Barley Yellow Dwarf in West Asia and North Africa

Proceedings of a Workshop
Rabat, Morocco, 19-21 November 1989

A. Comeau
K.M. Makkouk

editors

International Center for Agricultural Research in the Dry Areas

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organized by the International Center for Agricultural Research in the Dry Areas (ICARDA)
and
International Development Research Centre (IDRC)
held in Rabat, Morocco, 19-21 November 1989

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Foreword

In recent years, widespread infections of cereals with barley yellow dwarf virus (BYDV) in North Africa have attracted some attention among research and extension workers. As work on cereal virus diseases in the region is fairly limited, the International Center for Agricultural Research in the Dry Areas (ICARDA), in collaboration with Agriculture Canada and Laval University and with the full support of the International Development Research Centre (IDRC), organized a workshop on BYDV in Rabat, Morocco, in November 1989 to bring together scientists from the West Asia and North Africa (WANA) region with those from other institutions worldwide. The main objective of the workshop was to discuss the latest research developments and how to make use of them in formulating future research on BYDV in the WANA region in general and in North Africa in particular, where BYDV is causing serious losses in cereal crops. Future collaboration between ICARDA, Agriculture Canada, Laval University, Chile and scientists of the national programs in North African countries was also discussed at the workshop.

The contributions of all scientists who presented their findings at the workshop are included in this volume. Collectively, the papers provide ample coverage of BYDV and the promising approaches which should be considered in efforts to limit the spread of the virus and minimize the losses it causes.

ICARDA greatly appreciates the assistance given by the IDRC in sponsoring the workshop and financing this publication. The proceedings represent a valuable contribution to the knowledge about BYDV in the WANA region and are indicative of the regional and international cooperation targeted towards solving a problem of economic importance to several countries of the region served by ICARDA.

NASRAT R. FADDA
Director General, ICARDA

Preface

Meetings dealing with barley yellow dwarf virus (BYDV) are still something of a novelty. This aphid-borne luteovirus affecting all cereal species was described as early as 1951, but only since enzyme-linked immunosorbent assay (ELISA) test kits became commercially available has BYDV received serious attention. The 1987 meeting on BYDV organized by the Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT) in Italy attracted over 100 scientists, indicating the interest generated by these recent developments. We are still breaking new ground in developing new methods, establishing traditions and increasing general awareness of the need for research on luteoviruses.

Notwithstanding the usual caution about generalizations, it must be said that although a number of cereal crop problems have been solved during the past 50 years, the BYDV problem has, if anything, worsened. The reasons for this are not always clear or well documented, even in North America. Work in South America, where the BYDV problem erupted quite suddenly during the 1970s, shows that a concerted research effort does pay dividends. Strong local commitment in Chile and Brazil supported plant breeding, crop management and biological control research and, within a decade, this resulted in the creation of new cultivars and the introduction of aphid parasites, leading to a reduction in yield losses. Successful BYDV management through plant breeding has also been demonstrated in California, USA with the development of some barley cultivars and in other North American regions with the development of oats cultivars.

The choice of Rabat, Morocco as the venue for this meeting was very appropriate, as this part of North Africa is one area where the BYDV problem is now known to have economic importance. We extend our sincere thanks to the Moroccan scientists and staff of the International Center for Agricultural Research in the Dry Areas (ICARDA) based in Morocco for making the local arrangements for the meeting and to the numerous scientists from many countries who accepted the challenge of bringing everyone up to date on the status of BYDV and on current research aimed at its control.

It is also fitting to mention the pioneer role of the International Development Research Centre (IDRC) in financially supporting the research upon which much BYDV control is based. This was done through the breeding of virus-tolerant cereals, for which the international project at Laval University, Canada shared responsibility for a decade with ICARDA and Chilean scientists. Additional work on aphid resistance was done in Egypt by ICARDA and Egyptian scientists, with European support. Many products of the research encouraged by the IDRC and ICARDA appear in the following pages.

In the past, BYDV was often confused with other stresses such as drought or poor soil fertility. Knowledge about BYDV must be spread throughout the agricultural community via

agricultural schools and modern information media. We hope that all of you will enjoy this workshop, and will gain some insight that will help you in your future agricultural work.

To ensure stability of the world's cereal supply, we fully support the IDRC's philosophy of encouraging methods of pest control that are inexpensive and have a low impact on farmers and the ecosystem. These methods often boil down to plant breeding and other forms of biological control. Granted that in emergency situations other methods must also be given consideration, the long-term environmental objective should always have high priority in agriculture. Despite all the uncertainties about future food needs, agriculturalists must be ready to meet demand without adversely affecting the very resources that produce the food — soil and water. A better understanding of this basic principle is the key to our collective future.

To conclude, we sincerely thank the participants and the many institutions that provided financial support, particularly the IDRC which covered most of the costs of the meeting and the publication of the proceedings.

ANDRE COMEAU and KHALED MAKKOUK

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Introduction

Barley Yellow Dwarf Virus Epidemiology: A Study in Ecological Complexity

M.E. IRWIN and J.M. THRESH

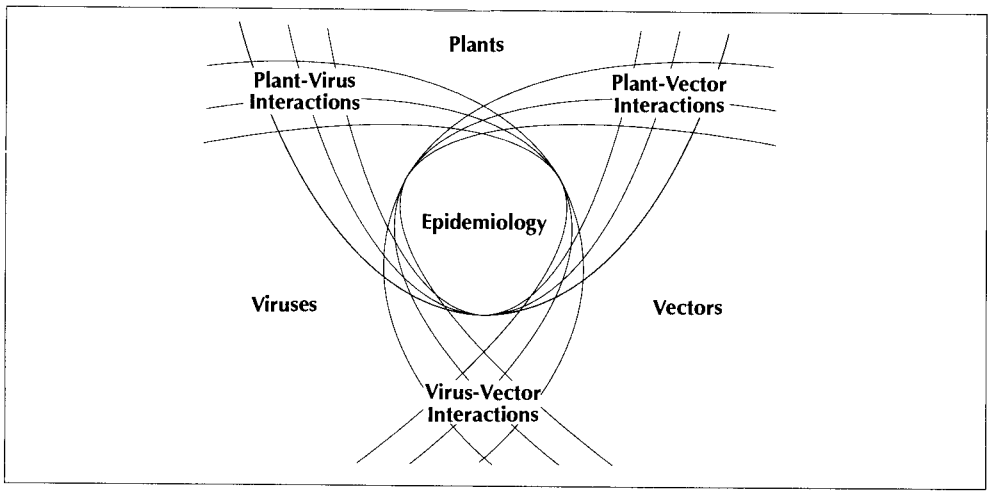
SUMMARY

Plant virus epidemics are induced and sustained through ecological associations linking environments with host plants, viruses and vectors. These associations are enhanced through the specific interactions between host plants and viruses, viruses and their vectors, and vectors and their host plants. When each of these interactive elements is, in itself, multifaceted, ecological complexity is greatly increased. This is the case with barley yellow dwarf virus (BYDV), an extraordinarily complex pathosystem involving several luteoviruses and luteovirus strains acting singly and in combination. Worldwide, over 100 species of the family Gramineae are natural hosts of BYDV, and at least 23 aphid species are known vectors of one or more strains of the virus. Past reviews have tended to emphasize the interactions involving the virus and its host plants and the virus and its vectors. This paper focuses on the complexities resulting from vector interactions with their environment and with the natural host plants of the viruses and the vectors. Strong emphasis is placed on the role of vector refuges and vector movement in BYDV epidemics.

Most pathosystems encompass interwoven biological relationships between a pathogen and its hosts in a shared environment. If additional elements such as the vectors of the pathogen are involved, as is the case with many plant virus systems, the complexity of the pathosystem increases substantially. This is further complicated when the virus is widespread in several crop and perennial plant species and has several distinct variants, or the variants are spread selectively by several vector species. All these elements, when interacting concurrently, create exceptionally complex ecological pathosystems. The disease complex known as barley yellow dwarf epitomizes such a system.

Investigating virus variants, their host plant and their vectors, and elucidating the interactions between these elements in diverse and fluctuating environments is an extremely difficult task, but it is one that must be undertaken in order to improve our understanding of the pathosystem. This requires a major multidisciplinary effort. The team should include not only researchers with expertise in plant/virus, virus/vector and plant/vector interactions (*see* Figure 1 *overleaf*), but also those who understand physical environments and their influence on biological processes and those who are able to analyze, interpret and model these associations. Through such multidisciplinary efforts, the scientific principles that govern barley yellow

Figure 1 **Conceptual diagram of the component parts and disciplines involved in the study of BYDV epidemiology**



dwarf virus (BYDV) epidemics can be established and placed in appropriate ecological and economic contexts in order to facilitate the forecasting and management of local and regional epidemics.

The literature on BYDV and its vector species is extensive — possibly greater than the literature for any other plant virus pathosystem — and contains many conflicting views. Much of the known data concerning the ecology of BYDV are widely quoted and have been discussed in previous reviews (e.g. Burnett, 1990). This paper is not intended to be a comprehensive re-evaluation of BYDV epidemiology. Rather, it focuses on some of the more controversial issues and attempts to put many of the known facts into an epidemiological context. Because we believe that vector movement has been inadequately studied and has not been incorporated into the foundation of BYDV epidemiology, emphasis is given here to the role of vectors.

COMPONENTS OF THE BYDV PATHOSYSTEM

Barley yellow dwarf is the most widespread and economically important virus disease of cereals worldwide (Plumb, 1983). It affects over 100 species in the family Gramineae, including barley, wheat, oats, sorghum, rye, triticale, maize, rice and many wild grasses (Slykhuis et al., 1967). Wild annual and perennial grasses, graminaceous weeds and volunteer cereals play an important role in the epidemiology of BYDV, serving as hosts and thus reservoirs of the virus complex.

To understand the epidemiology of BYDV, it is imperative that we understand the biotic components involved. The BYDV pathosystem comprises three biotic components: the luteoviruses that form the BYDV complex; the various aphid species that carry BYDV; and the grasses and other plant species that are hosts of both the luteoviruses and their vectors.

BYDV variants, their vectors and their host plants

The disease is caused by a range of luteoviruses, only some of which are closely related serologically and share common aphid vectors (Plumb, 1983). The various isolates are grouped into strains on the basis of their transmissibility by over 20 species of aphids in a circulative, persistent manner. Once the virus is acquired, the vector is potentially infective for life; however, BYDV is inefficiently transmitted when vector inoculation access periods are relatively short (less than 24 hours). Thus, BYDV epidemics in cereals are almost exclusively attributable to aphid species that colonize the plants (that is, feed for a considerable length of time, usually become established, and reproduce), rather than to itinerants that pass through and simply probe the plants while in transit.

Based upon the principal aphid species transmitting different isolates of BYDV, Rochow (1970) characterized and designated five strains found in New York State, USA. He gave each strain an acronym derived from the initial letters of its main vector species:

- MAV — transmitted specifically by *Sitobion avenae* (Fabr.), previously placed in the genus *Macrosiphum*;
- RPV — transmitted specifically by *Rhopalosiphum padi* (L.);
- RMV — transmitted specifically by *R. maidis* (Fitch.);
- SGV — transmitted specifically by *Schizaphis graminum* (Rond.);
- PAV — transmitted non-specifically by *R. padi* (an efficient vector) and *S. avenae* (a less efficient vector).

These strain designations have been adopted almost universally. However, because isolates of the strains vary, it has become common to use the suffix '-like' (for example, 'RPV-like' in the case of an isolate that fits the RPV designation but has not been fully characterized). A well-characterized isolate is usually designated according to locality (for example, 'RPV-IL' for a specific isolate of RPV from the state of Illinois, USA). In this paper, we also use the term 'variant' as a modification of some strain designations (for example, 'RPV variant' for an isolate that most closely resembles the RPV strain but has not been well-characterized and is not among the isolates upon which Rochow based the original designation).

The major vectors that colonize cereals have dissimilar biologies (Blackman and Eastop, 1985). *S. avenae* is monoecious and holocyclic, developing entirely on grasses. *R. padi* and *Metopolophium dirhodum* (Walker) are heteroecious and holocyclic, colonizing cereals and other graminaceous species as secondary hosts after overwintering on their primary woody hosts, *Prunus padus* and *Rosa* spp., respectively; in warmer climates, however, *M. dirhodum* can overwinter anholocyclically (Blackman and Eastop, 1985). *S. graminum* infests barley, wheat, sorghum, maize and many grass species, and reproduces anholocyclically in North America, where it overwinters mainly in the southern states of the USA (Irwin and Thresh, 1988); however, in parts of northern Europe and perhaps even North America it can overwinter holocyclically on graminaceous hosts (Blackman and Eastop, 1985). *R. maidis* is entirely anholocyclic, developing on a wide range of grasses, including barley, maize and sorghum, but

in central North America it does not overwinter north of southern Illinois and Arkansas (Voegtlin et al., 1987).

Although most isolates of BYDV infect many graminaceous species, there is evidence that some of them are adapted to particular host plants. Rochow et al. (1965) and Rochow (1984) noted that isolates transmitted non-specifically cause more severe symptoms than isolates transmitted specifically. Baltenberger et al. (1987) observed that a cultivar responded differently to an RPV variant and a PAV variant. It is also clear that mixed infections abound in the field, suggesting that cross protection is not an important factor limiting superfection. A survey in Pennsylvania, USA by Gildow et al. (1987) found that 16% of the BYDV-infected plants contained more than one strain of BYDV, and Baltenberger et al. (1987) concluded that dual infection by RPV and PAV variants caused more severe symptoms than either one alone. Comeau (pers. comm.) observed that when more than one strain of BYDV was spreading through fields, a greater proportion of the plants contained mixed infections during severe epidemics than during mild ones. This could have important economic, as well as epidemiological, implications.

In susceptible plants, the systemic movement of BYDV occurs 1-3 days after infection, depending on the length of the inoculation access period (Gill, 1968; Carrigan et al., 1983). Plant species, cultivar, temperature and, possibly, the BYDV variant involved all affect the rate of systemic movement; the rates are greater in susceptible cultivars than in more tolerant ones (Jensen, 1973).

Temporal changes in virus concentration during the course of infection may vary with BYDV strain. Skaria et al. (1984) found that, in pairs cultivars of wheat, barley and oats, concentrations of PAV-P antigen over a 30-day period differed slightly according to plant species but invariably reached peak titer 12 days after inoculation. As indirect evidence of the differences in virus concentration between BYDV isolates, Gill (1969a, 1969b) found that aphid transmission of a MAV variant in oats fluctuated cyclically over a 38-day period, whereas transmission of a non-specific SGV variant in oats over a 33-day period reached a single peak 9-14 days after inoculation.

Plant age at the time of virus inoculation affects the likelihood and course of infection (Swenson, 1963). For example, Eweida et al. (1988) observed that virus antigen concentrations in oats which had been inoculated at the 1- to 2-leaf stage with a severe PAV variant reached maximum levels in the roots after 7-8 days, and the concentrations were 3-4 times greater than in the leaves. Concentrations in similar oat plants inoculated at the 4- to 5-leaf stage reached maximum levels in the roots after 10 days and in the leaves after 18 days, but the concentration in the leaves was 2-5 five times greater than in the roots.

Time of infection relative to plant phenology has economic implications in that it restricts the time available for an epidemic to develop. Gildow and Frank (1988) confirmed that early infections of oats with a PAV variant led to greater yield reductions than slightly later infections. However, time of infection appears to be governed more by the timing of vector activity and date of sowing than by virus/plant interactions.

Changes in infectious virus concentrations are likely to influence epidemics. Infectious virus titer could certainly alter the probability of a vector acquiring and then transmitting a virus isolate. The importance of this factor in the progress of disease compared with the many factors involved, however, is presumably quite small. This is because, as long as a minimum titer is

present and the vector feeds for an adequate length of time, the probability of successful acquisition and transmission of that isolate is high, particularly with efficient vectors (Hewings and Eastman, pers. comm.). Thus, feeding, movement and other vector-related behavioral activities appear to be of greater importance in generating epidemics than do the intricate virus/host interrelationships.

Luteoviruses have a high degree of vector specificity. The data suggest that virus-recognizing receptors located on the cell membranes of the salivary glands may determine which luteoviruses can be transmitted by which aphid species. Because of the intimate association of luteoviruses with aphid tissues, these viruses are totally dependent on aphid behavior for their survival and spread (Gildow, 1990).

The status of BYDV strain designation

Based on serology, cytopathology (Gill and Chong, 1979) and dsRNA 'fingerprints' (Gildow et al., 1983), BYDV strains can be separated into two groups: Group 1 — PAV, MAV and SGV variants; and Group 2 — RPV and RMV variants. Because of the chemical and genetic integrity of the two groups, formal designation as distinct luteoviruses appears valid and fundamental to future investigations.

Other than these two distinct luteoviruses, the validity of the current classification of strains based on vector specificity is equivocal. Apart from the four aphid species mentioned above, at least 19 additional species can transmit one or more isolates of BYDV (A'Brook, 1981). When more vector species are tested against the innumerable BYDV isolates around the world, what will be the impact on the current classification? We postulate that as new isolates are characterized, their positions in the current classification will become ambiguous, and the separation of strains may require restructuring.

Already, the strain designations now used seem to be breaking down. The type isolate of PAV, described from New York State, is not transmitted by *R. maidis* (Rochow, 1970), while variants of the same strain in parts of Europe and the Mediterranean do appear to be transmitted by *R. maidis* (Makkouk et al., 1990), although perhaps by different genotypes. The type isolate of RPV, also described from New York State, is transmitted specifically by *R. padi* (Rochow, 1970), while a variant strain from California was found recently to be transmitted non-specifically by two additional aphid species, *S. avenae* and *S. graminum* (Creamer and Falk, 1989). Not only is the classification of BYDV breaking down because of specific variant interactions with specific vector species, it is also apparently being altered when variants of different strains occur together. One variant of a vector-specific strain can be transmitted by an additional aphid species if an appropriate variant of a companion strain is also present in the host plant, a phenomenon caused by genomic masking or perhaps phenotypic mixing. Thus, *R. padi* transmits most variants of RPV specifically and PAV non-specifically, but it can transmit variants of RMV, MAV and SGV in the presence of RPV variants and, occasionally, PAV variants. Several such examples have been reported (Rochow, 1982; Rochow et al., 1987). Indeed, many isolates may have evolved quite recently and not yet diverged much. This makes placing some of the variants in discrete strain groups extremely difficult and currently inappropriate.

BYDV detection in the field

BYDV is restricted to phloem tissues and, overall, it occurs in very low concentrations in plants. Virus symptoms are often difficult to detect in the field. For example, a survey of grasses in Scotland, UK established the prevalence of symptomless BYDV infections of PAV, RPV and MAV variants in ryegrass (Holmes, 1989).

The use of enzyme-linked immunosorbent assay (ELISA) is an efficient means of detecting the virus and has been important in confirming the presence and abundance not only of BYDV but also of its various designated strains. ELISA, using polyclonal antisera, is currently the preferred technique (Lister and Rochow, 1979), although it is fairly costly and labor intensive (it is inexpensive once the procedure is established). Recent work on the production of monoclonal antibodies and cDNA probes of selected BYDV strains has led to the development of more sensitive methods of detection (Miller et al., 1988a, 1988b; de Pace et al., 1990), but these methods are even more labor intensive and costly. Accurate, timely and cost-effective identification and characterization of the viruses and virus variants involved in the BYDV pathosystem are fundamental to the understanding and study of epidemics on both local and regional scales.

COMPLEXITIES OF THE BYDV PATHOSYSTEM

Understanding how the biotic components of the BYDV pathosystem interact is difficult because of the intricate and multifaceted nature of the associations. This is particularly so when the environment is treated as a series of factors that govern how and at what rate these components interact.

Effect of BYDV on plant biology

BYDV greatly influences the growth and metabolism of its host plants. Depending on the particular strain and its virulence, infection may contribute to winter-kill in cold, temperate regions; induce plant stunting; inhibit root growth; reduce or prevent flower production; or increase host susceptibility to opportunistic pathogens, drought and other stresses (Burnett, 1984).

The production of autumn-sown cereals in temperate climates is severely affected by winter stresses that interact with BYDV. Most winter cereals are more resistant to BYDV than are those sown in the spring, but the presence of BYDV contributes substantially to winter-kill (Comeau and Jedlinski, 1990). Under controlled environmental conditions, Paliwal and Andrews (1990) found that the effect of BYDV infection on plant tolerance of low temperatures was most severe in oats and barley, less so in wheat, and negligible in rye, although rye can sustain high virus concentrations. It may be significant that infected ryegrass produces a greater number of tillers and a higher ratio of vegetative to fertile tillers than healthy plants (Catherall, 1966). BYDV causes a rise in the critical threshold temperature at which 50% of the plants are killed at 4-8°C in barley and 2-4°C in wheat. This is extremely important considering that a

change of only 0.5°C can significantly affect the long-term survival of these crops (Paliwal and Andrews, 1990). BYDV infection reduces wheat cold-hardiness by about 3.5°C. It also reduces ice tolerance during early low-temperature growth but increases it after 4 months at low temperatures (Andrews and Paliwal, 1983).

Delserone et al. (1987) found that feeding by non-infective aphids on a winter barley cultivar (Pennrad) neither reduced top and root growth nor increased crown injury nearly as much as feeding by infective aphids. This is also true of wheat and oats, leading Comeau (pers. comm.) to postulate that in eastern Canada non-infective aphids cause relatively little damage to cereals. Because BYDV reduces root growth more than shoot growth, its debilitating effects may not be obvious (Catherall and Parry, 1987). During drought-ridden summers and without irrigation, plants infected with BYDV may not acquire sufficient water and nutrients to sustain growth and yield because of the impaired root structure. Thus BYDV infection can have disastrous consequences on cereal production in drought years.

Overall plant 'fitness' can also be affected by the interaction of BYDV and other pathogens. Sward and Kollmorgen (1986) and Sward (1990) found that BYDV and take-all fungus (*Gaeumannomyces graminus* var. *tritici*) each reduced grain yield and increased the number of 'deadheads' in wheat in Australia; the combined effect of BYDV and take-all fungus appeared greater, however, than the individual effect of each pathogen. Similarly, Comeau and Pelletier (1976) found that the yield losses resulting from leaf blotch, caused by *Septoria avenae*, on BYDV-infected oats were twice those of oats suffering from leaf blotch alone, and they concluded that BYDV predisposes oat plants to damage by *S. avenae*. According to Price and Stubbs (1963), the ability of root pathogens to induce premature ripening is enhanced in BYDV-infected wheat plants, suggesting that BYDV predisposes wheat to root diseases.

Other evidence suggests that plant fitness is not always decreased by BYDV interacting with other pathogens. Although in some cases BYDV initially inhibits the expression of powdery mildew (*Erysiphe graminis*) Potter and Jones (1981), in a study comparing BYDV-infected with virus-free plants, concluded that the effect of powdery mildew was not ultimately different.

Effect of host plant/environment interaction on vector biology

Plant species and cultivar, and the location of specific feeding sites, influence vector fecundity (Leather and Dixon, 1981, 1982; Foster et al., 1988). In field and greenhouse tests, the number of *R. padi* alatae has been correlated positively with plant size and density (Ahman et al., 1985). Plant growth stage also has a significant effect on aphid fecundity. Kieckhefer and Gellner (1988) tested *S. graminum*, *S. avenae*, *R. padi* and *R. maidis* under growth chamber conditions for fecundity on several hosts at differing growth stages. They reported that both *R. padi* and *S. graminum* had higher rates of reproduction on headed spring wheat than at earlier stages of growth, and that *R. padi* and *R. maidis* colonized older maize plants rather than the seedling stages, probably because of the initial protective effect of Dimboa (2,4-dihydroxy-7-methoxy-1,4-benzoxazine-3-one) (Kogan, 1975). Moreover, *S. graminum*, *R. padi* and *R. maidis* preferred young sorghum to older stages; the growth stage of barley affected the fecundity of *R. maidis* but not of *R. padi*, *S. avenae* or *S. graminum*; and no differences in the fecundity of

S. graminum, *R. padi* or *S. avenae* were found between aphids placed on vernalized and non-vernalized winter wheat. Watt (1979) and Walters and Dixon (1982) found that *S. avenae* preferred young heading stages of wheat to ripening grain.

In Australia, climatic factors affect the distribution of *R. maidis*, *Sitobion* spp., *M. dirhodum*, *R. padi* and *R. rufiabdominalis* directly by circumscribing the physical habitat, and indirectly by influencing the composition of the local flora. Johnstone et al. (1990) suggested that different grass species vary in their susceptibility to BYDV variants and in the likelihood of being colonized by different vector species. They also observed that BYDV variants that are vector specific tend to be common in regions where one vector species predominates, while variants that are not vector specific appear to be most common, together with mixed infections, in areas where more than one vector species is prevalent.

Effect of BYDV on vector biology

Luteoviruses affect aphid biology in several ways, including feeding efficiency, morphology, reproduction and the production of alates (Gildow, 1990). Miller and Coon (1964) found that viruliferous aphids had an increased developmental rate, longevity and reproduction period, and produced more progeny and consumed 13.8% less oxygen than non-viruliferous aphids. Araya and Foster (1987) showed that longevity decreased when *R. padi* fed on BYDV-infected, rather than uninfected, wheat; however, total reproductive capacity appeared to increase when *R. padi* fed on virus-infected wheat but not when it fed on virus-infected oats. In studies involving the use of an electronic monitoring sytem, Montllor and Gildow (1986) found that *S. graminum* fed better when oats were infected with an RPV variant of BYDV, although *R. padi* seemed to feed equally well on infected and uninfected oats. According to Fereres et al. (1989), *S. avenae* had a shorter development time, greater fecundity and a faster intrinsic rate of natural increase when feeding on BYDV-infected wheat than when feeding on uninfected plants of the same cultivar. These studies suggest a mutual interaction between BYDV and its vectors.

Gildow (1980) showed that a consistently higher percentage of winged progeny was produced on oats infected with BYDV compared with uninfected plants, regardless of aphid species, morphology of parent aphid or the BYDV isolate used. The final adult morph of an aphid was regulated by placing the first instar nymphs on BYDV-infected plants for a short time. When *S. avenae* and *R. padi* were reared on BYDV-infected plants, a far higher percentage of the eclosing adults were alatae than was the case with similar rearings on uninfected plants; this proved to be the case for field-collected aphids as well as those from laboratory colonies. Later, Montllor and Gildow (1986) observed that although the proportion of *R. padi* that developed into alatae on BYDV-infected oats was greater than on healthy oats, *S. graminum* showed no such response. Studies conducted by Ajayi (1986) showed that BYDV infection inreased the total amino acid content of leaves at three growth stages of spring wheat; the effect was greatest in the earlier stages, but alanine and glutamine were always more abundant in infected leaves than in healthy ones. Senescing oat leaves also appear to induce alate production, leading Gildow (1980) to postulate that changed nitrogen metabolism, resulting in increased amino acid concentrations in diseased or senescing plants, could trigger alate production. This shift in winged-morph production suggests that aphids from BYDV-infected

plants are more prone to disperse (Gildow, 1983), enhancing the potential increase in the rate of disease progress.

Coon (1959) demonstrated that an increase in amino acid concentration accelerates alate production in *R. insertum* on oats supplied with nitrogen fertilizer and also significantly increases progeny production. Markkula and Laurema (1964) showed that the reproduction of *R. padi* increases with the greater concentrations of free amino acids associated with BYDV-infection in oats, but that reproduction is unaffected for *S. avenae* and *M. dirhodum*. They argued, therefore, that changes in free amino acids alone cannot explain all the changes in aphid reproduction.

Cereal cultivars, bred for their high-yielding capacity, require high levels of fertilizer input. Because added nitrogen often increases aphid fecundity and alate production, it might well prove to be a key component in the explosive increase throughout the world in populations of cereal aphids and, hence, in BYDV incidence (Baranyovits, 1973).

Evolution of BYDV strains

Because most BYDV variants infect many graminaceous species, relationships involving BYDV and individual host species do not appear to be significant factors in the evolution of this pathosystem. The abundance and activities of the predominant vector species, in particular, seem to be of far greater importance. Indeed, the selection of BYDV isolates and their evolution towards dominance locally or regionally may well be dependent upon the types, abundance and activity patterns of vector species that occur in that area or that regularly migrate there from elsewhere, especially when considered in relation to the timing and scale of local cropping patterns. Thus, it appears that the vectors determine the rates and direction of the evolution taking place within the BYDV complex on micro- and perhaps macro-regional scales.

UNDERSTANDING THE FACTORS THAT DRIVE BYDV EPIDEMICS

A number of key issues need to be better understood if BYDV epidemics are to be managed. Three issues of crucial importance are the primary inoculum sources, how BYDV variants and their vectors survive unfavorable periods, and how the vectors move and disseminate the virus.

Primary inoculum sources

BYDV is neither seed nor mechanically transmitted. Although infective aphids can retain the ability to transmit the virus after moulting and throughout their lifespans, there is no 'vertical' transmission to the progeny. The current view is that aphids carry the virus into a newly sown field from some other host plant of the same or a different species that harbors the virus. Distinct variants, in fact, could be carried by vectors to a field from different sources. Therefore, an epidemic must begin by spreading from one or more virus reservoirs after a crop is sown. The primary inoculum sources may be local, regional or distant (Irwin and Thresh, 1988). It is important to realize that a plant, whether it is wild or not, may harbor the virus without

contributing to further spread. For spread to occur, a vector must move the virus from the reservoir to other hosts.

Surveys conducted in parts of the USA and Europe indicate that overwintering reservoirs of BYDV in wild grasses near cereal fields do not seem to contribute substantially to BYDV epidemics in adjacent crops. This is inferred because the predominant strain variant constituting the epidemic in the cereal crop often differs from that constituting the majority of infections in the wild, perennial grasses nearby. For example, grasses are a perennial source of BYDV in England, but the isolates from cereals often differ in their geographical distribution from those of grasses (Plumb, 1977). In studies conducted in Indiana, USA by Fargette et al. (1982), up to 50% of grasses surveyed contained PAV, MAV, RPV or some combination of these strain variants, whereas nearby cereals contained a preponderance of only PAV variants. This suggests that nearby wild grasses may not be the most important source of the virus attacking cereals in this region. In Canada, overwintering *R. padi* emerging from *P. padus* trees in the spring were not viruliferous until they had fed on infected plants (Slykhuis et al., 1967). Thus, immigrating *R. padi* derived from eggs cannot initiate epidemics unless they first spend some time feeding on BYDV-infected plants.

PAV variants commonly infect wild graminaceous plants in Spain (Jorda et al., 1990). However, contrary to the situation in Indiana, autumn and winter BYDV infections in cereals in Spain were found to be predominantly RPV variants (Moriones et al., 1989). In a survey of several countries in West Asia and North Africa, PAV variants were found to be the most prevalent during the 1985-86 season, although vector-specific assays showed that RMV and RPV variants also occurred in the region (Makkouk et al., 1990). These facts strongly caution against assuming that local reservoirs invariably serve as primary inoculum sources for epidemics in nearby cereal crops.

Vector biology, operating in the context of the environment, is the overriding factor determining the effectiveness of primary inoculum sources. Plumb (1977) suggested that aphid biology, weather and host availability determine which BYDV isolates spread from grasses to cereals and when this spread occurs.

The temporal juxtaposition of crop phenology and patterns of aphid activity also influence which of the virus reservoirs serve as the primary inoculum sources. For instance, *S. avenae* tends to be associated with the first BYDV infections of spring-sown cereals in Canada because *R. padi* populations decline before these crops are planted (Slykhuis et al., 1967). McGrath et al. (1987) and McGrath and Bale (1989) also implicated *S. avenae* as the primary vector of BYDV in winter barley in northern England. Thus, in this case, BYDV was carried from reservoirs to the crops by *S. avenae* and not by *R. padi*.

The temporal gap

In most areas where host plants of BYDV are grown, some climatic or other limitation prohibits continuous cropping and, hence, the continuous spread of the virus. The limitations can be very cold winters in temperate regions or desolate, dry seasons in semi-tropical ones, both of which can restrict the survival of viable, virus-infected host plants and, consequently, the virus variants and aphid vectors. Any climatic regime that tends to break the cropping sequence with a wide temporal gap can impose rigorous barriers to the continuity of BYDV epidemics.

In areas with harsh winters, winter and early spring cereals are usually sown in the autumn. This allows autumn migrants to introduce the virus to young crops before conditions deteriorate and growth is interrupted. The plants resume growth in the spring and serve as excellent reservoirs to initiate spring epidemics. A high incidence of BYDV in overwintering wheat and rye, for instance, indicated that winter cereal reservoirs of BYDV are common in Canada (Slykhuis et al., 1967). In 1982 and 1983, winter wheat and barley were found to be heavily infected, predominantly with a PAV variant, but RPV and RMV variants also occurred (Paliwal and Comeau, 1984). Mild winters in temperate zones enable some vectors, as well as BYDV, to overseason in cereal crops. In southern England, where winters are usually mild, *R. padi* overwinters anholocyclically in great abundance on graminaceous plants, but *R. insertum* and *R. maidis* do not appear to do so (Hand, 1989). According to Milinko and Nagy (1990), a mild winter often allows vectors to persist in cereal crops in central Europe, resulting in severe BYDV epidemics in the spring.

In the subtropical, dry climates of the Mediterranean region the summer drought appears to be the most important barrier to the carryover of BYDV and its vectors between successive cereal crops (Plumb, 1990). Wild grasses are known to play an important role in the overseasoning ecology of BYDV in many parts of the world where epidemiology studies have been conducted. In rainfed areas of West Asia and North Africa, wild grasses and graminaceous crops that survive the hot, dry summers are few and are probably not significant primary inoculum sources of BYDV epidemics in autumn-sown cereals. Perennial wild grasses in the moister, cooler highlands may serve as primary inoculum sources during the summer drought in the lowlands, but this needs to be substantiated through rigorous experimentation.

Major bridging crops such as maize also appear to play an important role in the carryover of BYDV through the summer drought in the Mediterranean region (Plumb, 1990). In irrigated areas of Italy, maize, one of the crops most frequently colonized in summer by *R. padi*, appears to play a decisive role as a virus inoculum source; Coceano and Peressini (1989) found that about 9% of the aphids colonizing the crop were infective with BYDV. Similarly, Refatti et al. (1990) found that 0.5-7.0% of the apterous *R. padi* randomly collected from maize in five localities in northern Italy transmitted BYDV. In the laboratory experiments reported by Osler et al. (1985), *R. padi* readily transmitted a PAV variant from maize to maize and from maize to oats. Drawing on these results and on their own findings, Coceano and Peressini (1989) suggested that the movement of *R. padi* from maize to barley or wheat could have a great influence on BYDV epidemics in those cereal crops.

Knowledge of the role of maize and sorghum in the ecology of BYDV in West Asia and North Africa is lacking. However, Makkouk et al. (1990) have suggested that maize, found to be infected by a PAV variant in Syria and Tunisia, and sorghum, found to be infected with BYDV in Tunisia, may be summer hosts. Thus, there is mounting evidence to implicate locally grown irrigated maize and sorghum as primary inoculum sources for autumn-sown crops of winter cereals.

The importance of irrigated maize as a bridging host is not confined to drier regions. In a survey of winter wheat in Washington State, USA, 20% of the samples were infected with BYDV, and irrigated maize that supported aphid vectors in the summer, along with maize planted in early autumn, had the highest BYDV incidence (Wyatt et al., 1988). In the eastern part of the state, winter grain crops become infected with BYDV soon after seedlings emerge

in the autumn. Brown et al. (1984) identified irrigated maize as a reservoir of both BYDV and its aphid vectors during the period between summer harvest and autumn sowing of winter cereals; BYDV occurred in 58-65% of the maize fields surveyed, and in all maize cultivars, hybrids and lines tested. The isolates of BYDV from eastern Washington maize appeared to be PAV variants (Brown et al., 1984), whereas RMV variants predominated in maize during the 1981 BYDV epidemic in eastern Canada (Paliwal, 1982). In eastern Washington State, infective *S. avenae* occurred in maize fields in June and July, while *R. padi* heavily infested maize in July, August and September, and over 60% of the individuals were infective (Brown et al., 1984).

Halbert et al. (1990) have cautioned that the ability to predict BYDV epidemics would depend upon the ability to measure vector flight intensity and primary inoculum pressure, reasserting Kennedy's (1950) dictum that vector activity in a crop is far more important than sheer numbers. They reported that the percentage transmission by *R. padi* collected from small grain cereals was similar to that measured for this aphid from maize, and that the rate of transmission by *R. padi* from suction traps was higher than that by aphids collected from either crop. They concluded that a measure of the inoculum reservoir in maize might be a good predictor of primary inoculum in cereals in irrigated areas of the Pacific Northwest, USA.

Recent evidence suggests that although *R. padi* may play a prominent role in transmitting BYDV from maize to cereal crops in the Pacific Northwest, *R. maidis* may not. Blackman et al. (1990) found that samples of *R. maidis* from maize in Idaho, USA, were all 2n = 8 karyotypes, whereas those from barley and barnyard grass (*Echinochloa crus-galli*) were all 2n = 10 and those from wheat were mainly 2n = 10, with some samples being 2n = 9 or 2n = 8. As these karyotypes of *R. maidis* seem to discriminate between crops, Blackman et al. (1990) suggested that it was unlikely that the maize karyotype would transmit BYDV to cereals or that the cereal karyotypes would transmit the virus to maize. They postulated that barnyard grass may be a more important primary source than maize of BYDV isolates carried by *R. maidis* to cereals in the Pacific Northwest.

The role of karyotypes of *R. maidis* in the Pacific Northwest may not occur in all geographical regions. Makkouk et al. (1990) found that approximately 30% of the *R. maidis* aphids tested under laboratory conditions in Syria could transmit BYDV, a far greater number than the 2.4% infection rate reported in similar North American tests, although four distinct 'biotypes' of *R. maidis* differed in their abilities to transmit a single isolate of BYDV (Saksena et al., 1964). Therefore, *R. maidis* may play a prominent role in BYDV epidemics in West Asia and North Africa where maize precedes and follows cereal crops (Makkouk et al., 1990), while in North America this species may be insignificant. This hypothesis must be tested by determining the karyotypes of *R. maidis* on crops in the Mediterranean region and then by following the movement of selected populations to determine whether they disperse and thus carry virus between maize and cereals.

Vector movement

The movement of vectors is responsible for BYDV epidemics, influences spatial and temporal patterns of infection in fields and determines which fields become infected. How aphids

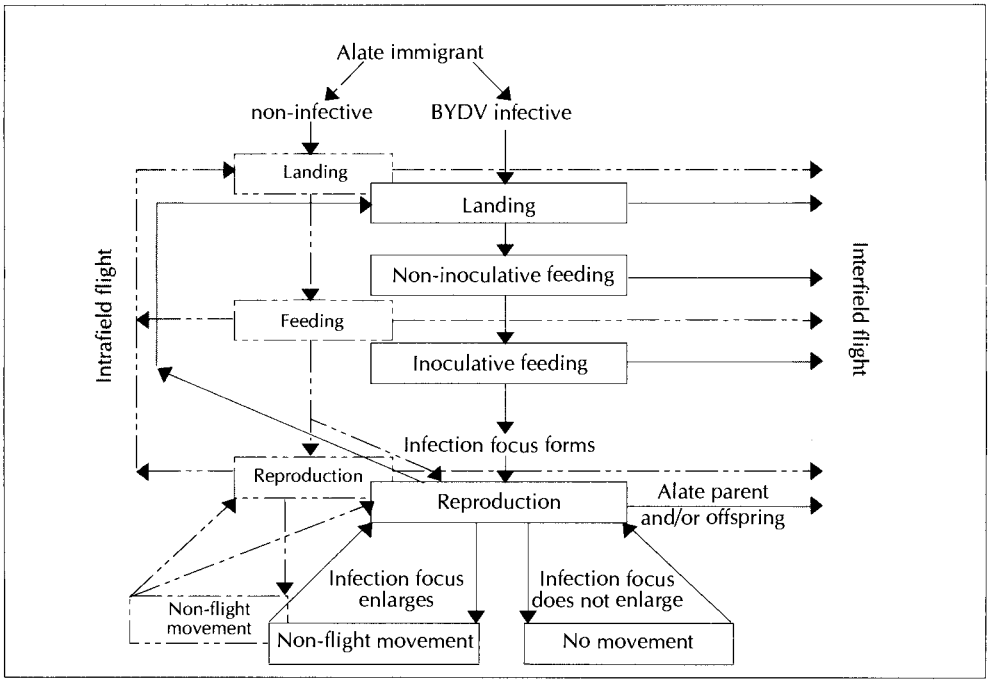
respond to environmental disturbances and physiologically induced cues regulates, to a large extent, how, when and how far they move. This is a crucially important and yet often neglected aspect of BYDV epidemiology.

An alate, BYDV-infective vector landing on a susceptible crop plant can, if it feeds for long enough, infect the plant and so initiate a new virus focus. If it reproduces, its apterous offspring can walk short distances to neighboring plants, transmitting the virus and thus enlarging the focus. At any time during this sequence, infective alates can fly to other plants in the field, initiating new foci, or fly out of the field to initiate new foci in neighboring or more distant fields, depending on the alates' physiological status in relation to flight activity and on the prevailing meteorological conditions (*see* Figure 2). These four modes of virus spread — enlarging existing foci and developing new foci in the same field, in nearby fields or in distant fields — must be clearly distinguished because each leads to a different pattern of spread and requires a different management strategy.

The four modes of virus spread generally correspond with types of vector movement: walking, short or moderately long host-seeking flights, and long-distance migratory flights. However, vector movement as such may not lead to virus spread because a vector may not be infective or may not land, acquire or transmit the virus. Thus, a vector might migrate far, but spread BYDV only locally.

Aphids present in a field can acquire BYDV in four ways: if they are born and develop on an infected plant; if they walk onto and colonize an infected plant and establish on it; if an

Figure 2 Flow diagram of BYDV spread including the inter- and intrafield components of epidemics



infective aphid transmits the virus to the plant on which they occur; or if they fly to and establish (or at least feed for a considerable length of time) on an infected plant. Thus, identical aphid colonies can be in close proximity, one producing offspring that, because of the plant on which they are born, become viruliferous, the other producing offspring that are likely to remain non-viruliferous. Only when the virus incidence in the field becomes high and when there is substantial movement of aphids is it likely that an aphid not born on an infected plant will become viruliferous. Moreover, a viruliferous aphid is not necessarily infective. To become infective, the virus isolate in the vector must be compatible with the genetic makeup of that aphid species. Therefore, an aphid enters the virus cycle only after overcoming many ecological, behavioral, physiological and genetic barriers. This implies that there are many points for intervention in the virus cycle.

Enlarging existing BYDV foci

The enlargement of BYDV foci may be caused by infective apterae or alatae walking between plants. It can also be caused by alatae flying from an infected plant to a nearby uninfected one although, from our observations, this option appears rather remote. In commercial cereal crops, plant densities are high and the flight distance between plants only 2 cm or less. Presumably, the energy required for an alate to take off and fly such distances is far greater than the energy needed to walk, particularly when the canopy is interconnected. Moreover, our observations suggest that when an aphid takes flight, it flies several centimeters before it responds to alighting stimuli. Thus, the minimal flight distance would take the alate aphid at least tens of centimeters away from the source plant, unless it chooses to circle back.

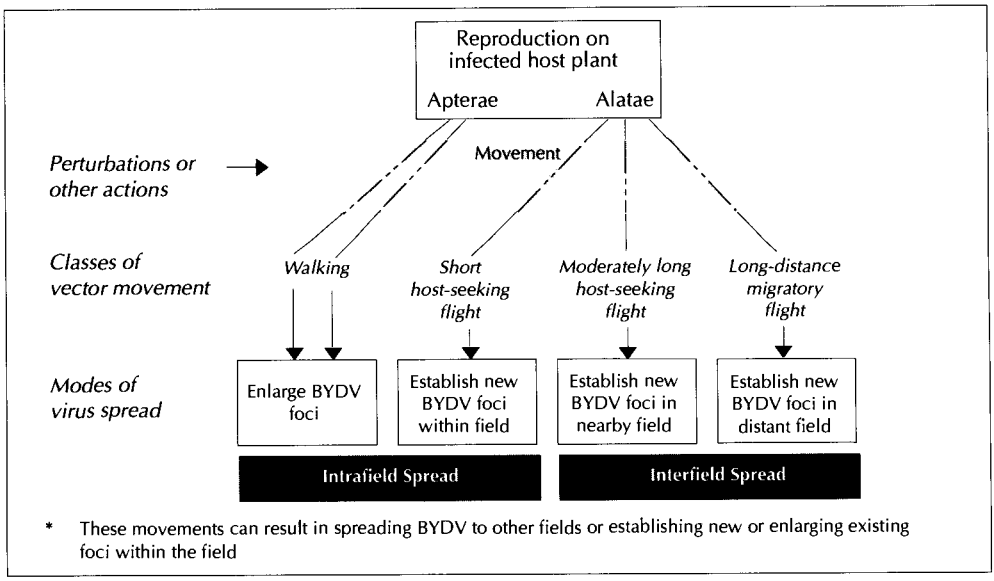
Several research findings confirm that aphids walking within cereal crops are one way in which BYDV is spread to neighboring plants. According to Conti et al. (1990), periods of mild weather stimulate aphid movement in cereal crops in Italy during the winter, leading to enlargement of existing foci. Halbert and Pike (1985) noted a similar phenomenon in winter wheat and barley fields in central Washington State, USA, and determined that 3.4-14.5% of the alate aphids collected from winter cereals during the autumn migration transmitted BYDV. In November, after the peak aphid flights had occurred for the year, there was an increase in the proportion of infected plants, suggesting active intrafield spread. Pitfall trap collections demonstrated active walking by aphids, and trap plants became infected, confirming that virus spread was occurring. Post-migration surveys of apterae and nymphs established that the numbers of infective apterae correlated well with concurrent increases in BYDV spread. Winter spread in the form of enlarged BYDV foci can occur only in areas where aphids overseason parthenogenetically.

Although existing BYDV foci can expand in winter during mild periods, it is mainly a spring and summer phenomenon. In studies in Quebec, Canada, Comeau and Dubuc (1977) noted that enlargement of existing foci was a major factor leading to a high incidence of BYDV and, therefore, to epidemics in summer cereal crops. In Australia, after initiation of cereal stem elongation, increases in virus incidence were thought to arise almost exclusively from local movement by apterae between plants (Johnstone et al., 1990). Why do nymphs, apterae and even alatae leave a host plant? They appear to do so because of 'signals' from the host plant that

it is stressed. The physiological status of an alate depends largely on prior environmental conditioning during its lifetime and on the conditioning of its parent. Plant-induced stress is a major factor influencing aphids. Thus, it seems that the physiological status of the host plant can trigger movement, especially as it influences the aphid's own physiological condition. Much spread of BYDV occurs when apterae leave overcrowded plants for nearby hosts. Orlob (1963) noted that *S. graminum* multiplied more rapidly when it was attended by ants than when unattended, leading to earlier overcrowding and quicker exodus. He also found that ant-mediated enlargement of BYDV foci was typically confined to field edges.

Environmental disturbances can dislodge aphids or cause them to colonize new plants (*see* Figure 3). The disturbances may be very subtle (such as wind swaying grass stems on which aphids occur) or they may be more overt (such as predators causing the aphids to emit an alarm pheromone, dislodge and disperse). Roitberg et al. (1979) indicated how these disturbances might influence aphid movement. They determined the influence of dense populations of a predator in a field colonized by an alarm pheromone-producing aphid, *Acyrthosiphon pisum*. The resulting enlargement of an infestation focus was greater at high than at low densities of the predator, although the numbers of aphids may have been reduced by predation.

Figure 3 Conceptual diagram of aphid movement resulting from various environmental disturbances



Developing new BYDV foci

To date, it has been almost impossible to establish with any degree of confidence whether a new virus focus in a cereal field is initiated by an infective alate that developed in the field, by an alate that acquired the virus in the field, or by an infective aphid immigrating from a neighboring

field or from afar. The inability to resolve this issue has impeded the understanding of BYDV epidemiology and has contributed to the limited success in controlling the disease.

Flight energetics studies, sometimes referred to as 'lipid depletion' or 'lipid utilization', have established procedures for determining the length of time an aphid has flown (Cockbain, 1961; Liquido and Irwin, 1986). These studies, coupled with studies on vector flight dynamics (Irwin and Thresh, 1988), may eventually make it possible to discriminate between the different types of vector flight. However, this type of research is only just beginning and it is currently impossible to accurately determine vector flight duration.

There are divergent views on vector flight classes and corresponding types of BYDV spread in cereal crops. This divergence may arise from the fact that studies have been conducted in very different regions. Several scientists believe that BYDV spreads mainly on a local scale. According to Tatchell et al. (1988), working in England, non-sexual alates introduce BYDV from comparatively local sources. Johnstone et al. (1990) suggested that most of the initial BYDV foci in cereal fields in Australia are caused by aphids flying short distances from reservoirs of infection in nearby grasses. This view was supported by Plumb (1990) who found little evidence of long-distance movements of aphids in Europe.

By contrast, several scientists have attributed local epidemics to long-distance migration. Paliwal (1982) argued that local reservoirs such as grasses, winter wheat and maize were unimportant as virus sources for eastern Canada's cereal crops during the 1981 BYDV epidemic; virus inoculum introduced by aphids from elsewhere was considered to be the main source of infection. Paliwal and Comeau (1984) attributed Canada's 1983 BYDV epidemic to a large aphid migration into autumn-sown crops in October 1982. In Spain, Moriones et al. (1989) determined that the more prevalent PAV variant in cereals was associated with high late-spring populations of *S. avenae* and *M. dirhodum* which, they postulated, migrated from distant areas. Conti et al. (1990) also concluded that migrating aphids brought BYDV into autumn-sown cereals in Italy; they further determined that the infectivity of these incoming migrants was relatively low and initial foci generally scattered and sparse. Elsewhere, there is evidence to suggest that *S. avenae* moves considerable distances (Loxdale et al., 1985); in some years it moves into Scandinavia from mainland Europe, causing outbreaks of a MAV variant in areas where variants transmitted by *R. padi* usually predominate (Plumb, 1990).

Paliwal and Comeau (1984) found little evidence of BYDV movement from autumn-sown winter cereals to spring-sown grains during 1983 in eastern Canada. This might be explained by the observations of Slykhuis et al. (1967) that populations of *R. padi* declined before cereals were sown in the spring; thus, populations of *S. avenae*, which may have been scarce during 1983, appear to be associated with the first BYDV infections in spring-sown cereals.

In their studies in England, Tatchell et al. (1988) found that, in autumn, a greater number of *R. padi* alatae are caught in suction traps at a height of 12.2 m than at 1.5 m, whereas in the reverse occurs in the spring and summer. This suggested that alatae moved out of the fields during the autumn and into the fields during the spring and summer. That aphids emigrate during crop maturation was substantiated by Milinko and Nagy (1990), who observed that when cereal crops began to ripen in June in Hungary, aphids migrated to immature maize fields and to volunteer cereal plants, the most important summer hosts of BYDV vectors in the country. These findings imply that emigration may be fairly local in scope but this is far from proven. Although Taylor (1986) has provided a detailed account of aphid migration and virus

spread, the fact remains that, while aphids can migrate hundreds and sometimes thousands of kilometers (Johnson, 1967, 1969; Thresh, 1983; Hendrie et al., 1986; Irwin and Thresh, 1988), only circumstantial evidence links these migrations to long-distance transportation of BYDV.

Another long-standing controversy surrounding BYDV epidemiology concerns the possible attractiveness of BYDV-infected foliage to vectors. Some authors assert that the yellowing caused by BYDV attracts vectors and this, in turn, could promote epidemics. It is difficult in field experiments to separate the effects of foliage color from the stunting associated with infection and the consequent change in apparency and ground cover. In England, BYDV-infected plants were found to support far higher numbers of *S. avenae* and *M. dirhodum* than non-infected plants, and this was attributed to the attractiveness of the yellow infected plants (Ajayi and Dewar, 1983); in flight chamber experiments using alates of both species, more specimens were attracted to BYDV-infected leaves than to healthy ones. However, Kieckhefer et al. (1976) had observed earlier that *S. graminum*, *S. avenae* and *R. padi* preferred to settle on heathy green leaves rather than BYDV-infected ones. This agrees with observations in soybean fields, where more individuals of several aphid species tended to land on healthy, dark green plants than on an isoline that was deficient in chlorophyll (Irwin and Kampmeier, 1989). The larger number of aphids reported by Ajayi and Dewar (1983) could be explained by population increases and the greater numbers of alates on infected plants, but this does not explain the results of their flight chamber experiments. Thus, the relative attractiveness of diseased and healthy cereal plants remains an unresolved question of considerable importance.

MANAGING BYDV EPIDEMICS

Three broad approaches have been adopted in attempts to control BYDV: breeding for host plant resistance (mainly to the virus but also to the vector), applying chemical pesticides to reduce vector populations, and manipulating the crop environment to minimize or retard epidemics. Each approach has achieved some success that may contribute to managing BYDV epidemics on local, regional or global scales.

Breeding for host plant resistance

Breeding for resistance to the virus has long been considered an appropriate control measure. However, few sources of BYDV resistance have been discovered, at least within the Triticeae, although some BYDV tolerance has been described (Larkin et al., 1990). The known sources of resistance include the Yd_3 and Yd_2 genes, and resistance from the wheatgrass *Thinopyrum intermedium* (*Thinopyrum* is a senior synonym of *Agropyron*). In several *Thinopyrum* species, the resistance tends to be the result of the failure of the virus to replicate and sometimes by the inability of the vector to locate phloem cells (Shukle et al., 1987). The Yd_2 gene, transferred from Ethiopian landraces, confers a degree of resistance to BYDV in oats, manifested by mild symptoms and limited replication; although this gene seems to be linked with undesirable agronomic characteristics such as lodging, it appears that, in some instances, this problem can be overcome (Comeau and Jedlinski, 1990). McGuire and Qualset (1990) have successfully transferred the Yd_2 gene from barley cultivars to a Chinese spring wheat, *Triticum aestivum*.

In essence, genes conferring tolerance or resistance to BYDV appear rather limited, with some apparently effective only against certain variants or specific BYDV strains (Larkin et al., 1990). Furthermore, after repeated attempts to transfer these genes to agronomically acceptable cultivars, there is relatively little to show for these efforts other than some oat and barley cultivars and the potential for improved wheat cultivars.

New approaches may soon change this situation. Low virus multiplication or true resistance to BYDV, found in perennial grasses, appears to be transferrable to wheat using molecular techniques (Xin et al., 1988; Guang-he et al., 1990), through the identification of a specific gene and through cytological and molecular hybridization studies. These studies have demonstrated that the wheat variety Zhong 4 carries BYDV resistance on a set of seven pairs of non-wheat chromosomes derived from a combination of the E and X genomes found in *Thinopyrum intermedium*.

An important feature concerning genetic tolerance or resistance to BYDV in cereals is that, while the Ethiopian Yd_2 gene seems effective in many areas, at least to certain variants, the degree of tolerance or resistance appears to differ from one region to another (Burnett and Mezzalama, 1990). A line of durum wheat found to have a relatively high level of BYDV resistance or tolerance in Canada did not show the same degree of resistance when grown at the International Center for Agricultural Research in the Dry Areas (ICARDA) in Syria (Comeau, pers. comm.). Therefore, locality-specific aspects influence the effectiveness of the resistance because different BYDV variants, and perhaps also different vectors, are involved.

There do not appear to have been any attempts to incorporate the vector resistance characteristics of certain grasses into agronomically adapted cereal cultivars, although vector-tolerant and vector-resistant genotypes have been identified. For example, resistance to *Diuraphis noxia*, an aphid that colonizes cereals, has been detected; however, this species does not seem to be an important vector of BYDV (du Toit, 1990). Tsumuki et al. (1989) suggested that surface wax on leaves, which is an inherited trait, is an important component of barley resistance to colonizing aphids, particularly *R. padi*. They drew this conclusion from results showting that resistance levels correlate positively with surface wax rather than with other traits, such as leaf color. Because BYDV is spread predominantly by vectors that colonize cereals, and because there is a reasonable expectation that some, if not most, of the spread in certain fields is associated with vectors walking or making short, in-field flights, incorporating vector-resistance genes into agronomically acceptable cultivars is a worthwhile goal. However, breeding for tolerance of vectors (that is, breeding for the ability of a host to sustain an infestation of vectors without the associated yield reductions) is unlikely to be appropriate.

Thus, two approaches appear to have high potential: attempting to incorporate BYDV-resistance genes into agronomically acceptable cereal cultivars, which may involve molecular engineering techniques; and attempting to incorporate vector-resistance genes into agronomically acceptable cereal cultivars, using conventional breeding methods or molecular engineering.

Applying chemical pesticides

Pesticides are frequently used to reduce vector populations in cereal fields. Whether this practice routinely and effectively reduces or retards BYDV epidemics in such fields is unclear.

Because virus spread is caused by vector movement, the importance of aphidicides in reducing or delaying epidemics depends largely on the type of vector movement occurring at and soon after the time of the application. If the vectors are walking (that is, enlarging existing virus foci), then the effectiveness of the application will depend upon the extent to which walking is decreased. Some insecticides, because they disturb colonized aphids, invoke rapid movement (usually walking), and thus may increase the rate of foci enlargement. If the aphidicide acts rapidly and kills the aphids before they have time to move, foci enlargement should be both delayed and reduced. Aphidicides are often applied in such a way that they do not reach the niches occupied by some aphid species (for instance, a species may be subterranean for parts of its life cycle, or protected within tightly coiled leaf whorls, or occur on the undersides of lower leaves).

The timing of pesticide applications is also important. In the temperate zones of Australia, climatic factors affect the distribution of *R. maidis*, *M. dirhodum* and *Sitobion* species, depending on the time of application. Johnstone et al. (1990) observed that a single aphidicide applied to autumn-sown crops during the winter appeared to be beneficial where foci of infection and infestation occurred; therefore, the extent of virus infection in the spring seemed to be related to the effectiveness and timing of aphid control the previous autumn. Studies in England indicated that an application too early in the autumn allowed reinfestation by aphids before the onset of winter, whereas intrafield infections had proliferated before late sprays were applied (McGrath et al., 1987). Other important considerations are how aphidicides might alter the relationships between vectors and natural enemies, particularly with regard to population dynamics and vector behavior, and whether vectors become resistant to a specific chemical or to an entire class of chemicals. These interactions have immense repercussions to the system as a whole and to the control of BYDV, particularly when considered in the light of potential long-term management strategies that take account of cultural practices.

Certainly, more knowledge is needed on the effectiveness of pesticides in limiting vector movement, on biological control interactions, on pesticide resistance and on how each of these issues influences the subsequent spread of BYDV. A much greater understanding is needed to provide alternatives to the 'blanket' pesticide spray used routinely by many farmers (Holmes, 1989) and to avoid irreversible mistakes that could result from the untimely or improper use of these potent chemicals. It must be appreciated that prolonged or routine use of chemical pesticides will engender vectors immune to their lethal effects. In short, chemical pesticides are powerful weapons in our arsenal for controlling BYDV epidemics, but they must be used wisely and as a last resort rather than a front-line defence.

Manipulating crop environments

Modifying cropping practices has long played an important role in the management of virus epidemics. Modifications can include alterations in sowing dates, crop rotations, plant density, sanitation procedures and even regulatory measures that enforce regional sanitation practices or synchronization of crop phenologies and temporal gaps between crop growing seasons. Such tactics generally target vectors, in an attempt to manipulate their overseasoning habitat, movement, phenology, reproduction and establishment in order to delay or reduce BYDV

epidemics; they can also be used to reduce alternative virus reservoirs, especially through sanitary practices that eliminate weeds and volunteer cereals that persist between growing seasons.

Sowing date is perhaps the best example of how cropping practices can be modified to minimize BYDV epidemics. Plumb (1984) showed that, in England, BYDV can be reduced considerably if autumn cereals are sown after the major aphid flights; however, a delay in the autumn sowing will reduce potential yield. Similarly, while later sowing of spring cereals can reduce potential BYDV incidence, potential yield will be lower because the crop has less time to reach maturity (Plumb, 1984). Tatchell et al. (1988) observed that sexual alate forms of the vector species predominate during the autumn in England. They also reported that only asexual alates were trapped in summer and that they were more than eight times as infective as those alates (mainly sexual) trapped in the autumn (74% compared with 9%). The sexual forms migrate to their primary hosts and do not contribute to BYDV spread in the autumn. Thus, autumn-sown cereals emerging before mid-September, prior to the transition of migrant aphids into a predominantly sexual population, are exposed to colonization by non-sexual alates and to the associated greater risk of virus spread. Johnstone et al. (1990) argued that a judicious choice of sowing date for wheat in relation to the major autumn and spring peaks of aphid flight activity can reduce BYDV epidemics in Australia. Jorda et al. (1987) monitored flights of *R. padi* into rice in Spain and concluded that delays in planting reduced BYDV epidemics in the rice crop.

Weeds can influence how BYDV epidemics start and progress through time and space, clearly demonstrating the intricate nature of the interactions between vector species, virus complexes and their hosts within a changing environment. From studies conducted in New Zealand, Smith (1963) found that the most severe incidence of BYDV in cereal crops occurred at the margins of fields alongside grasses and around ryegrass clumps regenerating from the grass ley. In Tasmania, studies by Guy (1988) indicated that *R. padi* is the dominant vector of BYDV and colonizes different weeds to different extents; this species was responsible for inducing different levels of incidence of BYDV in crops. Moreover, different strains of BYDV were found to be prevalent in different weed hosts — a PAV variant on fesue (*Festuca* spp.), and ryegrass (*Lolium* spp.) and an RPV variant on cocksfoot (*Dactylis* spp.) and canary grass (*Phalaris* spp.).

On the basis of experiences in France, Lapierre and Moreau (1986) suggested that the risk of BYDV spread is enhanced by intensive rotations only when prophylactic, sanitation measures are lacking and when the total crop area is being increased. This indicates that intensive rotations, in normal crop production allotments and accompanied by appropriate sanitation practices, could partially disrupt the epidemiological cycle. Plumb (1984) contended that, in crop rotations studied in England, if the previous crop had been a potential host of BYDV, the following cereal would have a greater virus incidence than if a non-host crop had been grown. This is further evidence that intrafield movement of BYDV is prevalent and important for spring-sown cereals in England. It also suggests that the course of epidemics is influenced not only by the practice of crop rotation itself but also by the type of crops in the rotation.

The incidence of BYDV has been reported to be greater in oat fields when wide row spacing rather than close spacing is used, when the fields are sown later rather than earlier and when they

are near unsprayed apple orchards and *P. padus* (Slykhuis et al., 1959). Alate aphids of several species often alight preferentially on plants at wide spacing (Irwin and Kampmeier, 1989), leading to greater rates of spread. It is also likely that, at wide spacings or decreased plant density, infective apterae and nymphs walking between plants reach fewer plants, thus curtailing the spread from existing foci. However, a low plant density also means that a greater proportion of the total stand will be infected by a similar number of infective immigrants entering the field, thus increasing the ratio of infected to uninfected plants.

The observation that virus incidence is greater in later sown crops is likely to be a function of the timing of vector flights relative to the timing of crop phenology, as discussed previously. Apple orchards and associated ground vegetation, hedges or windbreaks probably provide a sanctuary for natural enemies of vectors as well as a potential source of both vectors and virus. Under the circumstances reported by Slykhuis et al. (1959), relatively natural areas adjacent to cereals were evidently more important as reservoirs of the virus or vectors than of natural enemies; however, this aspect of the dynamics of virus epidemics is little understood. Holmes (1989) argued that the current infectivity indexing scheme which was developed by Plumb (1984) and colleagues at the Rothamsted Experimental Station, England does not take into account aphid population dynamics as influenced by aphid predators and diseases. Nearby trees of *P. padus*, a primary host of *R. padi*, could provide an early spring source of vectors, which would enhance spread of BYDV. Moreover, such an area could also be associated with virus reservoirs and secondary hosts of the vectors, and these could further fuel epidemics. Thus, with complex systems as described by Slykhuis et al. (1959), rigorous experimentation is needed to identify the main epidemiological factors involved.

Management strategy and forecasting epidemics

Epidemiological information is essential to develop truly effective BYDV control strategies (Irwin and Thresh, 1988). One important ingredient is information of the type of vector movement during epidemics; without this, management tactics cannot be targeted on the weakest links of the epidemiological cycle. This information must be understood in the context of the influence it has on epidemics under different management systems. The key to good control is to integrate the various tactics into a cohesive strategy that ultimately reduces the impact of BYDV on crop yield, not only over the short term but also over successive seasons, while at the same time safeguarding the environment and wildlife.

Strategies being used in France provide an example of how many epidemiological factors need to be integrated to forecast BYDV epidemics. Vector reservoirs, suction trap catches and field observations are considered, as are the location of alternative host plants in relation to the fields to be protected, the wind direction during aphid flights and the percentage of aphids that are viruliferous (Bayon and Ayrault, 1990). However, even this does not enable the system to be manipulated in order to retard or delay epidemics. So far, only a few issues have been addressed — appropriate timing of autumn-applied aphidicides, resistance or tolerance factors bred into agronomically acceptable cultivars, time of sowing date in relation to vector flight activity, and degree of rotation. There is still much to be learned not only about the epidemiology of BYDV but also about implementing what is known.

CONCLUSION

The epidemiology of a pathosystem as complex as BYDV is exceptionally difficult to elucidate because it is influenced by so many interrelated activities, only a few of which are known and measurable. It is one thing to know that a certain proportion of immigrating alate aphids are viruliferous, but quite another to know whether this incoming potential inoculum will lead to epidemics, as an inordinate number of interwoven biological and physical interactions intercede.

In writing this paper, it became apparent that, even with the wealth of information on this important and fascinating pathosystem, very little of its ecology is fully understood. Why? We believe the answer lies in two significant aspects of the biology of the pathosystem: the virus complex itself; and how vectors move and disseminate the virus among plants. The complexity of the luteoviruses causing barley yellow dwarf disease around the world has long been apparent, but only now are they being fully characterized. A coherent classification is still lacking, and the strain designation system now used globally is fraught with problems. This restricts an understanding of the pathosystem's ecology because the various luteoviruses behave differently, and until there is a better understanding of this ecology it will be impossible to develop definitive principles to account for BYDV epidemics.

Without an understanding of what makes vectors move, how far they travel and what causes them to settle, feed and reproduce, it is difficult to explain how BYDV epidemics progress, for it is the specific behavioral traits of vectors that drive and sustain epidemics. Simply designating aphids as vectors of BYDV is also misleading because species differ in terms of how they transmit the virus. Moreover, within each species there is a diverse range of populations and biotypes; each of these, in turn, has its own specific vector capabilities, responds slightly differently to external stimuli and is potentially keyed to settle on different plant genotypes.

All of this makes it questionable whether it will ever be possible fully to understand BYDV epidemics. Nevertheless, with a better understanding of the major biological components that constitute the pathosystem, the interaction of these components with given environmental factors, primary inoculum sources, how temporal gaps are overcome and how vectors disperse, coupled with competent modeling efforts, predicting epidemics should eventually become routine. Developing this knowledge base will also enable researchers to construct sound management strategies.

BYDV epidemiology is indeed a study in ecological complexity. It presents a challenge that must be met if the disease is to be managed. Assuming that adequate resources are provided for experimentation, we believe that within a decade much of the knowledge needed to understand and control this pathosystem will be available and that effective measures will be operating in at least some developed countries.

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PART 1

International and national programs on barley yellow dwarf virus

1.1

The Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT) Barley Yellow Dwarf Program

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SUMMARY

Cereal lines are screened at the Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT) in Mexico for resistance to barley yellow dwarf virus (BYDV). The lines are planted in small plots exposed to natural BYDV epidemics; those which seem resistant may be tested in experiments involving infestation with greenhouse-reared viruliferous aphids carrying a MAV-like isolate. Lines showing visual resistance in Mexico have been distributed worldwide. Resistance varies from site to site but some lines of bread wheat, durum wheat, barley and triticale are visually resistant at most sites. The Wheat Program has been trapping aphids in Mexico since 1986 at sites at El Batan and in the Toluca valley. The cereal-infesting species caught include *Metopolophium dirhodum*, *Rhopalosiphum maidis*, *R. padi*, *Diuraphis noxia*, *Sitobion avenae* and *Schizaphis graminum*. In 1987 and 1988 the most prevalent species caught at El Batan were *M. dirhodum* and *R. padi*, respectively. The most common species caught at Toluca in 1988 was *R. maidis*. In the past 5 years most isolates detected have been MAV-like. However, sampling has generally been carried out late in the growing season. Studies involving ELISA tests have shown that there is a higher proportion of PAV-like isolates in winter-sown cereals than in summer-sown cereals.

The barley yellow dwarf program at the Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT) was initiated in 1985 with the overall aim of facilitating the transfer of technology from developed countries to developing countries in order to reduce cereal yield losses caused by barley yellow dwarf virus (BYDV). It is funded by the Dipartimento Cooperazione Allo Sviluppo (DCAS) of the Ministry of Foreign Affairs, Italy. The objectives of the program are:

- to establish and/or strengthen relationships between developed and developing countries where BYDV causes significant economic losses;
- to offer training opportunities to scientists from developing countries where BYDV causes significant economic losses;

- to organize workshops and promote information dissemination through publications;
- to screen germplasm extensively to develop BYDV-resistant/tolerant materials, and distribute this germplasm to national program collaborators;
- to determine the genetic basis of BYDV resistance;
- to conduct epidemiological studies on BYDV in Mexico;
- to identify the range of BYDV isolates worldwide.

An international research network, in which Italian institutions play an important role, has been formed to further the development and exchange of germplasm and research procedures for controlling BYDV, especially in developing countries. Currently, a number of methods are being used around the world in efforts to control BYDV, with varying degrees of impact. In some regions, control can be partially effected by adjusting the planting time to avoid the period when aphids are most likely to infest cereal seedlings. Occasionally, partial control may be achieved by the judicious use of insecticides for aphid control. In some countries, biological control of aphids has reduced the incidence of BYDV. However, none of these methods is completely satisfactory, and the most effective control method is probably plant resistance or tolerance.

Germplasm screening is a core element of the CIMMYT program. Host plant resistance to BYDV, conferred by the Yd_2 gene transferred from Ethiopian landraces, has been demonstrated for barley, initially in California, USA and now in other countries. Similar resistance genes have not yet been identified in other cereals, but testing to date has not been exhaustive. This paper outlines the work being undertaken by the CIMMYT program on germplasm screening, as well as on yield loss studies, aphid trapping and the identification of BYDV isolates.

GERMPLASM SCREENING

Since 1980, CIMMYT has used its Atizapan research station (2640 m above sea level) in the Toluca valley, Mexico, where natural epidemics of BYDV occur, for screening both winter and spring materials. Because of land limitations, observations in winter are made on breeders' plots. Entries are hand sown 15-20 cm apart (to increase the intensity of BYDV infection) in two-row plots, 1 m long. The plots are separated by 50 cm and there are two replications. Symptoms of BYDV are assessed on a scale of 0-9 (0 = resistant, 9 = fully susceptible) (Qualset, 1984). The plots are sprayed with fungicides once every 2 weeks to eliminate the symptoms of other foliar diseases, enabling us to observe BYDV symptoms more easily.

Currently, the program is concentrating on screening advanced lines produced by CIMMYT breeding programs and on materials reported, by a network of cooperators working elsewhere, to be resistant to BYDV. Materials which appear to be resistant are distributed to cooperators and the data are fed back into the CIMMYT program. What we call resistance to BYDV has been termed 'slow yellowing' (Fox et al., 1990) and may include true resistance or tolerance to BYDV. The mechanism of resistance in many of the selected lines has not yet been determined.

Preliminary results from the CIMMYT program have been reported by Burnett et al. (1984) and Burnett and Mezzalama (1989). Here, only data from selected nurseries is presented. Tables 1 (*below*), 2 and 3 (*overleaf*) provide selected data from the second BYDV screening nursery for bread wheat (6 sites), durum wheat (4 sites) and triticale (4 sites); Table 4 (*page 38*) provides data from the fifth BYDV screening nursery for barley. These tables show the mean scores and range of scores across sites. While several lines show useful resistance across sites, in many cases there is an apparent variation in resistance across sites, with lines showing resistance at one site but susceptibility at another. This may signify differences in BYDV isolates between sites. The variation highlights the need for multilocational testing of material for BYDV resistance and should make us cautious about the universal utility of a resistant line selected at any one site. Nevertheless, it should be borne in mind that the Yd_2 resistance gene in barley, which is proving effective in many parts of the world, was selected at one site (Schaller, 1984).

Many winter wheat nurseries have been scored visually for resistance to BYDV at Toluca. Table 5 (*page 38*) lists the lines that have exhibited resistance over a number of years. The best of these lines are being used as parents in efforts to transfer BYDV resistance to spring wheat. There has been a limited distribution of some lines which hold particular promise.

Table 1 Mean scores of BYDV symptoms in check bread wheat cultivars and selected lines screened across six sites[a]

Bread wheat	Mean score	Range of scores
Checks:		
ANZA	3.8	1-6
BOW	4.7	1-8
ATLAS 68 (+ Yd_2)	4.3	1-7
Lines:		
SDY/CNRC/3/AU/UP 301/BOW	1.8	1-3
ALV 110/2*IAS 54/6/TP/4/ TZPP/SN64/NAPO/3/CN 067/5/PF 6968	1.9	1-6
VS 3600/MRL/3/BOW//YR/TRF'	2.0	1-3
AMD/HN4/3/GTO/7C//BB/CN 067/5/ PVN/4/BB/CNO//HAR/3/ORZ/6/TAN/SNB	2.1	1-4
MYNA	2.6	1-4
PF 79782	2.6	1-4
NING 8331	4.4	4-6

Note: a Score range at the six sites: Njoro, Kenya (0-7); Molo, Kenya (1-7); Beijing, China (1-5); Palmerston North, New Zealand (2-6); Santiago, Chile (1-7); Marino, Colombia (1-7).

Table 2 **Mean scores of BYDV symptoms in check durum wheat cultivars and selected lines screened across four sites[a]**

Durum wheat	Mean score	Range of scores
Checks:		
ATLAS 57 (- Yd_2)	6.5	3-9
ATLAS 68 (+ Yd_2)	4.3	2-8
ANZA	4.0	2-5
Lines:		
SAPI/TEAL	3.0	2-4
CHEN	3.3	2-4
AFN/IBIS//COO/3/GOO	3.3	2-4
CARC	3.5	2-4
YAV	3.8	2-5
MOA	3.8	3-4
GEDIZ/CIT 71	3.8	3-4
M 75	6.0	4-8

Note: a Score range at the four sites: Cape Province, South Africa (2-9); Beijing, China (3-5); Palmerston North, New Zealand (3-7); Santiago, Chile (2-7).

Approximately 50 lines of winter barley with BYDV scores of 5 or below have been identified. Some of these lines (such as 'Post', a winter barley from Missouri, USA) are known to be resistant to BYDV in other parts of the world but are not known to contain the Yd_2 gene. They have been crossed with lines known to contain this gene in an effort to combine both types of resistance.

The material being produced by the wide cross program at CIMMYT has been screened. Some lines involving crosses with *Elymus*, *Triticum*, *Aegilops* and *Thinopyrum* show promise but further testing is required. We are also screening some early generation segregating materials of bread wheat and durum wheat. We infected these lines with our Mexican MAV-like isolate, using greenhouse-reared viruliferous aphids applied with a Bazzoka applicator (Mihm, pers. comm.), and the preliminary results look encouraging.

YIELD LOSS STUDIES

Small-plot yield loss studies have been carried out in Mexico since 1986. A randomized split plot design with up to eight replications is used, with the cultivars as the subplot and treatments

Table 3 Mean scores of BYDV symptoms in check triticale cultivars and selected lines screened across four sites[a]

Triticale	Mean score	Range of scores
Checks:		
ATLAS 57 (- Yd_2)	6.8	4-9
ATLAS 68 (+ Yd_2)	3.7	1-9
Lines:		
STIER	1.8	1-3
PTR/CASTOR/BTA	2.0	1-3
GNU	2.0	1-3
TAPIR/PND/RM	2.1	1-3
TATU	2.3	1-4
YOGUI	2.3	1-4
FS 1795/LNC	2.3	1-4
M 2A//IRA/CAL/3/IGA	6.5	4-9

Note: a Score range at the four sites: Cape Province, South Africa (1-9); Beijing, China (1-4); Palmerston North, New Zealand (2-7); and Santiago, Chile (2-7).

as the main plot. There are three treatments: natural infection; artificial infection with greenhouse-reared viruliferous aphids at the 3- to 4-leaf stage; and a control protected by insecticides. In the earlier trials a PAV-like isolate of BYDV, with *Rhopalosiphum padi* (L.) as the vector, was used; currently, an MAV-like isolate, with *Metopolophium dirhodum* (Walker) as the vector, is being used as this appears to be the predominate isolate.

Some of the results from these trials are presented in Table 6 (*overleaf*). The more resistant the lines are to BYDV, the closer to 1:0 is the ratio of yield from sprayed plots to yield from infected plots. Some lines have exhibited a good level of resistance but, again, further testing is required.

APHID TRAPPING STUDIES

In 1986 studies were initiated in barley fields to evaluate the efficiency of yellow pan traps, horizontal mosaic green traps, fishing line traps and yellow sticky traps for trapping aphids (Jaime, 1988; Jaime et al., 1990) (*see* Table 7 *overleaf*). For the first three types of traps, four replicates were used in both 1986 and 1987; only two yellow sticky traps were available in 1986, but four were available in the following year. The yellow pan and horizontal mosaic green traps

Table 4 **Mean scores of BYDV symptoms in check barley cultivars and selected lines screened across six sites[a]**

Barley	Mean score	Range of scores
Checks		
ATLAS 57 (- Yd_2)	6.7	5-8
ATLAS 68 (+ Yd_2)	3.9	2-6
SUTTER + Yd_2	3.1	0-7
Lines		
PI 2325/MAF102//COSSACK	2.2	0-6
78W 40785	2.2	0-4
CI 39061 (+ Yd_2)	2.3	1-4
API/CM67/3/EMIR/NACKA// MGH 6355/4/H 2513API/CM 67/ORE (+Yd_2)	2.5	1-4
79W 41762	2.7	1-4
TERAN 78	2.8	2-3
P.STO	2.8	1-5
DS 4887	3.2	0-6
SUTTER*2/NUMAR (+ Yd_2)	3.3	1-7
ARUPO	7.2	6-8

Note: a Score range at the six sites: Njoro, Kenya (3-9); Molo, Kenya (0-8); Beijing, China (1-7); Davis, California (2 8); Palmerston North, New Zealand (1-8); Santiago, Chile (1-8).

Table 5 **Winter bread wheat lines that have shown BYDV resistance at Toluca, Mexico, over a number of years**

Lines or cultivars	No. of years	Lines or cultivars	No. of years
NS 974/NB 69565	6	F44.72	4
PYANE	6	77W 093	2
OK 77164	6	PONY'S'	2
STURDY	6	F9.70/MAYA	2
ANZA/SUT/CTK	5	F12.71/COC	2
NR 72.837 (ADAM)	4		

Table 6 Ratio of yield from sprayed plots to yield from BYDV-infected plots in small-plot trials on barley, wheat and winter wheat

Line or cultivar	Yield ratio
Barley (El Batan, Mexico, 1986)[a]:	
ATLAS 68 (+ Yd_2; resistant)	1.13
ATLAS 57 (- Yd_2; susceptible)	1.52
CERRO PRIETO	1.30
CENTINELA	1.50
Wheat (El Batan, Mexico, 1988)[b]:	
BOBWHITE (susceptible)	1.30
ANZA (resistant)	1.04
FAN 1	1.15
LIRA	1.25
PRL/TONI	1.16
TRAP 1	1.28
VS73.600/MRL/3/BOW//YR/TRF	1.00
Winter wheat (Atizapan, Mexico, 1988)[b]:	
BOBWHITE (susceptible)	1.39
ANZA (resistant)	1.09
NR 72.837 (ADAM)	1.39
ANZA/SUT//CTK	1.10
PAYNE	1.12

Note: a PAV-like isolate of BYDV transmitted by *Rhopalosiphum padi*.
b MAV-like isolate of BYDV transmitted by *Metopolophium dirhodum*.

Table 7 Common cereal aphids (*Rhopalosiphum, Metopolophium* and *Sitobion* spp.) captured by four types of traps, 1986 and 1987[a]

Trap type	Total no. aphids		*R. padi*		*R. maidis*		*M. dirhodum*		*S. avenae*	
	1986	1987	1986	1987	1986	1987	1986	1987	1986	1987
Yellow pan	129.6	211.3	1.4	0.6	0.2	7.7	3.5	6.4	1.3	2.0
Horizontal mosaic green	30.3	34.5	1.2	1.8	0.5	8.0	3.7	2.8	2.0	1.5
Fishing line	32.1	78.1	13.3	9.9	5.2	37.8	1.3	3.5	0.6	2.4
Yellow sticky	117.1	1762.7	40.5	51.7	28.6	1521.8	7.3	45.0	3.0	33.0

Note: a All traps corrected to the size of horizontal mosaic green trap.

were positioned horizontally, while the fishing line traps and the yellow sticky traps were placed vertically. All traps were placed at crop height, 10 m apart; the height was adjusted throughout the growing season so that the base of each trap was level with the top of the barley canopy. Aphids caught in the yellow pan traps, horizontal mosaic green traps and fishing line traps were counted twice a week; those on the yellow sticky traps were counted once a week.

The trapping studies provided information only on the relative numbers of cereal aphids caught. To assess which aphid species were capable of transmitting BYDV, Rothamsted low-level infectivity traps were used to capture alate aphids at CIMMYT's research station at El Batan, Texcoco (2240 m above sea level) in 1987 and 1988 and at Toluca in 1988. The trap was run continuously at El Batan and for 2 days each week at Toluca. At both sites the catches were collected at 8.30 a.m. The aphids were separated from other insects and were classified. Specimens of aphid species commonly infesting cereals were allowed to feed individually on oat seedlings at the 1- to 2-leaf stage, for 48 hours, to test their ability to transmit BYDV. In the case of large catches, a subsample of aphids was tested. At the end of the 48-hour period the survival and reproduction of the various species were recorded and the seedlings were sprayed with a systemic insecticide. Infective aphids were detected by symptom observation and enzyme-linked immunosorbent assay (ELISA) tests on the indicator plants.

The following cereal aphid species were captured at both sites: *R. padi*, *R. maidis* (Fitch.), *R. rufiabdominalis* (Sasaki), *Sitobion avenae* (Fabr.), *Schizaphis graminum* (Rond.), *M. dirhodum* and *Diuraphis noxia* (Mordw.) (*see* Table 8). MAV-like isolates of BYDV were the most frequently transmitted; the most common vector of these isolates was *M. dirhodum* (*see* Table 9). Although *S. avenae* also transmitted MAV-like isolates, the numbers of this species captured were much lower. Other isolates transmitted were PAV-like (only in mixed infections by *M. dirhodum*) and RMV-like (transmitted by *R. maidis*). *R. padi* was caught frequently, but it did not transmit BYDV.

We have also tested a wind sock trap (Ashby, 1976) to capture live aphids and we plan to compare the numbers of infective aphids captured by this trap with the number captured in the Rothamsted low-level infectivity traps. Although the wind sock trap captures few aphids and requires wind for its operation, it is cheap to manufacture and manage.

Table 8 **Number of cereal aphids caught at El Batan, 1987 and 1988, and at Toluca, 1988, with a Rothamsted low-level infectivity trap**

Aphid species	El Batan 1987	El Batan 1988	Toluca 1988[a]
Metopolophium dirhodum	1975	1610	542
Sitobion avenae	21	574	285
Rhopalosiphum padi	1658	4977	985
R. maidis	1755	2140	1374
R. rufiabdominalis	9	54	19
Schizaphis graminum	4	344	12
Diuraphis noxia	635	911	204

Note: a Values adjusted to 365 days.

Table 9 **Number of cereal aphids tested on indicator plants, number of infective aphids and BYDV isolates detected with ELISA at El Batan, 1987 and 1988, and Toluca, 1988**

	No. of aphids tested for infectivity/no. of infective aphids			
	El Batan		Toluca	Type of
Aphid species	1987	1988	1988	BYDV
Metopolophium dirhodum	137/41	85/13	81/36	MAV
	137/2	—	—	MAV + PAV
Sitobion avenae	18/2	48/3	10/2	MAV
Rhopalosiphum padi	199/0	210/0	32/0	—
R. maidis	284/7	103/7	41/0	RMV
R. rufiabdominalis	5/0	4/0	2/0	—
Schizaphis graminum	—	7/0	2/0	—
Diuraphis noxia	13/0	47/0	28/0	—

IDENTIFYING BYDV ISOLATES

The identification of BYDV isolates present in Mexico has been carried out using ELISA tests. Initially, air-dried leaf samples were sent to cooperating laboratories (mainly those at Purdue University, USA and Rothamsted Experimental Station, UK), but most samples are now tested in the CIMMYT laboratory in Mexico. Samples are tested with an indirect ELISA procedure using antisera for the MAV-, PAV- and RPV-like isolates (MAFF Laboratory, Harpenden, UK); recently, antisera for the RMV and SGV isolates have been produced for the CIMMYT program by Purdue University, and these will be included in future tests. Samples which exhibit OD values at A_{405nm}, higher than 3 times the healthy control value, are considered positive for BYDV infection.

Samples have been tested from Oregon, Monterrey, Celaya, Poza Rica, Toluca and El Batan. The most common isolate in these tests was MAV-like, although there have been some differences between laboratories. In the winter cereals at Toluca there was a higher incidence of PAV-like isolates.

In cooperation with Purdue University, CIMMYT is currently conducting a survey of isolates of BYDV worldwide. Leaves from both symptomatic and non-symptomatic cereal plants are being collected, air-dried and forwarded to Purdue University to be tested for the presence of BYDV by ELISA. The results from this survey and other studies will be used to produce a detailed world distribution map of BYDV isolates.

Variation of BYDV isolates in the field

CIMMYT's breeding programs at Toluca use two cycles of selection. In the winter cycle, seeding may take place between November and January; in the summer cycle it takes place in late May or early June. Between 1984 and 1986, the most common BYDV isolate detected at

Toluca was MAV-like. During this period, however, sampling was carried out mainly in late spring or summer on summer-sown crops. In 1987, sampling began on both winter (December-July) and summer (May-October) crops and a higher proportion of PAV- and RPV-like isolates were recovered in winter than in summer.

In view of this finding, a study was conducted to determine whether a change in the presence of the various BYDV isolates was occurring during the year and to assess the occurrence of mixed infections. Four cereal populations at Toluca were sampled: a bread wheat population sown on 21 November 1987 (early winter); a bread wheat population sown on 20 January 1988 (late winter); a bread wheat population sown on 6 May 1988 (summer); and a barley population sown on 10 May 1988 (summer). In each case plants were spaced 10-15 cm apart and individual plants were marked at 5 m intervals in the 10 central rows of a 2 ha field.

Leaf samples were collected from each plant at different stages of the growing season. For the plants sown in November, the sample size was increased by 50 plants from the initial 180 at each sampling date, but for those planted in January and May the sample size was kept constant. The first sampling date in each population was about 2 months after sowing (complete tillering). All the samples, retained as air-dried leaves, were tested using the indirect ELISA procedure and antisera mentioned above; symptoms were also recorded in the field at each sampling date.

In the November-sown bread wheat population, the incidence of MAV-like isolates increased from 4% at the first sampling date (9 February) to 55% at the last sampling date (6 June). The incidence of PAV-like isolates reached a maximum of 23% in April but decreased to 2% at the last two sampling dates. The incidence of RPV-like isolates reached a maximum of 12% in March and remained constant thereafter. Several combinations of mixed infections occurred between February and June; the highest number of PAV + RPV and MAV + RPV infections occurred in April and June, respectively (*see* Table 10).

In the January-sown population the highest number of MAV-like isolates occurred during April, but the number of PAV, RPV and of mixed infections was very low, as shown in Table 11 . The summer-sown populations of bread wheat and barley showed a similar pattern. In the bread wheat population, although the plants had been sprayed with a systemic insecticide, the

Table 10 **Number of MAV-, PAV- and RPV-like isolates and mixed infections, detected by ELISA, in a winter bread wheat population sown on 12 November 1987 at Atizapan, Mexico**

Date of collection	No. of plants tested	No. of positives detected by ELISA						
		MAV	PAV	RPV	PAV+ RPV	MAV+ RPV	MAV+ PAV	MAV+ PAV+ RPV
9 February	180	6	2	9	0	0	0	0
8 March	230	10	33	17	10	1	0	0
5 April	280	57	46	20	12	1	7	0
4 May	330	107	21	18	4	9	4	1
6 June	370	176	1	3	1	20	2	4

Table 11 **Number of MAV-, PAV- and RPV-like isolates and mixed infections, detected by ELISA, in a winter bread wheat population sown on 20 January 1988 at Atizapan, Mexico**

Date of collection	No. of plants tested	No. of positives detected by ELISA			
		MAV	PAV	RPV	MAV + RPV
8 March	100	1	0	0	0
21 April	100	40	1	1	1
23 May	100	59	1	0	2
27 June	63[a]	45	0	1	6

Note: a Only 63 samples were tested as the crop was senescing at this stage and senescent samples were not tested.

Table 12 **Number of MAV-, PAV- and RPV-like isolates and mixed infections, detected by ELISA, in a summer bread wheat population sown on 6 May 1988 at Atizapan, Mexico**

Date of collection	No. of plants tested	No. of positives detected by ELISA			
		MAV	PAV	RPV	MAV + RPV
20 July	180	37	9	0	3
2 September	180	63	0	0	0

Note: a The crop was sprayed with a systemic insecticide.

Table 13 **Number of MAV-, PAV- and RPV-like isolates and mixed infections, detected by ELISA, in a summer barley population sown on 10 May 1988 at Atizapan, Mexico**

Date of collection	No. of plants tested	No. of positives detected by ELISA					
		MAV	PAV	RPV	MAV+ PAV	MAV+ RPV	MAV+ PAV+ RPV
20 July	150	26	1	1	1	0	0
2 September	150	110	0	0	0	11	2

incidence of BYDV was high (35% in September); the predominant isolate in this population was MAV-like (*see* Table 12). In the barley population the incidence of BYDV was 82% in September and the predominant isolate was MAV. Out of the 123 infections recorded in this sample, 13 were mixed infections (*see* Table 13). The most characteristic expression of BYDV symptoms for all populations which were sown in November and January was recorded during mid-March. After 21 April the symptoms were indistinguishable from general yellowing resulting from senescence.

The results obtained in the early November-sown population and in the January- and May-sown populations showed a difference in the proportion of isolates detected using the ELISA test. In the November-sown population there was a higher proportion of PAV- and RPV-like isolates detected than in the January- and May-sown populations. It is likely that earlier sowing exposed populations to a different range of BYDV isolates. This confirms observations made in the 1987 cycles, but needs to be studied further. Understanding the variation of the infection in the field during a growing season can be very important when a germplasm screening program is conducted under conditions of natural infection.

CONCLUSION

The success of the CIMMYT program relies heavily on the network of cooperating institutions and scientists involved in the effort to reduce yield losses caused by BYDV. While the CIMMYT program has concentrated on selecting parent plants that appear to be resistant to BYDV in Mexico, many other institutions have materials that have been selected under different screening systems and in different environments; there are differences, too, in the BYDV isolates and aphid vectors that have been identified. Recent research has furthered our understanding of the genetic components for BYDV resistance or tolerance. The challenge is to make use of the knowledge and expertise now available and to test promising materials across a broad spectrum of sites. In its attempt to meet this challenge, the CIMMYT program will strive to strengthen and expand the network through which it screens and distributes materials.

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1.2

The Barley Yellow Dwarf Virus Program in Chile

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SUMMARY

During the early 1970s aphids became the most important entomological problem in wheat production in Chile. Barley yellow dwarf virus (BYDV) was first recorded in the country in 1972, and yield losses caused by this virus were particularly high between 1973 and 1978 and in 1982. At first, the problem was addressed by the widespread use of non-selective insecticides, which seriously affected natural biological control mechanisms. In 1975, the Instituto de Investigaciones Agropecuarias (INIA) initiated an integrated control system which included breeding for tolerance to BYDV, modifying cultural practices and using selective insecticides. The impact of BYDV is now less severe in Chile, aphid populations have decreased, and farmers no longer resort to using insecticides as intensively as they did in the 1970s.

The aphid species known to exist in Chile prior to 1966 were *Rhopalosiphum padi* (L.), *R. maidis* (Fitch.) and *Schizaphis graminum* (Rond.) (Zuñiga, 1985). In 1967, two more species were recorded, *Metopolophium dirhodum* (Walker) and *Sitobion avenae* (Fabr.). However, it was not known at this stage that these aphids were vectors of an important virus disease of cereals; when large aphid populations were observed in wheat fields, yield losses were attributed almost entirely to the damage caused by the aphids feeding on the wheat plants.

Barley yellow dwarf virus (BYDV) was first identified in Chile in 1972 by pathogenicity tests conducted with *M. dirhodum* and *R. padi* as the vectors and virus-infected oat plants as the inoculum source (Tollenar and Hepp, 1972). Electron-microscopy characterization identified virus particles present in phloem cells of barley and wheat as BYDV (Caglevic and Urbina, 1976). Using the enyzyme-linked immunosorbent assay (ELISA), a mixture of PAV- and MAV-like isolates were shown to be prevalent in southern Chile, while a PAV-like variant was predominant in the northern areas of the country (Herrera, 1984). Surveys conducted in 1987 and 1988 indicated that PAV-like isolates were the most important and prevalent throughout the country (*see* Table 1 *overleaf*). Recently, RPV- and RMV-like variants have been identified (Zerené, Herrera and Lister, pers. comm.). The incidence of BYDV in Chile in the 1986-89 period, based on results of ELISA tests, is summarized in Table 2 (*overleaf*).

Table 1 **Distribution of BYDV isolates determined from samples collected at three sites in Chile, 1987 and 1988[a]**

Collection site	No. of samples	% of BYDV isolates				
		PAV	MAV	RPV	RMV	SGV
Santiago (La Platina Experimental Station)	56	94.9	1.7	1.7	1.7	0.0
Chillan (Quilamapu Experimental Station)	27	96.4	3.6	0.0	0.0	0.0
Temuco (Carillanca Experimental Station)	28	85.7	0.0	10.7	3.6	0.0

Note: a Identification of isolates made by Dr R.M. Lister, Purdue University, USA.

Table 2 **Results of surveys conducted in different regions of Chile to evaluate natural infection by BYDV, 1986-89**

Region	1986-87		1987-88		1988-89	
	Samples collected	No. positives detected by ELISA[a]	Fields surveyed	No. positives detected by ELISA	Fields surveyed	No. positives detected by ELISA
Region III	—	—	6	1 (17%)	3	2 (67%)
Region IV	240	116 (48%)	7	6 (86%)	8	5 (63%)
Region V	6	6 (100%)	19	9 (47%)	9	2 (22%)
Metropolitan Area	98	56 (57%)	5	3 (60%)	10	3 (30%)
Region VI	12	11 (92%)	6	4 (67%)	13	8 (68%)
Region VII	—	—	—	—	8	6 (75%)
Region VIII	3	3 (100%)	2	2 (100%)	11	6 (55%)
Region IX	50	50 (100%)	1	0 (0%)	19	14 (74%)
Region X			2	1 (50%)	7	1 (14%)

Note: a Samples were considered positive if they had twice the absorbance value of the healthy check.

When the aphid-BYDV complex began to emerge in the early 1970s as the most important entomological problem in small-grain cereal production in Chile, the farmers' initial response was to combat the problem with the intensive use of a wide range of non-systemic chemical insecticides. These measures caused considerable damage to the natural biological control system, resulting in the development of large aphid population peaks at critical growth stages of the wheat crop. Subsequent research on systemic selective insecticides, critical population thresholds and insecticide doses persuaded farmers to avoid the indiscriminate use of chemical treatments. By 1975 it was clear that an integrated approach to the aphid-BYDV problem was

necessary in order to preserve the natural biological control resources, reduce environmental contamination of agricultural systems and ensure long-term stable control.

After a brief outline of the estimated wheat yield losses in Chile over the past 15 years, this paper describes the main components of the research program on integrated control being undertaken by the Instituto de Investigaciones Agropecuarias (INIA).

YIELD LOSS ASSESSMENT

Wheat yield losses resulting from BYDV in Chile have varied widely according to several factors, including wheat species (bread or durum wheat), growth habit (spring, winter, facultative), management factors (such as fertilization and date of sowing), regional differences (dryland or irrigated wheat cultivation) and climatological factors (such as drought occurrence and rainfall patterns). For 1975, 1976 and 1977, Herrera and Quiroz (1984) estimated losses of between 10 and 60% for different areas of the country. For the peak epidemic 1975-76 season, Caglevic (1978) estimated that yield losses ranged between 20 and 40%; the monetary value of losses during this season was estimated at US$ 15-20 million dollars (van der Bosh, 1976).

Using hectoliter weight to analyse the effect of BYDV on experimental material at La Platina Experimental Station, Cortazar (1987) reported yield losses of 15%, 31.5%, 17%, 17% and 9% for 1973, 1975, 1977, 1978 and 1979, respectively. In trials involving a standard set of 15 cultivars, conducted over 10 years (1976-86), Herrera and Quiros (1988) reported an average yield loss of almost 11%. Since 1979, with the exception of 1982, estimated yield losses at farm level have been lower in most areas of the country. Table 3 (*overleaf*) shows the effect of natural BYDV infection on wheat yields in different areas of Chile in the 1987-88 growing season.

INTEGRATED CONTROL OF BYDV

Between 1972 and 1975, when the use of insecticides was main defence practised by farmers to control the particularly high populations of aphids invading their wheat fields, it became increasingly clear that chemical sprays were not adequate to control the aphid-BYDV complex. Againt this background, INIA developed an integrated control approach comprising the following elements:

- Biological control of aphids by way of accelerated introduction, massive rearing, and distribution of bioregulators; evaluation, multiplication and distribution of native bioregulators;
- Studies on chemical control, with the emphasis on systemic selective insecticides and the critical dosage to be applied, in order to avoid damaging biological control mechanisms;
- Adoption of cultural practices, such as early seeding, to avoid coincidence of late plantings with aphid population peaks; control of weeds and wild hosts of aphids, acting as virus reservoirs; and development of a better understanding of the vectors' population dynamics;
- Breeding for tolerance or resistance, following an evaluation of available genetic materials.

Table 3 **Effect of natural infection by BYDV on yield and test weight of wheat in different regions of Chile, 1987-88**

Location	Yield (quintal/ha)		Hectoliter weight (kg/hl)	
	Permanent protection	Natural infection	Permanent protection	Natural infection
Metropolitan Area				
Spring wheat (La Platina)	55.85 a[a]	50.79 b	83.763 a	83.00 b
Litueche, Region VI				
Spring wheat (La Platina)	57.29 a	49.97 b	82.55 a	81.85 a
Talca, Region VII				
Spring wheat (Quilamapu)	23.05 a	22.97 a	84.27 a	83.26a
Los Angeles, Region VIII				
Spring wheat (Quilamapu)	80.03 a	79.50 b	84.57 a	84.33 a
Los Angeles, Region VIII				
Winter wheat (Quilamapu)	59.20 a	54.60 a	82.20 a	82.20 a
Chillan, Region VIII				
Spring wheat (Quilamapu)	68.00 a	68.40 a	82.80 a	82.90 a
Chillan, Region VIII				
Winter wheat (Quilamapu)	75.20 a	74.10 a	82.50 a	82.40 a
San Clemente, Region VIII				
Spring wheat (Carillanca)	51.80 a	15.70 a	83.70 a	84.10 a
Temuco, Region IX				
Spring wheat (Carillanca)	67.53 a	56.14 b	79.53 a	79.05 a
Temuco, Region IX				
Winter wheat (Carillanca)	69.96 a	61.15 b	77.64 a	76.91 a

Note: a Values in the same column followed by the same letter do not differ significantly at $p > 0.05$.

The breeding program concentrated on seven main areas: developing selection methods (under field and greenhouse conditions); screening serotypes present in Chile and determining their distribution; establishing a system of artificial inoculation that would operate efficiently for field testing of advanced lines and varieties as well as segregating materials; developing procedures for selecting tolerant or resistant germplasm based on yield or yield-related characteristics; introducing and screening genetic materials reported to be tolerant or resistant to BYDV in other areas of the world; exchanging germplasm with other institutions and participating in collaborative research efforts; and crossing selected materials with well-adapted local genotypes, as well as with foreign sources of tolerance and resistance, in order to develop improved cultivars and create new variability. The research on developing tolerant or resistant cultivars involved the following activities:

- Introduction of foreign genetic material from various sources to be tested in Chile under natural and artificial inoculation conditions, and selection of the best material for further analysis and inclusion in BYDV crossing blocks as parent genotypes;
- Development of a crossing program among tolerant genotypes to increase tolerance levels; this included selection of transgressive segregants and crossing with tolerant and adapted materials to select new varieties and cultivars;
- Screening breeding material entering yield trials and other advanced lines in the Artificially Inoculated Nursery (AIN); this involved evaluating breeding germplasm under field and greenhouse conditions, including early generations of segregating lines, and using specific deterrents against other diseases in order to correctly assess the BYDV effect (*see* Table 4).

Table 4 Cereal genotypes evaluated for BYDV tolerance of at La Platina Experimental Station, Chile, 1986-89

Type of nursery	No. of genotypes 1986-87	1987-88	1988-89	Total
National nurseries:				
Bread wheat	348	393	461	1202
Durum wheat	182	224	185	591
Barley		28	30	58
Triticale			22	22
International nurseries (CIMMYT):				
Bread wheat	146	92	107	345
Durum wheat	33	34	54	121
Barley	157	86	85	328
Triticale		68	88	156
Segregating lines:				
Bread and durum wheat (F_2-F_6)	200	296	644	1140
Recurrent selection for BYDV:				
Bread wheat		55	99	154
Total	1066	1276	1775	4117

Table 5 Number of cereal genotypes selected for tolerance or resistance to BYDV at La Platina Experimental Station, Chile, 1989

Material	No. of genotypes tested For 3 or more years	For 2 years	For 1 year
Bread wheat	34	39	114
Durum wheat	12	10	65
Barley	5	11	32
Triticale	0	6	31

Table 6 **Yield (g/0.6m²) and hectoliter weight (kg/hl) of durum wheat germplasm selected over three or more seasons for BYDV tolerance under artificial inoculation (AI) at La Platina Experimental Station, Chile, 1989**

Line or cultivar	% difference over average of all AI genotypes[a]		% loss resulting from AI	
	Yield	Weight	Yield	Weight
Tolerant check:				
Hercules x GTA'S' CD 1247 D 2Y	28.90	1.58	25.92	1.29
Susceptible check:				
Quilafén	-8.93	-1.16	23.56	1.92
Selected lines or cultivars:				
(CM-9704-39M-2Y-4M-1Y-OY) x CR'S'-215a-IIIC A-18521-1P-2P-2P,PLAC 1485	52.75	3.07	6.58	1.49
Crane's' CM-9704-39M-2Y-4M-1Y-DY A.18512-2P-3P-2P	45.80	1.01	8.32	2.01
NILE CD-74117-1L-1P-0AP	24.60	3.01	18.47	1.94
21563/AA'S'//D.DW S-15/3/CR'S' A-18475-2P-2P-2P	40.30	0.73	18.60	1.12
YAV'S' CM-9799-126M-1M-5Y-OM-8AV	12.67	2.53	38.00	2.33
BIT-SIB=21563/ANHJN-GA/2/ FLAMINGO SIP SCAR'S' CM-10162-76M-OY	9.47	2.32	27.28	2.02
YAV'S' CM-9799-126M-1M-4Y-0Y	7.17	2.50	26.10	1.04
PI 178083/FRIG'S' x HO CD-9660-9M-2Y-3M-1Y-OM	7.77	2.08	35.54	1.19
CR'S'-GS'S' x HO CD-9660-9M-2Y-2M-0Y	6.88	1.45	29.53	1.85
SNA 3	4.54	1.39	33.79	2.69
WIN'S'-AA'S' x STIL'S' CD-34011-3Y-1M-1Y-0M	-1.93	3.11	37.52	1.48

Note: a For all artificially inoculated durum wheat genotypes, the average yield over 3 years was 24.6 g/0.6m² and the average weight was 81.38 kg/hl.

Table 7 **Performance under permanent protection (PP) and artificial inoculation (AI) of barley germplasm selected for resistance to BYDV at La Platina Experimental Station, Chile in the 1987-88 and 1988-89 growing seasons**

Line or cultivar	Yield ($g/0.6m^2$) PP	Yield ($g/0.6m^2$) AI	Hectoliter weight (kg/hl) PP	Hectoliter weight (kg/hl) AI	% loss Yield	% loss Hl wt
Resistant check:						
Atlas 68	411.5	342.4	67.07	66.03	16.78	1.56
Susceptible check:						
Atlas 57	287.3	85.9	65.40	57.68	70.10	11.80
Selected lines or cultivars:						
HOR 728 CI 11577	189.7	191.3	75.26	76.18	0.00	0.00
BREA'S'/BEN CMB-75-522-4Y-500B -0Y-500B-501Y-OB	287.0	268.2	71.47	70.83	6.55	0.90
SOT/ABN//GAS/ORE'S' CMB-79A-10B0A-500B 1Y-1B-0Y	286.3	298.2	71.14	69.40	0.00	2.45
ASSE/CN/GUSS	253.1	209.2	78.00	76.63	17.34	1.76
M66-151/MANKER// 2P'2H'/3/DZ02-553 CMB-73A/1109-H-6B- 1Y-500B-0Y	297.1	233.5	70.89	68.15	21.41	3.87

The AIN included two treatments, with three replications each (Zerené and Ramirez, 1989). The first treatment involved permanent protection (PP) by systemic insecticide sprayed at 15-day intervals; the second treatment involved artificial inoculations (AI) with viruliferous aphids at stage 31 of Zadoks scale. At Zadoks 55, visual leaf symptoms were scored on a scale of 0-9 (0 = no symptoms, 9 = very susceptible). At harvest, grain yield and hectoliter weight were recorded for both treatments, and the PP-AI differences for both measurements were calculated. The differences were compared with those shown by tolerant and susceptible checks in order to select the genotypes which were to be included in the AIN in the following seasons (*see* Table 5, *page 51*).

A significant number of genotypes showing good BYDV tolerance have been selected in bread and durum spring wheat, winter bread wheat, barley and triticale. Tables 6 and 7 summarize the performance of selected durum wheat and barley germplasm, respectively, under artificial inoculation trials at La Platina Experimental Station. All new commercial cultivars released since 1985 have been tested in the AIN and found to have adequate BYDV tolerance (Zerené and Ramirez, 1989).

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1.3

The Agriculture Canada/Laval University Barley Yellow Dwarf Virus Program

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SUMMARY

The Agriculture Canada/Laval University research program on barley yellow dwarf virus (BYDV) was initiated in 1982, with support from the International Development Research Centre (IDRC). In collaboration with research institutions and individual plant breeders and pathologists worldwide, considerable progress has been made in the search for sources of BYDV tolerance and resistance. Some of the recent work in the program has focused on the interactions between BYDV and various environmental factors. Future research activities will include an investigation on whether or not selection for BYDV resistance could be used to improve other useful agronomic traits and thus extend the practical applications of current research efforts.

Agriculture Canada began research on barley yellow dwarf virus (BYDV) in 1972, after it had been recognized that BYDV was the real cause of drought-like symptoms in fields in Quebec Province. Germplasm was obtained from many of the pioneers in BYDV research in order to build up an elite nursery of BYDV tolerant or resistant material for spring and winter types of bread wheat, durum wheat, barley, oats, and triticale (Comeau, 1976). In 1977 an informal collaborative program on BYDV resistance was established between Laval University in Quebec and the Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT). In 1982, a joint program was set up by Laval University and Agriculture Canada, funded by the International Development Research Centre (IDRC). This program has worked closely with CIMMYT and the International Center for Agricultural Research in the Dry Areas (ICARDA) in efforts to identify sources of tolerance or resistance to BYDV.

PROGRESS THROUGH INTERNATIONAL COLLABORATION

The earlier work conducted by the Agriculture Canada/Laval University program focused on oats and barley but, with the involvement of CIMMYT and ICARDA, this was expanded to include bread wheat and durum wheat. The collection of germplasm from many sources, in

addition to the program's own elite nurseries, soon amounted to over 10 000 plots of spring cereals per year. Later, some winter types were added and a large number of segregating bulk progenies were inoculated to eliminate the BYDV-susceptible portion of the germplasm. The homozygous germplasm tested is shown in Table 1. In general, two to four repetitions were needed over 2 or 3 years for a proper assessment to be made of virus tolerance. To evaluate the BYDV reaction of the germplasm it was necessary to rear 5-20 million aphids per year, as each plot contained about 35 plants and each plant had to receive about 10 viruliferous aphids for uniform evaluation (Comeau, 1984). The surplus aphids, if any, were then given to local cereal breeders who, whenever possible, began to inoculate their bulk progenies of wheat, barley and oats in order to eliminate BYDV-susceptible lines in the early generations.

After 1985, collaboration with ICARDA increased and Chile became a partner in the program. During this phase research was conducted not only on identifying sources of resistance but also on verifying whether lines shown to be tolerant in Quebec would prove to be tolerant in other countries, in view of the importance of the interactions between BYDV and environmental factors such as soil types and climate. For wheat, it became obvious that South America (particularly Brazil) was a relatively good source of BYDV-tolerant genes, but it was difficult to transfer these genes into semidwarf wheat lines. This issue is dealt with in more detail in Paper 5.2 (Haber and Comeau, this proceedings).

PROGRAM ACHIEVEMENTS IN PLANT BREEDING

As early as 1974 it was known that perennial grasses were an excellent source of resistance to BYDV but the possibility of using these grasses to transfer resistance to cultivated cereals could not be investigated until 1986, when the program received grants to carry out this work. The research demonstrated clearly that the resistance in these grasses was far better than any resistance in cultivated species, and work began on transferring this resistance into wheat. The research also showed that aphid resistance was often present in the grasses.

The BYDV problem in oats in Quebec has essentially been solved, largely through the development of outstanding cultivars at Agriculture Canada's Sainte-Foy research station. The problem in barley was less important in 1971 than it is now, because the old cultivars showed some field tolerance which is lacking in more recently developed cultivars. The use of the Yd_2 gene from Ethiopian barleys is complicated by undesirable linkages, but some CIMMYT lines seem to have overcome this problem and since 1985 the program has given more attention to breeding BYDV tolerant or resistant barley, with a number of promising lines being developed. There has also been encouraging progress recently in BYDV research related to bread wheat.

FUTURE RESEARCH

The ongoing research projects at Laval University have helped expand the knowledge base in various areas of BYDV research (*see* Table 2 *overleaf*). Besides these specific projects, an important element of the work at Laval has been to conduct detailed observations, every year, of the field plots inoculated on a large scale under various field conditions.

Table 1 Cereal collections evaluated for BYDV tolerance in Quebec, Canada, 1977-89

Harvest year	No. of plots for each species (sources in parenthesis)	Total no. of plots[a]
1977	210 bread wheat (Quebec, New Zealand, California); 152 barley; 945 oats; 1200 *Avena sterilis*	2507
1978	60 *Triticum turgidum* (Ethiopia); 930 bread wheat (12th IBWSN[b], CIMMYT); 240 bread wheat (Qualset, Caetano, McEwan); 300 barley (Quebec, Winnipeg, USA); 1200 oats; 1465 *A. sterilis*; 60 triticale	4255
1979	20 *T. turgidum* (Ethiopia); 510 bread wheat (kept from previous year); 100 winter wheat; 33 interspecific wheat (Wells and Jedlinski); 15 barley; 465 oats; 477 wild *Avena* spp; (excluded from total: 60 perennial grasses)	1620
1980	69 wheat (diverse sources); 945 oats; 160 barley	1174
1981	466 durum wheat (12th IDSN[c], CIMMYT); 858 bread wheat (14th IBWSN, CIMMYT); 80 interspecific lines (Sando's collection); 144 *Aegilops* spp. and interspecific hybrids with *Aegilops* spp. (Kerber and Kimber); 2548 barley including the crossing block and 8th IBON[d] (CIMMYT); 566 wild *Avena* spp.; 424 oats; 160 winter wheat; 140 winter triticale	5386
1982	1171 durum wheat including 13th IDSN and PCs (CIMMYT), and 81-82 durum CB (ICARDA); 3060 bread wheat including 15th IBWSN and PCs (CIMMYT) and 81-82 RBW CB (ICARDA); 2670 barley including 9th IBON and PCs (CIMMYT) and 81-82 RB CB (ICARDA); 1320 oats; 200 winter wheat; (excluded from total: 500 perennial grasses)	8421
1983	2732 durum wheat including 14th IDSN and 82-83 PCs (CIMMYT) and 81-82 durum CB (ICARDA); 2325 bread wheat including 15th IBWSN and PCs (CIMMYT) and 82-83 RBW CB (ICARDA); 1774 barley including 9th IBON and PCs (CIMMYT) and 81-82 RB CB (ICARDA); 696 *Hordeum spontaneum*; 1756 oats; 1974 winter wheat including interspecific lines (Sando, Wells, Knott, Whelan, Cauderon, Jahier, McLean); 470 winter triticale	11 727
1984	773 durum wheat (mostly CIMMYT); 3321 spring wheat (CIMMYT, Mexico, Brazil, Canada, Japan, Turkey and others); 5462 barley including 83-84 KLDN[e] (CIMMYT) and RB CB (ICARDA); 547 triticale; 2807 oats; 731 winter wheat including many interspecific lines	13 641
1985	5869 bread wheat; 977 winter wheat; 1181 durum wheat; 4334 spring barley; 18 winter barley; 374 spring triticale; 30 winter triticale; 2602 oats	15 385
1986	6434 bread wheat; 657 winter wheat; 1315 durum wheat; 1786 spring barley; 109 winter barley; 1117 spring triticale; 14 winter triticale; 1200 oats	12 632

Table 1 (continued)

Harvest year	No. of plots for each species (sources in parenthesis)	Total no. of plots[a]
1987	2692 bread wheat; 800 winter wheat; 989 durum wheat; 1224 spring barley; 410 spring triticale; 1064 oats	7179
1988	4165 bread wheat; 800 winter wheat; 565 durum wheat; 1118 spring barley; 30 winter barley; 792 spring triticale; 766 oats	8236
1989	5869 bread wheat; 800 winter wheat; 1315 durum wheat; 1786 spring barley; 30 winter barley; 1117 spring triticale; 30 winter triticale; 2602 oats	13 549

Note: a Does not include the large number of segregating populations subjected to BYDV to eliminate the most susceptible plants, nor lines tested in the glasshouse. Virus-free check plots are also excluded.
b International Bread Wheat Screening Nursery.
c International Durum Wheat Screening Nursery.
d International Barley Observation Nursery.
e Key Location Disease Nursery.

Table 2 Theses completed at Laval University, Canada, on BYDV, and ongoing projects[a]

Theses completed:

Landry, B. 1982. Introgressing BYDV resistance genes from *Avena sterilis* into *Avena sativa*. MS thesis.
Collin, J. 1983. Evaluation of 61 winter wheat cross progenies, with and without BYDV inoculation in the fall. MS thesis.
Dion, Y. 1985. A 6 x 6 diallel analysis on barley, with and without BYDV and BSMV inoculations. MS thesis.
Cheour, F. 1987. Reaction of durum wheat (*Triticum durum*) to barley yellow dwarf virus. MS thesis.
Collin, J. 1987. Genetic resistance to barley yellow dwarf virus in triticale. PhD thesis.
Nkongolo, N.K.K. 1988. Transfer of BYDV resistance from triticale into wheat. PhD thesis.
Tremblay, C. 1988. Feasibility of transferring resistance to *Rhopalosiphum padi* from perennial grasses into wheat. MS thesis.
Plourde, A. 1989. Feasibility of using *Leymus* species as sources of resistance to barley yellow dwarf virus. PhD thesis.

Ongoing projects:

Although these research projects are partly in biotechnology, they all incorporate the goal of improving virus resistance and tolerance in cereals:

Theriault, C. Transfer of BYDV resistance from *Agrotricum* into wheat. PhD thesis.
Harper, L. Wheat-maize hybridization. MS thesis.
Maës, O. A study of embryo development in wheat. MS thesis.
Hamidou. D. *In vitro* selection of maize. MS thesis.
Duevi, M. Barley yellow dwarf virus development in *Sorghum* and *Pennisetum*. MS thesis.

Note: a All theses are in French apart from that by L. Harper, which is in English.

Table 3 Best sources of BYDV resistance and tolerance identified by the Agriculture Canada/Laval University research program

Species	Line	Category[a]	Owner or source
Six-row spring barley	8081 BQCB-10	VR	CIMMYT
	QB 235.6	VR	J.P. Dubuc
Two-row spring barley	Freja (Que. reselect.)	R	Public
	Corris	R	Aberystwyth
Winter barley	Wysor	VR	Virginia Polytech, USA
	Vixen	VR	Aberystwyth, UK
Spring oats	87 BYD OBS 29	VT	D. Brown (Winnipeg)
	QO 215.1	VT	J.P. Dubuc
	CI 9311	VT	USDA
	IL 79-4924	VT	C.M. Brown (Illinois)
	IL 85-1538	VT	C.M. Brown (Illinois)
	IL 86-5262	VT	C.M. Brown (Illinois)
	76s6-1454	VT	J.P. Dubuc
	Ogle	T	C.M. Brown (Illinois)
	OA 796-15	T	V. Burrows (Ottawa)
	TO 85025	T	Thompson's (Ontario)
	QO 209.48	T	J.P. Dubuc
Winter oats	Wintok	T	Public
Bread wheat	Maringa	T	Brazil
	IAS-20	T	Brazil
	Long Miai 10	T	Qi Shiyu (China)
	PF 79484	T	Brazil
	PF 82340	T	Brazil
	7th Lacos 40	T	Brazil
	Pdga/Nac//PF 7748	T	D. Knott (Zambia-Canada)
	LM10/IAS 20	T	A. Comeau
	810335-62D	T	C.O. Qualset
	PF 70354/Bow's'	MT	CIMMYT
Durum wheat	82pc Duros 476	MT	CIMMYT
Triticale	83 TF 519.31.1	R	A. Comeau
	Whale'S' 83cbst31	T	CIMMYT
Winter wheat	Augusta	T	Michigan
	Houser	T	Cornell, USA
Winter triticale	OAC Wintri	VT	Guelph, USA
	OAC Trillium	VT	Guelph, USA

Note: a R = resistant (virus multiplication reduced; little if any plant damage); T = tolerant (little damage despite a high virus concentration in plant); V = very ; M= moderately.

Some important findings have emerged from this gradual accumulation of knowledge about BYDV. It is clear, for example, that the effects of the virus are more devastating when the virus stress is combined with another stress, such as drought, cold or other diseases. Like a chameleon, the disease can take many guises according to soil types and climate, and yield

losses are not always accompanied by typical symptoms. This explains why the effects of the virus are often attributed to other causes. The severe drought in 1988 illustrated the potentially disastrous outcome of a combination of BYDV and drought. It seems that BYDV is a weakening agent, which may cause damage of its own as well as exacerbating damage resulting from other stress factors.

Such findings have led to a reorientation of the research program. Future research will focus not only on BYDV resistance, but also on the interaction of the virus with drought and with the fungal diseases that commonly mask the virus symptoms. Full use will also be made of the expertise developed over the years in germplasm evaluation, with more emphasis probably being given to interspecific derivatives that combine virus resistance with acceptable qualities that could make the lines attractive to plant breeders. Greater use will be made of enzyme-linked immunosorbent assay (ELISA); although this method has proved disappointing when used with conventional germplasm, it has been found to be a rapid, efficient tool to select interspecific wheat lines. A summary of the best sources of BYDV resistance and tolerance identified to date is given in Table 3 (*page 59*).

CONCLUSION

With the support of IDRC and the cooperation of many breeders and pathologists worldwide, the work undertaken by the Agriculture Canada/Laval University program has made a significant contribution to understanding BYDV (Comeau and St-Pierre, 1979-88). In collaboration with ICARDA, CIMMYT and the Chile program, work will continue on the development of resistant cultivars. However, as it is likely that the potential uses of BYDV selection could extend beyond the single goal of BYDV resistance, the program is now exploring whether or not BYDV selection could be used to improve, simultaneously, other useful agronomic traits, which would greatly extend the practical applications of this type of research.

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1.4

Barley Yellow Dwarf Research at the International Center for Agricultural Research in the Dry Areas (ICARDA)

K.M. MAKKOUK, O.F. MAMLUK and W. GHULAM

SUMMARY

Since 1986 the International Center for Agricultural Research in the Dry Areas (ICARDA) has conducted a number of field surveys on barley yellow dwarf virus (BYDV) in cereal crops in several countries in the West Asia/North Africa (WANA) region. The results obtained from serodiagnosis of the samples collected have provided considerable data on BYDV prevalence and the predominant BYDV isolates in the region. A program for screening cereals for BYDV resistance was initiated and the capacity to rear aphids for use in artificial BYDV inoculations was developed. Several barley lines, adapted to the region and showing a good level of BYDV resistance at sites in WANA countries and in Canada, were identified. Success with bread and durum wheat was more limited, although some breeding lines were found to be BYDV tolerant when inoculation was made during the tillering stage. Cereal wild relatives were recently tested to identify better sources of BYDV resistance than those found in cultivated wheat. A few accessions of *Agropyron* and *Aegilops* appeared to be immune to BYDV, and this will be investigated further by initiating intergeneric crosses.

Although barley yellow dwarf has been recognized as the most common and economically important disease of cereal grains worldwide (Plumb, 1983), until recently information on this virus in the West Asia/North Africa (WANA) region was limited. However, scattered reports based on field observations indicated that it was present in most WANA countries.

The progress made in developing techniques, such as enzyme-linked immunosorbent assay (ELISA), which permit a more sensitive detection of the virus, and the data obtained from surveys conducted by the International Center for Agricultural Research in the Dry Areas (ICARDA) in collaboration with national scientists has contributed to a clearer understanding of the relative importance of barley yellow dwarf virus (BYDV) in WANA countries, the prevalent strains, the common aphid vectors and possible alternate hosts of the virus. As plant genetic resistance to the virus is considered as being the most practical means of reducing losses

caused by BYDV infection, the primary objective of the barley yellow dwarf program at ICARDA is to identify sources of tolerance or resistance to BYDV. This paper summarizes the main research activities conducted at ICARDA on this virus in the 1986-89 period.

FIELD SURVEYS

Between 1986 and 1988, ICARDA reseachers conducted field surveys in Ethiopia, Jordan, Morocco, Syria and Tunisia and, through national scientists, obtained samples from those countries not visited. Hundreds of cereal samples were collected and tested serologically, using the ELISA technique, for the presence of BYDV; in these tests, both monoclonal and polyclonal antibodies were employed.

The survey results indicated that whereas BYDV incidence was low (2-10%) in some countries, such as Syria and Jordan; in others, such as Morocco and Tunisia, it was much higher (20-30%). The results of surveys conducted by national scientists are discussed in Part 2 of this proceedings. In the countries surveyed, the PAV type of BYDV was the most common (*see* Table 1), as is the case in most cereal growing areas of the world (Plumb, 1974; Rochow et al., 1986). More detailed results on the serotyping of BYDV in the WANA region have been published recently by Makkouk et al. (1989, 1990).

Table 1 **Serological typing of the cereal samples showing BYDV symptoms collected in Algeria, Jordan, Morocco, Syria and Tunisia, 1986-87[a]**

	No. of samples containing single or mixed BYDV isolates						
	Single infection				Mixed infection		
Year	PAV	MAV	RPV	F	PAV + MAV + RPV	PAV + MAV	PAV+RPV
1986	18	1	0	15	0	4	0
1987	16	0	5	2	2	3	5
% of total no. of samples evaluated	47.9	1.4	7.0	23.9	2.8	9.9	7.0

Note: a Two polyclonal and seven monoclonal antibodies were used in the tests. The polyclonal antibodies were F and B (Bioreba, Switzerland). The monoclonal antibodies were MAV91, MAC92, MACM2, MAFF2 (L. Torrance, MAFF, UK) and PAV-MC32-39, MAV-MC1-5 and RPB-MC7 (S. Wyatt, Washington State University, USA).

SCREENING FOR BYDV RESISTANCE

The main objective of screening cereals for BYDV resistance is to identify promising sources of resistance or tolerance which could be used in the crossing programs. Between 1986 and 1989 a number of cereal nurseries were evaluated for their reaction to BYDV (*see* Table 2).

Table 2 Cereal nurseries evaluated for their reaction to BYDV, Tel Hadya, Syria, 1986-89

Nursery	No. of entries evaluated 1986-87	1987-88	1988-89
Barley:			
Barley Key Location Disease Nursery	300	400	400
Barley Observation Nursery (HAA)[a]	—	—	128
Barley Observation Nursery (LRA)[b]	—	—	91
Barley Observation Nursery (MRA)[c]	—	—	84
CIMMYT Barley Yellow Dwarf Screening Nursery	—	83	83
Barley Crossing Block	—	48	—
Durum wheat:			
Durum Wheat Key Location Disease Nursery	250	200	240
Regional Durum Wheat Crossing Block	—	—	73
CIMMYT Barley Yellow Dwarf Screening Nursery	—	52	52
Regional Durum Yield Trial (LRA)	—	21	—
Regional Durum Yield Trial (MRA)	—	21	—
Bread wheat:			
Bread Wheat Key Location Disease Nursery	—	220	200
Bread Wheat Aleppo Crossing Block	—	—	238
Regional Bread Wheat Observation Nursery (LRA)	—	—	109
Regional Bread Wheat Crossing Block	169	—	173
CIMMYT Barley Yellow Dwarf Screening Nursery	—	105	105
Regional Bread Wheat Yield Trial (LRA)	—	23	—
Regional Bread Wheat Yield Trial (MRA)	—	23	—
Total	719	1196	1976

Note: a HAA = High altitude areas.
b LRA = Low rainfall areas.
c MRA = Moderate rainfall areas.

As BYDV natural infection in the WANA region varies considerably from one area to another (2-100%) and from one year to another, the proportion of plants that escape infection is relatively high. In such conditions, artificial inoculation using viruliferous aphids is the only way to reliably evaluate BYDV resistance or tolerance in cereals. The aphid-rearing method used at ICARDA follows that described by Comeau (1984). It is worth noting here that the most difficult part of this method is to keep aphid populations free from parasites and predators. It is essential to use proper cages and to exercise extreme care in handling the aphids. It is also important to rear virus-free aphids and viruliferous aphids separately and to introduce the virus to the virus-free colonies at the appropriate time (usually 6-8 weeks after introducing virus-free aphids to the host plant for multiplication purposes).

The BYDV isolate used was PAV, which is non-specifically transmitted by the aphid species *Rhopalosiphum padi* (L.) and *Sitobion avenae* (Fabr.). The main vector used in artificial inoculation was *R. padi*. Plants were inoculated during tillering and before stem elongation. Symptoms were scored on a scale of 0-9 (0 = no symptoms, 9 = severe symptoms); the readings

were taken after heading and before the plants changed color. Cereal lines with score of 8 or 9 were not harvested, but those with a score of 7 or less were harvested and the yield data and harvest index were determined. Cereal lines with a symptoms score of less than 6 and a yield and harvest index above average were retained and evaluated again the following year. The number of such lines did not exceed 2-3% of the total lines tested. Some of the cereal nurseries tested at ICARDA were also tested in Quebec, Canada.

The results of the evaluation of breeding lines in Quebec and Syria were similar. For example, the best bread wheat line from the Quebec Project (IAS 20) was also the best when evaluated at ICARDA (Comeau and Makkouk, 1988). At both sites, barley lines possessing the Yd_2 gene (a gene from Ethiopian barley which confers BYDV resistance) were superior, with the best lines scoring between 1 and 3 and the worst, Arabi Abiad, scoring 8. A comparison of the results of the barley trials in Quebec and Syria is given in Table 3.

Table 3 BYDV reaction of barley lines evaluated in Quebec, Canada and in Syria

Barley line	Symptom score[a]			
	Tel Hadya, Syria 1987	Lattakia, Syria 1988	Tel Hadya, Syria 1988	Quebec trials
Selected lines:				
8081 BCQB-10	—[b]	—	3	4.0
BKL 87-115	3	4	6	6.0
BKL 87-256	3	4	5	5.0
Shannon	—	—	4	5.2
BKL 85-237			3	6.2
Susceptible checks:				
Arabi Abiad	—	7	8	—
Harmal	—	—	—	8.0

Note: a Symptoms scored on a scale of 1-9 (0 = no symptoms; 5 moderately susceptible; 9 = very susceptible and dead before heading).
b Not tested.

MULTIPLE DISEASE RESISTANCE

Cereals are often affected simultaneously by several diseases, resulting in yield loss. In areas characterized by moderate rainfall (400-600 mm), such as North Africa, powdery mildew (*Erysiphe graminis* fs. *tritici*), *Septoria tritici* blotch and barley yellow dwarf are economically important diseases. Cereal germplasm with tolerance or resistance to all three pathogens is very useful to breeders of national programs for crop improvement.

Bread and durum wheat entries were evaluated for their reaction to these fungal diseases in multilocational tests in 'hot spots' under high disease pressure. Data on powdery mildew were obtained under natural infection from Deir Alla, Jordan and Sakarya, Turkey. Data on *Septoria* were obtained from Tel Hadya in Syria, from Guich and Merchouch in Morocco, from Beja in

Tunisia and from artificial inoculation with isolates of the pathogen prevailing in each country. BYDV data were obtained from artificial inoculation with a PAV isolate during the tillering stage at Tel Hadya. The diseases were scored on a scale of 0-9, as described above. The lines which had an average score of 5 or less for all three diseases are listed in Table 4; these lines were also those which produced above average grain yield after BYDV inoculation.

Table 4 **Reaction of bread and durum wheat lines to powdery mildew, *Septoria tritici* blotch and BYDV evaluated in multilocation testing, 1987-88**

Entry no.	Name or cross	Symptom score[a]		
		Powdery mildew	*Septoria*	BYDV
Bread wheat:				
1	GEN 81/YACO	4	4	4
2	VEE/3/R 37/GH 1121/KAL/BP	5	5	5
3	KVZ/CJ/MAD	0	5	4
4	A 041/EMU	4	4	5
5	VEE/NAC	1	5	5
6	FLN/ACC/ANA/3/PRL	2	5	4
7	FLN/ACC//ANA/3/PRL	0	5	4
8	VEE/NAC	2	5	5
9	RBS/ANZA/3/KVZ/MYS//YMH TOB/4/BOW	3	5	5
10	TTR/JUN	4	4	5
11	PRL/PEW	2	5	5
12	MAYA/SAP	4	4	5
13	PFAU/BANKS//BOW	3	5	4
14	SD 648.5/8156/3 CHR//SN 64/ K1/REND/4/CC/5/IWP 19	2	5	5
15	TR 380-16-3 A 614/CHAT	4	5	5
16	DGA/4/NAPO/TOB//8156 3/KAL/BB	0	5	5
17	2 CA 542C/SKOROSPELKA// NEUZUCHT/3/NAC 76	0	5	4
Durum wheat:				
18	MA-12	1	5	4
19	SCAR/GDOVZ 579/3/ GDOVZ 471/BR//PG	4	5	5
20	ENTE/MARIO//CANDO	4	4	5
21	GTA//D 21563/AA/3/STK/5/ FG/4/JO/61-130-115/3/GLL	1	5	5
22	LOUKOS 3	4	5	5
23	AKRACHE 1	5	5	4
24	HUI/YAV	4	5	5
25	STK/GEDIZ/3/PTL//S 15 CR/4/YAV 79	4	5	4
26	MARROUT	5	5	5

Note: a Symptoms scored on a scale of 1-9 (0 = no symptoms; 5 = moderately susceptible; 9 = very susceptible, dead before heading). Only lines with a score of 5 or less for all 3 diseases shown here.

BYDV RESISTANCE IN CEREAL WILD RELATIVES

With the progress made recently in interspecific and intergeneric hybridization, more emphasis is being placed on wild species as potential sources of useful genes in breeding programs. The level of BYDV tolerance in bread and durum wheat seems to be lower than that conferred by the Yd_2 gene in barley.

In trials conducted by ICARDA in 1988-89, 378 *Aegilops* accessions, 12 *Agropyron* accessions and 24 *Hordeum spontaneum* accessions were tested for their reaction to BYDV. The species were inoculated with a PAV isolate, using the vector *R. padi*. Observations were made 6-8 weeks after inoculation. To confirm the presence or absence of the virus, leaf samples

Table 5 Reaction of some accessions of wild relatives of wheat to BYDV infection and the genomes involved

Species	No. of accessions tested	No. of accessions found to be resistant[a]	Genomes
Aegilops triuncialis	119	22	CU
A. ovata	87	2	UM^o
A. biuncialis	43	2	UM^b
A. squarrosa	37	2	D
A. speltoides	27	3	S
A. triaristata	20	0	UM
A. umbellulata	15	3	U
A. peregrina	7	0	—
A. columnaris	5	0	UM^c
A. caudata	1	0	C
A. crassa	1	0	DM^{cR}
A. ventricosa	2	0	DM^v
A. cylindrica	1	0	CD
A. sharonensis	1	0	S^e
A. mutica	3	0	M^t
A. longissima	2	0	S^l
A. uniaristata	4	0	M^u
A. comosa	2	0	M
A. kotschyi	1	1	US^v
Agropyron cristatum	3	3	P
A. repens	1	1	
A. inerme	1	1	
A. intermedium	4	4	
A. elongatum	3	2	
Hordeum spontaneum	24	0	H
Triticum durum	—	—	AB
T. aestivum	—	—	ABD

Note: a An accession was considered resistant if no symptoms were produced and no virus was detected by ELISA.

were collected from all accessions (whether or not they showed symptoms) and tested by ELISA using an antiserum against the PAV isolate. The results of these trials are given in Table 5. Out of the 12 *Agropyron* accessions tested, 11 appeared to be immune in that they showed no symptoms and no virus was detected by ELISA. Earlier reports, however, had indicated that BYDV reaction in *Agropyron* species varied from apparent immunity to obvious symptoms (Bruel and Toko, 1957; Sharma et al., 1984; Comeau and Plourde, 1987).

Although the promising BYDV-resistant *Agropyron* and *Aegilops* accessions reported here will be subjected to further testing, our intention in this paper is to make researchers who are interested in sources of BYDV resistance in wheat wild relatives aware of the potential of this genetic material. The initial results indicate that some *Agropyron* and *Aegilops* accessions have high levels of BYDV resistance which do not exist in any known bread or durum wheat cultivar or breeding line, and they seem to possess genes that confer better resistance than the Yd_2 gene of barley. The possibility of producing agronomically useful plants by hybridizing these species with wheat has been reported by Cauderon (1966), Riley and Kimber (1966) and Comeau and Plourde (1987).

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PART 2

Reports from the West Asia/North Africa region

2.1

Epidemiology, Host Range and Strain Identification of Barley Yellow Dwarf Virus in West-Central Morocco

M. EL YAMANI

SUMMARY

Research conducted on the epidemiology of barley yellow dwarf virus (BYDV) in west-central Morocco indicated that the PAV strain of the virus was the most common (56%), followed by the MAV (35%) and RPV (9%) strains. The main vector of BYDV in the area was *Rhopalosiphum padi*, but at least nine other aphid species were also involved in the spread of the virus. The disease incidence monitored during the three growing seasons (1985-88) reached epiphytotic levels in 1986-87; the highest incidence occurred during the spring of each growing season. The disease was less severe in wheat than in barley. Maize and volunteer grasses played a significant role in the disease cycle by allowing the virus inoculum and vectors to survive the summer and early autumn conditions. Several grass species, including those common in west-central Morocco as well as those recently introduced for forage purposes, were shown to be susceptible to the virus, thus adding to the complexity of the disease ecology in the area.

Barley yellow dwarf disease occurs worldwide and is caused by a persistently transmitted virus in the luteovirus group (Rochow, 1970). The naturally occurring strains of barley yellow dwarf virus (BYDV) have been investigated in many regions of the world. Rochow (1969) defined four strains on the basis of aphid transmission specificity. Subsequently, Paliwal (1979) and Rochow and Carmichael (1979) found that results from serological tests used to differentiate the strains correlated with those from aphid transmission tests. In the Mediterranean region, however, it was not until 1980, when research began in Morocco, that BYDV attracted major attention. Since that time, several aspects of the disease have been investigated (El Yamani and Hill, 1990; Makkouk et al., 1990). This paper presents the results on the serological and biological characterization of BYDV strains, virus incidence and disease severity in cereals, and the host range of BYDV in west-central Morocco, a major cereal growing area in the Mediterranean region.

MATERIALS AND METHODS

Wheat, barley, oats and maize fields were surveyed by collecting single leaves from 50 plants at random, proceeding in a diagonal configuration. Leaves from each field were combined and samples of five leaves each were tested using the enzyme-linked immunsorbent assay (ELISA). Disease incidence was determined for each field using the formula below (Moran et al., 1983):

$$I\ (\%) = (1-Q^{1/N}) \times 100$$

where:

I = disease incidence
Q = proportion of leaf batches not infected with the virus
N = number of leaves per batch

Fields where the disease occurred in large areas were reported separately. Disease severity was scored on a scale of 0-9 (0 = no symptoms, 9 = severe symptoms) (Schaller and Qualset, 1980).

Aphid transmission of BYDV was effected according to the procedure described by Rochow (1969), using four aphid species — *Rhopalosiphum padi* (L.), *R. maidis* (Fitch.), *Sitobion (Macrosiphum) avenae* (Fabr.) and *Schizaphis graminum* (Rond.) — maintained as virus-free colonies on oats, *Avena sativa* L. (Clintland 64). Leaves from plant samples, usually with virus symptoms, were cut into pieces and divided into groups in order to conduct aphid transmission and ELISA tests, simultaneously. After incubation for 4-5 weeks, the inoculated plants were evaluated visually for symptoms and serologically by ELISA.

The double-antibody sandwich ELISA was used to test the samples (Clark and Adams, 1977; Gugerli, 1979; Lister and Rochow, 1979; Rochow and Carmichael, 1979; Diaco et al., 1986). The antisera were used at their optimal conditions of concentration, as determined by

Table 1 **Properties of antisera used to identify BYDV in cereal samples collected in Morocco, 1985-88**

Antiserum	Origin[a]	Type[b]	Known specificity to BYDV strains[c]			Antibody dilution or concentration (g/ml) used in study	
			MAV	PAV	RPV	Coating globulins	Conjugated globulins
B	Europe	PC	—	++	—	1/500	1/800
F	Europe	MC	++	—	—	1/400	1/600
MAV 3B10	USA	MC	++	++	++	1 - 4	—
MAV 2B12	USA	MC	++	—	—	—	4.00
MAV 7F6	USA	MV	++	—	++	—	0.25
MAV 6G7	USA	MC	—	—	++	—	0.03
PAV 3A11	USA	MC	—	++	—	—	4.00

Note: a Antisera B and F acquired from Bioreba, Switzerland, as kits prepared against the European strains; others produced and characterized at Iowa State University (Diaco et al., 1986, and unpubl. data).
b PC = polyclonal antibody; MC = monoclonal antibody.
c MAV = *Sitobion avenae*-specific strain, RPV = *Rhopalosiphum padi*-specific strain; PAV = vector non-specific strain.

the calculation of P/N ratios (Hill et al., 1981) (*see* Table 1). All samples were run at least in duplicate. Readings higher than the mean of the healthy sap by three standard deviations were considered as positive. All subsequent ELISA tests included buffer and healthy sap controls.

To investigate how BYDV and its vectors survive through the summer and the timing of virus spread in relation to cropping, plastic pots (16 cm diameter) containing 2-week old oat seedlings (10-20 seedlings per pot) were placed periodically at four sites (two pots per site) in west-central Morocco. The bait plants were maintained for 1 month at each site before they were brought back to the laboratory for examination. At the end of the month, the plants were caged and then examined in the laboratory for presence of virus symptoms and aphid species. The plants were then caged for another month to allow for further development of virus symptoms and the reproduction of vector species, after which a second assessment was made. Plant tissue was then harvested and tested by ELISA. The duration of this experiment was 19 months.

The virus host range study involved two groups of plants. In the first group, plants grown from seed in the greenhouse were inoculated with a non-specific PAV strain of BYDV, using *R. padi* as described above. The plants were sprayed with insecticide, observed for symptom development and subjected to ELISA tests using the PAV antiserum. The second group of plants consisted of symptomatic as well as symptomless grass species collected during field surveys or other occasions. The samples were evaluated by ELISA tests.

RESULTS

An analysis of the correlation between the ELISA results and aphid transmission specificity for the identification of BYDV strains showed that 26 of the 28 isolates studied contained only the non-specific PAV strain (*see* Table 2). Of the two samples remaining, one sample was doubly

Table 2 Comparative study of BYDV strains in west-central Morocco, using ELISA and aphid transmission tests

Isolate group	No. of samples/ group	A410 using enzyme-labelled globulins shown[a]						% aphid transmission using indicated aphid species[b]				Similarity to known BYDV strain[c]
		B	3A11	F	2B12	7F6	6G7	RP	RM	SA	SG	
A	26	2.50	0.85	0.04	0.10	0.12	0.07	60	32	25	15	PAV
B	1	0.76	0.08	0.37	0.04	0.07	0.04	53	0	14	28	PAV + MAV
C	1	1.36	0.20	0.06	0.10	0.41	0.52	43	3	33	27	PAV + RPV
Healthy check	—	0.06	0.09	0.10	0.05	0.13	0.08	0	0	0	0	Virus-free

Note: a Globulins B and 3A11 are polyclonal and monoclonal antibodies, respectively, specific to PAV; F and 2B12 are monoclonal antibodies specific to MAV; 7F6 a monoclonal antibody specific to MAV and RPV; 6G7 is a monoclonal antibody specific to RPV.

b Aphid species used were *Rhopalosiphum padi* (RP), *R. maidis* (RM), *Sitobion avenae* (SA) and *Schizaphis graminum* (SG); acquisition access period was 2 days and inoculation access was 5 days, using five aphids/seedling of *Avena sativa* cv. Clintland 64.

c MAV = *S. avenae*-specific strain; RPV = *R. padi*-specific strain; PAV = vector non-specific strain.

infected with the PAV and MAV strains and the other with the PAV and RPV strains. In all the transmission tests *R. padi* was the main vector in both single and double infections; the transmission rates by *R. maidis* and *S. graminum* were also fairly high. These results and others (data not shown) from recurrent aphid transmission tests showed a correlation of 80% with the ELISA results. Therefore, ELISA was used to test the majority of samples collected during field surveys.

The results of field survey revealed that the non-specific PAV strain was the most common, followed by the MAV and RPV strains, as indicated in Table 3. The relative presence of these three strains, alone or in combination, was 56% for PAV, 35% for MAV and 9% for RPV.

The highest disease incidence was observed in the 1985-86 and 1986-87 growing seasons (*see* Table 4). In 1988 there were numerous fields with a disease incidence of less than 10%.

Table 3 Occurrence of BYDV strains, singly or in mixed infection, detected by ELISA on field-collected samples from west-central Morocco

BYDV isolate[a]	No. of samples/plant species infected by BYDV isolates indicated				
	Wheats	Barley	Corn + Sorghum	Grasses[b]	Totals
PAV	140	73	24	25	262
MAV	53	32	6	14	105
RPV	7	7	0	0	14
PAV + MAV	44	32	23	27	126
PAV + RPV	6	1	2	7	16
MAV + RPV	7	2	0	0	9
PAV + MAV + RPV	15	4	3	13	35

Note: a PAV = vector non-specific strain; MAV = *S. avenae*-specific strain; RPV = *R. padi*-specific strain.
b The grass species included in this study are listed in Table 7.

Table 4 Incidence of BYDV in west-central Morocco, 1986-88

Disease incidence group	% fields/incidence group[a]		
	April 1986	April 1987	March 1988
Traces to 10%	36	39	59
11 to 20%	29	15	30
21 to 30%	00	08	08
Over 30%	36	15	02
Large patches[b]	00	25	01

Note: a Calculated on the basis of 14, 75 and 90 fields for April 1986, April 1987 and March 1988, respectively.
b Expanded areas of severely damaged plants throughout the fields.

Virus incidence in large patches of severely damaged plants was very common in the 1986-87 season. In general, the relative incidence of BYDV in barley fields was greater than that in wheat fields. Disease incidence was the highest for all crops in the 1987 growing season (*see* Figure 1). However, there was a general trend toward maximum disease incidence during the spring months. Infection of maize occurred in all three seasons, with the highest incidence in 1986. The data also demonstrated the role of wheat, barley and oat volunteers as potential oversummering reservoirs, as indicated in Figure 1.

In terms of disease severity, scored over two growing seasons, the highest scores (>4) were recorded for barley, followed by durum and bread wheats (*see* Table 5 *overleaf*). The disease was also more severe in 1987 than in 1988.

Figure 1 Incidence of BYDV infection in fields of maize, wheat, barley and oats in west-central Morocco in 1986, 1987 and 1988

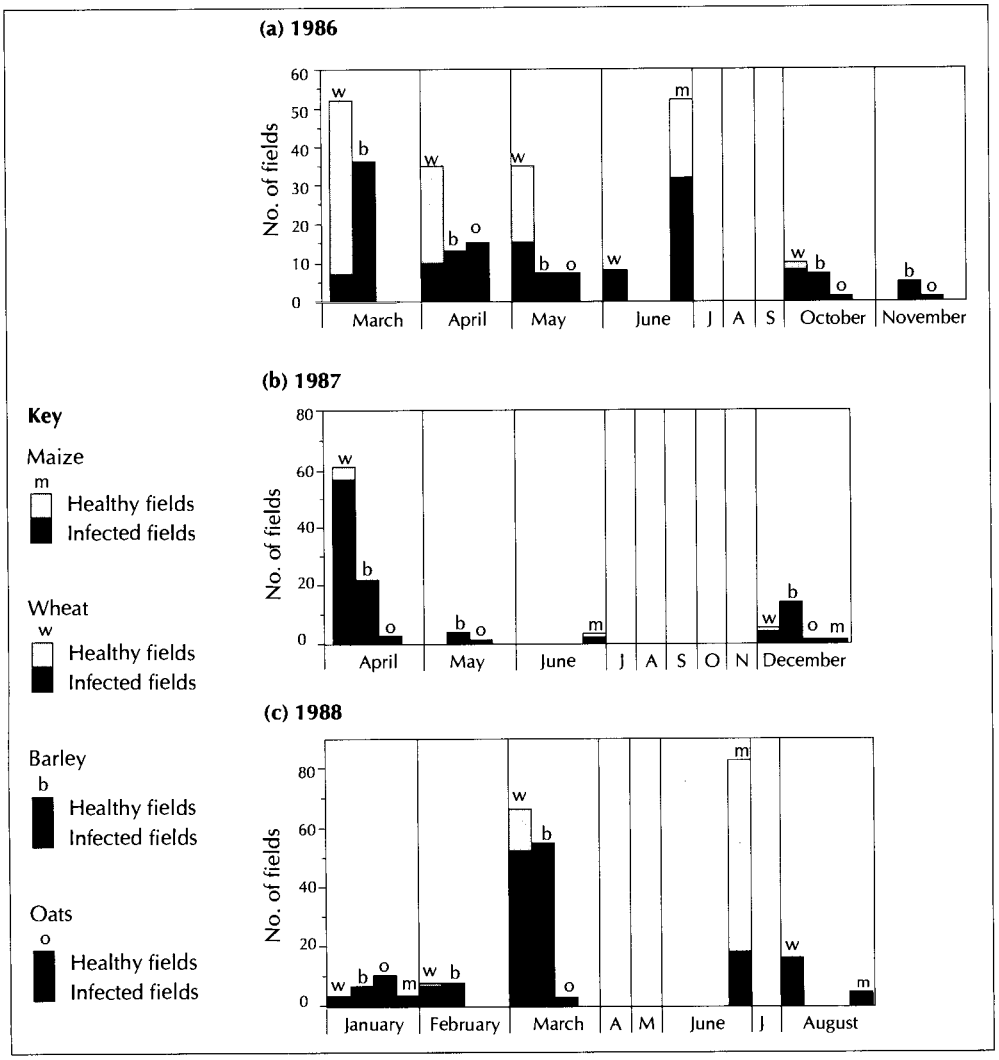

Table 5 Severity of BYDV incidence in small-grain cereals, west-central Morocco, 1987-88

Score[a]	% fields receiving severity score shown					
	Durum wheat		Bread wheat		Barley	
	Apr.1987	Mar. 1988	Apr. 1987	Mar. 1988	Apr. 1987	Mar. 1988
0	3	14	8	33	0	28
1	41	7	54	4	26	9
2	16	59	12	62	22	42
3	22	9	8	0	17	7
4	11	2	17	0	22	2
5	5	2	0	0	4	5
6	0	5	0	0	4	4
7	0	0	0	0	4	0
8	0	0	0	0	0	2
9	3	0	0	0	0	0
No. of fields	37	42	24	24	23	54

Note: a Scored on a scale of 0-9 (0 = no symptoms; 9 = very susceptible) (Schaller and Qualset, 1980).

Table 6 Occurrence of BYDV strains and aphid species on oat bait plants at four locations in west-central Morocco, 1986-88

Harvest date of bait plants	Species found on bait plants[a]							Isolates detected by ELISA[b]		
	RP	RM	SA	SG	MD	DN	UN	PAV	MAV	RPV
Dec 1986	+	-	-	-	-	-	-	+	+	-
Jan 1987	+	-	-	+	-	-	-	+	-	-
Feb 1987	+	-	-	-	-	-	-	+	-	-
Mar 1987	+	-	+	+	-	+	-	+	-	-
Apr 1987	+	-	-	+	-	-	-	+	+	-
May 1987	-	-	-	+	-	-	-	+	+	-
Jun 1987	+	-	-	-	-	-	-	+	+	+
Jul 1987	-	-	-	-	-	-	-	+	+	-
Aug 1987	-	-	-	-	-	-	-	+	+	-
Sep 1987	+	-	-	-	-	-	-	+	+	-
Oct 1987	+	-	-	-	-	-	-	+	+	-
Nov 1987	+	-	-	-	-	-	-	+	+	+
Dec 1987	+	-	-	-	-	-	-	+	+	+
Jan 1988	+	-	-	-	-	-	-	+	+	+
Feb 1988	+	+	-	-	-	-	-	+	+	+
Mar 1988	+	-	+	-	-	-	-	+	+	+
Apr 1988	+	+	+	-	+	-	-	+	+	+
May 1988	+	-	-	-	-	-	-	+	+	+
Jun 1988	+	-	-	-	-	-	+	+	+	+

Note: a RP = *Rhopalosiphum padi*; RM = *R. maidis*; SA = *Sitobion avenae*; SG = *Schizaphis graminum*; MD = *Metopolophium dirhodum*; DN = *Diuraphis noxia*; UN = unknown species. The presence of these species on the bait plants is designated by + and their absence by - .

b The double-antibody sandwich ELISA in this study used immunoreagents specific to the vector non-specific PAV, the *R. padi*-specific RPV and the *S. avenae*-specific MAV strains of BYDV. The presence of these strains on the bait plants is designated by + and their absence by - .

The results obtained from monitoring the survival of the virus-vector complex, using the oat bait plants, indicated that, in general, *R. padi* was present throughout the year, suggesting that it may be the most active virus vector. However, other aphid species were detected during the February-April period. The virus isolates detected in the bait plants over the 19-month duration of the survey were PAV (100%), MAV (84%) and RPV (47%). Seven different aphid species, including *Diuraphis noxia* (Mordv.), the Russian wheat aphid, were detected on the bait plants during the experiment (*see* Table 6).

All the grass species collected during the surveys contained at least one of the three virus strains (*see* Table 7); seven of these grasses were perennials. Mixed infection involving more than one BYDV strain was quite common, suggesting the potential for virus reservoirs and intermediate hosts. Data from the 17 grass species started from seed, all of which were perennials except *Stipa retorta*, showed that 10 species were susceptible, five were symptomless (*Agropyron dasystachyum*, *A. desertorum*, *Bromus inermis*, *Eragrostis lehmaniana* and *Stenotaphrum secundatum*) and only two (*Bouteloua curtipendula* and *Eragrostis intermedia*) were immune (*see* Table 8 *overleaf*).

Table 7 Grass hosts of BYDV collected from fields in west-central Morocco in the 1986-88 growing seasons

Grass species	Collection date (month/year)	ELISA reactions of antisera prepared against BYDV strains[a]		
		PAV	MAV	RPV
Aegilops ovata	4/87	+[b]	+	+
Arundo donax	10/86; 4/87; 2/88; 8/88	+	+	-
Bromus ridigus	4/87	+	-	-
Cenchrus longispinus	3/88	+	+	+
Cynodon dactylon	6, 7, 10/86; 12/87; 2/88	+	+	+
Digitaria sanguinalis	3/88	+	+	+
Echinochloa crus-galli	3/88	+	-	+
Hordeum murinum	3/88	+	-	+
Oryzopsis miliacea	12/87; 8/88	+	-	+
Paspalum dilatatum	3/88	+	-	+
Pennisetum villosum	6/88	+	-	+
Phalaris brachystachys	3/88	+	-	+
P. paradoxa	3/88	+	-	+
Phragmites communis	12/87; 8/88	+	+	-
Sorghum halepense	12/87; 1/88	-	+	-
Unknown species	11/86	+	+	-

Note: a The double-antibody sandwich ELISA in this study used antibodies prepared against the *Rhopalosiphum padi*-specific RPV, *Sitobion avenae*-specific MAV, and vector non-specific PAV strains.

b + and - designate positive and negative reactions, respectively.

Table 8 **Reactions of some pasture grasses in west-central Morocco to inoculation with BYDV**

Grass species	Expression of BYDV symptoms by inoculated plants[a]	No. of infected plants/ no. of plants tested[b]
Agropyron cristatum	yes	3/6
A. dasystachyum	no	1/2
A. desertorum	no	1/4
A. elongatum	yes	3/6
A. smithii	yes	2/3
A. trichophorum	yes	1/6
Bouteloua curtipendula	no	0/2
Bromus inermis	no	4/4
Dactylis glomerata	yes	3/4
Elymus junceus	yes	6/6
Eragrostis intermedia	no	3/5
E. lehmaniana	no	0/4
Oryzopsis sp.	yes	1/1
Phalaris tuberosa	yes	4/7
Sporobolus airoides	yes	4/6
Stenotaphrum secundatum	no	1/1
Stipa retorta	yes	—

Note: a Plants were inoculated with the PAV strain, carried by *Rhopalosiphum padi*, with 5 aphids/plant.
b The double-antibody sandwich ELISA was used, with PAV-specific immunoreagents.

CONCLUSION

The main findings from the research described above can be summarized as follows:

- PAV, MAV and RPV strains of BYDV are all present in west-central Morocco, with the PAV strain being the most common;
- the disease can reach epiphytotic levels in Morocco, as shown by the data collected in the 1986-87 growing season; the disease causes more severe symptoms in barley than in wheat;
- both the virus and the aphid vectors oversummer in maize and cereal volunteer plants;
- the aphid vector *R. padi*, found to be present in the area throughout the duration of the study, is the main BYDV vector, but other aphid species are responsible for secondary spread of the virus during spring;
- the area is rich in grasses susceptible to the identified naturally occurring BYDV isolates, with or without visible symptoms.

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2.2

Estimated Barley Yield Losses Attributable to Barley Yellow Dwarf Virus in Morocco

A. AMRI

SUMMARY

Barley is grown on over 2 million ha annually in Morocco. Foliar diseases consitute one of the major constraints to stable crop production in the country. Symptoms of barley yellow dwarf virus (BYDV) are common in many barley fields in different agroclimatic regions. In the study reported in this paper, aphid control by insecticides and near-isogenic lines was used to provide estimates of yield loss resulting from BYDV under artificial and natural infection conditions. During the 1982-83 season, the average grain yield losses following artificial inoculation and the use of Parathion were 14.1% and 31.3% at Merchouch and Guich, respectively. No significant loss was observed in the 1983-84 season under natural infection conditions. The BYDV-resistant isoline outyielded the susceptible isoline by 9% at Merchouch in 1982-83 and by 24% and 7% at Merchouch and Tessaout, respectively, in 1983-84. The effectiveness of the *Yd*$_2$ gene appears to depend on the level of infection and climatic conditions.

The low yields obtained from most barley fields in Morocco are attributed to the effects of prevailing foliar diseases (Amri and Mekni, 1989). Barley yellow dwarf virus (BYDV), a luteovirus which is transmitted by aphids, occurs in most cereal fields in the country, although its symptoms are often confused with the effects of some abiotic stresses (Yount et al., 1985; Comeau and Makkouk, 1988). The use of serological techniques have shown that BYDV is widespread in the semi-arid regions of Morocco and that it can attack all cereal species (El Yamani and Hill, 1990). Strain identification using the enzyme-linked immunosorbent assay (ELISA) and aphid transmission tests have indicated the predominance of a PAV-like strain of BYDV.

Under heavy inoculation, BYDV can be very damaging to cereals. Yount et al. (1985) estimated that yield loss resulting from BYDV in Montana, USA ranged between 44.9% and 74.5% for two-row barleys, six-row barleys, winter wheats and spring wheats. The average yield loss estimates for the bread wheat cultivars Nasma 149 and Saada, recorded over two seasons at the Sidi El Aydi, Jamaa Shaim and Tessaout Experimental Stations in Morocco, were

61% and 26% respectively, under artificial and natural infection conditions (El Yamani and Hill, 1990). At the Merchouch and Guich Experiment Stations, yield losses for barley cultivars, under natural infection conditions, were estimated to be 15% and 25%, respectively (El Holoui and Tagine, 1985). Smith and Sward (1982) found that inoculation before tiller initiation reduced grain yields by 9-90%, while inoculation at the stage of stem elongation reduced grain yield by only 6-9%. El Yamani and Hill (1991) observed a similar trend in Nasma 149, in which early inoculation reduced grain yield by 43% whereas late inoculation reduced it by only 29%. These infections also affected total biomass and all components of grain yield, including the 100-seed weight (Smith and Sward, 1982; Yount et al., 1985; El Yamani and Hill, 1991). Smith and Sward (1982) found a significant relationship between the extent of yield loss and the severity of the disease but this correlation was not significant in the study which was conducted by El Yamani and Hill 1991. Comeau and Makkouk (1988) concluded that plants affected by BYDV were more susceptible to attacks by other pests. Monneveux et al. (*see* Paper 5.1, this proceedings) have reached a similar conclusion; the results of their studies also indicate that moderate drought conditions and other abiotic stresses exacerbate the damage caused by BYDV.

Host plant resistance appears to be the most effective way to control BYDV. High levels of tolerance are conferred by the Yd_2 gene, derived from Ethiopian barley germplasm and now used in many cereal breeding programs throughout the world. The study reported in this paper, conducted in the 1982-83 and 1983-84 seasons and involving the use of insecticide control and near-isogenic lines, was initiated to provide an estimate of barley yield losses in Morocco and to determine the effectiveness of the Yd_2 gene under Morrocan conditions.

MATERIALS AND METHODS

Experiment using insecticide control

Five spring cultivars with differing reactions to BYDV were planted at the Guich and Merchouch Experiment Stations in 1982-83 and at Merchouch in 1983-84. Some of the characteristics of these cultivars are presented in Table 1. A randomized complete block design was used, with a split-plot arrangement of treatments and four replications. Each cultivar was planted in six 5 m-long rows, with a space of 0.3 m between the rows. The two main plots were BYDV treatments (inoculated and non-inoculated) and the subplots were cultivars. For the 1982-83 season, viruliferous aphids carrying the PAV isolate of BYDV were provided by Dr M. El Yamani.

Each plant in the non-inoculated plots was then sprayed with Parathion (6 ml of product in 10 l of water) at 2-weekly intervals, starting on the date that the aphids were spread on the inoculated plots. The inoculated plots were sprayed once, 6 days after infestation by aphids. For the 1983-84 experiment, only natural infestation was allowed on the inoculated plots. The aphids were controlled on the non-inoculated plots by spraying with Parathion, starting at the early tillering stage. BYDV severity was scored on a 0-9 scale, combining both the type of infection and the extent of the attack. Grain yield was estimated by harvesting the four central rows of each plot.

Table 1 Origin and characteristics of the cultivars used in trials to estimate yield loss resulting from BYDV, Morocco, 1982-84

Cultivar	Origin	No. of rows	Observations
Asni	INRA[a], Morocco	2	High yielding, susceptible to BYDV
Tissa	INRA, Morocco	2	High yielding, susceptible to BYDV
Arig 8	INRA, Morocco	6	High yielding, moderately susceptible to BYDV
Rabat 071	INRA, Morocco	6	Landrace, susceptible to BYDV
UC 76227 (Sut/Num)	California,USA	6	High yielding, possesses Yd_2 gene resistant to BYDV

Note: a Institut national de recherches agronomiques.

Experiment using near-isogenic lines

One pair of near-isogenic lines, Atlas 68 (+ Yd_2) and Atlas 57 (- Yd_2), was selected from a barley nursery supplied by the Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT). In the 1982-83 season, artificial inoculation was used following the same procedure as in the previous experiment but without the insecticide control. A randomized complete block design with four replications was used. In the 1983-84 season, the two lines were included in advanced yield trials planted at Merchouch and Tessaout Experiment Stations. Data on grain yield and BYDV reaction were recorded for each plot. The analysis of variance and appropriate comparisons were performed using the SAS procedures.

RESULTS AND DISCUSSION

Estimates of yield loss using insecticide control

The total rainfall for the 1982-83 season was 347.5 mm and 517.5 mm at Merchouch and Guich, respectively; in the following season the corresponding figures were 468.5 mm and 486.6 mm. The early drought at Merchouch in 1982-83 had a pronounced effect on plant growth; this was less marked in the 1983-84 season. These conditions did not allow a significant increase in the infective aphid populations after the artificial inoculations. At Merchouch, the average BYDV score for the non-inoculated plots was 4.3 and less than 30% of plants showed characteristic BYDV symptoms (*see* Table 2 *overleaf*). The BYDV score was higher at Guich and more plants showed clear BYDV symptoms.

Table 2 **Grain yield and BYDV scores of five barley cultivars evaluated under artificial inoculation at the Merchouch (M) and Guich (G) Experimental Stations, Morocco, in the 1982-83 growing season**

Cultivar	Reaction in non-inoculated plots[a]		Grain yield (kg/ha) Non-inoculated/ sprayed		Inoculated/ unsprayed		% yield loss	
	M	G	M	G	M	G	M	G
Asni	5.5	7.0	2270	2610	2020	1700	11.4	34.9
Tissa	5.5	7.0	1990	2720	1800	1800	9.5	33.8
Arig 8	3.0	5.0	2330	3680	1830	2640	21.4	28.3
Rabat 071	6.5	7.0	2720	4100	1820	2540	33.1	38.0
UC 76227	1.3	3.0	2270	3340	2490	2620	-9.7	21.5
Mean	4.3	5.8	2316	3290	1990	2660	14.1	31.3

CV (%) Merchouch = 15.4 CV (%) Guich = 21.0
LSD (0.05) Merchouch = 464.5 LSD (0.05) Guich = 582.7

Note: a Symptoms scored on a scale of 0-9 (0 = no symptoms; 9 = very susceptible).

The average grain yield losses attributable to BYDV were 14.1% and 31.3% at Merchouch and Guich, respectively. The yield loss at Guich was statistically significant and was higher than that at Merchouch because of a more severe attack at the former site. The cultivars used showed differences both in their BYDV scores and in the extent of the damage caused by the virus. The ranking of the cultivars for BYDV scores was similar for both sites. The resistant line UC 76227, having a Yd_2 gene, showed the lowest BYDV scores at both locations; it was not affected by BYDV at Merchouch but there was a 21.5% yield loss at Guich as a result of the more severe attack. This indicates that the effectiveness of the Yd_2 gene depends on the level of the infection. At both sites, the highest yield loss was shown by the landrace, Rabat 071. The newly released two-row barley cultivars Asni and Tissa showed intermediate and significant yield reductions, respectively.

These yield loss estimates were similar to those obtained by El Holoui and Tagine (1985) on the same set of cultivars under natural infection at Merchouch and Guich in the 1984-85 season. However, they were lower than those obtained by Yount et al. (1985) for barley in Montana, USA and by El Yamani and Hill (1990) for bread wheat in Morocco. The greater losses observed by these researchers could be explained by the more severe infections obtained after artificial inoculation.

In 1983-84, when only natural infection was allowed, there no significant grain yield differences between the inoculated and non-inoculated treatments. Only a few plants in each plot showed BYDV symptoms (*see* Table 3). The six-row barley cultivars Arig 8 and Rabat 071, along with the resistant cultivar UC 76227, did not suffer losses from natural infection; instead, there was an increase in grain yield which was attributed to the effects of the insecticide

on other pests or on plant growth. The two-row barley cultivars Asni and Tissa showed the highest losses. Subsquent observations in the field suggest that the two-row barleys are more damaged by BYDV than the six-row cultivars (unpublished data). The low estimates obtained in this study could be explained by a delayed natural infection which took place at stem elongation and by the low aphid population. These losses were similar to those obtained by El Yamani and Hill (1990) with late inoculation.

Table 3 **Grain yield and BYDV symptom scores of five barley cultivars evaluated under natural infection at Merchouch Experimental Station, Morocco, 1983-84**

Cultivar	Symptom score[a]	Grain yield (kg/ha) Non-inoculated/ sprayed	Non-inoculated/ unsprayed	% Yield loss
Asni	3.5	3275	2850	13.0
Tissa	3.5	3275	2925	10.7
Arig 8	3.0	2750	2750	-13.4
Rabat 071	3.0	2400	2412	0.0
UC 76227	1.0	2850	3125	-9.6
CV (%) = 15.4				
LSD (0.05) = 450.7				

Note: a Cultivars scored on a scale of 0-9 (0 = no symptoms; 9 = very susceptible).

Estimates of yield loss using near-isogenic lines

The near-isogenic lines Atlas 68 (+ Yd_2) and Atlas 57 (- Yd_2) did not differ in their reactions to powdery mildew and blotch; for BYDV, however, the scores were 3 and 7, respectively (*see* Table 4). Under artificial inoculation, the yield loss of 9% at Merchouch in 1982-83 was lower than the estimated loss when an insecticide was used. Under natural infection in the 1983-84

Table 4 **Grain yield and BYDV symptom scores of barley near-isogenic lines (Yd_2) isolated at Merchouch Experimental Station, Morocco, 1982-83**

Isoline	Symptom score[a] Powdery mildew	Net blotch	BYDV	Grain yield (kg/ha)	% yield loss
Atlas 68 (+ Yd_2)	6	4	3	5650	—
Atlas 57 (- Yd_2)	6	4	7	5190	9

Note: a Cultivars scored on a scale of 0-9 (0 = no symptoms; 9 = very susceptible).

season, the losses were 24% and 7% at Merchouch and Tessaout, respectively (*see* Table 5). The 7% and 9% losses were not significant. The BYDV-resistant isoline Atlas 68 showed symptoms of BYDV, indicating that the gene Yd_2 does not completely suppress infection.

Table 5 **Grain yield (kg/ha) of barley near-isogenic lines evaluated under natural BYDV infection at Merchouch and Tessaout Experimental Stations, Morocco, 1983-84**

Isoline	Merchouch	% yield loss	Tessaout	% yield loss
Atlas 68 (+ Yd_2)	4542	—	4667	—
Atlas 57 (- Yd_2)	3580	24	4350	7

CONCLUSION

Based on its widespread occurrence and impact on grain yields, BYDV can be considered an important constraint to barley production in Morocco. The use of the Yd_2 gene can reduce the impact of the virus, but its effectiveness depends on climatic conditions, infection level and genetic background.

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2.3

Survey of Barley Yellow Dwarf Virus in Small-Grain Cereals in the Ethiopian Highlands

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SUMMARY

Surveys were carried out in 1985-86 and 1988-89 growing seasons to determine the prevalence of barley yellow dwarf virus (BYDV) in the major small-grain cereal growing regions of the Ethiopian highlands. In most of the lcoations surveyed, a low incidence of BYDV symptoms such as yellowing, reddening, stunting and rosetting was observed. The survey results indicated that BYDV was prevalent in wheat, barley and oats in the Shewa, Arsi and Bale administrative regions (1800-3000 m above sea level). These results were confirmed by direct and indirect ELISA tests using antibodies against the PAV and MAV isolates of BYDV. Symptoms of the virus were also observed in cereals in the Gojam, Gonder, Hararge and Wellega administrative regions but the presence of BYDV was not confirmed serologically. The main aphid vectors recorded in the highlands were *Rhopalosiphum maidis*, *R. padi*, *Sitobion avenae*, *Schizaphis graminum* and *Diuraphis noxia*.

In Ethiopia small-grain cereals, including barley, wheat, oats and other grasses, are grown in areas which have an annual precipitation ranging from 300 mm to more than 1000 mm. In most areas they are cultivated in rotation with cool-season food legumes. All these cereals are hosts of barley yellow dwarf virus (BYDV) (Wiese, 1977; Fargette et al., 1982), which is persistently transmitted by aphids (Jedlinski, 1981). Symptoms of BYDV have been observed in cereals in Ethiopia (Stewart and Dagnachew, 1967; Torres, 1984).

Barleys which are highly resistant to BYDV have been collected from the country and an analysis of these samples showed variations in levels of resistance, with an increase in resistance among samples collected from higher elevations (Qualset, 1975). Although the virus has been considered to be of minor importance in Ethiopia, it is likely that different small-grain cereals grown together could act as reservoirs of BYDV and its aphid vectors. Several species of the known aphid vectors of BYDV, including *Rhopalosiphum maidis* (Fitch.), *R. padi* (L.), *Sitobion avenae* (Fabr.) and *Schizaphis graminum* (Rond.) have been recorded in the country (Crowe and Kemal, 1983).

In the past, information on BYDV in Ethiopia was based mainly on visual field diagnosis. More recently, surveys have been conducted and the wheat and barley samples collected during these surveys have been tested serologically at Purdue University, USA and at the International Center for Agricultural Research in the Dry Areas (ICARDA). The results of these surveys, reported here, confirm the presence of BYDV in almost all cereal crops grown in Ethiopia.

MATERIALS AND METHODS

Surveys of cereal fields were conducted in the 1985-86 and 1988-89 growing seasons in the Shewa, Arsi, Bale, Gojam and Gonder administrative regions of Ethiopia. In 1985-86, the disease diagnosis was based only on visual symptoms and few symptomatic plants were tested by enzyme-linked immunosorbent assay (ELISA). In 1988-89, 100 random samples were collected from each field visited; plants with symptoms suggestive of BYDV infection were also collected. The samples were tested by indirect ELISA, using a monoclonal antibody (MC32-49) provided by Dr S. Wyatt (USA), as well as by direct ELISA using the PAV (B) antiserum provided by Bioreba, Switzerland.

RESULTS AND DISCUSSION

In the 1985-86 season, a low incidence of BYDV-like symptoms (leaf yellowing, reddening, rosetting, stunting) was observed in most locations surveyed. The presence of BYDV antigen was confirmed in wheat and barley samples from Bekoji, Merero and Goffer (2500-3000 m above sea level) (*see* Table 1). The results indicated the presence of PAV-like serotypes.

Table 1 **Results of ELISA tests conducted to detect BYDV in barley and wheat samples collected in central Ethiopia, 1985-86**

Collection site	Crop	ELISA values (A405) PAV	MAV
Bekoji	Barley	0.208	0.594
Bekoji	Wheat	0.151	0.461
Goffer	Wheat	0.103	0.144
Merero	Wheat	0.185	0.607
Asasa	Wheat	0.045	0.137
Dixis	Wheat	0.036	0.152
Huruta	Wheat	0.039	0.137
PAV standard[a]		1.300	1.710
MAV standard[a]		0.093	1.931
RPV standard[a]		0.033	0.127
Healthy check (oats)		0.034	0.147

Note: a Fresh, recently infected tissue.

The 1988-89 survey covered a wider cereal growing area. The results of the ELISA tests conducted to detect BYDV in the cereal samples collected are summarized in Table 2. BYDV was detected in 19 of the 20 fields surveyed, an indication of its wide prevalence in Ethiopia. However, in 10 of these fields the virus was not detected in all leaf groups tested. For example, in the five samples from Arsi-Negele (25 leaves collected), BYDV was detected only in two, indicating that at least 60% of the collected leaves from that field thought to be BYDV-infected because of their yellowish color were in fact not infected. These findings emphasize the importance of carrying out local serological surveys on a large number of samples before reaching precise conclusions. The surveys also showed that aphids occurred in different crop stages and intensities and that their population varied from location to location. Similar observations have been made at research centers and state farms in Ethiopia.

The low incidence of BYDV might be attributed to reduced aphid activity or transmission efficiency. Barley and wheat fields in Ethiopia are cultivated with landraces that originated under a vast range of agroecological conditions. These genetically diverse landraces probably

Table 2 Results of ELISA tests conducted to detect BYDV in cereal leaves collected in central Ethiopia, 1988-89

Collection site	Crop	No. of leaf groups tested	No. of groups found to be BYDV positive Indirect ELISA (MC32-41)[a]	Direct ELISA PAV (B)[b]
Shewa Region:				
Ambo (PPRC)[c]	Barley	2	2	1
Altufa	Wheat	5	0	0
Near Ambo	Wheat	3	3	3
Chacha	Barley	10	6	7
	Oats	5	3	0
Dodota	Wheat	5	4	5
Arsi-Negelle	Wheat	5	2	2
Sheno	Barley	5	4	1
Sululta	Barley	5	5	5
Warabi	Oats	4	2	0
	Barley	5	5	5
Kasochangi	Barley	4	1	4
Debre Zeit	Barley	5	0	3
Arsi Region:				
Kulumsa	Wheat	5	4	5
Sagure	Wheat	5	3	0
Anagero	Wheat	5	4	0
Bekoji	Wheat	5	5	5
Asasa	Barley	3	0	3
Chancho	Barley	3	3	3

Note: a Monoclonal antibodies obtained from S. Wyatt, USA.
b Polyclonal antibodies obtained from Bioreba, Switzerland.
c Plant Protection Research Center, Ambo.

have an in-built tolerance to BYDV. In recent years there has been an increase in the intensive monocropping of geneticaly uniform varieties of small-grain cereals in the highlands of the Arsi and Bale regions, and it was in these areas that BYDV was found to be widely prevalent, although still with a low incidence. It is clear from the findings emerging from the recent surveys that a more detailed study of the distribution of BYDV and its isolates in Ethiopia needs to be conducted.

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2.4

Studies on Barley Yellow Dwarf Virus in Cereal Crops in Jordan

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SUMMARY

A study was initiated in 1987 to isolate and identify different strains of barley yellow dwarf virus (BYDV) in Jordan. Additional studies were conducted on the incidence, economic importance and epidemiology of the virus. The strains identified included MAV, PAV, RPV and RMV, either separately or in mixed infections. The incidence of BYDV reached 22%, 35%, 59% and 65% in wheat, barley, sorghum and corn, respectively. BYDV was detected in all wild grasses tested and this, coupled with the presence of the aphid vector species *Rhopalosiphum maidis*, *R. padi*, *Sitobion avenae* and *Schizaphis graminum*, may account for the high incidence of the disease found in cereals. BYDV reduced the plant height and grain yield in both wheat and barley. Among the wheat and barley cultivars tested, Hourani (wheat) and Acsad 176 (barley) showed the greatest tolerance of BYDV.

Wheat and barley are the most important cereal crops grown in Jordan. The area under these cereals covers some 187 000 ha and the average yield is 875 kg/ha for wheat and 667 kg/ha for barley. These low yields are attributable to a number of factors, including diseases. The most important disease affecting wheat and barley crops in Jordan is barley yellow dwarf. Prior to the study described in this paper, assessments of the incidence of barley yellow dwarf virus (BYDV) in Jordan were based solely on observations of field symptoms. This study was initiated to identify the BYDV strains present in the country and their vectors.

MATERIALS AND METHODS

For the direct enzyme-linked immunosorbent assay (ELISA) tests used in this study, antisera specific to B (PAV) and F (MAV) strains of BYDV were obtained from Bioreba, Switzerland. For the indirect ELISA tests, monoclonal antibodies against PAV, MAV and RPV strains were supplied by Dr S. Wyatt, USA. Goat anti-mouse alkaline phosphatase conjugate was purchased from Sigma Chemical Company.

Virus isolation

Leaf samples were collected in the Jordan Valley from stunted wild oat (*Avenae sterilis* L.) plants showing reddish-purple leaves. The virus strains were isolated using three aphid species, *Rhopalosiphum padi* (L.), *Sitobion (Macrosiphum) avenae* (Fabr.) and *R. maidis* (Fitch.) on the oat cultivar Clintland 64. Plants that reacted with symptoms similar to those found in the field were kept under glasshouse conditions at 20 ± 2°C. The identity of different strains was determined by serological tests using direct or indirect ELISA (Lister and Rochow, 1979; Koenig, 1981; Clark et al., 1986).

Efficiency of aphid transmission

Several species of wingless aphids were collected from cultivated or wild cereals in the Jordan Valley and were identified using the field identification key devised by Cohen (1974). To confirm the identification, specimens were sent to the British Museum, UK.

As the PAV strain is known to cause severe disease and to be efficiently transmitted by *R. padi* (Rochow, 1969), detailed aphid transmission experiments were carried out using PAV-infected plants and *R. padi* as the vector. Non-viruliferous apterous aphids were transferred with a camel-hair brush to oat plants infected with the PAV isolate for 2 days at 15°C. Ten aphids were transferred to 2-week-old healthy seedlings of Clintland 64 and allowed a 5-day inoculation feeding period. The aphids were then killed by insecticide and the plants were kept under glasshouse conditions at 20 ± 2°C and observed for symptom development.

Virus strain and vector prevalence

Virus strain and vector predominance was assessed in the Deir-Alla area, using Clintland 64 seedlings as the bait plant. The seedlings were planted in 12 x 13 cm pots, at the rate of one seedling per pot. Between 9 February 1987 and the end of the growing season (first week of May), 20-25 pots were placed weekly near wheat fields. After 1 week's exposure, aphids were identified and counted. The plants were then sprayed with insecticide and grown for 2 weeks in the glasshouse. Each plant was tested by ELISA for the presence of MAV, PAV and RPV strains.

BYDV incidence in cereals and wild grasses

The incidence of BYDV in cereal crops was assessed in 2184 leaf samples from corn, barley, sorghum and wheat plants. The samples had been collected at monthly intervals from fields randomly located along the Jordan Valley and in the highlands during the 1987 and 1988 growing seasons (*see* Table 1). Sampling was done by walking in the field in an 'X' pattern, collecting leaves. Five samples were taken from five randomly chosen locations in each field; each sample represented 10 different plants. All collected samples were tested by ELISA to detect the virus.

Table 1 Cereals samples collected in Jordan in the 1987-88 growing season

Year	Location	Crop	No. of samples collected[a]
1987	Irbid	Wheat	315
		Barley	45
	Karak	Wheat	287
	Madaba	Wheat	90
	Jordan Valley	Wheat	469
		Barley	151
	Jordan Valley and Irbid	Corn	85
	Jordan Valley and Irbid	Sorghum	139
1988	Irbid	Wheat	195
		Barley	67
	Jordan Valley	Wheat	264
		Barley	77

Note: a Corn and sorghum samples were collected between May and July 1987; wheat and barley samples were collected between January and May 1988.

A total of 240 leaf samples of the most common wild grasses were collected from random sites in the Jordan Valley. The grasses were identified by Dr. D. Al Eisawi, and included *Alopecurus myosuroides* Hudson, *Avena sterilis* L., *Cynodon dactylon* (L.) Pers., *Phalaris brachystachys* Link, *Lolium rigidum* Caud, *Sorghum halepense* (L.), *Polypogon monspeliensis* (L.) Desf., *Hordeum leporinum* Link., *Bromus rubens* L., *Eragrostis cilianensis* (All.) Vignlut, and *Stipa capensis* Thunb. ELISA was used to test all samples against antisera to PAV, MAV, RPV and RMV strains.

Economic analysis

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

Nine wheat cultivars (Sham 1, Korifla, F.8, Stork, Deir-Alla 2, Acsad 65, Hourani, Deir-Alla 6 and Deir-Alla 4) and four barley cultivars (Deir-Alla 106, Rum, Line 1 and Acsad 176) were seeded in methyl bromide fumigated soil in 12 x 13 cm plastic pots on 3 December 1987. A randomized complete block design was used. In each of the three replications, four plants of each cultivar were grown under glasshouse conditions at 20±2°C; half of them were inoculated at the 3-leaf stage by PAV, using *R. padi*. The aphids were then killed with an insecticide. The non-inoculated plants were infested with non-viruliferous aphids and acted as the control.

Over a period of 35 days after inoculation, plants were examined daily for symptom expression. Plant height, severity of symptoms and grain yield were recorded for individual plants of each cultivar. The paired comparison test was used to evaluate the effect of disease on plant height and grain yield within each cultivar, and Duncan's Multiple Range Test was used to evaluate reductions in plant height and grain yield.

RESULTS

Virus isolation

The MAV isolate transmitted by *S. avenae* produced mild symptoms and could not be transmitted by *R. padi*. The PAV isolate transmitted by both *R. padi* and *S. avenae* produced severe symptoms, and was used for further studies. The occurrence of other mild strains, such as RMV and RPV, was confirmed by serological or aphid transmission tests. *R. maidis* transmitted the RMV isolate, whereas *R. padi* transmitted the RPV isolate.

Efficiency of aphid transmission

Studies on the efficiency of four aphid species in transmitting the PAV strain indicated that *R. padi* was the most efficient vector, followed by *S. avenae* and *S. graminum* (*see* Table 2). An unexpected finding was that *R. maidis* did not transmit PAV to any of the tested plants, although studies in North America have shown that PAV isolates can be transmitted by *R. maidis* at a low frequency.

Table 2 Efficiency of four aphid species in transmitting the PAV isolate of BYDV

Aphid species	No. of infected plants/ no. of inoculated plants	% virus infection
Rhopalosiphum padi	15/16	94
Sitobion avenae	10/16	62
Schizaphis graminum	3/16	19
R. maidis	0/16	0
Check	0/16	0

Virus strain and vector prevalence

Three aphid species (*R. padi*, *S. graminum* and *S. avenae*) appeared suddenly on the Clintland 64 bait plants in the second week of February. Populations of *R. padi* and *S. graminum* reached high levels in the first and fourth weeks of April, and then decreased in the first week of May. The population of *S. avenae* showed slight increase in the first and third week of March. The relative abundance of the aphid species on the bait plants is given in Table 3. As shown in Table 4, 87% of the bait plants exposed during this period were found to be infected with BYDV.

BYDV incidence in cereals

The incidence of BYDV in wheat and barley samples collected during the 1987 growing season is summarized in Table 5 (*overleaf*). The collections were made in the Jordan Valley and in the highland areas of Irbid, Karak and Madaba.

Table 3 **Number of aphids collected from oat bait plants exposed for 1 week at Deir-Alla Experimental Station, Jordan, 1987**

Exposure date		No. of aphids collected/plant				Total
		Rhopalosiphum pali	*Schizaphis graminum*	*Sitobion avenae*	*Rhopalosiphum maidis*	
February	9	38	12	6	0	56
	16	39	9	11	1	60
	23	35	3	12	0	50
March	2	10	5	19	1	35
	9	6	11	5	0	22
	16	2	0	23	0	25
	23	25	3	6	0	34
	31	6	10	9	1	26
April	7	40	71	0	0	111
	14	29	24	0	0	53
	21	3	21	10	0	34
	28	63	74	9	0	146
May	5	13	4	2	0	19
Total		309	247	112	3	671

Table 4 **BYDV incidence detected by ELISA in the oat bait plants exposed for 1 week at Deir-Alla Experimental Station, Jordan, 1987[a]**

Exposure date		No. of plants	No. of plants infected with				Total no. positive samples (% of total no. tested)	
			MAV	PAV	RPV	PAV + RPV		
February	9	24	20	0	0	0	20	(83)
	16	23	21	0	0	0	21	(91)
	23	24	23	0	0	0	23	(96)
March	2	24	25	0	0	0	25	(100)
	9	22	0	0	0	22	22	(100)
	16	16	0	2	0	14	16	(100)
	23	23	0	1	0	21	22	(91)
	31	24	0	1	0	21	22	(91)
April	7	25	0	1	0	18	19	(76)
	14	16	0	1	3	8	12	(75)
	21	9	0	0	2	3	5	(55)
	28	22	0	0	1	17	18	(81)
May	5	8	0	2	0	1	3	(37)
Total		260	89	8	6	125	228	(87)

Note: a Two polyclonal antibodies (MAV and PAV) were used in the direct ELISA; two monoclonal antibodies (PAV and RPV) were used in the indirect ELISA.

Table 5 **BYDV incidence detected by ELISA in wheat and barley leaf samples collected in the Jordan Valley and Irbid regions, Jordan, 1987**

Crop and collection date and site		No. of samples	No. of plants infected with MAV	PAV	RPV	RPV + PAV	Total no. of positive samples (% of total no. tested)
Wheat:							
Jan	Jordan Valley	191	21	1	—[a]	—	22 (11.5)
Feb	Jordan Valley	95	26	3	—	—	29 (30.5)
Mar	Jordan Valley	75	42	3	—	—	45 (60.0)
Apr	Jordan Valley	108	70	8	1	—	79 (73.0)
Jan	Irbid	75	0	0	—	—	0 (0.0)
Feb	Irbid	80	6	1	—	—	7 (8.8)
Mar	Irbid	80	5	2	—	—	7 (8.8)
Apr	Irbid	80	12	0	—	—	12 (15.0)
Feb	Karak	100	0	0	—	—	0 (0.0)
Mar	Karak	100	0	0	—	—	0 (0.0)
Apr	Karak	87	10	0	—	—	10 (11.5)
Feb	Madaba	30	0	0	0	0	0 (0.0)
Mar	Madaba	35	1	0	0	0	1 (2.9)
Apr	Madaba	25	—	0	1	3	4 (16.0)
Barley:							
Jan	Jordan Valley	60	12	0	—	—	12 (20.0)
Feb	Jordan Valley	30	12	2	—	—	14 (47.0)
Mar	Jordan Valley	30	14	2	—	—	16 (53.0)
Apr	Jordan Valley	31	9	3	1	5	18 (58.0)
Jan	Irbid	10	0	0	—	—	0 (0.0)
Feb	Irbid	15	3	0	—	—	3 (20.0)
Mar	Irbid	10	2	0	—	—	2 (20.0)
Apr	Irbid	10	3	1	—	—	4 (20.0)

Note: a — = samples not tested.

In the Jordan Valley, the incidence of BYDV in wheat varied from 11.5% in January to 73% in April. Of the total number of infected plants, 90.8% were infected with MAV, 8.6% with PAV and 0.6% with RPV. The incidence of BYDV in barley in the valley varied from 20% in January to 58% in April. Of the infected plants, 78.3% were infected with MAV, 11.7% with PAV and 1.7% with RPV; the remaining samples showed mixed infections of RPV and PAV.

In Irbid, samples were collected from January to April. The virus was not detected in wheat or barley in January but by April the incidence of BYDV infection had reached 15% in wheat and 20% in barley. Of the infected wheat plants, 88.5% were infected with MAV and 11.5% with PAV. Of the infected barley plants, 88.9% were infected with MAV and 11.1% with PAV.

In Karak, samples were collected from February to April and BYDV incidence ranged from 0% in February to 11.5% in April; all infected samples were of the MAV type. In Madaba, samples were collected from February to April and BYDV infection ranged from 0% in February to 16% in April; a mixed infection of RPV and PAV was found in 60% of the samples, and MAV and RPV were found singly in 20% of the samples.

The incidence of BYDV in the samples of corn and sorghum which were collected during 1987 in the Jordan Valley and the Irbid area is summarized in Table 6. In the Jordan Valley, of the 71 samples of corn which were collected, 68% were found to be infected, 35.4% of them with PAV, 20.8% of them with RPV and the remainder with a mixed infection of PAV and RPV. In Irbid, of the 14 corn samples collected, 50% were infected, all of them with PAV. Of the 47 sorghum samples collected in the Jordan Valley, 47% were infected; the percentage of plants which were infected with PAV, RPV or a mixture of both types was 45.7%, 11.4% and 42.9%, respectively. In Irbid, of the 92 sorghum samples collected, 51% were infected; the percentage of plants infected with PAV, RPV or a mixture of both types was 46.8%, 25.5% and 27.7%, respectively.

Table 6 Seasonal incidence of BYDV in Jordan, 1987 and 1988

Year	Crop	Average seasonal incidence (%)	
		Jordan Valley	Irbid
1987	Wheat	37	6
	Barley	40	20
	Corn	68	50
	Sorghum	74	51
	Grasses	64	—
1988	Wheat	28	19
	Barley	42	22
	Grasses	72	—

The incidence of BYDV in wheat and barley samples which were collected in the Jordan Valley and the Irbid area during the 1988 season is summarized in Table 7 (*overleaf*). In the Jordan Valley, the percentage of wheat plants infected ranged from 1.4% in January to 49% in April; PAV, MAV and RMV strains of BYDV were detected in the samples. The percentage of barley plants infected ranged from 0% in January to 66.6% in April; PAV, MAV and RMV strains, either singly or in mixed infections, were detected. In Irbid, BYDV incidence in wheat ranged from 0% in January to 35% in April. In barley, it ranged from 13.3% in January to 22.7% in April.

The seasonal incidence of all BYDV isolates in cultivated cereals ranged between 37 and 74% in the Jordan Valley and between 6 and 51% in the highlands. The seasonal incidence in wheat and barley ranged between 28 and 42% in the Jordan Valley and between 19 and 22% in the highlands.

Table 7 **BYDV incidence detected by ELISA in wheat and barley leaf samples in the Jordan Valley and Irbid regions, Jordan, 1988**

Crop and collection date and site		No. of samples	No. of plants infected with MAV	PAV	RPV	Mixed	Total no. of positive samples (% of total no. tested)
Wheat:							
Jan.	Jordan valley	70	1	0	0	0	01 (1.4)
Feb.	Jordan valley	70	1	0	0	6	7 (27.0)
Mar.	Jordan valley	69	1	6	4	7	18 (40.5)
Apr.	Jordan valley	55	1	2	0	11	14 (49.0)
Jan.	Irbid	45	0	0	0	0	0 (0.0)
Feb.	Irbid	45	1	1	2	0	4 (8.8)
Mar.	Irbid	45	2	2	2	4	10 (22.2)
Apr.	Irbid	60	1	9	1	10	21 (35.0)
Barley:							
Jan.	Jordan valley	24	0	0	0	0	0 (0.0)
Feb.	Jordan valley	18	2	0	—[a]	2	4 (22.2)
Mar.	Jordan valley	20	0	1	3	4	8 (40.0)
Apr.	Jordan valley	15	0	2	1	7	10 (66.6)
Jan.	Irbid	15	1	0	1	10	2 (13.3)
Feb.	Irbid	15	0	0	0	0	0 (0.0)
Mar.	Irbid	15	0	1	0	0	1 (13.3)
Apr.	Irbid	22	0	1	1	3	5 (22.7)

Note a — = samples not tested.

BYDV incidence in wild grasses

BYDV incidence in the various grass species collected was determined by serological testing using direct and indirect ELISA. As shown in Table 6, BYDV was detected in 64% and 72% of the samples tested in 1987 and 1988, respectively. In general, all grasses tested were found to be infected with BYDV, with the incidence ranging from 38 to 75% in 1987 and from 42 to 100% in 1988 (*see* Table 8 *opposite and* Table 9 *overleaf*). In 1987 the MAV strain seemed to be the dominant type, followed by RPV and PAV either singly or in mixed infections, and the highest incidence of infection was recorded in *L. rigidium*, *A. sterilis* and *P. monspeliensis*. In 1988 a mixed infection of RPV and PAV seemed to be the most common, and the highest incidence of BYDV was recorded in *A. myosuroides*, *B. rubens* and *P. monspeliensis*. BYDV incidence early in the 1988 growing season (February-March) was 68.6%, whereas later in the season (April-May) it was 50%.

Economic importance of BYDV

The reductions in grain yield and plant height as a result of BYDV infection in wheat and barley cultivars are presented in Table 10 (*overleaf*). In wheat, the grain yield reduction ranged from

Table 8 BYDV incidence detected by ELISA in grass species collected in the Jordan Valley and the Irbid regions, Jordan, 1987[a]

Grass species (common name)	No. of samples	No. of plants infected with MAV	PAV	RPV	PAV + RPV	No. of positive samples (% of total no. tested)
Alopecurus myosuroides (Littoral grass)	10	3	0	0	3	6 (60)
Avena sterilis (Wild oat)	39	16	4	4	5	29 (74)
Bromus rubens (Opened-awned brome grass)	4	2	0	0	0	2 (50)
Cynodon dactylon (Bermuda grass)	16	5	1	0	3	8 (56)
Eragrostis cilianensis (Spreading love-grass)	13	5	0	0	0	5 (38)
Hordeum leporinum (Wild barley)	2	1	0	0	0	1 (50)
Lolium rigidum (Rigid rye-grass)	26	13	1	2	3	19 (73)
Phalaris brachystachys (Short-spike canary grass)	39	14	0	0	11	25 (64)
Polypogon monspeliensis (Annual beard-grass)	4	2	0	0	2	4 (75)
Sorghum halepense (Johnson grass)	15	1	1	2	5	9 (60)
Total	168	62	7	8	32	108 (64)

Note: a Two polyclonal antibodies (F and B) were used in the direct ELISA; two monoclonal antibodies (PAV and RPV) were used in the indirect ELISA.

41.61% in Hourani to 75.26% in Deir-Alla 2, compared to that of the healthy control. The plant height reductions were highest in the Sham 1, Deir-Alla 6 and Deir-Alla 4 cultivars and lowest in the Acsad 65 and F.8 cultivars.

Grain yield reduction in barley cultivars ranged from 40.6% in Acsad to 72% in Rum, compared to that of the healthy control. The yield reduction in Acsad was significantly lower than that in any of the other cultivars tested. Plant height reduction in barley cultivars ranged from 24.6% in Rum to 31.6% in Acsad, with no significant difference among the tested cultivars.

Table 9 **BYDV incidence detected by ELISA in grass species collected in the Jordan Valley and the Irbid regions, Jordan, 1988[a]**

Grass species	No. of samples	No. of plants infected with MAV	PAV	RPV	Mixed	No. of positive samples (% of total no. tested)
Alopecurus myosuroides	5	0	0	0	4	4 (80)
Avena sterilis	17	0	0	1	10	11 (65)
Bromus rubens	3	0	1	0	2	3 (100)
Cyndon dactylon	3	0	0	0	2	2 (67)
Hordeum leporinum	9	0	1	0	4	5 (55)
Lolium rigidum	9	0	1	1	4	6 (67)
Phalaris brachystachys	12	1	0	0	4	5 (42)
Polypogon monspeliensis	9	1	1	0	6	8 (89)
Sorghum halepense	4	2	0	0	0	2 (50)
Stipa capensis[a]	1	0	0	0	0	1 (100)
Total	72	4	4	2	36	47 (65)

Note: a Twisted-awned spear grass.

Table 10 **Percentage reduction in plant height and grain yield of wheat and barley cultivars in response to BYDV infection**

Cultivar	% reduction in plant height	% reduction in yield/plant
Wheat:		
Sham 1	25.5 a[a]	57.5 a, b
Korifla	13.9 b, c	63.1 a
F.8	9.8 c	67.1 a
Stork	17.5 a, b, c	57.0 a, b
Deir-Alla 2	21.7 a, b	75.3 a
Acsad 65	8.4 c	62.0 a, b
Hourani	13.2 b, c	41.2 b
Deir-Alla 6	25.5 a	56.9 a, b
Deir-Alla 4	25.6 a	73.6 a
Barley:		
Acsad 176	31.6 a	40.7 b
Line 1	25.0 a	65.7 a
Deir-Alla 106	25.1 a	70.2 a
Rum	24.6 a	72.0 a

Note: a Values in the same column followed by the same letter do not differ significantly at $p = 0.05$ (Duncan's Multiple Range Test). Statistical analysis of the wheat and barley cultivars are independent.

CONCLUSION

Based on the host range, aphid transmission tests and serological tests, four isolates of BYDV (PAV, MAV, RPV and RMV) were identified in Jordan, with the predominant ones being PAV and MAV. All the isolates appeared to have a similar host range, although they differed in symptom severity on the oat bait plants, Clintland 64. This finding supports the results obtained by Rochow (1969, 1970, 1979). However, there were clear differences in efficiency of transmission by specific aphid species, with *R. padi* being the most efficient in transmitting the PAV strain. The fact that *R. padi* transmitted the virus from all samples that were evaluated as RPV- or PAV-positive by ELISA substantiates the validity of ELISA as a tool for detecting BYDV in infected tissue.

BYDV was found to be common and widespread in wild grasses throughout the Jordan Valley and elsewhere in the country. The high incidence of BYDV in the grasses studied points to high inoculum potential which, in the presence of the insect vector, could play an important role in the epidemiology of BYDV in cereal crops. Corn and sorghum in the Jordan Valley also appeared to be important virus sources in late autumn for wheat and barley.

Wheat, barley, corn, sorghum and grasses (particularly perennial grasses) all showed some degree of BYDV infection and harbored aphid vectors, and thus they all play a role in the ecology of BYDV. Wheat and barley which are the main winter hosts, while sorghum and grasses act as oversummering hosts. It is likely that the perennial grasses constitute the primary inoculum source of BYDV for early planted cereal crops.

In terms of yield losses, the studies indicated that some cultivars (such as the wheat cultivar, Hourani, and the barley cultivar, Acsad 176) are more tolerant of the virus than others. In general, however, the BYDV-related yield losses in cereals observed in the studies indicate that the virus is economically important in Jordan.

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2.5

The Incidence of Barley Yellow Dwarf Virus in Barley in Egypt

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SUMMARY

In a study conducted in Egypt, barley yellow dwarf virus (BYDV) was recorded in various areas at different levels of incidence. The highest incidence was found in the Giza, Kalubia and Alexandria governorates; in the Assiout governorate no incidence was observed. Under field conditions, many barley cultivars and breeding lines showed a good level of BYDV tolerance. Results showed 794 out of the 928 entries evaluated had good levels of resistance. Five Egyptian barley varieties (Borg El-Arab, Marsa Matrouh, Bahtim 52, Giza 24 and Baladi 16) were found to be BYDV resistant under the field conditions. However, with the generally low BYDV incidence in the field, it was not possible to draw strong conclusions on the basis of natural infection.

The disease caused by barley yellow dwarf virus (BYDV) was first observed in barley fields in Egypt in 1977 (Abdel-Hak and Ghobrial, 1984). Since then, with the increase in aphid populations in some areas of the country, the problem has grown (Ghobrial et al., 1984a; Shafik et al., 1989). Many workers have reported that BYDV is capable of causing considerable damage to susceptible barley varieties (Oswald and Houston, 1951; Bruehl, 1961; Tetrault et al., 1963; Timian and Jensen, 1964; Comeau, 1984; Kinanci and Yakar, 1984; Schaller and Qualset, 1984). In general, it has been found in Egypt in late-seeded fields (December) (Abdel-Hak and Ghobrial, 1984). The most effective control measure seems to be the use of resistant cultivars (Suneson, 1955; Rasmusson and Schaller, 1959; Timian, 1975; Abdel-Hak and Ghobrial, 1984; Ghobrial et al., 1984b; Shafik et al., 1989). The study reported here was carried out during 1988 to assess the incidence of BYDV in barley fields in Egypt and to investigate the reaction of exotic genotypes and commercial varieties to BYDV under natural conditions.

MATERIALS AND METHODS

Samples of virus-infected plants were collected annually, in late March and early April, from growers' fields in various governorates in the delta, middle and southern regions of Egypt. The

collections took place at 30 km intervals, from about 50 fields in each governorate, and the average incidence of BYDV for each locality was calculated. In addition, a set of 47 different barley trap varieties were grown during the first week of December in four governorates. A survey was also conducted along the north coast, involving 14 locations (at Amria, King Mariout, El-Howoried, Bahig, Borg El-Arab, El-Hammam, Marsa Matrouh, El-Kasr, Abo-Lahow and Agiba) with a distance of about 10 km between each location. Three sets of barley trap varieties were planted at Nubaria, El-Kasr and Abo-Lahow.

To confirm the presence of the disease, virus-free apple grain aphids, *Rhopalosiphum padi* (L.), were placed on selected samples of barley in petri plates (Timian and Jensen, 1964). After 48 hours, the aphids were transferred to caged black hulless barley seedlings grown in clay pots (15 cm diameter) for 7 days. After a further 48 hours, the aphids were killed and the plants were incubated at 25°C for 7-10 days. Inoculated plants were inspected for disease symptoms.

To study the performance of barley genotypes under natural infection in the field, 928 crosses and 13 cultivars were planted at the Sakha, Sear El-Layan, Bahtim and Giza Experiment Stations, each station representing different climatic conditions. The genotypes were planted in single rows, 3.5 m long and 30 cm apart. The percentage of plants showing BYDV symptoms was recorded.

RESULTS AND DISCUSSION

Growers' fields

Based on the symptoms observed, the survey revealed that BYDV occurred in all governorates except Assiout. Infected plants were found in about 10% of the fields visited. The average incidence of BYDV in the governorates ranged from 0.06 to 0.70% (*see* Table 1).

BYDV was less prevalent in the 14 locations investigated along the north coast. Only two fields were found to have a BYDV infection (ranging from 0.5 to 2%). The average incidence of BYDV in the 14 locations was 0.02%.

Barley trap varieties

The results from the experiments involving barley trap varieties grown in four governorates and at three locations on the north coast were similar to those obtained from growers' fields (*see* Table 2). The reaction of cultivars varied according to location and the presence of *R. padi*. The highest incidence of BYDV was observed in the Alexandria, Giza and Kalubia governorates and along the north coast.

Screening for resistance

Through screening, the most susceptible genotypes were eliminated. Of the 928 crosses screened under natural infection, 794 showed no BYDV symptoms (*see* Table 3 *overleaf*). Of the 13 cultivars screened, the lowest incidence of BYDV occurred in Borg El-Arab, Marsa

Table 1 BYDV incidence in barley growers' fields, Egypt, 1988

Governorate	% of infected fields	Average BYDV incidence in infected fields (%)	Average BYDV incidence in governorates (%)
Delta region:			
Alexandria	9	2.5	0.23
Behira	8	2.5	0.20
Damietta	4	3.0	0.12
Kafr El-Sheikh	4	3.0	0.12
Dakahlia	3	2.0	0.06
Isamielia	4	2.0	0.08
Gharbia	6	2.5	0.15
Henoufia	7	3.0	0.21
Kalubia	10	3.0	0.30
Middle region:			
Giza	10	7.0	0.70
Fayoum	3	2.5	0.08
Minia	3	2.0	0.06
Southern region:			
Assiout	0	0	0.00

Table 2 Percentage and level of BYDV infection in barley trap varieties grown at various locations in Egypt, 1988

Governorate		% barley trap varieties infected	Range of BYDV symptom scores[a]
Delta region:	Alexandria	19.1	1-5
	Behira	10.6	1-5
	Damietta	8.5	1-2
	Kafr-El-Sheikh	8.5	1-5
	Sharkia	6.4	1-5
	Ismaielia	6.4	1-3
	Gharbia	8.5	1-3
	Henoufia	10.6	1-3
	Kalubia	12.8	2-5
Middle region:	Giza	17.0	2-5
	Fayoum	6.4	1-5
	Minia	4.2	1-5
Southern region:	Assiout	0.0	0
North coast:	Nubaria	12.8	2-5
	El-Kasr	12.8	2-5
	Abo-Lahow	12.8	2-5

Note: a Symptoms scored on a scale of 0-9 (0= no symptoms; 9 = severe symptoms).

Table 3 **Results of tests on barley genotypes to determine resistance to BYDV under Egyptian conditions**

Designation	No. of entries tested	No. of entries showing no infection
Key Location Disease Nursery	360	315
Crossing Block	145	140
Barley Observation Nursery (LRA)[a]	91	75
Barley Observation Nursery (MRA)	84	74
Barley Yield Trial (LRA)	24	12
Barley Yield Trial (MRA)	24	23
Breeding Materials	200	155

Note: a LRA = Low Rainfall Areas; MRA = Moderate Rainfall Areas

Table 4 **Percentage of BYDV incidence in Egyptian barley cultivars planted at four locations in Egypt, 1988**

Cultivar	Sakha[a]	Sear El-Layan	Bahtim	Giza
Giza 117	7	12	6	18
Giza 118	14	12	0	18
Giza 119	21	18	0	12
Giza 120	21	18	0	12
Giza 121	0	0	41	0
Giza 122	7	6	29	12
Giza 24	0	6	0	12
Giza 16	7	6	0	0
Palestine 10	0	6	12	6
Nabawi	14	6	6	0
Borg El-Arab	0	0	0	0
Marsa Matrouh	0	0	0	0
Bahtim 52	0	0	0	0

Note: a 70 samples were collected at Sakha; 85 samples were collected at the other locations.

Matrouh, Bahtim 52, Giza 24 and Baladi 16 (*see* Table 4). However, further testing under artificial inoculation is required to confirm whether these cultivars have a true resistance or tolerance to BYDV.

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2.6

Barley Yellow Dwarf Virus Research in Kenya

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SUMMARY

Barley yellow dwarf virus (BYDV) was first reported in Kenya in 1984, but only recently has serious damage caused by this virus been reported in barley, wheat and oat crops in the country. Research on BYDV in Kenya began in 1986. Epiphytotics associated with the virus complex were observed in 1986-89 in all the major cereal growing regions. The situation appeared to be aggravated by the continuous cultivation of cereals. Serological tests carried out at Rothamsted Experimental Station, UK on wheat and barley samples collected in 1986 indicated the presence of PAV, MAV and RPV isolates. Important aphid vectors of BYDV in Kenya are *Rhopalosiphum padi*, *R. maidis*, *R. insertum*, *Metopolophium dirhodum*, *M. festucae*, *Sitobion avenae*, *S. fragariae* and *Schizaphis graminum*. At altitudes of 2500 m or more above sea level *R. padi* appears to be the most important species, while *M. dirhodum* occurs in large numbers in all areas.

Barley yellow dwarf is now recognized as one of the most important diseases affecting a wide range of plants in the family Gramineae, including wheat, barley oats, rice and maize (Bruehl et al., 1959; Rochow, 1970). The barley yellow dwarf virus (BYDV) is persistently transmitted by over 20 aphid species and is widely distributed in all cereal growing areas of the world (Rochow, 1970). The symptoms of BYDV infection in barley include yellowing of the leaves (starting from the tip and progressing towards the base), stunted growth and poor grain filling. In oats, the leaves of infected plants become reddish-purple and the heads are often sterile. The virus has generally received little attention in relation to wheat because of the ambiguity of the symptoms it induces; the stunting and light green to yellowish (sometimes reddish) foliage caused by BYDV in wheat are often mistaken for nutritional or non-pathogenic disorders. Yield losses attributed to BYDV infection in the USA have been estimated at 1-3% annually, but at 35-74% under conditions which favor the development and spread of the virus (Burnett, 1984).

In Kenya, sporadic occurrences of BYDV have been noted over the years, but the importance of the virus as a yield-reducing factor in cereals has been obscured by the high incidence of rusts and other fungal diseases. Recent results from controlled experiments, however, indicate that losses in wheat and barley yields resulting from BYDV infection may be as high as 47% and 27%, respectively (Wangai, 1987).

MATERIALS AND METHODS

Commercial farms in Kenya were surveyed for the occurrence, severity and abundance of BYDV and the different species of aphid vectors. The study covered all the major cereal and barley producing areas (Nakuru, Mau Escarpment, Trans-Nzoia, Uasin Gishu and Timau).

Random sampling was done at intervals of 5 km or more, and the plants were visually assessed for BYDV symptoms. Aphids in the field were monitored by direct inspection of the crop and by the use of sweep-nets. Air-dried leaf samples selected at random from cereal crops and some common grasses in the cultivated fields were sent to Rothamsted Experimental Station, UK for serological tests. Each sample was tested in duplicate against antibodies to the PAV, MAV and RPV isolates of BYDV.

RESULTS AND DISCUSSION

BYDV-like symptoms were observed in all the regions surveyed (*see* Table 1). In the Trans-Nzoia and Uasin Gishu regions BYDV incidence was relatively low; however, wheat was the major crop surveyed in these areas and the difficulties of diagnosing BYDV by symptoms in wheat may have led to an underestimation of the prevalence of the virus.

In Mau Escarpment, where about 90% of Kenya's barley is grown, BYDV incidence was rated as severe. The aphid population were very high, with the most common species being *Rhopalosiphum padi* (L.), *R. maidis* (Fitch.), *Schizaphis graminum* (Rond.), *Sitobion avenae* (Fabr.) *S. fragariae* Walker and *Metopolophium dirhodum* (Walker). *R. padi* was the most abundant species in the Nakuru and Mau Escarpment areas, while *M. dirhodum* and *S. avenae* were the dominant species in East Mau and Timau, respectively. In Nakuru in 1987 and 1988, BYDV occurred in isolated patches; the 1987 crop season was dry, but in 1988 there was an excessively wet crop season, and these conditions may have adversely affected the aphid populations.

Three BYDV isolates have been identified so far (Wangai et al., unpubl.). In 1986 and 1987, PAV-like isolates were identified from Nakuru and Mau Escarpment and MAV-like isolates from Timau. Samples collected from these areas in 1988 showed a mixed-infection of PAV- and RPV-like isolates. Samples collected from the Uasin Gishu and Trans-Nzoia regions appeared to have a mixed infection of PAV, MAV and RPV isolates. These tests were not exhaustive and further tests are being carried out.

Eight species of grasses have been identified as wild or alternate hosts of BYDV in Kenya (Wangai et al., unpubl.): *Bromus pectinatus*, *Digitaria scalarum*, *Dactylis glomerata*, *Setaria* sp., *Hyperraneae* sp., *Panicum coloratum*, *Chloris gayana* and *Avenae fatua*. These grasses are commonly found in the cereal growing areas, either as weeds or pasture grasses, and could act as reservoirs for both the virus and the aphid vectors.

Rainfall distribution in Kenya varies from region to region, and thus the cereal growing seasons differ. The earliest planting (February-April) takes place in the Timau, Mt Kenya and lower Narok regions, while the latest planting (June-September) takes place in Mau Escarpment. Thus, there is a green crop of cereals in the field for most of the year. This allows aphids and virus survival throughout the year, facilitates prolonged local movement and provides a possible source of aphids which colonize green cereal crops in other areas after long-distance

Table 1 BYDV incidence and predominant aphid species and BYDV isolates in the main cereal growing areas of Kenya, 1988

Area	BYDV incidence (%)	Aphid species	BYDV isolates
Trans-Nzoia	0-20	*Sitobion avenae*	PAV
Uasin Gishu	0-20	*Metopolophium dirhodum*	PAV + MAV MAV
Timau	0-60	*M. dirhodum*	PAV + RPV
Nakuru	0-20	*Schizaphis graminum* *Rhopalosiphum padi*	MAV PAV + RPV
Mau Narok	>60	*R. padi*	PAV + RPV MAV
East Mau	>60	*M. dirhodum* *R. padi*	PAV + RPV MAV
West Mau	>60	*R. padi*	PAV + RPV MAV

migration. Locally, cereal aphids have also been found to go through the dry season on other gramineous host plants and to survive on volunteer wheat, barley or maize crops.

The severity of BYDV in Kenya has prompted barley farmers to embark on measures aimed at controlling aphid vectors. Insecticides are now being sprayed simultaneously with herbicides when the crop is at the tillering or early stem elongation stages. The effectiveness of these measures in controlling BYDV has yet to be evaluated.

In order to achieve effective control of the vectors of BYDV, it is important to be able to predict when viruliferous aphids are likely to infest a crop. To develop an aphid forecasting system, studies of the virus-vector-host relationship need to be intensified and more local information on aphid biology and migration patterns needs to be generated. Such a system will lead to improved BYDV control and thus maximize crop productivity and reduce the need for pesticides.

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PART 3

Breeding for resistance to barley yellow dwarf virus

3.1

Developing Host Plant Resistance to Barley Yellow Dwarf Virus: An Effective Control Strategy

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SUMMARY

Developing host plant resistance to barley yellow dwarf virus (BYDV) is essential in the efforts to control the disease caused by this virus. Controlling of the aphid vector with pesticides is impractical in most production systems; developing resistance to aphids does not prevent the aphids from injecting the virus; and modifying planting dates is not a uniformly successful control measure. This paper highlights past results obtained using traditional plant breeding approaches to control BYDV. Significant progress has been made in barley breeding programs worldwide by the use of the BYDV-resistant Yd_2 gene from Ethiopian barley. The gene is also effective, to a lesser degree, in single-chromosome addition lines of wheat. Resistance in oats has been successfully developed where the inheritance of resistance is probably multigenic. In wheat, while some varieties have shown measurable multigenic resistance, host plant resistance has generally remained elusive. However, continuous screening and evaluation of wheat materials in Canada, Chile and the USA has identified promising germplasm and new sources of resistance have been identified in wild relatives of wheat. Long-term breeding programs are required to transfer these genes to good locally adapted wheat varieties.

Barley yellow dwarf virus (BYDV), transmitted by aphids to many graminaceous species, is now known to cause yield losses in most small-grain cereal crops throughout the world. Wheat, barley, oats and triticale can be infected; maize and rice are also generally susceptible, but are not believed to be damaged by the virus in most production areas. BYDV was first identified in 1951 in the USA and subsequently in Europe, Australia and New Zealand. It was not until the late 1960s and 1970s that it was reported in South America, Africa and in other regions.

Many attempts have been made to estimate annual crop losses that could be attributed to BYDV. Pike (1990) reviewed published data from six to ten experiments for barley, oats and wheat. In general, the losses were greater for oats than for wheat and barley and depended upon the stage of growth at the time of inoculation (*see* Table 1 *overleaf*). However, even with the ubiquity of BYDV, its effects are sporadic and, to a large extent, unpredictable. The establishment and spread of the disease depends upon the movement of viruliferous aphids; the severity

Table 1 **Average annual yield loss attributable to BYDV in barley, oats and wheat when inoculated at different growth stages**

	Average % yield loss		
	Seedling	Tillering	Stem elongation
Barley	54	23	19
Oats	75	40	22
Wheat	50	29	14

of the disease, and hence the damage caused, is determined by many factors. Important findings have emerged from studies on the ecology, epidemiology and etiology of the disease. Among the most important are that there are vector-specific strains of BYDV and that the virus is not mechanically or seed-transmitted from plant to plant or from one plant generation to the next.

Some degree of control of BYDV can be effected by any or all of the following strategies: avoidance; escape; host plant resistance to the insect vector and/or the virus; and therapeutic treatments to plants to reduce intraplant virus replication or the injection of virus particles by the aphid vector. The use of systemic or contact pesticides can reduce BYDV infection and some cultural practices, such as adjusting sowing dates to avoid peak periods of aphid movement, have proved partially effective. However, these strategies have limitations: year-to-year climate variation greatly affects cropping patterns and aphid numbers, and chemicals used to control aphids do not act rapidly enough to prevent inoculation. Developing host plant resistance to the vector also has its limitations, as most insect resistance mechanisms allow momentary feeding and thereby enough time to inoculate plants.

The conclusion reached very soon after the discovery of BYDV (Bruehl, 1961) was that stable control of BYDV essentially depends upon host plant resistance to the virus. The purpose of this paper is to demonstrate that this strategy has proved effective in alleviating the damage caused by BYDV and to outline the research which still needs to be done in this area.

DISCOVERING HOST PLANT RESISTANCE

To implement the host plant resistance strategy for BYDV control, screening studies on genetic resources collections were initiated in barley, oats and wheat in the 1950s and 1960s in the USA and some other countries as the impact of the disease became evident.

Barley

The discovery of resistance in barley was made, by chance, at the University of California, Davis, USA, where the disease was first described. Among the annually grown parental line barley nursery were four introductions — CI 1227, CI 1237, CI 2376 and CI 3920-1 (Abate) — which survived the epiphytotic of 1951 with little damage (Schaller, pers. comm.). Subsequently, a systematic survey of the National Barley Collection at the United States

Department of Agriculture (USDA) was carried out. Additional resistant introductions from Ethiopia were found (Schaller et. al., 1963) and crosses were made in order to introduce resistance into California-adapted varieties.

These crosses failed initially because in the field crossing environment at Davis the resistant lines could not be used as female parents. Reciprocals were successful. The hybridization problem was found to be a post-fertilization floral sensitivity phenomenon controlled by a single gene (*fls*) (Qualset and Schaller, 1968). A single incompletely dominant resistance gene (Yd_2) was identified and transferred by backcrossing into several varieties in California (Schaller et al., 1970, 1974) and elsewhere (Catherall and Hayes, 1966).

Oats

The discovery of resistance in oats had rather different results. In Illinois, USA, Endo and Brown (1964) found some promising lines (such as Albion) in the USDA National Oat Collection but in subsequent plant breeding or genetic studies no obvious major genes were detected. By intercrossing the most resistant lines, researchers were able to select for resistance in progeny that exceeded the levels found in the parents (Brown and Jedlinski, 1978). This work provided the germplasm basis for several new varieties, such as the widely grown variety Ogle.

Resistance in oats is commonly found in varieties but usually at a low level (Qualset, 1967), probably because for many years before BYDV was described breeders selected against 'red leaf'. Baltenberger et al. (1988) evaluated C_0, C_1 and C_2 populations which had undergone one (C_1) or two (C_2) cycles or recurrent selection for BYDV in a population originating from intercrossing 17 lines, 10 of which had BYDV resistance. The selection was effective in improving the mean population BYDV score: 5.2, 5.0 and 4.3 for cycles C_0, C_1 and C_2. The geographically localized resistance found for barley was not found for oats. Comeau (1982) showed that resistance in *Avena sterilis* throughout the Mediterranean region was rather widely dispersed. *A. sterilis* selections believed to have major-gene resistance have been studied (Landry et al., 1984), thus broadening the genetic base for BYDV resistance breeding in oats.

Wheat

Resistance in wheat has been more difficult to assess and screening programs have yielded only a few possible sources of resistance, many of which showed susceptibility upon repeated testing. In the 1980s the USDA World Wheat Collection was screened in California but only a few lines showed promise, including Coker 55-9 (CI 13232) which proved to be the most useful in breeding in California (Qualset et al., 1973). A breeding line from Mexico, named Anza and released in California, showed a level of symptomatic resistance which was about equal to that observed in Coker 55-9. Partial control of BYDV with a soil-applied systemic insecticide (disolfoton) was used to assess host plant resistance (*see* Table 2 *overleaf*). The relative grain yield of an insecticide-treated variety to its untreated control (T/NT = 100% for a resistant variety and >100% for a susceptible variety) was used as an index of host plant resistance. Table 2 shows that Anza and Coker 55-9 had similar low values, but not as low as the BYDV-resistant barley (Atlas 68). These results confirmed that visual scoring for resistance

Table 2 **Effect of a soil-applied systemic insecticide on BYDV and grain yield of wheat varieties and Atlas 57 (susceptible) and Atlas 68 (resistant) barley[a]**

Variety	BYDV score[b]		Yield (kg/ha)		
	T[c]	NT	T	NT	T/NT(%)
Anza	1.8	3.0	3230	2690	120
Coker 55-9	1.7	2.6	2380	2000	119
Ramona 50	5.1	6.8	2390	1580	151
INIA 66R	3.8	5.4	3080	2590	119
Sonora 64	4.0	6.2	3040	2350	129
Lerma Rojo 64	4.6	6.1	2820	2330	121
Oviachis 65	2.6	4.2	2400	1750	137
Atlas 57	4.8	6.7	3450	2350	147
Atlas 68	1.8	2.0	4910	4620	106

Note: a Means over 4 years.
b Symptoms scored on a scale of 0-9 (0 = no visible symptoms; 9 = severe symptoms , discoloration and dwarfing).
c T = soil treated at planting; NT= no soil treatment.
Source: Pike, 1990

correctly identified resistance in Anza and Coker 55-9. However, the table also shows that INIA 66 and Lerma Rojo 64 had low resistance indices; these varieties had not beeen classified as resistant on the basis of visual scoring and this emphasized the need for quantitative assessment of BYDV resistance.

Host plant resistance has been investigated in a diallel cross of winter wheat (Cisar et al., 1982) showing quantitative inheritance of resistance, and the results obtained were similar to those obtained in California on Anza, Coker 55-9 and a Coker 55-9 derivative (Qualset et al., 1973; Topcu, 1975). More recently, some promising sources of resistance have been investigated. Metzger (pers. comm.) found that NS 879-4, a breeding line from Yugoslavia, was practically symptomless in a highly infected site in India. This line was investigated by Tola and Kronstad (1984) who found that the resistance attributed to NS 879-4 in crosses with winter wheats was quantitatively inherited. Similar results were obtained by other workers, including Lorens (pers. comm.) and Vogt (pers. comm.). In contrast, Tandon et al. (1990) have reported major gene resistance in NS 879-4 in India, the area where Metzger's observations were made initially.

For bread wheats, Chile has provided an important source of resistance to BYDV. Since about 1970 deliberate selection for resistance has been undertaken by the Instituto de Investigaciones Agropecuarias (INIA) breeding program (*see* Paper 1.2, this proceedings). Notable is the line known as Tolbay ('tolerant to barley yellow dwarf') which has shown little yield loss in controlled inoculation studies. Anza has also shown resistance in Chile. Two lines from the Quilamapu research station in Chile, introduced to California by C.W. Schaller, have been investigated in inheritance studies (Lorens, 1988); of these lines, Q 23-77 (Lancero INIA) has shown the best symptomatic resistance to BYDV; again, quantitative inheritance of resistance was identified. Intercrosses involving resistance sources have been studied at Davis.

There is growing evidence that combining the various sources of resistance by recurrent intercrossing and selection may well result in resistance at levels exceeding that conferred by Yd_2 in barley.

It has been more difficult to identify resistance in durum wheat (Cheour et al., 1989). In view of this, we tested the idea that resistance homologous to that found with barley may occur in Ethiopian tetraploid wheats (Qualset et al., 1977). Out of 584 landrace-genotypes, 40 with low visual symptom scores were selected after 5 years of sequential testing. None of the Ethiopian tetraploid wheats were as good as Yd_2 barley. About 10 of the selected lines were hybridized with Modoc durum wheat, and after several generations of selection for BYDV resistance and good agronomic type, lines with much greater resistance than Modoc have been isolated.

Because of the difficulty in identifying and manipulating BYDV resistance in wheat generally, several alternative approaches have been used to introduce resistance into adapted varieties. While it appears now that intraspecific variation for host plant resistance can be exploited successfully, widening the genetic base for resistance is desirable. The first attempt to do this involved disomically adding Yd_2 to hexaploid wheat (McGuire, 1984). Resistance to BYDV has been expressed in the disomic addition lines in spite of the possible interaction with 'susceptible' homoeoalleles on the three groups of wheat chromosomes (McGuire and Qualset, 1990). No substitution or recombinant lines have been isolated as yet, and thus the Yd_2 gene is still not readily usable in wheat breeding.

Triticale has been observed to have good symptomatic resistance (Comeau, 1984), presumably contributed by rye chromosomes. Information on resistance in rye is limited. Some bread wheats with wheat/rye translocations seemed to have some BYDV resistance. Triticale, therefore, may be a bridging species that could be exploited by BYDV resistance breeding.

Another approach, perhaps more exciting in terms of the results obtained from initial observations, is provided in several wheatgrass species. Three lines of evidence are now available: for several of these species, Sharma et al. (1984) did not detect BYDV in inoculated plants; a 56-chromosome amphiploid, Zhong 4, was identified with exceptionally good resistance, based on enzyme-linked immunosorbent assay (ELISA) for BYDV (Xin et al., 1988); and a 56-chromosome amphiploid of Chinese Spring wheat x *Lophopyrum elongatum* (E genome) showed apparent resistance as good as any previously observed in wheat (McGuire and Dvorak, unpubl.). Certainly, it would be a worth transferring these alien resistance genes to wheat.

Resistant varieties

A directory of released BYDV-resistant varieties has yet to be compiled. However, an informal review showed that 24 barley, 13 oats and 15 wheat varieties had a degree of resistance in the area in which they were released (Comeau and Qualset, unpubl.).

Visual scoring of symptoms as an indicator of BYDV resistance

There is some doubt as to the usefulness of visual scoring as an indicator of BYDV resistance. Certainly, we have experienced some disappointments but after several repetitions over

seasons we steadily eliminate lines with higher than desirable visual scores and ultimately conduct simple yield loss studies to confirm resistance. Results typical of those we have obtained using this procedure are given in Table 3 (Chicaiza, 1989).

Table 3 **Results of experiment conducted to assess accuracy of visual scoring, showing percentage insecticide-treated/non-treated and means for plant height, biomass and grain yield, and yield components in three cereal crosses**

Parent/progeny	Character							
	PH[a]	BW	GY	SN	KN	KS	KW	Mean
Cross 1								
Parent:								
Yecora Rojo	89	114[b]	171[b]	151[b]	170[b]	117	107	135[c]
Q23-77	114	104	112	95	108	119	106	108
Progeny (F_6):								
CM 82367-131YD	94	154	136	125	148	111	93	123
-134YD	100	110	94	101	111	95	88	100
-139YD	98	121	107	125	118	96	82	107
-140YD	111	84	89	91	86	94	102	94
-178YD	117[b]	109	93	80	94	106	98	100
Cross 2								
Parent:								
Sunbird	113	167[b]	195[b]	110	182[b]	138[b]	104	144[c]
NS 879-4	108	119	115	105	110	106	105	110
Progeny (F_6):								
CM 84496-1YD	93	112	115	117	127	109	90	109
-110YD	103	108	91	138	84	104	110[b]	105
-72YD	117[b]	108	164[b]	136	163[b]	120	104	138[c]
-94YD	82	117	127	129	123	96	105	111
Cross 3								
Parent:								
Anza	107	107	112	106	99	108	109	104
Q 23-77	114	104	112	95	108	119	106	108
Progeny (F_6):								
CM 8236 -18YD	93	129	114	122	102	82	112	108
-27YD	116	147[b]	159[b]	133	172[b]	129	96	137[c]
-41YD	95	90	125	88	126	133	97	108
-53YD	98	101	112	112	115	100	97	105
-53YD	111	110	108	93	98	106	113	106
-338YD	123[b]	191[b]	162[b]	163[b]	186[b]	108	94	147[c]

Note: a PH = plant height; BW = biomass; GY = grain yield; SN = spike number; KN = kernel number/plot; KS = kernel number/spike; KW = 200-kernel weight.
b Significantly different from 100 at p = 0.05.
c Judged to be susceptible without statistical test.

In the three crosses featured in Table 3, the F_3, F_4 and F_5 generations were visually scored. In the F_6 generation, hill plots were insecticide protected (T) or unprotected (NT). The plots were paired and replicated four times. Plant height, grain and biomass yields, and yield components were measured. The results were reported as indices, T/NT for each trait and the mean over all traits. The crosses shown in the table gave results as expected for the parents: susceptible — Yecora Rojo (135), Sunbird (144); resistant — Q 23-77 (108), NS 879-4 (110), Anza (104). If visual selection worked perfectly and if homozygosity for resistance was achieved by the F_5 generation, none of the F_6 lines should have differed in mean index from 100. That was the case in cross 1, but in crosses 2 and 3 some lines were judged to be susceptible; that is, 14 of the 17 visually selected lines proved to be resistant. Another interesting point arising from these results was that two susceptible lines appeared in the progeny of two resistant parents (cross 3), indicating that Anza and Q 23-77 differ in resistance genes. From these and other results, we believe that visual scoring is a useful selection criterion as long as it is not employed as the sole criterion.

Intraplant virus concentration an indicator of BYDV resistance

A second approach used to identify resistant plants is to assay BYDV concentration in plants after a specified post-inoculation period. BYDV concentration has been related to symptom expression (Jedlinski et al., 1977; Skaria et al., 1985). In cell sap BYDV concentration can be measured directly, or indirectly by immunochemical methods (ELISA) and virus RNA concentration as measured by hybridization with complementary DNA in cloned DNA probes. ELISA has been used as an indicator of host plant resistance in some studies involving alien species as sources of resistance (Sharma et al., 1984; Xin et al., 1988). Lorens et al. (1989) found heritable variation in PAV-RNA concentration using a cDNA clone. It appears from the rather limited data now available that intraplant BYDV concentration, and hence virus replication, is heritable. Following on from the results obtained by Lorens et al. (1989), we are conducting experiments to validate the use of a cDNA probe in breeding for resistance to BYDV.

SOME LESSONS LEARNED ABOUT SCREENING FOR BYDV RESISTANCE

On the basis of 30 years of studying BYDV in California and conducting short-term studies elsewhere in the USA and in other countries, some observations are offered here that may be useful to other researchers involved in BYDV resistance breeding programs.

- Visual assessment of symptoms is valuable (Qualset, 1984); plants which *repeatedly* show high symptom scores will probably not have resistance; those which show low symptom scores but which are in fact susceptible may be carried in an evaluation program but they will ultimately be eliminated when yield loss assessments are made.

- Visual scoring should be followed by controlled infection studies to verify resistance. Such studies may be simple insecticide spray protection assessments (as in Tables 3 and 4) or completely controlled inoculation studies.

- If host plant resistance assessments are to be made with controlled inoculations with a single BYDV type, it is essential to have information on the aphid vectors present in the breeding target area and hence on the relative frequency of BYDV types present. However, this is *not* essential if assessments are made in the field, over several seasons and sites in the breeding target area, with naturally occurring aphids and BYDV types.
- Extremely severe epiphytotics may render genotypes with useful resistance as being apparently highly susceptible. This is not generally a problem in barley, with Yd_2 being a rather strong resistance gene, but in wheat some rare and useful resistance may be discarded.
- Locally adapted cultivars should be carefully assessed for BYDV reaction. Some of them may provide useful, even if only partial, protection.
- Virus detection and quantification methods, such as ELISA, may not relate to host plant resistance and should be used in combination with visual and/or quantitative plant response data.

RESISTANT VARIETIES AND THEIR VALUE

BYDV is widely known to be yield-limiting and, as the discussion above shows, the tools (resistance genes and methods) for breeding BYDV-resistant varieties of barley, wheat and oats are available. Despite this, there are still relatively few breeding programs focusing specifically on BYDV resistance. There are several reasons for this, including: BYDV not recognized as a problem; misinterpretation of symptoms or no BYDV detection surveys; sporadic epiphytotics, with other diseases being regarded as more of a problem; resistance gene sources unknown to breeders; limited scope in breeding program because of limited resources; and inadequate knowledge of the disease cycle and modifying factors.

International recognition of BYDV grew as result of several workshops, including one in Quito, Ecuador in 1978 sponsored by the Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT) and Andean country programs. Participants at the International Winter Wheat Conference in Madrid, Spain in 1980 were introduced to the disease at several field sites in the country. From CIMMYT-sponsored international workshops in Italy in 1984, it became clear that BYDV was a widespread problem. Resistant varieties are now emerging from the few breeding programs and greater efforts are being made to reduce the effect of BYDV on world production of small-grain cereals.

Assessing the value of resistant varieties can best be done by comparing resistant and susceptible isolines under conditions of normal cultivation throughout a production region. Table 4 provides data from experiments in which California Mariout (CM, susceptible, $-Yd_2$), and its backcross derivative CM 72 (resistant, $+Yd_2$), were grown in yield trial plots in farmers' fields in California (Schaller and Qualset, 1980). In every case there was a significant advantage of CM 72 over CM. The two isolines do not differ in yield performance in the absence of BYDV. In the particular trials reported in Table 4, disease symptoms were visually present but certainly not at epidemic levels. The obvious conclusion was that BYDV was a yield-limiting factor of considerable economic value. Comeau (1987), in controlled inoculation studies, also noted that

Table 4 **Multiyear, multilocational grain yield performance of BYDV-resistant (CM 72) and BYDV-susceptible (California Mariout) isolines of barley in California, USA**

Location	No. of years	No. of trials	Grain yield (% CM)
Central Valley (north):			
Sutter County	3	3	106
Yolo County	3	5	110
Mean	—	—	108
Central Valley (south):			
Kings County	1	1	124
Fresno County	3	3	138
Mean	—	—	131
Southern California:			
Santa Barbara County	3	3	130
Riverside County	3	3	116
Orange County	1	1	125
Mean	—	—	124
Overall mean	—	19	119

Source: Schaller and Qualset, 1980

there were significant yield losses without extreme symptom expression. A series of BYDV-resistant barley varieties developed by C.W. Schaller and his colleagues at the University of California, Davis, has virtually replaced all the susceptible varieties.

Interesting results were obtained from winter barley experiments conducted by Parry and Habgood (1986) in Wales, UK. They compared Vixen (resistant, $+Yd_2$) with Igri (susceptible, $-Yd_2$) in field studies involving controlled timing and amount of aphid infestation. Vixen is a backcross derivative selected from Coracle x Igri; Coracle is a spring barley (Yd_2) developed in Wales. Vixen and Igri have similar grain yields in the absence of BYDV. The sowing dates were early, mid-season and late (5 and 19 September and 3 October); the inoculation dates were early, mid-season and late (19 September, 4 and 26 October); the inoculation dosages were control (no aphids), low (< 3 aphids per plant) and high (> 5 aphids per plant); and *Rhopalosiphum padi* (L.) was used as the vector for a mixture of mild and severe BYDV isolates. After a 3-day feeding period the aphids were killed with a systemic insecticide and the plots were sprayed again 3 weeks later to prevent further inoculation by naturally occurring aphids. The grain yield data, in terms of percentage of yield produced by the control, are given in Table 5 (*overleaf*). They show that even in conditions in which most damage to the crop was likely to occur (early and mid-season sowing, mid-season inoculation, high aphid dose) the resistant variety produced substantially higher yields than the susceptible variety. The mean over all treatments could be taken as representative of growers' conditions because of variable sowing dates and inoculation periods. In essence, the results showed that although Yd_2 did not eliminate yield loss, it gave the crop substantial protection against BYDV.

Table 5 **Grain yield performance of Igri (susceptible) and Vixen (resistant) winter barley varieties, in Wales, UK**

Sowing date	Inoculation date	Aphid dose	Igri	Vixen
Early season	19 September	low	90	98
		high	50[a]	89
	4 October	low	18[a]	89
		high	6[a]	64[a]
	26 October	low	88	105
		high	64[a]	93
Mid-season	4 October	low	22[a]	83
		high	8[a]	46[a]
	26 October	low	93	102
		high	68[a]	104
Late season	26 October	low	91	90
		high	68[a]	77[a]
Mean		low	65[a]	93
		high	44[a]	79

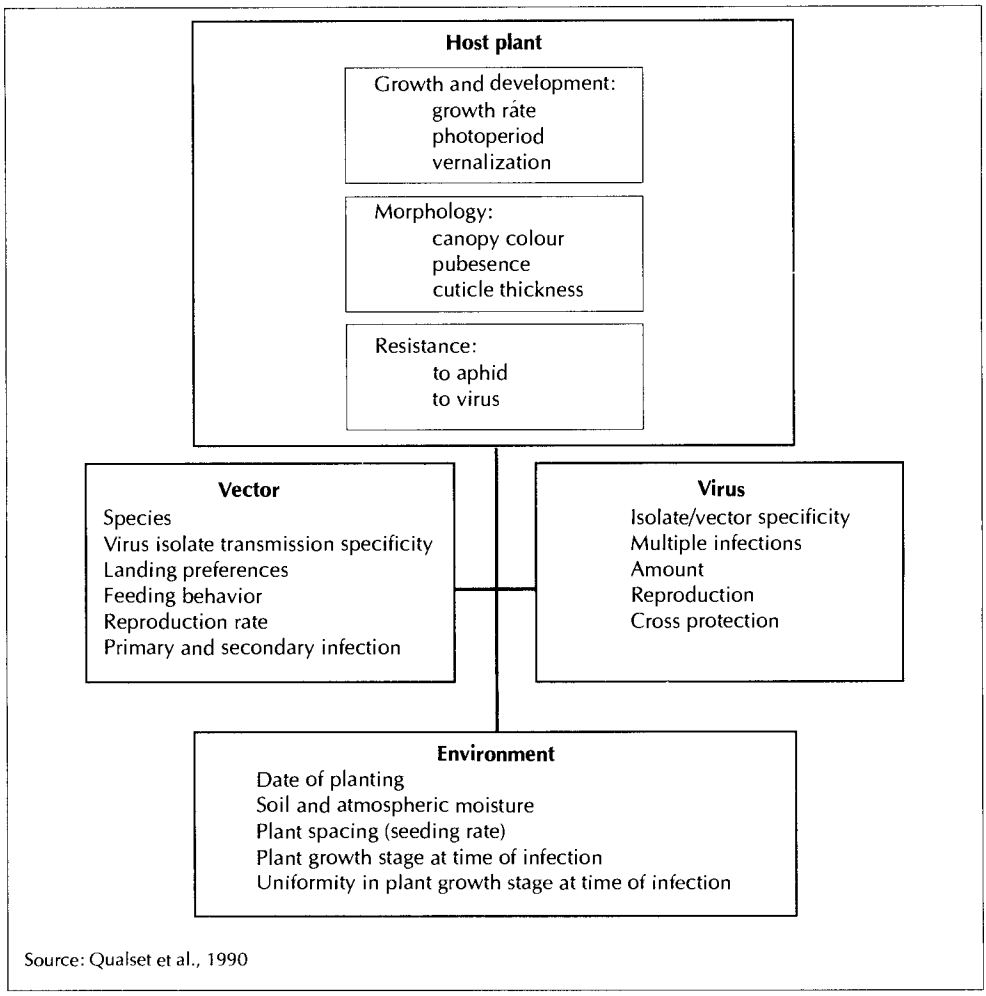
Note: a Significantly different from 100 at $p < 0.05$.

In Chile, a set of 10 standard varieties has been grown over a period of years with and without BYDV inoculation (Ramirez, 1990). Depending on the range of interrelated, contextual factors (featured in Figure 1) that affect the severity and control of BYDV, the value of average yield loss caused by BYDV (or, conversely, the value of potential benefits resulting from resistance to BYDV) may be determined.

To assess the value added to cereal production by using resistant varieties, the following simple procedure is recommended. First, consider the annual mean crop yield for a production region; then assume the benefit of resistance to BYDV as a percentage of the mean annual yield and compute the expected annual yield gain for each of these conditions. Table 6 shows a range in yields (0.5-4.0 t/ha) and in the effects of BYDV (5-25%). The added value (yield) to the crop is modest on a hectare basis in low-yield conditions, and probably not obvious or measurable on a field basis with a 5% advantage from BYDV resistance unless the mean yield is between 3 and 4 t/ha. However, the results become more dramatic when the added yield/ha is multiplied by the area under production. Three production areas, which could be illustrative of national production areas, are shown in Table 7 (*overleaf*). Even with the most conservative BYDV effect (5%) the benefits easily amount to an additional annual production of thousands of tons. To put a monetary value on breeding for resistance, these figures can be multiplied by the price of grain (*see* Table 8 *overleaf*).

Although the figures given above are hypothetical, they are realistic and they do illustrate the need for more emphasis to be placed on breeding for resistance. An extremely important

Figure 1 Interrelated host-vector-virus-environment factors that influence the severity of BYDV and its control



Source: Qualset et al., 1990

Table 6 Additional grain yield expected by using BYDV-resistant varieties

Current average annual yield (t/ha)	Effect of using BYDV-resistant varieties (% over average annual yield)				
	5	10	15	20	25
	kg/ha				
0.5	25	50	75	100	125
1.0	50	100	150	200	250
2.0	100	200	300	400	500
3.0	150	300	450	600	750
4.0	200	400	600	800	1000

Table 7 **Additional grain yield, attributable to using BYDV-resistant varieties, expected for production areas over average annual yields**

Area (ha)	Average annual yield (t/ha)	Effect of using BYDV-resistant varieties (% over average annual yield)				
		5	10	15	20	25
		kg/ha				
50 000	0.5	1250	2500	3750	5000	6250
	1.0	2500	5000	7500	10 000	12 500
	2.0	5000	10 000	15 000	20 000	25 000
	3.0	7500	15 000	225 000	30 000	37 500
	4.0	20 000	20 000	30 000	40 000	50 000
500 000	0.5	12 500	25 000	37 500	50 000	62 500
	1.0	25 000	50 000	75 000	100 000	125 000
	2.0	50 000	100 000	150 000	200 000	250 000
	3.0	75 000	150 000	335 000	300 000	375 000
	4.0	100 000	200 000	300 000	400 000	500 000
1 000 000	0.5	25 000	50 000	75 000	100 000	125 000
	1.0	50 000	100 000	150 000	200 000	250 000
	2.0	100 000	200 000	300 000	400 000	500 000
	3.0	150 000	300 000	450 000	600 000	750 000
	4.0	200 000	400 000	600 000	800 000	1 000 000

point is that the benefits are realized year after year without additional input costs. Variety development costs are investment costs which are non-recurring and are relatively small compared to the added value of host plant resistance. The variety introduction costs are not extra costs, as new seed must be periodically introduced to growers. The figures also assume linearity in yield increment as a benefit of host plant resistance, a reasonable assumption for most of the conditions illustrated if the available water and soil nutrients are sufficient to sustain the added productivity increments. This may not be true in drought years, but BYDV-infected plants are less able to thrive during periods of limited water availability than healthy plants (Comeau and Makkouk, 1988), and thus the benefit of host plant resistance may prevail under such conditions.

CONCLUSION

From the information now available, there is every indication that a rather modest investment in breeding for resistance to BYDV can have dramatic effects. Most countries which are affected by the virus do have breeding programs, and it is important that these programs place more emphasis on developing host plant resistance to BYDV. In addition, because there is evidence of host plant x virus isolate interactions in barley (Baltenberger et al., 1987), epidemiological studies need to be carried out. It would be useful to conduct annual surveys of

Table 8 **Value added annually to total production for different production areas for various levels of enhanced productivity attributable to the use of BYDV-resistant varieties (assuming value of grain to be US $ 100/t)[a]**

Area (ha)	Average annual yield (t/ha)	Effect of using BYDV-resistant varieties (% over average annual yield)				
		5	10	15	20	25
		US $ '000				
50 000	0.5	125	250	375	500	625
	1.0	250	500	750	1000	1250
	2.0	500	1000	1500	2000	2500
	3.0	750	1500	2250	3000	3750
	4.0	1000	2000	3000	4000	5000
500 000	0.5	1250	2500	3750	5000	6250
	1.0	2500	5000	7500	10 000	12 5000
	2.0	5000	10 000	15 000	20 000	25 000
	3.0	7500	15 000	22 500	30 000	37 000
	4.0	10 000	20 000	30 000	40 000	50 000
1 000 000	0.5	2500	5000	7500	10 000	12 500
	1.0	5 000	10 000	15 000	20 000	25 000
	2.0	10 000	20 000	30 000	40 000	50 000
	3.0	15 000	30 000	45 000	60 000	75 000
	4.0	20 000	40 000	60 000	80 000	100 000

Note: a 1986-87 wheat prices (US$/t) for selected countries represented at the BYDV workshop (CIMMYT, 1989): Algeria 467; Canada 82; Chile 166; Egypt 118; France 131; Jordan 351; Kenya 194; Libya 481; Mexico 95; Morocco 240; Syria 662; Tunisia 81; USA 79.

aphid species and virus types; this would be most effectively done through a battery of ELISA tests. Thus, an interdisciplinary approach should be adopted involving breeders, entomologists and virologists.

A critical need is to identify plant genetic resources with host plant resistance to BYDV. Experience to date suggests that sources of resistance discovered in one area generally can be useful in another area. All the promising sources should be assembled for evaluation in several test sites. The Yd_2 gene of barley has proved to be not only a useful source of BYDV resistance but also an easy monitoring tool for assessing the presence and severity of the disease. This gene should be backcrossed into locally adapted varieties of barley, both for direct use as varieties and for the purpose of disease monitoring. In wheat, resistance must be handled as a multigenic trait. Several sources of resistance should be intercrossed and crossed to local varieties for selection and reselection and, finally, for further intercrosses among selected resistant lines.

As BYDV poses a difficult challenge for plant breeders because of variable symptom expression and the complex host-vector-virus-environment interactions, repeated testing is needed. For this purpose, a network of researchers, representing the many countries affected by BYDV, should exchange materials and data annually.

In essence, the key elements of an effective approach to developing BYDV resistance in cereals are:

- epidemiological studies;
- interdisciplinary teams including entomologists, virologists and breeders;
- collection of international genetic resources with resistance to BYDV;
- international network for exchange and mutual evaluation of breeding lines;
- relatively long-term commitment (8-10 years, initially) to breeding for host plant resistance to BYDV.

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3.2

Breeding for Resistance to Barley Yellow Dwarf Virus: A Practical Approach

J.P. DUBUC and A. COMEAU

SUMMARY

Improving barley yellow dwarf virus (BYDV) tolerance and resistance in oats, barley, wheat and triticale was one of the main goals of a breeding program implemented at Agriculture Canada's Sainte-Foy Experiment Station. This goal was pursued through collaboration between breeder and pathologist, with BYDV-susceptible material often being eliminated very early from the segregating generation material. This approach yielded BYDV-tolerant cultivars of oats and triticale. A BYDV-tolerant cultivar of barley was also produced, but this was done without early-generation selection. This paper reviews the methodology and practical aspects of this research and discusses the positive contribution that BYDV selection, when properly organized, can make to a breeding program.

Cereal breeding began at Agriculture Canada's Sainte-Foy Experiment Station in 1957, with the initial focus on oats and barley. In the 1970s additional cereal plant breeders were recruited and in the early 1980s the program was expanded to cover all spring cereals — bread wheat, durum wheat, oats, barley and triticale. Since 1987, however, with changes in priorities and manpower, the program has concentrated mainly on bread wheat, with some work still being done on barley.

A breeding program develops numerous selection criteria to satisfy seed growers, cereal processors and farmers. Prior to incorporating resistance to barley yellow dwarf virus (BYDV), our criteria emphasized high yield and general adaptation to Canadian crop growing conditions, which vary from a maritime to a near-continental climate, with a growing season of about 100-115 days and soil conditions ranging from sandy loam to heavy clay and pH from 5 to 7. Specific targets were imposed by users. For barley, there were no strict specifications except high hectoliter weight, for feed uses; for oats, large plump seeds with high hectoliter weight and low hull content were requested; and for bread wheat, there were about 28 selection criteria to take into account.

So why would a breeder, already burdened by numerous selection criteria, agree to consider another problem, such as BYDV? The researchers gave the following reasons for taking on this extra load:

- Although aphids carrying the virus were generally not abundant, they were ever present, being blown into our area from the south-west. The virus itself was not visible everywhere but had been found on several species of cereals and grasses. Hidden damage was possible, as symptomless infection was common;
- As no research on BYDV had been conducted in our area, there was a real chance of success in producing something novel;
- The pathologist (Comeau) had an entomological background and was ready to develop better techniques for aphid rearing and BYDV inoculation. A more efficient technology was a prerequisite to integrating BYDV selection into the mainstream of the breeding program.
- The breeder (Dubuc) was experimenting with novel ideas on bulk-sorting technology to extract the tolerant genotypes from the inoculated bulks. BYDV selection was compatible with his new breeding methods.

To emphasize the fact that the results of plant breeding reflect the efficiency of methods and the validity of the decisions on the materials, the results of the BYDV research conducted over the past 17 years at Sainte-Foy are presented here in a format similar to that commonly used to report results from short-term research.

MATERIALS AND METHODS

Bulk selection

After several discussions between breeders and pathologists, we chose to select from bulks, discarding the widely used pedigree system. We did not have the manpower nor did we consider that genuine efforts within the pedigree system were warranted.

We chose to manipulate a broad genetic base instead of a high number of lines from a few crosses. High population requirements became evident after years of experience. If two pure lines were crossed, 2000 F_2 seeds were needed. More F_1 and F_2 seeds were needed if three parents were used, and over 30 F_1 seeds yielding 12 000 F_2 seeds were required when four parents were used. The F_2 was grown in bulk at half the commercial seeding rate to avoid competition with the short genotypes. We took advantage of the local facilities and land base, including three different altitudes above sea level at the research farm in La Pocatiere. The lowest level (50 m) was a fertile heavy clay with 1600 growing degree days (GDD); the second (150 m) was a rocky, dry sandy loam, and the third (250 m) was a very compact soil, a cold clay suffering from bad percolation and acidity (pH < 5.0), with only 1200 GDD.

To assess the yield plateau, thousands of F_5-F_7 lines from a broad base of crosses had to be produced each year, and grain yield was tested at the lowest cost. We avoided using statistical designs at the earlier stages up to the initial, non-replicated yield evaluation plot grown at only

one site. Rigorous selection with the least costly methods was the rule; we did not need to see a bad genotype twice to decide whether it should have been thrown out in the previous year. The unpredictable climate made hand-harvesting preferrable to machine harvesting, and the time lost was partly compensated by better seed purity. To increase efficiency, elimination was carried out by weighing before threshing and eliminating if the weight was less than 95% of the checks.

For efficient use of the growing facilities and rapid advance of generations, plants were grown in the field, in phytotrons or (later on) in a Californian winter nursery. Empirical evidence dictated that pure lines be established at different generations depending on the species: F_5-F_6 in oats, F_6-F_7 in barley and F_7-F_8 in triticale, from single-seed hills (30 x 30 cm apart). About 8000 spaced plants were narrowed down to 2500 lines, after elimination on the basis of agronomic traits (height, lodging and maturity). In wheat (which, like triticale, is a more recent project), a different system is being developed because of extreme selection pressure on grain quality characteristics. Lines are being established at the F_3 level to allow immediate selection for protein and hardness, which implies further purification later.

The F_3-F_6 lines were selected with the help of the pathologist, as discussed below. Statistical designs were used on the pure lines kept after the initial, non-replicated yield test. The number of sites and repetitions were increased gradually over the following 3 years.

BYDV selection technology

The methods for mass rearing of aphids and mass inoculation using the aphid spreading apparatus were developed locally. Among the most original was that used in the oats program, where the pathologist inoculated the breeder's own segregating progenies (usually in F_3 or F_4) with BYDV (and sometimes smuts) and also took notes on this inoculated material, instead of handling only the advanced breeding lines.

All sources of tolerance or resistance to BYDV were from abroad, with none of the resistant genitors being adapted to the local climate. We planned to manipulate hundreds of thousands of plants in the most efficient manner, in order to guarantee some possibility of success.

The F_3 and subsequent generations were grown at high planting density, about twice the commercial seeding rate, to select against excessive tillering ability. The bulks were grown first on the poor quality soil at an altitude of 250 m, where they were generally inoculated with BYDV (all species) and smuts (on oats). Further BYDV inoculation was sometimes carried out in the following year on the lower altitude soils. This inoculation of very early generations was a rather unique feature in a breeding program. During the first years of collaboration, the pathologist also made his own crosses and selected these even more rigorously for resistance.

Selection for grain quality parameters

The harvested F_2-F_4 grain was selected in three different ways. Grain width was selected with appropriate slot screens. The aerodynamics of the grain were improved by projecting grain near-horizontally, from a large moving belt, to a distance of about 3 m for the most aerodynamic kernels. Grain that failed to travel this distance was rejected. The density, or specific

gravity of the grain, was improved by flotation of bulk samples in a dense liquid. Floating seed was rejected; this method aimed at improving general disease resistance as well as hectoliter weight.

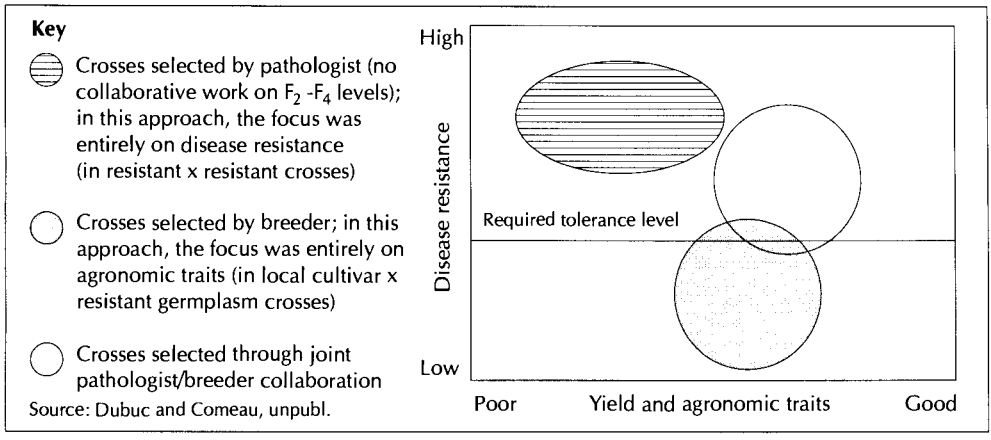
RESULTS AND DISCUSSION

The results obtained to date, in terms of quantity and quality of cultivars produced, reflect the value of the materials chosen and the methods used in the selection process.

Oat crosses made by the pathologist combined diverse sources of BYDV resistance, in the hope that this would result in gene pyramiding through rigorous virus selection. This approach was outstanding in improving BYDV resistance, but its usefulness is doubtful as the agronomic value of the lines with greater resistance was invariably well below standard. Crosses made by the breeder between local susceptible cultivars and BYDV-resistant lines were handled without virus selection; in these cases, it was always difficult to extract BYDV-tolerant lines from the crosses, as tolerance is related to three or four genes in oats and the number of tolerant lines in the progeny is invariably small (McKenzie et al., 1985). The third approach, however, rested on collaboration between the pathologist and breeder, with most of the crosses made by the breeder involving one BYDV-tolerant parent and being subjected to BYDV selection during segregation generations. This collaborative approach paid off. Lines isolated from bulks could not be susceptible to BYDV as all these unwanted genotypes had been eliminated. There was no need to use the time-consuming backcross method.

The performance of the three approaches used for oats is summarized in Figure 1. Collaboration may represent a compromise, but it proved to be highly efficient. Research aiming at introducing Yd_2 in barley, however, was more difficult; the resistant lines often had major agronomic defects, and work is still being conducted to eliminate the undesirable linkages. In the wheat and triticale projects there has been considerable and relatively problem-free progress.

Figure 1 Results obtained in oats program, using three BYDV selection approaches



Released cultivars

Among the cultivars released so far as a result of the work described above are 11 oat varieties registered in Canada and two registered in Tasmania. All those produced since 1985 have superior BYDV resistance (or tolerance), with good agronomic attributes (*see* Figure 2).

Figure 2 Comparison between BYDV-susceptible oats cultivars (most of which were produced before 1982) and BYDV-resistant cultivars (most of which were produced more recently).

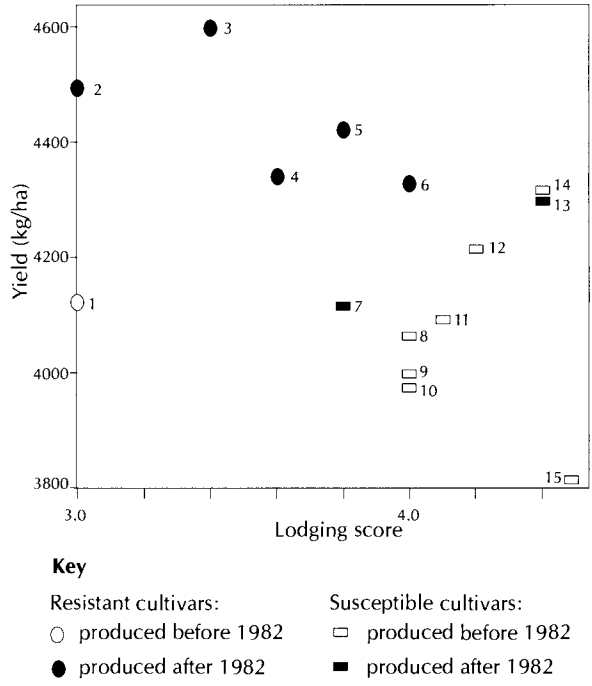
Resistant cultivars:		Release year
1	Oxford	1976
2	Ultima*	1988
3	Sylva*	1989
4	Appalaches*	1989
5	Capital*	1986
6	Nova*	1986

The new cultivars combine resistance with good agronomic traits, such as reduced lodging tendency

Susceptible cultivars:		
7	Marion*	1985
8	Manic*	1979
9	OAC Woodstock	1982
10	Kamouraska*	1982
11	Shaw*	1981
12	Lamar*	1979
13	Baldwin*	1985
14	Laurent	1977
15	Garry	1948

Garry was a very popular variety in 1970-75

* Cultivars developed by Agriculture Canada's Sainte-Foy breeding program



The cultivar Marion has intermediate BYDV reaction but useful field resistance to BYDV through escape, because of its early heading and maturity. In barley, we released two cultivars and helped a private company in producing another. These cultivars do not possess the Yd_2 gene and were produced without collaboration with a pathologist; however, they are reasonably tolerant to late BYDV infection. In triticale, all the initial parental lines were BYDV tolerant; from these, one variety was obtained which showed better yield, earliness and good hectoliter weight, without losing the BYDV tolerance. In bread wheat, the two advanced lines sumbitted for registration combine intermediate breadmaking quality with good yield and rather low scab symptoms. These lines are near average in BYDV tolerance, but BYDV remains an important selection parameter in the wheat program.

The released cultivars represent the product of 2500 crosses, ranging from single cross to eight-parent combinations. The best results were obtained using three parents. About 65% of these crosses were inoculated with BYDV during segregation, which means that, in 17 years, over 16 million plants have been inoculated. In the early years of the oats program, F_2-F_3 inoculation of bulks sometimes resulted in excessive loss of genetic variability and it became clear that oat plants were more susceptible than other species. In later years, we were more cautious and avoided inoculating oats before the beginning of stem elongation; the same precaution is needed with durum wheat, which is also very susceptible to BYDV.

Plant height had to be corrected by rejecting tall lines in generations grown without BYDV inoculation. For some reason, BYDV selection in bulk populations favored the survival of lines with long straw, which is not a highly prized trait in Quebec. Conversely, short-strawed lines were too often susceptible to BYDV. This unexpected problem was confirmed in all cereal species (Comeau and Jedlinski, 1990). To avoid this problem, the number of viruliferous aphids per plant had to be as precise as possible, but a compromise with the need for speed was required. It was evident that plants should never be inoculated before the beginning of stem elongation if mass selection by seed flotation was to be used. For bread wheat and durum wheat, a rate of 5-10 aphids per plant was adequate when using the PAV-type isolate 'Cloutier'. For triticale, up to 45 aphids per plant could be used.

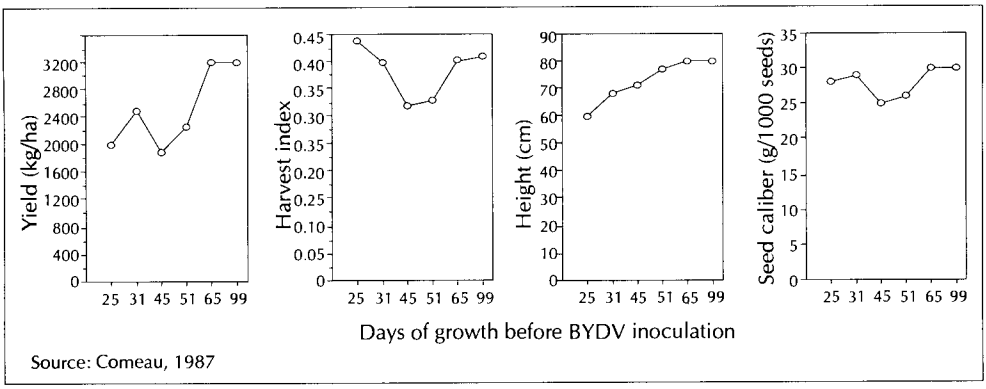
Selection for grain quality by flotation methods

Selection for grain width or aerodynamics might occasionally improve overall disease resistance but this approach did not suit the goals of our work. Selection by flotation of bulk samples in a dense liquid seemed a more useful approach, as this property is generally correlated with hectoliter weight, a trait used in Chile for BYDV selection (Ramirez, pers. comm.). Reduction of hectoliter weight caused by BYDV was confirmed for all BYDV-susceptible germplasm in our trials. Selection by flotation could therefore be used to reduce the frequency of BYDV-susceptible lines within a bulk. Special care should be taken, however, in ensuring the plants are inoculated late enough, just after the beginning of stem elongation. This is the critical period for inoculation if the aim is to reduce the grain quality rather than yield. Early inoculation reduces plant height and yield; later inoculation affects seed quality and harvest index, for all species (Comeau, 1987). This is illustrated by the response of bread wheat to BYDV inoculation at various times during the growing season (*see* Figure 3).

However, grain density is a complex trait with genetic and environmental components; any stress occurring during the growing season could modify it in fairly unpredictable ways. High-density grains simply have their components stored in a smaller volume. Density is almost completely independent of grain size, in contrast to other methods. For example, flotation could be particularly appropriate in the selection of small-grain cultivars if this trait is desired, as it does not discriminate against large seeds.

The flotation method has the drawback of selecting against high lipids in oats, and perhaps to some extent against high protein in wheat. In our oats program, the first drawback should have reduced the frequency of cultivars with high oil content but this did not prove to be a significant problem; in fact, the oil content of recent cultivars seems near normal. However, the

Figure 3 Response of bread wheat to BYDV inoculation at various times during the growing season



Source: Comeau, 1987

protein levels of recent cultivars is slightly lower than normal, by about 0.7% on average. This might stem from the use of the flotation method; another explanation may be that better grain filling in recent cultivars reduces protein level. Modern techniques allow low-cost protein determination; it would be easy to apply selection pressure on this trait to correct the observed tendency.

Flotation methods for bread wheat

As paramount importance is given to quality in bread wheat, an experiment was conducted to assess the risk of reducing grain quality when selecting for BYDV resistance using the flotation method. Three cultivars were used in this trial. Seeds were thrown into a mixture of organic solvents with a specific gravity of 1.38. The floating seed had, on average, 0.5% more protein (14.8%) than the dense seed (14.3%). However, the particle size index (PSI) of the floating seed was 52.7 units, whereas the dense seed had a PSI of 59.8, which meant that the dense seed was harder. These results suggest that selection of resistant germplasm by flotation could reduce protein slightly but increase the PSI, the former effect being undesirable and the latter desirable according to present Canadian criteria. We know that in other countries a softer grain with lower protein is generally requested by users. In either case, the risk of reducing the overall quality of selected bulks is not very high, considering that BYDV can reduce hectoliter weight by as much as 10% (St-Pierre, pers. comm.). This weight reduction is always accompanied by a significant reduction in the density of individual seeds, indicating that flotation can indeed discriminate between resistant and susceptible lines in a large bulk sample. Two or three cycles of such selection would make it more efficient. Protein levels can be easily selected through modern methods at later stages in the breeding process.

It would appear that the benefits of the flotation selection system far outweigh the disadvantages. Organic solvents may be unsuitable in some circumstances, being flammable, toxic or forbidden by law, but solutions containing magnesium sulfate (Comeau and Dubuc, 1977), other salts or sugar could also serve for flotation of seeds.

CONCLUSION

The success story described in this paper is the largely the result of collaboration between pathologist and breeder in developing and following a selection protocol. Essentially, at the breeding level, there must be only one set of germplasm, rather than one for the breeder and one for the pathologist. At the germplasm enhancement level, the pathologist should freely experiment with foreign cultivars and even wide hybridization, but lines from this group should be promoted to the breeder's crossing block only after yield trials or cytogenetic verification. This ensures that lines promoted to the crossing block can contribute positively to the program.

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3.3

Aphid Infestation and Damage in Wheat in Egypt

M.C. MOSAAD, A.A. SHAFI and R.H. MILLER

SUMMARY

A 8 x 8 diallel set consisting of parent lines and F_1 and F_2 generations of durum wheat were grown at Shandaweel in Sohag governorate, Egypt in the 1986-87 growing season to investigate aphid-related yield losses and plant tolerance of aphid infestations. In 1988-89 two wheat nurseries consisting of advanced bread wheat and durum wheat lines, supplied by the International Center for Agricultural Research in the Dry Areas (ICARDA), were sent to Shandaweel for field screening against aphids. A further 540 backcross lines were screened under naturally occurring aphid infestations. Aphid infestation in 1986-87 significantly affected plant height and thousand-kernel-weight, and there was an average overall yield reduction of 18.2%. Broad and narrow sense heritabilities ranged from intermediate to high for visual aphid infestation rating and plant damage. The results suggest that visual selection for aphid resistance may be valuable in early segregating generations. Of the 193 bread wheat entries screened in 1988-89, 15 were found to be moderately resistant; of the 240 durum wheat entries, eight were resistant and 65 moderately resistant. Among the backcross lines, four lines derived from a Bushland/Amigo T 101 x Sakha 69 cross and 14 derived from a Bushland/Amigo T 105 x Sakha 61 cross were moderately resistant.

Aphids are among the most damaging insect pests in wheat crops in Egypt. In recent years, aphid infestations in wheat have increased in Upper Egypt, resulting in grain yield losses of between 7.5 and 18.7% (Tantawi, 1985). Aphids damage their host plant by consuming plant fluids and carrying diseases, mainly viruses. In addition to the costs associated with insecticidal control, the insecticides themselves may pollute the environment if misused. One way to overcome this problem is to develop aphid-resistant wheat varieties that rarely require insecticide treatment.

The study described in this paper was designed to identify sources of aphid resistance in wheat by screening nurseries, assembled by Egyptian workers and by the International Center for Agricultural Research in the Dry Areas (ICARDA), against naturally occurring field populations of aphids at the Shandaweel Research Station in Sohag governorate, Egypt. We also sought to estimate yield losses and to measure changes in plant characteristics attributable to aphid infestation.

MATERIALS AND METHODS

An 8 x 8 diallel nursery, consisting of eight parent plants, 21 F_1 hybrids and 21 F_2 populations, was grown at Shandaweel in 1986-87 in a randomized complete block design with eight replications (*see* Table 1). Each of the parent plants and the F_1 hybrids were represented by one row per block. The rows were 1 m long and spaced 30 cm apart, with 10 cm between plants within each row. Borders were sown with the variety Dugoklesa, which is susceptible to aphid infestations. Weeds were controlled by hand. Four replications were kept free from aphid infestation by spraying malathion (mixed at 1:1 active ingredient/200 l water) three times during the growing season. Four replications were not sprayed and were allowed to be naturally infested by aphids.

Two wheat nurseries consisting of advanced breeding lines were supplied by ICARDA to workers in Egypt in 1988-89. The bread wheat nursery contained 193 entries and the durum wheat nursery contained 240 entries. In addition, 540 BC_3 lines from Egypt's nurseries were screened under naturally occurring aphid infestations at the Shandaweel Research Station.

Table 1 Parent, pedigree, source and descriptions of the eight genotypes used in experiments on aphid-infestation damage in wheat, Egypt, 1986-87

Parent (pedigree)	Source	Description
Gerardo vz 466-Gs's' (Gdo vz 466-B,Bal//Bye*Tc 60)	Line from Shandaweel durum wheat breeding program	Early, short, low yield, aphid tolerant
Bo's'-Gta's' (DT 216.156/Noghk/WLS/3/ RL 3442/LK/2/Tace/3*/Tc 60 Cr's'/4/t.pal./85309/ 1. Gle/2/*Tc 60/3/Gll's')	Line from Shandaweel durum wheat breeding program	Early, short, high yield, susceptible to aphids
Local 28	Egyptian local germplasm	Late, tall, moderate aphid tolerance
Local 43	Egyptian local germplasm	Moderately early, tall, medium yield, very susceptible to aphids
Local 44	Egyptian local germplasm	Late, tall, high yield, susceptible to aphids
Edmore (Edm.)	USA	Very late, tall, moderate yield, aphid tolerant
Jo's'*2/Gdo vz 394 (BYE*2/Tc 60//TAC 125E 3*Tc60 Gdo vz 394)	Mexico	Early, tall, moderate yield, aphid tolerant
Stellata's'(Stat's') (E 3728/3*CP//GZ/3/ cpe*3/GZ//Tc 60)	Mexico	Medium early, medium height, medium yield, susceptible to aphids

Plant tolerance to aphid infestation in 1986-87 was assessed by determining the amount of damage to the plant caused by the aphids. The damage was assessed on the same dates used for the aphid infestation assessment, according to following scale: 1 = no damage symptoms apparent (no damage); 2 = slight curling of the leaves, but no stunting or honeydew deposition visible (slight damage); 3 = obvious leaf curling, some leaves covered with honeydew and some stunting apparent (moderate damage); 4 = obvious leaf curling, heavy honeydew deposition on the leaves and many plants stunted (severe damage); 5 = obvious leaf curling, heavy honeydew deposition on leaves, severe stunting, and some plants killed (very severe damage).

The ratings for infestation and plant damage in 1986-87 were quantified by comparing ratings for individual lines to those of the check by computing $(X_{ij}/\bar{X}_i) \times 100$, where: X_i = the mean of the ith genotype in the insectide treatment and X_{ij} = the value of the ith genotype in the jth replication in the non-insecticide treatment. Statistical analyses were conducted according to Steele and Torrie (1980).

In both years infestation severity was recorded for each row of parent plants, F_1 hybrids and F_2 populations in each replication. In mid-February and on 1 and 21 March, plants were scored for aphid tolerance on a scale of 1-5, where: 1 = no aphids present on any plant (highly resistant); 2 = a few alate aphids present on a few plants (resistant); 3 = a few scattered colonies on many plants (moderately resistant); 4 = many scattered colonies on many plants (susceptible); and 5 = many aphid colonies, some containing alates, on all plants (highly susceptible).

RESULTS AND DISCUSSION

Fifteen of 193 bread wheat entries proved to be moderately resistant (*see* Table 2). Eight of 240 durum wheat entries were rated as resistant (*see* Table 3 *overleaf*), while an additional 65 entries

Table 2 **Bread wheat entries from the ICARDA nursery (WAT) showing moderate resistance to aphids under field infestation at Shandaweel, Egypt, 1988-89**

Entry no.	Name	Source	Serial no.
4	PRL'S'/4/TOB66'S'/3/CNO67/JAR66/KVZ	WAT89	104
18	BANK'S'/VE'S'	WAT89	118
19	TTM'S'/VEE'S'	WAT89	119
20	VEE'S'/YACO'S'	WAT89	120
21	VEE'S'/3/R37'GH1121//KAL/BB	WAT89	121
22	DGA/BJY'S'//DODO'S'	WAT89	122
23	KEA'S'/SNB'S'	WAT89	123
46	PRL'S'/CHOVA'S'	WAT89	222
65	VEE'S'/3/JUP73/EMU'S'//GJO'S'	WAT89	317
67	VEE'S'/KIRA'S'	WAT89	319
104	BOW'S'/3/YD'S'//BB/CHA	WAT89	508
105	BOW'S'/3/YD'S'//BB/CHA	WAT89	509
119	F35.70/MO//NAC	WAT89	523
122	JUP/BJY'S'//URES	WAT89	602
150	VEE'S'	WAT89	706

were rated as moderately resistant. In the field screenings of BC_3 lines, four lines derived from the Bushland/Amigo T 101 x Sakha 69 cross and 14 lines derived from the Bushland/Amigo T 105 x Sakha 61 cross were rated as resistant.

Table 3 **Durum wheat entries from the ICARDA nursery (DAT) showing resistance to aphids under natural infestation at Shandaweel, Egypt, 1988-89**

Entry no.	Name	Source	Serial no.
21	GS/FG//CNDO/3/DACK/KIF	DAT89	121
22	GS/FG/CNDO/3/DACK/KIF	DAT89	122
62	CHAHBA 88/MRB11	DAT89	314
64	CHAHBA 88/MRB11	DAT89	316
74	MRB3//FG/CIT	DAT89	402
78	MRB11/AMARELO DE BARBA BRANCO	DAT89	406
79	MRB3/4/BYE*2/TC//ZB/W/3/CIT	DAT89	407
80	MRB3/LAHN	DAT89	408

Aphid influence on plant development

Analysis of variance indicated that aphid infestation did not significantly ($p > .05$) affect heading date, spike length, number of spikes per plant, number of spikelets per spike or harvest index. However, genotypic differences in aphid damage were significant ($p < .05$) for plant height, thousand-kernel-weight (TKW), biological yield and grain yield per plant.

In aphid-infested plants, plant height was reduced by an average of 16.3 cm, TKW by 6.7%, biological yield by 15.9% and grain yield by 18.2%. Similar results had been reported by

Table 4 **Effect of aphid infestation, expressed as a percentage of the control, on plant height of parent plants (diagonal), F_1 hybrids (right of diagonal) and F_2 populations (left of diagonal)[a]**

Parent plant (P)	1	2	3	4	5	6	7	8	Mean of F_1 arrays
1 Gerardo vz 466-Gs's'	97.8	91.5	0	88.0	88.2	93.2	96.2	85.3	91.5
2 Bo's'-Gta's'	98.1	93.0	0	81.2	85.6	92.6	83.0	90.0	88.1
3 Local 28	96.0	97.4	93.1	0	0	0	0	0	0
4 Local 43	96.7	96.0	96.0	85.7	82.4	89.8	88.9	97.6	86.2
5 Local 44	95.4	97.1	96.3	94.9	92.2	91.5	89.2	85.6	87.9
6 Edmore	0	0	0	0	0	92.9	87.0	91.8	91.3
7 Jo's'*2Gdo vz 394	94.3	95.3	96.7	96.6	93.7	0	87.1	84.5	88.0
8 Stellata's' (Stat's')	94.5	94.8	97.4	94.5	94.1	0	92.8	86.7	87.4
Means of F_2 arrays	96.1	96.0	96.1	94.3	94.8	0	93.7	93.6	

Note: a $P \pm$ S.E. $= 2.6 \pm 0.2$; $F_1 \pm$ S.E. $= 2.4 \pm 0.1$; $F_2 \pm$ S.E. $= 95.0\ y \pm 0.4$; LSD 0.05 $F_1 = 5.9$; LSD 0.05 $F_2 = 4.1$.

Carrigan et al. (1981) for all of the above variables, by Kieckhefer and Kantack (1980) for TKW and grain yield, by McPherson (1983) and Du Toit and Walters (1984) for grain yield alone, and by Mosaad and Joppa (1987) for plant height.

Average aphid tolerance measured as a percentage of the controls is given in Tables 4, 5, 6 and 7 (*overleaf*). For parent plants, the averages ranged from 85.7 to 97.8% for plant height, from 85.5 to 98.0% for TKW, from 72.1 to 92.7% for biological yield and from 72.3 to 90.7% for grain yield. Among the cultivars, Gerardo vz 466-Gs's' appeared to be the least affected by

Table 5 **Effect of aphid infestation, expressed as a percentage of the control, on thousand-kernel-weight of parent plants (diagonal), F_1 hybrids (right of diagonal) and F_2 populations (left of diagonal) [a]**

Parent plant (P)	1	2	3	4	5	6	7	8	Mean of F_1 arrays
1 Gerardo vz 466-Gs's'	96.4	92.7	94.8	97.1	92.2	92.9	91.6	91.3	94.1
2 Bo's'-Gta's'	94.2	93.5	97.6	95.8	96.6	97.3	87.0	91.8	94.0
3 Local 28	94.6	95.7	98.0	96.2	87.5	97.8	97.6	95.7	95.5
4 Local 43	91.8	97.3	86.9	86.4	87.3	89.3	87.8	97.3	92.2
5 Local 44	92.8	91.8	94.1	82.9	85.5	93.9	80.3	97.0	91.1
6 Edmore	95.1	93.1	92.5	95.1	98.5	94.6	92.7	95.9	93.0
7 Jo's'*2Gdo vz 394	96.7	95.7	95.6	97.2	96.4	97.4	96.5	96.9	91.6
8 Stellata's' (Stat's')	89.9	94.8	96.2	96.2	86.1	86.7	97.7	91.2	94.6
Means of F_2 arrays	93.9	94.5	94.2	91.7	91.0	95.9	96.4	93.6	

Note: a P ± S.E. = 92.5 ± 1.6; F_1 ± S.E. = 93.7 ± 0.6; F_2 ± S.E. = 93.8 ± 0.6; LSD 0.05 F_1 = 4.9; LSD 0.05 F_2 = 5.7.

Table 6 **Effect of aphid infestation, expressed as a percentage of the control, on biological yield/plant of parent plants (diagonal), F_1 hybrids (right of diagonal) and F_2 populations (left of diagonal)[a]**

Parent plant (P)	1	2	3	4	5	6	7	8	Mean of F_1 arrays
1 Gerardo vz 466-Gs's'	92.7	90.3	0	88.0	95.4	90.5	87.9	84.6	88.5
2 Bo's'-Gt's'	89.0	72.1	0	82.1	89.7	89.6	86.8	88.2	85.5
3 Local 28	87.8	97.2	85.2	0	0	0	0	0	0
4 Local 43	87.1	84.4	97.4	75.5	82.9	85.5	88.4	87.7	84.3
5 Local 44	85.6	78.9	84.1	74.4	77.5	91.0	83.3	80.3	84.3
6 Edmore	78.4	97.2	89.2	89.2	80.3	85.9	89.2	90.7	89.0
7 Jo's'*2/Gdo vz 394	79.6	94.8	91.2	90.9	76.7	75.4	83.4	84.7	86.2
8 Stellata's'(Stat's')	96.5	72.0	85.3	81.9	82.5	77.8	87.0	79.2	85.1
Mean of F_2 arrays	86.0	85.7	89.7	83.9	79.9	84.2	84.8	82.7	

Note: a P ± S.E. = 81.4 ± 2.4; F_1 ± S.E. = 86.1 ± 0.7; F_2 ± S.E. = 84.6 ± 1.0; LSD 0.05 F_1 = 6.8; LSD 0.05 F_2 = 7.0.

Table 7 **Effects of aphid infestation, expressed as a percentage of the control, on grain yield of parent plants (diagonal), F_1 hybrids (right of diagonal) and F_2 populations (left of diagonal)[a]**

Parent plant (P)	1	2	3	4	5	6	7	8	Mean of F_1 arrays
1 Gerardo vz 466-Gs's'	90.7	91.7	0	91.3	82.9	84.0	90.8	84.4	88.2
2 Bo's'-Gta's'	89.0	72.3	0	80.0	78.1	80.0	86.0	83.4	82.3
3 Local 28	91.3	93.6	85.9	0	0	0	0	0	0
4 Local 43	73.2	79.4	93.1	79.4	84.5	81.8	93.0	88.0	86.0
5 Local 44	71.8	76.4	84.7	71.1	72.5	80.6	82.3	77.9	80.6
6 Edmore	87.3	93.9	90.4	82.6	73.0	87.2	82.4	90.1	83.1
7 Jo's'*2/Gdo vz 394	76.4	88.8	95.2	80.2	70.1	70.9	79.9	81.6	85.1
8 Stellata's'(Stat's')	86.2	77.3	91.4	78.9	84.3	74.4	74.4	79.1	83.6
Mean of F_2 arrays	83.0	83.3	90.7	79.2	73.7	82.7	79.7	80.5	

Note: a P ± S.E. = 79.6 ± 3.2; F_1 ± S.E. = 84.1 ± 1.0; F_2 ± S.E. = 81.6 ± 1.8; LSD 0.05 F_1 = 10.4; LSD 0.05 F_2 = 1.0.

Table 8 **Broad and narrow sense heritabilities of parameters measured in F_1 and F_2 generations, expressed as a percentage of the control, after aphid infestation**

	Heritability			
	Broad (%)		Narrow (%)	
	F_1	F_2	F_1	F_2
Plant height	57.6	34.1	32.3	13.9
Thousand-kernel weight	48.5	61.9	10.0	57.5
Biological yield	46.3	76.1	13.0	41.3
Grain yield	40.0	68.5	18.7	61.5
Infestation scale	64.2	78.6	56.0	75.2
Damage scale	52.1	69.8	32.1	67.9

aphid infestation, while Local 43, Local 44, and Stellata's' were the most susceptible. In F_1 hybrids TKW reduction ranged from 80.3 to 97.8%, biological yield from 80.3 to 91.0% and grain yield from 77.9 to 93%. The average reduction in F_2 populations ranged from 82.9 to 98.5% for TKW, from 74.4 to 97.4% for biological yield and from 70.1 to 95.2% for grain yield. In general, hybrids with tolerant or moderately tolerant parents, such as Gerardo vz 466-Gs's' and Edmore, also exhibited tolerance, while crosses with a susceptible parent did not.

Broad sense heritabilities were intermediate to high for all traits measured, suggesting that these traits were not greatly affected by environmental factors (*see* Table 8). Narrow sense heritabilities were low in F_1 hybrids and high in F_2 populations in all traits except plant height. These results suggest that selection for aphid tolerance may be accomplished during segregating generations.

Aphid infestation and damage ratings

The cultivars Edmore and Local 28 had the lowest aphid infestation and damage ratings, while Stellata's', Local 44 and Local 43 had the highest. The mean infestation rating for F_1 hybrids ranged from 1.6 to 3.5 (*see* Table 9); the damage scale ranged from 1.62 to 3.12 (*see* Table 10).

Generally, crosses with Edmore or Gerardo vz 466-GS's' as common parents exhibited lower infestation levels and lower damage than the other crosses, while crosses with Stellata's' or Local 44 as common parents had the highest infestation and damage ratings. The F_2 cross

Table 9 **Aphid infestation ratings of eight parent plants (diagonal), F_1 hybrids (right of diagonal) and F_2 populations (left of diagonal)[a]**

Parent plant (P)	1	2	3	4	5	6	7	8	Mean of F_1 arrays
1 Gerardo vz 466-Gs's'	2.3	2.5	1.9	3.2	2.9	1.6	2.3	2.5	2.5
2 Bo's'-Gta's'	2.7	2.6	1.8	2.7	3.0	1.6	2.0	3.4	2.5
3 Local 28	2.3	2.3	2.5	3.3	3.2	1.9	1.8	2.8	2.4
4 Local 43	3.3	3.3	3.3	3.6	3.3	2.8	3.0	2.8	3.1
5 Local 44	3.5	3.1	3.2	3.2	3.7	2.8	3.3	2.8	3.1
6 Edmore	2.5	2.6	2.3	3.0	2.8	2.0	2.0	2.8	2.2
7 Jo's'*2/Gdovz 394	2.5	2.6	2.2	3.1	3.6	2.4	3.0	3.5	2.6
8 Stellata's'(Stat's')	3.8	3.4	3.0	3.6	3.3	2.8	3.6	3.6	3.1
Mean of F_2 arrays	2.9	2.8	2.6	3.3	3.3	2.55	2.9	3.4	

Note: a P ± S.E. = 3.0 ± 0.2; F_1 ± S.E. = 2.7 ± 0.1; F_2 ± S.E. = 2.8 ± 0.1; LSD 0.05 F_1 = 0.6; LSD 0.05 F_2 = 0.4.

Table 10 **Aphid damage ratings of eight parent plants (diagonal), F_1 hybrids (right of diagonal) and F_2 populations (left of diagonal)[a]**

Parent plant (P)	1	2	3	4	5	6	7	8	Mean of F_1 arrays
1 Gerardo vz 466-Gs's'	2.2	2.3	2.0	2.1	2.3	1.6	2.3	2.3	2.1
2 Bo's'-Gta's'	2.1	2.3	1.6	2.1	2.4	1.6	1.9	2.8	2.1
3 Local 28	2.2	2.0	2.3	2.6	2.8	2.0	1.9	2.5	2.2
4 Local 43	2.6	2.9	3.2	3.1	3.0	2.1	2.9	3.1	2.7
5 Local 44	3.0	2.7	2.8	2.9	3.1	2.8	2.6	2.6	2.7
6 Edmore	2.0	2.0	2.0	2.8	2.5	1.7	1.8	2.3	2.0
7 Jo's'*2/Gdo vz 394	2.1	2.1	1.9	2.6	3.0	1.8	2.5	3.0	2.3
8 Stellata's'(Stat's')	3.5	2.8	3.1	3.4	2.9	2.4	2.7	3.8	2.8
Mean of F_2 arrays	2.5	2.4	2.4	2.9	2.7	2.1	2.4	3.03	

Note: a P ± S.E. = 2.6 ± 0.2; F_1 ± S.E. = 2.4 ± 0.1; F_2 ± S.E. = 2.6 ± 0.1; LSD 0.05 F_1 = 0.6; LSD 0.05 F_2 = 0.1.

Edmore x Jo's' *2/Gdo vz 394 had the lowest infestation and damage rating; the cross Gerardo vz 466-GS's' x Stellata's' had the highest infestation and damage ratings. The heritability estimates for aphid infestation and plant damage given in Table 8 suggest, again, that visual selection for tolerance or resistance to aphid infestation may be useful in early segregating populations.

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3.4

Host Plant Resistance to Aphids in Three Nile Valley Countries

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SUMMARY

Wheat and barley crops in Egypt, Sudan and Ethiopia are commonly infested by cereal aphids, resulting in economically significant yield losses. An aphid screening laboratory has been established in Giza, Egypt to screen wheat and barley lines from national programs and from the International Center for Agricultural Research in the Dry Areas (ICARDA) for resistance and tolerance to *Rhopalosiphum padi* and *Schizaphis graminum*, the most serious aphid pests in Egypt and Sudan, respectively. Laboratory tests showed that the progeny of crosses involving the commercial Egyptian varieties Giza 157, Sakha 61 and Sakha 69, as well as Bushland/Amigo lines obtained from the USA, were resistant to *S. graminum*. Some of these lines also showed potential resistance in the field to *R. padi*. A few *Hordeum spontaneum* lines were also resistant to these two aphid species in initial tests, as were lines of *Aegilops squarrosa* and *Triticum timopheevi* var. *timopheevi*. Further laboratory and field tests are planned to verify these findings. The screening procedure used examined plant tolerance to *S. graminum* toxins and plant resistance to *R. padi* population growth. The underlying physiological mechanisms of resistance are not yet known.

Several common aphid species attack wheat and barley in the Nile Valley countries of Egypt, Sudan and Egypt (*see* Table 1 *overleaf*). Four species predominate in Egypt — *Rhopalosiphum padi* (L.), *R. maidis* (Fitch.), *Sitobion avenae* (L.) and *Schizaphis graminum* (Rond.) — with *R. padi* the most serious pest of wheat in Middle and Upper Egypt and *R. maidis* the most serious pest of rainfed barley grown along the north-west coast and in the Sinai. Yield losses in wheat attributable to *R. padi* during severe outbreak years may reach 20% (Tantawi, 1985) but barley losses attributable to *R. maidis* are insignificant. Although *S. avenae* sometimes attains high populations on wheat, it causes only minor crop damage and is not generally considered a pest. Chemical insecticides are currently the only practical method used to control aphids, with many fields receiving up to three applications during the winter growing season.

In Sudan, *S. graminum* is the most serious insect pest of irrigated wheat and, as in Egypt, is controlled by one or more applications of chemical insecticides. Populations of *R. padi* are

Table 1 Aphid species commonly found on wheat and barley in Egypt, Sudan and Ethiopia

Scientific name	Common name
Diuraphis noxia (Mordv.)	Russian wheat aphid
Metopolophium dirhodum (Walker)	Rose wheat aphid
Rhopalosiphum maidis (Fitch.)	Corn leaf aphid
R. padi (L.)	Bird cherry oat aphid
Sitobium avenae (F.)	English grain aphid
Schizaphis graminum (Rond.)	Greenbug

often observed in wheat fields early in the season but they are rapidly displaced by *S. graminum* during the latter weeks of the growing season in late January and early February (ARC, 1988). Sudanese entomologists estimate that direct yield losses in wheat resulting from *S. graminum* may reach 30% in high infestation years in the Gezira Irrigation Scheme, south of Khartoum. Yield losses in the New Halfa Scheme near the Ethiopian border are lower, while in the traditional wheat growing areas of the north aphids seldom cause significant yield losses.

In the Ethiopian uplands, wheat and barley are most seriously damaged by *Diuraphis noxia* (Mordv.); small plot studies have indicated that barley yield losses may be as much as 70% (IAR, 1987). *D. noxia* infestations appear to be most severe in lightly drought-stressed barley grown above 2000 m.

DEVELOPING APHID RESISTANCE IN WHEAT AND BARLEY

Numerous genetic factors contribute to a plant's tolerance or resistance to aphid populations, including morphological and/or physiological traits that kill the aphids, reduce their ability to feed normally or repel them. External factors include weather, parasites, predators and agronomic practices used by the farmer (Dreyer and Campbell, 1987).

A general model depicting the mechanism by which aphids probe plant tissue to locate phloem cells has been proposed to account for resistance in several crop plants attacked by *S. graminum* (Dreyer and Campbell, 1983; Campbell and Dreyer, 1985). These workers suggest that a plant's resistance to an aphid species depends on the ability of the plant to withstand the action of the aphid's saliva, which contains pectinase, in depolymerizing the pectins of the plant's cell wall and middle lamellae. Biotypes are then distinguished according to their ability to depolymerize the increasingly complex plant pectins in more resistant lines. Although it does not account for all aphid-host relationships, this model helps explain some aphid-host relationships observed under natural conditions and provides a template for verifiable hypotheses.

Other aphid resistance mechanisms proposed include the presence of antixenotic secondary plant chemicals that are ingested by the aphid from the phloem. Examples of secondary chemicals that deter aphid feeding when added to artificial diets are gramine in barley (Corcuera, 1984; Zuñiga et al., 1985; Zuñiga and Corcuera, 1986) and Dimboa in wheat (Argandona et al., 1981; Argandona et al., 1980, 1983; Argandona and Corcuera, 1985).

However, honeydew analysis reported by Dreyer and Campbell (1987) indicates that these compounds may not be present in the phloem and would therefore not contribute to host plant resistance in the field.

Moderate levels of resistance in host plants may slow down aphid population growth by reducing reproductive rates or extending development to allow predators and parasites to exert a regulatory effect. If aphid populations are kept below the economic threshold, no additional control may be necessary (Starks et al., 1972). Breeding programs must continually keep ahead of the development of aphid biotypes, which may be accelerated if the resistance developed within a breeding lines is absolute (Eastop, 1973; Diehl and Bush, 1984).

In 1984 it was decided to construct an aphid screening laboratory to test wheat and barley lines from the Egyptian, Sudanese and Ethiopian national programs and those acquired from outside the region, including lines from the breeding program at the International Center for Agricultural Research in the Dry Areas (ICARDA). Since then, ICARDA has undertaken research on aphid-resistant lines in collaboration with these national programs.

Screening procedures used in the Giza aphid laboratory were modified from those described by Starks and Burton (1972) and have been described in detail by Elenin et al. (1989). The current annual capacity of the laboratory is approximately 5000 tests per year each for *R. padi* and *S. graminum*. The tests involve sowing 15 seeds of each line in soil-filled metal trays in rows 30 cm long; the plants are later thinned to 10 per row. Immediately after seedling emergence, aphids from stock cultures maintained in the laboratory, and replenished with field collections from time to time as needed, are introduced to the new seedlings by placing aphid-infested leaves on the soil between the seedling rows. The aphids quickly walk onto the young seedlings. The seedlings are examined after 2 days and additional aphids are added so that there are at least two on each seedling.

Damage ratings for *S. graminum* are made 15 days after aphid introduction (*see* Table 2). Plants with a score of 0-1 are considered tolerant, while those with a score of 2 or more are considered non-tolerant. Resistance to *R. padi* is assessed according to the total number of aphids present on the seedling at the time of rating. Lines with an average of less than 10 aphids per plant are considered resistant; those with 10-15 aphids per plant are considered moderately susceptible; and those with more than 15 aphids per plant are considered susceptible. Lines with a score of 0-1 for *S. graminum* or, in the case of *R. padi*, with 15 or less aphids per plant are

Table 2 Damage scoring system in wheat and barley for *Schizaphis graminum*, used in aphid resistance research in Egypt, Sudan and Ethiopia

Plant symptom	Score
No apparent damage	0
Red spots on leaves	1
Enlarged red spots on leaves, surrounded by yellow halo	2
Yellow patches on leaf	3
Whole leaf chlorotic	4
One or two leaves killed	5
Plant killed	6

subjected to two further tests in the laboratory, following the procedure described above. Those consistently selected in the three tests are then evaluated in field plots in Middle and Upper Egypt for resistance to *R. padi* and in the Gezira and New Halfa Irrigation Schemes, Sudan for resistance to *S. graminum*. Lines showing promise are recommended to breeders at ICARDA and to national programs in the region.

ACHIEVEMENTS AND FUTURE RESEARCH

Since the Giza laboratory began operation, over 10 000 wheat and barley lines have been tested. The vast majority of these were advanced breeding lines showing no tolerance or resistance to either aphid. However, in 1986-87 the wheat lines listed in Table 3 were used as parents in crosses whose offspring show promise for *S. graminum* resistance. The reactions of these lines and backcrosses to *S. graminum* may be attributable to two recessive genes, as suggested by Dahms et al. (1955) and Starks et al. (1981). Other workers have suggested that greenbug resistance was imparted by a single gene pair (Daniel and Porter, 1958; Painter and Peter, 1958; Gardenhire and Ghada, 1961; Curtis et al., 1963). These differences of opinion may arise from differences in techniques used to classify resistance, variation in the aphid biotypes used and variation in the number of modifying genes.

Table 3 Wheat lines used as parents in aphid resistance tests in Egypt, Sudan and Ethiopia, 1986-87

Parent	Pedigree
Bushland/Amigo T 101	Bushland T x F 79518-2 x 77A/5 /Amigo 11 T 101
Bushland/Amigo T 105	Bushland T x 38924-8 Amigo/27-105
Giza 157	Giza 155-PI64 x IR.64 /Tepp x Knott 11
Sakha 61	Inia-RL 4220 x 7C/YR'S': CM 15430-2S-6S
Sakha 69	Inia-RL 4220 x 7C/YR'S': CM 15430-2S-6S

The parental lines used in 1986-87 were evaluated in the field in 1988-89 for resistance to *R. padi*. Four lines of backcrossed (BC_3) Bushland/Amigo T 101 x Sakha 69 and 14 lines of backcrossed (BC_3) Bushland/Amigo T 105 x Sakha 69 were classified as moderately resistant, based on a low rate of spreading of the aphids among plants in the row. Other lines derived from backcrosses with both Bushland/Amigo lines and the three Egyptian varieties were rated as moderately resistant. These will be subjected to further field tests.

Several workers have reported aphid resistance in barley (Murty et al., 1968; Gill and Metcalf, 1977; Jain et al., 1984) and in wild barleys (Weibull, 1988). Our laboratory studies in Egypt have yet to verify these findings, although we have observed tolerance to *S. graminum* and *R. padi* in *Hordeum spontaneum*. In field trials conducted at Malawi in Middle Egypt in

1988-89, where barley is heavily attacked by *R. maidis*, none of the advanced breeding lines from ICARDA or from other national programs showed tolerance or resistance. Laboratory tests conducted in 1987-88 revealed four *H. spontaneum* lines with possible *S. graminum* tolerance and two *H. spontaneum* lines with possible *R. padi* resistance (Elenin et al.,1989) (*see* Table 4). In addition, a*Triticum timopheevi* var. *timopheevi* line andtwo lines of *T. aestivum*, of *T. durum* and of *Aegilops squarrosa* were identified as having moderate *R. padi* resistance. These lines are being re-evaluated to confirm resistance, although low germination has made it difficult to conduct tests on *A. squarrosa* and *T. timopheevi*.

Table 4 Reaction in barley lines tested for resistance to *Schizaphis graminum* in laboratory studies, 1987-88

Line	Resistant	Susceptible	Ratio
Bushland/Amigo T 105	10	0	—
Bushland/Amigo T 101	25	0	—
Giza 157	0	50	—
Sakha 61	0	50	—
Sakha 69	0	50	—
Bushland/Amigo T 105 x Giza 157	20	198	1:15
Bushland/Amigo T 105 x Sakha 69	24	281	1:15
Bushland/Amigo T 105 x Sakha 61	4	56	1:15
Bushland/Amigo T 105 x Giza 157	10[a]	83	1:15
	40[b]	88	1:3
Bushland/Amigo T 101 x Sakha 61	11[a]	88	1:15[c]
	29[b]	87	1:3
Bushland/Amigo T 101 x Sakha 69	13[a]	215	1:15
	4[b]	10	1:3

Note: a First cross.
b First backcross.
c $.05 < p < .10$.
Source: Adapted from Elenin et al., 1989

The mechanism of resistance against *R. padi* is not clear in the lines identified to date in the Giza laboratory. We suspect that leaf pubescence and cuticle toughness are important factors. Field observations also suggest that moderate resistance or tolerance to *R. padi* combined with earliness may allow a variety to escape most of the aphid population build-up while tolerating the low populations that do infest it. In Sudan, the problem is more difficult as the proximity of alternative hosts of *S. graminum* in fields surrounding wheat fields, coupled with the heavy use of insecticides in nearby cotton fields that reduce natural predator and parasite populations, provides a continuous source of aphids throughout the growing season. Using resistant varieties in combination with improved pest management practices on cotton will probably prove to be effective in controlling aphids on wheat in Sudan.

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PART 4

Methodologies for research on barley yellow dwarf virus

4.1

Barley Yellow Dwarf Virus Symptoms and ELISA Data in Relation to Biomass and Yield Loss

A. COMEAU, J. COLLIN and F. CHEOUR

SUMMARY

Symptoms of barley yellow dwarf virus (BYDV) are not appropriate as a diagnostic tool except in severe epidemic situations. The selection of tolerant or resistant cereal lines in plant breeding programs on the basis of symptoms is warranted only if a number of conditions are met. Virus infection must be done early with a high inoculum pressure, preferrably through artificial inoculation. The use of ELISA for breeding purposes is not practical for barley, durum wheat and bread wheat unless the material selected contains strong resistance genes of interspecific origin. However, ELISA is useful in distinguishing resistance from tolerance.

Determining the presence of barley yellow dwarf virus (BYDV) simply on the basis of the typical disease symptoms can be misleading. These symptoms may represent only the tip of the iceberg. Moreover, it is now clear that BYDV has often caused significant damage without being identified as the culprit. As long ago as 1974, Dr W.C. James and a BYDV specialist, Dr C.C. Gill, expressed concern about these issues. When Dr James was later appointed as Deputy Director General for Research at the Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT), he was among the first to recognize that research on BYDV deserved more support.

It was some time, however, before there was general recognition of the seriousness of BYDV and the need to control it. When a group of farmers were told by a virologist that their fields were fully infected, they denied that the disease existed and, fearing a drop in crop prices, threatened a lawsuit. All their fields needed, they said, was some nitrogen; indeed, by applying enough nitrogen they did manage to make the fields look greener. But BYDV symptoms are not always exactly like those in textbooks. Another virologist confirmed that the fields were about 100% infected. As BYDV-infected plants are less efficient in using nitrogen (Comeau and Barnett, 1979), it is unlikely that the potential yield of these fields was realized, despite the nitrogen added.

This paper presents a definition of BYDV symptoms and discusses the symptomatology of the virus in relation to yield losses, and compares evaluations based on symptoms with those determined by enzyme-linked immunosorbent assay (ELISA).

THE NATURE OF BYDV SYMPTOMS

The approach adopted in assessing symptoms of fungal diseases is often used, inappropriately, to assess BYDV symptoms. In the case of rusts, for example, diagnosis hinges on the fact that visible rust pustules are almost quantitatively related to the amount of this pathogen present in any cereal species; the interpretation of BYDV symptoms, however, is not nearly so straightforward (*see* Table 1). Many factors (including cereal species, genotypes, virus strains, environment and aphid population) influence the visibility of BYDV symptoms and thus interpreting these symptoms should be done with extreme caution. In addition, the symptoms of other stresses or viruses often resemble BYDV symptoms.

The comparisons presented in Table 1 are based on 18 years of field experience with natural epidemics and artificial inoculations (Comeau, unpubl.). Under the growing conditions in Quebec, Canada, bread and durum wheat present a special problem as the BYDV symptoms are often rather ephemeral and sometimes impossible to see. In all species except oats and

Table 1 Comparison of symptom diagnosis of BYDV and rusts in four cereal species

Symptom diagnosis	Rusts	BYDV			
		Oats	Barley	Bread wheat	Durum wheat
Ease of visual diagnosis	Very high	High	Moderate to high	Low to moderate	Very low
Quantitative estimate of pathogen	Easy	Difficult	Difficult	Difficult	Difficult
Symptom level correlated to grain yield loss	Very often	Often	Sometimes	Sometimes	Sometimes
Symptom visibility depending on biomass loss	Very rarely	Rarely	Often	Very often	Very often
Duration of symptoms (days)	20-50	0-50	0-25	0-10	0-10
Cryptic infestation	Rare	Frequent	Frequent	Frequent	Frequent
Masking of symptoms by secondary pathogens	No	Yes	Yes	Yes	Yes
Symptom expression modified by drought or soil type	Rarely	Often	Very often	Very often	Very often

barley, the field symptoms have poor diagnostic value, and ELISA verification is needed. In barley, infection of young plantlets (Zadoks 09-29) by a severe strain can be diagnosed visually but not with total confidence, as other viruses may have similar symptoms (von Wechmar, 1990). For all species, symptoms are a series of visible indicators of general physiological disturbances caused by a viral attack on the phloem, which is a key organ carrying the photosynthates. These indicators, often unrelated to the amount of BYDV in the plant, include reductions in root growth, plant height, biomass, tiller number, floret number, floret fertility and seed size. They may or may not include the partial loss of chlorophyll, which may or may not be accompanied by the evolution of yellow, red, purple or brown pigmentation and secondary infection by certain fungi that are often pathogens with saprophytic ability.

Environmental conditions can cause remarkable variation in the expression of symptoms in winter cereals. Collin (1983) reported that, in a large-scale field trial involving 61 winter wheat crosses, when the plants were seeded early in the autumn, symptom expression later in the growing season was poor but at least the symptoms present were as expected, and a yield loss of 24.0% was measured; but when winter wheat was seeded later, although subsequent symptom expression was even poorer there was still a yield loss of 20.5% (*see* Table 2). The trial was repeated in the following year, and although late seeding gave BYDV-related yield losses as high as those from early seeding, the correlation with symptoms was again too low to be of any use when the wheat was late-seeded. As shown in Table 3 (*overleaf*), while the low correla-tions were often statistically significant, their low values suggest probable failure of attempts to select BYDV-tolerant winter wheat on the basis of visual symptoms. In artificial inoculation trials involving durum wheat (Comeau and St-Pierre, 1984), the difficulty in obtaining symptom expression was sometimes a problem in that it was not possible to record symptoms in certain years. The same problem occurred in trials which were conducted with potted plants kept in a screen cage, under field temperature conditions, where a grain yield loss exceeding 50% was observed despite the absence of the so-called typical leaf tip yellowing (Cheour et. al., 1989).

Stresses affecting root growth, such as drought or the physicochemical properties of the soil, have a major influence on BYDV symptom expression. For example, many 'Buck Buck' lines are generally moderately tolerant, but in the 1988 drought they appeared to be as susceptible as all other semidwarf lines. These observations have led us to put more emphasis on developing

Table 2 Yield loss from BYDV in trials involving 61 winter wheat crosses

Year	Seeding date	Symptom visibility	Yield		Yield loss (%)
			Check	Inoculated	
1980-81	normal[a]	Poor	267	203	24.0
1980-81	late[b]	Very poor	187	148	20.5
1981-82	normal[a]	Fair	244	170	30.3
1981-82	late[c]	Poor	176	119	32.2

Note: a Plants inoculated at the 4-5 leaf stage.
b Plants inoculated at the 1 leaf stage.
c Plants inoculated at the 1-2 leaf stage.
Source: Collin, 1983

Table 3 Correlation between visual symptom score and grain yield of inoculated plots of winter wheat

Year	Seeding date	Time of symptom evaluation — May	June
1980-81	Normal	-0.20[a]	0.13[a]
1980-81	Late	-0.04n.s.[c]	0.11[b]
1981-82	Normal	—	-0.61[a]
1981-82	Late	—	-0.27[a]

Note: a Significant at $p < 0.01$.
b Significant at $p < 0.05$.
c Not significant.
Source: Collin, 1983

short-statured wheat able to resist BYDV under any climatic conditions; the problems of managing BYDV trials containing short-statured lines are discussed later in this paper.

In field surveys, what is reported as BYDV symptoms may well be BYDV; however, the symptoms could result from a combination of BYDV and other stresses, or from purely physicochemical factors, or even from another virus (von Wechmar, 1990). If symptoms are abundant, severe yield loss has probably occurred, but the viral cause of the symptoms must be verified by ELISA. Conversely, the absence of BYDV symptoms does not indicate the absence of BYDV infection. ELISA on a large bulk sample is always worthwhile as a first step in any survey, whether symptoms are present or not. If symptoms are present, a more detailed ELISA survey is warranted, as relatively significant BYDV-induced losses may be occurring even when symptoms are scarce or absent.

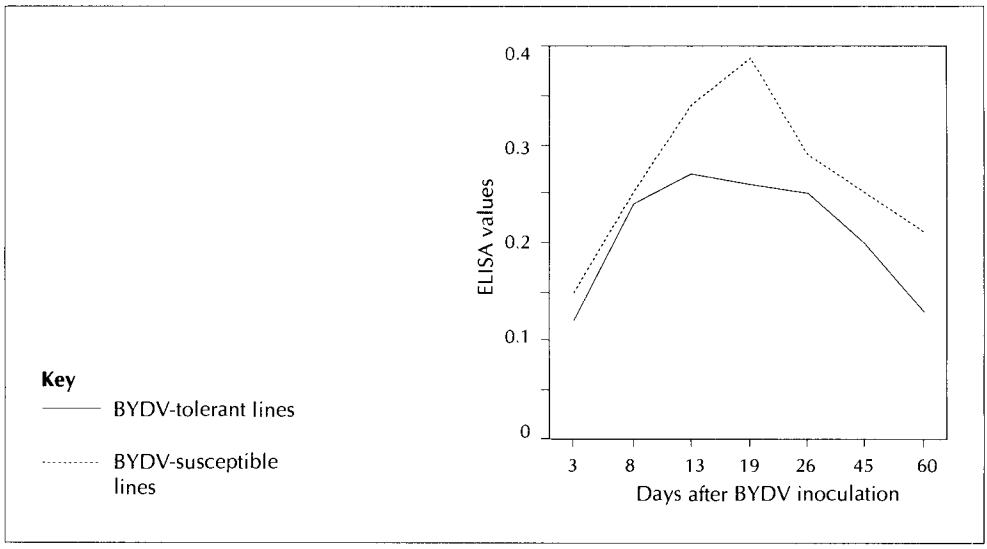
USING ELISA TO DETECT BYDV

ELISA has contributed greatly to our knowledge of BYDV worldwide. It is the tool that has allowed an accurate geographical mapping of the presence of BYDV and a recognition of the main isolates worldwide.

Extending the use of ELISA to the plant breeding level was initially fairly inefficient. Earlier studies showed some correlation between ELISA values and tolerance of cultivars in the field (Skaria et al., 1985). In our studies, the ELISA readings of the most susceptible durum lines were, on average, higher than the ELISA readings of the moderately tolerant lines 12th IDSN 227 and Boohai; however, each line showed a peak virus concentration at a different time, and thus ELISA evaluations based on a single date of sampling could have been totally misleading. The practical use of ELISA necessitates pooling information from many sampling dates, to eliminate the error which results from a single sampling. In two trials with durum wheat, we tested seven different dates, from early sampling (3-8 days post-inoculation) to very late sampling (60 days). In the first trial, the tolerant lines had somewhat lower average ELISA values, but the difference between tolerant and susceptible was rather small and useless for practical application (*see* Figure 1). Significant yield loss was recorded, but typical symptoms

were scarce. In the second trial, fairly typical symptoms were obtained (*see* Figure 2 *overleaf*). It is not clear why symptom levels differed so much between trials; as noted above, variability of symptom expression is a common problem with durum wheat (Cheour et al., 1989). Interestingly, the symptoms developed slowly but at a steady rate in the susceptible lines, and were at a maximum when the ELISA value was at its minimum, 60 days after inoculation. This indicates that it could be very misleading to wait until symptoms reach maximum expression before collecting samples for virus titer determination by ELISA.

Figure 1 **Average ELISA values for BYDV-tolerant durum wheat lines (12th IDSN 227 and Boohai) and for BYDV-susceptible lines (12th IDSN 74 and La Dulce), at 3-60 days after inoculation**



A further attempt was made to correlate the BYDV reaction with ELISA values at the species level. This involved inoculating plantlets of six species and comparing the values at 14 and 26 days after inoculation. In a few cases, such as with Corris (Yd_2 gene), a BYDV-resistant barley line, and 83TF 519.31.1, a BYDV-tolerant triticale line, the ELISA values were quite low and thus provided potentially useful information at both sampling dates (*see* Figure 3 *overleaf*). The susceptible barley lines, 850L 303 and Abee, had rather high values after 26 days; this would tend to confirm the conclusions reported by Skaria et al. (1985), who were able to identify resistant barley lines using ELISA. However, the very susceptible oat line, Lamar, had a very low ELISA value after 14 days, the very tolerant bread wheat, Long Miai 10, had a high ELISA value after 26 days, and the tolerant rye had high ELISA at both sampling dates. The BYDV-susceptible triticale line, Mapache, had lower ELISA values than the BYDV-tolerant wheat lines, IAS-20 and Long Miai 10. The sweet corn, Seneca 60, had an ELISA value barely higher than the significance threshold after 14 days, but a very significant value after 26 days.

These results do not enable us to draw any firm conclusions, but they are important in dispelling the common illusion that ELISA is an easy and foolproof method to use in breeding

Figure 2 **Comparison between symptom score and ELISA values, showing the averages for two BYDV-tolerant durum wheat lines (12th IDSN 227and Boohai) and two BYDV-susceptible lines (12th IDSN 74 and La Dulce) at 3-60 days after inoculation**

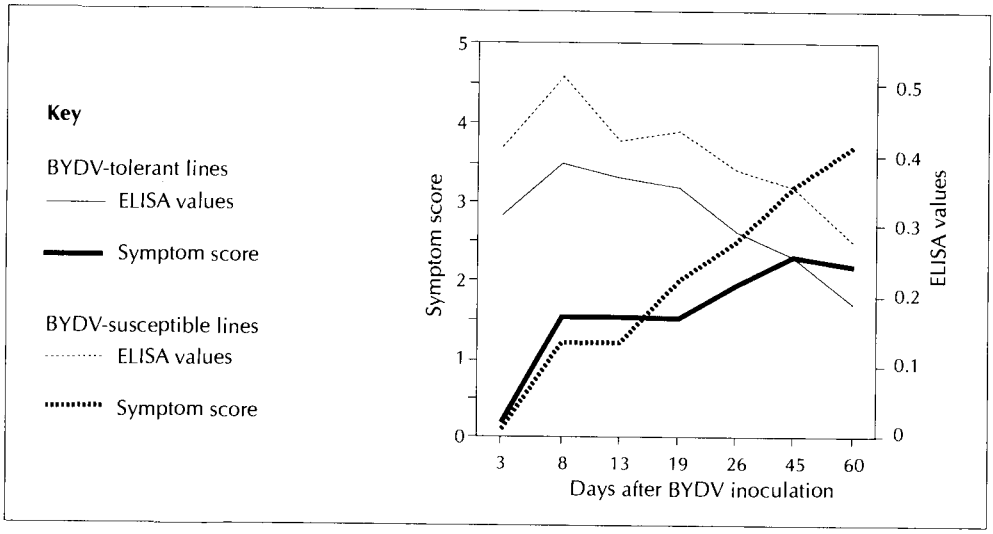
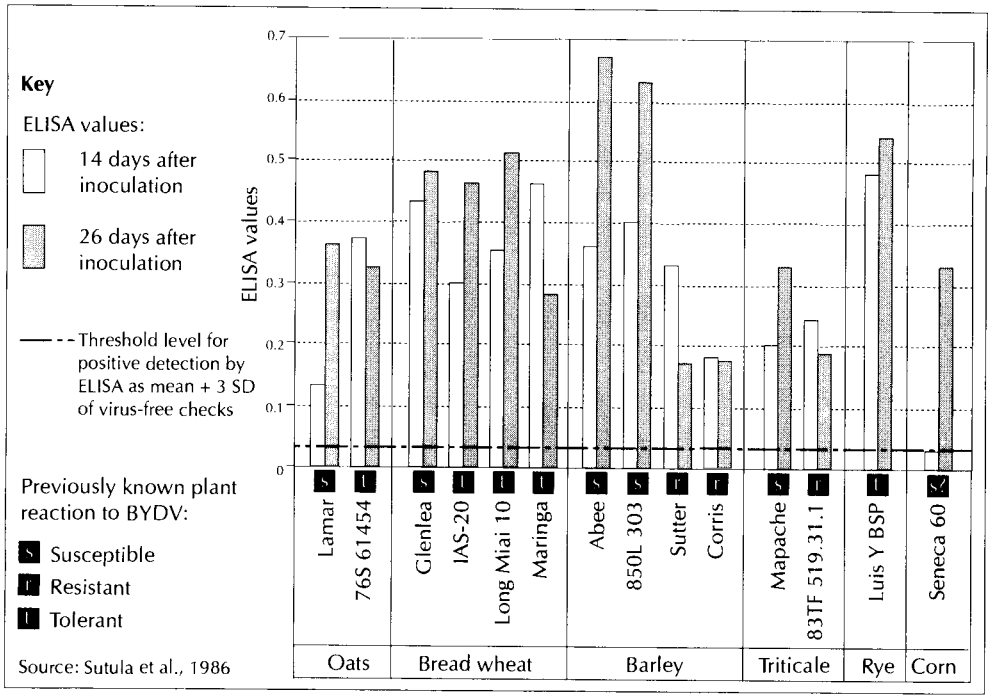


Figure 3 **Comparison of ELISA values at 14 days and 26 days after BYDV inoculation of plantlets of six cereal species**



for BYDV resistance. It is also worth stressing that the need for many sampling dates increases the cost of large-scale use of ELISA, and that it has not been shown yet that ELISA could be used in a practical manner in any cereal breeding program involving only domesticated cereals as parental material. However, the use of ELISA is very practical in the study of resistance in hybrids of wheat and perennial grasses (Sharma et al., 1984); as these grasses are immune, or at least far more resistant than the barleys carrying Yd_2, ELISA gives clear identification of the lines possessing resistance genes derived from *Agropyron*, *Leymus* or other species.

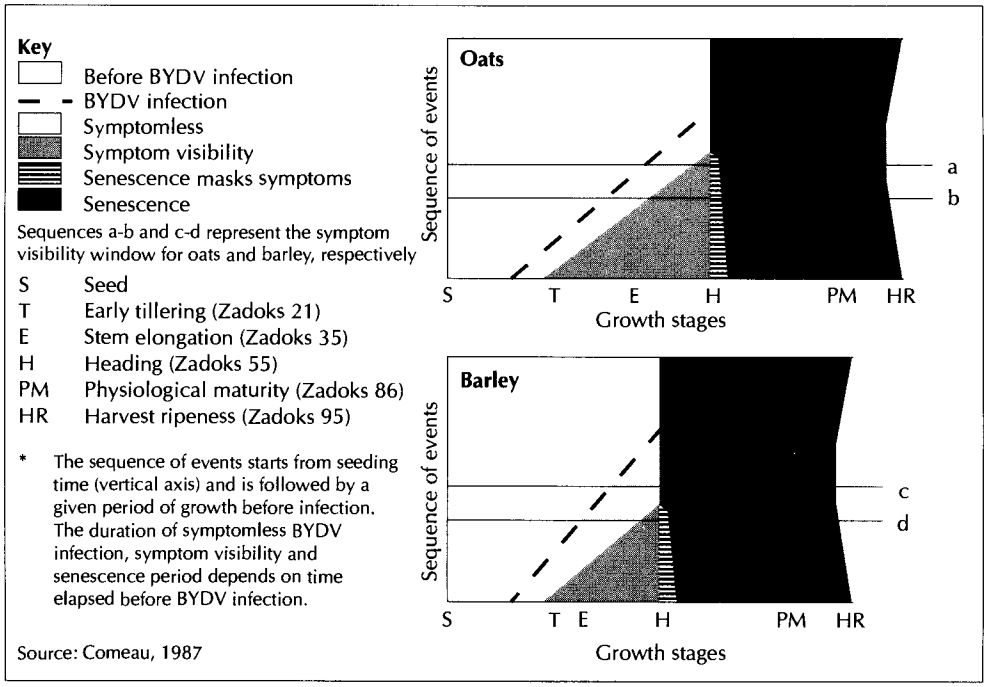
In essence, the real value of ELISA is that it distinguishes between true resistance, which is accompanied by low virus content, and tolerance, which is the ability of plants to produce adequate yields despite high virus content (Cooper and Jones, 1983).

INTERPRETING SYMPTOM READINGS

Delay between inoculation and symptom expression

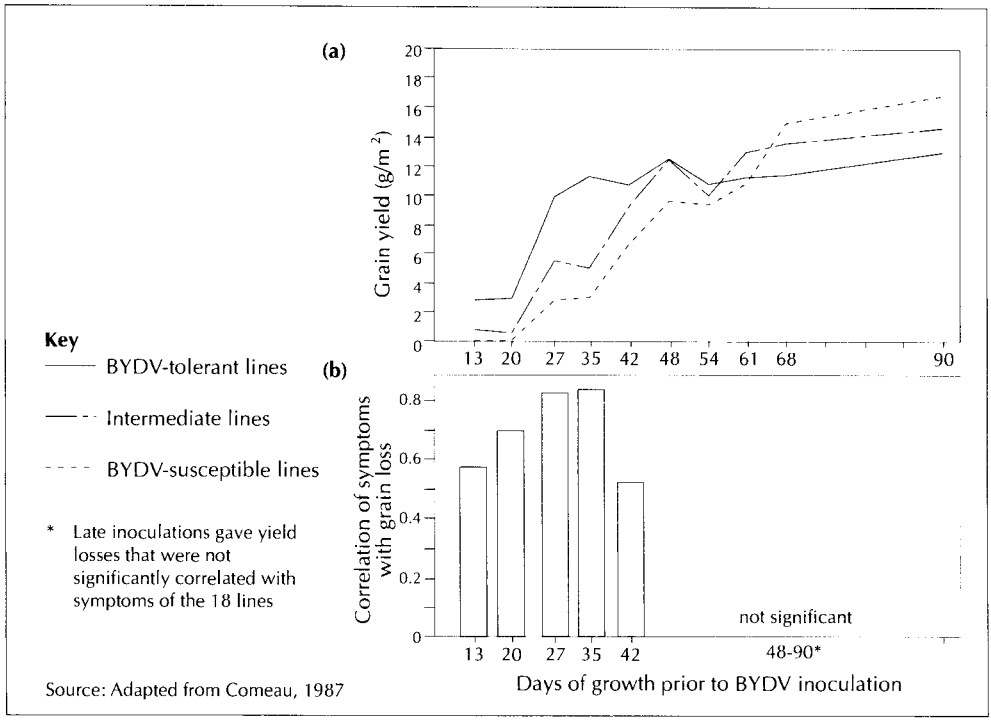
If plants are inoculated too late, it becomes impossible to distinguish symptoms from natural senescence (*see* Figure 4). Our studies in Quebec have shown that the most common natural epidemics coincide with a natural infection in July, which is near flowering time, 55 to 70 days after seeding; as symptoms may take 2 weeks to appear on oats, the ideal period for symptom

Figure 4 Symptom visibility model for oats and barley, showing sequence of events determined by time elapsed prior to BYDV inoculation*, based on observations in Quebec, Canada



observation (the visibility window) is fairly long, from 10 July to 10 August. In barley, as symptom expression is slower, symptoms would appear at grain-filling stage, on about 26 July; in this case the visibility window is very short, from July 26 to July 31, before senescence gradually masks the symptoms. Needless to say, if aphids arrive in June, heavy and visible damage may occur on all species, but if they do not arrive until in late July, the subsequent epidemic and damage will be symptomless. The visibility window model featured in Figure 4 was based on representative experimental data using artificial inoculations. As indicated in Figure 5, the damage associated with symptomless infection is not negligible at all. Similar problems were noted in winter wheat, and confirm the need for ELISA studies when evaluating the presence of BYDV. Oats was the only species for which symptoms were a reliable basis for BYDV assessment, but the recent introduction of tolerant cultivars might make symptomatology less useful in the future for this species.

Figure 5 **Average grain yield (a) and correlation of symptoms with grain yield loss (b) in 18 oats lines (6 tolerant, 4 intermediate and 8 susceptible) inoculated with BYDV at various dates during the growing season**



Timing of symptom evaluation

In breeding programs artificial inoculation is generally done between the 4-leaf stage and early tillering. In the case of oats and barley, symptom readings could be taken as late as 3-4 weeks after inoculation. Between 1976 to 1982 we took symptom readings at different stages, to verify

whether early or late notation was better correlated with the grain yield and grain quality of BYDV-infected plants. It soon became obvious that readings taken near maturity, after most chlorophyll had gone, seemed to provide the most reliable information about tolerance. After many years of comparitive studies, our colleagues at Winnipeg endorsed our recommendation that readings for oats should be taken very late (Haber, pers. comm.). In 1983, we took symptom readings for winter triticales at three different dates, and the latest date was the only one that gave the best correlations with all quantitative traits ($r = 0.50$ or better), confirming that in winter cereals, too, a very long delay is needed between BYDV inoculation and the notation of symptoms (Collin, 1987).

A notable feature emerging from our studies was that the time of symptom expression varied considerably among cultivars. One cultivar might have looked healthy until the grain-filling stage, but then failed to fill or showed heavy floret sterility. Another might have shown a significant amount of leaf yellowing but then regained an acceptable level of health, produced a reasonably green flag leaf and maintained good spike fertility and grain filling. This underlines the importance of basing assessments on end results rather than day-to-day observations. It is also important to remember that less visible symptoms, such as decreased quality of seed and forage, may have major economic significance.

Symptom scoring

Symptom scoring involves absorbing information, quite rapidly, about plant shape, tillering, height, floret sterility and color and integrating this information into a disease notation system, such as the scale devised by Qualset (1984). However, the observer must interpret this visual information with some degree of flexibility; in Quebec, for example, notations for bread wheat and durum wheat are particularly difficult because dwarfing and discoloration level may differ considerably from year to year.

Plant height represents a problem that deserves special mention. The eye records plant height but has no means of distinguishing between a genetic dwarf and a plant that is shorter because of virus infection. It is worth noting at this point that there are very few semidwarf wheat lines that deserve the label 'tolerant' if these lines are subjected to artificial inoculation, using 10-20 aphids/plant, and compared to the tall BYDV-tolerant line, Mariunga; when semidwarf lines are compared to each other, however, some (such as 14th IBWSN 45) are clearly more tolerant than others (such as Siete Cerros and Bow's). Our first attempt to deal with the plant height problem was to use a mathematical formula whereby symptoms, grain yield and harvest index of infected plants were combined into a tolerance index (Comeau, 1984). Later, we discovered that plant height readings could be used conveniently as a co-variate, at least within nurseries where there were strong genetic differences in plant height. To obtain an even more precise assessment, the elite material was divided into three groups — tall, medium and short lines — to avoid unwanted competition between tall and dwarf lines. This provided a new way of identifying accurately which lines within each height category possessed the best tolerance. A few replicates of virus-free plots should be grown in order to obtain a precise evaluation of genetic height.

To demonstrate the reality of the problem in bread wheat, data from 1985 and 1986 on the 28 best lines of all categories were analyzed. Symptoms were not distinct enough in 1985 and

therefore were not noted, but they were sufficiently visible in 1986 (*see* Table 4). Taken at face value, these data suggest that one could select for BYDV tolerance on the basis of plant height, as this correlates well with the visual symptom reading. However, to avoid confusing a tolerant plant with a genetically tall plant, the plant height of virus-free checks must be recorded. The height data could be used in many ways, such as in calculating the difference in height or the height ratio of inoculated and healthy plots (Collin et al., 1990).

Table 4 Correlation between various indicators of BYDV tolerance in bread wheat trials conducted over 2 years[a]

	1985 Plant height	1985 Grain yield	1986 Plant height	1986 Grain yield
1985 grain yield	0.206 n.s.[b]			
1986 plant height	0.928[c]	0.214 n.s.		
1986 grain yield	0.521[c]	0.341[d]	0.577[c]	
1986 symptoms	-0.768[c]	-0.213 n.s.	-0.834[c]	-0.642[c]

Note: a The germplasm included 40 lines of bread wheat with moderate to good BYDV tolerance; plants had been inoculated between Zadoks 25 and 32.
b Not significant.
c Significant at $p < 0.0001$.
d Significant at $p < 0.025$.

In a comprehensive general study of the correlation between various indicators of BYDV tolerance or resistance in triticale, Collin et al. (1990) showed that many different criteria were reasonably well correlated with symptoms and could be used by the plant breeder. Symptom scores correlated very strongly with the plant height, biomass and grain yield of inoculated plants, but also with the height ratio or difference (comparing diseased plants with healthy plants). The key to the success of this study was the use of abundant inoculum of viruliferous aphids. As triticale has greater BYDV tolerance than bread wheat, the abundant inoculum (20-40 aphids/plant) increased the differences between susceptible and tolerant lines.

However, it should not be forgotten that symptoms are only a crude visual tool used to estimate quantitative damage and that these symptoms are variable. A true picture of the effect of BYDV is gained if quantitative data are accurate; that is, accurate measurements should be made of the loss in biomass, grain yield and grain quality as a result of BYDV. Obtaining these data, however, is expensive. A less expensive compromise would be to calculate the biomass ratio, comparing infected plants with healthy plants, but the inoculation intensity must be appropriate (Collin et al., 1990). An alternative measure would be to learn how to rear more aphids so that heavier artificial inoculation could be used to induce more clear-cut symptoms. The best way to induce symptoms in triticale, durum wheat and bread wheat is by inoculating with a severe strain of BYDV and using a large number of viruliferous aphids. This produces a stronger shock effect and intensifies symptoms. Five aphids per plant is often not enough; we recommend from 10-20 aphids/plant for a good screening trial. At these higher levels, aphid damage may occur on young plants (2-4 leaves) and the selection may favor aphid-resistant as well as BYDV-resistant genotypes, but this is not a serious drawback because aphid resistance

could be valuable in itself. If this still does not produce strong symptoms, a mixture containing severe PAV and RPV strains could be used instead of a pure BYDV strain; this mixture, when transmitted by *Rhopalosiphum padi* (L.), usually produces the most severe symptoms. Aphid rearing can be done on a consistent basis for experiments with BYDV; our aphid production is about 10 million aphids in June and the same amount in September for winter cereals.

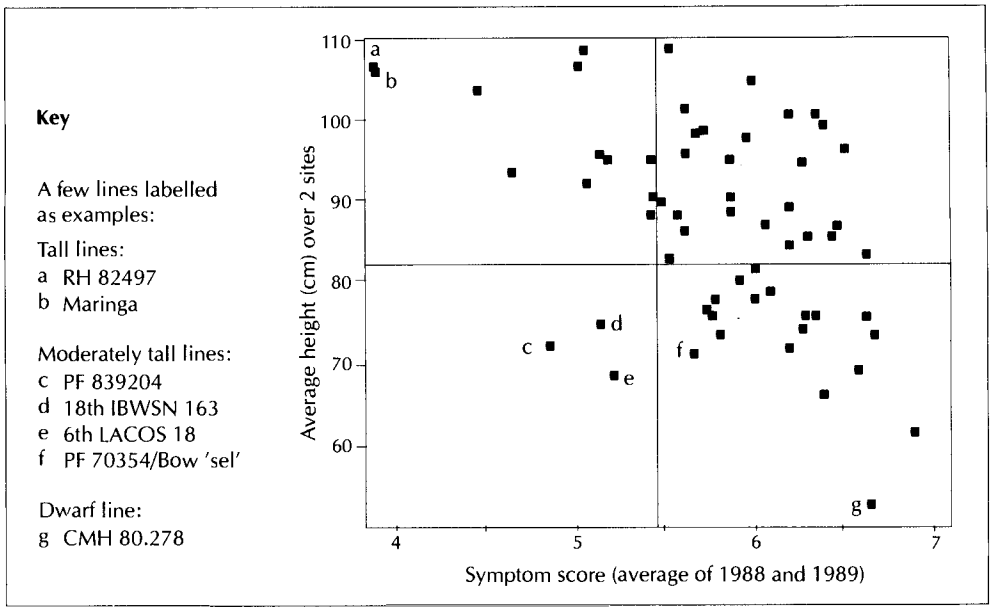
We also recommend that data be gathered over many years before conclusions are drawn, because there is an important interaction between BYDV and climate. Drought intensifies symptoms and sometimes make tolerance genes less effective. Excessive rain could drown aphids or concentrate them in water puddles, increasing experimental error. Nevertheless, in 17 years of work with BYDV, there was only one year (1978) in which our efforts were really wasted; in that year, the virus strain chosen was too mild and the inoculation too late.

Correlation between genetic height and BYDV tolerance

The statement earlier that semidwarf wheat is more BYDV susceptible than tall wheat was not borne out by the correlations shown in Table 4. These data might prove only that BYDV reduced plant height and that height information was very important in the eyes of the person who assigned the symptom values.

In order to gain a better interpretation of these tendencies, we compared the plant height at two sites of virus-free multiplication plots in 1989 with the symptom and biomass data obtained from BYDV-inoculated plots in 1988 and 1989 (*see* Figure 6). The wheat lines involved were

Figure 6 Comparison of plant height data obtained from two BYDV-free sites in 1989 with average symptom data obtained from BYDV-inoculation trials conducted in 1988 and 1989 using wheat lines identified to date as the most BYDV-tolerant



those from elite nurseries containing only the most tolerant lines found over the past 10 years for each height category; susceptible lines were not included. The correlations between healthy plant height and symptoms were significant (from r = -0.351 to r = 0.468, at $p < 0.001$), indicating that short stature is indeed linked to higher BYDV sensitivity (*see* Table 5). If susceptible lines of different statures had been included, it would probably have increased the correlations observed, as most short-statured lines are far more BYDV susceptible than the ones used in this trial. The desired short-statured lines with good BYDV tolerance are quite rare; moreover, the most stable tolerance from year to year has been observed with the tall genotypes RH 82497 and Maringa. The shorter lines tend to lose BYDV tolerance in dry years; in the 1988

Table 5 **Correlation between plant height of virus-free plants and the symptoms and biomass of BYDV-inoculated plots, for 65 lines previously identified as virus tolerant[a]**

	BYDV-inoculated plots					
	Symptoms			Biomass		
	1988	1989	Mean	1988	1989	Mean
1989 virus-free plant height	-0.351[b]	-0.466[c]	-0.468[c]	0.517[c]	0.307[b]	0.534[c]

Note: a Lines in this trial represented th e best sources of tolerance available in various plant height groups. The virus-free height was the average of data from two sites; other data were from one site.
b Significant at $p < 0.01$.
c Significant at $p < 0.0001$.

Table 6 **Comparison of height (cm), BYDV symptoms, yield (kg/ha) and BYDV tolerance in bread wheat lines classified in order of virus-free plant height**

Line	BYDV-inoculated			Virus-free		Tolerance[a]
	Height	Symptom	Yield	Height	Yield	(%)
Ciano 79	60	7.0	1599	58	3057	52
Tesia 79	64	6.9	1727	60	2408	72
Alondra 4546	57	7.3	877	63	3049	29
SWM 12686.20.33	69	5.7	3205	68	3810	84
14th IBWSN 182	73	5.8	2023	72	2412	84
NEAC 120	72	5.9	1739	73	2957	59
8182PCH 678	72	5.4	2464	77	3233	76
Thornbird's	75	5.6	1687	81	2985	57
Long Miai 10	81	4.7	2875	85	3139	93
Mascarenhas	77	5.3	2364	87	2965	80
BH 1146	89	4.6	2680	93	2821	95
Maringa	93	4.5	2564	89	2431	105
IAS-20	91	5.3	2724	91	2636	103

Note: a Calculated as: inoculated yield/virus-free yield; correlation between tolerance and virus-free plant height is 0.713 ($p < 0.008$).
Source: Comeau and St-Pierre, 1987

drought year, short-statured lines in Quebec were so devastated by BYDV that many had near zero yield (Comeau and St-Pierre, 1988).

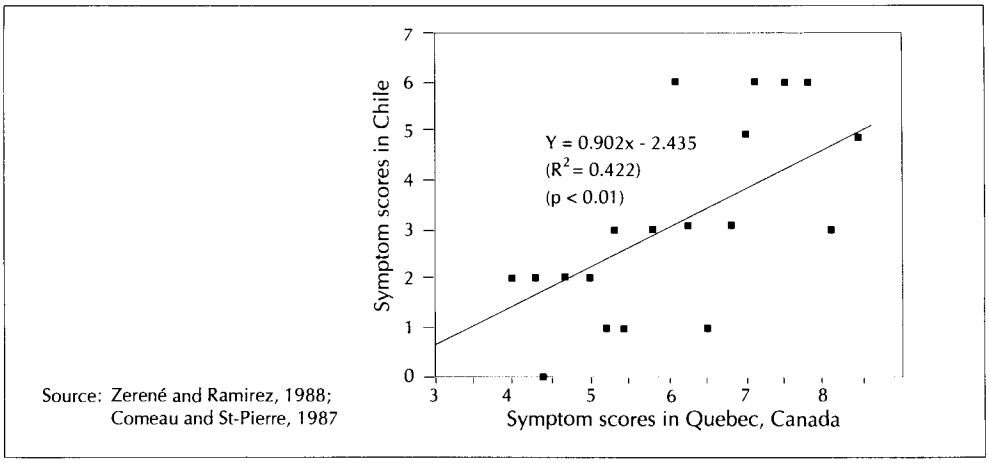
Yield loss studies including virus-free checks were also conducted. In these studies, the tall lines Maringa and IAS-20 were more BYDV-tolerant than any of the shorter wheat lines (*see* Table 6). This reaffirms the need to use genetic plant height as a co-variate in future studies of BYDV tolerance in wheat.

CONCLUSION

The eye is a poor tool for diagnosing BYDV infection in cereals, except for oats. Although visual scoring provides a means of rapidly assessing BYDV incidence, it should always be followed up by ELISA tests. Early infection of barley, for example, is quite visible, but it should be double-checked with ELISA, and plant sampling should disregard the presence or absence of symptoms. Care is also needed when using symptom data in breeding programs. In theory, symptom data are the cheapest source of information, but they are not the most reliable, as the following illustration shows. An untrained observer assessed the resistant triticale check Wintri as susceptible because it had some yellowed leaf tips, and classified a severely dwarfed but green genotype as resistant; at the time, this dwarfed genotype was suffering a yield loss of about 75%. The observer had taken notes too soon and had put the emphasis on the wrong trait. Although height reduction must be among the criteria used in visual assessments, it is important to remember that genetic height factors are significantly correlated to BYDV tolerance.

Artificial inoculation is usually preferrable as it produces more uniform and clear-cut symptoms. Correlations obtained between artificial inoculations are generally quite satisfactory from year to year or between distant locations, particularly in the case of oats (McKenzie et al., 1985) and barley (*see* Figure 7). However, sometimes the correlation is poor; attempts should then be made to identify the causes, as this could be related to the presence of different BYDV strains or to the presence of other viruses as well as BYDV. Multilocational trials can

Figure 7 Comparison of symptom scores obtained in Chile and Quebec, Canada for BYDV-inoculated barley grown under different conditions and photoperiods



Source: Zerené and Ramirez, 1988; Comeau and St-Pierre, 1987

also teach us a great deal about the practical value of any given source of BYDV tolerance or resistance for plant breeders worldwide.

When data from natural epidemics are obtained, it is important to assess the uniformity of the infestation, as the disease may sometimes be distributed in a very erratic manner, making the data less valuable. Leaf samples should be obtained for strain identification and, if possible, symptom scores should be accompanied by quantitative data. In essence, however, when artificial inoculation or natural infection is severe and uniform, symptoms represent an excellent tool for the breeder and pathologist.

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4.2

Advantages and Limitations of Some Methods for Barley Yellow Dwarf Virus Detection and Diagnosis

R.E. KLEIN and R.M. LISTER

SUMMARY

Barley yellow dwarf virus (BYDV) comprises a group of interrelated luteoviruses which typically have specific host and aphid vector relationships, with the result that a BYDV strain tolerated by one cultivar may cause severe symptoms in another. It is important, therefore, that epidemiological and resistance studies are concerned not only with virus detection and assay, but also with identifying the viruses involved. Immunological and genomic probes (cDNA's) that have been related to biological properties currently offer more convenient virus identification methods than checking vector specificity. The immunological methods most commonly used include various types of ELISA involving polyclonal and/or monoclonal antibodies, while the use of cDNA's may offer greater sensitivity and a broader basis for selecting discriminating or general probes.

Two important components in the control and management of barley yellow dwarf virus (BYDV) are the development of resistant or tolerant cereal varieties (Cooper and Jones, 1983) and understanding BYDV epidemiology. The term 'BYDV' actually includes a cluster of different viruses, which in turn comprise various strains with a variety of vector relationships and other properties. Cereal host susceptibility depends on the interactions of cultivars with the specific BYDV strain involved. Cultivars tolerating certain BYDV strains with little apparent effect may be severely affected by infection with other strains. This has clear implications for breeding programs. In addition, vector specificity is an important factor in epidemiological studies relating BYDV occurrence to vector populations.

Although the identification of BYDV strains is rooted in their vector specificities (Rochow, 1970a), serotypic differentiation has, in many instances, proved to run parallel to these (Rochow, 1979), perhaps because both vector specificity and immunological properties are based on virus capsid structure (Rochow, 1970b). Serological tests can also provide quantitative information which allows for a distinction to be made between tolerance and resistance

(Skaria et al., 1985). As genomic probes become more widely available, these provide alternative diagnostic tools for diagnosis and assay by nucleic acid (NA) hybridization reactions (Maule et al., 1983; Koenig et al., 1988).

Among the serological and NA hybridization techniques applicable to BYDV detection are enzyme-linked immunosorbent assay (ELISA), dot immunoassay (DIA) and nucleic acid spot hybridization (NASH). Protocols for these procedures are summarized in various references, including Maule et al. (1983), Clark et al. (1986) and Koenig et al. (1988). Each technique has advantages and limitations and a researcher's choice of technique should reflect such factors as the goals of the planned research and available facilities and resources. This paper describes some of the advantages and limitations of these techniques, based on work conducted at Purdue University, USA.

ENZYME-LINKED IMMUNOSORBENT ASSAY

At Purdue University, polyclonal antisera have been developed in rabbits to isolates of the five North American BYDV strains described by Rochow (1979): MAV, PAV, SGV, RPV and RMV (Hammond et al., 1989). Representative antisera were tested in a variety of ELISA formats, including double-antibody sandwich (DAS), protein A sandwich (DAS), F(ab')-fragment, and biotin-labelled ELISAs. Antiserum specificity varied with the ELISA format (*see* Table 1). For example, anti-PAV antiserum could not reliably distinguish between PAV and MAV when used as the second antibody in indirect ELISA, but when the same antiserum was conjugated to alkaline phosphate and used in DAS-ELISA, the two strains were easily distinguished (note that the term 'DAS-ELISA' is used here to denote double-antibody sandwich tests involving homologous *polyclonal* antisera). Antiserum specificity in DAS-ELISA seems to result in part from the glutaraldehyde treatment of the antigen-specific antibodies during conjugate preparation for direct ELISA.

Although the other ELISA formats can be useful under certain conditions, DAS-ELISA is one the most rapid and offers a useful degree of specificity. The above antisera have also been

Table 1 **ELISA values in indirect ELISA and DAS-ELISA tests of leaf tissue infected with PAV or MAV serotypes**

	Antigen[a]			
Second antibody[b]	**PAV**	**MAV**	**Non-infected control**	**ELISA value ratios PAV/MAV**
Antiserum	.797	.494	.075	1.6
Ig G	.770	.361	.043	2.1
F(ab')2	1.184	.292	.046	4.1
Ig Biotin	.850	.222	.068	3.8
None (DAS-ELISA)	1.400	.165	.100	8.5

Note: a Leaf extract at 1:10, w/v in 0.1 M phosphate, pH 7.
b Coating antibody was a monoclonal antibody (AF 8) reacting with both MAV and PAV serotypes.

tested in this way against BYDV isolates from around the world. In most cases, samples submitted for testing are symptomatic samples and have not been aphid-transmitted in the laboratory. Thus, it is difficult to be certain whether the failure of some to react in DAS-ELISA with at least one antiserum is attributable to the absence of BYDV or is indicative of a new BYDV strain, differing serotypically from those used for antiserum production. However, some of the samples from most areas do react with the antisera and, to date, previously undescribed BYDV strains do not appear to be a major problem in DAS-ELISA testing for BYDV using our polyclonal antisera.

DAS-ELISA offers many advantages to the user. In addition to possessing the required sensitivity, it discriminates between the major BYDV serotypes (MAV, PAV, SGV, RPV and RMV). The procedure is rapid, with results being obtained within 24 hours. In general, ELISA tests tend to tolerate temperature fluctuations and varying incubation times. Thus, DAS-ELISA can be easily adapted to the research location and requires a minimum amount of equipment. Qualitative results are easily assessed by inspection, and quantitative results can be obtained with the appropriate ELISA reader equipment. However, DAS-ELISA also has some disadvantages. It relies on polyclonal antisera that frequently are developed in an unrelated laboratory and may be in limited supply; the development of antisera, particularly BYDV antisera, is a laborious procedure. DAS-ELISA also requires the conjugation of specific serotype Ig with an appropriate enzyme (usually alkaline phosphatase). Antisera vary according to such factors as the animal in which the antiserum is produced and the time of production; the efficiency of the conjugation reaction can also vary. Thus, tests must be optimized for each new antiserum and conjugation.

Monoclonal antibody (Mab)-based ELISA using indirect procedures (for example, polyclonal antibody capture, followed by detection with a Mab and a conjugate detecting the Mab) offers a possible replacement for DAS-ELISA (speed, sensitivity and reliability under a variety of conditions) without the disadvantages of a variable polyclonal antiserum for detection and the need to conjugate the viral antibody with an enzyme. Standardized Mab-detecting conjugates are widely available commercially. In theory, once a cell line producing a Mab is established, an inexhaustible supply of identical antibody is available, in contrast to the situation with polyclonal antibodies, which are always finite and essentially variable in quality. However, whereas polyclonal antisera contain a range of antibodies directed to the various epitopes characterizing a serotype, the reactivity of each Mab is directed only to a specific epitope, which may or may not be widely representative. Isolates classed within a certain serotype in tests with polyclonal antisera may therefore react differentially with selected Mabs (Lister and Sward, 1988). This extreme specificity needs to be taken into account in developing panels of Mabs for use in surveys.

We have recently tested a wide range of Mabs for their ability to detect and discriminate between BYDV isolates, particularly PAV-like isolates (that is, from samples which reacted with PAV polyclonal antiserum in DAS-ELISA). No Mab reacted with all the samples (*see* Table 2 *overleaf*). The diagnostically more reliable Mabs are those which also react with MAV and/or SGV serotypes and their ability to discriminate between BYDV strains is limited. PAV-specific Mabs failed to react with all samples, but samples which failed to react with the Mab MAC91 (Torrance et al., 1986), which has been widely used to detect PAV serotypes, seem to be geographically confined to the western USA. It seems clear that Mab-based ELISA tests may

Table 2 **Ability of various monoclonal antibodies (known to react with PAV or MAV + PAV serotypes) to detect serotypes in leaf samples, previously identified as PAV-infected by DAS-ELISA, with a PAV polyclonal antiserum**

Monoclonal antibody[a]	Diagnostic reliability[b]			
	Positive	Negative	Total	% correct
1C2 (PAV)	103	26	129	80
MAC91 (PAV)	113	16	129	88
AF8 (PAV + MAV)	119	10	129	93
MAV3 (PAV + MAV)	58	5	63	92

Note: a 1C2 and AF8 were developed by Diaco et al. (1986), MAC91 by Torrance et al. (1986) and MAV3 by Hsu et al. (1984).
b Tests conducted using TAS-ELISA, with the PAV polyclonal Ig as the coating antibody.

be less reliable than DAS-ELISA, and that the degree of unreliability varies unpredictably with the serotypes of BYDV present. This lack of reliability cannot be assumed to be negligible until demonstrated to be so.

Although Mab-based ELISA tests eliminate the need for virus-specific Ig conjugates, most Mab tests are performed in a triple-antibody sandwich ELISA format (TAS-ELISA). These tests require a coating (antigen-capturing) antibody (a polyclonal Ig preparation is generally used), and thus the need for polyclonal antisera is not entirely eliminated. We have tried direct antigen coating, using plant extracts, but this has not proved successful with ELISA plate formats, presumably because they are not sensitive enough and the viral antigen is not sufficiently concentrated in extracts (obviously, the procedure does work when concentrated, purified antigen preparations are used).

DOT IMMUNOASSAY

DIA is performed on a paper-like support (usually nitrocellulose or nylon) with a substrate providing a precipitable enzyme product. In general, DIA closely resembles ELISA on microtiter plates, and many ELISA procedures are equally applicable to DIA. With some viruses, DIA conducted following protocols paralleling DAS-ELISA has proved to be more sensitive than DAS-ELISA on microtiter plates (for example, Banttari and Goodwin, 1985), but in our work with BYDV and that reported by Pereira (1986) this has not been the case.

However, because the supports used have a very high binding capacity for proteins, the DIA format offers advantages in tests involving antigen binding on the solid phase, as in indirect ELISA procedures, and we have been interested in it for BYDV testing primarily for this reason. Antigen can be directly spotted onto the support or filtered through it under vacuum with the aid of a manifold allowing placement of samples in various arrangements, including that corresponding to the 96-well array of typical microtiter ELISA plates. In such tests conducted at Purdue University, DIA with antigen directly applied to the support was as sensitive as DAS-ELISA but far less discriminating (*see* Table 3). Polyclonal antisera developed against MAV, raised in rabbits by intradermal injection, detected other BYDV strains equally well in this DIA,

Table 3 Activity of a polyclonal anti-MAV antiserum in DIA and ELISA tests of purified BYDV isolates

Sample[a]	ELISA values	
	DIA[b]	DAS-ELISA[c]
MAV (Intact)	1.695	1.140
MAV (Dissociated)	1.315	.055
RPV (Intact)	1.604	.092
RPV (Dissociated)	1.857	.089
RMV (Intact)	1.145	.034
RMV (Dissociated)	1.234	.003

Note: a Samples of 500 μg/ml purified virus; dissociation was in carbonate buffer, pH 9.6, overnight at 4°C according to procedure described by Diaco et al. (1986).
b For DIA values, reacted dots were removed and incubated in p-nitrophenyl phosphate substrate; ELISA values were determined as for DAS-ELISA.
c Differences noted were similar when either direct or indirect ELISA tests were used.

regardless of their serological distinctiveness as determined by other tests. Other antisera also show much wider activities in this DIA than in other immunoassays. We feel this may be the result of a combination of increased protein binding capacity with virus denaturation or deformation during binding to the support, which presumably allows highly conserved internal epitopes to become exposed during the immunoassay. Clearly, however, DIA offers potential for use in procedures involving antigen coating, thus dispensing with the need for an antiserum-binding antibody layer. In theory, therefore, the use of appropriate Mabs as the detecting antibody in DIA could overcome any need for using polyclonal antisera. In practice, however, this is proving difficult to confirm; our tests with BYDV for the Mabs tried so far have not worked well in DIA.

In essence, our experience indicates that DIA conducted with polyclonal antisera, and direct application of the virus to the nitrocellulose membrane, offers a potential broad-spectrum test capable of detecting a wide range of BYDV isolates. The test can be performed more rapidly than ELISA and can generally be completed within 1 day. However, the test is still experimental, and its application to routine diagnosis would be premature. One drawback is that DIA requires treatment of the sample extract to remove plant pigments which interfere with visualization of the results on the supporting membrane. Even then, it is often difficult to distinguish visually between the infected samples and the healthy controls. Additional time-consuming procedures or expensive equipment are necessary to quantify the results. For example, to do this in our research we have removed the 'dots' with a cork borer, incubated them in substrate and then read the results in an ELISA reader in the usual way. Other approaches could include the use of densitometry or reflectometry. Finally, from the work conducted to date, DIA involving antigen applied directly to the support appears to lack virtually any ability to discriminate between BYDV isolates. To the extent that strain identification is an integral part of BYDV surveys and control programs, such DIA is more a laboratory research tool than a routine diagnostic procedure for BYDV identification.

NUCLEIC ACID SPOT HYBRIDIZATION

The recent advances in the molecular biology of plant viruses have generated new detection techniques based on hybridization reactions between viral genomic segments and cloned copy DNA's (cDNA's) homologous to them. A simple format for conducting such hybridizations is NASH, which involves conducting hybridization reactions on a membrane (such as nitrocellulose or nylon) as in the DIA procedure (for example, Maule et al., 1983). The major theoretical advantage of NASH over serological tests is that NASH can be based on any selected parts of the entire virus genome rather than only on the coat protein. When it is based on genome portions other than that encoding the coat protein, sensitivity to differences among isolates will not depend solely on serotype relationships. Usually the cDNA probes used are labelled with ^{32}P but alternatives to isotopic labelling are now being sought, with some success in enzymatic or biotin labelling methods (Habili et al., 1987; Roy et al., 1988; Eweida et al., 1989).

At Purdue University we have genomic libraries for isolates of the MAV, PAV and RPV serotypes of BYDV (Barbara et al., 1987; Lister et al., 1990). Radioisotope-labelled cDNA probes representing the genome of each of these isolates have been tested against both purified virus and infected-plant extracts for five isolates representing the PAV, MAV, RPV, SGV and RMV serotypes (Fattouh, 1988; Fattouh et al., 1990; Lister et al., 1990). Probes prepared from clones derived from the MAV and PAV serotypes tend to recognize each other but not SGV, RPV or RMV serotypes. Probes prepared from clones derived from the RPV isolate hybridized only with RPV. Because of the cross-hybridization between PAV and MAV, discrimination between them required carefully chosen cDNA's representing dissimilar genomic regions. Interestingly, MAV probes did not hybridize with SGV in these experiments, although the two isolates are closely related serologically. Neither did RPV probes hybridize with RMV. It is not clear whether this was indicative of an underlying dissimilarity between these strains, despite their serological relationships, or whether it was attributable to physical factors (stringency) in the procedures used for the hybridization reaction; however, these hybridizations involved commonly used stringencies.

In the work conducted at Purdue, NASH has proved to be applicable with fresh or dry leaf samples, making it as versatile as ELISA in this regard (Lister et al., 1985; Fattouh, 1988). However, as currently applied, it is not a technique widely suitable for rapid and routine BYDV diagnosis, although it certainly has applications for suitably equipped diagnostic laboratories. Many of the chemicals used in the technique are hazardous and must be handled and disposed of with great care. This is true regardless of whether the probe is radioactively (r-probe) or non-radioactively (nr-probe) labelled. In our experiments, use of r-probes required less sample preparation time than was the case with nr-probes, but sample preparation is nevertheless more complicated than with DAS-ELISA. The useful shelf-life of r-probes is less than 1 month because of the decay of radioactivity, and thus these probes must be prepared regularly. In contrast, nr-probes may have shelf-lives of 1 year or more. Results can be achieved in 2 days with nr-probes), but up to 4 days may be required with r-probes because of the required exposure of X-ray sensitive film. R-probes can be easily quantified, whereas nr-probes are difficult and time-consuming to quantify, as in DIA.

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4.3

The Use of Artificial Inoculation with Viruliferous Aphids in Barley Yellow Dwarf Virus Research

A. COMEAU

SUMMARY

Appropriate aphid-rearing techniques can yield large populations of aphids, carrying barley yellow dwarf virus (BYDV), annually. The development of methods of bulk handling of aphids in Quebec, Canada has made possible the inoculation of large numbers of small plots with viruliferous aphids to evaluate cereals for BYDV resistance. With minor modifications, many of these techniques could be used in other parts of the world to help in plant breeding for resistance to the virus or the aphids. This paper describes the problems likely to be encountered during aphid rearing and possible ways of overcoming them.

A key element of barley yellow dwarf virus (BYDV) research in Quebec, Canada is the large-scale rearing of viruliferous aphids. This process allows one to deposit a relatively uniform number of aphids on the plants to be evaluated for BYDV resistance or tolerance. It gives uniformity of virus strain, and mixtures of known strains can also be used for selection purposes. Natural infection is generally less suitable for plant breeding work. It is uneven, contains unknown strains or mixtures of these strains, and is often poorly synchronized with the most appropriate plant growth stage for selection.

A variation of the principle of artificial inoculation is used in California, USA, where trap rows retain natural aphid populations that can be transferred to the BYDV trial area by pulling out the plants in the rows and spreading these over plots to be evaluated for BYDV (Qualset, 1984). However, there are few sites in the world that could apply this method every year, and thus many scientists have now adopted aphid-rearing methods similar to the method used in Quebec.

The details of the technique have been described in other publications (Comeau 1976, 1984). This paper summarizes the principal causes of problems that complicate aphid rearing and suggests ways of overcoming these difficulties.

THEORETICAL BASIS OF THE METHOD

The justification for artificial inoculation is that the effects of BYDV on plant height, grain yield, biomass and grain quality are closely related to the timing of infection (Comeau, 1984), or, to be more precise, to the growth stage of the plant when it becomes infected (Zerené and Ramirez, 1988). Early BYDV inoculation, from Zadoks 15 to 25, reduces biomass, height and grain yield. Later inoculation, from Zadoks 30 to 40, causes more damage to grain quality although the actual yield losses are less significant. Some applications, however, such as the removal of BYDV-susceptible segregants from F_3 populations, do necessitate BYDV inoculation at Zadoks 30-40 if the breeder plans to select on the basis of seed quality characteristics such as seed size, specific gravity or aerodynamic properties. For general selection work, the use of early inoculation has many advantages, despite the fact that lines with intermediate tolerance levels may be strongly disadvantaged. Early inoculation is best when the objective is to identify major genes, such as the barley Yd_2 gene.

The rearing of viruliferous aphids can be done as a one-step process by infesting plants with viruliferous aphids. But this method seldom works. If it is applied to young plants, the plants die too quickly; if it is applied to older plants, the aphid population seldom builds up adequately before the plants fill up their seeds. The rearing process developed in Quebec involves three steps. In the first step, plants are grown free from viruses and aphids, until a suitable plant biomass is produced, at about Zadoks 30. In the second step, these plants are infested with virus-free aphids which can reproduce for 10 days or more without reducing plant growth. In the final step, a few viruliferous aphids are introduced in the rearing premises, and the disease is then spread gradually through the plant population over the following week. Between 1980 and 1989, this process allowed the regular production of 10-20 million viruliferous aphids for experimental purposes and for the needs of the breeders. Large surfaces such as greenhouses or screenhouses are needed for such a project.

MATERIALS AND EQUIPMENT

Aphid rearing, harvesting and handling and the use of the aphid spreader have been described previously (Comeau, 1984). The aphid spreader, depicted in Figure 1, is an effective tool for rapid, efficient work but it is important to remember that aphids are small, soft-bodied insects that suffer rapidly from desiccation, excess heat and radiation. Other equipment needed includes: talcum powder, about 500 g/year; aphid-collecting trays and small shallow boxes to store aphids; one or two ice chests to protect the aphids (already packaged in boxes) from sunshine and heat; wet towels to keep moisture inside the ice chest at appropriate levels; and equipment for the proper care of the plants.

The main BYDV vector used in our work is *Rhopalosiphum padi* (L.). It reproduces rapidly on many species of cereals and is important worldwide. If one chooses to rear another aphid species, special care must be given to the temperature requirements (*see* Table 1). It would be impossible, for example, to rear *Metopolophium dirhodum* (Walker) unless the cool temperature needed is maintained at all times.

Figure 1 Aphid spreader used in BYDV program, Quebec, Canada

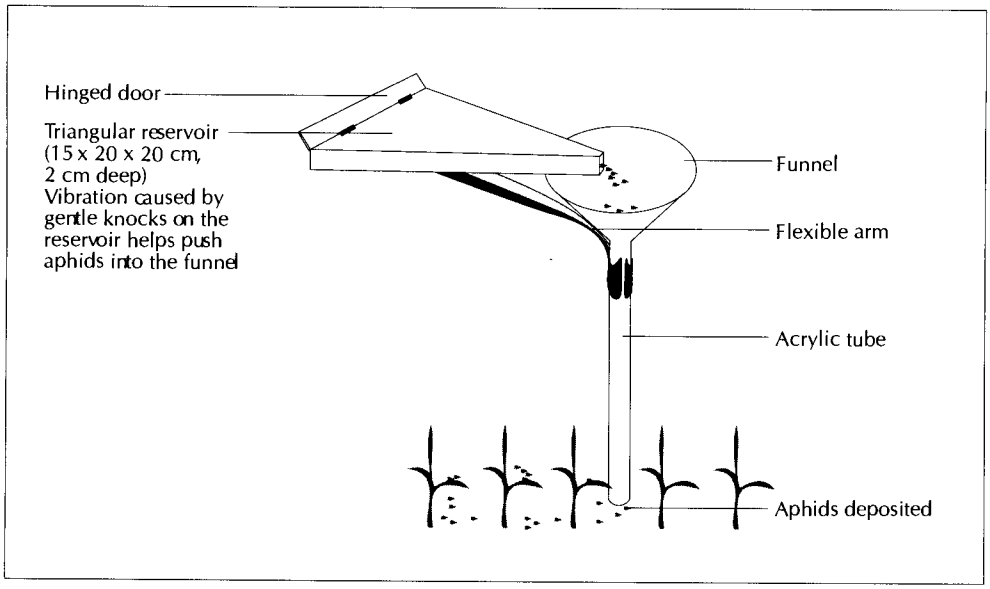

Table 1 Temperature requirements in rearing aphids to be used for transmitting BYDV[a]

Aphid species	Temperature (°C)		
	Optimal	Harmful	Deadly
Metopolophium dirhodum	12-15	25	29
Rhopalosiphum padi	15-20	27	32
R. maidis	18-23	30	36
Sitobion avenae	15-20	27	32

Note: a Heat resistance may differ according to the geographic origin of the strain; some strains of *R. padi* observed in Syria have better heat resistance. *Schizaphis graminum*, which is rare in Quebec, is known to be fairly heat resistant.

Winter oats are a good host for all species except for *R. maidis* (Fitch.). Virus strains must be selected with caution; preliminary trials are recommended before large-scale use. Virus strain mixtures (such as PAV + RPV) may be useful for certain purposes, especially if the sole objective is breeding. Virus-free aphid colonies must be started with very young aphids, within 50 minutes after birth. These young nymphs are very delicate and need to be handled with care.

PROBLEMS AND POSSIBLE SOLUTIONS

There is a significant cost involved in the need to maintain virus-free and virus-infected clones throughout the year. If the isolates used for research are lost, it may be very difficult to replace

them with identical ones. Currently, we renew the source cultures every 3 weeks. It is a good idea to keep colonies on live plants in the refrigerator (1-2°C) (von Wechmar, 1990), although if the humidity and oxygen levels are not right, the aphids and plants will die out. A number of other problems are listed in Table 2, accompanied by the recommended cure.

From our experience, the most challenging problem is the control of parasites. Predators can be controlled with a contact insecticide such as Sevin (Carbaryl) if care is taken to produce very large droplets. The uneven deposit will kill only the mobile species such as the ladybird beetles (coccinellids), but it is difficult to kill parasites without also killing the aphids. It seems that the only solutions are to fumigate the soil with formaldehyde, which implies a long waiting time before plant re-entry, or to use the insecticide Phosdrin (Mevinphos) before planting. This should destroy the pupae of parasites before the beginning of the rearing process. However, if the screen or plastic walls are not completely insect-proof, this effort will be wasted. Enough parasites may enter through a small hole to kill millions of aphids in a few weeks. We equipped our rearing greenhouse with a double-door system and good-quality screens, but the parasite problem always occurred at some stage, generally after the trials had been completed but occasionally while they were still in progress.

Fungi and viruses harmful to aphids may also cause problems. A general fungicide such as pentachloronitrobenzene could be used before planting, and all volunteer green plants in the premises should be destroyed. During aphid rearing, it is more difficult to get rid of undesired fungi; frequent use of the fungicides Vigil and Corbel may be helpful. The use of benomyl must be avoided as it kills the aphids. Rearing aphids in the same facility year after year tends to result in a gradual increase in problems as biological control agents keep accumulating inside the unit; the best approach here may be to use someone else's greenhouse for a year, if this option exists.

The latest part of the annual production of aphids includes a lot of alate forms. Initially, we made no use of these forms but we now recover them and keep them refrigerated for 1-2 days before using them. If aged properly, they eventually lose their flight muscles and become sedentary enough to be just as good as wingless aphids.

Aphids that have been distributed in the field for experimental purposes are no threat to neighboring farmers' fields if the timing of distribution is correct. The number of aphids which are carried by the jet winds in late June reaches billions in our area, and therefore a few million aphids released will not represent measurable risk. However, this factor may deserve some consideration in other areas, especially if parasites and predators are rare. In Quebec, predators eat the aphids voraciously soon after field release. We generally do not have to spray to kill them.

Soon after the virus is introduced in the greenhouse, the root system of the host plants becomes very inefficient. This detail is of paramount importance, and to address it the use a complete foliar fertilizer formula is essential. If a commercial formula is not available, the following home-made formula can be used: in 5 l water, dissolve (in mg): 500 KNO_3, 200 NH_4NO_3, 400 $MgSO_4.7H_2O$, 900 $CaNO_3.4H_2O$, 120 KH_2PO_4, 75 KCl, 0.2 Na EDTA, 0.2 $FeCl_3$, 0.02 H_3BO_3, 0.05 $MnCl_2.4H_2O$, 0.02 $ZnCl_2$, 0.01 $Na_2MoO_4.2H_2O$, 0.01 $CuCl_2.2H_2O$ and 0.001 $CoCl_2$. The final pH is adjusted to 4.7 with HCl and KOH. This foliar fertilizer should be used twice a week. If the leaves are pale green, the foliar fertilizer should be alternated with another foliar spray made of ammonium carbonate or ammonium nitrate (1000 mg/L) to increase the nitrogen level of plants and improve chlorophyll.

Table 2 Common problems (listed chronologically) encountered in aphid rearing, and possible solutions

Problem	Possible solution
Plants die prematurely when aphids are abundant (osmotic shock caused by honeydew)	Wash leaves every 2 or 3 days with a gentle water spray; wash twice at a 10-minute interval; treatment should begin before problem occurs.
Hymenopterous parasites and small diptera (cecidomyids) attack aphids in the main rearing facility	Add a double-door system designed to reduce unwanted entry of insects; seal any holes with masking tape in the first weeks; install an automated watering and temperature control system to reduce the number of trips to the rearing facility; inspect all screens and walls, and repair holes; verify screen mesh width.
Predators (coccinellids, syrphids) eat aphids in the main rearing facility	Prevention measures as above; contact insecticide (Sevin, Carbaryl, Gardona) at a low dose is useful against some species and will not kill too many aphids if a spray of big droplets is used, avoiding full coverage of plants; wash off residue 3 days later.
Aphids very small, with excess number of alates (occurs at the end of the rearing period and is related to overcrowding, heat and drought)	Scheduling of the aphid-rearing process should be adjusted to avoid hot period of the year; passive or active ventilation with automatic controls is useful to reduce the heat problems; a fine spray of water cools the plants and should be applied very frequently on hot days; alates could be harvested wherever they congregate, kept refrigerated at 5°C for 24-48 hours and then used as inoculum (they lose flight ability).
Plant biomass too low in the main rearing facility	Plant the host plants sooner and delay virus entry; increase soil temperature for germination by putting a polythene sheet over the plants from Zadoks 0 to 15; analyze soil to correct any deficiencies; ensure that soil moisture is ideal throughout the day; use foliar fertilizer twice a week, after virus entry.
Too many aphids, too soon	In subsequent aphid rearing, adjust the schedule; to avoid waste, aphids may be harvested and kept for a few days in flat trays at 0°C; some of the aphids could be killed with a small amount of natural pyrethrins (although the product is not residual, this should be done with extreme care).
Aphids die when inside the aphid spreader	To reduce excess radiation or high temperature inside the spreader, wrap the aphid reservoir with aluminium foil or a thick wet towel.
Aphids die quickly when touching the soil surface in the field during the spreading operation	As soil surfaces get too hot in the midday sun, work early in on the morning or at sunset, or sprinkle a small amount of water on the surface just before depositing the aphids; work on cool days if possible; soil temperature should be assessed frequently by hand.

Table 2 (continued)

Problem	Possible solution
Aphids get stuck together in the collecting tray or inside the boxes used for transportation	Use enough talcum powder to coat all surfaces before the aphids are collected; use more powder in the boxes during transportation; put ice packs outside the insulated ice chest used to carry aphids, rather than inside (if used inside, they cause condensation and the aphids drown).
Aphids die during transportation inside the boxes kept in the insulated ice chest	Cover the boxes with a wet blanket; never leave the ice chest open in the sunshine; put a wet towel inside the ice chest but outside the boxes.
Contamination of virus-source colonies with unwanted virus strains	To ensure safety of the plants, it essential to use a double-layered screen with more than 3 mm between screens, so that the aphids running loose cannot probe through the screen and contaminate plants inside a cage.
Poor longevity of source colonies	Verify temperature and moisture inside cages; in Brazil, spraying colonies with Vigil or Corbel (10 times recommended rate for cereals) prolonged the longevity of aphid colonies (Reis and Gassen, pers. comm.); sometimes an aphid colony may become infected with an insect pathogen, in which case this colony should be discarded and replaced with fresh aphid stock.

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4.4

Purification of Barley Yellow Dwarf Virus from Dried Leaf Tissue, and the Production of Antisera

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SUMMARY

At Purdue University, USA, virus was purified and antisera were produced from dried field-collected oats tissue, sent from Toluca, Mexico and infected with an undefined mixture of barley yellow dwarf virus (BYDV) isolates. In double-antibody sandwich ELISA tests, the antisera reacted strongly with MAV and RPV serotypes, moderately with PAV and RMV serotypes and only slightly with SGV serotypes. The antisera were also useful as a source of general-purpose trapping antibodies (for example, in triple-antibody sandwich ELISA tests with specific MAV, PAV and RPV monoclonal antibodies). This work demonstrates that where facilities for virus purification and antiserum production are not available, these procedures can be applied to dried infected leaf tissue at another location, thus avoiding the risks of transporting live materials between countries. Antisera thus prepared could be particularly useful as diagnostic reagents where locally important BYDV types differ from currently characterized isolates for which antisera are already available.

Barley yellow dwarf virus (BYDV) isolates have been well characterized in North America (Gill, 1969; Rochow, 1970, 1979; Rochow et al., 1986), in the UK (Plumb, 1974; Torrance et al., 1986; Pead and Torrance, 1988), in Australia (Johnstone et al., 1990) and elsewhere. These isolates correspond to groups represented by five isolates distinguished initially on the basis of vector specificity (Rochow, 1970, 1979) and later by serological methods (Rochow and Carmichael, 1979): Group 1 — MAV, PAV and SGV, which are serologically related; and Group 2 — RPV and RMV. However, recent reports, such as those from China (Zhou and Zhang, 1990), indicate that isolates from other parts of the world may differ serologically from these five serotypes. Many countries lack the facilities for virus purification and antiserum production, and this precludes the characterization of local isolates. There is a real possibility, therefore, that isolates differing from those currently recognized may occur in many locations in which cereal hosts susceptible to BYDV are grown. This would be particularly important in survey programs using detection reagents differentiating luteoviruses from other viruses.

The enzyme-linked immunosorbent assay (ELISA) activity of BYDV has been shown to persist in air-dried leaf samples shipped by mail worldwide (Lister et al., 1985). Transportation of dry leaf samples between countries poses no quarantine risk as BYDV is not sap transmissible. In order to see if this could be used as a basis for purifying BYDV isolates from another country, producing antisera against them and thereby characterizing them serotypically, we decided to attempt the purification of BYDV from dry leaf tissue collected in Mexico and mailed to our laboratory at Purdue University, USA.

MATERIALS AND METHODS

Oats tissue infected with a undefined mixture of BYDV isolates (Mex-1 BYDV) was collected in the field at Toluca, Mexico, air-dried and mailed to Purdue University for purification and antiserum production. Purification followed the procedure described by Hammond et al. (1983), except that the tissue was first pulverized in liquid nitrogen before extraction in 0.5M phosphate, pH 6.0, with repeated blending. This was followed by chloroform clarification, precipitation with polyethylene glycol (PEG 6000), concentration by ultracentrifugation and separation on rate zonal sucrose density gradients.

A New Zealand white rabbit was injected intramuscularly with 39 µg of virus (E 0.1% 1 cm 260 nm = 8.0), emulsified with Freund's complete adjuvant. This was followed by booster injections of 45, 59 and 68 µg of virus emulsified with Freund's incomplete adjuvant at 16, 28 and 68 days, respectively, after the initial immunization. The antisera produced were designated 'Mex-1'.

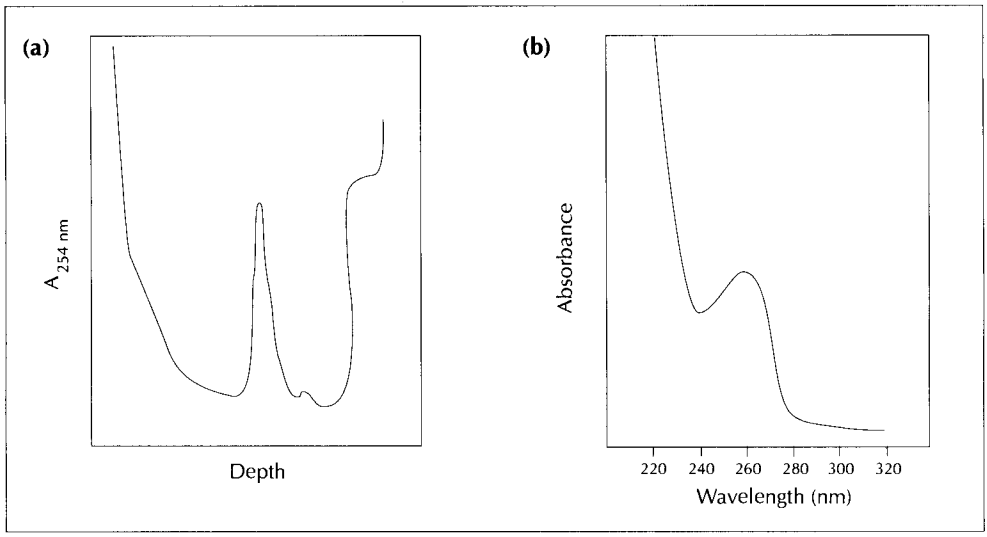
Serum from a bleed taken 50 days after the initial immunization was fractionated by ammonium sulfate precipitation and the Ig thus prepared (Mex-1:50) was conjugated with alkaline phosphatase (Clark et al., 1986) for use in double-antibody sandwich ELISA (DAS-ELISA). The ELISA procedures followed, using Dynatch Immulon 2 plates, were as described by Fargette et al. (1982). All values presented in the tables are averages for duplicate wells. Fractionated Ig from this bleed was also tested as a source of trapping antibodies in triple-antibody sandwich ELISA (TAS-ELISA) with MAV-, PAV- and RPV-specific monoclonal antibodies (Mabs) — MAFF2, MAV91 and MAV92, respectively — developed at the Ministry of Agriculture and Fisheries Harpenden Laboratory, UK (Torrance et al., 1986; Pead and Torrance, 1988). In TAS-ELISA, antigen is trapped by polyclonal antibodies coated on the ELISA plate, and detected by an appropriate Mab, followed by a commercially available anti-Mab conjugate. Other BYDV isolates used were as described by Vincent et al. (*see* Paper 4.5, this proceedings).

RESULTS AND DISCUSSION

A yield of 250 µg of purified virus was obtained from 100 g of dry leaf tissue. UV-absorbing peaks seen in UV analysis profiles of rate zonal sucrose density gradients, used to separate the virus from other constituents in partially purified preparations, showed a similar degree of purification to that obtained with fresh or frozen leaf tissue (Hammond et al., 1983). The

preparation had a $A_{260\,nm}/A_{280\,nm}$ ratio of 1.55 and a UV absorbance spectrum typical of BYDV, with a maximum at 260 nm and a minimum at 240 nm (*see* Figure 1).

Figure 1 **UV absorbance profile ($A_{254\,nm}$) of sucrose rate zonal density gradient separation (37 000 rpm, for 1.5 hours, Spinco SW 41 rotor) of a preparation of BYDVs (Mex-1), purified from dry leaf tissue (a); UV absorbance over the $A_{220\,nm}$-$A_{320\,nm}$ range of Mex-1 (b)**



In DAS-ELISA, Ig from the serum which was collected 50 days after initial immunization (Mex-1:50) reacted strongly with RPV, moderately with MAV and weakly with RMV and PAV; SGV was barely detectable (*see* Table 1 *overleaf*). This Ig and the Ig's from seven other bleeds, representing collections made 50-105 days after the initial immunization, also trapped MAV, PAV, RPV and RMV isolates when used as a coating antibody in DAS-ELISA tests on sap extracts from plants infected with each isolate, employing their homologous antibodies as conjugates (*see* Table 2 *overleaf*). These tests indicated no appreciable changes in the relative ability of the Ig's to trap antigens of these isolates over the period during which they were collected.

Fractionated Mex-1:50 was also shown to be a useful source of general-purpose trapping antibodies with MAV, PAV and RPV monoclonal antibodies in TAS-ELISA (*see* Table 3 *overleaf*). It appeared to be as efficient for trapping the virus as the homologous polyclonal sera of each serotype.

These results show that BYDV can be successfully purified from dry leaf tissue and used to produce antisera. The yield and purity of the virus preparation obtained in this study was comparable with those produced routinely from fresh or frozen tissue in our laboratory, as indicated in Figure 1. However, we have had less success in attempts to purify single BYDV isolates from Mexico, bulked up in greenhouse-grown oats and dried prior to sending to Purdue. Preparations from these materials contained relatively more host constituents than preparations

Table 1 **ELISA values for sap extracts containing MAV, PAV, SGV, RPV and RMV isolates, obtained in DAS-ELISA tests at Purdue University, USA, with Ig's indicated used as coats and conjugates[a]**

Isolate	Ig used as coat and conjugate[b]					
	Mex-1	MAV	PAV	SGV	RPV	RMV
MAV	0.25	0.41	0.03	0.00	0.00	0.00
PAV	0.16	0.04	0.32	0.00	0.00	0.00
SGV	0.05	0.12	0.09	0.66	0.00	0.00
RPV	0.77	0.00	0.00	0.00	0.92	0.00
RMV	0.25	0.00	0.00	0.00	0.00	0.44
Healthy control	0.02	0.00	0.01	0.01	0.00	0.00

Note: a Concentration of coating Ig — 1µg/ml; dilution of conjugates — 1: 1000. The same sap extracts (1:10, w/v leaf: 0.1 M phosphate, pH 7.0) were used for each test, but antigen concentrations were unknown.

b ELISA values represent A_{405} absorbances for 1 mg/ml of p-nitrophenyl phosphate after 30 minutes.

Table 2 **ELISA values in DAS-ELISA tests using Ig's from successive bleeds from a rabbit injected with Mex-1, as coating antibody to trap BYDV isolates, and conjugates homologous to the virus isolates**

Antigen/conjugate combination[a]	Mex-1 Ig's used							
	1(50)[b]	2(62)	3(79)	4(86)	5(92)	6(96)	7(88)	8(105)
MAV/MAV	1.04[c]	0.92	0.97	0.91	1.08	1.02	1.04	1.16
PAV/PAV	0.72	0.77	0.82	0.78	0.90	0.87	0.88	1.09
RPV/RPV	1.00	0.94	0.96	0.94	1.02	1.00	1.01	1.09
RMV/RMV	1.23	1.21	1.25	1.19	1.30	1.30	1.30	1.46
SGV/SGV	0.18	0.17	0.18	0.17	0.19	0.18	0.17	0.19

Note:: a Antigens were sap from leaves of oats (Clintland 64) infected for about 2 weeks, and extracted at 1:10, w/v with 0.1 M phosphate, pH 7.0. Conjugates were at 1:500 in extracts from non-infected plants made similarly. Value ranges obtained in control tests with extracts from healthy oats for each combination were: MAV, 0.00-0.00; PAV, 0.04-0.07; RPV, 0.01-0.02; RMV, 0.01-0.02; and SGV 0.00-0.02.

b Number of days after initial immunization given in parentheses.

c Data presented are for coating Ig's used at 1:1000. Dilution series included in each experiment indicated a linear response to dilution in this range.

from fresh or frozen tissue. This may have been because of higher temperatures in the greenhouse conditions used; it is well known that BYDV is more productive at cool temperates. Nevertheless, even these preparations would still be usable to produce antisera in rabbits. Techniques are available for absorbing the resulting antisera against extracts from healthy tissue to reduce undesirable 'background' reactions.

The work outlined above illustrates the possibility of purifying and characterizing BYDV isolates from parts of the world where facilities for virus purification and antiserum production

Table 3 **Reaction of sap extracts of MAV, PAV and RPV after 1 hour with MAFF2, MAC91 and MAC92 monoclonal antibodies using Mex-1 or homologous antibodies for trapping**

Trapping Ig	Sap extract	Monoclonal antibody[a] MAFF2	MAC91	MAV92
Mex-1	MAV	0.47	0.00	0.00
	PAV	0.09	0.10	0.00
	RPV	0.03	0.00	0.20
	healthy	0.02	0.00	0.00
MAV	MAV	0.55	0.00	0.00
PAV	PAV	0.06	0.12	0.00
RPV	RPV	0.02	0.00	0.13

Note: a Concentration: 1 µg/ml. Goat anti-rat alkaline phosphatase conjugate (Sigma Chemical Company) was used at 1:1000.

are not available. Researchers in these areas could collect naturally infected plants from the field, dry the tissue and send it to a laboratory which does have the necessary facilities. Although this might often result in an antiserum to a 'mixed' infection (as in the work described here), such antisera can be extremely useful for detecting and surveying the occurrence of local isolates (for example, Doupnik et al., 1982). Moreover, where more detailed discrimination is required, and appropriate facilities are available, attempts could be made at the 'home' location to separate isolates by transmission with selected vector species, and to propagate them under insect-proof conditions, preferably in cool temperatures, for shipping as dry leaf.

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4.5

Biotechnology: A New Weapon against Barley Yellow Dwarf Virus

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SUMMARY

This paper reviews recent work on the genomic structure of barley yellow dwarf virus (BYDV) and other luteoviruses. This work has enabled the identification of genomic segments likely to encode functional proteins and has generated specific cDNA probes for diagnosis. Segments of the genome-encoding viral coat proteins are of special interest, for they can be used as a basis for developing transgenic plants that may exhibit a cross protection-like resistance through the constitutive, inheritable expression of such proteins. At Purdue University, USA, this possibility is being examined with isolates of BYDV representing the MAV, PAV and RPV serotypes. Classical cross-protection interactions between these and other isolates indicate excellent prospects for genetically engineered cross-protection.

The term 'biotechnology' includes the study of genomic structure and expression. For plant viruses, such studies provide the information required for a basic understanding of genomic functions and viral relationships, which leads, in turn, to developing tools for diagnosis, management and control. Such information is rapidly emerging for barley yellow dwarf virus (BYDV), and the luteoviruses in general, through work being done in several laboratories. In this paper we summarize BYDV studies of this kind currently being conducted in our laboratory at Purdue University, USA.

CHARACTERISTICS OF BYDV

Luteoviruses cause yellowing on a wide range of host plants (Matthews, 1982). They are obligately transmitted by specific aphid vectors in a persistent, circulative manner and are limited to the phloem tissue of the plant. BYDV, the type member of the luteoviruses, consists of a group of related viruses that infect barley, oats, wheat, rice and other Gramineae hosts (Rochow, 1970a).

Isolates of BYDV have been named according to their specific aphid vectors and can be placed into two major groups based on serological relationships (Rochow, 1970a; Rochow and Duffus, 1981), cytopathological ultrastructure of infected cells (Gill and Chong, 1979), and dsRNA profiles obtained from infected tissue (Gildow et al., 1983). Group 1 includes: MAV, transmitted by *Sitobion (Macrosiphum) avenae* (Fabr.); PAV, transmitted by *S. avenae* and *Rhopalosiphum padi* (L.); and SGV, transmitted by *Schizaphis graminum* (Rond.). Group 2 includes: RMV, transmitted by *R. maidis* (Fitch.); and RPV, transmitted by *R. padi* (Rochow, 1970a; Rochow and Duffus, 1981). RPV is also related serologically to another luteovirus, beet western yellow virus (Rochow and Duffus, 1978). The differences in serological and aphid vector relationships among BYDV isolates are presumed to reflect differences in viral coat proteins (Rochow, 1970b).

In our experiments we have used isolates of BYDV representing the MAV, PAV, SGV, RPV and RMV serotypes, namely: Rochow's MAV (hereafter referred to as MAV-NY); MAV-PS1, an isolate obtained by subculture of MAV-NY; a P-PAV-like isolate from Indiana (Hammond et al., 1983); and subcultures of Rochow's SGV, RPV and RMV isolates (Rochow, 1970a). MAV-PS1 and MAV-NY were distinguished only by reactions to different monoclonal antibodies (Lister and Lei, 1987). Although the isolates in Group 1 are related serologically, P-PAV and MAV-PS1 are more closely related than SGV is to either of them. The Group 2 isolates RPV and RMV are only distantly related to Group 1 isolates, but are moderately related to each other (Webby and Lister, 1989).

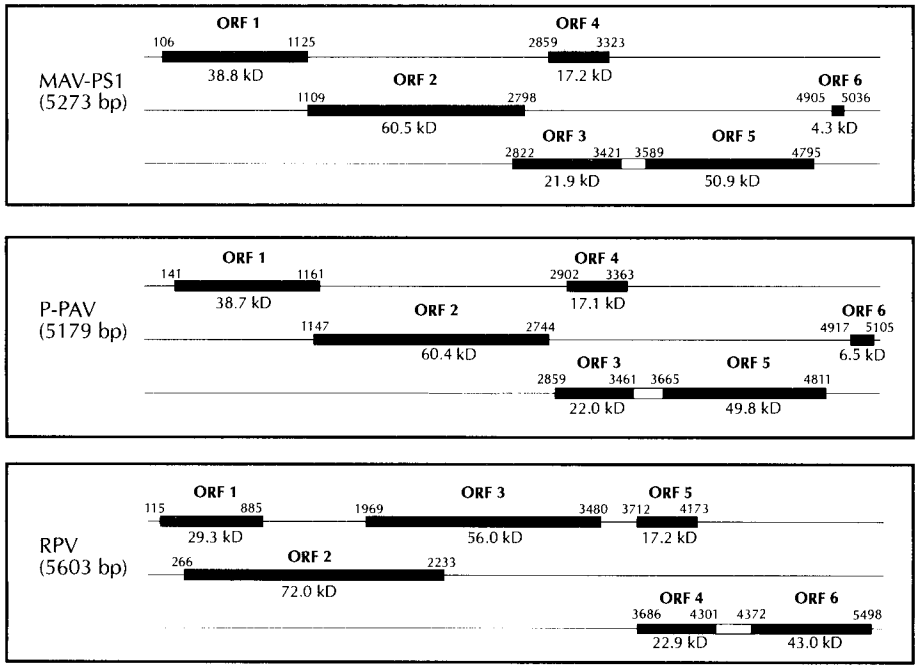
GENOMIC SEQUENCING AND ORGANIZATION

As a basis for nucleotide sequence determinations, cDNA libraries were prepared from the RNAs of the MAV-PS1, P-PAV and RPV isolates and cloned into plasmid and bacteriophage vectors (Barbara et al., 1987). From these libraries, overlapping clones representing the genome of each viral isolate were identified by restriction analysis and by hybridization. Polyclonal antisera prepared against each isolate identified regions within each genome which produced immunologically recognizable *lac*Z fusion proteins in the bacteriophage expression vector lambda gt 11. Clones expressing these fusion proteins presumably encode the viral coat protein (CP) gene. Restriction maps representing each genome were generated by single- and double-restriction enzyme digests and by Southern hybridization. These mapped cDNA's enabled detection and discrimination of BYDV isolates by nucleic acid hybridization reactions on nitrocellulose (*see* Paper 4.2, this proceedings). Plasmid DNA from clones representing the MAV-PS1, P-PAV and RPV genomes were sequenced by the dideoxy chain termination method (Sanger et al., 1977).

Based on the nucleotide sequence data from clones representing the antigenic regions, and on open reading frames (ORFs) capable of encoding the appropriate sized proteins, we have identified the CP gene for each of these BYDV isolates (*see* Figure 1). *In vitro* translation of synthetic mRNA derived from the RPV CP coding region yielded a polypeptide which co-migrated in polyacrylamide gel electrophoresis (PAGE) with the native viral CP. The CP sequence identified for P-PAV was found to be very similar to that identified by Miller et al. (1988) for a PAV-like isolate from Victoria, Australia (hereafter referred to as Vic-PAV).

Indeed, a comparison of the nucleotide and amino acid sequences of known luteovirus CP coding regions (Vincent et al., 1990) revealed a high degree of similarity (*see* Table 1 *overleaf*). While the PAV isolates contain almost identical CP sequences (95-97% identity), the RPV isolate shares greater identity with the other luteoviruses than with the MAV and PAV isolates; in particular, it shares 66% amino acid homology with potato leaf roll virus.

Figure 1 Identification of open reading frames (ORFs) in the genomes of BYDV



* Each line represents one reading frame within the viral genome; the boxes represent the OFRs identified within the reading frame. The numbers at the beginning and end of each shaded box represent the base position from the 5' end of the genome for the putative translation initiation and termination codons of each putative coding region, respectively. The open box at the end of the ≈22 kD ORFs represents the potential readthrough region following the coat protein termination codon and preceding the first ATG of the 43 or ≈50 kD ORF.

From the nucleotide sequences, we have also deduced the organization of each of these BYDV genomes, as shown in Figure 1. The MAV-PS1 and P-PAV isolates contain five major ORFs and one minor ORF, as in Vic-PAV (Miller et al., 1988). In contrast, the RPV isolate has six major ORFs, in a genome organization similar to that identified for beet western yellow virus (Veidt et al., 1988) and potato leaf roll virus (Mayo et al., 1989). Based on the identification of ORFs, it was possible to identify the putative coding regions for the RNA-dependent RNA replicase, the CP coding region and the VPg; the VPg is a small protein of unknown function covalently attached to 5'-end of the BYDV genome (Murphy et al., 1989).

Table 1 **Nucleotide and amino acid sequence homology between MAV-PS1, P-PAV, RPV and other luteovirus CP coding regions[a]**

	MAV-PS1	P-PAV	RPV	Vic-PAV	BWYV[b]	PLRV[c]	PLRV[d]
				Nucleotide homology (%)			
MAV-PS1	—	76.6	54.8	76.1	57.0	53.0	53.1
P-PAV	—	—	56.4	95.0	57.4	57.6	57.6
RPV	—	—	—	55.2	65.4	69.4	69.1
				Deduced amino acid homology (%)			
MAV-PS1	—	71.3	47.8	71.8	48.1	46.9	47.4
P-PAV	—	—	50.7	97.0	48.1	47.4	47.8
RPV	—	—	—	48.4	66.2	65.6	66.2

Note: a Reciprocal comparisons between isolates and self-comparisons are not presented and are indicated by — .
b Beet western yellow virus.
c Potato leaf roll virus; values based on Kawchuk et al., 1987.
d Values based on Prill et al., 1989.

The replicase coding regions were predicted on the basis of identity to the consensus sequence of other viral replicases (Kamer and Agros, 1984). It has been proposed that these replicases are synthesized via a -1 frameshift of the ribosome, resulting in a fusion of two ORFs to produce the replicase (Trifonov, 1987; Miller et al., 1988; Veidt et al., 1988).

Figure 1 also shows that the apparent coding region for the VPg is located completely within the CP coding region, although in a different reading frame. This organization is found in all luteoviruses that have been sequenced. Following the CP termination codon is an ORF for a 43 or ≈50 kD protein. For Vic-PAV, Miller et al. (1988) have postulated that a readthrough of the CP termination codon would produce a ≈70 kD protein composed of the CP and the ≈50 kD protein. Using serotype-specific polyclonal antisera, our Western analyses of purified MAV-PS1, P-PAV and RPV subjected to SDS-polyacrylamide gel electrophoresis routinely identified proteins of 22.5 kD, 17 kD and ≈50 kD for the MAV and PAV isolates, and 22.5, 17 and ≈65 kD for the RPV isolate. The 17 kD protein corresponds to the size of the VPg, while the 22.5 kD protein (the predominant polypeptide) is the coat protein. The immunological recognition of proteins which are larger that 22.5 kD in purified virus preparations indicates that such proteins are present in intact virions. The 50 kD proteins could correspond to the ORF-found 3' of the MAV and PAV CP regions. MAV-PS1 and P-PAV clones producing immunologically recognizable fusion proteins with the lambda gt 11 expression vector mapped to the 50 kD ORF region of their genomes. No ORF for a 65 kD polypeptide occurs in the RPV genome. The 50 kD proteins of MAV-PS1 and P-PAV may represent their 50 kD ORFs or may result from degradation of a larger (72.5 kD) protein comprising the 22.5 kD and 50 kD polypeptides.

The true roles and relationships of these proteins are unknown, but we plan to study their possible structural significance, as well as their significance, if any, in governing aphid transmission and/or serotype specificity.

CROSS PROTECTION

There is considerable interest in the possible use of viral coat protein genes to induce cross protection-like resistance in transgenic plants, and we have therefore begun to evaluate the potential to establish such cross protection-like effects as a means of resistance to BYDV. Classical cross protection is the effect whereby infection of the host by one virus prevents, or reduces, infection by a second, related virus. While this type of cross protection is already in practical use for inducing resistance in greenhouse tomatoes to tobacco mosaic virus and in citrus to citrus tristerza virus, such a procedure is obviously not applicable to controlling obligately aphid transmitted viruses, such as BYDV, in annual crops.

One possible explanation for the mechanism of cross protection is that the presence in infected cells of the CP of the primary protecting virus inhibits the uncoating of the challenge virus, and therefore its infectivity. It has been shown that transgenic plants expressing viral CP genes exhibit a cross protection-like type of viral resistance (Abel et al.,1986; Loesch-Fries et al., 1987; Tumer et al., 1987). This approach to inducing resistance by genetic engineering has proved successful in both laboratory and field experiments, and should overcome problems inherent in classical cross protection. We are therefore attempting to transform cereal plants with viral CP genes to investigate this possibility in BYDV.

Classical cross protection studies

We have conducted a series of experiments examining classical cross protection interactions between the isolates described above, representing the MAV, PAV, RPV, SGV and RMV serotypes of BYDV. In these experiments, seedlings of Clintland 64 oat plants were inoculated with one (protecting) isolate of BYDV and then, after various incubation times, with another (challenge) isolate. Plants singly inoculated with either isolate, and mock-inoculated plants, were used as positive and negative controls, respectively. The plants were sampled, divided into shoot and root samples, and tested for virus by enzyme-linked immunosorbent assay (ELISA) and, in some cases, by cDNA dot blot hybridization assay.

The results, given in Table 2 (*overleaf*), show that, overall, the efficiency of cross protection is consistent with serological relatedness between the isolates. Interpreting the significance of viral relationships in cross protection will require further genomic analysis of the isolates involved, but it appears that the more closely related the isolates, the more efficient is cross protection. Thus, the results suggest that coat protein genes from isolates in Group 1 might be capable of eliciting a cross protection-like effect in transgenic plants for a range of viruses in this group, but this would be less likely with respect to viruses in Group 2. Results from ELISA and cDNA hybridizations were consistent, indicating that cross protection affects both capsid and RNA synthesis.

Genetically engineered cross protection

As a first step towards evaluating genetically engineered cross protection, the RPV CP gene was subcloned into a vector capable of expressing it in plants. Transformation vectors were

Table 2 Summarized results of cross-protection studies conducted at Purdue University, USA, on BYDV isolates

Paired inocula	Serological relationship	Cross-protection	Efficiency of the cross-protection
MAV-PS1 and MAV-NY	Very strong	Yes	High
MAV-PS1 and P-PAV	Strong	Yes	Moderate
SGV and MAV-PS1	Moderate	Yes	Low
SGV and P-PAV	Low to moderate	Yes	Low
RPV and RMV	Low	No	— [a]
RPV and P-PAV	Little	No	—
RPV and MAV-PS1	Little	No	—
RMV and MAV-PS1	Little	No	—

Note: a Not detected.

constructed for use in either *Agrobacterium*-mediated Ti plasmid transformation of dicots, or microballistic ('gene gun') transformation of monocots whereby DNA coated on tungsten or gold microprojectiles is projected into cells. Both vectors contain the RPV CP coding region flanked 5' by the 35S CaMV promoter and 3' by the nopaline synthase polyadenylation signal. For the microballistic transformation experiments with cereals, the RPV coat protein transformation constructs were prepared in a high copy number plasmid (pGEM-3Z, Promega, Wisconsin, USA) capable of providing high yields of DNA. We are currently constructing similar transformation vectors for the MAV and PAV isolates.

While transformation and regeneration are more difficult with monocots than dicots, the required technologies are emerging rapidly, and we plan to test the procedures available for use with cereal seed, plants, plant parts, and protoplast. Meanwhile, as a model system to evaluate CP expression and genetically engineered cross protection, we have initiated transformation of potatoes with the RPV CP gene using the Ti-based transformation vector described above, pBIN19 (Bevan, 1984). In constructs of other viral CP genes, this vector has been found to provide sufficient levels of viral CP synthesis in transgenic dicot plants to produce a cross protection-like effect (Abel et al., 1986; Tumer et al., 1987). In the dicot species tested, such 'engineered' cross protection was obtained against closely and distantly related viruses. In general, it has been shown that genetically engineered cross protection can be achieved if there is greater than 60% sequence identity between the CP sequences (Beachy, pers. comm.). A comparison of the RPV CP sequence with the known CP sequences of luteoviruses revealed greater than 65% sequence identity with potato leaf roll virus (Vincent et al., 1990), indicating that cross protection against potato leaf roll virus may be effected by BYDV CP gene expression in potatoes. To this end, potato transformants have been identified after selection for antibiotic resistance and await further growth prior to evaluation of CP expression.

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4.6

Interspecific Hybridization: A New Reservoir of Resistance to Barley Yellow Dwarf Virus

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SUMMARY

The interspecific hybrids of wheat with perennial relatives display a large variability in the F_1 generation. Backcrossing to wheat is often difficult, but in some cases the transfer of resistance genes to wheat lines having 2n = 42 chromosomes has been achieved. These lines seem to be more resistant to barley yellow dwarf virus (BYDV) than the Ethiopian barleys that rely on the Yd_2 gene and show very strong repression of virus multiplication. Resistance to the aphid species *Rhopalosiphum padi* is also found in these hybrids. The paper presents a general overview of BYDV resistance in the Triticeae.

Although the disease caused by barley yellow dwarf virus (BYDV) is not the most visually detectable disease in the world, it is regarded by many scientists as the most damaging disease of cereals. It deserves major investment in research, including investment in costly biotechnological research.

Cultivated cereal species contain a range of BYDV tolerance genes but resistance genes are rare. It should be emphasised here that whereas 'tolerance' implies absence of damage, 'resistance' means limitation of pathogen reproduction. The Yd_2 gene in barley is generally referred to as a resistance gene, but comparisons of susceptible and resistant lines sometimes reveal only a modest difference in virus content. Rye resistance genes are similar to the barley Yd_2 gene in that the level of virus repression is quite variable from one trial to the next (Comeau and Croullebois, unpubl.). Studies in triticale showed that a small number of genes control resistance and tolerance (Collin et al., 1990); in the Agriculture Canada/Laval University program we are now attempting to prove that these genes are on the rye genome.

The barley Yd_2 gene has given long-lasting protection to barley crops in California, USA (Schaller, 1984). Attempts have been made to transfer this barley gene into bread wheat (McGuire and Qualset, 1990; Comeau and Fedak unpubl.). Interspecific hybrids often show reduced expression or full repression of genes (Kimber, 1983), and the genes which are

suppressed may be precisely those resistance genes that would justify the crossing effort. Because our tests using enzyme-linked immunosorbent assay (ELISA) showed that the Yd_2 gene did not always reduce the BYDV content of plants in a clearcut manner, it was feared that the expression of this gene in the wheat background could be difficult to obtain; therefore, one of our main goals was to conduct a broad survey of the Triticeae for resistance to BYDV. Perennial species are the main source of strong, unequivocal resistance, which may reach immune levels in some cases. Recent findings indicate that some *Aegilops* species may possess useful resistance (Makkouk, pers. comm.). Aphid resistance is also present in various Triticeae, as well as in *Avena macrostachya* (Weibull, 1986, 1987, 1988).

This paper summarizes the results of our attempts to transfer virus resistance from alien species to cultivated cereals.

BYDV RESISTANCE AND TOLERANCE AVAILABLE IN THE GENE POOLS

Primary gene pool

This gene pool is represented by the cultivated species and their closest relatives. As noted earlier, there are a few genes available in *Triticum aestivum* lines, but close inspection of the pedigree of these lines indicated that most BYDV tolerance genes found within *T. aestivum* could be of interspecific origin — namely, from rye or *Thinopyrum* (Comeau and Plourde, 1987). Trials conducted in 1981 on 100 *Aegilops* lines, mainly *Ae. tauschii* (*squarrosa*) and 25 *Triticum monococcum* and *T. urartu* lines showed that the A and D genome species were very susceptible to BYDV. Further work on species with the AB genome showed that all lines of all species were susceptible to BYDV (Cheour et al., 1989; Comeau, unpubl.). In 1989, a few accessions of *Aegilops* supplied by the International Center for Agricultural Research in the Dry Areas (ICARDA) were evaluated with ELISA and on the basis of symptoms. Lines A 400240 (*Ae. triuncialis*) and A 400140 (*Ae. neglecta*) were judged to be BYDV resistant. Rye and triticale remain logical sources of tolerance (Oswald and Houston, 1953), but the wheat lines containing rye chromosomes or arms generally show only part of the tolerance evident in the rye parent (Nkongolo, 1988). Attempts to create tolerant wheat from such crosses are still in progress.

In barley (*Hordeum vulgare*), the Yd_2 resistance gene from Ethiopian lines is adequate against PAV-like isolates, but it is less useful against RPV and other isolates (Skaria et al., 1985). A survey of 686 *H. spontaneum* lines from the United States Department of Agriculture (USDA) collection in 1983-84 revealed no tolerance or resistance equivalent to that conferred by Yd_2 (Comeau and St-Pierre, unpubl). In oats (*Avena sativa*), spring germplasm was obtained from Illinois, Michigan and New Zealand. Having the same ACD genome combination as cultivated oats, *A. sterilis* and *A. occidentalis* are an important but largely untapped reservoir of tolerance (Comeau, 1984; Landry et al., 1984). The winter oat Wintok would be a good source of BYDV tolerance (Comeau, unpubl.).

To summarize, tolerance was found in the primary gene pool of cultivated cereals. Resistance, however, was very rare.

Secondary gene pool

The discussion here relates mainly to the transfer of resistance into wheat (*Triticum* spp.), with a few comments on barley and oats.

The merits of the perennial *Agrotricum*, *Thinopyrum*, *Agropyron*, *Elytrigia*, *Leymus* and *Psathyrostachys* species include BYDV immunity and aphid resistance (Sharma et al., 1984; Comeau et al., 1985; Fedak et al., 1986; Comeau and Plourde, 1987; Shukle et al., 1987; Plourde et al., 1988, 1989a, 1989b; Xin et al., 1988; Tremblay et al. 1989; Larkin et al., 1990). However, there are many difficulties. Production of the F_1 generation is not easy to accomplish, and generally requires embryo culture. In order to cross wheat with *Leymus* and *Psathyrostachys*, we had to develop our own *in ovulo* embryo rescue method (Plourde et al., 1988). Interspecific F_1 plants simply became sterile laboratory curiosities, and the production of backcrosses and amphiploids remained a formidable task. We concluded that current methods were inadequate, and that new methods were needed to overcome this second bottleneck. A number of other biotechnological methods were then investigated, and wheat-like progenies were eventually obtained from all genera listed above.

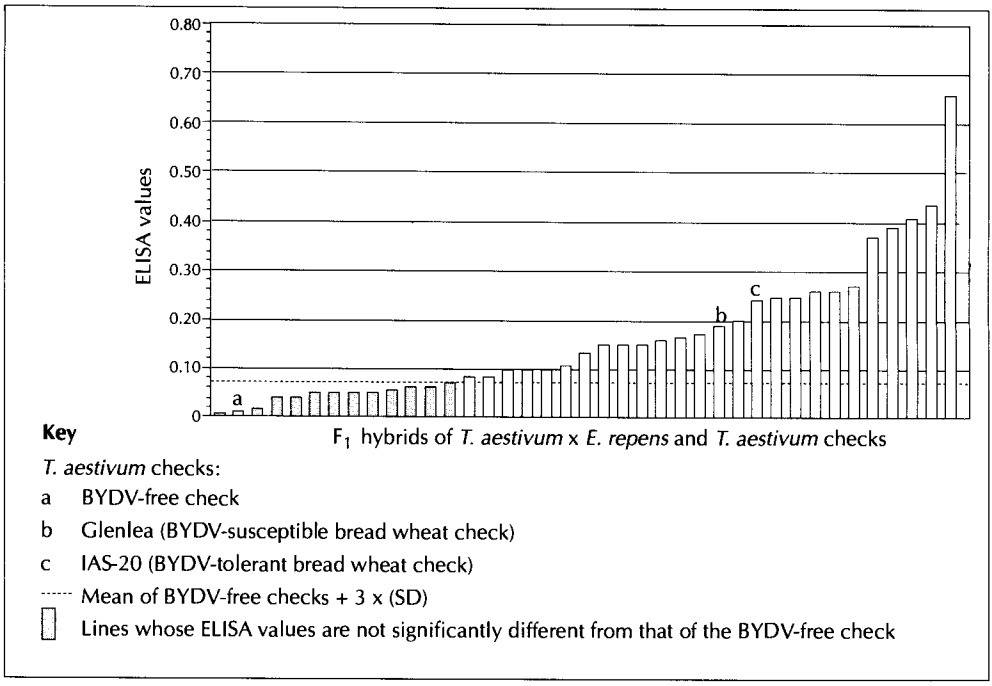
The last task will be to effect gene transfer from the alien chromosome into the wheat chromosome, with proper expression of tolerance or resistance. This implies analysis of the meiosis of many lines; it will probably also be necessary to use irradiation, *Ph1b* gene and other means to induce exchange of genetic material between chromosomes. The gene suppression effect observed by Kimber (1983) seems to be a rather common phenomenon; more investigation is needed on ways of reversing gene suppression mechanisms. In fact, none of 600 primary spring triticale lines synthesized at Agriculture Canada's Sainte-Foy Research Station were classified as BYDV tolerant. However, some secondary triticale lines from the Centre Internacional de Mejoramiento de Maíz y Trigo (CIMMYT) and from Guelph University showed excellent tolerance, which indicates that reversal of the gene suppression effect exists. The use of a broader range of parents was recommended in order to solve this problem (Kimber, 1983).

RESULTS OF WHEAT HYBRIDIZATION STUDIES

Wheat x *Thinopyrum* and wheat x *Elytrigia*

The vast majority of F_1 plants from wheat x *Thinopyrum ponticum* were classified as intermediate or susceptible on a symptom basis (Comeau and Plourde, 1987). However, about 2% of them were resistant. In wheat x *Elytrigia repens*, similar results were obtained. The wild parent *E. repens* is a resistant species, for which the ELISA value of 50 plants was uniformly negative (Comeau, unpubl.); however, 66% of the wheat x *E. repens* lines gave positive ELISA values (*see* Figure 1 *overleaf*). It was hoped that a first selection step at the F_1 level would help identify wheat x alien lines containing genes that would retain their expression when transferred into a wheat genetic background. This hope was partly fulfilled when a small number of wheat x *Thinopyrum* addition and substitution lines were obtained with almost total resistance from the alien parent.

Figure 1 **ELISA values of 36 F_1 hybrids of *Triticum aestivum* x *Elytrigia repens* and three checks**



Wheat x *Agrotricum* and wheat x other amphiploids

The genus *Agrotricum* contains amphiploids, generally 2n = 56, which are derived mainly from wheat x *Thinopyrum*. It is possible that other lines, received from the USSR without pedigree information, might contain a genome from *Leymus* or other species. Most of the amphiploids investigated for BYDV reaction were rather wheat-like, indicating a dominance of wheat genomes, but the seed was more slender. In general, they had a low or intermediate resistance level similar to wheat, but a few, including OK 7211542 (Cisar et al., 1982), Zhong 4 (Xin et al., 1988), and lines obtained from the USSR, were considered resistant and were crossed to wheat. Despite variations in the experimental conditions used for ELISA, these lines showed stable BYDV resistance in all field and laboratory trials in 1988-89 (*see* Table 1).

The morphology of OK 7211542 was similar to the wheat x *Thinopyrum* amphiploids we had produced at Sainte-Foy. This line was used extensively in crosses and backcrosses to the winter wheat cultivar Yorkstar. After rigorous selection for three generations, the bulk selection for better seed type through slot screens separated the segregating progenies in two distinct groups. The narrow-seeded material gave about 5-10% of plants with BYDV resistance in the following trial. The plump, wheat-like seeds generally turned out to be BYDV-susceptible wheat (2n = 42) and only about 12 lines with useful BYDV resistance were isolated from about 10 000 plump seeds of the F_4 generation of Yorkstar x OK 7211542. These wheat-like lines (2n = 42 to 2n = 44) represent material that deserves further study, as the useful genes have been somewhat separated from the wild traits, and the spike fertility seems high.

Table 1 **ELISA values of wheat lines and amphiploids, 13 days after BYDV infection of plantlets at the 4-leaf stage**

Line or cultivar	Origin	ELISA value	Chromosome value
Yok 17	Laval University	0.02	2n = 42
PGR 18752	USSR	0.05	2n = 56
PGR 18750	USSR	0.06	2n = 56
PGR 18749	USSR	0.06	2n = 56
PGR 18751	USSR	0.07	2n = 56
Zhong 4	China	0.08	2n = 56
OK 7211542	USA	0.09	2n = 56
PGR 18754	USSR	0.23	2n = 56
PW 327-1	Canada	0.27	2n = 56
T-Ai-7-135-6	Canada	0.32	2n = 56
Yorkstar	USA	0.35	2n = 42
SD 16415	Germany	0.38	2n = 56
IAS-20 (tolerant)	Brazil	0.52	2n = 42
Glenlea (susceptible)	Canada	0.67	2n = 42

Wheat x *Leymus*

The wheat x *Leymus* crosses seemed promising initially. When tested at the F_1 level, 92% of them proved to be BYDV resistant (Comeau and Plourde, 1987). However, the production of amphiploids proved impossible, and backcrossing was very difficult for *L. innovatus* and *L. multicaulis* and impossible for *L. angustus* 8x and *L. angustus* 12x. Our work was then reorientated towards developing new approaches in order to obtain the desired addition lines. Some of these approaches yielded plants with a wheat-like phenotype and 2n = 42 to 2n = 49, but the low repeatability will delay publication of the methods and results.

The line Inn 8R3, derived from wheat x *L. innovatus*, has 2n = 42, is wheat-like and fertile, and represents the best tolerance and resistance ever observed in a semidwarf background (*see* Table 2). As this line looks so similar to wheat, it is necessary to prove that the genes come from

Table 2 **Grain yield and virus symptoms of *Agrotricum* (2n = 56) and winter wheat (2n = 42) lines artificially infected with BYDV at the 3-leaf stage**

Line	Yield ($g/0.6m^2$)	Symptom score	Grain type
OK 7211542 (R)[a]	45.8	1.0	*Agrotricum*, narrow
Elmo (MT)	16.8	4.5	Winter wheat, plump
Augusta (MT)	15.4	4.0	Winter wheat, plump
Valor (S)	12.3	6.0	Winter wheat, plump
Lennox (S)	7.3	5.5	Winter wheat, plump
Inn 8R3[b] (R-MR)	50.7	2.5	Winter wheat, plump

Note: a R = resistant; MR = moderately resistant; MT = moderately tolerant; S = susceptible.
b Derived from Fukuho/*Leymus innovatus*.

L. innovatus. To demonstrate this, it may be necessary to use a precise technique such as the *in situ* DNA hybridization method.

Aphid and virus resistance in other cereal genera

Wild species of barley might offer a potential source of improved aphid resistance (Weibull 1987, 1988), but gene transfer from these species to cultivated barley is known to be very difficult (Fedak, pers. comm.). In oats, the perennial species *Avena macrostachya* could prove to be resistant to both the virus (Comeau, 1984) and the vector (Weibull 1986, 1988) if the difficulties of gene transfer could be overcome (Leggett, 1985).

OVERVIEW OF BYDV RESISTANCE IN THE TRITICEAE

The visual summary of our research on BYDV in the Triticeae provided in Figures 2 and 3 should prove useful in identifying possible directions for future research.

There is a loose but not negligible relationship between BYDV tolerance and aphid resistance within the Triticeae; however, this is not so evident within particular genera (*see* Figure 2) and genetic variability within a genus is often small. There is also some correlation between BYDV tolerance and ELISA values within the Triticeae; again, this is less evident within particular genera, and thus selection based on ELISA is generally not the best way to identify tolerant lines (*see* Figure 3).

From the overall pattern illustrated in the figures, we can hypothesize that the genes for BYDV tolerance, BYDV resistance and aphid resistance belong to three separate, independently inherited groups. If two of these three traits were controlled by one unique gene, they

Figure 2 Relationship between aphid resistance (*Rhopalosiphum padi*) and virus tolerance for tested species within the Triticeae

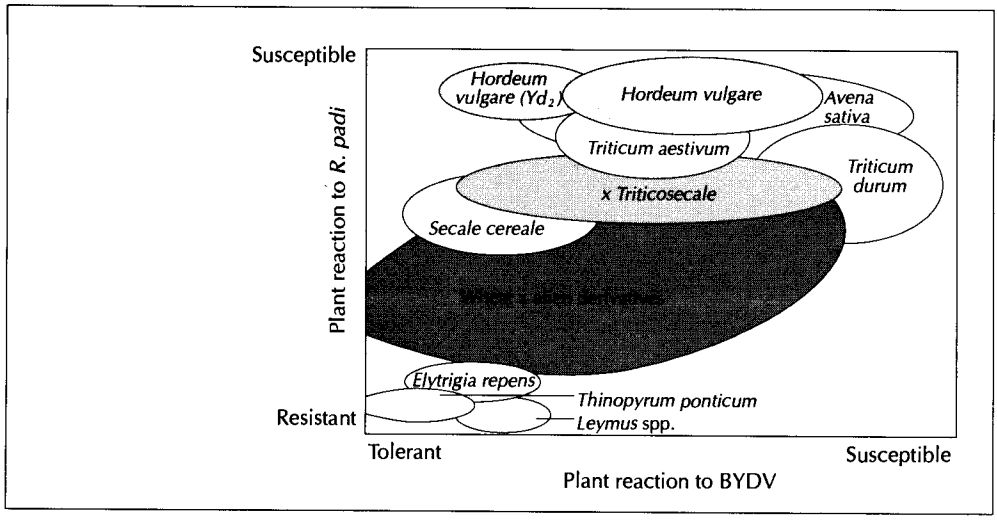
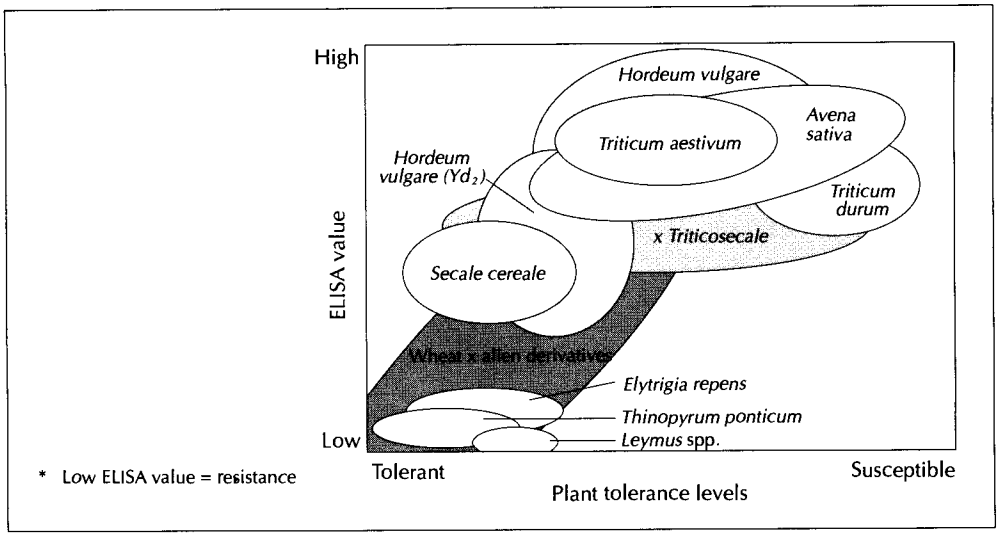


Figure 3 **Relationship between plant tolerance levels and ELISA values which relate to resistance against virus multiplication in the plant, for tested species within the Triticeae**



would behave as fully linked, and the correlation would be very strong. But in artificial inoculation trials, interspecific aphid-resistant lines were not necessarily tolerant or resistant to BYDV; moreover, many species such as bread wheat contained many lines displaying good virus tolerance without significant resistance. In practice, the categorization could be useful: BYDV tolerance would control damage, BYDV resistance would reduce the virus reservoir, and aphid resistance would reduce the movement of disease. As more than one gene is likely to be involved for each trait, the reservoir of genes useful against BYDV and potentially available within the Triticeae seems very large, but the accumulation of the three groups of genes in perennial species is far greater than that in annual species.

This factor is particularly important in terms of the adaptive value of the genes, related to the survival and overall fitness of annual and perennial species. The wild and cultivated cereal species are mostly spring types, generally evolving from dry areas which were not very suitable for aphids and BYDV (Zeven and Zhukovsky, 1975). Their short life cycle significantly increases the likelihood of virus escape, and therefore they do not have to accumulate a large arsenal of genes against BYDV and aphids. The winter species of *Secale* would fit somewhere in between the perennial and the annual species. For the perennials, the long life cycle reduces the chances of virus escape, as plants must face successive waves of aphid infestation. Virus infection affects cereal physiology in a number of ways, reducing overall metabolic efficiency and increasing susceptibility to fungal diseases, so that competitive vigor would be reduced in stress situations and winter-kill or death from drought would often follow. Indeed, studies have shown that the effect of BYDV on winter-kill in winter barley is dramatic (Paliwal and Andrews, 1990; Comeau, unpubl). For these reasons, BYDV susceptibility in a perennial would be highly detrimental, and it is likely that few senstitive plants survived the evolutionary

process that shaped the key genomes. The aphids probably also affected plant survival (Wellso et al., 1985), with the result that aphid-susceptible plants were also selected against in the perennial genera.

CONCLUSION

It would appear that perennial species in the Triticeae represent a rich source of resistance genes for cereal improvement. Efficient exploitation of alien germplasm is not easy, however, and there is considerable room for improvement in the commonly used methods. A much higher yield of useful plants could be obtained if the various problematic stages mentioned above were tackled individually. Gene suppression mechanisms also deserve research. Regeneration from calli, in solid or liquid media, and androgenesis are promising research avenues that are currently being investigated in order to accelerate the production of advanced progenies. Despite the laborious aspects of the work, we were able to produce over 2000 F_1 lines that were used in backcrosses to wheat, yielding over 200 lines endowed with a level of resistance considered to be higher than existing levels.

Extracting genes from perennial Triticeae will prove to be a major challenge for many years to come, and the potential payoff is very high. However, it would be wrong to concentrate only on genes giving immunity or strong resistance, simply because it is always risky to put all your eggs into one basket. Tolerance genes may have specific advantages, and thus we cannot justify discarding them. We have also a lot to learn about the agronomic traits and meiotic behavior of lines derived from wide hybridization. If unexpected problems should occur, the new technology based on restriction fragment length polymorphism (RFLP) might be an appropriate method for extracting useful gene(s) from closely linked deleterious genes.

Acknowledgments

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PART 5

Multiple stress resistance

5.1

Barley Yellow Dwarf Virus Tolerance in Drought Situations

P. MONNEVEUX, C.A. ST-PIERRE and A. COMEAU

SUMMARY

Drought is often accompanied by an increase in the damage caused by barley yellow dwarf virus (BYDV). Drought and BYDV both represent severe stresses when they occur separately; when they occur together, the combined effect on BYDV-susceptible cultivars is devastating. This paper puts forward the hypothesis that BYDV tolerance selection could be much easier to achieve than drought tolerance selection, and that some indirect progress in drought tolerance could be achieved through BYDV selection. The factual basis of this hypothesis is presented, together with suggestions for plant breeding strategies.

In recent years, the genetic tools available to plant breeders have become fairly diversified. Breeders have moved from using traditional methods such as pedigree or backcross selection (Jensen, 1970) to the use of doubled haploids and interspecific hybrids. Within the next decade, more powerful molecular genetic tools might be available for Gramineae. However, producing variants is not always the plant breeder's main problem. Efficient and cost-effective screens are urgently needed to identify the best agronomic genotypes, with the desired levels of stress and disease resistance. This paper outlines the results of some of the work on barley yellow dwarf virus (BYDV) conducted under the Agriculture Canada/Laval University project; it then reviews progress in breeding for drought resistance, discusses interactions between BYDV and drought, and suggests future directions for research.

BYDV TOLERANCE OR RESISTANCE

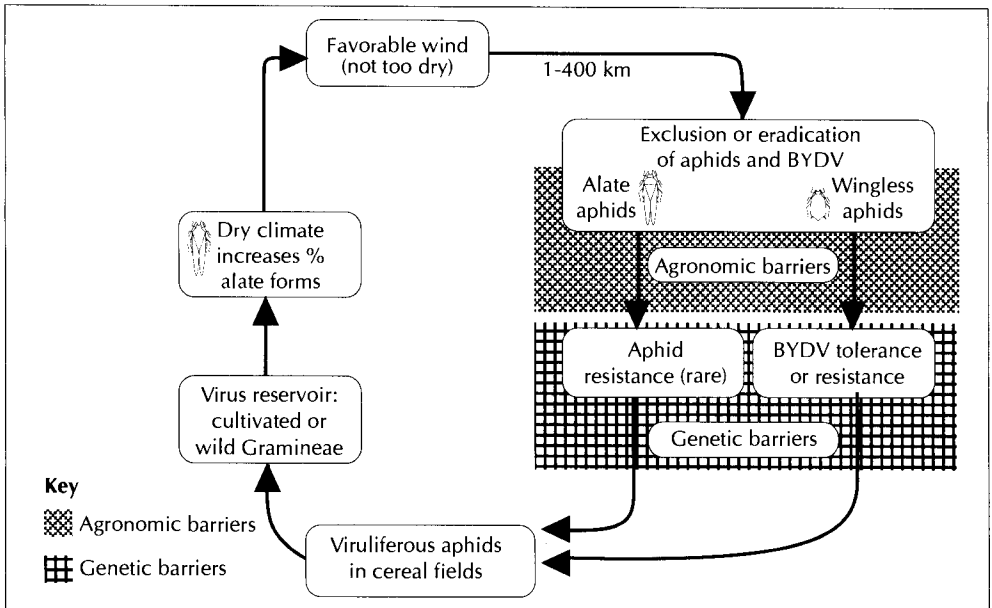
In artificial inoculation trials, BYDV caused damage to the majority of cereal species, although the most tolerant or resistant cultivars suffered little yield loss. In practice, tolerance and resistance were difficult to distinguish except by the use of enzyme-linked immunosorbent assay (ELISA). According to the official definition provided by pathologists, if a cultivar shows

tolerance in the field but is characterized by high ELISA values, it should be classified as tolerant; if it shows shows tolerance in the field and is characterized by low ELISA values, it should be classified as resistant. In our work, however, the ELISA values for many cultivars were intermediate, and therefore these cultivars could not be classified as resistant' or tolerant. For this reason, the term 'tolerance' is used here in its broadest sense to mean 'field tolerance', which may sometimes include effects of resistance genes.

We found that BYDV-tolerant cultivars or lines possessed specific features as a group, and these features were found across all cereal species. The BYDV-tolerant cultivars tended to be high-yielding, tall or moderately tall and relatively clean-looking; a number of them gave quite high yields in drought years. As a group, BYDV-susceptible cultivars or lines tended to be low yielding, variable in plant height (often rather short), and prone to attacks by *Septoria* and *Helminthosporium*; yield and grain quality were never adequate in drought years. Some of these observations were made many years ago but they have been supported by data from recent work. We also found that BYDV-resistant winter cereals in Canada tended to be more winterhardy; examples include Augusta and Houser winter wheat, OAC Elmira and Wysor winter barleys, and Wintok winter oats. All these observations underline the importance of devoting more attention to breeding for BYDV tolerance.

The annual cycle of BYDV can be impeded by the use of agronomic or genetic barriers (*see* Figure 1). As aphids are obligatory vectors, one might attempt to avoid the disease by seeding

Figure 1 The use of agronomic and genetic barriers to impede the annual cycle of BYDV



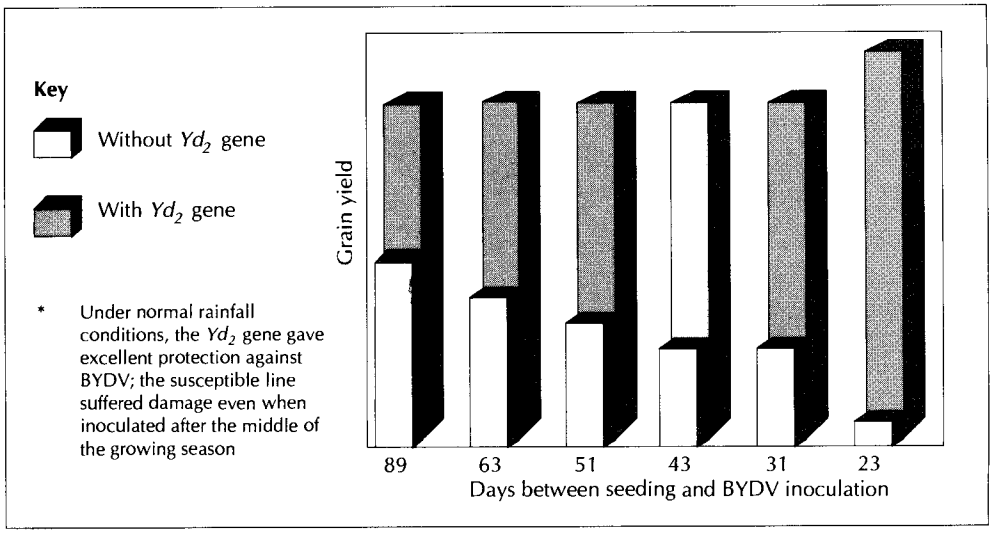
* The annual cycle of BYDV involves aphid migrations that may be triggered at the beginning of a dry period. The use of agronomic barriers such as adjustments in seeding dates may reduce virus damage but the best practical results have come from the use of genetic resistance against BYDV

at dates that minimize the likelihood of infection in young, highly susceptible plantlets. Chemical protection against the virus is impossible; using insecticide against aphids is possible but this is expensive, dangerous for the farmer and bad for the environment. Moreover, when major aphid migrations occur, insecticide may be less effective, as aphids may feed for many hours and transmit the virus before they die. Biological control through the introduction of parasites gave interesting results in South America, but without solving the BYDV problem.

This emphasizes the importance of the second type of barrier, genetic tolerance or resistance to the virus or to the aphid. This may be even more useful in drought situations. As drought reduces yield, using insecticide would be more difficult to justify on economic grounds. Drought leads to an increase in alate aphids, which may travel in great numbers over hundreds of kilometers if air moisture is adequate; however, in the most severe drought, aphid migration is impeded by aphid desiccation. Resistance to aphids would be useful in that it could limit the spread of the vector which causes the most severe damage, *Rhopalosiphum padi* (L.). To date, resistance to *R. padi* has been found only in interspecific hybrids (Tremblay et al., 1989).

BYDV resistance and tolerance genes have been found in barley, oats, bread wheat and triticale. Within the cultivated species, the only true resistance gene known is the barley Yd_2 gene (*see* Figure 2). Resistance genes are now being transferred from *Agropyron* and *Elymus* into wheat (Plourde, 1988; Plourde et al., 1988). Fortunately, the tolerance genes are not so rare and there are a number of lines which suffer low damage although they become quite infected with BYDV. The genetics of tolerance is generally complex. In *Triticum durum*, a thorough search of germplasm worldwide revealed little useful tolerance (Cheour et al., 1989). It appears, therefore, that the whole spectrum of tolerance and resistance can be found within cultivated cereals and their wild relatives. The highest susceptibility is found in oats, barley, durum wheat and a few triticale lines. Useful tolerance or resistance is found in Yd_2 barleys, rye, a few triticale

Figure 2 Relative grain yields of BYDV-infected barley lines, with and without the Yd_2 resistance gene, from trials in Quebec, Canada, 1979



lines and some bread wheat lines from Brazil, China and Africa. Immunity is often found in perennial wild relatives of cultivated cereals.

Selection for BYDV tolerance can be conducted uniformly using the screening method developed by Comeau (1984). This allows artificial inoculation over a full hectare and potentially more; it can be applied to homozygous lines or segregating populations. Using this method, we successfully screened germplasm from conventional breeding lines as well as from lines created by novel technologies. Symptoms notation was a useful selection tool, but had to be used with caution. The correlation of symptoms with yield loss was not always adequate.

Symptoms also varied according to the environment, as shown in the comparison between a normal year and a dry year in Table 1. It is necessary to use a high dose of artificial inoculum to obtain reliable symptom expression. In natural infection conditions, symptoms may be less widespread, less uniform and more ephemeral. Damage levels as high as 25% have been observed in artificial inoculation trials where no typical BYDV symptoms were observed. This hidden damage was related to a late inoculation of BYDV on spring or winter wheat.

Table 1 **BYDV symptom scores in tolerant and susceptible cereal lines grown in 1987 (normal rainfall year) and 1988 (drought year) in artificial inoculation trials at Laval University, Quebec, Canada**

Species	Line or cultivar	Reaction[a]	Symptom score[b] 1987	1988
Six-row barley	QB 235.6	T	4.2	1.8
	QB 172.15	T	3.9	1.8
	Bedford	S	6.8	7.7
Two-row barley	Freja	T	5.7	2.3
	Corris	T	5.2	5.0
	Abee	S	7.8	8.5
Oats	Q.O. 209.48	T	4.4	2.9
	Ogle	T	4.3	5.8
	Lamar	S	8.2	8.5
Bread wheat (tall)	Maringa	T	4.7	3.7
	IAS-20	T	5.7	5.6
	12th IBSWN 459	S	8.2	7.3
Bread wheat (short)	PF 70354/Bow's'	MT	6.7	4.7
	8182 PcHari-678	MT	4.7	6.0
	12th IBSWN 459	S	7.5	7.8
Durum wheat	82 PcDuros 476	T	3.7	5.0
	Belikh 2	MT	6.7	6.0
	12th IDSN 74	S	7.5	7.8
Triticale	83 TF 519.31.1	T	4.2	4.0
	Whale's' 83cbst31	T	5.3	6.3
	Mapache	S	7.7	8.3

Note: a From previous trials, lines classified as tolerant (T), moderately tolerant (MT) or susceptible (S).
b Symptoms scored on a scale of 0-9 (0 = no symptoms or visible damage; 9 = very severe biomass and grain yield loss).

GENETICS OF DROUGHT TOLERANCE

About 40% of the world's cereal growing areas are located in arid or semi-arid regions, but as much as 60% of the total area suffers temporary drought almost every year. As a corollary, efficient use of available water may be just as important as drought tolerance, because water availability is the most serious limitation to yield worldwide.

Breeding for drought tolerance could be viewed as breeding for high yield under dry conditions (Hurd, 1969). This approach involves lumping together the many individual factors which cause yield loss. Moreover, drought can affect many stages in plant growth, and genes that are useful against early drought may give no protection at flowering time. A second approach (known as 'defect elimination') is to eliminate the individual genetic deficiencies which result in drought sensitivity at given growth stages. This approach is simpler genetically, or at least in terms of measuring progress (Frankel, 1947). Nevertheless, breeding for drought tolerance is very difficult and may constitute the greatest challenge for plant breeders (Buddenhagen, 1983; Rasmusson and Gegenbach, 1983)

Baker (1968) stated that many single genes had relatively major (5%) effects on yield. This applies even in drought situations. Unfortunately, breeding for short straw and high harvest index has tended to reduce root length, resulting in increased drought sensitivity (Gorny and Larsson, 1989). Several other single morphological traits (such as earliness, leaf rolling, wax, length and diameter of roots) and physiological traits (such as fine adjustment of daily photosynthesis, opening of stomatae, osmotic regulation, early flowering and high proline) have been related to drought tolerance (Monneveux, 1989). The correlation between a given trait and overall drought tolerance may be of little practical value; for example, Naino et al. (1980) reported that, in sorghum, heat tolerance was poorly related to drought tolerance. In summary, the breeding approach could be empirical or analytical. As traits are numerous, the analytical 'defect elimination' approach might still require a very long process to produce a plant with fewer drought sensitivity traits. On the other hand, the empirical method of breeding for better performance in a dry environment would not help one understand the causes of the losses. The variability of drought stress within a site, between sites and over time can become a major impediment to progress when this empirical method is used (Buddenhagen, 1983).

A synthetic approach, used by various research institutions including the International Center for Agricultural Research in the Dry Areas (ICARDA), relies on the use of many dry sites for empirical work and on the observation of correlations with traits hypothetically associated with drought tolerance (Ceccarelli et al., 1987; Clarke, 1987). The need for new sources of tolerance has been emphasized by ICARDA, with the suggestion that wild species might deserve further study (Ceccarelli et al., 1987).

INTERACTIONS BETWEEN BYDV AND DROUGHT

The variability of drought tolerance data from year to year may be partly related to the presence or absence of BYDV. The importance of BYDV is often not recognized because the symptoms remain unnoticed or are hidden by other factors. For example, in the severe drought that occurred in North America in 1988, there was evidence that BYDV strongly increased drought

damage in certain areas; the drought increased the production of alates but was not severe enough to desiccate the aphids during migration. For some cultivars, the drought x BYDV interaction was devastating. This significant interaction allowed workers in the Laval University BYDV project to identify genotypes resistant to the double stress of BYDV and drought.

Although breeding traditionally selects a cross against a single stress or a single disease, it has been advocated that there should be simultaneous selection against BYDV, drought and fungal diseases (Comeau and Makkouk, 1988). Taking into account the findings reported by Simmonds (1981), we suggest three rules for the study of genotype x environment effects:

- the evaluation system should reflect agricultural reality;
- the selection and evaluation system should use a wide range of selected environments, including low-input and low-yielding sites;
- broadening the genetic base should become a major goal, particularly if previous selection has accumulated germplasm with responsiveness to high input rather than the desired plasticity and adaptation to stress.

Evaluation system should reflect agricultural reality

The likelihood of drought, BYDV or a combination of both stresses varies from one agricultural area to another. As indicated earlier, the combination of BYDV with moderate drought is quite common. Extreme drought conditions impede aphid reproduction and migration because these small insects are heat sensitive and possess a very small reserve of water in their bodies. However, the beginning of a drought period leads to an increase in the alate population within 6 days, followed by mass migration when conditions are humid enough, with favorable winds. Passive transport, sometimes over hundreds of kilometers, can rapidly bring an epidemic to another area. A cool, humid spring induces aphid build-up; a subsequent mid-season drought can produce the worst epidemic.

The selection strategy for moderately dry areas should be based on the economic principle that it is unlikely that local farmers could afford a high input system. The low-input strategy requires progress in drought resistance and water utilization efficiency. Even in areas where BYDV is considered less important than drought, BYDV selection is warranted because it may eliminate, at a low cost, some of the physiological deficiencies that result in drought sensitivity. It also eliminates the need for pesticides and may reduce the need for fertilizer. In pursuing these goals the breeder should adjust the drought x BYDV stress to fit the local situation. Reducing the intensity of the BYDV stress can be achieved by inoculating near the middle of the growing season, or by avoiding the use of the most severe BYDV strains for artificial inoculation.

Selection and evaluation system should use a wide range of environments

During the period of our research in Quebec, we did not have a year in which dry and normal conditions prevailed in the same year, but we were able to make comparisons between normal years and drought years. Typical observations of drought x BYDV damage are shown in Figures 3 to 7. In the absence of drought, the Yd_2 gene of barley was effective throughout the season (*see*

Figure 3). In the presence of drought, this gene remained useful, except in the worst case represented by a very early drought (*see* Figure 4). However, in this case, virus inoculations

Figure 3 Linear relationship of maximum yield with available water in bread wheat

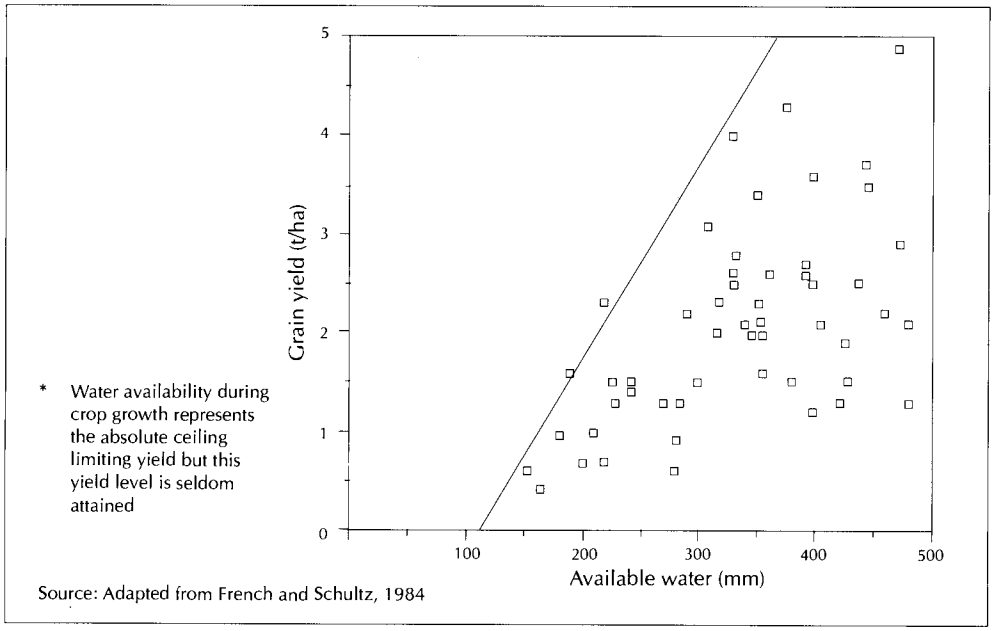
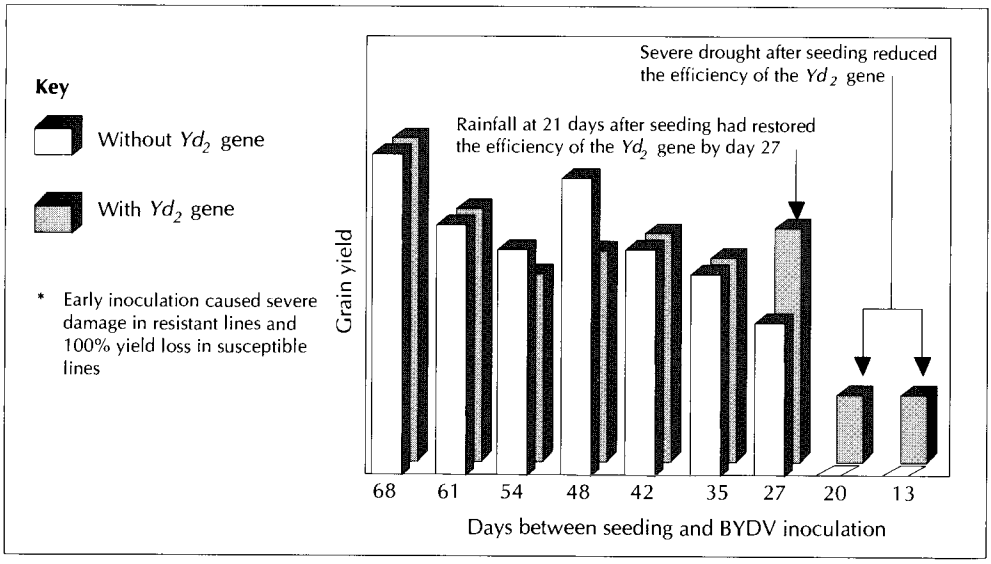


Figure 4 Relative yield losses in barley lines, with and without the Yd_2 resistance gene, caused by artificial BYDV inoculation in trials in Quebec, Canada in 1975, when drought conditions prevailed



carried out at various dates after seeding showed that the effectiveness of Yd_2 was regained after a rainfall which occurred 21 days after planting.

The comparison of a normal rainfall year (1987) with a dry year (1988) showed a major BYDV x drought interaction for all cereal species in trials where plants were artifically inoculated with BYDV. If interaction was negligible, the relative virus damage level would have been similar for both years, but what we saw was a disastrous seed yield of BYDV-susceptible cultivars in 1988. In fact, the yield of BYDV-susceptible bread wheat, durum wheat, barley, oats and triticale came close to zero because of the double stress (*see* Figures 5, 6 and 7). The BYDV-tolerant lines suffered various levels of loss from drought, but never total disaster. This suggests that the BYDV-tolerant lines should be more stable in normal field

Figure 5 Relative grain yield of BYDV-tolerant and BYDV-susceptible bread wheat lines, in a normal year and a drought year

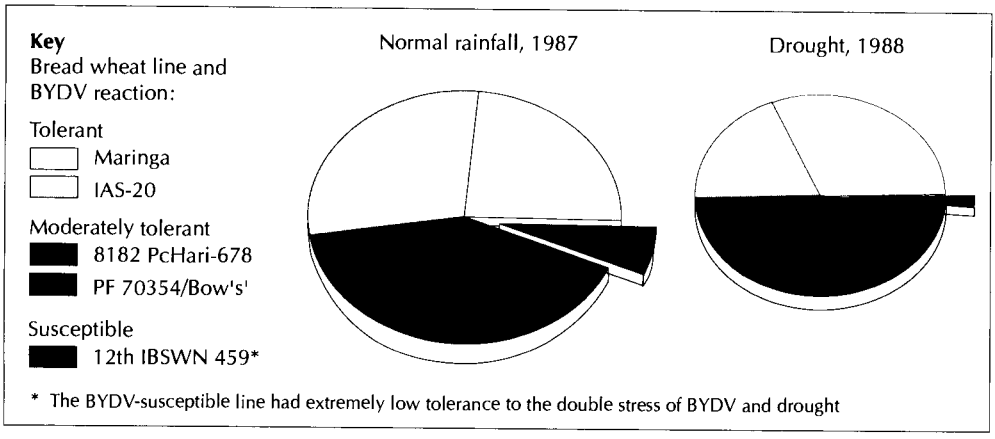


Figure 6 Relative grain yield of BYDV-tolerant and BYDV-susceptible durum wheat lines, in a normal year and a drought year

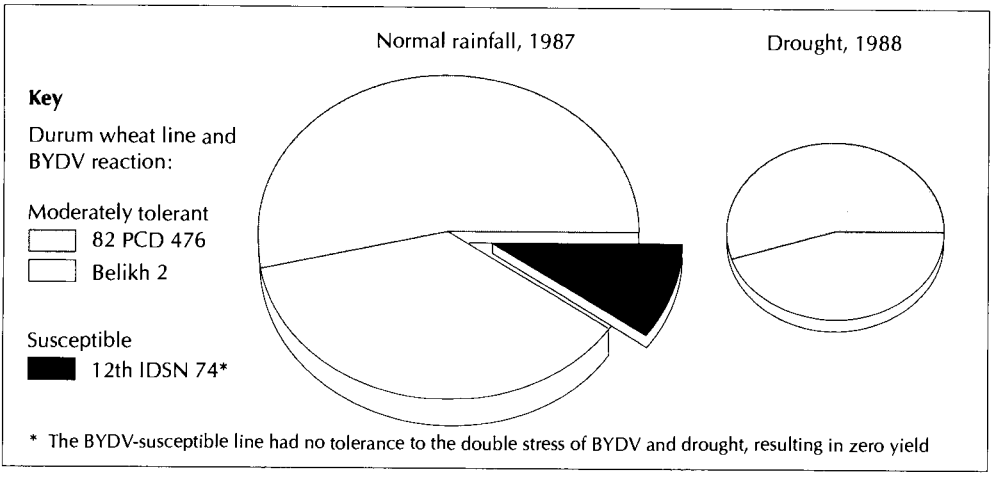
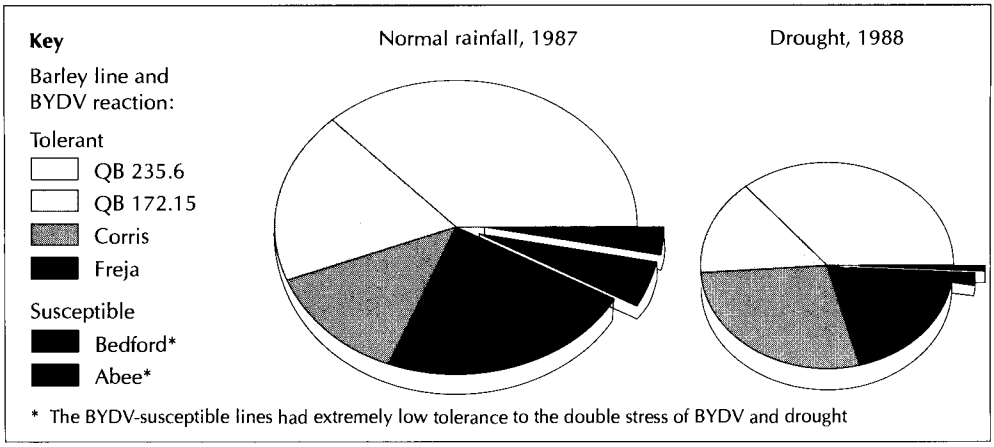


Figure 7 Relative grain yield of BYDV-tolerant and BYDV-susceptible barley lines, in a normal year and a drought year



situations where drought x BYDV situations are likely to occur. BYDV is known to cause more damage to roots than to aerial parts of the plant (Kainz and Hendrix, 1981), which may explain a good part of the observed BYDV x drought interaction.

Goal should be to broaden the genetic base

The probability of improving the root system through BYDV selection is especially worthy of investigation. An aggressive root system, endowed with disease resistance, is important to plant productivity in dry, low-input areas (Taylor and Nguyen, 1987).

After 18 years of research on BYDV, the Agriculture Canada/Laval University project has accumulated what may be the most diversified germplasm in the world for BYDV tolerance and resistance (Comeau and St-Pierre, 1979-88). The selection has been done mainly through artificial inoculation of pure lines and segregation populations in the field. Some ELISA studies have been conducted recently but the considerable progress achieved from field inoculation could never be matched with ELISA selection, which is not only far more expensive but also not very accurate in predicting tolerance in most lines of cereals. One major argument in favor of the BYDV selection approach is the high annual rate of progress with respect to a large number of desirable traits: resistance to physical stress, general disease resistance, and stability of grain quality.

Three factors were cited above as being essential to high yield in semi-arid conditions: ability to extract water from the soil; ability to use water efficiently and without waste to produce biomass; and ability to translocate photosynthate to the grain despite water stress (Monneveux, 1989). Of these three factors, two can be selected with BYDV. The virus can eliminate the plants with the least aggressive root systems (Comeau, unpubl.). Plants with marginal ability to translocate should also be eliminated, as the virus attacks the phloem and inhibits translocation (Esau, 1957; Jensen and Van Sambeek, 1972). It is worth mentioning that these two mechanisms would have no obvious negative side-effect on genetic yield potential

in sites or years where good moisture conditions prevail. Correlation between BYDV tolerance and drought tolerance is quite high among the very best lines selected for BYDV tolerance in every category of plant height.

This selection of the best BYDV-tolerant genotypes would have poorly represented the semidwarf category if plant height had not been taken into account. The well-known correlation between BYDV tolerance and plant height (Comeau and St-Pierre, 1988) is not desirable in humid environments but can probably be considered neutral or helpful in drier environments. In other words, the only unwanted side-effect of a rigorous selection in bulk populations with BYDV could be the elimination of a number of semidwarf genotypes, as these often suffered excessive damage in BYDV trials (Comeau and Jedlinski, 1990). These short plant types are generally not ideal under dry conditions, and thus BYDV selection cannot be considered detrimental in any way when breeding for dry sites.

CONCLUSION

The discovery of interactions between BYDV and drought emphasizes the need for further research in this area. The first trials should include studies of the morphology and physiology of cereals in four environments: non-stressed, BYDV-inoculated, drought-stressed and double stressed. Trials with isolated and combined stress factors, using many genotypes, are essential to understand the true nature of field-observed drought effects. An effort should be made to identify the precise factors that control BYDV tolerance and drought tolerance in order to improve the decisions about matching complementary parental genotypes for the crossing blocks of conventional cereal breeding projects (Rives, 1984). The trials should be backed up by field studies to assess the prevalence of the double stress. One of our principal hypotheses is that BYDV selection might be useful even for very dry areas where BYDV is not a frequent problem. In this situation, the ability of BYDV selection to select for physiological and morphological traits linked with drought tolerance is the most relevant question. Among physiological factors, photosynthesis (Jensen and Van Sambeek, 1972), translocation and proline levels may be of special interest. Proline accumulates under drought conditions (Hanson et al., 1977; Monneveux and Nemmar, 1986; Benlaribi and Monneveux, 1988) but also under virus infection (Perdrizet and Martin, 1960). The amount of callose blocking the phloem in BYDV-infected plants should also be investigated.

On the practical side, there is a need for new genes and a more efficient selection methodology. BYDV-tolerant parents may supply new, useful genes, and BYDV selection might become part of a better selection method against drought. Selection of a number of segregating populations of wheat and barley with BYDV is needed to demonstrate that the virus can serve as a simple, efficient selection tool to improve drought tolerance. It is expected that this selection, possibly in combination with moderate drought stress, would lead to rapid progress because the heritability and repeatability of BYDV tolerance is quite high (Landry et al., 1984) and this tolerance correlates with drought tolerance.

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5.2

Effect of Barley Yellow Dwarf Virus on the Root System of Barley

S. HABER and A. COMEAU

SUMMARY

The first effect of barley yellow dwarf virus (BYDV) in barley occurs at root level and, within 4 days after inoculation, daily root growth is severely inhibited. This inhibition lasts for many weeks. It is likely that the other symptoms of BYDV, as well as the interactions of BYDV with soil characteristics and environmental factors, are mainly secondary effects of the damage caused to the root system. This paper reports on a study conducted in Canada to assess the effect of BYDV on roots in order to better understand the interactions between BYDV and the environment.

The root system of a plant is the hidden and less well-known part of the plant, but it is just as essential to plant health as the aerial parts. The growth of the root system matches the growth of aerial parts (MacKey, 1973). The partition of photosynthate into phloem vessels directed to root or aerial meristems is a critical physiological adjustment of the plant, which maintains the equilibrium between shoot and root. Phloem viruses such as barley yellow dwarf virus (BYDV) reproduce exclusively in phloem cells, causing damage to this important system which is responsible for transferring the products of photosynthesis to the sink areas (Esau, 1957). As the aerial meristems of the young plant are much closer to the source, it is perhaps logical to expect that they would suffer less than the more distant root system.

This hypothesis was supported by Kainz and Hendrix (1981), who observed the growth of BYDV-infected oats and barley in a mist chamber. However, besides this work, little experimental evidence was available in the literature to support any general theory on damage inflicted by BYDV to the roots of cereals. A recent study showed that the roots of oats clearly represent the initial site of virus replication, before the invasion of aerial parts, in plants inoculated at the 4- to 5-leaf stage (Eweida et al., 1988). This prompted us to study the root damage caused by BYDV in barley, which could have important implications for understanding the interactions between BYDV and the environment.

MATERIALS AND METHODS

The first trial was conducted in Winnipeg, Canada using three susceptible cultivars (Bonanza, Argyle and Ellice) and five resistant lines containing the Yd_2 gene (Schaller et al., 1964). The source of Yd_2 was CI 3208-4, which was evaluated with four breeding lines containing the same gene (B 626-46-25, W 816-16-1, W 8116-22-2 and W 8315-77-1). Plants grown in pots in local clay soil were inoculated with a PAV-like isolate, as described by Comeau (1984). They were grown to maturity at 17-24°C under 20 000 lux with a 16-hour photoperiod. The soil was then washed off as carefully as possible. Roots and aerial parts were weighed dry.

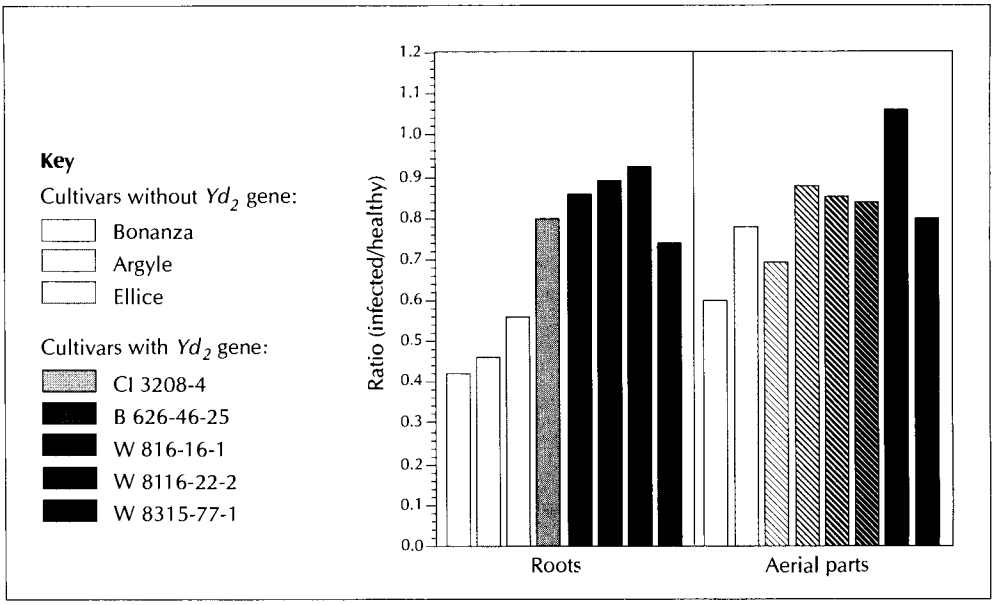
A second trial was conducted in Quebec with the susceptible cultivar Chapais and the resistant lines Corris and 8081BQCB 10. Corris possesses the Yd_2 gene, while 8081BQCB 10 is an Ethiopian line derived from the Hyproly material developed at the Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT); 8081 BQCB10 is as resistant as the Yd_2 lines and may contain the Yd_2 gene (Comeau and St-Pierre, 1988). The plants were infected with BYDV at the 1-leaf stage. This trial involved six replications of selected uniform single plants, grown in hydroponic conditions in a dilute commercial 20-20-20 N-P-K formula (0.25 g/L) with all microelements. The plants received a trickle of nutrient liquid every 30 minutes. Root growth was measured every 2 or 3 days until the 28th day, when the trial was terminated.

RESULTS AND DISCUSSION

In the Winnipeg trial, washing the gumbo-type clay away from the roots was not easy and, despite precautions, some loss of fine roots could not be avoided. In virus-free condition, the dry weight of the roots recovered averaged 0.42 g/plant for susceptible lines and 0.43 g/plant for the resistant lines. In the BYDV-inoculated treatment, the dry weight of the roots recovered averaged 0.20 g/plant for the susceptible lines and 0.36 g/plant for the resistant lines. Some effects were also visible on aerial parts, but the susceptible cultivar Argyle had more aerial dry matter (23.7 g/plant) than the resistant W 8315-77-1 (22.8 g/plant), despite the large differences visible at the root level between infected and healthy plants (*see* Figure 1). It was clear from this trial that BYDV damage to roots is indeed very severe. The trial also showed that data obtained from studying the roots provide a good indication of the presence or absence of the Yd_2 gene in barley. The effect on the roots was such that BYDV would be expected to reduce drought tolerance in a field situation, as it is known that rapid establishment of the root system is important for drought resistance (Taylor and Nguyen, 1987).

In the Quebec trial, in hydroponic conditions, roots were visible throughout the trial. The daily root growth of the susceptible cultivar Chapais became rather negligible on about the 4th day after inoculation, whereas the resistant plants kept growing steadily at a rate of 0.8-1.8 cm of fresh root per day, despite BYDV infection. This difference in growth rate was highly significant ($p < 0.001$). About 14 days after inoculation, the susceptible plants began to emit numerous fine, slow-growing lateral branches, but the overall length of the root system did not increase in any significant manner. In the resistant lines, such lateral branching was very rare in the distal 5 cm, near the root meristems. Although the root damage was very quick to show up in susceptible lines, there were no foliar symptoms until 11-13 days after inoculation, as

Figure 1 **Ratio of BYDV-infected/healthy dry mass of various barley cultivars tested in trials in Winnipeg, Canada, showing that root data is more clearly related to the Yd_2 resistance gene than data on aerial parts**



previously observed for barley (Comeau, 1987). This trial showed that virus damage appears first in the root system, and later on in the aerial parts. The research conducted by Eweida et al. (1988) on oats, using the enzyme-linked immunosorbent assay (ELISA), showed that BYDV multiplied in the roots before invading the aerial parts; our observations on roots would lead us to suspect that roots are also a primary site of multiplication in barley.

We concluded that a significant part of BYDV losses may be indirect. The virus first damages the roots, with the result that the root system is too short to supply the plants with water and nutrients. In the second phase, the aerial parts become malnourished, more or less dwarfed, and lose resistance to other stresses such as drought, disease and unfavorable soil properties. Reduced absorption of soil nutrients by BYDV-infected oats has been demonstrated by Comeau and Barnett (1979). In a third phase, many stresses can severely affect a plant that has lost its general resistance, and the yield loss is the combined result of the complex interaction between virus, climate, soil and fungal diseases. This three-phase damage scenario would explain why symptoms differ so much from year to year, and why they were so severe when drought occurred in Quebec in 1988. In this case, the root injury presumably became a far more important component of the final damage.

Future research directions should include a study of the possibility of using artificial BYDV selection as a tool to help geneticists select better root systems. Rapid root establishment would be of special value for dry areas (Taylor and Nguyen, 1987). If plants that are uniformly BYDV susceptible were inoculated between the Zadoks 15-30 growth stages, it seems likely that the highest yields would be obtained from those plants that had established their root system most rapidly. In the literature available, it is taken for granted that such differences are minor but that

they do exist. In the absence of BYDV, differences in root length depend partly on genotype. In trials on barley reported by Gorny and Larsson (1989), the broad-sense heritability of the trait was 0.79-0.89 and the narrow-sense heritability was 0.20-0.29. However, in these and other trials, the correlation of root length with plant height was generally very significant, ranging between 0.27 and 0.45 (MacKey, 1973; Gorny and Larsson, 1989). This correlation was even stronger in oats (r = 0.50) (MacKey, 1988). This indicates that selecting for better root systems might favor tall lines. Initial studies on this new idea, within a group of barley lines lacking the Yd_2 gene, showed that there may perhaps be more variability in six-row barleys than in two-row barley in terms of rapid root growth in virus-free conditions. The popular six-row cultivar Chapais was among those which resisted this treatment better than most cultivars, and Chapais is a moderately short-strawed cultivar (Comeau and Dubuc, unpubl.).

Plants possessing the Yd_2 gene or other resistance genes would be very useful, as this gene prevents inhibition of root growth in the case of a BYDV epidemic. The problems involved in breeding for BYDV resistance in barley have been discussed by Hayes et al. (1971) and Comeau and Jedlinski (1990). The Yd_2 gene is not necessarily easy to include in a cultivar; negative correlations with yield were observed in some early trials (Comeau and Jedlinski, 1990). Despite this difficulty, the Winnipeg breeding lines tested in the above trial had fairly good overall agronomic attributes. In the Quebec trial, most lines with Yd_2 were too tall and lodged badly. However, the line 8081BQCB 10 is not prone to lodging, and should become an important source of resistance in future breeding efforts. As the problem of the link between Yd_2 and unacceptable agronomic traits has probably been overcome, cautious optimism about creating BYDV-resistant barleys is now justified.

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5.3

Interactions between Barley Yellow Dwarf Virus, Aphids, Plants and Fungal Diseases: An Ecological Model

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SUMMARY

The study of ecological and epidemiological relationships between barley yellow dwarf virus (BYDV) and fungal diseases seems fully warranted. The virus often increases aphid reproduction and migrations. It also appears to increase general contamination of cereal spikes by fungi, although the reasons for this are not fully understood. The aphids may carry spores, but the virus-infected plants are predisposed to fungal attack. This predisposition seems related to the dwarfing effect of BYDV. More attention needs to be given by cereal breeders to multiple disease resistance, against barley yellow dwarf and fungal diseases.

Studies of the interaction of barley yellow dwarf virus (BYDV) with fungal diseases were initiated at Sainte-Foy Research Station in Quebec, Canada in 1974. The early studies confirmed that BYDV predisposes oats to *Septoria* (Comeau and Pelletier, 1976). Later, it became clear that the importance of such interactions had been underestimated (Comeau and Jedlinski, 1990), and we began to accumulate data on multiple disease resistance. Exploratory studies showed that BYDV resistance or tolerance was often accompanied by resistance to fungal diseases (Comeau and Makkouk, 1988). Two conflicting hypotheses could be derived from these preliminary observations. The first is that BYDV selection pressure also selects for tolerance or resistance to many fungal diseases. The second is that previous selection in disease-prone environments is in itself the only cause of the abundance of lines able to resist several diseases, including that caused by BYDV. The second hypothesis implies that selection for BYDV resistance alone would not necessarily improve the frequency of resistance to fungal diseases.

Current research in this area is not advanced enough to establish which hypothesis is valid. However, more information on the ecological interactions between BYDV, aphids, plants and

fungal diseases is emerging. This paper outlines the progress made recently towards understanding these interactions.

ELEMENTS OF THE ECOLOGICAL MODEL

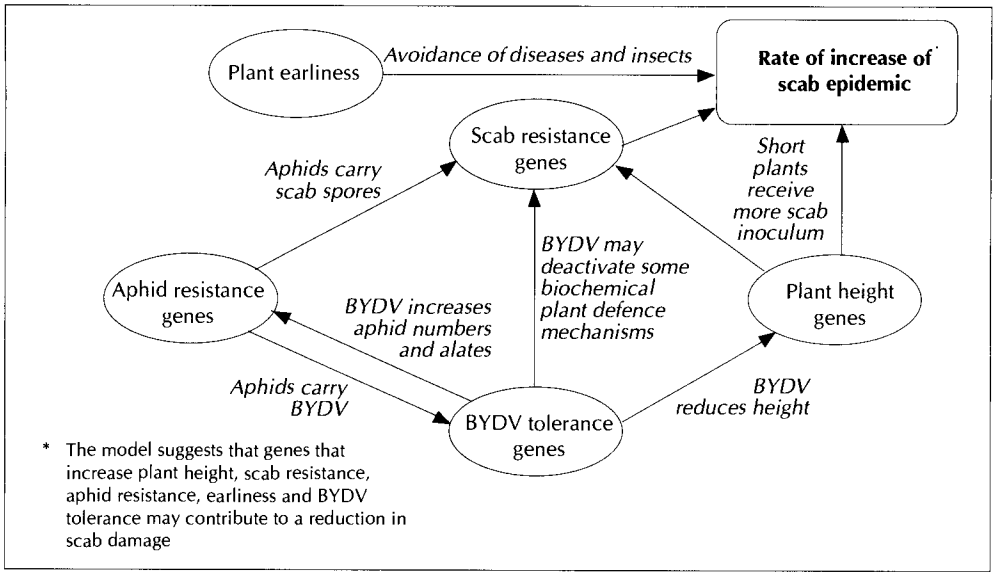
A disease of major importance to cereal growers in Quebec is scab, caused by *Fusarium graminearum* f. sp. *roseum*. In most years, the disease is not particularly widespread, but occasionally it does reach serious levels. Scab reduces grain quality and may also cause accumulation of toxins in the grain of certain cultivars. In 1978 we began making informal notes on scab epidemics, and were puzzled by the fact that they seemed to be closely linked with BYDV epidemics, temporally and spatially. The scab infestation of wheat in one of our BYDV selection nurseries was verified in 1988, and the lines most susceptible to BYDV carried a higher level of *Fusarium* contamination (Couture, pers. comm.).

In a study of scab infestation of grain conducted over many years and sites, a correlation of -0.61 ($p < 0.05$) was found between floret contamination by *Fusarium* and plant height (Couture, 1982). Earlier research on another wheat pathogen had concluded that late maturing and short strawed cultivars usually suffered more damage and infection by *Septoria* (Tavella, 1978). It is well known that BYDV reduces the height of cereals, so this in itself could account for the higher floret contamination observed in our BYDV nurseries. BYDV also increases aphid reproduction (Ajayi and Dewar, 1983; Ajayi, 1986) and the relative abundance of alate forms (Gildow, 1980). The most important piece of missing information was the possibility that aphids themselves carried spores of fungal plant pathogens. Preliminary trials have confirmed the presence of *Fusarium* on aphids (Comeau, unpubl.). We hope to undertake broader research on these interactions in the coming years. The literature already contains convincing evidence that insects can transmit fungal plant diseases. Huang et al. (1981) found that 35-86% of winged pea aphids were contaminated with *Verticillium*, and Harper and Huang (1984) have shown that the alfalfa weevil is also an important disease carrier.

Drawing on available evidence, we constructed a model of the complex interactions of BYDV with aphids, wheat plants and scab (*see* Figure 1). This ecological model predicts that, initially, BYDV infestation may accelerate aphid build-up and alate frequency. The aphids may then carry spores of scab over large distances during migrations but also over small distances within a field. However, an important part of the inoculum may come from within the field, either from inoculum developed at the soil level from seedborne fungi or from the previous year's residues in the soil itself (Couture, 1982). Rain splash and wind contamination may be the most obvious reasons why the cultivars that suffer height reduction have more florets contaminated with fungal spores coming from the ground level, but it is also possible that some biochemical mechanisms of plant defense against fungi could be impaired by BYDV.

Insecticide protection should reduce the level of fungal attack in situations where the model applies. One trial conducted in Quebec in 1986 gave a significant reduction of general fungal infection (*Fusarium*, *Septoria* and *Alternaria*) in treatments that were protected with the aphidicide Pirimor (Comeau, unpubl.). However, this indirect effect of insecticide should not be considered as the general rule, at least not on such a limited database. Supplementary trials are needed to assess this effect.

Figure 1 A proposed model of the interactions between parameters influencing the rate of increase of scab (*Fusarium* head blight) in the field*



THE IDEOTYPE FOR MULTIPLE DISEASE RESISTANCE

The maximum natural protection against scab would be obtained through a combination of five genetic traits: BYDV tolerance, aphid resistance and scab resistance in moderately tall, early maturing cultivars. Aphid resistance is rare, but a combination of the other traits was quite common in germplasm from Brazil. The popular cultivar Maringa is a key example; it is tall, resists scab (Miller et al., 1985) and shows moderate tolerance of BYDV (Comeau and Makkouk, 1988).

A common mechanism of defence against many diseases is earliness. Early maturity reduces attack from scab (Couture, 1982) and *Septoria* (Tavella, 1978), and also helps prevent BYDV damage (Comeau and Jedlinski, 1990). It is therefore a desirable trait, as long as it does not interfere with agronomic performance. However, it seems that earliness is generally a less valuable trait than plant height, in terms of its ability to minimize disease damage.

Unfortunately, tall straw has many drawbacks. Water is wasted because it is used for straw rather than grain yield production. Tall plants reduce work speed and may have poor seed quality because of lodging. This would suggests that the ideal would be to have rather short cultivars with high general disease resistance, but it is acknowledged that the shorter cultivars face a higher disease pressure at the floret level. It is also difficult to create short-statured, BYDV-tolerant wheat (Comeau and Jedlinski, 1990; Comeau, unpubl.), although why this is so is not clear. In view of the above observations, the creation of dwarf wheat with outstanding resistance to many diseases may remain a difficult goal for some time. The puzzle is that there is no obvious reason why short plants should be more prone to BYDV damage. The distance

from soil to floret level is, in the case of BYDV, totally irrelevant. Further research is needed to solve this puzzle.

In the meantime, a compromise is needed. The optimum plant height is probably a rather narrow range in which plants are tall enough to reduce disease, but short enough to be useful in agriculture. But it would be wrong to think that selection for disease resistance could be replaced by selecting only for height and maturity. At equal genetic height, various wheat lines showed significant differences in BYDV tolerance. For example, the old bread wheat landrace Red Fife suffered far more damage than Maringa, although virus-free Red Fife is about 4 cm taller than Maringa (Comeau and St-Pierre, 1988). In the 18th International Triticale Screening Nursery (ITSN) at the Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT), the germplasm actually fell into two distinct groups; the shorter lines tended to be BYDV susceptible, whereas the tall lines contained susceptible as well as tolerant lines (*see* Figure 2). We cite this triticale example because of the large amount of data available; however, similar conclusions about height have been reached with regard to wheat, barley and oats (Comeau, unpubl.).

If the first hypothesis is correct — that is, that BYDV selection pressure also selects for tolerance or resistance to many fungal diseases — then BYDV could potentially become a useful tool for breeding short-strawed wheat cultivars with multiple disease resistance.

CORRELATION BETWEEN BYDV TOLERANCE AND FUNGAL DISEASE RESISTANCE

Although it is not easy to interpret correlations between traits within a given cereal breeding nursery, studies have shown that BYDV tolerance is often accompanied by resistance to fungal diseases (Comeau and Makkouk, 1988). In Quebec, resistance to *Septoria* was found mainly in wheat lines with BYDV tolerance (Comeau and Jedlinski, 1990). In the 18th ITSN, significant correlations between BYDV symptoms and fungal disease symptoms were observed (*see* Table 1).

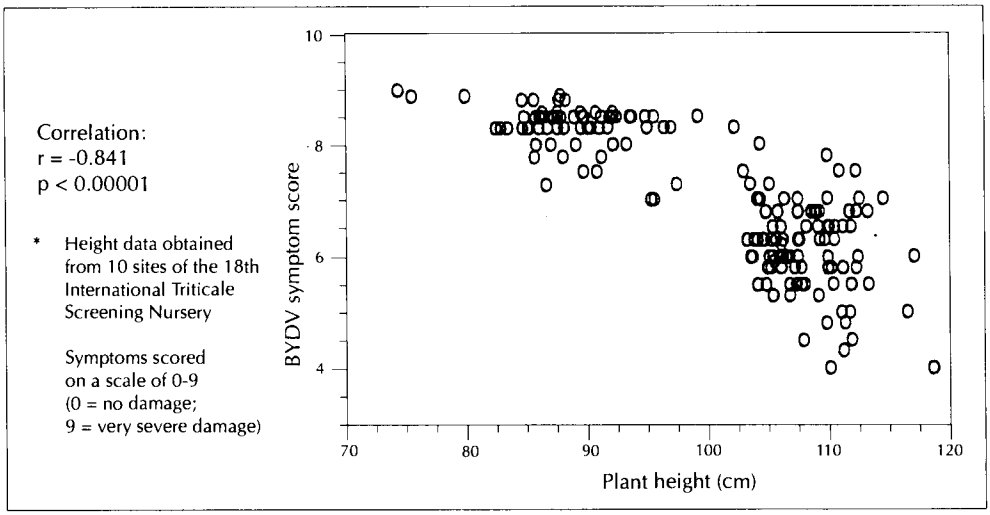
In our trials, the genetically shorter lines from the 18th ITSN had BYDV symptom values ranging from 7 to 9. Taller lines varied considerably in symptom level, but the average was only 6 (*see* Figure 2). The correlation of genetic height with BYDV symptoms was very high ($r = -0.841$, $p < 0.00001$). Specific trials are needed to provide a convincing interpretation of these correlations, but when the findings obtained by Tavella (1978) and Couture (1982) are taken into consideration, there is good reason to believe that certain genetic traits may protect against many diseases, and thus that multiple disease selection could be an interesting new approach for plant breeding. However, the possibility of selecting indirectly for resistance to many diseases may depend on the nature of the original germplasm. In progenies derived from Brazilian wheat, it was easy to retrieve multiple disease resistance after a rigorous selection of bulk plots with BYDV. In one series of CIMMYT progenies where the source of scab resistance was from China, the susceptibility to BYDV was too high and the BYDV selection resulted in near total destruction of the germplasm (Comeau, unpubl.). One conclusion from these trials is that multiple disease selection may work better when tolerance or resistance to all principal

Table 1 **Correlation of data from observations on disease symptoms and agronomic traits in the 18th International Triticale Screening Nursery (CIMMYT) with data from observations in Quebec, Canada for the same nursery[a]**

Disease symptom and agronomic trait	Correlation with plant height	Correlation with Quebec BYDV	Correlation with heading date
Disease symptom (origin):			
Septoria tritici (6 sites)	-0.326[b]	0.401[b]	-0.406[b]
Helminthosporium sp. (Yugoslavia)	-0.436[b]	0.446[b]	-0.397[b]
Helminthosporium teres (South Africa)	-0.705[b]	0.710[b]	-0.600[b]
All *Helminthosporium* entries (5 sites)	-0.295[c]	0.398[b]	-0.471
BYDV (Colombia, New Zealand)	-0.376[b]	0.407[b]	-0.200[d]
Agronomic trait:			
Plant height	—	-0.841[b]	0.404[b]
Heading date	0.404[b]	-0.565[b]	—

Note: a Source of data: Quebec BYDV, Comeau and St-Pierre 1988; all other data, Abdalla et al., 1989. The nursery included 175 lines. The BYDV inoculation in Quebec was quite severe, using a PAV strain of BYDV. The spot blotch data from Brazil was also correlated with the Quebec BYDV symptoms, but the correlation was low ($r = 0.187$, $p < 0.01$ for all BYDV data).
b Significant difference at $p < 0.00001$.
c Significant difference at $p < 0.0001$.
d Significant difference at $p < 0.01$.

Figure 2 **Relationship between genetic plant height and BYDV tolerance in triticale, based on data from trials in Quebec, Canada**



pathogens is present in the parental lines. It is also possible that the multiple stresses may have to be moderated or used with special precaution on shorter strawed germplasm.

Rye and *Agropyron*, both known for their good resistance to BYDV, are also quite resistant to take-all fungus (*Gaeumannomyces graminis* var. *tritici*) (Halloran, 1974), which appears to cause more damage when BYDV is present (Sward and Kollmorgen, 1986). This could mean that during the evolutionary processes that shaped these genera, both diseases were present and serious enough to eliminate almost completely the susceptible genotypes. In Brazil, the disease situation is very complex, and was formerly viewed as a serious obstacle to plant breeding, and yet from this hotbed of disease came valuable, unique germplasm that is proving useful in many breeding programs worldwide (Hettel, 1989).

Perennial grasses are often BYDV resistant or immune and could be a source of multiple disease resistance (Comeau and Plourde, 1987). In transferring BYDV resistance to wheat from perennial species, in some cases plants with high susceptibility to *Septoria* were obtained; in other cases, however, the resistance transferred to wheat appeared to be accompanied by resistance to *Septoria* and *Fusarium*. These are only casual observations, and it will be necessary to devise specific trials to assess whether or not selection for BYDV resistance results in improving tolerance or resistance to fungal diseases. There is the possibility that biochemical mechanisms would be responsible for multiple disease resistance, and such non-specific mechanisms do exist (for example, the pathogenesis-related proteins) (Carr and Klessig, 1989). Most interspecific wheat derivatives that showed BYDV resistance happened to be tall types, the only exception being the line Inn 8R3, derived from wheat/*Leymus innovatus*.

CONCLUSION

It is now possible to identify a few genetic traits that may be factors in multiple disease resistance. These traits include plant height, earliness, aphid resistance, BYDV resistance and tolerance, and pathogenesis-related proteins. From this knowledge, a model of probable interactions between BYDV, plants, aphids and fungal diseases has been developed. However, other factors could exist, especially at the biochemical level. Aphid contamination by plant pathogens, and the efficiency of transfer of this inoculum from aphids to plants, is also a subject that deserves immediate research attention. Premature conclusions are dangerous. Understanding the complexity of the interactions that influence BYDV requires an intensive collaborative research effort.

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Concluding Remarks

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The presentations given by researchers from the West Asia and North Africa (WANA) region at this workshop clearly indicated that awareness about barley yellow dwarf virus (BYDV) has increased significantly. In some countries, such as Morocco, the economic importance of the virus has been established. In others, the information is relatively new. Some information on BYDV in Jordan was available prior to the workshop, but the data presented at the workshop showed that during the 1987 growing season the incidence of BYDV reached 70%. The report from Kenya showed that a minor disease can suddenly become important; in 1987 the yield loss in wheat resulting from BYDV reached 40%. The presentations also showed that the availability of BYDV diagnostic tools in a number of countries has now made it possible to assess BYDV incidence more accurately and that more extensive surveys are required in the future. During the workshop, accounts of international and national BYDV programs were presented, thus exposing the participants to the progress made around the world in breeding cereals resistant to BYDV.

The program in Chile illustrated how progress can be achieved when there is government-level support for developing an integrated control strategy. Research in Canada demonstrated the advantage of involving a virologist/pathologist early in the breeding cycle. The elimination of susceptible material in the early generations of the breeding program proved very practical and saved a considerable amount of time and money.

Methodologies required for research on BYDV were also presented. Experience showed that artificial inoculation with viruliferous aphids is essential in screening for BYDV resistance in areas where epidemics are not common every year. To ensure that truly resistant lines are selected, inoculations of plant material need to be made when plants are young (3- to 5-leaf stage). In most locations in the WANA region, aphids are not common in the field at this growth stage and artificial inoculation is essential for efficient screening.

Research results presented at the workshop indicated that the virus can be successfully purified from desiccated leaves of BYDV-infected plants. Therefore, in countries which lack the laboratory facilities for virus purification, it is still possible to produce BYDV antisera against local isolates by harvesting plants showing disease symptoms, desiccating them at room temperature and then shipping them to a collaborating laboratory for virus purification and antiserum production.

Participants emphasized the need to establish a multilocational BYDV trial for the three key species: durum wheat, bread wheat and barley. Researchers who have considerable experience with BYDV agreed to contribute some of their best lines of these three species to

a common nursery, from which the lines would then be distributed to all interested parties for evaluation under local conditions. It was suggested that ICARDA be responsible for this nursery and the distribution of lines.

A better knowledge of BYDV is needed to ensure stability of the world's cereal supply. In North Africa, the importance of cereals is rapidly increasing, and a reliable food supply cannot be guaranteed after the end of the century. Within a global strategy for food production, the level of BYDV damage should be assessed annually now that the technology to make such assessments is available.

We hope that this meeting will be remembered as an important milestone in coordinating international efforts against BYDV. Let us share the goal of eliminating BYDV epidemics through the use of better cultivars, agronomic practices and other acceptable methods.

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