Transgressive segregation for resistance in barley to net blotch
by Bruce Patrick Bordelon

A thesis submitted in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in Plant Pathology
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Abstract:
Research was initiated to demonstrate transgressive segregation in barley for resistance to net blotch, caused by Pyvenophora teres Drechsl. F2 plants from crosses between susceptible cultivars were selected under disease conditions in the field. Their F3 progenies were screened and selected for seedling resistance to a single isolate of P. teres. The selected plants were grown to maturity in the greenhouse and the F4 progenies were screened for seedling resistance to the same isolate. Additional F2 progenies were screened for resistance in both the field and growth chamber.

The results showed that very little seedling resistance was expressed in the F3 progenies. However, many of the F progenies expressed high levels of resistance. A method to quantify levels of resistance was established and comparisons were made between the F4 lines and their parents. A total of 109 F4. lines from nine crosses were tested; 84 had means significantly less than the midparent mean, and 69 had means significantly less than the midparent and low-parent means. This proved the occurrence of transgressive segregation for net blotch resistance in barley. The increased resistance was apparently due to the additive effects of minor genes. Some of the best F4 lines were tested to a combination of isolates representing a wide range of virulence types. The reaction of the lines to the combination of isolates was similar to their reactions to a single isolate, indicating that the resistance was nonspecific in action. Screening for resistance in the field and growth chamber revealed that transgressive segregation was easiest to detect under seedling conditions in the growth chamber. The possibility of utilizing this type of resistance in commercial cultivars is discussed.

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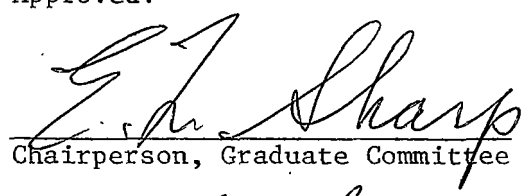

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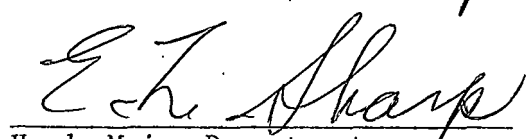

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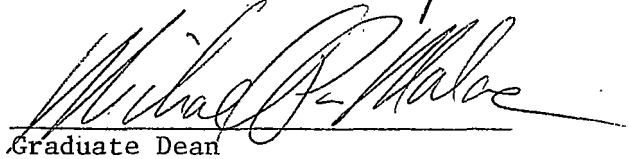

Graduate Dean

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TABLE OF CONTENTS

LIST OF TABLES

Table

ABSTRACT

Research was initiated to demonstrate transgressive segregation in barley for resistance to net blotch, caused by *Pyrenophora teres* Drechsl. F_2 plants from crosses between susceptible cultivars were selected under disease conditions in the field. Their F_3 progenies were screened and selected for seedling resistance to a single isolate of *P. teres*. The selected plants were grown to maturity in the greenhouse and the F_4 progenies were screened for seedling resistance to the same isolate. Additional F_2 progenies were screened for resistance in both the field and growth chamber.

The results showed that very little seedling resistance was expressed in the F_3 progenies. However, many of the F progenies expressed high levels of resistance. A method to quantify levels of resistance was established and comparisons were made between the F_4 lines and their parents. A total of 109 F_4 lines from nine crosses were tested; 84 had means significantly less than the mid-parent mean, and 69 had means significantly less than the mid-parent and low-parent means. This proved the occurrence of transgressive segregation for net blotch resistance in barley. The increased resistance was apparently due to the additive effects of minor genes. Some of the best F_4 lines were tested to a combination of isolates representing a wide range of virulence types. The reaction of the lines to the combination of isolates was similar to their reactions to a single isolate, indicating that the resistance was nonspecific in action. Screening for resistance in the field and growth chamber revealed that transgressive segregation was easiest to detect under seedling conditions in the growth chamber. The possibility of utilizing this type of resistance in commercial cultivars is discussed.

Chapter 1

INTRODUCTION

Net blotch disease of barley, caused by *Pyrenophora teres* Drechsl., is a common disease that occurs wherever the crop is grown. It is most prevalent in the temperate, humid regions of the world and in areas where barley is grown during the cool season (Dickson, 1956).

P. teres is predominately a leaf parasite, is seed-borne, and is a weak competitive saprophyte. Disease persistence is dependent on survival in infested debris and grain. Cultural practices, such as destruction of inoculum and the use of resistant cultivars are the primary means of control.

Changes in agricultural practices in recent years have favored the build up of inoculum. Minimum tillage, close rotations, and intensive cropping favor the accumulation of infested debris and allow the pathogen to exist from year to year. These new farming practices are generally more beneficial than harmful, so the control of net blotch must rely on the use of resistant cultivars.

P. teres is a highly variable organism that occurs as numerous virulence types. The sexual stage commonly occurs on the host. The probability of asexual recombination through heterokaryosis and parasexuality appears high. Because it is such a highly variable organism, long lasting resistance may be difficult to achieve. Single, major genes for resistance are not likely to remain

effective where the disease is common. Minor gene, nonspecific resistance is being used to control several plant diseases, especially those caused by highly variable organisms.

The objectives of the research described in this thesis were: (1) to demonstrate transgressive segregation for net blotch resistance in barley due to the additive effects of minor genes, (2) to determine if adequate levels of resistance could be established in lines with additive genes, and (3) to develop a system of consistent seedling inoculation and symptom quantification.

Chapter 2

LITERATURE REVIEW

Economic Importance

Changes in agricultural practices in recent years have led to a change in the status of many diseases, particularly leaf diseases of cereals (Shipton, et al., 1973). Despite its widespread occurrence, net blotch has been considered of minor importance in years past, but it is now being recognized as a destructive pathogen. Alone, or in combination with other diseases, net blotch has caused serious yield losses in many areas of the world.

In the United States, net blotch occurs in all significant barley growing areas. It was introduced with barley into North America (Dickson, 1956). Although net blotch can cause considerable loss, it is considered of minor importance in many areas compared to other diseases (Schaller and Wiebe, 1952). Schaller (1955) reported net blotch to be common in California, especially in early sown fields, and it was occasionally severe enough to cause complete necrosis of the leaves by flowering time. During the 1977-78 growing season, net blotch was so severe in California that fields were burned rather than harvested (Bjarko, 1979). Net blotch is considered to be of major importance in some barley areas, especially in Minnesota and Wisconsin (Shipteon, et al., 1973). This disease has produced detrimental effects on yield and malting quality of barley grown in northcentral Montana (Bjarko, 1979).

Barley diseases have become more prevalent in Canada since the 1940's (Buchannon and Wallace, 1962). Net blotch, together with spot blotch, caused by *Cochliobolus sativus* (Ito and Kuribay.) Drechsl., barley scald, caused by *Rhynchosporium secalis* (Oud.) J. J. Davis, and Septoria-speckled leaf blotch, caused by *Septoria passerinii* Sacc. caused considerable damage in the prairie provinces in the 1950's (Shipton, et al., 1973). Creelman (1965) noted that net blotch and scald were widespread and frequently severe in central and northern Alberta. McDonald and Buchannon (1964) reported a severe epidemic of net blotch in Manitoba in 1963. Besides North America, net blotch is of economic importance in South America, Europe, the Middle East, Africa, New Zealand and Australia (Shipton, et al., 1973).

The effect of net blotch on grain yield and quality has been reported by various authors. Shipton (1966 in Shipton, et al., 1973) noted that moderate infection on Beecher barley led to a 17% yield reduction in the field and a reduction in 1000 kernel weight and malt extract yield. Piening and Kaufman (1969) found yield reductions of 53% following destruction of two-thirds of the leaves, due mainly to a marked reduction in number of kernels per spike. Smedegard-Petersen (1976) reported yield losses of 9% and 11% in the barley cultivars Wing and Lauda, respectively, due to a reduction in grain size and weight. Caudel and Wilcoxson (1975) reported yield losses of 26% due to net blotch.

Causal Organism

Net blotch disease is caused by the fungus *Pyrenophora teres* Drechsl. The perfect stage is in the subdivision Ascomycotina (Ascomycetes), class Loculoascomycetes based on the fact that the asci are bitunicate, and the ascocarp is an ascostroma. The ascostromata are perithecioid pseudothecia with pseudoparaphyses, placing it in the order Pleosporales, family Pleosporaceae. This is a large group of ascomycetes which includes some economically important plant pathogens such as *Pyrenophora, Cochliobolus, Pleospora,* and *Leptosphaeria* (Alexopoulos and Mims, 1979; Webster, 1980).

The imperfect stage, *Drechslera teres* (Sacc.) Shoem., syn. *Helminthosporium teres* Sacc. is placed in the form-class Hyphomycetes, form-family Dematiaceae because of the absence of pycnidia or acervuli and the dark pigmentation of the hyphae and conidia (Alexopoulos and Mims, 1979; Webster, 1980). The conidia are phragmospores, transversely septate with 4-5 septa, light yellowish brown, cylindric, with an inflated basal cell. They measure 95-120 x 19-21 µm (Shoemaker, 1962). The conidia develop at the tips of successive lateral proliferations of the conidiophores and are pseudopleurogenous. The conidial germ tubes are lateral and may arise from intercalary as well as terminal cells (Luttrell, 1977).

P. teres also produces pycnidial-like structures on host tissue and in culture. They are globose to pear-shaped, and yellow to brown in

color. The spores are small (1.0-1.9 x 1.4-3.2 μm), hyaline, and spherical to ellipsoidal. Their function is not known, although it is thought that they may act as spermatia (McDonald, 1963). The heterothallic nature of *P. teres* has been shown by McDonald (1963). Cultures of each of the two mating types were obtained from each of several areas. Most isolates produced pycnidia and protoperithecia on natural media indicating that the species is bi-sexual, hermaphroditic, and self-sterile.

Etiology

Of the three types of spores; conidia, ascospores, and pycnidiospores, only the conidia and ascospores can infect host tissue. All of the spore types will germinate and grow on artificial media and the mycelium produced from each will infect host tissue (Smedegard-Petersen, 1972). The conidia and ascospores of *P. teres* germinate readily at room temperature and form hyaline germ tubes within one-half to two hours. Each cell of the multiseptate spores is potentially germinable, but germ tubes usually arise from the end cells of conidia and the central cells of ascospores. The germ tubes form appressoria-like structures before penetration. The penetration hyphae pass through the epidermal cells and enlarge slightly after passing through the lower cell wall. Hyphal growth proceeds intercellularly with cell death occurring in advance of the fungus (Keeling and Banttari, 1975; Shipton, et al., 1973). The respiration rate of susceptible barley leaves increases significantly over non-infected leaves. The respiration rate reaches a

maximum level coinciding with the appearance of visible symptoms (Smedegard-Petersen, 1976). Two toxins, both low molecular weight peptides, are produced by *P. teres*. Both toxins incite most of the symptoms that the pathogen causes, but they do not seem to determine pathogenicity; rather they contibute to the virulence of the organism (Smedegard-Petersen, 1976, 1977).

Symptomology

External symptoms on host tissue may be evident within two days of inoculation. They appear as minute, dark brown spots or streaks. With time the lesions enlarge, producing longitudinal and transverse streaks that form a reticulate, net-like pattern within an otherwise light brown lesion from which the common name of the disease is derived (Dickson, 1956). A zone of chlorosis typically forms around the lesions. Its size is dependent on the susceptibility of the host and the virulence of the isolate.

The symptoms may develop on leaves, sheaths, or culms. As infection progresses the lesions coalesce longitudinally, forming dark brown limited stripes with irregular margins. The net-like appearance is only evident along the margins of older lesions. In advanced stages of development a series of several stripes extend parallel along the blade. At this stage the disease is almost indistinguishable from barley stripe caused by *Pyrenophora graminea* Ito and Kuribay.

Isolates of *P. teres* have been collected that do not produce the

characteristic net lesions on host plants, but instead produce spot lesions very similar to the leaf symptoms produced by *Cochliobolus sativus*. The spot form produces well defined dark brown, elliptical or circular lesions. In crosses between the net and spot forms of *P. teres*, Smedegard-Petersen (1976) found that symptom expression is controlled by two independent allelic pairs of genes. He suggests the designation of *P. teres* forma *teres* for the net form and *P. teres* forma *maculata* for the spot form.

In addition to net and spot forms, certain highly virulent isolates of *P. teres*, due to high capacity for toxin production, may produce 'unspecific' symptoms. These are characterized by chlorosis and a diffuse, general water soaking followed by a rapid necrosis of infected tissue, but with little expression of the usual net and spot lesions. This can greatly complicate diagnosis in the field (Smedegard-Petersen, 1976).

Symptoms on resistant cultivars initially appear very similar to those on susceptible cultivars. However, the lesions elongate very little or not at all on the resistant varieties. Keeling and Banttari (1975) found no differences in spore germination, germ tube growth, or number of penetrations on leaves of resistant and susceptible barley. Growth of the fungus after penetration was, however, significantly less in resistant cultivars in which many infections did not progress beyond the penetrated cell. They proposed that a heat labile antifungal substance(s) is produced in resistant barley as a result of infection by *P. teres*.

Epidemiology

Conidiophores and conidia develop on the leaves after lesions appear, arising from between epidermal cells or stomata. This leads to secondary spread of the disease. High humidity appears necessary for their production (Shipton, et al., 1973). Sporulation may occur from lesions on resistant cultivars as well as susceptible ones; however, more time is required for sporulation to occur and the amount of sporulation is significantly less on resistant cultivars (Keeling and Banttari, 1975).

The seed-borne nature of *P. teres* is well known (Shipton, et al., 1973). The extent to which seedling infection from seed-borne inoculum occurs is dependent on several factors, including the environment. It has been demonstrated that infection of the primary leaf from seed-borne inoculum occurs at relatively low temperatures, ie: 10-15^{o}C and to a lesser extent at higher temperatures (Shipton, et al., 1973). Kenneth (in Shipton, et al., 1973) found that the caryopsis must be infected for seedling infection to develop. The significance of seed-borne inoculum as a source of primary inoculum has been studied. Piening (1968) showed that infested seed produced 0.5 to 1.5% seedling infection while heat or chemically treated seed produced 0.0 to 0.15% infected seedlings. However, when infected primary leaves were not removed from the plants, twice as many of the plants were infected 28 days after emergence than in plots where infected leaves were removed

as soon as noticed.

P. teres has a low competitive saprophytic ability and no soil phase. Ammon (1963) found that *Drechslera* spp. were able to survive saprophytically on organic material only a few weeks while two species of *Bipolaris* were capable of surviving longer periods in the soil. Nevertheless, contaminated stubble and straw appear to be the major source of primary inoculum. Piening (1968) found that in field plots where the infested straw from the past year's crop was lightly disced, 42% of all the emerging plants were infected, as compared to only 8% of the plants infected when the stubble was plowed under. This indicates that exposed residue is a good source of inoculum. It was not determined whether ascospores or conidia were responsible for the infections, but other observations of lesions on volunteer barley indicate that about half of the net blotch infections were initiated by ascospores.

The pseudothecia of *P. teres* form on the culms, nodes of the culms, and the sheath. In general, these ascocarps mature slowly, taking as long as two months in culture and up to six months in the field (Shipton, et al., 1973). In many areas fertile ascocarps are not abundant. It appears that the environment as well as the occurrence of compatible mating types may limit their formation. The sterile ascocarps, however, are still potential sources of inoculum because conidiophores and conidia develop on the ascocarps under

favorable conditions. Piening (1961) found both mature and immature ascocarps prevalent in parts of Canada that were capable of providing inoculum two years after harvest. Even immature ascocarps appear to successfully resist weathering. It seems, however, that ascospores present a greater hazard as a potential source of new virulence rather than as a source of primary inoculum.

Variability of the Pathogen

Isolates of *P. teres* have been found to differ sharply in virulence (Pon, 1949). This is evident when comparing reported sources of resistance to *P. teres*. Schaller and Wiebe (1952) were first to screen extensively for resistance. They tested 4,256 barley lines to California isolates of *P. teres*. Twenty-five of these lines proved to be highly resistant, most of them originating from Manchuria. Buchannon and McDonald (1965) tested 6,174 lines for resistance to isolates from Canada, Mexico, and the United States. They found 40 lines with resistance. The highest percentage originated in Ethiopia. They also found that some of the lines reported by Schaller and Wiebe (1952) to be resistant, were susceptible to Canada isolates. Caddel and Wilcoxson (1975) found that lines resistant to *P. teres* in other parts of the world were susceptible or moderately susceptible in Morocco. This indicates a difference in virulence among the isolates.

Bjarko (1979) tested 26 isolates of *P. teres*, 15 from the Middle East and 11 from Montana. He was able to separate the Middle East

isolates into seven virulence types using five differential barley lines and the Montana isolates into five virulence types using four differential barley lines. Khan and Boyd (1969a) separated isolates from western Australia into three virulence types using two differential varieties. McDonald and Buchannon (1962) identified two virulence types among isolates from Canada, Mexico, North Dakota, and California. Three virulence types have been reported in Canada; the normal net-type, a new virulent net-type, and a spot-type (Tekauz and Mills, 1974; Tekauz and Buchannon, 1977). It is believed that the spot form was introduced into the area near Winnepeg, Manitoba on contaminated barley seed from Europe (Tekauz and Buchannon, 1977). Smedegard-Petersen (1976) found that the spot form occurs abundantly in Denmark and is more prevalent than the net form. The spot form isolates are generally less virulent than the net form isolates.

P. teres has also been shown to be highly variable in characteristics other than virulence. Kenneth, et al. (1967) as in Shipton, et al. (1973) found that progeny of monoconidial and hyphal tip isolates continue to display considerable variability in cultural characteristics and specific pathogenicity after single spore subculturing for at least six generations. McDonald (1967) as in Shipton, et al. (1973) believed that particular clones were adapted to different environments. This was confirmed by the findings of Khan, et al. (1967) that the optimum temperatures for spore production and leaf infection were

higher for isolates collected on summer grown crops than for isolates collected on crops grown over mild winter periods. They have suggested the existence of temperature ecotypes among pathogenic fungi.

The high variability in the species *P. teres* is likely due to the occurrence of the sexual stage in nature and the possibility of genetic recombination through heterokaryosis and parasexuality. Parasexuality has been demonstrated in some genera of the family Pleosporaceae, e.g., *Leptosphaeria* and *Cochliobolus*, although it has not yet been shown in *Pyrenophora* (Tinline, 1962; Tinline and MacNeil, 1969). Studies by Shands and Dickson (1934) did suggest heterokaryosis in *Pyrenophora graminea*, although the evidence was not conclusive (Parmeter, et al., 1963). Although the sexual stage of *P. teres* has not been found abundantly in some areas, considerable variability exists among isolates collected in these areas. The asexual stage (*Drechslera teres)* is quite similar to that of *Cochliobolus sativus (Bipolaris sorokiniana)* in which heterokaryosis and parasexuality have been shown (Tinline, 1962), so it is likely that the same mechanisms could be operating in *P. teres*. The role of parasexuality and heterokaryosis in changing the virulence and host range of pathogens has been reported (Tinline and MacNeil, 1969).

The high variability of *P. teres* complicates genetic studies on resistance and has important implications in breeding programs. Tekauz and Mills (1974) found new virulence types in Canada that were pathogenic

on two previously resistant commercial cultivars. C.I. 5791, a commonly used source of resistance, was less resistant to the new virulence types. Consequently, breeding programs must utilize broad based resistance to attain effectiveness against all virulence types.

Another factor which complicates the identification of resistance is the variability of symptom expression. Several factors have been shown to influence the symptom expression by *P. teres*. These include light intensity, pre- and post-inoculation temperature, pre- and post-inoculation wetting of the foliage, nutritional status of the host and pathogen, age of the host and pathogen, and concentration of inoculum. The effect of most of these factors is specific to certain cultivars. The expression of resistance was more sensitive to environmental influence in cultivars of Manchurian origin than in Ethiopian cultivars (Khan and Boyd, 1969b). In addition, the penetrance and expressivity of genes conditioning host resistance can be influenced by the genetic background of the host (Khan and Boyd, 1969b; Khan, 1969; Shipton, et al., 1973). The variability of symptom expression has important implications in breeding programs such as the choice of recurrent (susceptible) parents and the role of the environment in selection.

Chapter 3

SELECTING TRANSGRESSIVE SEGREGATES FOR DISEASE RESISTANCE

One of the main objectives of the research reported in this thesis was to demonstrate transgressive segregation for resistance to net blotch in barley. In order to do this, plants needed to be selected in the first year so that advanced generations could be obtained. Therefore, plants were selected from existing F_2 populations. These were chosen because of their availability, and because the parents of the populations were susceptible or intermediate in the seedling stage to all five of the Montana virulence types of *P. teres* described by Bjarko (1979). This meant that they did not have major genes effective against the isolates used to screen the populations. Therefore, any minor genes would not be masked and would be detectable. The selection was done under field conditions.

Materials and Methods

Twenty-three F_2 populations of barley were screened for resistance in the 1979 net blotch disease nursery. The populations contained barley developed in a Montana State University-Agency for International Development project utilizing male sterile facilitated recurrent selection, and barley selected by Bjarko (1979). Fifteen of the populations were from crosses used to establish some of the base recurrent selection populations of barley. They had a common female

parent, Manchuria (CI 2330), which had male sterile gene (msg 10). The male parents were various two-row and six-row commercial cultivars chosen for their agronomic qualities. The seed for these populations came from a bulk of several F_1 plants. The remaining eight populations were material from Bjarko's research project. This latter group originated from crosses between the commercial cultivars Betzes, Georgie, Tifang, Hypana, and Firlbeck's III. Each population was the progeny of one or two F_1 plants. Approximately 400 seeds of each population were planted in four 20-ft rows.

The plots were inoculated 32 days after planting. The inoculum consisted of 9-10 day old cultures of *P. teres* grown on V-8 juice agar, blended in distilled water. Cultures from 10 plates were blended for 20-30 seconds in one liter of distilled water, then strained through two layers of cheesecloth. The inoculum contained approximately 3×10^4 spores per ml plus some mycelial fragments. Six isolates, representing all five Montana virulence types (Bjarko, 1979), were mixed in equal quantities to make up the final inoculum. The isolates were: Pt R (type A), Pt B (type B), Mt 77-5I (type C), Mt 77-3 (type D), Mt 77-6 (type E), and Ftb 78-1 (type B).

The plots were inoculated by spraying the prepared inoculum onto the plants with a 'Solo' backpack sprayer at a rate of approximately one liter per 20 ft of row. The plots were inoculated in the late evening, then covered with plastic sheets overnight to provide approximately 12 hrs

of high humidity for spore germination and penetration. The sheets were removed the following morning before the air temperature under the plastic reached 80°F. General observations of the disease reaction of each population were made starting 17 days after inoculation. The reaction types, on a scale of 0-4 (described in Chapter 4), and the extent of spread of the symptoms up the plants were recorded for each population so that a basis for single plant selection could be established. Several plants were selected as resistant or susceptible, relative to the other plants in the population, and tagged for harvest. Care was taken to avoid selecting escapes by choosing only plants that had obvious net blotch symptoms.

Results and Discussion

Table 3-1 lists each population, a summary of the general observations, and the basis for selection in that population. Weather conditions following inoculation were not favorable for spread of the disease so very little secondary infection occurred. The plants were in the tillering stage when inoculated and the fungus did not spread as the plants elongated, so the symptoms remained confined to the lowest leaves of the plants in most plots. This complicated evaluation because the infected leaves began to senesce early in the growing season, leaving only a few easily readable lesions on the plants. General observations showed that 11 of the 23 populations were resistant, two were intermediate, six were susceptible, and four contained both

resistant and susceptible plants. There appeared to have been some environmental variation within and among the plots and disease escapes were present in many populations. The plots were not replicated, so quantitative comparisons among the populations was not possible. Also, the low level of disease that resulted from the inoculation made direct measurement of resistance difficult.

The principle goal of this experiment was to select plants with minor gene, additive resistance from F_2 populations of crosses between cultivars that apparently did not have major genes for resistance to net blotch. The F_2 populations were used as material to select from, rather than material to be quantitatively evaluated. Some, but not all, of the parent cultivars were planted in the field, so some speculation can be made about transgressive segregation in the populations. Table 3-2 lists the crosses, the field reactions of the parents, and the general field reaction of the F_2 populations. The field reactions of the parents were from data from 1977, 1978, 1979, and 1980. Although all of the parent cultivars were susceptible or intermediate to the five Montana virulence types in the seedling stage, some had mature plant resistance. For example: Firlbeck's III, Larker, and Hypana were resistant or intermediate-to-resistant in the field, whereas they were susceptible or intermediate-to-susceptible as seedlings. Apparently, these cultivars have contributed their mature plant resistance to the F_2 populations of which they were parents. Two exceptions are BB F_2's

#20 and #22 in which Betzes-derived lines were the female parents. Apparently, Betzes' susceptibility is dominant or epistatic to the resistance of Firlbeck's III and Hypana.

Transgressive segregation was observed in the populations #1, #8, #10, #11, #12, #14, #15, #18, and #19. The low level of disease in the nursery and the fact that the parents' reactions were taken from several years' data may have biased these results.

Table 3-1. General observations of the reactions of F_2 barley populations inoculated with a combination of isolates of *Pyrenophora teres* under field conditions.

Cross: BB F_2 #1 Manchuria (CI 2330 msg 10) x Georgie

Observations: Mostly resistant (type 1-2) reactions with no spread up the plants; many escapes. Selected the most susceptible plants and a random sample of resistant plants.

Cross: BB F_2 #2 Manchuria (CI 2330 msg 10) x Firlbeck's III

Observations: Approximately one-half of the plants susceptible (type 3-4 reactions) with good spread up the plants and one-half of the plants resistant (type 1-2 reactions) with little or no spread up the plants. Selected the most resistant and most susceptible plants.

Cross: BB F_2 #3 Manchuria (CI 2330 msg 10) x Hector

Observations: Mostly susceptible (type 3-4 reactions) with good spread up the plants. Selected the most resistant and most susceptible plants.

Cross: BB F_2 #6 Manchuria (CI 2330 msg 10) x Bruens Wisa

Observations: Mostly intermediate (type 2-4 reactions) with some spread up the most susceptible plants. Selected the most resistant and most susceptible plants.

Cross: BB F_2 #7 Manchuria (CI 2330 msg 10) x Maris Mink

Observations: Mostly intermediate (type 2-3 reactions) with a little spread up some plants. Selected the most resistant and most susceptible plants.

Cross: BB F_2 #8 Manchuria (CI 2330 msg 10) x Virio

Observations: Mostly resistant (type 1-2 reactions) with no spread up the plants; many escapes. Selected random samples of resistant and susceptible plants.

Table 3-1. Cont.

Cross:	BB F_2 #9 Manchuria (CI 2330 msg 10) x Palliser
Observations:	Mostly susceptible (type 3-4 reactions) with good spread up the plants. Selected the most resistant and most susceptible plants.
Cross:	BB F_2 #10 Manchuria (CI 2330 msg 10) x Ingrid
Observations:	Approximately one-half of the plants susceptible (type 3-4 reactions) with good spread up the plants and one-half of the plants resistant (type 1-3 reactions) with little or no spread up the plants. Selected the most resistant and most susceptible plants.
Cross:	BB F_2 #11 Manchuria (CI 2330 msg 10) x Vanguard
Observations:	Mostly resistant (type 1-2 reactions) with no spread up the plant and a few susceptible plants (type 3-4 reactions) with some spread up the plants. Selected the most susceptible plants and a random sample of resistant plants.
Cross:	BB F_2 #12 Manchuria (CI 2330 msg 10) x Klages
Observations:	Approximately one-half of the plants resistant (type 1-2 reactions) with little or no spread up the plants and one-half of the plants susceptible (type 3-4 reactions) with good spread up the plants. Selected the most resistant and most susceptible plants.
Cross:	BB F_2 #13 Manchuria (CI 2330 msg 10) x Compana
Observations:	Mostly susceptible (type 3-4 reactions) with extensive spread up the plants; many escapes. Selected the most resistant plants and a random sample of susceptible plants.

Table 3-1. Cont.

Cross:	BB F_2 #14 Manchuria (CI 2330 msg 10) x Freja
Observations:	Mostly resistant (type 1-2 reactions) with no good spread up the plants; many escapes. Selected random samples of resistant and susceptible plants.
Cross:	BB F_2 #15 Manchuria (CI 2330 msg 10) x Beecher
Observations:	Mostly resistant (type 1-2 reactions) with no spread up the plants; many escapes. Selected random samples of resistant and susceptible plants.
Cross:	BB F_2 #16 Manchuria (CI 2330 msg 10) x Zephyr
Observations:	Mostly susceptible (type 2-4 reactions) with some spread up the plants. Selected the most resistant plants and a random sample of susceptible plants.
Cross:	BB F_2 #17 Manchuria (CI 2330 msg 10) x Larker
Observations:	Mostly resistant (type 1-2 reactions) with no spread up the plants; many escapes. Selected random samples of resistant and susceptible plants.
Cross:	BB F_2 #18 Betzes ert-a msg 23 x Georgia
Observations:	Approximately one-half the plants resistant (type 1-2 reactions) with little or no spread up the plants, and one-half susceptible (type 3-4 reactions) with good spread up the plants. Selected the most resistant and most susceptible plants.
Cross:	BB F_2 #19 Tifang x Georgie
Observations:	Mostly resistant (type 1-2 reactions) with no spread up the plants. Selected random samples of resistant and susceptible plants.

Table 3-1. Cont.

Cross: BB F_2 #20	Betzes ert-a msg 23 x Hypana
Observations:	Mostly susceptible (type 3-4 reactions) with good spread up the plants. Selected the most resistant plants and a random sample of susceptible plants.
Cross: BB F_2 #21	Georgie x Hypana
Observations:	Mostly resistant (type 1-2 reactions) with little or no spread up the plants, and a few susceptible (type 2-4 reactions) with some spread up the plant. Selected the most susceptible plants and a random sample of resistant plants.
Cross: BB F_2 #22	Betzes msg ω x Firlbeck's III
Observations:	Mostly susceptible (type 2-4 reactions) with good spread up the plants. Selected the most resistant plants and a random selection of susceptible plants.
Cross: BB F_2 #23	Georgie x Firlbeck's III
Observations:	Mostly resistant (type 1-2 reactions) with no spread up the plants; many escapes. Selected random samples of resistant and susceptible plants.
Cross: BB F_2 #24	Tifang x Firlbeck's III
Observations:	Mostly resistant (type 1-2 reactions) with no spread up the plants; many escapes. Selected random samples of resistant and susceptible plants.
Cross: BB F_2 #25	Firlbeck's III x Hypana
Observations:	Mostly resistant (type 1-2 reactions) with no spread up the plants; many escapes. Selected random samples of resistant and susceptible plants.

Table 3-2. Reactions of F_2 barley populations and their parents to *Pyrenophora teres* under field conditions.

BB F_2 #1	Manchuria (CI 2330 msg 10)	X	Georgie
	I-S[a]	F_2	I-S
		R[b]	
BB F_2 #2	CI 2330	X	Firlbeck's III
	I-S	F_2	I-R
		R&S	
BB F_2 #3	CI 2330	X	Hector
	I-S	F_2	S
		S	
BB F_2 #6	CI 2330	X	Bruens Wisa
	I-S	F_2	I
		I	
BB F_2 #7	CI 2330	X	Maris Mink
	I-S	F_2	I-S
		I	
BB F_2 #8	CI 2330	X	Virio
	I-S	F_2	I-S
		R[b]	
BB F_2 #9	CI 2330	X	Palliser
	I-S	F_2	I-S
		S	

Table 3-2 Cont.

BB F_2 #10	CI 2330	X	Ingrid
	I-S	$\frac{F_2}{R\&S^b}$	I
BB F_2 #11	CI 2330	X	Vanguard
	I-S	$\frac{F_2}{R\&S^b}$	S
BB F_2 #12	CI 2330	X	Klages
	I-S	$\frac{F_2}{R\&S^b}$	S
BB F_2 #13	CI 2330	X	Compana
	I-S	$\frac{F_2}{S}$	S
BB F_2 #14	CI 2330	X	Freja
	I-S	$\frac{F_2}{R^b}$	I-S
BB F_2 #15	CI 2330	X	Beecher
	I-S	$\frac{F_2}{R^b}$	I
BB F_2 #16	CI 2330	X	Zephyr
	I-S	$\frac{F_2}{S}$	S

Table 3-2. Cont.

BB F_2 #17	CI 2330	X	Larker
	I-S	F_2	I-R
		R	
BB F_2 #18	Betzes ert-a msg 23	X	Georgie
	S	F_2	I-S
		R&S[b]	
BB F_2 #19	Tifang	X	Georgie
	I	F_2	I-S
		R[b]	
BB F_2 #20	Betzes ert-a msg 23	X	Hypana
	S	F_2	I-R
		S	
BB F_2 #21	Georgie	X	Hypana
	I-S	F_2	I-R
		S	
BB F_2 #22	Betzes msg ω	X	Firlbeck's III
	S	F_2	I-R
		S	
BB F_2 #23	Georgie	X	Firlbeck's III
	I-S	F_2	I-R
		R	

Table 3-2. Cont.

BB F_2 #24	Tifang	X	Firlbeck's III
	I	F_2	I-R
		R	
BB F_2 #25	Firlbeck's III	X	Hypana
	I-R	F_2	I-R
		R	

[a]Field reactions based on reaction type (0-4) and extent of symptom spread on the plants.

[b]Reaction of the F_2 population indicates transgressive segregation for resistance.

Chapter 4

F_3 PROGENY TESTING

The F_3 progenies of some of the previously selected F_2 plants were screened for seedling resistance to net blotch. Time and space limitations prohibited the progeny testing of all selected plants, so a few resistant and susceptible progenies were tested from each of 11 of the 23 populations. Both resistant and susceptible progenies were tested to compare selection for resistance in the F_2 generation to no selection, or negative selection as was the case of the susceptible progenies. Transgressive segregation was expected in the progenies of plants with minor genes for resistance.

Materials and Methods

The F_3 progenies from previously selected F_2 plants were grown in a controlled environment room (CER) at 15/24°C on a 12 hr photoperiod (26,900 lux combined incandescent and cool-white fluorescent light). All seeds from a single F_2 plant were planted in autoclaved field soil in racks of 'cone-tainer super-cells,' four to seven seeds per cone. Each rack was 24" x 12" x 7" and held 98 cones. The cones are slightly tapered, 1½" top diameter and 8" deep.

All progenies of each cross were inoculated when eight to 10 days old with a single, highly virulent isolate of *P. teres*. Isolate Pt R was used to screen the progenies of the populations: BB F_2: #1, #2, #3, #6, #7, #9. Isolate Mt 77-5I was used to screen the progenies of the populations: BB F_2: #18, #19, #22, #23, #24. The fungus was

cultured on V-8 juice agar in an incubation chamber at 17-18°C and eight hrs of light (20 watt cool-white fluorescent). The isolates were initially established by single spore transfers from infested leaf tissue on water agar to V-8 juice agar plates. Subsequent culturing was done by transferring small masses of mycelia and spores to four spots on fresh agar plates with a dissecting needle. Cultures to be used as inocula, were started on the same day that the barley to be inoculated was planted.

The inoculum was prepared by flooding the plates of eight to 10 day old cultures with distilled water and scraping the colonies with a microscope slide. The spores were rinsed through four layers of cheesecloth, twice, to remove mycelial fragments. The spore concentrations were measured using a Howard Mold Counting Chamber (Hausser Scientific) and the inoculum standardized at 15,000 spores per ml. Approximately 0.2 ml of 1% Tween 20 was added to each 100 ml of inoculum as a surfactant. The inoculum was applied using a DeVilbiss No. 15 atomizer driven by compressed air at 15-20 psi. Approximately 100 ml of inoculum was applied to each rack of cones. After inoculation the racks were placed in an unlighted dew-simulation chamber at 24-27°C for 24 hrs, then returned to the CER. Seven days after inoculation each plant was rated for net blotch resistance using a scale of 0-4:

0: no observable infection.

1: pin-point to very slightly elongated lesions, no chlorosis.

2: slightly elongated lesions, slight chlorosis.

3: elongated lesions, criss-crossed with net-like venation, moderate chlorosis.

4: well developed, netted lesions, extensive chlorosis and necrosis.

Mesothetic reactions were often observed. They were indicated by listing the different reaction types observed, separated by a comma, in the order of abundance in which they occurred. Each plant was classified as resistant, intermediate, or susceptible as follows:

Resistant: Plants with type 1 or type 2 reactions, or a combination of type 1 and 2 reactions.

Intermediate: Plants with type 3 reactions, or a combination of type 2 and type 3 reactions.

Susceptible: Plants with type 4 reactions, or a combination of type 3 and 4 reactions.

The plants classified resistant or intermediate were saved for seed production by transplanting into four-inch plastic pots. These plants were grown for one week in the CER to establish good roots, then grown in a CER at 40-45^{o}F and 12 hrs light for three to four weeks to strengthen the plants and promote tillering. The plants were then transplanted into eight inch plastic pots, four plants per pot, and

grown to maturity in a greenhouse at 55/70°F night/day temperatures and natural daylight supplemented with 14 hrs of fluorescent and incandescent light.

Results and Discussion

The results of this experiment are summarized in Table 4-1. The progeny are listed according to the F_2 population number, followed by the letter R or S designating the classification of the F_2 plant, resistant or susceptible, followed by a number arbitrarily assigned to each progeny as they were tested to maintain autonomy among the F_3 progenies. For example: 3R-4 designates the F_3 progeny of a single F_2 plant from the population BB F_2 #3 (Manchuria x Hector) that was selected as resistant in the field, and is the fourth resistant plant progeny from the population to be tested.

The number of plants in each class, resistant, intermediate or susceptible were counted and the percentage of the total number of plants was computed. Many of the progenies were 100% susceptible. None of the progenies contained any resistant plants, but some of the progenies contained intermediate plants. These ranged from less than 1%, to over 10% intermediate. The mean of the '% Intermediate' class is listed for the resistant and susceptible groups of progenies of each cross. The mean of the resistant group of each cross is equal to or greater than the mean of the susceptible group of the same cross. This was expected since the F_2 plants were selected for resistance and

susceptibility. Of course, the plants were selected under low levels of disease and often at random, so the effectiveness of the selection is subject to question. It is possible that selection under severe disease conditions would result in F_3 progenies that would have higher percentages of resistant and intermediate plants than those tested in this study. The fact that some of the susceptible progenies contained intermediate plants might be due to the selection, or could indicate that resistance is likely to show up in later generations even though it was not apparent in the F_2.

Table 4-1. Disease classification of the F_3 progenies of barley inoculated with a single isolate of *Pyrenophora teres*.

F_3 Progeny	Total # Plants	% Resistant	% Intermediate	% Susceptible
1R-1	306	0.0	0.0	100.0
1R-2	530	0.0	1.1	98.9
1R-3	282	0.0	1.4	98.6
1R-4	435	0.0	0.0	100.0
			$\bar{x}$=0.6[a]	
1S-1	271	0.0	0.0	100.0
1S-2	530	0.0	0.6	99.4
1S-3	236	0.0	1.3	98.7
1S-4	943	0.0	0.0	100.0
1S-5	362	0.0	0.3	99.7
1S-6	175	0.0	0.0	100.0
			$\bar{x}$=0.4	
2R-1	818	0.0	0.1	99.9
2R-2	300	0.0	0.0	100.0
2R-3	1432	0.0	0.2	99.8
2R-4	193	0.0	0.0	100.0
2R-5	535	0.0	0.2	99.8
2R-6	209	0.0	0.0	100.0
2R-7	347	0.0	1.7	98.3
			$\bar{x}$=0.3	
2S-1	247	0.0	0.0	100.0
2S-2	268	0.0	0.4	99.6
2S-3	473	0.0	0.0	100.0
2S-4	885	0.0	0.2	99.8
2S-5	522	0.0	0.0	100.0
			$\bar{x}$=0.1	
3R-1	604	0.0	0.3	99.7
3R-2	752	0.0	0.1	99.9
3R-3	241	0.0	0.0	100.0
3R-4	312	0.0	3.5	96.5
3R-5	100	0.0	0.0	100.0
3R-6	112	0.0	0.0	100.0
			$\bar{x}$=0.7	

Table 4-1. Cont.

F_3 Progeny	Total # Plants	% Resistant	% Intermediate	% Susceptible
3S-1	545	0.0	0.0	100.0
3S-2	324	0.0	0.0	100.0
3S-3	498	0.0	0.0	100.0
3S-4	246	0.0	0.0	100.0
3S-5	326	0.0	0.0	100.0
3S-6	163	0.0	0.0	100.0
3S-7	232	0.0	0.0	100.0
			$\bar{x}$=0.0	
6R-1	589	0.0	0.0	100.0
6R-2	329	0.0	0.0	100.0
6R-3	389	0.0	0.0	100.0
			$\bar{x}$=0.0	
6S-1	672	0.0	0.0	100.0
6S-2	293	0.0	0.0	100.0
6S-3	497	0.0	0.0	100.0
			$\bar{x}$=0.0	
7R-1	493	0.0	0.0	100.0
7R-2	44	0.0	0.0	100.0
7R-3	976	0.0	0.0	100.0
7R-4	464	0.0	0.0	100.0
7R-5	26	0.0	0.0	100.0
7R-6	192	0.0	0.0	100.0
7R-7	157	0.0	0.0	100.0
7R-8	87	0.0	0.0	100.0
			$\bar{x}$=0.0	
7S-1	400	0.0	0.0	100.0
7S-2	52	0.0	0.0	100.0
7S-3	149	0.0	0.0	100.0
7S-4	227	0.0	0.0	100.0
			$\bar{x}$=0.0	

Table 4-1. Cont.

F_3 Progeny	Total # Plants	% Resistant	% Intermediate	% Susceptible
9R-1	207	0.0	0.0	100.0
9R-2	361	0.0	1.1	98.9
9R-3	777	0.0	3.4	96.6
9R-4	365	0.0	4.1	95.9
9R-5	282	0.0	2.5	97.5
9R-6	444	0.0	0.0	100.0
9R-7	280	0.0	1.8	98.2
9R-8	196	0.0	1.0	99.0
9R-9	605	0.0	0.8	98.2
9R-10	247	0.0	4.5	95.5
9R-11	206	0.0	0.0	100.0
9R-12	186	0.0	0.0	100.0
9R-13	378	0.0	0.0	100.0
			$\bar{x}$=1.5	
9S-1	185	0.0	2.2	97.8
9S-2	359	0.0	2.2	97.8
9S-3	258	0.0	0.0	100.0
			$\bar{x}$=1.5	
18R-1	1154	0.0	0.2	99.8
18R-2	248	0.0	0.4	99.6
18R-3	252	0.0	0.0	100.0
18R-4	833	0.0	0.0	100.0
18R-5	1023	0.0	0.6	99.4
18R-6	1220	0.0	0.0	100.0
18R-7	262	0.0	0.0	100.0
18R-8	325	0.0	0.0	100.0
			$\bar{x}$=0.2	
18S-1	297	0.0	0.0	100.0
18S-2	314	0.0	0.0	100.0
18S-3	431	0.0	0.0	100.0
18S-4	237	0.0	0.0	100.0
18S-5	357	0.0	0.0	100.0
			$\bar{x}$=0.0	