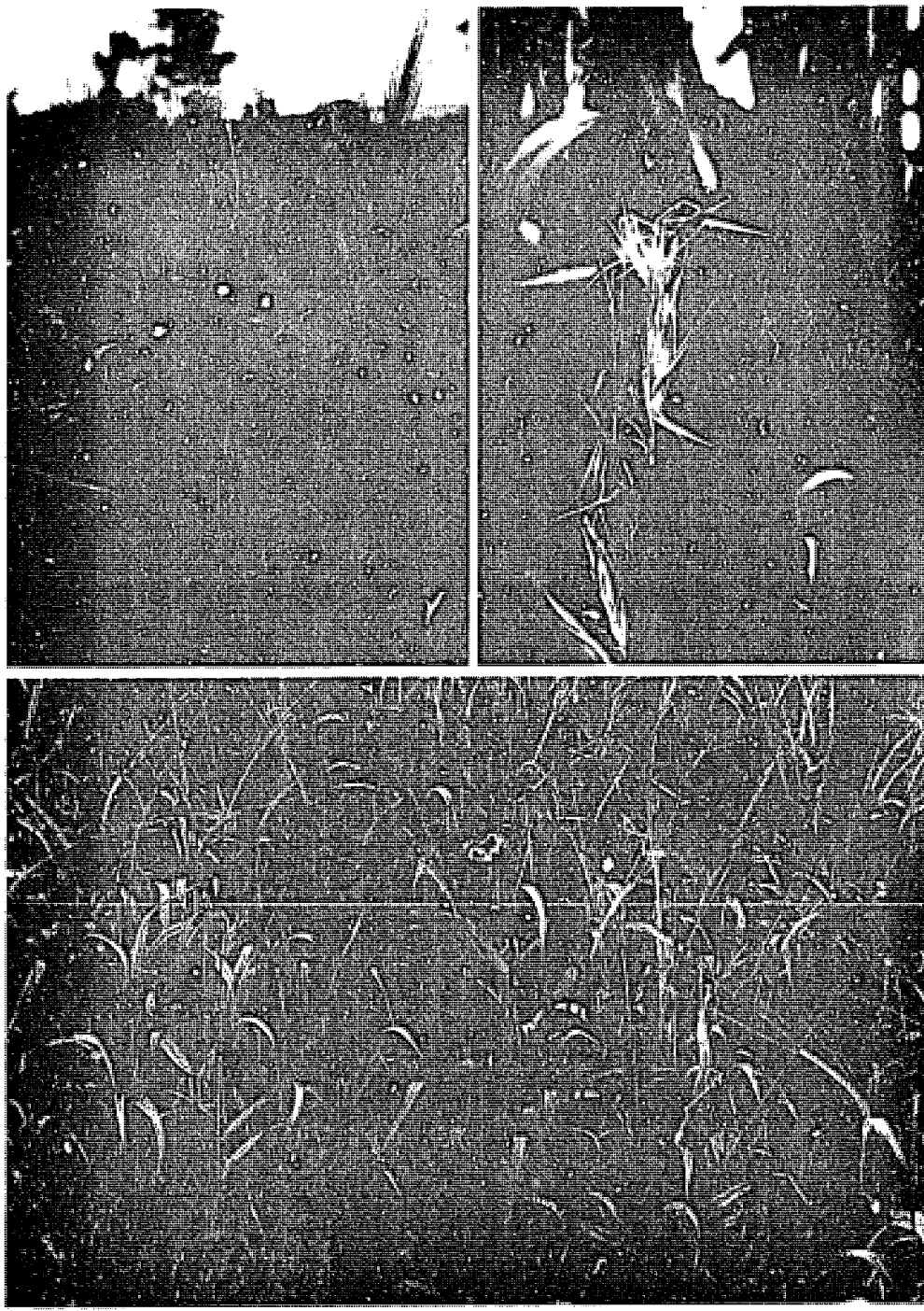

Clockwise from upper left: Barley yellow dwarf in wheat, oats, and barley (Photos by P.A. Burnett).

PN-ABF-323

ISN 66634

World Perspectives on Barley Yellow Dwarf

Proceedings of the International Workshop
July 6-11, 1987
Udine, Italy

P.A. Burnett, technical editor

Sponsored by:
Dipartimento Cooperazione Allo Sviluppo
International Maize and Wheat Improvement Center

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CIMMYT receives core support through the CGIAR from a number of sources, including the international aid agencies of Australia, Austria, Brazil, Canada, China, Denmark, the Federal Republic of Germany, Finland, France, India, Ireland, Italy, Japan, Mexico, the Netherlands, Norway, the Philippines, Spain, Switzerland, the United Kingdom, and the USA, and from the European Economic Commission, Ford Foundation, Inter-American Development Bank, OPEC Fund for International Development, UNDP, and World Bank. CIMMYT also receives non-CGIAR extra-core support from Belgium, the International Development Research Centre, the Rockefeller Foundation, and many of the core donors listed above.

Responsibility for this publication rests solely with CIMMYT.

Correct Citation: Burnett, P.A., ed. 1990. World Perspectives on Barley Yellow Dwarf. CIMMYT, Mexico, D.F., Mexico.

ISBN 968-6127-39-9

On the cover: Typical barley yellow dwarf infection in a farmer's field in Ecuador. (Photo by Hugo Vivar)

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Preface

P.A. Burnett Wheat Program, CIMMYT, Mexico

What is Barley Yellow Dwarf?

Barley yellow dwarf (BYD) is a cereal disease caused by an aphid-transmitted virus belonging to the luteovirus group, members of which cause the yellows diseases. The virus, known as barley yellow dwarf virus (BYDV), is actually a group of closely related viruses. BYDV is persistently transmitted, meaning that once an aphid acquires the virus, it will transmit it for life. The virus is not known to multiply in the insects; newborn nymphs (young aphids) are virus-free and acquire BYDV by feeding on infected plants.

BYD has been recorded in most areas of the world and its distribution is documented in these proceedings. Losses due to the disease vary, ranging from 1% to 3% annually, though in some years and some locations, losses may be as high as 20-30%. In some cases, crops have been totally destroyed.

Because its presence is often masked by other diseases, such as the rusts or other foliar diseases, the effects of BYD often become much more apparent once resistance to these diseases has been achieved. The virus partially plugs the phloem, interfering with translocation. Infection can severely stunt plants, inhibit root formation, delay heading, and reduce yield. Disease symptoms vary depending on the crop species or cultivar affected. On oats (*Avena sativa* L.) and some wheats (*Triticum* spp.), the leaves of diseased plants show a yellowing or reddening, but often the symptoms of BYD in bread wheat (*T. aestivum*), durum wheat (*T. turgidum* var. durum), and triticale (X *Triticosecale*) are not particularly apparent, and even experienced cereal workers may have difficulty recognizing them.

Formerly, the only practical method for diagnosing BYD was by aphid transmissions to indicator plants with the resulting development of typical disease symptoms. With the development of the enzyme-linked immunosorbent assay (ELISA), an alternative method of diagnosing the disease became available. In many parts of the world, however, researchers do not have the facilities to carry out either aphid transfers or ELISA, and BYDV must still be diagnosed on the basis of symptoms. One of the disquieting facts emerging from recent reports is the number of asymptomatic BYDV-infected cereal plants that are being discovered. However, in most places where BYD-resistant materials are selected, selection is being done to some extent on symptoms. This area of research requires a great deal of additional study.

The United Nations Development Programme (UNDP) sponsored a BYD workshop at CIMMYT December 6-8, 1983. The publication of the proceedings of this workshop in 1984 provided a benchmark of the disease's status.

In 1985 the Dipartimento Cooperazione Allo Sviluppo (DCAS) of the Republic of Italy awarded a technical assistance grant to CIMMYT to develop and implement an integrated pest management program for the control of BYDV.

This project aims to reduce losses caused by this virus disease by supporting the transfer of technology from developed country institutions (including Italian institutions directly funded by the grant) to developing countries, via CIMMYT. Since BYD is both significant and widespread in its distribution, the development of resistant germplasm could increase cereal production in developing and developed countries by decreasing the losses it currently causes.

The project's overall goal is to establish an international network of researchers familiar with BYDV, in which Italian institutions will play a role. The network aims to strengthen the development and exchange of germplasm and research procedures needed to achieve effective control of BYDV, especially in developing countries.

To successfully transfer the technology developed by this project to developing nations, a number of interrelated and complementary activities are envisioned:

1) Establish and/or strengthen institutional relationships among developed and developing country research institutions;

2) Offer training opportunities for national program scientists in developing countries where BYDV causes significant economic losses in production;

3) Screen germplasm extensively and hybridize to develop BYDV-resistant/tolerant germplasm, and establishing a delivery mechanism to get this germplasm to national program collaborators.

4) Study the epidemiology of BYDV and its aphid vectors.

To this end, programs on BYDV research have been or are being supported in Argentina, Chile, China, Ecuador, Kenya, and Zimbabwe. The aim has been to assist programs with partial, not total, funding of a project.

Two scientists, one in the U.S. and one in England, have been supported for graduate studies. Two masters candidates at Chapingo University, Mexico, have been involved with the program. Six participants in the CIMMYT crop improvement training course (breeding and pathology) have been supported. In addition, some other short-term training has been carried out.

In February 1986, associate scientist Monica Mezzalama joined the project to work mainly on ELISA and aphid trapping.

During the third year of the project, an international workshop was held July 6-11, 1987 in Udine, Italy. The funding for many of the participants attending this workshop came from the project. These proceedings are a collection of the papers and posters presented. As shown in the table of contents, these presentations have been divided into four sections-1) world situation, 2) virology, 3) ecology and epidemiology, and 4) control.

Dedications

These proceedings are dedicated to the following researchers, all of whom have made considerable contributions to the study and understanding of barley yellow dwarf virus:

The late Dr. Henryk Jedlinski
Dr. Clifford C. Gill
Dr. William F. Rochow
Dr. Charles W. Schaller

The late Dr. Henryk (Henry) Jedlinski

Henry was born in Bialystok, Poland, 15 February 1924. He died of cardiac arrest 4 April 1987. After beginning studies in Germany, he emmigrated to the USA and obtained his degrees from the University of Nebraska. He was appointed research plant pathologist with the USDA in November 1958; in 1959 he became associated with the Department of Plant Pathology at the University of Illinois at Urbana/Champaign. He later became associate professor of plant pathology.

Henry was a recognized authority on BYDV. With his colleague, C.M. Brown, a cereal breeder at the University of Illinois, he incorporated BYD resistance into oats and more recently wheat. He monitored the epidemiology of rusts, other airborne fungi, and foot and root rot fungi of cereals for interaction with BYDV. Henry had a continuous interest in the aphid vectors of BYDV and their specificity. His findings were fundamental to describing biotype variation in BYDV and have contributed to our understanding of vector, cross protection, and synergism among the BYDV isolates in nature.

Henry is missed by all who knew him or worked with him—for both his friendship and contributions to BYD research.

Dr. Clifford C. Gill

Cliff was born 7 October 1921 in Cape Town, South Africa. He earned his BSc degree from the University of Capetown and his PhD from Berkeley. He is now retired and lives in Winnipeg.

In 1964 Cliff went to the Winnipeg Research Station of Agriculture Canada where he initiated a program on cereal virus research. Of the many aspects of BYDV in which he worked, two areas were particularly important: 1) the discovery *Schizaphis graminum*-specific isolates of BYDV and 2) the characterization (with James Chong) of the differences in ultra structure alterations among variants of BYDV. The latter work served as one of the bases for current understanding of the taxonomy of the luteoviruses. His collaboration with cereal breeders at the Winnipeg Research Station helped in development of oat cultivars with superior BYDV resistance.

Dr. William F. Rochow

Bill was born in Lancaster, Pennsylvania, on 12 March 1927. He earned his degrees at Franklin and Marshall College. He spent 1955 at the Virus Laboratory at the University of California at Berkeley. Also during 1955, he joined USDA to initiate a study of oat viruses in cooperation with the Department of Plant Pathology at Cornell. He was later appointed professor of plant pathology. He is now retired and lives in Ithaca, New York.

Bill's findings have had a major influence on BYDV research on the luteoviruses, and on other persistently transmitted viruses. Bill is largely responsible for discovery and description of vector specificity of BYDV, which enabled him to identify the occurrence of dependent virus transmission from mixed infections using different BYDV isolates. Bill demonstrated that transcapsidation

influences BYDV epidemiology and helped develop and utilize bioassay techniques based on feeding aphids on purified virus through membranes and through microinjection of virus into anesthetized aphids. His leadership and willingness to assist other researchers have stimulated BYDV programs in many parts of the world.

Dr. Charles W. Schaller

A native of Wisconsin, Charles earned his degrees from the University of Wisconsin. In 1946 he obtained a position with the Department of Agronomy at the University of California at Davis where he spent his career until retirement in 1986 at Davis. Between 1968 and 1986, he released nine barley cultivars, which account for 80% of the barley grown in California. Charlie is best known to BYD workers for his incorporation of the *Yd2* gene into commercial barley cultivars from Ethiopian material. This material now has been widely tested in different regions of the world where the *Yd2* gene bestows resistance to some BYDV isolates. The gene is used in many breeding programs. Charlie has always put his energies into activities that most benefit his students and plant breeding programs.

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Acknowledgments

I would like to thank the following groups and individuals for their help in contributing to the success of this barley yellow dwarf workshop and the publication of the proceedings:

- The participants without whom there would have been no workshop.
- The Dipartimento Cooperazione Allo Suiluppo (DCAS) of the Ministry of Foreign Affairs of the Republic of Italy for funding the workshop.
- The directing staff of CIMMYT for giving me the opportunity to carry out such a project.
- Dr. Arthur Klatt, who gave me a great deal of help and support leading to and during the workshop.
- The program committee, who assisted in putting together the schedule of events.
- Dr. Elvio Refatti and his local committee in Udine for the work they did to make the meeting such an enjoyable occasion.
- Dr. Roger Blackman and Dr. Victor Eastop for their demonstrations of aphid identification at the workshop.
- Dr. Paul Gugerli for the ELISA demonstrations at the workshop.
- Janet Keyser, the wordsmith whose competence and humor ensured the completion of these published proceedings.
- Linda Ainsworth, whose assistance prior to and during the workshop was indispensable.
- Martha Larios, my secretary, who capably handled the many extra jobs and correspondence associated with such an undertaking.
- CIMMYT Information Services, especially Gene Hettel for his help and encouragement in every phase of this work and Miguel Mellado E. and his group of Rafael de la Colina F., José Manuel Fouilloux B., and Bertha Regalado M. for design and layout of this publication; and Maricela A. de Ramos, and Ma. Teresa Pedroza P. for typesetting.
- Drs. Paul Fox, Narceo Bajet, Dana Eaton, and Howard Eagles for their helpful suggestions and comments on some of the manuscripts.
- Jonathan Robinson for assistance with proof reading.
- Ms. Monica Mezzalama, my workmate, for her help, tolerance, encouragement, and humor while I was engaged in this project
- Dr. Harvey C. Smith who first introduced me to the study of barley yellow dwarf.
- My wife, Alison, and our children Ben, Samuel, and Anne for their love, help and understanding while this work was going on.
- "The Barley Yellow Dwarf," for his enthusiasm for the whole project.

It has been a time consuming but interesting task.

P.A. Burnett
Workshop organizer and
technical editor

Welcome from Prof. Franco Frilli, Rector of the University of Udine

I am pleased to give a cordial welcome to the participants of this workshop, which has gathered together scientists from many parts of the world here in Udine.

Your host, the University of Udine, was founded in 1978 and is made up of seven different faculties. The Agricultural Science Faculty, with its three degree programs in agricultural science, food science, and animal husbandry, and combined with Florence, has more different agricultural courses than any other institution in the country. I am proud to add that three of the four rectors here at the University have been chosen from among professors of the Agriculture Faculty. This is indicative of the trust that the other faculties place in our agriculture professors.

The first Department of Plant Protection in any Italian institution has been set up in our Faculty. This is an important innovation since it has been traditional to keep plant pathologists separated from entomologists and even plant virologists and mycologists.

Prof. E. Refatti and his co-workers, especially Prof. R. Osler, set up an active workgroup in 1980 with international connections that has given the University of Udine renown outside of Italy.

I would like to congratulate my Udine colleagues on their efforts to organize this workshop and bring it to Udine and I would like to extend to all the participants the warmest of welcomes from the University and best wishes that the meeting will be pleasant and profitable.

Welcome from Prof. E. Refatti, Chairman of the Udine Organizing Committee

In the name of the Udine Organizing Committee, I would like to thank the authorities of the University of Udine for being present during this opening session of the Barley Yellow Dwarf workshop. I would also like to extend a cordial welcome to the scientists from some 35 countries representing five continents who are participating in this event.

The high number of contributed papers and posters on this important disease of cereals, the high scientific level of their content, and the renown of their authors are certainly a good start for the success of the workshop.

In addition to facilitating information exchange, the workshop will update the state of research on BYD and give indications of the lines to follow for future studies. The transfer of results to the end users will also be important.

Prof. G.T. Scarascia-Mugnozza, rector of the Tuscia University (Viterbo) and well known agricultural geneticist, could not reach Udine to represent the Italian Ministry of Foreign Affairs at this opening session. Therefore, I am charged to welcome all of you in the name of the Ministry. It is through the "Dipartimento Cooperazione allo Sviluppo (DCAS)" that our Ministry has partially sponsored this workshop and is supporting research on Barley Yellow Dwarf both at CIMMYT and to some degree in other countries. Such an effort assists research programs in developing countries, where the disease is causing serious problems owing to the fact that cereals provide over half of the total caloric intake for human nutrition.

The organizing committee expresses its gratitude to: the Region Friuli-Venezia Giulia Administration, the University of Udine, the Chamber of Commerce and Agriculture of Udine, the Cassa di Risparmo of Udine and Pordenone, the Province of Udine Administration, the Collegio Bertoni and the Malgnani Institute for their various contributions to the workshop's organization.

I. World Situation

The "Yellow Plague" of Cereals, Barley Yellow Dwarf Virus

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Abstract

Barley yellow dwarf virus (BYDV) is distributed worldwide, and infects most cereals and grasses. It is a phloem-restricted pathogen, causing yellowing, reddening, and brittleness of leaves, dwarfing, and reduction in size and number of ears and grains. BYDV is a luteovirus with small isometric particles containing an ssRNA genome, and is transmitted persistently by more than 20 aphid species. Five virus isolates have been distinguished and divided into two subgroups on the basis of cytopathology and serology. Recent serological evidence also indicates that BYDV isolates are related to other luteoviruses, suggesting that a continuous, overlapping range of viruses may be implicated in the barley yellow dwarf syndrome. Until future research clarifies this point, the term BYDV continues to be used to indicate the agent(s) involved. Perennial wild or cultivated grasses constitute a large and permanent virus pool. Primary and secondary virus spread depends on the aphid vector reproduction and flight which, in turn, are influenced by climatic conditions. Recent research on monitoring and control of aphid vectors and on development of resistant cereal cultivars has improved the prospect of minimizing losses from BYDV infections.

Plant viruses reduce the quantity and quality of crop yields and may even cause the death of their host plants. Plant viruses of major economic significance spread rapidly, are difficult to control, and affect food and industrial crops. In addition, they are often ubiquitous and variable and are capable of producing variants that can survive in previously resistant cultivars. Many plant viruses are transmitted efficiently by a range of vectors, leading to sudden outbreaks in new geographic areas.

Barley yellow dwarf virus (BYDV) is certainly one such virus: it is distributed worldwide, infects practically all members of the Gramineae—including all the most important cereals and grasses—and possesses all the above attributes of destructive plant viruses (Plumb 1983; Rochow et al. 1986). For these reasons, it has been called the "yellow plague" of cereals (Duffus 1977a).

As with some other viruses, the symptoms caused by BYDV are often not sufficiently specific to allow the visual identification of infected plants. The dwarfing and leaf yellowing induced by this phloem-specific pathogen can easily be confused with damage due to frost, wet weather, waterlogging, nitrogen deficiency, and several other non-infectious agents. This is probably why the disease was not attributed to a viral infection until the middle of this century (Oswald and Houston 1951), even though disorders of cereals, most of which are now considered to have been BYDV infections, had frequently been noted (Galloway and Southwood 1890; Manns 1909: see Bruehl 1961; Barrus 1937; McKinney 1950; Johnston 1951).

Considering that most cereals and grasses are susceptible to BYDV, the disease might have been more appropriately named "cereal" yellow dwarf, particularly because this would have emphasized its economic importance (Burnett 1984). Serological evidence indicates that BYDV is related to several other luteoviruses, suggesting a continuous overlapping range of luteoviruses (Duffus 1977b; D'Arcy 1986). "Barley yellow dwarf" may be considered as a "convenient, all-embracing name for diseases with similar symptoms and effects that are caused by

persistently aphid-transmitted viruses only some of which are serologically related" (Plumb 1983). Until future research clarifies this point, the term BYDV continues to be used to indicate the agent(s) involved. Perhaps it would be better to use the plural form "barley yellow dwarf viruses."

The Virus

BYDV belongs to the luteovirus group, of which the MAV isolate is the type member (Matthews 1982). Particles are isometric, 24-25 nm in diameter, with sedimentation coefficients of 115-118 S. The virions contain a single component of single-stranded RNA, M_r 2.0 MDa, and have one major polypetide subunit of about 24 kDa (Rochow and Israel 1977; Hewings and D'Arcy 1986).

As BYDV infects all cultivated and wild Gramineae, it may be the most common cereal virus (Slykhuis 1967), and certainly one of the most widespread plant viruses in the world. Crops that suffer the most serious damage are autumn-sown cereals. Infected barley (*Hordeum vulgare* L.) shows diffuse, bright yellow discoloration starting from the leaf tip, and dwarfing. Oats (*Avena sativa* L.) show purpling or reddening of leaves, and stiffening of the whole plant. BYD symptoms on wheat (*Triticum aestivum* L.), rye (*Secale cereale* L.), and triticale (X *Triticosecale* Wittmack) include dwarfing and leaf yellowing, which are generally less severe than in barley. Among the spring-sown cereals, which can also be severely affected, rice (*Oryza sativa* L.) shows intense leaf yellowing and stunting and maize (*Zea mays* L.) symptoms include fine chlorotic spots or streaks on young leaves, and premature purpling of basal leaves. Symptoms may be restricted to certain tillers only. Yield is reduced, often drastically, through a reduction in the number of fertile tillers and also by a reduction of kernel size and number (Doodson and Saunders 1970; Pocsai and Szirmai 1985; Yount 1985).

Transmission

BYDV is transmitted by aphids in a persistent (circulative, non-propagative) manner, but is not transmitted from infected females to their progeny. No other means of natural transmission are known. Comprehensive lists of the aphid vectors have been published by A'Brook (1981), 23 species, and Jedlinski (1981), 18 species. Most of these species infest Gramineae. The relative importance of each as a vector varies considerably from one area to another. An aphid species may be an efficient vector in one country but not in another, depending on its natural cycle, and particularly on whether it has alternative host plants (heteroecious) or not (autoecious). As an example, *Rhopalosiphum padi* (L.) has alternate hosts in Britain (*Prunus padus* L., several Gramineae) but not in New Zealand (Plumb 1983), while in Italy it can overwinter both as eggs on primary hosts (*P. padus* and *P. serrulata* Lindl.) or as immature forms on secondary hosts (cereals and grasses) (Conti 1983). The aphids that migrate early from grasses may carry the virus, while those that migrate later from primary hosts do not.

High vector specificity is another attribute of BYDV, and the following virus isolates have been distinguished on this basis:

- RPV, specifically transmitted by *R. padi*
- RMV, specifically transmitted by *R. maidis* (Fitch)
- MAV, specifically transmitted by *Sitobion (=Macrosiphum) avenae* (Fabricius)
- SGV, specifically transmitted by *Schizaphis graminum* (Rondani)
- PAV, nonspecifically transmitted by *R. padi* and *S. avenae*

Other variants, transmitted specifically or nonspecifically by different aphid species have also been reported (Gill 1969; Paliwal 1982; Zhou et al. 1984).

The five BYDV isolates officially recognized have been split into two groups according to their serological

relationships: the first group includes RPV and RMV isolates, and the second includes MAV and PAV isolates. The viruses within each group are closely serologically interrelated and distinct from those in the other group. The SGV variant of BYDV described by Gill (1969) seems to have some antigens in common with the PAV isolate (Rochow and Carmichael 1979).

Recent investigations have clarified some of the mechanisms of circulative transmission and vector specificity of BYDV. Studies on the RPV isolate and its vector *R. padi* have shown that the virus is transported from the gut lumen into the hemocoel of aphid vectors through the hindgut cells but apparently not through the stomach or intestinal regions of the midgut (Gildow 1985). In earlier work, aphids of the species *S. avenae* were exposed to acquisition of the transmissible MAV isolate, and of the non-transmissible RPV isolate. The fate of virions of each isolate in the aphid was then followed by indirect labeling with ferritin-conjugated antibody. Particles of both isolates were detected in the accessory salivary glands but not in adjacent tissues; particles of both viruses were also clearly visible beyond the basal lamina surrounding the accessory gland. In contrast, only particles of the transmissible MAV isolate were detected in the intracellular canals draining the accessory gland, in lysosome-like vesicles in the cytoplasm, and in other cytoplasmic structures. This indicated that the selection of transmissible and non-transmissible isolates occurs at the gland plasmalemma, which underlies the basal lamina (Gildow and Rochow 1980).

The vector specificity of BYDV isolates is complicated by a phenomenon called "dependent transmission," resulting from transcapsidation or phenotypic mixing. The nucleic acid (RNA) is the component that induces disease and, in persistent aphid transmission, the coat protein is the component interacting with vectors to determine transmissibility. When two different BYDV isolates multiply simultaneously in the same plant, the RNA of one isolate may become encapsidated in the protein of the other, and vice-versa (transcapsidation); another possibility is that the RNA of one virus may be encapsidated by protein subunits from both viruses (phenotypic mixing). Both conditions could lead to the transmissibility of the RNA by vectors of inappropriate specificity. There is good evidence for both the phenomenon and the mechanism (Rochow 1977).

Epidemiology

Generally, perennial grasses form the main natural reservoir of BYDV, but in some countries one or more particular species play a predominant role. Several *Lolium* species in Britain and Scandinavia, and tall fescue (*Festuca arundinacea* Schreb.) in Missouri, USA, are well-known examples. In the Mediterranean area and in southwest England, maize is the principal virus source for *R. padi*, which migrates during fall and infects autumn-sown cereals (Dedryver and Robert 1981; Plumb 1983; Osler et al. 1985). The most important source of rice giallume, a BYDV isolate in Italy spread naturally by *R. padi* (Belli et al. 1986), is the perennial weed *Leersia oryzoides* (L.) Swartz (Osler et al. 1980). Apparently the first epiphytotics of rice giallume in Italy were favored by the introduction of crop mechanization. The massive use of herbicides and the continuous use of mechanical cultivators caused the gradual disappearance of weeds that reproduce only by seed, and favored the dissemination of *L. oryzoides*, which multiplies both by seed and by stolons (Conti 1983).

BYDV is introduced every year in autumn-sown cereal crops by migrant aphids, which establish their colonies in the cereals. The proportion of infective migrant aphids is relatively small (Plumb 1981; Loi et al. 1989) and even when large migrations occur, primary infection is generally scattered and sparse. From these early infection foci, secondary spread can occur during winter, when occasional periods of mild weather may stimulate aphid movement and feeding. In spring, the number of cereal-infesting

aphids increases due to rapid parthenogenetic multiplication and the virus spreads, even though the plants are older and more resistant. The appearance of winged aphid forms, migrating actively from crop to crop, also increases the possibilities for virus spread. Overwintered eggs on primary hosts may contribute other winged, migrant aphid vectors to the spring dispersal of BYDV in the field.

Due to the high concentration of host plants and vectors, and efficient transmission, BYDV causes frequent epiphytotics resulting in dramatic losses. Average annual losses due to BYDV in the USA from 1951 to 1960 were estimated at 1.6%, or $6,331,000, for barley, and at 3.8%, or $35,913,000, for oats. In particular, in 1959, 28% of the total oat production in Illinois was lost, and field experiments showed that the wheat yield was reduced by 63% after autumn BYDV infection, and by 41% after spring infection. In Indiana, the average yield losses per year are estimated to be about 10% in wheat.

Epiphytotics of BYDV in Canada occur about every 4 years. In October 1982, 100% of the winter cereals were infected with BYDV in the Quebec area. In Britain, BYDV is the most important virus disease of cereals. Surveys of spring-sown barley from 1967 to 1977, and of winter-sown wheat from 1971 to 1977, showed that up to 90% of crops were infected in any one year.

Epiphytotics of rice giallume were first reported in Italy in 1967 and continued for 10 to 12 years, until resistant rice varieties were introduced. In Italy, wheat and barley suffered the first severe BYDV infections in 1977-78; in 1977, about 120 hectares sown to barley in northeastern Italy had to be plowed under and sown again in the spring, due to 100% BYDV infection.

Other severe epiphytotics of BYDV have been reported in Ecuador (1983), Peru (1980), Spain (1983), Egypt (1961), Morocco (1980), Syria (1982), Turkey (1983), and the People's Republic of China (1970, 1973, 1978) (cf. CIMMYT 1984).

Control

Different strategies to control BYDV include:

Resistance—Sources of tolerance were detected in barley during the early BYDV outbreaks. This type of resistance is controlled in barley by one incompletely dominant gene of Ethiopian origin, named *Yd2*. Resistance in oats apparently depends on multiple gene systems, while in wheat it has been detected in different sources, and is controlled by different genes.

Husbandry—BYDV infection can be avoided or limited in winter cereals by monitoring the migrating aphid vectors in autumn and sowing cereals to emerge after the autumn population peak has passed.

Chemicals—Aphicides formulated as granules or sprays are sometimes very efficient in controlling virus spread; they are necessary when weather conditions and vector densities suggest heavy infection.

To ensure the best control, knowledge of BYDV epidemiology in different regions of the world must continually be updated. This is especially necessary because of the extreme variability of the viruses involved in the disease.

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Situation Review of Barley Yellow Dwarf in Canada

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Abstract

*Surveys in recent years show PAV-like isolates of barley yellow dwarf luteovirus (BYDV) to be the predominant type infecting cultivated cereal grains in North America. Disease outbreaks were localized in most regions in 1984 and 1985. In 1986, BYDV caused damage to all spring cereal species throughout eastern Canada. In addition to direct disease losses, BYDV has been shown to cause losses by reduction of cold hardiness in winter wheat (*Triticum aestivum *L.) and pre-disposition to scab and septoria. Most effort in breeding for BYDV resistance has been applied to oats (*Avena sativa *L.). As measured against the standard for resistance set by the Illinois variety, Ogle, several resistant cultivars have been released. Efforts continue to incorporate the* Yd_2 *gene from Ethiopian barley into adapted barley lines.*

Surveys in recent years in small-grain producing regions of North America show vector-nonspecific (PAV-like) isolates of barley yellow dwarf lutoevirus (BYDV) as the predominant variant infecting crops. This contrasts with the situation reported from New York, southern Ontario, and Manitoba in the 1960s of variation from one year to the other of the predominant BYDV variants. With the increasingly widespread use of enzyme-linked immunosorbent assay (ELISA), larger numbers of isolates from surveys have been evaluated, increasing confidence that survey results are broadly representative of actual distribution in the field.

Losses, Prevalent Vectors, and Virus Strains

Western Canada

In 1984-1986, yield losses due to barley yellow dwarf (BYD) occurred almost only in late-sown spring barley (*Hordeum vulgare* L.), and oat (*Avena sativa* L.) crops. The incidence of disease as estimated by surveys was low in 1984 and 1985, although BYDV was isolated from a higher proportion of fields in 1985 than in 1984. Aphid vectors arriving in early July in barley, oat, and wheat (*Triticum aestivum* L.) fields surveyed in eastern, central, and southern Manitoba were more numerous and a few days earlier in arriving in 1985 than in 1984. In some areas of central Saskatchewan, late planting resulted in occasional high incidences of BYD in oats, and in one dramatic instance resulted in the complete loss of a late-sown field of spring barley. In 1986, arrivals of aphids as early as mid-June combined with unusually late planting due to wet weather, led to expectations—which did not materialize—of a BYD epidemic for the eastern prairies of Canada. In all 3 years, aphid populations, proportion of fields with BYDV infections, and disease severity were much lower than levels reported in epidemic years such as 1974 (Gill 1975).

In 1984-1987, the majority among detected vector species were *Sitobion avenae* (Fabricius); in southern Manitoba about one-fifth of the vector aphids were (*Rhopalosiphum padi* L.). As in previous years, a vector-nonspecific variant (PAV-like) of BYDV made up the majority of the isolates obtained in field surveys. The *R. maidis* (Fitch)-specific variant (RMV-like) of BYDV was infrequently isolated from winter wheat in Manitoba in 1985 and 1986. An *S. avenae*-specific isolate (MAV-like) was identified in one instance from barley in central Alberta in 1985.

In 1986, *Schizaphis graminum* (Rondani) was reported in heavy infestations covering 1 million ha in Alberta and western and central Saskatchewan. *S. graminum* damage was first visible in early July, with the most serious damage

occurring in July and early August (J. Byers, personal communication). Although the aphid was first found in Alberta over 50 years ago, there is no evidence that it can overwinter in the province. Normally the insect is an annual problem only in the southern United States and is believed to be carried into western Canada by weather systems from the south each year. The *S. graminum* problem in Alberta was associated with later-than-normal planting of grain, early appearance of a heavy migration of aphids, and subsequent weather that favored a large buildup of aphid populations. There was little damage from BYDV in the area infested with *S. graminum* as the aphids did not appear to be transmitting the disease. Almost pure cultures of *S. graminum* were found in the dryland areas of southern Alberta but *R. padi* and *R. maidis* were also present in the irrigated areas and there was some BYD on barley in those areas (J. Byers, personal communication).

Eastern Canada

In contrast to the epidemic incidence of BYD in winter wheat and barley in 1982-83 (Paliwal 1984), disease outbreaks were sporadic and localized in most regions in 1984 and 1985. There is a general "background" level of between 4 and 10% infection in both winter and spring cereals in Ontario and western Quebec (Y.C. Paliwal, personal communication). Substantial losses in spring cereals due to infections much above this level occurred only in late-sown crops, or in some pockets of low-lying land.

In 1986, BYD caused damage to all spring cereal species throughout eastern Canada, reaching severe epidemic levels with losses of 60% on susceptible oats and two-row barley in the northwestern growing regions of Quebec. Losses of about 35% were observed in some fields of spring wheat in southwestern Quebec (A. Comeau, personal communication). Reports of heavy direct losses in spring wheat due to BYD are infrequent in Canada, but recent evidence that BYDV infection predisposes wheat to attacks by scab and septoria raises concerns about higher levels of indirect losses. In addition, higher than usual levels of BYDV in maize were recorded; in a limited sampling in eastern Ontario, up to 30% of plants in some fields were infected. *R. maidis*-specific (RMV-like), vector-nonspecific (PAV-like), and *S. graminum*-specific (SGV-like) variants were identified; RMV- and PAV-like variants were about equally prevalent and made up the majority of isolates (Y.C. Paliwal, personal communication).

Management and Control of BYD

Most effort in breeding for BYDV resistance has been applied to oats. As measured against the standard for tolerance set by the Illinois cultivar Ogle, several tolerant cultivars have been released. The varieties Nova and Cardinal, more tolerant than previously available cultivars for eastern Canada, yielded well in the epidemic of 1986 (A. Comeau, personal communication). Resistant varieties are being developed for western Canada. Robert, a new variety derived from complex crosses involving North American and Australian *Avena sativa* germplasm as well as *A. sterilis* L. accessions, equals Ogle in BYDV resistance and was registered in 1987 (P.D. Brown, unpublished).

An international barley yellow dwarf oat nursery coordinated by A. Comeau, Ste. Foy, Quebec, began its first trials in 1987. Advanced breeding material submitted by programs from Quebec, Manitoba, Wisconsin, Illinois, Indiana, California, and New Zealand are being

evaluated by the participating programs. This initiative follows from the results of a cooperative study of the inheritance of tolerance to BYDV in oats, which showed a significant correlation of reactions to BYDV among three sites: Palmerston North, New Zealand; Winnipeg, Manitoba; and Ste-Foy, Quebec (McKenzie et al. 1985).

Efforts continue to incorporate the *Yd2* gene from Ethiopian barley into adapted barley lines, because this gene confers desirable single-gene resistance (Schaller et al. 1963). In controlled yield trials that assessed the effects of artificial inoculation with a Montana isolate of PAV-like BYDV, Sutter spring barley was found to be resistant to disease in that it suffered no significant reduction in plant height, tillers per plant, seeds per head, 1000-seed weight, or yield per plant compared to uninoculated controls (Yount et al. 1985). Sutter (CI 1237 x Winter Tennessee) has the *Yd2* gene for BYDV resistance from its CI 1237 parent (Schaller et al. 1973).

A major difficulty in deploying the *Yd2* gene, the need to remove the spindly growth habit of the Ethiopian parents, is being addressed in several ways. One is by male sterile-facilitated recurrent selection in a population containing four different sources of *Yd2* (Crosslin et al. 1986). By this approach, it is expected that agronomically suitable lines could be developed that incorporate one or more sources of the *Yd2* gene. The germplasm has been registered as BYDV-tolerant barley composite cross XLIV and is available from the USDA Small Grains Collection (Crosslin et al. 1986).

An alternative approach, involving the crossing of an Ethiopian parent containing the *Yd2* gene (CI-32-08-4) into high quality lines adapted for western Canada, followed by recurrent selection for BYD resistance and desirable agronomic traits by the pedigree method, is making steady progress (R. Metcalfe and S. Haber, unpublished). Desirable traits of early maturity and high yield and grain quality, as well as BYD resistance, have been successfully incorporated into recent selections, but lodging resistance equal to check cultivars has still to meet the standards required for commercial release.

Efforts to identify BYDV resistance and incorporate it into wheat germplasm have been less extensive than for oats or barley. Indeed, susceptibility/resistance to BYDV or tolerance to BYD is still rarely described in registrations for new wheat cultivars. Anza, a short-statured spring wheat identified earlier as possessing tolerance to BYD (Qualset et al. 1973), was found to suffer yield losses similar to other, susceptible, spring wheat cultivars in a controlled trial in Montana, although symptom onset was delayed by 4 to 5 days (Yount et al. 1985).

The importance of identifying and incorporating BYD resistance into wheat has been heightened by observations that the greatest losses, especially in winter wheats, are due to indirect effects such as reduction of cold hardiness and predisposition to scab and septoria (Andrews and Paliwal 1986). With the paucity of resistance sources in wheat, attention has turned to some of the wild relatives of wheat. Crosses between wheat and such wild relatives as *Elymus* spp. have yielded offspring with excellent BYDV resistance, but much work lies ahead before this resistance can be incorporated into useful varieties (A. Comeau, unpublished).

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Current Status of Barley Yellow Dwarf in the United States: A Regional Situation Report

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* Dr. Gildow did not present this paper at the workshop, but was asked to summarize for these proceedings the USA situation.

Abstract

Barley yellow dwarf (BYD) can be observed annually in small grains throughout the cereal growing regions of the United States. Epiphytotics have generally been localized within limited areas of individual states and have occurred sporadically from year to year. Widespread yield losses attributable to BYD have not been sustained consistently by growers of any specific region. Increased recognition of BYD as a virus-induced disease associated with aphid spread, improved cultivar resistance and tolerance, and appropriate planting recommendations have helped reduce potential losses to BYD. Perhaps for these reasons and recent annual environmental conditions unfavorable to virus spread, BYD has not been of major economic concern in the United States during the past few years. Detailed epidemiological information is available only from areas (New York, Pennsylvania, Indiana, Illinois, Missouri, Colorado, Idaho, Montana, California, and Washington) where major BYD research laboratories are located. The status of the research is summarized.

Although severe yield losses have not been directly attributed to barley yellow dwarf virus (BYDV) infection in the United States in recent years, there is an increasing awareness that the potential for future BYDV epiphytotics still exists if appropriate environmental conditions occur. There is also a growing recognition that BYDV may contribute to small grain yield losses indirectly through effects on host plant physiology associated with cereal crop responses to environmental stresses and to other pathogens. For these reasons, active BYDV research programs are supported in the major small grain producing areas.

In this review I will attempt to briefly outline some of the known parameters influencing BYD epidemiology in several small grain growing regions, and summarize selected areas of BYDV research currently being conducted in laboratories throughout the United States. Readers interested in more detailed information may refer to the various references cited and directly contact the researchers involved.

Previously published reviews of BYDV in the USA are available in Barley Yellow Dwarf: A Proceedings of the Workshop, CIMMYT, 1984. Because of the large number of entomology, plant virology, and small grain breeding programs involved to various degrees in BYDV research, no attempt was made to describe all possible programs. I apologize in advance to my colleagues whose work may not be included because of space and publication deadlines, and hope the reader will recognize this as only a preliminary attempt to summarize information on BYDV research in the United States.

Northeastern Region

New York

Studies of BYDV in the eastern portions of the U.S. have been centered in the state of New York. Work by W. Rochow at Cornell University in Ithaca over the past 30 years was responsible for identifying many of the basic components involved in BYDV epidemiology, as well as for increasing our knowledge of virus-vector interactions. The five serologically distinct BYD luteovirus type-isolates (MAV, PAV, SGV, RPV, and RMV) were first identified and characterized in New York (Rochow 1969). Recent work at the Cornell lab has involved studies of monoclonal antibodies to BYDV (Hsu et al. 1984), use of chicken antibodies for

identification of SGV (Hu et al. 1985), studies of *Schizaphis graminum* (Rondani) transmission efficiency (Zhou and Rochow 1984), and continued investigation of the mechanism involved in dependent transmission of luteoviruses from mixed infections (Hu and Rochow 1986). Dr. Stewart Gray has recently taken over direction of the U.S. Department of Agriculture-Agriculture Research Service (USDA-ARS) laboratory at Cornell following the retirement of W.F. Rochow in 1986. Dr. Gray plans to focus his research efforts on BYDV epidemiology.

Although the MAV isolate predominated in field surveys during the 1960s, surveys of small grains in New York in 1984 and 1985 by Rochow indicated that three isolate-types (PAV, RPV, and RMV) occurred in barley (*Hordeum vulgare* L.), oats (*Avena sativa* L.), and wheat (*Triticum aestivum* L.) (Rochow et al. 1986). The PAV-like isolates were most common, and isolates similar to MAV and SGV were not detected by either aphid transmission bioassays or by enzyme-linked immunosorbent assay (ELISA). For unknown reasons the predominant isolate in New York has shifted from the MAV- to the PAV-like isolate. SGV-like isolates have been recovered in only 3 of the 29 years in which small grains were surveyed. Mixed infections were frequently detected, which suggested that dependent transmission of different isolate types may play an important role in BYDV survival and spread in this region. The most common aphid species observed on small grains are *Rhopalosiphum padi* (L.), *R. maidis* (Fitch), and *Sitobion avenae* (Fabricius). *Schizaphis graminum* and *Metopolophium dirhodum* (Walker) have been collected, but are not common.

No quantitative data are available on small grain yield losses due to BYDV in New York State, however, a 10 to 50% incidence of BYD in winter wheat and spring oats has been observed over the past several years in the central part of the state (G. Bergstrom, personal communication). Typically BYD has a spotty distribution from county to county. Studies have recently been initiated by A. Powers (Dept. Ecology and Systematics, Cornell University) to monitor BYDV aphid vectors in the field and to study aphid behavior in relation to small grain cultivars. Recent work by Powers has clearly indicated that maize (*Zea mays* L.) served as an important alternate host for *R. padi* and a reservoir for PAV during the summer months (July-September) after small grain harvest. Transmission of PAV by field collected *R. padi* was as high as 30% during September, and PAV was found to overwinter in fall planted wheat (A. Powers, personal communication). Work is continuing on the monitoring of transmission rates by field aphids and on testing cultural techniques for reducing BYD incidence.

Pennsylvania

Recent studies at Pennsylvania State University by Gildow and coworkers (Gildow et al. 1987) have identified the components of BYD in three environmentally distinct growing regions of the state (western, central, and southeastern regions). Pennsylvania isolates identified as PAV-, RPV-, MAV-, and RMV-PA types were recovered from spring oats and barley and fall planted barley and wheat by aphid transmission and by ELISA. Polyclonal antibodies to the New York isolates of BYDV were supplied by W.F. Rochow. Isolates similar to SGV were not recovered from any of the three regions surveyed, and MAV-like isolates were not recovered from the southeastern region of the state.

Only *R. padi* and *S. avenae* were observed on spring cereals and the PAV-PA isolates predominated. The most common aphid observed on fall planted small grains in October and November was *R. maidis*, and RMV-like isolates were most commonly recovered from fall planted cereals. No studies of BYDV in maize have been done. The incidence of BYD in spring cereals in Pennsylvania has been observed to be very low since 1983. Spring weather conditions have not favored aphid population growth during the critical early stages of cereal development. The existence of at least three major vector aphid species and the four isolate-types they transmit suggests that, given appropriate conditions, BYD epiphytotics could occur throughout the grain growing regions of the state.

Replicated field trials under controlled conditions have been done to assess the potential yield loss resulting from high incidences of BYDV infection to oats at susceptible growth stages (Gildow and Frank 1988). When Noble oats, a commonly grown cultivar, were inoculated in the field by *R. padi* viruliferous for PAV at 4 or 6 weeks after planting, all yield components were reduced relative to uninoculated control plots. Under ideal weather conditions and with nearly 100% incidence of infection at 4 weeks, yields were reduced by 87%. Inoculations at 6 weeks after planting resulted in only about a 30% yield reduction. These results clearly emphasized the importance of stage of crop development at time of infection and the critical importance of recommended planting dates in reducing potential yield losses. Such large yield losses have not been common in Pennsylvania for several years, probably due to the low incidence of BYDV, adherence to recommended planting dates, and availability of more tolerant cultivars. These tests do indicate the potential for yield losses in oats that could occur under conditions ideal for BYDV spread. For example, a few commercial fields of late planted spring oats in 1985 were observed to have a high incidence of BYDV-MAV-PA and sustained estimated yield losses of about 70% relative to previous harvests.

Current research at Pennsylvania State University is focused on studies of BYDV vector-specificity mechanisms regulating virus transmission (Gildow 1987), continued studies of BYDV epidemiology in the state, studies of the interactions between BYDV and fungal pathogens of small grains (Sommerfeld et al. 1987) and studies on the biology and structure of an aphid infecting virus, *Rhopalosiphum padi* virus (RhPV) in cooperation with C. D'Arcy at the Univ. of Illinois, (Gildow and D'Arcy 1985, 1987).

Southeastern United States

In general, very little is known about BYDV in the southern states. Most of the small grain area is planted to winter wheat and BYDV symptoms are not always obvious or diagnostic. Barley and oats are of minimal economic importance to the region. This author is unaware of any detailed studies of BYDV having been reported from this area. Information obtained from colleagues in three representative states indicates our current level of knowledge about BYDV in the southeastern USA.

Alabama

Symptoms of BYD have been observed annually since 1961 in small grain variety trials planted at 12 locations throughout the state (R. Gudauskas, personal communication). Wheat typically shows leaf discoloration, yellowing, and stunting of plants; however, tests are not routinely conducted to verify BYDV infection. Samples of oat and wheat tissue were sent to W.F. Rochow at Cornell University for testing in 1975. All infected samples were found to be infected with PAV-like isolates. This is the only confirmation of BYDV in Alabama to date. A full range of cereal grain aphid species, including *R. padi*, *R. maidis*, *S. avenae*, and *S. graminum*, occur in the state. Tests of insecticides applied at planting or as foliar sprays to oats resulted in decreased incidence of BYD and increased yields, compared to untreated control plots (Gudauskas and Estes 1978).

South Carolina

Based on characteristic symptoms of yellowing and stunting, BYD is believed to occur in fields of small grains annually. The occurrence, however, is sporadic in location each year and consistent losses due to infection are not known. In the spring of 1987, the incidence of BYD was believed to be higher than usual, with about 15% of the small grain fields observed showing symptoms of BYDV infection. However, total yield losses due to BYD were estimated to be no more than 1 to 2%. In 1976, 12 leaf tissue samples from plants showing typical BYDV symptoms were sent to Cornell University for testing. All samples were infected with PAV-like isolates. Samples sent in 1987 to C. D'Arcy at the University of Illinois were also found to be infected with PAV-like isolates. To date no other isolates have been identified (O.W. Barnett, personal communication).

Louisiana
Although Louisiana has about 700,000 hectares of soft red winter wheat in production, BYDV is not currently believed to have an economic impact. In many years the weather patterns are unfavorable to aphid populations due to rapid increases in temperatures in spring. Symptoms of BYDV have been observed in fields, however, the isolates that occur there have not been identified (L. Anzalone, Jr., personal communication).

Clearly BYDV does occur in the southern portions of the USA. The only isolate type identified to date was PAV. Many of the aphid vector species occur in this area, suggesting that other isolate types may occur. Further testing is necessary to verify this hypothesis. The mild winter and spring temperatures might be expected to support early aphid activity and enhance BYDV spread under appropriate conditions. It is expected that more information will become available in the near future.

Central United States

A preliminary report listing some BYDV isolate types identified by ELISA from small grain plants collected from nine midwestern and north central states has been previously presented (Clement et al. 1984).

Indiana
Small grain cereals are important crops in this region. For example, soft red winter wheat is the third most important crop in Indiana, with 450,000 ha in production. Although quantitative yield loss data are not yet available, BYDV is considered to be of economic importance in all small grain crops.

Recent epidemiological surveys by R. Lister and coworkers at Purdue University have identified isolates similar to PAV, RPV, and MAV in Indiana (Clement et al. 1986). Isolates similar to RMV and SGV have not yet been identified. Aphid vectors commonly observed in the field include *R. padi*, *R. maidis*, and *S. avenae*. In the spring, *R. padi* was the most common species present in small grain fields, and in the fall both *R. padi* and *R. maidis* were observed to increase on maize before migrating to fall planted wheat. Isolates similar to PAV, PAV-Purdue (PAV-P), were most commonly identified in small grains in Indiana. Although wheat was mostly asymptomatic, over half of the plants tested by ELISA tested positive for BYDV infection, indicating that visual surveys greatly underestimated the incidence of infection. When assayed for BYDV by ELISA, about 80% of the infected winter wheat plants were found to be infected with PAV-like isolates, and 10 to 20% by RPV-like isolates singly or in mixed infections. In these studies MAV-like isolates were not tested for. Surveys of spring oats in June indicated a 48% incidence of infection, with 43% infected with PAV- and 9% with RPV-like isolates.

Studies of perennial grasses as virus reservoirs indicated that 50% of the grass samples tested were infected with various BYDV isolate types (Fargette et al. 1982). About 80% were infected with RPV-like isolates and 8 to 16% with PAV-like isolates. Depending on the location in the state, 0 to 6% of the samples were infected with MAV-like isolates. These results suggested that in Indiana local BYDV-infected grasses are probably not the most important sources for viruliferous aphids infesting small grains fields. Although RPV-like isolates were most common in the grasses surrounding fields, PAV-like isolates predominated within the fields. At the present time the major sources of viruliferous aphids and PAV are unknown. Volunteer oats, wheat, and maize are believed to be important hosts of vectors and BYDV for bridging between the spring and fall planted small grain crops.

Current research on BYD at Purdue University includes studies of diagnostic techniques for isolate identification and characteristics (Lister et al. 1985; Barbara et al. 1987), studies of various parameters influencing BYDV epidemiology, and development of BYDV resistant small grain varieties (Skaria et al. 1985; Shukle et al. 1987). The program involves researchers from virology (R. Lister), entomology (J. Foster and R. Shukle), plant breeding (H. Ohm, G. Shaner, and H. Sharma) and molecular biology (B. Larkins).

Illinois
BYD in Illinois is believed to be most important in fall planted wheat and spring oats. The incidence of BYDV varies annually. Visual surveys relying on characteristic symptoms have suggested an annual infection rate of 10 to 20% in small grains. In 1987, many growers in Illinois increased their area of oats, which allowed increased visualization of symptoms of BYDV and its distribution within the state. In surveys of spring oats in 1987, up to 50% of the randomly sampled plants were found to be infected with BYDV when tested by ELISA with polyclonal antibodies made against the PAV-Illinois (PAV-IL) and RPV-Illinois (RPV-IL) isolates of BYDV. Other isolates were not tested for in this survey. The BYDV isolate types common in small grains of Illinois, ranked in order of predominance, include PAV, RPV, MAV, and RMV. The MAV- and RMV-like isolates are rare, and SGV-like isolates have not yet been identified. The two most commonly observed aphids in the spring are *R. padi* and *S. avenae*, and the third most common species is *S. graminum*. *R. maidis* is only rarely observed on spring cereals. Studies of maize and fall planted cereals have not yet been done (C. D'Arcy, personal communications).

The University of Illinois is the site of an oat breeding program for BYDV resistance, which has been run by C.M. Brown in cooperation with the late Henry Jedlinski of USDA. Recently F. Kolb has joined this breeding program and A. Hewings, USDA, has taken up the position formerly held by H. Jedlinski. The spring oat cultivar "Ogle," which has shown good tolerance to field infection by BYDV, was released from this program and is currently widely grown in the eastern and midwestern states.

Current research on BYDV in Illinois is being conducted by C. D'Arcy at the University of Illinois. Her research program is focused on studies of the relationships among luteoviruses (D'Arcy 1986; Hewings and D'Arcy 1986), improving luteovirus purification techniques (D'Arcy et al. 1983), continuing studies of the epidemiology of BYDV in Illinois, and epidemiological studies of RhPV virus in *R. padi*, in cooperation with F.E. Gildow at Penn State University (Gildow and D'Arcy 1985, 1987).

Missouri
BYD is common throughout Missouri; however, little is known about the details of the BYDV-vector complexes involved in BYDV epidemiology. The major cereal grain aphid species colonizing small grains annually are *R. padi* and *S. graminum*. Although the predominate BYDV isolate types occurring in the state have not been serologically characterized, it is clear that forage grasses provide a significant reservoir for BYDV. When 136 symptomless tall fescue (*Festuca arundinacea* Schreber) plants were collected from 90 counties and tested by aphid transmission bioassays to oats, 60% were found to be infected with *R. padi*-transmissible isolates of BYDV (Grafton et al. 1982b). This would suggest that PAV- or RPV-like isolates were probably involved. Transmission tests of 210 aphid colonies from field collected aphids indicated that 90% were viruliferous for BYDV when fed on oats. Fall infections of winter barley of BYDV reduced winter survival of plants by 83%, and reduced yield by up to 96% compared to uninoculated control plots (Grafton et al. 1982a). Four barley cultivars rated for tolerance to BYDV and listed in order of decreasing tolerance are Post > Perry > Harrison > Durra. Current research at the University of Missouri is focused on studies of resistance of winter barley crosses containing the Ethiopian Yd_2 gene for tolerance (J. Poehlman, personal communication), and studies of inheritance of BYDV resistance in oats (Gellner and Sechler 1986).

Western United States

Idaho
Epiphytotics of BYD occurred in different areas of Idaho in 1977, 1979, 1981, and 1985. In 1986 and 1987 the incidence of BYD in small grains was comparatively low, especially in spring cereals. All five

characterized BYDV isolate types have been recovered and identified by aphid transmission bioassays and ELISA from commercial fields of wheat and barley (Forster and Rochow 1983). Ranked by order of currently perceived importance, the isolates are SGV, PAV, RPV, MAV, and RMV. Although BYD occurs at significant levels in spring planted barley, it is considered to be of major economic importance on winter wheat.

The epidemiology of BYDV in Idaho differs significantly from that described for many other areas of the country. Isolates similar to SGV occur consistently throughout the state and play a predominate role in BYD epiphytotics in certain areas. For example, samples collected from small grains in the north and southwestern areas of Idaho in 1981 were found to be infected with each of the five isolate types. In 1985, however, most winter wheat plants collected from the high altitude, dryland valleys of southeastern portions of the state were infected with SGV-like isolates (Rochow et al. 1987). Of all infected plants sampled, 94% were infected with SGV-like isolates and 6% with PAV- and RPV-like isolates. These winter wheat fields were heavily infested with both *R. padi* and *S. graminum* during the fall, but *S. graminum* was not observed the following spring. Although a large portion of the aphid population consisted of *R. padi*, the *R. padi*-vectored isolates (RPV- and PAV-like) were not common in the host plants. In western Idaho, which is at a lower elevation, maize and wheat are commonly grown and PAV- and RPV-like isolates predominate. The MAV- and RMV-like isolates are occasionally encountered, but are rare in the small grains. RMV-like isolates are most frequently identified in barnyard grass (*Echinochloa crus galli* (L.) P. Beauv.), with up to 25% of the tested plants being infected (R. Forster and S. Halbert, personal communication).

Incidence of BYD in Idaho varies from year to year. In irrigated wheat, for instance, up to 80% of the plants may show symptoms of BYDV infection in some years. In 1987, however, only about 1% of the plants were infected, based on symptom expression. During years of severe epiphytotics resulting in 80% incidence of BYDV infection, yield losses have been estimated to approach 30% in irrigated wheat and 70% in dryland wheat. Irrigated winter wheat inoculated with PAV in controlled studies yielded 50% less than noninoculated controls. It was estimated that yield loss in wheat may approximate one half of the percentage of BYD incidence observed in the field.

Current control recommendations include delayed planting dates in the fall to avoid peak aphid populations on the newly emerging wheat seedlings, and in-furrow application of granular systemic insecticides at planting. These recommendations have been very effective in reducing the incidence of BYDV in Idaho.

Research at the University of Idaho by G. Bishop, S. Halbert, and R. Johnston is focused on monitoring aphid populations as part of a cooperative western regional aphid monitoring network aimed at developing aphid vector forecasting models and identifying components of the BYDV-aphid systems. Work at Kimberly, Idaho, by R. Forster is directed at surveys for identification of BYDV isolate types in small grains and maize and studies of BYDV epidemiology.

Montana

Epiphytotics of BYD occurred in winter wheat in 1980 and 1981 due to early seeding of fall planted crops and unusually mild autumn temperatures, which enhanced aphid activity. Evaluation of yield losses in 1980, based on 10 year averages of grain storage records, indicated a 30% loss of yield. Current estimated losses in 1986 and 1987 were only 15% and 5%, respectively, in the absence of a major BYDV epiphytotic (T. Carroll, personal communications). Surveys of small grains beginning in 1978 identified Montana BYDV isolate types as being similar to PAV, RPV, RMV, and MAV (Yount and Carroll 1983). All isolate identifications were done using a combination of aphid transmission bioassays and ELISA. Isolates identified as PAV-Montana (PAV-MT) were most prevalent, however, RMV-MT isolates were also very common in spring barley. Some of the RMV-MT

isolates differ serologically from the RMV-New York isolate characterized by W.F. Rochow, and are transmitted occasionally by *R. padi* and *S. graminum*, as well as by *R. maidis*. Aphids ranked by suspected importance as BYDV vectors are *R. maidis*, *R. padi*, *S. avenae*, and *S. graminum*. In 1986, *S. graminum* was the most prevalent aphid species in irrigated fields of barley in central Montana. It is believed, but unconfirmed, that SGV-like isolates may occur in fields when large populations of *S. graminum* occur. Field inoculation trials comparing PAV-MT inoculated and noninoculated test plots indicated an average yield reduction in winter wheat of 67%, in spring wheat of 74%, and in spring barley of 44 to 65% (Yount et al. 1985).

Research at Montana State University is directed at continuing studies of BYDV epidemiology by isolate characterization and aphid trapping, and selection for BYDV-tolerant small grain cultivars (Crosslin et al. 1986).

California

Barley yellow dwarf was first identified as a virus-induced disease after major epiphytotics in small grains occurred throughout cereal growing regions of California in the early 1950s (Oswald and Houston 1951). At the present time, however, BYD is not of major economic concern and serious yield losses due to BYD have not occurred for several years. A major control recommendation for growers is to delay planting in the fall until major aphid flights have peaked. In addition, barley with the *Yd2* gene for resistance is commonly grown throughout the state. Wheat cultivars "Anza" and "Yolo" have been selected for tolerance to BYDV and perform well annually. A major breeding program for BYDV resistance under the direction of C. Schaller and C. Qualset has existed at the University of California-Davis for many years and is well known (Qualset 1984).

In a preliminary survey of California small grains done in 1980-1982, three BYDV isolate types were identified, PAV-California (CA), RPV-CA, and MAV-CA (Gildow and Rochow 1983). The PAV-CA isolates predominated in most areas. MAV-CA occurred most commonly in coastal areas. In greenhouse tests, RPV-CA induced the most severe symptoms and PAV-CA milder symptoms in several California cultivars of barley and oats. Aphid vectors observed annually in fields were *R. padi*, *S. avenae*, and *M. dirhodum*. In irrigated fields *R. padi* occurred throughout the growing period, but *S. avenae* were most common early in the spring and *M. dirhodum* in late spring. The RMV and SGV isolates were not tested for because *R. maidis* and *S. graminum* were not observed in small grain fields during the study.

Surveys for BYDV in California rice crops in 1981 (Gildow, unpublished) failed to detect BYDV by aphid recovery bioassays to oats, and symptoms of BYDV in field grown rice plants were not observed. Rice cultivar "Cal Rose" did develop characteristic symptoms of yellowing and stunting when fed on for 3 days by *R. padi* previously acquisition-fed for 2 days on oats infected with the RPV-CA and PAV-CA isolates. These results suggested that California rice cultivars may be susceptible to regional BYDV isolates, but were escaping infection due to planting dates and cultural techniques.

Current epidemiological studies of BYDV by B. Falk and coworkers at UC-Davis are examining small grains and wild cereals for differential distribution of BYDV serotypes both geographically and by host species. Surveys from the Imperial Valley of southern California to the Tule Lake region of northern California using DAS-ELISA have indicated that PAV-CA isolates are most common, followed by RPV-CA and then MAV-CA. An unusual isolate of RPV-CA transmitted by both *R. padi* and *S. avenae* has been recently described (Creamer and Falk 1987).

Research on BYDV at UC-Davis includes studies of BYDV epidemiology, studies of BYDV replication and interactions with host genes for resistance, quantification of yield loss of small grain cultivars in response to infection by different BYDV isolate types, and continued use of classical selection techniques and novel techniques for developing BYDV-resistant small grains (Bryce Falk, personal communications).

Washington
Problems with BYD in cereals are generally associated with fall planted crops of wheat in Washington, especially in the irrigated central zones. The irrigated fields provide green plants during the dry summer period between wheat crops, which can maintain large aphid populations. The important infection period is believed to occur during the early autumn (September-October) when aphids appear to be most abundant in the fields. Cereals planted after September are usually minimally affected by BYDV infection. During the spring, aphids are less abundant, with the exception of *S. avenae*, which may increase in late spring on developing heads of grain. The small grain aphids ranked by currently perceived importance are *R. padi*, *M. dirhodum*, *S. avenae*, *S. graminum*, and R. maidis. The percentage of trapped cereal aphids transmitting BYDV annually varies from 1 to 10%. The recent discovery of RMV-like isolates in Washington suggests that R. maidis may be more important than previously expected. The increased importance of BYDV in this region is correlated to increased area under irrigation and increased maize planting. Maize serves as an excellent summer host of R. padi; surveys in 1980 recovered BYDV from 86% of the maize fields tested (Brown et al. 1984). Of the R. padi collected from these fields, 38% were viruliferous for BYDV. Yield reductions attributed to BYDV range from 3 to 14%, based on 9 cultivars of fall planted barley and wheat (K. Pike, personal communication).

Current research is focused on continued studies of the epidemiology of BYDV within the state, especially as it relates to the wheat-maize cycle of aphids and virus. Another major research emphasis involves cooperation in a USDA Western Regional Project to trap and monitor aphid populations, with a goal of developing forecasting systems.

Colorado
Studies have been initiated to identify isolates of BYDV infecting small grains throughout the state. During 1986 samples of fall and spring planted barley and wheat were sent to Gaylord Mink's lab at Washington State University for ELISA analysis. When tested against an antiserum recognizing both MAV- and PAV-like isolates, 14 of 40 samples were positive for infection. In 1987, 41 of 68 samples sent to Washington were positive for MAV or PAV, and 4 of 11 samples sent to Lister's lab at Purdue University were positive for PAV, but not MAV or RPV. So far, SGV and RMV isolates have not been tested for. The Russian wheat aphid *Diuraphis noxia* (Mordvilko) and BYDV were prevalent in the eastern plains of Colorado in 1987. Transmission tests of *D. noxia* acquisition fed on BYDV-infected (probably PAV) plants failed to detect BYDV transmission by this species. Work will continue on identification of BYDV isolates through cooperation with other BYDV laboratories (E.A. Milus and W.M. Brown, personal communications).

Overview
This summary of BYDV in various regions of the United States indicates a growing awareness of the potential importance of BYDV to small grain production, and of the extreme complexity of the virus-isolate: aphid biotype: host cultivar: environmental interactions involved in BYDV disease epidemics. There is an increased interest in the role of maize in survival of aphid vectors and luteoviruses between spring and fall cereals, in the effect of BYDV on winter survival of fall planted crops, and in the synergistic and antagonistic interactions of BYDV with other plant pathogens co-infecting small grains. Although PAV-like isolates are the most common types in most regions examined, some distinct regional differences become apparent when examined more closely. For instance, a variety of SGV-like isolates are unusually common in parts of Idaho, while unusual RMV-like isolates are of importance in Montana. The recent discovery of an RPV-CA isolate transmitted by both R. padi and S. avenae in California indicates that much remains to be known and understood about the range of BYD luteoviruses and the molecular aspects regulating their biological properties. New programs are being initiated to study the molecular biology of BYDV and to use new bioengineering techniques for developing BYDV resistant small grain varieties.

Unfortunately, at this time few resistance genes have been identified and characterized, and little is known about the resistance or tolerance mechanisms involved in qualitative multigenic inheritance of BYDV resistance in oats and wheat. Nothing is known about specific virus isolate/host gene interactions, and much remains to be learned about the details of virus survival, location of vector reservoirs, and virus-vector interactions involved in BYDV epidemiology. For example, relatively little is known about BYDV in the southern states, as well as in some of the large midwestern states in which small grains are major crops. Most of our knowledge about BYDV in the United States is centered in a few states where major laboratories focusing on BYDV research are located. Increased regional, national, and international cooperation in the future will facilitate more rapid acquisition of information concerning isolate and vector characterization, reduce duplication of the complicated and expensive facilities needed for quality BYDV research and plant breeding, and increase our understanding of BYDV epidemiology on a larger regional basis. These types of information and cooperation will probably be necessary for instituting effective control measures.

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Barley Yellow Dwarf Virus and Aphids in Mexico

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Abstract

The isolates of barley yellow dwarf virus (BYDV) present in Mexico have been typed using enzyme-linked immunosorbent assay (ELISA). This was done by either sending dried leaf samples to cooperating laboratories or by testing them in our own laboratory. Differences were observed among laboratories, but the prevalent isolate of BYDV was MAV-like, which may make Mexico unique. The prevalence and distribution of the aphid species in Mexico is summarized.

Barley yellow dwarf has been observed in Mexico for more than 30 years (Navarro 1984). During the last 3 years we have sampled cereal crops for the presence of barley yellow dwarf virus (BYDV). Initially, air-dried leaf samples were tested by enzyme-linked immunosorbent assay (ELISA) by R.M. Lister of Purdue University, USA, using polyclonal antibodies for PAV and RPV and a monoclonal antibody for MAV (MAV1), and by I. Barker of Rothamsted Experimental Station, England, using monoclonal antibody 91 for PAV, monoclonal antibody 92 for RPV, and monoclonal antibody MAFF2 for MAV.

A few samples have also been tested by the following cooperators: G.I. Mink, Washington State University, Prosser, Washington, USA, using a monoclonal antibody for a PAV-like isolate prepared by S. Wyatt, Washington State University, Pullman, USA; M.B. von Wechmar of the University of Cape Town, South Africa, using double antibody sandwich (DAS) ELISA against a South African BYDV antiserum, anti brome mosaic virus (BMV)-type strain, anti cucumber mosaic virus (CMV-Tob), anti CMV-Nel (2 two local CMV isolates), anti *Rhopalosiphum padi* virus (RhPV), and anti barley stripe mosaic virus (BSMV); and W.L. Gerlach and P.M. Waterhouse of Commonwealth Scientific and Industrial Research Organization of Canberra, Australia, using a number of samples with an antiserum made with R. Sward's VI isolate of BYDV, which is now known to be a mixture of PAV- and RPV-like isolates.

More recently we have carried out ELISA in Mexico using the monoclonal antibodies used by I. Barker. These were manufactured by L. Torrance, from the Ministry of Agriculture Food and Fisheries (MAFF) in England.

From this study, we have constructed a picture of the common BYDV isolates in Mexico. Figure 1 shows the locations sampled.

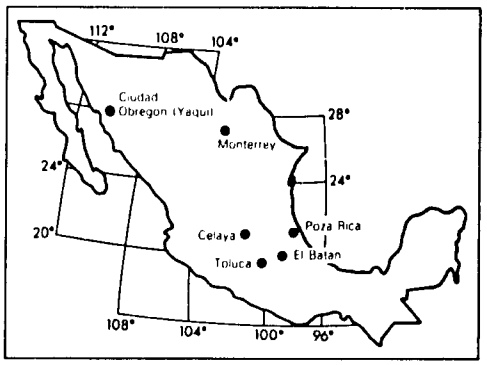

Figure 1. Sites sampled for BYDV in Mexico.

The results of ELISA tests conducted by R.M. Lister, I. Barker, and ourselves on air-dried leaf samples from winter and spring material collected in Mexico between 1984 and 1987 are given in Table 1. Between 1984 and 1986, samples came exclusively from Toluca and El Batan, but in the winter of 1987, many other areas of Mexico were sampled.

In the winter of 1984, in both Toluca and El Batan, the prevalent isolate was MAV-like. In the summer samples from Toluca, the prevalent isolate again was

MAV-like. During 1985 the number of PAV-like isolates increased, but there were still many MAV-like isolates. During 1986 MAV-like isolates again became predominant. In 1986, it appears that more PAV-like isolates appeared in Toluca.

Twenty-two dried leaf samples from winter material grown at Toluca in 1985 were tested in three laboratories by ELISA (Table 2), but different results were obtained by the laboratories. Apparently, the Australian tests failed to detect some PAV-like isolates that were detected elsewhere.

An additional 33 dried leaf samples from summer-grown material from Toluca were tested in four cooperating laboratories with ELISA and again there was variation among laboratories (Table 3). Apparently the BYDV antibody from South Africa would not detect the Mexican isolates of BYDV. However, many other viruses were found in our samples by the South African tests.

From the results obtained so far there are two points that should be stressed. The first is that there is not total agreement in the determination of isolates with the antisera used in different laboratories. To clarify this situation many more isolates from different areas of the world should be tested against the range of available antisera.

The second point is that, overall, the prevalent isolate in Mexico is MAV-like, which may make Mexico unique. However further surveys are necessary in other parts of the world.

Navarro (1984) reported the following aphid species on cereals in Mexico: *Rhopalosiphum padi* (L.), *R. maidis* (Fitch), *R. rufiabdominalis* (Sasaki), *Schizaphis graminum* (Rondani), *Metopolophium dirhodum* (Walker), *Sitobion avenae* (Fabricius), and *Diuraphis noxia* (Mordvilko). We often observe *R. rufiabdominalis* on the roots of the cereal crops in Yaqui Valley, and the occasional specimen has been taken

Table 1. Dried leaf samples from cereal samples tested for the presence of barley yellow dwarf virus by ELISA 1984-1987

Cooperator	Source of plant material	Number of samples	Number of positives	Virus isolates						
				MAV	PAV	RPV	MAV PAV	MAV RPV	PAV RPV	RPV MAV PAV
Lister	Winter Toluca 84	76	75	45	1	—	27	2	—	—
Lister	Winter El Batan 84	121	119	111	—	—	8	—	—	—
Lister	Summer Toluca 1984	16	16	12	—	—	—	4	—	—
Barker	Summer Toluca 1984	61	31	16	2	7	3	2	1	—
Lister	Winter Toluca 1985	26	19	5	7	—	7	—	—	—
Barker	Winter Toluca 1985	26	20	5	13	—	2	—	—	—
Lister	Summer Toluca 1985	41	31	14	—	—	15	2	—	—
Barker	Summer Toluca 1985	41	19	10	6	1	—	2	—	—
Lister	Winter Toluca 1986	82	30	22	2	—	2	4	—	—
Barker	Winter Toluca 1986	91	52	40	3	—	8	1	—	—
Barker & Mezzalama (tested at CIMMYT)	Summer Toluca 1986	128	127	79	16	1	23	4	3	1
	Winter Yaqui 1987	26	5	5	—	—	—	—	—	—
Mezzalama (tested at CIMMYT)	Winter Monterrey 1987	6	5	1	2	—	2	—	—	—
	Winter Celaya 1987	66	47	47	—	—	—	—	—	—
	Winter Toluca 1987	105	75	33	20	5	3	2	11	1
	Winter El Batan 1987	5	5	4	—	—	1	—	—	—
	Winter Poza Rica 1987	2	1	1	—	—	—	—	—	—

in our traps near El Batan. *S. graminum* has only been observed commonly in the north of Mexico or in the area near Celaya. We have not yet tested these two species for their ability to transmit BYDV.

So far the most common species found on crops is *M. dirhodum*, followed by *R. maidis*, *R. padi*, and, lastly, *S. avenae*. In the trapping study of last year, *R. padi* was the most common, followed by *M. dirhodum* (Jaime et al. 1989). These species may act as vectors of BYDV. *M. dirhodum* appears to be the most common vector of MAV-like isolates in Mexico, rather than *S. avenae*. (Mezzalama and Burnett 1989).

In the winter in both the high valleys surrounding Mexico City and the Bajio area surrounding Celaya, *D. noxia* is very common. It seems that this aphid is much more common or more of a problem under very low rainfall. In the summer when all of the rain falls in the high valleys, it is unusual to find a large amount of *D. noxia* damage unless there is a drought. In the winter, however, when small areas of cereals may be grown under irrigation in this region, they become severely infested with *D. noxia* even though we capture very few of this aphid species at this time in our suction traps. Perhaps the very heavy summer rain showers that this area receives act as a population control mechanism.

So far we have not been able to show positive transmission of BYDV by *D. noxia*, but we are continuing this work. The observations on aphids are preliminary and our current trapping studies will provide us with much more information.

Table 2. Dried leaf samples from winter material grown in Toluca tested for presence of barley yellow dwarf virus using ELISA by cooperators in 1985

	Cooperator		
Sample number	R.M. Lister	I. Barker	W.L. Gerlach
1	PAV	PAV	Weak PAV
2	PAV	PAV	PAV
3	MAV + PAV	MAV + PAV	—
4	MAV + PAV	MAV + PAV	—
5	—	—	—
6	PAV	—	PAV
7	MAV + PAV	MAV	PAV
8	MAV + PAV	MAV + PAV	PAV
9	MAV + PAV	PAV	—
10	MAV	—	—
11	MAV + PAV	PAV	—
12	PAV	PAV	PAV
13	PAV	PAV	—
14	MAV + PAV	MAV	PAV
15	—	PAV	PAV
16	MAV	PAV	PAV
17	—	PAV	PAV
18	MAV	PAV	PAV
19	MAV	MAV	PAV
20	—	—	—
21	—	—	—
22	PAV	PAV	—
	17 Positives	17 Positives	12 Positives Antisera for PAV and RPV used

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Table 3. Dried leaf samples collected from summer grown material in Toluca and tested for presence of viruses by cooperators

Sample number	Cooperator R.M. Lister	I. Barker	M.B. von Wechmar	G.I. Mink
1	MAV + PAV	MAV	—	PAV
2	MAV + PAV	PAV	—	PAV
3	MAV + PAV	MAV	—	PAV
4	MAV + PAV	MAV	—	PAV
5	MAV + PAV	PAV	—	PAV
6	MAV + PAV	MAV	—	PAV
7	MAV + PAV	MAV	—	PAV
8	—	—	BMV	PAV
9	—	—	BMV,CMV-Nel., RhPV	?
10	MAV	—	BMV, RhPV	PAV
11	—	—	RhPV, CMV-Tob., CMV-Nel	—
12	MAV + PAV	—	BMV,BSMV	PAV
13	MAV + PAV	PAV	—	?
14	MAV + PAV	—	?	NT
15	MAV + PAV	—	RhPV	NT
16	—	—	BMV	NT
17	MAV	—	—	NT
18	MAV	MAV	BMV	NT
19	MAV	MAV	RhPV	PAV
20	MAV	N.T.	—	PAV
21	MAV	MAV	BMV	NT
22	MAV + PAV	—	BMV	NT
23	MAV + PAV	PAV	BMV	NT
24	MAV	—	BMV	—
25	MAV	—	NT	PAV
26	MAV	—	NT	PAV
27	MAV	—	NT	PAV
28	MAV	MAV	—	NT
29	MAV + RPV	MAV	—	NT
30	MAV + RPV	MAV	—	NT
31	MAV	MAV + RPV	—	NT
32	—	—	—	PAV
33	—	PAV	BMV	NT

NT = Not tested.

Barley Yellow Dwarf in the Andean Countries of South America

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Abstract

In small grain cereal growing areas of Bolivia, Colombia, Ecuador, and Peru, barley yellow dwarf (BYD) appears to be the disease causing the greatest overall yield loss. It is widely distributed and severe attacks occur every season. Loss estimates range up to 30%. Enzyme-linked immunosorbent assay (ELISA) studies confirm that visual assessment of symptoms is effective throughout the Andes. MAV-, PAV-, and RPV-like serotypes have been identified and Metopolophium dirhodum *(Walker),* Sitobion avenae *(Fabricius),* Rhopalosiphum padi *(L.),* R. maidis *(Fitch), and* Schizaphis graminum *(Rondani) are the most common cereal aphids. Consistent genetic differences in susceptibility of varieties are observed and the exploitation of these in breeding for resistance or tolerance represents the only practical control measure.*

The Current Situation

With generally adequate levels of rust resistance now employed, barley yellow dwarf (BYD) has displaced the rusts as the disease complex causing the greatest overall yield loss in small grain producing areas in the Andean countries—Bolivia, Colombia, Ecuador, and Peru. The bulk of production occurs in mountainous regions between 2000 and 4000 meters above sea level. The diversity of micro-climates, sowing dates, and other cultural practices combined with out-of-season forage production means that cereals are grown year-round in many places and control or avoidance of aphids is impossible. Genetic resistance to BYD is the only feasible method of control. In most parts, BYD symptoms can be seen every year, developing from time to time into major epidemics generally associated with the drier than average conditions thought to favor aphid dispersal. Some of the major attacks observed were:

1977—northern Ecuador
1980—Huaylas Valley, Peru
1985—northern Ecuador
1987—southern Colombia
1987—Huaylas Valley, Peru

To date, field screening and selection under variable natural infection has been the usual strategy to counter BYD. However the barley breeding program of the Colombian breweries in the south of that country artificially increases stocks of the aphid *Sitobion avenae* (Fabricius) for field inoculation. In this region an old barley (*Hordeum vulgare* L.) variety, Galeras, maintains a high level of symptomatic resistance. In Ecuador, Dubin et al. (1984) conducted loss assessment studies with wheat and noted that losses to BYD of between 10 and 30% are common.

Visual Assessment and ELISA Tests

Rochow and Duffus (1981) reported that the expression of symptoms of BYD in the field is favored by high light intensity and cool temperatures, common conditions in the Andean highlands. When samples with symptoms have been analyzed, results of enzyme-linked immunosorbent assay (ELISA) analyses have generally confirmed the presence of BYDV. In 1981 Dubin et al. (1984) sent samples from Ecuador for analysis by Richard Lister at Purdue University (USA), and although only PAV and RPV antisera were used, 64% of the samples were positive. From 1985, the sampling was extended to four countries—Ecuador, Peru, Colombia, and Bolivia—and samples were again mailed to Lister at Purdue and also to Ian Barker at Rothamsted Experimental Station (UK).

Except for some anomalous results from Peru in 1987, there has been quite good agreement between the two laboratories, which tested for the MAV-, PAV-, and RPV-like isolates. The MAV type was most frequently detected, followed by PAV and then RPV types. Starting in 1986, paired samples of leaves with and without symptoms were taken from each line or variety tested. Additionally, it was decided to split all leaves along the midrib and send half of each leaf to Purdue and the other half to Rothamsted. Excluding 1987 results from Peru, there has been excellent agreement between field observation of the presence or absence of the disease and ELISA tests, reinforcing the hypothesis that Andean conditions lend themselves to visual assessment when the disease is present.

Twenty-two samples with typical BYD symptoms were sent in 1985 and 1986 and all were positive for BYDV at either Purdue or Rothamsted, but generally at both laboratories. Of the 6 paired samples without symptoms, all were negative at Purdue while at Rothamsted four were clearly negative and two gave inconclusive reactions.

Sources of Resistance and Breeding Strategies

As well as the reduction in symptoms of the Colombian barley variety Galeras, there are other consistent, observable, genetic differences. For instance, in Ecuadorean wheats (*Triticum aestivum* L.) the variety Chimborazo always appears more susceptible than Altar, while Tungurahua exhibits the most resistance of the three (Table 1). This is in accord with the observation of Dubin et al. (1984) that the percentage yield loss due to BYD was greater in Chimborazo than in Altar, although the difference was not great. From BYD field scores taken at several dates, it appears that some wheats maintain a low, or more resistant, score throughout the growth cycle, while others progress rapidly from low to high scores. The differential rate of development of BYD symptoms suggests that more resistant lines in this respect exhibit what may be described as "slow yellowing." In Ecuadorean barleys, Teran 79 and Dorada appear more susceptible than Duchicela (Table 1). In southern Colombia in 1987, Atlas 68 with the Yd_2 gene for BYD resistance was susceptible, but consistently less so than its near isogenic sister line, Atlas 57 (S. Fuentes, personal communication). Genetic bases of the observable differences are generally unknown in the Andean countries and cannot be exploited fully in breeding programs because the selection pressure possible at any site due to natural infection varies greatly from year to year.

To rectify this situation, a cooperative effort by the Instituto Nacional de Investigaciones Agropecuarias, the University of California at Davis, and CIMMYT is envisaged for Ecuador. Plans involve artificial inoculation and focus on the epidemiology and genetic control of BYD. An antiserum or complementary DNA probe for the SGV serotype may be developed.

Aphid Species

In the Andean countries the most common cereal aphids are *Metopolophium dirhodum* (Walker), *S. avenae*, *Rhopalosiphum padi* (L.), *R. maidis* (Fitch), and *Schizaphis graminum* (Rondani).

In lowland Bolivia, heavy infestation by *S. graminum* is common in wheat crops and *R. rufiabdominalis* (Sasaki), *R. maidis*, and *R. padi* also occur (Simoni 1987). In this region, where warmer temperatures favor the spread of arthropod predators, biological control of aphids is being researched, primarily to combat their direct damage. While some BYD-like symptoms have been observed in oats (*Avena sativa* L.), wheat generally does not exhibit symptoms and for this reason samples have not been analyzed. However sampling is planned to determine whether BYDV may be present without exhibiting the characteristic symptoms noted in the highlands.

Myzus persicae (Sulzer) is common in many crops in Peru and *Sipha flava* (Forbes) has been recorded on barley in Colombia.

Anomalous Results from Peru in 1987

In light of the previous agreement between field observation and laboratory tests, the 1987 results from Peru (Table 2) were to a degree anomalous. Possibly, there was a low level MAV-type infection present, but with visible symptoms mainly due to another causal organism that was either not BYDV or was a serotype, such as SGV or RMV, undetected by the antisera used. Further testing combined with more detailed identification of the aphids involved may clarify the situation.

Other Viruses and Virus-like Diseases

Other viral diseases in the region include barley stripe mosaic virus (BSMV) (Gutierrez et al., 1987). Nariño Dwarf disease, which is transmitted by the leafhopper *Cicadulina pastusae* Rup. & DeLg., is poorly understood and is believed to occur only in southern Colombia and northern Ecuador, where it can damage cereal crops. The causal agent has not been identified, although viruses, mycoplasms, and toxins have been suggested. There is some evidence to suggest that the leaf enations are produced by a toxin and are not directly related to the agent causing the dwarfing (E. Castro, personal communication). Leaf symptoms characteristic of Nariño Dwarf were observed without dwarfing in

Table 1. BYD symptoms on a 0-9 scale (Qualset 1984) at Santa Catalina Experiment Station, Ecuador

Wheat	1984	1985
TUNGURAHUA	1	2
KAL//KAL/BB/3/KAL/BB//MRS	2	2
E-73-1118/3/BB/NOR67//CNO/7C	2	2
BUHO/4/SON64/TZPP//Y50/NP/3/LAC617(67A)	0	1
BON/YR/3/*T. aest*//KAL/BB	1	2
IAS64/ALDAN	1	1
PF70354/ALONDRA	1	2
PF70354/MUS	2	1
VEERY	2	2
ALTAR (check)	7	6
CHIMBORAZO	8	7
Barley		
CN48/CI8985	2	2
DUCHICELA	1	0
BA//GAL//FUN/4/AVT/3/BZ/AS46//W/5/CN42/CI8985	2	1
SHYRI3	1	1
ABYSSINIAN VANTMORE	2	0
E-SC-II-72-670-13e-1e-2e-2E	1	0
SHRYRI4	2	1
CI1240/ARAMIR	2	2
DORADA (check)	8	7
TERAN 79 (check)	7	6

the Huaylas Valley of Peru in 1986 and 1987, but further investigation is needed to confirm whether the disease occurs in Peru. Ian Barker (personal communication) identified viral rods suggestive of wheat soilborne mosaic virus (WSBMV) in a triticale (X *Triticosecale* Wittmack) sample from Ecuador and a wheat from Peru during analyses for BYDV. It has been tentatively hypothesized that such particles may be implicated in causing the symptom commonly called "physiological flecking" in the region.

Acknowledgments

Thanks are extended to Richard Lister and Ian Barker for their ELISA analyses and to Pilar, who split the leaves.

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Table 2. ELISA results from Purdue and Rothamsted of 11 split leaf samples from Peru in 1987

	With symptoms		Without symptoms	
	Purdue	Rothamsted	Purdue	Rothamsted
1.	-	-	-	-
2.	-	-	-	-
3.	-	MAV	-	-
4.	-	-	-	-
5.	MAV	-	MAV	-
6.	MAV	MAV	MAV?	-
7.	MAV	-	-	-
8.	-	PAV	MAV?	-
9.	-	-	MAV	+?
10.	MAV	MAV?	MAV	-
11.	-	-	-	-

A Review of Barley Yellow Dwarf Virus in the Southern Cone Countries of South America

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Abstract

Aphids and barley yellow dwarf (BYD) have been known in the Southern Cone countries of South America (Argentina, Bolivia, Brazil, Chile, Paraguay, and Uruguay) for many years, but the aphids' role as vectors of barley yellow dwarf virus (BYDV) in cereals was not apparent until the late 1960s. The aphid vectors most widely distributed throughout the region are Metopolophium dirhodum *(Walker),* Sitobion avenae *(Fabricius),* Rhopalosiphum padi *(L.), and* Schizaphis graminum *(Rondani). BYD was positively identified in 1967 (Brazil) and 1972 (Chile). During 1974/75 and 1975/76 severe BYD epidemics developed, with losses in the range of 10 to 60%. From 1976/77 onward, there have been occasional localized outbreaks and losses of production. In Brazil, Chile, and Argentina, PAV- and MAV-like isolates of BYDV predominate, along with mixtures of isolates not yet well characterized. Integrated vector control programs have been conducted in Brazil, Argentina, and Chile since the 1970s. Tolerant germplasm has been selected by artificial inoculation of screening nurseries.*

The Southern Cone countries of South America—Argentina, Bolivia, Brazil, Chile, Paraguay, and Uruguay—grow nearly 10.5 million ha of winter cereals annually for grain production. Another 3.5 million ha are sown as "verdeos" for grazing or, in some cases, for initial grazing and later for grain harvesting. Production is estimated to be around 18.5 million tons. Almost 90% of this area is sown to wheat (*Triticum aestivum* L.) and the rest to oats (*Avena sativa* L.), barley (*Hordeum vulgare* L.), rye (*Secale cereale* L.), and triticale (X *Triticosecale* Wittmack). Grain production of these secondary cereals fluctuates between 500,000 and 600,000 tons (IICA 1986).

Argentina is the major producer and exports nearly 8 million tons of wheat each year. Brazil imports around 4 million tons yearly while the other countries import minor quantities, or are now self-sufficient (IICA 1986).

Aphid Vectors

Aphids and barley yellow dwarf virus (BYDV) have been recognized in the Southern Cone for many years. *Schizaphis graminum* (Rondani) was found as early as 1937 at La Estanzuela Experiment Station in Uruguay (Zerbino 1981). BYD-like symptoms were noted by Beeckman in 1929 at Bage, Rio Grande do Sul, Brazil, and the causal agent was characterized in 1967 by Caetano (Caetano 1968, 1972, 1981).

The main aphid vectors reported for the Southern Cone countries are listed in Table 1.

Table 1. Main aphid species reported as vectors

Species	Argentina	Bolivia	Brazil	Chile	Paraguay	Uruguay
M. dirhodum	X	X	X	X		X
R. padi	X		X	X	X	
S. graminum	X	X	X	X	X	
S. avenae			X	X	X	X
R. rufiabdominalis	X	X	X			
R. maidis			X		X	

Source: Informe de Reunión de Especialistas Nacionales en Pulgones. La Platina Exp. Sta., Santiago, Chile, 1982

Aphid species of minor or of unknown importance as vectors in BYDV transmission are *Sitobion avenae* (Fabricius) and *Rhopalosiphum maidis* (Fitch) in Argentina; *Sipha flava* (Forbes) in Brazil; *R. maidis*, *R. rufiabdominalis* (Sasaki), and *Geoica setulosa* (Passerini) in Chile; and *S. graminum* and *R. padi* (L.) in Uruguay (IICA 1976).

BYD Symptoms and Identification

Workers in Brazil were the first to positively relate BYD symptoms to the causal agent, in 1967, in Rio Grande do Sul. Later on, the disease was observed in all the wheat growing areas of the country (Convenio IICA-Cono Sur/BID 1982). Caetano (1984) indicated that no studies with serology had been conducted on BYD isolates in Brazil, but vector-specific as well as vector-nonspecific isolates had been detected, transmitted by *R. padi*, *R. maidis*, *S. avenae*, *Metopolophium dirhodum* (Walker), and *S. graminum*. He observed variation in symptom severity for vector-specific isolates, evidence of pathogenic variability. Further studies will be necessary to determine sources of tolerance or resistance.

In Chile, the virus was identified in 1972 by transmission tests to wheat using *M. dirhodum* and *R. padi* as vectors and infected oat plants as the source of inoculum (Tollenar and Hepp 1972). The virus was observed in phloem cells of barley and wheat with an electron microscope in 1976 (Caglevic and Urbina 1976). A mixture of PAV- and MAV-like isolates is prevalent in the southern area of Chile, while the PAV-like variants is predominant in the north (Herrera and Quiroz 1984). Using the enzyme-linked immunosorbent assay (ELISA), an isolate from oats, cv. Nehuén, was identified as PAV-like (Herrera 1984). No other variants have been described for Chile.

Leaf samples of oats, wheat, and barley from La Estanzuela Experiment Station, Uruguay, were tested by ELISA using anti-PAV serum. All these samples showed a positive reaction (Herrera 1986). This was the first serological determination of BYDV for Uruguay. For Brazil, Herrera detected a positive reaction using anti-MAV serum (Quiroz and Herrera 1986). Reports from Argentina (1986) have described transmission of a PAV-like variant by *S. avenae*. There is no information of known BYD variants from other Southern Cone countries. (PROCISUR 1986).

Frequency of Epidemics

Since the appearance of *M. dirhodum* and *S. avenae* as BYD vectors in the Southern Cone during the late 1960s and early 1970s, epidemics were observed in Brazil from 1967 to 1971, and BYD has been endemic since then, occurring with varying severity in the different wheat growing areas of this country (Caetano 1982).

Argentina reported widespread occurrence of symptoms in 1976. In 1981 the disease was epiphytotic in the Balcarce area and high losses were observed at other locations (Parisi 1982; De Biasi 1984).

For Chile the most serious epidemic occurred in 1975, but there were also severe losses due to BYD in 1976 and 1977 (Caglevic 1977; Cortázar 1984b; Madariaga 1986).

There are no recent data on epidemics from Bolivia, Paraguay, or Uruguay. It seems that BYD has been more severe in Brazil and Chile than in Argentina.

Losses Due to BYD

Economic losses due to BYD have been reported from Argentina, Brazil, and Chile. Although there are no figures available for Bolivia, Paraguay, and Uruguay, there are strong indications that BYD is causing damage to the winter cereals production in these countries.

In Argentina yield losses associated with the aphid-virus complex averaged 10% during 1971 to 1975, a production loss of 2.4 million tons (IICA 1976). There are no specific losses attributed to BYD in recent years, although high severity symptoms were seen in commercial areas in 1981 and in 1986 (De Biasi 1984, 1986).

For Brazil, Caetano (1982) estimated damage to crop production at 20 to 30% in the main wheat region, resulting in a loss of between 3.1 and 5.4 million tons of wheat during the 1967-1977 period.

In Chile, estimates of losses ranged from 10 to 60% for different areas of the country during 1975, 1976, and 1977 (Herrera and Quiroz 1984). Caglevic (1978) estimated 20 to 40% losses during the 1975/76 epidemic, and Van der Bosch (1976) evaluated the damage during this epidemic to be between $15 million and $20 million.

Cortázar (1984a, b) has analyzed losses caused by BYD by estimating the effect of the disease on the hectoliter weight of wheat. This parameter has proved to be an effective tool to compare tolerance or susceptibility under Chilean conditions. At La Platina Experiment Station, he detected losses of 20, 15, 13, and 7% for the years 1975, 1976, 1977, and 1978 respectively. No damages were determined between 1979 and 1981. For the last 5 years, from 1982 to 1986, losses were 19, 9, 9, 5, and 12%, at La Platina Experiment Station for the group of cultivars that has been used to evaluate BYD incidence from 1976 to 1986. The average yield loss for the 15 cultivars in this trial was 11% for the period 1976-1985 (Quiroz and Herrera 1986).

Control

Integrated vector control programs and the use of tolerant or resistant germplasm in breeding work have been carried on since the 1970s in Brazil, Chile, and Argentina. Bolivia and Uruguay are now training people and starting their own integrated control programs.

The testing of germplasm from different sources in the Southern Cone has resulted in the selection of some very outstanding material. In 1983 a cooperative nursery was started within the framework of the PROCISUR (Programa Cooperativa de Investigacion Agricola) Cooperative Research Project and coordinated by the Wheat Program INIA/CIMMYT, Chile. This nursery is called LACOS (Southern Cone Wheat Advanced Lines). These selected lines are C.A. St. Pierre, at the University of Laval, Quebec, Canadá (Comeau and St. Pierre 1984, 1985, 1986).

This project has shown that germplasm from Brazil is a highly promising source of tolerance to BYDV. Some lines from Argentina, Chile, and Uruguay are also mentioned as having good resistance to this disease.

The Present Situation

In a 1986 meeting of the Southern Cone countries, at Pergamino Experiment Station, Argentina, on virology and entomology of winter cereals (Madariaga and Gerding 1986), there was agreement on the need to look for efficient, inexpensive, and simple methods of BYD diagnosis to aid in selection of tolerant germplasm in large-scale tests.

It was proposed that Chile, where ELISA tests have been operated since 1983, could analyze samples from other Southern Cone countries. This work is now being started. At the same time, the samples will be sent to INTA-Argentina and Purdue-USA, where Dr. Lister has offered to examine some diseased material from Argentina (De Biasi 1986).

Other points discussed at the Pergamino meeting were the need for training research workers in centers where advanced investigations of BYDV and vectors are currently carried on and to start or encourage research related to the production of antisera, since Southern Cone countries do not have this capability at this time, and reagents from outside sources are quite expensive. It was agreed that countries like Bolivia, Paraguay, and Uruguay need to make simple field trials under natural infection conditions, to evaluate and quantify yield losses caused by BYD. This is important to help in finding funds to expand BYD-aphid research.

On another aspect, it was agreed to operate cooperative BYD diagnosis and evaluation tests in all Southern Cone countries, under uniform design and specific treatments of permanent protection and unprotected controls. These trials will include a set of known reaction cultivars, plus three genotypes of the participant country (tolerant, susceptible, and commercial) (Madariaga and Gerding 1986).

There are several aspects of research on BYD that need to be emphasized at the present time and in the future. Some of these needs are summarized here.

- Further reliable sources of tolerance need to be found to be used in breeding new tolerant cultivars and elite BYD-resistant germplasm.
- Accurate estimates of the effects of BYD on yield and yield components are needed.
- Rating scales for foliar symptoms need to be standardized.
- Epidemiology studies of the aphid-BYD complex and its interaction with environmental factors are necessary.
- Artificial inoculation techniques and facilities, both at field and greenhouse levels, need to be improved, including the mass-rearing of vectors under controlled conditions to facilitate evaluation of germplasm and related studies.
- Biological and integrated control programs should be expanded to all countries in the Southern Cone.

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Barley Yellow Dwarf Virus in Chile

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Abstract

Since the widespread epidemic of barley yellow dwarf in Chile in 1975, the disease has decreased in severity. Research on the aphid-barley yellow dwarf virus complex is described.

A widespread epidemic of barley yellow dwarf (BYD) occurred in Chile during 1975, causing wheat yield losses of up to 30%. The disease decreased in severity in the following seasons and losses in the order of 5 to 10% were recorded. During the 1986/87 crop season, BYD increased in severity and production losses of up to 10% were observed in the central irrigated area of the country.

The decrease in the occurrence of BYD during the years following the peak epidemic of 1975 has been mainly attributed to lower aphid populations and the release of tolerant local cultivars.

Studies (on the aphid-BYDV complex) by the Instituto Nacional de Investigaciones Agropecuarias's wheat project during the past 10 years have been focused on determining the aphid population fluctuations, the effect of bioregulators on aphid control, and the virus incidence under natural infection at different sites in the wheat production area. In addition, the screening of material using artificial inoculation has been carried on in order to obtain parental lines and cultivars with higher levels of resistance. These lines and cultivars have then been utilized in the breeding program for resistance.

Barley Yellow Dwarf in Argentina

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Abstract

*Only recently has serious damage in barley (*Hordeum vulgare *L.) and oat (*Avena sativa *L.) forage crops been reported to be caused by barley yellow dwarf (BYD), and losses on wheat (*Triticum aestivum *L.) have not been assessed. In 1981 an epiphytotic associated with the virus complex was observed in two areas of Buenos Aires Province. Tests using enzyme-linked immunosorbent assay on wheat, barley, and oat samples from Buenos Aires Province in 1984 and 1985 showed the presence of both PAV- and MAV-like isolates, with PAV-like being prevalent. The three important aphid vectors of BYD are* Schizaphis graminum *(Rondani),* Metopolophium dirhodum *(Walker) and* Rhopalosiphum padi *(L.).*

The occurrence of barley yellow dwarf (BYD) in Argentina was confirmed in 1978 by transmission using *Metopolophium dirhodum* (Walker) as the vector (De Biasi et al. 1978). Evidence of its presence had been reported previously (Borlaug and Gibler 1964; Tomaso 1975). During 1984 and 1986 samples of wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), and oats (*Avena sativa* L.) from Argentina were tested by R.M. Lister of Purdue University for barley yellow dwarf virus (BYDV) using enzyme-linked immunosorbent assay (ELISA) with positive results.

Distribution

Symptoms normally associated with BYD have been observed throughout the small grains production region (Figure 1) and also in marginal areas, such as Chaco Province in the northwest of the country. During 1984 and 1985 the main surveyed area was Buenos Aires Province, where an increasing incidence was regularly associated with the presence of different vectors. However, both incidence and vector population dropped during the 1986 season, possibly due to the frequency of rainfall.

BYDV Isolates

Sixteen samples of wheat, barley, and oats in 1984 and 28 in 1986 were collected from different commercial cereal fields located in Buenos Aires Province and tested for the presence of BYDV by Lister. A PAV-like isolate was found in 63 and 65% of the samples in 1984 and 1986, respectively. An MAV-like isolate was found on two samples in mixed infection with PAV in 1984. Antisera against PAV-, MAV-, and RPV-like isolates were used in these tests. The negative results obtained from the rest of the sample could be due to the presence of other BYDV isolates or the result of confusing symptoms.

We suspect that an SGV-like isolate could be the main isolate involved in the severe damage observed on barley and oats forage crops sown early in fall, because *Schizaphis graminum* (Rondani) is the aphid observed in these crops.

There are no available data in relation to the presence and distribution of BYDV isolates from other areas of the cereal region.

Vectors

In Argentina six cereal aphid species have been recorded: *S. graminum*, *M. dirhodum*, *Sitobion avenae* (Fabricius), *Rhopalosiphum padi* (L.), *R. rufiabdominalis* (Sasaki), and *R. maidis* (Fitch). Transmissions of BYDV have been obtained with all these vectors except *R. maidis*. However, few attempts have been made with *R. maidis* up to now. At present, *S. graminum* is considered the most important vector for oats and barley, which are colonized early after the emergence of seedlings. This aphid is normally restricted to the south and the southwestern areas of the Argentine cereal growing region.

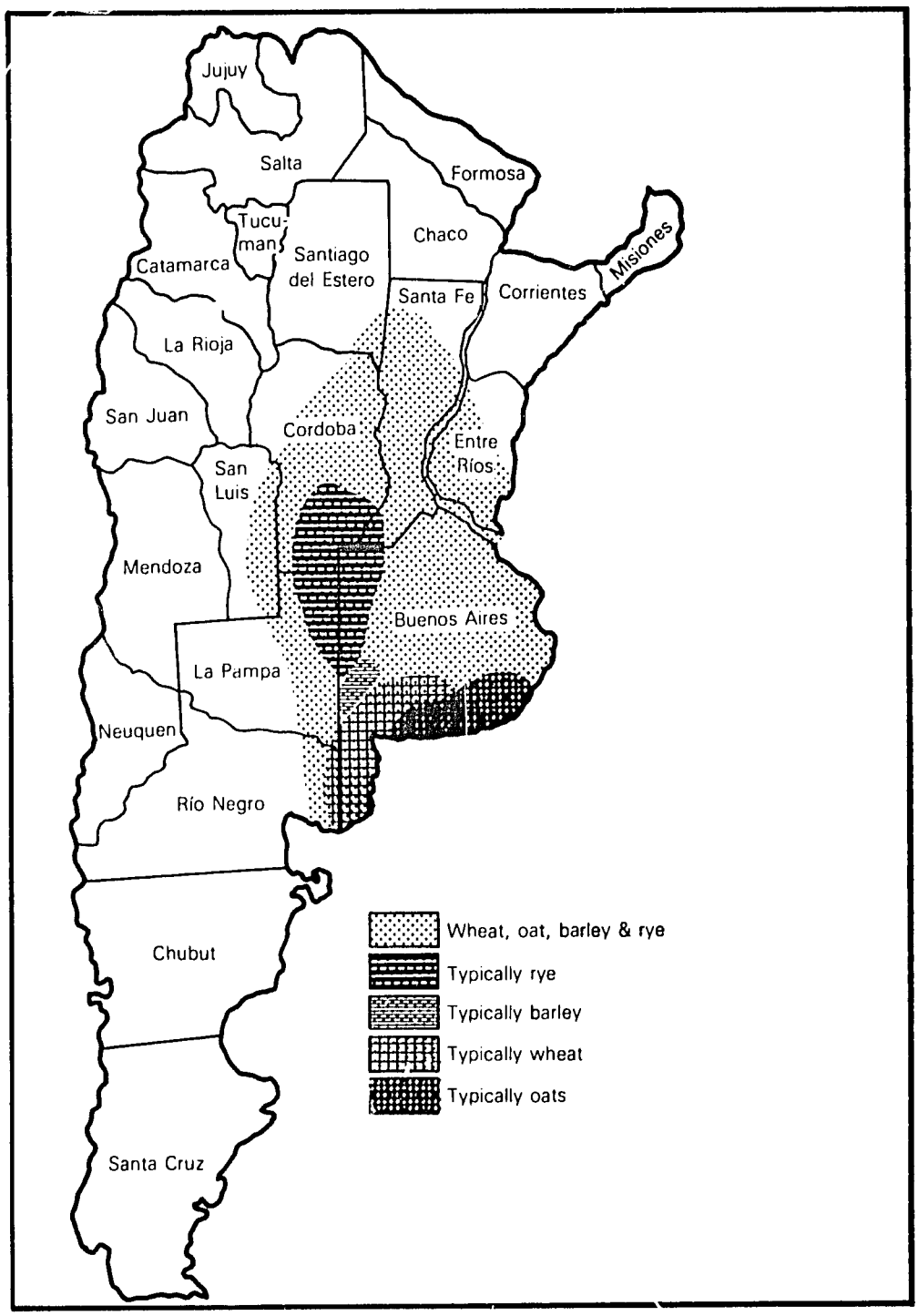

Figure 1. Argentina small grains regions.

characterized by cold and dry weather during winter. However, it is also present in the central and east central areas, which have higher temperatures and rainfall.

Heavy losses due to *S. graminum* were reported in 1937, 1951, 1952, and 1966 (INTA 1975). No evidence of its capability for transmitting BYDV was available until 1971 when C.A. Martinez (personal communication) used this aphid to obtain infection from oats to barley at Pergamino Experiment Station, north of Buenos Aires.

M. dirhodum, the second most important vector, suddenly appeared feeding on small grains in 1968. Its population reached explosive levels in 1969 and it was reported causing damage in wheat crops in 1971, 1972, and 1974 (INTA 1975).

In 1981 a high incidence of BYDV on durum wheat (*Triticum turgidum* L. var *durum*) in Balcarce (southeast of Buenos Aires) was related to the presence of *M. dirhodum*. Some fields and many experimental plots of bread wheat, oats, and barley were also observed severely damaged in Castelar (northeast of Buenos Aires), whereas in the southwest the incidence was only occasionally high. This aphid is still widely distributed in the cereal growing region, but its population decreased after the mid-1970s.

The *R. padi* population level has increased noticeably during the last few years, and it should be considered a potential vector. However, the cereal samples sent to R.M. Lister in 1984 and 1986 yielded no positive reaction to RPV in the ELISA test.

In 1972 severe damage produced by *S. avenae* was observed through the cereal growing area. In 1984 an MAV-like isolate was identified by Lister from oat samples collected at Balcarce.

R. rufiabdominalis was also found as a BYDV vector in a wheat nursery conducted during the summer at Balcarce. This aphid, along with *S. graminum*, is present in the province of Chaco as well, where a high incidence of BYDV was recorded on wheat plots at Saenz Peña Experiment Station in 1985.

Since 1979, entomologists from 12 experiment stations of Instituto Nacional de Tecnologia Agropecuaria (INTA) have developed a project on the identification of aphid species and biotypes, establishment of biological parameters, population dynamics, evaluation of losses, and integrated control measures. However, at present these studies are limited, due to the preponderance of other pests and the scarcity of financial resources.

Losses

Only recently was serious damage due to BYD on early sown fall oat and barley forage crops observed. This was related to a high level of *S. graminum*.

On the other hand, the effect of BYDV on wheat under prevailing conditions in the normal cereal growing season is not known.

In 1984 and 1985 fields trials conducted on five wheat varieties infected under controlled conditions using *M. dirhodum* as a vector showed that all cultivars exhibited conspicuous symptoms of the disease. Taking into account both symptoms and yield components, one cultivar in this study was slightly less affected.

Future Research

Major efforts on BYDV research work are limited due to the lack of resources in INTA. Our aim in attending the BYDV Workshop, besides the benefit of exchanging information or views with others researchers, is to establish any kind of cooperation and also to obtain international financial support to develop research work on the main areas indicated below:

- efficient diagnostic methods
- distribution of isolates in the whole cereal region
- crop losses on wheat
- control strategies

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Barley Yellow Dwarf in Britain

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Abstract

Barley yellow dwarf virus (BYDV) is the most important virus disease of cereals in Britain, with early sown winter crops particularly at risk. The Rothamsted type BYDV isolates can be divided into three groups, which are serologically similar to the PAV, RPV, and MAV isolates of Rochow and also share similar aphid transmission specificities. British field isolates can also be divided serologically into the same three groups, all of which commonly occur. The results of testing plants infected singly with trap-caught alatae from throughout Britain are given for 1984 and 1986. Current work includes the characterization of a range of field isolates to examine any variation in serology and aphid transmission specificity present in British isolates.

Barley yellow dwarf (BYD) is the most important virus disease of cereals in Britain with up to 90% of crops infected in some years (King 1977). The principal aphid vectors of BYDV are ubiquitous, and Britain's extensive areas of perennial grasses, most of which are also infected (Holmes 1985), provide important reservoirs of virus and vectors. The change in recent years from spring to winter barley (*Hordeum vulgare* L.) and the trend to earlier sowing (September) of winter cereals have put these crops at more risk. The epidemiology of the disease in Britain has already been described in an earlier situation report (Plumb 1984) and in more detail by Plumb (1981, 1986).

Current Situation

Yield losses attributable to BYDV of 30 to 40% have been measured in field trials of winter barley under natural infection at Rothamsted (Dawson et al. 1986) and much greater losses have been recorded elsewhere (Doodson and Saunders 1970). Losses nationally, in British cereal crops, are not known. Yield reductions of up to 26% have been reported for various ryegrass species (Catherall and Parry 1987).

Control

Current control of BYDV in cereals in Britain depends largely upon aphicide sprays, although a winter barley (cv. Vixen), which contains the *Yd2* gene (Parry and Habgood 1986), has been provisionally recommended for use where BYDV is a frequent problem. It is now commercially available. A forecasting system for BYDV risk is in operation and advice is given in October and November, on a regional basis, about which crops require autumn aphicide spraying (Plumb 1986).

The potential of naturally occurring compounds (known as antifeedants) that affect the behavior of the vectors, as alternatives to the broad spectrum insecticides currently used, is being investigated at Rothamsted. In 1984/5 field trials of winter barley, antifeedants derived from aphid alarm pheromone decreased virus infection and increased yields. In the 1985/6 trials, another antifeedant (polygodial), obtained from the water pepper *Polygonum hydropiper* L., decreased virus infection from 43 to 25% and increased yields from 3.83 to 5.22 t/ha under high disease pressure (Dawson et al. 1986).

Isolate Classification

Recent work at Rothamsted has looked at the classification of British BYDV isolates. The Rothamsted type isolates (Plumb 1974) can be divided into three groups, which are serologically indistinguishable in enzyme-linked immunosorbent assay (ELISA) from the PAV, RPV, and MAV isolates of Rochow (1969) and share similar transmission

specificities (Table 1). Serological differentiation was achieved using three strain-specific monoclonal antibodies supplied by L. Torrance, MAFF Harpenden Lab, Hatching Green, Harpenden, Herts. (Torrance et al. 1986; A. Pead and L. Torrance, personal communication). A fourth Rothamsted isolate, "B" (Plumb 1974), was shown to be a mixture of a PAV-like and an RPV-like isolate. British field isolates can also be divided serologically into the same three groups, all of which commonly occur in England and Wales. The results of testing plants infected singly with trap-caught live alatae from throughout Britain are shown in Table 2.

Table 1. Comparison of three UK BYDV isolates with three North American isolates using three monoclonal antibodies in ELISA and transmission by various aphid species

Isolate		Absorbance values in indirect ELISA[a]			Transmission frequency[b,c] (%)				
		MAC 91	MAC 92	MAFF 2	*Rp.*[d]	*Sa.*	*Md.*	*Mf.*	*Rm.*
G	(UK)[e]	1.10	0.01	0.01	72	20	26	2	16
R568	(UK)	0.03	0.36	0.01	66	0	9	21	13
F	(UK)	0.04	0.02	1.06	9	55	38	20	2
P-PAV	(USA)	0.96	0.01	0.04	63	21	-	-	-
RPV	(USA)	0.02	0.30	0.00	100	0	-	-	0
MAV	(USA)	0.01	0.01	1.14	3	100	-	-	0
Healthy sap		0.01	0.01	0.01					

[a] Means of duplicate wells. Mixed rabbit polyclonal anti-BYDV coating. Positives underlined.

[b] Data for U.K. isolates from G. Herrera (unpublished). Each figure based on 50 single aphids.

[c] Data for U.S. isolates for Rochow isolates (Rochow 1969). Ten aphids per plant except PAV which was from single aphid transfers.

[d] *Rp: Rhopalosiphum padi; Rm: R. maidis; Sa: Sitobion avenae; Md: Metopolophium dirhodum; Mf: M. festucae.*

[e] U.K. isolates were fresh leaves, extracted 1/15 (w/v). U.S. isolates were dried leaves, extracted 1/20 (w/v) and obtained from R. Lister (Purdue). RPV and MAV are Rochow isolates maintained at Purdue and P-PAV is Purdue PAV (Hammond *et al.* 1983).

Table 2. Frequencies of BYDV strains in two studies of British field isolates

Study	No. of plants[a] with given BYDV strain				
	PAV-like	RPV-like	MAV-like	PAV+RPV	Total
1984 (Summer + autumn)[b]	27(30)	16(18)	42(47)	5(6)	90(100)
1986 (Autumn only)[c]	13(41)	14(44)	4(13)	1(3)	32(100)

Figures in parentheses are percentages.

[a] Plants inoculated with single trap caught aphids from a number of sites throughout England and Wales.

[b] Torrance et al. (1986).

[c] D. Wright (unpublished), MAFF Harpenden Labs., Hatching Green, Harpenden, Herts., U.K.

The larger numbers of MAV-like isolates detected in the 1984 study of isolates of BYDV transmitted by aphids caught alive in suction traps was because summer flying *Sitobion avenae* (Fabricius) were included. In 1986 only autumn trap catches, which are mainly *Rhopalosiphum padi* (L.), were tested. Holmes (1985) found a predominance of PAV-like strains (35 and 44% of samples in May and July respectively) over MAV-like strains (9 and 10% of samples in May and July respectively) in perennial ryegrasses from farms around Auchincruive, Ayr, in Scotland

The classification of BYDV devised by Rochow (1969) has proved useful in describing the British BYDV isolates examined so far. Future work at Rothamsted will examine the serology, virulence, and transmission characteristics (using five aphid species) of a wider range of British isolates to look for variation within groups and check the validity of the classification for British isolates. A small number of isolates have already been found that do not react with any of the three monoclonal antibodies mentioned above though the biological significance of variation at this level remains unknown. Future survey work should discover any regional variation in BYDV strain abundance within Britain and follow any changes over time.

Acknowledgment

The help of S.M.D. Forde for part of this work is gratefully acknowledged.

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The Barley Yellow Dwarf Virus Situation in Western Europe

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Abstract

Barley yellow dwarf (BYD) is omnipresent in western Europe, being more or less important depending on weather conditions, latitude, and crop cultivated. Its importance increases north to south. Rhopalosiphum padi *(L.) is the most widespread and important aphid vector. Three isolates—PAV-, RPV-, and MAV-like-can be found; PAV-like is the most severe, being ubiquitous. Depending on latitude, sowing date, and weather conditions, aphicide treatments are very often necessary to avoid yield reduction. For this purpose a forecasting system was developed in central France. Rice (*Oryza sativa *L.) cultivars resistant to BYD have been released in Italy.*

The importance of barley yellow dwarf virus (BYDV) increases from north to south in western Europe because the conditions for aphid multiplication and overwintering are better in the south. The problem is important in areas where barley (*Hordeum vulgare* L.), wheat (*Triticum aestivum* L.), oats (*Avena sativa* L.), maize (*Zea mays* L.), or rice (*Oryza sativa* L.) are grown.

In 1958 BYDV was reported from Belgium (Roland 1960), France (Bogavac et al. 1968), and Italy (Osler 1984). Since then BYDV has been recorded in all the other countries of western Europe. The main outbreaks observed have been in Italy (on rice in 1972-73-74, on barley in 1977 and 1978), in Belgium (Cavelier and Maroquin 1978, 1984), in France on barley in 1975-76, in Sweden on oats (Lindsten 1978), and in Switzerland on barley and oats (Gugerli and Derron 1981).

The Situation

In the northern part of France, beginning 50 km north of Paris, BYDV is often present in early sown winter barley or in late-sown spring barley and oats. However it is not present every year. Barley yellow dwarf (BYD) is not observed on wheat and maize; wheat being grown mainly after sugar beets and sown late in the fall (Cavelier and Maroquin 1984), and maize being cultivated for silage, using generally tolerant cultivars. The maize crop is grown from May to October.

Aphids generally start flying in June, and *Rhopalosiphum padi* (L.) is the main BYDV vector. During wintertime aphid populations disappear and are very low the following spring unless temperatures during winter are mild. Under normal conditions (severe winters) *R. padi* arrive in maize fields very late in the season (end of June, beginning of July), by which time the maize has 6 or 7 leaves, and is less susceptible to BYD infection. No effect is observed when maize is infected with BYD at this growth stage.

Aphid migrations continue until mid-November. If winter barley is sown of the end September, the vectors (mainly *R. padi*, *Sitobion avenae* (Fabricius), and sometimes *R. maidis* (Fitch)) can infest and multiply on young cereals until the first frost, allowing a large percentage of the plants to be infected with BYDV (Cavelier and Maroquin 1978, 1984). In the spring barley and oat crops sown in the northern part of western Europe, the migrating aphids from the south generally arrive too late to induce epidemics of BYD unless the crops are sown very late.

From 50 km north of Paris to the south of Europe, the situation is more critical every year, mainly due to larger aphid populations, but also because of the overlapping of winter and summer cereal crops (maize and rice). Forage grasses in all of western Europe are infected with BYDV.

The vectors
The main aphids found in western Europe in order of importance are: *R. padi*, *S. avenae*, *Metopolophium dirhodum* (Walker), *Schizaphis graminum* (Rondani), and *R. maidis*. *R. maidis* is found more frequently in the north in years with higher autumn temperatures.

R. padi generally come from maize (Moreau et al. 1986; Moreau and Lapierre 1977), mainly in the south, and from forage grasses, wild Gramineae, and barley volunteers (Dedryver and Robert 1980).

When there are severe winters the population of *R. padi* is usually decimated, and in spring new populations start from the eggs laid in the fall on *Prunus padus L. R. padi* coming from eggs are not viruliferous.

S. avenae, *M. dirhodum*, and *S. graminum* are present throughout the year, migrating in June from barley to wheat, then to maize, other grasses, or volunteer barley, where they wait for the new shoots of winter cereals.

R. maidis is always present in the south, mainly in the grain-maize growing areas. This aphid migrates north during the months with warm temperatures. Fortunately it is an inefficient vector of the BYDV isolates present (Bayon and Ayrault 1983).

The isolates occurring
PAV-like isolates are the most prevalent, the most severe, and the most dangerous, being nonspecifically transmitted. MAV-like isolates are the second most important in percent and severity, the frequency of this isolate decreases north to south. The RPV-like isolate is ubiquitous, like the PAV-like, but fortunately it is not severe. These three isolates often occur in mixtures of two or three. PAV- and RPV-like isolates have been found infecting maize in southern France (Signoret and Alliot 1980). SGV- and RMV-like isolates have not yet been found.

ELISA tests and aphid transmission should be used to distinguish isolates (Maroquin et al. 1985). To produce specific antisera for ELISA, pure virus isolates are required; this work has been undertaken in some laboratories of western Europe.

Control
Modification of the sowing date may be used for winter barley and spring cereals to avoid infestations by aphids at a very susceptible growth stage of the cereals (Cavelier and Maroquin 1984).

Chemical control of aphid populations in small grain cereals is done in several areas. In the north of France and in Belgium, one insecticide treatment may be applied toward the end of October or the beginning November. South of Paris, two or three applications of aphicides are often necessary to avoid yield reduction. In the Mediterranean region, where durum wheat (*Triticum turgidum* L. *var durum*), which is generally more susceptible to BYDV than bread wheat, is largely cultivated, the situation is more critical.

A forecasting system has been developed in the Poitou-Charentes region of France (Bayon et al. 1983; Bayon and Ayrault 1983) for chemical control of BYDV in order to limit the number of treatments (see also Bayon, these Proceedings) and to determine the date of application.

The best solution is with resistant cultivars. For several years rice cultivars have been selected for resistance in Italy (Moletti and Osler 1978).

Work on selecting resistant maize and ryegrass lines is in progress in several countries in Western Europe (Federal Republic of Germany, Belgium, France and Italy).

Acknowledgments
We are indebted to the following colleagues who gave us information: A. Kurppa (Finland), P. Oxelfelt (Sweden), W. Huth (Federal Republic of Germany), M. Conti (Italy).

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Barley Yellow Dwarf in Spain

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Abstract

Barley yellow dwarf virus (BYDV) appears to be widespread in Spain although it does not cause consistently serious epidemics. A survey of the isolates in the cereal growing areas was carried out and the different isolates characterized through serial aphid transmissions and enzyme-linked immunosorbent assay. The prevalent isolate is similar to PAV. In rice the isolate found is of the RPV type. A comparative study of Spanish and American BYDV isolates is underway.

In Spain, barley yellow dwarf virus (BYDV) does not cause serious epidemics, although it is present in most cereal growing areas. Barley yellow dwarf (BYD) appears only in small patches within the fields and along the borders and is not economically important at the present time. The isolate infecting rice, however, was a serious problem for many years before the sowing dates were changed (Jordá et al. 1987).

The present work deals with the distribution of the virus isolates in the cereal growing areas, including areas of rice culture.

Material and Methods

Material suspected of being infected with barley yellow dwarf virus was obtained from the important cereal areas and analyzed. When possible, the areas from which promising samples were obtained or where the Spanish Extension Service suspected BYD were visited and cereal and other graminaceous plants were sampled. The disease symptoms and distribution were examined and data on previous incidence in the area were collected. The vector population was also examined and sampled.

Colonies of the vectors *Rhopalosiphum padi* (L.), *R. maidis* (Fitch), *Sitobion avenae* (Fabricius), *Metopolophium dirhodum* (Walker), and *Schizaphis graminum* (Rondani) were used to test virus transmission. These colonies were maintained on healthy plants. The aphid species were fed on the samples obtained from the field and were then allowed to feed on *Avena sativa* L. seedlings as test plants. The plant material sampled from the field was examined routinely with an electron microscope for any recognizable virus particles.

The virus was transmitted serially at least eight times, and the final isolate was checked by transmission with a range of vector species and by serology.

To produce antisera, virus was purified using the technique described by Rochow et al. (1971). Double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) was used for testing samples. The blank response was reduced through incubation of the virus antisera with healthy plant purified extracts (Lister and Rochow 1979; Osler 1984; Rochow et al. 1971; Rochow and Carmichael 1979).

Results

The aphid transmissions showed that the general isolate present in winter cereals was transmitted by both *R. padi* and *S. avenae*, while the rice isolate was only transmitted by *R. padi*.

The first serology study was carried out by absorbing Spanish antisera with American isolates and using the

mixture as a sample to be tested in a normal DAS-ELISA with American antisera (Table 1).

Reciprocally purified extracts from field samples, American strains, and the Spanish isolates that had been characterized by aphid transfer were checked with different antisera (Table 2). These results confirmed those shown in Table 1. The isolates from Lérida and Sevilla showed a close relation with PAV, whereas the rice isolate Enrojat 7, characterized by aphid transfer, appeared to be very similar to RPV. Other isolates, such as Enrojat 1, showed the presence of a mixture of isolates.

The most commonly found isolate was a PAV-like type, and the Spanish rice strain was similar to the RPV-like type in general aspects.

Table 1. Evaluation of some Spanish antisera with American strains using DAS-ELISA

Antisera to purified BYDV American serotypes \ Spanish antisera	Reaction with a mixture of the virus and Spanish antisera (Absorbance 405nm)			
	PBS	055	sn	060
RPV	1.362	1.345	1.380	1.312
MAV	1.426	1.077	0.843	0.367
PAV	1.230	0.002	0.001	0.000
RMV	0.511	0.496	0.517	0.517

PBS = phosphate-saline buffer.
055, sn, and 060 = Spanish antisera against different BYDV isolates.

Identical strains will produce a "0" reading.
Test performed in W.F. Rochow's laboratory at Cornell, USA.

Table 2. Evaluation of some Spanish isolates with American and Spanish antiseras using DAS-ELISA

Sample	Reaction with specific antibodies of BYDV (Absorbance 405nm)					
	American				Spanish	
	RPV	MAV	PAV	RMV	060[a]	Enrojat[b]
Lerida II	0.003	0.066	0.322	0.002	1.950	0.272
Enrojat 7	0.374	0.005	0.003	0.025	0.207	0.572
Enrojat 1	0.248	0.105	0.103	0.171	–	–
Healthy oats	0.022	0.003	0.001	0.004	0.139	0.172
RPV	0.748	0.006	0.008	0.039	–	–
MAV	0.000	0.514	0.069	0.015	–	–
PAV	0.001	0.120	0.582	0.021	–	–
RMV	0.005	0.003	0.000	0.241	–	–

[a] antiserum against infected plants from Sevilla (PAV-like).
[b] antiserum against infected rice from Valencia.

A survey of BYDV distribution throughout Spain was carried out both by aphid transmission and with ELISA. The vectors *R. padi* and *S. avenae* and antisera against Lérida and Sevilla isolates (equivalent to PAV) and rice Enrojat (related to RPV) were used. Table 3 lists the results. Full agreement between ELISA and aphid transmission tests was obtained.

Table 3. BYDV infections of cereals from different areas of Spain

Area	Crop	ELISA	Aphid Transmission test	
			R.p.	S.a.
Ciudad Real	Barley	—	—	—
	Wheat	—	—	—
Cordoba	Barley	+ a	—	—
	Triticale	+ a	+	+
Granada	Wheat	+ a	+	+
Huelva	Wheat	—	+	+
Jaén	Oats	+ a	nd	nd
Lérida	Barley	+ a	+	+
	Maize	+ b	+	—
	Oats	—	—	—
	Wheat	+ a	+ —	+
Madrid	Wheat	+ a	nd	nd
Murcia	Barley	+ b	+	—
Navarra	Barley	+ a	+	nd
Sevilla	Barley	+ a	+	+
	Triticale	+ a	+	+
	Wheat	+ a	+	+
Soria	Barley	+ a	—	+
	Wheat	+ a	—	+
Teruel	Barley	—	—	—
Toleda	Barley	+ b	+	—
Valencia	Maize	—	—	—
	Rice	+ b	+	—
Valladolid	Barley	nd	+	+
	Wheat	nd	+	+

a = antisera type PAV, b = antisera type RPV.

R.P. = *Rhopalosiphum padi*, S.a. = *Sitobion avenae*. nd = not determined. Other transmissions were made with both *Schizaphis graminum* and *Rhopalosiphum maidis* but all were consistently negative except some from Lérida, + = transmission, — = no transmission.

The Lérida-Sevilla isolate was the dominant isolate and appeared not to be very damaging. The isolate transmitted exclusively by *R. padi* was found very rarely but the damage caused was considerable. An *S. avenae* transmitted strain was obtained once but not found again.

Two surveys of weeds for the presence of BYDV were carried out using ELISA, with aphid transmission being used occasionally as a check. The PAV-like strain was found in the common graminaceous plants recognized as hosts. Some non-graminaceous hosts were found to be infected with the rice isolate. This infection must be confirmed through detailed studies, because there is the possibility that other serologically related luteoviruses were being detected.

Acknowledgments

We thank W.F. Rochow for his collaboration on the Table 1 data and F. Montes for his collaboration in the work and his encouragement.

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Occurrence of Barley Yellow Dwarf Virus in Three Major Cereal-growing Regions of Spain

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Abstract

Enzyme-linked immunosorbent assay (ELISA)-based surveys during the three years 1985-1987, in three major cereal-growing areas of Spain, confirmed the presence of barley yellow dwarf virus (BYDV). Samples of small grain cereals and grasses with and without barley yellow dwarf-like symptoms were sent to Purdue University where they were screened by ELISA. The results indicated the presence of PAV-, MAV-, and RPV-like isolates of BYDV. PAV-like isolates were the most common type, and were present in all the sampled areas. Isolates like RPV were detected only in samples from the central region at El Encin, Madrid. Only one single sample, collected from El Encin in 1987, was unequivocally diagnosed as containing an MAV-like isolate. The possible impact of BYDV in these major cereal-growing regions is discussed with special reference to its epidemiology.

The luteoviruses grouped under the name barley yellow dwarf virus (BYDV) comprise the major virus disease problem in small grains worldwide (Burnett 1984). All the major cereals are susceptible, including barley (*Hordeum vulgare* L.), oats (*Avena sativa* L.), wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.), and rye (*Secale cereale* L.). Most isolates of BYDV examined so far appear to resemble one of 5 types, exemplified by 5 isolates distinguished by acronyms based on their primary vectors (Rochow 1970), as follows: RPV, transmitted principally by *Rhopalosiphum padi* (L.); RMV, by *R. maidis* (Fitch); MAV, by *Sitobion (=Macrosiphum) avenae* (Fabricius); SGV, by *Schizaphis graminum* (Rondani); and PAV by both *R. padi* and *S. avenae*.

These types are also distinguishable serologically, especially by enzyme-linked immunosorbent assay (ELISA), and such tests are now widely used for diagnosing (Lister and Rochow 1979; Rochow 1979; Rochow and Carmichael 1979). We report here on the occurrence and distribution of BYDV in three major cereal-growing areas in Spain, as revealed by ELISA-based surveys done in 1984-1987. The occurrence of BYD in several cereal-growing regions of Spain has previously been noted in several reports, primarily based on symptomatology (Alfaro and Montes 1984).

Materials and Methods

Sampling procedure

During the 1984-85 and 1985-86 cereal-growing seasons, plant samples were collected in 15 different localities among the three main cereal-growing areas of Spain. The sampling points were selected according to previous observations of barley yellow dwarf (BYD)-like symptoms made by various research and extension colleagues. As far as possible, cereal samples were selected from plants with BYD-like symptoms, but these were often difficult to define; and, for example, most of these collected at El Encin in 1986 (Table 1) were symptomless, as symptomatic plants were uncommon then. The grass samples collected were from symptomless plants representing various locations.

Most samples were of cereals: soft wheat, *Triticum aestivum*; durum wheat, *T. turgidum* L. var *durum*; barley; oats; and triticale, X *Triticosecale* Wittmack. The remaining samples were the following grasses collected from field borders or hedgerows: *Hordeum murinum*, *Festuca arundinacea* Schreber, *Bromus* sp., *Phalaris* sp., and wild oats, *Avena* sp.

Each sample was air-dried during 2 to 3 days at room temperature (around 20°C). The dried samples were then packed in

paper envelopes and mailed to Purdue University, where they were tested for the presence of PAV-, RPV-, and MAV-like isolates by ELISA. The effectiveness of ELISA in testing such material was established previously (Lister et al. 1985).

In 1987, a similar approach was made in sampling several areas in the central region, at El Encin and Aranjuez, near Madrid. This sampling was done in the course of a single visit by the first author. Again, leaves were packaged and mailed to Purdue University for testing.

ELISA test procedure

The virus isolates used as standards in each ELISA test plate were: MAV and RPV, which were subcultures of Rochow's isolates (Rochow 1969, 1970), maintained by mass-transfer between batches of oats, cv Clintland 64, during 6 years at Purdue University; and Purdue-PAV (P-PAV), a PAV-like isolate described previously (Hammond et al. 1983). Antisera used as sources of immunoglobulins (Ig's) for ELISA were a polyclonal (pc) MAV antiserum kindly supplied by Dr. W.F. Rochow and pc antisera to P-PAV and RPV developed at Purdue University. For testing the 1986 and 1987 samples a monoclonal (mc) antiserum to MAV (mc MAV Ig-1), kindly supplied by Dr. H.T. Hsu, became available, and was used as a crucial test for MAV-like virus.

The reactivity of the polyclonal antisera in ELISA was as previously described (Fargette et al. 1982). Those to P-PAV and RPV specifically detected homologous isolates; that to MAV also showed some cross reactivity with P-PAV. The mc MAV Ig-1 was specific to MAV as reported by Hsu et al. (1984). Polyclonal and monoclonal Ig's were used in standard double antibody and indirect sandwich ELISA's applied to BYDV as previously described, with alkaline phosphatase as enzyme and p-nitrophenyl phosphate as substrate (Clark et al. 1986; Lister and Rochow 1979).

Table 1. Incidence of BYDV in samples collected from cereals and grasses during the 1986 survey in central, southwestern, and northeastern Spain, as determined by ELISA with immunoglobulin (Ig's) from polyclonal (P-PAV and RPV) and monoclonal (MAV) antisera

Sampling location	Collection date	No. of samples (grasses)	ELISA-positive with Ig indicated		
			P-PAV	RPV	MAV
El Encin (Madrid)	April 22-June 12	158(32)	2	0	0
Aranjuez (Madrid)	May 6	8(0)	0	0	0
Etsia (Lerida)	May 7	15(0)	1	0	0
INIA (Cordoba)	March 5	8(0)	0	0	0
INIA (Cordoba)	April 24	4(0)	2	0	0
Carmona (Sevilla)	March 6	10(0)	0	0	0
Palmar De Troya (Sevilla)	April 25	12(1)	6(1)	0	0
Tomejil (Sevilla)	April 25	9(0)	5	0	0
Total		224	16	0	0

Most samples from El Encin were symptomless. Most cereal samples from elsewhere showed symptoms suggesting BYDV infection. Numbers not in brackets are totals; numbers in brackets are numbers of grass samples.

Duplicate wells of Immulon plates (Dynatech Inc.) were used for each sample, and the results were averaged. ELISA values (A405 nm) exceeding three times that for healthy plant extracts were regarded as positive. This high value was chosen to reduce the likelihood of false positives.

Results and Discussion

The results of the 1985 survey (Table 2) indicated that PAV-like isolates were present in all of the regions sampled. RPV-like isolates were found only in the central area close to Madrid (El Encin). Table 3, summarizing results for samples collected at El Encin in May 1985, illustrates a typical range of ELISA values obtained with immunoglobulins from the pc antisera used in this survey. Although "background" levels (i.e., values for control extracts from healthy plants) varied, positive values were generally clearly defined by exceeding 3x background. This criterion, however, confused P-PAV and MAV because of the cross-reactivity of P-PAV with pc MAV-IG, but interestingly, this did not occur with most of the PAV-like isolates identified in these samples, which reacted more specifically in ELISA than did P-PAV. Samples 12 and 14 in this series, which showed reactivity with pc MAV-Ig as well as with pc PAV-Ig, were later retested with mc MAV-1. Both were negative and so were assumed to contain PAV-like isolates behaving like P-PAV in ELISA.

Results of the 1986 survey (Table 1) indicated that the southwestern region (Sevilla and Cordoba) was the most affected by BYDV. In this survey, mc MAV-1 was used throughout for diagnosing MAV-like isolates. All of the positive samples collected in the southwestern region appeared to contain PAV-like viruses. In the central area, fewer samples were positive in the 1986 collections than in the 1985 collections, probably because of the low aphid population levels due to prevailing cold temperature during the spring (A. Fereres, unpublished data). Samples reacting positive with pcP-PAV-Ig were detected in the northeastern region close to Lerida in both 1985 and 1986 (Tables 1 and 2). In 1986, PAV-like isolates were again the most abundant type in all the sampled areas, although some samples from the central area reacted positively with pc RPV-Ig.

Table 2. Incidence of BYDV in samples collected from cereals and grasses during the 1985 survey in central, southwestern, and northwestern Spain, as determined by ELISA with immunoglobulins (Ig's) from polyclonal (pc) antisera

Sampling location	Collection date	No. of samples	ELISA-positive with Ig indicated pc PAV	pc RPV	pc MAV
El Encin (Madrid)	May 9-June 13	91	46	4	3[a]
Aranjuez (Madrid)	May 16	12	2	0	0
INIA Valladolid	May 21	14	4	0	0
Almacelles, Torregrosa (Lerida)	May 30	20	3	0	0
Almacelles, Sidamunt (Lerida)	May 31	12	0	0	0
La Rinconada (Sevilla)	May 10	5	2	0	0
Total		154	57	4	3

Cereal samples showed symptoms suggesting BYDV infection. Grass sample did not.

[a] Reactions were positive with pc MAV-Ig, but negative with monoclonal MAV-1-Ig (see text).

Overall, PAV-like isolates were detected in soft and winter wheats, oats, barley, triticale, and also in a grass, *Phalaris* sp.—the latter in the Sevilla area (Table 1). RPV-like isolates were found in oats and soft wheat samples.

Most cereal samples collected in the 1987 survey at El Encin showed at least some indication of BYD symptoms, although these were not obvious, and the general impression was that BYD was uncommon. However, most of the crops seen had been planted in November 1986, after fall aphid migration had occurred. Some grass samples (not yet identified) had leaf reddening but most were symptomless. The most interesting record in the collection was of an MAV-like isolate (Table 4) from a plant of selection NC 018871. Figure 1 presents the distribution in all 15 locations sampled of the three different types of isolate (PAV-, MAV-, and RPV-like), found in the 3-year survey.

Regarding the general epidemiology of BYDV in Spain, the first *S. avenae* alates in central Spain are usually detected in mid-April (Castanera, in press), a few days before the first samples positive for BYDV results were collected in the present study. *S. avenae* is the major cereal aphid in the central region (Castanera and Gutierrez 1985) and is likely to be most important as a BYDV vector during spring. On the other hand, the highest population peaks of *R. padi* occur during autumn, and are usually reached in October on perennial grasses and wheat volunteers (A. Fereres and P. Castanera, unpublished data), when cereal crops have not yet emerged. This means that *R. padi* is probably the main vector of BYDV during autumn in this area. Grasses and cereal volunteers may serve as a reservoir of the disease during winter and early spring. Both aphid species seem to be anholocyclic in this area and are able to reproduce during the winter on wheat volunteers and grasses (Fereres and Castanera 1985). Therefore,

Table 3. ELISA of cereal samples from El Encin, Madrid (collected 5-9-85) with immunoglobulins from polyclonal antisera to the MAV, P-PAV, and RPV isolates

Sample number or type	ELISA value with Ig's indicated		
	P-PAV	RPV	MAV
1	.142*	.486*	.056
2	.117*	.107*	.045
3	.173*	.223	.037
4	.050	.116	.059
5	.179*	.123	.048
6	.092*	.144	.044
7	.144*	.125	.051
8	.264*	.062	.029
9	1.731*	.519*	.041
10	.237*	.317*	.053
11	1.849*	.034*	.022
12	.428*	.400*	.205*[a]
13	.142*	.115	.027
14	1.519*	.103	.125*[a]
15	.737*	.126	.052
16	1.864*	.065	.030
MAV control	.026	.065	.702*
P-PAV control	.591*	.067	.152*
RPV control	.012	1.032*	.041
Healthy control	.021	.105	.040

Asterisked values are regarded as positive. All values listed were determined in the same ELISA plate.

[a] Samples reacting like the P-PAV control in relation to P-PAV and MAV Ig's.

Table 4. Incidence of BYDV in samples collected from cereals and grasses during the 1987 survey in tho central region of Spain, as determined by ELISA with immunoglobulins (Ig's) from polyclonal (pc) and monoclonal (mc) antisera

Sampling location	Plant type	No. of samples	ELISA-positive with Ig indicated pc P-PAV	pc RPV	mc MAV
El Encin	cereals	58	16	1 (1)	1[a]
El Encin	grasses	10	4	3 (1)	0
Aranjuez	cereals	28	12	2 (1)	0
Aranjuez	grasses	2	2	1 (1)	0
Totals		98	34	7 (4)	1

Most cereals collected showed BYDV-like symptoms; most grasses did not Numbers in parentheses are those also reacting positively with pc P-PAV Ig; i.e. mixed infections.

[a] Mean ELISA value was 0.340, compared with values for MAV check and non-infected control extracts of 0.950 and 0.050, respectively.

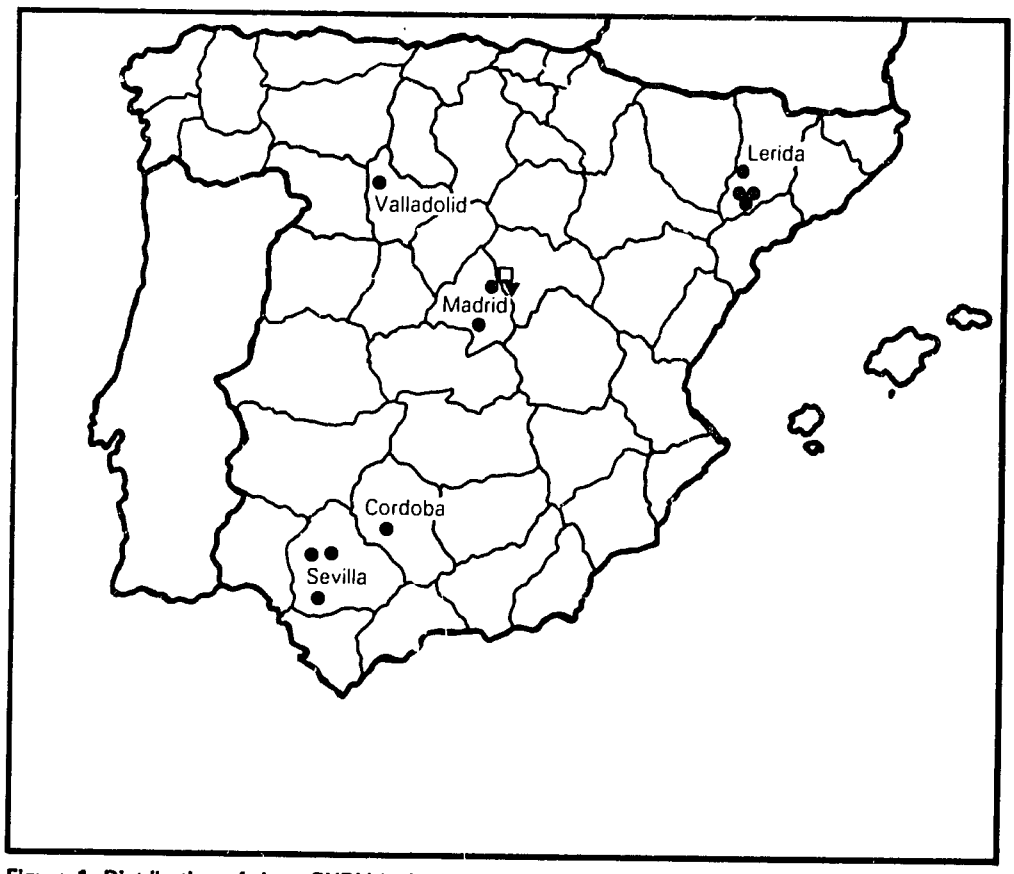

Figure 1. Distribution of three BYDV isolate types in Spain: PAV (•), RPV (□), and MAV (▲).

they are able to migrate during early spring, spreading the disease to adjacent cereal fields.

The epidemiology of the disease in the northwestern region seems to follow a pattern similar to that described above. *S. avenae* and *R. padi* are also the major cereal aphids found in this region, although maize (*Zea mays* L.) seems to play a more important role as a secondary host for aphids and virus during the summer (Pons 1986).

Because mild winters will help the spread of aphid vectors and the virus, BYDV has the potential for being especially important in southwestern Spain. BYDV appears also to occur widely in other major Spanish cereal-growing areas that were not surveyed. More extensive surveys of cereals and grasses are required to understand the epidemiology of BYDV in Spain, and measures for prevention or control should be considered.

Acknowledgments

We wish to thank various colleagues for help in locating and collecting samples, Jo Anne McFatridge for technical assistance, and the U.S.-Spain Joint Committee for Scientific and Technological Cooperation for funding the project.

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Barley Yellow Dwarf Virus Occurrence in Portugal

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Abstract

Over the past decade, agronomists working on cereals in the south of Portugal have noticed plants showing symptoms of barley yellow dwarf virus (BYDV) infection. In a survey of the aphids colonizing cereal crops, the BYDV vector Rhopalosiphum padi *(L.) was the predominant species in the first months of the year and* Sitobion avenae *(Fabricius) was also detected. In a preliminary study using the enzyme-linked immunosorbent assay, samples of wheat (*Triticum aestivum *L.), barley (*Hordeum vulgare *L.), oats (*Avena sativa *L.), and triticale (X* Triticosecale *Wittmack) plants were collected from farms located in southern Portugal and tested with a PAV antiserum. BYDV infection was found in several wheat, barley, oats, and triticale varieties.*

The south of Portugal is the most important cereal growing region of the nation. Over 60% of the area planted to wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), and oats (*Avena sativa* L.) is located there, and more than 70% of total yields are produced in the districts of Beja, Evora, and Portalegre.

For more than a decade, agronomists involved in crop improvement research programs have noticed plant symptoms suggestive of barley yellow dwarf (BYD). Those symptoms were leaf discolorations —yellow in barley and deep red to purple in oats—as well as various degrees of plant stunting.

At the same time, entomologists regularly surveying those areas identified the aphid species colonizing wheat, barley, and oats (Ilharco et al. 1982), including barley yellow dwarf virus (BYDV) vectors *Diuraphis noxia* (Mordvilko), *Metopolophium dirhodum* (Walker), *Rhopalosiphum maidis* (Fitch), *R. padi* (L.), *Schizaphis graminum* (Rondani), and *Sitobion avenae* (Fabricius). Such surveys showed a variation in aphid incidence over the last 13 years, with 1974 showing the highest population of aphids followed closely by 1982 (Table 1) (Ilharco et al. 1982; Lourenço and Pinto 1981 and in press; Pinto and Lourenço 1985). In 1974 a severe loss in wheat yield (ca. 80%) occurred in certain areas of Alentejo, where the vector *R. padi* was particularly abundant. The lowest incidence for the 13-year period was in 1979. In 1987, low to moderate aphid populations were observed.

Taking into account the biology of the most common aphid vector, *R. padi*, under our conditions, and the importance of the timing and the intensity of fall and winter rains (Ilharco et al. 1982; Lourenço and Oliveira 1983), an aphid population outbreak may occur in 1988 if high intensity winter rains come early in the season.

In 1987 enzyme-linked immunosorbent assays (ELISA) were initiated to determine BYDV infections.

The immunoglobulins (IgG) were prepared from an antiserum produced against a "type B" strain of BYDV (nomenclature of Plumb 1974), with

Table 1. Relative incidence of aphids in cereal crops (1974-1987)

Year	Incidence
1974	HH
1975	H
1976	L
1977	L
1978	L
1979	L
1980	M
1981	H
1982	L
1983	L
1984	L
1985	L
1986	L
1987	M

H = > 300/20 m^2.
M = 100-300 aphids/20 m^2.
L = > 100 aphids/20 m^2.

serological and transmission properties similar to the PAV strain (nomenclature of Rochow 1969), which is common in France and Switzerland. Freshly harvested leaf tissue, 3 to 5 g, was generally used, but frozen or dry leaf materials were also tried without an apparent difference in results.

The ELISA procedure followed closely that indicated by Clark and Adams (1974). Samples were ground in 2 vol of phosphate buffered saline containing 0.05% Tween 20 and 2% Polyvinylpyrolidon. Aliquots of 200 microliters were applied onto the wells of either freshly IgG-coated ELISA plates or on previously coated plates kept frozen at -20°C. The conjugate addition and substrate hydrolysis steps were carried out for 5 hours at 37°C and 1 hour at room temperature. The positive samples were determined by visual inspection of the yellow color developed in the wells or by reading the absorbance of the well contents in a spectrophotometer.

The results so far obtained showed BYDV infection in cultivars of wheat, barley, oats, and triticale (X *Triticosecale* Wittmack) (Table 2).

The preliminary results indicate infection levels of close to 10% for the Nazareno cultivar of wheat grown in the districts of Evora.

Work is now under way to determine levels of infection due to PAV as well as other strains of BYDV. Efforts will also be made to purify local strains of BYDV to produce a broad-spectrum antiserum for BYD diagnosis. The use of antisera produced against only one strain may significantly underestimate actual infection levels in the fields.

Tabla 2. Cereal cultivars infected with BYDV-B strain

Wheat	Barley	Oats	Triticale
Aranda	Beka	Avon	Bacum
Campodoro		Boa-Fe	Borba
Cocorit		São-Romão	
Tejo		Swan	
Nazareno			

Acknowledgments
Thanks are expressed to Mr. F. Rei, graduate student (University of Evora), for technical assistance in ELISA tests, Drs. F. Ilharco and A. Lourenço (Estação Agronômica Nacional, Oeiras) for information on aphid populations, and Drs. M. Barradas and F. Bagulho (Estação Nacional de Melhoramento de Plantas, Elvas) for information on crop symptomatology in past years.

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Barley Yellow Dwarf in Eastern Europe

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Abstract

Barley yellow dwarf (BYD) is a common disease of cereals, particularly winter barley (Hordeum vulgare *L.) and winter wheat (*Triticum aestivum *L.), in all East European countries; epidemics have been reported in Hungary and the German Democratic Republic. The principal aphid vectors are* Rhopalosiphum padi *(L.), and* Sitobion avenae *(Fabricius). PAV- and MAV-like isolates of barley yellow dwarf virus (BYDV) have been identified. The epidemiology of BYD in Hungary differs from that of Western Europe, and, in general, epidemics can be expected after summers that have been moist (with no extremes of rainfall or drought) and when autumns are warm and moist. The most important summer reservoirs of BYDV are volunteer plants, maize (*Zea mays *L.), and the meadow grasses. Cultural practices and resistance breeding are recommended for control.*

Barley yellow dwarf (BYD) is a common disease of cereals in all East European countries. Szirmai (1967) first reported this disease in Hungary. An epidemic occurred in winter barley (*Hordeum vulgare* L.) in Fejér County of Hungary in 1982 (Pocsai et al. 1983).

Barley yellow dwarf virus (BYDV) infects wheat (*Triticum aestivum* L.) (Szunics and Szunics 1984), maize (*Zea mays* L.) (Milinkó et al. 1984), and rice (*Oryza sativa* L.) (Pocsai et al. 1985), as well as barley (*Hordeum vulgare* L.).

There was a severe epidemic of BYD in winter barley in the German Democratic Republic (GDR) in 1984 (Proeseler et al. 1984; Kleinhempel and Nowak 1985). Therefore, in the GDR, routine diagnostic tests have been developed. These include bioassay using aphid vectors, enzyme-linked immunosorbent assay (ELISA), electron microscopy (EM), and immunosorbent electron microscopy (ISEM) (Haase et al. 1985; Stanarius and Proll 1986).

Since the detection of BYD in Poland in 1978, this disease has been observed in barley and oats (*Avena sativa* L.) in several districts of that country (Hoppe et al. 1983).

Szuchov et al. (1962) first described BYD from the USSR. Recently it has become a serious disease in winter wheat, barley, and triticale (X *Triticosecale* Wittmack) in the USSR (Nikolenko and Omelichenko 1985).

Damage

In spite of incomplete evidence, we believe that BYD is economically the most important virus disease of cereals in the East European countries. Autumn-sown cereals are damaged by this virus particularly if they are infected when young. Infection will reduce plant height, number of heads, number of grains, and 1000-grain weight. Very susceptible winter wheat varieties and lines may die in the following spring, and, in this case, the yield loss is 100% (Nagy and Milinkó 1986).

In Hungary Pocsai and Szirmai (1985) tested 14 barley cultivars for reaction to BYD; yields were reduced 57 to 83%.

In the 1984 epidemic in GDR, many hectares of winter barley had to be plowed up.

It is well known that there are large differences in virus incidence among years and among regions. However, we are of the opinion that BYD is growing to be a more and more important disease in our countries.

Vectors and Virus

The principal aphid vectors are *Rhopalosiphum padi* (L.) and *Sitobion (=Macrosiphum) avenae* (Fabricius) (Milinkó et al. 1983a, b; Kastirr et al. 1985; Kovács et al. 1987).

A BYDV isolate from GDR reacted with a PAV antiserum (Proll et al. 1985). On the other hand 10 BYDV isolates from West Hungary reacted strongly with the Swedish MAV and poorly with PAV antiserum in enzyme-linked immunosorbent assays (ELISA) (K. Lindsten and I. Milinkó, unpublished).

Epidemiology in Hungary

According to Nagy and Milinkó (1986), the epidemiology of BYDV in Hungary differs from that described in North European countries (Lindsten 1964; Plumb 1977) and France (Dedryver and Robert 1981). Moreover, it also seems to differ from that known in North America (Paliwal 1982). These differences may be due to the extreme continental climatic conditions of Hungary.

The weather during both summer and autumn may play a critical role in the survival and spread of BYDV. From our investigations it seems that a mild summer (not very dry and not very rainy) followed by a warm and dry autumn will cause a high level of BYDV infection of winter cereals. Furthermore, following a mild winter, when it is possible for the aphid vectors to overwinter in the cereal crop, and even when there is a moderately rainy spring, generally there will be a BYD epidemic.

Table 1. Effect of sowing data and density of winter barley stand on barley yellow dwarf infection, Táplánszentkereszt, Hungary, 1983-84

Variety	Plant density (m^2)	Sowing date Score BYD Incidence 0-5 Scale		
		23 September	5 October	19 October
GK 22 border on north edge of plot	ca. 500 seedlings	2.8	1.3	0.5
GK 22	300 seedlings	2.7	0.8	0.2
GK 22	500 seedlings	2.2	0.3	0.1
GK 22	700 seedlings	2.1	0.4	0.3
Kompolti/korai	500 seedlings	1.2	0.2	0.0
GK 22 border on south edge of plot	ca. 500 seedlings	2.5	0.9	0.3

Table 2. BYDV resistance evaluation of winter wheat varieties, Martonávár, Hungary, 1981-84

Varieties	1981	1982	1983	1984
		0 - 5 scale		
Very susceptible				
Martonvásár 9	5	4	5	2
Martonvásár 5	4	3	5	3
GK Boglár	-	3	5	3
GK Csongor	-	3	5	2
Moderately susceptible				
GK Tiszatáj	-	2	4	1
Martonvásár 6	-	2	3	-
Tolerant				
Martonvásár 8	-	0	1	0
Martonvásár 10	-	0	1	0
Jubilejnaja 50	-	0	0	0
Martonvásár 4	-	0	0	0

In early June, when the cereals begin to ripen, aphids migrate to the green maize fields and to volunteer cereal plants. In Hungary the most important summer reservoirs are the cereal volunteers, maize fields, and grasses. *Lolium* spp. proved to be a host in the GDR (Pfeffer and Pfeffer 1985).

Control

Integrated control of BYDV has recently been carried on in the GDR, Hungary, and the USSR.

Cultural—From our experiences the cultural practices that are important are: destruction of volunteer plants, late sowing in the autumn and early sowing in the spring, and insuring a compact crop stand. Table 1 illustrates this for barley.

Chemical—Treating the soil with granular insecticides and/or spraying with a systemic insecticide to control aphids is not widely used in Eastern Europe.

Resistance breeding—We have evaluated a large number of lines for BYDV and/or aphid resistance. Among the Hungarian winter wheat varieties,some are tolerant (Table 2). Hungarian barley varieties are generally susceptible or very susceptible to BYD. In the GDR, three lines of triticale were resistant to this virus (Kegler et al. 1985).

In our opinion, the main control method for BYD will be breeding for aphid and virus resistance.

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Situation Review of Barley Yellow Dwarf Virus in West Asia and North Africa

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Abstract

*Symptoms of barley yellow dwarf (BYD) have been observed on cereals in nearly all countries of West Asia and North Africa. Its incidence, however, has varied during the last 15 years. Observations from field surveys are summarized. Since symptoms of barley yellow dwarf virus (BYDV) are of low diagnostic value, especially in wheat (*Triticum aestivum *L.), more precise qualitative and quantitative detection was derived by vector transmission and serology. In 1985 and 1986, preliminary surveys by enzyme-linked immunosorbent assay (ELISA) indicated that BYDV incidence in the regions surveyed in Syria, Morocco, and Tunisia was around 7, 22, and 24%, respectively. By vector transmission PAV-, RPV-, and RMV-like isolates of BYDV were identified in Morocco and the PAV-like isolate in Syria. By serology PAV-like isolates were identified in Ethiopia, Lebanon, Morocco, Syria, and Tunisia, and MAV-like isolates were identified from Morocco and Tunisia. The PAV-like type was the most common in all countries surveyed. Screening for BYDV resistance by natural infection has been carried out in a number of countries of the region during the last few years. Screening for resistance by aphid inoculation was initiated in Syria in 1986 at the International Center for Agricultural Research in the Dry Areas (ICARDA). Such screening is expected to follow in other countries of the region soon.*

Barley yellow dwarf virus (BYDV) occurs in most countries where wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) are grown. BYDV has been reported in a number of countries in West Asia and North Africa (Bremer 1974; El-Yamani 1980; Slykhuis 1962), but until recently no intensive research has been carried out.

General awareness of the economic importance of barley yellow dwarf (BYD) on cereals has encouraged researchers to intensify work in a number of countries of West Asia and North Africa. In addition, because the International Center for Agricultural Research in the Dry Areas (ICARDA) has a world mandate for barley improvement (delegated by the Consultative Group for International Agricultural Research, the CGIAR), its virus program has given high priority to BYDV. Collaboration with a number of national programs was initiated in 1986.

This paper reviews work done on BYDV in the ICARDA region and may be regarded as the first region-wide summary of information on the occurrence/incidence of BYDV, common vectors, strain identification, possible wild hosts, and selection for BYDV resistance.

Field Surveys

BYDV incidence was reported to differ among locations in each country and among countries of the region. Field observations revealed that BYDV incidence was low (0 to 5%) in Algeria, Egypt, Jordan, Lebanon, Libya, Syria, and Turkey, but was higher (10 to 30%) in Tunisia and Morocco (Abdel-Hak 1984; Bremer 1974; El-Yamani 1980; Kinaci and Yakar 1984; Mamluk and van Leur 1984; and observations of present authors).

In Algeria, BYDV was observed annually at low incidence in a number of locations such as Guelma, Constantine, and Annaba. At Sidi Bel Abbas it was first observed in 1982. BYDV symptoms were found on the barley cultivars Saida and Tichdrett.

In Egypt, BYDV was first reported in 1962 (Slykhuis 1962) to be scattered in the delta area near Cairo. Slykhuis also reported the virus to infect barley in Jordan (Jarash area).

In Libya, BYD symptoms have been observed on cereals at a number of locations since 1970. Its incidence seems to be higher in irrigated than in rainfed regions. During the 1984/1985 growing season, higher BYD incidence than previous years was observed and yield losses due to the disease were recorded.

In Lebanon and Syria, BYDV incidence observed over the last few years has been low. Recent serological tests indicated that BYDV incidence in Syria varied between 2.5 and 22%, with an average of 7.1% (Table 1). However, the fields surveyed cover only a very small part of the cereal growing areas of that country.

BYDV has been reported in cereal crops in Tunisia and Morocco (El-Yamani 1980) for the last 15 to 20 years. Incidence during the 1985/1986 growing season averaged 21.8% in Morocco and 24.5% in Tunisia (Table 1). Such high incidences suggest that BYDV deserves further attention in these two countries.

The only loss assessment in the region comes from Morocco. During the 1981/1982 growing season, an experimental assessment on crop loss was conducted at the Sidi El-Aydi Station (Chaouia). A local bread wheat variety (Nesma 149) was inoculated with a PAV-like isolate at two different stages of Feekes' scale (Stages 1-2 and 5-6). Losses of 44 and 30% resulted from the early and late inoculations, respectively. These data suggest BYD is a limiting factor in cereal production in the region. More work on assessment of potential losses induced by BYD in the cereal cultivars currently used in the region needs to be carried out.

Aphid Transmission and Strain Identification

The BYDV aphid vectors *Rhopalosiphum padi* (L.), *R. maidis* (Fitch), *Sitobion avenae* (Fabricius) and *Schizaphis graminum* (Rondani) were reported on cereals and wild grasses in all countries of West Asia and North Africa (Elnegar et el. 1980; El-Yamani 1980; Slykhuis 1962). Recently we observed *Metopolophium dirhodum* (Walker) on cereals in Syria. Based on vector specificity, PAV-, RMV- and RPV-like isolates of BYDV were identified in Morocco and PAV-like isolates in Syria. The aphid-nonspecific PAV-like isolate of BYDV was the most prevalent.

A study on transmission efficiency of three different PAV-like isolates from Syria revealed *R. padi* to be the most efficient vector, followed by *S. avenae*

Table 1. Incidence of BYDV in randomly collected leaf samples from cereal fields in Morocco, Syria, and Tunisia during the 1985/1986 growing season. Results were based on group testing of leaves by ELISA

Country	Number of fields surveyed	Number of leaves tested[a]	% of BYDV incidence Average	Range
Morocco	14	1590	21.8	1.4-50
Syria	10	4160	7.1	2.5-22
Tunisia	11	1375	24.5	2.2-100

[a] Samples were tested in groups of 5 leaves.

and *S. graminum*. However, transmission efficiency of these three isolates by *R. maidis* was high (30%) (Table 2). *R. maidis* was reported to transmit PAV-like isolates at an extremely low rate (2.4%) (Rochow 1982). *R. maidis* may therefore be important in the ecology of the virus in the region, especially in irrigated areas where maize (*Zea mays* L.) is grown during summer. In such areas, *R. maidis* is likely to transmit PAV-like isolates from cereals to maize in late spring and back to cereals during early fall.

Serology permits greater differentiation of BYDV types or strains than does differential transmission by aphids. In Syria, at least two serologically different PAV-like types were identified on the basis of reactivity with two BYDV antisera (Table 3). In addition, a BYDV isolate from Morocco, which reacted with an MAV monoclonal antiserum (MAC-M2) (L. Torrance, MAFF, Harpenden, England), did not react with an MAV polyclonal antiserum (Inotech F), which is unusual. Another group of BYDV isolates from Syria that reacted with the MAV polyclonal antiserum (Inotech F) did not react with the MAV or PAV monoclonals prepared against British BYDV isolates, which is also unusual. Such variability among BYDV isolates has been recently reported elsewhere (Johnstone and Guy 1986). A BYDV isolate from Japan reacted strongly with a PAV-New York isolate antiserum in ELISA but not with an antiserum against a PAV isolate from Tasmania (0A6), even though PAV-NY and 0A6 are known to be serologically similar. Another case is the RMV-like isolate identified in Montana on the basis of vector specificity that did not react with the RMV-New York antiserum (Yount and Carroll 1983). The presence of more BYDV types than the five defined earlier (Rochow et al. 1987) increases the possibility of having mixed infections in field isolates, a problem which needs further work to clarify and resolve.

Alternate Hosts

Wild grasses, which act as the main hosts for BYDV and its vectors, play an important role in the ecology of BYDV (Coon 1959; Paliwal 1982). Little work has been conducted in the region to identify such natural sources of inoculum. In Morocco, using artificial inoculation, the following species were found susceptible to BYDV and thus may be potential reservoirs of the virus: *Cynodon dactylon* (L.) Pers., *Dactylis glomerata* L., and *Lolium italicum* A.

Table 2. Efficiency of transmission of three PAV-like isolates of barley yellow dwarf virus (BYDV) from Syria by four aphid vectors

Isolate	Vector	Ratio of number of plants infected to number of plants inoculated	% BYDV infection
Isolate 1	*R. padi*	51/69	73.9
	S. graminum	14/32	43.7
	S. avenae	23/53	43.3
	R. maidis	9/40	22.5
Isolate 2	*R. padi*	31/40	77.5
	S. graminum	15/44	34.0
	S. avenae	17/41	41.5
	R. maidis	13/43	30.2
Isolate 3	*R. padi*	31/47	66.0
	S. graminum	9/9	100.0
	S. avenae	33/44	75.0
	R. maidis	8/13	61.5

Braun (symptomless carriers); *Bouteloua curtipendula* (Michx.) Torr., *Bromus inermis* Leyss., *B. mollis* L., *Festuca arundinacea* Schreb., *Lolium perenne* L., and *Phleum pratense* L. (hosts with symptoms). In Egypt, the wild plants *Bromus catharticus* Vahl, *Hordeum murinum*, and *Panicum* sp. were found to be natural BYDV hosts (Elnagar et al. 1980). More work is needed on the ecology of BYDV in the region.

In rainfed areas, graminaceous crops and wild grasses that can survive the hot dry summer are scarce and are not likely to significantly contribute to the onset of BYDV epidemics in the fall when cereals are sown. However, in irrigated areas the situation is different. The presence of maize, found susceptible to a PAV-like isolate in Syria and Tunisia, and sorghum (*Sorghum vulgare* Pers.), found susceptible in Tunisia, is likely to increase the inoculation pressure of BYDV with time, as has been reported earlier from eastern Washington (Brown et al. 1984) and South Dakota (Stoner 1977). Both crops are grown as irrigated summer crops in cereal growing areas. The role of such crops in irrigated areas on BYDV spread to winter cereals in the region should be monitored over the coming few years.

Testing for BYDV Resistance

Resistant varieties appear to offer the best practical approach for BYDV control. In most current breeding programs selection is by submission to natural BYDV infection. Since incidence of natural infection is variable (2 to 100%) in the region (Table 1) and inconsistent, the proportion of plants escaping infection may be high. To overcome this problem, lines of barley, bread wheat (*Triticum aestivum* L.), and durum wheat (*Triticum turgidum* L. var. *durum*) were tested in collaboration with Laval University/Agriculture Canada, using aphid inoculation. Few barley and wheat lines seem to have good resistance to BYDV. During the 1985/1986 growing season, screening for BYDV resistance using artificial inoculation with aphids started at the ICARDA station near Aleppo, Syria. Breeding material tested there is also tested in Canada. Some of the breeding lines expressed BYDV resistance in both locations. More screening work under uniform and well-defined infection pressure is expected to be carried out in a number of countries in the region during the coming few years.

Acknowledgments

Thanks are expressed to Dr. Mustapha H. El-Shater, Agricultural Research Center, Tripoli, Libya, and Mr. Rashid Sayoud, IDGC-station, Guelma, Algeria, for providing information on BYDV from their respective countries. Thanks are also extended to Drs. Ian Barker and S. Forde, Rothamsted Experimental Station, England for testing some of the BYDV isolates. The authors also would like to thank Drs. L. Bos, IPO, Wageningen, The Netherlands, and R. Miller, ICARDA, Aleppo, Syria, for revising the manuscript and making many useful comments.

Table 3. Three PAV-like isolates from Tunisia and Syria with different binding characteristics when tested by direct and indirect ELISA

Sample Code	Country	Host	Sample A405/Healthy A405 Direct ELISA (F polyclonal)	Indirect ELISA (MC 32-39)[a]
SW 27-86	Syria	Wheat	22.7	0.7
TO 3-85	Tunisia	Oats	6.5	3.2
SW 9-85	Syria	Wheat	0.6	59.2

[a] In indirect ELISA, samples were added to the wells directly without IgG coating. PAV monoclonal antiserum used was provided by Dr. S. Wyatt, WSU, Pullman, Wash., USA.

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Barley Yellow Dwarf Virus, Other Small Grain Viruses, and *Diuraphis noxia* in Israel

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Abstract

Virus disease problems in cereal breeding and research programs were investigated in Israel during the spring of 1986. Samples were collected from indigenous wild growing cereals, from breeders, and from other experimental field trials. Diuraphis noxia *(Mordvilko) was found on* Hordeum *spp. under natural conditions, but it was not a pest in Israel. Double-antibody sandwich enzyme-linked immunosorbent assays were done with antisera against barley yellow dwarf virus (BYDV), brome mosaic virus (BMV), and cucumber mosaic virus (CMV). Results showed that BYDV was present and that CMV in cereals caused yellowing, reddening, and dwarfing symptoms similar to BYDV. BMV was detected in breeder material, but identification was inconclusive. One outbreak of wheat streak mosaic virus in a greenhouse was identified.*

From February to April 1986, a survey of virus diseases occurring in small grains and naturally growing wild wheat (*Triticum* sp.) and barley (*Hordeum* sp.) in Israel was conducted. Most specimens were obtained from various trials and experimental plots. Identification of virus diseases was based on the initial assessment of symptoms, followed by double-antibody sandwich enzyme-linked immunosorbent assays (DAS-ELISA) (Clarke and Bar-Joseph 1984) and sap and aphid transmission tests. Electron microscopy was used occasionally to confirm results obtained with other tests.

All samples collected were tested concurrently with antisera against brome mosaic virus (BMV), barley yellow dwarf virus (BYDV) (see Rybicki et al., these Proceedings), and three different isolates of cucumber mosaic virus (CMV). With the exception of one anti-CMV serum, which was raised against CMV-6 in Israel, all other antisera came from the laboratory of the senior author. Local Israeli wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) cultivars were used for sap and aphid transmission tests. Cucumber (*Cucumis sativus* L. cv. Bet Alpha) and *Chenopodium quinoa* Willd. were included to assist with the identification of CMV.

Barley Yellow Dwarf Virus (BYDV)

BYDV-like symptoms were noted in oats (*Avena sativa* L.) and barley cultivars grown in breeding and other experimental plots. Yellow dwarfed plants were occasionally also noted in wild growing *Hordeum spontaneum* K. Koch, *H. bulbosum* L, and *Triticum dicoccoides* (Koern. ex Asch. & Graebner) Aarons. DAS-ELISA with a PAV-like antiserum confirmed the presence of BYDV. *Rhopalosiphum padi* (L.) that occurred in small numbers in field-grown wheat, barley, and oats, transmitted the BYDV from field collected samples under experimental conditions.

Numerous samples collected for symptoms of yellow leaves and/or stunted growth did not react with the PAV-like antiserum. Antisera to other BYDV strains were not available, thus leaving the question open, whether other BYDV strains occur in Israel.

Cucumber Mosaic Virus (CMV)

In South Africa small grains with BYDV-like symptoms can be infected with CMV (von Wechmar and Rybicki 1985; von Wechmar 1986, these Proceedings). To check if CMV also infected small grains in Israel, three CMV antisera were included in all DAS-ELISA tests. One of these antisera was raised against CMV-6

(Prices strain) and was used routinely to detect CMV in the virus laboratory at the Volcani Centre; the other two antisera were raised to naturally occurring isolates from tobacco (*Nicotiana tabacum* L.) and lupines (*Lupinus* L. spp.) in South Africa (Lupuwana et al. 1985). The antisera reacted positively with a large number of samples with yellow and red leaf discolorations and dwarfing symptoms. The antisera did not react equally strongly with all samples, indicating strain differentiation and probably different origins of infection. In many cases both BYDV and CMV were present in the samples. Others were positive for CMV or BYDV only.

Sap and aphid transmissions with some isolates gave rise to yellow leaves testing positive in further DAS-ELISA tests. A triticale (X *Triticosecale* Wittmack) cultivar, Inbar, appeared to harbor a high incidence of CMV. At the time of flowering, numerous dead white ears were noted. In DAS-ELISA tests these plants reacted strongly positive with all three CMV antisera, but were negative for BYDV and BMV. Infected plants were noted in all plots of this cultivar grown at various localities. The cause of the white ear symptom had thus far been unknown and its significance had therefore been overlooked. In a breeding program it was noted that this white ear syndrome persisted throughout several generations.

Similar white dead ears were noted in the first generations of natural selections of *T. turgidum*. Single plant selections in a breeding program showed strong yellow and red leaf discoloration and in several cases also severe dwarfing. In several samples the OD 405 reading was as strong as the positive control, indicating a high level of virus in the diseased plants.

These tests appear to indicate that dead white ears in triticale cv. Inbar and in *T. dicoccoides* are associated with CMV infection. A similar observation was investigated in South Africa where wheat with dead white ears was shown to be infected with CMV (von Wechmar 1986).

Brome Mosaic Virus (BMV)

Antisera to BMV-type strain was included in all tests conducted. No incidence of natural BMV infection was noted. Several samples taken from breeding material reacted positive. This identification was inconclusive.

Barley Stripe Mosaic Virus (BSMV)

Rural Arab communities grow old, non-commercial barley cultivars for home use. A high incidence of barley stripe mosaic virus (BSMV) was noted in such barley. Streak symptoms on leaves were typical for BSMV. Diagnosis was based on symptoms only. This identification was confirmed by E. Antignos (personal communication).

Wheat Streak Mosaic Virus (WSMV)

One incidence of wheat streak mosaic virus (WSMV) was found, in a greenhouse in the presence of large numbers of its natural mite vector, *Eriophyes* (=*Aceria*) *tulipae* Keifer. Identification was based on virus morphology, transmission by sap inoculation, and by *E. tulipae* and M_r on PAGE (S. Cohen, unpublished). Although *E. tulipae* had been noted in Israel previously (Prof. Gerson, University of Jerusalem, personal communication), it had not been recorded to be associated with wheat streak mosaic virus in field-grown wheat.

Diuraphis noxia

Unlike the invasion and destruction of wheat fields in the Orange Free State by *Diuraphis noxia* (Mordvilko) (von Wechmar and Rybicki 1981), small colonies of this aphid can be found on naturally growing *Hordeum* species in Israel. In one incidence the aphid was noted on *H. spontaneum* plants initially collected for apparent BYD-like symptoms. At the time of collection, no aphids were noticed on the stunted yellow plants, but after they were transplanted and kept in a cool, shaded greenhouse, a fast-multiplying colony appeared on the young new shoots.

D. noxia was not found in association with cultivated small grains in the spring and early summer of 1986. This observation was confirmed by Prof. I. Harpaz, University of Jerusalem, who had earlier assisted with the identification of this aphid in South Africa. According to Prof. Harpaz, *D. noxia* has been known to occur in Israel for a long time, but has never been responsible for serious infestations in cultivated small grains.

Summary

Results obtained in this preliminary investigation provided evidence that a PAV-like strain of BYDV occurred in Israel. One incidence was found where *D. noxia* aphids were associated with *H. bulbosum* infected with BYDV and CMV. A BYDV isolate from cultivated oats was serially transmitted by *R. padi*. Transmission of this isolate with *D. noxia* was not attempted. Cultivated and wild growing cereals with apparent BYD-like symptoms reacted positively with anti-CMV sera. One incidence was examined where wild *H. spontaneum* plants appeared to be BYD-diseased, but were shown to be infected with CMV. *D. noxia* aphids were associated with these plants. Symptoms of dead white ears in wheat, triticale, and *T. dicoccoides* were associated with CMV infection. Natural infections of BMV were not detected, but barley streak mosaic virus was prevalent in older non-commercial barley cultivars grown on a small scale for home use in Arab communities. Wheat streak mosaic virus and its vector, *E. tulipae*, were identified in a greenhouse situation.

Acknowledgment

The travel grants made available by the Wheat Board and the University of Cape Town are gratefully acknowledged.

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Barley Yellow Dwarf Virus in Iran*

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* In view of the information it contains, this paper has been included in these Proceedings although the authors were unable to be present at the workshop.

Abstract

*Barley yellow dwarf (BYD) has been observed in barley (*Hordeum vulgare *L.) and durum wheat (*Triticum turgidum *L.* var. durum*) in Iran, but few symptoms have been seen in bread wheat (*Triticum aestivum *L.). Weed grasses are the principal hosts. BYD epidemics occurred in 1980 and 1987. Common aphid species found in Iran are* Rhopalosiphum maidis *(Fitch),* R. padi *(L.),* Schizaphis graminum *(Rondani),* Sitobion avenae *(Fabricius), and* Metopolophium dirhodum *(Walker). RPV-, RMV-, and SGV-like isolates are thought to be present. The symptoms of BYD vary with host, species, host age, virus isolate, and climatic conditions. Delayed planting seems to be the most effective means of control at the present time.*

Iran is situated between 25° and 40°N and 44° and 63°E. Of its total area of 165 million hectares, 25 million are at present used for agricultural purposes. Of this, wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) cover about 8.2 million hectares (33%). Wheat is the staple food of the population, and barley is the main grain fodder. The main diseases of wheat and barley are stripe, stem, and leaf rusts, bunts (covered and loose smut), mildew, *Helminthosporium sativum*, *H. teres*, *H. gramineum*, *Rynchosporium secale*, septoria, and barley yellow dwarf (BYD).

BYD Distribution in Iran

The occurrence of BYD was first reported on winter barley. It has been observed often in international nurseries, especially in durum wheat (*Triticum turgidum* L. var *durum*) and barley plots, but few symptoms have been observed in bread wheat (*Triticum aestivum* L.). A severe epidemic of the disease was observed in 1980; it was more severe in winter cereal regions than in the warmer areas. The losses varied from place to place and even in different parts of the same infected farm. During 1987 another severe epidemic of BYD occurred, which caused severe damage in winter regions. Estimated losses were 5 to 7%.

Host Range and Epidemiology

Of the cultivated graminae—wheat, barley, oats (*Avena sativa* L.), triticale (X *Triticosecale* Wittmack), rye (*Secale cereale* L.), maize (*Zea mays* L.), sorghum (*Sorghum vulgare* Pers.), and rice (*Oryza sativa* L.)—and weed grasses—ryegrass (*Lolium* sp.), timothy (*Phleum pratense* L.), bluegrass (*Poa* sp.), etc.—that are the hosts of the barley yellow dwarf virus (BYDV), weed grasses are the most important. Even though BYD is a new disease in Iran, we believe that, similar to the rusts, epidemics occur every 6 to 7 years (e.g., the first epidemic of BYD was in 1980 and the second in 1987).

Vectors

The common aphid species found in Iran are *Rhopalosiphum maidis* (Fitch), *R. padi* (L.), *Schizaphis graminum* (Rondani), *Sitobion avenae* (Fabricius), and *Metopolophium dirhodum* (Walker). Other aphid species need to be tested for their ability to transmit BYDV. It is thought that RPV-, RMV-, and SGV-like isolates of BYDV are present in Iran.

Disease Symptoms

In Iran the symptoms of BYD vary due to host species, host age, virus isolate, and climatic conditions. Usually the root system of the diseased plant does not develop, heading is late or no heads are produced, yellow leaves are observed in 6-row barley and wheat, and reddening of leaves in 2-row barley has been seen frequently. In the field, wheat shows

better resistance to BYDV than barley. Often 20 to 25 days after the first symptoms are observed, epidemic conditions will be seen, and, finally, in severe cases, the whole field will be infected.

Control

Chemical control (insecticides), weed control (herbicides), and improved agronomic practices are the most effective controls used in Iran. Time of planting and eradication of residues of the previous crop could be effective control measures.

Trials conducted to find the best sowing time for wheat and barley at different experimental stations in Iran showed that early planted plots were highly infected by BYDV and yielded about 1.5 tons less than later planting dates. This difference could be mainly due to BYD.

Resistant cultivars are thought to be the best control means, but unfortunately the resistant barley cultivars from California, Sutter and CM. 67, do not show resistance in Iran, having exhibited susceptibility in both 1980 and 1987. Control of aphid vectors by predators or parasites could be useful in Iran. *Adalia bipunctata* (L.) and *Coccinella septempunctata* L. show promise. Plowing and eradicating the residue of previous plants and weeds 1 to 2 months before sowing is very effective.

On-going Program and Needs

The program for the future at the Seed and Plant Improvement Institute in Karadj includes the following research objectives:

1. Study epidemic occurrence of BYD
2. Identify the virus isolates
3. Identify vectors
4. Screen local wheat and barley collections to identify sources of resistance to BYDV and incorporate these in improved cultivars
5. Test adaptability of the different resistance sources in different climatic zones
6. Study genetics of resistance sources
7. Train qualified workers to strengthen the research

Barley Yellow Dwarf Virus Situation Report for Eastern Africa with Special Emphasis on Kenya

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Abstract

*In Kenya, BYD was first noticed on barley (*Hordeum vulgare *L.) in 1964. A severe outbreak on wheat (*Triticum aestivum *L.) and barley was noted in 1985-86. PAV- and MAV-like isolates of barley yellow dwarf virus (BYDV) were present. Several known aphid vectors have been identified in Kenya. BYD was first reported in Ethiopia in 1967, and a major outbreak was reported in 1985. Seriological tests confirmed the presence of PAV- and MAV-like isolates. BYDV has also been observed in Tanzania, Burundi, Mozambique, and Zimbabwe, but is not an obvious yield constraint.*

Economic losses due to barley yellow dwarf (BYD) occur in East Africa periodically. However, little work has been done on the disease, and there are very few related studies. Most available information is based on visual field diagnosis, often an insufficient basis for reliable diagnosis of viral diseases.

In Kenya, BYD presents a serious problem for cereal crops. The disease symptoms were first noticed on barley in 1964, although no laboratory identification was made. Since then, sporadic outbreaks of the disease have been noticed on cereal crops. A severe outbreak of BYD on wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) was noted in 1985-86 in the Timau and Mau Narok areas in Kenya. Samples of wheat, barley, oats, and some grasses collected in the two regions were tested for barley yellow dwarf virus (BYDV) by R. Plumb of Rothamsted Experimental Station, UK, using enzyme-linked immunosorbent assay (ELISA). Each sample was tested against antibodies to PAV, MAV, and RPV isolates of BYDV. The presence of a PAV-like isolate was confirmed in wheat and barley from Mau Narok and MAV-like isolates were detected in samples from the Timau region.

Several species of cereal aphids that are known to be vectors of BYDV have been identified in Kenya; the most common are *Rhopalosiphum padi* (L.), *R. rufiabdominalis* (Sasaki), *R. maidis* (Fitch), *Metopolophium dirhodum* (Walker), *Schizaphis graminum* (Rondani), *Sitobion avenae* (Fabricius), and *Myzus persicae* (Sulzer).

BYD was first reported in Ethiopia in 1967. An outbreak of the disease in the major cereal growing areas in Ethiopia was reported in 1985 (Agranovsky et al. 1985); BYDV symptoms were found in cereals in most locations. Serological tests confirmed the presence of PAV- and MAV-like isolates of BYDV. None of the samples tested showed positive reactions with an antibody to the RPV isolate. Species of aphids found in Ethiopia that have been reported as vectors of BYDV are *R. padi*, *R. maidis*, *R. rufiabdominalis*, *S. avenae*, *S. graminum*, and *M. persicae*.

BYDV has also been observed in Tanzania, Burundi, Mozambique, and Zimbabwe. No systematic or quantitative studies are known to have been conducted to estimate losses due to BYD in these areas, an indication that the disease is not an obvious yield constraint. However, the presence of the virus and the vectors within the region suggests that BYDV may cause problems when cropping patterns change.

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Barley Yellow Dwarf in Southern Africa

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Abstract

Barley yellow dwarf virus (BYDV) occurs in small grains in the Western Cape, a winter rainfall region, and in the Eastern Orange Free State in South Africa. The situation is confused by the presence of other viruses such as cucumber mosaic virus and brome mosaic virus. BYDV infection was found along with these viruses, but at low incidence. PAV-like and SGV-like BYDV isolates occur in this area. The main aphid species implicated in the transmission of BYDV in South Africa are Rhopalosiphum padi *(L.),* Diuraphis noxia *(Mordvilko),* Schizaphis graminum *(Rondani),* Sitobion avenae *(Fabricius), and* R. maidis *(Fitch). BYD currently appears not to be an economically significant disease in South Africa. It has not been evaluated in other southern African countries mainly because of lack of trained staff.*

Since 1983 we have monitored the incidence of PAV-like barley yellow dwarf virus (BYDV) strains in small grain production regions in South Africa. The two main production regions are the eastern Orange Free State and the South Western Cape. These regions are geographically separated and climatically distinct.

In the Western Cape, a winter rainfall region with an essentially Mediterranean climate, BYDV occurs mainly in wheat (*Triticum aestivum* L.) and oats (*Avena sativa* L.) along the Cape Western coast, or Swartland, as it is known locally. Characteristic symptoms in oats, in which the disease is most noticeable, and other small grains were correlated with infection by the virus as well as enzyme-linked immunosorbent assay (ELISA) testing (BYDV-PAV-type). Although present, barley yellow dwarf (BYD) does not appear to be a problem in this region. This can be attributed to the long, dry, and hot summer months (November to March) when virtually no alternate virus or aphid hosts are available to bridge the interseasonal period until the onset of the first rains in late autumn, when small grains are sown.

Aphid populations build up toward August-September, which coincides with longer, sunny days. This increased light intensity brings out BYD symptoms, which last until the onset of warm weather when temperatures exceed 20°C. The warm weather is often brought on by sudden hot "Bergwinds," when leaves of diseased plants lose their characteristic coloring and die off rapidly. Unless symptoms are noted in this relatively short period, it is difficult to detect natural infections of BYDV in this region.

In the South Western coastal region, known as the "Ruens" (which refers to the hilly landscape), the situation is confused by the presence of other viruses such as cucumber mosaic virus (CMV) and brome mosaic virus (BMV), which produce yellowing symptoms in small grains and probably cause more economically significant crop losses than BYDV. This statement is based on tests conducted on randomly collected diseased plants over several seasons.

In recent years there has been a considerable shift toward growing more malting barley (*Hordeum vulgare* L.) in this region. Few typical BYD symptoms have been noticed in the barley cultivar Clipper currently grown, but there was a high incidence of CMV infection. When present, BYDV symptoms are usually discernible for a longer time than in the Swartland. This can be related to the higher humidity and greater cloud cover in this region.

The wheat producing region in the Eastern Orange Free State, our major production area, has a particularly high incidence of BMV, often occurring together with a CMV-like virus. BYDV

infection occurs along with these viruses but at a low incidence. Detection is made difficult by the presence of very high concentrations of BMV. BYDV does not appear to be a problem in this region. The BYDV isolates in this area were identified as PAV-like and SGV-like; we have not yet detected RPV-like strains in South Africa.

The main aphid species implicated in the transmission of BYDV in South Africa are *Rhopalosiphum padi* (L.), *Diuraphis noxia* (Mordvilko), *Schizaphis graminum* (Rondani), and *R. maidis* (Fitch).

BYDV appears not to cause economically significant diseases in South African small grains. This, in all probability, is due to the climatic conditions prevailing in the different regions.

Several changes in the pattern of this disease have been noted in South Africa since the early 1960s. A photographic record of this pattern is available that illustrates that BYD was readily recognizable in wheat, barley, and oat cultivars popular at the time when the Western Cape region was still the major small grain production center. Cultivars popular then have all been replaced by new improved ones, which express BYD symptoms less strongly. Whether they are less susceptible has not yet been examined in any other manner than visual assessment and by the mere fact that it is currently difficult to find incidences of infection at field level.

Random sampling on occasional field trips followed by ELISA tests showed that many apparently BYD-diseased plants are infected with CMV and BMV and react negatively with our antisera. Typical BYDV symptoms can, however, be found in breeding plots where a wide spectrum of genetic variability (often including older cultivars) is concentrated in a small locality, offsetting differences in symptom expression. The barley cultivar Clipper—now the leader for the malting industry—appears to have good field resistance. Similarly, as a laboratory test plant, it is not ideal for characteristic BYDV symptom expression; we chose it as our major test cultivar simply because good quality virus-free seed was available. New oat cultivars currently in use also do not display the characteristic discoloration and dwarfing as noted in older cultivars.

It should also be noted that in addition to having more tolerant or resistant cultivars, we now employ more stringent criteria for assessing the presence of BYDV. Where in the late 1960s aphid transmission studies were evaluated on visual assessment, we now use more refined methods like ELISA, immuno-electroblotting, mono-specific antibodies and other biophysical and biochemical criteria to determine the identity of infectious agents. In our own work this enabled us to establish whether BYD-like symptoms were caused by BYDV, barley stripe mosaic virus, CMV, or BMV, all of which cause dwarfing and leaf discoloration in our climatic conditions and, incidentally, can all be transmitted by aphids, albeit in small concentration (M.B. von Wechmar, M. Leisge, and R. Chauchan, unpublished results). Three of these viruses are also seedborne, which, when present in low concentration and low incidence, become amplified when colonized by nonviruliferous aphids.

With regard to BYDV in other southern African countries, only very limited information is available. The South African Regional Council for the Conservation and Utilization of the Soil (SARCCUS) runs an aid program called Southern African Regional Wheat Evaluation and Improvement Nursery (SARWEIN), which aims to assist participating countries (Malawi, Namibia, Botswana, Lesoto, Swaziland, and Mozambique) to select wheat cultivars adapted to their local growing conditions. For disease evaluation the emphasis is on stem rust and leaf rust resistance and to a lesser extent on powdery mildew. Virus diseases such as BYD are not considered for evaluation mainly because plant pathologists or virologists familiar with BYD symptoms are not available to assist with scoring for BYD incidence.

Barley Yellow Dwarf in Australia and New Zealand

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Abstract

Barley yellow dwarf (BYD) is now recognized as a significant disease of cereals throughout Australia. Considerable information has been gathered on its epidemiology, both for cereals and pasture grasses. Polyclonal and monoclonal antisera are available. Enzyme-linked immunosorbent assay is now in common use and specific and general cDNA probes have been produced. Non-radioactive probes using Photobiotin are also available. There has been renewed interest by cereal breeders to incorporate Yd2 resistance into local barley (Hordeum vulgare *L.) cultivars in Tasmania and Victoria. BYD was recognized and well documented as a major disease of cereals in New Zealand in the early 1960s. In more recent years, the research effort has concentrated on introducing better sources of resistance into barley, wheat (*Triticum aestivum *L.), and oats (*Avena sativa *L.).*

This situation report summarizes the objectives and results of research programs into barley yellow dwarf virus (BYDV) in Australia and New Zealand undertaken since the 1983 Workshop in Mexico. It should be read in conjunction with a number of other individual papers reporting Australian research results as well as the paper on epidemiology and control of BYDV in Australia and New Zealand (Johnstone et al., these Proceedings) because there is, by necessity, considerable overlap.

Australia

It is now recognized that barley yellow dwarf (BYD) is a significant disease of cereals throughout Australia. There has been a dramatic increase in the amount of research into BYDV and an increased awareness by cereal breeders, agronomists, and extension groups of the effects of the disease. At least some of the increased interest in BYDV appears to have been stimulated by the 1983 BYDV Workshop in Mexico. Most cereals are grown in areas of dryland farming (250 to 500 mm annual rainfall) (Figure 1), where BYDV is a sporadic and often local problem. However, the trend to expand cereal growing in higher rainfall areas where BYDV is documented as endemic (Sward 1986) has provided a further catalyst to research.

Virus epidemiology and vector relationships

Guy and Johnstone (1985) and Guy et al. (1986, 1987) have examined the incidence and types of BYDV in Tasmanian pastures using enzyme-linked immunosorbent assay (ELISA) and transmissions with aphids. Guy et al. (1986) found that 13% of young pastures (most less than 2 years old) from 5 sites were infected, the predominant isolate being the PAV type. Guy et al. (1987) in a second study examined annual and perennial grasses and found 9% were infected, although certain sub-families of grasses, such as the Bambusoideae, had a much higher incidence of BYDV (up to 31%). Again they found that the PAV types were predominant, and in addition, a significant number of mixed infections of PAV and RPV types were present. They reported that *Rhopalosiphum padi* (L.) transmitted the PAV and RPV types either alone or together and *Sitobion fragariae* (Walker) transmitted the PAV type alone, and occasionally RPV when present in mixed infections with PAV.

Johnstone et al. (1984), Johnstone and Guy (1986), and Johnstone and Barbetti (in press) have also examined other aspects of the epidemiology of BYD in Tasmania, such as the variation in incidence between years in spring-sown barley (*Hordeum vulgare* L.), the relative incidence of different BYDV types in cereals, the effects on productivity of pasture grasses, and the activity and field behavior of vector species.

The incidence and types of BYDV in cereals and grasses in Victoria and other states of mainland Australia have been examined using ELISA and transmission with aphids. Sward and Lister (1987) examined batches of samples from wheat (*Triticum aestivum* L.) crops from all major wheat growing districts of Victoria. ELISA showed that 38% of crops examined were infected and 11% of crops had a BYDV incidence greater than 10% even though the crops were sampled early in the season before spring aphid flights. PAV types were predominant, followed by RPV types. The statewide average incidence in wheat was estimated to be 2%.

In a further survey of cereals and grasses from mainland Australia, Sward and Lister (1988) identified four BYDV types on the basis of serological relationships, namely PAV (again the predominant type), RPV, MAV, and RMV. The vector relationships for RPV and RMV types were essentially the same as for the North American isolates, but the PAV type was transmitted predominantly by *R. padi* and rarely by the local *Sitobion* species (*S. miscanti* (Takahashi) not *S. avenae* (Fabricius)) (Sward and Rochow, in press). The type identified serologically as MAV-related exhibited an anomalous vector relationship in that its major vector species was *R. padi* (Lister and Sward 1988).

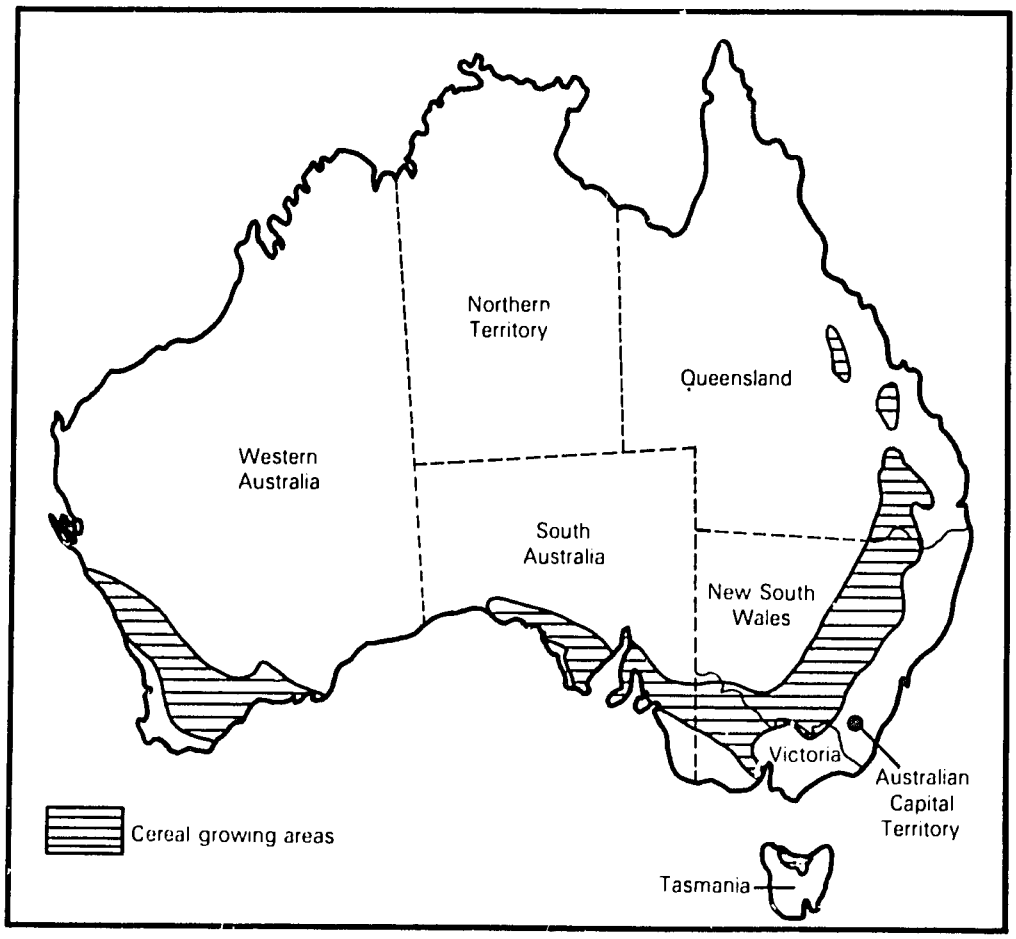

Figure 1. Cereal growing areas of Australia.

P. Ridland and R. Sward (unpublished) in Victoria have investigated the biology and vector relations of 6 species of cereal aphids. Of particular interest is the frequent occurrence of *R. rufiabdominalis* (Sasaki) in southern Victoria and the finding that it is an important vector of at least two BYDV types. Ridland and Carver (1987) also recorded *R. insertum* (Walker) for the first time in Australia and P. Ridland and R. Sward (unpublished) have shown that it also is a vector of BYDV, although of only minor importance. Studies are continuing on the biology of *R. insertum* and *R. rufiabdominalis* as well as of other vector aphid species.

McLean et al. (1984) used space-planted oats (*Avena sativa* L.) and two near isogenic barley cultivars, one BYDV resistant and the other susceptible, to estimate potential losses due to BYD at 15 sites in Western Australia. The incidence of BYDV infected oats ranged from 0 to 100% and the yield divergence between the two barley cultivars ranged from 21 to 81%. To date, only PAV types have been identified from Western Australia by ELISA (Sward and Lister 1988).

In Queensland, R.S. Greber (personal communication) has examined the occurrence of BYDV types in cereals and grasses and has found that, in general, isolates from wheat and barley are transmitted by *R. padi*, and serological examination of these has shown that they are of the PAV type (Sward and Lister 1988). In a proportion of oats and in certain grasses such as *Urochloa panicoides* Pal. Beauv., the predominant isolate is transmitted by *R. maidis* (Fitch) and has been shown by Sward and Lister (1988) to be of the RMV type. The RMV types occur mainly as summer-autumn epidemics in the grasses.

Metopolophium dirhodum (Walker), first recorded in Australia in southern New South Wales (Carver 1984) and soon after in Victoria and Tasmania, has been shown to be a good vector of PAV types but a poor vector of RPV types (Waterhouse and Helms 1985). Surveys to determine the incidence of BYDV in wheat in southern New South Wales in the 1986/87 season and the effect of BYDV on herbage and grain yield in the 1985/86 season in wheat and oats in a high rainfall area of New South Wales are being conducted (K. Helms and P. Waterhouse, personal communication).

Further important and often overlooked aspects of BYD epidemiology are the interactions with other disease organisms. Sward and Kollmorgen (1986) studied the combined infection of BYDV and take-all fungus (*Gaeumannomyces graminis* var. *tritici*) on the growth and yield of wheat and found significant interactions. These could be important in areas where both diseases are common (Sward and Kollmorgen, these Proceedings).

Diagnostic methods

ELISA is used for BYDV diagnosis. Locally produced as well as North American and British antisera are used (Table 1).

Table 1. Antisera that have been produced to Australian BYDV isolates

Serotypes	Antibody type	Reference or authority
PAV	polyclonal	Guy et al. 1986
PAV/RPV mixture	polyclonal	Sward and Lister 1987
PAV	monoclonals	Hewish et al. 1987
RPV	monoclonals	D. Hewish and R. Sward (unpublished data)
RPV	polyclonal	R. Sward (unpublished data)

A program is currently under way to produce polyclonal and monoclonal antisera to the Victorian MAV and RMV types and polyclonal antiserum to an RPV type from Tasmania.

A range of aphid species are reared in colonies by P. Guy and G. Johnstone in Tasmania, R. Sward in Victoria, and K. Helms and P. Waterhouse in the Australian Capital Territory, to facilitate transmission studies.

Nucleic acid probes have been produced using cloned cDNA fragments of the genome of the PAV type separated from the Victorian PAV- and RPV-like mixed isolate (Waterhouse et al. 1986). Probes made from some clones are specific for the PAV type, while others can detect a range of BYDV types and may be useful for diagnosis of previously uncharacterized BYD viruses. cDNA probes have been labelled with ^{32}P for use in the detection of BYDV by Waterhouse et al. (1986) and have been tagged with biotin using the Photobiotin reaction (Habili et al. 1987). It appears that the nucleic acid probes are at least 25 times more sensitive than ELISA, but the present disadvantage is that tissue extracts require more preparation for testing.

Using recombinant DNA techniques, W. Gerlach, A. Miller, R. Cheng, and P. Waterhouse (personal communication) are studying the genome organization, function, and evolution of BYDV types. The complete sequence of the Victorian PAV type has been determined and compared with various regions of the GPV and GAV types from China. The Victorian RPV type is being sequenced by T. Hercus, N. Habili and R. Symons (South Australia, personal communication).

Control

Research into methods of control has concentrated on breeding resistant or tolerant material. Associated with this is a considerable research effort into methods of identifying and measuring resistance and tolerance. In Tasmania, G. Johnstone and P. Guy (personal communication) are examining the effects of BYDV in tolerant (cv. Shannon) and sensitive (cv. Proctor, Triumph) barley cultivars, and virus content in infected oats and barley in relation to apparent tolerance of breeding lines and new cultivars under evaluation. W. Vertigan and others (personal communication) in Tasmania are involved in breeding and selecting for tolerance in barley, oats, triticale (X *Triticosecale* Wittmack), and wheat. Differences in susceptibility of pasture species are also being examined and single plant selections screened for tolerance (or resistance).

In Victoria, breeding to incorporate *Yd2* resistance into agronomically suitable barley cultivars is being carried out (G. Halloran, personal communication). In collaboration with this program, attempts are being made to identify specific products of the *Yd2* gene to provide a test that will assist in rapidly identifying lines carrying that resistance gene (P. Holloway and R. Heath, personal communication). The cDNA probes of Waterhouse et al. (1986) are also being used to assess virus content and monitor virus resistance in order to better understand the mechanism of resistance conferred by the *Yd2* gene (P. Holloway personal communication). Also in Victoria, wheat breeding lines are being screened for tolerance to BYDV (R. Young, P. Martin, and L. O'Brien, personal communication). Martin (1986) has examined a range of diploid and tetraploid ancestral species of *Triticum aestivum* and has found that two genes control the tolerance identified in a wild *T. turgidum* L. var. *dicoccoides* from Israel.

In the Australian Capital Territory, R. Brettell, P. Waterhouse, P. Larkin and R. Banks (personal communication) are examining BYDV resistance found in *Thinopyrum intermedium* (Host) that has been back-crossed by the Chinese to produce wheat cultivars such as Zhong 4. They have identified the chromosome location of the gene, and virus assays have indicated that the gene from *T. intermedium* may perform better in wheat than does the *Yd2* gene from C.I. 3208 in barley.

In South Australia, wheat and oats are being screened and selected for tolerance to BYDV by planting breeders' lines in areas known to have high levels of BYD each season (A. Rathjen, T. Wallwork, and C. Barr, personal communication). Preliminary tests on the predominantly *R. padi* transmitted isolates present in the high risk areas indicate that PAV types are common, although it is not known at this stage whether other types are present as well.

In Victoria, the effects of BYD on annual and perennial ryegrass lines are being examined and detailed measurements of growth rates and carbohydrate and nitrogen levels of roots and shoots have been used to estimate the degree of tolerance or susceptibility (D.R. Eagling, R. Sward, and G. Halloran, unpublished).

Various methods are being assessed for the control of the aphid vectors of BYDV. In Tasmania, the effects of synthetic pyrethroids on the primary and secondary spread of aphids are being examined (G. Johnstone, personal communication). In a number of sites in eastern Australia, various groups are monitoring the possible biological control of *M. dirhodum* by the hymenopterous parasite, *Aphidius rhopalosiphi* De Stefani, imported from Chile and New Zealand and released by the Commonwealth Scientific and Industrial Organization (CSIRO), Division of Entomology.

New Zealand

BYD was identified in grasses and cereals in New Zealand in the early 1960s and various aspects were documented. The principal features of the New Zealand situation were outlined in the 1983 Situation Report from the Mexico Workshop.

More recently, most work on the North Island has been carried out at the Department of Scientific and Industrial Research (DSIR) Aorangi research farm near Palmerston North in the Manawatu (southern North Island), where regular infestation of cereals with viruliferous *R. padi* occurs during the spring (October) sowing.

Currently the field evaluation of BYD infection of cereals is carried out by J.M. McEwan of the DSIR Crop Research Division Station in Palmerston North, while in the past Latch (1977) ((Plant Diseases Division DSIR, Palmerston) has studied BYDV in pasture grasses, and recently P. McGregor (Entomology Division DSIR, Palmerston North) has initiated a study on the aphid vectors of BYDV. Projects presently being carried out include: field evaluation of all locally produced cereal breeding stocks for BYDV reaction; disease screening of nurseries of advanced cereal breeding stocks from all Crop Research Division breeding projects for BYD reactions; scoring of CIMMYT BYDV Nurseries in bread wheat, barley, durum wheat (*Triticum turgidum* L. var. *durum*) and triticale for BYD reaction; scoring of CIMMYT Trials and Screening Nurseries in bread wheat, durum wheat, barley, and triticale for BYD reaction; participation in the establishment of an International BYD screening nursery in oats; incorporation of the Yd_2 gene throughout the barley breeding material and a search for alternative sources of genetic resistance in barley; and sampling of BYDV infected materials for BYDV strain identification at Rothamsted (UK) and Purdue University (USA) using ELISA techniques.

At Lincoln on the South Island, Stufkens and Farrell (1986, 1987) have examined the use of insecticides, plant resistance, and management practices for the control of BYDV in cereals. In 1986 when disease levels were less than 5%, pyrethroid sprayed at growth stages (GS) 12 to 22 reduced early BYD intensity by only about 50% in spring- and autumn-sown barley. A further pyrethroid spray at GS 30 in the latter crop greatly reduced late BYD intensity. Pyrethroid sprayed at GS 12 to 22 reduced BYD more than did drilled Terbufos granules in both sowings. The disease level of Fleet barley was 25 to 33% of that of Triumph or Mata.

J. Farrell and M. Stufkens (personal communication) have also shown that various BYDV isolates are transmitted by *R. padi*, *R. maidis*, *S. fragariae*, or *M. dirhodum*. Serological examination of New Zealand isolates has been carried

out (R. Sward, P. Guy, and W. Rochow, unpublished) in Australia and the USA and RPV-, MAV-, PAV-, and RMV-related types have been identified.

The main control methods recommended for the South Island are late sowing, insecticide applied at GS 25 to 30, or growing of cultivars with resistance. Work is continuing on the vector relationships with respect to BYDV type and on the importance of various aphid species at different times of the season.

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Status of Barley Yellow Dwarf Virus Research in Asia with Special Emphasis on India

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Abstract

*Barley yellow dwarf virus (BYDV) was first reported in the northern Himalayan regions in 1963 and sporadic appearances have also been recorded in other parts of the Himalayas. However, the disease has never posed any serious threat to the wheat (*Triticum aestivum *L.) crop in this region. A very severe but extremely localized BYDV epidemic in the late 1970s at a research station in the Uttar Pradesh hills (apparently aggravated by the cultivation of off-season wheat crops during summer) provided an opportunity to screen wheat germplasm for tolerance to BYDV and led to the identification of one resistant line, NS 879/4. Systematic selection in crosses of NS 879/4, carried out during the epidemic years, led to the development of four improved lines. Studies on BYDV host range and vector overwintering sites and migratory patterns have been conducted in China.*

India

In India four virus diseases have been reported to occur under natural conditions. These are wheat striate mosaic virus (Nagaich and Sinha 1974) wheat spindle streak mosaic virus (Ahlawat et al. 1976), wheat streak mosaic virus (Ray Chaudhary and Ganguly 1972), and barley yellow dwarf (BYD) (Nagaich and Vashisth 1963). However, in spite of their occurrence, these viruses have not posed any serious threat to the wheat (*Triticum aestivum* L.) crop except for sporadic appearances and occasional damage.

In India barley yellow dwarf virus (BYDV) was first reported on wheat and barley (*Hordeum vulgare* L.) by Nagaich and Vashisth (1963) from the North Himalayan regions. Attempts to transmit the disease through plant sap, soil, and seed were unsuccessful. Several species of unidentified leafhoppers collected from wheat and barley fields and surrounding pastures also did not transmit the disease to healthy wheat, barley, and oat (*Avena sativa* L.) plants. Nagaich and Vashisth, however, succeeded in transmitting the disease to oats, *Bromus*, *Lolium*, wheat, and barley seedlings by using three aphid species, *Sitobion avenae* (Fabricius), *Rhopalosiphum maidis* (Fitch) and *R. padi* (L.). The infected wheat plants turned yellow in about 2 to 3 weeks. The yellowing progressed toward the base from the tip of the lamina and a characteristic yellow color replaced the normal green pigment of the leaves. The affected plants remained stunted in all cases and had stiff, erect leaves.

Since then, BYDV-affected plants have been recorded by the authors at several places in the Himalayan regions including Bhowali, Almora, and Mukteswar in the Uttar Pradesh hills. However, the disease has not been found to assume any serious proportion. Beginning in 1975/76 very severe but localized, high-intensity BYD infection was recorded for a few years at farms of the Central Potato Research Institute (CPRI) regional center at Mukteswar, located at about 2,000 m above sea level in the Uttar Pradesh hills of the central Himalayas. Farmers' fields surrounding this center also had BYDV infection. During the worst years the wheat and barley crop was so severely damaged that no grain could be harvested in the varietal yield evaluation trials and other disease screening nurseries organized by Vivekananda Parvatiya Krishi Anusandhan Shala (VPKAS) at this center. During this period the entire crop of wheat and barley had to be plowed under as the plants remained very stunted and little grain or straw could be harvested. All triticale (X *Triticosecale* Wittmack) strains in various trials and nurseries showed a very low intensity of infection and several remained almost free.

An examination of the records maintained by the senior author showed that a few dwarf plants had been recorded sporadically in this region but were not taken seriously due to very low frequency of their appearance and lack of information on the disease. A severe situation began to develop gradually over a period of few years after an extended and intensified off-season wheat and barley nursery program was started in the summer of 1970. In this nursery, wheat and barley plants were continuously available throughout the year, i.e., the normal season crop from October to May and off-season crop from June to October. The cultivation of the off-season crops was reduced/stopped in 1980/1981 and the intensity of BYD-affected plants returned to normal (rare) and normal wheat cultivation again became feasible.

These events showed that BYD assumed severe proportions due to the cultivation of wheat year-round. They also indicated that the alternative hosts on which the disease normally survived during the off-season were probably not as effective carriers as the wheat and barley plants. It is, however, obvious that BYD can pose a serious problem in most hilly regions where wheat is grown throughout the year.

China

BYD has been reported to infect wheat, oats, barley, and even maize (*Zea mays* L.) by several workers from China. A group of researchers organized for control of virus diseases in wheat and oats in N. Shanxi ascertained the overwintering sites and migratory patterns of the four main aphid vectors of BYDV to determine a strategy for the control of this disease. Wu and Wang (1984) succeeded in isolating and identifying the pathogen from the maize red leaf disease, which occurs widely on maize, wheat, and broom corn millet (*Sorghum bicolor* (L.) Moench) in north, northwest, east, northeast and southwest China as BYDV. *Cymbopogon* Springel has long been mistaken as the overwintering host of *Schizaphis graminum* (Rondani) and the source of BYDV perpetuation in China. Investigations carried out by Wang et al. (1984) during 1981 to 1983 showed that the aphids on *Cymbopogon* were not the same as those on wheat. No overwintering host for the latter was found and it was considered that *S. graminum* on wheat was an immigrant from places outside the region. Li et al. (1985) studied the host range for BYDV using three varieties of *Triticum aestivum*, two of *T. turgidum*, and 13 other *Triticum* species such as *T. (aegilopoides* (Link) Bal. ex Koern.) = *T. boeoticum* subsp. *boeticum*, *T. sphaeroccum* Percival, *T. (orientale* Percival) = *T. turanicum* Jakubz, and *T. dicoccoides* (Koern. ex Asch. & Graebner) Aarons. They also included in their studies 13 varieties of *Setaria italica* (L.) P. Beauv., *H. vulgare*, *Avena sativa*, and *A. nuda* L., 6 forms of *Zea mays*, and 2 varieties of *Andropogon* L. and concluded that the virus has a wide host range.

Disease Expression

Watson and Mulligan (1960) have suggested the use of a visual score of BYD infection symptoms on a 0 to 9 scale. However, in the investigations at VPKAS the diseased plants were counted in percentages. "F" (free) was used to denote the complete absence of the disease, while strains showing less than 5% diseased plants were classified as resistant (R). Cultures with between 5 and 10% diseased plants were classified as moderately resistant (MR), and those with more than 10% intensity were categorized as susceptible (S).

Identification of Sources of Resistance

In addition to the large number of wheat strains that were screened for BYD reaction over several years from 1974-75 onward, 1308 spring and winter wheat, 35 barley, and 185 triticale lines were evaluated more systematically in a BYD screening nursery under natural epidemic conditions at Mukteswar during 1980-81. None of the lines was found to be resistant. A Yugoslav wheat line, NS

879/4, which had been earlier identified as resistant (Tandon et al. 1980) from a set of 24 winter wheats received from the Shimla center of the Indian Agricultural Research Institute and included as a check, was the only line that continued to show a high degree of resistance to BYD infection. This line was reported free by Singh et al. (1979); however, it has continued to show a small percentage (less than 5%) of infected plants in our screenings.

By and large, the triticale cultivars were found less affected by BYD than wheat and barley lines. Seven triticale lines—TL4, TL14, TL688, JNK42, UPT7449, UPT71195, and UPT 78268—showed a high degree of resistance. All the barley cultivars screened were found susceptible.

Genetics of Resistance

The F_2 generation of four wheat crosses involving NS 879/4 as the common resistant parent were scored under natural conditions for segregation during 1978-79, which was one of the very high disease epidemic years. Mode of segregation in the F_2 generation of these crosses indicated the operation of one or two dominant genes for resistance to BYDV (Table 1).

In the first two crosses, there was a monogenic segregation, while in the latter two a digenic segregation with complementary epistatis was evident. Simple inheritance of resistance to BYD was further supported by the easy recovery of resistant progenies in F_3 and later generations of several other crosses of NS 879/4 with susceptible parents such as VL 404, CPAN 1418, CPAN 1436, CPAN 1283, CPAN 1529, CPAN 1533, CPAN 1535, HW 171, UP 319, Girija, Shailja, HB 117-107, Kalyansona, Maldoa, Rossulka, Victor, Heinis VII, Yorkwin, and Flavina, which were grown with the objective of identifying appropriate resistant segregates.

Breeding BYDV resistant varieties of wheat

Soon after the confirmation of resistance to BYDV in NS 879/4 from observations made from 1975-76 to 1977-78, an active breeding program was taken up at VPKAS to develop improved wheat strains combining resistance to BYDV. All available breeding materials involving NS 879/4, developed specifically for BYDV resistance or otherwise, were grown at Mukteswar during the 1978-79 crop season. Sowing was done in the 3rd week of October to get high disease intensity. A large number of resistant selections were recovered from the crosses indicated above. These were replanted in 1979, 1980, and 1981 to confirm resistance and select for true breeding resistance cultivars combining other desirable features.

High natural disease incidence continued to provide a good opportunity for an effective screening of breeding materials and selection of BYDV resistant lines. The pedigree method of selection was practiced to handle all segregating materials. A screening of 650 resistant progenies originating from 90 crosses resulted in the identification of 30 desirable lines in advance generation

Table 1. Segregation for resistance to barley yellow dwarf virus in the F_2 generation of four wheat crosses

Cross	Observed frequency		Expected frequency		Expected ratio
	R	S	R	S	
SENTINAL x NS 879/4	87	23	82.5	27.5	3R:1S
HW 167 x NS 879/4	117	53	127.5	42.5	3R:1S
LEE x NS 879/4	109	67	99.0	77.0	9R:7S
CO AN 1695 x NS 879/4	57	56	63.6	49.4	9R:7S

R = Resistant, S = Susceptible.

materials, out of which four strains, namely VL 490 (NS 879/4 x Girija), VL 492 (NS 879/4 x Girija), VL 493 (NS 879/4 x Shailja), and VL 498 (NS 879/4 x UP 319), were found to combine several desirable features with resistance to BYD. Among these VL 493 and VL 498 combine BYD resistance with high yield, high degree of resistance to rusts, and good grain quality. In seven field trials conducted over 4 years, under rainfed conditions in the northwestern hills, both VL 493 and VL 498 have yielded 25 to 35% higher than the donor parent NS 879/4. NS 879/4 has also shown good adaptability to the north Indian hills, but its grain type is red, which is not acceptable to Indian consumers.

The BYDV problem has subsided since the off-season summer cropping was discontinued. Therefore, the new resistant strains have not been formally released for general cultivation. However, these strains continue to be grown on a limited scale around the site where the BYD epidemic occurred and are being maintained as potential genetic stock at VPKAS.

Acknowledgments

We are grateful to Dr. B.B. Nagaich, ex-Director, CPRI, Shimla, who confirmed the nature of the disease as BYDV by a personal visit at our request. We are also grateful to Dr. L.B. Goel, Senior Wheat Pathologist in the Wheat Directorate, for useful suggestions for improvement of the manuscript. Thanks are due to Dr. K.D. Koranne, Director, VPKAS Almora, for providing necessary facilities for the preparation of the manuscript.

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Barley Yellow Dwarf in Pakistan

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Abstract

*In Pakistan, barley yellow dwarf (BYD) occurs in all the major cereals. Surveys during the last 2 years have shown that almost all the commercial wheat (*Triticum aestivum *L.) varieties presently under cultivation in Pakistan are infected by barley yellow dwarf virus (BYDV). The incidence ranges between 0.5 and 1.0%. The disease is more prevalent in the plains than in the hills. Aphids, including the known BYDV vector,* Sitobion avenae *(Fabricius), have also been found in fairly high densities in areas where BYD was observed. BYD has also been observed in various international wheat nurseries. Studies on BYD are now being undertaken.*

All the major hosts of barley yellow dwarf virus (BYDV), i.e., wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), and oats (*Avena sativa* L.), are cultivated in Pakistan. Wheat is the staple food and occupies the largest area; barley and oats are grown on a small scale. During 1986-87, wheat covered an area of 7.3 million ha in Pakistan.

Symptoms of barley yellow dwarf (BYD)-like disease were first observed in Pakistan in 1964 near the Pakistan-Afghan border on a single plant of local wheat in a 4.5-ha field (M. Aslam, unpublished). These symptoms were not observed again in Pakistan until recently. During the last 3 to 4 years BYD-like symptoms have been observed at different places in wheat fields. The symptoms were sporadic and always had to be searched for. During 1985-86, BYD became suddenly visible and did not need to be looked for. It was more pronounced in north central Pakistan. Surveys conducted by the Crop Diseases Research Institute of the Pakistan Agricultural Research Council during the last 2 years have shown that almost all the commercial wheat varieties presently under cultivation in Pakistan are infected with barley yellow dwarf virus (BYDV). The incidence ranges between 0.5 and 1.0% in various fields. The disease is more prevalent in the plains than in the hilly areas.

In 1986, a heavy incidence of aphids was found on wheat, among which a known vector of BYDV (*Sitobion avenae* (Fabricius)) was commonly identified. Although no systematic observations were taken, in general, fairly high densities of vectors were found in areas where BYD was observed.

Until 1987, disease identification was based only on symptoms observation. During 1987, the presence of BYDV was confirmed at Rothamsted Experiment Station, England, in samples of leaves from diseased plants of wheat, barley, triticale (X *Triticosecale* Wittmack), and oats collected from different localities (Table 1). (We are grateful to them for this cooperation.) In 1987 BYD was more pronounced in Northern Punjab and in North West Frontier Province. The analyses carried out at Rothamsted indicated high levels of virus in the infected tissues. These analyses also indicated the presence of both PAV- and RPV-like strains of the virus.

BYD has also been observed in various International Wheat Nurseries, such as those from CIMMYT and ICARDA. The present situation is a cause of concern and calls for greater attention and international cooperation. The Crop Diseases Research Institute, Islamabad, is now undertaking systematic studies on BYD.

Table 1. Localities where BYD was collected and confirmed during April 1987

Locality	Crop
Chakwal	Bread wheat PAK-81
Gujrat	Oats
Gujrat	Bread wheat PAK-81
Islamabad	Triticale NIAB-T 183
Islamabad	Fodder oats
Pirsabak	Barley
Sialkot	Wheat
Taxila	Bread wheat PAK-81

II. Virology

Luteoviruses—One System, Many Variations

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Abstract

The viruses that induce symptoms once blamed on aging or nutrient deficiencies—stunting of infected plants and patterns of interveinal yellowing or reddening, and rolling and brittleness of infected leaves-and are transmitted by aphids in a persistent manner, are an interrelated group of viruses termed luteoviruses. Described originally from a number of different crop species from throughout the world as unique and distinct plant viruses, modern technology has shown that the 35 to 40 distinct entities represent only about 9 major distinct serotypes.

Most of the definite luteoviruses have very narrow and specific host ranges involving one, or perhaps two, plant families. In contrast, beet western yellows virus (BWYV) infects all plant subclasses, orders, and families susceptible to the other luteoviruses. Primeval aphids were polyphagous, and primitive yellowing viruses probably infected a wide range of hosts. Transmission into plant groups with host-specific vectors probably gave rise to the monofamily viruses such as barley yellow dwarf. Because they provide the only means for the spread of the virus in nature and are the key to understanding the ecology of the diseases, the focus on aphids and specificity of transmission has been important to the study of these yellows diseases. Electron microscopy, serology, and molecular technology have thus far shown that the luteoviruses are interrelated through their probable origin-BWYV. The ecologically distinct members of the group may or may not be similar to each other but form a continuum of viruses with serological, cytological, and epidemiological similarities to BWYV.

The interactions between luteoviruses and related and unrelated viruses in mixed infections may also significantly effect the epidemiology and origin of these viruses. Pea enation mosaic virus appears to have some affinities to the luteoviruses. It may represent an evolutionary link between luteoviruses and mechanically transmissible small, spherical viruses, or perhaps a link between luteoviruses and the aphid-transmitted helper-dependent virus complexes.

The yellowing and reddening of fields that took place in so many crops throughout the world as growing seasons progressed has long been blamed on natural factors such as aging or nutrient deficiencies or excesses. Potato leaf roll (PLR), although known since the middle 1700s, was not recognized as a specific disease of potato (*Solanum tuberosum* L.) until 1905 (Appel 1907), and until the transmission by aphids was shown (Quanjer et al. 1916; Botjes 1920) was thought to have various causes. Orton (1914) wrote, "The literature on leaf roll has become so voluminous that few will undertake to peruse all the contributions, which are, indeed, of very uneven merit, and anyone who attempts it is likely to emerge with his concepts of the disease more confused and hazy than at the start." This condition, of course, was due to the imperfect recognition of the PLR symptoms and to the many and varied opinions as to its true cause. PLR was attributed to either excesses or deficiencies of mineral elements in soils or in fertilizers, the use of either unripe or overripe tubers for seed or of tubers from prematurely ripened plants, or varietal degenerations.

There is ample evidence of barley yellow dwarf virus (BYDV)-induced disease epidemics of barley (*Hordeum vulgare* L.) and oats (*Avena sativa* L.) in the later 1800s in the United States (Bruehl 1961), prior by half-century to the significant determination of Oswald and Houston

(1951) that the yellowing and reddening disease was induced by a virus. Even their first reaction, "The abnormal condition on all three cereal crops was directly the result of some set of peculiar environmental conditions, probably involving temperature, soil moisture and soil fertility," related to the general difficulty that agriculturists have had, to relate these diseases to infectious causes. As Bruehl (1961) pointed out with barley yellow dwarf, but would also be true for yellowing diseases of potato, beet (*Beta vulgaris* L.), legumes (Leguminosae Juss.), lettuce (*Lactuca sativa* L.), etc., "As an old disease on major crops that receive the perennial close scrutiny of plant pathologists, it seemed incredible that it could have been overlooked for long."

Early ripening, drought, excessive moisture, nutritional deficiencies, or soil conditions were thought to be the cause of the yellowing of sugarbeet fields recorded from the Netherlands as early as 1912 (Coons 1952) and from Denmark in 1914 (Gram 1942). The disease was not implicated as a virus disease until Roland (1936) and Van Schreven (1936) showed that the causal agent was transmitted by aphids.

The concept of millions of hectares of subterranean clovers affected by yellowing viruses was developed as recently as the late 1960s and early 1970s (Johnstone and McLean 1987). The fact that the symptoms mimic magnesium deficiency (Millikan 1953) and infected plants have only half the magnesium content of healthy plants (Kellock 1971) lends emphasis to the difficulties that agriculturists have had in implicating an infectious nature for these diseases.

But as difficult as this concept of virtually universal viruses causing nutrient-deficiency-like symptoms has been for the agricultural community, the symptoms, along with their transmission by aphids in a persistent manner, have proved to be very reliable criteria for virus classification.

With few or no exceptions, viruses that induce stunting of infected plants and patterns of interveinal yellowing or reddening that develop acropetally, that show rolling and brittleness of infected leaves, and that are transmitted by aphids in a persistent manner are an interrelated group of viruses termed luteoviruses.

The major characteristics of the luteovirus group have recently been reviewed (Rochow and Duffus 1981; Waterhouse et al. 1988). We wish to discuss biological, historical, and relationship characteristics of the members of this group that may lead to new concepts of this very important and successful group of plants pathogens and to new avenues of research.

Members of the Luteovirus Group

Some 36 members and likely members of the luteoviruses are listed in Table 1. Continuing studies show that most luteoviruses are serologically related and new molecular methods may indicate new relationships or differences. Thus, we should view the information in Table 1 as a frequently changing list of viruses, recognizing that we have an incomplete knowledge of their relationships.

We have grouped the definite members into only nine distinct viruses, but have included with those viruses closely related strains or synonyms, which may differ in host range, vectors, and epidemiology. For instance, soybean dwarf virus (SDV), subterranean clover red leaf virus (SCRLV), and subterranean clover red leaf virus-California (SCRLV-C) differ markedly in host range, and in the case of SCRLV-California, even in vector specificity, but serologically they are indistinct (Johnstone et al. 1984a). We have eliminated some viruses previously classified as potential luteoviruses because their properties no longer seem to fit. These include subterranean clover stunt and filaree red leaf viruses.

Table 1. Members and likely members of the luteovirus group, with an important vector and a recent reference

Members	Closely related strains or synonyms	Vector	Vector Index	References
Barley yellow dwarf-MAV (BYDV-MAV)		*Sitobion avenae*	7	Gill 1967
	Barley yellow dwarf-PAV (BYDV-PAV)	*S. avenae, Rhopalosiphum padi*	7	Gill and Chong 1976
	Barley yellow dwarf-SGV (BYDV-SGV)	*Schizaphis graminum*	0.36	Gill and Chong 1979
Barley yellow dwarf-RPV (BYDV-RPV)		*R. padi*	7	Rochow et al. 1971
	Barley yellow dwarf-RMV (BYDV-RMV)	*R. maidis*	0.36	Rochow & Carmichael 1979
	Rice giallume (RGV)	*R. padi*	7	Osler et al. 1974
Bean leaf roll (BLRV)		*Acrythosiphon pisum*	0.36	Ashby & Huttinga 1979
	Legume yellows (LYV)	*A. pisum*	0.36	Duffus 1979
	Michigan alfalfa (MiAV)	*A. pisum*	0.36	Thottappilly et al. 1977
	Pea leaf roll (PeLRV)	*A. pisum*	0.36	Ashby & Huttinga 1979 Duffus 1972
Beet western yellows (BWYV)				
	Beet mild yellowing (BMYV)	*Myzus persicae* Sulzer	100	Duffus and Russell 1975
	Malva yellows (MYV)	*M. persicae*	100	Costa et al. 1959
	Turnip yellows (TuYV)	*M. persicae*	100	Duffus and Russell 1972
	Groundnut rosette assistor (GRAV)	*Aphis craccivora*	70	Casper et al. 1983
	Physalis mild chlorosis	*M. persicae*	100	MacKinnon 1965
Carrot red leaf (CRLV)		*Cavariella aegopodii*	0.36	Waterhouse and Murant 1982
Indonesian soybean dwarf (ISDV)		*Aphis glycines Matsumura*	0.21	Iwaki et al. 1980
Potato leaf roll (PLRV)		*M persicae*	100	Rowhani & Stace-Smith 1979
	Solanum yellows (SYV)	*M. persicae*	100	Milbrath and Duffus 1978
	Tobacco necrotic dwarf (TNDV)	*M. persicae*	100	Kubo and Takanami 1979
	Tomato yellow top (TYTV)	*M. persicae*	100	Thomas 1984

Soybean dwarf virus (SDV)		*Aulacorthum solani*	100	Tamada & Kojima 1977
	Subterranean clover red leaf (SCRLV)	*A. solani*	100	Johnstone et al. 1982
	Subterranean clover red leaf-C (SCRLV-C)	*Acyrthosiphon pisum*	0.36	Johnstone et al. 1984
Strawberry mild yellow edge (SMYEV)		*Chaetosiphon fragaefolii*	0.06	Martin & Converse 1985
Probable Members				
Banana bunchy top (BBTV)		*Pentolonia nigronervosa*	7	Dale et al. 1986
Milk vetch dwarf (MVDV)		*Aphis craccivora*	70	Inouye et al. 1968
Tobacco vein distorting (TVDV)		*M. persicae*	100	Smith 1946
Tobacco yellow vein assistor (TYVAV)		*M. persicae*	100	Adams and Hull 1972
Possible Members				
Beet yellow net (BYNV)		*M. persicae*	100	Sylvester 1958
Celery yellow spot (CYSV)		*Hyadaphis foeniculi*	0.36	Freitag and Severn 1945
Cotton anthocyanosis (CAV)		*Aphis gossypil*	100	Costa 1956
Physalis vein blotch (PVBV)		*M. persicae*	100	Mackinnon & Lawson 1966
Raspberry leaf curl (RLCV)		*Aphis rubicola*	0.06	Stace-Smith 1961
Tomato yellow net (TYNV)		*M. persicae*	100	Sylvester 1954

Vector index based on host range and the type of aphid host (Kennedy et al. 1962) and assuming an average plant family consists of 31.2 genera; Example: *M. persicae* occurs on over 50 plant families or 1562 genera = Index 100; *A. rubicola* on 1 plant genus = Index 0.06.

The recent work on beet western yellows virus (BWYV), potato leaf roll virus (PLRV), solanum yellows virus (SYV), and tomato yellow top virus (TYTV) suggests a serological and biological continuum of viruses, strains, or variants between BWYV on one end and PLRV on the other (Duffus 1981; Milbrath and Duffus 1978; Hassan et al. 1985).

Properties

Luteoviruses, at least superficially, appear to be structurally similar to many other small icosahedral single-stranded ssRNA plant viruses. The virions are approximately 25 to 30 nm in diameter and the ca. 6000 base genome is a single species of ssRNA of most likely positive-sense polarity (Waterhouse et al. 1988). The genomic RNA is encapsidated by a single species of capsid protein of M_r 24 to 26 kDa and is a probable T = 3 structure (Waterhouse et al. 1988).

All luteoviruses are transmitted by aphid vectors in a persistent-circulative manner. This is characterized by acquisition and inoculation thresholds of several minutes to a few hours, and retention is for days to life. There is a very short latent period measured in hours between acquisition and the subsequent ability to transmit, and there is no strong evidence for replication of luteoviruses in their aphid vectors. Luteoviruses are primarily phloem-limited in their plant hosts, and occur in relatively low titers in infected tissues. As a group, their molecular details are not as well studied as higher titer, mechanically transmitted viruses.

The New York RPV and MAV isolates of BYDV were the first luteoviruses to be partially characterized and have a partial analysis done of their genomes and coat proteins (Scalla and Rochow 1977; Brakke and Rochow 1974). It was not an easy task to obtain the amounts of virions needed for the characterizations, and the major obstacle to characterizing these and other luteoviruses was the ability to extract the virions from the phloem tissues so that sufficient quantities were available for analysis. A significant development in luteovirus purification was the discovery by Takanami and Kubo (1979a) that enzyme mixtures (cellulases and pectinases) could significantly aid in releasing virions of tobacco necrotic dwarf virus (TNDV) and PLRV (for a list of acronyms, see Table 1) from the phloem. Soon purification methods were developed for other luteoviruses; several others, including BWYV, SCRLV, and PLRV were subsequently partially characterized and found to be similar to the BYDVs at least in RNA and protein molecular weight (Falk and Duffus 1984; Waterhouse and Helms 1984; Takanami and Kubo 1979b).

The ability to infect tobacco (*Nicotiana tabacum* L.) protoplasts with TNDV allowed Kubo and Takanami (1979b) to investigate the effects of some metabolic inhibitors on BYDV replication and protein synthesis. Their observation that TNDV was unaffected by actinomycin D led them to suggest that it replicates without any DNA intermediate, but likely via RNA intermediates.

Gildow et al. (1983) provided further evidence in this regard, at least for BYDV. They found that double-stranded RNA molecules could be readily isolated from tissues of plants infected by five different isolates of BYDV. The major, highest molecular weight species corresponded to twice the molecular weight of virion RNA and was interpreted by them to probably represent genomic replicative form dsRNA. The significance of the other, lower molecular weight dsRNAs seen in these preparations was not known, but interestingly, the dsRNA patterns observed after polyacrylamide gel electrophoresis showed that the patterns for RMV and RPV were quite similar to each other, and obviously different from the patterns of PAV, MAV, and SGV. These latter dsRNA patterns were very similar to each other.

Thus, like previous analyses such as serological and cytopathological tests, dsRNA analysis showed these five BYDVs to fall into two distinct groups. More recent studies have demonstrated that for other luteoviruses such as BWYV and SDV, dsRNAs can be extracted and identified from infected tissues, and these are a further property that can be used to identify and compare various luteoviruses (Valverde et al., in press; Hewings et al. 1986).

Recently, molecular cloning of complementary DNAs to luteovirus genomic RNAs has been achieved (Waterhouse et al. 1986; Falk and Anderson 1987; Barbara et al. 1986). These have been used in hybridization tests to compare nucleic acid sequence relationships. Two groups have reported results using cDNA clones made to BYDV. Waterhouse et al. (1986) reported that cDNA:viral RNA hybridization results generally agreed with serological results for determining relationships among luteoviruses, however they found that some of their BYDV cDNA clones were somewhat general, and reacted with several different luteoviruses that they examined. Barbara et al. (1986) have reported that cDNA clones to the 3' end of MAV RNA are specific to MAV, while those cDNA clones to other parts of the genome also are more likely to react with PAV. Recent studies with cDNA clones made by random priming on virion RNAs of the ST9 isolate of BWYV have shown that these cDNA clones react significantly only with BWYV and not with the several other luteoviruses that have so far been tested (Falk and Anderson 1987). However, the cDNA clones that react with BWYV genomic RNA react with all BWYV isolates so far tested.

The capsid proteins of luteoviruses are thought to be intimately involved in determining the ability of a given luteovirus to be transmitted by a given aphid vector. The capsid proteins for many of the luteoviruses have been compared by methods such as SDS-PAGE and various serological tests. Again, BYDV was the first luteovirus to be examined for the molecular weight of its capsid protein (Scalla and Rochow 1977). Several luteoviruses have been subsequently similarly analyzed, and it is generally accepted that luteoviruses have a single major species of capsid protein of M_r 24 kDa (Waterhouse et al. 1987). Minor bands are often seen in SDS-PAGE analyzed virion proteins of luteoviruses (Scalla and Rochow 1977), but the significance, if any, of these bands is unknown at present. Amino acid analysis of the major capsid proteins of TNDV and SDV have shown ca. 219 amino acid residues (Waterhouse et al., 1988). Serological comparisons of the luteoviruses have shown varying degrees of interrelationships, and some of these are discussed later.

Besides the virion capsid protein, an additional protein has been identified for PLRV (Mayo et al. 1982). This has been determined to be a genome-linked protein at the 5' end of the virion RNA. The estimated molecular weight of this protein is ca. 7000. *In vitro* translation analyses of luteovirus genomic RNAs have not as yet been able to identify any more definitive virus-specific proteins (Mayo et al. 1982).

Other Plant Viruses

There are viruses belonging to four plant virus groups that are transmitted by aphids in a persistent manner; the luteovirus group, the rhabdovirus group, the artificial group including subterranean clover stunt virus (SCSV) (Grylls and Butler 1959) and filaree red leaf virus (FRLV) (Fraser 1951), and the monotypic pea enation mosaic virus (PEMV). Rhabdoviruses exhibit a distinctly different type of transmission behavior from luteoviruses. Those that have been studied have been shown to infect and multiply in their vectors, and thus are persistent-propagative as opposed to persistent-circulative. The rhabdoviruses have few similarities with the luteovirus group.

The causal agents of FRLV and SCSV have not yet been identified. The syndromes of these diseases are similar, but seem to be distinct and probably unrelated to the other groups of persistently transmitted viruses. There seems to be only one virus group that may have some affinity to the luteoviruses, and this is represented by PEMV. This is a persistent-circulative aphid-transmitted small icosahedral ssRNA virus. The main aphid vector is the pea aphid, *Acyrthosiphon pisum* (Harris).

PEMV is not a luteovirus; it is placed taxonomically in its own group, yet it has several properties that bring up questions about its relationships to the luteoviruses. It, like the luteoviruses, has

adopted persistent-circulative aphid transmission as its means of dispersal and survival. Unlike the luteoviruses, PEMV can be efficiently inoculated by aphids to host plants during very short inoculation access periods (Toros et al. 1978) and it has been suggested that the plant nonvascular tissues may actually be where infection takes place. These data, and the fact that it also is efficiently mechanically transmissible demonstrate that PEMV is biologically different from the luteoviruses.

The molecular composition of the PEMV virions also is quite different from that found in the luteoviruses. The purified virions sediment as two distinct nucleoprotein components. The nucleoproteins each contain ssRNAs, and two to three major ssRNAs are reported for PEMV (Adams et al. 1979). When PEMV is repeatedly mechanically transferred, isolates can readily lose their ability to be aphid transmitted (Clarke and Bath 1977; Hull 1977).

Recent comparative characterizations of aphid transmissible and non-aphid transmissible isolates indicate a specific virion capsid protein is lacking on the non-aphid transmissible isolates. Thus, like luteoviruses, the protein composition of the PEMV virion determines aphid transmissibility, but unlike the luteoviruses the PEMV aphid transmission-related protein has an M_r of 56 kDa. Interestingly, in the original characterization of BYDV capsid proteins by Scalla and Rochow (1977), they noted minor amounts of proteins of approximately this molecular weight, and for MAV and RPV these minor proteins had different molecular weights.

Another similarity to the luteoviruses that is exhibited by PEMV is that it can act as a helper virus for the aphid transmission of the helper-dependent virus, bean yellow vein banding virus (BYVBV) (Cockbain et al. 1986). This is discussed in more detail later.

The exact relationship, if any, of PEMV to the luteoviruses is unknown at present. However, whether it may represent some evolutionary link between mechanically transmissible small spherical plant viruses and luteoviruses, or perhaps some link between the aphid-transmitted helper-dependent virus complexes and the luteoviruses is interesting to consider.

Virus Complexes

Mixed infections of plant viruses in nature are probably more common than infections by single viruses. From an epidemiological standpoint, the pure cultures as studied in the laboratory rarely occur alone in nature. Several types of interactions between related and unrelated viruses involving the luteoviruses may affect the epidemiology and the origins of these viruses. The lack of, or low level of, interference phenomena between isolates, strains, or different luteoviruses is important in their distribution, and perhaps, origins. Duffus (1981) reported BWYV in leaf roll-affected potatoes from California. The virus was also associated with the disease in Tasmania (Duffus and Johnstone 1982). Because of strict plant quarantine and certification schemes operating in Tasmania since the early 1930s, these isolates of BWYV were probably representative of complexes that were common before world-wide certification schemes. It is possible that potatoes have served as a very important factor in the distribution of this virus through the world. One of the first commercial ELISA kits for PLRV produced in Germany, contained antibodies to both BWYV and PLRV (Duffus and Casper, unpublished).

BWYV frequently occurs in mixtures with SDV in Tasmania, particularly in legumes (Johnstone et al. 1984b). Luteovirus mixtures in bur medic (*Medicago polymorpha* L.), faba bean (*Vicia faba* L.), and garbanzo bean (*Cicer arietinum* L.) in California are very common.

Biologically, the role of the luteovirus protein capsid in vector transmissibility/specificity is well documented. As a general rule, aphid vector specificity is high in the luteoviruses, and perhaps greatest in some of the BYDVs. A clue as to the significance of the virion capsid in vector specificity was detected when a breakdown in specific transmission of

MAV occurred in mixed infections of RPV and MAV. Some MAV was transmitted efficiently by *Rhopalosiphum padi* (L.) from the doubly infected plants. This *R. padi*-transmitted MAV however, could be neutralized by using antiserum to RPV.

Antiserum to MAV only neutralized *Sitobion avenae* (Fabricius)-transmitted MAV and did not affect the *R. padi*-transmitted MAV. This one-way breakdown in vector specificity was interpreted to result from encapsidation of the MAV RNA by the RPV protein capsid (Rochow 1965, 1970). This phenomenon has been termed transcapsidation or genomic masking. Similar breakdowns in specificity of aphid transmission have been detected between other BYDVs in double infections. The significance of this mechanism in luteovirus distribution, epidemiology, and evolution is not presently known, but it surely has played a role.

A somewhat similar situation has been found for several aphid-transmitted helper-dependent virus complexes (Falk and Duffus 1981). These are specific diseases caused by double infections of a luteovirus (the helper virus) and a helper-dependent virus. Unlike the BYDV situations, the helper-dependent virus is not a luteovirus and is unrelated to the helper virus, and differs in a major biological property in that it is readily mechanically transmissible. The helper-dependent viruses are not aphid transmissible when they infect plants by themselves, hence the name helper-dependent viruses. Examples of the helper-dependent virus complexes can been seen in Table 2.

Evidence supporting the transcapsidation mechanism for the helper-dependent viruses as a means to gain their aphid transmissibility has been obtained for some of the different complexes. It was noticed by Adams and Hull (1972) that tobacco yellow vein virus (TYVV), which normally is transmitted by *Myzus persicae* (Sulzer) when in association with tobacco yellow vein assistor virus (TYVAV), could change aphid vector specificity and be efficiently transmitted by *Aphis craccivora* Koch when in a mixed association with groundnut rosette assistor virus (GRAV).

If virion protein capsid determines aphid vector transmissibility, then we might conclude that when TYVV is in a mixed infection with TYVAV, it has the protein capsid of the helper virus (TYVAV), and subsequently, it is transmissible by the TYVAV aphid vector, *M. persicae*. Then when TYVV is separated from TYVAV and put in a mixed infection with GRAV, which has a different capsid protein and another aphid species as its vector, if TYVV RNA can become genomically masked by the GRAV protein capsid, it could then also become transmissible by the GRAV aphid vector.

More direct evidence along these lines has been obtained for two other complexes, the lettuce speckles and carrot motley dwarf virus complexes. Elnagar and Murant (1978) showed that the helper-dependent virus of the motley dwarf complex, carrot mottle virus (CMotV), was significantly more stable and resistant to physical extremes when it was in the mixed infection with the luteovirus helper, carrot red leaf virus (CRLV), than when CMotV infected plants by itself. They interpreted this as evidence for genomic masking of the CMotV virion RNA by the stable CRLV capsid protein in the mixed infection.

For the lettuce speckles complex, Falk et al. (1979) showed similar evidence on the stability of the helper-dependent virus of this complex, lettuce speckles mottle virus (LSMV). However, they also showed that the aphid-transmissible LSMV from plants doubly infected by BWYV and LSMV could be effectively neutralized by using antiserum to BWYV. This provided more evidence in support of the genomic masking hypothesis. Later, Waterhouse and Murant (1983) also showed that aphid-transmissible CMotV from plants infected by CRLV and CMotV could be neutralized by antiserum to CRLV. They also showed that CMotV could utilize other luteoviruses as helpers, and the resulting aphid vector specificity was determined by the particular luteovirus in the mixed infection.

As it has been well documented that luteoviruses often occur in the field as mixed infections with other luteoviruses

Table 2. Interactions of luteoviruses in mixed infections

Helper	Dependent	Aphid Vector	Reference
Group I. Luteovirus - Luteovirus			
BYDV-RPV	BYDV-MAV	*Rhopalosiphum padi*	Rochow 1965, 1970
	BYDV-RMV		Rochow 1982
	BYDV-SGV		Rochow 1982
BYDV-PAV	BYDV-MAV	*R. padi*	Rochow 1982
	BYDV-RPV	*Sitobion fragariae*	Guy et al. 1987
	BYDV-RMV	*R. padi*	Rochow 1982
BYDV-RMV	BYDV-MAV	*R. maidis*	Rochow 1975
	BYDV-RPV		Rochow 1982
Group II. Luteovirus - Helper Dependent Virus			
Helper	**Dependent**	**Aphid Vector**	**Reference**
BWYV	LSMV	*Myzus persicae*	Falk et al. 1979
	CMotV	*M. persicae*	Waterhouse & Murant 1983
PLRV	CMotV	*M. persicae*	Waterhouse & Murant 1983
TUYV	LSMV	*M. persicae*	Falk et al. 1979
TYVAV	TYVV	*M. persicae*	Adams & Hull 1972
TVDV	TMotV	*M. persicae*	Smith 1946 a, b
CRLV	CMotV	*Cavariella aegopodii*	Waterhouse & Murant 1983
GRAV	GRV	*Aphis craccivora*	Adams & Hull 1972
	TYVV	*A. craccivora*	Adams & Hull 1972
BLRV	BYVBV	*Acyrthosiphon pisum*	Cockbain et al. 1986
PEMV[a]	BYVBV	*A. pisum*	Cocbain et al. 1987
Group III. Luteovirus - Satellite or Satellite-like Agent			
Helper	**Dependent**	**Aphid Vector**	**Reference**
BWYV (ST9)	(ST9) "extra"RNA	*M. persicae*	Falk & Duffus 1984
TNDV	Low M_r	*M. persicae*	Takanami & Kubo 1979

[a] PEMV is not a luteovirus.

(Waterhouse et al. 1988; Rochow 1965; Falk and Duffus 1981), or with other, unrelated plant viruses, the mechanism adopted by the helper-dependent viruses, which requires that they be mixed infections with luteoviruses for their resulting aphid transmission and survival, seems to be well adapted and thus fits easily with the biology of luteoviruses.

Satellite-like Agents and Luteoviruses

Besides the genomic RNAs of luteoviruses, and helper-dependent virus RNAs, there is evidence that some luteoviruses have satellite-like RNAs, which are encapsidated in the luteovirus protein capsids, and these satellite-like or extra RNAs are subsequently aphid transmitted by the luteovirus aphid vector. Takanami and Kubo (1979b) reported very small ssRNAs to be associated with the purified virion RNA of TNDV. Two distinct forms of RNA were detected on polyacrylamide gels, and both were slightly smaller that the CARNA 5 RNA.

Recently, Falk and Duffus (1984) have shown that an isolate of BWYV that gives a very severe reaction in shepherds' purse (*Capsella bursa-pastoris* (L.) Medikus) has an additional ssRNA that is not found in isolates of BWYV that gave typical symptoms on *C. bursa-pastoris*. It has been demonstrated by cDNA cloning and hybridization analysis that this extra virion-associated RNA is not related to the BWYV genomic ssRNA (Falk and Anderson 1987). None of the other BWYV or other luteovirus isolates that we have so far screened has an extra RNA that hybridizes with the cDNA clone to the extra RNA associated with the ST9 BWYV isolate (Valverde et al., in press).

With the abundance of satellite viruses and RNAs found among the other groups of plant viruses, the limited searching so far done among the luteoviruses, and the evidence for satellite-like RNAs that already has been discovered, it is very likely that more such agents will be found among the luteoviruses. The effects of these associated agents in mixed infections with luteoviruses on the resulting diseases will be interesting, for in some cases satellites appear to enhance diseases and in others to almost control diseases and, as such, function as natural biological control agents for plant viruses (Francki 1985).

Origins and Relationships of Luteoviruses

Weed patches in California, Tasmania, or Britain, barley fields in the Dakotas, potatoes in Germany, and legumes in Oregon all have one thing in common; the occurrence of luteoviruses in various combinations of mixed infections. Indeed, when examining these weeds and crops, there appears to be one system with a number of variations in host range, vector specificity, and epidemiology. The mixed infections of luteoviruses, and of luteoviruses with other unrelated plant viruses, have probably played a major role in luteovirus evolution and in the evolution of some other viruses.

The luteoviruses, described originally from a number of different crop species from all over the world, looked in most instances to be unique and distinct plant viruses causing epidemiologically different diseases. As our knowledge of the viruses has expanded through chemical and serological approaches, the closeness and interrelatedness of the viruses has become more and more apparent, until the some 35 to 40 distinct entities have been reduced to approximately nine major distinct serogroups. The other described viruses, in most instances, represent intermediate forms or epidemiologically distinct variants. In several instances, diseases in individual host groups, such as the Gramineae, with one described disease, have been shown to be caused by distinct viruses. Barley yellow dwarf (BYD) is now thought to be caused by several viruses that make up two separate virus subgroups in their vector relations, host ranges, and serological properties. The same is true for the legume infecting viruses, which now seem to represent three distinct viruses. Indeed, this is what would be expected if the origin of the luteoviruses were from a single source.

The BWYV group represents an extremely varied complex of virus strains or types differing in host ranges and epidemiological patterns. Little work has been done on vector specificity within this group, but we would expect a great deal of such specificity to occur in nature. Specific types such as turnip yellows virus (TuYV), beet mild yellowing virus (BMYV), and malva yellows virus (MYV) have been separated out by the specific diseases or epidemiological patterns that they represent. With several polyphagous aphid species as vectors, it is easy to postulate that individual isolates of this BWYV group may have been transmitted to the Leguminosae, others to the Solanaceae and still others to the Gramineae, etc. As primeval aphids were probably polyphagous and monophagy is a recent development in aphid evolution (Dixon 1985), it is easy to suggest that vector movement with monophagous species over a number of generations may have perpetuated and refined vector- and host-specific luteovirus types. Further evolution could also occur within the group, such as BYDV-MAV types could have originated from BYDV-RPV types, or perhaps via a separate introduction from another luteovirus group.

Evolutionary changes seem to be happening now with the legume infecting luteoviruses. Bean leaf roll virus (BLRV) occurs together with, and in addition to BWYV and SDV in bur medic, faba bean, and garbanzo bean in California (Johnstone et al. 1984a). If this complex in legumes had been studied in depth with the approaches utilized for BYDV, it could have been described as was BYDV, as a complex of virus isolates with different vector specificities, host ranges, and serotypes.

Tests in Tasmania up to 1980 indicated that all SDV-like isolates recovered from the field were transmitted exclusively by *Aulacorthum solani* (Kaltenbach) (Johnstone 1978; Johnstone and Duffus 1984; Johnstone and Patten 1981). *A. pisum* became established in Tasmania in 1980 and has since displaced *A. solani* as a major component of the aphid fauna. We now find that most SDV isolates recovered from the field are vectored specifically by *A. pisum*. Unlike the BYD viruses, these *A. pisum*-specific isolates are serologically indistinguishable from those vectored solely by *A. solani*. The recent isolates of SDV in Tasmania therefore closely resemble those found in California near Salinas where *A. pisum* is common on legumes and *A. solani* is rare (Johnstone et al. 1984a).

Ultrastructural differences demonstrated by Gill and Chong (1976, 1979) suggested that six variants of BYDV could be divided into two subgroups on the basis of cytopathological alterations induced by the viruses. The division of BYDV isolates into two groups is in agreement with a similar separation based on serological properties of the isolates (Rochow and Carmichael 1979) and on separation by dsRNA analysis (Gildow et al. 1983). Gill and Chong (1979) indicated the similarities between the ultrastructural alterations induced by BYDV-RPV and BWYV likewise indicated a closer relationship between these viruses, than between BYDV-MAV and BWYV, in a similar way to their reported serological relationships (Duffus and Rochow 1978).

L.L. Hoefert (personal communication) has indicated that those conclusions are perhaps too simplified and that ultrastructural alterations induced by BWYV encompass changes induced by both MAV and RPV types. For instance, with BYDV-MAV, single-membraned vesicles with fibrils appear in the cytoplasm, whereas with BYDV-RPV, vesicles with fibrils are enclosed in a second membrane within the cytoplasm. With BWYV, single-membraned vesicles are present when a few virus particles are in the sieve elements and the plasmodesmata of sieve element vascular parenchyma. Double-membraned vesicles follow rapidly after single-membraned vesicles become enclosed in endoplasmic reticulum, which produces the double membrane. Both single- and double-membraned vesicles may be found in the same cell.

Later with MAV, the nucleus becomes distorted and clumping of heterochromatin occurs followed by the appearance of filaments in the nucleoplasm. Virus particles appear in the nucleus much later. With RPV, virus particles appear around an undistorted nucleus, there is no clumping of heterochromatin and no filaments in the nucleoplasm. Again with BWYV, the ultrastructural alterations are similar to both the MAV and RPV types. The nucleus becomes distorted, but clumping of the heterochromatin occurs and virus particles and filaments can be found in the nucleus. Thus it appears that if indeed the ultrastructural differences induced by MAV and RPV are real, they are not distinct from changes that occur with BWYV. This may indicate origins of both types from BWYV, as was previously discussed.

The use of host range information to classify viruses has clear limitations and has been discussed at length in relation to luteoviruses of legumes (Johnstone et al. 1984b). However, the type of symptom is related to pathogenesis and perhaps also to the mechanism of vector transmission. These two factors utilized in comparisons of viruses over the past 40 years have differentiated the luteoviruses from all other virus groups. Some virus groups, closteroviruses, and some ungrouped whitefly-transmitted viruses have similar symptoms, whereas other virus groups have mocked the circulative persistent aphid transmission relationships. But thus far if plants show interveinal yellowing and the virus inducing those symptoms is transmitted persistently by aphids, it is a luteovirus.

Also, it appears that classification based on host range information may, in a group like the luteoviruses, reflect evolutionary relationships. This has been supported by limited serological and genome hybridization comparisons (Waterhouse et al. 1986). Most of the definite luteoviruses have very narrow and specific host ranges involving one or perhaps two plant families (Figure 1). BWYV, on the other hand, infects all plant subclasses, orders, and families infected by other luteoviruses.

If the major vectors of the individual luteoviruses are considered, the major reason for the narrow and specific host ranges of many of these viruses is readily understood. For example, if an artificial vector index is constructed based on the host plants of the aphids (Kennedy et al. 1962) using an "average" plant family consisting of 31 genera, we arrive at an index of 100 for *M. persicae* but only 0.06 for *Aphis rubicola* (Table 1). The former aphid is, of course, the vector of BWYV (infecting >25 families) and the latter aphid is the vector of Raspberry leaf curl (RLCV) (infecting only 1 genus). We can see that the vector index and plant host range show strong similarities.

It is interesting to note that two vectors of extreme importance in two distinct family-limited luteoviruses, BYDV and BLRV, namely *R. padi* in monocotyledonous families (Index 7) and *A. pisum* with host plants mostly in the Leguminosae (Index 0.36), are occasionally found on *Capsella bursa-pastoris*, the only plant species known that all BWYV isolates infect.

The fact that BWYV, transmitted by several polyphagous aphid species, infects all the plant families infected by the other luteoviruses, may indicate that the origin of the distinct one-family viruses was from BWYV. Once introduced into these plant groups, host-specific vectors carried variants through enough generations that they were distinct in host range, vector specificity, and serology. It is interesting to note that variants of SDV vectored by a polyphagous aphid species, *A. solani*, have a relatively wide host range, but a variant vectored specifically by *A. pisum* infects only the Leguminosae.

In addition to a spectrum of variation within any one group of luteoviruses, a serological link occurs among luteoviruses once thought to be distinct and different. A number of serological procedures have been applied to luteoviruses. The first was a type of infectivity neutralization developed by Gold and Duffus (1967). This technique

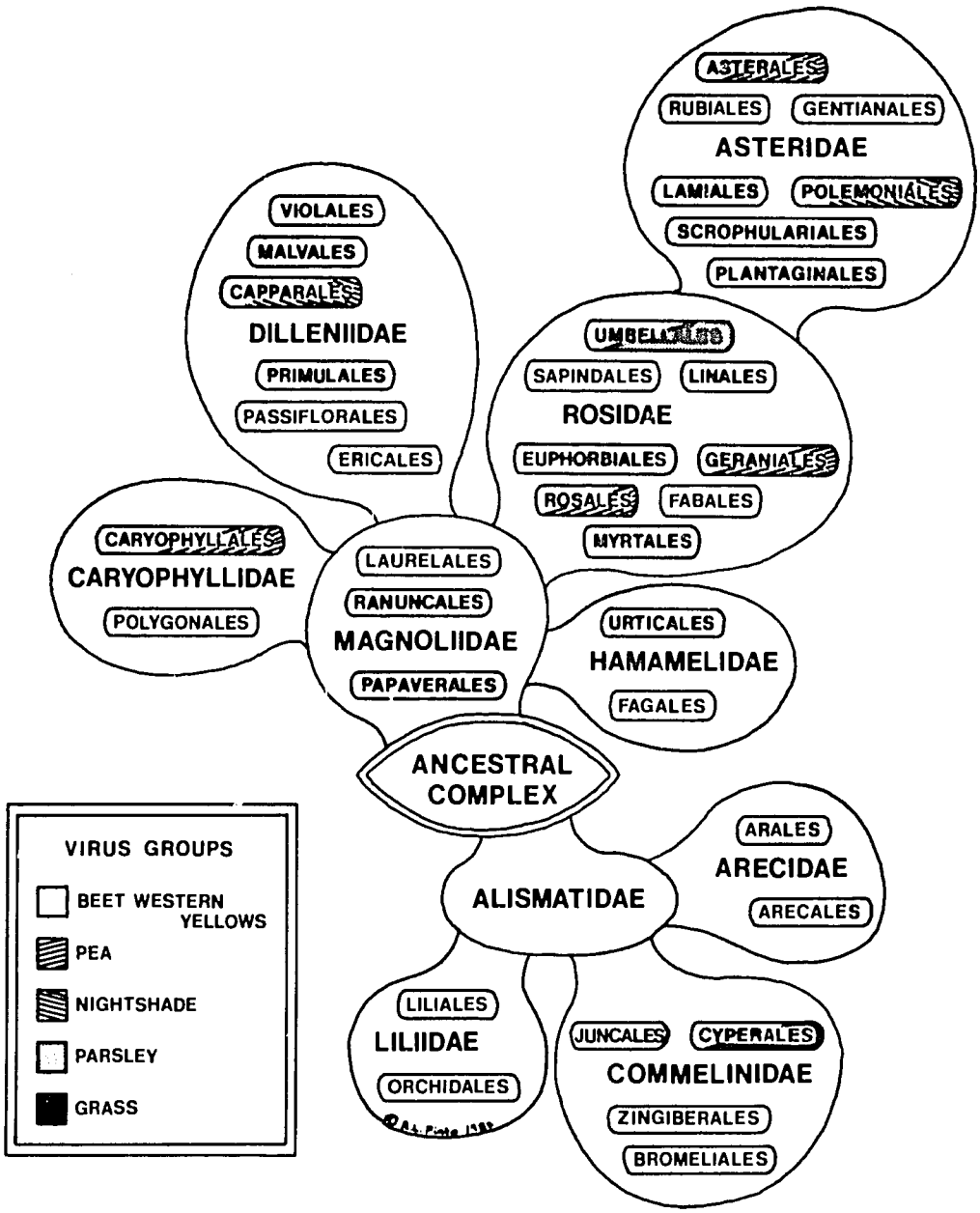

Figure 1. Host ranges of luteoviruses in selected orders of plants.

and variations thereof, utilizing antigen antibody reactions with a bioassay, could be effectively used with low titered antisera and crude virus preparations. Insects were allowed to feed through membranes on the antigen-antibody complexes or centrifuged preparations of them. The aphids were unable to transmit virus from the preparations if the reaction was with a related antiserum.

The early work in serology of luteoviruses was limited more by low concentration of virus in antigen preparations than by low titers of antisera. For example, antisera developed in 1961 for BYDV were not found to be active until several years later when this sensitive serological procedure was utilized (Rochow and Ball 1967). A variation in which the virus-antibody reaction is analyzed by measuring unreacted virus in centrifuged sucrose gradients permits quantitative studies of virus samples without a bioassay (Ball and Brakke 1969).

The early work with these procedures using low dilutions of BYDV antiserum permitted serological comparisons that were difficult or impossible by conventional techniques. The early results indicated that BWYV was related serologically to all members of the luteovirus group tested, viruses at first thought to be quite distinct and different (Duffus and Russell 1972, 1975; Duffus 1977; Duffus and Rochow 1978; Rochow and Duffus 1978).

As the antigen-antibody reaction technique has been refined by using antiserum dilutions (Johnstone et al. 1982) and other more specific methods such as ELISA (Casper 1977; Rowhani and Stace-Smith 1979), the luteoviruses seem to form a serological family tree with BWYV as the main branch, the sub-branches are essentially clusters of host, vector, and serologically specific viruses that may or may not be related to each other, but are related to BWYV. It would appear that the luteovirus tree at present has nine major branches representing BWYV, PLRV, BLRV, SDV, ISDV, CRLV, BYD-RPV, BYD-MAV, and strawberry mild yellow edge virus (SMYEV).

Conclusions

New technology is suggesting what early predictions of neutralization serology hinted, that the luteoviruses are essentially one system with many variations in host reaction and coat protein. Host reaction differences appear to be controlled by genome differences in some cases and by coat protein and thus vector specificity in other cases. Much of the emphasis in a monofamily virus such as BYDV has been with coat protein and vector specificity, however, the multi-family viruses such as BWYV have numerous apparently identical serological strains that differ greatly in host reactions in the areas of virulence, host range, and immunity. It would be surprising if all BYDV isolates of any given serotype had the same host range of wild and crop plants and the same virulence on a range of these host plants.

A critical evaluation of resistance to luteoviruses appears to be needed. There is some evidence of immunity to some luteoviruses within a species (Duffus and Milbrath 1977; Duffus 1979). There is other evidence with BYDV that symptomatic resistance is associated with reduced virus productivity. The effect is, however, cultivar and virus-type specific (Lister et al. 1984). Within serologically identical isolates of BWYV, there are vast differences in host reaction or immune reactions. This has not been looked at critically with most luteoviruses.

Luteoviruses are viruses that are adapted to long distance dispersal and survival by relying completely and intimately on aphids for transmission. In our presently primitive understanding of the transmission mechanisms of luteoviruses, we know that the virion capsid is involved in specificity of aphid vectors. That luteoviruses are successful plant pathogens must be accepted, and thus it is not surprising that other agents (satellites, helper-dependent plant viruses) would then also successfully exploit and utilize the transmission mechanisms used by luteoviruses for their own transmission and survival.

It is easy to speculate on the relationships of the luteoviruses to each other. We have done some speculation here, but the facts are that luteovirus ecology is a complex system involving aphids, plant hosts, and even other viruses and virus-like entities. All of these factors have played some role in the evolution of the luteoviruses. It will be interesting, as data become available, to look at coat protein sequence relationships of the various luteoviruses to attempt to dig deeper into the mechanisms that allow these important and successful plant pathogens to be intimately associated with and transmitted by their aphid vectors.

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Molecular Biology of Barley Yellow Dwarf Virus

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Abstract

Using recombinant DNA techniques, we have begun analysis of the genome organization, function, and evolution of barley yellow dwarf virus (BYDV). The complete nucleotide sequence of an Australian PAV serotype of BYDV has been determined. Open reading frames are evident and some genes have been identified. Different regions of the Chinese GPV and GAV isolates of BYDV show varying levels of homology to the Australian PAV sequence. Northern analysis of infected plants suggests that subgenomic RNAs of length 2.8 kb and 0.8 kb are produced during infection with the PAV isolate. Electroporation has been used to introduce viral RNA into protoplasts to establish infection in cell cultures. This approach will be used to further define the infection process, including stages of the replication cycle, effects of mutations on infection, and nucleic acid molecules that interfere with infection.

"Little is known of the chemical and physical properties," wrote Jones and Clifford (1983) in introducing their description of barley yellow dwarf virus (BYDV) in their book, *Cereal Diseases*.

We have begun an analysis of the genome organization of BYDV to obtain basic information on the genetic structure and evolution of the virus. This work also has application in the production of cloned cDNA sequences, which can be used as diagnostic probes. In the longer term, it will form the basis for synthesis of gene constructions that confer BYDV resistance to genetically transformed plants.

Genome Organization of an Australian PAV Isolate

We have prepared a series of cDNA clones from genomic RNA of an Australian PAV serotype. This serotype was separated from an Australian isolate that contained a PAV+RPV mixture (Waterhouse et al. 1986). The clones have been sequenced and found to encompass the entire 5,673 bases of the RNA genome. Up to 95% of the sequence has been verified from both strands of the cDNA and, in many cases, sequencing of different overlapping clones and direct sequencing of viral RNA. Computer analysis of the sequence has been used to identify significant open reading frames (ORFs) likely to encode proteins. These are shown in Figure 1.

For some of these ORFs a function can be proposed. The first and second ORFs nearest to the 5' end of the genome encode an RNA-dependent RNA polymerase, required for replication of the virus RNA. This gene has been identified by primary structural features of its inferred amino acid sequence; it has the conserved amino acid domains seen in RNA polymerase genes of other plant and animal viruses (Kamer and Argos 1984). These two ORFs are separated by a frameshift whose presence has been ascertained in a number of sequencing experiments involving both cDNA strands. One surprising result of computer comparisons of this gene with the RNA polymerase genes of other plant viruses is that, except for an N' terminal portion, the amino acid sequence has more than 32% homology with the analogous gene of carnation mottle virus (CarMV) (Guilley et al. 1985). These gene homologies argue for a previously unsuspected close evolutionary relationship between BYDV and CarMV.

The coat protein gene assignment is tentative. However, it encodes a protein of 22 kDa, which is similar in size to that found by Scalla and Rochow (1977). Additionally, its amino acid composition is similar to that of other luteovirus coat proteins (Kubo 1981; P.M. Waterhouse

and A. Kortt, unpublished). Furthermore, it shares structural features with the coat proteins of CarMV and southern bean mosaic virus (SBMV) (Hermodson et al. 1982), which suggests a taxonomic relationship with them. At their N' termini the PAV and SBMV proteins are proline rich, basic, and lack aromatic and acidic residues. Computer analysis also reveals a central conserved region shared by the coat proteins of all three viruses. We are presently checking whether this is the coat protein by 1) obtaining amino acid sequence data of coat protein fragments of the PAV serotype, and 2) immunological testing of bacterial strains engineered to express the putative coat protein gene product.

It is possible that an extended protein product is also expressed from the coat protein gene. This arises from the presence of a 48 kDa ORF immediately following after the UAG termination triplet of the coat protein gene. "Read-through" proteins, which result from suppression of termination triplets, have been observed for other plant viruses such as tobacco mosaic virus (TMV) (Goelet et al. 1982). If read-through did occur, then it would be predicted that high molecular weight proteins might be found at low levels in virion protein preparations of BYDV. Such proteins have been reported (Scalla and Rochow 1977), but it remains to be determined whether they actually originate as read-through products of the coat protein gene.

A number of small ORFs, including one in the 3' region of the genome, are also shown in Figure 1. Any of these could encode the 5' virus protein linked to the genome (VPg), which would be expected to have this size (6.0 to 6.5 kDa) if BYDV is similar to potato leaf roll virus, another luteovirus, whose VPg has been characterized (Mayo et al. 1982).

The genome of an Australian RPV isolate is being sequenced at the University of Adelaide to determine nucleotide divergence and evolutionary relationships of the two predominant Australian BYDV serotypes.

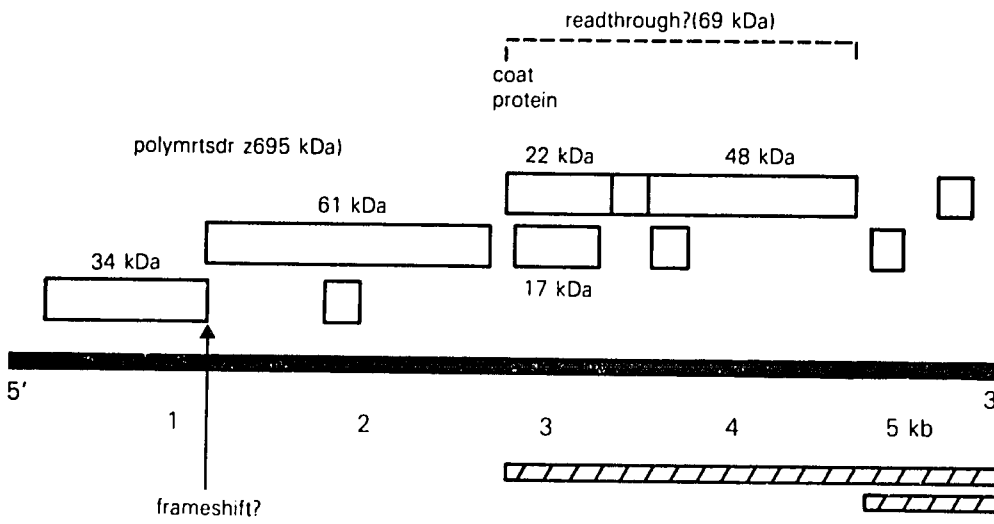

Figure 1. Proposed genome organization and subgenomic RNAs of an Australian PAV serotype of BYDV. The solid bar represents the 5.7 kb RNA genome. Open boxes above the line show the open reading frames (ORFs) and sizes of their encoded proteins. The ORFs represented by small boxes encode proteins of molecular weight = 6 kDa - 6.5 kDa). Diagonally marked segments below the line represent the subgenomic RNAs deduced from Northern analysis of infected plants. (Abbreviations: kb = kilobase, kDa = kilodaltons).

Subgenomic RNAs
One method of gene expression used by single stranded RNA viruses involves the use of less-than-full-length subgenomic mRNAs. Each subgenomic RNA is 3' coterminal and it expresses the most 5' gene within it.

We have detected subgenomic RNAs in total RNA extracted from PAV infected plants by Northern hybridization analysis. Less-than-full-length RNAs of 2.8 kb and 0.8 kb were detected, as well as full-length RNA. The hybridization to various cDNA probes from different parts of the PAV sequence is consistent with these RNAs being 3' co-terminal as expected for a virus with subgenomic strategy of gene expression. Subgenomic RNAs can often be detected as conspicuous double stranded RNAs (dsRNAs) in extracts from infected plants. Gildow et al. (1983) have described a series of dsRNAs associated with infection with different serotypes. For the PAV serotype the sizes of the major dsRNAs that they observed were similar to those that we detected by Northern analysis.

Two additional points should be made about our results. Firstly, there was no subgenomic RNA that would act as the message for expression of a new gene following the frameshift region of the RNA polymerase gene. This lends further support to the suggestion that this frameshift is suppressed during translation. Secondly, we did not detect a subgenomic RNA corresponding to a gene starting at the position of the read-through ORF immediately following the putative coat protein gene. This supports the suggestion made above that this region is expressed as a read-through protein.

Sequence divergence in the BYDV genome
The genome organization shown in Figure 1 is based on the sequence of cDNA clones prepared from one of our laboratory strains of BYDV. This PAV serotype was separated from a BYDV field isolate originally collected by R. Sward (Department of Agriculture and Rural Affairs, Burnley, Victoria, Australia), and probably originated from a unique or small size founder population, because we isolated it by twice passaging the virus at limiting dilution (Waterhouse et al. 1986). Consistent with this we found very little nucleotide sequence heterogeneity among overlapping clones from different parts of the genome, viz., 1 nucleotide difference in a total of 2,600 bases sequenced.

PAV-specific clones prepared from the original BYDV isolate showed divergence from the laboratory strain. A 1.6% base sequence variation was seen in a total of 3,119 bases sequenced. Most nucleotide changes in the RNA polymerase and putative coat protein genes were silent mutations, i.e., they did not alter amino acid sequence. This suggests that it is likely that a natural population of BYDV has sequence heterogeneity, and that although mutations are tolerated, selection pressure maintains the amino acid sequence of these ORFs. It also provides further indication that the identification of these two ORFs as functional genes is correct.

Toward the 3' end of the genome the laboratory PAV strain and the PAV sequences from clones of the original BYDV mixed isolate show greater sequence diversity. None of the base changes leads to frame reading changes in the 48 kDa read-through ORF as expected for a sequence encoding a protein.

Therefore, we can suggest that field isolates of BYDV consist of a population of sequences that maintain genetic information content but are divergent at the nucleotide sequence level.

To begin a comparison of genome divergence between BYDV serotypes, we have sequenced cloned cDNAs from two Chinese BYDV serotypes, designated GPV and GAV (Zhou et al. 1984). The level of divergence varies when different regions of the genome are compared. cDNAs encoding parts of the RNA polymerase and coat protein genes can be identified by homology with their counterpart genes in the PAV sequence. Within the RNA polymerase genes, a clone from one of the Chinese serotypes showed 95% sequence homology with the PAV sequence. In parts of the coat protein

genes that have been compared, the serotypes show approximately 75% sequence homology. In contrast, cDNAs from other regions of the GAV and/or GPV isolates showed no recognizable homology with the PAV sequence.

Applications of Molecular Biology of BYDV

Studies of genome organization of BYDV isolates provide basic information about the replication strategy and evolution of BYDV. There are also two areas in which the information and clones obtained from molecular biological studies can be applied in research on BYDV. These are the production of nucleic acid probes for the detection of BYDV, and attempts to produce synthetic resistance genes that will be effective in genetically transformed plants.

Nucleic acid probes for BYDV detection

Enzyme-linked immunosorbent assay (ELISA) (Clark and Adams 1977) has become established as a rapid and simple technique for the diagnosis of BYDV infection of plants. However, it detects only known serotypes, and standardized antisera are not widely available. The development of monoclonal antibodies could overcome these problems (Diaco et al. 1986).

An alternative approach to produce unlimited supplies of standardized probes is the use of nucleic acid probes. The technique relies on nucleic acid hybridization, the formation of duplexes between two complementary nucleic acid sequences. A sample of plant extract is bound to a membrane filter and hybridized with labelled nucleic acid probe prepared from cloned cDNA from the virus genome. Non-hybridized probe sequences are washed from the filter and the remaining probe sequences, which have hybridized to virus genome sequences in the plant sample, are assayed.

Waterhouse et al. (1986) and Barbara et al. (1987) have already described the use of ^{32}P labelled cDNA sequences to detect BYDV in infected plant samples. For general usage, non-radioactive probes would obviously have an advantage. In this regard, Habili et al. (1987) have described the detection of BYDV using photobiotin™ labelled probes. This non-radioactive probe, which forms the basis of a PAV-specific detection kit produced by BRESA Ltd. (Biochemistry Dept., University of Adelaide, South Australia 5001), is as sensitive as ^{32}P labelled probes.

We wish to stress that there is still much development that can be done with nucleic acid probes. The sensitivity at present is 25 times greater than classical double antibody sandwich ELISA (Waterhouse and Habili, unpublished). However, the probe used by Habili et al. (1987) covers only 1/3 of the PAV genome; presumably cloned sequences covering more of the genome will be more sensitive. Also, the extraction and hybridization procedures affect the sensitivity. Further refinements will be made in these areas.

Ultimately, only when two or more BYDV serotypes have been sequenced will we have the information needed for optimum design of nucleic acid probes. This will provide for highly sensitive, serotype-specific probes as well as effective general probes (based on conserved DNA sequences between different isolates or combinations of cloned cDNAs), for detection of a range of BYDV isolates including previously uncharacterized serotypes and related luteoviruses.

Synthesis of BYDV resistance genes

Two approaches that can be used to synthesize new virus resistance genes based on cloned cDNA sequences have recently been demonstrated. In bacteria the presence of high levels of complementary sequences to the virus genome can interfere with virus propagation during infection (Coleman et al. 1985). In plants, Powell Abel et al. (1986) and Tumer et al. (1987) have produced transgenic tobaccos (*Nicotiana tabacum* L.) that contain the TMV and alfalfa mosaic virus (AlMV) coat protein genes as plant gene constructions. Plants containing these constructions constitutively produce the coat proteins

without any detrimental effects and, when inoculated appropriately with TMV or AlMV, they show resistance or delayed onset of symptoms.

With recent reports of regeneration of rice (*Oryza sativa* L.) and transformation of rye (*Secale cereale* L.) (Cocking and Davey 1987), it should not be long before a range of cereals are amenable to genetic transformation. We can then adopt similar approaches, testing cloned segments of BYDV genomes and related sequences for their ability to confer resistance.

Prior to transformation and regeneration of transformed plants, it will be expedient to test gene constructions or transcripts against BYDV infection in cell culture systems. We have used electroporation to introduce BYDV RNA into barley leaf protoplasts to establish infection. However the level of infection is low (0.1 to 1%), similar to the levels observed for BYDV infection of cereal protoplasts using whole virus and poly-L-ornithine (Barnett et al. 1981). It will be important to increase infection levels by optimizing techniques before testing interference by gene constructions.

Acknowledgments

Financial support has been provided by the Australian Government National Biotechnology Program Research Grants Scheme in a joint project between our group and the Biochemistry Department of the University of Adelaide, where we are collaborating with Drs. R. Symons, N. Habili, T. Hercus, and J. McInnes. The visit of Dr. Cheng Zhuomin, Institute of Plant Protection, Beijing, was supported by a grant from the Australian Centre for International Agricultural Research (ACIAR). Dr. P. Larkin is collaborating with the protoplast infection experiments.

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Barley Yellow Dwarf Virus-Aphid Vector Interactions Associated with Virus Transmission and Vector Specificity

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Abstract

Barley yellow dwarf viruses (BYDV) and their aphid vectors are associated in a variety of complex interactions that influence both virus and aphid survival and spread. As luteoviruses infect and replicate in phloem tissues of plant hosts, it is probable that they can only be transmitted by phloem-feeding aphids.

Recent studies have elucidated the circulative route of BYDV through aphid vectors. Aphids ingest BYDV while feeding on phloem of infected plants. Virus acquisition occurs when virus is actively transported by the aphids' hindgut cells through the gut wall and is liberated into the body cavity. To be transmitted, virions in the hemolymph must come in contact with the aphid's accessory salivary gland, be transported through the gland, and be expelled into the salivary duct.

Luteoviruses have a high degree of vector specificity and are transmitted only by specific species of cereal aphids. Data suggest that virus-recognizing receptors located on cell membranes of the salivary gland may determine which luteoviruses can be transmitted by each aphid species. Because of this intimate association of luteoviruses with aphid cells, these viruses are totally dependent on aphid behavior for survival and spread. Although there is no evidence that luteoviruses infect aphid vectors, these viruses do have effects on aphid epidemiology. Results of several studies indicated that aphid feeding efficiency, reproduction, and morphology were affected by BYDV-infected host plants.

Increased reproductive potential and development of alate migrants aid aphid dispersal and survival and increase virus spread. These factors suggest that luteoviruses and aphids have co-evolved for mutual benefit.

The name "barley yellow dwarf virus" describes a group of related luteoviruses that share a common host range and induce similar symptoms of chlorosis and stunting in graminaceous hosts. The genetic relationships among different BYD viruses are not well understood. These viruses are generally referred to as isolate-types in reference to five distinct BYDV isolates (RPV, RMV, MAV, PAV, and SGV) that have been partially characterized. These five isolates were first recognized by the species of aphids that transmitted each isolate (Rochow 1969; Johnson and Rochow 1972). We now know these isolates differ in several characteristics, such as capsid protein structure (Scalla and Rochow 1977), which allows serological identification of different BYD viruses (Rochow and Carmichael 1979), cytopathology induced in hosts (Gill and Chong 1979), symptom severity (Baltenberger et al. 1987; Gildow and Rochow 1983; Rochow 1969), and RNAs associated with BYDV replication (Gildow et al. 1983).

In general, BYD viruses infect and replicate in phloem tissues of infected hosts (Jensen 1969). It is probably because of this phloem-specificity that BYD viruses are transmitted to plants only by aphids, which feed specifically in plant phloem. Because of the mutual association of virus and aphids with host phloem cells, BYD viruses are completely dependent on their aphid vectors for spread to new plant hosts. These viruses are not transmitted by seed, but may survive in perennial grasses in the absence of aphid vectors.

The host range and rate of BYDV spread among graminaceous host plants are dependent upon aphid epidemiology and the environmental parameters influencing aphid behavior and survival. In the absence of suitable virus hosts, the aphid may also act as a virus reservoir, contributing to virus survival until new plant hosts are located. There is no evidence at this time to suggest that BYDV infects or replicates in aphid vectors, but an intimate cellular association between the virus and vector does exist.

Plant-BYDV-Vector Interactions

A complex relationship controls the interactions among BYD viruses, their aphid vectors, and their mutual host plants (Figure 1). Successful completion of these three types of interactions, in the appropriate time frame and under certain environmental conditions, can result in BYDV epidemics. The virus-plant interaction occurs only after the aphid vector successfully locates and penetrates host plant sieve tube elements of the phloem. Penetration of sieve elements by stylets of homopteran phloem-feeding insects is the only method known for inoculating virus to plant sieve tube elements without inducing drastic cell responses resulting in disruption of translocation and cell necrosis. The aphid transmits BYDV to the phloem during feeding. This mechanism allows the virus to successfully avoid structural barriers to infection. Once inoculated into the phloem cytoplasm, the genetic interactions between virus and host regulate the ability of the virus to infect, replicate, and induce symptoms in the plant.

A second type of interaction involving aphid-host plant relationships influences vector feeding behavior, dispersal, and survival. Host plant effects on ability of cereal aphids to locate and penetrate phloem are well documented (Montllor et al. 1983). Age-related physiological differences among plants of the same species are known to influence aphid fecundity and morphology (Dixon and Glen 1971; Watt 1979).

The focus of this review will be on the third interaction, involving BYDV and the aphid vector. The virus-vector interactions may be described as two classes, direct interactions and indirect interactions. Direct interactions occur during the virus transmission process when virions must successfully pass

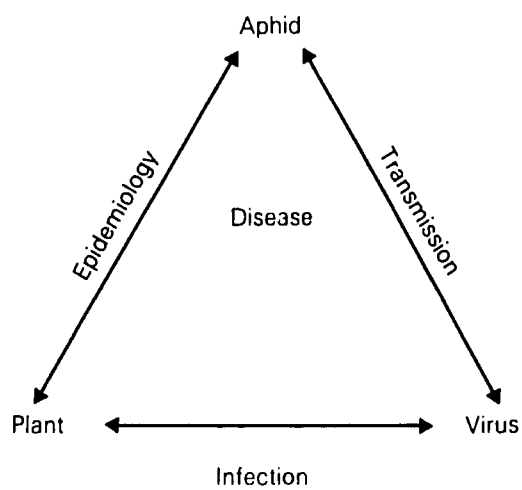

Figure 1. The complex of three types of interactions among the virus (barley yellow dwarf luteoviruses), the vector (Aphididae), and their shared host plant (Graminaceae), which may lead to virus transmission, infection, and disease spread when the appropriate environmental parameters occur.

through several aphid tissues in order for transmission to occur. These interactions generally involve highly specific virus-membrane recognition, packaging, and transport mechanisms. Indirect interactions result from BYDV effects on the virus-aphid-host plant following virus infection. These interactions influence aphid fitness.

Direct Virus-Vector Interactions

The route of BYDV through aphid vectors is outlined in Figure 2. In order for an aphid to transmit BYDV, the virus must first be ingested into the aphid's stylet food canal while the aphid is feeding on BYDV-infected phloem cells. Once ingested, virions suspended in phloem sap flow through the aphid's foregut and midgut to the hindgut. At the hindgut, virions may be actively transported through the cells making up the gut wall and liberated into the aphid's body cavity (hemocoel). This is the process of virus acquisition. Unacquired virions remain in the hindgut lumen, pass out of the aphid in the honeydew, and cannot be transmitted. Acquired virions become suspended in the aphid's blood (hemolymph) and circulate throughout the hemocoel. For this reason, this type of transmission mechanism is referred to as circulative transmission. The virus remains infectious while suspended in the hemolymph and the aphid's body becomes a temporary reservoir for the virus. The virus may persist in the aphid body and be transmitted for many days or weeks, if the aphid is an appropriate vector species. This type of transmission is also referred to as persistent transmission.

Transmission of BYDV occurs when virions in the hemolymph come into contact with the accessory salivary gland, pass through the gland cells, and are released into the salivary duct. The salivary duct drains the salivary gland and is connected directly to the salivary

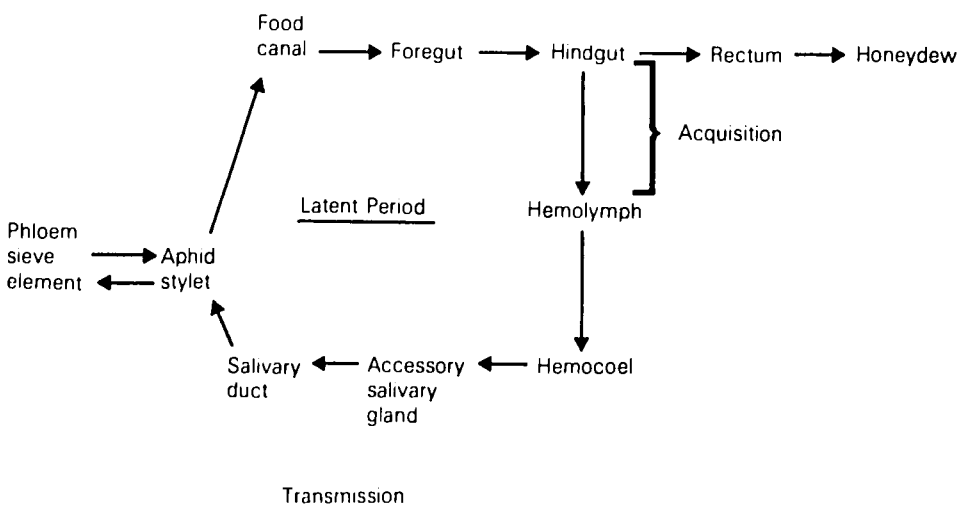

Figure 2. The circulative route of barley yellow dwarf viruses and other luteoviruses through their aphid vectors. Luteoviruses are ingested from infected phloem cells into the food canal centrally located in the stylets. Sites of virus-membrane interactions regulating virus acquisition and transmission occur specifically at the hindgut and accessory salivary gland. Virions not transported through the hindgut epithelium to the hemocoel cannot be transmitted and are voided in the honeydew. The latent period, during which the aphid is unable to transmit the virus, is the period beginning when the virus is first ingested and lasting until the virus enters the salivary duct of the stylet and is transmitted to plants during feeding.

canal in the stylet. Virions are excreted, along with salivary cell secretions, into plants during feeding. Aphids lack the malpighian tubules found in many insect species, which function in excretion of metabolic waste products. It is believed that the aphid salivary glands may partially fulfill this role (Miles 1972). If this is true, then it may not be surprising that BYDV in the hemolymph can be recognized as a foreign protein and be eliminated from the aphid's body through the salivary gland excretory-secretory mechanism.

Virus acquisition

Virus acquisition begins with penetration of the aphid's stylet into an infected phloem cell, followed by the initiation of feeding. Little is known about this process in relation to BYDV acquisition or transmission. Early work indicated that some aphids could acquire BYDV when allowed to feed on infected plants for as little as 30 min (Watson and Mulligan 1960). Efficient transmission of BYDV by at least 50% of an aphid test population, however, required an acquisition feeding period of up to 24 hours (Rochow 1963).

Recent investigations utilizing electronic monitoring of single aphids indicated that the time required for aphids to locate phloem and begin feeding varied among aphid species. *Sitobion avenae* (Fabricius) required an average time of about 30 min to locate phloem and begin feeding on Clintland 64 oats (*Avena sativa* L.). After 90 min, however, only 65% of the aphids tested had successfully initiated feeding (Scheller and Shukle 1986). In another study (Montllor and Gildow 1986), *Rhopalosiphum padi* (L.) required 60 min, while *Schizaphis graminum* (Rondani) required 200 min to successfully penetrate phloem when fed on California Red oats ((*Avena byzantina* Koch) = *A. sativa* L).

These results correspond to similar studies of *Myzus persicae* (Sulzer), vector of another luteovirus, potato leafroll virus, feeding on potato (Leonard and Holbrook 1978). Results of electronic monitoring studies indicate that aphids may begin acquiring virus within minutes of initiating feeding on infected phloem. The reported increased ability of aphids to transmit virus with increased acquisition feeding periods was probably related to greater virus concentration in the hemolymph, which increased the probability of transmission.

Once an aphid has initiated BYDV acquisition by feeding on infected phloem, a period of time elapses before the aphid can transmit the virus. This is the time required for the virus to circulate through the aphid's body from the food canal to the salivary duct, and is referred to as the latent period. In general, a 24- to 48-hour period occurs between the time aphids begin BYDV acquisition and when they are capable of transmitting virus (Rochow 1963). This latent period (LP) may vary with aphid species, BYDV isolate, and temperature. The LP_{50}, defined as the time required for 50% of an aphid test population to transmit virus, for *R. padi* transmitting a PAV-like isolate was 62 hours at 15°C, but only 35 hours at 20°C (Broek and Gill 1980). For *R. Padi* transmitting an RPV-like isolate the LP_{50} was only 50 hours at 15°C and 35 hours at 20°C. These differences in LP_{50} time probably reflect inherent differences in efficiency of virus recognition and transport through aphid tissues and the effects of temperature on the rate of these cellular processes.

The key to BYDV acquisition is the ability of virus particles to be transported through the gut wall barrier into the hemocoel. Recent ultrastructural observations have provided some insight into this process (Gildow 1985). When virus-free *R. Padi* were fed on RPV-infected oats, virus particles were associated only with the membrane lining the hindgut region of the alimentary canal. This virus-membrane interaction appeared to be very specific, since virus was not associated with the midgut region (stomach and intestine) in any of 40 aphids examined. From the static electron microscope images, a mechanism for BYDV acquisition was hypothesized.

Ultrastructural evidence suggests that BYDV particles suspended in the hindgut lumen attach to the hindgut cell

membrane (apical plasmalemma). This attachment elicits endocytotic uptake of individual virus particles into small coated vesicles that are capable of transporting the virus into the hindgut cell cytoplasm. The coated vesicles containing BYDV appear to fuse with larger vesicles (receptosomes or endosomes), concentrating the virus particles in these membrane structures. Elongate vesicles (tubular vesicles) containing linear arrays of BYDV particles develop from the receptosomes and migrate toward the cell membrane (basal plasmalemma) interfacing with the hemocoel. The tubular vesicles appear to fuse with the basal plasmalemma, liberating the virus into the hemocoel. Similar observations have recently been made with *R. padi* acquiring MAV and *S. graminum* fed on RPV (Figure 3). Details of this process are poorly understood and current research is directed at studies of the virus recognition mechanism, which elicits acquisition and occurs at the gut cell membrane. It is clear that there is a higher degree of specificity regulating BYDV transmission by aphids than regulating acquisition, since some nonvector aphid species can acquire some BYDV isolates that they cannot transmit (Rochow and Pang 1961).

Virus transmission

Much is known about BYDV transmission due to the work of W.F. Rochow over the past 30 years (Rochow 1958; Rochow et al. 1987). Table 1 illustrates the unique

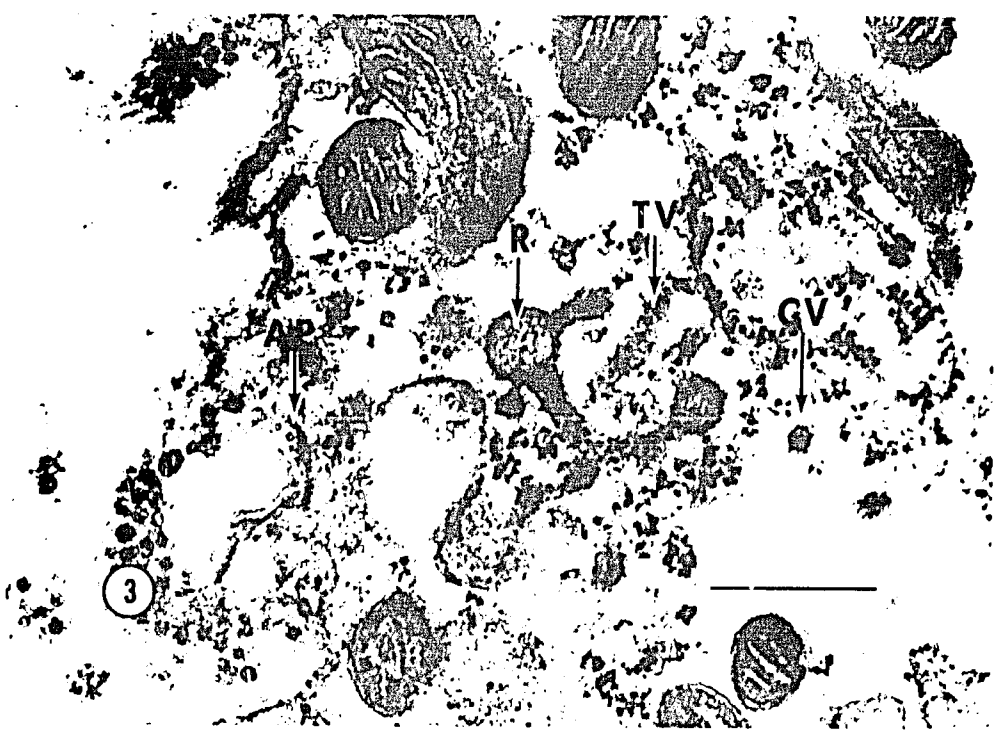

Figure 3. Virions of the New York RPV isolate of barley yellow dwarf virus (BYDV-RPV-NY) in membrane-bound vesicles within the cytoplasm of a hindgut cell of *Schizaphis graminum* fed 5 days on RPV-infected oats. Virus in the gut lumen is taken through the apical plasmalemma (AP) by endocytosis and transported individually across the cytoplasm in coated vesicles (CV). The coated vesicles are believed to release the virus into larger vesicles called receptosomes (R), which give rise to tubular vesicles (TV) containing virus, or coated vesicles may fuse directly with existing tubular vesicles. Tubular vesicles eventually fuse with the basal plasmalemma releasing the virions into the aphid's body cavity (hemocoel). Bar = 500 nm.

feature of BYDV-vector specificity that first allowed the identification of the various BYDV isolate-types (Gill 1967; Rochow 1969; Paliwal 1980; Gildow and Rochow 1983). Each BYDV isolate-type is transmitted by specific aphid species. Some isolates, such as RMV, are very specific and are known to be transmitted by only one aphid species. Other isolates, such as PAV, are transmitted by several species and have been inappropriately referred to as nonspecific isolates. The fact that some BYDV vector species do not efficiently transmit isolates such as PAV indicates that specificity does exist.

Results of transmission interference studies suggest that *R. padi*, which transmits both RPV and PAV, may possess distinct populations of cell receptors recognizing and eliciting transmission of these two isolates by separate regulatory mechanisms (Gildow and Rochow 1980a). The ability of an aphid species to transmit more than one isolate, or for an isolate to be transmitted by more than one aphid species, does not indicate nonspecific transmission. The ability of an isolate to be transmitted was found to be dependent upon the recognition of that isolate by regulatory mechanisms associated with the aphid salivary gland. The basis for this recognition involves key differences in the coat protein structure among the BYDV isolate-types. When highly concentrated purified preparations of BYDV isolates were microinjected into nonvector aphid species, transmission did not result because particles of these isolates were not recognized and transported through the salivary gland (Rochow 1969).

This concept for BYDV vector-specificity was supported by the discovery of dependent transmission of BYDV from mixed infections (Rochow 1977). Dependent transmission occurs when two distinct BYDV isolates replicate simultaneously in the same plant cell. The nucleic acid (RNA) of one isolate occasionally becomes encapsidated by the coat protein of the other isolate during virion assembly. This results in a hybrid BYDV particle. Such particles are transmitted in a vector-specific manner determined only by the coat protein, without regard to the type of nucleic acid it contains.

The biochemical mechanism for specificity has not been definitively identified and much remains to be learned. At the present time, it is hypothesized that receptor proteins

Table 1. Vector transmission patterns of specific barley yellow dwarf virus isolate-types by some major species of cereal grain aphids

	Ability to transmit BYDV isolate				
Aphid species	**MAV**	**PAV**	**RMV**	**RPV**	**SGV**
M. dirhodum	+	+	-	-	-
R. maidis	-	-	+	-	-
R. padi	-	+	-	+	-
R. rufiabdominalis	-	+	+	+	-
S. graminum	-	+	-	+	+
S. avenae	+	+	-	-	-

(+) indicates the aphid species has been reported to consistently transmit the BYDV isolate in single aphid tests. Transmission efficiency varies with the virus-vector combination. Specificity is not absolute and individual aphids of a nonvector species may occasionally transmit a normally nonvectored isolate (Gildow and Rochow 1983, Gill 1967, Paliwal 1980, Rochow 1969).

capable of binding only specific BYDV isolates may exist associated with membrane systems involved in BYDV transport through the salivary gland. Virions binding to these receptors would be taken into the cell and transported through the salivary gland cytoplasm for release into the salivary duct. Virions of nontransmitted isolates would not be recognized by the receptors and would remain suspended in the hemocoel.

Additional support for the virus-specific receptor concept came from studies of transmission interference by the MAV and PAV isolates in their common vector *S. avenae* (Gildow and Rochow 1980a). The MAV and PAV isolates are serologically related, indicating some similarities in coat protein structure. When aphids were allowed to feed on MAV-infected, and then on PAV-infected plants, transmission of PAV was lower than in aphids fed only on PAV. When purified MAV and PAV were mixed in various ratios and injected simultaneously into aphids, subsequent PAV transmission efficiency decreased as the MAV concentration increased (Gildow and Rochow 1980b). These results suggested that MAV and PAV could be competing for recognition, attachment, and transmission through the salivary gland by a regulatory receptor mechanism capable of recognizing both MAV and PAV. Transmission interference did not occur in *R. padi* between RPV and PAV, possibly due to the fact that RPV and PAV do not share significant protein coat similarities.

Ultrastructural observations of viruliferous *R. padi* and *S. avenae* have provided some evidence concerning the site of the BYDV-specificity mechanism and for the cellular mechanisms involved in transmission (Gildow 1987). To be transmitted, virions suspended in the hemolymph must diffuse through the extracellular basal lamina that surrounds the accessory salivary gland and come into contact with the accessory gland secretory cell membrane (basal plasmalemma). BYDV virions are commonly observed on this membrane, often within membrane invaginations extending into the cytoplasm.

The precise mechanism for how virions are taken into the accessory gland is unknown, but may involve a reversal of the process for virus transport at the hindgut. Virions are observed in the accessory gland cell cytoplasm enclosed in tubular vesicles (Figure 4), usually located near the microvilli-lined canals that branch through the accessory gland cytoplasm. The membrane lining of these canals is actually the apical plasmalemma, and the lumen of these canals connect directly to the salivary duct leading out of the aphid. Coated vesicles containing individual BYDV virions bud from the tubular vesicles, transport the virions to the canals, fuse to the canal membrane (exocytosis), and liberate the virion into the canal lumen. The virion can then flow with salivary secretions through the salivary duct and into the plant during feeding.

The mechanism for vector-specificity is currently believed to function at the accessory salivary gland basal plasmalemma bordering the hemocoel (Gildow and Rochow 1980c). In *S. avenae*, virions of the transmitted MAV-NY isolates were consistently observed on the plasmalemma and within the cytoplasmic vesicles associated with microvilli-lined canals. Particles of the RPV-NY isolate, which is not transmitted by *S. avenae*, were observed concentrated outside the plasmalemma, but never within the cell or salivary canals. A similar mechanism for luteovirus transmission was described for *M. persicae* (Gildow 1982).

These examples serve to illustrate the complex nature of the direct interactions between BYDVs and their aphid vectors.

Indirect Virus-Vector Interactions

Indirect aphid-virus interactions are influenced by the effect of BYDV infection on host plant biochemistry and physiology. These virus-induced plant alterations can change aphid behavior and development, which in turn influence BYDV epidemiology. Aphids fed on BYDV-infected cereals have been

reported to differ in morphology, reproductive potential, and feeding behavior from aphids fed on healthy plants under laboratory conditions (Gildow 1984). Very little is known about these interactions under field conditions.

Morphology

Populations of cereal aphids observed in small grains during the growing season consist of parthenogenetic females capable of rapid asexual reproduction. An adult aphid may easily produce 4 to 6 nymphs per day, and each nymph begins development with the capacity to mature as either a nonwinged (apterous) or winged (alate) adult. In general, apterous adults are less mobile and tend to produce more offspring than alate adults. Mature alate adults are predisposed to flight and participate in long-range dispersal (Dixon 1973). Thus, energy utilization is channeled between population increase and population distribution by apterae and alatae and both mechanisms contribute to species survival.

Several factors are known to influence whether nymphs mature as apterae or alatae. The best studied of these factors are population density and nutrition (Lees 1966; Schaefers 1972). Plant age (growth stage) is believed to affect aphid morphology indirectly by influencing aphid nutrition (Dixon and Glen 1971). Dean (1978) reported observing only apterae of *S. avenae* in fields of barley until mid-June, and after mid-July only alates were recovered. This observation was substantiated by Watt and Dixon (1981), who reported an increasing portion of alates developing on wheat at later growth stages in July.

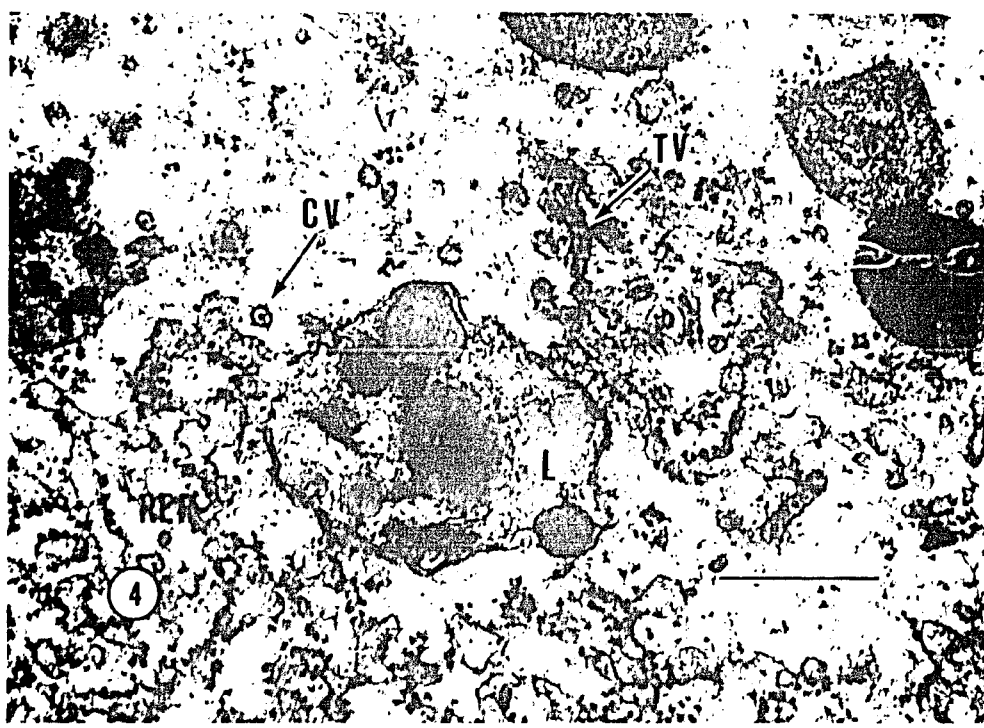

Figure 4. Virions of BYDV-RPV-NY in tubular vesicles (TV) and a coated vesicle (CV) in the cytoplasm of an accessory salivary gland secretory cell of *Rhopalosiphum padi* fed 10 days on RPV-infected oats. The mechanisms for virus transport into the cell and for accumulation of virions in tubular vesicles is unknown. Small coated vesicles containing single virions to the salivary canal membrane where they are released from the cell for transmission during feeding. Rough endoplasmic reticulum (RER), lysosome (L), mitochondrion (M). Bar = 500 nm.

BYDV infection of small grains also influences aphid morphology. Populations of *S. avenae* and *R. padi* respond to feeding on BYDV-infected oats and barley by producing a greater proportion of winged adults. Under laboratory conditions, in which *S. avenae* were first exposed to crowding stimulation and then reared on healthy or BYDV-infected oats, 30 and 70%, respectively, of 1,200 aphids matured as alates in 10 experiments (Gildow 1980). Parallel results were obtained with *R. padi* reared on healthy or BYDV-infected plants of two oat and two barley (*Hordeum vulgare* L.) cultivars. Forty and 74%, respectively, of 120 *R. padi* matured as alates on healthy or BYDV-infected barley cv. Prato (Gildow 1983). When first- and second-instar nymphs of *R. padi* were collected in the field from healthy or BYDV-infected barley and reared on healthy plants, 25 and 87%, respectively, matured as alates. Ajayi and Dewar (1983) reported a significantly greater proportion of fourth-instar *S. avenae* developed as alates on BYDV-infected oats (10.8%), than on healthy oats (4.9%) under field conditions.

Not all aphid species uniformly responded to feeding on infected plants by producing an increased proportion of winged forms. In a recent study (Montllor and Gildow 1986) comparing *S. graminum* and *R. padi*, 43 and 80%, respectively, of approximately 600 *R. padi* matured as alates on healthy or BYDV-infected oats, while almost none of the 270 *S. graminum* developed as alates.

Population density

The physiological condition of the host plant influences aphid reproductive potential or fecundity. Apterous *S. avenae* have been reported to have higher reproductive rates on developing panicles and young leaves than on mature or senescing leaves of oats (Watt 1979). Aphid size (weight) and number of nymphs produced per adult were greatest for aphids reared on panicles and young leaves, compared to mature leaves. *S. avenae* on young leaves matured sooner than those on mature leaves (8.4 vs. 10 days).

BYDV-infection of small grains also influences aphid fecundity. Populations of *S. graminum* reproducing on California Red oats infected with RIV-NY were 8 times larger than those developing on healthy oats over a 2-week period (Montllor and Gildow 1986). Healthy oats were found to be a poor host for *S. graminum*. Host suitability improved, however, following BYDV infection. Fecundity of *S. avenae* (Ajayi and Dewar 1983; Miller and Coons 1964) and *R. padi* (Markkula and Laurema 1964) has been reported to be greater on BYDV-infected cereals than on healthy plants, under both laboratory and field conditions.

Increased attraction

In addition to aphid population increases in BYDV-infected cereals resulting from increased reproduction, aphids may also be preferentially attracted to fields containing BYDV-infected plants. This concept was supported by field population studies and laboratory flight chamber experiments utilizing *S. avenae* and *Metopolophium dirhodum* (Walker) (Ajayi and Dewar 1983). More alate migrant aphids collected in BYDV-infected oat and barley fields showing severe yellowing than in healthy fields. Similar differences did not occur in alate populations between infected or healthy wheat fields that did not develop severe BYDV symptoms.

Feeding behavior

The increased development of winged forms and reproductive potential of aphids on BYDV-infected cereals reflects responses to a wide range of host plant alterations. Nutritional influences on aphid growth and development are most often cited as being responsible for the observed changes in the aphids. Following BYDV infection, several biochemical changes occur in the plant resulting in altered carbohydrate and nitrogen metabolism, reduced metabolite translocation, and phloem necrosis (Jensen 1972). In general, BYDV-infected leaves physiologically resemble senescent tissue with increased soluble carbohydrate and free amino acids (Beevers 1976). The response of the

aphid may be due to quality of the ingested phloem sap, or to effects of infection on feeding efficiency. Differences in feeding behavior, measured as number of stylet probes, time required for stylets to penetrate to phloem, and total phloem ingestion time, have been demonstrated for biotypes of *S. graminum* on resistant and susceptible sorghum (Montllor et al. 1983).

To test whether or not BYDV-infection influences aphid feeding behavior, the probing and feeding behavior of *S. graminum* biotype C was electronically monitored when aphids were placed on healthy oats or oats infected with the RPV-NY isolate of BYDV (Montllor and Gildow 1986). *S. graminum* fed better on infected oats than on healthy oats, as indicated by fewer number of probes to phloems (2.5 vs. 3.7), less time required to establish a phloem ingestion period greater than 15 min (200 vs. 300 min), and the duration of phloem ingestion (200 vs. 100 min) over 7 hours.

The time required to locate the phloem did not differ between treatments. Only 24% of stylet contacts with phloem were followed by long ingestion periods on healthy oats, compared to 53% on BYDV-infected oats. The aphids spent more time probing and ingested for shorter periods on the healthy oats. These data suggest that BYDV-infection improved the suitability of this oat variety as a host for *S. graminum* by increasing the amount of time the aphids spent feeding. These observations were similar to the differences observed for *S. graminum* on susceptible or resistant sorghum varieties (Montllor et al. 1983).

Conclusion

The direct aphid-BYDV interactions involved in virus acquisition and vector-specific transmission result from an intimate association between the vector cell components and plant virus, and suggest a high degree of compatibility. The direct interactions allow transmission of the virus to host phloem tissue, in which it specifically replicates. The aphid vectors acting as short-term virus reservoirs also increase the probability of virus survival and spread over long distances. These factors give a selective advantage to BYDV.

The indirect aphid-virus interactions apparently favor vector survival by increasing aphid fitness. Evidence indicates that for some species, under certain environmental conditions, feeding on BYDV-infected cereals results in more efficient feeding, improved nutrition, increased reproduction, and increased probability of development of winged migrants. Improved feeding efficiency favors acquisition of virus, while increased population size and wing development favor aphid survival and virus spread. The ability of the aphid to carry the virus to new host plants in a persistent manner ensures continued plant infection by the virus and an improved host suitability for the aphids. This might be especially important if the aphid is forced to feed on less desirable plant species.

The complex of aphid-BYDV interactions influencing virus transmission and aphid epidemiology suggests that BYDV and cereal aphids have co-evolved into a mutually beneficial association.

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Characterization of South African Isolates of Barley Yellow Dwarf Virus

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Abstract

A detailed characterization of two South African isolates is given. One isolate is transmitted by Schizaphis graminum *(Rondani) and is SGV-like. The other is transmitted by* Rhopalosiphum padi *(L.) and* Diuraphis noxia *(Mordvilko), and is related to PAV-like isolates. The latter has isometric particles 25 + 0.8 nm in diameter and apparent buoyant density of 1.435 g/cm^3 in cesium chloride and a protein of* M_r *24.5 kDa that degrades to 22.5 kDa on storage. It also contains ssRNA of* M_r *1.8 MD and produces dsRNA of* M_r *1.8 MD and produces dsRNA of* M_r *3.4 MD in infected plants. Both isolates appear relatively vector nonspecific.*

Barley yellow dwarf (BYD) was first described in South Africa in the western Cape Province (von Wechmar 1967; von Wechmar and Wassermann 1968). Viruslike particles were isolated from infected plants at the time, but were not characterized (von Wechmar, unpublished). More recently, our laboratory has described the aphid transmission, propagation, isolation, and partial characterization of barley yellow dwarf virus (BYDV) isolates transmitted by *Schizaphis graminum* (Rondani) (Rybicki 1984) and by either *Rhopalosiphum padi* (L.) or *Diuraphis noxia* (Mordvilko) (Rybicki and von Wechmar 1982a; von Wechmar and Rybicki 1984a,b), from naturally diseased Gramineae in the eastern Orange Free State (OFS), South Africa.

The *S. graminum*-transmitted disease agent was the first to be isolated and propagated in this laboratory, during preliminary investigations of the "Free State Streak" disease phenomenon (see below). However, *S. graminum* occurs only late in the growing season (when it is warm), and not in any great concentration in the eastern OFS, and so was not considered to be an important natural vector of disease in this, the main wheat growing area (Rybicki 1984; M.B. von Wechmar, unpublished). The characteristics of the virus are detailed here to put them on record.

The second virus isolate was associated with the "Free State Streak" syndrome first noticed in 1978 (von Wechmar and Rybicki 1981), and its apparent transmission by the aphid *D. noxia* (not yet reported to transmit BYDV) was of great interest, given the known importance of the aphid as a pest in its own right (Walters et al. 1980; Erdelen 1981; von Wechmar and Rybicki 1981; Gilchrist et al. 1984). We therefore considered it essential to properly characterize the virus in order to identify it more closely in terms of its vector preference and specificity, Rochow (1970, 1979).

S. graminum-Transmissible Isolate

Transmission and propagation

S. graminum from a non-viruliferous clone kept in our laboratory were fed on detached leaves of diseased field-collected Gramineae suspected of being infected with BYDV, collected in the OFS from the Small Grain Centre, Bethlehem, in the spring of 1978. The aphids were used to transmit the virus to 5-day-old barley (*Hordeum vulgare* L.), wheat (*Triticum aestivum* L.), and oat (*Avena sativa* L.), seedlings. After 3 days the inoculated plants were sprayed with an aphicide, transferred to a plant growth room at 20°C for 21 days, then harvested and stored at 4°C. This process was repeated until enough material had accumulated for purification. Symptoms typically appeared after 14 to 21 days: barley, oats, and wheat were stunted and barley leaves exhibited bright yellowing, while oat leaves showed salmon pink discoloration. Maize leaves had faint chlorotic line patterns.

Studies conducted in parallel indicated that both *D. noxia* and *R. padi* were also capable of transmitting this disease (M.B. von Wechmar and V.E. Coyne, unpublished), and that both aphids were implicated in the disease under natural conditions (Rybicki 1984).

Purification and characterization

The extraction procedure employed was one adapted from that of Rochow and Brakke (1964) and Brakke and Rochow (1974): plants were homogenized with potassium phosphate buffer, pH 7.0, extracts were clarified with 20% (v/v) chloroform, and precipitated with 9% (w/v) PEG MW 6000 and 2.5% (w/v) NaCl. PEG precipitates were resuspended in phosphate buffer, and concentrated by two cycles of differential ultracentrifugation to about 1000x the original sap concentration, and further purified by fractionation on rate-zonal sucrose gradients. Yield of virus was about 75 mg/kg plant tissue. Use of cellulolytic enzymes such as Driselase (Fluka) and Spark-L-pc (Miles Labs) resulted in far less pigmented plant debris in the PEG precipitates, but did not appreciably increase yields; the chloroform clarification was found to be essential for removal of soluble pigments and plant proteins; PEG precipitation was essential for concentration of the virus from sap.

The preparations were characterized by analytical sucrose gradient centrifugation, UV spectroscopy, electron microscopy, serology, and infectivity assay. Preparations produced a single UV-absorbing "peak" on sucrose gradients sedimenting at about 115 Svedberg, and contained a single species of isometric particles of mean diameter 24.8 ± 0.5 nm (Figure 1). UV scans were typical for a spherical nucleoprotein of high nucleic acid content.

Purified virus particles could be acquired via membrane feeding by *S. graminum* and transmitted to healthy plants. Aphid transmission tests on extracted virus using other aphids were not performed. An antiserum raised against the virus

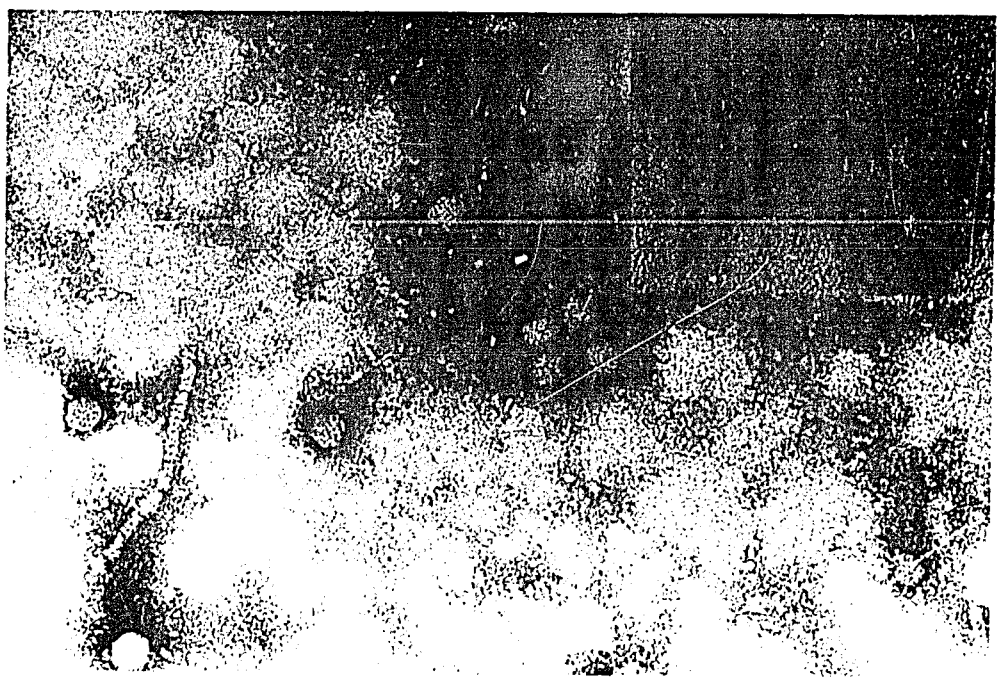

Figure 1. Electron micrograph of *Schizaphis graminum*-transmissible isolate of barley yellow dwarf virus. Particles were purified by sucrose gradient centrifugation, and stained with neutral phosphotungstate. Size bar indicates 100 nm. Magnification of the inset is 2.5x that of the larger print. Photographs by A. Timme, University of Cape Town Medical School.

reacted homologously in indirect ELISA, and with a British PAV-like isolate and the other South African (SA) isolate described here in Western blots. Antisera specific for a Canadian SGV isolate (code 6718; C.C. Gill, Agriculture Canada) and a British antiserum to a PAV-RPV mixture ("strain B"; M.F. Clark, East Malling, UK) both reacted in Western blot with extracts of leaf samples frozen for 8 years (not shown). The virus did not react with monoclonal antibodies (McAb) to British MAV-, RPV-, or PAV-like isolates.

The initial success of the transmission by the two other aphid species from field-collected material could indicate that the BYDV isolated was not particularly vector-specific. Another possibility is that an "SGV-like" isolate was sieved out of a mixed natural infection of several dependent isolates by S. graminum. However, this could not be tested as no further vector studies were performed after the membrane-feeding and inoculation.

It was therefore not possible to definitively identify the virus isolate in terms of Rochow's criteria of aphid specificity; however, the properties of our isolate appear to be most similar to the SGV nonspecific isolate of Gill (1969). It is also serologically related to PAV isolates, as expected (Hsu et al. 1985), although antigenically distinct in terms of reactivity with an McAb. As mentioned above, *S. graminum* are not particularly numerous in the eastern OFS in the late growing season; thus the virus described here may be incidental to the disease problems in this area.

R. Padi and *D. Noxia*-Transmissible Isolate

Transmission and propagation

In March 1980, a sample of severely-diseased wheat was obtained from the Small Grain Centre, Bethlehem, OFS. The summer-grown wheat had been heavily infested from January with *D. noxia*, and exhibited the characteristic "Free State Streak" symptoms attributed to the phytotoxicity of the aphid. Fully grown plants were yellow, had many dead tillers, and heading was reduced. Preliminary investigations revealed that the plants were heavily infected with brome mosaic virus (BMV): more than 2 g of virus could be extracted per kg of plants (von Wechmar and Rybicki 1981; Rybicki 1984).

The use of *D. noxia* and *R. padi* for the transmission of an agent causing yellowing symptoms from these plants has been described (von Wechmar and Rybicki 1981, 1984a). Both aphid species proved to be efficient transmitters of the virus, so the easier-to-maintain *R. padi* was used for routine propagation (Rybicki and von Wechmar 1982a).

Purification and characterization

Rybicki and von Wechmar (1982a) showed that BYDV was co-transmitted with BMV in the laboratory, and could be co-purified with BMV and the aphid pathogen *R. padi* virus (RhPV). Repeated transfers to barley free of seed-transmitted BMV and cucumber mosaic virus (CMV) (von Wechmar, these Proceedings) have allowed the isolation of BYDV (von Wechmar, unpublished). Yield of BYDV was $\pm$ 100 μg/kg diseased leaf. The original BMV/BYDV complex is, however, still being maintained after 7 years of repeated aphid transmission.

Preparations of this virus isolate were characterized by electron microscopy, analytical ultracentrifugation, sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), isopycnic banding in cesium chloride, the dsRNA extracted from infected plants, compound indirect ELISA using McAbs, and immunoelectroblot (Western blot) immunoassay (Rybicki and von Wechmar 1982b) using polyclonal antibodies. Purified virions sedimented at 114 Svedberg, appeared to have a diameter of 25 $\pm$ 0.8 nm and initially appeared to have a single capsid protein of M_r 22 kDa (Rybicki 1984). Subsequently it was found that fresher preparations contained two proteins, of M_r 22.5 and 24.5 kDa. Both reacted with antiserum to the virus in Western blots (Figure 2). The antiserum also recognized a $\pm$ 24 kDa protein in an extract of plants infected with a UK isolate of PAV. The virus reacted in Western blots with antiserum to Gill's isolate 6718 (SGV-like), but not with antiserum to his isolate 6524 (RPV-like).

Sandwich-indirect ELISA tests with McAbs obtained from L. Torrance (MAFF, Harpenden, UK) were performed as described by Torrance et al. (1986) with virus from extracts of plants separately infected with our two isolates, and a UK PAV-like isolate from R. Plumb (Rothamsted, UK)). The SA isolate and the UK PAV reacted with McAb MAC 91, but not with McAb MAC 92 or MAFF 2 (specific for UK PAV-, RPV-, and MAV-like isolates, respectively). The SA SGV-like isolate and extract of healthy plants did not react. Virus banded in CsCl at a density of 1.435 g/cm^3, although particles were not stable on prolonged exposure. A sample of 100 g of diseased barley was used for dsRNA extraction as described by Gildow et al. (1983): 3 bands—M_r's 3.3, 2.0, and 0.7 MDa—were seen on ethidium bromide-stained polyacrylamide gels. The value for the largest dsRNA is in good agreement with the ssRNA M_r of 1.8 MDa determined earlier (Rybicki 1984).

Identification of the isolate as PAV-like appears to be an obvious conclusion: the virus reacts with polyclonal antibodies raised to UK PAV-like and Canadian and SA SGV isolates, but not with antibodies to Canadian RPV-like, and reacts with McAbs specific for a UK PAV-like isolate. The dsRNA profile from infected plants is similar to that of the SGV-MAV-PAV group (Gildow et al. 1983) The aphid transmissibility is relatively nonspecific, but is most efficient by *R. padi* (M.B. von Wechmar, unpublished).

New findings are the apparent change of the coat protein and transmissibility by *D. noxia*. The virus isolate could be differentiated from the SGV-like isolate described above by symptoms on maize (M.B. von Wechmar, unpublished), and serological reactions with a range of antisera. The virus (and perhaps other strains) are definitely implicated in disease complexes of small grains in South Africa (see also Situation Report, these Proceedings). It is felt, however, that BYDV is not particularly important when compared to other viruses such as BMV and CMV occurring in the major wheat and barley-growing areas (M.B. von Wechmar, these Proceedings).

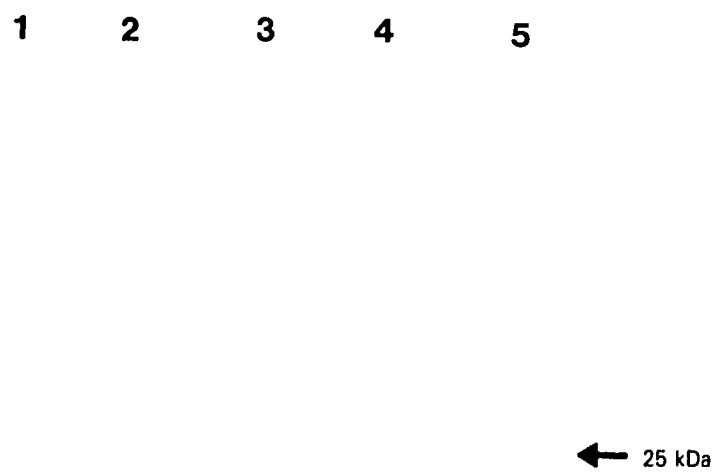

Figure 2. Western blot of various electrophoretically-fractionated samples probed with antiserum to *R. padi*- and *D. noxia*-transmissible barley yellow dwarf isolate. Samples, left to right (5 lanes): (1) Brome mosaic virus; (2) and (3) sucrose gradient fractions sedimenting at 80 Svedbergs (x2); (4) BYDV from a sucrose gradient; (5) *R. padi* virus of aphids. The antibody binding was detected using peroxidase-labelled goat-anti-rabbit and 4-chloro-1-naphtol substrate.

Acknowledgments
We wish to thank Ramola Chauhan, Karen Rous, and Janine Dekker for technical assistance, and the Wheat Board, the Council for Scientific and Industrial Research, and the University of Cape Town for supporting this work.

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Influence of Host Virus Content on the Acquisition and Transmission of Barley Yellow Dwarf Virus

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Abstract

*Oats (*Avena sativa *L.), wheat (*Triticum aestivum *L.), and barley (*Hordeum vulgare *L.) showed wide variations in barley yellow dwarf virus (BYDV) content, as assessed by enzyme-linked immunosorbent assay (ELISA), among different leaves on the same plant, and also between leaves in the same position (i.e., of the same age) on different plants. Selected entire plants and individual leaves were compared as virus sources for the acquisition and transmission of BYDV isolates—Purdue (P)-PAV, MAV, and RPV—by specific (efficient) or nonspecific (inefficient) vectors. Overall, there was no convincing evidence of differences in virus acquisition efficiency due to differences in virus content in leaves or plants.*

By contrast, in experiments where aphids fed on purified virus preparations through membranes, correlation between virus concentration and virus acquisition (as assessed by ELISA of aphid extracts and by transmission tests) was evident for both efficient and inefficient vectors. Also, aphids apparently acquired virus more efficiently when feeding on virus preparations through membranes than by feeding on individual leaves containing the same overall virus concentration. The combined results suggest that total BYDV content is not the primary factor affecting the acquisition of BYDV from plants by vectors, but that acquisition is probably strongly influenced by other factors, especially uneven distribution of virus within the leaf.

Immunity to barley yellow dwarf virus (BYDV) is not known in cereals, but in some cases resistance has been associated with relatively reduced virus productivity as compared to that in sensitive or "susceptible" plants (Jedlinski et al. 1977; Skaria et al. 1985). It has also been suggested that reduced virus titer in such resistant cultivars could reduce BYDV spread if it was associated with reduced virus transmission (Jedlinski et al. 1977; Lister et al. 1984). We have examined this possibility in relation to the acquisition of several isolates of BYDV by various vectors.

Transmissibility from Whole Plants

Skaria et al. (1985) found that among near isogenic pairs of barleys (*Hordeum vulgare* L.) differing by the presence or absence of the *Yd2* gene (i.e., *Yd2*+ or *Yd2*-, respectively) those without the *Yd2* gene produced relatively more of the Purdue (P)-PAV isolate of BYDV (Hammond et al. 1983) than their *Yd2*+ counterparts. We therefore compared P-PAV infected plants of the *Yd2*+ members of each pair with their *Yd2*- counterparts, and with Clintland 64 oats (*Avena sativa* L.), as sources of the P-PAV isolate for *Rhopalosiphum padi* (L.). Three acquisition periods (2, 6, or 48 hours) were used, each followed by an inoculation period of 2 days. The virus content of the entire above ground portion of the source plants was also compared by enzyme-linked immunosorbent assay (ELISA).

The results confirmed those of Skaria et al. (1985) regarding relative virus content. However, the efficiency of P-PAV acquisition by *R. padi*, although usually somewhat greater on average from source plants with relatively higher virus content, was significantly so only for the Briggs/Prato and Atlas 57/Atlas 68 pairs, and only with respect to the 2-hour acquisition feeds. For longer acquisition times, differences in acquisition efficiency were apparently overshadowed by other variables (Figure 1).

Virus Content of Individual Leaves

In the above experiments, aphids were not restricted to specific leaves, and some previous experiments with both circulative and nonpersistent viruses indicate that different leaves on the same plant can differ as sources of virus (Sylvester 1953, 1956; MacKinnon 1962; Swenson 1963). We therefore checked whether some of the variability noted could be related to differences in the virus content of individual leaves. We also compared the relative efficiency of leaves as sources of virus independent of virus content.

In all the combinations of host, virus isolate, and time after infection that were examined, virus content as indicated by ELISA varied greatly with leaf position and age. Data for Clintland 64 oats infected with the P-PAV, MAV, and RPV isolates of BYDV illustrate this variability (Table 1). Thus, at the time when virus content was expected to be highest (Skaria et al. 1985), ELISA values for leaves of plants infected with the P-PAV isolate ranged from 0.030 to 1.834, those for leaves of plants infected with the MAV isolate ranged from 0.103 to 1.772, and those for leaves of plants infected with the RPV isolate ranged from 0.040 to 1.709 (Table 1).

Overall, the data clearly indicated that the wide variation in virus content among leaves is likely to invalidate comparisons of virus acquisition efficiency by aphids feeding on whole plants. Accordingly, comparisons were attempted by following virus acquisition from detached leaves, subsequently assayed by ELISA.

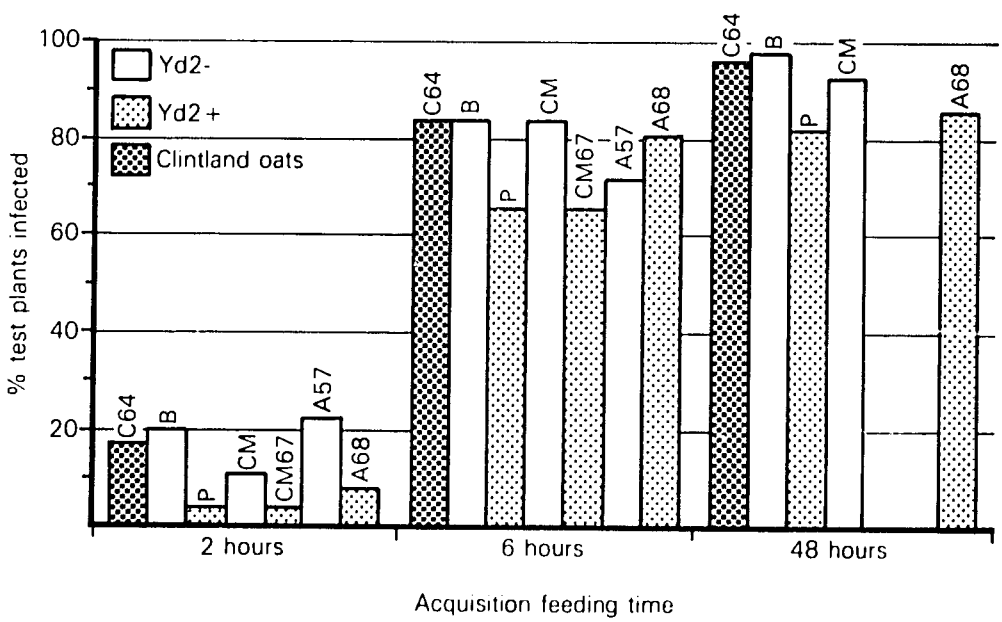

Figure 1. Relative transmissibility by *R. padi* of barley yellow dwarf virus (P-PAV isolate) from source plants with relatively high or low total virus content, to Clintland 64 test plants. Acquisition feeds on source plant were 2,6, and 48 hours and the transmission test feed was 48 hours. Ten source plants per cultivar and five test plants per source plant were used. C64 = Clintland 64, B = Briggs (Yd_2), P = Prato (Yd_2+), CM = California Mariout (Yd_2), CM67 = CM67 (Yd_2+), A57 = Atlas 57 (Yd_2-), A68 = Atlas 68 (Yd_2+). California Mariout and Atlas 57 barley were not tested for the 48 hour acquisiton feeding time.

Transmissibility from Individual Detached Leaves

For most experiments, Clintland 64 oats were the source of leaves for virus acquisition of the three BYDV isolates, because this was the cereal cultivar that showed the highest variations in virus content among individual leaves on the source plant.

The BYDV isolates used were P-PAV and the MAV and RPV isolates of Rochow (1969). For MAV and RPV, their respective specific vectors, *Sitobion avenae* (Fabricius) and *R. padi*, were used. For P-PAV, the transmission studies were done with both *R. padi* (an "efficient" vector) and *S. avenae* (an "inefficient" vector).

Eight viruliferous *R. padi* or *S. avenae* were used to infest each 6-day-old seedling source plant with the BYDV isolate. Five source plants of each cultivar were used for each virus isolate, acquisition feeding time, and repetition. Each batch of five source plants provided about 15 individual leaves for testing after optimal virus incubation periods, which were 12, 18, or 22 days for the P-PAV, MAV, and RPV isolates, respectively (Skaria et al. 1985). Aphids were killed with nicotine sulfate after an inoculation period of 2 days. Plants were grown in growth-chambers at 20° ± 1°C, with a 14-hour photoperiod.

Virus acquisition efficiency from individual source leaves was examined as follows. After the appropriate virus incubation period, individual leaves were cut with a razor blade from each source plant, under water, and immediately placed with their cut ends in tap water in small glass tubes. Virus-free *R. padi* or *S. avenae* were then placed on each individual source leaf to acquire virus. The acquisition feeding times tested were as follows:

- Acquisition of P-PAV by *R. padi*: from Clintland 64 oats—1, 2, 3, 4, 6, and 18 hours; from Briggs and Prato barleys—1, 2, 3, 4, and 6 hours; from Atlas 57, Atlas 68, California Mariout, and CM67 barleys—3 hours.

Table 1. Variations in barley yellow dwarf virus content (P-PAV, MAV, and RPV isolates) among individual leaves of Clintland 64 oats as indexed by ELISA

Isolate incubation period	Mean ELISA values Leaf position (old) 1	2	3	4	(young) 5
P-PAV/12 days	0.966[a]	0.599	0.407		
SD	(0.366)	(0.310)	(0.176)		
Range	1.834-0.014	1.720-0.101	0.938-0.030		
MAV/18 days	1.299[b]	0.305	0.525		
SD	(0.299)	(0.151)	(0.293)		
Range	1.772-0.593	0.611-0.103	1.419-0.203		
RPV/22 days	0.940[c]	0.859	0.618	0.512	0.362
SD	(0.510)	(0.501)	(0.314)	(0.249)	(0.207)
Range	1.709-0.033	1.366-0.025	1.297-0.033	1.037-0.040	0.946-0.117

[a] Mean ELISA values for duplicate wells in 6 separate experiments on a total of 144 plants. Mean ELISA value (+ SD) for healthy controls was 0.032 (+ 0.004).

[b] Mean ELISA values for duplicate wells in tests of 20 plants. Mean ELISA value (+ SD) for healthy controls was 0.020 (+ 0.008).

[c] Mean ELISA values for duplicate wells in tests of 20 plants. Mean ELISA value (+ SD) for healthy controls was 0.047 (+ 0.006).

When antigen concentration was reduced by one-half, ELISA values were reduced by about one-third.

- Acquisition of P-PAV by *S. avenae*: from Clintland 64 oats—3, 6, 12, 18, and 48 hours.
- Acquisition of MAV by *S. avenae*: from Clintland 64 oats—3 and 6 hours.
- Acquisition of RPV by *R. padi*: from Clintland 64 oats—3 and 6 hours; from Abe and Elmo wheats (*Triticum aestivum* L.)—6 hours.

After acquisition feeds, two aphids were transferred to each 6-day-old Clintland 64 test plant. Four tests plants were used for each source leaf, but sometimes only enough aphids survived the acquisition feed for three or, rarely, two test plants to be used per source leaf. The aphid inoculation feeds were 2 days, at room temperature, with a low light intensity to encourage settling. The inoculated test plants were then sprayed with 0.2% pyrethrin, and kept in a greenhouse for 12 to 15 days to allow the virus to develop. Control plants were kept in the same conditions.

ELISA and Statistical Analyses of Extracts

All individual source leaves were tested quantitatively (i.e., to estimate comparative virus content) by double antibody sandwich (DAS)-ELISA (Lister and Rochow 1979). Each test plant was tested qualitatively by DAS-ELISA, to determine the percentage of infection.

The probability of BYDV transmission by single aphids (P_t) was calculated by the maximum likelihood estimator (Gibbs and Gower 1960; Swallow 1985) of:

$$P_t = 1-(1-S/N)^{1/k}$$

in which:

N = total number of test plants used for each source leaf;
S = number of test plants which became infected; and
k = number of aphids per test plant (k = 2).

The relationship between P_t and virus content (i.e., ELISA value) was determined for each cereal cultivar and each acquisition feeding time as one treatment. Because the number of test plants used for each source leaf varied (i.e., N = 4, 3, or 2), each treatment was also analyzed after grouping ELISA values for the individual leaves in increments of 0.200, except for values below 0.100, which were grouped as "Group 1." Group 1 therefore included all individual leaves with very low or no detectable virus (ELISA value $\leq$ 0.099). Group 2 included ELISA values between 0.100 and 0.300, Group 3 included ELISA values between 0.301 and 0.500, etc. Correlation required that $P \leq 0.05$, but consideration was also given to the r-value (correlation coefficient) and the sample size (degrees of freedom = df). Correlations between P_t and the ELISA values of each group of individual leaves were then examined.

Overall, there was no convincing evidence of differences in virus acquisition efficiency due to differences in virus content of leaves. For example, of 31 sets of data from tests of various combinations of host, virus, vector, and acquisition feeding time, 23 showed no correlation and 7 showed poor correlations (i.e., significant at the 1% level). In only 1 experiment did a good correlation occur (i.e., significant at the 5% level). This was in the transmission of the P-PAV isolate by its inefficient vector *S. avenae*, with an 18-hour acquisition feed. The 7 low correlations occurred in transmissions of isolates by efficient vectors.

In similar work, but assessing virus content by purification, Foxe and Rochow (1975) showed that efficient vectors of the PAV, MAV, or RPV isolates transmitted equally well from old or young leaves, even though the latter contained approximately three times more virus than old leaves. However, they found that transmission of the PAV isolate by the inefficient vector *S. avenae*

was correlated with leaf age, and therefore, presumably, with virus content. In our experiments, even this correlation was not detected in experiments with the P-PAV isolate and *S. avenae* (Table 2). Further, there was no evidence of intrinsic differences in virus acquisition efficiency due to differences in the overall virus productivity of cultivars.

It appears likely that an overriding limiting factor in virus acquisition in vivo is that the virus is unevenly distributed within the host phloem. This has been demonstrated in barley (Jensen 1969), and therefore probably applies for other cereals. Thus, even where ELISA indicates relatively high virus content within the leaf, the limiting factor in virus acquisition could be whether aphids

Table 2. Relative acquisition of barley yellow dwarf virus by aphids fed on individual old or young leaves of Clintland 64 oats

Virus/ vector	Position of virus source leaf (old) 1	2	(young) 3
	% test plants infected (no.)		
P-PAV *R. padi*	28 (223)	25 (229)	36 (254)
P-PAV *S. avenae*	42 (158)	36 (184)	45 (123)
MAV *S. avenae*	66 (77)	70 (76)	69 (72)
RPV *R. padi*	68 (58)	48 (67)	64 (58)

Infections were assessed by ELISA.

Table 3. Comparative efficiency in acquisition of the P-PAV isolate of *R. padi* and *S. avenae* fed through membranes on purified virus preparations

P-PAV concentration (μg/ml)[a]	No. samples/ species[b]	Percentage of aphid samples containing detectable virus (mean ELISA values) for these samples[c] *R. padi*	*S. avenae*
5	21	0 (0.058 ± 0.021)	0 (0.038 ± 0.011)
18	24	17 (0.112 ± 0.007)	4 (0.108 ± 0.023)
41	16	81 (0.147 ± 0.047)	81 (0.162 ± 0.065)
70	8	100 (0.274 ± 0.052)	88 (0.149 ± 0.057)

[a] Preparations were in 20% sucrose in 0.1 M phosphate buffer, pH 7.0

[b] Each sample consisted of 2 aphids ground in 0.25 ml buffer. The acquisition feeding on virus concentrates was for 18 h at 21°C. Mean ELISA values for untreated aphids were 0.05 for *R. padi* and 0.04 for *S. avenae*.

[c] Mean values ± standard deviation for the positive samples.

actually probe the phloem that contains virus. Conversely, the fact that relatively high acquisition efficiencies were sometimes obtained with source leaves whose extracts gave relatively low ELISA values, suggests again that whether vectors fed in phloem tissue containing virus was more crucial for virus acquisition than the overall virus content of the leaf.

Virus Acquisition from Purified Preparations

The conclusions reached above were consistent with the results of experiments in which virus acquisition by aphids feeding through Parafilm membranes on sucrose solutions (about 20% w/v) containing virus was assessed by ELISA and by transmission efficiency. Thus, virus acquisition efficiency as assessed by ELISA of aphid extracts was correlated with the concentration of virus present in the preparations (Table 3). Similarly, transmission efficiency also increased with increasing virus content in the solutions (Table 4). However, in a third series of experiments, when *R. padi* fed on leaves of Clintland 64, extracts of which contained an average of 60 μg/ml of the P-PAV isolate, a lower proportion of them acquired ELISA-detectable virus than when aphids fed through a membrane on a preparation of the same concentration (Table 5).

Taken together, these experiments suggest that *in vivo* feeding by aphids on tissue with a certain overall virus concentration is less successful for virus acquisition than *in vitro* feeding on the same concentration of virus. Again, a likely reason for this is that virus is not uniformly distributed through the phloem of the leaf.

Table 4. Comparative efficiency in transmission to test plants of the P-PAV isolate by *R. padi* and *S. avenae* fed through membranes on purified virus preparations

P-PAV concentration (μg/ml)[a]	*R. padi*	*S. avenae*
	% test plants infected (no.)	
5	0 (28)	0 (30)
18	18 (28)	3 (34)
41	22 (23)	13 (23)
70	50 (12)	33 (12)

Test plants were Clintland 64 oats infested with 2 aphids each for a 2-day inoculation test feeding at 21°C. The acquisition feeding on virus concentrates was for 18 h at 21°C. Infections were determined by ELISA.

[a] Preparations were in 20% sucrose in 0.1 M phosphate buffer, pH 7.0.

Acknowledgments

This contribution reviews recent work conducted in partial fulfillment of the Ph.D. degree by the first author. Special thanks are due to INVOTAN for their partial support. Expert technical assistance by Jo Anne McFatridge is also acknowledged.

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Table 5. Detection of P-PAV by ELISA in samples of *R. padi* fed on infected Clintland 64 oats or by membrane feeding on virus concentrates

Mode of acquisition	Virus concentration (μg/ml)	No. of aphids in sample 2	5
		No. positive samples/total	
Membrane feeding	60	9/12	8/8
Clintland 64 oats	60	2/10	2/5

The Clintland 64 oats were 17 days old and had been infected for 11 days. Acquisition feeding was 18 hours at 21°C.

Purified virus preparations were in 20% sucrose in 0.1 M phosphate buffer, pH 7.0.

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Other Viruses Causing Barley Yellow Dwarf-like Disease in Small Grains in South Africa

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Abstract

Our experience is that dwarfing, sterile ears, yellowing, and excessive tillering in South African wheat are more often associated with brome mosaic virus and cucumber mosaic virus infections than with barley yellow dwarf virus infection. This paper describes the investigation of one such incidence where severe disease due to confounding viruses caused a yield loss of greater than 50%.

Previous reports from this laboratory have described virus diseases that are similar to or mimic barley yellow dwarf virus (BYDV) disease in wheat (*Triticum aestivum* L.). These include disease caused by brome mosaic virus (BMV) and cucumber mosaic virus (CMV) (von Wechmar et al. 1984a; von Wechmar and Rybicki 1985). It is our experience that the barley yellow dwarf (BYD)-like yellowing and dwarfing symptoms in wheat in South Africa are more often associated with infection by CMV and BMV, with only occasional incidence of BYDV (see Situation Report and Rybicki et al., these Proceedings).

This report details an investigation of a disease incident noted in the eastern Transvaal highveld, where BYD-like symptoms were originally noticed and CMV was found by subsequent testing.

Disease Investigation

The Chrissiesmeer district in the eastern highveld of the Transvaal is a new wheat growing area (since 1983); I inspected the first crop from this region after a report that widespread disease symptoms were noted after ear emergence. Symptoms noticed were the following: yellowed or white, dead or sterile ears on stunted shoots (2/3 to 1/2 size of healthy neighbors), and yellowed flag leaves and shoots, which died off. In 1983, R. Plumb (Rothamsted Experimental Station, Harpenden, UK) and I had noticed similarly diseased wheat in the Orange Free State and the Western Cape, and presumed it to be infected with BYDV. It was subsequently shown to be infected with CMV, which had possibly been transmitted through seed (von Wechmar, unpublished; von Wechmar et al. 1984a). BYDV could not be tested for at that time.

The disease described here could be transmitted by the aphids *Rhopalosiphum padi* (L.) and *Diuraphis noxia* (Mordvilko). In the light of our previous experiences, the diseased material from the field was tested by double antibody sandwich-enzyme-linked immunosorbent assay (DAS-ELISA) and Ouchterlony double immunodiffusion tests using anti-BMV and anti-CMV sera (3 each) and anti-BYDV serum (PAV-specific). Single shoots representing 13 field-collected plants, not all of which appeared diseased, were analyzed. Plants were crushed in phosphate buffered saline (PBS), and the sap either tested directly, or concentrated by ultracentrifugation before testing.

Results

Table 1 shows the results of testing single shoots by DAS-ELISA for BMV. The low incidence of BMV relative to symptom occurrence (17 infected/140 tested) prompted the subsequent screening of more plants with antisera to BMV isolates (x 3), CMV isolates (x 3), and a BYDV (PAV-like) isolate (Table 2). There was a low incidence of BYDV (4/52), a high incidence of CMV with 2 of the antisera, and no detectable CMV with the other CMV antiserum or BMV among the plants and shoots tested. CMV was detected together with BYDV in two separate plants. The BYDV was presumed to have been transmitted by aphids from grasses to the crop. The

CMV probably came from contaminated seed: Table 3 shows the results of testing the seed used for sowing the experimental field for CMV contamination. The diseased plants appeared to have been spread evenly throughout the field, as one would expect from seed transmission; subsequent spread from these foci could have been via aphids.

Discussion

This report describes an isolated disease occurrence, which happened to have been thoroughly investigated for the presence of three viruses. Similar incidents have occurred in the region of study and elsewhere that have not been as closely studied. In these cases, diagnosis on the basis of visual symptoms has varied from "BYD

Table 1. Results of DAS-ELISA to determine the presence of brome mosaic virus in single shoots of diseased plants

Plant no.	Symptoms of plants/shoots (length of shoot)	No. positive/ no. tested anti-BMV
1	Plants appearing normal	0/21
2	Plants with yellow shoots	
	a. Green normal (140 cm)	0/3
	b. Medium, yellow	2/8
	c. Short, dead ears	1/7
		3/18
3	Stunted plants	
	a. Green, normal, long	0/4
	b. Medium, long	1/8
	c. Short	0/9
		1/21
4	Diseased appearance	
	a. Green, normal (130 cm)	0/10
	b. Green, yellow ears (100 cm)	1/20
	c. Green, yellow ears (77 cm)	3/7
	d. Short, no ears	1/5
		5/42
5	Diseased appearance	
	a. Green, normal (140 cm)	7/15
	b. Green, yellow ears (100 cm)	1/6
	c. Short, no ears	0/1
		8/22
6	Diseased appearance	
	a. Green, normal (108 cm)	0/3
	b. Green, short (65 cm)	0/3
	c. Long (108 cm) yellow/white ears	0/3
	d. Short (75 cm) yellow/white ears	0/3
	e. Shoot (60 cm) no ears	0/4
		0/16
	Total	17/40

Table 2. Results of DAS-ELISA to determine the presence of brome mosaic virus, cucumber mosaic virus, and BYDV in single shoots of diseased plants

Plant no.	No. of shoots positive/no. of shoots tested				
	anti-BMV	anti-CMV-1	anti-CMV-2	anti-CMV-3	anti-BYDV
7	0/14	10/14	12/14	0/14	3/14
8	0/9	6/9	9/9	0/9	0/9
9	0/8	1/8	2/8	0/8	1/8
10	0/3	2/3	3/3	0/3	0/3
11	0/6	3/6	4/6	0/6	0/6
12	0/7	5/7	7/7	0/7	0/7
13	0/5	4/5	5/5	0/5	0/5
Total	0/52	31/52	42/52	0/52	4/52

Table 3. Radial-immune-diffusion test to determine the presence of brome mosaic virus and cucumber mosaic virus in the original seed sown at Chrissiesmeer

Seed Reference	No. positive/no. tested	
	BMV	CMV
Seedlot 1	0/100	2/100 CMV-2
	0/100	4/100 CMV-3
Seedlot 2	3/100	1/100 CMV-2
	0/100	10/100 CMV-3

disease" through root disease to cold stress. In the present case, fungi did not appear to be involved. Viruses were definitely present, as proved by the original finding of a low incidence of BMV infection. However, it was a surprise to find as much CMV as we did, in plants with symptoms more readily associated with BYDV infection, and which, in most cases, contained no BYDV. Yield loss was estimated at 50%, meaning that BYD-like diseases may be devastating even when BYDV is only peripherally involved.

Our experience, in this country, has been that BYDV is not as often associated with severe disease of wheat as are BMV and CMV. The latter two viruses can cause symptoms in wheat that mimic BYD symptoms in the absence of BYDV, although the three viruses can also occur as mixtures.

We have found that BMV and CMV may occur at very low concentrations in infected plants, especially when these have been infected by seed or aphid transmission (Rybicki 1984; von Wechmar et al. 1984b). Accordingly, we often find it as difficult to detect these viruses in field-collected wheat as it is to detect BYDV, meaning that less sensitive techniques normally used for high-yielding viruses may not always work in these cases. My final conclusion is that BYDV is not as much a problem in South African wheat as are other viruses, which can cause very similar diseases.

Acknowledgments
I wish to thank Karen Hussey for technical assistance and the Wheatboard and the University of Cape Town for supporting this work.

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Virus-like ("Cryptic") Particles Present in Healthy Cereal Tissue: Partial Characterization and Preparation of Specific Monoclonal Antibody

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Abstract

*Virus-like particles were isolated in low yields (1 to 20 μg/kg) from leaf and root tissue of a number of lines of barley (*Hordeum vulgare *L.), oats (*Avena sativa *L.), and wheat (*Triticum aestivum *L.) grown under conditions that excluded the introduction of known viruses by seed, soil, water contact, or insects. Extracted particles separated by rate-zonal centrifugation into discrete peaks were spherical (13.5 nm/50 S; 18 nm/72 S; 25 nm/118 S), geminate (24 x 14 nm/72 S), and "triplet" (36 x 14 nm/90 S). Geminate and triplet particles banded in gradients of cesium sulfate at 1.29 g/cm^{-3}; a band of 10%-shrunken particles formed at 1.31 g/cm^{-3}. Removal of salt and reconcentration of equilibrium-banded fractions yielded only 18 nm spherical particles, showing geminate and triplet morphologies to be probable artefacts. Monoclonal antibodies were used to affinity-purify the 25 nm spherical particles, which contained single-strand (ss) RNA of 1.4 MDa. Spherical 18 nm particles derived from fractions of geminate and triplet particles contained ssRNA of 1.4 and 0.72 MDa.*

Barley yellow dwarf virus (BYDV) is notoriously difficult to purify (Rochow and Duffus 1981). Among the variants described by Rochow (Rochow and Jedlinski 1970), isolates specifically transmitted by *Rhopalosiphum maidis* (Fitch) (RMV-like) have proved especially difficult to purify. In an effort to purify a sufficient amount of virions of a Manitoba RMV-like isolate for the production of an antiserum, large quantities (> 2 kg) of infected oat (*Avena sativa* L.) leaf and root tissue were processed and their extracts clarified and concentrated to a volume that could be layered onto a single 12 ml sucrose gradient; infectious RMV-like BYDV was recovered as determined by the results of membrane feeding assays, but most of the purified particles were 24 x 14 nm geminate particles (Haber and Gill 1984).

When similar quantities of oat tissue infected with isolates of other variants were similarly processed and analyzed, an array of spherical, geminate, and "triplet" particles was observed. Since it had been observed that aphids may be infected with a virus that is distinct from BYDV and is not transmitted to the aphid's plant host (D'Arcy et al. 1981), the possibility that the novel virus-like particles might be aphid viruses was investigated. Unexpectedly, the novel particles appeared in extracts from aphid-free plants, as well as plants that had been fed on by aphids which were non-viruliferous and viruliferous with respect to BYDV. Steam-sterilized soil was routinely used for growing plants, but as an additional precaution, the experiments were repeated using only root and leaf tissue grown in vermiculite and nourished with sterilized nutrient salt solutions.

In this paper, evidence from partial characterization will be presented to show that these novel particles are indeed viruses *sensu* Kassanis (1984). One of these particles, co-sedimenting with BYDV virions, has been affinity-purified with a monoclonal antibody (McAb) prepared against it. The possibility that this 25 nm particle may present confounding effects in experiments employing polyclonal antisera against BYDV, and this particle's relationship to brome mosaic virus (BMV), are discussed.

Materials and Methods

Extraction and purification of particles from root and leaf tissue of oats, barley (*Hordeum vulgare* L.), or wheat (*Triticum aestivum* L.) were carried out by methods

similar to those described for the purification of BYDV (e.g., Hammond et al. 1983). Tissue was pulverized in liquid nitrogen, blended in 0.1 M phosphate buffer, pH 6.5, and the fibrous tissue residue subjected to a second cycle of pulverization and blending. Extracted sap was clarified with 30% chloroform/1% Trition X-100 and the clarified sap subjected to several cycles of differential centrifugation, followed by centrifugation through a 1/3 volume of a cushion of 20% sucrose. With extract volume reduced to 1 ml or less, samples were layered on sucrose gradients (10 to 40% in 0.1 M phosphate pH 6.5) and centrifuged at 31,500 rpm for 120 min at 4°C in a Beckman SW 40 rotor. In most cases, fractions comprising portions of the 12 ml gradient from a depth of 2 to 8 ml were collected, pelleted by high speed centrifugation, resuspended in 0.5 ml phosphate buffer and rerun in sucrose density gradient centrifugation (SDGC) as above. Gradients were then scanned in an ISCO UA-2 absorbance monitor and 0.6 ml fractions were collected for storage at -80°C.

Parallel sucrose gradients in which purified satellite tobacco necrosis virus (sTNV) or top, middle, and bottom components of tobacco ringspot virus (TRSV) were analyzed, provided reference standards for sedimentation velocities.

Determinations of isopycnic buoyant densities in cesium sulfate were carried out in self-forming gradients prepared as follows: 0.5 ml of SDGC-purified particles were layered on 4 ml of a 12.5 to 50% gradient of cesium sulfate and centrifuged in a Beckman SW 50 rotor at 35,000 rpm for 24 hours at 20°C. Gradients were scanned in an ISCO UA-2 absorbance monitor, 0.3 ml fractions collected, and the density of each fraction determined by refractive index.

Particles were examined by electron microscopy, employing 0.2% phosphotungstate pH 7.2 as the negative stain for particles fractionated by sucrose or isopycnic gradients, and 2% uranyl acetate pH 7.0 for particles purified by affinity chromatography (see below).

Monoclonal antibodies (McAb) were prepared according to protocols described by McKenzie and Tremaine (1986). Only preparations of particles isolated from plant tissue that had never been exposed to BYDV or aphids were used to prime the initial immune response in mice. Since the amount of available particles was small, hybridomas were tested by indirect enzyme-linked immunosorbent assay (ELISA) using pooled rather than fractionated particles. The strongest positively reacting monoclones were injected into mice to induce ascitic tumors, and immunoglobulin (IgM in all cases) was purified from ascitic fluids by gel exclusion chromatography.

To identify to which class(es) of particles the McAb had been prepared, the specificity of the purified IgM antibodies was determined by affinity chromatography. IgM was covalently linked to column gel matrix and pooled particles were eluted through the column (Pharmacia 1979); particles with specific affinity were released from matrix-linked antibodies with glycine-HCl pH 2.25.

Nucleic acid components of purified particles were determined by nuclease digestions and submarine electrophoresis in agarose gels. RNase T1 and DNase I were used to determine susceptibility to ribonuclease and deoxyribonuclease attack, respectively. RNA from southern bean mosaic virus (SBMV) and a Hind III digest of lambda DNA were used as standards for RNA and DNA, respectively. Double-stranded (ds) RNA from H9 mycovirus was used to monitor the digestion of dsRNA by RNase T1 under low salt conditions and non-digestion under high salt conditions.

Results and Discussion

The following viruslike particles were observed in sucrose gradient fractions after two rounds of SDGC: a) 13.5 nm spheres sedimenting at ca. 50 S; b) 18 nm spheres/72 S; c) 25 nm spheres/118 S); d) 24 x 14 nm geminate particles/72 S; and e) 36 x 14 nm "triplet" particles/90 S.

Evidence from the determination of isopycnic buoyant densities of the geminate and triplet particles indicates that, unlike members of the geminivirus

group, the twin- and triple-sphere particles are probably artefactual. Geminate and triplet particles banded in gradients of cesium sulfate at 1.29 g/cm^3; a minor peak of particles whose dimensions appeared to have shrunk 10% formed at 1.31 g/cm^3. Removal of salt from the equilibrium-banded fractions, followed by reconcentration by high speed centrifugation yielded spherical particles of 18 nm diameter. In addition, SDGC fractions of geminate and triplet particles after storage at -80° C for several months revealed only spherical particles of 18 nm diameter.

The notion that the geminate (24 x 14 nm) and triplet (36 x 14 nm) particles are artefactual derivatives of the 18 nm spheres is also consistent with the observation that the geminate and triplet particles were not observed in all preparations but the 18 nm spheres were.

Like oats, barley and durum wheat (*Triticum turgidum* L. var. *durum*) yielded low quantities (1 to 10 μg/kg) of the novel, cryptic, viruslike particles. Not all types of particle described above were always observed; the 13.5 nm spherical particle was observed in extracts from oat and barley tissues, but not from durum wheat. The relative yields of the different classes of particles also differed: in barley, 25 nm spherical particles made up 80 to 90% of the observed particles, but in durum wheat, more than 90% were 18 nm spheres.

The viral (*sensu* Kassanis) character of the 18 nm spheres and their geminate and triplet derivatives, as well as that of the 25 nm spherical particles, is evident from their UV-absorbance profiles and the nature of their physical constituents (Tables 1 to 3). Insufficient amounts of purified 13.5 nm spherical particles have so far precluded further physical characterization of those particles. Spheres of 18 nm derived from preparations of geminate and triplet particles (cf. above) as well as "original" preparations of 18 nm spheres contain single-stranded (ss) RNA of ca. 1.40 and 0.85 MDa, further evidence that geminate and triplet particles are artefacts of the 18 nm spheres.

The observation in extracts from ostensibly healthy plants of 25 nm virus-like spheres co-sedimenting with BYDV indicates the need to be able to "disentangle" any 25 nm "confounding" particles that might be present in preparations of BYDV particles. An McAb was prepared against the "confounding" particles. Only particles that had been extracted from healthy plants were used to prime the mouse immune system. The specificity of the obtained McAb was determined by micro-scale affinity chromatography, an approach that minimized the possibility of losses. After a pool of particles similar to that used originally to prime the mouse immune system was passed through a column of McAb covalently linked to support matrix, individual beads were removed from the column and subjected to various treatments to determine which would release intact particles detectable by electron microscopy. Brief treatment with glycine-HCl pH 2.25 released intact 25 nm spherical particles and identified the McAb as being specific for this kind of particle.

Table 1. Absorbance characteristics of virus-like particles isolated from uninfected cereal tissue

Particle type	Amax (nm)	Amin (nm)	Amax/ Amin	A260/ A280
18 nm sphere	261	243	1.31	1.42
14 x 24 nm geminate	261	243	1.31	1.42
14 x 36 nm triplet	260	242	1.31	1.43
25 nm sphere (co-sediments with BYDV)	259	240	1.41	1.78

The "confounding" 25 nm particles subsequently eluted from the column with glycine-HCl pH 2.25 contained ssRNA of ca. 1.42 MDa. Three major bands and a fourth minor band were revealed by silver-stain PAGE analysis of the proteins of the "confounding" particle (Table 2). It is thus clear that the "confounding" particles are viral and distinct from BYDV. As we were able to extract (albeit at yields no higher than 20 μg/kg) the "confounding" particles from uninfected oat and barley tissue, polyclonal antisera prepared against low-

Table 2. Polypeptide species visualized by SDS-Page followed by silver staining. Molecular sizes estimated by interpolat in from migrations of known size standards

Particle type	Size in daltons	
18 nm sphere	27,100	main band
	21,200	minor band
14 x 24 nm geminate	27,100	main band
	21,200	minor band
14 x 36 nm triplet	27,100	main band
	21,200	minor band
25 nm sphere	37,700	è major bands staining
(co-sediments with BYDV)	31,000	è with approximately
	28,500	è equal intensity
	25,100	minor band

Table 3. Characterization of size and type of nucleic acid encapsidated in viruslike particles isolated from uninfected cereal tissue. Sizes (in megadaltons) estimated by interpolation of bands from Hind III digest of lambda DNA, and RNA of southern bean mosaic virus in agarose gel electrophoresis

Particle type or reference nucleic acid	buffer	RNase T1 high salt	RNase T1 low salt	DNase I
SBMV	1.41	"	"	1.41
Hind III digest of lambda DNA	array of bands 13.86 to 0.30			"
H9 mycovirus double-stranded RNA	array of bands 5.5 to 0.37		" "	array of bands 5.5 to 0.37
18 nm sphere (original morphologhy)	1.40 0.85	"	"	1.40 0.85
18 nm sphere (derived from pool of 14 x 24 nm geminate 14 x 36 nm triplet)	1.40 0.85	"	"	1.40 0.85
25 nm sphere (co-sediments with BYDV)	1.42	"	"	1.42

" = abolition of bands after nuclease treatment.

yielding isolates of BYDV might be contaminated with antibodies against those "confounding" particles.

Diagnoses of BYDV infection by analysis of symptoms and aphid transmission have recently been critically re-examined by comparison with enzyme-assisted immunoelectroblotting (von Wechmar and Rybicki 1985). In many cases, this sensitive serological test indicated the presence of BMV antigen and not BYDV antigen in samples previously diagnosed by other workers as BYDV-infected on the basis of symptoms and transmission. Our findings of "cryptic" and "confounding" viruses (*sensu* Kassanis) in extracts from ostensibly healthy cereal tissue suggest that additional evidence may be needed to reach a firm conclusion that BMV antigen is detected in extracts of plants incorrectly diagnosed as infected with BYDV. The use of McAb to both BMV and BYDV in the analyses by enzyme-assisted immunoelectroblotting would ensure that only antigens of either of those two viruses were detected. Additional, independent tests such as analysis for dsRNA and infection of dicotyledonous indicator hosts would leave no doubt that BMV can mimic BYDV infection.

In 1985 and 1986, BMV was diagnosed in wheat, barley, and triticale (*X Triticosecale* Wittmack) in experimental plots at two locations in Manitoba, Canada (Haber and Hamilton, 1989). These infections differed from those described in South Africa in that symptoms in the field did not resemble BYD, and high concentrations of BMV particles were isolated directly from field-infected plants. Epidemiological evidence indicated that the BMV found in Manitoba was transmitted mechanically from single sources, probably by mowing and the movement of implements. Attempts to transmit the Manitoba BMV isolate by the cereal aphids *Rhopalosiphum padi* (L.), *R. maidis*, and *Sitobion avenae* (Fabricius) in the manner described by Rybicki (1984) have, so far, been unsuccessful.

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Russian Wheat Aphid as a Vector of Brome Mosaic Virus in North America

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Abstract

Diuraphis noxia *(Mordvilko) is a damaging pest of small grains in several regions of the world. Within weeks of its identification in the United States in 1986, the aphid was found in five states and across several million hectares.* D. noxia *is reported to vector several small grain viruses including brome mosaic virus (BMV) and barley yellow dwarf virus (BYDV).*

Nonpersistent and persistent modes of transmission were tested with a Texas clone of D. noxia *and a Nebraska isolate of BMV on Baart wheat (*Triticum aestivum *L.) and Henry barley (*Hordeum vulgare *L.). No symptoms were observed on any test seedlings following insect feeding. After 28 days the leaf tips of the youngest leaves of each test seedling were collected for enzyme-linked immunosorbent assay (ELISA) analysis. An extract of the remaining leaf material was rubbed onto the leaves of uninfected Henry seedlings.*

All transmissions of BMV by D. noxia *were apparently persistent.* D. noxia *appears to transmit BMV inefficiently in a persistent manner, but the potential threat of such transmission is increased by the large populations that develop on wheat and barley.*

As early as 1900, *Diuraphis noxia* (Mordvilko) was considered a pest of small grains in Russia. Since that time the aphid has been reported as a pest in the Middle East, and in North African and European countries bordering the Mediterranean (USDA-ARS 1963). The aphid has a toxic saliva, which produces symptoms that can be mistaken for viral infections (von Wechmar and Rybicki 1981). Infested plants have characteristic tightly rolled leafwhorls, an initially prostrate and later unnaturally upright growth habit, and white streaks along the leaf midribs. Stunting, sterility, and premature death are usually observed.

D. noxia first appeared in South Africa in 1978 and has been implicated as the vector of a three-virus complex—brome mosaic virus (BMV), barley yellow dwarf virus (BYDV) and an aphid enterovirus, *Rhopalosiphum padi* virus (RhPV)—causing the Freestate streak disease (von Wechmar and Rybicki 1981). This was the first report of an aphid vector of BMV.

In March of 1986 *D. noxia* was collected and identified near Lubbock, Texas. Within weeks of the first collection and confirmation, aphid infestations were found in hundreds of counties over a five-state area and covering millions of hectares of barley (*Hordeum vulgare* L.) and wheat (*Triticum aestivum* L.). The distribution and dispersal of the aphid have been monitored very closely in the affected areas; it has overwintered as far north as Colorado and is currently found in nine states in the central and southern plains of the United States (Russian Wheat Aphid News 1987).

Following a meeting of the New Pest Advisory Group (Animal and Plant Health Inspection Service) in early May 1986 to coordinate information and research on *D. noxia*, the United States Department of Agriculture was asked to investigate and confirm the reports of plant virus transmission by the new pest through its Agricultural Research Service Foreign Disease-Weed Science Research Unit in Frederick, Maryland. Of particular interest were the virus-vector relationships of barley stripe mosaic virus (BSMV), BYDV, BMV, RhPV, and *D. noxia*. We report here on the BMV transmission studies.

Materials and Methods

A population of *D. noxia* collected in Texas was kindly provided by M. Rose, Texas A&M University, College Station,

Texas. At Frederick, new colonies were established from approximately 20 first-instar nymphs born in the absence of plant material. The BMV strain (Lane 1977) and the homologous antiserum were gifts from L. Lane and E. Ball of the University of Nebraska, Lincoln. Double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) was as described by Hewings and d'Arcy (1984), except the tissue samples and conjugate were prepared in 0.05 M sodium phosphate buffer, pH 6, instead of phosphate buffered saline (PBS). The aphid and the virus have been maintained on Baart wheat or Henry barley in a containment facility glasshouse at 18 to 28°C with natural daylight supplemented by high-output, cool-white fluorescent tubes for 15 h/day.

Initial experiments were designed to determine if *D. noxia* vectors BMV in either a nonpersistent or persistent manner. The inoculum source was Baart wheat or Henry barley plants 16 to 22 days after inoculation and exhibiting chlorotic streaks typical of BMV infection. Inoculum for positive controls for all experiments was prepared by macerating samples of infected leaf tissue in 0.05 M sodium phosphate, pH 6 (1:5, w/v), and rubbing the tissue extract onto 2-week-old Henry barley seedlings previously dusted with carborundum (Table 1). Negative controls were treated in the same way as experimental plants except that *D. noxia* was allowed access to uninfected Baart wheat or Henry barley.

Nonpersistent transmission
Leaf pieces of infected Baart were placed on moist filter paper in 100 mm plastic petri dishes and infested with *D. noxia*. Each aphid was allowed to probe for 0.5 to 5 min, then transferred individually to 2-week-old Henry barley seedlings. Ten aphids were allowed access to each test seedling. After a 72-hour inoculation access period, the aphids were killed with granular Furadan (carbofuran). The experiment was repeated 4 times and replicated 8 or 9 times.

Persistent transmission
Persistent transmission was tested in three different ways: *D. noxia* was allowed a 72-hour acquisition access period on infected Baart wheat or Henry barley leaf pieces; a 72-hour acquisition period on infected intact Baart or Henry plants; or a 24-hour acquisition period on infected Henry leaf pieces. Between 50 and 100 aphids were transferred from the inoculum source to each uninfected Henry seedling and confined in cellulose butyrate tube cages for a 72-hour inoculation access period. Cages were then removed and the pots treated with Furadan. The experiment was repeated 7 times and each treatment was replicated at least 6 times.

After inoculation the test plants were observed regularly for symptoms of BMV infection. Four weeks after the start of the inoculation access period, each plant was harvested, divided into two 1-g samples and assayed in two ways. Infectivity back-assay inoculum was prepared from one 1-g sample of leaf tissue prepared as the positive controls described above. The second 1-g sample was pulverized in liquid nitrogen, ground in buffer (1:5, w/v) with a Tissumizer (rotary homogenizer) and analyzed by DAS-ELISA.

The six back-assay plants testing positive for BMV were retained for inoculum for the second set of persistent transmission tests using the methodology described above, except that the aphids were killed with the synthetic pyrethroid, Sumithrin. Test plants were harvested and analyzed by ELISA.

Results
The BMV strain used in this study produces a strong chlorotic streaking reaction on Henry barley and Baart wheat 7 days after mechanical inoculation. As the infection progresses, the symptoms fade on mature leaves but are conspicuous on each developing leaf. In the initial series of experiments the Baart and Henry positive controls all became infected and showed typical symptoms. When back-assayed onto healthy seedlings or analyzed by ELISA, they all gave positive reactions (Table 2).

Nonpersistent transmission
Considerable chlorotic blotching and leafwhorl rolling, which were probably reactions to the vector's phytotoxic saliva, were observed in test plants in the

nonpersistent transmission tests. A few days after the aphids were removed, the plants appeared to recover somewhat. Subsequent growth was symptomless. After 28 days no BMV symptoms were observed. Back-assay and ELISA results were all negative (Table 2).

Persistent transmission

No symptoms typical of BMV infection were observed on any of the 50 Baart or 11 Henry plants that were inoculated with BMV by *D. noxia*. Toxic reactions occurred when aphids were present, but plants grew normally when the aphids

Table 1. Transmission flowchart for brome mosaic virus and *D. noxia*

Mechanical methodology	Persistent methodology (1)	(2)	(3)	Nonpersistent methodology
Inoculum extracted from same infected leaves as used in nonpersistent tests (Rubbed on several seedling leaves)	Infected leaf pieces infested with *D. noxia* (72-h AAP)	Infected (intact) plant infested with *D. noxia* (72-h AAP)	Infected leaf pieces infested with *D. noxia* (24-h AAP)	Infected leaf pieces infested with *D. noxia* (0.5-5.0 min (AAP)
↓ Observed for 28 days	Aphids transferred to wheat or barley seedlings for a 72- to 120-h IAP			↓ Aphids transferred to wheat or barley seedlings for a 72-h IAP
↓ 1 g samples harvested for back-assay and ELISA	↓ Treated with Furadan or Sumithrin			↓ Treated with Furadan
XXXXXXXXXXXXXXXX	↓ Observed for 28 days			↓ Observed for 28 days
	↓ Harvested for back-assay and/or ELISA			↓ Harvested for back-assay and/or ELISA
	↓ Leaf pieces from 6 positive assay plants from methods (1) and (3) infested with *D. noxia* for a 72-h AAP			XXXXXXXXXXXX
	↓ Leaf pieces with 50+ aphids transferred to barley seedlings for a 72-h IAP			
	↓ Aphids killed with Sumithrin			
	↓ Plants observed for 28 days			
	↓ Leaves harvested for ELISA (positive plants used for inoculum for third series of experiments)			
	↓			

AAP = Aquisition access period, IAP = Inoculum access period.

were removed. In the first four experiments in which the inoculum source was Baart wheat, all back-assays and all but one ELISA assay were negative. In the last three experiments the inoculum source was Henry barley. Of the 19 plants tested, none showed symptoms, yet 5 of the 19 tested positive by back-assay. The same 5 plus 2 additional plants were positive by ELISA (Table 2).

In the second series of persistent transmission tests all 35 barley plants inoculated with 50 or more *D. noxia* were asymptomatic 28 days after inoculation. However, 11 of the 35 plants were positive by ELISA.

Discussion

BMV is mechanically transmissible and there is a report of nematode transmission. Prior to a report by von Wechmar and Rybicki (1981), there had been no evidence that the virus could be transmitted by an arthropod vector (Lane 1977).

Many plant viruses are mechanically transmissible and are vectored in a nonpersistent manner by aphids. There are no confirmed reports of viruses that are both mechanically transmitted and persistently transmitted by aphids. Von Wechmar and Rybicki (1984) showed that plants inoculated with BMV by *D. noxia* had atypical symptoms and a reduced concentration of virus, which may result from an infection that is phloem-limited. When asymptomatic plants were used for inoculum in mechanical transmission assays, some plants showed typical BMV symptoms.

This preliminary report also indicates a different mode of transmission for BMV by *D. noxia* and possibly other cereal aphids. The data presented suggest that *D. noxia* transmits BMV very inefficiently. However, in the field, aphid populations increase rapidly in the rolled leafwhorls, often exceeding several hundred individuals per plant (Russian Wheat Aphid News 1987). Until better information is available on the efficiency of transmission of BMV by *D. noxia*, one can only speculate on the importance of aphids in the ecology and epidemiology of BMV.

A number of studies relating to *D. noxia* are planned at Frederick including electron microscopy of the relationship of BMV to the vector, cultivar effects, and

Table 2. Results of initial transmission experiments with *D. noxia* and brome mosaic virus (BMV)

Inoculum source	Inoculation type[a]	No. of reps.	No. of plants[b]	Back -assay[c]	ELISA[d]
Baart wheat	M	3	19/19	19/19	19/19
Baart wheat	N-P	4	0/25	0/25	0/25
Baart wheat & Henry barley	P-72	4	0/42	1/42	1/42
Henry barley	P-24	3	0/19	5/19	7/19

[a] M = mechanical, N-P = non-persistent, P-72 = persistent (72-h AAP acquisition access period); P-24 = persistent (24-h acquisition access period)

[b] No. of plants with symptoms/total number of plants at harvest

[c] No. of plants giving positive BMV symptoms by mechanical inoculation/total number of plants assayed.

[d] No. of plants testing positive by ELISA/total number of plants (positive = an absorbance value greater than the mean of the healthy controls + 4 standard deviations)

age of infection as it relates to the effect of virus concentration on vector transmission and efficiency. Transmission studies have been initiated on the ability of *D. noxia* to transmit BYDV strains and other important grain viruses.

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Monospecific Antibody Preparation for Use in the Detection of Viruses

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Abstract

Applications and modifications of a micro-immunoabsorbent technique for the purification of antibodies specific for plant virus capsid protein units for diagnosis of virus diseases in small grains are presented. It is suggested that monospecific antibodies may be used instead of monoclonals for many diagnostic routines.

Enzyme-assisted immunoelectroblotting or "Western" blotting has been used for some time in our laboratory for the investigation of viruses of cereals (Rybicki and von Wechmar 1982; von Wechmar et al. 1984; Rybicki 1984; Chauhan 1985; Knox 1986). The technique entails the separation of viral and host polypeptides by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), transverse electrophoretic transfer (electroblotting) of the resolved polypeptides onto an absorbent membrane, and detection of specific proteins by indirect enzyme- (or other amplified) immunoassay (Towbin et al. 1979). Specific polypeptide "bands" may be distinguished by molecular weight or mobility differences, where an antiserum recognizes more than one antigen.

An obvious extension of this technique is to use electrophoretically separated and blotted protein(s) as specific immunoadsorbent(s) for the preparation of monospecific antibodies (=antibodies reacting with only one protein species) from polyclonal, multiple-reactive antisera (Olmsted 1981; Rybicki 1986). Very briefly, this entails using excised strips of membrane with a single protein band adsorbed onto them to "fish out" specific antibodies from a serum that may contain antibodies to many different polypeptides. The attached antibodies may then be eluted from the immobilized protein, concentrated, and used in specific detection assays.

The technique has been tested in our laboratory with brome mosaic virus (BMV), plant and bacterial proteins, and the capsid proteins of the aphid pathogenic *Rhopalosiphum padi* virus (RhPV) (Rybicki 1986; J. Parker and C.W. Williamson, personal communication), and found to work very well. Monospecific antibodies have been used to differentiate strains of maize dwarf mosaic virus (MDMV-A and -B) in double infections (Chauhan 1985; R. Chauhan and M.B. von Wechmar, unpublished). We have recently applied this technique specifically to investigating virus problems in small grains; we present this application, and a useful simpler adaptation of the technique.

Detection of a Strain of Cucumber Mosaic Virus in Wheat

It was recently found (P. Lupuwana and E.A. Knox, personal communication) that antisera produced in our laboratory to BMV purified from wheat (*Triticum aestivum* L.) often contained antibodies that reacted specifically with cucumber mosaic virus (CMV) in immunoelectroblots ("Western" blots). Accordingly, extracts of various cereals including wheat were screened for CMV by means of double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) and Western blot tests (von Wechmar, these Proceedings and unpublished; Knox 1986).

Positive results were obtained with antisera raised against CMV grown in *Nicotiana tabacum* L. or *N. glutinosa*; however, CMV antisera always reacted with many plant proteins in Western blots of infested cereal samples, including the characteristic +25 kDa CMV coat protein. This made it difficult to decide whether the often faint reactions were in fact specific for CMV. In addition, it

proved extremely difficult to purify the apparently very unstable CMV, or to satisfactorily transmit it except by aphids (M.B. von Wechmar, unpublished). Accordingly, we decided to make monospecific antibodies from a CMV antiserum, prove that these reacted only with coat protein, and use them for detection of CMV in wheat.

Methods and results

Purified CMV strain Tob (local tobacco strain), propagated in tobacco, was electrophoresed and electroblotted as described (Rybicki 1986). The 25 kDa protein corresponding to CMV capsid protein was located with reference to immuno-stained control lanes, and a thin strip excised. Unoccupied protein binding sites were blocked, then the strip was used several times to select antibodies out of a CMV antiserum. Monospecific antibodies were eluted in 0.1M glycine-HCl pH 2.9, dialysed, and concentrated.

The reactivity of the antibodies with different strains of CMV was tested by Western blotting (Figure 1). The antibodies reacted with a wide range of CMVs, spanning the antigenic range found in this country (Lupuwana 1985; M.B. von Wechmar, unpublished). Tests using these antibodies have shown that CMV is present in wheat in this country (von Wechmar 1989).

Conclusions

The use of monospecific antibodies as described here and elsewhere (Rybicki 1986) adds a guarantee of specificity to tests that otherwise may not necessarily be as specific as desired. For example, we are now confident that ELISA tests performed using a given antibody preparation will detect only one species of antigen as defined by Western blot tests—and not several, as published earlier (Rybicki and von Wechmar 1982). Other laboratories may not be so sure,

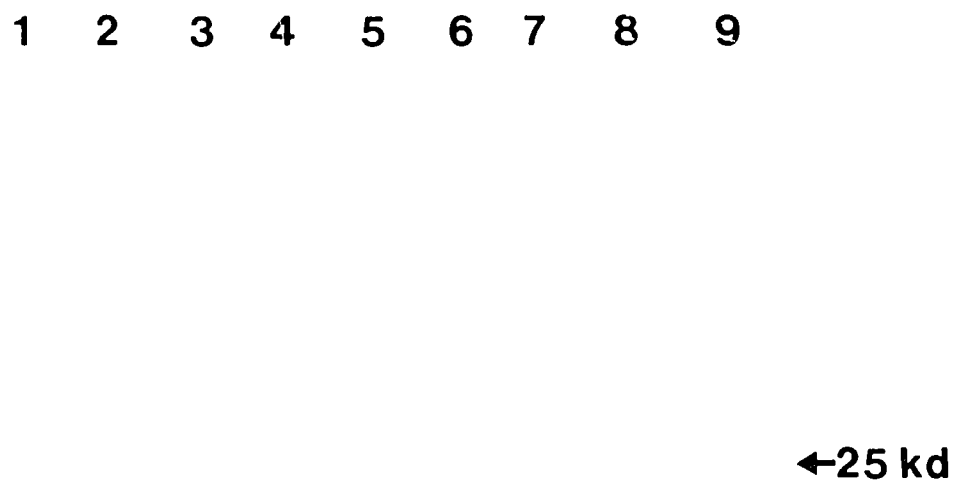

Figure 1. Western blot of samples of different isolates of cucumber mosaic virus probed with monospecific antibodies purified from electrophoretically-purified virus capsid protein.

Antibodies to CMV-Tobacco were prepared as described in the text. Samples: Lanes: (1) lupin strain, infected; (2) as (1), diluted; (3) CMV-Y (yellow strain); (4) CMV- Tob (purified); (5) CMV-K (Australia), infected sap; (6)-(9) sap from infected lupins.

Note (1), the lack of any reaction with host plant proteins; (2), weak reaction or lack of reaction with certain strains: this is a feature of CMV serology, even in indirect immunoassays, and necessitates the use of several antisera to detect all antigenic variants.

especially if they do not routinely use Western blot tests to check both their antigen purity and their antiserum specificity.

Immunospecific electron microscopy tests for BYDV may also have problems, given the presence of aphid viruses of similar size to BYDV in aphid vectors and in plant hosts. We have shown that antisera to BYDV often react with RhPV (C. Williamson; M.B. von Wechmar, unpublished). The use of monoclonal antibodies (McAb) bypasses all of these problems—but McAbs do not necessarily detect all strains of the virus, are difficult and expensive to prepare, and extremely expensive to buy.

The "Lunchbox" Immunoabsorbent Technique

We have explored the possibilities of using nitrocellulose and other adsorptive membranes as immunoadsorbents for simple, reliable "low tech" procedures for the purification of a specific antibody (E.P. Rybicki and J.T. Burger, unpublished). The technique that evolved derives from the procedure described above, but does not require the prior fractionation of an antigen mixture by electrophoresis. The procedures require only very basic laboratory equipment, including nitrocellulose membrane, simple benched centrifuges, a plastic freezer container or "lunch box," and some form of simple immunoassay system. We describe the application of the technique as explored using as model antigens BMV of cereals and *Ornithogalum* mosaic virus (OMV) (a poty-like virus of ornamentals) (J.T. Burger, unpublished). Rabbit antisera to both contained a variety of antibodies to plant antigens. The suitability of the method for the small-scale preparation of antibodies for immunoassays such as ELISA, immunodot blot, and Western blotting is discussed.

Absorption of antigens to membrane ("blotting")

Nitrocellulose paper (0.45um pore; Schleicher and Schuell) was cut into 10 x 10 cm squares, which fit into 12.5 x 17 x 8 cm plastic freezer boxes ("lunchboxes") with room for movement during shaking. Other membranes such as polyvinyllidene fluoride (Millipore) or other nylon based material can also be used (E.P. Rybicki, unpublished). For BMV antibody preparation, semipurified virus—made by resuspension of centrifugally pelleted clarified plant extract in 0.1M phosphate pH 7.0 (1/10 original volume)—was used to coat nitrocellulose. For OMV experiments, purified virus (J.T. Burger and M.B. von Wechmar, unpublished) in 0.1M Tris-HCl pH 9.0 was used. Water-wetted nitrocellulose was added to ± 25 ml volume of extract, and the boxes agitated at room temperature (22°C) for at least 2 hours.

Alternatively, the boxes were left on the bench and agitated by hand every 10 min. It did not appear necessary to "fix" antigen to the membrane by heat or other treatment. Membranes were washed in 3x changes of saline containing 0.05% (v/v) Tween-20 or Triton X-100 for 5 min, then "blocked" overnight using 20 ml phosphate-buffered saline (PBS) containing 0.05% Tween or Triton and 2% (w/v) bovine serum albumin or 5% (w/v) skimmed milk powder (blocking buffer).

Absorption of anti-host plant antibodies from serum

Sera to be absorbed were diluted 1/10-1/50 (depending on titre, pre-tested using a simple precipitin assay) in saline/detergent, and 20 ml added to a drained blot of healthy plant extract in a freezer box. The box was agitated at room temperature for 2 hours, and the serum was then poured off. This is also a useful way to absorb serum dilutions immediately prior to their use in Western blot tests (Lupuwana 1985; E.P. Rybicki, unpublished). An alternative option was to include the liquid plant sap extract as a 1/10 dilution in the serum dilution, and to incubate this with shaking for 2 hours.

Attachment and elution of specific antibodies

Appropriately diluted absorbed serum, or serum containing plant sap extract, was added to a drained virus extract blot in a freezer box. This was incubated as described above, following which the blot was thoroughly washed. The blot was rinsed in deionized water (or tap water), drained and lightly blotted on paper towel, and placed in 20 ml of "elution

buffer" (0.1M glycine/HCl, pH 2.9). The box was agitated for 10 min, the liquid drained off and immediately neutralized by addition of a predetermined amount of 0.1M NaOH. Essentially all specifically bound antibody may be removed from a blot by a single elution (Rybicki 1986 and unpublished), with yields of up to 1 mg for BMV extracts. The antibody attachment and elution steps may be repeated up to three times with the same BMV-infected sap blot with only slight drop in yield, as long as a fresh dilution of serum is used each time (E.P. Rybicki, unpublished). Antibody preparations were concentrated by dialysis against water and lyophilisation. Preparations were resuspended in small volumes of saline.

Immunoassay techniques
Antibody preparations were tested for specific activity by indirect ELISA (Rybicki and von Wechmar 1981) and Western blotting (Rybicki and von Wechmar 1982).

Results

As mentioned above, 1 mg or more antibody could be prepared from a single elution of a single BMV-infected sap extract-co ·ted membrane; this is sufficient to prepare enzyme conjugates for DAS-ELISA, and would provide materials for many assays. Yield for OMV was lower, as the yield of virus is lower (± 3 mg/kg). However, re-use of the antigen-coated membrane enables accumulation of enough antibody to allow many serological tests. In both cases, if antisera were absorbed with host plant antigens prior to application to blots, eluted antibodies reacted almost exclusively with virus coat protein, with very little "background" reaction with other polypeptides in Western blot tests (Figure 2). Eluted anti-BMV antibody could be diluted by a factor of 1/50 for use in Western blots, and up to 1/500 for indirect ELISA. This is a far better yield than can be obtained in the technique described above, with individual excised polypeptides.

Conclusions

The technique as described is a very simple immunoabsorbent-type method, with the important proviso being that very easily obtainable and relatively cheap materials may be used. Nitrocellulose or other membrane is the only expensive component, and that is far cheaper, far easier to use, and far better as an adsorbent than CNBr-Sepharose, or other comparable column chromatography materials commonly

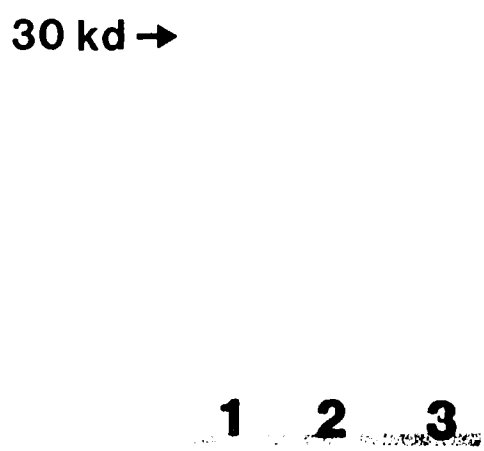

Figure 2. Western blot of three isolates of *Ornithogalum* mosaic virus probed with monospecific antibodies purified from virus adsorbed onto a nitrocellulose sheet.

Crudely purified virus isolates: left to right, OMV; OMV ex *Lachenalia* sp.; OMV 'wild' isolate. Antibodies eluted from virus (OMV) adsorbed onto nitrocellulose as described in the text, and detected using alkaline phosphatase-labelled goat-anti-rabbit antibodies and Nitro blue tetrazolium complex substrate.

used as immunoadsorbents. Up to 100 ug protein/cm^2 may be adsorbed onto nitrocellulose; polyvinylidene fluoride's capacity is even higher.

The use of crude extracts to purify antibodies is important when no facilities exist for purification of low-yielding viruses like OMV or BYDV: clarified sap extracts could be concentrated by PEG treatment; non-infected extracts could be used to absorb antisera; and infected extracts to purify monospecific antibodies. Even if antibody preparations are not exactly monospecific, their preparation in this way represents an important purification and concentration step, as relatively specific antibodies are purified in one step from raw serum, with a consequent increase in activity of the preparation once most of the extraneous serum proteins and immunoglobulins are removed.

This approach to antibody preparation may also be useful in the preparation of antibodies to unknown pathogens, or even to pathogenesis-induced proteins. Proteins present in extracts of diseased plants and not in healthy extracts may be used to purify specifically reactive antibodies from sera raised against the diseased extracts. We have found, for example, that rabbits injected with virus purified from small grains occasionally produce antibodies to other pathogens present due to seed infection and not ordinarily detectable. Repeated injection of extract, however, results in "biological amplification" of the contaminant by the immune system of the rabbit and the production of sufficient antibodies to react with trace amounts of the contaminant (Rybicki and von Wechmar 1982; Rybicki 1984).

Acknowledgments

We wish to thank Karen Rous for technical assistance, and the Council for Scientific and Industrial Research, the Wheat Board, the Department of Agriculture and the University of Cape Town for funding this work.

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PAV RNA Concentration Differences Among Field Resistant and Susceptible Wheat Cultivars

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Abstract

*Development of wheat cultivars (*Triticum aestivum *L.) resistant to barley yellow dwarf virus (BYDV) has often relied on visual scoring of symptoms under field conditions. To better understand the relationship of BYDV and host reaction, northern dot blot analysis was used to determine if four visually resistant cultivars had lower viral RNA concentrations relative to two susceptible cultivars. Plants were infected with the PAV isolate and grown in a growth chamber. Crude plant extracts were blotted on nitrocellulose and hybridized with ^{32}P labeled probe of the pPA8 (a PAV clone provided by Wayne Gerlach of CSIRO). A dilution series of purified plasmid and scintillation counting were used to determine PAV RNA concentration. Seri 82, a susceptible cultivar, was found to have a statistically greater PAV RNA concentration relative to NS 879/4. F_2 plants from a cross between these parents were grown in the greenhouse and the PAV RNA concentration determined for individual plants. The F_2 mean and distribution contrasted with 1985 field data. F_3 data for PAV RNA concentration are being collected to determine inheritance of resistance and effects of selection.*

Breeding cereals for resistance to barley yellow dwarf virus (BYDV) has depended on the use of leaf discoloration, dwarfing, reduced tillering, or yield reductions to assess BYDV susceptibility. A combination of these effects may also be used to assess the pathological effects of BYDV. Making selections based upon these criteria works well if resistance is controlled by genes with large effects. The *Yd2* gene in barley (*Hordeum vulgare* L.) is the only major gene resistance found among barley, wheat (*Triticum aestivum* L.), and oat (*Avena sativa* L.) crops (Rasmusson and Schaller 1959; Qualset 1984).

Studies on BYDV resistance in oats have shown that heritable resistance is quantitative in nature and that additive genetic effects explain most of the genetic variability (Jedlinski et al. 1977; Landry et al. 1984; McKenzie et al. 1985; Gellner and Sechler 1986). Introgression of resistance genes from related species to cultivated oats may allow selection for greater resistance (Landry et al. 1984). Quantitative resistance involving many genes with small effects and primarily with additive gene action has also been reported in common wheat (Qualset et al. 1973; Topcu 1975; Cisar et al. 1982; Tola and Kronstad 1984). Several *Agropyron* species have shown high levels of resistance to BYDV (Sharma et al. 1984). The introgression of resistance genes from related *Agropyron* species to wheat may allow selection for greater resistance to BYDV or for reduced aphid feeding and BYDV inoculation (Shukle et al. 1987).

The quantitative nature of resistance to BYDV in oats and wheat makes visual selection for resistant lines difficult due to variation in symptom expression (Qualset 1984). Environmental effects, genotype-specific symptom expression, maturity differences, and differential host reactions to different BYDV types all contribute to the difficulty of selecting for resistance based on symptoms.

This variability in symptom expression has provided the impetus for producing other methodologies to study the host/pathogen relationship and to assess resistance or susceptibility. In one study, intraplant translocation of BYDV was measured, but no differences for restriction of virus movement could be identified in the cultivars studied (Carrigan et al. 1983). Restriction of virus replication or accumulation that leads to reduced viral concentration has also been studied. Symptomatic resistance is not

always directly related to a lower concentration of the virus, but for some cultivar/BYDV type combinations reduced BYDV concentration has been found (Skaria et al. 1985).

Methods used to measure BYDV concentration have included virus purification (Jedlinski et al. 1977), and more recently enzyme-linked immunosorbent assay (ELISA) has been used to compare BYDV antigen titer as an index of symptomatic resistance (Skaria et al. 1985). A combination of recent studies has shown that the Yd_2 gene of barley may be specific in conferring resistance to the PAV type of BYDV in both symptomatic expression (Baltenberger et al. 1987) and in reduction of virus content (Skaria et al. 1985).

A methodology has recently been developed for the detection of viral nucleic acids in crude plant sap by nucleic acid hybridization. This has proved to be a sensitive and rapid means of detection for potato virus X (Baulcombe et al. 1984). Recent cloning of complementary DNAs for the Australian RPV and PAV types of BYDV allows detection of these specific BYDV types by northern dot blot assay using ^{32}P labeled recombinant plasmid probes (Waterhouse et al. 1986). Combining this method with a dilution series of known plasmid DNA concentration and scintillation counting allows calculation of relative BYDV RNA concentrations for cultivars from crude plant extracts. The concentration of viral RNA can then be compared for different cultivars to determine which cultivars with symptomatic resistance also restrict the BYDV RNA concentration of the specific BYDV type. Progress is reported here in evaluating the use of cloned complementary BYDV DNA to assess virus concentration and possible use of that information in breeding for BYD resistant plants.

Materials and Methods

The six bread wheat cultivars used in this study and their expression of visual symptoms due to natural BYDV infection in the field at Davis, California are listed in Table 1. A completely randomized experiment with four replicates (4 plants for each cultivar) was conducted in a growth chamber to study the relative differences in PAV RNA concentration for the six cultivars. Plants were infected with the PAV New York isolate of BYDV by *Rhopalosiphum padi* (L.) at the 2-leaf stage and grown in a growth chamber (10-h 8.5°C nights and 14-h 22.2°C days). Whole shoots of cultivars were sampled 9 and 15 days after the end of transmission (DAT). Plant sap was extracted with a leaf roller and 0.5 ml sodium phosphate buffer (0.1 M, pH 7.2) and then clarified with 0.5 ml chloroform (Waterhouse et al. 1986). Samples of 0.050 ml were spotted on nitrocellulose with a Bio Rad dot blot apparatus. A number of subsamples were spotted on duplicate nitrocellulose sheets and placed in reserve. Final calculations included a factor for adjustment of concentration for shoots with fresh weights less than 1 g. Healthy plants for each cultivar were included as blanks.

Table 1. Six wheat cultivars and their expression of visual symptoms when subjected to natural infection of BYDV at Davis, California

Cultivar	Expression of visual symptoms	Mean visual score (1987) (0 to 9)[a]
NS 879/4	resistant	2.7
Quilamapu 23-7	resistant	2.6
CI 13232	moderate resistance	6.0
Anza	moderate resistance	5.2
Seri 82	susceptible	6.8
Yecora Rojo	susceptible	7.0

[a] BYDV scoring system as described by Qualset (1984).

F_2 plants and parents of the cross NS 879/4 x Seri 82 were infected at the 4-leaf stage and grown in the greenhouse using the methods mentioned above. Upper leaves of F_2 plants were sampled 21 DAT. Fresh tissue of 0.5 g was used for extraction with 0.3 ml phosphate buffer and 0.3 ml chloroform. Samples of 0.050 ml were spotted on nitrocellulose with 3 subsamples spotted for each plant. Five plants of each parent were infected to determine BYDV RNA concentration for the parent cultivars. Healthy parent and F_2 plants were included as blanks.

The pPA8 BYDV cDNA clone used in these experiments was supplied by Wayne Gerlach (CSIRO, Canberra, Australia). The pPA8 clone is a PuC8 vector-based plasmid containing an insert of approximately 1.9 Kb of complementary DNA from an Australian PAV serotype. Standard nick translation and hybridization protocols were followed to detect PAV RNA sequences on the membranes. Blots were first autoradiographed and then individual dots were cut out with a paper punch, placed in scintillation fluid, and specific radioactivity determined. Viral RNA concentration was determined by a regression equation from a dilution series of pPA8 DNA. Figure 1 shows an example of a dilution series with its autoradiograph and regression equation. The prediction equation was then used to calculate the concentration of RNA based on specific radioactivity (counts per minute) as determined by a scintillation counter.

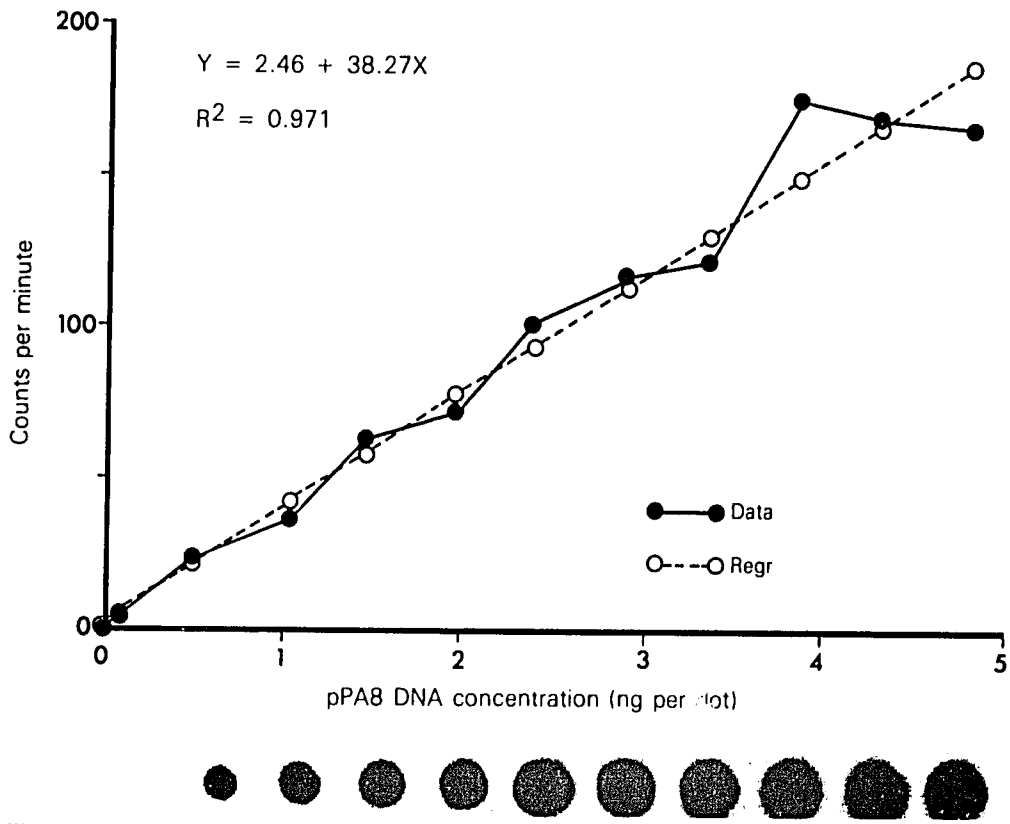

Figure 1. Autoradiograph of a standard dilution series of pPA8 DNA with a graph of the specific activity (counts per minute) for each concentration as determined by a scintillation counter. A regression line and its equation are included.

Results

An autoradiograph of samples from the first and second sampling dates for the six wheat cultivars is shown in Figure 2. Differences among cultivars were not readily visually apparent, but were statistically significant. Table 2 lists the mean PAV RNA concentration for each of the cultivars at each of the sampling dates. Seri 82 had a significantly higher concentration of viral RNA compared to the other five cultivars at 9 DAT. Samples taken 15 DAT showed a significantly higher concentration of viral RNA for Seri 82 compared to the more resistant cultivars (NS 879/4 and Quilamapu 23-7), but not compared to the other cultivars.

It should be kept in mind that these results apply only for the PAV type of BYDV. If a different BYDV type were to be used, the host response might be quite different. In this study NS 879/4 (resistant with regard to visual symptoms, Table 1) demonstrated the expected response of reduced PAV RNA concentration relative to Seri 82 (susceptible with regard to visual symptoms). An F_2 population from the cross NS 879/4 x Seri 82 was used to study the inheritance of this host response for reduced PAV RNA concentration.

Table 2. Mean detectable PAV RNA concentration for six wheat cultivars at two sampling dates determined by northern dot blot analysis. Each mean consists of four random plants sampled for each cultivar for each sampling date

Cultivars	Sampling date 9 DAT	15 DAT
	ng/g	ng/g
NS 879/4	4.40 b	1.37 b
Quilamapu 23-7	3.46 b	1.39 b
CI 13232	3.26 b	1.69 ab
Anza	4.82 b	1.58 ab
Seri 82	7.45 a	2.46 a
Yecora Rojo	3.41 b	1.57 ab

Means within columns marked with different letters are significantly different. The Waller-Duncan multiple comparison of means tests was used to determine statistical significance.

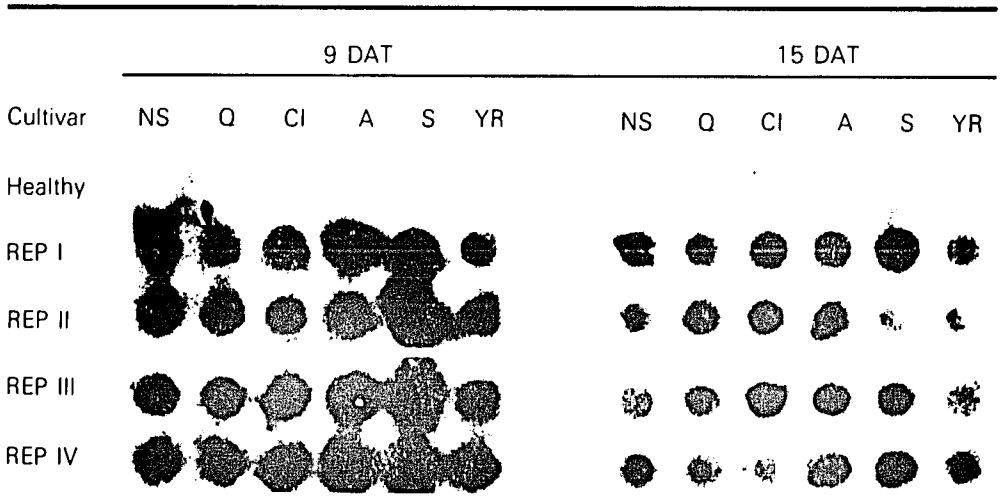

Figure 2. Autoradiograph of samples for the first and second sampling dates for six cultivars: NS 879/4 (NS), Quilamapu 23-7 (Q), CI 13232 (CI), Anza (A), Seri 82 (S), and Yecora Rojo (YR).

Frequency distributions of visual symptom scores for a different F_2 population of this same cross and parents subjected to field infection and visually scored in 1985 are shown in Figure 3. The F_2 mean is lower than the midparent value and is closer to the more resistant parent (NS 879/4) with regard to symptom expression. Figure 4 shows the F_2 distribution of 122 plants for detectable PAV RNA concentration. Though the distribution for these F_2 plants is similar in shape to those scored for visual symptoms (Figure 3), the relationship between the parental means and F_2 mean are quite different. In Figure 4 the F_2 mean is greater than the mean for the more susceptible parent (Seri 82).

The sample size for the parents may have been inadequate to give a good measure of BYDV RNA concentration in the greenhouse. The data from Table 2 indicate that the mean for Seri 82 should have been about twice that of NS 879/4, bringing it much closer to the mean for the F_2 plants. Some previous work shows that fluctuating temperatures in greenhouse conditions lead to greater variability in BYDV concentration compared to the more constant temperatures found in growth chambers (Skaria et al. 1985). These assays will be continued with F_3 progeny to study the inheritance of resistance as defined by reduced BYDV RNA concentration and to correlate these data with field symptom expression.

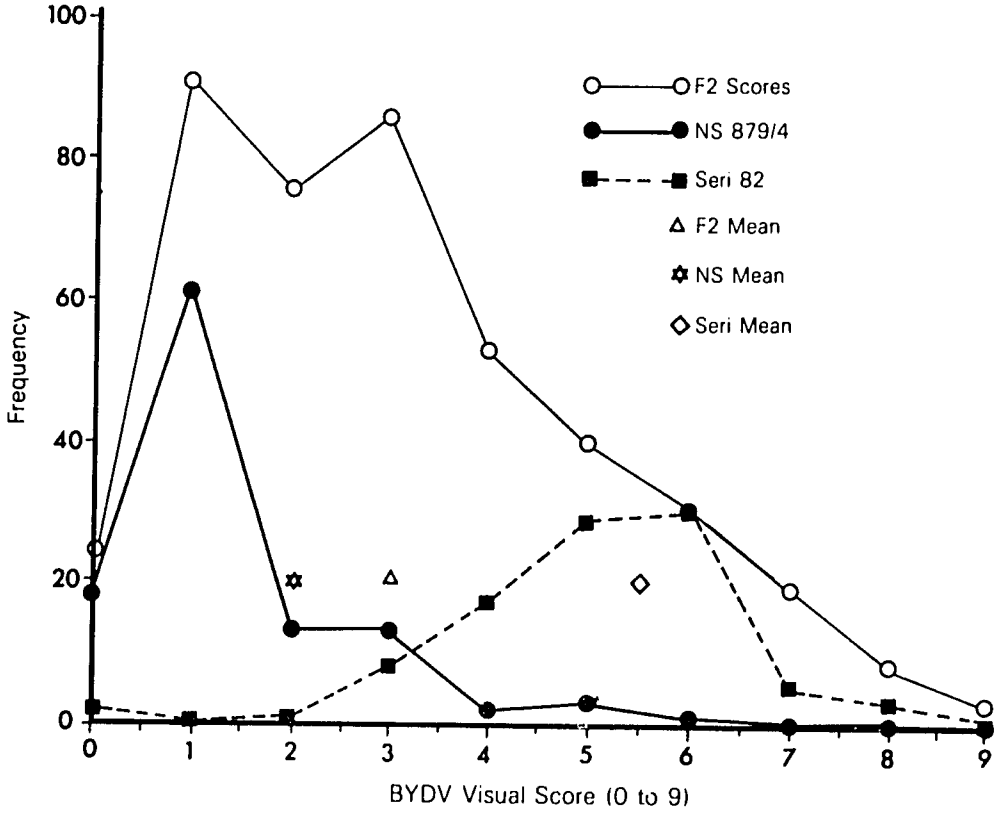

Figure 3. Frequency distributions and means of BYDV visual symptoms for an F_2 population from the cross NS 879/4 x Seri and for the respective parents.

Conclusions

Northern dot blot analysis allows sensitive determination of BYDV RNA concentrations in BYDV-infected plants. It can be used to measure relative differences among cultivars and it may be used nondestructively (only a few upper leaves sampled) on individual plants of segregating populations. A great advantage of this method is that resistance (reduced viral concentration) to a specific BYDV type can be identified. If this method correlates well with field resistance, as these preliminary data suggest, then breeding could be done for resistance to the most prevalent BYDV type in an area or resistances to different BYDV types could be recombined to develop cultivars with greater resistance to a number of BYDV types. Resistant lines could also be developed faster because several generations could be grown in the greenhouse. In the future, more sensitive measurements of BYDV RNA concentration should be possible, utilizing clones that contain greater amounts of BYDV cDNA and better hybridization techniques.

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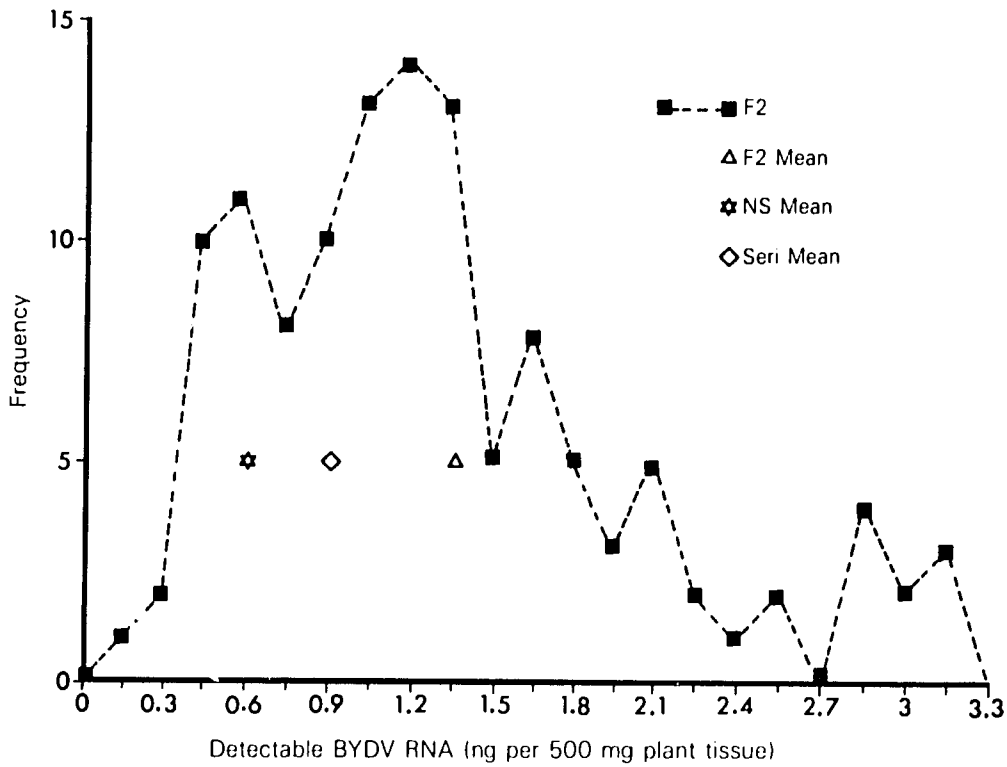

Figure 4. Frequency distribution and mean of detectable BYDV RNA and F_2 population from the cross NS 879/4 x Seri 82 and the mean for the respective parents. Five plants of each parent were used to determine the means for parental BYDV RNA concentration.

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Recovery of an Isolate of Barley Yellow Dwarf Virus from Barley in Northwest Italy

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Abstract

*An isolate of barley yellow dwarf virus (BYDV) was recovered from dwarfed and yellowed winter barley (*Hordeum vulgare *L.) in northwest Italy by serial transmissions with* Rhopalosiphum padi *(L.) to* Avena sativa *L. As indicators of the process of selection, three parameters were used: the proportion of plants infected by* R. padi *and* Sitobion avenae *(Fabricius) in consecutive transmission tests; the reactions of the infected plants of each transmission test with the sera against RPV- and MAV-like isolates; and the variability of the ELISA reactions within each group of plants tested. The isolate recovered was transmitted by* R. padi *and* S. avenae *with efficiencies of 92 and 11%, respectively. It also reacted consistently with the sera against the MAV isolate (relatively high absorbance values) and the RPV isolate (relatively lower values) in ELISA.*

Several isolates of barley yellow dwarf virus (BYDV) have been described from different parts of the world, on the basis either of their specific or nonspecific transmission by aphid vectors (Plumb 1974; Matsubara et al. 1985) or of their serological relationships (Hammond et al. 1983; Waterhouse and Helms 1985; Eweida 1986) to the isolates RPV, MAV, PAV, MRV (Rochow 1969), and SGV (Rochow 1979). In Italy the rice "giallume" isolate has been biologically characterized (Osler 1974) and classified as "PAV-like" (Belli et al. 1986), and the presence of a PAV-like isolate in barley (*Hordeum vulgare* L.) has been reported from northeast Italy (Osler et al. 1984).

In this work the isolation of a vector nonspecific isolate of BYDV from barley from northwest Italy is reported. The separation from the potential mixture in field samples was obtained by consecutive transmissions with the two aphid species, *Rhopalosiphum padi* (L.) and *Sitobion avenae* (Fabricius). The selection was monitored by enzyme-linked immunosorbent assay (ELISA) using sera against RPV- and MAV-like isolates.

Materials and Methods

Aphid rearing and virus transmission

Aphids of the two species *R. padi* and *S. avenae* were reared in insect cages on BYDV-free barley and wheat (*Triticum aestivum* L.) plants at 20 to 22°C in a controlled glasshouse.

The virus was first transmitted from field-collected barley with symptoms of yellowing and dwarfing to (*Avena byzantina* Koch) = *Avena sativa* L. using *R. padi* as vector.

Consecutive transmissions

The aphids were given an acquisition access period of 3 days on one infected plant, using the same plant as a virus source for both species. For transmission tests, aphids were individually caged for 3 days on pots containing one plant of *A. sativa* at the one-leaf stage. After a 3-day inoculation feed, the test plants were sprayed with insecticide and observed for symptoms in the glasshouse.

Preparation of antigens

After the appearance of BYDV symptoms, leaves of healthy and infected *A. sativa* were homogenized separately in phosphate-buffered saline containing 0.05% Tween-20 (PBS-T), using 2 ml of PBS-T for 1 g of plant material.

ELISA

ELISA was performed using the method of Clark and Adams (1977). The purified antibodies from sera against the RPV and MAV isolates and their corresponding alkaline phosphatase conjugates were kindly supplied by J.E. Duffus (USDA, Salinas, California). Microelisa plates were used. Absorbance was read at 405 nm in a MR 580 Dynatech Microelisa Autoreader; the reference well was filled with substrate solution only.

Only the 60 inner wells of each plate were used, the border rows being filled with water. One half of each plate was used to test samples with RPV antiserum, the other half to test the same samples against MAV antiserum. Duplicate wells were used for each test. Each plate contained duplicate samples of from 5 to 10 healthy *A. sativa* of the same age as the plant to be tested.

Data assessment and statistical analysis

The ELISA reactions of the infected material were evaluated from the ratio of the absorbance value of each sample over the geometric mean of the absorbance values of the healthy controls. The reactions with the two antisera produced two ratios: one relative to MAV antiserum (RMA) and one to RPV antiserum (RRP). Since these values are affected by the unknown relationships of the isolate to the two isolates against which the sera were produced, the ratio RMA/RRP was used as a relative measure of relationship.

The RMA/RRP ratios obtained for the plants of an aphid transmission were used to select the source plant for the next transmission. Generally plants with the lowest ratio were used.

Similar ratios (HMA and HRP) were calculated for the absorbance values of the healthy controls, dividing each value by the geometric mean value of healthy controls. The HMA/HRP ratio of each control was then calculated. These ratios have a theoretical mean of 1 and the variance of their distribution is a measure of the variability of the system.

The variances of the distributions of RMA/RRP and HMA/HRP were compared by means of the F test, after log transformation of the data, since the standard deviations were roughly proportional to the mean values (Armitage 1971). This transformation also corrected the asymmetry of the distribution of ratios.

Results

Aphid transmissions

After an initial increase in transmission efficiency of the isolate by *R. padi* and a parallel decrease in efficiency by *S. avenae*, the transmission rates with the two vectors used reached 92% with *R. padi* and 11% with *S. avenae*, and maintained similar proportions through eight consecutive transmissions in over 2 years (Figure 1).

The overall rates of transmission were 0.922 ($\pm$ 0.020) with *R. padi* (165 infected plants over 179 tested) and 0.115 ($\pm$ 0.024) with *S. avenae* (20 infected plants over 174 tested) (values in parenthesis are the standard errors of the proportions). Transmission test 11 was not included in the overall proportion since the rate of transmission was significantly lower ($P < 0.01$) than the overall rate of transmission. This low transmission rate was related to a very low survival rate of the aphids at the end of the virus acquisition period, for unknown reasons.

ELISA

The values of ratios RMA and RRP were highly variable, but there was an initial trend of reduction of RRP ratios down to a value of about 1 (i.e., reaction of infected plants with RPV-antiserum not clearly distinguishable from reactions of the healthy material), while the RMA ratios, although decreasing, were always high enough to permit discrimination of infected samples from healthy ones ($2 < RMA < 7$).

The results of ELISA expressed as a logarithm of the ratios RMA/RRP are summarized in Figure 2. During the first phase of selection (AT 1 to 7), the ratio RMA/RRPs were highly variable, and also depended on the vector used. From this point onward the value tended to a limit (about 0.4), independently of the vector used (see, for example AT 9 and 9a) and the selection of source plants with a low RMA/RRP ratio. An exception is represented by the AT 8, for which a different conjugate of MAV antiserum was used in ELISA.

The F tests for variance (Armitage 1971) gave nonsignificant results from AT 5 onward.

Discussion

After the initial variability, stable rates of transmission were obtained with the two vectors, *R. padi* and *S. avenae*. The rates of transmission obtained (92% with *R. padi* and 11% with *S. avenae*), compared to the rates obtained by Rochow (1969) with the same species of vectors, seem to exclude the presence of a single vector-specific isolate. Although both RPV and MAV isolates have very low transmission rates with *S. avenae* and *R. padi*, respectively (Rochow 1982a), these rates are considerably lower than those obtained with the isolate described here, hereafter called "Orzo Carmagnola" (OC) (orzo = barley). The transmission rates obtained seem also to exclude the presence of a mixed infection by two or more isolates, either vector-specific or not, since in this case the transmission efficiency of *S. avenae* would be higher (Rochow 1982a).

The ELISA results with the two antisera (RMA and RRP ratios) show that there is an appreciable reaction of the OC isolate only with the MAV antiserum. Our

Transmission number	Results of transmissions with the vector: *R. padi*	*S. avenae*	Transmission number
1	12/22		
2	22/33		
3	20/20	8/20	3a
4	11/20		
5	18/20	4/20	5a
6	20/20		
7	36/39	4/40	7a
8	17/20	0/12	8a
9	20/20	3/20	9a
10	17/20	3/20	10a
11	6/18	3/22	11a
12	37/40	3/40	12a
total (5 to 12)	165/179	20/174	
general rates of transmission and S.E.	0.922 (±0.020)	0.115 (±0.024)	

Figure 1. Results of consecutive transmissions from *Avena byzantina* to *A. byzantina* with the aphids *R. padi* and *S. avenae*. Solid lines connect consecutive transmissions. Results are expressed as number of plants infected over number of plants infested (one aphid per plant).

— Serial transmission 11 was not counted for the general rate of transmission, since its rate was significantly different from the general mean (see text).

— Figures in parentheses are the standard errors of the proportions.

results show that the range of ELISA values, when expressed as ratio of absorbance of infected samples over mean absorbance of healthy controls, are within the range of values reported by Lister and Rochow (1979) and by Rochow (1982b) testing the PAV isolate with anti-MAV globulins.

The use of the ratios of reactions of the same antigen to two antisera was established at the beginning of the selection to help the choice of the source plant for the next aphid transmission.

The stability of the variance of the RMA/RRP ratios and the fact that, after a few passages, it became not significantly different from the variance of the ratios of healthy controls, show that a certain homogeneity of the antigen has been reached.

The convergence of the mean value of ratios toward a definite value, notwithstanding the use as source for virus transmission of plants with low ratio values, shows that these low values were due to the variability of the system

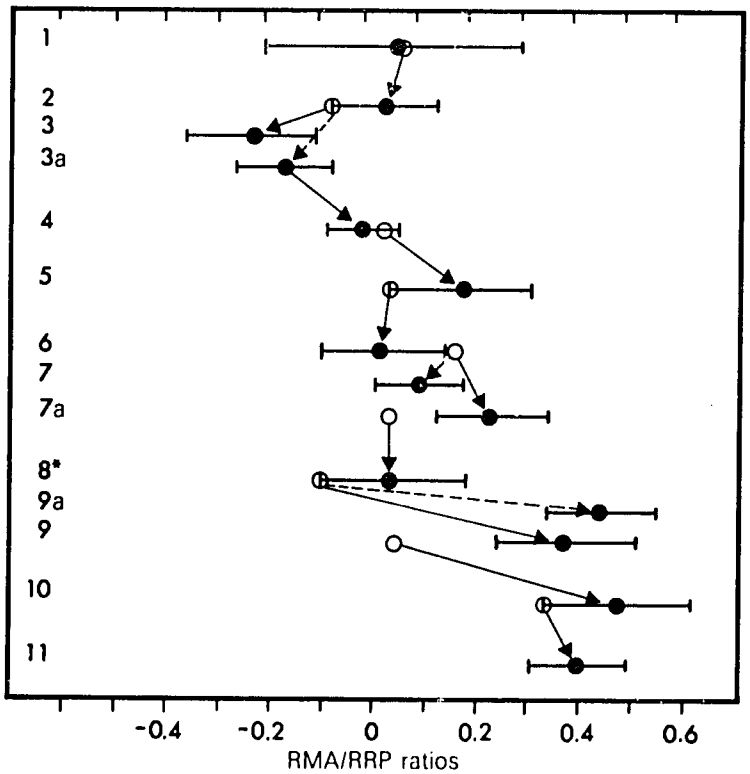

Figure 2. Mean values of the ratios RMA/RPP (expressed as logarithm) (x) and their standard deviations (horizontal lines) for the ELISA results of infected plants of consecutive aphid transmissions (numbers in column).

Solid lines indicate transmission with *R. padi*.

Broken lines indicate transmissions with *S. avenae*.

The symbol (o) indicates the value of RMA/RRP of the plant used as a source of virus for the next aphid transmission.

*** A different MAV conjugate was used for ELISA of plants of transmission 8.**

rather than to a mixed infection. In the absence of the homologous antigens and of the PAV antiserum, no quantitative meaning can be attached to the mean value of ratios obtained, except that there is some cross-reaction of the OC isolate with the MAV antiserum.

Although more biological characterization is needed, since only two of the common aphid species known to be vectors of different BYDV isolates have been tested, it is concluded that an isolate of the vector nonspecific type has been isolated. Based on the transmission rates, this isolate seems to differ from Rochow's PAV isolate and from the rice "giallume" isolated in Italy (Osler 1974), unless differences in vectoring efficiency among different aphid biotypes of the same species are involved (Swenson 1968).

Acknowledgment

This work was partially supported by CNR, Italy special grant I.P.R.A.-Subproject 2.

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The Relationship Between Barley Yellow Dwarf Virus Content in Aphids and Their Ability to Transmit

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Abstract

*Forecasting potential barley yellow dwarf virus (BYDV) outbreaks in the United Kingdom involves the daily trapping of cereal aphids and caging them individually on oat (*Avena sativa *L.) seedlings to estimate the percentage carrying virus. These plants cannot be diagnosed as infected much before 14 days after inoculation, and the direct testing of the aphids for virus presence might save this time and effort. Using an enzyme-amplified enzyme-linked immunosorbent assay (ELISA) technique, it was possible to detect an RPV-like BYDV isolate in single aphids and thus study the relationship between virus content in the aphid and its ability to transmit the virus to oat seedlings. Virus acquisition and retention in the aphid over time was also measured for the same RPV-like isolate comparing both efficient* Rhopalosiphum padi *(L.) and inefficient* Sitobion avenae *(Fabricius) vector aphid species.*

Methods used to forecast barley yellow dwarf virus (BYDV) outbreaks in England and Wales include the daily trapping of live cereal aphids and caging them on oat (*Avena sativa* L.) seedlings to estimate the percentage of infective aphids (Plumb et al. 1981). Symptoms and infection cannot be seen in these plants much earlier than 2 or 3 weeks after inoculations. Direct testing, by enzyme-linked immunosorbent assay (ELISA), of the aphids for virus offers the possibility of saving time and effort, enabling the forecast to be produced earlier. Among other advantages, direct testing does not need aphid-proof glasshouses and would allow many more aphids to be tested.

Detection of BYDV in single aphids was achieved by Paliwal (1982) using serologically specific electron microscopy (SSEM = ISEM) and by Torrance and Jones (1982) using an ELISA system with a fluorogenic substrate (MUP-ELISA). More recently, enzyme amplification has been used to increase the sensitivity of alkaline phosphatase-based ELISA systems to provide a sensitive assay also capable of detecting BYDV in single aphids, but not requiring an expensive fluorometric plate reader (Torrance 1987).

Paliwal (1982) and Torrance et al. (1986a) have studied the relationship between the presence of BYDV in single aphids and their ability to transmit the virus. The former study showed good correlation between the detection of three isolates of BYDV in the respective aphid species that transmit them efficiently and transmission by those aphids. However, an isolate efficiently transmitted by *Sitobion avenae* (Fabricius) was also detected in three inefficient vector aphid species. The second study compared the detection of BYDV in trapped migrant alatae using the MUP-ELISA with parallel transmission tests over 3 years. While numbers of autumn-trapped *Rhopalosiphum* spp. assessed as viruliferous by ELISA were similar to those transmitting virus, ten times more aphids (*Sitobion* spp.) trapped in spring and summer were assessed as viruliferous than actually transmitted.

This report summarizes the results of a further study of the relationship between BYDV content of single aphids, measured by enzyme-amplified ELISA, and their ability to transmit the virus.

Methods

An RPV-like BYDV isolate (R568), efficiently transmitted by *R. padi* (L.) and inefficiently transmitted by *S. avenae* was used. Healthy aphid clones were originally supplied by E.A. Lennon. Acquisition feeds were for 2 days on BYDV infected oats. Infection feeds were for 2 days on 8- to 10-day-old oat seedlings, cv Maris Tabard. Plants were tested by ELISA using the method of Torrance et al. (1986b). Aphids were tested for virus with the enzyme-

amplified ELISA essentially as described by Torrance (1987) modified by the addition of casein (3 g/liter) in the washing buffer. A polyclonal anti-BYDV antiserum kindly supplied by T.P. Pirone (Doupnik et al. 1982) was used to coat plates and an RPV-like specific monoclonal antibody, MAC 92 (Torrance et al. 1986b), was used as the second antibody in testing both plants and aphids.

Results

In the first set of experiments (Table 1), the ability of the two vector species to acquire and retain BYDV during a 2-day feed on healthy oats was compared with parallel transmission tests. Table 1 shows that both *R. padi* and *S. avenae* acquired the virus and that virus was still detectable in both aphid species after a 2-day feed. As expected, *R. padi* transmitted efficiently before and after the 2-day feed on healthy plants while transmission by *S. avenae* remained poor throughout. This ability to detect virus in aphids after a 2-day feed on healthy plants allowed tests of individual aphids after an infection feed and thus a comparison of the ELISA value for each aphid with its ability to transmit.

Table 2 shows the results of a second set of experiments, where individual *R. padi* were removed from numbered plants after 2-day infection feeds, immediately frozen and later tested for virus content. Only aphids that were alive and feeding at the time of removal were included. The correlation (Table 2) between detection of virus and transmission was very high (90%) even though there were great differences in virus content between individual aphids as assessed by absorbance at 492 nm (I. Barker and L. Torrance unpublished). Virus was also detected in ten individuals that failed to transmit, three of which had relatively high virus contents. Two other individuals transmitted virus but did not contain detectable amounts of virus.

Table 1. Comparison of the ability of two species of aphid to acquire and retain an RPV-like BYDV isolate

Aphid species	No. of aphids positive[a] by ELISA/no. tested	
	Tested immediately after acquisition feed	Tested after further two days on healthy oats
R. padi	38/40 (31/39)	35/40 (23/40)
S. avenae	27/40 (3/41)	34/41 (3/45)

Figures in parentheses are no. of aphids transmitting over no. tested for single aphids from the same experiment.

[a] Positive ELISA result taken as healthy mean + t(p=0.01). δ (for a one-tailed test).

Table 2. Comparison of the numbers of individual *R. padi* carrying virus as detected by ELISA with their ability to transmit RPV-like BYDV isolate to oats

Total no. of aphids tested	No. of aphids			
	Positive[a] by ELISA and transmitting	Negative by ELISA and not transmitting	Positive by ELISA and not transmitting	Negative by ELISA and transmitting
112 (100)	97 (87)	3 (3)	10 (9)	2 (2)

Combined results from three experiments. Figures in parentheses are percentages.

[a] Positive threshold as for Table 1.

Discussion

The enzyme amplified ELISA appears to be sensitive enough for the routine detection of an RPV-like BYDV isolate in individual aphids. The ability of *R. padi* to acquire BYDV isolates they transmit inefficiently, and *S. avenae* (Paliwal 1982), might partly explain the discrepancy between numbers of aphids positive by ELISA and parallel transmission tests reported by Torrance et al. (1986). A proportion of the spring and summer migrant *S. avenae* in that study might have been carrying detectable amounts of PAV-like isolates, which are relatively poorly transmitted by *S. avenae*.

Any further testing of aphids by ELISA for forecasting will require a knowledge of the relative efficiency of transmission of isolates by the different aphid species caught. This might enable a weighting system to be used when assessing their importance in forecasting the disease. However, as *R. padi* is largely responsible for the introduction and secondary spread of BYDV into United Kingdom cereal crops in the autumn, and because PAV- and RPV-like isolates (both efficiently transmitted by *R. padi*) are likely to be the predominant isolates transmitted, direct testing for PAV- and RPV-like isolates should be a feasible alternative to infectivity testing.

Direct testing of these *R. padi* would however prevent the separation of virginoparae from gynoparae, which have different roles in the epidemiology of the disease (Tatchell et al., in press). Strain-specific reagents such as the monoclonal antibody used in this study and others described by Torrance et al. (1986b) and M. Pead and L. Torrance (unpublished) would also be needed. Further work, using amplified ELISA to test field-caught aphids used in infectivity testing, is required.

Acknowledgments

The help of S.M.D. Forde, E.A. Lennon, R.A. Gutteridge, and the glasshouse staff of the Plant Pathology department, Rothamsted, is gratefully acknowledged. The financial support of the Overseas Development Administration for part of this work (IB) is also acknowledged.

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Kinetics of Barley Yellow Dwarf Infections in Maize

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Abstract

The kinetics of maize (Zea mays L.) infection by barley yellow dwarf virus (BYDV) was studied in a glasshouse. Young plants (5 to 7 leaves) were infested with viruliferous Rhopalosiphum padi *(L.) carrying a PAV isolate of BYDV. Sampling for enzyme-linked immunosorbent assay (ELISA) was carried out at a range of times after inoculation. Ten days after inoculation the optical density (OD) values obtained with ELISA were high in the roots (OD = .750) and very low in the leaves (OD = .090). Later (30 or 40 days), the OD values were high both in the roots and in the upper leaves. After 40 days, the OD values decreased in the whole plant. Another experiment showed that the aphids can acquire the virus from these plants for a long time after inoculation (70 days).*

Maize (*Zea mays* L.) is a widespread cultivated plant in the world. It is considered as a natural host for barley yellow dwarf virus (BYDV) by Stoner (1977) and Brown et al. (1983).

In France, areas planted with maize have increased considerably during the past 20 years. Furthermore, at the end of the summer, when all the other cereals have been harvested, maize is still available to aphids.

In 1985, a preliminary assessment of BYDV infection in maize under field conditions showed that this plant is a good reservoir of the virus in the west of France. In summer, aphids colonized the maize crops. In late September, large populations of *Rhopalosiphum padi* (L.) developed in the fields (more than 1000 aphids per plant), which resulted in BYDV infection being common (22% of the plants tested were infected) (Henry et al., in press). Assessment of BYDV infection levels requires simple, rapid, easily operated, and accurate sampling methods. Since it is impossible to test an entire plant because of its size, it is necessary to sample the part of the plant where virus is most likely to be easily detected. The distribution of the virus in maize was investigated: 1) to optimize the sampling, and 2) to compare this distribution to that of the aphids on the plant.

Methods

Maize (cv Dea) was grown in a glasshouse at a temperature of 24 to 30°C. The BYDV strain used was a PAV-like isolate from near Rennes.

Inoculation

Ten viruliferous aphids (*R. padi*) were transferred from infected wheat to maize seedlings (5- to 7-leaf stage). Plants were sprayed with an insecticide 5 days later. The inoculated plants were grown in the glasshouse until harvest. Five maize plants were sampled at 10-day intervals after inoculation, and subsamples of root and each individual leaf were assayed for BYDV by enzyme-linked immunosorbent assay (ELISA).

Back-transmission tests

Twenty non-viruliferous *R. padi* were allowed a 24-hour acquisition period on infected maize leaves. Then they were transferred to barley (*Hordeum vulgare* L.) seedlings (cv Capri) and killed after 48 hours. The test plants were collected 10 days later and tested by ELISA.

ELISA test

The ELISA technique used here was as described by Clark and Adams (1977). Antibodies (IgG) were provided by BIOREBA (Switzerland). The use of ELISA on maize had to be modified

because the leaves of young healthy plants (4 to 5 leaves) gave a strong reaction in ELISA-optical density (OD) > .900 (Table 1). To reduce this background, it was necessary to replace PBS-T-PVP by another extraction buffer (.06 M Phosphate + 1% milk powder). This buffer reduced background reactions from young leaves but did not lower OD values for infected plants (Table 1). Such high background reactions, which did not show with older plants (more than 6 leaves), are probably due to young plant tissues reacting to the antibodies. This modified extraction buffer was used in all assessment tests.

Results and Discussion

Kinetics of virus multiplication

Twelve days after inoculation, virus was only detected in the roots and younger leaves (mainly leaves 11 and 12). The OD values were higher in roots, possibly due to a more rapid multiplication of virus. Thereafter, virus migrated toward younger leaves, and after 20 days it was found in the whole plant. The OD values were highest 30 days after inoculation. Forty days after inoculation (panicle showing well), they began to decrease and went down to very low values 70 days after inoculation. This latter period corresponded to the maize growing more slowly. Thus it seems obvious that virus multiplication correlated well with the maize growth rate. Virus was recovered from leaves emerged after inoculation, showing a systemic infection as described by Stoner (1977).

Acquisition of BYDV from maize

Back-transmission experiments were performed from previously inoculated maize (Table 2). Aphids acquired BYDV from maize anytime after inoculation, even after 70 days, when OD values were very low.

Table 2. Transmission tests of *R. padi* fed on BYDV infected maize

Time since inoculation	Transmission tests [a] Total	Positive	Percentage of positive tests
20 days	32	5	15.6%
30 days	40	22	55.0%
40 days	17	5	29.4%
50 days	24	11	45.8%
60 days	33	10	30.3%
70 days	18	15	83.3%

[a] Aphids were fed on leaf 10, 20 days after inoculation and on leaf 13-14 for the other tests.

Table 1. Comparison of two extraction buffers read at A405 nm for ELISA for barley yellow dwarf virus

Samples	Plant growth stage	PBS-T-PVP buffer (pH = 7.4)	Phosphate buffer (pH = 7.2)
Healthy maize	4 leaves	.557	.004
Healthy maize	4-5 leaves	.924	.005
Healthy maize	6 leaves	.012	.002
Healthy maize	7 leaves	.014	.005
Infected maize	8 leaves	1.099	.993
roots			
leaves		.284	.035

Values above 0.1 were considered positive.

Conclusions

This study showed the variation of BYDV in maize tissue over time. The OD values were highest in leaves 11 and 12 (below and above the cob, respectively), and virus assessment was done from either of these leaves. However, since the temperature range, plant growth stage at inoculation, cultivar, and isolate of BYDV were not investigated, these results may be specific to this study.

Back-transmission tests performed with aphids reared on BYDV-infected maize plants showed that they could acquire the virus from these plants for the life of the inoculated plant, and transmit it to barley seedlings. This means that in the field in October BYDV could be readily transmitted to young cereal seedlings by aphids flying from infected maize plants.

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Transmission Characteristics of a Nonspecific Isolate of Barley Yellow Dwarf Virus Isolated from Maize

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Abstract

Serial transmission experiments on Avena sativa *L. were carried out with a PAV-like isolate of barley yellow dwarf virus (BYDV) using* Rhopalosiphum padi *(L.),* Metopolophium dirhodum (Walker), and Sitobion avenae *(Fabricius). Data are reported on the length of the median latent period (LP_{50}), the highest daily transmission rates, the percentage of individuals transmitting the virus at least once during the serial passages, the total transmission rates of the inoculative vectors tested, the weighted retention periods of infectivity, and the incubation periods of the disease in* A. byzantina. *The transmission curves are compared with the longevity and the rate of reproduction of the three aphid species. BYD damage was greater when* A. sativa *was inoculated by* R. padi *than when it was inoculated by* M. dirhodum *or* S. avenae. *All three species of aphids gradually lost their infectivity during successive serial transmissions. Their inoculative potential was restored when they reacquired the virus from suitable sources of inoculum (recharging).*

Barley yellow dwarf virus (BYDV) is a luteovirus transmitted in a persistent, circulative manner by its aphid vectors (Rochow 1959, 1963; Mueller and Rochow 1961; Paliwal and Sinha 1970; Van der Brook and Gill 1980). Transmission of BYDV has been investigated by various authors (Rochow 1959, 1963; Watson and Mulligan 1960; Gill 1970; Paliwal and Sinha 1970; Van der Brook and Gill 1980; Guang He Zhou and Rochow 1984).

The present research was undertaken to characterize the transmission of a nonspecific isolate of BYDV using three different aphid species as vectors.

The transmission characteristics of BYDV were compared with the fecundity and longevity of the three species of aphids and with the symptoms expressed by the test plants. This paper reports the median latent period (LP_{50}), defined as the time required for 50% of an aphid test population to transmit virus; the mean weighted retention period of infectivity; the daily and the total transmission rates of the three aphid species; the longevity, rates of reproduction, and "recharging" capacities of the three aphid species; the mean incubation period of the disease in the plant; and the damage to the plant due to the three different aphid species tested.

The terms used by Sylvester and Richardson (1966) and Sylvester and Osler (1977) have been adopted.

Materials and Methods

The variant of BYDV nonspecifically transmitted by *Rhopalosiphum padi* (L.), *Sitobion avenae* (Fabricius), and *Metopolophium dirhodum* (Walker), originally derived from maize and referred to as maize (M)-PAV (Osler et al. 1985), was used.

Virus-free aphids were propagated on caged oats (*Avena (byzantina)* = *sativa* L.), at 20°C. Nymphs of the same age (average 48 hours) were obtained by allowing maternal apterae to produce nymphs on successive sets of oat plants for periods of 24 hours.

Virus sources were symptomatic oat plants inoculated 15 to 20 days previously by *R. padi*. Test plants were oat plants at the one-leaf stage, transplanted into pots at least 2 days before inoculation.

The 12-hour acquisition feed was on virus infected detached oat leaves in petri dishes, in a growth chamber at 20°C with constant light. Single aphids were then transferred to individually caged oat seedlings in a growth chamber at 20°C at intervals of 12 hours (1st transfer), 24 hours (2nd to 25th transfer) and then at 48-hour intervals until death.

The experiment was started with 60, 60, and 66 individuals of *R. padi*, *M. dirhodum* and *S. avenae*, respectively.

After the removal of the aphids, the plants were routinely treated with insecticide and kept in a greenhouse at approximately 20°C until the development of symptoms. Daily records were taken of virus transmission, production of nymphs, longevity of the aphids, and symptom expression of the test plants. These data were then analyzed statistically according to Sylvester and Richardson (1966), Sylvester (1967), and Sylvester and Osler (1977) for circulative aphid transmitted viruses.

To demonstrate the possibility of restoring the rate of transmission of BYDV in a population of aphids whose inoculative potential had declined (recharging), a second serial transmission experiment was carried out.

On the basis of a preliminary experiment, it was determined that the rate of transmission of the aphid species started to decline consistently 6 to 7 days following acquisition.

At the 7th transfer, the populations of the three aphid species were split into two groups. Half continued to be serially transferred to new test plants while the remainder were allowed a second access period of 12 hours on BYDV-infected oat plants and then serially transferred as above, until their death.

Results

The results obtained for the retention of infectivity, fertility, and survival of the aphid vectors are shown in Figure 1.

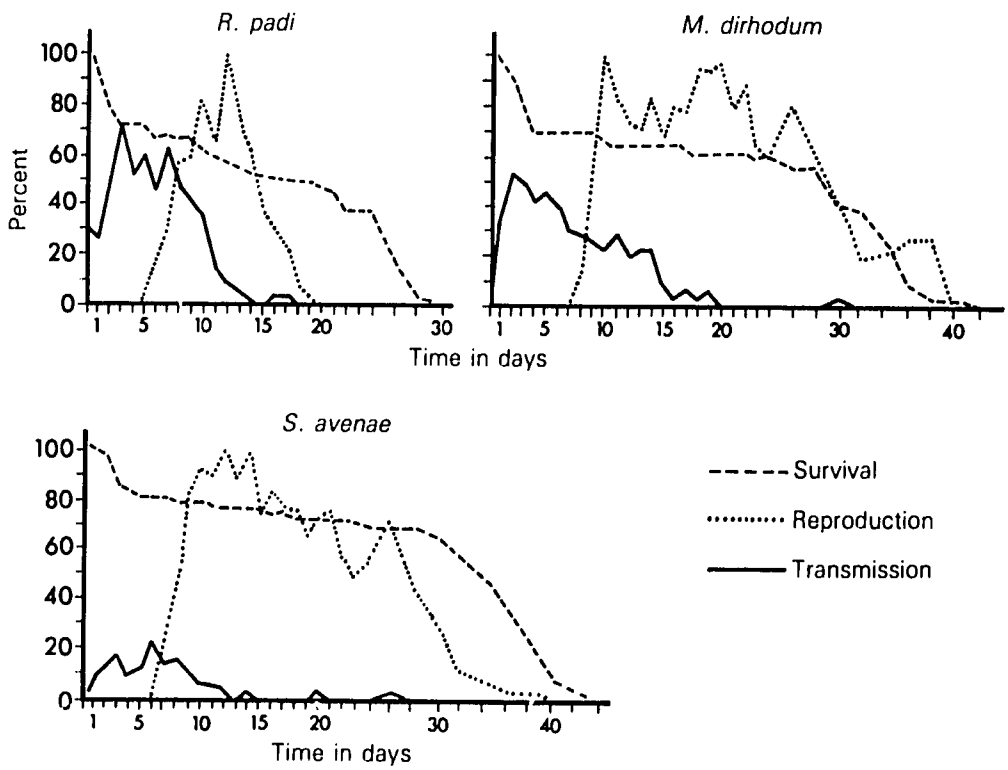

Figure 1. Survival, reproduction, and barley yellow dwarf virus transmission curves obtained at 20°C with the aphids *R. padi*, *M. dirhodum*, and *S. avenae*.

The highest daily transmission rates were 73, 53, and 23%, for *R. padi*, *M. dirhodum*, and *S. avenae*, respectively (calculated on the maximum number of transmissions/day divided by the inoculative vectors tested), and took place during the 4th, 3rd and 7th passage (= 3.5, 2.5, and 6.5 days after acquisition). The total percentages of inoculative aphids (= aphids that transmitted the virus at least once during the serial transfers) were 82, 79, and 53, respectively.

The mean transmission rates (based on the total number of plants infected out of those individually infested during all the serial transfers) were 27, 19, and 5%. The mean weighted retention periods of infectivity were 8.6, 9.7, and 8.0 days, while the maximum retention periods were 18, 32, and 28 days.

The average numbers of nymphs for each adult female *R. padi*, *M. dirhodum*, and *S. avenae* were 47, 51, and 54, respectively. The mean incubation periods of the disease in the plants were 13.2, 15.7, and 17.0 days when inoculated by *R. padi*, *M. dirhodum*, and *S. avenae*. The mean fresh weight of the oat plants inoculated with BYDV by *R. padi* was lower (2.05 g) than that of plants inoculated by *M. dirhodum* (2.31 g), *S. avenae* (2.66 g), or the control (5.5 g).

In the "recharging" experiment, it was demonstrated that *R. padi*, *M. dirhodum*, and *S. avenae* reached their maximum transmission rates of 39, 35, and 51% in 3, 5, and 5 days after the end of the first acquisition access. Six days after acquisition, the probability of transmission by the three aphid species declined to 0.20, 0.13, and 0.06.

After a further acquisition feed on virus-infected plants, all three aphid species had their inoculative potential restored. For *R. padi* and *M. dirhodum*, the second maximum transmission rate was even higher than after the first virus acquisition.

A measurable latent period occurred after the first acquisition feed as well as after the second one.

The retention and the net transmission rates (no. of plants infected per aphid) were higher for the "recharged" aphids than for the aphids that acquired the virus only once.

The latent periods of BYDV in the three species of aphid vectors were compared. The median latent periods of the virus calculated for *R. padi*, *M. dirhodum*, and *S. avenae* were 16, 25, and 66 hours, respectively.

Discussion

The data presented confirmed that the most efficient aphid species in transmitting this isolate of BYDV is *R. padi* followed by *M. dirhodum* and then *S. avenae*.

The present results also confirm that the isolate of BYDV from maize is nonspecific, similar to the PAV-like reported by Rochow and Duffus (1981) and "B" by Plumb (1974).

The LP_{50} of BYDV in *R. padi* was found to be 9 and 50 hours shorter than in *M. dirhodum* and *S. avenae*, respectively.

Van der Brook and Gill (1980) reported a longer LP_{50} in a nonspecific strain of BYDV transmitted by *R. padi* than the one described here. As they mention, the LP_{50} may be affected by several factors, such as the use of different aphid clones, the age of the vectors, the particular virus isolate, and the environmental conditions.

It is interesting to note the length of the LP_{50} is inversely correlated to the efficiency of the vector and the incubation period of the disease in the plant. For example, the latent period was shortest in *R. padi*, the most efficient vector, and longest in *S. avenae*, the least efficient one; the shortest incubation period of the disease in the plant and the most serious damage are induced by the most efficient vector, *R. padi*. The opposite is true for *S. avenae*, while *M. dirhodum* occupies an intermediate position.

Does this depend on dosage effect? Sylvester et al. (1970) demonstrated a similar correlation between the LP_{50} and

the probability of infectivity of an aphid and the relative amount of sowthistle yellow vein virus used as inoculum.

The maximum retention period of infectivity seems to be correlated with the longevity and with the vitality (i.e., reproductivity) of the aphid species. In the present investigation, the BYDV infectivity persisted longer in *M. dirhodum* than in *R. padi*. In fact, the survival of *R. padi* was short, and it aged quickly.

The positive "recharging" of infectivity demonstrated with BYDV in all three aphid species indicates that the phenomenon is correlated with the virus and is probably independent of the aphids used. Besides BYD, other examples of "rechargeable" viruses are known (Sylvester and Osler 1977; Sylvester and Richardson 1966). It would appear that infectivity restoration is a common characteristic of circulative viruses.

Acknowledgments
The authors thank Giacinto Valdevit and Alberto Loschi for their technical assistance.

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Studies of Swedish Barley Yellow Dwarf Virus Isolates Using Cloned cDNA Probes

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Abstract

Genome fragments of a barley yellow dwarf virus (BYDV) MAV serotype were cloned in plasmid pUC19. Clones that hybridized with only MAV serotypes as well as clones that hybridized with both MAV and PAV serotypes were produced. The ^{32}P-labeled probes readily detected BYDV in plant sap and in extracts from single viruliferous aphids.

Materials and Methods

RNA from an isolate specifically transmitted by *Sitobion avenae* (Fabricius) and serologically of the MAV type (Eweida and Oxelfelt 1985) was used as template for synthesizing cDNA by random priming (Maniatis et al. 1982). Second strand synthesis was carried out with ribonuclease H and DNA polymerase I according to Gubler and Hoffman (1983). After methylation and addition of T4 DNA polymerase, the product was ligated to linkers (Carlsson et al. 1985) and inserted at the EcoRI-site of plasmid pUC19. The plasmid was then used to transform *E. coli* strain Jm83 (Vieira and Messing 1982) and plated on X-gal substrate. White colonies were propagated, and among them we isolated plasmids containing sequences homologous to BYDV RNA. Four, designated A16, C10, C12, and C22, were used in this study. Two clones, pBY 82 (PAV-specific), and pBY 412 (RPV-specific), kindly supplied by P. Waterhouse (Waterhouse et al. 1986) were also used. The ^{32}P-labeled plasmid probes were prepared by nick translation (Arrand 1985).

Results and Discussion

In filter hybridization tests A16 was found to be specific for the homologous MAV serotype RNA, whereas C10, C12, and C22 hybridized equally well with the RNAs of the MAV and PAV serotypes. The pBY 82 hybridized strongly with the MAV serotype, but not with the RNA of the Swedish PAV serotype used in the study. The insert of pBY 82 hybridized strongly with a probe prepared from C22. The pBY 412 did not hybridize with any of the isolates tested.

None of the probes hybridized with TMV RNA or with total nucleic acids from healthy oats.

Nick translated clone C10 was used to probe filters onto which had been applied partially purified PAV and MAV serotypes, RNA from the same serotypes, sap from oats infected with PAV and MAV serotypes, and extracts from *Rhopalosiphum padi* (L.) and *Sitobion avenae* (Fabricius) viruliferous with PAV and MAV, respectively. The sap was diluted 1:1 with buffer and 10 μl applied to the filter. The aphid extracts were prepared by grinding 10 aphids in 500 μl buffer; 10 μl was blotted onto the filter, thus each dot corresponded to one fifth of an aphid. The probe hybridized very strongly in all cases.

Among cDNA clones prepared from RNA of an MAV serotype, some are MAV serotype-specific, whereas others hybridize with both PAV and MAV serotypes in agreement with the findings of Waterhouse et al. (1986). Somewhat surprising is the finding that the clone pBY 82, specific for Australian PAV, failed to hybridize with the Swedish PAV serotype, although it did hybridize with the MAV serotype.

The cDNA probes readily detected BYDV in plant sap and in extracts of single aphids.

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Barley Yellow Dwarf Virus RNA Has a Protein Covalently Linked to Its 5' Terminus

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Abstract

The RNA of an Illinois isolate of barley yellow dwarf virus (BYDV) transmitted specifically by Rhopalosiphum padi *(L.) was examined to determine the status of its 5' terminus. Initial studies were to determine if the 5' terminus of BYDV RNA was blocked, i.e., contained any derivatization other than phosphate(s). BYDV RNA, satellite tobacco necrosis virus RNA (STNV RNA, positive control) and tobacco mosaic virus RNA (TMV RNA, negative control) were treated with calf intestinal phosphatase, then T4 polynucleotide kinase and [γ-^{32}P] ATP were added. The 5' terminus of STNV RNA was consistently labeled with ^{32}P, however, neither BYDV RNA nor TMV RNA showed any sign of terminal labeling. We then attempted to determine if BYDV RNA contained a protein covalently linked to its 5' terminus (VPg). We therefore labeled BYDV RNA, cowpea mosaic virus RNA (CPMV RNA, positive control) and TMV RNA (negative control) with ^{125}I using the ^{125}I-Bolton-Hunter reagent. Precipitation of these reactions with 5% trichloroacetic acid showed that the CPMV RNA and BYDV RNA were labeled with ^{125}I, but the negative control TMV RNA was not. The extent of ^{125}I label in the treated BYDV RNA and CPMV RNA was reduced following treatment with proteases. Gel analyses of the ^{125}I labeled materials detected CPMV RNA VPg M_r 5 kDa and an apparent VPg of M_r 17 kDa from BYDV RNA, as well as the appropriate viral coat proteins.*

Barley yellow dwarf virus (BYDV), a member of the luteovirus group of plant viruses, contains single-stranded RNA with an estimated molecular weight of 2.0 MDa and a single coat protein component of 24.45 kDa. Little has been done to define the chemical properties of BYDV. Here we report that an Illinois isolate of BYDV transmitted specifically by *Rhopalosiphum padi* L. has a 17 kDa virion protein-genome linked (VPg) covalently attached to the 5' terminus of its RNA.

Materials and Methods

BYDV RNA (75μg) and satellite tobacco necrosis virus (STNV) RNA (16μg) were dephosphorylated according to the method of Donis-Keller et al. (1977) using 1 unit of calf intestinal phosphatase and incubated at 37°C for 20 min. The phosphatase reaction was stopped by a single phenol extraction and the RNAs were precipitated by 2 vol of cold absolute ethanol and 3.0 M sodium acetate (NaAc), pH 6.0, added to a final concentration of 0.1 M. The resultant pellet was dried under vacuum, re-suspended in sterile water and incubated at 55°C for 3 min before being rapidly cooled on ice (Leung et al. 1979). The dephosphorylated 5' terminus of BYDV RNA and STNV RNA was labeled with ^{32}P (Maniatis et al. 1982) using 10 units of T_4 polynucleotide kinase, 1μM "cold" ATP, 150μCi [γ-^{32}P] ATP (sp. act. 2000 Ci/mmole) and incubated at 37°C for 20 min. The reaction was stopped by a single phenol extraction. Samples were analyzed by gel electrophoresis using 1.75% agarose with a 40 mM Tris-acetate, 2 mM EDTA buffer system and then visualized by ethidium bromide staining and autoradiography.

Cowpea mosaic virus (CPMV) RNA (64μg), tobacco mosaic virus (TMV) RNA (75μg), BYDV RNA (75μg), and BYDV coat protein (20μg) were treated with ^{125}I-Bolton-Hunter reagent according to Harris et al. (1978). The resultant ^{125}I-labeled materials were separated from unbound ^{125}I-Bolton-Hunter reagent by gel filtration through Sephadex G25 and precipitation with 2 vol of cold absolute ethanol (viral RNA) or acetone (BYDV coat protein). The amount of ^{125}I-labeling was determined by acid precipitation of ^{125}I-labeled viral RNA with 5% TCA after digestion with RNases A and T1 (5μg) at 37°C for 3 hours or pronase at a final concentration of 100μg/ml at 37°C for 3

hours. Ribosomal RNA (30μg) and bovine serum albumin (30μg) were added as carriers, and each sample was collected on a millipore filter. Radioactivity was quantitated using a Beckman Gamma 8000 counter.

The ^{125}I-labeled BYDV coat protein and RNase-digested RNA were incubated overnight at 4°C with 20μg of immunoglobulin (Ig) made to BYDV coat protein. The Ig-protein complex was precipitated by protein A-Sepharose. The mixture was centrifuged 1 min (Microfuge) and proteins in the supernatant were precipitated with 2 vol of cold acetone. The radiolabeled proteins in the protein A-Sepharose-Ig complex were dissociated from the Ig by heating in dissociation buffer (Laemmli 1970). The ^{125}I-labeled materials were analyzed in all experiments by SDS-polyacrylamide gel electrophoresis (Laemmli 1970) with a 3% stacking gel and an 18% resolving gel and visualized by autoradiography.

After dephosphorylation and ^{32}P labeling with T_4 polynucleotide kinase and [γ-^{32}P] APT, BYDV RNA and STNV RNA migrate as single major bands when subjected to agarose gel electrophoresis. Autoradiography shows that the 5' terminus of STNV RNA is radiolabeled with ^{32}P; however, no radiolabeling is associated with BYDV RNA. This establishes that the 5' terminus of BYDV RNA is some derivative other than phosphate(s).

The presence of acid precipitable ^{125}I labeling is observed for BYDV RNA and CPMV RNA; however, little ^{125}I-labeling is associated with TMV RNA (Table 1). The ^{125}I-labeling associated with BYDV RNA and CPMV RNA is reduced upon treatment with pronase, indicating the radiolabeled material is protein.

The nature of this ^{125}I-labeled material was further characterized by SDS-polyacrylamide gel electrophoresis. The radiolabeled materials released from RNase-digested ^{125}I-labeled CPMV RNA migrate as protein components of approximately 5 kDa, the reported value for CPMV VPg (Daubert et al. 1978), and approximately 25 and 45 kDa, the M_r of CPMV coat protein expected as contaminants of CPMV RNA. All these radiolabeled materials disappear when these samples are also treated with pronase. The ^{125}I-labeled material associated with BYDV RNA, released after RNase treatment, migrates as a single major band of approximately 17 kDa, which is destroyed upon treatment with pronase. Therefore, BYDV RNA contains an apparent 5' terminal VPg with an M_r 17 kDa.

Table 1. Precipitation of ^{125}I-labeled-BYDV RNA, -CPMV RNA, and -TMV RNA after treatment with ribonucleases A and T1 and pronase

Treatment	TCA-precipitable[a] radioactivity (cpm)	% control
BYDV control	5690	100
BYDV + RNases	3493	58
BYDV + pronase	1609	27
CPMV control	1290	100
CPMV + RNases	7394	57
CPMV + pronase	249	2
TMV control	273	100
TMV + RNases	230	84
TMV + pronase	98	35

CPMV RNA = positive control for 5' terminal protein, TMV RNA = negative control for 5' terminal protein. Treatment was with RNase A (5 μg), RNase T1 (5 μg) and pronase (100 μg/ml) at 37°C for 3 hr.

[a] Precipitation using 5% TCA with the addition or ribosomal RNA (30 μg) and bovine serum albumin (30 μg) as carriers.

The ^{125}I-labeled 17 kDa protein was shown to be distinct from BYDV coat protein by immunoprecipitation assays. The antibody to BYDV coat protein precipitates ^{125}I-labeled BYDV coat protein but does not precipitate the ^{125}I-labeled 17 kDa protein. This establishes that the 17 kDa protein attached to BYDV RNA is not BYDV coat protein.

This study establishes that BYDV RNA from an isolate transmitted specifically by *R.padi* contains an apparent 17 kDa VPg. Another member of the luteovirus group, potato leafroll virus, has been shown to have a 7 kDa VPg (Mayo et al. 1982). This is the first evidence for the presence of a VPg associated with any of the BYDV strains. The nature of the 5' terminus, i.e., capped or protein-linked, of viral RNAs from viruses within a given plant virus group appears to be similar (van Vloten-Doting and Neeleman 1982). The nature of the 5' terminal structure may be consistent; however, in the case of the 5' terminal protein, the size may vary a great deal. This has been shown with the potyviruses—tobacco etch virus (6 kDa) (Hari 1981) and tobacco vein mottling virus (24 kDa) (Siaw et al. 1985)—and now with the luteoviruses.

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Stability of ELISA Activity in Frozen Samples of Barley Yellow Dwarf Virus-Infected Leaf and Leaf Extracts

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Abstract

*When replicate samples of barley yellow dwarf virus-infected intact oat (*Avena sativa *L.) leaf, leaf extracts in phosphate buffer, or chloroform-clarified leaf extracts, were stored frozen at -20°C, -80°C, or in liquid nitrogen, their enzyme-linked immunosorbent assay (ELISA) activities fluctuated over time and according to the virus isolate involved. With the Purdue (P)-PAV isolate, no significant decrease of ELISA activity was noted with leaf, crude leaf extracts, or clarified extracts stored for up to 4 weeks at -20°C, -80°C, or in liquid nitrogen, except that clarified extracts stored at -20°C deteriorated after 1 week. When tested after 8 weeks, however, ELISA activity was reduced to values that remained similar thereafter for up to 14 weeks, the longest period of storage tested. With the MAV and RPV isolates, significant deterioration in ELISA activity was noted in most samples after 4 weeks of storage, after which ELISA activity appeared to stabilize, although in some cases an increase in activity was observed in samples stored for longer periods. Again, deterioration was most evident after storage of clarified extracts at -20°C, which was overall the least effective treatment for preserving ELISA activity.*

It is often necessary to store samples for enzyme-linked immunosorbent assay (ELISA) so that comparative tests can be done when convenient. Among the methods available for such sample storage, freezing—as tissue, tissue extracts, or as partially purified preparations—is understandably the one most commonly used for virus preservation. For serological assays in general, including ELISA, it is necessary that the virus capsid remain either unaltered during storage, or be maintained in a form that still reacts with the detecting antiserum. However, the stability of ELISA reactivity during storage may vary according to virus and storage conditions. For example, the ELISA activity of extracts of *Prunus* spp. leaves infected with plum pox virus decreased after just 24 hours at -14°C, but no further decline had occurred after 4 months at -14°C (Adams 1978). Also, when potato (*Solanum tuberosum* L.) and tobacco (*Nicotiana tabacum* L.) leaves infected with potato leafroll virus, or potato viruses A, S, X, or Y, were stored at 25, 4, -20, or -70°C, variable infectivity and ELISA values were obtained (Singh and Somerville 1983). When infected leaves or sap was frozen at -20°C, ELISA values were lowered for all viruses tested except potato virus X. Refrigeration of leaves at 4°C, or freezing at -70°C, preserved the infectivity and serological activity during the storage period tested (12 days).

For barley yellow dwarf virus (BYDV), crude oat (*Avena sativa* L.) extracts at -20°C maintained good ELISA activity for as long as 1 year (Clement 1984), although no comparisons were made with the values for initial extracts. Rochow et al. (1976) detected no loss of infectivity in purified preparations of the MAV and RPV isolates of BYDV frozen in liquid nitrogen or stored lyophilized at -4, -70 or -190°C for 4 years. However, the membrane-feeding procedure used to test infectivity in their work does not accurately assess differences in infectivity, so some virus degradation may have occurred during storage. In experiments where comparative measurements were attempted, ELISA values for extracts of cereals infected with the PAV, MAV, or RPV isolates, prepared from freshly harvested tissue or from tissue stored overnight at 4 or -20°C, were similar (Lister and Rochow 1979; Rochow 1979). Also, storage of BYDV-infected leaf tissue for 4 days at -20 or -80°C involved no significant loss of virus as measured by ELISA (Skaria 1984). However, according to Doupnik et

al. (1982), when extracted sap of cereals infected with BYDV was tested by ELISA before storage and after 4 and 28 days at -20°C, the ELISA values progressively increased for the infected samples and decreased for the healthy controls.

The stability of ELISA activity in BYDV-infected samples is in fact difficult to assess precisely, because no standards for comparison that can be guaranteed not to change over time are available. However, we report here the results of experiments in which stored samples were assayed sequentially by identical ELISA procedures, using the same coat and conjugate concentrations, and carefully standardizing all other aspects of the procedure.

Materials and Methods

Three isolates of BYDV were examined: Purdue (P)-PAV (Hammond et al. 1983), MAV, and RPV (Rochow 1970). For each isolate, approximately 100 g of infected oat (cv. Clintland 64) "shoot" tissue (= all above-ground parts) were harvested (2 weeks after inoculation for the P-PAV and MAV isolates, and 3 weeks after inoculation for the RPV isolate), and cut into 0.5 to 2.0 cm pieces, which were mixed thoroughly and then divided in two batches. From one, 1-g tissue samples (four repetitions for each storage condition) were heat sealed in small polyethylene bags and stored in various conditions. The other batch of infected tissue was homogenized 1:20 (w/v) in 0.1 M potassium phosphate buffer, pH 7.0, in a blender. The homogenate was squeezed through cheesecloth and divided in two parts. From one batch, 1-ml samples of crude sap in "Eppendorf" tubes (four repetitions for each storage condition) were stored at different temperatures. The other batch of crude sap was clarified by mixing 1:1 (v/v) with chloroform, blending at low speed for 30 seconds, storing at 4°C for 20 to 30 min, and then centrifuging at 10,000 rpm for 10 min in a Sorvall SS-34 rotor. The supernatant (= clarified sap) was collected, divided in 1-ml samples in Eppendorf tubes (four repetitions for each storage condition), and stored together with the other tissue and crude sap samples. Samples of healthy tissue and crude sap were stored at the same conditions as the controls. Purified virus preparations of the three BYDV isolates stored at -80°C were also used as controls in the ELISA tests.

All tissue and extracts were tested by ELISA just before storage or immediately upon thawing after storage for 1, 4, 8, or 14 weeks, frozen at -20°C, -80°C, or immersed in liquid nitrogen in a 15-liter container. Frozen crude and clarified extracts were thawed and used in ELISA. Frozen tissue samples were ground into a powder with a mortar and pestle. The powder was further ground in a small amount of 0.1 M phosphate buffer, pH 7.0, to produce a paste, and then diluted in the same buffer to a final dilution of 1:20 (w/v). The extracts were then tested by ELISA.

A standard virus dilution curve was developed in all ELISA tests to establish the relationship between antigen dilution and ELISA values. All samples were tested in duplicate wells. The procedure used was double antibody sandwich (DAS)-ELISA, as described elsewhere (Lister and Rochow 1979). Mean ELISA values (A405 nm) of the four repetitions of the different treatments were compared by multiple-way analysis of variance (ANOVA).

Results and Discussion

When replicate samples of the three BYDV isolates in infected intact leaf, leaf extracts in phosphate buffer, or chloroform-clarified leaf extracts were stored frozen at -20°C, -80°C, or in liquid nitrogen, their ELISA activities fluctuated over time and according to the virus isolate involved. With the P-PAV isolate, no significant decrease of ELISA activity was noted with leaf or crude leaf extracts stored for up to 4 weeks at -20°C, -80°C, or in liquid nitrogen (Table 1). When tested after 8 weeks, however, ELISA activity was reduced to values that remained similar thereafter for up to 14 weeks, the longest period of storage tested. Similarly, ELISA activity was maintained in samples of clarified extracts stored at -80°C or in liquid nitrogen during 4 weeks and then was

reduced. However, that of clarified extracts stored at -20°C deteriorated after only 1 week to a relatively low value which was maintained in later tests.

With the MAV and RPV isolates, some degree of deterioration in ELISA activity was noted in most samples after 1 week of storage, but storage for 4 weeks resulted in more severe reductions (Tables 2 and 3). For unknown reasons, these reductions were followed by an increase in activity in samples stored for longer periods. Again, storage of clarified sap at -20°C was the least effective treatment for preserving ELISA activity.

Assuming that all variations due to the assay conditions were avoided, ELISA activity was maintained relatively unchanged for only up to 4 weeks when samples of leaf or crude extracts of leaf infected with the P-PAV isolate were frozen at -20°C, -80°C, or in liquid nitrogen. Similar samples containing the MAV or RPV isolates deteriorated more rapidly, but interestingly, after the initial decrease in ELISA activity of the samples containing the MAV or RPV isolates, some enhancement of activity was indicated after 8 or 14 weeks of storage. Reasons for this are not clear, but Doupnik et al. (1982) also found that the ELISA values for crude sap preparations containing a Kentucky isolate of BYDV were enhanced by storage at -20°C for 28 days.

The least satisfactory way of storing samples for maintaining ELISA activity was as chloroform-clarified sap at -20°C, and this was especially true of the MAV isolate. Storage by freezing at -20°C could be expected to reduce enzymatic degradations of virus less efficiently than storage at lower temperatures, and storage at -20°C of potato or tobacco leaves infected with several potato viruses has been found inferior for preservation of ELISA activity to storage at -70°C (Singh and Sommerville 1983).

The effects of various storage methods on subsequent ELISA activity are clearly complex, and will depend on various factors, such as the efficiency with which the antisera used "recognize" (i.e., react with) virions modified by storage, and whether substances inhibiting ELISA are produced during storage.

Table 1. Effect of freezing leaf samples and leaf extracts on the detection of the P-PAV isolate of barley yellow dwarf virus by ELISA

	Mean ELISA value (SD) Sample type and treatment								
Storage period (weeks)	Leaf			Crude sap			Clarified sap		
	N_2	-80°	-20°	N_2	-80°	-20°	N_2	-80°	-20°
0	1.198 (0.097)	1.198 (0.097)	1.198 (0.097)	0.964 (0.010)	0.964 (0.010)	0.964 (0.010)	1.089 (0.016)	1.089 (0.016)	1.089 (0.016)
1	1.197 (0.045)	1.205 (0.100)	1.015 (0.098)	1.021 (0.037)	0.944 (0.044)	0.924 (0.044)	1.022 (0.038)	0.985 (0.030)	0.249 (0.056)
4	1.125 (0.047)	1.198 (0.054)	1.102 (0.045)	1.070 (0.021)	0.988 (0.013)	0.924 (0.014)	1.051 (0.010)	0.958 (0.035)	0.237 (0.029)
8	0.778 (0.057)	0.763 (0.093)	0.791 (0.084)	0.731 (0.010)	0.701 (0.011)	0.640 (0.024)	0.761 (0.022)	0.768 (0.030)	0.137 (0.021)
14	0.768 (0.104)	0.789 (0.067)	0.710 (0.028)	0.712 (0.021)	0.697 (0.013)	0.615 (0.015)	0.817 (0.029)	0.747 (0.017)	0.121 (0.018)

Numbers are mean ELISA values (with standard deviations, SD, in parentheses) of 4 replications, each tested in duplicate wells. Mean ELISA value (± SD) for healthy controls was 0.029 (± 0.004).

Table 2. Effect of freezing leaf samples and leaf extracts on the detection of the P-PAV isolate of barley yellow dwarf virus by ELISA

	Mean ELISA value (SD) Sample type and treatment								
	Leaf			Crude sap			Clarified sap		
Storage period (weeks)	N_2	-80°	-20°	N_2	-80°	-20°	N_2	-80°	-20°
0	1.808 (0.107)	1.808 (0.107)	1.808 (0.107)	1.559 (0.035)	1.559 (0.035)	1.559 (0.035)	1.711 (0.079)	1.711 (0.079)	1.711 (0.079)
1	1.764 (0.076)	1.716 (0.167)	1.638 (0.153)	1.456 (0.017)	1.470 (0.033)	1.453 (0.016)	1.708 (0.020)	1.525 (0.071)	0.471 (0.110)
4	1.612 (0.130)	1.445 (0.073)	1.103 (0.146)	1.043 (0.36)	1.045 (0.036)	1.057 (0.029)	1.400 (0.024)	1.045 (0.063)	0.223 (0.024)
8	1.428 (0.146)	1.392 (0.170)	1.002 (0.190)	1.014 (0.015)	1.052 (0.012)	1.014 (0.017)	1.349 (0.008)	1.140 (0.015)	0.194 (0.021)
14	1.598 (0.139)	1.553 (0.184)	1.470 (0.219)	1.223 (0.022)	1.244 (0.018)	1.225 (0.031)	1.479 (0.059)	1.392 (0.024)	0.188 (0.015)

Numbers are mean ELISA values (with standard deviations, SD, in parentheses) of 4 replications, each tested in duplicate wells. Mean ELISA value (± SD) for healthy controls was 0.066 (± 0.014).

Table 3. Effect of freezing leaf samples and leaf extracts on the detection of the P-PAV isolate of barley yellow dwarf virus by ELISA

	Mean ELISA value (SD) Sample type and treatment								
	Leaf			Crude sap			Clarified sap		
Storage period (weeks)	N_2	-80°	-20°	N_2	-80°	-20°	N_2	-80°	-20°
0	1.273 (0.158)	1.273 (0.158)	1.273 (0.158)	1.350 (0.010)	1.350 (0.010)	1.350 (0.010)	1.811 (0.013)	1.811 (0.013)	1.811 (0.013)
1	1.335 (0.121)	1.004 (0.036)	1.098 (0 142)	1.190 (0.038)	1.154 (0.023)	1.222 (0.034)	1.648 (0.032)	1.641 (0.031)	1.304 (0.048)
4	0.877 (0.068)	0.850 (0.145)	0.651 (0.057)	0.828 (0.018)	0.787 (0.010)	0.819 (0.019)	1.253 (0.044)	1.193 (0.033)	0.786 (0.071)
8	0.750 (0.134)	0.746 (0.078)	0.689 (0.138)	0.903 (0.022)	0.890 (0.017)	0.892 (0.021)	1.314 (0.028)	1.313 (0.027)	0.804 (0.036)
14	0.910 (0.048)	0.920 (0.114)	0.759 (0.102)	0.965 (0.015)	0.982 (0.015)	0.975 (0.011)	1.474 (0.017)	1.472 (0.011)	0.968 (0.043)

Numbers are mean ELISA values (with standard deviations, SD, in parentheses) of 4 replications, each tested in duplicate wells. Mean ELISA value (± SD) for healthy controls was 0.049 (± 0.009).

Acknowledgments
This contribution reviews work conducted in partial fulfillment of the Ph.D. degree by the first author. Special thanks are due to INVOTAN for their partial support. Expert technical assistance by Jo Anne McFatridge is also acknowledged.

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Development and Use of cDNA Probes in Studies of Barley Yellow Dwarf Virus

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Abstract

Libraries of cDNA clones were produced from the RNAs of the MAV, RPV, and Purdue (P)-PAV isolates of barley yellow dwarf virus (BYDV) in bacteriophage λgtll, by random priming. Screening with antisera to the isolates allowed selection of the recombinants capable of expressing capsid protein. With MAV, subcloning into the plasmid pUC18, followed by restriction endonuclease mapping, identified overlapping inserts collectively representing at least 85% (a total of 5.1 kbp) of the BYDV genome. Immunologically positive clones shared a common region of approximately 1000 bp, located between 750 bp and 1750 bp from the 3' terminus of the genome. Nucleotide sequencing of this region is under way. Similar strategies for genome analysis are being used with the other isolates.

Sequence homology among various isolates of BYDV is being examined by using selected cDNA clones as probes in viral nucleic acid hybridization studies. For example, hybridization specificity with MAV cDNAs was influenced by the origin of the clones within the genome. Those from the putative coat protein-coding region hybridized well only to MAV-like isolates; those from elsewhere hybridized also with PAV-like isolates. None hybridized significantly to RPV. When used in diagnosis, the sensitivity of detection was related to probe size; the larger clones (to 2.2 kbp) detected as little as 70 pg of purified virus (1.4 ng/ml in a 50-μl sample). Studies on infected plant tissue indicated that the sensitivity of virus detection in plant extracts by dot-blot hybridization can be greater than that of ELISA, although inhibition of the reaction by sap constituents occurs at low dilutions. Preparations from frozen or air-dried tissue reacted well, suggesting the possibility of diagnosis of frozen-stored or dry-shipped samples by use of appropriate cDNA probes.

Currently, enzyme-linked immunosorbent assay (ELISA) is the most widely used method for diagnosing and discriminating between isolates of barley yellow dwarf virus (BYDV). However, serological reactions reflect properties of only a proportion of the viral genome, i.e., that which codes for the coat protein. The use of labeled, cloned complementary DNAs (cDNAs) as probes for detecting virus by dot-blot hybridization could provide a sensitive alternative to serology, capable of discriminating between isolates, or correlating them, not only by the coat protein-coding region of the genome, but also other parts. We have produced cDNA clones to several isolates of BYDV, and begun to assess their usefulness as probes for the detection of homologous and heterologous isolates of the virus. The following represents a condensation of a full account of part of this work (Barbara et al. 1987), which should be referred to for methological details), together with recent information on the utility of the cDNAs as heterologous and homologous probes.

Materials and Methods

Virus isolates

The isolates used were the MAV and RPV isolates of Rochow (1970) and the P-PAV isolate of Hammond et al. (1983), which is similar to the PAV isolate of Rochow (1970). All were maintained in *Avena sativa* L., cv. Clintland 64, in constant environment chambers at 18 to 20°C, and transferred as required with the appropriate vectors: *Sitobion avenae* (Fabricius) for MAV; *Rhopalosiphum padi* (L.) for RPV and P-PAV. The BYDV cultures were tested regularly by ELISA (Lister and Rochow 1979), using antisera capable of distinguishing the isolates, to ensure that no cross-contamination had occurred.

Virus purification and RNA extraction
BYDV was purified as described by Hammond et al. (1983). RNA was extracted by dissociation of the particles in either 0.1 M Tris-HGl, pH 9.5 (Brakke and Rochow 1974) on ice for 2 to 5 min, or 0.5% sodium dodecyl sulphate and 100 g/ml proteinase K for 30 min at 37°C. The RNA was then deproteinized by extracting twice with phenol/ chloroform and once with chloroform, and was concentrated by precipitation from 66% ethanol. Yields were similar by either procedure and gave products with similar UV spectra.

The cDNA cloning strategy
The genome of BYDV consists of one single-stranded, positive sense linear RNA with a molecular weight of 1.85-2 x 10^6 (5.6-6.0 kbp; Brakke and Rochow 1974). By analogy with potato leafroll virus, we assume that the RNA is not polyadenylated at the 3' terminus (Mayo et al. 1982; Goldbach 1986). Cloning strategy was therefore to produce a first strand cDNA by random priming with calf thymus DNA, so that most of the genome of each isolate would be equally represented in its library of clones. After second strand synthesis, the ds cDNA was methylated to prevent internal restriction at a later stage, and Eco RI compatible linkers were attached to both termini and digested with Eco RI. The tailed cDNA was then cloned into the unique Eco RI site in the bacteriophage λgtll (Young and Davis 1983). This vector was chosen as the initial cloning vehicle primarily because of its high cloning efficiency. It has the additional advantage of allowing expression of the cloned sequences as a chimeric protein (composed of encoded foreign protein and the λgt11 β-galactosidase protein), so that clones containing those sequences coding for viral coat protein can be identified serologically. Synthesis, cloning of the cDNAs into the bacteriophage λgt11, and clone selection were carried out largely as described by Huynh et al. (1985). Selected inserts prepared from plate stocks of λgt11 (Maniatis et al. 1982) were subcloned into pUC18 (Vieira and Messing 1982) for further study and use as probes. Hybridization probes were made by nick translation of plasmid DNA (Maniatis et al. 1982), usually to a specific activity of ca. 2 x 10^8 cpm/μg DNA.

Dot-blot hybridization
Purified virus of known concentration (estimated spectrophotometrically), tRNA, and pUC18, were diluted as required in 0.1 M sodium phosphate, pH 6.5, prior to immobilization on nitrocellulose. Samples (50μl) were applied with the aid of a hybri-dot manifold (BRL) onto nitrocellulose that had been wetted in 2X SSC (1X SSC = 0.015 M sodium citrate, 0.15 M sodium chloride). Blots were air-dried, baked *in vacuo* for 2 hours at 80°C and then pre-hybridized for 20 hours at 68°C in 5X SSC, 50 mM sodium phosphate, pH 6.5, 250 μg/ml calf thymus DNA, 0.1% SDS and 5X Denhardt's solution (Thomas 1980). Hybridization was in 5X SSC, 20 mM sodium phosphate, pH 6.5, 100 μg/ml calf thymus DNA, 0.1% SDS, 1X Denhardt's solution, and 5% dextran sulfate at 68°C for 24 hours. Blots were washed twice at room temperature for 10 min and twice at 68°C for 10 min in 1X SSC, 0.1% SDS. After washing, blots were exposed to film with intensifying screens at -80°C.

Leaf tissue was ground with the aid of carborundum in 0.1 M sodium phosphate, pH 6.5 (1 g tissue/2 ml of buffer). The extract was expressed through cheesecloth and clarified by a brief (2 min) spin in a microfuge. Hybridization was essentially as described above except: 1) the hybridization buffer contained 1X SSC instead of 5X SSC; and 2) the blots were washed twice for 20 min at room temperature, and then twice at 68°C for 30 min in 0.1 X SSC, 0.1% SDS.

ELISA
Standard methods (Lister and Rochow 1979) were used for double antibody sandwich ELISA.

Results

Restriction mapping of MAV

Subcloned inserts were mapped using a set of restriction enzymes, and a composite representation approximately 5.1 kbp long, equivalent to 85% of the genome, was constructed (Figure 1). The relative orientations of the segments of the map were deduced as described by Barbara et al. (1987).

Because random priming was used during the initial cDNA synthesis, the 3' terminus of the genome would not be represented in the library. It is not known whether any of the clones contain the 5' terminal sequence of the genome.

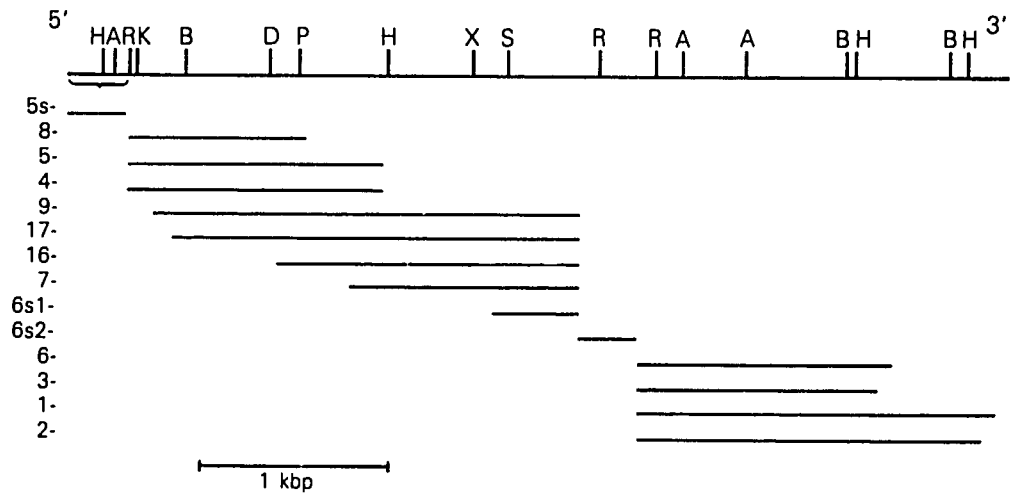

Figure 1. Restriction endonuclease map of the MAV isolate of barley yellow dwarf virus. The enzymes used were as follows: A = Acc I; B = Bam HI; D = Hind III; H = Hinc II; K = Kpn I; P = Pst I; R = Eco RI; S = Sac I; X - Xba I. Locations of 14 immunologically negative clones used to construct the map are shown. The orientation of the Eco RI fragment in brackets is not known.

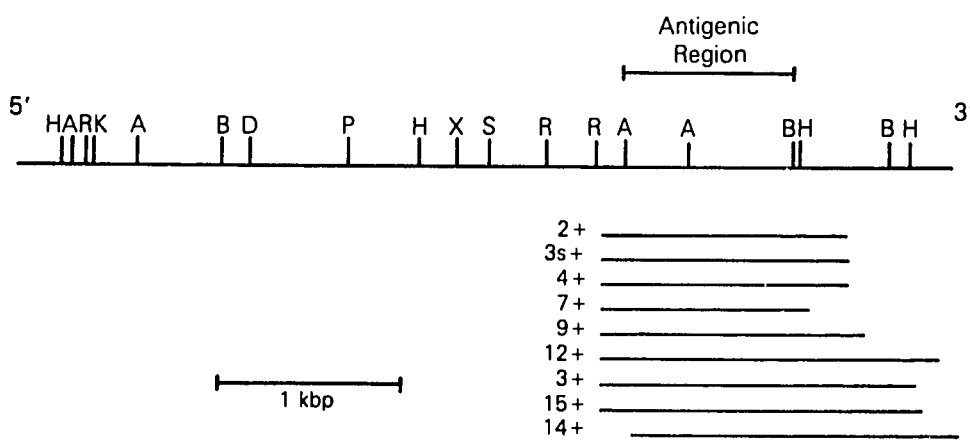

Figure 2. Locations of 9 immunologically positive cDNA clones on the MAV restriction endonuclease map. (Enzymes as in Figure 1). Bar above map defines the region common to the immunologically positive clones.

Those clones found capable of expressing the coat protein (Figure 2) shared a common region of 1000 bp, lying between 750 and 1750 bp from the 3' end of the restriction map. As only about 650 bp would be required for a protein of this size (MW 23,500; Scalla and Rochow 1977), most of the coat protein sequence probably occurs within this common region, but as yet, we cannot precisely ascribe a position on the genome to the coat protein gene.

Behavior of MAV probes in detecting BYDV

The ability to detect and distinguish different BYDV isolates in dot-blot hybridization was assessed in most detail with MAV-derived clones collectively representing the entire length of the mapped genome (Figures 3 and 4). The sensitivity of detection for purified virus in dot-blot hybridization was proportional to the size of cDNA insert up to 1.5 kbp for all probes tested. Usually a total of 200 pg of homologous purified virus (50μl of 4 ng/ml) was readily detected, and at best, 70 pg (50μl of 1.4 ng/ml) of virus gave a clearly visible reaction. The ability to detect the P-PAV isolate varied with the clone used as a probe. Clones which originated from the putative coat protein-coding area and bordering region (Figure 2) were most specific, hybridizing well only to the homologous isolate,

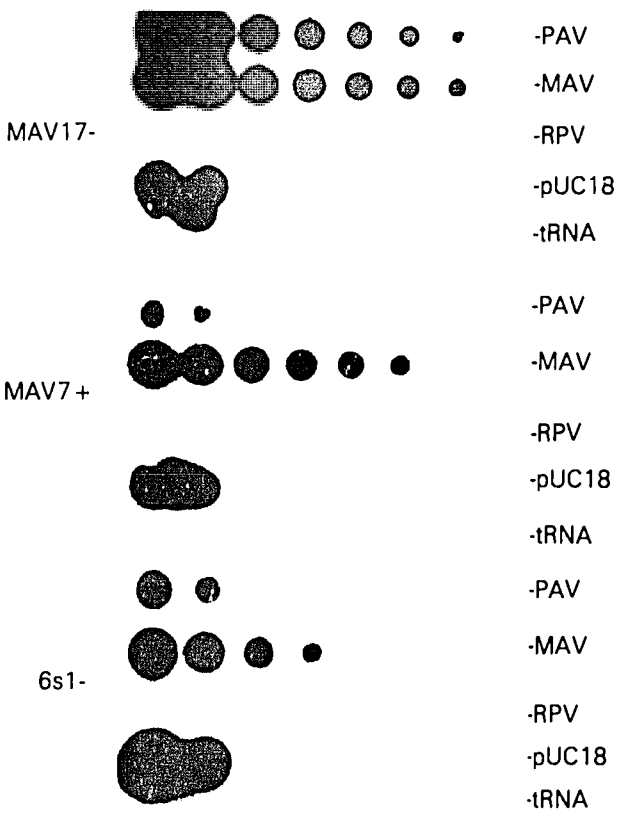

Figure 3. Dot-blot hybridization of barley yellow dwarf virus isolates with various MAV cDNA clones. The viruses and control nucleic acids indicated were probed with cDNA clones representing different parts of the MAV genome (see Figures 1 and 2). Transfer RNA at 1 μg/ml, and puC 18 DNA at 0.5 μg/ml and 0.25 μg/ml, were used as negative controls, respectively. 5 X 10^5 cpm of probe was used per ml of hybridization buffer.

whereas clones from the remainder of the genome reacted equally well to both MAV and P-PAV isolates (Figures 3 and 4). No clones hybridized to the RPV isolate even when a virus concentration of 1 μg/ml (50μl) was used.

The detection of virus in infected plant tissue was investigated by dot-blot hybridization, using an immunologically negative clone, 17- (2.2 kbp), as the probe. Optimum signal density occurred with diluted extracts (Figure 5), suggesting the presence of inhibitory substances in concentrated sap. Virus was detected in extracts of infected plants to a dilution end point of 1/256. In comparison, routine DAS ELISA detected virus in the same extracts to a dilution end point of about 1/64 (Figure 5).

P-PAV and RPV cDNAs as probes
Mapping of the P-PAV and RPV genomes is in progress. Meanwhile, clones regarded as representative of each genome have been used as diagnostic probes in experiments similar to those described above. For example, of six P-PAV clones used as probes (P7, P11, P26, P42, P45 and P51), two (P26 and P45) were specific to the P-PAV isolate and the remaining four also hybridized with MAV. By analogy with the results with MAV clones, we assume that P26 and P45 were derived from a less conserved region of the genome than the other four clones, perhaps involving the region specifying coat protein. None of the clones hybridized with the RPV isolate, and similarly, none of three RPV clones (representing about 4.8 kbp of the genome) tested as probes hybridized with P-PAV or MAV. These results (Figure 6) thus support those obtained with the MAV clones in indicating no genomic homologies between RPV and the other two isolates, but contrast with those of Waterhouse et al. (1986).

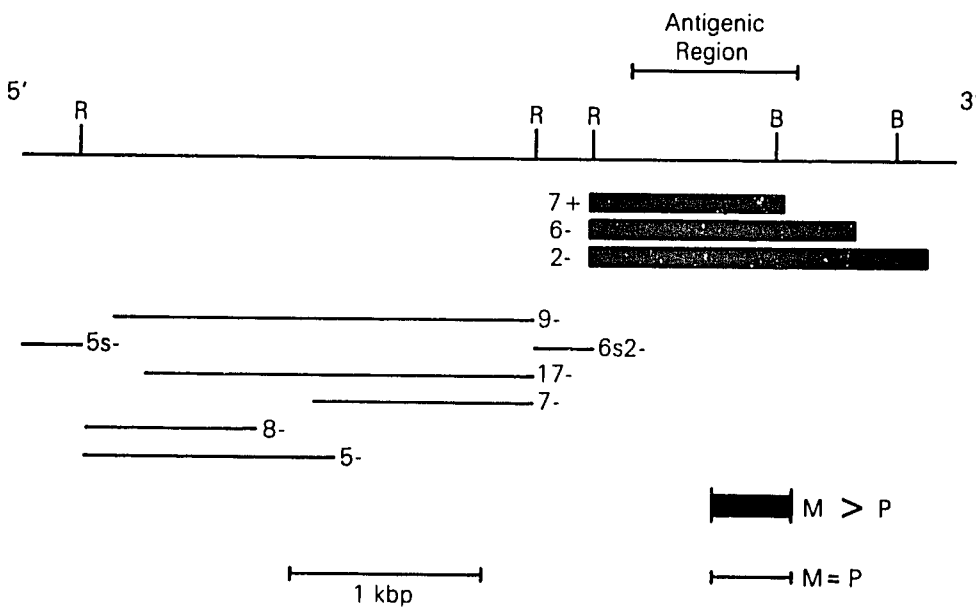

Figure 4. Specificity of hybridization to BYDV isolates. The relative specificities of 10 MAV clones as classified by dot-blot hybridization were as follows: M = P, detection of MAV and P-PAV was equivalent; M > P, detection of MAV was more than 27-fold greater than for P-PAV. No clones reacted to RPV (Enzymes as in Figure 1).

General utility of the clone in diagnosis

The sensitivities of the P-PAV and RPV clones in detecting homologous virus were similar to those of the MAV clones. The inhibitory effect of sap referred to above was greatly reduced by clarifying sap extracts by treatment with chloroform. Also, since the inhibitory effect was significant only with relatively concentrated extracts, it is unlikely to present problems in diagnosis.

Infections were detected in extracts from leaf air-dried for 1 week at room temperature or at 37°C in the laboratory, about as readily as in extracts from fresh leaf. However, virus was not detected in dry leaf samples received by mail from colleagues in Mexico, even though MAV-like isolates were detected in the same material by ELISA. This result suggests difficulties in releasing RNA from virions in such tissue, or possibly RNA degradation, and these possibilities are

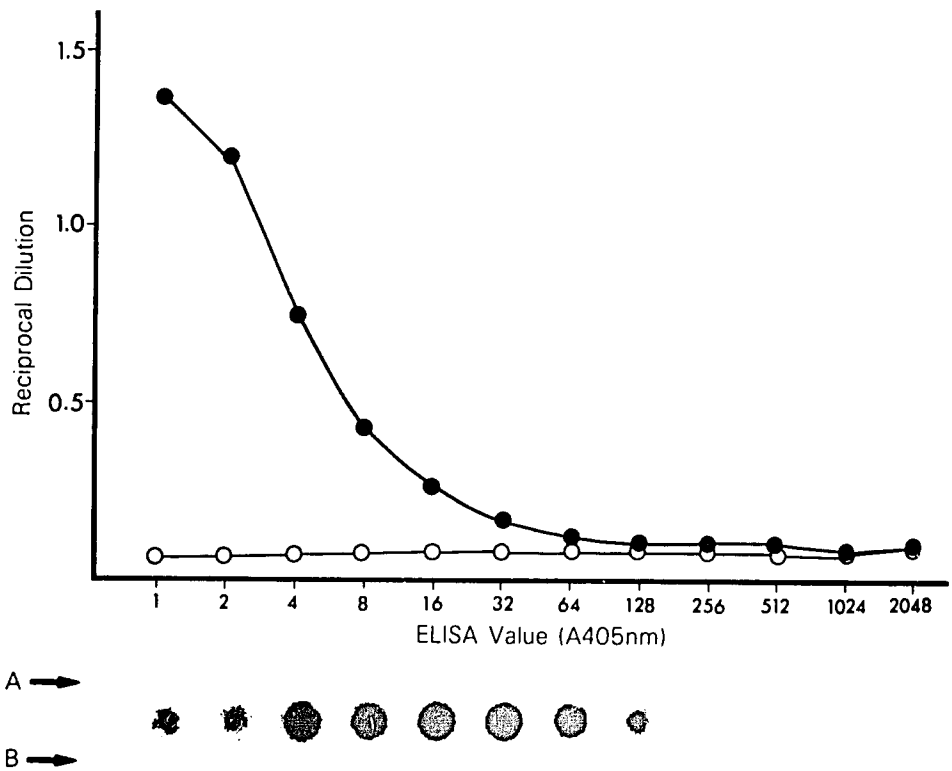

Figure 5. Comparison of dot-blot hybridization (50 μl samples) and double antibody sandwich ELISA (200 μl samples) in the detection of virus in plants. Data are shown for 2-fold serial dilutions of the same plant extracts made at 1:2, w/w, leaf to buffer. Above, ELISA: O–O -non-infected plant extracts, ●–● -infected plant extract. Below, dot blot hybridizations: 4-day exposure with 5 X 10^5 cpm of MAV 17- cDNA per ml of hybridization buffer. A = dilutions of infected plant extract; B = dilutions of healthy plant extract. Note that the optimum signal in dot-blot hybridization occurred at the 1/16 dilution.

under investigation. Tests with a sensitive MAV probe of single aphids (*S. avenae*) raised on MAV-infected plants have given promising results. Overall, the greatest problems with use of the probes for diagnosis are those associated with the handling and short life of the ^{32}P used as a label. Future work will therefore include study of alternative non-radioactive labels. Meanwhile sequencing studies are in progress that may aid in the understanding of aspects of genomic structure and function.

Acknowledgments

We thank the Society of General Microbiology for a travel grant to the second author, the Public Varieties of Indiana Board for partial funding, and Jo Anne McFatridge for technical assistance.

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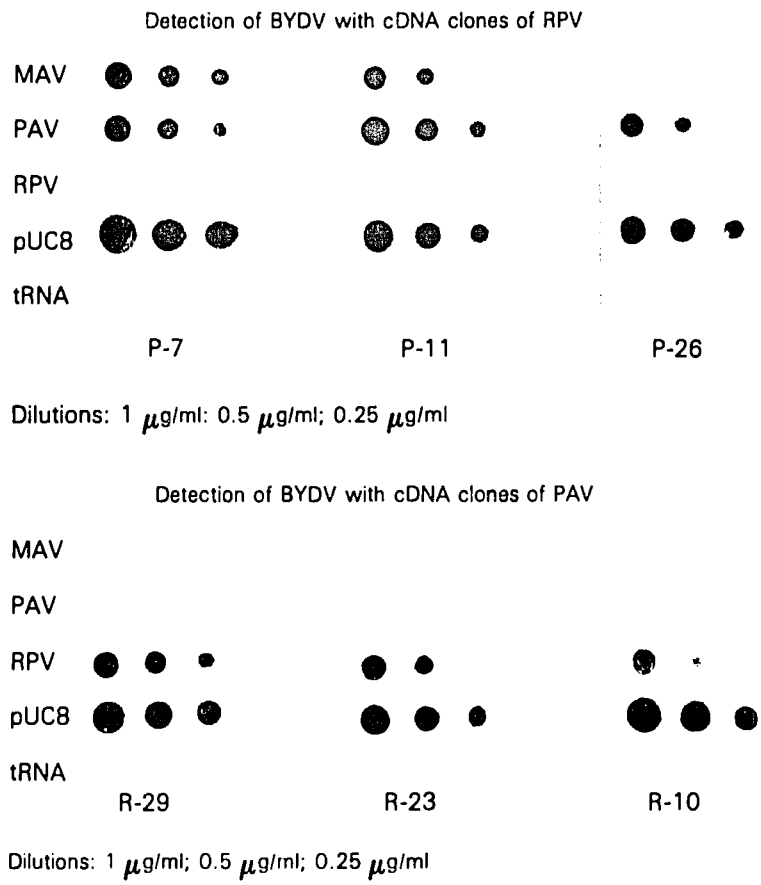

Figure 6. Dot-blot hybridizations of barley yellow dwarf virus isolates with various P-PAV and RPV cDNA clones. Viruses were used in 2-fold dilution series from 1 μg/ml. Other details are as in Figure 3.

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Differentiation of Barley Yellow Dwarf Virus with Monoclonal Antibodies by Immunosorbent Electron Microscopy

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Abstract

Three strain-specific monoclonal antibodies (McAb), MAC 91, MAC 92, and MAFF2, were compared with two polyclonal antisera for the diagnosis of BYDV strains in immunosorbent electron microscopy. Used at an optimal dilution (1 μg/ml), the three McAbs specifically trapped the Rothamsted PAV-, RPV- and MAV-like isolates, unlike the polyclonal antisera, which showed considerable heterologous reaction. The test provides a simple and sensitive diagnostic method for BYDV, suitable for small numbers of samples, and is useful in confirming doubtful enzyme-linked immunosorbent assay results.

BYDV is routinely diagnosed in our laboratory using an indirect enzyme-linked immunosorbent assay (ELISA) system, and strain differentiation into PAV-like, RPV-like, and MAV-like types (Rochow 1970) is achieved by probing with three strain-specific McAbs. In our experience, immunosorbent electron microscopy (ISEM) using polyclonal antisera is more sensitive than the corresponding ELISA test and is useful for confirming doubtful ELISA results. As a general diagnostic method, ISEM also has the advantages of being rapid and economical and not requiring conjugated or second species antisera. The purpose of this study was to extend the use of the three strain-specific monoclonal antibodies to ISEM for strain differentiation of BYDV. Diaco et al. (1986) reported the use of monoclonal antibodies in the specific detection of BYDV in ISEM.

This paper is the first report of the use of monoclonal antibodies in the differentiation of BYDV strains in infected plant sap by ISEM.

Method

Coating grids with antisera

The method used was a modification of the ISEM protocol described by Roberts and Harrison (1979). Three strain-specific McAbs, MAC 91 and MAC 92 (Torrance et al. 1986b), MAFF 2 (M.T. Pead and L. Torrance, unpublished), and a control McAb against Potato Virus X (Torrance et al. 1986a) were supplied by L. Torrance. Two polyclonal antisera were used in comparative tests: one against BYDV-type F (MAV-like), obtained from Bioreba AG and the other against field-infected BYDV plants from Kentucky, USA (Doupnik et al. 1982), which was donated by T.P. Pirone. The McAbs and partially purified polyclonal antisera were diluted and used at an optimal dilution of 1 μg/ml in 0.06 M phosphate buffer (Na_2HPO_4/KH_2PO_4, pH 6.5)

Three replicate 25-μl drops of each McAb were placed on dental wax on moist filter paper in a Petri dish. A 200 mesh nitrocellulose-coated carbon stabilized copper grid was floated on each drop and incubated at 30°C for 2 hours. Each grid was then washed for 15 min twice in 3 ml of phosphate buffer and dried on filter paper.

Preparation of leaf extracts

Four isolates of BYDV maintained at Rothamsted in a spring oat cv Maris Tabard were tested, namely: BYDV-G (PAV-like), BYDV-R-568 (RPV-like), BYDV-B (PAV + RPV-like mixture) (I. Barker and S.M.D. Forde, unpublished), and BYDV-F (MAV-like). Infected leaf material (0.1 g fresh wt) of each strain was extracted, 10 days after inoculation, with 0.9 ml of phosphate buffer and centrifuged at 10,000 g for 3 min in a microfuge. The supernatant was removed and 25 μl drops were placed on a wax mat as above. The antibody coated grids were put on the relevant sample drops using clean forceps and incubated in a covered Petri dish overnight at 4°C.

Negative staining of virus particles
Each grid was washed with 20 drops of 0.03 M phosphate buffer, drained, and negatively stained with 10 drops of a 2% sodium phosphotungstate solution, pH 7.0, and dried.

Electron microscopy
The grids were observed using a Siemens 102 transmission electron microscope at a magnification of 10,000. Numbers of BYDV particles per field of view were counted from six randomly chosen fields for each grid.

Results and Conclusions

The results (Table 1) showed that the three anti-BYDV McAbs, MAC 91, MAC 92 and MAFF2, specifically trapped the Rothamsted type isolates G-(PAV-like), R-568 (RPV-like), and F (MAV-like). The Rothamsted B isolate was trapped by both MAC 91 and MAC 92, which is consistent with it being a mixture of PAV- and RPV-like strains. Occasional particles (always less than two particles per field) were seen in testing the F isolate with MAC 91 and MAC 92 as well as the R-568 isolate with MAC 91. The reason for this occasional nonspecific trapping remains unclear. It is suggested that mean results less than two particles per field should be regarded as negative. The control rat monoclonal anti-PVX antibody showed no such reaction. The two polyclonal antisera used showed considerable cross-reaction among the isolates tested, as was expected. The Bioreba F antiserum trapped both the PAV and MAV-like isolates and the Kentucky antiserum trapped both the PAV- and RPV-like isolates and the MAV-like isolate weakly.

The McAbs successfully differentiated between the BYDV isolates tested in ISEM and the method is particularly useful in confirming doubtful ELISA results.

Acknowledgments

Many thanks to Sheila Roberts and Roy Woods for their help and advice on electron microscopy, Elizabeth Lennon and Rosie Gutteridge for supplying the BYDV isolates, and the Plant Pathology glasshouse staff. We also thank Tom Pirone for donating the Kentucky antiserum and Mike Adams for his help with the statistics.

This work was funded by the Overseas Development Administration.

Table 1. Results of the mean number of particles trapped by the antibody-antigen reaction

	Monoclonal antibodies				Polyclonal Antisera	
BYDV isolates	PVX	MAC 91	MAC 92	MAFF 2	BYDV Ky	BYDV F
G-(PAV)	0	15.0	0	0	18	29.1
	(0)	(1.145)	(0)	(0)	(1.261)	(1.465)
R-568-(RPV)	0	0.3	22.9	0	22.3	0
	(0)	(0.1)	(1.375)	(0)	(1.359)	(0)
B-(PAV + RPV)	0	17.4	23.1	0	22.8	28.4
	(0)	(1.24)	(1.343)	(0)	(1.294)	(1.455)
F-(MAV)	0	0.2	0.1	14.9	4.3	61.7
	(0)	(0.059)	(0.041)	(1.184)	(0.718)	(1.785)
Healthy sap	0	0	0	0	0	0
Control	(0)	(0)	(0)	(0)	(0)	(0)

Notes: The above values are mean numbers of virus particles per field from 3 grids (6 fields per grid). Values in the parentheses are $\log_{10}$ transformed means + 1. Values without parentheses are untransformed means to 1 decimal place. The S.E.D. (log. values) for isolate-antibody interaction was 0.106.

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The Biology and Taxonomy of the Aphids Transmitting Barley Yellow Dwarf Virus

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Abstract

All the cereal-feeding aphids are potential vectors of cereal viruses, as indeed are aphids feeding on other Gramineae and Cyperaceae. At least 24 species of aphids belonging to 15 different genera have been shown experimentally to transmit viruses of the barley yellow dwarf group. This paper reviews the biologies of the main Gramineae-feeding genera, with more detailed consideration of the principal vectors of barley yellow dwarf virus. Several of the vectors belong to complexes of species that are difficult to separate, for example Melanaphis sacchari/sorghi, Rhopalosiphum maidis *and* Sitobion fragariae/miscanthi. *These problems of species recognition and their agricultural implications are discussed.*

The objective of this paper is to summarize present knowledge of those aspects of the biology and taxonomy of cereal aphids that influence their ability to transmit barley yellow dwarf virus. Many aspects of aphid biology are relevant to virus transmission. We shall be mainly concerned with updating and filling any gaps in previous reviews (Plumb 1983; Gildow 1984). About the taxonomy of cereal aphids we will have more to say, as we wish to draw particular attention to the problems and uncertainties that exist in both the conceptual and the practical recognition of "meaningful taxonomic units" in most cereal aphids. By "meaningful taxonomic units" we mean groups of individuals or populations that have in common a set of correlated traits, such that the knowledge of one trait enables one to predict others. In those aphids that have an obligatory annual sexual phase, the smallest meaningful taxonomic units may be the species. On the other hand, in those aphids that reproduce continuously throughout the year by parthenogenesis, the smallest meaningful taxonomic unit may be a single clonal lineage, or a set of clonal lineages derived from a single clonal lineage, or a set of clonal lineages derived from a common ancestor and therefore having shared properties.

The term "biotype," as applied to aphids by economic entomologists, cannot be regarded as a meaningful taxonomic unit, because biotypes are recognized by only the single trait that happens to be of interest to the observer, such as the ability to colonize a certain crop variety. A biotype may be a single clone, but on the other hand, it might consist of any number of clones with just the single trait in common, especially if the trait in question is determined at a single genetic locus. The designation of a population as "biotype A" does not necessarily imply any relationship with other populations of "biotype A," nor does it enable one to predict any other properties for the population, other than the one by which it was recognized. Studies on biotypes, if they fail to give full consideration to this limitation, can result in endless confusion in the literature.

We will deal first, in alphabetical order, with the aphid genera containing the principal vectors of barley yellow dwarf virus (BYDV), and then discuss the other aphids that may possibly bring virus into the crop.

Cereal Aphids Transmitting BYDV Within the Crop

Anoecia (and other root aphids)

Some species of the main aphid subfamily Aphidinae colonize Gramineae at or below soil level (e.g., *Rhopalosiphum insertum* (Walker), *R. rufiabdominalis* (Sasaki), *Aphis maidiradicis* Forbes), but the true root-feeding groups are in two other subfamilies, the Anoeciinae (*Anoecia* spp) and the Pemphiginae (e.g., *Tetraneura*, *Forda*, *Aploneura*, *Geoica*). Aphids of these subfamilies can be recognized by

their very short antennal terminal process (much shorter than the base of the last antennal segment) and siphunculi reduced to small cones, pores, or even absent.

Anoecia species have a sexual phase on *Cornus* in Europe and North America, but anholocycly (overwintering of parthogenetic morphs) on roots of Gramineae is common and probably predominates in the Mediterranean region and in South America. The apterae on roots are more active than Pemphiginae, with longer legs and antennae, and usually have a dark dorsal shield. The alatae migrating to and from *Cornus* have a conspicuously large dark pterostigmal spot on the forewing. The species are difficult to differentiate. *Anoecia corni* (F.), the only species shown to be a BYDV vector (A'Brook and Dewar 1980, Conti and Casetta 1983), occurs in Europe eastward to Iran and the Himalayas, and in North America. Similar aphids also occur in Korea and Japan.

Tetraneura have *Ulmus* spp. as primary hosts, and the Fordini (*Forda*, *Geoica*, *Aploneura*) have a two-year life cycle with a sexual phase on *Pistacia* in the Mediterranean region and the Middle East. The apterae of all these genera are plump, sluggish aphids with short legs and antennae. Anholocycly on the roots of Gramineae is common, especially where the primary hosts are unavailable. The migrations to and from the primary hosts are probably of little importance in BYDV transmission, and it is not known whether the alatae produced by anholocyclic populations transmit virus.

Diuraphis

Diuraphis is a genus of about 10 species of small, elongate, wax-covered aphids with inconspicuous siphunculi, living in the rolled leaves of Gramineae in Europe and North America. The subgenus *Diuraphis* bears a conspicuous backwardly directed supracaudal process (Figure 1), which is barely indicated or lacking in the subgenus *Holcaphis*. Only three species occur on crop plants, and only one (*D. noxia* (Mordvilko)) is recorded as a virus vector.

Diuraphis (Holcaphis) frequens (Walker), widespread on *Agropyron repens* (L.) Beauvois in Europe and eastward to Turkey, was introduced into North America, where it has been collected on wheat (*Triticum aestivum* L.) and *Echinochloa crus-galli* (L.) Beauvois as well as on *Agropyron*, and it perhaps occurs also on other grasses. It was found in Quebec in 1956, New Brunswick in 1964, Manitoba in 1965, Washington in 1983 and Idaho in 1985.

Diuraphis (Holcaphis) tritici (Gillette) is the only *Diuraphis* known only from North America, and can be distinguished from *D. frequens* by its longer last rostral segment and other morphological features (see key below). It occurs on wheat, but is also recorded from *Agropyron occidentale* (Scribn.), which is possibly its native host.

Diuraphis (Diuraphis) noxia (Mordvilko), Russian wheat aphid, occurs mainly on barley (*Hordeum vulgare* L.) and wheat and more rarely on other cereals and grasses. First described from Russia, it is now found in southern Europe, Spain to Turkey, North and South Africa, the Middle East, Pakistan, and Argentina,

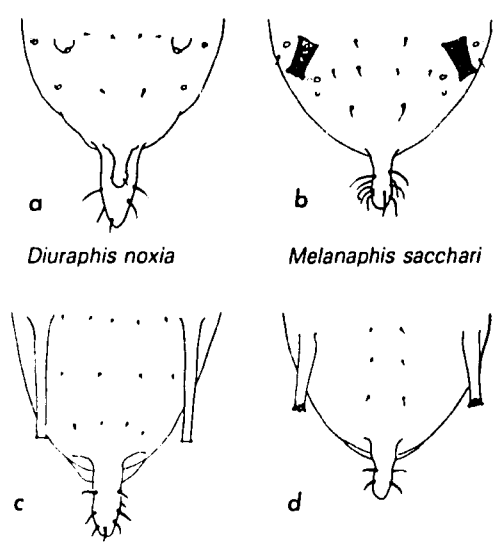

Figure 1. Rear of abdomen in dorsal view of aptera of four cereal aphids.

and has recently extended its range into Mexico and the USA. *D. noxia* has been reported as a vector of brome mosaic virus, barley stripe mosaic virus, cucumber mosaic virus, and BYDV. (von Wechmar and Rybicki 1981; von Wechmar et al. 1984). Walters (1984) reviews information about *D. noxia* and provides many references. Gilchrist et al. (1984) give an account of the problems associated with *D. noxia* in Mexico.

Key to the *Diuraphis* found on wheat:

1. Supracaudal process well-developed. Siphunculi about as long as broad. Lateral abdominal tubercles present on segments 3-5. Short-haired aphid, even the posterio-dorsal cephalic and 8th abdominal tergite hairs only 8-12 μm long ... *D. noxia*.

- Supracaudal process absent or only slightly indicated. Siphunculi little more than pores on pigmented bases. Lateral abdominal tubercles absent. Longer-haired aphids, hairs on 8th abdominal tergite 20-35 μm long... 2

2. Ultimate rostral segment 0.85-1.15 times as long as 2nd segment of hind tarsus. Eighth abdominal tergite bearing 7-11 hairs, the spinal (median) hairs about 20 μm long ... *D. tritici*.

- Ultimate rostral segment 0.55-0.75 times as long as second segment of hind tarsus. Eighth abdominal tergite bearing 4-6 (occasionally 7) hairs, the spinal ones being 25-35 μm long ... *D. frequens*.

Melanaphis

Melanaphis is an Old World genus of about 20 species of small, broadly oval aphids with short siphunculi (Figure 1), mostly associated with *Arundinaria* and the other bamboos in the Far East. A few species have Pyroidea as primary host plants, like members of the related genera *Rhopalosiphum* and *Schizaphis*. *Melanaphis (=Longiunguis)* sorghi (Theobald) has usually been regarded as a synonym of *M. sacchari* (Zehntner), but aphids from sorghum generally have a shorter antennal terminal process relative to length of hind tibia than those from sugar cane (Figure 2), and it now seems more likely that the two are distinct taxa, although not absolutely specific to the hosts indicated by their names. The separation needs to be confirmed by experimental studies. The true situation may well be more complicated, as this group, like *Rhopalosiphum maidis* (Fitch) discussed below, is mainly or entirely anholocyclic. Color, general size, hair lengths, and numbers of lateral abdominal tubercles are very variable and probably subject to environmental conditions, particularly nutrition. *M. indosacchari* David appears to be one such environmental form (of *M. sacchari*).

Melanaphis sacchari (Zehntner) lives on sugar cane (*Saccharum*) in South Africa, Asia, Australia, and Central and South America. It seems to have arrived in Central America (Guyana) about 1932 and spread south gradually, reaching Uruguay and Argentina only recently (Delfino 1985). Apart from sugar cane, it sometimes colonizes *E. crus-galli* and rice (*Oryza sativa* L.), and specimens from sorghum in Brazil in the British Museum (Natural History) collection seem to be this aphid rather than *M. sorghi*.

Melanaphis sorghi (Theobald) is common on *Sorghum halepense* Pers. in the Middle East and on *S. vulgare* Pers. in Africa and Asia, also sometimes colonizing sugar cane in Africa and maize in Southeast Asia.

Metopolophium

Species of *Metopolophium* are readily distinguished from other aphids on cereals by their pale tapering siphunculi, relatively long antennae, and rather slender body shape. The affinities of this genus are in some doubt. Morphologically the species are very like *Acyrthosiphon*, but biologically and karyologically they resemble *Sitobion*. Of the 18 described species, 15 have Gramineae as either their secondary or only host plants. The host-alternating species overwinter as eggs on *Rosa*. The few species without graminaceous hosts perhaps do not really belong in *Metopolophium*. The genus is predominantly European, and Stroyan (1982, 1984) and Heie (1986) have

comprehensively reviewed the European species so far recognized, including the important cereal pest *M. dirhodum* (Walker), which now has a worldwide distribution. The only other species regularly infesting cereal crops is *M. festucae cerealium* Stroyan. The non-pest species are morphologically very hard to distinguish from these two, but each restrict their feeding to one or two grass species in particular habitats. *M. frisicum* Hille Ris Lambers, which lives on *Poa* spp. in damp places, and *M. albidum* Hille Ris Lambers, which is specific to *Arrhenatherum elatius* Mert. and Koch, can transmit BYDV (A'Brook and Dewar 1980), and there is no reason to suppose that other species of *Metopolophium* are not also potential vectors wherever they occur.

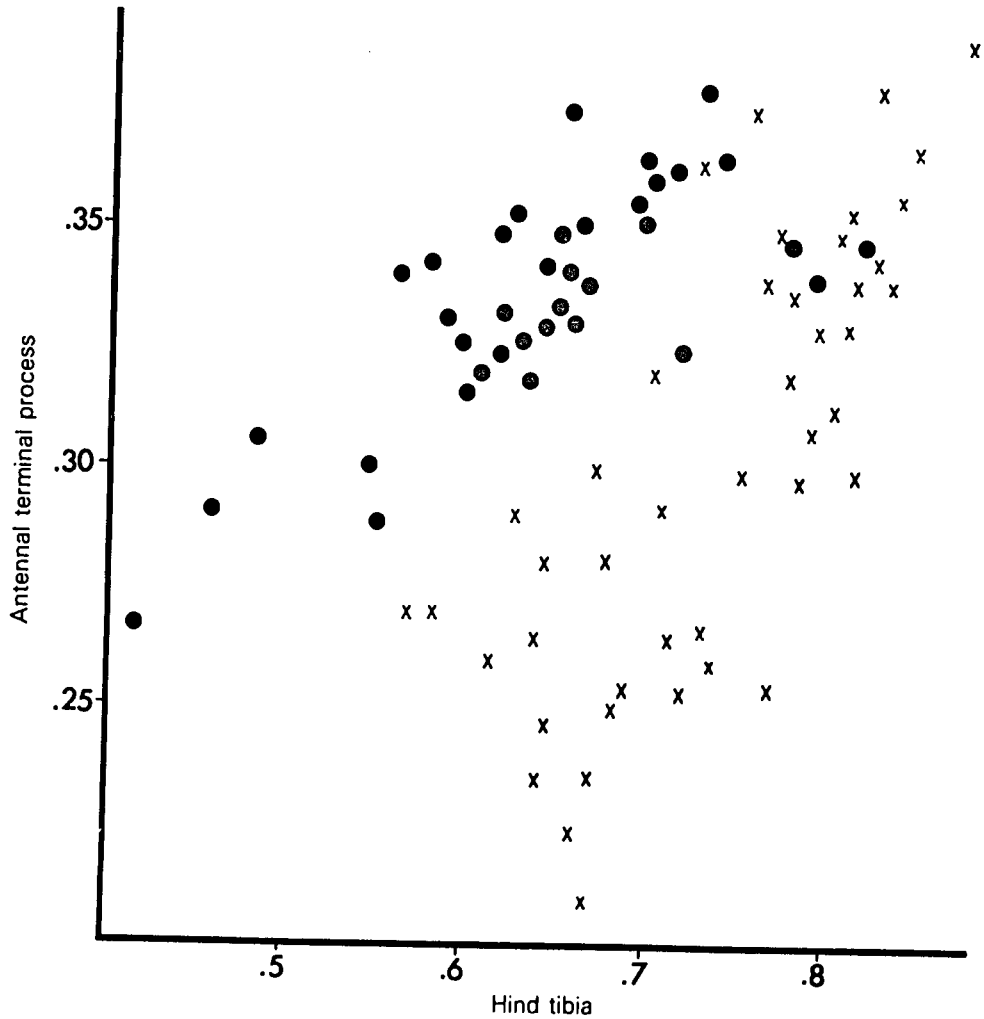

Figure 2. Plot of the measurements (in mm) of the length of the antennal terminal process against the length of the hind tibia for apterae of *Melanaphis* from sorghum (crosses) and sugar cane (circles) from many parts of the world, indicating the probable existence of two species.

M. dirhodum overwinters as eggs on wild or cultivated *Rosa* spp in cold temperate climates, but anholocycly—overwintering of parthenogenetic morphs—is now widespread wherever winter temperatures permit. Numerous species of Gramineae, and some other monocots such as *Iris*, are colonized, so alatae of *M. dirhodum* can usually find hosts at a suitable growth stage at all seasons. In northwest Europe, where holocycly predominates, the migration to cereals is normally too late to have a significant effect on yield. However, like *Sitobion avenae* (Fabricius), *M. dirhodum* is a rather active aphid, generally forming small colonies that readily produce more alatae (Cannon 1985), so that it may be important in spreading virus both through and between crops. Alatae leaving crops may also spread virus to late-maturing wild Gramineae, maize, and volunteer cereals.

Cannon (1986) studied populations of *M. dirhodum* over three summers in England, including an exceptionally large outbreak in 1979, and Dewar and Carter (1984) developed a decision tree for assessment of the risk of outbreaks. Development of spring populations on *Rosa* has recently been studied in Germany (Gruppe 1985) and the Netherlands (Hand and Hand 1986). Weber (1985b) set up 120 clones of *M. dirhodum*, all holocyclic, from cereal crops in Germany and found a wide variation in their ability to colonize barley, wheat, and oats (*Avena sativa* L.). The presence or absence of a sexual generation presumably has a major effect on population genetics and a parallel study in an area where *M. dirhodum* overwinters anholocyclically could be informative.

M. dirhodum recently reached Australia and New Zealand. In New South Wales it has been shown to be an efficient vector of a PAV-like isolate (Waterhouse and Helms 1985). In New Zealand it occurs on a succession of cereals and grasses throughout the year (Stufkens and Farrell 1985).

On cereals, living *M. dirhodum* are rather easy to distinguish from *M. festucae cerealium* by the broad bright green longitudinal dorsal stripe present in both immature and adult apterae and by the dusky apices to antennal segments III, IV and V. *M. fasciatum* Stroyen, the only other *Metopolophium* with these features, migrates between *Rosa canina* L. and *Arrhenatherum elatius* and has not been recorded from cereals.

Metopolophium festucae cerealium Stroyan is the name applied to the large, long-legged form of *M. festucae* (Theobald) that colonizes cereals in Europe and sometimes builds up damaging populations on pasture grasses. Work with laboratory cultures and detailed morphometric studies have established that the differences from the exclusively grass-feeding form are not merely due to better nutrition (Stroyan 1982), but the genetic relationships between the two forms and their respective host plant ranges are not yet clearly established. Both are predominantly anholocyclic, but both are also known to produce sexual morphs occasionally on grasses, so hybridization may occur.

Plumb's (1974) record of transmission of three isolates of BYDV by *M. festucae* should presumably be applied to *M. festucae cerealium*. This aphid is regarded as of generally low pest status on cereal crops in Europe. There is even some evidence that the early presence of *M. festucae cerealium* in the crop may be beneficial, allowing the buildup of populations of parasitoids that later attack *Sitobion avenae*. However, in laboratory experiments wheat was more susceptible to it than any grasses (Dent and Wratten 1986). The paradox is unexplained and it seems possible that in other circumstances (for example, if introduced outside Europe, or on other cereal cultivars) its attacks on cereals could become more serious.

Apterae of *M. festucae cerealium* tend to be larger, paler, and yellower than those of *M. festucae s. str.*, and pink populations are unknown. Alatae typically have dark and well-developed transverse dorsal abdominal bands. The best single morphometric character for distinguishing the two forms of *festucae*

is the length of the hind tibia, but a discriminant function involving six characters is needed to provide a reliable separation (Stroyan 1982).

Rhopalosiphum

Rhopalosiphum have strong taxonomic affinities with *Melanaphis* and *Schizaphis*, and the species share a similar biology with these two genera, typically having a sexual phase on *Prunus* or Pyroidea and migrating for the summer to monocotyledenous plants, mainly Gramineae. It is a difficult genus to define morphologically; a characteristic feature is the embossed polygonal pattern of small, bead-like or irregularly shaped nodules on the dorsal abdominal cuticle (Figure 3f), but this is difficult to see in unprepared specimens. The cereal-feeding species have rather characteristically shaped siphunculi: cylindrical, vasiform, or slightly swollen distally, but with a constriction just proximal to a well-developed apical flange (Figure 3g-j). The siphunculi and cauda of *Rhopalosiphum* species are dark and the cauda is rather short, always shorter than the siphunculi.

Of about 13 recognized species of *Rhopalosiphum*, 4 are proven vectors of BYDV (*R. insertum* (Walker), *R. maidis* (Fitch), *R. padi* (L.), and *R. rufiabdominalis* (Sasaki)). The biology of some of the other species is little known and they may include potential vectors. *R. padiformis* Richards, described from British Columbia and distinguishable from *R. padi* by the long, pointed hairs on the posterior abdominal tergites (Richards 1962), has been collected on wheat in Montana, USA. Its life cycle is unknown.

Rhopalosiphum insertum (Walker) overwinters as eggs on Pyroidea (*Malus*, *Pyrus*, *Crataegus*, *Sorbus*, *Cotoneaster*) and migrates to various Gramineae (especially *Poa* and *Festuca*), where it normally feeds at the base of the stem or below ground and is probably frequently overlooked. It will colonize wheat, barley, and oats in the laboratory, but the only field record from cereals that we know of is from Victoria, Australia, in 1983 -- its first appearance in Australia (M. Carver, personal communication). Anholocyclic overwintering does not normally occur.

The synonymy of this aphid is very confused; the names *R. crataegellum* Theobald and *R. oxyacanthae* Schrank have been applied to it by European workers, and North American taxonomists have been reluctant to accept that their *R. fitchii* Sanderson is the same species. The uncertainty has arisen partly because the subterranean populations are virtually unknown in North America, and alatae transferred from primary hosts to Gramineae in the laboratory have tended to form colonies above ground. The apterae from such aerial colonies are shorter-haired than those from subterranean populations of *R. insertum* in Europe, and also in life show some color differences (Smith and Richards 1963). Both hair length and color may be affected by environmental conditions during development, and especially by the differences in light and humidity above and below ground. Sun and Robinson (1965) showed that the range of Pyroidea used as primary hosts was as wide in Canada as in Europe, and Robinson and Chen (1969) obtained subterranean laboratory colonies on barley roots in Canada. The only record from a secondary host plant in the field in North America is from the "basal shoots" of *Alopecurus pratensis* L. (Smith and Richards 1963). This is strange considering the very large numbers that leave primary hosts such as *Crataegus*. Further work on this problem would clearly be desirable.

The first tests on BYDV transmission by *R. insertum* were conducted with aphids from primary host populations on *Crataegus* and *Malus* (Orlob and Arny 1960), of which only oviparae (in autumn) and immature progeny of the fundatrix (in spring) could transmit. Failure of gynoparae, males, and fundatrices to act as BYDV vectors may be explained by their non-acceptance of barley as a host plant. However, in the single test conducted with alate fundatrigeniae (= spring migrants) from *Malus*, these also failed to transmit. In comparative tests with *R. padi* (Smith and Richards 1963), *R. insertum* was less efficient at transmitting all but 1 of 15 isolates, but the fact that most of these isolates had previously passed through *R. padi* needs to be taken into account. In Britain, large numbers of infective gynoparae of *R. insertum* are flying in

the autumn of some years (A'Brook and Dewar 1980), but these are migrating to primary hosts and it seems unlikely that they would spread virus in autumn-sown cereal crops.

Rhopalosiphum maidis (Fitch) is apparently permanently parthenogenetic on Gramineae throughout the world. Males occur sporadically in various parts of the world, but sexual females and overwintering in the egg stage have never been observed. Although readily recognizable by its narrow shape, short siphunculi (Figure 3j) and short terminal process to the antenna (Figure 3e), *R. maidis* is nevertheless a problem for the taxonomist, as there is increasing evidence that the name is being applied

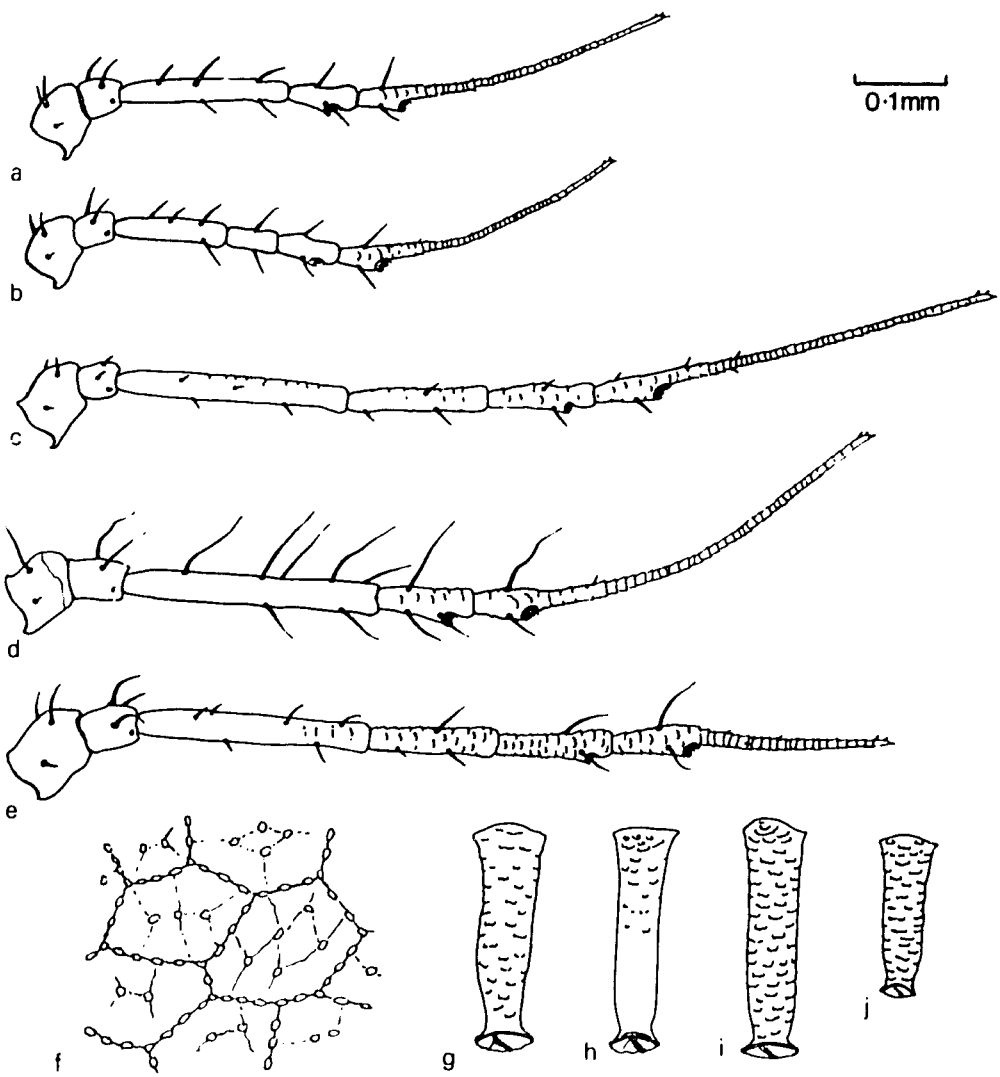

Figure 3. Some morphological characteristics of *Rhopalosiphum* spp. apterae. (a-e) Antennae of apterae of (a) *insertum*, 5-segmented form; (b) *insertum*, 6-segmented form; (c) *padi*; (d) *rufiabdominalis*; (e) *maidis*; (f) Dorsal abdominal sculpturing characteristic of *Rhopalosiphum* spp. (g-j) Left siphunculus of apterae of grass-feeding generations of (g) *insertum*; (h) *padi*; (i) *rufiabdominalis*; (j) *maidis*.

to a number of separate taxonomic entities isolated by their permanent parthenogenesis. But as the number of separate lineages (= clones) that may exist throughout the world is virtually unlimited, how necessary or useful is it to recognize these that occur, for example, in any one year in Kansas, USA?

A partial answer seems to be that, while it is clearly impractical to define the properties of every clone of *R. maidis* from every part of the world, populations can be grouped into certain major divisions, recognizable particularly according to their karyotype and their association with certain host plants. A form with 10 chromosomes is found predominantly on barley and panicoid grasses in the northern hemisphere. It has a slightly longer terminal process to the antenna (Figure 4), and can be distinguished by multivariate analysis from populations with 8 or 9 chromosomes that occur throughout the world on maize and sorghum (Blackman et al. 1987, Brown and Blackman 1988). As in the case of *Melanaphis sorghi/sacchari* discussed previously, the

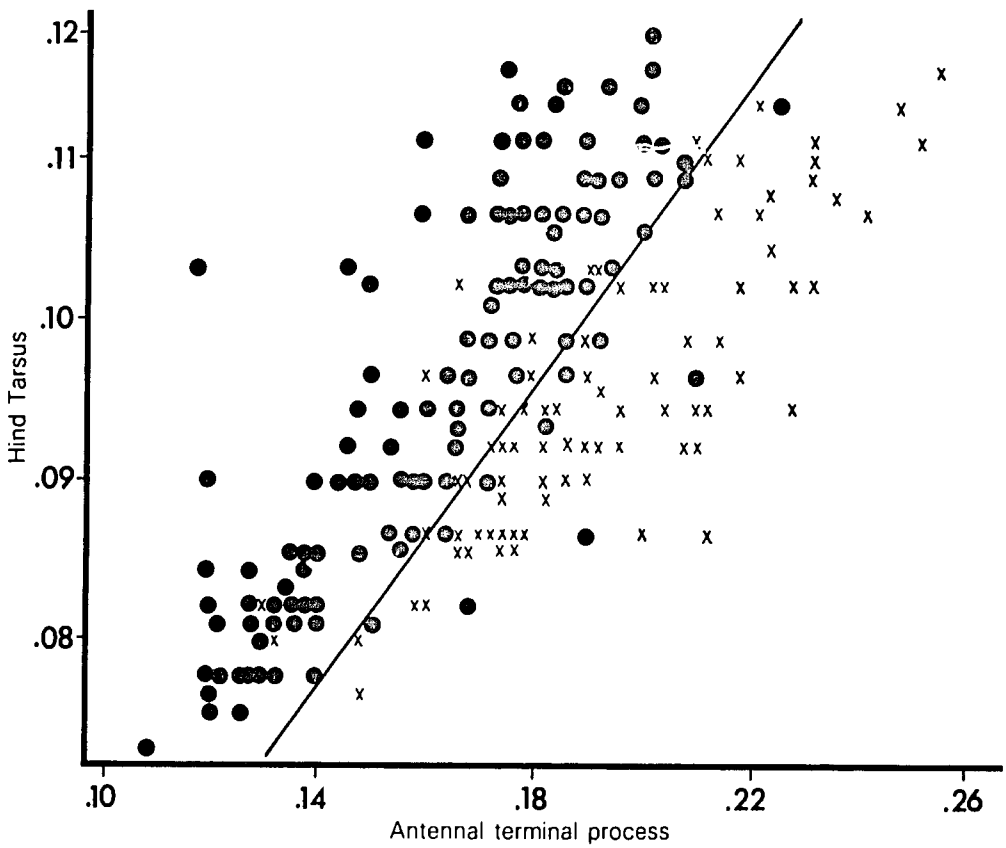

Figure 4. Plot of the measurements (in mm) of the length of the second segment of the hind tarsus against the length of the antennal terminal process for apterae of *Rhopalosiphum maidis* from all parts of the world, showng how those with 10 chromosomes (crosses) tend to have a longer antennal terminal process than those with 8 or 9 chromosomes (circles).

host plant association is not absolute and seems to depend to some extent on which hosts are available. Nevertheless anyone working with *R. maidis* needs to take into account the possibility, and in some cases the probability, that populations on different hosts would be better regarded as separate taxonomic entities. More karyotypic and morphometric studies need to be conducted on a world-wide scale in order to clarify this rather complex situation. At the same time, studies at a more local level can use enzyme electrophoresis to investigate clonal structure of *maidis* populations, and the extent of their migration (see, for example, Steiner et al. 1985).

Rhopalosiphum padi (L.) is rather specific in its choice of primary hosts, migrating mainly to *Prunus padus* L. in Europe and to *P. virginiana* L. in North America. Its secondary hosts are numerous species of Gramineae as well as many other monocotyledenous plants, and some dicotyledenous weeds such as *Capsella bursapastoris* (L.) Medic. *Lolium perenne* L. is a particularly favored host. The sexual phase on *Prunus* is dispensed with wherever winter conditions permit.

Numerous studies on the ecology and population dynamics of *R. padi* in recent years have addressed such topics as the timing of sexuales production (Ward et al. 1984); selection of primary hosts by gynoparae (Leather 1986); overwintering survival of eggs on *P. padus* (Hand and Hand 1986); overwintering survival of parthenogenetic generations on grasses and weeds (Dedryver and Gellé 1982), and on cereals (Wiktelius and Ekbom 1985); development of populations on autumn-sown cereals (Pike and Schaffner 1985); development of spring populations on the primary host (Dedryver 1983); fecundity of spring migrants (Wiktelius and Chiverton 1985); and the finding (Ahman et al. 1985) and selection (Leather and Dixon 1982) of secondary host plants. Spring migrants from *Prunus* are reported to have a greater readiness to colonize cereals than alatae from grasses (Leather and Dixon 1982); and if this is generally true it could help to explain why *R. padi* in Europe is most important as a pest of spring-sown

Leather and Dixon's results is difficult to assess without more information about the origin of the test aphids and the number of clones involved.

Another consideration seems to have been left out of all these studies. When a pest aphid has a variable life cycle, maintaining the "options" from year to year of either sexual reproduction followed by overwintering as eggs, or anholocyclic overwintering by continuous parthenogenesis, it is important to try to determine how the system works genetically, in order to understand how the integrity of the species as a whole is maintained. Is there any gene flow between the clones that overwinter parthenogenetically and those that produce sexual forms in autumn? Without some means of "dipping into the gene pool," anholocyclic populations would consist of isolated lineages that might be expected to show some divergence of genetic structure and properties from those that continue to have an annual sexual phase. In *Myzus persicae* (Sulzer), the production of males in autumn by otherwise anholocyclic clones (androcycly) provides the means for maintaining some gene flow, as well as a possible mechanism for maintaining the alternative methods of overwintering from year to year (Blackman 1974). In *R. padi* and other important cereal pests that frequently overwinter anholocyclically (*Metopolophium dirhodum*, *Schizaphis graminum* (Rondani), *Sitobion avenae*) we still cannot answer this basic question about their population genetics.

Apterae of *R. padi* are distinguishable from those of *R. insertum* and *R. rufiabdominalis* by their short antennal hairs (although, as discussed for *R. insertum*, hair length is very dependent on rearing conditions) and always 6-segmented antennae (Figure 3a-d). Methods of discriminating the various alate morphs from those of *R. insertum* are provided by Stroyan (1984).

Rhopalosiphum rufiabdominalis (Sasaki) has a sexual phase on various *Prunus* spp. in Japan, but it is permanently parthenogenetic on roots of rice and many other Gramineae in most parts of

the world, especially warmer regions. It also colonizes the roots of some Solanaceae. In North America it appears to have recently extended its range northward into Canada, where it has been shown to transmit RPV-like, RMV—like and nonspecific isolates of BYDV to cereals (Paliwal 1980). It has been confused in the past with *R. padi*, but is readily distinguished from this species and from *R. insertum* by its much longer antennal and dorsal body hairs. The terminal process of the antenna is also rather noticeably curved (Figure 3d). The ecology of *R. rufiabdominalis* as a pest of rice is reviewed by Yano et al. 1983, but little is known of its biology and importance as a BYDV vector.

Schizaphis

The 40-or-so species of *Schizaphis* have affinities with *Melanaphis* and *Rhopalosiphum*, but the alatae differ by invariably having forewings with once-branched media (always twice-branched in *Melanaphis*, and usually so in *Rhopalosiphum*). In cereal-feeding *Schizaphis*, both cauda and siphunculi are mainly pale in color, and the siphunculi are longer than the cauda and taper from base to apex with only a small apical flange (Figure 1). Like the other two genera, *Schizaphis* undoubtedly have a primitive association with Pyroidea as primary hosts, but most species have lost this association and are monoecious on Gramineae or Cyperaceae.

Schizaphis graminum (Rondani) provides a very good example of the limitations of the biotype concept. The available evidence suggests that *S. graminum* is a Palaearctic species that has been introduced into North America on (at least) two separate occasions, the first in about 1882 and the second in about 1968. In both cases there were severe economic consequences for cereal growers in the Great Plains area of the USA, probably because strong, adaptable genotypes were being presented with large acreages of susceptible host plants free of natural enemies. There were obvious founder effects; the second introduction ("biotype C") differed morphologically from the first in certain respects, rapidly established itself as a major pest of sorghum, and later acquired insecticidal resistance.

In about 1980, populations of *S. graminum* in the Great Plains area were found to have the ability to colonize varieties of wheat and sorghum that were previously resistant to other known biotypes, and were designated as "biotype E" (Porter et al. 1982). Recently, the host plant responses of samples of *S. graminum* from Oklahoma, Ohio, and Maryland were compared with the three extant Great Plains biotypes (B, C, and E), and found not to conform with any of them, the Ohio culture being regarded as sufficiently distinct to be designated a new biotype, "F" (Kindler and Spomer 1986).

S. graminum is regularly holocyclic in the northern USA (Webster and Phillips 1912), overwintering particularly on *Poa pratensis* L. (a host plant of palaearctic origin, like the aphid). Further south, biotypes C and E both readily produce sexuales and eggs in the laboratory under appropriate conditions, but completion of egg diapause requires over 3 weeks at temperatures at or below 1.7°C (Puterka and Slosser 1986). In the Great Plains area, with conditions mainly favoring anholocyclic overwintering rather than egg development, large crop monocultures will favor single, well-adapted aphid genotypes and thus promote the observed "biotype phenomenon." A much-needed genetic approach has recently been adopted by Eisenbach and Mittler (1987a, b), who have obtained some evidence that a) males of biotype E prefer to mate with their own oviparae rather than the oviparae of biotype C, and b) the ability of biotype E to colonize sorghum IS 809 may be maternally inherited (although the numbers of clones studied were too small to rule out Mendelian inheritance). These findings indicate other factors that may be contributing to the maintenance of the independent status of the Great Plains biotypes.

Multivariate morphometrics has also recently been used to separate samples identified by their host plant responses as biotypes B, C, and E (Fargo et al. 1986; Inayatullah et al. 1987). However, the method of canonical variates analysis is a very powerful tool that can separate individual genotypes, so there is no guarantee that the morphological characters correlated with host plant

responses in the three samples studied would be the same if samples from other sources were analyzed. What is needed is a more extensive analysis of clones established from numerous sources, including comparison between regions where anholocyclic overwintering predominates and areas with cold winters where a holocycle is the general rule, to see whether clones grouped according to the recognized biotypes. Electrophoretic studies could also contribute information about the number of "meaningful taxonomic units" involved.

Apart from the North American biotype problem, there are a number of close relatives of *S. graminum* that are very similar in morphology but more specific in their host plant relationships. *S. hypersiphonata* Basu has a longer antennal terminal process, longer siphunculi, and longer hairs on the 8th abdominal tergite than *S. graminum* and occurs in the Indian subcontinent, Southeast Asia, and Australia. Although most frequently found on *Digitaria*, it has been collected on cereals in Australia and the Philippines, but it is not recorded as a BYDV vector and is not a damaging pest. Orlob (1961) found an aphid similar to *S. graminum* feeding specifically on *Phleum pratense* L. in New Brunswick, Canada, and this aphid transmitted BYDV with rather low efficiency in laboratory tests. Other close relatives of *S. graminum* currently regarded as subspecies occur respectively on *Agrostis* spp. and *Holcus* spp. in Europe (Stroyan 1984).

Sipha

Sipha are small aphids with thick, spine-like hairs and short stump-shaped siphunculi. They live all the year on Gramineae. *Sipha glyceriae* (Kaltenbach), which can be injurious to rice crops, and *S. agropyronensis* (Gillette) have been tested as BYDV vectors and found not to transmit, but the three other species found commonly on cereals have not, to our knowledge, been tested. They are: *S. maydis* Passerini (in Europe and Asia eastward to India and Pakistan, and introduced into South Africa); *S. flava* Forbes (in North and South America); and *S. elegans* del Guercio (=*agropyrella*) (in Europe, Central Asia and introduced into North America). *Sipha* tend to infest cereal crops mainly in warm, dry climates. In central Europe *S. elegans* is more abundant in dry summers and on sparse stands of cereals (Honek 1985).

Sitobion

About 75 species are currently placed in the genus *Sitobion*. About 20 species are associated with Gramineae, and several of these overwinter on Rosaceae (*Rosa* or *Rubus*) or other plants (*Akebia*). The known cereal virus vectors may be recognized by their tapering black siphunculi bearing an apical band of polygonal reticulation. They are generally distinguishable as three species (*S. avenae*, *S. miscanthi* (Takahashi) and *S.fragariae* (Walker)) by the shapes and relative lengths of the cauda and siphunculi (Figure 5). In Europe *S. avenae* and *S. fragariae* are distinct, but *S. miscanthi* in the Far East and

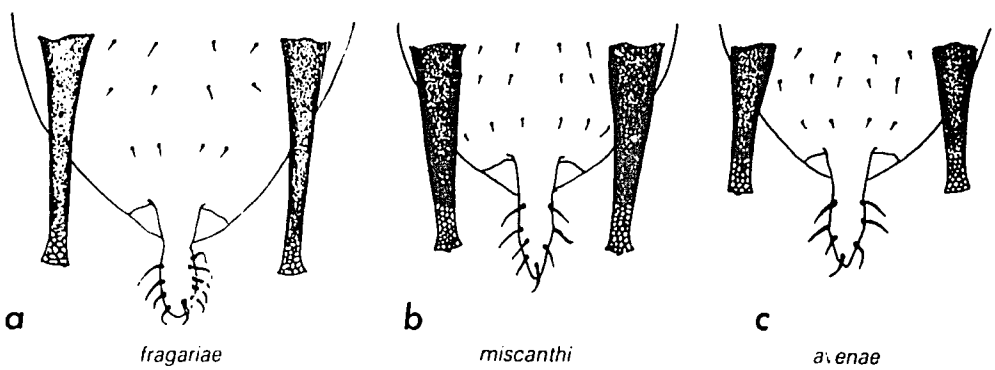

Figure 5. Rear of abdomen in dorsal view of typical apterae of cereal-feeding *Sitobion* spp.

Australia is somewhat intermediate between these two in its morphology (e.g., Figure 6), and has been confused with both of them.

Sitobion avenae (F.) occurs on many Gramineae including barley, wheat, and oats as well as some Cyperceae and Juncaceae, and it is occasionally found on the leaves of cultivated *Gladiolus* and

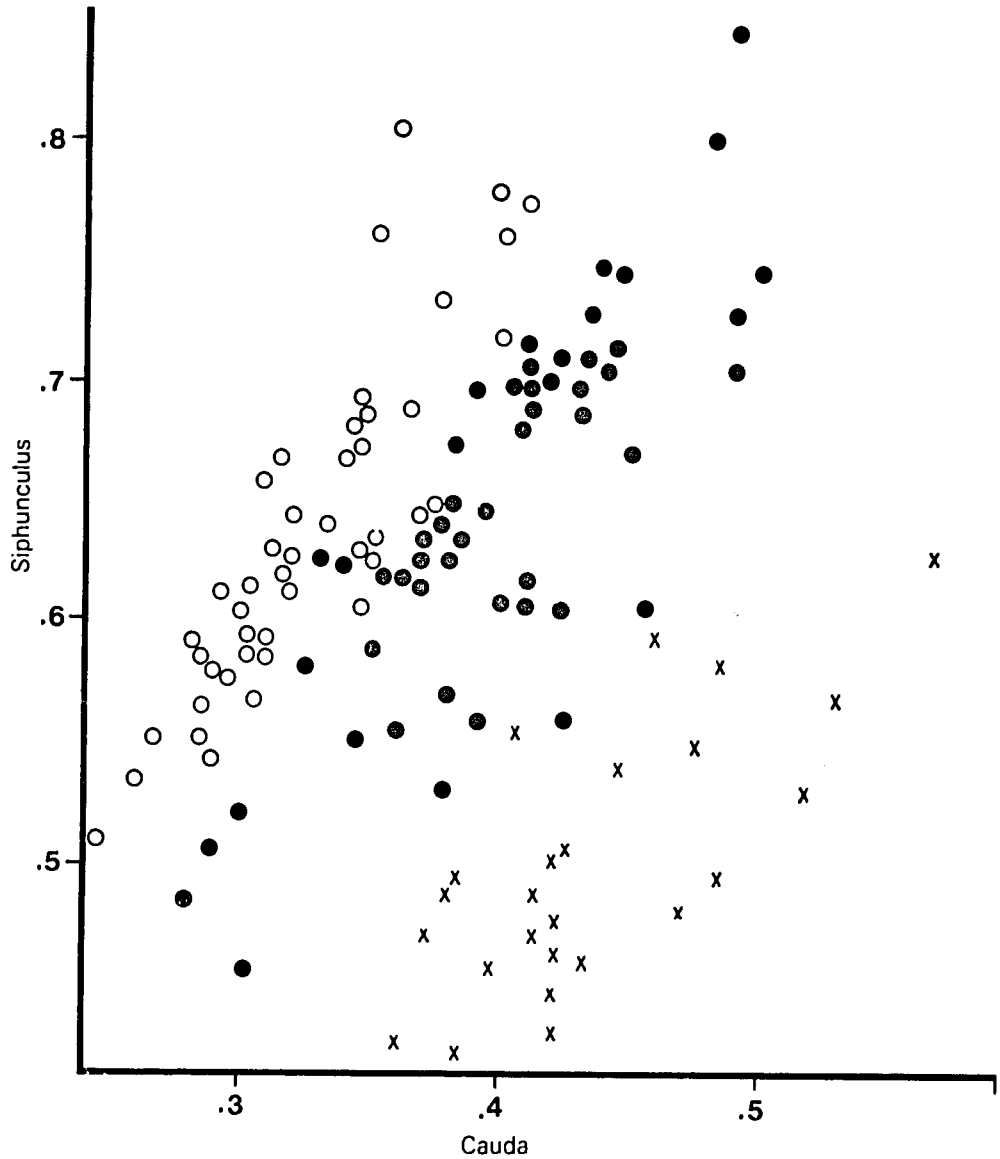

Figure 6. Plot of the measurement (in mm) of length of siphunculus against length of cauda for alatae of *Sitobion* spp. from various parts of the world, showing differences between *fragariae* (open circles), *miscanthi* (black circles) and *avenae* (crosses).

Iris (Iridaceae). It is widespread in Europe and the Mediterranean region, and eastward to Iran. It has spread through North and South America and was more recently introduced into Africa south of the Sahara (first records from Kenya 1964, South Africa 1971, and Zimbabwe 1976).

A form with rather paler bases to the siphunculi occurs in the Mediterranean region and eastward to Pakistan, Kashmir, Punjab, and Uttar Pradesh. The taxonomic status of these populations is uncertain, as British *S. avenae* reared at high temperatures retain the dark bases to the siphunculi. Possibly it is a single anholocyclic clone.

Far Eastern and Australasian records of *S. avenae* mostly or entirely apply to other species, principally *S. miscanthi*.

Although sexuales of *S. avenae* occur, many populations overwinter parthenogenetically in temperate climates, even through quite cold winters (Hand and Hand 1986). Weber (1985a) found that populations in a river valley contained anholocyclic clones, destined to overwinter parthenogenetically, whereas genotypes from more exposed areas all produced sexuales. He also found genetic adaptation to food plants. Other recent studies of variation within *S. avenae* were by Walters and Dixon (1982), on ovariole number and behavior, and by Loxdale and Tarr et al. (1985a, b) on enzyme systems.

The enormous literature on *S. avenae* includes recent studies of fecundity (Watt 1984), temperature requirements (Lykouressis 1985), production of sexual morphs (Hand and Wratten 1985), feeding behavior relevant to virus transmission (Scheller and Shukle 1986), and simulation models of the effect of natural enemies (Vorley and Wratten 1985). Population forecasting has received much attention; e.g., Watt et al. (1984), Dewar and Carter (1984), Vorley and Wratten (1985) and Entwhistle and Dixon (1986). Watt et al. (1984) discuss the economics of control practice. Every year the Review of Applied Entomology summarizes numerous articles concerning *S. avenae*.

Sitobion fragariae (Walker) overwinters in the egg stage on *Rubus* and probably sometimes on *Rosa*, and occurs in the summer on many Gramineae including wheat, barley, and oats. It may also be found on Juncaceae, on the flower stalks of Cyperaceae, and on the leaves of some Liliaceae (e.g., *Fritillaria*). It is widespread in Europe and the Mediterranean region, eastward to Iran. Since at least 1941 it has occurred in the USA west of the Rockies (California, Oregon, Washington), where it overwinters on *Rubus* and is common on *Bromus* in early summer. More recently it was introduced to South Africa (first record 1969), Tasmania (1965), mainland Australia (1972), the Antipodes (1969), and New Zealand (1971).

Some large alatae from Australian populations (and one specimen from New Zealand) bear secondary rhinaria on the 4th antennal segment, a character unknown for *S. fragariae* from anywhere else in the world (108 specimens from 48 samples from 11 countries from 1847 onward were examined). The genotype introduced to Australasia is therefore unusual and there must be some doubt about its identity.

Sitobion miscanthi (Takahashi) occurs on many Gramineae including barley, wheat, and oats, and also on some herbaceous dicotyledons, including *Capsella* and *Polygonum hydropiper* L. It occurs in Japan, China, India, Bangladesh, Malaya, Indonesia, Philippines, Australia, New Zealand, Fiji, Hawaii, and Tonga. *S. akebiae* (Shinji), which overwinters in the egg stage on *Akebia* and other plants in Japan (Miyazaki 1971), is closely related to and possibly identical with *S. miscanthi*.

S. miscanthi was common in Australia and New Zealand in 1959 and 1960, and all samples received from this area by the British Museum (Natural History) between 1928 and 1964 were *S. miscanthi*. Between 1965 and 1971, however, it seems to have been largely replaced by *S. fragariae*. Since 1965 two of the three samples of *Sitobion* received from Australia and all of the many samples from New Zealand have been *S. fragariae*.

Possible Virus Sources and their Vectors outside the Crop

Certain aphid species have been recorded as experimental vectors of members of the BYD group of viruses, but are unlikely to be of any importance in the field because their normal hosts are not Gramineae. They include: *Aphis glycines* Matsumura, which feed on *Glycine soya* Max. and other legumes in eastern Asia and some Pacific islands; *Aulacorthum (Neomyzus) circumflexum* Buckton, which colonizes many monocotyledons, particularly Araceae and their relatives, as well as some dicotyledons, but rarely Gramineae; *Cavariella salicicola* Matsumura, which migrates between *Salix* and a few genera of Umbelliferae in the Far East; *Ceruraphis eriophori* (Walker), which migrates between *Viburnum* and mainly Cyperaceae in Europe and North America; *Myzus persicae* (Sulzer), very polyphagous and sometimes found on grasses such as *Lolium perenne*, but unlikely to remain on cereals long enough to be an effective vector; and *Tuberocephalus momonis* (Matsumura), which migrates between *Prunus* and an unknown host in the Far East.

Rhopalomyzus poae (Gill) is a Gramineae-feeder and a recorded vector of BYDV (Orlob et al. 1961), but unlikely to be of any significance in crop habitats. It is a small, shiny brown-black aphid living on the basal parts of grasses close to soil level and often on etiolated stems under stones.

Perhaps more worthy of consideration are the numerous Gramineae-feeding aphids that have not been tested for BYDV transmission. About 250 species in more than 50 genera feed on Gramineae. Two of the main groups of genera, in the subfamilies Hormaphidinae (*Astegopteryx*, *Ceratovacuna*, *Pseudoregma*, etc.) and Drepanosiphinae (*Takecallis*, *Phyllaphidoides*, etc.) only occur on bamboos. The role of bamboos as reservoirs of BYDV in areas where they are used as windbreaks for horticultural crops has not been investigated. Such bamboo windbreaks are often also colonized by *Rhopalosiphum padi* and *R. maidis* living under the old leaf bases.

Members of the small family Israelaphidinae feed entirely on Gramineae. There are three species known only from the Mediterranean region and their alatae seem to be rare, so they are unlikely to be significant as virus vectors. In addition there are about 16 other small genera of Aphidinae, each with a few species living on Gramineae. Some of these aphids live on the leaves or flower stems but others feed inconspicuously on the leaf bases in turf. Any of these are potential introducers of BYD-type viruses from permanent pastures and wild grasses to cereals.

Concluding Remarks

Traditional taxonomic methods erect a classification framework and provide check-lists of species based on perceived morphological differences. However, some groups of organisms are studied more intensively on account of their economic or medical importance, and in these groups it is almost invariably the case that taxonomic relationships at the species level need further revision, because the conventionally recognized species are not in reality the lowest "meaningful taxonomic units."

The well-developed polymorphism of aphids has meant that experimental methods, especially rearing and host plant transfers, have for many years played a major part in addition to morphology for species recognition. Nevertheless, most of the more important crop pests aphids are highly adaptable, relatively polyphagous and widely distributed, and therefore subject to diverse selection pressures. Genotypes best fitted to the time and place will always tend to be favored, and in warm climates permanent parthenogenesis may cause populations to consist of one or a few selected genotypes (the "biotype phenomenon").

It is axiomatic in biology that the taxonomic status of a particular clone or population needs to be defined as rigorously as possible. We have tried to show in this paper how we believe that the necessary rigor is lacking in the case of most cereal aphids. More work needs to be done. In particular, more knowledge is required of the genetic structure of cereal aphid populations, the

nature and consequences of life cycle variation, and the extent of specialization to particular host plants. Experimental studies in which comparisons are made between clonal laboratory populations, multivariate morphometrics, electrophoresis, DNA comparisons, and karyological studies all have a part to play in acquiring the necessary information.

Acknowledgments

We are grateful to all those who have provided samples for cytological and morphological studies. Their help will be individually acknowledged in fuller accounts to be published elsewhere.

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The Epidemiology of Barley Yellow Dwarf in Europe

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Abstract

Although susceptible hosts are present in most of this area, knowledge of the presence of barley yellow dwarf virus (BYDV) is limited to regions where small grain cereals are widely grown. Based on the classification of W.F. Rochow, only MAV-, PAV- and RPV-like strains are known to occur. Although Rhopalosiphum maidis *(Fitch) and* Schizaphis graminum *(Rondani) are present, the RMV- and SGV-like strains have not been recorded. The epidemiology of BYDV is influenced principally by local cropping patterns and the relative occurrence and importance of holocyclic and anholocyclic forms of the main aphid vectors.*

In northern regions the dominant seasonal influence is winter cold and survival of vectors as eggs on rosaceous or graminaceous hosts is most important, whereas in the Mediterranean area the most important influence is summer drought, and here bridging crops such as maize appear to play an important part in the carryover of vectors and virus from one cereal to the next. There is no good evidence for long distance transport of aphids, although there is some evidence that Sitobion avenae *(Fabricius) may move into Scandinavia is some years causing outbreaks of MAV-like isolates in areas where* R. padi *(L.) transmitted strains usually predominate. The epidemiology of BYDV is illustrated in relation to regional differences in cropping practices, aphid populations, and climate.*

Europe is an area that is difficult to define geographically and politically. To a Briton "Europe" starts on the other side of the English Channel, to a European civil servant in Brussels it is the twelve member countries of the European Economic Community. Even the United Nations's Food and Agriculture Organization (FAO) has difficulty in defining the area, often including USSR with "centrally planned economies" rather than with Europe. A more generally acceptable definition is that area of land stretching from the southernmost part of Greece (35°N) to the North Cape (71°N) and from the west coast of Ireland (10°W) almost to the Ural Mountains in USSR (45°E), an area 1/14th of the earth's land surface, stretching 4000 km north to south and 5600 km east to west (Figure 1).

The climate of the region ranges from the typical continental climate of hot summers and very cold winters in Kiev to the Mediterranean climate of mild, wet winters and hot dry summers of Naples and Seville, and from the cold winters and warm summers of Scandinavia (Stockholm) to the wet mild winters and cool wet summers of the northwest (Cambridge) (Table 1).

Conditions cause an equally varied response in the distribution and biology of the principal aphid vectors of barley yellow dwarf virus (BYDV) and have an overriding effect on the crops that can be grown. Such a range is perhaps no more exceptional than a comparable land mass, nevertheless, there are climatic influences that differ from those experienced, say, in North America. The predominant movement of weather systems is from west to east. They develop in the Atlantic and move across Europe, farther north or south depending on the season. Those systems have a dominant influence on the weather in western Europe, but their influence wanes farther east. The prevailing wind direction, west or southwest, runs approximately at right angles to the seasonal changes. Thus agricultural changes in southern Europe have little influence in northern regions. The transport by winds of aphids from Mediterranean regions where cereals ripen in May/June/July to Britain or southern Scandinavia where harvest is 2 to 3 months later seems to be a rare event (Dewar et al. 1980), although movements over shorter distances can be important.

The political complexity of Europe is probably as great a difficulty in obtaining a complete picture of barley yellow dwarf (BYD) as the climatic, geographical, and agricultural complexity. Information on the occurrence and importance of the disease and of the vectors and their movements is very uneven. It is difficult to know whether the absence of comment in the literature on the occurrence and importance of the disease is a true reflection of the position or a lack of recognition of the problem. However, a disease identified as BYD appears to have been reported in one or another form in all countries in Europe and generally appears to cause losses, of decreasing severity, on barley (*Hordeum vulgare* L.) and oats (*Avena sativa* L.), wheat (*Triticum aestivum* L.), and maize (*Zea mays* L.); losses on grasses are recognized but more difficult to define.

In general, the disease has been studied most in countries where average yields are highest (Table 2). Thus interest has generally been greatest in northern and northwestern areas and only more recently in Mediterranean regions. However, this also reflects the predominant crops grown—oats and barley in the north, wheat and barley in central regions, and wheat and maize in the south—and the importance of susceptible crops. Another important influence on BYD epidemiology is the

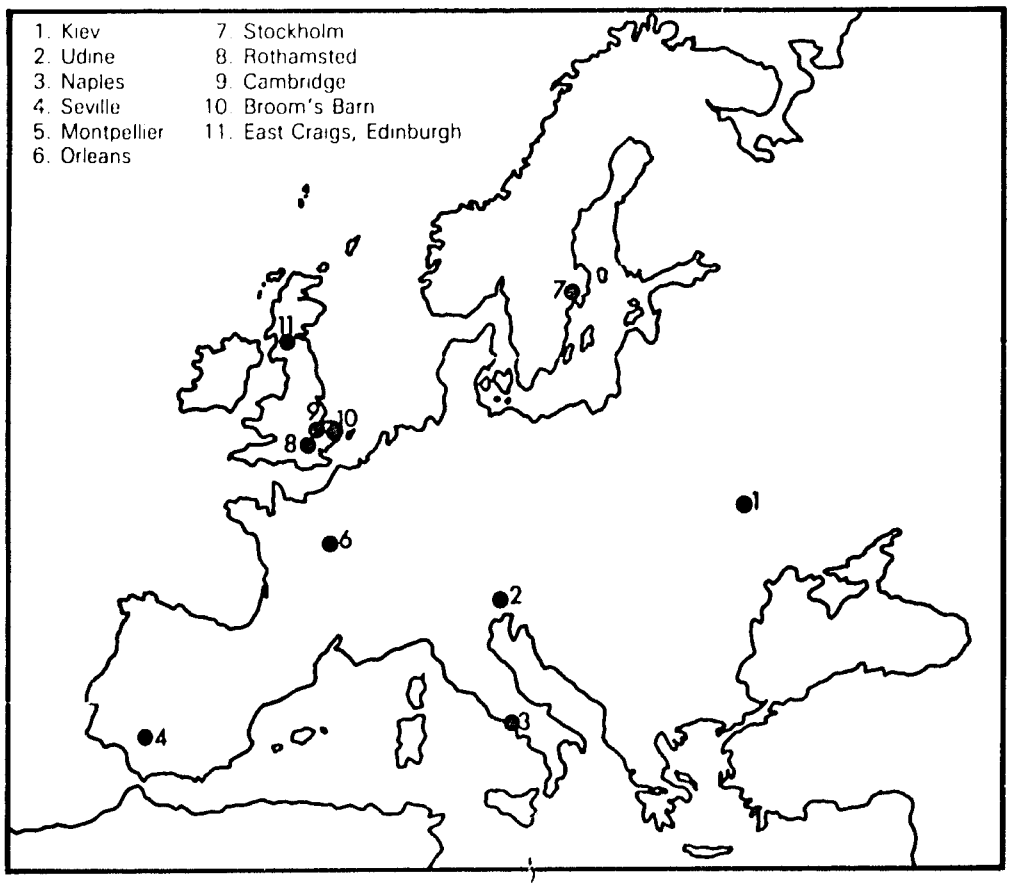

Figure 1. Outline map of Europe showing places mentioned in the text.

relative area of susceptible annual crops (cereals) and perennial reservoir (grass) to the agricultural economy of each country (Table 3).

Because of these wide differences of interest it is perhaps not surprising that there has been little coordination of results. The entomologists have been rather more cooperative than the pathologists; they have established a network of suction traps, some of the results of which are described later. However, there have recently been moves to try to establish the relationships between the various isolates of BYDV that are maintained in laboratories working on BYDV.

In the comments that follow an attempt is made to draw together some of the disparate information on BYD epidemiology in Europe, both in an attempt to provide a more coherent picture than before and also to see if any general principles can be established.

Vectors

Of the 23 potential vectors listed by A'Brook (1981), perhaps the largest representation in one area is found in Italy (Udine, Figure 1), where Peressini and Coceano (1986) listed 14 species (Table 4) known to be vectors of BYDV that had been caught in a suction trap. However, few of them have been recognized as sufficiently common as

Table 1. Weather data from representative European sites

	Cambridge (UK)		Stockholm (Sweden)		Naples (Italy)		Seville (Spain)		Kiev (USSR)	
Month	Mean °C	Rain mm	Mean °C	Rain mm	Mean °C	Rain mm	Mean °C	Rain mm	Mean °C	Rain mm
J	3.5	49	-2.9	43	8.5	122	10.3	73	-6.1	43
F	3.9	35	-3.1	30	8.9	88	11.6	59	-5.2	39
M	6.1	36	-0.7	26	10.8	63	14.1	90	-0.5	35
A	8.7	37	4.4	31	13.8	59	16.4	51	7.6	46
M	11.9	45	10.1	34	17.4	51	19.2	36	14.7	56
J	15.1	45	14.9	45	21.4	21	23.4	9	18.6	66
J	17.0	58	17.8	61	23.6	16	26.3	1	20.4	70
A	16.7	55	16.6	76	23.8	27	26.3	5	19.3	72
S	14.3	51	12.2	60	21.0	92	23.7	25	14.2	47
O	10.3	51	7.1	48	16.9	118	19.2	66	7.5	47
N	6.7	54	2.8	53	13.0	129	14.5	68	1.4	53
D	4.5	42	0.1	48	9.9	136	1.0	76	-2.9	41
Total		558		555		922		559		615

Table 2. Mean yields of wheat from the most and least productive European countries in 1985 (FAO production yearbook)

Country	Yield (t/ha)
Portugal	1.24
Greece	2.00
Spain	2.63
UK	6.15
Ireland	6.25
Netherlands	6.65

Table 3. Areas of representative countries growing arable and pasture crops, 1985 (FAO production yearbook)

Country	Arable	Pasture 000/ha	Arable:Pasture
Ireland	970	4865	0.2
Greece	2948	5255	0.6
UK	6930	11650	0.6
Norway	855	90	9.5
Denmark	2624	228	11.5
Finland	2335	130	18.0

vectors to justify detailed study. Thus, throughout Europe three species *Rhopalosiphum padi* (L.), *Sitobion avenae* (Fabricius) and *Metopolophium dirhodum* (Walker) predominate, and only *R. padi* can claim to be of importance everywhere. Vectors of occasional local importance in the warmer regions are *Schizaphis graminum* (Rondani) and *Rhopalosiphum maidis* (Fitch).

However, even for the three most common species their numbers and distribution throughout the year differ widely from region to region. Everywhere in Europe winter temperatures (Table 1) restrict aphid development and movement, although in Mediterranean regions this restriction is much less than farther north. A typical distribution of the

Table 4. Aphid species known to be vectors of BYDV caught in a suction trap in northeast Italy (from Peressini and Coceano 1986)

Anoecia corni
Hyalopterus pruni
Metopolophium albidum
M. dirhodum
M. festucae
Myzus persicae
Rhopalosiphum insertum
R. maidis
R. padi
R. poae
Schizaphis graminum
Sipha elegans
Sitobion avenae
S. fragariae

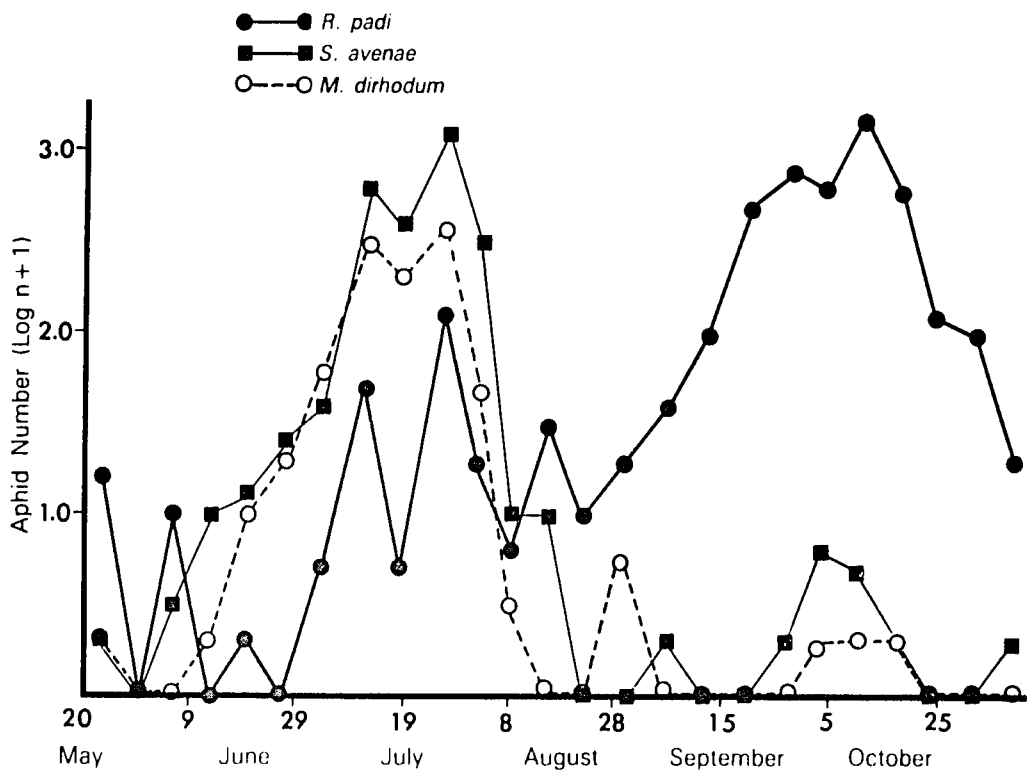

Figure 2. Number of cereal aphids caught in the Rothamsted Insect Survey suction trap at Rothamsted in 1985.

principal BYDV vectors as recorded in suction traps operating at 12.2 m at Rothamsted is given in Figure 2. Three distinct migrations can be distinguished, broadly defined as spring, summer, and autumn. However, not all species occur equally frequently in each migration. For example, *M. dirhodum* is presented in significant numbers only during the summer migration at Rothamsted (Figure 2). In 1979, when *M. dirhodum* was especially numerous in western Europe, a similar dispersal pattern was seen at Orleans (France), Broom's Barn (England) and East Craigs (Scotland) to that seen in Figure 2; in southern France the numbers and occurrence were more variable (Dewar et al. 1980). The observations made in that year also support the view that aphids caught in suction traps were principally derived from local populations rather than from more southerly regions where crops ripen earlier.

In Britain, the most numerous summer species is *S. avenae* or *M. dirhodum*, but in the autumn *Rhopalosiphum* species are often 100 times the number of all other BYDV vector species. This is a pattern that is also true for some regions of western France, but in Scandinavia (Wiktelius 1982) and northern Italy (Conti and Casetta 1983) *R. padi* is the most numerous aphid at all times of the year, although in Scandinavia its relative preponderance at different seasons differs widely from year to year.

Not only do the numbers and distribution differ in different regions but so does the morph, especially the presence of sexual forms in late summer and autumn. All three of the principal vector species have a sexual cycle in Europe, but its extent and importance in surviving over winter differs from north to south. *R. padi* and *M. dirhodum* have rosaceous species as primary hosts, *Prunus padus* L. and *Rosa* spp. respectively. *S. avenae* can complete its life cycle on Gramineae, although supporting evidence through the presence of eggs and males is limited and the work of Loxdale et al. (1985) in the UK suggests that *S. avenae* does not have a sexual cycle there, which may indicate that it rarely overwinters in this way throughout Europe.

The primary host of *R. padi* is present in all European countries (Tutin et al. 1968) but is most frequent naturally in northern and upland regions, although planted widely as an ornamental elsewhere. There is no shortage of the primary hosts of *M. dirhodum* but little knowledge of the importance of the sexual cycle. *M. dirhodum* can survive over winter on Gramineae as far north as southern England (George 1974) and the sexual cycle is probably rare or absent farther south. Vickerman and Wratten (1979) give further details of cereal aphid life cycles.

The life cycle of virus vectors is important in the epidemiology of BYD for a number of reasons. Survival as viviparae allows rapid multiplication given favorable conditions in spring, and on a host that could be infected with BYD. Survival as eggs breaks the virus cycle and delays migration. The sexual cycle also provides a mechanism for overcoming variable conditions. Clearly in Scandinavia aphids can only survive as eggs. In much of western Europe winters are not so consistently severe and a mixture of sexual and viviparous survival allows the aphids to cope with, and exploit, whatever conditions occur. In the Mediterranean region, where viviparous survival appears to be the rule, it is unclear whether the possibility of a sexual cycle exists, and unusually cold weather in these regions may have greater adverse effects than farther north.

In Britain, measurements of the BYDV infectivity of the early aphid migrants have shown how the species differ in when they are caught in suction traps and when they carry virus (Table 5). Clearly in spring *R. padi* are much more likely to infect crops when young than

Table 5. Mean date of first capture and first infective aphid (1971-80)

Species	First Capture	First Infective	Interval
R. padi	19 May	30 May	11 days
S. avenae	23 May	19 June	27 days
M. dirhodum	3 June	6 July	33 days

are the other two species, and the absence of infective M. *dirhodum* before July, by which time crop growth is well advanced, indicates that this aphid is relatively unimportant in virus epidemiology even when present in large numbers as in 1979.

Although it is generally accepted that persistent transmission by aphids is the only method of virus spread, it should be noted that there have been reports from Europe of transmission through seed (Szirmai 1979) and by the larvae of the shoot-boring fly, *Oscinella frit* (L.) (Jess and Mowat 1986). However, the extent and importance of such transmission is uncertain. BYDV is detectable in fresh green and dried seeds of oats but this should not be confused with seed transmission (Eweida 1985).

Virus Isolates

Based on the classification of Rochow (1979) three isolates of BYDV are known to be present in Europe, the PAV-, MAV- and RPV-like isolates. However, knowledge of the presence of these isolates is very patchy, and despite a great increase in the use of serological detection, much of the classification of isolates is still based entirely on vector transmission. Although this provides valuable epidemiological knowledge, it seems increasingly clear that within the type isolates there is variation in vector and serological characteristics. Such variation seems particularly marked in those isolates designated as "PAV." The polyclonal antisera available, including those available commercially, do not distinguish between isolates within a group.

The availability of monoclonal antibodies (Torrance et al. 1986) has enabled a much closer examination of isolates (Table 6). None of our "type" isolates is exclusively transmitted by one aphid species and it is interesting that *M. dirhodum*, a species not used in Rochow's tests, is an almost equally efficient vector of all isolates. The two field isolates are interesting because BB 307 would be classified as PAV-like based on serology but is only inefficiently transmitted by *S. avenae* and relatively efficiently transmitted by *R. padi*. W 725 reacts with none of the monoclonals and is very efficiently transmitted by *R. maidis*. This raises the possibility that this may be an RMV-like isolate. It was originally isolated from southern England, where *R. maidis* occasionally occurs, usually on barley, on which populations can be very large. Current, coordinated efforts within Europe to use these monoclonals to examine relationships between the 'type' isolates from different laboratories may identify more isolates in which biological and serological designations do not match.

Although information is patchy, it does appear that the greatest variety of isolates occurs in western areas, especially UK and France, where at least all three isolates occur. However, this

Table 6. Reactions of the three 'type' UK strains and two field isolates to monoclonal antibodies and their efficiency of transmission by vectors (data from G. Herrera)

UK isolate	Rochow isolate	ELISA[a] MAC 91	MAC 92	MAFF 2	% transmission R.p.	S.a.	M.d.	R.m.	M.f.
G	PAV	1.911	0.022	0.068	72	20	26	16	2
F	MAV	0.012	0.006	0.451	9	55	38	2	20
R	RPV	0.020	1.274	0.019	66	0	21	13	9
BB 307	–	0.970	0.022	0.030	74	5	4	32	0
W 725	–	0.026	0.027	0.042	28	10	52	86	17

R.p. = *R. padi*, S.a. = *S. avenae*, M.d. = *M. dirhodum*, R.m. = *R. maidis*, M.f. = *M. festucae*.

[a] Enzyme-linked immunoabsorbent assay. Optical density at 405 nm.

may merely reflect how detailed examination of isolates has been. Nevertheless it does appear that the most common isolates in Scandinavia are *R. padi*-related, PAV-like (Sweden) (Eweida et al. 1985), RPV- and PAV-like (Norway) (T. Munthe, personal communication), which is not surprising, as *R. padi* is by far the most common vector. MAV-like isolates do occur but sporadically and may be brought by immigrant *S. avenae* from farther south (Denmark?). In the Mediterranean region MAV-like isolates again seem rare with PAV-like isolates predominating and transmission tests indicating the possible presence of RPV-like isolates (Panayotou 1980). This absence of MAV-like isolates is probably largely due to the difficulty experienced by *S. avenae* in surviving the hot dry summers, when most Gramineae are dry and unsuitable hosts. Maize, which supports populations of *R. padi*, is rarely colonized by *S. avenae*.

The isolate of BYDV known as "giallume" in northern Italy is especially associated with rice (*Oryza sativa* L.) (Osler 1984). It appears to be related to the PAV-like isolate, but further testing is required to establish if it is a locally important variant that differs serologically from other PAV-like isolates and has a unique epidemiological cycle involving rice and the associated grass, *Leersia oryzoides* L., or whether rice is just another host of a common isolate.

Plant Hosts

As illustrated by Table 3 there is no shortage of perennial and annual hosts for BYDV. Most attention has been given to the incidence of BYD in cereals, but when surveys have been made of perennial grasses they have always revealed widespread infection (Doodson 1967; Lindsten and Gerhardsen 1969; Huth 1971; Holmes 1977) and often the presence of isolates not present in the cereals growing in the same area (Plumb 1978), presumably because exposure over many years results, eventually, in infection of grasses by all isolates present in a wide geographic area, whereas in any one year the virus isolate(s) that infect cereals will be dictated by the immediately prevailing conditions.

Therefore, perennial grasslands in Europe can be considered a ubiquitous source of virus, even though some species present in natural grasslands and hedgerows may be immune. Grasses exploited agriculturally, to give short- or long-term pastures, especially *Lolium* spp., seem particularly susceptible. Yield losses in grasses are difficult to quantify but they can be substantial; and because infection can change the plant's growth habit there is also an interaction between virus infection and management, that is, whether the grass is grazed or conserved (Catherall 1966).

Studies of virus-induced damage in cereals show that when inoculated at the same growth stage oats and barley are generally damaged more than wheat; oats and barley (Plumb 1986) can be killed by severe isolates of the virus if infected when young, but wheat survives. However, there are interactions between virus isolates and host species so such effects are not always consistent. Damage to maize (Panayotou 1977; Eweida et al. 1983) seems to be less than in small grain cereals, but can be serious, and maize seems to become difficult to infect more rapidly than other Gramineae. Infected grasses and maize seem to support smaller concentrations of virus than wheat, barley, and oats, and appear to be poorer sources of virus for aphid acquisition, although they are often good hosts for some vectors. Whether these observations are connected is uncertain.

Aphids certainly demonstrate feeding preferences. *R. padi* seems fairly catholic in its tastes, whereas, in UK, *R. maidis* is rarely found on hosts other than barley. Dean (1973) claimed that *R. padi* preferred wheat, *S. avenae* oats, and *M. dirhodum* barley, however, such preferences must be slight as they are not clearly manifest in crops and seem unlikely to influence virus transmission. On grasses feeding preferences seem more distinct, but again these are differences of degree and seem unlikely to be epidemiologically significant.

Crop Infection

In most of Europe the cereal crops principally at risk from BYD are those sown in autumn. The extent of that risk is determined by four main factors

- Crop sowing date
- Populations of potential virus vectors
- Populations of infective vectors
- Spread of virus within crops

Autumn-sown crops

Sowing date—Sowing date is often determined by the availability of land; one crop has to be harvested before the next can be sown. In these regions, mainly in the northwest, the policy often adopted is to sow as soon as possible, for fear that delay may decrease yield potential and in some circumstances may prevent sowing. The pressure to sow as early as possible has increased over the last 10 to 15 years as there has been a substantial change, especially for barley, from spring- to winter-sown crops, and the area of wheat sown in autumn has also increased substantially (Jenkins 1985). Thus, in Britain, 20 years ago autumn-sown barley was an unusual crop and wheat was sown in mid-October. Now more barley is sown in autumn than spring and a normal sowing date for most barley crops would be in September.

Such flexibility of sowing date is only possible in a maritime climate where moisture is rarely limiting, but this change is the principal reason why BYD is now recognized as such a serious problem in these regions. Indeed such is the perceived risk of BYD that in southern Britain and some areas of western France and in Belgium, insecticide treatment of early sown crops is now almost routine. Local topographical or cropping practices influence virus infection but, in general, crops sown after the end of September in Britain, and rather later in France, are rarely infected sufficiently extensively to cause crop loss (Table 7). However, even September-sown crops do not always need treatment, and results from Rothamsted have indicated that in only 5 of the last 17 years was treatment to control BYD justified.

The option of sowing late to avoid infection is rarely taken because of the yield penalty and because the cost of pesticides required to control infection effectively is cheap, relative to the potential yield loss that might result from a severe attack.

Potential vector populations—European workers are fortunate in having an extensive network of suction traps, sampling aphid populations at 12 m above ground, that provide a continuous record of alate aphids present at each site. The most concentrated distribution of these traps is in Britain, where weekly bulletins are issued by the Rothamsted Insect Survey listing 33 aphid species caught in each of 22 traps. Seven of the listed species are known vectors of isolates of BYDV. Figure 2 shows clearly the risk associated with the large migration of *R. padi* often seen in the autumn. Favorable conditions for aphid flight, temperatures above 10 to 12°C, last for longer in the south, and in some regions aphids are active virtually the whole year. In northern regions flights cease by the end of September and potential vector populations are usually larger in summer than in autumn (Wiktelius 1987).

Infective vectors—In northern and western regions the autumn migration is primarily a return migration to primary hosts, but virginoparae are present as well as gynoparae and males. The relative proportions of these morphs have a large influence on the risk of infection by BYD. The source of these aphids is mainly grasses and stubble regrowths in northern and western regions, but in the central regions, especially France, and

Table 7. Infection by barley yellow dwarf virus of crops sown at different times in September and October at Rothamsted

Sowing date	Virus infection (%)[a]
13 September	21.2
23 September	4.5
2 October	3.0
11 October	0.5
23 October	0.0

[a] Mean of 4 plots.

the south, maize is most important. In some regions, depending on crop sowing date, cereal volunteers can act as an intermediate host between maize and the autumn-sown crop (Dedryver and Robert 1981).

In southern Europe the principal method of survival over winter is as viviparae and generally autumn migrations are smaller than farther north. Therefore from south to north there is a complete gradation from anholocyclic to holocyclic life cycles. The significance of this for virus transmission is that sexual forms of *R. padi*, the principal autumn migrant in all regions, are biologically unlikely to infect cereal crops irrespective of whether they acquired BYDV from the host on which they were produced. Therefore, the proportion of the potential vectors that can infect Gramineae would be expected to decrease northward. Information on infectivity has been obtained in detail only from UK (Plumb et al. 1986) and the mean percentage of *R. padi* caught in suction traps from 1 September that infected test plants was less than 2% over a 10-year period, whereas in northwest Italy 14% were infective (Conti and Casetta 1983).

The UK results can be compared with results from 1986 when only 0.4% of aphids were infective, 9% reproduced on test plants and only 5% were alate exules (Tatchell et al. 1988). Clearly the potential number of aphid vectors can bear little relationship to the numbers that are infective. Both figures are essential to an assessment of the risk of virus infection.

The above data all relate to migrant aphid populations. In some circumstances the route of infection may be more direct. Plowed grass has long been recognized as posing a threat to a following cereal crop. The destruction of the grass by chemical or cultivation before the cereal is sown is vital if the risk of direct transfer of aphids, potentially carrying BYDV, to the emerging cereal seedlings, is to be avoided. The increased area of winter barley has increased the risk of direct transfer by what is sometimes known as the "green-bridge." Winter barley is usually harvested earlier than other cereals, in July in Britain, and often, because of the demands of other work, barley stubbles are not cultivated until the other cereals are harvested and preparations are being made for sowing autumn crops in September. In the intervening two months stubbles can regrow, shed grain can germinate and grow rapidly and grass weeds flourish. This provides a very attractive habitat for aphids leaving spring barley and wheat that is still ripening. Thus, when these barley stubbles are cultivated, aphids and virus infection can be widespread and, if the virus hosts are not killed, may spread to the newly sown swards.

Secondary spread—In most regions it appears that primary infection of crops is rarely widespread enough to cause severe damage on its own. However, in favorable circumstances dispersal from initial foci of infection can be rapid, resulting in characteristic patches in crops. The extent of this spread is presumably controlled by the influence of weather on the aphids, and their predators and parasites. In Britain most of the patchy virus spread is due to *R. padi*, a relatively sedentary aphid that presumably disperses, via apterae and alatae, only when populations have built up on the plant originally infested. The effectiveness of aphicides applied at the end of the autumn migration in preventing secondary spread indicates that most aphid dispersal occurs in deteriorating weather and may be caused by heavy rain and widely differing day and night temperatures, which make aphids move up and down plants, and not necessarily the same ones.

By contrast, *S. avenae* transmission, especially in spring-sown crops, is more often associated with scattered, random infections. Some patches do develop but generally seem smaller than those caused by *R. padi*. *M. dirhodum* does not seem to be associated with any particular dispersal pattern.

In most regions of Europe winter weather is cool enough to slow down aphid development and restrict aphid flight, and in many north and northwestern regions survival on crops is the exception

rather than the rule. The importance of those aphids that do survive in spreading virus within the crop the following spring and summer is uncertain. In northwestern regions it appears to be of little consequence, indeed the survival of aphids often means that their predators and parasites can also survive and this prevents, or restricts, the explosive development of aphids that enter crops, as in Scandinavia, free of natural controls.

For example, in Britain, aphids were long considered only a damaging pest species because large numbers were often present on leaves and ears during summer. In southern regions there seems less concern about direct damage, presumably reflecting generally more balanced populations. In both France (Dedryver and Robert 1981) and Hungary (Szirmai 1974) aphids can overwinter on cereal volunteers and can move into spring-sown crops the following year.

Spring-sown crops

The same four criteria described for autumn-sown crops influence BYD infection on spring cereals. However, it is later, rather than earlier sowing that increases the risk of infection. In maritime regions such as Britain the normal sowing date is March-April. Sowing is usually earlier in the drier, cooler east than in the wetter, milder west, but because of the milder conditions aphid activity is usually earlier in the west. Consequently, it is here that BYD damage is more frequently seen when young crops are exposed to infective vectors.

Crops sown in March usually escape serious infection as cereal aphid flight rarely begins before the middle of May and only infective *R. padi* are usually found before June (Table 5). However, it is usually *S. avenae* and *M. dirhodum* that are the most numerous species and they are trapped 10 to 14 and 21 to 30 days later than *R. padi* (Table 5). Crops sown in late April can be infested by aphids and infected by BYDV when young (Jenkyn and Plumb 1983) and both aphid populations and virus infection are much greater on these later sown crops (Table 8). In other areas of Europe, especially Scandinavia, there is a much more rapid invasion of spring-sown crops. *R. padi* is again the first migrant, but is also the most numerous in trap catches during the summer (Wiktelius 1982). After the initial infestation of crops, numbers increase rapidly and virus infection can be widespread and damaging.

Table 8. Numbers of aphids/100 shoots and incidence of barley yellow dwarf virus (infected plants/10m^2) in plots sown on different dates at Rothamsted

Sowing date	GS[a]	Aphids 15 June 59/49	28 June 75/73	5 July 83/77	BYDV 14 June 59/49
4 March 1976		150	129	6	2.6
13 April 1976		221	189	21	12.3
	GS	25 June 49/43	11 July 69/65	21 July 77/75	8 July 65/61
8 March 1977		10	52	21	3.8
25 April 1977		7	64	37	8.8

Source: Jenkyn and Plumb 1983.

[a] Growth stages (Zadoks et al., 1974).

Concluding Remarks

BYD is widely present in Europe, although information on the occurrence of infection, isolates, and crop loss is very uneven. Sources of virus are widespread and where they are perennial, as in grasses in the wetter regions, they are relatively stable. In these circumstances the isolate of virus that infects cereals and the damage that it causes is determined by local weather and its influence on vector biology and populations. Where the source of virus is an annual crop, such as maize, there is inevitably much variation from year to year. Perhaps in these regions, where most aphids over winter as viviparae, the predominant influence on BYD epidemiology is the source of virus, rather than the biology of the vector. Nevertheless, in all regions the extent of virus infection is determined by availability and susceptibility of crops and the potential numbers and proportion infective of virus vectors.

The dominant influences on vector biology and populations are the cold winters of the north and northwest and the consequent sexual cycle required for survival, and the hot dry summers of the south.

Europe's approach to the control of BYD has principally been to control vectors with insecticides. More recently the exploitation of resistance has been a more realistic option and an autumn-sown barley incorporating the *Yd2* gene is now available in Britain (Parry and Habgood 1986), although it may not completely replace the need for spraying where inoculation pressure is high (Plumb 1986), and much effort is now being devoted to breeding programs for cereals, maize, and grasses. However, more knowledge is needed about isolate distribution and interaction with cultivars to direct breeding programs. It seems likely that for at least the next 10 to 15 years pesticides will remain the principal method of control and it is essential, on economic and environmental grounds, that their use is based on a sound knowledge of BYD epidemiology.

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Epidemiology and Control of Barley Yellow Dwarf Viruses in Australia and New Zealand

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Abstract

*Diseases of barley (*Hordeum vulgare *L.), oats (*Avena sativa *L.), and wheat (*Triticum aestivum *L.) due to infection with barley yellow dwarf viruses (BYDVs) are widespread in Australia and New Zealand (Australasia). Barley yellow dwarf (BYD) is considered to be the most damaging disease of cereals in New Zealand and it reduces the value of production in Australia by about US$40 million annually. Incidence of infection is greatest in cool-temperate regions where the annual winter-dominant rainfall exceeds 500 mm and perennial grasses persist to serve as reservoirs of infection.*

BYD serotypes MAV, RMV, RPV, and PAV have been identified in Australasia. The vector specificities of these serotypes are generally similar to those reported from North America. However Sitobion avenae *(Fabricius) does not occur, but other* Sitobion *spp. do. The relative incidence of the four serotypes in each cropping region reflects the abundance there of the major vectors species,* Sitobion *spp.,* Metopolophium dirhodum *(Walker),* Rhopalosiphum maidis *(Fitch),* R. padi *(L.), and* R. rufiabdominalis *(Sasaki). Climatic factors affect the distribution of these vectors directly, as well as indirectly through influencing the composition of the local flora. The different grass species vary in their susceptibility to BYDVs and the likelihood of being colonized by different vectors. Isolates that are vector-specific are common in regions where one vector species predominates, while vector nonspecific isolates are most common together with mixed infections in areas where more than one vector species is prevalent.*

Most primary spread of viruses into cereals is over short distances from reservoirs of infection in grasses nearby. Crops are only susceptible to heavy infestation by alatae during their early growth stages and increases in virus incidence after initiation of stem elongation are almost exclusively due to movement by apterae between plants.

Considerable success in the control of BYD has been achieved by judicious choice of sowing date in relation to the major autumn and spring peaks of aphid flights activity. A single aphicide application to autumn-sown crops during the winter is beneficial where foci of infection and infestation have established, while synthetic pyrethroids can provide protection against primary infection. Long-term solutions to the control of BYD in Australasia are being sought through breeding, biological control programs and integrated crop management practices.

Cereal grain production is one of the major agricultural activities in Australia. The country's total grain production since 1982 has averaged 22 million tons annually, worth almost US$3 billion. Wheat (*Triticum aestivum* L.) is by far the most important crop and constitutes about 75% of the total production. It is followed in importance by barley (*Hordeum vulgare* L.), grain sorghum (*Sorghum vulgare* Pers.), oats (*Avena sativa* L.), and rice (*Oryza sativa* L.).

Total grain production in New Zealand is only 5 to 10% of that in Australia, and barley is more important than wheat. Most of the production is consumed locally, but some barley has been exported in recent years. Nevertheless, grain production is an important agricultural activity in some areas of New Zealand.

The histories of agriculture in Australia and New Zealand (Australasia) have demonstrated that diseases affecting cereal production can have important impacts on the economies of the two countries. Barley yellow dwarf (BYD) is currently considered the most damaging disease of New Zealand cereal crops and in Australia it is considered to be a major factor limiting production, particularly in the high rainfall areas (> 500 mm annually) where perennial pasture grasses persist (Gardner and O'Brien 1986).

Australia and New Zealand were discovered during an expedition to the South Pacific region led by Captain James Cook in 1769-70 from Europe. British colonization of Australia began shortly thereafter, at Sydney in New South Wales in 1788, and the first settlements of New Zealand took place from Australia 50 years later. The early settlers found that the flora and fauna in their new homelands were quite unlike those known to them in Europe. None of the small grain or coarse grain cereals was to be found, nor any of the pasture grasses used for grazing in the northern hemisphere. However the native flora did include a number of Poaceae species, which were probably free from aphids, including all known vectors of barley yellow dwarf viruses (BYDV) (Burbidge and Jacobs 1984; Eastop 1966).

Wheat, barley, and oats seed were introduced by the first European colonialists together with grasses present as weeds in soil containing potted trees and shrubs. These weeds probably harbored both BYDV and vectors. However the first reports of infestation of cereals with aphids were made considerably later, *Rhopalosiphum maidis* (Fitch) on maize in Queensland and New Zealand in the latter part of the nineteenth century (Cottier 1953) and *R. padi* (L.) on wheat in New South Wales in 1904 (Froggatt 1905).

The first report of BYD affecting cereals in New Zealand was made on the basis of symptoms observed in 1953 (Smith 1955) and in Australia at Tamworth, New South Wales, in 1956 (Smith 1957). These tentative diagnoses were subsequently confirmed in the late 1950s by aphid transmission tests using *R. maidis* and *R. padi* in New South Wales, and *R. padi* alone in New Zealand, Tasmania, and Victoria (Butler et al. 1960; Geard 1960; Price and Stubbs 1963; Smith 1963).

The ecology of BYDV in Australasia is unique. It represents the culmination of interactions in new environments for 200 years between a group of introduced viruses, their introduced vectors and natural enemies, introduced crop and pasture species that host the viruses and vectors, and the native flora.

Aphid Vectors

Vector species in Australasia

Known aphid vectors of BYDV in Australia and New Zealand include *Metopolophium dirhodum* (Walker), *Rhopalosiphum insertum* (Walker), *R. maidis*, *R. padi*, *R. rufiabdominalis* (Sasaki) and *Sitobion* spp.

M. dirhodum is a recent introduction to Australasia. It was first found in New Zealand in the Canterbury region of the South Island in 1982 (Stufkens and Farrell 1985) and in Australia at Bemboka in southeastern New South Wales in 1984 (Carver 1984). This species has spread rapidly and become a major component of the aphid fauna on cereals in Canterbury (New Zealand), New South Wales, Tasmania, and Victoria. *R. insertum* was also recorded recently in Australia for the first time (Ridland and Carver 1987).

R. maidis, *R. padi*, and *R. rufiabdominalis* each formed significant proportions of the total aphid catch during a survey of alate aphids flying over eastern Australia in the early 1960s (Hughes et al. 1965). *R. padi* has been considered the most important vector in small grains, but in the past 2 years it has been displaced by *M. dirhodum* as the most common species in many cereal crops, particularly barley, in Canterbury (Stufkens and Farrell 1987a) and Tasmania. *R. maidis* occurs most commonly on maize (*Zea mays* L.) and related Panicoideae but to a rather limited extent on small grains. The

possible importance of *R. rufiabdominalis* in the epidemiology of the disease in some regions was overlooked, because of its subterranean habit, until recent studies in southern Victoria indicated that it comprised a significant proportion of the total catch of *Rhopalosiphum* spp.

The situation with respect to *Sitobion* spp. is unclear. Eastop (1966) indicated that the early references to *Sitobion avenae* (Fabricius) in Australasia (Cottier 1953) were based on mistaken identifications that should have been *Sitobion miscanthi* (Takahashi), a species of Asian origin. More recently V.F. Eastop advised (personal communication) that several collections of *Sitobion* from Tasmania were *S. fragariae* (Walker) (Guy et al. 1986). In addition, recent comparative examinations of *Sitobion* material from New South Wales and Victoria indicated that some specimens were like *S. fragariae* from Tasmania and the others appeared to consist of two genetic forms of *S. miscanthi* (D.F. Hales, personal communication). The abundance of these various species and forms of *Sitobion* in New South Wales and Victoria relative to *R. padi* is not known. *S. fragariae* is a recent introduction and *S. avenae* does not occur in Australasia.

Schizaphis graminum (Rondani), the prime vector of SGV-like isolates of BYD in the Northern Hemisphere, does not occur in Australasia. *Diuraphis noxia* (Mordvilko) and *Metopolophium festucae* (Theobald) have also not established in the region.

Distribution of vector species

The northern extremities of the small grain cereal production regions in Australasia have a mild subtropical climate characterized by summer-dominant rainfall and mean daily temperatures of 10 to 15°C and 20 to 25°C in winter and summer respectively. At the southern extremities of production the conditions are cool temperate with mean daily winter and summer temperatures of 5 to 10°C and 15 to 20°C and a winter-dominant rainfall. These conditions, especially in winter, are much milder than in many northern hemisphere regions of cereal production. Extended periods of sub-zero (°C) temperatures are not encountered although severe overnight frosts are common in the southernmost regions during the winter. The cereal aphids seem able to survive these frosts by moving below ground and the holocyclic heteroecious vector species remain on their secondary hosts as virginoparae throughout the year.

In the cooler regions, biannual cycles of alate flight activity are most pronounced, with the major peak of flight occurring in the spring (October-November) and a minor peak in the autumn (April-May). These peaks of flight activity become progressively less pronounced northward, as do absolute numbers of aphids found flying (Hughes et al. 1965).

The observed distributions of the different vector species correlate with different climatic regions. This is probably due to differences in optimal threshold temperatures for development and survival of the different species. The *Sitobion* spp., best adapted to cool conditions among the vector species in Australasia, occur most frequently in the southernmost production areas, while *R. maidis* predominates in the northernmost latitudes where cereals are grown. *M. dirhodum* populations have been most explosive in cool temperate regions of Canterbury and southeastern Australia on barley, while *R. rufiabdominalis* has been found most frequently in intermediate climatic regions. *R. padi* appears best adapted to the broadest range of climatic conditions and is found in all cereal production regions but it is less tolerant of the semitropical climate of southeast Queensland than is *R. maidis*.

Populations of most of the BYDV species shift with changes of season. For example, *R. padi* is common on cereals in southeast Queensland during the winter, but not in summer when *R. maidis* is most frequently found. Conversely, *R. maidis* occurs commonly in Canterbury, Victoria, and Tasmania on maize and barley during and directly following the warmer months, but is rarely found there in winter and early spring.

The occurrence of the various vector species by region is also influenced by the distribution of their preferred host species. For example, species of Panicoideae, mainly with C_4 photosynthetic systems, are most commonly found in the warmest regions where cereals are grown, and many of these species are favored hosts of *R. maidis*. On the other hand, species of Pooideae, C_3 species, flourish in more temperate regions and are preferentially colonized by *M. dirhodum*, *R.padi*, and *Sitobion* spp. However these aphid species adapted to cool temperate climate differ with respect to their preferred hosts (Vickerman and Wratten 1979), and the numbers of *R. padi*, *M. dirhodum*, and *Sitobion* spp. in a district can be substantially affected by the relative abundance of their respective favored host species in the pastures.

Dispersal of vectors
The establishment of primary BYD infections in Australasian cereal crops generally results from relatively short range movement of vector alatae. Outbreaks of infection in crops can nearly always be traced to sources of infection and infestation nearby. Long distance migration of viruliferous vectors in low level jet streams is not implicated in BYD outbreaks in Australasia as it is in North America (Wallin and Loonan 1971). However sporadic episodes of long distance dispersal have occurred in Australasia with dramatic consequences. For example, *Sitobion miscanthi* was reported to have established in New Zealand as a result of dispersal from eastern Australia in low level jet streams, a distance of more than 2000 km (Close and Tomlinson 1975; Lowe 1969). Conversely, it has been suggested that *M. dirhodum* may have established in eastern Australia by dispersal in wind currents from New Zealand (Carver 1984). In the case of *R. maidis* and *R. rufiabdominalis*, both species are anholocyclic, and may have established in Australia by extension from Asia.

Long distance dispersal also occurs within Australia, and virus epidemics in Tasmania occasionally result from viruliferous alatae being blown more than 400 km from mainland Australia (Johnstone 1984). *M. dirhodum* was collected in yellow water pan traps at several sites in northern Tasmania shortly after it was found in southeastern New South Wales (Johnstone and Spratt, unpublished information, 1984), again suggesting long distance dispersal. There is also evidence that some aphid species adapted to cool temperate climates may retreat to Tasmania to over-summer and reinvade the mainland states when conditions cool during the autumn (M.J. Way, personal communication).

Viruses

Serotypes in Australasia
Four serotypes have been identified in Australasia. They correspond to the MAV, PAV, RMV, and RPV New York type isolates described from North America (Guy et al. 1986; Johnstone 1984; Sward and Lister 1987, 1988; Waterhouse et al. 1986). The vector specificities of isolates of the four serotypes from Australia and New Zealand were generally similar to those from North America. An exception was that isolates from Victoria serologically like North American MAV isolates were transmitted most efficiently by *R. padi* and poorly by Victorian *Sitobion miscanthi* (Lister and Sward 1988).

SGV-like isolates, transmitted most efficiently by *S. graminum*, have not been identified in Australasia. Their absence probably reflects the absence of the prime vector species. Similarly, GPV-like isolates, transmitted efficiently by both *R. padi* and *S. graminum* in China (Zhang et al. 1983; Zhou et al. 1984), have not been found in either Australia or New Zealand.

Distribution of serotypes
The distribution of the four serotypes of BYDV found in Australia and New Zealand varies among the different cereal production areas. It reflects the activity and abundance of the major vector species in those areas in the same way that serotype distribution matches vector occurrence in China (Zhou et al 1984). Thus, information on the incidence of different serotypes in a district provides valuable clues to the activity of different vector species. The Australasian surveys for viruses and vectors indicated that vector-specific virus isolates were

common in regions where one species dominated the overall aphid population while vector non-specific types were more prevalent in areas where there were more diverse species mixes of vectors and hosts.

Southeast Queensland is one of the northernmost areas where small grains are grown in Australia. Wheat, barley, and oats are grown there during the winter months. *R. padi* may infest these crops, leading to a low incidence of BYD associated with infection by isolates of the vector nonspecific PAV serotype; some of the isolates from oats were transmitted by *R. maidis*. During the summer when temperatures and humidity are high, *R. padi* occurs in much lower numbers in this region and cereal cropping reverts to maize, sweet corn, and sorghum. These maize and sorghum crops do not become infected with BYDV even though they are frequently heavily infested by *R. maidis*. A panicoid grass, *Urochloa panicoides*, grown profusely as a weed in the maize crops and infection with RMV-like isolates in this grass is common, sometimes reaching 60%. It appears that the maize cultivars grown in Queensland are resistant to infection with Australian RMV-like isolates.

In Tasmania, the southernmost small grain production area in Australia, *Sitobion* spp. and *R. padi* both commonly infest cereals (Martyn 1983; Guy et al. 1987). The development of winged forms in these two species is not coincident and patterns of flight activity differ during the year, which probably leads to the common occurrence of vector nonspecific PAV-like isolates transmissible by both *R. padi* and *Sitobion* spp., but not by *R. maidis*, which is rare. Numbers of *R. padi* in Tasmania are normally higher than those *Sitobion* spp. and vector specific RPV-like isolates also occur in cereals, in a ratio about 1:20, RPV-like:PAV-like (Guy et al. 1986, 1987). Interestingly, no MAV-like isolates have been identified from Tasmania.

In southern Victoria, where conditions are warmer, the abundance of *Sitobion* spp. relative to *R. padi* is less than in Tasmania and a greater proportion of the infections in cereals are of the RPV-like isolate (20%). However vector nonspecific PAV-like isolates still predominate, while occasionally isolates are found that are of either the MAV or RMV serotype. The relative proportions of *R. maidis*, *R. padi*, and *Sitobion* spp. on cereals in Victoria vary depending upon whether the crop is barley, wheat, or oats. These vector colonizing preferences are reflected in the incidence of infection with different viruses; RMV-like isolates were found only in barley, PAV- and RPV-like isolates were the most common types found in wheat, and MAV- and PAV-like isolates together with PAV-like/RPV-like mixed infections were most common in oats.

Examination of suction trap catches in Victoria in 1977-78 indicated that, of all grain aphids caught, 84% were *R. padi*, 13% were *Sitobion* spp. and 2% were *R. maidis*; the proportion of each species that was infective (serotype not determined) was 38, 24, and 6%, respectively (Smith and Plumb 1981).

Tests in Ithaca, New York, Hobart, Tasmania; Melbourne, Victoria; and Harpenden, UK; on samples of cereals with BYD from New Zealand have confirmed the presence of MAV-, RMV-, RPV- and PAV-like isolates in that country. However the numbers of plants tested were limited. MAV-like isolates were most common in 1985, while PAV-like isolates predominated in 1986. Results from serotyping were in accord with data on the occurrence of aphids on cereals in New Zealand. *M. dirhodum*, *R. padi* and *Sitobion* spp. are all found commonly, but variably, on wheat and oats while in addition, *R. maidis* infests barley. *M. dirhodum* transmitted MAV- and PAV-like isolates from New Zealand, PAV-like but not RPV-like isolates from mainland Australia (Waterhouse and Helms 1985), and PAV-like isolates plus PAV-like/RPV-like mixtures from Tasmania.

Epidemics in Cereal Crops

Sources of infection and infestation

Pasture, weed, and roadside grasses are considered to be the main sources of infection and infestation for spread to cereal crops in Australia and New

Zealand. Between-crop spread is unimportant because winged forms do not appear soon enough in populations on even the earliest autumn-sown crops to be of any consequence, even for spring-sown crops. Perennial ryegrass (*Lolium perenne* L.) is the most common pasture species grown in the high rainfall areas of southern Australia and New Zealand where BYD is most common. Perennial ryegrass plants are frequently infected with BYDV and also commonly inhabited by vectors, especially *R. padi* and *Sitobion* spp. A survey in New Zealand indicated that 6- to 30-year-old perennial ryegrass pastures often contained more than 50% of plants infected with BYDV isolates transmissible by *R. padi*, with the highest recorded instance being 84% (Latch 1977). The infection level in five Tasmanian perennial ryegrass pastures more than 4 years old averaged 36% and exceeded 70% in one instance (Guy et al. 1986).

Most of the infections in Tasmanian perennial ryegrass pastures were of the PAV type, although significant numbers of RPV infections and PAV/RPV mixtures were also found (Guy et al. 1987). Studies on the perennial ryegrass plants in a pasture species experiment sampled intensively for 3 years indicated that 63% of the infected tillers contained PAV alone, 14% contained RPV alone and 23% contained PAV/RPV mixtures. Similar infection levels were found in the tall fescue (*Festuca arundinacea* Schrev.) plots, but the situation with cocksfoot (*Dactylis glomerata* L.) and phalaris (*Phalaris aquatica* L.) was quite different; RPV was far more common in these two species than PAV. These differences were consistent with the results from a large-scale survey of Tasmanian Poaceae that suggested that different species of grasses vary in their susceptibility to PAV and RPV.

In general, PAV was most commonly found in species of the Pooideae sub-family while RPV infections predominated in the Panicoideae (Guy et al 1987). However these differences were not absolute and some species of Pooideae—cocksfoot, Yorkshire fog (*Holcus lanatus* L.), Kentucky bluegrass (*Poa pratensis*)—were infected exclusively, or almost so, with RPV. These results indicated potential sources of resistance to PAV and RPV among various species of Poaceae.

Virus mixtures, particularly PAV/RPV mixtures, were commonly found during surveys of cereals and grasses and occurred far more frequently than would have been expected had each virus infected plants independently. This indicated that there are selective advantages to BYDV mixtures because of enhanced opportunities for transmission by aphids as a result of heterologous encapsidation (Johnstone and Guy 1986).

The grassland vegetation in the subtropical region of southeast Queensland differs from that farther south, and there PAV-like isolates and *R. padi* "over-summer" primarily on the pooid Bromus catharticus Vahl, while RMV-like isolates "overwinter" primarily on the panicoid *Paspalum dilatatum* Poiret.

There has been a trend in recent years toward the use of a wider range of perennial pasture grasses in the temperate higher rainfall areas of Australia and New Zealand in order to overcome a range of other disease, pest, and physiological problems encountered with perennial ryegrass in some situations. This will probably lead to an increase in the incidence and/or diversity of BYDVs in the pasture grasses, serving as reservoirs of infection for spread to cereal crops, because the prime vector species (*M. dirhodum*, *R. maidis*, *R. padi*, and *Sitobion* sp..) have distinct host preferences among the Poaceae, as do the various viruses themselves (Guy et al. 1987).

Development of epidemics

The majority of small grain cereal crops in Australasia are sown in the autumn using cultivars that do not have a vernalization requirement, while the remainder are sown using the same cultivars during the spring. Irrespective of sowing date, crops are most susceptible to infestation with aphids shortly after emergence. Winged aphids

settle and colonize cereal crops relatively rarely after a complete ground cover has been established. The degree of infestation of crops by viruliferous alatae and the establishment of primary infection and infestation foci is therefore dependent upon the intensity of local vector aphid flight activity during the first few weeks following crop emergence.

The peak of autumn aphid flight activity is normally much smaller than the spring flight, and autumn-sown crops are therefore less liable to contract large numbers of primary infections. However this is not always the case, especially if crops are sown very early so that their exposure to the autumn flight during April and May is maximized. More often, less than 5% of the plants in a crop become infested with viruliferous alatae during the autumn. The apterous progeny of these primary infectors increase and spread slowly during the winter, and then rapidly during the spring as temperatures rise and the plants become larger. This vector activity during the spring often results in rapid rates of virus spread.

Populations of *R. padi* develop mostly on oats, less commonly on barley and are rarely seen on wheat, but percentage infestation levels may nevertheless still be high. This species has been the most common cereal aphid and is considered to be most important vector overall. However, *M. dirhodum* has been especially important since its introduction in Canterbury and Tasmania, where barley is the major cereal crop. Populations of this species in many barley crops in 1985 and 1986 were much larger than those of *R. padi*. In Tasmania more than 80% of the *M. dirhodum* alatae settling on a barley crop shortly after emergence carried PAV, but the source(s) of these aphids was not established. However, *M. dirhodum* was not found infesting perennial ryegrass in Tasmania, although it was common on cocksfoot, phalaris, and several *Bromus* spp. (S. M. Leighton and G.R. Johnstone, unpublished). *R. maidis* and *Sitobion* spp. have significant local importance as vectors in areas where they are abundant.

R. rufiabdominalis, a vector as efficient as *R. padi* of MAV-like and PAV-like isolates from Victoria (Ridland and Sward, unpublished), comprised 40% of the alate cereal aphid catch (the *R. padi* catch was 55%) in net traps positioned over cereal crops in southern Victoria from 1983 to 1986. These findings suggested that the importance of this species as a vector may well have been overlooked in the past even though significant numbers were also trapped in New South Wales and Victoria during an earlier survey of alate aphids in eastern Australia (Hughes et al. 1965). This species transmitted vector nonspecific PAV-like isolates from Illinois (Jedlinski 1981).

Spring-sown crops often emerge either just before, or during, the major peak of aphid flight activity that occurs from September to November depending upon the region. High levels of primary infection periodically develop in crops as a result, causing large losses in production. Infection rates in spring-sown cereals in Australasia are among the highest in the world and virus incidence may exceed 80% within four weeks of emergence at susceptible sites in epidemic years. Secondary spread of virus in spring-sown crops generally seems to be minor and of little consequence.

Disease incidence

High levels of infection with BYDV are mostly confined to the temperate regions, where the prime aphid vectors are most abundant and where the annual rainfall exceeds 500 mm. In these regions perennial pasture grasses persist and serve as local reservoirs of virus infection and aphid infestation. The regions involved include the cereal cropping areas in New Zealand, Tasmania, the southwest of Western Australia, the south coast of New South Wales, southern and central Victoria, and northeastern Victoria, a low rainfall area where pastures are irrigated. Infected plants can generally be found in most crops in these areas in most seasons.

Precise information on the frequency and severity of epidemics is not available. Problems are greatest in those crops sown earliest in the autumn and in

spring. Epidemics develop on average about 3 years in 5 in northern Tasmania, where most of the State's cereal production is concentrated. Natural infection in breeders' plots sown there in the spring is sufficiently high to permit steady progress in the selection of tolerant seedlines. The breeding program resulted in the development of the barley cv. Shannon, near isogenic to Proctor except for containing the *Yd2* gene for BYD resistance. The significance of BYD in Tasmania can be gauged from the fact that Shannon out-yielded Proctor by 28% overall in 30 field trials during five consecutive seasons (Vertigan 1980).

Similar results to those from Tasmania were obtained by McLean et al. (1984) in comparisons of the cultivars Proctor and Shannon over two seasons in southwestern Western Australia. The yield divergence between the two cultivars in favor of Shannon exceeded 50% at 9 of the 15 experimental sites and reflected the occurrence of BYDV infection. Epidemics in New Zealand occurred frequently in the late 1950s and 1960s. During this period a large number of experiments were conducted to assess the benefit from spraying autumn-sown crops in late winter to destroy infestations of vectors before the onset of virus spread in the spring. Yield increases occurred as a result of aphid control and reduced virus incidence in 44 out of 120 trials over ten seasons from 1958 to 1969 (Close 1969). Epidemics occurred about 3 years in 5 over this period. More recently the frequency of BYD outbreaks has been less, about 1 year in 5, due to the adoption of control measures involving changes in time of sowing.

Instances of virus disease control in Australasia as a result of aphicide treatment since the pioneering New Zealand work have been reported in recent years by workers from several regions (Hart 1978; Johnstone et al. 1987; McLean et al. 1984; Stufkens and Farrell 1987a). The reports help document the incidence and effects of BYD in cereals through demonstrating the increases in productivity that result from treatments effecting control of virus spread.

In Victoria, epidemics occur in the main wheat growing areas about 1 year in 5. Immunoassays on samples collected from representative Victorian wheat crops in September 1986 indicated that 10 out of 26 crops were infected and 3 out of 26 had an incidence greater than 10% (Sward and Lister 1987).

Epidemics of BYD do not seem to occur in Queensland and incidence of infection in crops in southeast Queensland in recent years has not exceeded 1%.

There have been no reports from Australasia of BYDV infection in maize, sweet corn, rice, or sorghum.

Effects of infection

The effects of infection have been discussed generally in sections on incidence and control. PAV-like isolates are most common and these also produce the most severe effects in oats, wheat, and barley when compared with MAV-like, RMV-like and RPV-like isolates. The most severe disease results from mixed infections. Mixtures of PAV-like and RPV-like isolates occur commonly in southeastern Australia. Infection with a Victorian isolate representing a mixed PAV-like/RPV-like infection reduced the yield of wheat cv. Olympic by 80% (Smith and Sward 1982), of wheat cv. Condor by 40% (Sward and Kollmorgen 1986), and of barley cv. Parwan by 60% (Sward 1983). Yield losses of 20% were reported for Victorian wheat crops in some seasons in disease-prone areas (Price 1970), and in 1980 losses in wheat due to BYD were estimated at US$30 million based on incidence of infection in oat indicator plots planted throughout the State.

The effects of virus infection are often compounded in the field because plants affected by BYD are more susceptible to debilitation by other pathogens such as the take-all fungus (*Gaeumannomyces graminis* var. *tritici* Walker) (Sward and Kollmorgen 1986).

Recent indications to the Wheat Research Council of Australia by local breeders and virologists conservatively suggested

that total losses in productivity of wheat due to BYD in Australia were about 2% on average over all crops and all years. Percentage loss figures for barley and oats, which are more frequently grown in the higher rainfall areas, would be considerably greater than that for wheat. These assessments suggest that BYD reduces the value of cereal production in Australia on average by about US$40 million annually.

Control of epidemics

Smith (1963) demonstrated that losses due to BYD in autumn-sown cereal crops could be alleviated by a single application in late winter or earlier of a systemic organophosphate such as demeton-S-methyl or dimethoate. The treatment eradicated foci of infestation with vectors established during the autumn, reduced virus spread from these foci during the spring, and often led to significant yield increases as a result. Similar conclusions were indicated from experiments in Tasmania involving aphicides applied to autumn-sown wheat (Martyn 1983).

The results from these experiments confirmed perceived understanding of the epidemiology of the disease and the relative roles of alatae and apterae in virus spread in autumn-sown crops. Following these experiments, growers in New Zealand were encouraged to inspect their crops for aphids during the winter after the autumn peak of aphid flight activity had ceased and to apply an aphicide if infestations were noted. This practice became popular with farmers but Lowe (1967) subsequently demonstrated that the BYD problem could largely be overcome by sowing late, after the end of May, so that crops emerged after the peak of aphid flight activity in the autumn had declined. This procedure is now the current practice and has led to a decrease in the incidence of epidemics.

The pattern of infection in spring-sown crops is different from that in autumn-sown crops, where most of the infected plants are clustered as a result of secondary spread of apterae. In spring-sown crops most infections are primary, resulting from viruliferous alatae flying into recently emerged crops from infection sources nearby, probing on the young plants, and infecting them.

Aphicides such as the carbamates and organophosphates do not effectively control primary spread because virus in inoculated into treated plants before sufficient insecticide has been imbibed to kill the vectors. Results from experiments on spring-sown cereal crops using such aphicides in New Zealand were therefore erratic (Close 1969). However it has been reported that a synthetic pyrethroid, deltamethrin, had a rapid "knock-down" effect on aphids and prevented spread by *Myzus persicae* (Sulzer) of persistent, semi-persistent, and non-persistent viruses (Gibson et al., 1982). Barrett et al. (1981) reported that two other pyrethroids effectively controlled BYD in the field in Europe. Experiments in New Zealand and Tasmania have confirmed that some synthetic pyrethroids effectively control primary infections in spring-sown cereal crops (Johnstone et al. 1987; Stufkens and Farrell 1987a).

Deferment of the sowing date has also been used to control virus infection in spring-sown crops in the North Island of New Zealand, but although virus infection levels were reduced, the potential yields of the crops were reduced considerably using this strategy.

The infection rates reported for BYDV in Australasia in both autumn- and spring-sown crops seem to be higher and occur earlier than practically anywhere else in the world. This reflects the mild temperate climatic conditions in New Zealand and in the high rainfall coastal areas of Australia, where perennial grasses persist and the development of large vector populations is favored. In addition, most of the vectors, all introduced, are not constrained by parasites, pathogens, and predators to the same extent that they are in their countries of origin. It appears that the BYD vectors were introduced and established in Australia without their normal complements of natural enemies. Lacewings, syrphids, and coccinellids are

seen in cereal crops exerting pressure on aphid populations but rarely in a way that would have any significant effect on virus spread. Occasionally, spectacular epidemics of fungal diseases are seen in aphid populations in wet seasons, e.g., epidemics of *Erynia neoaphids* in *M. dirhodum* populations in Tasmania (Johnstone and Leighton, unpublished). The main vector, *R. padi*, is parasitized by *Aphidius colemani* and *A. similis*; these natural enemies, plus coccinellids and spiders, provide good control of *R. padi* in the subtropical cereal growing region of southeast Queensland.

Some work is in progress to systematically introduce and release exotic pathogens and parasites of BYDV vectors in Australasia. As part of this program, an aphidiid wasp (*Aphidius rhopalosiphi* De Stafeni) was recently released at several sites in southern Australia. *A. rhopalosiphi* has also been established in the South Island of New Zealand and subsequently released (Stufkens and Farrell 1987b).

Long-term solutions to control BYD in cereals will inevitably rely on development of resistant cultivars. A good deal of progress has already been made in this respect for barley, oats and wheat in both Australia and New Zealand (R. Sward, these Proceedings). However, there is scope for much larger gains in the future through further breeding and cultivar development. Work is progressing at several centers in both countries toward these objectives (R. Sward, these proceedings). These approaches involve both conventional breeding methods and novel biotechnological techniques including the production of synthetic resistance genes and transfer of BYD resistance from *Agropyron* spp. to wheat.

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Ecology of Cereal Aphids Transmitting Barley Yellow Dwarf Virus in Kenya

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Abstract

Aphid movements in Kenya were monitored through aerial sampling using two types of sticky traps. The aphid species frequently found that attack cereal crops and can transmit barley yellow dwarf virus include Schizaphis graminum *(Rondani),* Metopolophium dirhodum *(Walker),* Rhopalosiphum padi *(L.),* R. maidis *(Fitch), and* Sitobion avenae *(Fabricius). These species occur in all wheat (*Triticum aestivum *L.) growing areas (between 1,800 and 3,000 m above sea level). The cropping system favors prolonged local movements of aphids and also provides green crop in other wheat areas for colonization after long distance migratory flights. Aphids not involved in long distance migration may survive on alternate gramineous host plants or volunteer plants of wheat and barley (*Hordeum vulgare *L.).*

The problem of aphids infesting small cereals, particularly wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.), in Kenya is a frequent one. Although Walker (1954) estimated there would be about two outbreaks of cereal aphids (*Schizaphis graminum* (Rondani) and *Rhopalosiphum maidis* (Fitch)) in a century, several outbreaks have since then been reported (Wanjama 1979). The factors that led to these outbreaks have not been studied and conclusions based on weather records alone (Walker 1954) may not explain the complexity of aphid ecology. In the higher latitudes aphids overwinter as eggs, but under tropical conditions they do not enter a sexual phase (Blackman 1979). The most difficult weather that aphids go through under tropical conditions is the dry period. Eastop (1983) suggested that aphids go through this dry spell in the surviving patches of green vegetation.

Migrating aphids have been reported to travel long distances (Taylor and Palmer 1972; Taylor et al. 1979). When the movement of aphids is monitored, it may be possible to forecast future infestation or recommend planting dates that evade severe attacks (Kieckhefer et al. 1974; Wanjama 1986). Forecasting the population levels of aphids that are vectors of plant disease facilitates a simultaneous forecast of the diseases. The work reported here is a preliminary study seeking to provide information relating to the factors that help the cereal aphids to survive on a 12-month basis and so to perpetuate barley yellow dwarf virus (BYDV) in Kenya.

Monitoring

The work followed the techniques of an earlier program, 1983 to 1985, that monitored *S. graminum* using sticky traps at Njoro (Wanjama 1986). In that study, sampling was done at heights of 1.5, 3.0, 4.5, and 6 m using clear plastic sheets smeared on both sides with a sticky commercial preparation used for trapping aphids and other insects (Tanglefoot), mounted on a metal pipe 3.8 cm in diameter and 6 m high. A pulley at the top and a rope connecting the wires that held the plastic pieces helped to raise and lower the traps.

In the 1986 study, cylindrical metal food cans were used to make traps for catching aphids in wheat fields. The cans were painted yellow, covered with a clear plastic smeared with Tanglefoot, and positioned on wooden pegs so as to be 1 m high. Six such traps were used at Njoro. In fields distant from Njoro, sampling was limited to sweep netting and direct observations of aphid colonies. The latter method was used to determine the migratory status of the aphid populations.

Aphid Species Involved

Eighteen species of aphids have been reported to transmit BYDV (Jedlinski 1981), but only a few of these are considered important in Kenya. The major species infesting cereal crops here are *S. graminum*, *R. padi* (L.), *R. maidis*, *Metopolophium dirhodum* (Walker), and *Sitobion avenae* (Fabricius). All these species are found in all the wheat growing areas of the country, but are more prolific at lower elevations. At higher elevations, above 2,700 m above

sea level, BYDV is severe despite the low numbers of the vectors, which is an indication that while the original colonizing population of aphids may be very high, the rate of population growth is very low.

Cropping Patterns

Several wheat areas exist in Kenya where wheat and barley are planted at different times (Figure 1), most areas having only one crop in a year. At Timau two crops are planted per year, the first in

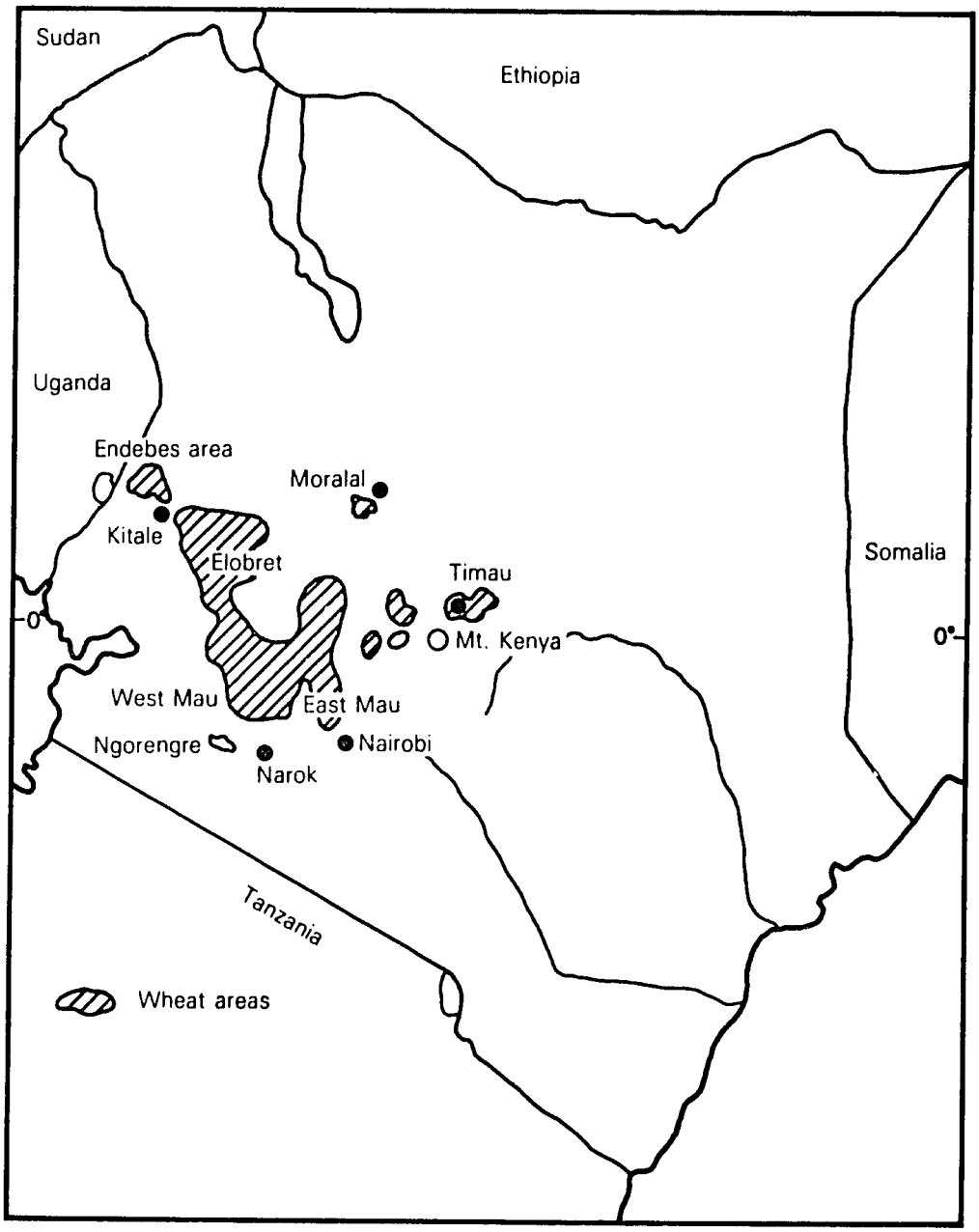

Figure 1. Wheat growing areas in Kenya.

February. Planting also begins in February in lower Narok (Ngorengore). In Nakuru, Maralal, and Eldoret planting is done between April and June. In the higher areas of Mau Narok, Endebes and Timau (second crop) planting starts from June to the end of August, although in Timau planting extends to October. This planting sequence provides some green crop of wheat and barley in the country throughout the year.

Alternate Hosts

There is no evidence yet that cereal aphids in the tropics have herbaceous alternate hosts. In the areas covered by the survey some green grasses were found at most times throughout the year. Aphids have been found to survive on *Bromus pectinatus*, *Dractylis glomerata* L., *Poa leptoclada*, *Avena fatua* L. and *A. sterilis* L. in Njoro, Mau Narok, and Timau. All five major species have been found to survive on these grass species. Besides the wild grasses, volunteer cereal crops may act as aphid reservoirs after crop harvest.

Possible Aphid Movements

Although no quantitative work has been done to assess the proportion of each aphid species in the population, all the major species occurred in the different wheat areas. Since in each area planting was at different times and continued for about 3 months, green crops for aphid colonization were provided for a long period. Many more aphid generations were produced than would be the case if planting in the same area were done simultaneously.

After crop harvest in lower Narok, aphids were abundant on cereal crops in the Njoro area and winged forms were being produced when crops in Mau Narok area (East and West Mau) were at seedling stage. However in Mau Narok the aphids, though widespread, did not produce large populations. In Timau, green crop was available throughout almost the entire year and aphids dispersed from one field to another. In February 1987 when planting was starting in lower Narok, aphid colonies in fields at Timau were producing winged forms. In addition there were also young crops at Timau. Aphids involved in long distance migration also may be taking off from each field as the population builds up or as the crop matures. The aerial population of aphids is therefore constantly replenished so that a trapping exercise can catch aphids for the greater part of the year. *S. graminum*, for example, is caught at Njoro from June to March (Wanjama 1986).

Some aphid movement hypotheses can be advanced based on aphid monitoring and known aphid migratory tendencies:

- Within the country aphids are able to survive throughout the year and the infestations seen at the beginning of each crop season may be originating from other areas within the country. Aphids migrating long distances are carried on the wind, and although winds at the equator are not very strong the distances between the various wheat areas are not great. After the initial population has colonized a new crop, dispersal flights of succeeding generations spread the aphid infestation locally. There is a cycle of movement among the wheat growing areas in Kenya and dispersal flights between fields in the same area.

- Since aphids can migrate over long distances (Taylor and Palmer 1972), they may be reaching Kenya from distant areas outside the country. As the inter-tropical convergence zone shifts poleward, monsoon winds develop between it and the equator (Pedgley 1982). These seasonal winds may transport airborne aphids across the Kenya borders from the north or south. There is wheat growing in these areas that could be a source of migrating aphids.

These two hypotheses are not considered as alternatives but as possible ways that these populations are maintained in Kenya.

Conclusions

From the results of the preliminary work, the major considerations in studying the ecology of the cereal aphids in Kenya are:

- The wide variation of climatic conditions in the wheat growing areas in Kenya is a significant factor in the spatial and temporal distribution of the aphids year round.
- Aphid movements between the different ecological zones may be influenced by the cropping pattern in Kenya. More detailed study is required in order to map out the sequence of the aphid movements.
- In order to understand the importance of long distance migration of cereal aphids, knowledge of aphid population dynamics in neighboring countries is necessary.
- Alternate grass host plants may be playing an important role in the survival of the cereal aphids when the cereal crop is harvested. These plants could be acting as a host for BYDV.
- When the pattern of aphid infestation on crops is well documented, specific recommendations on dates of planting, methods of monitoring infestations, and control on a local basis could be developed.

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Aphid Vectors of Barley Yellow Dwarf Virus in Hungary

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Abstract

During 1980-86, the aphid species Sitobion avenae *(Fabricius),* Rhopalosiphum padi *(L.), and* Metopolophium dirhodum *(Walker) were the most prevalent aphids on cereal crops grown in Hungary. Other species occurred in a relatively low frequency.* S. avenae *started to appear by mid-May on winter wheat (*Triticum aestivum *L.) and winter barley (*Hordeum vulgare *L.), and colonies, mainly present on the spikes, reached maximum during the second half of June. Winter wheat, winter and spring barley, and oats (*Avena sativa *L.) were equally important host plants for* R. padi, *which was dominant throughout the season. The first winged individuals started to appear by mid-May, reaching maximum during the second 10 days of June.* M. dirhodum *were often present on leaves of winter wheat, winter barley, oats, and maize (*Zea mays *L.), but not on rye (*Secale cereale *L.).* R. padi *had the greatest fluctuations in annual numbers. There was no barley yellow dwarf epidemic in 1985 due to the severe winter of 1984-85, followed by unfavorable conditions for aphid multiplication in the spring.*

In Hungary, barley yellow dwarf virus (BYDV) is ranked as the second, after maize dwarf mosaic virus, economically important Gramineae virus pathogen. Since 1980 we have been investigating the dynamics of cereal aphid populations on winter and spring small grains, maize (*Zea mays* L.), and sorghum (*Sorghum bicolor* (L.) Moench).

Sitobion avenae (Fabricius), *Rhopalosiphum padi* (L.), and *Metopolophium dirhodum* (Walker) are the dominant aphid species in cereal plants in Hungary. In addition, *R. maidis* (Fitch), *Aphis fabae* Scopoli, and *R. insertum* (Walker) occur in lower numbers. The total number of each of these virus vector aphid species varies greatly from year to year and from one locality to another (Figure 1).

Data on the aphid species occurring on cereal plants in Hungary have been published by Szalay-Marzsó (1970), V. Rakk (1978), Kozma (1979, 1980), V. Rakk and Milinkó (1981), Milinkó et al. (1983a, b, c), V. Rakk et al. (1985), and Milinkó et al. (1986).

The present investigations were carried out in commercial fields of cereal varieties grown in Hungary as well as in experimental variety comparison plots. In each investigation 10 sampling sites were examined. For each site the number of each aphid species present on five randomly chosen plants was determined.

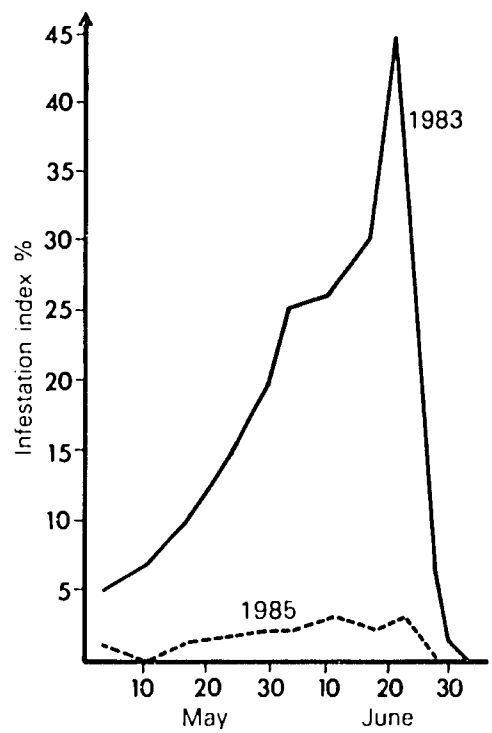

Figure 1. Infestation index of aphids on winter wheat in Keszthely in the spring 1983 and 1985.

The individual number of aphid species was determined according to the degrees given by Banks scale (van Emden 1972). An infestation index was calculated from the values determined by this scale.

For *S. avenae*, winter wheat (*Triticum aestivum* L.) and winter barley (*Hordeum vulgare* L.) were the most important host plants. On these plants, the first winged individuals started to appear by mid-May. Under favorable conditions, the number of *S. avenae* present at the time of wheat flowering multiplied 40 to 50 times by the time the plant reached the wax ripening stage. The maximum number of this species occurred during the second half of June. Colonies of *S. avenae* were usually present on the spikes; in the case of oats (*Avena sativa* L.) they were present on the panicles. On maize, colonies of this species were found on the surface of the leaves. Lower numbers of *S. avenae* were recorded on rye (*Secale cereale* L.), spring barley, oats, and maize fields. In Hungary, this species may be one of the most important vectors of BYDV.

For *R. padi*, winter wheat, winter and spring barley, oats, and maize were equally important host plants. The first winged individuals started to appear by the middle of May. At the beginning, colonies were found on the lower third of the plant stems. As the plant ripened, this species was found on the spikes and panicles. The population reached its maximum by the second 10 days of June. On maize, *R. padi* individuals started to appear during the first days of June and reached their maximum number during the last 10 days of the same month. They produced large colonies on the leaves and inflorescences as well as on the enveloping leaves of the maize ears. The population decreased during mid-summer but increased once again in September. In spring barley and oats *R. padi* was the dominant species over the whole growing season. On maize *M. dirhodum* and *R. padi* were the dominant species at the beginning of summer, while during autumn *R. padi* dominated alone. The autumn multiplication of *R. padi* first took place on maize and volunteer plants of small grain cereals, and later it was found on seedlings of winter cereals. Accordingly, this aphid species is considered to be of great importance in the epidemiology of BYD.

Individuals of *M. dirhodum* were regularly present on leaves of winter wheat, winter and spring barley, oats, and maize, but not on rye. The well developed colonies were found on veins near the leaf midrib. The first winged individuals started to appear by the beginning of May on both winter wheat and winter barley, the maximum number was reached at the end of May. On maize, however, this maximum was found during the last 10 days of June.

R. maidis occurred on both the leaves and heads of spring barley and oats. The maximum number on both these host plants was reached at the end of June or the beginning of July. Individuals of this species were also regularly present on the volunteers of winter barley. During autumn (end of September and the first days of October), the characteristic dark green color of *R. maidis* individuals could be seen in fields of maize and sorghum.

A. fabae appeared as single individuals or small colonies on maize plants after the middle of summer. Small colonies of *R. insertum* were recorded on maize in 1982.

During the period of population increase, aphid enemies started to appear. After the aphid numbers reached their maximum, they often decreased suddenly because of the activity of parasites and predators, the maturing of their host plants, climatic conditions (dry weather), and their migrating character. Among the aphid vectors studied, *R. padi* was found to have the largest fluctuation in its numbers.

During the present study, the season of 1984-85 was the most unfavorable for aphid multiplication. The severe long winter (average temperature of January -5°C, February -4°C) made it impossible for the aphids to overwinter as anholocyclic asexually reproducing females. In addition, the spring of 1985

was climatically unfavorable for aphid multiplication. Accordingly, 1985 was not a BYD epidemic year in Hungary. By way of contrast, large numbers of aphids survived and severe BYDV infections were recorded in 1983 and 1984. The relationship between climatic conditions and multiplication of the aphid species that vector BYDV is the foundation for improving the prediction of epidemics of BYDV and for making recommendations on possible control measures.

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Migrations of Aphid Vectors of Barley Yellow Dwarf Virus in Northeast and Northwest Italy

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Abstract

The flight activity of aphid species known as vectors of barley yellow dwarf virus (BYDV) was studied in the northwest and the northeast of Italy from 1982 to 1986. The winged, migrating forms were captured daily with two Rothamsted Insect Survey suction traps positioned at 12.2 m above ground level, at Carmagnola, near Turin, and at Pozzuolo del Friuli, near Udine. Numbers of individuals captured were compared weekly for Rhopalosiphum padi *(L.) (the most frequent species) and for all vectors together. Weekly mean temperature and weekly precipitation were recorded for the two localities. During the last 2 years, data so obtained were used to advise farmers when to sow winter wheat (*Triticum aestivum *L.) and barley (*Hordeum vulgare *L.) in order to avoid the major aphid flights and BYDV infections. A good estimate of the total population of autumn migrating vectors in northern Italy could be obtained by counting* R. padi, R. maidis *(Fitch),* R. insertum *(Walker),* Sitobion avenae *(Fabricius),* Anoecia corni *(Fabricius), and* Myzus persicae *(Sulzer).*

In the late 1970s the autumn cereal crops in northern Italy suffered from severe outbreaks of barley yellow dwarf (BYD), particularly in barley (*Hordeum vulgare* L.) and wheat (*Triticum aestivum* L.) (Conti and Casetta 1983).

As a consequence of the increasing importance of BYD as a "yellow plague," epidemiological studies were started in 1981 in both northeast and northwest Italy. These studies were aimed at gaining a better understanding of the epidemiology of BYD in these areas, in an attempt to control the virus.

As a part of this research project, the migration of the aphid species known to be vectors of barley yellow dwarf virus (BYDV) were monitored in northern Italy by means of two traps of the Rothamsted Insect Survey type.

In this paper data from 5 years of catches (1982-1986) are reported, considering both *Rhopalosiphum padi* (L.) alone and the other 13 species of BYDV vectors. A preliminary analysis of data is also reported for the two sites where trapping was carried out in relation to meteorological data.

On the basis of such data an attempt is also made to find a group of aphid species representative of the total vectors. The numbers of these aphids would be used as an aid to advise farmers on the best time of sowing winter cereals. During the last few years, this advice has been offered, but using data on catches of the whole aphid vector population.

Materials and Methods

Migrating aphids were caught at 12.2 m by two Rothamsted Insect Survey suction traps (Taylor 1974) modified for studying aphid migrations (Dewar et al. 1980). These were operated continuously from March to December at Carmagnola, ca. 30 km from Torino (northwest Italy) (Instituto di Fitovirologia Applicata-I.F.V.A.) and at Pozzuolo del Friuli (northeast Italy) (Centro Regionale di Sperimentazione Agraria-C.R.S.A.). The aphids were separated from other insects and identified under light stereomicroscopes. All the species listed by A'Brook (1981) were considered to be potential vectors of BYDV. Daily captures were pooled on a standard week basis (Taylor et al. 1981) for comparisons of data from different years.

Weekly captures at the two sites and for the years 1983-1986 were compared for the total of potential BYDV vectors and for *R. padi* alone

In order to find a group of species representing a consistent proportion of the total vectors, two aphid groups were

considered: one included *R. padi*, *R. insertum* (Walker), *R. maidis* (Fitch), and *Sitobion avenae* (Fabricius), the other included the above mentioned species plus *Anoecia cerni* (Fabricius) and *Myzus persicae* (Sulzer). The total number of vectors was calculated from the number of aphids in the group using the equation:

$$CTV = int\ (GV/P + 0.5)$$

where CTV is the calculated number of vectors, GV is the number of aphids in the group, P is the ratio of GV over the total of vectors caught, and "int" is the integer part of the result of the calculation.

Daily values for maximum and minimum temperatures (°C) and rainfall (mm) were taken from meteorological data collected from the localities where the traps were operating. Mean temperatures were derived from the maximum and minimum temperature. Data were then pooled on a standard week basis using weekly mean temperatures and weekly cumulative rainfall. Data for Carmagnola were kindly supplied by the Instituto de Coltivazioni Erbacee—University of Torino, while at Pozzuolo they were taken from the C.R.S.A. meteorological station.

Results

Migration of *R. padi*

Catches of *R. padi* are reported in Figures 1 to 10 as solid black expressed as logarithm of the number of individual aphids caught plus 1 (Log(N+1)). Data from the northwest, for 1982 only, included also *R. insertum*, whose

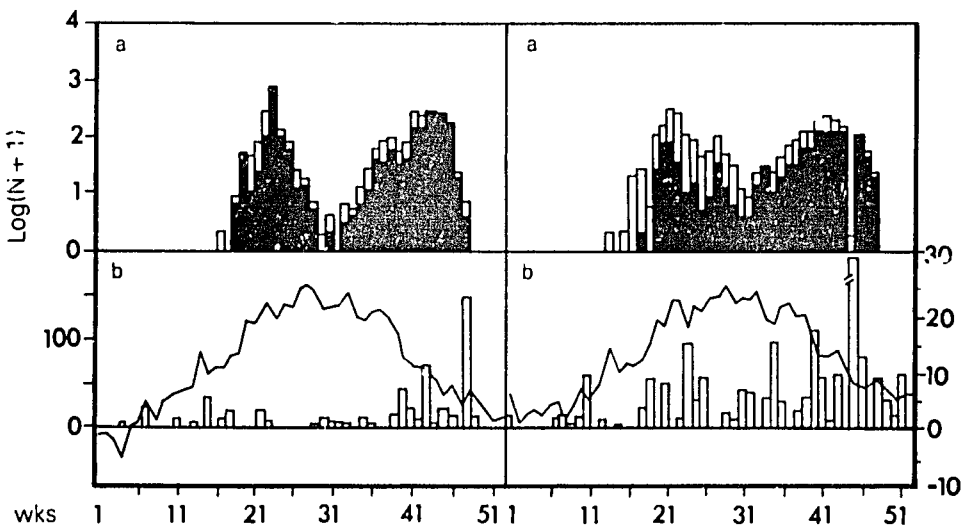

Figure 1. Aphid catches, mean weekly temperature and weekly rainfall in northwest Italy in 1982. (a) Catches of *R. padi* + *R. insertum* (in black) and of other BYDV vector species (in white) according to the list of Table 2, expressed as Log (N + 1). *H. pruni*, *M. albidum*, *M. persicae* and *R. insertum* are not included in the counts. (b) Mean weekly temperatures (line) in °C and weekly rainfall (bars) in mm. Both in (a) and (b) standard weeks on the X-axis.

Figure 2. Aphid catches, mean weekly temperatures and weekly rainfall in northeast Italy in 1982. (a) Catches of *R. padi* (in black) and of the other BYDV vector species (white in bars), according to the list of Table 2. (b) Mean weekly temperature (line) in °C and weekly rainfall (bars) in mm. Both in (a) and (b) standard weeks on the X-axis.

contribution to the total number of vectors was usually very low. Differences from year to year and between the two localities considered are summarized in Table 1, by listing seven points in time that characterize the annual aphid migrations, namely: the time of the first catch of *R. padi*, the time when the fifth aphid of a species was caught (Bouchery et al. 1985), the time of the spring migration peak, the time of the summer minimum, the autumn peak, the week when the fifth *R. padi* from the last was caught in the traps (Thomas et al. 1983), and the week when the last aphid was caught in the traps.

Table 1. Timing of *R. padi* migration in northwest (NW) and northeast (NE) Italy. The time of the year is expressed as standard weeks

	Standard week of catches in years									
	1982		1983		1984		1985		1986	
	NW	NE	NW	NE	NW	NE	NW	NE	NW	NE
First *R. padi*	19	13	16	15	18	15	18	21	19	20
Fifth-from-first	19	20	17	19	19	22	21	23	20	23
Spring peak	24	22	24	25	27	28	26	28	26	25
Summer minimum	29-31	32	32	32	34-36	34	28	33-34	32-34	31
Autumn peak	44	42	41	42	44	43	42	42	42	41
Fifth-from-last	47	48	46	46	48	48	46	47	47	49
Last *R. padi*	48	48	47	48	48	48	46	48	48	50

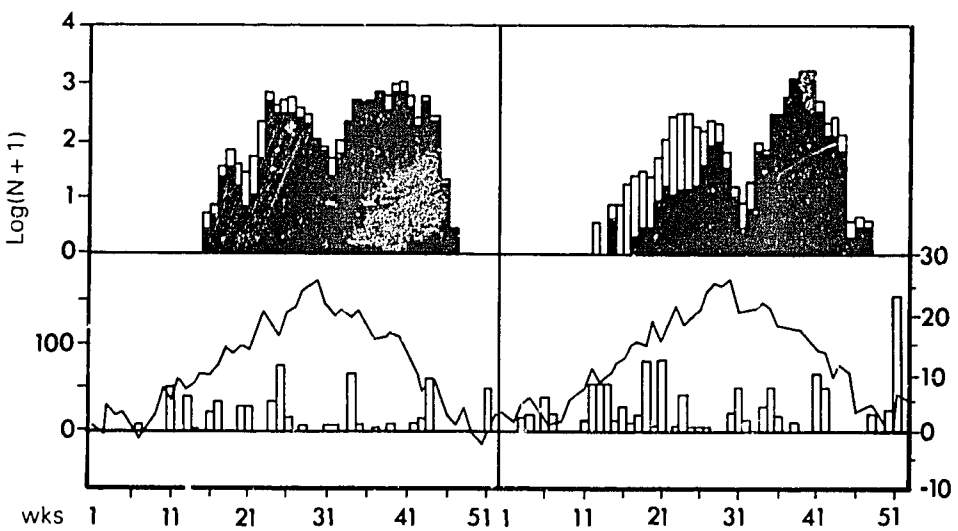

Figure 3. Aphid catches, mean weekly temperatures and weekly rainfall in northwest Italy in 1983. Symbols and units as in Figure 2.

Figure 4. Aphid catches, mean weekly temperatures and weekly rainfall in northeast Italy in 1983. Symbols and units as in Figure 2.

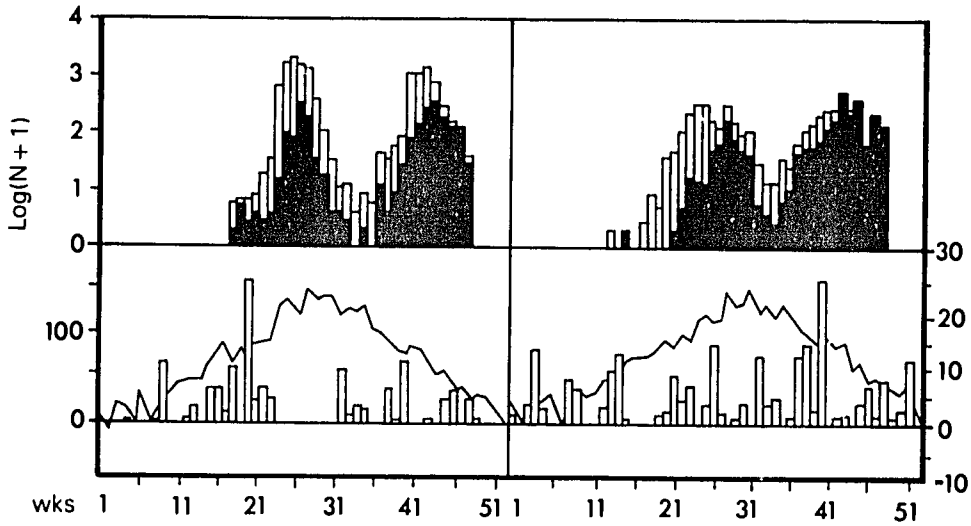

Figure 5. Aphid catches, mean weekly temperatures, and weekly rainfall in northwest Italy in 1984. Symbols and units as in Figure 2.

Figure 6. Aphid catches, mean weekly temperatures, and weekly rainfall in northeast Italy in 1984. Symbols and units as in Figure 2.

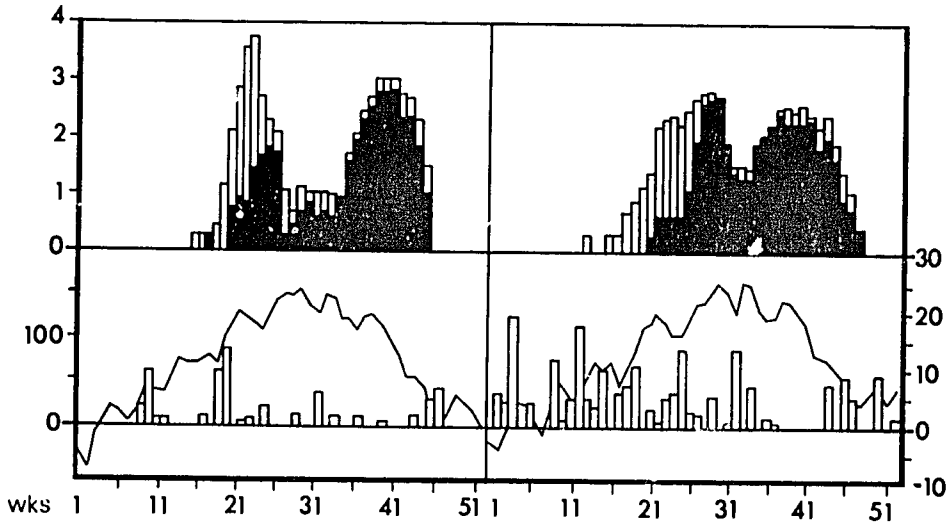

Figure 7. Aphid catches, mean weekly temperatures, and weekly rainfall in northwest Italy in 1985. Symbols and units as in Figure 2.

Figure 8. Aphid catches, mean weekly temperatures, and weekly rainfall in northeast Italy in 1985. Symbols and units as in Figure 2.

There was a certain variation in the times of the first catches, but, on average, the spring migrations in the northeast began a little later, as shown by the time of the fifth aphid caught, whereas the other times were almost the same, with a few exceptions.

Total of vectors

Fourteen species of the 23 listed by A'Brook (1981) were caught in the traps at least once in the two sites (Table 2).

The height of bars in histograms in Figures 1 though 10, represents the total of the 14 vector species (expressed as Log(N + 1)), while the contribution of the 13 species other than *R. padi* is indicated by the white area in the bars.

Metopolophium albidum Hille Ris Lambers and *Sipha elegans* (del Guercio) were never found in the catches from the trap in the northwest, while they were occasionally present at a very low level in the northeast.

The same seven times of migrations as for *R. padi* are summarized for the other potential vectors of BYDV in Table 3. *Hyalopterus pruni* (Geoffroy), *M. albidum*, *M. persicae* and *R. insertum* were not included in the aphid species to be

Table 2. Aphid species, potential vectors of barley yellow dwarf virus, caught at least once in northwest or northern Italy

Anoecia corni
Hyalopterus pruni
Metopolophium albidum
M. dirhodum
M. festucae
Myzus persicae
Rhopalosiphum insertum
R. maidis
R. padi
R. poae
Schizaphis graminum
Sipha elegans
Sitobion avenae
S. fragariae

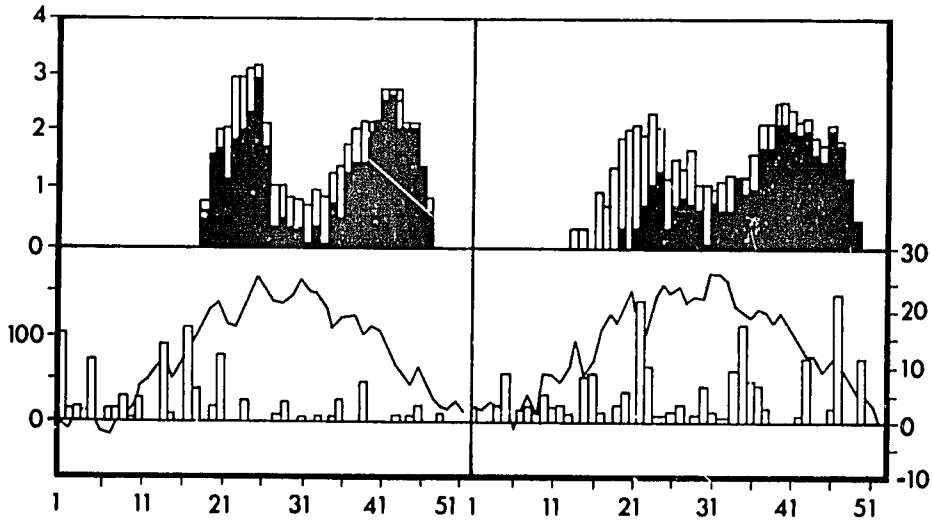

Figure 9. Aphid catches, mean weekly temperatures, and weekly rainfall in northwest Italy in 1986. Symbols and units as in Figure 2.

Figure 10. Aphid catches, mean weekly temperatures, and weekly rainfall in northeast Italy in 1986. Symbols and units as in Figure 2.

identified in samples caught in the northwest during 1982. Therefore the corresponding value of total vectors is not available (NA).

The only obvious difference between the two locations was an earlier beginning of migrations in the northeast, while the average values of other parameters considered did not differ (except for a very early summer minimum in northwest Italy in 1985), and no regular pattern was found comparing year by year the data from the two areas.

The aphid species first caught in the traps varied greatly from year to year and from site to site, revealing no constant pattern.

Grouping of species

The grouping of *R. padi*, *R. insertum*, *R. maidis*, and *S. avenae* was found unsuitable to estimate the total of potential vectors of BYDV, as the total of these four species did not represent a consistent proportion of the total vectors. Adding *A. corni* and *M. persicae* to the group made the proportion consistent for the different years and for the two areas, for the autumn migration, i.e., from week 32 to 50 (September 3-December 16 included). The mean proportion of the total of vectors represented by the six species was P = 0.9826 (calculated from week 36 to week 50; weeks 51 and 52 were omitted because no aphids were caught during these 2 weeks).

The regression, of calculated values against the observed values for the two areas was:

CTV = -0.2101 (+/- 0.6536) + 1.001 (+/-0.0014) OTV(R = 0.999; D.F. = 118)

where CTV is the number of vectors calculated and OTV is the observed number of vectors; the figures in brackets are the standard errors of parameters, R is the coefficient of regression and D.F. the degrees of freedom.

As an example of the estimates obtained with the above equation, Figure 11 shows the calculated numbers of vectors for the year 1985 plotted against the observed values of catches in northwest Italy. The solid line is the theoretical line.

Discussion

Our results indicate that *R. padi* is the species found most frequently and consistently in trap catches in both the areas studied. This agrees with preliminary reports for northwest Italy in the years 1981 and 1982 (Conti and Casetta 1983), and for northeast Italy in 1982 and 1983 (Coceano 1984). The number of individuals of the other species caught is quite variable from year to year and from one area to the other, as shown by the absence of *M. albidum* and *S. elegans* in northwest Italy.

Table 3. Timing of the migrations of potential BYDV vectors other than *R. padi* caught in the two traps in northwest (NW) and northeast (NE) Italy. The time is expressed as standard weeks

	Standard week of catches in years									
	1982		1983		1984		1985		1986	
	NW[a]	NE	NW	NE	NW	NE	NW	NE	NW	NE
First vector	NA	14	16	13	18	13	16	13	19	14
Fifth-from-first	NA	17	17	15	19	18	20	18	21	17
Spring peak	NA	22	24	25	26	24	24	28	26	24
Summer minimum	NA	32	32	32	34	33-34	29	34	32	32
Autumn peak	NA	42	41	40	43	43	41	41	42	41
Fifth-from-last	NA	48	45	46	48	48	46	47	46	48
Last vector	NA	48	46	48	48	48	46	47	48	48

[a] Since not all the 13 species of Table 2 were considered in 1982 in the northwest, the corresponding values are not available (NA).

The most evident difference between the two areas was an earlier beginning (3 to 5 weeks earlier) and establishing (1 to 4 weeks) of migrations of BYDV vectors other than *R. padi* in the northeast compared to the northwest.

Although relating meteorological data and migrations is always difficult (A'Brook 1981), a difference in the weekly mean temperatures between the two areas was detected, the winter mean temperatures of the northeast being consistently higher, as the northeast greatly benefits from the influence of the Adriatic Sea. Thomas et al. (1983) found a significant correlation between the first catch of *Phorodon humuli* (Schrank) in suction traps with the sunshine and rainfall in January and the mean temperature in February. Since the weekly mean temperature is influenced by both sunshine and rainfall, it is possible that difference in time of the first catch is related to the different winter temperatures. For some unknown reason, *R. padi* showed a reverse tendency in the time of beginning of migrations in both sites.

The end of migrations of *R. padi* and of all BYDV vectors did not differ in the 2 sites, in the years examined, except in 1986, when the last *R. padi* was caught 2 weeks later in northeast (1 week difference is not considered significant, because of the pooling of data on a standard week basis). Thomas et al. (1983) correlate the time of the end of migration of *P. humuli* to the maximum and minimum temperature of June. From examining the summer mean temperatures, it was found that these did not differ significantly for the two localities examined.

The regression of calculated number of vectors against the observed number of catches showed that it is possible to have a good estimate of the total population of autumn migrating vectors in northern Italy by limiting the counts to only six aphid species—*R. padi*, *R. maidis*, *R. insertum*, *S. avenae*, *A. corni*, and *M. persicae*. The species considered include the most important Gramineae-infesting species and the most important BYDV vectors to autumn-sown cereals in northern Italy.

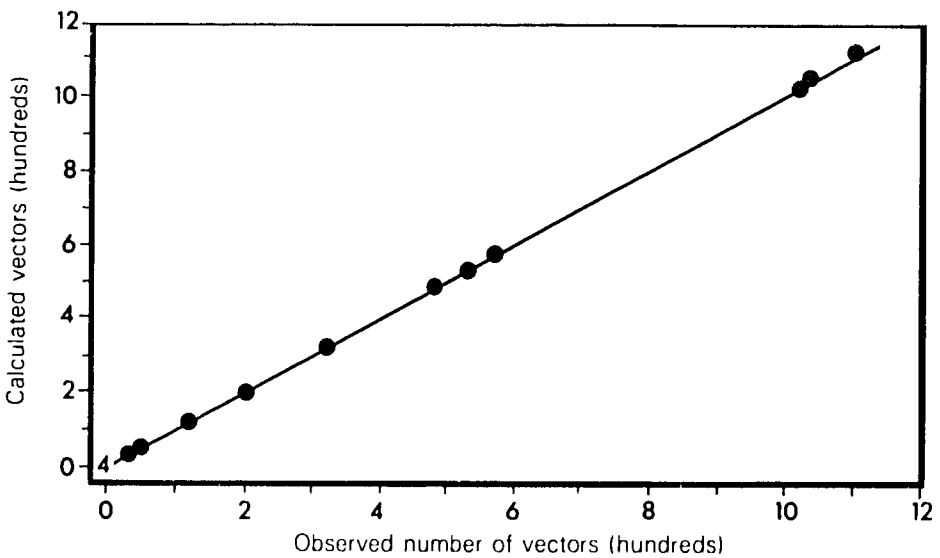

Figure 11. Calculated number of vectors against the observed number of vectors in catches in Carmagnola (NW) for weeks 36-50 in year 1985. The number '4' indicates four indistinguishable points, otherwise marked (●). Line represents the theoretical values. Both X- and Y-axes are linear and values are expressed in hundreds.

The data of total catches of the 14 species were used during the last 2 years in the northwest (I.F.V.A., unpublished data) and during the last 4 years in the northeast (Coceano 1984) to advise farmers when to sow winter wheat and barley in order to escape heavy aphid infestations and so avoid BYDV infections. If the above relation between catches of the six aphid species and the total of vectors can be confirmed in other areas in northern Italy, it will be possible to extend the BYDV-prevention service to all the local cereal-growing areas using this limited number of aphid species. This would considerably reduce the work and the training needed to supply this service, and further the control of BYDV.

Acknowledgments

The work was partially supported by CNR, Italy Special grant I.F.R.A.-Sub-project 1.

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Natural Infectivity of Migrating Aphid Vectors of Barley Yellow Dwarf Virus in Northern Italy

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Abstract

The infectivity of winged migrating aphid vectors of barley yellow dwarf virus (BYDV) was investigated from 1981 to 1986 in two localities in northwestern and northeastern Italy (Carmagnola, Turin, and Pozzuolo, Udine) by using two types of suction traps that capture live aphids. Flying aphids were captured, identified, and tested individually for BYDV transmission on either barley or oats, which were then checked for virus presence by both observation of symptoms and ELISA. Of nine aphid species collected near Turin, five transmitted BYDV to the indicator plants, namely: Anoecia corni *(Fabricius),* Rhopalosiphum insertum *(Walker),* R. maidis *(Fitch),* R. padi *(L.), and* Sitobion avenae *(Fabricius). Of 13 species collected near Udine,* A. corni, Hyalopterus pruni *(Geoffroy),* Metopolophium albidum *Hille Ris Lambers,* R. maidis, *and* R. padi *proved to be infective. The highest seasonal infectivity of the whole aphid population was observed every year in October-November when heavy BYDV infections occur on winter barley and wheat. Of the several aphid species found to transmit BYDV,* R. padi *appeared to be the most important.*

Barley yellow dwarf virus (BYDV) was first reported in Italy in rice (*Oryza sativa* L.) (Belli et al. 1974), in which it caused serious epiphytotics in the 1960s and was known under the name "giallume" (= yellowing). In subsequent years, the virus was also reported in barley (*Hordeum vulgare* L.), wheat (*Triticum aestivum* L.), oats (*Avena sativa* L.) (Conti 1978; Giunchedi and Credi 1978; Snidaro 1978), and more recently, in maize (*Zea mays* L.) (Loi et al. 1985; Osler et al. 1985). The most severe damage occurs at present in winter barley, particularly in crops which are sown early in the autumn.

Epiphytotics of BYDV in winter cereals greatly depend on two main factors: the number of aphids that migrate in autumn and the level of their infectivity, i.e., the percent of BYDV-carriers in the migrating aphid populations. Both these parameters can be estimated by the use of suction traps, which allows both the checking of the daily number of flying aphids and the evaluation of their infectivity when captured alive. For practical purposes, on the basis of aphid catches and infectivity tests, it can then be decided whether virus control has to be done by different means, such as delaying the date of sowing or spraying with insecticides (Plumb 1977; Kendall and Smith 1981a,b).

From 1981 to 1986, by using suction traps of two types, we studied the infectivity of the potential aphid vectors of BYDV in northern Italy. The results are reported in this paper.

Materials and Methods

Two types of traps were used at both Carmagnola (Torino) northwestern Italy, and Pozzuolo (Udine), northeastern Italy: one operating at about 2 m above the ground, and another of the Rothamsted Insect Survey-type, working at a height of 12 m. Only one type of trap was used each year in each locality, except in 1985

when both types of traps were used at Pozzuolo. Aphids were captured 2 to 3 days per week, and 2 to 4 times each day, with a total trap working time of 2 to 3 hours per day.

The aphids were collected in a bottle containing a few fresh virus-free leaves of barley. Immediately after the end of captures, the potential BYDV vector species (Kennedy *et al.* 1962; Plumb 1976; A'Brook and Dewar 1980) were identified under a light microscope and each individual was transferred singly onto greenhouse-grown barley or oats at the first leaf stage. The aphids were checked every day for survival during a 3-day inoculation feeding period at 20°C.. (In a few cases, the aphids were identified after the infectivity test had been completed.) After the inoculation, the plants were sprayed with an insecticide, and grown in the glasshouse for at least 40 days and checked for BYD symptoms.

The diagnosis was completed by double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA), using antisera to a RPV-like or a MAV-like isolate in Torino and to a PAV-like isolate in Udine.

Results

The annual number of aphids caught by the suction traps in the two areas investigated and the proportion of infective individuals are reported in Table 1. In Figure 1, the monthly totals of potential vectors of BYDV and infective individuals captured alive in suction traps are reported.

R. padi was the species caught most often and one of those found most frequently infective (Tables 1 and 2).

Altogether, 13 species of potential aphid vectors of BYDV were captured: 9 in Torino and 13 in Udine. The aphid species showing the highest level of infectivity in the two areas considered were, in order of infectivity, *R. maidis* (Fitch), *R. padi* (L.), *R. insertum* (Walker), *Sitobion avenae* (Fabricius), and *Anoecia corni* (Fabricius) in Torino; *Metopolophium albidum* Hille Ris Lambers, *R. padi*, *M. dirhodum* (Walker), *Hyalopterus pruni* (Geoffroy), *R. maidis*, and *A. corni* in Udine. It must be noted, however, that the percentage of infective aphids was determined on a quite different number of individuals for each species (Table 1).

Table 1. Yearly numbers of aphids, potential vectors of BYDV, caught alive by different suction

	Carmagnola-Torino				
	1981 A	1982 A	1984 A	1985 B	Total Torino
	No. infective aphids/No. trapped individuals (%)				
Anoecia corni		3/70 (4%)	0/96	0/13	3/179 (2%)
Hylaopterus pruni			0/56	0/1	0/57
M. albidum					
M. dirhodum			0/24	0/1	0/25
M. festucae					
Myzus persicae			0/2	0/14	0/16
Rhopalosiphum insertum			0/7	2/23 (9%)	2/30 (7%)
R. maidis	1/2 (50%)	2/4 (50%)	0/2	2/25 (8%)	5/33 (15%)
R. padi	13/93 (14%)	3/37 (8%)	3/54 (6%)	1/117 (1%)	20/301 (7%)
R. poae					
Schizaphis graminum				0/4	0/4
Sitobion avenae	1/14 (7%)	0/4	0/1	0/8	1/27 (4%)
S. fragariae					
Total	15/109 (14%)	8/115 (7%)	3/242 (1%)	5/206 (2%)	31/672 (5%)

A = Suction trap about 2 m high.
B = R.I.S. type trap about 12 m high.

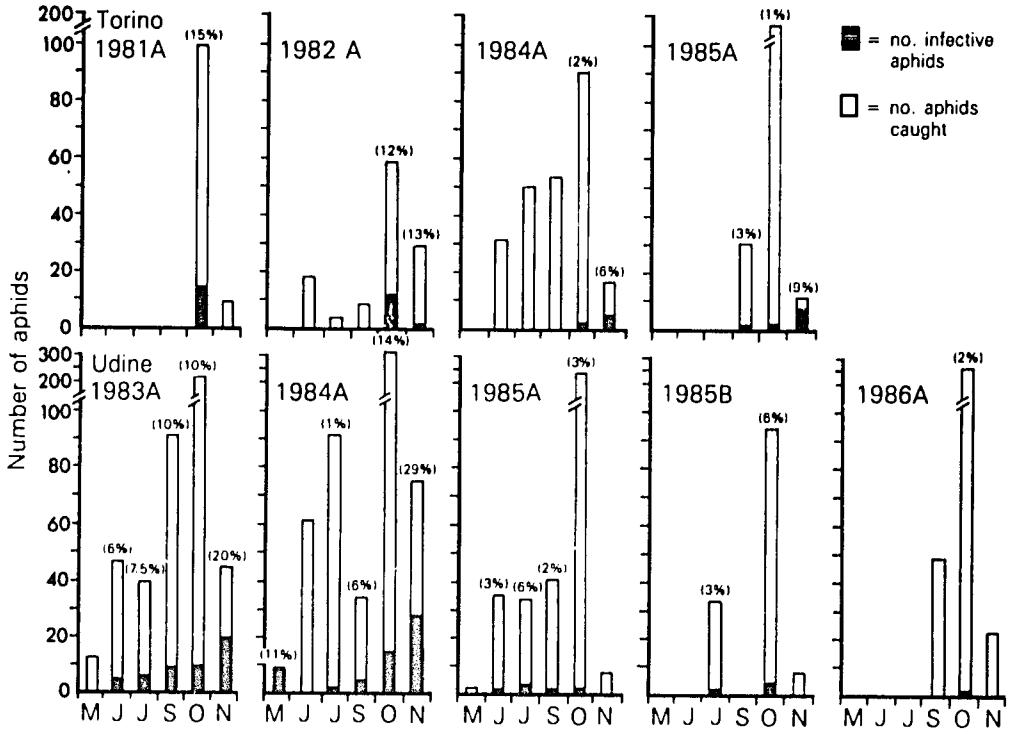

Figure 1. Monthly numbers of infective aphids captured by "live suction traps" compared with the monthly totals of potential BYDV vector species caught.

A = trap about 2 m high. B = R.I.S. type trap about 12 m high.

traps and tested for their infectivity

Pozzuolo-Udine						
1983 A	1984 A	1985 A	1985 B	1986 B	Total Udine	
No. infective aphids/No. trapped individuals (%)						
1/57 (2%)	0/126	0/122	1/42 (2%)	0/167	2/514	(0.4%)
1/24 (4%)	0/4	0/5	1/21 (5%)		2/54	(4%)
0/2	1/1 (100%)				1/3	(33%)
2/10 (20%)	0/29	0/2			2/41	(5%)
0/2					0/2	
0/5	0/4				0/9	
0/2	0/2			0/4	0/8	
0/24	1/8 (12%)	0/11	0/6	0/15	1/64	(2%)
41/319 (13%)	69/384 (18%)	12/203 (6%)	5/64 (8%)	6/138 (4%)	133/1108	(12%)
0/2	0/1				0/3	
	0/1			0/1	0/2	
0/12	0/23	0/15	0/1	0/1	0/52	
0/2					0/2	
45/461 (10%)	71/582 (12%)	12/358 (3%)	7/134 (5%)	6/236 (2%)	141/1862	(8%)

Table 2. Monthly total of *R. padi* caught by "live suction traps" and tested for their infectivity on *A. sativa*

	Carmagnola-Torino				
	1981 A	1982 A	1984 A	1985 B	Total Torino
May					
June		0/13			0/13
July			0/6		0/6
September		0/2	0/1	0/16	0/19
October	13/87 (15%)	2/16 (12%)	2/33 (6%)	0/94	17/230 (7%)
November	0/6	1/6 (17%)	1/14 (7%)	1/7 (14%)	3/33 (9%)
Total	13/93 (14%)	3/37 (8%)	3/54 (5%)	1/117 (1%)	20/301 (7%)

A = Suction trap about 2 m high.
B = R.I.S. type trap about 12 m high.

The average rate of survival of the alatae—with the only exception being *A. corni*—on the test plants after 1, 2, and 3 inoculation days was 98%, 96%, and 89%, respectively. Of all aphids tested from 1981 to 1986, 5% transmitted BYDV in Torino and 8% in Udine. The number of the alates captured and the level of their natural infectivity proceeded in parallel during the season, being low in May; increasing in June; and reaching their maximum in October-November (Figure 1).

Of the total number of plants that were considered to be BYDV-infected, 11% did not show symptoms but were ELISA-positive; another 11% showing typical BYDV symptoms of plants did not react positively in ELISA.

Discussion

Although *R. maidis* and *M. albidum* were aphid species that transmitted BYDV to the test plants in the highest percentages, in northwestern and northeastern Italy, respectively, *R. padi* seems to be the most important virus vector in both the areas studied. It was the species caught most frequently and was often infective (Tables 1 and 2).

The number and high level of infectivity of the migrant aphid populations in October-November explain why the winter cereals may suffer heavy BYDV infections in northern Italy. This is similar to the observations in West Wales of A'Brook and Dewar (1980).

The level of infectivity of the different aphid species varied greatly from year to year in both the localities considered (Table 1). In northwestern Italy, five of nine aphid species, considered potential BYDV vectors, were found to naturally transmit the virus while, in northeastern Italy, 5 of 13 captured aphid species transmitted BYDV to the test plants.

The presence of different species of aphid as vector of BYDV in the two locations studied (Table 1) may suggest that different epidemiological conditions exist in the two areas. However, to clarify this point as well as other aspects of the local BYDV epidemiology, more extensive investigations are needed.

Acknowledgments

The authors thank Giacinto Valdevit for collaboration in the identification of aphid species and Alberto Loschi for technical assistance.

Pozzuolo-Udine					
1983 A	1984 A	1985 A	1985 B	1986 B	Total Udine
0/3					0/3
1/6 (17%)	0/10	1/1 (100%)			2/17 (12%)
1/23 (4%)	1/77 (1%)	2/25 (8%)	0/6		4/131 (3%)
9/75 (12%)	2/15 (13%)	1/29 (3%)		0/24	12/143 (8%)
21/179 (12%)	44/214 (21%)	8/144 (5%)	5/52 (10%)	6/101 (6%)	84/690 (12%)
9/33 (27%)	22/68 (32%)	0/4	0/6	0/13	31/24 (25%)
41/319 (13%)	69/384 (18%)	12/203 (6%)	5/64 (8%)	6/138 (4%)	133/1108 (12%)

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Infectivity Index and Barley Yellow Dwarf Virus Infection in Friuli-Venezia Giulia

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Abstract

*Barley yellow dwarf virus (BYDV) has been causing severe yield losses in autumn-sown cereals in Friuli-Venezia Giulia, northeastern Italy, since 1978. Since 1983, the correlation between migrations of aphid vectors, their natural infectivity, and infection in barley (*Hordeum vulgare *L.) crops has been studied. An infectivity index was obtained by multiplying the number of the aphids caught in a Rothamsted Insect Survey trap by their infectivity. To evaluate the BYDV incidence in barley fields, one plot of the cv. Astrix was sown in mid-October each year in close proximity to the suction traps. The BYDV presence in both* Avena sativa *L. test plants and the barley plot was determined by ELISA (enzyme-linked immunosorbent assay), using an antiserum for the PAV-like isolate of the virus. Every year, the correlation between the infectivity index and the primary BYDV infections in the experimental field was evaluated. The results are discussed, with particular regard to the predominant role of the aphid* Rhopalosiphum padi *(L.) as vector, the pattern of the secondary virus spread, and the related yield losses.*

The first report of widespread barley yellow dwarf virus (BYDV) infection in the Friuli-Venezia Giulia Region, northeast Italy, was reported in 1978 (Conti 1978). Since then the virus has caused considerable damage to autumn-winter grains. The "BYDV Problem" (Plumb 1983) includes the study of the virus-vector-host relationships (Osler et al. 1984), as well as attempts to control the disease (Peressini and Coceano 1986; Snidaro 1984).

The aim of this research was to survey the infections of BYDV in an experimental field of barley (*Hordeum vulgare* L.) during crop growth in order to identify the primary infection and the consequent secondary spread, if any, due to the activity and infectivity of the wingless vectors.

The infectivity index (Plumb et al. 1982) for the various years was calculated and an attempt was made to verify its usefulness and applicability in our environmental conditions. This work helps in forecasting and determining control strategies for BYDV infections in autumn-sown grains.

Materials and Methods

Quantification of BYDV infections

Since 1983, in an area where BYD is epidemic, a plot of the susceptible barley cultivar Astrix has been planted. This plot was seeded each year in mid-October. Single plants as samples for laboratory tests were gathered at random. In the first year (1983/84) four samplings were made, each consisting of 200 samples along the diagonals of the field. In the following 3 years, sampling was carried out bi-weekly, from the second- to third-leaf stage up to heading, taking 4 plants in succession in the row, in 12 sectors of the plot, for a total of 48 plants per survey. Approximately 40 samplings were carried out each year.

The samples collected (portions of 0.5 g per plant) were conserved at -20°C until they were used. For evaluation of the infection the double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) was carried out on raw sap, using an anti-BYDV PAV-type serum supplied by Bioreba (Switzerland) (Clark and Adams 1977; Peressini 1985; Plumb 1974). It was decided to consider the BYDV infections found approximately 20 days after the conclusion of the autumn migration of the potential aphid vectors as primary.

The data relating to migrations and infectivity of the potential aphid vectors of BYDV in the Friuli-Venezia Giulia region are derived from work carried out previously (see Caciagli et al.; Loi et al., these Proceedings).

Infectivity indexes

For each year of research, the infectivity indexes were calculated according to Plumb et al. (1982). These authors pointed out that to obtain the infectivity index, the most important components are the number of migrating potential aphid vectors, their level of infectivity in the period considered, and the vegetative stage of the susceptible crop. The infectivity index is obtained by multiplying the number of aphids caught in the Rothamsted Insect Survey trap (12.20 m) by the corresponding infectivity rate.

For the purpose of the present work two indices were used: one from the standard 40th week (Taylor et al. 1981) (Autumn Infectivity Index), corresponding to the beginning of the ordinary autumn sowing period in our region; the other from the emergence date of the plants in our experimental plot (Emergence Infectivity Index). The period of time between emergence and the end of the vectors' flight was defined as "exposure period"; it was expressed in days and represented the risk period for the autumn-sown crop. Therefore, multiplying the Emergence Infectivity Index by the exposure days of the barley Astrix cultivar, the Specific Infectivity Index was calculated for each of the 4 years of trials. In Figure 1 the infectivity indexes are shown calculated on a weekly basis.

The Specific Infectivity Index is therefore related to the relative primary infection levels and to the yield obtained in the various research years (Figure 2).

Yield

The yield data quoted here are based on the production of four common cultivars of barley (Alpha, Arma, Barberousse, and Igri) grown in the same area and with the same time of sowing as the Astrix experimental field. These cultivars were chosen because they are the most frequently grown in the area, and even though they are all susceptible to BYDV, Alpha and Barberousse are considered more tolerant than Arma and Igri.

Results

The data relating to the calculation of the Autumn Infectivity Index are shown in Table 1. They illustrate the variability between years in the number of migratory aphids and the infectivity percentages of the potential BYDV vectors. A considerable proportion (from 25 to 60%) of the total captures was made after week 40 (see Caciagli et al., these Proceedings). The average infectivity of the vector aphids varied from a minimum of 2.9% in 1986 to 16.8% in 1984 (see Loi et al., these Proceedings).

The Specific Infectivity Index calculated from the date of emergence of plants in the experimental plot is given in Table 2. As can be noted from Table 1, the infectivity index reflects variations in the relationship between the number of vectors and their infectivity between years. The exposure period was quite constant in the 4 years of the study.

The infection of BYDV in the field is represented by Figure 1, where the evolution of the infections in the 4 years is shown. Finally, in Figure 2 the three main parameters are indicated: Specific Infectivity Index, primary infection level, and average yields obtained.

Discussion

An understanding of factors affecting variation in aphid populations is important when confronted with other

Table 1. Autumnal infectivity index (AII)

Year	Aphid catches (a)	Mean infectivity (b)	AII (axb)
1983	4389	0.099	435
1984	2684	0.168	451
1985	1696	0.033	56
1986	1640	0.029	48

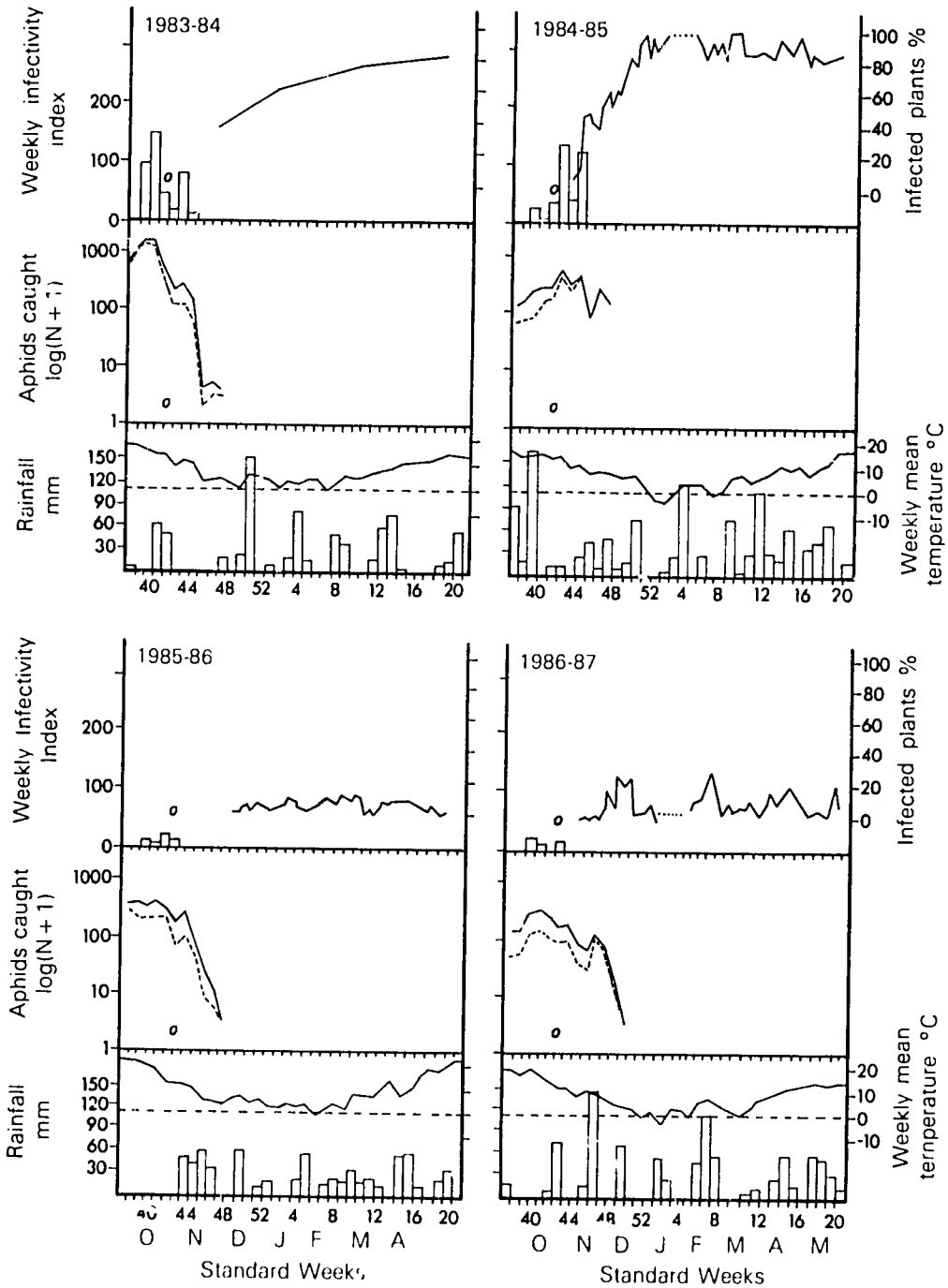

Figure 1. Catch of migrant aphids, weekly infectivity index, and evolution of BYDV infection on barley (years 1983-1987).

o = emergence. ····· = no sampling.

agro-climatical situations. Abiotic factors can influence the characteristics of aphid migrations, by advancing or delaying the flight period and, in part, even the proportions of the flight.

The Autumn Infectivity Index provides an initial indication of the different situations encountered in our region. In the first 2 years, 1983 and 1984, high values were found; the greater number of captures in 1983 being compensated by increased infectivity in 1984. In 1985 and in 1986 the Autumn Infectivity Index values were low, and substantial differences were not registered between the two parameters that determine them (Table 1).

When the emergence date of the experimental crop is considered, the profile of the various years changes significantly. In 1984 a high proportion of aphid migrations (approximately 38%) took place after the barley emergence. Meanwhile the aphid infectivity increased considerably: the direct consequence was a much higher Specific Infectivity Index than the one obtained in 1983. The situation in 1986 was different: a much smaller number of migrating aphids were recorded after the emergence (approximately 20% of the annual total) with very low infectivity (average of the period = 1.4%) resulted in the lowest Specific Infectivity Index of the 4 years (Table 2).

The infectivity indices calculated in this manner vary significantly from those of Table 1 and do not differ from those calculated on a weekly basis for the same periods (Figure 1). In this regard data relating to the second year (1984-85) appear quite interesting. In the first stages following the emergence (standard weeks 45 and 46) during a few days the percentage of infected plants increased from approximately 10 to 50%. The weekly infectivity index contributes to the explanation of this phenomenon. The heavy migrations, accompanied by a high

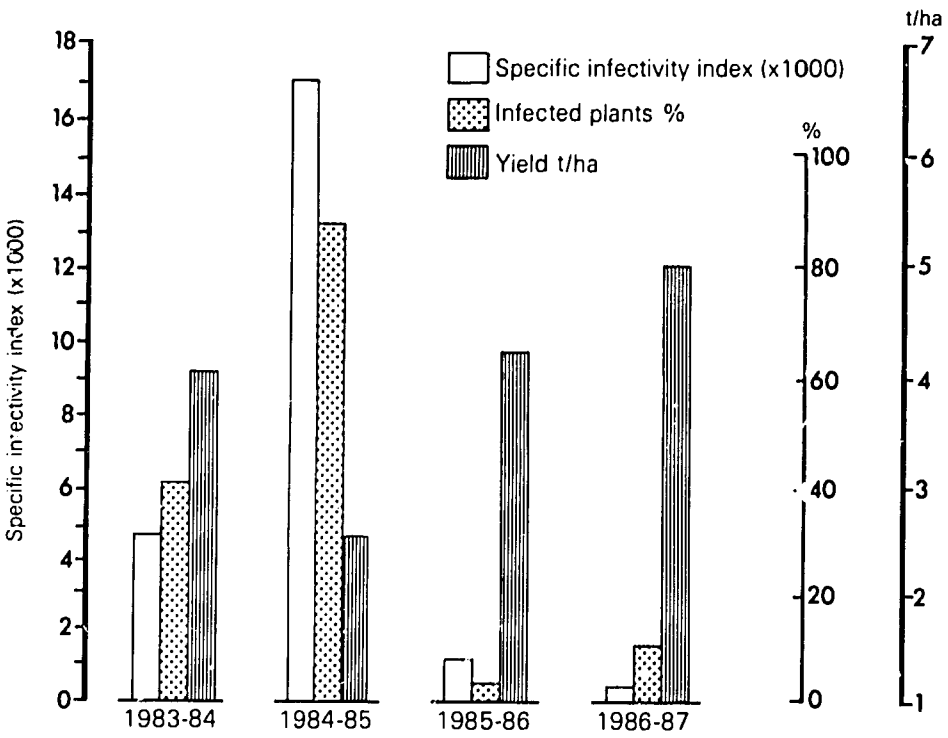

Figure 2. Comparison of specific infectivity index, primary infection level, and barley yield (years 1983-87).

infectivity, noticed in the two previous weeks, provide quite substantial infectivity indices: in particular the index of week 43 which is equal to approximately 1/3 of the total Autumn Infectivity Index.

Only in 1983/84 were aphids observed on the barley plants during the whole course of sampling. In the following seasons their presence was observed only in the autumn. This is supported by the fact that in the first year BYDV infections increased during the whole vegetative period. In the second year, by contrast, infections reached their maximum level at approximately 20 days after the end of the flight period, before a period of intense cold (first 10 days of January 1985). In 1985/86 and 1986/87 the infections were quite contained, with little or no secondary spread. In 1986/87 the infection was found to be slightly higher than in the previous year, even though the Specific Infectivity Index was lower. A possible explanation can be supplied by the greater number of *R. padi* present in that year.

Even with only 4 years of experimentation, a reasonable relationship was noted between the Specific Infectivity Index and the primary infection level and between the latter and the average yield obtained in the various years.

When the time of the aphid flights is known, the time of sowing can be adjusted to avoid exposing the emerging crop to the aphids (Coceano 1984). Our results confirm that for controlling BYDV infections in autumn-sown grains, it is important to closely monitor the crop for aphid infestations after sowing. Then according to the infection pressure, calculated as the infectivity index, it may be necessary to carry out direct control measures to stop the spread of BYDV due to colonization by wingless aphids.

Acknowledgments

We wish to thank Prof. M. Conti, Instituto di Fitovirologia Applicata del C.N.R., Turin, for his help during the whole course of the research, colleague M. Snidaro (C.R.S.A.) for his cooperation in conducting field studies, and A. Canalaz, C. Polito and G. Valdevit for their technical assistance.

Research work supported by C.N.R. Sub-Project 1, Paper N. 1501.

Table 2. Specific Infectivity Index (SII)

Year	Aphid catches (a)	Mean infectivity (b)	Exposure period (days) (c)	SII (axbxc)
		All species		
1983	770	0.136	44	4620
1984	1909	0.207	43	16985
1985	469	0.063	36	1080
1986	622	0.014	40	360
		Only *R. padi*		
1983	369	0.176	44	2860
1984	1660	0.241	43	17200
1985	192	0.071	36	504
1986	372	0.023	40	360

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The 1985 Barley Yellow Dwarf Epidemic in Winter Wheat Involving Barley Yellow Dwarf Virus Transmitted by *Schizaphis graminum* and Wheat Streak Mosaic

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Abstract

The first confirmed widespread barley yellow dwarf (BYD) epidemic in winter wheat in southern Idaho occurred in the 1977-78 crop. A virus similar to barley yellow dwarf virus (BYDV)-SGV was detected by enzyme-linked immunosorbent assay (ELISA) and aphid transmission in 2 of 3 wheat samples tested. Subsequent widespread BYD epidemics affected irrigated winter wheat crops in 1979-80 and 1981-82. BYDV-SGV was one of the viruses detected in these epidemics. A severe epidemic of BYD and wheat streak mosaic (WSM) occurred in 1984-85 in dryland winter wheat in southeastern Idaho. Large numbers of Schizaphis graminum *(Rondani) and* Rhopalosiphum padi *(L.) infested young wheat plants that fall, but 94% of the barley yellow dwarf virus (BYDV) detected in numerous samples by ELISA and aphid transmission tests was transmitted by* S. graminum, *indicating that the predominant virus was similar to BYDV-SGV. Comparison of four pairs of severely affected and minimally affected adjacent fields revealed that the latter were seeded about 4 weeks later or were treated with the systemic insecticide phorate at seeding time. Studies revealed that the phorate treatment substantially reduced the incidence of BYD but had no effect on the incidence of WSM.*

The first verified epidemic of barley yellow dwarf (BYD) in irrigated winter wheat (*Triticum aestivum* L.) in southern Idaho occurred in 1978. Subsequent epidemics affecting irrigated winter wheat occurred in 1979-80 and 1981-82. Although only 3 to 12 wheat samples were tested by vector transfer and enzyme-linked immunosorbent assay (ELISA) each year from 1978-1981, luteoviruses similar to barley yellow dwarf virus (BYDV)-SGV were identified each year (Rochow et al. 1987). Epidemics of BYD in spring wheat and barley (*Hordeum vulgare* L.) occur less frequently, usually when seeding is delayed due to protracted wet weather. Wheat streak mosaic (WSM) has been detected sporadically in Idaho but has previously caused widespread economic losses only in 1960, when fields in Bonneville and Franklin Counties were affected.

In October 1984 much nonirrigated winter wheat in the Arbon and Rockland Valleys of Power County in southeast Idaho appeared severely chlorotic. Observations showed moderate to heavy infestations of *Schizaphis graminum* (Rondani) and *Rhopalosiphum padi* (L.). The disease was tentatively diagnosed as BYD, which was subsequently confirmed in four of six plant samples taken from six fields showing symptoms submitted to W.F. Rochow at Cornell University in Ithaca, New York. Further analysis of chlorotic plants from the two fields in which no BYDV was detected revealed the presence of many *Eriophyes (=Aceria) tulipae* Keifer, the mite vector of wheat streak mosaic virus (WSMV). Plant samples from these two fields and from one of the four BYD fields were sent to J.H. Riesselman and T.W. Carroll at Montana State University, who confirmed WSM. It then became apparent that the epidemic involved two diseases, and further observations showed the incidence of each was sufficient to cause appreciable yield losses.

Wheat producers in this dryland, summer-fallow area usually seed between late August and mid-September. However, because of severe snowmold losses (due primarily to infection by *Typhula* sp.) the preceding years, many growers switched from their usual hard red cultivars to the snowmold tolerant,

soft white cultivar, Sprague, and started seeding in early August. Coincidentally, spring wheat seeded in the area matured later than normal in 1984 due to delayed spring seedings, and was still green when the fall wheat emerged. Thus, the early fall seeding and the continuous green wheat provided favorable conditions for both diseases.

Our purpose is to report the uniqueness of this epidemic caused by SGV-like BYDV and WSMV and to document the effects of seeding dates and phorate treatments on disease incidence.

Materials and Methods

Observations were made on several occasions during the fall of 1984 and spring of 1985 to evaluate the extent and severity of the epidemic. To determine the incidence of the two diseases and establish subsequent yield losses, eight fields were selected in Arbon Valley for intensive study. These consisted of four pairs, with one field in each pair having severe symptoms and the other with mild or no apparent symptoms. Fields in three pairs were adjacent or separated by a road. Fields in the fourth pair were about 200 meters apart. Later we learned that two of the pairs included phorate treated and untreated fields with similar seeding dates. Phorate is a systemic organophosphorus insecticide applied in the seed furrow at seeding time for aphid control (Forster et al. 1983). The other two pairs were not insecticide treated but had greatly different seeding dates.

To determine disease incidence, a sample of 50 randomly selected plants in mid-tillering stage (Feekes growth stage 3) was taken from each field in November for indexing on virus indicator plants. Each sample was indexed for BYDV on oats (*Avena sativa* L. cv. California Red) using *S. graminum* and *R. padi*, the species associated with the epidemic. Checks including a known SGV-like BYDV isolate and virus-free aphids were employed. Characteristic symptoms were expressed in the indicator plants after 2 weeks.

The same series of plant samples was indexed for WSMV by mechanical transmission. Leaf pieces from test plants were ground in a mortar with a small amount of distilled water. The mixture was rubbed lightly on one side of a wheat seedling with a carborundum coated cotton swab. Virus incidence was recorded after 3 weeks.

Results and Discussion

Field observations revealed that early-seeded fields were more severely affected by both WSM and BYD and that phorate treatments reduced BYD but not WSM in early seedings. This observation is supported by the results of disease indexing of plants from the selected fields (Table 1). The mean incidence of BYD was 23% in the two non-insecticide-treated fields seeded around August 15 and only 3% for the two seeded around September 10. The actual incidence in the fields was probably higher, however,

Table 1. Results of greenhouse indexing of wheat plants from fields in Arbon Valley, Power County, using mechanical inoculation for wheat streak mosaic and aphid inoculation for barley yellow dwarf

Field No.	Treatment[a]	Seeding date	Wheat streak mosaic	Disease incidence (%) Barley yellow dwarf S. graminum	R. padi	Total
1	Phorate	Aug 10	78	2	0	2
2	Untreated	Aug 10	80	13	2	15
3	Phorate	Aug 10-15	46	4	0	4
4	Untreated	Aug 10-15	76	33	0	33
5	Untreated	Sept 10	22	0	0	0
6	Untreated	Aug 15	54	9	2	11
7	Untreated	Sept 10-15	32	2	2	4
8	Untreated	Aug 10-15	46	22	4	26

[a] Phorate treatments were applied in furrow at planting at the rate of 1.12 kg ai/ha.

since only 15 of 32 check indicator plants infested with *S. graminum* that had previously fed on plants known to be infected with BYDV-SGV became infected. Similarly, the mean incidence of WSM detected was 63% in the six early-seeded fields and only 27% in the two late-seeded fields.

About 94% of the BYDV detected in field plants was transmitted by *S. graminum*, indicating that the predominant virus was similar to BYDV-SGV. This result is substantiated by the fact that the BYDV in the initial four infected samples submitted from the epidemic area was serologically similar to BYDV-SGV.

The virus transmitted by *R. padi* could have been either PAV-like BYDV or RPV-like. BYDV PAV is efficiently transmitted by *R. padi* and inefficiently by *S. graminum*, while BYDV RPV tends to be specifically transmitted by *R. padi*. BYDV RPV is occasionally transmitted by *S. graminum*. There was no evidence of mixed infections of the BYD viruses since *S. graminum* and *R. padi* did not transmit virus from the same plant. Mixed infections of WSMV and BYDV, however, were common. Under circumstances of this epidemic, the systemic insecticide phorate was apparently highly effective in reducing BYD spread, but apparently had little effect on WSM incidence.

The WSM epidemic was undoubtedly precipitated by early seeding of the winter wheat crop, which emerged while many fields of the spring wheat crop were immature. Wet, unusually cool, spring weather delayed seeding and, hence, maturity of the spring crop. Although no observations were made on the spring crop, there is reason to believe that *E. tulipae* transmitted the WSMV from the spring wheat to early-seeded winter wheat seedlings. The moderate levels of WSM observed in the two later seeded winter wheat fields (Table 1) suggest the mite populations continued to develop and spread the virus through the early fall period.

Overlapping of the spring and fall crops may have also contributed to the BYD spread. SGV-like virus, transmitted by *S. graminum*, was apparently the main virus in the epidemic. *S. graminum* can overwinter as eggs in the winter crop. After a period of aphid colony development, migrating forms could transfer SGV-like BYDV from the winter to spring crops and subsequently to the emerging winter crop. In this way, the SGV-like BYDV would be perpetuated. By contrast, *R. padi* overwinters on *Prunus virginiana* L. Spring migrant forms from this host would be BYDV-free but could acquire the virus by feeding on BYDV-infected wild grasses or cereals. A period of colonization would be required before significant numbers of alatae would be produced to spread the virus to the winter wheat crop. Although significant virus spread would be possible, the route would certainly be less direct than for SGV-like BYDV.

All eight fields in this study, as well as dozens of others similarly affected, were plowed in the spring after it became apparent that yields would be severely depressed, and it was not possible to obtain crop loss data from these fields. However, several other similarly affected fields in the area that were not plowed yielded 340 to 540 kg/ha. This contrasts with a potential 3300 to 4000 kg/ha yield for the cultivar Sprague in that area. We believe that the WSM accounted for a much larger proportion of the yield loss than the BYD.

This is apparently a unique region in view of the infrequent detections of SGV-like isolates elsewhere.

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Maize, a Natural and Experimental Host of Barley Yellow Dwarf Virus in Northern Italy

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Abstract

*During recent years a gradual increase in maize (*Zea mays *L.) plants showing barley yellow dwarf virus (BYDV) symptoms has been noticed in northern Italy, mainly in late-sown crops. Severe damage was observed in some inbred lines, sometimes causing a total crop loss. Results obtained by surveys using enzyme-linked immunosorbent assay (ELISA) and by aphid transmissions confirmed that an isolate of BYDV (M-PAV) is the agent inducing the described symptoms.* Rhopalosiphum padi *(L.) is the most efficient vector of the M-PAV isolate, which is also transmissible by* Sitobion avenae *(Fabricius) and by* Metopolophium dirhodum *(Walker). Transmissions with* R. padi *proved that there are maize lines that show BYD symptoms both in the greenhouse and in the field; other lines only show symptoms in the field, and yet other lines never develop symptoms. Symptomless plants were also detected by ELISA among maize inbred lines and hybrids with natural infection. The possible role of maize in the epidemiology of BYD is considered. In particular it was observed that maize is an excellent autumn host for aphids, especially for* R. padi. *The percentage of* R. padi *collected at random from maize plants in five localities that transmitted BYDV ranged from 0.5 to 7%.*

During the last 10 years a gradual increase in maize (*Zea mays* L.) plants showing barley yellow dwarf (BYD) symptoms has been noticed in northern Italy. Nevertheless, up to now the disease has not played an important economic role in maize hybrids. However, some of the inbred lines of maize used for production of commercial hybrids have been found to be very susceptible to barley yellow dwarf virus (BYDV), to the extent that sometimes it is not possible to produce seeds in areas where the disease is epidemic (Loi et al. 1985; Osler et al. 1985).

BYDV has been reported to affect maize in several European and American countries, both from experimental (Allen 1957; Watson and Mulligan 1960; Stoner 1965, 1977; Panayotou 1977) and natural infections (Stoner 1977). Symptomatic and asymptomatic BYDV infections in maize have been reported (Loi et al. 1985; Osler et al. 1985). Various isolates of BYDV have been detected in maize plants in the countries where they have been studied (Panayotou 1977; Stoner 1977; Bayon et al. 1981; Paliwal 1982; Eweida et al. 1983; Brown et al. 1984).

Considering the economic importance of maize and the epidemiology of BYD in the Friuli-Venezia Giulia region, a study was undertaken to determine the extent of the disease in maize fields of the region, the most common and typical symptoms on maize, the precise etiology of the disease, the responses of some maize hybrids and inbred lines to virus infection both in the field and in the greenhouse, and the role of maize in the epidemiology of BYD. The results are discussed below.

Materials and Methods

During 1981 to 1986, natural distribution and incidence of BYD in maize were investigated in several areas of the Friuli-Venezia Giulia region, in collections of hybrids and inbred lines. The plots were periodically checked, starting from seedling emergence and continuing until August.

In October 1984 about 100 young potted plants of barley (*Hordeum vulgare* L.) and oats (*Avena (byzantina) =sativa* L.) and 5 plants of each of 15 different genotypes of maize were exposed as bait plants to natural infections of BYDV. One to two months after the beginning of the exposure, 2 or 3 plants per genotype were analyzed for BYDV infection by enzyme-linked immunosorbent assay (ELISA).

The symptomatology of BYD on maize was checked in hybrid and inbred lines, both in the greenhouse and in the field.

The etiology of the disease was determined both by infectivity recovery tests carried out by aphids and by ELISA, using antiserum to a PAV-like isolate. Transmission trials on *A. sativa* and maize inbred line 33-16 were conducted with the vector *Rhopalosiphum padi* (L.), using six maize inbred lines and seven hybrids with natural infections and all showing BYD symptoms, as sources of inoculum. Transmissions were also carried out with the aphid species *R. padi*, *Sitobion avenae* (Fabricius) and *Metopolophium dirhodum* (Walker) from oats experimentally infected with a strain of BYDV isolated from maize, i.e., M-PAV, to oats and maize 33-16.

The aphids acquired virus by feeding on detached leaves in containers in the greenhouse, at 20°C for 2 days. Plants were inoculated in the greenhouse with viruliferous aphids (three per plant for oats and ten for maize 33-16) to feed for 3 to 5 days. Maize test plants were inoculated at the four-leaf stage or soon after germination. The positive infections of the test plants were assessed by both symptom expression and ELISA reaction.

ELISA was also used on maize plants (hybrids and inbred lines) grown in the field and showing BYD symptoms. Additionally, symptomless plants were checked by ELISA in order to detect latent BYDV infections.

To determine if environmental conditions affected symptom expression in maize, plants of 13 different inbreds were inoculated by *R. padi* in the greenhouse and half of them were transplanted immediately into field plots. After symptom appearance, all the plants, including the symptomless ones, were tested by ELISA.

The role of maize in the epidemiology of BYD was investigated in areas of Friuli where both maize and barley crops were present. In October 900 *R. padi* individuals were collected at random from 300 plants of commercial maize hybrids, in five different localities where the disease was epidemic, and fed on oats to check their infectivity (3 aphids per test plant).

Results

From 1981 to 1986 BYD was constantly noted in the Friuli-Venezia Giulia region, both on maize hybrids and inbred lines cultivated in different areas. The first symptoms could be detected from the end of May (inbred lines) to the middle of June (hybrids). The percentage of plants showing BYD symptoms ranged from zero in the tolerant genotypes to almost 100% in some inbred lines, i.e., 33-16, NC222, W64a.

None of the young maize bait plants exposed to natural infections in October showed BYD symptoms before frost (beginning of December), but ELISA reactions were positive in 21 of 30 cases. All the oat and barley plants showed BYD symptoms and the reaction to ELISA was positive in the 6 samples examined.

ELISA-confirmed BYD symptoms in maize are: red-purple discoloration, starting from the tip and the margins of the basal leaves; parallel red-purple stripes; marginal reddening; leaf necrosis; and stunting. The type of symptoms observed in the greenhouse were similar to those developed in the field, but they were generally less severe.

The results obtained in experimental transmissions by aphids are summarized in Table 1. BYDV was recovered by a high percentage of *R. padi* from the six maize inbred lines and seven maize hybrids that showed typical symptoms. A high percentage of positive transmissions was also obtained from oats and maize 33-16 previously artificially inoculated with the M-PAV strain to oat and maize 33-16 test plants. *R. padi* was the most efficient vector of BYDV recovered from maize, followed by *M. dirhodum* and then by *S. avenae*.

There was agreement between symptom expression and ELISA results for oats but diagnosis based on ELISA is more sensitive than symptom appearance in the maize line 33-16.

With field samples of 65 inbred lines and 33 hybrids of maize showing BYD-like symptoms 56 (86%) and 29 (88%) plants, respectively, gave a positive reaction when tested by ELISA. Nine symptomless inbreds and 1/30 of the symptomless hybrids in the field proved to be infected by BYDV.

The comparative trials among the 13 inbred lines inoculated and split into two groups (greenhouse and field) gave the following results: a) five lines (B64, B68, Lo876o2, Mo17, N7B) did not show any symptoms either in the greenhouse or in the field, but 84% (42/50) of the greenhouse and 54% (29/54) of the field reacted positively in ELISA; b) four lines (B73, B77, C123, Va26) developed symptoms only in the field (92% of the plants), ELISA reaction was positive for 98% of the plants grown in the greenhouse and 100% of those grown in the field; c) four lines (NC222, Pa70, W64a, 33-16) developed symptoms both in the greenhouse (74%) and in the field (96%); ELISA reaction was positive for 100% of the former and 98% of the latter.

The percentage of *R. padi* collected at random in October from commercial hybrids in five localities of the Friuli-Venezia Giulia region that transmitted BYDV ranged from 0.5 to 7%. It was also observed that colonies of aphids, mainly *R. padi*, are quite common and often large on maize in the fall.

Discussion

Field and greenhouse observations, as well as transmission trials and ELISA tests, have confirmed that marginal reddening of the basal leaves, stunting, and precocious necrosis are typical symptoms of BYD on maize.

The disease is present in all the maize areas of the Friuli-Venezia Giulia region, as well as in northern Italy (Belli et al. 1980; Osler et al. 1985). Inbred lines are more sensitive than maize hybrids.

Table 1. Results obtained in aphid transmission of BYDV (M-PAV) from maize and *A. sativa* to maize and *A. sativa*

		Number of test plants with positive reaction (relative percentage)			
		A. sativa		Maize 33-16	
Source of inoculum	Aphid species	Symptom expression	ELISA positive	Symptom expression	ELISA positive
Six inbred lines with natural symptoms	*R. padi*	30/36 (83%)	30/36 (83%)	42/81 (52%)	70/81 (87%)
Seven hybrids with natural infection	*R. padi*	27/35 (77%)	27/35 (77%)	16/41 (39%)	21/41 (51%)
A. sativa artificially inoculated	*R. padi*	45/90 (50%) (50%)	27/30 (90%) (90%)	15/31 (48%) (48%)	28/31 (90%) (90%)
	S. avenae	27/57 (47%)	5/10 (50%)	1/22 (5%)	7/22 (32%)
	M. dirhodum	33/57 (58%)	5/10 (50%)	1/22 (5%)	9/23 (39%)
Maize 33-16 artificially inoculated	*R. padi*	13/20 (65%)	14/20 (70%)	7/14 (50%)	13/14 (93%)

Transmission of BYDV by aphid vectors from maize hybrids and inbred lines to maize and oats and vice versa occurs. *R. padi* is the most efficient vector, followed by *M. dirhodum* and then by *S. avenae*. The aphid species *Schizaphis graminum*, *R. maidis*, and *M. festucae* have not been found to transmit the M-PAV isolate present in Friuli in maize plants.

ELISA tests on different inbreds and hybrids of maize have proved that the M-PAV isolate is widespread in maize crops of the Friuli-Venezia Giulia region. On maize 33-16 ELISA reaction is a more sensitive diagnostic method than symptom expression. Practically no difference was noted between the two methods when applied to oats.

Genotype and environmental conditions can play an important role in BYD symptom expression on maize. Symptom occurrence and intensity are generally more frequent and severe in plants grown in the field than in the greenhouse. We suggest the results obtained support the conclusion that the symptomless lines and hybrids of maize are tolerant but not resistant.

From the epidemiological point of view, maize seems to be an important host of BYDV. In fact, in October maize plants are quite frequently infested by large colonies of *R. padi* and the incidence of the disease at that time can reach 7%. A high level of BYDV infection in the fall was shown by exposing potted plants of maize, oats, and barley to natural infection.

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Barley Yellow Dwarf Infectivity of *Rhopalosiphum padi* in Maize as an Estimate of Primary Inoculum Pressure in Irrigated Winter Wheat

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Abstract

Barley yellow dwarf (BYD) has become an increasingly important limiting factor in irrigated small grain production in the Pacific Northwest of the USA. Capability to predict BYD outbreaks depends upon measuring vector flight intensity and primary inoculum pressure. Rhopalosiphum padi *(L.) collected from maize (*Zea mays *L.), from newly emerged small grains and from a suction trap modified for live collection were assayed for BYDV transmission. Percent transmission by* R. padi *collected on small grains was the same or slightly higher than by* R. padi *collected in maize. Percent transmission by* R. padi *in the suction trap was considerably higher than by* R. padi *collected on plants. It appears from our data that a measure of the inoculum reservoir in maize could be a good predictor of primary inoculum in cereals in irrigated areas in the Pacific Northwest.*

Barley yellow dwarf virus (BYDV) has increasingly been implicated in yield loss in irrigated small grain production in the Pacific Northwest of the USA (Blackmer 1986; Forster et al. 1983; Halbert and Pike 1985). Spread of the disease can be reduced by delayed planting of fall grains and/or use of systemic insecticides to control aphid vectors; but since protective measures are not required every year, it would be useful to be able to predict potential for epidemics.

Although aphid numbers are important, potential for virus spread is also contingent upon the percent of the aphids that are viruliferous. A suction trap network is doing a satisfactory job of monitoring grain aphid flights (Halbert et al. in press; Pike and Bishop 1987), but a means of monitoring primary inoculum pressure is needed. Inoculum level can be measured by assaying aphids collected in suction traps modified for live collection (Plumb and Lennon 1982), but this method is too labor-intensive for area-wide use. *Rhopalosiphum padi* (L.), the most important BYDV vector in irrigated grain, builds up to populations of 60 million/ha on maize (Blackmer 1986). Maize (*Zea mays* L.) is also a symptomless carrier of BYDV (Brown et al. 1984). Experiments were conducted to ascertain whether percent transmission by *R. padi* collected in maize could be related to percent transmission by *R. padi* in flight and on newly emerged grain. Aphids collected in an adapted suction trap were used as a standard of comparison in 3 of the 4 years.

Materials and Methods

R. padi was collected from maize and grain in Prosser, Washington, in 1983, from maize, small grains, and a suction trap adapted for live collection in Prosser in 1984, and from maize, small grains, and an adapted suction trap in Canyon County, Idaho, in 1985 and 1986. All aphids were assayed for BYDV transmission.

Results and Discussion

Percent transmission by resident *R. padi* collected from maize and by immigrant *R. padi* collected from grain in 1983 was much higher than in the other 3 years (Table 1). Percent transmission by *R. padi* collected in the suction trap was

Table 1. Percent transmission of BYDV by *R. padi* collected on maize, small grains, or in a suction trap

	Maize (all morphs)	Small grains (alates only)	Trap
WA 1983	11.5	10.5	–
WA 1984	0.0	2.7	7.2
ID 1985	1.2	2.4	6.0
ID 1986	0.6	1.3	6.9

consistently higher than percent transmission by *R. padi* collected on plants. In the experiments done in Idaho, male *R. padi* were found to transmit BYDV particularly frequently. Fall migrant forms may be inherently better vectors, or they may visit more plants than alate exules.

It appears from these data that a measure of the inoculum reservoir in maize could be a good predictor of primary inoculum pressure in irrigated areas. Using the transmission by *R. padi* collected from maize as a predictor also has the advantage of providing a mid-summer indicator of the potential for an autumn epidemic.

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Comparisons of Serological and Vector Relationships Among MAV-like Isolates of Barley Yellow Dwarf Virus from Australia and the USA

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Abstract

Some barley yellow dwarf (BYDV) isolates obtained from cereals collected in Victoria, Australia, during 1985-86, were serologically similar to the MAV isolate of W.F. Rochow, but seemed more akin to the PAV isolate of Rochow in vector relationships. Most serological comparisons used the Purdue culture of Rochow's MAV, initially obtained from Cornell but maintained by transfer by Sitobion avenae *(Fabricius) during 6 years. However, detailed examination of the Purdue culture with monoclonal antibodies revealed that a change in its serological behavior had occurred during culture, although the characteristics of efficient transmission by* S. avenae *and not* Rhopalosiphum padi *(L.) had been maintained.*

This apparent change in serological behavior of the type MAV of Rochow during subculture was unexpected, and would not have been noted with the polyclonal antisera available to us, to which both the Rochow and Purdue cultures react similarly. Among possible reasons is selection from a mixture of MAV-like viruses having generally similar vector relationships. The results emphasize that, because aphid transmission is obligatory for transfer of BYDV, it is impossible to obtain "pure" cultures, for all BYDV isolates are likely to comprise mixtures of viruses that happen to be similarly transmissible by the vector(s) used for passage. In this way, the Australian MAV-like isolates, which differ in vector relationships from the USA MAVs, may have been selected from introduced MAV-like infections by the aphid vectors that predominate in Australia.

For practical reasons, vector transmissibility was used in early studies of the luteoviruses grouped as barley yellow dwarf virus (BYDV) (Rochow 1970) as a fundamental characteristic distinguishing isolates. Illustrative of this are the five isolates given acronyms based on their principal aphid vectors, as follows: RPV, transmitted principally by *Rhopalosiphum padi* (L.); RMV by *R. maidis* (Fitch); MAV by *Sitobion (then known as Macrosiphum) avenae* (Fabricius); SGV by *Schizaphis graminum*; (Rondani) and PAV by both *R. padi* and *S. avenae* (Rochow 1969; Gill 1969). These exemplify five categories of BYDV that appear to accommodate many isolates. More recently it has been shown that serological tests, especially enzyme-linked immunosorbent assay (ELISA) (Lister and Rochow 1979), can diagnose and differentiate isolates into the same categories as those given by vector studies (Rochow and Carmichael 1979; Rochow 1979, 1982a). Because serological testing is easier to apply, it is currently the predominant diagnostic method used for BYDV.

However, each category of BYDV as defined above probably includes variants differing in detail regarding such properties as virulence, serological behavior, and vector relationships. Such differences could have important implications for the epidemiology and economic effects of BYDV in a particular area, and also for serological diagnosis. We report here on the properties of MAV-like isolates from Australia and also derived from Rochow's MAV-type isolate that illustrate this potential. More extensive accounts of the results appear elsewhere (Lister and Sward, in press).

Materials and Methods

Virus isolates

The isolates used as standards were: MAV, obtained from dried oat (*Avenae sativa* L.) leaf tissue from subcultures of Rochow's isolate, maintained by mass transfer of *S. avenae* between batches of plants during 6 years at Purdue, as described previously (Lister et al. 1985);

Purdue-PAV (P-PAV) (Hammond et al. 1983), similarly obtained from dried leaf tissue from subcultures maintained at Purdue; and V1-PAV, obtained from a subculture of a PAV-like isolate, separated from the mixture of PAV- and RPV-like isolates collected in Victoria, Australia, and maintained at Burnley Gardens (Waterhouse et al. 1986). Oat cultivars Algeribee and Clintland 64 were used for isolate culture and non-infected ("healthy") control tissue at Burnley Gardens and Purdue, respectively.

ELISA

Table 1 lists the polyclonal (pc) and monoclonal (mc) antisera used as sources of immunoglobulins (Ig's) for ELISA tests. Some of the serological specificities of these Ig's have been described in the references listed. Regarding the behavior of Ig's from antisera to the MAV, the pc MAV-Ig has been shown to react heterologously with PAV-like isolates in ELISA tests (Fargette et al. 1982), though less so than homologously. The Ig's from monoclonal antisera to MAV (mc MAV-Ig's-1 and -3) are reported to react strongly with MAV, although some cross-reactivity with PAV occurred with mc MAV-Ig3 (Hsu et al. 1984).

ELISA was done with 200-μl samples in Immulon M-129 B microtiter plates (Dynatech Inc.), by standard double antibody sandwich (DAS) or indirect (ID) procedures for the pc Ig's and mc Ig's, respectively (Lister and Rochow 1979; Clark et al. 1986). Because only small supplies of the mc MAV-Ig's are available, our procedure for diagnosing MAV-like isolates is first to test with pc MAV.Ig, then to subject samples reacting positively to retesting with mc MAV.Ig-1.

Most sample extracts were made by grinding fresh or dry leaf in liquid nitrogen, then re-grinding in 0.1 M phosphate buffer, pH 7.0, at 1:10, w/v (Lister et al., 1985). Timing of the steps in ELISA varied, but for convenience was usually as follows: DAS-coating, 2 to 3 hours (37°C); sample application, 4 to 6 hours (37°C); conjugate, overnight 4°C); substrate 30 to 60 min (20°C); ID-coating, 2 to 3 hours (37°C); sample application, 4 to 6 hours (37°C); second (monoclonal) antibody, overnight (4°C); anti-mouse conjugate (Sigma), 2 hours (37°C); and substrate 30 to 60 min (20°C). Test results (A_{405} nm) were read in a Dynatech microElisa minireader. Duplicate wells were used for each

Table 1. Antisera usad as sources of immunoglobulins (Ig's) for ELISA

Ig Type/ Designation	Eliciting antigen	Source or reference
Polyclonal		
pc VI-PAV	Mixture of RPV-like and PAV-like isolates from Victoria, Australia	Sward and Lister 1987
pc IL-PAV	PAV-like isolate from Illinois, USA	Darcy and Hewings 1986
pc P-PAV	PAV-like isolate from Indiana, USA	Hammond et al. 1983
pc TAS-PAV	PAV-like isolate from Tasmania, Australia	Guy et al. 1986
pc MAV	Type MAV of Rochow	Rochow 1969
Monoclonal		
mc MAV-1	Type MAV of Rochow	Hsu et al. 1984
mc MAV-3	Type MAV of Rochow	Hsu et al. 1984

sample and results were averaged. Extracts from non-infected oats were used as controls.

Results and Discussion

Australian isolates

In course of ELISA screening of cereal survey samples collected in Victoria, Australia, with MAV- and PAV-Ig's, some were found that reacted positively with both Ig's, but more strongly with the MAV-Ig's than with the PAV-Ig. The behavior of the standard PAV-like isolate used as a check was the reverse of this (Table 2).

Use of the monoclonal antibody Ig's clarified interpretation of these results by differentiating the isolates into two groups, one (Group A) comprising isolates reacting like the PAV-like standard, and another (Group B) reacting like the MAV standard (Table 2). This behavior remained consistent in subsequent tests of the plants, which were maintained in the greenhouse for several weeks. By these serological criteria, therefore, Group A isolates could be designated as PAV-like, and Group B isolates as MAV-like.

As *S. avenae* has not been recorded in Australia, and because of quarantine restrictions, it was not possible to confirm the serological diagnosis by vector studies. However, tests done at Burnley Gardens showed clearly that a representative MAV-like isolate from Victoria was transmitted very efficiently by *R. padi* (75 transmissions in 96 tests), but relatively inefficiently by the *Sitobion sp.* available for testing, namely *S. miscanthi* (Takahashi) (3 transmissions in 52 tests). Moreover, plants infected by *R. padi* reacted negatively in ELISA with IL.PAV.Ig, indicating that transmission by *R. padi* was not due to transcapsidation (Rochow 1982b) of MAV-like genomes with PAV-like coat protein, such as might be possible in a mixed infection.

Because the finding of isolates behaving serologically like MAV was novel for Australia, their reactions and those of the MAV standard were compared in ELISA with several of the Ig's available to us for diagnosis. Values for DAS tests with various pc.Ig's and representative antigens are given in Table 3. Values for the V1-PAV isolate were as expected for a PAV-like isolate. The coating and

Table 2. ELISA of samples from Balliang collection 1, of September 1985

Sample designation (Source)	Reactions with indicated polyclonal (pc) or monoclonal (mc) immunoglobulins (Ig's)					Diagnosis
	Expt. 1		Expt.2			
	pc IL-PAV	pc MAV	mc MAV-1	mc MAV-3	pc IL-PAV	
li/1 (barley)	1.71	.42	.02	.19	.54	PAV-like
li/2 (barley)	1.82	.44	.03	.28	.76	PAV-like
lj/1 (oats)	.55	.12	.02	.25	.84	PAV-like
2a/1 (oats)	.16	.72	.70	.02	.03	MAV-like
4a/1 (oats)	.12	.55	.58	.01	.01	MAV-like
4a/2 (oats)	.13	.35	.68	.01	.00	MAV-like
4a/3 (oats)	1.81	.43	.00	.48	.95	PAV-like
4b/2 (barley)	.10	.44	.69	.02	.03	MAV-like
VI-PAV standard	1.12	.11	.02	.48	1.70	–
MAV standard	.14	1.40	.54	.06	.08	–
Health control	.02	.02	.01	.02	.04	–

All samples listed were negative in ELISA with RPV and RMV Ig's.

Ig's indicated are conjugates or second antibodies, all used at 1/1000 dilution. Polyclonal IL-PAV-Ig was used as coating Ig with IL-PAV conjugate. Polyclonal MAV Ig was used as coating Ig with MAV conjugate and monoclonal MAV-1 or MAV-3 second antibodies, respectively.

conjugated pc.Ig's from antisera to PAV-like isolates reacted efficiently with V1-PAV, whereas pc.Ig from the MAV antiserum reacted efficiently with it only when used as coating Ig. (We assume that heterologous reactions of this Ig used as conjugate were impaired by the glutaraldehyde treatment used for enzyme labelling). In the reciprocal tests of MAV antigen with various Ig's, the results indicated that pc.Ig's from antisera to the PAV-like isolates V1-PAV and IL-PAV did not react efficiently with MAV, whether they were used as coat or conjugate. Interestingly however, pc.Ig from antiserum to the TAS-PAV isolate apparently bound MAV as strongly as did pc MAV.Ig, when used either as coat or conjugate.

Similar experiments, with mc Ig's used as second antibody in indirect ELISA, confirmed that the Australian MAV-like isolates behaved like MAV in tests with a range of pc Ig's used as coat for trapping antigens with the two mc Ig's, mc MAV-1 and MAV-3, used as second antibody (Table 4). In this case, the Australian MAV-like isolates were apparently trapped almost as efficiently by the pc TAS-PAV.Ig as by the pc MAV.Ig, although both the pc V1-PAV.Ig and pc IL-PAV.Ig also trapped them more efficiently than they trapped MAV. These results confirmed the serological affinities of the Victorian MAV-like isolates and the MAV standard. They also indicated that the TAS-PAV isolate had closer serological affinities with MAV than did

Table 3. Comparative activity of selected antigens in ELISA with homologous and heterologous coating and conjugate Ig's

Antigen (conjugate Ig)	Coating Ig			
	VI-PAV	IL-PAV	TAS-PAV	MAV
VI-PAV				
(VI-PAV)	.42*	.53	.51	.45
(IL-PAV)	.61	1.09*	1.02	.78
(TAS-PAV)	1.16	1.56	1.65*	1.35
(MAV)	.20	.28	.27	.29*
MAV				
(VI-PAV)	.02*	.03	.10	.12
(IL-PAV)	.02	.03*	.11	.15
(TAS-PAV)	.05	.13	.70*	.88
(MAV)	.06	.13	.91	1.23*
2a/1				
(VI-PAV)	.05*	.13	.46	.49
(IL-PAV)	.04	.08*	.29	.34
(TAS-PAV)	.08	.23	.99*	1.08
(MAV)	.06	.17	.75	.85*
Healthy				
(V1-PAV)	.02*	.01	.02	.02
(IL-PAV)	.02	.01*	.01	.02
(TAS-PAV)	.01	.01	.01*	.02
(MAV)	.02	.03	.02	.04*

* = Reactions involving homologous coat and conjugate Ig's.
[a] Similar results were obtained in this experiment with isolate 4a/1, Table 2, and a further MAV-like isolate from Victoria.

the other PAV-like isolates that were used for pc antiserum production, or possibly that this isolate was a mixture of PAV- and MAV-like isolates. We were unable to determine this, as the TAS-PAV isolate itself was not available to us. At any rate, it is clear that use of the pc TAS-PAV.Ig alone in screening samples would probably fail to distinguish between PAV-like isolates and the Victorian MAV-like isolates.

Rochow MAV isolates

Reference to Table 4 shows that the MAV standard used, like the Victorian MAV-like isolates, did not react significantly with mc MAV.Ig3. This is in contrast to the results of Hsu et al. (1984), who used reaction with Rochow's MAV isolate as the criterion for selecting mc MAV antibodies. This anomalous serological behavior, together with anomalous vector relationships of the Victorian MAV-like isolates, prompted further examination at Purdue of the Purdue subculture of Rochow's MAV.

In serological comparisons, the behavior of the Purdue subculture was compared with that of MAV newly-acquired from W.F. Rochow's stock culture maintained at Cornell. Frozen-stored samples from earlier Purdue subcultures of the original isolate for Rochow were also available, and these were included in the comparisons. The results (Table 5) confirmed that Rochow's MAV stock culture reacted with both mc MAV-1 and mc MAV-3, but that the current Purdue subculture reacted only with mc MAV-1. Moreover it appeared that although earlier subcultures made at Purdue behaved like the Rochow MAV stock culture in this regard, more recent ones had lost the ability to react efficiently with mc MAV-3.

Rochow's MAV is characterized by transmissibility by *S. avenae* and not by *R. padi*. This was also true of the current Purdue culture of MAV in tests of its transmissibility done at Purdue. It was

Table 4. Comparative activity of selected antigens in indirect ELISAs with monoclonal antibodies to the MAV isolates

Antigen (Second antibody)	Coating pc Ig's			
	VI-PAV	IL-PAV	TAS-PAV	MAV
VI-PAV				
(MAV-1)	.01	.01	.01	.02
(MAV-3)	.68	.76	.81	.71
(MAV)				
(MAV-1)	.02	.07	.42	.57
(MAV-3)	.01	.01	.01	.01
2a/1[a]				
(MAV-1)	.16	.31	.89	.99
(MAV-3)	.01	.00	.02	.02
Healthy				
(MAV-1)	.00	.00	.00	.01
(MAV-3)	.00	.01	.00	.01

Monoclonal antibodies were used as second antibodies after antigens were bound with the coating antibodies indicated.

[a] Similar results were obtained in this experiment with isolate 4a/1, Table 2, and a further MAV-like isolate from Victoria.

transmitted by 17 of 30 *S. avenae* raised on infected Clintland 64 oats and transferred singly to individual Clintland 64 oat test plants, but by 0 of 30 *R.padi* treated similarly.

The results described illustrate important features of BYDV, and probably of luteoviruses in general. Firstly, the fact that aphid transmission is required for BYDV transfer makes it impossible to obtain "pure" cultures. All BYDV "isolates" are likely to comprise mixtures of viruses that are similarly transmissible by the vector(s) used for passage, and with the host and conditions used. Chance selection by the vector, or selection pressures due to various factors, may therefore affect the specific type of virus that predominates in a culture. In this way, change in the serological behavior, though not vector specificity, apparently occurred in the MAV isolate as subcultured at Purdue over several years. Also, conceivably the Victorian MAV-like isolates, which differ in vector relationships from Rochow's MAV, and thus probably from other MAV-like isolates, may have been selected from introduced MAV-like infections by the aphid vectors that predominate in Australia.

Secondly, although serological differentiation has come to be regarded as a more convenient way of discriminating between isolates than vector relationships, serological screening will require the use of a range of antisera, especially if monoclonal antibodies are used. Further, if serological screening is to be relevant to the epidemiological situation, it will be important, at some point in screening programs, to relate serological results to vector relationships.

Acknowledgments

We sincerely thank Miss Bernadette Deane for technical assistance, various colleagues who supplied antisera, and the Wheat Industry Research Council for funding the project.

Table 5. Comparative activity of selected antigens in ELISAs with monoclonal antibodies to the MAV isolates

Isolate (Source)	ELISA with Ig's indicated			
	Plate 1-Monoclonal Ig's		Plate 2-Polyclonal	
	MAV-1	MAV-3	MAV	P-PAV
MAV (newly acquired from W.F. Rochow)	0.47	0.74	0.52	0.14
MAV (cultured 6 years at Purdue)	0.54	0.08	0.83	0.11
P.PAV (cultured at Purdue)	0.06	0.89	0.23	1.02
Healthy	0.06	0.08	0.07	0.09

All ELISAs were done at the same time, with the same extracts. Tests with monoclonal Ig's were done in a different plate from those with polyclonal Ig's. Procedures used were DAS and ID ELISAs, as described in the text.

[a] Tests of frozen samples dated 7/15/83 and 8/11/83 were positive with both monoclonal Ig's. Those for all 8 subsequent samples available, collected at intervals between 6/7/85-3/5/86, were positive only with MAV-1.

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Cereal Aphid Vectors: A Western Regional (USA) Monitoring System

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Abstract

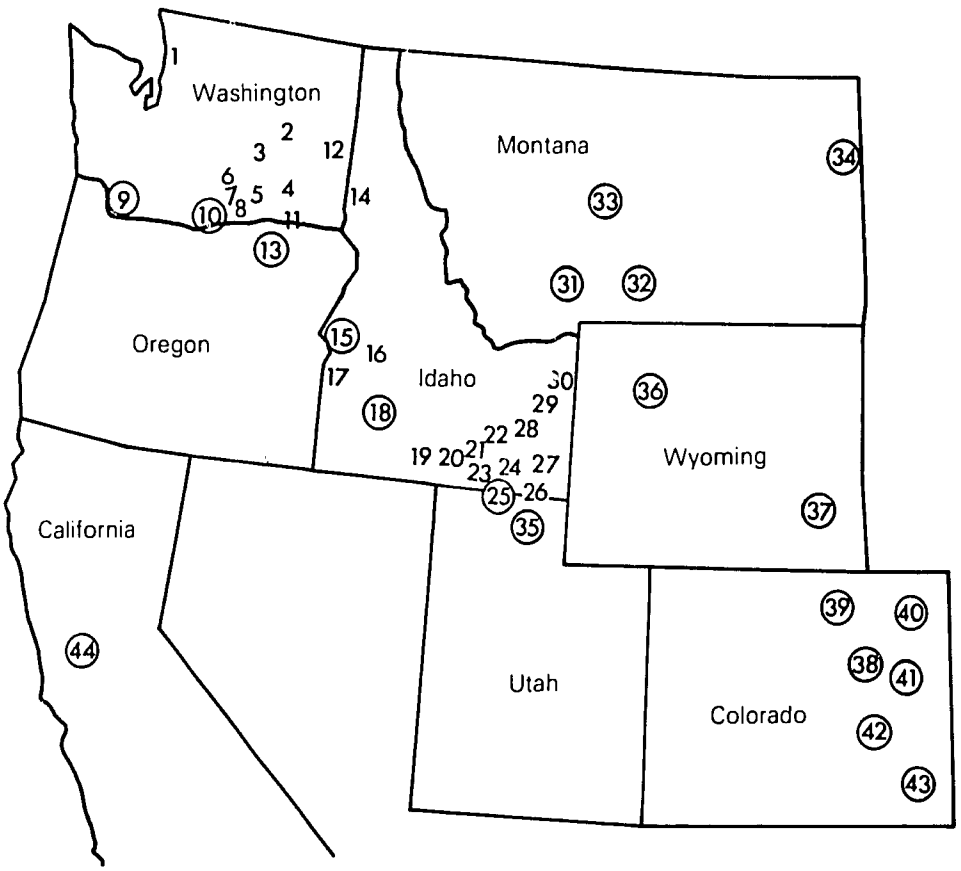
A regional system of insect suction traps to monitor cereal and other economic aphids, established over a period of 3 years (1985-87), consists of 44 traps covering areas in the western USA. The network is part of a long-term commitment to monitor, characterize, and study winged aphid activity on a regional scale. During the 1985-86 growing seasons, more than 170 aphid species were sampled in suction traps in Washington and Idaho. Of the total network catch, an average of 84% (51 species) were aphids of economic concern; 61% (10 species) of the total catch were cereal aphids.

A regional system of insect suction traps to monitor cereal and other economic aphids, consisting of 25 traps (10 in Washington, 15 in Idaho) in 1986, stretched from northwestern Washington to eastern Idaho. During the spring of 1987, the system was expanded to 44 traps to cover areas in 8 western states (Figure 1). The network is part of a long-term commitment to monitor, characterize, and study winged aphid activity on a broad regional scale, similar to the EURAPHID system of Europe. The traps are designed with a fan suspended below an air-filtering wire mesh enclosed cone, housed at the base of a 6 m, 30.5 cm diameter polyvinal chloride cylinder; overall trap height is 8 m.

The traps are situated in a wide variety of agroecosystems, precipitation zones (< 40 to > 80 cm annual precip.), and elevations (30 to 1830 m). About 8 million ha of small grains are grown in the western states, of which some 70% are in semi-arid (< 50 cm annual precip.) grain-fallow areas. Production methods have changed substantially through the years: in the late 1800s, more than 90% of the small grains were spring-seeded; today, more than 90% are fall-seeded. The change, due largely to improvements in cultivars (e.g., increased hardiness, stiff-straw, better emergence), soil moisture management, and deep-furrow planting, has led to generally higher yields. However, the fall seedings (particularly those planted before the end of September) frequently incur high levels of cereal aphids and virus infection.

During the 1985 and 1986 growing seasons, more than 150,000 aphids (170+ spp.) were sampled in suction traps in Washington and Idaho (Table 1). Of the total network catch, an average of 84% (51 spp.) were aphids of economic concern; 61% (10 spp.) of the total catch were cereal aphids (*Aphis armoraciae* Cowen, *Metopolophium dirhodum* (Walker), *Rhopalosiphum padi* (L.), *R. maidis* (Fitch), *R. insertum* (Walker), *R. rufiabdominalis* (Sasaki), *Schizaphis graminum* (Rondani), *Sitobion avenae* (Fabricius), *S. fragariae* (Walker), and *Sipha elegans* del Guercio). The large area of cereals no doubt contributed to the high percentage of cereal aphids captured. On a region-wide basis, the occurrence and proportion of species changed constantly, and was frequently variable between areas. The general population trends and species percentage values for Washington and Idaho (1985 and 1986) are shown in Figure 2 and Tables 2 and 3.

Beginning in 1986 (July to November), the results on the economic species were summarized and distributed weekly to selected growers, fieldmen, extension agents, and scientists as a pest and crop management tool. When appropriate, interpretive comments were included. This information, combined with in-field



Washington
1. Mt. Vernon
2. Wilbur
3. Ephrata
4. Connell
5. Rattlesnake
6. Prosser
7. Horse Heaven
8. Paterson
9. Vancouver
10. Bickleton
11. Prescott
12. Rosalia

Oregon
13. Pendleton

Idaho
14. Moscow
15. Payette
16. Parma
17. Wilden
18. Mtn. Home
19. Kimberly
20. Burley
21. Neeley area
22. Aberdeen
23. Rockland
24. Arbon Valley
25. Holbrook
26. Preston
27. Soda Springs
28. Shelly
29. Ririe
30. Tetonia

Montana
31. Bozeman
32. Huntley
33. Moccasin
34. Sidney

Utah
35. Logan

Wyoming
36. Worland
37. Wheatland

Colorado
38. Strasburg
39. Ft. Collins
40. Akron
41. Limon
42. Rocky Ford
43. Walsh

California
44. Davis

Figure 1. Aphid suction trap sites in the western United States (numbers in circles, new for 1987).

checks by growers, provided a background for intelligent decision making on when to plant and/or the need and timing of aphidicide treatments.

The network has also facilitated tracking the movement of the Russian wheat aphid, *Diuraphis noxia* (Mordvilko), a serious new pest of Colorado and southern Wyoming. Its rapid dispersal northward from Texas in 1986 suggests that it will spread farther in the future.

Table 1. Aphid suction trap catch, Washington and Idaho, 1985-86

State	Year	No. traps operat-ing	Cereal aphids		Other economic aphids		Non-economic aphids		Total
Wash.	1985	6	10,667	(55.2)	5,518	(28.6)	3,124	(16.2)	19,309
Wash.	1986	10	17,749	(49.0)	9,394	(25.9)	9,116	(25.1)	36,259
Idaho	1985	8	34,577	(69.1)	10.551	(21.1)	4,877	(9.8)	50,005
Idaho	1986	14	29,870	(62.8)	10,592	(22.3)	7,097	(14.9)	47,559

Percent of total catch in parentheses.

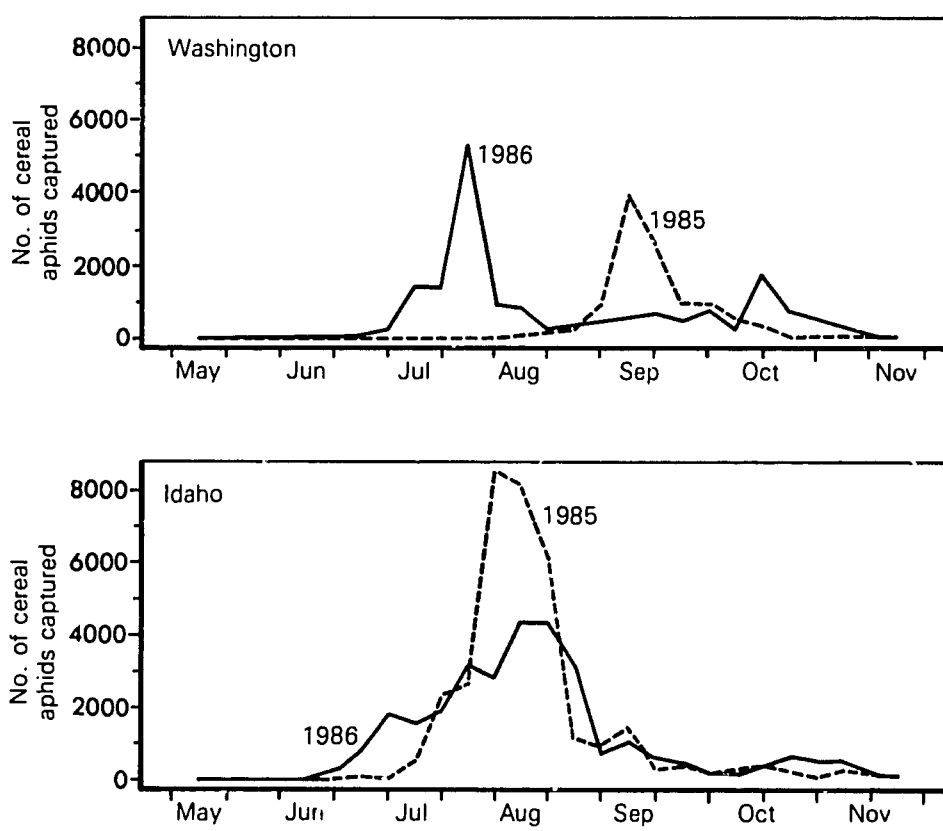


Figure 2. Cereal aphid trends in Washington and Idaho, 1985, 1986.

Table 2. Cereal aphids captured in suction traps, Washington and Idaho, 1985-1986

	No. of trapped cereal aphids					
State	Jun	Jul	Aug	Sep	Oct	Season
Wash. 1985 total	7	62	2,611	7,510	433	10,667
(% of total spp.)	(8)	(10)	(70)	(69)	(15)	(55)
Wash. 1986 total	173	9,289	1,874	2,715	3,537	17,749
(% of total spp.)	(19)	(71)	(54)	(55)	(30)	(49)
Idaho 1985 total	182	14,544	17,557	1,065	1,116	34,577
(% of total spp.)	(24)	(67)	(60)	(24)	(19)	(69)
Idaho 1986 total	1,635	10,975	13,358	1,562	2,128	29,870
(% of total spp.)	(31)	(69)	(67)	(37)	(32)	(63)

Table 3. Cereal aphid species as a percentage of the total trapped cereal aphids, Washington and Idaho, 1985-1986 (May-Oct.)

	% Cereal aphids spp. of total cereal aphid catch					
State	*R. padi*	*R. maidis*	*S. avenae*	*M. dirhodum*	*S. graminum*	Other cereal aphid spp.[a]
Wash. (1985) avg.	90.2	5.6	1.4	1.6	0.4	0.9
Wash. (1986) avg.	50.8	3.3	32.4	8.8	2.5	2.0
Idaho (1985) avg.	29.7	22.2	4.4	3.4	39.1	1.2
	24.3	8.6	8.6	31.6	26.4	0.5

[a] *Aphis armoraciae, Rhopalosiphum insertum, R. rufiabdominalis, S. fragariae,* and *Sipha elegans.*

Estimating Potential Crop Colonization by Barley Yellow Dwarf Virus Vectors from Suction Trap Samples

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Abstract

In England, barley yellow dwarf virus (BYDV) is a problem associated mainly with autumn-sown cereals. The total number of two important vector species, Rhopalosiphum padi *(L.) and* Sitobion avenae *(Fabricius), often differ greatly in suction trap samples in autumn. Three alate morphs of the dioecious* R. padi *are present in autumn, but only alate exules will colonize cereals, while gynoparae and males are in search of the primary host,* Prunus padus *L. In contrast, all alate morphs of the monoecious* S. avenae *will colonize cereals. Hence, differences in densities on crops, at this time, are usually much less pronounced.*

Host plant choice trials of female R. padi *alatae trapped alive indicated that, at Rothamsted, alate exules were largely replaced by gynoparae during the first half of September.* R. padi *alate exules, the morph that spreads BYDV in crops, therefore form only a small proportion of the total* R. padi *migrants in autumn. The relative proportions of* R. padi *alate exules to* S. avenae *alatae, in suction trap samples in autumn, were similar to the proportions found on crops.*

Suction trap samples in Britain indicate that cereal aphids form a significant proportion of the aerial aphid fauna, both in late summer when emigrating from crops, and in the autumn when infection of emerging crops with barley yellow dwarf virus (BYDV) may occur (Taylor et al. 1981, 1982). *Rhopalosiphum padi* (L.) is one of the most numerous aphids recorded in suction traps in autumn, but the aerial population is comprised of three morphs. Two of them, males and gynoparae, are in search of the primary host, *Prunus padus* L. (Dixon 1971), while alate exules, morphologically very similar to gynoparae (Rogerson 1948), colonize and reproduce on cereals (Dean 1974) and are probably the most important vectors. *Sitobion avenae* (Fabricius) also occurs on cereals in the autumn (Dewar and Carter 1984), but is less common in suction trap samples. As it is monoecious, all alatae are capable of colonizing cereals and introducing virus.

Thus, to determine the relative importance of alatae *R. padi* and *S. avenae* in the epidemiology of BYDV, it is necessary to know the proportion of *R. padi* that are alate exules in autumn. This paper describes work on the biology of migrant morphs of *R. padi* to determine this proportion.

Materials and Methods

Suction traps have monitored aerial aphid populations at heights of 1.5 and 12.2 m above ground level at Rothamsted since 1972 and 1964, respectively. The 12.2 m trap is part of a network of traps throughout Britain operated by the Rothamsted Insect Survey (Taylor 1974, 1986). Two additional suction traps were operated at Rothamsted from the end of July to early November 1986 at 1.5 (see Plumb 1976 for details of trap) and 12.2 m above ground level.

Insects were trapped live and samples collected twice daily. Samples were sorted in a Perspex box placed by a window, and up to 50 female *R. padi*/trap/day were retained after the species and sex had been checked using a binocular microscope. These aphids were placed individually on moist filter paper in clear polystyrene boxes (7.8 x 4.6 x 21 cm) containing a leaf disc of *P. padus* (20 mm diameter) and a strip of winter barley (*Hordeum vulgare* L.) leaf, cv Igri, of similar area. The boxes were left for 48 hours in a controlled environment room (18°C; 18:6 h light:dark). Each box was then examined to determine the host plant on which the female had settled, the number of nymphs on each host plant and the morph of the nymphs (oviparae,

produced by gynoparae, are pale yellow-green as young nymphs, with a conical posterior abdomen and cauda, while virginoparae, produced by alate exules, are darker green with reddish patches around the siphunculi, and a more rounded end to the abdomen (Tatchell et al. 1988). These data usually confirmed the morph of the alate female tested.

Results

Initially all *R. padi* tested were alate exules, the first gynopara not being recorded until 19 August (at 12.2 m). During the first half of September the proportion of gynoparae increased rapidly at 12.2 m, and two weeks later at 1.5 m (Figure 1). By the end of September no alate exules were found, but in early October they were again present in both traps in small proportions.

The ratio of the two female morphs sampled at the two heights (difference of the log n = 1) indicates that the density of gynoparae increased with height, at least to 12.2 m, whereas that of alate exules was similar at the two heights (Figure 2).

Discussion

The infectivity index is a measure of the risk of infection by BYDV of autumn-sown cereals by migrant aphids in England and Wales (Plumb 1983). It is calculated weekly and is the product of the number of each vector species, recorded in 12.2 m Rothamsted insect Survey suction trap samples, and the proportion of each species (*R. padi* and *R. insertum* (Walker) combined) found to be infective when trapped alive at 1.5 m (Plumb 1976). *S. avenae* has only rarely

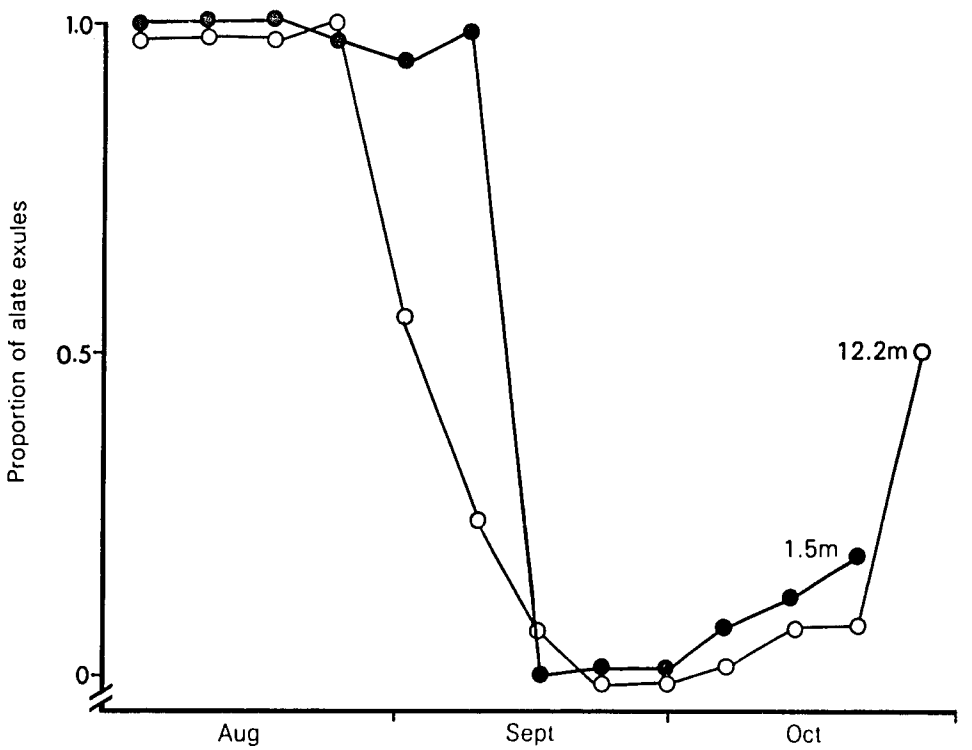

Figure 1. The proportion of alate female *R. padi* that were alate exules at 12.2 and 1.5 m determined from host plant choice tests.

been recorded as infective, so the index is determined by *Rhopalosiphum* species. The importance of *R. padi* is further increased as it is known to carry a severe strain of the virus (Plumb 1974). However, the infectivity index gives equal weighting to all morphs of *R. padi* irrespective of the host plant they are in search of.

Findings from the present study, if typical of most autumns, indicate the morph composition of the autumn aerial populations of *R. padi* and their behaviors have important consequences for the assessment of risk from BYDV. Cereals emerging in early September are very likely to be colonized by *R. padi*, as the majority of alatae at this time are exules, and the risk of BYDV is correspondingly high. The risk for crops emerging later depends on the proportion of exules in the aerial population, with the phenology of the changeover being largely determined by daylength (and hence latitude) and temperature (Dixon and Glen 1971).

This decline in the proportion of alatae capable of colonizing cereals explains why densities of *R. padi* and *S. avenae* on cereals are more similar than suction trap samples would indicate (Dewar and Carter 1984). In 1986 the ratio of *R. padi* to *S. avenae* alatae sampled in the 12.2 m Rothamsted suction trap was 282:1 whereas on barley it was 8:1. This latter ratio was very similar to 12:1 that Tatchell et al. (1988) estimated for alate exule *R. padi* to *S. avenae* alatae in suction trap samples for 1986. In addition, the density of gynoparae increased with height, at least up to 12.2 m, whereas the density of alatae exules was similar. Thus, the proportion of each morph was different at the two heights sampled (1.5 and 12.2 m).

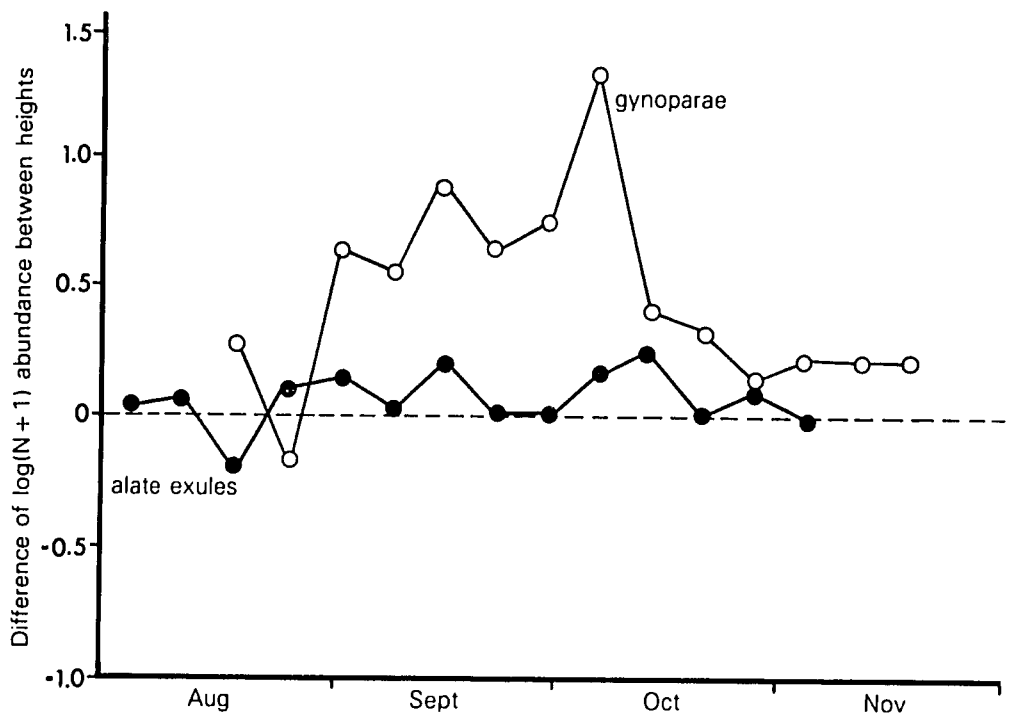

Figure 2. The ratio of the aerial abundance of *R. padi* alate exules and gynoparae at 12.2 and 1.5 m. A value of 1.0 indicates the density at 12.2 m was 10 times greater than at 1.5 m.

Once the effects of latitude and phenology on the morph composition of migrant vector aphid populations have been established and their flight behaviors determined, it may be possible to refine the infectivity index so it only takes account of those morphs that introduce BYDV in autumn-sown cereals.

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Identification of the Variants of Barley Yellow Dwarf Virus in China

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Abstract

Since 1983 four isolates of barley yellow dwarf virus (BYDV) in China have been identified by a series of experiments involving comparative transmission by four aphid species and enzyme-linked immunosorbent assay (ELISA) tests using antisera for MAV, PAV, RPV, and RMV, from the USA and the UK. These isolates are: GPV, transmitted nonspecifically by Schizaphis graminum *(Rondani) and* Rhopalosiphum padi *(L.), a new isolate of BYDV that does not react to the antisera for MAV, PAV, RPV, or RMV; GAV, transmitted nonspecifically by* S. graminum *and* Sitobion avenae *(Fabricius), related to the MAV and PAV variants; PAGV, transmitted nonspecifically by* R. padi, R. maidis *(Fitch) and* S. avenae, *similar to the PAV isolate; and RMV, transmitted specifically by* R. maidis, *very similar to the North American RMV isolate.* S. graminum *is the most important vector of BYDV in China.*

Luteoviruses that cause barley yellow dwarf (BYD) of cereal crops are very important in northern and northwestern China, in the provinces of Henan, Shanxi, Shaanxi, Gansu, Hebei, and Shandong as well as Ningxia and Nei Monggol autonomous regions. Yield losses in wheat caused by BYD have been estimated at 20 to 30% in years of severe infection; in 1970, 500,000 tons of wheat (*Triticum aestivum* L.) were lost to BYD in the provinces of Shaanxi and Gansu and the Ningxia autonomous region alone. To date, five aphid species—*Schizaphis graminum* (Rondani), *Sitobion avenae* (Fabricius), *Rhopalosiphum padi* (L.), *Metopolophium dirhodum* (Walker), and *R. maidis* (Fitch)—have been identified as virus vectors. Among them, *S. graminum* is the most important vector in China.

Since 1983, four isolates of BYDV have been identified based on results of comparative transmission tests with four aphid species and enzyme-linked immunosorbent assays (ELISA). They have been named:

- GPV, transmitted nonspecifically by *S. graminum* and *R. padi*;
- GAV, transmitted nonspecifically by *S. graminum* and *S. avenae*;
- PAGV, transmitted nonspecifically by *R. padi*, *S. avenae* and *S. graminum*; and
- RMV, transmitted specifically by *R. maidis*.

Comparative transmission—The patterns of transmission by four aphid species in 17 serial transfers during a period of 3 years (Table 1) were remarkably constant.

Table 1. Comparative transmission by 4 aphid species of 4 isolates of BYDV

Strains of BYDV	Percentage of plants that became infected with BYDV in parallel tests with 4 aphid species			
	S. graminum	*S. avenae*	*R. padi*	*R. maidis*
GPV	90.2	0	67.0	0
GAV	81.2	96.6	0	0
PAGV	43.1	77.8	96.2	0
RMV	0	0	0	88.0
Control	0	0	0	0

Each percentage was based on 80 to 133 test plants. Controls were 105 plants for each aphid species.

Membrane feeding tests—When aphids were allowed to feed through stretched parafilm on partially purified virus preparations, the results were the same as those obtained when aphids were fed on infected plants (Table 2).

Influence of temperature—Vector specificity was compared at different temperatures both for acquisition and inoculation feeding. Tests included acquisition feeding for 2 days at 15, 20, and 25°C, followed by 3 to 5 days of inoculation test feeding at 15, 20, and 25°C. The results indicated that temperature did not affect vector specificity.

ELISA comparisons—Dried samples, collected from diseased plants in the greenhouse after 25 to 30 days inoculation, were sent to W.F. Rochow, Cornell University, USA, and R.T. Plumb, Rothamsted Experimental Station, UK, for ELISA assays. Results were as follows: GAV was similar to MAV both in the USA and the UK. PAGV only reacted with monoclonal antibody MAC 91, which reacts to the PAV isolate in the UK. GPV did not react with any of the 5 antisera available to BYDV in the USA, but had a very weak reaction with monoclonal antibody MAFF2. The reaction was too weak to be recognized as MAV in the UK. (Table 3).

Table 2. Transmission of 4 isolates of BYDV by 4 aphid species allowed to acquire virus by feeding through membrane on partially purified preparation

BYDV isolates	Transmission by aphid species			
	S. graminum	*S. avenae*	*R. padi*	*R. maidis*
GPV	10/10	0	6/10	0/9
GAV	9/10	9/10	0	0/10
PAGV	12/27	9/22	10/25	0/21
RMV	0/14	0/19	0/13	12/23
Controls	0/20	0/20	0/20	0/20

Numerator is number of plants that became infected. Denominator is number of plants infested with 8-10 aphids per plant.

Table 3. ELISA reaction of 3-g samples with IgG in USA and UK

Isolates	USA					UK		
	RPV	MAV	PAV	RMV	SGV	Mac91	Mac92	MAFC2
GPV	0.012	0.010	0.004	0.010	0.025	0.020	0.020	0.029
GAV	0.006	0.256	0.038	0.019	0.132	0.024	0.019	0.291
PAGV						0.355	0.007	0.022
RMV						0.007	0.004	0.022
Virus check	0.352	0.232	0.425	0.132	0.056			
healthy	0.020	0.007	0.003	0.009	0.039	0.004	0.002	0.003
RPV						0.026	0.681	0.030
RPV + PAV						0.410	0.396	0.030
PAV						0.567	0.010	0.004
MAV						0.016	0.012	0.459

Comparison of virulence—Coast Black oat (*Avena (byzantina K. Koch) =sativa* L.) plants infected by RMV develop very mild symptoms; the latent period is much longer than that of the other 3 strains. Oats infected by GAV and GPV develop moderately severe symptoms; and oats infected by PAGV are the most severely affected (Table 4).

Conclusions

GAV is similar to MAV; PAGV is similar to PAV; GPV, which is distinct from MAV and PAGV, is an isolate of BYDV and, so far as is currently known, is unique to China. *S. graminum*, which is able to transmit three isolates of BYDV very effectively, is the most important aphid vector of BYDV in China.

Table 4. Comparison of the effect of BYDV isolates on Coast Black oats 45 days after inoculation

Isolates	No. plants measured	No. tillers/ plant	Height of plants (cm)	Length of root (cm)	No. of roots
Healthy	10	3.5	55	21	10
RMV	17	2.3	41	15	8
GPV	33	1.7	33	9	6
GAV	37	1.8	27	7	4
PAGV	39	2.7	25	9	6

Detection and Diagnosis of Barley Yellow Dwarf Virus in Northern Italy

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Abstract

*Large numbers of leaf samples taken from cereal crops in the Po Valley (northern Italy) over several years have been tested for barley yellow dwarf virus (BYDV) by aphid transmission and enzyme-linked immunosorbent assay (ELISA). BYDV was detected throughout the year in barley (*Hordeum vulgare *L.) (from November to April), wheat (*Triticum aestivum *L.) (from February to May), rice (*Oryza sativa *L.) (June and July), maize (*Zea mays *L.) (from June to October) and oats (*Avena sativa *L.) (from October to April). The two methods of detection gave similar results. A PAV-like isolate of BYDV seems to be the most common in northern Italy.*

Research on barley yellow dwarf virus (BYDV) was initiated at our Institute about 20 years ago with the study of rice "giallume" (yellows), a severe disease of rice (*Oryza sativa* L.) that was new for Italy at that time. After several years of investigation, the main points of the disease etiology and epidemiology have been elucidated. Today we know that rice giallume is caused by an isolate of BYDV that is transmitted mainly by the aphids *Rhopalosiphum padi* (L.) and *Sitobion avenae* (Fabricius), and that the virus overwinters in *Leersia oryzoides* L. and other weeds, besides barley (*Hordeum vulgare* L.) and wheat (*Triticum aestivum* L.).

Virus Purification and Serology

Detection and diagnosis of BYDV were done initially on the basis of symptoms and of aphid transmission to test plants, with special regard to oats (*Avena (byzantina K. Koch) =sativa* L). Recently we found a suitable purification procedure for our rice giallume virus isolate (RGV) that produces virus yields of up to 100 μg/100 g tissue (Figure 1), and we prepared a homologous antiserum with titre 1:1024 (Belli et al. 1986).

We used this antiserum for a better identification of the rice isolate and for diagnostic purposes. In gel diffusion tests, pure preparations of RGV reacted with the homologous antiserum and with antisera to two different MAV (= *S. avenae* transmitted) isolates of BYDV, but did not react with an antiserum to the RPV (=*R. padi* transmitted) isolate of BYDV or with antisera to beet western yellows virus, potato leafroll virus, or pea leafroll virus. Therefore, we consider our rice isolate as belonging to group I of BYDV isolates, following the classification proposed by Gill and Chong (1979) and provisionally accepted by both Rochow (1982) and Francki et al. (1985). Considering these results together with those obtained by Osler (1984) from aphid transmission, we think that isolate RGV is a "PAV-like" isolate (sensu Rochow 1969) of BYDV. This classification has been confirmed by R.M. Lister (personal communication).

Frozen tissue
↓
Crushing in liquid nitrogen
↓
Homogenization in blender with phosphate buffer 0.1M + thioglycollic acid 0.1% + Driselase 0.5% pH 6 followed by maceration overnight
↓
Filtration through cheesecloth
↓
Clarification with n-butanol/chloroform 1:1 mixture followed by a low speed centrifugation
↓
Addition 8% PEG and 0.2 NaCl and centrifugation
↓
2 cycles of low and high speed centrifugations
↓
Layering on 10-40% sucrose DG and centrifugation
↓
Scanning on ISCO and collection of fractions corresponding to the peak
↓
Final virus pelletting

Figure 1. Virus purification procedure.

With the antiserum to RGV an enzyme-linked immunosorbent assay (ELISA) system has been set up for diagnostic purposes. Preliminary and comparative tests showed a complete agreement between the results obtained with the two methods of detection (aphid transmission and ELISA). Therefore, during the last 3 years we have mainly used the ELISA test for BYDV detection. The advantages of this method are that it is simple, quick, and sensitive, and that it can be used with dried or frozen material.

Sample Processing and Results

The samples collected from different cereal crops in several areas of the central Po valley were extracted for ELISA after storage (usually frozen, sometimes air-dried). The method we used for sample processing is shown in Figure 2, as well as the variations in procedure that were tested without finding any evident advantage. Usually 3 g of plant tissue were used in these tests.

ELISA was done with polyclonal antisera, by the usual double-antibody sandwich procedure, in Nunc "Immuno Plates I F."

Besides our antiserum to RGV, we used two commercial kits (Bioreba, Basel, Switzerland): one to a PAV-like isolate (type P) and the other to an MAV-like isolate (type F) of BYDV. A total of 82 samples were tested: 44 appeared to be infected and reacted positively with both antibodies to RGV and to PAV. No sample gave a positive reaction with antibodies to MAV.

BYDV was detected throughout the year in a range of cereal crops. BYDV could be found in barley from November to April, in wheat from February to May, in rice

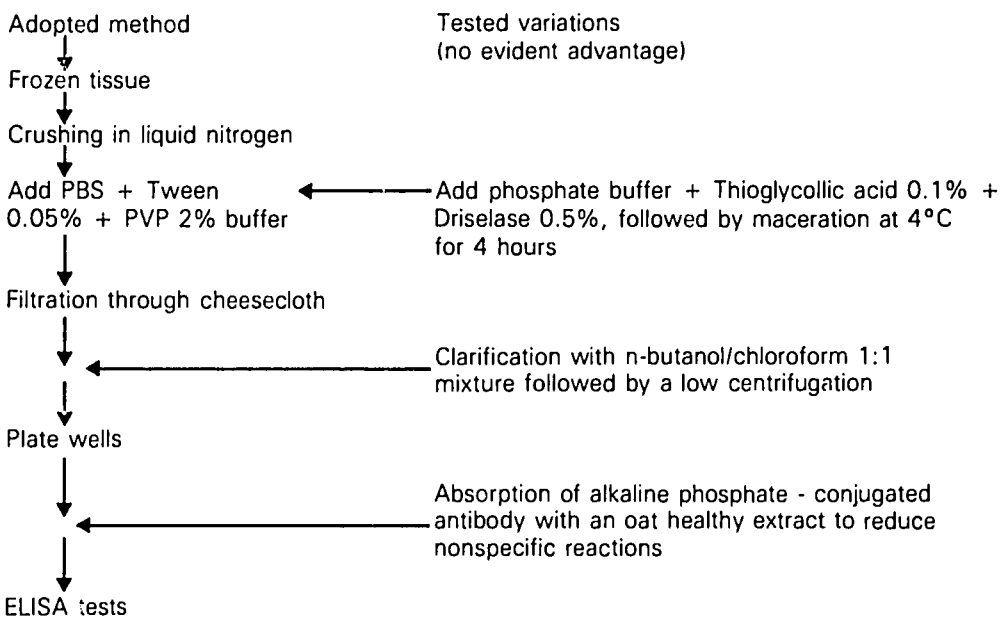

Figure 2. Procedure for testing field samples.

June and July, in maize (*Zea mays* L.) from June to October and in oats from October to April.

Conclusions

The main points and results of our recent work on BYDV can be summarized as follows:

- A suitable purification procedure for the rice isolate RGV has been established.
- On the basis of serological and aphid transmission tests the rice isolate is considered to be a PAV type.
- The methods we used for sample processing and ELISA are very suitable for detection and diagnosis of BYDV.
- In the central Po Valley BYDV is present throughout the year in different cereal crops.
- PAV seems to be the most common isolate in our area.

Acknowledgment

We thank C.E. Longoni for valuable technical assistance in virus propagation and aphid transmission.

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Vector Relationships of Isolates of Barley Yellow Dwarf Virus in Mexico

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Abstract

Isolates of barley yellow dwarf virus (BYDV) were detected by indirect enzyme-linked immunosorbent assay (ELISA). Three monoclonal antibodies, produced against the English MAV-, PAV- and RPV-like isolates, were used. The plants tested were infected with a range of species of cereal aphids collected from the field. Metopolophium dirhodum *(Walker) was a vector of a MAV-like isolate,* Sitobion avenae *(Fabricius) of MAV- and PAV-like isolates, and* Rhopalosiphum padi *(L.) of PAV- and RPV-like isolates.* R. maidis *(Fitch) did not transmit any BYDV isolates detectable by the monoclonal antibodies used, but mild symptoms suggested the presence of an RMV-like isolate. A number of mixed infections were detected.*

Barley yellow dwarf virus (BYDV) has been known in Mexico for the last 30 years (Navarro 1984), but only recently has the study of the efficiency of its vectors been initiated. Gilchrist et al. (1986), using transmission tests, proved that *Rhopalosiphum padi* (L.), *R. maidis* (Fitch), *Sitobion avenae* (Fabricius), and *Metopolophium dirhodum* (Walker) were vectors of BYDV.

Enzyme-linked immunosorbent assay (ELISA) for detection of BYDV was first used by Lister and Rochow (1979). It has proved a useful complementary tool to support biological tests (Rochow 1979, 1982).

Our work employed both ELISA and transmission tests with a range of aphid vectors to obtain information on certain BYDV isolates in Mexico.

Materials and Methods

Collection of aphids from the field

Cereal aphids were collected from plants of barley (*Hordeum vulgare* L.), wheat (*Triticum aestivum* L.), and oats (*Avena sativa* L.) showing symptoms of BYD in the field. These aphids were transported from the field to the laboratory in small plastic vials in a cool box. In the laboratory the vials were emptied into a sorting tray and a fine natural-hair brush was used to place each aphid on an oat seedling (cultivars: Paramo, Coast Black, Clintland 64) at the 2-leaf stage. Each seedling was covered from planting until the end of the aphid-feeding period by a plastic tube (20 cm long and 4 cm in diameter) to avoid contamination by stray aphids.

Each aphid tested was caged on the seedling for 5 days, at 18 to 20°C in a greenhouse with natural light. Feeding was terminated by spraying with a systemic insecticide (oxydemeton-methyl). Seedlings were then maintained in another greenhouse at 15 to 20°C.

Identification of virus isolates

ELISA test procedure—Indirect ELISA tests were carried out following the procedure described by Clark and Bar-Joseph (1984) and using three monoclonal antibodies prepared by L. Torrance in the MAFF Laboratory, Harpenden, UK. These were Mab. 91, prepared against the English PAV-like type, Mab. 92 against the English RPV-like type, and Mab. MAFF 2, prepared against the English MAV-like type.

Approximately 0.1 g of tissue from the leaf tip of the youngest expanded leaf from inoculated seedlings was tested. Extraction was in 1:20 (w/v) of PBS-Tween buffer (ph 7.4) with 2% polyvinylpyrrolidone (M_r 4.0 MDa) added.

Flat bottom plates (Immunolon 2-Dynatech 011 010-3450) were used. The reacted substrate p-nitrophenyl-phosphate was stopped with NaOH. Absorbances were read at 405 nm by an automatic ELISA-reader (Minireader II, Dynatech). Absorbance values higher than three times the healthy control value were considered positive (I. Barker, personal communication).

Vector transmission tests—Ten isolates of BYDV consisting of two PAV-like isolates, two MAV-like isolates, one RPV-like isolate, one PAV + MAV-like isolate and four MAV + RPV-like isolates were chosen for transmission tests. *M. dirhodum*, *S. avenae*, *R. padi*, and *R. maidis* were used as vectors.

Detached virus-infected leaves were put in plastic Petri dishes and non-viruliferous aphids were allowed an acquisition feeding period of 48 hours, at 18°C, in the dark (Rochow 1958). Then these aphids were placed on Clintland 64 oat seedlings and allowed an inoculation feeding period of 5 days. Aphids fed on virus-free leaves were used as controls. Either 1 aphid or 10 aphids per plant were used in the inoculation feeds.

Results and Discussion

Detection of different BYDV isolates
Of the 631 aphid specimens collected in the field from plants of oats, barley, and wheat, 295 were *M. dirhodum*, 147 *S. avenae*, 127 *R. padi*, 62 *R. maidis*.

The ELISA tests of the oat seedlings on which these aphids had fed detected an MAV-like isolate most frequently. This isolate was transmitted by a large proportion of *S. avenae* and *M. dirhodum* (Table 1). A low incidence of a PAV-like isolate was detected. This was transmitted by *S. avenae* and occasionally by *M. dirhodum*, but never by *R. padi*. The RPV-like isolate was transmitted only by *R. padi*.

The oat seedlings on which *R. maidis* fed were tested with ELISA, but always gave negative responses to the monoclonal antibodies available. However, some plants showed symptoms, probably indicating that they were infected with an RMV-like isolate, which we cannot detect.

Mixed infections of RPV- and MAV-like isolates have been found and transmitted by three vectors: *M. dirhodum*, *S. avenae*, and *R. padi* (Table 1). The same phenomenon was described commonly in the field in the USA, but there, only *R. padi* was a vector (Rochow and Gill 1978). In one case in this study, *M. dirhodum* transmitted PAV- and MAV-like isolates together.

All the BYDV infected oat plants exhibited the following symptoms: reddening of leaves, general stunting, spindly growth, and early heading. However, it was not possible to see differences among plants infected separately with the three different virus isolates.

Table 1. Number of aphids collected from the field and percentage of isolates transmitted during summer 1986

	M. dirhodum	*S. avenae*	*R. padi*	Total
Number of aphids tested	295	147	127	569
Number of positive transfers				
PAV-like	3	1	-	4
MAV-like	124	41	-	165
RPV-like	-	-	7	7
PAV- + MAV-like	1	-	-	1
RPV- + MAV-like	8	2	1	11
Total	136	44	8	188
Percent of aphids transmitting BYDV (all isolates)	47	30	6	

Vector specificity and efficiency
Some MAV-like isolates transmitted by either *S. avenae* or *M. dirhodum* in the initial transmission from the field reacted to the same monoclonal antibody, Mab. MAFF 2, produced against the English MAV-like isolate. Subsequent transmission experiments showed that both aphid species could transmit these MAV-like isolates.

The PAV-like isolates transmitted by *S. avenae* and *M. dirhodum* from the field were detected by the same monoclonal antibody, Mab. 91, produced against the English PAV-like type.

M. dirhodum was never able to spread the PAV-like isolates in transmission studies. Both *R. padi* and *S. avenae* could transmit a PAV-like isolate after initial transmission by either *M. dirhodum* or *S. avenae* (Figure 1).

The RPV-like isolate, transmitted by *R. padi* found in the field, and reacting to monoclonal Mab. 92 produced against the English RPV-like type, was found to be transmitted only by *R. padi*. When 100 single aphid transfers were carried out with *R. padi*, *S. avenae*, and *M. dirhodum* from a viruliferous source plant infected with an RPV-like isolate by *R. padi*, 36% of the *R. padi* and none of the *S. avenae* or *M. dirhodum* transmitted BYDV.

The single mixed infection of PAV- and MAV-like isolates was transmitted from the field by *M. dirhodum*. This mixture was used for comparative transfers by *S. avenae*, *M. dirhodum*, and *R. padi*. *M. dirhodum* only transmitted MAV-like isolate, *R. padi* only transmitted PAV-like isolate, and *S. avenae* transmitted both PAV- and MAV-like isolates. This could be a case of dependent transmission. Recently we have detected two more cases of this mixed infection from the field, but they have not yet been studied.

On some occasions *M. dirhodum*, *S. avenae*, and *R. padi* collected from the field transmitted MAV- and RPV-like isolates together when tested by ELISA. From all mixed infected test plants *M. dirhodum* was able to transmit the MAV-like isolate (Table 2). *S. avenae* transmitted MAV-like isolates from F393, F470, and F472 but not from F103. *R. padi* transmitted RPV-like isolates only from F103, originally transmitted by *R. padi* from the field.

We are currently studying the transmission characteristics of PAV-, MAV- and RPV-like isolates.

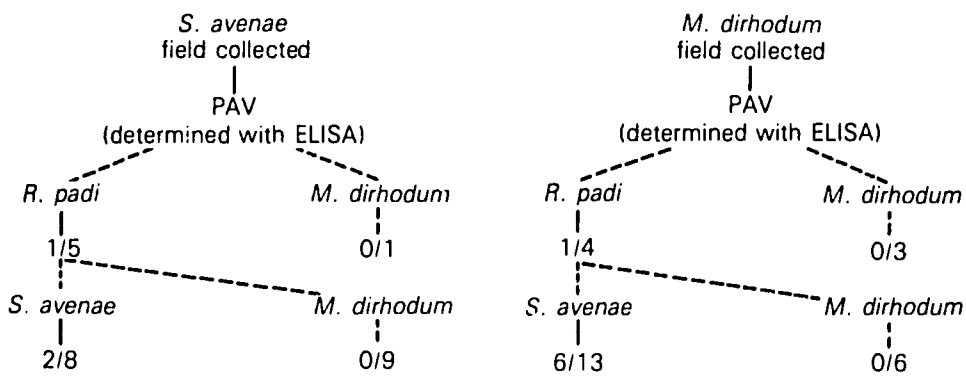

Figure 1. Vector specificity of two PAV-like isolates originally transmitted by cereal aphids collected from the field and determined with serial vector transmissions, and ELISA tests.

Table 2. Comparative transfers from plants with mixed infections of MAV- and RPV- like isolates

Virus sources classified by ELISA	*S. avenae*		*M. dirhodum*		*R. padi*	
	MAV	RPV	MAV	RPV	MAV	RPV
F393	2/11	0/11	4/4	0/4	0/13	0/13
F470	6/13	0/13	26/26	0/26	0/9	0/9
F472 (infected with MAV- and RPV- like isolates by *M. dirhodum* collected from the field, summer 1986)	6/6	0/6	2/3	0/3	0/12	0/12
F103 (infected with MAV- and RPV- like isolates by *R. padi* collected from the field, summer 1986)	0/5	0/5	2/2	0/2	0/6	2/6

10 aphids were used in each transfer.

Virus source plants and test plants used for transfers have been tested with ELISA and symptoms have been observed.

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Simulation Model of Barley Yellow Dwarf Virus Epidemiology

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Abstract

A model simulating the epidemiology of BYDV has been constructed. A comparison of the model's output with data from a field trial carried out during 1985/86 has shown that it does not accurately predict field observations. The model fails to predict the early appearance of alate and apterous aphids and underestimates the peak population density. Possible weaknesses in the model are discussed and future development proposed.

In England, primary infection of autumn-sown cereals with barley yellow dwarf virus (BYDV) is by migrant vector aphids, especially *Rhopalosiphum padi* (L.). Secondary spread of the virus results from the dispersal of their apterous offspring (Plumb 1983). Currently, advice in England on the need for and timing of an aphicide spray application, aimed at preventing this secondary spread, is based on a combination of a regional infectivity index (Plumb et al. 1986), field samples of aphid numbers, and a knowledge of previous disease incidence.

It was felt that by constructing a model simulating BYDV epidemiology it would be possible to identify key components of the system and investigate these further. Also, once the model had been validated, it could be incorporated into the current advisory scheme to improve control of the disease.

The Model

Published data were used to calculate parameters for the model, which describes the population dynamics of *R. padi* and the spread of virus in autumn-sown barley (*Hordeum vulgare* L.). Laboratory and field experiments were carried out to provide further information and validate the model.

The model consists of four sections: data input, the aphid subsystem, the virus subsystem, and output.

Data input

The following data are required to run the model: the day numbers for the start and finish of the simulation (day 1 = January 1st), daily maximum and minimum screen temperatures (°C), number of plants per square meter, the day numbers for the start and finish of alate immigration, and the daily number of *R. padi* alate exules in the nearest Rothamsted Insect Survey 12.2 m suction trap (Taylor 1974).

Aphid subsystem

The subsystem for the aphids consists of i) immigration, ii) development and survival, and iii) reproduction and morph determination.

Immigration—The number of alate exules colonizing a crop was estimated from the daily Rothamsted Insect Survey suction trap samples of female *R. padi* (Taylor 1974). The number that were gynoparae was determined from the proportion of *R. padi* that failed to reproduce in infectivity trials (R. Plumb, E. Lennon, and R. Gutteridge, unpublished results) and was subtracted from the total female *R. padi* trapped. Taylor and Palmer (1972) calculated that for every aphid sampled in a suction trap 237/ha would be expected to land, assuming random deposition, a flight time of 2 hours, and a density gradient of -1. Since the number of plants/m^2 is known, the number of alates landing per plant was calculated.

Development and survival—A logistic function of the proportion of aphid development against temperature (Dean 1974) was used to update development such that nymphs became adult when the proportion of development reached 1.0, while values less than this determined the nymphal instar.

As no laboratory data on aphid survival suitable for inclusion in the model were available, results from field observations were used. A linear relationship was calculated between the number of day-degrees below 2.8°C, the theoretical development threshold for *R. padi* (Dean 1974), and the difference between observed and predicted (assuming an exponential population increase) aphid field densities to give an estimate of aphid survival.

Reproduction and morph determination—A linear relationship between reproductive rate and temperature (Dean 1974) was used to calculate the number of nymphs produced per adult aphid per day. The morph composition of these nymphs was determined at birth using a linear relationship between the proportion of aphids that were alatiform and the total aphid density, using data from Rautapaa (1976).

Virus subsystem

Primary virus infection was estimated using the assumption that each infective aphid recorded in the suction trap would infect a plant, while secondary spread was estimated from aphid density and a function combining BYDV acquisition and transmission with the rate of aphid inter-plant movement. This function, the transmission coefficient (number of newly infected plants per infectious aphid per day), was calculated from laboratory studies in which infective apterous aphids were allowed to move freely between plants within a small arena. At intervals the aphids were killed and the amount of infection determined by visual inspection.

Output

The model output gave the numbers of aphids per plant in each instar of both morphs, total numbers of aphids per plant, daily infection of plants, and accumulated percentage of plants infected.

Simulations

Output from the model was compared with data from a field trial at Rothamsted during autumn and winter 1985/86. Plots of winter barley were sown in early September and allowed to be infested naturally with aphids. Weekly counts were made of the aphid population numbers, and the extent of virus infection was determined on three occasions.

The predicted population increase for total *R. padi* was less than observed, the predicted population peak was not as high and occurred 12 days later (Figure 1). The model also underestimated the high numbers of both alate (Figure 2) and apterous adults (Figure 3) found on the crop in early October and the low numbers of alatae later in the season. The size of the peak apterous adult aphid density was, however, predicted accurately, although it occurred 26 days after the observed peak density (Figure 3).

The model also underestimated virus infection. Observed infection in the field was 21%, while the model only predicted 2% (Figure 4).

Discussion

The predicted densities of total aphids, and the various instars, differed from those observed. There are a number of possible explanations for this. The proportion of alate exules in the suction trap samples may not have been estimated accurately, leading to errors in calculated colonization rates. A more reliable estimate may be obtained from host choice experiments of alate aphids caught live in a suction trap (Carter and Tatchell, these Proceedings). It was also assumed that immigrant alatae would not colonize the crop until plants had at least one leaf fully expanded (decimal growth stage 11, Zadoks et al. 1974). If alatae colonized the crop prior to this, the model would underestimate their numbers. A further assumption was that as soon as fourth-instar alatiform nymphs became adult, they emigrated. As temperatures fell below the flight threshold temperature of 9.8°C for *R. padi* (Raatikainen and Tinnila 1961), emigration may have been prevented, thus increasing the number of alate aphids found in the field.

A further error could be because the development time from birth to adult used in the model was determined from a

study keeping aphids under constant temperature (Dean 1974) and is longer than that in the field. Previous studies on the effect of temperature on aphid development have shown that development times are shorter for aphids kept under fluctuating temperatures, which are closer to field conditions, than those kept under constant conditions (Messenger 1964; Siddiqui et al. 1973). Hence, apterous adults would appear earlier than the model predicted.

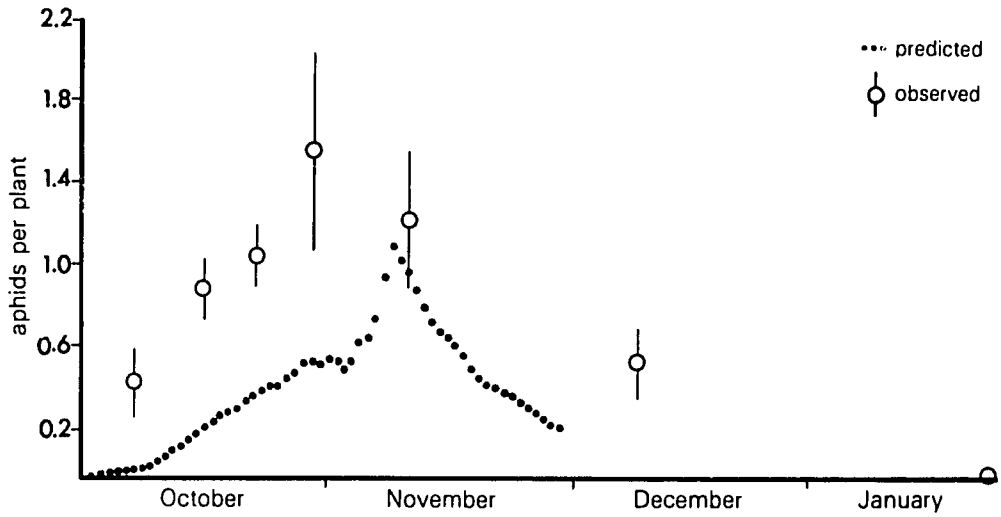

Figure 1. Observed and predicted trends in numbers of aphids per plant, Rothamsted 1985/6.

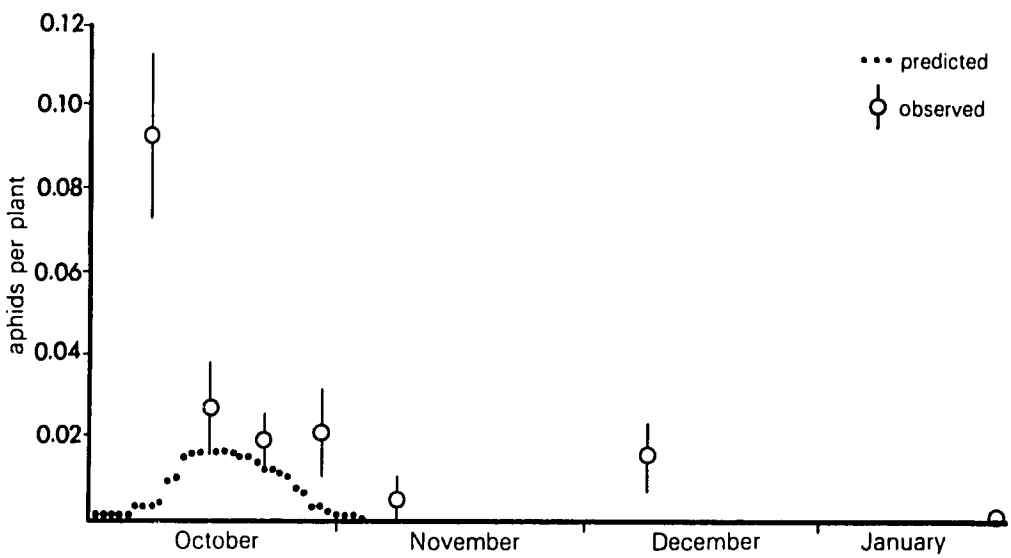

Figure 2. Observed and predicted trends in numbers of alate adult aphids per plant, Rothamsted 1985/6.

The transmission coefficient underestimated virus spread in the field in the autumn and winter of 1985/86. There are at least three possible explanations for this. Rainfall and high winds could increase virus spread by dislodging vectors, resulting in their moving to new host plants. Similar disturbance of aphids could also be caused by attack from a natural enemy. Roitberg et al. (1979) found that apterous pea aphids, *Acyrthosiphon pisum*

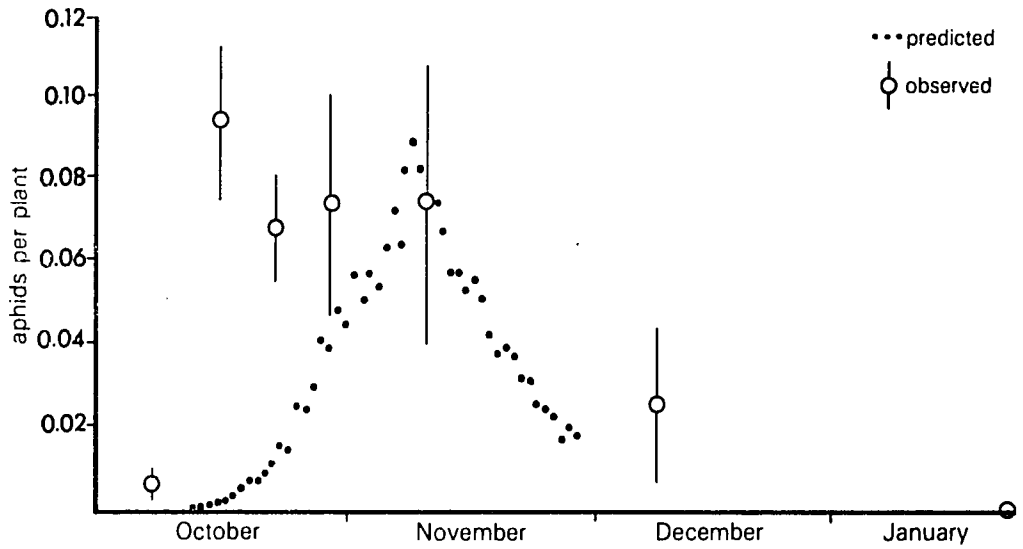

Figure 3. Observed and predicted trends in numbers of apterous adult aphids per plant, Rothamsted 1985/6.

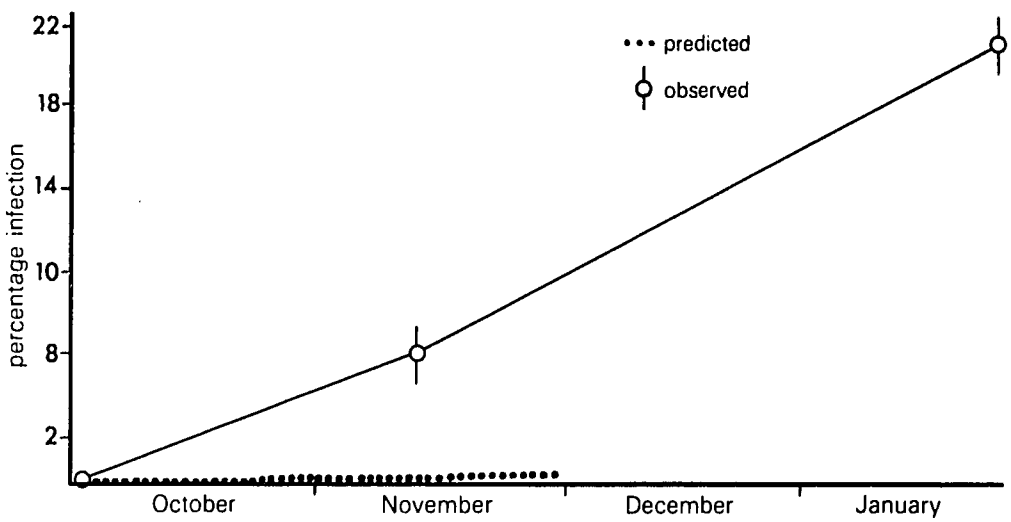

Figure 4. Observed and predicted trends in percentage of plants infected with BYDV, Rothamsted 1985/6.

(Harris), readily dispersed in the presence of coccinellids, but movement was rare if predators were absent. However, this species of aphids are known to fall from plants readily, unlike *R. padi* (G.M. Tatchell, personal communication), and this must be considered when drawing comparisons. Predators are known to feed on cereal aphids in the autumn (Sopp and Chiverton 1987), but there is no information on their effect on aphid dispersal. As the crop grows, contact between neighboring plants increases and aphid movement between plants would be much easier, facilitating virus spread. Since the model underestimated the number of aphids found in the field, this in itself would result in a lower incidence of virus spread.

The model requires further development to improve the accuracy of its predictions, but it has already highlighted weaknesses in our knowledge of BYDV epidemiology that can be overcome by further research. These include the attractiveness of crops at different growth stages to alate aphids, the effect of low fluctuating temperatures in development and survival rates of apterous aphids, and the movement of these aphid morphs between plants. Once the model has been sufficiently validated, it could then be used with the current decision-making scheme to improve control of the disease.

Acknowledgments

The author would like to thank Drs. N. Carter, P.C. Jepson, and G.M. Tatchell for constructive criticism and the Science and Engineering Research Council for financial support.

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The Effects of the Interaction of Barley Yellow Dwarf Virus and Take-All Fungus on the Growth and Yield of Wheat

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Abstract

*Field and glasshouse trials were carried out to determine the single and combined effects of barley yellow dwarf (BYD) and take-all fungus (*Gaeumannomyces graminis *var. tritici Walker) on the growth and yield of wheat (*Triticum aestivum *L.), cv. Condor. Inoculation with viruliferous aphids was done at two growth stages and three rates of take-all fungus were produced. Each pathogen reduced grain yield and increased the number of deadheads. In a number of cases, the combined effect of BYD and take-all fungus was greater than a simple additive effect of either pathogen alone. Grain yield per plot was reduced, while the incidence of deadheads was increased, particularly following infection with BYDV after tillering. The symptoms of BYD could be confused with take-all.*

Butler (1948) first described the effects of take-all, *Gaeumannomyces graminis* (Sacc) Arx and Olivier var. *tritici* Walker, in Australia in 1948 and it is now considered one of the most serious of the soil-borne diseases of wheat (*Triticum aestivum* L.) in many areas. The disease occurs in all major wheat districts of Victoria, and in seasons when climatic conditions are favorable, yield losses as high as 90% occur in individual crops (Kollmorgen 1982).

Barley yellow dwarf (BYD) was first confirmed in Australia in 1960 by Butler et al. (1960) and Geard (1960) through transmission tests to oat (*Avena sativa* L.) indicator plants. Since the initial confirmation, BYD has been identified in cereal crops from most parts of Australia and in wheat in Victoria. Infection prior to tillering can reduce grain yields by up to 80% (Smith and Sward 1982).

In the Mallee district (northwest Victoria), which produces about 30% of the state's wheat, take-all is a serious problem in most years, whereas epidemics of BYD only occur once in every 5 to 7 years. However, in the Wimmera district (west Victoria), where about 40% of the state's wheat is produced and rainfall is higher and the soils heavier than in the Mallee, both take-all and BYD are present each year. In some areas, such as the southern Wimmera, both take-all fungus and BYD are common (Edmonds 1983; Sward, unpublished data) and therefore a certain proportion of plants can be expected to have dual infections.

In preliminary glasshouse trials the combined effects of dual infection were serious. Detailed field and glasshouse studies were therefore established to determine the single and combined effects of BYD and take-all fungus on the growth and yield of wheat.

Glasshouse Study

Glasshouse studies were established using a sandy loam (described by Chambers 1971) amended with NPK fertilizer (6.9% N, 4.9% P, 8.0% K) at the rate of 3.1 g/liter. Oat grains sterilized by gamma irradiation (2.5 Megarad) were soaked in water overnight, drained, and autoclaved in 2.25-liter wine flagons at 100 Kpa for 45 min. The grains were inoculated with monosporous isolates of take-all fungus and incubated at 25°C until colonized by the fungus (4 to 6 weeks). Autoclaved inoculated oat grain was used in control treatments. Soil was infested at four rates of take-all fungus inoculum (G_0, G_1, G_2, G_3). These correspond to 0, 0.5, 1.4, 1.9% v/v of live fungal inoculum in soil, respectively. Each was supplemented with killed inoculum to bring the final percentage of total fungal inoculum in soil to 1.9%. Soil (1.3 liters) mixed with the respective inoculum, was placed in 2-liter plastic pots. Three wheat seeds (cv. Condor) were sown in each pot and thinned to one per pot after emergence.

A virulent barley yellow dwarf virus (BYDV) (V_1) comprising a mixture of PAV- and RPV-like types (Sward and Lister 1987) was used to produce three BYDV treatments:

B_0: five non-viruliferous *R. padi* applied at the two-leaf stage (decimal growth stage [GS] 12, Large 1954), 3 weeks after sowing

B_1: five BYDV-infective *R. padi* applied at the two-leaf stage (GS 12) 3 weeks after sowing

B_2: five BYDV-infective *R. padi* applied at early stem extension (GS 30), 7.5 weeks after sowing

The aphids fed for 60 hours and were removed by fumigating plants with dichlorvos, 0.5% (Vapona). The 18 replicates of each of the 12 treatments were randomized in three blocks on benches in an insect-screened, unheated glasshouse. Observations on leaf symptoms were made throughout, and at 9 weeks after sowing, one block of six replicates was harvested, the roots were washed free of soil, and top and root weights recorded. The rating scales used to estimate take-all fungus and BYV infection severity are given in Table 1.

At maturity, 9 of the 12 remaining replicates were selected randomly, harvested, and the grain yield per plant and 1000-grain weight measured, as well as the number of completely infertile heads and heads containing only a few, severely shriveled grains (deadheads).

Results

Nine weeks after sowing the majority of leaves of plants inoculated with BYDV at GS 12 (B_1) exhibited extensive discoloration characterized by interveinal chlorosis sometimes associated with a reddening or bronzing. By contrast, plants growing in soil amended with the highest rates of take-all fungus (G_2 and G_3) showed some yellowing of the leaf tips only, while the plants growing at rates G_0 and G_1 had no apparent leaf symptoms. Lesions were present on roots of plants grown in all pots where take-all fungus was applied. Infection with BYDV at GS 12 resulted in reductions in the weight of tops and roots of nearly 50% compared with the controls only 6 weeks post-inoculation (see Figures 1 and 2). Take-all fungus had no apparent effect on root weight of seedlings, but at the highest rate of inoculum (G_3) it significantly reduced the top weight.

The combined effects of BYDV (B_1) and take-all fungus on the weight of tops and roots were less than the sum of the individual effects. However, BYDV score allocated to plants inoculated at GS 12 and grown in soil inoculated with the highest rate of take-all fungus was significantly greater than where take-all fungus was absent. Similarly, the take-all fungus score of plants grown in soil inoculated at the two highest rates of take-all fungus and inoculated with BYDV at GS 12 was greater than where BYDV was absent. No effects of BYDV inoculation at GS 30 (B_2) were apparent because plants had been infected only 10 days previously.

Table 1. Rating scales used to estimate infection severity of take-all fungus and BYD infections

Take-all fungus						
Scale	0	1	2	3	4	5
Root area infected (%) (including subcrown internodes)	0	1-5	6-25	26-50	51-75	76-100

Barley yellow dwarf

0 No leaf discoloration, good plant vigor
1 Trace of leaf discoloration, good plant vigor
2 Moderate leaf discoloration, slight dwarfing
3 Extensive leaf discoloration, moderate dwarfing
4 Extensive leaf discoloration and necrosis, severe dwarfing

At maturity (see Table 2), both BYDV alone and take-all fungus alone caused a reduction in the grain weight per plant. In plants dually infected, however, the combined effects were less than additive for both these parameters. BYD alone and take-all fungus alone at the two highest levels (G_2 and G_3) caused a reduction in 1000-grain weight, but the combined effects of the two pathogens were less than additive. The number of deadheads per plant was increased by both BYD alone and take-all fungus (G_3) alone. Overall, BYDV inoculation at GS 30 increased the number of deadheads more than did inoculation at GS 12, while the combined effect of the two pathogens on deadhead production, although complex, generally was less than additive.

Field Study

A field experiment was established at Horsham, Victoria, with wheat (cv. Condor) sown in 6 m by 8 row plots and oat-grain inoculum and superphosphate positioned adjacent to the seed. There were four different levels (0, 1.6, 3.0 and 4.8 ml/m drill row) of live oat inoculum. Autoclaved inoculum was mixed with the live inoculum to bring the total volume to 4.8 ml/m drill row in all treatments.

Experimental design was a randomized-block factorial with four rates of take-all fungus inoculum and three BYDV treatments. The three BYDV treatments were:

- No aphids.
- BYDV infective *R. padi* applied at the two-leaf stage (GS 12) 3.5 weeks after sowing.

Figure 1. BYD without take-all.

Figure 2. BYD with take-all.

- BYDV infective *R. padi* applied at early stem extension (GS 30) 10.5 weeks after sowing.

Aphids were applied approximately two per plant and allowed to feed for 72 hours, then all plots were sprayed with demeton S methol, 1.1 liter/ha (Metasystox). The BYDV isolate previously described for the glasshouse study was used.

Observations on leaf symptoms were made throughout and plots were sampled at 12 weeks after sowing by removing 0.5 m and lengths of drill row from the inner six rows. Fifteen plants were selected from each plot sample, top and root weights were measured, and the BYD and take-all fungus scores were calculated. The presence or absence of BYDV was confirmed using enzyme-linked immunosorbent assay (ELISA)

Table 2. Glasshouse study—Effect of barley yellow dwarf virus (BYDV) and *G. graminis* var. *tritici* (take-all fungus) on wheat, cv. Condor, at maturity

	Grain weight per plant (g)			
Rate of take-all fungus inoculation (% v/v)	BYDV treatments B_0	B_1	B_2	Mean
G_0	5.81^{a}	2.48^{ef}	2.91^{def}	3.73^{A}
G_1	4.62^{bc}	3.06^{def}	3.72^{bcd}	3.80^{A}
G_2	3.56^{cde}	3.21^{def}	2.28^{f}	3.02^{B}
G_3	4.78^{b}	2.93^{def}	3.37^{def}	3.69^{A}
Mean	4.69^{X}	2.92^{Y}	3.07^{Y}	

	1000-grain weight (g)			
Rate of take-all fungus inoculation (% v/v)	BYDV treatments B_0	B_1	B_2	Mean
G_0	52.51^{a}	28.54^{a}	39.59^{bcd}	40.21^{A}
G_1	47.19^{ab}	34.65^{cde}	33.34^{de}	38.39^{A}
G_2	43.25^{bc}	34.21^{cde}	35.95^{cde}	37.81^{A}
G_3	41.15^{bcd}	28.34^{e}	37.13^{cde}	35.54^{A}
Mean	46.02^{X}	31.44^{Z}	36.50^{Y}	

	Number of infertile heads (deadheads) per plant			
Rate of take-all fungus inoculation (% v/v)	BYDV treatments B_0	B_1	B_2	Mean
G_0	0.39^{f}	1.61^{bcd}	2.17^{ab}	1.39^{AB}
G_1	0.78^{cdef}	1.56^{bcd}	0.94^{cdef}	1.09^{B}
G_2	0.72^{def}	0.50^{ef}	2.61^{a}	1.28^{AB}
G_3	2.00^{ab}	1.72^{abc}	1.39^{bcde}	1.70^{A}
Mean	0.97^{Y}	1.35^{Y}	1.78^{X}	

B_0; B_1; B_2; represent inoculation with non-viruliferous aphids; inoculation with BYDV-infected aphids at growth stage 12; inoculation with BYDV-infected aphids at growth stage 30, respectively.

G_0; G_1; G_2; G_3 represent 0; 0.5; 1.4; 1.9% v/v of take-all fungus-infected, live oat inoculum in the soil, respectively.

Duncan's Multiple Range Test; Figures followed by the same letter are not significantly different ($P < 0.05$).

according to the method of Sward and Lister (1987). At maturity, plot length was reduced to 5 m and plots harvested. The grain yield and 1000-grain weight were measured and the percentage of heads containing little or no grain (deadheads) was calculated.

Results for both the field and glasshouse study were analyzed using a one-way and two-way analysis of variance. Mean separations were calculated using Duncan's Multiple Range Test.

Results

Twelve weeks after sowing, plants inoculated with BYDV had extensive leaf discoloration characterized by a general chlorosis, which, at this stage, was similar to symptoms of take-all fungus. Leaves of plants with dual infections also exhibited extensive discoloration, often with pronounced bronzing or reddening and interveinal chlorosis. Alone, BYD caused a reduction in top weight of almost 50% following inoculation at GS 12 (B_1) and almost 30% following inoculation at GS 30 (B_2). Take-all fungus at the lowest rate (G_1) caused a reduction in top weight of almost 50% and of more than 60% at the two highest rates (G_2 and G_3). At this stage, in plants infected with both pathogens, the combined effects were less than the sum of individual effects. No differences between root weights were recorded for any BYDV or take-all fungus treatments at any stage during the field study. The take-all fungus score was consistent with the relative amounts of live inoculum incorporated into the soil, and there was no interaction between take-all fungus and BYD on the take-all score. The apparent severity of BYDV symptoms, on the other hand, was complicated by the presence of take-all fungus, and relatively high scores were recorded in plants infected by take-all fungus but not inoculated with BYDV.

At maturity (Table 3), BYD alone, inoculated prior to tillering (B_1), caused a significant reduction in grain yield per plot, whereas inoculation after tillering (B_2) did not. Take-all fungus caused a reduction ($P < 0.05$) in grain yield per plot at the highest rate (G_2 and G_3) when compared with the control (G_0), although the mean separations using Duncan's Multiple Range Test indicated no significant reduction in grain yield due to take-all fungus in the absence of BYDV. However, in the presence of BYDV, at the higher rates of take-all fungus (G_3B_1, G_2B_2, G_3B_2), a significant reduction in grain yield per plot was recorded. Neither BYDV nor take-all fungus alone affected the 1000-grain weight. However in plants dually infected by take-all fungus (G_2) and BYDV, inoculated at GS 30 (B_2), a significant reduction in 1000-grain weight was recorded. Inoculation of BYDV after tillering (B_2) caused a greater overall reduction than did inoculation prior to tillering.

Take-all fungus alone had no effect on deadhead production, whereas BYD alone increased the number of deadheads, but only following inoculation at GS 30 (B_2). A synergistic reaction of the two pathogens on deadhead production was recorded, and in plants dually infected the percentage of deadheads per plot was significantly increased over the controls.

Discussion

Both take-all fungus and BYD are recorded as highly pathogenic on wheat in Victoria (Price 1970; Smith and Sward 1982) and in this present study, grain yield losses near 60% resulted when high relative concentrations of take-all fungus inoculum were incorporated into the soil followed by inoculation with BYDV at the two-leaf stage (GS 12).

Each pathogen alone can markedly affect the growth of wheat, particularly the roots (Esau 1957; Chambers 1971), and it was not surprising that an interaction resulted when plants were dually infected. Growth and yield measurements, particularly from the field study, indicated that a number of synergistic responses occurred and affected parameters such as grain yield per plot, 1000-grain weight, and the percentage of deadheads per plot. This could have been at least partly due to the secondary effects of BYDV affecting the growth and proliferation of take-all fungus. BYDV replicates and accumulates in the cells of the phloem, namely the sieve elements, companion cells, and phloem parenchyma (Gill and Chong

1975). The primary effect on host tissues is the necrosis of the cells of the phloem and deposition of 'gum' in sieve and possibly xylem elements (Esau 1957). The subsequent secondary effect appears to be the development of a resistance to the translocation of photosynthate (Jensen 1972). The gross effects of this are seen in the roots as vastly reduced growth and increased total nitrogen (Orlob and Arny 1961).

Garrett (1967) demonstrated that the level of nitrogen *in vivo* and *in vitro* can exert a highly significant effect on the growth and survival of take-all fungus. The secondary effects of BYDV, particularly on the root nitrogen level, may conceivably lead to increased growth of the fungus in vivo. The take-all fungus scores for G_2B_1, G_3B_1, and G_3B_2 recorded in the glasshouse study 9 weeks after sowing, support this hypothesis,

Table 3. Field Study. Effect of barley yellow dwarf virus (BYDV) and *G. graminis* var. *tritici* (take-all fungus) on wheat, cv. Condor, at maturity

	Grain weight per plot (g)			
Rate of take-all fungus inoculation (ml/m)	BYDV treatments B_0	B_1	B_2	Mean
G_0	3252^{a}	1952^{cde}	2574^{abc}	2538^{A}
G_1	2838^{ab}	1688^{ef}	2064^{bcde}	2146^{AB}
G_2	2866^{ab}	1652^{ef}	1647^{ef}	1983^{BC}
G_3	2360^{abcd}	1220^{f}	1771^{de}	1721^{C}
Mean	2811^{X}	1605^{Z}	1984^{Y}	

	1000-grain weight (g)			
Rate of take-all fungus inoculation (ml/m)	BYDV treatments B_0	B_1	B_2	Mean
G_0	37.10^{ab}	35.66^{abc}	34.04^{bcd}	35.60^{A}
G_1	38.54^{a}	35.62^{abc}	33.44^{cd}	35.87^{A}
G_2	38.20^{a}	35.44^{abc}	31.80^{d}	35.15^{A}
G_3	38.40^{a}	36.58^{abc}	34.14^{bcd}	36.37^{A}
Mean	38.06^{X}	35.83^{Y}	33.36^{Z}	

	Infertile heads (deadheads) (%)			
Rate of take-all fungus inoculation (ml/m)	BYDV treatments B_0	B_1	B_2	Mean
G_0	0^{f}	0.2^{ef}	0.4^{cde}	0.2^{B}
G_1	0.2^{ef}	0.8^{abcd}	1.2^{ab}	0.7^{A}
G_2	0.3^{def}	0.6^{abcde}	1.3^{a}	0.6^{A}
G_3	0.2^{ef}	0.5^{bcde}	0.9^{abc}	0.5^{A}
Mean	0.1^{Z}	0.5^{Y}	0.9^{X}	

B_0; B_1; B_2; represent no aphids; inoculation with BYDV-infected aphids at growth stage 12; inoculation with BYDV-infected aphids at growth stage 30, respectively.

G_0; G_1; G_2; G_3 represent 0; 1.6; 3.0; 4.8; ml of take-all fungus-infected, live oat inoculum per meter of drill row, respectively.

Duncan's Multiple Range Test; Figures followed by the same letter are not significantly different ($P < 0.05$).

and an increased proliferation of take-all fungus was measured in the plants infected with BYDV compared with their respective B_0 controls.

In the glasshouse study, the BYD score, which was based on visual assessment of leaf symptoms, was generally consistent with the stage of growth of wheat plants at the time of inoculation. The results of the field study followed the same general trends as those of the glasshouse trial except in the early stages, around 12 weeks after sowing. Very important was the fact that in plants not inoculated with BYDV and confirmed BYDV negative by ELISA, high BYD scores were recorded where take-all fungus was present. This reflected an overlap of leaf symptoms that occurred when symptoms were first expressed, and is proof of the difficulties encountered in an attempted diagnosis of these two pathogens based solely on leaf symptoms at this stage of growth.

In the glasshouse study BYD and take-all fungus each caused deadhead formation. This was not so obvious in the field study, however, but may be at least partly explained by the very mild weather conditions and a lack of moisture stress that prevailed in western Victoria during flowering to ripening. It is noteworthy that BYDV inoculation in both glasshouse and field at the later growth stage. (GS 30) resulted in a higher percentage of deadheads than did inoculation at the seedling stage, even though grain yield was not as severely affected as in plants inoculated at the earlier stage (GS 12). The difference in numbers of deadheads reflected a major effect of later inoculation, which was manifested in the high proportion of secondary tillers that produced deadheads because they collapsed and shriveled. The main tillers reacted differently and did not collapse. The heads continued developing, and grainfilling occurred, although the 1000-grain weight was significantly lower than for the other treatments.

In the field study, the combined effect of the two pathogens on the percentage of deadheads was considerably greater than a simple additive effect of either pathogen alone. In the glasshouse study, the single and combined effects of BYDV and take-all fungus on grain yield were not as clear-cut. This is frequently the case with pot experiments and is presumably a consequence of a series of factors such as crowding of roots, repeated drying and wetting, and other environmental factors experienced in a glasshouse environment.

The results of the present study, showing that the combined effects of BYD and take-all fungus can be severe, have important implications for geographical areas such as the southern Wimmera, where the two pathogens are common in wheat. That the combined effects of BYD and take-all fungus were severe was not surprising as both pathogens affect the roots of wheat. Numerous other studies (McIntyre and Dodds 1979; Varughese and Griffiths 1983; Potter 1980, 1982; Comeau and Pelletier 1976) have reported the effects on plants of dual infection with a range of viruses and fungi. The responses recorded are many and varied, but in certain cases an interaction of the pathogens has resulted in severe reductions in plant growth and yield. These other studies and this present report emphasize the importance of careful consideration of the effects of control and management strategies, not just on a single target organism, but on other organisms that may also be affected.

Acknowledgments

Grateful thanks are made to Diane Collins, Wendy Lehman, Fiona Hewett, and Richard Smith for technical assistance, to Peter Franz for statistical advice, and to the Wheat Research Council for financial support for this project.

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Barley Yellow Dwarf Virus-Host Plant Interactions Affecting Winter Stress Tolerance in Cereals

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Abstract

*Production of winter cereals in temperate climates is greatly affected by winter stresses and disease incidence. In controlled environment studies, barley yellow dwarf virus (BYDV) infection reduced low temperature tolerance of plants most severely in oats (*Avena sativa *L.) and barley (*Hordeum vulgare *L.) and less severely in wheat (*Triticum aestivum *L.), but had no effect in rye (*Secale cereale *L.), although rye developed high concentrations of the virus. BYDV caused a rise in the LT_{50} (temperature at which 50% of the plants are killed) of 4 to 8°C in barley and 2 to 4°C in wheat; a change of even 0.5°C in LT_{50} can significantly affect long-term field survival of plants. Survival in ice of infected plants was reduced by 70 to 90% in barley and 30 to 40% in wheat.*

The effects of long (34 days) or short (18 days) disease development periods with and without preinfection cold hardening are discussed, as well as the effects of BYDV on low temperature tolerance and ice survival after flooding. More CO_2 and ethanol were accumulated in infected barley and wheat plants during ice encasement. Carbohydrate content of plant crowns and leaf bases is discussed.

Winter cereals in temperate climates are subjected to a number of environmental stresses that affect survival of plants through the winter. The most significant of these stresses include exposure to low subfreezing temperatures, ice encasement at high subfreezing temperatures, flooding at near freezing temperatures, and desiccation at subfreezing temperatures under little or no snow cover. Depending on the severity of the climate and the hardiness of the cultivars, overwintering damage may vary from loss of a small percentage of plants to a total kill.

Barley yellow dwarf virus (BYDV) causes the most important virus disease of cereals in North America and some other parts of the world. Autumn infections of the virus in winter cereals occur every year, with incidences high enough to affect crop performance. Field studies with artificial inoculations of BYDV have shown that the virus significantly reduces survival of plants through the winter (Endo and Brown 1962; Cisar et al. 1982; Fitzgerald and Stoner 1967; Grafton et al. 1982). Controlled environment studies compared the response of different cereals to simulated winter stresses under BYDV infection and elucidated the effects of different stress factors as modified by the virus infection. The results of these field and controlled environment studies are discussed below.

Overwintering Behavior of BYDV-Infected Cereals

Endo and Brown (1962) were the first to report that infection with BYDV reduced winter survival of cereals. Under winter conditions in Illinois, winterkill was increased by 30 to 40% in oat (*Avena sativa* L.) cultivars and by 30% in barley (*Hordeum vulgare* L.) due to BYDV. Plants inoculated early in the fall showed greater reduction in survival than those inoculated in late fall, although levels of infection (98 to 100%) resulting from different dates of inoculation were the same. Winter survival of wheat in another study in the same geographical area was reduced by 51% (Cisar et al. 1982). In a subsequent field study in Missouri, Grafton et al. (1982) showed that survival of BYDV susceptible barley cultivars, inoculated with the virus, was reduced by 49 to 81%, while survival of BYDV tolerant cultivars was reduced only

6.5 to 11%. In a similar study, the response of several Canadian cultivars of barley was investigated under the more severe winter stress conditions of eastern Canada. Winterkill in BYDV inoculated plots varied from 85 to 95% in virus susceptible cultivars but only 20 to 25% in tolerant cultivars. Nearly 100% of the uninoculated plots of both susceptible and tolerant cultivars survived (Comeau and St. Pierre, personal communication). It appears, therefore, that BYDV tolerant cultivars are able to express their winter hardiness potential in the presence of virus infection while susceptible ones are not.

Controlled Environment studies of BYDV and Winter Stress

Survival and yield potential of winter cereals is adversely affected by varying intensities of winter stresses in different geographic regions within the temperate zones. Furthermore, the combination and succession pattern of the winter stresses inflicted on a crop vary from year to year and greatly affect plant survival. Therefore, the role of BYDV infection as a modifier of the effects of winter stresses cannot be precisely determined from field studies. Moreover, considerable variation occurs in the prevalence of virus strain types from year to year, further complicating the understanding of BYDV effects (Paliwal 1982). Additional biotic stresses such as root pathogens, nematodes, and other viruses are also usually present under field conditions and interact with the abiotic stresses, making it difficult to ascertain the effects solely due to BYDV. Controlled environment studies facilitate investigation of interactions of defined BYDV stress with specific abiotic (winter) stresses, and elucidation of the factors determining the effects of infection by BYDV.

Reaction of BYDV infected cereals to winter stresses

Sub-freezing temperatures and ice encasement are the two most common and most damaging winter stresses in many parts of North America. The effects of these stresses on the response of BYDV (*Sitobion avenae* (Fabricius)-specific strain) MAV-like infected oats, barley, wheat (*Triticum aestivum* L.), and rye (*Secale cereale* L.) have been determined (Table 1) (Paliwal and Andrews 1979). Young plants were inoculated with the virus at 15° for 3 days and then the temperature was lowered gradually to a day/night temperature of 2°/0°C to initiate cold acclimation of plants. After 6 weeks of cold hardening, the plants were subjected to various levels of freezing and ice encasement stresses.

Oats suffered the greatest reduction in freezing tolerance (LT_{50} = temperature of 50% plant kill) and survival in ice encasement at -1°C, followed by barley and the wheat (Table 1). No detrimental effect of BYDV was observed on the rye cultivar used although it was systematically infected by the virus and developed BYDV concentrations comparable to those of wheat. The magnitude of the BYDV effect was greater in cultivars with lower cold hardiness potential than in those with higher inherent hardiness potential (Paliwal and Andrews 1979; Andrews and Paliwal 1983). It has been reported than an LT_{50} change of even 0.5°C has a significant effect on field survival of wheat (Fowler et al. 1981), and it is likely that similar consequences occur in other winter cereals.

Flooding of plants at near freezing temperature followed by ice encasement is a combination of winter stresses that frequently occurs in the cereal growing regions of eastern North America and affects plant survival. Barley and wheat infected with BYDV were examined for effects of low temperature flooding. Survival of barley plants in ice encasement is reduced by flooding that precedes icing while survival of wheat in ice is increased by flooding (Andrews and Pomeroy 1981). In BYDV infected barley, flooding (2°/0°C) for 2 weeks reduced ice tolerance (LT_{50} days = days of ice encasement at -1°C that kill 50% of the plants) by 4.5 days as compared to virus-free plants, where the LT_{50} was reduced by only 0.8 days. The additive effect of the biotic (BYDV) and the two abiotic stresses reduced the LT_{50} days of plants by more than 5 days. In virus-free wheat flooding increased survival of plants in

ice by 4.7 days, but this positive effect of flooding was nearly cancelled by BYDV in infected plants (Andrews and Paliwal 1986).

Factors affecting the BYDV interactions

A number of factors that commonly occur in nature can increase or decrease the effects of BYDV infection on stress tolerances. The length of the disease development period prior to cold hardening of plants, different strains of the virus, and pre-infection conditioning of the plants influence the expression of BYDV effects and subsequent cold hardening of plants. Infection with BYDV may occur at various stages of cold hardening of plants and various periods of disease development may follow, depending upon temperature patterns during the autumn and the time of infection of plants. Extended periods of near freezing temperatures after BYDV infection may also modify the influence of BYDV on subsequent plant responses to low temperature and icing stresses.

Disease development period—The effect of the disease development period was investigated in wheat (Andrews and Paliwal 1983). The disease development period was considered to extend from the time of inoculation of plants with BYDV until the temperature was lowered to 2°C day/0°C night to start cold hardening of plants. After inoculation with a BYDV vector nonspecific PAV-like isolate at 15°/15°C, temperature during disease development was lowered in three steps to 2°/0°C, the durations of the steps depending on the length of the development period being tested. Plants were subjected to 6 weeks of cold hardening (2°/0°C) after the disease development period and then tested against the cold stresses. A long disease development period of 34 days reduced

Table 1. Effect of barley yellow dwarf virus infection on tolerance to subfreezing temperatures and on survival after ice encasement of cereal plants subjected to cold hardening after virus infection

Cultivar		LT_{50}[a] temperature (°C)	% survival of plants after 4 and 7 days of ice encasement 4 days	7 days
Oats cv. Coast Black	NI	-11.6±0.5	0.0	0.0
	I	- 6.7±0.3	0.0	0.0
Barley cv. Dover	NI	-14.7±0.3	25.0±13.2	0.0
	I	- 8.5±0.6	7.5±2.4	0.0
Wheat cv. Fredrick	NI	-17.8±0.3	90.0±5.0	8.0±3.0
	I	-15.8±0.6	27.0±6.0	0.0
Wheat cv. Norstar	NI	-20.8±0.3	–	95.0
	I	-19.0±0.3	–	98.0
Wheat cv. Kharkov	NI	-26.3±0.3	100	97.0±3.3
	I	-24.0±0.1	100	98.0±1.4
Rye cv. Puma	NI	-31.0±0.4	100	100
	I	-30.0	100	100

Results are expressed as mean value of 4 replicates ± standard error of the mean in each case.

NI noninfected (control), I infected plants (BYDV).

[a] Temperature at which 50% of the test plants were killed.

LT_{50} in wheat cv. Frederick by 3.2°C, but an 18-day disease development period caused a reduction of only 0.8°C (Figure 1.). Survival of plants under ice encasement (10 days at -1°C) was reduced 32% by the short development period and 66% with the longer period. Clearly, the length of the disease development period influenced the effect of BYDV on acquisition of low

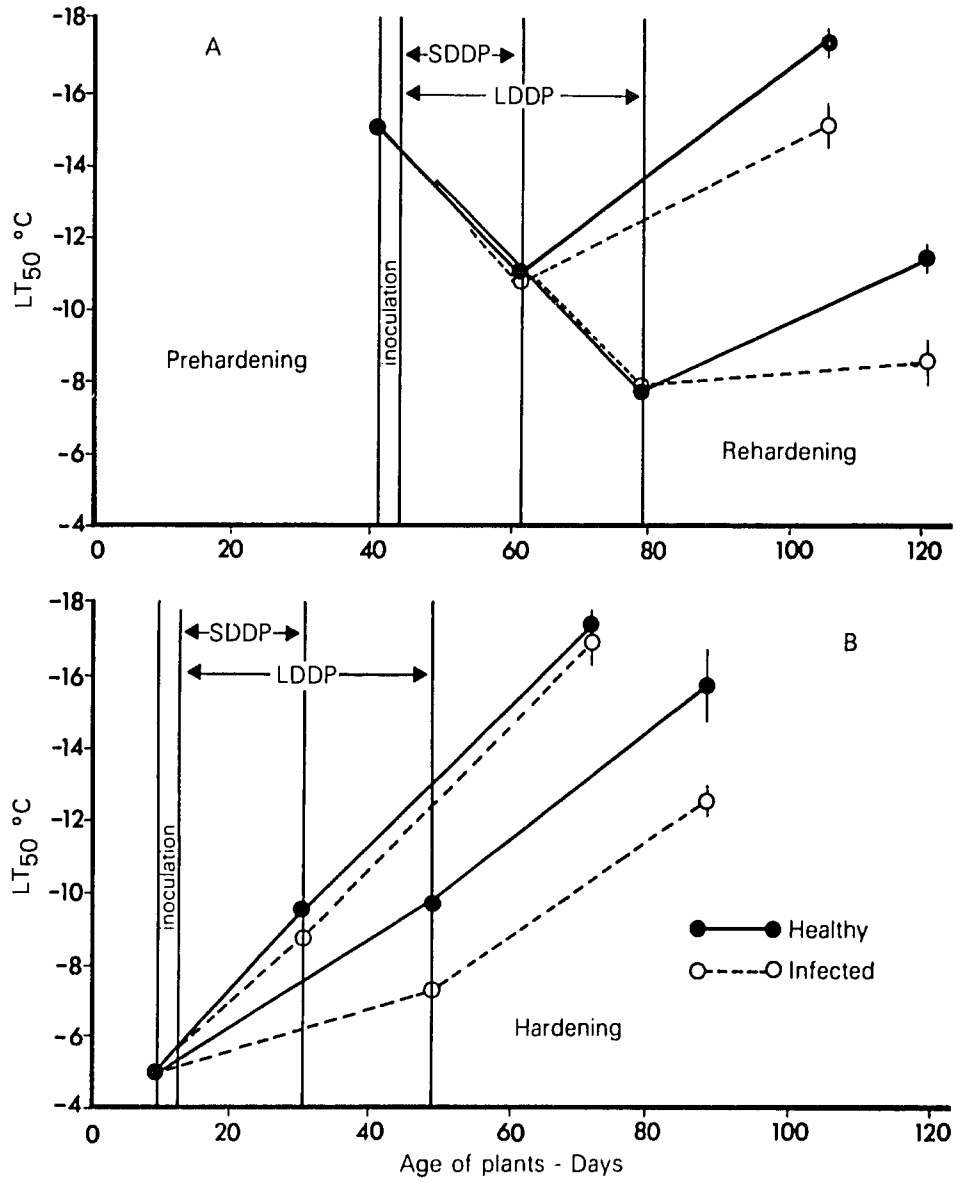

Figure 1. The cold hardiness of healthy and BYDV-infected Fredrick wheat with (A) and without (B) cold hardening before inoculation and with short (S) and long (L) disease development periods (DDP). Bars indicated ± standard error of four replicates.

temperature and ice tolerance by the plants. In barley cv. Dover even a change of 4 days in the length of the disease development period made a significant difference. LT_{50} temperatures were 6.2° and 2.1° warmer for BYDV infected plants given 21 and 17 days of disease development, respectively (Paliwal and Andrews 1979).

Virus isolate—Different isolates of the virus may differ in their modifying effect on stress tolerance of plants. A vector nonspecific (PAV-like) isolate of the virus reduced icing tolerance of the wheat plants by about 5 LT_{50} days and low temperature tolerance by 2.5°C (LT_{50}) as compared to healthy plants. The effect of a less virulent, *Rhopalosiphum maidis* (Fitch)-specific RMV-like isolate was not significantly different from the effect of the PAV-like isolate on low temperature tolerance, but the ice tolerance of plants was unaffected by the milder strain (Y. Paliwal and C. Andrews, unpublished results).

Preinfection conditioning—The effect of preinfection cold hardening of plants on virus induced modification of winter stress tolerance was investigated in wheat cv Frederick (Andrews and Paliwal 1983). Plants were subjected to 6 weeks of cold hardening (2°/0°C), which allowed plants to acquire their full potential of low temperature and icing tolerance. A short (18 days) or long (34 days) disease development period of BYDV at warmer temperatures (15°/15°C-2°/0°C) caused a similar degree of dehardening of both virus-free and infected plants. When plants were rehardened at 2°/0°C for another 6 weeks and tested against the two stresses, plants with the long disease development period failed to reharden, while those given the short disease development period rehardened to a LT_{50} level 2° warmer than the virus-free control plants. The difference in actual LT_{50} values attained by infected and healthy plants upon rehardening was the same with either of the disease development periods (Figure 1).

A comparison of results with pre-infection hardened and nonhardened plants (Figure 1) shows that pre-infection hardening had little effect on hardiness reduction due to BYDV in plants with the long disease development period, but in plants with the short disease development period the deleterious effect of virus infection was greater on prehardened plants. Furthermore, pre-infection hardening did not significantly modify the BYDV effect on survival of plants in ice. The dehardening and rehardening behavior of infected and virus-free plants (Figure 1) shows that final levels of hardiness of infected plants were 6 to 10°C warmer than the levels potentially attainable by this wheat cultivar.

Similar reductions in cold stress tolerance would be expected to occur in the field in years when normal, cool autumn conditions inducing cold hardiness in plants are interrupted by above average temperatures conducive to greater plant growth and disease development. These events, which are common in several parts of North America, would lead to drastic winterkill, particularly in early planted wheat.

Maintenance of initially acquired cold hardiness of plants over extended periods of mild cold stress was affected by BYDV infection. The LT_{50} of Frederick winter wheat maintained at 2°/0°C after a BYDV disease development period of 18 days showed, as expected, a reduced LT_{50} at 2 months after the disease development period, but in the subsequent 6 months at 2°/0°C there was no difference in tolerance to subfreezing temperatures between the virus-free and infected plants. During the first 4 months after the disease development period, the low temperature tolerance of virus-free plants decreased, but that of infected plants remained at the maximum attained level. Hardiness levels of both infected and healthy plants decreased at the same rate in the next 2 months.

Behavior of plants under ice encasement provided unexpected results. After 7 days of ice encasement, survival of infected plants at 2 and 4 months past the disease development period was 6% and 55% higher, respectively, than the virus-free control plants. At 6 months past the

disease development period, survival of all plants in ice was poor, but the virus-free plants showed 14% higher survival than the infected plants.

Thus, for a certain period during plant growth and development at low temperatures, BYDV infection induces increased ice tolerance in cereals while maintaining their tolerance to sub-freezing temperatures. Such a response to virus infection was previously seen in certain experimental conditions with Frederick wheat (Paliwal and Andrews 1979) and was also recorded in field conditions in South Dakota in three wheat cultivars where plants inoculated 30 days after seeding had higher winter survival than uninoculated plants. Plants inoculated 7 days after seeding also survived better than uninoculated plants, but the increase in survival was less pronounced (Fitzgerald and Stoner 1967).

Metabolic changes in plants under cold stress

A strong correlation between tolerance to subfreezing temperatures and dry matter content of plants is well established. The dry matter content of winter wheat plants increases as they acquire cold hardiness (Fowler et al. 1981). BYDV infection also results in higher dry matter in plants (Jensen 1968). However, increased dry matter as a response to BYDV infection did not result in increased cold hardiness of infected wheat plants as compared with virus-free control plants. Increased tolerance to ice encasement injury in BYDV infected plants was associated with higher dry matter content of infected plants (Andrews and Paliwal 1983). Based on Jensen's (1969) findings that BYDV infected plants accumulated more carbohydrate than virus-free plants, Andrews and Paliwal (1983) suggested that this carbohydrate energy reserve in plants may be utilized for anaerobic respiration during ice encasement. Thus, it is likely that excess carbohydrate in infected plants could promote better ice tolerance in such plants.

Levels of total nonstructural carbohydrate—Significant differences were observed in the levels of total nonstructural carbohydrates (TNSC) in the crowns of BYDV infected and virus-free plants after 6 weeks of cold hardening (2°/0°C) followed by two weeks of low temperature flooding (2°/0°C). In Dover barley without flooding, infected plants accumulated 27% less TNSC in their crowns than virus-free plants and the loss of TNSC during flooding was 44% greater in crowns of infected plants. In Fredrick wheat, crown TNSC levels were 22.5% lower in BYDV infected plants, but decreases during flooding were nearly the same as in healthy plants. The rates of carbohydrate utilization during ice encasement following flooding of plants were nearly the same for virus-free and infected plants (Andrews and Paliwal 1986). These results, therefore, do not indicate a direct role of carbohydrates in promoting survival of cereal plants during icing since BYDV infection in wheat cancels the positive effect of flooding on survival in ice.

Accumulation of CO_2 and ethanol—Changes in accumulation of CO_2 and ethanol, two main glycolytic end products, during flooding and ice encasement were also investigated in the crown tissue of Dover barley and Fredrick wheat. Generally, infected plants accumulated more CO_2 than healthy plants. During the 2-week flooding period that followed cold hardening, virus infection caused about 7% higher CO_2 accumulation than in healthy barley plants, but no significant differences attributable to BYDV were noted in CO_2 accumulation in wheat during flooding. After 4 days of ice encasement immediately following the flooding, the level of CO_2 in infected Fredrick and Dover plants was at a level 28 to 50% higher than in uninfected flooded or unflooded plants of both cereals. In 10 subsequent days of ice encasement, CO_2 accumulated more rapidly in crowns, of BYDV infected plants of flooded barley and flooded or unflooded wheat. Ethanol accumulation after a 2-week flooding period occurred in the flooded plants of

both cereals and was 43% lower in infected barley, but more than three times higher in infected wheat compared with healthy plants.

During an ice encasement period of 14 days, both the previous flooding and BYDV infection stimulated ethanol production in both cereals. However, the BYDV effect was much more pronounced in barley, causing up to 100% increase in ethanol levels of plant crowns (Andrews and Paliwal 1986). It is not clear in what way the virus infection stimulates the production of ethanol and, to a lesser extent, CO_2 during ice encasement, particularly as the rate of carbohydrate utilization by plants in ice does not appear to be stimulated by the virus. Possibly virus-directed accumulations of intermediary glycolytic metabolites are reduced to ethanol and CO_2 during ice encasement without further carbohydrate breakdown.

Also, differences in carbohydrate composition develop during cold hardening in the presence or absence of the virus infection. The authors have found that the fructan component, which has been reported to be associated with cold hardiness and low temperature growth in cereals and grasses (Trunova 1968; Olien and Lester 1985; Pollock and Jones 1979), accumulates to a much lower level in plant crowns and leaves in the presence of virus infection (C. Andrews and Y. Paliwal, unpublished results). The significance of such a major change remains unclear.

Prospects

It is now well established that BYDV interacts with its host plants, significantly affecting their response to winter stresses. While a number of effects of BYDV pathogenesis on acquisition, expression, and maintenance of tolerance to winter stresses in wheat and barley have been elucidated, the underlying physiological changes and biochemical processes at the cellular and molecular level are unknown.

Considering the worldwide distribution of BYDV, its widespread occurrence within cereal growing regions every year, and its large host range, insufficient attention has been given to the study of the many probable interactions of BYDV with various abiotic and biotic stresses that occur in nature and affect crop performance. BYDV is a phloem limited virus inciting a 'yellows' type of disease. Other cereal viruses such as wheat spindle streak mosaic and wheat streak mosaic that cause a mosaic type of disease coinfect the plants with BYDV. Effects of such mixed infections on plant responses to winter stresses need to be studied. Tolerance to other abiotic stresses such as water deficit, environmental pollution, etc., may be affected by BYDV infection. A thorough understanding of interactions between BYDV and the locally important abiotic stresses should be helpful in devising strategies for reducing the impact of abiotic stresses on plants as well as for better disease management.

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The late Dr. Yogesh C. Paliwal

After the workshop, we were saddened to hear of the death of Dr. Yogesh C. Paliwal, 46. He died suddenly of a heart attack at his home in Ottawa, Canada, on June 23, 1988. Yogesh was a researcher with Agriculture Canada, which he joined in 1967 after a 2-year post-doctoral with the Department at Ottawa. He received his BSc in 1959 from Agra University in India and his MSc and PhD degrees from IARI at New Delhi in 1961 and 1965.

Yogesh carried out research on both the applied and fundamental aspects of plant virus diseases. In June 1987 he was awarded the Gordon J. Green Award for Outstanding Achievement by the Canadian Phytopathological Society in recognition of his research contributions, which have led to the understanding of the diseases caused by plant viruses and strategies for control, particularly for BYDV. Those of us who knew Yogesh are saddened by his passing and will miss him as a friend and colleague.

Short- and Long-term Maintenance of Aphid Clones

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Abstract

Over the past 9 years we have developed certain aphid handling regimes that are essentially simple and nonlaborious. Our systems allow long-term maintenance of colonies/clones, and simultaneously ensure that established aphid clones are available for use in ongoing research projects. Maintaining aphids over extended periods at low temperatures without frequent transfer of either plants or aphids is discussed.

The distance between our Cape Town research laboratory and the Orange Free State (OFS) wheat (*Triticum aestivum* L.) fields (1,500 km), necessitated the development of aphid holding facilities to maintain aphid virus-vectors for research purposes; also to maintain the original aphid clones linked to certain cereal virus diseases researched since 1978, when *Diuraphis noxia* (Mordvilko) was first noted in the OFS-grown wheat.

The initial sampling trip to collect diseased wheat linked to the presence of *D. noxia* infestations coincided with cold weather in the high altitude wheat production region in the eastern OFS, which in winter averages freezing night and moderately warm day temperatures. Finding natural infestations of *D. noxia* under these extreme climatic conditions prompted the concept of keeping this aphid, and later also others, at low temperatures for maintenance and virus transfer. The availability of large walk-in coldrooms, operating at 4 to 6°C, facilitated storing *D. noxia* at temperatures similar to those encountered under natural conditions in the OFS winter. The reduced aphid mobility and population growth meant that colonies could easily be left unattended for 8 to 12 weeks on one set of host plants.

Several considerations led us to develop systems for easy and safe aphid maintenance that under our conditions proved to be functional and satisfactory. These will be discussed separately.

Incubators

D. noxia, a new aphid in the OFS wheat, was first encountered when its identity was not certain. As at that time the OFS was the only place where *D. noxia* infestation had been noted in South Africa, the Department of Agriculture considered it important to ensure that aphids taken to the University of Cape Town for experimental work be contained, to avoid infestation of the Cape Western and Southern Cape small grain production regions. To implement this request for quarantine, self-contained refrigerated incubators were made available (custom-made by Specht Scientific [Pty] Ltd.).

The incubators were fitted with sensitive temperature controls and timer-regulated light sources, which made it possible to select different day/night temperatures. They were also fitted with four shelves, each illuminated by two 2-ft (61 cm) fluorescent VHO Growlux light tubes attached to the center of the shelf or to the sides of the cabinet. The latter arrangement was preferred, as it facilitated cleaning. A *D. noxia* aphid clone was maintained in such an incubator for 9 years at a 7 to 10°C temperature regime and a 14/10 hour night/day cycle. The incubators were kept in a room partitioned off by double exit doors and controlled by negative air pressure and special air filters.

Quarantining *D. noxia* at the University became unnecessary, as the aphid spread to all major wheat producing regions in South Africa within 2 years after it was recorded. In itself, the quarantining was an interesting exercise, and it laid the foundation for exploring and adapting the facilities further and also for holding other aphid species.

Our barley yellow dwarf virus (BYDV) PAV-like isolate, transmitted by *Rhopalosiphum padi* (L.) as a complex of viruses with brome mosaic virus (BMV) and *R. padi* virus (RhPV), has been maintained in such an incubator since 1979. The BYDV SGV-like isolate was lost, as our *Schizaphis graminum* (Rondani) clone was sensitive to low temperatures and could not be maintained at 4 to 6°C, and additional insect incubators were not available at the time. Population growth of *S. graminum* at room temperature (21°C) was too rapid to maintain this aphid safely with any other species.

Insect incubators have since been used successfully to maintain clones of virus-free *R. padi; R. padi* infected with RhPV (7 years); *R. padi* (RhPV positive) transmitting BYDV PAV-like isolate; *R. maidis* (maize [Zea mays L.] clone) sensitive to low temperatures; *R. maidis* (barley [*Hordeum vulgare* L.] clone); *D. noxia;* a *Myzus persicae* (Sulzer) clone from wheat; and other aphid species.

Gauze Cages

Gauze cages 45 x 45 x 50 cm were used to accommodate nine potted seedling plants for aphid transmitted virus propagation. The cages were covered with nylon netting; one side was an open flap fitted to a wooden slat to secure it when closed. The cages were set on double story trolleys, and each level was fitted with four 8-ft VHO Growlux fluorescent tubes controlled by time switches to regulate the light/dark cycle. Cages were either kept at an average of 18 to 21°C for fast population growth, which necessitated a weekly change of host plants, or in a coldroom at 4 to 6°C to slow down population increase. The coldroom host plants required less frequent changing (8 to 12 week intervals). Cages were kept 25 cm apart with catch plants placed in between to attract stray aphids. Stray aphids were not a problem at 4 to 6°C.

Cages were also kept on window sills in front of large windows providing sufficient light. Catch plants between the cage and the window collected stray aphids. Host plants in these cages had to be changed on a weekly basis.

To encourage aphids to settle after host plant changes, reading lamps fitted with incandescent 60 w globes were focused from the outside onto the back of the cages. These lamps, when burned continuously, caused aphids to cluster in the warmth of the spotlight and encouraged a slightly faster population growth when compared to aphids in gauze cages without incandescent lighting. Such aphids usually appeared exceptionally large, which was the result of an accumulation of unborn nymphs at the low temperature. The added advantage of these aphid clusters was that upon transfer to 20°C they gave birth to numerous nymphs in rapid succession. This behavior was used to obtain non-viruliferous clones and clones of equal age.

Maintenance of New Collections and Backup for Clones

To ensure that aphids collected in different regions were maintained, clones (from single aphids) were started on barley seedling leaves on moist filter paper in Petri dishes. When several aphids had reached maturity, leaves and aphids were transferred to boiling tubes (large test tubes) containing sterile vermiculite, wetted with an inorganic nutrient solution, and 2 to 3 barley seedlings (5 cm long) grown from surface sterilized virus-free seed. The tubes were covered with fine weave nylon mesh and were kept at a slant of 60° in baskets on a light box, regulated by a time switch allowing a 10/14 hour light/dark cycle. The light box was kept in a coldroom at 4 to 6°C. Aphids in test tubes multiplied slowly and could be left unattended for 8 to 12 weeks, when transfers were made. Providing light from the bottom had the advantage that the leaves did not lift off or grow through the nylon netting.

This method allowed maintenance of 1) numerous different aphid clones and 2) aphid transmitted virus isolates, i.e., BYDV, BMV, and RhPV.

Canning bottles

As an alternative to the boiling tubes, 2-liter glass canning jars fitted with a two-part metal lid consisting of a flat disk held in place by a screw ring, were used. The flat disk was replaced by aphid-proof nylon gauze. The jars held clusters of seedlings rooted in vermiculite and could

support larger numbers of aphids. On average, transfer of aphids was done approximately every 3 months. The jars were kept on shelves in a coldroom at 4 to 6°C. Cold fluorescent VHO light was supplied from the rear (2 strips) and operated off an automatic time switch set at 10/14 hours light/dark.

The advantage of jars over the boiling tubes was that a larger number of aphids could be accommodated, allowing a faster start when aphids were required for experimental work. The jars were also wide enough for the aphids to remain on the seedlings in the center, preventing them from drowning in water condensation droplets on the sides of the jar.

Occasionally aphids were transferred after longer intervals, sometimes when the seedlings already had died off. Without fail, living aphids, usually a few alates, would still be found alive among the lower stems and roots. These starved aphids became active when transferred to room temperature and could easily be recovered.

Non-viruliferous clones

For virus transfer studies, virus-free aphids were used. These were obtained by collecting adults reared at 4 to 6°C under continuous spotlight, starving them for 2 hours at 4°C, and then settling them on virus-free barley seedling leaves, whereupon they would give birth to numerous nymphs in quick succession. At birth nymphs were transferred onto new uninfected leaves to avoid their feeding on the same leaf as their parent. Nymphs were reared on seedling leaves in large Petri dishes and when mature, were transferred with the leaf to clusters of seedlings either growing in soil or in canning bottles to avoid further contact and possible contamination with RhPV from non-sterile equipment.

Aphid clones started in this fashion were checked in enzyme-linked immunosorbent assay (ELISA) for the presence of various viruses with specific antisera. Similarly, long-term virus-free clones of *R. padi* were checked regularly to ascertain whether they were still free of the RhpV-virus or any other contamination. Host plants of virus-free control aphids were kept after aphid transfer and processed for virus extraction, the sap concentrated and tested for aphid and cereal viruses in ELISA. Finding positives in such tests led to the identification of seedborne viruses and helped identify reliable virus-free seed batches for maintaining non-viruliferous aphids.

Surplus aphids were also collected at the time of aphid transfer; aliquots were coded and frozen (-20°C) immediately. More recently aphids infected with RhPV have been stored at -70°C. This collection of frozen aphids has already proved to be an important link with past research in establishing the presence of RhPV in aphid species and detecting contaminated clones. RhPV was shown to retain its infectivity in such frozen aphids for 6 years and could be used to reinfect virus-free *R. padi* and *D. noxia* (C. Williamson and M.B. von Wechmar, unpublished.)

Wasps and red spiders

Our field collected aphids were often contaminated with parasitic wasps, which could decimate an aphid colony unless controlled. Maintaining aphids at low temperature controlled and eliminated this problem entirely. Red spiders occasionally became a problem in our dry air-conditioned environment. Although not a problem in cereal seedling hosts used for aphids, this pest too could be easily controlled and eliminated by switching to low holding temperatures.

Before we adopted low temperatures generally for aphid maintenance, wasps occasionally were a problem. The addition of a praying mantis to such an aphid colony soon reduced the number of wasps. In addition, a praying mantis would also control the number of alates in and outside aphid cages kept at room temperature. Forgotten praying mantises have emerged on occasion from a stint in the cold room and looked none the worse for the experience. We have had praying mantises on our window sill for extended time intervals—doing an excellent cleaning up job of escaped alate aphids. Regrettably, these insects are regarded with some superstition locally and often meet an unnatural death.

A Simple and Versatile System for Controlled Rearing of Cereal Aphids and Transmission of Barley Yellow Dwarf Virus Isolates

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Abstract

A system has been developed in which aphids are reared on young cereal seedlings grown on nutrient soaked cotton wool in disposable polystyrene cups with cardboard lids. This system has been used to maintain and rear cereal aphids in a secure and controlled manner, free of the risk of colonies becoming infective with BYDV, or of contamination by aphid parasitoids. The system has also been adapted to enable the testing of transmission rates of various BYDV isolates by a range of aphid species, and has been further used for the collection of infective aphids from the field in a condition in which they can be multiplied or an isolate test performed on the host seedling.

One of the fundamental properties used to differentiate the isolate groups of the barley yellow dwarf viruses (BYDVs) is vector specificity (Gill 1967; Rochow 1969). In any laboratory studying these viruses, it is necessary, therefore, to maintain colonies of particular cereal aphid species so the virus isolates can be routinely transmitted from source plants to test plants. In our laboratory, we routinely work with four aphid species: *Rhopalosiphum padi* (L.), *R. maidis* (Fitch), *Metopolophium dirhodum* (Walker) and *Sitobion fragariae* (Walker), although in certain experiments we have used up to seven different species.

The traditional aphid rearing systems of clip-cages or whole plant cages (Adams and van Emden 1972) are not always completely satisfactory. They are time consuming to set up, aphid numbers are difficult to regulate, and problems can occur due to colonization of the cultures by aphid parasitoids such as *Aphidius colemani* Viereck (Carver and Stary 1974) or through epizootics of entomophthoran fungi (Milner et al 1980).

We therefore developed a simple system for rearing a range of cereal aphids, which has also proved most useful for conducting transmission tests. In this report, we describe this rearing system and discuss its advantages and various uses.

Rearing System for Cereal Aphids

The standard rearing cup is a high-impact polystyrene cup of 40 mm diameter rim, 28 mm diameter base and 38 mm height (nominal capacity 35 ml) with a cardboard lid. A newly-germinated cereal seedling (Decimal Growth Stage 07) (Zadoks et al. 1974), that is, with the coleoptile just emerged from the caryopsis, is placed on a 0.45 g ball of cotton and cellulose fibre, which is saturated with 7 ml of modified Hoagland's solution (Hughes and Woolcock 1965) delivered by an automatic dispensing pipette (Figure 1).

To minimize contamination of the seedlings by *Aspergillus flavus*, cereal seed is dusted with captan prior to germination. The germination chambers are polystyrene food containers (K16) with a 120 mm diameter rim, 100 mm diameter base and 63 mm depth, and a tight-fitting lid. The treated seed is spread on a layer of blotting paper covering a 20 mm layer of cotton wool saturated with tap water. The covered chamber is stored at 20°C. The seedlings are suitable for aphids 3 days after sowing and are certain to be free of any non-seed-borne virus.

Maintaining Virus-Free Colonies

Aphid colonies free of non-transovarially transmitted virus can be founded by using newly-born progeny (which have not fed on infected plants). These progeny are reared individually in the cups. These colonies are then sub-

cultured every 7 days (at 20°C), when one newly-born nymph is placed into a cup and the parent is discarded. This system is very flexible since the numbers of aphids reared through each week is easily regulated. One aptera of *R. padi* will typically give birth to 8 to 9 nymphs in 24 hours at 20°C early in her reproductive life (Figure 2). Thus, from 1 to 9 cups can be set up per aptera per day.

Since every aphid is confined and the lid of each cup can be labelled easily, cross contamination of colonies should be entirely avoidable. We have been able to rear 7 species of cereal aphids on one shelf in a constant temperature room for 24 months without any mixing of species or introduction of BYDV occurring (Figure 3).

Virus Transmission Tests

The cup rearing system enables virus transmission tests to be conducted in a standardized manner using aphids of known and similar age in each test. The

Figure 2. One aptera of *R. padi* will typically give birth to 8 to 9 nymphs in 24 hours at 20°C.

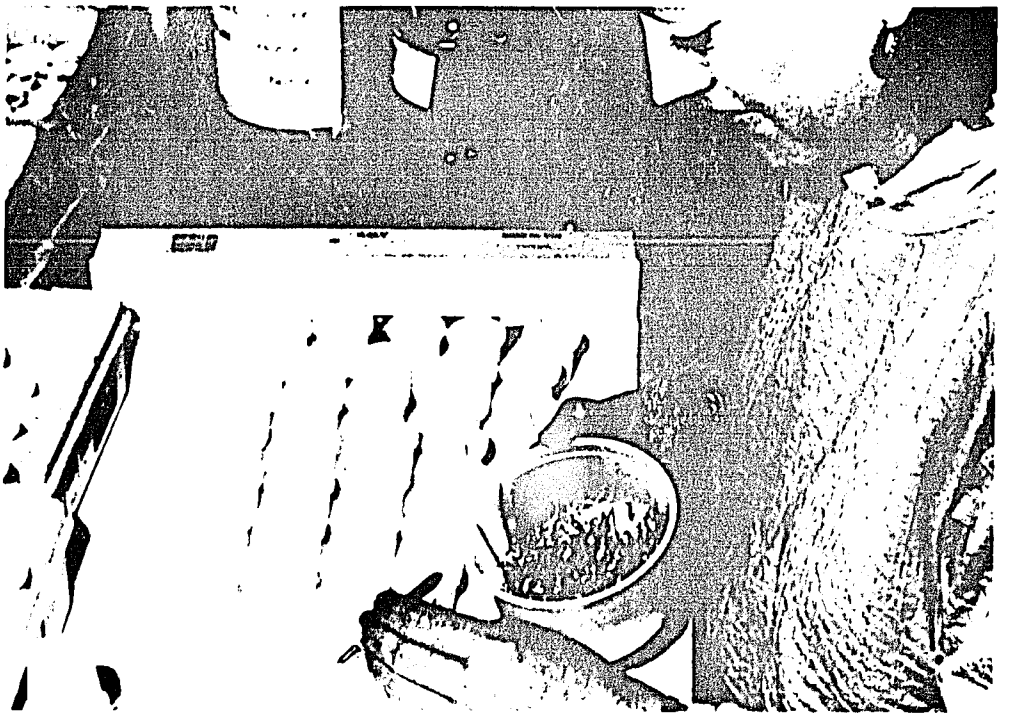

Figure 1. The standard rearing cup consists of a polystyrene cup with a cardboard lid. Newly germinated cereal seedlings are placed onto nutrient-soaked cotton wool in the base of the cup.

protocol we have developed for BYDV transmission tests with *Rhopalosiphum* spp. is outlined below. The aim is to complete the transmission feed while the aphid is still in the fourth instar. If left until adult, removal of its offspring is time consuming. The timings or temperatures can be altered if working with other species of aphids with different developmental rates.

Day 1: Newly-born nymphs (0 to 12 hours old) are placed into individual rearing cups and held for 7 days at 20°C.

Day 8: These individually reared apterae are collected and placed on 20 mm portions of healthy leaves (which are then placed in a closed K16 container as used for seed germination). Ten aphids are held in each container for 24 hours at 20°C.

Day 9: The apterae are removed and either discarded or placed on fresh healthy leaf material as before for production of more nymphs. The test leaves (known positives, field samples, healthy controls) are each divided into equal pieces (so each species of aphid can feed on equivalent pieces of the same leaf) and placed into labelled K16 containers. The nymphs produced in the previous 24 hours are placed onto the test leaves using a fine sable-hair artist's brush. These test containers are then placed at 17.5°C for 48 hours to allow for acquisition feeding.

Day 11: The nymphs are transferred to cups that contain the test seedling (in our case, *Avena sativa* L. cv. Algeribee). Three nymphs are placed on each seedling. The seedlings are held for a further 4 days at 20°C. The test leaves can then be stored in a freezer for subsequent testing with ELISA to complement the glasshouse observations.

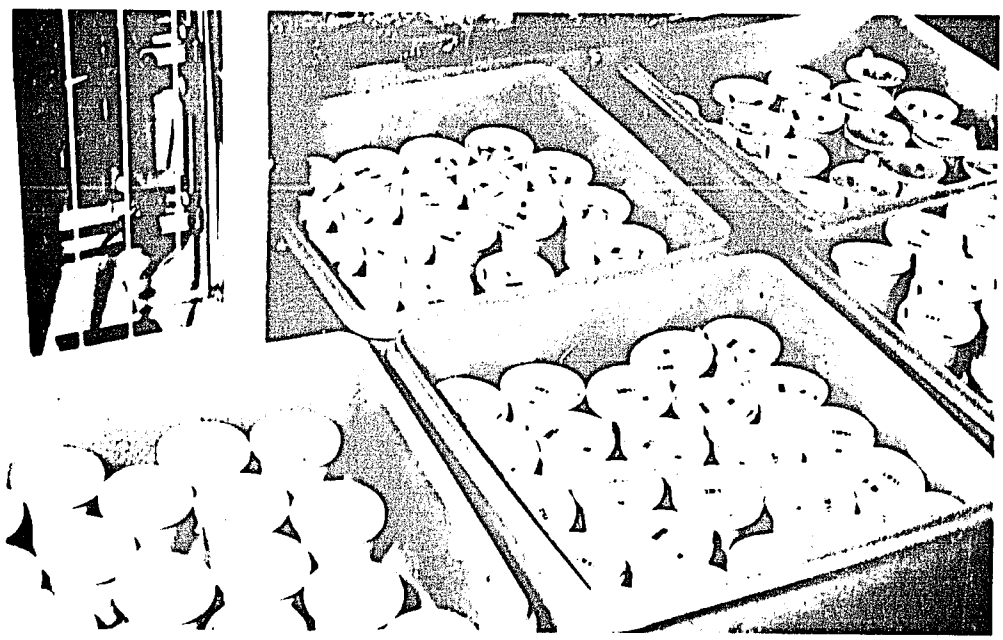

Figure 3. Aphids are confined in cups with the cardboard lids labelled. A range of aphid species is easily and securely reared in a small area of a constant temperature room.

Day 15: At this stage, the aphids are still in the 4th instar. The aphids are removed and killed. Their feeding position can be noted, if required, and any mortalities recorded. This census of vectors ensures freedom from cross-contamination. The now aphid-free seedlings are then transplanted into boxes containing potting mix for subsequent growth in the glasshouse. The seedlings transplant successfully, although careful watering (for example, with a squeeze bottle) is recommended for the next few days. We fumigate the glasshouse with dichlorvos twice weekly to prevent vagrant aphids from colonizing the test plants. Symptom development is recorded twice-weekly and plants are generally harvested 6 weeks after transplanting, with plant material being stored at -4°C for subsequent testing with ELISA.

In one 12-month period, 17 series of sequential transmissions were conducted with *R. padi* using a local PAV-like isolate, and 12 series of sequential transmissions were conducted with *R. padi* using a local RPV-like isolate. These isolates were separated from a virulent mixed isolate collected in southwest Victoria (Sward and Lister 1987). Transmission success for the PAV-like isolate averaged 47% and for the RPV-like isolate 80%.

The system has proved less reliable with *M. dirhodum*, which frequently failed to settle and feed satisfactorily on the detached test leaves or the very small test seedlings. In a trial using the local PAV-like isolate and *M. dirhodum*, a transmission success of 35% (7 positives from 20 transmissions) resulted from detached leaves, compared with 85% (28/41) when *M. dirhodum* acquired the virus by feeding on infected potted oat plants ($X_1^2 = 6.1$ $P < 0.05$). In contrast, *R. padi* had a transmission success of 85% (18/20) from the detached leaves and 86% (31/36) from the infected plants ($X_1^2 = 0.01$ $P > 0.05$). This result emphasizes the need for careful observations with any system of transmission tests using aphids.

Advantages and Other Uses of the System

The main advantages of rearing aphids using the cup system outlined here include:

1) The aphids can be easily observed and handled in the cups under a dissecting microscope.

2) The cups can be set up very quickly. For example, one person can prepare 100 cups ready for aphids in 10 minutes.

3) The problems of aphid parasitoids or entomophthoran fungi are entirely avoidable since the cups remain closed except for the short periods when aphids are sub-cultured. This is done in the relative security of the laboratory environment.

There are a number of other uses for the system. One is in determining the infectivity of field-collected aphids, an essential part of any epidemiological study of BYDV (Irwin and Ruesink 1986). Caged potted plants are cumbersome to transport and use in the field and there is a substantial risk of accidental contamination during the period of transport. There can also be difficulty in retrieving aphids that fall onto the soil surface of the pot.

The cups have proved ideal for field assays of aphids. They are conveniently and safely transported to the test site; individual aphids can be easily placed on the seedlings and then securely covered. The cardboard lid allows for rapid labelling as specimens are captured. Retrieval of the aphid to allow for positive identification is certain and auxiliary information on the level of parasitism by aphidiid wasps or infection by entomophthoran fungi can be gathered. Any offspring of the aphid can be subcultured if required. This can facilitate positive identification of alatae of *Rhopalosiphum* spp.

The cup system also proved most useful in intensive laboratory studies of two semi-subterranean cereal aphids, *R. ruflabdominalis* (Sasaki) and *R. insertum* (Walker). These aphids are easily observed and manipulated on the exposed roots of the seedlings in the cups. The studies involved the influence of temperature on the development and fertility of both species and the effect of crowding on wing formation. Furthermore, the cup system provides a simple and secure method for maintaining the number of clones of a species required for studies involving isozyme analysis, karyotype analysis, resistance to insecticides, or morphometric variation.

The only potential disadvantage of the system is that aphids may become adapted to the rearing conditions and their performance may no longer be representative of field-collected aphids. This in fact applies to all laboratory cultures of insects to some extent, and may be partly overcome by regularly collecting fresh cultures from the field to compare with the established laboratory culture.

Acknowledgments

We thank Bernadette Deane for technical assistance and the Wheat Research Council and the Australian Meat Research Council for financial support.

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Incidence of PAV-like and RPV-like Viruses in Four Pasture Species on Two Sites at Sandford, Tasmania

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Abstract

*Four pasture species sown at the same time in heavy and light soil conditions were sampled for barley yellow dwarf viruses. Only PAV-like, RPV-like, and mixed infections of these two viruses were detected from the two sites. Higher levels of infection were detected in samples from the heavy soil site. The highest levels of infection (from predominantly PAV-like viruses) were detected in fescue (*Festuca arundinacea *Schreb) (21%) and ryegrass (*Lolium perenne *L.) (27%). Levels of infection were lower in cocksfoot (*Dactylis glomerata *L.) (6%) and phalaris (*Phalaris aquatica *L.) (7%), and both were predominantly infected with RPV-like viruses.* Rhopalosiphum padi *(L.) was collected from all four species, while small numbers of* Sitobion fragariae *(Walker) were collected only from fescue.*

Most surveys of barley yellow dwarf virus (BYDV) infected pastures sample a number of pastures of different ages to estimate the incidence of infection (e.g., Guy et al. 1986; Latch 1977). This study of two pastures sown on the same day examines the effects of different site characteristics and different host species on levels of infection.

Materials and Methods

Four grass species—cocksfoot (*Dactylis glomerata* L. cv. Porto), fescue (*Festuca arundinacea* Schreb cv. Demeter), phalaris (*Phalaris aquatica* L. cv. Australian), and perennial ryegrass (*Lolium perenne* L. cv. Victorian)—were sampled every four to six weeks (July 1983 to December 1985) from two sets of replicated plots at Sandford, Tasmania. One set was established on a dark brown clay (heavy soil site) and the other on a gray unconsolidated sand (light soil site). The two sites were 200 m apart.

At each sampling 25 tillers were collected at random from plants of each species at both soil sites.

Each tiller was ground in buffer and tested for the presence of MAV, PAV, and RPV viruses using a modified double antibody sandwich enzyme-linked immunosorbent assay (ELISA) (see Guy et al. 1986 for details).

Additional subsamples from 200 of the plants were triturated in groups of five in phosphate buffer and inoculated to seedlings of grass-virus indicator species.

Results

Only PAV, RPV, and mixed infections of these two viruses were detected in the samples from the two sites. No other aphid transmitted or mechanically transmissible viruses were detected.

Most of the infected grasses showed no symptoms of virus infection in these closely grazed pastures. A few ryegrass plants were stunted and produced unusually large numbers of tillers. The effects of BYD viruses were more obvious under the less restrictive growth conditions of the glasshouse. During spring infected (PAV, RPV, mixed infections) fescue plants developed yellow mottles in their older leaves. Occasionally, infected (PAV) phalaris plants showed conspicuous yellowing and stunting. Infected cocksfoot plants showed no conspicuous symptoms. Ryegrass plants occasionally showed severe stunting when infected (PAV, RPV, mixed infections). PAV infection of ryegrass reduced dry weights by an average of 16% in yield experiments.

Larger numbers of aphids were collected from the heavy soil site samples than from the light soil site samples (Table 1). More aphids were collected from fescue samples than from the other three species. *Rhopalosiphum padi* (L.) was the most commonly encountered aphid collected from all four pasture species. Small numbers of *Sitobion fragariae*

(Walker) were collected only from fescue and one *Metopolophium dirhodum* (Walker) was collected from phalaris.

Glasshouse tests showed that *M. dirhodum* and only some clones of *S. fragariae* (Guy et al. 1987) transmitted PAV viruses and mixed infections of PAV and RPV viruses. *R. padi* transmitted PAV, RPV, and mixed infections.

Higher levels of infection were detected in samples from the heavy soil site than in samples from the light soil site (Table 1). The most common type of infection was with PAV viruses (57%) followed by RPV (26%) and mixed infections (17%).

The highest levels of infection were detected in fescue (21%) and ryegrass (27%), and both of these species were predominantly infected with PAV viruses and with moderate levels of RPV viruses and mixed infections. The overall levels of infection were lower in cocksfoot (6%) and phalaris (7%) and both were predominantly infected with RPV viruses. There were moderate levels of PAV infections and low levels of mixed infections in these two species.

Discussion

Previous studies have concentrated on sampling different pastures of different ages (Catherall 1963; Guy et al. 1986; Latch 1977). However, this study shows that differences in age do not account for all the variation in virus incidence between pastures. Both of the Sandford pastures were sown on the same day and yet there were marked differences (Table 1) in the levels of infection at the two sites and in the levels and types of infection in the four species.

Table 1. The incidence of barley yellow dwarf viruses and aphids in pasture grasses at two sites, Sandford, Tasmania

Heavy soil site

Grass species	No. of samples infected with PAV-like	P+R	RPV-like	BYD totals	No. aphids collected *R. padi/S. fragariae*
Cocksfoot	8		36	44	11
Fescue	108	30	15	153	22/1
Phalaris	16	2	26	44	8
Ryegrass	78	28	23	129	9
Totals	210	60	100	370	50/1

Total no. of samples collected: 2100

Light soil site

	No. of samples infected with PAV	P+R	RPV	BYD totals	No. aphids collected *R. padi/S. fragariae*
Cocksfoot	1	1	17	19	5
Fescue	43	13	13	69	4/4
Phalaris	4		25	29	4/(1 Md)
Ryegrass	98	36	16	150	1
Totals	146	50	71	267	14/5

Total no. of samples collected: 2000

Md: *M. dirhodum*.

There was a broad correlation between the number of aphids collected and the levels of infection at the two sites. Generally, there were larger populations of aphids on the physically more stable, and less drought prone, heavy soil site (McQuillan et al. 1986). However, there was no obvious correlation between aphid number and levels of infection in the individual species, suggesting that there are marked differences in the susceptibility of the four species to PAV and RPV viruses.

This suggests that resistance to PAV viruses is a higher priority for fescue and ryegrass breeding programs and that resistance to RPV viruses may be more important for cocksfoot and phalaris breeding programs. Increased awareness of microclimate variations may be useful in identifying sites that are prone to infection with barley yellow dwarf viruses.

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Preliminary Reports on the Overwintering of *Rhopalosiphum padi* in Central Italy

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Abstract

The results of a preliminary investigation on the overwintering of an anholocyclic population of Rhopalosiphum padi *(L.) are presented and discussed. Sampling was carried out from November 1986 to April 1987 on cereal crops in the area of Lazio, Italy, where the typical primary host plant,* Prunus padus *L., is absent. The aphid was observed overwintering as virginoparae mainly on the aerial parts of the plants (leaf and stem bases) and only occasionally on the roots.*

Rhopalosiphum padi (L.) generally has a dioecious holocycle on *Prunus padus* L. as a primary host and a range of species of both cultivated and wild Gramineae as secondary hosts. In the areas where the primary host is absent, the aphid continues its life cycle on secondary hosts (Vidano 1959).

The biology of *R. padi* in central Italy, where *P. padus* is absent (Pignatti 1982), is unknown. To improve the knowledge of the life-cycle of the aphid in this area, a program of sampling in cereal crops in the area of Lazio was undertaken. Preliminary samplings on overwintering populations of *R. padi* were made. The aim of this study was to elucidate the phenology of the overwintering forms and note their location on the plant.

Methods

Sampling was carried out from November 1986 to April 1987 in the area of Bullicame near Viterbo (Lazio) on volunteers of the 1986 cereal crops, including both *Avena* spp. and barley (*Hordeum vulgare* L.) in two fields of approximately 0.25 ha. These fields are approximately 300 m above sea level and belong to the mesaxheric region, hypomesaxheric subregion under a temperate climate of the "A" type (coldest month's average temperature between 0 and 10°C; average yearly rainfall between 700 and 1000 mm; highest rainfall concentration in spring) (Tommaselli et al. 1973).

Samples were taken every 15 to 20 days. Each sample was collected after a visual observation of about 45 min. The samples were taken from throughout the field. To verify the preference of the aphid on the roots of cereals, a sample of 15 plants with the roots attached was collected from each field at each sampling time. The root system was examined in the laboratory. The instars of *R. padi* were identified according to Villanueva and Strong (1964).

Discussion

The aphids collected were both winged and wingless virginoparae as well as the four nymphal stages. (Table 1). Wingless virginoparae were present during the whole sampling period and their numbers varied little throughout the period. The number of winged virginoparae found progressively decreased and none was found from January until the end of the sampling period. The number of young instars diminished and almost disappeared during January and February, especially I, II, and IV stage nymphs.

All the forms were observed mainly on the epigeal organs of the plants (lower and upper face of the leaf blade, sheath, culm, and stem bases). No preferences were observed for the position of the individuals on the different plant parts. In a few cases only, the aphid was observed on the underground part of the plants (one individual of II young instar in December 1986, and one in January 1987).

Table 1. Numerical distribution of *R. padi* samples during November 1986-April 1987

	1986				1987					
	Nov.		Dec.		Jan.		Febr.		March	April
Forms	13	27	16	20	14	27	18	5	26	16
Wingless virginoparae	2	9	3	9	3	2	7	3	1	7
Winged virginoparae	10	7	1	4	–	–	–	–	–	–
Nymphs I stage	15	14	–	14	–	–	–	4		6
Young II stage	13	2	–	4	–	–	–	4	–	2
Young III stage	5	4	2	1	1	–	1	2	–	–
Young IV stage	5	3	4	5	–	–	2	7	–	4

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Level of Some Nutrients in Rice Giallume Virus Infected Plants

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Abstract

The content of eight elements (N, P, K, Ca, Mg, Zn, Fe, and Mn) was evaluated in straw of ripened rice plants that had been artificially inoculated with the rice giallume virus (RGV) using the aphid Rhopalosiphum padi *(L.) as vector at the first leaf stage and at tiller initiation. The results obtained in 11 Italian varieties pointed out a great difference both between the infected plants and the check and between the plants inoculated at different phenological stages. Since concentrations of the eight nutrients were very different in the healthy plants compared with the diseased ones, RGV is believed to affect the metabolism of these elements.*

In the 1970s, "giallume"—a rice disease caused by rice giallume virus (RGV), a nonspecific isolate of the barley yellow dwarf virus (BYDV) (Osler 1984; Belli et al. 1986)—became the most important disease of rice in Italy (Baldacci et al. 1970; Belli et al. 1974; Osler et al. 1977; Osler 1980; Moletti et al. 1989).

This study was carried out to measure the effects of virus infection on eight elements in rice plants.

Materials and Methods

The plant material used is reported by Moletti et al.(these Proceeding). The amount of potassium, calcium, magnesium, zinc, iron, and manganese content in the plant material was determined by atomic absorption spectrophotometry (Perkin Helmer mod. 560), after wet digestion of the plant material (1 g of straw with 5 ml of HNO_3, 65% + 2 ml of $HClO_4$, 70%). Nitrogen was determined according to Fossati and Mazzini (1979), phosphorus according to Yoshida et al. (1976).

Results

The results showed differences in the concentrations of the analyzed mineral nutrients between diseased and control plants, and between plants inoculated at the first leaf stage and those inoculated at tiller initiation (Table 1). For most nutrients the concentrations were related to the disease severity, which was greater in the plants inoculated at the younger growth stage (Moletti et al., these Proceedings). In plants inoculated at tiller initiation, except for magnesium and zinc, the nutrient concentrations were intermediate between those of the control and the first-leaf-inoculated plants.

For the 11 cultivars tested, the mean values of nitrogen, phosphorus, and manganese were, respectively, 112, 130, and 91% higher in the plants inoculated at the first-leaf stage, and 69, 60, and 51% higher in the plants inoculated at tiller initiation, than in the control. For potassium, calcium, and iron, in contrast, the values were, respectively, 24, 28, and 22% lower in the first-leaf-stage inoculated plants, and 12, 6 and 1%

Table 1. Mean dry matter yield and nitrogen, phosphorus, potassium, calcium, magnesium, zinc, iron, and manganese concentrations in straw of RGV artificially infected rice plants at two phenological stages (first leaf and tiller initiation) and in straw of control plants of 11 varieties

	Inoculation stage	Dry matter yield g/plant	Element concentrations[a] N ppm	P ppm	K ppm	Ca ppm	Mg ppm	Zn ppm	Fe ppm	Mn ppm
Mean	1st leaf	4.5	10481	2010	14360	3385	2412	68	142	889
	Tiller in.	6.9	8353	1398	16685	4414	2826	93	180	738
	Control	10.3	4954	875	18802	4706	2252	75	182	466

[a] Parts per million (ppm) on dry weight basis, mean of 3 replications.

lower in the plants inoculated at tiller initiation, than in the control. For magnesium and zinc the highest values were those of the plants inoculated at tiller initiation, 25% higher than in the control. For the first-leaf-stage inoculation, values were 7% higher for magnesium and 9% lower for zinc in the infected plants than in the control ones.

Discussion

In inoculated, BYDV susceptible wheat plants, metabolic disorders, such as massive carbohydrate accumulation, loss of chlorophyll and photosynthetic activity, and increased respiration rate, have been reported (Jensen 1972; Jensen and Van Sambeek 1972). Because of the intimate and extensive role of mineral nutrients in plant functions, some disorders can be expected. In this study a high negative correlation over all varieties was found between the dry matter yield per plant and the content of nitrogen ($r = -0.852^{***}$), phosphorus ($r = -0.830^{***}$), and to a lesser extent manganese ($r = -0.667^{***}$) and magnesium ($r = -0.373^{*}$). A positive correlation was found with potassium ($r = +0.821^{***}$), calcium ($r = +0.691^{***}$), and to a lesser extent with iron ($r = +0.410^{*}$). The mineral disorders found in the RGV infected rice plants are quite similar to the nutrient mineral disorders found in some plant stresses, i.e., water stress (Viets 1972). Orlob and Arny (1961) reported that BYDV alters the water relations of its hosts.

It would be interesting to test whether these disorders of the mineral metabolism could be reduced by changing the mineral nutrition of RGV infected plants and whether such treatment could alleviate the damage caused by the disease.

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Barley Yellow Dwarf Virus: Epidemiology and Risk Forecasting

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Abstract

The main criteria that should be taken into account to forecast the risks of barley yellow dwarf virus infection in cereals are discussed. These include the roles of vector reservoirs, suction traps, and field observations. Also presented are the details of a program to aid farmers in deciding when to apply chemicals. New possibilities for improving the forecasting of aphid flights, pinpointing high risk areas and telematic aids for rapid transmission of data, are discussed.

Aphid Host Plants (Reservoirs)

The importance of host plants (reservoirs) of *Rhopalosiphum padi* (L.), the main vector of barley yellow dwarf virus (BYDV) in autumn, was studied during the autumns of 1980 and 1981 in southwestern France (Table 1). In any area, the number of viruliferous aphids found on cereal crops depended primarily on the nature and abundance of reservoirs nearby before the cereals became prone to attack.

The proportion of the aphids being viruliferous can vary from place to place because it is possible to have a break in the vector/virus/host plant relationship. For example, during the drought of late summer 1985 in southwestern France, aphids leaving maize as the crop dried could not find other host plants (Gramineae) because small grains had not yet been seeded. As a result, cereals emerging after the cessation of aphid flights were not infected with BYDV. This also resulted in aphid populations being virtually free of BYDV the following autumn (1986).

Risk Forecasting

The risk of aphid infestation in an emerging cereal crop depends on:

- The location of other host plants in the cereal growing area;
- The wind direction during aphid flights (toward the cereal crop);
- The percentage of the total aphid population that is viruliferous. Aphid abundance is variable according to the quantity of nearby host plants.

Based on studies conducted between 1976 and 1982 with either natural or artificial infestations and under different environmental conditions, a model was established for decision making as to when to use chemical control against the aphid vectors, primarily *R. padi* (Bayon and Ayrault 1983).

This model was improved through further tests in 1983 and 1984. It was computerized as a program called "IPHYTO" and is now available to

Table 1. Synthesis of results obtained in autumn 1980 and 1981

Vector	Origin	Rate of virus infection	Consequences on cereal
Rhopalosiphum padi	Volunteer cereals	High	-short treatment delay -low threshold (10 to 15%)
	Maize and/or rye grass	Low	-long treatment delay -or high threshold

French farmers and technicians (Figure 1). It can be accessed through the French videotex system called "MINITEL" (Bayon and Ayrault 1987). This system allows immediate transmission of data to one or more recipients.

Other Studies in Progress

Other studies are in progress and looking at the role of several technological innovations that may help in predicting future BYDV incidence.

Aerial imagery—Imagery from the SPOT satellite can show reservoir fields that may be infested with aphids (Bayon *et al.* 1983). The SPOT system, conceived and designed by the French Centre National d'Etudes Spatiales (CNES), consists essentially of an earth observation satellite and ground stations that receive the data. The first satellite in this system uses three spectral bands in the visible and near-infrared portions of the spectrum. The color images have a

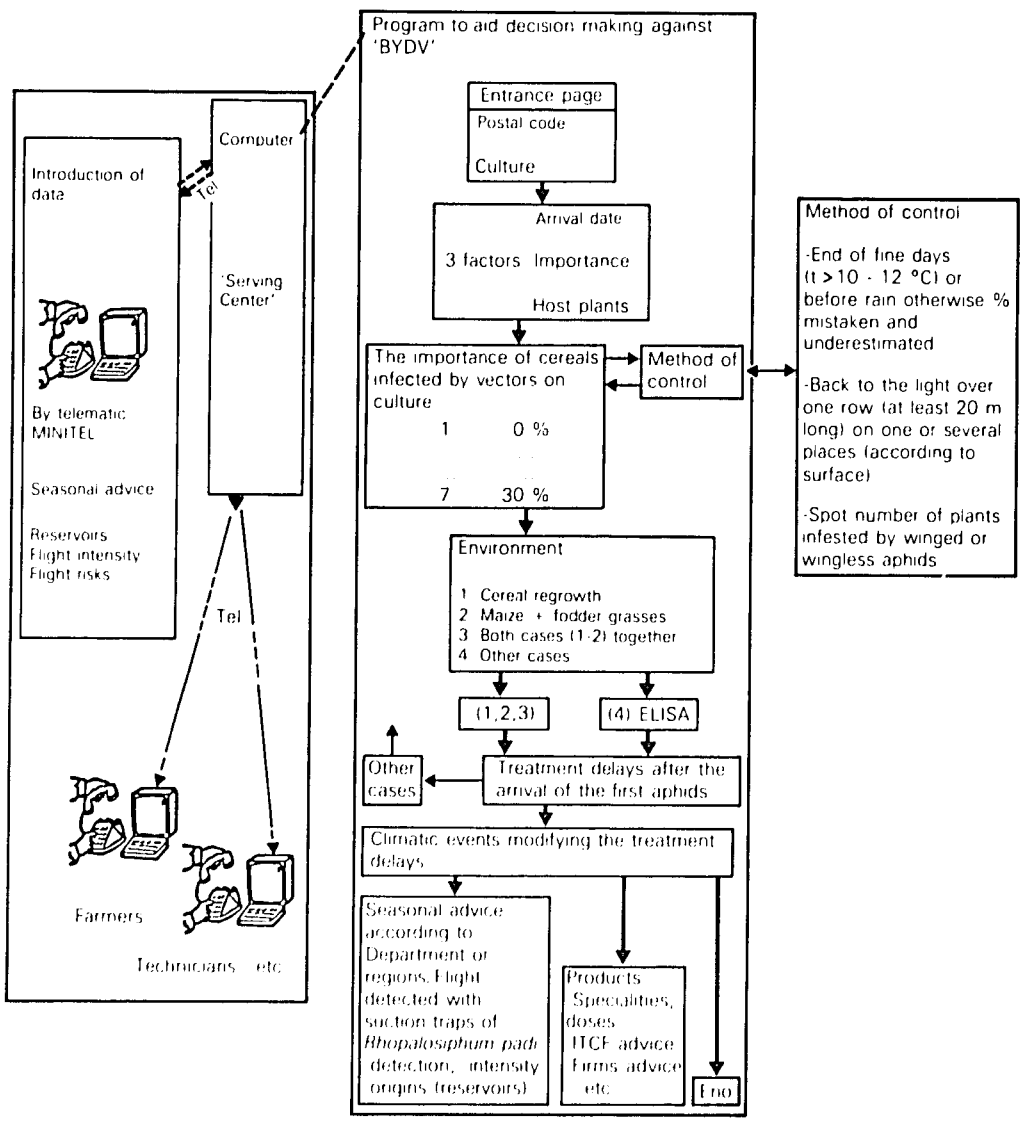
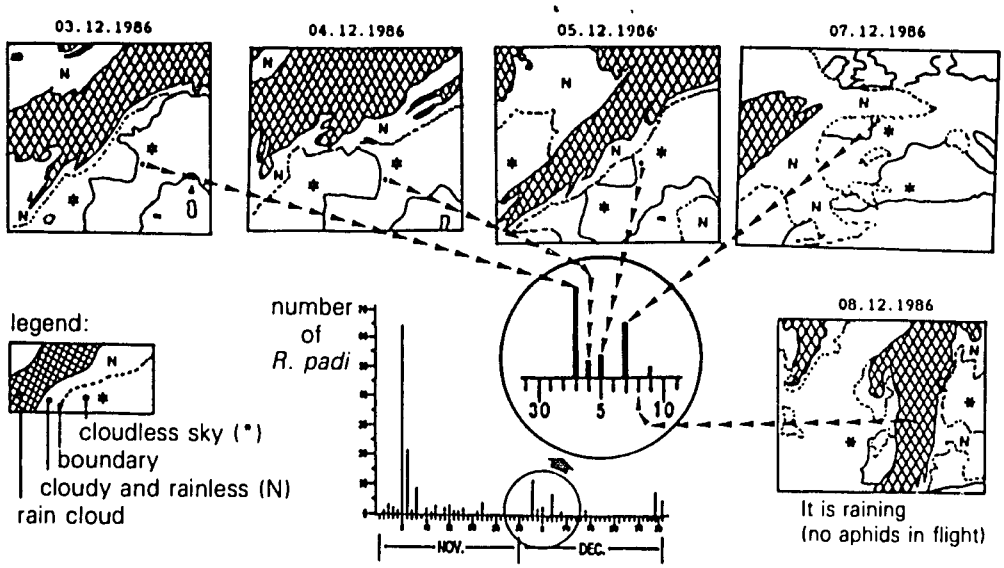


Figure 1. Decision making model for BYDV control.

ground resolution of 20 m, the black and white images, 10 m. This technology is suitable for observing the small fields common in many European countries.

Aphid flight forecasts—Imagery from the European Meteorological Satellite (METEOSAT) can be used to forecast risks of aphid flights based on aphids captured by suction traps in Tusson-Algre (Figure 2). The traps are part of a network termed AGRAPHID in France and EURAPHID in Europe.

Surface temperature charts—Surface temperature charts from the National Oceanographic and Atmospheric Administration (NOAA) satellite may be used to locate areas with risk of aphid flights. For example, there is great risk of



Example of telematic messages as a result of using this imagery

Message: (Angouleme, 4 Dec., 1986, 10:30 a.m.)

According to satellite imagery, high probability of flights today 4 Dec. and tomorrow 5 Dec... before arrival of rain.

Control if possible, sample in the afternoon (between 14 and 16 h), noting the percentage of plants infested by aphids.

Alert all observers possessing MINITEL.

Message: (Angouleme, 9 Dec. 1986, 9:00 a.m.)

Captures in suction- rap:

date	*R. padi*	*R. maidis*	*S. avenae*	*M. dirhodum*
8 Dec.	0	0	0	0

Remarks: The presence of flights on 4, 5 and especially 7 Dec. confirm our forecasts of 4 Dec. Probability of flights ... before arrival of rain in the morning 8 Dec.

Figure 2. Relationship between the European meteorological images returned by METEOSAT satellite and the captures of *R. padi* from Tusson-Algre's suction trap.

aphid flights in areas where aphids are active in crops and the surface temperatures range from 10 to 12°C (Figure 3a). Where surface temperatures range from 3 to 4°C (Figure 3b), there is little risk of aphid flights even if aphids are active in the crops. Data returned by the Advanced Very High-Resolution Radiometer (AVHRR) on board the NOAA satellite are processed by the CNES for subsequent mapping of the surface temperatures.

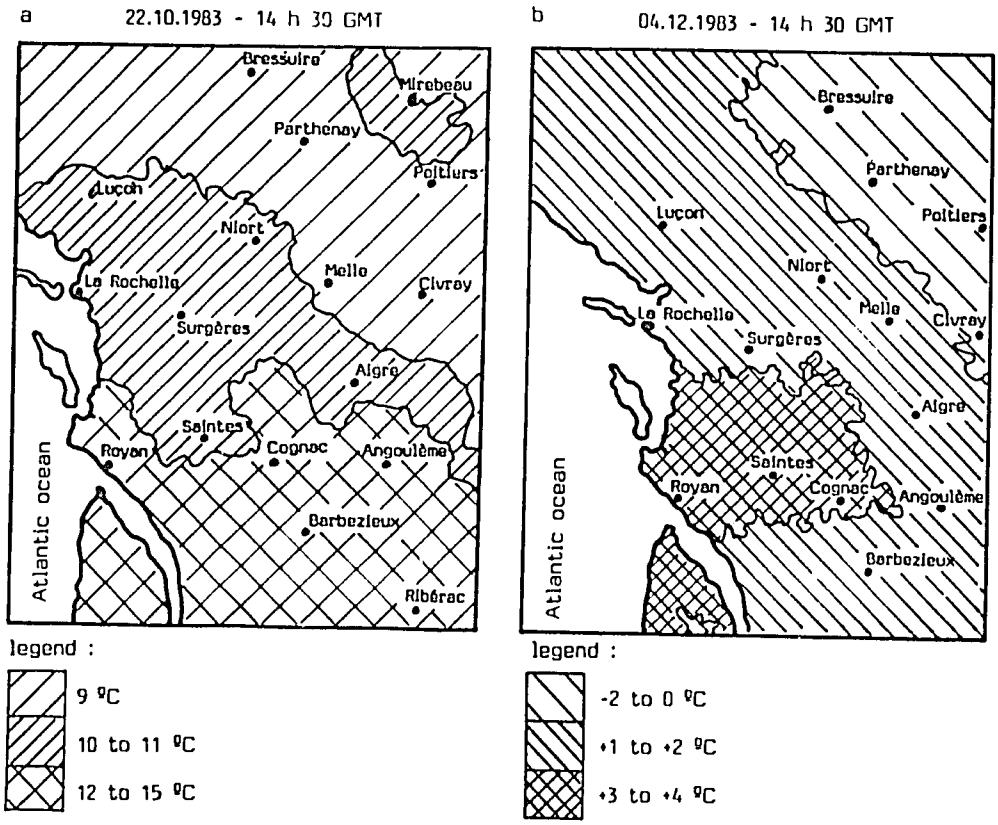


Figure 3. Surface temperature charts from Poitou-Charentes area extracted from NOAA satellite imagery processed by CNES - Toulouse, France.

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Ultrastructural Alterations in Maize Plants Exhibiting Different Foliar Symptoms of Barley Yellow Dwarf Virus

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Abstract

*A strain of barley yellow dwarf virus from maize (*Zea mays *L.), M-BYDV, causes different maize genotypes to react with distinct foliar symptoms, either reddening or fine chlorotic irregular spotting.* Rhopalosiphum padi *(L.) was used as a vector to transmit M-BYDV to two maize inbred lines and one hybrid, as well as to* Avena sativa *L. Symptom expression was compared with enzyme-linked immunosorbent assay (ELIZA). Whatever the source of inoculum, the symptoms were constant with genotype. Samples from infected leaf and root tissue from both healthy and infected plants were observed with an electron microscope. In all infected lines a very high number of large starch granules were observed in the chloroplasts of the bundle sheath cells. Other ultrastructure alterations observed depended on cultivar.*

While transmitting an isolate of barley yellow dwarf virus isolated from maize (*Zea mays* L.), M-BYDV, in Northern Italy with aphids, we observed that maize reacts to the virus with two distinct foliar symptoms according to the genotype: (1) reddening; (2) fine chlorotic irregular spots. The former has been widely demonstrated to be associated with M-BYDV (Osler et al. 1984; Osler et al. 1985a, b).

The aim of the present work was to demonstrate experimentally the relationship between M-BYDV infections and the appearance of fine chlorotic irregular spots noticed sometimes on maize and to compare the ultrastructural alterations in leaves showing reddening or fine chlorite spot type symptoms.

Materials and Methods

Transmission experiments—A series of experimental transmissions was carried out using apterous *Rhopalosiphum padi* (L.) from a healthy colony. For acquisition the aphids were fed on BYDV-infected maize inbred lines 33-16 and A619 and the hybrid Carnia. The acquisition feed was 48 hours at 20°C, in the greenhouse; the infected aphids were then transferred to *Avena (byzantina K. Koch) =sativa* L. and to the maize test plants mentioned above. The transmission procedures adopted were as described by Osler et al. (1985a).

A number of test plants were exposed to aphids from healthy *R. padi* colonies in order to check for possible direct damage from the aphids and to see if other viruses were transmitted to Carnia and A619. A transmission cycle was also completed using *R. padi*: from 33-16 to oats to Carnia to oats to A619 to oats to 33-16.

Electron Microscopy—In order to compare the ultrastructural alterations caused by BYDV, leaf and root portions were cut into 1 to 2 mm pieces, 8 to 9 weeks after inoculation, from both healthy and infected plants. The samples were fixed in phosphate buffered 3% glutaraldehyde, pH 6.9, for 2 hours, washed in buffer, post-fixed in phosphate 1% osmium tetroxide for 2 hours, dehydrated in ethanol, and embedded in Araldite. The leaf samples were selected in such a way as to follow the development of the symptoms in relation to the age of the tissues; therefore they were collected along the main axis of the leaves in the basal, median, and apical regions. Ultrathin sections were stained with lead citrate and examined in a Hitachi 300 electron microscope, at the accelerating voltage of 80kV.

Results and Discussion

These transmission experiments showed it was possible to transfer M-BYDV from the maize inbred lines 33-16 (leaf reddening) and A619 (chlorotic irregular spots), as well as from hybrid Carnia and

from oats, to 33-16, A619, Carnia, and oats. Using 10 exposed individuals of *R. padi* per plant, the average reactions of the inoculated plants—represented both as symptom expression and enzyme-linked immunosorbent assay (ELISA) positive answers—were, respectively, maize A619, 75 and 80%; maize Carnia, 11 and 83%; maize 33-16, 23 and 64%; oats, 98 and 98% (using 3 aphids per plant). . The shortest incubation period occurred in A619 (chlorotic spots) and the longest in 33-16 (leaf reddening). Whatever the source of inoculum, the symptoms induced on the various test plants were always reddening on 33-16 and oats and fine chlorotic spots on A619 and Carnia.

An abnormal accumulation of starch granules was observed in the chloroplasts of the bundle sheath cells of all tested plants, as described in previous research for the line 33-16 (Favali et al. 1984; Favali et al. 1985). We also observed the deposition of callose-like material around the pores of the sieve plate, both in the leaves and in the roots, which seemed to be a general event at the later stages of the infection. These phenomena were interpreted as general effects of the virus infection, responsible, at least in part, for the stunting of the plants. Several inclusion bodies, crystalline or amorphous, different in size and shape, were observed to be localized in the phloem cells, mainly in the line 33-16 (Figure 1). Some of these inclusions were observed only in the sieve tubes of 33-16 (Figure 2); they are identical to the structures seen in pangola stunt virus infected leaves (Giannotti and Milne 1977).

Figure 1. Maize line 33-16 infected by BYDV: several crystals and inclusions are visible in the phloem cells (x17,000).

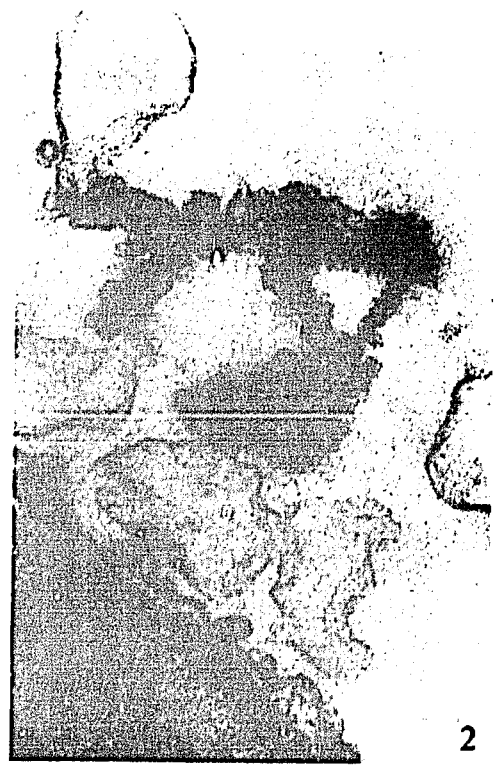

Figure 2. High magnification of the inclusions of Figure 1 (double arrow) (x68,000).

In the root cells of the inbred line A619, several ribosomes were visible in close proximity to particles showing a diameter of 60-80 nm with thin fibrous strands radiating into the cytoplasm (Figure 3). Similar particles were observed in the root cells of wheat. (Jordan and Cooper 1986).

In the hybrid Carnia, the most prominent alterations were the crystalline-like fibrils both in the leaf and in the root, close to the nucleolus (Figure 4, arrow). These results confirm those obtained during the electron microscopic observations of other lines (33-16, FR18, B84 and W64A) previously reported (Favali et al. 1985). It seems that alterations such as the thylakoid disorganization, the starch accumulation in the bundle sheath chloroplasts, and the obliteration of the pores of the sieve plates by callose, could be responsible, at least in part, for the stunting of the infected plants, while the peculiar alterations could be responsible for the different foliar symptoms. It seems that the genotype of each line of maize tested may interact with the BYDV to cause different alterations, some of them present in all the lines, others peculiar for each line.

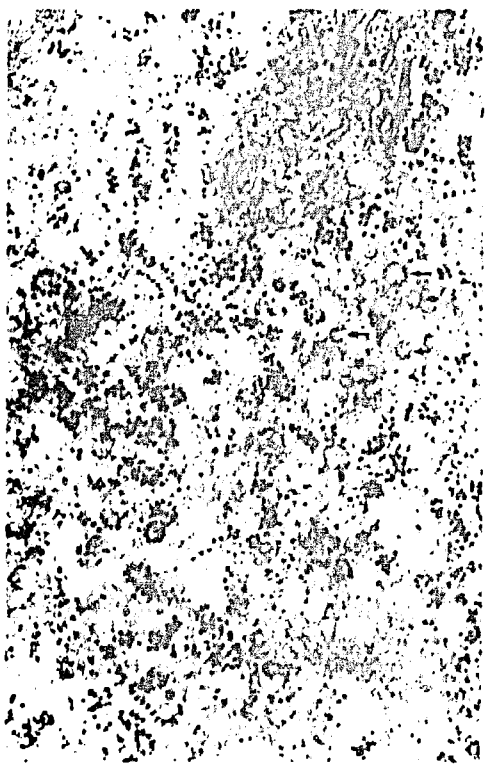

Figure 3. Maize line A619 infected by BYDV, particles (arrows) in the cytoplasm of root cells, showing thin fibrous strands radiating in the cytoplasm (x40,000).

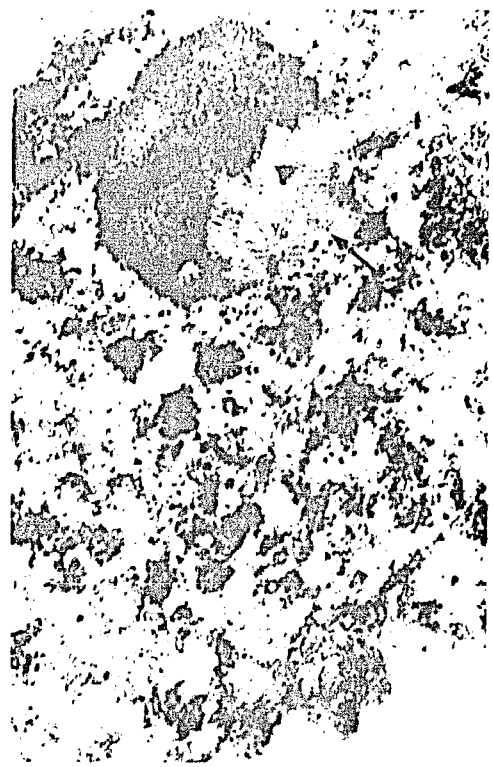

Figure 4. Maize hybrid Carnia infected by BYDV: note the crystalline-like fibrils (arrow) close to the nucleolus (x30,000).

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Cereal Aphid Captures in Lombardy

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Abstract
Data on captures with a Rothamsted suction trap of aphids infesting cereals in northern Italy are reported. Samples were taken daily from spring to autumn in the years 1983 to 1986. Fluctuations in the number of insects captured were observed.

The damage caused by aphids that vector cereal virus diseases is affected by the environment (Walters and Dixon 1983). This present work was carried out to ascertain which aphid vectors of barley yellow dwarf virus (BYDV) have the highest density in the central area of the Po Valley.

Materials and Methods
Various traps were tested for their ability to intercept or to attract flying aphids. These included sticky traps, yellow water trays, tungsten or UV lamps, fixed and movable nets, and suction traps (Taylor and Palmer 1972; Johnson 1950a, b; Taylor 1955, 1962). The Rothamsted type suction trap, which is used at many aphid survey stations throughout Europe (Patti 1985), as well as in Italy, proved to be the most reliable. A Rothamsted type suction trap was therefore set up in the Lombardy area to catch the aphid species that are possible vectors of BYDV. The area in which it was installed included crops of tomato (*Lycopersicon lycopersicum* (L.) Karsten), beetroot (*Beta vulgaris* L.), and cereals—barley (*Hordeum vulgare* L.), wheat (*Triticum aestivum* L.), and maize (*Zea mays* L.).

The trap was operated from 0900 to 1700 every day from May to December, 1983 to 1986.

The insects trapped were taken to the Institute of Entomology of the University of Milan and the different species separated. Attention was focused on the aphids that were possible vectors of BYDV.

Results
The most frequent aphids captured were *Rhopalosiphum padi* (L.), *R. maidis* (Fitch), *R. insertum* (Walker), and *Sitobion avenae* (Fabricius). Figures 1-4 show the captures on a species basis. Two peaks of captures were found, the first in May and June, the second in October and November. The results in 1983 were different from the 3 other years in which sampling was carried out. The reason for this could be either that the climatic factors that year were so different that the population peaks were different or that these results reflect some initial operating problems with the trap (Colombo and Limonta 1986).

In 1985 and 1986 there was a reduction in the number of *R. padi* caught when compared to 1984. However there were increases in the number of the other three species trapped.

The most widespread species is clearly *R. padi*, but *R. maidis*, *R. insertum*, and *S. avenae* can be very common in certain years. The large catches recorded in October and November suggest that late plantings of barley and wheat, so as to avoid this migration, would be useful, because seedling plants are the most susceptible to BYDV (Süss and Colombo 1985; Passerini and Coceano 1986).

Building on the results of this 4-year survey, a program is planned to achieve the following objectives:

- To elucidate the relations among infestation of BYDV caused by migrating aphids, the spread within these crops due to apterous migration, and the captures with the Rothamsted survey trap; and
- To establish the relationship between local climatic factors and the number of alate aphids captured.

The work is aimed at determining what conditions favor or reduce aphid presence in the central area of the Po Valley.

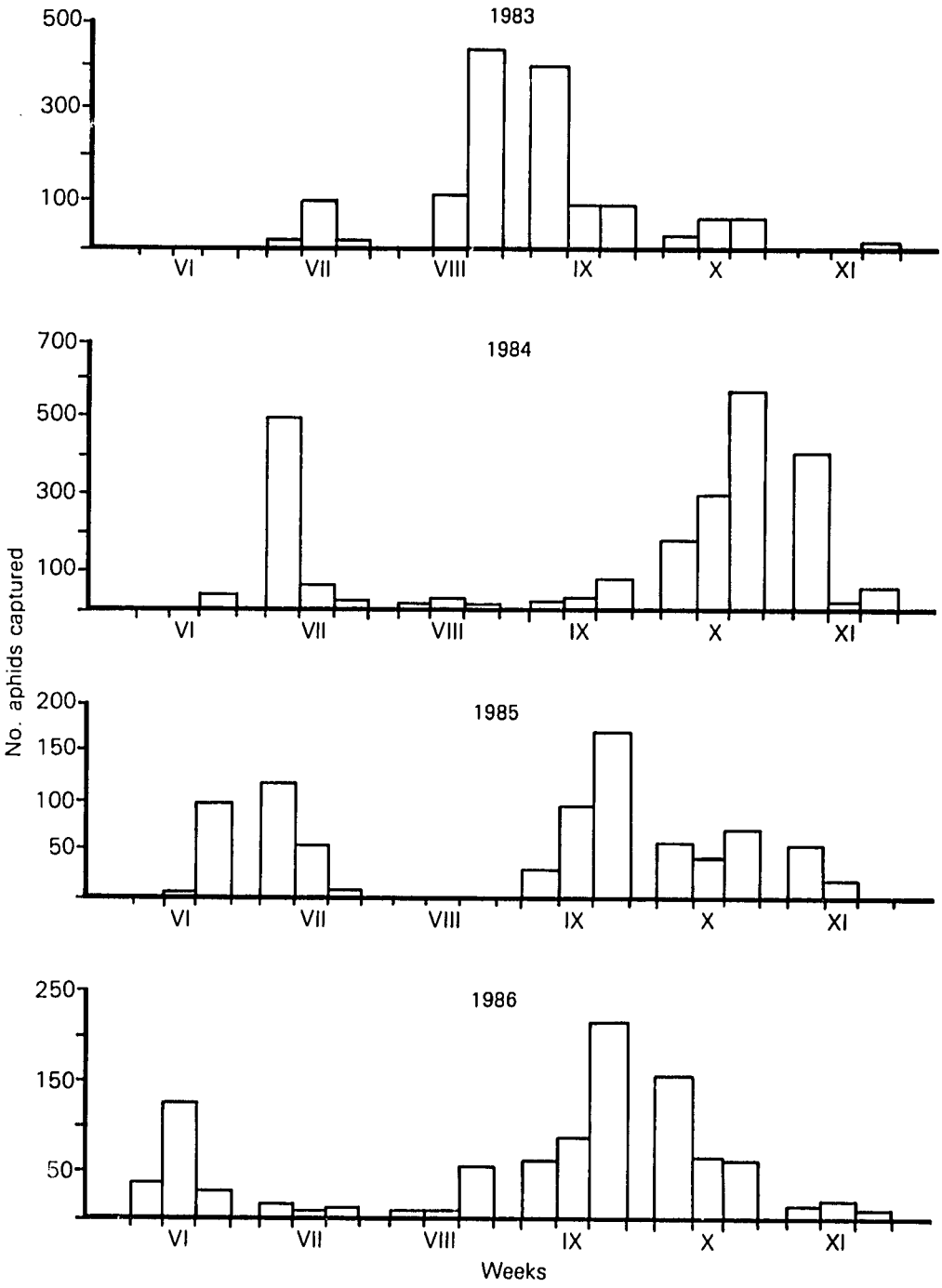

Figure 1. Weekly totals of *R. padi* caught in a Rothamsted type suction trap in Lombardy, 1983-1986.

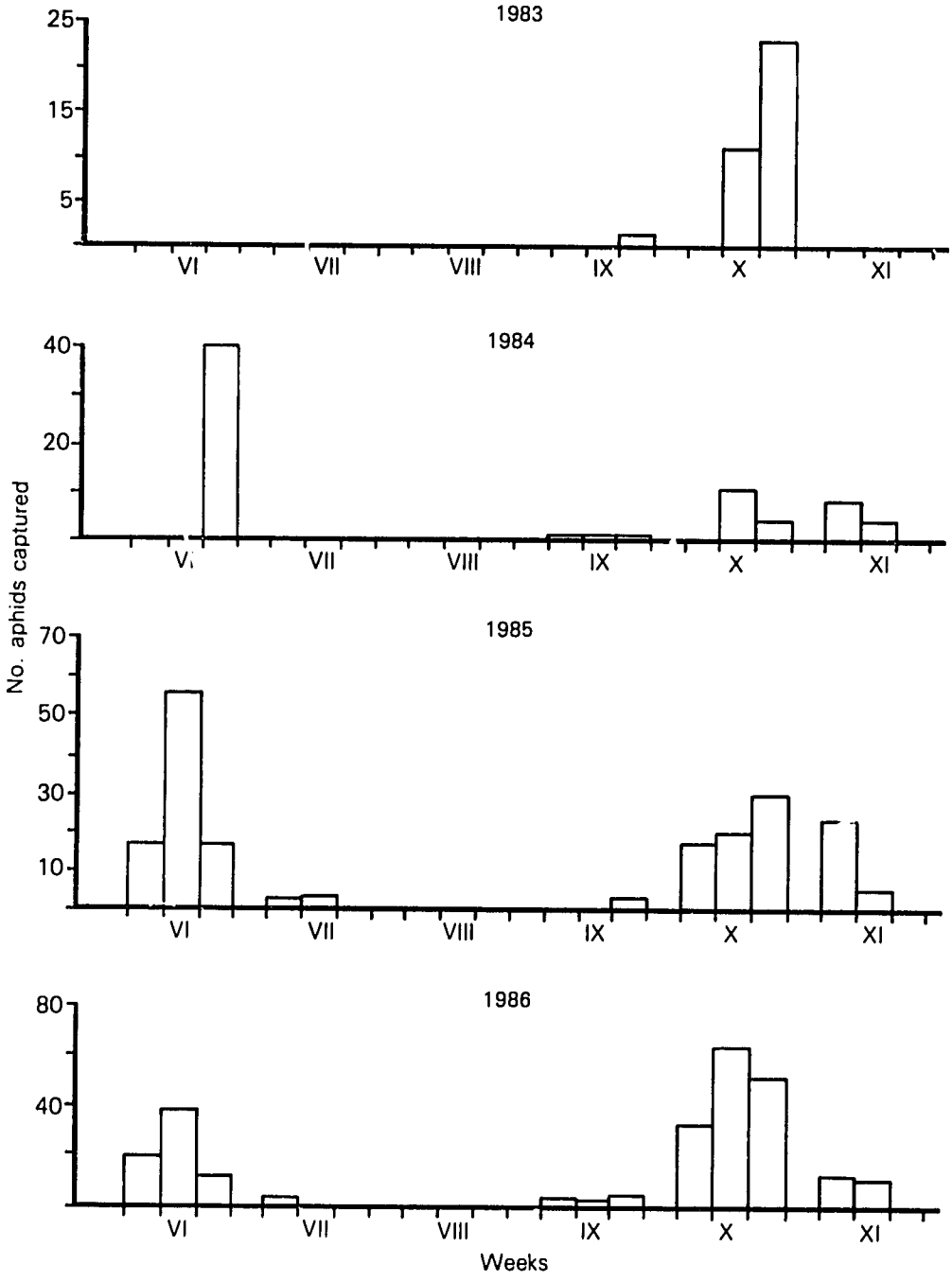

Figure 2. Weekly totals of *S. avenae* caught in a Rothamsted type suction trap in Lombardy, 1983-1986.

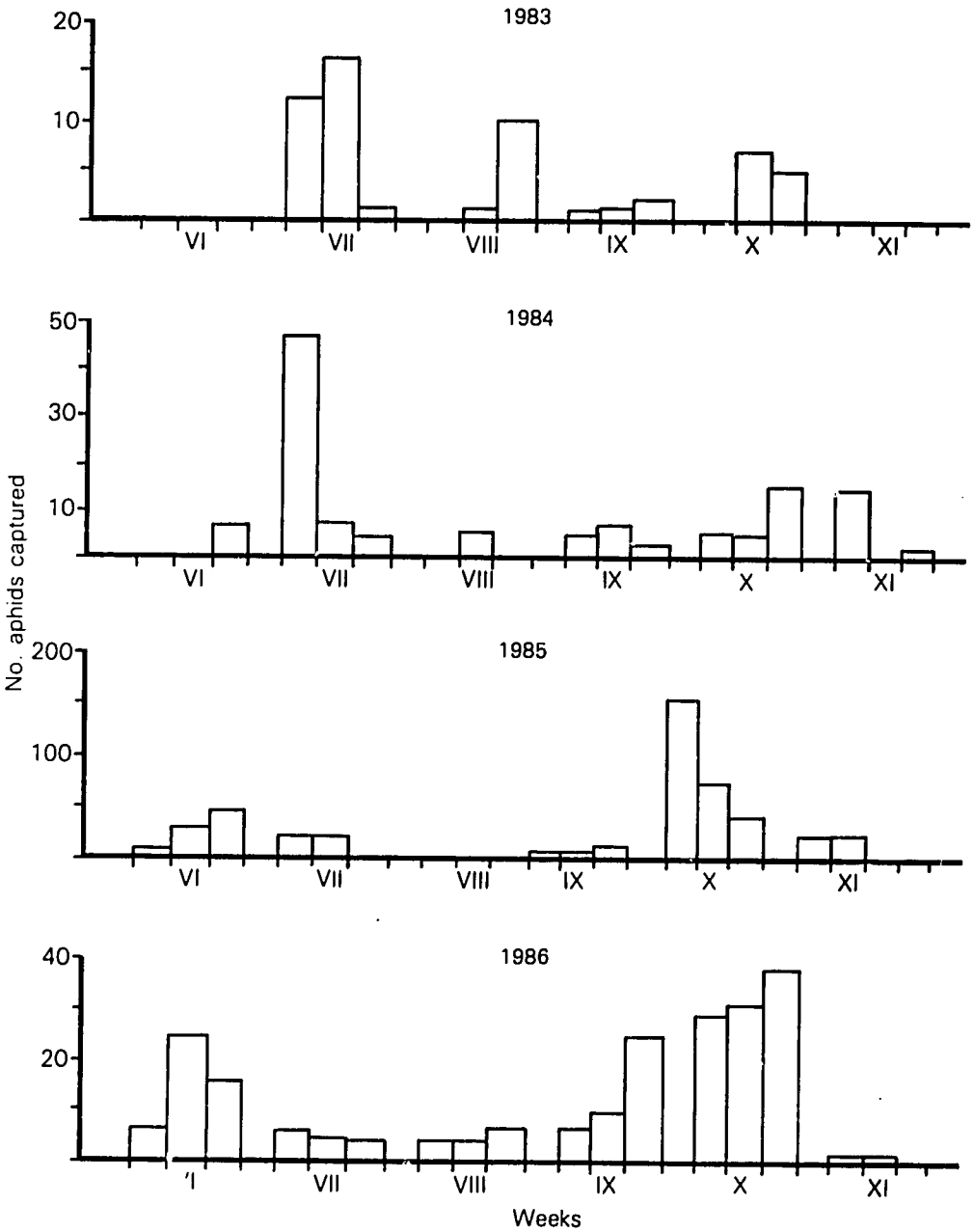

Figure 3. Weekly totals of *R. maidis* caught in a Rothamsted type suction trap in Lombardy, 1983-1986.

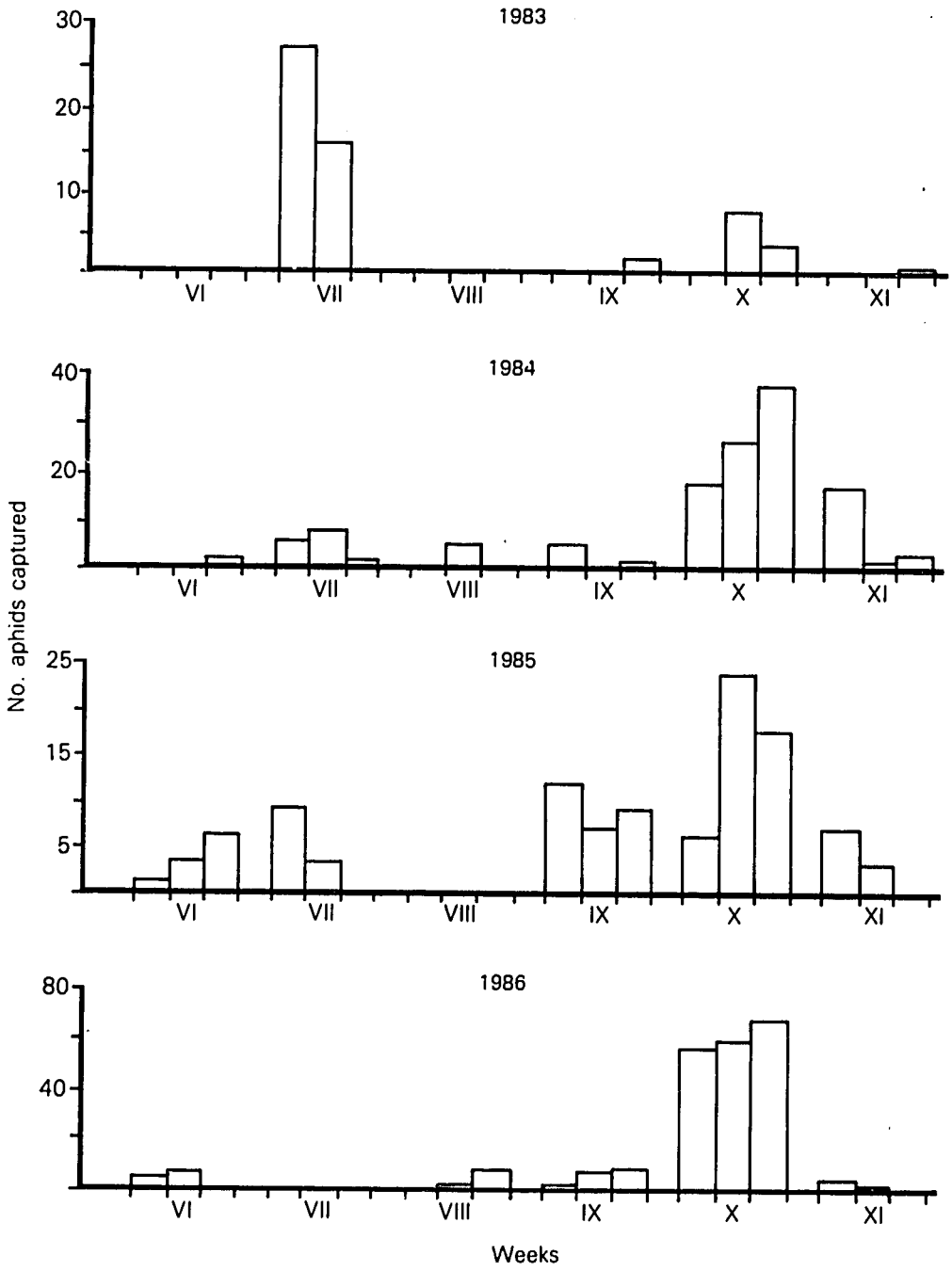

Figure 4. Weekly totals of *R. insertum* caught in a Rothamsted type suction trap in Lombardy, 1983-1986.

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Evaluation of Cereal Aphid Traps in Barley in the Valley of Mexico

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Abstract

*Yellow pan traps, horizontal mosaic green traps, fishing line traps, and yellow sticky traps were used to trap aphids in a barley (*Hordeum vulgare *L.) field in Mexico. The objective of this experiment was to determine the best method of assessing populations of aphids capable of transmitting barley yellow dwarf virus (BYDV). Based on the species caught by each trap, the fishing line and yellow sticky traps were the most effective for estimating BYDV vector populations. However, the yellow sticky traps damaged the aphids, so the fishing line traps are recommended for assessing BYDV vector populations in Mexico.*

Barley yellow dwarf virus (BYDV) is persistently transmitted by up to 23 aphid species (A'Brook 1981). The most important vectors are *Rhopalosiphum padi* (L.), *R. maidis* (Fitch), *Sitobion avenae* (Fabricius), *Schizaphis graminum* (Rondani), and *Metopolophium dirhodum (Walker).*

BYDV is present in Mexico (Navarro 1984), but little is known about the aphid species that transmit the virus or the variation in their numbers throughout the season. To identify the incidence of possible BYDV aphid vectors in the Valley of Mexico and the most effective ways of catching these aphid species, four types of traps were assessed.

Materials and Methods

On July 12, 1986, four types of traps for catching winged aphids were placed in a barley (*Hordeum vulgare L.)* field in Montecillos, Mexico:

- Yellow pan traps consisting of trays 30 x 40 cm and 8 cm deep, painted yellow and filled with water (Rodriguez and Rodriguez 1981)
- Horizontal mosaic green traps consisting of transparent plastic containers 11 x 11 cm and 4 cm deep with an ermine lime glazed tile placed in the bottom, and filled with water (Irwin 1980 and Irwin and Shultz 1981)
- Fishing line traps made with an aluminum frame 30 cm x 30 cm strung vertically with transparent nylon fishing line (7 kg breaking strain) at 3 mm intervals and sprayed with petroleum jelly (Labonne et al. 1983)
- Yellow sticky traps consisting of a piece of yellow cardboard 20 x 15 cm with a sticky surface.

All traps were located at crop height and spaced 10 m apart. The yellow pan and horizontal mosaic green traps were positioned horizontally, while the fishing line traps and the yellow sticky traps were placed vertically.

The height of the traps was changed throughout the crop growing season so that the base of all traps stayed approximately level with the top of the barley canopy.

Aphids trapped in the yellow pan, horizontal mosaic green traps, and the fishing line traps were counted twice each week and those on the yellow sticky traps were counted once each week.

Four replicates of the yellow pan trap, the horizontal mosaic green trap, and the fishing line trap were used, but only two yellow sticky traps were available.

The traps were placed in the field on July 12 and the first aphids were caught on July 19.

Results and Discussion

The yellow pan caught significantly more individuals and species of aphids (1286 individuals of 30 species) than the other types of traps (Table 1). This trap does not give an estimate of the population of potential BYDV vectors present in the crop, however, since the captured species (principally *Aphis* spp., *Hayhurstia atriplicis* L., and *Capitophorus hippophaes* (Walker)) have never been reported as BYDV vectors, nor as feeding on cereals. These species were probably captured because of an attraction to the yellow color of the trap (Alverson et al. 1977).

The fishing line and yellow sticky traps caught a significantly higher number of *R. padi* and *R. maidis* (Table 1), which are potential BYDV vectors.

Another potential BYDV vector, *M. dirhodum*, was captured in greatest quantity by the yellow pan traps (Table 1); however, there was no significant difference between the number caught in yellow pan traps and the number caught on the yellow sticky traps. Again, this species is probably attracted tc yellow. However, the number caught in the yellow sticky trap was not significantly higher than the number caught in the horizontal mosaic green trap or the fishing line trap.

The greatest number of *S. avenae*, another potential vector species, was trapped by the yellow pans. However the number caught in the yellow pans was not significantly different from the number caught in the other traps.

The largest number of aphids captured was during the period between late August and late September (Table 2), the period when barley crops usually mature. Because large numbers of aphids arrive quite late in the crop's growing cycle, damage from BYDV should not be severe for crops grown at the normal time. For late-sown crops, damage could be more severe due to the larger number of aphids present when the crop is susceptible.

Based on the species caught by each trap, the fishing line and yellow sticky traps are most effective for estimating potential BYDV vector populations. The yellow sticky traps, however, damage the captured aphids, sometimes making identification difficult. The fishing line traps, besides being easy to use and economical to build, do not damage the captured aphids and are recommended for assessing population sizes of BYDV vectors in Mexico.

The horizontal mosaic green traps caught very few aphids. This trap was originally designed for use in soybeans to obtain absolute records of aphids alighting on the soybean canopy. It was designed to have a similar reflective spectrogram to young soybean leaves of the cultivar Williams. The low catches recorded in barley could mean that the reflectance of this trap is different from that of barley, and so aphids may avoid this trap. Alternatively the number of aphids that actually land on the canopy is far smaller than the number that is in the air above the canopy. An analysis of the aphids captured per cm^2 in each trap will also be done.

Table 1. Common cereal aphids captured by four trap types

Trap type	Total aphids	*R. padi*	*R. maidis*	*M. dirhodum*	*S. avenae*
Yellow pan	1285.7a	13.7b	1.5c	34.7a	11.2a
Horizontal mosaic green	30.3b	1.2b	0.5c	3.7b	2.0a
Fishing line	239.0b	99.2a	38.7b	9.7b	4.7a
Yellow sticky	290.5b	100.5a	71.0a	18.0ab	7.5a

Numbers followed by the same letter are not significantly different from each other at the 5% level.

Table 2. Mean numbers of aphids that are potential vectors of barley yellow dwarf virus trapped by different trap types in a barley field in Montecillos, Mexico

	1st Month 19 July-18 August	2nd Month 19 August-19 Sept.	3rd Month 19 Sept.-11 Oct.
		Yellow Pan—mean 4 traps	
R. padi	1.00	12.25	1.50
R. maidis	0.50	0.75	0.25
M. dirhodum	1.25	33.25	0.25
S. avenae	0.50	9.50	1.25
		Horizontal mosaic green—mean 4 traps	
R. padi	0.75	0.50	0.00
R. maidis	0.25	0.25	0.00
M. dirhodum	1.00	2.75	0.00
S. avenae	0.50	1.00	0.25
		Fishing line trap—mean 4 traps	
R. padi	17.75	99.50	2.25
R. maidis	23.25	16.00	1.25
M. dirhodum	0.75	8.50	0.50
S. avenae	0.50	4.00	0.25
		Yellow sticky trap—mean 2 traps	
R. padi	17.5	73.5	10.5
R, maidis	41.0	18.0	12.0
M dirhodum	1.5	15.0	1.5
S. avenae	1.5	3.5	4.0

A problem with the design of this study was that the traps were positioned too close together (10 m apart) and so the yellow traps could have attracted aphids away from the other traps.

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Distinct Reactions of Maize to Barley Yellow Dwarf Virus and Confusing Foliar Symptoms

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Abstract

*The most common symptoms occurring on over 1,000 maize (*Zea mays *L.) genotypes due both to natural and experimental barley yellow dwarf virus (BYDV) infections were leaf reddening on the tips of the basal leaves and longitudinal stripes. Fine chlorotic spots and broken chlorotic stripes were also observed. Similar discolorations were induced on the younger leaves by phosphorus deficiency. Frost induces a transient reddening on young virus-free maize plants, but the experimental induction of such damage to maize has not yet been successful. Leafhoppers,* Zyginidia pullula *Boheman, cause chlorotic spots on maize, also, but the distribution on the leaves is different. Leaf malformations observed in 9 genotypes were not correlated with BYDV infections.*

Maize (*Zea mays* L.) has been recognized as a host of barley yellow dwarf virus (BYDV) either in the screenhouse, after artificial inoculations, or in the field (Panayotou 1977; Stoner 1977; Carroll 1984; Brown et al. 1984; Pearson and Robb 1984; Loi et al. 1985). The symptoms in BYDV-infected maize plants differ according to genotype, virus strain, and environmental conditions. The most common symptoms are: leaf reddening; interveinal flecking, streaking, or fine chlorotic irregular spots; leaf malformations; necroses; and stunting of the plants (Panayotou 1977; Stoner 1977; Belli et al. 1980; Signoret and Alliot 1981; Eweida et al. 1983; Brown et al. 1984; Favali et al., in press; Loi et al. 1985; Osler et al. 1985).

Other causes, i.e., frost, nutrient deficiency, and insect damage, can induce similar symptoms to barley yellow dwarf (BYD) in maize (Bertolini et al. 1982; Biondi et al. 1983; Pearson and Robb 1984). This paper examines the reactions of maize to different causes, artificially induced or observed.

Barley Yellow Dwarf Symptoms

During the years from 1982 to 1987, 1,036 maize genotypes were exposed to natural BYDV infections (Loi et al. 1986) or inoculated artificially (Loi et al. 1985) by using a PAV-like isolate of BYDV from maize (M-BYDV) and the aphid vector *Rhopalosiphum padi* (L.). The most common symptoms observed were uniform leaf reddening on the tips of the basal leaves and longitudinal stripes (Osler et al. 1985). Fine chlorotic irregular spots and broken stripes were found in leaves of maize plants A619, F_7, and Carnia (Favali, these Proceedings) grown in the greenhouse, in the screenhouse, and in the open field.

Inbred line A619 reacts to the infection only with fine chlorotic spots, while the lines F_7 (kindly supplied by Prof. P.A. Signoret, France) and Carnia develop chlorotic spots (6 to 10 days after infection) followed by leaf reddening (20 to 30 days after inoculation).

The yellowing on the tips of basal leaves of maize sometimes observed in the field has not been proved to be related to BYDV infections.

Confusing Foliar Symptoms

Frost

Under natural conditions in northern Italy, several maize inbred lines (W153R, CM105, H96, Mo17) are known to be sensitive to frost (Bertolini et al. 1982; C. Lorenzoni and M. Snidaro, personal communication). In Friuli early reddening has been frequently observed in the field in maize inbred lines that have not been infested by aphids. The typical reddening was generally accompanied by leaf twisting and stunting of the plant. Attempts were made to artificially induce leaf reddening in plants of several maize lines by frost treatment. Temperatures in the screenhouse ranged from 3 to 13°C (alternating), in day-night lighting conditions.

Leaf chlorosis, necroses, and stunting of the treated plants were obtained, but leaf reddening similar to that naturally occurring in the field was not induced.

Nutrient deficiency
The maize inbred line 33-16 grown in pots in the greenhouse may show leaf reddening in plants not infected with BYDV. The distribution of the leaf discoloration is quite similar to that observed in BYDV-infected plants, but the apical leaves are those affected. Therefore, it is possible to distinguish reddening induced by phosphorus deficiency in maize plants and symptoms of BYDV even when they occur in the same plant. Physiological reddening can be prevented or delayed by growing virus-free maize plants in larger pots.

Insect injury
Chlorotic spots on maize leaves are common, mostly in July and August, due to feeding of the leafhopper (*Zyginidia pullula* Boheman, Vidano 1981). The symptoms are similar to those attributed to BYD, but the distribution on the leaves is different. The aphid species that are potential vectors of BYDV do not induce reddening on maize, at least in normal greenhouse conditions.

Leaf malformations
Leaves showing three apices have been observed from time to time in 9 different maize genotypes both inoculated or uninoculated with BYDV. This symptom has been noticed in plants grown in the greenhouse, in the screenhouse, and in the field. In the screenhouse the malformation was detected in 64 out of 474 (14%) maize plants not infected with BYDV and in 64 out of 425 (15%) BYDV-infected plants.

The negative results obtained from enzyme-linked immunosorbent assay (ELISA) of 13 uninoculated maize plants of four different lines showing leaf malformations in an insect-proof greenhouse suggest that this symptom is not caused by BYD.

Discussion
This analysis shows that maize can react to different stresses with similar symptoms and, on the other hand, to the same causal agent with different types of symptoms. Leaf reddening, for example, is induced by BYDV, P deficiency, and frost. It has been reported to be caused by herbicides, broken midrib, and by infectious agents such as maize chlorotic dwarf and stunt as well, but these agents are not known in Italy.

Diagnosis based on the observed symptoms has to be considered carefully, especially in the field. Aphid transmissions, serology, and electron microscopy have to be used as diagnostic confirmation for BYDV. The problem is more complicated when different causes contribute to the syndrome, as in the case of BYDV and/or P deficiency, and frost. This work will be continued in order to study in depth the reactions of maize to different single and combined causes.

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IV. Control

A Review of Barley Yellow Dwarf Virus Grain Yield Losses

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Abstract

*Studies have been conducted worldwide to document the extent of plant damage caused by BYDV. Its impact on wheat (*Triticum aestivum *L.), barley (*Hordeum vulgare *L.), and oats (*Avena sativa *L.) is variable, depending upon such factors as aphid vector species and infectivity, virus isolate(s), and plant stage infected. A summary of the grain yield losses from selected studies in Europe, Canada, USA, Ecuador, and Australia is provided. Included is information on vector species, artificial inoculation, natural infestation/infection, crop type, and growth stage infected, based principally on published studies from field and greenhouse experimentation.*

Studies have been conducted worldwide on small grains to demonstrate the extent of plant damage caused by barley yellow dwarf virus (BYDV). Crop height, heads per plant, head size, floret fertility, kernel number, kernel size, grain quality, hardiness, and grain yield are among characters that may be affected by BYDV. In this review, only the virus impact on grain yield is summarized; a complete review of affected plant components is under preparation and will be published separately.

The literature is replete with reports on cereal aphid-grain crop relationships, but only reports that addressed both aphid-virus and grain yields are included here. For comparison purposes, yield data (g/plant, kg/ha, kernel wt./plot, etc.) from virus infected plots were standardized to a "percentage of the uninfected." The summarized yield losses are based largely on replicated trials of infected and uninfected plants of wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), and oats (*Avena sativa* L.) from research conducted in Europe, Canada, Ecuador, USA, and Australia.

In a majority of the published studies, virus infection in trial plots was assured by inoculation using cultured viruliferous aphids. Although the specific virus isolates under study were not always specified, the aphid vectors were, which suggests indirectly what isolates were involved. *Rhopalosiphum padi* (L.) was the vector species most often used.

Yield loss percentages, based on artificial inoculation of wheat, barley, and oats, are presented in Tables 1, 2, and 3 for field studies, and in Table 4 for greenhouse studies. The results are arranged according to the initial plant stage infected. Based on overall averages, seedling infection caused the greatest yield reductions, while stem extension, pre-boot, and boot stage infections caused the least. Although there was some commonality among the studies reviewed, there was also considerable variation, not only between, but also within studies. The range in yields for a given growth stage (single site year) was probably influenced most by plant genotype, whereas differences between sites, site years, or separate studies, involved not only genotype, but also virus isolates and growing conditions. On a percentage basis, overall yield losses were greater in oats than either barley or wheat; barley tended to be the second most injured crop.

The results in Tables 1 to 4 represent yield losses associated with BYDV transmitted by *R. padi*. Table 5 presents yield losses associated with BYDV transmitted by *R. maidis* (Fitch) and *Sitobion avenae* (Fabricius). In all of these studies, virus infection occurred from introduced viruliferous aphids. With the artificial inoculation, yield reductions should be considered as worst case situations, since essentially every aphid was infective and every plant was infected. Under natural conditions, it is not likely that every emigrant would carry virus or that every plant in a field

Table 1. Barley yellow dwarf virus effects on grain yield in wheat (*T. aestivum*) resulting from artificial introduction of viruliferous *R. padi* into field plots

Country	Crop yr	Plant stage infected	No. lines/ varieties eval.	% Yield reduction from uninfected Range	x	Reference
Australia	76/78	Seedling	1	24.7 - 56.9	45.6	Smith & Sward 1982
England	64/65	Seedling	2	21.9 - 51.9	36.6[a]	Doodson & Saunders 1970b
England	66	Seedling	2	9.1 - 37.1	23.1[a]	Doodson & Saunders 1970b
Finland	62	Seedling	1		43.7	Bremer 1965
USA, Ill.	78/79	Seedling	288		74.2	Cisar et al. 1982
USA, Ind.	77/78	Seedling	7	27.0 - 80.0	57.1	Carrigan et al. 1981
USA, Ind.	78/79	Seedling	6	24.0 - 35.0	32.0[a]	Carrigan et al. 1981
USA, Kan	64/65	Seedling	12	26.1 - 51.6	42.6	Palmer & Sill 1966
USA, S.D.	63/64	Seedling	3	75.4 - 87.4	81.0	Fitzgerald & Stoner 1967
USA, S.D.	66	Seedling	10	47.3 - 76.3	60.8	Jensen et al. 1971
			332		49.7	
England	57	E. Tillering	2	-5.7 - 8.3	1.3	Watson & Mulligan 1960
England	58	E. Tillering	1	40.0 - 64.1	52.0	Watson & Mulligan 1960
England	57	Tillering	2	-8.6 - 5.6	0.7	Watson & Mulligan 1960
England	58	Tillering	1	10.0 - 43.6	31.8	Watson & Mulligan 1960
USA, S.D.	63/64	Tillering[b]	3	57.5 - 65.6	62.2	Fitzgerald & Stoner 1967
USA, Kan.	64/65	Tillering[b]	12	-12.6 - 34.7	14.4	Palmer & Sill 1966
USA, Ill.	78/79	Tillering[b]	288		47.5	Cisar et al. 1982
USA, Ind.	78/79	Tillering[b]	6	16.0 - 41.0	26.0	Carrigan et al. 1981
			315		29.3	
Australia	76/78	Stem ext.	1	0.7 - 5.9	2.5	Smith & Sward 1982
England	65/66	Stem ext.	2	23.0 - 19.3	21.1[a]	Doodson & Saunders 1970b
England	68/69	Stem ext.	4	28.3 - 13.8	22.5[a]	Doodson & Saunders 1970b
England	68/69	Pre-boot	4	6.1 - 18.[illegible]	11.6[a]	Doodson & Saunders 1970b
England	69	Pre-boot	4	9.8 - 41.0	17.9[a]	Doodson & Saunders 1970b
England	60	Pre-boot	1		9.8[a]	Doodson & Saunders 1970b
			16		14.2	

[a] Values estimated from histograms of healthy and infected cultivars.

[b] Stage estimated; crop planted in the fall, infested in the spring.

E. = Early.

would be infected. Thus, expected yield losses from natural infection are not, on the average, likely to be as severe as in the studies above. Available data on natural BYDV-caused grain losses are presented in Table 6. In these studies, natural infection caused less average yield loss in the wheat, barley, and oats than artificial infection if in the latter the virus was introduced in the seedling or tillering stage. For the Washington State trials, losses were associated with seedling infection of late August to early September plantings.

There has been little question that BYDV causes losses in small grains. The review demonstrates this through documented reference of virus-caused grain losses worldwide.

Table 2. Barley yellow dwarf virus effects on grain yield in barley (*H. vulgare*) resulting from artificial introduction of viruliferous *R. padi* into field plots

Country	Crop yr	Plant stage infected	No. lines/ varieties eval.	% Yield reduction from uninfected Range	x	Reference
Can., Man.	–	Seedling	2	12.0 - 91.6	50.8	Comeau & Dubuc 1978
Can., Ont.	58	Seedling	2	-28.4 - 58.1	14.8	Slykhuis et al. 1959
England	65/66	Seedling	2	31.3 - 37.5	34.4[a]	Doodson & Saunders 1970b
England	65	Seedling	4	42.9 - 95.7	74.1[a]	Doodson & Saunders 1970b
England	–	Seedling	4	64.8 - 86.3	72.8	Boulton & Catherall 1980
Finland	62	Seedling	1		51.5	Bremer 1965
USA, Minn.	64	Seedling	1	91.2 - 95.2	93.2	Banttari 1965
USA, Mo.	78/89	Seedling	8	0.0 - 99.0	44.7[a]	Grafton et al. 1982
			25		54.5	
England	67/68	E. tillering	4	13.3 - 49.1	34.8[a]	Doodson & Saunders 1970b
England	66	E. tillering	2	30.1 - 56.5	43.3[a]	Doodson & Saunders 1970b
England	57	E. tillering	2	2.6 - 17.5	10.0	Watson & Mulligan 1960
England	58	E. tillering	1	16.7 - 34.1	38.9	Watson & Mulligan 1960
Can., Ont.	58	Tillering	2	14.0 - 19.8	16.9	Slykhuis et al. 1959
England	57	Tillering	2	-5.1 - 15.0	0.6	Watson & Mulligan 1960
England	57	Tillering	1	0.0 - 33.3	13.9	Watson & Mulligan 1960
			14		22.6	
England	65/66	Stem ext.	2	9.0 - 29.6	19.3[a]	Doodson & Saunders 1970b
England	67/68	Stem ext.	4	0.0 - 21.3	12.8[a]	Doodson & Saunders 1970b
England	68/69	Stem ext.	3	22.0 - 32.5	27.7[a]	Doodson & Saunders 1970b
England	65	Stem ext.	5	25.6 - 40.4	33.2[a]	Doodson & Saunders 1970b
England	69	Stem ext.	2	28.8 - 41.3	35.0[a]	Doodson & Saunders 1970b
England	68	Pre-boot	4	2.8 - 15.0	11.1[a]	Doodson & Saunders 1970b
England	68/69	Pre-boot	3	4.6 - 13.1	9.2[a]	Doodson & Saunders 1970b
Can., Ont.	58	Boot	2	-12.3 - 15.7	1.7	Slykhuis et al. 1959
			25		18.7	

[a] Values estimated from histograms of healthy and infected cultivars.

E. = Early.

Table 3. Barley yellow dwarf virus effects on grain yield in oats (*A. sativa*) resulting from artificial introduction of viruliferous *R. padi* into field plots

Country	Crop yr	Plant stage infected	No. lines/ varieties eval.	% Yield reduction from uninfected Range	x	Reference
Can., Man.	–	Seedling	5	30.0 - 100.0	54.7	Comeau & Dubuc 1978
Can., Ont.	58	Seedling	2	72.6 - 74.1	73.3	Slykhuis et al. 1959
England	65/66	Seedling	2	50.0 - 81.3	65.6[a]	Doodson & Saunders 1970b
England	65	Seedling	2	77.8 - 97.1	87.4[a]	Doodson & Saunders 1970b
Finland	62	Seedling	1		80.6	Bremer 1965
USA, Ill.	56	Seedling	3	75.8 - 94.4	88.2	Endo 1957
			15		75.0	
England	67/68	E. tillering	4	37.4 - 59.1	47.7[a]	Doodson & Saunders 1970b
England	66	E. tillering	2	42.3 - 63.5	52.9[a]	Doodson & Saunders 1970b
England	57	E. tillering	2	19.2 - 35.3	27.2	Watson & Mulligan 1960
England	58	E. tillering	1	55.9 - 80.0	67.9	Watson & Mulligan 1960
Can., Ont.	58	Tillering	2	42.0 - 45.8	43.9	Slykhuis et al. 1959
England	57	Tillering	2	15.4 - 26.5	20.4	Watson & Mulligan 1960
England	58	Tillering	1	-5.9 - 47.1	22.8	Watson & Mulligan 1960
			14		40.4	
England	65/66	Stem ext.	2	23.7 - 35.7	29.7[a]	Doodson & Saunders 1970b
England	67/68	Stem ext.	4	1.8 - 16.1	7.8[a]	Doodson & Saunders 1970b
England	68/69	Stem ext.	2	21.2 - 24.0	22.5[a]	Doodson & Saunders 1970b
England	65	Stem ext.	2	42.7 - 57.1	49.9[a]	Doodson & Saunders 1970b
England	69	Stem ext.	2	25.0 - 41.0	33.0[a]	Doodson & Saunders 1970b
England	68	Pre-boot	2	5.1 - 37.3	21.2[a]	Doodson & Saunders 1970b
England	68/69	Pre-boot	2	5.2-7.0	6.1[a]	Doodson & Saunders 1970b
Can., Ont.	58	Boot	2	10.4 - 20.5	15.4	Slykhuis et al. 1959
USA, Ill.	56	Boot	3	10.4 - 21.8	15.7	Endo 1957
			21		22.4	

[a] Values estimated from histograms of healthy and infected cultivars.

Table 4. Barley yellow dwarf virus effects on grain yield in wheat, barley, and oats resulting from artificial introduction of viruliferous *R. padi* onto plants in the greenhouse or growth chamber

Country	Crop	Plant stage infected	No. lines/ varieties eval.	% Yield reduction from uninfected Range	x	Reference
Can., Man.	Wheat	Seedling	1		17.7	Burnett & Gill 1976
England	Wheat	Seedling	5	55.6 - 82.7	64.5[a]	Doodson & Saunders 1970a
England	Wheat	E. till.	8	4.0 - 62.7	34.8[a]	Doodson & Saunders 1970a
			14		39.0	
Can., Man.	Barley	Seedl.	1		76.6	Burnett & Gill 1976
England	Barley	Seedl.	9	6.9 - 93.7	59.8[a]	Doodson & Saunders 1970b
England	Barley	Seedl.[b]	5	1.0 - 98.1	61.5	Boulton & Catherall 1980
USA, Minn.	Barley	Seedl.	2	27.6 - 100.0	76.9	Banttari 1965
			17		68.7	
Can., Man.	Oats	Seedl.	22	20.3 - 98.0	71.0	Gill & McKenzie 1977
Can., Man.	Oats	Seedl.	1	20.3 - 98.0	88.6	Burnett & Gill 1976
England	Oats	Seedl.	4	55.1 - 98.0	81.8[a]	Doodson & Saunders 1970a
England	Oats	E. till.	4	34.2 - 96.9	73.9[a]	Doodson & Saunders 1970a
Can., Man.	Oats	Till	1	73.0 - 78.0	75.3	Gill & Comeau [c]
Can., Man.	Oats	Stem ext.	3	34.0 - 46.0	44.0	Gill & Comeau [c]
			35		72.4	

[a] Values estimated from histograms of healthy and infected cultivars.
[b] Pot experiment conducted largely under a wire mesh cage outdoors.
[c] Reported in Gill & McKenzie (1977)

Table 5. Barley yellow dwarf virus effects on grain yield in wheat, barley, and oats resulting from artificial introduction of viruliferous *R. maidis* or *S. avenae* onto plants in the field or greenhouse

Country	Crop	Green-house or field	Aphid vector spp.[a]	Plant stage infected	No. lines/ varieties eval.	% Yield reduction from un-infected	Reference
Can., Man.	Wheat	Greenh.	Rm	Seedling	1	10.8	Burnett & Gill 1976
Can., Man.	Barley	Greenh.	Rm	Seedling	1	20.2	Burnett & Gill 1976
Can., Man.	Barley	Field	Sa	Seedling	2	28.5	Comeau & Dubuc 1978
Can., Man.	Oats	Greenh.	Rm	Seedling	1	38.4	Gill & McKenzie 1977
Can., Man.	Oats	Greenh.	Rm	Seedling	1	60.4	Burnett & Gill 1976
Can., Man.	Oats	Greenh.	Rm	Tillering	1	79.0	Gill & Comeau[b]
Can., Man.	Oats	Greenh.	Rm	Stem ext.	1	51.0	Gill & Comeau[b]
Can., Man.	Oats	Field	Sa	Seedling	5	24.1	Comeau & Dubuc 1978
Can., Man.	Oats	Greenh.	Sa	Seedling	10	79.7	Gill & McKenzie 1977

[a] Rm = *R. maidis*, Sa = *S. avenae*.
[b] Reported in Gill & McKenzie 1977.

Table 6. Barley yellow dwarf virus effects on grain yield in wheat, barley, and oats resulting from natural field infection; virus transmitted by *R. padi*

Country	Crop	Crop yr.	No. lines/ var. eval.	% Yield reduction from uninfected Range	x	Reference
Ecuador	Wheat	83	1	11.0 - 31.0		Dubin et al. 1984
Finland	Wheat	74	1		5.8	Rautapaa & Uoti 1976
Finland	Wheat	75	1		0.9	Rautapaa & Uoti 1976
USA, Ind.	Wheat	75/76	6	15.0 - 37.0	23.0[a]	Carrigan et al. 1981
USA, Wash.	Wheat	82/83	1		31.1	Pike unpubl. data
USA, Wash.	Wheat	83/84	1	15.6 - 25.1	20.4	Pike unpubl. data
USA, Wash.	Wheat	84/85	12	12.6 - 28.4	20.5	Pike unpubl. data
USA, Wash.	Wheat	85/86	12	2.7 - 5.5	4.1	Pike unpubl. data
			35		16.5	
Finland	Barley	71	2	3.5 - 29.5	16.5	Rautapaa & Uoti 1976
Finland	Barley	74	2	11.5 - 12.0	11.7	Rautapaa & Uoti 1976
USA, Wash.	Barley	85/86	5	5.6 - 21.1	15.8	Pike unpubl. data
			9		14.7	
Canada	Oats	69	2	58.0 - 71.0	64.5	Martens & McDonald 1970
Finland	Oats	71	1		23.7	Rautapaa & Uoti 1976
Finland	Oats	74	1		6.2	Rautapaa & Uoti 1976
Finland	Oats	75	1		4.4	Rautapaa & Uoti 1976
			5		24.7	

[a] Based on yields from uninfected plots planted 20 days later than infected plots.

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Biological Control of Cereal Aphids in the Southern Cone of South America

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Abstract

After the appearance of cereal aphids in Chile in 1967 and in Argentina, Brazil, Uruguay, and Paraguay one or two seasons later, a rapid buildup of populations occurred, forcing farmers to spray wide-spectrum pesticides several times per season. A permanent control strategy involving a broad, ecosystem approach was undertaken in Brazil and Chile, while in Argentina some preliminary activities were initiated. Control of the cereal aphids was obtained in Chile, Brazil, and Argentina after the introduction and establishment of natural enemies. Since 1981-82 the use of chemicals for aphid control has been rare in the Southern Cone countries, except in some states in northern Brazil. About US$52 million are saved annually in reduced pesticide costs in Argentina, Brazil, and Chile. The total cost of the project was less than US$350,000. Since 1981 a substantial drop in the source of barley yellow dwarf virus inoculum has been recorded.

Chile, in 1967, was the first country in South America from which *Sitobion avenae* (Fabricius) and *Metopolophium dirhodum* (Walker) were reported attacking wheat (*Triticum aestivum* L.) (Lara and Zúñiga 1969; Zúñiga 1986a). These aphid species appeared in Argentina and Brazil in 1968 and 1969, respectively (Zúñiga and Suzuki 1976), and populations increased rapidly thereafter, with over 100 individuals per tiller being observed (Aranda 1975; Beltran 1972; Caballero 1972; Lamborot and Guerrero 1979; Parisi 1975; Perea 1975; Zúñiga 1971, 1978). These large populations were mainly due to the absence of natural enemy parasitoids (van den Bosch 1976) and the effects of frequent and extensive spraying of wide spectrum pesticides, which limited the effectiveness of endemic predators and pathogens (Eichler 1976; González 1979, 1981; Perea 1975; Zúñiga and Suzuki 1976; Zúñiga 1976, 1978).

The cost of chemical control increased gradually while wheat production remained low, indicating that this strategy of spraying was inadequate (Baumgaertner 1978). Continuing infestations occurred in the wake of widespread insecticide usage and it has been suggested that the problem could eventually worsen as the aphids developed resistance to the chemicals (Irribarra 1973; van den Bosch 1976; Zúñiga 1979). The same pattern was observed in Uruguay and Paraguay, while in Bolivia and Peru the problem was less pronounced, due to the small area of wheat cultivation.

In 1969, preliminary experiments started in Chile involving an integrated pest management (IPM) approach (Zúñiga 1986a). A biologically oriented program was developed, within a broad, agroecosystem approach, which has been named Multilateral Biological Control (Figure 1). Initially this project in Chile was within a project sponsored by the United Nations Food and Agriculture Organization (FAO). Two aspects were studied—the role of endemic natural enemies and the introduction of potentially effective species into the crop (resource) system. Every effort was made to protect and augment populations of natural enemies already present in Chile (Zúñiga 1974, 1976) and Brazil, particularly in Rio Grande do Sul (Zúñiga 1983a). Sixteen endemic species of parasitoids, pathogens, and predators were found to be present in Argentine and Brazil (Zúñiga 1983a) and 25 in Chile (Zúñiga 1986b).

A major reason for epidemic levels of these aphids species was that they were recent introductions to South America, and they had escaped their adapted and effective bioregulators, namely parasitoids. These parasitoids effectively suppress them in their native habitat of Europe and the Middle East (van den

Bosch 1976). Species of small parasitic wasps of the Aphidiidae family offered the greatest potential for natural control (Stary 1972, 1976).

With the assistance of FAO, the United Nations Development Programme, and the University of California, a cooperative biological control program was initiated in Chile and Brazil. A wider program was also initiated under the Inter-American Institute for Cooperation on Agriculture (IICA) umbrella, involving all the Southern Cone countries. A number of population studies and some classical biological control activities were pursued in Argentina (E. Botto, personal communication; Parisi 1975).

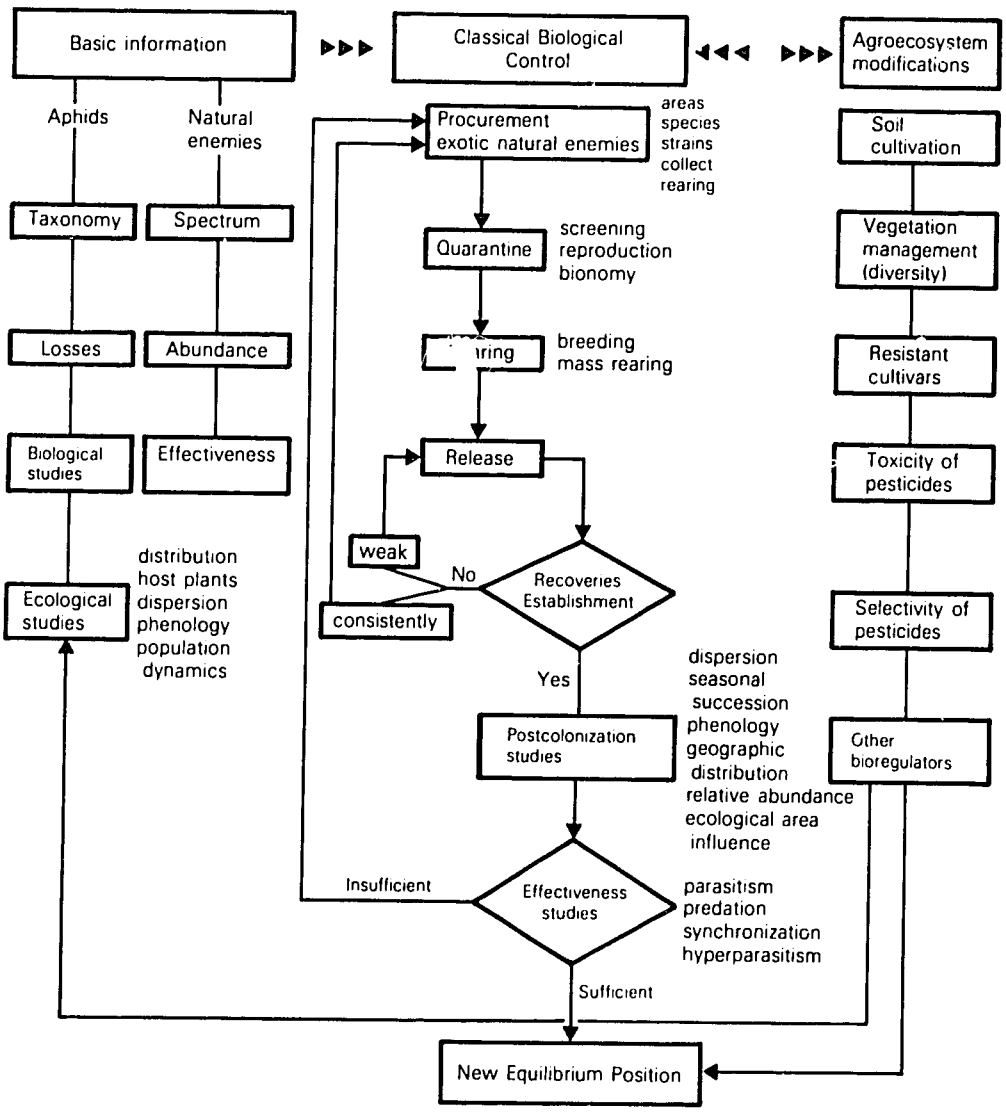

Figure 1. Diagram of the multilateral biological control project against the cereal aphids.

The general hypothesis on which this program was based was that if specific bioregulatory species were introduced and established in the cereal agroecosystems, effective economic control of aphid populations could be obtained. In addition, a substantial reduction in the source of inoculum of barley yellow dwarf virus (BYDV) could be expected (Zúñiga 1986b).

In Argentina, Brazil, and Chile large quarantine and rearing laboratories were set up (E. Botto, personal communication; Zúñiga 1983a, 1986b).

Introduction of Natural Enemies

Introduction of exotic natural enemy species was initiated in Chile, Brazil, and Argentine in 1975, 1978, and 1980, respectively (Botto personal communication; Zúñiga 1983a, 1986b). Chile began with the introduction and study of five coccinellid species, while Brazil began with two species. Chile and Brazil started the introduction of parasitoids in 1976 and 1978, respectively. A list of the introduced predators and parasites is given in Table 1.

Rearing of Predator and Parasitoid Species

A large-scale production system was established to rear predators and parasitoids. This consisted of rearing rooms maintained at a range of environmental conditions, containing cages made of perpex or mesh, which were filled with potted plants. These plants were infested with aphids and then used to infest larger cages or houses (3 x 6 m) made of mesh, which contained small plots of wheat plants. When the plots were heavily infested with aphids, they were exposed to colonies of parasitoids. When mummies began to be observed, the plants were cut and the mummies collected. Each house was able to yield up to 300,000 parasitoids. In addition, large colonies of each aphid and each parasitoid species were maintained in isolated rooms. The parasitoids were released in the field using potted plants or wheat leaves carrying colonies of parasitized aphids.

Argentine reared a total of 11,000 parasitoid specimens (E. Botto, personal communication), Brazil released 1,000,000 up until 1980 (Zúñiga 1983a), and Chile released 7,500,000 parasitoids and 500,000 predators (Zúñiga 1986b). The species that eventually established are indicated in Table 1.

Results and Discussion

An evaluation of the degree of control and aphid abundance was conducted for 10 years in Chile and 3 years in Brazil (Zúñiga 1983a), and a preliminary evaluation was carried out in Argentina. The results showed that in these countries *M. dirhodum* and *S. avenae* were under complete control. In Uruguay A. Terra et al. (unpublished) showed that the aphid abundance in 1981 in a field in Montevideo, where biological control agents were present, reached a maximum of 5.3 aphids per tiller in the case of *M. dirhodum* and only 0.8 per tiller for *S. avenae*, probably because of the suppressive action of parasitoids and predators. During this peak aphid population period over 1.5 parasitized *M. dirhodum* and 0.5 parasitized *S. avenae* per tiller were found.

In the northern state of Santa Catarina (Brazil), broad spectrum chemicals are still sprayed regularly, hindering the establishment and/or the action of effective parasitoids against *M. dirhodum*, *S. avenae*, and *Schizaphis graminum* (Rondani). In 1980 in Rio Grande do Sul (Brazil), where an IPM program has been established, only 23% of the wheat growers were still spraying (Tambasco 1981), and currently almost no insecticide spraying is carried on in this region. In comparison, in Parana, where there is no IPM program, 95% of the farmers are still spraying insecticides on their wheat crops.

In Argentina, the aphid population dropped from a peak of 80 *M. dirhodum* per tiller to less than 2 per tiller during the period of 1974 to 1980 (Parisi 1981). Finally, Uruguay and Paraguay have been favored by the natural dispersion of parasitoids that cross the borders from neighboring countries, and now the cereal crops are free from economic damage from aphids (A. Terra, personal communication).

A high level of parasitism has been demonstrated for different localities and countries by dissecting the aphids and counting the individual parasitoid larvae. The information obtained supports the hypothesis that the introduced natural enemies were the main factor in determining the new equilibrium position of cereal aphid populations in these countries. Furthermore, surveys revealed that the level of parasitism of the colonizing individuals that arrived into the crop and the migrating individuals at the end of the season was usually nearly 100%. In Rio Grande do Sul the number of aphids was reduced to the point that it was not possible to find surviving aphids during the summer (Zúñiga 1983a).

Table 1. Natural enemy species for cereal aphid control introduced in the Southern Cone of South America

	Argentina		Brazil		Chile	
	I	E	I	E	I	E
Predators						
Coccinellidae:						
Coccinella septempunctata L.			x		x	
Cocconella transversoguttata Falderman					x	
Coleomegilla maculata (De Geer)						
Coccinella maculata					x	
Hippodamia quinquesignata (Kirby)			x			
Hippodamia variegata (Goeze)					x	x
Liodalia flavomaculata (De Geer)						
Parasitoids						
Aphelinidae:						
Aphelinus abdominalis Dalman			x			x
Aphelinus asychis Walker			x		x	
Aphelinus varipes (Foerster)			x		x	
Aphidiidae:						
Aphidius ervi Haliday	—	?	x	?	x	x
Aphidius picipes Nees			x			
Aphidius rhopalosiphi De Stefani		x[a]	x	x	x	x
Aphidius uzbekistanicus Luzhetzki		x[a]	x	x	x	?
Ephedrus plagiator Nees	x		x		x	
Monoctonus nervosus (Haliday)					x	
Praon gallicum Stary	x		x		x	x
Praon volucre (Haliday)		x[a]	x	x	x	x
Toxares sp.		x				

I = introduced, E = established.
[a] Introduced through natural dispersion and then established.

Figures of parasitization of aphids obtained during the evaluation periods are important although the role of parasitoids determined by dissection of aphids only partially represents their actual value (Zúñiga 1983b).

In addition to the regulatory effect of the parasitoids, mention should be made of the successful establishment of the ladybird *Hippodamia variegata*, introduced from South Africa into Chile in 1975. This coccinellid is now widely distributed and abundant throughout the country, feeding on many aphids species on different plants and in different agroecosystems.

Surveys were conducted in Brazil and Chile to determine the new level of cereal aphids present in reservoirs other than cereal fields or cultivated pastures. These showed that aphid populations dropped drastically in the Andean vegetation where they survive during summer and part of autumn in Chile and on gramineous plants along the borders of the rivers in Rio Grande do Sul in Brazil (Quiroz et al. 1986; Zúñiga 1983a). In spite of the abundance of possible grass hosts of BYDV in the Andean region, only a low percentage have been found to be infected with BYDV (C. Quiroz, personal communication). This suggests that the natural agents introduced to control the aphids had an indirect influence on the percent of these grass hosts that are becoming infested with BYDV, probably because nearly all of the aphids migrating from the wheat fields were parasitized and this interfered with their ability to transmit BYDV.

Economic Impact of the Biological Control Program

The reduction in the yield of cereals caused by aphid attack is difficult to estimate. Approximate figures have been calculated based on the cost of two annual treatments with insecticide, which is a rather conservative figure for most of the countries in the Southern Cone. The possible resurgence of secondary pests and the cost of equipment and fuel were excluded from the estimates. Depending on the area cultivated each year, Argentina is annually saving approximately US$28,000,000, Brazil US$11,000,000, Chile US$11,000,000 to $15,000,000, Paraguay US$360,000, and Uruguay US$6,400,000. This amounts to as much as US$60,000,000 saved annually in the five countries together. If this figure is compared to the cost of the biological control program, which, until 1982, was approximately US$84,255 in Brazil and US$250,000 in Chile, a favorable cost/benefit ratio of the biological control strategy is demonstrated.

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Genetics of Host Plant Resistance to Barley Yellow Dwarf Virus

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Abstract

Host plant resistance (HPR) to Barley Yellow Dwarf Virus (BYDV), in various degrees, has been observed in barley (Hordeum vulgare L.), *wheat* (Triticum aestivum), *triticale* (X Triticosecale), *oats* (Avena sativa L.), *rice* (Oryza sativa), *rye* (Secale cereale), *and maize* (Zea mays L.). *A major gene (Yd_2) and a minor gene (yd_1) were found in barley. Additional genes with minor HPR effects are suspected in barley. Major HPR genes have not been identified in wheat, triticale, or oats. We hypothesize that such genes exist, but detection is difficult because of the following factors: 1) polyploid species have homoeologous loci that may obscure single HPR gene effects; 2) barley yellow dwarf (BYD) reaction is assessed visually or by yield-loss measurements both of which are environmentally sensitive, resulting in low repeatability of reaction types; 3) HPR genes may be specific to BYDV isolates so that multiple or unknown virus isolate infections may obscure individual HPR gene effects; and 4) feeding preferences of aphid species in genetically heterogeneous plant populations may affect the degree and timing of infection. Further resolution of the genetics of HPR requires consideration of all of these factors; when breeding for HPR varieties, these factors must also be recognized, but less so if recurrent selection is practiced.*

The hosts of the barley yellow dwarf virus (BYDV) are widely represented in the Gramineae, and this virus is especially noteworthy for reducing the productivity of cereal grain crops. The disease caused by BYDV, barley yellow dwarf (BYD) is mainly recognized for its effects on barley *(Hordeum vulgare* L.), oats *(Avena sativa* L.), and wheat *(Triticum aestivum)*, but it is also known to attack triticale *(X Triticosecale)*, rye *(Secale cereale)*, rice *(Oryza sativa)*, and maize *(Zea mays* L.). Since the discovery of BYD in the early 1950s, it has been widely accepted that the use of host plant resistance (HPR) was the most efficacious control strategy for BYD, even though avoidance of epiphytotics is sometimes possible by adjusting the sowing date so that seedling plants are not infested by viruliferous aphids. Plant breeders first searched for sources of resistance and then included promising lines in hybridization and selection programs. In most instances, the breeding efforts were initiated without access to good information about the genetic basis of HPR. Now, more than 30 years since BYD resistance became an important breeding objective, there is a lack of detailed information on the genetics of HPR in wheat and oats, but reasonably good data on barley. Since BYD on maize and rice has become important only recently, there is little work published.

BYD is one of the most difficult of the cereal diseases to investigate for HPR because of complex biological interactions introduced by the host plant, the virus, and the aphid vectors. Each of these biological entities is greatly affected by variation in environmental factors, further complicating the analysis of HPR (Figure 1). It is not possible to review how all these many factors influence the BYD severity, in fact most of the interacting factors shown in Figure 1 have not been studied in detail. Because of the complexity of BYD, it has not been a favored disease for genetic investigations. Genetic studies have been usually conducted with the goal to provide useful insight for plant breeding to reduce economic losses. Considerable advances in BYD research have been made possible by the use of the enzyme-linked immunosorbent assay (ELISA)

technique and by nucleic acid hybridization for diagnosing the presence of BYDV and quantifying the amount of virus present in the plant and more direct measures of virus RNA concentration. Detailed genetical analysis of the host-pathogen interaction requires a defined virus type that is stable in its virulence, aphid vector colonies that are genetically uniform and transmit BYDV at a repeatable frequency, and a method for introducing a known amount of virus into plants grown under well defined conditions. These requirements are not easily met and are not essential in serving the needs of a plant breeder. This paper will be mainly concerned with the genetics of HPR with resolution sufficient to aid in breeding resistant cultivars.

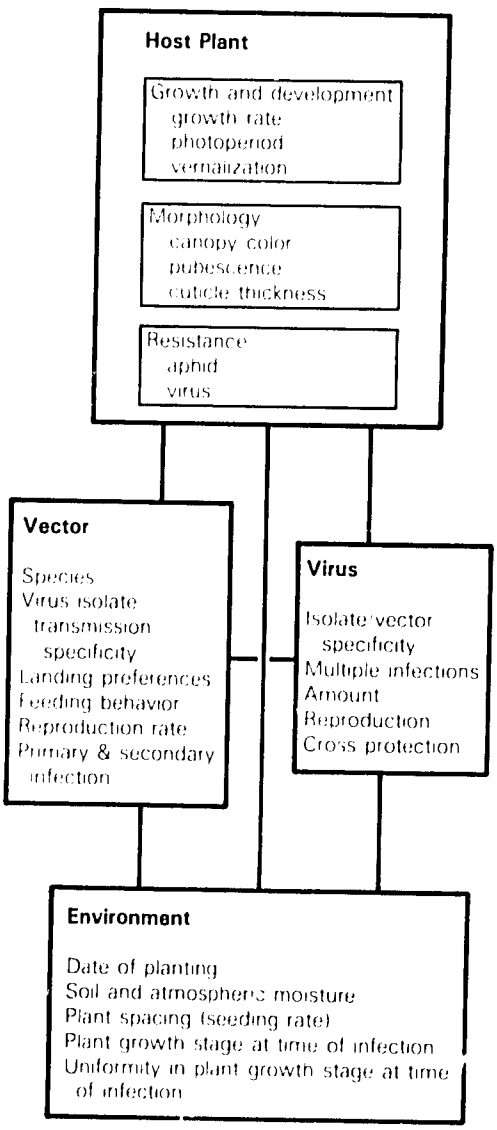

Figure 1. Some interacting host, vector, virus, and environmental factors that influence the effects of the barley yellow dwarf virus disease on cereal crops.

The breeder requires answers to the following questions:

- What plant, virus, and vector relationships are indicative of HPR?
- Do HPR genes exist in cultivars, landraces, or wild and cultivated relatives?
- Is HPR conditioned by single genes or multigene complexes?
- Can the gene or gene complexes be manipulated and combined into suitable agronomic types by readily available gene manipulation strategies?

In addition, the breeder would like to be able to judge whether HPR, after introduction into cultivars, will be durable. The following sections present some discussion and evidence concerning the these questions.

Manifestation of HPR

Plant responses

BYD was discovered and described on the basis of yellowing and dwarfing in barley. The symptoms also include tiller mortality and reduced grain number (sterility). Some genotypes of barley, oats, and wheat also show leaf reddening. After visual observation, the degree of symptom expression has been scored numerically on a 0 (no symptoms) to 9 (severe symptoms) scale (Qualset 1984). These scores have been assumed to relate to the degree of HPR. For barley this was satisfactory (Rasmusson and Schaller 1959) as verified by genetic analysis, but a major HPR gene was present, so large differences in symptom expressions were seen. With wheat and oats, the visual symptom expression is not so easily shown to be related to

resistance. Evaluation of grain yield and yield component expression in infected and noninfected plants has generally supported the validity of visual scoring of symptom expression (Topcu 1975; Baltenberger et al. 1987), but there have also been some exceptions where yield losses are measured with very limited symptom expression and vice versa. Visual scoring under controlled inoculation conditions, followed by extensive progeny-testing and yield loss measurements have been successfully used in several plant breeding programs.

Vector behavior

Successful transmission of BYDV depends upon a complex interaction among three biological systems: plant, virus, and aphid. A viruliferous aphid must find and land on a susceptible plant; the physical and chemical properties of the plant must permit the aphid to insert its stylets into a phloem sieve element, where it salivates the virus into the plant's phloem. Because the virus is transmitted only by aphid feeding, aphid behavior is a source of variability that must be taken into account. For example, cultivars may escape infection because they have plant characteristics that are unattractive or repelling to aphids under field-testing conditions. These cultivars cannot readily be distinguished from those that are attractive to aphids, readily fed upon, and inoculated with virus, but are asymptomatic virus hosts. Perhaps of greater importance, plants may be resistant to phloem contact by aphids, and hence to virus transmission. This represents a largely unexplored potential for manifestation of HPR.

Host plant-finding, sampling, and feeding are the primary aphid activities that affect HPR to BYD. The aphid vector has been largely ignored, primarily because a short feeding period can result in virus transmission even if there is a host resistance mechanism to the aphid, as exists for greenbug-resistant wheat. However, we entertain the possibility of HPR to some aspects of aphid activities as a means to reduce BYD effects. Some of the relevant observations follow.

Plant finding—Aphids may migrate long distances. Their movement is usually passive as they catch rides on wind currents. However, they can land at will on a particular plant or surface (Kennedy and Fosbrooke 1972). It is primarily a visual cue that governs the end of passive movement and the landing on a plant. Unlike many other insect species, olfaction does not seem to play a role in plant finding. From a distance, visual detection of light-dark patterns and perception of intensity differences at the horizon line characterize how an airborne aphid detects plants (Prokopy and Owens 1983). Response to color at this stage is unlikely because insects generally have low visual acuity except at close range.

At close range, aphids do respond positively to specific foliage colors, especially yellow. Visual sensitivity extends from 350 to 600 nm, peaking at 550 nm (Prokopy and Owens 1983). Many aphids discriminate between host and nonhost plants, or between plants or portions of plants in particular physiological conditions (i.e., infected with BYDV) on the basis of yellow hue and the saturation or intensity of reflected light. For example, the aphid, *Hyalopterus pruni* looking for *Phraomites* plants as summer hosts lands in greater numbers on plants with unsaturated yellow than on plants with saturated yellow and appear to use this same saturation discrimination basis for discerning unsaturated *Phraomites* hosts from nonhosts (Moericke 1969). Aphid species that feed on hosts whose foliar hues are saturated are not attracted to highly unsaturated yellow surfaces. Irwin and Goodman (1981) demonstrated that, in 16 x 16-m blocks of soybeans, two of five aphid species had higher landing rates on dark green than light green foliage. Furthermore, there was a positive correlation between aphid landing rates and field spread of the nonpersistently transmitted soybean mosaic virus (Irwin and Goodman 1981; Schultz et al. 1985).

The kaleidoscope of colors present in field plots containing many cereal cultivars may influence the landing rates of aphids on particular plants. Under these conditions, where aphids have a wide range of choices, certain cultivars may consistently escape or have lower

infection due to an unattractive color. However, the landing patterns may be quite different in the "no-choice" monocultures of modern agriculture. Nevertheless, genetic manipulation of plant color has potential as a means for influencing aphid landing patterns and field spread of BYDV. On the other hand, aphid response to plant colors is a variable to consider when evaluating plants for BYD resistance. Under field conditions with many cultivars of different colors planted side by side, a cultivar may consistently escape infection. We have often observed in the field lower BYD symptom expression in wheats with bright green color, expressed in plants with less cuticular wax on the leaf surface.

Sampling and feeding behavior—After landing on a plant, aphids perform a characteristic sampling behavior that consists of several short probes into the epidermal and mesophyll cells. If the mechanical and chemical plant cues perceived by mechano- and chemo-receptors, respectively (McLean and Kinsey 1984) are favorable, stylet insertion proceeds until a phloem sieve element is penetrated. If plant cues continue to be favorable, phloem ingestion begins (defined as phloem ingestion lasting longer than 15 minutes (Montllor et al. 1983). Hence, plant characteristics that inhibit probing, such as glandular trichomes or heavy pubescence, may discourage further sampling and probing (Tingey and Gibson 1978). The chemistry of the intercellular spaces of cell walls may also "turn off" further probing (Dreyer and Campbell 1984).

Halting aphid probing may be very effective in preventing BYDV transmission, particularly in light of new findings by Scheller and Shukle (1986), who showed that penetration of one sieve element resulted in a 65% chance of transmission independent of the duration of phloem contact. The chance of transmission increased with the number of sieve element penetrations. This indicates that, as one would expect, salivation into the sieve element is the critical event in transmission. Hence, plant characteristics that prevent phloem contact could be an expression of HPR.

Host plant resistance to aphids has been shown to be a result of lengthening the time taken by aphids to reach the phloem (Dreyer and Campbell 1984; Dreyer at al. 1984; Montllor et al. 1983; Campbell et al. 1982). This phenomenon of decreased phloem contact by aphid vectors has also been related to beet yellows virus (also a luteovirus similar to BYDV) resistance in sugar beets (Haniotakis and Lange 1974). A similar shift from phloem feeding to xylem feeding has been noted in studies of leafhopper feeding on highly resistant rice varieties (Heinrichs and Rapusas 1984). This shift in feeding behavior also resulted in greatly reduced transmission of rice tungro, a semipersistently transmitted virus. Feeding efficiency also depends upon plant health as shown by Montllor and Gildow (1986). They showed that *Schizaphis graminum* fed better on BYDV-infected than on healthy oats; whereas no differences were found for *Rhopalosiphum padi* L.

Because behaviors such as salivation and ingestion occur within leaf tissues, a technique is needed to determine exactly when and where these behavioral events are occurring. McLean and Kinsey (1964) introduced a technique of electronically monitoring the probing behavior of aphids that makes it possible to quantify the frequency and duration of aphid salivation and ingestion, as well as the position of the stylet tips at a given time (Kawabe et al. 1981). Distinctive sequences of waveforms diagnostic of salivation into and ingestion from the phloem have been demonstrated for many aphid species (McLean and Kinsey 1967; Campbell et al. 1982; Kennedy et al. 1978; Nault and Styer 1972; Scheller and Shukle 1986). Electronic monitoring of aphid feeding has proven a useful tool in screening both monocotyledons and dicotyledons for resistance to aphid feeding (Dreyer and Campbell 1984; Dreyer et al. 1984; Montllor et al. 1983; Campbell et al. 1982; Kennedy et al. 1978; Haniotakis and Lange 1974; Nielson and Don 1974; McLean and Kinsey 1968). Only recently, electronic monitoring of aphid feeding has emerged as a potentially useful tool for screening plants for resistance to aphid phloem contact and hence resistance to BYDV

transmission (Scheller and Shukle 1986; Ullman et al. 1988). Ullman et al. (1988) monitored aphid feeding behavior to determine if BYD resistance expressed by the *Yd2* gene in California Mariout barley is a function of aphid-plant interactions or plant-virus interactions. Analysis of the primary aphid behavior governing transmission efficiency, such as, number of sieve element contacts and time to the first sieve element contact, demonstrated that there was no significant difference between isolines of California Mariout barley with and without the *Yd2* gene. This work suggests that the tolerance imparted by the *Yd2* gene results from plant-virus interactions rather than aphid-plant interactions. Kieckhefer et al. (1980) found no relationship of HPR in oats with aphid attractiveness or support of aphid populations, also indicating that plant virus relationships predominate in HPR. However, Shukle et al. (1987), while studying BYD resistance in several wheatgrass species, found that aphids had restricted ability to locate phloem cells in some species. However, most of the resistant species showed restricted virus multiplication.

Virus concentration

The amount of virus in a plant after a specified time after inoculation can be ascertained. There has been some effort to determine if the amount of virus present is related to damage (yield loss) caused by BYDV and to visual symptom expression. Jedlinski et al. (1977) infected greenhouse-grown oat plants with BYDV-PAV and found that resistant sibs had 28 mg/kg (fresh weight) of virus compared with 47 mg/kg in susceptible sibs. These results were compared to field studies (3 years) where mean grain yield of the same resistant sibs was 23.4 g/plot compared to 8.9 g/plot for the susceptible sibs after inoculation with PAV. Thus there was 68% more virus recovered and 62% lower grain yield of the susceptible lines than the resistant lines.

Virus accumulation, as measured by ELISA, has been taken as an indication of HPR in wheat and wheatgrass x wheat amphiploids (Xin et al. 1988). Lorens et al. (1989) used a BYDV-PAV cDNA clone to assay for virus concentration in greenhouse-inoculated and noninoculated wheat plants and found a reasonably good relationship to visual scores on field-grown plants. Furthermore, in an F_2 population of a resistant x susceptible hybrid, plants with low or high virus concentration were selected for F_3 progeny-testing. The F_3 plants had virus concentrations highly correlated to the amount observed in F_2 plants. In both the oats and wheat studies, virus concentration was heritable and probably related to HPR. However, ELISA and cDNA clones to assay virus titer in plants must be validated by field analysis to confirm if HPR is detected to the extent useful in plant breeding.

Vector-virus specificity

The classical studies by Rochow (1979) elucidated vector-virus relationships that still provide the basis for classification of BYDV isolates. Five major isolates were identified by the aphid species that selectively transmit BYDV: RPV (*Rhopalosiphum* padi), RMV (*R. maidis* Fitch), MAV (*Sitobion avenae*), SGV (*Shizaphis graminum*), and PAV (R. padi and *S. avenae*). Serological studies have shown that PAV and MAV are closely related, but distinct from RPV (Aapola and Rochow 1971). Furthermore, RPV is more closely related to beet western yellows virus (BWYV) than to PAV and MAV (Rochow and Duffus 1978) that emphasizes genetic variability within BYDV. This variability has direct bearing on the approaches to detecting HPR and breeding resistant varieties.

HPR has been detected, for the most part, in studies of field-grown plants. Some workers have inoculated plants with one isolate in the field test sites, e.g., at Illinois (Jedlinski et al. 1977), by a single isolate in the greenhouse followed by transplantation of inoculated plants to the field, e.g., in Indiana (Baltenberger et al. 1987), or by field inoculation of unknown extant isolates, e.g., in California (Schaller et al. 1963).

Several workers have shown BYDV isolate x crop genotype interactions. Recent results of Baltenberger et al. (1987) with the barley varieties California Mariout (CM) (susceptible to BYD, *yd2*) and CM 67 (resistant to *Yd2*) are

important because they demonstrate the interaction of a single HPR gene with RPV and PAV. Visual symptom scores and grain yield reduction for plants inoculated by RPV or PAV (Table 1) showed that CM 67 *(Yd2)* had HPR to both RPV and PAV, but CM 67 *(yd2)* had apparent HPR to RPV only. CM 67 was developed in California by selecting for field resistance to BYD. CM 67 is clearly a susceptible variety there, but the responses to RPV and PAV suggest that PAV was the predominant field isolate. Recent results support that idea (Gildow and Rochow 1983).

These results suggest that, while HPR studies need to be cognizant of extant isolates in the field, selection in the presence of a mixture of isolates may give variable results, but is clearly necessary in plant breeding programs.

Environmental effects

Variation in symptom expression among BYDV-infected plants has confounded studies of HPR and no doubt obscured genetic interpretation of segregation in resistant x susceptible hybrid populations. It will not be possible to review the relevant data here, but we point out that certain practices are useful to evaluate HPR. Figure 1 gives a list of environmental factors that can influence BYD. Date of planting can be critical because seedling plants infested with viruliferous aphids show more severe symptoms than plants infested later, such as during culm elongation. For HPR evaluation in the field depending on natural infection, it has been useful to plant the test genotypes a few weeks before peak aphid flights (Qualset 1984). It is also useful to plant "aphid traps" of susceptible cultivars at various times to ensure that large numbers of aphids are available to infest HPR evaluation plots.

Controlled inoculations are most reliable in the field. Paired plots of infested and noninfested plants have been extensively used in Illinois (Jedlinski 1984) and Indiana. At Indiana a very useful practice has been to inoculate plants in the greenhouse with subsequent transplanting of inoculated and control plants to the field (Baltenberger et al. 1987). After transplantation, both inoculated and control plants are protected from further infection by insecticide applications.

The degree of symptom expression is affected by the number of viruliferous aphids feeding on the plants (Burnett and Gill 1976), therefore, uniform infestation is highly desirable. Comeau (1984) has developed a system of greenhouse rearing of aphids and uniform infestation of field-grown plants. He applies talc-coated aphids through a small tube to individual plants.

Since the growth stage of plants at time of inoculation by BYDV is critical, segregation for winter habit or time of flowering in populations infested by aphids at one time can result in variable BYD symptoms that are not manifestations of HPR. This was clearly demonstrated in barley by Jones and Catherall (1970), who found that plants with the Yd_2 resistance gene varied in level of HPR depending on stage and rate of plant development. In these cases, homozygous lines must be evaluated with inoculations timed according to

Table 1. BYD symptom score and grain yield reduction of California Mariout (CM) without *Yd2* and CM 67 with *Yd2* barley after inoculation with two BYDV isolates[a]

Variety	Symptom score[b] RPV	PAV	Control	Grain yield reduction, % RPV	PAV
CM (*yd2*)	4.5	6.8	1.3	12	80
CM 67 (*Yd2*)	4.5	3.8	1.0	34	37

[a] Baltenberger et al. (1987).
[b] 0 = no symptoms, 9 = severe symptoms.

growth stage in order to interpret HPR. Plant spacing also influences plant development rate. Under natural conditions widely spaced plants often show more severe symptoms than closely spaced plants. This further complicates evaluation for HPR because genotypes with a useful level of resistance in normal crop density may appear as highly susceptible under wide spacing.

Sources of HPR

BYD surely existed for many years prior to its description by Oswald and Houston (1953). It is quite possible that natural selection for resistance occurred in landraces and breeders plots. However, in areas where BYD caused severe yield reductions, the cultivars being grown were generally very susceptible. Searches among world collections of cultivated barley, oats, and wheat have identified HPR with various degrees of success. In barley a geographic-specific gene was found, *Yd2* (Rasmusson and Schaller 1959), which occurred only in Ethiopian barleys, at especially high frequencies in barley collected at high elevations (Qualset 1975). Additional barleys were known to have low levels of resistance, such as some "Manchurian" types (Schaller et al. 1963) or Rojo barley, reported to have a recessive HPR gene, yd1 (Suneson 1955).

Numerous screening trials of oats have been conducted (Jedlinski 1984) and a rather small number of hexaploid varieties were identified with HPR. Very high HPR was found in Saia, a diploid *(Avena strigosa)*, but this cultivar has not been used in breeding of hexaploid oats. HPR was also identified in the hexaploid *A. fatua* (Rines et al. 1979) and *A. sterilis* (Comeau 1982).

In wheat, the situation is rather similar to oats. Extensive evaluation of the world collection resulted in the identification of a small number of tolerant, cultivated tetraploid and hexaploid cultivars and landraces (Qualset et al. 1973, 1977). Recently, extensive evaluation of CIMMYT and California breeding lines among the spring wheats has identified several new sources of HPR (unpublished results). Good HPR is found in several cultivars developed in Chile (reported in these proceedings). Especially noteworthy is the cultivar NS879/4 originating in Novi Sad, Yugoslavia, and identified as potentially resistant in India. Tola and Kronstad (1984) proved that it had heritable resistance in crosses with both winter and spring wheats.

Even the best HPR in wheat is not as high as found in barley or oats. This has prompted the search for resistance among wild relatives and the transfer of Yd_2 from barley to wheat. Several wheatgrass species appear to be good sources of resistance to BYDV because no virus could be recovered by aphid transmission tests or ELISA (Hazelwood 1983; Sharma et al. 1984; Shukle et al. 1987). Results reported in these proceedings and by Xin et al. (1988) show that Zhong 4, a 56-chromosome *Triticum aestivum-Trichopyrum intermedium (syn. Thinopyrum intermedium)* amphiploid, had exceptionally good resistance. In other materials, two of seven single chromosome substitutions from the diploid *Lophopyrum elongatum* to Chinese Spring showed field resistance at Davis, California, almost equal to the amphiploid from which they were derived (McGuire et al., unpublished).

Among cultivated relatives of wheat, rye, and triticale are potential donors of HPR to wheat. Comeau and St-Pierre (1984) and others have identified triticale cultivars with BYD resistance, presumably contributed by genes on rye chromosomes. Rye is variable for BYD reaction in California, but apparently generally quite resistant elsewhere. No heritability studies have been done with rye to assess the value of this source for HPR to BYD. Triticale has been studied considerably less. Collin et al. (1989) suggest that the resistance in triticale is simply inherited and that it is superior to that found in wheat. The *Yd2* gene from barley expresses resistance in wheat-barley chromosome 3 addition lines (McGuire and Qualset 1989). Thus, for wheat it appears that there is now a suitable gene pool for breeding resistant cultivars. That was not the case even 4 years ago.

BYD was only recently described as a disease of maize and rice, so there is limited information available about HPR

in these crops. Loi et al. (1986) and Lorenzoni (1989) have shown genetic differences in HPR among inbred lines and hybrids of maize under field evaluations in Italy. Also, in Italy, differences among rice cultivars have been found (Osler 1984) which are under investigation for HPR (Osler 1989).

HPR Genes

Host plant resistance was broadly defined by Fraser (1986) "as any inhibition of virus multiplication, or of its pathogenic effects on the host." There are certainly large phenotypic responses by host plants to BYDV infection. A major point of this review has been to discuss some of the BYDV x host x environment interactions that obviously impact on the design of studies directed toward discovering the genetic basis of HPR to BYDV. The focus here is on the host plant response to the virus, but obviously a complete understanding of HPR depends upon information about the genetics of virulence in the virus.

Virus resistance in plants is often found to be oligogenic, but, as Fraser (1986) points out, the major genes are easiest to identify and are more likely to be reported. In cases of observed polygenic segregations the effects of environment may preclude the detection of major genes.

We have taken the hypothesis that HPR to BYDV is controlled by individually detectable genes. The discovery of *Yd2* in Ethiopian barley certainly supports this hypothesis, but up to now major genes have not been clearly identified in wheat or oats. In barley, *yd1* was proposed as a recessive resistance gene (Suneson 1955), but the level on resistance conferred was so low that this gene was not studied further. The *Yd2* gene was discovered in Ethiopian barley (Rasmusson and Schaller 1959). This gene conferred easily identifiable resistance even though in inheritance studies it proved to be additive or incompletely dominant. *Yd2* was mapped to chromosome 3, linked to club (dense) spike (15.9 cM) and xantha (14.5 cM), and a gene for resistance to Rhyncosporium secalis (27.6 cM) (Schaller et al. 1964). *Yd2* was identified by visual scoring of field-grown plants in natural, but induced epiphytotics (Qualset 1984). F_2 genotypes were confirmed by F_3 and F_4 progeny testing. This was an essential step to confirm gene identification and in the linkage analysis because of the various factors that influence symptom expression. Tests for allelism of *Yd2* and the search for additional loci have failed to detect additional genetic diversity in HPR for BYD in barley. Twenty Ethiopian barleys (see Schaller 1984 for review) all had *Yd2*. The basis for observed differences in resistance among barleys carrying *Yd2* is not known, allelic variability at the *Yd2* locus is not fully excluded.

Yd2 showed variable expression depending on the background genotype. For example, in Great Britain Catherall and Hayes (1966) found limited effectiveness of *Yd2* in late or slow-developing cultivars. This gene is found in both early and late maturing Ethiopian barley, but predominantly in late cultivars originating at high elevations (Qualset 1975). This suggests that the ineffectiveness of *Yd2* in late-maturing European germplasm may be associated with the genetic background of that material. In contrast, Schaller (1984) in California transferred *Yd2* to a late-maturing cultivar, Sutter, which has very strong resistance to BYD.

In oats Jedlinski (1984) stated that the resistance is, "relatively simply inherited, usually in a quantitative manner, with the nature of inheritance depending on the genotype of the parents and the characters." Several workers proved that HPR was heritable (Brown and Poehlman 1962; Endo and Brown 1964; Weerapat et al. 1974; Cooper and Sorrells 1983; Gellner and Sechler 1986). Some of these studies support Jedlinski's "relatively simply inherited" conclusion. Intercrosses between cultivars having measurable HPR have produced transgressive segregates with greater resistance to BYD than the parents (Brown and Jedlinksi 1978). In a multisite study of random F_4-derived F_5 lines from four crosses, McKenzie et al. (1985) presented segregation data that were suggestive of major-gene segregation. Since near-homozygous lines were studied, the expected segregation ratios for major genes were simplified. At

one location (Glenlea) a single BYDV isolate (PAV) was used to inoculate the lines that should further simplify the segregation patterns. Our analysis of the detailed segregation data from Glenlea, in relation to parental responses, shows with reasonably high assurance that the lines from three of the four crosses segregated 3 resistant:1 susceptible and one cross segregated 1 resistant:1 susceptible as expected for two- and one-gene segregation for HPR. A second study (Landry et al. 1984) of resistant *A. sterilis* germplasm in crosses with Lamar, a highly susceptible *A. sativa* cultivar, showed rather complex segregation patterns for visual BYD response scores in the F_2. No F_3 progenies were studied. A quantitative genetic interpretation of generation means (P_1, P_2, F_1, and F_2) showed predominantly additive gene action for HPR. The number of genes segregating was estimated by the effective factor approach to be 3 or 4 in the *A. sterilis* x *A. sativa* crosses and 2 in a sativa x sativa cross. Landry et al. (1984) suggested that *A. sterilis* provided HPR genes not found in *A. sativa*. The available data for oats are supportive, but do not prove conclusively that major genes for HPR to BYD have been found.

Several studies of HPR in wheat have been done, all with hexaploid *Triticum aestivum*. As mentioned earlier, the putative sources of HPR genes in wheat have BYD symptom expression and in general do not appear to be as resistant as barley with *Yd2*. Nevertheless, heritable resistance has been identified (Qualset et al. 1973; Topcu 1975; Cisar et al. 1982; Tola and Kronstad 1984) in both winter and spring wheats. Qualset et al. (1973) showed that Anza, a resistant spring wheat, had dominant gene action for HPR as measured by visual symptom scores in most of 54 F_1 hybrids. In that same study, segregation distributions for F_3 line means gave continuous distributions in crosses of Anza x susceptible parents, whereas in a cross involving two resistant parents a bimodal segregation pattern was obtained. Topcu (1975) found similar results. Later, Tola and Kronstad (1984) showed bimodal segregation in two winter wheat crosses, both with F_2 plants and F_3 line means. In a 12-parent diallel cross, Cisar et al. (1982) found significant general and specific combining ability effects based on F_2 and parental data. No major gene segregation models were applied in these studies, even though the data suggest that a small number of HPR genes may have been segregating. In those studies, highly heterozygous generations were evaluated without critical progeny tests. Thus, we rather reluctantly must defer a positive conclusion for major-gene HPR to BYD in wheat.

The identification of HPR genes in cultivated oats and bread wheat is complicated by the hexaploid nature of these species. Duplicate or triplicate loci are expected on homoeologous chromosomes, for example on chromosomes 1A, 1B, and 1D in wheat. Disomic inheritance is expected because these species are diploidized in their chromosome pairing behavior at meiosis. The effect of polyploidy is to multiply the number of homoeologous loci, resulting in up to three different alleles at a single locus in a hexaploid. If wheat or oats is susceptible, i.e., have "susceptibility" alleles at all three homoeologous loci, a single HPR allele from another source must be dominant to three other genes for HPR to be expressed. Therefore, it is not surprising that the genetic basis for resistance in wheat and oats has not been clearly elucidated.

Utilization of HPR

Yd2 has dramatic effects on barley production in areas where this gene has been used in varieties. For example, in California, CM 67 averaged 19% higher grain yield than the susceptible cultivar California Mariout in 19 trials over 3 years (C.W. Schaller, unpublished data). *Yd2* can be rather easily transferred by backcrossing to locally adapted types. It has been widely used in the ICARDA (International Center for Agricultural Research in the Dry Areas)-CIMMYT international breeding programs, so by now this gene is widely dispersed in the world's barley germplasm. *Yd2* was found in Ethiopian landraces, but there were no undesirable characters that were not easily eliminated in the selection program. However, some of the Ethiopian donor cultivars could not readily be used as female parents because of a single-gene condition called floral sensitivity

(Qualset and Schaller 1968) that prevented hybrid seed development. Reciprocal crosses were successful.

Concern has been raised over the fact that only a single HPR gene was found and that barley was vulnerable to attack by new BYDV strains or isolates. Resistance was discovered in the mid-1950s. Some reversals in BYD reaction of Ethiopian barleys have been observed at Davis, California, and Palmerston North, New Zealand, in natural infection of field-grown plants (C.W. Schaller and J.M. McEwan, unpublished results). There have been occasional reports of *Yd2*-carrying cultivars showing severe BYD symptoms. Reference was made earlier to the observation in Great Britain that *Yd2* was not effective in the genetic background of late-maturing barleys. Thus there is increasing evidence that *Yd2* might be vulnerable. Baltenberger et al. (1987) showed vector-virus specific responses of California Mariout and CM 67 which suggest that *Yd2* may be an isolate-specific HPR gene. *Yd2* was especially not effective in plants infected with both RPV and PAV. BYDV isolates have generally not been identified in the areas where *Yd2* is being used, so it is quite possible that the apparent susceptibility in barleys with *Yd2* is due to variable virus isolates.

With wheat and oats, the situation is quite different from barley; however, the best HPR in these crops is usually not as good as conferred by *Yd2* in barley. Host-virus specific responses have not been so easily observed, although, for example Ogle oats is more resistant to one BYDV isolate than another (J. Griesbach et al., unpublished results). This might be explained by the fact that HPR as presently known in wheat and oats is multigenic and the accumulated resistance genes may reduce virus x plant genotype interactions. Breeding for resistance in both wheat and oats depends more on population breeding methodology than in barley. The general strategy is to combine HPR from as many sources as possible through multiple hybridizations and selection. The work of Brown and Jedlinski (1978) clearly showed that HPR could be realized at higher levels than found in the best parents. Because of low heritability, selection repeated over two or more generations is needed to recover HPR from segregating populations. Several hybridization cycles will be most effective in eventually obtaining a high level of resistance (Qualset et al. 1973).

In the wheat breeding program at Davis, a disjunct recurrent selection program has been in progress for about 20 years. In this program, first the unadapted HPR source CI 13232 was hybridized with locally adapted cultivars. BYD resistance was recovered and a second source of resistance was introduced from the adapted variety Anza. Again improved HPR was realized and the most advanced lines have been crossed to unadapted NS879/4, a third source of HPR. At the same time, both Anza and NS879/4 were individually used as parents with susceptible cultivars. The best lines from these crosses are now being intermated in a more traditional recurrent selection breeding procedure. This strategy is not unlike traditional wheat breeding programs that have successfully improved performance and disease resistance over several decades (MacKey and Qualset 1986).

A modified form of this strategy is recommended for barley where major and minor HPR genes should be combined. Qualset (1984) suggested parallel programs be conducted to select for resistance due to *Yd2* and from other sources, such as the Manchurian barleys. It is important to select for HPR genes having small effects in the absence of *Yd2*. When measurable resistance is obtained it can be combined with *Yd2* and then quite possibly HPR greater and more durable than already realized in cultivars carrying *Yd2* will be obtained.

This discussion has concentrated on using intraspecific genetic resources for BYD resistance. In oats, *A. sterilis* x *A. sativa* and *A. fatua* x *A. sativa* hybrids are easily made, thus the HPR observed in *A. sterilis* can be transferred to *A. sativa* types. *A. sterilis* has been successfully used in oat improvement already so this should be a successful approach to reach higher levels of HPR in oat.

For wheat, it was pointed out earlier that several newly identified sources of BYD resistance were found in rather distantly related species. Knott (1986) showed that more than 15 cultivars have been obtained from five alien species and nine other successful interspecific gene transfers have been made that have not yet been introduced into commercial varieties. It can be expected that BYD resistance from wheatgrass species can be used successfully in wheat improvement. However, some problems of agronomic performance and grain quality can be anticipated, based on experience with other interspecific gene transfers. Again recurrent selection is indicated as an effective, even if long-term, breeding strategy.

As a final note, transfer of alien HPR genes would be greatly facilitated by molecular-based gene transformation systems. Because of the already demonstrated value of interspecific genetic resources, development of the required technology should receive high priority.

Acknowledgments

The authors acknowledge financial support from USAID, CIMMYT, The Rockefeller Foundation, California Crop Improvement Association, California Wheat Commission, and the Western U.S. Regional Project on Integrated Pest Management (W-161). This support has made it possible to investigate the vagaries of BYD. The late Joseph A. Rupert in 1969 gave initial encouragement for continuation of BYD research because of its international importance. Arthur Klatt, Clive James, and the late Glenn Anderson of CIMMYT were instrumental in facilitating the research at Davis and providing the impetus for an international BYD research network.

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Distribution of Barley Yellow Dwarf Virus Isolates in Pennsylvania and the Effect of the PAV Isolate on Yield of Oats

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Abstract

From 1984 to 1986 isolates of barley yellow dwarf virus (BYDV) were identified in small grains collected from three cereal management regions of Pennsylvania. Of 300 plants, 82, 19, 9, and 4% were infected with isolates resembling PAV, RPV, MAV, and RMV, respectively, based on enzyme immunoassays and aphid transmission bioassay tests. Of these plants, 16% were infected with two isolates, suggesting that infection resulted from inoculation by two aphid species, or by dependent virus transmission from doubly infected source plants. Only 5% of 329 alate Rhopalosiphum padi *(L.) and* Sitobion avenae *(Fabricius) collected in the field from asymptomatic oats (*Avena sativa *L.) were viruliferous for BYDV. Field inoculations of oats at 4 or 6 weeks after planting with a PAV isolate significantly reduced the number of panicles/m, seed/m, and seed/panicle. The 1000-kernel weight was not affected. Yields in plots infected with viruliferous aphids at 4 or 6 weeks after planting were reduced by 86 and 28%, respectively, compared to uninfested controls. Significant differences were observed between 1985 and 1986 relative to annual fluctuations in environmental parameters influencing aphid vector biology.*

Barley yellow dwarf virus (BYDV) occurs annually in spring and fall planted small grains in Pennsylvania. Although BYDV incidence has not been of economic importance in recent years, epiphytotics do occasionally occur resulting in consistent yield losses. Pennsylvania is divided geographically into three environmentally distinct grain management areas. Region 1 has a mild climate and includes the southeastern portion of the state, region 2 includes the centrally located counties, and region 3 consists of the western and northern counties, which are generally at higher elevations and have cooler climates. Recommended planting dates and cultivars differ for each region. Incidence of BYDV can vary year to year from region to region. Besides the yield loss directly attributed to BYDV infection, recent studies have indicated that BYDV may reduce winter survival of fall planted grains (Delserone and Frank 1985) and influence infection by fungal pathogens (Sommerfeld et al. 1987).

The purposes of the studies reported here were to identify isolates of BYDV occurring in small grains of Pennsylvania, to determine their geographical distribution, and to determine whether or not the most prevalent isolate was affecting yield of oats under field conditions.

Materials and Methods

Small grains were sampled from eight counties of Pennsylvania, representing the three management areas, from 1984 to 1986. Most collections were of spring oats planted in April of each year. BYDV isolates were identified by enzyme-linked immunosorbent assays (ELISA) utilizing polyclonal antibodies specific for the New York RPV, RMV, MAV, and PAV isolates supplied by W.F. Rochow (Cornell University). Only plants showing yellowing or stunting symptoms typical of BYDV infection were selected for testing. In addition, some samples were tested for virus recovery by aphid transmission bioassays utilizing *Rhopalosiphum padi* (L.), *R. maidis* (Fitch), *Sitobion avenae* (Fabricius), and *Schizaphis graminum* (Rondani) (Rochow 1969). Field collected aphids were tested for their ability to transmit BYDV during a 5-day inoculation feeding on 7-day-old seedlings of California Red oats (*Avena byzantina* K. Koch =*Avena sativa* L.).

Effects of BYDV on yield components of Noble oats (*A. sativa*) a commonly grown cultivar, were determined in field plots inoculated with the New York PAV type-isolate of BYDV (PAV-NY). Test plots were sprayed with systemic acephate insecticide (0.56 kg/ha) on a 10-day schedule, except for a 2-week period before plants were infested with aphids for inoculation. In addition, all plots were treated with the fungicide mancozeb (2.2 kg/ha) to prevent foliar fungal infections. Oat test plots were surrounded on all sides by plots of barley (*Hordeum vulgare* L.) to provide a barrier between treatments.

Treatments consisted of: no inoculation, inoculation of 4-week-old oats, and inoculation of 6-week-old oats with the PAV-NY isolate of BYDV. Treatments were applied to one meter of drill row in the center of each 5-row plot. Plants were infested with *R. padi* acquisition fed 48 hours on PAV-NY infected oats. Approximately 200 aphids were applied to each test plot and the plots were caged to contain the aphids for 3 days. All plants were sprayed with acephate 7 days after initiating the inoculation feeding. Plants were rated at GS-9 (Feekes scale) for disease severity (Doodson and Saunders 1970). When mature, each of four replicates for each treatment was harvested and the components of yield were determined by counting the number of tillers/m, heads/m, seeds/head, and total yield. Data were subjected to analysis of variance and mean separation with a Waller-Duncan k-ratio t-test.

Results

Four isolates of BYDV were identified by ELISA in Pennsylvania small grains (Table 1). The SGV isolate was not identified by ELISA or by aphid bioassay. Of 282 plants tested, 225 (80%) were found to be infected. All four isolates were recovered from each of the three management regions, with the exception of MAV, which was not identified in region 1. PAV-like isolates were the most common isolates recovered in each region during each of the 3 years. When data from ELISA and aphid bioassays were pooled, 82, 19, 9, and 4% of 300 plants were infected with PAV, RPV, MAV, and RMV, respectively. Of these, 16% were doubly infected with combinations of PAV + RPV (10%), PAV + RMV (2%), MAV + RPV (3%), and MAV + RMV (1%). When 329 alate *R. padi* and *S. avenae* were collected from asymptomatic oats in early June from nine fields in the three regions and tested for BYDV transmission, 15, 1, and 1 transmitted PAV, RPV, and MAV, respectively. One *R. padi* transmitted RPV and PAV simultaneously.

Table 1. Identification of barley yellow dwarf virus isolates in three environmentally distinct cereal growing regions of Pennsylvania by enzyme immunosorbent assay, 1984-86

Region	Total no. plants tested	No. plants infected with the BYDV isolate indicated[a]			
		RPV	RMV	MAV	PAV
1	51	2	2	0	33
2	111	21	4	11	80
3	120	28	1	14	65
Total No.	282	51	7	25	178
Percentage		18	2	9	63

Region 1 (Lancaster Co.), region 2 (Centre, Columbia, Lycoming, and Union Co.), and region 3 (Somerset and Westmoreland Co.).

[a] Data include counts from 16% of the plants that were doubly infected with two isolate types.

Results of field inoculation studies in 1985 (Table 2) indicated that age of plant at time of inoculation significantly influenced yield components. The yield reduction was 87 and 27% relative to controls for treatments inoculated at 4 and 6 weeks, respectively. In 1985 the weather was favorable for aphid feeding. Four days after the plots were infested aphid counts indicated that 13 to 16 aphids were feeding on each plant and all plants were infested. When this experiment was repeated in 1986, the weather during the 4-week inoculation was abnormally cold and aphids remained inactive for much of the inoculation period. The yield reduction in 1986 was 23 and 28% for the 4- and 6-week treatments, respectively. An average of only 5 to 6 aphids were observed feeding on plants and during the 4-week inoculation no aphids were observed on 12% of the plants. These results demonstrate the influence of environment on BYDV incidence through effects on vector behavior and survival.

Discussion

Results of field surveys indicate that a range of BYDV isolate types occurs in all three regions of Pennsylvania; however, PAV isolates are the most common. For this reason the PAV-NY isolate was selected for field evaluation of crop loss related to age of plant at inoculation. In this study the oat cultivar, isolate type, date of inoculation, and vector population were carefully controlled. No other BYDV isolates were recovered from the plots during the season, and foliar pathogens were not observed. For these reasons the observed differences in yield among treatments were believed to be due to the age of the plants at the time of inoculation. Yield losses of 87% obtained in 1985 by inoculation at 4 weeks of age were severe and are not typical under Pennsylvania growing conditions. These plants were uniformly severely stunted, with much leaf reddening. Plants in the 1985 6-week treatment and both treatments in 1986 showed less severe symptoms. These plants showed little stunting or reddening, and only mild chlorosis. Yield losses from these treatments of 23 to 28% are considered more typical for our growing conditions. The 87% yield reduction of the 4-week treatment, however, does indicate the potential for yield loss under ideal environmental conditions favoring early aphid dispersal and population growth.

The discovery that 16% of the field survey plants were doubly infected suggests that care should be taken when selecting for BYDV tolerance under field conditions, or when using unidentified BYDV isolates. Cultivars respond differently to various BYDV isolate-types (Baltenberger et al. 1987; Gildow and Rochow 1983). The incidence of mixed infections also increases the possibility that dependent virus transmission (Rochow 1982) may play a significant role in BYDV epidemiology in Pennsylvania.

Table 2. Effect of infection with the PAV isolate of barley yellow dwarf virus on yield components of Noble oats (*A. sativa*)

Yield components	Age of plants at time of inoculation 4 wk	6 wk	Control
Disease severity	6.6	4.7	0.0
Yield (g/m)	10.6	57.6	79.4
Heads/m	48.3	89.3	102.0
Seed/head	6.7	21.9	24.3
1000-kernel weight (g)	32.4	29.4	32.0

Disease severity values are the means of 100 samples from 4 replications based on a 0-8 scale with 8 being maximum stunting and chlorosis (Doodson and Saunders 1970). Yield component values are the means of four replicates. All values were significantly different based on Waller-Duncan t-test, except for TKW.

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Barley Yellow Dwarf In Morocco: Occurrence and Crop Loss Assessment

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Abstract

*The PAV-like isolate of barley yellow dwarf virus (BYDV) predominated in infected wheat (*Triticum aestivum *L.), oat (*Avena sativa *L.) and barley (*Hordeum vulgare *L.) samples collected from fields in the Rabat, Casablanca, and Marrakech areas of Morocco and tested by aphid transmission in 1981. One PAV-like isolate from the Rabat area was used as inoculum in a crop loss assessment trial in the Chaouia area in 1982. Bread wheat variety Nesma 149 suffered yield losses of 44 and 30% following early and late inoculations. The total yield, 1000-kernel weight, and head weight were adversely affected by the inoculations.*

Barley yellow dwarf (BYD), first described by Oswald and Houston (1951) in California as a problem of cereals and grasses, has been reported from many parts of the world (Rochow 1970). It is caused by a persistent virus with isometric particles (24 nm diameter), which is transmitted by aphids. Five isolates of barley yellow dwarf virus (BYDV) have been distinguished on the basis of the specificity of aphid transmission (Rochow 1969; Gill 1969; Rochow and Muller 1971). Three isolates were identified in Morocco (El Yamani 1980). The nonspecific (PAV-like) isolate had a higher incidence than the *Rhopalosiphum padi* (L.)-specific (RPV-like) and the *R. maidis* (Fitch)-specific (RMV-like) isolates. *Sipha (Rungsia) maydis* Passerini was another aphid vector identified in Morocco.

Damage by BYD epiphytotics is described in the literature. In 1969, losses in barley (*Hordeum vulgare* L.) yields due to BYD were estimated to be about 34% in Manitoba, Canada (Fisher et al. 1976). Gill (1980) in Canada reported losses of about 7% in wheat (*Triticum aestivum* L.) yields in 1978. In France, Bayon and Ayrault (1977) reported losses of 2.5 to 3.0 t/ha for spring cereals and 2.0 to 3.5 t/ha for winter cereals. In 1977, losses due to this disease in Australia were estimated to be 28 to 54% of the yield, depending on the time of infection (Smith et al. 1977).

In the Tessaout area of Morocco, J. Burleigh, Professor at the Institute of Agronomy and Veterinary Sciences, Hassan II, (personal communication) estimated BYD incidence of 75% in the bread wheat variety Nesma 149 and 90% in the durum wheat (*Triticum turgidum* L. rom. *durum*) variety Kyperounda. The present study follows up aspects of BYD in Morocco.

Materials and Methods

Various samples of oats (*Avena sativa* L.), barley, and wheat with BYD-like symptoms were collected in the Rabat, Casablanca, and Marrakech areas of Morocco during the 1981-82 growing season. These samples were used as sources of virus in aphid transmission studies with *R. padi*, *R. maidis*, *Sitobion (=Macrosiphum) avenae* (Fabricius), and *Schizaphis graminum* (Rondani). These species were maintained as virus free cultures in our laboratory throughout this study. All species were given a 4-day acquisition feed on detached leaves (Rochow 1969) and a 3-day inoculation period on healthy seedlings of Coast Black oats. The plants were then treated with nicotine insecticide to rid them of the aphids, and transferred to a greenhouse for symptom development.

Isolates were identified through transmissions with the four aphid species. After the first inoculation, the infected plants were used as sources for later acquisitions. Subsequent reactions were recorded. In a yield loss assessment trial at the Sidi El Aydi Experiment Station, *R. padi* that had been reared on diseased oats were used to inoculate Nesma 149 with a PAV-like isolate. Aphids on diseased leaf pieces were distributed throughout the plots to be inoculated. A randomized complete block

with four replications and four treatments was used. The plots were eight rows wide and 6 m long. The four treatments were:

1) Early inoculation: plants were at stages 1-2 on Feeke's scale (Large 1954)

2) Late inoculation: plants were at stages 5-6

3) Insecticide treatment: Roxion (dimethoate 40%) was used every 15 days until the heading stage

4) Natural check: untreated plots

The following parameters were measured: number of tillers per linear m, number of diseased plants per m^2, number of heads with blasted florets per m^2, plot yield, head weight, and 1000-kernel weight.

Results

Symptom and isolate identification

Transmissions from the collected samples to Coast Black oats are shown in Table 1. *R. padi* transmitted all the six isolates quite efficiently. *S. graminum* and *R. maidis* transmitted all isolates but one, and *S. avenae* transmitted all but two.

Subsequent transmissions from plants originally inoculated with isolate R-821 yielded the results in Table 2. These recurrent transmissions showed that *R. padi*, *R. maidis*, and *S. avenae* kept their ability to transmit the virus while *S. graminum* exhibited some irregularity in its performance.

Control plants consisting of Coast Black oats caged with healthy aphids of the same species showed no positive symptoms throughout this study.

Loss assessment

The data from the yield loss assessment trial are summarized in Table 3. Early inoculation gave the highest number of plants with symptoms per m^2 and had a significant effect on total yield (44% reduction) and on all yield components except the number of tillers per linear meter. Late inoculation, on the other hand, had a significant effect only on total yield (30% reduction), head weight, and 1000-kernel weight. No statistically significant difference was found between the insecticide treatment and the natural check for any of the parameters assessed. The regression analysis yielded a weak negative correlation between the number of diseased plants and yield losses ($r = -0.36$).

Table 1. Sample origins and aphid transmission results

Sample code	Sample origin	*R. padi*	*R. maidis*	*S. avenae*	*S. graminum*
		no. plants infected/no. inoculated			
R-821	Rabat area	11/12	8/12	11/12	10/12
R-822	Rabat area	7/12	6/12	8/16	6/12
C-823	Casablanca area	8/12	0/12	0/12	1/12
C-824	Casablanca area	12/12	6/12	7/12	5/12
M-825	Marrakech area	11/12	6/12	4/12	0/12
M-826	Marrakech area	10/12	7/12	0/12	4/12

Discussion

The aphid transmission tests proved that the virus was the causal agent of the symptoms observed on the collected samples. The difference in ability of the aphid species to transmit isolates of BYDV was similar in Morocco to that reported by Oswald and Houston (1952) and Rochow (1969, 1979) from the USA, Gill (1969, 1970) from Canada, and Fisher and coworkers (1977) from Europe.

Our isolates R-821, R-822, C-824, and M-825 seem to resemble the nonspecific isolate (PAV-like) of Rochow (1969), transmitted nonspecifically by *R. padi*, *S. avenae*, *R. maidis*, and *S. graminum*. Data presented in Table 2 show that the R-821 isolate is a nonspecific strain. The other three isolates in the group are probably similar. No transmissions were performed with the isolates C-823 and M-826, but these seemed to be similar to the *R. padi*-specific isolate (RPV-like) of Rochow (1969). It seems that the BYDV isolates in Morocco are similar to isolates known elsewhere. The incidence of the PAV-like isolate in Morocco is quite high (four out of six isolates studied), which could be a serious problem in the cultivation of cereals, given its nonspecific transmission.

Table 2. Results of recurrent transmissions from the sample R-821 originating from Rabat area

Plant sources obtained after inoculation by	No. plants infected/ no. inoculated	Recurrent transmission by:			
		R. padi	*R. maidis*	*S. avenae*	*S. graminum*
R. padi	11/12	10/12	6/12	10/12	7/10
R. maidis	8/12	8/12	6/12	10/12	8/12
S. avenae	11/12	12/12	9/12	10/12	9/12
S. graminum	10/12	8/12	0/12	7/12	0/12

Table 3. Effect of BYDV infections on the yield and yield components of the bread wheat NESMA 149

Treatments	Diseased plants/ m^2 at stage 9[a]	Tiller no./m	No. of heads w/ blasted florets/m^2	Head weight (g)	1000-kernel weight (g)	Total yield (t/ha)
Early inoculation (at plant stages 1-2[a])	17**	166	20**	0.79**	33.13**	1.07**
Late inoculation (at plant stages 5-6[a])	5	161	10	0.99**	40.50**	1.33**
Insecticide treated	1	163	10	1.25	47.81	1.89
Natural check	2	148	12	1.15	45.07	1.70

[a] Feeke's scale (Large 1954).
** A statistically significant difference between the inoculation treatment and the insecticide treatment at 1% level.

The loss study showed the importance of early infections on total yield and yield components. The results of this study were similar to those obtained by Smith et al. (1977) in Australia, however the number of tillers was not affected in our study. The low coefficient found with regression analysis in our experiment suggests the difficulty of using external symptoms to predict yield losses under our conditions. This is different from Smith et al. (1977) who found high correlation coefficients.

Acknowledgments

The support and understanding of the INRA director and sub-directors in general are acknowledged. We also appreciate the help and good care given to this work by our technicians and the Sidi El Aydi Experiment Station staff.

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Effects of Barley Yellow Dwarf Virus on Cereals in Kenya

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Abstract

Field trials were conducted in Njoro to determine the effects of barley yellow dwarf virus on the growth and yield of some recently released varieties of wheat (Triticum aestivum *L.) and barley* (Hordeum vulgare *L.), commercially grown in Kenya. A field-collected isolate of the virus was used to infect three varieties of each crop using viruliferous* Rhopalosiphum padi (L.). *All the varieties tested were classified as susceptible to the virus, and suffered losses in plant height, 1000-grain weight, and grain yield.*

Barley yellow dwarf (BYD) is a severe disease in cereals with a world-wide distribution (Rochow 1970). The main crops attacked include wheat *(Triticum aestivum* L.), barley *(Hordeum vulgare* L.), oats (*Avena sativa* L.), and several species of pasture grasses (Rochow 1970). The disease is transmitted by over 18 species of aphids (Jedlinski 1981) although the main vectors are *Rhopalosiphum padi* (L.), *R. maidis* (Fitch), *Sitobion avenae* (Fabricius), *Metopolophium dirhodum* (Walker), and *Schizaphis graminum* (Rondani) (Gildow 1984).

BYD has been reported as a sporadic disease in Kenya (Torres 1984), but in 1985-86 severe infection was noted in some parts of the country, mainly on barley and oats.

Information on the losses due to BYD in Kenya is lacking. Therefore this work was initiated to study the effect of barley yellow dwarf virus (BYDV) on yield of various cultivars of cereals grown in Kenya.

Materials and Methods

Virus-free colonies of *R. padi* were started from an original aphid collected from wheat in the field. The aphids were maintained on caged oats (Suregrain) in the glasshouse.

A field isolate of BYDV was collected from barley in Njoro and was also maintained on oats (Lampton) in the glasshouses. Viruliferous aphid colonies were obtained by allowing the aphids a 3-day acquisition feed on the infected oats.

Field plots were planted at the National Plant Breeding Station, Njoro, using the following varieties: (wheat) Kenya Kulungu, Kenya Tumbili, and Kenya Popo; (barley) Bima, Tumaini, and Kenya Research.

The experiment for each cultivar was planted in three replicates in a randomized block, split-plot design with varieties as main plots and healthy controls and BYDV-inoculated treatments as sub-plots.

Two 2-m rows of each variety were planted 35 cm apart to form a plot. The plots were separated by two guard rows of oats. At least five viruliferous aphids were placed on each plant at tillering stage (GS 22, Zadok et al. 1974). Both the inoculated and healthy plants were sprayed with malathion after a 7-day inoculation period, and then after every 2 weeks to minimize virus transmission by the aphids.

Symptoms of BYDV were recorded for all the plots at 2-week intervals. Ten individual plants were randomly selected from the center 1.6 m of the two-row plots. Data collected from the plants included grain yield, 1000-grain weight, seeds per head and plant height. The effect of BYDV was measured as the difference between the healthy control and the corresponding inoculated plots of the different parameters. (Yount et al. 1985).

Results

The disease symptoms observed on barley were similar to those described by Rochow (1970) and Mathre (1982). Infection was uniform throughout the inoculated barley plots, with all the plants becoming infected. Symptoms appeared 21 days after inoculation in all three barley varieties tested, while the uninoculated plants remained symptomless. The inoculated plots showed intense leaf yellowing and moderately severe dwarfing. On a scale of 0 to 9, where 0 = no symptoms and 9 = severe dwarfing, complete yellowing, and considerable sterility (Qualset 1984), the barley plots had an average score of 6.

For barley, the mean losses in grain yield due to BYDV were as follows: Kenya Research 54.7%, Tumaini 26.4%, and Bima 16.5% (Table 1).

In the wheat experiments, all the plants in the inoculated plots became uniformly infected while the control plants remained healthy. Leaf chlorosis was observed 20 days after inoculation in Kenya Kulungu; symptom development lagged for about 15 to 20 days in Kenya Popo and Kenya Tumbili. Leaves in the infected plants developed leaf tip chlorosis followed by yellowing along the margin, which gradually extended the full length of the leaf. Leaf chlorosis was most severe in Kenya Kulungu at the onset of symptoms, scoring 8 (Qualset 1984), but the crop recovered considerably as it neared maturity, getting an average score of 5. Infested plots of Kenya Popo and Kenya Tumbili retained an average score of 5 on the same scale for symptom expression, from the onset of symptoms to maturity.

Grain yield was significantly reduced in the three wheat varieties tested (Table 1), all being affected with an average reduction in yield of 46.7%.

Discussion

On the basis of symptom severity and reduction in yield and height, the cultivars tested were classified as being susceptible to BYD.

Although all the inoculated barleys had moderately severe symptoms (average score, 6), Bima had the lowest reduction in both yield and 1000-grain weight, 16.4 and 8.0% respectively. This is in comparison to an older variety, Kenya Research, which experienced the highest reduction in yield and 1000-grain weight, 54.7 and 18.8%, respectively, due to virus infection. High yield reduction incurred in barley plots artificially infected with BYDV have also been

Table 1. Effect of barley yellow dwarf virus on growth and yield of barley and wheat varieties commercially grown in Kenya

	Plant height (cm)		Seeds/ head		1000-grain weight (g)		Yield (90-kg bags/ha)	
Variety	C	I	C	I	C	I	C	I
Barley								
Kenya Research	113.7	100 *	31	29	42.6	34.6*	12.8	5.8
Tumaini	105.2	87.9*	30	28	49.0	44.9	38.0	28.0*
Bima	101.1	89.1	26	27	46.2	42.5	29.4	24.5
Mean	106.7	92.3**	29	28	45.9	40.7*	26.7	19.4*
Wheat								
K. Popo	123.1	120.7	38	22**	46.4	41.3**	21.2	12.5*
K. Tumbili	125.4	119.9	35	24*	39.5	37.1	31.6	15.2**
K. Kulungu	112.4	95.6**	35	18**	39.9	38.8	17.9	10.1*
Mean	120.3	112.10**	36	21**	41.9	39.1**	23.5	12.6**

C = control, I = inoculated. Differences between control and inoculated means are significant.
* P = 0.05 and ** P = 0.01 using LSD.

reported as follows; 14-26%, Burnett and Gill 1976; 39%, Watson and Mulligan 1960; and 44.7% Yount et al. (1985). Mean yield reduction in inoculated wheat was 46.7%. Similar results have been reported in which the yield of spring wheat was lowered by 36% in experimental fields where a virulent isolate of BYDV had been inoculated (Watson and Mulligan 1960).

Plant stunting occurred in the wheat varieties, although it was the short variety, K. Kulungu, that was significantly more dwarfed by the virus than the taller varieties, K. Popo and K. Tumbili.

There was one main difference between the responses of wheat and those of barley. Yield losses of wheat were much higher than those of barley despite the symptoms of BYD in wheat being much less noticeable than in barley. It is therefore likely that Kenya wheat farmers suffer considerable losses each year from this virus without realizing it.

More experimental work with Kenya wheat and barley varieties is therefore needed in order to estimate more accurately the losses incurred by natural infection of various isolates of BYDV in these crops.

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A Cereal Germplasm, "Zhong 4," for Resistance to Barley Yellow Dwarf Virus in China

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Abstract

From 1983 to 1986, 14,000 samples of cereal germplasm from domestic and foreign sources were screened for resistance to barley yellow dwarf virus (BYDV) in the field using the hill plot technique. Selected germplasm was inoculated with the GPV, GAV, and PAGV variants of BYDV by Schizaphis graminum *(Rondani),* Sitobion avenae *(Fabricius), and* Rhopalosiphum padi *(L.), respectively. Zhong 4, a cross of two Chinese wheats (*Triticum aestivum *L.), Ke Qiang and Nan Da 2419, with* Thinopyrum intermedium *(Host), and the barley cultivars Post, Atlas 68, and Sutter were good sources of resistance to BYDV. Zhong 4 showed mild symptoms and contained a low concentration of virus as shown by enzyme-linked immunosorbent assay.*

Effective control of barley yellow dwarf virus (BYDV) by genetic resistance has been successfully demonstrated in barley (*Hordeum vulgare* L.). However, information on the inheritance of resistance to BYDV in wheat (*Triticum aestivum* L.) is still very scarce. Immunity has not been found, and no major gene conditioning resistance has been identified.

During 1983-1986, we collected about 14,000 entries of cereal crops from domestic sources and 66 foreign countries. Among them, Post, Atlas 68, and Sutter barley could be recognized as resistant to BYDV. Anza wheat, which was highly resistant to BYDV in the USA, was susceptible to BYDV in China.

We obtained some germplasm of a line called Zhong 4 from Guo Li yuan in the Institute of Plant Protection, Gansu Academy of Agricultural Sciences, in 1984. This was a line that was produced in 1953 by Sun Shan-cheng by crossing *Thinopyrum intermedium* (Host) with an F_5 derived from the cross of Ke Qiang and Nan Da 2419, two Chinese wheats. After inoculation with viruliferous *Schizaphis graminum* (Rondani), the Zhong 4 entries expressed such mild symptoms that they looked like healthy plants. To confirm the results, we carried out further experiments.

Screening in the Field

Zhong 4, together with other entries, was seeded in hill plots (10 seeds per hill) as is usual for our field testing. All procedures were the same for every entry except that there were two replications of Zhong 4. The disease score was 0.0, 1.0, and 0.5 in 1985, 1986, and 1987, respectively, on a 0-9 scale (Qualset 1984). In the field, even though some plants of Zhong 4 were infected with BYD, the symptoms were very mild; Zhong 4 looked like a green island surrounded by a yellow sea.

Screening with Three Strains of BYDV

The GPV, GAV, and PAGV isolates of BYDV (Zhou and Zhang, these Proceedings) were used as virus sources and *S. graminum*, *Sitobion avenae* (Fabricius), and *Rhopalosiphum padi* (L.) as virus vectors, respectively. Leaves with typical BYDV symptoms were detached from infected plants and put into covered dishes, infested with aphids, and placed in growth chambers at 15°C for a 2-day virus acquisition feed. From 45 to 50 viruliferous aphids were transferred to each Zhong 4 hill and allowed to feed for 7 to 10 days. There were no noticeable differences observed among the effects of the three strains of BYDV in the 2 years of testing. These strains of BYDV were highly virulent on all other germplasm being tested.

Enzyme-linked Immunosorbent Assay (ELISA)

The external symptoms of Zhong 4 after infection with BYDV were very mild. Enzyme-linked immunosorbent assay (ELISA) was used for measuring virus concentration. After inoculation with GAV, 0.5 g of detached leaves were pulverized with buffer. Antibodies of MAV supplied by W.F. Rochow and GAV made in our laboratory were purified and conjugated with alkaline phosphate. The antibody-antigen-conjugated IgG procedure was used.

The results indicated that the virus concentration in infected plants was very low. Similar results with Zhong 4 have been obtained in the CSIRO Plant Industry Laboratory, in Canberra, Australia.

Because of these results, we concluded that Zhong 4 was not resistant to infection, but resistant to reproduction of virus.

Thinopyrum intermedium, One of the Parents of Zhong 4

Plants grown from seeds of *T. intermedium* obtained from Sun Shancheng were inoculated with *S. graminum* that were infected with GPV and GAV isolates of BYDV. No symptoms were observed. To test *T. intermedium* as a host of BYDV, we fed virus-free *S. graminum* on detached leaves from *T. intermedium* for 2 days at 15°C, 30 days after inoculation with BYDV. A control of oats inoculated with the same virus isolates at the same time, which showed typical symptoms, was also checked for infection using the same method. After this acquisition feed, the *S. graminum* were transferred to Coast Black oats. At the same time, the detached leaves were tested for BYDV by means of ELISA. Both results showed that *T. intermedium* was not infected with BYDV. Therefore, it is suggested that crossing *T. intermedium* and wheat has led to the resistance gene from *T. intermedium* being introgressed into the progeny-Zhong 4, giving a high level of resistance to BYDV.

Biological and serological tests as described are being used to screen the progeny of crosses between wheat and Zhong 4.

We have noticed that Zhong 4 is susceptible to infestation by *S. graminum*.

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Breeding Program for Resistance to Yellow Dwarf Virus in Winter Barley

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Abstract

*Breeding for barley (*Hordeum vulgare *L.) genotypes tolerant or resistant to barley yellow dwarf virus (BYDV) was started in Italy in 1982. Two resistant lines from the International Center for Agricultural Research in the Dry Areas (ICARDA) were crossed with four high yielding winter six-row susceptible cultivars. Field observations in segregating populations indicate that selection considering plant height and grains/spike could be efficient for improving specific resistance in winter barley. Commercial varieties and experimental lines of winter barley derived from existing breeding programs for yield and other agronomic traits were evaluated over a period of 2 years under field conditions for tolerance of genotypes to BYDV by visual assessment based on disease severity symptoms. Three tolerant six-row genotypes and three high yielding commercial varieties are being used for the development of a synthetic population by recurrent selection.*

A shift in barley (*Hordeum vulgare* L.) production to the highly fertile soils of the north of Italy followed the introduction in 1960 of new winter varieties from England, Germany, and France, which were more profitable than wheat (*Triticum aestivum* L.) or other forage crops in fertile soil. This has been accompanied by some reduction in area in the more traditional areas of the south. Despite the increased area and a remarkable increase in yield per unit area, barley production is still less than the domestic requirement (Stanca et al. 1986).

Since 1970, farmers in the northeast of Italy have sown winter barley at the end of September or the beginning of October to optimize the variety performance from early sowing. Aphids coming from cereal volunteers or maize (*Zea mays* L.) to barley seedlings in early autumn seriously damage the crop by transmitting barley yellow dwarf virus (BYDV). The first severe damage to winter barley in the northeast was observed in the spring of 1978, with yield losses in some fields of nearly 90% (Conti 1978; Snidaro 1978).

Although two genes for specific resistance to BYDV in barley have been identified, the incompletely dominant gene Yd_2 has been used in the production of BYDV resistant cultivars (Rasmusson and Schaller 1959; Rochow 1961; Schaller et al. 1964; Poehlman et al. 1981; Grafton et al. 1982).

Since 1982, two breeding programs have been initiated: 1) to transfer the Yd_2 gene into winter barley cultivars and 2) to develop a synthetic population with the final aim of accumulating genes for tolerance to BYDV.

Materials and Methods

Transferring the Yd_2 gene

In 1984 six resistant lines carrying the Yd_2 gene, kindly supplied from the International Center for Agricultural Research in the Dry Areas (ICARDA), were evaluated under field conditions. Four of them were discarded for poor field performance, and two (IBYT 29 and CBB 130) were used for the crossing program. Four six-row high yielding susceptible winter barley varieties were crossed with the two ICARDA lines.

In 1985, 10 F_2 populations of about 2000 plants were seeded at the end of September as spaced plants. Visual

selection was done at tillering and flowering for earliness (days from sowing), plant height (cm), and grains/spike. In 1986-87, 120 F_3 selected progenies for each cross were evaluated in field plots and selected for plant height and grains/spike.

Developing a synthetic population
A total of 400 lines and commercial varieties were tested for reaction to BYDV over a 2-year period (1982-83). The materials were sown at Tauriano at the end of September as single 1 m^2 plots (8 rows) in a field adjacent to maize (*Zea mays* L.). This provided natural aphid flights from maize plants and volunteers to barley plots. Tests on *Avena (byzantina* K. Koch) =*sativa* L. demonstrated that aphids collected from barley seedlings in October and November were transmitting BYDV. A control plot of each variety was treated with a chemical product. Classification for disease reaction was made visually at tillering (for leaf yellowing) and after flowering (for dwarfism), using a scale from 0 to 9 in which 0 = immune and 9 = highly susceptible. The final estimate was based on a combination of these two scores (leaf yellowing and dwarfism). Two winter varieties and three breeding lines were considered tolerant. All possible crosses among these five genotypes, without reciprocals, were made to develop a synthetic population using recurrent selection.

Results and Discussion

Resistance
The two selected resistant lines from ICARDA (CBB 130 and IBYT 29) were not immune, but proved to be sufficiently resistant. There were no stunted plants or leaf yellowing at tillering stage, as in the commercial varieties. The reduction in plant height and grains/spike due to virus infection was very high in the six-row cultivars Arma, Gerbel, and Plaisant (Table 1). Barberousse performed well even though at seedling stage it showed the same amount of yellowing as the other varieties.

The distribution of the F_2 population for plant height showed a variation from susceptible to resistant. Selection was practiced within the interval from the mean value of the resistant parent to the mean value of the protected susceptible parent (Figure 1). Eight percent of the F_2 plants were selected.

In 1986-87 the F_3 progenies were evaluated in Pozzuolo. The infection of BYDV was uniform, as in F_2, allowing the selection of progenies on the basis of plant height, grains/spike, and leaf yellowing. For plant height, 12% of F_3 progenies were selected and none of them showed stunting. The selected progenies were mainly distributed in the interval between the resistant parent and the protected susceptible one (Figure 2).

Table 1. Reaction to barley yellow dwarf in resistant lines and susceptible varieties of winter barley for plant height, grains/spike, and leaf yellowing

Genotypes	Plant height (healthy) (cm)	Reduction in diseased plant (cm)	Grain/spike (healthy) (no.)	Reduction in diseased plant (no.)	Leaf yellowing visual score (0-9)[a]
IBYT 29	94	7	68	8	0
CBB 130	88	13	66	6	0
Arma	97	51	54	35	8
Barberousse	95	36	54	19	6
Gerbel	102	45	58	40	9
Plaisant	107	52	64	40	7
SE	4	11	4	9	

[a] 0 = immune; 9 = susceptible.

The same progenies were evaluated for grains/spike and significant differences among crosses were found. No significant correlation between plant height and grains/spike has been discovered. This indicates that resistant progenies are not necessarily taller. On the basis of our results, it seems that selection in F_3 considering plant height and grains/spike could be efficient for improving specific BYDV resistance in winter barley.

Tolerance

The program for nonspecific resistance was started by evaluating 400 lines and commercial varieties. After 2 years of field evaluation, four varieties and six advanced lines were considered tolerant. The reduction of plant height due to aphid infection ranged from 15 to 36 cm (Table 2). The reductions in grains/spike were less evident, ranging from 5 to 19. Three tolerant six-row genotypes and three high yielding commercial varieties are being used for the development of a synthetic population via recurrent selection. The philosophy of this cyclical breeding scheme is to promote recombination among linked alleles, to increase the frequencies of favorable genes, and to optimize the genetic background of different desirable genes.

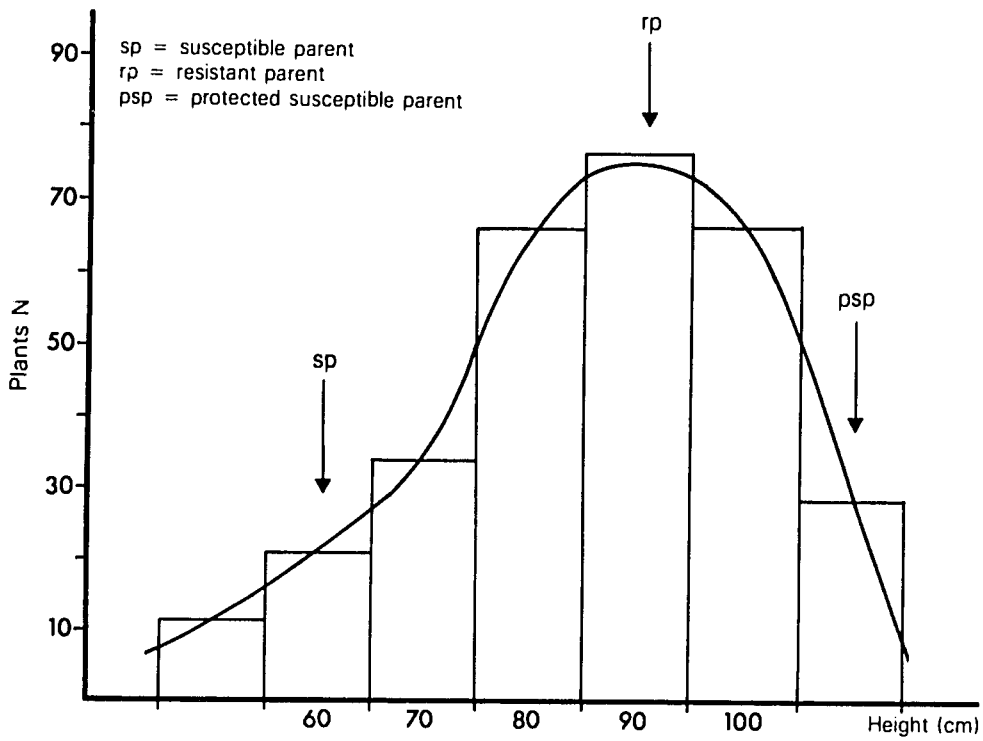

Figure 1. Frequency distribution of F_2 plants from crosses 'resistant x susceptible' for plant height.

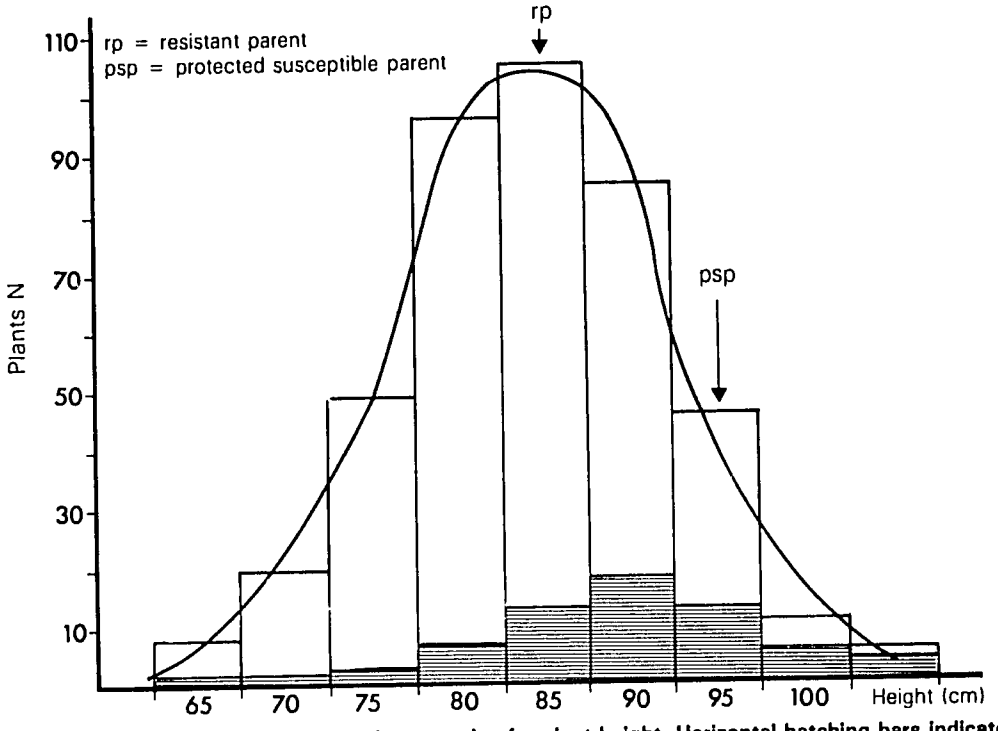

Figure 2. Frequency distribution of F_3 progenies for plant height. Horizontal hatching bars indicate selected progenies.

Table 2. Barley yellow dwarf reaction in tolerant lines and varieties of barley for plant height, grain/spike, and leaf yellowing

Genotypes	Plant height (healthy) (cm)	Reduction in diseased plant (cm)	Grain/spike (healthy) (no.)	Reduction in diseased plant (no.)	Leaf yellowing visual score (0-9)[a]
			Six-row		
Barberousse	95	36	54	19	6
Ogra	110	28	74	6	4
Fior 426	91	28	70	10	6
Fior 440	94	25	56	14	4
Fior 64.38	100	32	66	9	4
SE		3	5	3	
			Two-row		
Alpha	95	15	24	8	2
Nicola	96	35	24	6	5
Fior 272	94	20	24	5	5
Fior 447	95	29	26	8	5
A46	70	15	26	7	5
SE	7	6	1	1	

[a] 0 = immune; 9 = susceptible.

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Tolerance to Barley Yellow Dwarf Virus in Maize

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Abstract

More than 1200 inbred lines of maize (Zea mays L.) were observed to evaluate tolerance to barley yellow dwarf virus (BYDV) under natural infection in the field in Italy. Susceptibility was expressed by a score corresponding to the percentage of plants showing typical BYDV symptoms in a plot. A series of crosses between inbreds with different degrees of tolerance was also studied. The data obtained and the limitations of the results are discussed.

Maize (*Zea mays* L.) infected with barley yellow dwarf virus (BYDV), besides acting as a reservoir for the virus (Stoner 1977), suffers yield losses (Pearson and Robb 1984). Therefore we tested germplasm of this species to ascertain if suitable sources of tolerance or resistance are available.

A wide collection of inbred lines coming from temperate areas of Europe and North America were tested. Experiments aimed at elucidating the inheritance of BYDV tolerance were also performed.

Materials and Methods

Inbred lines supplied by research institutions of different countries were screened in the field under natural BYDV infection. About 1200 inbreds have been tested; information is available for about 1000. Under our conditions, at least 2 years of observations are required for an evaluation of the genotype response to BYDV. Together with the inbreds, a number of crosses (one 6 x 6 diallel and several topcross series) involving lines with different degrees of BYDV susceptibility were studied to elucidate the tolerance character in the hybrids.

The fields were located in the Friuli region of Italy, in areas well known for the frequency of natural BYDV infections (1982-1984: Tauriano; 1985-86: Pozzuolo) (Osler et al. 1984). In 1982, 950 inbreds were subjected to a preliminary screening in single plots of 15 plants. Subsequently a selected number of inbreds (150 to 250 per year) were evaluated in plots of 20 to 24 plants with two or more replications. These inbreds included the lines commonly used as parents of the commercial hybrids and those most interesting for BYDV reaction. Every 20 plots four inbreds that were highly susceptible to BYDV, maize dwarf mosaic virus, or both (33-16, W22 or B77, H56, H102) were inserted as testers to check intensity and uniformity of virus incidence in the field.

Maize lines for testing were planted at the end of April or the beginning of May; the plots were observed from the end of June until the end of August, at intervals of 20 days. The plants presenting symptoms attributable to barley yellow dwarf (BYD) were recorded as diseased and their frequency in the plot, expressed as percentage, was considered an index of susceptibility.

Results

On the basis of these criteria, a differentiation for susceptibility was possible among the inbreds. Most demonstrated some level of susceptibility, even if percentages higher than 20% were rare. The highest values are reported in Table 1 for inbreds for which more than 100 observations were collected over at least 4 years. With a lower number of data it is difficult to give a meaningful BYDV score, owing to the fluctuations of infection observed on the

same genotypes among years and among plots within years (Table 2). For the same reason, highly tolerant inbreds can be hard to recognize. Table 3 lists the lines that showed no BYDV symptoms in all the years of observation. Some other lines had a very low frequency of diseased plants (Mo17: 1 in 208; A239, AF33, AR220, F522, Lo876o2, N6, T143: 1 in about 150). Regarding this behavior, we can also presume that the aphids are differentially attracted by the various maize inbreds.

Table 1. Inbreds highly susceptible to BYDV (5 years of observations)

Inbred	No. plants examined	Positive cases no.	%
B52	123	75	61.0
B77	542	333	61.4
H102	254	97	38.2
T251	128	53	41.4
Va45	155	35	22.5
W22	734	149	20.3
W64A	126	49	38.9
33-16	1010	284	28.1

The severity of BYDV symptoms in hybrids is generally lower than in inbreds. This is evident when F_1 progeny are compared to the respective parent lines in the same field. The examples shown in Table 4 are extracted from Loi et al. (1986). The F_1 expresses a degree of tolerance to BYDV approaching that of the more tolerant parent or better. Among the hybrids under examination, those currently cultivated in the U.S. Corn Belt and in similar climatic areas showed a high level of tolerance.

Discussion

The screening of maize germplasm for BYDV tolerance exploiting natural infection can be successful. However several years of observations are required

Table 2. Variation in the frequency of plants with BYDV symptoms in the experiment fields (4 plots taken at random within each year)

Inbred	plot	1982	1983	1984	1985	1986
		no. plants showing symptoms/total no. plants				
W22	I	0/15	6/20	4/20	2/20	0/10
	II	10/15	0/20	0/20	3/15	0/20
	III	0/15	0/20	2/20	0/20	5/21
	IV	0/15	11/17	12/20	0/20	5/20
33-16	I	8/11	9/18	6/20	2/20	2/23
	II	10/12	14/16	0/20	1/15	0/21
	III	8/15	10/18	1/20	0/20	3/20
	IV	6/12	1/20	4/20	1/15	3/22

Table 3. Inbreds classified as tolerant to BYDV (number of plants without symptoms in at least 4 years of observation)

Inbred	Examined plants (no.)	Inbred	Examined plants (no.)
A541	131	N28	169
B68	162	NC234	143
C144	163	Pall	153
K41	174	SC84A	174
K302	172	T220	166
Ky226	162	T250	176
Mo23W	170	T264	167
N7B	171		

for a precise evaluation of the tolerance level of a genotype. This is due to the annual fluctuations in intensity of BYD that occur even in locations where it seems epidemic. For example, in the plots at Pozzuolo, in 1985, few plants showed BYD symptoms, even though the adjacent barley (*Hordeum vulgare* L.) crop was heavily affected by the disease and the potential vector aphids were abundant in the maize experiment field.

In spite of these difficulties, our research has identified from a large collection of maize inbreds, a series of genotypes both for complete tolerance to BYDV and for relatively high susceptibility to BYDV. Inbreds of the second group can be used as testers for monitoring BYDV spread both in open field and in greenhouse experiments. Unfortunately most of the inbreds that are highly susceptible to BYDV in the field (B52, B77, H102, W64A) are also susceptible to maize dwarf mosaic virus (MDMV), and this may have some confounding effect on symptom expression. The lines W22 and 33-16 are resistant to MDMV, but are less susceptible to BYDV.

Particularly important is the finding that some lines currently employed in hybrid formulation are tolerant to BYDV. The tolerance character, though quantitative in its expression, behaves as a dominant factor (gene) in most of the F_1 crosses considered. Therefore, one tolerant parent is sufficient to obtain a tolerant hybrid. All the commercial hybrids considered in this study showed a satisfactory degree of tolerance under our environmental conditions.

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Table 4. Susceptibility to BYDV in some F_1 crosses and their parents (P_1 and P_2)

Cross	Plants with symptoms (%)		
	P_1	P_2	F_1
B14A x B73	20.5	22.7	4.0
B73 x B84	22.7	14.5	6.3
B14A x Mo17	20.5	0.6	0.0
B73 x Mo17	22.7	0.6	1.0
B73 x Va36	22.7	2.6	3.0
B84 x Va36	14.5	2.6	3.3

Source: Loi et al. (1986).

Analysis of Genetic Resistance to Barley Yellow Dwarf Virus in Triticale and Evaluation of Various Estimators of Resistance

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Abstract

Genetic studies on barley yellow dwarf virus (BYDV) resistance of spring and winter triticale (X Triticosecale *Wittmack) were conducted using parental lines representing the full range of variability of the species. In winter types, various estimators of resistance were compared to define which method gave the most valid information for the least expense of time. The final biomass of plants inoculated with a rather large number of aphids carrying a virulent PAV-like isolate was in itself a good estimator of resistance, and visual symptom notations were also useful if the artificial BYDV inoculation was uniform and severe enough to induce pronounced dwarfing. The range of genetic variability was broader in winter triticale than in spring triticale, and winter types offered the best resistance. Evidence was found for additivity, dominance, and interactions. As the resistance in triticale is simply inherited and definitely superior to the resistance in wheat (*Triticum aestivum *L.), triticale would now seem like a key source of genes for wheat breeding through interspecific hybridization.*

Triticale (X *Triticosecale* Wittmack) is a man-made amphiploid resulting from the cross between wheat (*Triticum* spp.) and rye (*Secale cereale* L.). In wheat, no outstanding source of resistance to barley yellow dwarf virus (BYDV) has been discovered (Tola and Kronstad 1984). Rye, however, is considered resistant to BYDV (Bruehl 1961; Comeau 1986). The response to BYDV observed in triticale varies from resistance levels similar to those observed in rye to susceptibility reactions comparable to that of durum wheat (*Triticum turgidum* L. var. *durum*) (Comeau et al. 1983; Comeau 1986).

Triticale was first considered to be generally resistant to BYDV (Larter 1974), but interest in the disease was then very limited. However, with the increase in importance of the new crop, the importance of BYDV in triticale progressively became apparent. It is now widely recognized that barley yellow dwarf (BYD) is the major virus disease observed on triticale (CIMMYT 1979; Skovmand et al. 1985).

Genetic variability for BYDV resistance has been reported by several workers and many resistant lines have been identified (CIMMYT 1979; Singh et al. 1979; Apablaza and Celis 1982; Comeau and St-Pierre 1982, 1983; Comeau et al. 1983; Burnett 1984; Kegler et al. 1985). Most of the screening work for BYDV resistance has been accomplished on spring triticales. BYDV resistance is better in triticale than in wheat (Apablaza and Celis 1982; Comeau and St-Pierre 1983; Comeau 1986), and the best winter triticales have shown resistance levels superior to those observed in spring triticale (Comeau and St-Pierre 1983, 1984). However, nothing is known of the genetic basis of the resistance observed in triticale. A good knowledge of the genetics of resistance to BYDV in triticale is essential in adopting strategy for detection and transfer of sources of resistance either within triticale or from triticale to wheat, in which additional sources of resistance are urgently needed (Qualset 1984).

Genetic resistance to BYDV was studied in triticale at Laval University during 1984 to 1986. Two of our objectives were to determine the importance of genetic variation found in winter triticale and to investigate the genetic basis of the variation observed in BYDV resistance in triticale, both in spring and winter types. Following some preliminary observations on winter triticale, we hypothesized that spring and winter triticales differ in their range of response to viral infection,

which is likely as spring and winter triticales have probably followed different paths during the first years of their respective synthesis. In fact, the genetic contribution of the wheat parent may well have been greater in spring triticale, since most spring ryes are not outstanding agronomically. Winter ryes, however, show much more interesting agronomic characteristics, such as very good winter survival ability and vigorous growth; they are more desirable as parents in the synthesis of a winter triticale. The main purpose of the following discussion is, therefore, to report the results obtained from winter triticale screening, including comparison of some indicators of BYDV resistance usable in triticale screening and evaluation of the range of variation observed in winter triticale for BYDV resistance. We also report preliminary results obtained from genetic studies in order to compare spring and winter triticales.

Screening for BYDV Resistance in Winter Triticale

The screening experiment for BYDV resistance involved 148 winter triticale lines coming from the Guelph University cereal breeding program. The lines were evaluated in presence and absence of artificial inoculation in three different environments: 1) in an unheated plastic-covered glasshouse, also named "tunnel," in 1984-85, 2) in the field in 1984-85, and 3) in the field in 1985-86.

In the fall of 1984 the genotypes were seeded by hand in the tunnel on 12 and 13 September in 5-plant hills, 30 cm apart, in a honeycomb design. Seeding was done directly in the soil. We commonly grow winter cereals in a tunnel to protect the plants from winter damage. In mid-December, the plants were completely covered with glass wool blankets, which were removed by mid-April, when the outside temperature warmed. This methodology allows us to evaluate BYDV resistance in winter cereals independently of other types of winter stresses, which are usually confounded with virus reaction in the field.

In order to ensure good control of aphid movements, the tunnel was divided in two parts and the inoculation treatment was randomly assigned to one of the parts. Four repetitions were nested inside each of the two treatments, which were BYDV inoculation and control. A 250-micron mesh was used as partition to prevent aphid escapes from the inoculated side and, one day before inoculation, the control plots were sprayed with pirimicarb. Plants at the two-leaf stage were inoculated 5 October with a mixture of BYDV isolates (90% PAV-like: 10% MAV-like) carried by *Rhopalosiphum padi* L. A mean of 225 aphids per hill were counted on 11 October, when the aphids were killed by spraying the insecticide. Visual scores were observed 19 July 1985.

In the field, only 106 genotypes were seeded on 29 August 1984 at a density of 40 seeds per 65-cm row in a split-plot design where 3 repetitions were nested within each inoculation treatment. Plants were inoculated at the 2-leaf stage, 19 September, using the same virus isolates as in the tunnel. However, aphids were not killed by spraying in the field since low temperatures in the fall restrain aphid activity. Visual scores were noted on 31 October.

In 1985, the experiment, including all 148 genotypes, was seeded on 3 September in a split-plot design with six replications. One of the replications had to be eliminated due to uneven winter damage. Plants were inoculated on 20 September using a virus mixture similar to that used the previous year. No visual assessment of disease resistance was made on this experiment.

Possible criteria for judging resistance

Many indicators of virus resistance can be used to evaluate genotypes when both inoculated and uninoculated plots are grown. These different measures are all estimated from some component of plant biomass production and are therefore indirect measurements of genetic

resistance. A good indicator of virus resistance, useful for screening, must be simple, economical, and accurate. We compared 12 different measures of genetic resistance using the data obtained from winter triticale screening under three environments in order to determine if visual scores and biomass yield of inoculated plots, the most commonly used criteria, are good measurements of virus resistance for screening purposes.

Table 1 shows the correlations observed among these indicators. The indicators are listed in order of increasing complexity and cost. Symptom scores (SCORE) are generally easily observed when growing lines under either natural or artificial inoculation. Height of inoculated plants (HEIGHT-I) only requires a simple measurement. Biomass yield of infected plots (BIOM-I) requires harvest of all the material under evaluation, and threshing will be necessary to obtain grain yield (GRAIN-I). The harvest index of inoculated lines (H.I.-I) can be determined from the last two variables. The seven other measurements, however, require that both inoculated and uninoculated plots be grown. The difference in height observed under both treatments can either be expressed as the ratio of height of inoculated plots on height of control plots (HEIGHT-R) or the difference between them (HEIGHT-D). In a similar way, the ratio and difference observed for biomass yield (BIOM-R and BIOM-D) and grain yield (GRAIN-R and GRAIN-D) can be used as indicators of resistance. Finally, the tolerance index (TOLIND) can be determined from the ratio of harvest index of inoculated plots on harvest index of uninoculated plots.

From Table 1 we can see that all variables were significantly correlated to visual score and inoculated biomass yield with the exception of tolerance index in the field in 1984-85. For that year, correlations were higher in the tunnel than in the field. This difference can first be explained by the fact that the intensity of inoculation, based on the initial number of aphids applied per plant, was higher in the tunnel. It can also be attributed to environmental

Table 1. Correlations observed in winter triticale among the different indicators of genetic resistance, listed in order of increasing complexity and cost

	Score	Height-I	Biom-I	Grain-I	H.I.-I	Height-R	Height-D	Biom-R	Biom-D	Grain-R	Grain-D	Tolind
• Tunnel 1984-85[a]												
Score	1.0	-0.85**	-0.82**	-0.78**	-0.63**	-0.84**	0.83**	-0.27**	0.64**	-0.46**	0.57**	-0.49**
Biom-I	-0.82**	0.79**	1.0	0.97**	0.56**	0.69**	-0.70**	0.31**	-0.77**	0.56**	-0.72**	0.43**
log(Biom-I)	-0.92**	0.84**	–	–	0.65**	0.08**	-0.80**	-	–	–	–	0.49**
• Field 1984-85[b]												
Score	1.0	-0.31*	-0.42**	-0.45**	-0.20	-0.33**	0.36**	-0.20**	0.35**	-0.12*	0.36**	n.s.
Biom-I	-0.42**	0.61**	1.0	0.96**	0.24**	0.49**	-0.47**	0.29**	-0.57**	0.16**	-0.52**	n.s.
• Field 1985-86[c]												
Biom-I	-	0.79**	1.0	0.97**	–	0.58**	-0.51**	0.75**	-0.64**	0.87**	-0.74**	–

Score = visual score; Height-I = height of inoculated plants; Biom-I = inoculated biomass yield; Grain-I = inoculated grain yield; H.I.-I = inoculated harvest index; Height-R = inoculated height/control height; Height-D = control height - inoculated height; Biom-R = inoculated biomass/control biomass; Biom-D = control biomass - inoculated biomass; Grain-R = inoculated grain yield/control grain yield; Grain-D = control grain yield - inoculated grain yield; Tolind = inoculated harvest index/control harvest index.

n.s.: non significant, *: significant at the 0.05 level, **: significant at the 0.01 level.
[a] the number of observations ranged from 549 to 579.
[b] the number of observations ranged from 306 to 318.
[c] the number of observations ranged from 95-110 (Grain-I, Grain-R, Grain-D) to 739 (other variables).

effects. Most differences in performance observed among the entries in the tunnel were caused by the virus. In the field, the variation caused by the virus was confounded with variation from winter damage effects. The magnitude of correlations observed in the field in 1985-86 was comparable to that seen in the tunnel in the year before.

Overall, visual scores were correlated with height, biomass, and grain yield under inoculation, but the closeness of the relation was much better in the tunnel than in the field in 1984-85. A logarithmic transformation of inoculated biomass yield improved some of the correlations observed in the tunnel. The correlations observed in the tunnel involved visual scores, which were noted 3 weeks before harvest, while in the field they were noted at tillering stage, in the fall.

It is well known that correlations between symptoms and yield losses vary from year to year in a rather unpredictable way. Moreover, symptom expression is in dynamic evolution and varies with time. However, there is a good indication that the correlation between visual score and biomass or grain yield under inoculation will improve considerably if the intensity of inoculation is sufficiently high to promote the expression of a reasonable range of dwarfism and to affect tillering. In other words, the intensity of inoculation must be high enough, so that visual scores can integrate observations on dwarfism and general vigor of the plant as reflected by tillering and relative size of the spikes. Leaf discoloration should also be integrated in the symptom scores, but this character may be more variable on a time axis as new leaves may appear greener or more chlorotic than the previous ones.

Correlations observed between biomass yield and grain yield under inoculation varied from 0.96 to 0.97 and were slightly higher than in control plots (0.90-0.94). The correlations between the two variables and visual scores were also very similar. Therefore, it seemed that threshing the inoculated material solely to obtain grain yield data was not worth the extra work since both variables gave very similar estimates. Harvest index correlated less well with visual score than either biomass or grain yield under inoculation.

Until now, in our breeding work, we had relied on symptom scores and on the biomass of inoculated plots to select for resistance. This experiment showed that the indirect estimations of resistance (ratios or differences) involving comparisons with control plots did not correlate better with the symptom score and with the inoculated plot biomass than the estimations obtained directly from the inoculated plots. Ratios are used solely to obtain more accurate estimates of genetic resistance, independent of potential performance in control plots. Therefore, performance estimated from such a ratio is not correlated to performance in control plots, and provides a uniform basis for comparison of genetic resistance.

Differences give an estimate of the absolute decrease in performance attributable to viral infection. Difference estimates of resistance are more likely to be dependent on potential performance in absence of viral infection since, as potential performance increases, absolute differences will likely increase for a fixed proportion of losses. It can be seen from Table 1 that ratios and differences for height correlated equally well with scores and inoculated biomass yield, while ratios for biomass and grain yield correlated less well than differences for these characters, except in 1985-86. The explanation for this is mainly statistical: the use of a ratio of two variables is satisfactory if the relation between those variables, for instance inoculated biomass yield and control biomass yield, is linear and goes through the origin, implying that the variables are highly correlated. Correlations observed between inoculated biomass yield and control biomass yield were all significant but rather low, varying from 0.10 to 0.26 (Table 2). The effect of using ratios or differences for this character resulted in an increase of correlation with control performance, which is the opposite of the desired effect. The same conclusions applied to grain yield, a character highly correlated to biomass yield.

Correlations observed between inoculated height and control height were higher, varying from 0.49 to 0.59. Ratios and differences for height were generally less correlated with control height than inoculated height and were therefore considered better indicators of genetic resistance. Finally, tolerance index showed the poorest correlations with visual score and biomass yield under inoculation (Table 1). Tolerance index may not be a good indicator of varietal resistance to BYDV in triticale, contrary to what was observed in oats (*Avena sativa* L.) (Cooper and Sorrells 1983).

Evaluation of criteria as indicators of genetic resistance

If we grow both inoculated and control plots in a screening test, the ratio of mean inoculated biomass to mean control biomass (BIOM-I/C) is an objective measure that gives accurate information on the true resistance of a line; this ratio is a final judgment on the value of the line, while symptom scores are subjective, indirect, and not final. BIOM-I/C would appear to be the best practical measure of resistance available. Since the ultimate aim of screening for BYDV resistance is the ranking of genotypes according to their respective degree of resistance, which allows the selection of a given proportion of the best ones, we compared the rankings obtained from the various criteria with the ranking obtained from BIOM-I/C.

Table 3 shows rank correlations observed between the ranking of entries given by BIOM-I/C and the ranking obtained from the 12 indicators of resistance listed previously. First, we see that all rankings are highly correlated. Correlations between rankings obtained from ratio of the means and mean of the ratios for biomass yield are different from 1.0, indicating a slight bias.

However, the main conclusion that can be drawn from Table 3 is that visual scores and inoculated biomass yield gave rankings that were very well correlated with the ranking obtained from BIOM-I/C, when the intensity of inoculation was sufficiently high. In the tunnel, the rankings obtained from visual score and inoculated biomass yield were very close to the ranking given by BIOM-I/C, and the extra work needed to obtain more complex variables was not fully justified. Likewise, inoculated biomass yield was also very well correlated in the field in 1985-86. Only in the field in 1984-85 were the correlations observed for scores and inoculated biomass yield really lower than obtained from ratios and differences estimated from biomass and grain yield. In general, harvest index under inoculation and tolerance index gave the poorest correlations.

To conclude on the various indicators of resistance, it seems that visual scores and inoculated biomass yield, which are widely used as measures of genetic resistance, are very useful tools for the

Table 2. Correlations observed in winter triticale between variables in control plots and the same variables under inoculation expressed as absolute inoculated values, ratios (inoculated/control), and differences (control-inoculated)

	Tunnel 1984-85			Field 1984-85			Field 1985-86		
	Inoculated values	Ratios	Differences	Inoculated values	Ratios	Differences	Inoculated values	Ratios	Differences
Control biomass yield	0.10*	-0.24**	0.55**	0.26**	-0.40**	0.61**	0.23**	-0.36**	0.60**
Control grain yield	0.12**	-0.31**	0.61**	0.25**	-0.35**	0.65**	-0.13 n.s.	-0.47**	0.75**
Control height	0.49**	0.01 n.s.	0.02 n.s.	0.58**	0.01 n.s.	0.16**	0.59**	-0.17**	0.30**

n.s. not significant, * significant at the 0.05 level, ** significant at the 0.01 level

purpose of ranking entries and making selection in winter triticale, when inoculation intensity is appropriate. With lower intensities of inoculation, inoculated biomass yield remains the cheapest estimator of genetic resistance available for selection and still gives rankings correlated with those obtained from more complex variables requiring inoculated and control plots.

Genetic variability for BYDV resistance in winter triticale

We observed a wide range of variability for BYDV resistance among genotypes of winter triticale. The mean performance of resistant and susceptible genotypes, as determined by BIOM-I/C on percentage basis, is shown in Table 4. In 1984-85, the range of performances was similar in the tunnel and in the field. The best genotypes showed very good levels of resistance, while the most susceptible ones showed a major reduction of biomass. The best 10% of the resistant genotypes yielded under inoculation from 131 to 155% of their performance in control plots. This overestimation of genetic resistance was partly due to the fact that inoculated and control plots were grouped in main inoculation treatments and that spacing between plots, especially in the tunnel, was very tight. This resulted in a very intense

Table 3. Spearman correlations observed between the ranking of winter triticale lines given by Biom-I/C and the rankings obtained from different indicators of genetic resistance

	Score	Height-I	Biom-I	Grain-I	H.I.-I	Height-R	Height-D	Biom-R	Biom-D	Grain-R	Grain-D	Tollind
• Tunnel 1984-85[a]												
Biom-I/C	0.90**	0.79**	0.92**	0.88**	0.57**	0.87**	0.86**	0.97**	0.93**	0.95**	0.88**	0.66**
• Field 1984-85:												
Biom-I/C	0.54**	0.40**	0.69**	0.68**	0.25**	0.63**	0.64**	0.96**	0.95**	0.94**	0.91**	0.43**
• Field 1985-86:												
Biom-I/C	(b)	0.70**	0.85**	(b)	(b)	0.85**	0.82**	0.97**	0.93**	(b)	(b)	(b)

Biom-I/C ratio of mean biomass yield under artificial inoculation to mean biomass yield in control plots; other abbreviations are defined in Table 1

[a] the number of observations number of genotypes = 148 for tunnel 1984-85 and field 1985-86, and 106 for field 1984-85.

[b] not measured in this environment

** significant at the 0.01 level

Table 4. Mean relative performance of resistant and susceptible genotypes of winter triticale as measured by BIOM-I/C, expressed on a percentage basis

	Tunnel 1984-85 (148 genotypes)	Field 1984-85 (106 genotypes)	Field 1985-86 (148 genotypes)
Highest genotype	234	228	125
Highest 10%	155	131	105
All genotypes	66	61	65
Lowest 10%	11	14	32
Lowest genotype	3	4	23

BIOM-I/C ratio of mean inoculated biomass to mean control biomass.

competition in control plots, where all uninoculated genotypes competed for nutrients and light, while on the inoculated side resistant genotypes grew very aggressively, facing no competition from susceptible neighbors. Therefore, these evaluations have a relative rather than absolute value for comparison purposes.

The 10% most susceptible genotypes gave biomass yields of about 4% of those in control plots. The mean performance of all inoculated genotypes varied from 61 to 66% of the mean performance without inoculation.

In 1985-86, the range of variation observed was more realistic, varying from 105% for the 10% best genotypes to 32% for the 10% poorest genotypes. Mean performance of all genotypes observed under inoculation was 65% of that observed in control plots.

Triticale no. 46 (CIM 13-E1-E3-E5-EB), GWT-281, OAC Wintri, M69-81 (sister line to OAC Wintri), GWT-112, and triticale no. 44 (75 S x W E17-EB) were among the most resistant genotypes and performed well in the three environments. These six lines had relatively stable performances, giving a high mean biomass yield under artificial inoculation over the three environments. They were also favorably classified in each of the three environments for their genetic resistance, as measured by BIOM-I/C.

Comparison of Spring and Winter Triticales

We investigated the genetic basis of variation found in both spring and winter triticales for BYDV resistance. In 1985, the F_2 and backcross progenies originating from crosses among one resistant spring triticale, Z-9, one moderately resistant, Beagle, and one susceptible, Mapache (=Cananea 79=M2A"S"), were visually rated for BYDV tolerance under artificial inoculation caused by "Cloutier" isolate (PAV like) carried by *R. padi* at two sites (Figure 1). In 1986, the same observations were made on F_2 and backcross progenies from crosses among the resistant winter triticale lines OAC Wintri and GWT-112 and the susceptible GWT-133 (Figure 2). Preliminary results from these genetic studies are reported in Table 5.

The range of variation observed for BYDV resistance was different in spring and winter parents used in genetic studies. In spring triticale, biomass yield of the most resistant parent under artificial inoculation was 55% of yield in absence of inoculation. In winter triticale, the most resistant parent was much more resistant, yielding 92% of control. However, the susceptible winter parent was more susceptible than the spring parent, as shown by their respective performance. These results suggest that the protection conferred by the genes is better in winter than in spring triticales.

Estimations of the minimal number of segregating factors according to the Castle-Wright index method (Wright 1968) were also different in spring and winter triticales. A small number of genes seems to be involved in control of genetic variability for BYDV resistance in triticale. However the estimates of gene number were lower in spring than in winter triticale.

Broad sense heritability estimates were higher in spring than in winter triticale, which is in agreement with the estimates of number of genes (Simmonds 1979). As the number of genes involved increases, heritability estimates should decrease.

Finally, genetic effects estimated according to Mather and Jinks'(1982) generation means analysis were complex and varied depending on the type of triticale, the specific cross, and even the environment involved. For each type, we observed significant additive, dominance, and interaction effects at least once.

Thus, adequate sources of resistance are available in triticale, mainly in winter types. These sources of resistance are easily detectable by screening under

Figure 1. Spring triticale lines used as parents in genetic studies carried out in 1985 at La Pocatière, Quebec. From left to right: Z-9, resistant, control (témoin) and inoculated (BYDV); Beagle, moderately resistant, control and inoculated; and Mapache, susceptible, control, and inoculated.

Table 5. Comparison of genetic resistance observed in spring and winter triticales in genetic studies carried out in 1985 and 1986

	Spring triticale	Winter triticale
Range of genetic variation[a]	54.5-25.9%	91.9-10.9%
Minimum number of genes[b]	0.8-0.9	1.7-2.4
Broad sense heritability	0.85-0.95	0.40-0.72
Types of genetic effects	Additivity Dominance Interactions	Additivity Dominance Interactions

[a] as estimated by the ratio of mean inoculated biomass yield to mean control biomass yield, expressed on percentage basis

[b] estimates are biased downward

proper natural or artificial inoculation. On account of the estimates of minimum number of genes and broad sense heritabilities, breeding for BYDV resistance in triticale should not present any serious problem. A backcross method modification involving convergent crosses could be appropriate in spring triticale to transfer resistance to well adapted genotypes. However, some form of recurrent selection more strongly oriented toward population development may be more suitable to manipulate resistance in winter triticale.

Tola and Kronstad (1984) confirmed that BYDV resistance in wheat is quantitative. In triticale, only a small number of genes seem to be involved, and the resistance level of the best triticales is clearly superior to that of the best wheats; for these two reasons, resistance from triticale represents a key source of genes for rapid improvement of BYDV resistance in wheat through interspecific hybridization.

Figure 2. Winter triticale lines used as parents in genetic studies carried out in 1985-86 in unheated plastic-covered glasshouse (tunnels) at Sainte-Foy, Québec. From left to right: OAC Wintri, resistant, control (témoin) and inoculated (BYDV); GWT-112, resistant, control and inoculated; and GWT-133, susceptible, control, and inoculated.

Conclusions

1) Visual scores and biomass yield of inoculated material are very good indicators of BYDV resistance for the purpose of ranking entries and selecting in large populations; however, intense inoculation is essential for efficient work.

2) A wide range of variability in BYDV resistance was observed in winter triticale. Very good levels of resistance, superior to those observed in wheat, are available for breeding purposes.

3) Spring and winter triticales are different with respect to BYDV resistance. Winter types offer better levels of resistance. The estimated minimum number of genes is higher in winter types, which is supported by lower heritability estimates.

4) Genetic resistance is available in triticale and sources of resistance can easily be detected. Therefore, according to heritability estimates and estimated minimum number of genes involved in the control of this variability, breeding for BYDV resistance looks very promising in triticale.

Acknowledgments
Thanks are expressed to Drs. V. Poysa and E. Reinbergs for providing most of the winter triticale lines used in this work.

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Identification, Characterization, and Utilization of Sources of Resistance to Barley Yellow Dwarf Virus

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Abstract

*The enzyme-linked immunosorbent assay (ELISA) technique was used with a local isolate of the PAV serotype to screen wild grasses for resistance to barley yellow dwarf virus (BYDV). Thirteen species of grass showed resistance in that they could maintain low levels of the virus for one month after inoculation. The F_1 hybrids with wheat (*Triticum aestivum *L.) showed good resistance. Particularly promising was a partial amphiploid from China called Zhong 4, which showed very low levels of PAV in ELISA. Probes with various ribosomal spacer sequences and 5S ribosomal sequences of DNA isolated from Zhong 4 suggested that some alien chromosomes present are closely related to the E genome. The partial amphiploid TAF 46 from Y. Cauderon and the derived single chromosome addition line, L1, showed good resistance to virus multiplication (PAV and RPV-like). We are attempting to introgress these resistances to wheat using cell culture induced non-homologous exchange or the* ph *effect.*

Few sources of resistance to barley yellow dwarf (BYDV) have been described in the Triticeae. These include the barley (*Hordeum vulgare* L.) CI-3906 (*Yd3* gene), barley CI-3208 (*Yd2* gene) (Rasmusson and Schaller 1959), and *Thinopyrum intermedium* (Host) Barkworth and Dewey (syn: *Agropyron intermedium*) (Sharma et al. 1984). Some tolerance has been described in wheat (*Triticum aestivum* L.) (Qualset et al. 1973; Cisar et al. 1982), but no major resistance has been found. Recently, B. Gill, K. Morris, and R. Appels (personal communication) found addition lines from *Elymus ciliaris* (Trin.) Tzvelev and *E. trachycaulus* (Link) Gould ex Shinn. that carry genes for resistance to the MAV- and RPV-like isolates of BYDV.

We have screened a number of accessions of grasses in an attempt to find sources of resistance to BYDV. The screening was done by feeding viruliferous *Rhopalosiphum padi* (L.) carrying an Australian PAV serotype (Waterhouse et al. 1986) on the test seedlings and measuring virus levels in the infected plants 4 weeks after the feeding. Virus levels determined in this way generally correlate with the degree of susceptibility in the field. Table 1 lists the accessions and Table 2 lists hybrids to wheat or lines derived from hybrids. In total 52 accessions representing 32 species have been examined using the PAV serotype of BYDV in the enzyme-linked immunosorbent assay (ELISA) screen. The aphid numbers were examined on all individual seedlings in these assays and ELISA only used if aphids were confirmed to be attached. Some caution is needed with three of these species, where the aphid numbers on the plants seemed to be low (e.g., *Elymus ciliaris*, *Elytrigia repens* (L.) Nevski, and (*Agropyron spicatum*) =*Elytrigia spicata* (Pursh) D.R. Dewey. In these cases the low virus levels may be partly due to feeding suppression resulting in low dose inoculation rather than inhibition of virus multiplication.

Table 3 summarizes the species we consider to have most potential as sources of resistance at least to the PAV serotype. Not all accessions of a given species will have the same reaction. For example, the two accessions of *T. junceum* were susceptible and moderately resistant. Also the two accessions of *T. intermedium* involved in the partial amphiploids Zhong 4 and TAF 46 appear to be different. Both are

Table 1. Species screened for BYDV resistance (PAV isolate)

Species	Accession	Origin	Genome	Aphid feeding	PAV-ELISA result
Critesion bogdanii	PI 314696	USSR	H	low	S
C. brevisubulatum	A-24	Iran	H		MR
Thinopyrum junceiforme	CPI 104061	France	J_1J_2		R
T. scirpeum	CS-5-6170	Aegean	E_1E_2		S
T. intermedium	PI 414668	Greece	E_1E_2x		R
T. junceum	CA 432		Exy		S
T. junceum	CA 435		Exy		MR
Agropyron spicatum	TA 2219	US	S	low	R
A. sp.	CPI 109890	China			MR
Elymus ciliaris	HE 2-2130	Japan	Sy	low	R
E. ciliaris	TA 2006		Sy	low	R
E. sibiricus L.	PI 429797	USSR	SH		R
E. canadensis L.	PI 232249	US	SH		S
E. caninus (L.)	PI 314612	USSR	SH		S
E. scabrus	86611	Australia	SHy		S
E. scabrus	AGf	Australia	SHy		S
E. scabrus	86136	Australia	SHy		S
E. scabrus	CPI 107 964	Australia	SHy		S
Elytrigia repens	D 2634	China	S_1S_2x	low	R
E. pycnatha	RS-3-21	France			S
E. pungens	RS-3-3140	France	Exyz		MR
E. elongata	CPI 107489		E		S
Leymus racemosus (Lam.)	RS-6-2630	USSR	JN		MR
L. racemosus	CPI 109895		JN		S
L. karataviensis	PI 314671	USSR	JN		R
Pascopyrum smithii	RS-11-1720	US	SHJN		R
Dasypyrum villosum	AWC 17953		V		S
D. villosum	CPI 107733		V		S
Triticum tauschii	192-1518		D		S
T. tauschii	185-1487-7		D		S
T. tauschii	D-9		D		S
T. tauschii	AC 7504		D		S
Aegilops umbellulata	CA 439		U		S
Ae. umbellulata	CA 440		U		S
Ae. umbellulata	CPI 109882		U		S
Ae. variabilis	CPI 109885		BU		S
Ae. uniaristata	CPI 109883		M^u		S
Ae. uniaristata	CPI 109884		M^u		S
Ae. ventricosa	CPI 109886		DM^v		S
Ae. ventricosa	CPI 109881		DM^v		S

Lower case designations for genomes indicate they are unknown. The use of the same lower case designation in different species does not imply homology.

R = resistant, MR = moderately resistant, S = susceptible.

Table 2. Alien hybrids to wheat and derived lines

Description	Accession	Cytology	Origin	PAV-ELISA
CS/*A. scirpeum*//*T. aestivum* ($E_1E_1E_2E_2$)	CPI 109887	partial amphiploid 2n = 8x = 56 mainly	Mujeeb-Kazi CIMMYT	S
CS/*A. curvifolium*//*T. aestivum* ($E_1E_1E_2E_2$)	CPI 109591	partial amphiploid 2n = 8x = 56 mainly	Mujeeb-Kazi CIMMYT	S
T. turgidum/*A. pulcherrimum*	CPI 109592	amphiploid 2n = 10x = 70	Mujeeb-Kazi CIMMYT	R
T. turgidum/*A. acutum*	CPI 109893	amphiploid 2n = 10x = 70	Mujeeb-Kazi CIMMYT	R
T. turgidum/*T. junceiforme* ($J_1J_1J_2J_2$)	CPI 109894	amphiploid 2n = 8x = 56	Mujeeb-Kazi CIMMYT	S
CS//*A. repens*/*A. desertorum* ($S_1S_1S_2S_2$xx)(PPPP)	CPI 109889	2n = 10x = 70	Mujeeb-Kazi CIMMYT	MR
CS/*E. elongata* (EE)	CA 155	amphiploid	Dvorak UC-Davis	S
CS/*A. junceum*	CPI 109130	amphiploid 2n = 8x = 56	Forster/Miller PBI	MR
Zhong 4 (*Th. intermedium*/*T. aestivum*) ($E_1E_1E_2E_2$xx)	CPI 107735	partial amphiploid 2n = 8x = 56	Q. Shiyu Sun Shancheng China	R
TAF 46 (*Th. intermedium*/*T. aestivum*)	CPI 109386	partial amphiploid 2n = 8x = 56	Cauderon France	R
Th. intermedium/*T. aestivum*	WE 84924	F_1 2n = 42	Chen Xiao China	R
Th. intermedium/*T. aestivum*	CPI 109562	F_1 2n = 42	Chen Xiao China	R

CS = Chinese spring wheat.

resistant to the PAV serotype, but only TAF 46 and the derived single addition line, L1, are resistant to the RPV-like isolate. It is also noteworthy that diverse genomes appear to be carrying BYDV resistance (Table 3). Most of these species have already been successfully crossed to wheat (hexaploid or tetraploid), so there is some prospect of access to this resistant germplasm.

We have examined Zhong 4 in detail to determine the genomes present and thus identify this source of resistance. *T. intermedium* is a hexaploid with two genomes closely related to each other and to the E genome of *T. elongatum* (Host). Its three genomes are designated $E_1E_1E_2E_2XX$ (Dewey 1984). Seed of the original *T. intermedium* used in the cross to wheat was not available, but it is believed to be of USSR origin.

Zhong 4 is a stable partial amphiploid with 2n = 56 chromosomes. Meiotic examination of F_1 hybrids between Zhong 4 and wheat usually revealed 20-21 bivalents with usually 6-8 univalents. N-banded mitotic spreads indicated the presence of the A, B, and D genomes and non-wheat chromosomes. Therefore Zhong 4 appears to have all the normal wheat chromosomes and one alien genome. The identity of this alien genome was established with DNA sequence probes.

Ribosomal spacer region sequences isolated from the E genome of *T. elongatum* hybridized strongly to Zhong 4 DNA. Relative homology was determined by the temperature at which the probe bound to the filter-immobilized DNA. Zhong 4 has as high an affinity to this probe as does *T. elongatum* itself. In *T. elongatum* the spacer sequences assay the *Nor* loci on chromosomes 5E and 6E. An additional E-genome probe was used for the 5S ribosomal gene, which is located on chromosome 1E. This also had high homology to Zhong 4 DNA.

The unknown genome of *T. intermedium* designated X is known to have sequences homologous to the rye (*Secale cereale* L.) "350-family" telomeric sequences (Lapitan et al. 1987). These sequences are located on the ends of all the X genome chromosomes. When Zhong 4 was assayed with one probe of this family (pSchet3), no homology was found. However, a different probe, pSchet1, which represents a triplet of repeats of this family, did have homology to Zhong 4 DNA.

We would tentatively conclude from these various lines of evidence that the genome designation of Zhong 4 is AA BB DD E*E*. The E* genome is likely to be composite of the E_1 and E_2 genomes, but perhaps with a translocation carrying a segment of an X genome chromosome. The E_1 and E_2 genomes have been

Table 3. Promising species for sources of BYD (PAV) resistance

Species	Genomes
* *Thinopyrum junceiforme*	J_1J_2
* *T. intermedium*	E_1E_2x
* *T. junceum*	Exy
* *Elymus ciliaris*	Sy
E. sibiricus	SH
* *Elytrigia repens*	S_1S_2x
* *E. pungens*	Exyz
* *Leymus racemosus*	JN
L. karataviensis	JN
Pascopyrum smithii	SHJN
Agropyron spicatum	S
* *A. pulcherrimum*	?
* *A. acutum*	?

* Successful crosses to wheat have been made in a number of laboratories.

shown to autosyndetically pair in intergeneric hybrids (Stebbins and Pun 1953) and autoalloploids (Dewey 1962). This capacity for E_1 and E_2 chromosomes to pair would account for their preferential retention (relative to X chromosomes) in partial amphiploids derived from *T. intermedium* x wheat.

The partial amphiploid TAF 46 from Y. Cauderon, France, is likely to have a similar genomic composition to Zhong 4. The disomic individual addition lines derived from TAF 46 were also examined. Line L1 was the only one of the six different lines available that showed resistance to the PAV serotype. L1 was also resistant to the RPV-like isolate. There is evidence that the E genome chromosomes in L1 are homoeologous to the group 7 chromosomes of wheat. This evidence includes the capacity for the L1 non-wheat chromosome to substitute for group 7 wheat chromosomes (The and Baker 1970), additional leaf peroxidase isozyme bands in L1 (Figueiras et al. 1986), and a red coleoptile characteristic in L1 but not the background wheat, Vilmorin. The red coleoptile gene is a convenient marker for the alien chromosome and appears to be on the opposite arm to the BYDV resistance gene.

The prospects of deploying this E genome source of resistance for wheat improvement are greatly enhanced by the location of the resistance on a single alien chromosome. *T. intermedium* E_1 or E_2 genome chromosomes do not ordinarily pair with the wheat genome chromosomes. However there are at least four methods whereby genetic exchange between the E genome and wheat genomes might be obtained:

- radiation induced breakage and fusion (Knott 1961)
- homoeologous pairing induced by the *ph* gene (and/or deleted chromosome 5B) (Wang et al. 1977; Riley 1974)
- homoeologous pairing induced by genes from *Aegilops speltoides* Tausch crossed into the *Thinopyrum* x wheat derivates (Cauderon and Kyan 1974)
- non-homologous exchange (both homoeologous and non-homoeologous) resulting from cell culture-induced breakage and fusion (Larkin and Scowcroft 1981; Lapitan et al. 1984; Larkin 1985; Davies et al. 1986).

Currently the second and last of these approaches is being tried with the L1 addition line. Seed derived from *ph* mutant/L1//N5BT4D/3/CS and produced by R. McIntosh, Sydney University, is being screened by ELISA for BYDV resistance. Families are being sought with segregation patterns indicating the resistance has been transferred to a wheat chromosome.

Monosomic additions produced by crossing L1 with wheat are being cultured *in vitro* from immature embryos and immature spikes. These will be regenerated, selfed, and families screened for BYDV resistance segregations suggestive of introgression onto a wheat chromosome.

Suspected introgressions will be confirmed using E genome-specific dispersed, repetitive DNA probes. These probes will be biotin-labelled and used to hybridize *in situ* to chromosome preparations in order to visualize segments of E chromatin in wheat chromosomes (Rayburn and Gill 1985; Lapitan et al. 1986).

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The Use of Cloned Hybridization Probes to Detect Barley Yellow Dwarf Virus Infection in Tetraploid Wheat Germplasm

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Abstract

*A PAV-like isolate of barley yellow dwarf virus (BYDV) was used in controlled inoculation experiments in the greenhouse to determine the intensity of the autoradiographic signal from positive Northern dot-blot hybridization of leaf sample extract of infected and uninfected plants of tetraploid wheat (*Triticum turgidum *L.). Radioactive probes gave more consistent results than biotin probes. The proportion of entries with reduced virus content in collections from Ethiopia, Iran, Iraq, and Turkey were 24.3, 28.6, 8.7 and 10.5%, respectively. No other differential symptoms were expressed on the infested plants in the greenhouse. Therefore, the further evaluation of the tetraploid wheat entries for tolerance should involve (a) field testing and (b) the use of additional criteria to evaluate tolerance to BYDV.*

Currently, barley yellow dwarf virus (BYDV) in the wheat (*Triticum aestivum* L.) plant can be detected by several alternative diagnostic procedures: a) visual symptoms, b) aphid acquisition feeding-transmission tests, c) serological detection, and d) dot-blot hybridization using nucleic acid probes.

BYDV symptoms (different degrees of plant dwarfing and leaf discoloration) are not readily recognizable in infected wheat plants grown in certain conditions (i.e., greenhouses). Enzyme-linked immunosorbent assay (ELISA) (Lister and Rochow 1979) and serological specific electron microscopy (SSEM) (Paliwal 1977) are widely used procedures for detection of BYDV in plant tissue, although the results rely on the quality of the antisera batch available for each BYDV luteovirus serotype. BYDV serotypes can be detected quickly and easily with the dot-blot hybridization technique and it can be applied to large numbers of small samples of host tissues with the possible advantage of sensitivity and a degree of specificity, depending on the hybridization condition or on the sequence used as probe. Waterhouse et al. (1986) produced serotype-specific probes from cloned cDNA sequences of BYDV.

Because of the lack of good sources of resistance to BYDV in tetraploid wheat, we have undertaken a survey of available collections of tetraploid wheat (*Triticum turgidum* L.) and of wild diploid relatives from Ethiopia, Iran, Iraq, and Turkey to evaluate the efficiency of a pBY82 DNA probe specific for detecting a PAV-like isolate of BYDV. The aim was to develop an assay procedure based on dot-blot hybridization technique that would determine the ability of isolates of BYDV to replicate in tissue of different tetraploid wheat entries. The assumption is that lower amounts of BYDV viral particles detected in the infected host tissue indicate a lower ability of the virus to replicate within the host, indicating resistance to the virus.

Material and Methods

Tetraploid wheat entries

Four groups of introductions were studied. The first group consisted of 531 collections from Ethiopia, resulting from expeditions made by researchers of the Germplasm Institute, CNR, Bari, Italy, in Ethiopia during 1972-1973. These entries were already evaluated for morphological traits (Spagnoletti Zeuli et al. 1984) and showed great diversity. Some of them were heterogeneous for kernel color. The second group comprised 51 accessions from Iraq, the third group was 70 accessions from Iran, and the fourth group was 152 accessions from Turkey. All the entries have been included in the USDA National Small Grain collections, and accessions were kindly provided by the CNR-Germplasm Institute, Bari, Italy. The entries were studied in two periods: those from Ethiopia in October 1986, and those from Iran, Iraq, and Turkey in March 1987.

Controlled inoculation
Four seeds from each introduction were separately planted in small pots filled with a mixture of soil and commercial compost in a greenhouse kept at 16°C with 16 hours of supplemental light. The pots with seedlings of each entry were randomly allotted to two benches (two seedlings per bench). The seedlings of one bench were used for BYDV inoculation and those of the other bench represented the uninfected negative control. When the seedlings were at the two-leaf stage they were infested for 3 days with three viruliferous *Rhopalosiphum padi* (L.), which were kindly provided by R. Osler of the Istituto di Difesa delle Piante, University of Udine. Before being used for the controlled infestation experiment, these aphids were allowed a 3-day acquisition feeding period on (*Avena byzantina*) =*Avena sativa* L. leaf tissue infected with a PAV-like isolate of BYDV originally isolated from naturally inoculated maize plants by R. Osler. After infestation the inoculated and noninoculated plants were covered with transparent plastic tubes, which were closed at the top with an aphid-proof net.

Leaf and root tissue samples and aphid samples
Leaf samples were collected from each plant 13 days after infestation. The upper third of the developing third leaf was detached from each treated and untreated (control) plant. Each leaf piece was diced into 5 mm sections, put into a 1-ml microcentrifuge tube and stored at -80°C. Root samples were taken from some inoculated plants 13 days after infestation. The roots of one plant were diced into 5 mm sections and stored in the same way as the leaf samples. To ascertain the sensitivity of the assay procedure in detecting virus particles in aphids, a range of two to ten viruliferous aphids was used to prepare aphid extracts.

Sample extract and nitrocellulose filter preparation
In all experiments 70 mg of leaf or root tissue were ground directly in the microcentrifuge tube for one minute with the aid of a glass rod in the presence of 150 μl 0.1M sodium phosphate buffer, pH 7.0 (at a tissue buffer ratio of 1.2 w/v). Then 2 volumes of chloroform were added and a further 30 seconds grinding was performed followed by centrifuging at 14,000 rpm for 1 minute. The supernatant was recovered and transferred to a new centrifuge tube and immediately spotted on an 11 x 8 cm nitrocellulose filter previously marked every 1 cm by numbering with a blue ball point pen and soaked in 2 x SSC (sodium saline citrate buffer). Five 1-μl extracts were used to spot each sample. A 1:1 mixture of chloroform-butanol was used to prepare aphids extracts.

Preparation of pBY82 plasmid probe
The pBY82 plasmid containing a cDNA insert that hybridizes only with the PAV serotype RNA was kindly provided by W.L. Gerlach, P.M. Waterhouse, W.A. Miller, and K. Helms of the Division of Plant Industry, CSIRO, Canberra, Australia, and used as probe (Waterhouse et al. 1986). A nick translation kit (Amersham) was used to prepare a ^{32}P-labelled plasmid probe at specific activity of 1 x 10^8 cpm/μg of plasmid DNA; 1 μg of a radioactive probe was used to hybridize 20 nitrocellulose filters spotted with about 1600 different sample extracts.

One μg of plasmid DNA was probed with biotin using a BRL nonradioactive nucleic acid detection system kit, to compare the ability of radioactive and biotinilated probes in detecting the PAV-like isolate of BYDV in wheat plant extract. This probe was sufficient to hybridize only two nitrocellulose filters, each spotted with 80 sample extracts.

Northern dot-blot hybridization
Filters were prehybridized for 60 min at 42°C in a 6-ml solution containing 5 x SSPE (sodium saline phosphate EDTA buffer), 50% (v/v) formamide, 5x Denhardt's reagent, 10% (v/v) polyethyleneglycol (PEG), 20 μg sonicated and heat-denaturated salmon sperm DNA. This prehybridization solution was replaced with 4 ml of fresh solution to which heat denatured nick-translated radioactive or biotinilated probe was added. Hybridization proceeded for 8 hours at 42°C. The filters were then washed twice in 5x SSPE at 42°C for 15 min, twice in 1x SSPE, 0.1% SDS

(sodium dodecyl sulfate) at 42°C for 15 min, and once in 1x SSPE, 0.1% SDS at room temperature for 15 min. Filters were briefly dried and exposed to Kodak X-Omat film 50-282 for 48 hours at 80°C using Dupont Cronex cassette.

Data record
The intensity of the autoradiographic signals was used to infer the presence and relative amount of virus in the sampled leaf. Readings were made by visual judgment of the intensity of a signal. Sample spots that gave signals relatively darker than the negative control were considered positive for BYDV. Entries were divided into two groups: those for which there were autoradiographic signals (from sample extract of at least one out of the two infected seedlings examined) similar to the very light signal of the corresponding negative control (group -), and those for which there were autoradiographic signals (from sample extract of at least one out of the two infected seedlings examined) slightly darker (+), darker (+ +), and much darker (+ + +) than those corresponding to sample extract of the negative control (Figure 1).

Results

Development of the assay procedure
Conditions to maximize the intensity of the autoradiographic signal due to the RNA viral component of the sample extract and reduce the noise due to the other components of the sample extract were checked.

Clarification of the sap extract with chloroform and its rapid use for spotting gave much better results (clear positive hybridization signal) than unclarified or clarified sap sample extract kept several days at -80°C (Figure 2). However, there were similar intensities of autoradiographic signals in both sample extracts obtained from infected leaf kept at -80°C for several days and unfrozen sample extracts obtained from tissues of infected plants.

To detect the presence of the PAV-like isolate of BYDV in aphids, at least four viruliferous aphids were needed in one aphid sample extract (spot A_{13} in Figure 2). When only two viruliferous aphids were used to prepare aphid sample extract, no virus detection was possible (spot A_{14} in Figure 2).

The sap extracted from healthy plants always gave a signal, however, the signal was significantly lighter than the signal from infected plants. The increase in signal from infected plants was more than 30% when compared to healthy plants. There were always stronger autoradiographic signals from spots of extracts from roots compared to spots of extracts from leaves of the same plant (Figure 3).

The use of PEG in the hybridization mixture allowed a reduction of the prehybridization time and produced a dramatic increase in signal from the spots of sample extracts of infected material (either leaves or aphids); when

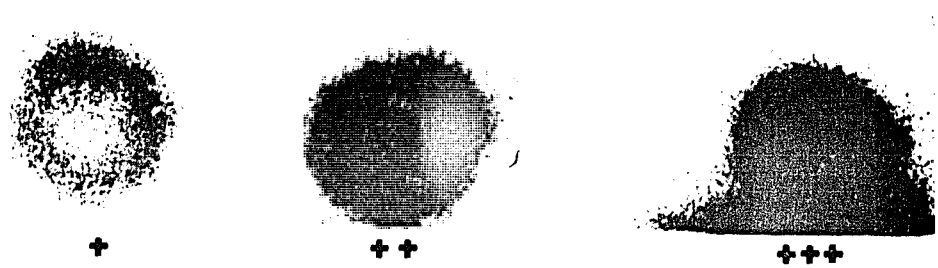


Figure 1. Intensity of autoradiographic signal from positive hybridization (with ^{32}P-labeled pBY82 DNA probe) of leaf sample extracts scored as (+), (+ +), and (+ + +).

(a) Northern dot-blot hybridized NC filter

(b) Autoradiography

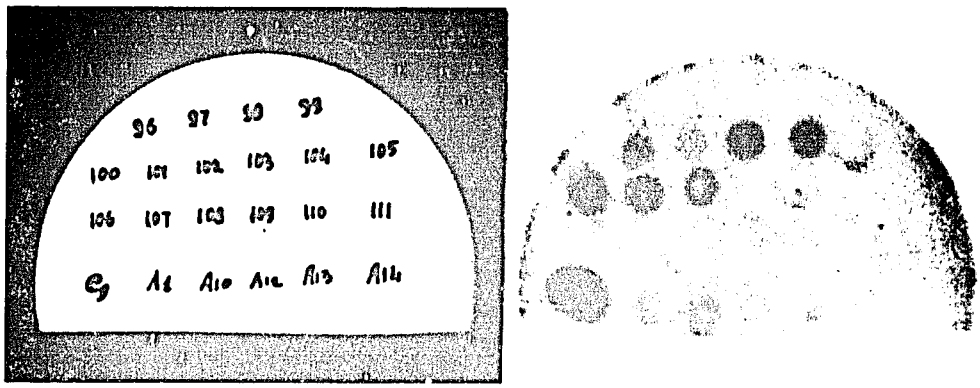


Figure 2. (a) Nitrocellulose filter used in Northern dot-blot hybridization with ^{32}P-labeled pBY82 DNA probe, and (b) autoradiography of positive hybridized spots. Spots on the filter were prepared with 5 μl of: fresh clarified sample extract (spots 96 to 99), fresh clarified sample extract from frozen leaves kept at -80° for 20 days (spots 100 to 102), straight phosphate buffer (spots 103 to 105) and frozen clarified sample extract kept at -80°C for 7 days (spots 106 to 111) from leaves of PAV infected plants; 1/100 dilution of pBY82 DNA (spot C_9, positive control); chloroform-butanol extract of 10 (spot A_1), 8 (spot A_{10}), 6 (spot A_{13}), and 2 (spot A_{14}) viruliferous aphids.

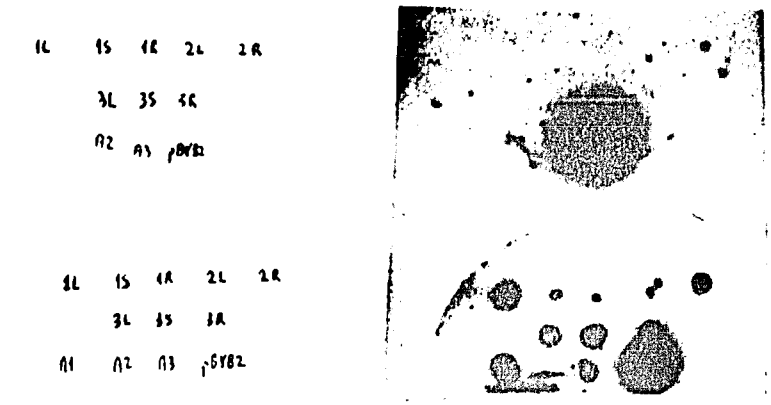
(a)

Northern dot-blot hybridized nitrocellulose filters

(b)

Autoradiography

No PEG



No PEG

+ PEG

+ PEG

Figure 3. (a) Nitrocellulose filter used in Northern dot-blot hybridization with ^{32}P-labeled pBY82 DNA probe in the presence (+PEG) or absence (no PEG) of polyethyleneglycol, and (b) autoradiography of positive hybridized spots containing 5μl of sample extract from leaf (L), stem (S), and root (R) of PAV infected plant 1 (spots 1L, 1S, and 1R respectively), plant 2 (spots 2L and 2R), and plant 3 (spots 3L, 3S, and 3R); three different 4-aphid extracts (spots A1, A2, and A3); 1/100 dilution of pBY82 DNA (positive control).

PEG was not used the spots of infected material did not show any signal at all (Figure 3). PEG has the same function as dextran sulfate in standard Northern blot hybridization experiments and is less expensive. The adopted detection procedure allowed one operator to prepare three filters (80 sample spots per filter) in one working day.

Biotinilated probe gave good chromogenic signal on the hybridized filter only when it was used at a concentration of 0.1 μg/ml of hybridization mixture. However, in a comparison of the type of signal produced by sample extracts spotted on duplicate filters, one of which was used for Northern dot-blot hybridization with radioactive probe and the other for Northern dot-blot hybridization with biotinilated probe, there were only 69% of the samples with concordant types of signals on both detection systems (either very light or very strong signal), while for the other 31% of the samples the signals were not comparable (Figure 4).

Screening results

For 24% of the Ethiopian tetraploid wheat entries tested (indicated as [-]) scoring class in Figure 5) there were no differences between the autoradiographic signals obtained from spots of sample extract of infected plants and those from noninfected negative control wheat plants. This suggests that the amount of virus present in those entries was insufficient to be detected by the method used. The signal from the negative control plants appeared as a light shadow of gray on the autoradiographic film. The frequency of entries from Iran, Iraq, and Turkey included in the (-) scoring class was 29, 8 and 11%, respectively.

Although the entries not included in the (-) scoring class showed a continuous distribution of intensity of autoradiographic signals, they were grouped in three scoring classes indicated as +, + +, and + + +, according to the increasing intensity of the autoradiographic signal after comparison with signals from the negative control. Different amounts of virus particles were present among entries on the basis of intensity of autoradiographic signals. The entries in class (-) would be more desirable for further screening for possible sources of BYDV tolerance.

None of the infected plants showed leaf discoloration. However, 40 days after inoculation (plants at the booting stage) the infected plants showed stunting and were, on average, 4 cm shorter than the non-infected plants. However, no appreciable difference in symptom appearance and plant height was detected between plants classified as (-) and (+) in Northern dot blot.

Discussion and Conclusions

The main problem in breeding for increased resistance to BYDV is finding good sources of resistance in plants to be used as parental material. A correlated problem is finding criteria to identify the resistant phenotypes accurately.

The biochemical techniques (ELISA, Northern dot-blot hybridization, filter paper immunobinding assay, Western blotting, etc.), which allow quantitative and qualitative detection of plant viruses, may become necessary assay procedures to be used in preliminary germplasm screening surveys for resistance to BYDV. These require accurate inoculation of plants and precise sample extract preparation (i.e., for small sample extract to be tested, the ratio leaf tissue: extracting buffer must be constant and the leaf tissue must come from a leaf of the same age and at the same position in every tested plant) to avoid large sampling errors. It is also assuming that there is little variation in virus titer between plant accessions so that it is possible to compare between plants. Additionally, other criteria such as leaf yellowing, stunting, tiller number, and yield should be applied for further field testing of the entries considered resistant by the biochemical techniques used.

In tetraploid wheat, the level of resistance found in the collections screened so far is not very high (Qualset et al. 1977). Therefore it is necessary to continue the search for tetraploid wheat

entries that are resistant to each BYDV isolate. The use of an effective biochemical criterion to estimate resistance during the screening process may be worthwhile.

Our study showed that Northern dot-blot hybridization can be used to detect the PAV-like isolate of BYDV in viruliferous aphids and in infected plant tissue using radioactive nick-translated pBY82 plasmid DNA as a probe that hybridizes only with PAV serotype RNA. The assay

(a)

Autoradiographic diagnosis

(b)

Chromogenic diagnosis

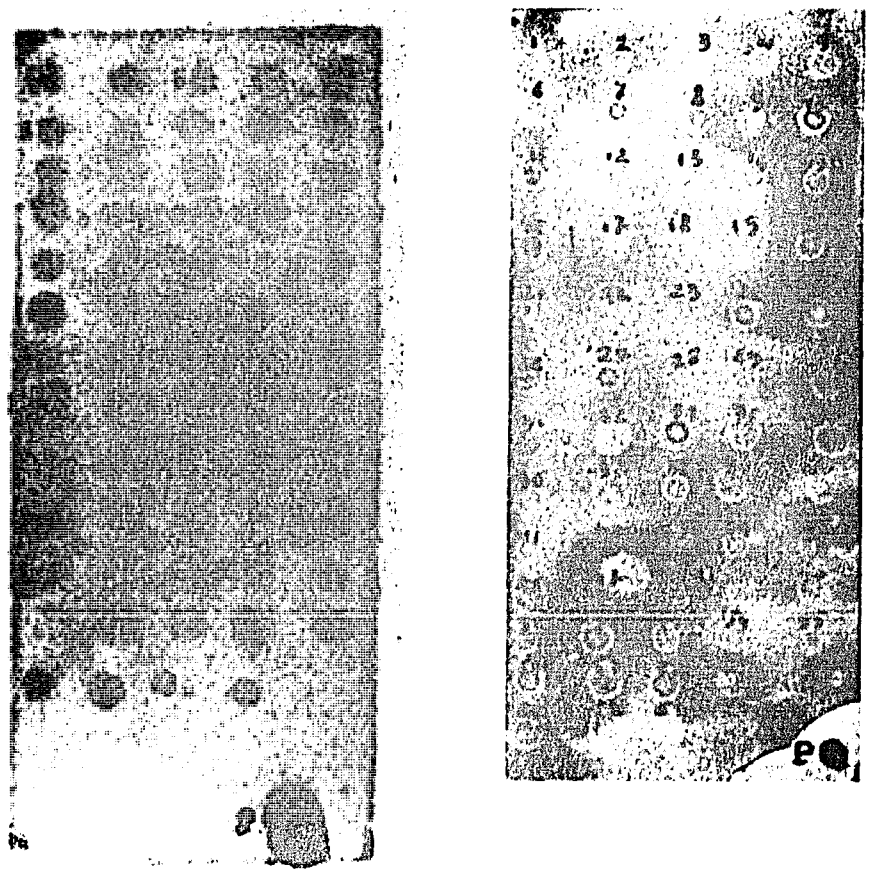


Figure 4. Positive hybridization on filters used in Northern dot-blot hybridization with (a) ^{32}P-labeled pBY82 DNA probe (as revealed from autoradiography) and (b) biotinilated pBY82 probe (as revealed from chromogenic reaction) of sample extract from leaves of infected plants (dots from 1 to 64) and from two leaves of the uninfected plant (dots 28 and 29, negative control). 1,3,6,15,17,18,19,23,30,31,33,39,44,54, and 63 gave similar intensities of autoradiographic and chromogenic signals and were included in the (+) scoring class. Dot 30 is an example of a nonconcordant signal: (+ +) in autoradiographic and (-) in chromogenic diagnosis. Dot P was from a 1/100 dilution of pBY82 DNA (positive control).

procedure was sensitive and rapid, and the results were amenable for qualitative and quantitative scorings.

The survey of tetraploid wheat entries was based on infection with a PAV-like isolate of BYDV because PAV-like are the most widespread serotypes of BYDV in many durum wheat growing areas. The lack of leaf discoloration for all the entries after infection with the PAV-like isolate made it impossible to use leaf symptoms as a further screening criteria. Stunting was present, but its expression did not discriminate significantly any entry.

In a previous screening survey of tetraploid wheat collections from Ethiopia, evaluated in the field using visual symptom scores for leaf discoloration and dwarfing (Qualset et al. 1977), the proportion of resistant entries was 20% of those identified in this study. This may indicate that the currently described technique has to be used in association with resistance screening in the field. Further testing of the (-) class entries in the field in controlled inoculation experiments is planned.

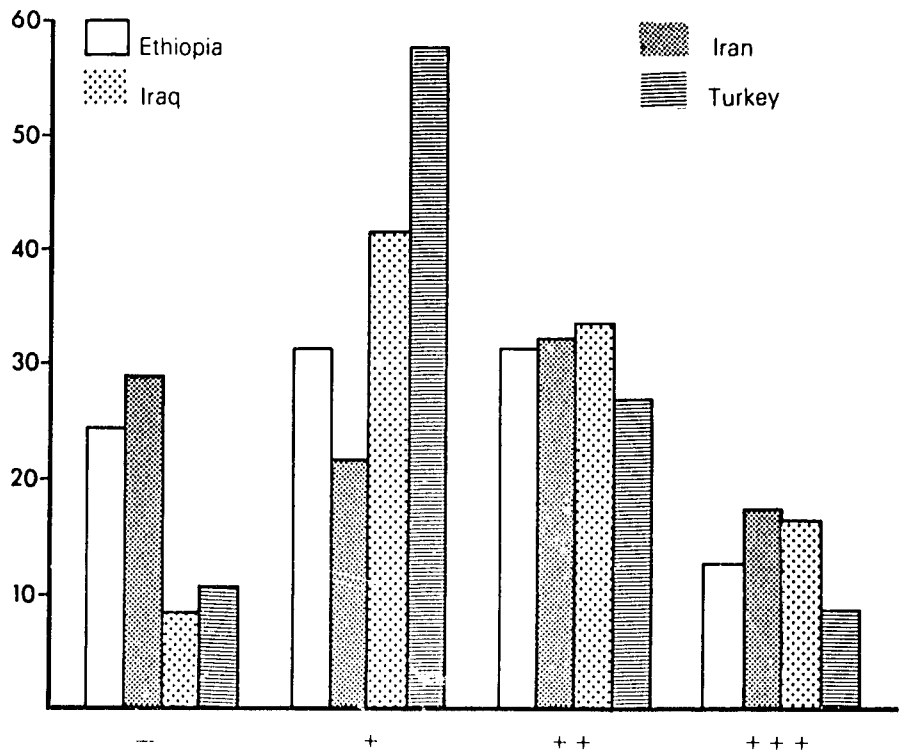


Figure 5. Frequency distribution of PAV BYDV infected tetraploid wheat collections scored for intensity of autoradiographic signal from leaf sample extract after Northern dot-blot hybridization with pBY82 radioactive probe specific for PAV isolate. The scoring classes were divided according to the intensity of autoradiographic signal as: light (-), slightly darker (+), darker (+ +), and much darker (+ + +) in relation to the signal of corresponding uninfected (negative control) plants.

In conclusion, the study showed that differential autoradiographic signals (from positive hybridization of leaf sample extract with a PAV serotype DNA probe), were expressed by tetraploid wheat entries infected with a PAV-like isolate of BYDV. This indicated differential reaction of the entries to infection with a PAV-like isolate and may be of use to identify sources of resistance to PAV-like isolates of BYDV. Because no other differential symptoms were expressed in the infected plants in the greenhouse, the further evaluation for tolerance of the (-) class entries should involve a) field testing under controlled inoculation with the PAV isolate of BYDV and b) the use of additional criteria to evaluate resistance to BYDV such as plant height, spike fertility, kernel weight, and grain yield per plant.

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Inheritance of Resistance to Giallume in Rice

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Abstract
*"Giallume" is a disease of rice caused by the rice giallume virus (RGV), a nonspecific strain of the barley yellow dwarf virus. The study of the inheritance of resistance to giallume in rice (*Oryza sativa *L.) was carried out on the basis of two susceptible/resistant crosses. The test plants, artificially inoculated at the first-leaf stage using the aphid* Rhopalosiphum padi *(L.) as vector, were classified on the basis of symptom expression. It was impossible, under the conditions of this experiment, to clearly identify the heterozygotes. The data agree with the hypothesis of one incompletely dominant gene governing resistance.*

"Giallume" is a disease of rice (*Oryza sativa* L.) caused by the rice giallume virus (RGV), a nonspecific isolate of barley yellow dwarf virus (BYDV) (Osler 1984; Belli et al. 1986).

This disease was observed the first time in 1955 (Corbetta 1967), but it was not until the 1970s that it became an important problem for cultivated rice in Italy (Baldacci et al. 1970; Belli et al. 1974; Osler et al. 1977; Osler 1980; Moletti et al. 1989).

The source of resistance to giallume, on the basis of symptom expression, is the old Italian variety Vialone Nero, sometimes known as Vialone or Nero di Vialone. Resistance has also been found in some Italian varieties having that genotype as an ancestor (Moletti and Osler 1978; Moletti et al. 1979).

All the high yielding rice varieties presently cultivated in Italy are susceptible to this virus disease. Understanding the genetic basis of resistance to giallume is important to breeders who wish to introduce this character into the Italian cultivated rice varieties.

Materials and Methods
The P_1, P_2, F_1, F_2, BCP_1, and BCP_2 generations of two susceptible/resistant cross combinations, Mondiale/Arborio and Cripto/Arborio, were used in this study.

In addition, the second backcross of six susceptible/resistant cross combinations—Radon*3/Rodio, Torio*3/Veneria, Cripto*3/Veneria, Cripto*3/Vialone Nano, Padano*3/Navile, and Titanio*3/Veneria—was also analyzed.

The seedlings were artificially inoculated in the greenhouse at the first-leaf stage using three *Rhopalosiphum padi* (L.) per plant as vectors. The plants were then grown outside the greenhouse, using the procedure described by Moletti and Osler (1978).

The plants were classified for resistance on the basis of symptom expression (Corbetta 1967; Baldacci et al. 1970).

Results
In the heterozygotes, symptoms appeared late and slowly and it was more difficult to classify the diseased plants than in the susceptible homozygous genotypes. Therefore, it was impossible, under our conditions, to clearly identify, on the basis of symptom expression alone, the heterozygotes.

The results of the artificial infection of the cross combinations (susceptible/resistant) Mondiale/Arborio and Cripto/Arborio are reported in Table 1.

At the second scoring, 3 months after inoculation, 86% of susceptible parent Mondiale and 100% of Cripto showed disease symptoms, while no plants of the resistant parent Arborio showed symptoms.

The F_1 for Mondiale/Arborio and Cripto/Arborio showed the same trend for both the cross combinations, increasing from 13 and 19% diseased plants, respectively, at the first scoring, 1 month after inoculation, to 67 and 55%, respectively, at the second.

In the F_2 generation, both cross combinations showed a ratio of approximately 3 resistant: 1 susceptible at the first scoring and a ratio of approximately 1 resistant: 3 susceptible at the second scoring.

The response of the backcrosses to the susceptible parents was quite similar for both the cross combinations analyzed, while the backcrosses to the resistant parent Arborio gave different results at the second scoring: 45% diseased plants for Mondiale/Arborio and only 5% for Cripto/Arborio.

In the study involving the second backcrosses (Table 2) 57 to 92% of plants, depending on the backcrosses, showed symptoms of giallume, while 55

Table 1. Plant reaction to RGV artificial inoculation in two susceptible/resistant cross combinations: Mondiale/Arborio and Cripto/Arborio

Generation and score	No.[a]	No. of plants total	No. of plants diseased	% diseased plants observed	% diseased plants expected[b]	ratio 3:1 X2	ratio 3:1 P	ratio 1:3 X2	ratio 1:3 P
MONDIALE(P1)	1	14	6	43	100				
	2	14	12	86					
ARBORIO(P2)	1	15	0	0	0				
	2	15	0	0					
MONDIALE/ARBORIO									
F_1	1	46	6	13	0-100				
	2	46	31	67					
F_2	1	301	82	27	25-75	0.870	0.30-0.40		
	2	287	193	67				6.411	0.025-0.01
BCP_1	1	98	24	25	50-100				
	2	96	70	73					
BCP_2	1	94	2	2	0-50				
	2	93	42	45					
CRIPTO(P1)	1	48	18	38	100				
	2	48	48	100					
ARBORIO(P2)	1	15	0	0	0				
	2	15	0	0					
CRIPTO/ARBORIO									
F_1	1	86	16	19	0-100				
	2	86	47	55					
F_2	1	224	72	32	25-75	6.095	0.025-0.01		
	2	224	158	71				2.380	0.10-0.20
BCP_1	1	88	26	30	50-100				
	2	88	67	76					
BCP_2	1	74	1	1	0-50				
	2	74	4	5					

[a] 1 = 1 month after inoculation; 2 = 3 months after inoculation.
[b] on the basis of a single incompletely dominant gene and being not possible to identify the heterozygotes.

to 98% of the susceptible parents showed symptoms. It should be pointed out that, in analyzing these materials, the behavior of the backcross vs its own susceptible parent is more important than the percentage value per se. The percentage of plants with symptoms in each backcross and in its own susceptible parent was generally quite similar.

The mean percentage of diseased plants for the two inoculations was clearly different: 82% for backcrosses and 84% for susceptible parents in inoculation I compared with 58% for backcrosses and 67% for susceptible parents in inoculation II. Considering that inoculation II was made 1 week later than inoculation I on seedlings at the same growth stage, these data suggest that there may be a strong effect on symptom expression caused by the interaction of the genotype with the environmental conditions.

Discussion

The data are not easy to interpret, but the hypothesis of a single incompletely dominant gene governing resistance enables us to explain the results.

Table 2. Plant reaction to RGV artificial inoculation in six second backcrosses and the susceptible parents

Generation (Susceptible parent)	No. of plants total	No. of plants diseased	% diseased plants	Inoculation[a]
RADON*3/RODIO	150	117	78	I
(RADON)	97	95	98	I
TORIO*3/VENERIA	162	126	78	I
(TORIO)	83	46	55	I
CRIPTO*3/VENERIA	132	121	92	I
(CRIPTO)	100	95	95	I
CRIPTO*3/VIALONE NANO	154	87	57	II
(CRIPTO)	129	88	68	II
PADANO*3/NAVILE	144	100	69	II
(PADANO)	96	62	65	II
TITANIO*3/VENERIA	72	46	64	II
(TITANIO)	56	37	66	II
Total				
Inoculation I backcrosses	444	364	82	
Susceptible parents	280	236	84	
Total				
Inoculation II backcrosses	370	215	58	
Susceptible parents	281	187	67	
Total				
Backcrosses	814	579	71	
Susceptible parents	561	423	75	

[a] Inoculation I was made 1 week earlier than inoculation II; both with seedlings in first-leaf stage.

Let us consider the hypothesis that one incompletely dominant gene is governing resistance. The same genetic condition has been reported for BYDV in barley (*Hordeum vulgare* L.) (Rasmusson and Schaller 1959; Damsteegt and Bruehl 1964; Schaller et al. 1964). The interaction of incomplete dominance with genetic background, environmental conditions, source of inoculum, and plant stage explains the variability found in the symptom expression in the heterozygotes. A different expression of the symptoms in homozygous vs heterozygous plants was also reported for BYDV-infected barley (Rasmusson and Schaller 1959; Schaller et al. 1964) and the effect of a number of factors on barley yellow dwarf symptom expression has been reported in barley (Catherall et al. 1970; Jones and Catherall 1970) and in rice for RGV (Osler and Moletti 1984). In the heterozygotes the symptom expression will be more irregular or variable if the development of the virus is delayed or subjected to other constraints.

The fact that the heterozygotes may be classified as symptomatologically resistant or symptomatologically susceptible and that this classification can be modified due to a number of interactions, the segregation rates should define a range rather than the exact theoretical value. Consequently, for the study involving second backcrosses the behavior, as found, should be comparable to that of the susceptible parent in the same test.

The hypothesis of a more complex genetic basis governing resistance must also be considered. However, the resistance to RGV is found in many Italian rice varieties and breeding lines that have been produced without selection under disease pressure, but having as a common ancestor the old cultivar Vialone Nero, the only source of resistance to giallume found as yet (Moletti and Osler 1979), which suggests that it must be very easily transferred. In addition, a simple dominant genetic basis has also been reported for the resistance to other virus diseases in rice (Khush 1977).

Because it is impossible to clearly distinguish the heterozygotes, the breeding work for RGV resistance may be of low efficiency.

An attempt to discriminate the heterozygotes on the basis of the time from the inoculation to the symptom expression and the analysis of segregating populations grown in open rice fields under natural infection conditions can be made.

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The Barley Yellow Dwarf Screening Program at CIMMYT

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Abstract

*Screening of cereal lines for barley yellow dwarf resistance has begun in the CIMMYT wheat program in Mexico. Currently the screening program tests cereal lines in small, space-planted plots that are exposed to naturally occurring epidemics of barley yellow dwarf virus. We have the capability of infesting plots with greenhouse-reared viruliferous aphids. Lines that show visual resistance in Mexico have been distributed to selected sites worldwide. There is a great deal of among-site variation in resistance, but some lines of bread wheat (*Triticum aestivum *L.), durum wheat (*Triticum turgidum *L. var* durum*), barley (*Hordeum vulgare *L.) and triticale (X* Triticosecale *Wittmack) are resistant at most sites.*

Barley yellow dwarf (BYD) is currently the most important and widespread virus disease of small grains. Barley yellow dwarf virus (BYDV) attacks all small grain cereals including bread wheat (*Triticum aestivum* L.), durum wheat (*Triticum turgidum* L. var *durum*), barley (*Hordeum vulgare* L.), oats (*Avena sativa* L.), and triticale (X *Triticosecale* Wittmack). The virus also infects at least 100 other grass species including maize (*Zea mays* L.) and rice (*Oryza sativa* L.), and these hosts can act as reservoirs for the virus and for aphids.

BYD has been recorded in most areas of the world. Losses due to the disease vary, estimates ranging from 1 to 3% annually are attributed to BYDV in the USA, although in some years and some locations, losses may be as high as 20 to 30%. In some cases, crops have been totally destroyed.

Because its presence is often masked by other diseases, such as the rusts, BYD often becomes apparent only when resistance to these diseases has been developed.

The BYD research project at CIMMYT supports the transfer of technology currently available in developed countries to developing countries in an effort to reduce losses caused by BYD. The project is funded by the Dipartimento Cooperazione Allo Sviluppo (DCAS) of the Ministry of Foreign Affairs of the Republic of Italy and was initiated in 1985. An international research network, in which Italian institutions play an important role, is being formed to further the development and exchange of germplasm and research procedures for controlling BYDV, especially in developing countries.

To successfully transfer the technology developed through this project to developing nations, a number of interrelated objectives are being pursued:

- Establishing and/or strengthening relationships among developed and developing countries where BYDV causes significant economics losses
- Offering training opportunities for scientists from developing countries where BYDV causes significant economic losses
- Screening germplasm extensively to develop BYDV resistant/tolerant materials, and delivering this germplasm to national program collaborators
- Conducting epidemiological studies on BYD in Mexico
- Identifying the range of BYDV isolates in the world

In some regions, control of BYD can be partially effected by adjusting planting time to avoid the period when aphids are most likely to infest cereal seedlings. Occasionally, partial control may be obtained by the judicious use of insecticides for aphid control, thereby limiting the spread of BYDV. In some

countries, biological control of aphids has reduced the incidence of BYD. However, none of these methods is completely satisfactory, and the most effective control is probably plant resistance.

Germplasm development and distribution are two of the essential tasks of CIMMYT. Host plant resistance to BYDV has been demonstrated for barley, initially in California and now in other countries, conditioned by a single gene (*Yd2*) transferred from Ethiopian landrace varieties. This control method is being pursued in the CIMMYT program. Similar resistance genes have not yet been identified in other cereals, but testing to date has not been exhaustive.

Initial efforts concentrated on germplasm screening, in particular advanced lines produced by CIMMYT breeding programs and selected materials from various cooperators.

Germplasm Screening

Since 1980, CIMMYT has utilized Toluca, Mexico, which has natural BYD epidemics, for screening winter and spring materials. BYD observations in the winter are taken on the breeders' plots because of limitations of available land. Previously, there had been selection for BYD resistance because breeders did not retain lines that showed stunting and discoloration.

Since 1984 we have been successfully using Toluca as a spring BYD screening site by sowing in mid-June. This represents late sowing for this site. The plants are spaced 15 to 20 cm apart to increase the intensity of BYDV infection. In the spring plantings, entries are hand-seeded in two-row plots of 1 m, with 5 to 7 seeds/m. Plots are separated by a 50-cm gap and two replicates are sown. We have experimented with using single rows of 2 m, but the two-row plots have proved superior. Observations on symptoms are taken using a 0 to 9 scale where 0 is resistant and 9 is fully susceptible (Qualset 1984). We are experimenting with spraying the plots every 2 weeks with fungicides to eliminate the foliar symptoms of other diseases, enabling us to evaluate BYD symptoms more easily.

Currently the program is concentrating on screening advanced lines of the CIMMYT breeding programs plus materials reported to be resistant to BYDV elsewhere. Material appearing resistant to BYDV in our tests is selected and maintained for retesting and for distribution to other sites where BYDV is a problem.

Results

Spring bread wheat

The preliminary bread wheat nursery for BYD was obtained from an initial screening in Toluca during the winter cycle of 1981/82. It contained 89 entries that had appeared resistant to BYDV. Two check cultivars, Anza and Nacozari, with known resistance, were included every 20 entries. Usable data were only obtained from three sites: Canada—A.I. Comeau; New Zealand—J.M. McEwan; and USA—C.O. Qualset. The eight lines that appeared to exhibit resistance at all sites were FLN/ACC//ANA, PRL'S' (two lines), JUP/EMU'S'//GJO'S' (three lines), DODO'S', and ERA/MN69146//PVN76'S'. There was apparent variation of resistance across sites, with many reversals being apparent where lines that appeared resistant at one of the sites were susceptible at another. This illustrates the variable nature of BYD and the need for multi-site testing for BYDV resistance.

Screening of CIMMYT's advanced lines began in earnest in Toluca in the summer of 1984 and continued in the summer of 1985. In the first year, 228 lines were selected in Toluca and in the subsequent year these were retested in Toluca and in Davis, California. Lines that exhibited resistance at Toluca in 1984, 1985, and 1986 and at Davis in 1985 are listed in Table 1.

Additional lines with resistance for 2 years in Toluca and with good plant type for the breeding program are ALDAN'S'/CNT9, BUC'S'/CHRC'S', TRT'S', NEPAL 133, FCT'S', HAHN'S'/LIRA'S', and DOVE'S'/BUC'S'.

A first official BYD screening nursery for bread wheat, containing 146 lines, was assembled for distribution to selected sites where BYD is usually present. This nursery was composed of selections from the original BYD nursery, plus selections from the 228 lines that were selected in Toluca in 1984. Preliminary data from this nursery at two sites are shown in Table 2.

During 1985 and 1986, approximately 3000 spring bread wheat lines per year were screened in the program and selections retained. The second BYD bread wheat screening nursery, containing 92 entries, was sent out; the preliminary data on resistant entries from two sites are shown in Table 3.

Winter bread wheat

Many winter wheat nurseries have been scored visually for resistance to BYD in Toluca. The lines that have exhibited resistance over a number of years include NS974/NB69565, PAYNE, OK 77164, STURDY, ANZA/SUT//CTK, NR72.837,

Table 1. Bread wheat selections that showed resistance to BYD in Toluca in 1984, 1985, and 1986 and at Davis, California, USA, in 1985

TONI*
AMD'S'/HN4//COC
F12.71/COC*
LIRA'S'*
BJY'S'/COC*
R37/GHL121.//KAL/BB/3/KLT'S'
THB'S'
E7408/PAM'S'//HORK'S'/PF73226
ALV110/2*IAS54/6/TP/4/TZPP/SN64//NAPO/3/CNO 67/5/PF6968
SAP'S'/MON'S'
RPB 709.71/COC
PRL'S'*
PAR/H 567.71
FLK'S'/HORK'S'*
BUCK MAPUCHE
RSK/TIRESEL//COC

* These looked particularly good in 1986.

Table 2. Entries in the first barley yellow dwarf bread wheat nursery with scores that showed resistance at Santiago, Chile, and Quito, Ecuador

Line	Sites	
	Santiago	Quito
CEP80111	3	2
ALD'S'/PIMA/3/CMH74A.630/BUI'S'//CMH74A.630	3	2
SAP'S'/MON'S'	3	3
BUC'S'/PVN'S'	4.5	2
ANB'S'/JUP	4.5	2
TRAP #1	4.5	4
YACO'S'	3	2
PVN'S'/MN70121	3	3
PF72640/PF7326//PF7065/Ald'S'	3	3
PF79765	3	2
RC7201/2*BR2	3	1
RPB709.71/COC	1.5	—
YR/TRF'S'	3	2

Scoring is done on a 0-9 scale, where 0= resistant and 9 = fully susceptible.

F44.72, 77WO93, PONY'S', F9.70/MAYA, and F12.71/COC. The best lines will be used as parents in Mexico aimed at transferring BYD resistance from these winter lines to spring wheat. Some of these lines look particularly good.

Durum wheat

Resistance in durum wheat seems to be limited. For 3 years most of the nurseries have been checked, but only a few durum lines with resistance were found. Entries that showed resistance at Toluca over 3 years are M 75, GS'S'/CR'S'// SBAB1/3/HO/4/ MEXI'S'/5/MEMO'S', STINT'S', GIA'S', LOON'S', and MEMO'S'/YAV79. Durum lines that were selected at Davis and showed good resistance at Toluca in 1986 included MEXI75/CHUMPI 2//FAB'S'/3/P66.270 /4/GOO'S' (2 lines), MEMO'S'/ALBE'S', MEMO'S'/MEX75, and SCA'S'/4/ZB /MHMDM RARI//S15 CR'S'/3/MEXI'S' /5/FRIG'S'. Winter materials have been checked and the two lines that looked the best were CAM 20 and PI 330551 (T.M.). Our work with durums is a little disappointing; we have materials that look good for 2 years and then appear susceptible.

The first BYD screening nursery for durum, 29 entries, was distributed to sites that normally experience BYD epidemics for further testing. Three lines—CIT 71, AFN'S'/IBIS'S'//COO'S' /3/GOO'S', and YAV'S'/FG'S'//ROH'S'—have looked good in Chile and at Sevilla and Lleida in Spain.

A wider range of germplasm needs to be screened before really good durum resistance can be identified, however we have now got some promising lines.

Barley

Barley lines for inclusion in the BYD screening nurseries are originally selected in Toluca. There have been five BYDV barley screening nurseries distributed.

The first nursery consisted of 169 entries (129 spring and 40 winter barleys) and was distributed in 1982. Usable data were received from three cooperators: Canada—A.I. Comeau; New Zealand—J.M. McEwan; and Spain—J. Hernando Velasco. Table 4 lists the spring materials that exhibited resistance at all

Table 3. Preliminary scores of the second barley yellow dwarf screening nursery bread wheat 1986-87

Cultivar	Sites Palmerston North New Zealand (mean 2 scores)	Pasto Colombia (initial score)
DOVE'S'/BUC'S'	3	2
LIRA'S'	2.5	2
THB'S'	2.5	2
ALV110/2*IAS54/6/TP/4/TZPP/SN64// NAPO/3/CNO67/5/PF6968	2.5	2
PRL'S'	2.5	2
KEA'S'/GH'S'	3.0	3
HAHN'S'/LIRA'S'	3.0	1
SDY//CHRC'S'/3/AU/UP301//BOW'S'	2	2
VS73.600/MRL'S'/3/BOW'S'//YR/TRF'S'	2	1
TRAP #1	2.5	1
PF339//MRS/COC	3.5	1
HAHN'S'*2/PRL'S'	2.5	2
Checks		
ATLAS 57	6.6	8.3
ATLAS 68	2.1	5.0
ANZA	3.1	3.3
BOW'S'	4.0	6.3

Scoring is done on a 0-9 scale, where 0 = resistant and 9 = fully susceptible.

Table 4. Lines from the first barley BYD screening nursery that appeared resistant at three sites: Saint-Foy, Quebec (Canada); Palmerston North (New Zealand); and Madrid (Spain)

PROMESA
CACO'S'
SUTTER
BEN 4D
CHINO'S'
ASSE/NACKTA//VILLA ROBLEDO/3/PYD
OJIL'S' (2 lines)
LIGNEE 640
API/CM67//APM/1B65/3/API/CM67//II266* L2966.69/4/BEN
ORE'S'//API/CM67
ORE'S'//INDIAN DWARF/CM67
API/CM67//AGER
ALAMOS'S'

sites. Again there were many notable reversals and this again accentuates the necessity for multi-location testing.

The Yd_2 gene was obtained from Ethiopian landraces of barley and appears to be a strong and durable gene for resistance to BYD. It was first exploited by C.W. Schaller of the University of California at Davis and now is used worldwide to provide resistance for BYDV. The second barley BYD screening nursery consisted of 43 entries that were not known to contain the *Yd_2* gene for resistance. Table 5 lists the 13 best lines with the scores from the eight sites where usable data were obtained. Even within this limited group scores vary greatly across sites. For example, Nigrinudum and PI382411 scored 7 (susceptible) at site 2, but at the seven

Table 5. Scores of selected lines indicating resistance from the second barley BYD screening nursery at eight sites: Tunis, Tunisia (1); Beijing, China (2); Montpellier, France (3); Lleida, Spain (4); Madrid, Spain (5); Toluca, Mexico (6); Pendleton, USA (7); and Palmerston North, New Zealand (8)

	Sites								
	1	2	3	4	5	6	7	8	Mean
PI2325/MAF102//COSSACK	3	4	1	1	0	2	3	2	2.0
79W41762	3	1	1	0	1	4	6	3	2.4
TERAN 78	3	4	1	0	5	2	3	2	2.5
G134/APM//CI08971/3/ API/11012.2//P71386	5	6	1	0	1	3	3	4	2.9
DUCHICELA	5	3	2	1	3	3	4	2	2.9
LIGNEE 640	5	3	—	1	0	5	3	3	2.9
GUS	5	5	2	1	3	3	3	2	3.0
NIGRINUDUM	3	7	1	3	1	5	3	1	3.0
78W40785	5	5	2	0	5	4	3	1	3.1
PI382411	3	7	1	5	3	2	3	1	3.1
TRIUMF	9	8	2	1	3	5*	7	7	5.3
ATLAS 57	9	5	4	6	3	6	8	5	5.8
ATLAS 68	5	5	1	7	1	4	3	2	3.5

* More often 7 at this site.

Scoring is done on a 0-9 scale, where 0 = resistant and 9 = fully susceptible.

other sites they showed resistance. This accentuates the need for across site testing; resistance at one site does not necessarily mean resistance at another site. Many other lines had low scores at five or six sites but received high scores at others.

Data from three of the checks that were included in the second BYD screening nursery, Triumf and the isogenic lines Atlas 57 (-*Yd2*) and Atlas 68 (+*Yd2*), are included in Table 5. Also included were California Mariout (-*Yd2*) and CM67 (+*Yd2*), but these two lines do not appear to be good indicator test lines because they mature too rapidly and the onset of maturity can often be mistaken for BYD susceptibility. These check lines also reveal the great variation in BYD scores.

Table 6 lists the preliminary results from the fourth barley yellow dwarf screening nursery.

The best entries from the crossing block at Toluca for reaction to BYD in 1984 were DORADA, CI2325/CI12225//BOY•2/3• SURB, ESC.II.72.83.3E.5E.1E, CEDRO'S', SUTTER, PROMESA, SUTTER•2/NUMAR, and API/CM67//AGER. These have been tested again over the last 2 years. They exhibit good resistance in Mexico and some also exhibit good resistance at other sites.

We have carried out screening on lines that have been coming from the barley program and on lines included in the International Barley Observation Nursery (IBON). Little resistance was found until 1986. The resistance that is appearing in the IBON is coming from crossing directed toward resistance.

Winter barley

During the last three winter cycles at Toluca (1983/84, 1984/85, 1985/86), approximately 50 barley lines have been identified that had BYDV scores of 5 or below. Some of these lines are known to be resistant to BYDV in other areas of the world but are not known to contain the *Yd2* gene, e.g., Post, a winter barley from Missouri. These lines will be crossed with lines known to contain the *Yd2* gene in an effort to combine both types of resistance.

Triticale

In general, triticale seems to have a high level of resistance, which is probably coming from the rye. Over the years, a number of lines have been selected, some of which appear to have a level of resistance at other sites (Table 7).

Wide crosses

The material being produced by M. Kazi's program at CIMMYT has been screened and some lines involving crosses with *Elymus*, *Triticum*, *Aegilops*, and *Thinopyrum* are showing promise. However, results are preliminary and

Table 6. Preliminary scores from the fourth barley yellow dwarf screening nursery

Entry number in nursery	Sevilla Spain	Lleida Spain	Palmerston North New Zealand	Peru	Beijing China	Santiago Chile
4	2	5	2	2	5	3
19	--	4	1	2	2	3
27	0	5	3	3	2	5
32	0	5	2	2	2	5
38	--	7	3	4	2	5
42	4	7	1	3	3	5
49	2	6	2	3	3	5
58	0	4	4	4	5	5
61	--	5	2	0	2	3
116	1	6	2	2	2	2

Scoring is done on a 0-9 scale, where 0 = resistant and 9 = fully susceptible.

further testing is required. Screening accessions of the above species is continuing at CIMMYT.

Conclusion

The CIMMYT BYD program so far has concentrated on selecting parents that appear resistant to BYDV in Mexico. Many other research establishments throughout the world have conducted BYD screening for a number of years and the last 2 years we have obtained lines from Davis, California, and Sainte Foy Quebec, Canada, that have been selected at those sites and now these are being used in our program.

Current data are preliminary and further testing is required at a greater range of sites before reliable data are obtained. The strength of the CIMMYT program is the network of cooperators that have been screening materials for BYD resistance.

CIMMYT will act as a collator of this information and a distribution center for materials, as well as conduct its own program. It will continue to send out barley yellow dwarf screening nurseries on a regular basis.

Acknowledgments

Special tribute is paid to the CIMMYT breeders Drs. S. Rajaram, P. Brajcich, H. Vivar, G. Varughese, and A. Mujeeb-Kazi for their support and assistance with this project. Thanks are also due to Ing. Juan Vallejo, Javier Varela, Marcos Lugo, Pedro Rosas, and Juan Meneses for their technical help. The cooperators of CIMMYT, who have taken time to make notes on distributed nurseries, are also thanked.

Reference

Qualset, C.O. 1984. Evaluation and breeding methods for barley yellow dwarf resistance. *In* Barley Yellow Dwarf. CIMMYT, Mexico. Pp. 72-82.

Table 7. Preliminary results of the first triticale barley yellow dwarf screening nursery

Cultivar	Sites: Santa Catalina, Quito, Ecuador	Sites: Sevilla, Spain
WHALE'S'	2	0
PTR'S'/Castor'S'//BTA'S'	2	0
GNU'S' (2 lines)	2	1
PND'S'/ABN'S'//2*IA	2	0
PTR'S'//CML'S'/FS 1377/3/IA//CIN'S'/FS658*	2	2
LMG'S'/POL'S'	2	1
PND'S'/LNC'S'//TGE'S'	2	0
HARE 134	2	1
DGO'S'	2	0

Scoring is done on a 0-9 scale, where 0 = resistant and 9 = fully susceptible.

Successful Breeding for Barley Yellow Dwarf Resistance or Tolerance: A Systematic Approach Related to Other Agronomic Characteristics

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* Deceased

Abstract

Resistance to barley yellow dwarf virus (BYDV) is frequently linked with characteristics, both beneficial and detrimental to the final agronomic value of the cultivars. BYDV resistance or tolerance is discussed in terms of the traits found associated with resistance—growth habit, earliness, yield, height, lodging resistance, grain quality, other disease resistances, and root system characteristics. In winter triticale (X Triticosecale *Wittmack), winter barley (*Hordeum vulgare *L.), and winter wheat (*Triticum aestivum *L.), the winter survival is often indirectly improved by barley yellow dwarf virus resistance genes. Most winter cereals are more resistant to BYDV than spring-sown cereals, but BYDV, when present, contributes to winterkill. In all cereal species, earliness is a form of field resistance; however, breeding for earliness is not a highly successful avenue to breed for resistance to BYDV.*

*In oats (*Avena sativa *L.), virus resistant lines were formerly characterized by low yield, excessive height, and poor seed quality, until the breeding program in Illinois produced high-yielding virus resistant lines with good lodging resistance and good seed quality. Initial attempts to breed BYDV resistant barleys in Quebec, Canada, have not been particularly successful because the principal resistance gene* Yd_2 *is linked with factors that cause lodging and reduce the seed quality. However, there are now CIMMYT-bred dwarf barleys possessing the* Yd_2 *gene and from this enhanced resistant germplasm, successful barley breeding can be expected. In spring triticale, barley yellow dwarf resistance is typically accompanied by high yield and relatively tall plant size. In spring wheat, resistance to the virus is mostly found in Brazilian lines with excessive plant height but also with good resistance to fungal diseases such as septoria and fusarium. A better knowledge of the above linkages should be useful in planning the breeding of BYDV-resistant or tolerant cultivars. The current challenge should be to improve the resistance of dwarf plant types especially in bread wheat.*

Cereal breeders have found both defects and useful qualities in the germplasm suggested to them as sources of resistance or tolerance to barley yellow dwarf (BYD). Over the years, it became apparent that there was a common pattern in these qualities and defects, and that it was worth using a systematic approach of close collaboration with the plant breeders to take full advantage of the useful characteristics and eliminate the negative traits.

The present resistance breeding work in Sainte-Foy deals with bread wheat (*Triticum aestivum* L.), durum wheat (*Triticum turgidum* L. var. *durum*), barley (*Hordeum vulgare* L.), triticale (X *Triticosecale* Wittmack), oats (*Avena sativa* L.), rye (*Secale cereale* L.), and interspecific derivatives; the Illinois project involves oats and winter wheat. We will discuss here barley yellow dwarf virus (BYDV) resistance or tolerance in terms of its value to plant breeding, considering the traits we found associated with resistance. These traits include growth habit, earliness, yield, height, lodging resistance, grain quality, disease resistance, and root system characteristics.

This review of the indirect effects of breeding for resistance or tolerance will emphasize the results obtained by the Illinois project on oats, which was one of the pioneering efforts to control BYD after the disease was discovered in 1953. The breeding of barley, wheat, and

triticale will also be discussed, for spring and winter types, addressing the concerns of plant breeders about the side effects of BYDV selection. Breeding methodology will be considered only briefly as it has been adequately discussed elsewhere (Qualset et al. 1973; Comeau 1986; Tola and Kronstad 1984; CIMMYT 1984).

A theory was recently put forward that disease resistance mechanisms could consume enough energy to reduce the yield potential of a cultivar (Smedegaard-Petersen and Tolstrup 1985). BYDV particles represent one part per million in a diseased plant, therefore, if this hypothesis is true, this is indeed a minute amount of viral energy causing a tremendous amount of damage. If energy relationships cannot account for the activity of the attacking virus, one fails to understand why the reaction of the plant, which may be disease resistance or susceptibility, should be interpreted with reference to energy considerations.

We will note the fact that this theory was proposed for fungal diseases, but considering the ideological impact of such a theory we will investigate below the relationships between BYDV resistance and yield, and show that BYDV resistance or tolerance is likely to be associated with high yield.

Resistance and Tolerance

Some confusion exists over the use of the terms resistance and tolerance. Resistance is the ability of a plant to suppress or retard the reproduction of the virus, which should be proved by assays of virus content over time. Tolerance is the ability to endure infection without showing severe disease, and the discussion of tolerance must always be related to a known degree of infection of the host tissue (Federation of British Plant Pathologists 1973). These definitions help to explain why BYDV specialists generally cannot qualify their plants in terms of resistance or tolerance: the virus content of the plants reaches a sharp peak, which may last less than a week, followed by a significant decrease; these events can be modified by complex interactions of plant genes, viral genes, and environment. We will attempt to use the terms tolerance and resistance in conformity with the above definitions whenever available data allow us to choose the proper term.

Growth Habit and BYDV Reaction

As a rule, perennial grasses possess relatively good BYDV resistance or tolerance and sometimes immunity (Comeau and Plourde 1987). Winter wheats, sown in the fall, sometimes show better tolerance than the wheats sown in the spring. Winter triticale is more resistant than spring triticale (Collin et al. 1989). It has been shown that one of the principal effects of BYDV is to reduce the root system (Kainz and Hendrix 1981), and this effect becomes more growth-limiting in spring wheat, which has a more shallow root system.

However, in winter cereals, interaction for BYDV with winterkill agents can turn the BYDV problem into a mortality factor. In Canada, BYDV-infected winter barley has low odds of survival. BYDV reduces the cold tolerance of wheat (Andrews and Paliwal 1983; Paliwal and Andrews 1979, 1989). For this reason, the artificial BYDV inoculation of winter barley, winter triticale, and winter wheat in the fall would be expected to cause the winterkill of a good part of the germplasm. We are currently testing this hypothesis in Sainte-Foy (C.A. Sainte-Pierre, personal communication).

Earliness and BYDV Reaction

In a recent study it was shown that all cereal species become quite tolerant to BYDV during the grain-filling period (Comeau 1987). As in practice the BYD epidemics often flare up after the date of flowering, there are many cases where, in natural conditions, the earliness of the cultivar can provide a form of escape from BYDV damage.

In the early days of the Illinois program, a very early oat cultivar, Lang, was released and this cultivar sometimes yielded well in natural epidemics although it was not really BYDV-resistant. More recently, another early oat cultivar was released in Quebec; this cultivar, Marion, displayed the same ability to escape part of the BYDV damage in natural epidemics in Quebec. However, when the epidemic occurs a bit

earlier in the season, this escape-type tolerance is of no value. The ideal is certainly to combine good BYDV resistance or tolerance with some earliness, as achieved in the oat cultivar Ogle, developed by C.M. Brown and H. Jedslinski in Illinois.

In the UK it was shown that late barley was more severely damaged by BYDV (Jones and Catherall 1970). In wheat and triticale the interaction of earliness with BYDV reaction has not been sufficiently documented.

The idea that breeding for earliness could solve the BYDV problem is unrealistic. Most early cultivars are intrinsically low-yielding types, with a few noteworthy exceptions such as the oat cultivar Ogle. There is only limited potential for progress in BYDV tolerance through the development of earlier cultivars.

The artificial inoculation of bulk populations with BYDV sometimes results in the elimination of late-ripening oat types, without penalty on the middle-range maturity types. This should be viewed by plant breeders as a fringe benefit obtained from BYDV inoculation.

Yield and BYDV Reaction

In the early part of the Illinois work, BYDV resistant lines did not seem very high yielding, and in speculating about the difficulty of creating an acceptable oat cultivar, one might have elaborated prematurely the theory that resistance was associated with low yield (Jedlinski 1972). However, the breeding of the cultivar Ogle by the Illinois group showed that this negative association did not exist. Although there are indeed some sources of BYDV resistance that result in a high percentage of low-yielding progeny, it is possible to discover BYDV resistant or tolerant lines that do not create this problem.

The Illinois BYDV resistance project was initiated by R. Endo and C.M. Brown in 1955 just after the discovery of the existence of the virus, and H. Jedlinski joined in 1959 (Brown and Jedlinski 1960). For the next 25 years, a very significant part of the useful information and virus-resistant oat germplasm in the USA came from the Illinois project. It is interesting to follow the correlation between BYDV resistance and the yield of the cultivars in the Uniform Midseason Oat Nursery (UMSON), which is the nursery where candidate cultivars for northeastern USA are tested before release to farmers. In the 21 years of records assessed, the correlation between yield and symptoms was almost always negative (Figure 1). Correlation was most significant for years of epidemic such as 1971, 1976, 1983, and 1986.

However, there is another trend observed in more recent years, as significant correlations are becoming more frequent. This is, in our opinion, not linked to an increasing level of BYDV infections in the USA, but to the more frequent occurrence of oat lines displaying an association of BYDV resistance with high yield potential, a feature initially observed in Ogle, which entered the trials in 1975. Removing the Ogle data from these trials reduces the correlation value, and the difference is significant at the 0.01 level (Table 1). However, as other high yielding, BYDV resistant oat lines were included in the 1976-1985 trials, the trend toward more significant correlations in recent years remains visible even after deleting the Ogle data.

Canadian breeders, inspired by the success of the Illinois project, started two programs to breed BYDV resistant oats, one in Sainte-Foy in 1972 and one in Winnipeg in 1975. The first BYDV resistant lines from the Canadian projects were too low yielding, and in the Eastern and Western Co-op trials, which evaluate cultivars before release, there was frequently a positive correlation between symptoms and yield, at least before 1983. This meant that before 1983 the most susceptible lines had the best yield. This may not be the case in the future, as Canadian scientists are now producing high yielding, BYDV resistant cultivars, both in Sainte-Foy and Winnipeg.

The trend toward more significant correlation of susceptibility with yield was also the subject of a correlation study. For the UMSON trials, there is a correlation of -0.58 between the age of the Illinois breeding project and the susceptibility-yield correlations

themselves, this being significant at the 0.01 level (Figure 1). For the pooled data from the two Canadian projects, where 11 years are available to confirm the trend, the correlation was -0.54, also significant at the 0.01 level. Therefore, not only do we see BYDV susceptibility associated with low yield, but there is a statistically significant trend over the years in that same direction, in both the UMSON and the Canadian trials.

In barley, most if not all of the very resistant lines possess the Yd_2 gene. Correlations between the yield and the symptoms of barley are available for only one CIMMYT trial, and there was a significant tendency: fewer BYDV symptoms in Quebec trials were correlated with better yield in the CIMMYT international trial (Table 2).

Correlation studies such as above are easy to interpret in the case of oats and barley, as these species generally display obvious BYD symptoms when infected. In other species, the symptoms are less visible. However, the biomass and grain yield of susceptible durum wheat, common wheat, and triticale are strongly decreased (50 to 90%) by BYD. Therefore, an attempt was made to correlate these estimators of BYD reaction in Sainte-Foy with the yield potential as evaluated in the international trials for CIMMYT. The positive correlations obtained confirmed that lines that resisted or tolerated BYDV infection in Sainte-Foy were often lines endowed with good yield in international trials. (Table 2). A similar trend also occurred once for a durum trial, the 12th IDSN. The highest correlation was obtained in triticale, the species

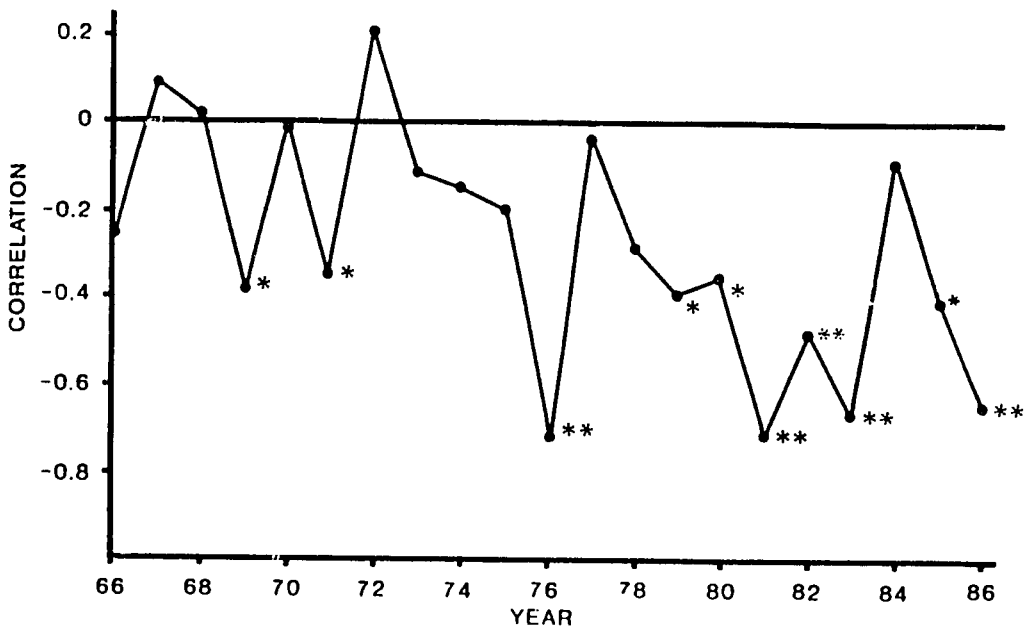

Figure 1. Evolution over 21 years of correlations (r) of average yield at all reporting stations (average of 12 to 22 sites) with average symptoms recorded with artificial or natural BYDV infection (average of 1 to 6 data sets) for the Uniform Midseason Oat Nursery, a trial which varied in size from 26 to 36 cultivars. The negative correlations mean that higher yield is frequent in lines showing less symptoms. The data from natural infections had to be weighted to obtain a similar scale for averaging purposes. Significant r-values are indicated with* (0.05 level) or ** (0.01 level).

displaying the highest diversity for BYD reaction, some lines being very resistant, others intermediate, others very susceptible. It is comforting to see that for all cereal species the trend is totally contrary to the Smedegaard-Petersen and Tolstrup hypothesis.

It now appears to us that breeding for BYDV resistance or tolerance is equivalent to breeding not only for better yield stability but also for better yield worldwide. Two hypotheses were proposed to explain this fact: either invisible BYDV damage is much higher

Table 1. Effect of the presence or absence of one BYDV-resistant cultivar, Ogle, on the correlation between average BYDV symptoms and the average yield evaluated in the Uniform Midseason Oat Performance Nursery (USA)

Year	Number of stations reporting: BYDV data	Number of stations reporting: Yield	Number of oat lines	Correlation between symptoms[a] and yield: Including Ogle data	Correlation between symptoms[a] and yield: Ogle data excluded	Difference[b]
1976[c]	5	12	36	-0.71**	-0.69**	-0.02
1977	1	18	30	-0.04	+0.14	-0.18
1978	4	20	30	-0.29	-0.20	-0.09
1979	4	20	28	-0.40*	-0.40*	0.00
1980	4	19	35	-0.36*	-0.29	-0.07
1981	4	16	36	-0.71**	-0.67**	-0.04
1982	6	20	33	-0.49**	-0.36*	-0.13
1983	6	21	36	-0.67**	-0.63**	-0.04
1984	3	19	35	-0.10	-0.03	-0.07
1985	3	21	35	-0.41*	-0.38*	-0.03
Average	-	-	-	-0.42	-0 35	-0.07

* Significant at the 0.05 level.
** Significant at the 0.01 level.
a Weighted average symptom score, calculated for all stations reporting symptoms from artificial inoculation or percent natural infection.
b The effect of excluding Ogle is a reduction of correlation that is significant at the 0.01 level, according to the Wilcoxon Signed Rank Test (Snedecor and Cochran 1967).
c Three sites with heavy BYDV infection were not included in the yield average of 1976.

Table 2. Correlation between international yield data from CIMMYT trials and characters used to evaluate BYDV resistance in Sainte-Foy

CIMMYT nursery	Species	No. of lines	Characters used to estimate BYDV reaction in Sainte-Foy	Correlation with CIMMYT international yield average	Number of international locations in CIMMYT yield average
14th IBWSN	spring wheat	431	grain yield	0.16*	65
15th IBWSN	spring wheat	206	grain yield	0.17*	16
12th IDSN	durum	233	grain yield	0.29**	26
13th IDSN	durum	235	biomass	0.01	7
12th ITSN	spring triticale	285	grain yield	0.46**	50
13th ITSN	spring triticale	172	biomass	0.61**	24
8th IBON	spring barley	286	symptoms	-0.36**	8

* Significant at the 0.05 level.
** Significant at the 0.01 level.
Symptom scale: 0 = healthy-looking, 9 = very diseased; naked barley lines not included.

than expected, so that resistant or tolerant cultivars obtain a yield advantage, or else the BYD-induced phloem damage mimics or increases other stresses, penalizing the genotypes that have lower physiological efficiency, poor translocation, and inferior stress resistance (C.A. St-Pierre, personal communication).

However, a word of caution is justified. In oats, extreme selection pressure for BYDV resistance or tolerance has not produced the highest yielding oat lines. This extreme selection pressure, obtained by inoculating plants at the one- or two-leaf stage, appeared to favor tall oat types, such as the experimental line AA 68-75, which have a low harvest index and poor yielding potential in Sainte-Foy. Many of the most resistant or tolerant oat lines released as germplasm by Jedlinski (CI 9303 to CI 9315) display similar problems. The best practical results are therefore obtained by relatively severe, but not excessive, selection pressure, and by close collaboration with the plant breeders in order to select, among many sources of BYDV resistance, the ones that offer a real potential for the production of high yielding, BYDV resistant cultivars.

Plant Height: A Natural Tolerance Factor?

It was only quite recently that we realized how widespread is the association between tall plant size and BYDV tolerance. In wheats, oats, barley, triticale, even in perennial grasses, the tallest lines, cultivars, or species are commonly less damaged by BYDV. We also found in Sainte-Foy that in attempting to breed BYDV-tolerant dwarf oats and wheat, discarding the tall segregants often results in losing all the tolerant material in the population. Some sources of BYDV resistance such as the oat line AA 68-75 were rejected from the breeding program in Sainte-Foy because of this unacceptable linkage. In spring wheat, we recently developed ways to produce reliable symptoms by using a severe BYDV isolate on plots seeded quite early (before mid-May) and inoculated before stem elongation. However, when symptoms are severe, the dwarf types are the ones that suffer the most and are eliminated.

For the 1986 and 1987 Sainte-Foy trials performed on the best BYDV resistant bread wheat, we tested dwarf, semi-dwarf, and tall wheat lines separately, to reduce competition effects and also to assess more precisely the selection methods to be used in each height class. As observed in 1986, correlations between various estimators of BYDV resistance differ according to the height class: the height of BYDV-inoculated lines was positively correlated to BYDV resistance (evaluated by symptoms, or by the yield of BYDV-inoculated plots) in dwarf and semi-dwarf lines, but not in tall lines. On the other hand, the harvest index was negatively correlated to symptoms only for the tall lines (Table 3).

In another trial, the two most BYDV-resistant dwarfs and the five most BYDV-resistant tall wheat lines were compared in BYDV-inoculated and non-inoculated plots. It was confirmed that the best BYDV resistance or tolerance in dwarf wheats does not match the higher resistance levels found in the tall types (Table 4). Dwarf BYDV-resistant lines remain for now rare and difficult to breed.

We observed in 1973-1975 in Sainte-Foy that the Yd_2 gene from various Ethiopian accessions was linked with factors that caused excessive height, severe lodging, and reduced yield and seed quality under virus-free growing conditions. We have not yet had enough experience with the CIMMYT-bred dwarf barleys possessing the Yd_2 gene, but it appears that most of the deleterious linkages may have been removed.

The exact reason for the frequent association of BYDV tolerance with tall plant types is not obvious. Perhaps BYDV reduces the rate of extension of the peduncle, thus causing more floret sterility in dwarf types, especially when BYDV occurs together with drought. Perhaps the interaction between virus and fungal pathogens is more damaging for short wheats, which carry their heads relatively close to the soil surface, which is a major source of fungal inoculum. Perhaps, also, dwarf wheats have a slightly less aggressive root system, a feature that would decrease BYDV

tolerance; however, nothing was found in the literature except speculation on the subject.

Relationship with the Root System

The most BYDV-resistant cereal species is rye, followed by triticale. The aggressive root system of these species certainly acts to increase their BYD tolerance. The first effect of BYD is to reduce the translocation of photosynthate, thus starving the root system (Kainz and Hendrix 1981). Species or cultivars endowed with the most vigorous root systems suffer proportionally less in such a case. Barley roots more rapidly than wheat, and this may explain why certain barley lines possessing *Yd2* are much more tolerant or resistant to BYDV than any bread wheat or durum wheat under moderate drought stress, as was observed in Sainte-Foy in 1983 (A. Comeau, unpublished).

The effect of artificial inoculation with BYDV should theoretically favor the recovery of deep-rooted lines, especially in drought situations. However, no studies have been undertaken on the subject. Many Brazilian lines adapted to low-phosphorus or acid soils turned out to be quite resistant to BYDV; the bread wheat lines IAS-20, Maringa, and Mascarenhas are good examples (Comeau and St-Pierre 1986). Some more interactions of BYDV with the root

Table 3. Correlation between characters that can be used as estimators of resistance within three virus-inoculated groups of BYDV-resistant spring wheat lines representing dwarf, semi-dwarf, and tall types (Quebec, 1986)

		Pearson's correlation coefficient (r)					
Group	n	Height and symptoms	Height and yield	Height and harvest index	Symptoms and yield	Symptoms and harvest index	Yield and harvest index
Dwarf	30	-0.75**	+0.40*	-0.12	-0.47**	+0.08	+.63**
Semi-dwarf	32	-0.66**	+0.67**	+0.27	-0.80**	-0.31	+.60**
Tall	26	-0.48	+0.36	-0.05	-0.72**	-0.55**	+.55**

* Significant at the 0.05 level
** Significant at the 0.01 level.

Table 4. Comparison of the characterisitcs of the most resistant short and tall cultivars of spring wheat, with and without BYDV inoculation (Quebec, 1986)

	Average height	Symptom score[a]	Average yield (kg/ha)	Average biomass (kg/ha)	Average harvest index
Two short cultivars[b]					
BYDV-inoculated	84.5	4.7	4224	11692	0.37
Not inoculated	80.9	-	5292	13131	0.41
Ratio inoculated/ not inoculated	1.04	-	0.80	0.89	0.90
Five tall cultivars[c]					
BYDV-inoculated	107	2.2	5083	14836	0.34
Not inoculated	108	-	5344	14012	0.38
Ratio inoculated/ not inoculated	0.99	-	0.95	1.06	0.90

a Scale: 0 = healthy, 9 = very diseased (low biomass, poor tillering, discoloration).
b The cultivars were 14th IBWSN-182 and NEAC 120.
c The cul vars were IAS-20, Pel 72390, Long Miai 10, Maringa, and 81-82 PC Hari 678.

system will be considered in the section dealing with the fungal pathogens, which are often associated with BYDV epidemics.

Grain Quality

We lack research in grain quality in resistant or tolerant sources, but we have not yet discovered a BYDV-resistant bread wheat or durum wheat endowed with a breadmaking quality that matches the Canadian standards. For this character lines that meet the bread making criteria of other regions have been found. However, it would be timely to evaluate the quality of a number of BYDV resistant bread wheats, to provide better advice to the breeders.

Chilean scientists demonstrated that BYDV-resistant wheats have better test weight and better looking grain under natural epidemics of BYDV. In oats and barley, although the first resistant cultivars released had less than ideal grain quality, many new cultivars have good or excellent quality; examples would be OT 238 from Canada, and Ogle oats and Gus barley from the USA.

Resistance to Fungi and BYDV Reaction

Many examples have been found where BYDV predisposes cereals to severe attacks by pathogens such as *Septoria*, take-all (*Gaeumannomyces graminis* (Sacc) Arx and Olivier var. *tritici* Walker), and ergot (*Claviceps purpurea* (Fr.) Tul.) (Comeau and Pelletier 1976; Sward and Kollmorgen 1986; Jedlinski 1983). We believe the importance of this interaction has been underrated. In 1986, we observed that BYDV infection of BYDV-susceptible wheat resulted in a much higher incidence of septoria and scab (*Fusarium graminearum* Schw.). On the other hand, BYDV-resistant cultivars remained quite clean looking (Figure 2).

More evidence of this interaction comes from the septoria observations in the Quebec wheat breeding project report of 1986. In this report, only 8 lines were declared resistant to septoria in the Quebec area, and out of these 8 lines, 6 were BYDV-resistant accessions obtained from the BYDV selection project. A chi-square test on the contingency table of association between BYDV resistance and septoria resistance yields a value of 17.3, significant at the 0.001 level. This does not mean that septoria resistance is identical with BYDV resistance; however, in Quebec conditions in the summer of 1986, where some natural BYDV infection was present, there was a significant association between BYDV resistance and septoria resistance under field conditions. The *Septoria*, *Alternaria*, and other fungi actually masked the presence of the virus in the field. Similar phenomena were reported by M.M. Kohli at the 1980 CIMMYT BYDV workshop about bread wheat in Chile.

In our most recent BYDV trials (Comeau and St-Pierre 1986), correlations were highly significant ($P < 0.0001$) between the fungal attack score on heads (visual note) and the BYDV symptoms; the correlations ranged from 0.66 for average-height wheat lines to 0.70 in tall wheat lines. Correlations of fungal attack score with yield of BYDV-inoculated plots were even more significant, being -0.81 and -0.90 respectively in the above trials. This is the proof that BYDV seriously weakens the resistance of wheat to fungi.

Cereal diseases seldom occur alone in nature. The tradition of studies and breeding projects on single diseases may therefore be inconsistent with the natural situations in countries where diseases are abundant. Combined inoculations of BYDV, scab, and septoria may be worth a try as a breeding approach, and even more complex stress combinations may be used in plant breeding, provided stresses are applied uniformly to each plant (Buddenhagen 1983).

The Value of Breeding for BYDV Resistance or Tolerance

The first practical results from breeding for BYDV resistance should be a stabilization of the yield in years of BYDV epidemics and an improvement of the sanitary quality of the grain of all species of cereals, since solving the BYDV problem reduces the undesirable interactions of BYDV with fungal pathogens. Winter hardiness of winter cereals should also be improved by BYDV selection, especially in the case of winter barley.

Interesting fringe benefits from BYDV inoculation of bulk populations might also include a slight improvement in earliness, a better translocation, and a better root system. It seems plausible that for certain crosses, BYDV selection will increase the average yield potential of the derived lines. However the choice of the proper parents and a good BYDV-inoculation method are prerequisites to success.

Breeding methods must be adapted to the genetic nature of the resistance or tolerance. Backcross may work with the *Yd2* gene of barley if intermediate symptoms are obtained on the heterozygous lines. The reaction of the heterozygous lines varies according to the environment. For other species, recurrent selection methods are recommended (Tola and Kronstad 1984), and the starting point of a program should preferably involve the use of a "Resistant/Agronomic//Agronomic" backcross before any selfing (Comeau 1986).

Sufficient numbers of genes for resistance/tolerance to the PAV-like isolates of BYDV have now been found for all species, except for durum wheat.

However, in bread wheat, BYDV-resistant dwarf wheats are still very scarce, and our early attempts to transfer resistance or tolerance from tall wheats to dwarf ones have met unexpected difficulties. These are the problem areas that deserve intensive research, and it is hoped that within a few years the same progress that was achieved in oats, for example

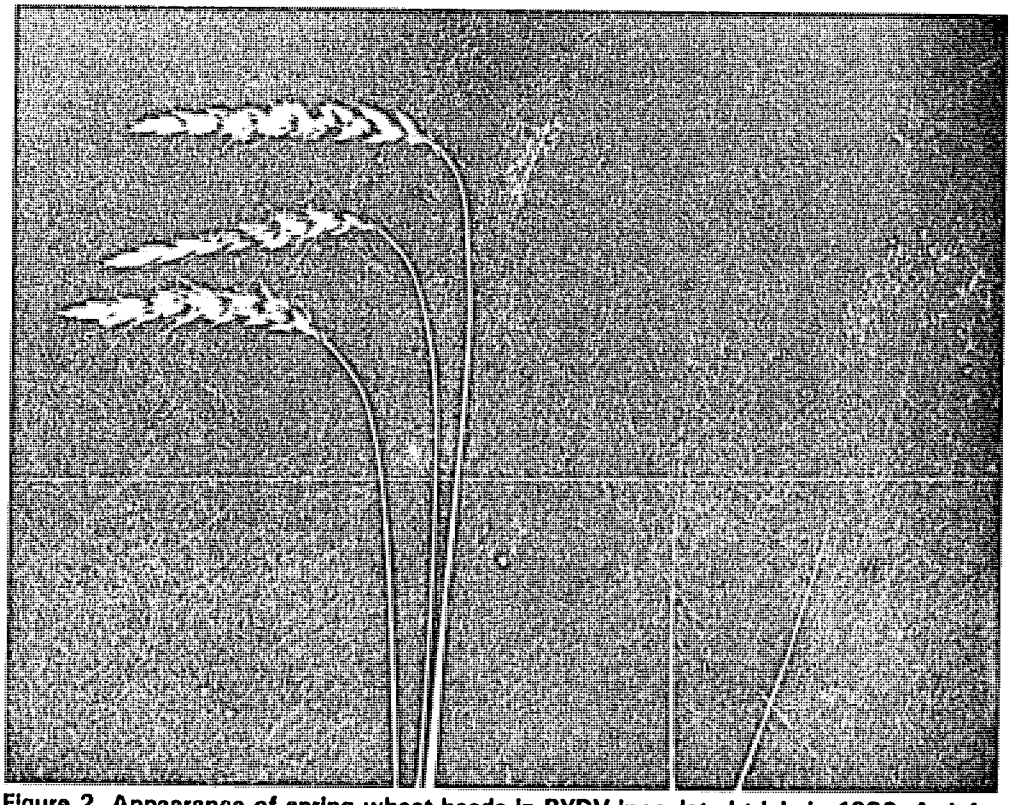

Figure 2. Appearance of spring wheat heads in BYDV-inoculated trials in 1986. At left, three heads of BYDV-resistant Brazilian cultivar IAS-20, normal in size and clean-looking. At right, three heads of typical dwarf wheat types showing yield reduction and invasion by pathogenic and saprophytic fungi, resulting in a grey and black appearance.

with the cultivar Ogle, which features a relatively short, very strong straw, outstanding seed quality, excellent yield, earliness, and good resistance, will be obtained in bread wheat. Ogle has become a key parental line for oat breeding worldwide.

The overall conclusion about the value of BYDV resistance or tolerance is definitely positive. Due to the costs of chemical control, BYDV resistance or tolerance should be favored, especially for developing countries. Let us remember that the proof has already been made in many countries in North and South America that this is a plant breeding approach that pays off. BYDV being a problem in most cereal-growing areas, a more serious effort should be spent on this virus worldwide, and plant breeding is the method that can give positive, long-lasting, and ecologically sound progress.

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Resistance to *Diuraphis noxia* in Wheat: Screening Techniques and Identification of Resistance

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Abstract

The appearance of Diuraphis noxia *(Mordvilko) (Hemiptera: Aphididae) in South Africa in 1978 as a major pest of wheat led to a search for sources of resistance for use in breeding programs. Resistance was identified in* Triticum monococcum *L. but proved difficult to transfer to* T. aestivum *L. Recently renewed efforts were made to find sources of resistance in* T. aestivum. *Genotypes from the countries of origin of* D. noxia *were screened using a new greenhouse technique. Two* T. aestivum *entries were identified as resistant to* D. noxia. *Further tests revealed high levels of antibiotic and antixenotic resistance to* D. noxia *in both entries. A backcross program has now been initiated to incorporate the resistance in local cultivars. Preliminary results of inheritance studies indicate that resistance in both genotypes is controlled by a single dominant factor.*

The Russian wheat aphid, *Diuraphis noxia* (Mordvilko) (Hemiptera: Aphididae) is a key pest of wheat (*Triticum aestivum* L.) in South Africa and can cause substantial yield losses (du Toit and Walters 1984). This pest is indigenous to southern Russia and certain Middle East countries. It has also been reported from Mexico, in 1980 (Gilchrist et al. 1984), and as recently as 1986 from the southern parts of the United States (Webster et al. 1987).

D. noxia was first recorded in South Africa in 1978 (Walters et al. 1980) and a comprehensive research program was initiated toward the end of 1979. Research included studies on the biology, ecology, damage assessment, chemical control, economic threshold, plant resistance, and associated viruses of the pest. Results obtained up to early 1982 were published in Technical Communication No. 191 of the Department of Agriculture in South Africa. Sound control recommendations based on ecological, damage assessment, and economic threshold studies are now available to the South African wheat producers.

The most obvious symptoms of *D. noxia* damage on small grains, especially wheat and barley (*Hordeum vulgare* L.), are longitudinal yellowish white and purple streaks on the leaves and a tubelike rolling of infested leaves. Initially these symptoms were called "Free State streak" disease. They are not, however, caused by a pathogen but by the secretion of a toxic substance during the feeding process (Hewitt et al. 1984; Kriel et al. 1986). Several other *Diuraphis* spp. are known to cause discoloration and rolling of the leaves on different host plants (Parker 1916; Borner et al. 1957; Bremer and Raatikainen 1975).

A complicating factor, however, is the ability of *D. noxia* and other locally occurring wheat aphids to transmit brome mosaic virus (BMV), barley stripe mosaic virus, cucumber mosaic virus, and barley yellow dwarf virus (BYDV) to small grains (von Wechmar 1984; von Wechmar et al. 1984). In the eastern Orange Free State, BMV seems to be omnipresent and studies to determine the relationship between virus incidence and wheat yield are currently being undertaken. A clear distinction should be made between yield losses caused by *D. noxia* per se and those caused by the transmission of one or more viruses. The term "Free State streak disease" is thus confusing and is no longer used in South Africa.

All South African wheat cultivars are susceptible to *D. noxia*. The primary methods of control are cultural practices, especially the elimination during summer of volunteer wheat, which is the most important source of infestation, and chemical control. The high cost and extensive use of insecticides has led to renewed efforts to find germplasm with resistance to *D. noxia* that can be utilized in a breeding program. Resistance to *D.*

noxia was previously identified in *Triticum monococcum* L. (Butts and Pakendorf 1984) and amphiploids of *T. monococcum/T. turgidum* L. var. *durum* (du Toit and van Niekerk 1985) but proved difficult to transfer to *T. aestivum*. Recently emphasis has therefore been placed on finding resistant sources in exotic *T. aestivum* germplasm.

Procedure

General screening technique
It is generally accepted that if material is to be sought that is resistant to an introduced insect pest species, germplasm from the native distribution area of the pest should be screened, as resistance may have developed as a result of many years of natural selection (Dahms 1972). In 1986 wheat entries from Middle East countries and the USSR were screened in the greenhouse for resistance to *D. noxia*. All entries were obtained from the germplasm collection at the Small Grain Center, Bethlehem, South Africa.

A modification of the screening technique described by Starks and Burton (1977) was used. Entries were planted in rows in plastic flats 30 x 27 x 10 cm with six test rows, one susceptible check row, and one resistant check row per flat. Entry rows were randomly allocated and 4 cm apart. Each row consisted of 12 to 15 plants. Plants were grown under a temperature regime of 22°C (day) and 15°C (night).

At the one-leaf stage, apterous *D. noxia* were collected from greenhouse cultures on wheat (cv. Betta) and transferred to 5 cm glass vials at a rate of approximately 60 aphids of various instars per vial. The contents of each vial were then scattered over a plant row to ensure an even distribution of aphids. Three weeks after infestation, plant damage was rated visually by scoring individual rows on a 1 to 6 scale:

1 = small isolated chlorotic spots on the leaves, highly resistant;

2 = larger isolated chlorotic spots on the leaves, resistant;

3 = chlorotic spots tending to become streaky, moderately resistant;

4 = mild streaks visible and leaves tending to roll lengthwise, moderately susceptible;

5 = prominent white/yellow streaks present and leaves tightly rolled, susceptible;

6 = severe white/yellow streaks, leaves tightly rolled and starting to die from the tips, highly susceptible.

Symptoms on the two youngest leaves were considered for scoring purposes.

Characterization of resistance
Six resistant entries identified during general screening were again planted in greenhouse flats with four replicates per entry. Susceptible checks (cv. Betta, Tugela, and Scheepers 69) and a resistant check (*T. monococcum*, A544) were also included. The same procedure as earlier described was followed, but plants from each entry were scored individually. The scores were subjected to an analysis of variance and means were compared by using Tukey's test.

According to Painter (1951) three components of resistance to insects can be distinguished, namely antibiosis, tolerance, and nonpreference. The term antixenosis (Kogan and Ortman 1978) is used here instead of nonpreference. Tests were also conducted to determine antibiotic and antixenotic resistance in the six entries.

Antibiosis—Five test plants from each resistant entry as well as five susceptible check plants (cv. Betta) and five resistant check plants (*T. monococcum*) were grown individually in pots and infested with four adult apterous *D. noxia*. As soon as young nymphs were noticed, the adults and some of the nymphs were removed to leave about six nymphs per plant. The same procedure was repeated with the second generation aphids. As soon as the second generation aphids started to reproduce, two aphids per plant from all entries were removed and dissected under a stereo-microscope (Dewar 1977). In total, 10 aphids (= 10 replicates) per entry were dissected and the number of embryos per aphid was determined. Only embryos with

pigmented eyes were counted and mean embryo counts were compared using Tukey's test.

Antixenosis—One plant from each entry as well as one Betta plant (susceptible check) and one *T. monococcum* plant (resistant check) were grown in a circle near the edge of a 170 mm diameter pot in the greenhouse. This test had four replications (pots) per entry. At the one-leaf stage 50 adult apterous aphids were released in the center of each pot. The number of aphids per plant for each entry was determined 24 hours later and the means compared by using Tukey's test.

Heritability of resistance
Crosses between identified resistant *T. aestivum* entries and local susceptible cultivars and subsequent backcrosses were made in order to determine the heritability of resistance.

Results and Discussion

Varying levels of resistance were found in three *T. aestivum*, one *T. monococcum*, and two emmer wheat (*T. dicoccum* Schrank) entries (Table 1).

Table 1. Damage rating and identity of *Triticum* genotypes selected in the first screening test

Species	SA no.[a]	Damage rating[b]	Country of origin
Betta (susceptible)	—	6	—
T. aestivum	16B4	3	Iran
T. aestivum	2199	3	USSR
T. aestivum	2129	4	Rumania
T. dicoccum	1242	4	USSR
T. dicoccum	1904	4	USSR
T. monococcum	836	3	USSR
T. monococcum (A544) (resistant)	—	2	—

[a] Small Grain Center germplasm collection.
[b] 1 = highly resistant; 6 = highly susceptible.

Table 2. Resistance reactions of wheat seedlings to *D. noxia* in greenhouse tests

Entry	Damage rating[a]	Antibiosis (embryos/aphid)	Antixenosis (aphids/plant)
Betta	5.7a	13.6a	8.3a
Scheepers 69	5.6a	—	—
Tungela	5.5a	—	—
SA 2129	4.6b	10.7b	5.0ab
SA 1904	3.9bc	11.1b	3.3b
SA 1242	3.6cd	9.4cd	2.0b
SA 16B4	3.0d	8.6de	2.5b
SA 836	2.9d	8.8de	2.0b
T. monococcum			
(A544)	1.8e	7.7e	2.0b
SA 2199	1.4e	9.1d	4.0b
LSD_T ($p = 0.05$)	0.9	1.4	4.3

Means followed by the same letter in a column are not significantly different ($p = 0.05$).

[a] 1 = highly resistant; 6 = highly susceptible.

The damage ratings, number of embryos per aphid (antibiosis), and number of aphids per plant (antixenosis) for each entry are presented in Table 2.

SA 2199, SA 836, and SA 1684 had the lowest damage ratings and also the highest levels of antibiotic resistance. None of the six test entries differed significantly from the resistant *T. monococcum* (A544) in the antixenotic test. Except for SA 2129 all the other test entries had significantly fewer aphids per plant after 24 hours than the susceptible Betta.

The visual rating scale proved to be a reliable method for identifying resistant sources. The mean number of embryos per aphid and the mean number of aphids per plant gave clear evidence of variable levels of antibiotic as well as antixenotic resistance in all six test entries. Taking the data of all three tests into account, SA 2199, SA 1684 (both *T. aestivum*), and SA 836 (*T. monococcum*) showed superior levels of resistance to *D. noxia*.

Crosses between SA 2199, SA 1684 (both resistant), and local susceptible cultivars resulted in F_1 plants that were all resistant to *D. noxia*, indicating the resistance to be dominant. Backcrosses were made to the susceptible parents and BC1, F_2, and parent plants were tested simultaneously. A good fit for a 1:1 ratio of resistance and susceptibility was obtained on all BC1 populations and a 3:1 on all F_2 populations. The testcross ratio and the F_2 data are in agreement with inheritance controlled by a single dominant gene in both resistance entries.

A backcross program is now being conducted to incorporate the *D. noxia* resistance into our local cultivars.

Tests to verify the level of resistance in adult plants under greenhouse and field conditions will also be carried out.

Acknowledgment

Thanks are expressed to M.C. Walters, Winter Rainfall Region, Department of Agriculture, for critical reading of the manuscript and useful comments.

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Agronomic Techniques for Preventing Barley Yellow Dwarf Damage in Winter Cereal

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Abstract

*Times of sowing, insecticide treatment, and cultivar tolerance were studied as potential control methods for barley yellow dwarf (BYD) in autumn-sown barley (*Hordeum vulgare *L.) and wheat (*Triticum aestivum *L.) in northeast Italy. Tests in three locations over 4 years demonstrated that BYD damage can be substantially avoided without yield losses due to frost by sowing between October 15 and 30. Spraying with insecticides limited the spread of barley yellow dwarf virus (BYDV) by eliminating the aphid vectors, making it possible for farmers to sow at the beginning of October when the environment is more favorable for wheat, but not necessarily barley, crop development. The majority of commercial barley cultivars appeared susceptible to BYD. Yield loss between treated and untreated plots for the 23 cultivars tested was 53%. Wheat cultivars with average tolerance were numerous and a few had good resistance.*

Approximately 200,000 ha of the Friuli-Venezia Giulia region of Italy are arable. The most common crops are cereals, soybeans (*Glycine max* (L.) Merr.), and forage crops. In the last 20 years farmer preference for one crop or the other has varied. Among the grains, the largest area is planted to maize (*Zea mays* L.), but since 1977 the barley (*Hordeum vulgare* L.) area has also been considerable, greater than wheat (*Triticum aestivum* L.). Barley is preferred among autumn-sown crops because of its relatively short growing season. In our environment, barley is ready for harvest about 10 days before wheat, and it is possible to grow a second crop in the same year.

With the increase in the barley area, the incidence of disease, in particular barley yellow dwarf (BYD), has increased. Since the first report of barley yellow dwarf virus (BYDV) (Conti 1978; Snidaro 1978), damage due to the disease has been observed regularly, but its severity has varied from area to area. Since 1978, three lines of agronomic research have been studied to assess the possibility of preventing the damage caused by BYD both to barley and to wheat. These are:

1) the times of autumn sowing
2) the effectiveness of insecticide treatments for controlling aphid vectors of BYDV
3) the tolerance to BYD currently present in commercial cultivars

Materials and Methods

Between the years 1979-83, three localities—Spilimbergo, Torviscosa, and Moimacco—were selected for the experiments, representing different soil and climatic conditions and BYD intensities.

In the first 2 years of trials, six cultivars of barley and three of wheat were used, and in the following 3 years seven cultivars of each were used.

The trials had three sowing times: the first coincided with the earliest sowings in the region (beginning of October), the second corresponded to the most frequent date of sowing (end of October), and the third was delayed to mid-November. As Figure 1 shows, the average temperature drops during October and reaches below 10°C by the beginning of November.

Each plot consisted of 10 rows 7.5 m long, for a total area of 10.2 m^2; 350 seeds per m^2 were planted for barley and 500 for wheat. The range of sowing dates is shown in Table 1. In a single trial the subsequent sowings were separated by at least 15 days.

In 1983, trials on the effectiveness of aphicides were started as an adjunct to the times of sowing research. In two locations (Besiliano and Torviscosa) the

number of the plots was doubled in the first planting to compare the plots protected with chemical insecticide (Deltamethrin, 0.3 liter/ha) and untreated test plots. The insecticide was applied when the plants were at the 2nd- to 3rd-leaf stage and, when necessary, the treatment was repeated 10 days later. For these trials 12 cultivars of barley and 11 of wheat were used.

In a further locality, Pozzuolo del Friuli, 4 cultivars of barley and 2 of wheat known to have different tolerances to BYDV were compared in three ways: a) exposed to aphids; b) treated with insecticidal products; and c) covered with an aphid-proof net. The insecticide was again applied at the 2nd- to 3rd-leaf stage. The covered plot was protected with a small tunnel of the aphid-proof net from immediately after the sowing until mid-November.

A trial was also started in 1983 at Pozzuolo del Friuli and Torviscosa to screen the cultivars of barley and wheat presently cultivated in Italy for tolerance to BYD. At the beginning of October, 23 cultivars of barley and 23 of wheat were sown in plots of 10.2 m^2, in square lattices with replications. Three replications were exposed to aphid infection, and three were protected by insecticide treatments.

By comparing the yield of a cultivar in treated and untreated plots, an indication of its susceptibility to BYDV can be obtained.

Table 1. Periods within which the sowings were carried out, in the different years and locations, for the three sowing times

Sowing times	Sowing dates
First	25/ 9 - 9/10
Second	22/10 - 4/11
Third	8/11 - 19/11

Results and Discussion

The trials in the first 5-year period pointed out the severity of the BYDV infections on early-sown barley and wheat crops. In 1981 in Spilimbergo the average yields were as low as 0.23 t/ha in barley and 0.58 t/ha in wheat. On an average, the first sowings of barley produced lower yields than the second sowings (end of October) and were quite variable among years and locations. In

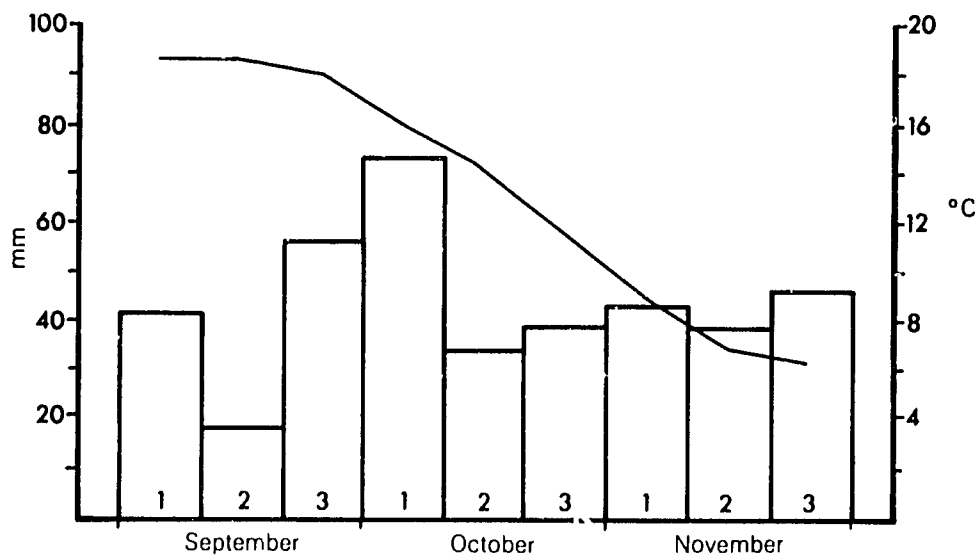

Figure 1. Ten-day average temperature (line) and rainfall (columns) for the period of autumn sowing in Pozzuolo del Friuli, means of the years 1979-1986.

Figure 2 we can see that for the period 1979 to 1983 the absolute difference in grain yield for barley was 2.59, 1.97, and 0.81 t/ha. in Spilimbergo, Moimacco, and Torviscosa, respectively. Wheat, on an average, was damaged less by BYDV than barley, with a drop in the same localities of 1.22, 0.89, and 0.97 t/ha. Damage was highest in 1980 and 1981 in all locations. The differences between times of sowing were very high for some barley cultivars: Arma, Astrix, and Igri appeared particularly sensitive and in 1981, in Spilimbergo, died completely.

The data show that the second sowings (at the end of October), on the whole, prevented BYDV infection by avoiding the flights of viruliferous aphids. Both barley and wheat appeared practically undamaged by BYDV and only on rare occasions was there some cold damage in the winter due to seeding at this time. These results are similar to those of La Pierre et al. (1977) and Plumb (1977).

The mid-November (third) sowings, even if not damaged by BYD, were not completely satisfactory due to a considerable reduction of yield caused by cold.

The third sowings of barley yielded 0.10, 0.78 and 0.98 t/ha less than the second sowings at Spilimbergo, Moimacco, and Torviscosa, respectively. The parallel yield losses for wheat were 0.87, 0.50 and 0.53 t/ha.

Variations in yield depended mainly on the number of ears per m^2, particularly in barley. This number was reduced by the poor development of plants infected by BYDV in the first sowing and by low tillering in the third sowing.

The presence of aphids (mainly *Rhopalosiphum padi* (L.)) in autumn on the young plants of the first sowing was variable in relation to years and localities. The highest frequency was registered in Spilimbergo (more than 20 aphids per plant, in 1981), while in Torviscosa the number was always moderate. In Moimacco, an average of 2 to 3 aphids was present on the plant at the 3rd- to 4th-leaf stage. There were very few or no aphids on the seedlings of the successive sowings.

Figure 3 illustrates the grain yields obtained with different times of sowing at two sites in 2 years when there was a

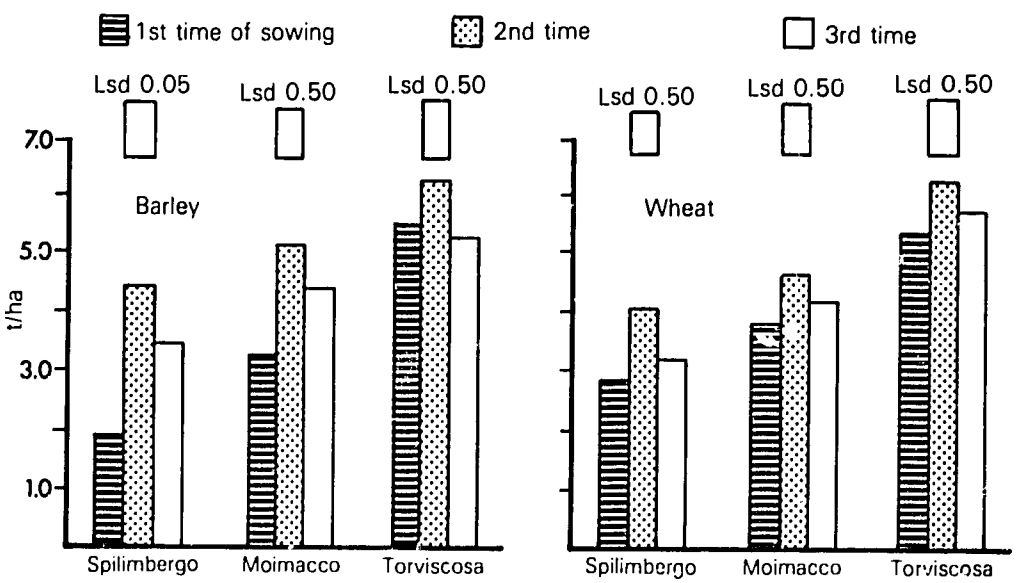

Figure 2. Grain yield for barley and wheat in three locations, with three times of sowing, average of several cultivars in the years 1979-1983.

high incidence of BYDV. In addition, the yield loss attributable to the virus can be calculated by comparing the treatments with and without insecticide. The average losses in Torviscosa and Basiliano were 1.39 and 1.58 t/ha for barley and 1.20 and 1.65 t/ha for wheat, respectively.

Therefore, we conclude that chemicals are effective in the control of aphids on the crops and such treatment will prevent heavy production losses due to BYDV. These results are similar to the findings of Horrellou and Evans (1979), Lescar (1981), Plumb (1981), and Grafton et al. (1982).

In the absence of BYDV infection, the yield differences between the first and the second sowings were not significant for barley, in either of the trial localities. However, for barley we do not suggest early sowing to increase grain yield, because of the risk of BYDV infection (Osler at al. 1984). On the other hand, wheat yields in the most fertile areas are considerably improved by early sowing, but great care must be taken to control aphids.

The delayed sowings of mid-November were undamaged by virus, but yields were lower than with the second sowing.

The effectiveness of the protection obtained by insecticide treatments was confirmed by trials conducted in Pozzuolo 1983-85. The barley and wheat yields appear similar (7.5 t/ha) when protected by an aphid-proof net during the first vegetative stages. Differences between the two species appeared in the plots exposed to the aphids: barley is more sensitive to BYDV, producing 3.25 t/ha in comparison with the 5.32 t/ha produced by wheat (Figure 4).

The reactions to insecticide treatments were also different. The differences between treated and covered trials were practically nil in wheat, while in barley a drop of in yield .82 t/ha was observed. This is probably due to incomplete protection by the insecticide.

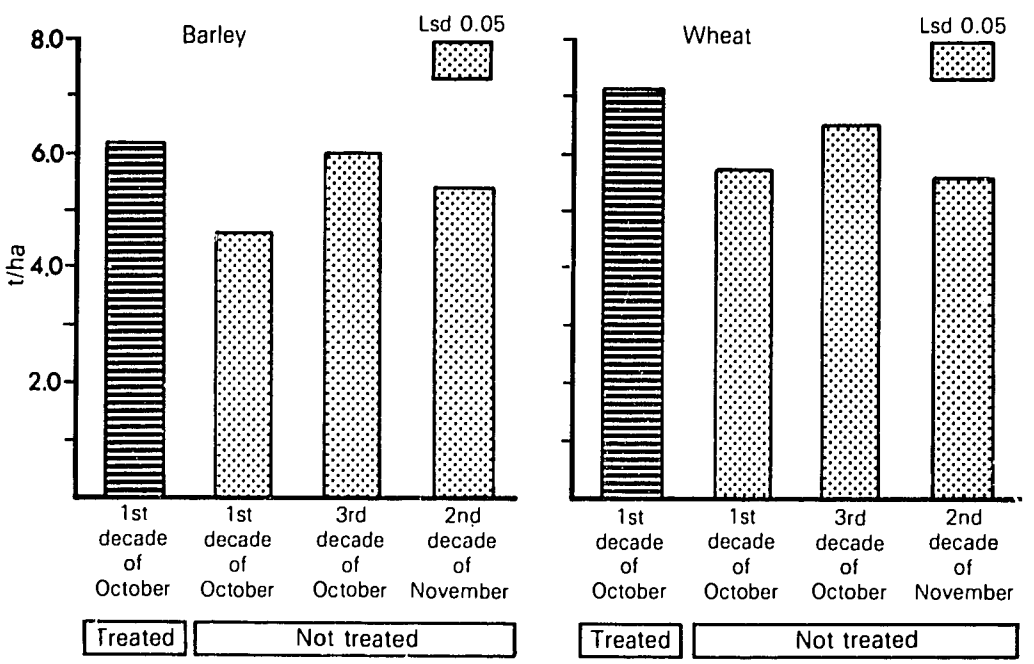

Figure 3. Effect of different sowing times and insecticide protection on grain yield in barley and wheat, average of several varieties in two locations and 2 years.

Differences also emerged from an analysis of the number of plants infected by BYDV carried out with enzyme-linked immunosorbent assay (ELISA) (S. Peressini, personal communication).

In the studies to evaluate the tolerance in available commercial cultivars of barley and wheat, the yield reduction expressed as percentage was considered more useful than absolute loss in t/ha; the data were transformed into a synthetic score, taking into account the economic significance of the damage (Table 2). A cultivar, therefore, is defined as excellent when loss is under 5% of the maximum yield, the fluctuation in yield considered as normal. Cultivars suffering yield losses of above 40% were classified as susceptible.

In this study, barley was found to be more susceptible than wheat to BYDV. This is similar to the findings of Yount et al. (1985). Wheat genotypes with average tolerance were numerous, and a few had very good resistance. (Dorado, Oderzo, Orso, and Loreto had losses of 10% or less.) The majority of barley cultivars appeared susceptible, the average yield loss for the 23 cultivars was 33%. Only a few exhibited a medium or good level of tolerance: the most tolerant cultivars were Alpha and Nico.

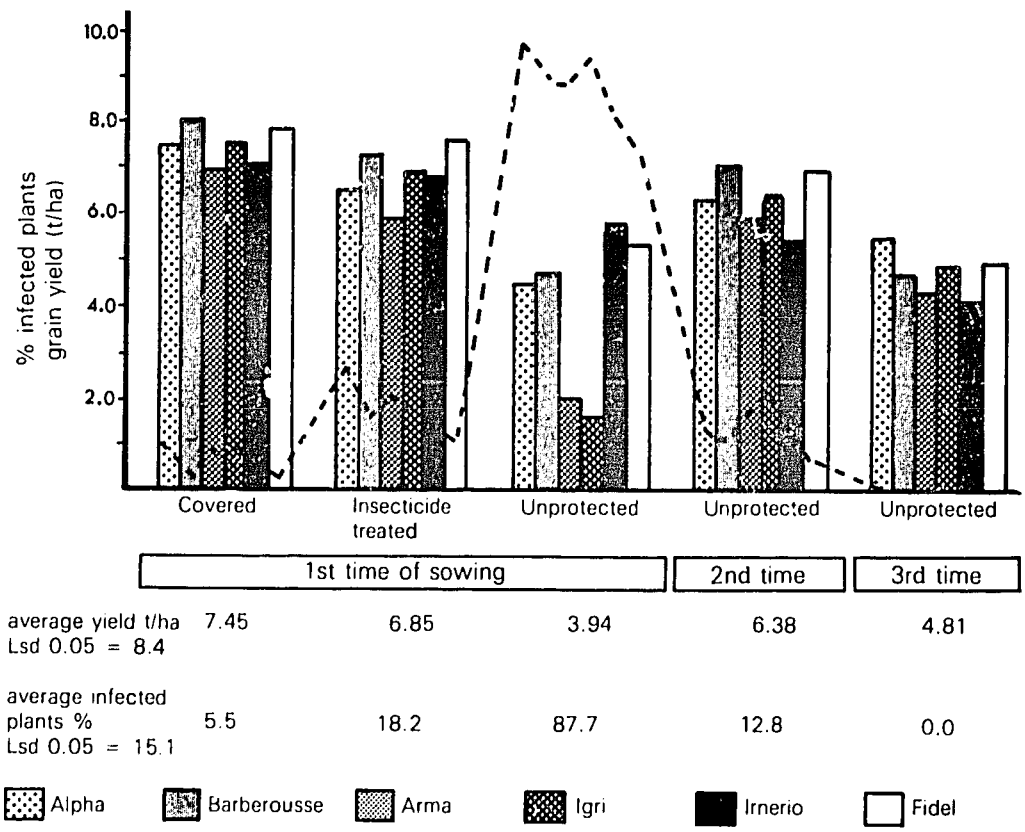

Figure 4. Grain yield of cultivars of barley (4 columns on left) and wheat (2 columns on right) for three times of sowing in Pozzuolo del Friuli. The dashed line indicates the percentage of BYD-infected plants, average of the years 1984-1985.

Table 2. Tolerance to BYDV in barley and wheat, estimated by the difference between plots treated with insecticides and untreated. Mean of two locations (Pozzuolo and Torviscosa), 3 years

Cultivar	Yield (t/ha) treated	Yield (t/ha) untreated	Loss %	Tolerance score[a]
Barley				
IGRI	6.7	3.9	42	VB
ARDA	6.7	4.7	30	L
BARBEROUSSE	6.7	5.7	15	M
ROBUR	6.4	4.7	27	L
SELVAGGIO	6.4	4.8	25	M
ETRUSCO	6.2	4.1	34	L
GERBEL	6.2	3.8	39	L
JAIDOR	6.2	3.8	39	L
PLAISANT	6.0	3.3	45	VB
NICO	5.9	5.3	10	G
JEFF	5.9	4.5	24	M
PIRATE	5.9	1.0	83	VB
THIBAUT	5.9	4.2	29	L
MIRCO	5.8	4.0	31	L
TANIA	5.8	4.7	19	M
ALPHA	5.8	5.3	9	G
AGER	5.6	4.3	23	M
PANDA	5.6	3.0	46	VB
ELDORADO	5.4	4.8	11	M
CIRO	5.3	3.7	30	L
NOVO PERGA	5.0	1.3	74	VB
ARMA	4.9	3.2	35	L
OPALE	3.9	2.6	33	L
Average	5.8	3.9	33	
Wheat				
CHIARANO	7.3	6.3	14	M
DORADO	7.3	6.6	10	G
LEOPARDO	7.2	5.9	18	M
CENTAURO	7.2	6.2	14	M
PANDAS	7.0	5.8	17	M
ODERZO	7.0	6.3	10	G
GEMINI	6.9	5.1	26	L
GALLO	6.9	5.5	20	M
CONCORDIA	6.9	5.7	17	M
ETRURIA	6.7	5.7	15	M
TOMMASO	6.6	5.7	14	M
AURELIO	6.6	5.4	18	M
SALIENTE	6.5	5.7	12	M
ORSO	6.5	5.8	10	G
LARIO	6.4	5.3	17	M
IRNERIO	6.4	5.4	16	M
CONSTANTINO	6.4	5.2	19	M
GLADIO	6.2	5.3	15	M
LORETO	6.2	5.8	6	G
MANIAL	6.1	4.5	26	L
MEC	5.9	4.9	17	M
GRANAROLO	5.7	4.9	14	M
SALMONE	4.8	4.1	15	M
Average	6.6	5.5	16	
Significance of the analysis of variance	••	••	••	

[a]VB = very bad, L = low, M = moderate, G = good, E = excellent.

The results of these studies have led to the following recommendations for the region:

1) Sow from 10 October, seed wheat first, followed by tolerant, high yielding cultivars of barley.

2) Spray with an insecticide at the 2nd- to 3rd-leaf stage, when aphids are noticed on the crops.

For long-term control, it appears advisable to select cultivars highly tolerant to BYDV.

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Some Agronomic Traits Affected by Rice Giallume Virus Inoculated at Two Phenological Stages in 11 Italian Rice Varieties

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Abstract

*Plant height, panicle length, tiller number, panicle and straw yield, 1000-grain weight, and seed germination of 11 Italian rice (*Oryza sativa *L.) varieties differing in their susceptibility to the rice giallume virus (RGV, an isolate of barley yellow dwarf virus) were evaluated. Inoculation was with* Rhopalosiphum padi *(L.) at two stages of plant growth (first leaf and tillering). The most severe damage occurred when plants were inoculated at the first leaf stage, but the virus considerably reduced the plant growth, grain and straw yield, 1000-grain weight, and seed germination at both growth stages.*

Rice giallume is a serious disease caused by rice giallume virus (RGV), an isolate of barley yellow dwarf virus (BYDV) (Osler 1984; Belli et al. 1986; L. Torrance, personal communication).

From 1970 to 1980 the incidence of rice giallume increased until it became the main disease problem of rice (*Oryza sativa* L.) in Italy. From 1981 to 1985, giallume damage decreased considerably, probably due to utilization of less susceptible varieties in areas where natural infection and losses in crops were high; eradication of the weed *Leersia oryzoides* L., which is the main reservoir of the virus in rice fields; and reduced numbers of the aphid *Rhopalosiphum padi* (L.), which is the main vector of RGV in rice fields. In 1986 the population of *R. padi* increased greatly and in some areas rice fields were again damaged by RGV.

Because of the economic importance of rice in Italy and of the damage caused by RGV, this disease was the subject of many studies (Amici et al. 1974; Belli et al. 1974; Osler et al. 1974, 1977, 1980, 1984; Osler 1980, 1984; Moletti et al. 1979; Belli et al. 1986), which have identified and characterized the virus, elucidated its epidemiology and found ways to reduce its spreading and damage.

In a study of transmissions with the aphid *R. padi* using different rice cultivators, it was shown that, when inoculated early, the disease incubation period is shorter and the percentages of infected plants are higher than when inoculated late (Osler and Moletti 1984).

This work continues the previous research and evaluates the damage caused by RGV in some Italian rice varieties inoculated at two growth stages, first leaf and tiller initiation.

Materials and Methods

Eleven rice cultivars having different degrees of susceptibility to RGV (Moletti and Osler 1978) were evaluated. Rice plants were grown in pots 30 cm in diameter and 50 cm high (10 plants per pot), and kept in a greenhouse (18 to 24°C, about 80% RH, with supplementary light).

Inoculation was carried out when the plants were 10 days old (first-leaf stage) or 25 days old (tiller initiation) with *R. padi* that had had a 3-day acquisition period on infected rice plants of the cultivar Balilla in a growth chamber. Each test plant was inoculated for a 3-day period with three viruliferous aphids, using leaf cages. The pots were then moved outdoors and periodically treated with insecticides to avoid natural infections.

For each rice variety tested, 60 plants (6 pots) were inoculated at the first-leaf stage and 60 at tiller initiation. A 60-plant uninoculated control was grown. The experiment was arranged in a split-plot design with six replications, where the varieties were the plot and the two inoculations and the control were the subplots.

At maturity the plants were harvested and the following data were collected: culm and panicle length, tiller number, dry matter yield, grain yield, grain straw ratio, 1000-grain weight, and seed germination.

Results

The results (Table 1) showed that the viral infection at both phenological stages damaged all the varieties noticeably, but the infection at the first-leaf stage caused both a higher percentage of infected plants and a higher reduction in the measured agronomic traits.

Table 1. Effect of RGV inoculated at two phenological stages (first leaf and tiller initiation) on some agronomic traits in 11 Italian rice varieties

		Plant height		Tillers/ plant		Dry matter yield g/plant		Grain yield/ g/plant		Seed germinability	
Variety	Inoculation stage	cm	% control	n.	% control	g	% control	g	% control	%	% control
Balilla	I stage	55.5	75.7	0.1	4.2	2.8	27.2	0.1	2.0	70.2	76.6
	II stage	65.0	88.7	1.1	45.8	5.5	53.4	1.7	34.7	87.3	95.2
	control	73.3	100.0	2.4	100.0	10.3	100.0	4.9	100.0	91.7	100.0
Cripto	I stage	62.5	88.8	0.7	43.8	5.2	53.1	0.5	12.2	90.0	91.8
	II stage	61.9	87.9	1.0	67.5	6.6	67.3	2.0	48.8	92.3	94.2
	control	70.4	100.0	1.6	100.0	9.8	100.0	4.1	100.0	98.0	100.0
Europa	I stage	62.8	74.1	0.9	69.2	3.9	41.5	0.1	2.6	48.8	51.2
	II stage	72.6	85.6	1.1	84.6	7.3	77.7	2.5	64.1	95.0	99.7
	control	84.8	100.0	1.3	100.0	9.4	100.0	3.9	100.0	95.3	100.0
Monticelli	I stage	77.0	86.8	0.6	31.6	5.4	40.3	0.7	13.7	92.0	94.6
	II stage	74.7	84.2	1.2	63.2	7.4	55.2	2.6	51.0	96.7	99.4
	control	88.7	100.0	1.9	100.0	13.4	100.0	5.1	100.0	97.3	100.0
Padano	I stage	69.8	89.9	0.1	5.3	4.5	40.9	0.2	5.3	28.7	29.1
	II stage	69.6	89.7	1.0	52.6	7.7	70.0	1.9	50.0	90.3	91.5
	control	77.6	100.0	1.9	100.0	11.0	100.0	3.8	100.0	98.7	100.0
Radom	I stage	72.0	85.2	0.1	6.7	4.1	38.3	0.5	11.1	87.8	95.4
	II stage	67.5	79.9	0.9	53.3	7.3	68.2	2.1	46.7	81.7	88.8
	control	84.5	100.0	1.5	100.0	10.7	100.0	4.5	100.0	92.0	100.0
Ribe	I stage	69.7	86.0	0.1	9.1	4.0	44.0	0.3	7.7	89.5	91.6
	II stage	65.3	80.6	0.7	63.6	6.6	72.5	1.7	43.6	95.0	97.2
	control	81.0	100.0	1.1	100.0	9.1	100.0	3.9	100.0	97.7	100.0
Ringo	I stage	60.1	76.0	0.7	70.0	4.3	51.8	0.1	3.2	58.0	59.0
	II stage	61.0	77.1	0.8	80.0	5.7	68.7	1.4	45.2	95.3	96.9
	control	79.1	100.0	1.0	100.0	8.3	100.0	3.1	100.0	98.3	100.0
Roma	I stage	76.2	92.0	0.5	41.7	5.8	63.7	0.3	8.3	87.3	89.1
	II stage	72.5	87.6	0.8	66.7	7.0	76.9	2.4	66.7	93.3	95.2
	control	82.8	100.0	1.2	100.0	9.1	100.0	3.6	100.0	98.0	100.0
Rosa Marchetti	I stage	73.9	83.4	0.3	15.0	4.1	36.3	0.4	7.5	82.0	83.7
	II stage	80.3	90.6	1.2	60.0	7.4	65.5	2.4	45.3	93.3	95.2
	control	88.6	100.0	2.0	100.0	11.3	100.0	5.3	100.0	98.0	100.0
S. Andrea	I stage	74.6	85.1	0.5	31.3	5.2	45.6	0.8	15.4	88.2	97.2
	II stage	72.7	82.9	1.1	68.3	7.4	64.9	2.3	44.2	88.3	97.4
	control	87.7	100.0	1.6	100.0	11.4	100.0	5.2	100.0	90.7	100.0
Mean	I stage	68.5 b	83.8	0.4 c	25.0	4.5 c	43.7	0.4 c	9.3	74.8 c	77.9
	II stage	69.4 b	84.9	1.0 b	62.5	6.9 b	67.0	2.1 b	48.8	91.7 b	95.5
	control	81.7 a	100.0	1.6 a	100.0	10.3 a	100.0	4.3 a	100.0	96.0 a	100.0

Figures followed by the same letter are not significantly different at the 5% level using Duncan's multiple range test.

The average reductions in the agronomic traits in the diseased plants, compared with the healthy ones, of the 11 rice varieties inoculated at the first-leaf stage or tiller initiation were: culm length, 16 and 17%; panicle length, 16 and 8%; tiller number, 75 and 38%; dry matter yield 56 and 33%; grain yield, 91 and 51%; grain straw, 89 and 41%; 1000-grain weight, 30 and 7%; and seed germinability, 22 and 6%, respectively.

Among the varieties, there was great variability in the responses of the different agronomic traits at either growth stage inoculated and, consequently, it was difficult to rank them according to susceptibility. Generally, the more damaged varieties were Balilla and Padano, which showed both high susceptibility and high sensitivity to RGV. The least damaged cultivars were S. Andrea and Roma. This result agrees with observations made in rice fields in naturally infected areas.

Discussion

All the traits measured were severely reduced. Grain yield, the most important trait measured, showed a range of decreases from 85% (S. Andrea) to 98% (Balilla) when inoculation was at the first-leaf stage and from 33% (Roma) to 65% (Balilla) when inoculation was done at tiller initiation.

For some varieties the observed decrease of seed germination due to inoculation with RGV should be taken into account when rice is being cultivated for seed production.

It is evident that the earlier the infection, the greater the damage it causes. This agrees with the results obtained with BYDV infections in barley (*Hordeum vulgare* L.), oats (*Avena sativa* L.), and wheat (*Triticum aestivum* L.) (Oswald and Houston 1953; Endo and Brown 1957; Fitzgerald and Stoner 1967; Smith and Sward 1982).

The greater reductions caused by inoculation at the first-leaf stage of plant growth explains why in rice fields the most damaged plants are those nearest to the natural source of infection (*L. oryzoides*). They are inoculated by apterous aphids moving from *L. oryzoides* to rice when the rice is at the initial stages of growth.

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Chemical Control of Barley Yellow Dwarf in Spring Oats

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Abstract

*In 1981 a series of field trials was started to investigate the effect of different pesticides on the spread of barley yellow dwarf virus (BYDV) in spring oats (*Avena sativa *L.) in Sweden. The aphids were controlled by spraying the plots once or twice at different early growth stages of the plants. BYDV-infected plants occurred most frequently in the experimental fields in 1983 and 1986. In 1983 the mean yield increased 16% and 38% when the plots were sprayed twice with ethiofencarb and fenvalerate, respectively, and in 1986 the mean yield increased 18% and 19% when the plots were sprayed twice with deltamethrin and oxidemetonmethyl, respectively. However, the number of aphids per culm was often significantly higher in plots treated with deltamethrin than in untreated plots.*

In some areas of Sweden barley yellow dwarf (BYD) frequently causes damage on spring oats (*Avena sativa* L.), especially in years when the sowing is delayed. Most of the varieties grown are very susceptible to BYD and thus chemical treatment of the virus-transmitting aphids is, at present, the best method to control the disease. There are several European reports of experiments where BYD in winter barley (*Hordeum vulgare* L.) was controlled by spraying the aphids in the autumn. A few reports are known in which the virus was controlled in spring sown barley or oats (Jenkyn and Plumb 1983).

In 1981 a series of field trials was started to investigate the effect of different pesticides on the spread of BYD in spring oats. The trials were conducted in Smaaland, a county in the Southern Highlands of Sweden.

Materials and Methods

The virus-transmitting aphids were controlled by spraying the plots once or twice with ethiofencarb (Croneton E), fenvalerate (Sumicidin 10 FW), deltamethrin (Decis), or oxidemeton-methyl (Metasystox R100) at different early growth stages of the oat plants. The plants were sprayed the first time at the 1- to 2-leaf stage, the 2- to 3-leaf stage, or the 4-leaf stage. The second spraying was carried out 2 to 3 weeks after the first one.

The treatments were in a randomized block design with four replicates. The size of the plots was 60 m^2. The number of aphids was counted on 10 or 20 culms in each plot. BYD incidence was assessed by counting the number of plants per m^2 showing symptoms in each plot.

The results of the trials were statistically analyzed using analysis of variance, with comparison of means by the LSR-method.

Results

Only the results of 1983 and 1986 will be reported here, since infection by barley yellow dwarf virus (BYDV) was common in the experimental fields only in these years. In 1983 the damage of BYD was especially severe. *Sitobion avenae* (Fabricius) was the most common aphid species in oats and BYDV isolates transmitted specifically by *S. avenae* predominated (Eweida 1986). In 1986 *Rhopalosiphum padi* (L.) was the most common aphid species and isolates of BYDV mainly transmitted by *R. padi* predominated.

A summary of six trials in 1983 is shown in Table 1. The plots were treated with ethiofencarb or fenvalerate. The number of aphids was relatively small, however, significant differences between treated and untreated plots could be observed. The number of BYDV infected plants increased considerably in the latter part of July and significant differences between treated and untreated plots were recorded. The yield was low throughout,

owing to a long period of dry weather. All types of treatments provided an increase in yield from 16 to 38%, but only in plots treated once or twice with fenvalerate was the increase, 28 and 38% respectively, significant.

In 1986 the trials were treated with fenvalerate, deltamethrin and oxidemetonmethyl. A summary of six trials is shown in Table 2. The aphids were more numerous than in 1983, but significant differences between treated and untreated plots were few. The number of BYDV infected plants was, at the first count taken in the middle of July, significantly different in treated than in untreated plots. The next BYDV infected plant count taken at the end of July showed no significant differences owing to great variation in infection between (the single) trials. All three insecticides resulted in an increase in yield, but only in plots treated twice with deltamethrin and oxidemetonmethyl was the increase, 18 and 19% respectively, significant.

Discussion

In our experiments ethiofencarb, fenvalerate, deltamethrin, and oxidemetonmethyl significantly reduced the infection of BYDV, when applied early and before aphids were even detected.

Table 1. Control of barley yellow dwarf virus in spring oats, summary of six trials in 1983

Treatment		Rate g a.i./ha	No. of aphids/culm Week no. 26	27	28	BYDV infected plants/m^2 Week no. 28	30	Yield t/ha	Relative yield
A Untreated			1.7	3.9	4.0	2.2	47	2.30	100
B Fenvalerate	I	100	0.5	1.5	3.1	1.2	35	2.74	119
C Ethiofencarb	II	500	0.7	2.3	2.6	1.4	41	2.68	116
D Ethiofencarb	II + III	500	0.4	1.0	1.8	1.1	38	2.68	116
E Fenvalerate	II	100	0.3	0.9	2.6	0.9	33	2.95	128
F Fenvalerate	II + III	100	0.2	0.3	0.6	0.7	26	3.17	138
Significance level			0.998	1.000	0.990	0.999	1.000	1.000	
Coeff. of variation (%)			96.4	70.9	57.4	41.0	10.0	9.0	
Significant differences			A ≠ BDEF	A ≠ BDEF	A ≠ BDEF	A ≠ BDEF	A ≠ BDEF C ≠ E F ≠ BCDE	A ≠ EF F ≠ BC	

Week no. 26 = 27 June – 3 July
Spraying time: I = 1-2 leaf stage, II = 2-3 leaf stage, III = 2-3 weeks after II

Table 2. Control of barley yellow dwarf virus in spring oats, summary of six trials in 1986

Treatment		Rate g a.i./ha	No. of aphids/culm Week no. 26	27	28	BYDV infected plants/m^2 Week no. 28	30	Yield t/ha	Relative yield
A Untreated			1.2	7.2	1.6	3.0	15.5	3.00	100
B Fenvalerate	I	100	0.9	7.6	2.2	2.1	9.2	3.34	111
C Deltamethrin	I	10	1.1	9.3	1.0	2.3	13.1	3.17	106
D Deltamethrin	II	10	0.8	6.3	4.8	1.7	6.5	3.17	106
E Deltamethrin	II + III	10	0.7	5.3	2.2	1.7	5.9	3.55	118
F Oxidemetonmethyl	II + III	100	0.5	1.1	0.3	1.8	4.7	3.58	119
Significance level			0.976	0.965	0.975	1.000	0.736	0.998	
Coeff. of variation (%)			39.4	66.4	106.7	20.1	98.6	7.3	
Significant differences			A = F	C = F	D = F	A = BDEF		A = EF	

Week no. 26 = 23 – 29 June
Spraying time: I = 1-2 leaf stage, II = 4 leaf stage, III = 2-3 weeks after II

In 1986 the aphids often were more numerous in plots treated with deltamethrin than in untreated plots, especially at later countings. This was more obvious in the single trials where significantly more aphids were often found in plots treated with deltamethrin. The number of aphids was generally much lower in plots treated with oxidemetonmethyl than in untreated plots. Nevertheless, in our trials the BYDV incidence and the yield were about the same in plots sprayed with deltamethrin as in those sprayed with oxidemetonmethyl, which provided a better and longer control of the aphids.

The effect of deltamethrin, a synthetic pyrethroid, on the number of aphids was surprising. However, Jenkyn and Plumb (1983) one year found an increase in the number of aphids in plots of barley treated with dimethoate, an organophosphorous insecticide, and suggested that the spray also killed predators and parasites.

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Barley Yellow Dwarf Virus: Losses, Virus-Plant Relationship, Chemical Control of Vectors

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Astract

Losses due to barley yellow dwarf virus (BYDV) are presented. The virus-plant relationship, especially the effect on yield of the titer of BYDV in the plant at various growth stages as measured by enzyme-linked immunosorbent assay, is discussed, as well as the chemical control of aphid vectors.

Damage and the Relationship between Yield and Symptoms

In 1975, in the north of the Charente area of southwestern France, the average decreases in grain yield, due to barley yellow dwarf (BYD), were:

2.5-3.0 t/ha for spring barley,
2.0-3.5 t/ha for winter barley,
2.0-2.5 t/ha for winter wheat.

More than 20,000 ha of winter wheat and 17,000 ha of winter and spring barley were affected. The value of the losses due to BYD has been estimated at 63 million francs.

In field tests, losses of up to 5 t/ha due to BYD have been verified on winter barley under natural infection. The relationship between yield loss and the percentage of plants with symptoms of BYD shows that an economic threshold is reached when 10-15% of plants exhibit symptoms (Figure 1).

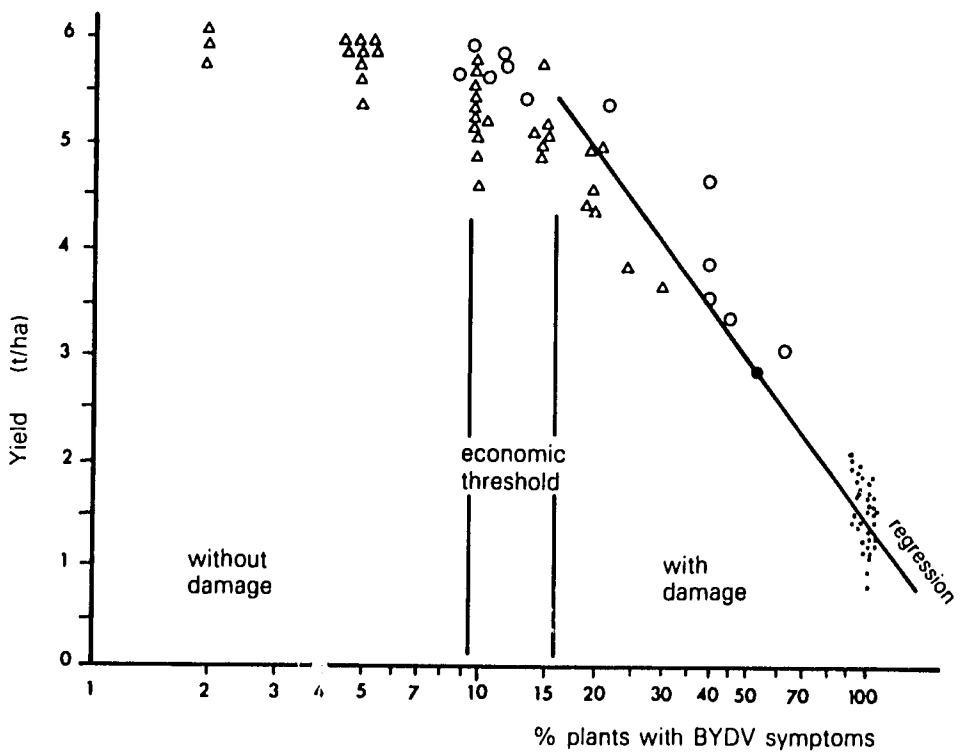

Figure 1. Relationship of yield and percentage of plants with BYD symptoms in barley.

Relationship of Yield to Amount of BYDV in Plant

Under natural field conditions, the yield-virus quantity relationship, in the enzyme-linked immunosorbent assay (ELISA) test, is a straight line of regression,

$$Y = mX + b$$

in which Y = yield in t/ha, X = quantity of virus in the plant, in Optical Density units adjusted to a standard reference, and b = yield in t/ha of the cereal free from virus. During the 1980-81 season, the following results were obtained:

Astrix winter barley—
$Y = -1.547\ x + 7.542$ with $r = -0.9$
(coefficient of linear correlation)

Talent winter wheat—
$Y = 0.560\ x + 7.693$ with $r = -0.97$
(coefficient of linear correlation)

These regression lines show that winter barley is generally much more sensitive to barley yellow dwarf virus (BYDV) than winter wheat.

The effect of BYD on 1000-grain weight of winter wheat varies according to the intensity of infection. The 1000-grain weight of BYD-free winter wheat is 52 g while that for a severely infected crop is 18 g.

Evolution of Virus Content in Plants during Growth

During 1984-85 a study on the titer of BYDV at different dates during the growing season, as measured by ELISA was conducted in the winter barley cultiver Capri (Figure 2). There were differences in the titer of virus present at different growth stages. The amount of virus was low in early vegetative stages and early tillering and increased later up until ear emergence in late tillering.

Chemical Control of Vectors

A study on the effect of aphicides on *Rhopalosiphum padi* show varied results according to the rate of chemical used (Bayon *et al.* 1981). Tests in naturally infested fields confirmed a correlation between cereal yields and the virus titers in plants.

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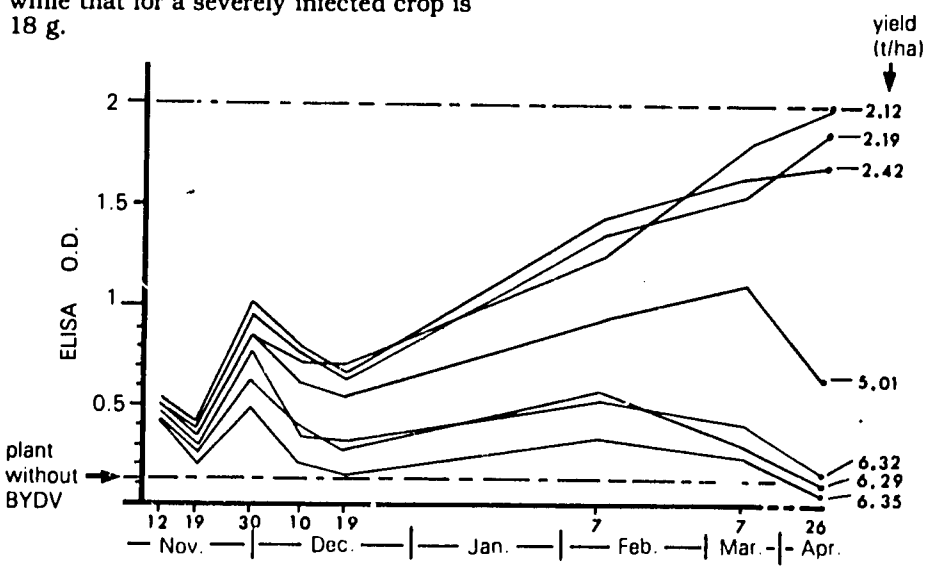

Figure 2. Evolution of virus in plant following the vegetation (winter barley cv. CAPRI) 1984-85.

Vector Infectivity as an Aid to Forecasting Barley Yellow Dwarf Virus Incidence

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Abstract

Although aphids are essential for virus transmission, the number of aphids caught in suction traps or on crops is not always directly correlated with the risk of virus infection. This is especially true in autumn when a large portion of the migrant population is male or gynoparae. In the UK we have supplemented the raw aphid data by measuring the infectivity of the aphid vectors with transmission tests. The resulting Infectivity Index represents the risk of infection of BYDV. A threshold value has been determined that gives a reliable guide to virus infection locally to Rothamsted and has been adopted elsewhere to aid decisions on the need to use insecticidal control measures. Alternative, serological methods of detecting virus in aphids have been tried, but the relationship between these tests and infectivity is not yet clear enough for such methods to be used instead of the biological tests.

One of the traditional approaches of entomologists to a pest problem is to establish damage thresholds based on the numbers of a particular pest species at a critical plant growth stage. For cereal aphids in the UK the critical stage has been defined as flowering and the threshold number as five, with the prospect of numbers increasing (George 1975). However, when the aphids can transmit viruses, the numbers present on the crop or caught in suction traps are only a measure of the potential risk of virus infection. A thousand aphids, none of which transmits virus, are less important than one hundred that do. As there is no way of detecting infective virus vectors morphologically, the presence of virus and the vectors' ability to transmit it must be determined using indirect methods such as serology or direct bioassays, i.e., allowing the vectors to feed on, and infect, susceptible hosts.

Assessing Viruliferous and Infective Aphids

A distinction should be made between aphids that are viruliferous, i.e., they carry virus that they may or may not transmit to plant hosts, and those that are infective, i.e., they both carry and transmit virus.

Barley yellow dwarf virus (BYDV) can be detected in aphids by serology (Torrance et al. 1986) and by cDNA probes, but as these methods only detect viruliferous aphids, there is not always good agreement with the proportion infective (Torrance et al. 1986). However, improvements in the detection system to eliminate some of the nonspecific reactions may improve the agreement. In general the serological method detected virus in a larger proportion of aphids than transmitted virus to test plants.

The principal disadvantage of the assay by transmission is the length of time from trapping the aphid to knowing if it has transmitted BYDV. Its advantages are that it mimics what happens in crops and also allows other epidemiologically important measurements to be made, such as survival on the test plant, reproduction, and closer examination of the vector specificity of the virus transmitted. The time to positive identification of virus transmission can also be shortened by testing the test plant by enzyme-linked immunosorbent assay 7 to 10 days after the beginning of the 2- to 3-day infection feeding period.

The system that we currently use is based on that described by Plumb (1976, 1986). Aphids are caught alive in a suction trap sampling at 1.7 m above the ground. The catch is emptied at 0900 and 1600 and the cereal aphids separated and identified, where possible, to species. Each aphid is caged on a single seedling of a susceptible oat (*Avena sativa* L.) cultivar (Blenda or Maris Tabard) at no older than the one-leaf stage. The aphid is allowed to feed for 2 to 3 days before its survival and reproduction are

recorded. If the aphid is recovered, it is preserved for future checks on its identity if necessary. Test plants are kept in a well-lit, cool (15 to 20°C) glasshouse for 4 weeks and the appearance of symptoms recorded.

The Need for Forecasting

In Britain barley yellow dwarf (BYD) is most damaging to crops sown in the autumn (September and October) and because of the perceived risk of BYDV many of the earlier sown crops, especially barley (*Hordeum vulgare* L.) sown in September, are sprayed routinely with insecticide at the end of October or beginning of November, to control the spread of BYDV. Therefore, it is at this time especially that the farmer needs guidance on whether or not crops are sufficiently at risk from BYDV to justify insecticide treatment.

Table 1 gives the numbers of *Rhopalosiphum padi* (L.), *Sitobion avenae* (Fabricius), and *Metopolophium dirhodum* (Walker) caught at Rothamsted in the suction trap of the Rothamsted Insect Survey (Taylor 1973), from 1 September in each of the last 12 years and the proportion infective. There is a range of two orders of magnitude in the numbers caught, and from 0 to 8.6% were infective each year. The small proportion that are infective probably reflects the biology of the most numerous aphid, *R. padi*, which is holocyclic in our climate and of which an increasing proportion during the autumn migration are sexual forms, gynoparae and males (Tatchell 1988).

In an attempt to reflect more accurately the risk of virus infection, the potential number of vectors each week is multiplied by the proportion infective, the product being called an Infectivity Index. When this index is accumulated each week, the figure in the right hand column of Table 1 is given. It has been found that this figure gives a more accurate forecast of virus damage and therefore the benefit to be obtained from a pesticide treatment than aphid numbers in the suction trap, or aphids found in the crop (Plumb 1986, 1987). For example, in autumn 1978 the third largest total of potential vectors was recorded, few were infective, the Index was 19, and there was little or no virus infection in autumn cereal crops. However, in 1980 and 1982 aphids were relatively numerous, 5 to 7% were infective, and virus infection was quite widespread. Analysis of results over several years has shown that the threshold value of the index, above which crops will benefit from aphicide treatment is about 50 (Plumb et al. 1986). In some years sheer numbers of vectors can compensate for inefficient transmission, as in 1985. What is also interesting is that in the past 12 years, when the area of autumn-sown cereals

Table 1. Numbers of vector species (*R. padi*, *S. avenae*, and *M. dirhodum*) caught in the Insect Survey trap at Rothamsted, the proportion trapped alive that were infective, and the total infectivity index, in autumn 1975-1986

Year	Number Trapped	% Infective	Infectivity Index (cumulative weekly)
1975	44	0.0	0
1976	430	0.0	0
1977	2195	0.0	0
1978	2639	1.2	19
1979	1614	2.6	35
1980	2341	7.3	195
1981	111	8.6	15
1982	2612	5.4	145
1983	398	0.3	4
1984	766	4.5	21
1985	4993	1.1	95
1986	1397	0.4	12

has increased and much has been sown in September, only in 3 years did the index indicate that treatment would be beneficial, a result largely supported by observation.

The system currently used is very simple and can be improved, nevertheless it has demonstrated the importance of aphid infectivity as a component in forecasting systems, and one that should be taken into account when studying BYD epidemiology.

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Transfer of the *Yd2* Barley Yellow Dwarf Virus Resistance Gene from Barley to Wheat

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Abstract

*Disomic addition lines of barley (*Hordeum vulgare *L.) chromosome 3, which carries the* Yd_2 *gene, have been obtained in Chinese Spring wheat (*Triticum aestivum *L.) from two barley cultivars, Atlas 68 and CI 3208-4. In barley, the* Yd_2 *gene confers resistance to the barley yellow dwarf virus (BYDV). The disomic addition lines were obtained only from the selfed progeny of monotelodisomic additions (2n = 42 + 1 + t). Substitution and recombinant lines are still under development. The disomic addition of* Yd_2 *to wheat conferred at best a small improvement in BYDV resistance based on visual symptom scoring in one field trial. Since* Yd_2 *is not a completely dominant gene in barley, the observed result was expected. Expression of* Yd_2 *is expected to be improved in wheat substitution and translocation lines. We are optimistic that it will be useful in wheat breeding, especially in wheat lines having resistance from other sources.*

While some protection against barley yellow dwarf virus (BYDV) can be realized by crop management practices (Bruehl 1961), resistant varieties offer the greatest opportunity for reduction of yield losses in the face of the increasing worldwide incidence of the virus. In California, for example, annual increases in productivity of barley (*Hordeum vulgare* L.) varieties due to the incorporation of the BYDV resistance gene have been conservatively estimated at 200,000 tons (Schaller 1984). In wheat (*Triticum aestivum* L.), however, no major gene has been found. The wheat genotypes with measurable, heritable resistance that have been identified, express it as a quantitative rather than a qualitative character (Qualset et al. 1973; Topcu 1975).

The Yd_2 gene in barley was identified in entries of the United States Department of Agriculture (USDA) World Collection of Barley that originated from Ethiopia (Schaller et al. 1963). Genetic analyses showed that a single gene conferred resistance (Rasmusson and Schaller 1959). Studies of resistant x resistant crosses failed to reveal susceptible recombinants, suggesting that the same single gene confers resistance in all resistant parents so far tested (Schaller et al. 1964; Damsteegt and Bruehl 1964; Sieveking 1969; Catherall et al. 1970; Hayes et al. 1971). Linkage tests showed that *Yd2* was located on barley chromosome 3 (Schaller et al. 1964). The long-term goal of the research program described here is to provide breeding material for bread wheat that possesses *Yd2*, transferred from barley.

Materials and Methods

The barley genotypes used as donors of *Yd2* were the cultivar Atlas 68 and the Ethiopian landrace identified in the USDA World Collection as CI 3208-4. The recipient hexaploid wheat genotype was Chinese Spring.

The overall procedure began with crosses between barley as pollen parent and wheat as maternal parent. The F_1 plants were treated with colchicine to double the chromosome number and were subsequently backcrossed to Chinese Spring. The backcross progeny were screened to identify monosomic addition lines (2n = 43) of barley chromosome 3. The screening of these derivatives and of the initial F_1 plants to identify the barley chromosomes was done by C-banding and confirmed for the derivatives by starch gel electrophoresis for an esterase isoenzyme located on chromosome 3. These steps were described earlier (McGuire 1984).

After the monosomic additions are identified, the subsequent steps of the procedure are the production of stable disomic addition lines (2n = 44), the production of disomic substitution lines (2n = 42), the determination of whether the gene is expressed in the wheat

background, the induction of recombination between the barley chromosome and a wheat chromosome, and finally, selection of recombinants that express the transferred gene. Additional objectives are the search for genetic markers tightly linked to *Yd2*, which would facilitate the identification of recombinants, and the substitution of barley chromosome 3 into an additional wheat genotype (cultivar Anza) to assess the effect of different wheat genetic backgrounds on the expression of *Yd2*.

A disomic addition line of barley chromosome 3 from cultivar Betzes in Chinese Spring developed in Australia (Islam et al. 1981) was available for comparison purposes. Betzes lacks *Yd2* and thus is susceptible to BYDV.

Results

Production of disomic addition lines

It was anticipated that selfing of monosomic addition lines would produce disomics. This proved not to be the case (Table 1): 450 plants monosomic for barley chromosome 3 yielded no disomics. However, progeny of these selfed monosomic additions did include some monotelodisomic plants, 2n = 43 + t, which had a complete barley chromosome 3 plus a long or short arm barley 3 telosome, presumably derived from misdivision of barley chromosome 3 in its monosomic state. It was from these plants that the disomic additions were finally derived. Initially, 70 BC_2F_3 monotelodisomic additions of barley chromosome 3 from Atlas 68 plus the barley chromosome 3 short arm were screened and 21 ditelosomic additions but no disomic additions were recovered. Subsequently, 81 BC_2F_4 monotelodisomics were screened and a single Atlas 68 barley chromosome 3 disomic addition (Figure 1a) was recovered along with 29 ditelosomic additions. This disomic addition is stable; 16 of the 17 screened progeny have been disomic as well. Similarly, two disomic additions of barley chromosome 3 from CI 3208-4 (Figure 1b) were recovered from 50 screened BC_2F_3 monotelodisomics (a complete barley chromosome 3 plus the long telosome). One of these disomics is more stable than the other, 60 progeny of one yielded only 10 disomics, while 77 progeny of the other yielded 67 disomics.

Various other derivatives were also screened: 393 double monosomic addition lines (which had two nonhomologous barley chromosomes) yielded two ditelosomic plants with the short arm of barley chromosome 3, but no completely disomic plants. None of the 120 screened monotelosomics (2n = 42 + t) yielded any ditelosomic plants (2n = 42 + 2t).

Screening of disomic addition lines for *Yd2* expression

In the University of California at Davis 1987 BYDV nursery, a three-replicate test of single, 2-m rows was established. The genotypes tested were Chinese Spring; three disomic addition lines of barley chromosome 3 in Chinese Spring—one line with chromosome 3 from Betzes, one with chromosome 3 from Atlas 68, and one with chromosome 3 from landrace CI 3208-4—and a ditelosomic addition in Chinese Spring of the short arm of chromosome 3 from Atlas 68. The rows

Table 1. Frequency of recovery of disomic and ditelosomic plants from progeny of various types of monosomic addition lines

Type of addition line	Chromosome number	Number of plants screened	Disomics recovered	Ditelosomics recovered
Monosomic	43	450	0	0
Monotelosomic	42 + t	120	0	0
Monotelodisomic	43 + t	501	5	52
Double monosomic	44	393	0	2

were scored with the 0 to 9, resistant to susceptible scale of Qualset (1984), and row scores were averaged over the three replicates (Table 2). The overall mean row score for the genotypes lacking Yd_2 (the first three genotypes of Table 2) was 6.1, and the score for the two genotypes with two doses of Yd_2 (the last two in Table 2) was 5.0. These two mean scores differ significantly at the 10% probability level as judged by Student's t test.

Production of Anza aneuploids

To produce substitution lines of barley chromosome 3 in Anza, it is necessary to have lines of Anza monosomic for the homoeologous group 3 wheat chromosomes which will be replaced by the barley chromosomes. Production of these monosomics began with initial crosses between Anza and each of the group 3 monosomic lines of Chinese Spring (monosomics 3A, 3B, and 3D). These crosses yielded plants lacking the Chinese Spring group 3 monosome but having a single homologous Anza chromosome. These progeny segregated for the presence of the Anza group 3 chromosome in each case. Four generations of backcrossing to Anza have been completed.

C-band screening

The C-band karyotype of barley is distinct from that of wheat, allowing the barley chromosomes to be distinguished from those of wheat in the same cell. Thus, C-banding was used from the beginning; first, to verify that the F_1 embryos that had more than 21 chromosomes had barley chromosomes (McGuire 1984). Subsequently, C-banding

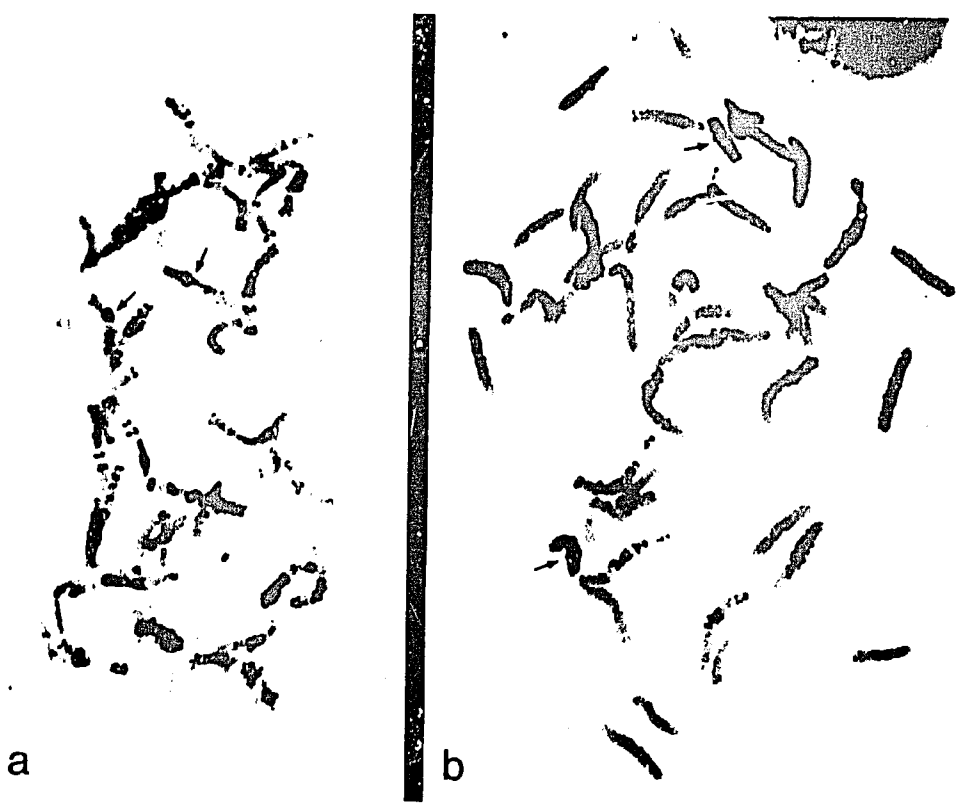

Figure 1. C-banded root-tip cells of the disomic additions (2n = 44) with a pair of barley chromosome 3 (arrows) from (a) Atlas 68 and (b) CI 3208-4.

was used to identify the derivatives from the backcrossing of the colchicine-treated F_1 plants that had barley chromosome 3. In the disomic addition lines (2n = 44), the added pair of barley chromosomes can be identified distinctly from the rest of the wheat chromosomes (Figure 1).

Linkage relationships among *Yd2*, the distal C-band, and esterase isoenzyme loci

The C-band karyotype of barley chromosomes can vary in several respects from one barley genotype to another (Linde-Laursen 1978). A conspicuous difference is the presence or absence of the long arm distal band (Figure 2), termed 3ℓ3 by Linde-Laursen (1979). There is indirect evidence that *Yd2* is on the long arm of chromosome 3 (Tsuchiya 1984; Takahashi 1983). The recent linkage map of barley chromosome 3 by Takahashi (1983) suggests that *Yd2* is closely proximal to the 3ℓ3 C-Band. Since this band is present in Atlas 68 and absent in barley cultivar Betzes, which does not possess *Yd2*, this linkage can be estimated.

Two loci, *Est-1* and *Est-4*, (or *Est 1* and *Est 3* in the nomenclature of Kahler and Allard 1981) are tightly linked and are distally located on the long arm of barley chromosome 3 (Hvid and Nielsen 1977). However, there was essentially free recombination between these loci and the

Table 2. Mean row score for BYD symptoms of Chinese Spring (CS) and various addition lines of barley chromosomes in a 1986-87 three-replicate test

Genotype	Copies of *Yd2*	Chromosome number	Mean row score (Range)
CS	0	42	5.3 (4-6)
DA Betzes 3	0	44	6.3 (6-7)
DtA Atlas 68 3s	0	42 + 2t	6.7 (6-7)
MtDA CI 3208-4 3 + 3s	1	43 + t	5.7 (5-6)
DA Atlas 68 3	2	44	4.3 (4-5)
DA CI 3208-4 3	2	44	5.8 (4-7)
Standard error:			0.53

A = disomic addition; DtA = ditelosomic addition; MtDA = monotelodisomic addition; s = short chromosome arm.

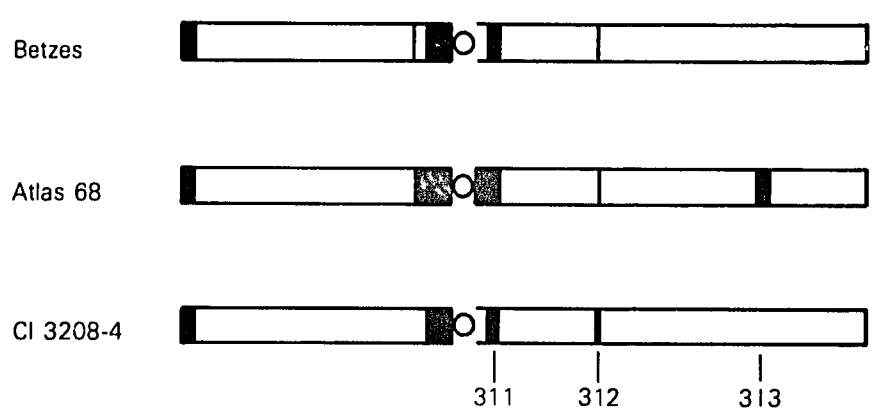

Figure 2. Diagrammatic representation of C-banding patterns of barley chromosome 3 in three genotypes. Band 3/3 was considered as a maker for *Yd2* in Atlas 68.

3ℓ3 C-band (Linde-Laursen 1982). Betzes and Atlas 68 differ in the alleles present at these two esterase loci, so the linkage between the esterase loci and *Yd2* can also be estimated.

A cross was made between Betzes and Atlas 68 and the segregation of both the 3ℓ3 band and the alleles at the esterase loci was scored in 113 F_2 plants from this cross. These results confirmed the free recombination between the 3ℓ3 band and the esterase loci found by Linde-Laursen (1982). Since the expression of *Yd2* cannot be scored reliably on a single plant basis, it was necessary to progeny test these same F_2 plants. A preliminary screening of 39 F_3 families for BYD reaction indicated free recombination between *Yd2* and the esterase loci. The 3ℓ3 band and *Yd2* were linked, but an estimate of the strength of that linkage must await completion of analysis of 120 F_3 lines that was initiated in 1987.

Production of barley chromosome 3 substitution lines in Chinese Spring
Crosses between the Chinese Spring group 3 monosomics and the two disomic addition lines have been made. Screening of the resulting progeny for the desired 2n=41 plants that have barley chromosome 3 is under way. These will be selfed and their progeny will be screened for disomic substitutions, 2n=42 having barley chromosome 3.

Discussion

The lack of disomic progeny from selfed monosomic plants indicates that there is poor transmission of barley chromosome 3 through the pollen line in wheat. The regularity of meiosis was improved in monotelodisomic plants since barley chromosome 3 had a pairing partner, its homologous telosome, thus, it was possible to obtain disomic addition lines.

With the poor transmission of barley chromosome 3 through the pollen line from a monosomic state, the production of a disomic substitution from a monosomic substitution may be a problem. Very large numbers may have to be screened.

In barley, *Yd2* shows partial dominance to additivity in conferring resistance, depending on the genetic background. Our initial observations on the expression of Yd2 in Chinese Spring have shown that this gene is not completely dominant in this genetic background either. The addition of two "resistance" alleles from barley in the two addition lines resulted in a slightly improved BYD resistance reaction (Table 2), as would be expected if *Yd2* were a partially dominant gene. If there exists a pair of "susceptibility" genes in each genome of wheat, homoeallelic to the *Yd2* locus of barley, then expression of *Yd2* in wheat might be improved in a substitution or translocation line because there would then be four, instead of six, susceptibility alleles present with the two *Yd2* resistance alleles.

Further evaluations of the addition lines will be made to confirm the effects of *Yd2* in wheat. We remain optimistic that *Yd2* will be a valuable gene for improving the resistance of wheat to BYD, especially when used in conjunction with existing wheat genes that have partial resistance to BYD. Efforts are being continued to develop substitution and recombination lines in wheat that contain *Yd2*.

Acknowledgment

This work has been conducted with support of United States Department of Agriculture Competitive Grant 84-CRCR-1-1544.

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Correlation of Ratings in Oat Disease Nurseries: High Rank Correlations Are the Next Best Thing to True Uniform Disease Ratings

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Abstract

*In an ideal uniform rating system, different evaluators arrive at similar absolute numerical ratings for the same "amount" of disease. This is rarely achieved with ratings for barley yellow dwarf (BYD) disease, but a rating system is successful if it enables the breeder to select the best sources of resistance consistently and reliably. Analyses of 1985 and 1986 ratings for BYD damage in about 25 advanced oat (*Avena sativa *L.) breeding lines from eastern and western disease nurseries at Sainte-Foy, Quebec, and Glenlea, Manitoba, showed rank correlations near 0.90. By ensuring that virulent inoculum was uniformly applied at early plant growth stages, and that ratings were performed "blindly" with replication at both mid and late season, highly similar relative rankings were achieved. This means that if isolates of barley yellow dwarf virus are uniform country-wide, selection for BYD resistance could be conducted at one site.*

Barley yellow dwarf (BYD) is the most serious virus disease of cereals in Canada. Most control efforts are directed toward incorporating sources of resistance into new varieties and selecting for tolerance by screening in disease nurseries. At present, this is principally done in cooperation with oat (*Avena sativa* L.) breeding programs at Winnipeg, Manitoba, and Sainte-Foy, Quebec.

In an ideal uniform rating system for disease nurseries, different evaluators would arrive at similar, absolute numerical ratings for the same "amount" of disease. In practice, this is rarely achieved for BYD ratings because of the influences of local and seasonal conditions on the complex of symptoms (leaf discoloration, stunting, reduced vigor and tillering, blasting, etc.) that the evaluator takes into account in arriving at a rating, as well as the unavoidable variations among individual evaluators in interpreting those complexes of symptoms. Any rating system succeeds to the extent that it provides information useful to the breeder in making selections. The object of the work reported here was to determine the extent of correlation of ratings under conditions of strong, deliberately applied, disease pressure for the same oat lines screened in nurseries at different locations by different evaluators.

Materials and Methods

Aphid inoculum was prepared according to methods already described (Comeau 1976). In 1985, a cocktail of virulent Quebec barley yellow dwarf virus (BYDV) isolates was used for both the Quebec and Manitoba disease nurseries. In 1986, a highly virulent isolate of a vector-nonspecific (PAV-like) BYDV variant obtained in a 1985 survey for BYDV in western Canada was used in the Manitoba nursery, while the Quebec nursery used similar inoculum to that of 1985. Each nursery contained entries for the Eastern and Western Co-op trials in randomized complete blocks replicated four times. Aphid inoculum was applied to seedlings at the two-leaf stage, followed by a second application one week later. In 1986, part of the Manitoba nursery experienced flooding and some of the ratings were not based on four replications.

Disease was rated on a 1 to 9 scale (1 = best, 9 = worst plant response) at the boot stage and early dough stage in Manitoba, and at the dough stage in Quebec. Evaluation was "blind" in that the evaluator did not know the identity of the material being rated, eliminating the bias of "expected" performance of a given line.

After tabulation of disease ratings, the rankings of varieties in the Eastern and Western Co-op tests were correlated a) between locations and b) between years,

by Spearman's rank correlation, a test which makes no assumptions about any underlying normal distribution of the data (Snedecor 1956).

Results and Discussion

The rank correlations between: a) two different locations (with two different evaluators) (Table 1) and b) two different years (with different weather conditions) (Table 2) are highly significant. Ratings taken at Glenlea at both the boot and mid-dough stages were significantly correlated with those taken at Ste. Foy at the mid-dough stage (Tables 1 and 2). The rankings of oat lines for evaluation were very similar at different locations and in different years. Not only did both

Table 1. Rank correlation of barley yellow dwarf disease ratings in oat nurseries between different locations in same year

Reading	No. of entries	r_s	significant at 1% level if r_s >
1985 Western Co-op test: Ste. Foy reading *vs* 1st Glenlea reading (boot stage)	25	0.890	0.505
1985 Western Co-op test: Ste. Foy reading *vs* 2nd Glenlea reading (dough stage)	25	0.914	0.505
1985 Western Co-op test: Ste. Foy reading *vs* mean of 2 Glenlea readings	25	0.908	0.505
1986 Western Co-op test: Ste. Foy reading *vs* 2nd Glenlea reading (dough stage)	25	0.881	0.505

Table 2. Rank correlation of barley yellow dwarf disease ratings in oat nurseries between different years in same location

Reading	No. of entries	r_s	significant at 1% level if r_s >
Common entries of 1985 & 86 Western Co-op 1st Glenlea reading (boot stage)	12	0.802	0.708
Common entries of 1985 & 86 Western Co-op 2nd Glenlea reading (dough stage)	12	0.880	0.708
Common entries of 1985 & 86 Western Co-op mean of 2 Glenlea readings	12	0.841	0.708
Common entries of 1985 & 86 Eastern Co-op 2nd Glenlea reading (dough stage)	10	0.940	0.765
Common entries of 1985 & 86 Eastern & Western Co-op combined 2nd Glenlea reading (dough stage)	22	0.880	0.537

nursery trials pick the same clear winners and losers, but they also ranked very similarly those lines with middling levels of susceptibility/resistance.

These results are not necessarily at variance with the observation (reported in Paliwal 1982) that breeders' lines displaying resistance to local BYDV variants in one region do not always show resistance in another. The advanced material submitted for testing in the Western and Eastern Co-op trials is limited to about 25 lines; a regional variation in resistance that could have been detected in the 1986 trials (where distinct regional inocula were used) may simply have not been apparent among any of the small number of lines tested. In addition, vector nonspecific (PAV-like) BYDV variants have predominated in recent years in all North American regions where periodic characterizations for vector specificities of isolates from crops (maize, *Zea mays* L., excepted) have been carried out. Under such circumstances an oat line resistant to virulent PAV-like isolates from Alberta might very well also show resistance to virulent—and PAV-like—BYDV isolates from Quebec. Should there be a return to a situation of annual variation of predominant BYDV variants as occurred in the later 1960s in Manitoba (Gill 1969), high correlations of resistance ratings between years and regions might not be observed.

Further light would be shed on these questions by analyses similar to those reported here, but using data obtained from an international cooperative screening system (McKenzie et al. 1985). A first step in this direction has been taken with the start of an International BYDV Oat Nursery this year that will evaluate advanced lines submitted by programs from CIMMYT, Quebec, Manitoba, Illinois, Indiana, Wisconsin, California, and New Zealand.

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Preliminary Comparisons of Barley Yellow Dwarf Scores on CIMMYT Nurseries in Contrasting Environments Using Nonparametic Correlations

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Abstract

Of 58 pair-wise environment combinations with the same genotypes, 17 gave positive, significant Spearman's rank correlations for visual barley yellow dwarf scores. Spearman's method gave significantly higher coefficients than Pearson's product moment method indicating the utility of the nonparametric test. The development of an evaluation system that minimizes differences among scorers and maximizes genetic differences and environmental effects due to viral variation is suggested.

Resistance to barley yellow dwarf virus (BYDV) is continuously distributed, but is difficult to evaluate quantitatively in plant breeding programs, which often use a 0-9 visual scale (Qualset 1984).

CIMMYT screening nurseries are evaluated for BYDV resistance in Mexico before world distribution. The objective of this analysis was to investigate the quantification of relationships among locations and particularly to determine if BYD screening in Mexico reflects reactions in other parts of the world.

Ideal conditions for obtaining high correlation among genotypes over environments include the following. Observations by different individuals are unbiased. Scores are normally distributed within environments. Environments do not affect symptom expression. Disease scores are taken at the same physiological stage. Nutrient deficiencies and/or other diseases do not confound BYD expression. Virus infection is homogeneous across entries.

Materials and Methods

Bread wheat (*Triticum aestivum* L.), durum wheat (*Triticum turgidum* L. var *durum*) and barley (*Hordeum vulgare* L.) screening nurseries were sent to various parts of the world for BYD evaluation (Table 1). Entries were scored generally on a 0-9 scale (Qualset 1984) by cooperators at each site.

Table 1. Locations where nurseries were evaluated for their reaction to infection with barley yellow dwarf

Nursery	No. ontries	Locations
First barley BYD screening	169	St. Foy, Canada; Madrid, Spain; Palmerston North, New Zealand
Second barley BYD screening	43	Beijing, China; Lincolnshine, England; Montpellier, France; Lleida, Spain; Madrid, Spain, Toluca, Mexico; Davis, USA; Palmerston North, New Zealand; Béja, Tunisia
Third barley BYD screening	115	Lleida, Spain; Madrid, Spain; Palmerston North, New Zealand
Preliminary bread wheat BYD screening	89	St. Foy, Canada; Davis, USA; Palmerston North, New Zealand
Bread wheat selections	228	Toluca, Mexico; Davis, USA

Product moment correlations and Spearman's nonparametric correlations were calculated for pairwise combinations of locations.

Results and Discussion

Only the third barley screening nursery did not produce a significant interlocation correlation (Table 4). For the other five nurseries (Tables 2, 3, 5, and 6) at least one quarter of the location combinations showed significant positive correlations which were generally increased by using Spearman's rank correlation.

It is unlikely that any of the ideal conditions outlined above were met, and possibly scoring plants at different stages of development within a nursery at a

Table 2. Spearman's rank correlation coefficients for the first barley yellow dwarf screening nursery, barley

	Locations MS	PNNZ
SFQC	0.14†	0.18*
MS		0.17*

†, * significant at 0.10 and 0.05 probability levels, respectively.

SFQC - St. Foy, Quebec, Canada.
MS - Madrid, Spain.
PNNZ - Palmerston North, New Zealand.

Table 3. Spearman's rank correlation coefficients for the second barley yellow dwarf screening nursery, barley

	Locations							
	BC	LE	MF	LS	MS	TM	DC USA	PNNZ
BT	.06 NS	.08 NS	.60***	.15 NS	-.15 NS	.25†	.25†	.34*
BC		.08 NS	.14 NS	.26†	.18 NS	.02 NS	.25†	.13 NS
LE			.06 NS	.14 NS	.11 NS	-.02 NS	.09 NS	.12 NS
MF				.16 NS	-.16 NS	.28 NS	.21 NS	.20 NS
LS					.12 NS	.31*	.01 NS	-.17 NS
MS						-.19 NS	.22 NS	.02 NS
TM							.03 NS	.11 NS
DCUSA								.51***

NS, not significant; †, *, ***, significant at 0.10, 0.05 and 0.001 probability levels, respectively.

BT - Béja, Tunisia
BC - Beijing, China
LE - Lincolnshire, England.
MF - Montpellier, France.
LS - Lleida, Spain.
MS - Madrid, Spain.
TM - Toluca, Mexico.
DCUSA - Davis, California, USA.
PNNZ - Palmeston North, New Zealand.

given time was the most serious flaw. Therefore, it was highly encouraging to find significant correlations, despite the noise and imperfections of the system. For instance the significant correlation between Davis, California, USA (second score) and Palmerston North, New Zealand (Table 5) may suggest that virus isolates in these two areas are similar, but differ from Quebec isolate(s). Because of noise resulting from imperfections in the scoring system, the use of correlations in this situation is more akin to their use in marketing surveys rather than for rigorous agronomic studies. Highly controlled agronomic experiments usually require numerically larger, statistically significant correlations to draw inferences while marketing surveys may depend on much smaller values to detect trends.

If location specific strain differences caused major rank ranges within germplasm, then significant negative correlations might occur. However, none

Table 4. Spearman's rank correlation coefficients for the third barley yellow dwarf screening nursery, barley

	Locations	
	MS	PNNZ
LS	-0.04 NS	-0.09 NS
MS		0.05 NS

NS, not significant.

LS - Lleida, Spain.
MS - Madrid, Spain.
PNNZ - Palmerston North, New Zealand.

Table 5. Spearman's rank correlation coefficients for the preliminary screening nursery, bread wheat

	Locations		
	PNNZ	DCUSA1	DCUSA2
SFQC	0.02 NS	0.13 NS	0.007 NS
PNNZ		0.10 NS	0.22 *
DCUSA1			0.64 ***

NS, not significant.
*, ***, significant at the 0.05 and 0.001 probability levels, respectively.

SFQC - St. Foy, Quebec, Canada.
PNNZ - Palmerston North, New Zealand.
DCUSA1 - Davis, California, USA - first score
DCUSA2 - Davis, California, USA - second score

Table 6. Spearman's rank correlation coefficients for Toluca, Mexico, and Davis, California for Toluca bread wheat selections

	DC J6	TM S24	TM O3	TM O8
DC M28	0.74 ***	0.05 NS	0.03 NS	-0.03 NS
DC J6		0.09 NS	0.05 NS	0.07 NS
TM S24			0.62 ***	0.56 ***
TM O3				0.65 ***

DC - Davis, California, USA.
TM - Toluca, Mexico.

M28 - May 28
J6 - June 6
S24 - September 24
O3 - October 3
O8 - October 8
NS - not significant
*** significant at 0.001 probability level.

was observed. Although these are preliminary studies with tentative conclusions requiring validation, the lack of significant negative correlations may result from generalized strain non-specific resistance factors which would tend to increase correlation among locations, in additional to more strain specific factors which could contribute to either positive or negative correlations among locations.

We feel these preliminary results justify further more detailed work to refine the correlation technique to the extent that it can be used with confidence in making epidemiological inferences to complement those coming from serological studies. For economic reasons, serology is not feasible at many screening locations returning visual scores.

Relating field scores to aphid trap data and to greenhouse scores at key locations under highly controlled inoculation with ELISA confirmation may assist in calibrating correlation techniques.

Methods of combining scores across years should be investigated along with methods for adjustment of scores to take account of maturity differences within nurseries. Cluster and Factor analyses may prove useful for interpreting the structure within larger correlations matrices produced as BYD nurseries continue to be more widely grown. Without such multivariate techniques, relationships among varieties and among sites, become increasing difficult as the size of the correlation matrix increases. Multivariate analysis of correlations among locations may assist in forming epidemiological units.

Different relationships among wheat varieties and among barleys might be expected from more comprehensive studies, because of the different genetic base for resistance in the two crops, with wheat believed to depend upon minor genes and barley having at least one functional major gene.

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Preliminary Report on Parasitoids and Predators of *Rhopalosiphum padi*

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Abstract

Preliminary research on the biology of Rhopalosiphum padi *(L.) in the area around Viterbo, Italy, is reported. Three parasitoids were identified and studied.* Diaeretiella rapae *Stary (Hym. Aphidiidae) was found attacking* R. padi *for the first time it Italy. A preliminary list of parasitoids and predators of* R. padi *for the world is included.*

In order to evaluate the potential of biological control of the aphid vectors of barley yellow dwarf virus (BYDV), a preliminary study on parasitoids and predators of *Rhopalosiphum padi* (L.) in the Viterbo area of Italy was initiated. A bibliographic search was conducted on the parasitoids and predators of *R. padi* in the world.

An aphid population sample was taken from 100 plants of wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) near Viterbo. Three species of Hymenoptera Aphidiidae were found— *Aphidius rhopalosiphi* De St.[1], *A. matricariae* Hal., and *Diaeretiella rapae* Stary. The record of *D. rapae* on *R. padi* is new for Italy.

All three species of parasitoids were collected in the period from January to July 1987. During the first sampling, aphid population density on the epigeous parts of plants was very low (1 to 2 aphids per plant).

In spring (May-June), aphid samples were taken from barley and the following percentages of parasitization were estimated: 34.2% by *A. rhopalosiphi*, 39.5% by *A. matricariae*, and 26.3% by *D. rapae*. In contrast, the parasitization during autumn was only by *A. matricariae*.

Hymenoptera hyperparasites of Aphidiidae, probably Charipinae, (Cynipidae) emerged from about 1% of collected mummies.

In another Italian area (Piemonte), hyperparasites Megaspilidae Megaspilinae were obtained (Olmi 1969).

Parasitoids and predators of *R. padi*, worldwide, are listed in Tables 1 and 2.

[1] Species *incertae sedis et nomen dubium*. Described from Sicily as a parasite of Rhopalosiphum spp., De Stefani-Pérez, 1902.

Table 1. Parasitoids of *R. padi* worldwide

Name	Year	Geographic distribution
Hymenoptera: Ichneumonoide, Fam. Aphiidae		
Adialytus arvicola (Stary)	1961	Holarctic
Aphidius avenae Haliday	1834	Palaearctic
A. avenaphis (Fitch)	1861	Nearctic
A. equiseticola Stary	1963	Czechoslovakia, Italy. On *R. padi* in Italy
A. ervi Haliday	1834	Palaearctic introduced to Nearctic
A. frumentarius Latteur	1979	Belgium, England

Table 1. (continued)

Name	Year	Geographic distribution
A. matricariae Haliday	1834	Holarctic, Neotropical, south Zimbabwe, Australia
A. obscuripes Ashmead	1888	Nearctic, Hawauu
A. pascuorum Marshall	1896	Europe
A. picipes (Nees)	1811	Europe
A. rhopalosiphi De Stefani Pérez	1902	
Species incertae sedis et nomen dubium		
A. uzbekistanicus Luzhetzki	1960	Asiatic USSR, England
Binodoxys angelicae (Haliday)	1833	European subregion
B. centaureae (Haliday)	1833	Europe, USSR
Diaeretiella rapae (M'Intosh)	1855	Cosmopolitan
Ephedrus lacertosus (Haliday)	1833	Holarctic
E. persicae Froggatt	1904	Cosmopolitan
syn *E. palaestinensis*	1959	Mackauer
E. palaestinensis Mackauer (syn with *persicae* by Mackauer 1963, accepted by Gardenfors 1986)	1959	Cyprus, Lebanon, Israel, Jordan, introduced in California in 1965
E. plagiator (Nees)	1811	Palaearctic
Lysiphlebus testaceipes (Cresson)	1880	Nearctic, Neotropics, Oceania, Ethiopia?
Monoctonus cerasi (Marshall)	1896	Europe
Praon abjectum (Haliday)	1833	Palaearctic, W. Nearctic
P. americanum (Ashmead)	1888	USA
P. necans Mackauer	1959	Palaearctic, on *R. padi* in Italy
P. volucre (Haliday)	1833	Palaearctic, USA
Trioxys auctus (Haliday)	1833	Palaearctic
Hymenoptera: Ceraphronoidea, Fam. Megaspilidae		
Dendrocerus sp.		on *R. padi* in Britain[a]
D. aphidum (Kieffer)	1907	on *R. padi* in USSR[a]

Table 1. (continued)

Name	Year	Geographic distribution
Hymenoptera: Chalcidoidea, Fam. Pteromalidae (suspected hyperparasites)		
Asaphes vulgaris Walker	1834	Europe, Greenland?, Canada?, USA? Argentina
Pachyneuron aphidis (Bouché)	1834	
P. siphonophorae (Ashmead)	1886	USA
Hymenoptera: Chalcidoidea, Fam. Encyrtidae		
Syrphophagus aphidivorus (Mayr)	1875	On *R. padi* in USSR[a]
S. aeruginosis (Dalmar)	1820	On *R. padi* in USSR[a]
Hymenoptera: Chalcidoidea, Fam. Aphelinidae		
Aphelinus abdominalis (Dalmar) syn *Aphelinus flavipes* (Foerster)	1820	On *R. padi* in Italy[a]

[a] The country of the only finding on *R. padi*.

Table 2. Predators of *R. padi* (L.) worldwide

Name	Year	Geographic distribution
Diptera: Syrphidae		
Epistrophe nitidicollis (Meigen)	1822	Europe, Japan
Episyrphus balteatus (De Geer)	*1776*	*Cosmopolitan*
Meliscaeva cinctella (Zetterstedt)	1843	Europe, Algeria, Morocco
Melongyna (Meligramma) triangulifera (Zetterstedt)	1843	Europe
Metasyrphus corollae (Fabricius)	1794	Europe, North Africa, northwest and east Asia
M. luniger (Meigen)	1822	Europe, North Africa, Canary Islands, Asia
Pipiza bimaculata Meigen	1822	Europe
Platycheirus ambiguus (Fallen)	1817	Europe, Greenland, Siberia, India
Scaeva pyrastri (L.)	1758	Europe, Canary Islands, Madeira, North Africa, W. and N. Asia, China, America

Table 2. (continued)

Name	Year	Geographic distribution
Syrphus ribesii (L.)	1758	Europe, North Africa, Asia, America
S. torvus Osten Sacken	1875	Europe, Greenland, China, Japan, North America
S. vitripennis Meigen	1822	Europe, Japan
Coleoptera: Coccinellidae		
Adalia bipuntata (L.)	1758	Azores, Europe, Asia, Siberia
Aiolocaria hexaspilota (Hope)	1831	India, China, Japan
Anatis ocellata (L.)	1758	Palaearctic, introduced in USA
Calvia decemguttata (L.)	1767	Palaearctic
C. (*Anisocalvia*) *duodecimmaculata* (Gebler)	1832	Sikkim, Japan, Himalaya, USA
Chilocorus kuwanae Silvestri	1909	On *R. padi* in Japan[a]
Harmonia axyridis (Pallas)	1773	Siberia, Japan, China, introduced in California, Hawaii
Coccinella septempunctata L.	1758	Palaearctic, Oriental
Hippodamia tredecimpunctata (L.)	1758	Palaearctic, Nearctic
Oenopia conglobata (L.)	1758	Palaearctic, North America
Propylea japonica (Thunberg)	1781	East Palaearctic, Oriental
P. quatuordecimpunctata (L.)	1758	Palaearctic
Scymus subvillosus (Goeze)		On *R. padi* in Morocco[a]
Neuroptera: Chrysopidae		
Chrysopa sp.		on *R. padi* in Japan[a]

Sources: Currado and Olmi 1976; Ferriere 1965; Graham 1969; Iablokoff-Khnzorian 1982; Laska and Stary 1980; Mackauer and Stary 1967; Olmi 1969; Powell 1982; Stary 1976.
[a] The country of the only finding on *R. padi*.

Acknowledgments
The author acknowledges with appreciation Prof. E. Tremblay and Dr. F. Pennacchio (Istituto di Entomologia, Università di Portici, Italia) for the Hymenoptera Aphidiidae determinations.

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The Effect of Barley Yellow Dwarf Virus on Barley Yields in Mexico

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Abstract

The Yd_2 *gene confers resistance to barley yellow dwarf virus (BYDV). Isogenic lines Atlas 68 (+*Yd_2*) and Atlas 57 (-*Yd_2*) from California and two common barley cultivars from Mexico, Centinela and Cerro Prieto, were exposed to the following treatments: infection with a PAV-like BYDV isolate vectored by* Rhopalosiphum padi *(L.) at the three- to four-leaf stage; infection with the same isolate at flowering; spraying with systemic insecticides; and natural infection. Significant yield decreases were observed with all the barley cultivars except Atlas 68.*

Barley yellow dwarf virus (BYDV) is one of the most important viral diseases of small grains worldwide (Burnett 1984). In California, a field comparison of the yield of isogenic barley *(Hordeum vulgare* L.) lines, CM67 with and California Mariout without the Yd_2 gene for BYDV resistance, was conducted. Over 19 locations, CM67 yielded 19% above its susceptible isoline, with individual location differences as high as 60% (Schaller and Qualset 1984).

In Mexico, although BYDV has been known for over 30 years (Navarro 1984), its effect on cereal yields has not been studied. The present work was undertaken to determine the effect of BYDV on barley cultivars under field conditions in Mexico.

Materials and Methods

The barley cultivars used were Centinela and Cerro prieto, widely grown in Mexico, and two isogenic California lines, Atlas 68 (resistant -Yd_2) and Atlas 57 (susceptible -Yd_2).

The treatments were natural infection, artificial infection at the three- to four-leaf stage, artificial infection at flowering, and a control protected with insecticides.

A randomized split plot design with eight replicates was used with the four cultivars as the subplots and treatments as the main plot. Each cultivar plot was two 5-m rows. Borders of Paramo oats *(Avena sativa* L.) were sown between the plots.

Plots were infected with a PAV-like isolate of BYDV using *Rhopalosiphum padi* (L.) as the vector. These aphids were reared in a greenhouse at 20°C and fed viruliferous Paramo oats. Aphids were shaken from the oats and mixed with talcum powder to keep honeydew from sticking them together (Comeau 1984). Approximately three aphids per plant were applied and allowed to feed for 15 days before being sprayed with oxydemeton-methyl (6.8 ml/liter of water).

Two dates of infection were used. The first was the three- to four-leaf stage, 20 days after seeding, and the second was at the mean flowering date of the cultivars, 56 days after seeding. Neither of these treatments was protected before inoculation. These two treatments were subsequently sprayed every 2 weeks to stop infestation by other aphids. In the control treatment oxydemeton-methyl was applied at 2-week intervals throughout the crop cycle to eliminate aphid inoculation. The fourth treatment

left the plots untreated to reflect losses caused by natural infections of BYDV. The whole experiment was sprayed every 2 weeks, alternating the fungicides Triadimefon and Propiconazole, to eliminate fungal diseases.

Mature plant height was measured, and grain from the central 4 m of the plots was harvested. The 1000-grain weight was also measured. Prior to harvest 20 spikes were harvested for each plot to determine the number of grains per spike.

Results and Discussion

Virus infection at the seedling stage reduced yield significantly in the cultivars Centinela, Cerro Prieto, and Atlas 57. There was no significant effect on the cultivar Atlas 68 (Table 1). Infection at flowering also caused significant yield losses with Centinela and Cerro Prieto but not with Atlas 57, which matured earlier.

There was also a significant yield reduction with natural infection for Cerro Prieto. This may reflect losses to natural BYDV infection consistent with the other cultivars, except Atlas 68, showing a yield reduction that was not statistically significant. Further study is required on this aspect.

Only seedling infection caused a significant reduction in 1000-grain weight for cultivars Centinela, Cerro Prieto, and Atlas 57 (Table 2). Again, Atlas 68 was not affected. We also detected a significant reduction in 1000-grain weight in the naturally infected check Cerro Prieto.

Plant height was also significantly reduced for the seedling inoculation in the cultivars Centinela, Cerro Prieto, and Atlas 57. The number of seeds per spike showed no significant difference for any of the treatments.

Table 1. Effect of barley yellow dwarf virus on yield of four barley cultivars

Cultivar	Inoculated as seedling	Inoculated at flowering	Natural infection	Sprayed with insecticide
		Yield (g/plot)		
Centinela	665.1**	884.3*	917.3	997.9
Cerro Prieto	827.4**	912.8*	867.4*	1070.9
Atlas 57	654.3**	905.1	926.2	999.5
Atlas 68	811.7	895.6	901.3	915.0

* Significant at 5% level.
** Significant at 1% level.

Table 2. Effect of barley yellow dwarf virus on grain weight of four barley cultivars

Cultivar	Inoculated as seedling	Inoculated at flowering	Natural infection	Sprayed with insecticide
		1000-grain weight (g)		
Centinela	36.0*	39.6	39.4	40.9
Cerro Prieto	36.2*	37.3	35.8*	38.4
Atlas 57	38.5*	42.8	41.6	42.4
Atlas 68	39.8	41.5	41.4	41.9

* Significant at the 5% level.

Conclusion

A PAV-like variant of BYDV found in Mexico, efficiently transmitted by *R. padi*. It can reduce barley yield, especially with seedling inoculation. This suggests that commercial losses may be greatest from early infection. The yield losses of Mexico's most widely grown barley cultivars Centinela and Cerro Prieto, under inoculation were large, 33% and 28%, respectively. This suggests the potential impact of BYD and the need to focus on incidence and losses in commercial crops.

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When Is a Cultivar Reaction to Barley Yellow Dwarf Virus Significant?

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Abstract

Potential sources of error in assessing the reaction of cereal cultivars to barley yellow dwarf virus infection—infection method, interaction with drought, fungi, or winter stress, virus isolate, aphid species and number, and presence of genetic variability—are discussed.

Information gathered from infecting cultivars with barley yellow dwarf virus (BYDV) is significant when the factors of variation (Table 1) are controlled. The significance of BYDV data can be interpreted by researchers with respect to these factors. We want to show here the attention given to the control of such error factors in our trials.

Virus Infection Methods

As plant growth stage at infection has a major effect, germination uniformity is even more necessary in BYDV trials than in conventional breeding (Table 2). In natural infections one has no control over plant growth stage, aphid numbers, or virus isolate. Artificial inoculation using known virus isolates and depositing 5 to 20 aphids per plant gives uniform, repeatable trials.

Table 1. Factors affecting the significance of results in BYDV testing of spring and winter cereals

For all cereals:

- Seeding date and germination uniformity
- Plant growth at inoculation time
- Virus strains or mixtures
- Uniform aphid distribution
- Drought severity
- BYDV and pathogenic fungi interaction
- Choice of an estimator of BYDV reaction
- Proper checks, resistant and susceptible

Specific to winter cereals:

- Winter cold, flooding, and ice encasement
- Number of freeze-thaw cycles
- Presence of snow molds
- Other uncontrolled factors

Interaction with Drought

Repeatability of BYDV reaction in our trials is generally good. In trials where resistant and susceptible lines are adequately represented, year-to-year correlations ranged from 0.64 to 0.98 over 1984-1986. However, in 1983 severe drought just after germination made resistance genes inefficient except in a few cultivars possessing combined drought resistance and BYDV resistance. Correlations between 1982 and 1983 data were all close to 0.10 and non-significant, as both BYDV and drought resistance were essential to performance in 1983.

Virus Isolates

Our study of four virus isolates (*Rhopalosiphum padi* (L.)-specific, *R. maidis* (Fitch)-specific, *Sitobion avenae*

Table 2. Grain yield of bread wheat (*T. aestivum*) inoculated with BYDV at different growth stages

	Days after seeding elapsed before BYDV inoculation[a]		
	23	31	43
	Kilos		
Karamu	2472	2328	1775
L19	1797	2528	2372
Glenlea[b]	1084	1615	1231

Source: La Pocatière 1979

[a] Heading occurred about 60 days after seeding.
[b] Susceptible cultivar

(Fabricius)-specific, and nonspecific showed evidence of cultivar x BYDV isolate interaction (Tables 3, 4), but nevertheless there were highly significant correlations between virus isolates. As cultivar-specific interactions exist, virus isolates must be properly identified.

Aphid Species and Numbers

Viral infection makes plants more sensitive to aphid damage. Aphid species and numbers may influence cultivar reaction in a severe natural epidemic.

Existence of Genetic Variability

No reliable year-to-year correlation on BYDV reaction can be obtained in species that do not contain a sufficient range of genetic variability for resistance. For example, all rye (*Secale cereale* L.) lines are resistant, and almost all durum (*Triticum turgidum* L. var. *durum*) lines are susceptible. Triticale (X *Triticosecale* Wittmack) and barley (*Hordeum vulgare* L.) have the broadest genetic variability for BYDV reaction, and in these species the year-to-year correlations of BYDV

Table 3. Effect of various BYDV isolates on the tolerance ratio (average yield of inoculated divided by average yield of noninoculated) for 6 oat (*Avena sativa* L.) cultivars

	Tolerance ratio Virus isolate				
Oat cultivar	Non-specific	*R. padi* specific	*R. maidis* specific	*S. avenae* specific	Average of 4 isolates
Dorval	15	2	52	41	28
FF 64-74	77	65	101	74	79
Garry	13	6	46	33	24
Hudson	19	26	5	39	22
Ill 71-1161	93	67	98	68	82
Jaycee	81	51	84	83	75

Source: Comeau and Gill, unpublished. Winnipeg 1976.

Table 4. Correlation between tolerance ratios obtained with 4 different virus isolates on 20 oat lines

Virus isolate[a]	*R. padi* specific	*R. maidis* specific	*S. avenae* specific
Non-specific	0.85 (0.001)	0.70 (0.0006)	0.73 (0.0002)
R. padi specific		0.65 (0.002)	0.82 (0.0001)
R. maidis specific			0.71 (0.0004)

Source: Comeau and Gill, unpublished 1976.

[a] The nonspecific strain was most severe, and the *R. maidis*-specific one was least severe.

( ) = Probability level.

reaction are the most significant. Bread wheat (*Triticum aestivum* L.) has intermediate genetic variability.

Interactions with Fungi

BYDV predisposes cereals to damage by septoria, scab, take-all, and other fungi. These interactions affect yield and grain quality.

Choice of an Estimator of BYDV Reaction

Classical symptoms are useful for oat and barley trials. In tests on wheat and triticale, less attention should be paid to leaf discoloration, which is often misleading. As BYDV affects tillering, height, and grain filling, biomass data and comparisons with checks are recommended.

Interactions with Winter Stress in Winter Cereals

BYDV testing of winter cereals is difficult in Quebec because BYDV x winter stress interactions are important. The virus increases snow mold damage and reduces resistance to cold, flooding, ice encasement, and freeze-thaw stress. The use of glasswool as artificial protection against winter stress in a plastic covered greenhouse allows us to eliminate winter damage effects from our BYDV trials.

Conclusions

1) Multilocation tests should depend on artificial inoculation with known virus isolates.

2) Natural infection data have complementary value if observations are supplied on uniformity of infection, aphid species, virus strains, and climatic conditions.

3) Susceptible and resistant checks must be used.

4) Proper estimators of resistance must be chosen after careful evaluation.

5) Interactions with diseases and winter stresses must be known and controlled.

6) The significance of BYDV data on cultivars should be interpreted by a team of experts.

Acknowledgment

We would like to thank the International Development Research Centre for their support of our work.

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