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Chapter 10

Fusarium Head Blight of Barley: Impact, Epidemics, Management, and Strategies for Identifying and Utilizing Genetic Resistance

Brian J. Steffenson

Fusarium head blight is one of the most devastating and insidious diseases of barley (*Hordeum vulgare*). It is caused by a number of different *Fusarium* species (e.g., *F. graminearum* [telomorph: *Gibberella zeae*], *F. culmorum*, *F. avenaceum* [telomorph: *Gibberella avenacea*], *F. sporotrichioides*, and *F. poae*) (Abramson et al., 1998; Chen et al., 1982; Clear et al., 1996; Koizumi et al., 1991; Martin et al., 1991; Mihuta-Grimm and Forster, 1989; Parry et al., 1995; Perkowski et al., 1995; Salas et al., 1999; Sturtz and Johnston, 1983), many of which are known to produce various mycotoxins that are hazardous to humans and animals (Abramson et al., 1998; Joffé, 1986; Perkowski et al., 1995, 1997b; Salas et al., 1999; Usleber et al., 1996). Head blight has been a relatively minor and sporadic disease problem of barley in the USA and other countries for many years. Over the past decade, however, it has re-emerged as the most important factor reducing the yield and quality of the crop in several production areas of North America, South America, Europe, and Asia. The head blight epidemics of the 1990s in the upper Midwest region of the USA were particularly devastating and caused severe economic losses, grain processing problems for producers and end-users alike, food/feed safety concerns, and human hardship. Because of head blight, the upper Midwest region is no longer considered a reliable producer of premium malting barley, and imports from Canada have risen dramatically to meet the needs of the brewing industry.

The number of studies on head blight of wheat far exceeds the number on barley. This is largely due to the greater economic importance of wheat as a crop, but also to the greater significance of head blight on wheat over barley. Although wheat and barley are attacked by the same head blight pathogens, they differ in their response to the disease. In general, barley is less susceptible to head blight than wheat. This difference is most apparent in susceptible wheats where the pathogen can spread from a single infection focus and kill the entire spike. Extensive disease spread in barley spikes is rare. Head blight also impacts the yield of wheat more than barley, but the opposite is true with regard to mycotoxin accumulation. High levels of mycotoxins can accumulate in the outside tissues (i.e., lemma and palea) of florets. Because the lemma and palea are retained on barley kernels through harvesting and end-use processing, mycotoxin levels are generally higher than in wheat where the seed hull is shed during threshing. Several spike morphology (e.g., row type, kernel density, and spike angle) and agronomic traits (e.g., heading date and plant height) may

affect the level of head blight occurring on barley. Of particular note is the row type character (two- vs. six-row) in barley, which does not exist in wheat. Two-rowed types generally exhibit less head blight than six-rowed types. Moreover, the most resistant barleys identified thus far are all two-rowed types. It is not known whether the lower head blight severity in two-rowed types is due to linkage with resistance genes or possibly pleiotropy. The same is true for other spike morphology and agronomic traits. Like wheat, no immunity to head blight has been identified in barley. However, several sources of partial resistance are available. Genetic and molecular mapping studies have shown that head blight resistance is controlled by a number of loci with relatively small effects. Molecular marker assisted selection may be an important tool for hastening the development of barley cultivars with head blight resistance.

This chapter reviews pertinent literature on the pathology of head blight of barley, synthesizes the latest research results, and assesses prospects for disease management through the development of resistant cultivars.

Impact of the Disease on Barley Producers and Users

Head blight is very important to producers because it can significantly reduce both the yield and quality of the crop. Infections occurring on plants at flowering or the early stages of kernel development can cause yield losses approaching 100% because most spikelets will be sterile and the kernels extremely thin. In contrast, infections occurring during the late stages of kernel development will often have little impact on yield. Precise yield loss estimates are difficult to obtain for head blight in the field because disease control in fungicide-treated plots is often incomplete, and the presence of other foliar diseases may confound the results. In controlled field inoculation tests, infection by *F. graminearum*, *F. culmorum*, and *F. sporotrichioides* resulted in yield reductions of 41%, 47%, and 42%, respectively (Perkowski et al., 1995). These three *Fusarium* species also caused a 15 to 18% reduction in kernel number and a 31 to 36% reduction in thousand-kernel weight. Similar results were obtained for barley inoculated with two different mycotoxin-producing isolates of *F. graminearum* in the field (Perkowski et al., 1997b). Since head blight pathogens attack the part of the plant (i.e., the kernels) that is ultimately utilized for food and feed, even minor infections can have a marked impact on quality. This is especially true with regard to the mycotoxins produced by many head blight pathogens. Mycotoxin contamination of grain is the most insidious aspect of this disease. Many producers have experienced the frustration of having raised a "good" barley crop with high yield, test weight, and plump kernels only to have their grain severely discounted at the elevator because of mycotoxin contamination. In addition to income losses for mycotoxin contamination, barley producers also can suffer grain quality discounts for low test weight, a high percentage of thin kernels, and a high incidence of blighted kernels (US Department of Agriculture [USDA]). Head blight also can affect

seed quality. Producers who save seed infected with *Fusarium* for the sowing of next season's crop may experience reduced seed germination and a higher incidence of seedling blight and root rot (Christensen, 1963; Dickson, 1942).

Head blight is a great concern to the end-users of barley, not only to the malting and brewing industries, but also to companies involved in utilization of the crop for animal feed and human food. Maltsters will not buy barley that is severely affected with head blight because it will often have lower kernel plumpness, malt extract, and germination, which are important malting quality factors (see Ch. 15 by Schwarz). Additionally, infection may contribute to excessively high levels of total wort (soluble) nitrogen and free amino nitrogen (FAN) in malt. Mycotoxins are of paramount importance to maltsters because most brewers will not purchase malt with detectable levels (above 0.5 ppm) of deoxynivalenol (DON), one of the most important mycotoxins found in grain throughout the world (Tanaka et al., 1988; Ch. 6 by Mirocha). Brewers are concerned about *Fusarium*-infected malt because it can contribute to off-flavors in beer, various processing problems in the brew-house, and beer gushing (Schwarz et al., 1996; Ch. 15 by Schwarz). Moreover, mycotoxins such as DON may ultimately end up in beer (Schwarz et al., 1995b; Ch. 15 by Schwarz). Although the brewing industry is concerned about possible human health risks posed by mycotoxins such as DON, their biggest problem may be one of public perception. If the public perceives that a "toxin" might be present in beer, the fallout from such negative publicity would have devastating consequences for the industry.

Companies utilizing barley for human food (flour, soups, etc.) have many of the same concerns about mycotoxins as the brewing industry. Since most food companies use pearled or hulless barley in their products, they can sometimes utilize barley grain with DON levels that are unacceptable to the brewing industry. Most of the DON in barley kernels is present in the hull; thus, the use of pearled kernels (Clear et al., 1997) or hulless cultivars (Clear et al., 1997; Steffenson et al., 1996b) will significantly reduce the level of this mycotoxin. The US Food & Drug Administration (FDA) established an advisory tolerance limit of 1 ppm DON in food products (Trucksess, 1995). Thus, most food companies will only buy barley grain that can be converted to flour or pearls meeting the FDA tolerance limit. In addition to the specification for DON, many food companies selectively source barley that is plump, sound, and free of blight. Head blight can adversely affect each of these quality factors (i.e., kernel size, integrity, and health), resulting in the grain being unusable for various food products.

The acute and chronic effects of DON ingestion in humans are not well documented. Anecdotal information suggests that it may be associated with outbreaks of "red-mold" toxicoses in people who ingested *Fusarium*-infected grain in Japan (rice), China (maize and wheat), and India (wheat) (Beardall and Miller, 1994; Kuiper-Goodman, 1994). These people experienced the acute toxicosis symptoms of nausea, vomiting, diarrhea, abdominal pain, headache,

dizziness, and fever (Kuiper-Goodman, 1994). In mice, chronic ingestion of DON may contribute to auto-immune-like effects, which are similar to human immunoglobulin A (IgA) nephropathy (Rotter et al., 1996). It is clear that additional toxicology studies are warranted to assess the potential of DON, as well as other mycotoxins, in causing disease in humans.

In the animal feed industry, barley quality specifications are not as stringent as in the brewing and food sectors. Nevertheless, standards are required for test weight and, in some cases, DON concentration in grain. Several studies have demonstrated that head blight can markedly reduce test weight and kernel weight (Dickson, 1942; Perkowski et al., 1995; Perkowski et al., 1997b) and lead to concomitant losses in the feed value factors of digestive starch energy, crude protein, and crude fat (DiCostanzo et al., 1994; Ch. 15 by Schwarz). Additionally, *Fusarium*-infected grain (barley, maize, etc.), along with its associated mycotoxins, can cause health problems (e.g., mycoses and mycotoxicoses) when fed to some farm animals, particularly swine (DiCostanzo et al., 1994; Joffé, 1986; Trenholm et al., 1994). Feed refusal, vomiting, and hyperestrogenism are some of the symptoms exhibited by swine fed with *Fusarium*-infected grain containing low to moderate levels of DON (Joffé, 1986; Trenholm et al., 1994). In contrast, ruminant animals generally are not affected by barley grain containing DON. Anderson et al. (1996) fed gestating heifers, lactating heifers, and feedlot steers rations of barley containing 9 to 13 ppm of DON and found no negative effects on the animals.

Symptoms of the Disease and Signs of the Pathogen

Barley spikes completely enclosed within the flag leaf sheath or "boot" are protected from infection by *Fusarium*. Florets can be infected as soon as the spikes emerge from the flag leaf sheath (Prom et al., 1999). Heavily infected spikes darken and appear compressed because the spikelets are small and press tightly to the rachis (Dickson, 1956). Individual kernels infected with *Fusarium* are usually discolored tan to dark brown, especially at the base (Dickson and Mains, 1929; Dickson, 1942; Steffenson, 1998). Head blight symptoms can often be confused with kernel blights caused by other pathogens such as *Cochliobolus sativus* and *Alternaria* species (Christensen, 1963; Dickson, 1956; Mathre, 1997; Miles et al., 1989); thus, isolation tests are often required to determine the organism associated with the disease.

In addition to these symptoms, signs of the pathogen also can be observed on barley kernels. A pinkish to salmon-colored mass of fungal mycelium and conidia can form on infected kernels under some environmental conditions (Dickson and Mains, 1929). In some instances, blue-black perithecia of the *G. zeae* (perfect) stage will develop on the surface of kernels (Dickson, 1942; Mathre, 1997).

Distribution and Importance of Head Blight on Barley

Regions Where Head Blight is Endemic and Severe Epidemics have Occurred

Head blight is known to be endemic and severe on barley in northeast Asia. During the early part of the 20th century, head blight was considered one of the most destructive diseases of cereals in the Pacific coast region of Siberia (Atanasoff, 1920). The same is true today as barley is widely cultivated in the region and is often severely infected with the disease (O. Afanasenko, personal communication). In China, head blight is considered the most important disease of barley and wheat in the lower Yangtze River Valley and is found as far west as Shaanxi, Ningxia, and Qinghai Provinces and as far north as Heilongjiang Province (Cook, 1981; Ch. 11 by Bai et al.). From 1950 to 1990, seven severe and 14 moderate epidemics occurred on wheat (Wang, 1997; Ch. 11 by Bai et al.) and likely to some degree on barley as well, in the Yangtze River Valley. In Japan, head blight is a major disease of barley, and farmers experience severe epidemics every 11 years on average (Vestal, 1964). In 1963 and 1998, head blight was especially widespread and severe (Ban, 2001; T. Ban, personal communication). The 1963 epidemic affected almost every barley production area of the country (over 400,000 Ha or 72% of the production area) causing yield losses of over 96% in some fields. In 1998, yield losses in two-rowed and hulless barley were estimated at 67% and 96%, respectively, in the Kyushu district of southwestern Japan. In South Korea, head blight occurs on barley nearly every year, although only three major epidemics have occurred since the turn of the century—in 1901, 1928, and 1963 (Vestal, 1964). The last epidemic was particularly notable because of its severity and impact on people. At that time, South Koreans depended heavily on barley as their major food grain between the time when the previous season's rice supply had run out and the new rice crop was harvested. In 1963, head blight destroyed over one half of South Korea's barley crop (Vestal, 1964) causing yield losses of 80 to 100% in some locales (Kim et al., 1993). In response to this serious situation, emergency shipments of grain were imported to prevent food shortage problems. Epidemics of head blight continue to plague barley in the southern provinces of South Korea (W.J. Lee, personal communication). In the Western Hemisphere, head blight is endemic on cereals in the United States (Atanasoff, 1920; Dickson, 1942; Dickson and Mains, 1929; McMullen et al., 1997; Ch. 1 by Stack), and severe epidemics have periodically occurred on barley (see next section).

Regions Where Head Blight is Present and Occasional Outbreaks have Occurred

The regions of the world where head blight of barley occurs have been poorly documented. This may be due to the low incidence and sporadic nature of the disease, a lack of knowledge concerning the identity of the disease (especially during the early part of the 20th century), the dearth of published information on head blight outbreaks, and perhaps an unwillingness of officials

to report the disease because of the possible effect on exports and public perceptions of a safe food supply. In some cases, information on the distribution and severity of head blight can be obtained indirectly from data on the frequency of *Fusarium* species isolated from harvested barley seed (e.g., from state seed testing laboratories) (Crosier and Waters, 1959; Gordon, 1959; Ichinoe et al., 1985; Koizumi et al., 1991; Salas et al., 1999), mycotoxin surveys of grain samples (Gareis et al., 1989; Kim et al., 1993; Perkowski et al., 1997a; Schwarz et al., 1995a; Tanaka et al., 1988; Yoshizawa and Jin, 1995), and outbreaks of primary gushing in breweries (Casey, 1996).

Because of the importance and wide cultivation of wheat, far more information is available on the distribution of and losses to head blight. Parry et al. (1995) give a detailed summary of the geographical distribution of head blight in small grain cereals, primarily wheat. In most areas where head blight of wheat is a continual problem, there likely will be some infection on barley if the crop is grown to any extent in the region; however, this assumption may not hold true for all cereal production areas because barley is generally less susceptible than wheat and can escape infection with its early maturity.

In Europe, head blight is widely distributed (Cassini, 1981; Maric, 1981; Parry et al., 1995) and sometimes severe on wheat from the United Kingdom in the west (Bennett, 1930; Parry et al., 1995) to the Ukraine and Baltic states in the east and from Scandinavia in the north (Skadhauge et al., 1997) to Italy, Yugoslavia, and Bulgaria in the south (Maric, 1981; Parry et al., 1995). In many of these European countries, head blight of barley also may be present when moist weather conditions occur during the flowering and heading period (Maric, 1981; Skadhauge et al., 1997). This supposition is supported by data on the presence of Fusarium mycotoxins in barley grain from some countries (e.g., Gareis et al., 1989; Hietamiemi and Kumpulainen, 1991; Tanaka et al., 1988; Tanaka et al., 1990). Head blight can be an occasional problem on barley in western France (O. Robert, personal communication) and has emerged in the 1990s as an important disease in Poland (the Lublin and Zamosc regions) (Perkowski et al., 1995; Perkowski et al., 1997a, 1997b; J. Chelkowski, personal communication) and the Czech Republic (J. Spunar, personal communication). In many European countries, barley is treated with fungicides to reduce foliar diseases such as powdery mildew, net blotch, and leaf rust. Some of these fungicides also can reduce head blight infection; thus, the potential impact of the disease on barley in Europe is probably greater than actually occurs.

In South America, head blight has caused significant losses on barley over the last decade in the Rio Grande do Sul region of Brazil (G. Arias, personal communication). It also has been an occasional problem in Ecuador, southern Colombia (H. Vivar, personal communication), and Uruguay (S. German and S. Stewart, personal communication). Head blight is sometimes found on barley in New Zealand (Sayer, 1992) and is extremely rare and isolated in Australia (W.J.R. Boyd, L. Burgess, D. Moody, H. Wallwork, and G. Wildermuth,

personal communication). In countries surrounding the Mediterranean Sea (i.e., from Morocco to Egypt in north Africa; from Portugal to Greece in southern Europe, and parts of Turkey, Syria, Lebanon, and Israel) and in South Africa where barley is grown in winter rainfall areas, head blight appears to be rare and of little significance (W. Boshoff, J. Molina Cano; J. van Leur, and H. Toubia-Rahme, personal communication). Head blight can become an important problem in some of these areas if highly favorable weather conditions prevail or risky management practices are used. For example, head blight outbreaks of wheat were recorded in New South Wales, Australia in 1999 as a result of unusually wet weather (Manning et al., 2000) and in South Africa in 2000 because of rotation with maize under sprinkler irrigation (Z.A. Pretorius, personal communication). In North America, head blight has emerged since 1993 as the most important disease of barley in the southern part of Manitoba in Canada (Abramson et al., 1998; Clear et al., 1996; McCallum et al., 1999; Tekauz et al., 1995, 1996) and the high plateau region of central Mexico (Vivar, 1996) in addition to the Upper Midwest region of the United States (Steffenson, 1998; Steffenson, 1999) (see next section).

Head Blight of Barley in the United States

Barley was introduced into the United States by the first settlers on the east and west coasts. Major centers of barley production arose later in both New York and California (Weaver, 1950). After the late 1850s, barley production started to increase substantially in the Midwestern states of Ohio, Michigan, Illinois, Indiana, Iowa, Wisconsin, and Minnesota (Weaver, 1950). It was in this region that head blight was first widely reported on barley, although it likely was present earlier in the New England and Mid-Atlantic states. Dickson (1942) stated that head blight of barley was most severe in the more humid and southern areas of barley production in the Midwest, although periodic outbreaks of the disease did occur during wet seasons in the typically drier prairie regions of northwestern Minnesota and the eastern Dakotas. Head blight was considered rare or not present in the semi-arid plains and inter-mountain regions of the west (Dickson, 1942).

The devastating head blight epidemic of wheat in 1919 (see Ch. 1 by Stack) also caused heavy losses in barley across a wide area (Johnson et al., 1920; Mundkur, 1934; Weniger, 1923). In this epidemic, one million bushels of barley and rye were lost in North Dakota alone (Weniger, 1923). Between 1928 and 1937, head blight caused localized losses in barley almost every year in the Midwest and in some eastern states, except 1934 and 1936, which were drought years (Dickson, 1942). Annual average losses for this period were greatest in states with the largest barley production (Minnesota, Wisconsin, Iowa, Illinois, and the Dakotas) ranging from 101,000 bushels for South Dakota to 590,000 for Minnesota (Dickson, 1942). The head blight epidemics of 1928 (Burnett and Reddy, 1937; Christensen et al., 1929; Dickson and Mains, 1929; Mundkur, 1934; Weaver, 1950), 1932 (Mundkur, 1934), and 1935 (Dickson,

1942; Burnett and Reddy, 1937) were particularly severe on barley. In spring 1928, barley cultivars were widely planted in the southern Corn Belt states to replace winter wheat acreage that had been severely winter-killed (Dickson and Mains, 1929; Ch. 1 by Stack). These barley cultivars were not adapted to this region and flowered during a period of humid weather, which favored infection and resulted in one of the most severe epidemics ever reported in barley. Losses were variable across the Midwest (Mundkur, 1934), but ranged up to 20% in Indiana (Mains et al., 1929). The 1928 epidemic was a watershed event, because it was the primary factor leading to the virtual elimination of barley production in the southern part of the Corn Belt states (Harlan, 1934). Head blight also contributed to the decline of barley cultivation in the eastern and central Corn Belt states (Burnett and Reddy, 1937; Wiebe, 1979) as well as southern Minnesota (Christensen, 1963; Ch. 1 by Stack) and Wisconsin (Steffenson, 1998); however, other factors such as a succession of seasons with low crop yields, spot blotch epidemics (caused by *C. sativus*), and competing crops (especially hybrid maize) also led to this trend (Weaver, 1950).

Perhaps nowhere has barley production been so closely tied to head blight as in Iowa. During the early part of the 20th century, most barley in Iowa was cultivated in rotation after maize, and it was a common practice not to plow under maize stubble prior to sowing barley (Burnett and Reddy, 1937). As Weaver (1950) states:

> In Iowa, a state lying almost entirely within the Corn Belt, scab plays an important, and sometimes dominant, role in determining the size and value of the barley crop in any particular series of years. The most striking feature brought out by a line graph showing the total acreage devoted to barley in this state, is the remarkable succession of high and low points occurring at regular four or five year intervals. For two or three years the state as a whole may be relatively free from serious attacks of the *Gibberella* fungus, and the barley acreage steadily increases. Then a season follows in which the critical combination of weather conditions at heading time is extremely favorable to the growth of the scab-producing fungus, and the previously somewhat latent reservoir of disease, almost omnipresent throughout the corn lands of the state, provides the basis for a widely destructive epidemic outbreak. Seasons follow in which a greatly reduced acreage is sown to barley, and then as the epidemic passes, expansion begins again and the cycle is repeated. Between 1900 and 1930, the barley crop in Iowa passed through this complete series of events at least six times.

After World War II, barley production became centered in northwestern Minnesota and the eastern Dakotas in the upper Midwest and Montana, Idaho, and Washington in the west. The upper Midwest states, in particular, developed a reputation as the leading center for premium malting barley production in the United States. This reputation was tarnished by the series of severe head blight epidemics starting in 1993.

Head blight epidemics in the upper Midwest during the 1990s. During the first four decades of the 20th century, head blight was rare on barley grown in the western Dakotas, but occurred periodically in epidemic proportions in the eastern Dakotas and northwestern Minnesota during years of excessive precipitation (Dickson, 1942). From the mid 1940s to the early 1990s, minor epidemics of head blight occasionally occurred on wheat in the region (Wilcoxson et al., 1988), but they never impacted barley to any great extent (R.D. Wilcoxson, V.D. Pederson, and R.G. Timian, personal communication). Thus, no effort was made to breed barley cultivars specifically for resistance. The severe epidemics of the 1990s forced breeders and pathologists to redirect efforts into ameliorating the impact of this devastating disease on cereal crops. The first of these major epidemics occurred in 1993 and took most producers and scientists by surprise. Above normal levels of precipitation in July (the time when the crop was heading) produced conditions that favored the development and spread of head blight throughout the upper Midwest (Dill-Macky, 1997; McMullen et al., 1997; Steffenson, 1998), especially the Red River Valley area of northwestern Minnesota, eastern North Dakota, northeastern South Dakota (Salas et al., 1999), and southern Manitoba, Canada. Analysis of grain samples from the Midwest Barley Quality Survey by North Dakota State University (NDSU) revealed that head blight and DON were widespread in barley across the region. Mean head blight severity and DON concentration ranged from 8% and less than 0.5 ppm in northwestern North Dakota to 39% and 7.9 ppm in northeastern South Dakota (Salas et al., 1999). Average barley yields in northeastern North Dakota dropped from 75 bushels/acre in 1992 to 45 bushels/acre in 1993, largely because of head blight (McMullen et al., 1997). Over the entire region, yield losses in barley in 1993 were estimated at 1.6 million metric tons (McMullen et al., 1997) with a value of about $122 million. Head blight was so severe in some fields that growers abandoned their crops and burned them. In fact, about 500,000 acres or 17% of the 2.9 million planted barley acres in North Dakota were not harvested in 1993 (Anonymous, 1999).

Head blight epidemics occurred repeatedly during the succeeding seven seasons (1994-2000) with the Red River Valley region again being most affected (Salas et al., 1999; Tekauz et al., 1996; B.J. Steffenson, unpublished data). Mean head blight severity and DON concentration within crop reporting districts of the Red River Valley ranged from 16 to 51% and 3.9 to 21.8 ppm, respectively, in 1994; 12 to 27% and 3.3 to 10.8 ppm in 1995; 12 to 39% and less than 0.5 to 8.3 ppm in 1996 (Salas et al., 1999); 22 to 40% and 1.2 to 7.0 ppm in 1997; and 10 to 20% and 1.0 to 6.6 ppm in 1998 (B.J. Steffenson, unpublished data). The US General Accounting Office estimated that about $200 million in revenues were lost by barley farmers in North Dakota alone from 1993 to 97 (Anonymous, 1999). About 70% of the revenue losses ($139 million) was due to a reduction in yield, and about 30% ($61 million) was due to price discounts for DON contamination. For 1993 to 1998, a net revenue loss of $406 million was estimated by J. Mittleider of the North Dakota Barley

Council (Windels, 2000) for the tri-state area of Minnesota, North Dakota, and South Dakota, including losses from nonharvested acres. In addition to barley, the hard red spring and durum wheat crops were likewise devastated by these epidemics. Cumulative economic losses of over $1.1 billion were estimated for the two wheat crops in the tri-state region from 1993 to 99 (Anonymous, 1999; Johnson et al., 1998; B.J. Steffenson, unpublished data). The upper Midwest was not the only region affected. Indeed, head blight re-emerged with a vengeance across North America in the 1990's causing epidemics on cereals in 26 states and four Canadian provinces (McMullen et al., 1997; Windels, 2000). Total losses caused by head blight for all cereals in the USA are estimated at nearly $3 billion in the 1990s (Windels, 2000). Considering the devastating effects on crop yield and quality, the possible health risks associated with mycotoxins in infected grain, the catastrophic economic losses incurred by producers and endusers, and resulting human hardship, the head blight epidemics of the 1990s must be considered among the worst agricultural disasters ever recorded for small grain cereal crops in the history of the United States.

The head blight epidemics of the 1990s radically changed the fabric of the barley industry and agricultural communities in the tri-state region (Windels, 2000; Ch. 18 by McMullen). North Dakota had previously been the leading state in the nation for production of premium malting barley. Prior to 1993, 60 to 70% of North Dakota's barley crop was sold at a premium malting barley price (Anonymous, 1999), which can range up to 63% higher than the feed barley price (J. Mittleider, personal communication). After the 1993 epidemic, only 30% of the state's barley crop was sold at a malting price. With eight consecutive years (1993-2000) of head blight and DON contamination problems, the upper Midwest was no longer considered a reliable producer of premium malting barley for domestic markets (Steffenson, 1998). Consequently, brewers in the USA looked elsewhere to source malting barley with very low or nondetectable levels of DON. Most of them looked north of the border to Canada. During the head blight epidemics of 1993 to 1997, barley exports from Canada into the United States increased about four-fold. By 1997, one quarter of all malting barley used by brewers in the United States came from Canada—a five-fold increase over the pre-epidemic years (Anonymous, 1999). Generally, a shortage of premium domestic malting barley would result in an increase for the price paid per bushel of any remaining available stocks; however, this was not the case during the epidemic years. Instead, the price paid for malting barley actually decreased, primarily from the glut of barley being imported from Canada. Average net cash returns per farm in most of the counties hardest hit by the disease (i.e., northeastern North Dakota and northwestern Minnesota) dropped below the federal poverty income guide for a family of five (Windels, 2000). Many farmers were faced with the heart-wrenching decision of remaining on the farm and going deeper into debt under the ever-growing uncertainties of the grain markets or giving up their beloved livelihood and liquidating what was

left of their remaining assets. The Farm Service Agency (FSA) of the USDA estimated that about 768 farmers (~14%) in the major barley producing regions of North Dakota stopped farming, primarily from the consequence of the epidemics (Anonymous, 1999). This led to record increases in the number of farm auctions during the epidemic years, but in many cases, local customers did not have the cash to purchase any farm equipment (Windels, 2000; Ch. 18 by McMullen), and so it was sold to out-state buyers. The economic impact of head blight surged through the small agricultural communities causing businesses such as implement dealers to corner cafes to close their doors. The small farming towns in the region had changed dramatically because a fungal disease destroyed their crops.

Factors Contributing to Head Blight Epidemics

As with all plant disease epidemics, the key factors contributing to the head blight outbreaks were: 1) widespread cultivation of susceptible cultivars, 2) abundance of virulent pathogen inoculum, and 3) favorable weather conditions for disease development.

First, most of the commercial barley cultivars grown worldwide are fairly susceptible to head blight. This is especially true for six-rowed cultivars grown in the upper Midwest region of the USA and southern Manitoba in Canada. Prior to the 1990s, only a few barley improvement programs in the world were breeding for resistance to head blight, and no highly resistant cultivar with outstanding yielding ability and quality had been released. Thus, without adequate resistance, the crop is at risk when inoculum is present and the weather conditions are favorable.

Second, abundant pathogen inoculum (primarily ascospores of *G. zeae* [Khonga and Sutton, 1986]) is often present in the field (Dickson and Mains, 1929). Fusarium head blight pathogens overwinter primarily in crop debris (Sutton, 1982) of wheat, barley, and maize. The current abundance of inoculum in many areas of the world is related to the increased use of conservation tillage (low or no-till) practices that leave increased amounts of debris on the soil surface (Dill-Macky, 1997; Dill-Macky and Jones, 2000; Steffenson, 1998; Wilcoxson et al., 1988).

Third, the weather conditions were favorable for disease development. In general, head blight is favored by warm and moist conditions (Dickson, 1942; Sutton, 1982), although the severe 1993 epidemic in the upper Midwest occurred during one of the coolest summers on record (Enz and Brenk, 1994). This is not surprising given that inoculum production (i.e., development of ascospores and conidia) and infection can take place over a fairly wide range of temperatures from 16 to 31°C and 20 to 30°C, respectively (Sutton, 1982). The importance of moist weather conditions in contributing to epidemics cannot be understated. In the semi-arid, inter-mountain cereal-growing regions of the USA (i.e., Colorado, Idaho, Montana, and Wyoming), head blight of barley is very

rare (Dickson, 1942; B.J. Steffenson, unpublished data), even though *Fusarium* inoculum is present in fields and the pathogens can be isolated from symptomless kernels (B. Salas and B.J. Steffenson, unpublished data). In 1982 and 1984, head blight epidemics occurred in sprinkler-irrigated fields of barley and wheat in Idaho (Mihuta-Grimm and Forster, 1989). These epidemics coincided with unusually frequent rainfall during anthesis and early heading of the crops. Significantly, nearby rill-irrigated fields of barley and wheat had little or no infection. Thus, in the semi-arid regions of Idaho, head blight epidemics may occur when sprinkler irrigation, coupled with frequent rainfall, provide a wetness period of sufficient duration for *Fusarium* infection (Mihuta-Grimm and Forster, 1989).

Strategies for Head Blight Management

Four primary strategies for management of head blight include biological control, cultural practices, fungicide application, and disease resistance. A discussion of each strategy and its potential for management of head blight in barley is given next.

Biological Control

The biological control strategy involves the application of other microorganisms (usually bacteria) to the spikes of plants to limit Fusarium infection and its adverse effects on barley. This strategy requires extensive in vitro screenings to identify aggressive microorganisms that can compete well or be strongly antagonistic against *Fusarium*, studies on how to best deploy the biological control agent on the crop at the proper time, and rigorous field tests for assessing the efficacy of the agent in suppressing disease development and production of mycotoxins like DON. Moreover, this biological control agent must not contribute to any adverse effects on barley during the malting or brewing process. Despite these challenges, management of head blight by biological agents merits more attention based on some recent successes in wheat (see Ch. 14 by Luz et al.)

Cultural Practices

In most regions, head blight epidemics are initiated by inoculum (mostly ascospores) produced in crop residues on or just below the soil surface (Khonga and Sutton, 1986). Any measure that reduces this colonized crop residue also will reduce inoculum potential of the pathogen. Plowing (Dill-Macky and Jones, 2000) and/or burning can significantly reduce the amount of *Fusarium*-infected crop residue in the field. However, these practices have drawbacks. For example, moldboard plowing can leave the soil surface bare, leading to greater erosion and loss of valuable soil moisture. Moreover, plowing is a costly energy- and time-consuming practice. The burning of fields is detrimental to air quality, and some states (e.g., California) are implementing a ban on its practice.

Another effective cultural management practice is crop rotation. The rotation of cereals with host crops that are not congenial to *F. graminearum* (e.g., soybeans, dry beans, sunflowers and canola) can reduce the level of inoculum available for initiating epidemics (Dill-Macky and Jones, 2000).

Fungicide Application

Several fungicides are registered for use on barley and have some efficacy against head blight (Jones, 2000; Ueda and Yoshizawa, 1988). Unfortunately, most of these compounds have not been highly effective or consistent in their control of the disease in barley (Jones, 2000; Martin et al., 1991; Wilcoxson, 1996; McMullen et al., 1999, pp. 69-70 in proc. National Fusarium Head Blight Forum, 5-7 December 1999, Sioux Falls, SD). Moreover, fungicide treatments have not reliably reduced DON concentrations in grain to levels acceptable to the brewing industry (Jones, 2000; J. Lukach and B.J. Steffenson, unpublished data). With new developments in fungicide chemistry, as well as refinements in application technology (Hofman et al., 2000; Wilcoxson, 1996; Ch. 13 by Mesterházy), it should be possible to achieve better disease control and mycotoxin reductions with fungicide treatments.

Other drawbacks to fungicides as a management strategy include additional costs to producers, possibile fungicide residues on harvested grain, and development of fungicide-tolerant strains of *Fusarium*. Given the low commodity prices for barley and the relatively high cost of fungicide applications, few producers in the USA can afford extra inputs without a guarantee for a good return. Fungicides are most effective against head blight when applied to the crop after complete head emergence (McMullen et al., 1999, pp. 69-70 in proc. National Fusarium Head Blight Forum, Sioux Falls, SD, 5-7 December 1999). Applications of fungicides late into the heading stage can sometimes lead to residues in harvested grain. This is a potential problem for producers because the malting industry will not purchase barley with any pesticide residues. Finally, widespread and continuous use of fungicides for head blight control could lead to the development of tolerant *Fusarium* strains. In Japan, Wu and Takeda (1997) found a fairly high in vitro mutation rate ($\sim 10^{-5}$ to 10^{-6}) for topsin tolerance in several *Fusarium* isolates. Moreover, topsin tolerance in these *Fusarium* isolates paralleled tolerance to another systemic fungicide, benomyl. Fungicides should be used judiciously and in combination with other management strategies to minimize the development of tolerant isolates of *Fusarium*.

Disease Resistance

The deployment of resistant cultivars is the most cost effective and environmentally sound means of managing head blight, especially when coupled with the inoculum reduction practices of crop residue management and rotation. Unfortunately, development of a resistant malting barley cultivar with all of the desirable agronomic and quality traits may take up to 15 years because of the

complexity of the task. In the barley improvement programs of the upper Midwest region, thousands of plants from many segregating populations are evaluated in the field each year to select breeding lines with outstanding agronomic characters (e.g., high yield potential, lodging resistance, plump kernels, etc.) and disease resistance (to spot blotch, net blotch, stem rust, etc.) (Steffenson, 1999). Lines selected for superior performance from these trials are evaluated for various malting quality traits (e.g., malt extract, protein, diastatic power, etc.). From these tests, candidates for release are selected and evaluated for more than 20 quality traits over several years (i.e., pilot and plant scale tests) before they are recommended for malting by the American Malting Barley Association (AMBA) (Wych and Rasmusson, 1983). Many of the important agronomic and quality traits required in a malting barley cultivar are under polygenic control (Hayes et al., 1993). The selection and transfer of these many genes into a single "package" called a malting barley cultivar will become even more difficult by having to incorporate head blight resistance, which is controlled by a number of genes with relatively small effects (de la Peña et al., 1999; Ma et al., 2000; Zhu et al., 1999).

Screening and Breeding Barley for Head Blight Resistance

Difficulties in Screening Barley for Head Blight Resistance and Low DON Concentration

In addition to the difficulties involved in handling the complex genetic trait of head blight resistance in breeding programs, almost every facet of screening for resistance and low DON concentration in barley is time consuming, labor intensive, and expensive. Since head blight is a disease that attacks the spikes, barley plants must be grown to the adult plant stage for evaluation. These evaluations require about 3 to 4 months to complete in the field or greenhouse. In contrast, resistance testing to many other barley diseases (e.g., stem rust, leaf rust, powdery mildew, net blotch, spot blotch, etc.) can be completed on seedlings in about 3 or 4 weeks (Steffenson et al., 1995; Steffenson et al., 1996a). Head blight resistance screening in the field is labor intensive, requiring multiple inoculations, detailed disease assessments, and processing of grain samples for various postharvest analyses. Mycotoxin assays and labor contribute to the high cost of these screening evaluations. An explanation of the steps and difficulties involved in screening barley germplasm for resistance and low DON concentration in the NDSU barley improvement program is given next.

Field screening for resistance. Field evaluations are essential for assessing the natural development of head blight and accumulation of DON in barley accessions. However, disease nurseries are difficult to establish, requiring large amounts of pathogen inoculum, several successive inoculations during the season, staking of plants if lodging is a potential problem, and an overhead irrigation system to provide moist conditions for *Fusarium* infection.

Agronomic and morphological traits can affect the amount of disease occurring on barley (Couture, 1982; Shands, 1933, 1935; Steffenson et al., 1996b); therefore, assessments of heading date, plant height, spike morphology, etc. are made in conjunction with head blight assessments. The most accurate way to assess head blight severity in the field is to actually count the number of infected kernels and divide that sum by the total number of kernels within the spike (Prom et al., 1996). This calculation allows for direct comparison of disease severities between two- and six-rowed barley accessions. Head blight is typically assessed on 10 to 20 spikes from each barley entry depending on the level of infection in the nursery (Steffenson, 1998). The magnitude of this task becomes evident when one considers that thousands of lines are screened per season.

Greenhouse screening for resistance. Evaluation of barley germplasm for head blight resistance in the greenhouse requires a large amount of bench space and about the same amount of time (3 to 4 months) to complete as field evaluations. Thus, the number of lines that can be evaluated per season is limited. To obtain consistent results, all plants should be grown as uniformly as possible and inoculated at the same time and developmental stage. This requires several sequential plantings of the test accessions and ties up limited greenhouse space for much of the autumn/winter disease screening season.

DON assays. The amount of DON in barley grain is of paramount importance to the malting and brewing industries. When barley accessions from field or greenhouse inoculation tests are mature, all spikes are hand-harvested, threshed, cleaned, and weighed in preparation for DON assays. At NDSU, barley samples are analyzed with a gas chromatograph with an electron capture detector (GC-ECD) (Tacke and Casper, 1996) to accurately assay DON concentrations. Given the number of samples (~7,000) screened each year, the cost of breeding for low DON concentration can be extremely high (Steffenson, 1998).

Mycoflora analyses. Several different *Fusarium* species cause head blight of barley (Abramson et al., 1998; Salas et al., 1999). Moreover, other common pathogens such as *C. sativus* and *Alternaria* species can cause kernel blight symptoms that are similar to those caused by *Fusarium*. To determine the identity of kernel blight pathogens in both inoculated and uninoculated nurseries in the field, mycoflora assays are routinely made from random accessions in each nursery. Mycoflora assays are time consuming and laborious because 50 to 100 kernels from each sample are plated onto nutrient media, and resulting fungal colonies are identified to species by microscopy (Salas et al., 1999).

Gushing assays. Gushing is a major concern of brewers, but the factor(s) causing it have not been fully elucidated (Ch. 15 by Schwarz). As advanced breeding lines with head blight resistance become available, it may be necessary to assay them for their propensity to cause gushing after they are exposed to *Fusarium* inoculum in the field, malted, and made into beer. These assays (see

Ch. 15 by Schwarz), like the other protocols used in screening, are laborious, time consuming, and expensive.

Germplasm Screening Efforts

Evaluation of barley cultivars, landraces, and miscellaneous accessions. Several research groups in the USA and East Asia have evaluated barley germplasm collections for reaction to head blight. Over 100 sources of resistance were identified from these studies, but none exhibited immunity to the disease (Chen et al., 1991; Gocho and Hirai, 1987; Shands, 1933, 1935; Takeda and Heta, 1989; Zhou et al., 1991; U.M. Scholz and B.J. Steffenson, unpublished data).

In the USA, the search for barley accessions with head blight resistance began in earnest shortly after the devastating epidemic of 1928 under the direction of J.J. Christensen at the University of Minnesota and R.G. Shands at the University of Wisconsin. Christensen et al. (1929) and Immer and Christensen (1943) reported that the six-rowed accessions CI 2492, CI 1613, Improved Manchuria, Korsbyg, and Peatland and the two-rowed accession Svansota exhibited some resistance to head blight in Minnesota. Shands extensively evaluated germplasms beginning in 1930 (Shands, 1933, 1935, 1940) and confirmed the resistance of Svansota (Shands, 1933) and that of Chevron (Shands, 1939), a line selected from the same seed lot where Peatland was derived. After the early 1940s, research on head blight of barley waned in the USA because the disease became less important. This situation changed after the severe epidemic of 1993 as new research initiatives were launched to identify head blight resistance and breed resistant barley cultivars.

In the upper Midwest region of the USA, six-rowed barley is the preferred type for malting. Unfortunately, six-rowed types are generally more susceptible to head blight than two-rowed types (Chen et al., 1991; Shands, 1933; Takeda and Heta, 1989; Zhou et al., 1991; B.J. Steffenson, unpublished data). Several excellent sources of resistance have been identified in a two-rowed background (Prom et al., 1996, 1997; Takeda and Heta, 1989); however, in breeding, it has been difficult to recover six-rowed lines with the same level of resistance as the two-rowed sources. To identify additional sources of resistance in a six-rowed background (other than Chevron and Peatland), researchers at NDSU evaluated over 8,200 accessions of six-rowed spring barley from the USDA Small Grains Germplasm Collection in the field (Scholz et al., 1999; U.M. Scholz, B.J. Steffenson, and R.D. Horsley, unpublished data). Only 13 accessions were identified with resistance equal to or greater than that of Chevron (i.e., <25% infection). These resistant accessions originated from Canada, China, Ethiopia, Romania, and the USA.

In Japan, several research groups have evaluated barley germplasm for head blight resistance. Heta and Hiura (1963) identified 23 resistant accessions from over 1,500 screened. Gocho and Hirai (1987) evaluated 1,181 two-rowed accessions and 1,384 six-rowed accessions for resistance in the field and/or by

artificial inoculation in the greenhouse. The two-rowed accessions Isaria, Old Cromarty (both from Europe), Kanto Nijo 2 (Japan), Lichiti (Africa), Samaria (Oceania), and Svansota (United States) were most resistant. The six-rowed Japanese accessions Bizen Wase, Baitori 105, Banjaku, Ao Hadaka, and Ariura Hadaka were moderately resistant. Germplasm from Japan and East Asia exhibited the widest range of variation for head blight reaction. Takeda and Heta (1989) evaluated 4,881 barley accessions from around the world for resistance. A high level of variation for head blight severity was detected in the germplasm, but most accessions were susceptible. Overall, two-rowed accessions were more resistant than six-rowed accessions. Resistant accessions were mostly from China, Japan, Korea, and Europe and rarely from Africa and southwest Asia. Twenty-three accessions exhibited consistently low head blight scores of 4 or less (i.e., less than 20% infection) when tested over multiple seasons. All were of the two-rowed type, and nine were previously reported resistant by Heta and Hiura (1963).

In China, Zhou et al. (1991) evaluated nearly 8,000 barley accessions from China and other countries in the field and identified 27 resistant accessions, most of which were from the Yangtze River basin, a region where head blight is endemic. Resistant accessions also were identified from Japan, Sweden, and Denmark. Statistical analysis of data from the entire screened collection indicated two-rowed and hulless types were generally more resistant than six-rowed and hulled types. Chen et al. (1991) screened 4,163 barley accessions from various institutes and universities in China. Fourteen accessions from the Yangtze River basin region were resistant and exhibited less than 5% head blight infection. Overall, hulled types were more resistant than hulless types, and two-rowed types were more resistant than six-rowed hulled or hulless types.

Evaluation of wild Hordeum *species and allied genera. Hordeum vulgare* subsp. *spontaneum* readily hybridizes with barley and thus serves as a primary gene pool for head blight resistance genes. Over 500 accessions of *H. vulgare* subsp. *spontaneum* were evaluated for head blight in the field at Hangzhou, China. Disease severity varied moderately among the accessions, but resistance was not any higher than already reported for two-rowed barley accessions (B.J. Steffenson, unpublished data). *Hordeum bulbosum* or bulbous barley grass also can hybridize with barley, but the identification and characterization of recombinant progeny is not routine (Pickering, 2000). Putative *H. vulgare* × *H. bulbosum* recombinants are initially identified by their unique morphology (e.g., leaf pubescence) or disease resistance in the field. Further confirmation of *H. bulbosum* introgressions is then made by cytogenetic analysis, Giemsa C-banding, isozyme analysis, molecular methods, and/or sequential genomic in situ hybridization (GISH) and fluorescence in situ hybridization (FISH) (Pickering, 2000). *Hordeum bulbosum* was reported to possess resistance to head blight by Xu and Kasha (1992), but details of the evaluation or resistant clones were not given. Several accessions of this species were tested in China, but the extremely late heading of lines precluded assessment of their resistance

Regardless of the specific growth stage chosen, all accessions must be inoculated at the same stage in order for valid comparisons to be made in the level of head blight resistance. This can be done in the greenhouse by staggering plantings of accessions with different heading dates. In field evaluations, this is a continual problem. To reduce the variation associated with differences in flowering time among barley accessions evaluated in the field, Takeda and Heta (1989) developed the "cut-spike" screening technique. For this evaluation, barley accessions are initially grown in uniform plots in the field. As the different lines approach the flowering stage, individual plants are collected by cutting the stems below the second node and placing them in a container with constant overflowing water. The cut-spikes of plants are spray inoculated with a suspension of ascospores, placed in a high humidity (100% RH) environment at 25°C for 2 days, and then incubated under the controlled conditions of a growth chamber. This screening protocol is laborious to perform, but offers the best means of controlling most of the variables affecting this host-parasite interaction. The ICARDA/CIMMYT program also inoculates barley at flowering, but on intact plants in the field using the single floret method (Vivar et al., 1997). The methods used by Takeda and Heta (1989) and Vivar et al. (1997) allow for uniform inoculation of field-grown plants at a specific growth stage; however, a successive series of labor-intensive inoculations are required. The stage at which barley plants are infected cannot be readily controlled using the grain spawn and mist field methods because after the grain and rice substrates are placed in the field, ascospores are released indiscriminately over a period of days. However, the grain spawn and mist field techniques are convenient to use when labor is limited and/or travel time to the nurseries is great (see Ch. 8 by Dill-Macky).

Influence of inoculum concentration. Barley lines with moderately high resistance may exhibit elevated infection levels when subjected to heavy inoculum concentrations. CI 4196 and Chevron are regarded as the most resistant two-rowed and six-rowed barley lines, respectively, in the field (Prom et al., 1997; Steffenson, 1999). In evaluations conducted over 12 stations/year in North Dakota, CI 4196 and Chevron exhibited infection levels ranging from 0 to 7.6% (average = 2.3%) and 0 to 17.8% (average = 3.5%), respectively, using the grain spawn method of inoculation (Prom et al., 1997; Steffenson, 1999; B.J. Steffenson and U.M. Scholz, unpublished data). The corresponding level of disease on the susceptible control Stander was 6.2 to 62.3% (average = 24.9%). In a companion study in South Dakota, the range and average infection levels of CI 4196, Chevron, and Stander were 20.9 to 64.3% (average = 49.1%), 28.2 to 55.8% (average = 37.5%), and 23.3 to 40.4% (average = 35.2%), respectively, when subjected to both the grain spawn and spray inoculation methods (Y. Jin, unpublished data). The high infection levels found on CI 4196 and Chevron in South Dakota were likely due to an excessively high inoculum load (Y. Jin, personal communication). These results indicate that barley accessions should

be screened under moderate disease levels in the field. Control over inoculum levels can be best achieved with the spray or single floret inoculation method.

Infectivity of different propagules. Several studies have been conducted to determine the relative infectivity of various infective propagules, especially macroconidia and ascospores of *F. gramimearum* and *G. zeae*, respectively (see Ch. 8 by Dill-Macky). In single floret inoculations of wheat, the infectivity of the two spore types was quantitatively similar (Stack, 1989). The relative infectivity of macroconidia and ascospores also was compared in spray inoculation tests of Stander barley in the greenhouse and field. Infectivity of macroconidia was statistically similar to ascospores in both the greenhouse (30.4% vs. 33.2%) and field (6.5% vs. 5.4%) (U.M. Scholz and B.J. Steffenson, unpublished data). Thus, the choice of spore type for inoculation may depend largely on the ease by which researchers can produce and handle them.

Infection and incubation period. After inoculations are made in the greenhouse or field, plants are usually provided with a moist period (in dew/mist chambers or by irrigation) to promote infection. The duration of this moist period may affect the level of disease on plants. Moist periods of 16 to 24 hr at 21 to 25°C are usually adequate for obtaining good infection after greenhouse inoculations (B.J. Steffenson, unpublished data). Longer (>30 hr) moist periods can lead to surface mycelial growth of the fungus and subsequent infection by the mycelium growing across kernels in the spike. This type of mycelial infection also has been observed on portions of spikes not fully emerged from the flag leaf sheath and on fully emerged spikes exposed to moist conditions for a prolonged period in the field (B.J. Steffenson, unpublished data). The level of head blight infection induced by mycelium under such conditions is often much higher than that observed on plants under natural epidemics. In the field, differences in ambient weather conditions during the infection and subsequent incubation period (i.e., dry vs. wet and/or low vs. high temperature) may contribute to substantial variation in head blight development on accessions with the same level of resistance, but different maturities. This effect will occur in all field evaluation tests regardless of the inoculation method. Use of the cut-spike evaluation protocol (Takeda and Heta, 1989) should eliminate most of the variation from this factor.

Correlation of results using different inoculation techniques. When barley lines are inoculated using different techniques or under different conditions in separate experiments, the resulting head blight severities are generally not highly correlated. For example, Takeda and Heta (1989) found a fairly weak correlation of 0.62 between disease scores of barley lines in a test using spray-inoculated cut-spikes compared to mist field-inoculated plants. Zhu et al. (1999) compared disease severities of doubled haploid lines from a mapping study of head blight resistance in the Gobernadora/CMB643 population in tests at four locations and with different inoculation methods. Spray and single floret inoculations were used in Mexico (Toluca), whereas the grain spawn method was used in China (Shanghai) and North Dakota (Fargo and Langdon). Results

from the spray inoculation were not significantly correlated with those from the single floret inoculation in Mexico or with those from the grain spawn inoculation at Langdon. Correlations were significant, but very low between the results from the spray inoculation in Toluca and those of the grain spawn inoculation in Shanghai (r = 0.22) and Fargo (r = 0.32). In the comparisons of Zhu et al. (1999), the highest correlation between results with different inoculation methods for the grain spawn inoculation at Langdon and the single floret inoculation at Toluca (r = 0.45). Correlations between results from tests using the grain spawn inoculation at different locations also were statistically significant, but fairly low (r = 0.30-0.60) with the highest value obtained between tests at Langdon and Fargo. From these studies, the choice of inoculation technique clearly can have a marked effect on the relative severities of head blight on barley in different tests. Further research is needed to refine these inoculation methods and reduce variability associated with the interaction of barley and its head blight pathogens.

Comparison of head blight reactions of barley accessions tested in greenhouse and field. The agreement between screening tests conducted in the greenhouse and field is an important consideration for breeders wanting to use the former venue for winter or "off-season" evaluation tests. At NDSU, limited tests were conducted on 12 six-rowed accessions (plus controls) exhibiting the highest level of head blight resistance as identified from the field. In general, accessions with the lowest infection in the field also had low disease levels in greenhouse inoculation tests (U.M. Scholz and B.J. Steffenson, unpublished data). The susceptible and moderately susceptible controls of cultivar Stander and line CI 5414 exhibited high and moderate head blight severities, respectively, in both evaluations. From these preliminary results, the agreement between greenhouse and field evaluations appears to be fairly good in germplasm possessing a high level of resistance.

Resistance to Initial Infection and to Spread

In wheat, Schroeder and Christensen (1963) described two types of resistance to head blight: type I resistance operates against initial pathogen infection, and type II resistance operates against spread of the pathogen in the spike after an initial infection site is established. As mentioned previously, type I resistance is usually measured by spray inoculating spikes with a suspension of macroconidia or ascospores and then assessing the number of infected spikelets. In contrast, type II resistance is usually measured by inoculating a single floret within the center of a spike and then assessing the spread of infection from that initial site (Schroeder and Christensen, 1963). Several additional types of resistance have been proposed, including resistance based on the ability of certain wheats to degrade or conjugate pathogen-produced mycotoxins such as DON or to tolerate high levels of these toxins (Miller et al., 1985; Wang and Miller, 1988). (See also Ch. 3 by Bushnell et al., Ch. 9 by Mesterházy).

Compared to wheat, most barley accessions appear to possess a high level of type II resistance because extensive vertical spread within a spike seldom occurs in the field, even under favorable environmental conditions (B.J. Steffenson, unpublished data). Lateral disease spread does, however, occur rather frequently among the three kernels at a rachis node in six-rowed genotypes (Atanasoff, 1920; B.J. Steffenson, unpublished data). When barley germplasm with a high level of type II resistance is evaluated in the field using the grain spawn or mist field inoculation methods, disease assessments detect mostly type I resistance. Barley accessions with resistance to initial infection have been described in several studies using these inoculation methods (e.g., Takeda and Heta, 1989; Prom et al., 1996, 1997; Scholz et al., 1999).

Capettini (1999) assessed type II resistance of barley in the greenhouse by inoculating plants with the single floret technique and then incubating them at 100% RH for 72 hr. Significant differences were observed for the amount of spread in the spike (this included lateral spread from the central to lateral spikelets) among six-rowed cultivars, suggesting that genetic variability exists for this type of resistance. However, the overall mean number of kernels infected by spread after inoculation was only 7.3 (12% of kernels in a spike); thus, disease spread was not extensive. Cappettini (1999) stated that his results should be interpreted with caution because of the extreme level of variability in the experiments. Additional greenhouse evaluations are needed to fully elucidate the type and level of head blight resistance in barley accessions selected in the field.

As described earlier, assessments for disease spread in the spike are routinely made in the field using the single floret technique in the ICARDA/CIMMYT barley improvement program. From these evaluations, several cultivars (e.g., Shyri and Atahualpa) and derived lines were found to carry resistance to pathogen spread (Vivar et al., 1997). Moreover, several investigators have found that resistance to initial infection is independent of resistance to spread in some barley lines (Cappettini, 1999; Vivar et al., 1997). The possible presence in barley of yet other types of resistance (i.e., those relating to mycotoxins or possibly tolerance to head blight) is currently under investigation at ICARDA/CIMMYT in Mexico (L. Gilchrist, personal communication).

Agronomic Traits and Spike Characteristics in Relation to Head Blight Development

Many factors are thought to influence the development of head blight on barley including the agronomic traits of heading date (Shands, 1933) and plant height (Couture, 1982), and various spike characters such as row type (Shands, 1933), kernel density (Chen et al., 1991; Shands, 1933; Takeda, 1990), and spike angle (Shands, 1935). To assess the possible contribution of such factors to disease development, near-isogenic lines (NILs) for some of the traits were evaluated to head blight in the field (Steffenson et al., 1996b). NILs are pairs of

genotypes that putatively differ for only one trait (i.e., a single gene in many cases). The assumption that NILs differ for only a single trait is greatly oversimplified given the amount of linkage drag that can occur in the development of such lines (Young and Tanksley, 1989). Nevertheless, NILs are the best set of genetic stocks available for studying the effect of a "single trait" in a uniform genetic background. In addition to the NILs, data from molecular marker maps (de la Peña et al., 1999; Ma et al., 2000; Zhu et al., 1999) and conventional genetic analyses (Takeda, 1990) of several barley crosses were investigated to assess the association of various agronomic and spike morphology traits with head blight infection levels.

Heading date and flowering time: early versus late. The association of late heading date and low head blight infection in barley was reported in the early screening studies of Shands (1933). In a comparison of NILs for heading date, Steffenson et al. (1996b) found that the level of infection in the early heading NIL was nearly six times greater than the late heading NIL. The association of these traits also was confirmed in two molecular mapping studies involving the resistant parent Chevron, as most of the QTLs identified for heading date were coincident with QTLs contributing to head blight reaction (de la Peña et al., 1999; Ma et al., 2000). As in the NIL study of Steffenson et al. (1996b), late heading progeny exhibited lower infection. Due to low map resolution in the molecular mapping studies, it was not possible to determine whether the coincident QTLs were due to linkage (i.e., heading date and head blight reaction are controlled by different QTLs located in close proximity to each other on the same chromosome) or pleiotropy (i.e., QTLs for heading date also control the distinct and seemingly unrelated phenotype of head blight reaction). If pleiotropy is indeed involved, the observed trend may be from the shorter exposure of late heading accessions to inoculum, thereby resulting in less infection. It is interesting to note that the opposite trend occurs in China where spring type barleys are sown in the autumn. In the Yangtze River Valley region, early-maturing cultivars generally sustain less infection than late-maturing cultivars because they escape the warm, humid spring weather that is favorable for head blight development. In China, early maturity is a character that is exploited in barley improvement programs to escape head blight infection (Cook, 1981).

In a study of flowering time, Takeda and Heta (1989) found no correlation with head blight assessment scores using the spray (on cut-spikes) (-0.14) or mist field (-0.09) inoculation methods on 258 barley accessions. Flowering time among the 23 most resistant accessions differed by almost 2 weeks; however, the analysis of variance revealed that flowering time was not a significant factor influencing the head blight score.

Height: Short versus tall stature. In general, tall-statured plants exhibit lower levels of head blight infection than short-statured plants. This commonly observed effect may be partly due to the greater distance of spikes on tall plants from the most concentrated strata of inoculum originating on the soil surface.

Couture (1982) found a strong negative correlation of -0.90 between plant height and head blight infection in barley. A similar trend was found in three molecular mapping populations as plant height QTLs coincided with at least one QTL for head blight reaction (de la Peña et al., 1999; Ma et al., 2000; Zhu et al., 1999). Again, the low resolution of the molecular maps did not distinguish between linkage or pleiotropy. Recent data from a Foster (medium statured, susceptible) × CI 4196 (tall statured, resistant) cross suggest that linkage may be involved because several of the tallest progeny in the population exhibited very high levels of head blight (R.D. Horsley and B.J. Steffenson, unpublished data).

Lodging: Resistance versus susceptibility. Lodging can have a pronounced effect on head blight infection (Shands, 1933). Lodged plants will exhibit higher disease severities because they remain wetter from dew or rainfall for a longer period of time and are closer to the inoculum source on the soil surface. This effect can even occur in the most resistant germplasm. In a row of accession CI 4196 (one of the most resistant lines identified to date), half of the plants lodged for several days in the field before being staked upright. These lodged plants exhibited head blight severities ranging from 20 to 35% compared to less than 5% for plants that did not lodge (B.J. Steffenson, unpublished data).

Row type: Two-rowed versus six-rowed. Several extensive germplasm screening studies have shown that six-rowed genotypes generally exhibit higher levels of infection than two-rowed genotypes (Chen et al., 1991; Shands, 1933; Takeda and Heta, 1989; Zhou et al., 1991). This same trend also was noted in anecdotal observations of barley cultivars in commercial production. In the Yangtze River Valley of China, head blight severity was correlated with row type in barley; six-rowed cultivars exhibited higher disease severities and sustained more damage than two-rowed cultivars (S. Ding, personal communication). In Japan, six-rowed cultivars sustained much higher yield losses than two-rowed cultivars during the severe head blight epidemics of the 1960s. The susceptibility of these six-rowed cultivars was a major factor contributing to the change to two-rowed cultivars in the Kyushu region of southern Japan (T. Ban, personal communication).

The *vrs1* locus, which controls the development of lateral spikelets, and the *int-c* locus, which controls the size and fertility of lateral spikelets, are the primary determinants of two-rowed and six-rowed spikes in barley. Most six-rowed cultivars have the *vrs1.a* and *Int-c.a* alleles and three fertile spikelets at each rachis node, whereas most two-rowed cultivars have the *Vrs1.b* and *int-c.b* alleles and one fertile spikelet at each rachis node (Davis et al., 1997). Takeda (1990) investigated the level of head blight infection in progeny derived from two-rowed × six-rowed parents. Two-rowed progeny from the F_2, F_3, and F_4 generations exhibited the lowest disease severity (4.5, 3.9, and 4.0, respectively) followed by the heterozygotes (5.0, 4.3, and 4.5), then by the six-rowed progeny (5.5, 5.9, and 6.1). In a comparison of NILs differing for row type, Steffenson et al. (1996b) found that six-rowed NILs had 1.1 to 1.3 times more head blight

infection than two-rowed NILs. The higher disease levels in six-rowed accessions may be due to the higher density of fertile spikelets. This architecture may provide a more favorable environment for infection and facilitate disease spread, especially from spikelet to spikelet within a rachis node as is frequently observed in the field (Atanasoff, 1920; B.J. Steffenson, unpublished data).

F. graminearum readily infects and colonizes sterile spikelets, regardless of whether the condition is caused by environmental stresses or genetic factors (B.J. Steffenson, unpublished data). Extensive areas of infected sterile tissue may contribute to increased infection of fertile spikelets. Gilchrist et al. (1996) reported an association between reduced lateral glumes (i.e., deficiens types), which are sterile and head blight resistance. Zhu et al. (1999) corroborated this initial observation by finding coincident QTLs for lateral floret size and head blight reaction in the Gobernadora/CMB643 population.

Kernel density of spike: Low versus high. Steffenson et al. (1996b) found that NILs with dense spikes exhibited 1.1 to 1.3 times more head blight infection than NILs with lax spikes in both a two-rowed and six-rowed genetic background. In molecular mapping studies, Ma et al. (2000) and Zhu et al. (1999) found coincident QTLs for kernel density and head blight reaction in the Chevron/Stander (a six-rowed × six-rowed cross) and Gobernadora/CMB643 (a two-rowed × two-rowed cross) populations. If pleiotropy is involved in this case, the higher level of head blight observed in the dense spike accessions may be due to the architecture providing a more favorable environment for infection and spikelet to spikelet spread. It should be noted that the dense spike character will not always contribute to elevated levels of head blight infection since Takeda and Heta (1989) identified a number of highly resistant lines with dense spikes.

Spike angle: Erect versus nodding. Barley can differ greatly with regard to spike angle during grain fill. Some accessions have spikes that remain vertical or nearly so until maturity, whereas others have spikes that nod downward, sometimes to angles exceeding 100° from the vertical. Shands (1935) reported that the nodding spike phenotype was often associated with a lower level of head blight infection. Steffenson et al. (1996b) likewise found that a nodding spike NIL had significantly less (1.4 times) infection than an erect spike NIL. In a cross between Chevron (head blight resistant with an extremely nodding spike) and Stander (head blight susceptible with a semi-erect spike), Ma et al. (2000) identified a single QTL for spike angle that coincided with a QTL for head blight reaction on chromosome 2H. The question of whether these coincident QTLs are due to linkage or pleiotropy is unresolved. If pleiotropy is involved, the higher infection on erect spikes may be due to the greater retention of moisture (in the form of dew or rain) than on nodding spikes, which tend to shed free water more readily.

Hulled/hulless kernels and awn/awnless spikes. The hulled/hulless and awn/awnless traits are also thought to influence head blight infection in barley.

From the evaluation of a large number of barley accessions, Zhou et al. (1991) reported that hulless types were generally more resistant to head blight than hulled types, whereas Chen et al. (1991) reported the opposite. Steffenson et al. (1996b) found the hulled component had higher infection levels than the hulless component in three of five pairs of hulled/hulless NILs tested. Dickson (1942) reported that awnless (hooded) barley types were very susceptible to head blight. The same observation was confirmed in the head blight screening of over 5,000 six-rowed barleys (B.J. Steffenson, unpublished data). This is in contrast to wheat for which awnless types generally have less infection than awned types (Ch. 9 by Mesterházy).

Anther exsertion. Previous studies in wheat have shown that compounds present in anthers are stimulatory to hyphal extension of *F. graminearum* and may lead to greater infection rates (Strange and Smith, 1978; Strange et al., 1978). Thus, plants that do not exsert their anthers, or do so to a limited extent, may sustain less head blight infection as was found for barley by Vivar et al. (1997). Under some growing conditions, barley will undergo anthesis and anther exsertion after the spike has emerged from the flag leaf sheath, whereas in other environments, these events will occur while the spike is still enclosed within the sheath. The selection of lines that flower in the sheath was used as a breeding strategy to avoid head blight infection in China (Cook, 1981), but the results of this effort have not been reported.

Extensive germplasm screening efforts have shown that, in general, tall, late heading two-rowed genotypes with nodding spikes are often more resistant to head blight than short, early heading six-rowed genotypes with erect spikes (B.J. Steffenson, unpublished data). These observations suggest that one or more of these traits may affect the level of head blight infection and therefore contribute to resistance or, perhaps more correctly, disease escape. Molecular mapping studies provided corroborative evidence for these observations as QTLs for heading date, plant height, kernel density, and/or spike angle were coincident with QTLs contributing to head blight reaction and also low DON concentration (de la Peña et al., 1999; Ma et al., 2000; Zhu et al., 1999). To definitively resolve the contribution of various characters to head blight development and the question of linkage or pleiotropy, high-resolution molecular maps for the target regions should be constructed in conjunction with controlled greenhouse disease evaluations. By making several successive plantings of progeny in the greenhouse, it will be feasible to inoculate an entire mapping population in one or two runs, thereby removing the possible confounding effect of heading date on head blight infection. Moreover, by uniformly spraying inoculum directly onto the spike, the possible confounding effect of plant height can be removed.

Pathogenic Variability of Fusarium Head Blight Pathogens and Host-Parasite Interactions

Variability for pathogenicity and virulence in *F. graminearum* and other *Fusarium* species is a major concern to researchers involved in breeding for resistance. The most desirable resistance is that which is effective against all head blight pathogens and their genotypes. Tu (1929) investigated the pathogenic variation of *Fusarium* isolates on both barley and wheat in the field. He made pathogenicity assessments based on the amount of infection induced by each isolate on the two hosts. Form 1 of *F. graminearum* consistently induced twice as much infection as form 3 of this species on barley cultivar Minnsturdi and many of the wheat cultivars tested. Variation for pathogenicity among isolates within *F. culmorum* and *F. avenaceum* also was found. Tu (1929) was one of the first investigators to provide some evidence for pathogenic variation in *Fusarium* head blight pathogens and suggested that this factor be considered when screening for resistance in barley and wheat.

In a more recent study, Takeda and Kanatani (1991) assessed the virulence of 20 *F. graminearum* strains collected from Japan and the USA on 20 selected accessions of barley. Using the spray inoculation method on cut-spikes (Takeda and Heta, 1989), a high degree of variation was found for virulence in the *F. graminearum* isolates and for resistance in the barley accessions; however, no statistically significant fungal isolate × host accession interactions were detected. This suggests the absence of distinct pathotypes of *F. graminearum* and the lack of gene-for-gene interactions. Similar results were found with wheat. From a critical review of the literature, Mesterházy (Ch. 9) concluded that there was no strong evidence for the existence of vertical pathotypes in either *F. graminearum* or *F. culmorum* on wheat.

In another study, Takeda et al. (1995) investigated the host-parasite interactions of *Fusarium* species (104 isolates including *F. graminearum*, *F. avenaceum*, *F. sporotrichioides*, *F. acuminatum*, and *F. culmorum*) on lines of both barley and wheat. Statistically significant interactions for infection rate were detected in the ANOVA for fungal isolate × host (wheat vs. barley), fungal isolate × barley line, and fungal isolate × wheat line interactions, but the magnitude of most effects was very small. Principal component analysis revealed that the first component representing general pathogenicity on wheat or barley accounted for over 89% of the total variation. The second principal component representing specific pathogenicity between wheat and barley accounted for only 5 to 6% of the variation. Although two isolates of *F. graminearum* (SHIN-996 and ARC-2124-1) exhibited much higher pathogenicity on barley over wheat, the second principal component again accounted for only 4% of the total variation. These analyses suggest that host specific variation is very minor in comparison with general pathogenicity.

The relative pathogenicity of *Fusarium* species infecting barley can vary markedly. Salas et al. (1999) inoculated the susceptible barley cultivar Stander

in the field with a composite of three isolates of four different *Fusarium* species. The head blight severity on Stander ranged from 7 to 21% for *F. graminearum*, 4 to 11% for *F. avenaceum*, 2 to 11% for *F. sporotrichioides*, and 3 to 6% for *F. poae* over a 2-year investigation. The relative pathogenicity of the same *Fusarium* species, plus *F. culmorum*, was investigated in another study with Stander in the field. The highest percentages of infection were obtained with *F. graminearum* (27-49%) and *F. culmorum* (20-55%), followed in descending order by *F. avenaceum* (12-19%), *F. sporotrichioides* (6-11%), and *F. poae* (2-4%) (U.M. Scholz and B.J. Steffenson, unpublished data).

Genetics and Heritability of Head Blight Resistance and Low DON Concentration

Several studies on the genetics of head blight resistance have been made in Japan and the USA. Takeda (1990) made a series of crosses between the susceptible two-rowed line CI 3752 or the resistant two-rowed line Russia 6 and several six-rowed lines with moderate levels of head blight resistance. Progeny in the F_2, F_3, and F_4 generations were then evaluated for resistance to initial infection using the spray inoculation method on cut-spikes. The continuous variation observed for disease severity in the F_2 generation suggested that resistance was under quantitative genetic control in each of the populations. Estimates of heritability were made based on the genetic gain/selection differential ratio and were 0.25 and 0.33 for the F_2 to F_3 and F_3 to F_4 selection response, respectively. Parent-offspring correlations also were calculated in the populations. The correlation between the F_3 plant score and F_4 line means ranged from 0.12 to 0.72 with a pooled mean of 0.46 for all five populations. Correlations between F_3 line means and F_4 line means ranged from 0.14 to 0.74 with a pooled mean of 0.51.

In another study, Takeda and Wu (1996) assessed the inheritance of resistance using F_1s from 8 × 8 and 6 × 6 complete diallel crosses. The results indicated that resistance to initial infection was conferred mostly by additive genes, although dominant gene effects also were statistically significant. In some accessions, maternal effects were statistically significant suggesting the possible role of cytoplasmic factors. Narrow and broad sense heritabilities were about 0.4 and 0.4 to 0.6, respectively, based on the variance components. F_1 progeny of the 8 × 8 and 6 × 6 diallel were crossed (i.e., "top crossed") to the susceptible line Ethiopian 402 and the moderately resistant line Harbin, respectively. Data from the diallel and the top crosses indicated that the direction and magnitude of dominant gene effects varied with respect to the parental combinations used. The basis of head blight resistance in this material was considered to be under complex genetic control.

In Minnesota, Capettini (1999) investigated the heritability of overall resistance (i.e., to initial infection and also spread) in four barley populations derived from six-rowed resistant Chevron and GD2-27 and two-rowed resistant Gobernadora and Zhedar 1 in the field. Estimated heritabilities were based on

line means and varied considerably among locations within a year and among years within a location. Across all environments, the highest heritability for overall head blight resistance was observed in the cross involving GD2-27 (0.76), whereas the lowest heritability was observed in crosses involving Chevron and Zhedar 1 (both 0.48). Heritabilities also were estimated for low DON concentration in the grain. The heritability of low DON concentration in crosses involving Chevron and GD2-27 was 0.50 and 0.43, respectively, lower than those found for head blight resistance in the same crosses (Cappetini, 1999). The two-rowed line CI 4196 is regarded as one of the most resistant barley lines to head blight (Prom et al., 1997; Steffenson, 1999; Takeda and Heta, 1989) and exhibits among the lowest levels of DON under moderate disease pressure in the field (Prom et al., 1997; Steffenson, 1999, unpublished data). Urrea Flórez (2000) crossed CI 4196 with the susceptible six-rowed cultivar Foster and found a heritability of 0.65 for overall head blight resistance in progeny using parent-offspring regression. The heritability of DON concentration was much lower at 0.46.

The phenotypic frequency distribution for head blight resistance and DON concentration in the molecular mapping populations investigated by de la Peña et al. (1999), Ma et al. (2000), and Zhu et al. (1999) all suggested quantitative inheritance for these traits. In field evaluations, Ma et al. (2000) estimated the heritability of overall head blight resistance and DON concentration in the Chevron/Stander population to be 0.31 and 0.25, respectively. Zhu et al. (1999) found heritabilities of 0.50 for resistance to initial infection (spray inoculation method), 0.81 for resistance to spread (single floret inoculation), and 0.78 for overall head blight resistance (grain spawn method) in the Gobernadora/CMB643 population evaluated in the field.

From these genetic studies, it is evident that head blight resistance in barley is under polygenic control and its heritability can vary greatly. The wide range of heritability values (0.31 to 0.81) found across studies may reflect differences in the resistant and susceptible parents used, the inoculation method, and the environment. Overall, it should be feasible to develop barley cultivars with head blight resistance from most of the studied sources. The generally lower heritability values obtained for DON concentration across studies indicates that breeding for this trait will be more difficult.

Molecular Mapping of Loci Controlling Head Blight Resistance and Low DON Concentration

The construction of molecular marker maps is the most efficient method for positioning loci controlling head blight resistance and low DON concentration on the barley genome. A summary of information and the chromosomal position of QTLs identified for head blight resistance and low DON concentration are given in Table 1 and Fig. 1. De la Peña et al. (1999) and Ma et al. (2000) developed molecular maps to position loci contributing to both head blight resistance and low DON concentration in crosses with the six-rowed resistance

source, Chevron. In a recombinant inbred line population of Chevron/M69, de la Peña et al. (1999) identified ten QTLs for resistance, three on chromosome 2H, three on chromosome 7H, and one each on chromosomes 1H, 3H, 4H, and 5H. Each QTL was detected in only one of the three environments tested. Seven of the resistance QTLs were contributed by Chevron and three by M69. In one environment where four resistance QTLs were identified, the multi-locus model explained 45.9% of the variance. The highest variance explained by a single resistance QTL was 16.0%. Several, but not all, identified QTLs for head blight resistance coincided with QTLs for low DON concentration. For low DON concentration, two QTLs were identified on chromosome 2H, and one each on chromosomes 5H and 7H. All four were contributed by Chevron. Head blight severity was negatively correlated with heading date and plant height; still, several identified QTLs for head blight resistance mapped independently of QTLs controlling these two agronomic traits (de la Peña et al., 1999)

In a Chevron/Stander doubled haploid population, Ma et al. (2000) identified nine QTLs for head blight resistance on chromosomes 2H (two QTLs identified), 3H (three QTLs identified), 5H (two QTLs identified), 6H, and 7H (Table 1, Fig. 1). All but three of these QTLs were contributed by Chevron. One QTL on chromosome 2H was identified in five of the six environments tested. In contrast, four QTLs were detected in only one environment—three of which were contributed by the susceptible parent Stander. The amount of phenotypic variance explained by a single QTL ranged from 7.4 to 27.4%. Within an environment, the multi-locus models explained from 30 to 43.9% of the phenotypic variance. Nine QTLs were identified for low DON concentration, and all but one (on chromosome 1H) coincided with QTLs for head blight resistance. This result suggests that the same factors contributing to lower head blight infection also contribute to lower levels of DON. As was found in the study by de la Peña et al. (1999), most of the QTLs for head blight reaction and DON concentration coincided with QTLs for heading date and plant height (Ma et al. 2000). Nearly all of the QTLs for head blight resistance identified by Ma et al. (2000) mapped to the same general chromosomal regions as those found by de la Peña et al. (1999). This is not surprising given that both studies used Chevron as the resistant parent. Different results were obtained between the two studies for several of the QTLs contributing to head blight, DON, heading date, and height. This may be due to genotype × environment interactions, genotypic contribution of the susceptible parent, or perhaps the type of population used (recombinant inbred line versus doubled haploid) (Ma et al., 2000).

A third mapping effort was conducted on a doubled haploid population derived from the two-rowed parents Gobernadora and CMB643 (Zhu et al., 1999). In this study, separate tests were made to assess resistance to initial infection and also to spread. QTLs contributing to head blight resistance were detected on every chromosome except 5H; however, most were not significant at P=0.05 (Table 1, Fig. 1). Two QTLs explaining the largest phenotypic variance for resistance to spread were positioned on chromosomes 2H (33% variance)

Table 1. Summary of information on quantitative trait loci (QTLs) identified for Fusarium head blight (FHB) resistance and low deoxynivalenol concentration by molecular mapping in three barley crosses (See Fig. 1 for map of chromosomal positions

Cross[1]	Trait[2]	Chromo-some	Flanking Markers or Closest Marker	No. of Environ. Det./Total[3]	Range in Pheno. Var. Expl. by Indiv. Alleles[3]	Source of Resistance Allele	Coincident Traits
Chevron/Stander	FHB res.	1 (7H)	MWG36b - MWG836	3/6	12.8 - 21.3	Chevron	Heading date
Chevron/Stander	DON conc.	1 (7H)	WG789a - MWG836	4/5	8.9 - 23.1	Chevron	Heading date
Chevron/Stander	FHB res.	2 (2H)	BCD339c - BCD1407	5/6	11.8 - 20.7	Chevron	Height, heading date
Chevron/Stander	DON conc.	2 (2H)	BCD339c - BCD1407	4/5	9.6 - 21.6	Chevron	Height, heading date
Chevron/Stander	FHB resis.	2 (2H)	BCD307b - CDO684b	1/6[4]	27.4[4]	Chevron	Spike density, spike angle
Chevron/Stander	DON conc.	2 (2H)	BCD307b - CDO684b	1/5	18.9	Chevron	Spike density, spike angle
Chevron/Stander	FHB res.	3 (3H)	ABG316a - CDO395	2/6	10.8 - 16.0	Chevron	Height
Chevron/Stander	DON conc.	3 (3H)	ABG316a - CDO395	1/5	8.5	Chevron	Height
Chevron/Stander	FHB res.	3 (3H)	BCD512c - CDO105	1/6	7.5	Stander	Heading date
Chevron/Stander	DON conc.	3 (3H)	BCD512c - CDO105	1/5	7.8	Stander	Heading date
Chevron/Stander	FHB res.	3 (3H)	WG420b - ABC172	1/6	9.5	Stander	None
Chevron/Stander	DON conc.	3 (3H)	WG420b - ABC172	1/5	8.2	Stander	None
Chevron/Stander	DON conc.	5 (1H)	CDO431 - cMWG706	2/5	6.5 - 11.1	Chevron	Spike density
Chevron/Stander	FHB res.	6 (6H)	WG719d - CDO785d	2/6	9.7 - 10.0	Chevron	Height, kernel plumpness
Chevron/Stander	DON conc.	6 (6H)	WG719d - CDO785d	3/5	7.4-11.9	Chevron	Height, kernel plumpness
Chevron/Stander	FHB res.	7 (5H)	MWG502 - BG739	1/6	17.3	Stander	Height
Chevron/Stander	DON conc.	7 (5H)	MWG502 - BG739	1/5	20.1	Stander	Height
Chevron/Stander	FHB res.	7 (5H)	BCD1449 - MWG533a	3/6[4]	7.4 - 26.2[4]	Chevron	Heading date
Chevron/M69	FHB res.	1 (7H)	MWG530 - MWG564	1/3	10.0	Chevron	None
Chevron/M69	FHB res.	1 (7H)	MWG836 - ABG476	1/3	10.0	M69	Heading date
Chevron/M69	DON conc.	1 (7H)	MWG836 - ABG476	1/2	17.0	Chevron	Heading date, height
Chevron/M69	FHB res.	1 (7H)	ABG476 - BCD98b	1/3	0.6	M69	Height
Chevron/M69	DON conc.	2 (2H)	ABC311 - MWG858	2/2	6.3 - 23.8	Chevron	None
Chevron/M69	FHB res.	2 (2H)	ABG459-MWG520a	1/3	7.2	Chevron	None
Chevron/M69	FHB res.	2 (2H)	MWG887-ABC306	1/3	13.5	Chevron	Heading date
Chevron/M69	DON conc.	2 (2H)	ABC306-BCD1087b	1/2	25.8	Chevron	None
Chevron/M69	FHB res.	2 (2H)	KSUF15-ABG497a	1/3	16.0	M69	None
Chevron/M69	FHB res.	3 (3H)	ABC171-CDO395	1/3	8.4	Chevron	None
Chevron/M69	FHB res.	4 (4H)	ABG705b-ABC303	1/3	4.4	Chevron	None
Chevron/M69	FHB res.	5 (1H)	ABG452-ABG74	1/3	7.1	Chevron	None
Chevron/M69	FHB res.	7 (5H)	CDO400-CDO59b	1/3	8.6	Chevron	None
Chevron/M69	DON conc.	7 (5H)	ABC302-MWG503b	1/2	11.2	Chevron	None
Goberna.[5]/CMB643	FHB res.	2 (2H)	MWG503[6]	1/4[7]	33.0[9]	CMB643	Spike density, lat.[10] floret size
Goberna./CMB643	DON conc.	2 (2H)	RZ740[6]	1/2[8]	8[8]	CMB643	Spike density, lat. floret size
Goberna./CMB643	FHB res.	3 (3H)	ABG004[6]	1/4[7]	16.0[11]	CMB643	None
Goberna./CMB643	FHB res.	4 (4H)	CDO542[6]	2/4[7]	4.0 - 12.0[9]	Goberna.	Spike density
Goberna./CMB643	DON conc.	4 (4H)	Phy2[6]	2/2[8]	7 - 13[8]	Goberna.	Spike density, lat. floret size

[1] Data summaries for Chevron/Stander and Chevron/M69 crosses are from Ma et al. (2000) and de la Peña et al. (1999), respectively. These populations were evaluated in the field using grain spawn inoculation or natural inoculum for infection. Since there appears to be a relatively high level of resistance to spread in both sets of parents, the identified QTLs probably describe resistance to initial infection. Data summary for Gobernadora/CMB643 cross is from Zhu et al. (1999). This population was evaluated in the field using grain spawn, spray inoculation, and single floret inoculation. Grain spawn and spray inoculation methods measure, to a large extent, resistance to initial infection, whereas single floret inoculation method measures resistance to spread (Zhu et al. 1999).

[2] Fusarium head blight resistance, deoxynivalenol concentration

[3] Number of Environments Detected/Total: Range in Phenotypic Variation Explained by Individual Alleles

[4] A QTL was revealed in one environment after fixing the effect of the main QTL, then rescanning the genome for other QTLs (Ma et al. 2000).

[5] Gobernadora

[6] Only the single marker closest to the peak QTL value was given in Zhu et al. (1999).

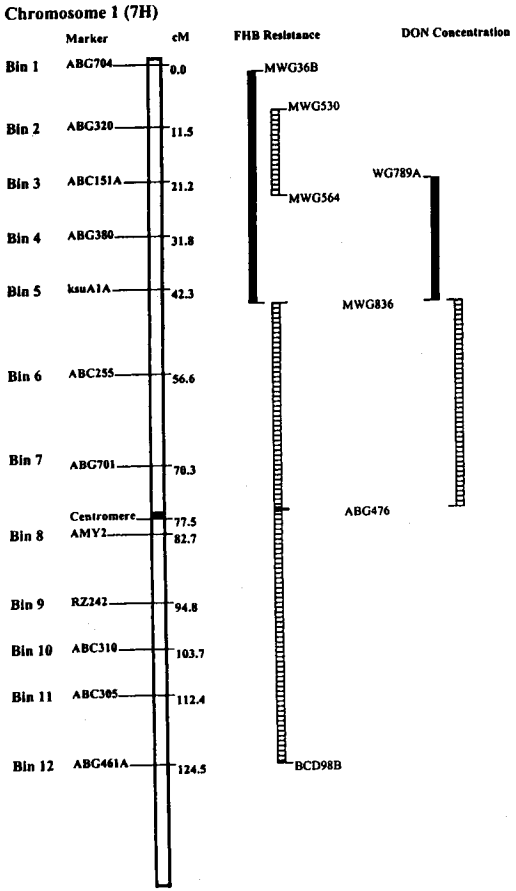
[7] Different inoculation methods were used in the four environments: grain spawn method at two locations in North Dakota and one location in China, the spray inoculation method in Mexico, and the single floret method in Mexico (Zhu et al. 1999).

[8] DON concentration was assessed only in the North Dakota environments where the grain spawn inoculation method was used.

[9] Identified QTL most likely describes resistance to spread based on the single floret inoculation method (Zhu et al. 1999).

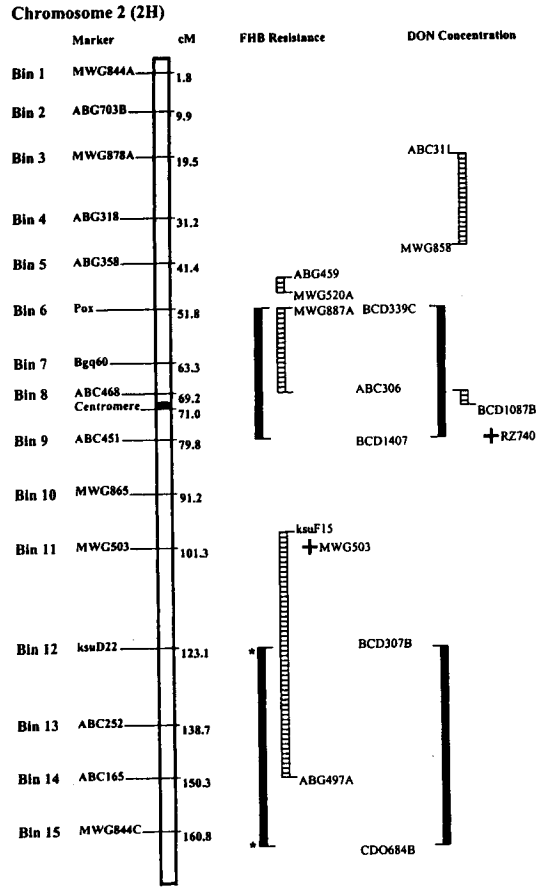
[10] Lateral floret size

[11] Identified QTL confers resistance to initial infection based on the spray inoculation method (Zhu et al. 1999).



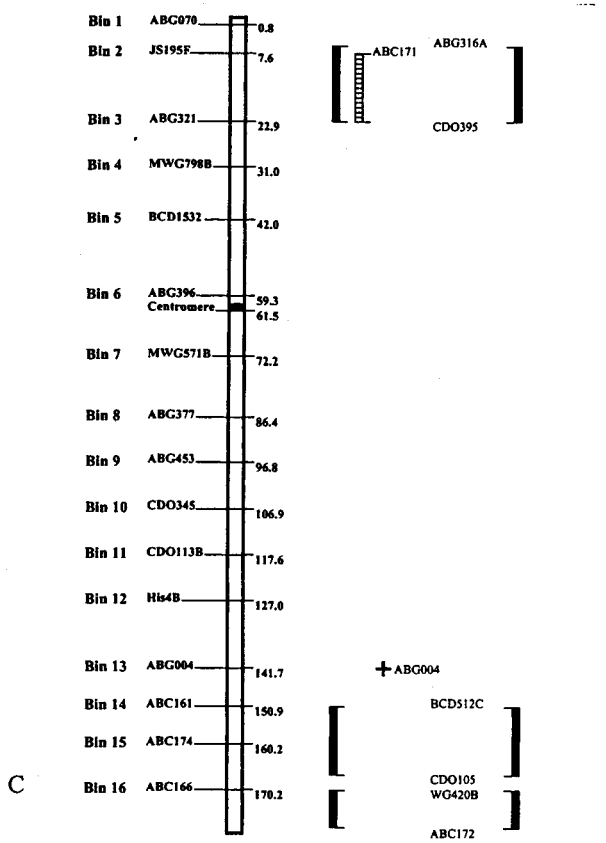
A

Fig. 1A-G. Chromosomal positions of quantitative trait loci (QTLs) contributing to Fusarium head blight (FHB) resistance and low deoxynivalenol (DON) concentration in three barley crosses: Chevron/Stander (represented by solid bars), Chevron/M69 (dashed bars), and Gobernadora/CMB643 (crosses). The relative position of flanking markers (for Chevron/Stander and Chevron/M69) or closest marker (for Gobernadora/CMB643) for each QTL in relation to the barley "bin" map was estimated from data contained in several sources: the Washington State University Barley Genomics website (<http://barleygenomics.wsu.edu>, curator: A. Kleinhofs); North American Barley Genomics Project website (<www.css.orst.edu/barley/NABGMP/nabgmp.htm>, curator P. Hayes), and Graingenes website (<http://wheat.pw.usda.gov/>, curator: D. Matthews). Three markers (CDO431, WG719D, and CDO785D), denoted with a question mark, could not be reliably positioned within a specific bin based on available mapping information. *(continued on next page)*



B

Fig. 1A-G. *(continued from previous page)* Positions of several flanking markers reported by de la Peña et al. (1999) did not correspond to other published barley maps (A. Kleinhofs, personal communication). These markers (in bold and underlined) are positioned based on original published information of de la Peña et al. (1999). The QTL designated with asterisks (*) was revealed in one environment after fixing the effect of the main QTL, then rescanning the genome for other QTLs (Ma et al., 2000). Additional information on the identified QTLs is given in Table 1.



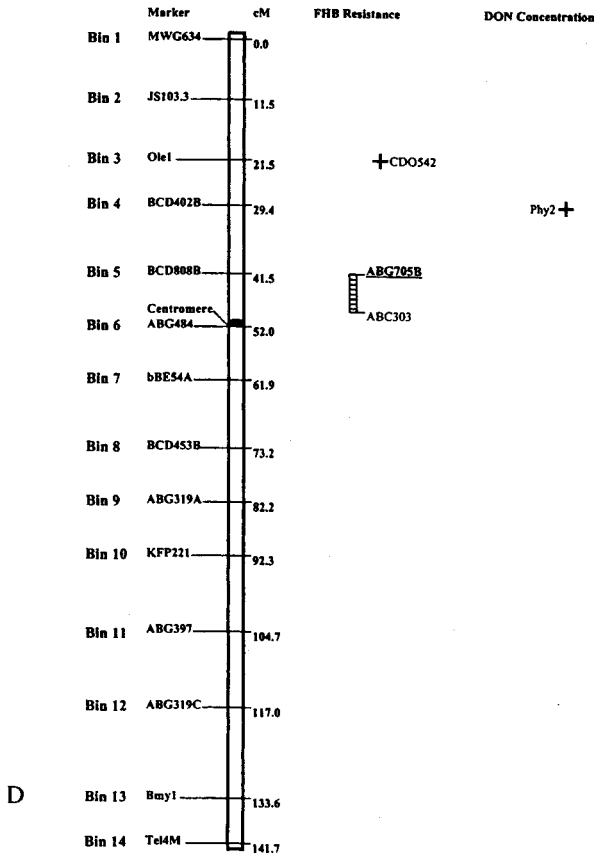
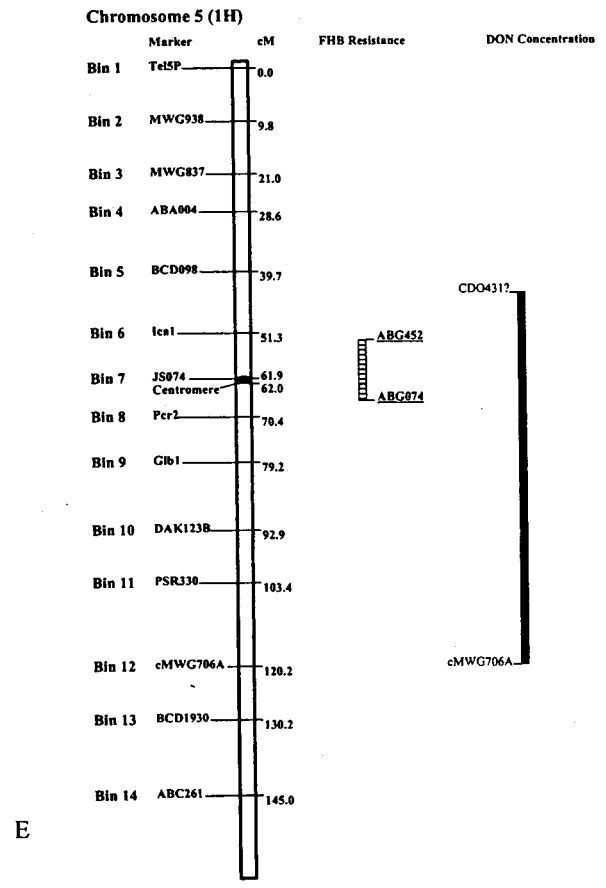


Fig. 1A-G. *See pages 274 - 275 for figure legend.*



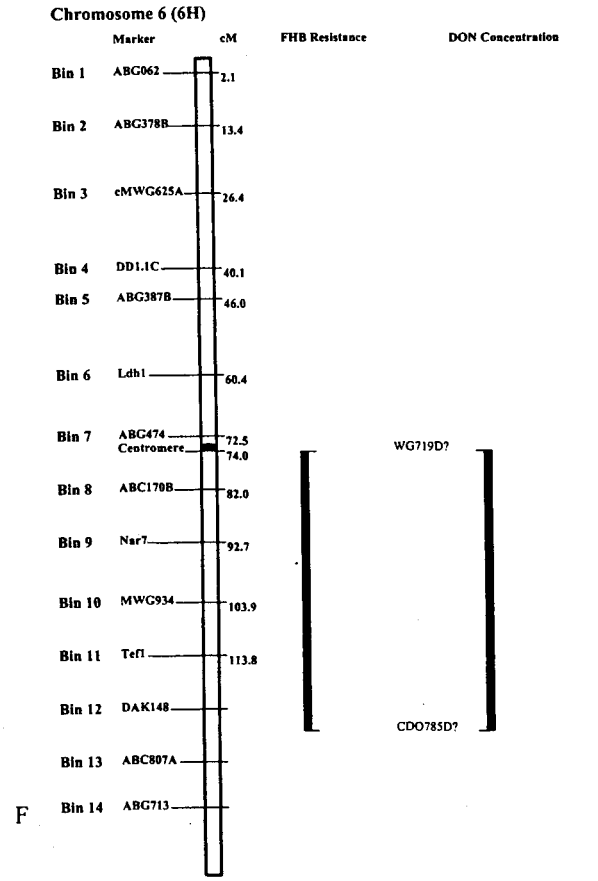
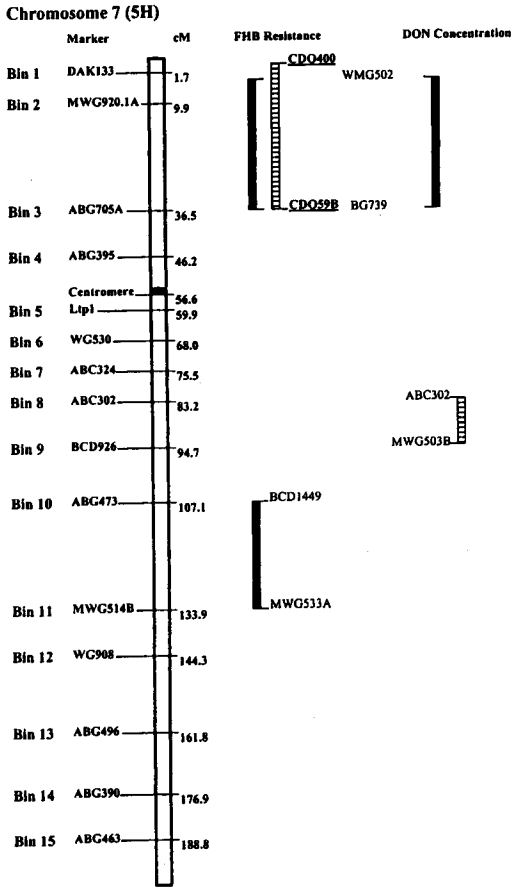


Fig. 1A-G. *See pages 274 - 275 for figure legend.*



G

Fig. 1A-G. *See pages 274 - 275 for figure legend.*

and 4H (12% variance). A QTL conferring resistance to initial infection on chromosome 3H explained 16% of the variance. Most of the other individual QTLs explained less than 10% of the variance. Within an environment, the multi-locus models explained 8 to 60% of the total variance. Resistance QTLs identified in the Gobernadora/CMB643 population were all coincident with QTLs for plant height, inflorescence density, and/or lateral floret size, with the exception of two small effect QTLs on chromosomes 1H and 6H. It is interesting to note that heading date was not associated with head blight resistance in contrast to other mapping studies (de la Peña et al., 1999; Ma et al., 2000).

The molecular mapping studies completed thus far indicate that head blight resistance is indeed a complex quantitative trait controlled, in most cases, by a number of loci with relatively small effects that are scattered across the barley genome. Moreover, most of the QTLs for head blight resistance are coincident with QTLs controlling various spike morphology (row type, lateral floret size, inflorescence density, etc.) and agronomic traits (heading date, height, etc.) as previously discussed.

Breeding Strategies and Head Blight Resistant Barley Cultivars

Relatively few barley accessions have been identified with high levels of resistance to head blight, and as in wheat, none of them exhibit immunity. Moreover, most of the resistant barley accessions have undesirable agronomic (e.g., low yield, weak straw, susceptibility to other diseases, etc.) and malt quality (e.g., low extract and diastatic power, etc.) traits (Urrea Flórez, 2000). At least four breeding cycles will be required to develop acceptable malting barley cultivars with head blight resistance from these unadapted lines (Urrea Flórez, 2000). A modified pedigree method is being used to breed for head blight resistance in North Dakota, Minnesota, Mexico (ICARDA/CIMMYT), Japan, and the Czech Republic. A recurrent selection scheme, which may be effective for combining resistance genes from different sources, is being initiated in North Dakota; however, to achieve reasonable progress within a short period of time, the base material used in the recurrent selection scheme should be relatively advanced in terms of agronomic and quality traits.

Doubled haploid populations are increasingly being utilized for barley breeding in many countries around the world (Pickering and Devaux, 1992). These populations offer several advantages over conventional populations (i.e., F_2, F_3 progeny, etc.) for head blight resistance breeding. First, since doubled haploid lines are completely homozygous, the variable expression of resistance genes in the heterozygous condition can be avoided. Second, the "immortal" nature of doubled haploid lines allows for repeated head blight and DON phenotyping across locations and years. This is a very important aspect given the variable nature of resistance expression in barley. Recombinant inbred line populations offer the same advantages as doubled haploid populations, but take longer to develop.

In breeding malting barley cultivars, it is important to incorporate the highest level of head blight resistance possible because this will contribute, in most cases, to the lowest DON concentration. To achieve this objective, genes conferring different types of resistance from diverse sources should be pyramided into breeding lines as is being done in the ICARDA/CIMMYT barley improvement program in Mexico (Vivar et al., 1997). This strategy is designed to yield transgressive segregants, i.e., progeny with higher levels of head blight resistance than the parents. Sumai 3, one of the most resistant wheat lines known, was derived from a cross between two moderately susceptible parents (see Ch. 11 by Bai et al.). In the upper Midwest region of the USA, most of the current cultivars and breeding lines possess an adequate level of resistance to spread; thus, breeding for resistance to initial infection (i.e., type as in wheat) is a higher priority. As described earlier, this type of resistance can be best assessed in the field using the spray inoculation technique (on cut spikes or on whole plants), but general inferences may also be obtained with grain spawn inoculation (Prom et al., 1996, 1997; Takeda and Heta, 1989). In the field, multiple replicates, locations, and years are required to obtain reliable results on the performance of breeding lines because of the variable nature of resistance expression and of DON accumulation. Campbell and Lipps (1998) investigated sources of experimental variation in head blight assessments of wheat in screening nurseries. They found the standard error for head blight infection was reduced most by additional environments and next by additional replicates. Because the addition of another environment was five times more costly than adding another replicate, the most cost-effective way to increase precision in disease assessment was through the addition of replicates. The final decision regarding the division of resources among the different sampling levels for head blight assessments will depend largely upon time, labor, and financial resources, but also the overall research objective (e.g., routine screening vs. phenotyping for molecular mapping studies) as well.

It is important to include the appropriate controls at frequent intervals throughout the screening nurseries. At the minimum, each nursery should have resistant and susceptible controls, preferably in both a two- and six-rowed genetic background. Ideally, it would be best to include controls with low, intermediate, and high levels of resistance, each with different maturities (e.g., early-, mid-, and late-season). Disease levels within a nursery can vary greatly despite efforts to ensure uniformity. The planting of controls for every 25 to 50 entries should be sufficient to monitor the variability of head blight infection across a nursery.

In screening for broad-based resistance, it is important to consider the range of variation in the pathogen populations. Since species other than *F. graminearum* can cause head blight as discussed earlier, it may be necessary to establish separate screening nurseries for each of the primary pathogen species. Moreover, the choice of pathogen isolates for screening is also an important consideration. As described previously, most of the studies conducted on the

pathogenic variability of *F. graminearum* have reported the absence of distinct race-specific or vertical pathotypes; however, isolates differing in aggressiveness have been detected (Ch. 9 by Mesterházy). To reduce the variation associated with pathogen genotype, Mesterházy (1995) recommended the use of a mixture of various aggressive isolates for inoculation of wheat. The same strategy is appropriate for barley.

Despite all of the difficulties associated with screening and breeding for resistance, significant progress is being made toward the development of six-rowed malting barley cultivars with head blight resistance for the upper Midwest region of the USA. At NDSU, reliable methods for creating head blight epidemics and assessing resistance in the field have been developed. This has led to the identification of accessions that consistently exhibit low levels of head blight infection and DON across different locations and years (Steffenson, 1998, 1999). Resistance genes from these sources are being combined into adapted germplasm in an accelerated breeding program.

An accelerated head blight resistance breeding program. At NDSU, an accelerated program to breed for head blight resistance was initiated in 1995. The primary goal of this program was to develop, within the shortest possible time, an adapted six-rowed malting barley cultivar with a high level of resistance to head blight and the accumulation of DON. This program was established by: 1) using winter field screening nurseries in China; 2) expanding efforts to screen breeding lines; and 3) developing molecular marker maps to position genes controlling resistance with the ultimate goal of using marker-assisted selection.

As mentioned previously, field screening nurseries have proven invaluable for identifying germplasm with resistance to head blight and to the accumulation of DON. Unfortunately, in North Dakota, only one field screening generation can be obtained per year. By using winter screening nurseries, two field screening generations can be obtained per year. China was selected as the site for such an off-season nursery because head blight is endemic and often severe in the lower Yangtze River Valley (Cook, 1981; Ch. 11 by Bai et al.). In practice, the Chinese nurseries have proven ideal as off-season screening sites because 1) uniform and severe disease epidemics were induced almost every year; 2) *F. graminearum* was essentially the only pathogen present in the nurseries, so possible confounding effects of other head blight diseases were eliminated; and 3) winter and spring barleys headed about the same time so relative resistance levels could be compared between the two types. Moreover, the timing of disease assessments in China (early to mid-May) allowed for a "shuttle" system of breeding. That is, lines selected as being resistant in China could be harvested and shuttled back to North Dakota for further evaluation in the field, thereby hastening the advancement of breeding lines with resistance. In general, accessions exhibiting the highest levels of resistance in North Dakota also were among the most resistant in China (Steffenson, 1998, 1999; B.J. Steffenson and R.D. Horsley, unpublished data). Thus, the value and

effectiveness of the winter screening nurseries in China were validated in terms of selecting for high levels of stable resistance. With moderately resistant accessions, the agreement in disease reaction between the two sites was not as high.

The emphasis placed on breeding for head blight resistance in the NDSU barley improvement program since 1993 has come at the cost of evaluating and advancing other potentially valuable breeding lines. Combining all of the favorable alleles that make a superior malting barley cultivar with those contributing to head blight resistance is a great challenge. The more progeny that are screened in segregating populations, the better the chance of recovering a line that carries all of the desired characters. To obtain a reasonable chance of recovering such lines, the number of breeding lines evaluated each year for head blight resistance was increased from about 2,000 to 12,000. This higher through-put has substantially increased labor and material costs for breeding, but should yield several superior parents for further crossing.

Marker-assisted selection holds great promise for the efficient transfer of QTLs controlling resistance to head blight and to the accumulation of DON in barley. If closely linked flanking markers can be identified for a resistance QTL, they can be exploited for the indirect selection of the trait without the need for disease and mycotoxin phenotyping, which are expensive, time consuming, and prone to environmental influences. A marker-assisted selection program is being developed at NDSU based on the resistance sources of Chevron and CI 4196.

Barley cultivars with resistance to head blight. Although head blight is an important disease of barley in several regions of the world, few cultivars with resistance have been developed and released to producers. The ICARDA/CIMMYT barley improvement program based in Mexico cooperated with the Shanghai Academy of Agricultural Sciences to develop the barley cultivar Gobernadora for China in 1987. Gobernadora (known as Zhenmai-1 in China) yields well and possesses resistance to head blight and barley yellow mosaic, two of the most important diseases in the country (Vivar, 1987). It was cultivated on over 100,000 hectares in eastern central China (Vivar et al., 1997). ICARDA/CIMMYT also is developing barley cultivars with head blight resistance for the Andean region of South America in cooperation with several national programs (e.g., INIAP in Ecuador). Shyri (a hulled type) and Atahualpa (a hulless type) are two released Ecuadorian cultivars that carry some resistance (Vivar, 1996; Vivar et al., 1997).

In Japan, there are a number of barley improvement programs breeding for head blight resistance, particularly in the southern part of the country. From these programs, several two-rowed cultivars (e.g., Nishino Gold, Nishinochikara, and Mikamo Golden) with moderate resistance were released (see Yamashita, 1987, 1988 for summary). Gocho and Hirai (1987) evaluated 63 Japanese two-rowed, six-rowed, and hulless cultivars for head blight reaction and identified several (e.g., Hosimasari, Daisen Gold, and New Golden) with fairly high levels of resistance. The Japanese landrace cultivar Golden Melon is thought to be the source of head blight resistance in many of the modern two-rowed malting cultivars (Takeda and Heta, 1989). Development of resistant six-rowed cultivars remains a challenge for breeders in Japan.

At the University of Minnesota, there has been a long-term effort to breed barley for resistance to kernel discoloration (i.e., kernel blight) (Immer and Christensen, 1943), a disease caused primarily by *Cochliobolus sativus*, but also *Alternaria alternata* and various species of *Fusarium* as well (Miles et al., 1989; Wilcoxson et al., 1980). Screening for resistance to this disease was done by repeatedly inoculating plants with *C. sativus* in the field, providing overhead irrigation, and then assessing the degree of kernel discoloration based on a 1 to 5 scale (Gebhardt et al., 1992; Miles et al., 1989; Wilcoxson et al., 1980). One of the primary resistance sources used in this program was Chevron. During the course of selecting for resistance to kernel discoloration caused by *C. sativus* in Chevron-derived populations, progress also was unknowingly made in advancing progeny with head blight resistance (de la Peña et al., 1999; R.D. Wilcoxson, personal communication). This long-term breeding effort culminated in the release of MNBrite, a cultivar with a high level of kernel discoloration resistance and an intermediate level of head blight resistance (de la Peña et al., 1999; Rasmusson et al., 1999). The progress achieved in breeding for resistance to both diseases is not surprising since Immer and Christensen (1943) reported that, in general, barley lines exhibiting resistance to kernel blight (=discoloration) caused by *C. sativus* also are resistant to head blight. Indeed, this relationship was corroborated by the identification of coincident QTLs for kernel discoloration resistance and head blight resistance on chromosome 2H in the Chevron/M69 population (de la Peña et al., 1999). It is very possible that some of the same QTLs conferring resistance to kernel discoloration may also control resistance to head blight. Additional disease evaluations and construction of a higher resolution molecular map of the Chevron/M69 population are required to resolve this question. Different investigators have documented the low incidence and severity of kernel discoloration and head blight in Chevron over many locations and years (e.g., de la Peña et al., 1999; Miles et al., 1989; Prom et al., 1996; Shands, 1939; Wilcoxson et al., 1980). These studies indicate that Chevron possesses a high level of "general" resistance to fungal infection of the kernels.

As discussed previously, germplasm evaluations (Chen et al., 1991; Shands, 1933; Takeda and Heta, 1989; Zhou et al., 1991) and anecdotal observations of commercial barley cultivars in production (T. Ban and S. Ding, personal communication) indicate that six-rowed barley accessions are generally more susceptible to head blight than two-rowed accessions. During the head blight epidemics of the 1990s in the USA, six-rowed cultivars, the type preferred for malting in the upper Midwest, suffered severe yield and quality losses (McMullen et al., 1997; Steffenson, 1998, 1999). Two-rowed cultivars offer a possible means for producing acceptable malting quality barley in this region because they generally sustain less head blight damage and accumulate lower levels of DON. Conlon, a two-rowed cultivar released by the North Dakota

Agricultural Experiment Station in 1996, was recommended as a malting barley cultivar in 2000. It is the first two-rowed cultivar recommended for malting by the AMBA in the upper Midwest. Conlon is moderately resistant to head blight and generally exhibits lower DON levels than widely grown six-rowed cultivars under moderate disease pressure (Steffenson, 1998, unpublished data). If head blight continues to be a problem in the upper Midwest, Conlon and other two-rowed cultivars like it may become important sources of premium malting barley with low or nondetectable levels of DON.

Application of Biotechnology for the Development of Barley Cultivars Resistant to Head Blight

Several recent developments in biotechnology and molecular biology hold some promise for hastening the development of barley cultivars with head blight resistance. The first was the transformation of barley. Particle bombardment (Ritala et al., 1994; Wan and Lemaux, 1994) and an *Agrobacterium*-mediated protocol (Tingay et al., 1997) have been successfully used to transform barley. Unfortunately, the efficiency of these methods is low (range of 0-5.6% with most less than 1%) (Lemaux et al., 1999), and the number of barley lines amenable to transformation is limited (see Ch. 12 by Muehlbauer and Bushnell). Nevertheless, several laboratories around the world are actively working on increasing the efficiency of these transformation protocols on barley cultivars of agronomic importance (Dahleen et al., 2000). Success in this endeavor could ultimately lead to the transformation of an already desirable malting barley cultivar with genes that inhibit head blight pathogens and the production of their mycotoxins.

A second major development was the discovery and characterization of various genes conferring resistance to fungal pathogens.

Skadhauge et al. (1997) discovered a proanthocyanidin-free mutant of barley that shows extreme resistance to *Fusarium* in vitro. The hyphae of *F. graminearum*, *F. culmorum*, and *F. poae* were unable to penetrate the testa layer of this mutant's developing caryopsis under in vitro infection experiments, apparently from the accumulation of dihydroquercetin, a potent inhibitor of *Fusarium* growth. In field tests, this mutant proved to be susceptible to head blight (B.J. Steffenson, unpublished data). Further research is needed to explore more fully the effect of dihydroquercetin on *Fusarium* infections of barley.

The exploitation of different classes of antifungal proteins may be one of the most fruitful lines of research for developing head blight resistant barley cultivars. Candidate antifungal proteins include chitinases, glucanases, thionins, osmotins, and thaumatin-like proteins (Dahleen et al., 2000; see Ch. 12 by Muehlbauer and Bushnell). A rice thaumatin-like protein was introduced into a susceptible wheat cultivar by particle bombardment, and transgenic plants with the protein exhibited delayed head blight symptoms after inoculation with *F. graminearum* (Chen et al., 1999). Additionally, a research group at Monsanto

reported a significant enhancement of head blight resistance in transgenic wheat plants with another introduced antifungal protein (Hakimi et al., 1997). The results in wheat are encouraging, but far more research must be done to optimize the expression of these genes for the effective control of head blight.

Several other potentially useful categories of genes for head blight control include 1) those inducing systemic acquired resistance or hypersensitivity, 2) those coding for enzymes involved in various defense reaction pathways, and 3) those coding for factors acting against various mycotoxins that might serve as possible pathogenicity factors for *Fusarium* (see Ch. 12 by Muehlbauer and Bushnell).

To be completely effective, these genes must be expressed in all vulnerable tissues of the spike and halt most, if not all, *Fusarium* infections. Additionally, these genes must not detrimentally affect either the agronomic performance or quality of barley cultivars, especially those used in malting. Still, the most important test will be the performance of transgenic barley plants under head blight epidemics in the field.

Summary and Conclusions

Fusarium head blight is, without question, one of the most devastating and insidious diseases of barley. Although the disease may not cause severe yield loss in every case, low levels of infection can have serious consequences for the brewing, food, and swine industries from the contamination of grain with mycotoxins such as DON. Head blight has been a relatively minor and sporadic disease problem of barley in the United States and other countries for many years. Over the past decade, however, head blight has re-emerged as the most important factor reducing the yield and quality of barley in many regions of the world. The epidemics of 1993 to 2000 in the United States caused economic losses that were unparalleled in the history of barley production in the region. The primary factors contributing to these epidemics were changes in cropping practices, the wide cultivation of susceptible cultivars, and moist weather conditions during heading and grain filling (Steffenson, 1999). Head blight epidemics will likely occur with some frequency in the future if inoculum levels of *Fusarium* remain high in fields and susceptible cultivars continue to be widely grown. The weather conditions will ultimately determine how widespread and severe the epidemics become.

Head blight is one of the most difficult diseases to control in barley. Additionally, the quality and processing problems associated with *Fusarium*-infected grain are extremely difficult, if not impossible, to manage for end-users. Thus, it is important to prevent the disease from becoming established on kernels in the field. Head blight may be best controlled through an integrated approach of cultural practices, fungicide application, and deployment of resistant cultivars. Breeding for head blight resistance is difficult. Almost every facet of screening for resistance and low DON concentration is time consuming,

labor intensive, and expensive. Additional research is needed to refine greenhouse and field screening methods for assessing different resistance types and reduce the extreme variability associated with the head blight-barley interaction. Genetic studies indicate that head blight resistance is conferred by many genes with small effects scattered across the barley genome. Moreover, molecular mapping studies revealed that most QTLs for head blight reaction coincide with QTLs controlling various agronomic and spike morphology traits. The effect that agronomic and spike morphology traits have on head blight infection is poorly understood. Further studies are needed to differentiate between the possible disease escape mechanisms conferred by these traits and "true" host resistance. Such information will facilitate the efficient development of cultivars with resistance mechanisms that act directly against the pathogen. Still, from a practical breeding perspective, any trait that lessens the impact of the disease and its adverse effect on quality is desirable and should be considered in the development of cultivars.

Despite all of the difficulties in screening and breeding for head blight resistance, progress is being made in developing resistant six-rowed malting barley cultivars for the upper Midwest region of the USA. Several diverse six-rowed and two-rowed accessions that consistently exhibit low levels of head blight infection and DON have been identified and are being used as the primary resistance sources in the barley improvement program. The resistance QTLs from these sources are being transferred into adapted germplasm with malting quality by conventional breeding methods and by use of marker-assisted selection as part of an accelerated resistance breeding effort. Finally, success in several lines of biotechnology research may lead to the transformation of an already desirable malting barley cultivar with genes that inhibit *Fusarium* spp. and the production of their mycotoxins. Through all of these resistance breeding efforts and various on-farm management strategies, it should be possible to reduce the devastating effects of head blight and reliably produce quality malting barley once again.

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