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Chemical Control and Determination of Fungal Causal Agents of Wilt Disease of Onion in Tekirdağ Province

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ABSTRACT

This research was carried out in Tekirdağ central province which is an important onion production area. The determination of the ratio of the existence and pathogenicities and chemical control of fungal agents of wilt disease in onion were investigetad in this area. Fungal agents of wilt disease, ***Fusarium oxysporum*** *Schl.. 42.91 % and 53.55 %,* ***Botrytis allii*** *Munn 23.88 % and 10.07 %,* ***Fusarium acuminatum*** *(Ellis) Everh. 16.05 % and 10.82 % were determined both in onion roots and bulbs respectively. It was confirmed, after the pathogenicity tests, that* ***B. allii*** *(92.86 %),* ***F. oxysporum*** *(68.08 %),* ***F. acuminatum*** *(38.61 %) were the pathogens. As a result of fungicide applications against the disease, it was found that the fungicides contain benomyl and vinclozolin inhibited both root rot and rot on bulbs in onion when applied to onion sets and sets + foliage. The results are being recorded for the first time in Türkiye except for the rates of existence of* ***Fusarium*** *spp in onion rotts.*

INTRODUCTION

Wilt disease which causes economical losses in many different plants, have been also known for onion for a long time. For several countries it was reported that among wilt disease agents, **Fusarium oxysporum** f. sp. **cepae** caused pre-and post emergence death and basal rot (Naik and Burden, 1981; Takakuwra **et al.**, 1981; Lacy and Roberts, 1982; Kodama, 1983)and **Botrytis** spp. caused neck rot (Barnoczkine-Stoilova, 1984; Stewart and Mansfield, 1984), **Sclerotium cepivorum** caused white rot (Coley-Smith **et al.**, 1987), **Pyrenochaeta terrestris** caused pink root rot (Ferrera, 1990) in onion. Additionaly **Rhizoctonia solani** and **Pythium butleri** were also recorded to be the agents of the disease (Shuag and Rana, 1984). To date, only a limited number of reports have appeared, on wilt disease of onion in Türkiye (Öz, 1984). The method used for the determination of the wilt disease agents, previously, was the inoculation of wounded bulbs or soaking the sets and seedlings in spore suspensions, from which **F. oxysporum** and **B. allii** were found to be the pathogens (Stewart and Mans-

field, 1984; Alderman **et al.**, 1985; Presley, 1985; Szolay and Barnoczki-Stoilova, 1987; Roberti **et al.**, 1989).

Spraying only seeds or onion sets or leaves with different fungicides were recommended to control neck rot and basal rot by **Botrytis** spp. and **F. oxysporum** respectively (Ashour **et al.**, 1980; Hajdu and Nagyimre, 1980; Rod and Janyska, 1980; Naik and Burden, 1981).

During the last decades, although the wilt disease of onion was widespread in Tekirdağ, the disease did not receive enough attention from researchers. The objectives of this work were to determine fungal causal agents of the wilt disease and their pathogenicities, as well as to investigate the control methods by different fungicide applications.

MATERIALS and METHODS

1. Survey and isolation of fungi from diseased plants.

In 1993, the surveys were performed during the cultivation period of onion (February-August) in one location from a total of 20 onion fields. The diseased plant samples were collected with 15 day intervals and the isolations were made from bulbs and roots.

Small pieces of infected tissues (1-2 mm) were removed from roots and bulbs and washed in tap water, then in sterile distilled water and blott dried on sterile paper towels, then they were cultured on water agar medium. Fungi species which could not be identified on water agar medium were further cultured on Potato Sacchorose Agar (PSA). The number of different fungi growing on the media was determined for roots and bulbs seperately by using microscope.b The chi-square analysis was carried out on the data. Of the isolate population, a total of 22 isolates, 10 of **Fusarium oxysporum**, 10 of **F. acuminatum** and 2 of **Botrytis allii**, were chosen randomly for pathogenicity tests.

2. Pathogenicity test.

Plastic pots in 12 cm diameter, sterilized soil (1/3 soil + 1/3 sand + 1/3 manure) and a local variety the cv. Firuzköy, which is known to be sensitive to wilt disease agents were used in the test. Fungi isolated from the roots and bulbs were grown on PSA in 8.5 cm diameter petridishes. When the cultures of **Fusarium oxysporum, F. acuminatum** and **Botrytis allii** extended to the edges of the petridishes they were removed together with the agar and each culture was placed in a seperate pot and were immediately covered by a layer of sterile soil in 1 cm thikness. Surface sterilized, five onion sets were planted to the pots and an additional layer of sterile soil in 3 cm thickness was placed over the sets. Pots were watered with tap water as required.

3. Fungicide applications

The cultivar Firüzköy and the fungicides containing the following active ingredients were applied in doses (as preparation) given in bracktes, the first dose being applied to leaves and the second to onion sets: benomly, 50 %, WP, (60 g/100 1 water, 200 g/100 kg), vinclozolin, 50 %, WP, (75 g/100 1 water, 100 g/100 kg) and mancozeb, 80 %, WP, (200 g/100 1 water, 200 g/100 kg). The following methods were used in the application of the fungicidies: a. spraying the onion sets (SS) b. spraying the foliage but not the onion sets (SF) c. spraying both the foliage and the sets (SS + SF). For the onion sets treatments using powder formulated fungicides, the sets firstly, were moistened, then the fungicidies were sprinkled down evenly on the sets which were immediately mixed well. The foliage was sprayed twice, first at the 3-4 leaf stage, the second 14 days later.

The randomised block experimental design with three replications,.each of which containing 120 sets in plots of 4 m2, was used. The rots ratio, which were for the bulbs and the roots, was determined by counting the number of rotten bulbs and bulbs carrying rotten roots seperately for each onion sample during the vegetation period as well as at the time of harvest.

RESULTS

1. The rotting caused by various wilt disease agents were observed in the onion roots and bulbs. Also, pre-and post-emergence stage death of the sets occured in the field. The outer leaves of the diseased plants were observed, at the 4-5 leaf stage, to turn yellowish but the healthy leaves remained green. When the diseased plants were removed from the soil and examined, the roots were observed to have turned brown. However, some plants had rots on the bulbs although their roots were healty. The rots on the bulbs were in the form of neck rot and basal rot which eventually resulted in undevoloped or small bulbs. The fungi detected in the bulbs and roots and their rates of the existence are given in the Table 1.

Table 1: The rates of the existence of the fungi detected in onion roots and bulbs

	Number of plants	F. oxy (%)	F. acum. (%)	B. allii (%)	Sap. Fung. (%)	Total Fun. (%)
Roots	536	42.91	16.05*	23.88*	3.73	86.57**
Bulbs	536	53.55**	10.82	10.07	2.05	76.49

* $p < 0.05$ ** $p < 0.01$

F. oxy.: *Fusarium oxysporum*; F. acum.: *Fusarium acuminatum*; *B. allii: Botrytis allii;* Sap. Fung.: Saprophyte Fungi; Total Fun.: Total Fungi

Fusarium species were isolated in rotten roots and bulbs of onion. Among them, **F. oxysporum** was present at the highest rate being 53.55 % and 42.91 % in bulbs and roots respectively. Its rate of existence in bulbs was found to be statistically significant. **F. acuminatum** was present at a higher rate in rotten roots (16.05 %) than rotten bulbs.

Botrytis allii was also isolated represanting a higher ratio in the rotten roots (23.88 %), than in the rotten bulbs (10.07 %).

Saprophyte fungi had a rather low ratio for both roots and bulbs. All fungi were present in a significantly high rate of 86.57 % in rotten roots.

2. It was determined, from the pathogenicity tests, that the causal agents of the disease were **Botrytis allii** ranking the highest; **F. oxysporum** the second and **F. acuminatum** the third (Table 2). It was concluded that the three fungi could directly be transmitted to unwounded onion sets from soil.

Table 2: Pathogenicity of the isolated fungi

Fungi	Number of isolates	Pathogenicity (%) Min.	Max
B. allii	2	0	92.86*
F. oxysporum	10	0	68.08
F. acuminatum	10	0	39.61

* Mean of 3 pots with 5 plants in each

3. The incidence rates of bulb rot from the fungicide applications experiment are given in the Table 3. As seen in the Table 3, the mean lowest rate of bulb rot (1.73 %) belongs to vinclozolin. Benomyl is in the second rankt, at the same times, rate of the

Table 3: The incidence rates of bulb rot (%)*

Applications	Fungicides			Control
	Vinclozolin	Benomly	Mancozeb	
SS	2.01	2.58	16.38	20.56
SF	2.03	5.12	13.04	29.20
SS + SF	1.15	1.73	10.09	29.31
Mean	1.73 d	3.17 c	13.39 b	26.36 a

* Each value is the mean of three replications.
SS: Spraying the onion sets, SF: Spraying the foliage

bulb rot (3.17 %) is not too high. Both vinclozolin and benomyl are significantly more effective than mancozeb. Although application and interaction effects are not significant, bulb rot is lower in SS + SF treatment of benomly and vinclozolin than SS and SF treatments.

The incidence rates of root rot are also given in the Table 4.

The lowest rate of root rot was obtained from the SS + SF (1.14 %) mand SS (2.58 %) treatments of benomly. Vinclozolin ranks the second for the same applications. The root rot has significantly higher ratios in SF applications than the SS and SS + SF when benomyl and vinclozolin were applied.

Table 4. The incidence rates of root rot (%)*

Applications	Fungicides**			Control
	Vinclozolin	Benomly	Mancozeb	
SS	2.86 b	2.58 b	8.05 b	34.58
SF	8.39 a	6.27 a	15.94 a	32.74
SS + SF	3.16 b	1.14 b	4.04 c	21.55
Mean	4.81	3.34	9.34	29.62

* Each value is the mean of three replications. ** Means within each column value followed by the same letter are not significantly different (p: 0.05) according to LSD test.
SS: Spraying the onion sets, SF: Spraying the foliage

DISCUSSION

Fussarium spp. were found to be the most common agent on both rotten and bulbs of onion. In Türkiye, Öz (1984) isolated **Fusarium** spp. (**F. oxysporum**, 21.78 %., **F. acuminatum** 5.94 %) from onion roots only. However, this research has recorded the existence of **Fusarium** spp. in onion bulbs for the first time in Türkiye. The rates of existence of **F. oxysporum** and **F. acuminatum** (42.91 % and 16.05 % respectively) in the roots were found higher than the ratios previously reported by Öz (1984). In other countries it was reported that **F. oxysporum** caused basal rot, pre-and post emergence death in onion (Takakuwra **et al.**, 1981; Naik and Burden, 1981; Kodama, 1983).

The rate of existence of **B. allii** was reported also for the first time in onion roots and bulbs in Türkiye during the vegetation period, however there are earlier reports that **Botrytis** spp. caused neck rot on onion bulbs in the field abroad (Barnoczkine-Stoilova, 1984; Stewart and Mansfield, 1984) and under storage conditions (Ishizaka and Yanagita, 1981; Maude **et al.**, 1984; Presley, 1985; Özmen, 1991).

Any other species reported in other countries was not observed (Shuag and Rana, 1984; Coley-Smith **et al.**, 1987; Ferrera, 1990).

Microscobic characteristics of the **Fusarium oxysporum** Schl., **F. acuminatum** (Ellis) Everh. and **Botrytis allii** Munn were also similar to the previous descriptions (Karaca, 1974; Soran, 1975; Kranz **et al.**, 1977; Presley, 1985).

Among the isolated fungi, **B. allii** became the pathogen at the highest ratio. **B. allii** should also be taken into consideration since its pathogenicity was high and sporulation was observed directly on the bulbs. In other countries pathogenicity tests for **F. oxysporum**, involved soaking intact onion sets or seedlings into spore suspension, and for **B. allii** the inoculation was realised by treating wounded bulbs with spor suspension (Stewart and Mansfield, 1984; Szolay and Barnoczki-Stoilova, 1987; Roberti **et al.**, 1989). It was determined, however, in the present research that these agents (**Fusarium oxysporum, F. acuminatum, B. allii**) expressed their pathogenicity on onion set especially after they were directly inoculated into soil instead of the plant material itself which was determined for the first time in Türkiye. The method was previously employed for pathogenicity tests of **Fusarium spp.** on melon (Soran, 1975).

In the control experiments, bulb and root rot in onion were lower for SS (spraying onion sets) and SS + SF (spraying the onion sets+spraying both the foliage and the sets) with benomly and vinclozolin. In the only foliar spraying application without SS treatment, the rate of root rot was especially higher.

Earlier studies concentrated only on seed or onion sets or foliar spraying to control wilt disease in onion (Ashour **et al.**, 1980; Ali and Shabrawy, 1980; El-Shehaby **et al.**, 1987; Naik and Burden, 1981; Kodama, 1983). In these studies, it was noticed that onion sets or seedlings spraying with procymidone (El-Shehaby **et al.**, 1987), benomly + mancozeb (Naik and Burden, 1981) benomly (Kodama, 1983) and benomly and carbendazim (Ali and Shabrawy, 1980) inhibited **B. alllii and F. oxysporum** in onion. In our research, root rot in particular was lower in SS + SF treatment with benomly, bulb rot, in the same application with benomly and vinclozolin.

In conclusion, it is recommended that spraying the onion sets (with powder formulated fungicides) or spraying the onion sets+spraying the foliage with benomly or vinclozolin could be applied effectively against wilt disease of onion. The later is more advisable if the disease is more intense.

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ÖZET

TEKİRDAĞ İLİNDE SOĞANDA SOLGUNLUK HASTALIĞI FUNGAL ETMENLERİNİN TESPİTİ VE KİMYASAL SAVAŞIMI

Bu çalışmada, soğan üretiminin büyük önem taşıdığı Tekirdağ ili merkezde solgunluk hastalığı fungal etmenlerinin bulunma oranları, patojenisiteleri ve kimyasal

kontrol olanakları araştırılmıştır. Solgunluk hastalığı fungal etmenlerinden **Fusarium oxysporum** Schl.köklerden % 42.91 ve yumrulardan %53.55 oranında, **Botrytis allii** Munn. köklerden %23.88 ve yumrulardan % 10.07 oranında, **Fusarium acuminatum** (Ellis) Everh. ise köklerden % 16.05 ve yumrulardan % 10.82 oranında izole edilmiştir. Patojenisite testlerinde, **B. allii** (% 92.86), **F. oxysporum** (% 68.08) ve **F. acuminatum** (% 38.61)'un patojen oldukları tespit edilmiştir. Bu hastalığa karşı yapılan fungisit uygulamaları sonucunda benomly ve vinclozolin aktif maddeli fungisitlerin, arpacık ilaçlaması ve arpacık+ yaprak ilaçlaması uygulamalarında hem kök çürüklüğü hem de yumru çürüklüğünü önledikleri belirlenmiştir.

Bu araştırmada, **Fusarium** spp'nin soğan köklerinden izolasyonu dışındaki diğer tüm bulgular Türkiye için ilk kayıttır.

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Anastomosis Groups of **Rhizoctonia solani** Kühn and Binucleate **Rhizoctonia** Isolates from Various Crops in Türkiye

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ABSTRACT

*One hundred fifty three **Rhizoctonia** isolates were obtained from various crops in 9 provinces of Türkiye. Of these isolates, 60 % were multinucleate and 40 % were binucleate. Anastomosis tests showed that the multinucleate **Rhizoctonia solani** isolates were from one of these groups; AG-1, AG-2 type 1, AG-2, AG-3, AG-4, AG-5, AG-9, AG-10, and that the binucleate **Rhizoctonia** belonged to AG-A, AG-E, AG-F, AG-G, AG-I and AG-K. In this study, among the **R. solani** groups; AG-1, AG-9 and AG-10, and all the binucleate **Rhizoctonia** groups were recorded for the first time in Türkiye.*

INTRODUCTION

Rhizoctonia solani Kühn (teleomorph **Thanatephorus cucumeris** (Frank) Donk) is a common pathogen of various crops, however there is not any study on binucleate **Rhizoctonia** (teleomorph **Ceratobasidium** spp. Rogers) in Türkiye.

Rhizoctonia is divided into two major groups, multinucleate and binucleate. Multinucleate **R. solani** isolates are grouped by anastomosis between hyphae into AG-1 to AG-10 and a bridging isolate AG-BI (Ogoshi, 1975; Kuninaga **et al.**, 1978; Homma et al., 1983; Neate and Warcup, 1985; Carling **et al.**, 1987; Ogoshi **et al.**, 1990). Binucleate **Rhizoctonia** isolates are also grouped by anastomosis into AG-A to AG-S (Ogoshi **et al.**, 1979; Ogoshi **et al.**, 1983; Sneh **et al.**, 1991).

The objective of this study is to determine the anastomosis groups of **R. solani** and binucleate **Rhizoctonia** isolates from various crops in Türkiye.

MATERIALS and METHODS

Collection and isolation

Isolates of **Rhizoctonia** species were obtained from various crops in nine provinces (Table 1) of Türkiye during 1990-1994. Small pieces of plant tissues were surface-disinfected in 0.5 % sodium hypochlorite for 1 min. and placed on 1.5 % water agar containing 50 mg/l streptomycin sulfate (Demirci and Döken, 1993). After 48-72 hours

of incubation at room temperature, reisolation from the margin of each colony were placed on water agar or potato dextrose agar (PDA). **Rhizoctonia** isolates obtained in this way were transferred on PDA slants and stored at 10 °C.

Table 1: The sampling provinces in Türkiye and the anastomosis groups of **Rhizoctonia solani** isolates.

Province	No. of isolates	Anastomosis Groups (AG)							
		AG-1	AG-2 type 1	AG-2 type 2	AG-3	AG-4	AG-5	AG-9	AG-10
Erzurum	36	1	7	-	9	15	3	1	-
Iğdır	20	-	2	-	-	17	1	-	-
Erzincan	13	-	-	-	-	12	-	-	1
Trabzon	10	-	2	1	-	6	1	-	-
Artvin	5	-	1	-	-	4	-	-	-
Amasya	3	-	-	-	-	3	-	-	-
Tokat	2	-	1	-	1	-	-	-	-
Rize	2	-	-	-	-	1	1	-	-
Gümüşhane	1	-	-	-	1	-	-	-	-
Total	92	1	13	1	11	58	6	1	1

Identification of **Rhizoctonia**

Isolates were identified on the basis of characteristics of their vegetative hyphae (Ogoshi, 1975), whether binucleate or multinucleate (Bandoni, 1979), requirement for thiamine (Rovira **et al.**., 1986) and hyphal anastomosis with tester isolates.

Anastomosis group typing

Multinucleate and binucleate isolates were paired with tester isolates of **R. solani** (AG-1, AG-2 type 1, AG-2 type 2, AG-3, AG-4, AG-5, AG-6, AG-7, AG-8, AG-9, AG-10 and AG-BI) and binucleate **Rhizoctonia** (AG-A, AG-Ba, AG-Bb, AG-C, AG-D, AG-E, AG-F, AG-G, AG-I, AG-K, AG-L, AG-N- AG-O, AG-P and AG-Q) respectively. Mycelial disks (5 mm in diameter) from tester and unknown isolates were placed 2-4 cm apart on 2% water agar in 9 cm diameter petri dishes (Parmeter **et al.**, 1969).Anastomosis was determined with phase-contrast microscope without staining after the petri dishes were incubated for 48-72 hours at room temperature.

RESULTS and DISCUSSION

In this study total 153 isolates were obtained. Ninety two of these isolates were multinucleate R. **solani** and 61 were binucleate **Rhizoctonia**. Among the isolates of R. **solani** recovered from 16 plant species in nine provinces, one was AG-1, 13 were AG-2 type 1, one was AG-2 type 2, 11 were AG-3, 58 were AG-4, six were AG-5, one was AG-9 and one was AG-10. The provinces and the plant species from which **R. solani** isolates recovered are given in Table 1 and 2 respectively according to the anastomosis groups of these isolates. AG-2 type 1, AG-2 type 2, AG-3, AG-4 and AG-5 identified in the present investigation have been isolated previously from various plant species in Türkiye (Tuncer and Erdiller, 1990; Demirci and Döken, 1993).

Table 2: The origin and the anastomosis groups of 92 isolates of **Rhizoctonia solani** from Türkiye.

Host	Source	Anastomosis Groups (AG)							
		AG-1	AG-2 type 1	AG-2 type 2	AG-3	AG-4	AG-5	AG-9	AG-10
Alfalfa	Root	-	2	-	-	7	-	-	-
Apple seedling	Stem	-	-	-	-	3	-	-	1
Bean	Hypocotyl	-	-	-	-	8	2	-	-
Bean	Seed	1	-	-	-	-	-	-	-
Cabbage	Root	-	2	1	-	2	-	-	-
Carrot	Tuber	-	-	-	-	1	-	-	-
Corn	Root	-	3	-	-	2	1	-	-
Cucumber	Root	-	-	-	1	3	-	-	-
Eggplant	Root	-	2	-	-	1	-	-	-
Garlic	Stem	-	-	-	-	1	-	-	-
Leek	Stem	-	1	-	-	-	-	-	-
Pepper	Hypocotyl	-	1	-	-	13	-	-	-
Potato	Stem	-	1	-	9	1	1	-	-
Rye	Root	-	-	-	-	1	-	-	-
Squash	Root	-	1	-	-	-	-	-	-
Sugar beet	Tuber	-	-	-	-	2	1	1	-
Tomato	Root	-	-	-	1	13	1	-	

However in the present study existence of AG-1, AG-9 and AG-10 isolated from bean, sugar beet and apple seedling respectively were determined in Türkiye for the first time. Among them AG-9 and AG-10 were only obtained in U. S. A. both from soil and various plants (Carling **et al.**, 1987; Ogoshi **et al.**, 1990). In Türkiye besides the known hosts of the previously determined anastomosis groups, in this study AG-2 type 1 was isolated from alfalfa, cabbage, corn, eggplant, leek and squash, AG-type 2 from cabbage, AG-3 from cucumber and tomato, AG-4 from apple seedling, bean, cabbage, carrot, corn, cucumber, eggplant, garlic, rye and sugar beet, AG-5 from corn and tomato, for the first time. When the provinces where the isolates were obtained and their host plants are taken into consideration then it can be interpreted that AG-4 is the most widely distributed anastomosis group and has a broad host range.

The provinces and the plant species from which binucleate **Rhizoctonia** isolates obtained are shown in Table 3 and 4 respectively in respect to the anastomosis groups of these isolates. Fifteeen of the 61 isolates were AG-A, three were AG-E, seven were AG-F, three were AG-G, three were AG-I and 30 were AG-T. In this study the anastomosis groups of binucleate **Rhizoctonia** and the plants they isolated were found for the first time in Türkiye. On the other hand from Table 3 and 4 it can be seen that AG-A and AG-K are widespread and their host ranges are broad.

Table 3: The sampling provinces in Türkiye and the anastomosis groups of binucleate **Rhizoctonia** isolates.

Province	No. of isolates	Anastomosis Groups (AG)					
		AG-A	AG-E	AG-F	AG-G	AG-I	AG-K
Erzurum	30	3	2	-	2	1	22
Erzincan	11	-	1	2	-	2	6
Trabzon	11	6	-	3	1	-	1
Iğdır	2	-	-	2	-	-	-
Artvin	2	2	-	-	-	-	-
Amasya	2	2	-	-	-	-	-
Gümüşhane	3	2	-	-	-	-	1
Total	61	15	3	7	3	3	30

Table 4: The origin and the anastomosis groups of 61 isolates of binucleate **Rhizoctonia** from Türkiye.

Host	Source	Anastomosis Groups (AG)					
		AG-A	AG-E	AG-F	AG-G	AG-I	AG-K
Alfalfa	Root	-	-	-	-	-	5
Bean	Hypocotyl	3	1	-	-	2	11
Cabbage	Root	1	-	3	-	-	-
Corn	Root	1	-	-	-	1	2
Cucumber	Root	1	-	-	1	-	-
Eggplant	Root	-	-	2	-	-	-
Leek	Stem	1	-	-	-	-	-
Onion	Stem	-	-	1	-	-	-
Parsley	Root	2	-	-	-	-	-
Pepper	Hypocotyl	3	-	1	-	-	-
Potato	Stem	2	2	-	1	-	8
Rye	Root	-	-	-	-	-	1
Sguash	Root	-	-	-	-	-	1
Sugar beet	Tuber	-	-	-	-	-	1
Sweet basil	Root	-	-	-	-	-	1
Tomato	Root	1	-	-	1	-	-

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ÖZET

ÇEŞİTLİ KÜLTÜR BİTKİLERİNDEN ELDE EDİLEN **RHIZOCTONIA SOLANI** KÜHN VE İKİ NUKLEUSLU **RHIZOCTONIA** İZOLATLARININ ANASTOMOSİS GRUPLARI

Türkiye'de 9 ilden toplanan çeşitli kültür bitkilerinden 153 **Rhizoctonia** izolatı elde edilmiştir. Bu izolatların %60'ının çok nukleuslu, % 40'ının iki nukleuslu olduğu saptanmıştır. Anastomosis testleri sonucunda çok nukleuslu olan **Rhizoctonia solani** izolatlarının AG-1, AG-2 tip 1, AG-2 tip 2, AG-3, AG-4, AG-5, AG-9 ve AG-10'a, iki

nukleuslu **Rhizoctonia**'ların ise AG-A, AG-E, AG-F, AG-G, AG-I ve AG-K'a ait oldukları belirlenmiştir. **R. solani**'nin AG-1, AG-9 ve AG-10 ile iki nukleuslu **Rhizoctonia**'ların çalışmada saptanan gruplarının Türkiye'de bulunduğu bu çalışma ile ilk kez ortaya konmuştur.

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Biology and Chemical Control of **Melampyrum arvense** L. In Cereal Fields in Central Anatolia

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ABSTRACT

***Melampyrum arvense** L., a hemiparasite weed of cereal belongs to Scrophulariaceae, and is a common weed in Bolu and Kütahya provinces.*

This weed is common in winter cereal fields. It sometimes appears in stuble. It prefers the soils have lime, clay and lime. In the survey, made in 1976 the average density of the weed in Kütahya province was 17, 19/m^2.

*In the literature review it has not been found a chemical against **M. arvense**. Chemicals were used against this weed in this experiment, in practice are common and broad spectrumed ones.*

*In the years 1978 and 1979 in order to find effective chemicals against this weed Bromoxynil + MCPA (1.60 L/ha), Bromoxynil + MCPA + Methabenzthiazuron (I, 60 L+2 kg/ha), Methabenzthiazuron + Diclorprop (2,5 kg/ha) and 2,4-D isooctyl ester + 2,4, 5, -T isooctyl ester (I, 45 L/ha) were applied to the wheat fields at tillering stage in Kütahya. The experimental design was Randomised Block Design with 3 replicates. Bromoxynil + MCPA and Bromoxynil + MCPA + Methabenzthiazuron gave 95.4% and 91.8% in 1978, 99. I% and 91.8% effectivenes in 1979 respectively. Methabenzthiazuron + Diclorprop and 2,4-D isooctyl ester+2,4,5-T isooctyl ester were not sufficiently effective. Bromoxynil + MCPA + Methabenzthiazuron mixture showed 23.3% phytotoxicity on barley in 1979. For the control of **M. arvense** Bromoxynil + MCPA was only chemical that would be given to practice.*

INTRODUCTION

Melampyrum arvense is an annual, broad leaved and semi parasite weed of cereal(Hegi, 1918). It takes nutrient from wheat roots and from soil. Plants exert inhibitors before haustoria are developed. This weed is common in winter cereal fields which have rainfall. It prefers the soils with lime and clay and lime. It was found at 2340 m altitute in Erzurum province (Herbarium of Botanical Department, Ankara Üniv.). It was wide spread in Gazel Yakup and Çoban villages of Kütahya-Tavşanlı district and in Elmacık, Kükürt villages of Kütahya-Ayvalı district. It was found in winter cereal (Davis, 1978). Güncan (1982) determined the density of **M. arvense** in Erzurum-Kars high

plateau as an average of 0.0335% as weight and 0,1056% as numerical value, and as a maximum of 0,1683% as weight and 0.5307% as a numerical value. In addition at Karadeniz mountain pass, the ratio of **M. arvense** that mixes into wheat as weight is 0.0129%.

About 800 ha field infested with **M. arvense.** In the literature review it was not found a recommended chemical against **M. arvense.** Chemicals were used in the experiments againts this weed were common and broad spectrumed ones.

MATERIALS and METHODS

For survey at every 10kms the car was stopped and **M. arvense** /m^2 was counted in fields and arithmetical average was culculated.

Mixture of **M. arvense** and wheat seeds were sown in institute experiment field in October. In every 10Mm^2 plot three rows mixture of seeds (130 g) were sown. Replicate number was 9. After emergence each week and each 15 th day phenology were followed.

Experiments were carried out in Ağaçköy of Kütahya in the years 1978 and 1979. In the experimental area the canopy of **M. arvense** was 40% in 1978, 20% in 1979. Experiments were conducted in randomised block design with 3 replicates. Each plot size was 20 m2. Herbicides were used in experiments were Methabenzthiazuron+Diclorprop (2,5 kg/ha), 2,4, -D isooctyl ester +2,4, 5-T isooctyl ester, Bromoxynil+MCPA (1,60 L/ha),Bromxynil+MCPA+ Methabenzthiazuron (1,601+2 kg/ha). They were given in Table 1.

Table 1: Chemicals were used in 1978 and 1979 experiments

HERBICIDES				Recommended dose hectare	
Trade name	Firm	Common name	For-mulata-liton	Active ingre-dient	Preparate
Tribunil combi	Bayer	Methabenzthiazuron 17,5% + Diclorprop 50%	WP	1,68 kg	2,5 kg
Ester combi	Bayer	2,4-D isooctyl ester 34% +2,4,5-T isooctyl ester 7, 4%	Sol.	0.6 kg	1.45 L
Brominal plus	Tarkim	Bromoxynil+ MCPA=64.I%	Em.	1,025L	1.6L
Brominal plus + Tribunil	Tarkim Bayer	Bromoxynil+MCPA 64.I%+Methabenz-thiazuron 70%	Em. WP	1,025L 1,4 kg	1,6L .2 kg

In the years 1978 and 1979 herbicides were applied with a 2L 4,5 atmosphere pressured holder hand sprayer. Herbicides were used during tillering stage of wheat and 4 th leaves stage of **M. arvense**. Herbicides applied with 500L/ha water as post-emergence. Weather temperature during application was recorded. The date of post-emergence application was 11th of May 1978, evaluations were made on 29 th of May, 1978 and 15 th of June, 1978. In 1979application was made on 3rd of May, 1979, effects of herbicides evaluated on 18th of May 1979 and on 1st of June, 1979. Evaluations were made according to I-9 EWRC scale.

1-9 (EWRC) scale		
1	99.I%	Complete death
2	97.7%	
3	95.4%	
4	91.8%	
		enough
5	86.0%	
6	76.7%	
7	61.8%	
8	38.2%	
9	0.0%	no effect

RESULTS

The average of **M. arvense** in Kütahya was 17,19/m^2 as result of survey.

M. arvense overwinters on the roots of wheats by holding on root with its haustoria (Figure 1). In early March a couple of cotyledon emerged to soil surface, at the end of March 2 couple leaves in the middle of April opposite 3 couple leaves appeared. During this stage wheats were four leaved stage, after 2 and 3 couple leaves and leaves tooted at the bottom appeared in May (Figure 2). Description of **M. arvense** was made with its tooted leaves.

Before 15 th of July the weed dried completely. Capsules were opened and seeds were scattered (Figure 3). It given reddish colour to flour. Bread became indigestible. The seeds contained rhinanthine (Bonnier, 1934).

Inflorescens began from apex to downward at mid of May, Ovary had two lobes and in each lobe there was a seed (Bonnier, 1934). **M. arvense** matured at the same time with cereal seeds (Figure 3).

The effects of herbicides on **M. arvense** in 1978 were given Table 2. The mixture of Bromoxynil + MCPA + Methabenzthizuron gave 99,1% effect on **M. arvense**. Bromoxynil + MCPA gave satisfactory control (95,4%). Methabenzthiazuron + Diclorprop and 2,4-D isooctyl ester +2,4,5-T isooctyl ester did not control this weed.

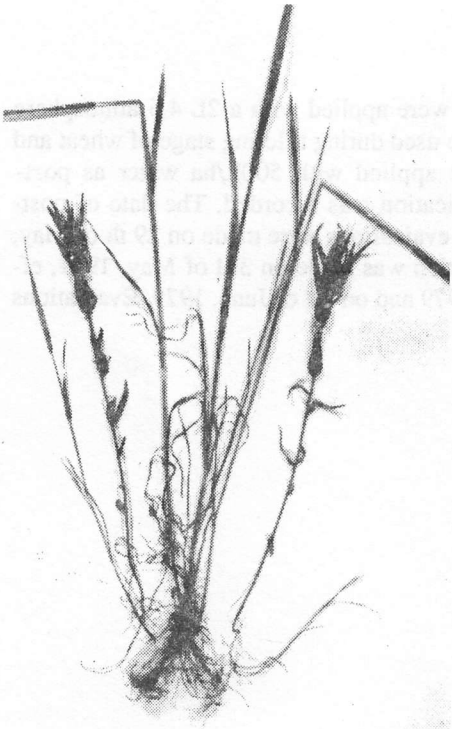

Figure 1. The parasitism of **Melampyrum arvense**

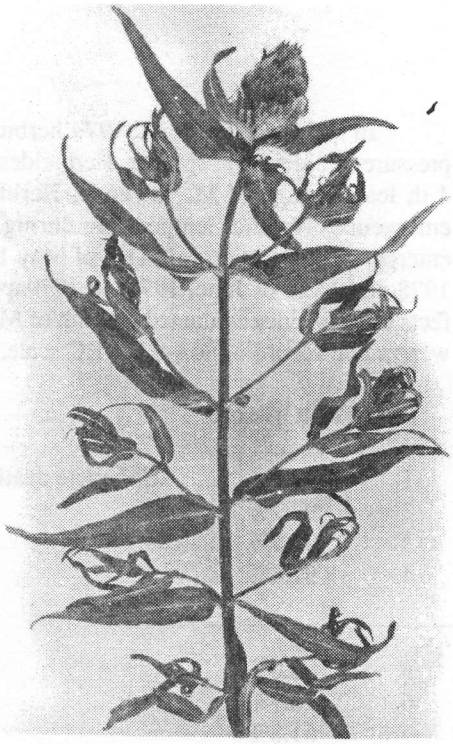

Figure 2. Leaves of **Melampyrum arvense**

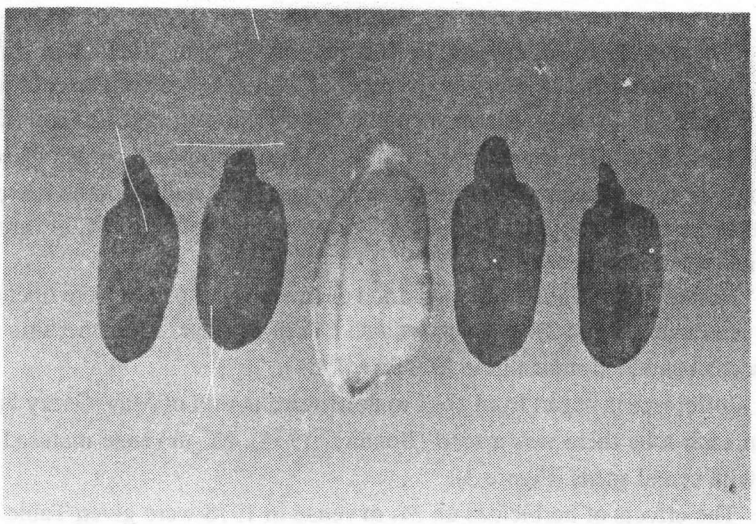

Figure 3. Seeds of **M. arvense** and seed of wheat (in the middle)

Table 2: The effects of herbicides on **M. arvense** in 1978

Herbicides	Rate/ha	Percent effect on M. arvenSe	Phytotoxicity %
Bromoxynil + MCPA = 64.1%	1,60 L	95.4	0
Bromoxynil + MCPA = 64.1% + Methabenzthiazuron 70%	1,60 L + 2.0 kg	99.1	0
Methabinzthiazuron 17.5 + Diclorprop 50%	2.5 kg	86.0	0
2,4-D isooctyl ester 34% + 2,4, 5-T isooctyl ester 7,4%	1.45 L	61.8	0

Phytotoxicity was not observed. Same herbicides were used in 1979 against **M. arvense, Geranium tuberosum, Bifora radians.** Data were given in Table 3. Bromoxynil-MCPA, Bromoxynil+MCPA+Methabenzthiazuron gave good control **M. arvense** and **Geranium tuberosum** but 23.3% phytotoxicity was observed with Bromoxynil+MCPA+Methabenzthiazuron.

Weather temperature during application 18°C in 1978, 18,5°C in 1979.

Table 3: The effects of herbicides on **M. arvense, Geranium tuberosum, Bifora radians** in 1979.

Herbicides	Rates/ha	*M. arvense* %	*Geranium tuberosum* %	*Bifora radians* %	*Phytotoxicity* %
Bromoxinil + MCPA = 64.I%	1.60 L	91.8	95.4	0	0
Bromoxinil + MCPA + Methabenzthiazuron	1.6 L + 2.0 kg	91.8	91.8	61.8	23.3
Methabenzthiazuron 17.5%+ Diclorprop 50%	2.5 kg	76.7	61.8	0	4.6
2,4-D isooctyl ester+ 2,4,5-T isooctyl ester	1.45 L	76.7	86.0	61.8	0

DISCUSSION

Bromoxynil + MCPA 64.1% and Bromoxynil + MCPA 64.1% + Methabenzthiazuron 70 % gave 95.4% and 99.1% effectivenesS respectively in the year of 1978. Methabenzthiazuron 17.5% + Diclorprop 50% and 2,4-D isooctyl ester 34%+2,4, 5-T isooctyl ester 7,4% gave 86%, 61.8% effectiveness respectively.

In the year 1979 Bromoxynil + MCPA 64.1 and Bromoxynil + MCPA 64.1% + Methabenzthiazuron 70% gave 91.8% effectiveness against **M. arvense**. But Bromoxynil + MCPA64.1 + Methabenhzthiazuron 70% showed phytotoxicity against barley. Methabenzthiazuron 7.5%+ Diclorprop 50% and 2,4-D isooctyl ester 34%+2,4,5-T isooctyl ester 7,4% did not give sufficient effect. According to two years trials Bromoxynil + MCPA, 64,1% was found advisible against **M. arvense**.

M. arvense was seen at plateaus like Erzurum-Kars and Kütahya plateaus. This weed was more dense in Kütahya was compared to the research of Güncan (1982).

ÖZET

ORTA ANADOLU'DA HUBUBATTA PEMPE OT (*Melampyrum arvense* L.)'UN BİYOLOJİSİ VE KİMYASAL MÜCADELESİ

Pembe ot (**Melampyrum arvense** L.) kışlık hububatın yarı paraziti (hemiparasite) bir yabancı ottur. Scrophulariaceae familyasına ait Bolu ve Kütahya'da yaygın bir yabancı ottur. Kireçli ve killi kireçli toprakları tercih eder. Kütahya'da ortalama yaygınlığı m2 de 17.19 olarak bulunmuştur.

Literatür taramasında Pembeota önerilen bir herbisite rastlanmamıştır. Denemelerde kullanılan ilaçlar geniş spektrumlu ilaçlardır. 1978 ve 1979 yıllarında Pembeot'a karşı etkili herbisitleri saptamak amacıyla Bromoxynil +MCPA64.I% (I.6 L/ha), Bromoxynil+MCPA64.I%+Methabenzthiazuron70% (I.60 L+2 kg/ha), Methabenzthiazuron 17.5%+Diclorprop 50% (2.5 g/ha) ve 2,4-D isooctyl ester 34%+2,4,5-T isooctyl ester 7.4% (I.45 L/ha) denemeye alınmıştır. Kütahya'da ilaçlar buğdayın kardeşlenme devresinde uygulanmışlardır. Denemeler tesadüf blokları deneme desenine göre 3 tekerrürlü olarak kurulmuştur. 1978 yılında Bromoxynil+MCPA 64.I% %95.4, Bromoxynil+MCPA%64.I+Methabenzthiazuron %70 %91.8 oranlarında etkili olmuşlardır. 1979 yılında aynı ilaçlar sırasıyla %99.1 ve %91.8 etkili bulunmuşlardır. Methabenzthiazuron %17.5+Diclorprop %50 ile 2,4-D isooctyl ester %34+2,4,5-T isooctyl ester %7.4 yeterli etki verememişlerdir. 1979 yılında Bromoxynil + MCPA %64.I +Methabenzthiazuron %70 %23.3 oranında fitotoksite göstermiştir. Pembeot'un kontrolu için Bromoxynil+MCPA %64.I tavsiye edilebilir bulunmuştur.

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THE NINTH CONGRESS OF THE MEDITERRANEAN PHYTOPATHOLOGICAL UNION

September 18-23, Kuşadası- Aydın, Türkiye

The Mediterranean Phytopathological Union (MPU) organizes its International Phytopathological Congress every 3-4 years in one Mediterranean Country. In 1994, the 9th Congress of the Mediterranean Phytopathological Union held at Kuşadası, Türkiye, from September 18 to 23 by Turkish Phytopathological Society in collaboration with the Mediterranean Phytopathological Union. The Papers presented during the Congress were published as proceedings. In this issue of The Journal of Turkish Phytopathology, the papers of kerynotespeakers which belong to "Advances in biotechnology applied to plant pathology", "Biological control of plant diseases", "Management of plant diseases" and "Concluding Remarks of the Congress" were presented.

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On the Application of Advanced Detection Techniques in Plant Pathology

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INTRODUCTION

The diagnosis of virus and virus-like diseases is often complicated by two very important factors, (1) because of their small size, transparent bodies etc. most of these pathogens cannot be seen with regular compound microscopes, and, due to their distribution in the diseased plant, frequently they cannot be found and observed even with electron microscope; (2) the symptoms of many diseases they cause in field are nonspecific and resemble each other and those caused on plants by many environmental factors, by insect damage or by other pathogens of the root system. Quite frequently a plant may be attacked by two or more types of symptoms. The most important aspect of such a situation is that the presence of the additional pathogens is recognized. Once this found out, the diagnosis of the disease(s) and the identification of the pathogen(s) may be possible. Especially when little is known about the disease in question, the value of less specific tests in the early testing stages has to be recognized. As with specific tests, other viruses not tested for will be overlooked, at least initially and it may be much later before undetected agents are finally implicated as contributors or the true cause of a disease problem.

Of course, several virus and virus-like citrus diseases develop very distinct symptoms in field and these diseases can be diagnosed and the pathogen identified easily and with an acceptable degree of accuracy. Often however, the detection of plant pathogens relies on the development of specific diagnostic tools, which can be used to identify an agent, to study the spatial and temporal spread of the disease, and to monitor the sanitary situation of a crop in a region.

BIOLOGICAL ASSAYS

In spite of the great progress that has been made in the development of detection tools, such techniques do not exist for many of economic important plant pathogens just because the causal agent is not characterized yet. Citrus blight is an excellent example for this situation. Although reported from Florida in the 1890's the cause of this destruc-

tive disease is not known up to now. As for blight, for many serious citrus diseases suitable detection tools are not available. In these cases, pathogens can be only diagnosed by biological assays. Now many indicator plants exist for the detection of almost all known virus and virus-like diseases within a reasonable short time (ROISTACHER 1991).

The same is to say for new appearing diseases, e.g., citrus chlorotic dwarf virus in Türkiye (KORKMAZ **et al.**, 1994a, 1994b, ÇINAR **et al.**, 1995). For this whitefly-transmitted virus, biological indexing is the only possibility for diagnosis till the pathogen has been characterized and suitable and rapid detection tools have been developed, which may, however, require several years. Most important, biological assays are often more sensitive than most of the biochemical detection tools and avoid the use of hazard chemicals. On the other hand, biological assays are time consuming (up to several years), restricted to the only a few samples, and expensive since they require a lot labor work.

To overcome these problems, rapid, easy to handle and reliable detection tools for the use in plant pathology have been developed within the last 20 years. Such rapid detection methods are now used for quarantine purposes and larger-scale surveys as well as for sanitation programs but still together with biological assays.

SEROLOGY ASSAYS

The obviously first dramatic progress in the development of detection tools was the introduction of enzyme-linked immunosorbent assay (ELISA) in plant pathology by CLARKS & ADAMS (1977). Properly used, ELISA is a sensitive, accurate and rapid detection method. It is especially effective where large number of samples must be assayed and where results are needed rapidly.

Several variations of ELISA have been developed in which polyclonal or monoclonal antibodies bound to solid phase are used to trap the antigen and enzyme labeled antibodies combined with specific substrates to visualize the trapped antigens. The sensivity of ELISA has been greatly enhanced through secondary antibodies and biotin-streptavidin linkages. Each of these ELISA systems differs in specificity, sensitivity and handling so that suitable ELISA systems have to be established for each pathogen and in each laboratory depending on its specific aims and facilities.

One has to assume that with the selection of monoclonal antibodies that react either to generically shared or type specific epitopes, it is now possible to define, enumerate, and systematize epitopes among pathogens, isolates, or epitopes within pathogen groups as well as to characterize the antibodies produced against these epitopes. Thus, the use of ELISA to characterize virus strains, to follow translocation of particular isolates in cross-protection studies, to detect pathogens in plants transformed with virus coat potein genes, and to monitor infection in challenged transgenic plants, grew dra-

matically in the last years (e.g., GARNSEY **et al.** 1989, PERMAR **et al.**, 1990, CAMBRA **et al.**, 1993, ZEBZAMI **et al.**, 1993).

The drawback of conventional ELISA is the need of much sample preparation and its long assay time. This technique requires well-equipped laboratories, and once that the pathogen is trapped in the plate continuous processing is necessary. Immunoblot procedures are a form of ELISA where the antigen is bound to a membrane that has protein binding properties and the antigen is detected directly or indirectly with a labeled probe. An improved immunoblot technique was recently described by LIN **et al.** (1990) where the tissue sample is blotted directly on the membrane. This direct tissue blotting assay (DTBIA) is rapid and sensitive, requires no sample preparation and provides information on distribution and localization of the pathogen in the host tissue (PERMAR **et al.** 1992, GARNSEY **et al.** 1993). Blotted membranes can be stored for up to six months before assay at room temperature without any lost in sensitivity and thus, DTBIA provides a very convenient method to ship a sample for testing. This method is extremely convenient for field survey work in remote sites and for laboratories with lack of sophisticated facilities (MAKKOUK **et al.** 1994).

NUCLEIC ACID HYBRIDIZATION

Serological techniques detect pathogens by specific recognition of coat or membrane proteins by specific antibodies developed in animals against these proteins. Molecular hybridization techniques detect nucleic acids by specific recognition of their nucleotide sequences. The specific pairing of the bases composing nucleic acids is the basis for the formation of hybrids (double-stranded nature) between complementary molecules and, thus, for the use of molecular hybridization as a diagnostic technique.

Most molecular hybridization detection systems base on a solid support hybridization, the sample being permanently immobilized on a membrane and use complementary DNA probes or in vitro transcribed complementary DNA labeled, e.g., radioactively or with biotin for detection. Nucleic acid hybridization developed to a very potent technique that can be used for the identification of DNA and RNA pathogens with varying degree of homology and to estimate relative amounts of nucleic acid with known homology.

Although applied for the detection of a lot virus and to the characterization of virus strains (ROSNER **et al.** 1986, SEMORILE **et al.** 1993), molecular hybridization is very important in the detection and characterization of complex pathogens like MLOs and BLO. The detection of MLOs and BLOs in suspected plants and insect vectors by DNA-probes, showed that this technique can be efficiently used to study the epidemiology of greening disease of citrus (BLO), Witches' broom disease of lime (MLO), and **Spiroplasma citri,** the causal agent of citrus stubborn disease (CSD) (BOVÉ **et al.** 1987, VILLECHANOUX **et al.** 1992, BOVÉ **et al.** 1993a, 1993b, GARNIER **et al.**

1993). This technique was proved to be more sensitive than ELISA (BOVÉ **et al.** 1987). Crush plots of insects directly onto membranes requires little sample preparation and blotted membranes can be shipped safely to another location for testing. Thus, this method is also available for researchers who have no facilities to hybridize samples.

Once suitable DNA fragments of a pathogen of interest have been cloned and sequenced, gene amplification can easily be developed if a technique more sensitive than DNA hybridization is required to detect pathogens.

POLYMERASE CHAIN REACTION

In the short history of molecular biology, few new techniques provide such a dramatic progress in detection of plant pathogens approached both to basic and applied research. The capacity to amplify specific segments of DNA, made possible by the polymerase chain reaction represents such a development.

The polymerase chain reaction (PCR) is an in vitro method for the enzymatic synthesis of specific DNA sequences, using two oligonucleotide primers that hybridize to the opposite strands and flank the region of interest in the target DNA. PCR provides a simple, ingenious method to exponentially amplify specific DNA sequence (SAIKI et al. 1988). Repetitive series of cycles involving template denaturation, primer annealing, and the extension of the annealed primers by DNA polymerase results in the accumulation of a specific fragment defined by the primers.

The use of PCR grew rapidly in plant pathology with the introduction of **Thermus aquaticus** (Taq)-DNA polymerase in 1988, which reduces PCR costs and allows automated thermal cycling. In a recent review on PCR and plant disease diagnosis, HENSON and FRENCH (1993) listed more than 50 plant pathogens including viroids, viruses, MLOs, bacteria, fungi and nematodes in which amplification of target sequences by PCR have been already achieved. However, since the date of this report the number of pathogens for which PCR detection methods have been developed incerased dramatically. This technique has been adapted for different virus and virus-like citrus diseases such as citrus tristeza virus (CTV), several citrus viroids and the citrus stubborn disease pathogen (YANG **et al.** 1992, LEVY and HADIDI 1993, SAILLARD **et al.** 1993, NOLASCO **et al.** 1993, 1994).

PCR offers several advantages compared to serologic or hybridization methods:

(i) organisms need not to be cultured before their detection by PCR

(ii) PCR is very sensitive with the theoretical potential to detect a single target molecule

(iii) PCR is rapid and adaptable to many uses.

Depending on the choice of primers, PCR enables the detection of a single pathogen or many members of a group of related pathogens. However, one important drawback of PCR is that amplified products have to be visualized by agarose gel electrophor-

esis and the obtained results have to be often confirmed by southern blot. This is not only time consuming but hinders processing many plant samples. To solve this problem, recently spectrofluorometry for the detection of amplified products was achieved, which is also reported to increase significantly PCR sensitivity (NOLASCO **et al.** 1993).

Another shortcome of conventional PCR is the difficulty to confirm the presence of viable pathogens in diseased plants that produce positive PCR reactions but from which the pathogen cannot be cultured (HARTUNG **et al.** 1993). Detection methods that would detect pathogen antibodies in addition to, or instead of, their nucleic acid are possibly better indicators of pathogen viability. Furthermore, as the presence of crude plant extract often inhibits PCR and decreases the sensitivity, a procedure called immuno-capture PCR has been developed which simplifies sample preparation and enhances the specificty and sensitivity of conventional PCR (WETZEL **et al.** 1992, NOLASCO **et al.** 1993, SAILLARD et al. 1993). In immuno-capture PCR, pathogens present in crude plant extracts are captured by polyclonal antibodies coating the wall of the tube in which the PCR is carried out. After the capture step, the plant extract is poured off and the pathogens trapped in the tube are submitted to PCR reaction without DNA isolation. Immuno-capture of pathogen cells by coated antibodies and elimination of the plant extracts increased the sensitivity by a factor of 10 (**S. citri**) to 250 (Plum Pox virus) compared to conventional PCR (WETZEL **et al.** 1992, SAILLARD **et al.** 1993).

Already there exists a possibility to combine immuno-capture and RAPD fingerprinting, which requires specific antibodies but not specific primers, providing a powerful tool in studies on host-pathogen inactions and in screening and selecting plant genotypes resistant to certain pathogens. Because of its sensitivity, PCR will continue to be used diagnostically to detect genoms or antigens of microorganisms that are scare, difficult to culture or not to culture at all. Pathogen screening of seeds, micropropagated tissue culture, or vegetatively propagated plants will be assisted by PCR or related techniques. Research to date has just begun to develop specific applications, and it is likely that the setting up of PCR-based diagnostic tests will grow rapidly soon. As PCR methods for the detection of plant pathogens become available, more research will focus on using these as tools to study pathogen populations, biology, ecology and epidemiology, variability, and host-pathogen interactions. The recently achieved possibility to perform PCR, including IC-PCR and IC/RT-PCR in a single ELISA plate with the quantification of amplified products by spectrofluorometry instead of their separation by electrophoresis will lead to full automation of this technique, being not more complicated and not more laborious than conventional ELISA (NOLASCO **et al.** 1993).

THE USE OF DETECTION TOOLS

Scientist involved in the detection and diagnosis of pathogens in diseased plants may today refer to many powerful and quick tools, adaptable for nearly all fields of in-

terest in plant pathology or protection. However, the choice of which techniques to use and in what order, and for what aim to use them is often a difficult one. This a sensitive question and a legal one as well, but it is a question need to be addressed. Of course, it is overemphasized to use PCR in a field survey on a simple virus for which adequate ELISA kits are available. On the other hand, the application of PCR is reasonable in quarantine or sanitary programs where many combined samples are tested or where only very few amounts of plant tissue are available for the detection of pathogens. The use of monoclonal antibodies in ELISA is recommendable if strains of, e.g., citrus tristeza virus (CTV) should be detected or to follow the translocation of particular CTV-isolates in cross-protection studies. It is a waste of money if only the diagnosis of CTV in the frame of an eradication program is anticipated. However, it is not the scope of the paper to discuss the problems related to the individual use of a detection tool. The question on the use of detection tools should be emphasized in a broader frame.

The application of detection methods can be separated into two major fields (Fig. 1):

(i) survey orientated approach
(ii) empirical orientated system

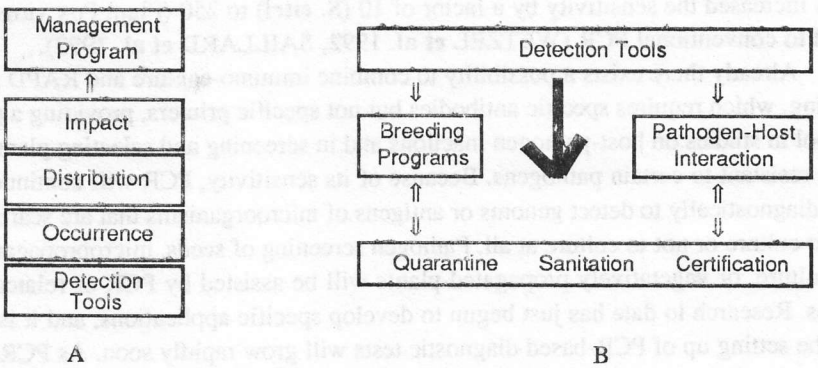

Figure 1. Flowchart presenting the principle strategies on the use of detection tools in a survey orientated approach (A) and in an empirical orientated system (B)

In the survey orientated approach, the application of detection tools is almost exclusively concentrated on the determination of a pathogen in a certain crop. Depending on the extent of such a survey, some information on the distribution of the pathogen in certain region might come out. Especially in non-developed countries the use of detection tools, most commonly DAS-ELISA, is mainly restricted to a survey orientated approach. However, it should be pointed out that field survey contribute less in developing disease management programs.

Moreover, the development of a disease management program is even not anticipated. Of course, the use of ELISA for detecting, e.g., citrus tristeza virus has permitted intensive and extensive surveys of large areas of citrus in a short time. Surveys conducted in Israel, California, Spain, Italy and recently in Cyprus uncovered many CTV-infected trees. However, such surveys are of less use if not combined with the destruction of infected trees. Only a program using a reliable technique for detection, followed by eradication of trees found infected may help to control the spread of destructive diseases like CTV.

In order to establish a long term, profitable program for all levels, first a very well managed scheme having concise concepts with well-determined milestones should be put into action. An excellent example for a such concept is the citrus variety improvement program in Spain, matching exactly the idea of an empirical orientated system in plant protection (NAVARRO et al. 1988).

Controlling virus and virus-like diseases necessitate the use of healthy and high quality planting material. Like for many other crops, the production of healthy citrus trees requires the establishment of three different but related programs: quarantine, sanitation and certification. In the framework of such programs, detection assays are necessary and powerful tools, but they are really just that: tools. A main step in any citrus budwood improvement program is the selection of individual trees of different cultivars according to horticultural criteria. Indexing of those trees, by using different detection tools, will allow one to get an accurate knowledge of pathogens present in an area. In the Spanish citrus sanitation program this step provided very comprehensive information about the incidence of different diseases, the type of isolates of each disease and the detection of severe isolates of CTV introduced illegally with satsuma mandarins imported from Japan. Indexing of mother trees was also the basis for the establishment of the collection of virus and virus-like isolates which is actively being used for research on these pathogens (NAVARRO, 1993).

The substantial different outcome of the survey orientated approach compared to the empirical orientated system is clearly demonstrated for Sharka disease of stone fruits in Italy and CTV in Turkey (Fig. 2). Sharka disease was probably introduced by plum pox infected budwood imported from Yugoslavia into the coastal region of southern Italy in 1987. During a survey in 1988 eighteen-thousand trees were indexed by ELISA for Sharka disease and 762 trees in 12 orchards were found infected with the plum pox virus. All infected trees were eradicated.

Within a few years the scientists involved in the program could rise public and producer awareness about this disease resulting in the establishment of a certification program for all stone fruit viruses in Apulia. All viruses found in stone fruits were eradicated by thermotherapy and with this virus free material, scientist, nurserymen and growers convinced the government to enact a national law for mandatory certification

for stone fruit viruses. This law became reality for all of Italy in 1993 (ROISTACHER 1993).

This program established in Italy for stone fruit viruses will help to prevent not only the indroduction of diseases into the stone fruit production area but prevent their dissemination into the mainstream of budwood supply. The stone fruit virus sanitation and mandatory certification program is wonderful example of an empirical orientated plant protection management system.

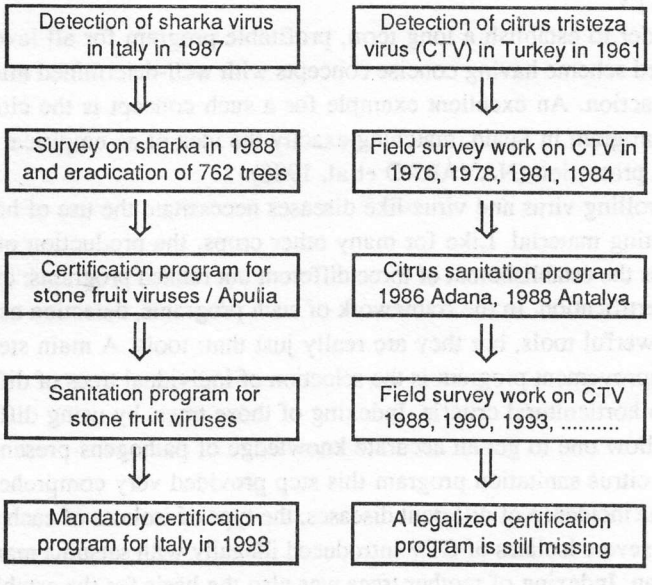

Figure 1. Different outcomes of the survey oriantated approach and the empirical orientated system in plant protection: the Sharka disease control program in Italy and the citrus tristeza detection "program" in Turkey

Just the opposite is to say for the control of citrus tristeza virus in Turkey. In spite of that CTV was already reported in 1961, no serious efforts were made to establish a short term eradication or long term eradication or long term certification program (Fig. 2). As it is typical for a survey orientated approach, several studies proved the occurence of CTV again and again, however, not even one CTV infected tree was eradicated within the last 34 years. Also the establishment of two budwood improvement programs Turkey in 1986 (Adana) and 1988 (Antalya) did not change much. Both programs have the capacity to provide plentifully of virus free citrus budwood and nursery trees of more than 70 economic imported citrus varieties. However, due to the lack of a legalized mandatory certification program in Turkey less than 10% of all nursery trees

planted are virus-free. Although the Turkish sanitation laws allow the eradication of virus infected trees, there is no one to find bringing these laws into action. Nevertheless, there are still scientists surveying on CTV in Turkey providing not much more information than that there is CTV in Turkey. It is noteworthy to know that the same scientists strongy address that the Turkish citrus industry would greatly benefit from the eradication of infected trees and the establishing of a certification program. Plant protection representatives, scientists and citrus growers and politicians in Turkey are obviously not aware of the tristeza threat they face due to their specific sanitary situation and traditional horticultural practice. Still it is not understood that it is important to maintain the percentage of contaminated trees below the critical level of 1%. Various specialists estimate that once the percentage of tristeza infected trees has reached 2% or more, the epidemic is likely to expand inexorably.

Scientists in Turkey and Italy have access to powerful and quick detection tools, which ought to be used to improve the sanitary situation in any crop. The certification program in Italy and its lack in Turkey has nothing to do with knowledge in plant protection or access to sufficient detection tools in either of both countries but with differences in the principle idea of **plant protection.** Besides running a successful survey and eradication program for plum pox infected trees in Italy, scientist convinced the politicians of the need for legalizing mandatory certification. ROISTACHER (1993), who summarized the outstanding plum pox program in Italy stated "... **Credit must be given to those scientist who gave up their time and energy to address this practical problem instead of working on specific problems which would advanced their academic carees...**[1]". In Turkey no scientist or plant protection representative has obviously the energy or the time to do this job, may be because they are busy with surveying.

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Biological Control of Plant Diseases: A Possible Mission*

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INTRODUCTION

The first definite indications that saprophytic soil fungi such as *Trichoderma* and *Gliocladium* were responsible for the inhibition or destruction of plant pathogens like *Rhizoctonia* and *Pythium* were made by Weindling in the early 1930's. These studies remained of academic interest until the publication of Rachel Carson's Silent Spring in 1962 in which the dangers of pesticide application were brought to public notice. Shortly thereafter, plant pathologists focused on approaches to achieve biological control with reduced dependence on synthetic fungicides by: a) soil management practices (use of organic amendments, crop rotation, and sublethal chemical and physical treatments) and b) mass introduction of biological control agents to soils, seeds, foliage and even on already harvested fruits.

Remarkably large amounts of funds reaching many billions of US$ have been spent during the last 30 years not only in the research centres but also by private sectors with the aim of studying and developing sound and applicable biocontrol of several serious plant diseases. It is awkward but today if anyone of us steps into an Agribusiness pharmacy shop he/she will be able to purchase hundreds of formulations of fungicides. However there are very slim chances to spot a formulated biocontrol agent to be used for the control of a plant pathogen. But scientists are still struggling hard to find out ways of biological control although their commercial application is well behind our expectations.

Why biological control?

Is there any urgent need for alternative solutions in plant disease control? What are the major reasons?

1. Increased public, scientific and state concern over food safety caused by fungicide residues in plant produce and their impact on human health. The American Research Council in a 1987 report indicated that fungicides pose %60 of the ongological risk from the use of pesticides together

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2. Increased public, scientific and state concern over Environmental impacts caused by the use of various chemicals (water, soil pollution, etc.)

3. Development of resistance to fungicides this means loss of efficacy and eventually withdrawal from the market of very important and effective up to now fungicides.

4. Synthetic fungicides usually produce consistent performance and broad geographical utility. But there are no effective chemicals for many soilborne pathogens. Furthermore minor crops often lack registered fungicides since the market size does not justify the cost of testing, regulatory approval, manufacture and marketing.

This review paper describes certain cases for possibilities of applying biological control against plant diseases by using formulations available in the market. It also refers to the inherent difficulties which currently create pessimism but also presents evidence suggesting optimism for the future of biological control of plant diseases. Possibly there are thousands of scientists (pathologists, microbiologists, bacteriologists, biochemists or molecular biologists) working on biological control of Plant Diseases. At least most of them are convinced that regardless of the existing difficulties biological control of plant diseases, including the use of genes transferred from natural antagonists to crop plants, has a potential to become the major component of pest management of the 21st century. This could be achieved with no significant compromise in the quantity or quality of plant products, the environment or the expectations of the society because offers promise for the suppression mainly of soilborne diseases many of which are not effectively controlled by conventional chemical products.

Below are the most recognisable and studied modes of action of biocontrol agents. Mechanisms on which the whole research effort has been concentrated to develop the available biocontrol formulations but also to build the future of biocontrol of plant diseases.

Mechanisms of action of biocontrol agents

Four are the main mechanisms of action in the antagonistic activity of biocontrol agents although other mechanism may also operate.

Antibiosis

Recombinant DNA technology has revolutionised research in biological control of Plant Disesases by providing techniques to identify traits responsible for disease suppression and to clone, characterise and transfer these important biocontrol genes. In the last years application of this technology along with the use of modern analytical techniques has provided unequivocal evidence that production of antibiotics siderophores and or hydrogen cyanide are the primary mechanism of pathogen suppression in many Fluorescent pseudomonads systems. In other cases however antifungal metabolites contribute rather than account for all of the biocontrol activity of antagonistic strains of bacteria.

Parasitism

The most commonly associated disease control mechanism is the mycoparasitism of fungal pathogens.

Competition

Competition for iron is another major mechanism by which fluorescent pseudomonads may inhibit the growth of plant pathogens. They are characterised by the production of yellow-green pigments termed pyoverdines or pseudobactins that fluoresce under UV irradiation and function as siderophores. Pyoverdines produced in the rhizosphere by fluorescent pseudomonads are thought to sequester iron in a form that is unavailable to target pathogens. It is also demonstrated that competition for carbon such as in the case of saprophytic **Fusarium oxysporum** or nitrogen could be involved in the biological control of certain soilborne pathogens.

Induced Resistance

Certain Plant Growth promoting rhizobacteria may increase crop productivity by growth promotion or biological control based on the production of bacterial metabolites (antibiotics or siderophores). They may also affect host physiology by enhancing production of host defense-related compounds indicating potential of inducing disease resistance. Induced disease resistance phenomena have been also observed with saprophytic isolates of **Fusarium oxysporum.**

BIOCONTROL AGENTS

FUNGAL BIOCONTROL AGENTS

Biological control agents mainly belong to fungi including yeasts and to bacteria. As for the fungal antagonists here are the fungal biological control agents tested or used in the field with potential of formulation.

Table 1: Fungal biological control agents tested or used in the field with potential of formulation.

Antagonist	Pathogen	Disease	Host
1. *Epicoccum purpurescens*	*Sclerotinia sclerotiorum*	White mould	Beans
2. *Fusarium oxysporum*	*Fusarium oxysporum*	Fusarium wilt	Various hosts
3. *Gliocladium virens*	*Rhizoctonia solani & Pythium ultimum*	damping - off	Cotton
4. *Penicillium oxalicum*	*Pythium ultimum*	damping - off	Chickpea
5. *Rhizoctonia spp.*	*Rhizoctonia solani*	crown and root rot	Sugar beets
6. *Sporidesmium sclerotivorum*	*Sclerotinia sclerotiorum*	lettuce drop	Lettuce
7. *Talaromyces flavus*	*Verticillium dahliae*	wilt	Egglplant
8. *Trichoderma spp.*	*Rhizoctonia solani*	damping-off	Various hosts
	Sclerotium rolfsii	root rot, blight	
	Sclerotium cepivorum	white rot	Onion
	Pythium ultimum	damping-off	Peas
	Rhizoctonia solani	black scurf, stem canker	Potatoes
9. *Verticillium biguttatum*	*Rhizoctonia solani*	black scurf, stem canker	Potatoes

Table 2: Fungal antagonists or hyperparasites to Powdery mildews

A. ANTAGONIST	POWDERY MILDEW
1. Tilletia pallescens	*Erysiphe graminis f. sp. hordei*
2. Tilletidopsis sp.	*Sphaerotheca fuligenea*
3. Tilletiopsis albescens	*Sphaerotheca fuligenea*
4. Tilletiopsis minor	*Sphaerotheca fuligenea*
5. Acremonium alternatum	*Sphaerotheca fuligenea*
6. Aphanocladium album	*Sphaerotheca fuligenea*
7. Cladosporium cladosporiodes	*Erysiphe polygoni*
8. Curvularia lunata	*Erysiphe polygoni*
9. Stephanoascus rugulosus (Sporothrix rugulosa)	*Sphaerotheca fuligenea of cucumbers*

Sporothrix is the most promosing case under glasshouse conditions.

B. HYPERPARASITE	POWDERY MILDEW
1. Ampelomyces quisqualis	*Cystotheca wrightii*
2. Ampelomyces quisqualis	*Erysiphe polygoni*
3. Ampelomyces quisqualis	*Microsphaera euonimi-japonicae*
4. Ampelomyces quisqualis	*Sphaerotheca fuligenea*

AVAILABLE COMMERCIAL FUNGAL PRODUCTS

In spite of many years of research with several biocontrol fungi there are only a few formulations which have been commercialised. Fungal antagonists registered to be used or applied commercially are given in below.

1. A mixture of isolates of *Trichoderma harzianum and T. polysporum* registered for use as a mycofungicide to control tree wounding decay and wood rot. Specifically it is claimed to control *Heterobasidion annosum* and *Chondrostereum purpureum* in wounds of Ornamentals, shade trees and forest trees. The trade name is Binab T (Binab Bio-Innovation AB-Sweden) and is registered in the USA.

2. A **Trichoderma harzianum** biotype prepared by protoplast fusion called AG2 and produced by the Cornell Research Foundation in the USA protects against a wide range of soilborne pathogenic fungi on overall crops. It controls a wide range of plant pathogenic fungi including *Pythium ultimum, Rhizoctonia solani, Fusarium graminearum, Botrytis cinerea, Sclerotinia homeocarpa,* and *Guignardia bidwelli* but not *Phytophthora* spp. It is effective on a wide range of crops and can be used as a seed

treatment, a granule for in-furrow or broadcast application to soil, or as a spray to control fruit or foliar pathogens.

3. A race of *Trichoderma harzianum* called race 39 is used by Makheteshim Chemical Works Ltd. in Israel for formulation with the trade name Trichodex. This is recommended against *Botrytis* **cinerea** of tomato or grapes. The formulation is also recommended to be alternated with dicarboximides.

4. *A Trichoderma lignorum* isolate in Russia multiplied by growing the antagonist on sterilized turf is recommended for use against *Rhizoctonia solani*, *Fusarium spp.* and *Colletotrichum spp.* which cause root and hypocotyl rot of cucumbers and tomatoes. The formulations is called Trichodermin-3.

5. An isolate G1-21 of *Gliocladium virens* was the first registered fungal formulation developed in the United States. The trade name is GlioGardTM (W.R. Grace & Co. Conn.) It is a solid prill composed of fermenter-produced biomass of the isolate powdered wheat bran and sodium alginate suspension which is jelled dried and applied to greenhouse horticultural soilless growing media to prevent damping-off of seedling caused by *Pythium* and *Rhizoctonia.*

6. A formulation of *Pythium oligandrum* is produced for the suppression of *Pythium ultimum* under the trade name Polygandron in the Czech Republic. A suspension of mycelia and oospores is coated onto sugar beet seed before sowing. Alternatively *P. oligandrum* is grown on vermiculite/cornmeal and pelleted onto cress and sugar beet seed.

7. Spores of the basidiomycete *Phlebia gigantea* belonging to Hydnaceae which are rehydrated in water and poured or painted over tree stumps to prevent the spread of root rot and heart decay caused by the pathogenic basidiomycete, *Heterobasidion annosum* (PG Suspension) Great Britain.

8. *Coniothyrium minitans* a mycoparasite on sclerotia of pathogens such as *Sclerotinia* and *Sclerotium* is used to produce Micon in Russia and Coniothyrin in Hungary respectively. These formulations reduce dampingg-off disease in greenhouses and stalk rot and wilt of sunflowers caused by **Sclerotinia sclerotiorum.**

Table 3: Fungal antagonists registered to be used or applied commercially

ANTAGONIST	TRADE NAME	COUNTRY
1. Trichoderma harzianum and *T. polysporum*	Binab T	USA
2. Trichoderma harzianum	AG2	USA
3. Trichoderma harzianum	Trichodex	Israel
4. Trichoderma lignorum	Trichodermin-3	Russia
5. Gliocladium virens G1-1	GlioGard™	USA
6. Pythium oligandrum	Polygandron	Czech R
7. Phlebia gigantea	PG Suspension	Great Britain
8. Coniothyrium minitans	Micon	Russia
9. Coniothyrium minitans	Coniothyrin	Hungary

YEASTS AS BIOCONTROL AGENTS

Yeasts have been found capable of protecting fruits against postharvest rots. Some of these yeasts have been patented and tested on a large scale under commercial conditions. Attempts are now being made to develop and market antagonistic yeasts as postharvest treatments to control rots of fruits and vegetables. Table 4. refers to pathogens causing post harvest rots of tomato controlled by the yeast *Pichia quilliermondii*. The effectiveness one of these yeasts could reach almost 100%.

Table 4: Pathogens causing post harvest rots of tomato controlled by the yeast *Pichia quilliermondii*.

Pathogens controlled	Antagonist (US-7)	% Control
Rhizopus stolonifer	----	87
Botrytis cinerea	----	93
Alternaria alternata	----	91

Table 5: Pathogens causing post harvest decay of fruits controlled by the same yeast *Pichia quilliermondii*.

Pathogens controlled	HOST
Rhizopus stolonifer	GRAPES
Penicillium digitatum	GRAPEFRUIT
Penicillium expansum	PEARS
Botrytis cinerea	APPLES

BACTERIAL AS BIOCONTROL AGENTS

Many genera of bacteria have been selected from the phyllosphere or rhizosphere and identified as antagonists useful in biological control of foliar or soilborne disease of plants.

Unfortunately of these a handful is currently used in commercial agriculture.

1. *Agrobacterium radiobacter* strain K84 has been used commercially in various parts of the world for more than a decade for the biological control of crown gall disesase caused by *Agrobacterium tumefaciens*. And it is the remarkable success of K84 which has much stimulated enthusiasm in the potential of bacterial antagonists for biological control of plant diseases.

2. *Streptomyces griseoviridis* strain K-61 is the active ingredient of Mycostop developed by Kemira Espoo Finland. Mycostop is marketed in Europe primarily of for suppression of disease of Ornamentals and vegetable crops caused by *Fusarium* spp.

3. A strain of *Bacillus subtilis* was registered by USA EPA in 1992 as a product called Kodiak for use as a seed treatment for suppression of diseases of several crops caused by **Rhizoctonia solani** and *Fusarium solani*..

4. A strain of **Pseudomonas cepacia** also was registered by EPA in 1992 under the trade name Blue Circle for suppression of dumping off disease of a variety of vegetable and field crops caused by *Rhizoctonia* sp. and *Fusarium* sp.

5. A mixture of three strains of *Pseudomonas* spp. was registered by the EPA in 1992 as a product called Frostguard for the control of frost damage to several deciduous tree and vegetable crops and fire blight disease of pear and apple.

6. A strain of *Pseudomonas fluorescens* was sold as Dagger-G for suppression of dumping off disease of cotton but is no longer available commercially.

Table 6: Bacterial antagonists used commercially

ANTAGONIST	TRADE NAME
1. *Agrobacterium radiobacter* strain K84	NoGall
2. *Streptomyces griseoviridis* strain K-61	Mycostop
3. A strain of *Bacillus subtilis*	Kodiak
4. A strain of *Pseudomonas cepacia*	Blue Circle
5. A mixture of three strains of *Pseudomonas spp.*	Frostguard
6. A strain of *Pseudomonas fluorescens*	Dagger-G

DISCUSSION

After giving the high lights of the present status of biological control of plant disease I would like to summarize and underline that Successful use of biological control of plant diseases requires:

1. Effective biocontrol agents

2. Production and formulation methods that give rise to high yields of biomass consisting of appropriate efficacious propagules of high viability and stability and

3. Delivery systems that provide a conducive milieu and minimize growth of competitive microflora.

Now it is generally accepted that full employment of biological control will require the management of populations or genes of thousands if not tens of thousands of site-or disease-spesific biological control agents. Furthermore it will require changes in the institutional infrastructure, scientific resources and socioeconomic resolve to develop this still largely untapped biological and genetic resource in time to make this vision a reality in the 21 st century.

The early 2000 will be a period of substantial change in the methods used for pest management. It is logical to support the view that we cannot move immediately from our current methods to innovate ones and in parallel continue to meet production needs of managed ecosystem.

Looking ahead there are a lot of pessimistic but also several optimist views for the future of biological control of plant diseases.

Pessimistic views for the future of biological control

1. Unfortunately there are thousands of plant diseases to be controlled in thousands of plants with thousands of varieties or hybrids. It is extremely difficult and costs a lot of money to find, study, test, formulate and use commercially in the field

2. There are extreme differences among climatic conditions, edaphic parameters and cultural practices followed around the world. So, a successful antagonist in one place might fail complete elsewhere

3. The approach of the 'single bullet'

It is known that chemical companies are testing thousands of compounds with fungicidal potential. They will be pleased to find out just one which will result in the production of a new fungicide. On the contrary scientists working on biological control of plant pathogens are usually testing some tens and rarely a hundred of isolates trying to spot one capable of controlling a disease. It is obvious that the probabilities to find a successful antagonist are very slim

4. Finally there is an apparent lack of biological products or formulations able to provide convincing evidence for an effective biological control with very few exceptions.

5. Public concern of environmentalists on the use of genetically engineered products. As for the optimistic views 1. Breaking through in testing methods. It is known that the procedure of the in vitro tests with antagonists is not always working in vivo. For example antibiotic producing agents are not always efficient in preventing disease development. It is obvious that the expected breakthroughs in testing methods will accelerate research on biological control of plant disesases.

2. Intense involvement of genetic enginering people in producing resistant plants through incorporation of genes for resistance originated from antagonists may offer new possibilities of confronting plant diseases.

3. There are also hopes that Integrated disesase management will help to exploit the effectiveness of a formulation along with the limited use of effective fungicides.

4. Another promising case regards the Multifaceted Biological Control strategy applied to Biological Control of Post Harvest Diseases. Indeed Resent research data demonstrate that biocontrol of postharvest diseases is more feasible due to the controlled conditions under which it could be applied compared with other plant disease systems. Furthermore, it has been shown that a multiple mode of action approach has advantages over the use of solitary biological control method. Thus, combining antagonistic yeast *Pichia quilliermondii* which:a) competes with the pathogen **Botrytis cinerea** for nutrients, b) induces resistance responses in the host and c) parasitizes the pathogen with reduced doses of TBZ fungicide (only 10% of the recommended rate) plus various additives such as $CaCl_2$. This salt enhances the effectiveness of the antagonist and reduces the population of the yeast required to achieve control of post harvest diseases in citrus.

5. Finally successful partnership between Research Laboratories and Industry will increase the optimism.

After analysing negative and positive parameters of biological control of plant diseases let's try to refer to those factors necessary for the future success of biological control.

1. Scientists working on biological control of plant diseases are confident that there are thousands of microbial biocontrol agents already discovered or soon to be discovered in nature. An unusual problem in plant pathology relates to the fact that new interesting microbial biocontrol agents with novel mechanisms of control turn up so frequently. Until recently the tendency was to collect or screen 10,50 or at most a 100 candidate organisms, pick up one or two and then focus for the next several years on interesting mechanisms of biocontrol used by these organisms. This has been valuable in building up science but has limited progress toward use because the strains have performed inconsistently. On average, we can expect better results working with the best of a thousand than the best of 10 or 100 candidate strains.

2. One of the most important factors is funding for the required research and development. The government sources are always restricted. Private companies also invest large amounts of money. Unfortunately, without new and more efficient ways for companies to deal with the multiple niche-market products the future of biological control will depend mainly on public-supported research and extension which may not be available. One possibility for funding may be by broadening the host range of antagonists through genetic manipulation. This would expand the market size and increase the economical attractiveness of these biocontrol agents. A second possibility to increase economic attractiveness of biological control agents for private investments is through identification of traits in the agents that can be transfer to crop plants. Such traits could be use to develop elite lines of crop plants for use in either public or private plant breeding programms.

3. A great concern at present about biological control of plant diseases is expressed over the genetically manipulated biocontrol agents although transgenic plants have also been a source of public concern. Unfortunately without public support and confidence regulator agencies will be forced to move cautiously and will require more data for approvals. Furthermore private investment will be more tentative.

4. Major changes will also be required among users of biocontrol technologies.

a. More and better information will be needed on best management practices to enhance, optimise or at least conserve the benefits of indigenous biocontrol agents.

b. More and better education is needed to teach users to deliver and process this information.

c. More cooperation and coordination will be important among users, researchers, extension plant pathologists and advisors of agribusiness company representatives.

5. The users of biological control should be the strongest links together with science and education in several fronts that must advance simultaneously for full employment of this technology.

The major requirements for must users are:

- That the antagonist works successfully - is economical - for an increasing percentage of users that is economically sound and sustainable.

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Evolving Expectations for Integrated Disease Management: Advantage Mediterranea

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INTRODUCTION

As the world population becomes increasingly urbanized and unfamiliar with the concepts and practices of modern agricultural production, the mass media (newspapers, television, radio) have become the major source of public information in this area. Many of the reports which have been widely disseminated in recent years have portrayed production agriculture as relying on repeated drenching of crops and soil with highly toxic pesticides, resulting in poisonous contamination of food products and soil, air, and water resources.

Being understandably repulsed by these unpleasant images of tainted food supplies and natural resources, a large segment of the public has voiced a desire to purchase and consume products which are not sprayed with pesticides, and which have not, through their production, contributed to environmental pollution. Furthermore, concerned citizens and their governmental representatives have supported pesticide usage laws so restrictive that producers fear unnecessary economic losses due to diseases and pests.

Several agricultural production and/or pest management philosophies have emerged to address these issues, among which are organic or ecological production, sustainable or low input agriculture, and integrated pest management. Each of these philosophies aims to reduce the pesticide load in the environment to varying extents, using somewhat different approaches. To add to the philosophical diversity, each system harbors several differing schools of thought. In general terms:

- "Organic" or "ecological" production seeks to promote a harmonious, naturally balanced agroecosystem through optimization of organic materials present in the soil. Plants grown in a harmonious balance are thought to be naturally healthy and resistant to diseases and pests (1). If fungicides or other pesticides are needed, they must be derived from "natural" sources and not from a synthetic industrial process.

- "Sustainable" and "low input sustainable agriculture" are blanket terms for agricultural systems which aim to improve agricultural as well as natural resource productivity, and often include elements of social engineering of rural society (3). Reductions in pesticide use and food safety issues usually are included in these philosophies.

- "Integrated pest management" (IPM) is intended to provide an economically and environmentally viable compromise between conventional pest management systems (which may include unnecessary or excessive pesticide sprays) and organic production (where effective pest management measures may not be employed due to lack of allowable materials or philosophical aversion).

Although there has been considerable debate over the direction of IPM during the past few years, the widely accepted expectation of IPM appears to be evolving more toward "organic" agriculture than "conventional" or "status quo" farming practices. This paper will document the apparent shift in IPM philosophy with respect to managing plant diseases, and propose the rationale for such a shift generally benefiting agricultural production in Mediterranean or desert climatic zones characterized by warm temperatures and minimal precipitation during the growing season.

DIRECTIONS IN IPM

IPM has succeeded in earning widespread acceptance as the preferred basis for regional and national pest management policies by governmental agencies. As such, it has been subjected to continual pressure toward adjustment and reforging by proponents at the two poles of thought: unrestricted pesticide use (agrichemical interests) or prohibited pesticide use (environmental activists). Adoption of a definition favorable to one's viewpoint can mean tacit government and popular approval of that philosophy, and lead to shifts in public and private sector funding and legislation. Organized public interest groups in the arena of pesticide use and farming practices understand that legal mandate is the most efficient means of effecting rapid and widespread change in the agricultural sector.

IPM was originally proposed as a management system for insects and other arthropod pests, and early proponents largely ignored plant diseases, the biology of which did not fit in well with many of the entomological concepts they were trying to promote. The IPM theory formally entered into the plant disease management arena much later. Numerous definitions of IPM have been published and supported by various entities during the 35 years since the was proposed. In 1959, California entomologist V. Stern and associates (18) defined their seminal "Integrated control" concept as:

"Applied pest control which combines and integrates biological and chemical control. Chemical control is used as necessary and in a manner which is least disruptive to biological control".

More recently, some authors have criticized the evolution of IPM philosophy for being heavily weighted toward simply timing or forecasting pesticide applications, rather than integrating other non-chemical methods such as biological, physical, and cultural management strategies (4). However, other scientists see definite trends in IPM philosophy toward reduced emphasis on pesticides have occurred over the years (9). Recently proposed definitions of "biologically intensive" (8) and "ecologically-based" (2) IPM appear to be shifting away from pesticide use. A recent definition (8) proposes that "biologically intensive IPM":

"... relies primarily on biological control, host resistance, cultural management and the judicious use of environmentally safe pesticides."

In formulating biologically and economically viable strategies for integrated disease management, the importance of climate cannot be overemphasized. Production areas with climatic conditions characterized by short, cool growing seasons and/or those with frequent precipitation events and high humidity typically are subject to more fungal and bacterial diseases of above-ground plant parts than locations with relatively arid and warm growing seasons. However, there are foliar diseases adapted to virtually all climatic regions which are currently difficult to manage without regular pesticide applications.

ADVANTAGE MEDITERRANEA?

Many physical and cultural strategies which alter the crop ecosystem may produce an additional, successive cascade of pesticidal effects encompassing elements of both biological control and induced host plant resistance. In unfavorable climates where environmental conditions resulting in high disease pressure frequently occur, effects of physical and cultural disease management methods can be insufficient to provide economic benefit. However, in production areas where the impact of adverse environmental conditions on plant disease in moderate or low, efficacy of physical and cultural disease management methods can approach, or even exceed the benefits obtained by pesticide applications. In irrigated and dryland agriculture in Mediterranean, desert, and steppe climates, the interrelated strategies of cultural and physical management measures can can be increasingly used to biological and economic advantage.

An excellent example of this is canopy management (basal leaf removal) for diseases of wine grapes in California. Excision of leaves around the cluster zone of the grape canopy shortly after bloom increases airflow and light penetration into the interior of the vines (6). The result is excellent non-chemical control of Botrytis bunch rot, sour rot, and rots caused by **Aspergillus niger** and **Penicillium** spp. (6, 14, 15). The practice has reduced fungicide applications for bunch rots by 67% in the coastal valleys, and eliminated the need for rot sprays in the warmer interior valleys, except in unusual weather conditions. Within a 10- year period since development began, producers of

about 40% of the wine grape acreage in California have adopted this practice for their annual cultural programs (13). Interestingly, other benefits have been found to accrue from leaf removal, including improved wine quality (from enhanced flavor and color characteristics), lower populations of certain arthropod pests, including leafhoppers (**Erythroneura** spp.), and improved spray coverage in the cluster zone when pesticide applications are needed (14, 15). Leaf removal in wine grapes has also been tested in a temperate climatic zone in North America which does not have a dry growing season. In those experiments, leaf removal failed to provide economic control of Botrytis bunch rot during a year with a rainy growing season (7).

Another example of a physical control measure having advantage in many Mediterranean, desert, and steppe climatic areas is soil solarization. Within the climatic and economic limitations (high value horticultural crops), solarization has proven to be an effective, alternative soil disinfestation technique for a wide spectrum of crops and pests (10-12, 17). It is currently underused in commercial agriculture for several reasons: i) because growers who routinely disinfest soil are accustomed to using chemical fumigants; ii) as solarization requires 4-6 weeks to accomplish during summer months, it must be carefully worked into a crop rotation scheme; and iii) treatment predictability (confidence of return on investment) can vary in marginal climatic areas because it is a passive, solar radiation-dependent measure (17).

One of the most intensive usages has been in greenhouse vegetable production in Japan, where over 3,400 ha were under routine solarization in 1988(11). Growers in certain Mediterranean locations, such as Sicily and Crete, are also using greenhouse solarization to commercial advantage.

Another developing application for solarization technology is in prophylactic and therapeutic treatment of soilborne diseases and pests of perennial fruit, nut, and vine crops. Solarization has been successfully used to prevent (16) and cure (19) Verticillium wilt in susceptible tree crops, while minimizing irrigation water requirements (5). Commercial permanent crop growers in California are currently testing solarization for use in almond, citrus, olive, and pistachio.

With the impending regulatory restrictions on use of methyl bromide and other soil fumigant chemicals, solarization has the potential to become a major soil disinfestation technique, particularly in greenhouses and in open fields in locations with warmer climates. Recent studies have shown that the reliability of solarization can be enhanced by combining with low dosages of pesticides such as metham sodium, or with organic residues (10) or fertilizers.

Many other innovative cultural and physical disease management strategies, including use of resistant varieties, crop rotation, field sanitation, and fertilizer and irrigation water management, can be used to economic advantage under conditions where disease pressure is not excessive. Implementation of successful "biologically intensive" or

"ecologically-based" integrated disease management strategies will be easier and more economical in climates which are less conducive to pathogen development. In fact, preferential agricultural production in less conducive climates can be considered to be a component of integrated disease management in itself. If public sentiment continues to favor crop disease management strategies which result in reduced pesticide use, Mediterranea has the opportunity to lead the way.

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Concluding Remarks Of 9th Congress Of The Mediterranean Phytopathological Union

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I wish to thank very much the Organizing Committee of the 9th Congress of the Mediterranean Phytopathological Union for having invited me to address you some concluding remarks at the end of this meeting.

This is an honor for me, but it is also a very difficult task because of the diversity of the topics which were presented in the 150 oral and poster presentations.

One of the originalities of this Congress is to have put together scientists of so many different fields of research and of so many different countries in order to share their experience of crop protection in the Mediterranean environment.

It was also an unique occasion for us to appreciate the quality and the diversity of the researches conducted in crop protection in our hosting country, Türkiye.

I will now point out briefly some new developments in Phytopathology which were highlighed in this meeting.

In the field of diagnosis we have seen a double evolution:

- a trend towards more single and cheapest techniques: in this respect the tissue blot immunoassay which was described in several presentations will provide an easy means to survey viral diseases (and why not in the future some fungal or bacterial diseases). This will surely help to achieve a proper evaluation of the major pathological problems occuring in the fields and subsequently help to develop adapted control strategies.

- We have also observed a second trend towards the need for the development of new techniques which would enable us to go beyond the "species" level in diagnosis. Indeed several papers pointed out the need for rapid and single ways to properly identify pathotypes or races of a given pathogen.

No doubt than in the near future-may be in our next Congress-we will have reports on molecular techniques allowing a single identification of pathotypes or races. This will allow to achieve a more efficient deployment of resistances as suggested in several presentations.

A relatively recent trend in Plant Pathology is the better-and I would say the more spontaneous-interactions with Plant Breeders, not only for the identification of resistance genes but also for the development of strategies which will ensure a better durabiliy of resistance genes (including those introduced through genetic engineering).

Another important issue which was discussed in this meeting was the potential of biological- I would rather say microbiological- control as an attractive alternative to pesticide use. Several presentations clearly pointed out that this approach is not any more a scientist dream, but is now in full development with several commercial applications.

The third major component of IPM remains cultural practices. We have seen that they can significantly reduce or slowdown disease spread, but also, interestingly reduce the amount of pesticides to be applied in the fields.

The occurrence of new pathogens in our countries, as it was reported several times, must remind us that plant diseases represent for many crops an ever changing problem, which requires from us the capacity to adapt to new approaches and to regularly revise our dogma on plant disease incidences. Obviously our meeting will have contributed to important exchanges and discussions in this respect.

Finally, I wish to thank both the Organizing Committee and the Phytopathological Mediterranean Union to have offered us, in beautifull Kufladas›, so close to the Mediterranean Sea, a new opportunity to gather, to exchange information and to make contacts for future cooperations.

I wish also to thank all the participants for their contributions which made this meeting so interesting. I wish to extend my thanks to the Organizing Committee for all the work done, which made this meeting a full success...

September 23rd 1994

NOTICE TO CONTRIBUTORS

1. Papers offered for publication should be original contributions dealing with the mycology, bacteriology, virology, herbology toxicology and nematology.

2. Manuscripts must be written in English, German or French.

3. Papers accepted for the Journal of Turkish Phytopathology may not be published elsewhere, in any form or language.

4. In addition to ressearch papers, the journal publishes letters the editor, book reviews and short communications that the author does not intend to publish in more detail at a later date.

5. Papers must have a short abstract which will be printed in the beginning, introduction, materials and methods, results, discussion, summary, acknowledgment (if necessary) and literature cited.

6. All papers are revieved by scientists qualified to judge the validity of the research. Acceptance or rejection, however, is the decision of the subject editor. Acceptance of papers is based solely on their sicentific merit. A rejected manuscript is sent back to its author. Accepted manuscripts are published appreximatoly in the order they are received.

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8. Al responsibility of published papers belongs to its author.

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7. Yazar veya yazarlar grubuna yirmibeş adet ayrı basım gönderilir. Ayrıca telif hakkı ödenmez.

8. Yayınlanan yazıların tüm sorumluluğu yazı sahiplerine aittir.

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