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Investigations on the Tristeza (Quick Decline) Virus Disease in the Satsuma Mandarins; its Definition, Crop Losses and Determination of the Strains in Izmir Province

Turhan AZERİ¹ and İbrahim KARACA²

ABSTRACT

The incidence of the tristeza virus were 15,56 % in Central County (Merkez ilçe), 17,05 % in Seferihisar, 16,66 % in Bornova, 15,00 % in Karşıyaka, 20,00 % in Menemen 22,5 % in Urla county. The general incidence was 17,79 % for Izmir province. Some Satsuma orchard had 30 % infection.

Tristeza affected Satsuma mandarins (*C. unshiu* Marc.) on sour orange rootstocks displayed stunting, general decline, gradual decline, dieback and death of the trees. The other characteristic symptoms included overgrowth and occurrence of the severe pinhollings (honey combing) on the inner surface of the sour orange bark. Satsuma mandarin trees on *Poncirus trifoliata* L. Raf. Which infected with the severe strains of tristeza displayed yellowing, mild stunting suppression of the Scion and trifoliata rootstock and some gradual decline.

Five different strains of the tristeza virus (mild, moderate and severe strains) were found in our Satsuma mandarins. The severe strain caused veinlet-netting and vein corking on the lime plants. The mild and moderate strains of tristeza were more widely distributed than the severe strains in the Satsuma orchards.

¹) Regional Plant Protection Research Inst. Bornova, İZMİR/TURKEY.

²) Department of Phytopathology and Agricultural Botany,
Agricultural Faculty of Ege Univ. Bornova, İZMİR/TURKEY.

TRISTEZA VIRUS DISEASE

Sieve tube necrosis immediately below the bud union were observed in the sour orange bark floem stained with Congo red+lacmoid. Sieve elements near the cambium were normal and functioning in some sections. Callus deposits were also observed on the sieve plates near the Cambium. The same anatomical symptoms were also occurred in mild degree in bark floem of the *P. trifoliata* and Satsuma Scion.

The results of the correlations between the strains of tristeza virus and the yield of the 177 indexed Satsuma trees from the 19 orchards showed that, the correlation coefficients (r) were negative and significantly high from $r = -0,7180$ to $r = -0,9925$. For 14 Satsuma orchards, buded on *P. trifoliata*, the equation was $Y = 137,6807 - 17,0417X$. For 5 Satsuma orchards, buded on sour orange the equations was $Y = 181,2851 - 28,8X$. "F" Values were significant according to 5 % for 3 for remained 16 orchards. The losses of the yield were significant on the diseased Satsuma trees buded on sour orange and infected with the severe strains of tristeza virus.

INTRODUCTION

Satsuma mandarin is the main Citrus variety grown extensively in the Izmir province located at the Aegean Coast of Turkey. In recent years Satsuma plantations have been increased greatly and approximately more than I million Satsuma plants were planted in this region, with the plantation of the 100.000 Satsuma plants each year. *P. trifoliata* is the most common rootstock although sour orange is also used in some plantings.

Tristeza, also quick decline or bud-union decline, has existed for many years in the tropical Citrus growing countries. It is known that, the effects of the tristeza virus were recognized as stock scion incompatibilities prior to 1900 in south Africa

as reported by Wallace (1957) Mc Clean (1963, 1974) and Klotz et al (1972). Tristeza caused extensive losses between 1930 and 1940 in Argentina, Brazil and California. More than 20 Million Citrus trees on sour orange rootstocks were completely destroyed in Argentina, Brazil and other South America countries, and about 3 million in Southern California as reported by Wallace (1957), Klotz et al (1972). Tristeza is present world wide, however. in most Mayer lemons, Satsuma mandarins and in certain tangerin selections. It has been reported that, Satsuma mandarins are infected with tristeza in Italy and in the other parts of the Satsuma growing Mediterranean area particularly in North Africa.

Because of tristeza infections, some of the Satsuma trees in North Africa have been severely dwarfed as, reported by Reichert (1959), Reichert et al (1960). Tristeza was reported also in Cyprus, Southern France, Greece, Spain and in Israel by the same authors. In Japan, almost all Satsuma trees tested for tristeza showed positive reaction as reported by Yamada and Tanaka (1969). A severe strain of tristeza, apparently the same strain that found in south Africa, Brazil was found in Texas in a Satsuma orange, Sueoka Satsuma Introduction from Japan as reported by Olson (1959), Olson and Sleeth (1956).

The experiment results from the citrus growing countries led us to determine the presence and the strains of tristeza virus in Our Satsuma mandarins. Since 1969, several stunted and the declined Satsuma trees in the Izmir province have been tested for tristeza using Mexican lime test plants. The present study describes the symptom, crop losses and determination of the different strains of tristeza in Izmir province. Experiments were carried out during the year of 1969-1976 at the Regional Plant Protection Research Institute, Bornova, İZMİR.

MATERIALS AND METHODS

The survey was made between 1974-1975 in the six Satsuma producing counties namely Central county (Merkez ilçe) Seferihisar, Bornova, Karşıyaka, Menemen and Urla to describe the rates of incidence of the tristeza virus. A total number of 2200 Satsuma mandarin trees 8-year-old and older bedded on *Poncirus trifoliata* and sour orange rootstocks in 110 different Satsuma orchards were examined at random for tristeza in the survey. Since 1971 Satsuma mandarin trees have been tested with Mexican limes in the survey area for symptom and strain determinations of tristeza before the survey studies. During the survey, Satsuma manda-

rins on *P. trifoliata* and sour orange rootstocks were examined for the typical symptoms of tristeza virus which described by Bitters and Parker (1952), Calavan et al (1962) Reichert et al (1960), Harpaz (1964) Yamada and Tanaka (1964), Knorr (1966) and Pujol et al (1972). In the experiments, Satsuma mandarins were indexed with the Mexican lime (*Citrus aurantifolia* Swing.) test plants. M. lime indicator seedlings were grown in 10-inch black color plastic pots containing soil, sand and peat (2:1:1). Budwoods and the leaves which obtained from the Satsuma trees were collected during the survey for the inoculation test. Side

grafts, chip bud grafts and especially leaf insert grafts (Fig. 1) were used to provide more virus infections as described by Wallace (1968) and Cohen (1972). Two pencil - size lime seedlings were used in each test. Four or five leaf insert graft were inserted in each lime plant. One non-inoculated lime seedling was left as control. The temperature varied from 20°C to 27°C during the indexing period in the glasshouse. All indicator plants were checked every week for symptoms as described by McClean (1962) and Wallace (1968).

A scale by McClean (1963) was used to determine the different strains of tristeza. The scale contained 7 different strain categories except the first category that was showed for negative reaction of the lime plant (tristeza free).

The other categories showed from the occasional very small vein-clearings (very mild strain), clearings more localised to form vein fleckings and extensive clearing of veins (moderate strains) to the All veins cleared = veinlet netting and vein corking (severe strain) of the Mexican lime indicator plants. In the categories, different degrees of the stem pittings on the inoculated lime plants which described by McClean (1963) from the occasional small pits (very mild strain) to the extensive and severe pitting on the lime plants were used in the categories with the vein-clearings.

Sectioning and staining pathological phloem :

During the survey and the examinations a small piece of bark samples, approximately 5 mm. tangentially x 30mm longitudinally was cut from the bud-union of the indexed or examined Satsuma trees. Samples included scion and rootstock bark. Randolph's modified Navashing solution was used as a fixative as described by Johansen (1940) and Scheider (1959). The sectioning was done on a sliding microtom equipped with a freezing attachment. Sections were stained with a mixture of Congo red and lacmoid, hematoxyline and lacmoid. Staining was done according to the procedure described by Batzer and Schneider (1960), Schneider (1954, 1959). Sieve tube necrosis Callus deposits on the sieve-plates and the other anatomical disturbances in the bark phloem of the tristeza infected Satsuma scion, sour orange and the *P. trifoliata* rootstocks were investigated.

Experiments were carried out also to determine the crop losses from the tristeza virus. 177 Satsuma mandarin trees (129 trees buded on *P. trifoliata* and 48 trees buded on sour orange rootstocks) at the different ages (from 9 to 18 years old) in 19 different orchards were used in the crop losses experiments. During the field observations these trees were examined for exocortis, Satsuma Dwarf psorosis and the xyloporosis diseases as reported by Azeri

(1972, 1973). These Satsuma trees have also been indexed for the tristeza virus strains. Indexed and virus free 49 Satsuma trees of the 177 trees were used in calculation of the crop losses for the tristeza affected trees.

The correlations between the different strains of the tristeza virus and the crop losses of the totally 128 Satsuma trees in 19 orchards were calculated.

RESULTS

Tristeza virus was found widely distributed in Satsuma mandarins and especially more destructive on the Satsuma mandarins buded on sour orange rootstock.

Survey studies:

Survey studies and the indexing

tests in the Izmir province indicated that, the rates of the incidence of tristeza virus were different in degree from 15 % to 22 % percent in the counties. The general rates of incidence was 17,79 % for Izmir province as shown in table 1.

Table 1. The rate of incidence of the tristeza virus in Satsuma mandarins in the different counties.

County Name	The number of the orchards and the trees examined for tristeza		The number of affected Satsuma trees	The number of unaffected Satsuma trees (virus-free)	The rates of incidence of the tristeza virus (%)
	Number of Orchards	Number of trees			
Merkez İlçe (Central County)	84	1680	262	1418	15.56
Seferihisar	17	340	58	282	17.05
Bornova	3	60	10	50	16.66
Karşıyaka	3	60	9	51	15.00
Menemen	1	20	4	16	20.00
Urla	2	40	9	31	22.5
Total	110	2200	352	1848	17.78*

*) The general rate of incidence of tristeza for Izmir area.

TRISTEZA VIRUS DISEASE

The rate was 30 % in some severely tristeza affected Satsuma orchard in which sour orange had been used as the rootstock.

Symptoms of tristeza:

Tristeza affected Satsuma trees/ on sour orange rootstocks displayed stunting and general decline with partial defoliation and dieback in the field observation (Fig. 2.A,B). Some 19-20 years old Satsuma mandarins on sour orange were 90 to 150 cm. height and showed dieback. Other characteristic symptoms were suppression of normal seasonal flushes

of growth and leaf discoloration. The leaves on the infected trees were olive-green to yellow green in color. Some infected Satsuma trees showed severe yellowing with gradual decline. The other symptoms of tristeza virus were over growth of the scion, honeycombing or pinholes on the inner surface of the sour orange bark (Fig.2.C). Pegs (also much enlarged) occurred on the woody cylinder from which honeycombed bark has been removed. The bark below the bud union was abnormally thicker than the normal bark. The small fibrous roots around the root system of the severely tristeza affected some sour

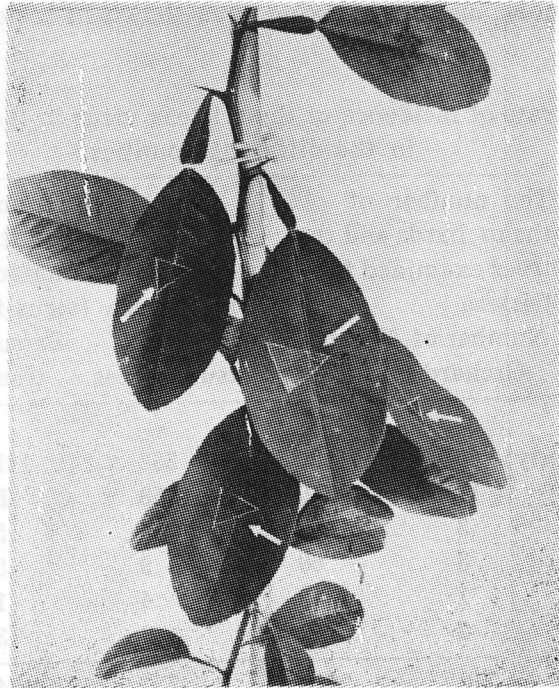


Fig. 1. Leaf insert grafts on the Mexican lime indicator leaves.

orange rootstocks were died (Fig. 2. B). The sieve tubes of the severely tristeza affected sour orange rootstock showed also severe necrosis in the microscopis examination.

Some Satsuma trees on sour orange which were severely infected with tristeza showing severe invers-pittings and dieback in 1974 were died in 1977. Sour orange rootstocks of the dead trees showed poor growing shoots. The tristeza virus strains were diagnosed from these Satsuma trees by indexing test with Mexican lime. During the symptom observation in the orchards tristeza affected Satsuma mandarins on sour orange did not show collapse as reported from the Tropical Countries and Southern California. Diseased trees showed gradual and chronic decline as the severe Symptoms of the tristeza in the experiment region. These chronic declined trees have been lived for several years because of the new functional sieve tubes in the sour orange floem. Satsuma mandarin trees on *P. trifoliata* rootstocks which were infected with the severe strain of tristeza displayed yellowing mild stunting, small leaves of some branches, suppression of growth of the scion and the *P. trifoliata* rootstock, poor growing of the sprouts, some gradual decline of the trees. Pinhollings (honeycombing) were seen on the cambial face of the bark (Fig. 3.A) and tiny pin-like pegs on the wood of the Satsuma scion buded

on sour orange rootstock (Fig. 3.B). Some tristeza infected Satsuma scion on sour orange rootstock showed dead bark or shelling bark and honeycombing symptoms.

Microscopic examination:

Microscopic examination revealed that, the cytoplasm of parenchyma cells showed darkly staining material (Chromatic cells) (Fig.4.A) in the tristeza infected sour orange bark floem. Sieve tube necrosis immediately below the bud-union were observed also in the sour orange bark phloem stained with hematoxylin : lacmoid or Congo red+lacmoid (Fig. 4.B). These food-conducting tubes in sour orange bark floem near the bud union were destroyed as showed in Fig. 4.A and Fig. 4.B, and lost their functions. Sieve elements near the cambium were normal and functioning. Hypertrophied (enlarged) and the divided paranchyma cells also occurred in the bark phloem of the tristeza infected sour orange (Fig. 4.C). Callus depositions were observed on the sieve plates near the cambium (Fig. 5.A). The same anatomical symptoms (Callus deposits) also occurred to a mild degree in the bark phloem of the *P. trifoliata* rootstock (Fig. 5.B) and the Satsuma scion. The bark floem of the tristeza uninfected (tristeza free) sour orange did not show callus deposits and sieve tube-necrosis as shown in Fig. 5.C.

Indexing and Strains of tristeza:

Since 1970, more than 300 Satsuma mandarin trees buded on *P. trifoliata* and sour orange rootstocks have been indexed for strain determination of tristeza virus. The results obtained by indexing Satsuma mandarins with lime, showed 5 strains of the tristeza virus (mild, moderate and severe strains) according to their symptomatology on Mexican lime. The severe strains of tristeza caused gradual decline of the Satsuma trees, severe vein-clearing (Fig. 6.A), and the severe stem pitting on the lime plants. The mild and moderate strains of the tristeza (Fig. 6.B,C) were widely distributed then the severe strains in the Satsuma orchards. Mexican lime plants developed vein - clearing symptoms 21 to 30 days after the side graft inoculations and 6-8 weeks after leaf insert graft inoculations. The severe strains of tristeza also caused stunting, yellowing and defoliation of the leaves on terminal twigs, very small and upright cupped leaves, vein-corking in the advanced stage and extensive stem pittings in Mexican limes. The indexing tests revealed that, the severe strain of tristeza was present in our Satsuma mandarins.

Satsuma mandarins which are buded on sour orange and affected by a severe strain showed declining and death of the Satsuma trees (Satsuma scions). The results of correlations between the strains of tristeza virus and the yield of 176 indexed Satsuma trees (93 diseased trees buded on *P. trifoliata*, 35 diseased trees buded on sour orange and 49 were virus free) from the 19 orchards revealed that, correlation coefficients (r) were negative and significantly high from ($r=-0.7180$) to ($r=-0.9925$). For 14 Satsuma orchards, in which Satsuma trees were buded on *P. trifoliata* correlation (r) was -0.7180 and the equation was $Y=137.6804 - 170417$. For 5 Satsuma orchards buded on sour orange rootstocks correlation was $r=-0.9002$ and the equation was $Y=181.2851-28.85 x$. According to the variance analysis between crop losses and the virus strains, "F" values were significant according to 5 % for 3 orchard from 19 orchards, and were significant according to 1 % for remained 16 orchards. The crop losses were significant on the diseased Satsuma trees buded on sour orange and infected with the severe strains of the tristeza virus.

DISCUSSION

Satsuma mandarin trees buded on sour orange rootstocks in the experiment area displayed typical gradual decline, dieback and death of the trees as reported by Bitters and Parker (1952), Harpaz (1964), Reichert et al (1960), Knorr (1966). Our indexing experiments revealed that, most of the severely tristeza affected trees that have been showed gradual decline and dying with the severe invers pitting on the sour orange bark were carrying the sevre strains of the virus. Tristeza affected trees with sour orange rotstocks did not show Collapse (quick-decline) as described by Bitters and parker (1952) Wallace (1957) and Mendel (1965). Collapse type symptom of the tristeza virus were reported from the many tropical countries and the southern California by the same authors. The most efficient vector of the tristeza *Toxoptera citricidus* Kirk. that caused the severe epidemic was common in the tropical countries. When the tristeza was transmitted in the tropical countries by means of the *Toxoptera citricidus*, caused colapse and quick-decline all kinds of Citrus trees on sour orange rootstocks as reported by Wallace (1957) and Mendel (1965). Fortunately, the most efficient vector of tristeza *Toxoptera citriicidus* Kirk. is not present in the Mediterranean countries as reported by Harpaz (1964), Reichert(1959), Reichert et al(1960).

In Japan, almost all of the Satsuma mandarins are infected with the tristeza virus because of the wide distribution of the Aphid through the country. According to Klotz et al (1972) and Wallace(1957), tristeza virus had been destructive in southern California to Citrus trees buded on sour orange because of the epidemy of the virus by *Aphis gossypii*. *A. gossypii* so far has not been adapted to Satsuma mandarins in Turkey as reported by Azeri (1972) and Dolar (1976). In Florida, *A. spiraeicola* and *T. aurantii* were also capable of transmitting tristeza as reported by Klotz et al (1972). It is the authors opinion that, because of the presence of the severe strains of tristeza virus in our Satsuma mandarins, tristeza may be very dangerous in future if *Aphis gossypii* is adapted in our Citrus. Severe strains of the tristeza was also reported from Satsuma mandarins in Texas introduced from Japan by Olson and Mc Donald (1955) Olson and Sleeth (1956) and Olson (1956). As reported by Yamada and Tanaka (1969), most of the Satsuma trees in Japon were infected by the severe strains of the virus.

In our experiment area, Satsuma mandarin trees buded on *P. trifoliata* showed mild stunting and some gradual decline of the trees. It is not possible to eradicate completely the tristeza affected Satsuma on *P. trifoliata*, because of the tristeza

affected *P. trifoliata* rootstocks did not show invertebrate infestations. Our indexing tests revealed that severe strains of the tristeza virus that caused decline and death of the Satsuma on sour orange were found in our Satsuma as reported from the several country by Olson (1956), Olson and Sleeth (1956), McClean (1963), Yamada and Tanaka (1969). Severe strains of the tristeza virus caused to 94.64 % crop losses when the Satsuma trees were declined and buded on sour orange rootstocks, and 70 %

crop losses of the severely affected declined Satsuma buded on *P. trifoliata*. Dolar (1976) also reported crop losses from 79,2 % to 99,4 % in Citrus trees buded on sour orange rootstock.

Eradication is necessary in our Citrus orchards. It is necessary also to initiate short-term and long-term indexing and the bud-wood registration program to establish virus free Citrus foundation and mother blocks in Turkey.

ÖZET

İZMİR İLİNDEKİ SATSUMA MANDARİNLERİNDE GÖÇÜREN

(Tristeza= Quick Decline) VİRUS HASTALIĞININ TANIMI,

ZARAR DERECEŚİ, DAĞILIŐI VE FARKLI İRKLARININ

SAPTANMASI ÜZERİNDE ARAŐTIRMALAR

Göçüren virusunun İzmir ilindeki Satsuma mandarinlerindeki durumunu saptamak amacı ile, 1969 yılından itibaren Mexican lime bitkisi ile endeksleme çalışmaları uygulanmıştır. Sürveyde yaşları 8 ve daha yukarı olan üç yapraklı ve turuncu anacı üzerine aşıllı 110 Satsuma bahçesinde toplam olarak 2200 ağaç Göçüren Hastalığı yönünden incelenmiştir. Verim Denemesi için, 19 ayrı Satsuma plantasyonunda 176 ağaç üzerinde denemeler yürütülmüştür.

Verim denemesine alınan 176 ağaç göçüren virus ırkları yönünden de sera koşullarında M. lime test bitkileri ile endekslenmiştir. Denemeye alınan 19 Satsuma plantasyonunda saptanan Göçüren virus ırkları ile ürün arasındaki korelasyonlar saptanmıştır.

İzmir ilinde Satsuma üretimi yapılan aşağıdaki ilçelerde Ağustos 1974 tarihinden itibaren uygulanan sürveyde, Merkez ilçede % 15,56 Seferihisar'da % 17,05, Bornova'da %

15,66, Karşıyaka'da % 15,0 Menemen de % 20,0 ve Urla'da % 22,5 Göçüren Hastalığı yönünden yakalanma oranları saptanmıştır. İzmir ili için hesabedilen yakalanma oranı % 17.79 dur.

Simptomatolojik gözlemlerde, turunç anacı üzerine aşılı Satsuma mandarinlerinde Göçüren Virusunun tipik belirtisi olan, bodurluk, yavaş göçme, geriye doğru ölüm ve aşı yerine kadar ani kurumalar görülmüştü. İnfekteli ağaçlarda ayrıca, kloroz ufak ve soluk yeşil renkte yapraklar ve üründe azalma olmuştur. Virusun şiddetli ırkları ile infekteli ağaçlarda belirtilerin daha şiddetli olduğu görülmüştür. Göçüren viruslu Satsuma mandarin ağacının aşı yerinin üst kısmında dışarı doğru büyüme (Overgrowth) belirtisi saptanmıştır. Enfekteli ağaçların aşı yerinden alınan kabuk kesitlerinde turunca ait kabuk alt yüzeyinde aşı yerinden itibaren aşağı doğru içine batırılmış gibi ince çukurcuklar (pinholing = pitting = honey combing) ile odun yüzeyinde bu çukurcukların karşıtı olan balık dişi şeklinde çıkıntılara (pegs) rastlanmıştır.

Uç yapraklı anaç üzerine aşılı ve Göçüren virusu ile infekteli Satsuma mandarinlerinde bodurluk, sürgünlerde durgunluk, yaprak ufalması, yapraklarda donuk yeşil ve sarımsı yeşil renk bozulmaları, ağaçta genel durgunluk ve aşı yerine yakın kabuk alt yüzeyinde hafif çukurcuklara (pinhollings) rastlanmıştır.

Lâboratuvarda uygulanan doku boyama testlerinde, Göçüren virusunun, turunç anacının floem parankima hücrelerinde ve kalburlu iletim borularında nekrozlar oluşturarak kalburlu boruları inaktif duruma getirdiği ve kambiuma yakın yeni oluşmuş kalburlu borularının üzerinde kallus oluşturduğu görülmüştür. Turunç floeminde oluşan kalbur nekrozlaşmalarının ilerlemesi durumunda Satsuma mandarin ağaçlarının ani olarak kuruduğu, fakat turunç anacının alt kısımdan yeni zayıf sürgünler verdiği saptanmıştır.

Araştırma bölgelerindeki Satsuma mandarinlerimizde göçüren virusunun zayıftan en kuvvetli irkına kadar 5 ayrı irkinin bulunduğu Meksika lime testleri ile saptanmıştır. Satsuma mandarinlerimizde Göçüren virusunun zayıf ve orta derecedeki ırklarının şiddetli ırklarından daha yaygın olduğu lime testleri ile anlaşılmıştır. Göçüren virusunun şiddetli ırkları lime bitkilerinin yaprak damarlarında çok şiddetli damar beyazlaşmaları oluşturmuştur.

Göçüren virusunun Tropikal turunçgil afidi (*Toxoptera citricidus*) ve *Aphis gossypii* ile bilhassa turunç anacı üzerine aşılı turunçgillerde ani ölüm şeklinde oluşturduğu Collapse belirtisine bizde rastlanmamıştır. Fakat bizdeki satsumalarda turunç anacına kadar olan (aşı yerine kadar) aşı kısmının (mandarinin) yavaş yavaş öldüğü ve tamamen kuruduğu saptanmıştır.

TRISTEZA VIRUS DISEASE

Göçüren virus ırkları ile ürün arasındaki korelasyonların saptanması için, araştırma bölgesinde üçyapraklı ve turunç anacı üzerine aşılı 19 Satsuma mandarin bahçesinde toplam 176 ağaç üzerinde yapılan hesaplamalar sonucu, saptanan virus ırkları ile ürün arasında önemli derecede ve negatif bir korelasyon bulunmuş ve korelasyon kat sayılarının $r = -0,7180$ ile $r = -0,9925$ arasın-

da değiştiği saptanmıştır. Bahçeler için uygulanan varyans analizinde bulunan "F" değerleri 19 bahçenin 3'ünde % 5'e göre önemli geriye kalan 16 bahçede ise, % 1'e göre önemli derecede farklı olduğu görülmüştür. Göçüren virusunun kuvvetli ırkları ile infekteli turunç üzerine aşılı Satsuma mandarin ağaçlarında önemli derecede ürün azalmaları saptanmıştır.

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TRISTEZA VIRUS DISEASE

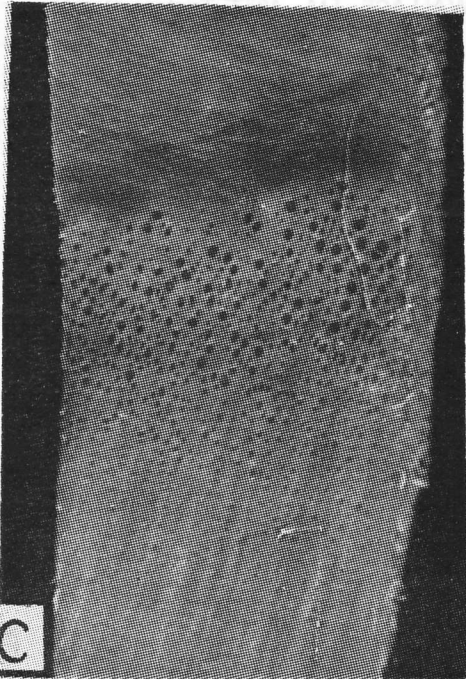
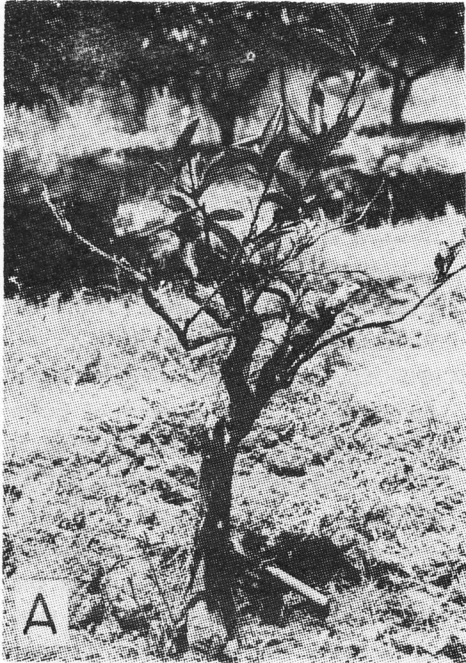


Fig. 2. Typical symptoms of tristeza virus on Satsuma mandarins budded on sour orange rootstocks. A, severely tristeza affected declined satsuma tree. B, chronic decline and dead of the fiber roots: C, pits (honeycombing) on the inner surface of the sour orange bark.

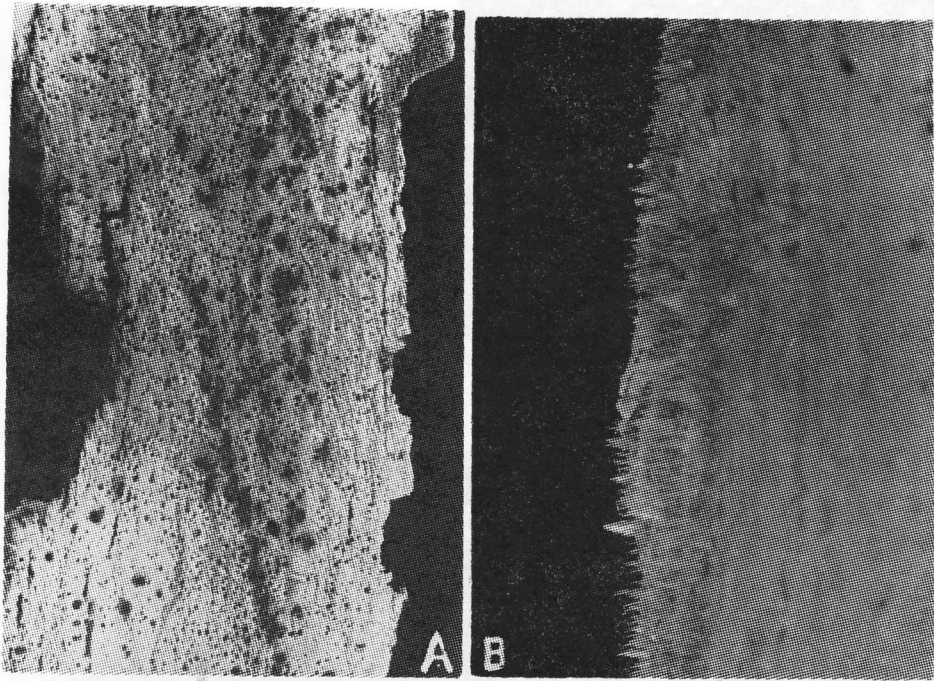


Fig. 3. Symptoms of the tristeza virus on the Satsuma scion; A, Pits or honeycombing in the inner face of the Satsuma scion over the bud union; B, pegs on the woody cylinder of the Satsuma scion.

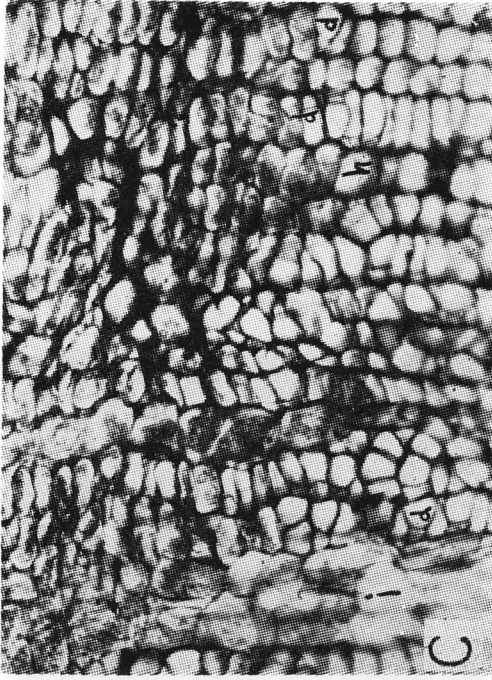
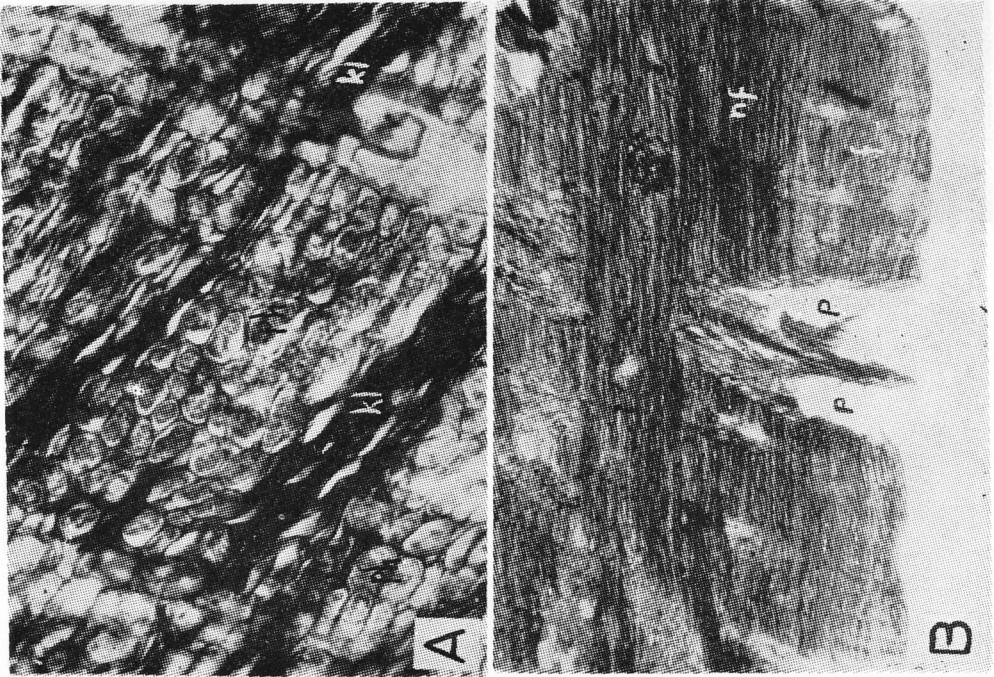


Fig. 4. Floem of the tristeza-infected sour orange bark sections. A, chromatic parenchyma cells (ph) and necrosis of the sieve tubes. B, functioning (f) and nonfunctioning (nf) sieve tubes, the pit (p) and the cambium (c) are shown. C, Hypertrophied (h) and the divided parenchyma cells (d).

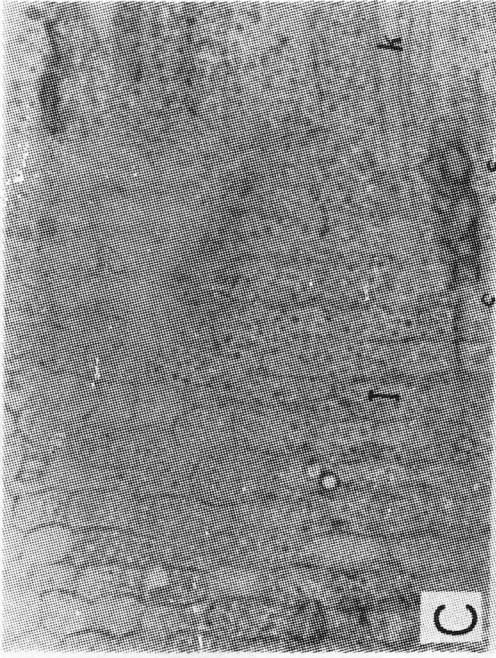
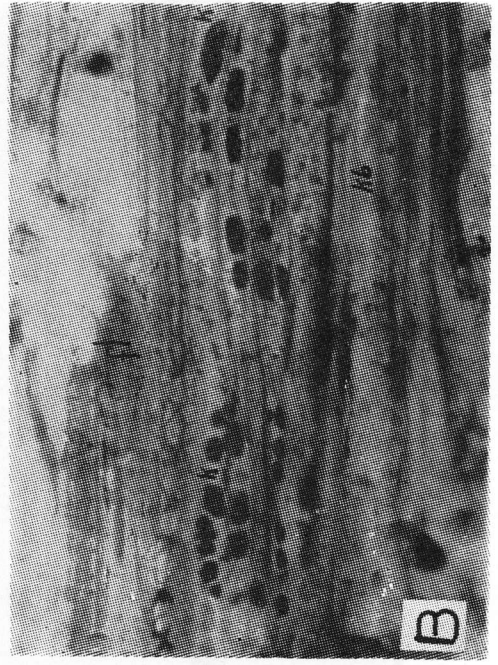
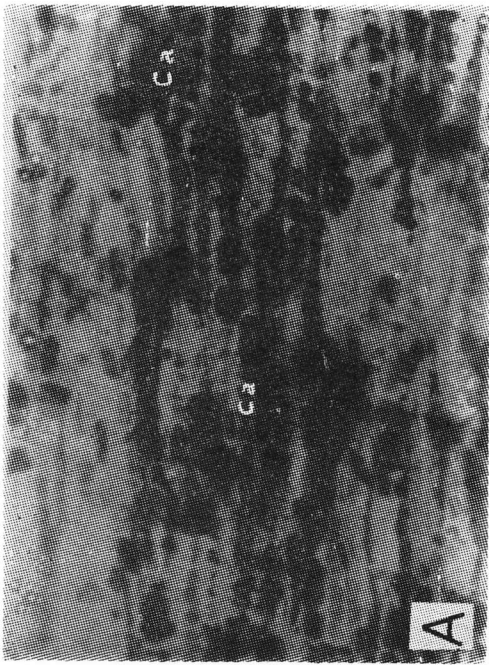
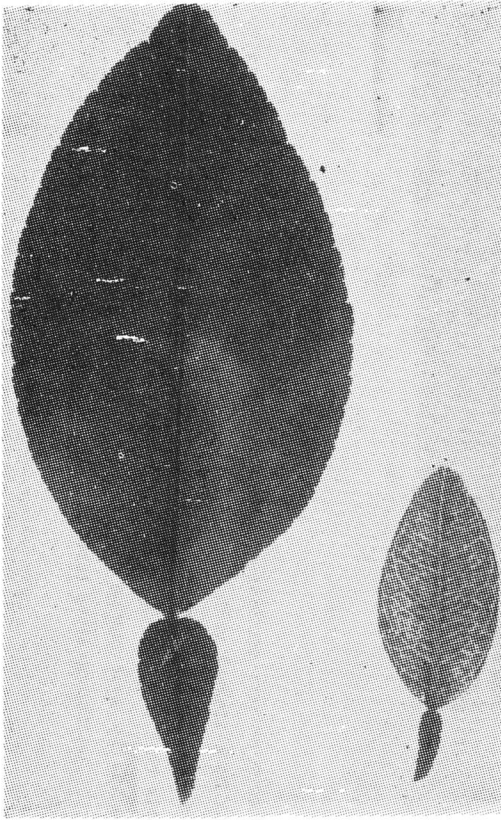
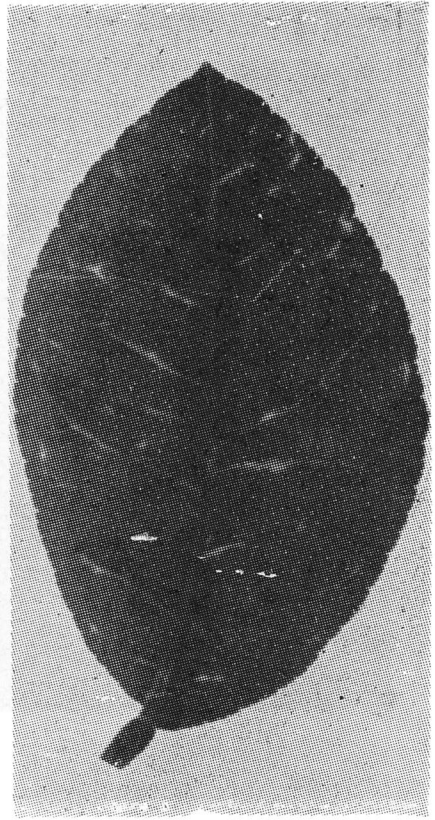


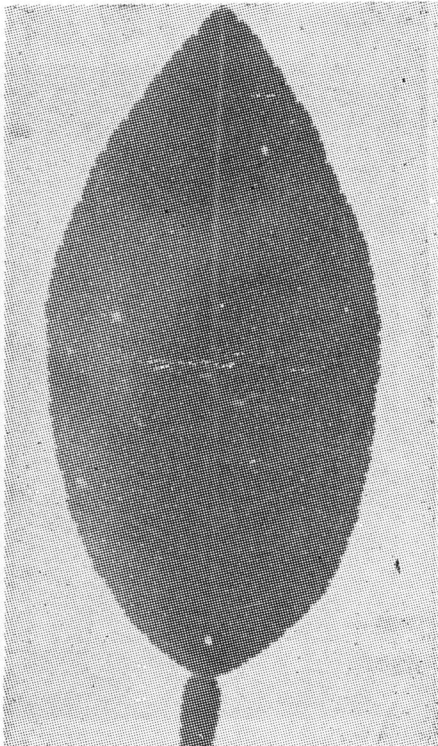
Fig. 5. Radial phloem sections of *tristeza* infected sour orange and *P. trifoliata* rootstock bark stained with Congo red and lacmoid. A, Callus deposits on the sieve plates in sour orange phloem (Ca). B, Callus on the sieve plates in *P. trifoliata* bark phloem (k), sieve plates (kb). C, Radial sections of phloem of the healthy sour orange rootstock showed clear phloem ray cells (l), functioning sieve tubes (k), cambium is at below (c).



A



B



C

Fig. 6. The leaves of the Mexican lime infected with the different tristeza virus strains. A, very small leaves showing All-clearing= veinlet netting (severe strain), large leaf at the left is control from the uninfected lime. B, Clearings more localised (Mild strains). C, Occasional small clearings (very mild strain).

Histology of Penetration of Resistant and Susceptible Sorghum Leaves by *Colletotrichum graminicolum*

Ashok MISHRA and B. Singh SIRADHANA

University of Udaipur, Rajasthan College of Agriculture, Department of Plant Pathology, Udaipur, INDIA.

ABSTRACT

When sorghum leaves (susceptible and resistant) are inoculated with conidia of *Colletotrichum graminicolum* the single celled spores germinate and produce appressoria. The penetration by infection peg is generally through cuticle and rarely through stomata. The initial stages of penetration is almost similar in susceptible and resistant leaves. However, further progress of the pathogen is checked by a zone barrier in resistant leaves. In susceptible leaves the pathogen colonized in the host cells and mycelium is also seen in xylem.

INTRODUCTION

Study of host-parasite relationship helps in understanding the parasitism which may provide a base for controlling the disease. There is little information on the host-parasite relationship of *Colletotrichum graminicolum* (Ces.) Wils and sorghum leaves. Zwillenberg (1959) observed that maize leaves were rapidly pervaded by conidia of *C. gra-*

minicolum and the acervuli developed after 2 - 10 days. Ultrastructural study of penetration of maize leaves by this pathogen was studied by Politis and Wheeler (1973). Present study deals with the histopathological study of anthracnose of sorghum (*Sorghum bicolor* (L.) Moench) in susceptible and resistant cultivars.

MATERIALS AND METHODS

Latch and Hansen's (1962) method was followed to study the host penetration mechanism. Fresh green leaves of susceptible (local) and resistant (CSH-5) were removed from the plants grown in pots,

washed with sterile water and were inoculated by rubbing spore and mycelial suspension of *C. graminicolum* on both the surfaces of leaves. Such leaves were then placed on slides in petridishes lined with moist

filter paper. Samples of the leaves were taken at the intervals of 4, 8, 12, 24, 48 and 72 hours after inoculation and were placed in a hot solution of glacial acetic acid and 95 % ethanol (1:1 v/v). The solution was gradually boiled until the leaves lost chlorophyll. The 80 treated leaves were then dipped for 30 minutes in a chloral hydrate solution. Cleared leaves were stained on slides with lactophenol cotton blue and then examined under microscope.

For histological studies plants grown in pots were inoculated by

usual method. The inoculated leaves were killed and fixed in formalin acetic acid (90 ml of 70 % ethyl alcohol, 5 ml glacial acetic acid and 5 ml of formalin) after every 24 hours of inoculation till the appearance of symptoms. The fixed leaf pieces were properly dehydrated and embedded in paraffin wax as described by Johansen (1940). The sections of 10 to 15 μ thickness were cut by a hand operated rotary microtome, stained with safranin and fast green combinations and mounted in a drop of canada balsam.

RESULTS

It was observed that conidia of *C. graminicolum* germinated readily on the leaf surface. Generally germination started after 4 hours of inoculation and the germ tubes were slender on susceptible leaves while stocky on resistant leaves. On susceptible leaves the germ tubes continued to grow. The tips of the germ tubes were swollen which were evidently appressoria. Appressoria were frequently observed near the points where epidermal cells join. Penetration through stomata was also observed. A sharp cut on the cuticle and epidermal cells by infection peg was observed.

After penetration the hyphae enlarged and proliferated in mesophyll region of the susceptible variety. In resistant variety the penet-

ration may or may not occur. The germ tubes get enlarged and swollen. This may be due to some biochemical reaction. However, even if penetration occurred in resistant leaves, in advance of the mycelium a reddish pigment was produced and absorbed by the cell walls. The red zone constituted a barrier to the progress of the invading mycelium (Fig. 1). In susceptible leaves mycelium progressed further and passed down in the palisade cells resulting in the disintegration of the chloroplasts and the cytoplasm (Fig 2). Parenchymatous tissues were severely affected. In later stages the pathogen had colonized a majority of cells resulting in their disintegration (Fig 3). In some cases hypha were seen in xylem. (Fig 4).

DISCUSSION

Colletotrichum sp. are generally considered to be a pathogen which penetrates directly through cuticle. In the ultra-structural study of penetration of maize leaves, Politis and Wheeler (1973) and of oat leaves Politis (1976) observed that the fungus makes a sharp cut of the cuticle and does not exert mechanical pressure as they did not observe any kind of depression in epidermal layer at the point of penetration. In our studies it was observed that the pathogen could penetrate the cuticle and also through stomata. The penetration in susceptible and resistant sorghum leaves was almost similar. However, in the resistant variety further growth of the pathogen was checked and it could not colonize the host cells. A reddish zone was formed in advance of the mycelium in resistant leaves perhaps

because of accumulation of some antifungal substances. Preliminary studies have shown that phenols and some toxic metabolites are present or formed consequent to infection in such cases. Reddish pigment constituting a barrier was also observed by Edgerton and Carvajal (1944) in red rot of sugarcane. Zwillenberg (1959) examined the leaves of *Lolium perenne* L, *Dactylis glomerata* L. and wheat after inoculating *C. graminicolum* and found a thickening of the outer epidermal cell walls at the site of appressoria.

In the susceptible cultivar the pathogen colonized the host cells. Disintegration of chloroplasts occurred. After ramification of the mycelium beneath the epidermis acervuli developed. The symptoms developed fully in 7-10 days of inoculation.

ÖZET

DAYANIKLI ve DUYARLI SORGUM YAPRAKLARINDA

Colletotrichum graminicolum'un PENETRASYON HİSTOLOJİSİ

Dayanıklı ve duyarlı sorgum yaprakları *Colletotrichum graminicolum*'un konileri ile inokule edildiğinde, tek hücreli sporlar çimlenir ve appressoryum oluşur. Penetrasyon genel olarak enfeksiyon çivisi ile kutikuladan, bazen de stomalardan gerçekleşir. Penetrasyonun ilk evreleri

dayanıklı ve duyarlı yapraklarda aynıdır. Ancak etmenin daha sonraki gelişmeleri dayanıklı bitkilerde kırmızı renkteki bir sınır ile engellenir. Duyarlı yapraklarda ise etmen konukçu hücrelerinde koloni oluşturur ve miselyum ksilemde de görülebilir.

COLLETOTRICHUM GRAMINICOLUM

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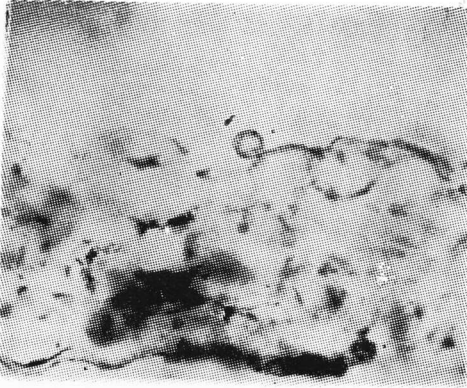


Fig. 1

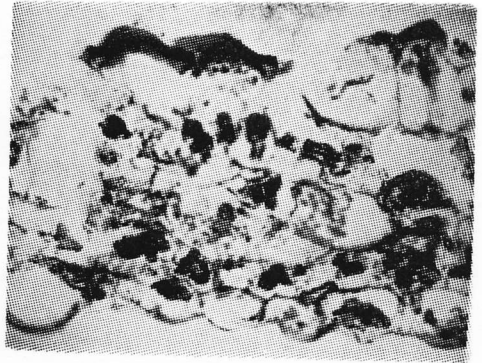


Fig. 2

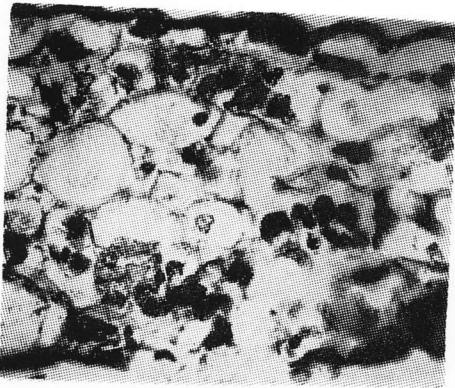


Fig. 3

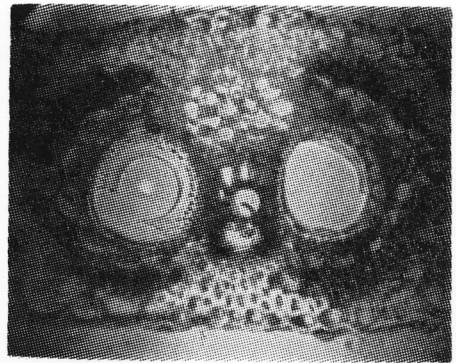


Fig. 4

Fig. 1. T.S. of a resistant leaf (CSH-5) showing red zone in advance of the mycelium x 450

Fig. 2—3. T.S. of leaf showing disintegration of host cells and colonization by the pathogen x 820

Fig. 4. T.S. of leaf showing mycelium in the xylem and disintegration of chloroplasts x 820

Anfälligkeit Verschiedener Sorten von *Lycopersicon esculentum* Mill. gegenüber *Pseudomonas tomato* (Okabe) Alstätt

Özden ÇINAR

Lehrstuhl für Pflanzenschutz, Landwirtschaftliche
Fakultät der Çukurova Universität, ADANA, TURKEI

ZUSAMMENFASSUNG

9 Tomatensorten wurden auf Anfälligkeit gegenüber *P. tomato* hinsichtlich der Symptomentwicklung und der Vermehrung der Bakterien in den Blättern untersucht. Nach den hervorgerufenen Symptomen und der Bakterienvermehrung in Pflanzenblättern zeigten sich die Sorten Antalya 1F₁ und Antalya 2F₂ gegenüber Rakibi schwach anfällig. Die Unterschiede in der Symptomentwicklung bei den 9 verschiedenen Sorten waren besonders eindeutig am 4. Tag nach der Infektion. Nach der Bakterienzählbestimmung in infizierten Blättern lassen sich die Anfälligkeitsunterschiede erst am 4. Tag nach der Infektion sehr klar erkennen.

EINLEITUNG

Als Ursache einer 1975 im Mittelmeer beobachteten Krankheit auf Tomaten wurde *Pseudomonas tomato* diagnostiziert (Çınar, 1977). Die Symptome dieser Krankheit sind im wesentlichen gekennzeichnet durch braeunliche bis schwarze Flecken auf den Blättern, den Stängeln,

den Blatt-, Blüten- und Fruchstielen, den Kelchen, den Kelchblättern sowie durch kleine schwarze Bläschen auf den Früchten (Abb. 1, 2).

Während sich die Krankheit in den Jahren ihres Auftretens epidemisch ausbreitete, ist der Befall 1978 nur noch sporadisch. In einigen Pub-

likationen wird erwahnt, dass **P. tomato** durch infiziertes Saatgut übertragen werden und im Boden in Abwesenheit der Wirtspflanzen überleben kann (Bosshard - Heer und Vogelsanger, 1977). Da man mit dieser Bakterienkrankheit chemisch noch nicht bekaempfen kann, sind die Produktion von gesundem Saatgut und die Züchtung resistenter Sorten die bisher wichtigsten Bekämpfungsmassnahmen. Aus die-

sem Grund wurden 9 verschiedene Tomatensorten, die in Mittelmeergebiet angebaut werden, auf Anfälligkeit gegenüber **P. tomato** untersucht. Bei den Sorten, die aufgrund der Symptomausprägung weniger anfällig erschienen, wurde der Anfälligkeitsgrad genau bestimmt, indem die Vermehrung der Bakterien in den Blättern quantitativ erfasst wurde.

MATERIAL UND METHODEN

Die Blattunterseite von 9 verschiedenen Tomatensorten (Antalya 1F₁, Antalya 2F₂, Monfavet 63/5, Pearson, Potantote, Rakibi, Super Marmende, U.C. 156, VF 145) wurden mit drei Tage alten Bakterienkulturen (ca. 10⁸ Zellen/ml) mit Hilfe einer Spritzpistole infiziert. Die Pflanzen wurden dann im Folienhohtunnel (ø 25°C, max 35°C und min 10°C, 40-90 % Luftfeuchtigkeit) aufgestellt. Sie waren 3 Monate alt.

Zur quantitativen Bestimmung der Bakterienvermehrung der schwach anfälligen (Antalya 1F₁ und Antalya 2F₂) und anfälligen (Rakibi) Tomatensorten wurden zu verschiedenen Zeiten nach der Inoku-

lation aus den infizierten Blättern mit einem sterilen Korkbohrer von 5,4 mm Durchmesser Blattscheibchen entnommen. Jede Probe bestand aus 30 Scheibchen von verschiedenen Pflanzen. Die Scheibchen, die also eine Blattfläche von 6,87 cm² repräsentierten, wurden in 15 ml sterilem Wasser 1 min lang homogenisiert. Aus dem Homogenat wurde 1 ml, einer Blattfläche von 0,45 cm² entsprechend, entnommen und mit 9 ml Wasser oder Agar vermischt. Die anschliessend in Agar hergestellte Verdünnungsreihe wurde plattiert und die Kolonienzahl nach 3 Tagen bestimmt (Çınar und Rudolph, 1972). Es wurden 3 Wiederholungen durchgeführt.

ERGEBNISSE

9 Sorten von *Lycopersicon esculentum* wurden auf Anfälligkeit

gegenüber **P. tomato** getestet. Nach den bei künstlicher Inokulation her-

vorgerufenen Symptomen wurden 2 Gruppen verschieden hoher Anfaelligkeit gebildet (Tab 1). Die Einteilung in die 2 Gruppen erfolgte nach den unterschiedlichen Symptomen zu verschiedenen Zeiten nach der Infektion, wie sie aus Tabelle 2 hervorgehen.

Zur Untersuchung der Vermehrungsrate von *P. tomato* dienten zwei schwach anfaellige Sorten (Antalya 1F₁ und Antalya (2F₂) sowie eine anfaellige Sorte (Rakibi). Die Symptomentwicklung auf diesen Sorten zeigt die unterschiedliche Anfaelligkeit recht deutlich.

Tabelle 1. Die Anfaelligkeit von Tomatensorten gegenüber *P. tomato*

Anfaelligkeitsgrad	Wirtspflanzen Tomatensorten
Schwach anfaellig	Antalya 1F ₁ , Antalya 2F ₂
Anfaellig	Monfaet 63/5, Pearson, Potantote, Rakibi, Super Marmande, U.C. 156, VF 145

Tabelle 2. Symptomentwicklung bei Tomatensorten verschieden hoher Anfaelligkeit

Tage nach der Inokulation	Schwach anfaellig	anfaellig
1		
2	braune oder hellgrüne Flecken	hellgrüne Flecken
4	kleine braune eingetrocknete Flecken und kleine frische Flecken	wenige kleine braune eingetrocknete Flecken und frische Flecken
7	mittlere braune eingetrocknete Flecken und wenige frische Flecken	grosse braune eingetrocknete Flecken und viele frische Flecken mit chlorotischem Hof
15	grosse braune eingetrocknete Flecken und wenige kleine schwarze Flecken	grosse vertrocknete Flecken und schwarze Flecken, Z.T. Blätter abgefallen

Erwartungsgemaess erreichte die Bakterienvermehrung in der anfaelligen Sorte Rakibi die höchsten Werte (vgl. Tab 3). Aber zwischen den beiden weniger anfaelligen Sorten ergab sich auch einen Unter-

schied. Die Bakterien wurden in der Sorte Antalya 1F₁ trotz etwa gleicher Symptomentwicklung wie in der Sorte Antalya 2F₂ etwas staerker gehemmt.

Tabelle 3. Vermehrung "in vivo" von *P. tomato* in Tomatensortenblaettern mit unterschiedlicher Anfaelligkeit

Sorten	Tage nach der Inokulation				
	1	2	3	4	7
Antalya 1F ₁	1,9 x 10 ⁴	5,0 x 10 ⁵	5,1 x 10 ⁵	1,3 x 10 ⁵	1,2 x 10 ⁵
Antalya 2F ₂	1,8 x 10 ⁴	3,8 x 10 ⁵	6,7 x 10 ⁵	6,8 x 10 ⁵	1,1 x 10 ⁵
Rakibi	2,0 x 10 ⁴	1,1 x 10 ⁶	2,1 x 10 ⁶	6,1 x 10 ⁶	6,6 x 10 ⁵

Die unterschiedliche Vermehrung der Bakterien in den anfaelligen und schwach anfaelligen Pflanzen kommt so sehr deutlich zum Ausdruck. Dies zeigte sich bereits am 2. Tag p.i. und besonders deutlich auch am 3. Tag p.i. (Abb. 3). Nach dem 3. Tag p.i. war die Bakterienvermehrung in Sorte Antalya 1F₁, nach dem

4. Tag. p.i. in den Sorten Antalya 2F₂ und Rakibi gegenüber der logarithmischen Vermehrungsrate der ersten Tage deutlich abgefallen.

Der Versuch zeigte eine recht gute Übereinstimmung zwischen den im Blatt gefundenen Bakteriendichten und den aeusserlich sichtbaren Symptomen.

DISKUSSION

Soweit uns bekannt ist, wurde bisher nicht die Anfaelligkeit verschiedener Tomatensorten gegenüber *P. tomato* berichtet.

Die Untersuchungen über die Bakterienvermehrung "in vivo" er-

gaben für "Antalya 1F₁" eine deutlichere Verlagsamung als für die aeusserlich aehnlich reagierende "Antalya 2F₂". Bei "Antalya 1F₁" scheint die Reaktion des Blattgewebes schneller abzulaufen, denn ab 3

d.p.i. war die Bakterienkonzentration deutlich niedriger als bei den anderen Sorten. Die *Pseudomonas syringae* infizierten 2 schwach anfaelligen Bohnensorten ergaben nach der Bakterienvermehrung auch einen deutlichen Unterschied. (Delgado, 1974). Am Anfang vermehrten sich die Bakterien in 3 Blättersorten gleich schnell, und erst mit dem Beginn der Immunreaktion in schwach anfaelligen Sorten kam es zu einer Verlangsamung der Bakterienvermehrung. Dieses Ergebnis entspricht Allington und Chamberlain (1949), die die Vermehrung von *Xanthomonas phaseoli* und *Pseudomonas glyceaine* in Blättern von *Phaseolus vulgaris* und *Glycine max* analysierten. Nach dem 3 Tag p.i. wurde ein deutlicher Abfall der

Bakterienkonzentration in der Sorte Antalya 1F₁ beobachtet. Aehnliche Ergebnisse haben Stall und Cook (1966) nach Infektion von Paprikapflanzen. *Xanthomonas vesicatoria* gefunden. Bei den verschiedenen Bakterienkrankheiten wurden aehnliche Unterschiede in der Bakterienvermehrung (zwischen 10-100. fach) von anderen Autoren bei dem Vergleich anfaelliger und resistenter Sorten festgestellt. (Diachun und Troutman, 1954; Stall und Cook, 1966; Çinar und Rudolph, 1972; Delgado, 1974). Mit Feldversuchen muss man die Ergebnisse der Gewachshaus - Untersuchungen dahingehend prüfen, ob die von uns als schwach anfaellig qualifizierten Tomatensorten zur Unterdrückung der Schwarzfleckenkrankheit ausreichen.

ÖZET

FARKLI *Lycopersion esculentum* Mill. ÇEŞİTLERİNİN *Pseudomonas* Tomato (Okabe) Alstatt'a KARŞI DUYARLILIKLARI

9 Domates çeşidinin *P. tomato* ya karşı duyarlılıkları, yapraklardaki simptom gelişmesine ve bakteri hücre sayısına göre araştırılmıştır.

Yapraklardaki simptom gelişmesi ve bakteri hücre sayısına göre duyarlılık saptanmasında, Antalya 1F₁ ve Antalya 2F₂ çeşitleri Rakibi'ye

kıyasla az duyarlı bulunmuştur. 9 Farklı domates çeşidinde simptom gelişmesindeki en belirgin fark enfeksiyondan sonra 4. günde gözlenmiştir. Aynı şekilde enfekteli yapraklarda bakteri hücre sayısına göre duyarlılık farkı en açık olarak enfeksiyondan sonra 4. günde saptanmıştır.

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ÖZET

FARKLI İYPERPERSON ESCULENTUM MİLİ ÇEŞİTLERİNİN PSEUDOMONAS TOMATO (OKABE) ALSTATT'İN KARŞI DUYARLILIKLARI

9 Domates çeşidinin *P. tomato*-ya karşı duyarlılıkları, yapraklarda-ki simptom gelişmesine ve bakteriyel hücre sayısına göre araştırılmıştır. Yapraklardaki simptom gelişme-si ve bakteriyel hücre sayısına göre du-yarlılık farkları en açık olarak en-ferlik simptomundan sonra 4. gründe sa-ya-tilmiştir. Aynı şekilde enfekteli yap-raklarda bakteriyel hücre sayısına göre farklar da bakteriyel hücre sayısına göre araştırılmıştır.

9 Domates çeşidinin *P. tomato*-ya karşı duyarlılıkları, yapraklarda-ki simptom gelişmesine ve bakteriyel hücre sayısına göre araştırılmıştır. Yapraklardaki simptom gelişme-si ve bakteriyel hücre sayısına göre du-yarlılık farkları en açık olarak en-ferlik simptomundan sonra 4. gründe sa-ya-tilmiştir. Aynı şekilde enfekteli yap-raklarda bakteriyel hücre sayısına göre farklar da bakteriyel hücre sayısına göre araştırılmıştır.

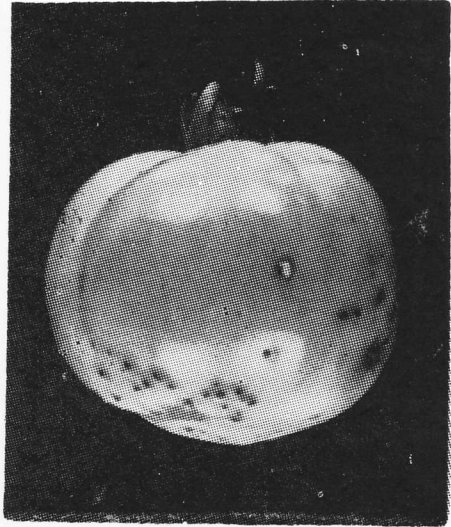
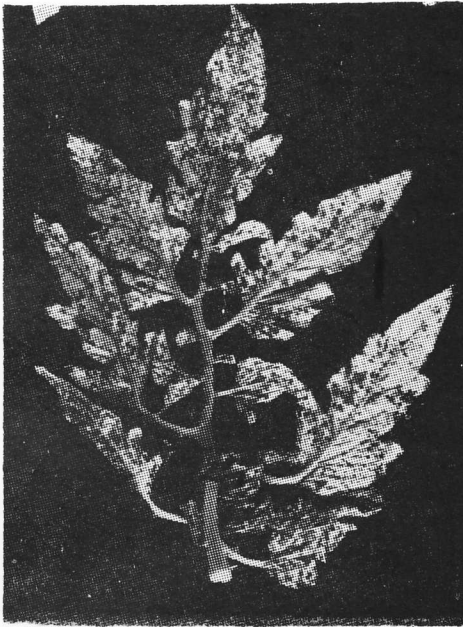


Abb. 1. Flecke durch *P. tomato* auf Blättern von Tomaten aus einem natürlich infizierten Feldbestand.

Abb. 2. Flecke auf der Frucht durch *P. tomato* natürlich infiziert.

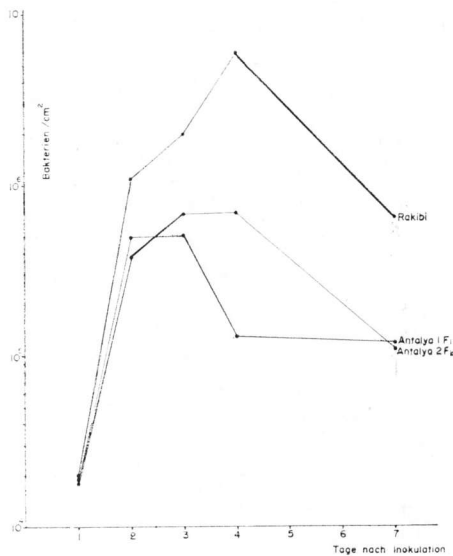


Abb. 3. Vermehrung von *P. tomato* im Blattgewebe von 3 Tomatensorten verschiedener Anfaelligkeit.

Untersuchungen über Isolation des Erregers der Schwarzbeinigkeit (*Erwinia atroseptica* van Hall. der Kartoffeln und seine biochemischen und serologischen Eigenschaften ¹⁾)

Necatî BAYKAL² und Yavuz Emin ÖKTEM³

ZUSAMMENFASSUNG

Der Erreger der Schwarzbeinigkeit (*Erwinia atroseptica* van Hall) an den Saatgutknollen in der Umgebung von Bolu wurde auf einem Selektivenaerboden isoliert, und ihre biochemische und serologische Eigenschaften untersucht. Isolation wurde auf dem Stewart-Selektivenaerboden verwirklicht und dabei wurde auf Naehrboden typische Kraterkolonien beobachtet. Das Bolu-Isolat (5a) hatte dieselbe morphologischen Eigenschaften wie das Originalisolat (Nr. 549 von NCPPB) und gleiche serologische und biochemische Reaktionen gezeigt, auch bei den Infektionsversuchen an den Kartoffelpflanzen typische Faeule verursacht. Bei dem Objekttraeger-agglutinationstest zeigte das Bolu Isolat gegen bis zu 1/40 verdünnte Antiserum positive Reaktion. Aehnliche Ergebnisse wurden auch bei dem Pflanzenextrakt beobachtet.

EINLEITUNG

Der Kartoffelanbau in der Türkei kommt mit etwa 2.490.000 Tonnen Ertrag im Jahre 1975 an (Anonymus, 1975).

Die Schwarzbeinigkeit, die als eine wichtige Kartoffelkrankheit angesehen wird, wurde in der Türkei zum erstenmal von Gassner im Jahre 1938

1) Vortrag gehalten auf dem II. Phytopath. Kongress in Ankara.

2) Lehrstuhl für Phytopathologie, Landw. Fakultät der Ankara-Univ.

3) Institut für Pflanzenschutz, Ankara.

ERWINIA ATROSEPTICA

(Bremer, et al. 1952) festgestellt. Danach wurde von Karel und Karahan (1962) beobachtet dass die Krankheit in Kartoffelanbau von Mittelanatolien bis zu 35 % Schaden verursacht. Die Krankheit wurde auch von Karaca (1966) in Schwarzenmeergebiet und in Mittelanatolien gesehen.

Die beste Abwehrmassnahme ist einwandfreie Knollen zu verwenden

Der Erste Zweck dieser Untersuchung ist die Isolationsmethode des Erregers aufzustellen und seine morphologische und biochemische Eigenschaften zu bestimmen. Die zweite Aufgabe war die serologische Methode zu finden, die die Identifizierung des Erregers im eingeführten Material bei der türkischen Quarantäne und Saatgutzuchtzentrale ermöglichen kann.

MATERIAL UND METHODEN

Isolationstechnik

Die Oberfläche der erkrankten Stengel wurde mit Leitungswasser gut gewaschen, abgetrocknet und in einem Sterilporzellanmörser unter Zusatz von sterilem Wasser kraeftig zerrieben. Aus diesem Extrakt wurde ein Tröpfchen mit Hilfe einer Impföse genommen und auf Agar (Stewart-Naehrboden) ausgestrichen. Nach 48 stundiger Bebrütung bei 27°C wurden die ausgewachsenen Bakterienkolonien wieder auf Stewart-Naehrboden übergeimpft (Stewart, 1962). Stewart-Naehrboden besteht aus:

A. Mac Conkey Agar: 69.5 g Mac Conkey Agar, 5.3 g $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$, 1000 ml dest. Wasser.

B. Pectitlösung: 2 g. Sodium polypectate, 6 ml Ethanol (% 96), 0.1 g EDTA, 100 ml dest. Wasser (pH=7.2).

10 ml von Pectitlösung wurde über Mac Conkey Agar zugesetzt.

Pathogenitaet

Die Bakterien wurden auf Nutrient Agar (NA) Naehrboden kultiviert, nach 24 Stunden eine Baktériensuspension vorbereitet, und an die Kartoffelpflanzen nach Graham und Dowson (1960 a) infiziert. Die geimpfte Pflanzen wurden in Inkubator bei 90-95 % Luftfeuchtigkeit und bei 24°C aufgestellt.

Physiologische Eigenschaften

Oxidasetest (Nach Kovacs, 1956): Als Reagens wurde % 1 ige waessrige Lösung von Tertamethyl - parphenylene - diamine dihydrochlorid benutzt.

Katalasetest: Es wurde % 3 iger H_2O_2 benutzt.

Wachstum auf der Kartoffelscheibe: Von den 2 taegigen Kulturen wurden die Isolate auf den Kartoffelscheiben geimpft, bei 27°C 48 Stunden inkubiert.

Ausnutzung von Glucose. Der nach Wachstumstest der Bakterien wurde Hugh und Liefson (1953) durchgeführt.

Saccharosetest: Naehrlösung, bestehend aus 10 g Pepton, 5 g Lab-Lemco, 40 g Saccharose und 1000 ml dest. Waser (pH = 7.0). Nach dem Beimpfen wurde die Naehrlösung bei 25°C 2 Tage geschüttelt. Anschliessend zu dieser Lösung Benedigt-Reagens versetzt und die Farbveraenderung beobachtet.

Pectinverflüssigungstest (Nach Graham und Dowson, 1960 b): Der mit Bakterien beimften Pectinnaehrboden wurde bei 27°C inkubiert, je 24 Stunden bis zu 14 Tagen geprüft.

H₂S Bildung Nach Anonymus, 1971): Es wurde bei diesem Test der

fertige Naehrboden TSI (Oxoid) verwendet.

Gluconatstest (Nach Graham und Dowson, 1960 b): Nach der 48 stundigen Bebrütung wurde die Lösung mit Benedickt-reagens geprüft.

Saeure-und Gasbildung aus Zuckern: Nach Cowan und Steel (1970) wurde Bromtymolblau und Pepton (mit Wasser) benutzt. Zum Sterilisation der Zuckern wurde das Bakterienfilter verwendet. Die Prüfung der Gasbildung erfolgte durch Dunhamröhrchen.

Serologie: Als Antigen wurde eine Kultur von *Erwinia atroseptica* von der National Collection of Plant Pathogenic Bacteria - England (Nr. 549) bezogen. Nach der 24 stündigen Bebrütung der Kultur auf NA wurden die Bakterien mit physiologischem Serum gewaschen und eine Konzentration von ca 10⁹ Zellen/ml hergestellt. Mit dieser Bakteriensuspension wurde ein Jahr alte Neuzeland Kaninchen nach wie Tabelle 1 injiziert.

Tabelle 1. Injektionsplan und Antigensmenge durch verwendete Injektion

Tage	Injektionsgestalt	Antigensmenge (ml)
0	subcutan	1.0
7	intravenös	0.2
10	intravenös	0.5
14	intravenös	1.0
17	intravenös	2.0
21	Blutentnahme	

ERGEBNISSE

Isolation und Pathogenitaet

Der Erreger der Schwarzbeinigkeit bildete auf Stewart-Naehrboden charakteristische kraterformige und rosafarbige Kolonien.

Mit dem gewonnenen Isolat (Bolu, 5a) wurden die Kartoffelstengeln inokuliert, um die Pathogenitaet des Isolats zu pruefen. Die Injektionspunkte zeigten nach 3-4 Tagen auffallende schwarzbraune oder dunklere bis rein schwarze Verfaerbung. Manche Pflanzen waren an diesen

Stengelpunkten abgebrochen (Abb. 1 und 2).

Von den kranken kartoffelpflanzen wurden Ruckisolationen durchgefuehrt, und mit diesen Ruckisolaten wurden die kartoffellstengel von *E. atroseptica* (Nr. 549, NCPPB) wieder infiziert. Diese Ruckisolaten zeigten die gleichen Krankheits Symptome wie die Originalkultur.

Physiologische Eigenschaften

Die Ergebnisse wurden im Tabellen 2 und 3 zusammengefasst.

Tabelle 2. Verschiedene Reaktionen bei anthesischen und eigenen Isolaten

Reaktion	<i>E. carotovora</i> (549 - NCPPB)	Eigenes Isolat (5a - Bolu)
Oxidase	—	—
Katalase	+	+
Wachstum auf der Kartoffelscheibe	+	+
Ausnutzung von Glucose	F	F
Sucrosetest	+	+
Pectinverfluessigungstet	+	+
H ₂ S - Bildung	—	—
Gluconatstest	—	—
+ : Reaktion positiv		
— : Reaktion negativ		
F : Fermentativ		

Tabelle 3. Säure- und Gasbildung aus Zuckern durch Original- Kultur und eigenes Isolat

Zuckern	<i>E. carotovora</i> (549 - NCPPB)	Eigenes Isolat (5a - Bolu)
Lactose	+	+
Glucose	+g	+g
Salicin	+	+
Trehalose	+	+
Maltose	+	+

+ : Reaktion positiv

g : Gasbildung

Serologie

Die Injektionen wurden wie nach Tabella 1 durchgeführt. Vier Tage nach letzter Injektion wurde von Ohrvenen des Kaninchens Blut entnommen und die Antikorkonzentrationen geprüft. Wenn Antiserumtiter mehr als 1/1600 war, wurde die Injektion nicht mehr fortgesetzt. Ganzes Blut des Kaninchens wurde entnommen und abzentrifugiert.

Mit den eigenen und originalen Isolaten wurde Objektträger-Agglutinationstest durchgeführt.

Die Antiseren reagierten bis zur 1/40 Verdünnung als positiv (Abb. 3).

Mit den Extrakten von kranken Pflanzen: Nach dem Waschen mit Leitungswasser wurden die Kartoffelpflanzenstengeln mit Filterpapier abgetrocknet. Die erkrankten Stellen der Pflanzen wurden durch Mill gepresst, und ein paar Tropfen davon wurde auf bis zu 1/5 verdünntes Antiserum gegeben und mit Impfföse gemischt. In kurzer Zeit wurde eine starke Agglutination beobachtet. Bei den mit gesundem Pflanzenextrakt durchgeführten Versuchen wurde keine Agglutination festgestellt.

DISKUSSION

Die aus dem Material von Bolu isolierte Bakterienkultur zeigte gleiche morphologische, biochemische

und serologische Eigenschaften wie die originale Kultur von *E. atroseptica* (Nr. 549 von NCPPB). Diese Er-

gebniisse bestaetigten die Arbeiten von Breed et al. (1957), Graham und Dowson (1960 a,b).

Die gewonnenen Anteseren könn-ten bis zu 1/40 verdünnt werden. Die beiden Kulturen reagierten serolo-gisch als positiv. Jedoch teilte Gra-ham (1963) mit, dass die Nassfaeule-bakterien als Antigene hinsichtlich der chemischen Zusammensetzung heterogen anzunehmen sind. Aus

diesen Grunden soll man neben dem serologischen Test auch die Pathoge-nitaetsteste durchführen.

Beim serologischen Test kann der Pflanzenextrakt verwendet wer-den. Im Rahmen der Krankheitsdiag-nose kann diese Methode als brauch-bar angesehen werden. Diese Ergeb-nisse können in Saatgutzuchzentrale und beim Quarantaeneinspektion eine praktische Verwendung finden.

ÖZET

PATATES DİP YANIKLIĞI ETMENİ (*Erwinia atroseptica* van Hall.)'NİN İZOLASYONU, BİYOKİMYASAL ÖZELLİKLERİ VE SEROLOJİSİ ÜZERİNDE ÇALIŞMALAR

Patates Dip Yanıklığı Etmeni *Erwinia atroseptica* van Hall., Bolu Bölgesi tohumluk patateslerinden selektif bir besiyeri ile izole edilmiş ve bakterinin morfolojik, biyokimyasal ve serolojik özellikleri araştırılmıştır. İzolasyonda selektif Stewart ortamı kullanılmış, etmen bu ortamda tipik krater şeklinde kolonilerini oluşturmuştur. Bolu izolatu (5a), orijinal *E. atroseptica* (No 549-NCPPB) izolatu ile aynı morfolojik, biyokim-

yasal ve serolojik davranışları göstermiştir. Keza patates bitkilerine yapılan yapay inokulasyonda da tipik yumuşak çürüklüğü oluşturmuştur.

Lâm agglutinasyon testinde Bolu izolatu, orijinal *E. atroseptica* kültürü ile beraber, 1/40'a kadar sulandırılmış antiserumla pozitif reaksiyon vermiştir. Aynı olumlu sonuç, bitki özsuyu ile yapılan agglutinasyon testinde de görülmüştür.

DISKUSION

und serologische Eigenschaften wie die originale Kultur von *E. atroseptica* (Nr. 549 von NCPPB). Diese Er-

Die aus dem Material von Bolu isolierte Bakterienkultur zeigte die morphologische, biochemische

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ERWINIA ATROSEPTICA



Abb. 1. Wurzelhalsfäule der Kartoffel durch natürliche Infektion (Bo'u, 1977).

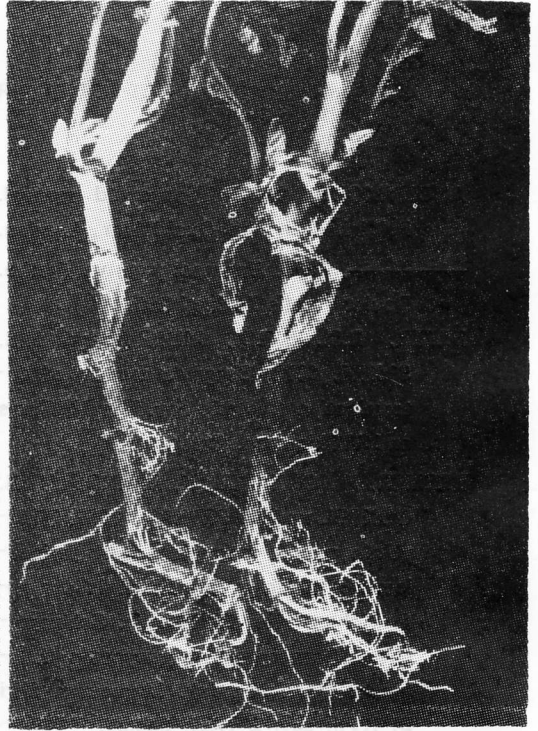


Abb. 2. Stengelfäule der Kartoffel durch künstliche Infektion

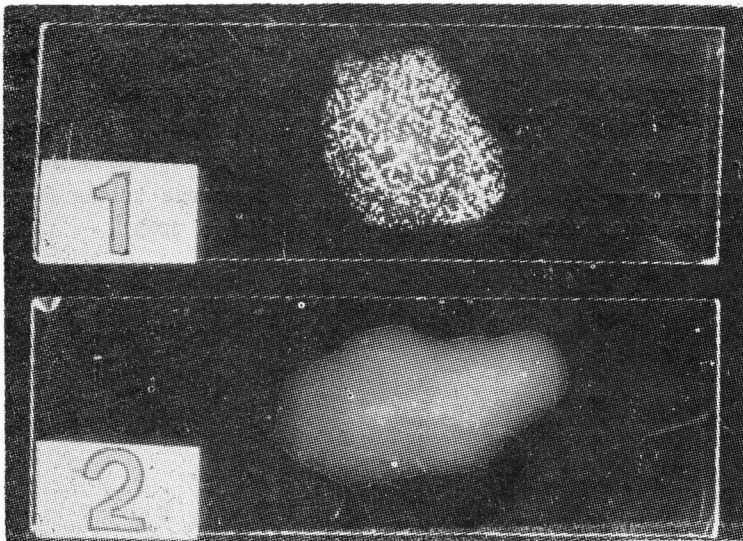


Abb. 3. Positivreaktion (1) beim Objektträger — Agg'utinationstests von Bo'u-Isolat und Kontroll (2).

veränderung die dem Pilz Nähr-
stoffe aus den Protoplasten liefern
würde, konnte in der aktiven Aus-
breitung des Pilzes bei der Inokulation
eine Rolle spielen. Inwieweit die enzy-
matische Aktivität durch die wirtspezifische
Inokulation mit *S. tritici* beeinflusst wird
und inwieweit die enzymatische Aktivität
des Pilzes durch die wirtspezifische Inokulation
beeinflusst wird, ist Gegenstand der vorliegenden
Untersuchungen. Danach ist es denkbar, dass sich
der Pilz den interzellularen Bereich der
Zellwände des Wirtspflanzen aneignet.

Über Enzymatische Aktivität Von *Septoria tritici* Rob. Ex Desm. In Bezug Auf Anfälligkeit Von Weizensorten

Von Ersin ONOĞUR

Institut für Phytopathologie und landwirtschaftliche Botanik,
Ege Universität, Bornova/İZMİR

form: Methanol (1:1) wurde das Se-
die Vorbehandlung der Testpflanzen
und zwar resistenten (*Caribo*), an-

ZUSAMMENFASSUNG

In den vorliegenden Untersuchungen wurde geprüft, ob die Reaktionen von Weizensorten unterschiedlicher Anfälligkeit gegenüber *Septoria tritici* auf der enzymatischen Fähigkeit des Pilzes beruhen, die Zellwände aufzuschließen.

Dabei wurde festgestellt, dass der Erreger in vitro zellwandabbauende (β -Glucosidase, α - und β -Galactosidase, β -Xylosidase) bildet, jedoch die Resistenz nicht auf einer sorten-bedingten Stimulation der Enzymproduktion zurückzuführen ist.

Die Enzymaktivität kann lediglich zur Unterscheidung in Wirt und Nicht-Wirt dienen.

EINLEITUNG

Es ist vermutet worden, dass die chemische Zusammensetzung der Zellwand über Resistenz und Anfälligkeit entscheiden kann. So berichteten Bateman et al. (1969), dass *Rhizoctonia solani* an isoliertem Zellwandmaterial anfälliger Bohnenpflanzen bezüglich Bildung polysac-

charidabbauender Enzyme aktiver war, so dass die Zellwände stärker degradiert wurden als die älterer und resistenterer Pflanzen.

Wie in eigenen Untersuchungen auch festgestellt werden konnte, wächst *S. tritici* fast ausschliesslich im Interzellularraum. Eine Permeabilitäts

veränderung, die dem Pilz Nährstoffe aus den Protoplasten liefern würde, konnte in der aktiven Ausbreitungsphase sowohl bei der anfälligen als auch bei der resistenten Sorte etwa bis zum 10. Tag nach der Inokulation nicht beobachtet werden. Danach ist es denkbar, dass sich der Pilz den interzellularen Bereich

als Nahrungsquelle erschliesst. Daher sollte geprüft werden, ob die Enzymaktivität durch die wirtsspezifische Zellwandzusammensetzung beeinflusst wird und inwieweit die enzymatisch aufgeschlossenen Zellwandstoffe vom Pilz genutzt werden können.

MATERIAL UND METHODEN

Isolierung des Zellwandmaterials

Die Vorbehandlung der Testpflanzen und zwar resistenten (Caribo), anfälligen (Florian) und Nichtwirt-Haferpflanzen (Tiger) und die anschließende Isolierung des Zellwandmaterials geschah nach den Vorschriften von Nevins et al. (1968).

Nach einer Dunkelperiode zur Reduzierung des Stärkegehaltes in den Zellen und damit zur Vermeidung der Stärkekontamination, wurden die 2. und 3. Blätter abgeschnitten und in Polyäthylenbeutel bis zur Verwendung bei -20°C aufbewahrt. Die Isolierung fand in einem Kühlraum bei 4°C statt. Das eingefrorene Material wurde in kleine Stücke geschnitten, mit 10 Vol. (v:w) PO_4 -Puffer (pH 7,0) in einem Waring-Blender homogenisiert und anschließend 2x mit dem gleichen Puffer und 1x mit aqua dest. bei 5000 U/min. für 15 min. zentrifugiert. Nach jeweils dreimaligem Waschen mit 10 Vol. (v:w) Aceton und 10 Vol. (v:w) Chloro-

form: Methanol (1:1) wurde das Sediment bei Zimmertemperatur getrocknet. In diesen Lösungsmitteln nicht gelöstes Material diente als einzige C-Quelle in den nachfolgenden Versuchen.

Kultivierung des Pilzes

Als Kultursubstrat befanden sich in 300 ml Kolben je 100 mg Zellwandmaterial, 0,2 g NH_4NO_3 , 0,05 ml Spurenelementlösung und 50 ml aqua dest. Nach Sterilisation bei 115°C für 30 min. wurden die Kolben mit 0,25 ml einer auf 30×10^6 Konidien/ml eingestellten Konidien suspension beimpft und als Schüttelkultur (40 Hüben/min.) bei 20°C mit 15 Stunden Lichtperiode am Tag aufbewahrt. Eine Bakterienkontamination wurde auf Nutrient-Broth-Agar bei 30°C stichprobenweise geprüft.

Bestimmung der Enzymaktivitäten

Die β -Glucoseaktivität wurde in Abhängigkeit von der Zeit (4, 6, 8, 10, 12

14, 18, 22 und 30 Tage nach der Impfung) und die Aktivitäten der übrigen Enzyme 22 Tage nach der Impfung in Anlehnung an Lüning (1975) bestimmt. Hierbei wurde 0,5 ml Kulturflüssigkeit mit 1,5 ml 1/10 verdünntem Na_2HPO_4 /Citronensäure-Puffer (McIlvaine, pH 6.0) in einem Reagenzglas gemischt und in einem Wasserbad bei 35°C für 5 min. equilibriert. Anschliessend wurde 0.5 ml entsprechende Substratlösung (α - und β -Formen des p-Nitrophenyl-Glucopyranosides und des p-Nitrophenyl-Galactopyranosides, jeweils 15 mg/5 ml aq. dest., und p-Nitrophenyl- β -Xylopyranosid, 13,5 mg/5 ml aq. dest): einpipettiert und nach Schütteln im Wasserbad für weitere 60 min. inkubiert. Durch Zugabe von 2,5 ml NaOH-Glycin-Puffer (pH 10) wurde der Reaktionsablauf gestoppt. In diesem alkalischen Bereich trat das durch die katalytische Wirkung von Enzymen abgespaltene p-Nitrophenol in Form des gelben p-Nitrophenolats auf. Die Farbintensität wurde nach gründlichem Schütteln

β -Glucosidaseaktivität

Nach 8-tägiger Anlaufzeit stieg die β -Glucosidaseaktivität plötzlich in den Kulturen mit Zellwandmaterial beider Sorten stark an (Abb.1). Diese Aktivitätserhöhung war jedoch stärker an 'Florian'-Zellwandmaterial.

und Filtrieren im Spektralphotometer bei 430 nm bestimmt. Eine Eichkurve diente zur Ermittlung der freigesetzten Menge an p-Nitrophenol in μmol durch 0,5 ml Kulturflüssigkeit in 1 Stunde bei 35°C als Mass für die Enzymaktivitäten des Pilzes.

Bestimmung des Wachstums in Kulturen

Der quantitative Vergleich der Pilzmasse in Schüttelkulturen mit isoliertem Zellwandmaterial wurde durch Ermittlung des Chitingehaltes des Myzels mit Hilfe der Glucosamin-Bestimmung durchgeführt (Ride und Drysdale, 1972). Hierzu wurde der Kolbeninhalt dreimal mit Wasser gewaschen und im Ultra-Turrax mit aqua dest. homogenisiert und auf 10 ml eingengt. Die weitere Behandlung der Probe erfolgte nach der Vorschrift der Autoren. Die entstehende blaue Färbung wurde im Spektralphotometer bei 650 nm gemessen und anhand einer Eichkurve der Glucosamin-Gehalt ermittelt.

ERGEBNISSE

Mit der Alterung trat dann in Kulturen eine Aktivitätsverminderung auf.

An Haferzellwandmaterial wies der Pilz eine erheblich geringere β -Glucosidaseaktivität auf als die am Zellwandmaterial seiner Wirte.

SEPTORIA TRITICI

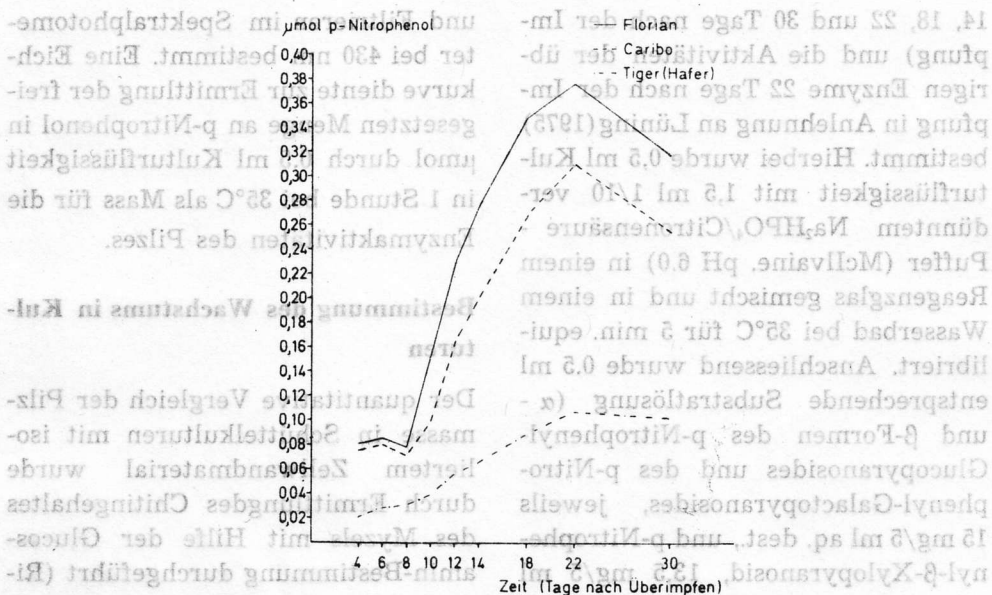


Abb. 1.: β -Glucosidaseaktivität von *S. tritici* auf Zellwandmaterial der Testsorten 'Caribo', 'Florian' und 'Tiger' (Hafer) in Abhängigkeit von der Zeit. Enzymaktivität ermittelt an umgesetzten $\mu\text{mol p-Nitrophenol/Std.}$

Die Übrigen Enzyme

Die Aktivitäten der übrigen Enzyme wurden am 22. Tag nach dem Überimpfen in 'Caribo' - und 'Florian' - Zellwandkulturen bestimmt (Tab.1). Eine β -Glucosidaseaktivität wurde nicht festgestellt. Die α - und β -Glucosidase - aktivitäten waren niedrig. Eine hohe Aktivität wurde für die β -Xylosidase gefunden, sie war aber etwa 61 % niedriger als die β -Glucosidaseaktivität.

Wachstum in Kulturen

Nach Feststellung der sortenspezifischen Induktion vor allem von β -Glu-

cosidaseaktivität an 'Florian' - Zellwandmaterial stellte sich die Frage, ob der Pilz parallel zu der erhöhten Aktivität auch besser gewachsen war. Dabei stellte sich heraus, dass *S. tritici* auf dem Zellwandmaterial beider Sorten gleich gut wuchs, dagegen schlecht auf Haferzellwandmaterial (Tab. 2).

Die geringere β -Glucosidaseaktivität auf 'Caribo' - Zellwandmaterial hat also keinen Einfluss auf das Wachstum. Das schlechte Wachstum auf Haferzellwandmaterial könnte auf der niedrigen β -Glucosidaseaktivität auf diesem Substrat beruhen.

Tab. 1: Aktivitäten von α -Glucosidase, α - und β -Galactosidase und β -Xylosidase von *S. tritici* in Kulturen mit Zellwandmaterial von 'Caribo' und 'Florian'. Enzymaktivität ermittelt an umgesetzten $\mu\text{mol p-Nitrophenol}/0,5 \text{ ml Kulturflüssigkeit}/\text{Stunde}$ ($n=5$)

Enzyme	Caribo	Florian
α -Glucosidase	—	—
α -Galactosidase	0,070	0,078
β -Galactosidase	0,025	0,031
β -Xylosidase	0,200	0,220

Tab. 2: Quantitative Bestimmung der Pilzmasse von *S. tritici* in Kulturen mit Zellwandmaterial der Testsorten 'Caribo', 'Florian' und 'Tiger' (Hafer) in $\mu\text{g Glucosamin}/1,5 \text{ ml Probe}$ aus 5:1 eingengtem Kolbeninhalt ($n=5$)

	Caribo	Florian	Tiger
Glucosamingehalt	23,12	22,66	4,32

DISKUSSION

Die erfolgreiche Besiedlung des pflanzlichen Gewebes hängt auch von der enzymatischen Fähigkeit von Erregern ab. Ausgehend von dieser Tatsache wurde untersucht, ob die Sortenunterschiede bei der Septoria-Erkrankung durch enzymatische Aufschliessung des Zellwandmaterials zu erklären waren.

Hydrolytische Enzyme scheinen bei der Septoria-Erkrankung eine Rolle zu spielen. Ein Nachweis dafür kann im Wachstum des Pilzes auf isoliertem Zellwandmaterial gesehen werden und beweist die Fähigkeit von *S. tritici*, Zellwandstoffe zur Energiegewinnung aufzuschliessen. Auf Zellwandmaterial beider Sorten scheidet

er α - und β -Galactosidase, β -Glucosidase und β -Xylosidase aus, Enzyme, die ihre Substrate zu den entsprechenden Monosacchariden spalten.

Die β -Glucosidase- und β -Xylosidase-Aktivitäten waren an dem Zellwandmaterial von 'Florian' höher als an dem von 'Caribo'. Zöge man die Fähigkeit des Pilzes, Zellwandmaterial energetisch vollständig aufzuschliessen, als Resistenzkriterium in Betracht, so liessen die vorliegenden Ergebnisse eher den Schluss zu, dass die Sorte 'Caribo' für *S. tritici* eine geeignetere Wirtspflanze wäre. Auf Zellwandmaterial dieser Sorte wächst der Pilz bei geringerem en-

zymatischen 'Aufwand' ebenso gut, wie auf dem Material der Sorte 'Florian', wie durch die Bestimmung des Glucosamin-Gehaltes nachgewiesen wurde. Auf Zellwandmaterial von Hafer, einem Nichtwirt, jedoch zeigt *S. tritici* weder entsprechende Enzymaktivität noch Wachstum. Eine Unterscheidung in Wirt und Nichtwirt wäre also mit diesem Kriterium möglich. Albersheim und Anderson-Prouty (1975) stellten fest, dass eine sortenspezifische Induktion zellwandabbauender Enzyme keine Differenzierung in anfällige und resistente Sorte ermöglicht. Diese Feststellung wird durch die vorliegenden Ergebnisse gestützt.

ÖZET

Septoria tritici Rob. ex Desm. YE KARŞI BUĞDAY ÇEŞİTLERİNİN DUYARLILIĞI İLE ETMENİN ENZİM AKTİVİTESİ ARASINDAKİ İLİŞKİLER ÜZERİNDE ARAŞTIRMALAR

Bitki dokusunda bir fungal patojenin başarılı bir biçimde yayılması diğer faktörler yanında, onun polisakkarit yapısında olan hücre elementlerini hidrolitik enzimleri yardımıyla monosakkaritlere kadar indirgeyip gerekli enerjiyi temin edebilme yeteneğine de bağlıdır. Konukçu hücre çeperinin kimyasal bileşimi söz konusu enzimlerin induksiyonunda anahtar rolü oynamakta ve bu dayanıklılık veya duyarlılığı saptayan bir faktör olabilmektedir.

Bu noktadan hareket edilerek ye leke oluşumuna kadar konukçu parankima dokusunda önemli bir permeabilite değişikliği olmaması gözlemine dayanılarak, in vitro koşullarda izole edilmiş hücre çeperi materyeli üzerinde *Septoria tritici*'nin α - ve β -Glukosidase, α - ve β -Galaktosidase ve β -Xylosidase aktiviteleri araştırıldı.

Dayanıklı ve duyarlı buğday çeşitlerine ait bitkilerin hücre çeperi materyeli üzerinde etmen farklı bir

E. ONOĞUR

β -Glukosidase aktivitesi, ancak eşit bir gelişme gösterdi. Diğer enzim aktivitelerinde önemli bir fark saptanmadı. Etmene doğal konukçu olmayan yulaf bitkisine ait materyal üzerinde düşük bir β -Glukosidase aktivitesi ve oldukça az bir gelişme gözlemlendi.

Buradan söz konusu etmene karşı buğday çeşitlerinin gösterdiği re-

aksiyon ayrıcalığının konukçu hücre çeperinin kimyasal bileşimine ve dolayısı ile enzim induksiyonuna dayandırılmıyacağı kanısına varıldı. Ancak farklı genus'tan bitkiler arasındaki reaksiyon ayrıcalığının polisakaritleri parçalayan enzimlerin induksiyonu ile ilgili olabileceği sonucuna varıldı.

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Investigation on *Gymnosporangium* spp. in Eastern and Southeastern Areas of Turkey

Necmettin DİNÇ¹ and M. Asil YILMAZ²

ABSTRACT

Occurrence of different species of *Gymnosporangium* on Rosaceae and their distribution have been studied in eastern and southeastern areas of Turkey. Four species, *G. confusum* Plowr. *G. tremelloides* Hart, *G. fuscum* DC. and *G. clavariaeforme* (Jack) DC. have been identified on Rosaceae at different localities.

INTRODUCTION

In this study, it was proposed to find out the different species of *Gymnosporangium* attacking Rosaceae in eastern and southeastern areas of Turkey. According to the biological observations made in this study, the disease causes damage in four different ways:

1. Fruit droppings due to fruit infection.
2. Unsatisfactory quality due to infected and deformed fruits.
3. Smaller fruit sizes due to premature defoliation.
4. Reduced future productivity due to weakened trees.

1) Regional Plant Protection Research Institute, Adana—TURKEY

2) Dep. of Plant Protection, Agricultural Faculty, University of Çukurova, Adana—TURKEY

GYMNOSPORANGIUM SPECIES

MATERIALS AND METHODS

Rosaceae plants, *Pyrus malus* L., *P. elaeagnifolia* Pall., *Cydonia vulgaris* L., and *Crataegus* spp., were carefully examined in Adana, Hatay, Maraş, Malatya, Elazığ and Tunceli provinces. Special attention was given to the areas where apple and pear growing had economic importance. Cedar trees (*Juniperus excelsa* Bieb. *J. oxycedrus* L., *J. foeditissima* Wild.) near by the apple and pear plantations were also examined. Above

mentioned areas were visited in winter, spring and summer for biological observations and findings were recorded.

Characteristics of spermagonium basidium spermatium and aeciospores were used for taxonomic studies and identifications of different species were done according to the earlier studies especially to Bernaux (1956).

RESULTS AND DISCUSSION

Gymnosporangium tremelloides Hart: This species was found very widespread and destructive on apple in Çokak, Çığışar, Afşin, Arifiye, Elbistan, Gökşun and Zorkun areas. In Çokak, 40 percent of the apple trees were infected since apple orchards are located in cedar woods, this disease is more destructive in Çığışar. The number of lesions found on leaves varied from 1 to 100.

Sori on *J. communis* L. are large brown and tremelloid shaped and are shorter and wider than the sori of *G. fuscum* D.C.

Gymnosporangium fuscum D.C.: This is the most frequently encountered species in Maraş. It is also found in Malatya, Elazığ, Tunceli and Erzincan. It mainly infects *Pyrus communis* L. and *P. elaeagnifolia* in Rosaceae. It causes premature defo-

liation and reduced fruit quality on pears. Generally, damage to pear is between 4.26 to 100 percent.

Telial sori which contain several thousands of teliospores develop at 20°T and are dark brown in color. Basidiospores are formed only after they have been subjected to rain at least for half an hour. *J. oxycedrus* *J. excelsa* Bieb. have been found as telial host of this pathogen (Dinç, 1974). Dark and light colored teliospores are almost same to each other.

Gymnosporangium confusum Plowr.: This pathogen is quince rust, attacks *Cydonia vulgaris* L. It has been also reported from *Crataegus monogyna* Jack. and *Mespilus germanica* L. (Karaca, 1965) and *Malus* sp. (Anderson, 1956). The damage of *G. confusum* Plowr. is restricted to the small local quince orchards in

Tanır, Elazığ, Şüşnaz, Harput and Tunceli.

Although this pathogen may infect the fruit and fruit stalk, the main damage is brought chiefly by leaf infection. Infection especially along the midrib and on leaf stem caused leaf-curling. The deformed leaves on the plants are the result of curling and galls formed on leaves. The long aecial horn appear on fruits. Formation of pseudoperidia takes place by mid July and reaches maturity earlier than that of pear rust. First infection has been observed on september. 1st in Şüşnaz.

G. confusum Plowr. is very similar to **G. clavariaeforme** (Jack) D.C. but the cells of pseudoperidial paraphysis are shorter in **G. confusum** Plowr. at aecial stage.

Sori on cedrus are reddish, brown and larger. Darker teliospores are

shorter than lighter ones.

Gymnosporangium clavariaeforme (Jack) D.C.=(**G. gracile** Pat): This pathogen has been reported on **Cerataegus monogyna** Jack. **C. orientalis** Pall. in Turkey (Karaca, 1965). It has been found on pears in Gölbaşı village of Doğanşehir in this study. It has been found on **Crataegus** spp. in Bahçe, Adana, Tellidere, İskenderun, Arapgir and Osmanpaşa, Malatya. Aecial sori are yellowish, and pseudoperidia first appear by mid June.

Orange yellow telial sori contain both dark and light colored teliospores. The shape of aecia cups are very similar to that of **G. confusum** Plowr. But can be differentiated by the typical shape of pseudoperidia under the microscope. Pseudoperidia have longer paraphysis and protruding cels on it are roundish.

ÖZET

DOĞU VE GÜNEYDOĞU ANADOLU'DA GYMNOSPORANGİUMLAR ÜZERİNDE BİR ÇALIŞMA

Doğu ve Güneydoğu Anadolu'da yapılan bu çalışmada Rosaceae üzerinde tesbit edilen Gymnosporangium türleri ve yayılışları kısa olarak incelenmiştir. **G. tremelloides** Hart.: Kahramanmaraş (Çokak, Çığışar, Afşin, Arifiye, Elbistan, Göksun) Hatay (Zorkun) bölgelerinde; **G. fusum** D.C.: Kahramanmaraş, Malatya Elazığ, Tunceli, Erzincan'da; **G. confusum** Plowr. Kahramanmaraş (Ta-

nır köyünde), Elazığ (Şüşnaz, Harput) ve Tunceli'de; **G. clavariaeforme** (Jack) D.C. ise Malatya Doğanşehir ve ayrıca Arapgirin Osmanpaşa mıntkasında), Hatay (İskenderun Tellidere köyünde), Adana (Bahçe)'de tesbit edilmişlerdir. Ayrıca **G. clavariaeforme** (Jack) D.C. Malatya'nın Doğanşehir ilçesi Gölbaşı köyünde armutlarda da görülmüştür.

GYMNOSPORANGIUM SPECIES

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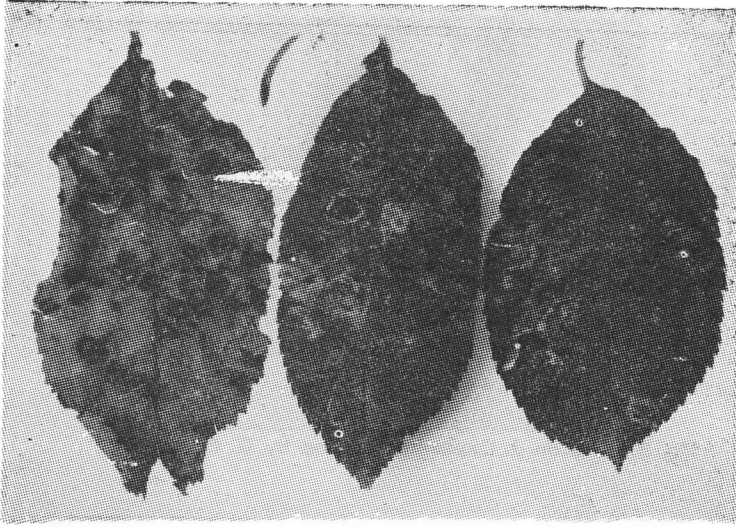


Fig. 1. Damage caused by *G. tremelloides* Hart. on apple leaves

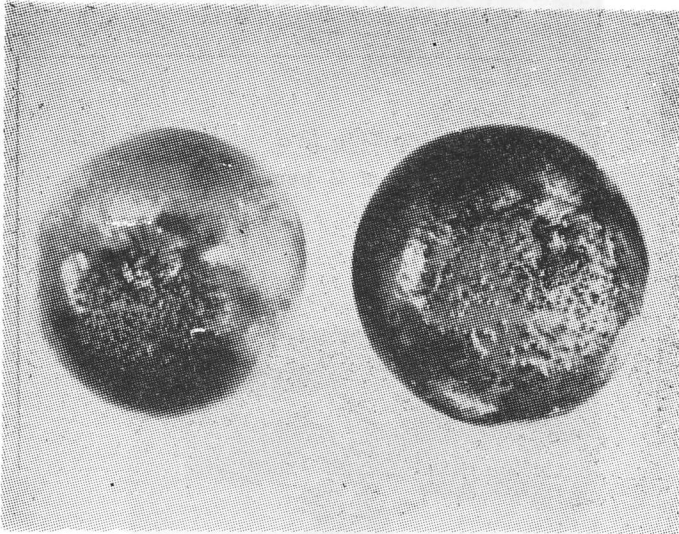


Fig. 2. Infected apple fruit by *G. tremelloides* Hart.

Fig. 3. Ascus cups of *G. clavosporium* (Jack) D.C. on *G. monogyna* Jack.

GYMNOSPORANGIUM SPECIES



Fig. 3. Galls on *J. Oxycedrus* caused by *G. fuscum* D.C.

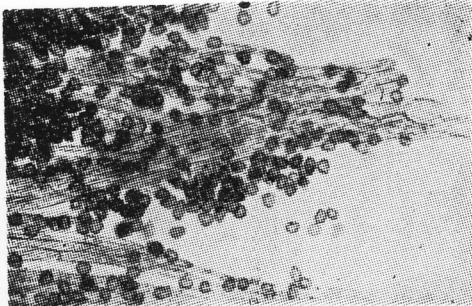


Fig. 4. Aeciospores of *G. tremuloides* Hart.



Fig. 5. Aecia cups of *G. clavariaeforme* (Jack) D.C. on *C. monogyna* Jack.

Investigations on the Effects of Some Herbicides on the Growth and Virulence of *Rhizoctonia solani* and *Trichoderma viride*

Emel SEZGIN

Regional Plant Protection Research Institute, Bornova, Izmir, TURKEY.

ABSTRACT

Growth of the *R. solani* in the presence of herbicides (trifluralin, EPTC and aretit) was different than control, but there was no significant difference in the growth of *T. viride* between the control and herbicide treatments. The virulence of *R. solani* increased by herbicides. In sterilized soil, *R. solani* grown on PDA and PDA + herbicides caused severe injury on the cotton seedlings, but the pre-emergence damping-off was generally higher than control.

INTRODUCTION

Herbicides have a significant effect on various plant diseases. Herbicides may change the incidence of plant diseases through the effect they have on the pathogen, the host, or microorganisms in the environment.

Certain herbicides decrease some diseases; some of these have also been reported to increase the incidence of certain other diseases. The

mechanism of increase and the incidence of disease may be due to direct stimulatory effect on the pathogen, increased virulence of the pathogen, increased susceptibility of the host, and suppression of microorganisms antagonistic to the pathogen.

Many of the studies on the interaction of herbicides and soil microorganism have been concerned with

herbicides persistence. Katan and Eshel (1973) in a recent review listed 20 pathogens which interacted with herbicides to increase plant diseases. The list includes air-borne diseases, vascular wilt diseases, and soil-borne diseases such as seedling damping-off caused by *Rhizoctonia solani* Kuehn.

R. solani is a common and important soil pathogen in Aegean region and it is responsible for damping-off diseases in seedlings of many crops. The possibility of interaction between herbicides applied to cotton and *R. solani* has been postulated by several workers (Kennedy et al 1959; Rodriguez-Kabana et al, 1966).

Pickard and Standifer (1966) have shown that seedlings growing in soil treated with trifluralin were more susceptible to damping-off. Ka-

tan and Eshel (1972) found that diphenamid increased damping-off on pepper caused by *R. solani*. Chandler and Santelmann (1968) reported that trifluralin and prometryne in combination with *R. solani* reduced the weight of cotton plants in growth chambers. Under field conditions, however, only trifluralin treatments significantly reduced the percent of surviving seedlings and inhibited the growth on cotton plants in *R. solani* infested soil. Neubauer (1973), reported increased growth of *R. solani* in trifluralin treated soil.

This study was carried out in order to determine the effects of trifluralin, EPTC and aretite on *R. solani* and *Trichoderma viride*. *T. viride* is parasitic and antagonistic on *R. solani* and several other fungi.

MATERIALS AND METHODS

Trifluralin (a, a, a. trifluore-2,6, dinitro N, N-di-propyl-p-toluidine), EPTC (s-Ethyl dipropylthiocarbamate) and aretite (Dinitro-alkylphenyl aasetat (Dinosebacetat) were used in test. The stock solutions of herbicides were prepared in 95 % ethyl alcohol, and quantities of this were incorporated into petri dishes containing 20 ml of a basal medium (PDA) to provide concentrations of 0, 25, 50, 75, 100 and 150 µg/ml.

The test fungi were planted in petri dishes containing PDA + Her-

bicides. The fungi planted dishes were incubated at 25°C for 7 days and diameters of the colony growth of the fungi was measured in millimeters during the incubation periods.

The influence of the herbicides on the antagonistic effect of *T. viride* was determined by planting *T. viride* and *R. solani* on PDA medium. Previously, *T. viride* was grown on PDA + Herbicides and *R. solani* on PDA medium only. The cultures were incubated at 25°C for 7 days.

Czapek's solution was used to

observe the effects of herbicides on virulence of *R. solani*. Each tube was contained 25 ml of czapek's solution. These tubes were inoculated with a mycelial disc of 5 mm diameter from a 7 day-old culture of *R. solani* grown on PDA + Herbicides. Then healthy cotton seedlings at 2-4 leaves stage were dipped in each tubes. The tubes were incubated at 25°C, for 7 days.

The experiments of the effects of herbicides on disease incidence were carried out in the pots. The pots were filled with sterilized soil. Into each pot 1/4 petri dishes of *R. solani* grown on PDA + Herbicides was incorporated. Five days after these application, 20 cotton seeds were sown in to each pot.

The number of the healthy seedlings was determined in 15 days after showing.

RESULTS AND DISCUSSION

In the experiments done in petri-dishes the growth of *R. solani* in the presence of aretit, the mycelial growth of *R. solani* was slow than the others. The highest concentration of aretit (150 µg/ml) was inhibited the mycelial growth of *R. solani* in culture medium. On the otherhand, aretit caused an important morphogenic change on hyphal growth of *R. solani*. The appearance of the colony was powdery and the hyphae of *R. solani* after branch much more profusely than in normal growth.

All of the concentrations of EPTC was stimulated the growth of the *R. solani* at the beginning whereas, this effect did not continue during the tests.

Trifluralin, also increased the growth of *R. solani* more than control except the 100 µg/ml dosage (Fi-

gure 1,2,3). The stimulation of the growth of *R. solani* by herbicides in culture has been reported in some studies. For example, Altman (1969) found that *R. solani* was stimulated by 25 herbicides in culture media.

The sclerotial production of *R. solani* in culture media was stimulated when the concentration of trifluralin and EPTC increased. Tang (1970) reported that trifluralin increased production and germination of chlamydo spores of *Fusarium oxysporum* f. sp. *vasinfectum* in soil. *R. solani* is also saprophyte in soil, and it survives in the soil with sclerotia during the unfavorable conditions. Therefore, the stimulatory effect of trifluralin and EPTC is very important from the view of the persistence of *R. solani* in soil.

The effect of herbicides on *T. viride* was not significant. The growth of *T. viride* on agar containing herbicides was not different than control. However, herbicides especially aretite caused important morphogenic changes on *T. viride*. Herbicides affected spore production and pigmentation of *T. viride*.

The experiments showed that herbicides did not influence the antagonistic effect of *T. viride* (Figure 4).

In our tests the virulence of *R. solani* grown on herbicides supplemented medium was increased. In test tubes *R. solani* caused lesions, but the root rot injury on the treated ones was more severe than the con-

trols and seedlings were died suddenly (Figure 6).

In sterilized soil, *R. solani* grown on PDA and PDA + Herbicides, caused severe injury on the cotton seedlings, but the pre - emergence damping-off was higher than control (Figure 8). The increase in disease incidence due to herbicides has been reported. Donald et al (1976) was found that the application of 3,4 and 4,5 Kg/ha of EPTC resulted in increasing root rot injury to navy bean grown in soil with a natural or artificial *Fusarium solani* infestation. Chandler and Santelmann (1968) found that under field conditions, trifluralin treatments significantly reduced the percent of surviving seedlings and inhibited cotton plant growth in *Rhizoctonia* infested soil.

ÖZET

BAZI HERBİSİDLERİN RHIZOCTONIA SOLANI İLE TRICHODERMA VIRIDE'NİN GELİŞME VE VİRÜLENSLERİNE ETKİLERİ ÜZERİNDE ARAŞTIRMALAR

Araştırma bölgemizde çok yaygın ve pekçok kültür bitkisinde çökerten hastalığına sebep olan *R. solani* ve onun önemli antagonistlerinden olan *T. viride*'ye Trifluralin, EPTAN ve ARETİT adlı herbisidlerin etkilerini araştırmak üzere yapılmıştır.

Adı geçen herbisidler mililitrede 0, 25, 50, 75, 100 ve 150 µg aktif madde olacak şekilde PDA besi ortamına

ilave edilmişler ve herbisid içeren bu ortamlara test fungusları ekilmişlerdir. Bir haftalık inkubasyon süresince her gün fungusların gelişme hızları, koloni çapları ölçülmek suretiyle izlenmiştir. Herbisidlerin fungusların virülenslerine olan etkileri czapek besi solüsyonu ihtiva eden tüplere herbisidli ortamlarda geliştirilen fungus parçacıkları vermek ve bu tüp-

lere 3. yaprak devresinde sağlam pamuk fideleri batırmak suretiyle araştırılmıştır. Ayrıca steril saksı ve topraklara da herbisidli ortamlarda geliştirilen *R. solani* bulaştırmak ve bu topraklara pamuk tohumu ekmek suretiyle de herbisidlerin hastalık çıkışı üzerindeki etkileri araştırılmıştır.

Deneme sonuçlarına göre her üç herbisid de *R. solani*'nin gelişme hızını ve morfolojik yapısını etkilemişler, virülensini arttırmışlardır. *T. v-ride*'nin gelişme hızı ve antagonistik etkisi üzerinde herbisidlerin herhangi bir etkisi olmamış ancak morfolojik yapısında değişikliğe sebep olmuşlardır.

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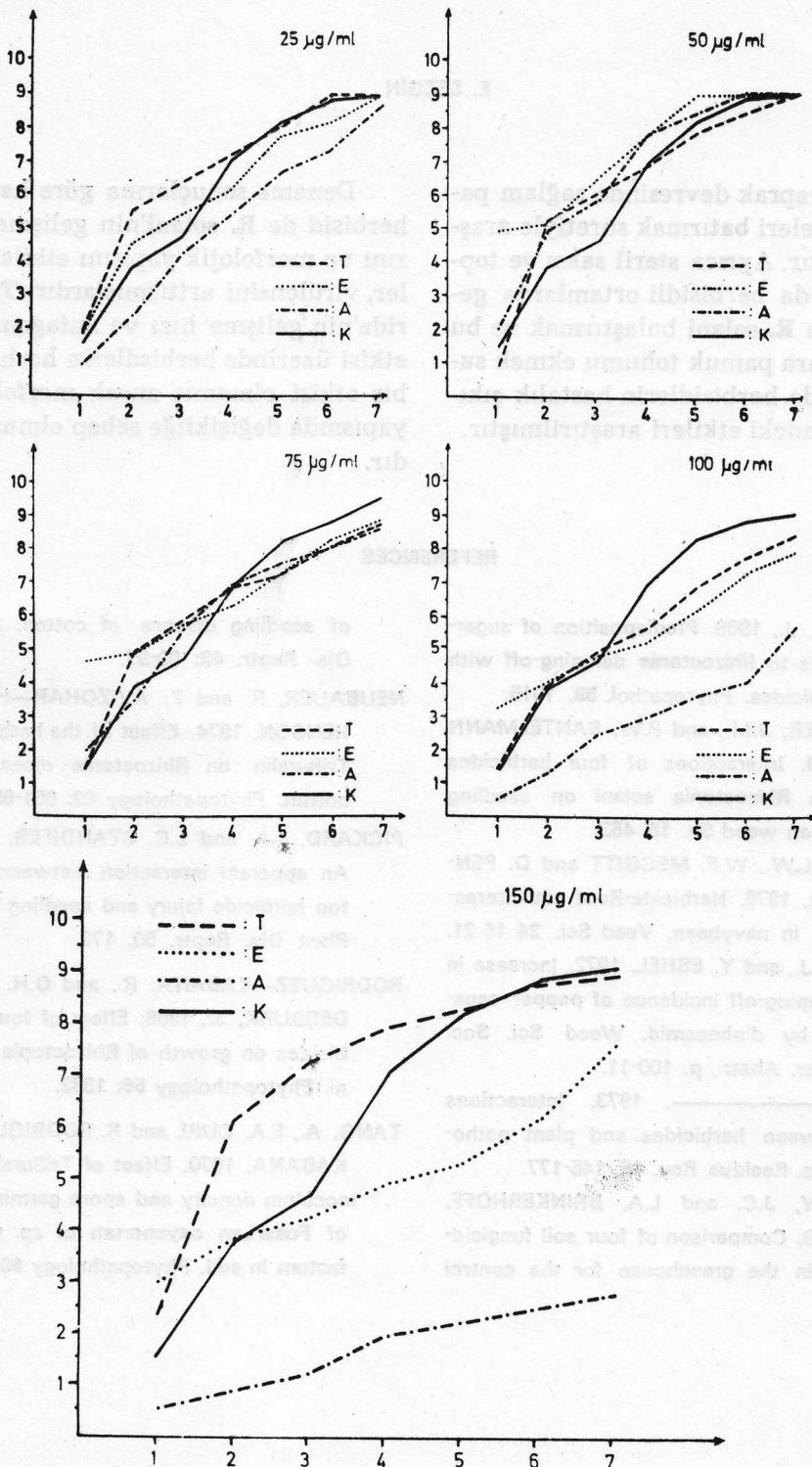


Figure 1, 2, and 3: Effect of the 25, 50, 75, 100 and 150 µg/ml dosages of herbicides on hyphal growth of *R. solani* Trifluralin (T), EPTC (E), Aretit (A), and Control (K).

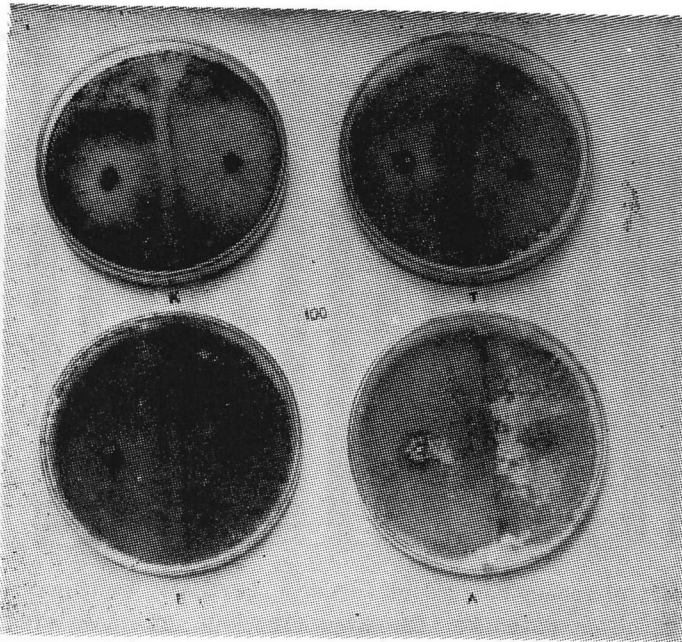


Figure 4: Effect of the 100 $\mu\text{g/ml}$ dosages of herbicides on antagonistic effect of *T. viride* on *R. solani* Control(K), Trifluralin(T), EPTC(E) and Aretit(A).

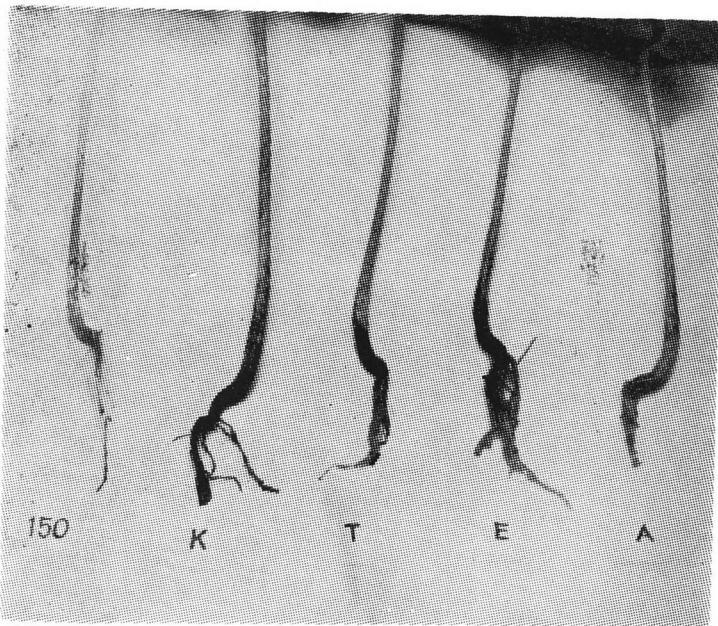


Figure 5: Effect of the 150 $\mu\text{g/ml}$ dosages of herbicides on virulence of *R. solani*. Control(K), Trifluralin(T), EPTC(E) and Aretit(A).

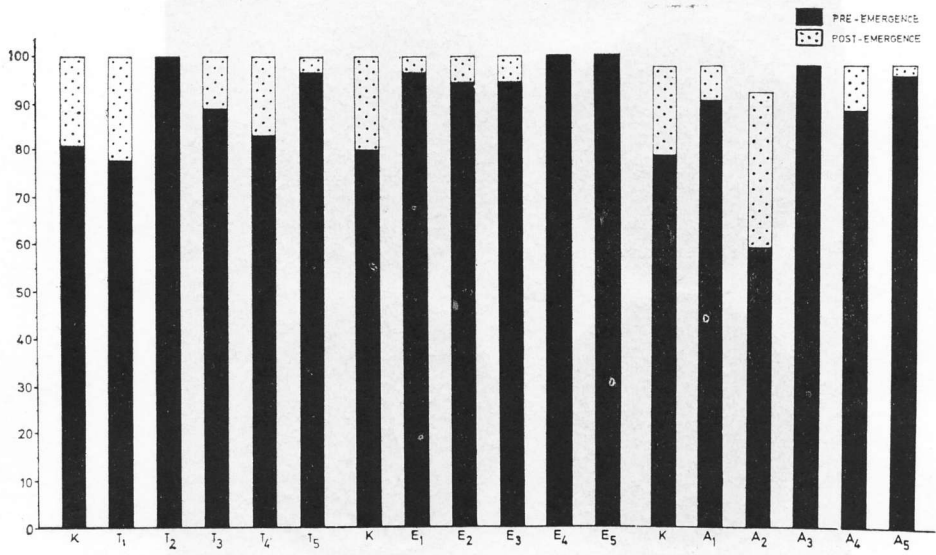


Figure 6: Effect of various dosages of Trifluralin, EPTC and Aretit on incidence of disease. 25 $\mu\text{g/ml}$ (T1,E1,A1), 50 $\mu\text{g/ml}$ (T2,E2,A2), 75 $\mu\text{g/ml}$ (T3,E3,A3), 100 $\mu\text{g/ml}$ (T4,E4,A4) and 150 $\mu\text{g/ml}$ (T5,E5,A5).



Figure 5: Effect of the 150 $\mu\text{g/ml}$ dosage of herbicides on virulence of R. solani. Control(K), Trifluralin(T), EPTC(E) and Aretit(A).

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All Correspondance Should Be Made To

TÜRKİYE FİTOPATOLOJİ DERNEĞİ

Ege Üniversitesi Ziraat Fakültesi

Bitki Koruma Bölümü

Bornova : İzmir,

TURKEY