₁ /SSF	St	25 <	-	61/55	-
St ₂ /SSF	St	2	-	62/53	-	S ₃ /SSF+NSF	St	12	7	70/57	64/59	GD ₁ /SSF	St	25	3	67/58	59/54
St ₂ /SSF	St	7	3	64/53	64/57	S ₃ /SSF+NSF	S	-	-	-	-	GD ₁ /SSF	CD	25 <	25 <	68/61	59/56
St ₂ /NSF	St	20	5	64/53	60/54	S ₁ /SSF+NSF	St	25	1	62/58	46/48	GD ₁ /NSF	CD	25 <	25 <	67/60	69/65
St ₂ /NSF	D	-	-	-	-	S ₃ /SSF+NSF	St	25 <	14	65/58	66/63	GD ₁ /NSF	CD	25 <	24	67/60	64/61
St ₂ /NSF	St	3	2	66/59	73/59	S ₃ /SSF+NSF	St	-	5	-	63/60	GD ₁ /NSF	CD	14	25 <	65/61	62/58
St ₂ /NSF	St	1	-	73/63	-	S ₃ /SSF+NSF	D	-	-	-	-	GD ₁ /NSF	St	4	10	72/67	59/54
St ₂ /NSF	D	-	-	-	-	S ₃ /SSF+NSF	D	-	-	-	-	GD ₁ /NSF	CD	6	20	74/67	61/56
St ₂ /SSF	D	-	-	-	-	S ₃ /SSF+NSF	S	-	-	-	-	GD ₁ /SSF	S	-	-	-	-
St ₂ /SSF	St	11	4	69/57	64/60	S ₃ /SSF+NSF	St	15	17	70/66	65/61	GD ₁ /SSF	CD	15	16	66/60	68/63
St ₂ /SSF	St	-	-	-	-	S ₃ /SSF+NSF	D	-	-	-	-	GD ₁ /SSF	St	-	25 <	-	67/63
St ₂ /SSF	St	1	-	80/68	-	S ₃ /SSF+NSF	St	2	12	79/64	68/61	GD ₁ /SSF	CD	25 <	22	64/60	66/62
St ₂ /SSF	St	-	-	-	-	S ₃ /SSF+NSF	St	2	13	79/72	68/60	GD ₁ /SSF	CD	25 <	25 <	69/62	66/62

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Con't (Table 1.)

NP _s	Kind of fruit	amount		MFS (mm)		NP _s	Kind of fruit	amount		MFS (mm)		NP _s	Kind of fruit	amount		MFS (mm)	
		24.9.91	23.9.92	24.9.91	23.9.92			24.9.91	23.9.92	24.9.91	23.9.92			24.9.91	23.9.92	24.9.91	23.9.92
S ₄	S	-	-	-	-	GD ₂ /SSF	GD	-	1	-	60/52	S ₅ /VC+SSF	S	-	-	-	-
S ₄	S	-	-	-	-	GD ₂ /SSF	GD	-	11	-	63/59	S ₅ /VC+SSF	D	-	-	-	-
S ₄	S	-	-	-	-	GD ₂ /SSF	GD	-	25 <	-	58/54	S ₅ /VC+SSF	S	-	-	-	-
S ₄	S	-	-	-	-	GD ₂ /NSF	GD	6	19	66/59	63/61	S ₅ /VC+SSF	VC	-	9	-	37/31
S ₄	D	-	-	-	-	GD ₂	GD	6	25 <	67/61	59/54	S ₅ /VC+NSF	VC	25 <	8	38/31	36/30
S ₄ /GD + NSF	GD	10	8	67/61	64/61	GD ₂ /LL	GD	24	25 <	68/58	69/62	S ₅ /VC+NSF	VC	22	-	41/35	-
S ₄ /GD + NSF	GD	21	21	65/60	66/61	GD ₂ /LL	IL	15	1	66/61	56/45	S ₅ /VC+NSF	VC	-	7	-	36/30
S ₄ /GD + SSF	GD	4	20	68/59	64/58	GD ₂ /GD	GD	18	13	68/61	68/60	S ₅ /VC	VC	25 <	-	40/34	-
S ₄ /GD + SSF	D	-	-	-	-	GD ₂ /GD	GD	12	25 <	71/64	71/65	S ₅ /VC+SSF	D	-	-	-	-
S ₄ /GD + SSF	GD	6	25 <	61/56	60/55	GD ₂ /VC	D	-	-	-	-	S ₅ /SSF	S	-	-	-	-
S ₄ /GD + SSF	GD	6	25 >	67/60	65/59	GD ₂ /VC	VC	21	3	41/35	43/33	S ₅	S	-	-	-	-
S ₄ /GD + NSF	D	-	-	-	-	GD ₂ /Spy	GD	12	25 <	66/61	67/62	S ₅	S	-	-	-	-
S ₄ /GD + NSF	GD	13	2	63/54	64/53	GD ₂ /Spy	GD	25 <	14	65/60	66/62	S ₅	S	-	-	-	-
S ₄ /GD + NSF	D	-	-	-	-	GD ₂	GD	2	19	69/59	60/55	S ₅	S	-	-	-	-
S ₄ /GD + NSF	GD	6	25 <	68/61	71/56	GD ₂	GD	5	24	73/69	62/59	S ₅	S	-	-	-	-

Table 2. Classification of the trees produced fruits and mean fruit size (MFS: length / height) per tree in 1991 and 1992

NPs ⁽¹⁾	Total ⁽²⁾ NPs		1991		1992		NPs ⁽¹⁾	Total ⁽²⁾ NPs		1991		1992	
	NPs Produced	MFS (mm)	NPs Produced	MFS (mm)	NPs Produced	MFS (mm)		NPs Produced	MFS (mm)	NPs Produced	MFS (mm)	NPs Produced	MFS (mm)
Starking (st) fruit producing NP _s (trees)													
St ₁ / NSF	6/6		3	62/57	3	59/53	GD ₁ / NSF	4/5		4	68/52	4	64/60
St ₂ / NSF	3/5		3	67/58	2	66/57	GD ₂ / NSF	1/1		1	66/59	1	63/61
St ₃ / SSF	6/6		2	67/53	4	64/59	GD ₁ / SSF	4/10		4	67/61	4	65/61
St ₂ / SSF	7/10		7	69/58	2	64/59	GD ₂ / SSF	3/3		-	-	3	60/55
St ₁ / Spy + NSF	1/2		-	-	1	62/60	GD ₂	3/3		3	70/63	3	60/59
S ₂ / NSF	4/5		4	62/56	4	54/52	GD ₂ / LL	1/2		1	68/58	1	69/52
S ₂ / LL + SSF	1/6		1	75/67	1	64/61	GD ₂ / GD	2/2		2	70/63	2	70/63
S ₃ / SSF + NSF	11/15		8	71/62	9	71/58	GD ₂ / Spy	2/2		2	66/61	2	67/62
GD ₁ / NSF	1/5		1	72/67	1	59/54	S ₄ / GD + NSF	4/6		4	66/59	4	66/58
GD ₁ / SSF	5/10		4	64/58	4	65/60	S ₄ / GD + SSF	3/4		3	65/58	3	63/57
Lord Lambourne (LL) fruit producing NP _s (trees)													
S ₁ / LL + NSF	2/2		2	75/62	2	77/63	S ₅ / VC + NSF	3/3		2	40/33	2	36/30
S ₂ / LL + NSF	2/4		2	76/61	2	74/60	S ₅ / VC + SSF	1/5		-	-	1	37/31
S ₁ / LL + SSF	4/4		3	72/56	4	72/57	S ₅ / VC	1/2		1	40/34	-	-
S ₂ / LL + SSF	2/6		2	70/54	2	71/59	GD ₂ / VC	1/2		1	41/35	1	43/33
S ₁ / LL	1/2		1	72/55	1	72/58	Spy 227 fruit producing NPs (trees)						
GD ₂ / LL	1/2		1	66/61	1	56/45	S ₁ / Spy + SSF	5/5		4	24/22	5	24/22

(1) NPs: Nursery plants. The first initials indicate the rootstocks, seconds and thirds indicate the buds taken from inoculum sources or indicators.

NSF: Normal sized fruit producing tree, SSF: Small sized fruit producing tree, S: Seedling.

(2) numerator: Same kind of fruit producing NPs, denominator: total of budded NPs.

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GD is a cultured apple variety and is known as a good indicator for apple green crinkle and proliferation diseases (Mc Grum et al., 1960; Anonymous, 1983 and Nemeth, 1986). The combinations made with GD did not show any symptoms of bushy shoots, stimulation of axillary buds and leaf abnormalities which indicates that the two inoculum sources were free of the above mentioned diseases. MFSs of GD fruits in different combinations changed from 60/65 mm and 70/63 mm showed that any smalling did not occur in GD₁ /NSF, GD₂ /NSF, GD₁ /SSF, GD₂ /SSF, GD₂ /LL, GD₂ /GD, GD₂ /Spy, S₄ / GD+NSF and S₄ / GD+SSF (Table 2) and confirmed the absence of the above diseases.

Chat fruit, literally, means dwarf and small sized fruits and symptom expression is manifest in the production of small, dwarfed fruit (Mc Grum et al., 1960). Chat fruit disease has been detected by using Lord Lambourne in some rootstock clones (Posnette and Cropley, 1965) and the mean weight of fruit was reduced 23% in Tydeman's Early Worcester apple trees (Cropley and Posnette, 1968). As mentioned before, the level of the fruit size can easily and directly be expressed by length and height of the fruit instead of weight. Owing to this, MFS was taken into consideration to determine the fruit size. There was not big difference between MFSs of S₁ / LL and GD₁ / LL. On the other hand, Chat fruit causes dark green mottles (about 5 mm radius) on the small sized fruits (Nemeth, 1986) that was not observed in related combinations of this study. Consequently, It was concluded that SSFPT was not infected with Chat fruit disease.

Another virus disease of apple, Stem pitting, is affecting the developing wood and bark tissues to make grooves in wood, yellow- red colour of leaves and small sized fruits in some cases (Baumann and Lemmich, 1965; Anonymous, 1983; Nemeth, 1986). The indicators of stem pitting used in this study, VC and Spy produced normal sized fruits that the MFSs changed from 36/30 mm to 40/34 for the combinations of S₅ / VC+NSF, S₅ / VC + SSF, S₅ / VC, and 43/33 mm for GD₁/ VC (Table 2). It is very clear that the similar MFSs were taken for S₅ / VC+NSF (36/30 mm) and for S₅ / VC+SSF (37/31 mm) in 1992. If there was stem pitting in SSFPT_s, there would be symptoms in foliage and in fruit sizes in combination made with VC and Spy buddings. This indicates that the SSFPTs were free of stem pitting disease and the symptomless combination of S₁ / Spy+SSF also produced normal sized fruits (24/22 mm) confirmed the absence of stem pitting.

As a result, six years of field indexing, and ultrathin sectioning and electron microscope examining of leaf samples showed that viruses and mycoplasmas do not cause small sized apple fruit and SSFPTs are free of any bud (graft) transmissible patogen as discussed above.

In replying the question to "What can be the reason of the trouble in SSFPTs

other than virus and mycoplasmas in Isparta apple orchards ?" , some colleagues and I suggest that the problem be investigated in terms of physiological, ecological factors.

ÖZET

Isparta ili elma ağaçlarında meyvelerin küçük kalma nedenlerinin virüs (green crinkle, Stem pitting) ve mikoplazma (Chat fruit, proliferation) yönünden araştırıldığı bu çalışmada: Lord Lambourne, Golden Delicious, Virginia Crab ve Spy 227 elma çeşitleri indikatör olarak kullanılmış, "tek göz", "çift göz" aşılama ile çeşitli kombinasyonlar arazide indeksleme çalışmaları yapılmıştır. Yaklaşık altı yıl süren gözlemler ve son iki yılda hasat edilen meyvelerin sayım, ölçümlerine ait değerlendirmeler ve yaparak örneklerinin ince kesit (ultra thin sectioning), elektron mikroskop incelemeleri neticesinde meyvelerin küçük kalmasına aşı ile taşınan virüs ve mikoplazma ile diğer etmenlerin neden olmadığı ve konunun bitki fizyolojisi, ekoloji vb yönlerden kapsamlı olarak incelenmesinin gerekli olduğu kanısına varılmıştır.

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Figure 1. Small sized fruits
Starkrimson apple



Figure 2. Normal sized
Starking fruit
in the combination
of S_2 / NSF

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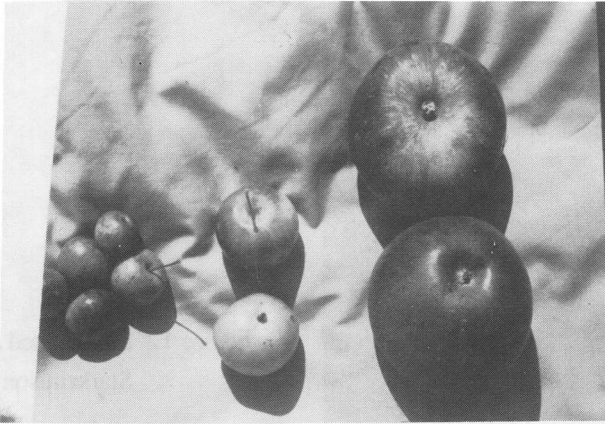


Figure 3. Normal sized apple fruits beared in some combinations of LL, VC and Spy indicators (from left to right).



Figure 4. Normal sized LL fruits of combination of S_1 / LL+SSF.



Figure 5. Normal sized VC
fruits of
Combination of
 S_5 /VC.



Figure 6. Normal sized Spy
fruits of
Combination of
 S_1 / Spy + SSF.

Fungal Diseases of Sunflower in Aegean Region of Türkiye

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ABSTRACT

*It was found that **Macrophomina phaseolina**, **Plasmopara helianthi** and **Sclerotinia sclerotiorum** were potential pathogens on sunflower during the surveys in Aegean Region in 1991.*

***Alternaria sp.**, **Puccinia helianthi**, **Verticillium dahliae**, **Rhizopus sp.** and **Botrytis cinerea** were determined to be less important on sunflower.*

INTRODUCTION

Sunflower (**Helianthus annuus L.**) is one of the main crops for oil seeds in Türkiye. It is responsible for 45, 6% of the total oil seed production. According to the data of 1988, total sunflower cultivation area was 750.000 ha., total sunflower yield was 1.150.000 tons and average yield was 153,3 kg/da (Anon., 1990).

Vniimk- 1646 and Vniimk- 8931 cultivars of sunflower have been grown for long in Türkiye; however, hybride cultivars with high oil have been grown in order to meet vegetable oil shortage since 1984. The rate of them in total sunflower cultivation was 3%, 14% and 48% in 1984, 1985 and 1986 respectively (Akar, 1986). Recently they have been dominant gradually in sunflower production. On the other hand, it is known that new diseases which are not problem previously appear when hybride cultivars are grown in the countries growing sunflower. For example, two of these diseases are **Phomopsis helianthi** and **Phoma macdonaldi** (Iliescu et al., 1982; Vörös et al., 1983; Cobia and Zimmer, 1985; Progetti et al., 1988). These examples may be increased.

In 1970s, sunflower diseases were determined as **Plasmopara helianthi**, **Puccinia helianthi**, **Sclerotinia sclerotiorum**, **Botrytis cinerea**, **Alternaria sp.**, **Helminthosporium sp.**, **Rhizopus sp.**, and **Septoria sp.** in Thrace Region while Vniimk cultivar was growing (Yücer and Karaca, 1978). Nevertheless, it is not known sunflower diseases in Aegean Region. As mentioned above, it is of importance to determine the fungal diseases of sunflower in order that hybride cultivars have been grown recently. Therefore, surveys were done to determine the fungal pathogens which can be problem, disease incidence of sunflowers and prevalence of the fungal diseases in Aegean Region during 1991.

MATERIALS and METHODS

Surveys covered Balıkesir and Çanakkale provinces. Observations were done in these provinces and 9 districts. Fields which were observed were 100 (Table 1).

Table 1. Sunflower cultivation areas and the observed fields during surveys in 1991.

The observed places		Cultivation Area (ha)	Number of the Observed Fields
Provinces	Districts		
Balıkesir	Central	6.197	7
	Bandırma	17.213	21
	Gönen	13.081	16
	Manyas	11.016	13
	Susurluk	9.250	11
Total		56.757	68
Çanakkale	Central	3.239	4
	Biga	13.494	16
	Eceabat	1.079	0
	Ezine	2.159	3
	Gelibolu	6.777	9
Total		26.748	32
General total		83.505	100

Surveys were carried out at the flowering stage in order to determine the pathogens found on the above ground- parts of sunflower.

At each sample unit, 100 plants from 10 ha of fields, 150 plants from 11-100 ha of fields, 225 plants from above 100 ha of fields were observed. For this, 20 plants from 5 different places, 15 plants from 10 different places and 15 plants from 15 different places were sampled randomly from center and margins of each unit. They were counted as healthy or diseased plants.

Rough identifications of the diseases were made macroscopically from the symptoms on the plants. Definite identifications were made microscopically after isolation and pure culture preparations of the pathogens. For this, the diseased plant parts, except *Plasmopara* and *Puccinia*, were placed in petri dishes containing 20 ml 2% PDA after they were surface-sterilized, and they were incubated for 14 days in 20 C°.

Disease incidence of sunflowers in each field and disease prevalence in the region were determined.

RESULTS and DISCUSSION

During the surveys in Aegean Region, 8 fungal pathogen were found. The disease incidence of sunflowers and the prevalence of the diseases are given in Table 2 and 3 respectively.

Table 2. The fungi found in the surveyed area and disease incidence of sunflowers in each sample center (%)

Sampling centers	Fungi ^x							
	Pl	Ma	Sc	Al	Pu	Ve	Rh	Bo
Balıkesir Central	0	0	1,8	0	0	0	0	0
Bandırma	0	8,3	2,0	0,7	0	0	0	0
Gönen	6,7	1,7	7,1	0	0	0	0	0,2
Manyas	5,9	1,5	0,1	1,3	0	0	0,9	0
Susurluk	3,0	0,7	0,4	1,1	3,5	0	0	0
Çanakkale Central	0,1	0	0,3	0	0	0	0	0
Biga	0,1	0	0,1	0	2,2	0,1	0	0
Ezine	0,1	0	0,1	0	0	1,7	0	0
Gelibolu	0	0,5	1,3	3,3	0	0	0	0

^xPl: *Plasmopara helianthi*, Ma: *Macrophomina phaseolina*, Sc: *Sclerotinia sclerotiorum*, Al: *Alternaria* sp., Pu: *Puccinia helianthi*, Ve: *Verticillium dahliae*, Rh: *Rhizopus* sp., Bo: *Botrytis cinerea*

When considering the prevalence of the fungal pathogens, it is seen that *M. phaseolina* is the first important pathogen (Table 3). It causes charcoal rot on sunflower. The pathogen was found on sunflowers in the USSR in the 1930s but was first recognized in Yugoslavia after 1960 and in various Mediterranean countries after 1970 (Sackston, 1981). *M. phaseolina* grows best and attacks at temperatures near or above 30 C°. Although seedlings can be killed rapidly under suitable experimental conditions and in the field, the pathogen's effects are not usually evident until plants are beginning to mature (Sackston, 1981; Zizzerini et al., 1985; El-din et al., 1986; Sacdashaivaiah et al., 1986). If moisture supplies are adequate, sunflowers may not show

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symptoms of charcoal rot even if the pathogen is on and in the roots. Plants that suffer drought stress and high temperatures may ripen prematurely, with small heads, poorly filled seed, and much reduced yields (Sackston, 1981; Cobia and Zimmer, 1985). Except Balıkesir Central, Çanakkale Central, Biga and Ezine, it was found that the pathogen was present in the sampling centers.

Table 3. Prevalence of the fungal pathogens in the surveyed area (%)

Sampling centers	Fungi ^x							
	Ma	Sc	Pl	Al	Pu	Ve	Rh	Bo
Balıkesir Central	0	14,2	0	0	0	0	0	0
Bandırma	61,9	23,8	0	9,5	0	0	0	0
Gönen	43,7	25,0	43,7	0	0	0	0	6,2
Manyas	53,8	15,3	23,0	15,3	0	0	7,6	0
Susurluk	72,7	45,4	63,6	27,2	18,1	0	0	0
Çanakkale Central	0	100	25,0	0	0	0	0	0
Biga	0	12,5	6,2	0	6,2	6,2	0	0
Ezine	0	33,3	33,3	0	0	33,3	0	0
Gelibolu	22,2	44,4	0	11,1	0	0	0	0
Surveyed area	37	28	20	8	3	2	1	1

x Ma: *Macrophomina phaseolina*, Pl: *Plasmopara helianthi*, Sc: *Sclerotinia sclerotiorum*, Al: *Alternaria* sp., Pu: *Puccinia helianthi*, Ve: *Verticillium dahliae*, Rh: *Rhizopus* sp., Bo: *Botrytis cinerea*

Management measures include crop rotation and burial of crop debris. There is no effective chemical to pathogen, so it is likely to problem for the region in the future.

M. phaseolina is followed by **S. sclerotiorum**. It causes root and basal stem rot and wilt of sunflowers and head rot. This pathogen was determined in all surveyed area. It was generally more severely on heavy soils. The wide host range and lack of specialization of the pathogen make it difficult to control by crop rotation. The probability of selecting resistant strains within a host species is also reduced. Differences in susceptibility among breeding lines have been demonstrated in France and Yugoslavia, however, and are being exploited in breeding programs (Ljubick et al., 1983; Fomina, 1987). The possibility of biological control using hyperparasitic fungi is being investigated (Zizzerini and Tosi, 1985; Zizzerini et al., 1987).

Plasmopara helianthi was the third common and effective fungus in the surveyed area. It causes downy mildew on sunflowers. It was described in Türkiye in 1958 (Karel, 1958). Its potential for destructiveness when moisture is adequate after sowing has made downy mildew one of the major diseases of sunflowers. The pathogen has been found in most areas where sunflowers are widely grown (Leppik, 1962). It is seedborne, but most infection arises from soilborne inoculum (Delanoe, 1972). The fact that the disease incidence was low in the surveyed area could be due to seed treatment. However, its prevalence shows that the pathogen can not be neglected, so seed treatment must be done against this disease.

Alternaria sp. was isolated from leaf lesions in the surveyed area. Leaf spots were generally located on the lower leaves. Later leaf lesions may coalesce and cause leaves to wither. Heavy leaf infection can result in defoliation (Carson, 1985). Leaf spots of sunflower can be controlled by spraying with appropriate fungicides. The use of this control measure will be determined more by economic considerations than by its effectiveness. It came to a conclusion that the fungus was not any problem in the surveyed area.

Puccinia helianthi Schw. (Rust) caused little injury. Fungicide control of rust is possible but usually not economical. The only practical way to control sunflower rust can be with resistant varieties. It was seen that the pathogen was not in economical level in the region.

V. dahlia Kleb. (*Verticillium* wilt) was determined at two districts. It is known to be present in most major sunflower-producing areas of the world (Iliescu and Csep, 1982; Lamarque, 1983; Bruniard et al., 1984). Growing sunflowers repeatedly or in short rotations may increase inoculum levels markedly and may result in severe outbreaks of *Verticillium* wilt, but longer rotations do not guarantee freedom from the disease (Sackston, 1981; Cobia and Zimmer, 1985). The pathogen was seen not to be in economical level in the region.

B. cinerea and **Rhizopus** sp. were found only on head rots of sunflowers. The fact that both pathogens were local might be due to climatic conditions of the region.

Consequently, it is difficult to answer whether these diseases will become limiting factors in sunflower production on a wide scale in the region. However, some important considerations are obvious. If sunflower is treated as a speculative crop, to be grown year after year on the same fields while cash returns are good, the results almost certainly will be disastrous. If long rotations and other sound cultural practices are followed, the long-term prospects for the crop may be good.

ÖZET

EGE BÖLGESİ AYÇİÇEĞİ ÜRETİM ALANLARINDA GÖRÜLEN
FUNGAL HASTALIKLAR

Ege Bölgesinde 1991 yılında yapılan surveyler sırasında, **Macrophomina phaseolina**, **Plasmopara helianthi** ve **Sclerotinia sclerotiorum**'un ayçiçeği için potansiyel patojenler olduğu belirlenmiştir.

Alternaria sp., **Puccinia helianthi**, **Verticillium dahliae**, **Rhizopus sp.** ve **Botrytis cinerea** funguslarının ayçiçeği üretiminde verim kaybına neden olacak düzeyde ve etkide olmadıkları görülmüştür.

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