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## Gummy Pitting, A Destructive Virus Disease of *Poncirus trifoliata* Rootstock in Izmir Province of Turkey

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### ABSTRACT

A destructive disease of trifoliata orange have been observed in Satsuma mandarins during the survey studies in Izmir Region and indexing trials have been done for the citrus virus diseases. The indexing tests for tristeza, exocortis, xyloporosis and the other virus diseases the anatomical tests and the field observations revealed that gummy pitting had different symptoms from those of the aforementioned diseases. The main visual symptoms of the disease were gum pockets in the bark and wood of trifoliata orange rootstock, and the extended typical gummy pitting from the bud-union to the soil level. These symptoms were different from those of tristeza, exocortis, xyloporosis and cristacortis viruses. The incidence was 50 % in some severely affected orchards and Satsuma trees showed declining and dying symptoms.

### INTRODUCTION

Because of its tolerance to tristeza and resistance to foot rot, its adaptation to heavy soil and the excellent quality fruit produced by Satsuma trees grown on it, trifoliata orange (*Poncirus trifoliata* (L) Raf.) is a widely used rootstock in Izmir. However, typical symptoms of gummy pitting virus have been observed on trifoliata rootstock under the Satsuma trees since 1975 by the surveys and the indexing experiments for tristeza, exocortis, xyloporosis and the other virus di-

seases of citrus (Azeri, 1973; Azeri and Heper, 1978).

Since then, the relationships of the gummy pitting with the other known viruses which caused dwarfness, barkscallings, gumming, declining and stem pitting incited by the recently reported Cristacortis (Azeri, 1979) have been examined. Gummy pitting affected trees have been tested by indexing and the Child's Color test during the virus studies.

## MATERIALS and METHODS

Totally 30 Satsuma mandarins at 10 to 15 years old which showed the expressing symptoms of gummy pitting on their trifoliata stocks were selected for tristeza, exocortis, xyloporosis, psorosis, stubborn index tests since 1973. The following indicator plants were used in indexing; Etrog citron (*Citrus medico* Linn.) Arizona 861 for exocortis; Orlando tangelo (*C. paradisi* Macf. x *C. reticulata* Blanco) for xyloporosis; Madam vinous (*C. sinensis* (L) Osb.) sweet orange, Duncan and Marsh grapefruit (*C.*

*paradisi* Macf.) for Stubborn; Mexican lime (*C. aurantifolia* (Christm) for tristeza; sexton tangelo (*C. reticulata* x *C. paradisi*) and Dweet tangor (*C. reticulata* Blanco x *C. sinensis* (L) Osb.) for psorosis (Concave-Gum) virus were used as previously reported by Azeri and Heper (1978). The bark floem of the trifoliata stocks were also examined by Child's color test for detection of exocortis aldehyde reaction in phloem as described by Azeri (1973).

## RESULTS and DISCUSSION

**Symptoms:** The typical visual symptoms of gummy pitting virus are gum pockets in the bark (Fig. 3) and wood of trifoliata orange rootstocks (Fig. 4). When the bark of the virus affected trifoliata stock is removed below the bud-union, typical gummy pittings extended from the bud-union to the soil level can be seen as shown in (Fig. 1). Gum impagnated pegs different from xyloporosis and cristacortis pegs have been observed under the bark surface of trifoliata stock (Fig. 2).

In severely affected trees, trifoliata rootstocks have developed very deep gummy pittings and the bands of gum from the bark towards the center as shown in (Fig. 5). At this stage of the disease, trees show declining or frequently dying symptoms.

The differences of the gummy

pitting from tristeza and xyloporosis are normal and clean scion bark and wood development above the bud-union when the first disease causes heavy gumming below the bud-union as shown in (Fig. 3). The visual symptoms of gummy pitting on the Satsuma scion are mild or severe dwarfness and declining (Fig. 6) at the later stage when the Satsuma trees reach 10 or 15 year old.

**Indexing tests.** - Indexing trials with 30 Satsuma trees affected by typical gummy pitting have revealed that, this disease and its symptoms have not been associated with those tristeza, xyloporosis, exocortis, psorosis and stubborn disease as previously described by Azeri (1973, 1978, 1979, 1981). Some gummy pitting and exocortis-infected Satsuma trees showed both gummy pitting and typical exocor-

tis scally bark symptoms. Gummy pitting-infected satsuma trees free from exocortis, xyloporosis, tristeza and psorosis have developed only typical gummy pitting symptoms on trifoliata stock. When the satsuma are infected with xyloporosis, typical gummy pegs have been noticed above the bud union different from gummy pitting pegs and pitting which developed under the bud-union as shown in Fig. 1 and 2.

**Color tests.**-Bark specimens from the gummy-pitting affected 30 trees have been examined by child's color test (Azeri, 1973) for identifications of anatomical affects of gummy pitting and exocortis diseases in the bark phloem of trifoliata stock. When the trifoliata stocks were infected with only gummy pitting, phloroglucinol/HCL treated-bark specimens have

showed typical gum impagnations in the trifoliata bark as shown in Fig. 4. During the complex infections with exocortis and gummy pitting, it was possible to see both phloem ray cells aldehyde reaction of exocortis and gummy pitting reaction and phloem gum impagnatioen of gummy pitting.

**Incidence.**-The large affect of gummy pitting virus has been observed on trifoliata stocks in 9 Satsuma plantations in different localities Seferihisar, Gümüldür and Central County. The examined Satsuma trees were between 10-15 yeard old. The rate of incidence of gummy pitting virus were very high (50 %) at the 3 orchards in Seferihisar county as shown in Table 1. All of the gummy pitting affected trees showed typical symptoms of the virus on their trifoliata stocks.

Table 1. The rates of incidence of gummy pitting in the different localities.

Localities	The number of the Satsuma Orchards and the trees examined for gummy pitting		The number of The gummy pitting affected trees	The incidence of gummy pitting %
	Number of Orchard	Number of trees		
Central County	3	300	80	26,6
Seferihisar	3	300	150	50
Gümüldür	3	300	75	25
Total	9	900	205	

Schwarz and McClean (1969) reported that, gummy pitting was not insect transmissible and the disease had quite different symptoms from those of tristeza, xyloporosis, exocortis, tatter-leaf virus diseases. The authors also reported 71-82 % incidence of gummy pitting in South Africa for different citrus bud on trifoliata orange rootstock. Fraser et al. (1976) also reported that, many varieties of citrus carried gummy pitting inducing factor and produced gummy pitting symptoms when bud on trifoliata rootstocks. The same authors revealed that, high temperature could affect the symptoms development and the symptom severity on trifoliata orange was related with the different strains of gummy pitting virus. The

authors reported that, gummy pitting could develop its symptoms not associated with exocortis, xyloporosis and tristeza but mixture of strains of gummy pitting and exocortis may occur commonly in some trees. Reuther et al (1978) also reported gummy pitting was not spread by natural means, but this virus was normally spread only by the movement of infected propagative material.

For control measures, it can be recommended use of virus free clean parent material from healthy Satsuma trees. Clean stock is useful also in limiting or preventing damage from the other known virus diseases. Eradication of the unfruitful and declined trees, and replanting with virus free plants is necessary in a short time.

## Ö Z E T

### İZMİR İLİNDEKİ ÜÇYAPRAKLI ANAÇLARINDA ZARARLI BİR VİRUS HASTALIĞI «ZAMKLI ÇUKURLUK» (GUMMY-PITTING)

Satsuma mandarini İzmir iline bağlı bazı sahil muntikalarında geniş bir üretim alanına sahiptir. Satsuma mandarini bu yörelerdeki toprak yapılarına ve kök çürüklüğüne dayanıklı olan ve kaliteli meyve verimine olumlu etkisi olan üçyapraklı anacı üzerine aşılanmaktadır. Üçyapraklı anacın birçok iyi özellikleri yanında bilhassa exocortis hastalığına ve son yıllardaki araştırmalarda gummy pitting hastalığına karşı duyarlı olduğu bilinmektedir. İzmir ilindeki turunçgil ağaçlarındaki virütik hastalıkların saptanması için 1973 yılından beri ya-

pılan endeksleme testleri ile tarla gözlemleri sonunda, exocortisten sonra gummy pitting hastalığının üçyapraklı anaçlarda zamklı çukurluklar meydana getirerek 10 ve 15 yaşındaki ağaçlarda ölümler meydana getirdiği saptanmıştır. Hastalık üçyapraklı anacın üst kısmındaki aşı kısmında herhangi bir anatomik belirti oluşturmadığı halde, aşı yerinin hemen altından kök tacinca kadar üçyapraklı anacının kabuk ve odun dokusunu zamklandırmakta ve diğer viruslerden farklı çukurluk belirtileri oluşturmaktadır. Hastalık bazı Satsuma bahçele-

rinde % 50'ye kadar yaygınlık göstermekte olup, 10 ve 15 yaşındaki ağaçlarda aşırı bodurluk, göçme ve ölüm belirtileri göstermektedir.

Zamklı çukurluk hastalığının üretimde kullanılan aşı gözü ve kalem-

leriyle geçmesi nedeniyle, yeni fi-dan üretimlerinin virüssüz ağaçlar-dan yapılması ve ayrıca hastalıklı verimsiz ağaçların sökülerek yerle-rine sağlıklı ağaçların dikilmesi ge-rekmektedir.

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## GUMMY PITTING



Figure 1. Gummy pitting virus affected trifoliata orange rootstock showing typical pits of the disease.

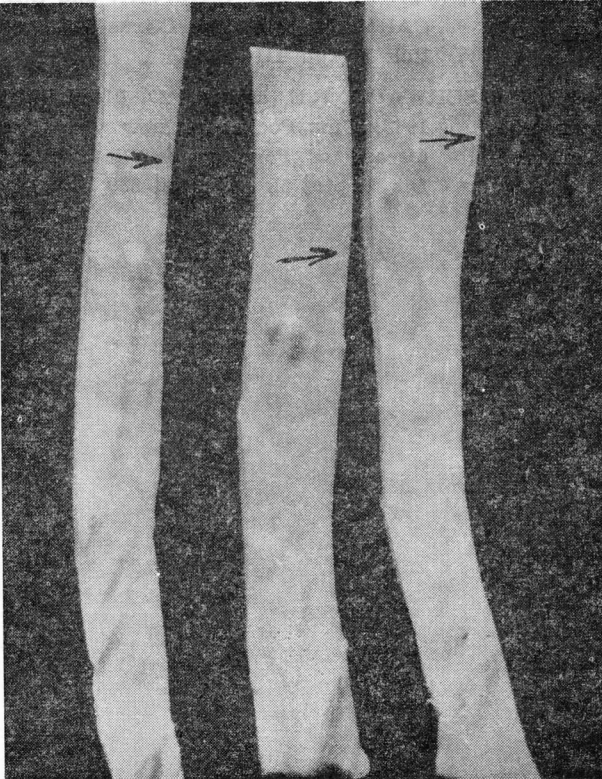


Figure 2. Gum impregnated pegs of gummy pitting on the lower bark surface of the trifoliata stock. The Satsuma section above the bud union (arrows) were normal (clean).



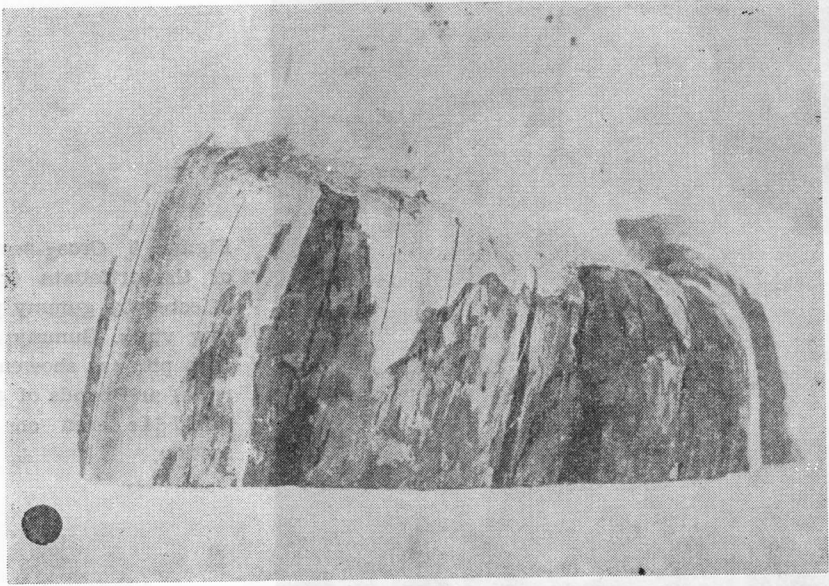


Figure 3. Heavy gum lesions and pits on the wood of trifoliata orange rootstock after removing the bark wood and the bark tissues of the Satsuma scion is clean.



Figure 4. Radial-longitudinal section from the bark specimen of the gummy pitting affected trifoliata stock treated with phloroginichol+HCl; (a) phloem leaf fibres; heavy gum impagnated dead floem tissue different from the aldehyde reactions of exocortis.

## GUMMY PITTING

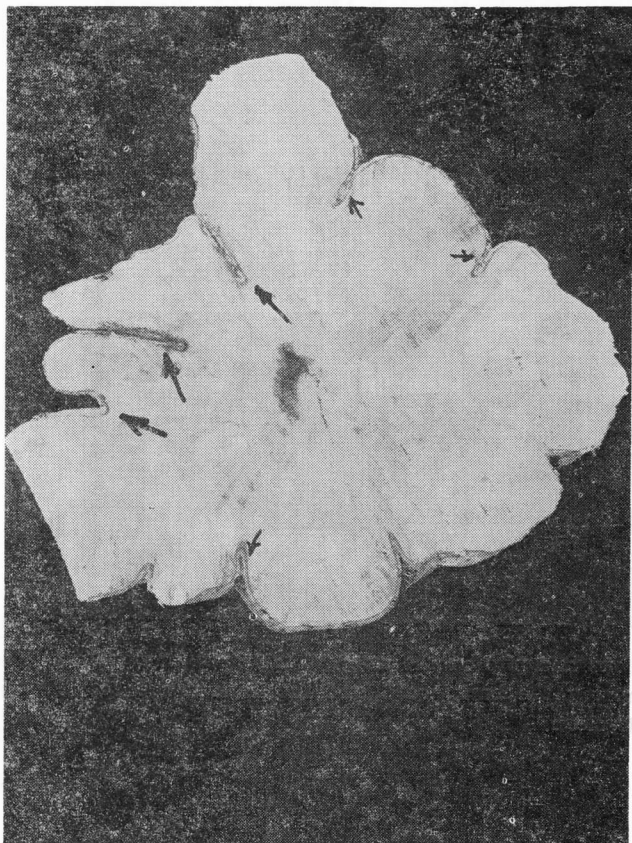


Figure 5. Cross-section of the trifoliata trunk affected by gummy pitting virus. Gummy and deep pits (as shown by rows) and bands of gum could be seen on the section.

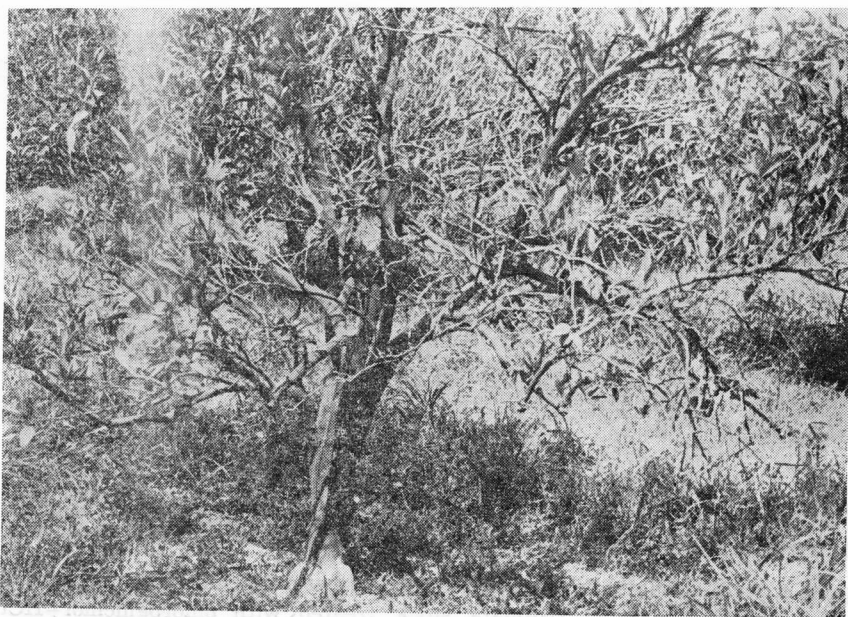


Figure 6. Satsuma tree on gummy pitting affected trifoliata orange stock. Satsuma trees shows dwarfness and declining from the single virus.

## Preliminary Investigation of Potato Diseases Caused by Mycoplasmalike Organisms (MLO) in Erzurum Region

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### ABSTRACT

Three different kinds of potato disease caused by mycoplasmalike organisms (MLO) were described in Erzurum region by evaluating their visible symptoms in field conditions. These are namely; hair sprout on tubers, witches' broom and stolbur diseases on potato plants. The incidence of witches' broom infection is very scare in the region, however, this is the first report about the presence of it in Turkey. The hair sprout has at least 4.1 % rate of disease incidence in the seed stock of potato growers. On the other hand the rate of disease incidence of potato stolbur could increase to the level of 86.3 % which is probably disseminated by two species of leafhopper vectors (*Hyalosthes obsoletus* Signoret and *Abhrodes bicinctus* Schrank) efficiently. So, in spite of its suitable ecological, topographical and climatical conditions for the seed potato production in Erzurum area, these vector-borne pathogenic diseases of MLO decrease the chance of the successful seed potato production.

### INTRODUCTION

As Ilisulu (1957) evaluated that the commercial potato production is possible everywhere in Turkey, seed potatoes, however, could produced in several regions, like Erzurum, Kars and Bolu. He emphasized that Erzurum region has two highlands for this purpose, namely Pasinler and Erzurum plains. Special topographical, ecological and climatical features of these plains are almost ideal for the seed potato production which requires very

special environmental conditions. For this reason Erzurum area has attracted close attentions of public and private sectors for the seed potato production. Even though several attempts were made for the production of certified seed potatoes in isolated fields and locations of both Pasinler and Erzurum plains since 1975. Despite of the careful field controls for those diseases on potato plants caused by fungi, bacteria and viruses which were in-

dexed and rogued immediately, all those attempts were unsuccessful and disappointing because of some other prevailing diseases of potatoes. All those failed attempts imply that the seed potato production is going to be very risky business venture in this area.

Present disease problems of Erzurum potatoes can be summarized as follow; dry rots of potato tubers in storages caused by *Fusarium* spp. were reported by Gündüz (1977) are widespread and very destructive for tubers in storage. Döken (1977) also identified and reported another fungal infection, black dot caused by *Colletotrichum atramentarium* (B. et. Br.) Taub has been a quite harmful disease in the field conditions. According to Yüksel (1978) tuber rot nematode *Ditylenchus destructor* Thorne is another local problem in Erzurum region. A number of virus diseases of potatoes caused by potato virus X (PVX), potato virus Y (PVY), potato leaf roll virus (PLRV) and

potato virus S (PVS) were identified and reported by Çıtır (1980) in field conditions and they have degenerated all the potato seed stocks of growers in this area (Çıtır, 1982). All of those potato diseases in Erzurum region have been established themselves thoroughly, including their efficient vectors, which pose a serious threat to the seed potato production (Çıtır, 1984).

In spite of using potato seeds which were free from all those diseases of potato revealed that some other disease problems are present in Erzurum area. Because of these unknown infections valuable foundation and registered seed potato material for the seed potato production have been lost since 1975.

The aims of this study were to determine those potato diseases caused by MLO and their rate of infections which were probably responsible for the recent disappointments of seed potato production in Erzurum area.

#### MATERIALS and METHODS

In order to determine seed-borne potato diseases caused by MLO, seed potato tubers were sampled according to Bora and Karaca (1970), from the growers of 29 villages in Pasinler and Erzurum plains. The sampled potato tubers were placed in greenhouse section in which the mean temperature is about 20°C in April. Two weeks later the tubers which were not sprouted or sprouted hairy, were counted and recorded. So the rate of hair sprout

infection was determined as in % in the Erzurum area.

Observations for the witches' broom infections on potatoes were done through out the field trips in Pasinler and Erzurum plains. The suspected potato plants were examined for the characteristic symptoms of the MLO infection, and described.

The vector-borne potato diseases caused by MLO were studied by sowing disease-free registered Cosima

and Isola seed tubers, obtained from Bolu and Erzurum, potato research stations of Ege Regional Agricultural Research Institute, Menemen, Izmir. Experimental field plots containing 280 plants from the each variety were set up 100 by 40 cm distances in the Research Farm of Atatürk University in Erzurum plain during the years of 1981, 1982 and 1983. The recording date of stolbur disease incidence and the rate of infection were determined during these experiments. Furthermore in 1984 by setting up another field experiment, in which the plots containing 44 plants each, the rate

of stolbur infection, the date disease incidence and the effect of disease on tuber yield were determined in both Erzurum and Pasinler Plains. The research farm of Eastern Anatolian Regional Agricultural Research Institute in Pasinler Plain was also used for this purpose. In order to avoid the contaminations and interactions of virus diseases of potatoes, the suspected plants were checked by mechanical sap inoculations made from these plants to the virus indicator plants listed in Table 1. So virus infected plants were indexed and rogued.

Table 1. Virus indicator plants used for the indexing of potato viruses in Pasinler and Erzurum plains.

Name of indicator plant	Variety	Name of virus
<i>Gomphrena globosa</i> L.	Mixed colors	PVX
<i>Chenopodium amaranticolor</i> Coste + Reyn.	—	PVY
<i>Chenopodium quinoa</i> Willd.	—	PVS
<i>Datura stramonium</i> L.	—	PVX
<i>Datura metel</i> L.	—	PVS
<i>Nicotiana tabacum</i> L.	White-Burley	PVY

## RESULTS

As a result of the investigations made on seed-borne hair sprout disease incidence on potato seed material was determined as shown in Fig. 1., and its rate of disease incidence was summarized in Table 2. Despite of the rigid hard appearances of some infected tubers they could never sprout. Some of them,

however, exhibited long spindle or hairy sprouts in contrast to thick and short sprouts of healthy tubers. Such as infected tubers never grown up as healthy plants. The average rate of hair sprout infection on seed potatoes occurs as 4.1 % in Erzurum region.

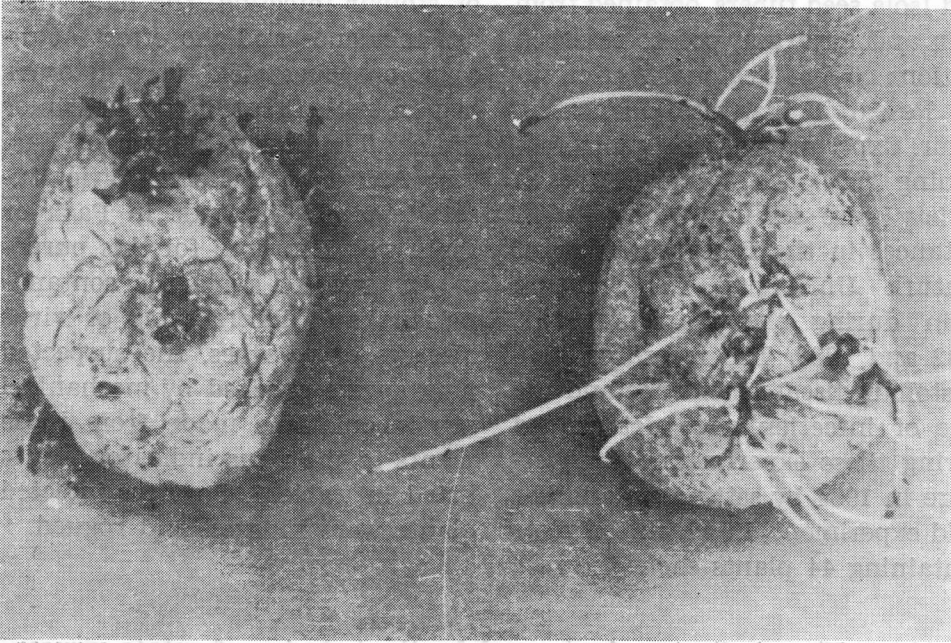


Figure 1. Hair sprout infection on seed potato tubers caused by Mycoplasma-like organisms (MLO). Healthy tuber is on the left.

Table 2. The infection rate of seed-borne hair sprout disease on potatoes caused by MLO in Erzurum region.

Location of potato tubers sampled	Number of villages	Number of tubers tested	Number of infected tuber	Rate of inf. %
Erzurum Plain	13	323	12	3.71
Pasinler Plain	16	485	21	4.33
Total	29	808	33	4.14

The witches' broom of potato was another disease caused by MLOs, was found rarely in Erzurum area. The infected tubers produced great number of thin and long shoots

when they used as a seed material (Fig. 2). These shoots and buds abnormally crowded and never produced any healthy plants or tubers.



Figure 2. Disease symptoms of witches' broom in Cosima potato collected in Pasinler plain in 1980.

A vector-borne disease, stolbur of potato was found as the most destructive infection caused by MLO in Erzurum region. The infection was recorded only by sowing disease-free registered potato seed tubers, in order to avoid interactions with other vector borne potato diseases. Somehow registered seed potatoes were contaminated with viruses and other pathogenic diseases in a few instances in field experiments. Only one plant was infected by tuber rot nematode *Ditylenchus destructor* Thorne. The potato plants have been infected mostly by stolbur disease in the field plots. Three-year of field experiments in Erzurum plain indicated that, the visible symptoms of stolbur are appeared 90 days after sowing during

the first half of August. The average rate of infection was over 80 %. The results of field experiments conducted in 1984 was summarized in Table 3. The visible symptoms of potato stolbur infection were appeared once again during the first half of August in both plains. The rate of infection, however, indicated slight differences which were not significantly important among the locations and varieties. On the other hand the reduction of tuber yields were significantly different between two locations.

The stolbur infected cosima and isola plants exhibited identical symptoms as shown in Fig. 3. Upper leaflets rolled and developed purple tips on the stems, shorter

### MYCOPLASMALIKE ORGANISMS

and thicker internodes large adventive buds and aerial tubers were recognized as exhibited characteristic symptoms. Some of the plants wilted and died prematurely. Po-

tato tubers harvested from the infected plants were soft and gummy in texture when they were pressed Fig. 4.

Table 3. The infection rate of potato stolbur disease caused by Mycoplasma-like organism (MLO) in Erzurum area and its effect on the tuber yield in 1984.

Location of field plots	Potato cultivars	Rate of inf. %	Tuber yield reduction on per plant %
Erzurum Plain	Isola	86.3	46
(Atatürk Univ. Farm)	Cosima	81.8	53
Pasinler Plain	Isola	81.8	30
(E.A.R.A.R.I. Station)	Cosima	72.7	35

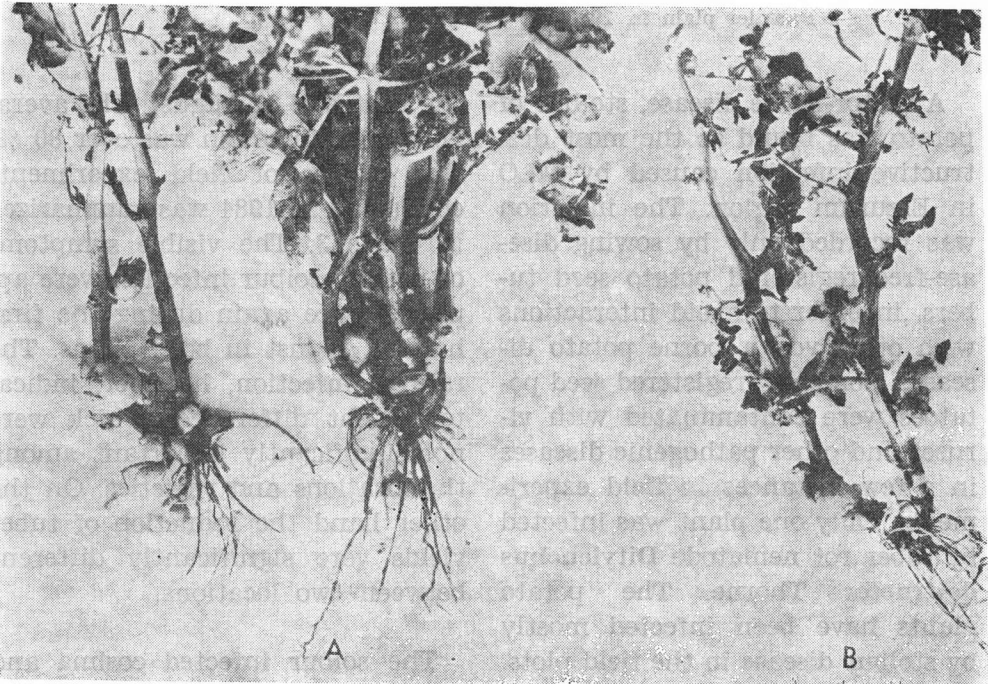


Figure 3. Characteristic symptoms of stolbur disease caused by Mycoplasma-like organism (MLO) on two potato cultivars. A. Isola, B. Cosima.



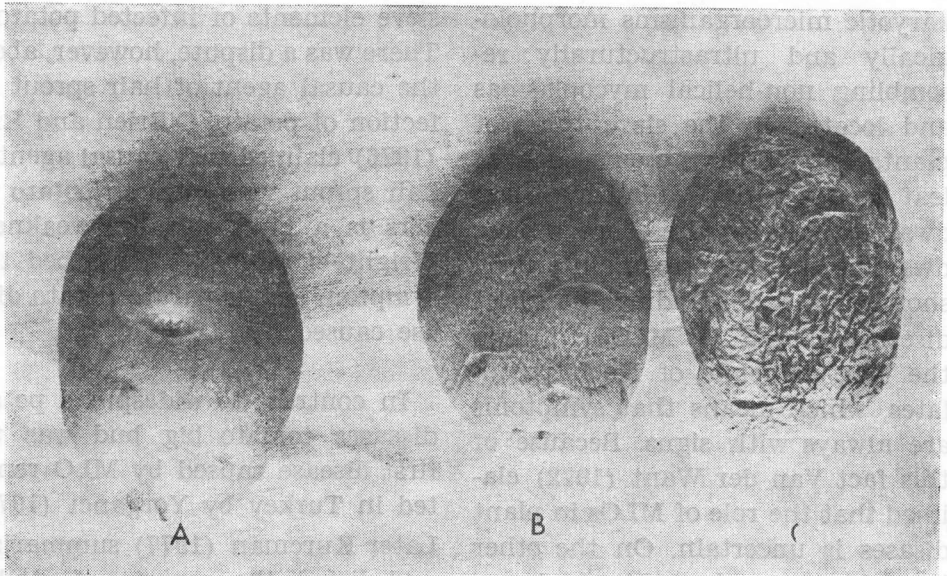


Figure 4. Stolbur disease symptoms on Isola potato tubers. A. Tuber from healthy plant, B, C. Tubers from infected plant.

#### DISCUSSION

If an agricultural area has to be selected for the seed production, a number of features including disease conditions of potato have to be examined thoroughly. Erzurum was named as a seed potato production area due to its ideal topographical, ecological and climatical features by Ilisulu (1957). The conditions of potato diseases, however, were overlooked for this selection. Beside the reported potato diseases, the results of this investigations revealed some unknown disease infections in Erzurum area. In addition to some pathogens of viruses, bacteria and fungi, MLOs are also responsible agents of some other potato diseases like stolbur, hair sprout and witches' broom. The incidence of stolbur di-

sease of potato was reported in Bolu by Sahtiyancı (1966). It was also reported in Erzurum area by Sahtiyancı and Varlı (1966)\* under the name of Stolbur virus disease of potato. After discovery of mycoplasma-like organisms (MLO), have been the real causal agents of some of the plant virus diseases by Doi and his coworkers in 1967, a number of plant virus diseases have been reexamined etiologically. In this first report in Japanese Doi and his coworkers studied the potato witches' broom infection and found MLO in the phloem elements of infected potato plants. Bové (1984) reviewed that the term MLO never implies the genus *Mycoplasma*, but refers to pleiomorphic, wall-less pro-

(\*) Ş. Sahtiyancı and G. Varlı, 1966. Annual Report of Potato Res. Project No. 107815. R.P.P. Res. Inst. Erenköy, İstanbul, Turkey

karyotic microorganisms morphologically and ultrastructurally resembling non-helical mycoplasmas and located in the sieve tubes of plants carrying symptoms such as leaf yellows, leaf mottling, leaf dwarfing, flower virescence, flower dwarfing, witches broom, internode shortening and stunting. So plant diseases caused by MLOs fit only the first principle of Koch postulates, which means that symptoms are always with signs. Because of this fact Van der Want (1972) claimed that the role of MLOs in plant diseases is uncertain. On the other hand Maramorosh (1974)\* had suggested that the first principle of Koch postulate about the symptoms and signs union for the identity of cause of plant diseases are enough evidence, to attribute to MLOs are the causal agents of some virus diseases, like aster yellows and witches' broom. Even though he had suggested that may be many plant, animal and human diseases caused by unknown identities have to be reexamined etiologically, which might be caused either by MLOs or viroids.

As a results of etiological studies made on potato stolbur disease by Semancik and Peterson (1971) revealed that the causal agent was not a virus but a tetracycline sensitive MLO. Nagaich et al (1974) confirmed those reports of stolbur and witches' broom infections of potato by determining MLOs in the

sieve elements of infected potatoes. There was a dispute, however, about the causal agent of hair sprout infection of potato. O'Brien and Rich (1976) claimed that causal agent of hair sprout symptoms on potato tubers is a kind of tuber weakness. Wright et al (1981) described this symptom as a separate potato disease caused by MLO.

In contrast to widespread potato diseases tomato big bud was the first disease caused by MLO reported in Turkey by Yorgancı (1976). Later Kurçman (1977) summarized and listed the reports of stolbur and hair sprout infections of potato caused by MLOs in different parts of Turkey. The witches' broom infection of potato, however, never reported before in Turkey. So, this could be considered the first report of witches, broom disease in Erzurum as well as in Turkey. This potato infection was rarely found in Erzurum area similar to Beemster and Rozendaal (1972)'s rare incidence report of witches' broom of potato in other countries.

Four different leafhopper species were identified and reported as vectors of stolbur pathogen of potato. Among them *Hyalesthes obsoletus* Signoret was described the most efficient one by Beemster and Rozendaal (1972) and Wright et al (1981). *H. obsoletus* was identified by Lodos and Kalkandelen (1980) as widespread in Erzurum area and

(\*) K. Maramorosh, 1974. Lecture Outlines in Plant Virology. Rutgers Univ. New Brunswick N.J., U.S.A.

the other parts of Turkey. *Abhrodes bicinctus* Schrank is another vector of stolbur that was also identified in Erzurum area by Lodos and Kalkandelen (1982). There is no report about *Ophiola flavopicta* in Erzurum area which is described as a vector of potato witches' broom by Beemester and Rozendaal (1972).

*Convolvulus arvensis* L was described as the most efficient overwintering host of potato stolbur MLO among the 350 species of host plants by Wright et al (1981). Because of its obligate parasitic character MLO of stolbur needs an over wintering host. *C. arvensis* is an ideal overwintering host species because of its living stolons during

the winter. Günçan (1976) identified *C. arvensis* as a quite widespread weed problem in Erzurum area. So all those factors; pathogen, overwintering host, vectors and environmental conditions are suitable for the prevailing epidemics of stolbur disease of potato in Erzurum area.

Nevertheless, potato diseases caused by MLO's in Erzurum area needs more investigations to be done. At least the pathogens have to be studied by utilizing electron microscope in the sieve elements of infected potato plants for the confirmation of these symptomatological evidences.

## Ö Z E T

### ERZURUM BÖLGESİNDE MİKOPLAZMA BENZERİ ORGANİZMA (MLO)'LARIN NEDEN OLDUKLARI PATATES HASTALIKLARI HAKKINDA ÖN ARAŞTIRMALAR

Erzurum ve çevresindeki patates üretim alanlarında mikoplazma benzeri organizma (MLO)'ların neden oldukları üç ayrı patates hastalığı bunların sergiledikleri belirtilere göre tanımlanmışlardır. Bunlar sırası ile yumruda kılcal sürgün, cadı-süpürgesi ve stolbur hastalıklarıdır. Bölgedeki patateslerde cadı-süpürgesine çok az rastlanmakla beraber tanımlanan örneklerle bu patates hastalığının bulunuşu ilk defa rapor edilmektedir. Kılcal sürgün hastalığı ise üreticilerin kullandıkları patates tohumluklarında ancak % 4.1 oranında

görülmeştir. Öte yandan bölgede yıllara göre değişmekle birlikte bünyeme mevsimi içerisinde iki ayrı böcek vektörü (*Hyaesthes obsoletus* Signoret ve *Abhrodes bicinctus* Schrank) tarafından etkin bir şekilde bulaştırıldığı sanılan stolbur hastalığı oranının % 86.3 seviyesine kadar yükselebildiği saptanmıştır. Türkiye'de sertifikalı tohumluk patates üretiminin yapılabileği uygun ekolojik, topografik ve iklim koşullarını içeren Erzurum için bu durum, üretimde başarı şansını azaltan bir sonuçtur.

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Studies on the Pathogenicity and the Identification by using Serological Methods of a Bacterial Agent (**Corynebacterium michiganense** pv. **michiganense** 'Smith' Jensen) which causes Tomato Wilt in greenhouses of Antalya province

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### ABSTRACT

The pathogenicity of the bacterial agent isolated from the stems and petioles of Tomato plants that show typical symptoms of Bacterial Canker disease was determined by root inoculation technique, and it was identified as **Corynebacterium michiganense** pv. **michiganense** 'Smith' Jensen by using an anti-**C. michiganense** pv. **michiganense** serum that had been produced in a rabbit.

### INTRODUCTION

Antalya province is an important vegetable growing center with 29458 hectares of growing area and a production of 779921 tons of vegetables both fields and greenhouses, 264171 tons of this total vegetable production belong to tomato yield (Anonymous, 1982).

Bacterial Canker of Tomato (**Corynebacterium michiganense** pv. **michiganense** 'Smith' Jensen) is a disease that appears when tomato plants begin to flowering and it causes serious damages. According to Karaca (1977), "Bremer et Özkan (1950) has determined this disease for the first time in Turkey.

Later Bremer et al. (1952) and Karahan (1965) reported the disease in Central and South Anatolia". Karaca and Saygılı (1982) found out that **C. michiganense** pv. **michiganense** is effective in Manisa, Balıkesir and Çanakkale. The symptoms of the disease have been observed as wilting of tomatoes in greenhouses of Antalya province in recent years. The disease is spreading out quickly. Serological techniques can aid in the detection of Bacterial Canker of Tomato (Morton and Thyri, 1966), and this study has been carried out in order to identify the agent exactly to a degree.

## MATERIALS AND METHODS

In order to isolate the bacterium, the stems and petioles of infected plants were crushed in sterile water. This suspension was diluted 100 and 10000 times, and 0.1 ml inoculum was transferred onto SNA (Standart 1 Nutrient Agar, Merck 7881) from the last two dilutions with a micropipette and spread to the agar surface. The suitable ones among the colonies that develop were transferred onto agar slants one by one.

For the pathogenicity test, the isolate numbered B.5/5 was used. A suspension containing about  $10^{5-6}$  bacteria cells per ml was made from the 2 days old cultures of the bacterium. The roots of the seedlings with 3-6 leaves were dipped into this suspension for 6 hours, and then transplanted to the pots. The temperature range was about 18-27°C.

In order to obtain the antiserum, the method of Kiraly et al. (1974) was used by modifying partially. For this purpose, a culture of *C. michiganense* pv. *michiganense* numbered C.1 (NCPPB 1468) and

a healthy domesticated rabbit were used. A suspension of the bacterium with a final concentration of about  $3 \times 10^8$  cells per ml in physiological saline (0.85 % NaCl) was prepared from 24 hours old SNA slants. This antigen material was injected to the rabbit subcutaneously 6 times totally. These injections were made at 3 days intervals and at the doses of 0.5, 0.5, 1, 2, 2, 4 ml respectively. The animal was sacrificed 6 days after the last injection and its blood was placed in a glass measure. After a resting of 2 hours, we put it into the refrigerator. The following day the serum accumulated above the blood was centrifugated for 5 minutes at 2000 rpm. So the clot particules was removed. In order to prevent the activity of microorganisms, phenol was added to the antiserum.

Slide agglutination and tube agglutination tests according to the techniques of Kiraly et al. (1974) were also made with this obtained antiserum, by using the C.1 and B.5/5 numbered cultures and the others that are present in our collection.

## RESULTS and DISCUSSION

At the end of the pathogenicity test, tomato seedlings that were inoculated with the bacterium numbered B.5/5 began to wilt 7-10 days after the inoculation. They showed the typical initial symptoms of Bacterial Canker. These plants en-

tirely dried up by time. The control plants did not show any symptoms of the disease.

The results of the serological tests to identify the bacterium numbered B.5/5 are given in Table 1.

Table 1. The reactions of anti-*C. michiganense* pv. *michiganense* with the bacterium numbered C.1 (NCPFB 1468), the tomato wilt agent numbered B.5/5 and some bacteria cultures

ISOLATES	Slide agglu.	Tube agglu.
C.1 ( <i>C. michiganense</i> pv. <i>michiganense</i> )	+	+
B.5/5 (Orig. cult.)	+	+
<i>C. michiganense</i> pv. <i>sepedonicum</i>	—	—
<i>E. carotovora</i> pv. <i>carotovora</i>	—	—
<i>P. syringae</i>	—	—
<i>X. campestris</i> pv. <i>vesicatoria</i>	—	—

As it was seen in Table 1, both the bacterium numbered C.1 that we used to obtain antiserum and the bacterium numbered B.5/5 (original culture) gave positive results in the tests. The others, *C. michiganense* pv. *sepedonicum*, *Erwinia carotovora* pv. *carotovora* Dye, *Pseudomonas syringae* van Hall, *Xanthomonas campestris* pv. *vesicatoria* 'Doidge' Dowson did not give any reactions.

Non-specific reactions being observed in slide agglutination tests occasionally reduces the sensitivity of these tests (Öktem, 1982). On the other hand, the same author has reported that all of the *C. michiganense* pv. *michiganense* isolates gave positive reactions but *Agrobacterium* sp., *Erwinia* sp. and *Pseudomonas* sp. did not produce any agglutination, with an anti-*C. michiganense* pv. *michiganense* serum obtained by himself. There is a similarity between these results and ours, and consequently we can say that the antiserum is specific for *C. michiganense* pv. *michiganense*.

The titre of antiserum was found same, but fairly low in the tube agglutination tests for both bacteria, C.1 (Check) and B.5/5 (Orig. cult.).

In the serological studies, the positive reaction of slide agglutination test brings into light the serological relationship of the pathogens. Tube agglutination and agar diffusion tests also determine the degree of this relation (Kiraly et al., 1974). But unfortunately in this study we couldn't make agar diffusion test because the antiserum titre was very low in tube agglutination test. However the results that we obtained from slide agglutination test showed that *C. michiganense* pv. *michiganense* isolate numbered C.1 and tomato wilt isolate numbered B.5/5 were serologically related. Obtaining the antiserum titre at the same level in the tube agglutination test showed that both of the bacteria are identical.

Thus it is determined that the causal agent of Bacterial Wilts on Tomatoes in greenhouses of Antal-



ya is *C. michiganense* pv. *michiganense* 'Smith' Jensen, and it is necessary to be taken the suitable control measures for this disease.

Ö Z E T

ANTALYA İLİ SERALARINDA ZARAR YAPAN DOMATES  
BAKTERİYEL SOLGUNLUĞU ETMENİ (*C. michiganense* pv.  
*michiganense* 'Smith' Jensen)'NİN PATOJENİSİTESİ VE SEROLOJİK  
YÖNTEMLERLE TEŞHİSİ ÜZERİNDE ÇALIŞMALAR

Domates Bakteriyel Solgunluğu hastalığının tipik belirtilerini gösteren solgun domates bitkilerinin gövde ve yaprak saplarından izole edilen etmenin, kök inokulasyon

yöntemiyle patojenisitesi belirlendi ve bir ev tavşanında elde edilen Anti-*C. michiganense* pv. *michiganense* serumu yardımıyla teşhisi yapıldı.

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## Licht- und elektronenmikroskopische Untersuchungen an Wurzeln mit Fusariumarten infizierter Pflanzen (**Cucumis melo**)

Haluk SORAN\*

Muhsin ÖZEL\*\*

### ZUSAMMENFASSUNG

Wurzelproben der im Laborversuch mit verschiedenen Fusariumarten (**F. oxysporum**, **F. tabacinum**, **F. culmorum**, **F. equiseti** und **F. solani**) infizierten Honigmelonen (**Cucumis melo**) wurden licht- und elektronenmikroskopisch untersucht.

**F. oxysporum** war als einzige Art bereits fünf Tage nach Infektion bis in Xylemzellen des Leitbündels eingedrungen und wurde folglich als Erreger der Vergilbungserkrankung bei Honigmelonen bestätigt.

**F. tabacinum** dagegen zeigte starke Vermehrung nur im Bereich des Rindenparenchyms (Gewebespezifität) und wurde primär als Erreger der Wurzelfäule und sekundär der welke identifiziert.

**F. culmorum**, **F. equiseti** und **F. solani** wurden in den Wurzelproben der infizierten Pflanzen nicht festgestellt und folglich für Honigmelonen als nicht pathogen bestätigt.

### EINLEITUNG

In den Anbaugebieten am Marmara-Meer und in Mittelanatolien wird allein mehr als die Hälfte der gesamten türkischen Honigmelonenproduktion geerntet (Anonymus, 1975). Untersuchungen in dieser Gegend ergaben, daß die durch Fusariumarten verursachte welke und Wurzelfäule der Honigmelone (**Cucumis melo**) sehr wichtige Faktoren sind, die den Ertrag jährlich stark beeinträchtigen (Soran, 1973).

Von erkrankten Pflanzen wurden folgende weit verbreitete Fusariumarten isoliert: **F. oxysporum**, **F. tabacinum**, **F. culmorum**, **F. equiseti** und **F. solani** (Soran, 1979). Die folgenden Infektionsversuche mit diesen Fusariumarten aus mehreren unterschiedlichen Isolaten zeigten, daß eine Pathogenität mit makroskopisch sichtbaren Symptomen nur durch **F. oxysporum** und **F. tabacinum** induziert

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wurde. Die mit *F. culmorum*, *F. equiseti* und *F. solani* infizierten Pflanzen blieben dagegen symptomlos (Soran, 1979).

Durch die vorliegende licht- und

elektronenmikroskopische Untersuchungen wurden die oben genannten fünf Fusariumarten nach Infektion an Wurzeln von Honigmelonensämlingen histopathologisch verglichen.

#### MATERIAL and METHODEN

**Pilzisolat:** Die für die Infektion benutzten Fusariumarten (*F. culmorum*, *F. equiseti*, *F. solani*, *F. oxysporum* und *F. tabacium*) waren während umfangreicher Untersuchungen über die Ursachen der Welkekrankheit bei Honigmelonen zwischen 1970 und 1975 in der Türkei isoliert worden (Soran, 1979).

**Pilzinfektion:** Die Samen der Honigmelone *Cucumis melo*, Sorte Hasanbey, wurden zuerst in 7,5 %  $H_2O_2$  10 Minuten gehalten und danach steril getrocknet. Die Aussaat dieser vorbehandelten Samen erfolgte in mit Potato Dextrose Agar (PDA) als Nährboden beschichteten Petrischalen (9 cm). In jede Petrischale wurden 8-10 Samen rund herum 1 cm vom Rand entfernt ausgesät. Ein periodischer Lichtschrank simulierte bei 25°C die Wachstumsbedingungen für die Sämlinge. Nach deutlicher Wurzelbildung wurde jede Petrischale in der Mitte mit einer Fusariumart beimpft und das Wachstum des Pilzhofes bis zu den randständigen Pflanzen verfolgt. 5 Tage nach Beimpfung der Pflanzenwurzeln mit

dem jeweiligen Pilzstamm wurden die Pflanzen aus den Schalen herausgenommen, gewaschen und die entnommenen Wurzelproben für Licht- u. Elektronenmikroskopie (EM) vorbereitet.

**Licht- u. EM-Präparation:** Die Wurzelproben von infizierten und nicht infizierten Pflanzen wurden in 2,5 % Glutaraldehyd (Serva) in 0,1 M Phosphatpuffer pH 7,2 mindestens 2 Std. bis mehrere Tage (Sabatini et al., 1963) und in 2 %  $O_5O_4$  (Merck) in A.bi-dest 2 Std. (Wohlfarth-Botterman, 1957) fixiert, in steigender Alkoholreihe Alkoholreihe entwässert und in Epon 812 + Araldit Mischung (Mollenhauer, 1964) eingebettet. Die Semidünnschnitte (0,5-1  $\mu$  m) für die Lichtmikroskopie wurden mit Methylenblau + Azur II (Merck) nach Richardson et al. (1960) gefärbt und mit dem Fotomikroskop II (Zeiss) unter Verwendung des Phasenkontrastverfahrens ausgewertet. Die Ultradünnschnitte für die Elektronenmikroskopie wurden mit Bleizitrat nach Venable et al. (1965) kontrastiert und im Elmiskop 101 (Siemens) untersucht.

#### ERGEBNISSE

**Kontrollpflanze:** Zunächst wurde der anatomische Wurzelbau einer nicht infizierten jungen Pflanze (*Cucumis melo*) im Bereich des

Hypokotyls an Querschnitten lichtmikroskopisch untersucht. Der Infektionsablauf konnte danach bei infizierten Pflanzen mit verschiede-

nen *Fusarium*arten orientiert verfolgt werden. Abb. 1 zeigt deutlich in diesem Bereich, von innen nach außen, Xylem (X), Phloem (Ph), zwischen diesen das Kambium (C), langgestreckte Endothelzellen (En) um das Leitbündel herum, Rinde (R) von großen und unregelmäßigen Zellen aufgebaut und mehrschichtige Epidermis (Ep).

**Wachstum der *Fusarium*arten in wurzeln infizierter Pflanzen:** Die mit *F. culmorum*, *F. equiseti* und *F. solani* infizierten jungen Pflanzen zeigten zum Zeitpunkt der Probenentnahme keine Symptome an Blättern oder an Wurzeln. Licht- und elektronenmikroskopisch konnten diese Pilzarten in keinem der oben genannten Wurzelbereichen festgestellt werden, auch nicht in Epidermiszellen. Zum aktiven Eindringen in die Wurzel waren sie offensichtlich nicht in der Lage (Abb. 2 a, b, u. c).

Die mit *F. tabacinum* infizierten jungen Pflanzen zeigten dagegen bei der Probenentnahme kleine, braune, makroskopisch schwach sichtbare Areale nur an Wurzeln. Licht- und elektronenmikroskopisch konnte ein starkes Wachstum mit

sehr ausgeprägter Besiedelung im Rindenbereich festgestellt wurden (Abb. 3 a). In Rindenparenchymzellen waren oft mehrere Hyphenanschnitte in einer Zelle (Abb. 3 b) und ein aktives Eindringen in die Nachbarzellen zu beobachten (Abb. 3 c). Das Eindringen in eine Zelle ging immer mit Einengung der Hyphendicke und Segmentbildung an der Eindringungsstelle einher (Abb. 3 c). *F. tabacinum* konnte jedoch im Leitbündel in Xylem, Phloem und Leitbündelparenchymzellen nicht beobachtet werden.

Die mit *F. oxysporum* infizierten jungen Pflanzen zeigten leichte Vergilbungserscheinungen an Blättern, die Wurzeln dagegen waren symptomfrei. *F. oxysporum* war der einzige, bis tief in Xylemzellen im Leitbündel eingedrungene Art (Abb. 4 a, b). Das Hyphenwachstum in der Rinde und im Leitbündelbereich war normal (Abb. 2 d) und nicht so ausgeprägt wie bei *F. tabacinum*. *F. oxysporum* zeigte auch in den Xylemzellen ein normales Wachstum und veränderte die Feinstruktur dieser Zellen nicht, zumindest nicht bis zum Zeitpunkt der Probenentnahme.

## DISKUSSION

Bei welke und Wurzelfäuleerkrankungen durch phytopathogene Pilze werden meist viele Pilzarten und -stämme aus erkrankten Pflanzen und Kulturböden isoliert. Von den vielfältigen und diskutablen Isolierungsmethoden muß eine geeignete angewendet werden, um den eigentlichen Erreger selektiert erfassen

zu können (Miller, 1945; Stoddard, 1947; Wensley and McKeen, 1962, 1963; Douglas, 1967). Es werden jedoch oft bei einer Erkrankung alle isolierten Pilzarten und -stämme als Erreger angesehen (Karahan, 1971). Aus diesem Grund sind histopathologische Untersuchungen meist unumgänglich, wenn es da-

rum geht, den eigentlichen Erreger zu bestimmen und Wirt-Parasit Interaktionen mit zytologischen Veränderungen zu verfolgen. Der Infektionsvorgang und das sich Ausbreiten eines pathogenen Agents kann in verschiedenen Wirtsgeweben nur durch Histopathologie gezeigt werden.

Nach den hier vorliegenden Ergebnissen können bei der Welke und Wurzelfäuleerkrankung der Honigmelone (*Cucumis melo*) die Fusariumarten *F. culmorum*, *F. equiseti* und *F. solani* nicht die eigentlichen Erreger sein. Sie sind vielmehr als Isolate anzusehen, die durch eine Sekundärinfektion oder saprophytisches Wachstum bedingt sind. Die Ergebnisse von Soran (1979), wonach diese Stämme keine makroskopisch sichtbaren Symptome induzierten und folglich nicht pathogen sein dürften, werden hier bestätigt (Abb. 2 a, b, c).

*F. tabacinum* dagegen dringt sehr schnell in die Wurzel ein und breitet sich im Bereich des Rindenparenchyms mit sehr starkem Wachstum aus, ohne weiter in andere Bereiche der Wurzel vorzudringen. Das deutet auf eine Gewebespezifität des *F. tabacinum* für Rindenparenchymzellen (Abb. 3 a, b, c) hin. Diese Infektions- und Wachstumseigenschaft des Pilzes muß so interpretiert werden, daß *F. tabacinum* für früh auftretende Wurzelfäule verantwortlich ist und als Folge dieser durch Zerstörungen im

Wurzelbereich die Vergilbung an Blättern bedingt. Einzig die Fusariumart, *F. oxysporum*, wurde bereits fünf Tage nach Infektion in Xylemzellen des Leitbündels festgestellt (Abb. 4 a, b). Das Wachstum des *F. oxysporum* war im Rindenbereich mäßig bis normal, jedoch im Leitbündel sehr ausgeprägt (Abb. 2 d). Dieser Befund zeigt, daß *F. oxysporum* den Rindenbereich mit wenig seitlichem Wachstum schnell überquert und sich erst im Leitbündelbereich, besonders in Xylemzellen, ausbreitet. Die schnelle Besiedelung dieser Zellen durch ständig wachsende Pilzhyphen muß zur Folge haben, daß der Wassertransport von den Wurzeln zu den oberirdischen Teilen der Pflanze erst vermindert und später unterbrochen wird. Für früh auftretende Welkeerkrankung der Honigmelone, ohne Fäulnisbildung an den Wurzeln, muß nach den hier vorliegenden Ergebnissen *F. oxysporum* allein verantwortlich sein.

Im Freiland, auf einem mit Fusariumarten kontaminierten Kulturboden, sind die Verhältnisse verwickelter. Während Welkeerkrankung durch *F. oxysporum* und Wurzelfäule durch *F. tabacinum* getrennt oder gleichzeitig auftreten können, werden andere, an sich nichtpathogene Fusariumarten und Bodenpilze auf den bereits geschädigten Wurzeln der erkrankten Pflanzen gute Wachstumsbedingungen vorfinden.

Ö Z E T

FUSARIUM TÜRLERİYLE İNFEKTELİ BİTKİLERİN  
(Cucumis melo) KÖKLERİNDE IŞIK VE ELEKTRON  
MİKROSKOBU İLE ARAŞTIRMALAR

Solgunluk belirtileri gösteren kavunlardan elde edilen *F. culmorum*, *F. equiseti*, *F. solani* ve *F. tabacinum*'un bitki köklerine girişleri yerleştiği dokular ve hücre içersindeki yayılma şekilleri ışık ve elektron mikroskopta ayrıntılı biçimde incelenmiştir. Bunlardan *F. culmorum*, *F. equiseti* ve *F. solani* epidermisi aşır kök dokusu içine gireme-

mişlerdir. *F. tabacinum* kökün korteks kısmında yayılma göstermiş. *F. oxysporum* ise dıştan içe doğru tüm dokulara yayılmış ve iletim doku hücrelerine yerleşmiştir.

*F. oxysporum* hücre duvarını geçerken şekil değişikliğine uğramadığı halde, *F. tabacinum* hücre duvarını, daraltıp, bölme oluşturarak geçmektedir.

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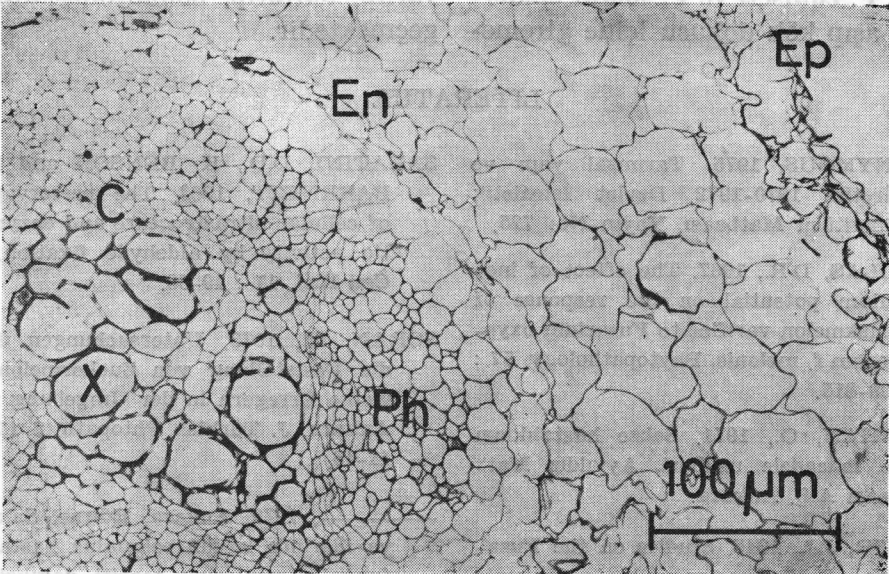


Abb. 1: Wurzelquerschnitt einer nicht infizierten Pflanze; Licht- mikroskopie - Phasenkontrast, Vergr.:  $\times 250$ . Abkürzungen: Ep = Epidermis, En = Endodermis, R = Rinde, C = Cambium, Ph = Phloem, X = Xylem.

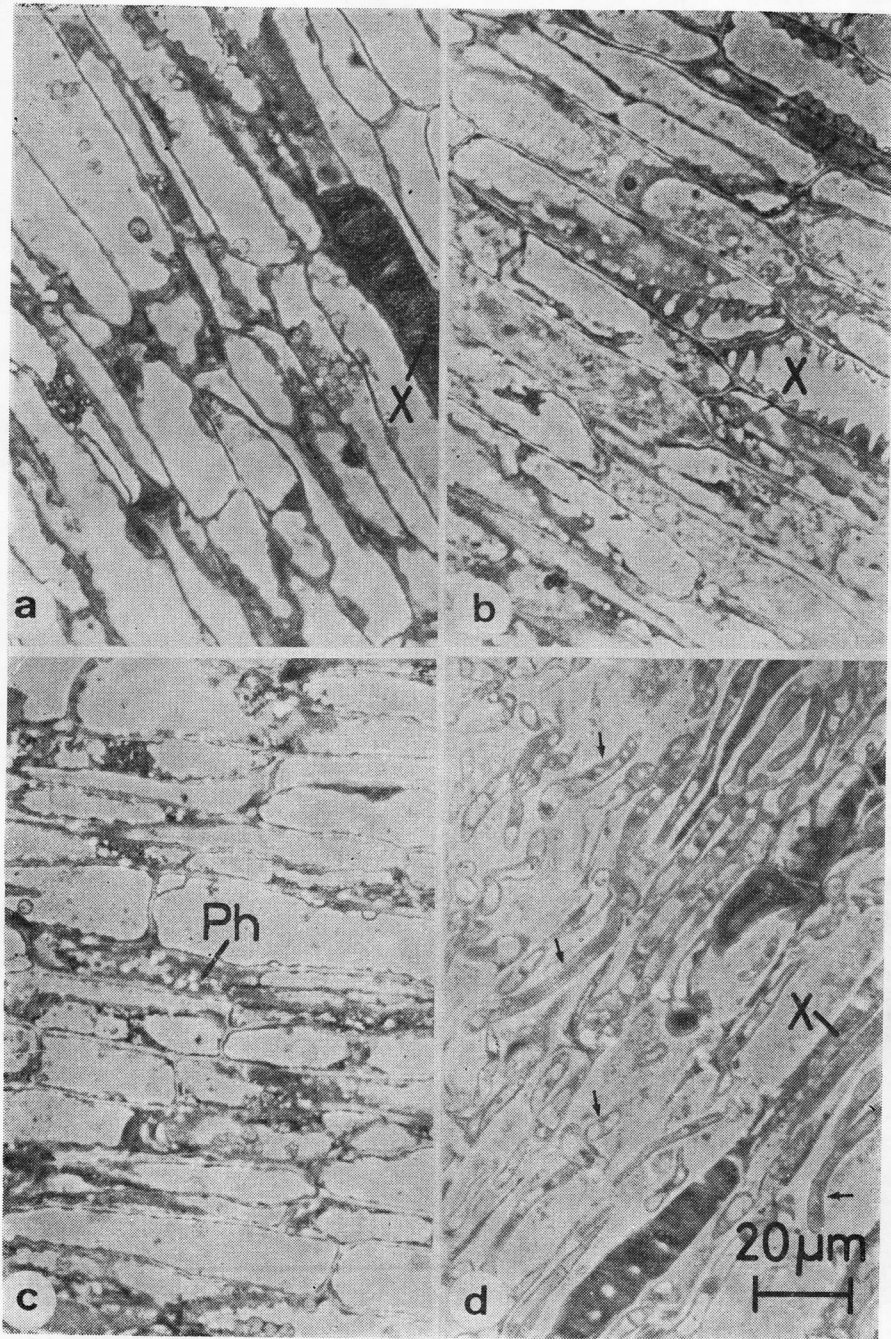


Abb. 2: Längsschnitte durch Leitbündelbereiche der mit verschiedenen Fusariumarten infizierten Pflanzen: a) *F. culmorum*, b) *F. equiseti*, c) *F. solani*, d) *F. oxysporum*. Nur *F. oxysporum* zeigt in d) ein dichtes Hyphenwachstum im Leitbündelbereich (Pfeile). Lichtmikroskopie-Phasenkontrast, Vergr.:  $\times 600$ .



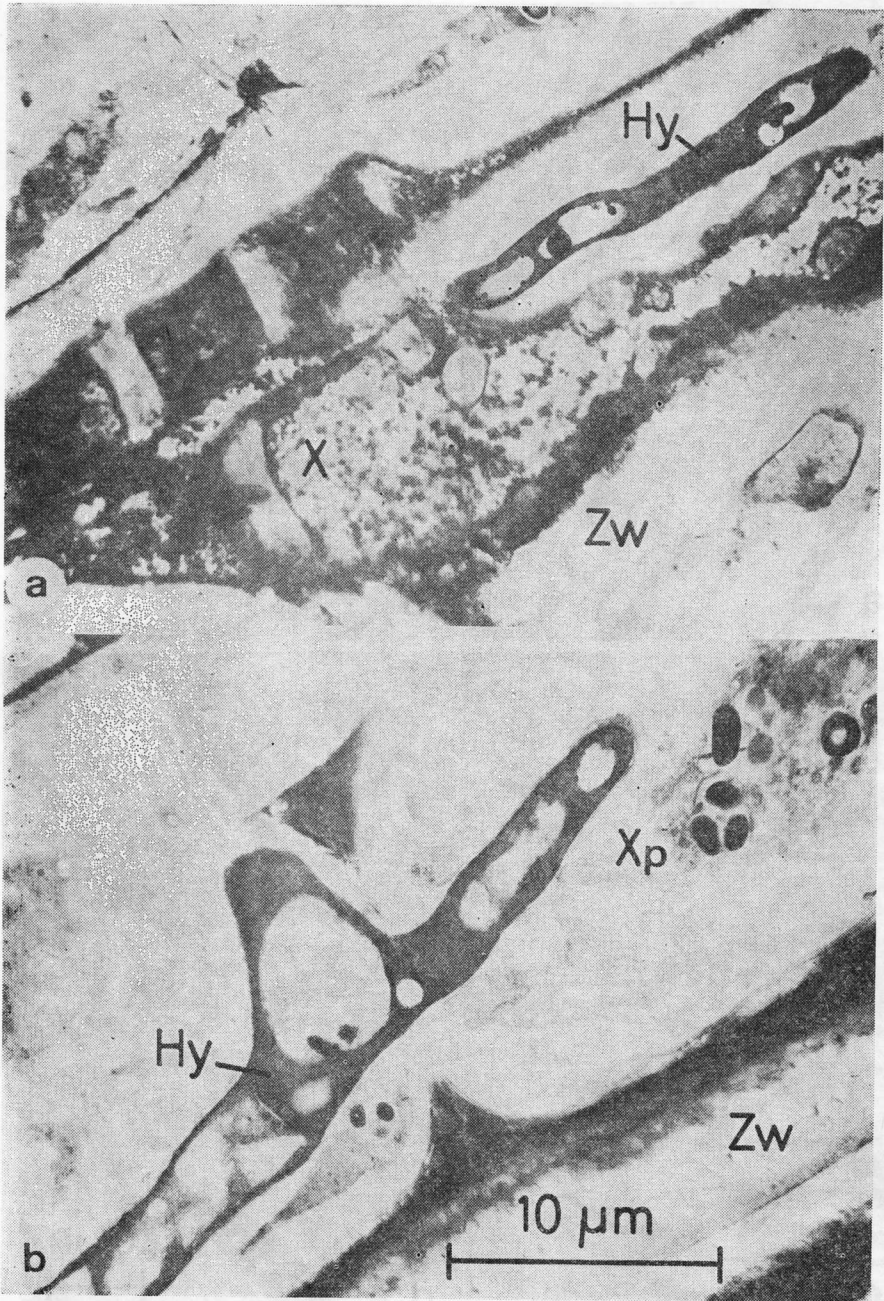


Abb. 3: Ausbreitung und dichtes Wachstum von *F. tabacinum* im Rindenbereich; a) Längsschnitt mit sehr dichtem Hyphenwachstum, Lichtmikroskopie-Phasenkontrast, Vergr.:  $\times 550$ . b) Querschnitt einiger Rindenparenchym-Zellen mit mehreren Hyphen (Hy), Vergr.:  $\times 7.000$ . c) Eindringen der Hyphe (Hy) von einer Parenchymzelle in die andere mit Segmentbildung (Pfeil), Vergr.:  $\times 13.500$ , (Zw = Zellwand, Pl = Plasmalemma).

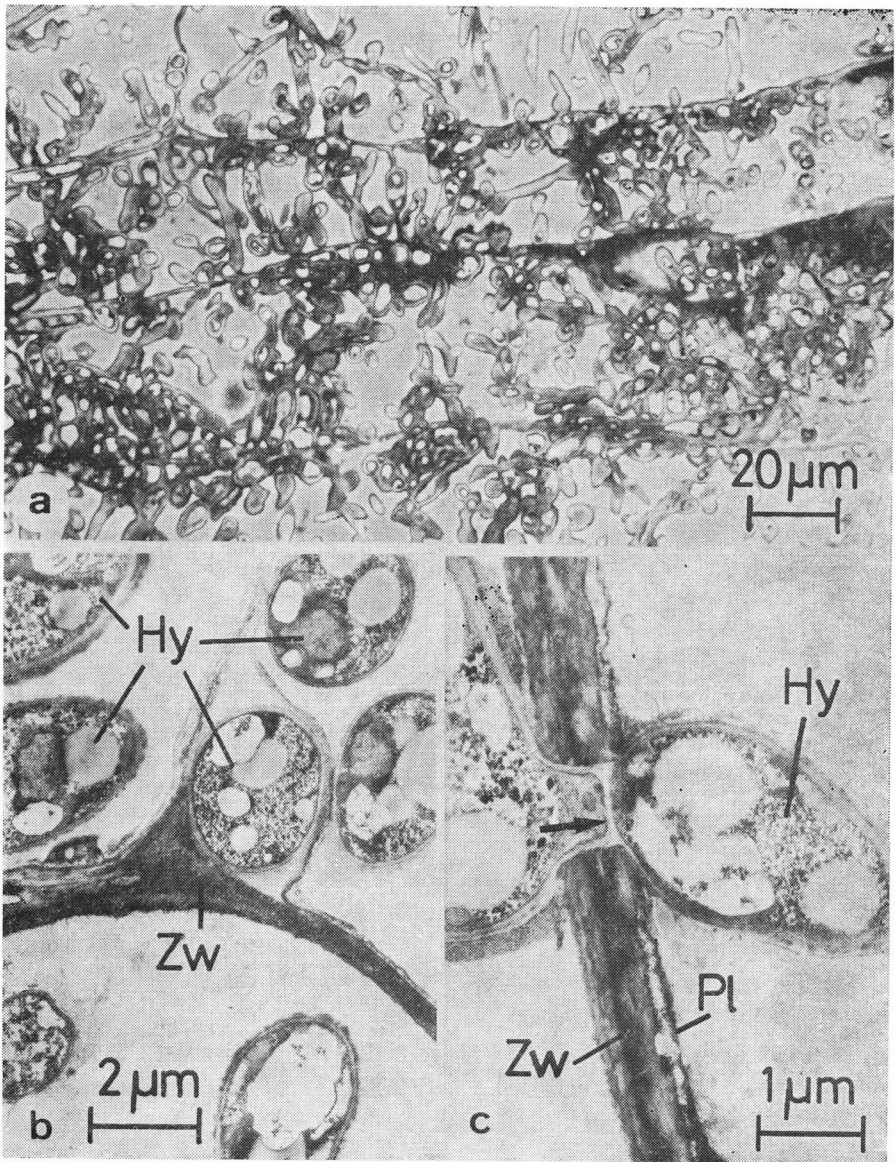


Abb. 4: Längsschnitte durch das Xylem einer mit *F. oxysporum* infizierten Pflanze, a) Hyphenwachstum (Hy) direkt in einer Xylem-Zelle (X), b) in einer noch nicht ausdifferenzierten Xylemparenchym-Zelle (Xp). Vergr.:  $\times 3.600$ .

## Schwarzbeinigkeit an Weizen in der Türkei

Gülay TURHAN\*

Ersin ONOĞUR\*

Atilla ATAÇ\*\*

## ZUSAMMENFASSUNG

Im Süd-Osten der Türkei wurde 1984 in einem Weizenfeld Symptomen beobachtet, die auf die Schwarzbeinigkeit hindeuteten. Durch Isolationen und Pathogenitätstest wurde das Vorhandensein von *Gaeumannomyces graminis* var. *tritici* in der Türkei nochmals bestätigt. Der mitisolierte Pilz *Cephalosporium* sp. erwies sich dabei als nicht Pathogen. Die Verwendung von  $\text{AgNO}_3$  als Sterilisationsmittel wurde für die weiteren Isolationsversuche empfohlen.

## EINLEITUNG

Schwarzbeinigkeit, hervorgerufen durch *Gaeumannomyces graminis* (Sacc.) Arx et Olivier var. *tritici* Walker, gehört zu den wichtigsten Fusskrankheiten bei Getreide sowohl in den kühlfeuchten als auch in den trocken-warmen Klimazonen. Der Erreger, zu dessen Wirtspflanzenkreis vor allem Weizen, Gerste und Roggen gehören, befällt hauptsächlich das Wurzelsystem und die Halmbasis durch seine Laufhyphen. Die erkrankten Pflanzen zeigen durch fortschreitende Zerstörung des Wurzelsystems Wachstumsverzögerungen und Notreifeerscheinungen (Gerlagh 1968, Nilsson 1969, Jörgensen und Jensen 1970, Walker 1975, Fischer 1977).

Die Bekämpfung des Erregers ist durch Fruchtfolge möglich. Er besitzt eine geringe saprophytische

Konkurrenzfähigkeit und ist gegen mikrobielle Einflüsse im Boden sehr anfällig. Bereits durch einjährigen Anbau eines Nichtwirtes ist eine Befallsminderung zu erzielen. Auch bei langjährigen Monokultur kommt es zu einem geringeren Befall. Die spezifische Antagonisten des Erregers, die während der Weizen- oder Gerstenmonokultur durch den Parasit induziert und stimuliert werden, führen bereits nach 3 Jahren zu einem «Decline-Effect», der sich in einem Rückgang des bis dahin ansteigenden Befalls äussert (Diercks et al. 1970, Walker 1975).

Über das Vorkommen dieser Krankheit in den verschiedenen Getreideanbaugebieten der Türkei liegen bereits Angaben vor. So berichtet Iren (1981), dass während

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einer Befallserhebung bezüglich Getreidekrankheiten 1971-1972, die Schwarzbeinigkeits im Süd-Osten der Türkei beobachtet wurde. Nach Kinacı (1985), kommt diese Krankheit auch in Zentral Anatolien vor. In der Marmara-Region einschliesslich Thrazien (Nord-West

Türkei) wird der Befall in den letzten Jahren häufiger beobachtet (Iren 1985).

In dieser Arbeit wird über die Ergebnisse der Isolationen aus den Pflanzen mit Schwarzbeinigkeits-symptomen und den Pathogenitätstest berichtet.

### MATERIAL und METHODEN

**Pflanzenmaterial:** Die kranken Pflanzen der Weizensorte «Orso» wurden in Reyhanlı, Süd-Ost Türkei, im April 1984 gesammelt. Die Krankheit war fast in der Hälfte der insgesamt 1000 Dekar grossen Anbaufläche zu beobachten.

Als Testpflanzen zur Pathogenitätsuntersuchung wurden die Pflanzen der Weizensorte «Cumhuriyet-75» verwendet.

**Isolationmethode:** Zur Isolation der Pilze wurden die Proben (Halmbasis mit Wurzeln) nach Waschen unter fliessendem Wasser in kleinere Teil zerstückelt und in 1 % igem  $\text{AgNO}_3$  1 Min. lang belassen. Die Isolation erfolgte auf PDA mit Streptomycine (100 mg/l) (Asher 1978).

Zur Gewinnung des Inokulums wurden die Isolate in 250 ml Erlenmayerkolben mit dem Gemisch von 135 g Sand, 15 g Maismehl und 20 ml PD bei  $25 \pm 1^\circ\text{C}$  im Dunkel 5 Wochen kultiviert. Diese «Stockkulturen» wurden zur Inokulation der Versuchserde verwendet. Hierzu

wurde das Inokulum mit einem sterilen Gemisch von Erde-Sand-Kompost (1:1:1) im Verhältnis von 1:20 (v/v) gut vermischt. Bei den Mischinokulationen mit *G. graminis* var. *tritici* und *Cephalosporium* sp. bestand das Inokulum aus einer Mischung der Inokula von beiden Pilzen (1:1), das wiederum in gleichen Verhältnis (1:20) mit der Versuchserde vermischt wurde. Nach dem Füllen von Tontöpfe ( $\varnothing$  12 cm) mit dem inokulierten Erdgemisch wurden die Körner der Weizensorte «Cumhuriyet-75» mit jeweils 15 Körner/Topf ausgelegt. Die Töpfe wurden in einer Klimakammer bei  $20 \pm 2^\circ\text{C}$  und bei einer Belichtungsdauer von 14 Stunden gehalten.

Der Versuch wurde in 7-facher Wiederholung als randomisierte Blockanlage durchgeführt.

Der Befall wurde in Prozent der befallenen Pflanzen/Topf im 4-Blattstadium, etwa 6 Wochen nach dem Auslegen angegeben.

### ERGEBNISSE

**Isolierte Pilze:** Aus der Halmbasis von kranken Pflanzen wurden 2 Pilze isoliert und diese wurden als *G. graminis* var. *tritici* und *Cepha-*

*losporium* sp. identifiziert.

**Pathogenitätstest:** Der Befall an den Pflanzen in den mit *G. graminis* var. *tritici*, *Cephalosporium* sp.

und *G. graminis* var. *tritici* + *Cephalosporium* sp. inokulierten Töpfen wurde bonitiert. Die mit *Gaeumannomyces* inokulierten Pflanzen zeigten einen allgemeinen Entwicklungsrückgang. Die ersten Blätter waren chlorotisch, verwelkt oder bereits abgestorben. Die ober-

en Blätter waren zum Teil ebenfalls verwelkt (Abb. 1). Die Halmbasis war nekrotisch, die Wurzeln waren mehr oder weniger verrottet. Das Wurzelvolumen war erheblich geringer als das der Kontrollpflanzen (Abb. 2).



Abb. 1. Mit *G. graminis* var. *tritici* inokulierter Topf (rechts) und Kontrolle (links).

Gae + Ceph.	28.8	11.8	16.8	14.3	22.0	20.4	15.8	20.2
Ggt	57.1	72.2	66.8	53.8	69.2	62.2	58.2	70.0
Ggr	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

\**G. graminis* var. *tritici*, \*\**Cephalosporium* sp.

In den folgenden Tagen wurde von anfangen sich zu erholen beobachtet, das manche mit *G. graminis* var. *tritici* inokulierten Pflanzen (Abb. 3) und konnten

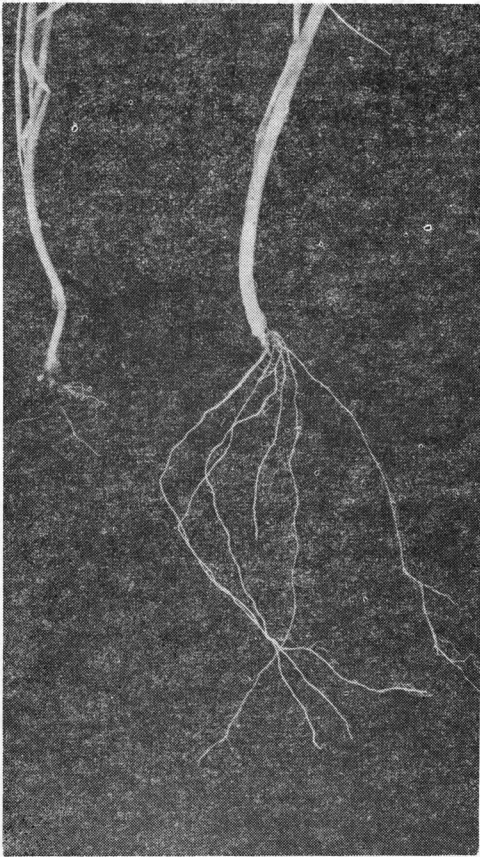


Abb. 2. Wurzelentwicklung an der mit *G. graminis* var. *tritici* inokulierten Pflanze (links), Kontrolle (rechts).

Die Inokulation mit *Cephalosporium* sp. verursachte keinerlei Befallssymptome. Der Prozentsatz der befallenen Pflanzen in den mit

beiden Pilzen im Verhältnis von 1:1 inokulierten Töpfen lag unter den nur mit *G. graminis* var. *tritici* inokulierten Töpfen (Tab. 1).

Tab. 1. Prozent der befallenen Pflanzen in den mit \*Ggt, Ggt + \*\*Ceph. und Ceph. inokulierten Töpfen.

Inokulation mit	Prozent der befallenen Pflanzen je Topf							
	X							
	X							
Ggt + Ceph.	28.6	11.8	16.6	14.3	25.0	29.4	15.8	20.2
Ggt	57.1	72.2	66.6	53.8	69.2	83.3	88.2	70.0
Ceph.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

\**G. graminis* var. *tritici*, \*\**Cephalosporium* sp.

In den folgenden Tagen wurde beobachtet, dass manche mit *G. graminis* var. *tritici* inokulierten Pflan-

zen anfangen sich zu erholen. Solche Pflanzen bildeten neue Seitenwurzeln (Abb. 3) und konnten

damit weiter wachsen bzw. gesund aussehende Blätter entwickeln. Sie blieben zurück jedoch in der Entwicklung im Vergleich mit den

Kontrollpflanzen. Die Anzahl solcher Pflanzen wurde nicht ermittelt.

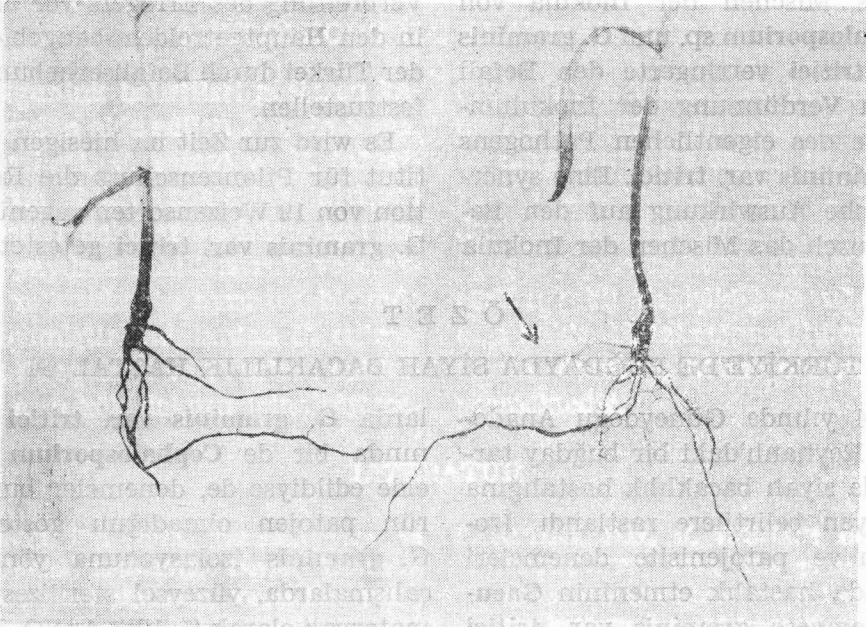


Abb. 3. Bildung neue Seitenwurzeln (Pfeil) an der kranken Pflanze (rechts), kranke Pflanze ohne neue Werzelbildung (links).

### DISKUSSION

Durch diese Arbeit konnte das Vorhandensein der Schwarzbeinigkeit in der Türkei, in diesem Falle mit Hilfe der Koch'schen Postulate bestätigt werden. Möglicherweise hätte der Erreger in der Türkei bereits vorher bei mehreren Befallserhebungen bezüglich der Getreidekrankheiten festgestellt werden können. Der Grund, warum der Erreger bisher nicht isoliert wurde, dürfte in der Isolationsmethode liegen. Oberflächliche Sterilisation mit den üblichen Mitteln, Chlorine-oder Quecksilberbasis kann zum negativen Ergebnissen führen (Nilsson 1969) wahrscheinlich be-

dingt durch die Abtötung mitunter auch *G. graminis* var. *tritici*. Sterilisation der Proben mit Chloramin-T erlaubte in der vorliegenden Arbeit keine Isolation des Pathogens. Es ist deshalb zu empfehlen,  $AgNO_3$  als spezifisches Sterilisationsmittel zur Isolation dieses Erregers in den betreffenden Versuchen einzusetzen.

*Cephalosporium* sp. war bei den mehrmaligen Isolationen während der Versuche fast immer ein mitisolierter Pilz. Da eine *Cephalosporium* Art, *C. gramineum* Nisik et Skata zu den Pathogenen an Getreide gehört, wurde dieses Isolat

auch auf seine Pathogenität hin getestet. Es erwies sich jedoch als nicht Pathogen.

Das Mischen der Inokula von *Cephalosporium* sp. und *G. graminis* var. *tritici* verringerte den Befall durch Verdünnung der Inokulummenge des eigentlichen Pathogens *G. graminis* var. *tritici*. Eine synergistische Auswirkung auf den Befall durch das Mischen der Inokula

war demnach nicht zu verzeichnen.

Die Schwarzbeinigkei t zählt zu den wichtigsten Pathogenen an Getreide. Es ist wünschenswert, die Verbreitung des Erregers vor allem in den Hauptgetreideanbaugebieten der Türkei durch Befallserhebungen festzustellen.

Es wird zur Zeit im hiesigen Institut für Pflanzenschutz die Reaktion von 19 Weizensorten gegenüber *G. graminis* var. *tritici* getestet.

## Ö Z E T

### TÜRKIYE'DE BUĞDAYDA SİYAH BACA KLILIK HASTALI ĞI

1984 yılında Güneydo ğu Anadolu'da Reyhanlı'daki bir bu ğday tarlasında siyah bacaklılık hastalı ğına benzeyen belirtilere rastlandı. İzolasyon ve patojenisite denemeleri sonunda hastalık etmeninin *Gaeumannomyces graminis* var. *tritici* oldu ğu kesinlik kazandı. İzolasyon-

larda *G. graminis* var. *tritici* yanında bir de *Cephalosporium* sp. elde edildi yse de, denemeler bu türün patojen olmadığını gösterdi. *G. graminis* izolasyonuna yönelik çalışmalarda, yüzeysel sterilizasyon materyali olarak % 1'lik AgNO<sub>3</sub> kullanılması tavsiye edildi.

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