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The Resistance Mechanism of a Barley Cultivar Yeşilköy 6678 to **Rhynchosporium secalis** (Oudem.) J.J. Davis

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ABSTRACT

The leaf washings and intercellular fluid from a susceptible barley cultivar Tokak stimulated the germination of conidia of **Rhynchosporium secalis** (Oudem.) J.J. Davis, while the ones obtained from a completely resistant barley cultivar Yeşilköy 6678 were partly retarded conidial germination. In all treatments there was no difference in the germination of conidia as a response to inoculation. On leaves of Yeşilköy 6678 the germination of conidia and appressorium formation were not influenced by the resistance of the cultivar. The resistance appearsduring the penetration of cuticula or just at the begining of subcuticular development. Cuticula as a physical barrier was not determined the resistance, unless it was attributed to the antifungal substances present in cuticula, cuticular layers or possibly in the outer epidermal walls.

INTRODUCTION

The introduction and development of Agricultural Tecnology have naturally led to the changes in the importance of some crops and their diseases such as leaf scald of barley caused by **Rhynchosporium secalis** (Oudem.) J.J. Davis. This disease of barley considered one of the major and serious disease of barley especially in clooer and humid areas and in the years having seasonal conditions favouring the disease development.

As with most leaf diseases the most effective and economic way of control is best achieved by growing resistant cultivars (Shipton and Tweedie, 1968; Marshall, 1973;

Karaca, 1974). So that there is always a requirement for a resistant barley cultivar, in this respect the ultimate aim must be to breed resistant cultivars or to look for a resistant cultivar between the existing ones. As a matter of fact Webster et.al., (1977) indicates the existance of resistant and immune cultivars against leaf scald of barley.

Our previous studies done on 22 barley cultivars by using the isolates of **R. secalis** obtained from the different barley growing areas of Erzurum Province showed that is probobly only one race prevailing in Erzurum conditions since the reaction of each cultivar to all isolates is same. A six row barley cultivar Yeşilköy 6678 is found to be completely resistant to this race of **R. secalis** (Döken, 1979).[1]

The aim of this study is to obtain information about the bases for resistance in plants of Yeşilköy 6678 against **R. secalis** in compare with a susceptible barley cultivar Tokak which is the main cultivar of the province and on the other hand to emphasise the complete resistance of Yeşilköy 6678.

MATERIAL and METHODS

Two barley cultivars, completely resistant six row cultivar Yeşilköy 6678, susceptible two row cultivar Tokak and scald pathogen **R. secalis** which was maintained on lima bean agar at 18°C were used in these experiments. The barley plants were raised in 10 cm pots up to 5 th. leaf stage. Some of these plants were inoculated by spraying 10^6spore/ml. conidia suspension to leaves until run-off. A surfactant Tween 20 was added at the rate of 2,3 drops to every 100 ml. suspension to improve the adherence properties of spores. Following inoculation plants were covered with polythene bags for 48 hours to maintain high humidity.

For obtaining intercellular fluid and leaf washings firstly plants which are inoculated (24 hours before) and not were placed in shallow trays of water and covered with polythene bags to produce guttation fluid and leaf exudates. After 48 hours the thirth and fourth leaves were excised in 5 cm length and placed in a tube which formed part of a leaf washing system as shown below (Fig. 1). On leaves 100 ml of distilled water were slowly passed and the washings were collected after three times of recirculation during 24 hours.

[1] Döken, M.T., 1979. Erzurum'da arpada izole edilen **Rhynchosporium secalis** (Oudem.) J.J. Davis'in morfolojisi, biyolojisi, zarar durumu ve savaş yöntemleri üzerinde çalışmalar. Atatürk Üniversitesi, Ziraat Fak. Bitki Koruma Bölümü. Doçentlik Tezi, Erzurum, Turkey.

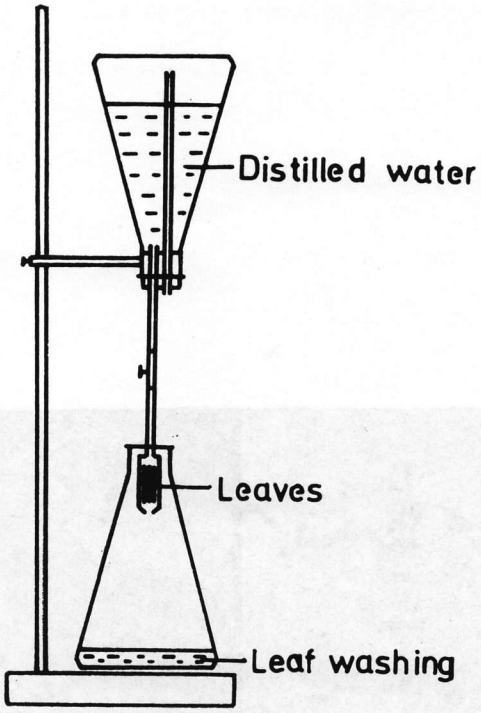

Fig.1. Leaf washing system

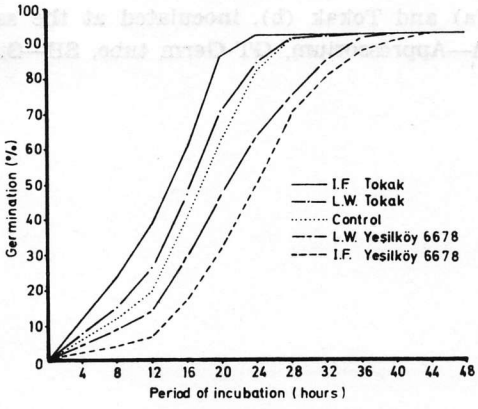

Fig.2. The germination of conidia in the leaf washings (L.W.) and intercellular fluid (I.F.) of barley cultivars Yeşilköy 6678 and Tokak

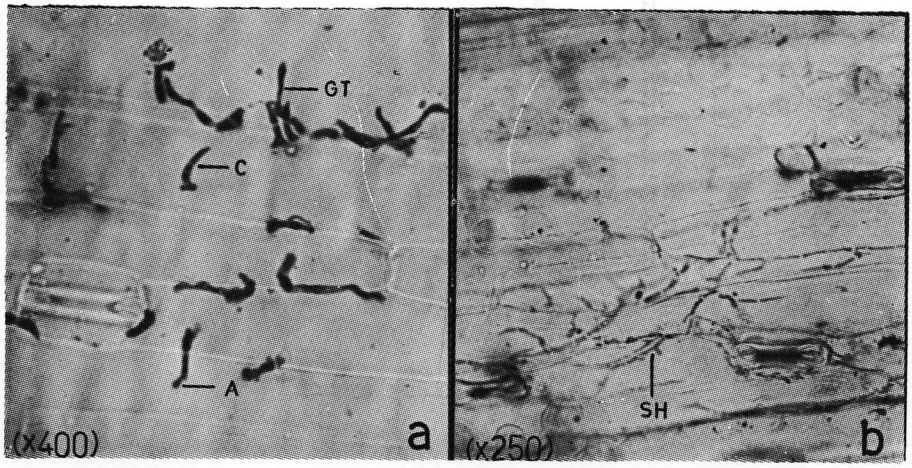

Fig.3 Stages in the development of **R. secalis** on the leaves of Yeşilköy 6678 (a) and Tokak (b), inoculated at the same time. C—Conidium, A—Appressorium, GT-Germ tube, SH—Subcuticular hypha

To remove intercellular fluid from leaves same type of leaves excised from the same plants were firmly attached with polythene tape to inside of centrifuge tubes such a way which prevents the leaves moving into centrifugal fluid. The leaves were centrifuged at about 1900 g RCF (Relative centrifugal force) for 20 minutes. The fluid collected in the bottom of centrifuge tubes and the leaf washings were passed through bacteriological filters. Then 0.3 ml. of intercellular fluid, leaf washings from both inoculated and uninoculated barley cultivars and sterile distilled water as control were separately dropped on each cellophane disc placed on water agar in petri dishes. Conidia taken from a sporulating culture were spread on every cellophane disc with a sterile needle. Then they were transferred to an incubator operating at 18°C. From every treatment four cellophane discs were taken in every four hours for two days. Under microscope in four microscope fields altogether 200 conidia were assesed for germination.

To determine host-pathogen interraction from the point of resistance, leaves from both inoculated cultivars were detached at four hour intervals in the first 48 hours period after inoculation, and then at 12 hour intervals for 20 days. For direct observation the excised leaves were immediately cleared in lactophenol + methanol + chololoform solution and then stained with analin blue (Döken, 1981). The slides prepared in lactophenol were examined under microscope.

For the determine whether cuticula acts as a physical barrier or not, to the penetration of the fungus, the leaves of Yeşilköy 6678 were gently rubbed by carborandum and then inoculated as indicated above. The plants were examined continuously for two months for the presence of lesions.

RESULTS and DISCUSSION

In the leaf washings, intercellular fluid of both barley cultivars and in distilled water different levels of germination were recorded in each period of incubation, but there was no difference as a response to inoculation. In all treatments conidia germination were reached up to % 93 in about 44 hours, unless no further increase were noted. The results were summarised in Fig.2.

The leaf washings and intercellular fluid of a susceptible barley cultivar Tokak were stimulated conidia germination in compare to control and to the resistant cultivar's. The increased germination of spores appear to be an effect of substances present in. As a matter of fact Ayres and Owen (1970) showed that the germination of spores of **R. secalis** is stimulated by nutrients at the leaf surface and

according to Goatley and Lewis (1966), these substances are organic acids (including amino acids), sugar, vitamins and inorganic materials. The intercellular fluid of Tokak promoted germination more than it's leaf washings probably due to difference in the concentration of the substances. Because the substances in the leaf washings were collected by running distilled water on them which of-course decreases the concentration of stimulating substances.

The effect of the leaf washings and intercellular fluid of Yeşilköy 6678 was different than Tokak's, thus the spore germination was partly retarded. Here the influence of intercellular fluid was also higher, possibly due to the reason mentioned above. The germination of conidia was not completely inhibited. But on attached leaves of Yeşilköy 6678 the permanent release of inhibitor substances may completely supress the germination of conidia. Unless in direct observation no inhibition of spore germination was detected on leaves, germ tube production and appressorium formation was as in Tokak leaves. In is apparent that the resistance has no effect on conidia germination and appressorium formation as indicated by Fowler and Owen (1971). On susceptible cultivar Tokak after appressorium formation the cuticula was penetrated and subcuticular hyphae were grew especially along the grooves between the epidermal cells and were branched profusely to form mycelial mats to start infection, but in Yeşilköy 6678 no subcuticular development was observed (Fig. 3). So it is concluded that the resistance has no effect on conidia germination and appressorium formation as mentioned by Fowler and Owen (1971). The resistance of Yeşilköy 6678 appears during the cuticula penetration or just at the begining of subcuticular development. As a matter of fact, according to Fowler and Owen (1971), the earliest point at which resistance to **R. secalis** is manifested in barley is at the penetration of cuticula.

Cuticula may act as a physical barrier in preventing pathogen from entering. However in Yeşilköy 6678 cuticula was not regarded as a cause of high resistance to the fact that no sign of infection ever been obtained from heavy inoculations done in different ecological conditions at various development stages (Döken, 1979), altough the cuticula thickness is affected by enverimental conditions (Juniper, 1960), age or developing stage of plants (Wood, 1967). On the other hand when cuticula of leaves were abraded to alter physical properties of cuticula, the resistance of Yeşilköy 6678 still remained unaltered.

Since there was no hyphal establishment in subcuticular position and cuticula as a physical obstacle was not accounted for the high resistance, this resistance is mainly

attributed to antifungal substances present in cuticula, cuticular layers or in the outer epidermal walls, possibly accumulating between epidermis, cuticula and acting during penetration or just at the begining of subcuticular development, there completely inhibiting the further development of the fungus. The delaying effect of leaf washings and intercellular fluid of Yeşilköy 6678 on conidia germination could be the influence of these antifungal substances diffusing into intercellular fluid and on to leaf surfaces.

ÖZET

YEŞİLKÖY 6678 ARPA ÇEŞİDİNDE **Rhynchosporium secalis** (Oudem.) J.J. Davis'e KARŞI GÖRÜLEN DAYANIKLILIK MEKANİZMASI

Arpa yaprak lekesi hastalığı etmeni olan **Rhynchosporium secalis** (Oudem.) J.J. Davis'e hassas olan Tokak arpa çeşidi yapraklarından elde edilen yıkama suyu ve hücreler arası su konidi'lerin çimlenmesini teşvik etmesine karşın, tam dayanıklı çeşitli olan Yeşilköy 6678 den elde edilenler ise geciktirmiş, fakat tamamen önlememiştir. Her iki çeşidin de hücreler arası suyu daha etkili bulunmuştur. İnokulasyon sonucu bu arpa çeşitlerinin yapraklarından elde edilen hücreler arası suyun ve yıkama suyunun teşvik edici veya geciktirici özelliğinde bir değişiklik olmamıştır. Yeşilköy 6678 de görülen dayanıklılık yaprakları üzerinde konidi çimlenmesine ve appressorium oluşumuna etki etmemekte, ancak kutikulanın penetrasyonu veya hemen takiben kutikula altı gelişmenin başladığı an ortaya çıkmaktadır. Fiziksel bir engel olarak kutikulanın pek rolü olmayıp, dayanıklılığın kutikula, kutikular katlar veya dış epidermal duvarlarda bulunan antifungal maddelerin fungus'un gelişmesini tamamen önleyici niteliğinden ileri geldiği varsayılmaktadır.

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Confirming the Factors and the Ratio of Bacterial Diseases of Potatoes in Izmir and its Surroundings and Investigation on the Reactions of Important Potato Varieties of the Region

Mehmet GÜNDOĞDU[1] and İbrahim KARACA[2]

ABSTRACT

This study has been done between the years 1972 and 1975 in order to confirm the factors of bacterial diseases in İzmir and its surroundings and the reactions of important varieties of the region.

It was determined that the prevalence of **E. atroseptica** and **E. carotovora** was a negligible quantity but average prevalence of **S. scabies** was 35.27 % in the potato growing areas of İzmir and its surroundings. This survey has put forward that manure has encouraging effect on **S. scabies**. The most affected potato variety is Sarıkız and for this reason, the production of it has been reduced.

INTRODUCTION

The best potato seed is produced in Kars, Erzurum, Uludağ, Bozdağ, Trabzon and on the mountains and high plains of Bolu (İncekara, 1965).

The production of Potato in Turkey is increasing year by year. According to Agricultural statistics for 1973 potato was produced in an area of 180.000 hectars and the production was 2.200.000 tons. In Ege Region from a producing area of 15.572 hectars, 244.087 tons of potato has been obtained, in 1973 (Devlet İstatistik Enstitüsü, 1975). In the region where did my personal researches and surveys, that is in an about İzmir these potato varieties are produced in the proportions pointed out below: Ari 60 %; Sarıkız 20 %; Ostra 10 %; Alpha %.

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Till today, potato bottom burnt (**Erwinia atroseptica** (Van Hall) Jennison); Wet rottenness (**Erwinia carotovora** (Jones) Holland, and potato scab (**Streptomyces scabies** (Thax) Waks et Henrici) is confirmed in the Region (Karaca 1964; Karahan 1971; Kâya and Gündoğdu 1972)

The aim this study is to confirm the proportional amounts of bacterial diseases of potato and the sensitivity of the potato varieties which are produced in the region. Surveys are made in the most important potato producing centers: Ödemiş, Kiraz, Bornova, Karşıyaka and Menemen.

MATERIAL and METHODS

In the laboratory tests for artificial inoculation and the reactions of varieties to the bacteria Ari, Alpha, Brava, Feldhelson, Ostra and Sarıkız patotoes are used. In 5 districts, 64 potato fields, that represent the potato production area is taken for survey.

From the potato heaps which are selected for seed, 100 potatoes per heap are taken cut and controlled. In the field at three random places, one row controlled and healthy and diseased plants are counted. In the harvest in three rows, 15 potatoes per row are controlled from cutside as well as inside by cutting in half.

The scabby potatoes are disinfected surfacely with sublimate, after that, the bacterium is isolated from the lesion. In the isolation, Yeast-Extract-pepton medium is used. The stems of infected potato plants are used by planting in same medium after being washed in % 70 alcohol (Corbaz 1964). The growing colonies, the are taken to Stolp-Agar Medium.

The bacteria are classified according to cultural and biochemical properties (Burkholder and Smith, 1949; Elliott 1951; Stapp 1956; Karaca 1966).

At the patogenicity studies, the potato plants are sown in 12 cm Ø pots. With the inoculation injector, isolated **E. atroseptica** and the original **E. atroseptica** are inoculated to the stems of those plants. Potatoes are cut in two, Sterilized and then, to these surfaces, the isolated **E. carotovora** and the original **E. carotovora** are inoculated.

To the little lumps, **S. scabies** and the original **S. scabies** are inoculated according to Busch and Gilpatrick method (Lawrence 1956). The reactions of potato varieties to the factors; The studies are made with the idendified bacteria Black and white isolates are taken from **S. scabies** which formed two different colonies and from **E. atroseptica**, one from each, using the same method as in artificial inoculation.

RESULTS and DISCUSSION

As a result, in year 1972, **Streptomyces scabies** is found at a proportion of 100 %, in the potatoes which are isolated from the ones taken while surveying. Beside this, 3 **E. atroseptica** and 2 **E. carotovora** are isolated. At the and of tests, these bacterial cultures gave the same results as the original ones.

S. scabies isolates are examined for their cultural properties after being distinguished to two groups as A (Black) and B (White) according to their colonial structure and colours. The appearances of the colony types are seen in (Fig 1)

In the year 1972, 3 **E. atroseptica** and 2 **E. carotovora** was in minimum quantity in İzmir and its surroundings and so, nothing can be said about the ratio. The existency ratios of potato Scab **(S. scabies)** can be seen at (Table 1).

Table 1.

The proportion of existency of **S. scabies** in İzmir and its surroundings in 1972

Observation Place	The ratio of disease (%) 4 surveys.
Ödemiş	36.07
Kiraz	43.51
Karşıyaka	36.30
Bornova	22.81
Menemen	20.30
The provincial average	35.27

At the end of the surveys and studies, **S. scabies** is found maximally in Sarıkız variety, followed by Brava.

S. scabies shows different effects in manured fields and the fertilized ones. When the potato seeds taken from a field, and sown in the same field, **S. scabies** occure more wide spread.

The reactions of potato varieties to the diseases, the potato varieties set up four groups, for their reactions against **E. atropseptica.** The most sensitive group contains Brava, Ari, Ostra; and Feldhelson fol-

lows them and makes the second group, Sarıkız the third and Alpha, the fourth and Fig. 2 shows the reaction of Brava, Feldhelson and Ostra to **E. atroseptica.**

And against **E. carotovora,** six diffirent groups can be seen. From the most sensitive to the less; Brava, Ari, Ostra, Alpha, Sarıkız and Feldhelson (Fig 3).

For the reactions against **S. scabies;** potatoes can be grouped for the sensitivity degrees, like this; Sarıkız, Brava, Ari, Feldhelson, Ostra and Alpha (Fig 4).

In the research area **E. atroseptica** and **E. carotovora** were less amount but **S. scabies** was at high percentage naturally, because the producer were not using the seed potato brought from other regions. As the result of this situation until the present study was done the producers were using their own seed-potatoes after taking all cultural precautions thus limiting the ratio of diseases.

Potato scab disease **(S. scabies)** was found to be more prevelant than the others. The reason for this sowing the same seed-potato obtained from same fields and as Güner (1961 recorded the farmers used alkaline naturel manure which sitimulated the disease occurence (Karaca 1966).

ÖZET

İZMİR VE ÇEVRESİNDE PATATESLERDE GÖRÜLEN BAKTERİ HASTALIKLARININ VE ETMENLERİNİN TESBİTİ VE BÖLGENİN ÖNEMLİ PATATES ÇEŞİTLERİNİN REAKSİYONLARI ÜZERİNE ARAŞTIRMALAR

Bu araştırma, İzmir ve çevresinde patateslerde bakteri hastalıklarının oranı ve izole edilen türlere patates çeşitlerinin reaksiyonlarını saptamak amacıyla yapılmıştır. Çalışma 1972-1975 yıllarında Bornova, Bölge Zirai Mücadele Araştırma Enstitüsünde yürütülmüştür.

İzmir ve çevresi patateslerinde oranlanmıyacak kadar az **E. carotovora** ve **E. atroseptica**'nın yanı sıra; aşağıdaki ilçelere göre yüzde bulunuş oranı verilen miktarda **S. scabies** saptanmıştır.

İLÇELER :	BULUNUŞ ORANLARI (%)
Ödemiş	36,07
Kiraz	43,51
Karşıyaka	36,30
Bornova	22,81
Menemen	20,30
İl Ortalaması	35,27

Çalışmalar sırasında **S scabies** hayvan gübresi atılan topraklarda daha fazla saptanmıştır. Patates çeşitlerinin **E. carotovora** ve **E. atroseptica** ve **S. scabies'e** reaksiyonları denemelerin sonucunda Sarıkız, Brava, Ari, Alpha, Ostra ve Feldhelson kendi aralarında tek başlarına grup oluşturmuşlardır.

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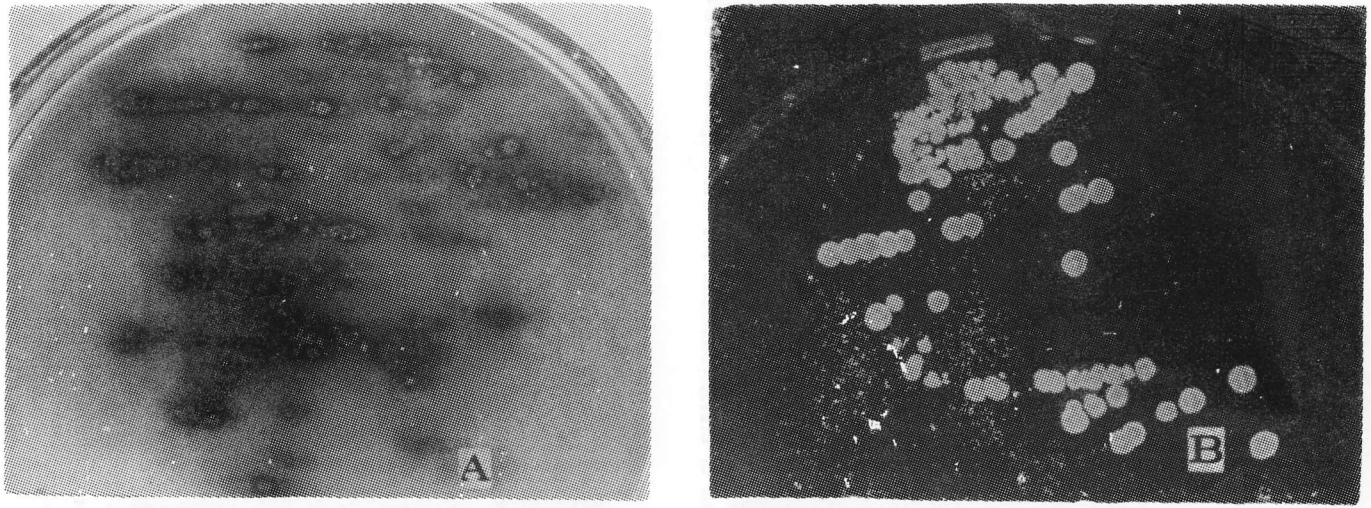

Fig. 1. Colony types of **S. scabies.**

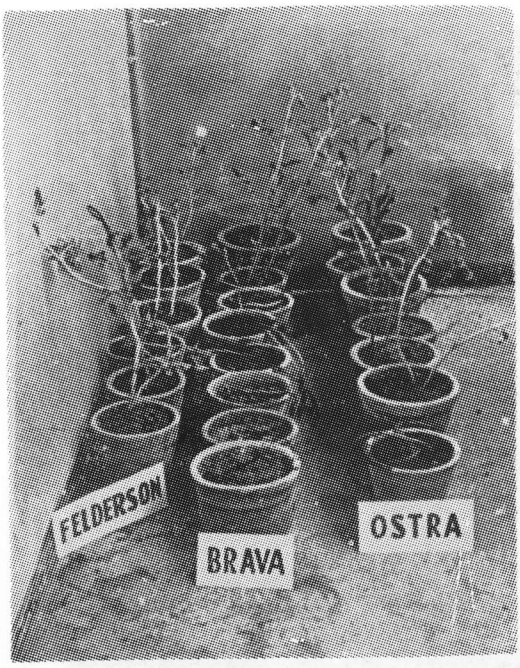

Fig. 2. The reactions of Brava, Feldhelson and Ostra against **E. atroseptica.**

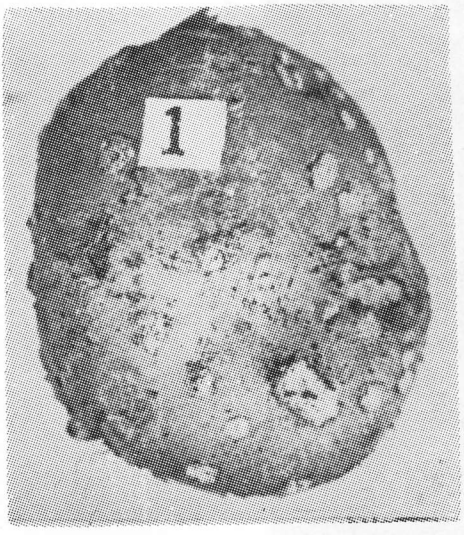

Fig. 3. The reactions of the potato varieties against **E. carotovora**

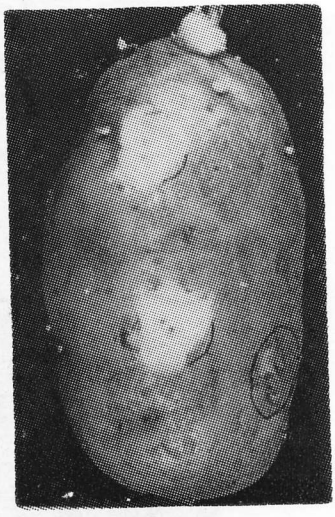

Fig. 4, The reactions of the potato varieties against **S. scabies**

J. Turkish Phytopath., Vol. 10, Num. 2-3, 79-87, 1981.

Preliminary Report of Tomato Spotted Wilt Virus (TSWV) and is Epidemy on Tobacco in the Çanakkale Region of Turkey

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ABSTRACT

This is the preliminary report of Tomato Spotted Wilt Virus (Kromnek virus; Lycopersicon virus 3) in Çanakkale from Turkey. A survey was made between 1980-1981 in tobacco fields where the virus made epidemy and caused serious damage. The typical symptoms of TSWV, concentric rings with a central spot, large plaque like lessions with concentric zones or necrotic tissue, necrotic lines mainly along the side of veins, apical necrosis, stunting and leaf malformation have been observed on the infected tobacco plants. Sap inoculation tests with sensitive herbaceous host plants and physical property tests in the labrotory have been revealed that, the causal virus is Tobacco spotted wilt virus. The virus showed very high incidence 95-100 % in some tobacco fields. It has been experimentally shown that, thrips is responsible from the epidemy of TSWV in the survey areas.

INTRODUCTION

A serious disease causing severe necrosis and malformation of tobacco plants was first noticed in 1979 in the tobacco fields in Çanakkale region. Since than, the virus made epidemy and caused economic losses in a number of the tobacco fields in this areas. Investigations have been made in 1980-1981 on indexing tests and the thrips transmission tests for determination of the causal virus.

MATERIAL and METHODS

1. Indexin tests with herbaceous hosts :

During the field observation in July 1980-1981, leaf samples col-

lected from the severely infected tobacco plants with Tobacco Spotted Wilt Virus (TSWV) placed seperately in the polietilen, and kept in the cool ice-box. Leaf tissues were triturated in 0.02 M phosphate buffer, ph 7.0 containing 0.01 M sodium sulfite (for stabilizing of the virus). The prepared inoculum were rubbed on the carborandum dasted (500-mesh) leaves of the following indicator test plants. After inoculations leaves were washed with water.

The following herbacious host plants were used in sap inoculation test :

Petunia hybrida Minstral, **Nicotiana glutinosa, Nicotiana tabacum** Samsun NN, **N. tabacum** (Turkish) **N. tabacum** Samsun, **N. clevelandii, N. tabacum** xanty, **Cucumis sativus** (Cucumber), **Lycopersicon esculentum** (tomato), **Vinca rosea, Tropaelum majus** (Nasturtium), **Phaseolus vulgaris L. Vigna sinensis** (Cowpea), **Chenopodium amaranticolor, C. quinoa** and **Datura stromanium.**

The identity of TSWV was made by the charecteristic symptoms produced on the first 11 herbaceous test plants. The sources of inoculum also were detected for tobacco mosaic virus (TMV) and tobacco Ringspot virus (TRSV). Two or three test plants were used in each inoculation. The symptoms observations on the test plants started 2 days after the sap inoculations. Herbaceous test plants Kept in a climate controled chamber at 25°C under alternating 12 hr. light and dark period.

2. Transmission of disease by thrips :

During the field observations in the survey areas in July 1981, the young leaves which showed silvered appearence of the vein and contained many adults and the nymphs of the thrips were collected from the heavely infected tobacco plants and Kept in a box. The adults and the nymphs of the feeding thrips were observed through a stereoscopic microscope and especially the adults were immediately transfered to the host plants such as **N. tabacum** Samsun, **N. tabacum** (Turkish) and **Tropaelum majus** with a moist camel's hair bruch. At least 20 to 30 adult thrips and some nymphs were placed on the host. Thrips were also periodically observed on the test plants with a x20 hand lens. Test plants Kept in a climate controled chamber at 25-26°C and 60 % related humidity under alternating 12 hr. Light and dark period, Symptom observation made on the developping leaves of the infected host plants.

3. Physical property in crude sap :

Undiluted sap from systemically infected **N. tabacum** leaves was used for determination of thermal inactivation point (TIP) and longevity **in vitro.** Termal inactivation studies were conducted by grinding the tissue just prior to heatin. Each 1 ml sap sample was heated in a

water bath for 10 min at 40-45 and 50°C, cooled immedietely in an ice bath and inoculated on **Petunia hybrida** test plants. Undiluted crushed sap kept **in vitro** at room temperature 5 hours and inoculated **Petunia** and **Tropaelum majus** test plants.

RESULT and DISCUSSION

1. Symptomatology of the disease :

Several distinct types of symptoms were observed on the tobacco plants (local variety nemaly Agonya) during the field observations. The characteristic symtoms of the Tomato Spotted Wilt virus (TSWV) were; typical concentric rings with a central spot, large plaque like lessions composed of concentric zones or necrotic tissue, necrotic lines mainly along the side of the veins (Fig. 1.), apical necrosis «tip blight», malformation of the leaves of the systemically infected plants. In July 1980-1981, after the rainy days during the early june, the systemic symptoms developed rapidely troughout the tobacco fields in the survey areas. Systemically infected upper leavês of the tobacco plants developed many concentric necrotic rings. Leaf surface of the systemically infected tobacco almost entirely covered with small necrotic rings as showen in fig.2.

Progress of the disease in this systemic stage was rapid. Thrips populations were very high. Tobacco plants generally died completely or died down except for the central shoot. In the late July, because of the hot weather conditions, sometimes diseased tobacco plants recovered from the disease and produced some healthy small leaves on the upperside of the tobacco plants. But the disease had been considered as a very descructive disease on tobacco plants throughout the fields.

2. Incidence of TSWV in the survey areas :

Survey and field investigations were made in July 1980-1981 in tobacco growing counties and villages of Çanakkale Province where the disease showed very high incidence and was very descructive. Totaly 35 tobacco field were examined during the survey as showen in table 1.

Table 1. The rate of incidence of TSWV in several localities of the Çanakkale province.

Localities	Number of tobacco field examined	The rates of incidence of TSWV (%)	The situation of thrips
Kalkım	6	80	+a
Akça koyun	7	85	+a
Karabey	8	95	+a
Yenice	5	45	+b
Davut köy	5	20	+b
Çınarcık	5	2	—

+a : Thrips population was very high.
+b : » » was moderate.

The incidence of TSWV were very high in Kalkım, Akça koyun and Karabey as showen in Table 1. because of the involving the good ecology of thrips vector. In some tobacco field all of the plants have been infested by thrips. We noticed 100 % incidence in some tobacco fields in Akçakoyun and Karabey village near Kalkım. Incidence was very moderate in Çınarcık because of the absence of the thrips vector.

3. Indexing results :

The symptoms on the several herbaceous host plants sap inoculated from the disease tobacco plants were as discribed following.

Petunia hybrida Minstrel: 2 or 4 days after the sap inoculations, **Petunia** plants developed typical local necrotic lessions of TSWV as showen in Fig.3. Local necrotic circular rings spots were very severe in many inoculated petunia. Circular spots showed reddish-brown margin and a paler center as showen in Fig.3, Generally, virus was not systemic in the many inoculated petunia plants as reported by Smith (1951, 1957), Ivancheva (1959), Gibbs etal (1970), Chrisochoou (1981) and Mickovski (1981).

Nicotiana glutinosa was very sensitive to the sap inoculations. Thypical large local necrotic lesions developed in 3 to 4 days after the inoculations as showen in Fig.4. Later on, the lesions increased in size and formed concentsic necrotic zones abont 2 to 3 mm. in diameter. Following these typical symptoms, some **N. glutinosa** plants showed letal systemic necrosis and diying as reported by Smith (1951, 1957), Gumpf and Weathers (1972), Kohler and Klinkowski (1954), Gibbs et al. (1970).

N. tabacum Samsun NN (tobacco) : Local necrotic lesions developed 4 to 5 days ater the inoculations (fig.5) folowed by systemic necrotic pattern concentric rings (fig.6.) and leaf deformations. The same symptom also occured on **N. clevelandii.** Inoculated **N. clevelandii** plant died after 10 days **N. tabacum** xanty developed large necrotic local lesions 3 to 4 days after the sap inoculations as showen in fig.7 **N. tabacum** Samsun developed typical local necrotic ring spot 3-4 days after the inoculations. Later, some concentric ring spot occured on the leaves. Systemically infected upper leaves also developed typical concentric rings on the leaf surface.

Tropaeolum majus (Nasturtium) : Sap inoculated leaves were symptomless, 10 to 15 days after the inoculations, systemic mosaic pattern, vein clearing of young de veloped leaves, distortion and cupping of the leaves, yellowish spots and some mottling, stunting and many pale necrotic spots covered on the leaf surface of the plants (fig.8) as reported by Smith (1951, 1957), Pritchard (1949), Gibbs et al. (1970) Nasturtium plant was found very sensetive and good test plant for TSWV. **Cucumis sativus** cucumber test plant developed local chlorotic spots with necrotic centers 4-5 days after the inoculations as reported by Smith (1975), Gumpf and Weathers (1972), and Gibbs et al. (1970).

Vinca rosea : about 2 weeks after the sap inoculations, local necrotic spots (black coloured), yellowing and some leaf deformations developed.

Lycopersicon esculentum : about 2 weeks after the sap inoculations of the 10-15 cm high tomato plants showed some concentric rings on the young leaves, slightly curling downwards. The most characteristic symptoms was typical bronzing of the leaves covered throughout the leaf surface completey. Infected tomato plants were stunted comparing with the normal plants. In the late stage of the disease, bronz color stunted plants were killed. Some tomato plants inoculated in the old age have not been killed. The fruit of some younger infected tomato plants developed typical concentric Circles, paler red and yellow coloring in the surface as showen in fig.9.

Datura stramonium test plants showed typical concentric ring spot and vein necrosis on the sap inoculated leaves 4-5 days after the sap inoculations tests as described by Smith (1957).

Phaseolus vulgaris L., **Vigna sinensis** (Cowpea), **Chenopodium amaranticolor** and **C. quinoa** have not been developed symptoms, Sap inoculated some **N. tabacum** plants developed characteristic symptoms of Tobacco Mosaic virus with TSWV. No symptoms of Tobacco Ringspot Virus (TRSV) as reported by Gibbs et al (1970) have been observed on the inoculated test plants.

4. Physical property results :

Virus lost its infectivily in sap heated for 10 min at 40-45 and 50°C and, in the cruded sap Kept **in vitro** at room temperature 5 hours. Inoculated **Petunia hybrida** and **Tropaelum majus** test plants with treated sap did not produce any symptoms of TSWV as comparing with the control.

The indexing and the physical property results in this experiment have been revealed that, the causal virus is TSWV in the survey areas. According to our observations in the entemology laboratory the adult thrips was very small dark brown or yellowish colored and0.8 - 0.9 mm in length similar to onion thrips, **Thrips tabaci** Lindeman as reported by Pritchard (1949), Chrisochoou (1981), Mickovski (1981) and Lodos (1981), Cengiz (1974) and Lodos (1981) previously reported that, **Thrips tabaci** L. is wide distributed and caused large injury is known as «white vein» in tobacco, and has wide host range in Turkey.

Thrips transmitted **N. tabacum** tests plants also showed characteristic leaf symptoms of TSWV as showen in fig 10 as reported by Zawirska (1979) and Gibbs et al. (1970). **Tropaelum majus** plants also developed typical TSWV symptoms 8 to 10 days later after the thrips transmission as reported by Pritchard (1949). Experiment results showed that thrips is responsible from the epidemy of TSWV.

Tomato Spotted Wilt Virus is known very destructive disease especially on tobacco and tomato plants. The virus has very wide host range such as in 166 plant species in 34 families, including 7 monocotylodoneus families described by Klinowski and Uschdraweit 1952; Smith 1957; Best 1968; Mickovski 1981; Chrisochoou 1981. TSWV was first recognized by Brittlebank in 1919 on tomato, later confirmed that the causal is a virus by samuel et al. 1930. Virus made epidemy in France and caused big damage 1937 reported by Mickosvki (981). The presence and the damage of TSWV was reported in Bulgaria in 1952, in Greece and Poland 1956, in Chechoslovakia 1958 in Yugoslavia 1963. In Bulgaria, TSWV caused big damage, incidence of the disease was 90 to 95 % in certain region and losses was 1000 ton tobacco plants (Ivancheva-1959). In Yugoslavia it caused big yield losses 90 % in 1969 reported by Mickovski (1969). TSWV have been caused big damage in Greece and Yugoslavia and tobacco yield losses was up to 50 % or more in some places as recently reported by Chrisochoou (1981) and Mickovski (1981).

According to our experiment, TSWV was very descructive in the tobacco growing some districts in the Çanakkale province as reported in this paper. Yield losses due by the virus estimated up to 50 % in the some localities (this data have been given by Çanakkale Mo-

nopoly directorate). It is the author's opinion that, because of the TSWV is very dangerous and lack of resistance among the cultivated tobacco varieties, control measures in the Çanakkale region must of Killing the thrips. Killing overwintering thrips in the fields and Complete protection of the seedbeds and in the field is very important to the succesful control of the disease.

ÖZET

TÜRKİYE'DE DOMATES LEKELİ SOLGUNLUK VİRUSUNA AİT İLK RAPOR VE ÇANAKKALE İLİNDEKİ TÜTÜNLERDE EPİDEMİ DURUMU

1979 yılından beri Çanakkale ilinin bazı tütün üretim bölgelerinde epidemi meydana getirerek büyük bir ürün kaybına neden olan virus hastalığının saptanması amacı ile, 1980 yılından itibaren çeşitli otsu endikatör bitkileri ile endeksleme testleri ve infekteli bitki suyu ile **in Vitro** testleri uygulanmıştır. Ayrıca, söz konusu epidemiyi meydana getiren virusun vektörünün saptanması amacıyla Thrips ile nakil denemeleri de uygulanmıştır.

Uygulanan endeksleme testlerinde **Petunia hybrida, N. glutinosa, N. tabacum** Samsun NN, **N. tabacum** xanty, **Tropaolum majus** (Nasturtium), **Cucumis sativus, Vinca rosea, Datura stramonim** gibi otsu endikatör bitkileri üzerinde Domates Lekeli Solgunluk Virusu (TSWV)'nun karakteristik belirtileri elde edilmiştir. **In Vitro'da** uygulanan denemelerde, Virus, hastalıklı bitki suyunda 40-45°C'de 10 dakikada, ayrıca oda sıcaklığında 5 saat süreyle bekletilen hastalıklı bitki suyunda inaktif hale gelmiştir. Araştırma bölgesinde epideminin oluştuğu bölgelerde çok yoğun olan Thrips'in **Thrips tabaci** olduğu ve yapılan nakil denemelerinde TSWV' nün epidemi meydana getirmesinde rol oynadığı ve araştırma bölgesinde büyük bir potansiyele sahip olduğu saptanmıştır.

Domates Lekeli Solgunluk Virusunun Balkan ülkelerinde başta Yugoslavya, Bulgaristan, Yunanistan ve Çekovlovakya'da tütünlerde büyük ürün kayıpları meydana getirdiği literatürde kayıtlıdır. Balkan ülkelerinde olduğu gibi, ülkemizdeki epidemiyi de **Thrips tabaci** meydana getirmektedir. Bu virusun araştırma bölgesindeki tütünlerde meydana getirdiği büyük zararlar yanında,, tütün tarlaları civarındaki domateslerde de aynı derecede zarar yaptığı, ayrıca Manisa ilimizin salçalık domates üretim alanlarında tipik hastalık belirtileri gösterdiği ve zararlar yaptığı endeksleme ve laboratuvar testleri ile saptanmıştır. Virusun domateslerde tohum yolu ile geçebildiği lireratürde kayıtlıdır. Bu nedenle, çok geniş bir konukçu dizisi olan ve Thrips ile büyük bir yayılma potansiyeline sahip olan bu virusa karşı çok sıkı karantina önlemleri alınması gerekmektedir.

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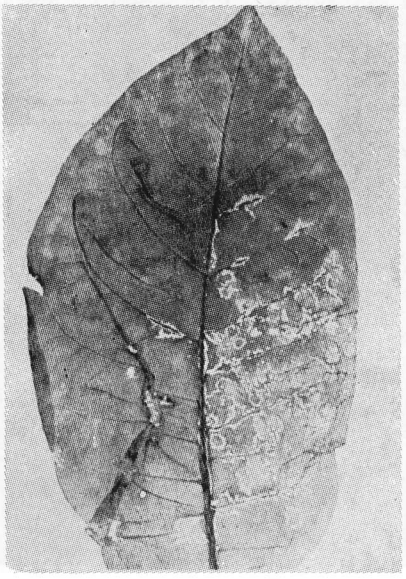

Fig. 1. Leaf of tobacco plant infected with TSWV, showing typical symptom of the virus.

Fig. 2. Leaf of tobacco plant systemically infected by TSWV, showing characteristic rings and necrosis.

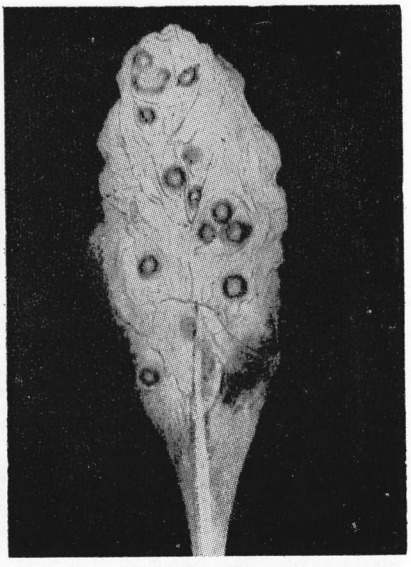

Fig. 3. Local necrotic of TSWV, on petunia leaf, 3 days after the sap inoculations

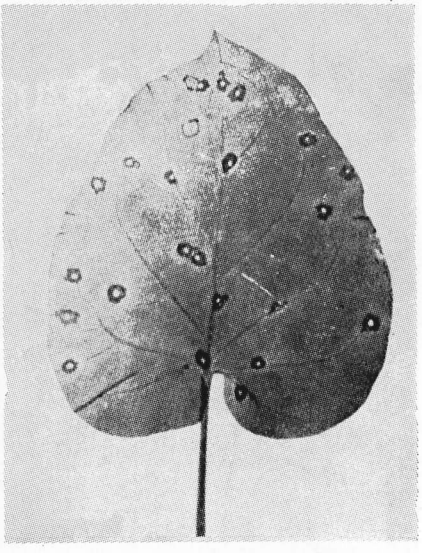

Fig. 4. Local necrotic lesions, of TSWV on **N. glutinosa**.

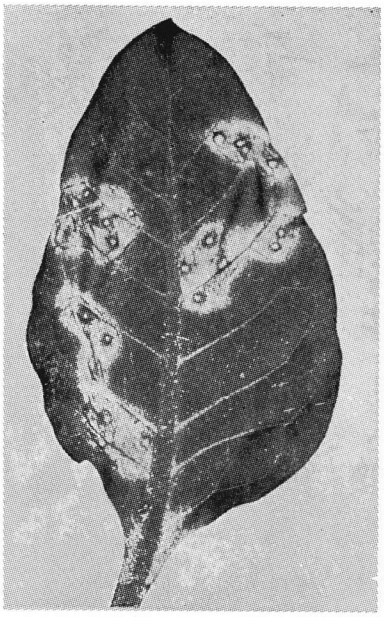

Fig. 5. Typical local necrotic lesions of TWSV on **N. tabacum** Samsun

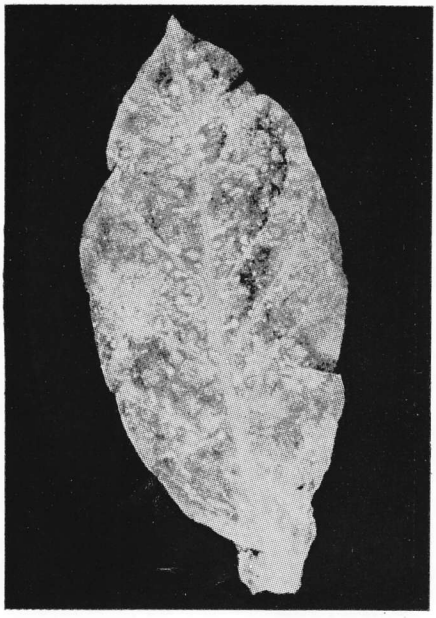

Fig. 6. Leaf **N. tabacum**, sap inoculated with TSWV showing systemic concentric rings of the virus

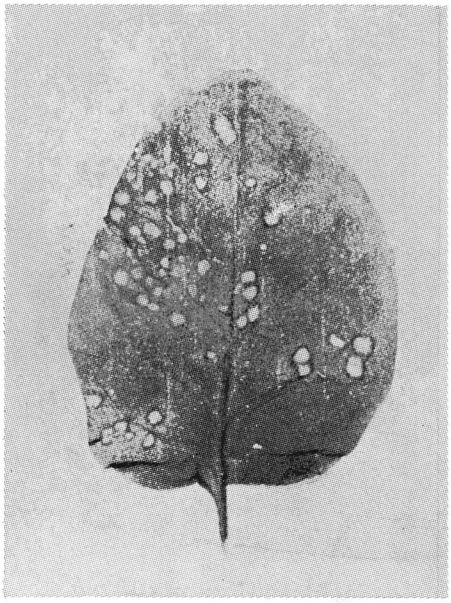

Fig. 7. Characteristic local necrotic lesions of TSWV on **N. tabacum** xanty.

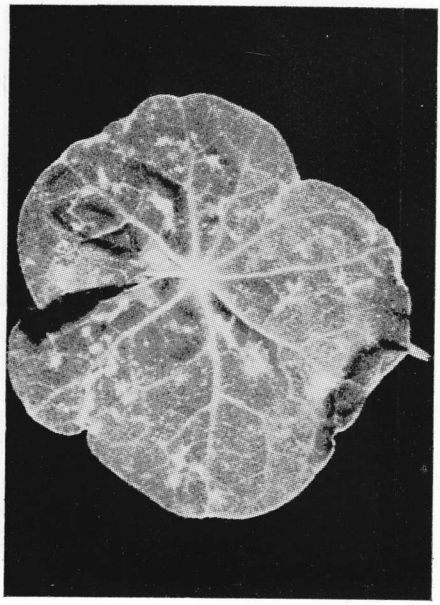

Fig. 8. Systemic symptoms of TSWV on **Tropaeolum majus**

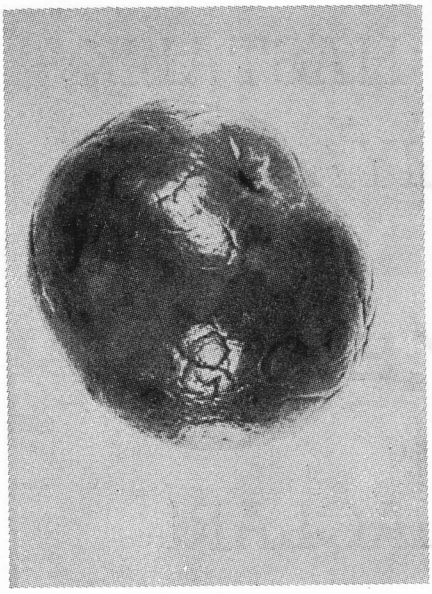

Fig. 9. Fruit of TSWV infected tomato plant showing characteristic rings, paler red and yellowing coloring on the fruit surface.

Fig. 10. Systemically infected **N. tabacum** leaf by thrips, showing characteristic concentric rings of TSWV.

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Pathogenicity Tests of Some **Pestalotia** Species on Various Ornamental Plants

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ABSTRACT

Various **Pestalotia** spp. were isolated from the diseased leaves of **Anthurium crassineivium, Cymbidium hybridium, Oxalis hedysaroides, Rosa** sp., **Chrysanthemum** sp., **Scindapsus aureus, Gerbera jamesonii** and **Chamerops excelsa.** Four isolates obtained from **Chyrsanthemum, Rosa, Scindapsus** and **Gerbera** were tested in respect to their pathogenicity by applying of mass inoculation technique to the uninjured and artifically injured leaves. All of the isolates tested were found to be pathogenic under the experimental conditions.

INTRODUCTION

Some species of **Pestalotia** are known to cause damages on various ornamental plants. DODGE and RICKETT (1948) and PAPE (1964) were reported that **Pestalozzia palmarum** Cke. **P. phoenicis** Grev.ua and **Pestalotia palmarum** Cke. **P. palmicola** caused leaf blight on Palmae and **Pestalozzia rhipsalidis** Grill.caused spot disease on Opuntia. DODGE and RICKETT (1948) and EMMANOUIL (1970) noted that on certain Camelias **Pestalotia quepini** Desm. caused spot disease. **P. rhododendri** Guba. was isolated by STATHIS (1970) from the leaves of Rhodendron. In India, **P. algeriensis** (Sacc Berl). Guba. and **Pestalotiopsis effecta** were reported on **Magnolia grandiflora** and **Cymbidium gemianum** respectively by MITRA and TANDON (1966), and by SRIVASTAVA et al (1980).

Survey studies were conducted on the diseases of ornamental plants in İzmir and its surroundings during 1979-1980 and several species of **Pestalotia** have been observed on various ornamental plants.

This paper deals with the pathogenicity tests of **Pestalotia** species on their host plants.

MATERIAL and METHODS

Pestalotia spp. were isolated from the diseased leaves of **Anthurium** sp., Orchid, **Oxalis hedysaroides**, Rosa sp., **Chrysanthemum** sp., **Scindapsus aureus, Gerbera jamesonii** and **Chamerops excelsa.**

Pestalotia sp. isolated from **Chamerops excelsa** was identified as **P. palmarum** Cke. Therefore, it was not included in the pathogenicity tests. On the other hand the isolates obtained from **Oxalis hedysaroides, Anthurium** sp. and Orchid were also excluded from the experiments, because of their rarity.

Isolations were made according to commonly used methods on PDA medium. Two methods of artifical inoculation were employed in the pathogenicity tests and inoculations were made both on to the injured and uninjured surfaces of the tested leaves. The surface of leaves were washed with sterilized distilled water before inoculations and the injury of leaves were made by using cardorandum dust.

1. Mass inoculation method

A small piece of inoculum containing spores and mycelium was placed on the injured and un injured surface of the separate leaves and the inoculated area was covered with a moist cotton pad. Injured and uninjured areas of the leaves of the control plants were covered with moist cotton pads only.

2. Spore suspension method

The suspension of the spores were prepared by homogenizing the whole culture in sterilized distilled water in a blender. This suspension was sprayed on the injured and uninjured surfaces of the separate leaves. The control plants were sprayed with sterilized distilled water only.

Following the inoculations the plants were incubated in a room which was maintained at 25 + 2°C and the necessary moisture was provided by daily water sprayings.

RESULTS and DISCUSSION

On the host plants, about 7-10 days after the inoculations yellowish-green areas were first observed on the leaf surface where the inoculum was applied. Later, this area became a dull brown lesion, then, turned to a dark brown coloured patch. There was no symptom on the control plants.

Mass inoculation method was more effective than the spraying method and the infections were more succesful on the injured surfaces.

Re-isolations were made from artifically inoculated leaves and then re-isolates and original isolates were examined and compared under the microscope.

Spore sizes were measured in both original and re-isolates. The results were indicated that the average size of the spores was in confidence limit.

Pathogenicity tests of the four isolates were conducted artificially by mass inoculation method with or without injuring the leaves. All of the **Pestalotia** spp. were pathogenic under the experimental conditions and they could infect the host plants provided that they were previously injured. There was no infection in the absence of injury. TANDON and BHARGAUVA (1960) and TANDON and CHANDRA (1963) also observed that uninjured leaves were not infected by **Pestalotia sp.**

Ö Z E T

ÇEŞİTLİ SÜS BITKİLERİNDE BAZI Pestalotia TÜRLERİNİN PATOJENİSİTESİ

1979-1980 yılları arasında İzmir ve çevresinde ticari amaçla çiçek üretimi yapılan alanlarda yapılan surveyler sonucu bazı süs bitkilerinde (Flamingo, Orkide, Oxalis, Gül, Krizantem, Fatos, Gerbera ve Palmiye) yaprak lekelerinden **Pestalotia** türleri izole edilmiştir. Bunlardan Flamingo, Orkide ve Oxalis izolatları konukçu bitkiler temin edilemediğinden, Palmiye izolatı ise Palmiyelerde yaprak yanıklığı oluşturduğu literatürde kayıtlı **P. palmarum** Cke. olarak tanımlandığından patojenisite testlerine alınmamışlardır. Diğer konukçularla iki yönteme göre yürütülen patojenisite testlerinde izolatlar kendi konukçularında lezyon oluşturmuşlar ve re-izole edilmişlerdir. Kullanılan yöntemlerden parça inokulasyon yöntemi ve zedelenmiş yapraklar inokulasyonda başarılı olmuştur.

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Relationship of Pumpkin Mosaic Virus with its Aphid Vector, **Aphis gossypii** Glov.

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ABSTRACT

Studies conducted on the relationship of Pumpkin mosaic virus (PWP) with its aphid vector, **Aphis gossypii** Glov. indicated that the preacquisition fasting of aphid vector was essantial for successful transmission of PMV. No transmission was observed unless aphids were starved for a minimum of 10 minutes. The optimum preacquisition fasting period was found to be 90 minutes. The aphids acquried and transmitted the virus in a very short feeding period of 20 and 10 seconds respectively. Longer acquisition and inoculation feeding periods decreased the transmission efficiency of the aphid vector. The optimum acquisition and inoculation feeding periods were found to be 10-15 and 15-30 minutes respectively. Even a single viruliferous aphid was capable of transmitting the virus to healthy pumpkin plants. However, the maximum transmission was obtained when groups of 10 aphids per plant were employed. The infectivity of aphid vector was lost when they were given post-acquisition fasting beyond 2 hours. In serial transfers of single viruliferous aphids to healthy test plants, it was observed that when the interval of feeding on successive plants was 10 minutes and above, the aphids transmitted the virus to the first plant of the series only. In case of short feeding intervals of 2 and 5 minutes on each test plant in the series, however, the third and second plants could be infected respectively but not the subsequent ones, indicating thereby that the virus in typically of the non-persistent type. Both alate and apterous forms were found to be almost equally efficient in transmitting the virus.

INTRODUCTION

Among the most important, most complex and most extensively distributed agent of plant virus vectors, aphids, leaf hoppers, whiteflies and thrips have attracted worldwide attention and their relationship to the viruses they transmit have been studied in many cases. Black (1959) pointed out that the vectors of any plant virus are almost restricted to one of the major taxa, such as the aphids, the leaf hoppers, the whiteflies, the thrips or the nematodes and a plant virus is almost transmitted by only one of the principal types of transmission, that is, either the circulative, the stylet-borne or the propagative type.

Aphids form the largest group of the vectors of plant viruses studied. Transmission by aphids has been reviewed by various authors such as (Bawden, 1957; Carter, 1961; Maramorosch, 1963; Rochow, 1961; Smith, 1957, 1958; Sylvester, 1958; 1962).

During 1976-77, a new disease of pumpkin (**Cucurbita maxima** Poir and **C. moschata** Duch.) was noticed around Bangalore (Karnataka State). Preliminary studies on this virus revealed that this virus was transmitted by aphid (**Aphis gossypii**) under natural conditions (Singh, 1980). Since this aphid is anatural vector of this virus, it was considered worthwhile to study the relationship of this virus its aphid vector (**A. gossypii**) and the results of these studies have been presented in this communication.

MATERIAL and METHODS

The pumpkin mosaic virus (PMV) culture was maintained in an insect proof glasshouse on healthy pumpkin (**C. maxima**) plants. Healthy colonies of aphid, **A. gossypii** was maintained on healthy okra (**Abelmoschus esculentus** Moench) plants. All the transmission tests were made from recently infected pumpkin plants as virus source. In all the experiments young healthy pumpkin (**C. maxima**) plants var. 'Arka Suryamukhi' at two leaf stage were used as test plants. In all the inoculation studies adult aphids were used except otherwise stated. During the feeding of the aphids, the test plants were covered with lantern globes with muslin cloth fixed on the top. The aphids, after specified treatment, were placed on the test plants with the aid of a camel hair brusk No. 1. Aphids were killed at the end of the requried feeding period by spraying the plants with 0.05 % Dimethoate. In case of short feeding periods of less than 5 minutes, the individual aphids were watched through a magnifying lens

and the time of feeding was determined by means of a stop watch by noting the time when aphids had inserted their stylets.

RESULTS

1. Effect of preacquisition fasting period of the aphid vector on the transmission efficiency of PMV :

A large number of aphids (**A. gossypii**) were collected from the culture plant and were starved in a glass vial for 5, 10, 15, 25, 40, 60, 90 minutes and 2, 4, 8 and 24 hours. Batches of 15 aphids from each of above mentioned categories were given acquisition feeding for 10 minutes before transferring them to feed on healthy test plants. After acquisition feeding all the aphids in each batch were transferred to a set of 10 healthy pumpkin plants each for inoculation feeding. They were allowed to remain on test plants for overnight. Finally all the aphids were killed by spraying test plants with 0.05 % Dimethoate solution. The controls were maintained simultaneously by giving the acquisition feeding with non-starved aphids. The results are presented in table-1. It is quite evident from the data presented in table-1 that no transmission was obtained unless aphids were given preacquisition starvation. A low percentage of transmission (16.66) was obtained when they were given preacquisition fasting of 5 minutes. When the fasting period was increased from 5-90 mts, there was an increase in the ability of the aphids to the virus transmission, beyond which the percentage of infection decreased gradually. The maximum infection (100.00 %) was observed at 90 minutes of fasting.

2. Effect of different acquisition feeding periods of the aphid vector on the transmission efficiency of PMV :

Aphids (**A. gossypii**) were collected from the source plant and were given preacquisition fasting for 90 minutes (90 minutes preacquisition fasting was found to be an optimum period for maximum transmission). Batches of 15 aphids each were given an acquisition feeding period of 5, 10, 20, 40, 60 seconds and 1, 3, 5, 10, 15, 20, 30, 60, 90 minutes respectively on virus diseased plant before transferring them to test plants for inoculation feeding. The aphids were allowed to remain on the test plants overnight. It is evident from the data presented in table-2 that the aphids acquired the virus from infected plants in a very short feeding period of 10 seconds. However, the maximum infection (100.00 %) was achieved when the aphids were allowed to acquire virus for 10-15 minutes. Acquisition feeding beyond optimum (i.e. 10-15 minutes) reduced the transmission efficiency of the aphids gradually.

3. Effect of inoculation feeding period of the aphids on the transmission efficiency of PMV :

A number of aphids were starved for 90 minutes and were given acquisition feeding on virus infected pumpkin leaves for 15 minutes. Aphids were distributed in 13 batches consisting of 15 aphids each, and then transferred to test plants for inoculation feeding for 10 and 30 sec., 1, 5, 10, 15, 30 min. and 1, 2, 4, 6, 12 and 24 hr. respectively. The details are presented in table-3. It was observed that aphids transmitted PMV to an extent of 6.66 % in as short as 10 seconds of inoculation feed on the test plants. However, the percentage of transmission increased gradually with an increase in inoculation feeding period upto 30 minutes. A period between 15-30 minutes was found to be an optimum inoculation feeding period for cent per cent transmission efficiency of aphids.

4. Relation to number of viruliferous aphids per plant to the percentage transmission of PMV :

A large number of aphids were starved for 90 minutes and then divided in 10 groups consisting of 1, 2, 3, 5, 10, 15, 20, 30, 40 and 50 aphids of each group were given acquisition feeding for 10 minutes on pumpkin leaves severely infected with PMV. Viruliferous aphids from each group were transferred to healthy pumpkin plants separately for test feeding.

The data presented in table-4 show that even a single viruliferous aphid could transmit the virus to an extent of 6.66 per cent. Cent per cent transmission was obtained when 10, 15 and 20 aphids per plant were used for transmission.

5. Effect of post-acquisition starvation of viruliferous aphids on the transmission of PMV :

A number of aphids were starved for 90 minutes and then they were given on acquisition feeding on PMV infected pumpkin plant for 10 minutes. Aphids were then divided into 9 groups consisting of 15 aphids each and were given different post, acquisition starvation viz., 10, 20, 40, 60, 90 min. and 2, 4, 6, and 12 hours respectively. Group of 15 aphids were transferred to healthy pumpkin plants for inoculation feeding. The results presented in table-5 indicate that the aphids could retain the virus in their mouth parts upto 2 hours only, after removing them from acquisition source. The reduction in transmission ability started with 10 minuted of postacquisition fasting and continued upto 2 hours. No transmission was observed after 2 hours of fasting.

6. Non-persistence of PMV in the aphid vector in successive transfers :

The experiments were conducted to determine how long the viruliferous aphids would remain in-

fective in successive transfers to healthy plants without access to a fresh infection source. For this purpose aphids were given preacqusition fasting and acquisition feeding as mentioned earlier. The individual aphids were then transferred in succession to a series of five healthy test plants. Different feeding intervals were given to different series such as 2, 5, 10, 15, 30 min. and 1, 2, 4 hours respectively.

The aphids infected 3rd plant of the series when a single viruliferous aphid was transferred at intervals of 2 and 5 minutes and upto 2nd plant in the series when aphids were transferred at 10 minute interval. When the aphids were transferred at intervals of 15 min., 30 min., 1, 2, and 4 hours, only the first plant of the series got the infection. The virus-vector relationship was found to be of non-persistent type (Table-6).

7. Comparative efficiency of alate (winged) and apterous (non-winged) forms of aphids in the transmission of PMV :

A large number of alate and apterous forms of aphids were collected and starved as mentioned earlier and given optimum acquisition feeding on virus infected leaves. Finally they were transferred in batches of 10 each to healthy test plants for inoculation feeding. From the results it is clear that under laboratory conditions both the forms, were equally efficient vectors of the virus.

DISCUSSION

The mechanism whereby non-persistent viruses are transmitted have been the subject of continuous debate since. Hoggan (1931, 1933, 1935) proposed that these viruses are transmitted mechanically on the insect's stylets. During the present studies it was observed that the preacquisition starvation increased the efficiency of the aphid vector **(A. gossypii)**; shorter the preacquisition fasting, lesser was the percentage transmission of PMV. Optimum period of 90 minutes of starvation was required by the aphids for maximum transmission of PMV. No transmission was observed unless aphids were subjected to the preacquisition starvation for a minimum of 10 minutes. These findings are in concurrence with those reported by Watson (1936), Watson and Roberts (1939), Bradley (1952), Sylvester (1949, 1950), Bhargav (1951), Miller (1952) and Singh **et al.** (1975).

In the present investigations the results obtained cannot be interpreted with certainity but they are compatible with the inactivator hypothesis putforth by Watson and Roberts (1939, 1940). As per this hypothesis, during the process of transmission from one plant to another the virus comes in contact

with some substance which partially or wholly destroys its infectivity. It is presumed that this substance either ceases to be produced during starvation of insects or is produced at a much lower rate so that when an aphid is given a short feeding time after starvation, the amount of inactivation is not sufficient to inactivate the virus taken in, thus resulting in more infectivity. But with prolonged infection feeding the effect of fasting disappears and the amount of inactivator increases resulting in the inactivation of a greater amount of virus. On the other hand if the aphids are not starved, the amount of inactivator already present in insect at its maximum and inactivates larger proportion of the virus taken in, during the short infection feeding period. Bradley (1961), observed that aphids require some time to retract the stylet into the labium when feeding is interrupted by removal from the host plant. Although they may appear to feed or probe immediately on the diseased plants, they cannot do so until the stylets are retracted and thus do not acquire virus. A period without feeding allows the aphids to retract their stylets.

The experiments conducted on the effect of acquisition feeding of the aphid vector on the transmission of PMV have shown that the PMV could be acquired in a very short period of 10 seconds (Table 2). This indicates that the acquisition threshold varies inversely as the efficiency of transmission. The optimum acquisition feeding period was found to be 5 minutes. Increasing the acquisition feeding period beyond the optimum diseased the percentage transmission of the virus. Similar results were reported by Day and Irzykiewicz (1954), Bradley (1954), Nariani and Sastry (1962), Nagarajan and Ramakrishnan (1971), Singh (1970-72) and Singh **et al.** (1975).

The fall in the percentage of infection with longer feeding periods have been explained by Watson and Roberts (1939) and Day and Irzykiewicz (1954) on the basis of production of inhibitors in insects during feeding. Sylvester (1949) showed that the beet mosaic virus could be acquired in single short probe (15-60 seconds). The process of probing itself may scour the virus previously acquired from the stylets (Bradlley, 1959). According to Bradley (1954), however, the formation of salivery sheath during longer feeding intervals prevents the aphids from becoming infective. Bradley, 1959 also suggested that constant probing by aphids caused them to lose infevtivity primarily because the virus is scoured from the stylets as they penetrate.

The investigations carried out on the effect of inoculation feeding period of the aphid vector in the percentage infection of PMV indicated that **A. gossypii** could transmit the virus in a very short ino-

culation feeding period of 10 seconds. The percentage of infection, however, increased with increase in feeding period upto 1 hour. Longer inoculation feeding decreased the percentage transmission (Table-3). It is presumed that more efficient vector carry a larger quantity of virus in its mouth part and at every probe a larger charge of virus is delivered into the susceptible host. Nariani and Sastry (1962) working with chilli mosaic virus observed similar results and the explaination given by them was that most of the aphids, that can cause infection, do so within first hour and further increase in the duration of test feeding does not seem to increase the number of infections. Nagarajan and Ramakrishnan (1971) reported similar results with bittergourd mosaic virus and its five aphid vectors. Similar findings were reported by Singh (1970, 1972, Singh **et al.** (1975).

The inoculation step in stylet-borne transmission is similar in many ways to the acquisition process. Inoculation has been reported by several workers to occur after probes as short as 5 seconds on the test plants. First probe longer than about 15-30 seconds do not increase the probability of infection (Bradlley, 1952). Presumably, in these early probes, virus is usually introduced between the transverse walls of epidermal cells. Nevertheless, introduction intracellularly into epidermal cells, or into underlying mesophyll cells, is by no means ruled out.

Studies on the relationship of number of viruliferous aphids to the percentage transmission of PMV revealed that even a single viruliferous aphid was capable of percentage (Table-4). However, the percentage of transmission increased with an increase in the number of viruliferous aphids per plant to a maximum of 20. Further increase in number of aphids per plant decreased the percentage transmission of PMV. The results of the present findings are in concurrence with those reported by Freitag and Severin (1945), Nariani and Sastry (1962), Nagarajan and Ramakrishnan, (1971) and Sing **et al.** (1975). The results confirm view of Watson (1936) and Storey (1939) that the infections produced are local and independent for each aphid and not the result of accumulation of sub-infective doses from different members of a group and the low percentage of infections obtained with single aphid does not indicate a fixed low standart of efficiency on the vectors but is due to fluctuations in the infection capacity which can be increased or decreased according to the conditions of the experiment.

The experiments carried out on the effect of post-infection starvation on the transmission of PMV indicated that as the fasting period was increased, the percentage transmission decreased gradu-

ally and no transmission was observed beyond 2 hours of post-acquisition fasting (Table-5). Such decreases in infection have also been reported by Watson and Roberts (1939, 1940), Singh (1970), Nagarajan and Ramakrishnan (1971), and Singh et al. (1975).

With stylet-borne viruses, aphids are usually able to infect plants immediately after the acquisition, but lose their ability to infect more or less rapidly. Thus, in the present studies there appears no possibility of virus replication in the vector. Generally speaking, aphids carrying a styletborne virus remain infective somewhat longer off the plant than on it. This may be due in part to lack of probing but as Bradlley (1952) pointed out that captive aphids are by no means inactive. In the present studies, it is seen that the aphids lost their infection ability after 2 hours. This may be attributed due to lack of delayed feeding or without acess for infectivity.

In the present studies in the serial transfers of viruliferous aphids, it was noticed that the aphids cease to be infective very soon while feeding on test plants. The aphids infected 3rd plant of the series when they were allowed to feed 2 minutes (Table-6). Persistence of PMV in **A. gossypii** was longer during fasting than feeding. This corroborates earlier studies on cucumber mosaic virus (Doolittle and Walker, 1928; Bhargava, 1951), Potato virus Y (Smith, 1931) and lettuce mosaic virus (Kassanis, 1947). Nagarajan and Ramakrishnan (1971) reported that persistence of bittergourd mosaic virus in its aphid vectors was longer during fasting than feeding. Day and Irzyekeiwicz (1954) observed that the duration of persistence of infectivity of aphids has a bearing on the inactivation hypothesis. During fasting, the virus would have less opportunity of coming in contact with salivary inhibitor. During feeding some virus is wiped off by the stylets but the very survival time of virus during feeding indicates that they are subjected to an additional inhibiting action. The results of the present study on persistence of virus in the aphid vector seems to lend support to the inactivator hypothesis. The stylets come to hold less and less virus after successive probe so that the possibility of a successful inoculation is reduced.

The main reason for attempting to determine how long aphids retain the ability to infect, is in relation to the spread of the virus in field. In most of the experiment, conditions have not been particularly close to those that might exist under field conditions.

The comparative efficiency of both alate and apterous forms of **A. gossypii** in the transmission of PMV has been determined in the present studies. The results indicated that there was no significant difference in the ability of these forms as far as transmission of

PMV was concerned. These results closely resemble with those reported by Nariani and Sastry (1962), Singh (1970, 1972), Nagarajan and Ramakrishnan (1971), and Singh et al. (1975).

Based on these findings on the relationship of PMV with its aphid vector (**A. gossypii**), it is concluded that PMV is transmitted by **A. gossypii** aphid in a "non-persistent" manner and it is 'stylet-borne'.

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ÖZET

KABAK MOZAYIK VİRUSU VE AFİD VEKTÖRÜ (**Aphis gossypii** Glov.) ARASINDAKİ İLİŞKİ

Kabak Mozayik Virüsu (PMV) ile Afid vektörü (**Aphis gossypii** Glov.) arasındaki ilişki üzerinde yapılan çalışmalar, virüsun başarılı bir şekilde taşınması için afid vektörünün virusu kazanma öncesinde aç bırakılmasının esas olduğunu göstermiştir. Afidler en az 10 dakika aç bırakılmadıkça taşınmanın olmadığı gözlenmiştir. Virusu kazanma öncesi optimum açlık periyodu 90 dakika olmuştur. Afidler vektörü çok kısa bir beslenme periyodunda (20 saniye) kazanır ve 10 saniyede taşırlar. Daha uzun kazanma ve inokulasyon beslenme periyodları afid vektörün taşıma etkinliğini azaltmıştır. Kazanma ve inokulasyon için optimum beslenme periyodları sırasıyla 10-15 ve 15-30 dakika olarak bulunmuştur. Bir tek virulent afid bile virusu sağlam kabak bitkilerine taşıma yeteneğindedir. Bununla beraber maksimum taşınma, her bitki için 10 afidlik gruplar kullanıldığı zaman elde edilmiştir. Afid vektörün infektif oluşu, virusu kazanmayı izleyen ve 2 saatin üzerinde olan bir açlık periyodu verildiğinde kaybolmaktadır. Tek bir virulent afit ile yapılan seri halindeki taşıma denemelerinde, birbirini izleyen bitkiler üzerindeki besleme aralığı 10 dakika ve daha fazla olduğunda afidin virusu sadece birinci seri bitkelere taşıdığı gözlenmiştir. Her bir serideki test bitkileri üzerinde 2 ve 5 dakika gibi kısa beslenme periyodlarında sırasıyla ikinci ve üçüncü bitki infekte olmuş fakat bunları izleyenler infekte olmamıştır. Bu durum virusun tipik olarak non-persistent tip olduğunu göstermektedir. Kanatlı ve kanatsız formlar hemen hemen eşit etkinlikte olarak virusu taşırlar.

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Table 1

Effect of preacquisition fasting period of the applied vector, **Aphis gossypii** on the transmission efficiency of PMV

Sl. No.	Preacquisition fasting period	Total No. of plants infected/ inoculated*	Percentage of infection	Mean of transformed values**
1.	No fasting	0/30	0.00	0.00
2.	5 minutes	5/30	16.66	21.90
3.	10 minutes	5/30	16.66	21.90
4.	15 minutes	7/30	23.33	32.09
5.	25 minutes	9/30	30.00	36.13
6.	40 minutes	17/30	56.66	51.91
7.	60 minutes	28/30	93.33	82.71
8.	90 minutes	30/30	100.00	00.00
9.	2 hours	25/30	83.33	70.50
10.	4 hours	20/30	66.66	58.22
11.	8 hours	12/30	40.00	42.09
12.	24 hours	8/30	26.66	34.18

* Average of 3 experiments

** Significant

SEM 3.45

CD at 5% 10.12

CD at 1% 13.76

Numerator : Number of plants infected

Denominator : Number of plants inoculated

Table 2

Effect of acquisition feeding period of the aphid vector, **A. gossypii** on the transmission afficiency of PMV

Sl. No.	Acquisition feeding period	Total number of plants infected/ inoculated*	Percentage of infection	Mean of transformed values**
1.	5 seconds	0/30	0.00	0.00
2.	10 seconds	2/30	6.66	19.49
3.	20 seconds	5/30	16.66	27.59
4.	40 seconds	6/30	20.00	30.00
5.	60 seconds	8/30	26.66	34.18
6.	3 minutes	16/30	53.33	49.82
7.	5 minutes	30/30	100.00	84.82
8.	10 minutes	30/30	100.00	84.26
9.	15 minutes	30/30	100.00	84.26
10.	20 minutes	25/30	83.33	70.50
11.	30 minutes	21/30	70.00	60.31
12.	60 minutes	11/30	36.66	40.17
13.	90 minutes	11/30	36.66	40.17

* Average of 3 experiments

** Significant

SEM 2.19

CD at 5% 6.39

CD at 1% 8.66

Numerator : Number of plants infected

Denominator : Number of plants inoculated

Table 3

Effect of inoculation feeding period of the aphid vector, **A. gossypii** on the transmission efficiency of PMV

Sl. No.	Inoculation feeding period	Total number of plants infected/ inoculated*	Percentage of infection	Mean of transformed values**
1.	10 seconds	2/30	6.66	19.49
2.	30 seconds	4/30	13.33	25.19
3.	1 minute	8/30	26.66	38.04
4.	5 minutes	13/30	43.33	44.04
5.	10 minutes	18/30	60.00	54.31
6.	15 minutes	30/30	100.00	84.26
7.	30 minutes	30/30	100.00	84.26
8.	1 hour	29/30	96.66	81.86
9.	2 hours	25/30	83.33	70.50
10.	4 hours	23/30	76.66	64.80
11.	6 hours	18/30	60.00	53.72
12.	12 hours	14/30	46.66	45.95
13.	24 hours	14/30	46.66	45.95

* Average of 3 experiments

** Significant

SEM 3.35

CD at 5% 9.80

CD at 1% 13.28

Numerator : Number of plants infected

Denominator : Number of plants inoculated

Table 4

Relation of number of viruliferous aphids per plant to the transmission of PMV

No. of aphids per plant	Total number of plants infected/ inoculated*	Percentage of infection	Mean of transformed values**
1	5/30	16.66	27.59
2	7/30	23.33	32.09
3	10/30	33.33	38.22
5	23/30	76.66	64.80
10	30/30	100.00	84.26
15	30/30	100.00	84.26
20	30/30	100.00	84.26
30	25/30	83.33	70.50
40	21/30	70.00	60.31
50	19/30	63.33	56.27

* Average of 3 experiments

** Significant

SEM	2.76
CD at 5%	8.21
CD at 1%	11.25

Numerator : Number of plants infected

Denominator : Number of plants inoculated

Table 5

Effect of post-infection starvation of aphid vector, **A. gossypii** on the transmission of PMV

Sl. No.	Post-infection starvation	Total number of plants infected/ inoculated*	Percentage of infection	Mean of transformed values**
1.	No fasting	30/30	100.00	90.00
2.	10 minutes	25/30	83.33	66.14
3.	20 minutes	19/30	63.33	52.77
4.	40 minutes	16/30	53.33	46.92
5.	60 minutes	10/30	33.33	35.21
6.	90 minutes	8/30	26.66	30.99
7.	2 hours	4/30	13.33	21.14
8.	4 hours	0/30	0.00	0.00
9.	6 hours	0/30	0.00	0.00
10.	12 hours	0/30	0.00	0.00

* Average of 3 experiments

** Significant

SEM 1.77

CD at 5% 5.22

CD at 1% 7.12

Numerator : Number of plants infected

Denominator : Number of plants inoculated

Table 6

Persistence of pumpkin mosaic virus in its aphid vector, **A. gssypii** in successive transfers

Sl. No.	Feeding period on test plant of the series	Infection produced on test plants in successive transfers (Serial number of plants tested) 1	2	3	4	5
1.	2 mts	+	+	+	—	—
2.	»	—	+	+	—	—
3.	»	+	—	+	—	—
4.	»	+	+	+	—	—
5.	»	+	+	—	—	—
6.	6 mts	+	—	—	—	—
7.	»	+	+	—	—	—
8.	»	+	—	—	—	—
9.	»	+	—	—	—	—
10.	»	+	—	—	—	—
11.	10 min	+	—	—	—	—
12.	»	+	+	—	—	—
13.	»	+	+	—	—	—
14.	»	+	+	—	—	—
15.	»	—	—	—	—	—
16.	15 mts	—	—	—	—	—
17.	»	+	—	—	—	—
18.	»	+	—	—	—	—
19.	»	+	—	—	—	—
20.	»	+	—	—	—	—
21.	30 mts	+	—	—	—	—
22.	»	—	—	—	—	—
23.	»	+	—	—	—	—
24.	»	+	—	—	—	—
25.	»	—	—	—	—	—
26	1 hour	+	—	—	—	—
27.	»	+	—	—	—	—
28.	»	+	—	—	—	—
29.	»	—	—	—	—	—
30.	»	—	—	—	—	—
31.	2 hrs	+	—	—	—	—
32.	»	+	—	—	—	—
33.	»	—	—	—	—	—
34.	»	+	—	—	—	—
35.	4 hrs	—	—	—	—	—
36.	»	+	—	—	—	—
37.	»	—	—	—	—	—
38.	»	+	—	—	—	—
39.	»	—	—	—	—	—
40.	»	—	—	—	—	—

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