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Necrotic Strain of Satsuma Dwarf Virus and Stubborn Disease On Satsuma Mandarin Trees in Izmir Province of Turkey

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ABSTRACT

For identification of the necrotic strain of Satsuma Dwarf virus, mechanical sap inoculation tests were carried out by using Red Kidney bean, blackeye cowpea and the strain diagnostic host white sesame. Red Kidney bean and the blackeye cowpea plants showed local lesions on the inoculated leaves, mottling and vein clearing on the upper leaves, necrotic streaks on petioles and stem. White sesame plants showed severe local lesions on the inoculated leaves; vein-clearing, necrosis, curling and malformation of the upper leaves with the necrotic strain of SDV. Satsuma trees showed severe symptoms of SDV when infected with necrotic strain.

Stubborn was observed in 1973 on Satsuma mandarins during the survey and the indexing studies. Satsuma mandarin trees which showed the typical stubborn symptoms were tested by using Madam vinous, Duncan and Marsh grapefruit as indicator plants. Short-term indexing in the glasshouse condition and the side graft inoculations have been applied in the test. 3-4 months after the graft inoculations, typical symptoms of Stubborn disease, small and upright chlorotic leaves, pale-green marginal and interveinal areas of the leaves, small and cupped leaves developed on the indicator seedlings.

INTRODUCTION

Since 1952, when Satsuma Dwarf virus was first reported by Yamada and Sawamura, many studies have been made on this virus in Japan. Satsuma Dwarf and Hassaku dwarf were important virus diseases of Satsuma in Japan as reported by Tanaka, Kishi and Yamada (1965). SDV was previously reported widely distributed on Satsuma mandarins in Izmir region (Azeri, 1973). Since then, field and the indexing trials have been done on identification of the necrotic strain of SDV.

During the periodic inspections, some Satsuma trees near the

Stubborn affected Washington navel oranges showed typical small and acorn shaped fruit, short internodes of the shoots, small leaves showing mottle, chlorosis and Zn deficiency like foliar symptoms. The results of these inspections led us to indexing studies on Stubborn disease.

MATERIALS AND METHODS

Totally 15 Satsuma mandarin trees at 15-20 year old which showed severe symptoms of Satsuma Dwarf virus (SDV) with narrow boat shape and dwarfed spoon shape leaves, shortened internodes giving the twing witches-broom appearance, smaller and immatured fruits have been tested by sap inoculation for identification of the necrotic strain of SDV (Miyakawa, 1972; Usugu and Saito, 1976). Ten Satsuma trees with the characteristic symptoms of Stubborn disease (*Spiroplasma citri* Saglio et al.) (little leaf or piny leaf, bunchy upright growth grown, stunted shoots, mottled leaves, small and acorn shape and malformed or deformed fruits) were graft inoculated for stubborn disease.

Mechanical inoculation test for SDV :

Soft and young shoots of SDV affected Satsuma trees shorter than 10 to 15 cm were collected in plastic bags for inoculum, Blackeye cowpea (*Vigna unguiculata* (L, Walp.), Red Kidney bean (*Phaseolus vulgaris*) and white sesame (*Sesamum indicum* L.) were used in the mechanical tests (Miyakawa, 1972; Usugian Saito, 1976). Infected sap was prepared by macerating young leaves with addition of an equal volume of a 0.05 M $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ and KH_2PO_4 buffer solutions, PH 6.98. Leaves of indicator plants were dusted with 500-mesh carborandum and rubbed with a small piece of absorbent cotton dipped in inoculum. The leaves were rinsed with tap water after the inoculations. The inoculated host plants were kept in the labrotory at the optimum temperature 28°C.

Side-graft inoculation for Stubborn :

Short-term indexing were made in the greenhouse conditions. One or two side graft inoculations were used in the indexing test as described by Calavan et al (1968). Pencilsized Madam vinous (*C. sinensis* (L) Osb.) sweet orange, Duncan and Marsh grapefruits (*C. paradisi* Macf.) seedlings were used in indexing tests. Indicator seedlings were inoculated with one or two side grafts from 50 to 100 mm. long during the spring. Three or four indicator seedlings were used in each test. Two piece of side grafts were grafted into each indicator seedling. Grafts were protected from drying by using the polyethylene after the cutting back of the indicator seedlings, A single unbranched shoot

was forced from near the inoculation site as shown in figure 6. Symptom inspection were made frequently, 2 months after the inoculations. Some indicator seedlings were inoculated with healthy side pieces for control.

RESULTS AND DISCUSSION

Symptoms :

Symptoms of SDV on Satsuma mandarins (*Citrus unshiu* Marc.) were previously reported by Azeri (1973). Its mild strains were also reported more distributed than the severe strain. According to our field observations and the indexing tests, the symptoms of the necrotic strain were : boat-and spoon-shaped malformed leaves (fig. 1.), leaf crinkling, short internodes and witches-broom appearance of the twigs and downward leaf curling. In some Satsuma mandarin trees these symptoms were not apparent, and many infected trees showed mild symptoms when the trees were infected with the mild strains. Necrotic strain caused persistent cupping with the other above mentioned symptoms. These symptoms were present on the lower part of the leaves. During the high temperature conditions in the hot summer. These symptoms were absent on the top leaves of the trees infected both the severe and mild strains. Diseased Satsuma trees that affected by necrotic strain of SDV showed poor growth and very poor quality and quantity of fruit production. We noticed that, severe leaf and the fruit symptoms were persistent at the lower parts of the trees throughout the summer when the trees were affected by necrotic strain. Yamada and Tanaka (1968) reported that, symptoms of SDV is affected by high temperature at the time of flushing being masked above 28°C for 12 hours every days, Miyakawa (1972) also reported that, when Satsuma trees were infected by the mild strains always displayed very mild symptoms of SDV.

In Izmir province, many Satsuma trees showed poor growth and the poor fruit quality but no apparently specific symptoms of SDV. The results of the indexing tests with these poorly growth Satsuma trees revealed that, many of them were found infected with Tristeza and Tristeza Seedling yellows. These Tristeza infected Satsuma trees also showed stem pitting on the trunk and the branches above the bud union resembling the Hassaku Dwarf (Caused by tristeza) symptoms as reported by Azeri and Karaca (1978, 1981). The same symptoms on Satsuma mandarins was also reported by Yamada and Tanaka (1968) and Kishi (1972) from Japan. We also determined that, Psorosis (symptom with oak leaf pattern in spring) and SDV infected Satsuma trees showed small and cupped, boat and crinkled shaped leaves on the same Satsuma trees affected by both viruses (fig. 2B).

SATSUMA DWARF VIRUS

Stubborn affected Satsuma trees have especially been observed near the Washington navel (*C. sinensis* (L), Osbeck) orange growth in the same Satsuma Orchard 100 % per cent of Washington and the other sweet orange trees in the Satsuma plantings were found to carry Stubborn. Most of them were worthless by showing typical symptoms of stubborn : stunting of tree, the leaves were cup-shaped, heart-shaped and mottled; unseasonal flowering, fruits of several ages at one time, small fruit and acorn shape like symptoms occurred on the observed sweet orange trees near the Satsuma trees.

Satsuma mandarins around these Stubborn affected sweet orange trees also showed the following symptoms of Stubborn disease: Deformed and cylinder-shaped and acorn shaped lopsided fruits, mature fruit with the small green fruits and unseasonal flowering simultaneously on the same Stubborn affected trees as shown in fig. 4. Although most Satsuma trees not effected by exocortis, displayed stunting and shortened intervals between leaf bases on most shoots and the typical vertically positioned or picket-fence leaves and long sprout with short internodes on the branches.

Results of the sap inoculation test for SDV :

Phaseolus vulgaris (Red Kidney bean) plants mechanically inoculated by the sap with severe necrotic strain affected Satsuma leaves developed chlorotic spots, clear mottling, vein-clearing, malformation of the leaf; the top of the Kidney bean exhibited chlorotic spots then turned into necrotic ring spots and terminal wilt as reported by Azeri (1973) and Tanaka et al (1965). A few days after the sap inoculations Blackeye Cowpea (*Vigna unguiculata* (L) Walp) plant leaves were boat-shaped, showed mottling, curling and vein-clearing. Systemically infected upper leaves did not grow. Infected Cowpeas with necrotic strain showed poor growth necrotic streaks on petioles and stems. Systemically infected upper leaves of all inoculated Blackeye cowpeas died in 6 to 10 days as reported by Miyakawa (1972).

White Sesame (*Sesemum indicum* L.) was found very suitable test plant for necrotic strain differentiation when infected with this strain developed yellowing and necrotic local lesions on inoculated leaves 2 weeks after inoculations as shown in figure 3. Inoculated or systemically infected upper leaves showed vein clearing curlings, malformation as reported by Tanaka et al (1965). In the later stage, the leaves showed necrosis from the tips, with vein necrosis. The petioles of inoculated sesame plants showed epinasty and downward curling, white sesame was found to be a suitable test plant for necrotic strain of SDV as described by Yamada and Tanaka (1968). The same authors reported that, the insect vectors of SDV is not known at present. Mec-

hanical inoculation tests revealed that, stunted Satsuma trees that had displayed very severe symptoms of SDV carried necrotic strain of the virus.

Indexing for Stubborn :

Graft inoculated Madam vinous, Duncan grapefruit and Marsh grapefruit indicator seedlings developed slight or severe stunting and short internodes 2 or 3 months after the inoculations. Grapefruit seedlings showed small, cupped and chlorotic leaves 3 months after the inoculations as seen in fig. 6; Marginal and interveinal areas near the tips of the new grown leaves were pale green. These symptoms were very severe after 6 or 8 months from the inoculations. Control seedlings grafted from the healthy Satsuma trees developed strong sprouts as shown in fig. 6 (in center). Madame vinous and Grapefruit seedlings developed very clear symptoms of Stubborn as reported by Calavan (1968).

Indexing tests revealed that, Satsuma mandarin trees surrounded by severely stubborn affected sweet orange especially Washington Navel were found Stubborn carriers. Stubborn disease is readily transmissible by grafting and the leaf hopper vectors *Circulifer tenellius* (Baher), and *Scaphytopius nitridus* (DeLong) as reported by Rana et al. (1975). In California stubborn was found rapidly spreaded naturally in most areas where the vectod populations were very high as reported by Calavan (1968).

Natural incidence of infection by *Spiroplasma citri* in sweet orange seedlings was reported (Calavan, 1976) 90 % in Moreno location in California containing several hundred diseased naturally infected stubborn trees surrounded the young planted citrus seedlings. The incidence of natural infection by *S. citri* was related with vector population and high incidence of *S. citri* infection. On California the natural incidence of *S. citri* infection in sweet orange in the Moreno place was apparently confined to the hot mounths of summer and early fall, June through October. Through a FAO aided project work carried out by Bove in Syria, it is brought into light that *Neocaliturus haematoceps* is a really efficient insect vector in terms of spread of Stubborn (Anonymous, 1985). It is stated by Lodos and Kalkandelen (1985) that, *N. haematoceps* is a country-wide spread species on a numerous host plants in Turkey. Lodos (1982) describes the species *N. haematoceps* as green or grenish-yellow color and 2,5 - 4.0 mm lenght. It is found also in Europe, Cyprus, Lebanon, Iran and Rusia. In Turkey however it is country-wide spread, but, found in Middle, west, South East and East Anatolia more density and afficient insect vector in terms of spread of Beet curly top virus.

For avoiding Stubborn transmission and distribution, we suggest eradication of the stubborn affected sweet orange trees and Satsuma trees in and around the Satsuma orchards. It is also necessary to initiate short-term and long-term indexing and the bud-wood registration programme to establish virus and Stubborn free Citrus foundation and mother blocks, and production of nursery trees in the areas where the vector is not present. Vector studies is also necessary for identification of natural incidence of *S. citri* infection in Satsuma mandarins in several satsuma growing locations in Izmir.

Ö Z E T

İZMİR İLİNDEKİ SATSUMA MANDARİNLERİNDE SATSUMA CÜCELİK VIRÜSÜNÜN NEKROTİK İRKİ İLE PALAMUTLAŞMA HASTALIĞININ DURUMU

İzmir ilindeki Satsuma mandarinlerinde Satsuma cücelik virüsü (SDV)'nin çok şiddetli belirtilerini gösteren Satsuma mandarinlerinden Red Kidney fasülyesi ile börülce ve beyaz susam otsu bitkileri üzerine uygulanan mekanik inokülasyonlar sonucu, bu ağaçların virusun nekrotik irki ile infekteli olduğu saptanmıştır. Nekrotik irk ile infekteli Satsuma ağaçları üzerinde çok şiddetli yaprak küçülmeleri, yapraklarda gondol kayığı ve ufak kaşık şekli, yapraklarda deformasyon ve bükülme, dallarda sürgünlerde kısa internodiumlar, sürgün vereme ve çalı süpürgesi görünümü alma, yapraklarda içe kapanma durumu, ağaçta çok şiddetli bodurluk gibi belirtiler görülmüştür. Zayıf ve orta derece ırklarla infekteli ağaçlarda daha zayıf belirtiler oluşmaktadır.

Palamutlaşma hastalığı için uygulanan endeksleme testlerinde genellikle bu hastalık ile infekteli olan ve tipik belirti gösteren Washington portakallarının civarında bulunan Satsuma mandarinlerinin Palamutlaşma Hastalığı ile infekteli olduğu saptanmıştır. Palamutlaşma hastalığının Washington portakallarından Satsuma mandarinlerine geçme durumu portakal ve mandarinin yan yana dikili olduğu bahçelerde kolaylıkla görülmekteydi. Ayrıca bu gözlemlerde bahçelerde tabii bir bulaşmanın bulunduğu da açıkça anlaşılmaktaydı. Nitekim güney doğu sınır komşumuz olan Suriye'de 1985 yılında Palamutlaşma hastalığının vektörü olarak saptanan *Neolaliturus haematoceps* vektörünün ülkemizde yaygın olarak bulunduğu bilinmektedir. Tabii bulaşmaların bu vektör ile olduğu ve gün geçtikçe daha tehlikeli boyutlara ulaşacağı bir gerçektir. Bu nedenle, infeksiyon kaynağı olan portakalların sökülmesi, mandarinle portakalın aynı bahçeye veya yakın mesafelere dikilmemesi ve ayrıca vektör olan emici böceklere karşı gerekli mücadele önlemlerinin bir an önce alınmasında fayda vardır.

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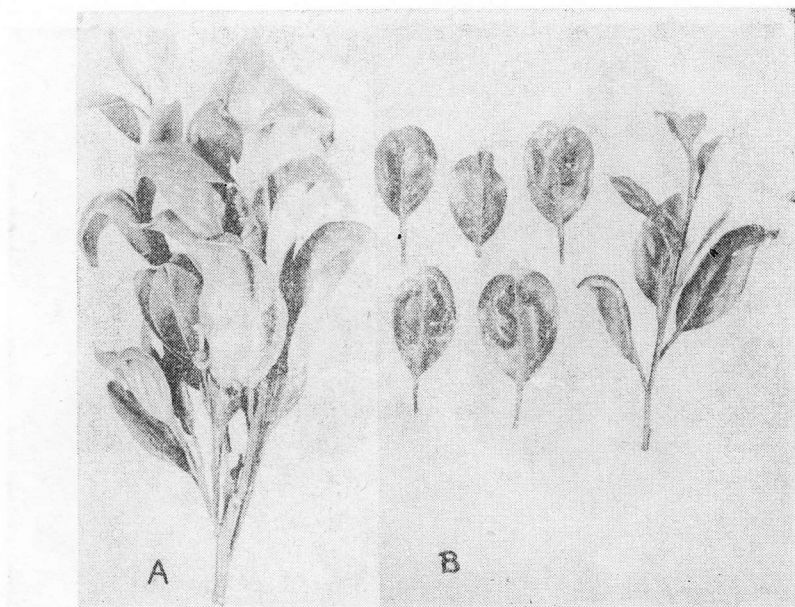


Figure 1. Leaf symptoms of SDV affected Satsuma mandarin tree infected with necrotic strain.

- A— Boat-shaped malformed leaves, leaf crinkling, short internodes and witches broom appearance.
- B— Boat and spoon-shaped malformed leaves.

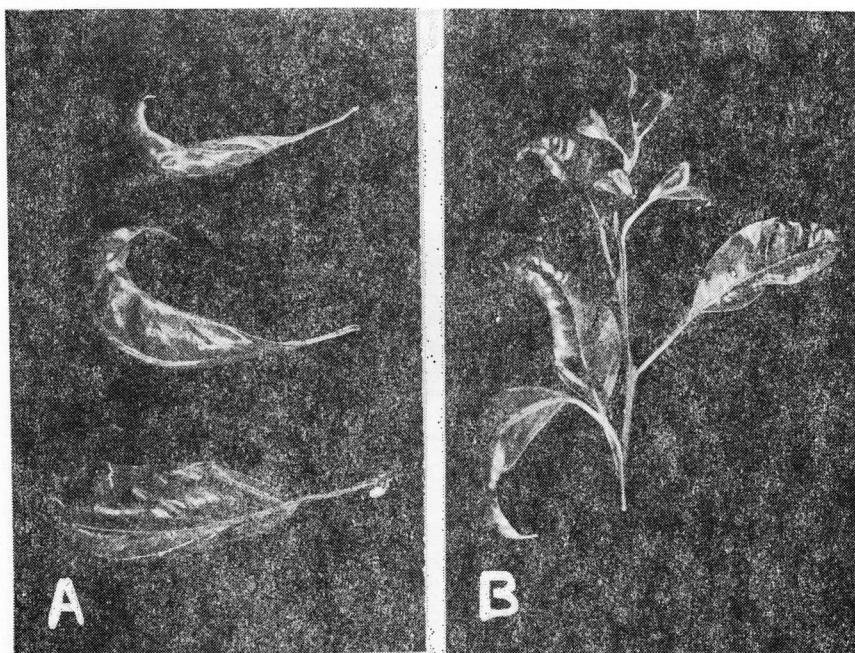


Figure 2. Leaf symptoms of SDV + other viruses on Satsuma mandarins.

- A— SDV necrotic strain + Tristeza severe strain infection.
- B— SDV + Psorosis symptoms (Psorosis crinkle leaf + boat and spoon shape symptom of SDV).

SATSUMA DWARF VIRUS

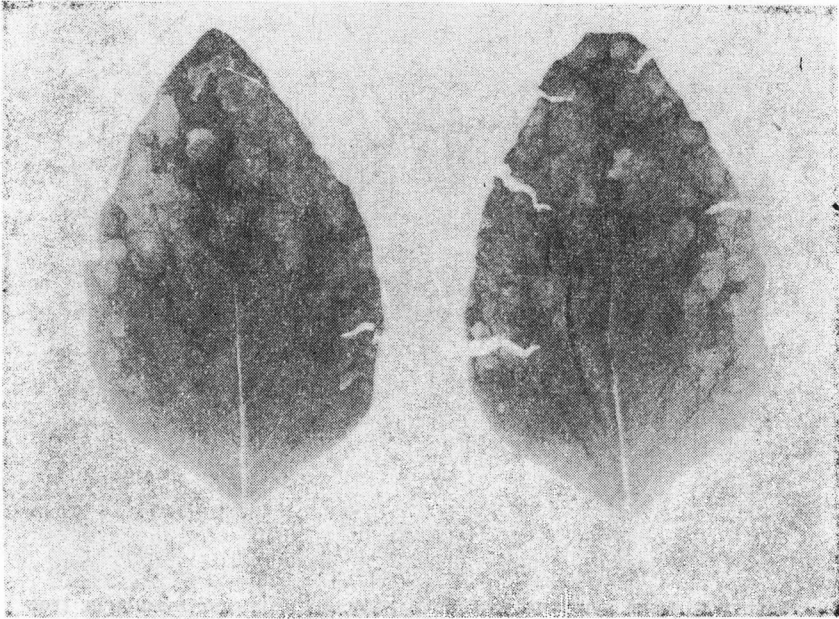


Figure 3. Sap-inoculated white sesame (*Sesamum indicum*) leaves from SDV (With necrotic strain) affected Satsuma trees. Note the necrotic spots on the inoculated leaves.

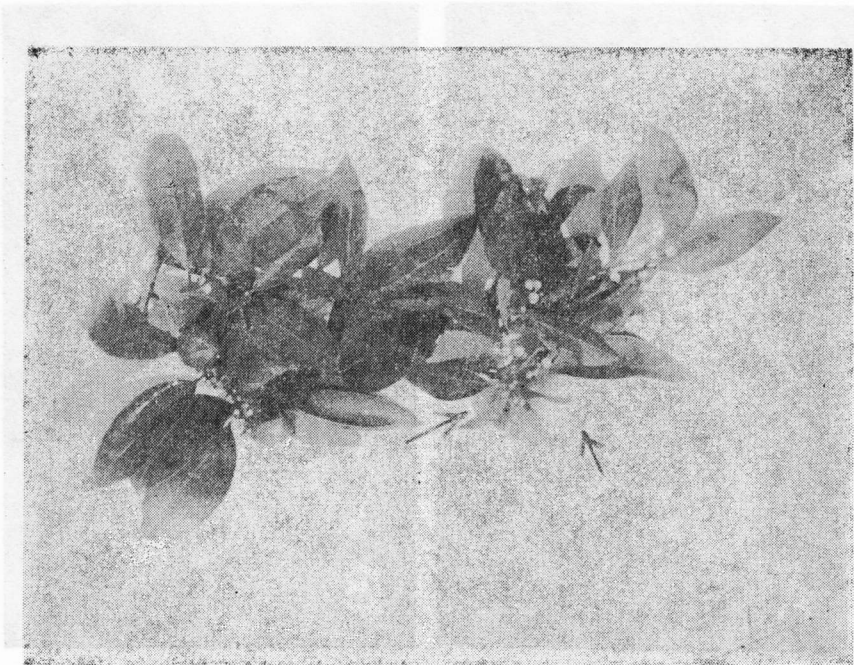


Figure 4. Mature fruits small and green fruits and flowers Shown with arrows, on the same stubborn affected Satsuma tree.

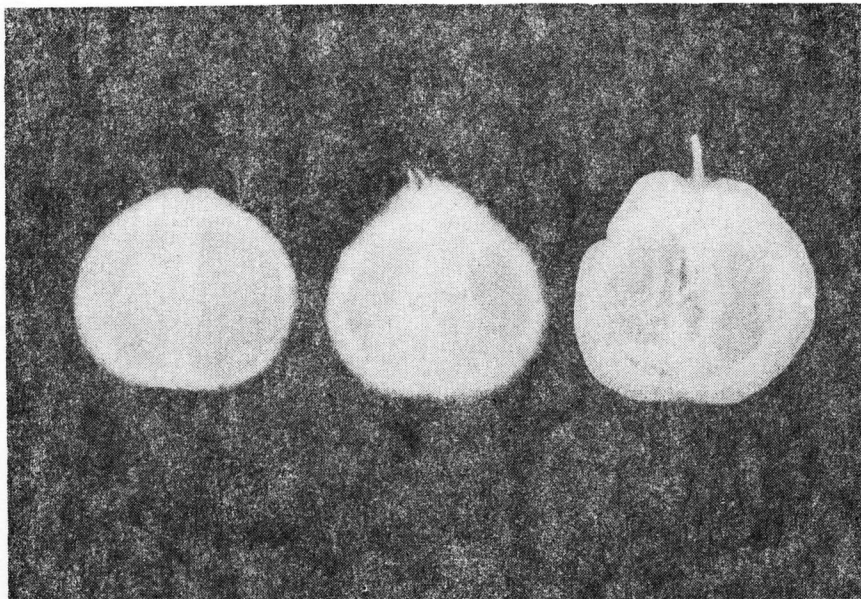


Figure 5. Fruit symptom of stubborn on the stubborn affected satsuma tree: left normal satsuma fruit from the unaffected tree, right, acorn shaped fruit of the stubborn affected satsuma tree.



Figure 6. Duncan grapefruit indicator seedlings; center healthy control, side grafted with healthy tissue; At the right and the left side, grapefruit seedlings side grafted from the stubborn affected satsuma tree; note short internodes and small mottled leaves.

Oversummering and Overwintering of the Wheat Rusts in East and Southeast Anatolia

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ABSTRACT

After wheat harvest, the rusted stubble, leaves and stems were kept in some fields at different elevations until the germination of winter wheats to determine whether the urediospores of rusts on stubble would survive or not. When the winter wheats germinate in fall, the susceptible wheat varieties were separately inoculated with each of the urediospores of rusts collected from these stubbles. In addition, susceptible varieties grown during the summer were inoculated with each of the urediospores of rusts and the development of rusts were observed to determine the survival of the diseases on volunteer wheats. Meanwhile, some observations were also made to determine the extent of rusts on grasses. When the rusted grasses were found, the susceptible wheats were inoculated with the urediospores collected from these grasses.

These experiments showed that the urediospores of stem and leaf rusts can survive on stubble, dry leaves and stems of wheat during the summer in some fields where the elevation is 1000 meter or higher, the period between the harvest and germination of winter wheat is shorter than 60 days. The urediospores of stripe rusts can survive the summer on spring wheat remainders in some places where the elevation is higher than 1550 meters and the period between the harvest of spring wheat and the germination of winter wheat is short (30-38 days).

INTRODUCTION

As it is known, the epidemics of wheat rusts occur on wheat areas in some countries and also in Turkey in some years and cause losses in the yield. A lot of investigations were carried out on wheat rust diseases in the world for the reasons of importance of wheat as food for mankind and the damages of the diseases on wheat. In addition, the oversummering and overwintering of the rusts were investigated. Some investigators recorded that the urediospores of the rusts can survive the summer on wheat stubbles, volunteer wheats and some

grasses at high elevations, and the rusts can also survive the winter as urediospores or mycelium on winter wheats (1, 3, 4, 5, 6, 7, 8, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20). There is no detailed work on this matter in Turkey except (9) investigation of epidemiology and alternate hosts of stem rust in middle Anatolia. This work was done to determine stripe rust (*Puccinia striiformis* West), leaf rust (*P. recondita* Rob. ex Desm. f. sp. *tritici*) and stem rust (*P. graminis* Pers. f. sp. *tritici* Eriks and Henn) whether survive the summer and winter in East and Southeast Anatolia or not.

MATERIAL and METHODS

The wheats grown in these areas, volunteer wheats, wild grasses, urediospores of stem rust, leaf rust and stripe rust of wheat and some susceptible wheats (Little Clup, Michigan Amber and Gains which are susceptible to stem rust, leaf rust and stripe rust respectively) were constituted the material of this study.

The urediospores of stem rust, leaf rust and stripe rust of wheat were individually collected from infected wheat and grass leaves with the aid of cyclone spore collector (3).

The vacuum-drying technique (16) was used for long term storage of urediospores. While spores were vacuum-dried for three hours, the ampoules containing spores were flame-sealed and then stored in refrigerator at 5°C.

«Brush» and «Dip» inoculation techniques (3) were used in this study. The leaves were lightly rubbed between clean, moistened fingers, and inoculation was then made by inverting the pots containing the sporulating cultures over the plants to be inoculated and lightly brushing them together. Inoculated plants were again moistened and placed in a moist chamber for the penetration (Brush technique). In dip inoculation technique, urediospores were floated on the surface of water in a cup. Potted seedling plants to be inoculated were first prepared by lightly rubbing the moistened leaf surface, inverting the pots, and dipping the leaves into the spore suspension. Urediospores cling to the leaves as they were pulled out of the water and placed in a moist chamber. Wheat rusts require free moisture for infection. The inoculated plants were kept in dew chamber at 13°C in the dark for 24 hours for stripe rusts penetration, and also at 18°C for stem and leaf rusts penetration. After dew period, the plants were placed in the growth chambers programmed at 13°C/17°C (night/day) for stripe rusts, and at 18°C/23°C for stem and leaf rusts with 1000 footcandles of light for 12 hours for symptom development.

To determine overwintering of rusts, the rusted winter and spring wheat stubbles, leaves and stems were kept at different elevations (in Bitlis, Tatvan and Muş in 1975; and in Bitlis, Elazığ, Sivrice and Diyarbakır in 1976) in natural conditions from the period of harvest to germination of winter wheats. Then they were brought to the laboratory and the susceptible seedling plants grown in pots were individually inoculated with these urediospores using «Dip» inoculation technique when the seedling plants were in the 3-4 leaf stage. After dew period, the plants were grown in the growth chamber until symptoms had fully developed. The percentage of germination of urediospores were also determined in both harvest time and germination of winter wheat. In addition, the rusts were searched on volunteer wheats and wild grasses during the summer. The seedling susceptible wheats were inoculated with these urediospores if they were found. Moreover, the seedling plants grown in big pots were individually inoculated with the urediospores collected in harvest time from different elevations (Diyarbakır, Elazığ, Sivrice and Bitlis in the fields in early and, late August). After dew period, they were kept in the same places and irrigated regularly until winter wheats germinate here. If the plants have pustules, the urediospores were collected from dry and green leaves and were brought to the laboratory when the winter wheats germinated in those areas. The seedling susceptible wheats grown in green house were individually inoculated with these urediospores in that time.

To determine the overwintering of wheat rusts, the winter wheats were individually inoculated with urediospores of three of wheat rusts in the field when the wheats were in the 3-4 leaf stage in 1976 and 1981. Inoculated plants were examined from time to time until early July of 1977 and 1982.

RESULTS and DISCUSSION

According to the experiments, the urediospores of stem and leaf rusts can survive the summer on dry stubble, stems and leaves of wheat in Elazığ, Sivrice, Muş, Bitlis and Tatvan, but not in Diyarbakır. However, the urediospores of stripe rust can overwinter on these remainders of spring wheats at the high elevations of Muş, Bitlis and Tatvan. The germinations of urediospores showed similar results (Table 1).

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Table 1. The summer survival of the urediospores of wheat rusts on remainders of wheat in different locations

Location	Elevation	Number of days from harvest to germination of winter wheat	The infectivity/and percentage of urediospores germination		
			<i>P. graminis</i>	<i>P. recondita</i>	<i>P. striiformis</i>
Diyarbakır	660	95 (Winter wheat)	—/0	—/0	—/0
Elazığ	1105	60 (» »)	+/10	+/5	—/0
Sivrice	1250	60 (» »)	+/10	+/5	—/0
Muş	1284	50 (» »)	+/15	+/10	—/0
İtliis	1550	50 (» »)	+/15	+/10	—/0
Bitlis	»	30-38 (Spring wheat)	+/40	+/40	+/30
Tatvan	1664	50 (Winter wheat)	+/15	+/10	—/0
Tatvan	»	30-38 (Spring wheat)	+/40	+/40	+/30

As it is seen from Table 1, the elevations where stem and rusts survive the summer are higher than 1000 meters, and the period between harvest and germination of winter wheat is 60 days or less. The urediospores of stripe rusts can oversummer on remainders of some spring wheat areas where the elevations are higher than 1550 meters and the period between the harvest of spring wheat and the germination of winter wheat is about 30-38 days. According to some investigators, the urediospores of stem rust can survive on dry stubble more than 40 days at 75°F (10). The urediospores of stripe rust can survive on stubble for at least 51 days in the field (16). Wheats produced at high elevations in some parts of the world are bridges between winter cereal crops for oversummering of stripe rust (5, 6, 7, 11, 13, 20).

The stripe rust was regularly found to oversummer on wheat at 6000 feet or higher in India (13). Present study showed similar results with those of previously mentioned works. The reasons why the urediospores of rusts do not oversummer on the remainders in Diyarbakır are probably due to the long period between harvest and germination of winter wheats (95 days), and also due to the low elevation and hot summer (Table 1 and 2).

Table 2. Some meteorological data (about 50 years) in some locations where the experiments were carried out*

Locations	Months	Monthly temperature (°C)			Total rainfall (mm)
		Max.	Min.	Average	
Diyarbakır	July	43.6	16.0	31.0	1.4
	August	43.2	14.2	30.4	1.1
	September	38.0	8.5	24.9	3.4
	October	32.1	0.4	17.3	28.2
Elazığ	July	39.1	13.5	27.2	3.7
	August	39.0	14.3	27.0	2.2
	September	34.4	8.8	22.0	8.8
	October	26.6	0.5	14.9	35.8
Muş	July	36.5	12.4	24.8	6.8
	August	36.1	12.4	24.8	4.6
	September	32.5	8.0	19.8	13.8
	October	25.2	1.0	12.4	67.5
Bitlis	July	34.5	10.3	22.4	5.0
	August	34.3	10.3	22.4	6.1
	September	30.2	6.4	17.5	16.5
	October	24.1	1.0	11.2	71.5
Tatvan	July	32.4	11.0	21.9	5.6
	August	32.2	10.4	21.8	4.6
	September	27.9	6.7	17.0	14.4
	October	21.6	0.2	10.5	74.9

* Ortalama, ekstrem sıcaklık ve yağış değerleri bülteni (Günlük-Aylık), Başbakanlık Devlet Meteoroloji Genel Müdürlüğü, Ankara-1984.

To determine the oversummering of the rusts on volunteer wheats, the potted plants were inoculated in the field in early and late August with the urediospores collected from harvested wheats, and consequently pustules of stem and leaf rusts occurred in Diyarbakır, Elazığ and Sivrice, while stripe rust and other two rusts occurred Bitlis. These results show that the stripe rust can survive on volunteer wheats only in some areas where the summer is cooler and the elevation is higher. However stem and leaf rusts can survive both in warmer and cooler areas at low and high elevations. Actually lower temperature in some areas especially at nights (Table 2) is favorable for infection if there is free moisture. But there is generally not much moisture and rainfall in Diyarbakır in summer. Also the weather is hot and period between harvest and germination of winter wheats is longer. In addition it is

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difficult to find active urediospores in natural conditions in such areas for infection. For these reasons, the oversummering of rusts on volunteer wheats in Diyarbakır is doubtful. But, all conditions in east Anatolia are more favorable than in Southeast for oversummering the rusts on volunteer wheats. Some investigators also showed that the rusts can survive on volunteer wheats in some areas in summer (13, 14, 18, 20).

Leaf rust pustules were found on *Cyperus rotundus* L. sent from Adana, and stripe rust was found on *Agropyron elengatum* (Host.) Pal. which was green until early October near running waters in Elazığ. When the susceptible wheat varieties were individually inoculated with these urediospores, the diseases actually occurred. These results showed that wheat rusts can oversummer on some grasses in some areas and infect the wheats sown in fall. Some investigators reported that stripe and other rusts of wheat survive on some grasses during the summer (1, 12, 15, 16, 18, 19). Present results show a close similarity with these records.

The pustules of three rusts were found on wheat leaves inoculated in autumn during the winter in Elazığ and Diyarbakır. However, some of these infected leaves died in spring, the new infections were seen on newly emerged leaves. The pustules on infected leaves disappeared in Bitlis in late autumn. But, they appeared again on infected and emerged leaves in late May and in June. These results show that the rusts can overwinter as urediospores on dead or living leaves in some areas. Also, they can overwinter as mycelium within leaves in some areas where the weather is cooler and elevation is higher. Some investigators also found similar results. Stripe rust can also survive as mycelium on winter wheats, volunteers and some grasses (8, 20). It can overwinter as urediospores and mycelium on dead or living leaves of wheat (2, 4, 16). Stem and leaf rust can also overwinter on wheats in some areas of Kansas (12, 14).

In conclusion, the rusts oversummer as urediospores on remainders of wheat, volunteers and some grasses in some areas where have high elevations and cool summer. They are carried away by wind from these areas to winter wheat areas in fall and overwinter on these wheats as urediospores or mycelium.

Ö Z E T

DOĞU VE GÜNEYDOĞU ANADOLUDA BUĞDAY PASLARININ YAZLAMA VE KIŞLAMASI

Bölgede pasların buğday kalıntılarında yazlamasını tesbit amacıyla hasat mevsimi rakımı farklı yerlerden toplanan paslı buğday yap-

rak ve sapsları kışlık buğdaylar çimleninceye kadar toplandıkları yerlerde muhafaza edilmişlerdir. Güzün kışlık buğdaylar çimlendiklerinde bu kalıntılardan alınan uredosporlar hassas çeşitlere ayrı ayrı inoküle edilmişlerdir. Ayrıca kendigelenler üzerinde pasların yazlamalarını incelemek için yaz boyunca yetiştirilen hassas çeşitlere her üç pas ayrı ayrı inoküle edilmiş ve hastalığın gelişimi incelenmiştir. Bu arada yaz boyunca yabancı graminelerde pas araması yapılmış ve bulunduğu buğdaya inoküle edilmiştir.

Pasların kışlamasını incelemek için güzün buğdaylar 3-4 yaprak dönemine geldiklerinde Elazığ, Diyarbakır ve Bitlis'de her üç pas uredosporlarıyla ayrı ayrı inoküle edilmişler ve hastalığın gelişimi incelenmiştir.

Yapılan çalışma sonunda rakımı 1000 m.den yüksek, yazları serin geçen ve hasatla kışlık buğdayların çimlenmesi arasındaki sürenin 60 günden az olduğu yerlerde kara ve kahverengi pasın buğday kalıntıları üzerinde; rakımı 1550 m.den yüksek, yazları daha serin geçen ve yazlık ekinlerin hasatı ile kışlık ekinlerin çimlenmesi arasındaki sürenin kısa olduğu (30-38 gün) yerlerde sarı pasın yazlık buğday kalıntıları üzerinde yazlayabildiği görülmüştür. Ayrıca kahverengi ve kara pasın bölgenin bir çok yerinde, sarı pasın ise rakımı daha yüksek (1550 m. den fazla) yerlerde kendigelenler ve bazı yabancı gramineler üzerinde uredosporlar halinde yazlayabildiği daha sonra kışlık ekilen buğdaylara geçerek bu buğdaylar üzerinde uredospor veya mycelium halinde kışı geçirdiği, böylece ara konukçuya ihtiyaç duymadan hayat çemberini tamamlayabildiği anlaşılmıştır.

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An Investigation On Survival of Soil-Borne Spores of Common Bunt And Infection of Wheat Plants

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ABSTRACT

This investigation was carried out to determine the survival of soil borne spores of common bunt and the infection of wheat plants by using the mixture of 8 bunt races and Heines VII wheat variety. The survival of the chlamidospores was investigated by keeping them in and on the soil and examining under the microscope. The wheat variety was sown in the inoculated soil and bunt infected and uninfected heads were counted to determine the pathogenicity of soil-borne spores.

Teliospores of common bunt on and in soil continued to germinate up to mid-January and mid-February, respectively. Bunt infection was determined on wheat sown in autumn in the same year. But, infection rate was lower than that of seed-borne common bunt (Table 2). However, bunt was not seen on wheat sown in the following year in the same inoculated soil. This work showed that the teliospores of common bunt in soil do not overwinter and do not infect the wheat.

INTRODUCTION

It is recorded that soil-borne spores of common bunt cause infection on wheat and the effectiveness of seed-treatment is lower in some countries such as in the United States and in West Germany (Hanna and Popp, 1934; Laurence, 1961; Sorauer, 1962; Kendrick et al, 1964 and Hoffmann, 1978*).

This investigation was carried out between 1981-1983 to determine the survival and pathogenicity of soil-borne spores of common bunt in the east of Turkey.

MATERIALS and METHODS

The mixture of common bunt races C-6, C-12, C-20, F-57, F-65, F-66, F-67 and F-68 determined by us (Finci et al., 1983) and the wheat variety Heines VII known susceptible to bunt (Metzger and Hoffmann,

* Hoffman, J.A., 1978. Results of seed-treatment Screening tests for control of common and dwarf bunt of wheat in 1978, Crop Research Laboratory, Utah State University-UMC 63, Logan, Utah (Unpublished report)

1978) were used in this experiment. These works were carried out in two parts.

In nature, the survival of the teliospores was investigated by making them wait in 10 cm depth of the ground and on the soil surface by the aids of Zagg's (1959) technique. The spore germinations were examined carefully under the microscope once a month.

To determine the pathogenicity of soil-borne spores, 1 g. chlamydo-spores were inoculated into the each m^2 soil at the university farm, Elaziğ, 1981. Uninoculated seeds were sown in some part of the inoculated soil within the same year and in some other parts of the inoculated soil in the next year.

Some plots were set down as control sowing inoculated seeds to the uninoculated soil, uninoculated seeds to the inoculated soil and uninoculated seeds to the uninoculated soil in sowing time. Experiments were carried out in randomized plot design in 4 replicates. Each plot was $1 \times 2 m = 2 m^2$. The percentages of bunt infection on each plot were determined by head counts. The average infection rates in the replicates are shown in Table 2.

RESULTS and DISCUSSION

As it is seen from Table 1, teliospores inoculated on and in soil in harvest time in 1981 continued to germinate up to mid-January and mid-February, respectively. It was not seen any germination after these periods even if the spores from soil were brought to the laboratory and were kept there in suitable conditions.

The disease was occurred when the seeds were sown in the same year in inoculated soil. But the rate of infected heads was lower than the plots where the inoculated seeds were sown in uninoculated soil. Whereas, the disease was not seen when the uninoculated seeds were sown in the next fall in the same inoculated soil (Table 2). This is in harmony with some literatures (Hanna and Popp, 1934; Laurence, 1961 and Sorauer, 1962).

These results are important for some areas where the fallow land is not be made and the wheats are sown successively. The effectiveness of seed treatment may be lower in these areas. As a matter of fact Hoffmann (1978) also showed that the effectiveness of seed treatment was lower when the treated seeds were sown in the inoculated soil in the same year.

Table 1. The germination of common bunt teliospores inoculated on and in the soil in harvest season in 1981

Checking dates	Germination		Germination after bringing from soil and Keeping at the laboratory	
	on soil	in soil	on soil	in soil
15.11.1981	+	+	+	+
15.12.1981	+	+	+	+
15.1.1982	+	+	—	+
15.2.1982	—	+	—	—
15.3.1982	—	—	—	—

(—) : No germination

(+) : Germination is a few

(++) : Germination is middle

(+++): Germination is much

Table 2. % infection of wheat by soil-borne spores of common bunt

Treatment	% Smutted heads of wheat	
	Sowed in fall, 1981 Counted in 1982	Sowed in fall, 1982 Counted in 1983
Untreated check (Uninoculated seeds and soil)	0	0
Check (Inoculated seeds in sowing time)	14	12
Check (Inoculated seeds in sowing time)	63	60
Inoculated soil in harvest season in 1981	13	0

Ö Z E T

TOPRAĞA KARIŞAN SÜRME SPORLARININ YAŞAMA SÜRELERİNİN
VE İNFEKSİYON YAPIP YAPMADIKLARININ TESPİTİ
ÜZERİNDE BİR ARAŞTIRMA

Toprağa karışan sürme sporlarının çimlenme sürelerini ve infeksiyon yapip yapmadıklarını, infeksiyon yapıyorlar ise bu infeksiyon yapma güçlerini ne zamana kadar sürdürdüklerini tespit etmek amacıyla bu çalışma yapılmıştır. Çalışmalarda sürmeye karşı hassas olarak bilinen ve A.B.Devletlerinden temin edilen Heines VII buğday varyetesi ile tarafımızca tespit edilen C-6, C-12, C-20, F-57, F-65, F-66, F-67 ve F-68 sürme ırklarının karışımı kullanılmıştır.

Çalışmalar sonunda, hasat sırasında toprağa bulaştırılan sporlardan toprak üstünde olanların Ocak ortasına kadar, 10 cm. toprak derinliğinde olanların Şubat ortasına kadar çimlenmelerini sürdürdükleri, bu tarihlerden sonra çimlenmeye rastlanmadığı görülmüştür.

Bulaştırılmış toprağa aynı yıl bulaşık olmayan tohum ekildiğinde ortalama % 13 hastalıklı başak tespit edilmiştir. Halbuki aynı bulaşık toprağa ertesi yıl ekim yapıldığında bu parsellerde hastalığa rastlanmamıştır.

Her iki deneme sonuçları, bulaşan toprağa aynı yıl ekim yapıldığında bitkilerin hastalanabileceğini, ertesi yıl ekim yapıldığında bu tehlikenin ortadan kalkacağını göstermektedir. Bu durum, nadas ve münavebe uygulanmayan ve üst üste buğday ekilen alanlar için önemli olup, bu gibi yerlerde tohum ilaçlarının etkinliğini azaltabileceğini göstermektedir.

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Host Speciation, Antagonists and Parasites of **Sclerotinia sclerotiorum** (Lib.) de Bary.

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ABSTRACT

Cross inoculation of the isolates of **Sclerotinia sclerotiorum** from bean, cabbage, cucumber, eggplant, tomato and sunflower and an isolate of **Sclerotinia minor** from lettuce to the above hosts did not show any speciation but various hosts expressed different percentages of disease.

Two thousand six hundred sclerotia of **Sclerotinia sclerotiorum** from tomato, cucumber and eggplant, and **Sclerotinia minor** from lettuce were incubated after surface sterilization with NaOCl and among the various organisms the following ones were found out as important parasites and antagonists to **Sclerotinia sclerotiorum**.

Mycelial parasites : **Aspergillus** spp., **Fusarium oxysporum**, **Fusarium sambucinum**, **Gliocladium virens**, **Mucor hiemalis** f. **hiemalis**, **Penicillium** spp., **Trichoderma harzianum**.

Sclerotial parasites : **Aspergillus** spp., **Gliocladium roseum**, **Gliocladium virens**, **Mucor hiemalis** f. **hiemalis**, **Penicillium** spp., **Trichoderma harzianum**.

Antagonists : **Bacillus** sp., **Cladosporium cladosporoides**, **Fusarium equiseti**, **Gliocladium roseum**, **Penicillium** spp.

INTRODUCTION

Sclerotinia sclerotiorum (Lib.) de Bary. is an important pathogen, causing diseases on various plants. Purdy (1979) mentioned that this pathogen caused disease on 383 plant species. Along with its widespread occurrence in the world, it is also a serious disease on specially greenhouse grown plants in the west and south of Turkey (Karaca 1968, Anonymous 1984).

The difficulty in controlling the disease, its resistance to fungicides and having a great number of hosts have influenced researchers to work on its biological control and host speciation. Host speciation have not aroused much interest so far. Only Price and Colhoun (1975 a and b) tried to find out the variability of the pathogen on various

plants. They cross inoculated 19 isolates of *S. sclerotiorum* from various hosts and did not find any variability. Even though they detected some difference in sclerotial size and ascus length, there was not any host speciation but different percentage of disease expression.

Various investigators have searched for the antagonists and parasites of *S. sclerotiorum* and so far, more than 25 agents have been identified. Among them, *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *A. ustus*, *Coniothyrium minitans*, *Fusarium solari*, *Gliocladium catenulatum*, *G. deliquescens*, *G. roseum*, *G. virens*, *Harzia acromonoides*, *Microsphaeropsis centaureae*, *Mucor hiemalis* f. *hiemalis*, *Penicillium citrinum*, *P. spinulosum*, *P. steckii*, *P. vermiculatum*, *Sporidesmium sclerotivorum*, *Stachybotrys* sp., *Teratosperma oligocladium*, *Trichoderma hamatum*, *T. koningii*, *T. viride*, some Actinomycetes, and bacteria are mostly encountered antagonists and parasites (Adams and Ayers 1979, Ayers and Adams 1981, 1983, Bedi 1961, Cole and Kendrick 1981, Domsch et al. 1980, Huang and Hoes 1976, Huang 1977, 1978, Rai and Sexena 1977, Su and Leu 1980, Su and Sun 1980, Trutmann et al. 1981, 1982 a, 1982 b, Tu 1980, Watson and Miltimore 1975).

In Turkey, biological control and host speciation of *S. sclerotiorum* has not been taken into consideration in a broad sense, for this reason this work was carried out.

MATERIALS and METHODS

In order to determine host speciation of *Sclerotinia sclerotiorum* six plants, bean, cabbage, cucumber, eggplant, sunflower and tomato were grown in sterile soils in polyethylene bags and cross inoculated by the isolates of the above plants. In addition to these lettuce and an isolate of *Sclerotinia minor* Jagger. was also tested. Seeds of the plants were disinfected by 1 % NaOCl for 10 min., then treated with thiram (4 g a.i./kg seed). The soil was disinfected by 2 % formaldehyde at the rate of 20 lit./0,5 m³ soil. Inoculation was made as Madjid et al (1983) as known agar block inoculation technique. Percentages of disease were calculated based on the maceration and death of the tissues of the inoculated points of the plants.

Probable antagonists and parasites of *S. sclerotiorum* were isolated from the sclerotia of the pathogen, collected 2600 sclerotia from green-house and fields of Antalya and İzmir provinces. For this aim, first, sclerotia of *S. sclerotiorum* were disinfected with 1 % NaOCl for 3 min. then incubated on moistened blotters for 7 days.

Antagonistic and parasitic effects of the isolates to *S. sclerotiorum* were tested on PDA by employing dual inoculation method. Culture

discs of both antagonists and the pathogen, 6 mm in diameter, were placed on the agar surface either 6 mm apart or in the center one the top of the other, being the pathogen under and surfaces facing each other.

Sclerotial parasitism was investigated by inoculating parasites to the culture grown sterile sclerotia. Inoculated sclerotia were placed on moistened blotter papers and petri dishes were sealed by sterile melted parafine. These sclerotia were incubated 24 days at $22 \pm 1^\circ\text{C}$, then they surface disinfected with 1 % NaOCl for 2 min., and plated on PDA. The temperature for the other incubations was also $22 \pm 1^\circ\text{C}$.

RESULTS

Host speciation.

Isolates of *Sclerotinia sclerotiorum* from different hosts and an isolate of *Sclerotinia minor* from lettuce caused different percentages of disease of different hosts. Results are shown in Table 1. As seen in the table, there was not a noticeable host speciation, but eggplant isolate of *S. sclerotiorum* generally produced a low percentage of disease.

Table 1. Percentages of disease of six *Sclerotinia sclerotiorum* isolates and isolate of *S. minor* on different host plants (50 plants at each treatment).

Pest Plants	Isolates of <i>S. sclerotiorum</i>						Isolate of <i>S. minor</i>
	Bean	Cabbage	Cucumber	Sunflower	Tomato	Eggplant	
Bean	100.00	100.00	33.33	100.00	100.00	00.00	100.00
Cabbage	92.59	76.19	47.61	95.65	71.42	4.54	60.00
Cucumber	87.57	85.71	81.81	100.00	83.33	66.66	75.00
Sunflower	86.36	100.00	96.42	95.45	89.47	22.72	100.00
Tomato	100.00	72.22	72.22	84.61	100.00	21.73	92.85
Eggplant	61.53	95.65	100.00	100.00	100.00	00.00	85.45
Lettuce	100.00	87.50	63.63	100.00	100.00	33.33	100.00

This isolate did not also show a uniform growth of the pathogen.

When the average percentages of disease of host plants were calculated, cucumber took the first rank and the others were sunflower, lettuce, eggplant, tomato, bean and cabbage respectively (Table 2).

Table 2. Mean disease intensity of plant species against all the isolates of *S. sclerotiorum*

Cucumber	Sunflower	Lettuce	Eggplant	Tomato	Bean	Cabbage
84.14	81.73	80.74	76.19	73.13	72.22	64.66

When the isolates of *S. sclerotiorum* and *S. minor* were taken into consideration and average diseases which were caused by them were calculated, it was found that sunflower isolate caused maximum disease intensity and isolates of tomato, bean, cabbage, cucumber, eggplant followed it respectively. The isolate of *Sclerotinia minor* caused diseases on all the plant species and mean diseases intensity of it was 89.04 % (Table 3).

Table 3. Mean percentage diseases of all inoculated plants by six *S. sclerotiorum* and one *S. minor* isolates

Isolates of <i>S. sclerotiorum</i>						Isolates of <i>S. minor</i>
Sunflower	Tomato	Bean	Cabbage	Cucumber	Eggplant	Lettuce
96.53	92.03	89.71	88.18	70.71	21.28	89.04

In these tests, *S. minor* produced identical small sclerotia on all the host plants while *S. sclerotiorum* yielded bigger and variable sclerotia (Figure 1).

Mycelial antagonists and parasites of *Sclerotinia sclerotiorum*

After incubating 2600 sclerotia of *S. sclerotiorum* various fungi and bacteria were isolated. These isolates were tested for antagonism and parasitism on culture by dual inoculations and on aseptically grown sclerotia on blotters.

Some fungi were found to be highly parasitic on *S. sclerotiorum* mycelia. These were *Trichoderma harzianum*, *Fusarium sambucinum*, *Fusarium oxysporum*, *Gliocladium virens* and *Mucor hiemalis* f. *hiemalis*. Among them *T. harzianum* and *F. oxysporum* completely inhibited sclerotial formation while *Gliocladium virens* was less effective (Fig. 2).

Some fungi were weakly parasitic. They were *Penicillium* spp. and *Aspergillus* spp. Along with their parasitic effect, some isolates of *Penicillium* showed strong antagonistic effect. Some *Penicillium* and *Aspergillus* isolates also prevented sclerotial formation.

The fungi and bacteria which produced an inhibition zone were *Bacillus* sp., *Gliocladium roseum*, *Penicillium* sp. (Isolate no 15), *Cladosporium cladosporoides*, *Penicillium* spp. (Isolate no 8), *Fusarium equiseti* respectively.

Sclerotial parasites

After 24 days of incubation of the inoculated sclerotia of *S. sclerotiorum* with various antagonists, the percentage recovery of parasites were determined by culturing the surface disinfected sclerotia on PDA. The fungi that were recovered more than 90 % intensity were *Aspergillus* spp., *Gliocladium roseum*, *Gliocladium virens*, *Mucor hiema-*

lis f. hiemalis, *Penicillium* spp., *Trichoderma harzianum*. *Microsphaeropsis centaureae*, a well known parasite, gave 20 % recovery. However it, disintegrated the sclerotia to some extent, but did not completely destroyed them (Fig. 3).

DISCUSSION

In our experiments, *Sclerotinia sclerotiorum* and *Sclerotinia minor* did not show any host speciation. Both of the pathogens affected all the seven test plants. However, some isolates of *S. sclerotiorum* were weakly pathogenic on most of the test plants. The same result was also obtained by Price and Colhoun (1975 a, b). In general, the most susceptible host was cucumber and the others were sunflower, lettuce, eggplant, tomato, bean and cabbage respectively. Under natural conditions, this order of susceptibility might be different, since we employed agarblock inoculation method, in that we inoculated mycelia of the pathogen directly on the cut surfaces of the plants. In nature, susceptible stages of the hosts might also be different. We recovered most of the antagonistic and parasitic microorganisms of *S. sclerotiorum* from its sclerotia except that of the well known parasites, *Teratosperma oligocladium*, *Sporidesmium sclerotivorum* and *Coniothyrium minitans*. Among the antagonists; *Bacillus* sp., *Penicillium* spp, and parasites; *Trichoderma harzianum*, *Fusarium sambucinum*, *F. oxysporum*, *Mucor hiemalis f. hiemalis*, *Gliocladium virens* and *G. roseum* were the most effective ones. Although *Microsphaeropsis centaureae* showed a low percentage of recovery from the sclerotia, in a longer incubation period it might be more effective since it caused intensive maceration in the effected sclerotia.

Possible use of the antagonistic and parasitic microorganisms should have to be investigated for biological control of the disease. Some antagonistic bacteria might have been missed since we neither used bacteriological isolation media nor showed much attention to the other bacteria.

Ö Z E T

Sclerotinia sclerotiorum (Lib. de Bary.)'UN KONUKÇUYA ÖZELLEŞMESİ, ANTAGONİST VE PARAZİTLERİNİN TESBİTİ

Ayçiçeği, domates, fasulye, lahana ve patlıcandan elde edilen *S. sclerotiorum* ve *S. minor* izolatları yukarıdaki konukçulara çapraz olarak inokule edildiklerinde herhangi bir konukçuya özelleşme göstermemişler, fakat farklı konukçularda değişik yüzdelerde hastalık oluşturmuşlardır.

S. SCLEROTIORUM

Domates, hıyar ve patlıcandan elde edilen *S. sclerotiorum* ve maruldan elde edilen *S. minor*'un 2600 dolayında sclerotiumlarının NaOCl ile yüzeysel dezenfeksiyonundan sonra inkubasyonu ile elde edilen değişik organizmalardan aşağıdakiler *S. sclerotiorum*'a karşı parazitik ve antagonistik etki bakımından önemli bulunmuşlardır.

Mycelial parazitler: *Aspergillus* spp., *Fusarium oxysporum*, *Fusarium sambucinum*, *Gliocladium virens*, *Mucor hiemalis* f. *hiemalis*, *Penicillium* spp., *Trichoderma harzianum*.

Sclerotial parazitler: *Aspergillus* spp., *Gliocladium roseum*, *Gliocladium virens*, *Mucor hiemalis* f. *hiemalis*, *Penicillium* spp., *Trichoderma harzianum*.

Antagonistler: *Bacillus* sp., *Cladosporium cladosporoides*, *Fusarium equiseti*, *Gliocladium roseum*, *Penicillium* spp.

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S. SCLEROTIORUM

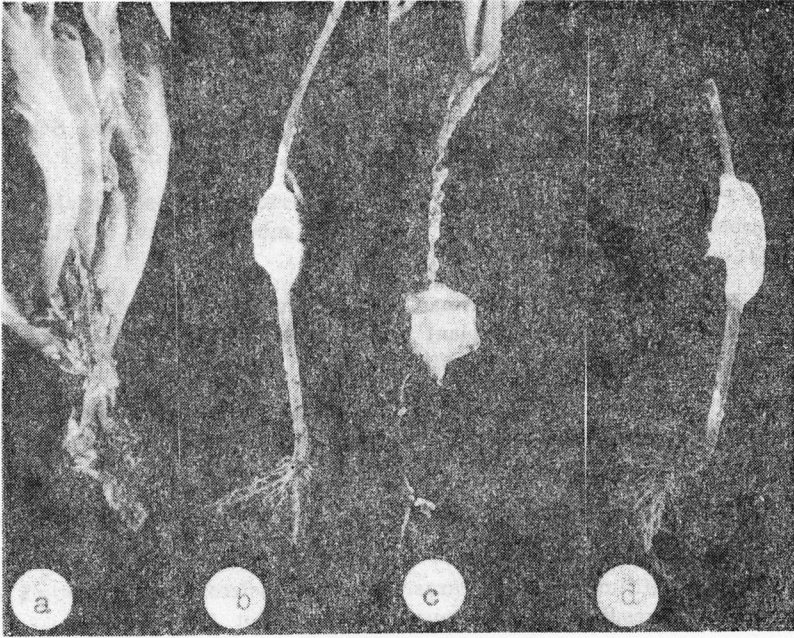


Fig. 1. Diseased plants caused by *S. minor* (a and b) *Sclerotinia sclerotiorum* (c and d) on lettuce and sunflower respectively.

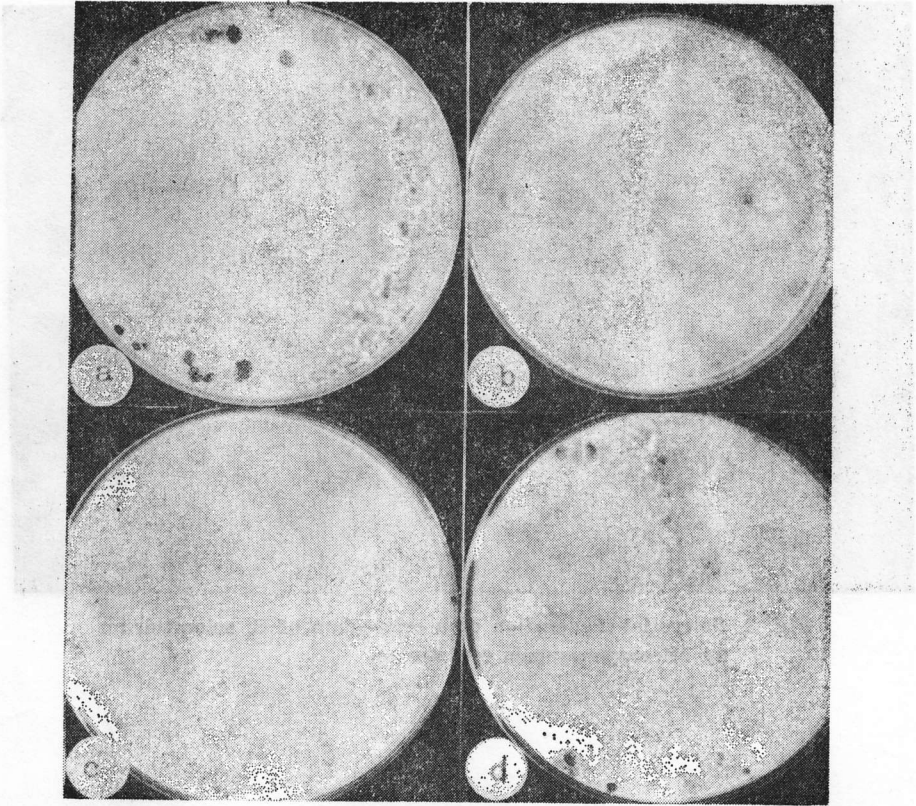


Fig. 2. Parasitism of *Sclerotium* mycelia by some parasites a) *S. sclerotiorum* alone, b, c and d) dual inoculation of cultures with *S. sclerotiorum* (left) and parasites (right) b) *Trichoderma harzianum*, c) *Fusarium oxysporum*, d) *Gliocladium virens*.

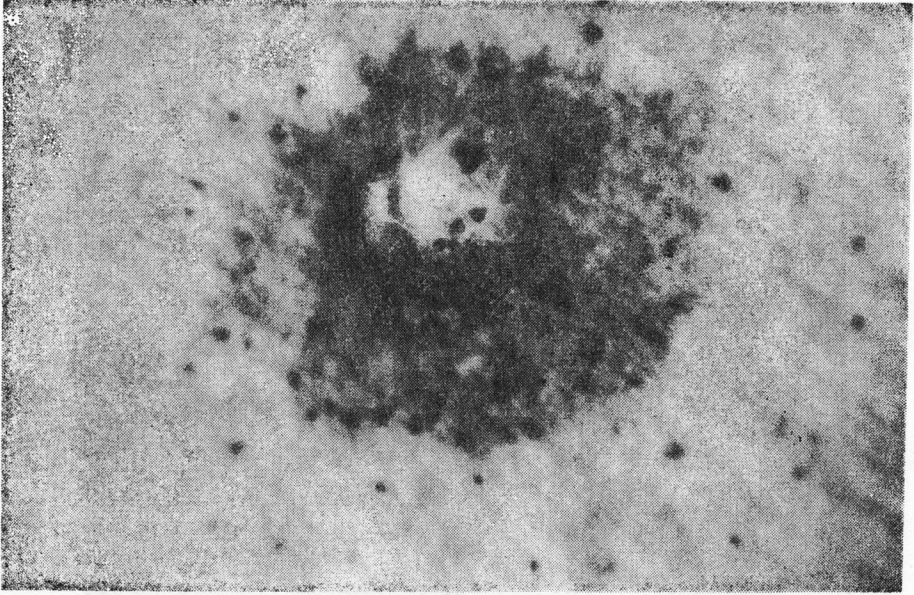


Fig. 3. Sclerotial maceration of a sclerotium of *S. sclerotiorum* by *Microsphaeropsis centaureae*.

Fig. 1. Fruiting of *Sclerotium mycelii* by some parasites: a) *S. sclerotiorum* alone; b and c) dual location of cultures with *S. sclerotiorum* (left) and parasite (right): b) *Trichoderma harzianum*; c) *Trichoderma reesei*; d) *Gibberinia virescens*.

Lytische Wirkung von **Aphanocladium album** auf die Uredosporen-Keimschlaeuche von **Puccinia graminis** f.sp. **tritici**

N. Kemal KOÇ¹

ZUSAMMENFASSUNG

Die lytische Wirkung von **Aphanocladium album** auf die Keimschlaeuche der Uredosporen von **Puccinia graminis** f.sp. **tritici** wurde mit Hilfe Raster- und Transmission Elektronen Mikroskopie untersucht. Die Keimschlaeuche wurden in Anwesenheit von **A. album** 7 Tage nach der Inkubation aufgelöst. Im Vergleich zu Sporenhalt und Sporenwand wiesen die Warzen der Uredosporen einen grösseren Widerstand auf.

EINLEITUNG

Koç und Kern (1980) stellten fest, dass der imperfekte Pilz **Aphanocladium album** während der Parasitierung auf den Uredolagern von **P. graminis** f.sp. **tritici** die Zellwand und die Keimporen der Uredosporen enzymatisch auflöst.

Mit den folgenden Untersuchungen soll abgeklärt werden, ob **A. album** auch die Keimschlaeuche der Uredosporen aufzulösen vermag.

MATERIAL UND METHODEN

Die reifen Uredosporen von **P. graminis** f.sp. **tritici** wurden mit sterilem Wasser gewaschen und auf sterilen Filterpapierrondellen (7 mm Ø) bei 22°C in feuchten Petrischalen während 24 h zur Keimung gebracht.

Die Papierrondellen mit gekeimten Uredosporen wurden in Reagenzglasern gegeben und mit 2 ml Konidien suspension (5.4 x 10⁶ konidien/ml dest. Wasser) von **A. album** aufgefüllt, die Kontrolle nur mit 2 ml dest. Wasser. Die Reagenzglasern wurden während einer Woche bei 24°C gehalten und danach alle 2 Tage nach lysierten Keimschlaeuchen zuerst unter dem Lichtmikroskop und dann mit dem Raster- und Transmission Elektronen Mikroskop untersucht.

Für die Herstellung der Aufnahmen wurde die früher beschriebene Methode verwendet (Koç et al., 1980).

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ERGEBNISSE UND DISKUSSION

Die Keimschlaeuche der Uredosporen von *P. graminis* f.sp. *tritici* wurden in Anwesenheit der Hyperparasiten *A. album* etwa 7 Tage nach der Inkubation aufgelöst, was wahrscheinlich auf die Wirkung von Enzymen zurückzuführen ist. Hingegen waren die Keimschlaeuche der mit dest. Wasser behandelten Uredosporen nicht beschadigt (Fig. 1). Die Lyse beginnt meistens beim Keimschlauchansatz. Nach der Lyse der Keimschlaeuches degenerierte auch das Cytoplasma und die Zellwand der Uredosporen und wurde ebenfalls aufgelöst (Fig. 2., 3. und 4.). Acha et al. (1965) wiesen nach, dass *V. lecanii* die Keimschlaeuche verschiedener *Melampsora* - und *Puccinia* - Arten aufzulösen vermag. Die Auflösung der Keimschlaeuche durch *A. album* geschieht in aehnlichen Zeitraeumen wie bei *V. lecanii*. Die Warzen und das Deckhaeutchen (Pellicle) setzten den enzymatischen Abbau grosseren Widerstand entgegen als Sporenwand und Cytoplasma (Fig. 4), was auf die Zusammensetzung der Sporenwand und Cytoplasma zurückzuführen ist (Littlefield and Bracker, 1971).

Über aehnliche Effekte berichteten auch Mendgen (1981) bei *V. lecanii* und *P. striiformis* sowie Haenssler et al. (1981) bei *V. lecanii* und *P. graminis* f.sp. *tritici* interaktionen. Berücksichtigen wir die nahe Verwandtschaft von *A. album* und *V. lecanii*, so wird die Annahme für *A. album* weiter gefestigt.

Ö Z E T

Aphanocladium album'UN **Puccinia graminis** f.sp. **tritici**
UREDOSPORLARININ ÇİM BORULARI ÜZERİNDEKİ LYTİK ETKİSİ

Hiperparazit *Aphanocladium album*'un *P. graminis* f.sp. *tritici* uredospor çimborucukları üzerindeki lytik etkisi scanning ve transmision elektron mikroskop ile incelendi. *A. album* spor süspansiyonu ile inkube edilen *P. graminis* uredospor çimborucukları, inkubasyondan 7 gün sonra tamamen parçalandı ve spor yüzeyindeki uzantıların, hücre duvarı ve içeriğine göre daha dayanıklı olduğu görüldü.

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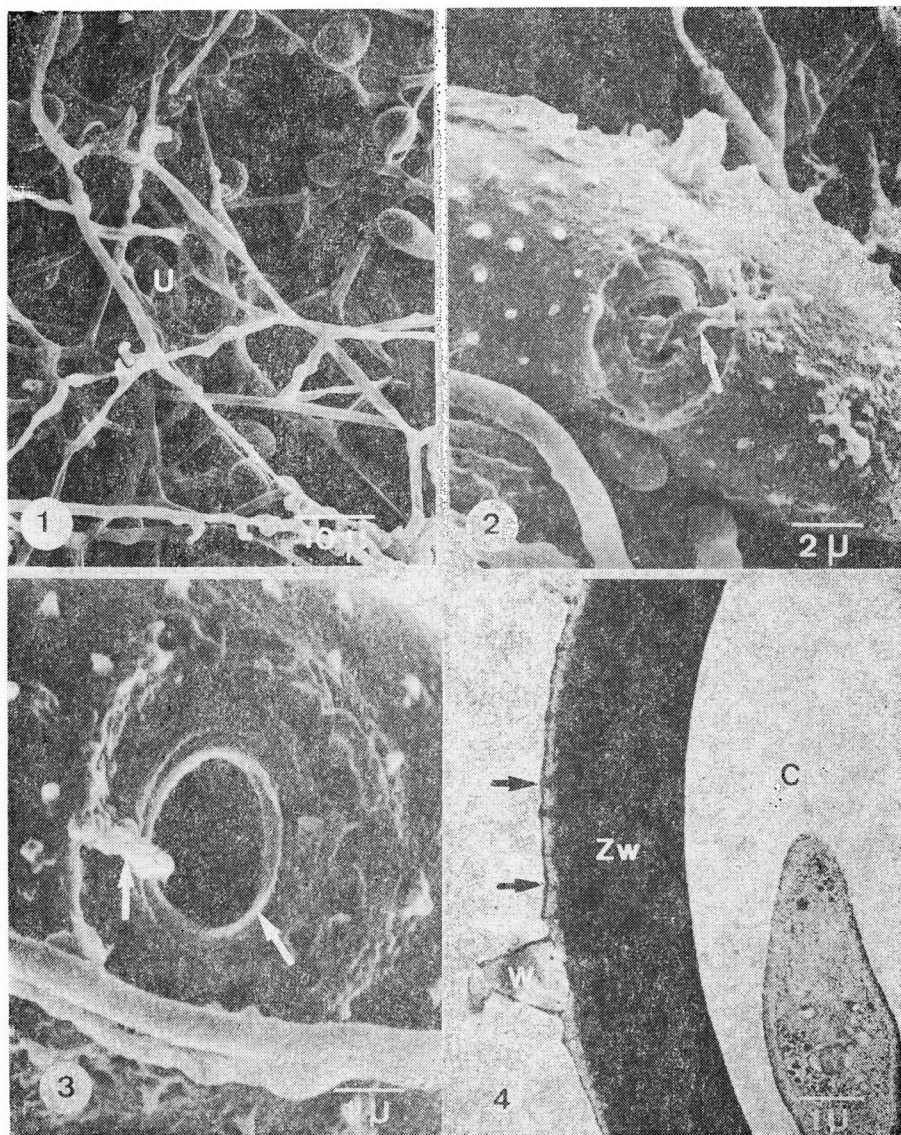


Fig. 1. Gekeimte Uredosporen (U) ohne *Aphanocladium album*.

Fig. 2. Beginnende Auflösung der Keimschlaeuche (Pfeil).

Fig. 3. Rest des lysierten Keimschlaeuches und enzymatisch angegriffener Keimschlauchansatz (Pfeil).

Fig. 4. Beschädigte Zellwand (Zw) und Cytoplasma (S) mit intacte Deckhaeutchen (Pellicle) (Pfeil) und Warzen (W).

Alternaria oleracea Milbraith on the Plants of Early Cauliflower Varieties grown for Seed Production

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ABSTRACT

During the studies to control of the crown rotings on the early cauliflower varieties primarily by the rainy weather, brownish-black, dry and sunken lesions have been observed on the crowns which inhibit also the formation of flower stalks. After isolations and pathogenicity tests the pathogen has been identified as **Alternaria oleracea** Milbraith.

INTRODUCTION

Cauliflower is one of the important winter vegetables in Turkey. Total production area and amount are 4000 ha and 65.000 - 70.000 tons per year, respectively. Approximately 75 % of the total production is realized in the Aegean Region, particularly in the İzmir, Manisa and Aydın provinces (1). The marketable crowns of the cauliflowers are susceptible to extreme climatic conditions like low temperature and heavy rains in the periods when the crowns complete their maturation for market. Hence, if they are not harvested on time, they lose quickly their market values. This event is of great importance for seed production. Having good seed yield is difficult because of the rotings on the crowns caused by low temperature and heavy rain. This problem is observed clearly on the early varieties matured in the autumn months.

Some experiments have being conducted on the control of the rotings on the crowns caused by extrem climatic conditions and or by pathogens using the «Brio olenia» cauliflower variety at The Horticultural Department of Agricultural Faculty of Aegean University. In these experiments some applications have being tested like covering the individual plants with the perforated polyethylen bags, tying the leaves over the crowns, cutting out some flower lobes at the center of the crowns and covering the plants with low plastic tunnels which have both sides opened.

During these experiments in december 1985 a lot of brownish-black, dry and sunken, soattered lesions on the crowns have been observed. The crowns showing these symptoms formed no flower stalks.

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Besides these symptoms, it has been also recorded that the plants covered with polyethylene bags have been infected by *Peronospora brassicae*, *Sclerotinia* sp. and *Botrytis* sp.

This paper reports the studies to identify the causal organism of the symptoms on the cauliflower crowns.

MATERIALS AND METHODS

Plant material used in the tests was provided by The Horticultural Department of The Agricultural Faculty. Isolations were made from the variety «Brio osenia» and pathogenicity tests were done on plants of «Matra».

PDA including Streptomycine sulphate served as isolation medium. While no sporulation of the cultures having *Alternaria* character was observed on this medium, Filter paper technique was used (7). The mycellium on the ager surface was scraped out and the rest of the culture pierced. The agar pieces were brought onto the sterile and wet filter paper in the petri dishes which were then kept at 24°C in dark for 3 days. The spores obtained by this method were used for the inoculum.

The inoculation of the fresh harvested «Matra» crowns was made by spraying after taking-off the leaves. The inoculum concentration was as 80.000 spores/ml. After inoculation the crowns were covered by polyethylene bags and kept at 24°C in a clima chamber. Humidity in the bags was provided by the wet cotton pieces.

The identification of the pathogen was based on the morphology of the colonies on PDA and spores. The results were compared with those reported in CMI Descriptions (2, 3).

RESULTS

The isolations from the infected crowns always gave the same fungus with typical *Alternaria*-appearance without sporulation. The sporulation occurred abundantly on the filter paper around the agar-pieces.

In the pathogenicity tests first symptoms occurred on the «Matra» crowns 4 days after inoculation as little, sunken, brownishblack lesions which corresponded well with those observed under the field conditions. After coalescence of the small lesions larger ones were formed on the crowns with time. The isolations from these lesions have revealed the presence of the same fungus.

The pathogen is identified as *Alternaria oleracea* Milbraith by de-

termining the morphological characteristics of the spores and of the colonies on the agar medium.

The conidia were averagely 22.7 u long, 9.67 u thick and have a beak with 4.2 u length and so the beak has approximately the 1/5 length of the conidium. Figure 2 shows the conidia of *A. oleracea* isolated in this study. The colonies on PDA are dark olivaceous in colour velvety and smooth.

DISCUSSION

According to the C M I Descriptions No: 163 (3) the conidia of *A. oleracea* have 1-11, mostly less than 6 transverse septa and few, up to 6 longitudinal septa. They are 18-130 u long, 8-30 u thick and have a beak with 1/6 length of the conidium. The colonies of the fungus are dark olivaceous brown to dark blackish brown in colour, velvety, smooth and effused. These morphological features were also recorded on the isolate used in this study.

Another pathogen on the Cruciferae *A. brassicae*, have different colony and spore features and it is easily distinguishable by its longer beak (2).

A. oleracea is a seed-borne pathogen. It causes more severe diseases on cauliflowers and it is more common and widespread than *A. brassicae* (Berk.) Sacc. (3, 5, 8). KARACA (6) reports that *A. oleracea* is present in Turkey and occurs besides Cruciferae also on other crop plants. The author gives the names *A. brassicicola* (Schw.) Wiltshire and *A. brassicae* (Berk.) Sacc, as synonymy for *A. oleracea* Milbraith. On the other hand, *A. brassicicola* is accepted as synonymy for *A. oleracea* but not for *A. brassicae* (2, 3, 5). The name *A. brassicae* represents also a species other than *A. brassicicola* or *A. oleracea*.

In our study we have used the name *A. oleracea* while the names «*brassicicola*» and «*brassicicae*» can be easily confused (6).

In the next studies it is planned to find out the relation between disease severity and growing technique in field and also in greenhouses with or without chemical control of the pathogen.

Ö Z E T

TOHUM ÜRETİMİ AMACIYLA YETİŞTİRİLEN ERKENCİ KARNİBAHAR ÇEŞİTLERİNDE *Alternaria oleracea* Milbraith.

Erkenci karnıbahar çeşitlerinin baş çürümelerini kontrol etmek için yapılan çalışmalar sırasında özellikle yağışlı havalarda başlar üzerinde çiçek saplarının da oluşumunu engelleyen kahverengimsi-siyah,

kuru ve batık lezyonlar görüldü. İzolasyon ve patojenisite çalışmalarından sonra patojen *Alternaria oleracea* Millbraith olarak tespit edildi.

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ÖZET

TOHUM ÜRETİMİ AMACIYLA YETİŞTİRİLEN ERKENÇİ KARNİBAHAR ÇEŞİTLERİNDE *Alternaria oleracea* Millbraith

Erkenç karnibahar çeşitlerinin baş görünüşlerini kontrol etmek için yapılan çalışmalar sırasında özellikle yağışlı havalarda başlar üzerinde çiçek saplarının da olgunlamanın engellenen kahverengimsi-şişmiş

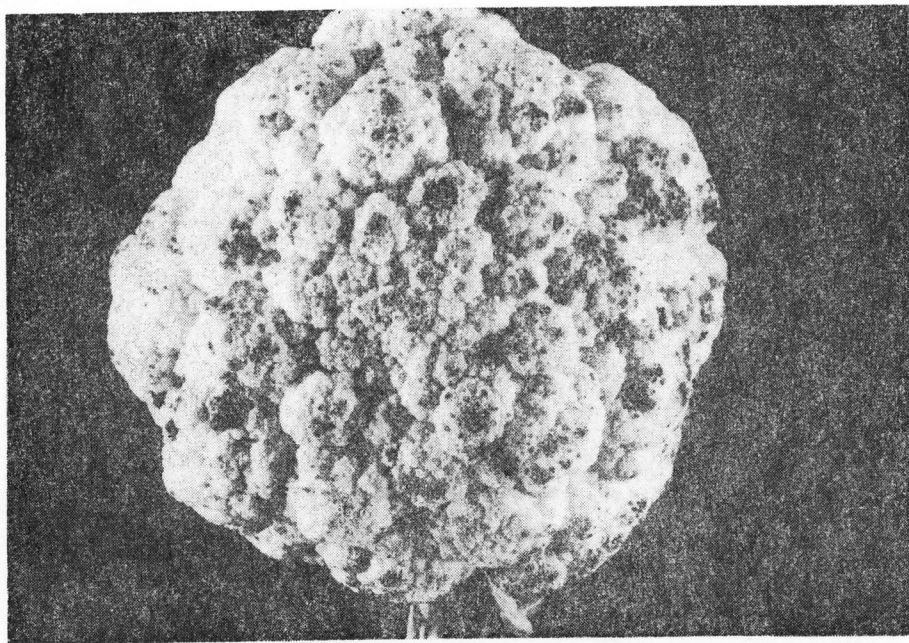


Fig. 1. Symptoms on the «Matra» crowns 10 days after inoculation with *A. oleracea*

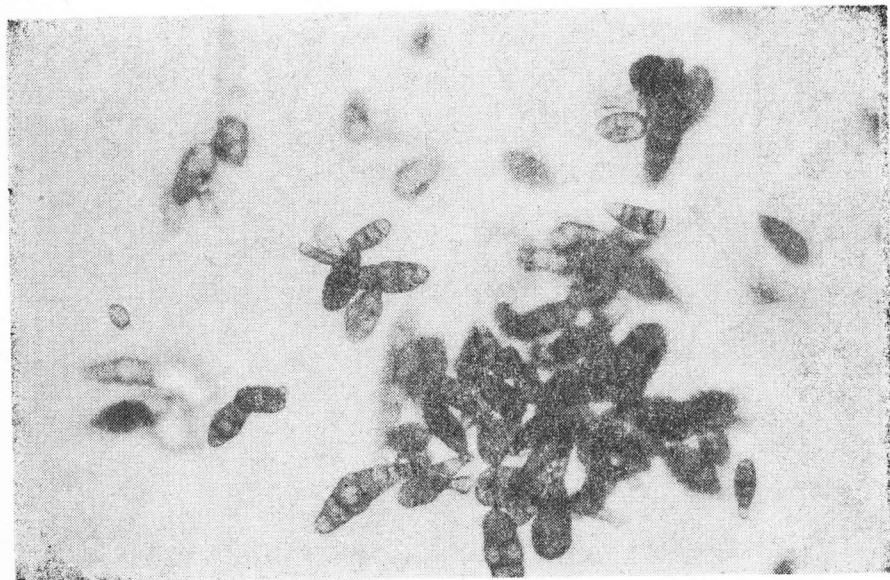


Fig. 2. Conidia of *A. oleracea* used in the pathogenicity tests

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