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MATERIAL UND METHODEN

titütien im Kirschanbau für Labor-  
untersuchungen aufbewahrt. In 15  
tagigem Abstand konnten die ein-  
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den Blattproben entnommen und in Plas-

## Nachweis des Blattbraeunerregers an Sauerkirschen In Afyon

ERGEBNISSE

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### ZUSAMMENFASSUNG

Um den Blattbraeunerreger an Sauerkirschen zu bestimmen, wurden im Jahre 1977 in Afyon die Kirschanbauegebiete untersucht. In allen Anbauegebieten wurde beobachtet, dass die Blattbraeunerkrankheit weitverbreitet war und grossen Schaden verursacht hatte.

An den gesammelten Blattproben wurde als Krankheitserreger **Gnomonia erythrostoma** festgestellt, und die morphologischen Merkmale des Pilzes beschrieben.

### EINLEITUNG

Dem Saurkirschanbau in der Türkei kommt mit etwa 33.000 Tonnen Ertrag im Jahre eine nicht unbeachtliche Bedeutung im Obstbau zu, besonders aber im Anbauegebiet Afyon, wo jährlich 14 % des Gesamtertrages geerntet werden (3). Hier sind die Kirschanbaue nicht homogen verteilt, sondern konzentrieren sich auf viele Gebiete und liegen in den Haenden der Kleinbauern, deren einzigen Lebensunterhalt sie darstellen. Den Bauern und dem örtlichen Pflanzenschutzamt nach trat erstmals im

Jahre 1965 eine Krankheit auf, die an Kirschanbaue fröhe Blattbraeunungen verursachte. Die Krankheit breitete sich von Jahr zu Jahr über alle Anbauegebiete in Afyon aus und führte zu immer grösserem Ertragsverlust. Besonders gross war der Schaden im Jahre 1976, als die Krankheit epidemisch auftrat.

In der vorliegenden Arbeit wird über das Vorkommen des Blattbraeunerregers an Kirschanbaue, seine Bestimmung und den Krankheitsverlauf berichtet.

MATERIAL UND METHODEN

Um den Krankheitsverlauf zu beobachten, wurden in den Monaten Mai- August 1977 die Kirschanbaugelände in Afyon besucht. Dabei wurden von verschiedenen Gebieten Blattproben entnommen und in Plas-

tiktüten im Kühlschrank für Laboruntersuchungen aufbewahrt. In 15 taeigigem Abstand konnten die einzelnen Proben dann auf ihrem Pilzbefall hin untersucht werden.

ERGEBNISSE

Die in Afyon durchgeführten Untersuchungen zeigten, dass die Blattbraeunerkkrankheit in allen Anbaugeländen weitverbreitet vorkam und grosse Schaden verursacht hatte. Die Sauerkirschen waren von der Krankheit staerker befallen als die Süsskirschen.

gross. Die Pykniosporen waren fadenfoermig, selten gebogen, 14 µm lang und 0,8 µm dick (Abb. 4).

Spaeter begann auch im Innern des Blattes die Entwicklung der Perithezien (Abb 5).

3.1. Krankheitsbild

Die Krankheit wurde erst Ende Mai bemerkbar, Auf den Blaettern traten anfangs noch undeutliche helle Flecken auf, die sich im Juli gelb und spaeter braun verfaerbtten (Abb. 1).

Die Perithezien wurden langsam entwickelt und waren erst im Januar 1978 reif. Sie waren 160 X 140 µm. gross und haben einen langen Hals (Abb. 6).

Die Aszi gelangen erst im April und Mai zur völligen Reife. Sie sind zylindrisch, 80 µm lang und 13 µm dick und haben an der Spitze eine natürliche Pore (Abb. 7).

Die erkrankten Blaetter rollten sich von den Raendern her zusammen und vertrockneten. Der grosste Teil der vertrockneten Blaetter bleibt an den Zweigen haengen. Im Frühjahr erschienen die neuen Blaetter zwischen den dueren Blaetter des letzten Jahres (Abb. 2).

Die Askosporen waren eliptisch, hyalin und 25 µm lang und 5 µm dick (Abb. 8).

3.2. Der Erreger

In dem abgestorbenen Blattgewebe erkannte man zahlreiche schwarze kugelige Pyknidien (Abb. 3).

Das Krankheitsbild und die oben beschriebenen morphologischen Merkmale von diesem auf dem Blaettern gewachsenen Pilz wurden mit denen in der Literatur verglichen und als Krankheitserreger *Gnomonia erythrostoma* (Pers) Auerw. erkannt (2, 15, 18).

Sie waren anfaenglich von der Epidermis bedeckt und 100 X 80 µm



## SCHLUSSBETRACHTUNG

**Gnomonia erythrostoma** trat erstmals im Jahre 1880 in Deutschland in der Umgebung von Hamburg epidemisch auf (5). Spaeter wurde darüber aus der Schweiz, (2,9), Frankreich (6, 8), Italien (5), Grossbritannien (21), Griechenland (7), Tschechoslowakei (1), und aus der UDSSR (4, 13) berichtet. Dies ist der erste Bericht über das Vorkommen des **Gnomonia erythrostoma** in der Türkei.

Aus den im Jahre 1977 in Afyon durchgeführten Beobachtungen geht hervor, dass die Krankheit nicht nur auf Sauerkirschen begrenzt ist. Auch die Süsskirschen werden von der Krankheit befallen. Obwohl die Bäume nebeneinander stehen, werden Süsskirschen weniger befallen als Sauerkirschen. Dagegen wird aus Deutschland und aus der Schweiz berichtet, dass die Krankheit fast ausschliesslich auf Süsskirschen begrenzt sei (2,11,15). Spaeter wurde aus der UDSSR Tschechoslowakei und Jugoslawien berichtet, dass die Aprikosen ebenfalls durch **G. erythrostoma** befallen werden (1, 4, 13, 16).

Obwohl die Krankheit im ganz Europa weit verbreitet ist, wurde ein

epidemisches Auftreten sehr selten beobachtet. **G. erythrostoma** tritt meist in den Tälern und besonders in regnerischen Jahren auf. Das heisst, um eine Epidemie auszulösen, braucht der Pilz höhere Luftfeuchtigkeit. Deswegen kommt es im Jahr 1976 in Afyon zu einer Epidemie.

Um der Ausbreitung der Krankheit vorzubeugen, ist das Absammeln der befallenen und an den Zweigen haengegebliebenen Blätter eine entscheidende Massnahme, weil sich der Pilzbefall nur auf die Blätter beschränkt (2, 15).

Nur muss darauf geachtet werden dass in allen Anbaugebieten diese Massnahme sorgfältig durchgeführt und die gesammelten Blätter vernichtet werden. Ausserdem werden auch verschiedene Mittel zur chemischen Bekämpfung empfohlen, die gute Ergebnisse bringen sollen: 2 % Bordobrühe, 1 % NDOC, 2 % Nitrofen, 0,4 % Zineb (2,13,21).

Unter türkischen Verhältnissen sollen weitere geeignete Bekämpfungsmassnahmen noch untersucht werden.

## ÖZET

## AFYON İLİ VIŞNELERİNDE YAPRAK KAHVERENGİLİĞİ HASTALIĞI ETMENİNİN SAPTANMASI

Afyon İli vişne alanlarında görülen Yaprak Kahverengileşmesi hasta-

lığının etmenini saptamak amacıyla 1977 yılında Bölgede incelemeler ya-

pılmıştır. Yapılan bu incelemelerde, hastalığın bütün bölgede yaygın ve zararlı olduğu görülmüştür. Toplanan hastalıklı bitki örneklerinin incelen-

mesi sonucu hastalık etmeninin **Gnomonia erythrostoma** olduğu anlaşılmış ve hastalığın belirtileri ve etmenin morfolojik özellikleri saptanmıştır.

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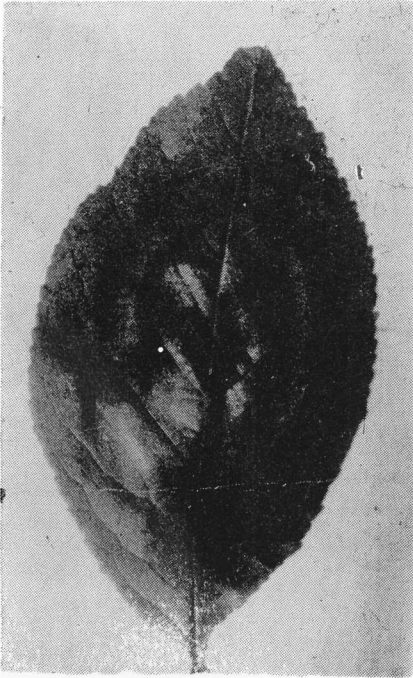


Abb. 1: Flecken auf den Blättern



Abb. 2: Die gerollten und vertrockneten  
Blätter an den Zweigen

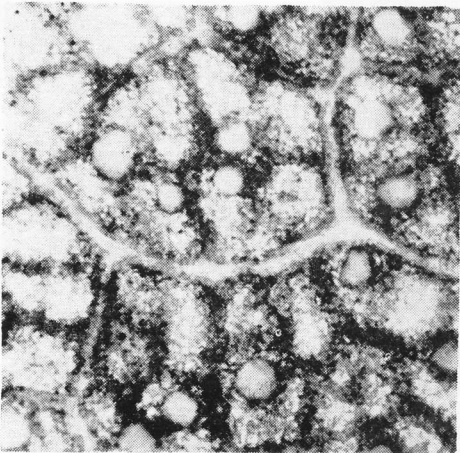


Abb. 3: Die Pycnidien in  
dem Blattgewebe 100 X

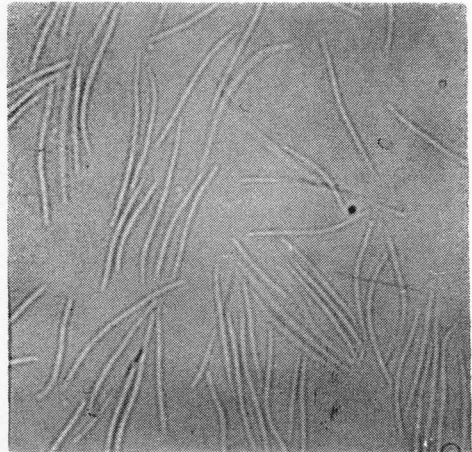


Abb. 4: Die Pycniosporen  
des Pilzes 2000 X



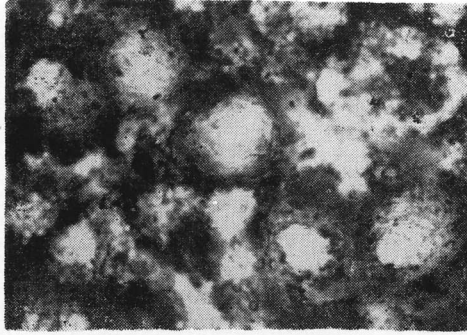


Abb. 5: Die Perithechien im  
Innern des Blattes 50 X

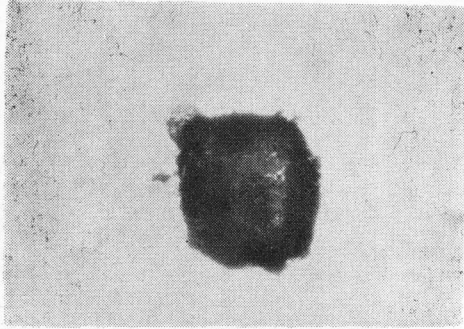


Abb. 6: Das Perithecium des Pilzes 80 X

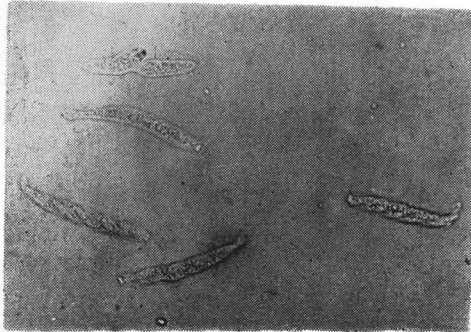


Abb. 7: Die Aszi des Pilzes 400 X

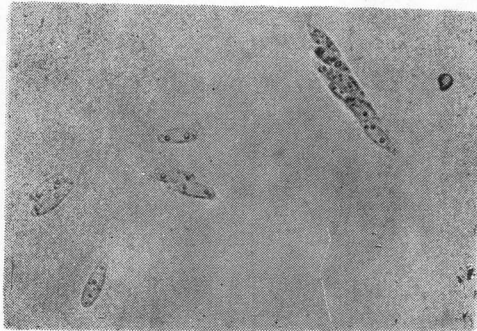


Abb. 8: Die Askosporen des Pilzes. 400 X



# Der bakterielle Maulbeerbrand verursacht durch *Pseudomonas mori* (Bayer et Lambert) Stevens

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## ZUSAMMENFASSUNG

Im Jahre 1976 wurde in Adana der Maulbeerbrand, verursacht durch *Pseudomonas mori*, auf Blättern und Zweigen der Maulbeerbäume festgestellt.

Aus erkrankten Blättern wurden Bakterienisolate gewonnen. Diese konnten im Vergleich mit authentischen Isolaten auf Grund der an künstlich infizierten Maulbeerbäumen, -blättern und -zweigen, hervorgerufenen Symptomen und ihrer physiologischen Eigenschaften eindeutig als *Pseudomonas mori* identifiziert werden.

## EINLEITUNG

Im Zeitraum zwischen Mai und Oktober-1976 wurden die Krankheitserscheinungen an den Blättern der Maulbeerbäume in Adana beobachtet, die den bakteriellen Symptomen ähneln. Die typischen Symptome dieser Krankheit sind durch die Abbildungen 1 und 2 wiedergegeben.

Auf den Blättern erschienen unregelmässig dunkelbraun gefärbte

nekrotische Flecke verschiedener Grösse (2-8 mm Durchmesser). Die Flecken waren von einem gelben Hof umgeben. Auf den Adern und Mittelrippen entstanden ebenfalls Flecken. Wie die Abbildungen 3 - 4 zeigen, können die Blätter durch die Wirkung dieser Krankheit missgestaltet sein. Bei dem fortgeschrittenen Krankheitsstadium trat Zerrei-

gen der Blattlamina auf, färbten sich schwer erkrankte Blätter gelb, die später abfielen. In der Türkei wurde der bakterielle Maulbeerbrand zum erstenmal von Bremer et al. (1947) beschrieben. Danach wurde von Türkoğlu und Öktem (1973) diese bakterielle Krankheit in Mittel-

Anatolien bestimmt.

In Mittelmeergebiet befinden sich die Maulbeerbaeume am Rand von Gemüsegärten und Strassen sowie in Hausgärten. Deswegen könnte man sagen, dass die Maulbeere keine wirtschaftliche Bedeutung in diesem Gebiet hat.

#### MATERIAL UND METHODEN

##### Isolationstechnik

Um die bakteriellen Erreger mikroskopisch zu untersuchen, wurden ein kleines Blattstückchen aus dem erkrankten Blatt heraus geschnitten, das neben gesundem Gewebe sowohl die Krankheitszone wie Teile des Blattfleckes enthielten. Bei der mikroskopischen Kontrolle der Präparate haben sich reichlich Bakterien gezeigt.

Um die pathogenen Bakterien aus den erkrankten Blattgeweben zu isolieren, wurde die folgende Arbeit durchgeführt:

a) Die Unter und Oberfläche der erkrankten Blätter wurde mit 70% iger Alkohol gereinigt. Aus dem Blatt wurden dreieckige Stückchen so ausgetrennt, dass die Spitze des Dreieckes im Zentrum eines Blattfleckes lag. Das Blattstückchen wurde mit einer abgeflamten Pinzette erfasst. Die Agarfläche wurde mit der Spitze des Dreieckes aufgestrichen.

Blattober- und -unterseite wurden zunächst gründlich mit 70% igem Alkohol gereinigt. Kleine befallene Stücke wurden ausgeschnitten und in einem Porzellanmörser, der mit 96% igem Alkohol gereinigt wurde, unter Zusatz von sterilem Wasser kräftig zerrieben. Aus diesem Extrakt wurde ein Tröpfchen mit Hilfe einer Impfpipette genommen und auf Agar aufgestrichen. Aus den nach diesen Methoden gewonnenen Kulturen wurden Reinkulturen nach dem Koch'schen Plattengussverfahren hergestellt.

##### Pathogenitätsprüfungen

Die Infektionsversuche wurden mit 20 Bakterienisolaten ausgeführt. Die Bakterienkulturen wurden auf Hefe-Extrakt-Pepton-Nährboden (Yeast-Extract 3 g, Peptone 10 g, NaCl 5, Agar 10, dest. Wasser 1000 ml, pH 7.0) kultiviert und waren zum Zeitpunkt der Infektion 3 Tage alt. Die Bakterien wurden vom Agar gespült und im Bausch-Lomb-Spektralphotometer die Extinktion bei 660

nm gemessen. Die Suspendierung wurde mit destilliertem Wasser soweit verdünnt, dass sich eine Extinktion von 0,06 ergab. Diese Suspendierung wurde mit Leitungswasser nochmal 1:10 verdünnt. Die zur Infektion verwendete Bakterienkonzentration betrug dann ca. 10<sup>8</sup> Zellen/ml.

Zum Versuch wurden belaubte Zweige von Maulbeeren und Blätter getopfter Tabakpflanzen benutzt. Die Blätter waren, soweit das bei solchen möglich ist, von gleicher Konsistenz und von gleichem Alter.

Für die Pathogenitätsprüfung wurden die Blätter und Zweige von Maulbeeren mit der Stecknadel ausgestrichen, anschliessend wurde die Bakteriensuspendierung auf die Blattunterseite und auf die Zweige mittels etwas Watte aufgebracht. Als Kontrolle wurde sterilisiertes Wasser verwendet. Danach wurden belaubten Zweige mit einer nassen Folie bedeckt und nach 48 Stunden wurden sie entfernt. Zum Nachweis der Infektiosität an Tabak wurde das von KLEMENT (1963) beschriebene Verfahren angewendet, bei dem die Infektion durch Injektion der Bakterien Suspendierungen (10<sup>8</sup> Zellen/ml) in die Interkostalfelder der Tabakblätter erfolgt.

#### Physiologische Eigenschaften

Um die Identifizierung des vorliegenden Erregers mit notwendiger Sicherheit durchführen zu können,

wurde eine Kultur von **Pseudomonas mori** von der National collection of plant pathogenic bacteria, plant pathology laboratory, Harpenden, England, bezogen und die kulturellen Eigenschaften beider Organismen verglichen.

Zur Kennzeichnung der verschiedenen Isolate wurde die Entwicklung und ihr Aussehen auf Hefextrakt-Pepton-Agar in Petrischalen bei 20°C nach 3-5 Tagen festgestellt.

Als weitere Merkmale wurden untersucht:

#### Fluoreszenz im Medium B (Nach KING et al. 1957)

Bei positiver Reaktion scheiden die Bakterien ein grünfluoreszierendes Pigment in das sie umgebende Medium.

#### Oxidasetest (Nach KOVACS, 1956)

Als Reagens wurde 1% iger wässriger Lösung von Tetramethylparaphenylendiamin-dihydrochlorid benutzt. Bei positiver Reaktion verfärbt sich der Ausstrich innerhalb von 5-10 sek nach KOVACS dunkel purpur.

#### Milchtest (Nach KÖSKER, 1971)

In 1000 ml Magermilch wurde 10 ml Bromkresol purpur (0,2% iger) zugesetzt. Bei einer alkalischen Reaktion bleibt die Farbe purpur.

**Gelatineverflüssigungstest**

(NIENHAUS, 1969)

Beimpfen der gelatinenährboden durch Stichkultur etwa auf 3 cm Tiefe. Prüfung auf Proteolyse erfolgt innerhalb von 7 Tagen täglich.

Nährboden am Einstich unverändert: negative, Nährboden trichförmig aufgelöst = positiv.

**Stärkehydrolysetest**

(NIENHAUS, 1969)

Sterile Petrischalen mit 15 ml Stärkeagar beschichten. Prüfung auf Stärkehydrolyse erfolgte nach 5 Tagen durch Überschichten der Platten mit Lugolscher Lösung.

**Katalasetest** (verändert nach MÜLLER und MELCHINGER, 1964)

Nährboden, bestehend aus 3 g Beef Extract, 5 g Pepton, 15 g Agar, 1000 ml dest. Wasser, wurde nach dem Beimpfen bei 25 °C bebrütet. Auf die Kolonien wurde 3% iger  $H_2O_2$  aufgetropft. Bei positiver Reaktion trat mehr oder weniger starke Blasenbildung durch freiwerdenden Sauerstoff auf.

**Kasainetest** (verändert nach JANKE und DICKSCHEIT, 1971)

Es wurde der gleiche Nährboden wie Katalasetest verwendet. Nur wurde 10% iger Magermilch zugesetzt.

Nachdem die Schalen gut bewachsen waren, wurden sie mit Testlö-

sung (15 g  $HgCl_2$ , 20 ml konz. HCl, 100 ml  $H_2O$ ) kurz übergossen. Bei positiver Reaktion erschienen aufgehellte Zonen neben den und um die Bakterienkolonien.

**Nitratreduktionstest** (NIENHAUS, 1969).

Nitratlösung (3 g Fleischextrakt, 5 g Pepton, 1 g Kaliumnitrat, 1000 ml dest. Wasser, pH 7,2) in Reagenzglasern wurde mit den zu prüfenden Bakterienkulturen beimpft und nach 24 stündigem Bebrüten täglich auf Nitritbildung geprüft.

Die Prüfung auf Nitritbildung erfolgte mit folgenden Lösungen:

A. 0,8 g Sulfanilsäure in 100 ml 5-n-Essigsäure gelöst.

B. In 20 ml kochendem dest. Wasser werden 0,5 g Naphthylamin gelöst, die Lösung vom Rückstand abgegossen und mit 5-n-Essigsäure auf 100 ml aufgefüllt.

Nach 2, 4, 6, und 10 Tagen etwa 1-2 ml von Lösung A und 1-2 ml von Lösung B jeweils zugeben.

Bei positiver Reaktion tritt sofort Rotfärbung auf.

**Indolbildung** (verändert nach STAPP, 1958)

Von dem Test-Nährboden nach Merck (5462) zur Nitrit- und Indolbestimmung wurde eine 1% ige Lösung hergestellt.

Die Prüfung auf Indolbildung erfolgte in, 2, 4, 6 und 10 Tagen. Im Falle einer positiven Reaktion tritt kirschrote Faerbung auf, wenn etwa 5 ml der beimpften Lösungen mit 5-10 Tropfen Indolreagens versetzt werden.

Zur Prüfung wurde Indolreagens nach EHRLICH verwendet:

#### **Ammoniakbildung** (Nach GARRETT et al. 19966)

Die Prüfung auf Ammoniakbildung erfolgte nach 7 Tagen. Im Falle einer positiven Reaktion tritt schwarze Faerbung auf. Zur Prüfung wurde Nesslerreagenz verwendet:

#### **H<sub>2</sub>S-Bildung** (Veraendert nach STAPP, 1958)

Von der Bouillon wurden jeweils 50 ml in 100 ml Erlenmeyer kolben gefüllt, sterilisiert und beimpft. Nach dem Beimpfen wurden alkalische Bleiacetatpapierstreifen so in die Kolben eingehaengt, dass der untere Rand sich etwa 1 cm über der Naehr-lösung befand.

Wird H<sub>2</sub>S gebildet, so faerbt sich der Bleiacetatpapierstreifen infolge Bleisulfidbildung je nach Intensitaet von unten nach oben denkelfarbraun bis schwarz.

#### **Voges - Proskauer - Reaktion** (Nach KÖŞKER, 1971)

Von den bebrüteten Kulturen wurden 100 ml mit 0,6 ml -Naphthol

(6 % ige alkoholische Lösung) und 0,2 ml 40 % KOH versetzt. Die Röhren wurden 10 Minute geschüttelt. Bei positiver Reaktion tritt rote Faerbung auf.

#### **Methylrot Reaktion** (Nach KÖŞKER, 1971)

Es wurde die gleiche Naehr-lösung wie Voges-Proskauer-Reaktion verwendet.

5 ml zu untersuchenden Kultur-flüssigkeit wurde mit 1 Tropfen Methylrot Lösung versetzt..

Saeurebildung führt zum Um-schlag des pH-Indikators Methylrot: rot (pH) gelb.

#### **Citrat Reaktion** (Nach KÖŞKER 1971)

Naehr-lösung, bestehend aus 5 g NaCl, 2,5 g MgSO<sub>4</sub> · 7H<sub>2</sub>O, 1 g NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub>, 1 g K<sub>2</sub>HPO<sub>4</sub>, 2 g Natrium-citrat, 1000 ml dest. Wasser, wurde durch Filterpapier filtriert und anschliessend 0,008 % Bromthymolblau und 2 % Agar zugesetzt.

Bei positiver Reaktion veraendert sich die grüne Farbe blau.

#### **Methylenblau Reaktion** (Veraendert nach KÖŞKER, 1971)

Naehr-lösung bestehend aus 5 g Glucose, 10 g Peptone, 0,004 g Methylenblau.

Im Falle einer positiven Reaktion hellt sich die blau Farbe von Naehr-



lösung auf. Im Gegenteil veraendert sich sie nicht.

**NaCl-Toleranz** (Nach ZEHR, 1971)

Difco Nutrient Broth enthielt 4 % NaCl.

Im Falle einer positiven Reaktion waechsen die Kulturen gut.

**Nutrient Broth + 5 % Saccarose** (Nach HEYNS, 1960)

Difco Nutrient Broth enthielt 5 % Saccarose.

Bei positiver Reaktion waechsen die Bakterien gut.

**Ausnutzung von Aethanol** (ABDOU, 1959)

pH-Wert von Aethanolmedium mittels NaOH auf 7,2 einstellen und im Autoklaven sterilisieren, jedem Röhrrchen 0,1 ml Aethonal zugeben.

Bei positiver Reaktion Waechst die Kultur.

**Wachstum in flüssigen Naehrs-substrakten** (nach STAPP, 1958)

Dunham's, Cohn's und Ashby's Lösungen wurden benutzt.

**Saeurebildung aus Zuckern** (nach DORNER, 1932).

Nach der Sterilisation wurde dem Naehrssubstrat die jeweilige Kohlenhydratverbindung in Form einer steril filtrierten Lösung zugesetzt. Es wurde jeweils 1 % ige Kohlenhydratlösung verwendet: Arabinose, Xylose, Glucose, Maltose, Saccharose, Lactose, Raffinose, Levulose.

Der zugesetzte Indikator Bromkresolpurpur, vaerendert sich violett bei niedrigerem pH-Wert wird er gelb.

## ERGEBNISSE

### Pathogenitaetsprüfungen

Die Ausgangskulturen waren 19 Bakterienisolate. Mit diesen Kulturen wurden zum ersten Mal die Tabakblaetter infiziert, um die pathogenen Isolate zu erkennen.

Von den injizierten 19 Bakterienisolaten hatten nur 6 Isolate auf Tabak positiv reagiert. Mit diesen positiv reagierten Kulturen wurden die Maulbeerblaetter und - Zweige infiziert. Die gebildeten Flecken erschienen wie in der Einleitung beschriebenen Symptomen.

Zur Erfüllung der Koch'schen Postulate wurden Rückisolationen von der infizierten Pflanzen durchgeführt. Mit den gewonnenen Isolaten wurden im Vergleich mit Ori-

nalkultur von **P. mori** Maulbeerblaetter infiziert.

Die verschiedenen Rückisolate zeigten die gleichen Krankheits-symptome wie die Originalkulturen. Im Virulenzgrad ergaben sich geringe Unterschiede.

### Physiologische Eigenschaften

Auf Hefeextrakt-Pepton-Agar bildeten die Isolate durchsichtige, weisse, runde, glatte, flache Kolonien. Die typischen Krankheitssymptome, wie sie auf dem Feld beobachtet worden waren, zeigten nur solche Isolate. Deswegen wurden für die weiteren Untersuchungen nur zwei typischen Isolate dieser Gruppe verwendet.

Die Ergebnisse wurden im Tabelle 1 und 2 zusammengefasst.

Tabelle 1. Verschiedene biochemische Reaktionen bei authentischen und eigenen Isolaten

	P. mori	Isolierte Kulturen
KING Medium B	+	+
KOVACS Oxidasetest	—	—
Milchtest	+	+
Gelatineverflüssigung	—	—
Stärkehydrolyse	—	—
Katalasetest	( )	( )
Kazeinetest	—	—
Nitratreduktion	—	—
Indolbildung	—	—
Ammoniakbildung	—	—
H <sub>2</sub> S bildung	( )	( )
VOGES—PROSKAUER Reaktion	—	—
Methylrot Reaktion	—	—
Citrat Reaktion	+	+
Methylenblau Reaktion	+	+
NaCl-Toleranz	( )	( )
NutrientBroth + 5 % Saccharose	+	+
Ausnutzung von Aethanol	—	—
COHN's Naehrlösung	—	—
DUNHAM's Naehrlösung	+	+
ASHBY's Naehrlösung	—	—

Tabelle 2. Saeurebildung aus Zuckern durch Orginal-Kultur und eigene Isolate

	P. mori	Eigene Isolate
Arabinose	+	+
Galactose	+	+
Glucose	+	+
Lactose	—	—
Levulose	+	+
Maltose	—	—
Raffinose	+	+
Saccharose	+	+
Xylose	+	+

+ : Reaktion positiv; ( ) : Reaktion fraglich; — : Reaktion negativ

**Diskussion**

Die von uns untersuchten Bakterienisolate konnten also auf Grund der an Maulbeerbaeumen hervorgerufenen Symptomen und der physiologischen Eigenschaften (Tab. 1 und 2) eindeutig als **Pseudomonas mori** identifiziert werden.

Die festgestellten physiologischen Eigenschaften unserer Isolate (Tab. 1) stimmten fast mit den für **P. mori** beschriebenen (BREED et al. 1960; STAPP, 1956, Türkoğlu und ÖKTEM 1973) überein. Aber bei unseren Isolaten waren Ammoniakbildung und Nitratreduktionsteste negativ. Diese Befunde stimmen mit denen von KLEMENT et.al., 1960 nicht überein. Die in unseren Testen zum Vergleich herangezogene Originalkultur von

**P.mori** waren ebenfalls negativ. Ausserdem haben wir eine schwache H<sub>2</sub>S-Bildung und ganz schwaches Wachstum in Nutrient-Broth + 4 % NaCl Naehrboden bei authentischen und eigenen Isolaten bestimmt. Während Breed et al. (1957). H<sub>2</sub>S-Bildung und NaCl - Toleranz bei **P. mori** negativ gefunden haben, müssen wir sie nach unseren Befunden fraglich bewerten. Ebenfalls wurde der Katalasetest bei unserer Untersuchung als fragliches Ergebnis bonitiert, weil sich einige Blasen gebildet haben. Dagegen wurde der Katalasetest von Türkoğlu und Öktem (1973) positiv gefunden. Zum Schluss können wir sagen, dass die Identifikation unserer Isolate als **P. mori** nicht gezweifelt werden.

**ÖZET****BAKTERİYEL DUT YANIKLIĞI (Pseudomonas mori**

“Bayer et Lambert Stevens”)

1976 yılında Adana'da **Pseudomonas mori** etmeninin neden olduğu dut yanıklığı hastalığı dutağacı yaprak ve dallarında saptandı. Hastalıklı dut yapraklarından izole edilen kültürler ve karşılaştırma kültürü olarak alınan orijinal kültürle yaprak

ve dallarda yapılan suni enfeksiyonlar sonucu oluşan semptomlar ve bunların fizyolojik özellikleri yönünden birbirlerine çok benzemesi nedeniyle, izolatlar **Pseudomonas mori** olarak tanılanmıştır.

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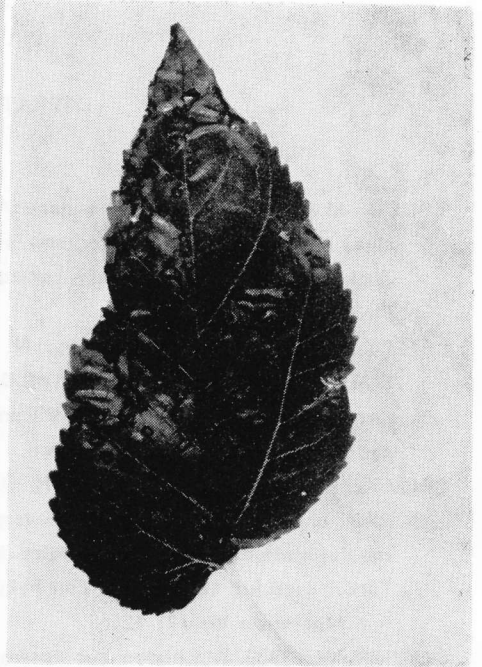


Abb. 1 und 2: Flecke auf den Blättern durch *P. mori* natürlich infiziert.

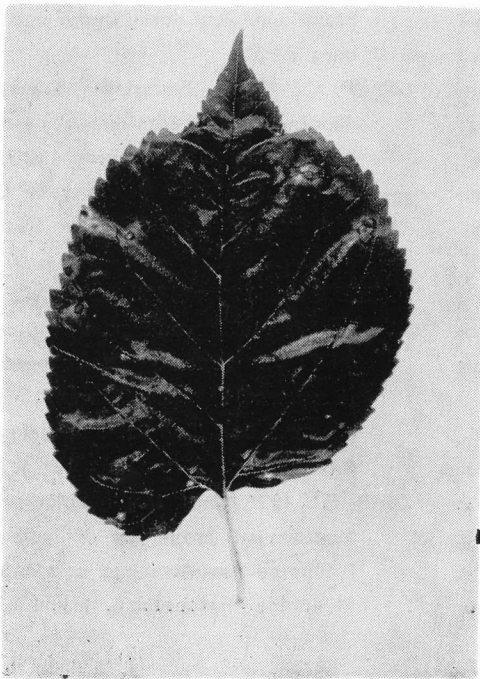


Abb. 3 und 4. Verkrümmungen auf Maulbeerblättern



## Potato Rot Nematode (*Ditylenchus destructor*) in Erzurum Province

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### INTRODUCTION

In Turkey no research has been conducted on the potato tuber rots caused by nematodes and even the distribution of these type of rots has not been determined. In 1976, decays on Hasankale and Erzurum originated potatoes were noticed in stores in a great scale. When the tuber samples were examined, potato rot nematode *Ditylenchus destructor* and another species from the same genus were found as the causal agents of these rots. In November 1977, *D. destructor* was encountered in Denizli on Afyonkarahisar originated potatoes delivered to market.

*D. destructor* had been accepted as a race of *Ditylenchus dipsaci* before Thorne (1945), described it as another species. This nematode is in-

festing most of the hosts of *D. dipsaci* so from the side of host preference feature, it's discrimination from the stem and bulb nematode is difficult. But an absence of a resistant period in the life cycle of *D. destructor* and it's sensitivity to drought differs it from *D. dipsaci*. Thorne (1961), suggests that it may over winter as eggs. At the same time it was put forward that this nematode survives in soil on fungal hosts and weeds. Hooper (1973), reported that this nematode is especially found in temperate regions and mainly localized in North America, many parts of Europe especially West and South Central U.S.S.R., Some Mediterranean countries, South Africa, Bangladesh and Hawaii.

## MATERIALS AND METHOD

Potato tuber samples were taken from the markets of Erzurum, the stores of potato growers in many provinces of Erzurum such as Sögütlü, Pasinler. The tuber samples were studied under stereo-microscope and the penetration and infestation ways

were tried to be determined. For obtaining the nematodes from the infested tubers, initially finely cuted tuber samples were placed in water for 24 hours and then passed through 30, 10, 200, 300 mesh screens.

## RESULTS

According to the surveys in 1976-77, during the harvest of potatoes in Erzurum provinces, the degree of infestation of *D. destructor* showed great variability. Sögütlü Village of Erzurum was found to be the most infested area, where % 20 of the yield were subjected to decay. In Erzurum under cool and moist store conditions, the nematodes are continue to increase their population and their spread causes the decay of healthy tubers nearby. Also the rotting of the tubers in the stores increase by the collective effect of fungal, bacterial diseases, acar (Acariidae) and saprophyte nematodes.

In stores the penetration of nematodes to healthy tubers were observed through lenticels and region of eyes. These results were also confirmed by other researchers.

When the skin of healthy seemed tubers were peeled tiny, white nematode colonies could be observed. During the storage period these colonies also increases and invades the healthy tissue, where the colour gra-

dually becomes darken. Consequently dark brown necrotic rots could be seen covering all the tuber. The distinct area existed between the healthy and decayed tissues is the place where the nematodes and eggs are found in abundance. This region to the inside appears white and granular. Rarely very few nematodes are present in the death tissues of the tubers. In a state of their presence in the death tissues it is assumed that the nematodes are feeding on fungus mycelia growing on the moist portions of death tissues. As the damage progresses the tissues dry and shrink and the skin becomes leathery and frequently cracks occur on. (Fig. 1).

Generally secondary invasions of microorganisms occur on nematode infested tubers. If the tubers are affected only by the nematode, the invaded areas showed slight depressed lesions and colour of the skin seems more darker. When this region opened a whitish, fibrous appearance could be seen. In this region the tissues are harder.

ÖZET

ERZURUM ÇEVRESİNDE PATATES YUMRU NEMATODU  
(*Ditylenchus destructor*)

Patates yumrularında zarar yapan patates yumru nematodu (*Ditylenchus destructor*) Erzurum çevresinde hasat zamanında tarlada 1976 yılında bulunmuştur. Patatesler depolarda muhafazaları esnasında bu nematodtan daha fazla zarar görmek-

tedir. Keza aynı nematoda 1977 yılında Denizli'de pazara arzedilen ve menşei Afyonkarahisar olduğu söylenen patates yumrularında da tesadüf edilmiştir. Hasat zamanında tarladaki zarar % 20 ye kadar yükselmektedir.

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DITYLENCHUS DESTRUCTOR

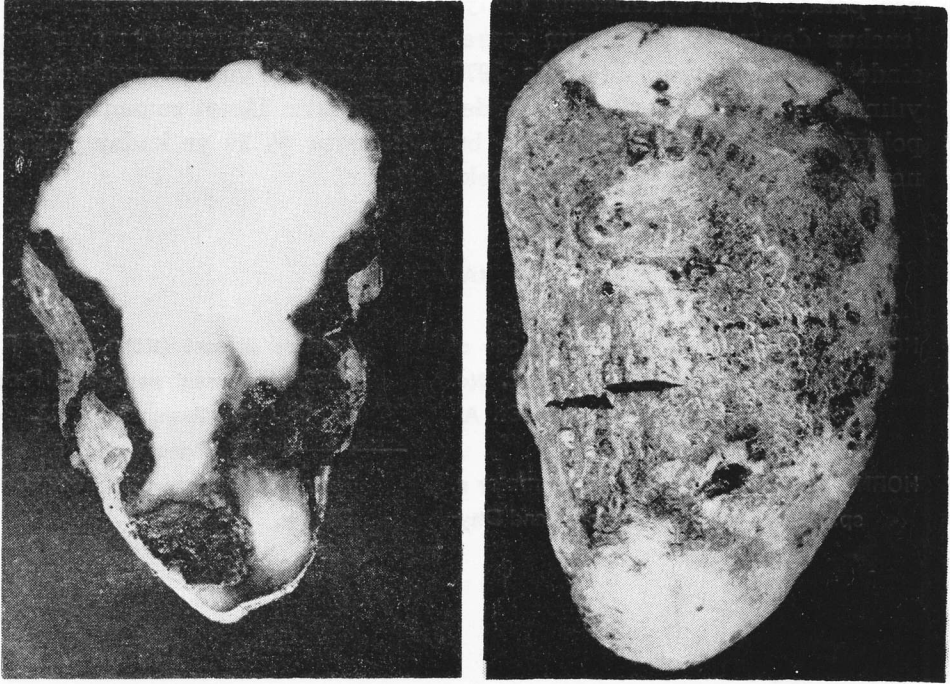


Fig. 1: *Ditylenchus destructor* destruction on potato tuber

# Investigations on the Optimum Growth Temperatures and the Effectiveness of Some Chemicals of the Phytopathogenic Bacteria

Sevil KAYA\* and Ibrahim KARACA\*\*

## ABSTRACT

Investigations on the optimum growth temperature of plant pathogenic bacteria and effect of some chemicals on them were carried out in 1967-1970 under laboratory conditions. Species undertest showed optimum growth at the following temperatures:

**P. phaseolicola** 22°C, **P. maculicola** 24°C, **P. marginalis**, **E. atroseptica** **P. morsprunorum** 25°C, **P. tabaci** 26°C, **P. lachrymans**, **E. carotovora**, **X. pelargonii** 27°C, **P. syringae**, **X. campestris**, **X. vesicatoria** 28°C, **A. tumefaciens**, **C. michiganense**, **P. pisi** 29°C, **E. amylovora** 32°C, **P. solanacearum** 35°C.

According to the results of the experiments which were carried out using the dry-disc method in vitro conditions, antibiotics like streptomycin, penicillin, kanamycin, terramycin were effective on the bacteria which were tested. Some of the fungicides although hot as much as antibiotics, were also significantly effective, preferably zirthane, dithane M-22, antracol and also sublimate which is an inorganic material were positively.

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## INTRODUCTION

Due to its suitable climatic conditions polyculture is dominantly in practice in Aegean Region and the bacterial diseases cause great losses by affecting the crop cultivated in this region.

Like all the other living organisms temperature is an essential factor on the growth and reproduction of bacteria.

Knowing the optimum growth temperatures of the bacteria helps a

great deal while growing them as pure cultures without losing their properties (Lambin and German, 1961).

On the other hand, the temperatures at which diseases can cause epidemics could also be established if the optimum growth temperatures are known.

This study deals with the chemicals tested in vitro conditions and that could be used against the bacterial diseases of plants.

## MATERIALS AND METHODS

The bacterial cultures used in this study were received from Dr. D. Rudolph (Göttingen); Dr. D. Knösel (Stuttgart-Hohenheim); Drs. H. Mass Geesteramis (Holland) and British Museum.

The bacteria were:

- Pseudomonas solanacearum* (Erw. Smith) Erw. Smith
- Pseudomonas marginalis* (Brown) Stevens
- Erwinia atroseptica* (van Hall) Jennison
- Pseudomonas pisi* Sackett
- Pseudomonas mors-prunorum* Wormald
- Erwinia carotovora* (Jones) Holland
- Agrobacterium tumefaciens* (Smith and Townsend) Conn.
- Xanthomonas vesicatoria* (Doidge) Dowson
- Xanthomonas campestris* (Pammel) Dowson
- Pseudomonas tabaci* (Wolf and Foster) Stevens
- Pseudomonas maculicola* (Mc Culloch) Stevens
- Pseudomonas phaseolicola* (Burkholder) Dowson
- Pseudomonas lachrymans* (Smith and Bryan) Carsner
- Xanthomonas pelargonii* (Brown) Starr and Burkholder
- Pseudomonas syringae* van Hall
- Erwinia amylovora* (Burill) Winslow et al.
- Xanthomonas phaseoli* (Erw. Smith) Dowson
- Corynebacterium michiganense*

In growing and keeping of these bacterial cultures, methods of Bilgehan (1965) and Dowson (1957) were applied.

The determinations of the optimal growth temperatures were based

on the methods given by Breed et al (1975) and Karaca (1966). It was decided to control the activity of bacteria at the temperatures that are listed in Table I.

Table I. Growth temperatures that were obtained from literature and temperatures that were checked for the effect on growth of tested bacteria.

Name of the bacteria	optimum growth temperatures mentioned in the literature (C°)	Temperatures examined in order to find the optimum growth temperature (C°)
<i>P. marginalis</i>	25—26	23, 25, 27, 29, 31
<i>E. atroseptica</i>	25	23, 25, 27, 29
<i>P. mors-prunorum</i>	25	23, 25, 27, 29
<i>P. pisi</i>	27—28	25, 27, 29, 31
<i>E. carotovora</i>	27	25, 27, 29, 31
<i>A. tumefaciens</i>	25—28	23, 25, 27, 29, 31
<i>P. solanacearum</i>	35—37	33, 35, 37, 39, 41
<i>X. vesicatoria</i>	30	26, 28, 30, 32, 34
<i>X. campestris</i>	28—30	26, 28, 30, 32, 34
<i>P. tabaci</i>	22—27	20, 22, 24, 26, 28, 30
<i>P. maculicola</i>	24—25	22, 24, 26, 28, 30
<i>P. phaseolicola</i>	20—23	18, 20, 22, 24, 26
<i>P. lachrymans</i>	25—27	23, 25, 27, 29, 31
<i>X. pelargonii</i>	27	25, 27, 29, 31
<i>P. syringae</i>	28—30	26, 28, 30, 32, 34
<i>E. amylovora</i>	30	28, 30, 32, 34
<i>X. phaseoli</i>	31	29, 31, 33, 35
<i>C. michiganense</i>	25—27	23, 25, 27, 29, 31

PHYTOPATHOGENIC BACTERIA

AYARCA and I. KARACA

Bacterial cultures grown under these temperatures were counted according to Breed's method. The method has been established with the help of Yaygin (1967).

Optimal growth temperature of every bacteria species has been derived from these numbers.

Chemical treatments in vitro conditions were designed according to randomized plots with three replicates. The low and high dosages of the organic and systemic fungicides and antibiotics tested were given in table 2.

Table 2. Chemicals used in trial, their active materials and dosages

Name	Nam and percentage of the a.i.	Formulation	Dosage gr/lit water
Antracol	Zinopropylen bisdithiocarbomat, 70	W.P.	1,5-2,5 gr.
Cupper fungiced	Cupper oksiklorid, 50	W.P.	3- 5 gr.
Dithane M-22	Manganez ethylen bisdithiocarbomate, 80	W.P.	2- 3 gr.
Orthocide 50	Captan, 50	W.P.	2,5-3,5 gr.
Karathane	Dinitro (1-methyl heptyl) fenil crotonate, 37	L.C.	0,3-0,6 gr.
Melprex	Dodine, 65	W.P.	60-100gr.
Brassicol	P C N B, 75	W.P.	3- 4 gr.
Plantvax	2,3-Dihydro-5-carboxanilido 6-methyl-1,4-oxathiin 4,4-Dioxide	W.P.	1,25-2,5 gr.
Benlate	Methyl-1-(Butylcarbamoyl)-1-2-benzimidazole carbomate 150	W.P.	0,3-0,6 gr.
Zirthane	Zinc dimethyldithiocarbomate, 80	W.P.	1,5- 3 gr.
Penicillin	Penicillin G potassium, 100	Crystalline	500.000-250.000-unite
Streptomycin	Streptomycin sulfate 100	Solution	1- 2 gr.
Terramycin	Oxytetracycline, 10	Solution	1- 2 gr.
Kanamycin	Kanamycin sulfate, 100	Solution	1- 2 gr.
Corrasive sublimate	Mercury chloride, 100	W.P.	1- gr.

In the trial, Dry disc method one of the diffusion methods, has been used (Bilgehan, 1965).

The treated discs were placed in each petri-dish where the bacteria were sown then these petri dishes

were inoculated for 24 hours at the optimum temperatures found for each bacterium. At the end of this period the effectiveness of each chemical was measured from the inhibition areas formed around the disc.

#### RESULTS AND DISCUSSION

Optimum growth temperatures of the bacteria under test were found as follows:

*P. phaseolicola* 22°C

*P. maculicola* 24°C

*E. atroseptica*, *P. mors-prunorum* ve *P. marginalis* 25°C

*P. tabaci* 26°C

*E. carotovora*, *P. lachrymans* ve *X. pelargonii* 27°C

*P. syringae*, *X. campestris* ve *X. vesicatoria* 28°C

*A. tumefaciens*, *P. pisi* ve *C. michiganense* 29°C

*X. phaseoli* 31°C

*E. amylovora* 32°C

*P. solanacearum* 35°C

Optimum growth temperatures found for *A. tumefaciens*, *P. lachrymans*, *X. vesicatoria*, *E. amylovora* and *P. tabaci* did not coincide with the figures mentioned in the literature (Johnson, 1921; Gardner and Kendrick, 1923, Patel, 1926; Riker, et al 1930; Coley, 1931; Keit et al 1936; Theis et al, 1950; Wiles and Walker, 1952). The reason for such a variation could be the difference in laboratory conditions. Thus. Chalmers (1955) points out the fact that there can be a  $\pm 90$  % difference among the bacteria numbers of the milk sample counted in different laboratories Properties such as the counting method,

the difference in the composition of the nutrient media, pH, etc. are also effective on the optimum temperature Especially, the difference in the composition of the nutrient media cause bacteria to grow at different rates under different temperature conditions.

Depending upon the countings it was determined that *P. marginalis*, *P. lachrymans*, *C. michiganense*, *X. pelargonii* and *P. maculicola* could reproduce above and below their optimum temperatures with approximately the same speed as they reproduce at the optimum point.



On the other hand, it was not possible to compare our results related to *P. pisi*, *X. phaseoli*, *E. carotovora*, *E. atroseptica*, and *P. mors-prunorum* with the literature, since we could not find any studies carried on these species.

The optimum growth temperature of *P. solanacearum* that reproduces at high temperature was found as 25-30°C, 30°C and 27°C by Meier and Link (1925), Nakata (1930) and Moreas (1947) respectively. During the present study optimum growth was obtained at 35°C which shows the similarity with the above mentioned results.

The effect of various chemicals on different species of bacteria was found significantly different at the level of 1 % according to the analysis of variance.

As there was a highly significant difference among the effect of chemicals on each species of bacteria the chemicals were grouped by orthogonal sampling (Steel and Torrie, 1960).

Until now, several chemicals containing different active ingredients were tested *in vivo* and *in vitro* conditions against the bacteria that studied in the present work too. Investigations on this subject that carried out by different research workers gave both positive and negative results (Wiles and Walker, 1952; Oksentiyan et al 1960; Graham and Zefrira, 1961; Keil and Wilson, 1962; Fponek and Klas, 1963, Jones and Parker, 1963; Nikitiva, 1963;

Jones, 1964; Beleva, 1964; Jenkins, 1964; Nauman, 1965; De Azevedo, 1965; Knösel and Thill, 1966; Ercoloni et al 1967; Kapustin, 1967; Fonnesbach, 1969).

As the conclusion penisilin, streptomycin, Kanamycin and terramycin like antibiotics recomended against these bacteria firstly and it was determined that the following preparations could also be used successfully.

- P. lachrymans* : Zirthane, Corrosive sublimate, Dithane M-22
- P. mors-prunorum* : Zirthane, Corrosive sublimate, Dithane M-22
- P. solanacearum*: Zirthane, Corrosive sublimate, Dithane M-22  
cupper fungiced.
- P. syringae* : Zirthane, Corrosive sublimate
- X. phaseoli* : Zirthane, Dithane M-22  
Karathane, Corrosive sublimate.
- X. campestris* : Corrosive sublimate, Zirthane, Antracol, Dithane M, 22, Karathane, Melprex, cupper fungiced.
- X. vesicatoria* : Corrosive sublimate, Zirthane, Dithane M,22,
- E. atroseptica* : Corrosive sublimate
- E. amylovora* : Zirthane, Dithane M, 22, supper fungiced.
- E. carotovora* : Antracol, corrosive sublimate, Dithane M-22, Zirt-hane, Karathane.
- A. tumefaciens* : Corrosive sublimate



## ÖZET

## BİTKİ PATOJENİ BAZI BAKTERİLERİN OPTİMUM GELİŞME SICAKLIKLARI VE BUNLARA BAZI İLAÇLARIN ETKİNLİKLERİ ÜZERİNDE ARAŞTIRMALAR

Bitki patojeni bazı bakterilerin optimum gelişme sıcaklıkları ve bazı ilaçların bunlar üzerindeki etkilerinin araştırılması ile ilgili çalışmalar 1967-1970 yılları arasında laboratuvar koşullarında Bornova Bölge Zirai Mücadele Araştırma Enstitüsünde yapılmıştır. Hollanda, Almanya ve İngiltere'den getirilen bazı bakteri türlerinin optimum gelişme noktalarını bulmak için yapılan çalışmalar sonucunda *P. phaseolicola*'nın 22°C de, *P. maculicola*'nın 24°C de, *P. marginalis*, *E. atroseptica* ve *P. mors-pronorum*'un 25°C de, *P. tabaci*'nin 26°C de, *P. lachrymans*, *E. carotovora* ve *X. pelargonii*'nin 27°C de, *P. syringae*, *X. campestris* ve *X. vesicatoria*'nın 28°C de, *A. tumefaciens*, *C. michiga-*

nense *P. pisi*'nin 29°C de, *E. amylovora*'nın 32°C de, *P. solanacearum*'un 35°C de optimum olarak gelişme göserdikleri saptanmıştır.

Bazı ilaçlar ile kuru disk metodu kullanmak suretiyle in vitro koşullarda yapılan denemelerin sonucuna göre de, streptomycin, penicillin Kanamycin, terramycin gibi antibiyotiklerin denemeye alınan bakteri türlerine karşı etkili oldukları, bunların yanında bazı fungusitlerin antibiyotikler kadar olmamakla beraber üzerlerinde durulabilecek derecede etki gösterdikleri, bunların içinde zirthane, dithane M,22 ve antracol'un tercih edilebileceği ayrıca anorganik bir madde olan sublimentinde olumlu etkide bulunduğu sonucuna varılmıştır.

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## Effect of Virus Infection Times on Yield of Five Tomato Varieties.

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### ABSTRACT

The effect of virus infection times on five tomato varieties was studied at two different stage. Artificial inoculations of tobacco mosaic virus and tomato streak virus were carried out at two different stages. Results indicated that late inoculations were less effective than earlier inoculations and the variety Red to was less affected among the other varieties.

### INTRODUCTION

It is well known that virus diseases cause great losses of yield either quantitatively or qualitatively. Tomato crop suffer from both. Quantitative losses are yield reduction such as smaller and fewer fruit per plant, whereas qualitative loss are usually involved in lower market values. Both cases produce an important economical loss.

According to the statistics 8.219.000 tons of vegetables were produced totally in Central Anatolia and 25 % of the total crop was tomato in 1973 (Anonymus, 1973).

Klinkowski (1968) reported that virus diseases of tomatoes cause 10 % loss of yield on average. If this is so, the estimated crop losses due to the viruses will reach up to 205.000 tons for tomato in the year of 1973 at least Reports related to crop losses of tomatoes are very few for Turkey. Yorganci (1975) worked on the yield loss of tomatoes caused by viruses and found that flowering stage of plants was more susceptible that green fruit stage and reaction of varieties were different against the infection. The inoculation stage was the main factor



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affecting crop losses. In the work of Tekinel (1973) it was reported that tomato plants infected with tomato mosaic virus by 26.7 % and with tomato streak virus by 0,6 %.

In the present work, susceptibility of five tomato varieties which were grown commercially in Central Anatolia were checked against the infection by two viruses at two different stages.

### MATERIALS AND METHODS

The viruses used in this work were tobacco mosaic virus (TMV) and tomato streak virus (TSV). They were obtained from naturally-infected tomato plants collected from fields and maintained in *Nicotiana tabacum* cv. White Burley. Regular inoculations to young stock plants were made to obtain viruses in high concentrations and inocula were prepared from infected mature leaves by macerating 1 gr fresh leaf-tissue with the addition of 4 cc 0.1 M phosphate buffer pH 7.0, then the pulp was squeezed through double muslin. "Celite" was used as abrasive and added to the inocula before inoculations

Seeds of five tomato varieties, namely VFN 8, Red top U.C. 156, Campbelle 2 and Es 24, were sown in

wooden-boxes and tomato seedlings were transplanted into the field about six weeks after seeding. Randomised Blocks experimental design was used with three replicates.

Separate plants of the same varieties were artificially inoculated TMV and TSV at two different stages. First inoculations (seedling stage) were made four weeks after transplanting and second inoculations (flower stage) were followed this two weeks later.

Reduction in yield was assayed by set of fruits for the first three clusters. Results were analysed according to Analysis of variance and "Duncan test" were applied whenever necessary.

### RESULTS AND DISCUSSION

After fruit setting was completed but before the ripening initiated the fruits set on the first three clusters

were counted. The average number of fruits were given on table 1.



Inoculat.	Stage Virus Varieties	I. Replicate		II. Replicate		III. Replicate		Mean	
		Cont.	Infect.	Cont.	Infect.	Cont.	Infect.	Cont.	Infect.
TMV	VFN 8	7.40	4.20	5.40	3.60	5.40	3.80	6.06	3.86
	Redtop	5.00	3.80	5.60	2.80	4.20	4.20	4.90	3.60
	Campbelle2	4.60	1.80	7.00	2.40	5.60	4.80	5.73	3.00
	U.C. 156	5.40	1.60	6.80	2.00	6.80	3.40	6.33	2.33
	Es 24	5.00	3.20	6.00	2.20	6.00	2.20	5.66	2.53
Seedling Stage	VFN 8	3.60	4.00	5.60	4.60	2.60	1.60	3.93	3.40
	Red top	4.20	4.40	9.40	3.80	9.40	3.60	7.66	3.93
	TSV Campbelle2	5.40	3.20	8.00	6.60	9.40	6.00	7.60	5.26
	U.C. 156	9.40	3.00	7.60	4.20	4.60	2.60	7.26	3.26
	Es 24	5.00	1.80	6.20	2.80	5.80	3.00	6.33	2.53
TMV	VFN 8	4.60	6.00	3.40	1.00	7.20	5.20	5.06	4.06
	Red top	12.80	7.80	7.20	5.00	10.00	3.20	10.00	5.33
	Campbelle2	6.20	3.40	3.40	2.20	8.20	3.20	6.60	2.93
	U.C. 156	8.60	5.20	6.00	4.60	7.60	6.20	7.40	6.46
	Es 24	8.20	6.40	5.20	3.20	6.60	8.80	6.66	6.13
Flowering Stage	VFN 8	6.20	5.60	3.40	3.80	6.40	3.40	5.33	4.26
	Red top	14.20	11.60	4.60	5.20	10.80	8.80	13.20	8.53
	TSV Campbelle2	8.40	5.00	5.40	5.00	7.60	5.20	7.13	5.06
	U.C. 156	9.00	5.80	8.60	4.00	6.40	4.80	8.00	4.86
	Es 24	8.60	3.00	7.20	3.00	8.00	4.80	7.93	3.60

When the results were analysed according to the analysis of variance a 1 % significant difference obtained between the yield of control and inoculated plants both in seedling stage inoculations and flowering stage inoculations.

There was no significant difference among the varieties inoculated at seedling stage whereas the varieties affected differently from the inoculations at flower stage. The difference

between the varieties inoculated with TMV at flower stage was found to be significant at the 1 % level. "Duncan test" was applied and the following results were obtained:

- Red top A
- U.C.156 AB
- Es 24 AB
- Campbelle 2 C
- VFN 8 C

Flower stage inoculations with TSV gave almost the same results as Red top being the most fruitful variety and others fell into the same group.

It is clearly shown that the tomato varieties under test were affected by the inoculations of both viruses either at seedling or at flowering stages. Alexander (1950) reported that TMV reduced the yield in glasshouse by 13.2 % and the greatest reduction occurred during early part of season. In the present study it was also observed that inoculations which were carried out at seedling stage resulted in greater losses. Fruit yield, or in other words, number of fruit decreased severely.

According to Crill et al. (1973)

decreased yields were due to significant reductions in number of fruit per plant as well as decreased weight of individual fruit. This was also observed during the course of this study where the fruit of early infected plants were smaller and fewer.

Alexander (1952) inoculated three different varieties of tomato plants with TMV at three different dates and found that later inoculations caused less reduction in yield. In the present study the plants were less affected from the flowering stage inoculations.

Reaction of the varieties found to be different against the late inoculations. Analysis of the results showed that the variety Red top was the least affected.

## ÖZET

### VIRUS ENFEKSİYON ZAMANLARININ DOMATES ÇEŞİTLERİNDE VERİME ETKİSİ

Orta Anadolu'da yetiştirilen VFN 8, Red top, Campbelle 2., U.C. 156 ve Es 24 domates çeşitlerini tütün mozayik virusu ve domates tek viruslu çizgi hastalığı inokulasyonlarına karşı reaksiyonlarının incelendiği bu çalışmada virus inokulasyonlarının verim üzerindeki etkisi ve dayanıklı çeşitler araştırılmıştır.

Çok faktörlü tesadüf blokları deneme desenine göre üç tekerrürlü olarak kurulan denemede gerek fide devresi, gerekse çiçek devresi inoku-

lasyonlarının verimi azalttığı saptanmıştır. Fide devresi inokulasyonlarıyla çiçek devresi inokulasyonları verim üzerinde farklı etkiler meydana getirmiş ve geç inokulasyonların daha az ürün kaybına sebep olduğu saptanmıştır.

Fide devresi inokulasyonları çeşitlerin verimleri arasında bir farklılık yaratmamış; fakat kontrol bitkilerin verimleriyle aşılı bitkilerin verimleri arasında % 1 seviyesinde önemli farklılık bulunmuştur. Çiçek

devresi inokulasyonlarında çeşitler arasında da farklılık bulunmuş ve inokulasyonlardan en az etkilenen çeşidin Red top çeşidi olduğu sap-

tanmıştır. U.C. 156 ve Es 24 çeşitleri ikinci grupu VFN 8 ve Campbelle 2 çeşitleri de üçüncü grubu meydana getirmiştir.

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# Investigations on Sunflower Diseases in Thrace, Their Rate of Existence Their Fungal Pathogens and Their Pathogenicities

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## ABSTRACT

The purpose of this study was to determine the fungal pathogens, which show symptoms on the above-ground parts of sunflower, their rate of existence and spread range, and to investigate their pathogenicity on sunflower fields of Thrace in 1975.

Observations were carried out in 227 fields, as a total number of Edirne, Tekirdağ, Kırklareli and Thrace part of Istanbul. Eight fungal species were determined during the surveys: *Plasmopara helianthi*, *Puccinia helianthi*, *Sclerotinia sclerotiorum*, *Botrytis cinerea*, *Alternaria* sp., *Helminthosporium* sp., *Rhizopus* sp., and *Septoria* sp.

All these fungi except *Plasmopara helianthi* were reported on sunflowers for the first time, in Turkey. All of these 8 fungi gave positive results on the pathogenicity tests.

## INTRODUCTION

Seed oil production, except olive oil, is mainly based on two seed oil crop, cotton and sunflower in Turkey. Sunflower is responsible for 48

% of the total oil seed production (5). Sunflower is not only important in oil industry, but also important in animal food industry, cellulose in-

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dustry, paint and soap industry and in medicine. Sunflower is mainly cultivated in the Thrace of Turkey, 73.7 % of the total cultivation area in 1970 and 74.5 % in 1974 were taken place in this part of the country. While total sunflower cultivation area was 360.000 Ha in 1970, it reached to 424.555 Ha with an 18 % increase in 1974. This increase became 19 % in Thrace.

It is necessary to obtain max. yield from a unit area instead of the enlargement of the cultivation area. According to the data of 1973 statistics, sunflower yield was 1164 kg/Ha in Turkey, while it was 1556 kg/Ha in Russia, 1774 kg/Ha in Bulgaria and 1938 kg/Ha in Yugoslavia (4). One of the reason of decrease in the yield is the increase of the effects of diseases.

Symptoms caused by the pathogens appear in different phenological stages. Therefore the genus of the pathogen will be different according to the isolation time (8).

The fungus **Plasmopara** is the most important one which causes great damage to sunflower. This fungus can infect the plant from the begining of germination of the seed, till flowering period (9). Some investigators have been reported that when sunflower infection by **P. helianthi** reached up to 80 %, the yield loss became 48 % which is an unnegligible figure (20).

**Puccinia** sp. can be seen during flowering period in every field where sunflower is cultivated. It is found mostly on the leaves although could be seen on every parts of the plants (9).

It is known that sunflower plants are very susceptible to **Sclerotinia** sp. which late planting was applied (21) **Sclerotia** could be formed in the stalks and adjacent to root of the dead plants (15).

**Septoria** sp. is only seen on the leaves (10). In every stages on the growth period of the plant, leaf lesions with dark margins caused by this fungus can be observed (9).

**Alternaria** sp. is generally, not seen until the flowering period (9). Infection can be observed on mature leaves as necrotic leaf lesions and a severe infection can cause defoliation (7). Symptoms can be seen on the stalks of seedlings and on mature plants heads (16).

**Rhizopus** sp. causes head rot which starts from the edge and moves to the center. Consequently the head appears a thorn-up-pattern (15-18). Most effective period of **Botrytis** sp. which causes grey rot is the time when the head matures (2-3).

Lesions on the leaves caused by **Helminthosporium** resemble to those lesions caused by **Alternaria** sp. (11).

## MATERIALS AND METHODS

This study covers Edirne, Tekirdağ, Kırklareli and Istanbul provinces of Thrace. Survey was carried out in these 4 provinces and 19 districts, including 111 villages. Fields that observed were 227.

Sunflower planting time differed in fields where survey was conducted, according to different climatic factors. Planting period starts from the end of March and continues until the end of May. The common sunflower variety which is cultivated in the area is VNIIMK 8931. Peredovick and VNIIMK 1646 varieties are cultivated in small amount.

This study was carried out in order to determine the pathogens found on the above ground-parts of sunflower and their sensitivity to these pathogens and the extension rate of the infected plants in the fields.

In our surveys 100 plants from each sample units were observed. For this, 20 plants were sampled randomly from center and margins of each units and these plants were counted as healthy or infected.

Rough-identifications of the fungi were made on isolations obtained from the symptom - giving parts of the plants. Definite identifications were made after isolation and pure culture preparations of the pathogens.

In pathogenicity tests, the fungi **Sclerotinia**, **Botrytis**, **Alternaria**, **Sep-toria**, **Rhizopus** and **Helminthospo-**

**rium** which were isolated from the surveyed area in 1975 and obligate parasites **Plasmopara** and **Puccinia** were tested. **Botrytis** and **Rhizopus** were tested in the field and the others in pots in the greenhouse.

Field and greenhouse tests were carried out according to random block design with 5 replications. The fungal isolates, except **Plasmopara**, **Puccinia** and **Sclerotinia**, were placed in petri dishes containing 20 ml 2 % PDA and they were incubated for 15 days in 20°C. There was infected plant debris in the pots where **Sclerotinia** was tested. Besides, sclerotia of the fungus were mixed to the soil during the seed planting in this tests (13). Plants used in pathogenicity tests were in two-true leaf stage. Isolates in the petri dishes were mixed with 0.5 liter sterile-distile water separately and spore suspensions were prepared. Anuredospore suspension of **Puccinia** is prepared from the infected leaves collected from the survey area. Inoculum for each isolate is applied by a fine brush or sprayed to the leaves of the plants (13).

Spore suspensions of **Rhizopus** and **Botrytis** were prepared by washing the surface of the agar in petri dishes and these suspensions were injected in to the bottom section of the sunflower heads by using a syringe (13,17).

Readings of the field and greenhouse tests were made 20 days after

the inoculation. The plants were classified as healthy and diseased.

Infection rate among sunflower plants in each field and the rate of

#### RESULTS AND DISCUSSION

The fungi found in sunflower fields during 1975 surveys and the rate of fungal genera in each sample center are given in table 1.

**Plasmopara helianthi** was the most common and effective fungus in the surveyed area. This fungus causes downy mildew on sunflowers and this disease was observed in every stage of the development of sunflowers. When the plants were infected during seedling stage, plants can not develop normally and can not produce head, then wither and dry. It was determined that plants infected in later stages, became stunted produced the head earlier than the healthy plants and their seeds on the head were empty (fig. 1). These plants, also, can be broken from the stem very easily, and their leaves become curled (fig. 2). It was observed that lower side of the leaves show a mass of sporangia and sporangio-phore of the fungus first along the veins, then this white cottony mass covers the whole surface (fig. 3,4). When stem was cut longitudinally, necrosis was observed in the xylem, phloem and the pith.

**Puccinia helianthi** Schw., the causal agent of sunflower rust, was

the extension of the fungi infecting sunflower plant in the region were determined (14).

observed in the surveyed area during the period of flowering time till harvest of the sunflowers. It was also observed on the sunflowers planted in late season, although carried no flowers. Rust pimples were formed first underside of the leaves (fig. 5). later it covered the whole plant. In some cases rust pimples were observed underside of the heads (fig. 6). This fungus is reported on sunflowers for the first time in Turkey.

**Sclerotinia sclerotiorum** was generally more effective on sunflowers grown on heavy soils. First symptom caused by **S. sclerotiorum** was withering, later, plants dry (fig. 7). The fungus wither the plant pith (fig. 8) and produce plenty of scleroti there.

**Alternaria** sp., **Helminthosporium** sp., **Septoria** sp. which were isolated from the surveyed area, caused only leaf lesions. Leaf lesions caused by **Septoria** sp. were found in two sections of the area. One of them, Yerlisu district of Keşan and Deveçatağı, village of Kırklareli province. Symptoms observed in both places were seen on young plants (fig. 9).

**Alteranria** leaf spot was common on sunflowers in the region. Leaf

Table 1. The fungi found in the sunflower field during 1975 surveys and the rate of fungal genera in each sample center of each genus

Fungal Genera	Sampling centers																		
	Edirne Merkez İlçe	Lalapaşa	Havsa	Keşan	Uzunköprü	Ipsala	Enez	Tekirdağ Merkez İlçe	Çorlu	Hayrabolu	Murath	Saray	Malkara	Kırklareli Merkez İlçe	Pınarhisar	Babaeski	Lüleburgaz	Silivri	Çatalca
Plasmopara	25.16	22.50	41.56	8.35	23.90	16.91	11.20	47.50	31.62	50.58	57.88	38.57	35.35	37.0	31.16	20.63	32.80	29.0	8.66
Puccinia	7.61	—	0.12	7.14	5.95	37.33	—	0.92	6.75	16.23	8.0	3.0	13.71	6.86	3.16	6.54	2.2	—	29.66
Sclerotinia	—	—	0.43	2.09	—	17.91	2.60	7.14	—	—	—	—	—	0.14	—	—	—	—	—
Alternaria	1.33	1.16	7.50	1.67	1.90	—	—	3.35	0.75	—	—	0.42	2.71	1.8	1.0	—	0.35	3.5	13.0
Helminthosporium	1.50	—	—	—	—	—	—	0.57	—	—	—	—	—	—	—	—	—	—	—
Rhizopus	—	—	—	—	—	1.5	—	—	—	—	—	—	—	—	—	—	—	—	—
Septoria	—	—	—	1.71	—	—	—	—	—	—	—	—	—	0.53	—	—	—	—	—
Botrytis	—	—	—	—	—	0.41	—	—	—	—	—	—	—	—	—	—	—	0.25	—



spots were generally located on the lower leaves. Later in the season these leaf lesions coalesce and the whole leaf dries (fig. 10).

**Helminthosporium** sp. was seen to cause different leaf lesions than **Alternaria** sp. (fig. 11). **Helminthosporium** sp. was found in Kayapa village of Edirne province and İncecik and Biyıklı village of Tekirdağ province.

**Botrytis cinerea** and **Rhizopus** sp. were found only on head infections of sunflowers (fig. 12, 13, 14, 15). **B. cinerea** was found only in Sarıcaali district of Lüleburgaz and in İpsala. **Botrytis** and **Rhizopus** symptoms on the underside of the sunflower head look alike.

Extension rate of the fungi determined in the surveyed area is given in table 2. It was observed that **Plasmopara helianthi** was disseminated to the whole area. **Puccinia helianthi**, **Alternaria** sp., **Sclerotinia sclerotiorum** were partially disseminated the area.

During surveys, 8 fungi were found causing infection on sunflower. Pathogenicity tests of these 8 fungi and the results they showed were given in table 3.

It was determined that firstly, **Plasmopara helianthi** and secondly **Puccinia helianthi** and **S. sclerotiorum** have the potential in causing epidemics in the region. **Alternaria**

sp. was not in economical level and infection potential was low for sunflower. Same can be said for the other fungi found in sunflower fields in Thrace.

It is quite that, pathogens which cause mildew, rust and stem rot can initiate epidemics if the preventive measures were not fulfilled. On the other hand, pathogens seem to be not important now may be a problem later. Preventive measures can be applied as for short term or long term. For the short term preventive measures, chemical applications are the first action; but, in chemical applications against **Plasmopara helianthi** in the countries where sunflower is grown, it is not reached to a definite conclusion (1, 19). However, studies continue on subject. Chemical control against **Sclerotinia sclerotiorum** and **Puccinia helianthi** were successful in some countries (13). It is necessary to study on this line in our country, too; because, especially rust, has reached to a level that can cause problems in the long run. In the first place, resistant varieties should be studied. Some investigators report promising results (2, 12). This type of studies should be undertaken in our country.

The other important point is that the seed given to the growers must be clean and certificated. Because, **Plasmopara**, **Puccinia** and **Sclerotinia** can be carried in/on the seed (6,12). This problem is important, too.



## SUNFLOWER DISEASES

Table 3. Infecting rate of the 8 fungi tested for pathogenicity

Fungi	Replications					Average
	I	II	III	IV	V	
<b>S. sclerotiorum</b>	66.6	44.4	77.7	77.7	33.3	59.9
<b>Alternaria sp.</b>	44.4	44.4	33.3	66.6	44.4	46.6
<b>Helminthosporium sp.</b>	22.2	33.3	11.1	33.3	22.2	24.4
<b>Septoria sp.</b>	0	11.1	0	0	11.1	4.4
<b>Botrytis cinerea</b>	100	100	83.3	83.3	83.3	89.9
<b>Rhizopus sp.</b>	100	100	100	100	100	100
<b>Plasmopara helianthi</b>	77.7	66.6	66.6	44.4	66.6	64.3
<b>Puccinia helianthi</b>	33.3	44.4	22.2	22.2	11.1	26.6

### ÖZET

#### TRAKYA BÖLGESİNDE AYÇİÇEKLERİNDE GÖRÜLEN HASTALIKLARIN ORANI, BELİRTİLERİ, FUNGAL ETMENLERİ VE ETMENLERİN PATOJENİSİTESİ ÜZERİNDE ARAŞTIRMALAR

Araştırma, 1975 yılında Trakya Bölgesi ayçiçeği ekiliş alanlarında ayçiçeklerinin toprak üstü kısımlarında belirti veren fungal etmenleri, bulunuş ve yaygınlık oranlarını ve patojenisitelerini araştırmak amacı ile yapılmıştır.

Araştırma bölgesine giren Edirne Tekirdağ, Kırklareli ve İstanbul (Trakya kesimi) illerinde toplam olarak 227 tarlada gözlem yapılmıştır. Surveyde 8 fungus saptanmıştır : **Plasmopara helianthi**, **Puccinia helianthi**, **Sclerotinia sclerotiorum**, **Botrytis cinerea**, **Alternaria sp.**, **Helminthosporium sp.**, **Rhizopus sp.** ve **Septoria sp.**

Saptanan funguslardan **Plasmopara helianthi** hariç diğerleri Yurdumuz için ayçiçeklerinde ilk kez rapor edilmektedir.

Patojenisite testine alınan 8 fungusun hepsi ayçiçeklerinde hastalık belirtisi oluşturmuşlardır. **Rhizopus sp.** verilen ayçiçeği tablalarının hepsinin hastalandığı görülmüştür. Bunu **Botrytis cinerea** (% 89.9) ve **Plasmopara helianthi** (% 64.3)'nin hastalandığı bitkilerin oranı izlemiştir. Bitkilerde en az hastalık belirtisi veren fungus % 4.4 ile **Septoria sp.** olmuştur.

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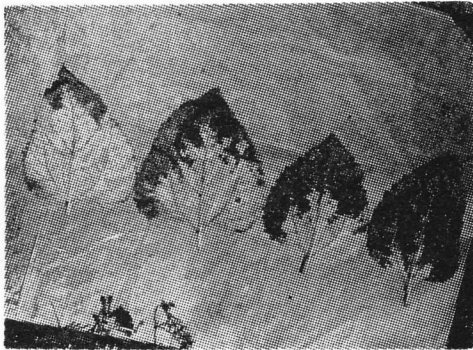




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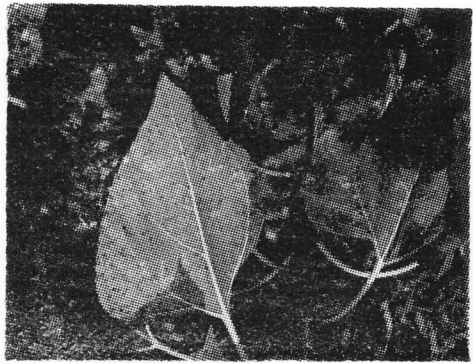
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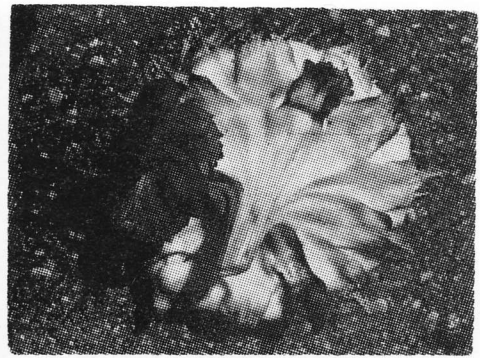
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Fig. 1. A stunted sunflower plant due to downy mildew

Fig. 2. Wrinklings on sunflower leaves due to downy mildew

Fig. 3. Cottony mass appearance of the downy mildew  
on the lower side of the leaves

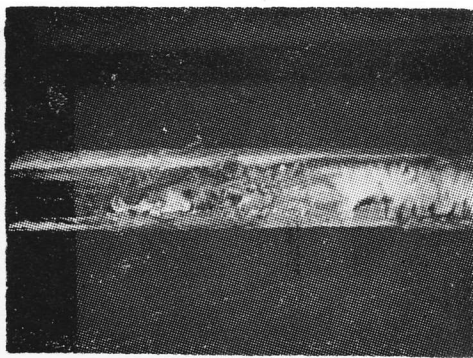
Fig. 4. A longitudinal section of an infected plant

Fig. 5. Rust pustules on the lower side of the leaves

Fig. 6. Rust pustules on the sunflower head



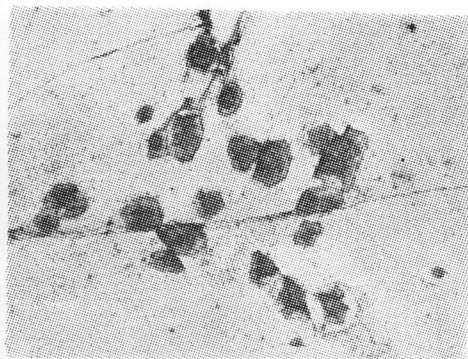
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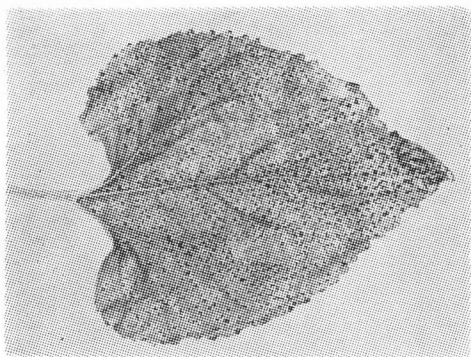
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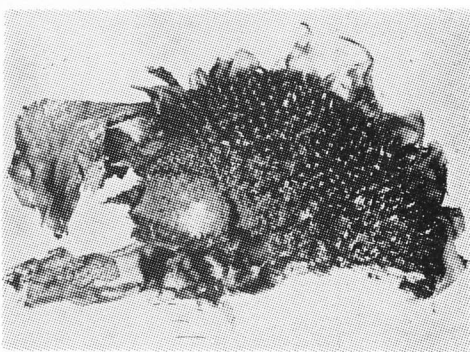
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Fig. 7. Wilting and drying on sunflowers cause by *S. sclerotiorum*

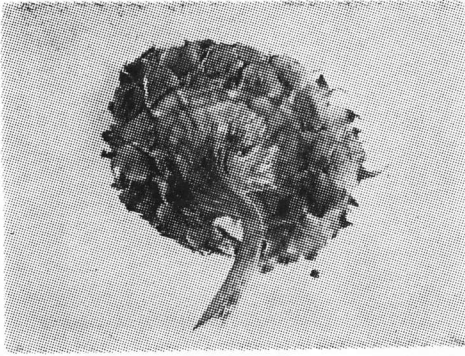
Fig. 8. Drying in the pith of the sunflower plant cause by *S. sclerotiorum*

Fig. 9. Leaf spots cause by *Septoria* sp. on sunflower plant

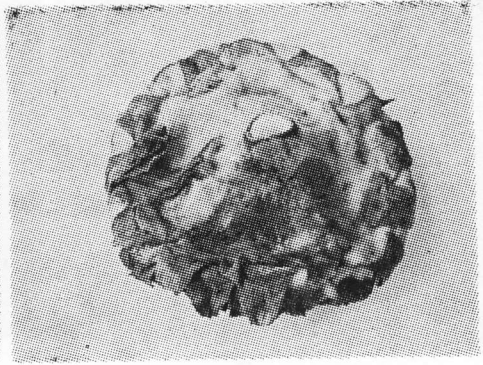
Fig. 10. Leaf spots cause by *Alternaria* sp. on sunflower leaf

Fig. 11. Leaf spots cause by *Helminthosporium* sp.

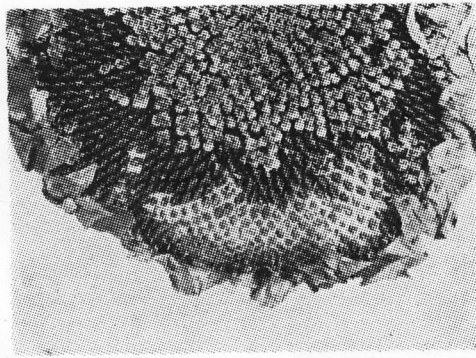
Fig. 12. Rotting cause by *B. cinerea* on the upper side of the head



13



14



15

Fig. 13. Rotting cause by *B. cinerea* on the lower side of the head

Fig. 14. Rotting cause by *Rhizopus* sp. on the lower side of the head

Fig. 15. Rotting cause by *Rhizopus* sp. on the upper side of the head

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