



Development of barley germplasm with tolerance to barley yellow dwarf virus (BYDV) and the effects of BYDV infection on isogenic barley cultivars
by James Mitchell Crosslin

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Plant Pathology
Montana State University
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Abstract:

Barley yellow dwarf (BYD) is a serious disease of barley (*Hordeum vulgare* L.) and causes major crop losses worldwide. Tolerance to barley yellow dwarf virus (BYDV), the causal agent, of BYD, offers a means of reducing losses to the disease. Genetic male sterility was used to facilitate the development of a six-row barley population with a high level of tolerance to BYDV. A large percentage of plants in the population exhibit no or only slight discoloration following inoculation with BYDV. The population was developed by crossing Composite Cross XXXIII A and B, Composite Cross XLIII, and Sutter barley. Tolerance to the virus was gained by incorporating the Yd2 gene into the population. This gene moderates the development of BYD symptoms and reduces yield losses in BYDV infected barley. The genetic background of the population is very diverse.

In order to more fully understand the effects of BYDV on host plants, barley cultivars with and without the Yd2 gene were inoculated with BYDV in field trials and greenhouse experiments. Results indicate that leaf chlorosis and stunting of roots and shoots due to BYD are reduced in cultivars containing Yd2. Cultivars without Yd2 may suffer 90-95% yield reductions when infected with BYDV, whereas genetically similar cultivars with Yd2 show little, if any, yield reduction.

Electron microscopy of BYDV inoculated plants revealed the presence of BYDV particles in phloem cells of root tissue from barley cultivars with and without the Yd2 gene. However, virus particles were not observed in root tissue of the Yd2-containing cultivar Atlas 68.

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A thesis submitted in partial fulfillment
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in

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Bozeman, Montana

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James Mitchell Crosslin

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ABSTRACT

Barley yellow dwarf (BYD) is a serious disease of barley (Hordeum vulgare L.) and causes major crop losses worldwide. Tolerance to barley yellow dwarf virus (BYDV), the causal agent of BYD, offers a means of reducing losses to the disease. Genetic male sterility was used to facilitate the development of a six-row barley population with a high level of tolerance to BYDV. A large percentage of plants in the population exhibit no or only slight discoloration following inoculation with BYDV. The population was developed by crossing Composite Cross XXXIIIA and B, Composite Cross XLIII, and Sutter barley. Tolerance to the virus was gained by incorporating the Yd2 gene into the population. This gene moderates the development of BYD symptoms and reduces yield losses in BYDV infected barley. The genetic background of the population is very diverse.

In order to more fully understand the effects of BYDV on host plants, barley cultivars with and without the Yd2 gene were inoculated with BYDV in field trials and greenhouse experiments. Results indicate that leaf chlorosis and stunting of roots and shoots due to BYD are reduced in cultivars containing Yd2. Cultivars without Yd2 may suffer 90-95% yield reductions when infected with BYDV, whereas genetically similar cultivars with Yd2 show little, if any, yield reduction.

Electron microscopy of BYDV inoculated plants revealed the presence of BYDV particles in phloem cells of root tissue from barley cultivars with and without the Yd2 gene. However, virus particles were not observed in root tissue of the Yd2-containing cultivar Atlas 68.

CHAPTER 1
INTRODUCTION

Barley yellow dwarf (BYD) is a disease affecting many crop species including barley, oats, wheat, and corn. The disease-causing agent has been termed barley yellow dwarf virus (BYDV).

BYDV is not mechanically transmissible or seed transmitted, but it is transmitted (vectored) by aphids in a persistent manner. The virus is limited to phloem tissue and generally causes "yellows" type symptoms and dwarfing in host plants. The virus particles are isometric in shape and about 25-30nm in diameter (Rochow and Duffus, 1981; Matthews, 1982).

The designation Yd2 has been suggested for a gene in barley that conditions both reduced symptom expression and reduced yield loss in BYDV infected plants. The tolerance to BYDV conferred by Yd2 was noted in several Ethiopian barley lines (Rasmusson and Schaller, 1959).

Some researchers have found, however, a poor correlation between chlorosis development and reduced yield under field conditions. Catherall and Hayes (1966) and Gill et al. (1969) found that cultivars exhibiting the most leaf chlorosis did not always produce the lowest yields. However, these researchers found that increased

leaf yellowing generally denoted susceptibility and susceptible cultivars exhibit reduced yields (Bruehl, 1961). These findings suggest that selection of lines that exhibit little discoloration will, in most cases, also select for reduced yield loss from BYD.

Researchers studying the effects of incorporating Yd2 into European barley cultivars found that its expression varied with the source of the gene and with the background genotype into which it was placed (Catherall and Hayes, 1966). These observations indicated that one possible approach to obtaining agronomically acceptable BYDV tolerant lines would be to incorporate several sources of Yd2 into a diverse background containing agronomically acceptable genotypes. BYDV tolerant plants within this diverse "gene pool" could then be selected for regionally desirable characteristics (Qualset and Suneson, 1966).

Incorporation of genes for disease resistance into susceptible cultivars and repeated backcrossing has led to the development of "isogenic" barley cultivars (Weibe, 1968). Isogenic refers to two cultivars that are considered very similar in genetic make-up, with the exception of a single gene or perhaps a very few genes. In the case of Yd2, a cultivar containing the gene is

resistant to BYD whereas the isogenic without Yd2 is susceptible. The two cultivars are otherwise similar (Schaller and Chim; 1969a, 1969b).

The effects of BYDV on the growth and yield of cereals have been studied extensively over the last 20-30 years (Plumb, 1983). A few investigators have noted a decrease in root growth of infected plants. However, data quantifying the reduction in root growth through the use of isogenic barley cultivars are lacking.

Electron microscopic (EM) observations of ultrathin tissue sections from BYDV infected susceptible hosts have revealed the presence of virus particles in phloem tissue. However, to date little, if any, EM work has been conducted on BYDV resistant cultivars. Also, most EM work on BYDV infected plants has involved observation of oat leaf tissue while largely ignoring root tissue and other host plant genera, such as barley (Gill and Chong, 1979).

The four objectives of the research reported herein are listed below:

1. Develop a six-rowed barley population containing several sources of Yd2 in a diverse genetic background.
2. Study the effect of BYDV infection on the yield of isogenic barley cultivars that contain or lack Yd2.

3. Evaluate the effects of BYDV infection on root and shoot growth of isogenic barley cultivars.

4. Study the occurrence of BYDV particles in root tissue of isogenic barley cultivars using electron microscopy.

Fulfillment of the first objective would yield BYDV tolerant germplasm that could be exploited by barley researchers around the world due to wide genetic diversity within the barley population.

Achievement of the second and third objectives would allow appraisal of the effects of BYDV infections on yield and growth of isogenics and may show a correlation of reduction in growth with a reduction in yield.

Completion of the last objective would allow comparative analysis of virus particle occurrence in infected isogenic cultivars possibly revealing differences in the cytological and morphological responses of resistant versus susceptible plants.

This information will enable us to more fully understand the nature of BYDV infections and the effects of infection on host plants.

CHAPTER 2

LITERATURE REVIEW

History and importance of barley yellow dwarf

The viral nature of the disease barley yellow dwarf (BYD) has been assumed since Oswald and Houston (1951) reported the aphid transmission of a widespread disease affecting barley, oats, and wheat in California. However it was not until Rochow and Brakke (1964) established a relationship between the presence of small isometric particles and infectivity, that the viral origin of BYD was confirmed. Barley yellow dwarf virus (BYDV) was also found to be the causal agent of "oat red leaf disease" by Takeshita (1956). Work by Oswald and Houston (1953a) and Rochow (1961) has shown that BYDV is transmitted in a persistent manner by aphids and is neither mechanically nor seed transmitted.

Barley yellow dwarf can cause major economic losses, as was reported by Oswald and Houston (1953a), when an estimated 10% of the 1951 California barley crop was lost to this disease. Browning et al. (1959) reported that 12% of the oat crop in Iowa that year was lost due to yellow dwarf. This loss represented approximately 394,000 metric tons (Bruehl, 1961). Similar losses were reported in several other states between 1951 and

1960 (Bruehl, 1961). Recently, localized epiphytotics of BYD have occurred in Montana (Yount and Carroll, 1983) and Idaho (Forster and Rochow, 1983).

Losses due to BYD were also considerable in Canada in 1974. Gill (1975) reported a loss in oats of over 30,000 metric tons and in Conquest and Herta barleys an additional 42,000 were lost.

In addition to the United States and Canada, BYDV is known to cause losses in Britain (Doodson and Saunders, 1970), New Zealand (Smith, 1963b), and China (Zhang et al., 1983), to name a few areas of importance. Rochow and Duffus (1981) consider BYDV to be "the most common and widespread of all cereal viruses."

Host range and symptoms of BYDV

The most commonly observed symptoms of BYDV infection include yellowing and stunting. In barley the chlorosis typically appears first on the tips of older leaves, gradually moving down the blade until the entire leaf is bright yellow. Some barley cultivars may show a reddening of the leaves. There may be development of a noticeable green/yellow mottling, with yellow areas coalescing into a general bright yellow chlorosis. These symptoms are very similar to those of aster yellows in

barley (Banttari, 1965). The leaves often assume a stiff, erect posture and appear much thicker than healthy leaves. Some cultivars may develop serrated leaf margins. Experimental inoculations have shown that these symptoms begin to develop in 10-21 days, depending somewhat on the crop and cultivar (Oswald and Houston, 1953a; Bruehl, 1961; Rochow, 1961).

In oats, symptom development follows that of barley except that most oat cultivars exhibit varying degrees of reddening or purpling of the leaves (Takeshita, 1956). Also, water-soaked areas and streaks may appear in oats or barley (Rochow, 1961) and a sugary exudate may appear on the affected leaves (Esau, 1957b).

In addition to the leaf symptoms mentioned above, BYDV infection of susceptible cultivars results in marked stunting of roots and shoots (Bruehl, 1961; Kainz and Hendrix, 1981), reduction in number of seeds per spike in wheat and barley, blasting of florets in oats, and some cultivars show an increase in tillering (Oswald and Houston, 1953a; Rochow, 1961; Catherall and Hayes, 1966). The yield of a susceptible cultivar is dramatically reduced (Bruehl, 1961; Doodson and Saunders, 1970).

Host range studies of BYDV have shown that many members of the Gramineae are affected, including barley (Hordeum vulgare L.), oats (Avena sativa L.), wheat (Triticum aestivum L.), rye (Secale cereale L.), rice (Oryza sativa L.) and corn (Zea mays L.) (Bruehl, 1961).

Oswald and Houston (1953b) inoculated 55 grass species with the virus and found that 20 exhibited BYDV symptoms and from which aphids successfully recovered the virus. Sixteen species showed no symptoms, but aphids were able to recover the virus. The remaining 19 species failed to develop symptoms and virus could not be recovered. Notable symptomatic non-cereal hosts included Bromus inermis Leyss., Avena fatua L., and Festuca reflexa Buckl..

Rochow (1961) lists 84 species that have been found to be hosts of BYDV by one or more researchers. Some non-cereal grass hosts of possible epidemiological significance include Poa pratensis L., Agropyron intermedium Host., Lolium perenne L., and Phleum pratense L. (Bruehl, 1961). Recently, corn has been found to be an epidemiologically important host in Washington (Brown et al., 1984). Rochow and Duffus (1981) speculate that "since so many plant species do not develop symptoms of

BYDV infection, it would be unusual to find a grass species not susceptible to one or more isolates of BYDV."

According to Bruehl (1961), G. B. Orlob used English grain aphids to recover BYDV from dodder (Cuscuta campestris Yunck.) growing on diseased barley. In addition, Timian (1964) showed transmission of BYDV from diseased barley to healthy plants via dodder. Dodder however is not listed as a host of the virus by Bruehl (1961) or Rochow (1961).

Diagnosis of BYDV infections

As the symptoms of BYDV infection can be confused with other diseases or physiological disorders, diagnosis based solely on symptoms may be unreliable (Rochow, 1961).

Oswald and Houston (1951, 1953a, 1953b) used four species of aphids in studies on the transmission of BYDV. These researchers diagnosed BYD based on development of characteristic symptoms in susceptible cultivars following infestation of healthy plants by aphids that had fed on diseased plants. Symptoms normally appeared within two weeks of infestation with viruliferous aphids. Plants infested with aphids that had not fed on diseased plants remained symptom-free.

An improvement in the aphid transmission technique (Rochow, 1963) demonstrated that aphids could acquire the virus by feeding on detached leaves or leaf pieces. In this technique virus free aphids were allowed to feed in the dark for 1-2 days on leaf pieces in humidified dishes. The aphids were then transferred to oat seedlings with a moistened camel's hair brush. After a 3-5 day inoculation period the plants were sprayed or fumigated to kill the aphids and observed for characteristic BYD symptom development.

Rochow (1960) also found that BYDV could be acquired from liquid extracts of infected plants by the English grain aphid (Macrosiphum avenae Fabr.) feeding through animal membranes. Extracts were made by grinding infected oat tissue in a 10% sucrose solution.

Some serological tests that have been used for BYDV diagnosis include neutralization of virus transmission in plant extracts used for membrane feedings, micro-agar double diffusion and latex agglutination (Rochow, 1970). More recently enzyme linked immunosorbent assay (ELISA) techniques have been applied to BYDV (Lister and Rochow, 1979). Tests comparing the efficiency and accuracy of biological techniques (vector studies) versus ELISA for

BYDV diagnosis have shown ELISA to be a reliable, rapid, and sensitive technique. (Rochow, 1979, 1982).

An additional technique for BYDV diagnosis involves electron microscopic (EM) observation of virus particles trapped on an EM grid by antibodies against BYDV (Paliwal, 1977). This technique is termed serologically specific electron microscopy (SSEM) or immunosorbent electron microscopy (ISEM), and has been recently applied to BYDV using monoclonal antibodies (Diacio et al., 1984).

Virus isolates and vector specificity

Bruehl and Toko (1957) tested two isolates of BYDV and noted some variation in host range between the two. For example, some Poa species were susceptible to one isolate and not the other. They also found that Bromus inermis was "immune" to the two isolates (from Washington) they used but was susceptible to the California isolate used by Oswald and Houston (1953b). Obviously differences among BYDV isolates exist.

The early work of Oswald and Houston (1951, 1953a, 1953b) involved a BYDV isolate that was vectored by all four of the aphid species tested. The species used were Rhopalosiphum maidis Fitch, the corn leaf aphid; R. prunifoliae Fitch (= R. padi L.), the apple-grain or oat

bird-cherry aphid; Macrosiphum granarium Kirby (= M. avenae Fabr.), the grain aphid; and M. dirhodum Walker, the grass aphid. These researchers noted that the four species varied in their transmission efficiencies (1953a). Macrosiphum granarium was the most efficient vector (93.2%) and M. dirhodum the least efficient (26.8%).

A few years later, Allen (1957b) tested 43 isolates of BYDV for symptom development on Black Hulless, Rojo, and Atlas 46 barleys, and Coast Black oats (Avena byzantina K.Koch) using Rhopalosiphum prunifoliae Fitch as the vector. Allen divided the 43 isolates into seven "strain types" based on these tests and noted that most isolates produced severe discoloration and stunting on all the test species except Rojo barley. It was also noted that in plants simultaneously inoculated with mixtures of the strains, the separate strains could be later extracted in "pure" form.

Although Allen (1957b) noted that isolates of BYDV differed in their symptom expression, he did not attempt to differentiate these isolates based on vector specificities or lack thereof. Rochow (1959) tested 127 BYDV isolates for transmission by Rhopalosiphum fitchii Sand., M. avenae, R. maidis, and Toxoptera (= Schizaphis) graminum Rondani. BYDV was recovered from 68 of these

samples by M. avenae only, from 14 by R. fitchii only, and from 25 by both R. fitchii and M. avenae. The virus was also recovered from 12 samples by R. maidis only and from seven samples by R. maidis and other species. This work demonstrated that BYDV isolates differ markedly in their vector specificities.

Additional work by Rochow (1969) allowed hundreds of samples of BYDV to be classified into four groups based on differences in transmission by three aphid species. These groups consisted of PAV, vectored nonspecifically by R. padi (= R. fitchii in earlier work, Rochow, 1969) and M. avenae with a rare R. maidis transmission; RPV transmitted specifically by R. padi; MAV transmitted specifically by M. avenae; and RMV transmitted specifically by R. maidis. An additional isolate vectored specifically by Schizaphis graminum has been termed SGV (Johnson and Rochow, 1972).

Serological testing has shown that the RPV and RMV isolates are related, but are distinct from the PAV and MAV isolates, which are related. The SGV isolates appear to have some antigens in common with PAV (Rochow and Duffus, 1981).

Although these "types" of BYDV (PAV, MAV, SGV, RPV, and RMV) are widely recognized (Plumb, 1983), there are

no doubt others with different vector specificities and serological characteristics (Zhang et al., 1983).

Physical properties of the virus

Barley yellow dwarf virus (MAV type) was first purified by Rochow and Brakke (1964) and these workers were able to demonstrate a relationship between small (30nm) isometric particles and infectivity.

To date, BYDV isolates studied are composed of a single component of single-stranded RNA with a molecular weight of about 2.0×10^6 d. A protein with a molecular weight of about 24,000d has been isolated from the MAV and RPV types. Estimates of the size of BYDV particles vary from 30nm for shadowed preparations to 22-26nm in ultrathin sections of infected tissue (Rochow and Duffus, 1981).

Location of BYDV particles in infected plants

Allen (1957a) found that in sections of leaf tissue on which *R. padi* had fed, over 77% of the feeding tracts ended in the phloem, less than 1% in the xylem, and about 22% elsewhere. This was a strong indication that BYDV was associated with the phloem tissue.

Electron microscopy of BYDV infected barley leaf and root tissue by Jensen (1969) showed isometric, densely

staining particles about 24nm in diameter in the phloem. Healthy tissue contained no such particles. However, Jensen was not able to identify the type of phloem cells which contained masses of virus particles due to the disrupted nature of the cell's internal structure. Generally only one or two cells in an entire vascular bundle contained virus particles.

Amici et al. (1979) studied the occurrence of BYDV particles in roots of "rice giallume" infected Leersia oryzoides (Br.) Sol., a perennial grassy weed. These researchers found small densely staining, isometric particles in phloem tissue of leaves and roots. Large crystalline arrays of virus particles were observed in one phloem parenchyma cell from root tissue.

Work by the various researchers cited above would indicate that BYDV particles are confined to phloem tissue. However, Gill and Chong (1981) noted that under conditions of double infection with MAV and RPV isolates, oat xylem tissue was found to contain virus particles.

Anatomical and cytological effects of BYDV infection

Investigations by Esau (1957a) conducted at the light microscope level indicated that BYDV had profound

effects on host phloem tissue. Esau noted that in BYDV infected leaves, necrosis of the first sieve elements occurred before comparable effects in healthy plants were observed. Parenchyma cells near these early sieve elements often underwent early necrosis as well. In infected root tissue Esau also noted that parenchyma cells of the pericycle and phloem began degeneration prior to that of healthy tissue. Sieve elements in roots were observed to accumulate a "gummos" material and collapse prematurely. The cells next to the sieve elements were observed to undergo the most degenerative changes.

At the electron microscope level Gill and Chong (1975) studied the effects of BYDV infection on oat leaf tissue. As early as four days after inoculation, inclusions consisting of virus-like particles, slender filaments and vesicles containing fibrils were observed in phloem parenchyma, companion cells, and sieve elements. These researchers noted that the nuclei of infected cells became distorted and heterochromatin appeared "clumped." Within 11 days inclusions were observed in nearly all phloem cells.

Later work by these investigators (Gill and Chong, 1979) provided evidence for division of BYDV isolates

into two subgroups based on cytopathology. Criteria used for division of the isolates included alterations in the nucleus, where in the cell virus particles were first observed, and the type of fibril-containing vesicles found in the cytoplasm. These effects were observed in sieve elements, companion cells and phloem parenchyma.

Subgroup 1, made up of PAV-, MAV-, and SGV-like isolates caused infections that showed: 1) distorted nuclear outlines, then aggregation of densely staining material, 2) virus particles first appearing in the cytoplasm, and 3) the presence of single-membraned vesicles containing fibrils in the cytoplasm.

Subgroup 2, consisting of the RMV- and RPV-like isolates caused infections that exhibited: 1) nuclei which remained normal in outline with a slow disintegration of heterochromatin, 2) virus particles usually appearing first around the nucleolus, and 3) fibril-containing vesicles enclosed within a second membrane.

It is interesting to note that each of these two subgroups (Gill and Chong, 1979) is composed of isolates that are considered serologically related (Rochow and Duffus, 1981).

Control of BYDV

Some possible control strategies for BYDV include insecticide applications to control the aphid vectors, removal of virus source plants, use of resistant crop cultivars, and avoidance of periods of high vector populations by adjusting planting dates (Rochow and Duffus, 1981).

The use of insecticides to control BYDV by destroying aphid vectors has been somewhat successful. Caldwell et al. (1959) used Dimethoate to control aphids in germplasm nurseries. Smith (1963b) achieved BYDV control in tests conducted in New Zealand by the use of systemic organo-phosphorous sprays to kill R. padi. Yield increases of 5 to 24 bushels of wheat per acre were noted when aphids were controlled. However, Smith (1963b) also noted that Metasystox sprays applied to oats in 1956-58 failed to control BYDV. This researcher hypothesized that since oats are more susceptible to BYDV than is wheat (Bruehl, 1961), a low population of aphids was sufficient to cause severe infection.

Regarding insecticides, Plumb (1983) is of the opinion that "at present chemicals seem unlikely to prevent primary infection and their effect on secondary

spread could be swamped by a continuous influx of infective vectors."

As many common grass species are known hosts of BYDV (Rochow, 1961), elimination of non-cereal hosts may not be practical. In New Zealand the widespread occurrence of perennial grass pastures near cereal fields virtually assures availability of both vector and virus (Smith, 1963b).

Since young plants are the most susceptible to BYD injury (Rochow and Duffus, 1981), adjusting planting dates to avoid periods of high aphid numbers can reduce losses from BYDV. Extremely early fall sown grains are apt to become infested and suffer disease loss (Bruehl, 1961). By avoiding early fall and late spring plantings, losses may be minimized (Plumb, 1983; Rochow and Duffus, 1981; Yount and Carroll, 1983).

If the disease cannot be avoided, the only alternative is to plant disease resistant cultivars. Oswald and Houston (1953a) noted varietal differences in wheat, oats, and barley to BYDV infection. Although most oat cultivars are very susceptible to BYDV infection, some resistant cultivars have been noted (Browning et al., 1959; Jedlinski and Brown, 1959). In wheat several sources of resistance have been found, but this

resistance appears to be quantitative in nature and does not impart complete freedom from BYD (Qualset et al., 1973). Although there seems to be some resistance to BYDV in certain cultivars of wheat and oats, it is in barley that the best resistance has been found.

Tolerance to BYDV in barley

Rasmusson and Schaller (1959) suggested the designation Yd2 for a gene that conditioned reduced symptom expression in barley. This gene, which was found in several barleys of Ethiopian origin, was studied by crossing the resistant lines C.I. 1227, 1237, 2376, and Abate (C.I. 14119) with susceptible lines or with other resistant material. Results from the F₃ indicated that the four cultivars tested contained the same gene. Later work by Damsteegt and Bruehl (1964) indicated that Yd2 was incompletely dominant in its action. However, as parents and progenies often differ in their apparent level of resistance to BYDV the presence of different alleles or modifying factors has been suggested (Schaller, 1964; Catherall et al., 1970). In addition, Catherall and Hayes (1966) noted that the expression of Yd2 varied with the background genotype into which it was placed.

One approach to studying the effects of BYDV infection has involved the use of barley cultivars that are "identical" in all respects except for resistance or susceptibility to BYDV (McLean, et al., 1983; Skaria, et al., 1984a, 1984b). These genetically similar cultivars are often developed by backcrossing and although it cannot be said with certainty that they differ only in regards to one gene, Yd2, they are considered isogenics (Weibe, 1968).

Over the years a number of BYD resistant barleys have been developed by backcrossing a good agronomic type with a Yd2 source. These include Atlas 68 and CM67 (Schaller and Chim, 1969a, 1969b) and Sutter (Schaller et al., 1973).

Barley composite cross populations

The first report of cultivation of a mixture of barley hybrids, or composite, is that of Harlan and Martini (1929). A number of years later, Suneson (1951) discussed using a recently found genetic male sterility factor (Suneson, 1940) to produce hybrid barley. Utilizing the male sterile plants as females eliminated the time consuming and laborious process of emasculation, thus enabling workers to make a previously unheard of

number of crosses in a very short time. In this way Suneson and Wiebe (1962) were able to cross virtually the entire American World Collection of spring barleys to produce Composite Cross (CC) XXI.

Since genetic male sterility in barley was controlled by a simply inherited recessive gene, this allowed an easy means of maintaining sterility in a population (Suneson, 1951). Crossing a homozygous male fertile (MsMs) plant with a homozygous male sterile (msms) plant will result in heterozygous male fertile (Msms) individuals. By selfing an Msms, the progeny will segregate approximately three fertiles (MsMs, Msms, msMs) to one sterile (msms) (Poehlman, 1979). As natural outcrossing of male sterile plants with nearby fertiles will occur (Suneson, 1951) the hybrid seed set in such a manner will be Msms, and male sterility can be maintained in a population by harvesting only male sterile plants and collecting the outcrossed seed.

Recurrent selection populations for disease resistance

Composite cross populations, facilitated by the use of male steriles, have been exploited in plant pathology. By making a large number of initial crosses, screening for resistant plants, and allowing these plants to

outcross, a system called male sterile facilitated recurrent selection has been developed.

The development of a male sterile facilitated recurrent selection population (MSFRSP, also referred to as RSP) begins with the establishment of a base population that is segregating for both male sterility and the character for which it is to be selected, such as disease resistance. This first step is accomplished by crossing agronomically acceptable, genetically diverse cultivars as males with male sterile plants as females. A number of different sources of male sterility have been identified (Hockett and Eslick, 1971). Ten to twenty cultivars are often included in these first crosses. The resulting seed is bulked and the F_1 grown, forming the recurrent selection population (RSP) base.

The next step is to cross male sterile plants in the RSP base as females with a number of lines containing sources of resistance to a particular disease. Typically at least 250 crosses will be made.

The F_1 is grown in isolation to prevent introduction of unwanted genotypes from outside the RSP. In this "recombination" cycle, male fertile plants will outcross with steriles. Male sterile plants only are harvested and the outcrossed seed collected.

A sample of the outcrossed seed, usually 500g or more, is space planted in a disease nursery. Normally the plant density is about 1 plant per 5-15cm of row with rows 30cm apart. This spacing facilitates individual plant evaluation, increases tillering, and simplifies the roguing process.

After inoculation with the disease in question, or the occurrence of natural infections, highly susceptible plants are rogued out. In the first selection cycle only 10-20% of the plants are discarded to avoid early narrowing of the germplasm. In later cycles up to 50% or more of the susceptible plants can be rogued out. Male sterile plants are also removed from the disease nursery. This is done to prevent susceptible plants from outcrossing. After removal of susceptibles and steriles the remaining plants are bulk harvested.

The population now undergoes a recombination cycle where only male steriles are harvested. The outcrossed seed is then space planted in a disease nursery as previously described. These selection-recombination cycles are repeated as long as an increase in disease resistance is observed (H. E. Bockelman and R. T. Ramage, personal communications).

Male sterile facilitated recurrent selection populations developed in the above manner include Composite Cross (CC) XLIII, developed for scald and net blotch resistance (Bockelman et al., 1983), and CC XXXIIIA and B, developed for BYDV resistance (Thompson and Craddock, 1979).

Use of terms

According to Cooper and Jones (1983) the British Mycological Society describes a resistant organism as "possessing qualities that hinder the development of a given disease." The same investigators state that the Terminology Sub-committee of the Federation of British Plant Pathologists substitutes the word "pathogen" for "disease" in the above definition.

Regarding viruses, Cooper and Jones (1983) have concluded from publications by the American Phytopathological Society, the British Mycological Society, and the Federation of British Plant Pathologists, that tolerant "is a term used to describe a host that a specific virus can infect and in which it can replicate and invade without causing severe symptoms or greatly diminishing the rate or amount of plant growth or marketable yield."

In contrast, Schaller and Chim (1969b), in the registration of CM67 barley state that this cultivar is "tolerant" to BYDV "having the Yd2 gene for resistance from C.I. 2376". The same authors (Schaller and Chim, 1973) in registering Sutter barley state that the cultivar is "highly tolerant to the BYDV, having the Yd2 gene for resistance from C.I. 1237."

Owing to the difference of opinion regarding use of the terms resistant, tolerant, sensitive, etc. (see Bos, 1983; Tavantzis, 1984) I will use the term "tolerant" to describe plants that exhibit reduced symptom expression. By comparison, the term "resistant" will be used to describe plants resistant to BYD disease as evidenced by their apparent ability to produce a near normal yield although they are infected by BYDV. "Sensitive" will be considered the opposite of tolerant and "susceptible" the opposite of resistant.

CHAPTER 3

DEVELOPMENT OF BYDV TOLERANT BARLEY GERMPLASM

Materials and Methods1982 field season

In 1982 development of a 6-rowed barley male sterile facilitated recurrent selection population (RSP) with tolerance to BYDV was begun. In development of the population, tolerance to BYDV was judged by the lack of severe chlorosis development following BYDV inoculation. A preliminary report on this work has been published (Crosslin et al., 1984).

Parental lines for this population were chosen based on the need to incorporate BYD resistance, good agronomic qualities, and wide genetic diversity. One parent chosen was CC XXXIIIA and B (Thompson and Craddock, 1979), a 6-rowed RSP developed for BYD resistance having four sources of Yd2. The sources of Yd2 in CC XXXIII are C.I. 1227, 1237, 2376, and 14119 (formerly 3920-1). All of these are of Ethiopian origin (Oswald and Houston, 1953a). This population also contains male sterile genes (msg) 1 and msg 2. The second parent chosen was CC XLIII (Bockelman et al., 1983) a 6-rowed barley RSP developed for scald and net blotch resistance, but also contains

Yd2 from C.I. 14119 and 2376. CC XLIII is genetically very diverse and contains many good agronomic types in its background. This population also contains msg10. The last parent chosen was a Manchuria (C.I. 2330) msg10 x Sutter (C.I. 15475) F₂. Hereafter this hybrid will be referred to as M-S. Manchuria contributed msg10 to facilitate crossing, and Sutter is BYD resistant, having gained Yd2 from C.I. 1237. Sutter also possesses some scald, net blotch, and powdery mildew resistance (Schaller et al., 1973).

A seed mixture of CC XXXIIIA and B was made by combining approximately 66g each of A and B from Pullman, WA with 22g each of A and B from Bozeman, MT disease nurseries. This resulted in about 175g of seed. More Pullman seed was included due to the presence of heavier BYD selection pressure there in 1979 (year the seed was harvested) than in Bozeman. To this 175g of CC XXXIII, 325g of CC XLIII was added. This will be referred to as "population mixture". To the 500g of population mixture, 2kg of killed wheat was added to facilitate space planting.

This seed was planted 24 May, 1982 at the Fort Ellis Research Farm near Bozeman, MT. The material was planted in two blocks, each consisting of 40 rows 21m long and

30cm apart. A mixture of 165g M-S and 660g of killed wheat was planted in six 12m rows between the two population mixture blocks. The entire planting was surrounded by 12 rows of spring wheat.

Approximately 30 days after planting, the stand was estimated by counting the number of plants in three rows and averaging over all rows. By this method an estimated 8,300 plants were present in the population mixture blocks and 450 in the M-S rows.

From 19 July, 1982 to 30 July, 1982 crosses were made by trimming the florets back on male sterile heads, covering with a glassine bag, and "twirling" a pollen-bearing head inside the bag. Male sterile plants in the population mixture were crossed with male fertile M-S plants and male fertiles in the population mixture were crossed with male sterile M-S. A total of approximately 350 crosses were made.

Crossed heads were harvested and threshed in September. The 264 harvested heads of population mixture females x M-S males yielded 183g of seed. The 53 M-S females x population mixture males yielded 30g of seed. Also, male sterile plants in both the population mixture and the M-S were harvested, and the outcrossed seed collected. Male sterile plants in the M-S and population

mixture yielded 55g and 571g of outcrossed seed respectively.

In the fall of 1982, three seed lots were assembled for shipment to Arizona, designated isolations 8, 9, and 10. Isolation 8 consisted of the 30g of M-S females x population mixture males. Isolation 9 was the 183g of population mixture females x M-S males. Isolation 10 consisted of a mixture of 55g of outcrossed M-S seed and 400g of outcrossed seed from the population mixture blocks. These three seed lots were planted 100m from other barleys by Western Plant Breeders near Chandler, Arizona on 3 January, 1983.

1983 field season

Male sterile plants in isolations 8, 9, and 10 were harvested in May, 1983, and the outcrossed seed collected. In addition about 100 heads from male fertile plants in isolations 8 and 9 were collected.

A seed mixture was made consisting of 500g of isolation 10, 75g each of isolations 8 and 9, and 25g each of seeds from fertile plants in isolations 8 and 9. This mixture will be referred to as RSP-BYDV 1983, and was planted at the Arthur H. Post Field Research Laboratory near Bozeman, MT on 26 May, 1983. Killed

wheat was added to the seed to facilitate space planting. Following emergence the plants were hand thinned to about 2-3 plants per 30cm of row. This was done to facilitate individual plant evaluation. After thinning, the plant stand was estimated at 10,600.

In addition to planting the block of RSP-BYDV 1983, a replicated trial was conducted to test the reaction of RSP-BYDV parental populations to BYDV infection. The design was a randomized complete block with four blocks. Each block consisted of three rows per entry. Entries were M-S, CC XXXIIIA and B (same mixture used in initial planting), CC XLIII, isolation 10, and RSP-BYDV 1983. Three rows of Coast Black oats (Avena byzantina K.Koch) were planted between the blocks to act as susceptible indicators of BYDV infection. Rows were 6m long and 30cm apart. All entries were space planted and thinned to 2-3 plants per 30cm of row. The entire experimental area was surrounded by 12 rows of spring wheat to act as a "barrier" for the inoculative aphids that would be introduced.

The main population block and the replicated trials suffered some damage from cattle grazing and hail. For this reason yield data were not collected from the rows of parental populations.

BYDV isolates used in 1983

Two PAV-like isolates of BYDV were used in field inoculations in 1983. One isolate, MT-PAV, was recovered from a BYDV symptomatic barley plant in the Montana State University greenhouse, Range 4, in early 1983. It should be mentioned that this is not the same "MT-PAV" referred to by Yount (1982). This isolate from the greenhouse was found to be highly virulent on Coast Black oats and was vectored efficiently by both R. padi and M. avenae (J. M. Crosslin, unpublished). The second isolate, termed WA-PAV, was a PAV-like isolate from Washington provided by S. D. Wyatt at Washington State University. This isolate was also vectored efficiently by R. padi and M. avenae. Both of these isolates were found to give positive results in ELISA tests with immunoglobulins against a New York PAV isolate (W. F. Rochow, personal communication).

Aphid rearing 1983

Virus-free R. padi and M. avenae, reared at the Montana State University insectary, were allowed to feed on detached leaves of Coast Black oats infected with MT-PAV and WA-PAV using the method of Rochow (1963). Following the two day acquisition period, leaf pieces infested with aphids were placed in 20cm pots containing

Coast Black oat and Klages barley seedlings. About 60 pots, each containing about 100 seedlings were infested. Aphids were allowed to colonize the seedlings and were left undisturbed. Plants were grown under fluorescent lighting and watered as necessary. The temperature was maintained at about 20-25 C. Within two weeks of infestation some of the plants began to exhibit chlorosis and reddening typical of BYDV infection. Large numbers of aphids were present on all plants within 2-3 weeks.

Aphids were collected by using a technique similar to that of Comeau (1976) and Yount (1982). The aphid infested plants were shaken over a large piece of heavy paper dusted with talcum powder. The talcum prevents the aphids from sticking together in large masses. The aphids were placed in plastic dishes with tight fitting lids for transport to the field. The plants were returned to the isolation room to allow the aphids to increase for subsequent harvests.

Field plot infestations 1983

Plants were infested with viruliferous aphids when they reached the 3-5 leaf stage (stage 13-15, Tottman and Makepeace, 1979).

The replicated trials involving parental lines were infested with the inoculative aphids by shaking a few aphids out of the containers directly onto plants in the center row of each three row replicate.

Very early in the aphid spreading procedure it was realized that there were insufficient aphids to infest every plant in the RSP-BYDV 1983 block. Due to the shortage of aphids every third or fourth row of the population was infested by sprinkling aphids onto each plant.

Aphids were spread three additional times as they became available over the course of about three weeks. Center rows of the replicated trials were reinfested as before. An attempt was made to evenly disperse aphids throughout the RSP-BYDV 1983 block.

Following development of BYDV symptoms in the population and parental lines, a 0-3 rating scale was devised as an aid in determining symptom severity. A zero rating indicated that leaf chlorosis was not observed. Plants receiving a one rating showed leaf tip chlorosis on one or a few leaves. A rating of two indicated that most or all of the leaves were affected, but entire leaves were not chlorotic or necrotic. The three disease rating indicated that virtually the entire

plant was severely affected and entire leaves were often chlorotic or necrotic. A few plants given a three rating had apparently been killed by BYD.

Dwarfing was not considered in assigning a disease rating because of the presence of dwarfing genes in the population. Most of the dwarf genotypes would have originated in CC XXXIIIA, members of which had been selected for short, stiff straw, and rapid early growth (Thompson and Craddock, 1979).

Following the harvest of RSP-BYDV 1983 (see Results and Discussion) 500g of seed was sent to Arizona for natural recombination. Outcrossed seed harvested from male sterile plants was returned to Bozeman in May of 1984.

1984 field season

In 1984 the population (now called RSP-BYDV 1984) was planted as in 1983. The parental lines were not planted. However, in an attempt to compare the reactions of the 1983 and 1984 populations to BYDV infection, replicated plots were established. Two plots, one to be infested with viruliferous aphids and one noninfested control, were space planted in a randomized complete block design in four blocks. The 1983 and 1984

populations were planted in three 6m rows per block. Rows of Coast Black oats were included as susceptible indicators.

Aphid rearing in 1984 was similar to that in 1983, except that about 120 20cm pots of Klages and Coast Black oat seedlings were infested. In 1984 only the MT-PAV isolate was used. Inoculative aphids were collected as in 1983.

Owing to the larger number of aphids available in 1984, the entire plot of RSP-BYDV 1984 was infested within a four day period, when plants were in the 3-5 leaf stage. All plants within the population, about 11,000, had aphids sprinkled directly onto them. The replicated trials of RSP-BYDV 1983 and 1984 were infested with inoculative aphids by sprinkling them directly onto each plant in the center row of each three row replicate.

The replicated plots which served as controls were not infested with virus-free aphids. Neither the aphid-infested nor the control plots were sprayed with insecticide at any time. Twelve rows of spring wheat planted between the infested and noninfested plots were to serve as a barrier to the spread of aphids into the noninfested plots. However, late in the season a few plants in the control plots were BYD symptomatic.

Whether these plants were infected as a result of natural aphid movements or "leakage" of aphids from the infested plots is not known.

Reaction of the 1983 and 1984 populations to MT-PAV were compared by measuring disease reaction type (0-3), total dry weight/row, straw weight/row, grain weight/row, 1000 kernel weight, hectoliter weight, germination, and seed protein.

The disease rating of each plant, except the first and last, in the center row of each three row replicate was recorded. The row was then harvested by cutting the plants at the soil line with a sickle. Plants were bundled and allowed to dry.

When dry, the bundle was weighed and then threshed. In this way it was possible to obtain total dry weight/row, straw weight/row, and grain weight/row. Thousand kernel weight for each row was obtained by weighing 1000 seeds which had been counted previously with an electronic seed counter. Hectoliter weight was extrapolated from the weight of 100cc of grain and was determined for each harvested row.

Germination tests were performed using 400 seeds from bulked seed of the four replicates of each population/treatment combination. The tests were

conducted by the Montana State Seed Laboratory at Montana State University according to the Rules of the Association of Official Seed Analysts (Copeland, 1981).

Protein determinations were made on two samples of bulked seed from the four replicates of each population/treatment combination. The analyses were performed at the Montana State University Cereal Quality Laboratory using the infra-red reflectance technique. The apparatus used was a Technicon InfraAnalyzer 400 (Technicon Industrial Systems, Tarrytown, NY 10591).

The data obtained from measurement of the above parameters were analyzed using multi-factorial analysis of variance for a randomized complete block design.

About 340 plants showing 0 or 1 reaction types and possessing desirable characteristics such as large heads with well-filled seed, erect stature, and many tillers, were tagged. Prior to harvest individual heads from these plants were removed for subsequent head-row evaluation.

An estimated 5-10% of the plants in RSP-BYDV 1984 possessed short, stiff straw, and early growth habit. Single heads from about 60 plants with these characteristics, as well as 0 or 1 reaction types, were harvested.

At maturity the population block was bulk harvested with a plot combine. The 340 selected heads were individually threshed and the seed placed in envelopes. The 60 heads from the short, early plants were threshed and bulked.

Results and Discussion

Reaction of RSP-BYDV 1983 to BYDV, Bozeman 1983

Within two weeks of initial infestation with viruliferous aphids, some plants within the population had developed chlorosis and mottling typical of BYDV infestation.

Although symptom development began at about the same time for many plants throughout the population, within three or four weeks obvious differences were observed among plants in the intensity of chlorosis. The 0-3 rating scale previously described was applied as an aid in determining how many plants should be rogued from the population.

Forty-eight days post-infestation, plants within ten random 2m x 2m areas of the population block were evaluated. By this method a total of 423 plants were rated. Of this number approximately 9% were rated as 0, 21% as 1, 53% as 2, and 17% were rated 3 (Table 1). This evaluation showed that the inoculation procedures were indeed very effective as less than 10% of the plants were symptom free. Of the symptomless plants some may have escaped inoculation and others could have been extremely tolerant of the virus and failed to develop

Table 1. Disease reaction of BYDV inoculated RSP-BYDV 1983 and RSP-BYDV 1984 main population blocks.^{1/}

Population	0	% Plants with Reaction Type ^{2/}			# Plants Evaluated
		1	2	3	
RSP-BYDV 1983	9.2 ^{3/}	21.3	52.7	16.8	423
RSP-BYDV 1984	9.9	29.7	20.8	39.5	595

^{1/}RSP-BYDV 1983 was infested with Rhopalosiphum padi and Macrosiphum avenae carrying both MT-PAV and WA-PAV isolates of BYDV.

RSP-BYDV 1984 was infested with R. padi and M. avenae carrying MT-PAV.

Evaluations of ten random 2m x 2m areas of the population blocks were conducted 48 days after infestation in both years.

^{2/}Plants with a 0 reaction type showed no leaf chlorosis. A rating of 1 indicated that one or a few leaves showed leaf tip chlorosis. A 2 rating indicated that most or all leaves were affected but entire leaves were not chlorotic or necrotic. The 3 rating indicated that the entire plant was severely affected and entire leaves were chlorotic or necrotic.

^{3/}Statistical comparisons were not conducted on these data due to the use of different virus isolate mixtures in each of the years and the heavier early infestation of RSP-BYDV 1984 with inoculative aphids.

symptoms. Because the population would be subjected to BYDV inoculations in subsequent years, the presence of a few escapes was not considered important.

Noting that about 17% of the plants were severely affected and that 20% or fewer of the plants in an RSP should be discarded the first year (H. E. Bockelman, personal communication), it was decided to rogue all plants with a 3 reaction type. This was accomplished over the course of about ten days by walking down the rows and simply pulling the plants. The author was the only person involved in roguing in order to minimize differences in reading of symptoms. A running total of plants rogued was kept and at the end of the roguing process 1,697 plants showing a 3 reaction were removed. This was equivalent to about 16% of the plants in the population.

Some additional plants were rogued as well. Perhaps 100 smut infected plants and "black barleys" were removed. Approximately 500 male sterile plants were removed as well. Removing the male sterile plants reduced the amount of ergot harvested (most of the male steriles were heavily infected with ergot, Claviceps purpurea (Fr.)Tul.), and reduced the amount of outcrossed seed that would be harvested. Some of these outcrosses

could have been from the type 3 rogues or highly susceptible material planted nearby.

When the remaining plants were mature, they were bulk harvested with a plot combine. About 25kg of seed was obtained.

The seed was cleaned with an air blower so that the lightest 20% were removed. This was done to help select for BYDV tolerant types by removing small, shriveled seed set on the more sensitive, but unrogued, plants. One of the factors involved in yield loss due to BYDV is a reduction in kernel weight (Bruehl, 1961; Yount, 1982). Also, larger seeds may produce more vigorous plants (Kaufmann et al., 1963).

A 500g sample of the "cleaned" seed was sent to Arizona and planted near Chandler by personnel of Western Plant Breeders on 20 December, 1983. In late April of 1984 male sterile plants were harvested by personnel from Montana State University and the outcrossed seed returned to Bozeman.

Reaction of RSP-BYDV parental lines to BYDV, Bozeman 1983

Following inoculation with a mixture of MT-PAV and WA-PAV, the disease reactions of replicated rows of RSP-BYDV 1983 and the populations used as parents were determined. The rating scale used was the same as for

the main population block. Table 2 shows the reaction of the parentals and RSP-BYDV 1983 28 days after inoculation.

CC XXXIIIA and B showed the highest level of tolerance to BYDV. This result might be expected as CC XXXIIIA and B has undergone several cycles of selection for BYDV tolerance (Thompson and Craddock, 1979; and T. W. Carroll, personal communication).

The values for CC XLIII and RSP-BYDV 1983 are quite similar and this is not surprising as CC XLIII was the major parent. The relatively low tolerance of M-S is something of a surprise, however. Yount (1982) found Sutter to be highly tolerant to a BYDV isolate similar to MT-PAV. It is possible that crossing Manchuria with Sutter resulted in the loss of homozygosity of Yd2, and thus in a lower level of tolerance to BYDV. This reduction in the effectiveness of Yd2 in heterozygous plants was noted by Catherall and Hayes (1966) in crosses of resistant x susceptible cultivars.

Table 3 shows the reaction of the population and parental lines evaluated 38 days after infestation. The discoloration trends observed at 28 days remain. CC XXXIIIA and B still contained significantly more asymptomatic plants (0 reaction types) than the other

Table 2. Disease reaction of RSP-BYDV 1983 and parental populations 28 days after inoculation with a mixture of MT-PAV and WA-PAV isolates of BYDV, 1983. ^{1/}

Population	0	% Plants with Reaction Type ^{2/}			# Plants Evaluated
		1	2	3	
CC XXXIIIA,B	36c ^{3/}	24a	28a	12a	92
CC XLIII	2a	18a	63b	17a	100
Isolation 10	16b	16a	58b	10a	89
M-S F ₂	10ab	15a	57b	18a	54
RSP-BYDV 1983	7ab	15a	59b	18a	67

^{1/}Plants were infested with viruliferous Rhopalosiphum padi and Macrosiphum avenae in the 3-5 leaf stage.

Values are the means of all plants in four replicates of 6m, bordered rows.

^{2/}Plants with a 0 reaction type showed no leaf chlorosis. A rating of 1 indicated that one or a few leaves showed leaf tip chlorosis. A 2 rating indicated that most or all leaves were affected but entire leaves were not chlorotic or necrotic. The 3 rating indicated that the entire plant was severely affected and entire leaves were chlorotic or necrotic.

^{3/}Values followed by the same letter are not significantly different at P=0.05 using the LSD method. Statistical analyses were conducted prior to rounding.

Table 3. Disease reactions of RSP-BYDV 1983 and parental populations 38 days after inoculation with a mixture of MT-PAV and WA-PAV isolates of BYDV.^{1/}

Population	% Plants with Reaction Type ^{2/}				# Plants Evaluated
	0	1	2	3	
CC XXXIIIA,B	22b ^{3/}	31a	21a	26a	92
CC XLIII	1a	6a	52b	40a	100
Isolation 10	6a	17a	49b	28a	89
M-S F ₂	7a	9a	40b	45a	54
RSP-BYDV 1983	3a	10a	53b	35a	67

1/Plants were infested with viruliferous Rhopalosiphum padi and Macrosiphum avenae in the 3-5 leaf stage.

Values are the means of all plants in four replicates of 6m, bordered rows.

2/Plants with a 0 reaction type showed no leaf chlorosis. A rating of 1 indicated that one or a few leaves showed leaf tip chlorosis. A 2 rating indicated that most or all leaves were affected but entire leaves were not chlorotic or necrotic. The 3 rating indicated that the entire plant was severely affected and entire leaves were chlorotic or necrotic.

3/values followed by the same letter are not significantly different at P=0.05 using the LSD method. Statistical analyses were conducted prior to rounding.

lines, and over half of the plants were of reaction types 0 and 1. The number of severely affected plants (3 reaction type) had approximately doubled in all lines, indicating an increase in symptom severity with time.

Reaction of RSP-BYDV 1984 to BYDV, Bozeman, 1984

The outcrossed seed from Arizona 1983-1984, designated RSP-BYDV 1984, was planted and infested with R. padi and M. avenae carrying MT-PAV as previously described.

Within two weeks of infestation chlorosis typical of BYD began to develop. Symptom development appeared to be much more pronounced than in 1983. This was probably due to the fact that all of the plants in RSP-BYDV 1984 had been individually infested with viruliferous aphids, whereas those in RSP-BYDV 1983 had not. This difference in infestation probably meant that plants in the 1984 population had been inoculated at an earlier stage than in 1983. Generally the earlier that plants are inoculated with BYDV the more severe are the effects (Panayotou, 1979; Rochow and Duffus, 1981).

Although temperature plays an important role in BYD symptom development (Endo, 1957), the conditions in both 1983 and 1984 were conducive to symptom development. At

the Post Research Farm, the mean high and low temperatures for the 21 day period following infestation in 1983 were 23.7 and 7.5 C respectively. For the corresponding period in 1984, the mean temperatures were 25.2 and 7.5 C (data from the National Oceanic and Atmospheric Administration, Climatological Data for Montana). This indicates that any differences in symptom development between 1983 and 1984 were probably not due to temperature variations.

As in 1983, plants within ten 2m x 2m areas of the population block were rated on a 0-3 scale 48 days after infestation. Approximately 40% were given a 3 rating (Table 1). Considering the percentage of plants with a 3 reaction type, these results support the hypothesis that more plants in 1984 had been infected earlier in their development than in 1983.

The percentages of plants showing a 0 reaction type were very similar for both years, 9.2% in 1983 and 9.9% in 1984 (Table 1). This indicates that a high level of infection was obtained in both years. Since all plants in RSP-BYDV 1984 had inoculative aphids placed on them, these figures would seem to indicate that the 9-10% of the plants which remained symptomless were probably not escapes but rather symptomless hosts. Support of this

hypothesis was obtained by performing aphid transmission tests on tissue from five symptomless plants in RSP-BYDV 1984. The vector species used was R. padi but tests with M. avenae were also conducted in one case. From all the plants tested, aphids acquired and subsequently transmitted virus to healthy CBO seedlings, as evidenced by the characteristic BYD symptoms. These tests indicated that an isolate of BYDV vectored by both R. padi and M. avenae was present in the population. However, the infection could have been the result of naturally occurring viruliferous aphid flights and subsequent late inoculation of the plants. If inoculation had occurred sufficiently late in the development of plants in the population, symptoms may not have developed.

Owing to the development of reaction type 3 on about 40% of the plants in RSP-BYDV 1984 (Table 1), it was decided to rogue all of these severely affected plants. During the roguing all male sterile plants in the population were removed as well. As in 1983 smutted plants and plants with black kernels were also removed. Considerably fewer black barleys were observed in 1984 than in 1983. The remainder of the plants were harvested with a plot combine.

Following cleaning and deawning of the population seed, some was packaged in 500g lots and shipped to various cooperators around the world. To two population seed lots of 475g each, 25g of seed from the short, stiff, early plants was added to ensure inclusion of this phenotype in the population and to increase its relative frequency. One of these seed lots will be sent to Arizona for a recombination cycle in the winter of 1984-85. The other seed lot will be planted near Riverside, CA and subjected to natural BYDV infection.

The 340 heads of selected plants will be planted at either Davis or Riverside, CA in the winter of 1984-85. Half of the seed from each head may be planted at each of these two locations. Natural infections of BYDV are generally high in these areas and further selections of tolerant, adapted types will be possible.

Comparative reactions of RSP-BYDV 1983 and RSP-BYDV 1984 to MT-PAV, Bozeman, 1984

In addition to the evaluation of data from the two population blocks in 1983 and 1984 (Table 1), replicated trials of RSP-BYDV 1983 and 1984 were conducted in 1984. The results of these trials are shown in Tables 4, 5, and 6. Table 4 shows the disease

Table 4. Disease reaction of RSP-BYDV 1983 and RSP-BYDV 1984 in replicated field plots to MT-PAV inoculation, 1984.^{1/}

Population	0	% Plants with Reaction Type ^{2/}			# Plants Evaluated
		1	2	3	
RSP-BYDV 1983	3.6a ^{3/}	14.4a	21.0a	61.0a	161
RSP-BYDV 1984	6.2a	31.7b	16.3a	45.9a	162

^{1/}Plants were infested with viruliferous Rhopalosiphum padi and Macrosiphum avenae in the 3-5 leaf stage.

Values are the means of all plants in four replicates of 6m, bordered rows. Evaluation was made 43 days after infestation.

^{2/}Plants with a 0 reaction type showed no leaf chlorosis. A rating of 1 indicated that one or a few leaves showed leaf tip chlorosis. A 2 rating indicated that most or all leaves were affected but entire leaves were not chlorotic or necrotic. The 3 rating indicated that the entire plant was severely affected and entire leaves were chlorotic or necrotic.

^{3/}Values followed by the same letter are not significantly different at P=0.05 using the LSD method. Statistical analyses were conducted prior to rounding.

Table 5. Comparative performance of RSP-BYDV 1983 and RSP-BYDV 1984 inoculated with MT-PAV, 1984. Values for dry matter, straw, and grain per row, and 1000 kernel weight.^{1/}

Population	Dry Matter/ Row (g)	Straw/ Row (g)	Grain/ Row (g)	1000 Kernel Weight (g)
1983				
Control ^{2/}	843.7	590.4	253.3	41.4
Inoculated	440.0	307.6	132.4	35.7
Inoculated as % of control	52.2* ^{3/}	52.1*	52.3*	86.3*
1984				
Control	792.5	554.4	238.1	41.7
Inoculated	587.5	414.3	173.2	38.0
Inoculated as % of control	74.1*	74.7*	72.7*	91.1*

^{1/}Inoculated plants were infested with viruliferous Rhopalosiphum padi and Macrosiphum avenae in the 3-5 leaf stage.

Values are the means of four replicates of 6m, bordered rows.

All but first and last plants in each row were harvested.

^{2/}Control plots were not infested with aphids. Neither control nor inoculated plots were sprayed with insecticide at any time. A few plants within the control plots were BYD symptomatic.

^{3/A} (*) indicates that the difference between inoculated and control values are significant at P=0.05 by the LSD method. Values were calculated prior to rounding.

Table 6. Comparative performance of RSP-BYDV 1983 and RSP-BYDV 1984 inoculated with MT-PAV, 1984. Values for disease rating, hectoliter weight, seed protein, and germination.^{1/}

Population	Disease Rating (0-3)	Hectoliter Weight (kg)	Seed Protein (%)	Germination (%)
1983				
Control ^{2/}	0.3	56.2	12.8	98
Inoculated	2.5	54.0	13.7	99
Inoculated as % of control	- ^{3/}	96.1*	107.0	101
1984				
Control	0.3	56.2	13.0	99
Inoculated	2.0	54.5	13.0	98
Inoculated as % of control	- *	96.9*	100.0	99

^{1/}Inoculated plants were infested with viruliferous Rhopalosiphum padi and Macrosiphum avenae in the 3-5 leaf stage.

Values for disease rating are the means of ten plants from each of four replications of 6m bordered rows. Hectoliter weight was extrapolated from one 100cc sample from each of the four replicates. Seed protein and germination were determined on samples of bulked seed of the four replicates. Seed protein determined by infrared reflectance technique. Germination values are means of 400 seeds germinated according to AOSA rules.

^{2/}Control plots were not infested with aphids. Neither control nor infested plots were sprayed with insecticide at any time. A few plants within the control plots were BYD symptomatic.

^{3/}A (*) indicates that the difference between inoculated and control values are significant at P=0.05 by the LSD method. Values were calculated prior to rounding.

reaction of both populations to MT-PAV inoculation. About 17% of the plants in the main population block of RSP-BYDV 1983 exhibited a 3 reaction type (Table 1), whereas 61% exhibited a 3 reaction type in replicated trials in 1984 (Table 4). This difference is probably due to the infestation with viruliferous aphids of every plant in the replicated trials, but not in the main population block. The RSP-BYDV 1984 data are similar between the main block (Table 1) and the replicated trials (Table 4).

The two populations were also compared for effects other than disease reaction. Data for dry matter, straw, and grain production, and 1000 kernel weight are presented in Table 5. Both populations showed statistically significant reductions in all four of these parameters. In each case however, the 1984 population suffered less reduction than the 1983 population. This difference may be due to an increase in the level of tolerance in the 1984 population brought about by removal of highly susceptible plants in 1983.

Table 6 lists the mean values for disease rating, hectoliter weight, seed protein, and percent germination. Both populations showed significant increases in disease rating and significant decreases in hectoliter weights.

The protein and germination parameters were not significantly affected in either population. Germination tests were performed because at least one worker has noted a decrease in the germination of seeds from BYDV infected plants (Panayotou, 1978). This does not seem to be the case under the conditions of this study.

Only PAV-like isolates were used in inoculations of RSP-BYDV. Although differences in the virulence of virus isolates have been noted (Bruehl, 1961), lines containing Yd2 generally show tolerance to many isolates of BYDV (Jones and Catherall, 1970b). For this reason it is expected that RSP-BYDV, recently designated Composite Cross XLIV (D. H. Smith, personal communication), will show a high level of tolerance to most, or all, BYDV isolates. In this regard, testing in various regions of the world will provide data on the reaction of CC XLIV to different environmental conditions and BYDV isolates.

Composite Cross XLIV is genetically very diverse. A total of over 150 plants in each of the 1983 and 1984 populations were compared for disease reaction and yield parameters (Tables 4 - 6). However, this may constitute a relatively small sample of genotypes within the population. This small sample size may account for some of the differences observed in the 1983 and 1984

populations. Testing of subsequent generations of CC XLIV will be necessary in order to verify an increase in the level of BYDV tolerance within the population.

CHAPTER 4

EFFECTS OF BYDV INFECTION ON
ISOGENIC BARLEY CULTIVARSMaterials and Methods1983 field evaluations

In May of 1983 an experiment was devised to test the effects of BYDV infections on cultivars with or without the Yd2 gene for BYD resistance, but which are otherwise similar genetically.

The cultivars chosen were Atlas 68 (C.I. 13824), Atlas 57 (C.I. 10078), CM67 (C.I. 13782), and California Mariout (C.I. 1455). Atlas 68 and CM67 are resistant and California Mariout (CM) and Atlas 57 are susceptible to BYD. Atlas 68 was developed by crossing various Atlas lines and C.I. 1179 with "Abate" (C.I. 14119, formerly C.I. 3920-1), the Yd2 source. CM67 received Yd2 from C.I. 2376 and resulted from crosses of CM, Club Mariout, and C.I. 2376. In field trials, Atlas 68 was similar to Atlas 57 in all characteristics except BYDV and powdery mildew reaction. Also, CM67 was found similar to California Mariout in all characteristics except BYDV reaction (Schaller and Chim, 1969a, 1969b). Due to this

similarity, Atlas 68 and Atlas 57 will be considered an isogenic pair, as will CM67 and California Mariout. Seed of CM67, Atlas 68, and Atlas 57 was obtained from the Seed Room at Montana State University with the assistance of Ken Gilbertson. Seed of California Mariout was obtained from C. W. Schaller, University of California, Davis.

The four cultivars were individually space planted in three 3m rows in each of four blocks of a randomized complete block design. Two plots were planted, one to be infested with viruliferous aphids and the other to serve as a noninfested control. Three rows of Coast Black oats were planted between blocks in the infested and noninfested plots to serve as susceptible indicators. Plants in the inoculated plot were infested with viruliferous R. padi and M. avenae carrying MT-PAV and WA-PAV as described for the RSP-BYDV parental lines. Plants were first infested in the 3-5 leaf stage and at three subsequent times over the next three weeks.

However, disease development or yield data were not obtained due to severe grazing and trampling of the plots by cattle. In an attempt to salvage the experiment, the plants were clipped by hand to a height of seven centimeters above the soil line. This was done in hopes

of obtaining information from plant regrowth. Hail damage precluded the gathering of yield data from the regrowth.

Plants in the infested and noninfested plots were removed from the field and root tissue was examined by electron microscopy. This procedure will be described in detail below.

1984 field evaluations

The same isogenic cultivars were planted in a randomized complete block design similar to that in 1983 except that the rows were 6m in length. Viruliferous aphids were reared as in 1983 except this year only the MT-PAV isolate of BYDV was used. Infestation was carried out as in 1983 except that the plants were infested twice, with a four day interval between infestations. Neither the infested nor noninfested plots were sprayed with insecticide at any time.

Parameters measured for both the infested and non infested plots were disease rating, seed yield/row, total number of tillers/plant, number of fertile tillers/plant, number of seed/head, weight of seed/head, plant height, 1000 kernel weight, hectoliter weight, germination

percentage, and seed protein. A tiller was considered fertile in at least one seed was present in the head.

Disease rating, number of fertile and total tillers, number of seed/head and weight of seed/head were determined for each of the ten center plants in the middle row of each three row replicate. Disease rating and tiller numbers were determined 48 days after infestation. Number and weight of seed per head were determined by counting and weighing the seed produced by the primary tiller on each of the ten evaluated plants. Plant height was measured about 0.5m from each end and in the center of the middle row. Measurements were from the soil line to the top of the highest head, excluding awns. At maturity the entire middle row, except the first and last plants, was harvested and threshed. After counting and weighing, seed from the primary tiller of evaluated plants was added to the grain from the corresponding row. Grain yield/row, 1000 kernel weight and hectoliter weight were determined for each harvested row as described for the population. Most of the inoculated Atlas 57 and CM rows failed to produce sufficient seed for determining 1000 kernel weight and hectoliter weights as previously described. When this occurred, these values were extrapolated from all available seed. Germination and

seed protein were determined from bulked samples of the four replications as previously described.

The data were analyzed using a multi-factorial analysis of variance for a randomized complete block design.

Electron microscopy of field grown plant tissue

In 1983 two plants each of Atlas 68, Atlas 57, CM67, CM, and Coast Black oats (CBO) were dug from both the infested and noninfested field plots. One set of plants was dug 32 days after the first infestation and a second set 10 days later. Only BYDV symptomatic plants were removed from the infested plots. Plants removed from the noninfested plot appeared healthy. Soil was removed from the roots with cold running tap water.

Root tissue samples approximately 1mm in length were cut from the largest roots about 10-15mm from the root tip. Tissue was cut using a new razor blade while submerged in 3% glutaraldehyde in 0.1M potassium sodium phosphate buffer (PSPB), pH 7.2-7.4. Two to four tissue samples were taken from each of 3-4 roots, placed in glutaraldehyde-PSPB, vacuum infiltrated for 20 minutes, and stored at 4 C. After at least 24 hours the tissue was rinsed three times in PSPB and post-fixed for four hours with 2% osmium tetroxide in PSPB. Following osmium

fixation the tissue was washed three times in cold distilled water (pH 7.2-7.4). At this point preparative techniques for the 32 and 42 day material varied.

Following the water rinses, the 32 day material was dehydrated in two 10 minute treatments with acidified 2,2-dimethoxypropane (DMP). Tissue was then stored overnight in nonacidified DMP. The next day infiltration with Spurr's epoxy resin (Spurr, 1969) was begun by placing tissue in a 3:1 ratio (vol:vol) of DMP:resin for 30 minutes on a rotary shaker. The ratio was then changed to 1:1 DMP:resin for 60 minutes on a rotary shaker. Tissue was then placed in 100% resin overnight at 4 C. Following overnight infiltration tissue was placed in plastic capsules which were filled with resin. The resin was polymerized (hardened) in a 70 C oven overnight (12-15 hrs).

The 42 day material was dehydrated in a 50-100% graded ethanol series rather than DMP. After three 15 minute treatments in 100% ethanol the tissue was infiltrated using a 2:1 ethanol:resin mixture on a rotary shaker for one hour, transferred to 1:1 ethanol:resin for two hours on a shaker, and finally 100% resin on a shaker for one hour, and stored overnight at 4 C. The tissue

was then placed in plastic capsules and polymerized as before.

Tissue was sectioned with a Reichert OM-U2 ultramicrotome fitted with glass knives. Thick sections were stained with toluidine blue and observed with a light microscope prior to ultrathin sectioning. Ultrathin sections were placed on uncoated 300 mesh electron microscope grids, stained with uranyl acetate and lead citrate, and observed with a Zeiss EM9S-2 electron microscope. A few sections were observed with Zeiss EM10 or JOEL 100CX microscopes.

Electron microscopy of growth chamber grown plant tissue

Plastic cones measuring 21cm in length by 4cm wide at the top, with a total volume of about 165cc were planted with 1 seed/cone of either the isogenic barleys or Coast Black oats. Cones were filled to within about 6cm of the top with a 1:1 (vol:vol) mixture of steam pasteurized soil and vermiculite. One seed was placed in the center of each cone and about 3cm of the soil mixture was added.

Cones were placed under fluorescent lighting of approximately 1.0×10^4 ergs $\text{cm}^{-2}\text{sec}^{-1}$ in an isolation room maintained at about 20-25 C. When plants were in

the 1- to early 2- leaf stage they were covered with cylindrical clear plastic cages approximately 25cm long and 3cm in diameter.

Plants were divided into two treatments; infested with viruliferous aphids or infested with virus-free aphids. About five R. padi, either virus-free or carrying MT-PAV and WA-PAV, were placed inside the cages with a camel's hair brush. Tops of the cages were sealed with foam rubber plugs. The plants were maintained under fluorescent lighting at 20-25 C for five days, at which time the cages were removed and plants were fumigated with dichlorvos (Vapona) to kill the aphids. The plants were then placed in a growth chamber at 20 C day and 16 C night temperatures (± 2 C) with a 14 hour photoperiod. Incandescent and fluorescent lighting of approximately 1.0×10^4 ergs $\text{cm}^{-2}\text{sec}^{-1}$ at mid-canopy was provided.

Prior to harvest, an aphid transmission test (Rochow, 1963) using R. padi was conducted on each plant to verify the presence or absence of BYDV prior to preparation of tissue for electron microscopy.

Plants were harvested at either 24 or 42 days after the first day of aphid infestation. Roots were washed free of soil with cold running tap water. Tissue was excised as described for field grown material. Samples

consisted of root tips excised 1mm from the apex, 1mm sections from 10-15mm behind the root tip, and 1mm sections taken 10-15mm from the base of the roots. As with the field grown material, only the largest roots were sampled.

Tissue preparation was similar to that of the field grown plants. After glutaraldehyde and osmium fixation the tissue was dehydrated in a graded ethanol series and finally in two changes of propylene oxide (PO). Tissue was then placed in 2:1 PO:resin overnight on a rotary shaker and stored for an additional 12 hours at 4 C. Infiltration was continued using 1:1 and 1:2 PO:resin, each for 24 hours. Tissue was then transferred to 100% resin, vacuum infiltrated for 30 minutes, and placed on a rotary shaker overnight. Finally the tissue was transferred to fresh resin in plastic capsules and polymerized for 15 hours at 70 C.

Two to five plants of each cultivar/treatment/time were sampled. Three to five tissue samples per sampling site per plant were processed.

Tissue was sectioned, stained, and observed as previously described.

Effect of BYDV on root and shoot growth

During processing of tissue for the electron microscopy study, severely stunted root systems of BYDV infected susceptible cultivars were observed. Shoots were noticeably stunted but not as severely as roots. In an attempt to quantify this reduction in root and shoot growth the following investigation was conducted.

Single seeds of the barley cultivars Atlas 68, Atlas 57, CM67, CM, Pirolina (C.I. 9558), Unitan (C.I. 10421), Coracle (British Cb 1029), and Coast Black oats (CBO) were planted in plastic cones as described previously, except that a 1:1 soil:sand (vol:vol) mixture was used. Pirolina and Unitan are susceptible to BYD and Coracle is resistant.

In the first experiment Atlas 68, Atlas 57, CM67, CM, and CBO seedlings were divided into three treatments. One treatment consisted of BYDV inoculation using about five R. padi carrying MT-PAV and WA-PAV. The second treatment was infestation with about five virus-free R. padi. In the third treatment plants were kept aphid free. In all treatments the plants were caged as previously described.

In the second experiment the cultivars Pirolina, Unitan, and Coracle were used and only the BYDV

inoculated and aphid-free treatments were applied due to space limitations.

Treatments were started when plants were in the 1-2 leaf stage. After caging, the plants were kept in an isolation room for five days. Fluorescent lighting was provided and the room was maintained at about 20-25 C. At the end of the five day inoculation period the plants were fumigated with Vapona, moved to a growth chamber, and kept under conditions previously described. Plants were watered frequently, and fertilized with a 15-30-15 or 20-20-20 soluble fertilizer about twice a week.

The plants were harvested 24 or 42 days after infestation. Roots were washed free of soil in cold running tap water. The plants were placed into nylon mesh bags and dried at 100 C for 24-36 hours. After drying, the roots and shoots were weighed separately to the nearest 0.01g. Five plants/cultivar/treatment/time were harvested and weighed.

The data were analyzed using multi-factorial analysis of variance. Means were separated by using the least significant difference (LSD) method.

A correlation analysis was made using the yield and growth data obtained for the various cultivars in 1984, and the yield data of Yount (1982) for Pirolina, Unitan,

and Coracle. Data points consisted of grain yield and oven dry weights of inoculated plants expressed as percent of noninoculated controls. Correlation coefficients were determined by entering yield and dry weight data as pairs. The percentages were not arcsine transformed.

Results and Discussion

1983 field evaluation of isogenic barley lines.

Chlorosis typical of BYD was readily apparent in the infested plots three weeks after infestation. All cultivars, with or without Yd2, had developed some degree of chlorosis. The Coast Black oats had developed typical pinkish discoloration and growth had virtually ceased.

However, before disease readings were made the plots were severely trampled and grazed by cattle. This occurred 23 days after inoculation. Most of the plants in both the inoculated and noninoculated plots were grazed to within a few centimeters of the soil surface. Others were pulled out of the soil or trampled beyond usefulness.

As harvest time approached there was a chance of obtaining yield data from plant regrowth. However, a hail storm in late August caused sufficient damage to preclude gathering of useful data.

1984 field evaluations

In 1984 symptoms developed in the inoculated plots as they had in 1983. A few plants within the control plots developed BYD symptoms late in the season. This resulted from either natural viruliferous aphid flights

or by movement of aphids from the infested plots. The overall impact of these few symptomatic plants on parameter values for the control plots was considered insignificant.

The effects of MT-PAV inoculation on grain yield, 1000 kernel weight, hectoliter weight, seed protein, and germination are shown in Table 7. These data indicate that inoculated Atlas 57 and California Mariout (CM) suffered significant reductions in grain yield, 1000 kernel weight, and hectoliter weight. Reductions in yield of the two susceptible cultivars, Atlas 57 and CM, are striking. The yields of these two cultivars were reduced by over 90% by MT-PAV inoculation. These results agree with reductions suffered by other susceptible cultivars (Bruehl, 1961; Yount, 1982). The reduction in 1000 kernel weight of inoculated Atlas 68 was statistically significant ($P=0.05$) but was considerably less than the reduction suffered by CM and Atlas 57. CM67 showed significant changes only in germination. Why seeds of CM67 collected from BYDV inoculated plants would show significantly higher germination is unknown. The seed from inoculated CM also showed higher, but nonsignificant, germination as compared to the control. These results are surprising as at least one researcher

Table 7. Performance of Atlas 68, Atlas 57, CM67, and California Mariout barleys inoculated with MT-PAV isolate of barley yellow dwarf virus, Bozeman, 1984. Performance based on values obtained for grain yield per row, 1000 kernel weight, hectoliter weight of seed, seed protein, and seed germination.

Cultivar	Grain yield/ row(g)	1000 kernel weight(g)	Hecto- liter weight (kg)	Seed protein (%)	Germi- nation (%)
Atlas 68					
control ^{1/}	331.7	47.4	57.8	14.0	95
inoc.	316.5	44.7	58.8	12.8	93
% of control	95.4	94.4* ^{2/}	101.8	91.4	97.9
Atlas 57					
control	337.7	43.5	58.3	13.6	94
inoc.	32.3	31.9	55.3	14.2	95
% of control	9.5*	73.3*	94.8*	104.4	101.1
CM67					
control	191.5	45.0	58.3	17.6	70
inoc.	206.5	42.8	59.7	15.9	85
% of control	107.8	95.2	102.3	90.3	121.4*
Cal. Mariout					
control	229.2	43.4	58.7	18.8	75
inoc.	18.8	24.6	51.0	18.0	84
% of control	8.2*	56.5*	87.0*	95.7	112.0

^{1/}Data in control and inoculated rows are treatment means. Four rows/cultivar/treatment were harvested. Grain yield, 1000 kernel weight, and hectoliter weight (extrapolated from wt. of 100cc) were determined for each harvested row. Seed protein and germination are means of samples from bulked seeds of the four rows.

^{2/}Percent of control = inoc./control x 100. A (*) indicates that the differences between inoculated and control values are statistically significant at P=0.05 level by the LSD method. Values were calculated before rounding.

has noted a decrease in germination of seeds from BYDV infested plants (Panayotou, 1978). Another interesting result is the lack of relationship between seed protein and 1000 kernel weight. In barley, as kernel weight decreases there is generally a corresponding increase in percent protein due to relatively greater amounts of protein vs. starch in smaller seed (Leonard and Martin, 1963). However, Atlas 68, Atlas 57, and CM showed significant reductions in 1000 kernel weight but protein was not significantly affected. This may be due to the physiological effects of BYDV on host plants (Jensen, 1968).

The effects of MT-PAV inoculation on disease rating, tiller numbers, number and weight of seed per head, and plant height are shown in Table 8. The values for disease rating of controls are not zero due to the presence of a few BYD symptomatic plants in control plots. Inoculated plots showed a significant increase in symptom severity in all cultivars, but the resistant cultivars were less affected. Only the susceptible cultivars showed a significant decrease in fertile and total tiller numbers with BYDV inoculation. This significant decrease in tiller number of susceptible cultivars following BYDV inoculation agrees with results

Table 8. Performance of Atlas 68, Atlas 57, CM67, and California Mariout barleys inoculated with MT-PAV isolate of barley yellow dwarf virus, Bozeman, 1984. Performance based on values obtained for disease rating, fertile tillers/plant, total tillers, seed/head, weight of seed/head, and plant height.

Cultivar	Disease rating (0-3)	Fertile tillers/plant	Total tillers/plant	No. seed/head	Weight of seed/head (g)	Plt ht. (cm)
Atlas 68						
control	0.2	5.7	7.4	36.3	1.92	75.7
inoc.	1.2	5.1	6.7	37.4	1.91	69.0
% cont. ^{2/}	- *	89.0	90.7	103.0	99.50	91.1*
Atlas 57						
control	0.5	6.5	8.3	40.4	1.94	75.7
inoc.	3.0	2.1	2.8	14.2	0.49	49.6
% cont.	- *	32.2*	33.8*	35.1*	25.40*	65.6*
CM67						
control	0.3	6.1	7.4	29.0	1.50	54.8
inoc.	1.2	5.9	7.0	29.8	1.45	53.1
% cont.	- *	96.3	94.6	102.8	97.00	96.8
Cal. Mariout						
control	0.3	5.8	6.7	27.8	1.42	53.8
inoc.	2.9	2.0	3.4	17.6	0.49	38.2
% cont.	- *	34.8*	50.2*	63.2*	34.60*	71.0*

^{1/}Data in control and inoculated rows are treatment means. Four rows/cultivar/treatment were harvested. Plant height was measured at three points in each row. All other data are based on the ten center plants in each harvested row.

^{2/}% cont. = inoc./control x 100. A (*) indicates that the difference between control and inoculated values are significant at P=0.05 using the LSD method. Values were calculated before rounding.

obtained by Yount (1982) and Doodson and Saunders (1970). However, Panayotou (1979) and Oswald and Houston (1953a) noted an increase in total tiller number in some cultivars following BYDV inoculation. The number and weight of seed per head were significantly reduced in Atlas 57 and CM. At harvest time it had been observed that many heads of inoculated Atlas 57 and CM failed to produce any seed. In contrast, the number of seeds per head in Atlas 68 and CM67 were not significantly affected by BYDV inoculation. The effects of BYDV on number and weight of seeds produced are known to be major contributing factors in yield loss due to BYDV (Doodson and Saunders, 1970; Yount, 1982). Plant height was significantly reduced in all cultivars except CM67. The height reduction in Atlas 68, although statistically significant, was only about 10%.

In these experiments CM and Atlas 57 produced virtually no yield. In other experiments involving artificial epiphytotics, the yields of these two cultivars were reduced approximately 40-60% to that of their resistant counterparts. In these experiments however, (Schaller and Chim, 1969a, 1969b) the virus isolates used and plant growth stage when infested were not reported.

In recent investigations with Proctor (susceptible) and the isogenic Shannon (resistant), Proctor suffered a 95% yield loss when inoculated with BYDV in greenhouse experiments. In field experiments Proctor yielded 35-40% less than Shannon (McLean et al., 1983).

Effect of BYDV on root and shoot growth

Plants grown in cone containers and raised in a growth chamber were harvested and dried as previously described. The roots of the BYDV inoculated susceptible cultivars were severely stunted in comparison to the resistant cultivars. Moreover, they were darker in color and they formed a less dense root ball in the cone containers. In most cultivars the shoot growth of inoculated plants was not dramatically affected by BYDV infection, although Coast Black oats (CBO) and Unitan barley were severely stunted. In addition, leaf discoloration typical of BYD was most pronounced in CBO and Unitan. Atlas 57 developed leaf chlorosis but to a lesser extent than had been observed under field conditions. Perhaps this effect was due to lower light intensity in the growth chamber than in the field. The need for high light intensity to obtain optimum BYD symptom expression has been noted (Endo, 1957; Rochow, 1961).

Root and shoot weights of Atlas 68, Atlas 57, CM67, California Mariout (CM), and CBO 24 days after inoculation are shown in Table 9. Root weight is significantly reduced only in CBO and the two susceptible barley cultivars, CM and Atlas 57. Shoot weight at 24 days was significantly reduced only in CBO, confirming the value of this oat cultivar as a susceptible indicator of BYDV infection. Statistical analysis failed to reveal a significant difference in root or shoot weight between the aphid-free and the nonviruliferous aphid treatments.

In contrast to the data obtained at 24 days, root and shoot weights were significantly reduced in most cultivars 42 days after inoculation (Table 10). These data indicate that only Atlas 68 failed to suffer significant reductions in root weight in response to BYDV infection. At 42 days only the susceptible cultivars and CBO showed significant reductions in shoot weight. Statistical analysis revealed a significant reduction in root and shoot weight of CBO by nonviruliferous aphid feeding, and Atlas 68 showed a significant reduction in shoot weight from nonviruliferous aphid feeding as well. Conversely, CM, and Atlas 57 showed no significant

Table 9. Root and shoot dry weight of isogenic barley cultivars and Coast Black oats produced in a growth chamber 24 days after BYDV inoculation or infestation with nonviruliferous aphids or no infestation with aphids.^{1/}

Cultivar	Tissue	Dry Weight (g)			Inoc. as % of no aphids
		BYDV inoc.	Virus-free aphids	No aphids	
Atlas 68	root	.248 ^{2/}	.416	.324	76.5
Atlas 57	"	.104	.330	.304	34.2* ^{4/}
CM67	"	.234	.270	.294	79.6
Ca. Mariout	"	.158	.436	.356	44.4*
C.B. oats	"	.030	.212	.284	10.6*
Mean		.155a ^{3/}	.333b	.312b	
Atlas 68	shoot	.474	.560	.466	101.7
Atlas 57	"	.386	.442	.402	96.0
CM67	"	.470	.548	.556	84.5
Ca. Mariout	"	.498	.664	.636	78.3
C.B. oats	"	.172	.360	.484	35.5*
Mean		.400a	.511b	.509b	

^{1/}Plants in the 1-2 leaf stage were inoculated with a mixture of MT-PAV and WA-PAV BYDV isolates using about five *R. padi*/plant, infested with about five virus-free *R. padi*, or caged without aphids.

^{2/}Values are the means of 5 plants/cultivar/treatment.

^{3/}Values followed by the same letter are not significantly different at P=0.05 by the LSD method.

^{4/}A (*) indicates that the difference between the no aphids and BYDV inoculated treatments are significant at P=0.05 by the LSD method. Data for roots and shoots were analyzed separately.

Table 10. Root and shoot dry weight of isogenic barley cultivars and Coast Black oats produced in a growth chamber 42 days after BYDV inoculation or infestation with nonviruliferous aphids or no infestation with aphids.^{1/}

Cultivar	Tissue	Dry Weight (g)			Inoc. as % of no aphids
		BYDV inoc.	Virus-free aphids	No aphids	
Atlas 68	root	.714 ^{2/}	.740	.818	87.3
Atlas 57	"	.146	.888	1.036	14.1* ^{4/}
CM67	"	.530	.690	.764	69.4*
Ca. Mariout	"	.392	.628	.618	63.4*
C.B. oats	"	.062	.630 ^{#5/}	.870	7.1*
Mean		.369a ^{3/}	.715b	.812c	
Atlas 68	shoot	1.196	.982 [#]	1.372	87.2
Atlas 57	"	.824	1.054	1.202	68.6*
CM67	"	1.548	1.844	1.632	94.9
Ca. Mariout	"	1.480	1.848	1.914	77.3*
C.B. oats	"	.520	.850 [#]	1.158	44.9*
Mean		1.144a	1.326b	1.456c	

^{1/}Plants in the 1-2 leaf stage were inoculated with a mixture of MT-PAV and WA-PAV BYDV isolates using about five *R. padi*/plant, infested with about five virus-free *R. padi*, or caged without aphids.

^{2/}Values are the means of 5 plants/cultivar/treatment.

^{3/}Values followed by the same letter are not significantly different at P=0.05 by the LSD method.

^{4/}A (*) indicates that the difference between the no aphids and BYDV inoculated treatments are significant at P=0.05 by the LSD method. Data for roots and shoots were analyzed separately.

^{5/}A (#) indicates that the differences between the aphid-free and virus-free aphid treatments are significant at P=0.05 by the LSD method.

effects with nonviruliferous aphid infestation. Burnett and Gill (1976) failed to detect a difference in the yield of greenhouse-grown Herta barley with two days of feeding by 1, 20 or 100 nonviruliferous R. padi. However these researchers did not investigate the effects of aphid feeding on growth.

Data for root and shoot dry weights of the nonisogenic barley cultivars Pirolina, Unitan, and Coracle at 24 and 42 days post-inoculation are shown in Table 11. These data are similar to those obtained for the isogenics. At 24 days the inoculated susceptible cultivars, Pirolina and Unitan, had suffered dramatic reductions in root but not in shoot weights. Shoot weights of infected plants were not significantly different from the controls. The 42 day data indicate that all cultivars suffered significant reductions in root weight with inoculation, but Coracle, the resistant cultivar, showed the least reduction. Pirolina displayed relatively less root weight loss at 42 days than at 24. This may indicate something of a recovery from the initial infection. Indeed, some studies have shown that adequate moisture and high fertility can partially overcome the effects of BYDV infection (Oswald and Houston, 1953a; Bruehl, 1961).

Table 11. Root and shoot dry weight of growth chamber-grown Piroline, Unitan, and Coracle barleys 24 and 42 days after BYDV inoculation and BYDV-free tissue of the same age.^{1/}

Cultivar	Days after BYDV inoc.	Tissue	Dry Weight (g)		Inoc. as % of BYDV free plants
			BYDV inoc. plants	BYDV free plants	
Piroline	24	root	.136	.350	38.9* ^{2/}
Unitan	"	"	.148	.352	42.0*
Coracle	"	"	.236	.260	90.8
MEAN			.173a ^{3/}	.321b	
Piroline	24	shoot	.590	.550	107.3
Unitan	"	"	.594	.586	101.4
Coracle	"	"	.505	.460	109.1
MEAN			.562a	.532a	
Piroline	42	root	.476	.664	71.7*
Unitan	"	"	.136	.490	27.8*
Coracle	"	"	.668	.860	77.7*
MEAN			.427a	.671b	
Piroline	42	shoot	1.698	1.540	110.3
Unitan	"	"	.832	1.316	63.2
Coracle	"	"	1.502	1.536	97.8
MEAN			1.344a	1.464a	

^{1/}plants in the 1-2 leaf stage were infested with about five *R. padi* carrying a mixture of MT-PAV and WA-PAV isolates and caged (inoc.) or caged without aphids (BYDV-free).

^{2/}A (*) indicates that the difference between healthy and inoculated values is significant at P=0.05 by the LSD method.

^{3/}values followed by the same letter are not significantly different at P=0.05 by the LSD method.

Data for each tissue type and harvest date were analyzed separately.

Correlation of root and shoot reduction with yield loss

Work by Kainz and Hendrix (1981) indicated that barley plants grown in mist culture suffered reductions in root dry weight, root length, and root numbers when inoculated with BYDV. Although these researchers noted that a resistant barley line was significantly less affected than was Steptoe, a susceptible cultivar, they concluded that the degree of root suppression was "not necessarily correlated with BYDV resistance in barley." However, Grafton et al. (1982) found that winter barleys which were inoculated with BYDV suffered increased winter injury as compared to noninfected plants. It seems possible that reduced root growth may have contributed to the increase in winter injury.

In order to determine the relatedness of root growth and yield, data were compiled on several barley cultivars. The cultivars Pirolina, Unitan, and Coracle were selected for comparison with the isogenics because they had been tested under field conditions similar to those of the isogenics. Yount (1982) inoculated these three cultivars with an isolate similar to the MT-PAV isolate used in the field inoculations of the isogenics in 1984. He found that inoculated Pirolina, Unitan, and

Coracle yielded 9.0%, 39.5%, and 94.0% of noninoculated control plants respectively.

The results of correlation calculations are shown in Table 12. The data for 24 day root weight shows highly significant ($P=0.01$) correlation with yield. These data indicate that a significant correlation does not exist between the stunting of shoot growth by BYDV infection under growth chamber conditions and the yielding ability of that cultivar in the field. Indeed, the lack of a statistically significant relationship between leaf discoloration, foliar stunting and field performance has been demonstrated by other workers (Jones and Catherall, 1970a). However, the surprisingly good correlation between root growth of inoculated cultivars under controlled conditions and yield may offer a means of preliminary screening of germplasm for BYDV tolerance. Planting a large number of lines in containers, inoculating seedlings with BYDV, and assessing root growth at about three weeks could allow winter screening of advanced barley lines or cultivars prior to spring planting.

Table 12. Correlation of root and shoot dry weight with grain yield of seven barley cultivars inoculated with BYDV.^{1/}

Cultivar ^{2/}	<u>Inoculated as % of noninoculated control</u>				
	grain yield	24 day roots	24 day shoots	42 day roots	42 day shoots
Atlas 68	95.4	76.5	101.7	87.3	87.2
Atlas 57	9.5	34.2	96.0	14.1	68.6
CM67	107.8	79.6	84.5	69.4	94.9
Ca. Mariout	8.2	44.4	78.3	63.4	77.3
Piroline	9.0	38.9	107.3	71.7	110.3
Unitan	39.5	42.0	101.4	27.8	63.2
Coracle	94.0	90.8	109.1	77.7	97.8
Correlation ^{3/}	-	.943**	.120	.558	.304

^{1/}Plants in the 1-2 leaf stage were inoculated with a mixture of MT-PAV and WA-PAV isolates of BYDV. Shoot dry weights were determined 24 or 42 days after inoculation. Values are means of five plants/cultivar/time. Yield data were obtained from replicated field plots inoculated with PAV-like isolates in the 3-5 leaf stage.

^{2/}Yield data on Piroline, Unitan, and Coracle from Yount, 1982.

^{3/}A (**) indicates a highly significant (P=0.01) correlation.

Electron microscopy of BYDV infected root tissue

Studies involving electron microscopic observations of BYDV infected tissue have largely focused on the occurrence of virus particles in phloem cells of greenhouse grown oat leaf tissue (Gill and Chong, 1975, 1976, 1979, 1981). Jensen (1969) observed root and shoot tissue from BYDV infected Black Hulless barley, but the major emphasis was on leaf tissue.

The electron microscope (EM) studies described herein were undertaken in order to provide additional evidence that BYDV was indeed the cause of the yellowing and stunting of plants following aphid infestations in 1983. Observations of virus particles in tissue of symptomatic, noninfested plants would strengthen the argument that these symptoms were indeed virus induced and not due to other causes (Bruehl, 1961). The EM studies were also used to compare root tissue from BYDV inoculated resistant and susceptible isogenic barley cultivars.

The first root tissue samples sectioned and observed with the EM were obtained from barley roots 32 days after infestation with viruliferous aphids. These plants had been grown in replicated field trials in 1983. Sections were obtained of root tissue from inoculated plants of

Atlas 68, California Mariout (CM), and Coast Black oats (CBO).

Ultrathin sections of CM tissue revealed masses of virus particles in companion, phloem parenchyma, and pericycle cells. In addition, small "clumps" of virus particles were observed in sieve tube members. Cell types were identified from works by Heimsch (1951), Esau (1957a, 1957b), Bonnett (1968), Evert (1977), and Danilova and Telopova (1980).

Virus particles were identified based on size, morphology, and location in the tissue. The particles were found to be 21-25nm in diameter, round, often "hollow" in appearance, and seemed to be confined to the phloem areas. Figure 1 shows a mass of virus particles in CM67 root tissue. These characteristics fit the reported physical properties of BYDV particles (Rochow, 1970; Jensen, 1969; Matthews, 1982).

When tissue samples from 42 days post-inoculation were sectioned, the material proved to be of better quality than the 32 day material. Observation of tissue sections from inoculated Atlas 68, Atlas 57, CM67, CM, and CBO revealed the presence of virus particles in all cultivars except Atlas 68. Virus particles were observed in sieve tube members, companion cells, parenchyma, and

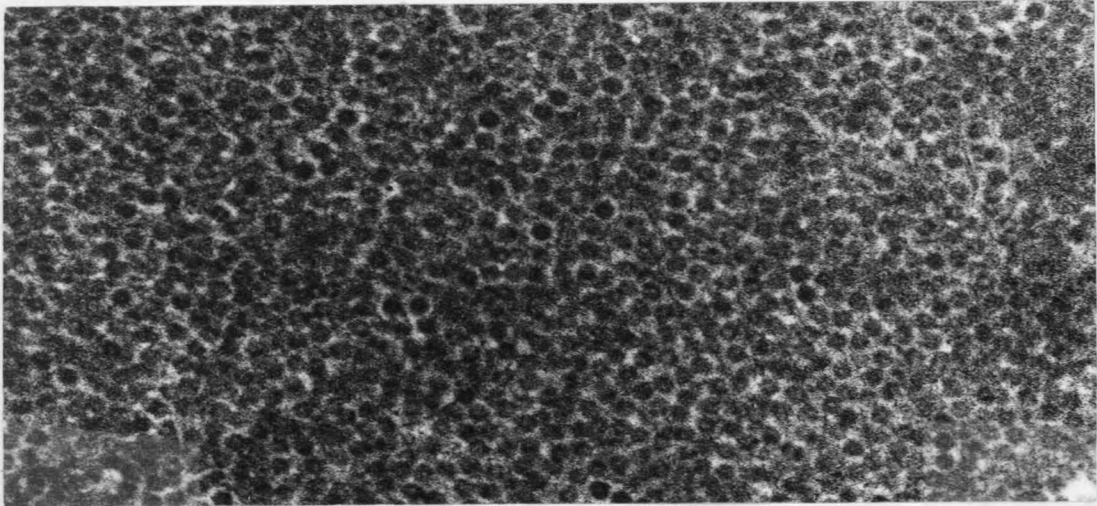


Figure 1. Electron micrograph of particles of barley yellow dwarf virus (BYDV) in root tissue of BYDV inoculated, field grown CM67 barley. Photographed using a Zeiss EM10. 126,000 x.

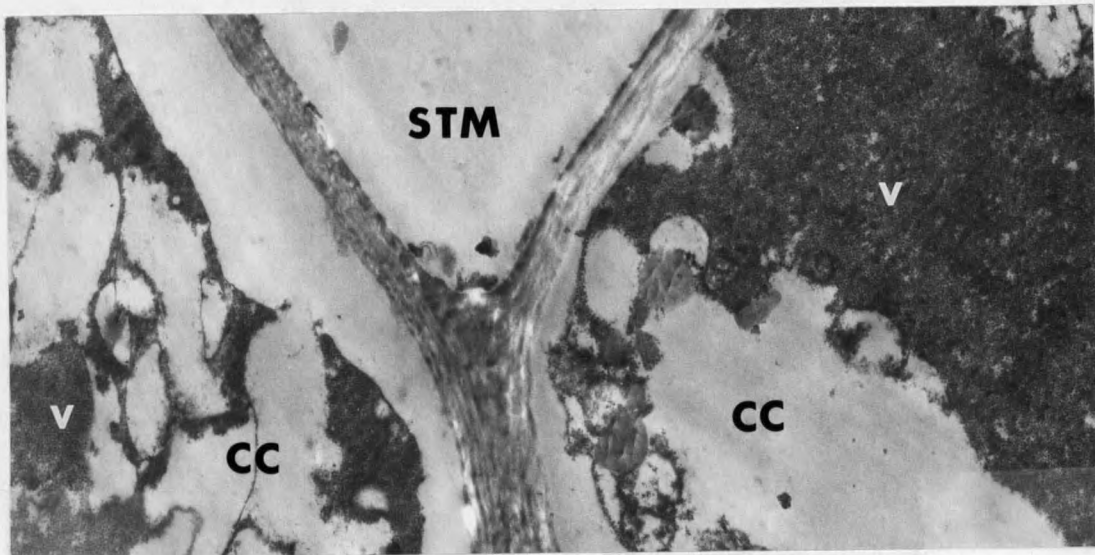


Figure 2. BYDV particles in root tissue of field grown CM67 barley. Photographed using a Zeiss EM9S2. 17,5000x. CC = companion cell, STM = sieve tube member, V = virus particles.

pericycle cells of Atlas 57. In CM67 virus particles were seen in companion cells, parenchyma, and pericycle cells. Figure 2 shows BYDV particles in companion cells of CM67. In the 42 day post-inoculation samples, virus particles in CM were confined to pericycle cells.

In addition to observations of tissue from BYDV inoculated plants, ultrathin sections were obtained from noninoculated Atlas 68, CM67, and CM. Virus particles were never observed in these tissues, thus supporting the previous identification of the small, isometric objects observed in inoculated plants as BYDV particles.

Root tips were the first growth chamber grown tissues observed. The samples had been excised from the plant and sectioned such that the observed tissues were from approximately 1mm from the root apex. In only one cultivar, Atlas 57, were virus particles observed in root tip tissue. The virus particles were observed in a small loose clump in a cell of unknown type.

Visualization of virus particles in root tip tissue may have been confounded by the relatively undifferentiated nature of the cells in this area (Heimsch, 1951; Esau, 1957a). With the exception of sieve tube members, most cells contained a normal complement of organelles including nuclei, mitochondria,

vesicles, and endoplasmic reticulum with associated ribosomes. In cells containing these various components it would be very difficult to discern a few, or even many, BYDV particles due to their diminutive size. These cytoplasm-filled cells in the root tip were in contrast to the previously observed field grown tissue from 10-15mm from the root tip. Cells in the field grown material were generally devoid of cell contents except for a few membrane fragments, occasional mitochondria, and the infrequent occurrence of nuclear material in some of the companion cells and parenchyma.

Observation of root tissue 10-15mm from the root tip of inoculated container grown plants revealed the presence of virus particles only in CM67 and CM. In both of these cultivars virus particles were observed in companion cells and parenchyma, but only in CM were particles observed in sieve tube members. Virus particles occurred as small clumps rather than the large masses observed in the field grown material. Perhaps the lower light intensity and different environmental conditions in the growth chamber as compared to the field resulted in reduced virus concentration (Endo, 1957; Catherall and Hayes, 1966).

In summarizing the EM observations it should be mentioned that tissue sections from only 15 inoculated and 11 noninoculated plants were observed. Also, varying numbers of tissue samples from each of these plants were sectioned and observed. Tissue samples from the basal region of the roots were not sectioned. Assuming that the tissue observed was a representative random sample, a few observations are noteworthy.

None of the Atlas 68 tissue observed contained identifiable virus particles, whereas large masses of virus particles were frequently observed in the isogenic cultivar Atlas 57. However, tissue of both CM67 and CM contained masses of virus particles. These results are of interest in light of the recent work of Skaria et al. (1984a). These researchers noted in shoot tissue of PAV inoculated resistant cultivars Atlas 68, CM67, and Prato, less viral antigen was detected by ELISA than in inoculated plants of the susceptible isogenic cultivars Atlas 57, CM, and Briggs respectively. The results of Skaria et al. (1984a) indicate Yd2 may reduce BYD severity by reducing virus replication. Electron microscopic observations of CM67 and CM failed to show a difference in the relative amount of virus present in these two cultivars. In contrast, virus particles were

commonly observed in Atlas 57 but not in Atlas 68. Also, virus particles were never observed in sieve tube members of Atlas 68 or CM67, but were occasionally observed in sieve tube members of Atlas 57 and CM.

Of additional interest were the observations of virus particles in pericycle cells of inoculated Atlas 57, CM67, and CM. To the author's knowledge this is the first report of the presence of BYDV particles in pericycle cells of BYD affected barley. The significance, if any, of this infection has not been determined.

CHAPTER 5

SUMMARY

A six-rowed spring barley population has been developed which exhibits a high level of tolerance to BYDV. Tolerance to BYDV is evidenced by reduced chlorosis development in a large percentage of plants in the population following BYDV inoculation. Tolerance to BYDV was attained by incorporation of four sources of the Yd2 gene. Recently the curator of the National Small Grains Collection in Beltsville, MD, assigned the designation Composite Cross XLIV to this population (D. H. Smith, personal communication).

Composite Cross (CC) XLIV was developed using male sterile facilitated recurrent selection. Parental lines consisted of CC XXXIIIA and B, CC XLIII, and Sutter barley. CC XXXIIIA and B was chosen because it contains four sources of Yd2 and is highly tolerant to BYDV. The second parent, CC XLIII, possesses a high level of resistance to scald and net blotch, but also contains two sources of Yd2. Genetically CC XLIII is very diverse and contains many agronomically acceptable cultivars in its background. Sutter was chosen as a parent due to its high level of tolerance to BYDV and good agronomic

characteristics. Sutter also possesses some resistance to scald, net blotch, and powdery mildew.

The F_1 seed from crosses of the above populations was grown in Arizona in 1983 and outcrossed seed was collected from male sterile plants. Outcrossed seed was planted in Bozeman and the plants were inoculated with BYDV. Following roguing of severely chlorotic plants, comprising about 16% of the population, bulk harvested seed was returned to Arizona. Collection of outcrossed seed and BYDV inoculation were repeated in 1984. Severely chlorotic plants comprising about 40% of the population were removed in 1984. Remaining plants were bulk harvested. Approximately 340 heads from highly tolerant plants had been removed from the population prior to harvest. Seed from these heads will be planted in Riverside and Davis, CA and selections of tolerant, agronomically promising plants will be made in 1985.

Comparisons of the 1983 and 1984 populations indicate that the 1984 population suffers less reduction in yield, 1000 kernel weight, and hectoliter weight following inoculation with BYDV. The 1984 population also developed less foliar discoloration than did the 1983 population, implying selection for increased tolerance to BYDV was effective.

In addition to development of the population, effects of BYDV infection on resistant and susceptible isogenic barley cultivars have been studied. Atlas 68, Atlas 57, CM67, and California Mariout (CM) were the cultivars tested. The susceptible cultivars Atlas 57 and CM suffered dramatic reductions in yield, number of tillers per plant, seed per head, 1000 kernel weight, hectoliter weight, and plant height. Isogenic cultivars with Yd2 (Atlas 68 and CM67) were much less affected by BYDV infection.

The effects of BYDV infection of root and shoot growth were studied using the isogenics and three additional barley cultivars; Pirolina, Unitan, and Coracle. The data indicate that susceptible cultivars suffer greater reduction in root and shoot dry weight than do the resistant cultivars. Correlation was highly significant between root dry weight of container grown plants 24 days after BYDV inoculation, and the yield of these seven barley cultivars in the field. There was no correlation between shoot growth and yield.

Electron microscopic observations of root tissue from BYDV inoculated plants revealed the presence of BYDV particles in phloem tissue and pericycle cells of Atlas 57, CM67, and California Mariout. Observations of tissue

from the isogenic pair of cultivars CM67 and California Mariout failed to reveal differences in the relative amount or frequency of virus particles detected in these two cultivars. Virus particles were frequently observed in Atlas 57, but were not detected in its isogenic Atlas 68. However, virus particles were not observed in the sieve tube members of resistant cultivars, but were occasionally observed in sieve tube members of susceptible cultivars.

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