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Occurrence and Distribution of *Spiroplasma citri* and Sesame Phyllody in Sesame in Southern Anatolia

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ABSTRACT

Two surveys were conducted to study the occurrence and distribution of *Spiroplasma citri* Saglio et al. and sesame phyllody phytoplasma in sesame from Antalya to Şanlıurfa in 1992 and again in 1993. Since both pathogens are transmitted by *Circulifer haematoceps* (M. et R.) (Homoptera, Cicadellidae), samples of this leafhopper were collected in representative sesame fields and their inoculativity was determined by ELISA and transmission tests to *Catharanthus roseus* (L.). Sesame phyllody was widespread in the provinces of Antalya and İçel, but no *S. citri*-infected plant was determined. In the provinces of Kahramanmaraş, Gaziantep-Kilis, and Şanlıurfa a very high rate of *S. citri*-infected plants were observed but sesame phyllody was rare. Both diseases were present in sesame in similar rates in Adana and Hatay. In none of the leafhopper samples collected in Antalya *S. citri* was detected and in all transmission tests anly the sesame phyllody phytoplasma was transmitted. *C. haematoceps* obtained from Adana and Hatay transmitted *S. citri* and the sesame phyllody phytoplasma in almost equal rates, while those leafhoppers collected in Kahramanmaraş, Gaziantep-Kilis, and Şanlıurfa only transmitted *S. citri* to indicator plants.

INTRODUCTION

Sesame *(Sesamum indicum* L.) is an important cultivated as second crop in the Aegean region, along the Mediterranean Sea and in southeast Türkiye. It is often affected by two destructive plant pathogens: *Spiroplasma citri* Saglio et al. and sesame phyllody phytoplasma (Türkoğlu and Fidan, 1985; Başpınar et al., 1993; Kersting et al. 1993). Both pathogens are transmitted by the same leafhopper vector, *Circulifer haematoceps* (M. et R.) (Homoptera, Cicadellidae) (Fos et al., 1986, Kersting and Şengonca, 1992, Salehi and Izadpanah, 1992; Kersting, 1993).

S. citri is also causal agent of citrus stubborn disease, a very severe disease of citrus, especially in the east Mediterranean region of Türkiye. Sesame is supposed to be an important host plant of *C. haematoceps* and the only known significant inoculum source of *S. citri* in the east Mediterranean region (Kersting et al., 1992; 1993). It has been suggested that the citrus stubborn disease pathogen is spread from sesame to citrus by *C. haematoceps* in late summer or early autumm (Kersting 1991).

Despite of the importance of *S. citri* and sesame phyllody as disease in sesame itself and in addition, as inoculum source of the citrus stubborn disease pathogen, very few is known about the distribution of both pathogens in sesame in Türkiye. However, this knowledge may prove useful towards a better understanding in the epidemiology of both pathogens and its vector insect *C. haematoceps.*

MATERIAL and METHODS

A survey was conducted by the authors in the sesame-growing area from Adana to Antalya in July 1992 and again from Adana to Antalya in July 1992 and again from Adana to Antalya and from Adana to Şanlıurfa in June and July 1993. Sesame plants were visually inspected for *S. citri*-symptoms (yellowing, stunting, proliferation) (Kersting et al. 1992) and sesame phyllody (virsescence, phyllody) (Vasudiva and Sahambi, 1959; Choopanya, 1971; Salehi and Izadpanah, 1992; Kersting, 1993) in as many fields as possible but at least one every ten to 15 kilometers, if a field was avaible. Every sesame field was visually checked for diseased plants for about five minutes. It was not attempt to estimate any rate of infection but only the presence of *S. citri* and sesame phyllody-diseased plants was recorded. Diseased plants from several locations were collected and later diagnosed for the presence of *S. citri* by ELISA and culture in a modified SMC-medium in the laboratory (Bove et al., 1983; Saillard and Bove, 1983).

In a number of fields, however, in at least one representative for each location, leafhoppers were collected by a mechanical insect aspirator (D-VAC). Each field was sampled for about 100 seconds. *C. haematoceps* were sucked from the collection net by a mouth aspirator directly in field and used either for transmission tests or were further processed for ELISA. A few male *C. haematoceps* of each location were dried and kept for species idendification later in the laboratory.

Leafhopper samples consisting of 5 to 10 individuals, depending on avaibility, were killed in a killing jar and directly macerated in ELISA sample buffer (pH 9.6) and stored in labeled plastic vials in an ice chest. Back in Adana the samples were either

directly used for ELISA or stored at -25°C for not more than 3 months. ELISA was conducted as described elsewhere (Clark et al., 1978; Kersting and Şengonca, 1992) using a polyclonal antiserum prepared against *S. citri* (type strain RA 82-HP) kindly provided by Prof. Dr. J.M. Bove (Laboratoire de Biologie Cellulaire et Moleculaire, Villenave-d' Ornon, France).

For transmission experiments, groups of ten *C.haematoceps* (males and females mixed) were directly transferred to young *Catharanthus roseus* (L.) plants for at least three days. The plants were covered by a plastic cylinder (10 x 15 cm) with three ventilation holes closed by fine gaze. No spesial attention was paid for incoluation access period conditions, since the receptor plants were kept in the bus under natural light. Upon return to Adana, leafhoppers were removed from the cages and all receptor plants were sprayed with a systemic insecticide and kept for at least 12 weeks in greenhouse at 25-35°C for developing of symptoms. Plants showing any symptoms attributable to *S. citri* or sesame phyllody phytoplasma were further analysed by ELISA and culture of *S. citri* to confirm their infection. Any sesame phyllody infection was only recorded visually.

RESULTS

According to the field inspections, no sesame plants exhibiting symptoms attributable to *S. citri* were determined in Antalya and İçel (Fig. 1). However, almost in every sesame field, plants were found showing clear cut symptoms of sesame phyllody. *S. citri* was not detected in any of the phyllody diseased plants neither by ELISA nor by culture of the pathogen. In contrast to Antalya and İçel, many *S. citri*-infected sesame plants were determined in Kahramanmaraş, Gaziantep-Kilis, and Şanlıurfa and this was confirmed both by ELISA and culture. Sesame phyllody was only sporadically observed in these provinces, but it was somewhat more common in Kahramanmaraş compared to Şanlıurfa. In Adana and Hatay sesame plants were infected with *S. citri* and sesame phyllody phytoplasma almost in equal number. Some of the phyllody-diseased sesame plants were concurrently infected with *S. citri* as determined by ELISA and culture.

A total number of 456 *C. haematoceps* samples were analyzed by ELISA for a *S. citri*-infection, out of which 102 samples (22.4 %) were tested positive (Table 1). The highest rate of *S. citri* harboring leafhoppers was determined in Şanlıurfa valueing on average 54.8 % with 23 of 34 infected samples in Bozova. A considerable high rate *S. citri*-infected *C.haematoceps* were also determined in Gaziantep-Kilis (33.3 %), Adana (27.1 %) and Hatay (15.2 %). In contrast, only one group of leafhoppers were found haboring *S. citri* in the province İçel and no *S. citri* was detected in any of 71 *C. haematoceps* samples collected in Antalya.

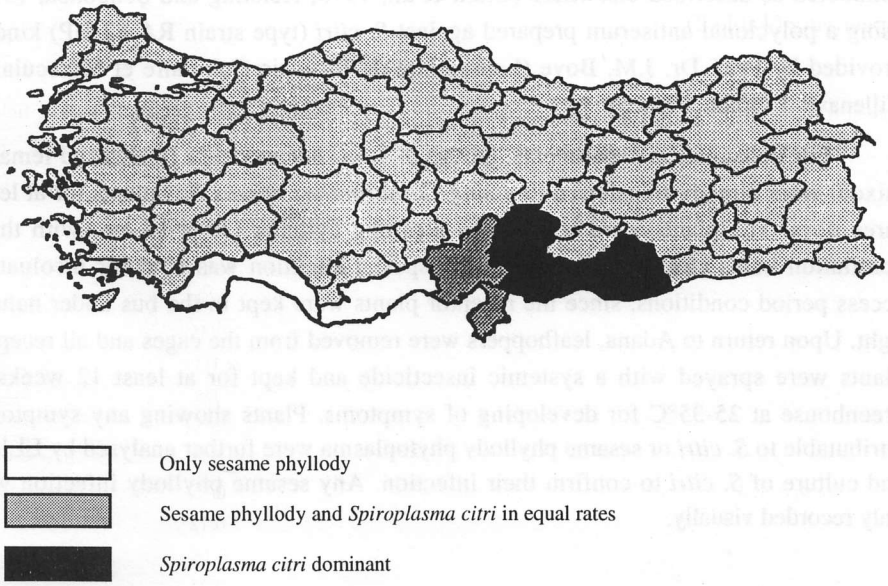

Fig. 1. Occurrence of *Spiroplasma citri* and sesame phyllody in sesame in different provinces in Southern Anatolia

The transmission tests resulted in 104 successful transmissions of *S. citri* or sesame phyllody phytoplasma (30.7 %) (Table 2). In 47 C. rosue plants (13.9 %) *S. citri* was detected by ELISA and its presence was confirmed by culture of the pathogen. On average 16.8 % of the indicator plants (57) developed clear symptoms of a phytoplasma infection (virescence, phyllody). Five of these plants, all obtained from transmission test using *C. haematoceps* collected in Adana were concurrently infected with *S. citri.* Four *C. roseus* plants, derived from transmission tests in Antalya developed leaf yellowing and they were stunted but no virescence or phyllody symptoms were observed. In none of these four plants *S. citri* was detected, neither by ELISA nor by culture of the pathogen.

In none of 93 transmission tests using *C. haematoceps* collected in Antalya and in none of 14 test with leafhoppers collected in İçel *S. citri* was transmitted to *C. roseus.* In all successful transmission experiments (30.8 %), *C. haematoceps* transmitted a phytoplasma to the indicator plants. In contrast, none of 70 transmission test using *C. haematoceps* collected in Kahramanmaraş, Gaziantep-Kilis and Şanlıurfa resulted in a sesame phyllody infected indicator plant, but *S. citri* was transmitted to 19 of 70 plants

tested (27.1 %). Leafhoppers collected in Adana and Hatay transmitted *S. citri* (22/162) and sesame phyllody phytoplasma (28/162) in almost equal rates of 14.8 % and 17.3 %, respectively (Table 2).

Table 1. Number and ratio of *Spiroplasma citri*-infected *Circulifer haematoceps* collected in sesame fields in Southern Anatolia

Province Location	Mean number of *C. haematoceps* per ELISA test	Number of positive ELISA tests/ number of ELISA tests	
Adana		51/188	(27.1 %)
Bacalı	9.4	14/87	
Çatalan	6.7	9/35	
Imamoğlu	10.0	1/3	
Kadirli	10.0	1/5	
Mustafalar	5.4	26/58	
Antalya		0/71	(0.0 %)
Gazipaşa	8.1	0/12	
Aksu	10.0	0/12	
Anamur	8.6	0/18	
Manavgat	9.3	0/21	
Serik	7.0	0/8	
Hatay		5/33	(15.2 %)
Dörtyol	10.0	4/17	
İskenderun	5.8	0/4	
Kırıkhan	6.3	1/6	
Yakacık	5.7	0/6	
İçel		1/25	(4.0 %)
Kurtuluş	8.4	0/8	
Olukbaşı	10.0	1/12	
Silifke	10.0	0/5	
G.Antep-Kilis		4/12	(33.3 %)
Kilis	10.0	4/12	
K.Maraş		9/34	(26.5 %)
K.Maraş	10.0	6/21	
Narlı	6.2	2/5	
Türkoğlu	7.0	1/8	
Şanlıurfa		34/62	(54.8 %)
Akziyaret	9.1	5/12	
Birecik	9.6	1/8	
Bozova	8.7	23/34	
Dutluca	8.4	5/8	
Total		102/456	(22.4 %)

Table 2. Transmission of *Spiroplasma citri* and sesame phyllody phytoplasma by *Circulifer haematoceps* collected in sesame fields in Southern Anatolia

Province	Number diseased plants/ number inoculated plants	*S. citri*		Phyllody	
		Number	%	Number	%
Antalya	28/93	0	0.0	28	30.1
İçel	5/14	0	0.0	5	35.7
Adana	49/149	27	18.1	22	14.8
Hatay	3/13	1	7.7	2	15.4
Kahramanmaraş	2/20	2	10.0	0	0.0
Gaziantep-Kilis	2/9	2	22.2	0	0.0
Şanlıurfa	15/41	15	36.6	0	0.0
Total	104/339	47	13.9	57	16.8

DISCUSSION

S. citri and sesame phyllody were not equal distributed in the sesame growing area of south Türkiye. No *S. citri*-infected sesame plant was found in Antalya and İçel and no successful transmission of this pathogen was achieved using. *C. haematoceps* collected in these western provinces. However, many phyllody diseased plants were observed and a phytoplasma was frequently transmitted to *C. roseus* plants. The situation was opposite in Kahramanmaraş, Gaziantep-Kilis and Şanlıurfa, where few phyllody-infected but many *S. citri*-infected sesame plants were observed. Consequently, *C. haematoceps* collected in these eastern provinces only transmitted *S. citri* to the indicator plants. In Adana and Hatay located in between the other two areas, approximately the same number of *S. citri*-and sesame phyllody-diseased plants was observed and filed collected leafhoppers transmitted both diseases to *C. roseus.*

This results was somewhat surprising, since sesame is more common in Antalya compared to east Turkey. The vector *C. haematoceps* was very abundant in all areas sampled. In spite of that, no *S. citri* was observed in Antalya and İçel and no transmission was achieved. Comparable to this situation, citrus stubborn disease caused by the same pathogen was rarely found in citrus orchards in the Antalya region during previous surveys (Salibe, 1986; Bove, 1995). In opposite, citrus stubborn disease is widespread in the east Mediteranenan region as its is *S. citri* in sesame (Roistacher 1990; Çınar et al., 1993). Even more than 500 km east citrus growing area, *S. citri* commonly occurred in sesame. A disease lonf known as sesame yellows in Iran was also recently found to be caused by *S. citri* (Salehi and Izadpanah, 1995), indicating a widedistribution of S. citri in he west Asian region. Whether *S. citri* is mainly a disease of sesame and later spread to citrus in areas were both crops are grown together remains

to be proven. It is long known that citrus is actually not a good host for *S. citri,* since a vector transmission from citrus to citrus or tho herbaceous plants is very difficult to achieve. Thus, citrus has to be considered as dead end for he pathogen.

Unfortunately we are at this moment not able to reasonably explain why no *S. citri* occurred on sesame in Antalya. There are only little climatic differences between Adana and Antalya that might serve as a possible explaining. One might argue that there is a geographical barrier between the western provinces İçel and Antalya and Adana and the provinces further east. As a result of this "isolation" a biotype of *C. haematoceps* might have become established in Antalya that lost its capability to transmit *S. citri.* However, there is no any prove for that. The only difference we observed among the sesame culture in Antalya or İçel and the eastern provinces was that in the western provinces sesame is irrigated but in the east not. In Adana *S. citri*-infected plants are more common in nonirrigated areas, but on the other hand sesame is only rarely grown in irrigated areas. It is even not clear how irrigation or dryland farming may affect the occurence of *S. citri* in sesame.

More detailed studies are necessary to explain these open questions. The authors suggest that a consideration of differences in local vector populations and the effect of agricultural practices on spread and occurence of both pathogens may contribute greatly towards a more general understanding of the epidemiology of pathogens transmitted by *C. haematoceps.*

ÖZET

Spiroplasma citri ve SUSAM PHYLLODY HASTALIKLARININ GÜNEY ANADOLU'DAKİ SUSAMLARDA DURUMU VE YAYILIŞI

Spiroplasma citri Saglio et al. ve Susam phyllody hastalığının susamlardaki durumu ve yayılışı Antalya'dan Şanlıurfa'ya kadar 1992 ve 1993 yıllarında yapılan iki surveyle ortaya konulmuştur. Söz konusu bu hastalıkların *Circulifer haematoceps* (M. et R.) (Homoptera, Cicadellidae) tarafından taşınabilmesi nedeniyle, bu cicadellid türün susam tarlalarını temsil edecek nitelikte örneklemesi yapılarak hastalık etmelerini bünyesinde bulundurup bulundurmadığı ELISA ile belirlenmiş ve test bitkisi *Catharanthus roseus* (L.) kullanılarak taşıma denemeleri yapılmıştır.

Antalya ve İçel illerinde Susam phyllody hastalığı yaygın olarak belirlenmesine karşın, *S. citri* ile infekteli herhangi bir susam bitkisi saptanmamıştır. Kahramanmaraş, Gaziantep-Kilis ve Şanlıurfa illerinde ise çok sayıda *S. citri* ile bulaşık bitki görülürken, Susam phyllody hastalğı çok az sayıdaki bitkide ortaya çıkmıştır. Adana ve Hatay

illerinde ise söz konusu iki hastalık, susamlarda birbirine yakın oranlarda belirlenmiştir. Antalya'da susam tarlalarından toplanan *C. haematoceps* bireylerinin ise biçbirisinde *S. citri* bulunamamış ve yapılan taşıma denemelerinde test bitkilerine sadece Susam phyllody hastalığının taşınabildiği ortaya çıkarılmıştır. Adana ve Hatay illerindeki susamlardan toplanan *C. haematoceps* örneklerinin ise, hem *S. citri* ve hem de Susam phyllody hatalıklarını test bitkilerine hemen hemen eşit oranlarda taşıdığı, buna karşın Kahramanmaraş, Gaziantep-Kiliş ve Şanlıurfa'dan toplanan *C. haematoceps*'lerin sadece *S. citri*'yi taşıdığı belirlenmiştir.

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Determination of the Races of *Fusarium oxysporum* f.sp. *ciceris* in Ankara Province, Turkey

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ABSTRACT

Thirty one isolates of *Fusarium oxysporum* f.sp. *ciceris* were isolated from affected plants collected from main chickpea areas in Ankara Province. A set of ten differential chickpea cultivars (JG-62, C-104, JG-74, CPS-1, BG-212, WR-315, Annigeri, Chafa, L-550, 850-3/27) was used to identify races of *F. oxysporum* f.sp. *ciceris*. The existence of three races (0, 2 and 3) from seven reported races of the fungus was determined in Ankara, Turkey.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is produced in sixty-two provinces of Turkey and approximately, 20 068 ha are sown annually in Ankara province of Turkey (Anonymous, 1994). Several diseases have been determined on chickpea in Ankara. It has been reported that root rot and wilt diseases of chickpea are an important disease group. Among these, *Fusarium oxysporum* Schlechtend. : Fr. f.sp. *ciceris* (Padwick) Matuo &K. Sato as wilt pathogen of chickpea is the most common pathogen (Dolar, 1996). Wilt of chickpea caused by *F.oxysporum* was first described by Padwick in 1940 and it has since been reported from several countries (Nene et al., 1996). Seven races of *F. oxysporum* f. sp. *ciceris* have been identified by their differential interactions with chickpea lines (Haware and Nene, 1982; Cabrera de la Colina et al., 1985; Trapero-Casas and Jimenez-Diaz, 1985; Phillips, 1988). Haware and Nene (1982) presented evidence of the existence of at least four races (race 1, 2, 3 and 4) of *F. oysporum* f. sp. *ciceris* in India. Two additional races (race 0 and 5) have been reported in Spain (Cabrera de la Colina et al., 1985). Phillips (1988) found the seventh race of *F. oxysporum* f.sp. *ciceris* in California called race 6. Race 0 occurs in California, Spain and Tunisia: races 1 and 6 were identified in California, Morocco and Spain: and race 5 was only found in California and Spain (Kaiser et al., 1994 and Hervas et al., 1995). Information on the

pathogenic variability in pathogen populations is useful in the breeding programs for resistance to the disease. The most effective and economical method of controlling Fusarium wilt of chickpea worldwide is by use of resistant cultivars. However, the effectiveness of resistant cultivars is threatened by the occurence of different pathogenic races (Kaiser et al., 1994). It is, therefore, necessary to identify the races of *Fusarium oxsporum* f.sp. *ciceris* for successful breeding of resistant cultivars. The races of *F. oxysporum* f.sp. *ciceris* have not been reported from Turkey.

The purpose of this paper was to determine the races of *F.oxysporum* f. sp. *ciceris* in Ankara province, Turkey.

MATERIALS and METHODS

Surveys and fungal isolation: Surveys were carried out in the main chickpea areas of Ankara province (Central Ankara, Ayaş, Çubuk, Haymana, Polatlı, Bala, Beypazarı, Kalecik, Şereflikoçhisar, Kızılcahamam, Güdül) from May through August from 1992 to 1994.

Fungal isolations were made from collar regions of the affected plants. Tissue pieces were surface-sterilized with sodium hypochlorite (1%) for 3 min and plated on water agar (WA), potatodextrose agar (PDA) and acid-potato-dextrose agar (APDA). Cultures were incubated at 22±1°C with a 12 h photoperiod of near UV light. Single spores isolations of the cultures were obtained and identified according to Booth (1971). All isolates were stored in test tubes including autoclaved sandy loam soil.

Pathogenicity test, inoculum preparation and inoculation metod: The pathogenicity of isolates of *F. oxysporum* was tested by using pot-culture inoculation method developed by Nene and Haware (1980). Susceptible cultivar AUG 424 obtained from NIAB (Nuclear Institute for Agricultire and Biology, Faisalabad, Pakistan) was used in all pathogenicity tests.

Inoculum of each test isolate was preparet from 7 day-old cultures on PDA. The inoculum was propagated for 14 days on 100 g of 9:1 sand chickpea meal medium in 250 ml conical flasks at 25±1°C with an illumination of 12 h per day. The inoculum in each flask was mixed thoroughly with 2 kg autoclaved soil in each pot. The soil in the pots was lightly watered after the inoculum was incoporated. Sowing was done 4-5 days later. Control plants were grown in a comparable mixture of noninfested sand-chickpea meal and autoclaved soil. Plants were grown in a growth room at 25±2°C/21±2°C (day/night) with a 12 h photoperiod of fluorescent light at approximately 11 000 lux. Plants were observed weekly for symptoms.

Determination of formae speciales of isolates: Eight legume species were used to determine the formae speciales of pathogenic isolates of *F. oxysporum*. These legumes were alfalfa (*Medicago sativa* L.), bean (*Phaseolus vulgaris* L.), chickpea-cultivar AUG 424 and local cultivar (*Cicer arietinum* L.) broadbean (*Vicia faba* L.), lentil (*Lens culinaris* L.) pea (*Pisum sativum* L.), soybean (*Glycine max* (L).) Merr.), cowpea (*Vigna unguiculata* (L.) Walp.)

Determination of races of *Fusarium oxysporum* f. sp. *ciceris*: A set of ten different chickpea cultivars (JG-62, C-104, JG-74, CPS-1, BG-212, WR-315, Annigeri, Chafa, L-550 and 850-3/27) was used to identify the races of *F. oxysporum* f.sp. *ciceris* (Haware and Nene, 1982). Seeds of ten chickpea cultivars were obtained from ICRISAT's Germplasm Resources Unit. Seeds of each test cultivar were surface disinfected by immersing in 1 % solution of sodium hypochlorite for 3 min before sowing into each pot ot culture-infested soil. Control pots were kept for each cultivar. Pots were maintained in growth room for six weeks, under the conditions indicated above. Each treatment was replicated six times (a pot containing seven plants constituted a replicate). All experiments were repeated three times.

Disease assesment: The observations on mortality because of wilt an root rot were recorded at weekly intervals up to 42 days. Disease reactions were classified according to percentage of dead plants as resistant (0-20 %), moderately susceptible (21-50 %) and susceptible (> 50 %) (Haware and Nene, 1982).

RESULTS and DISCUSSION

Thirty-one isolates of *F. oxysporum* f.sp. *ciceris* were classificated into three races (Table 1).

Races 0, 2 and 3 were identified in Ankara but other four races (races 1, 4, 5 and 6) were not found. Race 2 is the most pathogenic isolates group. Except cultivar WR-315 and 850-3/27, other eight cultivars are susceptible to it (Table 1). So far, race 2 and 3 have been reported only from India (Haware and Nene, 1982). The both two races caused the typical Fusarium wilt. Race 0 (14 of 31 isolates) was found to be widespread in the chickpea growing areas of Ankara, as compared with race 2 (11 of 31) and race 3 (6 of 31). Jimenez-Diaz and Trapero-Casas (1990) reported that race 0 was widely distributed in the Mediterranean basin and it showed limited pathogenecity to kabuli cultivars (large, ramhead-shaped, beige seeds). Race o caused foliar yellowing syndrome and its virulence was less than the other races. These results are in agreement with the findings of Cabrera de al Colina et al. (1985) and Trapero-Casas and Jimenez-Diaz (1985) who reported that race 0 was the least virulent of the seven races. Although race 0 is widespread in chickpea growing areas, it shows limited pathogenicity to kabuli cultivars which are widely grown in Turkey. For this reason it may not cause serious

problems in chickpea growing areas. However, development of resistant cultivars to highly pathogenic races 2 and 3 are necessary.

Table 1. Reaction [1] of ten differential cultivars of chickpea to different races of Fusarium oxysporum f. sp. ciceris,

Cultivar	Race O	Race 1	Race 2	Race 3	Race 4	Race 5	Race 6
JG 62	R	S	S	M	S	S	S
C 104	M	S	S	R	S	S	S-M
JG 74	R	R	S-M	R	R	S	R
CPS 1	R	R	S	S	M	S	R
BG 212	R	R	S	M	M	R	R
WR 315	R	R	R	S-M	R	R	R
Annigeri	-	S	S	S	S	-	R
Chafa	-	S	S	S-M	S	-	R
L 550	-	S	S	M	S	-	S-M
850-3/27	-	S	M	M	M	-	R
No. of isolates representing the race	14	not found	11	6	not found	not found	not found

1 R= resistant (0-20 % mortality); M=moderately susceptible (21-50 % mortality); S=susceptible (>50 % mortality)

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ÖZET

TÜRKİYE'DE ANKARA İLİNDE *Fusarium oxysporum* f.sp. *ciceris'İN* IRKLARININ BELİRLENMESİ

Ankara ili sınırları içindeki başlıca nohut ekim alanlarından toplanan hastalıklı bitkilerden yapılan izolasyonlar sonucunda 31 adet *Fusarium oxysporum* f.sp.*ciceris* izolatı elde edilmiştir. *F.oxysporum* f.sp. *ciceris* izolatlarının ırklarını belirlemek için 10 ayırıcı nohut çeşinden oluşan bir set (JG-62, C-104, JG-74, CPS-1, BG-212, WR-315, Annigeri, Chafa, L-550, 580-3/27) kullanılmıştır. *F. oxysporum* f.sp. *ciceris*'in şimdiye kadar tespit edildiği bildirilen yedi ırkından üçünün (0, 2 ve 3) Ankara ilindeki nohut ekim alanlarında bulunduğu tespit edilmiştir.

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Untersuchungen Über Die Netzfleckenkrankheit (*Drechslera teres* Shoem. f.sp. *teres* Smedeg. *D. teres* Shoem. f.sp. *maculata* Smedeg.) An Gerste

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ZUSAMMENFASSUNG

In den Monaten Mai-Juli 1994-1995 wurde Mittelanatolien auf die Gerstenkrankheit *Drechslera teres* (Sacc) Shoem. untersucht. Es wurde von 246 ausgesuchten Gerstenanbaufeldern bereits 210 Felder als con *D. teres* befallen festgestellt, d.h. in Mittelanatolien eine sehr groβe Intensität. So wurde in diesem Gebiet ein durchschnittlicher Befall von 93.35 % festgestellt. Auβerdem wurde mit dieser Arbeit zwei Pathotypen von *D. teres (D. teres* f. sp. *maculata* und *D. teres* f.sp. *teres)* gefunden.

EINLEITUNG

Es wurde bekannt, daβ *D. teres* die Gerste infiziert (Mc Donald und Buchonon 1964, Hartmann 1993). Die Erreger wurden fast überall auf den Gerstenanbauflächen der Welt nachgewiesen, die den wichtigsten Schaden an Gerste verursachen (Aktaş 1987, Deimel 1988, Brandl und Hoffmann 1990, Hartmann 1993). Nach Aktaş (1987) wurde in Mittelanatolien ein durchschnittlicher Befall an Gerste von 69.7 % festgestellt. Das Pathogen zeigt in der Türkei von Jahr zu Jahr eine zunehmende Verbreitung. In dieser vorliegenden Arbeit wird über die Verbreitung und wirtschaftliche Bedeutung des Erregers an Gerstenpflanzen in Mittelanatolien berichtet. Auβerdem wird erstmals das Vorkommen von zwei Pathotypen von *D.teres* in der Türkei berichtet. Als dominante Typ wurde *D.teres* f. sp. *maculata* (spot-typ) überall nachgewiesen, während der Pathotyp *D. teres* f. sp. *teres* (net-typ) manchmal in unterschiedlicher Form gering verbreitet gefunden wurde. Diese beide Typen wurden als neue Typ Smedegard-Petersen (1971) gennannt. Danach wurde diese Typ durch viele Autoren wie Tekauz and Mills (1974), Khan and Tekauz (1982) und Tekauz (1985) bestätigt.

MATERIAL UND METHODEN

1. Untersuchungen im Freiland

Um die von *D. teres* befallenen Gerstenpflanzen zu sammeln, wurden in den Monaten Mai-Juni 1994-1995 nach "Systematische-Vorbildungsmethode" (Bora und Karaca, 1970) neun Provinzen in Mittelanatoliens besucht. Je nach Groβe des Feldes wurde 1 bis 5 proben auf der Gerstenanbaufläche entnommen. In Untersuchungen wurden 246 Gerstenanbaufelder besucht. Danach wurden die in jedem Feld erkrankten Pflanzen und die prozentuale Krankheitsintensität von *D. teres.* bestimmt. Befallenen Blattflächen wurden unter Zuhilfnahme nach Bestimmungsschlüssel für Pilzkrankheiten an Getreide der Firma Ciba-Geigy GmbH-Angaben (Abb. 1) festgestellt.

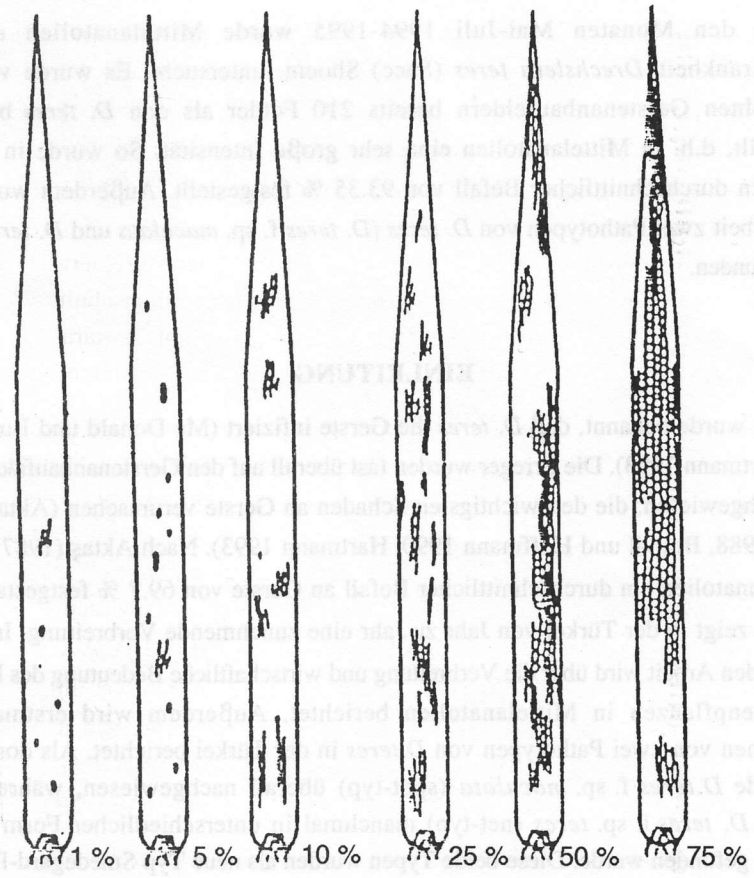

Abb. 1. Bestimmungsschlüssel zur Ermittlung der prozentuale Krankheitsintensität

2. Untersuchungen im Labor

Bei den Gerstenpflanzenproben wurden dunkelbraune Blattfleckenteile ca. 2 cm abgeschnitten. Danach wurde mit 1 % iger Chlorlauge (NaOCI) 2 min. oberflächlich sterilisiert und mit sterilem Wasser 3 mal gut gewaschen und in der Feuchtkammer gelassen, bei 22±1°C und 3-4 Tage inkubiert. Der Inkubationsraum wurde 12 Std.pro Tag unter 2000 lukses beleauchtet. *D. teres* wurde auf 20 % igem V 8-Agar (200 ml. Libby's Gemüsesaft + 2g CaCO3 + 20 gr Agar + 800 ml dest. Wasser) aufgeimpft und nach 7 Tage sehr gute Konideinbildung festgestellt. Die Klimabedingungen waren wie oben.

Ergebnisse und Diskussion

Im Untersuchungsgebiet wurde D. teres von 246 studierten Gerstenanbaufeldern in ingesamt 210 Feldem D. teres festgestellt. Von befallenen Gerstenanbaufeldern wurde pro Feld eine Gerstenblattprobe genommen. Wie die Tab 1 zeight, ist D. teres auf Gerstenanbau flächen in neun Provinzen gefunden worden. Aber in der Umgebung von Aksaray und Yozgat ist mit 100 % *D. teres* der stärkste Befall fesgestellt worden. In Mittelanatolien wurde bei Untersuchungen 1994-1995 zu 93.35 % befallene Gerstenanbaufelder mit diesem Erreger gefunden.

Die durschhnittliche Krankheitsintensität in Gerstenanbaufeldern lag zwichen 0.88-19.98 %, wie man aus der Tab. 1 ersehen kann. Eine durshschnittlich stärkere Krankheitsintensität von *D. teres* ist nur in der Umgebung von Ankara und Aksaray nachgewiesen werden. Sonst lag die durschschnittliche Krankheitsintensität bei 12.32 %.

Das Krankheitsbild von *D. teres* zeigt an Gerstenblättern gebräunte kleine punktförmige, manchmal ovale unregelmässig fleckenartige nekrotik läsionen. Diese Pathotyp war überall verbreitet und wird als spot-blotch (Spot-Typ) *D. teres* f.sp.*maculata* bezeichnet. Wie die Tab. 1 zeigt, ist bezeichnete *D.teres* f. sp. *maculata* in insgesamt 210 entnommenen Gerstenblattproben 197 mal nachgewiesen worden. Also ist diese Pathotyp 93.06 % in Mittelanatolien verbereitet (Abb. 2A). Auch ein anderes Krankheitsbild kann af Gerstenblattflächen als gelegentlich vereinigte Flecken beobachtet werden. Diese Pathotyp lag häufiger in Eskişehir-Sarıcakaya, aber nur wenig in Aksaray, Karaman und Konya, Diese Typ wird als net-blotch (Net-Typ) *D. teres* f.sp. *teres* bezeichnet und lag insgesamt in Mittelanatolien bei 6.94 % (Abb. 2B).

Zwischen den Konidien dieser beiden Isolatetypen wurden keine Unterschiede gefunden. Aber ich glaube, dab diese beiden Isolatetypen virulenzlich unterschiedlich sein können (Abb. 2C).

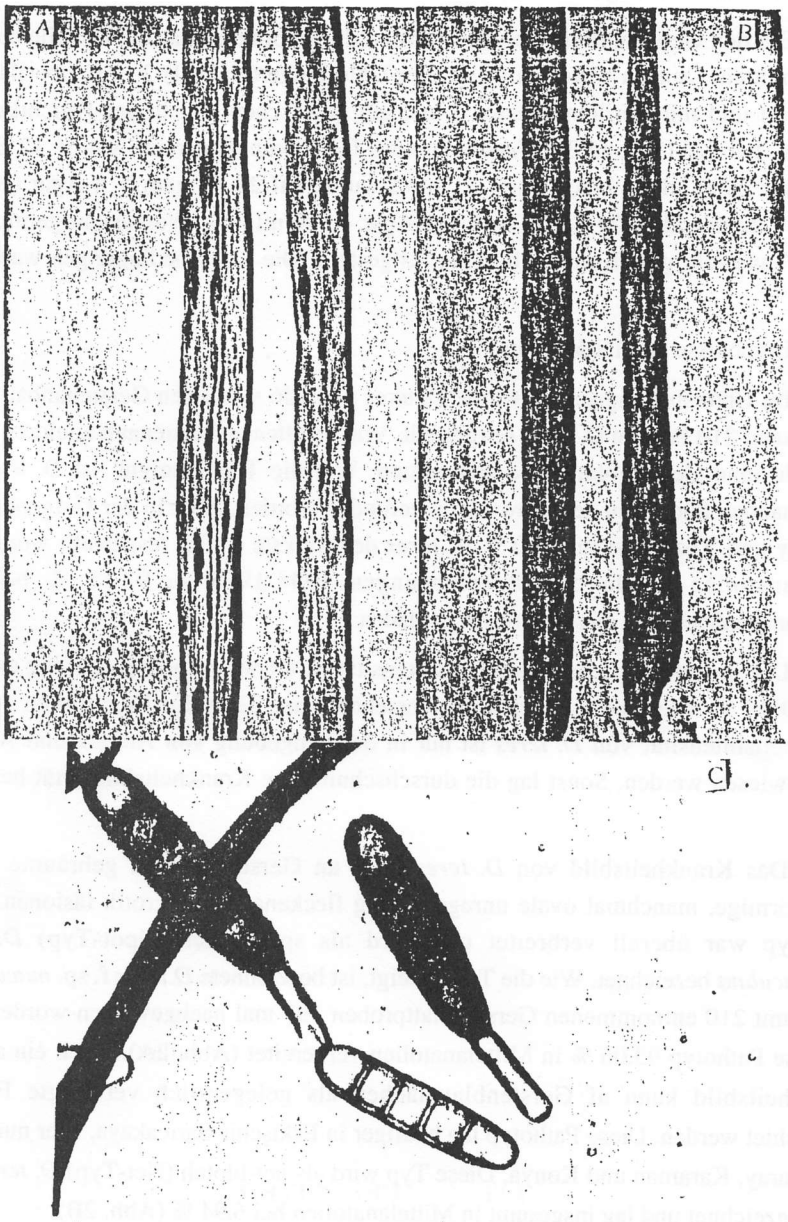

Abb. 2. Krankheitssymptome an Gerstenblaetter

A. *D. teres* f.sp. *maculata,* B. *D. teres,* C. Konidien von *D. teres*

Tabelle 1. Verbreitung der Pathotypen und durchschnittliche Krankheitsintensität von *Drechslera teres* in befallenen Gerstenanbaufeldern in Mittelanatolien

Provınz	Untersuc-hungen Feldfläche (da)	Zahl der befallenen Felder	Zahl der gesunden Felder	Stop-Typ	Net-Typ	Befallsver-hältniss im Feld (%)	Krankheitsin-tensität im Feld (%)
AKSARAY (1995)	966	15	1	13	2	100.00	14.98
ANKARA (1994-95)	2340	39	5	39	0	97.23	16.46
ESKİŞEHİR (1995)	2020	29	7	20	9	87.11	8.91
KARAMAN (1995)	1566	18	1	17	1	83.47	10.98
KIRIKKALE (1994)	823	12	3	12	0	95.13	12.47
KIRKŞEHİR (1994)	1541	16	3	16	0	97.43	12.20
KONYA (1995)	3956	54	10	53	1	97.65	12.90
SİVAS (1995)	820	10	5	10	0	63.91	8.85
YOZGAT (1995)	1510	17	1	17	0	100.00	10.66
Insgesamt	15542	210	36	197	13		
Befallene Gerstenanbaufelder (%)		85.36	14.64				
Verbreitung der Pathotypen (%)				93.80	6.20		

SUMMARY

During may till june 1994-1995 were barley fields in the central region of Anatolia were examined to find *Drechslera teres.* Already 210 field of totally 246 examined plots were infected with this disease, that means a very high percentage. The average infection was 93.35 % in this area. In addition two pathotypes of *D. teres (D. teres* f. sp. *maculata* and *D. teres* f. sp. *teres*) were found.

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Studies on Alfalfa Mosaic Virus of Alfalfa Growing Areas in Erzurum

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SUMMARY

In this study, alfalfa mosaic virus infection rates as predicted by observations were found to be varying between 10.91-14.20 % of the total alfalfa growing areas in Erzurum, in 1993. Leaf sap extracts which were obtained from the plants showing mosaic virus symptoms were transferred to the healty test plants by mechanical inoculation and the symptoms exhibited by the test plants have been observed. Diagnosis of the virus was realysed by the dsRNA (double stranded RNA which is a replicative form of the virus's RNA) isolation and analysis. Three different dsRNA isolation methods which were generally used in diagnosing viral infections were also investigated for the suitability of diagnosing alfalfa mosaic virus. in this study. dsRNA samples obtained by the three different isolation methods. were subjected to electrophoretic seperation in 1.2 % agar-gel medium and as a result of this seperation. dsRNA profiles having AMV (Alfalfa mosaic virus) spesific bands have been obtained. It was found that all the isolation methods tried were found to be also muitable for the isolation of AMV's dsRNA, but the most clear dsRNA profile have been obtained with the method suggested by Morris and Dodds.

INTRODUCTION

Because of ist high protein content and nutritive value, alfalfa both either as fresh and dry, occupies on of the most outstanding place among fodder plants in animal nutrition.

In regard to alfalfa growing potential. Erzurum and its vicinity is on top of the list among the counties of Eastern Anatolian Region (Anon, 1992). In agreement with this, amongst the fodder plants grown in Turkey alfalfa growing field area and alfalfa production rates were reported to be 52 % and 64 % respectively (Anon, 1992).

A like other crop plants. alfalfa is also affected by some plants diseases, pests, parazites and weeds. According to Smith (1957). Crill et al. (1970) and Beczner (1974) 3.18 and 24 different types of viruses can infect alfalfa plant respectively. Moreover, Edwarson and Christie (1986) have reported alfalfa as host plant for more than 28 viruses. The same authors also have stressed that alfalfa mosaic virus among all others is the only one which is most important and wide spread disease factor all over the world.

AMV (Alfalfa mosaic virus) which is most important and wide spread viral diseases of all others, can be transferred by various means: as mechanical inoculation with plant sap, with seeds, with aphids in a nonpersistent manner, with *cuscuta* and with weed seeds (Smith, 1957; Hull, 1969; Çalı and Erdiller, 1990).

Bailis and Olennu (1986) have reported have yield reduction rates between 14.8 % 22.8 % and 15.0 % - 18.1 % on fresh and dry weight bases respectively, caused by AMV infections on alfalfa fields.

Since dsRNA profiles which have obtained from the virus infected plants are specific to each virus or even virus strain (Morris and Dodds. 1979). For diagnosis of viral diseases or desorders various dsRNA isolation and analysis methods are suggested by investigators (Morris and Dodds, 1979; Valverde et al., 1990).

Pinck and Hirt (1972), Parent and Asselin (1984), Pietersen et al., (1985) have succeeded to isolate and analyse virus spesific dsRNA which is obtained from AMV inoculated tobacco leaves by a gel-electrophoretic method. As a result of the electrophoretic analysis, the investigators have found four different types of dsRNA profiles.

It was determined that alfalfa mosaic virus which was in alfalfa mosaic group. This is a monotypic group with alfalfa mosaic virus (AMV) as its sole member, although the virus does occur as numerous distinc strains. AMV praticles are built from a single species of polypeptide of Mr 24.3 x 10^3 to form quasi-spherical particles, about 18 nm in diameter, plus a series of bacilliform particles of the same width but differing lenghts. The particles encapsidate four major species of single-stranded, positive sense RNA with Mr from 1.04 x 10^6 to 0.28 x 10^6 (Francki et al., 1985; Pietersen et al., 1985; Matthews, 1993).

Research studies involving alfalfa viruses are rather restricted and quite scarce in Turkey. Erdiller and Lesemann (1985) have reported AMV infections of alfalfa grown around and about Ankara as a results of serologic tests, mechanical inoculation studies and immunosorbent-electron microscobic studies.

Çalı and Erdiller (1990) have diagnosed AMV infections of alfalfa which have grown in Central Anatolian geographic region by mechanical inoculation, by serologic test methods and by identification of physical properties of the virus.

AMV infections of alfalfa in the Aegean Region have also been diagnosed by Fidan (1992) with mechanical inoculation, serologic tests and with physical properties of the virus.

The main purposes of this research work were as follows; to transfer AMV which is wide spread in alfalfa growing areas in general and existency in Erzurum alfalfa growing areas was also a high possibility predicted by symptomatic observations, to healthy test plants by mechanical inoculation and to observe symptoms caused by the virus and to make diagnosis by dsRNA analysis.

In this study dsRNA, wich is a replicative form of viral RNA derived from virus's own genomic RNA, isolation was realysed by using three different methods and suitability of these methods for AMV were also examined. The diagnosis and identification of the virus have been tried by the electrophoretic analysis on agar gel medium of dsRNA samples obtained with three different methods.

MATERIALS and METHODS

A. Studies on Infected Plant Material

Survey studies have been made on alfalfa growing areas in Erzurum in 1993 sping and summer season. Leaf samples from alfalfa plants exhibiting viral-like symptoms were collected from 18 representative fields of 8 different villages of Erzurum. In leaf sampling and prediction of disease rates, suggestions by Bora and Karaca (1970) have been followed.

The inoculum which is prepared with 0.1 % mercaptoethanol and 1 ml buffered phosphate solution (0.01 M, pH: 7.2) have been applied at the rate of 1 ml per 1 gr of leaf material to the test plants by mechanical inoculation. Inoculated test plants have been kept in green -house conditions at 18-20°C for observing the disaesa symptoms (Bos and Jaspars. 1971; Smith, 1972; Noordam, 1973).

The isolate which have showed the most severe outstanding symptoms on the test plants was selected and used for mechanical inoculation and dsRNA isolation studies.

B. Studies for Nucleic Acid Isolation and Analysis

12-14 days after inoculation. *Nicotiana tabacum* Xanthii plants showed sistemic infection symptoms, have been used for virus isolation and multiplication (Pinck and Hirt, 1972; Parent and Asselin, 1984).

Three different methods were used for isolating nucleic acid. First of these is the method utilized and suggested by Morris and Doods (1979) for dsRNA isolation. The second one is the purification method which is used by Duran-Vila. Flores and Semancik (1986) for viroid extraction. The last one is the method which is used in producing dsRNA based on solubility differentation of LiCl (lithium chloride) by Diaz-Ruiz and Kaper (1978).

dsRNA samples are analysed at 1.2 % agar gel medium and in TBE buffer (0.089 M tris-borate, 0.089 M boric-acid , 0.002 M EDTA) by electrophoretic method (Maniatis et al., 1982). The electrophoresis was carried on for 3-4 hours at horizontal slab gel with 100 volt AC current. The gels which were stained with ethidium bromide were examined under UV--trans-illumination and were also photographed. The Hind III was used as the molecular weight standart.

RESULT and DISCUSSION

All alfalfa fields an areas in Erzurum observed for this study were found to be infected to some extend with AMV and the disease rates were predicted to be varying 10.91 % - 10.20 %.

Quantitative evaluations depending on symptom may vary to a great extent because of the factors as being masked of the AMV effects on alfalfa and changing of the symptoms depending on the growth stage (Hull, 1969; Van Regenmortel and Pinck, 1981). Disease rates in this study have been based on symptomatologic observations.

As a result of mechanical inoculations of test plants. *Chenopodium amanticolor* and *Chenopodium quinoa* plants have exhibited local lesions (Table 1). These results are in agreement with pervious reseach conclusions of other authors (Bos and Jaspars. 1971; Van Regenmortel and Pinck, 1981; Jaspars and Bos, 1980). On the other hand the rest of the test plants *Nicotiana tabacum* Samsun "NN", "White Burley", "Xanthii", *Nicotiana glutinosa* have shown systemic infections (Table 2) as in the same parallel to the previous research results (Hull, 1969; Pinck and Hirth, 1972; Pietersen et al., 1985; Heijtink and Jaspars, 1974; Esbroeck and Hiruki, 1990).

Having obtained similar or parallel results with previous research work as a result of mechanical inoculation of the test plants with inoculum from leaves which have shown mosaic symptoms have dawn at us that those mosaic symptoms observed on alfalfa growing areas in Erzurum may possibly be AMV.

Viral dsRNA samples of *N.tabacum* Xanthii plant leaves which are infected with AMV disorder have been obtained by three different methods as already been mentioned previously. As shown by the electrophoretic analysis of dsRNA samples at 1.2 % agar gel medium and as also have been evidenced as by various authors (Bol and Lak Kaashoek, 1974; Parent and Asselin. 1985; Pietersen at al., 1985) being connected to AMV, four banded viral dsRNA profiles have been obtained (Figure 1).

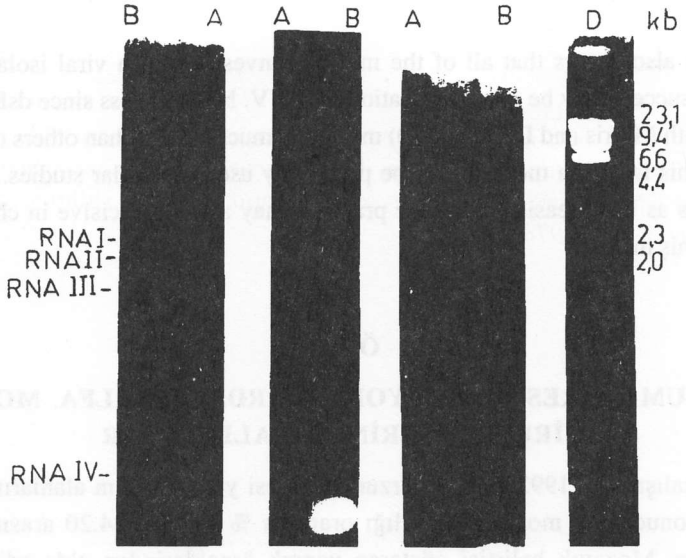

Figure 1. The electrophoretical of dsRNA obtained from *Nicotiana tabacum* Xanthii (A. healty, B. enfectious) by the methods of Morris and Dodds (1979) (I), Diaz-Ruiz and Kaper (1978) (II) and Duran-Vila et al. (1986) (III) d: λDNA-molecular weight standart.

Table 1. The symptoms of infected alfalfa samples on test plants

Test Plants	Stated Existing Time	Forming Symptoms
Chenopodium amaranticolor	5-7 days	NLL
Chenopodium quinoa	5-7 days	NLL
Datura stramonium	8-10 days	NLL-Deformation
Nicotiana tabacum	-	-
"Xanthii"	12-14 days	SE-Mo-NLL-CLL-Deformation
"Samsun NN"	12-14 days	SE-Mo-CLL-Deformation
"White Burley"	12-14 days	SE-Mo-CLL-Deformation
Nicotiana rustica	12-14 days	SE-Mo-NLL-Deformation
Nicotiana glutinosa	12-14 days	SE-Mo-CLL-Deformation
Nicotiana benthemiana	-	-
Gomphrena globosa	-	-
Phaseolus vulgaris	-	-
Vicia faba	-	-
Vigna sinensis	-	-

NLL: Necrotic Local Lesion
CLL: Chlorotic Local Lesion
Mo: Mosaic
Se: Systemic Infection

This also shows that all of the methods investigated in viral isolation in this study may successfully be used in isolation of AMV. Nevertheless since dsRNA profile obtained with Morris and Dodds (1979) method is much clearer than others or compared to others, this isolation method may be preferably used in similar studies. In addition such factors as being easier and more practical may also be decisive in choosing and preferring this method.

ÖZET

ERZURUM YÖRESİNDEKİ YONCALARDA ALFALFA MOZAYIK VİRUSU ÜZERİNDE ÇALIŞMALAR

Bu çalışmada, 1993 yılında, Erzurum yöresi yonca üretim alanlarında yapılan surveyler sonucunda, mozayık hastalığı oranının % 10.91 - 14.20 arasında olduğu saptanmıştır. Mozayık belirtisi gösteren yaprak örneklerinden elde edilen özsuyu mekaniksel inokulasyonla test bitkilerine taşınarak yonca mozayik virüsünün test konukçularında sergilediği belirtiler gözlenmiştir. Virusun tanısı, virusa ait RNA (Ribo Nükleik Asit)'nın replikatif formu olan dsRNA (çift iplikli RNA) izolasyonu ve analizi gerçekleştirilmiştir. Çalışmada, genel olarak virusların tanısında kullanılan üç ayrı dsRNA izolasyon yönteminin bu virus için uygulanabilirliği araştırılmıştır. Uygulanan üç izolasyon yöntemi ile elde edilen dsRNA'ların % 1.2'lik agar jelde elektroforetik ayrımı sonucu alfalfa mozayık virusu (AMV) ne özgü bantları içeren dsRNA profilleri elde edilmiştir. Uygulanan izolasyon yöntemlerinin her üçünün de AMV dsRNA izolasyonunda kullanılabileceği saptanmış ancak en belirgin dsRNA profili Morris ve Dodds'un önerdiği yöntemle elde edilmiştir.

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Accumulation of the Phytoalexin Medicarpin in Young and Old Leaves of Resistant and Susceptible Chickpea Cultivars to *Ascochyta rabiei* (Pass.) Labr.

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ABSTRACT

Accumulation of the phytoalexin medicarpin in young and old leaves of resistant (ILC 3279) and susceptible (ILC 1929) cultivars of chickpea to *Ascochyta rabiei* was determined by TLC and HPLC methods. Amount of medicarpin in young leaflets of both cultivars was more than old leaflets. There was a strong negative correlation between the leaf age and accumulation of medicarpin in resistant and susceptible cultivars (r= -0.98 and r= -0.97, respectively). There was also a high negative correlation (r= -0.94) between disease incidence values and accumulation of the phytoalexin.

INTRODUCTION

A number of plant species produce antimicrobial compounds, termed "phytoalexins" in response to infection by microorganisms or stress. Phytoalexins are assumed to play an important role in the plant defence response against fungal pathogens (Bailey and Mansfield, 1982; Darvill and Albersheim, 1984). The phytoalexins isolated from the leguminous plants, peas, beans and alfalfa have in common the pterocarpanoid ring structure (Perrin and Bottomley, 1962; Perrin, 1964; Smith et al., 1972). Pterocarpan phytoalexins medicarpin and maackiain have been identified in chickpea (*Cicer arietinum L.*) infected by some fungi (Kunzru and Sinha, 1970; Keen, 1975; Ingham, 1976; Denny and Van Etten, 1981; Köster et al., 1983; Weigand et al., 1986; Dolar and Gürcan, 1993).

Ascochyta rabiei (Pass.) Labr., a phytopathogenic deuteromycete parasite of chickpea is the causal agent of the serious blight of this important grain legume crop (Nene, 1982). The resistance of chickpea to *A. rabiei* is partly explained by the accelerated accumulation of the pterocarpan phytoalexins maackiain and medicarpin.

Large amounts of medicarpin and maackiain in leaves and stems of resistant cultivars to *A. rabiei* have accumulated whereas susceptible cultivars have produced small amounts of phytoalexins after fungus inoculation (Weigand et al., 1986; Keβmann and Barz, 1987; Dolar and Gürcan, 1993).

Leaf or plant age has a significant effect on disease reaction. Generally, young leaves are more resistant than older leaves but the reaction of resistance has been altered depending on plant species, cultivars, pathogens (Heath and Wood, 1969; Warren et al., 1971; Kim et al., 1989; Stewart, 1990; Dolar et al., 1994; Zheng and Sutton, 1994).

Consequently, the objectives of the present study were to determine the concentration of the phytoalexin medicarpin occuring in young and old leaflets of resistant and susceptible chickpea cultivars to *Ascochyta 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 for Agricultural Research in the Dry Areas (ICARDA) Aleppo, Syria. Seeds of each cultivar were surface sterilized with sodium hypochloride (1%) for 3 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 weer with a dilute solution of 20-20-20 (N-P-K). Plants were grown in growth chambers at 22±1°C with a relative humidity of 25-50 % and illuminated for 12 h per day with white fluorescent light (14 580 lux).

2. Fungal Material: The race 1 of *Ascochyta rabiei* used in this studied. Cultures of pathogen were maintained on Chickpea-Seed Meal Dextrose Agar (SCMDA: 40 g chickpea seed meal, 20 g dextrose, 20 g agar, 1 l distilled water) in 9 cm plastic petri dishes at 20±1°C and were illuminated for 12 h per day by 2 white fluorescent tubes (F20T12/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 two or three layers of cheesecloth in a funnel and adjusted to 1.2 x 10^5 spores/ml using a haemocytometer. Aerial parts of 30 day-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 four 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 (Dolar and Gürcan, 1993).

Samples of younger and older leaflet were taken 96 h after inoculation. Those from the most recent fully expanded leaves were designated as yound leaflets, whereas older leaflets were derived from the first or second oldest leaves.

4. Extraction and Isolation of Medicarpin: Collected leaflet samples from susceptible and resistant plants were immediately weighed and put in the absolute ethanol (Merck) and then stored at room temperature (22°C) in the dark overnight. Each replicate consisted of 0.5 g fr. weight of tissue in 8 ml of absolute ethanol. This ethanol was decanted and the leaflets were soaked in a second 8 ml aliquot of absolute ethanol overnight. Soaking procedure was replicated three times and the three ethanol fractions were combined and all of the ethanol was filtered through Whatman-1filter paper. The ethanol solution evaporated to dryness in vacuo at 40°C and the residue was dissolved in small volume (1.5 ml) of absolute ethanol. This ethanol evoporated in vacuo on a evopomix at 40°C and the residue was dissolved in 100 µl of absolute ethanol for chromatography (Verna J. Higgins, *unpublished data*).

5. Determination of Medicarpin on Thin Layer Chromatography: The extracts and standart medicarpin 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 (Mc Murchy and Higgins, 1984).

6. 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 4 mm x 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 and quantitation (Hewlett-Packard integrator) was achieved by external standardisation.

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

7. Disease Assessment: Disease incidence was determined as the percentage of leaflets infected 96 h after inoculation. All calculations were performed using the MSTAT statistic programme. Significance was determined at $p<0.05$, using Duncan's Multiple Range Test.

RESULTS

1. Disease Incidence of Leaflets of Different Ages: No lesions appeared on younger leaflets of resistant cultivars (ILC 3279) although lesions were observed less frequently (7.5 %) on older leaflets of the same cultivar 96 h after inoculation (Table 1). Both of the younger and older leaflets of resistant cultivar exhibited a pronounced number of hypersensitive spotst. Disease incidence values on younger and older leaflets of susceptible cultivar (ILC 1929) was found 35 % and 50 %, respectively (Table 1).

Table 1. Disease incidence values on young and old leaflets of resistant and susceptible chickpea cultivars 96 h after inoculation with *Ascochyta rabiei*

Cultivar Name	Leaflet Age	Disease Incidence* (% of leaflets infected)	
ILC 3279	Young	d	0.0
(Resistant)	Old	c	7.5
ILC 1929	Young	b	35.0
(Susceptible)	Old	a	50.0

* Calculation based on 200 leaflets. Values within one column followed by different letters are significantly different at p=0.05 (Duncan's Multiple Range Test)

2. Amount of Medicarpin in Diseased Leaves: TLC method was used to determine medicarpin in samples because of practical method for determination of phytoalexins. Extracts, including medicarpin gave the characteristic bright yellow-orange area and the Rf value corresponding to the standart when the diazotised p-nitroanilin sprayed on TLC of the samples.

Quantitative measurements of medicarpin were made by HPLC. Results of HPLC analysis obtained from younger and older leaflets of resistant and susceptible cultivars are shown in Table 2. The extracts of the leaflets of the susceptible cultivar (ILC 1929) contained smaller amonts of medicarpin than the resistant cultivar (ILC 3279). Younger leaflets of both cultivars were produced larger amonts of medicarpin than older leaflets. Leaves of control plants contained trace amonts of medicarpin.

The results of this study showed that a high negative correlation (r= -0.94) existed between disease incidence values and accumulation of phytoalexin. As the production of medicarpin increased, disease incidence values decreased. Likewise, the other strong negative correlations were found between leaf age and amount of medicarpin in resistant and susceptible cultivars (r=-0.98 and r= -0.97, respectively).

Table 2. Amount of medicarpin in young and old leaflets of resistant and susceptible chickpea cultivars 96 h after inoculation with *Ascochyta rabiei*

Cultivar Name	Leaflet Age	Amount of Medicarpin* (µg/g dry weight)	
ILC 3279	Young	a	142.2
(Resistant)	Old	b	105.8
ILC 1929	Young	c	76.4
(Susceptible)	Old	d	66.0

* Values are means of four replicates. Values within one column followed by different letters are significantly diferent at p=0.05 (Duncan's Multiple Range Test).

DISCUSSION

Four days after inoculation with race 1 of *Ascochyta rabiei,* normal lesions were produced on the young and old leaflets of susceptible (ILC 1929) cultivar. The lesions expanded more rapidly in the older leaflets as compared to younger leaflets. Young leaflets of resistant cultivar showed hypersensitivity or lack of symptoms. The old leaflets of the same cultivar exhibited hypersensitive spots and less frequently normal lesions. Some workers reported that leaf or plant age has a significant effect on disease reaction and susceptibility has altered depending on age. These changes in susceptibility of leaf or plant may be a result of physiological and morphological difference (Heath and Wood, 1969; Warren et al., 1971; Kim et al., 1989; Stewart, 1990; Zheng and Sutton, 1994).

Phytoalexins play a significant role in the defense mechanisms of higher plant toward phytopathogenic fungi (Bailey and Mansfield, 1982; Darvill and Albersheim, 1984). Generally, accumulation of phytoalexins in the resistant plants is much higher than the susceptible plants (Duczek and Higgins, 1976; Ebel, 1986; Weigand et al., 1986; Dolar and Gürcan, 1993). This is also the case in chickpea (Weigand et al., 1986; Keβmann and Barz, 1987; Dolar and Gürcan, 1993). In this study, a strong negative correlation was determined between leaf age and accumulation of medicarpin. The amount of medicarpin changed depending on the leaf age in resistant and susceptible chickpea cultivars. In addition, the existence of a negative correlation between the disease incidence values and the amount of medicarpin was demonstrated.

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ÖZET

Ascochyta rabiei (Pass.) Labr. 'A DAYANIKLI VE DUYARLI NOHUT ÇEŞİTLERİNİN GENÇ VE YAŞLI YAPRAKLARINDA MEDİCARPİN OLUŞUMU

Ascochyta rabiei (Pass.) Labr.'a dayanıklı (ILC 3279) ve duyarlı (ILC 1929) nohut çeşitlerinin genç ve yaşlı yapraklarında oluşan medicarpin miktarı TLC ve HPLC metodları ile belirlenmiştir. Her iki çeşidin genç yapraklarında phytoalexin miktarı yaşlı yapraklardakinden daha yüksek bulunmuştur. Dayanıklı ve duyarlı çeşitlerde medicarpin miktarı ile yaprak yaşı arasında negatif korelasyon (r= - 0.98 ve r= - 0.97 sırasıyla) bulunmasının yanısıra yapraklarda oluşan phytoalexin miktarı ile enfeksiyon oranı arasında da negatif bir korelasyon (r= -0.94) tespit edilmiştir.

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Total, Extractable and Bound ^{14}C-Trifluralin Residues in the Soil and Melon

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ABSTRACT

^{14}C-trifluralin residues in melon grown sandy-loam solis remained mainly in soil. Maximum ^{14}C-residues were found in 0-7.5 cm soil depths. Downward movement of the trifluralin or its metabolites was not observed at significant concentrations. Four months after the aplication, 5.2% and 8.9% of the intially applied radioactivity were found in the form of metabolites designated as TR-1 and TR-2, respectively, in the combined two depths.

In the analysis carried out after the harvest, maximum residues were found in the roots. They were 1.072 and 0.901 pm for the hairy roots and tap roots, respectively. The cortex of crown and stele of crown contained 0.489 and 0.239 ppm, respectively. Residues in the leaves and stem decreased towards the apex and they were 0.137 and 0.149 ppm in the lower leaves and lower stem, respectively. Fruits contained 0.008, 0.003 and 0.017 ppm ^{14}C-residues in the skin, flish and seeds of the fruit, respectively. ^{14}C-residues in root, stem and leaves mostly consisted of TR-1 and TR-2, but trace amount of TR-3, TR-4 and TR-21.

INTRODUCTION

Trifluralin (μ, μ, μ-trifluoro-2.6-dinitro-*N, N*-dipropyl-*p*-toluidine). is a selective preemergence, soil incorporated herbicide which controls of a wide variety of grass and broadleaf weeds in many agronomic and horticultural crops, including members of Cucarbitaceae. The residue of trifluralin has been investigated in soil and plants (Probst et al. 1967; Helling, 1976; Duseja and Holmes 1978; Golab et al., 1979). When root crops such as carrots onions and turnips were grown in soil containing trifluralin, trifluralin residues were found on the surface (Golab et al., 1967; Probst et al., 1967). Previous studies have demonstrated that most of the residue was found in the peel part of carrot (Tiryaki et al., 1996 and 1997).

In Turkey, the use of trifluralin in the cultivation of vegetables is 12367 kg a.i. per year (Amonymous, 1996). The tolerance limit for he trifluralin in Cucurbitaceae has been reported to be 0.05 ppm (Sitting, 1980).

Earlier studies showed that trifluralin markedly increased their resistance to vascular wilts caused by *Fusarium, Verticillium* species as well as *Rhizoctonia* (Grinstein et al., 1984; Cohen et al., 1986). *Fusarium* wilt is most important disease in the melon plantation in Turkey (Karahan et al., 1981; Yıldız, 1977).

Effects of trifluralin concentration at 0.5, 1 and 2 ppm levels on germination and emergence of some melon (*Cucumis melo* L.) varieties have also been investigated. In general an increase in trifluralin doses has caused a decrease of germination and emergence. This decrease has especialy found significant between the non-treated and 2 ppm-treatment level (Tiryaki and Maden, 1991).

The purpose of the current study was to investigate the persistence of ^{14}C-trifluralin in a soil under outdoor conditions. The uptake of the herbicide from the treated soil by melon were also studied. A number of metabolite of trifluralin in the soil and melon were identified and the amount of the bound residues was determined.

MATERIALS and METHODS

Chemicals

Trifluralin-Ring-UL-^{14}C, 100 μCi was supplied by Dow Elanco (Environmental Chemistry Lab., Greenfield, Indian USA) and Sigma Chemical Co. (via Turkish Atomic Energy Agency, Ankara Turkey). The radiochemical purity of the trifluralin was 98%. The reference standard of trifluralin (purity 99.5%) was a gift from Dow Elanco. The metabolites μ,μ,μ-trifluoro-2,6-dinitro *N*-propyl-p-toluidine (TR-2); μ,μ,μ-trifluoro-2.6-dinitro-p-toluidine (TR-3); μ,μ,μ-trifluoro-N^4, N^4 dipropyltoluene-3.4-triamine (TR-7); and 4-(dipropylamino)-3.5-dinitrobenzoic acid (TR-21) were supplied by Biosciences Research Lab, U.S. Dept of Agric, ARS, Fargo, USA.

Field experiment

Melon (Yuva variety) were grown in boxes at the Turkish Atomic Energy Authority, Ankara Nuclear Research and Training Centre's Experimental Farm, Ankara, Turkey. The experiments were carried out under the outdoor conditions in boxes measuring 60x60x60 cm, constructed from galvanized-steel. The base of the boxes contained holes to permit the drainage of excess water (Kohli et al. 1973). The inside of

the boxes (pots) was covered with polyethylene sheets. The bottom 25 mm of the boxes was packed with stone chips of about 25 mm diameter, and the stones were covered wih a 25 mm layer of well rotted turf. The boxes were sunk into pits such that the upper surface of the soil in boxes was at the level of the surrounding ground. Boxes were filled with soil-manure mixture, and ^{14}C-trifluralin was aplied at the recommended rates of 0.84 kg a.i./ha. The herbicide was incorporated into the top 7.5cm of the soil. After the trifluralin aplication, melon seeds were sown. Air temperature and rainfall were recorded during the vegetation period. A summary of climatic conditions, the analyses of soil, and further cultural details are included in Table 1.

Table 1. Cultural details for melon grown with ^{14}C-trifluralin treatment

Growth parameter	Details for site indicated	
	1991	1992
Application rate of ^{14}C-trifluralin	0.84 kg a.i./ha	0.84 kg a.i./ha
Depth of incorporation	7.5 cm	7.5 cm
Specific activity of ^{14}C-trifluralin	0.401 mCi/mg	1.141 µCi/mg
Variety	Yuva	Yuva
Date of sowing	May 14, 1991	June 5, 1992
Depth of sowing	3-4 cm	3-4 cm
Fertilizer (manure)	12.6 kg/pot	12.6 kg/pot
Soil		
Texture	Sandy loam	Sandy loam
Organic matter	1.87 %	1.87 %
Sand	46.70 %	46.70 %
Silt	17.95 %	17.95 %
Clay	35.35 %	35.35 %
pH	8.1 %	8.1 %
Watering	1-2 times a week in dry weather	2 times a week in dry weather
Total rainfall during crop growth, mm	110.7 (max.16.0; min 0.0)	69.3 (max. 15.3; min. 0.0)
Range of mean maximum daily temperature, °C	11-32	12-34
Date of harvest	October 23, 1991	October 2, 1992

Soil and crop sampling

Zero time soil samples were removed from the two pots immediately after application of ^{14}C-trifluralin and periodically thereafter at 1 monthly interval until harvest. Samples were collected at depths of 0-7.5 cm, and 7.5-15 cm. After the harvest the fruits were sampled as a skin, flesh and seed. The cortex and stele of crown tissues were also sampled. Root samples were separated as hairy root and tap root. Remaining root part was divided into two parts as the upper half and the lower half of the tap root. Leaves and stems were also sampled. Each branch was divided into three equal parts, then each part was separated into leave and stem. Leaves and stems in each part gathered from all branches were then analyzed separately.

All samples were analyzed or processed in duplicate. There were two pots each year indicating 2 replications. Therefore overall means for two years are reported throughout this paper. The residue level in soil and plant samples were explained based on oven dry weight and fresh weight, respectively.

Extraction

Harvesting time soil and plant samples were extracted with methanol using Soxhlet extraction apparatus which is described by L'annunziata (1979). The amount of samples which placed in paper thimble were about 40-50 g and 5-25 g soil and plant samples, respectively. The extracts then concentrated to a small volume using Rotary evaporator. The extracts were radioassayed to determine extractable ^{14}C-residues by using 1550 Tri Carb Liquid Scintillation Analyzer.

Determination of radioactivity

Soil and/or melon samples were combusted in a Harvey Biological Oxidizer, OX-600, to produce $^{14}CO_2$. Combustion products then counted to determine total and/or bound ^{14}C-residues.

Chromatography and analysis

Thin Layer Chromatography (TLC): TLC was performed with 20x20 cm and 5x20cm precoated silica gel F_{254} (Merck) chromatoplates with 0.25 mm gel thickness. The five reference standards were then spotted on the plates and then developed in a solvent system hexane: benzene (35:65). The developed plates were observed under UV light (λ=254 nm). Under these experimental conditions the following R_f values

were obtained: TR-1 R_f 0.88; TR-2 R_f 0.76; TR-3 R_f 0.42; Tr-7 Rf 0.24; and TR-21 R_f 0.10 (in benzene-ethylacetate, 35:65, solvent system). The extracts were then applied on plates at about 1 cm below the preabsorbent line and developed in hexane:benzene (35:65) and benzene-ethylacetate (35:65) solvent systems in containers which were lined with paper and saturated prior to use. The material in these spots was scrapped off the plate to scintillation vial and added Insta-Gel scinttilation cocktail for counting.

Column Chromatography (CC): Column chromatography was carried out with a glass column (10x1.5 cm) containing 4 g of florisil. Column was conditioned with 5 ml methanol by passing through column. Extracts and/or reference standard (1 mL) was applied to the column and eluted with hexane: benzene (2:1). The eluates were evaporated under air to just dryness, redissolved in 1 mL methanol and assayed in a 1550 Tri-Carb Liquid Scintillation Analyzer to determine extractable ^{14}C-residues.

Gas Chromatography (GC): The gas chromatography was a Varian Model 3700 equipped with ECD detector and glas column coated with OV-17. Column was operated at 150°C. The nitrogen carrier flow rate, injector port temperature and detector temperature were 50 psig, 250°C and 260°C, respectively. Under these experimental conditions the retention time for TR-1, and TR-2 were 3.2, and 3.6 min, respectively.

A schematic diagram for the analysis of ^{14}C-trifluralin and its metabolites residues in soil and melon samples in shown in Figure 1.

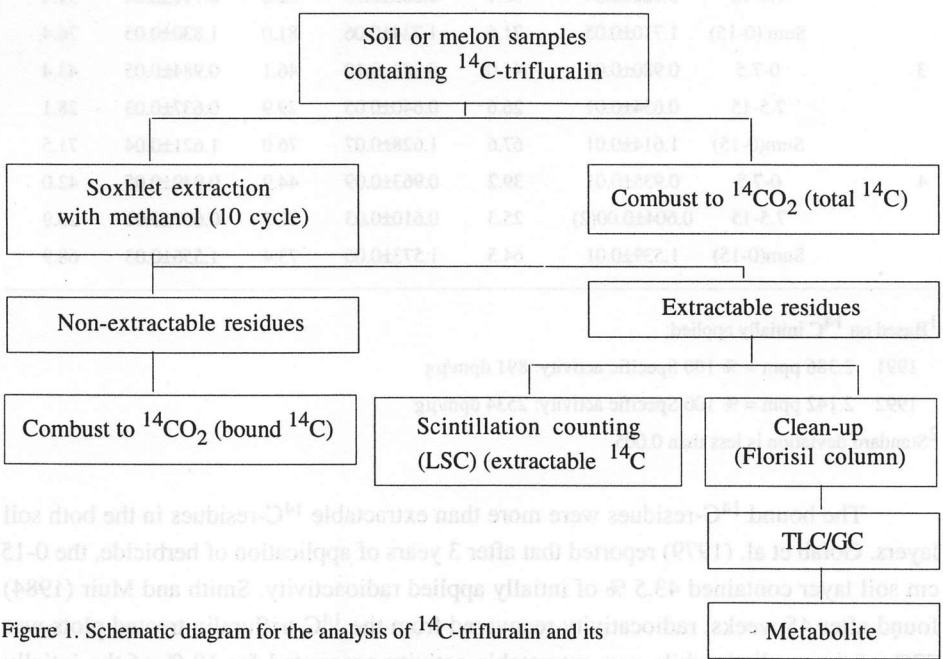

Figure 1. Schematic diagram for the analysis of ^{14}C-trifluralin and its metabolites residues in soil and melon samples.

RESULT and DISCUSSION

^{14}C-trifluralin in the soil

Soil combustion analysis of the two layer of soil treated with ^{14}C-trifluralin is shown in Table 2 for both 1991 and 1992 experiment. The data in Table 3 shows that after four months of application, the combined two layers of soil (0-15 cm) contained 1.556 ppm, 0.559 ppm and 0.817 ppm as total ^{14}C, extractable ^{14}C, and bound ^{14}C-residues, respectively.

Table 2. Total ^{14}C-residues in the soil over the growing period of melon[1]

Time of sampling (months)	Depth of soil (cm)	1991		1992		Average	
		ppm	%	ppm	%	ppm	%
Zero time	0-7.5	2.386±0.30	100.0	2.142±0.24	100.0	2.264±0.30	100.0
1	0-7.5	1.170±0.05	49.0	1.076±0.10	50.2	1.123±0.07	49.6
	7.5-15	0.740±0.03	31.0	0.740±0.06	34.5	0.740±0.04	32.7
	Sum (0-15)	1.910±0.04	80.0	1.816±0.08	84.7	1.863±0.05	82.3
2	0-7.5	0.990±0.05	41.5	1.049±0.08	49.0	1.019±0.06	45.0
	7.5-15	0.720±0.04	30.1	0.685±0.05	32.0	0.711±0.04	31.4
	Sum (0-15)	1.710±0.05	71.6	1.734±0.06	81.0	1.830±0.05	76.4
3	0-7.5	0.980±0.01	41.0	0.988±0.10	46.1	0.984±0.05	43.4
	7.5-15	0.634±0.01	26.6	0.640±0.05	29.9	0.637±0.03	28.1
	Sum(0-15)	1.614±0.01	67.6	1.628±0.07	76.0	1.621±0.04	71.5
4	0-7.5	0.935±0.01	39.2	0.963±0.09	44.9	0.949±0.05	42.0
	7.5-15	0.604±0.00(2)	25.3	0.610±0.03	28.5	0.607±0.01	26.9
	Sum(0-15)	1.539±0.01	64.5	1.573±0.06	73.4	1.556±0.03	68.9

[1]Based on ^{14}C initially applied;

1991 2.386 ppm = % 100 Specific activity: 891 dpm/μg

1992 2.142 ppm = % 100 Specific activity: 2534 dpm/μg

[2]Standard deviation is less than 0.005

The bound ^{14}C-residues were more than extractable ^{14}C-residues in the both soil layers. Golab et al. (1979) reported that after 3 years of application of herbicide, the 0-15 cm soil layer contained 43.5 % of intially applied radioactivity. Smith and Muir (1984) found after 45 weeks, radiocativity recovered from the ^{14}C-trifluralin treated plots was 77% of the applied, while non-extractable activity accounted for 10 % of the intially

applied. The persistence and movement of ^{14}C-trifluralin residues in soil changed with the time. After one month about 1.863 ppm of the intially applied radioactivity was still present in the sum of the two depths of the soil, while four months later only about 1.556 ppm of applied radioactivity was present (Table 2). Duseja and Holmes (1978) found that after 25 and 120 days application (0.75 lb/acre application rate), 48.3 % and 25.9 % of applied radioactiviy, respectively, was present in the sum of the two depths.

Table 3. ^{14}C-residues in the soil and melon plant samples at the harvesting time(1)

Sampling/(2)	Total residue ppm	Extractable residue ppm	Bound residue ppm
SOIL			
0-7.5cm	0.949±0.05	0.327±0.07	0.512±0.07
7.5-15 cm	0.607±0.01	0.232±0.03	0.305±0.03
Sum (0-15 cm)	1.556±0.03	0.559±0.05	0.817±0.05
MELON PLANT			
Hairy root	1.072±0.15	0.609±0.07	0.500±0.06
Tap root	0.901±0.10	0.348±0.05	0.408±0.08
Cortex of tap root-lower half	0.444±0.04	0.178±0.02	0.265±0.04
Stele of tap root-lower half	0.186±0.03	0.074±0.01	0.118±0.07
Cortex of tap root-upper half	0.632±0.07	0.213±0.09	0.353±0.06
Stele of tap root-upper half	0.174±0.04	0.057±0.00(3)	0.108±0.01
Cortex of crown	0.489±0.08	0.127±0.01	0.279±0.04
Stele of crown	0.239±0.05	0.076±0.01	0.148±0.02
Lower stems	0.149±0.01	0.059±0.00	0.067±0.01
Median stmes	0.049±0.02	0.038±0.01	0.013±0.01
Upper stems	0.043±0.01	0.030±0.01	0.011±0.01
Lower leaves	0.137±0.03	0.062±0.01	0.066±0.01
Median leaves	0.064±0.01	0.026±0.00	0.032±0.01
Upper leaves	0.048±0.01	0.024±0.01	0.023±0.03
FRUIT			
Skin	0.008±0.00	0.005±0.00	*(4)
Flesh	0.003±0.00	*	*
Seed	0.017±0.01	0.006±0.00	0.009±0.00

[1]Averages of two years
[2]For details see "Soil and Crop Sampling"
[3]Standard deviation is lees than 0.005
[4]Not detected

TOTAL, EXTRACTABLE AND BOUND ^{14}C-Trifluralin RESIDUES in the SOIL and MELON

Table 4. Identified extractable ^{14}C-residues in the soil and melon samples at the harvesting time[1]

Sampling[2]	Extractable ^{14}C-residues ppm	TR-1 (trifluralin) ppm	TR-2 ppm	TR-3 ppm	TR-7 ppm	TR-21 ppm	TOTAL ppm
SOIL							
0-7.5 cm	0.327±0.07	0.059±0.01	0.126±0.02	0.005±0.00[3]	*[4]	*	0.190±0.03
7.5-15 cm	0.232±0.03	0.060±0.01	0.074±0.01	0.004±0.00	*	0.004±0.00	0.142±0.02
Sum (0-15 cm)	0.559±0.05	0.119±0.01	0.200±0.01	0.009±0.00	*	0.004±0.00	0.332±0.02
MELON PLANT							
Hairy root	0.609±0.07	0.250±0.02	0.104±0.01	0.004±0.00	0.003±0.00	*	0.360±0.03
Tap root	0.348±0.05	0.134±0.01	0.058±0.01	0.003±0.00	0.004±0.00	*	0.199±0.01
Cortex of tap root-lower half	0.178±0.02	0.071±0.00	0.033±0.00	*	0.003±0.00	0.004±0.00	0.110±0.00
Stele of tap root-lower half	0.074±0.01	0.034±0.00	0.005±0.00	*	*	*	0.040±0.01
Cortex of tap root-upper half	0.213±0.09	0.086±0.01	0.037±0.00	0.005±0.00	*	*	0.128±0.02
Stele of tap root-upper half	0.057±0.004	0.010±0.00	0.005±0.00	*	*	*	0.015±0.01
Cortex of crown	0.127±0.01	0.021±0.00	0.008±0.00	*	*	*	0.029±0.00
Stele of crown	0.076±0.01	0.012±0.00	0.006±0.00	*	*	*	0.018±0.02
Lower stems	0.059±0.00	0.010±0.00	0.006±0.00	*	*	*	0.016±0.01
Median stems	0.038±0.01	*	*	*	*	*	*
Upper stems	0.030±0.01	*	*	*	*	*	*
Lower leaves	0.062±0.01	0.011±0.00	0.005±0.00	*	*	*	0.016±0.02
Median leaves	0.026±0.00	*	*	*	*	*	*
Upper leaves	0.024±0.01	*	*	*	*	*	*
FRUIT							
Skin	0.005±0.00	*	*	*	*	*	*
Flesh	0.002±0.00	*	*	*	*	*	*
Seed	0.006±0.00	*	*	*	*	*	*

[1]Averages of two years

[2]For details see "Soil and Crop Sampling"

[3]Standart deviation is less than 0.005

[4]Not detected

Movement of ^{14}C-trifluralin residues in soil

Soil samples, collected at the two depths from the post treated at the rate of 0.840 kg a.i./ha with ^{14}C-trifluralin by incorporation in the uper 7.5 cm soil layer, were analyzed for the presence of the trifluralin. The data indicate a small amount leaching of the herbicide (Table 2). Most of the applied herbicide was located in the incorporation depth. The 0.949-1.123 ppm and 0.607-0.740 ppm remained in the 0-7.5 cm and 7.5-15 cm soil layers, respectively (Table 2). Golab et al. (1979) observed that, at the rate of 1.68 kg a.i/ha application, after 12 and 24 month 91 % and 98.8 % of the radioactivity was present in the 0-15 cm zone and 76 % and 95 % of the activity in the zone of incorporation.

Total, extractable and bound ^{14}C-residues in the soil and melon

The total, extractable and bound ^{14}C residues in the soil and melon samples are given in Table 3. Four month later, soil and most of the melon plant samples contained more bound ^{14}C-residues than extractable ^{14}C-residues.

Most of the residue was found in the root part of melon. The data in Table 3 show that the hairy roots and tap roots contained 1.072 ppm and, 0.901 ppm respectively. Similar results have been reported by Probst et al. (1967), Golab et al. (1967 and 1979). They explained that root crops grown in soil treated with trifluralin, had shown no contain herbicide residues on the surface.

As it shown in Table 3, the residue levels of the skin, flesh and seed of melon fruit were less than the maximum residue level set up by EPA (Environmental Protection Agency) as a 0.05 ppm for Cucurbitaceae (Sitting, 1980). On the other hand, the cortex of crown and stele of crown contained 0.489 ppm and 0.239 ppm, respectively. These results confirm Grinstein et al. (1984)'s findings that trifluralin decreases the attack of soil-born fungal pathogens.

Identification of extractable ^{14}C-residues

Examination of soil extracts by TLC indicated the presence of TR-1 (trifluralin) and TR-2 (Table 4). Four month later, TR-2 concentrations were more than TR-1 concentrations. These results are parallel with Golab et al. (1979)'s findings. The results in Table 4 indicate the presence of trace amount of the metabolites TR-3, TR-7, and TR-21. Similar findings have been explained by Golab et al. (1967) and Probst et al. (1967).

As to plant samples, hairy root and tap root contained 0.250 ppm and 0.134 ppm respectively, as TR-1, and 0.104 ppm and 0.058 ppm TR-2. The 0.021 and 0.008 ppm were located in the cortex of crown of crown as TR-1 and TR-2, respectively, and 0.012 and 0.006 ppm in the stele of crown (Table 4). Small amount of TR-3, TR-7 and TR-21 were also found in the plant samples (Table 4).

ÖZET

TOPRAK VE KAVUN BİTKİSİNDE TOPLAM, EKSTRAKTE EDİLEBİLİR VE BAĞLI ^{14}C-TRİFLURALİN KALINTILARI

Kavun yetiştirilen kumlu-killi topraklarda ^{14}C-trifluralin kalıntısının çoğu 0-7.5 cm toprak katmanında bulunmuştur. Trifluralinin veya matebolitlerinin toprağın derinliklerine doğru hareketi fazla olmamıştır. Dört ay sonra her iki toprak derinliğinde başlangıçta uygulanan ^{14}C-trifluralinin % 5.2 si TR-1, % 8.9'u TR-2 olarak bulunmuştur.

Hasattan sonra yapılan analizlerde en fazla kalıntıya bitkinin köklerinde rastlanmıştır. Kılcal köklerde 1.072 ppm, kalın köklerde 0.901 ppm; kök boğazının kabuk kısmında 0.489 ppm, iç kısmında ise 0.239 ppm kalıntı bulunmuştur.

Bitkideki kalıntılar uç kısımlara doğru azalmıştır. Dip kısım yapraklarda 0.137 ppm, dip kısım saplarda ise 0.149 ppm kalıntı saptanmıştır. Kavun meyvesinde ise; kabukta 0.008 ppm, yenilebilir kısımda 0.003 ppm ve çekirdekte 0.017 ppm kalıntı tesbit edilmiştir Kök, yaprak ve sap ekstraktlarında esas olarak TR-1 ve TR-2 bulunmuştur. Ancak eser miktarlarda TR-3, TR-4v eTR-21 kalıntılarına da rastalnmıştır.

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NOTICE TO CONTRIBUTORS

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