OCCURRENCE OF BARLEY YELLOW DWARF VIRUS (BYDV) ON WHEAT IN JORDAN

BY

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Partial Fulfilment of the requirement

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IN

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UNIVERSITY OF JORDAN.

بسم الله الرحمن الرحيم

DEDICATED TO MY

MOTHER,

FATHER AND

BROTHERS.

UNIVERSITY OF JORDAN

FACULTY OF AGRICULTURE

DEPARTMENT OF PLANT PROTECTION

I hereby recommend that this thesis prepared under my direction by **Moh'd N. F El-Zou'bi** entitled :

OCCURRENCE OF BARLEY YELLOW DWARF VIRUS (BYDV) ON WHEAT IN JORDAN

be accepted as fulfilling the thesis requirement for the degree of

MASTER OF SCIENCE

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As members of the final examination commitee, We certify that we have read this thesis and agree that it may be presented for final defense.

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final approval and acceptance of this thesis is contingent on the candidate's adequate performance and defense thereof at the final oral examination.

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INTRODUCTION

Cereal grains are major source of food for human and animals. In terms of total yield production , wheat is the leading cereal grain in the world (Haldore et al.,1982). Other important cereal grains are barley, ,oat, and sorghum.

In Jordan although wheat occupied 61% of the area planted to grain crops, wheat production is only equal to 12% of consumption through 1978-1987 (Anonymous,1988). In 1986-1987 the total area planted to wheat and barley was 1245426 and 600601 donums*, respectively(Anonymous,1987). The average yield was 88 kg per donum for wheat,and 68 kg for barley. This compares very low with those in developed countries. Such low productivity is seemingly due to many factors among the most critical of which rainfall, improper cultural practices and pests.

Of the diseases reported on cereals, there were twenty viruses affecting wheat and barley. The most important viral disease is caused by barley yellow dwarf virus(BYDV) (Oswald and Houston,1953a).It has been recognized in three continents as a serious disease to the production of wheat, barley, and oat (Bruehl, 1961).This virus belongs to the luteovirus group (Matthews, 1981;

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* Donum= 1000 m2

Walkey, 1985), member of which can cause yellow disease.

Infection by BYDV resulted in severe stunting of infected plants, partial phloem plugging, root inhibition, and delaying or preventing heading and thus reducing the yield (Bruehl,1961;Esau,1957; Oswald and Houston,1953a; Panayotou, 1978).

The disease has a wide host range in the grass family, which includes all small grains of cereal, including wheat, barley, oats, maize, and sorghum (Oswald and Houston,1953b; Bruehl,1961; Stoner, 1977). The virus also infects the grasses, which act as reservoir for the virus and its aphid vector (Paliwal, 1982). The Barley yellow dwarf disease is caused by a virus transmitted by at least five different species of grain aphids in a persistent manner(Orlob et al., 1961; Paliwal and Sinha, 1970), but not by seed, soil, or sap transmission (Gill,1967; Jedlinski et al., 1974; Rochow and Duffus, 1981). BYDV was assumed to occur in Jordan on wheat and barley on the basis of field symptoms (Mink and AL-Musa, 1984).

This study was initiated to identify BYDV strains in Jordan and its vectors. In addition field incidence of BYDV on wheat, barley, corn, and sorghum was studied and the virus economic impact on wheat and barley cultivars was evaluated. Furthermore the virus reservoir on grasses was assessed.

LITERATURE REVIEW

Barley yellow dwarf virus (BYDV) caused a destructive disease of cereals that had been recognized in California in 1951 in most barley production areas in the state reaching epidemic proportions in several years (Oswald and Houston, 1953a). After that, BYDV has been recorded from most areas of the world wherever barley is grown (Bruehl, 1961). The virus has an extensive host range among Gramineae (Oswald and Houston, 1953b). Economic plant hosts of BYDV included barley, oats, wheat , rice, corn and rye. Other important hosts included widely distributed lawn, pasture, and grasses (Bruehl, 1961; Latch, 1980; Paliwal, 1982; Stoner, 1977 ; Oswald and Houston (1953b) reported that about 36 species of grasses were susceptible to BYDV, and observed that some of them were symptomless carriers .

Symptoms of BYDV vary with the cultivars, the age of the plant at the time of infection,the strain of the virus,and the environmental conditions (Bruehl, 1961; Orlob and Arny,1961; Oswald and Houston, 1953a).They included characteristic golden yellowing of barley leaves, chlorosis with occasional little reddening of wheat leaves. Oat leaves become reddish or purple in color (Allen, 1957; Bruehl, 1961). Young plants were either killed , or stunted so severely that they failed to head. Severity of BYDV ranged from mild symptoms

to total collapse and death of infected plant(Bruehl, 1961; Panayotou,1979). Symptoms of barley yellow dwarf virus were more pronounced under cool temperatures, long days, and high light intensity(Orlob and Arny, 1961).

Replication of the virus occurs primarily in phloem tissues (sieve elements, companion cell, and parenchyma) with consequent phloem necrosis (Esau, 1957; Jensen, 1969a). It does not multiply in its aphid vector (Paliwal and Sinha, 1970).

Barley yellow dwarf virus includes at least five isolates that had been grouped according to their vector specificity. The groups are designated by the initial letters of their principal vector. These isolates are transmitted separately by *Macrosiphum) (=Sitobion) avenae* (MAV), *Rhopalosiphum padi* (RPV), *R. padi* and *M. avenae* (PAV), or *R. maidis* (RMV), or *Schizaphis graminum* (SGV) (Johnson and Rochow, 1972; Rochow, 1969; 1979). The isolates could be grouped by serological methods to two groups, namely RPV and RMV isolates in one group and MAV, PAV, and SGV isolates in the other group. (Rochow and Carmichael, 1979; Paliwal, 1977).

The physiological alteration of cereal grains resulting from BYDV infection had been noted. The virus reduced the average yield by 53% ,the photosynthesis

rate by 45%, and the chlorophyll content by 80%. It also resulted in an accumulation of soluble carbohydrate (Sugar) and strach in the infected leaves (Jensen, 1972). BYDV induces ultrastructral changes in the infected phloem tissue (companion cells, and sieve elements) that included slender filaments, small vesicles containing fibrills, amorphous material in addition to the virus particles (Gill and Chong; 1975; 1976; 1979).In this regard there are distinctive differences among BYDV strains. Accordingly, BYDV strains could fell into two groups as previously mentioned. In the first group (MAV, PAV, SGV), single membraned vesicles containing fibrills appeared in the cytoplasm. This is referred to as MAV pattern. Moreover, this pattern involved distortion of the nuclear outline. Whereas in the RPV and RMV pattern a second membrane containing fibrils is present and the virus particles were observed first around the nucleus. This pattern is characterized by non distorted nucleous (Gill and Chong,1976; 1979).

In infected barley, the weight of 1000 seeds was reduced by 21.9% (Gill, 1970) and the fresh and dry weight of diseased plants were reduced by 28% and 25%, respectively (Jensen, 1972).

Only 14 aphid species have been reported to transmit barley yellow dwarf virus to cereals. The best known vectors of BYDV included *Metopolophium*

(Acyrthosiphum)dirhodum Walk., *R.maidis* Fitch ., *R. Padi* L., *M. avenae* Fab. and *S. graminum* Rondani. The virus is not transmitted by mechanical inoculation but in the persistent manner by aphid vector which remain viruliferous for 2-3 weeks (Rochow,1970). Aphids acquire BYDV by feeding for 2 days on infected plant, the virus must pass into the gut and then passes through the haemocoel of the aphid to the salivary glands via hemolymph. It is introduced into the plants through the salivary secretions (Gildow, 1982; Gildow and Rochow, 1980). BYDV can be also transmitted by the membrane-feeding technique (Rochow,1960).

BYDV belongs to the luteovirus group (Walkey, 1985,); The term BYDV includes several related isometric viruses of graminaceous plants (Rochow and Duffus, 1981), with particles of about 30nm in diameter, a sedimentation coefficient of 115 to 118^{S}, and a single stranded RNA of molecular weight $2.0X10^{6}$'daltons (Brakke and Rochow, 1974; Rochow et al ., 1971). The RNA content of BYDV was estimated to be 28% (Walkey, 1985). The molecular weight of coat protein has been estimated to be 23,500 daltons for the MAV and 24,450 daltons for RPV isolate (Scalla and Rochow, 1977).

The virus concentration is very low in infected tissue ranging from 20 ug to 106 ug /1000 g source tissue depending on the strain of the virus

(Rochow et al.,1971).The thermal inactivation point(TIP) in a sap of infected plant with BYDV ranged between 65 and 70 $\overset{o}{C}$,the dilution end point DEP is 10^{-3}. (Heagy and Rochow , 1965; Rochow, 1970). Clintland 64 Oat (*Avena Sativa L.*.cv. Clintland 64) is a good propagative host for barley yellow dwarf virus (Patterson and Schafer, 1978).

Serology is one of the most commonly used tools for identification of barley yellow dwarf virus. One sensitive serological tests that has been examined for usefulness in BYDV surveys is the enzyme-linked immunosorbent assay (ELISA), which was adapted for the detection of BYDV luteoviruses in plant tissue (Clark and Adams, 1977; Lister and Rochow,1979; Stobbs and Barker, 1985; Voller et al.,1976). Other useful tests for BYDV detection employing micro agar double diffusion (Aapola and Rochow,1971), and serologically specific electron microscopy (Paliwal,1977). Cross protection tests have been successfully used to demonstrate strain relationships of BYDV, so that systemic invasion by one strain can protect the plant against severe symptom caused by infection with a second related virus (Aapola and Rochow, 1971; Jedlinski and Brown, 1965).

The most appropriate method for the control of barley yellow dwarf virus depends upon the conditions under which a crop is grown and the

epidemiology of virus under those conditions. Losses caused by BYDV can be minimized as a result of elimination of virus source plants (Plum and Thresh, 1983), application of insecticides (Bruehl, 1961; Jedlinski et al., 1974), and changing the planting time to avoid maximum aphid population (Jedlinski et al. 1974). Overall, the use of resistant crop cultivars offers the best approach for control of BYDV(Carrigan et al 1981.; 1983).

MATERIALS AND METHODS

Virus Source. Leaf samples were collected from stunted wild oat (*Avena sterilis* L.)plant showing reddish to purple leaves in the Jordan valley. Virus isolation and strain separation was then made by aphid transmission using *R.padi, M. avenae and R. maidis* on Clintland* .64 oat plants. Details of transmission experiments will be discussed elsewhere. Plants that reacted with symptoms similar to those found in the field were kept under glasshouse conditions at 20 ± 2 °C. The identity of different strains that were isolated by the three aphid species was confirmed by serological tests using direct or indirect ELISA (Clark, et al, 1986; Koenig , 1981; Lister and Rochow, 1979). Details of ELISA test will be discussed later in the text.

Host Range. Six seedlings of each of wheat ' Deir Alla 4', barley 'Deir Alla 106', corn 'Jubilee', oat 'Clintland 64', and sorghum 'Local' at 3 leaf stage, grown in 13 x 16 cm diameter pots in the glasshouse at the rate of one seedling per pot, were inoculated by PAV strain using *R. Padi.* Three seedlings of each species were infested by nonviruliferous aphids as control. Plants were observed 3 weeks after inoculation for symptom development.

* Clintland 64' oat was obtained from Purdue University Agricultural experiment station, West Lafayette, IN.

Aphid Identification. Several species of wingless aphids had been collected from cultivated or wild cereals in the Jordan valley. Identification of these aphids was based on a key for identifying cereal aphids (Cohen,1974). Confirmation of the identity of aphids was done by sending specimens to the British museum.

Aphid transmission. Since the PAV strain can cause severe disease and is reported to be efficiently transmitted by *R. Padi* (Rochow,1969), detailed aphid transmission experiments were carried out using PAV infected plants and *R. padi* as a vector. Nonviruliferous apterious aphids were transferred with a camel- hair brush to oat plant infected with PAV isolate for 2 days at 15 $\overset{o}{C}$. Ten insects were transferred to two week old healthy seedling of *Avena sativa* L. cv.Clintland 64 and allowed 5 days inoculation feeding period. The aphids were then killed by Cypermethrin* and the plants were kept under glasshouse conditions at 20 $\pm$ 2 $\overset{o}{C}$ and observed for symptom development. All transmission experiments by aphids were done as described here unless otherwise mentioned.

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*Cypermethrin, (RS)-a- cyano -3- phenoxybenzyl(1RS)-cis, trans-3- (2-2- dichlorovinyl) -2,2 dimethyl cyclopropana carboxylate. ICI

Efficiency of transmission by aphid. Wingless aphids were collected from cultivated or wild cereals. The insects were identified as *R. padi* (L), *R. maidis* (Fitch),*M. avenae* (Fabricius), and*S. graminum* (Rondani) as described earlier. comparative transmission tests using these insects were carried out using the Clintland 64 oat cultivar infected with PAV strain as source of inoculum. For each aphid species 16 oat seedlings at third leaf stage were inoculated and kept under glasshouse conditions at 20±2°C for symptom development.

Transmission through membrane by aphids. Transmission through membranes was done to confirm results of ELISA with respect to BYDV-PAV infectivity. Nonviruliferous *R. Padi* previously starved at 15 °C for 3h, were placed in small feeding chambers topped with stretched parafilm membrane. Test inocula were placed on the oppsite side of the membrane so that aphids could feed through it on the virus preparation. Inocula were prepared by macerating infected tissue in a condition similar to that used for ELISA tests at the rate of 1g per ml in neutral 0.l M phosphate buffer containing 10% sucrose; the homogenate was clarified by low speed centrifugation at 4000 rpm for 20 minutes. Following an acquistion feeding period of 2 days at 15 °C, aphids were transferred to Clintland 64 oat seedlings, and were given 5 days

inoculation feeding period before they were killed with Cypermethrin. Similarly aphids that had been feeding on 10% sucrose were placed on oat seedlings to serve as control. Assay plants were kept in the glasshouse three weeks for symptom development.

Virus strain and vector prevalence. Virus strain and vector predominance was assessed in Deir Alla area, using Clintland 64 oat seedling as bait plant. The seedlings were planted in 12 x 13 cm diameter pots, at the rate of one seedling per pot. 20-25 pots were placed near wheat fields, starting Feb 9 at weekly intervals and extended to the end of the growing season (First week of May) of 1987. After one week exposure, aphids were identified and counted. The plants were sprayed with Cypermethrin and grown for two weeks in the glasshouse. Each plant was tested by ELISA for the presence of MAV, PAV, and RPV virus types.

Serology. Antisera specific to B (PAV), and F (MAV) types of barley yellow dwarf virus purchased from INOTECH company, were used for detection of virus by direct enzyme-linked immunosorbent assay (ELISA) tests. Moreover antisera to PAV, MAV, RPV, RMV, and 32-41* types of barley yellow dwarf

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* 32-41 Antisera was prepared against PAV, MAV and SGV types of BYDV

virus, kindley supplied by Steve Wyatt, were used for indirect enzyme-linked immunosorbent assay (ELISA) tests. Also Goat anti-mouse alkaline phosphatase conjugate was purchased from SIGMA chemical company.

Antigen preparation. Extraction of antigen for ELISA test was done by chopping 1 gram of fresh or frozen tissue at _ 20C°. Each sample was then homogenized in phosphate buffer saline containing 0.05% Tween 20, and 2% polyvinylpyrrolidone (PVP) at a tissue : buffer ratio of 1 : 10 (w/v).

Direct Enzyme-linked immunosorbent assay (ELISA). Double sandwich procedure was employed as described by Lister and Rochow 1979 . Wells of polystyrene Micro ELISA plates (Dynatech Laboratories) were coated by 200 ul per well of immuno-globulin (Ig) diluted in coating buffer (appendix 1), for three hours at 37C°, at the immuno-globulin : buffer ratio was of 0.0025:1 (V/V) coating buffer (Appendix 1). The plates were rinsed three times with PBS-Tween 20 (Appendix 1), and 200 uL of extract sap was added to each well. The plates were then incubated overnight at 4 C°. After further rinsing with PBS -Tween, 200 uL per well of enzyme - labelled gamma - globulin (conjugate) diluted in conjugate buffer (Appendix 1) at .0025:1* was added to each well to

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* Dilution 1:400 were used for both coating and conjugated antibodies

react with bound antigen during a further 4 h incubation at 37 °C.

Finally, unreacted conjugate was rinsed away three times with PBS-Tween 20. Specific antibody-antigen reactions was assessed by adding 200 uL P. nitrophenylphosphate at 1mg/ml in substrate buffer (Appendix 1) to each well. The plate was incubated at room temperature for the reaction to take place .

Reactions were stopped after 1 h by adding 50 uL per well of 3M sodium hydroxide. Assay was done by visual observation of the yellow color, or by reading absorbances (A 405nm)using EL 308 model reader, Bio-Tek instruments. Reactions that gave obvious yellow color or absorbances greater than twice the average for healthy control samples in the same plate were considered as positive .

Indirect enzyme-linked immunosorbent assay. Indirect ELISA procedure was employed as described by (Clark, et al., 1986; Koenig, 1981; Lommel et al., 1982). Wells of polystyrene Micro ELISA plates (Dynatech Laboratories) were coated by incubation 200 ul of extract sap to each well for three h at 37°C. After incubation the plates were rinsed three times with PBS-Tween 20 (Appendix 1), and 200 uL antisera diluted at 1:40 in conjugate buffer were added per well. The plate was incubated at 4°C overnight . After further rinsing,

200 uL per well of goat anti-mouse, alkaline phosphatase conjugate was added at a 1/1000 dilution in coating buffer for three h incubation at 37°C.

Finally, unreacted conjugate was rinsed away three times with PBS-tween 20.Antigen-antibody reactions was assessed by adding 200 uL P. nitrophenyphosphate at 1mg per 1ml in substrate buffer to each well. plate was incubated at room temperature for the reaction to take place. Reactions were stopped after 1h by adding 50 uL 3M sodium hydroxide per well. Assay was made by visual observation of the yellow color, or by reading absorbances (A 405nm) with model EL 308 reader, Bio -Tek instruments. Reactions that gave obvious yellow color or absorbances greater than twice the average for healthy control samples in the same plate were considered as positive.

Incidence Of The Disease. Incidence of BYDV in cereal crops was assessed in 2184 leaf samples from small grain plants (corn, barley, sorghum, wheat) collected from fields randomly located along the Jordan valley and highlands during the growing season of 1987, and 1988 (Table 1). Random sampling was done by walking in the field in an "x" pattern and taking samples at monthly intervals. Five samples were taken from five different location in each field chosen at random and each sample represented 10 different plants usually one leaf per plant. All collected samples were tested by ELISA to detect the virus.

Table 1. Number of samples collected from different cereal crops* growing in different areas in Jordan 1987-1988.

Year	Location	host	Number of samples collected
1987	Irbid	Wheat	315
		barley	45
	Karak	Wheat	287
	Madaba	Wheat	90
	Jordan valley	wheat	469
		barley	151
	Jordan valley and Irbid	Corn	85
	Jordan valley and Irbid	Sorghum	139
1988	Irbid	wheat	195
		barley	67
	Jordan valley	wheat	264
		barley	77
Total			2184

*All Wheat and barley samples were collected between January and May whereas corn and sorghum were collected between May and July 1987.

Estimation of incidence was done by applying this formula :

$$\text{Incidence \%} = \frac{\text{Number of infected samples}}{\text{Total Number of samples}} \times 100$$

Occurrence of barley yellow dwarf virus in wild grasses. A total of 240 leaf samples from grasses were sampled randomly from the Jordan valley. Samples were taken from the most common grasses in the study areas . Identification of these grasses was done by Dr Dawud Al-Eisawi.* These included *Alopecurus myosuroides* Hudson, *Avena sterilis* L., *Cynodon dactylon*(L), *Phalaris brachystachys* Link, *Lolium rigidum* Gaud, *Sorghum halepense* (L.) Pers., *Polypogon monspeliensis* (L.) Desf, *Hordeum Leporinum* Link. *Bromus rubens* L., *Eragrostis Cilianensis* (All.) Vign- Lut, and *Stipa capensis* Thunb. All samples were tested against antisera to PAV, MAV, RPV ,and RMV types of barley yelow dwarf virus, by ELISA.

Economic Importance. All commercial wheat and barley cultivars in Jordan were tested for their susceptibility, symptom severity, and yield reduction in response to infection by the PAV strain of barley yellow dwarf virus.

Nine cultivars of wheat including sham 1, Korifla, F. 8, Stork, Deir-Alla 2,

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* Dr. Dawud Al-Eisawi. Departemt of Biological Science. Faculty of Science. University of Jordan.

Acsad 65, Hourani, Deir-Alla 6, Deir-Alla 4, and four cultivars of barley including Deir-Alla 106, Rum, Line 1, and Acsad 176 were seeded in methyl bromide fumigated soil in 12 x 13 cm diameter plastic pots on the 3rd of December 1987. A randomized complete block design with three replicates was used. In each replication, four plants from each cultivar were grown under glasshouse conditions at 20±2 ^{o}C, half of them were inoculated by PAV using the aphid *R. padi* at the third leaf stage. The aphids were then killed with Cypermethrin. The remaining uninoculated plants were infested by nonvirul-iferouus aphids acted as control. plants were examined daily for symptom expression for a period of 35 days after inoculation.

Plant height*, severity of symptom , and grain yield were recorded for individual plants of each cultivar. The paired comparison test was done to study the effect of disease on plant height and grain yield within each cultivar. Duncan's Multiple Range Test was used to evaluate the reduction in plant height and grain yield among the wheat and barley cultivars under study.

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* Plant height = Distance from base of the plant to the top of the main culm

Results

Virus isolation. *M. avenae* transmitted an isolate that gave mild symptoms which could not be transmitted by *R. padi*; this will be referred to hereafter as (MAV), whereas *R. padi* transmitted another isolate that gave severe symptoms and was also transmitted by *M. avenae*. It will be referred to hereafter as (PAV). Since PAV is the strain which cause severe disease as described by others(Rochow, 1969;1970), it had been maintained for further studies . Occurrence of other mild strains as (RMV) and (RPV), was either recorded by serology or preferential aphid transmission. *R. maidis* transmitted RMV isolate, whereas *R. Padi* transmitted RPV isolate .

Host range and symptomology. Host range of the barley yellow dwarf virus is restricted to gramineae. All plants showed inital symptoms 7 - 26 days after inoculation by *R. Padi* vector. Symptoms vary with the crop as follows :

1) Barley (*Hordeum vulgare* L.).

Within 12-15 days following inoculation , leaves start to turn yellow usually at the tips . The yellowing progress downward , at first along the leaf margins . After that, the color of leaves developed a golden yellow color.

Infected plants are generally stunted , and the size of spike is reduced compared to that of the control plants.

2) Wheat (Triticum aestivum L.)

The first sign of infection could be observed 3 weeks after inoculation as a darker than normal green color of the older leaves with serrated edges and little redding of the lower leaves. Tillering of infected plants is suppressed , the plants are stunted, the size of spike is reduced , and the kernel is shrivelled.

3) Oats (*Avena sativa* L.)

The first symptoms of the disease appeared at the tip of the leaf 7-10 days after inoculation as reddish yellow color which will eventually cover the entire leaf. Infected plants are dwarfed and failed to head.

4) Corn (*Zea mays* L.)

Symptoms of BYDV in corn were slow to develop , appearing first in the lower mature leaves 25 days after inoculation . The lower leaves show yellowing to purpling with consequent drying up of their tips and margins . Stunting was observed and the size of the ear is less than the normal .

5) Sorghum (*Sorghum vulgare* Pers) .

Leaves of inoculated plants turn yellow to purple , the discoloration

begins at the tips and edges and progress down the leaf. The infected plants are also stunted .

Efficiency of transmission by aphid. The transmission efficiency of BYDV- PAV strain by four species of aphids was determined. Results (Table 2) indicated that *R. Padi* was the most efficient vector for PAV strain and *M. avenae* was the second most efficient vector, followed by *S. graminum. R. Maidis* did not transmit PAV strain to any of the tested plants.

Transmission through membrane. When the insect vector *R. Padi* was fed on inocula prepared by macerating 1gm PAV infected tissue in 1mL of o.1 M phosphate buffer (pH7), containing 10% Sucrose,it was able to transmit the virus with 100% efficiency . This corroborate with the results of ELISA for these samples (Table 3).

Virus strain and Vector prevalence. Three species of aphid population on bait plants (Clintland 64), scattered near grain crops, appeared suddenly in the second week of February. Population of *R. padi* and *S. graminum* reached high levels during the Ist and 4th weeks of April,then aphid population decreased in the first week of May , whereas the population of *M. avenae* showed slight increased in the first and third week of March (Fig 1).

Table 2. Transmission efficiency of PAV strain of barley yellow dwarf virus by four aphid species.

Aphid species	Number of infected plants / number of inoculated plants	% of virus infection
R. Padi	15/16	94%
M. avenae	10/16	62%
S. graminum	3/16	19%
R. maidis	0/16	0
control	0/16	0

Table 3. Testing cereal samples collected from the fields for barley yellow dwarf virus (BYDV) by enzyme-linked Immunosorbent assay(ELISA) and virus transmission through membrane with *R. Padi* .

Plant species	Number of samples	Number of infected samples using	
		ELISA	aphid transmission assay plants (*Avena sativa. cv.* Clintland 64)
Wheat	16	16	16
oat	2	2	2
barley	2	2	2
control	12	0	0

Fig 1 : Population of several species of aphids per plant collected from oat bait plants exposed one week at Dair-Alla during 1937.

The population of different aphid species including *R. Padi*, *S. graminum* , *M. avenae* and *R. Maidis* on bait plants was estimated to be 46.,36.8,16.7% and 0.4% of their total population, respectively (Table 4). Incidence of BYDV in bait plants did not correspond to the aphid population (Fig2). A total of 87% of the oat bait plants (Clintland 64) exposed during this period were found infected with BYDV , of which 55.1% were found mixedly infected with PAV and RPV, 2.6 % were of the RPV type, 3.1 % were of the PAV type , and 39.2 % were of the MAV (Table 5). RPV and PAV in mixedly infections were seemed to be predominant type .

Incidence of the disease. Incidence of BYDV in wheat in the Jordan valley during the growing season of 1987 varied from 11.5% in January to 73% in April. Of the total infected plants 90.8% were of the MAV type, and 8.6% were of the PAV type, and 0.6% were RPV type (Table 6). The total incidence of BYDV strains in barley in the Jordan valley varied from 20% in January to 58% in April. Of the total infected plants78.3% were of the MAV type, 11.7% were of the PAV type, and 1.7% were of the RPV type. The remaining samples were mixedly infected with RPV and PAV types (Table 7).

Incidence in the highlands was studied in Irbid, Karak, and Madaba

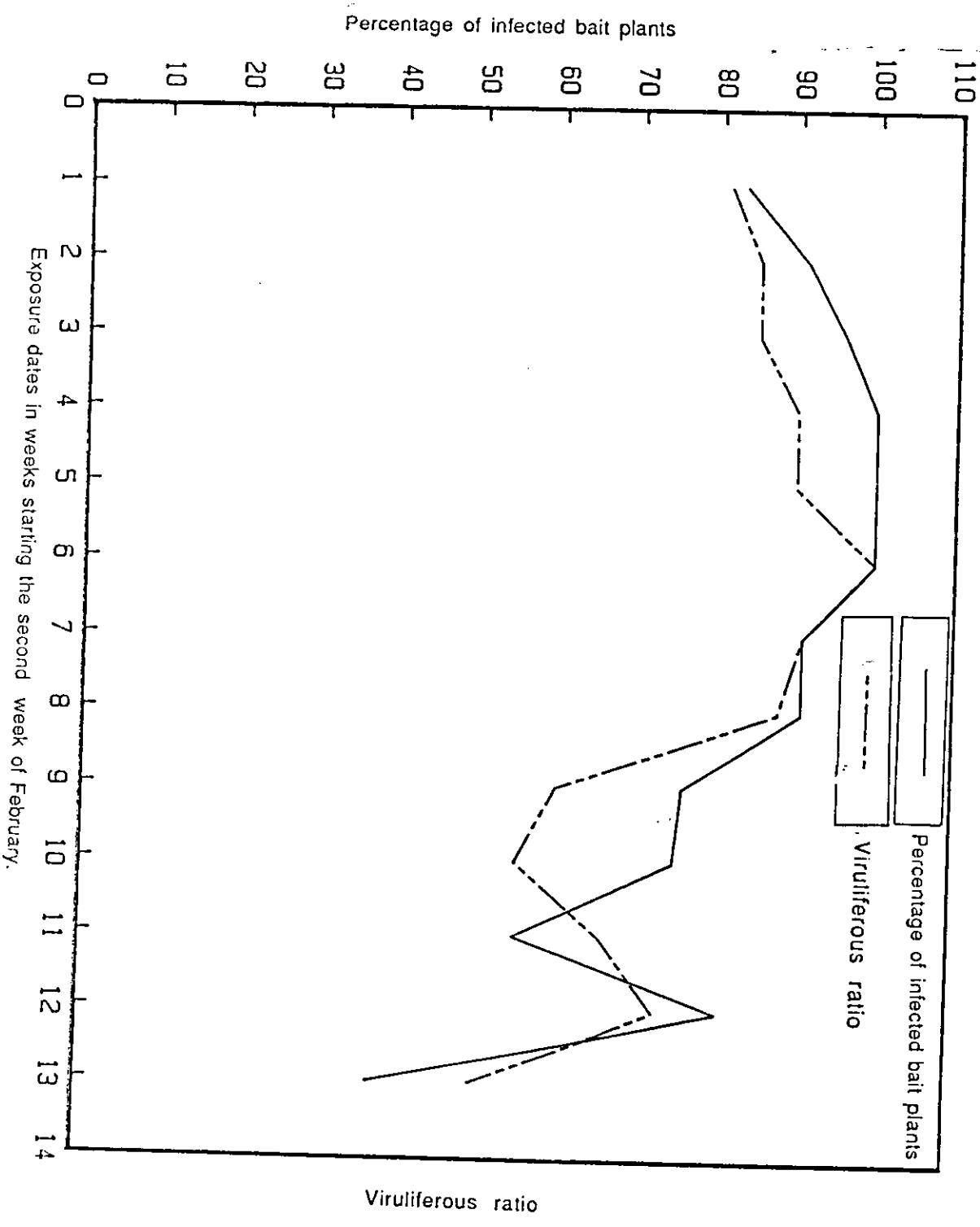

Fig 2 : Incidence of barley yellow dwarf virus and viruliferous ratio of aphid collected from oat bait plants exposed one week at Dair-Alla during 1987.

Table 4. Total number of aphids per plants collected from oat bait plants exposed for one week at Deir-Alla station during 1987.

Exposure date	*R.padi*	*S.graminum*	*M.avenea*	*R.maidis*	Total
February.					
9	38	12	6	0	56
16	39	9	11	1	60
23	35	3	12	0	50
March.					
2	10	5	19	1	35
9	6	11	5	0	22
16	2	0	23	0	25
23	25	3	6	0	34
31	6	10	9	1	26
April					
7	40	71	0	0	111
14	29	24	0	0	53
21	3	21	10	0	34
28	63	74	9	0	146
May					
5	13	4	2	0	19
Total	309	247	112	3	671
Percentage	46.5%	36.8%	16.7%	0.4%	

Table 5. Incidence of Isolates of barley yellow dwarf virus detected by ELISA* in the oat bait plants exposed for one week at Deir-Alla station during 1987 growing season.

Exposure date	number of plants	ELISA Positive for					virus infection
		MAV	PAV	RPV	PAV+RPV	Total+tve	(%)
February 9	24	20	0	0	0	20	83%
= 16	23	21	0	0	0	21	91%
= 23	24	23	0	0	0	23	96%
March 2	24	25	0	0	0	25	100%
= 9	22	0	0	0	22	22	100%
= 16	16	0	2	0	14	16	100%
= 23	23	0	0	0	21	21	91%
= 31	24	0	1	0	21	22	91%
April 7	25	0	1	0	18	19	76%
= 14	16	0	1	3	8	12	75%
= 21	9	0	0	2	3	5	55%
= 28	22	0	0	1	17	18	81%
May 5	8	0	2	0	1	3	37%
Total	260	89	7	6	125	227	87%
%		39,2%	3.1%	2.6%	55.1%		

* Two polyclonal antibodies (MAV and PAV) were used in the direct ELISA, and two monoclonal antisera PAV, and RPV in the indirect ELISA.

Table 6. Incidence of BYDV in wheat leaf samples from the Jordan valley in 1987.

Date of sample collection	total number of samples	ELISA positive for : MAV	PAV	RPV	Total	%
January	191	21	1	-	22	11.5%
February	95	26	3	-	29	30.5%
March	75	42	3	-	45	60%
April	108	70	8	1	79	73%
Total	469	159	15	1	175	37%
%		90.8%	8.6%	0.6%		

Monoclonal antisera (PAV, and RPV) were used in the indirect ELISA, and two polyclonal antibodies (F and B) were used in the direct ELISA.

**(-) Samples were not tested.

Table 7. Incidence of BYDV in barley leaf samples from the Jordan valley in 1987.

Date of sample collection	total number of samples	ELISA positive for : MAV	PAV	RPV	RPV and PAV	Total	%
January	60	12	0	-	-	12	20%
February	30	12	2	-	-	14	47%
March	30	14	2	-	-	16	53%
April	31	9	3	1	5	18	58%
Total	151	47	7	1	5	60	40%
%		78.3%	11.7%	1.7%	8.3%		

*Monoclonal antisera (PAV, and RPV) were used in the indirect ELISA, and two polyclonal antibodies (F and B) were used in the direct ELISA.

**(-) Samples were not tested.

areas. In Irbid, samples were collected from January to April during the growing season of 1987. The virus was not detected in January but started to build up slowly to 15% in April. Of the infected wheat samples 88.5% were of the MAV-type , and 11.5%of BYDV were of the PAV type (Table 8). In the barley fields the virus was not detected in January but start to build up to 40% in April. Of the infected barley samples 88.8% were of the MAV type, 11.2% were of the PAV type (Table 9).

In Karak, samples were collected from February to April. The percentage of BYDV ranged from 0% in February to 11.5% in April. And all infected samples were of the MAV type (Table 10).

In Madaba, samples were collected from February to April. The Percentage of BYDV ranged from 0% in February to 16% in April.Of the total samples 60% Were mixedly infected with RPV and PAV type ,whereas MAV and RPV were separately found in 20% (Table 11).

Incidence of BYDV in corn and sorghum during 1987 along the Jordan valley and Irbid area was determined . In the Jordan valley of 71 corn leaf samples collected, 48 (68%) samples were tested as positive for BYDV. The percentages of plants infected with PAV, RPV, and mixed Infection (PAV and

Table 8. Incidence of BYDV in wheat leaf samples from Irbid during 1987.

Date of sample collection	Total number of samples	ELISA positive for : PAV	MAV	TOTAL	%
January	75	0	0	0	0%
February	80	1	6	7	8.8%
March	80	2	5	7	8.8%
April	80	0	12	12	15%
Total	315	3	23	26	8%
%		11.5%	88.5%		

* Two polyclonal antibodies (F and B) were used in the direct ELISA

Table 9. Incidence of BYDV in barley leaf samples from Irbid during 1987.

Date of sample collection	Total number of samples	ELISA positive for : PAV	MAV	TOTAL	%
January	10	0	0	0	0%
February	15	0	3	3	20%
March	10	0	2	2	20%
April	10	1	3	4	40%
Total	45	1	8	9	20%
%		11.1%	88.9%		

* Two polyclonal antibodies (F and B) were used in the direct ELISA

Table 10. Incidence of BYDV in wheat leaf samples from Karak in 1987.

Date of sample collection	Total number of samples	ELISA positive for : PAV	MAV	TOTAL	%
February	100	0	0	0	0%
March	100	0	0	0	0%
April	87	0	10	10	11.5%
Total	287	0	10	10	3.5%
%		0%	100%		

*Two polyclonal antibodies (PAV and MAV) were used in the direct ELISA.

Table 11. Incidence of BYDV In wheat leaf Samples from Madaba in 1987.

Date of sample collection	Total number of samples	ELISA positive for : PAV	MAV	RPV	RPV and PAV	TOTAL	%
February	30	0	0	0	0	0	0%
March	35	0	1	0	0	1	2.9%
April	25	0	-	1	3	4	16%
Total	90	0	1	1	3	5	5.5%
%		0%	20%	20%	60%		

* Two monoclonal antisera (PAV and RPV) were used in the indirect ELISA, and one polyclonal antibodies MAV was used in the direct ELISA.

**(-) Samples were not tested.

RPV) were 35.4 % , 20.8% and 43.8% respectively (Table 12). In Irbid, of 14 samples collected, 7(50%) were tested as positive for BYDV(Table 12) and all infected samples were of the PAV type.

In addition, 47 sorghum samples were collected from the Jordan valley and tested for BYDV by ELISA. Thirty five (74%) samples were found positive . The percentages of plants infected with PAV,RPV and both (PAV and RPV) were 45.7%, 11.4% and 42.9% respectively (Table 13). In Irbid of 92 samples collected, 47 (51%) samples were positive for BYDV . The percentage of plants infected with PAV, RPV, and both (PAV and RPV) were 46.8% , 25.5%, 27.7%, respectivelly (Table 13).

Incidence of the disease in 1988. During the growing season of 1988 incidence of BYDV in wheat in the Jordan valley ranged from 1.4 % in January to 49% in April. 32-41, PAV MAV and RMV strains of BYDV comprised 46.7%, 10.7%, 5.3%, 5.3 of the total incidence of BYDV, respectively. The remaining samples were mixedly infected. (Table 14). In addition , incidence of BYDV in barley ranged from 0% in January to 80% in April. The percentage of plants infected with PAV, MAV , RMV and 32-41 strains of BYDV was 9.3% , 6.3%12. 5%, 31.3%, and 32% of the total infection, respectively . The remaining samples

Table12: Incidence of BYDV in Corn leaf samples from the Jordan valley and Irbid areas during growing season 1987.

Locations	Total number of samples	ELISA positive for : PAV	RPV	PAV and RPV	TOTAL	%
Jordan valley	71	17	10	21	48	68%
%		35.4%	20.8%	43.8%		
Irbid	14	7	0	0	7	50%
%		100%				
Total	85	24	10	21	55	65%

* Two monoclonal antisera (PAV and RPV) were used in the indirect ELISA.

Table13: Incidence of BYDV in sorghum leaf samples from the Jordan valley and Irbid areas during growing season 1987.

Locations	Total number of samples	ELISA positive for : PAV	RPV	PAV and RPV	TOTAL	%
Jordan valley	47	16	4	15	35	74%
%		45.7%	11.4%	42.9%		
Irbid	92	22	12	13	47	51%
%		46.8%	25.5%	27.7%		
Total	139	38	16	28	82	59%

* Two monoclonal antisera (PAV and RPV) were used in the indirect ELISA.

Table 14. Incidence of BYDV in wheat leaf samples from the Jordan valley during growing season 1988.

Date of sample Collection	Total number of samples	ELISA positive for : PAV	MAV	RMV	32-41	mixed infection**	TOTAL	%
January	70	0	1	0	0	0	1	1.4%
February	70	0	1	0	12	6	19	27%
March	69	6	1	4	10	7	28	40.5%
April	55	2	1	0	13	11	27	49%
Total	264	8	4	4	35	24	75	28%
%		10.7%	5.3%	5.3%	46.7%	32%		

* Four monoclonal antisera (PAV, MAV, RMV, 32-41) were used in the indirect ELISA.

** Two or three strains in the same plant.

were mixedly infected (Table 15).

Incidence in the highlands was studied in Irbid. The percentage of BYDV in wheat ranged from 0% in January to 36% in April of the year 1988. Of the infected samples 8%were identified as 32-41 type,31.3% were infected with the PAV type, 10.5% were infected with MAV type, and 13% were infected with RMV type. The remaining infected samples were mixedly infected with BYDV types. (Table 16).

The percentage of BYDV in barley ranged from 13.3% in January to 32% in April in 1988. Of the infected samples 46.7% were identified as 32-41 type, 13.3% were infected with the PAV type, 6.7% were infected with MAV type , and 13.3% were infected with RMV type. The remaining infected samples were mixedly infected with BYDV types. (Table 17). The seasonal incidence in the Jordan Valley of all BYDV isolates ranged from 37% to 74% in cultivated cereals. In the higlands the incidence ranged from 6%-51% in 1988, the seasonal incidence ranged from 28%-42% in the Jordan Valley and from 19%-22% in the highlands . BYDV was detected in 64% and 72% of the weeds tested in 1987 and 1988 ,respectively (Table 18).

Occurrence of BYDV in grasses. Occurrence of barley yellow dwarf virus

Table 15. Incidence of BYDV in barley leaf samples from the Jordan valley during growing season 1988.

Date of sample Collection	Total number of samples	ELISA positive for : PAV	MAV	RMV	32-41	mixed infection**	TOTAL	%
January	24	0	0	0	0	0	0	0%
February	18	0	2	-	4	2	8	44%
March	20	1	0	3	4	4	12	60%
April	15	2	0	1	2	7	12	80%
Total	77	3	2	4	10	13	32	42%
%		9.3%	6.3%	12.5%	31.3%	40.6%		

* Four monoclonal antisera (PAV, MAV, RMV, 32-41) were used in the indirect ELISA.

** Two or three strains in the same plant.

Table 16. Incidence of BYDV in wheat leaf samples from Irbid during growing season 1988.

Date of sample Collection	Total number of samples	ELISA positive for : PAV	MAV	RMV	32-41	mixed infection**	TOTAL	%
January	45	0	0	0	0	0	0	0%
February	45	1	1	2	1	0	5	11%
March	45	2	2	2	1	4	11	24%
April	60	9	1	1	1	10	22	36%
Total	195	12	4	5	3	14	38	19%
%		31.5%	10.5%	13%	8%	37%		

* Four monoclonal antisera (PAV, MAV, RMV, 32-41) were used in the indirect ELISA.

** Two or three strains in the same plant.

Table 17. Incidence of BYDV in barley leaf samples from Irbid during growing season 1988.

Date of sample Collection	Total number of samples	ELISA positive for : PAV	MAV	RMV	32-41	mixed infection**	TOTAL	%
January	15	0	1	1	0	0	2	13.3%
February	15	0	0	0	2	0	2	13.3%
March	15	1	0	0	3	0	4	26%
April	22	1	0	1	2	3	7	32.9%
Total	67	2	1	2	7	3	15	22%
%		13.3%	6.7%	13.3%	46.7%	20%		

* Four monoclonal antisera (PAV, MAV, RMV, 32-41) were used in the indirect ELISA.

** Two or three strains in the same plant.

Table18. seasonal incidence of barley yellow dwarf virus in Jordan in 1987 and 1988.

		Average seasonal incidence	
year	Crop	Jordan valley	Higlands
1987	Wheat	37%	6%
	Barley	40%	20%
	Corn	68%	50%
	Sorghum	74%	51%
	Grasses	64%	-
1988	Wheat	28%	19%
	Barley	42%	22%
	Grasses	72%	-

in different species of grasses was determined by serological testing using direct and indirect ELISA as described elsewhere in the text. Of 168 samples-tested, 108 reacted positively for BYDV. In general all tested grasses were found infected with BYDV at a ratio ranging from 38% to 75%. MAV types seemed to be the dominant type followed by RPV and PAV separately or mixed . Moreover, *Lolium rigidum, Avena sterilis, Polypogon monspeliensis* showed higher incidence of infections than the others grasses during 1987. Mixedly infections of virus strains occurred in 29.6% of infected samples (Table 19).

During the growing season of 1988, 72 samples of grasses from different species were collected. 72% of these samples reacted positively for BYDV using ELISA tests. RPV and PAV mixed infection seemed to be the dominant type. *Alopecurus myosuroides, Bromus rubens, and P. monspeliensis* showed higher incidence of infections than the others(Table 20). Incidence of BYDV in early season (February -March) was 68.6% whereas in late season (April-May) was 50% (Table 21) .

Economic Importance. Symptom expression of wheat barley and oats cultivars, rated according to Watson and Mulligan severity index (I960), are presented in (Table 22). All inoculated plants developed symptoms typical of

Table 19. Barley yellow dwarf virus (BYDV) infection detected by ELISA in grass species in 1987

Grass species	Common name	Number Plants tested	Number of Plants Infected with Isolate type					
			* MAV	PAV**	RPV**	PAV+ RPV	Total Positive	%
Alopecurus myosuroides	Littoral Grass	10	3	0	0	3	6	60 %
Avena sterilis	Wild oat	39	16	4	4	5	29	74%
Bromus rubens	Opened-awned Brome grass	4	2	0	0	0	2	50%
Cynodon dactylcn	Bermuda Grass	16	5	1	0	3	9	56%
Eragrostis Cilianensis	Spreading Love-Grass	13	5	0	0	0	5	38%
Hordeum Leporinum	Wild barley	2	1	0	0	0	1	50%..
Lolium rigidum.	Rigid Rye - Grass	26	13	1	2	3	19	73%
Phalaris brachystachys	Short-Spiked canaary Grass	39	14	0	0	11	25	64%
Polypogon monspeliensis	Annual Beardgrass	4	1	0	0	2	3	75 %
Sorghum halopense	Johnson Grass	15	1	1	2	5	9	60%
Total		168	61	7	8	32	108	64%
%			56.5%	6.5%	7.4%	29.6%	–	–

* Two Polyclonal antibodies (F and B) were used in the direct ELISA.

** Two monoclonal antisera (PAV, and RPV) used in the indirect ELISA .

Table 20 Barley yellow dwarf virus (BYDV) infections detected by (ELISA) in grass species in 1988.

Grass species	Common name	Number of plants infected with Isolate type							
		Number * Plants tested	32-41	RMV	PAV	MAV	Mixedly	Total Positive of BYDV	%
Alopecurus myosuroides	Littoral Grass	5	1	0	0	0	4	5	100
Avena sterilis	Wild oat	17	2	0	0	1	10	13	76
Bromus rubens	Opened-awned Brome grass	3	0	0	1	0	2	3	100
Cynodon dactylon	Bermuda Grass	3	0	0	0	0	2	2	67
Hordeum Leporinum	Wild barley	9	1	0	1	0	4	6	67
Lolium rigidum	Rigid Rye - Grass	9	1	0	1	1	4	7	78
Phalaris brachystachys	Short-Spiked Canary Grass	12	1	1	0	0	4	6	50
Polypogon monspeliensis	A nnual Beardgrass	9	0	1	1	0	6	8	89
Sorghum halepense	Johnson Grass	4	0	2	0	0	0	2	50
Stipa capensis	Twisted - awned spear Grass	1	0	0	0	0	0	1	100%
Total		72	6	4	4	2	36	52	72 %

Table 21. Occurrence of barlay yellow dwarf virus in grasses in the Jordan valley.

Grass species	Early season February-March	Late season April-May
Alopecurus myosuroides	3/6	3/4
Avenae sterilis.	21/25	8/14
Bromus rubens.	1/2	1/2
Cynodon dactylon.	5/7	4/9
Hordeum Leporinum	-	1/2
Eragrostis cilianensis	5/13	-
Lolium rigidum.	16/20	3/16
Phalaris brachystachys.	17/26	8/13
Polypogon monspellliensis	1/2	2/2
Sorghum halepense.	1/1	8/14
Total	70/102	38/76
%	68.6%	50%

Table 22. Severity of symptoms in wheat and barley cultivars incoulated with the PAV strain , and grown in the glasshouse.

	Cultivars Wheat	sympton Severity Rating *	Description
1	Sham 1	6	More extensive bright yellowing of leaves; moderate to poor plant vigor; dwarfing.
* 2	Korifla	5	Moderate to somewhat extensive yellowing of leaves; reduction in height.
* 3	F. 8	2	Trace amounts of yellowing of leaves; no sign of dwarfing; leaves became purple.
4	Stork	3	Moderate to low amount of yellowing ; no sign of dwarfing.
* 5	Deir Alla 2	3	Moderate amount of yellowing ; moderate plants vigor.
6	Acsad 65	3	Moderate amount of yellowing of leaves; moderate to good plant vigor.
7	Hourani	1	Low amount of yellowing of the tips of leaves; vigorous plant appearance.
8	Deir Alla 6	5	Moderate to extensive yellowing of the leaves; poor plants vigor
* 9	Deir Alla 4	5	Moderate to extensive yellowing of the leaves; reduced spike size; reduction in height.
	Barley		
1	A csad 65	3	Moderate of yellowing leaves; moderate plants vigor.
2	Line 1	2	Moderate of yellowing leaves; no sign of dwarfing ; vigorous plant appearcnce.
3	Deir Alla 106	4	High level of yellowing ; moderate plants vigor; reduced spike size.
4	Rum	5	Severe yellowing , small spike; moderate to poor plant vigor.
	Control plants	0	No visible symptom .
	Oat (Clintland 64)	7	Reddish to purple coloration; dwarfing ; no spikes.

* Visual score - a o to 7 rating of the overall symptom expression , a scale by Watson and Malligan 1960.

barley yellow dwarf virus. These symptoms varies from discoloration (yellowing or reddening) and dwarfing to very limited visual symptoms.

Grain yield reduction caused by BYDV in wheat cultivars ranged from 41.16% in Hourani to 75.26% in Deir-Alla 2. Reduction in the yield is not significantly different among all cultivars except for Hourani which exhibited the lowest yield reduction (Table 23) .

Effect of BYDV on plant height of wheat cultivar, is presented in (Table 23). There is no significant reduction in plant height among sham I, stork, Deir-Alla 2 , Deir-Alla 6 , and Deir- Alla 4 .Sham I, Deir-Alla 6 , and Deir Alla 4 ,attained the highest height reduction of 25.46% , 25.50%, 25.56%, respectively.

Acsad 65 and F.8 showed less reduction in plant height of 8.43%,9.83 of that in the control, respectively. Reduction of plant height of Korifla and Hourani was intermediate reaching 13.86% and 13.16% of that in healthy control.

Barley

Grain yield reduction caused by BYDV in barley cultivars ranged from 40.6% in Acsad to 72% in Rum As compared to that of the control. There is no significant difference among line I, Deir-Alla 106,and Rum. Yield reduction of

Table 23. Percent of reduction in grain yield and plant height of wheat cultivars in response to BYDV infeceion.

Wheat Cultivars	percent reduction in plant height/plant	Percent reduction in yield/plant.
Sham1	25.46 a	57.5 a b
Korifla	13.86 b c	63.06 a
F.8	9.83 c	67.13 a
Stork	17.5 a b c	56.96 a b
Deir-Alla 2	21.73 a b	75.26 a
Acsad 65	8.43 c	62. a b
Hourani	13.16 b c	41.16 b
Deir Alla 6	25.5 a	56.93 a b
Deir Alla 4	25.56 a	73.56 a

* Means followed by the same letter are not significantly different at the 5% level. Means separation is by Dancan's Multiple Range Test.

Acsad was significantly less than that of any of the remaining cultivars (Table 24).Effect of BYDV on plant height of barley cultivars is presented in (Table 24). Plant height reduction of barley cultivars ranged from 24.56% in Rum to 3l.60% in Acsad with no significant difference among the tested cultivars.

Table 24. percent of reduction in grain yield of barley cultivars in response to BYDV infection.

Barley Cultivar	Percent reduction in plant height/plant	Percent reduction in yield/ plant.
Acsad	31.6 a	40.66 b
Line 1	25.03a	65.73a
Deir Alla 106	25.06a	70.2 a
Rum	24.56a	72. a

* Means followed by the same letter are not significantly different at the 5% level. Means separation is by Dancan's Multiple Range Test.

DISCUSSION

Based on the host range , insect transmission tests , and serology (Enzyme - Linked immanosorbent assay) , four isolates of barley yellow dwarf virus were identified as PAV , MAV , RPV , and RMV. These isolates occurred separately or in mixed infection in the plants. Incidence of the virus ranged from 37% in wheat to 74% in sorghum in 1987. In 1988 , however, the incidence ranged from 28% in wheat to 72% in grasses. It is noted that the seasonal incidence of the virus in the Jordan Valley is higher than that in the highlands (Table 18). This may be due to either high inoculum sources or and to high aphid population.

Prevalence of different strains of BYDV in all cultivated cereal crops were 35.5% , 24% , 7.6% and 2.1% for MAV , PAV, RPV and RMV, respectively (Table 27). Infection with two strains in cereal crops is more frequent (18.5%) than those with three strains (4.7%) (Table 25) . The results indicated that PAV and MAV are the predominant strains under the Jordanian conditions. MAV and PAV are reported to cause mild and severe symptom, respectively (Rochow , 1969;1970). Mixed infection has critical impact on the disease response of infected plants. Synergistic effect manifested as severe symptoms had been

(Table 25). Prevalence of barley yellow dwarf virus isolates in infected cereal crops and grasses collected from different areas in Jordan during 1987 and 1988.

HOST	Percentage of Plants infected with isolate type													
	Single infection%				Mixed Infection%									
	MAV	PAV	RPV	RMV	32-41	RPV and PAV	MAV and RMV	PAV and RMV	PAV and 32-41	MAV and 32-41	RMV and 32-41	MAV and PAV	MAV,PAV and 32-41	PAV,MAV and RMV	PAV , RMV and 32-41
Cultivated Cereal Crops	35.5	24	7.6	2.1	7.6	15.3	0.7	0.9	0.1	0.5	0.5	0.4	2	1.2	1.5
Grass	39	7	5	2.3	4	20	1.2	2	5	0.6	-	-	0.6	8	5.5

* Infection with one strain in the same plant.(76.8%)

** Infection with two strain in the same plant.(18.5%)

*** Infection with three strain in the same plant.(4.7%)

reported where RPV and PAV isolates occur in the same plant as it is illustrated in (Table 5). This agrees with the findings of others (Aapola and Rochow , 1971). Infection with RPV resulted in mild symptoms. This strain also cross protected the plants from the challenging severe strain (Aapola and Rochow, 1971).

All BYDV isolates in Jordan have similar host range , but differ in symptom severity on oat indicator plants, Clintland 64. This agrees with the findings of others (Rochow , 1969 , 1970). However the major distinguishing criterion is the transmission by specific vectors. RPV , RMV , and MAV are transmitted specifically by the respective vectors *R.padi* , *R. maidis* , and *M. avenae,* whereas PAV is transmitted non specifically by *R.paid* , *M.. avenae* , and *S. graminum*.These results are in agreement with those found by others (Johson and Rochow , 1972 ; Rochow , 1969 ; 1979) . It is speculated that specificity in transmission of circulative Luteovirus by aphids is regulated by interaction between virus capsid protein and specific receptor sites in accessory salivary glands of the vector (Gildow , 1982; Gildow and Rochow , 1980).

Results in (Table 2) indicated that the vectors of BYDV including *R. padi* , *M. avenae* , *R. maidis* , and *S. graminum* were trapped on oat plants , with

R. padi being the most efficient in transmitting PAV strain than *M. avenae*. The fact that *R. padi* transmitted the virus (PAV) from all samples tested positive by ELISA for PAV and RPV substaintiated the validty of ELISA as tool for detecting BYDV in infected tissue .

BYDV was common and wide spread in grasses throughout the Jordan Valley and elsewhere in Jordan. The incidence of BYDV ranged from 64% to 74% in grasses during a survey in 1987 and 1988 (Tables 19 and 20) . Such high incidence of infected grass pointed to high inoculum potential that may assume , in the presence of insect vector an important role in the epidemiology of BYDV in cereal crops. In addition to grasses , corn and sorghum (Tables12 and13) in the late fall in the Jordan Valley are seemingly , important virus source for wheat and barley that overlapped with these crops .

In consideratiion of the fact that wheat, barley, corn, sorghum and grasses especially perennial grasses like (Johnson grass and Bermuda grass) showed various levels of BYDV infection and aphid vectors. These hosts constitute links in the disease cycle of the virus. The virus may cycle from wheat which act as an overwintering to both corn , sorghum and grasses which act as oversummering in Jordan. Moreover the perennial grasses are expected to

provide primary inoculum of BYDV infection for early planted cereal crops.

High incidence of BYDV on the bait plants that were exposed in early season (Fig 2) in presence of relatively low aphid population , is probably due to high viruliferous ratio (Fig 2) . It is likely that the high viruliferous ratio is attributed to high inoculum potential of the virus as represented in infected grasses (Table 21). The low incidence of BYDV on the bait plants that were exposed late in the season is probably due to relatively low viruliferous ratio. This could be expected only if cultivated cereal crops infected early in the season did not constitute improtant element in the inoculum potential which in turn may be expected if aphids colonize the cultivated cereal crops and tend not to migrate to other cereal fields. In this case infected grasses are expected to be the more important inoculum source. This may be supported by the fact that grasses usually are either perennial or germinate before planting cereal crops under the Jordan valley conditions. These grasses are expected to be exposed to water stress which may induce the aphids to migrate to nearby irrigated to cultivated cereal crops.

BYDV is economically important on cereal crops, since it reduces the grain yield by 41.16% to 75% in wheat (Table 23) and by 40.6% to 72% in barley

(Table 25). However, some wheat cultivars are more tolerant to the virus such as Hourani, whereas the most tolerant barley cultivar was Acsad 176.

In conclusion four strains of BYDV occur separately or in mixed infection in cultivated and noncultivated cereals in Jordan. Moreover grasses seems to constitute major inoculum source of the virus which in presence of the vector may result in relatively high disease incidence in cultivated cereal crops .

SUMMARY

"OCCURRENCE OF BARLEY YELLOW DWARF VIRUS (BYDV) ON WHEAT IN JORDAN"

A study was initiated in 1987 to isolate and identify different strains of barley yellow dwarf virus in Jordan. Moreover, studies on the incidence, economical importance and epidemiology of the disease were carried out. Strains of barley yellow dwarf virus including : MAV, PAV, RPV and RMV occurred separately or in mixed infection in the plants were identified on the basis of host range, symptomology, insect transmission and serology.

Incidence of barley yellow virus reached 22% , 35% , 65% , and 59% , in wheat , barley, corn and sorghum, respectively . The virus was recovered from all tested grasses . Grasses are seemingly the more critical virus source which in presence of *R. midis, R. Padi, M . avenae*, and S. *graminun* aphid vectors may lead to high seasonal incidence of the disease .

BYDV reduced the plant height and grain yield in both wheat and barley . The Hourani cultivar is more tolerant to the virus than other tested wheat cultivars, whereas Acsad 176 showed more tolerance than other barley cultivars .

ملخص

فيروس إصفرار وتقزم الشعير على القمح

أجريت دراسات على فيروس أصفرار وتقزم الشعير لمعرفة السلالات المختلفة لهذا الفيروس ، ومدى الاصابة بكل منها ووبائية الفيروس ، والاضرار الأقتصادية التي تسبب في احداثها .وقد ظهر الفيروس في أربع سلالات هي MAV, PAV, RMV, RPV تصيب النبات بصورة مفرده أو مجتمعه ، وقد تم تعريف هذه السلالات بناء على دراسة المدى العائلي التي تصيبها كل سلالة ، والأعراض المرضية الناشئة عن الاصابة بها ونوع المن ، الناقل لها والفحص السيرولوجي .

كما تبين ان نسبة ٢٢٪ ، ٣٤٪ ، ٦٥٪ ، ٥٩٪ من العينات التي جمعت من كل من محاصيل القمح ، والشعير ، والذرة الحلوة ، وذرة المكانس على الترتيب كانت مصابة بالفيروس . هذا وقد عزل الفيروس من جميع انواع الاعشاب التي جمعت ، الامر الذي يؤكد اعتبارها مصدراً للعدوى وذلك لوجود كل من الحشرات خاصة وأن انواع المن المختلفة *R. padi, R. maidis. M ;avenae , and S. graminum* كانت قد جمعت عنها . وتؤثر الاصابة بالفيروس على كمية الانتاج وطول نبات كل من محصولي القمح والشعير ، غير ان الصنف حوراني كان أعلى اصناف القمح المدروسة تحملاً لهذا الفيروس، بنيما كان الصنف أكساد ١٧٦ اكثر تحملاً من بقية اصناف الشعير المدروسة.

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Appendix 1)Buffer formulations used in ELISA tests.

phosphate Buffer Saline - tween formulations (PBS-T,pH7.4) .

Nacl	8.0 g.
KH_2PO_4	0.2 g.
$Na_2HPO_4 .12 H_2o$	2.9 g.
Kcl	0.2 g.
NaN_3	0.2 g.
Tween 20	0.5 ml.

Disolve it in distilled water to 1000 ml.
This buffer was used for washing ELISA plates .

Coating Buffer formulations. (pH 9.6)

$Na_2 CO_3$	1.59 g.
$NaHCO_3$	2.93 g.
NaN_3	0.2 g.

Disolve it in distilled water to 1000 ml.
Coating buffer was used to dilute gamma-globulin for coating ELISA plates .

Conjugate Buffer formulations (pH , 7.4)

NaCl	8.0 g.
KH_2PO_4	0.2 g.
$Na_2HPo_4. 12H_2O$	2.9 g.
KCl	0.2 g.
NaN_3	0.2 g.
Tween 20	0.5 ml.
polyvinylpyrrolidone	20. g.
Ovalbumin	2.0 g.

Disolve it in distilled water to 1000 ml.
Conjugate buffer was used for diluted two enzyme - labilled gamma globulin .

Substrate Buffer formulations (pH , 9.8)

Diethanolamine	97 ml.
Distilled Water	800 ml.
NaN_3	0.2 g

Adjust PH to 9.8 with concentrated Hcl. make up to 1 liter with distilled water.

Substrate buffer was used for diluted the P.nitrophenylphosphate.

Antigen (Extraction) Buffer formulations (pH 7.4).

Nacl	8.0 g
KH_2PO_4	02 g
$Na_2HPO_4.12H_2O$	2.9 g
Kcl	0.2 g
NaN_3	0.2 g
Tween 20	0.5 ml.
PVP	20 g

This buffer was used for grinding tissue for direct ELISA.

PEP Buffer formulations

PBS - Tween	1200 ml.
PolyvinylPyrrolidone	24 g
Egg albamin	2.4 g

PEP buffer was used for Grinding tissue for indirect ELISA.

APPendiX 2. Occurrence of Viruliferous aphids collected from the oat bait plants (C.64) at Deir - Alla area in 1987.

Exposure	Total	Number of Viruliferous	%
Fabruary 9	11	9	81%
16	13	11	85%
23	13	11	85%
March 2	10	9	90%
9	10	9	90%
16	10	10	100%
23	11	10	91%
31	16	14	88%
April 7	10	6	60%
14	9	5	55%
21	9	6	66%
28	11	8	73%
May 5	10	5	50%