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# Der Einfluss der Infektion mit *Xanthomonas phaseoli* var. *fuscans* (Burkh.) Starr et Burkh. auf die Peroxydase-Aktivitaet bei anfaelligen und resistenten Bohnensorten

Özden ÇINAR

Lehrstuhl für Pflanzenschutz, Landwirtschaftliche Fakultät der Çukurova Üniversitesi,

ADANA, TURKEI

## ZUSAMMENFASSUNG

In sieben Bohnensorten bzw. -zuchtlinien unterschiedlicher Anfaelligkeit wurde der Einfluss der Infektion auf die Peroxydase-Aktivitaet untersucht. Drei Tage nach Infektion wurde die höchste relative Peroxydase-Aktivitaet in der Zuchtlinie mit der höchsten Resistenz und die niedrigste Aktivitaet in der anfaelligsten Sorte gefunden. Zu diesem Zeitpunkt hatten nur die infizierten resistenten oder schwach resistenten Sorten eine höhere Peroxydase-Aktivitaet als die gesunden.

## EINLEITUNG

Die biochemischen Ursachen der Resistenz gegen Bakterienkrankheiten sind noch wenig bekannt. Wie bei anderen Pflanzenkrankheiten (KIRALY und FARKAS, 1962) hat man versucht, auch bei der Resistenz gegen Bakterien Beziehungen zur Peroxydase-Aktivitaet zu

finden (z.B. LOVREKOVICH et al. 1968). Durch die folgenden Versuche sollte festgestellt werden, ob zwischen dem Resistenzgrad der verschiedenen Bohnensorten und Zuchtlinien gegen *Xanthomonas phaseoli* var. *fuscans* und der Peroxydaseaktivitaet nach Infektion eine Korrelation besteht.

## MATERIAL und METHODE

Nach Anzucht der Bohnenpflanzen im Gewächshaus wurden die Fiederblätter der vier Wochen alten Pflanzen mit drei Tage alten Bakterienkulturen (ca.  $10^8$  zellen/ml) mit Hilfe einer Spritzpistole infiziert. Die Suspension wurde vorsichtig von der Blattunterseite in die Spaltöffnungen gepresst, ohne dass die Blätter verletzt wurden. Es wurde darauf geachtet, dass die gesamte Blattfläche gleichmäßig infiltriert wurde. Zur Kontrolle wurden die Blätter in gleicher Weise mit Wasser infiltriert. Die Pflanzen wurden dann im Gewächshaus ( $\varnothing$  20°C, max 28°C und min 12°C, 40-70 % Luftfeuchtigkeit) aufgestellt. Es wurden 5 verschiedene Sorten, bzw. Zuchtstämmen von *Phaseolus vulgaris* untersucht: 02 und 017, resistent; 09, schwach anfällig; Red Kidney, sehr anfällig. Zur Infektion diente das Bakterienisolat T<sub>4a5</sub> von *Xanthomonas phaseoli* var. *fuscans*.

**Aufarbeitung** : Jeder Versuche wurde mit zwei Wiederholungen durchgeführt. Zwei Stunden vor dem Probenehmen wurden die Pflanzen gründlich gegossen. Die Probe bestand aus 1 g Frischgewicht (Blatt ohne Stiel). Sie wurde im auf 0°C gekühlten Bühler-Homogenisator zusammen mit 6 ml Phosphat-Puffer (0,1 M pH 6,0) 1 min, homogenisiert. Der gewonnene Extrakt wurde 30 min bei 4°C und 9750 g zentrifugiert. Der Überstand wurde mit kaltem Extraktionspuffer 1:4 verdünnt. Die-

ser verdünnte Überstand diente zur Aktivitätsbestimmung.

**Proteinbestimmung** : 0,5 ml des unverdünnten Pflanzenextraktes wurden mit 0,5 ml Trichloressigsäure (10 %) vermischt und im Kühlraum aufbewahrt. Danach wurden die Proben 20 min bei 5500 g zentrifugiert. Nach der Dekantierung des Überstandes wurden die Niederschläge mit quartzdest. Wasser in Reagenzglasern gespült und 0,5 ml 10 NH<sub>2</sub>SO<sub>4</sub> hinzugefügt. Die Reagenzglaserner wurde in Aluminiumheizblöcken erst auf 75°C, später auf 100°C solange erwärmt, bis das ganze Wasser verdampft war, dann wurde die Temperatur bis auf 150°C erhöht. Um die Reaktion zu beschleunigen, wurde 30 % H<sub>2</sub>O<sub>2</sub> zugegeben. Vor der Zugabe von H<sub>2</sub>O<sub>2</sub> wurden die Proben auf 50°C abgekühlt und nach einem Tropfen Zugabe wieder auf 150 °C erwärmt, bis die Lösung klar war. Nach dem Abkühlen wurden zu jedem Röhrchen 2 ml quartzdest. Wasser gegeben und geschüttelt. Von jedem Röhrchen wurden für die Stickstoffbestimmung 0,25 ml entnommen.

Zur Stickstoffbestimmung (nach JOHNSON 1941) wurden Reagenzglas 1,75 ml quartzdest. Wasser, 2 ml Farbreagenz (das Farbreagenz enthält pro Liter: 4 g KI, 4 g H<sub>2</sub>I<sub>2</sub> und 1,75g Gum ghatti) und 3ml 2N NaOH gegeben und nach dem Schütteln 15 min stehen gelassen. Dann wurde die Extinktion im Spektralphotometer (Baush-Lomb Spectronic-20) bei 590

nm gemessen. Zur Herstellung einer Eichkurve wurden für jede Bestimmung 0,320 N<sub>2</sub> (als (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>) in der gleichen Weise mit Schwefelsäure erhitzt und dann mit Farbreagenz vermischt. Der Proteingehalt wurde durch Multiplikation der N-Werte mit dem Faktor 6,25 errechnet.

**Peroxydase - Bestimmung :** Die Aktivitätsbestimmung erfolgte im Spektralphotometer (Bausch - Lomb Spectronic-20) bei 470 nm folgenden Reaktionslösung: 3,6 ml H<sub>2</sub>O, 0,1 ml Guajacol (0,02 M), 1 ml Phosphatpuffer (0,1 M pH 6,0), 0,1 ml Pflanzenextrakt, 0,4 ml H<sub>2</sub>O<sub>2</sub> wurden für jeden Versuch frisch angesetzt. Vor Zugabe von H<sub>2</sub>O<sub>2</sub> wurde das Gemisch 30 sec in ein 37°C Wasserbad gestellt und die optische Dichte des Gemisches auf Null eingestellt, dann H<sub>2</sub>O<sub>2</sub> zugegeben, gleichzeitig die Stoppuhr in Gang gesetzt, geschüttelt und alle 30 bis zu 250 sec abgelesen.

**Auswertung :** Der Anstieg der Extinktion zwischen 1,5 und 2,5 Minuten nach Reaktionsstart wurde verglichen und auf Frischgewicht sowie Gesamtprotein bezogen, da die Extinktion in diesem Zeitraum linear anstieg.

#### ERGEBNISSE

Die Peroxydase - Aktivitäten wurden bei den oben beschriebenen Stämmen 02, 017, 09 und Red Kidney (RK), sowie der anfälligen Sorte PI 181 954 (181) untersucht. 12 Stunden nach Infektion (Abb. 1) zeigte sich noch keine Korrelation Resistenz und infektionsbedingter

Aktivitätsänderung. Innerhalb der nächsten 12 Stunden stieg die Peroxydase-Aktivität bei den beiden resistenten Stämmen (02 und 017) im Vergleich zu gesunden Pflanzen deutlich an (Abb. 1), bei den drei anderen fiel die relative Aktivität ab oder blieb gleich. Danach fiel die relative Peroxydase-Aktivität nur bei der hoch-anfälligen Sorte RK bis zum 5. Tag weiter ab, bei allen anderen Sorten nahm sie zu. Eine Korrelation zwischen Resistenz und infektionsbedingter Aktivitätszunahme zeigte sich besonders deutlich am 7. Tag nach Infektion. Am 3. und 5. Tag nach Infektion verhielt sich der Stamm 09 nicht ganz entsprechend der Resistenz, da er einen infektionsbedingten Peroxydase-Anstieg ähnlich wie 02 und 017 zeigte. Bei den übrigen Sorten entsprachen die Unterschiede der relativen Peroxydase-Aktivität den Unterschieden in der Resistenz.

#### DISKUSSION

Obwohl sich die Symptome nach Infektion mit *Xanthomonas phaseoli* var. *fuscans* im Vergleich zu anderen Bohnenbakteriosen relativ langsam entwickeln, kann man schon am 3. Tag nach Infektion auf Grund der Symptome Unterschiede in der Resistenz erkennen.

Wenn Unterschiede in der Peroxydase-Aktivität Beziehungen zur Resistenz haben, müssen sie daher bis zum 3. Tag in Erscheinung treten. Spätere Unterschiede in der infektionsbedingten Peroxydase-Aktivität

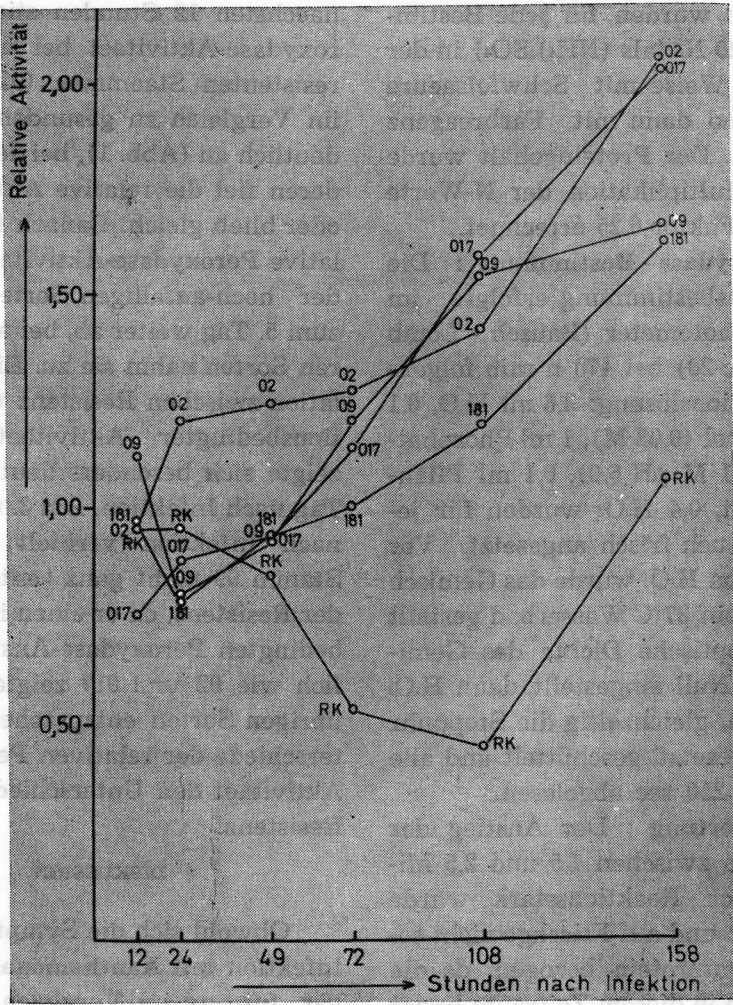


Abb. 1. Relative Aktivitaet der Peroxydase, bezogen auf mg Protein.

taet müssen als Sekundaererscheinungen aufgefasst werden.

Innerhalb dieser ersten drei Tage stieg die relative Peroxydase-Aktivitaet nur bei den beiden resistenten Staemmen 02 und 017 seit dem ersten gemessenen Zeitpunkt (12 Stunden nach Infektion) kontinuierlich an, waehrend sie bei den anderen Sorten abfiel oder gleich blieb.

Am 3. Tag nach Infektion zeigte sich bis auf den Stamm 09 eine Korrelation zwischen den Unterschieden in der relativan Peroxydase-Aktivitaet und Resistenz.

HERZMANN hatte 1958 bei mit Brennflecken befallenen Bohnen festgestellt, dass die Peroxydase-Aktivitaet der Kranken Pflanzen stets höher als die der gesunden war.

Auch bei vielen anderen Wirt-Parasit-Paaren wurden Beziehungen zwischen Peroxydaseanstieg und Resistenz vermutet (FARKAS und KIRALY (1962) und TOMYAMA (1963).

Bei Bakterienkrankheiten wurden ebenfalls Hinweise für Beziehungen zwischen Peroxydase-Aktivität und Resistenz gefunden: DEVERALL and WALKER (1963) fanden in gesunden Pflanzenextrakten von gegen *Ps. phaseolicola* resistenten Zuchtstämmen eine höhere  $O_2$ -Aufnahme im Vergleich zu anfälligen Zuchtstämmen. Später identifiziert EPON und DEVERALL (1968) eine Lipoxydase, die für die höhere  $O_2$ -Aufnahme verantwortlich war. Das Hydroperoxyd, das als Produkt der Lipoxydation von Linolensäure entsteht, kann durch die Cytchrom-C-Peroxydase verwertet werden (SAUNDERS et al. 1964). Die C-Peroxydase kann auch als normale Peroxydase gemessen werden und wurde durch unseren Test mit erfasst.

RUDOLP und STAHMANN (1964) fanden bei dem System *Phaseolus vulgaris/Ps. phaseolicola*, dass in infizierten und während der ersten Tage nach Infektion höher lag als in anfälligen Pflanzen.

LOVREKOVICH et al. (1968) fanden ebenfalls eine positive Korrelation zwischen Peroxydase-Aktivität und Resistenz im System *Nicotiana tabacum/Ps. tabaci*, die auch vom Alter der Pflanzen abhängig war. Durch Injektion von Handels-

präparaten von Peroxydase in die Blätter konnte die Resistenz erhöht werden. Ebenso konnten durch Injektion von zell-freien Bakterienpräparaten Peroxydase-Aktivität und Resistenz erhöht werden.

Beziehungen zwischen Peroxydase und Resistenz deuten sich auch in unseren Versuchen an.

Wir haben am 3. Tag nach der Infektion die höchste relative Peroxydase-Aktivität in dem resistenten Stamm 02 und die niedrigste Aktivität in der anfälligen Sorte Red Kidney gefunden. Zu diesem Zeitpunkt hatten nur die resistenten oder schwach resistenten Stämme (02, 017 und 09) in der erkrankten Pflanze eine höhere Peroxydase-Aktivität als in der gesunden. Es scheint daher so, als ob Peroxydase-Aktivität eine enge Beziehung zu einer Abwehrreaktion hat, deren Geschwindigkeit innerhalb der ersten drei Tage sicher eine Rolle spielt. Je früher das Wirtsgewebe gegen den Erreger reagiert, desto resistenter wird die Pflanze. Als Beispiel kann man 02 anführen. 02 reagiert schon 12 Stunden nach der Infektion. Demgegenüber zeigt die anfällige Sorte 181 erst ab 24 Std nach der Infektion eine positive Reaktion. Je stärker nun diese Reaktion ist, umso resistenter ist die Pflanze, so gezeigt am Vergleich 181, 09, 017. Die Frage bleibt jedoch offen, ob die erhöhte Peroxydase-Aktivität nur eine Folge der Resistenzreaktion oder die Ursache der Resistenz ist.

ÖZET

DUYARLI ve DAYANIKLI FASULYE ÇEŞİTLERİNDE *Xanthomonas phaseoli* var. *fuscans*'ın PEROKSİDAZ AKTİVİTESİ ÜZERİNE ETKİSİ İLE İLGİLİ ARAŞTIRMALAR

Duyarlı ve Dayanıklı Fasulye Çeşitlerinde *Xanthomonas phaseoli* var. *fuscans* (Burkh.) Starr et Burkh ile Enfeksiyonun Peroksidaz Aktivitesi Üzerine Etkisi farklı duyarlık gösteren 5 fasulye çeşidinde veya safhattında araştırılmıştır. Enfeksi -

yondan üç gün sonra en dayanıklı safhatta en yüksek ve en duyarlı fasulye çeşitinde en düşük peroksidaz aktivitesi bulunmuştur. Bu zaman içinde efekteli ve dayanıklı veya az dayanıklı çeşitler sağlamlara göre daha yüksek peroksidaz aktivitesi göstermiştir.

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## Die wichtigste Krankheiten der Kichererbse in Mittelanatolien \*

Haluk SORAN

Lehrstuhl für Pflanzenschutz, Landwirtschaftliche Fakultät der Ankara Universität,  
Ankara, TURKEY

### ZUSAMMENFASSUNG

Um die wichtigste Krankheit festzustellen, wurde im Jahre 1973 die Kichererbsenanbauggebiete der Provinzen von Ankara, Afyon, Burdur, Çorum, Eskişehir und Kütahya untersucht. An allen Gebieten wurde Brennfleckenkrankheit und Wurzelfäule als wichtigste Krankheiten beobachtet.

Aus den gesammelten Bodenproben und erkrankten Pflanzen wurde *Ascochyta rabiei* als Erreger der Brennfleckenkrankheit, *Pythium ultimum*, *Fusarium oxysporum* und *Fusarium acuminatum* als Erregern von Wurzelfäule isoliert.

Bei Pathogenitätstesten wurde festgestellt, dass die Isolate von *Ascochyta rabiei* stark pathogen sind. Die Pathogenität der *Pythium* Isolate schwankte zwischen 7-100 %. Die *Fusarium* Isolate haben eine Pathogenität von 0-50 % gezeigt.

### EINLEITUNG

Kichererbse (*Cicer arietinum*) wird in Mittelanatolien und auch in den gleichen rockenklimagebieten angebaut, um Bodenfruchtbarkeit zu schützen, oder zu steigern, und auch um Brachfelder zu benutzen. In der

Türkei wird jedes Jahr 180 000 Hektar Kichererbse angebaut und an diesen Gebieten 183 000 Tonnen Ertrag erhalten (1).

Aus diesen Ertrag wird ca 25 000 Tonnen exportiert. Mittelanatolien

\*) Vortrag gehalten auf dem ersten phytopathologischen Kongress in İzmir, 20-24. Oktober. 1975

bringt alleine 30 % des ganzen Ertrags und in diesem Gebiet wird der Ertrag oft durch die Krankheiten begrenzt. Besonders in den feuchten Jahren wie 1972 und 1975 konnte an vielen Anbaugebieten und auch an Versuchspartzen überhaupt keinen Ernteverfahren durchgeführt werden.

Auch in vielen Kichererbsenanbaugebieten der Erde wie Indien, Iran und Israel werden die Krankheiten als wichtigste Begrenzungsfaktor angesehen (7,10,11,13,14,15).

Eine Literaturüberblick laesst erkennen, dass sich je nach Umgebungsverhaeltnissen viele verschiedene Erregern bei der Krankheiten beteiligen (3,4,5,6,9,12).

In der Türkei waren zwar einige Krankheiten bei Kichererbse festgestellt (2, 8), doch wurden keine grundlegenden Untersuchungen darüber angestellt.

In dieser Arbeit wird versucht, die wichtigsten Krankheiten der Kichererbse im Mittelanatolien festzustellen.

#### MATERIAL und METHODIK

In den Monaten Juli und August, 1973 wurden in Mittelanatolien 30 Kichererbsenfelder ausgewaehlt. Aus diesen Feldern wurden Boden und erkrankter Pflanzen-Proben gesammelt und nach ihrer Pilzflora untersucht.

#### 2.1 Methoden für die Probenentnahmen

Bodenproben wurden mittels eines Handschaufeles aus 0-15 cm Tiefen auf Diaganalen durch die Felder vorgenommen. Die einzelne Probe umfasste etwa 2 L. Boden. In der gleichen Zeit wurde auch die erkrankten Pflanzen mit ihren ganzen Wurzeln gesammelt.

#### 2.2 Isolationstechnik

Die gesammelten Bodenproben wurden jeweils 3 Töpfen (8 cm ø)

Auf jeden Topf wurden 5 Samen ausgelegt, die 15 min. in 7,5 % H<sub>2</sub>O<sub>2</sub> sterilisiert waren.

Waehrend eines Monats wurden im 5 taegigem Abstand sichtbar kranke Pflanzen ausgenommen und nach ihren Pilzbesatz untersucht.

Die erkrankten Wurzeln wurden mit Leitungswasser gut gewaschen und in eine 40 ppm Aureomycin-Lösung (3-5 min.) eingetaucht. Nach mehrfachem weiteren Waschen mit sterilem Wasser wurden die Wurzeln in 1 mm lange Partikel geschnitten und auf Wasseragar (1,5 g Agar, 100 ml dest. Wasser) ausgelegt.

#### 2.3. Pathogenitaetsteste

##### 2.3.1 Für *Ascochyta rabiei*

Die Vermehrung der zu testenden Isolate erfolgte auf Kichererb-

senmehl-Agar (3 g Kichererbsenmehl, 1,5 g Agar, 100 ml dest. Wasser) in Petrischalen. Nachdem die reichlich Pyknidien gebildet waren, wurden mit sterilem Wasser Sporensuspension vorbereitet und mit einem Laborsprühgeraet auf 20 tage alten Pflanzen gespritzt.

### 2. 3. 2 Für die übrigen isolierten Pilze

Die Vermehrung der Pilze erfolgte auf PBA (1,5 g Biomalz, 1,5 g Agar, 0,25 g Pepton, 100 ml dest. Wasser) in Petrischalen. Nachdem diese

voll bewachsen waren, wurden Scheiben mit 7 cm von dem mit Pilzen bewachsenen Agar ausgeschnitten und in einem Blumentopf (8cm $\emptyset$ ) gelegt, der zu 2/3 mit gedampfter Komposterde gefüllt war, und mit einer 1 cm hohen Schicht der gleichen Komposterde bedeckt. Auf diese wurden pro Topf 5 mit H<sub>2</sub>O<sub>2</sub> desinfizierte Samen ausgelegt, mit weiteren 2 cm Komposterde bedeckt.

Während eines Monats wurden im 5 taegigem Abstand kranke Pflanzen ausgezählt.

## ERGEBNISSE

Die im Jahre 1973 in Mittelanatolien durchgeführten Beobachtungen haben gezeigt, dass an allen Anbaugebieten Brennfleckenkrankheit und die Wurzelfaeule schaedlich und weitverbreitet sind. Kichererbsenrost wurde nur in zwei Feldern in der Umgebung von Eskişehir beobachtet.

### 3. 1 Brennfleckenkrankheit der Kichererbse

Erreger der Brennfleckenkrankheit ist *Ascochyta rabiei* (Pass) Lab. Die Krankheit wird hauptsaechlich mit Samen übertragen. Legt man erkrankte Samen aus, so zeigt sich die Krankheit bei genügender Feuchtigkeit an den jungen Stengeln der Keimpflanzen (Abb. 1); haeufig gehen solche Keimpflanzen ein.

In vielen Faellen wird sie aber

bis zu einem gewissen Grade überwunden. An solchen Pflanzen bildet der Pilz Pyknidien und Pyknidiosporen (Abb. 2), von denen aus sich die Krankheit ausbreiten können.

Die Pyknidiosporen können allen oberirdischen Teile der Pflanzen infizieren.

An Blaettern findet man haeufig braune oder rotbraune, etwas eingesunkene Flecken, die zu weilen kreisrund sind, meist aber unregelmässige Form haben.

Die Grösse der Flecken schwankt von wenigen Millimetern bis etwa 1 cm Durchmesser, doch können auch mehrere Flecken miteinander verschmelzen (Abb. 3).

Ein ganz aehnliches Krankheitsbild zeigt sich an Hülsen (Abb. 4). Samen vielfach braune Flecken aufweisen.

DIE WICHTIGSTE KRANKHEITEN DER KICHERERBSE IN MITTELANATOLIEN

Die Krankheit breitet sich im Feld von Infektionsstelle aus und bildet Infektionsherde (Abb. 5).

Günstige Vorbedingungen für die Ausbreitung der Krankheit sind vor allem in feuchten Sommern gegeben. Bei erkrankten Pflanzen werden nach frühzeitigem Blattfall und Hülsen Infektionen nur kleine unreife Samen gebildet (Abb. 6).

### 3.2 Wurzelfaeule der Kichererbse

Wurzelfaeule ist die zweite wichtigste Krankheit bei allen Anbaugebieten. Auf fast allen Kichererbsenschlägen kann man bald nach dem Auflaufen junge Keimpflanzen finden, die gelb werden, umfallen und eingehen. Die nähere Untersuchung derartiger Pflanzen ergibt, dass Hauptwurzel und Sten-

gelgrund schwarze Flecken zeigen. Im späteren Stadien sieht man auf den Feldern einzelne gewelkte Pflanzen (Abb. 7).

Um die Wurzelfaeuleerregern festzustellen wurden unter Gewächshausbedingungen in 30 Bodenproben Kichererbsen ausgesäet und auch aus den Feldern erkrankten Pflanzen gesammelt und nach ihren Pilzbesatz untersucht. Die Ergebnisse sind aus der Tabelle 1 zu sehen. Aus unter Gewächshausbedingungen erkrankten 56 Pflanzen wurde 62% **Pythium**, 18% **Fusarium** und 20% andere Pilz-Gattungen isoliert. Dagegen wurden bei aus den Feldern gesammelten 50 erkrankten Pflanzen 14% **Pythium**, 68% **Fusarium** und 18% andere Pilz-Gattungen festgestellt.

Tabelle 1 : Beteiligung der Wurzelfaeuleerregern bei der Krankheit

Untersuchten Pflanzen (bzw. Partikeln)	Die Pilze							
	Pythium		Fusarium		Andere Pilzarten			
	Zahl	%	Zahl	%	Zahl	%		
Unter Gewächshaus Bedingungen	56	(56)	35	62	10	18	11	20
Unter Feld Bedingungen	50	(250)	34	14	169	68	47	18

3.3 Pathogenitaet der Isolate

Insgesamt wurden waehrend unserer Versuche 306 Isolate aus kranken Pflanzen gewonnen. Aus drei wahrscheinlich pathogenen Gruppen wurden jeweils eine groessere Zahl von Isolaten zufallsmaessig ausgewählt und getestet (Tab. 2). Die isolierten 10 **Ascochyta** Isolate erwiesen sich inder geprüften Auswahl saemtllich als hochpathogen.

Pathogenitaet der **Pythium** Iso-

late schwankte zwischen 7-100%. Jedoch lag bei 90% der Staemme eine Pathogenitaet von über 50 % vor. Eine mykologische Untersuchung hat gezeigt, dass die hoch pathogene Isolate **Pythium ultimum** waren.

Bei **Fusarium** Isolate variierte die Prozentzahl der Pathogenitaet von 0 bis maximal 50. Die pathogene Isolate wurden als **Fusarium oxysporum** festgestellt. Ein Isolat von **Fusarium acuminatum** hat 45 % Pathogenitaet gezeigt.

SCHLUSSBETRACHTUNG

Aus unseren Untersuchungen geht hervor, dass unter mittelanatolischen Bedingungen 2 Gruppen von Krankheiten bei Kichererbse Rolle spielen : Eine ist Brennfleckenkrankheit, die duch den Pilz **Ascochyta rabiei** hervorgerufen wird, jedes Jahr überal zu finden. Ihre Verbreitung

und Schaedigung sind stark mit dem sommerlichen Regen abhaengig. In regnerischen Jahren tritt die Krankheit in grösseren Masse auf und ruft schwere Schaden hervor. Dagegen bleibt bei trockenen Jahren mit einzelnen Pflanzen begrenzt.

Tabelle 2 : Pathogenitaet der Isolate

Pilze	Untersuchten Isolate	Prozentuale Proz. Anzahl von Isolaten mit über 5 % Pathogenitaet		
		Min	Max	
<b>Ascochyta</b>	10	100	100	100
<b>Pythium</b>	10	7	100	90
<b>Fusarium</b>	20	0	50	0

Von der in unseren Bedingungen festgesellten Wurzelfaeuleerregern **Pythium ultimum** spielt hauptsächlich am Anfangsstadien der Pflanze

als Wurzelbranderreger grosse Rolle, waehrend die **Fusarium**-arten noch mehr in spaeteren Stadien isoliert werden.

## Ö Z E T

## ORTA ANADOLU'DA ÖNEMLİ NOHUT HASTALIKLARI

Önemli hastalıkları saptamak amacıyla 1973 yılında Ankara, Afyon, Burdur, Çorum, Eskişehir ve Kütahya illeri nohut ekim alanları gezilmiş bütün bölgelerde Antraknoz ve Kök çürüklüğü hastalıklarının etkin ve yaygın olduğu görülmüştür.

Bölgelerden alınan hasta bitki ve toprak örneklerinin incelenmesinden Bölgede yaygın Antraknoz hastalığının etmeni **Ascochyta rabiei**, kökçü-

rüklüğü etmenlerinin de **Pythium ultimum**, **Fusarium oxysporum**, **Fusarium acuminatum** olduğu anlaşılmıştır.

Elde edilen izolatlarla uygulanan testlerde, bütün **Ascochyta** izolatlarının %100, **Pythium** izolatlarının %7 - 100, **Fusarium** izolatlarının da %0 - 50 oranları arasında patojen oldukları saptanmıştır.

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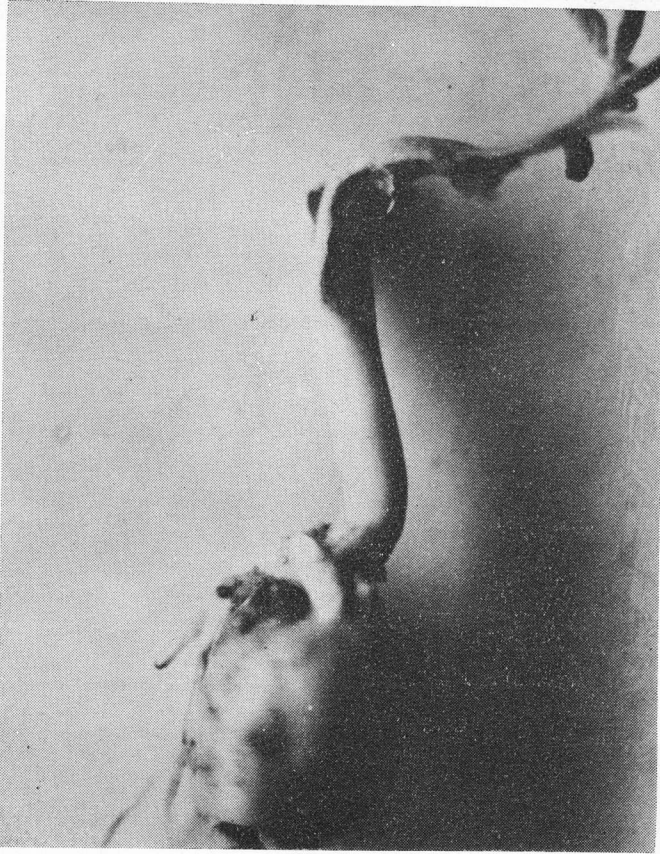


Abbildung 1 *Ascochyta rabiei* auf Kichererbsenkeimling

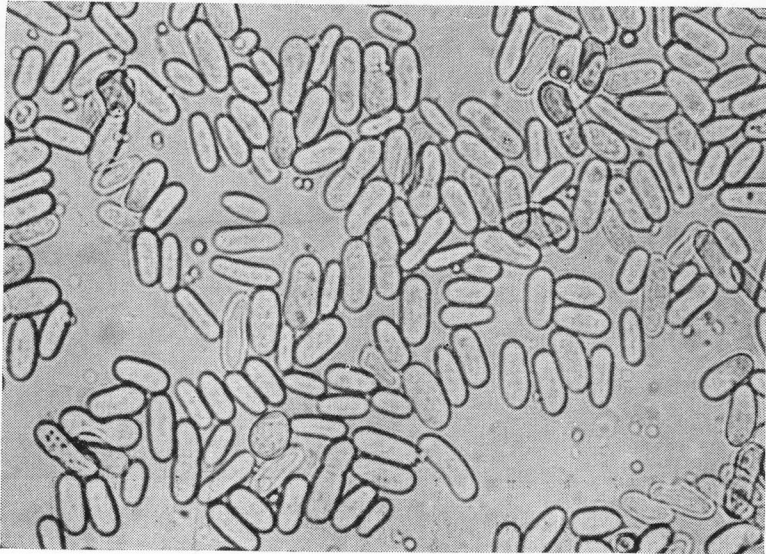


Abbildung 2 Pyknidiosporen von *Ascochyta rabiei*

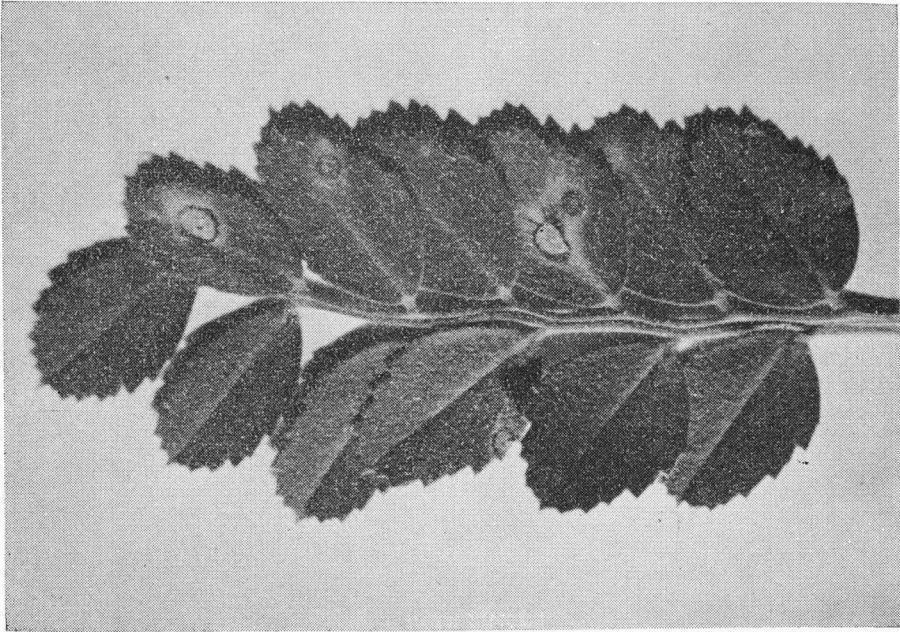


Abbildung 3

*Ascochyta rabiei* auf Kichererbsenblättern

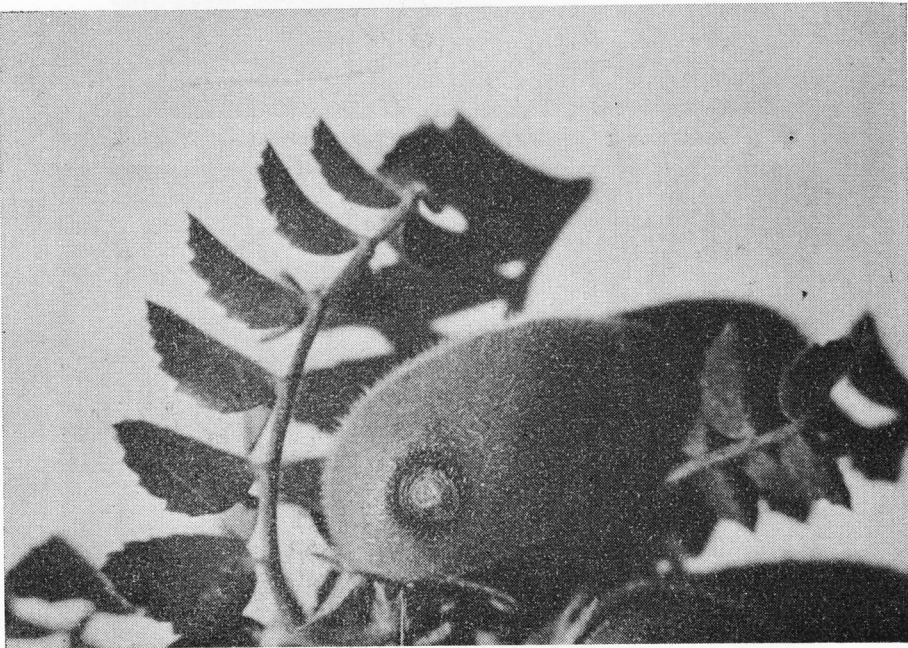


Abbildung 4

*Ascochyta rabiei* auf Kichererbsenhülsen



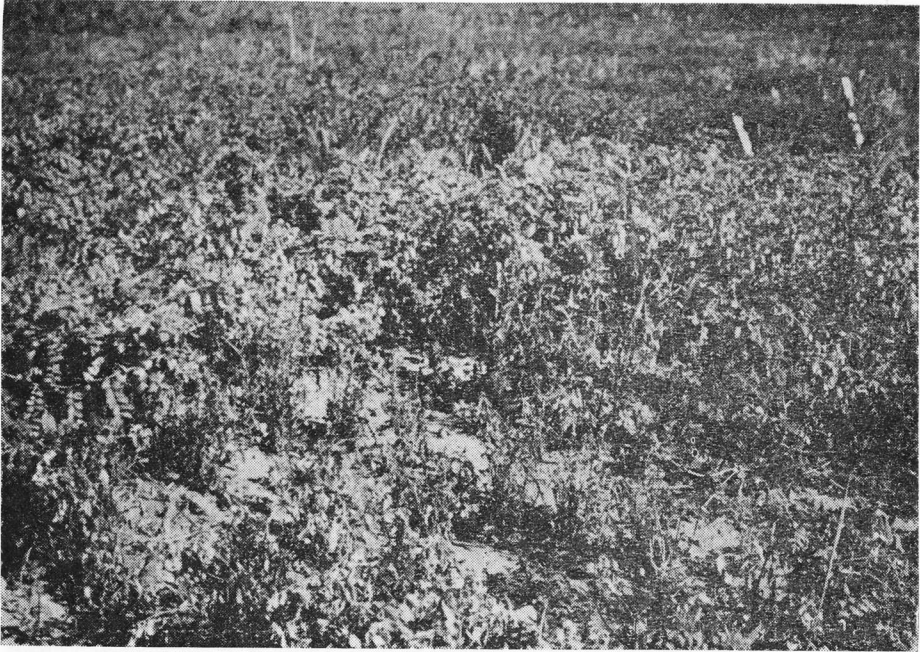


Abbildung 5

**Ascochyta rabiei** Infektionsherde im Feld

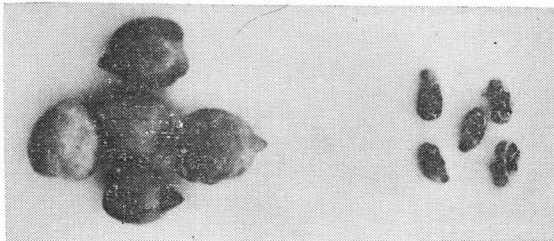


Abbildung 6

Aussehen der Kicherebsensamen nach der  
Infektion von **Ascochyta rabiei**  
Links: Gesund, Rechts: Infiziert



Abbildung 7

Kichererbsenwelke nach der Infektionen von  
Wurzelfaeulerregern

INTRODUCTION

Pear rust disease (*G. fuscum* D.C.) is distributed almost everywhere in this country. Therefore in this study, the economic importance of the disease is investigated in order to present the results to pear growers in Elazığ and Malatya provinces.

## Researches on Pear Rust Disease (*Gymnosporangium fuscum* D.C.) in Elazığ and Malatya

Necmettin DİNÇ(1) and İbrahim KARACA(2)

MATERIALS and METHODS

ABSTRACT

These experiments were carried out during 1967, 1968 and 1969 in the region of Elazığ and Malatya which are located in the eastern part of Turkey. The purpose of these experiments was to determine the morphology and biology of *Gymnosporangium fuscum* D.C. and also to make a survey on the distribution of the disease.

This investigation indicated that to percentage yield losses varied between 9.69 percent and 100 when the percentage of the infection was 4,26 % and 100 % respectively.

In the biological tests with early basidiospore infections generally occurred between April 15 and May 5 when the pear trees were mostly in blooming stage. Fruit infections occurred only on early pear varieties.

The incubation period of the fungus was determined as between 9 and 26 days, on the pear leaves in natural and artificial trials.

Appearance of spermagonia, tumours, pseudoperidia and aeciospores of the fungus were observed 22-29 days, 78-89 days, 131-141 days and 147-159 days after the infections respectively.

Under natural conditions aeciospores were carried by the wind to the juniper trees and persisted on these trees until spring. Incubation period of the fungus was determined as 5-6 months on junipers.

Increasing of the infection was related with the distance between pear and juniper trees, obstacles in this place, the direction of the wind while the basidiospores are being carried by the air and susceptibility of the pear varieties.

(1) : Regional Plant Protection Research Institute, Diyarbakır, TURKEY

(2) : Department of Phytopathology and Agricultural Botany University of Ege, İzmir, TURKEY

INTRODUCTION

Pear rust disease (*G. fuscum* D. C.) is distributed almost every part of Turkey (Fig. 1). It causes yield lost between 4,26% and 100% depend upon the density of the disease.

Some results were given by Bremer and Göksel(1950), Bremer(1954) Göksel(1952) and Levendoğlu(1959).

But there is not any completely study on the disease. Therefore in this study the role of the alternate hosts, economic importance of the disease and some other aspects have been investigated in order to present the biology of the disease for Elazığ and Malatya provinces.

MATERIALS and METHODS

Artificial inoculations have been made on *Prunus elaeagnifolia* Pall. and pear varieties as İmam, Biber, Gök, Kadıkafası, Beurre-cleraigo, Sarı, Williams, Kışbeyi, Kütdüßen, Bal Hocamız, Limon, Abbasi, Göksulu, Eğrisap. The researches on telial period of *G. fuscum* D.C. were done on a susceptible host *Juniperus oxycedrus* L. Countings for the distribution of the disease were made on different geographical and microclimatological areas which were accepted as a unit. The following scale was prepared and used during the survey :

- 0 - There is no spot on leaves.
- 1 - Two spots smaller than 10 mm. or one spot bigger than 10 mm. diameters (Spots covered 3 % area of leaves)
- 11 - 3 spots smaller than 10 mm. or 2 spots bigger than 10 mm. diameters (Spots covered 5 % area of leaves)
- 111 - 4 spots smaller than 10 mm. or 3 spots bigger than 10 mm.

diameters (Spots covered 10 % area of leaves).

IV - 5-15 spots. (Spots covered 25 % area of leaves)

V - 16-20 spots. (Spots covered 50 % area of leaves)

VI - 21-30 spots. (Spots covered 75% area of leaves)

VII - 31 and more spots. (Spots covered more than 75 % area of leaves).

In order to determine the correlation between fungal density and the degree of damage, experiments were carried out in the orchards.

The inoculum which used for artificial inoculations was obtained from the telial sorus of *J. oxycedrus* L. Inoculations made on pear trees were shown on Fig. 2.

Inoculum of aeciospores which were obtained from aecidia of pear leaves was inoculated to *J. oxycedrus* L., *J. excelsa* Bieb. and *J. foeditissima* Willd.

Mycelium were painted with (lactophenol + Cotton blue) and cuticula with (lactic acid + Sudan - III) also spermatia with cotton blue + acetic acid).

Preparations were made with

(gliserin + jelatin) and surrounded with Canada balsame.

Aeciospores were germinated in steril medium of bacto-agar. Teliospores and basidiospores were produced on telial galls in porselen potas.

**RESULTS**

The region where the survey was conducted can be classified according to the degree of the disease: Slightly infected areas: Darende, Hekimhan, Keban, Baskıl; moderately infected areas: Akçadağ, Yeşilyurt, Arapgir, Palu and severly infected area Doğanşehir. According to these results

the averages of the disease severity were 12,29 % for Malatya and 4.99 % for Elazığ.

This investigation indicated that yield losses vary between 9.69 % and 100 % when the percentages of the infection are 4,26 % - 100 % respectively.

In morphological studies the following measurements were found:

Spermogonia	156,85 X 129,25	microns (Fig.3)
Spermatia	2,50 X 6,45	»
Aeciospores	20,67 X 26,42	»
Tliosporos	26,45 X 45,73	» (with colour membrane)
»	20,02 X 47,10	» ( » incolour » )
Basidiospores	10,54 X 13,73	»

In the biological tests it was found that the early basidiospores infections occur under natural conditions between 15/April and 7/May. Generally during this period pear trees are mostly in blooming and their leaves are in normal shape.

The early infections are the most important ones occur in this period. It was determined that disseminations basidiospores and their infections on pear trees take place from the middle of April up to the end of

May and continued approximately for a period of 40 days.

Fruit infections occurred only on early pear varieties such as Limonarmudu. According to the observations performed at room temperature with inoculated pear leaves and shoots, previously placed in glass, the following results were obtained : Eighty five percent and 95 % of basidiospores were germinated after 12 and 24 hours respectively. But appressorium was produced at 80 percent 12 hours after the inoculation

When the leaves have become approximately 1 - 2 centimeters in length highest infections were occurred. This is related to the thickness of the cuticula noticed during the observations made on different stages of development of the leaves. When the leaves are approximately 1-2 centimeters in size the cuticula are not formed yet. The formation of the cuticula starts when the pear trees in pink stage and the thickness of the cuticula reaches to 2 microns when the fruit becomes 5 millimeters in size

In order to determine the incubation period of *G. fuscum* D.C. on the leaves of pear trees both artificial and natural trials and studies were carried-out and the incubation period is varied between 9 to 26 days. This period is shortened when the weather temperature increases. For instance the incubation period takes only 8 days at 18,5 C° in room conditions.

Spermogonium matured 22 - 29 days after infection. At least two spermogonia were found in one spot. Their top points were in the distance of 100 - 550 (224,80 ± 9,92) microns from each other. Taking this into account the spermogonia were facundate (-Spermatisize) each other, rather than themselves. In this event the spermatia spread to another spots leaves and other trees by insects.

It has been determined that tumours were formed 78-89 days after

the infections. This formation generally occurs from the 8<sup>th</sup> to the 31<sup>th</sup> July in the survey area.

The pseudoperidia appear 131-141 days after infections during August 27, September 19. But the ejections of the aeciospores occur 147-159 days after the infection which is between September 11 October 12 (Fig. 4).

The artificial inoculations were made on *J. oxycedrus* L. *J. excelsa* Bieb., *J. fuditissima* Wild. on September 9-13, 1968 and the galls appeared on only *J. oxycedrus* L. and *J. excelsa* Bieb. on September 3, 1969. Under the natural conditions after aeciospore ejections, they were carried by the wind to the juniper trees and they persisted on the junipers until spring.

Infections occurred in the spring and new galls appeared in September. According to this result incubation period of *G. fuscum* D.C. on the junipers is determined to be 5 - 6 months.

During the aeciospore germination experiments, a maximum percentage of germination (45 %) was obtained, when aeciospores were kept in incubators for six days at 3°C and after that, six days at 18°C.

Teliospore tongues were established in late February or early March eighteen months after aeciospore inoculations on the juniper trees. Maximum tongues (telial so -

rus) developed and appeared at 20°C and maximum teliospores on the spore tongues occurred at 18-20 C°.

Maximum basidiospores occurred when the teliospores were kept 1/2 hour in dry condition, or 1 hour in water and then 10 hours in dry conditions. Maximum basidiospores and teliospores germinated at 19 - 20 C° (Fig. 5-6).

It has been determined that the aecial hosts of *G. fuscum* D.C. are *Prunus communis* L., *P. elaeagrifolia* Pall and the telial hosts are *J. oxycedrus* L., *J. exelsa* Bieb.

The results of experiments of

the artificial infection showed that the pear variety of Man and Ahlat become susceptible to *G. fuscum* D. C. But Gök, Kadıkafası, Beurre-cleraigo, Sarı, Williams, Kışbeyi, Küt-düşen, Bal hocamız, Limon, Abbasi, Göksulu and Eğrisap were very susceptible.

A red colour occurred around the sporangium spots on the leaves of limon and Biber pear varieties. This redness is the characteristic indication for these infected varieties.

The Göksulu pear variety lost the most leaves and the Kadıkafası lost the fewest leaves among all infected varieties.

#### DISCUSSION

The density of the disease in Malatya is higher than Elazığ because pear orchards and juniper trees are more close to each other in Malatya than in Elazığ.

The density of pear rust on pear trees are depend upon : 1) The distance between junipers and pear trees. 2) Density of disease on junipers. 3) Air currents during the basidiospores ejection.

It has been established that the first basidiospores infections occurred in the period between middle of April and at the beginning of May. These results are in accordance with Bremer (1954), Göksel (1952), Bernaux (1956) and Levendoğlu (1959).

Infections continue 40 days, however Göksel (1952) has established 60 days in Ankara province. The elevation in Elazığ and Malatya is higher than Ankara province. For this reason, the winter period of Elazığ and Malatya is longer than Ankara and also spring period is shorter than Ankara, so infection period takes a short time.

When the cuticula of leaves become thin infections has not been increased. Also Cifferi (1945) and Bernaux (1956) have found the same results.

Bernaux (1956) has recorded that *G. fuscum* D.C. causes buds in-

fections, but this position could not be seen.

It was established that the incubation period occurred between 8-26 days. The same period has been recorded as 7 days by Oersted, 15-30 days by Cifferi, 7-8 days by Viennot-Bourgin (1949), 15-25 days by Bernaux (1956) and 13-15 days by Viennot Bourgin (1965).

The aecium tumours were shown 78 - 89 days after infections, however aeciospore ejections occurred between 11 September - 12 October. These results are the same of Bernaux (1917).

Telial sorus (Teliospores tonque) have to wait 30 minutes in water and then 10 hours in dry conditions for giving maximum basidiospores. Bernaux (1956) has shown the same results. But according to Bliss (1933) telial sorus must wait 30 minutes in water and 3 hours in dry conditions for *G. Juniperi-virginianae* Schw.

It was established that *P. communis* L., *P. elaeagnifolia* Pall. are aecial hosts. *J. oxycedrus* L. and *J. excelsa* Bieb. are telial hosts of *G. fuscum* D.C. in Malatya and Elazığ regions.

The cuticula is the most important factor on the resistance of aecial hosts against *G. fuscum* D.C. This result was given by Heald (1933) and Bernaux (1956) too.

It was not possible to separate the varieties of pear trees as immune

very resistant, resistant, susceptible, very susceptible. Because experimental species were only susceptible and very susceptible. According to my results, İmam armudu (A kind of pear variety) and Ahlat (*P. elaeagnifolia* Pall.) are susceptible, the others are very susceptible.

Gäumann (1950) has given that chlorotic leaves of pears are resistant against *G. fuscum* D.C. But there was not seen any chlorotic leaves in my experiments.

It was established that basidiospores of *G. fuscum* D.C. cause important infections when the distance of the pear trees and juniperus shorter than 1000 m. Viennot Bourgin (1949), Gäumann (1950), Bermer (1951-1954), Göksel (1953) and Bernaux (1956) also have recorded that important infections occur when this distance shorter than 1000 m. But Fischer (1949) says that the infections are not important when the distance more than 200 m.

The basidiospores can be carried as far away as 20 kilometers distance from its origin. But generally when the distance of the two hosts is above 1000 meters, the disease intensity is low and the damage is negligible.

It has been determined that the increase of the infections is subject to the following conditions: The distance between pear and junipers is very important. If the distance is shorter than 1000 meters, the degree



of infections and the damage will be increased. Obstacles like trees, hills, ext.

While the basidiospores are being carried by the air, in the case of directions of wind is toward the pear trees, the infections will be greater and if the member of infected junipers is quite high in this case infections of pear trees will reach a comparatively high level. The degree of infections increases in direct proportion to the susceptibility of the pear varieties.

For the control of pear rust the

following procedures should be carried out: The host junipers which are locally scattered around the pear plantations should be eradicated when they are 1000 meters apart from the pear trees. If the population of junipers is very large (as is the case in Kapideresi) it is suggested that pear trees not be grown in this areas or chemical control should be performed for the prevention of diseases.

Further investigations are necessary to determine suitable and economical chemical control programs.

#### Ö Z E T

### ELAZIĞ VE MALATYA İLLERİ ARMUTLARINDAKİ MEMELİ PAS HASTALIĞI ÜZERİNDE ARAŞTIRMALAR

1967-1969 yılları arasında yürütülen bu araştırmada armut memeli pas hastalığı etmeni olan *Gymnosporangium fuscum* D.C. fungusunun morfoloji ve biyolojisi incelenmiş aynı zamanda hastalığın dağılımı konusunda survey yapılmıştır.

Survey kapsamına alınan illerdeki bahçeler hastalık şiddetine göre sınıflandırılmış ve ortalama hastalık şiddeti Malatya Elâzığ da sırasıyla % 12,29 ve % 4,99 olarak saptanmıştır.

Hastalık şiddeti x verim kaybı ilişkilerinin denemelerde % 4,26-100.00 arasında değişen hastalık şiddeti değerlerine karşın ürün eksilişi-

nin sırasıyla % 9,69 - 100.00 arasında değiştiği belirlenmiştir.

Yaprak büyüklüğü ile enfeksiyon şiddeti arasında ilişki incelenmiş ve yapraklar 1 - 2 cm. çapında iken en yüksek düzeyde enfeksiyon saptanmış ve bu durum kutikula kalınlığına bağlanmıştır.

Yapay ve doğal bulaştırma denemelerinde etmen fungusun değişik dönemlerinin armut, ahlat ve ardıc ağaçlarında görünüşü süresel olarak incelenmiştir.

Elâzığ ve Malatya yörelerinde *G. fuscum*'un aecial konukçuları *Prunus communis* L. (Armut) ve *P. elaeagri-*

**folia** (Ahlat) ve telial konukçaları **J. oxycedris** L. ve **J. excelsa** Bieb. türündeki ardıç ağaçlarıdır.

Yapay bulaştırma testlerinde İmam çeşidi ile Ahlat duyarlı bulunurken denenen diğer 13 armut varietesi çok duyarlı olarak saptanmıştır.

Enfeksiyon artışı ile ardıç ağaçları arasındaki uzaklığa ve aradaki arazinin durumuna bağlı olmuştur. Bu uzaklık 1000 m. den fazla olduğunda ve arada ağaç, tepe, v.s. gibi

engeller bulunduğu enfeksiyon azalmaktadır.

Armut memeli pası ile savaş konusunda, armut ağaçlarından 1000 m. den uzakta olan ardıçların kesilmesi veya örneğin Kapıderesinde olduğu gibi ardıç ağaçlarının yoğun olduğu bölgelerde armut yetiştiriciliğinden vazgeçilmesi veya bu yörelerde lâhli kontrol yapılması önerilebilir. Ancak uygun ve ekonomik ilâçlama programları için yeni çalışmalar zorunludur.

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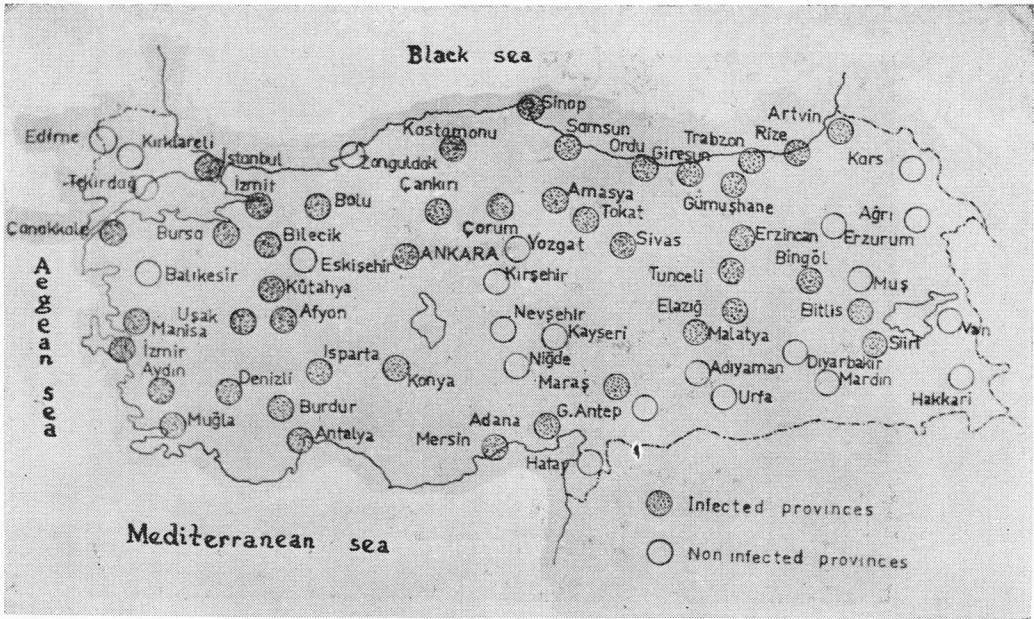


Fig. 1. Infected areas with *G. fuscum* D.C. in Turkey

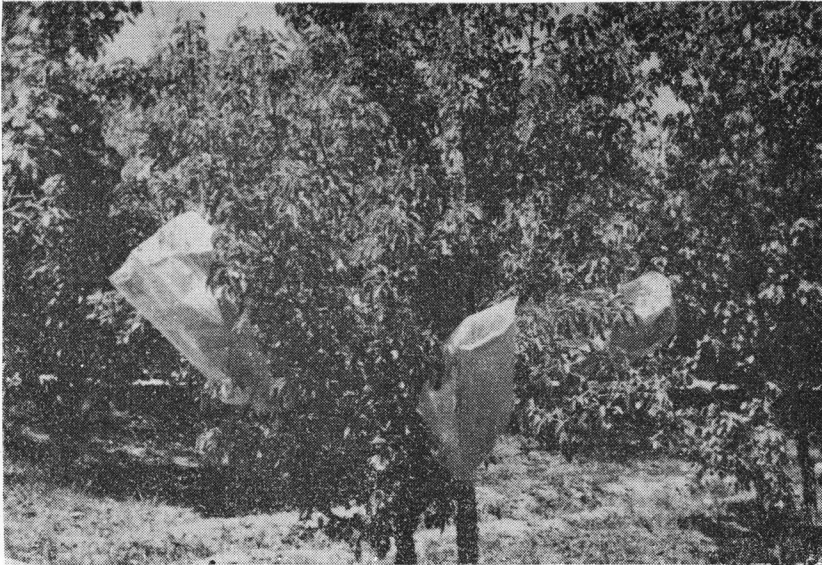


Fig. 2. The inoculation on pear trees.

GYMNOSPORANGIUM FUSCUM D.C.

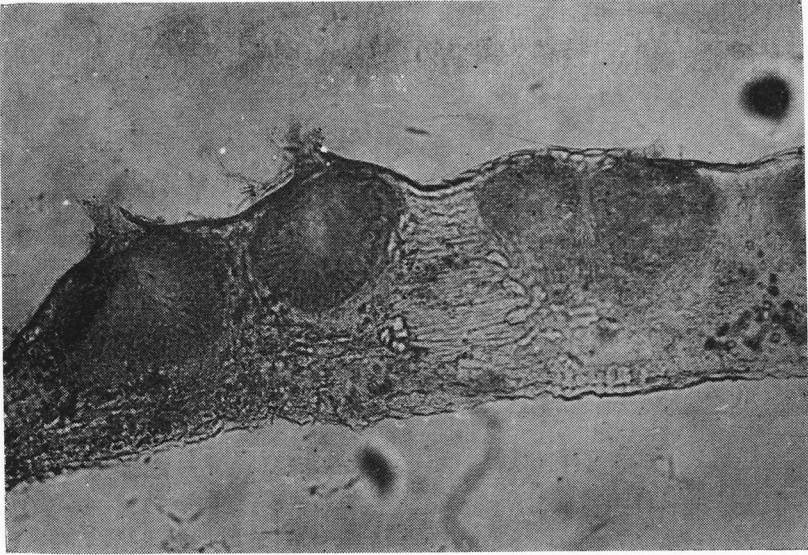


Fig. 3. Spermagonia on the leaves section (X450).

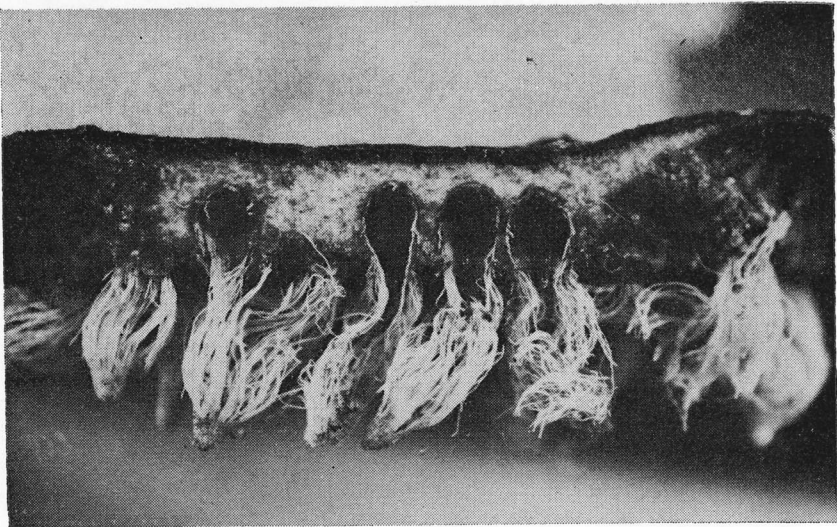


Fig. 4. The breadthwise section of the aecia (X10).

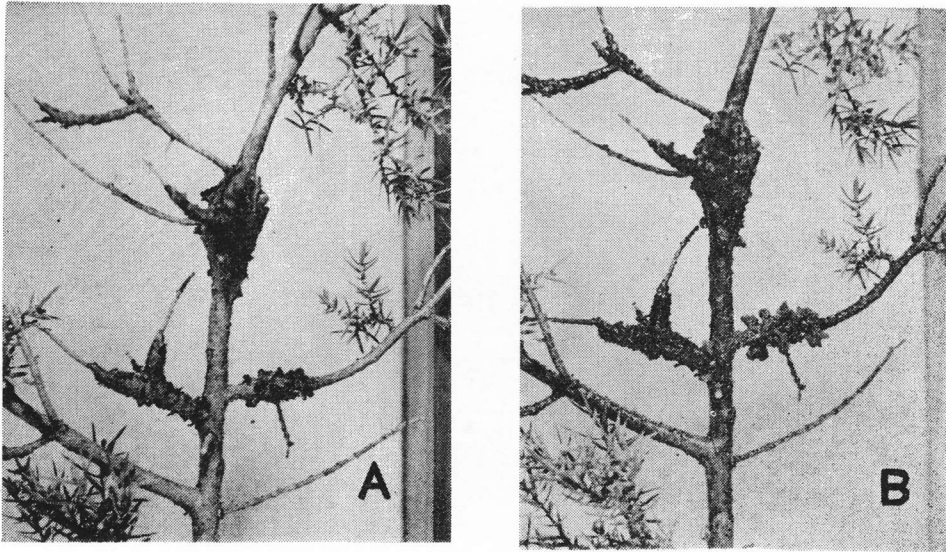


Fig. 5. The swelling of the wetted telial sorus (Approx X2)

A) Before being wetted

B) After being wetted

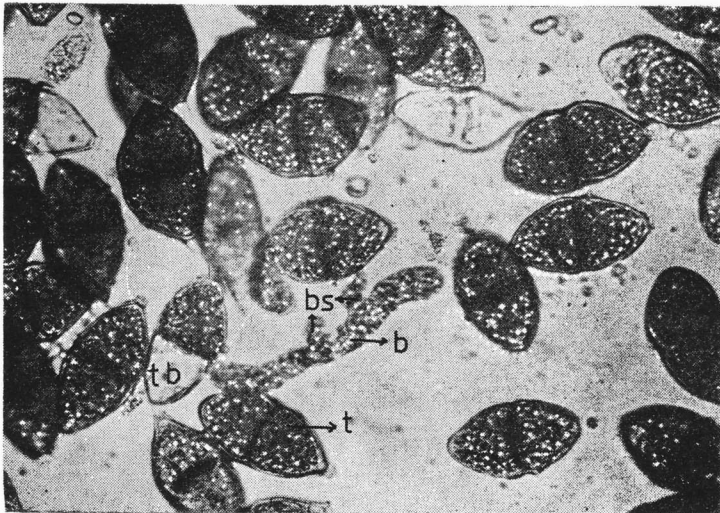


Fig. 6. The occurring of the basidiospores (X450).

(t): teliospores; (b): basidium; (bs): basidiospores

(tb): the emptied cell of the teliospore.

## Studies on the Inoculation Techniques of Covered Smut (*Ustilago hordei* "Pers." Lagerh) of Barley

Coşkun SAYDAM and Mustafa COPÇU

Regional Plant Protection Research Institute Bornova, İzmir, TURKEY

### ABSTRACT

This study was carried-out during 1974 and 1975 in order to obtain the most suitable inoculation technique of Covered Smut (*Ustilago hordei* «Pers.» Lagerh) of barley. In these experiments 3 and 11 inoculation methods were examined in 1974 and 1975 respectively.

Two inoculation techniques based on the husking of the glumes and treatment of the seeds in the spore suspension of the fungus gave the highest infection severity in 1974 and 1975 trials. According to the results, these inoculation techniques seemed to be the most reliable for covered smut of barley. But it should be taken into consideration that the use these methods is not so easy. Because the husking of barley is a difficult task particularly in large trials such as chemical control and breeding programmes will be consuming. Therefore the studies in 1975 were intensified on the dehusked barley seeds. For this purpose the effects of dry spores or spore suspension of the fungus on the infection were examined at the different stages of the germinating seeds. In this inspection increasing of the infection degree was taken as a basis of evaluating of the methods. But all of these new methods showed little or no infection.

There was no difference of using either dry spores or spore suspension in direct inoculations with dehusked seeds.

Inoculation made by dry spores was more effective than spore suspension at the beginning of the germination stage. However the degree of the infection was reduced under the level of natural infection.

## INTRODUCTION

The development of the industries of animal food and beer based on barley influenced its production and importance in our region as well as in Turkey (ANONYMUS, 1968, 1972). Increasing of the production will be possible partly by means of development of agricultural practices and partly by means of establishment of the protective seed treatment against certain diseases of barley.

One of the important diseases of barley is smut (*Ustilago* spp.) Infection degree was determined at 30 % in some barley fields (ÖĞÜT and COPÇU, 1973; SELÇUK, 1973).

Although three species of barley smuts (*U. nuda* «Jens» Rostr, *U. hordei* «Pers» Lagerh, *U. nigra* Tapke) were recorded in Turkey (BREMER, 1948; İREN 1962; KARACA, 1965) the semiloose smut (*U. nigra* Tapke) was more prevalent than covered smut in the Northern part of Turkey on the contrary of its occurrence in foreign countries (İREN, 1962).

The chemical control of covered smut of barley was given by various researchers (BREMER, 1948; DICKSON, 1956; İREN, 1962; KARACA, 1965; ALMURATOV, 1966; LOPATIN et al., 1969; REBENKO, 1970) as

seed treatment by organic mercury products and other seed dressings. Some of the authors suggested the effectiveness of water treatment of seeds (DOBREV, 1966; DIWERNICKI and MIKOLAJEWICZ, 1970).

According to the results obtained from the chemical trials made in Turkey some products such as Ceresan and Vitavax (ÇELİK and BABAÖĞLU, 1973) and Dithane M-45 (ŞENYÜREK et al., 1970) were recommended against the covered smut. Although some researchers (BREMER, 1948; DICKSON, 1956; İREN, 1962; KARACA, 1965) suggested that it will be possible to establish some breeding programmes on the local varieties of barley and to get resistant varieties, it is absolutely necessary to use seed dressings because of the physiological races of the fungus and non-availability of the completely resistant varieties against all races of the fungus (DICKSON, 1956).

This study aims to develop a method which is easy and suitable for artificial inoculation by the pathogen of covered smut in chemical control and breeding studies those will be carried-out in further experiments.

ÇELİK, Ç. and B. BABAÖĞLU, 1973». A project on the inoculation techniques and chemical control of covered smut of barley. No. A104.316 (Unpublished)

ÖĞÜT, M. and M. COPÇU, 1973 A project on the survey studies of wheat diseases. No. E. 104. 813/3 (Unpublished)

ŞENTÜRK, M. et al., 1970. A project on the species and severity of the barley smuts. No. A103. 304 Unpublished)

## MATERIALS and METHODS

This study was carried-out as a pot experiments in 1974 and 1975.

1 - The experiments carried-out in 1974:

The variety of Zafer and diseased ears collected from various parts of Ege Region in 1973 were used as material in this study.

1.1 - Direct inoculation by dry spores: Barley seed (100 gr) were inoculated at 03 % ratio of inoculum by shaking in a small containers for 5 min.

1.2 - Inoculation by spore suspension: The seeds of barley husked by hand, shaken in the spore suspension prepared at 01 % ratio for 1 min. and kept in this suspension for 15 min. After pouring the suspension the seeds were kept in this flask for 16-24 hours at the temperature of laboratory (19-20 C°). Later on the inoculated seeds were dried (TAPKE and BEVER, 1942).

1.3 - Modified inoculation technique : The seeds of barley soaked in tap water for 1/2 hour and husked by hand. These husked seeds were kept in the spore suspension prepared at 01 % inoculum ratio until the suspension level reduced to the 3/4 of the seeds level (It took approximately for 15 min). Later the inoculated seeds were dried between the papers

(SHIRIVASTAVA and SRIVASTAVA, 1971).

After the inoculation made by these methods mentioned above, the seeds were sown in pots. The pots in 50 cm diameter were filled with field soil and twenty five seeds were placed per pot. At the ripening stages, the infection degree was evaluated by counting healthy and diseased ears. Later the result were analysed to determine the differences between the methods.

2 - The Experiment in 1975 :

In this trial studies were intensified on the dehusked seeds to get an easy and suitable inoculation method. For this purpose 11 methods were investigated.

2.1 - Direct inoculation by dry spores: Dehusked seeds of barley were inoculated by the method mentioned in 1.1.

2.2 - Direct inoculation by spore suspension : Dehusked seeds were soaked in spore suspension prepared at 01 % inoculum ratio for 1/2 hour and then were dried.

2.3 - Inoculation by spore suspension : (TAPKE and BEVER, 1942).

2.4 - Modified inoculation technique : (SHIRIVASTAVA and SRIVASTAVA, 1971).



2.5 - Inoculation with dry spores at the beginning of the germination of the seeds: The seeds were germinated between wet papers when the radicle was 1-2 cm, and plumule 2-4 mm in length, germinated seeds were inoculated with dry spores as in 1.1.

2.6 - Inoculation with spore suspension at the beginning of the germination of the seeds: The seeds were germinated as in 2.5 and inoculated as in 2.2 by using spore suspension.

2.7 - Inoculation by spraying of spore suspension at the beginning of the germination of the seeds on the surface of the seed-bed : The seeds were placed on the surface of the pot soil. The papers covering the seeds were kept permanently moistened to stimulate the germination. When the phenology of the germinated seeds achieved the same stage mentioned above (2.5) spore suspension (0.03 % ratio and 200 cc for 5 pots) was sprayed. Later soil was poured on the seeds.

2.8 - Inoculation by spraying of spore suspension with the germinated seeds on the surface of soil: When the germinated seeds had radicle 2-4 cm and plumule 0.5-1.0cm in length spore suspension was sprayed as in 2.7

2.9 - Inoculation with spore suspension of germinated seeds : The seeds were germinated between wet papers. When the seeds had a phenology as in 2.8 were inoculated in a spore suspension as in 2.2.

2.10 - Inoculation with dry spores during germination : Dry spores of the fungus were placed between wet papers. The seeds were germinated between these papers until the phenology mentioned in 2.8.

2.11 - Directsowing of the barley seeds.

The methods were evaluated by analysing of the infection degrees obtained at the ripening stages of the plants.

RESULTS

1 - Results of 1974 trial:

The results of 3 inoculation methods were given in table 1

Table 1. The infection ratio of covered smut obtained by 3 different inoculation methods (1974)

INOCULATION TECHNIQUES	INFECTION RATIO (%)										Avera- ges	DUN- CAN TEST
	Replications											
	I	II	III	IV	V	VI	VII	VIII	IX	X		
1.3	63.2	70.0	53.8	83.3	75.0	53.8	37.5	100.0	68.4	93.8	69.9	A
1.2	50.0	77.3	64.3	64.7	60.0	28.6	54.5	43.5	68.8	61.9	57.4	A
1.1	13.8	3.3	11.5	7.4	0.0	10.7	0.0	0.0	7.7	4.3	5.9	B

According to these results the methods of Modified Inoculation Technique (1.3) and Inoculation by spore suspension (1.2) were more effective than the method in which dry spores were used on dehusked seeds. The averages of their infec-

tion ratios were 69.9 % and 5.9 % respectively

## 2 - Results of 1975 trial.

The infection ratios of 11 methods and their results of analyses were shown in table 2.

Table 2. The infection ratio of covered smut obtained by artificial inoculations (1975)

INOCULATION TECHNIQUES	INFECTION RATIO (%)					Averages	DUN- CAN
	Replications						
	I	II	III	IV	V		
2.3	39.1	40.0	26.1	32.0	32.4	33.92	A
2.4	15.4	34.8	50.0	31.8	31.3	32.66	A
2.11	20.7	14.8	8.7	39.2	16.7	20.01	A B
2.2	20.0	8.0	39.1	7.4	29.6	18.22	A B
2.1	20.0	8.0	19.0	18.2	11.1	15.26	A B
2.5	12.0	6.5	14.8	13.6	14.8	2.32	B C
2.7	4.5	0.0	9.5	4.2	0.0	3.64	C
2.9	0.0	8.0	0.0	8.0	0.0	3.20	D
2.6	4.0	3.7	0.0	0.0	0.0	1.52	D
2.10	0.0	0.0	0.0	0.0	4.0	0.80	D
2.8	0.0	0.0	0.0	3.3	0.0	0.66	D

The higher severities of the covered smut were obtained also by «Modified Inoculation Technique» and «Inoculation by spore suspen-

sion» as shown in Table 2. The averages of the infection ratios of these methods were 32,66 % and 33,92 % respectively.

## DISCUSSION

The inoculation methods based on husking of the glumes of barley seeds were the most effective in both 1974 and 1975 trials. But these met-

hods (TAPKE and BEVER, 1942; SHIRIVASTAVA and SRIVASTAVA, 1971) were not practical because of difficulty of husking of the seeds

in large experiments.

Therefore the studies were intensified to improve a new method that can give high infection ratio without husking the seeds. For this purpose the new inoculation methods were based on using dry or suspension of spores and treatment at the different stages of germination of the seeds without husking.

According to the results obtained on the infection degree of the methods there was not any difference between using dry spores and suspension of spores in direct inoculation techniques of the dehusked seeds of barley.

The various inoculation methods based on the germination of the seeds were ineffective. When the seeds were germinated either between papers or on the surface of the soil of the pots and inoculated by soaking in or spraying of the spore suspension the infection degrees were lower than the natural infection level obtained by direct sowing of barley seeds. When the seeds were inoculated at the beginning of the germination with dry spores resulted more infection than the spore suspension. At the later stage of the germination the methods gave the least infection. Reduction of the infection under the natural level may be due to the characters of our new methods. In these methods the seeds were placed in very much moistened environment (for the beginning of the ger-

mination for 40-hour and the other stage for 72-80-hour). This may influence the development of the infection as an unfavourable condition. Whereby the methods reduced the infection under the natural level as a controlling measure. This conclusion is also confirmed by the studies of DOBREV (1966) and DWERNICKI and MIKOLAJEWICZ (1970).

The differences of the results of two years experiment which the infection degree in 1974 was higher than 1975, may be explained with the different depth of the seed bed. Similar results were found by COMES and ENE (1965) and KARACA (1965).

Another variation are on the results of the dry spore inoculations. The higher ratio of the infection in 1975 may be related to the infestation of the seeds by natural infection in the field. The infection degree of control pots which sown without artificial inoculation confirmed to this result. The natural infestation in the field and association of the fungus to the seeds from harvesting to sowing will be also investigated in next studies.

As a result, there is not any significant difference between the two methods based on the husking of the seeds of barley, although the modified inoculation method (SHIRIVASTAVA and SRIVASTAVA, 1971) is more effective than the other. Because the infection ratio increased to

93,8 % and 100 % degrees in some replications of this method in 1974. Therefore this method may be recommended for small and particular trials. But the number of the seeds should be increased at 40-50 % ratio for sowing because of the embryo injuries during husking

The studies on the development of the resistant barley varieties and new chemical control experiments will require a lot of artificially inoculated seeds. Therefore the improving of new easy inoculation techniques will be necessary and the our studies will continue on covered smut of barley.

### Ö Z E T

#### ARPA KAPALI RASTIĞI (*Ustilago hordei* «Pers». Lagerh) NİN İNOKULASYON YÖNTEMLERİ ÜZERİNDE ÇALIŞMALAR

Arpa kapalı rastığı (*Ustilago hordei* «Pers». Lagerh) ile en uygun inokulasyon yöntemini saptamayı amaçlayan bu çalışmanın 1974 yılında 3, 1975 yılında ise 11 yöntem denenmiştir. Arpa kapalı rastığına duyarlı Zafer arpa çeşidi ile saksı denemesi şeklinde yürütülen bu çalışmada, hastalık oranlarının analizi ile yöntemler değerlendirilmiştir.

Gerek 1974 ve gerekse 1975 yılı çalışmalarında arpa kavuzlarını soyma ve spor süspansiyonu ile bulaştırma esasına dayanan iki yöntem, diğerlerinden daha yüksek hastalık oranı oluşturmuştur. Ancak kavuz soyma işlemi, bu yöntemlerin geniş kapsamlı çalışmalarda kullanımı güçleştirmektedir. Bu nedenle kavuzlu tohumlar üzerine yoğunlaştırılan çalışmalarda, doğrudan doğruya inokulasyon, tohum yatağı veya kurutma kâğıtlarında çimlendirilen tohumları çimlenmenin değişik evrele-

rinde, kuru veya süspansiyon halindeki sporlarla inokule etme esaslarına dayanan yöntemler incelenmiştir. Ancak bu yöntemlerin tümünde az sayıda enfeksiyon oluşmuştur.

Kavuzlu tohumların doğrudan doğruya inokulasyonunda, sporların kuru veya süspansiyon halinde bulaştırılması farksız enfeksiyon oranı oluşturmuştur

Çimlenmenin başlangıcında sporların kuru olarak bulaştırılması, süspansiyona oranla daha yüksek hastalık oranı oluşturmuştur.

Çimlenmenin ileri devrelerinde yapılan inokulasyonlarda hastalık oranı düşüktür. Bu yöntemlerdeki bu konuya gelecek çalışmalarda, arpa kapalı Rastığı'nın kontrolü yönünden ağırlık verilecektir.

Arpa kapalı rastığı ile yapılacak çalışmalarda güçlüklerine rağmen, güvenli olarak kullanılabilir ve yay inokulasyon yöntemleri, kavuz-

ların temizlenmesi esasına dayananlardır. Ancak kavuz tohumlarla, uygulanması kolay ve yüksek düzeyde

enfeksiyon oranı verebilecek yeni bulaştırma yöntemlerinin araştırılmasına devam edilecektir.

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## **Investigations on a New Bacterial Disease of Tomatoes in Ege\***

**Hikmet SAYGILI**

Department of Phytopathology and Agricultural Botany, University of Ege, Bornova-İzmir, **TURKEY**

In April 1975, one new bacterial disease symptoms were seen as black spots on leaves and stems in infested tomato seedling beds of Tukaş canning-factory, where is near Turgutlu and Salihli towns, particularly on tomato varieties D 1706 and ES 58, and the disease was reported to our Department by interested tomato growers. Afterwards some complaints were reported from tomato fields of Karacabey and M. Kemalpaşa towns (Bursa), and from fields of Tat canning-factory.

During the surveys between April and end of the July, the same symptoms were seen in most of tomato fields in Turgutlu and Salihli towns (Manisa), Kemalpaşa, (İzmir) Çanakkale and Karacabey, M. Kemalpaşa (Bursa). Isolations were made after sampling from the same areas. It was established that tomato

plant which particularly came from much infected seedling beds had abnormal growth and infected plants were more feeble than uninfected plants in the fields. In consequence of the disease, large planted tomato fields were pulled out and planted again for other cultural plants instead of tomato by the great numbers of growers. Symptoms of the disease: All aerial parts of infected tomato plants, such as leaves, fruits, flowers, petioles, sepals and stems have typical bacterial spots with irregular shapes in which have changing colours between brown and black. These spots are seen on naturally or artificially infected tomato plants.

The symptom of the disease is fairly clear on the leaves. In the early stages these spots have brown colour and after it goes to black and they appear like points. Generally

\*) This paper has been given at First Turkish Phytopathological Congress, İzmir, October 20-24, 1975.

the spots may be rounded by yellowish haloes (Figure 1,2).

These spots on the leaf are round - shaped or lengthy - shaped. When spots are numerous they are joined each and completely cover the surface, then leaves are faded and dried (Figure 3).

The spots are seen on the stem, twigs and also on petioles. Shapes of the spots are generally lengthy and superficial and sometimes near round-shaped (Figure 4). The symptoms of the disease are also seen on fruits, fruit stalks and sepals (Figure 5,6,7). The symptoms on sepals are the same with the stem symptoms (Figure 5) As for fruit symptoms, in the early stages they look as points and after growth they are developed as scabby black spots. These scabby spots are superficial and can be scraped easily (Figure 6).

Numerous spots joining each other on the fruits cause their deformation and so loss of their commercial value.

Samples from the cities and towns where the disease was seen were taken and examined in our laboratory. Isolations were made from diseased plant materials. SNA medium (Sucrose 5 %, Nutrient Broth, 0.8 % (DIFCO), and Bacto - Agar (DIFCO) 1.8 %) was used. Before isolations pieces of plant material

were sterilized in 70 % alcohol for half minute, and washed twice with steril water and pounded in steril mortars. From the suspension in the mortars caused bacterial agent was streaked into the SNA plates with a wire loop and the cultures were incubated at 26 C°.

In this way 20 isolates were obtained from diseased plants that came from different places. Hypersensitivity tests were made on tobacco, according to method of KLEMENT (1963) and variety of White Burley was used in the studies. Hypersensitivity tests gave the positive results.

The isolates were compared with described bacterial cultures by authorities like *Corynebacterium michiganense*, *Xanthomonas vesicatoria*, *Pseudomonas tomato* and *Pseudomonas viridislava*. For description of the agent some useful tests were made, such as oxidase test and levan formation were seen the SNA media and fluorescens pigments on the King B media. Results in Eskulinhidrolize, was positive.

According to results of the tests and symptomatological properties, this causal organism of the disease was a species of genus *Pseudomonas* in which gives fluorescens pigment. In the further studies causal bacterial species will be exactly described and also its properties will be more clear.

Ö Z E T

EGE BÖLGESİNDE DOMATESLERDE GÖRÜLEN BİR BAKTERİ  
HASTALIĞININ TANIMI ÜZERİNDE  
ARAŞTIRMALAR

1975 yılı ilkbaharında, Tukaş Konserve Fabrikasının Turgutlu ve Salihli'deki domates fideliklerinde, özellikle D706 ve ES 58 çeşidi domates fidelerinde lekeler şeklinde belirtiler görülmüştür. Daha sonraları aynı şikâyetler, Bursa ili Karacabey ve M. Kemalpaşa ilçelerinden ve Tat Konserve fabrikasından da gelmiştir. Nisan-Haziran 1975 döneminde bölgede yapılan incelemelerde, hastalığın fideden sonra tarla devresinde de bazı yerlerde yaygın olduğu ve bitkinin bütün toprak üstü organlarında zarar yaptığı saptanmıştır.

Hastalık belirtisi, kahverengiden siyaha kadar değişen renkte lekeler şeklinde olup, bu lekeler yaprak, sap, çiçek, ve meyve sapslarında görülmektedir. Meyvelerde ise, küçük yüzeysel kabarcıklar şeklindedir.

Yapılan laboratuvar incelemelerinde ve çeşitli biyokimyasal testler sonucunda, hastalığı oluşturan etmenin, fluorescens pigment veren **Pseudomonas** cinsinden bir bakteri olduğu saptanmıştır. Bakterinin tam teşhisinin yapılması ve diğer özelliklerinin belirlenmesi için çalışmalarda ilerde de devam edilecektir

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Figure 1. Natural infection on tomato leaflet and appearance of haloes around the spots.

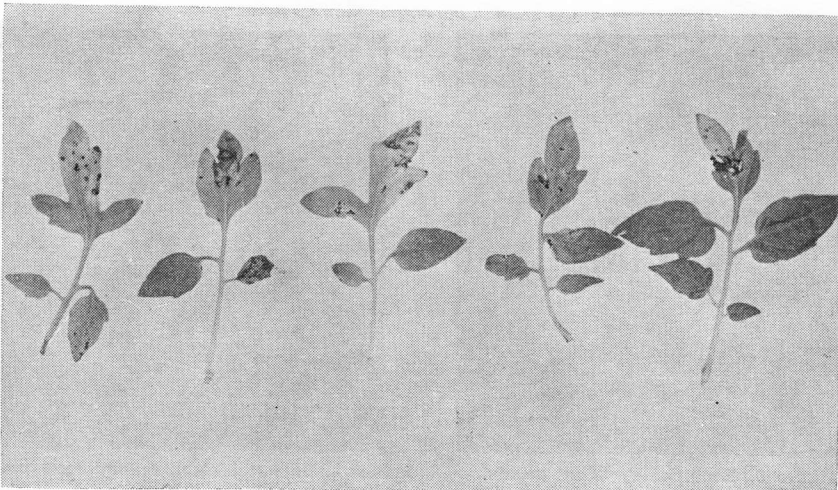


Figure 2. Appearance of leaf spots and haloes around them after artificial inoculation by spraying bacterial suspension.

A NEW BACTERIAL DISEASE OF TOMATO

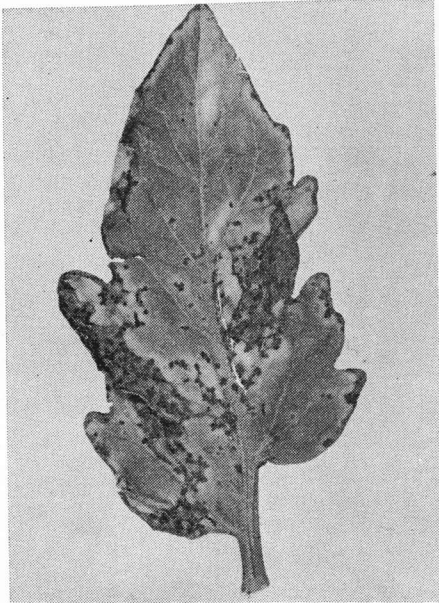


Figure 3. Numerous spots joined each other on the leaf.

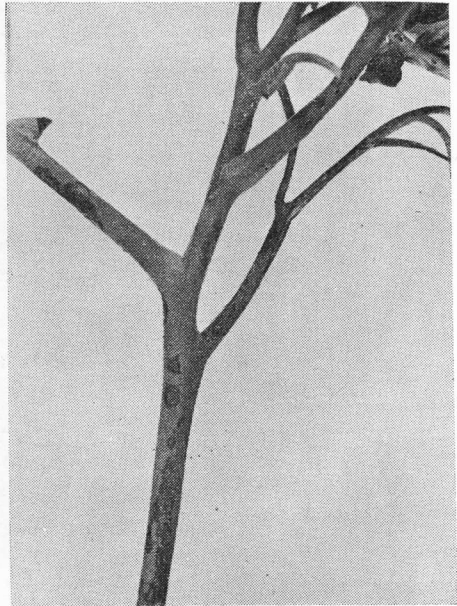


Figure 4. Spots of causal bacterial agent on stem, twigs and petiol.

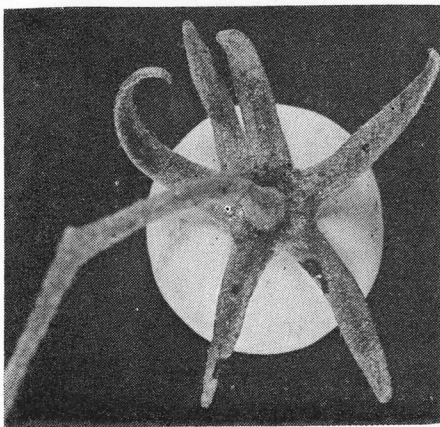


Figure 5. Bacterial spots on sepals.

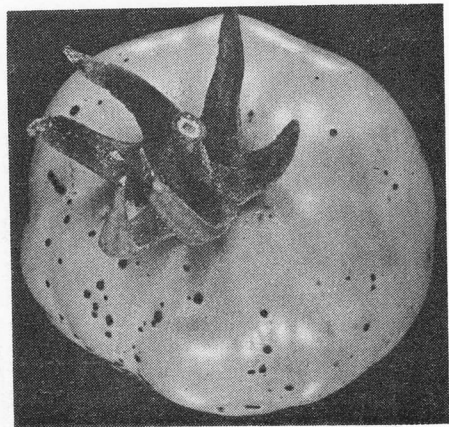


Figure 6. Bacterial black scabby-spots on fruit.

# Essais de la Lutte Contre la Cercosporiose des Bettraves Avec Les Fongicides Systemiques

Mehmet GÖBELEZ

Institut Sucrierie, Etimesut, Ankara, TURQUIE.

La cercosporiose cause des dommages importants à la culture des bettraves en Turquie (15.000 hect. à Adapazarı et à Susurluk).

Nous avons obtenus des bons resultats des essais d'applications des nouveaux fongicides. Les effets comparable des nouveaux fongicides avec celles des anciens sont à la suivante:

	Polarisation %	Rendement kg/h
Controle	15,39	68040
Brestan cons	16,04	71360
Enovit	17,16	72320
Benlate	17,14	69340

Les fongicides ont été appliqués à l'aide d'un atomiseur à dos aux dates suivante; le 5 et le 25 juillet et le 15 août; à 4 repétion d'une surface 100 m<sup>2</sup>(10x10)

Les tableaux I et II présentent les resultats des assais de 1972 et de 1973.

Tableau I. Les resultats obtenus d'essai de 1972

les fongicides en leurs doses p.I hectare	la resultats	
	Polarisation %	Rendement kg/h
1 - Témoin (contrôle) .....	14,73	52 980
2 - Enovit (% 70 trifonatemetil) 400 g.....	16,64	58 620
3 - Bavistin (% 50 Benzimidazole) 400 g	16,66	53 040
4 - Derosal Wp (% 60 Carbendazol) 400 g	16,76	61 860
5 - Derosal sol (% 20 Carbendazim) 1200 cc	16,99	55 860

Tableau II. L'essai de 1973

Les fongicides et leurs doses par hect.	La resultats	
	Polarisation %	Rendement kg/h
1 - Temoine .....	13,80	56 900
2 - Derosal Wp (la même 1972)	17,10	62 500
3 - Derosal sol. » » .....	17,20	67 200
4 - Bavistin » » .....	16,54	66 400
5 - Enovit » » .....	16,42	56 700

En 1974, nous avons reçu encore quelques nouveaux fongicides et nous en avons faits essai à les mêmes conditions. Mais en 1974 les conditions climatique n'étaient pas aussi favorable pour l'épidemie de cette maladie et la damage n'était pas à la même valeur à celle de 1972 et 1973

Les resutats d'essai de 1974 sont dans le tableau III.

Tableau III. Essais de 1974

1'er essai		
Les fongicides et leurs doses g pour hectare	La resultat	
	Polarisation %	Rendement kg/h
1 - Temoine .....	14,98	45 900
2 - Enovit (la même 1972)	16,15	51 200
3 - Calixin 400+Polyram. 1000 g. ....	14,93	47 200
4 - Tecto Wp (% 60 thiabendazole) 400 g	15,61	50 000
5 - Tectoflow sol. » 400 cc	15,38	47 500
6 - DPX Wp Du-Pont 2 kg .....	15,96	51 300
7 - Bavistin (la même 1972)	15,83	56 600
2'em essai		
1 - Temoine .....	14,40	45 500
2 - Enovit (1972) .....	15,72	51 000
3 - Derosal sol (1972)	15,73	54 000
4 - Derosal Wp (1972)	16,36	52 600
5 - DPX. wp. Du-Pont .....	15,32	51 100

Pour avoir une idée définitive, nous signalons les valeurs moyen de trois ans à la suivante:

Les fongicides	Les resultats	
	Polarisation %	Rendement kg/h
1 - Temoine .....	14,48	50 320
2 - Enovit	16,23	54 380
3 - Derosal wp	16,74	58 987
4 - Derosal sol.	16,64	59 020
5 - Bavistin .....	16,34	58 680

Nous signalons que les resultats obtenue avec les novels fongicides sont significatif. Nous raportons une augmentation à la polarisation de 1.75 à 2,26 et une augmentation du récolte au environs 4,060 au 8700 kg par hectare

Ö Z E T

ŞEKERPANCARINDA CERCOSPORA YAPRAK LEKESİNİN SİSTEMİK FUNGİSİTLERLE KONTROLU ÜZERİNDE BİR ÇALIŞMA

Şekerpancarındaki *Cercospora* yaprak leke hastalığına karşı sistemik ilâçlarla yürütülen üç yıllık (1972-74) çalışma sonunda, ilâçlamannın şeker oranı (polarizasyon yüzdesi) ve pancar verimini arttırdığı görülmüştür. Böylece ilâçlı parsellerde, kontrola oranla polarizasyon yüzdesinde 1,75-2,26 ve pancar veriminde 4060-8700 kg/ha değerleri arasında değişen artışlar saptanmıştır.

**All Correspondance Should Be Made To**  
**TÜRKİYE FİTOPATOLOJİ DERNEĞİ**

Ege Üniversitesi Ziraat Fakültesi

Bitki Koruma Bölümü

Bornova : İzmir,

TURKEY