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Studies on Relation Between Host and Pathogen of Sunflower Downy Mildew (**Plasmopara helianthi** Novot.)

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ABSTRACT

In the study, 72 cultivars and lines of sunflowers were tested for reaction to downy mildew. 17 cultivars out of 72 were in the resistant group.

*Fungal development in sunflower seedlings resistant and susceptible (Vniimk 8931) to **P. helianthi** was compared. There were mycelia and oospores in the sections of the root and hypocotyl of all resistant cultivars, but no fungal structures in the sections of the epicotyl.*

Phytoalexins were examined in resistant and susceptible seedlings to find whether they played a role in downy mildew resistance. It was understood that they were not playing any role in resistance.

INTRODUCTION

Downy mildew (**Plasmopara helianthi** Novot.) is the most important disease limiting sunflower production. The fact that pathogen is a soil and seed borne increase its importance (Cohn and Sackston, 1973).

Resistant hybrids could be obtained as a result of breeding studies because of the fact that no chemical control could be found satisfactory to this disease until the 1980 s (Zimmer and Kinman, 1972; Vear, 1974). Therefore, it is recommended to grow the resistant cultivars in order to prevent disease as cultural practise.

In the recent years, a lot of sunflower hybrids have introduced to Turkey as in other crops. So, in this study 72 cultivars and lines of sunflowers were tested for reaction to **P. helianthi** under controlled conditions.

In such studies, the plants with sporulation on the cotyledons after artificial inoculation were evaluated until 1981 (Zimmer, 1972). But the following studies clarified the suspicious sides of this evaluation (Sackston et al., 1976; Viranyi, 1978). For this reason Viranyi and Bartha's evaluation method (1981) which is based on various disease symptoms was used in this study. On the other hand to understand the reactions of the resistant cultivars, fungal development in sunflower seedlings resistant and susceptible to **P. helianthi** was examined, and to find whether phytoalexins play a role in downy mildew resistance, they were also examined in the resistant and susceptible seedlings.

MATERIALS and METHODS

The isolate having the highest virulence out of 13 *P. helianthi* isolates from Thrace, Aegean and Black Sea regions was used. The seeds belonging to 72 cultivars tested were obtained from Agricultural Research Institutes in Mene-men (Izmir) and Edime.

Experiments were carried out in plastic pots (11 cm diameter) and sterilized soil was used. Pots were placed on benches illuminated with 40 w fluorescent tubes. Light intensity was about 13.000 lux. Day length was 14 h and temperature was 20 ± 2 °C.

Inoculation method of Cohen and Sackston (1973) was used. Seeds were surface-sterilized for 5 min in 1% sodium hypochlorite, washed three times in tap water and germinated on moist filter paper at the room temperature. The third day, germinated seeds were immersed in a suspension of 100.000 sporangia/ml in distilled water, at 18 °C for 6 h, and then they were planted. Five pots were used for each cultivar, and ten germinated seeds were planted per pot. Ten day old infected seedlings were covered with plastic bags at 18 °C for about 20 h for sporulation profusely on cotyledons.

At cotyledonary and four-leaf stages, the number of plants with damping-off, sporulation on their leaves, leaf-chlorosis and hypocotyl lesion was recorded in each pot.

The behaviour of cultivars and lines of sunflowers was grouped according to Viranyi and Bartha (1981) (Table 1).

Table 1. Classification of sunflowers for resistance to *Plasmopara helianthi*

Group	Response to downy mildew	Disease symptoms	Extent of fungal invasion
I	Susceptible	damping off, sporulation on hypocotyl and true leaves leaf chlorosis sporulation on epicotyl pieces	entire plant
II	Moderately resistant	sporulation on hypocotyls and cotyledons	roots, hypocotyl and cotyle dons
III	Resistant	lesions and / or sporulation on hypocotyls	roots and hypocotyl
IV	Highly resistant	no symptoms	no fungal invasion

Manner of sporulation cotyledons of susceptible (Vniimk 8931) and resistant cultivars was recorded as profuse, weak or nil. Furthermore, plants were removed from pots and washed completely in tap water. Root, hypocotyl and epicotyl pieces of them were fixed in formal-acetic-alcohol (FAA) mixture for 48 h for histological work. They were shaken in 70 % alcohol. Free had sections from fixed material were stained with 0,05 percent cotton blue in lactophenol, and they were examined under light microscope.

Effect of root extracts of susceptible and resistant cultivars on germination of zoosporangia and disease ratio was determined as follows: 1 g of very small roots was put into small bottles and then placed in a deepfreeze 8,16,30 and 48 h after inoculation. Sørensen PO₄ buffer was prepared. Small roots were crushed in buffer at the ratio of 1:10 (w/w). Homogenat was filtered, and then kept in a refrigerator for 30 min.

Homogenat was poured as a thin film on water agar. Conidia of *Botrytis cinerea* were shaken on this film. Germinated and ungerminated conidia were counted under microscope 24 h later.

A suspension of 100.000 sporangia/ml in homogenat was prepared. Susceptible sunflower seeds (Vniimk 8931) were inoculated with this suspension. Disease ratio of plants was determined.

Effect of homogenat on germination of zoosporangia was found by determining the number of germinated and ungerminated conidia under microscope after zoosporangia were kept in homogenat for 8 h. Percentage of germination was calculated by mean of three counts.

RESULTS

Reactions of sunflower cultivars and lines to downy mildew (*P. helianthi*) were given in Table 2.

Table 2. Distribution of sunflower cultivars and lines for the degree of downy mildew resistance (in 50 plants)

Cultivar and line	% of plants			
	Susceptible	Moderately resistant	Resistant	Highly resistant
A-1004	80	16	4	0
Argentario	84	18	2	0
As-504	78	18	4	0
As-532	72	20	8	0

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Cultivar and line	% of plants			
	Susceptible	Moderately resistant	Resistant	Highly resistant
As-1004	74	20	6	0
Bolero	88	12	0	0
Challenger	80	12	8	0
Columbia	82	10	8	0
Discovery	90	10	0	0
Elia	0	0	100	0
Exp-448	84	14	2	0
Exp-449	80	16	4	0
Exp-445	82	13	0	0
Exp-468	78	18	4	0
Exp-1948	86	12	2	0
Exp-5526	82	16	2	0
Felix	86	8	6	0
Flame	84	12	4	0
Floram-305	0	2	98	0
Floram-328	0	40	60	0
Hemus	88	10	2	0
Hyb.219/79/Gi	0	26	74	0
Hyb.13/80	0	4	96	0
HS-20 B ₂ K ₄ S ₈	86	14	0	0
HS-20 B ₂ K ₃ S ₁₀	82	16	2	0
IH-10	76	18	6	0
Iregi-816 B	84	14	2	0
Mirasol	0	12	88	0
Novisad-20	80	16	4	0
Novisad-61	76	14	10	0
NS-H-15	14	0	86	0
NS-H-17	28	0	72	0
NS-H-27	8	0	92	0

Cultivar and line	% of plants			
	Susceptible	Moderately resistant	Resistant	Highly resistant
Pemir	78	16	6	0
Peredovik	86	10	4	0
Primasol	0	8	92	0
Record-1-1	84	14	2	0
Ro-25	74	16	10	0
Record	86	8	6	0
Ro-85	84	14	2	0
Rodeo	0	0	100	0
Romsun-53	0	86	14	0
Romsun-59	0	24	76	0
Romsun-90	0	28	72	0
Select	78	16	6	0
Semu 219/79	84	14	2	0
Semu 13/80	88	12	0	0
Sorem-80	80	16	4	0
Sunbred-212	82	14	4	0
Sunbred-246	76	16	8	0
Sunbred-262	84	10	6	0
Sunbred-280	72	22	6	0
Sunbred-285	80	14	6	0
Sunbred-2012	84	8	8	0
Seedstel-S-315	74	18	8	0
Super	82	12	6	0
Start	0	4	96	0
Topflor	86	12	2	0
Türk Ay 1	70	24	6	0
TR-52-TE	84	16	0	0
TR-26-TE	72	16	12	0
TR-7-TE	82	16	2	0

SUNFLOWER DOWNY MILDEW

Cultivar and line	% of plants			
	Susceptible	Moderately resistant	Resistant	Highly resistant
TR-15-TE	70	18	12	0
Upsol	74	18	8	0
Vulcano	70	20	10	0
Vniimk 8931 (B ₂ K ₂ S ₁₄)	84	16	0	0
Vniimk 8931 (B ₁ K ₂ S ₁₆)	86	12	2	0
Vniimk 8931 (B ₂ K ₁ S ₁₅)	82	14	4	0
Vniimk 8931	86	12	2	0
H-1	0	32	68	0
IS-7776-S	0	26	74	0
IS-7116	0	22	78	0

Data of examination done microscopically and macroscopically in susceptible (Vniimk 8931) and resistant cultivars was seen in Table 3.

Table 3. Observation of *P. helianthi* microscopically and macroscopically in sunflower seedlings susceptible (Vniimk 8931) and resistant to downy mildew

Cultivar	mycelium+oospores			sporulation on cotyledon		
	root	hypocotyl	epicotyl	profuse	weak	necrosis
Mirasol	+	+	-	-	-	+
Floram	+	+	-	-	+	-
Floram-328	+	+	-	-	+	-
Romsun-59	+	+	-	-	+	-
Romsun-90	+	+	-	-	+	-
Start	+	+	-	-	+	-
Primasol	+	+	-	-	-	+
Elia	+	+	-	-	-	-
Rodeo	+	+	-	-	-	-
Hyb. 13/80	+	+	-	-	+	-
Hyb. 219/79/Gi	+	+	-	-	+	+
H-1	+	+	-	-	+	-
IS-7775 S	+	+	-	-	+	-
IS-7116	+	+	-	-	+	-
Vniimk 8931 (susceptible)	+	+	+	+	-	-

Data of effect of root extracts of susceptible and resistant cultivars on conidia of *Botrytis cinerea* and germination of zoosporangia, and capacity of zoosporangia capable of infecting the susceptible cultivar in root extracts are seen in Table 4.

Table 4. Effect of root extracts of susceptible (Vniimk 8931) and resistant cultivars (Hyb. 219/79/Gi) inoculated by *P. helianthi* on conidia of *B. cinerea* and zoosporangia of *P. helianthi* and disease ratio caused by zoosporangia in root extracts

Period after inoculation (h)	<i>B. cinerea</i> % of germinated conidia	<i>P. helianthi</i> % of germinated zoosporangia	% of disease ratio on susceptible plant	
8	Susceptible	100	86	63,3
	Resistant	100	82	66,6
16	Susceptible	100	78	70,0
	Resistant	100	76	66,6
30	Susceptible	100	74	63,3
	Resistant	100	78	60,0
48	Susceptible	100	74	56,6
	Resistant	100	70	60,0
	Sterilized water	100	96	96,6
	Buffer	100	92	93,3

DISCUSSION

It was seen that 17 cultivars and lines out of 72 were in resistant group and 55 were in susceptible group. Cultivars and lines in resistant group were Elia, Floram 305,) floram (60, Hyb. 219/79/Gi, Hyb. 13/80, Mirasol, NS-H-15, NS-H-17, NS-H-27, Primasol, Rodeo, Romsun-59, Romsun-90, Start, H-1, IS-7775 S and IS-7116. Plants in these cultivars and lines were in resistant group at the ratio of 60 % and 100 %. Plants in other cultivars and lines were in susceptible group at the ratio of 70 % and 90 % (Table 2).

From these results, it follows that there is a complex relation between sunflower cultivars and lines and *P. helianthi*. These relations contain some intermediate forms.

Most of plant breeders evaluate practically all plants in moderately and high-

ly resistant groups as resistant. Whereas, the fact that such plants are infected by pathogen is practically of importance (Viranyi and Bartha, 1981).

Subsequent genetic studies have revealed that five genes (P1₁, P1₂, P1₃, P1₄, and P1₅) are responsible for downy mildew resistance (Sackston, 1981; Viranyi and Bartha, 1981; Fick and Auwarter, 1982). Because of the fact that it could not be learnt genes of cultivars and lines tested, it could not be discussed here on genes of them. However, it must be preferred the ones which are determined as resistant among cultivars and lines to be imported into Turkey. In addition it will be useful to employ these plants as gene source in resistant plant breeding.

Mycelium and oospores were found in the sections of root and hypocotyl of all resistant cultivars and lines. On the other hand, they were not seen in the section of epicotyl (Table 3). In the previous studies, mycelium was also seen in hypocotyl of resistant plants (Montes and Sackston, 1974; Viranyi, 1978).

Weak sporulation, necrosis and necrosis plus weak sporulation occurred on cotyledons of resistant cultivars and lines or no symptoms presented on them. Such observations on resistant plants were also determined by other researchers (Viranyi and Dobrovoskyi, 1980).

An important point necessary to be emphasized is that mycelium and oospores are present in resistant plants. Such plants may cause increase of inoculum in soil where they are grown. Besides these plants may increase sexual stage of pathogen as underlined by Sackston et al. (1976) and Viranyi (1978). So it may occur probability to be formed the races with high virulence.

From these findings, it follows that it can not be way to grow only resistant cultivar to downy mildew. Therefore, seed treatment must be taken into consideration.

Fungal growth is not seen in epicotyl of all resistant cultivars. Although hypocotyl-stem "transition", also called diaphragm, may serve as a mechanical barrier to fungal spread, it is put forward that this barrier can be passed in susceptible plants but can not be passed in resistant plants (Montes and Sackston, 1974). Viranyi and Mohamed (1985) also emphasized that effect of diaphragm on pathogen was much more evident in resistant plants.

Apparently, sunflower cultivars may be resistant to downy mildew due to a morphological characteristic (=diaphragm) which is present in plant before disease. A similar situation may be also a question of sunflower cultivars and lines tested in this study.

Although it is found that diaphragm plays an important role in resistant plants, it is known that other factors such as phenols, phytoalexins and hypersensitivity also act a part in resistant plants (Wehtje et al., 1979; Viranyi, 1980; Tena and Valbuena, 1983). But in this study, it could not be found that root extracts of resistant plants infected by *P. helianthi* had an effect on sporangia germinated

and then on fungal growth (Table 4). For this reason, it seems to be important of morphological barrier in plants which were found as resistant in this study.

ÖZET

AYÇİÇEĞİ MİLDİYÖSÜ (*Plasmopara helianthi* Novot.) 'NDE KONUKÇU PATOJEN İLİŞKİLERİ ÜZERİNDE GÖZLEMLER

Çalışmada, 72 ayçiçeği çeşit ve hattının *P. helianthi*'ye karşı davranışı belirlenmiştir. Testlenen 72 ayçiçeği çeşit ve hattından 17'si dayanıklı gruba, 55'i ise duyarlı gruba girmiştir. Dayanıklı gruba giren çeşit ve hatlar; Elia, Floram 305, Floram 60, Hyb. 219/79/Gi, Hyb. 13/80, Mirasol, NS-H-15, NS-H-17, NS-H-27, Primasol, Rodeo, Romsun-59, Romsun-90, Start, H-1, IS-7775 ve IS-7116'dır. Bu çeşit ve hatlardaki bitkiler, % 60 ile % 100 arasında değişen oranlarda dayanıklı gruba girmişlerdir. Diğer çeşit ve hatlarda ise bitkilerin % 70 ile % 90'ı duyarlı grup içerisinde yer almıştır.

Dayanıklı çeşit ve hatlar ile duyarlı çeşit (Vniimk 8931) karşılaştırmalı gözlemlere alınmıştır. Dayanıklı çeşit ve hatların tümünde kök ve hipokotil kesitlerinde miselyum ve oospora rastlanmıştır. Epikotil kesitlerinde ise miselyum ve oospor görülmemiştir.

Dayanıklılıkta fitoaleksinlerin rol oynayıp oynamadıkları incelenmiş, fitoaleksinlerin rolü konusunda bir bulguya rastlanmamıştır. Hastalık öncesi mevcut bir morfolojik özellik nedeniyle (diyafram) ayçiçeği çeşitlerinin dayanıklı olabildikleri sonucuna varılmıştır.

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In Vitro Investigations on the Antagonistic Effects of Several Isolates Against **Botrytis cinerea**.

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ABSTRACT

*In this study, the most potential antagonists that inhibited **B.cinerea** growth on the different media were investigated. The fungi, actinomycetes and bacteria isolated from plant phyllosphere and soil were evaluated for their potential as biocontrol agent of **B.cinerea**. Two microorganisms with the ability to reduce the growth of **B.cinerea** in vitro were identified as the preliminary evaluations. The antagonists; *Paecilomyces lilacinus* and *Bacillus firmus* showed promising effects on biocontrol of the pathogen.*

INTRODUCTION

Vegetables greenhouses are widely extended along the Aegean and Mediterranean regions. The particular greenhouse locations are İzmir in Aegean and Muğla, Antalya in Mediterranean region.

Plant diseases caused by several pests are the important problems of the greenhouses. One of the most frequent and cause considerably losses to crop plant is gray mold pathogen **Botrytis cinerea** Pers.

B. cinerea causes blossom blight, fruit rot, stem canker, leaf spot on a variety of vegetables, ornamentals, fruits and field crops in different parts of the world. The fungus overwinters in the soil as mycelium on decaying plant debris and as sclerotia which survival for about one year. The fungus grows well under cool and wet conditions. The current management program for gray mold is often unsuccessful because of not being available enough heating in the greenhouse in winter.

Controlling of the disease which includes frequent fungicide sprays in the greenhouse during the winter has failed because the fungus developed resistant strains to benzimidazole and dicarboximide fungicides. Fungicide resistance is widespread and can develop within one season. The fungus has a heterocharyotic character and 120 nuclei in the vegetative cells (Menzinger, 1965). The development of pathogen strains resistant to fungicides has become a major problem through the world (Dekker, 1982).

Consideration of these problems has resulted in an interest in biological control of **B. cinerea**. In this alternative control survival or activity of the pathogen is reduced by any other living microorganisms. Biological control may operate at any stage of the pathogen survival or disease development through antibiosis lysis, parasitism or competition (Papavizas, 1980).

The surface of aerial plant parts provides a habitat for epiphytic microorganisms many of which are capable of influencing the growth of pathogens. Thus the phyllosphere is the main area to search for the antagonistic effect.

MATERIALS and METHODS

During 1987 and 1989, the healthy plant parts and soils were collected from the 221 vegetable growing greenhouses of Aegean and Mediterranean regions.

Survey of microorganisms and their Isolation methods :

Isolations of fungi, actinomycetes and bacteria were made on the following selective media : Starch Casein Agar (William, 1971) for actinomycetes, Martin Medium (Martin, 1950) for fungi, SNA for bacteria.

Organisms to be tested for antagonistic activity were isolated from tomato, pepper, cucumber and eggplant leaves and soils. In vitro screening were conducted in three stages: First, the plant leaves were cut and washed in 200 ml sterilized water in a shaker for 30 min. After serial 10^{-5} dilutions washings were mixed in cooled sterilized selective media and shared into the petri plates (Stott, 1971). A second type of in vitro screening was conducted with soil, 25 g dry weight of soil was diluted until 10^{-5} and this dilutions were mixed into the previously mentioned media and added into the petri plates. Third, in order to isolate and assess the antagonistic microorganisms associated with sclerotia, 3 week old sclerotia of *B.cinerea* were produced under sunlight on a PDA medium. 15 sclerotia were placed in to a depth of 3 cm under the surface of 7 eggplant, 7 cucumber, 11 tomato and 8 pepper soil samples. Soils in the glass jars with sclerotia were incubated at 22°C for 5 weeks under 30 % water holding capacity (WHC). After incubation, sclerotia of *B.cinerea* were removed from soil, surface sterilized with 1 % sodium hypochlorite and incubated on 1 % water agar at 16 - 21°C in darkness. All organisms appearing on Sclerotia and surrounding agar were isolated and identified (Credle and Swasithamparana, 1985).

Determination of the microbial potential :

Various actinomycetes, fungal and bacterial isolates were tested with several methods for their antagonistic activity.

Triple agar layer : The first agar layer, 1.5 % water agar was poured into the plates at least 1 day before the second layer was added.

The second layer, the selective media contained the soil and leaf diluted suspensions. The plates were then incubated 3-4 days at $25 \pm 1^\circ\text{C}$. At the end of this period actinomycetes, bacteria and fungi are barely visible on the plates.

The third agar layer was added after incubation. The cultures of *B.cinerea* were suspended with a sterilized cheesecloth into the PDA medium. The plates were then incubated at 25°C for 6 days. Antagonistic organisms were purified on the inhibition zones as illustrated in Figure 1 (Herr, 1959).

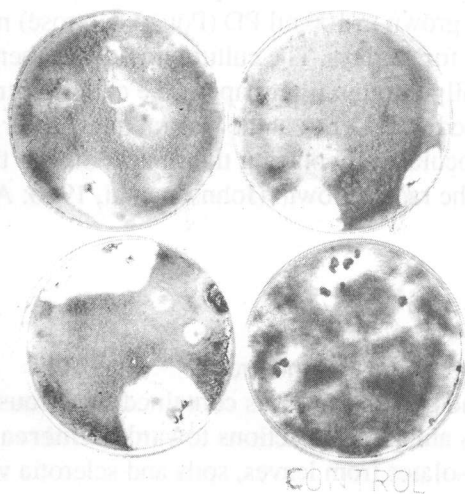


Fig. 1 : Determination of the inhibition zones using triple agar layer technique

Streak Method : This method is used for the antagonistic efficacy of microorganism. In streak method, the antagonist was streaked on to the selective media at one side of a petri plate. After 4-6 day of incubation at $27 \pm 1^\circ\text{C}$, the pathogen discs (5 mm dia.) out from the actively growing colony were placed on the other side. After incubation for 5-6 days, the zone of inhibition occurred between the two colonies was measured. The effective antagonists were selected as antibiotic producers and hyperparasite (Johnson et. al, 1960).

Agar-Ring Method : Firstly screened actinomycetes and bacteria were selected with an agar-ring method improved by Turhan and Grossman (1986). In this method on the bottom of the plates, a circular piece of aluminium foil is placed and an aluminium ring (60 - 70 mm dia., height 10 mm) is put on the foil. Then PDA cooled down to a temperature of $45 - 48^\circ\text{C}$ is poured into the inside of the ring by hand to a height of 5 mm. About 24 h later an isolate of actinomycetes, bacteria or fungi is inoculated onto the whole agar surface. After 48 h incubation at $27 \pm 1^\circ\text{C}$ the ring is removed with a sterile forceps, the agar layer is turned upside-down and the foil is pulled off. Six agar discs (5 mm diam.) obtained by punching out from the margins of actively growing colonies of *B.cinerea* are transferred to this side. After an incubation period of 5 to 10 days at appropriate temperature radial growth of the pathogen is measured and compared with the growth on control plates in order to assess the antibiotic efficacy of the antagonists.

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Determination of the antibiotic activity with culture filtrate :

The antagonistic organism was grown in 100 ml PD (Potato dextrose) medium in 250 ml erlenmayer at $25 \pm 1^\circ\text{C}$ for 15 days. The culture liquid was then obtained by filtering the culture aseptically through filter paper. The culture filtrate of the antagonist was added to sterilized warm PDA at the several rates. After the medium solidified, the plate was inoculated by placing the fungus disc in the centre. The culture was evaluated by the radial growth (Johnson et al, 1960; Arjunarao, 1971).

RESULTS

Isolation and screening of antagonists in vitro :

Washing from leaves and soil suspensions contained numerous microorganisms was exhibited various antagonistic actions toward *B.cinerea*. The potential of 756 microorganism isolates from leaves, soils and sclerotia was shown in Table 1.

Table 1 : Number of Actinomycetes, bacterial and fungal isolates

Microorganisms Isolated	Source of Isolation								Sclerotium	Total No
	Tomato		Pepper		Eggplant		Cucumber			
	Leaf	Soil	Leaf	Soil	Leaf	Soil	Leaf	Soil		
Actinomycetes	98	181	31	34	21	30	30	48	0	473
Fungus	50	55	6	11	5	69	19	1	16	232
Bacterium	8	12	5	1	0	5	11	2	7	51
Total	156	248	42	46	26	104	60	51	23	-
Total Number	404		88		130		111		23	756

Different isolates such as actinomycetes, fungi and bacteria were isolated in totally 756 microorganisms. The incidence rate of the isolates were 62.6, 30.7 and 6.7 % respectively.

Screening of antagonists in vitro :

The isolates that gave the best results in the triple agar layer were compared with each other of streak and agar ring method to choose for the best ones. The results of these tests were summarized in Table 2, 3, 4.

Table 2 : The antagonistic actinomycetes determined with the Streak and Agar-ring methods.

No	Number of isola.	Width of inhibition zone (mm) as streak method	Effectiveness of the isolates as Agar-ring method	No	Number of isola.	Width of inhibition zone (mm) as streak method	Effectiveness of the isolates as Agar-ring method
1	A-310	30	+++	24	A-295	18	+++
2	A-257	26	+++	25	A-626	18	+++
3	A-455	24	+++	26	A-647	18	+++
4	A-2	22	+++	27	A-653	18	+++
5	A-10	22	+++	28	A-672	18	+++
6	A-456	22	+++	29	A-674	18	+++
7	A-630	21	+++	30	A-197	17	++
8	A-4	20	+++	31	A-281	17	++
9	A-293	20	+++	32	A-288	17	++
10	A-330	20	+++	33	A-437	17	++
11	A-387	20	+++	34	A-625	17	++
12	A-486	20	+++	35	A-664	17	++
13	A-608	20	+++	36	A-83	16	++
14	A-615	20	+++	37	A-161	16	++
15	A-624	20	+++	38	A-206	16	++
16	A-297	19	+++	39	A-259	16	++
17	A-303	19	+++	40	A-289	16	++
18	A-53	18	+++	41	A-472	16	++
19	A-74	18	+++	42	A-5	15	++
20	A-191	18	+++	43	A-18	15	++
21	A-210	18	+++	44	A-62	15	++
22	A-221	18	+++	45	A-81	15	++
23	A-216	18	+++	46	A-82	15	++

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No	Number of isola.	Width of inhibition zone (mm) as streak method	Effectiveness of the isolates as Agar-ring method	No	Number of isola.	Width of inhibition zone (mm) as streak method	Effectiveness of the isolates as Agar-ring method
47	A-90	15	++	63	A-620	13	++
48	A-119	15	++	64	A-86	11	++
49	A-170	15	++	65	A-464	11	++
50	A-189	15	++	66	A-1	10	++
51	A-214	15	++	67	A-3	10	++
52	A-323	15	++	68	A-14	10	++
53	A-490	15	++	69	A-313	10	++
54	A-505	15	++	70	A-344	10	++
55	A-23	14	++	71	A-392	10	++
56	A-69	14	++	72	A-429	10	++
57	A-258	14	++	73	A-463	10	++
58	A-329	14	++	74	A-536	10	++
59	A-332	14	++	75	A-605	10	++
60	A-7	13	++	76	A-608	10	++
61	A-41	13	++	77	A-349	9	+
62	A-284	13	++	78	A-369	8	+
				79	A-441	8	+
				80	A-347	7	+
				81	A-359	6	+
				82	A-539	5	+

+ : Mycelial growth of B.c.
 ++ : Low " " "
 +++ : No mycelial growth of B.c

No effect : 0
 Low Antibiosis : Inhibition zone < 3 mm
 Definite " : " " 3-6 mm
 Strong " : " " 7-10 mm
 Very strong " : " " > 10 mm

Table 3 : The Antagonistic fungi determined with the Streak and Agar-ring method.

No	Number of isola	Width of inhibition zone (mm) as streak method	Effectiveness of the isolates as Agar-ring meth.	No	Number of isola.	Width of inhibition zone (mm) as streak method	Effectiveness of the isolates as Agar-ring meth.
1	F- 1	20	+++	7	F- 445	15	++
2	F- 534	20	+++	8	F- 537	15	++
3	F- 550	20	+++	9	F- 543	15	++
4	F- 109	18	+++	10	F- 560	15	++
5	F- 142	17	++	11	F- 16	12	++
6	F- 585	17	++	12	F- 565	12	++
No	Number of isola.	Width of inhibition zone (mm) as streak method	Effectiveness of the isolates as Agar-ring meth.	No	Number of isola.	Width of inhibition zone (mm) as streak method	Effectiveness of the isolates as Agar-ring meth.
13	F-515	11	++	29	F- 535	5	+
14	F- 3	10	++	30	F- 110	4	+
15	F-491	10	++	31	F- 113	4	+
16	F-509	10	++	32	F- 108	3	+
17	F-510	10	++	33	F- 111	3	+
18	F-542	10	++	34	F- 114	3	+
19	F-564	10	++	35	F- 118	3	+
20	F-587	10	++	36	F- 121	3	+
21	F- 4	9	+	37	F- 685	3	+
22	F- 5	9	+	38	F- 112	2	+
23	F- 2	8	+	39	F- 115	2	+
24	F- 7	8	+	40	F- 117	2	+
25	F-181	8	+	41	F- 122	2	+
26	F-450	8	+	42	F- 686	2	+
27	F-512	7	+	43	F- 116	1	+
28	F-555	7	+	44	F- 684	1	+

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Table 4: The antagonistic bacteria determined with the Streak and Agar-ring method.

No	Number of isola.	Width of inhibition zone (mm) as streak method	Effectiveness of the isolates as Agar-ring meth.	No	Number of isola	Width of inhibition zone (mm) as streak method	Effectiveness of the isolates as Agar-ring meth.
1	B - 17	30	+++	11	B- 496	16	++
2	B- 290	19	+++	12	B- 104	15	++
3	B- 220	18	+++	13	B- 105	14	++
4	B- 480	18	+++	14	B- 120	3	+
5	B- 482	18	+++	15	B- 125	3	+
6	B- 101	17	++	16	B- 687	3	+
7	B- 225	17	++	17	B- 688	3	+
8	B- 477	17	++	18	B- 123	2	+
9	B- 479	17	++	19	B- 124	2	+
10	B- 109	16	++	20	B- 689	2	+

When all isolates were evaluated in Table 2, 3, 4, it was achieved various effectivity range of 157 (20.8 %) of 756 isolates. The isolate activity is shown to be very similar in two tests as well. The ability of the antagonists to control *B. cinerea* at streak method will appear the condition clearly. The evaluation of the inhibition zones of all isolates was given in Table 5.

Table 5: Mode of Antibiotic activity by using the Streak Method

Type and Degree of Effectivity	Number of Isolates			Total Number
	Actinomycetes	Fungi	Bacteria	
No effect	391	177	31	599
Low Antibiosis	0	7	3	10
Definite Antibiosis	2	9	4	15
Strong Antibiosis	15	15	0	30
Very Strong Antibiosis	65	13	13	91
Hyperparasitic Effect	-	11	-	11
TOTAL	473	232	51	756

82 (17.3 %) of 473 actinomycetes showed various degrees of effect on *B. cinerea* on the contrary 391 (82.7 %) isolates had no effect. There were 55 (23,7 %) antagonistic fungal isolates which included hyperparasitic fungi. Bacteria were determined as 20 (39.2 %). The isolates shown antagonistic activity

were included in strong and very strong antibiosis groups. On the other hand, only one isolate of 16 fungi determined on the sclerotium shown strong activity of 7 bacteria was low antibiosis group. It was also determined 11 non effective hyperparasitic fungi in the tests.

After all of the isolates had been evaluated and ranked, a final comparison was carried out with the most promising isolates. These isolates were classified in accordance with their culture filtrate containing the inhibitory substances. The antagonists were grown in PD medium and filtered aseptically, then the culture filtrate of the antagonist was added to warm PDA at the rates of 1/4 and 1/10. As the test results: The culture filtrate of isolate F-1, diluted four and ten times with PDA, reduced the mycelial growth of *B. cinerea* at the rates of 71.68 and 58.47 % respectively. The reductions with isolate B-17 were 82.9 and 68.62 % in some respects. (Fig 2, 3).

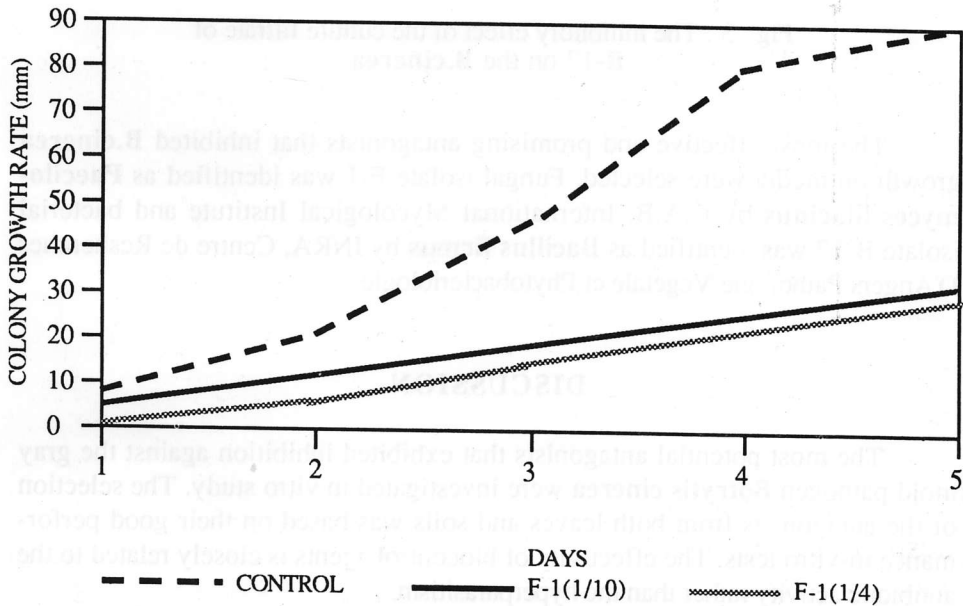


Fig 2 : The inhibitory effect of the culture filtrate of F-1 on the *B.cinerea*

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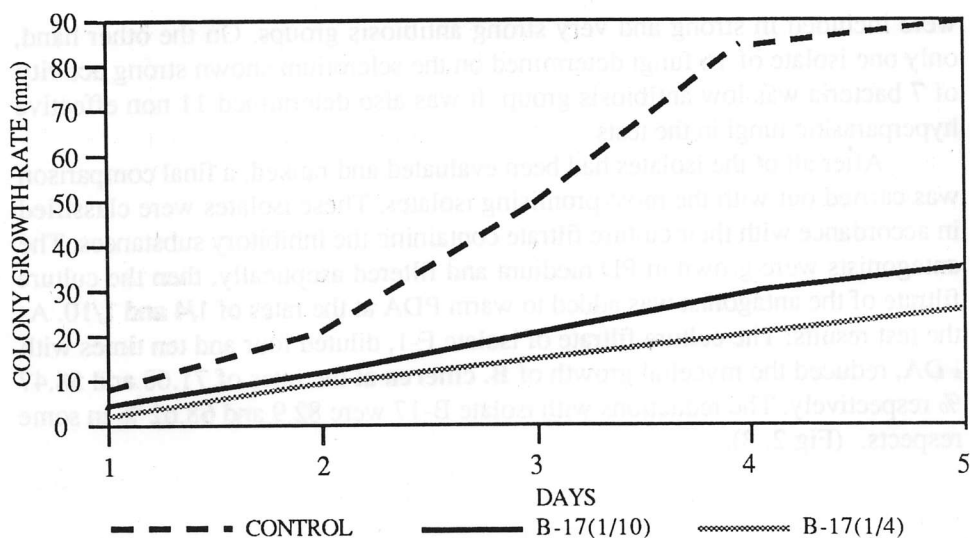


Fig 3 : The inhibitory effect of the culture filtrate of B-17 on the *B.cinerea*

The most effective and promising antagonists that inhibited *B.cinerea* growth on media were selected. Fungal isolate F-1 was identified as *Paecilomyces lilacinus* by C.A.B. International Mycological Institute and bacterial isolate B-17 was identified as *Bacillus firmus* by INRA, Centre de Resherches D'Angers Pathologie Vegetale et Phytobacteriologie.

DISCUSSION

The most potential antagonists that exhibited inhibition against the gray mold pathogen *Botrytis cinerea* were investigated in vitro study. The selection of the antagonists from both leaves and soils was based on their good performance in-vitro tests. The effectivity of biocontrol agents is closely related to the antibiotic activity rather than the hyperparasitism.

Antagonism is found between microorganisms on leaf and fruit surfaces and the relationship between *B.cinerea* and the microflora. Its inoculum potential is limited by its microbial heighbours which produce antibiotics. The antibiotic action of the promising isolates is connected with their ability to toxin production. Both of the isolates has strong inhibitive action toward to *B.cinerea*. *Paecilomyces lilacinus* was isolated from tomato and cucumber phyllosphere and eggplant and cucumber greenhouse soils. *Bacillus firmus* was also isolat-

ed from tomato phyllosphere. The antagonistic activity of two isolates was detected by other researchers (Blakeman and Fokkema, 1982; Rod, 1984; Edwards et al, 1989; Newhook, 1951).

Several in vitro methods for determination of the antagonistic activity by the screening large amount of leaf and soil flora was used to prevent the growth of **B.cinerea**.

B.cinerea is greatly inhibited by two antagonists; **Paecilomyces lilacinus** and **Bacillus firmus** which acts by antibiosis. These promising biocontrol agents will take consideration for further in-vivo tests to evaluate their potential applications.

ÖZET

Botrytis cinerea' YA KARŞI ÇEŞİTLİ İZOLATLARIN ANTAGONİSTİK ETKİLERİ ÜZERİNDE İN-VİTRO ARAŞTIRMALAR

Bu çalışmada, çeşitli ortamlar üzerinde **B.cinerea**'nın gelişmesini engelleyen birçok antagonist araştırılmıştır. Bitkilerin yeşil aksamı ve topraktan izole edilen fungus, actinomycetes ve bakteriler, **B.cinerea**'ya karşı olan antagonistik potansiyelleri yönünden değerlendirilmiştir. İlk değerlendirmelere göre **B.cinerea** gelişimini in-vitro da azaltma özelliğinde olan iki mikroorganizma tanımlanmıştır. **Paecilomyces lilacinus** ve **Bacillus firmus** olarak belirlenen bu antagonistler, patojenin biyolojik kontrolünde ümitvar olarak bulunmuşlardır.

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Effect Of Fungicidal Seed Treatment On Wilt Disease of Cumin

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ABSTRACT

Among the seven fungicides tested in 1983-84 and 1984-85, Bavistin and Benlate were proved effective in controlling the disease in both of years. The decrease in disease incidence resulted into maximum increase of cumin yield in the years in question by these two fungicides.

INTRODUCTION

Cumin (*Cuminum cyminum* L.) is an important cash crop of Rajasthan and Gujrat. Wilt of cumin induced by *Fusarium oxysporum* f. sp. *cumini* is a destructive disease of cumin in all cumin-growing areas of India. (Patel et al., 1957). Patel, et al., (1957) found that the loss varied between 5 to 60 per cent in Rajasthan. In later studies by Patel and Prasad (1963) the losses reported to be upto 96 per cent in Gujrat. In Rajasthan, Mathur and Mathur (1964) observed the losses ranging upto 70 to 80 per cent.

MATERIALS and METHODS

Looking to the severity of disease and losses caused by it, an experiment with cumin cv RS - 1 was conducted in randomised block design in 1983-84 and 1984-85 at college farm in jobner. Plot size was 2x2.5 m. The row to row and plant to plant distance was 15 cm and 5 cm respectively. The available soil moisture content ranged from 12 to 15 per cent and soil temperature upto 15 cm depth ranged from 22-25°C. Ten days before sowing, the plots were artificially inoculated with *Fusarium* inoculum. The spore concentration was 10^6 conidia per ml. The inoculum was obtained from 10-days old culture of the pathogen grown at 25-30°C in 500 ml flasks each of which contained 100 g sterilized maize meal medium (25 g grinded maize and 75 g moistened soil). Inoculum from three flasks was pooled and evenly mixed up with the soil of each plot.

Relative efficacy of 7 fungicides viz. Plantvax, Vitavax, Bavistin WP, Thiobendazole, Demosan 65W, Dithane M-45 and Benlate at the rate of 2 g/kg seed

were shaken together by seed dressing machines. This is essentially a hollow cylindrical metallic drum fitted at one end with a lid, which can be closed tightly. Eccentrically at either end, this rests on a stand and is provided with a handle. On turning the handle not only does the drum rotate, but because of the eccentric mounting the contents get a very thorough agitation. By such an action the seeds and the chemical inside get mixed thoroughly. The crop was sown in the first week of December in 1983-84 and in 1984-85. Each treatment consisted of four replications.

Since a diseased plant wilted completely as a result of pathogenesis, therefore, observations were confined to disease incidence only. Number of diseased plants were counted daily and the daily counts were added up and the total number of plants wilted was utilized in computing per cent disease incidence. The crop was harvested 105 days after planting. The data recorded

$$\text{Per cent disease incidence} = \frac{\text{Number of wilted plants}}{\text{Number of plants inoculated}} \times 100$$

for disease incidence and yield was subjected to analysis of variance for the evaluation of results.

RESULTS

Minimum disease incidence was recorded in both of years in plots sown with Bavistin-treated seeds (Table 1). Next best was Benlate treatment. In both of years, no significant difference in disease incidence was recorded between Bavistin and Benlate treatment but disease incidence in these treatments was significantly lower than the disease incidence in checks plots. In both the years, Demosan and Dithane M-45 proved least effective. Highest per cent disease control was obtained by Bavistin followed by Benlate in both of years, while least per cent disease control was obtained by Demosan and Dithane M-45.

All the fungicides rendered higher yields over check in both of years. Maximum yields were rendered by Bavistin followed by Benlate in both of years. The difference in yields rendered by these two fungicides was non significant. Yields from Bavistin and Benlate treatments were significantly higher than the yields from check in both of years. Although per cent yield increase over check was obtained by all the fungicides in both of years, but per cent yield increase was highest in Bavistin followed by Benlate in both of years.

DISCUSSION

Except a report by Singh et al (1972) concerning effectiveness of seed treatment with an organomercurial compounds, no information was available on

Table 1. Effect of seed treatment with fungicides on incidence of Fusarium wilt and yield of cumin

Fungicides	1983 - 84				1984 - 85			
	Disease incidence (%)	Disease control (%)	Yield g/plot	Yield increase (%)	Disease incidence (%)	Disease control (%)	Yield g/plot	Yield increase (%)
Plantavax	42.41 (40.60)	35.19	49.25	27.92	45.32 (42.29)	35.21	48.25	25.32
Vitavax	45.42 (42.34)	30.59	48.00	24.68	50.84 (45.46)	27.32	46.50	20.78
Bavistin	25.18 (30.04)	61.52	75.50	96.10	34.14 (35.67)	51.19	75.00	94.81
Thiobendazole TBZ	49.07 (44.44)	25.02	47.50	23.38	52.97 (46.70)	24.27	51.75	34.42
Demosan	55.95 (48.39)	14.50	46.25	20.13	60.41 (51.00)	13.64	48.75	26.62
Dithane M - 45	55.18 (47.96)	15.68	52.50	36.36	60.26 (50.89)	13.85	48.50	25.97
Benlate	30.28 (33.30)	53.73	71.00	84.42	37.23 (37.53)	46.78	64.50	67.53
Control	65.44 (53.97)	-	38.50	-	69.95 (56.74)	-	38.50	-
S. Em. \pm	1.4387		2.1047		1.1575		4.6076	
CD at 5 %	4.13		6.04		3.32		13.22	
CD at 1 %	5.76		8.43		4.64		18.44	
CV	6.74		7.86		5.06		17.48	

Plot size : 2 X 2.5 m

Data are mean of 4 replications

Figures in parentheses are angular values

disease management by chemical seed treatment. The present study indicates that the disease can be significantly reduced by seed treatment with Bavistin and Benlate. It is interesting to recall that seed treatment with these fungicides have been found effective against **Fusarium** wilt of other crops as reported by Kannaiyan and Nene (1974) and Chakrabarti and Basuchaudhary (1979 and 1980). The effect of Bavistin and Chakrabarti and Basuchaudhary (1979 and 1980). The effect of Bavistin and Benlate lasted for 35-40 days. Presence of these fungicides on the surface of seed checked the growth of the pathogen either present in seed or soil. The initial infection to plant by **Fusarium** was reduced by these fungicides, which resulted into decrease in disease incidence and increase in cumin yield.

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Effect Of Salicylic Acid on the Control Of Bacterial Speck of Tomato Caused by *Pseudomonas syringae* pv. *tomato*

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ABSTRACT

Seven applications of salicylic acid (0.36 to 7.24 mM) to tomato plants (Lycopersicon esculentum) at 2-day intervals prior to inoculation with Pseudomonas syringae pv. tomato reduced the severity of bacterial speck by up to 81.0 %. Treatments with 3.62 mM salicylic acid reduced the disease severity by 71.7 - 81.0 % according to control plants under greenhouse conditions. Applications by either irrigation or spray conferred the same degree of resistance of tomato plants to bacterial speck. Growth of P.s.pv. tomato was not affected when applied to bacterial growth medium at concentrations from 0.36 to 7.24 mM.

INTRODUCTION

Bacterial speck of tomato (*Lycopersicon esculentum* Mill) caused by *Pseudomonas syringae* pv. *tomato* (Okabe) Young et al (*P.s.pv.tomato*) causes substantial economical loss in tomato crops in many countries (4, 11, 23, 24, 29). Presently, three strategic are used to control the bacterial speck of tomato. In the first, copper compounds and streptomycin sulfate are applied to plants (3, 7, 8, 14, 29). In the second, resistant tomato cultivars developed to bacterial speck (5, 18, 19, 20). In the third, certain strains of fluorescent pseudomonad are applied to plants to antagonize which have been shown to *P.s.pv.tomato* (6, 7, 9).

Salicylic acid, a phenolic compound, regulates plant growth (22) and induces flowering (12, 25) in some plants. Salicylic acid and acetyl salicylic acid induce pathogenesis related proteins and confer resistance to plants to some viral diseases (16, 17, 26, 27, 28).

Below, we describe an evaluation of salicylic acid on the induction of the resistance of tomato plants *P.s.pv.tomato* infection under greenhouse conditions.

MATERIALS and METHODS

Bacterial strain and inoculum. The strain of *P.s.pv.tomato* used in this study was obtained from leaves of tomato seedlings infected with bacterial speck pathogen in Bursa, Turkey in 1983. This strain was cultured on King's medium B(13) for 48 hrs at 24-26 °C and the cells were washed from the agar surface with sterile distilled water and stored at +4 °C. The concentration of inocula was adjusted to 10⁹ colony-forming units per milliliter with sterile distilled water.

Tomato plants. Bacterial speck susceptible tomato cultivar H-2274 used in this study was obtained from Ministry of Agriculture and Forestry of Turkey. Tomato seeds were sown in trays and grown for 6 days in greenhouse. Seedlings were transferred to pots (15 seedlings per pot) and grown for 16 days at 20.8 + 2.1 °C (minimum 14.0 °C, maximum 28.5 °C) and 73.0 ± 5.5 % relative humidity in greenhouse. Eight plants per pot, at the same stage of development, were identified at 16 days and left undisturbed; all other plants were removed.

Application of salicylic acid to plants. Salicylic acid solutions were prepared in sterile distilled water at 0.36, 1.45, 3.62 and 7.24 mM concentrations and stored at +4 °C. Twenty-four day old tomato seedlings were treated seven times with salicylic acid by spray (10 ml/plant) or by irrigation (100 ml/pot) at 2-day intervals. Control plants were treated with sterile distilled water. Each concentration of salicylic acid was applied to five separate pots, each containing eight plants (40 plants total per each salicylic acid concentration). After salicylic acid applications, tomato leaves were sprayed three times with sterile distilled water at 2-day intervals.

Inoculation and estimation of disease severity. Plants were inoculated with a hand-held sprayer until water droplets formed on both the upperside and underside of leaves (approximately 10 ml inoculum per plant). Inoculated plants were kept at high humidity in separate polyethylene bags for 48 hrs, and then transferred to greenhouse until symptoms developed. Disease severity was recorded 15 days after inoculation using the following scale (20): 0 = no disease; 1 = 1-3 specks per leaf; 2 = 4-10 specks per leaf; 3 = more than 10 specks per leaf. The two uppermost leaves of each plant which were fully expanded and uniformly infected were used to determine the disease index. The data were subjected to statistical analysis using Tukey's multiple comparisons test.

RESULTS and DISCUSSION

The effect of salicylic acid concentration and method of application on the induction of resistance to *P.s.pv.tomato* infection are shown in Table 1. There

was no significant difference between irrigation and spray methods of salicylic acid application when resistance to *P.s.pv.tomato* infection was assessed (correlation coefficient = 0.994). The 3.62 mM concentration was the most effective in both irrigation and spray methods, resulting in significant reduction in disease severity. This salicylic acid concentration reduced disease severity by 81.0.

Table 1. Effect of salicylic acid concentration and method of application on the control of bacterial speck of tomato caused by *Pseudomonas syringae pv. tomato*.

Concentration of Salicylic Acid	Disease Index*		Reduction of Disease (%)	
	Spray	Irrigation	Spray	Irrigation
0 (Control)	2.142 a	2.248 a	-	-
0.36 mM	1.866 a	1.855 a	12.9	17.5
1.45 mM	1.452 ab	1.775 a	32.2	21.1
3.62 mM	0.407 b	0.636 b	81.0	71.7
7.24 mM	0.965 b	0.840 b	55.0	62.6

* Every index value was obtained from five replicates (each replicate consisted of 1 pot (8 plants). Means within each column values followed by the same letter are not significantly different ($p = 0.05$) according to Tukey's multiple comparisons procedure.

and 71.7 % when applied by spray or irrigation, respectively. Reductions of disease severity by salicylic acid concentrations below 3.62 mM were statistically insignificant. Also, there was no significant difference between the 3.62 and 7.24 mM salicylic acid concentrations. Phytotoxic effect and the increasing in disease severity were observed at 7.24 mM concentration of salicylic acid, and resulted in plants that were 10 - 15 % shorter than control plants. Probably the phytotoxic effect of 7.24 mM concentration of salicylic acid might reduce the resistance of tomato plants to bacterial speck pathogen.

The results of this study show that salicylic acid has a potential for use in the control of bacterial speck of tomato caused by *P.s.pv.tomato*. This is of

BACTERIAL SPECK OF TOMATO

importance, since control of bacterial speck of tomato by copper compounds has not been effective in some studies (10, 15, 21), and some strains of **P.s.pv.tomato** have been found to be resistant to copper compounds (1, 2). Future studies should focus on the effects of salicylic acid treatment on the control of bacterial speck of tomato under field conditions and on tomato yield. In addition, these studies should also investigate the effects of salicylic acid derivatives (e.g., acetyl salicylic acid) as well as the mechanism of salicylic acid-induced resistance to **P.s.pv.tomato** infection. The mechanism of action of salicylic acid in the induction of resistance to **P.s.pv.tomato** is not presently understood. Based on this study, salicylic acid had no toxic effect on growth of **P.s.pv.tomato** when applied in to growth medium at concentrations ranging from 0.36 to 7.24 mM. This suggests that salicylic acid action is exerted at the level of plant, e.g. induction of the synthesis of pathogenesis related proteins.

ÖZET

Pseudomonas Syringae pv.Tomato, NEDEN OLDUĞU DOMATES BAKTERİYEL LEKE HASTALIĞININ KONTROLÜ ÜZERİNE SALİSİLİK ASİDİN ETKİSİ

Salisilik Asidin **P.s.pv.tomato** ile inokule edilmeden önce domates bitkilerine (**Lycopersicum esculentum**) ikişer gün aralıklarla 0.36 - 7.24 mM arası konsantrasyonlarda 7 defa uygulması bakteriyel leke şiddetini % 81'e kadar düşürmüştür. Sera şartları altında 3.62 mM salisilik asit uygulamaları kontrol bitkilere göre hastalık şiddetini % 71.7 - 81.0 arasında düşürmüştür. Gerek sulama ve gerekse püskürtme yoluyla yapılan uygulamalar bakteriyel lekeye karşı domates bitkilerinin aynı derecede direnç kazanmalarına neden olmuştur. Salisilik asidin üreme besiyerine 0.36 - 7.24 mM konsantrasyonlar arasında ilavesi **P.s.pv.tomato**'nun üremesi üzerine etki etmemiştir.

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Untersuchungen Zur Verunkrautung In Zitrus In Abhängigkeit Von Bekämpfungsmassnahmen

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ZUSAMMENFASSUNG

*Die drei Gräser **Cynodon dactylon** (L.) Pers., **Cyperus rotundus** L. und **Sorghum halepense** (L.) Pers. sind die wichtigsten Schadgräser in Zitrusanbau der Türkei. Das Ziel dieser Arbeit war, die Wirkung von 14 Varianten aus verschiedenen Bodenbearbeitungsmaßnahmen, Herbizidanwendungen und deren Kombinationen sowie Bedeckung mit schwarzer Folie auf die ober- und unterirdische vegetative Entwicklung dieser Arten zu untersuchen.*

Bei der Bedeckung mit schwarzer Folie sowie bei der Kombinationsanwendung von Bromocil + Glyphosat wurde ausreichende Entwicklungshemmung dieser ausdauernden Unkrautarten festgestellt. Durch die intensive Bodenbearbeitung mit Grubber oder Scheibenegge können die drei Unkrautarten reduziert, jedoch nicht entscheidend zurückgedrängt werden. Mit dem Einsatz von Glyphosat in Kombinationen mit mechanischen Maßnahmen ließen sich die Arten nur unbefriedigend kontrollieren, die einzige positive Ausnahme war hier Herbizidanwendung in Kombination mit Grubbereinsatz.

Die Ergebnisse zeigen, daß man auf mechanische Maßnahmen möglichst verzichten sollte. Die Bedeckung mit Folie zur Unkrautkontrolle sollte weiter untersucht werden. Die Wirkung von Herbiziden wird weiter geprüft, auch um Möglichkeiten der Unkrautkontrolle in jungen Zitruspflanzungen aufzuzeigen.

EINLEITUNG

Zitruspflanzungen werden heute in der Türkei meistens im Abstand von 7 x 7 m angelegt und im Sommer alle 2 Wochen bewässert. Vor der Bewässerung findet eine relativ einseitige Bodenbearbeitung mit Grubber oder Scheibenegge statt. Diese Maßnahme führt jedoch mittel- bis langfristig bei gleichzeitiger Verteuerung der Arbeitskräfte zu einer zunehmenden Verschärfung des Unkrautproblems in Zitrus und macht die Erarbeitung verbesserter Bekämpfungsstrategien unter stärkerem Einbezug moderner Verfahren erforderlich.

Heute zählen in Zitrusgärten der Çukurova aufgrund der genannten Maßnahmen ausdauernde Gräser wie **Cynodon dactylon** (L.) Pers., **Cyperus rotundus** L., und **Sorghum halepense** (L.) Pers. zu den Problemunkräutern.

VERUNKRANUTUNG IN ZITRUSGÄRTEN

In Zitrusgärten wurden Versuche unter Einbeziehung von 14 Unkrautbekämpfungsvarianten durchgeführt. Die Auswertungen erfolgten unter Berücksichtigung der ober- und unterirdischen Entwicklung der drei oben genannten Arten in Abhängigkeit von den Bekämpfungsmaßnahmen. Die Varianten bestanden aus Einzelmaßnahmen bzw. Kombinationen von Herbizidanwendungen und Bodenbearbeitungen sowie Bodenbedeckung mit schwarzer Folie.

MATERIAL und METHODEN

Die Versuche wurden im Zitrusanbaugebiet in Alata-Çukurova durchgeführt. Die Parzellengröße der Varianten in den Zitrusreihen betrug 200 m² und zwischen den Zitrusreihen 400 m². Der Unkrautdeckungsgrad wurde am 1.2.1987 in den einzelnen Parzellen nach Arten aufgenommen. Neun Tage später, am 10.2.1987, wurde die Versuchsfläche ca. 30 cm Tiefe gepflügt.

Die Versuche wurden in 14 vierfach wiederholten Varianten angelegt. Die Anwendungshäufigkeiten der Maßnahmen zu jeder Variante sind in Tabelle 1 dargestellt. Die Maßnahmen erfolgten nach Bedarf bei einem Unkrautdeckungsgrad von 5-10 %. Die Anwendungstermine sind in den Abbildungen dargestellt.

Die Herbizidvarianten wurden mit einem Parzellenspritzgerät (Druck : 3-4 bar, Ausbringmenge : 600 l/ha) behandelt. Die Herbizide kamen in praxisüblicher Dosierung zur Anwendung : Bromacil 5,6 kg/ha, Glyphosat 4,6 kg/ha, Paraquat 0,4 l/ha.

Tab. 1: Anwendungshäufigkeiten der Varianten in und zwischen den Zitrusreihen

Nr. der Variante	Variante	Anwendungshäufigkeit (im Zweijahreszeitraum)
1.	Nur Herbizide	
2.	Nur Herbizide (in den Reihen)	1 x Bromacil + 2 x Glyphosat
3.	Nur Herbizide (zwischen den Reihen)	1 x Bromacil + 3 x Glyphosat
4.	Bedeckung mit schwarzer Folie (in den Reihen)	13 x Paraquat
5.	Scheibenegge (zwischen den Reihen)	1 x Folie
6.	Scheibenegge + Glyphosat (zwischen den Reihen)	9 x Scheibenegge
7.	Mähen (in den Reihen)	7 x Sch. egge + 2 x Glyphosat
8.	Mähen + Glyphosat (in den Reihen)	11 x Mähen
9.	Mähen (zwischen den Reihen)	8 x Mähen + 2 x Glyphosat
10.	Mähen + Glyphosat (zwischen den Reihen)	10 x Mähen
11.	Handhacke (in den Reihen)	7 x Mähen + 2 x Glyphosat
12.	Handhacke + Glyphosat (in den Reihen)	9 x Handhacke
13.	Grubber (zwischen den Reihen)	6 x Handhacke + 2 x Glyphosat
14.	Grubber + Glyphosat (zwischen den Reihen)	9 x Grubber
		7 x Grubber + 2 x Glyphosat

Die Versuchsdauer betrug zwei Jahre. Etwa alle zwei Monate wurde der Unkrautdeckungsgrad in den Einzelparzellen in % bonitiert. Das unter-irdische Wachstum der ausdauernden Unkrautarten wurde einmal am 25.12.1987 in jeder Parzelle auf einer Fläche von 1 m² bis 20 cm Tiefe erfaßt. Hierzu wurden die Nodien der Rhizome von *C. dactylon* und *S. halepense* sowie die Knollen von *C.rotundus* gezählt.

Die Versuchsfläche wurden im Sommer nach Bedarf ca. alle 2 Wochen bewässert. Alle weiteren Bewirtschaftungsmaßnahmen wurden praxisüblich durchgeführt.

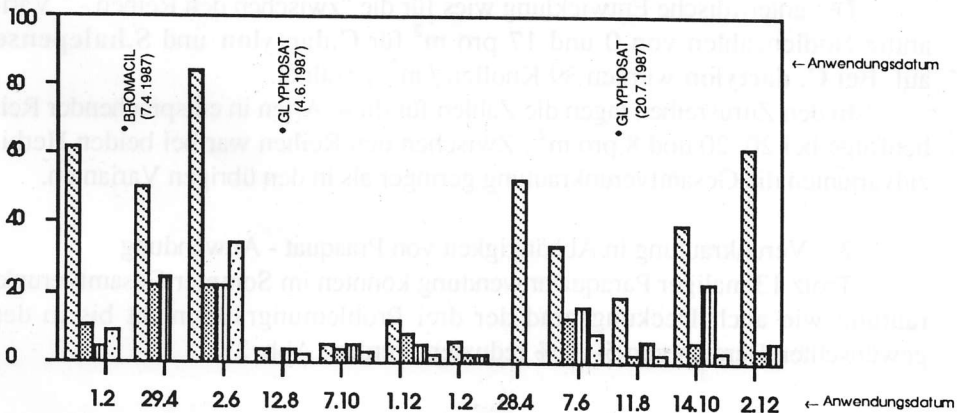
ERGEBNISSE

1. Verunkrautung in Abhängigkeit von Bromacil - + Glyphosat - Anwendung.

Alle drei ausdauernden Arten *C. dactylon*, *C. rotundus* und *S. halepense* zeigen durch die Bromacil - + Glyphosat Anwendungen im Vergleich mit Varianten wie Paraquat, Scheibenegge, Mähen, Handhacke und Grubber eine abnehmende oberirdische Entwicklung.

Einzelne Arten weisen ab Frühjahr 1987 über die verbleibende Versuchsdauer nicht mehr als 15 % Deckungsgrad auf (Abb. 1).

Deckungsgrad in % (in den Reihen)



Monate im 1987 und 1988

Abb. 1 : Verunkrautung in Abhängigkeit von der Variante "Glyphosat + Bromacil".

Deckungsgrad in % (zwischen den Reihen)

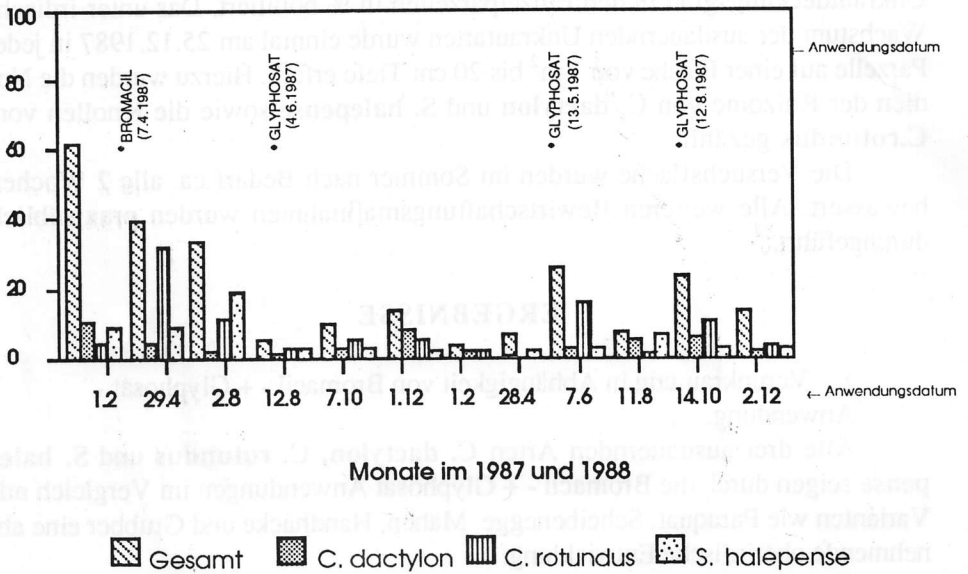


Abb. 1 : Verunkrautung in Abhängigkeit von der Variante "Glyphosat + Bromacil".

Die unterirdische Entwicklung wies für die "zwischen den Reihen - " Variante Nodienzahlen von 0 und 17 pro m² für *C.dactylon* und *S.halepense* auf. Bei *C. dactylon* wurden 39 Knollen / m² gezählt.

In den Zitrusreihen lagen die Zahlen für diese Arten in entsprechender Reihenfolge bei 20, 20 und 8 pro m². Zwischen den Reihen war bei beiden Herbizidvarianten die Gesamtverunkrautung geringer als in den übrigen Varianten.

2. Verunkrautung in Abhängigkeit von Praaquat - Anwendung

Trotz 13 maliger Paraquatanwendung konnten im Sommer Gesamtverunkrautung wie auch Deckungsgrad der drei Problemungräser nicht bis in den gewünschten Bereich von 5-10 % reduziert werden (Abb. 2).

Deckungsgrad in % (in den Reihen)

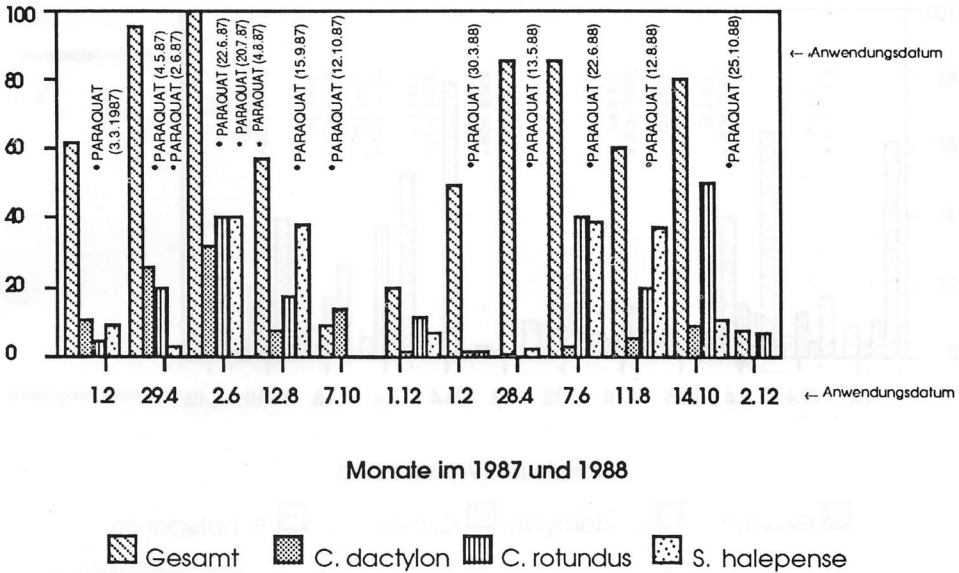


Abb. 2 : Verunkrautung in Abhängigkeit von der Variante "Paraquat"
 Bei der Paraquat-Variante war auch die unterirdische Nodienbildung ziemlich hoch. So wurden im Mittel bei *S. halepense* 153 und bei *C. dactylon* 55 Nodien / m² gezählt. *C.rotundus* bildete im Durchschnitt 25 Knollen/m².

3. Verunkrautung in Abhängigkeit von Abdeckung mit schwarzer Folie
 Bedeckung der Zitrus-Reihen mit schwarzer Folie zeigte auf die oberirdische und unterirdische Entwicklung eine ausreichende Wirkung. Oberirdisches Wachstum konnte bei keiner Art festgestellt werden. Die unterirdische Nodienbildung war hier im Vergleich zu allen weiteren Varianten am niedrigsten (*S.halepense* 1, *C. dactylon* 2 Nodien und *C.rotundus* 22 Knollen/m²).

4. Verunkrautung in Abhängigkeit von Scheibeneggen-Einsatz
 Die Ergebnisse (Abb.3) zeigen, daß der Einsatz der Scheibenegge allein über die zweijährige Versuchsdauer Arten wie *C. dactylon*, *C. rotundus* und *S. halepense* nicht unter Kontrolle bringen kann : Gegenüber der Gesamtverunkrautung zu Versuchsbeginn wurde nach Ablauf der zwei Jahre keine Verminderung erzielt.

VERUNKRANUTUNG IN ZITRUSGÄRTEN

Deckungsgrad in % (zwischen den Reihen)

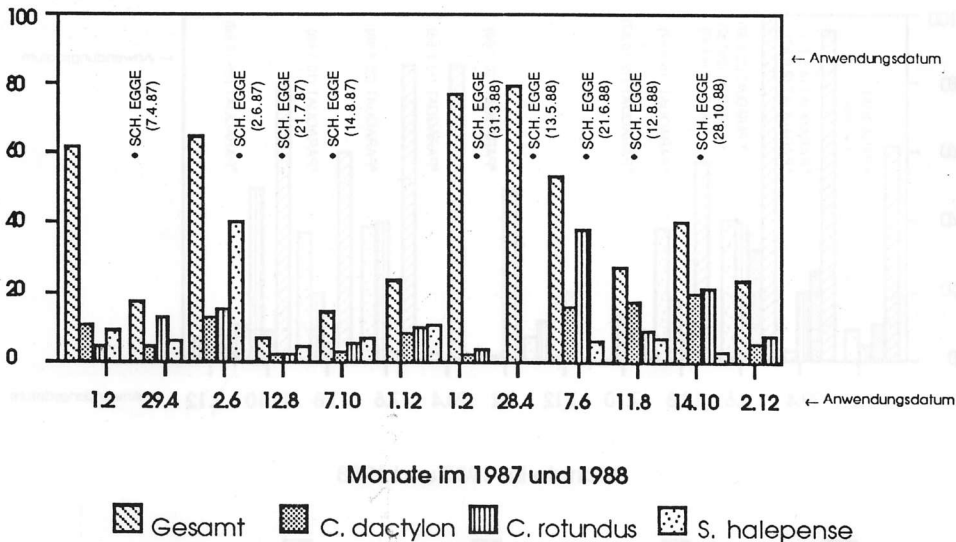


Abb. 3 : Verunkrautung in Abhängigkeit von Variante "Scheibenegge"

5. Verunkrautung in Abhängigkeit von Scheibeneggen - und Glyphosat - Einsatz

Glyphosat - Behandlung in Kombination mit Scheibeneggen - Einsatz hatte etwas bessere verunkrautungsmindernde Wirkung gegenüber den drei ausdauernden Arten als alleiniger Scheibeneggen-Einsatz (s. Abb. 3 u. 4).

Deckungsgrad in % (zwischen den Reihen)

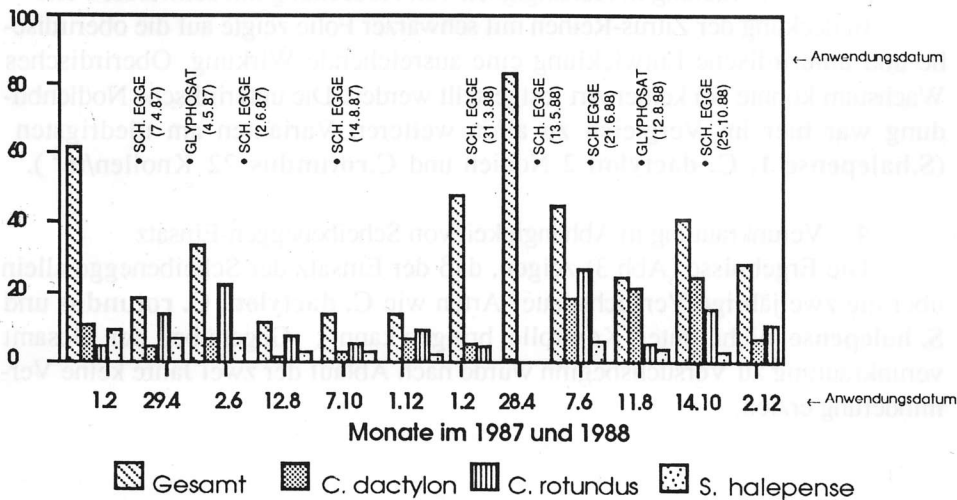


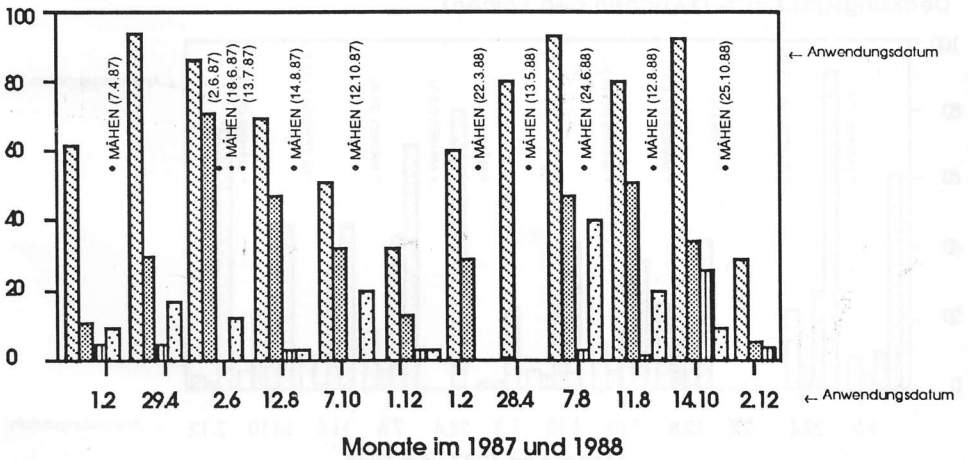
Abb. 4 : Verunkrautung in Abhängigkeit von Variante "Scheibenegge + Glyphosat"

Unterirdische Nodien - und Knollenbildung wurden bei dieser Kombination zweier Maßnahmen vermindert : bei *C. dactylon* um 37 Rhizomnodien/m² (von 40 auf 3), bei *S. halepense* um 25 Nodien/m² (von 25 auf 0) und bei *C. rotundus* um 14 Knollen/m² (von 63 auf 49). Auch hier hatte die zusätzliche Glyphosat-Anwendung eine Wirkung.

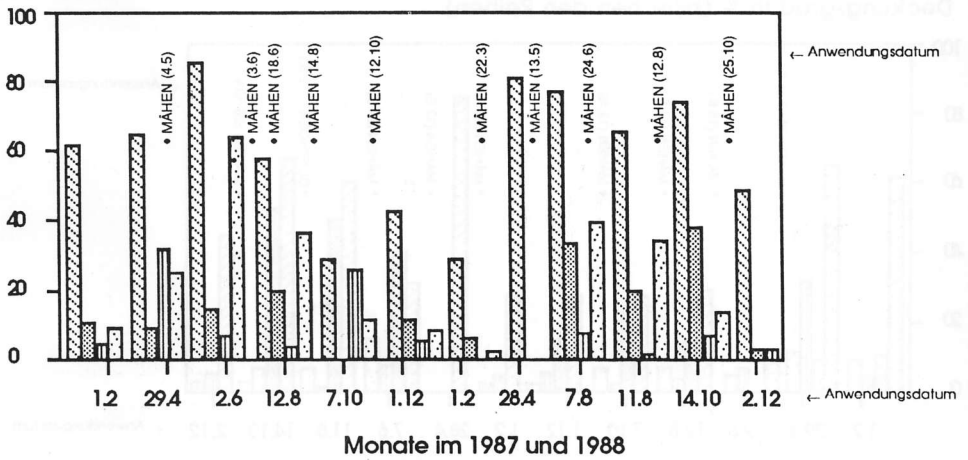
6. Verunkrautung in Abhängigkeit von Mähen

Die in Abbildung 5 dargestellten Ergebnisse zeigen, daß die Verunkrautung durch Mahd nicht reduziert wurde. Besonders nahm die niedrig-wüchsige *C.dactylon* in den zwei Jahren zu.

Deckungsgrad in % (in den Reihen)



Deckungsgrad in % (zwischen den Reihen)



Gesamt
 C. dactylon
 C. rotundus
 S. halepense

Abb. 5 : Verunkrautung in Abhängigkeit von Variante "Mähen"

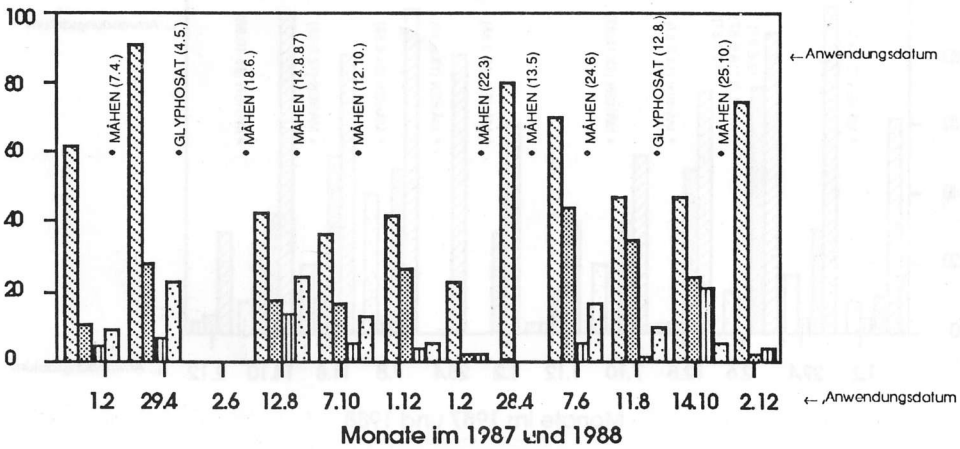
VERUNKRANUTUNG IN ZITRUSGÄRTEN

Im Vergleich zu den anderen Varianten wurde durch das Mähen das unterirdische Wachstum der drei ausdauernden Arten stimuliert : Zwischen den Reihen wurden pro m² bei *C. dactylon* 48 Rhizomnodien, bei *S. halepense* 120 Nodien und bei *C. rotundus* 44 Knollen gezählt. In den Reihen waren es bei *C. dactylon* 58 Nodien/m², bei *S. halepense* 153 Nodien/m² und bei *C. rotundus* 26 Knollen/m².

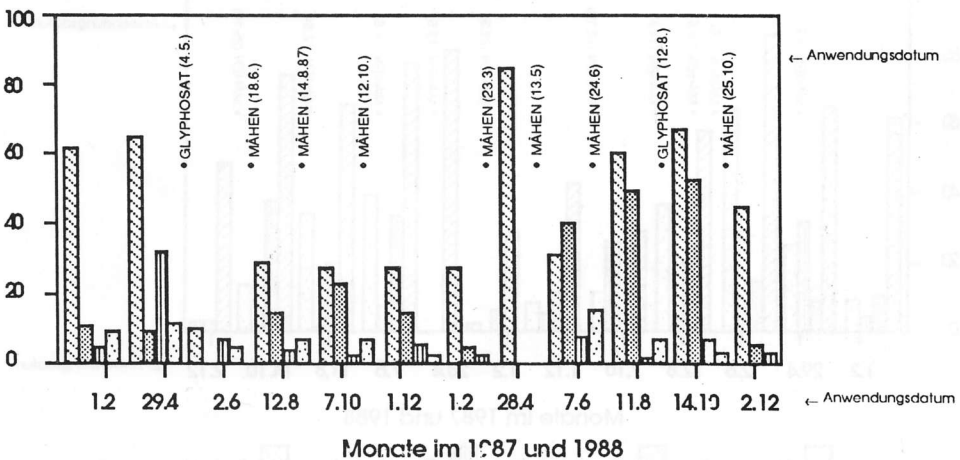
7. Verunkrautung in Abhängigkeit von Mähen in Kombinationen mit Glyphosat-Anwendung

Durch die Anwendung von Glyphosat in Kombinationen mit Mahd wurde die Verunkrautung der drei Arten um mehr als 50 % reduziert. Im unterirdischen Wachstum zeigten sich entsprechende Ergebnisse (Abb. 5 u. 6).

Deckungsgrad in % (zwischen den Reihen)



Deckungsgrad in % (zwischen den Reihen)



Gesamt
 C. dactylon
 C. rotundus
 S. halepense

Abb. 6 : Verunkrautung in Abhängigkeit von Variante "Mähens + Glyphosat"

8. Verunkrautung in Abhängigkeit von Handhacken-Einsatz

Die Wirkung der Handhacke auf die Verunkrautung in den Zitrusreihen war gegenüber den Varianten "Paraquat" oder "Mähen" besser (Abb. 2, 5 u. 7).

Deckungsgrad in % (in den Reihen)

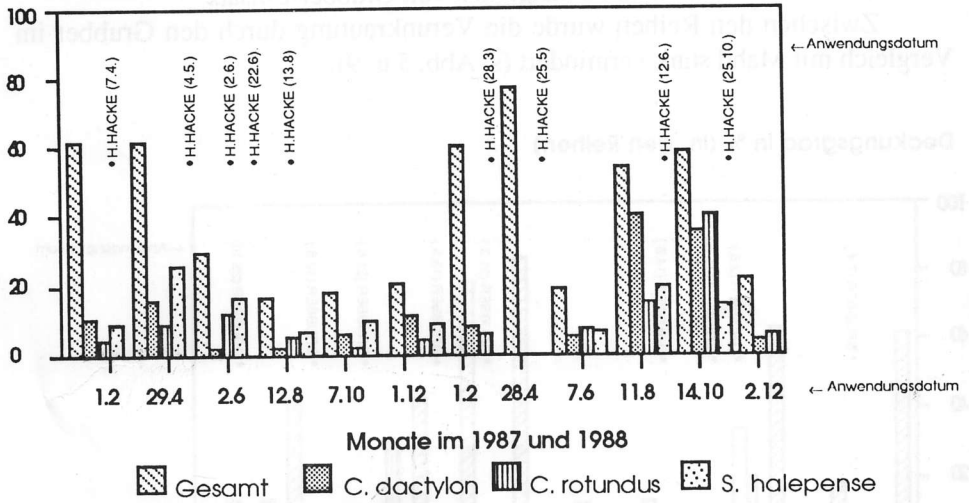


Abb. 7 : Verunkrautung in Abhängigkeit von Variante "Handhacke"

9. Verunkrautung in Abhängigkeit von Handhacken-Einsatz in Kombination mit Glyphosat-Applikation

In den Zitrusreihen war die Kombination "Handhacke + Glyphosat" wirkungsvoller als der alleinige Handhacken-Einsatz (s. Abb. 7 u. 8).

Deckungsgrad in % (in den Reihen)

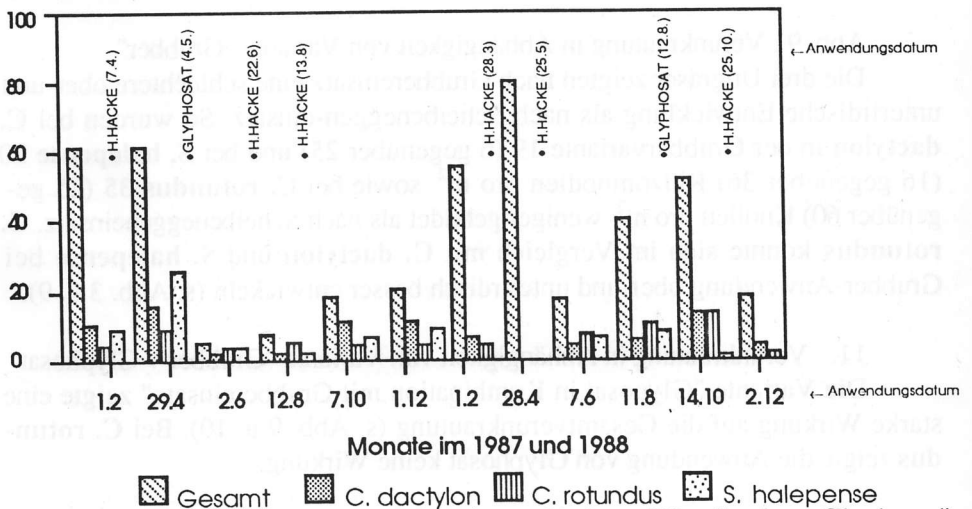


Abb. 8 : Verunkrautung in Abhängigkeit von Variante "Handhacke + Glyphosat"

VERUNKRANUTUNG IN ZITRUSGÄRTEN

Die Variante "Handhacke" reduzierte auch die unterirdische vegetative Entwicklung der drei ausdauernden Unkräuter stärker als die Varianten "Mähen" und "Paraquat."

10. Verunkrautung in Abhängigkeit von Grubber-Einsatz

Zwischen den Reihen wurde die Verunkrautung durch den Grubber im Vergleich mit Mahd stark vermindert (s. Abb. 5 u. 9).

Deckungsgrad in % (in den Reihen)

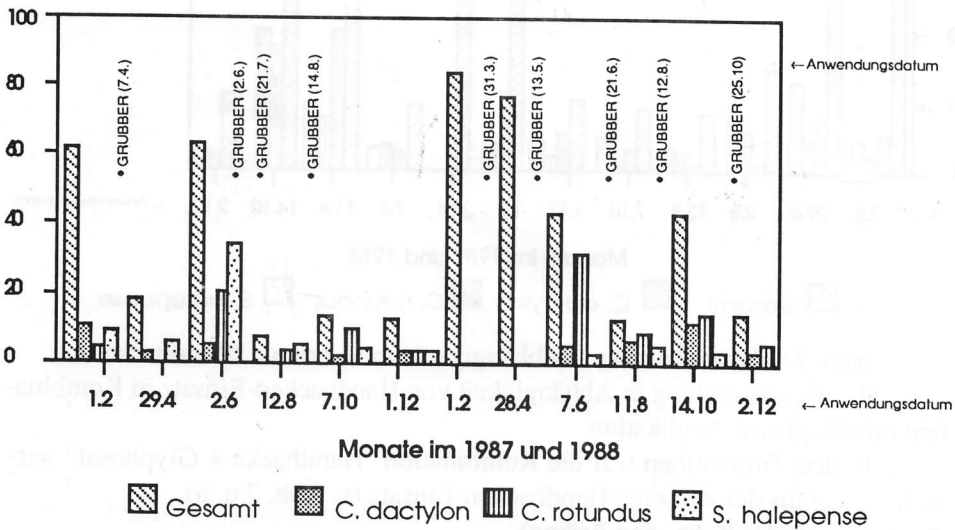


Abb. 9 : Verunkrautung in Abhängigkeit von Variante "Grubber"

Die drei Ungräser zeigten nach Grubbereinsatz eine schlechtere ober- und unterirdische Entwicklung als nach Scheibeneggen-einsatz. So wurden bei *C. dactylon* in der Grubbersvariante 19 (6 gegenüber 25) und bei *S. halepense* 20 (16 gegenüber 36) Rhizomnodien pro m^2 sowie bei *C. rotundus* 35 (25 gegenüber 60) Knollen pro m^2 weniger gebildet als nach Scheibeneggen-einsatz. *C. rotundus* konnte sich im Vergleich mit *C. dactylon* und *S. halepense* bei Grubber-Anwendung ober- und unterirdisch besser entwickeln (s. Abb. 3 u. 9).

11. Verunkrautung in Abhängigkeit von Variante "Grubber + Glyphosat"

Die Variante "Glyphosat in Kombination mit Grubbereinsatz" zeigte eine starke Wirkung auf die Gesamtverunkrautung (s. Abb. 9 u. 10). Bei *C. rotundus* zeigte die Anwendung von Glyphosat keine Wirkung.

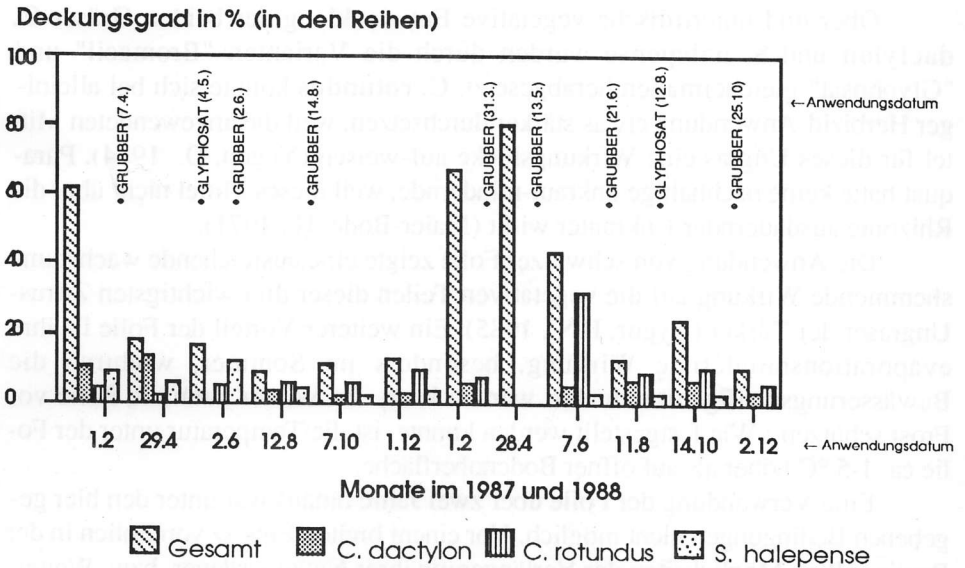


Abb. 10 : Verunkrautung in Abhängigkeit von Variante "Grubber + Glyphosat"

DISKUSSION

Zitruspflanzungen werden heute in der Çukurova meistens im Abstand von 7 x 7 m angelegt und im Sommer alle 2 Wochen bewässert. Vor der Bewässerung findet eine relativ einseitige Bodenbearbeitung mit Grubber und Scheibenegge Zur Unkrautbekämpfung statt. Dadurch werden die Wurzeln von Zitruspflanzen verletzt (Tuzcu, ö., 1984). Diese traditionellen Maßnahmen und die Verteuerung der Arbeitskräfte führen zu einer zunehmenden Verschärfung des Unkrautproblems im Zitrusanbau der Türkei.

Die im Zitrusanbau in der Türkei häufig vorkommenden ausdauernden Arten *C. dactylon*, *C. rotundus* und *S. halepense* gehören zu den zehn weltweit wichtigsten Unkräutern (Holm et. al., 1977).

Diese Umstände unterstreichen die Bedeutung einer sich an ökologisch angepaßte Bekämpfungsverfahren orientierenden Unkrautforschung.

Durch die Herbizidanwendungen wurde die Verunkrautungsstärke auf den gewünschten Deckungsgrad herabgesetzt. Bei alleinigem Herbizideinsatz wurde innerhalb der zweijährigen Versuchsdauer keine Bodenbearbeitung durchgeführt. Damit wurde zugleich die Verletzung von Stämmen und Wurzeln der Zitrusbäume stark reduziert, eine Ertragsverminderung durch mechanische Schäden dieser Art wurde verhindert.

Ober- und unterirdische vegetative Entwicklung der beiden Gräser *C. dactylon* und *S. halepense* wurden durch die Varianten "Bromacil" und "Glyphosat" gleichermaßen herabgesetzt. *C. rotundus* konnte sich bei alleiniger Herbizid-Anwendung etwas stärker durchsetzen, weil die angewendeten Mittel für dieses Ungras eine Wirkungslücke aufweisen (Yegen, O., 1984). Paraquat hatte keine nachhaltige unkrut-mindernde, weil dieses Mittel nicht über die Rhizome ausdauernder Unkräuter wirkt (Maier-Bode, H., 1971).

Die Anwendung von schwarzer Folie zeigte eine ausreichende wachstumshemmende Wirkung auf die vegetativen Teilen dieser drei wichtigsten Zitrus-Ungräser der Türkei (Uygur, F.N., 1985). Ein weiterer Vorteil der Folie ist ihre evaporationsmindernde Wirkung, besonders im Sommer, wodurch die Bewässerungshäufigkeit reduziert werden kann. Im Winter kann die Folie vor Frost schützen : Wie festgestellt werden konnte, ist die Temperatur unter der Folie ca. 1-5 °C höher als auf offener Bodenoberfläche.

Eine Verwendung der Folie über zwei Jahre hinaus war unter den hier gegebenen Bedingungen nicht möglich. Vor einem breiten Einsatz von Folien in der Praxis sollten Möglichkeiten der Verlängerung ihrer Nutzungsdauer, bzw. Weiterverwendungsmöglichkeiten geprüft werden.

Durch die intensive Bodenbearbeitung kann die Entwicklung von *C. dactylon* und *S. halepense* vermindert werden, nicht jedoch die von *C. rotundus*. Entscheidend zurückgedrängt werden können die beiden erstgenannten Arten jedoch mit dieser Maßnahme nicht. Auch mit den mechanischen Varianten wie Scheibeneggen- oder Grubbereinsatz konnte der Deckungsgrad der drei Arten nicht auf den gewünschten Bereich von 5-10 % herabgesetzt werden. *C. rotundus* wurde in ihrer Entwicklung anfangs durch die Bodenbearbeitungen sogar etwas stimuliert.

Mit der Anwendung von Glyphosat in Kombination mit dem Grubber-einsatz zwischen den Reihen konnte die Verunkrautung durch die drei genannten Arten unter Kontrolle gebracht werden. Mit anderen Kombinationsvarianten wurden derart positive Ergebnisse nicht erzielt.

Die Ergebnisse lassen sich auf die untenstehenden Empfehlungen für eine praktische Unkrautbekämpfung im Zitrusanbau zusammenfassen. Zugleich werden wichtige zukünftige Ziele in der Unkrautforschung der Türkei deutlich :

- Wenn möglich, sollte man auf mechanische Maßnahmen verzichten, sie zumindest aber auf den Einsatz des Grubber in Kombination mit Herbiziden reduzieren
- Die auch in Zukunft gegebene Notwendigkeit der Untersuchung von Herbiziden auf ihre unkrutunterdrückende Wirkung wird betont. Einen besonderen Stellenwert hat hier die Prüfung von Mitteln in jungen Zitruspflanzungen
- Neuere Bekämpfungsmethoden wie Bedeckung mit Folien sollten weiter untersucht und gegebenenfalls im Zitrusanbau als angepaßte Unkraut-

bekämpfungsmethoden eingesetzt werden.

ÖZET

TURUNÇGİLLERDE YABANCI OTLANMANIN UYGULANAN YABANCI OT MÜCADELE YÖNTEMLERİNE BAĞIMLILIĞININ ARAŞTIRILMASI

Cynodon dactylon (L.) Pers., **Cyperus rotundus** L. ve **Sorghum halepense** (L.) Pers. Türkiye turunçgil alanlarının en önemli üç yabancı otudur. Bu çalışmanın amacı, toprak işleme yöntemleri, herbisit uygulamaları ile bunların kobinasyonlarının ve siyah naylonla örtme gibi toplam 14 variantın bu üç türün toprak altı ve toprak üstü gelişmesine etkisinin araştırılmasıdır.

Siyah naylon ile toprak yüzeyini kaplama ve Bromacil + Glyphosat uygulamalarında bu devamlı yabancı otların gelişmesinde önemli derecede bir gerileme saptanmıştır. Kültüvator ve diskaro ile yapılan çok sık uygulamalar bu üç yabancı ot türünü azaltmış ancak yeterince etkili olmamıştır. Mekanik mücadelenin Glyphosat ile kombinasyonu genelde bu türlerin kontrolünde yetersiz olmuş, bunlardan sadece olumlu sonucu ise Kültüvator + Glyphosat uygulaması vermiştir.

Sonuçlar göstermiştir ki, turunçgilde yabancı ot kontrolü açısından tek başına yapılan mekanik bir mücadeleden mümkün olduğunca vazgeçilmelidir. Siyah naylonla yapılan uygulamada, araştırmaya devam edilmelidir. Özellikle genç bahçelerde uygulanabilecek herbisitler de araştırılmalı ve testlenmelidir.

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Die Ermittlung der Übertragungshäufigkeit von **Spiroplasma citri** Saglio et al. durch **Circulifer opacipennis** (Lethierry) anhand exponierter Indikatorpflanzen im Zitrusanbauggebiet an der Südostmittelmeerküste der Türkei*

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ZUSAMMENFASSUNG

In der vorliegenden Arbeit wurde die Übertragungshäufigkeit von Spiroplasma citri Saglio et al. durch Circulifer opacipennis (Lethierry) (Homoptera : Cicadellidae) mit Hilfe der exponierten Indikatorpflanze Catharanthus roseus L. an verschiedenen Standorten der Çukurova (kilikische Ebene) in den Jahren 1988 bis 1990 untersucht. Durch die Bonitur der Vektorpopulation wurde außerdem ein Zusammenhang zwischen Übertragungshäufigkeit und Auftreten des Vektors hergestellt.

Die Ergebnisse zeigten, daß es zwischen den Jahren stark schwankende Übertragungshäufigkeiten bzw. Infektionsraten von 11 % im Jahre 1988, ein bis zwei Prozent im Jahre 1989 und keiner Infektion im Jahre 1988, ein bis zwei Prozent im Jahre 1989 und keiner Infektion im Jahre 1990 gab. Ein Einfluß der geographischen Lage oder der Entfernung zu mit S. citri infizierten Zitrusanlagen auf die Infektionshäufigkeit war nicht festzustellen.

Es konnte gezeigt werden, daß die jährlichen Schwankungen in der Übertragungshäufigkeit durch eine in den Jahren unterschiedliche Einwanderungsrate des Vektors C. opacipennis in die C. roseus - Parzellen bedingt war. Im Jahre 1988 wurden noch durchschnittlich bis zu 10,3 C. opacipennis pro Boniturtermin erfaßt, während in den folgenden Jahren nur noch ein bis zwei Individuen bonitiert wurden.

EINLEITUNG

Der Zitrus zählt zu den wichtigsten Agrarprodukten der Çukurova (kilikische Ebene) an der Südostmittelmeerküste der Türkei. Seit Mitte der 70 er Jahre ist es in dieser Region mit großer staatlicher Unterstützung zu einer

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beträchtlichen Erweiterung der Zitrusanbaufläche gekommen. Zeitgleich mit dieser Flächenerweiterung war ein epidemieartiges Auftreten der Zitrusstauche, hervorgerufen durch den Prokaryonten *Spiroplasma citri* Saglio et al. (Mycoplasmatales : Spiroplasmataceae), zu beobachten (ÇAĞLAYAN 1987).

Diese Krankheit führt neben starken Ertragsausfällen zu einer Verminderung der Fruchtqualität. ÇAĞLAYAN et al. (1990) sehen in der Verwendung infizierten Pfropfmaterials und dem Anbau empfindlicher Sorten die Haptursachen für die epidemieartige Ausbreitung der Krankheit. Demgegenüber berichten CALAVAN et al. (1969), daß eine Verbreitung von *S. citri* durch Okulation zwar möglich, die Übertragungsrate jedoch sehr schwankend und im allgemeinen gering ist. So beobachtete CALAVAN in der Çukurova schon 1969 die Verbreitung der Zitrusstauche durch Insektenvektoren in Baumschulen (zit. nach CALAVAN & BOVE 1989).

Der bedeutendste Vektor der Zitrusstauche im Mittelmeergebiet ist *Circulifer opacipennis* (Lethierry) (Homoptera : Cicadellidae) (FOS et al. 1986, KERSTING 1991, KERSTING & ŞENGONCA 1991). Über deren jährliches Auftreten in diesem Gebiet ist jedoch wenig bekannt (BAŞPINAR 1990). Darüber hinaus fehlen Kenntnisse über die Übertragungshäufigkeit von *S. citri* durch *C. opacipennis* in verschiedenen Jahren, die das epidemieartige Auftreten der Zitrusstauche Mitte der 70 er Jahre in der Çukurova erklären könnten.

Ziel der vorliegenden Untersuchungen war es daher, mit Hilfe der exponierten Indikatorpflanze *Catharanthus roseus* L. die Übertragungshäufigkeit des Erregers der Zitrusstauche durch *C. opacipennis* an verschiedenen Standorten in aufeinanderfolgenden Jahren zu erfassen. Durch die Bonitur der Vektorpopulation auf diesen Standorten wurde außerdem versucht, einen Zusammenhang zwischen Übertragungshäufigkeit und Auftreten des Vektors herzustellen.

MATERIAL und METHODE

Die Ermittlung der Übertragungshäufigkeit von *S. citri* durch *C. opacipennis* erfolgte von 1988 bis 1990 an drei bzw. zwei verschiedenen Standorten in der Çukurova.

Die Versuchspartellen wurden an verschiedenen Orten der Çukurova in unterschiedlicher Entfernung zu mit *S. citri* infizierten Zitrusanlagen angelegt. Zwei Flächen befanden sich im inneren Teil der Çukurova, ca. 40 km entfernt von der Küste auf den Versuchsfeldern der Universität Çukurova in Balcalı. Eine dieser Flächen lag am Obstrand einer 12 Jahre alten, stark mit Zitrusstauche infizierten Süßorangenanlage (*Citrus sinensis* (L.) Osb., "Frost Washington

Navel") (Balcalı I), eine andere ca. 4 km entfernt vom nächsten Zitrusgarten am östlichen Hochufer des Seyhan-Stausees auf freiliegendem Feld (Balcalı II). Der dritte Versuchsstandort befand sich am Nordrand einer ebenfalls stark mit Zitrusstauche infizierten, ca. 10 Jahre alten Süßorangenanlage ("Frost Washington Navel") des staatlichen Obst- und Gemüsebauforschungsinstituts in Alata unmittelbar an der Küste am westlichen Rand der kilikischen Ebene (Alata) (Abb. 1). Aufgrund der klimatischen Einheitlichkeit des Untersuchungsgebietes wurden in den Jahren 1989 und 1990 nur noch die beiden Standorte in Balcalı beibehalten.

In der ersten Aprilwoche wurden je 100, im insektensicheren Gewächshaus angezogene, ca. 10 cm hohe *C. roseus* -Pflanzen in 16 m² große Parzellen an den verschiedenen Standorten ausgepflanzt.

Über die gesamte Vegetationsperiode, in den ersten beiden Jahren von April bis Oktober, im Jahre 1990 jedoch nur bis August, erfolgte eine visuelle Bonitur der Pflanzen auf einen

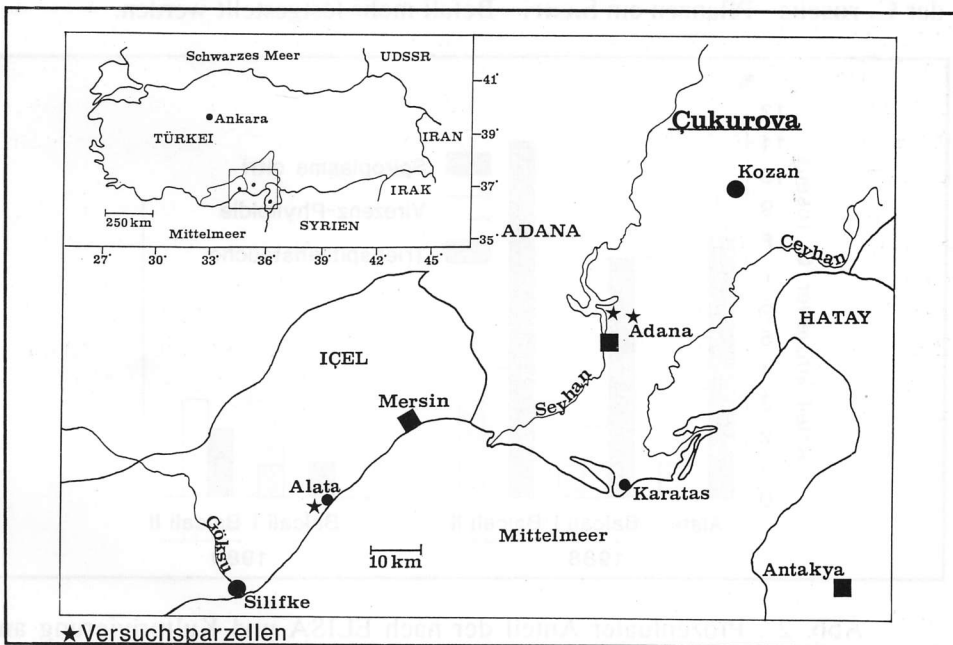


Abb. 1 : Geographische Lage des Çukurova - Gebietes sowie der Standorte der Versuche

S.citri - Befall. Blattproben aller symptomzeigender *C. roseus* wurden entnommen und im Labor mit ELISA (SAILLARD & BOVE 1983) sowie durch Kultivierung (BOVE et al. 1983) auf den Erreger hin untersucht (KERSTING et al. 1991).

SPIROPLASMA CITRI

Zur Feststellung des Auftretens von *C.opacipennis* fanden in den Jahren 1988 bis 1989 im Zeitraum April bis September und 1990 von April bis August in der ersten und dritten Woche eines Monats standardisierte D-VAC-Bonituren statt., Dazu wurden die Parzellen drei Minuten lang in größtmöglicher Bodennähe mit dem D-VAC abgesaugt. Die Probenahmen erfolgten jeweils zwischen 10.00 und 12.00 Uhr.

ERGEBNISSE

Die ersten Syptome eines *S.citri* - Befalls der Indikatorpflanzen konnten in jedem Jahr in der letzten Juniwoche beobachtet werden. Im Versuchsjahr 1988 zeigten sich maximal 11 % der *C.roseus* mit dem Erreger der Zitrusstauche infiziert (Balcali II). Im folgenden Jahr sank die Infektionsrate auf bis zu 1,0 % am Standort Balcali I stark ab (Abb. 2). Im Jahre 1990 konnte sogar bei keiner der *C. roseus* - Pflanzen ein *S.citri* - Befall mehr festgestellt werden.

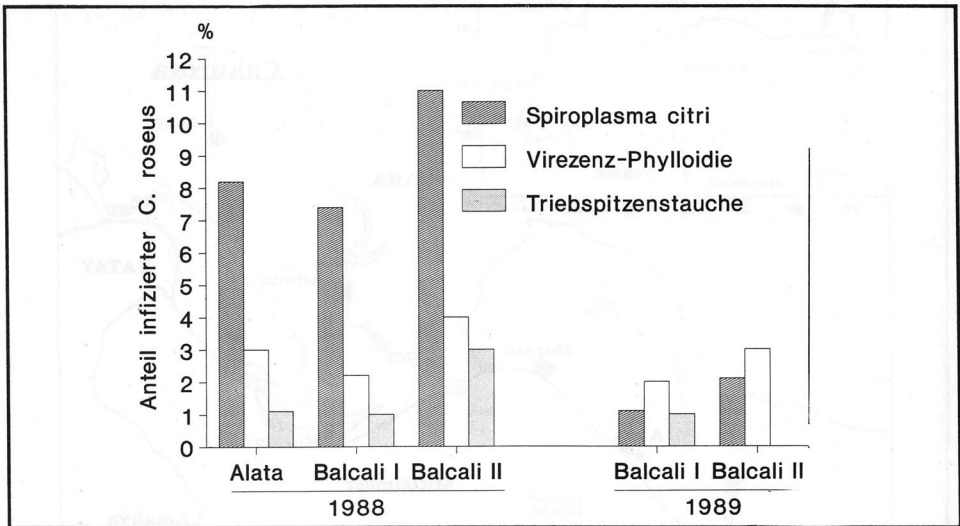


Abb. 2 : Prozentualer Anteil der nach ELISA und Kulturivierung an *Spiroplasma citri*, Viresenz-Phylloidie und Triebspitzenstauche erkrankten *Catharanthus roseus* an verschiedenen Standorten im Jahre 1988 und 1989

Im Gegensatz zu den Unterschieden bei der Infektionsrate in den verschiedenen Jahren gab es kaum Abweichungen zwischen den Versuchsstandorten. Die Standorte in Alata und Balcali I wiesen 1988 nur etwas geringere Infektionsraten auf als der Standort Balcali II der mehrere Kilometer

von Zitrusgärten entfernt lag. Im folgenden Jahr war dieser Sachverhalt, bei allgemein geringerer Infektionsrate, ebenfalls festzustellen (Abb. 2).

Neben den an *S.citri* erkrankten *C.roseus* wurden noch weitere Krankheitsbilder an den Versuchsstandorten beobachtet. Ein Teil der Indikatorpflanzen zeigte Viresenz-Phylloidie-Symptome, bei der sich Blüten unter Vergrünung zu Laubblättern zurückentwickeln während andere Pflanzen eine Triebspitzenstauche aufwiesen. Im Jahre 1988 traten auch hier die höchsten Infektionsraten auf, wohingegen der Befall im folgenden Jahr an allen Standorten stark abnahm (Abb. 2). Im Jahre 1990 waren solche Krankheitsbilder nicht mehr festzustellen.

C. opacipennis zeigte auf den *C. roseus* - Parzellen einen ähnlichen Einwanderungsverlauf (Abb. 3). Ein deutliches Maximum der Einwanderung trat in allen drei Untersuchungs Jahren im Mai auf. Der Einwanderungsverlauf zeigte hingegen zwischen den Jahren große Unterschiede. So waren im Mai 1988 mit durchschnittlich bis zu 10,3 Individuen deutlich mehr *C.opacipennis* erfaßt worden als in den beiden folgenden Jahren. Im Jahre 1989 und 1990 wurden maximal ein bzw. zwei Individuen in den D-VAC-Bonituren registriert.

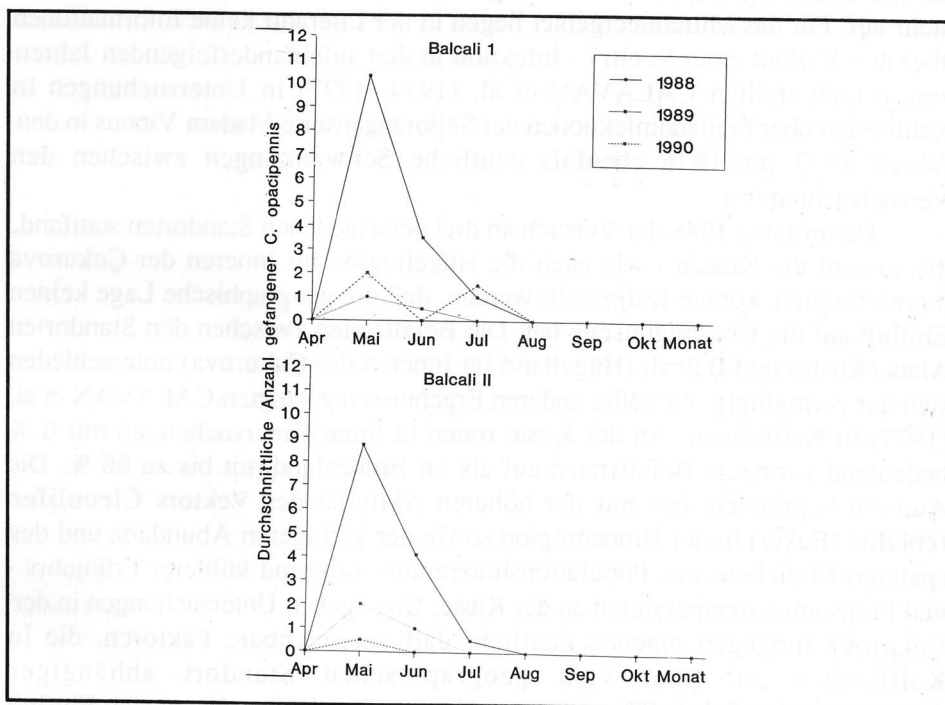


Abb. 3 : Einwanderungsverlauf von *Circulifer opacipennis* auf *Catharanthus roseus* an den Standorten Balcali I und II in den Jahren 1988 bis 1990

DISKUSSION

Mit Hilfe der Indikatorpflanze *C. roseus* war es möglich, die Übertragungsintensität von *S. citri* durch *C. opacipennis* in den verschiedenen Jahren zu ermitteln.

Die vorliegenden Untersuchungen zur Übertragungshäufigkeit ergaben im Versuchsjahr 1988 Infektionsraten von bis zu 11 % der Indikatorpflanzen. Mit diesem Ergebnis vergleichbare Befallsraten von 16 % ermittelten BOVE et al. (1979) in Marokko. Von sehr viel höheren Infektionsraten berichten CALAVAN et al. (1977), die in Kalifornien bis zu 86 % der exponierten *C. roseus* mit dem Zitrusstaucherreger infiziert fanden. Einen deutlich geringeren Anteil befallener *C. roseus* erwähnt BAŞPINAR (1990), der von je 100, in den Jahren 1985 und 1986 exponierten Indikatorpflanzen, nur eine einzige mit dem Zitrusstaucherreger infiziert fand.

In den eigenen Untersuchungen sanken die Infektionsraten auf den Indikatorparzellen in den folgenden Jahren deutlich ab; so traten im Jahre 1989 nur maximal zwei und im Jahre 1990 gar keine an *S. citri* erkrankten Pflanzen mehr auf. Für das Mittelmeergebiet liegen in der Literatur keine Informationen über den Verlauf einer *S. citri* - Infektion in den aufeinanderfolgenden Jahren vor, jedoch stellten CALAVAN et al. (1974, 1977) in Untersuchungen in Kalifornien über Freilandinfektionen der Süßorangensorte Madam Vinous in den Jahren 1973 bis 1976 ebenfalls deutliche Schwankungen zwischen den Versuchsjahren fest.

Da im Jahre 1988 der Versuch an drei verschiedenen Standorten stattfand, die sowohl die Küsten - wie auch die Hügelregion im Inneren der Çukurova repräsentierten, konnte festgestellt werden, daß die geographische Lage keinen Einfluß auf die Übertragungsrate hat. Die Befallsraten zwischen den Standorten Alata (Küste) und Balcalı (Hügelland im Inneren der Çukurova) unterschieden sich nur geringfügig. Zu völlig anderen Ergebnissen gelangten CALAVAN et al. (1977) in Kalifornien. An der Küste traten in ihren Untersuchungen mit 6 % bedeutend geringere Befallsraten auf als im Binnenland mit bis zu 86 %. Die Autoren begründen dies mit der höheren Aktivität des Vektors *Circulifer tenellus* (Baker) in der Binnenregion sowie der geringeren Abundanz und des späteren Erreichens des Populationsmaximums aufgrund kühlerer Frühjahrs- und Frühsommertemperaturen an der Küste. Die eigenen Untersuchungen in der Çukurova hingegen machen deutlich, daß vergleichbare Faktoren, die in Kalifornien zu einer vom geographischen Standort abhängigen Übertragungshäufigkeit führen, nicht vorliegen. Desgleichen konnte ein Einfluß der relativen Nähe zu infizierten Zitrusanlagen auf die Infektionsrate nicht festgestellt werden.

Hingegen zeigt die Betrachtung der Abundanzdynamik des Vektors *C.*

opacipennis, daß die in den Jahren zu beobachtende Abnahme des Anteils infizierter *C. roseus* eindeutig auf einen Abfall der Populationsdichte dieser Cicadellidenart zurückgeführt werden kann. Auch BOVE (1986) führt in seinen Untersuchungen die hohe Infektionsrate der *C. roseus* - Pflanzen auf eine hohe Abundanz von *C. opacipennis* zurück, die in seinen Gelbtafelfängen in den Parzellen eine sehr häufig vorkommende Art war. Dadurch die D-VAC-Fänge nahezu die gesamte Cicadellidenpopulation von den Parzellen entfernt wurde, zudem eine Vermehrung der *C. opacipennis* auf *C. roseus* nicht zu beobachten war, wurde im engeren Sinne nicht der Populationsverlauf ermittelt, sondern die Migration der Cicadellide auf die Indikatorpflanze. Es war also 1989 und 1990 eine geringere Zuwanderung dieser Art in den Parzellen zu beobachten. Die Abnahme des Zufluges im Mai könnte an der höheren Mortalität der überwinterten Weibchen aufgrund der kühlen Witterung in den Wintern 1989 und 1990 gelegen haben. Eine Migration von *C. opacipennis* war in den folgenden Monaten nicht mehr zu beobachten, wodurch der starke Abfall der Population zum Sommer hin erklärbar wird.

Auf allen Versuchsstandorten und in allen Versuchsjahren konnten ca. fünf bis sechs Wochen nach Erfassung der ersten *C. opacipennis* die ersten *C. roseus* - Pflanzen mit *S. citri* - Symptomen beobachtet werden. Nach KALOOSTIAN et al. (1975) treten nach einer Vektorinfektion die ersten Krankheitsbilder auf *C. roseus* innerhalb von fünf Wochen auf. Bei suboptimalen Temperaturen dürfte dieser Zeitraum eher noch länger sein (CALAVAN & BOVE 1989), so daß man in den eigenen Versuchen von einer im Mai erfolgten Infektion der Indikatorpflanzen ausgehen kann.

Zusammenfassend kann festgestellt werden, daß in der Çukuröva von einer jährlich stark schwankende Übertragungshäufigkeit auszugehen ist. Diese Schwankungen gehen einher mit einer unterschiedlich hohen Populationsdichte des Vektors *C. opacipennis*, so daß mit einer Erfassung der jährlich auftretenden inokulativen Vektorpopulation eventuell die Infektionsgefahr für Zitruspflanzen abgeschätzt werden kann.

ÖZET

İNDİKATÖR BİTKİ KULLANARAK GÜNEY DOĞU AKDENİZ
TURUNÇGİL BÖLGESİNDE *Spiroplasma citri* SAGLIO ET AL.'NİN
VEKTÖR BÖCEK *Circulifer opacipennis* (Lethierry) İLE TAŞINMA
BAŞARISININ SAPTANMASI

Bu çalışmada indikatör bitki *Catharanthus roseus* L.'u kullanarak turunçgil stubborn hastalığının etmeni olan *Spiroplasma citri* Saglio et al.'nin vektör böcek *Circulifer opacipennis* (Lethierry) ile taşınma başarıları 1988 ile

1990 yılları arasında Çukurova Bölgesinin çeşitli yörelerinde araştırılmıştır. Aynı zamanda vektör popülasyonunun saptanması sırasında yakalanan birey adedi ile **S. citri**'nin taşınma başarısı arasında bir ilişki kurulmaya çalışılmıştır.

Araştırma sonuçları vektör böceğin **S. citri**'yi taşıma başarısının yıldan yıla çok değiştiğini ve bu başarının diğer bir deyişle indikatör bitkilerdeki infeksiyon oranının 1988'de % 11, 1989'da % 1 olduğunu ve 1990 yılında da hiç bir infeksiyonun olmadığını göstermiştir. Yörenin coğrafi durumu yada hastalıklı turunçgil bahçesinin deneme yerine uzaklığı ise taşıma başarısına bir etki yapmamıştır.

Vektörün **S. citri**'yi taşıma başarısı üzerine en büyük etkinin, vektörün popülasyon yoğunluğu olduğu bulunmuştur. Taşıma başarısının yüksek olduğu 1988 yılında ortalama 10,3, bunu izleyen ve başarının düşük olduğu 1989 yılında ise sadece 1,0 vektör saptanmıştır.

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