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Studies on Citrus Stubborn Disease and Sesame Phyllody in Sesame and Their Related Leafhoper Vectors

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ABSTRACT

*The occurence of leafhoppers and the population dynamic of **Circulifer haematoceps** (M. & R.) and **Orosius orientalis** (Matsumura) (Homoptera: Cicadelliade) were studied on spring and summer sesame by D-VAC collection in Adana from May to September in 1991. Furthermore, the incidence of **Spiroplasma citri** Saglio et al. and sesame phyllody in infected sesame plants were observed in field. Both diseases were vectored by **C. haematoceps** and **O. orientalis**, respectively. The infection of **C. haematoceps** and sesame by **S. citri** was determined by ELISA in laboratory.*

*Leafhoppers were more dominant on spring sesame compared to summer crop, however, the number of **C. haematoceps** and **O. orientalis** in summer sesame outnumbered the amount of both species in spring sesame. The first **C. haematoceps** collected in May were all hibernating females and were harboring **S. citri**, as determined by by ELISA. The first **S. citri** infected sesame platns were detected in the mid of May, which probably infected just after germination in April.*

*With increasing population of **C. haematoceps** the number of **S. citri** - infected plants raised up 125 plants in summer sesame till mid of August. Most of the sesame plants were concurrent infected with **S. citri** plus sesame phyllody MLO, thus symptoms positively attributable to **S. citri** were rarely observed.*

*This study conclusively demonstrated the importance of sesame, especially of summer sesame, and the associated **C. haematoceps** population for the epidemiology of **S. citri**.*

INTRODUCTION

Stubborn disease, caused by ***Spiroplasma citri*** Saglio ***et al.*** (Mycoplasmatales: Spiroplasmatacea) is one of the most serious and destrutive diseases of citrus in the Eastren Mediterranean Region of Türkiye and the Near East (BOVE 1986,

ÇAĞLAYAN 1987, ÇINAR *et al.* 1993). The citrus stubborn disase pathogen (CSD) is transmitted by the leafhopper ***Circulifer haematoceps*** (M. & R.) (Homoptera: Cicadelliade) (FOS *et al.* 1986, KERSTING and ŞENGONCA 1992). Probable due to this vector transmission up to 50 % of all Navel orange trees are infected by the CSD pathogen in the Çukurova region, where about 85 % of the total Turkish citrus production is concentrated (GÜLLÜ 1989).

A number of non-citrus hosts of ***S. citri*** are known in the Çukurova region (UYGUR *et al.* 1991, KERSTING *et al.* 1992, BAŞPINAR *et al.* 1993). However, a recent study showed that only sesame (***Sesamum indicum*** (L)). serves as an excellent host, both for the vector ***C. haematoceps*** and the pathogen ***S. citri*** (KERSTING *et al.* 1993). Moreover sesame is frequently infected by sesame phyllody MLO (mycoplasma-like organism) in Asia, a disease known to be transmitted by the leafhoppers ***Orosius orientalis*** (Matsumura) and ***Orosius cellulosus*** (Lindberg) (VASUDIVA and SAHAMBI 1959, CHOOPANYA 1973). This disease was previously reported from Türkiye (TÜRKOĞLU and FİDAN 1985) and observed by the authors on nearly all sesame fields along the south coast of Türkiye and in South East Anatolia in 1990.

For the reason that sesame is an important crop in the Eastern Mediterranean Region, covering about 10.000 ha/year and because of its significance for the epidemiology of the CSD pathogen, the population changes of the most important leafhopper sepecies and the incidence of ***S. citri*** and sesame phyllody MLO were studied on sesame in the Çukurova region of Türkiye in 1991.

MATERIALS AND METHODS

Leafhoppers were collected both in spring and summer sesame in Adana thorughout the vegetation period from May to September in 1991. The sesame field consisted of four varieties, namely, Gölmarmara, Muganlı, Özberk-82, and a local variety. The spring sesame was sowed on April, 15th and the summer sesame on June, 25 th. The total experimental area included 112 rows of 50 plants each for both the spring and summer date.

Leafhopper samplings were conducted three times a month by a mechanical insect collector (D-VAC) and were standardized by sucking a single plant with 100 repetitions for three seconds.

S. citri was detected in sesame and in leafhoppers following the technique described by BOVE *et al.* (1987) and MARKHAM *et al.* (1983). For this purpose, sesame plants showing either yellowing, stunting or phyllody and the leafhopper associated with these plants were analyzed for the presence of ***S. citri.***

In addition, surveys for mollicute infected sesame and leafhoppers were conducted in Kozan and Silifke an important growing area of sesame in 1991.

RESULTS

During the one year study totaly 15 leafhopper species were determined on spring and summer sesame. As it is shown in table 1, ***Asymmetrasca decedens*** (Paoli), ***Empoasca decipiens*** Paoli morphological indistinguishable from each other - ***Cicadulina bipunctella*** (Matsumura), ***Macrosteles quadripunctulatus*** (Kbm.), ***C. haematoceps***, ***O. orientalis*** and ***Psammotettix provincialis*** (L). were common and occurred in comparatively high number. ***Aconurella prolixa*** (Lethierry), ***Anaceratagallia ribauti*** (Oss.)., ***A. laevis*** (Ribaut), ***Austroagallia sinuata*** (Ribaut), ***Balclutha hebe*** (Kirkaldy) as well as ***B. punctata*** (Fabricius), ***Batracomorphus glaber*** Haupt, ***Eucelis alsius*** Ribaut and ***Exitianus capicola*** (Stàl) were rare, and observed, in general, only in spring sesame. The number of leafhopper species on the first crop was as double as high (15 species) than in summer sesame (7 species). Much the same is to say for the abundance of leafhoppers with the exception of ***C. haematoceps*** and ***O. orientalis***, since more individuals were encountered in the summer crop.

For the reason that ***C. haematoceps*** and ***O. orientalis*** are important vector species, their populaton fluctuations on the spring and summer sesame crop as well as the number of ***S. citri*** and sesame phyllody infected plants are described in detail.

At the first sampling date, 26 ***C. haematoceps*** were collected, being all females and again 11 females were sampled ten days later. The first males were observed end of May. The ***C. haematoceps*** population reached a small peak beginning of June. From this date on, the number of ***C. haematoceps*** decreased until harvesting time end of August. On the spring sesame ***O. orientalis*** never reached high numbers starting with two individuals in May and only up to five individuals were sampled end of August (Fig. 1).

The first plant showing yellowing symptoms were discovered mid of May. Later in the season plants with virescence or phyllody were discovered. ***S. citri*** was detecded in all plants by ELISA, showing either yellowing, stunting or symptoms attributable to sesame phyllody. The number of infected plants increased slightly in spring sesame valuing 25 plants end of August (Fig. 1)

On the summer crop of sesame, 19 ***C. haematoceps*** were collected beginning of August. The population strongly increased and reached its maximum level end of August with about 46 individuals/per D-vac collection. Later the population severerly decreased and only 8 individuals were sampled mid of September. The first ***O. orientalis*** were observed end of July (10 individuals) and then he population grew gradually up to 27 individuals/per D-vac collection beginning of September (Fig. 2).

The firs plants showing yellowing or phyllody symptoms in the second crop were observed beginning of August. Later the number of infected plants increased dramatically and at end of sesame season up to 125 plants were found to be infected (Fig. 2).

Table 1 : Occurence and number of leafhoppers in spring and summer sesame in Adana in 1991.

Leafhopper species	Leafhopper in 17 D-vac samplings on sesame (spring crop)		Leafhopper in 8 D-vac samplings on sesame (summer crop)	
	Occurence	Number	Occurence	Number
Aconurella prolixa	1	1	0	0
Anaceratagallia laevis	1	1	0	0
Anaceratagallia ribauti	2	3	0	0
Asymmetrasca decedens + Empoasca deciepiens	17	1186	8	176
Austroagallia sinuata	1	1	3	0
Balclutha hebe	3	19	3	12
Balclutha punctata	2	10	–	4
Batracomorphus glaber	1	1	6	0
Cicadulina bipunctella	9	84	7	39
Circulifer opacipennis	14	98	0	124
Euscelis alsius	1	2	0	0
Exitianus capicola	3	3	6	0
Macrosteles quadripunctulatus	9	138	7	33
Orosius orientalis	10	28	8	108
Psammotettix provincialis	8	53	0	0

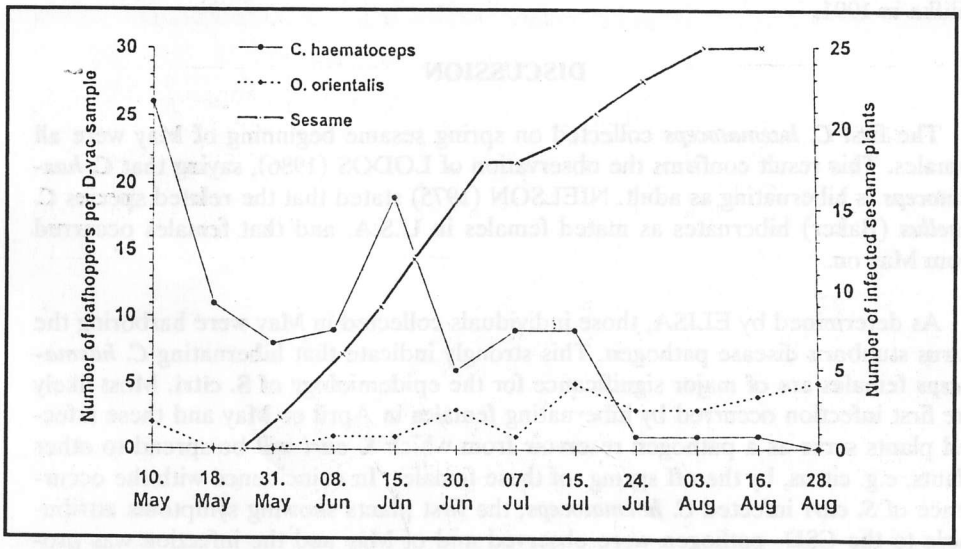

Figure 1 : Population changes of **Circulifer haematoceps** and **Orosius orientalis** as well as number of **Spirolasma citri** and sesame phyllody infected sesame plants in spring sesame in Adana in 1991.

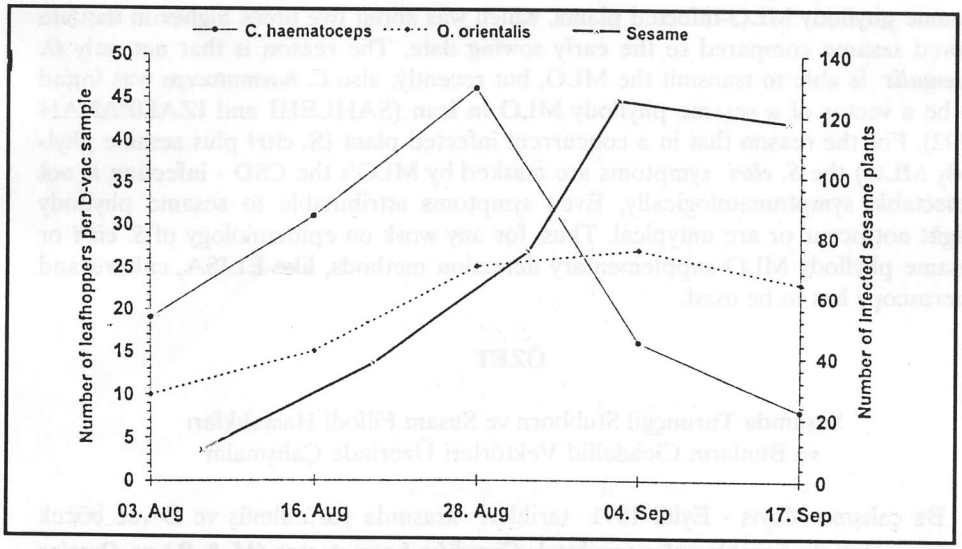

Figure 2 : Population changes of **Circuliferr opacipennis** and **Orosius orientalis** as well as number of **Spirolasma citri** and sesame phyllody infected sesame plants in summer sesame in Adana in 1991.

S. citri infected *C. haematoceps* and sesame was also common around Kozan and Silifke in 1991.

DISCUSSION

The first *C. haematoceps* collected on spring sesame beginning of May were all females. This result confirms the observation of LODOS (1986), saying that *C. haematoceps* is hibernating as adult. NIELSON (1975) stated that the related species *C. tenellus* (Baker) hibernates as mated females in U.S.A. and that females occurred from May on.

As determined by ELISA, those individuals collected in May were harboring the citrus stubborn disease pathogen. This strongly indicate that hibernating *C. haematoceps* females are of major significance for the epidemiology of **S. citri.** Most likely the first infection occurred by hibernating females in April or May and these infected plants serve as a pathogen reservoir from which *S. citri* will be spread to other plants, e.g. citrus, by the off spring of these females. In coincidence with the occurrence of *S. citri* infected *C. haematoceps*, the first plants showing symptoms attributable to the CSD pathogen were observed mid of May and the infection was proved by ELISA. These sesame plants were probably infected just after germination in Mid of April, before the first leafhopper samples were done.

The number of *C. haematoceps*, vectoring *S. citri* was much higher in summer sesame than in spring sesame. Much the same is to say for the number of *S. citri* - and sesame phyllody MLO-infected plants, which was about five times higher in the late sowed sesame compared to the early sowing date. The reason is that not only *O. orientalis* is able to transmit the MLO, but recently, also *C. haematoceps* was found to be a vector of a sesame phyllody MLO in Iran (SAHLEHI and IZADPANAH 1992). For the reason that in a concurrent infected plant (**S. citri** plus sesame phyllody MLO) the *S. citri* symptoms are masked by MLO's the CSD - infection is not detectable symptomatologically. Even symptoms attributable to sesame phyllody might not occur or are untypical. Thus, for any work on epidemiology of *S. citri* or sesame phyllody MLO supplementary detection methods, like ELISA, culture and microscopy has to be used.

ÖZET

Susamda Turunçgil Stubborn ve Susam Fillodi Hastalıkları
ve Bunların Cicadellid Vektörleri Üzerinde Çalışmalar

Bu çalışma Mayıs - Eylül 1991 tarihleri arasında yürütülmüş ve D-vac böcek toplama aleti ile örneklemeler yapılarak *Circulifer haematoceps (M & R.)* ve *Orosius orientalis* (Matsumura)'in birinci ve ikinci ürün susamda bulunma zamanları ve populasyon dalgalanmaları incelenmiş ve ayrıca. *Spiroplasma citri* Saglio *et al*

ve Susam fillodi hastalığının tarla içerisindeki yayılışı izlenmiştir. Bu hastalıklar sırasıyla *C. haematoceps* ve *O. orientalis* tarafından taşınmaktadır. *C. haematoceps* ve susamda *S. citri*'nin varlığı laboratuvarda ELISA testi ile saptanmıştır.

Cicadellidlerin birinci ürün susamda tür sayısı bakımından daha zengin olduğu, ancak *C. haematoceps* ve *O. orientalis*'in diğer türlere göre hem birinci ve hem de ikinci ürün susamda daha yüksek düzeyde populasyonlar oluşturdukları belirlenmiştir. Kışı geçiren dişi bireylerin bünyelerindeki *S. citri* ELISA testi ile ortaya konmuştur. Tarla içinde infekteli ilk bitki mayıs ortalarında saptanmış olup, bu bitkinin çinlenmeden hemen sonra vektörlerin hastalığı taşımasıyla infekteli hale gelebileceği düşünülmektedir.

C. haematoceps populasyonunun artmasıyla birlikte *S. citri* ile infekteli bitki sayısında da bir artış gözlenmiş olup, ağustos ortalarında 125 adet infekteli bitki ile bu sayı en yüksek düzeye ulaşmıştır. Bu bitkilerin büyük çoğunluğu hem *S. citri* ve hem de Fillodi etmeni ile infekteli bulunmuş sadece *S. citri* simptomu gösteren bitki sayısı son derece düşük saptanmıştır.

Bu çalışmayla susam bitkisinin özellikle ikinci ürün susamın gerek *S. citri* ile infektili çok sayıda bitkiyi ve gerekse yüksek populasyon düzeylerinde *C. haematoceps*'i birlikte bulundurması nedeniyle *S. citri*'nin epidemiyolojisinde çok önemli bir yeri olduğu ortaya konulmuştur.

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Seasonal Changes in Transmission of Citrus Stubborn Disease Pathogen, *Spiroplasma citri*, by Budwood Grafting

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ABSTRACT

The Mediterranean region is the most important citrus growing area of Türkiye and about 85 % of the total production is concentrated in this region. Almost all citrus trees are infected with at least one virus-like disease, the most important being citrus stubborn disease (CSD), caused by ***Spiroplasma citri*** *Saglio* ***et al.*** *The objective of the present study was the evaluation of the rate of transmission of* ***S.citri*** *by budwoods with regard to the season and the period of storing at cool temperature.*

Budwoods were collected in February, May, August, and November from ***S.citri*** *infected Atwood navel oranges. One third of the budwoods was grafted so sour orange the same day, the rest was stored in a refrigerator at 4 °C for one and two month, respectively. The rate of transmission of the CSD pathogen was evaluated symptomatologically, by biological indexing, and by ELISA.*

Out of 260 plants 15 showed symptoms attributable to ***S.citri****, 11 plants were tested positive by biological indexing and 17 plants were harboring the CSD pathogen as determined by ELISA. Over all 19 citrus plants were infected with* ***S.citri*** *according to biological indexing and ELISA, however only 4 plants were positive tested symptomatologically, by biological indexing, and by ELISA.*

As a result of this study it is stronogly suggested that in any budwood improvement program, biological indexing as the preferred method should be combined with ELISA or culture of the CSD pathogen in the detection of ***S.citri*** *infected trees.*

INTRODUCTION

Citrus is of ever increasing importance for Türkiye and the production reached about 1.5 million tons in 1991 (ANONYMOUS, 1991). Citrus plantations have mainly been established along the south coast of Türkiye, where about 85 % of the total citrus is produced. Unfortunately, many citrus orchards in the Eastern Mediterranean region are infected with several virus and virus-like diseases. One of the most important is citrus

stubborn disease (CSD), caused by ***Spiroplasma citri*** Saglio et al. (Mycoplasmatales: Spiroplasmataceae) (SALIBE 1986, Çınar ***et al.*** 1993).

Citrus stubborn disease was first observed in California in the beginning of the 20 th century (FAWCETT ***et al.*** 1944) and later on, the disease spread widely in many Mediterranean and Middle East countries (CALAVAN and BOVE 1989). The presence of a stubborn-like disease in Türkiye was reported for the first time in the 1950's (CHAPOT, 1959), reaching epidemic levels in the 1970's. ***S.citri*** was introduced into Türkiye probably through infected young citrus trees or budwoods. In the following years the disease spread rapidly and the common planting of navel oranges which are highly sensitive to ***S. citri.***

S. citri is vectored by leafhoppers, especially by species of the genus ***Circulifer.*** In Türkiye, ***Circulifer haematoceps*** complex (M. & R.) was found to be only vector of the stubborn disease pathogen (KERSTING and ŞENGONCA 1992). Besides by leafhoppers, the disease is transmitted by budwoods from infected trees or propagations. Howeer, it is reported that the transmission rate is quite variable and ranged from 0 to 100 % depending on variety, tissue and season (CALAVAN ***et al.*** 1969, GUMPF ***et al.*** 1986).

The purpose of this study was to determine the transmission rate of ***S. citri*** by using budwoods from stubborn infected field trees with regard to season and the peiod of storing at cool temperature.

MATERIALS AND METHODS

During this study, five stubborn infected Atwood Navel orange trees (***Citrus sinensis*** (L.) Osb.) at the Mersin - Alata Horticultural Research Institute had been chosen as budwood source. In May, August, November 1989 and in February 1990 at least 90 buds were collected at each date. Each of the 90 buds were separated into three groups. The first group was immediately grafted on sour orange and the other two groups were grafted following a one or two month storage in a refrigerator at 4^0C. For each different group 30 virus and stubborn free budwoods were grafted as control.

The budwoods were grafted to one year old sour orange nucellars and wrapped with polyethylene tape for three weeks. Thereafter the sour oranges were pruned 10 cm. above the graft. The scions were forced to grow as single shoot for six month at 32/27^{0}C (day/ night) temperature, 70 ± 5 % relative humidity and 16 h light in an insect-free greenhouse.

To determine the transmission rate of ***S. citri*** by budwoods, the citrus stubborn disease pathogen was detected in all test plants symptomatologicalyy, by biological indexing and enzyme-linked imunosorbent assay (ELISA). Biological indexing was done according a modified technigque from CALAVAN and CHRISTIANSEN (1965) by side-grafting a 5 cm long Madame Vinous shoot with three buds to each test plant. The shoots were covered

by a polyethylene bag for three weeks and later forced to grow as a single shoot. All test plants were kept at 32/27 °C (day/night) temperature, 70 ± 5 % relative humidity and 16 light in an insect-free greenhouse. The evalution of symptoms were started three month after grafting (ROISTACHER 1991). All plants were tested serologically for the presence of ***S.citri*** by a DAS-ELISA (CLARK and ADAMS 1977), using a polyclonal antibody against SPA-strain Israel. The results were evaluated in a Titertek Multiscan ELISA Reader at 405 nm; the plants were tested positive, if the difference between sample and healty control was higher than 0,1 OD (SAILLARD and BOVE 1983).

RESULTS AND DISCUSSION

In general, the survival rate of budwoods collected in May and August was compartively low. Only 43.3 % and 47.8 % of the grafts survived, while 98,9 % of those buds collected in November and February continued to grow. All buds taken from healthy, greenhouse-grown, Navel oranges survived. Budwoods collected in November or February and kept for one and two months at 4° C showed the same viability as those grafted at the same day. Notable more buds died after storing for one or two month, if collected in May or August (Tab. 1).

After a six months observation period only 15 of 260 trees showed symptoms attributable to stubborn disease (5,8 %). The symptoms observed varied from very severe to slight. The four plants revealing severe symptoms were small with shortened internodes and the leaves, have had symptoms of zinc deficiency. The eleven plants with slight characteristis were stunted, but no leaves symptoms were observed. Using biological indexing eleven plants (4,2 %) were tested positive for CSD pathogen and by ELISA in total 17 plants (6,5 %) were harboring ***S. citri.*** Over all 19 citrus plants (7,3 %) were infected with the stubborn disease pathogen according to the ELISA and biological indexing results (Tab. 1).

There was only a slight effect of season on the transmission rate of ***S. citri*** by budwoods. According to the ELISA and biological indexing results, the transmission rate ranged from 5,6 % to 10 % if the budwoods were collected in February and May, respectively. Over all the transmission rate was as low as 7,3 %. CALAVAN ***et al.*** (1969) observed a very low percentage of transmmission from budwoods grafted in August, October and January as well as a lower transmission rate when using buds instead of side grafts. These results clearly indicate that the CSD pathogen is distributed irregularly in many trees and that pathogen is inhibited or lost in most budwoods during much of the year.

Five plants revealing no typical characteristics of a ***S. citri*** infection were tested positive by biological indexing and ELISA. Although 15 plants showed typical symptoms of an ***S. citri*** infection, these observations were confirmed only for six plants by biological indexing or by ELISA. Out of eleven plants tested positive by biological indexing, nine reacted positive and two negative in the serological test. Furthermore, seven citrus trees which did not show any symptoms in biological indexing were tested positive by ELISA. Solely four plants found to be infected symptomatologically, by biological indexing, and by ELISA (Tab. 2).

Table 1. Number of bud woods survived and rate of transmission of *Spiroplasma citri* bybudwoods with regard to season and storing at 4 °C

Group No.	Budwood collection time	Grafting time	Survived budwoods*		Number of S.citri infected citrus trees							
					Symptomatologically		Biological indexing		ELISA		ELISA and biological indexing	
			No.	%	No.	%	No.	%	No.	%	No.	%
1-A	May	May	19	63.3	2		1		1		1	
1-B		June	20	66.7	3		2		3		3	
1-C		July	0	00.0	0		0		0		0	
Total			**39**	**43.3**	**5**	**13.0**	**3**	**5,0**	**4**	**10,0**	**4**	**10,0**
2-A	August	August	28	93.3	1				1		1	
2-B		September	3	10.0	0		0		0		0	
2-C		October	12	40.0	1		2		1		2	
Total			**43**	**47.8**	**2**	**4,7**	**2**	**4,7**	**2**	**4,7**	**3**	**7,0**
3-A	November	November	30	100.0	3		2		3		3	
3-B		December	30	100.0	1		1		2		2	
3-C		January	29	96.7	1		1		2		2	
Total			**89**	**98.9**	**5**	**5,6**	**4**	**4,5**	**7**	**7,9**	**7**	**7,9**
3-A	February	February	30	100.0	2		1		2		2	
3-B		March	30	100.0	1		1		1		2	
3-C		April	29	96.7	0		0		1		1	
Total			**89**	**98.9**	**3**	**14,6**	**2**	**2,3**	**4**	**4,5**	**5**	**5,6**
Total			**260**	**72,2**	**15**	**5,8**	**11**	**4,2**	**17**	**5,8**	**19**	**7,3**

*) In the control groups all bud woods survived.

Table 2: Citrus trees infected by ***Spiroplasma citri*** as determined symptomatologically, by biological indexing, and by ELISA. Trees tested positive by all three methods are idicated by bold numbers

Group No.	Budwood Collection time	Grafting time	Citrus plants (No.) infected with *S.citri*		
			Symptoms	Biological indexing	ELISA
1-A	May	May	1,12	8	8
1-B		June	1,5,9	2,9	1, 8, 9
1-C		July	-	-	-
2-A	August	August	21	-	13
2-B		September	-	-	-
2-C		October	7	6,7	7
3-A	November	November	13,**20,33**	**20,23**	8,**20,23**
3-B		December	19	18	18,30
3-C		Janurary	10	16	15,16
4-A	February	February	10,23	16	16,23
4-B		March	16	29	16
4-C		April	-	-	23

These results clearly indicate that stubborn is almost undetectable sympomatoloically in young citrus trees. Even plants with no symptoms may harbor the CSD pathogen and will help to spread *S. citri* if used as a budwood source. It is obvious that even the application of only one detection method might led to false negative, so that biological indexing should be combined either with ELISA or culture of the pathogen in any butwood improvement program.

ÖZET

TURUNÇGIL STUBBORN HASTALIĞI ETMENİ *Spıroplasma citri*'nin AŞIGÖZÜ YOLU İLE TAŞINMASINDAKİ MEVSİMSEL DEĞİŞİKLİKLER

Türkiye turunçgil tarımının % 85'inin yapıldığı Akdeniz Bölgesi'nde mevcut çok sayıdaki virüs ve virüs benzeri hastalıklardan en önemlilerinden biri de *Spiroplasma citri* etmeninin neden olduğu Stubborn hastalığıdır. Bu çalışmanın amacı hastalığın aşıgözü ile taşınmasında aşıgözlerinin ağaçtan alım zamanı ve düşük sıcaklıklarda (+ 4 °C) bekletme süresinin etkisini araştırmaktır.

Aşıgözleri Mayıs, Ağustos, Kasım 1989 ve Şubat 1990 tarihlerinde kesilmiş ve her grup içinde 3 kısma ayrılmıştır. Ayrılan kısımlardan birincisi hemen, ikincisi bir ay ve üçüncüsü iki ay soğukta depolandıktan sonra aşılanmıştır. Hastalığın taşınması sipmtomolojik, biyolojik indeksleme ve ELISA testine göre araştırılmıştır.

Mayıs ve Ağustos'ta alınan aşıgözlerinin tutma oranları düşük bulunmuştur. Altı aylık gözlemlerden sonra 260 bitkinin 15'inde zayıftan kuvvetliye kadar değişen simptomlar gözlenmiştir. Biyolojik indeksleme sonucu ise 260 bitkiden 11'i, ELISA testi çalışmaları sonucu ise 17 bitki etmen ile bulaşık olarak bulunmuştur. ELISA testi ve biyolojik indekslemeler sonucu ise 19 bitki bulaşık olarak saptanmıştır.

Aşıgözü alma zamanının etkisi çok az bulunmuş, en düşük değer Ağustos ayında alınan gözlerde belirlenmiştir.

Simptomolojik gözlemler, biyolojik indeksleme ve ELISA testinin her üçüne birden yanlızca 4 bitki pozitif sonuç vermiştir. Bu nedenle aşıgözü ıslah programlarında biyolojik indeksleme programının ELISA veya kültür testlerinden biri ile komoine edilmesi gerektiği sonucuna varılmıştır.

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The Role of the Phytoalexins on the Resistance to Chickpea Blight (*Ascochyta rabiei* (Pass.) Labr.) in Chickpeas

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ABSTRACT

*After inoculation with race 1 of **Ascochyta rabiei**, the accumulation of pterocarpan phytoalexins in the leaves and stems of the chickpea (**Cicer arietinum**) cultivars ILC 1929 (susceptible) and ILC 3279 (resistant) determined to be quantitatively by HPLC.. Medicarpin and maackiain in the leaves and stems of resistant cultivar accumulated 12 hours after inoculation. Maackiain did not accumulate in the stems and leaves of the susceptible plants although medicarpin started to accumulate 24 h after inoculation. Contents of medicarpin (22 µg/g. fr.w.) and maackiain (17.0 µg/g fr.w) in resistant cultivar was enough for the inhibition of fungal development but it was less (9.0 µg/g fr.w.) in the susceptible ones. The detection of maackiain in only resistant plants indicated that this compound has an important role in the resistance.*

INTRODUCTION

Chickpea (***Cicer arietinum*** L.) is a widespread crop plant in semiarid areas of Asian and North African countries. The pterocarpans medicarpin and maackiain have been identified as the main phytoalexeins of this plant (Keen, 1975) where they accumulate during infection with ***Helminthosporium carbonum*** Ullstrup (Ingham 1976) or ***Nectria haematococca*** (Denny and VanEtten, 1981).

The most important disease recorded on chickpea in Ascochyta blight which is caused by ***Ascochyta rabiei*** (Pass.) Labr. (Teliomorph ***Mycosphaeralla rabiei*** Kov.) There are many reports of serious losses of chickpea crops caused by Ascochyta blight (Nene, 1982). Singh and Reddy (1990) demonstrated that in favourable environmental conditions yield losses due to the disease can reach 100%. ***Ascochyta rabiei***-resistant cultivars of chickpea showed, among other properties (Nene, 1982) a much higher phenolic content of unidentified structures when compared to susceptible cultivars (Vir nad Grewal. 1974). Furthermore, inoculation of seed cavities of detached pods of ***Cicer arietinum*** with spor suspensions of ***A. rabiei*** led to the accumulation of an antifungal compound which was not chemically identified (Kunzru and Sinha, 1970)

The present study was undertaken to compare ***A. rabiei*** -resistant and a susceptible cultivar of chickpea with regard to the accumulation of phytoalexins during infection with spore suspensions of a virulent race (race 1) of ***A. rabiei.***

MATERIALS AND METHODS

1. Plant Material : Seeds of chickpea cultivars ILC 1929 (susceptible) and ILC 3279 (resistant) were obtained from the germplasm collection of the International Center of Agricultural Research in the Dry Areas (ICARDA) Aleppo, Syria. Seeds of each cultivar were surface sterilized with sodium hypochlorite (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. The plants, thinned to five per pot after germination, were watered daily and fertilized twice a week a dilute solution of 20 - 20 - 20 (N - P - K). Plants were grown in Conviron growth chambers at 22 ± 1°C with a relative humidity of 25 - 50% and illuminated for 12 h per day with white fluorescent light (14 850 lux).

2. Fungal Material : The race 1 of ***Ascochyta rabiei*** used in this studied. Cultures of pathogen were maintained on CSMDA (Chickpea Seed Meal Dextrose Agar) in 9 cm plastic petri dishes at 20 ± 1°C and were illuminated for 12 h per day by 2 white fluorescent tubes (F 20 T12/CW).

3. Inoculation of Plants : Spore suspensions of ***A. rabiei*** were prepared from 14 day-old cultures using sterile distilled water. The spore suspension was filtered through cheesecloth (3 layers) to remove mycelial fragments and it was adjusted to a concentration of 1.2×10^5 spores per ml. Aerial parts of 15 days old plants were sprayed with the spore suspension (to run off) using a pressure sprayer. Control plants were sprayed with sterile distilled water. After spraying, the plants were covered with transparent polyethylene bags for six days to maintain leaf wetness and incubated in a growth chamber with a 12 h photoperiod (14 850 lux) and day and night temperatures of approximately 20°C and 18°C respectively.

Samples of leaf and stem were taken 12, 24, 40, 60 and 90 h after inoculation. Collected plant samples were weighed and then put in the 100 % ethanol (EtOH).

4. Extration and Isolation of Medicarpin and Maackiain : Samples of leaf and stem were then cut from susceptible and resistant plants immediately imersed in 100 % EtOH and stored and room temperature (22°C) in the dark overnight. Each replicate consisted of 0.5 g. fr. weight of tissue in 10 ml of 100 % EtOH. This EtOH was decanted and the leaves and stems were soaked in a second 10 ml aliguot of 100 % EtOH overnight. Soaking procedure was replicated three times and the three EtOH fractions were combined and all of the EtOH was filtered through Whatman-1 filter paper. The ethanol solution evaporated to dryness in vacuo at 40°C. The dry residue was washed 3 times with 2 ml CCl_4/g tissue and CCl_4 was partitioned 3 times with 1 volume of 0.2 N NaOH. The NaOH fraction was acidified to pH 3 with 6 N HCL, then partitioned 3 times with 0.5 volume of CCl_4. The final CCl_4 fractions of extractas were evaporated in vacuo and the residue was diss

solved in small volume (1.5 ml) of absolute ethanol. This ethanol evaporated in vacou on a evopomix at 40°C and the residue was dissolved in 100 µl of 100 % EtOH for chromatography (Higgins and Smith, 1971: Higgins, 1972).

5. Determination of Phytoalexins on Thin Layer Chromatography : The extracts and standart phytoalexins were streaked on silica gel plates (0.25 mm precoated silica gel with F-254 indicator, Merck.) The plates were developed two times in n-pentane: ethyl ether: glacial acetic acid (75 : 25 : 1, V/V) (Smith ***et al.*** 1971) with the solvent allowed to move 6 cm the first time and 17 cm the second time. For detection of compound was used diazotized p-nitroaniline reagent (McMurchy and Higgins, 1984).

6. Spectrophotometric Method : The extracts of the leaf and stem and standart phytoalexins (medicarpin and maackiain) were streaked on TLC plate and the plate developed in solvent system. After drying, the compound was located by comparison with the standart under shotrwave UV light as spots appear as dark areas on the bright background. The appropriate area of silica was scraped off the plate and put in 2 ml EtOH and filtered through alass-wool to remove the silica. This material can be assayed by spectrophotometry or HPLC.

The samples were put in a 1 ml quartz cuvette and scaned the waveleghts 190-350 mm using pure EtOH in the reference cuvette and recorded the absorbance.

The amounts of medicarpin and maackiain per ml of extracts determined using the published extinction coefficients of the compounds and the following formula.

$A = Elc$. *where* A = *absorbance*

l = *length of light path (= 1 cm)*

c = *concentration in moles*

E = *molar absorbptivity*

Extinction coefficients:

Medicarpin log E = 3.89 at 287 nm.

Maackiain log E = 3.93 at 311 nm.

7. High Performance Liquid Chromatography (HPLC) Analysis : Chromatography (HPLC) separations were carried out with a Hewlett Packard 1090 Chromatography. Samples (10 µl) were separated using a Techsil C-18 column (250 x 4mm 10 µm. HPLC Technology Inc.) and a flow rate of 1 ml/min. A linear gradient of 45 % A in (A + B) to 55 % A in (A + B) in 20 min was applied. Solvent A was 100 % methanol and solvent B was 1 % acetic acid. Compounds were detected at 287 and 311 nm.

Samples eluted from TLC plates for HPLC and filtrated through Gelman Sciences Acro LC 3A filter (0.45 µm).

8. Effect of Medicarpin and Maackiain on Spore Germination and Germ Tube Growth of *Ascochyta rabiei* : A conidial suspension of ***A. rabiei*** was prepared in half strength Czapek-Dox broth (BBL). 20 µg/ml of medicarpin plus maackiain in a 1 : 1 ratio and five different concentrations (0, 10, 20, 40 and 60 µg/ml) of medicarpin and maackiain were prepared in absolute ethanol. The EtOH was evaporated off

and the residues dissolved in 2 μl of EtOH. Then 98 μl sterile liquid medium (50 % strength Czapek-Drox broth) containing the spore suspension of *A. rabiei* (5 x 10^3 spores / ml) was added. 20 μl of each of these treatments was put to the centre of wax pencil rings on sterile glass slides inside a petri plate humidity chamber. Drops (20 μl) of spore suspension in medium containing 2% ethanol served as the control. The slides were incubated at 25°C and the spores killed ant fixed at 14 hours by adding 5 μl of cotton blue in lactophenol. The germination and germ tube length of 100 spores for each siled was recorded. There were four slides of each treatment so 400 spores were examined in total per treatment.

RESULTS

1. Phytoalexin Accumulation : TLC method was used to determine medicarpin and maackiain in samples beacuse of practical method for determination of phytoalexins. Extracts, including medicarpin and maackiain gave the characteristic bright yellow-orange area ant the Rf value corresponding to the standart when the diazotised p-nitroanilin sprayed on TLC of the samples.

Quantitative HPLC measurements of medicarpin and maackiain were found more sensitive than spectrophotometric measurements. Results of HPLC analysis obtained from leaf and stem experiments are shown in Fig. 1 and Fig. 2.

The extracts of the leaf and stem of the susceptible cultivar (ILC 1929) contained smaller amounts of medicarpin than resistant cultivar (ILC 3279). Medicarpin in the leaves and stems of susceptible cultivar was produced very small amounts (0.65 and 0.05 μg/g fr. w. respectively) 24 hours after inoculation whereas accumulation of medicarpin in the leaves and stems of the resistant cultivar began 12 h after inocxion. The level of medicarpin in leaves and stems of resistant cultivar within 24 hours had reached concentrations of 6.59 and 8.6 μg/g fr.w. respectively. Amounts of medicarpin in both cultivar contiuned to increase depending on time. Maackiain was detected neither the extracts of stem nor leaf of the susceptible cultivar. However accumaliton of maackiain in the resistant cultivar began after 12 hours after application of spores and had reached rather high levels (17 μg/g fr.w.) within 24 hours.

In summary, the results obtained with the phytoalexins (Fig. 1 and Fig. 2) clearly show that the two chickpea cultivars possess remarkable differences with regard to phytoalexin accumulation during infection with *A. rabiei.*

2. The Effect of Medicarpin and Maackiain on Spore Germination and Germ Tube Growth of *Ascochyta rabiei* : The antifungal properties of medicarpin and maackiain at 0, 10, 20, 40, 60 μg/ml and 20 μg/ml medicarpin plus maackiain in a 1:1 ratio were determined by measuring its effect on spore germination and germ tube growth by the slide germination test (Table 1.)

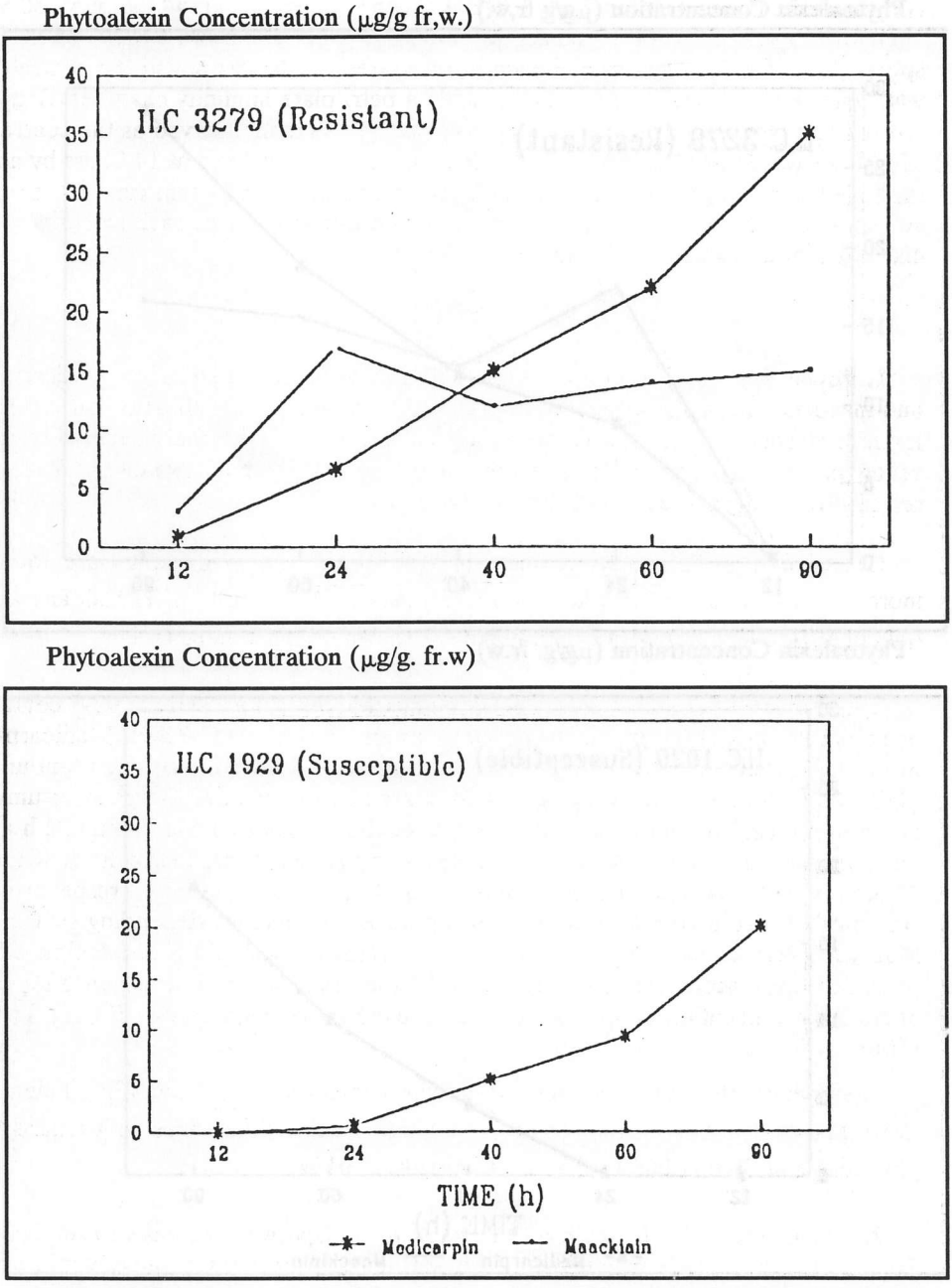

Fig.1 : Accumulation of the phytoalexins medicarpin and maackiain in the leaves of a susceptible (ILC 1929) and a resistant (ILC 3279) cultivar of chickpea after inoculation with spores of ***Ascochyta rabiei.***

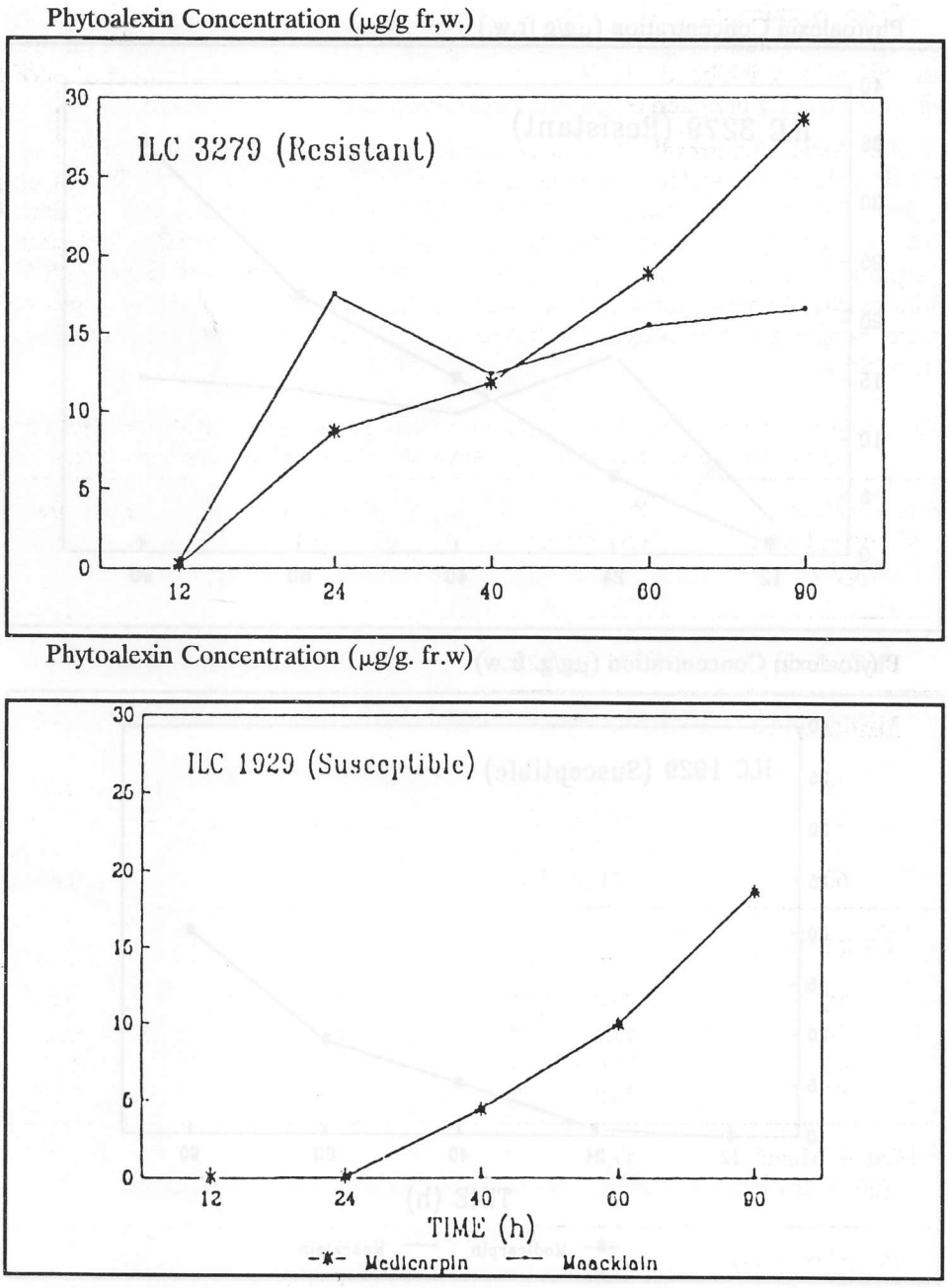

Fig.2 : Accumulation of the phytoalexins medicarpin and maackiain in the leaves of a susceptible (ILC 1929) and a resistant (ILC 3279) cultivar of chickpea after inoculation with spores of ***Ascochyta rabiei.***

Spore germination of *A. rabiei* after 14 hours incubation was not significantly inhibited by 10 and 20 μg/ml of medicarpin but at 60 μg/ml of medicarpin and maackiain were inhibited 50.6 % and 52 % respectively. Spore germination of *A. rabiei* was strongly inhibited by maackiain in comparision with medicarpin.

The germ tube growth of *A. rabiei* was inhibited by medicarpin, maackiain and medicarpin plus maackiain treatments. At the lowest concentration of medicarpin (10 μg/ml), the germ tube growth was inhibited 50.37 % whereas at the same concentration of maackiain was inhibited 65.94 %. Effect of maackiain and medicarpin at 60 μg/ml on germ tube elongation of *A. rabiei* was found same as 88.48 %. The results (Table 1) show that the spore germination and the germ tube growth were progressively inhibited by increasing concentrations of medicarpin and maackiain.

Table 1. Percentage of germination and germ tube length of *Ascochyta rabiei* spores treated with various concentrations of medicarpin and maackiain after 14 h incubation.

Concentration of Phytoalexins (μg/ml)	Percentage of Germination (%)	Germination as % of Control	Germ Tube Lenght (μm)
0 (Control)	75 ± 1.24	–	33.06 ± 2.41
Medicarpin			
10	69.5 ± 1.22	92.6	16.41 ± 1.09
20	62.0 ± 1.70	82.6	14.42 ± 1.70
40	55.5 ± 1.30	74.0	7.91 ± 0.78
60	37.0 ± 1.57	49.3	4.80 ± 0.40
Maackiain			
10	50.8 ± 1.25	67.73	11.26 ± 1.01
20	48.5 ± 1.04	64.66	8.98 ± 0.32
40	37.0 ± 1.56	49.3	7.40 ± 1.70
60	36.0 ± 1.33	48.0	4.82 ± 0.27
Med + Maac[a] (20 + 20)	47.0 ± 1.40	62.6	6.74 ± 1.83

[a]Med + Maac (1:1)

Microscopic observations at 14 hours supported the damaging effect of medicarpin and maackiain on germ tubes. Germ tube apices in control treatments and in

low concentration of medicarpin and maackiain treatments that had resumed growth, showed the normal turgid and "smooth" apperance of growing tips whereas many of those at the high concentrations that had not resumed growth appeared collapsed and "rough". Original germ tube collapsed and new growth frequently developed via a new grem tube or a new branch of the "old" germ tube.

DISCUSSION

Infection of ***Cicer arietinum*** cultivar ILC 3279 with spores of ***Ascochyta rabiei*** resulted in a pronounced accumulation of the phytoalexins medicarpin and maackiain. It is therefore safe to assume that the unidentified antifungal compound ("cicerin") described in an earlier study with chickpea and ***A. rabiei*** (Kunzru and Sinha 1970) has been a mixture of both phytoalexins. These antifungal compounds previously reported for unnamed chickpea cultivars when infected with other fungi (Ingham, 1976; Denny and VanEtten, 1981). The strikingly different levels of phytoalexins in the resistant and the susceptible variety of chickpea (Fig. 1 and 2) together with the established difference in resistance of cultivars ILC 1929 and ILC 3279 towards ***A. rabiei*** are in line with various other reports that rapid and high accumulation of such antifungal compounds appears to be an important trait of resistant plant (Kuc and Rush, 1985). The symptoms developed by cultivar ILC 3279 towards infection with spores of a virulent race of ***A. rabiei*** may be interpreted as a hypersensitive reaction because the formation of small necrotic spots has been quite obvious.

Chickpea cultivar ILC 1929 clearly failed to accumulate significant amounts of phytoalexins (Fig. 1 and 2); maackiain has even been absent from the infected stem and leaves. Our results are in general agreement with the observations by Weigand ***et al.***, 1986.

Phytoalexins play a significant role in the defence mechanisms of higher plant towards phytopathogenic fungi (Darvill and Albersheim, 1984; Hahn ***et al.***, 1985). Higgins and Smith (1971) reported that the quantities of medicarpin and maackiain produced by red clover leaves in response to inoculation with ***Helminthosporium turcicum*** appeared sufficient to adversely affect growth of ***H. turcicum*** on clover leaves. KeBmann and Barz (1987) demonstrated accumalation of medi-carpin and maackiain in cell suspension cultures of resistant cultivar to ***A. rabiei*** higher than in cell suspension cultures of susceptible cultivar.

This study showed the existence of a high correlation between the degree of resistance of chickpea varieties and accumaliton of phytoalexins.

ÖZET

Nohutlarda Antraknoza (*Ascochyta rabiei* (pass.) Labr.)'a Dayanıklılıkta Phytoalexinlerin Rolü

Ascochyta rabiei'nin virulent bir ırkı (ırk 1) ile inokule edilen dayanıklı (ILC 3279) ve duyarlı (ILC 1929) nohut çeşitlerinin yaprak ve gövdelerinde oluşan pterocarpan phytoalexinlerin miktarları HPLC ile saptanmıştır. ***Ascochyta rabiei*** ile inokule edilen dayanıklı çeşitlerin yaprak ve gövdelerinde pterocarpan phytoalexinlerden medicarpin ve maackiain'in her ikiside inokulasyondan 12 saat sonra oluşmaktadır. Duyarlı çeşitlerde maackiain'e rastlanmamasına karşın medicarpin birikimi inokulasyondan 24 saat sonra başlamaktadır. Dayanıklı bitkilerde medicarpin (22 μg/g taze ağırlık) ve maackain (17.0 μg/g taze ağırlık) miktarları fungal gelişmeyi engellemeye yeterli düzeylerde iken duyarlı çeşitlerde (9.0 μg/g taze ağırlık) yeterli düzeyde değildir. Maackiain'in sadece dayanıklı bitkilerde tespit edilmesi bu phytoalexinin dayanıklılıktaki rolünün önemli olduğunu göstermektedir.

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Occurence And Distribution Of Fungal Diseases On Lentil In Ankara Province*

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ABSTRACT

As a result of the surveys carried out on lentil in Ankara Province, it was found that the incidence of mean disease ratio was 37.0 % at seedling stage and 40.7 % at flowering-podding stages. Percentages of the isolated fungi were as follows; At seedling stage; ***Fusarium equiseti*** *4.2 %,* ***F. graminearum*** *16.2 %,* ***F. oxysproum*** *9.3 %,* ***F. solani*** *4.9 %.* ***Rhizoctonia solani*** *0.9 %,* ***Alternaria alternata*** *7.5 %,* ***Cladosporium*** *sp. 4.5 %, and* ***Helminthosporium*** *sp. 4.7 %, at flowering stage;* ***F. equiseti*** *3.0 %,* ***F. graminearum*** *10.8 %,* ***F. oxysporum*** *6.1 %,* ***F. solani*** *2.7 %,* ***R. solani*** *2.0 %,* ***A. alternata*** *16.8 %,* ***Cladosporium*** *sp. 7.2 %,* ***Helminthosporium*** *sp. 6.9 %,* ***Phoma medicaginis*** *4.9 %, and* ***Uluocladium atrum*** *2.9 %.*

In the pathogenicity tests, all isolated fungi except. ***Cladosporium*** *sp. and* ***Helminthosporium*** *sp. were found to be pathogenic on this host.*

INTRODUCTION

Lentil (***Lens culinaris***) is an important legumes crop because of its high protein contents. It is not only used as a green manure but also grown as a rational crop. Lentil is affected by a number of fungal diseases which cause root - rot, wilt, stem-lesion and leaf spots.

In Türkiye, ***Uromyces fabae*** (Pers.) de Bary was reported to cause little damage in İzmir, Ankara, Tunceli and Hatay because of its occurence at later stages of growth (Bremer, ***et al.*** 1947, 1952). Root-rots of lentil caused by ***Fusarium, Rhizoctonia*** and ***Pythium*** species were reported in Ankara province (Soran, 1979). Lentil blight was first observed in this region by İren ***et al.*** (1983). Who also made its comparison with the causal agents of pea blight.

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The fungal diseases of lentil and their distribution in Southeastern Anatolia were determined (Sağır, 1988).

The studies carried out in outher countries indicate that the leaf and pod blight can be caused by ***Alternaria alternata*** (Fr.) Keissler (Gupta and Das, 1964; Khare, 1981; Bellar and Kebabeh, 1983, Kaiser and Hannan, 1986) and ***Ascochyta lentis*** Bond. Y. Vassıl. (Mitidieri, 1978; Kaiser and Hannan, 1986). Similarly it was reported that the root-rots may be caused by ***Fusarium solani*** (Mart.) Sacc. (Shatla ***et al,*** 1975; Khare, 1981; Bhalla ***et al*** 1984; Al-Ahmad Mauselli, 1987), ***F. equiseti*** (Corda) Sacc. (Khare 1981, Bhalla ***et al,*** 1984), ***F. graminearum*** Schwabe (Mc Kenzie and Morrall, 1975; Khare, 1981; Bhalla et al, 1984), ***F. oxysporum*** Schlecht (Mc Kenzie and Morall, 1975, Bellar and Kebabeh, 1983; Bhalle ***et al,*** 1984; Al-Ahmad Mouselli, 1987), ***Rhizoctonia solani*** Kühn (Shatta ***et al,*** 1975; Mitidieri, 1978; Khare. 1981; Bellar and Kebabeh, 1983; Bhaller ***et al,*** 1984), ***Pythium ultimum*** Trow (Shatla et al, 1975), ***Sclerotinia sclerotiorum*** (Lib.) de By (Mitidieri, 1978) and ***Phoma medicaginis*** Malbr. Roum. Var. ***pinodella*** (Jones) Boerema (Bellar and Kebabeh, 1983; Kaiser and Hannon, 1986). It was reported that ***Erysiphe polygoni*** DC. causes powdery mildew on lentil (Mitidieri, 1978).

The aim of this study was to determine fungal diseases of lentil and their distribution in Ankara province as no such detailed study has been carried out for the last ten years.

MATERIALS AND METHODS

1. Survey and Isolation of Fungi from Diseased Plants

In 1990 the surveys were performed at two different stages of growth (at seedling and flowering-podding) in selected 6 towns of Ankara Province. The size of lentil growing area in each town, the number of fields surveyed are shown in Table 1.

Table 1. The lentil growing areas surveyed in Ankara province.

Towns	Lentil growing area (ha)	Number of field surveyed
Haymana	5.000	17
Şereflikoçhisar	2.500	8
Kalecik	2.500	8
Çubuk	1.700	5
Bala	1.000	3
Polatlı	650	2
Total	13.350	43

Small pieces of infected plants (2 - 5 mm) were out from roots, leaves and washed in tap water, soaked in 1 % NaOCL for 1 - 2 min; washed in sterile distilled water and blott dried on sterile paper towels. They were plated on Potato Dextrose Agar medium and identification of the fungi was made after incubation at 22 ± 2°C under fluorescent lights (12 hr. photoperiod). As fungi emerged, hyphal tips were transferred to PDA slants and stored at 4°C in dark.

Booth (1971) was referred in identifictaion of ***Fusarium*** species. Other fungi were identified to genus or species levels according to Ellis (1971). Domsch ***et al.,*** (1980) and Sutton 1980).

2. Pathogenicity Tests

In the test, plastic pots in 20 cm diameter and pasteurized garden soil were used. Fungi isolated from roots and foots of plants were grown on PDA (Potato dextrose agar) in 9 cm diameter petridishes. When they covered all the petridishes, cultures from 3 petridishes foreach pathogen, were removed and placed on the soil in pots. Then the cultures were covered with pasteurized soil in 1 cm. thickness. Ten surface sterilized lentil seeds were sown on these pots and additional 2 cm thick soil was placed over the seeds: Pots were irrigated with tap water as required.

Control pots were treated the same way except they were given only clean agar dishes.

Fungi isolated from leaves were cultured on PDA and 50 ml. spore suspensions of 1×10^6 spor/ml each isolated was sprayed on to 30 days old lentil seedlings in three pots. The pots were put in polyethylene bags for 48 hours to maintain high humidity. The pots were kept in green house at 20 ± 4°C and each pot was taken as a replicate.

Evaluaiton was made one month after inoculations for root-rot pathogens, the plants were uprooted and root-rot were examined. To confirm pathogenicty re-isolations from the diseased roots were also done. For leaf pathogens, plants regularly observed for their symptoms. The re-isolations were also done in the leaf pathogens.

RESULTS

The fungi detecded in lentil fields at seedling and flowering-podding stages in towns of Ankara and their distribution is given in table 2. ***Fusarium*** species were prevalent in all towns at both stages. Among them ***F. graminearum*** was present at the highest rate being (16.2 % and 10.8%). at seedling and flowering-podding stages respectively. It was followed by ***F. oxysporum*** (9.3 % - 8.1 %), ***F. solani*** (4.9 % - 2.7 %) and ***F. equiseti*** (4.2 % - 3.0 %).

Rhizoctonia solani one of the most important root-rot fungi was observed at the

first stage in Kalecik at the rate of 5 % but in Haymana and Şereflikoçhisar, this ratio was as 2.5 % and 5 % at second stage respectively. The distribution ratio of this pathogen at province level was 0.9 % 0.2 % at the first and the second periods respectively.

Table 2. The fungi detected in lentil fields at seedling, flowering-podding stages in Ankara Province and their distribution ratios.

Fungi	Isolated fungi from the towns (%)													
	1. Seedling stage						2. Flowering-podding stage							
	Haymana		Ş.koçhisar		Kalecik		Çubuk		Bala		Polatlı		Average	
	1*	2**	1	2	1	2	1	2	1	2	1	2	1	2
F. equiseti	4.7	2.8	2.5	2.9	5.9	4.7	4.0	1.5	1.9	–	4.2	3.5	4.2	3.0
F. graminearum	22.4	14.1	8.3	10.3	13.3	6.4	18.8	9.6	8.3	–	15.4	7.1	16.2	10.8
F. oxysporum	12.2	6.1	4.1	3.8	12.1	8.9	3.3	6.5	4.8	–	3.2	2.7	9.3	6.1
F. solani	5.3	3.3	6.3	2.1	4.6	1.5	4.0	3.5	3.3	–	2.8	4.0	4.9	2.7
R. solani	0.0	2.5	0.0	5.0	5.0	0.0	0.0	0.0	00	–	0.0	0.0	0.9	2.0
A. alternata	12.1	22.0	10.2	14.3	0.0	10.8	0.0	12.5	3.4	–	17.3	20.3	7.5	16.8
Cladosporium sp.	4.1	7.3	6.8	2.0	4.4	8.5	3.8	10.3	1.9	–	5.3	7.9	4.5	7.2
Halminthosporuium sp.	6.4	9.1	8.5	7.8	1.9	2.4	1.5	5.9	0.0	–	3.8	7.5	4.7	6.9
P. medicaginis	0.0	4.9	0.0	0.0	0.0	10.2	0.0	4.4	0.0	–	0.0	4.7	0.0	4.9
U. atrum	0.0	2.5	0.0	0.0	0.0	3.6	0.0	6.7	0.0	–	0.0	3.6	0.0	2.9

* Seedling stage

** Flowering-podding stage

Alternaria alternata which can cause disease was on stem and foliage of the plants was found in all of the towns, except Kalecik and Çubuk at a mean rate of 7.5 % in the first survey period but it was prevalent in all towns at a mean rate of 16.8 % in the second survey period, on the other hand ***Phoma medicaginis*** and ***Uluocladium atrum*** were found only in the second period in all the places, except Şereflikoçhisar where not found at all at the mean rates of 4.9 % and 2.9 % respectiveley.

In table 2, only figures of the seedling stage were given for town of Bala since this region could not be surveyed at the second stage.

Disease ratios based ont the towns are given in table 3.

Table 3. Lentil fungal disease ratios in towns of Ankara province.

Towns	Disease ratios (%)	
	Seedling stage	Flowering-podding stage
Haymana	53.8	52.3
Şereflikoçhisar	30.9	37.6
Kalecik	41.2	36.8
Çubuk	40.5	39.6
Bala	19.7	–
Polatlı	35.7	37.4
Average	37.0	40.7

As seen in the table 3 mean disease rate at seedling stage is 37 %. The maximum rate of disease was in Haymana (53.8 %) while the minimum rate was in Bala (19.7 %). However at the flowering-podding stage mean disease rate was 40.7 %, maximum and minimum disease ratios being in Haymana (52.3 %) and Kalecik (36.8 %) respectively.

Dwarf, discolored or wilted plants and the ones having brown discoloration on their root and crowns yielded ***Fusarium equiseti, F. graminearum, F. oxysporum, F. solani*** and ***Rhizoctonia solani, Alternaria alternata*** was isolated only from the above ground parts of the plants, specially from the brown spots near the leaf tips and completely blighted ones. ***Phoma medicaginis*** was recovered from the plants having small irregular and brown leaf spots. On the other hand ***Uluocladium atrum*** was isolated from chlorotic leaves. Pathogenicity of these fungi was proved (Table 4).

Table 4. Pathogenicity of the Isolated Fungi.

Fungi	Isolate Number	Patogenicity (%) Min.	Max.	Rates of isolates showing more then 50 % pathogenicity (%)
F. equiseti	10	0	60	20
F. graminearum	10	0	100	80
F. oxysporum	10	0	100	60
F. solani	10	0	70	60
R. solani	5	100	100	100
A. alternata	10	0	70	60
Cladosporium sp.	5	0	20	0
Helminthosporium sp.	5	0	10	0
P. medicaginis	10	0	80	60
U. atrum	5	0	60	15

As seen in the table 4, ***Rhizoctonia solani*** was the most virulent pathogen. ***Cladosporium*** sp. and ***Helminthosporium*** sp. are not found to be pathogenic.

DISCUSSION

Fusarium root-rots caused by various ***Fusarium*** species was found to be the most common disease on lentils in this region, and this was supported by the finding of Soran (1979). However although he also found ***Rhizoctonia*** very common in our work this was not the case.

Fusarium graminearum was recorded for the first time on lentil in Türkiye and it was found to be the most widespread disease agent in Ankara. This pathogen was

also described as a root-rot agent by other researchers on lentil (Khare 1981 and Bhalla *et al*, 1984). The occurence of ***Fusarium oxysporium, F. solani, F. equiseti*** on lentil were also mentioned by Soran (1979), Khare (1981), Bhalle *et al.* (1984) and Sağır (1988).

Alternaria alternata was the most widespread leaf pathogen in this province specially at the flowering-podding stage and it was also isolated by Sağır (1988) in the southeast Anatolia.

The reason why we could not isolate ***Phoma medicaginis*** at seedling stage is not known. However it is known to be a common root-rot and leaf spot agent and was recovered at all stages by Sağır (1988) in southeast Anatolia.

Rust (***Uromyces fabae***), mildew (***Peronospora lentis***), and antrachnose (***Ascochyta lentis***) which are important diseases of lentil have not been recovered in this work. This could be attributed to either the use of clean seeds or the fact that the cultivation of lentil has been recently started.

ÖZET

Ankara İlinde Yetiştirilen Mercimeklerde Görülen Fungal Hastalıkların Tespiti ve Yayılış Oranlarının Saptanması

Ankara ilinde fide ve çiçeklenme-kapsül döneminde mercimek ekiliş alanlarında yapılan sürveyler sonucunda, ortalama hastalık oranı fide döneminde % 37.0 ve çiçeklenme-kapsül döneminde % 40.7 bulunmuştur. İzole edilen fungusların yüzdeleri fide döneminde ***Fusarium equiseti*** % 4.2, ***F. graminearum*** % 16.2, ***F. oxysporum*** % 9.3, ***F. solani*** % 4.9, ***Rhizoctonia solani*** % 0.9, ***Alternaria alternata*** % 7.5, ***Cladosporium*** sp. %4.5 ve ***Helminthosporium*** sp. % 4.7, çiçeklenme-kapsül döneminde ise ***F. equiseti*** %3.0, ***F. graminearum*** % 10.8, ***F. oxysporum*** % 6.1, ***F. solani*** % 2.7, ***R. solani*** %2.0, ***A. alternata*** %16.8, ***Cladosporium*** sp. % 7.2, ***Phoma medicaginis*** % 4.9, **Helminthosporium** sp. % 6.9 ve ***Uluocladium atrum*** % 2.9 olarak saptanmıştır.

Patojenisite denemelerine göre, ***Cladosporium*** sp. ve ***Helminthosporium*** sp. dışındaki bütün funguslar mercimeklerde patojen olarak bulunmuştur.

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Researches on Some Biologic and Emerging Properties of Black Grass (*Alopecurus myosuroides* Hudson)

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ABSTRACT

*Several studies were carried out related with the germination and emerging properties of black grass (**Alopecurus myosuroides**). In the studies carried out to determine thef effect of the temperature on germination, the rates were found to change between 35-73 % at 5-30° C.Daily 12h dark + 12 h light application gave slightly higher germination (66 %) regarding the 24h dark application (61 %). In 25, 50 and 75 % soil benefical water levels, 68, 69 and 95 % plant emerging occured, respectively, being mostly in the first 10 days. In the studies related with the effect of seeding depths on plant emerging, it was found that the emerging rates were reduced toward the higher depths of the soil, and rest of the seed left in the soil were observed to be lost at the end of 20 months*

INTRODUCTION

Black grass (*Apopecurus myosuroides* Hudson) is one of the most important weed species of wheat fields in Central Anatolia Region, ranking in the 9 th position with the population density of 1,47 plant/sq.m (Taştan and Erçiş, 1993). The main reasons for rapid population increase is probably widely use of 2,4-D or similar spectrumed compounds for many years as well as its biologic properties. On the other hand, it is also reported that the overdose manure use, soil cultivations and long-term crop rotations have casued the black grass and some other monocotyledons to have much populated (Ferrari, ***et al***, 1985). In brief, changes in agronomic techniques have created new weed problems (Holm,1982).

Although several compounds have been developed against grass weeds, more detailed information is needed to develope integrated and more effective control systems. I order

to enlighten its biologic and emerging properties, this study had been carried out in Ankara province during 1989-1990. Experiments were set up to determine the effect of temperatures and light on germination; and effect of soil benefical water levels and soil depths on plant emerging. Alive seed rates after a period in soil depths also thought to be studied.

MATERIALS AND METHODS

Seeds used in trials were collected on 13.07.1989 and 25.07.1990 in Ankara province and kept in laboratory conditions until the trials set up.

I. Germination Trials

In the studies carried out to determine the effect of temperature and light on germination, trials were set up according to ISTA (International Seed testing Association) norms (Anonymous,1985), as four replicates each having 100 seed. 1,6 and 12 - month - old seeds were used in temperature studies, and only 6- monthold seeds in light studies.

Seeds were kept in distilled water for 10'and planted in petri dishes containing 3-fold-filter paper. Petri dishes were placed in germination chambers working at 5,10,15,20,25 and 30 °C temperature, and 15° C in light studies. Countings were started by the 7 th day of the trial and continued until the 30 th. Each seed produced 5 mm germination tube was assumed to be germinated and removed off the trial.

Temperature effect studies were carried out in dark conditions. In light studies, 3X20 watt flourescent lamps were used in the germination chambers and the petri dishes were covered by black or transparent polyethylen material to realize 24 h dark or 12 h dark + 12 h light conditions

II. Plant Emerging Trials

In the studies to research the effect of soil benefical water levels on plant emerging,the seeds were planted in pots on 11.04.1991, with the soil having 38 % field capacity, 20.2 % fading point; the physical structure 35 % sand, 22.2 % silt, 42.9 % clay and the benefical water levels of 25,50 and 75 %. Trials were set up in four replicates each pot having 25 seed. Polyethylen covers were used to prevent the water loss. The pots were placed in germination chambers working at 15°C in dark, and plant emergings were checked for 30 days.

In the studies carried out to determine the effect of seeding depths on plant emerging and rates of alive seed and seed loss, the seeds were planted in pots on 01.11.1989 in depths of the soil of 5, 10, 15, 20, and 25 cm. Trials were set up in three replicates each having 100 seed. The pots were buried in experimental field up to the top and checked for plant emerging for 20 months in necessary intervals. At the end of the trial rest of the seed left in the soil were planned to be checked for alive seed rates.

In both studies, the seed collected on 13.07.1989 were used.

RESULTS

1. Germination

The results of the studies carried out to determine the effect of temperature on germination are given in Table 1.

Table 1. Germination rates of black grass at various temperatures in dark conditions.

Seed age (month)	Temperature (°C)/Germination Rates (%)					
	5	10	15	20	25	30
1	55	78	75	73	77	32
6	46	61	77	64	72	32
12	56	67	64	52	71	41

Excluding 5 and 30 °C, enough good germination could be obtained in all temperatures, all more or less close to each other. Because of close variation it is hard to say anything about optimum germination temperature. In the age base, although there existed no prominent differences, one-month-old group had shown slightly higher germination.

Studies carried out to determine the effect of light on germination gave the following results shown in Table 2.

Table 2. Germination rates of 6-month-old black grass seed at dark and dark + light combination at 15°C.

Applications/daily	Germination rates %
24 h dark	61
12 h dark + 12 h light	66

Although the germination rates are close to each, it can be said that the light induced the germination.

II. Plant Emerging

The results of the pot studies carried out to determine the effect of soil benefical water levels on plant emergings are given in Table 3.

Table 3. Plant emerging rates of black grass in various soil benefical water levels

Soil benefical water levels	Days / Plant emerging rates (%)						Total
%	7	10	15	20	25	30	
25	36	30	2	0	0	0	68
50	0	63	4	1	1	0	69
75	50	40	2	2	1	0	95

From the table it is clear that the plant emerges were highly completed in the first 10 days and 75 % soil benefical water levels had given the best plant emerging rate with 95 %.

The studies related with the determination of the effect of seeding depths on plant emerging gave the following results shown Table 4.

Table 4. Rates of plant emerging in various seeding depths of soil in 20 months.

Seeding depths (cm)	Plant emerging rates (%)
5	16
10	6
15	0
20	0

Although plant emerging rates are very low, it is clear that the plant emerging rates are decreasing towards the higher depths of the soil.

At the end of the 20 months, the soil was dug up for the rest of the seed, however they all found to be lost, which meaned seed loss rate was 100 % in every depth. Therefore no stduy could be made to determine the alive seed rates.

DISCUSSION

Studies related with the determination of optimum germination temperature showed that black grass had no dormancy and could germinate enough well between 10°-25° C. In another study (Sauerborn *et al*, 1988) optimum germination temperature for the weed was reported to be 15°-25°C. Although an inner dormancy for one-month-old seed was reported (Williams, 1987), we got the highest germination rates at this group. However, the information that seed collections from different localities had generally variable germination rates (Naylor and Abdalla, 1993) should be concerned.

The positive effect of light on germination has seen also in our studies as reported by several literatures (Ferrari et al, 1985; Williams et al, 1984; Williams, 1987). In addition, 95-100 % germination rate could be reached by 8 h light + 16 h dark application (Naylor and Abdalla, 1983).

Since 75 % benefical water level gave 95 % plant emerging mostly in the first 10 days, it may be said that the soil humidity forces collective emerges.

Although a high percent plant emerging was expected at 2 and 5 cm soil depths, the results were not satisfactory. Probably 2 cm depth trial wolud give a very high percent plant emerge if it wasn't spoiled. Nevertheless, it was reported that the black grass had the highest emerging rates at 0-6 cm soil depths (Lovato and Viggiani, 1974). Similar to our trials, it is performed that the emerging rates were reduced toward the higher depths of the soil (Williams *et al*, 1984). Although it is also stated that the germination rate doesn't affected by soil depths (Zwerger and Hurle, 1989), we assume that the statement is related with "germination" but not" plant emerges".

At the end of 20 months, no seed could be detected, and seed loss was 100 % in every depth. The rate was 78-83 % in another study in a period of 2-3 years (Moss,1985) and it was also reported that the seed loss in the soil was highly realized in the first year and only a 10 % was left in four years in which the rate of alive ones was more than 70 % (Chadoeuf *et al* 1986). In our another study (Taştan *et al,* 1991) on distribution of the plant emerges to seasons, it was performed that the whole emerging had happened in the first spring and following autumn.

For the conclusion, it can be said that more detailed studies should be carried out related with the biology and the population dynamism of the weed in order to develope integrated control methods.

ÖZET

Tilki Kuyruğunun (*Alpoecurus myosuroides* Hudson) Bazı Biyolojik ve Çıkış Özellikleri Üzerinde Araştırmalar

Tilki kuyruğunun 1,6 ve 12 ay yaşlı tohumları, 5-30°Cde %35-73 çimlenme vermişlerdir. 24 saat karanlık ortamda % 61,12 saat karanlık + 12 saat aydınlık kombinasyonunda

ise % 66 çimlenme elde edilmiştir. Toprakta % 25 yarayışlı suda % 68, % 75% de ise % 95 çıkış gözlenmiş, çıkışların da genellikle ilk 10 gün içinde olduğu tesbit edilmiştir. Ekim derinliğinin çıkışa etkisi çalışmalarında derinlik arttıkça çıkış azalmıştır. 20 ay sonunda hiç bir derinlikte tohuma rastlanmamıştır.

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Big - Vein Virus Disease of Lettuce in Erzurum, TÜRKİYE*

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A disease symptomatologically defined as big-vein virus of lettuce (Fig. 1) was seen to be widely appeared on Yedikule, Royal Sluis, Salinas, Das-1011, Romulus MF, Goolguard and Great Lakes-118 lettuce cultivars in Erzurum during 1991 and 1992. ***Olpidium brassicae*** (Wor.) Dong known as the vector of big - bein virus (Lange and Insunza, 1977) causing typical vein - banding on leaves (Smith, 1972) was isolated from the roots of infected lettuce plants.

The disease was transmitted and original symptoms were developed on leaves of lettuce seedlings (Fig. 2) one month after the inoculations done by infesting the soil around the roots of healthy seedlings with zoospore suspension obtained from the roots of infected plants as described by Lin ***et al.*** (1970). Previously the presence of lettuce plants showing symptoms resembling those of big-vein virus disease was reported in the lettuce growing areas of İzmir attempts to transmit the disease to healthy lettuces were failed (Fidan and Türkoğlu, 1988).

Studies on big - vein virus and its vector ***O. brassicae*** have been conducting.

ÖZET

Erzurum'da Marullarda İri Damar Virus Hastalığı

1991 ve 1992 yıllarında Erzurum'da Yedikule, Royal Sluis, Salinas, Das-1011, Romulus MF, Goolguard ve Great Lakes - 118 marul çeşitlerinde simptomatolojik olarak tanısı yapılan marul iri damar virus hastalığının yaygın olarak ortaya çıktığı gözlenmiştir (Şekil 1). Marul yaprak damarlarında tipik damar açılmasına (Smith, 1972) neden olan marul iri damar virus hastalığı etmeninin (Lettuce big vein virus, LBVV), vektörü olarak bilinen ***Olpidium brassicae*** (Wor.) Dong. fungusu (Lange and Insunza, 1977) enfekteli marul köklerinden izole edilmiştir.

Bu amaçla, marul iri damar virus hastalığı ile enfekteli bitki köklerinden elde edilen fungus zoospor süspansiyonu sağlıklı marul fidelerinin kök bölgesine ilave edilerek (Lin ***et el.*** 1970) hastalık taşınmış ve orijinal simptomlar inokülasyondan bir ay sonra elde edilmiştir (Şekil 2). Daha önce Fidan ve Türkoğlu (1988), İzmir ili marul ekim alanlarında marullarda iri damar virüs hastalığı belirtisine benzer belir-

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tiyi gösteren bitkilerin bulunduğunu saptamışlar, fakat bu belirtileri gösteren marullardan sağlam marullara hastalık etmeni virusü taşıyamadıklarını bildirmişlerdir.

Marul iri damar virüs etmeni ve vektörü ile ilgili çalışmalar devam etmektedir.

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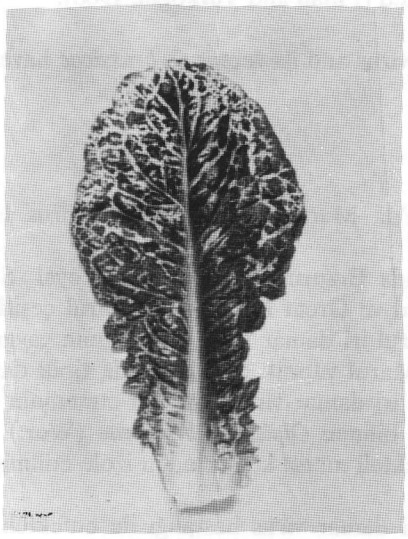

Fig. 1. Vein - banding of big - vein virus disease on lettuce leaf.

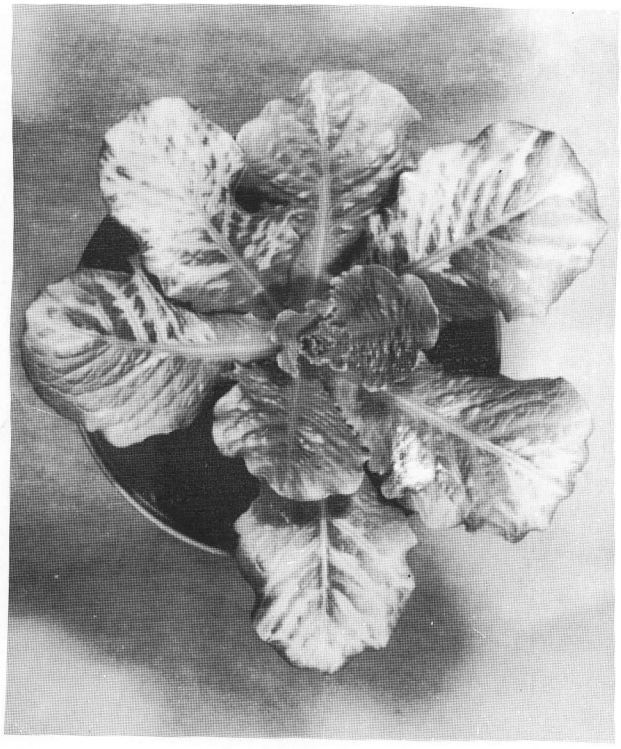

Fig. 2. Big - vein virus disease on young lettuce plant one month after inoculation.

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Recent Records On Virus Diseases Of Vegetables In Greenhouses

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In the greenhouses where tomatoes, peppers, eggplants and cucumbers are grown in Muğla over Aegean Region, some mosaic and necrotic lesions have been found on the leaves. In the further stages of plant growing , dead areas on the top of the shoots and on the side branches of the plants and deformations and necrotic lesions on the fruits have been observed

As a result of Elisa test, tomato spotted wild virus, tomato ring spot virus and tomato black ring virus on tomatoes; tomato ring spot virus on peppers; tomato ring spot virus and tomato black ring virus on cucumbers and tomato black ring virus on eggplants have been found. These diseases except tomato spotted wild virus are new records for Türkiye.

Descriptive studies with mechanical inoculation tests are being conducted.

Serada Yetiştirilen Sebzelerde Saptanan Yeni Virus Hastalıkları

Muğla ilinde seralarda yetiştirilen domates, biber, patlıcan ve hıyar gibi sebzelerde yapraklarda mozayık ve nekrotik lekeler, daha sonraki dönemlerde tepe sürgünlerinde ve yan dallarda kurumalar, meyvelerde şekil bozuklukları ve nekrotik lekeler görülmüştür.

Yapılan ELISA testi sonucunda domateslerde, Domates lekeli solgunluk virusu (Tomato spotted wild virus), Domates halka leke virusu (Tomato ring spot virus), Domates siyah halka virusu (Tomato black ring virus) biberlerde; Domates halka leke virusu ve Domates siyah halka virusu, Hıyarlarda Domates halka leke virusu ve Domates siyah halka virusu, Patlıcanda Domates siyah halka virusu gibi yeni virus hastalıkları tespit edilmiştir. Domates lekeli solgunluk virusu dışında diğer virus hastalıkları Türkiye için yeni kayıttır.

Mekanik inokulasyon testleri ile tanılama çalışmalarına devam edilmektedir.

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