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CONTENTS

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Results from Studies on Barley Stripe Mosaic Virus

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ABSTRACT

Using the seed-infected barley plants as source material, the dilution end-point of the clarified sap diluted with 0.1 M buffer phosphate was 10^{-3}

Serological studies confirmed that both crude and clarified sap of the virus gave a precipitation band with antiserum of 1:128, but not with normal serum.

The virus was still active after 7 days at room temperature, but infectivity of the virus was completely destroyed by heating the sap at 50°C for 12 minutes.

The infectivity of the virus was greatly affected by purification, probably because of the age of donor plants used to get inoculum for purification.

Electron microscopy of partially purified barley stripe mosaic virus (BSMV) showed that rod-shaped particles of BSMV was 130 nm. in length and 30 nm. in width and anisometric.

The most interesting thing was that **Chenopodium quinoa** has been determined as a new local lesion host of the virus. **N.tabacum** var. Samsun previously recorded as local lesion host of the virus, but no symptoms were determined on that plant with BSMV. Dikson and Hannchen barley plants gave good systemic reaction with the virus.

INTRODUCTION

BSMV (false stripe) has been found to occur naturally as a serious systemic disease of barley and wheat as well as being widely distributed. The disease has been known since 1910 as a false stripe and was considered to be a non-parasitic disorder of barley plants (1). It was first identified as a seed-borne and mechanically transmissible virus disease in 1950 by McKinney (13). Inoculation tests have shown that the disease also induces systemic infection on sweet corn, smooth crabgrass and/or local lesions on bromegrass (13). Among the dicotyledonous plants, the tobacco plant, variety Samsun, has given local lesion symptoms with BSMV (15).

Kassanis and Slykhuis (12) in Canada have proved that Italian rye grass, **Lolium multiflorum,** Lam, **Beta vulgaris, Chenopodium amaranticolor** and **Spinacea oleracea** L. were susceptible to the virus. The former three gave local lesion symptoms, but the latter reacted systemically. Many of the barley and wheat varieties have been found susceptible (4) and their reactions to infection with the virus differed greatly (12). Several strains of the virus have been found based on the variation in symptom expression and reactions of host (5, 11,17). Severe yield reduction, from 90 to 31 percent occurs in both barley and wheat plants infected with BSMV in the field (6,10,15).

Singh et al (18) reported that the seed transmission was influenced by the age of the plant at inoculation. The virus was revealed as carried inside the seed with infected seeds bein smaller than normal ones, suggesting partial control by screening (14). Approximately, 5 to 50 % of the seed from infected plants could carry the virus (1). The virus can also be transferred by contact with plant parts of diseased and healthy plants (7).

MATERIALS AND METHODS

Viral inocula was obtained from barley seedlings grown from seed infected with BSMV and maintained in plants of barley, **Hordeum vulgare,** L. var. Dickson.

Crude extracts of fresh barley leaves in 0.1 M phosphate buffer, pH 7.0 were mechanically inoculated to greenhouse-grown plants, using Carborundum. Inoculations were accom-

plished by rubbing the extract on the leaves with the forefinger. After inoculation, the plants were washed with tap water for a few seconds to remove the excess inoculum from the leaves and then kept in the greenhouse.

Two varieties of barley, Dickson and Hannchen, one variety of wheat, Baart, with 6 or 7 plants from each cultivar, were inoculated at the one leaf stage. In addition to these plants the other plant species **Chenopodium amaranticolor, Cucumis sativus** «Chicago pickling», **Gomphrena globosa, Lycopersicon esculentum** «Bonny beat», **Nicotiana tabacum** «Samsun NN», **Phaseolus vulgaris** «Bountiful», **Vigna sinensis** «Blackeye» and **Vinca rosea** «purity» were also used for host range and symptomatology studies with two plants of each species were inoculated on the primary and/or older leaves. Symptoms were read 1 to 3 weeks after inoculation. Using **C. amaranticolor** as a local lesion host attemps were made to recover the virus from all inoculated plants to reveal any latent infection.

Clarified extracts of the virus were used to determine the stability **in vitro,** dilution end-point and thermal inactivation-point of the virus.

Crude extracts were prepared by grinding 0.5 gr. tissue of systemically infected barley plants in 5 ml. of 0.1 M phosphate buffer, pH 7.0 and squeezing through the cheesecloth into a beaker, then clarified in a low speed centrifuge at 2000 rpm for 10 min.

When determining the longevity **in vitro,** 0.2 ml. of this stock solution was placed into each of 5 Kahn tubes. Two Carborundum dusted leaves of **C. amaranticolor** were inoculated after clarification. One hour later 2 more leaves of **C. amaranticolor** were inoculated with the stock solution of the virus in the second tube. Inoculations were made with the solution in the 3rd, 4th and 5th tubes, 1,2 and 7 days later, respectively.

Thermal inactivation point of the virus was determined by inoculating 2 leaves of **C. amaranticolor** with dilution of 2.5 ml. of the stock solution in 2.5 ml. of 0.1 M phosphate buffer, pH 7.0 and placing 1 ml. of this dilution into each of 5 Kahn tubes. After heating each tube at different temperature ranging from 50° to 80° C in 10 degree steps by half immersing in a thermostatically controlled water bath for 12 min. The tubes were removed and cooled under running tap water. Then 2 leaves of **C. amaranticolor** were inoculated for each temperature treatments. The fifth tube of the series was left at room temperature and rubbed onto 2 leaves after all inoculated.

Using clarified sap and 0.1 M phosphate buffer, pH 7.0 dilutions were made beginning with a dilution of 1:10, the series was carried to 1: 1.000.000 in 6 steps. Previously Carborundum dusted 2 leaves of **C. amaranticolor** were inoculated with each degree of dilutions separetly.

Both partially purified and leaf dip preparations of the virus were examined with a Philips EM - 300 electron microscope operating at 60 kv.

Partially purified virus was obtained by grinding systemically infected leaves of barley plants (w/v) in twice as much 0.1 M borate buffer, pH 7.5 with sodium thioglycollate. After being squeezed through cheesecloth and centrifuged at 2000 rpm for 10 min. the supernatant was saved and centrifuged, 30.000 g/45 min. in a 30 rotor of a Spinco Model L. ultracentrifuge. The pellet was resuspended in 0.01 M buffer, pH 7.5 and centrifuged at 2..000 g/min. and the supernatant was saved as partially purified virus. Then one drop of 1:100 dilution of the virus solution was placed on a Collodian-coated 200 mesh - copper grid. After this grid dried, it was placed in a vacuum evaporator and cast a shadow with a platinum: palladium alloy.

Crude preparations were made by leaf-dip preparations of the virus into distilled water on a Formvar coated 200 mesh- copper grid, then adding a droplet of sodium phosphotungstate for negative stain of running an epidermal strip of the virus infected leaf into 2 % formalin, then negative staining the material.

Crude and clarified virus preparations were used in Ouchterlony agar double-diffusion test. Extracts were prepared by grinding 4 grams of infected and/or healthy leaves of barley plants with a mortar and pestle in 8 ml. of physiological saline (0.85 % NaCl in 0.01 M phosphate buffer, pH 7.0) then squeezing each through cheesecloth. Two ml. of the virus and of the healthy extract were saved, remainders were clarified at 2.000 g/10 min. to obtain clarified extracts.

RESULTS AND DISCUSSION

Host range and symptomatology: Attempts were made to find local lesion and systemic hosts of BSMV. Of the plants tested, only **C. amaranticolor, C. quinoa***, Dickson and Hannchen barley and Baart

*) Inoculated only with partially purified virus.

wheat gave definite symptoms. The virus induced local chlorotic lesions in the leaves of **C. amaranticolor** and **C. quinoa.** These chlorotic lesions became necrotic and death of infected leaves ensued 3 to 4 weeks after inoculation. All the infected plants of barley varieties and fewer plants of wheat showed severe systemic chlorotic mosaic patterns. The chlorotic patterns consisted of a few to many yellowish green, short to long stripes running parallel to the veins of the leaves. Numerous species of the Graminae plants have been reported to be susceptible to BSMV (13,15,16,17, 19). However, the numbers of plant species outside the Graminae family were fairly small and only **N. tabacum** var. Samsun (14,15), **C. album** (16), **C. amaranticolor** (11,12), **Beta vulgaris, Lolium multiflorum,** L, **Spinacea oleracea** L. (12) have been reported as local and/or systemic hosts.

Although **N. tabacum** var. Samsun has been shown as a local lesion host (13,15), in the tests carried out by Kassanis and Slykhuis (12) no symptoms were detected with their BSMV isolates on that cultivar. Also, in this study no symptoms were observed on the leaves of **N. tabacum** «Samsun NN» infected with crude extract of BSMV. At least 3 reasons could account for absence of symptoms : strain of the virus ,age of the donor or age of the tobacco plants. The virus was recovered from the leaves of all inoculated barley plants, but none of the symptomless non - graminae plants.

In the test to determine how infectivity is affected when clarified sap of infected Dickson barley plants is diluted, the average number of leasion on **C. amaranticolor** leaves/ dilution is shown in figure (1).

Figure-1. Numbers of lesions of the leaves of **C. amaranticolor** with each degree of dilution of BSMV.

Degree of dilution	No of lesion on inoculated leaves
1:100	18
1:1.000	3
1:10.000	—
1:100.000	—
1:1.000.000	—

The data in (Figure-1) shows that the dilution end-point was reached at 10^{-3}; however it has been reported slightly beyond 10^{-4} (15) and $2x\ 10^{-3}$ (12). The differences in the dilution end-point seem more significant and reasonable as reported by Kassanis and Slykhuis (12) two plausible explanations can be offered: 1) concentration in the sap might change with age of the plants and environmental conditions under which the plants were grown after infection, as well as 2) effect of light intensity on the virus concentration.

Heating at 50° C for 12 minutes destroyed the infectivity of the virus. However the thermal death point has been reported for 65° C (12), 68° C (14) for 10 min. Two minute difference could possibly destroyed the infectivity of BSMV at 50° C.

The activation time of the virus at room temperature has been reported for 15 to 22 days (14). The virus was found to be active at room temperature for 7 days; it is still possible though, that after 7 days the virus was still active.

Electron microscopy: In this study short, thick, rod-shaped particles typical of anisometric viruses were found in 1:100 diluted partially purified preparation of BSMV from seed-infected barley leaves (Figure 2).

When the partially purified preparation in 1:10 dilution was inoculated to the leaves of **C. quinoa** it was found infective, inciting symptoms on **C. quinoa** identical to symptoms incited by crude leaf extract or clarified extracts on **C. amaranticolor.**

The length of 49 rod-shaped particles was measured and the particle lengths were sorted into 20 nm categories as indicated in the histogram (figure 3). The modal length of the particles was found 120 nm to 140 nm. The length of the particles ranged from 26 nm to 314 nm, but all particles were anisometric.The width of the particles was 30 nm.

The mean number of the electron micrograph taken at a magnification of 42.000 times to determine the length of the particle was found 130 nm. The mean length of the particles was calculated within the main distribution peak as indicated by (Figure 4).

Figure-4. Calculation of the mean length of the particles leng groups used to

No particles measured	calculate mean length (nm)	No of particles used to calculate length	mean length (nm)
49	123.80-135.71	16	129.86

The mean length falled very close to the mode. No particles obtained with the leaf dip preparation of the virus.

The length and width rod-shaped particles of the virus in leaf tissue of the infected plants have been reported as follow: 1) 130.7 nm in length and 30 nm in width (9), 2) 120 nm in length in either the cytoplasm or the nucleus (8), (3.) 126 nm in length (4.) 20 n min width and 135 to 175 nm in length (12).

When previously reported the length and width of the particles were compared to those found in this study no significant difference appeared.

Serology: Crude and clarified extracts of Dickson barley leaves mechanically infected with BSMV antiserum signifying a positive antigen-antibody reaction in the Ouchterlony agar double - diffusion test within 39-40 hours. Both crude and clarified sap gave a precipitin band with antiserum of 1:128, therefore, the titer of antisera was 1:128 or probable more. After one week, the position of the precipitin band shifted further away from the antigen resorvior and the precipitation band became larger.

ÖZET

ARPA ÇİZGİLİ MOZAİK VİRÜSÜ (BSMV) ÜZERİNDE ÇALIŞMALAR

Arpa çizgili mosaik virüsü (BSMV) ile enfekteli Dickson çeşidi tohumlarından üretilen arpa fideleri inokulum kaynağı olarak kullanılmış ve 0,1 M fosfat tamponla seyredilmiş olan virüslü bitkilerden alınan arıtılmış özsuyunun son seyreltme noktası 10^{-3} olarak saptanmıştır.

Serolojik çalışmalar ham ve arıtılmış virüslü bitki özsuyunun her ikisinde de 1 : 128 oranında seyretilmiş antiserumla bir çökerti bandı verirken normal serumla böyle bir band teşekkül etmemiştir.

Virüs oda sıcaklığında 7 gün sonra da aktif olmakla beraber 50 C° de 12 dakika ısıtıldığında tamamiyle tahrip olmuştur.

Virüs saflaştırıldığında enfektivitesinin etkilenmiş olması belki de kullanılan donor bitkilerin yaşından dolayı olabilir.

Kısmi olarak saflaştırılan Arpa Mozaik Virüsünün elektron mikroskobisi, çubuk şeklindeki partiküllerin 130 nm uzunluğunda, 30 nm genişliğinde ve anisometrik olduğunu göstermiştir.

Chenopodium quinoa bitkisinin yeni bir lokal lezyon konukçusu olarak saptanmasına karşı daha önce lokal lezyon konukçusu olarak kaydedilen **N. tabacum** var. Samsun'da herhangi bir simptom görülmemiştir.

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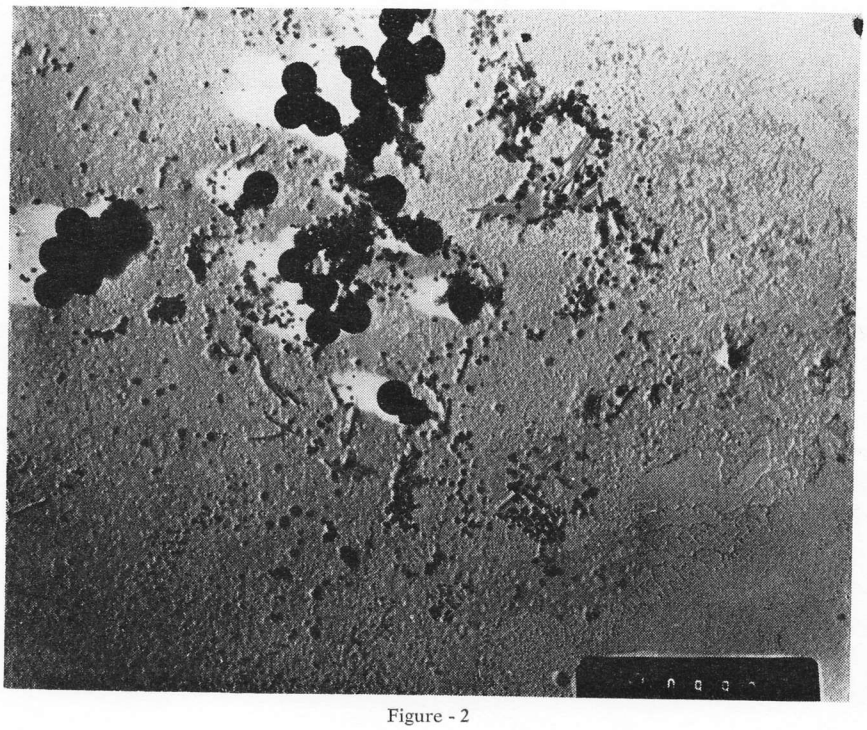

Figure - 2

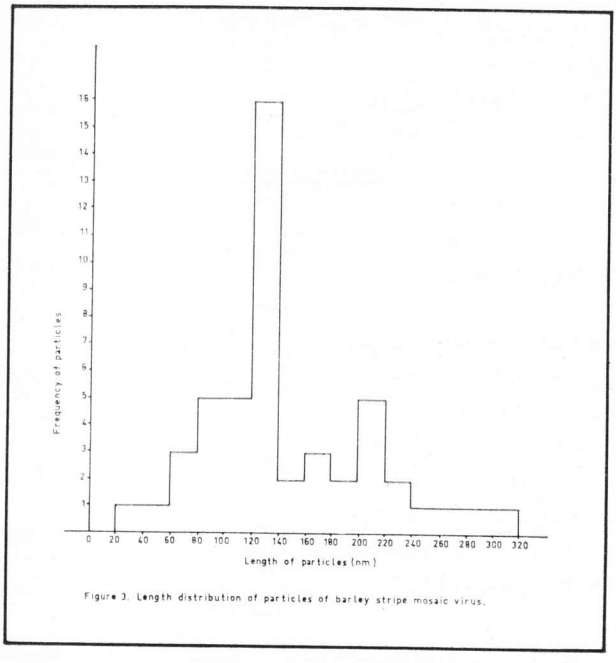

Figure 3. Length distribution of particles of barley stripe mosaic virus.

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Reduction of Virus Disease Effects on Tomato by Barriers in Çukurova Region [1]

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ABSTRACT

The effect of Tomato Yellow leaf curl virus on yield and other growth parameters of Linda and Super Marmande cultivars were determined. The mean yield was 2.7-2.8 folds higher in caged plants than the plants grown in open plots. The plants in open plots were stunted and showed considerable decrease in stem weight, stem lenght, root weight, root lenght, and soluble solid contents. The mineral element uptake of the leaves were also analyzed but no significant difference were found between caged and open plots.

INTRODUCTION

Virus diseases show a very wide distribution in southern Turkey. During the surveys conducted in greenhouses along the mediterrenean belt, we were not able to observe any single tomato plant free of virus. Even tomatoes grown from resistant seeds which were introduce by well known firms were infected by virus diseases. Since the virus diseases are very widespread, the tomato production in southern Turkey in much lower than expected.

The viruses detected from tomatoes grown in Çukurova region were

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TMV, CMV, PVY, TBSV, PVX and TYLCV (Yılmaz 1978, 1980). TMV and TYLCV were the most destructive ones compared to the others (Yılmaz et. al., 1979). Although TMV is transmitted by mechanical means and by seeds (Broadbent, 1964, Gooding, 1975; Gooding and Suggs, 1976), TYLCV is only transmitted by whiteflies, **Bemisia tabaci** Genn (Cohen and Nitzany, 1965; Verma et. al., (1975).

Yılmaz et. al. (1979) reported that the losses in yield caused by TYLCV could be reduced to some degree by modifying the planting times of seedlings. They concluded that tomato seedlings should be planted towards the end of November when whiteflies were absent. However the development of warm weather during the growing period resulted a rapid increase in whitefly population and therefore caused a spread of TYLCV.

The purpose of this study was to minimize the damage of whitefly-borne virus, TYLCV, using a cheesecloth barrier.

MATERIAL AND METHODS

The seedlings of Linda and Super Marmande cultivars were grown in Jiffy Pots, and the transplanted to the greenhouse at their 4-10 leaf stage on October 13, 1978. 12 plots of 10 seedlings were allocated for each cultivar. Six of these plots were covered with 2 m high finemesh cheesecloth cager after transplanting, and other 6 plots were left open. Fruits were harvested twice a week beginning February 13, 1979. Total yield per plant was recorded, and soluble solids and vitamin C contents of fruits were determined (Lees, 1971) for healthy and TYLCV infected plants. The plants were removed on April 2, 1980, and their stem weight, stem height, root weight, and root length were measured. The cages were taken away several weeks before the removal of plants.

The third and fourth leaves of healthy and TYLCV infected plants were taken as a sample at the flowering stage for determination of micro and macro elements (Broeshart and Redlick, 1961).

Randomized block desing was chosen with 2 treatments replicated 6 times for each cultivar and the results were analyzed statistically (Steel and Torrie, 1969).

RESULTS

Tomatoes grown in open plots showed systemic mosaic, yellowing and curling symptoms 2-4 weeks after transplanting to the greenhouse. The number of infected plants increased rapidly and there was not any single healthy plants without infection by the end of November. In infected plants, a mechanically transmitted, seed-borne virus (Gooding and Suggs, 1976), TMV, was also present in addition to TYLCV. But the percentage of TMV was much lower compared to the TYLCV in the plants grown in open plots of both varieties (Table 1). In contrast, the percentage of TMV infected plants grown under the cages was higher than that of TYLCV (Table 1).

Table-1. Percent of infected plants by TYLCV and TMV.

	Caged			Open		
Cultivars	Healthy	TYLCV	TMV	Healthy	TYLCV	TMV
Linda	54.2	1.7	44.1	none	66.7	33.3
Supermarmande	12.8	23.6	63.6	none	82.7	17.3

The symptoms of TYLCV on caged plants appeared only after removal the cages indicating that trans mission of the virus to the plants by whiteflies was prevented by cheesecloth barriers during the early stage of growth. This late infection of TYLCV did not show any effect on yield or other parameters measured in this study, because the plants reached to their maximal growth and their optimal yield was already harvested before infection took place.

Since the main purpose of this study was to compare the yield and other growth parameters of healthy and TYLCV infected plants, those which were infected by TMV were disregarded, and comparisons were made between the healthy plants grown in cages and TYLCV infected in open plots.

Effect of TYLCV on fruit weight an yield

The fruit size attained in TYLCV infected plants was significantly smaller than that of healthy plants. The increase in mean fruit weight of the plants grown in cages was approximately 2-fold for both cultivars (Table 2).

Table-2. The effect of TYLCV on mean fruit weight (gr) for linda and super Marmande cultivars[1].

Plants	Linda	Super Marmande
Healthy	79.9 a	124.9 a
TYLCV	40.0 b	59.3 b

1) Means in collums followed by the different letter are significantly different by LSD test, 5 % level.

The same trend was also apparent on mean yield per plant. The mean yield of plants grown in cages was 2.8-fold higher for Linda, and 2.7-fold for Super Marmande cultivar compared to the ones grown in open plots (Fig. 1).

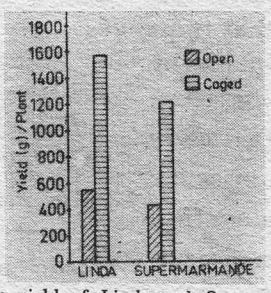

Fig. 1. The yield of Linda and Supermarmande cultivars grown in cage and open plots.

Effect of TYLCV on vitamin C and Soluble Solid content of fruits

As seen in Table 3, the vitamin C content of fruits of healthy and TYLCV infected plants did not show any significant differences for both cultivars. The fruits of TYLCV infected plants showed significantly lower values for percent soluble solids than that of healthy plants.

But in Linda cultivar, the increase in soluble solids in healthy plants war almost as twice as the Super Marmande cultivar (Table 3).

Table-3. The effect of TYLCV on Vitamin C and soluble solid contents of fruits of Linda and Supermarmande cultivars[1].

	Linda		Super Marmande	
Plants	Vitamin C (mg/100 ml)	Soluble Solids (%)	Vitamin C (mg/100 ml)	Soluble Solids (%)
Healthy	14.1 a	6.06 a	11.9 a	4.66 a
TYLCV	13.2 a	3.53 b	10.9 a	3.06 b

1) Means in collums followed by the same letter are not significantly different by LSD test 5 % level.

Effect of TYLCV Various Growth Parameters

Growth rate of TYLCV infected plants was markedly reduced in both cultivars. Infection by the virus at early stages of growth caused dwarfing of the whole plant. In both cultivars, healthy plants grew over 2 m in height but infected plants were stunted and growth siezed when plants were about 1 m tall (Table 4). The weight gain was also significantly different between healthy and TYLCV infected plants. The stem weight was around 300 g for infected plants but it was 1446 and 1170 g in healthy plants for Linda and Super Marmande cultivars, respectively (Table 4).

Although roots were reduced in growth, there was no statistical differences detected on root lenght be-

tween healthy and TYLCV infected plants for both cultivars. But the root weight of healthy plants of both cultivars were significantly higher than the root weight of TYLCV infected plants (Table 4).

Table-4. The effect of TYLCV on stem weight and height, and on root weight and length of Linda and Super Marmande cultivars[1].

Growth	Linda		Super Marmande	
Parameter	Healthy	TYLCV	Healthy	TYLCV
Stem Weight (g)	1446 a	293.3 b	1170.0 b	332.7 a
Stem Height (cm)	206.6 a	105.0 b	220.6 a	120.6 b
Root Weight (g)	48.3 a	17.6 b	36.6 a	25.3 b
Root Length (cm)	21.6 a	14.5 a	32.6 a	24.3 a

1) Means in rows followed by the same letter are not significantly different by LSD test, 5 % level.

Effect of TYLCV on macro and micro element uptake

Statistical analysis indicated that there was not any significant differences on macro and micro element uptake of leaves between healthy and TYLCV infected plants (Table 5). Although not significant the

Table-5. The effect of TYLCV on macro and micro element uptake of the leaves of Linda and Super Marmande cultivars.

	%					ppm			
Cultivar	N	P	K	Ca	Mg	Fe	Zn	Cu	Mn
Healthy Linda	3.63	0.29	0.79	0.77	0.55	66.7	28.0	26.3	96.5
TYLCV	3.18	0.36	0.80	0.84	0.35	60.0	48.2	20.0	78.0
Healthy Super Marmande	3.39	0.40	0.70	1.25	0.37	67.9	89.1	19.6	64.3
TYLCV	3.22	0.36	0.61	1.18	0.64	72.1	48.3	25.9	110.3

amount of Zn in TYLCV infected leaves of Linda cultivar was much higher than the others. But attribution of this difference to TYLCV was questionable.

DISCUSSION

Our data indicate that losses in TYLCV-infected tomato plants was mainly due to dwarfing of plant, and reduction in size and number of fruits, which agreed with the damage caused by most of whiteflyborne viruses (Costa, 1975).

As stated for whitefly-transmitted viruses by Costa (1976) TYLCV was more effectively transmitted by **Bemisia tabaci** and was more destructive when insects feed on young plants in our study. The damage was only observed in TYLCV infected plants grown in open plots. Although symptoms of TYLCV was observed in plants grown in cages late in the season, it did not cause demarkable differences than healthy plants.

Since whitefly - transmitted diseases are not seed-borne (Costa, 1976; Yılmaz, 1978), and TYLCV is not transmitted mechanically (Cohen and Nitzany, 1966), and no resistant varieties have been developed yet (Costa, 1975) a best approch to control TYLCV sholud depend on isolation of vectors from host plant. Control of whitefly-transmitted diseases by the application of insecticides has been tried with varying degrees of success. The ability of single individuals of whitefly adults to transmit TYLCV (Cohen and Nitzany 1966) limits the chemical control of whiteflies since all the individuals in the population have to be killed in order to achieve a TYLCV free productions. However some favorable results in the control of whitefly-transmitted leaf curl and yellow leaf curl of tomatoes with insecticides were reported from Israel (Melamed-Madjar et. al. 1970). Undoubtedly the best way of succesful control is to use the combination of various measures; like avoidance of the disease by selection of planting time elimination of disease agent reservoirs and vector control by chemicals or other means.

The results of our study indicates that succesful control of TYLCV can be achieved in Çukurova region especially for greenhouse grown tomatoes if dynamics of local whitefly population in relation with phenology of host plants are studied in detail. Planting tomatoes at the decline phase of whitefly populations screen-

ing the enterences and other openings with fine-mesh barriers, and careful observations of whitefly population development inside the greenhouse can easily prevent early infection of plants. Since crop losses induced by whitefly transmitted diseases are usually negligible if spread occurs late in the season (Costa, 1976) the tomato production can be increased considerably if the preventive measures mentioned above are taken at the early stage of growth,

ÖZET

ÇUKUROVA BÖLGESİNDE VİRUS HASTALIKLARININ DOMATESLER ÜZERİNDEKİ ETKİLERİNİN ÖRTÜ KULLANMAK SURETİYLE AZALTILMASI

Domates sarı yaprak kıvırcıklığı virusunun (TYLCV), ürün ve diğer gelişme değişkenleri üzerine olan etkisi Linda ve Super Marmande çeşitleri üzerinde saptanmıştır. Kafes içinde yetiştirilen bitkilerdeki ortalama ürün, açık parsellerde yetiştirilenlerden 2,7-2.8 kat yüksek bulunmuştur. Açık parsellerde yetiştirilen bitkiler bodurlaşmış ve gövde ağırlığı, gövde uzunluğu, kök ağırlığı, kök uzunluğu ve eriyebilir katı madde içeriği önemli eksiliş göstermiştir, Yaprakların mineral madde alımı analizlerinde kafes içinde veya açıkta yetişen bitkiler arasında önemli bir farklılık bulunmamıştır.

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The Distribution and the Damage of Bunts (Tilletia spp.) and Wheat Gal Nematode (Anguine tritici (Steinbuch) Chitwood) on Wheat in the Eastern Part of Anatolia

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ABSTRACT

The causal agents of wheat bunts in the Eastern Part of Anatolia are **Tilletia foetida** (Wallr.) Lira, Tilletia caries (D.C.) Tul. and **Tilletia contraversa** Kühn. in which **T.foetida** is distributed to almost all districts of the Eastern Part of Anatolia, while **T.caries** is restricted to Diyarbakır Urfa Region and to the southern districts of Upper Fırat and Murat Basin. On the other hand **T.contraversa** is encountered almost in all regions except Erzurum Kars Plateu. Wheat gal nematode **(Anguine tritici** (Steinbuch, Chitwood) which is prevalent in all regions is mostly found in Erzurum Kars Plateu and least in Samsun Amasya Basin. Basides the other factors the damage of bunts and wheat gal nematode fluctuate occording to irrigation and sowing season of wheat. Bunts are two or three times more in irrigated fields and on spring wheat, while wheat gal nematode couses more infection on winter wheat and in irrigated conditions.

INTRODUCTION

The most important crops of the Eastern Part of Anatolia are cereals in which wheat production is the highest, In our country bunts of wheat are important diseases besides various other diseases cousing yield reductions, According to the surveys done on the causal agents of common

bunts, **Tilletia foetida** (Wallr,) Liro is much more spreaded than **Tilletia caries** D,C,) Tul. (Gassner and Göydün, 1937). The yield losses estimated by Gassner and Göydün (1937) and Özkan (1956) were about 15-20 %. The clamydospores released from the broken bunted grains during harvest or thresing contaminated to healthy seeds. The pathogen which overwinters as clamydospores on healthy seeds could be easily and economically eliminated by seed treatments by using an appropriate fungicide. In the recent years the increase in the use of treated seeds resulted with a decrease in the yield losses of wheat.

Another bunt fungi is **Tilletia contraversa** Kühn., couses dwarf bunt of wheat. This disease is especially distributed to the high, mountainous 1300-2000 m) parts of Eastern Anatolia, Middle Anatolia, Mediterrenean Regions and in some years couses up to 80 % damage on wheat production (Özkan, 1971). Althouh the disease in not economically very destructive from the point of total wheat production of Turkey, unless efforts to control the disease will prevent it's distribution to clean areas basides reducing the damage. This disease is not effectively controlled by seed treatments, since the infection behaviour of this pathogen is different than the common bunt fungi (İren, 1962; Karaca, 1965).

The wheat Gal nematode **(Anguine tritici** (Steinbuch) Chitwood) was firstly mentioned in the paper of of Needham in 1743, is primarly destructive on wheat and secondly on barley and rye Southey, 1959). This disease is completely eliminated in countries where seed cleaning is applied (Southey, 1978). In China the damage of this nematode on wheat is around 0.25 % but in some fields the yield losses can be increase up to 30-69 % (Webster, 1972; Southey, 1978). Infested plants are stunted and show rolling, twisting and crinkling of the leaves. On ears the glumes are abnormally spreading and the awns are twisting and spreading as well. If galls which contain the second stage larvae are sown with seed then the second stage larvae emerge in moist soil, invade host seedlings and feed ectoparasitically on the tissues of young leaves near the growing point. In infested ears some or all of the grains are replaced by galls. The bright green or brown colored galls are easily seperated from the wheat grains and these galls are shed from the ears more readly than the grains. The galls may be confused with bunted grains (due to **Tilletia** spp.) unless the nematode galls are very hard and do not crush between fingers. In dry conditions these galls are remain viable for about 32 years (Norton,

1978). Each female lays more than 2500 eggs (Zuckerman and Colleques, 1971). The most effective control of these nematodes are seed cleaning and then crop rotation. In moist soils absence of host plants for one year eliminates most of the population, so few years of rotation completely free the soil from **A. tritici.** But under prolonged dry conditions they survive and keep their virulance for many years. In Yugoslavia and Rumania resistant wheat cultivars are found against **A. tritici** (Southey, 1978).

The aim of this work is to find out the distribution and damage of bunts and wheat gal nematode in the Eastern Part of Anatolia.

MATERIALS AND METHODS

To determine the distribution of wheat bunts, gal nematode and besides to obtain information on about their damage in the Eastern part of Anatolia, the wheat growing areas are divided into eight regions according to their climate, elevation and soil characters. The wheat samples were asked from the places which represent the region's character. During 1976 - 80 samples were brought from the 1126 villages of 78 counties (Map 1). In each village 150 gr. of wheat which is not cleaned by selectors were taken without considering them as winter or spring cultivar and their growing conditions. As a result 18 % of the whole samples were from irrigated conditions and 23.2 % were spring wheat. The population changes between the years resulted from the effect of climatic conditions were partly eliminated by taking wheat samples continiously for four years. In every sample the number of bunted grains and nematode galls were counted and weighted. Apart, the clamydospores from every bunted grain were observed under microscope. The wheat bunt fungi species vere distinguished according to the morphological characters of clamydospores. In every sample the percent of each species was also calculated. The distribution of bunt fungi species and their intensity were determined on county bases by taking the average of the results obtained in every county.

In order to get information about the damages of wheat gal nematode and wheat bunts, the number and weight ratios of nematode galls and bunted grains in wheat crop were determined. Here in, the weighted means were used as indicated by Bo-

ra and Karaca (1970) and in the weighted means the wheat production of the countries were taken into account. In calculating the ratios of weights, the 1000 grain weight of wheat was taken as 33 gr. close to the values obtained by Köycü (1979)[1] and Ertugay (1980)[2] in East Anatolia. The damage caused by bunts and wheat gal nematode were calculated seperately for being grown as winter or spring cultivar and being irrigated or not. In each county equal number of samples were taken for each condition. So the calculations were done on equal number of wheat samples taken from similar ecological conditions. The results were compared by proportional tests (Öztürk, 1978).

RESULTS AND DISCUSSION

a) The bunt fungi of wheat:

According to the samples brought from the villages of the Eastern Part of Anatolia, **T. foetida** distributed to the almost all regions as seen on map 1. The other common bunt fungus **T. caries** is especially prevalent in Diyarbakır Urfa Region where in Ergani and Hani it is the only species and in Hilvan, Viranşehir and Kızıltepe it is more than **T. foetida. T. caries** is also found partly in the southern region of Upper Fırat and Murat Basin. The dwarf bunt **(T. contraversa)** is encountered almost in all regions except Erzurum Kars Plateu. It is mainly spreaded in the Passage region of Black Sea, Van Lage Region and Upper Fırat Murat Basin. This species was found to be more prevalent than **T. foetida** only in Varto and Göksun.

The ratio of the number of bunted grains in harvested wheat varies according to the region where the maximum ratio is obtained in Upper Fırat and Murat Basin in which 0,80 % of the grains are smutted (Table,

1) Köycü, C., 1979. Çeşitli kaynaklardan temin edilen yerli ve yabancı bazı kışlık ekmeklik buğdaylarda **(Triticum aestivum,** L.) verim unsurları ve diğer morfolojik karekterler ile ekmeklik kalitesi üzerinde araştırmalar. Atatürk Ü. Zir. Fak. Tarla Bitkileri Bölümü Doçentlik Tezi, Erzurum-Turkey (In press).

2) Ertugay, Z. 1980. Doğu Anadolu Bölgesinde yetiştirilen Kırık buğdayının (Tr.aestivum L.var. belsii) ekmeklik kalitesi üzerinde araştırmalar. Atatürk Üni. Zir. Fak. Süt ve Gıda Teknolojisi Bölümü doktora tezi, Erzurum-Turkey (In press).

1). The minimum is obtained in Sivas Region (0,03 %).

The number of bunted grains in the crop may give us a partial information about the damage of bunts, unless it does not reflect the real values. Because as a result of to be broken during harvest or treshing, some of the bunted grains are lost, so the damage could not be calculated precisely. The mean results of three years show that the damage of bunts in the eastern part of Anatolia is at least more than 0.26 %.

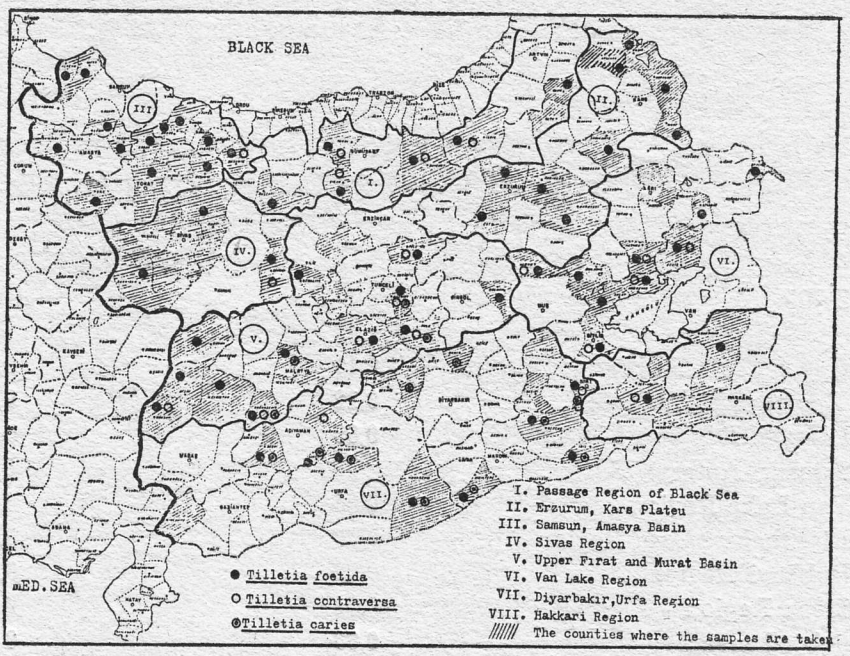
Table-1. The ratios of number and weight of bunted grains in the wheat crop in the various regions of the Eastern Part of Anatolia.

Regions	The ratio of bunted grains % Weight[1]	Number
1. Upper Fırat and Murat Basin	0,18	0,80
2.. Erzurum Kars Plateu	0,12	0,50
3. Passage Region of Black Sea	0,06	0,25
4. Samsun Amasya Basin	0,03	0,14
5. Van Lake Region	0,03	0,11
6. Diyarbakır, Urfa Region	0,02	0,10
7. Hakkari Region	0,02	0,09
8. Sivas Region	0,01	0,03
Weighted mean	0,06	0,26

1) 1000 grain weight of bunted grains is taken as 7.63 gr.

On the other hand the damage coused by bunts varies according to the sowing season and to be irrigated or not. As a matter of fact as seen on table 2, in spring sown wheat the 0.82 % of the grains are bunted and this ratio is 0,27 % in winter wheat. The difference between them is statistically important. The ratio of bunted grains in the wheat crop obtained from irrigated conditions is 0,20 % while it is 0.08 % in non irrigated and the difference is statistically important. Subsequently the infection of bunt fungi is two or three times more in irrigated condi-

tions and on spring wheat than on dry conditions and on winter wheat. The results show that in humid soils especially in spring sowing the bunt clamydospores find optimum conditions for germination and infection. As a matter of fact in Sivas Region one of the reason in the reduction of the damage coused by bunts could be dry cultivation of wheat.



Map. 1. The distribution of bunt fungi species between the counties where the samples are taken

Table-2. The mean ratios of bunted grains in wheat crop according to sowing season and irrigated or dry cultivation of wheat.

Sowing season	The ratio of bunted grains (%)	Cultivation type	The ratio of bunted grains (%)
Spring	0.82 a	Irrigated	0.20 a
Winter	0.27 b	Dry	0.08 b

In each row the difference between the groups having different letters are statistically importan.

The number of bunted grains mix into wheat seeds used for sowing changes according to the region as well. The highest number is obtained in Upper Fırat and Murat Basin where the average number of bunted grains is 3619 per decar, but in Sivas Region the value drops to 113. The mean number for the Eastern Part of Anatolia is 1176 (Table 3).

Table-3. The number of bunted grains sown together with wheat seeds to a decar of field in the various Regions of the Eastern Part of Anatolia.

Region	Bunted grains
Upper Fırat and Murat Basin	3619
Erzurum Kars Plateu	2264
Passage Region of Black sea	1152
Samsun Amasya Basin	632
Van Lake Region	516
Diyarbakır Urfa Region	441
Hakkari Region	407
Sivas Region	113
Weighted mean	1176

b) The wheat gal nematode **(Anguine tritici)** :

According to the calculations done on the harvested wheat crop which is not pass through selector, the maximum nematode galls were determined in Erzurum Kars Plateu where the ratio of number of galls is 0,58 %. The least number of nematode galls were obtained in Samsun and Amasya Basin 0,03 % (Table 4). It is more or less possible to determine the damage coused by wheat gall nematodes by calculating percentage of galls in crop. Because these galls do not crush during harvest and treshing, only few are drop on to ground and lost during harvest. So the percentage number of galls in crop shows the damage at least level. In the Eastern Part of Anatolia an avarage more than 0.20 % of the wheat yield is lost by wheat gall nematodes. On

Table-4. The ratios of number and weight of nematode galls in the wheat crop in the various regions of the Eastern Part of Anatolia

Regions	The ratio of nematode galls %	
	Weight*	Number
Erzurum Kars Plateu	0.16	0.58
Upper Fırat Murat Basin	0.07	0.24
Diyarbakır Urfa Region	0.07	0.23
Passage Region of Karadeniz	0.04	0.15
Van Lake Region	0.04	0.14
Hakkari Region	0.03	0.10
Sivas Region	0.03	0.09
Samsun Amasya Basin	0.01	0.03
Weighted mean	0.06	0.20

*) 1000 grain weight of nematode galls is 9.27 gr.

weight basis at least an average 0.06 % of the yield is nematode galls.

Besides the other factors the damage caused by wheat gal nematode fluctuates according to the sowing season of wheats and being irrigated or not. The destructiveness of the nematode on winter wheat is two or three times more than spring wheat and also the damage on wheats grown in irrigated conditions are more than the wheats grown under dry conditions. The differences in both are statistically important (Table 5). In humid soils the larvae inside nematode galls become active by absorbing water in optimum level and in such soils their movement increases which resulted with more infection. At the same time it is seen that in winter sowing, the prevailing conditions seems to be favourable for nematode infection. As a matter of fact Webster (1972) indicated that cool and humid soils are suitable for wheat gall nematode.

Since the galls are main infection court so in dissemination of this nematode the galls which mix into wheat seeds play important role. The number of galls found in wheat seeds is related with the contamination degree of the area. So the highest number of nematode galls is found

Table-5. The mean ratios of nematode galls in wheat crop according to sowing season and irrigated or dry cultivation of wheat

Sowing season	The ratio of nematode galls (%)	Cultivation Type	The ratio of nematode galls (%)
Winter	0.31 a	Irrigated	0.20 a
Spring	0.14 b	Dry	0.08 b

In each row the difference between the groups having different letters are statistically important.

in Erzurum Kars Plateu. In this region to a devar of field an average 2654 nematode galls are sown together with wheat seeds. The least number of galls are counted in Samsun, Amasya Basin (134 galls) (Table 6).

Table-6. The number of nematode galls sown together with wheat seeds to a decar of field in the various Regions of the Eastern of Anatolia

Erzurum Kars Plateu	2654
Upper Fırat and Murat Basin	1092
Diyarbakır, Urfa Region	1024
Passage Region of Black Sea	698
Van Lake Region	633
Hakkari Region	474
Sivas Region	398
Samsun, Amasya Basin	134
Weighted mean	907

In the Eastern Part of Anatolia, bunts and wheat gal nematodes couse reduction in wheat yield. The damage of bunts and wheat gal nematode changes according to the regions This variation between the regions is because of climatic conditions of the region, sowing season, irrigation status and seed cleaning. As a matter of fact bunts are two or three times more in irrigated fields and on spring wheat while wheat gal nematodes couses more infection on winter wheat and in irrigated conditions. So preventive measures should be taken especially in the regions where the conditions are favoring infection. If bunts and wheat gal nematodes are effectively controlled in the Eastern Part of Anatolia the wheat production can be increased at least 0.5 %.

ÖZET

ANADOLUNUN DOĞUSUNDA BUĞDAYDA ZARARLI OLAN SÜRME (**Tilletia** spp.) ve BUĞDAY GAL NEMATODU (**Anguine tritici** (Steinbuch)Chitwood)'NUN YAYILIŞI VE ZARAR DERECESİ

Anadolunun doğusunda buğdayda zararlı olan sürme mantarları **Tilletia foetida** (Wall.) Lira **Tilletia caries** (D.C.) Tul. ve **Tilletia contraversa** Kühn. olup bunlardan **T.foetida** Anadolunun doğusunda bütün bölgelerde hemen hemen yayılmıştır. **T. caries** Diyarbakır Urfa bölgesinde ve ayrıca Yukarı Fırat, Murat Havzasının güney kısımlarında yaygındır. **T. contraversa** Erzurum Kars yaylası hariç her tarafta rastlanmaktadır.

Sürme mantarlarının oluşturduğu kör taneler buğday ürünü içerisinde sayısal olarak en fazla karışma oranı Yukarı Fırat ve Murat havzasında (% 0.80), en az ise Sivas bölgesinde (% 0.03) saptanmıştır. Anadolunun doğusunda ise kör tane karışma oranı ortalama % 0.26 olarak bulunmuştur. Sürme mantarları yazlık ekim yapılan ve sulanabilen koşullarda kışlık ekim ve kıraca oranla 2-3 misli daha fazla zararlı olmaktadır. Diğer taraftan bölgede dekara taşınan kör tane sayısı ortalama 1176' dır.

Buğday gal nematodu (**Anguine tritici** (Steinbuch) Chitwood)'nun enfeksiyonu sonucu oluşturduğu gallerin buğday ürünü içerisine sayısal olarak en fazla Erzurum Kars yaylasında (% 0.58), en az ise Samsun Amasya havzasında (% 0.03) karış-

tığı saptanmıştır. Anadolunun doğusunda gallerin karışma oranı ortalama % 0.20 olarak bulunmuştur. Buğday gal nematodu en fazla sulanabilen arazilerde ve kışlık ekimlerde zararlı olup, bu koşullarda yazlık buğday ve kıraca oranla 2 mislinden fazla ürün kaybına neden olmaktadır. Bölgede dekara taşınan gal sayısı ortalama 907'dir.

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Increase in Sunflower Yield by Controlling Rust with Systemic and Non - Systemic Fungicides

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ABSTRACT

Loss in seed yield due to sunflower rust was successfully reduced by spraying systemic fungicides Benodanil and Vitavax even after establishment of the pathogen in the host, the percentage increase in yield being 51.08 and 51.17 respectively as compared to untreated control. Two sprays of Benodanil and Vitavax at 30 days interval were more effective in controlling rust than 3 sprays of non-systemic fungicides sprayed at 20 days interval. There was no significant difference in rust control between 3 sprays and 2 sprays of systemic fungicides.

INTRODUCTION

Sunflower rust caused by **Puccinia helianthi** Schw. is an important disease in India (Lal and Singh, 1977; Anilkumar and Urs, 1977 and Singh and Lal 1977 and 1978). It reduces yield of grain and oil considerably (Middleton and Obst, 1972). Non - systemic fungicides were found to control the disease when used as protective spray (Midleton and Obst, 1972; Ramaswamy and Mathar, 1973 and Singh, 1975). Efficacy of systemic fungicides along with non-systemic fungicides have been reported to control the disease even after the establishment of the fungus in cage house experiments (Lal and Singh, 1977; Singh and Lal, 1977 & 1978; Mathur **et al** 1978 and Mathur **et al,** 1980).

Those fungicides which were found effective in cage house experi-

ments, were tested in field during 1975-76 and 1976-77. The minimum number of sprays required for effective control of the disease after infection was also determined in the field in 1977-78 and 1978-79 and results of these field experiments are presented in this paper.

MATERIALS AND METHODS

The susceptible variety EC 68414 was used throughout the experiments during the years 1975-76, 1976-77, 1977-78 and 1978-79. All inoculations were done in the evening by using fresh uredospore suspension in wtater and spraying on 1 month old plants by the help of 1 litre capacity and sprayer. To ensure sucessful infection, inoculation was done 8 times within one week at intervals of 2-3

In 1975-76 and 1976-77, 4 systemic fungicides viz. Benodanil (2-Iodobenzoic acid anilide, 50 % WP), Vitavax (2,3-Dihydro-5-Carboxonilido-6 me - thyl-1, 4-oxathiin, 75 % WP), RH-124 (4-n-Butyl-1, 2,4- Trizol, 80 % water soluble liquid), Plantvax (2-3-Dihydro-5-carboxamilido-6-methyl-1, 4-oxa thiin-4, 4-dioxide) and 2 non-systemic funficides viz. Dithane M-45 (75 % Zinc ion and Manganese ethylene bisdithiocarbamate), Dithane Z - 78 (75 % Zinc ethylene bisdithiocarbamates), were sprayed on inoculated plants after appearance of initial symptoms, at concentrations of 0.1 % and 0.2 % respectively. In all, 3 sprayings of each of the fungicides were given at interval of 20 days. In 1978-79, one new systemic fungicide viz. Bayleton (1-4-Clorophenoxy)-3, 3-dimethyl-1-(1 H-1,2,4-Trizol-1-yl)-2-butanene) at 0.1 % concentration and one non-systemic fungicide viz. Syllit (n-dodecylguanadine acetate) at 0.2 % concentration were included besides the 4 systemic and 2 non-systemic fungicides used during the preceding years and the treatments were modified i.e., inoculated plants were sprayed with the fungicides once in the first treatment, twice in the secon treatment and thrice in the thirdtreatment; Syllit was however, used as given in third treatment. Interval between sprayings was 30 days.

Experiments were caried out in the field in both Rabi and Kharif seasons and results of rust control and yield were recorded except for the year 1977-78 when only the rust control results were taken; 0 to 5 rating was recorded based on percentage of leaf area infected. The infection index and percentage efficiency of disease control were calculated according to the following formulae (Horsfall and Hensberger, 1942):

$$\text{Infection index} = \frac{\text{Sum of individual rating}}{\text{Total number of plants}} \times \frac{100}{\text{Maximum disease rating}}$$

$$\text{Percent efficiency of disease control} = \frac{\text{Infection index in control} - \text{Infection index in treatments}}{\text{Infection index in control}} \times 100$$

RESULTS AND CONCLUSION

Benodanil followed by Vitavax and Plantvax were found to be effective in controlling rust during field trials of 1975-76 and 1976-77, both in the Rabi and Kharif seasons, percentages of efficiency of disease control being 68.78, 46.20 and 36.06 respectively whereas RH-124, Dithane M-45 and Dithane Z-78 were not as effective. Nevertheless, there was increase in seed yield in all the treatments, irrespective of the fungicides being systemic or nonsystemic, as compared to untreated control and highest percentages of increase in yield were 51.08 and 51.77 in case of Benodanil and Vitavax (Table 1). Similar trends were also found during field experiments of 1977-78 and 1978-79. In 1977-78, Benodanil and Plantvax were most effective in controlling rust when sprayed twice at intervals of 30 days, the rust control being 62.08 % and 66.77 % and thus gave better results than 3 sprays of Dithane Z - 78 and Dithane M - 46 where rust control was 50.01 % and 33.42 % respectively (Table 2).

In 1978-79 also systemic fungicides Benodanil, Plantvax and Vitavax proved to be better than all the nonsystemic fungicides. There was not much difference in controlling rust when systemic fungicides were sprayed either 3 times or two times at intervals of 30 days. Although there was no significant rust control when these systemic fungicides were spray ed only once during the entire growing season of the crop, yet there was significant increase in yield as compared to control (Table 3). Maximum increase in yield was found to be 76.66 % in case of Benodanil followed by Vitavax (56.75 %) when sprayed 3 times. In general, plant growth was better when sprayed with systemic fungicides.

From results of field trials of 4 years it is apparent that when there

was control of rust, there was increase in yield but there was no correlation with infection index and yield. In some treatments particularly with non-systemic fungicides, there was increase in yield as compared to untreated control even when there was no control of rust, some other diseases and/or epiphytic microflora might have been controlled by these fungicides, which otherwise would have harmful effects on the plants.

In case of systemic fungicides, although there was no rust control when plants were sprayed once, yet there was increase in yield which may possibly be on account of general improvement in plant growth which was apparent on visual observations.

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ÖZET

SİSTEMİK VE SİSTEMİK OLMAYAN FUNGİSİTLERLE AYÇİÇEĞİ PASI KONTROLUNDA ÜRÜN ARTIŞI

Ayçiçeği pasına karşı Benodanil ve Vitavax sistemik fungisitlerinin, patogenin konukçuya yerleşmesinden sonra bile uygulaması ile tohum ürününde meydana gelen kayıp başarılı bir şekilde azaltılmıştır. Kontrola kıyasla üründeki artış sırasıyla % 51.08 ve % 51.17 olmuştur. Pas hastalığının kontrolunda Benodanil ve Vitavax'ın 30 gün ara ile iki uygulaması, sistemik olmayan fungisitlerin 20 gün ara ile üç defa uygulanmasından daha etkili bulunmuştur. Sistemik fungisitlerin üç defa uygulanması ile iki defa uygulanması arasında önemli bir farklılık bulunmamıştır.

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Table-1. Sunflower rust infection and yield when sprayed with systemic non-systemic fungicides in field in Rabi and Kharif seasons, 1975-76 and 1976-77.

S.No.	Fungiicde	Infection index 1975-76 Kharif	Infection index 1976-77 Kharif	Infection index 1976-77 Rabi	Average infection index	Percentage efficiency of disease control	* Yield (Kg) 1975-76 Kharif	* Yield (Kg) 1976-77 Kharif	* Yield (Kg) 1976-77 Rabi	Average yield	Percent increase in yield
1.	Benodanil	14.74 (22.58)	11.23 (19.58)	19.59 (26.27)	15.18	68.78	7.145	6.462	6.900	6.835	51.08
2.	Vitavax	30.78 (33.70)	19.83 (26.44)	27.88 (31.87)	26.16	46.20	7.105	7.037	6.375	6.839	51.17
3.	RH-124	40.53 (39.54)	28.00 (31.95)	57.22 (49.15)	41.91	13.81	5.540	5.588	5.238	5.455	20.57
4.	Plantvax	—	21.88 (27.89)	39.71 (39.06)	39.79**	36.06	—	5.950	6.050	6.000**	22.62
5.	Dithane M-45	38.53 (38.37)	25.91 (30.60)	45.54 (42.44)	36.66	24.61	6.535	6.200	5.775	6.170	36.38
6.	Dithane Z-78	42.71 (40.82)	30.27 (33.38)	52.93 (46.68)	41.97	13.69	5.150	5.537	5.688	5.458	20.64
7.	Control	49.96 (44.98)	36.76 (37.32)	59.57 (50.52)	48.63 (48.16)**	—	3.785	4.837	4.950	4.524 (4.893)**	—
	S.Em±	0.67	0.64	0.63			0.484	0.409	0.445		
	C.D. 5 %	1.97	1.91	1.87			1.425	—	—		
	C.D. 1 %	2.69	2.61	2.57			—	—	—		

In parenthesis are angular values, * yield of 100 plants, ** average of 2 years

Table-2. Comparison of 2 sprays of sytemic fungicides with 3 sprays of non-systemic fungicides on rust infection in the field during Rabi 1977-78

S. No.	Fungicide	Concentration (%)	Infection index	Percentage efficiency of disease control
1.	Vitavax	0.1	44.99 (42.13)	43.76
2.	Plantvax	0.1	26.58 (31.04)	66.77
3.	Benodanil	0.1	30.33 (33.42)	62.08
4.	Difolatan	0.2	73.29 (58.88)	8.38
5.	Dithane M-45	0.2	53.26 (46.87)	33.42
6.	Dithane Z-78	0.2	39.99 (39.23)	50.01
7.	Control	—	80.00 (63.44)	—
	S.Em ±		0.602	
	C.D. 5%		1.86	
	C.D. 1%		2.60	

In parenthesis are angular values

Table-3. Relative effect of systemic and non-systemic fungicides on rust infection and yield when sprayed once, twice and thrice during Rabi 1978-79.

S. No.	Fungicide	Number of spray	Infection index	Percentage efficiency of disease control	Yield* (Kg)	Percent increase in yield
1.	Benodanil	1	34.98 (36.26)	27.62	17.580	58.37
		2	20.59 (26.99)	57.39	16.470	48.37
		3	15.19 (22.94)	68.57	19.610	76.66
2.	Bayleton	1	46.32 (42.89)	4.15	14.630	31.80
		2	29.82 (33.10)	38.29	16.710	50.54
		3	29.65 (32.99)	38.65	15.840	42.70
3.	Vitavax	1	46.72 (43.12)	3.33	16.620	49.72
		2	27.23 (31.46)	43.65	16.020	44.32
		3	26.54 (31.01)	45.08	17.400	56.75
4.	Plantvax	1	53.34 (46.92)	—	13.800	24.32
		2	20.71 (27.07)	57.14	13.280	19.63
		3	22.64 (28.41)	53.15	15.600	40.54
5.	Dithame M-45	1	45.31 (42.31)	6.24	10.740	—
		2	34.36 (35.89)	28.90	12.400	11.71
		3	31.17 (33.94)	35.50	13.860	24.86
6.	Dithane Z-78	1	39.33 (38.84)	18.62	11.880	7.02
		2	30.50 (33.52)	36.89	13.980	25.94
		3	32.93 (35.02)	31.86	15.000	35.13
7.	Syllit	3	30.78 (33.70)	36.31	13.000	17.11
8.	Control	—	48.33	—	11.100	—
		S.Em.	C.D. 5%	C.D. 1%	S.Em. C.D. 5%	C.D. 1%
	Treatment	0.642	1.841	2.470	0.656 1.881	2.523
	Spray	0.454	1.302	1.746	0.464 1.330	1.785
	Treatment X Spray	1.110	3.183	4.270	1.136 —	—

In Parenthesis are angular values, * Yield of 300 plants.

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The First Report of Stem Pitting and Fleck Disease on Turkish Grapevines

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ABSTRACT

This is the preliminary report of stem pitting or wood pitting (bois strié, legno riccio) and fleck diseases of grapevine in Turkey. Severe stem pitting and furrow like depression symptoms has been observed on the woody clylinder of **Vitis vinifera** L. var. Sultana (Tompson seedless) and its American hybrid rootstock R-99. Stem pitting symptoms were more prominent on R-99 rootstock which is commonly used in the survey area. The Satsuma scion was markedly thicher in diameter than the R-99 rootstock. Stem pitting has been found associated with Grapevine fanleaf virus (GFLV) by indexing test. The same symptoms have not been seen on Sultana grapes grown on their own roots.

Characteristic leaf symptom of fleck disease was also observed on **Vitis rupestris** var. St. George indicators graft inoculated from some disease Sultana grapevines.

INTRODUCTION

Turkey has a favorable climate for grape growing and according to Oraman (1970), has had a grape industry since 3500 B.C. Vineyard area is about more then 848.000 hectare.

Because of their good quality and light color, the raisins, particularly Sultana has acquired an enviable reputation in European markets. Sultana is the main grape variety grown extensively in İzmir and Manisa provinces. Vineyard area is about 32.052

hectare in İzmir and 43.454 hectare in Manisa. Production is 114.961 ton in İzmir and 325.364 ton in Manisa totaly 440.325 ton more then half of the total production of The Aegean region (629.004 ton).

In many vineyards in İzmir and Manisa, variety Sultana has been grown on American hybrid rootstocks because of the phylloxera problem. Besides, most of the Sultana grapevines in Manisa have been grown on their own roots.

Indexing experiment have been carried out since 1978 for detecting the virus diseases of grapevine. During the observations in vineyards, some declined and diseased Sultana on R-99 rootstocks showed stem pitting or wood-pitting symptom on the scion and the rootstock. These Sultana grapevines were indexed to determine what the causal virus is.

MATERIALS AND METHODS

Experimental observations were made in the grapevine growing area of Kemalpaşa, Menemen and Manisa between 1978 - 1980. Sultana grapevines grafted on R - 99 with typical stem-pitting symptom and the other diseased grapevines grafted on American rootstock and on its own roots were tagged and numbered for indexing experiment. Indexing tests have been applied by using both woody grapevine indicator and herbaceous host plants to determine the causal viruses. **Vitis rupestris** St. George and Mission were used in indexing to detect GFLV, vein-banding and fleck viruses. Baco 22 A used for leaf roll virus. **Chenopodium quinoa** was used as a herbaceous indicator.

Dorman cuttings from several Sultana grafted on R-99 with stem-pitting symptoms and other diseased Sultana were collected in January and March. One or two bud cuttings of St. George indicator free-from viruses was used in indexing. One chip - bud from the donor diseased Sultana was inserted into St.George indicator dorman cutting as shown in Fig. 6. Tip-bud grafting used for Baco 22 A. One Baco 22 A bud cuttung grafted on the indexed Sultana dorman cutting with one or two buds. After graftings, the cuttings of indicator plants and the other grafted cuttings were returned to the moist send beds, held at 27°-29°C for further callusing and root development. When roots and young indicator shoots have developed as in Fig. 6, grafted cuttings were potted in clay

or polietylen pots and helded in the greenhouse conditions (in 23°-24°C) until the symptom appearance.

In indexing with herbaceous indicators, dorman cuttings of the Sultana grapevines grafted on R - 99 showing severe wood-pitting and the other diseased Sultana, were planted in a cutting bed at 27° - 29° C in March and April. The cuttings rooted and produced sufficient tissues for sap inoculations on herbaceous. Approximately 1 g. young leaf tissues were triturated in 2,5 % nicotine + 0,2 M phosphate buffer pH 7,6 and the prepared inocula were rubbed onto carborundum dusted **Chenopodium quinoa** (two plant with 8-12 true leaves inoculated per inoculum). Average greenhouse and room temperatures were 23°-25°C during the indexing experiments in spring and early summer.

RESULTS AND DISCUSSION

Stem pitting symptom and indexing results:

In the observations, in several vineyards in İzmir, Manisa, Kemalpaşa and Menemen areas 10 of the 50 examined diseased Sultana on R-99 rootstock showed visible wood-pitting symptoms on the scion and the rootstock. External symptoms on these wood - pitting affected vines were: delayed pushing of the spring growth, die-back or some declining of the scion shoots, poor growing, very stunted shoots, the bud union often showed a marked difference between the diameters of rootstock and scion (over - growth just above the bud-union) as shown in Fig. 8. The latter being usually thinner. The leaves of Sultana were distorted and showed light musaic. Shoots also showed short internodes, double nodes, other malformations the same of those induced by GFLV infected vines. Some Sultana grapes were unfruitful, others few small clusters with shelled berries.

R.S.George indicator plants inoculated with buds from stem pitting affected vines developed typical leaf symptom of GELV as following: leaf asymetry, mosaic mottle, oil spots, deep marginal sinuses, reduction in size and deformities of the leaves (Fig. 2), dip marginal sinuses, in the petal sinus was larger then 180 degree as shown in Fig. 3.; Systemically infected St. George plants had sharply intented bushy and stunted leaves as in fig. 3.; irregular branching with mildly fasciated internodes, short internodes and double buds as describ-

ed by Giovanni and Hewitt (1963), Goheen and Hewitt (1962), and Vuittenez (1970). The carachteristic symptoms of GFLV have also been observed on Mission indicators as reported by Winkler et al. (1974) as in Fig.4.

Chenopodium quinoa herbaceous indicator plants inoculated from the cutting leaves of wood-pitting affected Sultana showed typical systemic vein - clearing symptoms of GFLV (Fig. 5.A.) 8 or 10 days after inoculations as reported by Quacquarelli and Martelli (1965), Vuittenez (1970) and Uyemoto et al. (1976). After 15 or 20 days, vein-clearing symptom disappeared completely as desribed by Vuittenez 1970). Local lesion with systemic vein-clearing were also observed in some inoculations (Fig. 5. B). Baco 22 A indicators inoculated from the same sources did not develope leaf symptoms of grapevine leafroll virus as described by Goheen and Hewitt (1964) and Goheen (1970).

Stem pitting symptom of grapvines were previously reported by Gibbs et al (1970) and Boubals (1977) Mavraganis et al. (1977). The authors reported that, stem pitting was graft transmitted by vegetative propagation and realated with grapevine fan leaf virus, but not related grapevine leaf-roll. Boubals (1977) also reported that, grapevines which heavely infected by GFLV showed very veak plants, daying-back and stem pitting of the rootstock and the scion. According to Winkler et al. (1974), wood -pitting disease was observed in California grapevines for the first time by Hewitt and Neja in 1971. The same disease also occured in Italy, Hungary, Israel and South Africa. The same author reported that, it causes decline with progressive reduction of crops. The disease can be spreaded by **Xiphinema index.**

Our indexing experiment with St. George, Mission, Baco 22 A and **C. quinoa** revealed that, stem-pitting symptom is related with GFLV.

Symptom of fleck on St.George indicator:

In indexing experiments, some inoculated St. George indicators developed typical chlorotic translucent vein break in the third and fourth order veins of young and medium aged leaves as showen in Fig.1. The vein breaks varied from one to three millimeters in length as reported by Hewitt et al. (1970), Martelli and Hewitt (1963). Flecks on a leaf varied from a few to many. Leaves with numerous flecks were twisted and wrinkled (Fig. 7). Indexing experiment showed that, some Sultana grapevines also carried fleck virus.

Hewitt et al. (1970) reported that fleck has been transmitted by graft

inoculation to several varieties of **Vitis vinifera** and to hybrid rootstocks. The disease was reported is latent in many varieties in California, has also been observed in indicator plants under tests in South Africa and in Australia. Fleck virus is under quarantine in the foreign countries as reported by Kahn et al. (1979).

It is the author opinion that, stem-pitting and fleck diseases of grapes can be avoided by using indexed virus - free scion and the rootstock varieties in new plantings.

ÖZET

TÜRKİYE'DE ASMALARDA FLECK VE GÖVDE ÇUKURLUK HASTALIKLARI

İzmir, Manisa ve Kemalpaşa bağ üretim alanlarında hapılan gözlemlerde, R-99 anaç üzerine aşılı çekirdeksiz üzüm asmalarında anaç ve aşı gövdesi üzerinde gövde çukurlaşma (wood pitting) hastalığının tipik belirtileri saptanmıştır. Yelpaze yaprak virusu (Fanleaf)'nun tipik belirtilerini gösteren bu omacların yapılan endeksleme testlerinde, endikatör olarak kullanılan «R» St. George, Mission ve **C. quinoa** üzerinde Yelpaze Yaprak virusunun tipik belirtileri görülmüştür. Baco 22 A üzerinde herhangi bir belirti görülmediğinden gövde çukurlaşma belirtilerinin Yaprak Kıvrılma (Leaf roll) virusu ile ilgili olmadığı anlaşılmıştır. Endeksleme denemelerinde, hastalıklı bazı çekirdeksiz omcalara ait testlerde endikatör olarak kullanılan «R» St. George endikatör bitkisinin yapraklarında fleck virusunun tipik belirtisi olan, yaprak yan damarlarında kesik çizgi şeklinde beyaz renk açılmaları (flecks) görülmüştür. Bazı çekirdeksiz omcaların bu virusla infekteli olduğu anlaşılmıştır. Bu virus karantinaya dahil olduğundan sertifikasyon çalışmalarında ve karantina endekslemelerinde dikkate alınması gerekmektedir.

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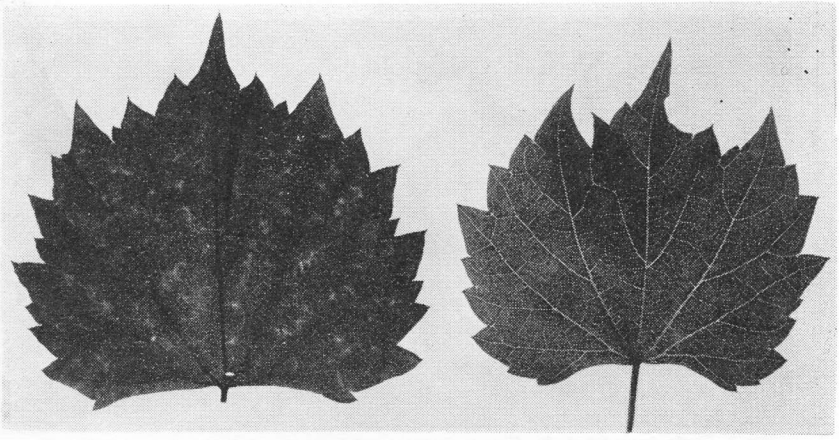

Fig. 1. Fleck symptoms on graft inoculated Rs. St. George. The leaf at the right is control from uninoculated St. George.

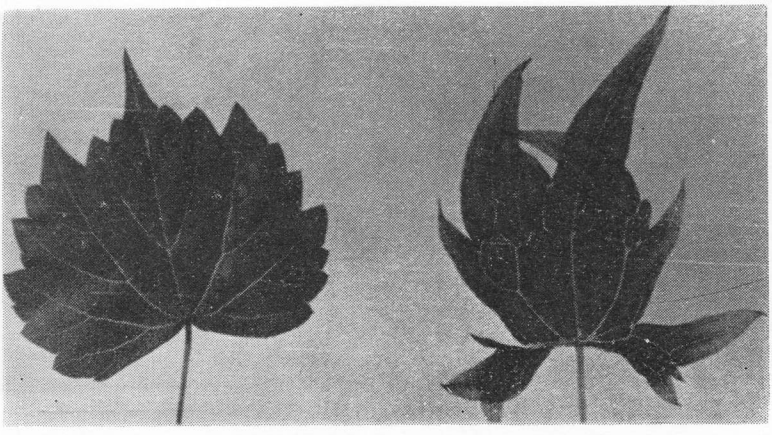

Fig. 2. Graft inoculated **Vitis rupestris** St. George shows typical leaf symptom of GFLV. The leaf at the left is control from uninoculated St. George

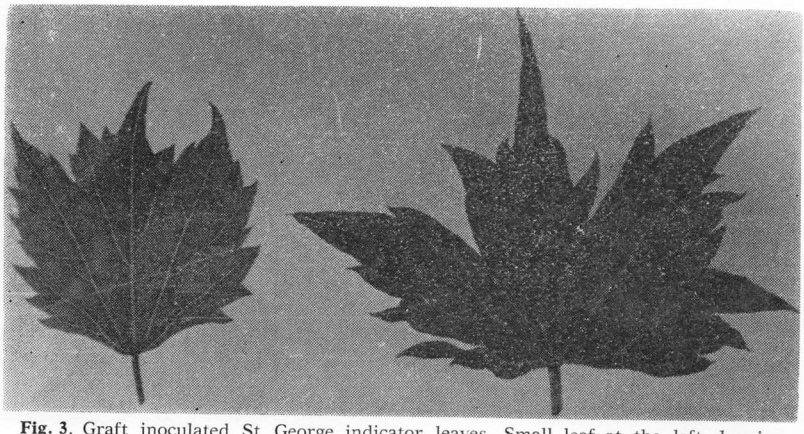

Fig. 3. Graft inoculated St. George indicator leaves. Small leaf at the left showing large sinus at the petiol and leaf deformation of GFLV.

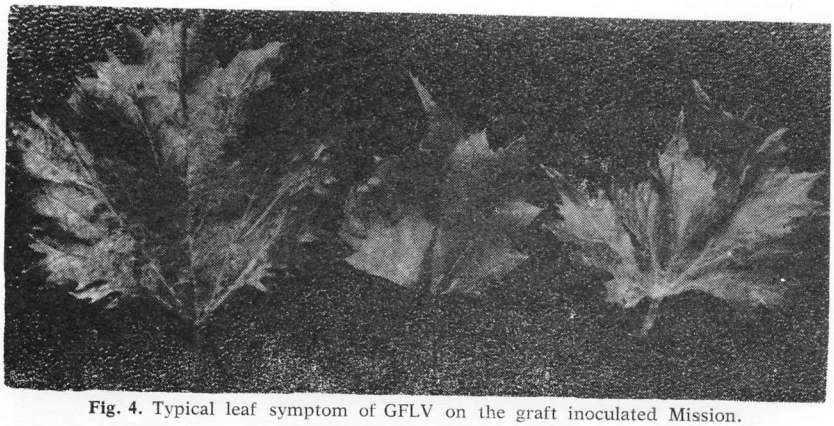

Fig. 4. Typical leaf symptom of GFLV on the graft inoculated Mission.

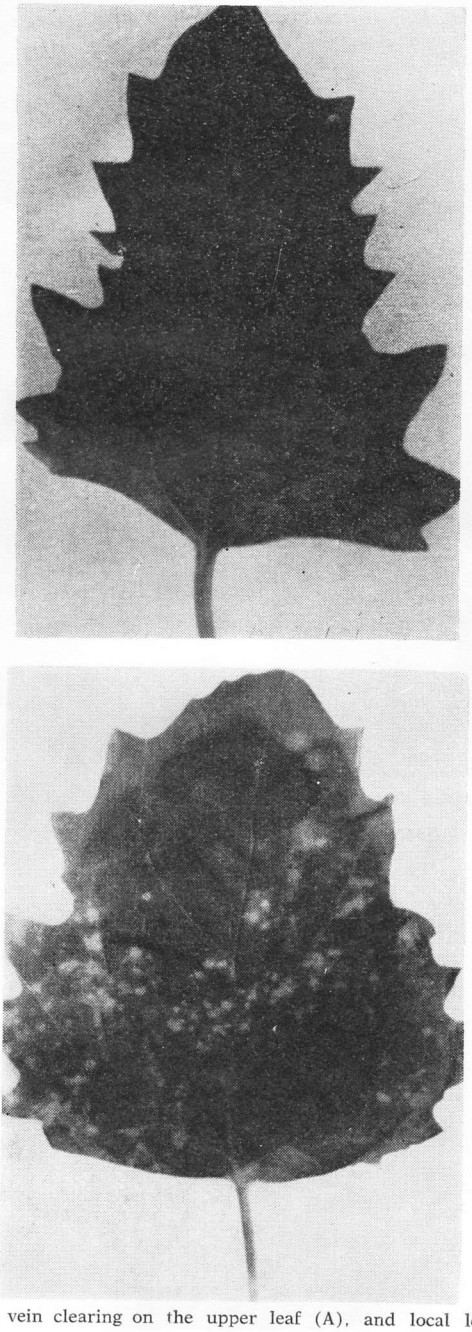

Fig. 5. Systemic vein clearing on the upper leaf (A), and local lesion symptoms (B) of GFLV on the sap inoculated leaves of the **C. quinoa.**

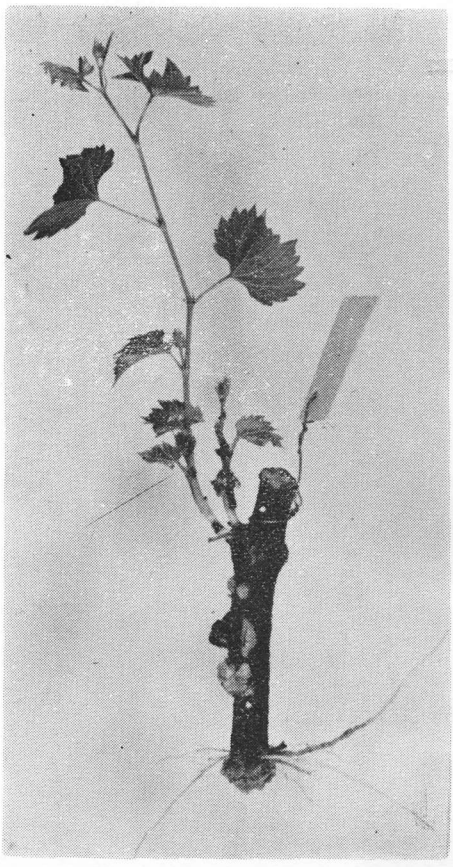

Fig. 6. Chip-bud graft inoculated dormant cutting of Rs. St. George indicator

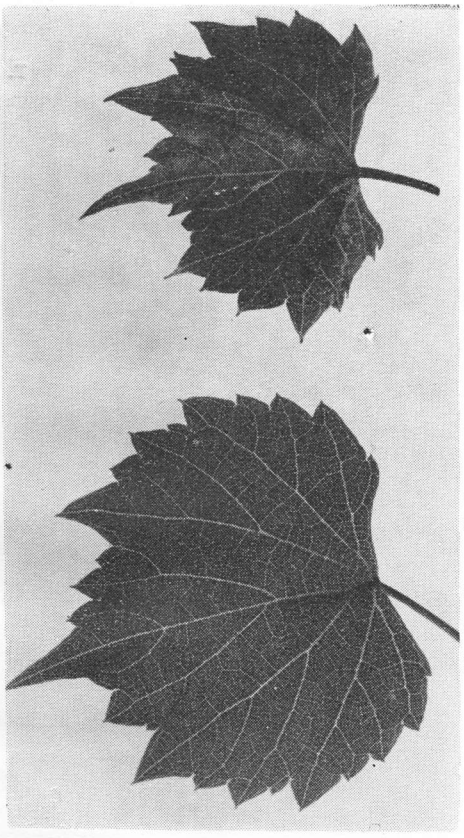

Fig. 7. Severe fleck symptoms on St. George.

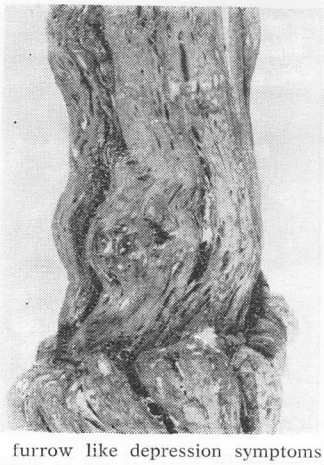

Fig. 8. Stem pitting and furrow like depression symptoms on R-99 rootstock and the Sultana scion, overgrow at the bud union.

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Utilization of Carbon, Nitrogen and Vitamins by Colletotrichum graminicolum Isolates

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ABSTRACT

The paper deals with the utilization of carbon, nitrogen and vitamins by three isolates (I_1, I_2 and I_3) of **Colletotrichum graminicolum** (Ces.) Wils., the incitant of anthracnose of sorghum. The studies were carried out in Richard's medium adjusted to pH 6.0 and indubated at 30+ 1C°. Fructose was best utilized by I_2 and I_2 while I_3 utilized more of maltose, among nitrogen sources peptone supported maximum growth of isolate I_2 and I_3. Isolate I_1 grew best on DL-Threonine. Ammonium nitrate and potasium nitrate favoured abundent sporulation of isolate I_1. Thiamine increased the dry weight of the three isolates and riboflavine supported abundant sporulaiton of isolate I_1 only. These results are an evidence that variability exist in nutritional requirement of **C. graminicolum** isolates.

INTRODUCTION

Knowledge of nutritional requirement of the pathogen helps in better understanding of host parasite relationship and variability in the isolates. **Colletotrichum graminicolum** (Ces.) Wils. attacks many graminaceus plants besides sorghum (**Sorghum bicolar** (L.) Moench). Pathogenic and cultural variability among the isolates of this pathogen from different hosts has been reported (Chowdhury 1936 and Chohan, 1967). Therefore, three isolates from sorghum were taken to study their nutritional requirement.

MATERIALS AND METHODS

Isolates I_1, I_2 and I_3 from sorghum were taken from different localities of Rajasthan (India). Cultures were maintained on potato dextrose agar. Richard's medium was used as basal medium in this study. Carbon and nitrogen compounds were incorporated separately at the same carbon/nitrogen level of the basal medium. The amount of vitamins added has been indicated in Table 3. Twenty ml of the medium was poured in 100 ml Erlenmeyer flasks. The medium was buffered to pH 6.0 before autoclaving at 15 psi for 15 minutes. In case of vitamins steam sterilization was done for 30 minutes for three consecutive days. The flasks were inoculated with 2 mm mycelial disc from one week old culture. The contents of flasks were filtered through previously dried and weighed Whatman (42) filter paper, after 10 days of inoculation. The filter papers with mycelial mat were dried in an electric oven at 60C for 24 hr and then cooled in a desiccator and weighed. Average of 4 replications was worked out and spores were counted in drops from a flask under microscope (10 x) and graded as follows: 0 = no spores; poor=1-7 spores; moderate= 8-15 spores; good=16-23 spores; abundant = above 24 spore.

RESULTS

Effect of different carbon sources on growth and sporulation:

The isolates did not grow in the absence of carbon source. Growth of the three isolates differed significantly from each other except the growth of isolate I_1 which was not significantly different on xylose, galactose and mannose. Maximum growth of isolate I_1 was on maltose, cellobiose and fructose and minimum on sorbose. Fructose was the best source for isolate I_2 and I_3 whereas sorbose for I_2 and mannitol for I_3 were the poorest source of carbon. Galactose, fructose, maltose and sucrose were excellent for sporulation of I_1 and lactose for I_2. Maltose and fructose supported good sporulation of I_3. Carbon sources for good sporulation of isolate I_1 were xylose, raffinose and mannitol; for isolate I_2, mannose, raffinose and for isolate I_3, sorbose, cellobiose, raffinose, xylose and glucose (Table 1).

Effect of nitrogen sources on growth and sporulation:

Results show that there were significant difference among the isolates in the utilization of nitrogen sources. Peptone was best of nitrogen followed by threonine and aspartic acid for isolates I_2 and I_3. For isolate I_1 best sources of nitrogen were threonine, methionine and peptone with no significant difference in these sources. Urea for isolate I_3, ammonium nitrate for isolates I_1 and I_2, and sodium nitrate for isolate I_1 were poor sources of nitrogen. In general sporulation was good on potassium nitrate followed by ammonium nitrate and asparagine. Isolate I_1 on sodium nitrate and I_3 on glycine and phenylalanine did not sporulate (Table 2).

Effect of vitamins on growth and sporulation : Thiamine and inositol were significant for supporting growth over control. For all the isolates thiamine was good followed by inositol, pyridoxine and nicotinic acid. Mean dry mycelial weight of three isolates were non - significant. Ascorbic acid was inhibitory to growth.

Thiamine and riboflavine increased the sporulation of isolate I_1 but not of I_2 and I_3. Nicotinic acid and ascorbic acid completely checked the sporulation of isolate I_3 (Table 3).

DISCUSSION

Among the various carbon sources tested fructose was best for the isolates in general for growth and sporulation. However, isolates differed in their utilization of different sources of carbon. Maltose was reported to be most nutritious for **Colletotrichum lini** (Tochinai, 1926). Difference in the carbon utilization by different fungi have been reported by the various workers (Durairaj, 1956; Mathur et al., 1950; Sahni et al. 1975).

There were differences in the utilization of nitrogen sources by different isolates of **G. graminicolum.** Peptone, a complex mixture of peptides and amino acids is reported to be a good source of nitrogen for **Colletotrichum** sp by Ramakrishman (1946), Mathur et al. (1950), and Sahni et al. (1975) is further corroborated by our results. Isolates of **C. graminicolum** utilized some of the vitamins supplied in the medium. Thiamine was utilized more by all the isolates followed by inositol. Tandon (1951) noted that thiamine supported

good growth of many fungi. Mathur et al. (1949) observed that different strains of **C. lindemuthianum** exhibited partial deficiencies for vitamins. Our results are in conformity with those of Misra and Mahmood (1961) and Ghouse and Khan (1963)

The results reveal that variability in the nutritional requirements exist among the isolates of **C. graminicolum** and perhaps may be an explaination for variation in virulence of the isolates.

The authors are grateful to Dr. H.N. Mehrotra, Dean, Rajasthan College of Agriculture, Udaipur for the facilities.

ÖZET

Colletotrichum graminicolum İZOLATLARININ GELİŞMESİNE AZOT, KARBON ve VİTAMİNLERİN ETKİSİ

Çalışmada üç (I_1, I_2, I_3) **C.graminicolum** izolatının gelişmesine farklı karbon, azot ve vitamin kaynaklarının etkileri incelenmiştir. Araştırmalar pH derecesi 6 olan Richard ortamında yürütülmüş ve kültürler 30∓ 1°C sıcaklıkta inkube edilmiştir. Karbon kaynakları içinde I_1 ve I_2 izolatları fruktozu tercih ederken I_3 için maltoz daha uygun bulunmuştur.

Yine I_1 ve I_3 peptone da maksimum büyüme göstermiş, I_1 için DL-Threonine uygun bulunmuştur. Amonyum nitrat ve Potasyum nitrat ise I_1 izolatında sporulasyonu artırıcı olmuştur. Thiamine üç izolatta da kuru kütleyi artırırken riboflavin sadece I_1 izolatında sporulasyonu teşvik etmiştir.

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Table-1. Effect of various carbon sources on growth and sporulation of **Colletotrichum graminicolum** isolates

Carbon sources	Dry mycelium weight (mg)			Mean for carbon sources	Sporulation		
	I_1	I_2	I_3		I_1	I_2	I_3
D-Glucose	325	320	280	308.3	+++	++	+
D-Xylose	235	196	262	231.0	++	++	+
Mannose	246	253	230	248.0	+++	+	++
D-Galactose	239	180	270	229.7	++++	++	++
D-Fructose	345	373	391	369.2	++++	+++	++
Lactose	243	203	289	245.0	+++	++++	++
Maltose	253	351	304	336.0	++++	+++	++
Sucrose	297	313	346	318.7	++++	++	++
Raffinose	285	268	190	247.7	++	+	+
Mannitol	204	215	178	199.0	++	++	++
Sorbose	192	166	208	188.7	++	+	+
Cellobiose	351	365	330	348.7	+++	++	+
Control	60	41	33	44.7	+	+	+

	Isolate	Carbon	Interaction
SEm	1.582	3.294	5.705
C.D. at 5%	4.45	9.27	16.06

\+ = poor
++ = moderate
+++ = good
++++ = abundant
— = no sporulation

Table-2. Effect of various nitrogen sources on growth and sporulation of **Colletotrichum graminicolum** isolates

Nitrogen sources	Dry mycelial weight (mg)			Mean for nitrogen source	Sporulation		
	I_1	I_2	I_3		I_1	I_2	I_3
Ammonium nitrate	222	171	240	211.0	++++	++	+++
Ammoni. sulphate	222	171	240	211.0	+	+	+
Sodium nitrate	222	206	243	225.3	++	+	++
Sodium nitrite	253	213	172	219.8	—	+	+
Potasium nitrate	274	291	308	291.0	++++	+++	++
Urea	259	209	115	194.3	+	+	+
Glycine	271	286	190	249.0	+	+	—
Peptone	33	370	388	363.7	+	++	+
L-Asparagine	312	327	285	308.0	+++	++	++
Aspartic acid	323	345	319	329.0	++	++	++
L-Histidine	317	323	298	311.6	++	++	+
DL-Tryptophan	232	234	251	239.0	+	++	+
DL-Threonine	339	351	337	342.3	++	+	+
L-Cysteine	291	312	305	302.7	++	++	+
Phenylalanine	318	331	301	316.7	+++	++	—
DL-Methionine	336	298	313	315.7	+++	+++	++
Control	223	201	190	204.7	+	+	—

	Isolate	Nitrogen	Interaction
SEm	1.043	2.530	4.381
C.D. at 5%	2.89	7.08	12.27

\+ = poor
++ = moderate
+++ = good
++++ = abundent
— = no sporulation

Table-3. Effect of vitamins on growth and sporulation of **C. graminicolum** isolates

Vitamin sources	Concentration per litre in/μg	Dry mycelial wt. (mg.) of isolates			Mean for vitamins	Sporulation		
		I_1	I_2	I_3		I_1	I_2	I_3
Thiamine	100	392	370	402	388.0	+++	+++	++
Riboflavine	100	275	270	290	278.0	++++	++	+
Folic acid	100	261	250	242	251.0	+	+	—
Nicotinic acid	100	291	280	248	273.0	+	+	—
Pyridoxine	50	293	260	253	268.7	++	+	+
Ascorbic acid	50	255	230	224	236.3	+	+	—
Biotin	5	258	252	240	250.0	+	+	+
Inositol	5 mg	300	305	325	310.0	++	++	+
Control (No vitamin)		287	290	302	292.0	++	+++	++

— = No sporulation
\+ = Poor
++ = Moderate
+++ = Good
++++ = Abundant

	Isolate	Vitamin	Interaction
S.Em.	3.288	5.696	9.866
C.D. at 5%	9.33	16.16	27.99

TABLE OF CONTENTS AND INDEX TO VOLUME SEVENTH

1980

TABLE OF CONTENTS

No. 1 — Jan.: 1980

No. 2 — May — Sep.: 1980

INDEX

All Correspondance Should Be Made To

FİTOPATOLOJİ DERNEĞİ

Ege Üniversitesi Ziraat Fakültesi

Fitopatoloji ve Zirai Botanik Kürsüsü

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