



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
Montana State University
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Abstract:

Research was initiated to demonstrate transgressive segregation in barley for resistance to net blotch, caused by *Pyrenophora teres* Drechs. 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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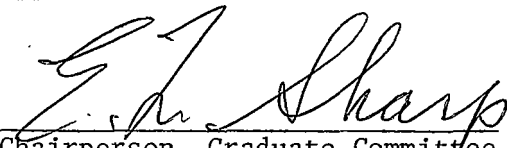
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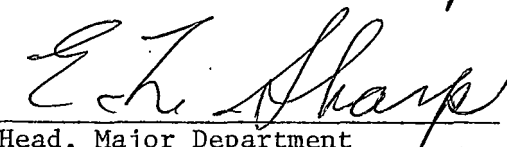
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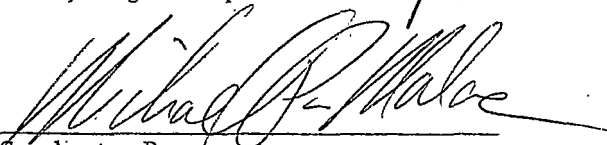
in

Plant Pathology

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Bozeman, Montana

January, 1981

ACKNOWLEDGMENT

I wish to express my love and admiration for my wife, Kathy, who supported us during my graduate studies and typed this thesis.

I also wish to thank the following people:

Dr. E. L. Sharp for his support while serving as my major professor.

Drs. D. E. Mathre, A. L. Scharen, and J. M. Martin for their help and advice while serving on my graduate committee and instructing me in coursework.

Dr. H. E. Bockelman for his help and advise on my research program.

My fellow graduate students for their friendship and support throughout my studies.

The U. S. Agency for International Development for financial support.

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ABSTRACT

Research was initiated to demonstrate transgressive segregation in barley for resistance to net blotch, caused by *Pyrenophora teres* Drechs. 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_4 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 (Shipton, 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.) Drechs1., 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* Drechs1. 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 μ m), 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 Bantari, 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 contribute 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°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 Winnipeg, 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 (Parmer, 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 F₂ barley populations inoculated with a combination of isolates of *Pyrenophora teres* under field conditions.

Cross: BB F₂ #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 F₂ #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 F₂ #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 F₂ #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 F₂ #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 F₂ #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 F₂ #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 F₂ #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 F₂ #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 F₂ #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 F₂ #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 F₂ #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 F₂ #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 F₂ #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 F₂ #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 F₂ #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 F₂ #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 F₂ barley populations and their parents to *Pyrenophora teres* under field conditions.

BB F ₂ #1	<u>Manchuria (CI 2330 msg 10)</u> I-S ^a	X $\frac{F_2}{R^b}$	<u>Georgie</u> I-S
BB F ₂ #2	<u>CI 2330</u> I-S	X $\frac{F_2}{R\&S}$	<u>Firlbeck's III</u> I-R
BB F ₂ #3	<u>CI 2330</u> I-S	X $\frac{F_2}{S}$	<u>Hector</u> S
BB F ₂ #6	<u>CI 2330</u> I-S	X $\frac{F_2}{I}$	<u>Bruens Wisa</u> I
BB F ₂ #7	<u>CI 2330</u> I-S	X $\frac{F_2}{I}$	<u>Maris Mink</u> I-S
BB F ₂ #8	<u>CI 2330</u> I-S	X $\frac{F_2}{R^b}$	<u>Virio</u> I-S
BB F ₂ #9	<u>CI 2330</u> I-S	X $\frac{F_2}{S}$	<u>Palliser</u> I-S

Table 3-2 Cont.

BB F ₂ #10	<u>CI 2330</u> I-S	X <u>F₂</u> R&S ^b	<u>Ingrid</u> I
BB F ₂ #11	<u>CI 2330</u> I-S	X <u>F₂</u> R&S ^b	<u>Vanguard</u> S
BB F ₂ #12	<u>CI 2330</u> I-S	X <u>F₂</u> R&S ^b	<u>Klages</u> S
BB F ₂ #13	<u>CI 2330</u> I-S	X <u>F₂</u> S	<u>Compana</u> S
BB F ₂ #14	<u>CI 2330</u> I-S	X <u>F₂</u> R ^b	<u>Freja</u> I-S
BB F ₂ #15	<u>CI 2330</u> I-S	X <u>F₂</u> R ^b	<u>Beecher</u> I
BB F ₂ #16	<u>CI 2330</u> I-S	X <u>F₂</u> S	<u>Zephyr</u> S

Table 3-2. Cont.

BB F ₂ #17	<u>CI 2330</u> I-S	X <u>F₂</u> R	<u>Larker</u> I-R
BB F ₂ #18	<u>Betzes ert-a msg 23</u> S	X <u>F₂</u> R&S ^b	<u>Georgie</u> I-S
BB F ₂ #19	<u>Tifang</u> I	X <u>F₂</u> R ^b	<u>Georgie</u> I-S
BB F ₂ #20	<u>Betzes ert-a msg 23</u> S	X <u>F₂</u> S	<u>Hypana</u> I-R
BB F ₂ #21	<u>Georgie</u> I-S	X <u>F₂</u> S	<u>Hypana</u> I-R
BB F ₂ #22	<u>Betzes msg ω</u> S	X <u>F₂</u> S	<u>Firlbeck's III</u> I-R
BB F ₂ #23	<u>Georgie</u> I-S	X <u>F₂</u> R	<u>Firlbeck's III</u> I-R

Table 3-2. Cont.

BB F ₂ #24	<u>Tifang</u>	X	<u>Firlbeck's III</u>
	I	<u>F₂</u>	I-R
		R	
BB F ₂ #25	<u>Firlbeck's III</u>	X	<u>Hypana</u>
	I-R	<u>F₂</u>	I-R
		R	

^aField reactions based on reaction type (0-4) and extent of symptom spread on the plants.

^bReaction of the F₂ population indicates transgressive segregation for resistance.

Chapter 4

F₃ PROGENY TESTING

The F₃ progenies of some of the previously selected F₂ 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 F₂ 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 F₃ progenies from previously selected F₂ 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 F₂ 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 F₂: #1, #2, #3, #6, #7, #9. Isolate Mt 77-5I was used to screen the progenies of the populations: BB F₂: #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°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 F₃ progenies of barley inoculated with a single isolate of *Pyrenophora teres*.

<u>F₃ Progeny</u>	<u>Total # Plants</u>	<u>% Resistant</u>	<u>% Intermediate</u>	<u>% Susceptible</u>
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
			<u>x=0.6^a</u>	
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
			<u>x=0.4</u>	
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
			<u>x=0.3</u>	
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
			<u>x=0.1</u>	
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
			<u>x=0.7</u>	

Table 4-1. Cont.

<u>F₂</u> Progeny	<u>Total # Plants</u>	<u>% Resistant</u>	<u>% Intermediate</u>	<u>% Susceptible</u>
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
			<u>0.0</u>	
			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
			<u>0.0</u>	
			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
			<u>0.0</u>	
			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
			<u>0.0</u>	
			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
			<u>0.0</u>	
			x=0.0	

Table 4-1. Cont.

<u>F₂</u> Progeny	<u>Total # Plants</u>	<u>% Resistant</u>	<u>% Intermediate</u>	<u>% Susceptible</u>
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
			<u>x=1.5</u>	
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
			<u>x=1.5</u>	
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
			<u>x=0.2</u>	
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
			<u>x=0.0</u>	

Table 4-1. Cont.

<u>F₃ Progeny</u>	<u>Total # Plants</u>	<u>% Resistant</u>	<u>% Intermediate</u>	<u>% Susceptible</u>
19R-1	143	0.0	0.0	100.0
19R-2	352	0.0	1.1	98.9
19R-3	295	0.0	1.7	98.3
19R-4	412	0.0	0.0	100.0
			<u>x=0.7</u>	
19S-1	305	0.0	0.0	100.0
19S-2	391	0.0	0.0	100.0
19S-3	518	0.0	0.0	100.0
19S-4	316	0.0	0.0	100.0
19S-5	478	0.0	0.0	100.0
			<u>x=0.0</u>	
22R-1	571	0.0	0.0	100.0
22R-2	361	0.0	0.0	100.0
22R-3	415	0.0	0.0	100.0
22R-4	420	0.0	2.1	97.9
22R-5	323	0.0	0.3	99.7
22R-6	392	0.0	0.0	100.0
22R-7	1126	0.0	0.4	99.6
22R-8	263	0.0	0.0	100.0
22R-9	234	0.0	0.0	100.0
			<u>x=0.3</u>	
22S-1	653	0.0	0.0	100.0
22S-2	536	0.0	0.0	100.0
22S-3	613	0.0	1.0	99.0
22S-4	441	0.0	0.0	100.0
			<u>x=0.3</u>	
23R-1	653	0.0	2.9	97.1
23R-2	306	0.0	0.0	100.0
23R-3	232	0.0	7.8	92.2
23R-4	247	0.0	0.0	100.0

Table 4-1. Cont.

<u>F₂</u> Progeny	<u>Total # Plants</u>	<u>% Resistant</u>	<u>% Intermediate</u>	<u>% Susceptible</u>
23R-5	83	0.0	0.0	100.0
23R-8	93	0.0	10.8	89.2
23R-9	503	0.0	3.0	97.0
			$\bar{x}=3.5$	
23S-1	332	0.0	0.0	100.0
23S-2	376	0.0	0.3	99.7
23S-5	616	0.0	0.5	99.5
			$\bar{x}=0.3$	
24R-1	239	0.0	8.4	91.6
24R-2	897	0.0	0.0	100.0
24R-3	269	0.0	0.0	100.0
24R-4	581	0.0	2.9	97.1
24R-5	203	0.0	1.0	99.0
			$\bar{x}=2.5$	
24S-1	393	0.0	0.5	99.5
24S-2	838	0.0	0.2	99.8
			$\bar{x}=0.4$	

^aMean % Intermediate for each group of resistant and susceptible progenies.

Chapter 5

F₄ PROGENY TESTING

The F₃ plants that had been selected for resistance and grown to maturity in the previous seedling screening work were harvested and the F₄ progenies screened for seedling resistance. It was anticipated that a higher percentage of the F₄ progenies would be resistant or intermediate than in the preceeding F₃ progenies.

Materials and Methods

The F₄ progenies were screened with the same isolate of *P. teres* that was used to screen the corresponding F₃ progenies, either Pt. R or Mt 77-51. The method of inoculation was the same as described in Chapter 4. The rating system was modified so that disease resistance could be quantitatively measured. The primary leaf of each plant was rated for its reaction type, as previously described, and for the percent of the leaf area that was affected by the lesions and the associated chlorosis and necrosis. Time was important when rating large numbers of plants, so visual estimates were made of the percent leaf area affected rather than direct measurements. The precision of the visual estimates was checked by tracing the leaf on a clear plastic grid, marking out the affected leaf area, and then counting the number of squares, total and affected. For the purpose of statistical analysis, the 0-4 reaction type was coded 1-10 and the percent leaf area affected was divided

by 10 to give a severity (Sev) rating.

<u>Reaction Type</u>	<u>Code</u>
4	10
4,3	9
3,4	8
3	7
3,2	6
2,3	5
2	4
2,1	3
1,2	2
1	1

This code was then used as a multiplier of the severity rating to get a 'Disease Index' rating for each plant. An example of the computation of 'Disease Index' ratings is as follows:

<u>Rxn Type</u>	<u>Code</u>	<u>% Leaf Area Affected</u>	<u>Sev</u>	<u>Disease Index</u>
4	10	40	4.0	40.0
3,4	8	25	2.5	20.0
2	4	10	1.0	4.0

The 'Disease Index' values were used to compute the mean and variance for each F_4 progeny and its parents. A comparison between the F_4 progenies and their parents was made using a t-test. Each rack of 'cone-tainers' was an experimental unit. At least 25 seeds of some of the F_4 progenies and their parents were planted in each rack. Ten plants of each parent cultivar were rated and used to calculate a mean and standard deviation. All F_4 plants that emerged were rated and used to calculate means and standard deviations for the progenies. The environmental variability within racks was minimized by planting

resistant and susceptible checks in the perimeter row of cones to buffer the test lines.

Results and Discussion

More than one rack was needed to screen all the progenies of each cross. The variation among the racks was found to be significant at the 5% level by comparing the means of the parent cultivars planted in each rack. Consequently, the comparisons of the F_4 progenies and their parents were made within each rack. The variances of the lines within a rack were sometimes found to be unequal using Hartley's maximum F test (David, 1952). This is partly because a higher variance is associated with higher means using this system of quantification. The comparisons can be made within a rack if the unequal variances and unequal sample sizes are taken into consideration. The method is described in section 4.14 of "Statistical Methods" by Snedecor and Cochran (1967). Linear comparisons were made using the 'Compare' program of MSUSTAT (1/1980 version, MSU Statistical Center, R. E. Lund). A one-tailed t-value was used to test if the mean 'Disease Index' value of the F_4 progenies was significantly less than that of the parents at the 5% level. Each F_4 progeny mean was compared to the average value of the two parents (mid-parent) and to the mean of the most resistant parent (low-parent). The results are listed in Table 5-1.

One hundred-nine F_4 lines were tested. Eighty-four of these had 'Disease Index' means that were significantly less than the mid-parent 'Disease Index' mean from the same experimental unit. Sixty-nine had means significantly less than the low-parent mean from the same experimental unit.

In addition to comparing the 'Disease Index' ratings of the populations, the percentage of the total number of plants in each progeny that were resistant, intermediate, and susceptible was computed. The criterion for classification was changed slightly from Chapter 4 to include the percent leaf area affected. The classification is as follows:

Resistant: Plants with type 1 or type 2 reactions, or a combination of type 1 and 2 reactions, i.e. 1,2 and 2,1, regardless of the percent leaf area affected.

Intermediate: Plants with type 3 reactions, or a combination of type 2 and type 3 reactions, i.e. 2,3 and 3,2, regardless of the percent leaf area affected; and plants with a combination of type 3 and 4 reactions, i.e. 3,4 only, with less than 30% leaf area affected.

Susceptible: Plants with type 4 reactions, or a combination of type 3 and 4 reactions, i.e. 4,3, regardless of the percent leaf area affected; or 3,4 reactions with 30% or higher leaf area affected.

The results are listed in Table 5-2 along with the same data for

the corresponding F_3 progenies. The percent resistant and intermediate is considerably higher in most of the F_4 progenies than in the F_3 progenies. This indicates that progress has been made and that resistance is more likely to show up in the later generations as the plants become more homozygous.

When the data from Tables 5-1 and 5-2 are compared, it can be seen that the linear comparisons of the F_4 progenies and their parents using 'Disease Index' means detect some differences that are not apparent when inspecting the disease classification of the progenies. For instance, F_4 :9R-5-A has a 'Disease Index' mean that is significantly less than the mid-parent mean and the low-parent mean, but the progeny is classified 100% susceptible. This situation occurs with other F_4 progenies as well. The reason is that a wide range of 'Disease Index' values occurs among plants in the susceptible class. For instance, a rating of type 3,4 and 30% leaf area affected represents one of the lowest ratings for plants in the susceptible class. This rating equals a 'Disease Index' value of 24.0. The highest rating for plants in the susceptible class would be type 4 and 100% leaf area affected, giving a 'Disease Index' value of 100.0. This wide range of values means that quantitative differences can be detected among susceptible plants. Differences of this type might indicate the presence of minor genes in numbers too low to

confer qualitative differences in resistance.

The opposite case is also apparent. That is, some F_4 lines may contain resistant and intermediate plants, but have 'Disease Index' means that are not significantly different from those of the parents. This occurs with a few F_4 progenies of the crosses Tifang x Georgie (BB F_2 #19) and Georgie x Firlbeck's III (BB F_2 #23). One reason for this situation is that these three cultivars exhibit a variability of symptom expression under standard inoculation conditions. Therefore, some of the plants of the parent cultivars were intermediate in reaction, giving a low 'Disease Index' value, so the mean of the parent lines was so low that only the means of the best F_4 lines were declared significantly less.

It should be noted that data for F_4 lines: 1R-2-A, 1R-2-B, 1R-2-C, and 9R-2-A are listed in Table 5-2, but do not appear in Table 5-1. These lines were some of the first to be tested and were rated for reaction types only, so 'Disease Index' values were not calculated. They were classified on the basis of reaction types as in Chapter 4.

The crosses from which F_4 lines were obtained, can be compared for their general effectiveness at combining genes for additive resistance. Comparisons of the F_4 lines and their parents by 'Disease Index' means (Table 5-1) is the best indication of

transgressive segregation and consequent success of the cross based on quantitative measurement. Data from Table 5-2 make an interesting illustration of progress through selection in a qualitative sense. However, this is not the best way to illustrate transgressive segregation because some of the parents are variable in their reaction, as mentioned. The quantitative comparisons listed in Table 5-1 take this variability into account by comparing the F_4 progenies with their parents within the same experimental unit.

The data from both tables can be helpful in making comparisons among the crosses for their general effectiveness at combining genes for additive resistance. These data also indicate the success of the selection within the F_2 and F_3 generations.

A summary of the comparisons among the crosses is as follows, with the crosses listed in the order of successfulness:

CI 2330 msg 10 x Hector (BB F_2 #3) - All F_4 lines (13/13) are better than the most resistant parent. The F_4 lines are 45.1% resistant and 19.9% intermediate, overall.

Tifang x Firlbeck's III (BB F_2 #24) - Twenty-two out of 24 F_4 lines are better than the most resistant parent. The F_4 lines are 2.2% resistant and 69.9% intermediate, overall.

Betzes ert-a msg 23 x Georgie (BB F_2 #18) - Seven out of eight F_4 lines are better than the most resistant parent. The F_4 lines are 0.0%

resistant and 19.6% intermediate, overall.

Tifang x Georgie (BBF₂ #19) - Seven out of 10 F₄ lines are better than the most resistant parent. The F₄ lines are 3.1% resistant and 42.5% intermediate, overall.

Betzes msg w x Firlbeck's III (BBF₂ #22) - Two out of three F₄ lines are better than the most resistant parent. The F₄ lines are 16.7% resistant and 36.7% intermediate, overall.

Georgie x Firlbeck's III (BBF₂ #23) - Eight out of 14 F₄ lines are better than the most resistant parent. The F₄ lines are 34.7% resistant and 39.3% intermediate, overall.

CI 2330 msg 10 x Firlbeck's III (BBF₂ #2) - Four out of eight F₄ lines are better than the most resistant parent. The F₄ lines are 5.4% resistant and 18.7% intermediate, overall.

CI 2330 msg 10 x Palliser (BBF₂ #9) - Six out of 20 F₄ lines are better than the most resistant parent. The F₄ lines are 0.0% resistant and 10.1% intermediate, overall.

CI 2330 msg 10 x Georgie (BBF₂ #1) - None of the F₄ lines (0/9) are better than the most resistant parent. The F₄ lines are 0.0% resistant and 4.3% intermediate, overall.

Some speculation about the combining ability of the parent cultivars can be made. Four of the crosses have a common female parent, CI 2330 msg 10. This cultivar was used as the female parent

of the crosses that make up the base recurrent selection populations because it was known to be a good combiner. The four male parents of these crosses can be compared for their combining ability with CI 2330. These cultivars are Georgie, Firlbeck's III, Hector, and Palliser. Hector was by far the best combiner of this group. One hundred percent of the F_4 lines that were tested from the cross CI 2330 x Hector (BB F_2 #3) were better than the most resistant parent. Most of these lines contained some plants that exhibited a high level of resistance. Firlbeck's III was the next best combiner with CI 2330. Fifty percent of the F_4 lines tested from this cross were better than the most resistant parent. Firlbeck's III was the male parent in three other crosses in this study. It was a better combiner with Tifang, Beszes msg ω , and Georgie than with CI 2330. Palliser and Georgie were not good combiners with CI 2330, their F_4 lines being mostly susceptible.

Tifang, Firlbeck's III, and Georgie are good combiners with each other. Georgie and Firlbeck's III were also good combiners with Betzes-derived cultivars. Interestingly, these cultivars exhibit a variability of symptom expression. The plants varied in their reaction from susceptible to intermediate within a single experiment and often varied considerably among experiments. This problem was not solved. It appears likely that these cultivars

contain genes that condition resistance and were probably sensitive to subtle environmental changes. They may be heterogeneous, as well.

Table 5-1. Results of linear comparisons of the 'Disease Index' means of the F_4 lines and their parents.

<u>F_4 lines</u>	<u>Significantly Less than MP (5%)</u>	<u>Significantly Less than LP (5%)</u>
1R-3-A	+ ^a	-
1R-3-B	-	-
1R-3-C	-	-
1R-3-D	-	-
1S-2-A	-	-
1S-2-B	-	-
1S-2-C	-	-
1S-3-A	-	-
1S-3-B	+	-
Total Lines = 9	2	0
2R-5-A	+	-
2R-7-A	+	-
2R-7-B	+	+
2R-7-C	+	+
2R-7-D	+	-
2R-7-E	+	+
2S-2-A	+	+
2S-4-A	+	-
Total Lines = 8	8	4
3R-1-A	+	+
3R-1-B	+	+
3R-2-A	+	+
3R-4-A	+	+
3R-4-B	+	+
3R-4-C	+	+
3R-4-D	+	+
3R-4-E	+	+
3R-4-F	+	+
3R-4-G	+	+
3R-4-H	+	+
3R-4-I	+	+
3R-4-J	+	+
Total Lines = 13	13	13

Table 5-1. Cont.

<u>F₄ lines</u>	<u>Significantly Less than MP (5%)</u>	<u>Significantly Less than LP (5%)</u>
9R-2-B	+	+
9R-3-A	+	+
9R-3-B	+	-
9R-3-C	-	-
9R-4-A	+	-
9R-5-A	+	+
9R-5-B	-	-
9R-5-C	-	-
9R-7-A	-	-
9R-8-A	-	-
9R-8-B	-	-
9R-9-A	-	-
9R-9-B	+	+
9R-9-C	+	-
9R-9-D	+	+
9R-10-A	-	-
9R-10-B	+	+
9R-10-C	-	-
9S-2-A	-	-
9S-2-B	-	-
Total Lines = 20	9	6
18R-1-A	-	-
18R-1-B	+	+
18R-2-A	+	+
18R-5-A	+	+
18R-5-B	+	+
18R-5-C	+	+
18R-5-D	+	+
18R-5-E	+	+
Total Lines = 8	7	7
19R-2-A	+	+
19R-2-B	+	+
19R-2-C	+	+

Table 5-1. Cont.

<u>F₄ lines</u>	<u>Significantly Less than MP (5%)</u>	<u>Significantly Less than LP (5%)</u>
19R-3-A	+	+
19R-3-B	-	-
19R-3-C	+	+
19R-3-D	+	+
19R-3-E	+	+
19R-5-A	-	-
19R-5-B	+	-
Total Lines = 10	8	7
22R-4-C	+	+
22R-4-F	+	-
22R-5-A	+	+
Total Lines = 3	3	2
23R-1-B	-	-
23R-1-D	+	+
23R-1-G	+	-
23R-1-H	+	+
23R-1-I	+	-
23R-3-C	+	+
23R-3-F	-	-
23R-3-G	+	+
23R-3-H	-	-
23R-3-J	+	+
23R-3-L	+	+
23R-8-A	+	+
23R-8-C	+	+
23S-2-A	-	-
Total Lines = 14	10	8
24R-1-A	+	+
24R-1-B	+	+
24R-1-C	+	+
24R-1-D	+	+
24R-1-E	+	+

Table 5 -1. Cont.

<u>F₄ lines</u>	<u>Significantly Less than MP (5%)</u>	<u>Significantly Less than LP (5%)</u>
24R-1-F	+	+
24R-1-G	+	+
24R-1-H	+	+
24R-1-J	+	+
24R-1-K	+	+
24R-1-L	+	+
24R-4-A	+	+
24R-4-B	+	+
24R-4-C	+	+
24R-4-D	+	+
24R-4-F	+	+
24R-4-G	+	+
24R-4-H	+	+
24R-4-J	+	+
24R-5-A	+	+
24R-5-B	+	+
24S-1-A	+	-
24S-1-B	+	-
Total Lines = 24	24	22

^a + = 'Disease Index' mean of the F₄ line is significantly less than the mid-parent (MP) 'Disease Index' mean or the low-parent (LP) 'Disease Index' mean at the 5% level.

- = 'Disease Index' mean of the F₄ line is not significantly less than the mid-parent (MP) 'Disease Index' mean or the low-parent (LP) 'Disease Index' mean at the 5% level.

Table 5-2. Comparison of the disease classification of F_3 progenies and the F_4 progenies from selected F_3 plants.

	F_3 Progenies				F_4 Progenies		
	% R ^a	% I	% S		% R	% I	% S
1R-2	0.0	1.1	98.9	1R-2-A	0.0	31.6	68.4
"	"	"	"	1R-2-B	"	16.5	83.5
"	"	"	"	1R-2-C	"	3.3	96.7
1R-3	0.0	1.4	98.6	1R-3-A	0.0	0.0	100.0
"	"	"	"	1R-3-B	"	"	"
"	"	"	"	1R-3-C	"	"	"
"	"	"	"	1R-3-D	"	"	"
1S-2	0.0	0.6	99.4	1S-2-A	0.0	0.0	100.0
"	"	"	"	1S-2-B	"	"	"
"	"	"	"	1S-2-C	"	"	"
1S-3	0.0	1.3	98.7	1S-3-A	0.0	0.0	100.0
"	"	"	"	1S-3-B	"	"	"
				$\bar{x}^b =$	0.0	4.3	95.7
2R-5	0.0	0.2	99.8	2R-5-A	0.0	13.9	86.1
2R-7	0.0	1.7	98.3	2R-7-A	0.0	0.0	100.0
"	"	"	"	2R-7-B	3.3	30.0	66.7
"	"	"	"	2R-7-C	0.0	48.3	51.7
"	"	"	"	2R-7-D	"	3.0	97.0
"	"	"	"	2R-7-E	"	19.0	81.0
2S-2	0.0	0.4	99.6	2S-2-A	40.0	32.0	28.0
2S-4	0.0	0.2	99.8	2S-4-A	0.0	3.7	96.3
				$\bar{x} =$	5.4	18.7	75.9
3R-1	0.0	0.3	99.7	3R-1-A	0.0	1.8	98.2
"	"	"	"	3R-1-B	"	0.0	100.0
3R-2	0.0	0.1	99.9	3R-2-A	33.3	55.6	11.1
3R-4	"	3.5	96.5	3R-4-A	41.4	31.0	27.6
"	"	"	"	3R-4-B	89.1	8.7	2.2
"	"	"	"	3R-4-C	78.6	17.9	3.5
"	"	"	"	3R-4-D	0.0	16.7	83.3
"	"	"	"	3R-4-E	2.7	45.9	51.4
"	"	"	"	3R-4-F	33.3	44.4	22.3
"	"	"	"	3R-4-G	62.5	9.4	28.1

Table 5-2. Cont.

	<u>F₃ Progenies</u>				<u>F₄ Progenies</u>		
	% R	% I	% S		% R	% I	% S
3R-4	0.0	3.5	96.5	3R-4-H	77.4	19.4	3.2
"	"	"	"	3R-4-I	93.5	0.0	6.5
"	"	"	"	3R-4-J	75.0	8.3	16.7
				$\bar{x} =$	45.1	19.9	35.0
9R-2	0.0	1.1	98.9	9R-2-A	0.0	47.7	52.3
"	"	"	"	9R-2-B	"	47.9	52.1
9R-3	0.0	3.4	96.6	9R-3-A	0.0	34.9	65.1
"	"	"	"	9R-3-B	"	11.6	88.4
"	"	"	"	9R-3-C	"	0.0	100.0
9R-4	0.0	4.1	95.9	9R-4-A	0.0	0.0	100.0
9R-5	0.0	2.5	97.5	9R-5-A	0.0	0.0	100.0
"	"	"	"	9R-5-B	"	"	"
"	"	"	"	9R-5-C	"	"	"
9R-7	0.0	1.8	98.2	9R-7-A	0.0	0.0	100.0
9R-8	0.0	1.0	99.0	9R-8-A	0.0	0.0	100.0
"	"	"	"	9R-8-B	"	"	"
9R-9	0.0	0.8	99.2	9R-9-A	0.0	0.0	100.0
"	"	"	"	9R-9-B	"	16.4	83.6
"	"	"	"	9R-9-C	"	1.9	98.1
"	"	"	"	9R-9-D	"	0.0	100.0
9R-10	0.0	4.5	95.5	9R-10-A	0.0	0.0	100.0
"	"	"	"	9R-10-B	"	"	"
"	"	"	"	9R-10-C	"	"	"
9S-2	0.0	2.2	97.8	9S-2-A	0.0	28.8	71.2
"	"	"	"	9S-2-B	"	22.7	77.3
				$\bar{x} =$	0.0	10.1	89.9
18R-1	0.0	0.2	99.8	18R-1-A	0.0	0.0	100.0
"	"	"	"	18R-1-B	"	7.5	92.5
18R-2	0.0	0.4	99.6	18R-2-A	0.0	6.3	93.7
18R-5	0.0	0.6	99.4	18R-5-A	0.0	66.7	33.3
"	"	"	"	18R-5-B	"	0.1	90.9
"	"	"	"	18R-5-C	"	5.6	94.4
"	"	"	"	18R-5-D	"	15.4	84.6
"	"	"	"	18R-5-E	"	46.2	53.8
				$\bar{x} =$	0.0	19.6	80.4

Table 5-2. Cont.

	F ₃ Progenies				F ₄ Progenies		
	% R	% I	% S		% R	% I	% S
19R-2	0.0	1.1	98.9	19R-2-A	0.0	44.0	56.0
"	"	"	"	19R-2-B	"	55.6	44.4
"	"	"	"	19R-2-C	11.1	33.3	55.6
19R-3	0.0	1.7	98.3	19R-3-A	0.0	59.1	40.9
"	"	"	"	19R-3-B	"	4.5	95.5
"	"	"	"	19R-3-C	4.5	77.3	18.2
"	"	"	"	19R-3-D	15.4	84.6	0.0
"	"	"	"	19R-3-E	0.0	50.0	50.0
19S-5	0.0	0.0	100.0	19S-5-A	0.0	16.7	83.3
"	"	"	"	19S-5-B	"	0.0	100.0
				$\bar{x} =$	3.1	42.5	54.4
22R-4	0.0	2.1	97.9	22R-4-C	0.0	0.0	100.0
"	"	"	"	22R-4-F	"	60.0	40.0
22R-5	0.0	0.3	99.7	22R-5-A	$\bar{x} =$	50.0	0.0
					16.7	36.7	46.7
23R-1	0.0	2.9	97.1	23R-1-B	0.0	25.0	75.0
"	"	"	"	23R-1-D	"	22.2	77.8
"	"	"	"	23R-1-G	31.3	43.8	24.9
"	"	"	"	23R-1-H	0.0	66.7	33.3
"	"	"	"	23R-1-I	"	36.4	63.6
23R-3	0.0	7.8	92.2	23R-3-C	100.0	0.0	0.0
"	"	"	"	23R-3-F	50.0	"	50.0
"	"	"	"	23R-3-G	85.7	"	14.3
"	"	"	"	23R-3-H	40.0	40.0	20.0
"	"	"	"	23R-3-J	63.6	27.3	9.1
"	"	"	"	23R-3-L	71.4	28.6	0.0
23R-8	0.0	10.8	89.2	23R-8-A	0.0	66.7	33.3
"	"	"	"	23R-8-C	33.3	33.3	"
23S-2	0.0	0.3	99.7	23S-2-A	$\bar{x} =$	10.0	60.0
					34.7	39.3	26.0
24R-1	0.0	8.4	91.6	24R-1-A	0.0	46.7	53.3
"	"	"	"	24R-1-B	"	60.0	40.0
"	"	"	"	24R-1-C	"	71.4	28.6

Table 5-2. Cont.

	<u>F₃ Progenies</u>				<u>F₄ Progenies</u>		
	% R	% I	% S		% R	% I	% S
24R-1	0.0	8.4	91.6	24R-1-D	0.0	73.9	26.1
"	"	"	"	24R-1-E	"	57.7	42.3
"	"	"	"	24R-1-F	12.5	83.3	4.2
"	"	"	"	24R-1-G	8.3	91.7	0.0
"	"	"	"	24R-1-H	0.0	70.6	29.4
"	"	"	"	24R-1-I	"	42.9	57.1
"	"	"	"	24R-1-J	"	61.9	38.1
"	"	"	"	24R-1-K	"	54.5	45.5
"	"	"	"	24R-1-L	"	28.6	71.4
24R-4	0.0	2.9	97.1	24R-4-A	4.3	91.3	4.3
"	"	"	"	24R-4-B	7.1	92.9	0.0
"	"	"	"	24R-4-C	0.0	100.0	"
"	"	"	"	24R-4-D	7.1	92.9	"
"	"	"	"	24R-4-F	0.0	73.9	26.1
"	"	"	"	24R-4-G	6.3	81.3	12.5
"	"	"	"	24R-4-H	7.1	85.7	7.1
"	"	"	"	24R-4-J	0.0	94.1	5.9
24R-5	0.0	1.0	99.0	24R-5-A	0.0	75.0	25.0
"	"	"	"	24R-5-B	"	100.0	0.0
24S-1	0.0	0.5	99.5	24S-1-A	0.0	35.3	64.7
"	"	"	"	24S-1-B	"	14.3	85.7
					<u>x =</u>	<u>2.2</u>	<u>69.9</u>
							<u>27.9</u>

^a% R, % I, % S are the percentages of the total progeny that were resistant, intermediate, or susceptible, respectively.

^bMeans of the F₄ lines of each cross for each disease class.

Chapter 6

TESTING F_4 PROGENIES TO A COMBINATION OF VIRULENCE TYPES

Screening the F_4 progenies for resistance to a single isolate of *B. tereus* showed that several of the progenies contained resistant and intermediate plants. It was assumed that this resistance was due to the additive effects of minor genes since the parents of the progenies were susceptible to the virulence types used to screen the progenies.

If the resistance was due to additive effects, then it should have been effective against several virulence types of the pathogen. To determine this, some of the best F_4 progenies were tested against a combination of isolates representing all five Montana virulence types.

Materials and Methods

Eleven of the previously tested F_4 progenies were screened for resistance to a combination of virulence types. Five isolates were used, one representing each of the five Montana virulence types described by Bjarko (1979). The isolates were: Pt R (type A), Ftb 78-1 (type B), Mt 77-5I (type C), Mt 77-3 (type D), and Mt 77-6 (type E). Inoculum was prepared with each isolate as described in Chapter 4. Equal quantities of inoculum from each isolate were mixed to form the final inoculum. The inoculation procedure is described in Chapter 4. The plants were rated as described in Chapter 5.

Results and Discussion

The results are listed in Table 6-1. The disease reactions of the progenies to the combination of isolates were similar to their reactions to the single isolate against which they were selected. There were some changes in the percentages in each disease reaction class and in the 'Disease Index' mean. These were probably due to the variability among experimental units and because different plants from a segregating F_4 population were in the tests. It would have been preferable to test the F_4 lines to each isolate separately, but there was not enough seed of the lines to allow this.

The results indicate that the resistance of the F_4 lines is nonspecific in action.

Table 6-1. Disease classification of F_4 progenies when inoculated with a single isolate and a combination of five different virulence types of *Pyrenophora teres*.

F_4 Lines	Single Isolate			Combination of Isolates		
	R	I	S	R	I	S
2R-7-B	3.3	30.0	66.7	9.1	54.5	36.4
2R-7-C	0.0	48.3	51.7	25.0	75.0	0.0
3R-4-B	89.1	8.7	2.2	54.5	22.7	22.7
3R-4-C	78.6	17.9	3.5	70.0	20.0	10.0
3R-4-E	2.7	45.9	51.4	21.0	63.2	15.8
3R-4-G	62.5	9.4	28.1	61.5	19.2	19.2
3R-4-H	77.4	19.4	3.2	70.4	7.4	22.2
3R-4-I	93.5	0.0	6.5	76.2	9.5	14.3
19R-3-C	4.5	77.3	18.2	81.8	18.2	0.0
19R-3-D	15.4	84.6	0.0	100.0	0.0	0.0
22R-5-A	50.0	50.0	0.0	100.0	0.0	0.0

Chapter 7

SPORE PRODUCTION STUDIES

Keeling and Bantarri (1975) found that there were no differences in spore germination, germ tube growth, or number of penetrations on leaves of resistant and susceptible barley. Sporulation of the fungus on excised leaves was found to differ significantly between resistant and susceptible cultivars. More time was required for sporulation to occur and the amount of sporulation was significantly less on resistant cultivars. A study was initiated to compare some of the F_4 progenies and their parents on the basis of sporulation on excised leaves.

Materials and Methods

Four F_4 progenies of each cross, BB F_2 #2 (CI 2330 x Firlbeck's III) and BB F_2 #3 (CI 2330 x Hector) were tested for the time required for sporulation to occur and the number of spores produced per unit area of leaf. Six leaves of each F_4 progeny and their parents were collected at the time of disease rating. A 5 cm section of each leaf containing lesions was surface sterilized for 30 sec in 0.5% NaClO. The leaf sections were placed on microscope slides which were placed in moist chambers. The moist chambers were placed in an incubation chamber at 17-18°C and eight hrs of light (20 watt cool-white fluorescent). The leaves were separated into three replications with two leaves of each F_4 progeny and their

parents in each replication. The leaves were inspected at 24 hr intervals for the occurrence of sporulation. The number of spores produced was determined five days after the start of incubation. The spores were counted through a stereoscope at 63X magnification. Counts were made on four 0.64 mm^2 areas of each leaf. The areas were chosen at random on each leaf piece. The average of the four counts was computed for each leaf and standardized to the number of spores per mm^2 . These means were used to compute an analysis of variance for the F_4 progenies and their parents. The individual leaves of each line were treated as subsamples in the analysis. Linear comparisons were made between the mean of each F_4 progeny and the mean of the two parents (mid-parent value) and between the mean of each F_4 progeny and the mean of the best parent (low-parent value).

Results and Discussion

The results are listed in Tables 7-1 and 7-2. The data for 'Time Required for Sporulation' show the number of leaves out of six on which sporulation had occurred at 24 hr intervals. In Table 7-1 (data for CI 2330 x Firlbeck's III) it can be seen that two of the F_4 lines had leaves which required a longer period for sporulation than Firlbeck's III, the low-parent. These two lines produced numbers of spores per mm^2 of leaf that were significantly less (5%) than the mid-parent value, but not less than the low-parent value.

In Table 7-2 (data for CI 2330 x Hector) it can be seen that all four F_4 lines had leaves which required a longer period for sporulation than either parent. These lines also produced fewer spores per mm^2 of leaf than either parent. The mean number of spores produced by each F_4 line was significantly less (5%) than the mid-parent and low-parent means.

It is probable that more differences could have been detected between the parents and F_4 progenies of CI 2330 x Firlbeck's III if the spore counts had been made at 72 or 96 hrs instead of 120 hrs. All leaves of both parents had produced spores by 48 hrs and probably had maximum sporulation at 72 hrs, so there would have been no difference in the number of spores produced if the counts had been made earlier. This would have represented natural conditions more closely, as well.

The F_4 lines of CI 2330 x Hector exhibited a high level of resistance; typically type 1 or type 2 lesions, so it was not surprising that they were better than the parents.

This technique was very time consuming, but it could be valuable in detecting differences among cultivars that exhibit intermediate or susceptible reactions. The plant characters that affect sporulation of the pathogen are typical of those usually associated with minor gene, nonspecific resistance. These

factors can be very important in retarding the development of an epidemic.

Table 7-1. Sporulation of *Pyrenophora teres* on excised leaves of F₄ barley lines and parents of the cross CI 2330 x Firlbeck's III.

	TIME REQUIRED FOR SPORULATION			
	<u>24 hrs</u>	<u>28 hrs</u>	<u>72 hrs</u>	<u>96 hrs</u>
P ₁ (CI 2330)	6/6 ^a			
P ₂ (Firlbeck's III)	0/6	6/6		
F ₄ -1(2R-5-A)	1/6	4/6	6/6	
F ₄ -2(2R-7-A)	4/6	6/6		
F ₄ -3(2R-7-B)	1/6	6/6		
F ₄ -4(2S-2-A)	1/6	4/6	4/6	6/6

<u>Line</u>	<u>AMOUNT OF SPORULATION</u>
	<u>Mean # Spores/mm²</u>
P ₁ (CI 2330)	33.2
P ₂ (Firlbeck's III)	17.3
F ₄ -1(2R-5-A)	11.7 *MP ^b
F ₄ -2(2R-7-A)	22.9
F ₄ -3(2R-7-B)	22.1
F ₄ -4(2S-2-A)	13.2 *MP

^aNumber of leaves with sporulation/total number of leaves.

^bSignificantly less than mid-parent mean (MP) or both mid-parent and low-parent means (MP & LP) at the 5% level.

Table 7-2. Sporulation of *Pyrenophora teres* on excised leaves of F₄ barley lines and parents of the cross CI 2330 x Hector.

	TIME REQUIRED FOR SPORULATION			
	24 hrs	28 hrs	72 hrs	96 hrs
P ₁ (CI 2330)	4/6	6/6 ^a		
P ₂ (Hector)	4/6	6/6		
F ₄ -1 (3R-4-G)	2/6	3/6	3/6	5/6
F ₄ -2 (3R-4-H)	1/6	1/6	2/6	4/6
F ₄ -3 (3R-4-I)	1/6	1/6	1/6	4/6
F ₄ -4 (3R-4-J)	2/6	2/6	5/6	5/6

Line	AMOUNT OF SPORULATION	
	Mean # Spores/mm ²	
P ₁ (CI 2330)	63.0	
P ₂ (Hector)	64.6	
F ₄ -1 (3R-4-G)	15.1 *MP & LP ^b	
F ₄ -2 (3R-4-H)	16.7 *MP & LP	
F ₄ -3 (3R-4-I)	9.1 *MP & LP	
F ₄ -4 (3R-4-J)	18.8 *MP & LP	

^aNumber of leaves with sporulation/total number of leaves.

^bSignificantly less than mid-parent mean (MP) or both mid-parent and low-parent means (MP & LP) at the 5% level.

Chapter 8

CROSSES OF COMMERCIAL CULTIVARS AND SCREENING F₂'s IN FIELD AND GREENHOUSE

Crosses were made between 29 susceptible commercial cultivars to determine which of these cultivars might be good sources of minor gene, nonspecific resistance to *P. teres*. The parentage of the cultivars was taken into consideration so that only cultivars from diverse backgrounds were crossed. The F₂ progenies were screened for resistance in the field and in the growth chamber to determine which technique was most likely to detect low levels of resistance in the F₂ generations.

Materials and Methods

The crosses were made in the summer of 1979. The cultivars were included in the recurrent selection population crossing block. One or two heads were used for each cross. The crosses were made at random among unrelated cultivars, depending on which cultivars were ready on a given day. A total of 81 crosses were made using 26 commercial cultivars, many of which are currently grown in Montana. The F₁ seed was sent to Arizona to be grown in the winter nurseries at Mesa. The four best F₁ plants were harvested individually. Approximately 1000 F₂ seeds from one or two F₁ plants of 20 of the crosses, were planted in the 1980 Bozeman disease nursery, each in a plot of four 60 ft. rows. The parent cultivars were planted in single

10 ft rows. The plots were inoculated two times with a combination of isolates (as described in Chapter 3); the first time 33 days after planting with approximately 40,000 spores per ml, and the second time eight days later with approximately 50,000 spores per ml. The plots received rain just prior to, and after both inoculations, so it was not necessary to cover the inoculated plots with plastic to maintain a high relative humidity. Low volume, sprinkler irrigation was used about one week after the second inoculation to wet the plots and promote secondary spread of disease. Disease resistance was evaluated when the plants were in the heading stage. Eight, 10-plant samples, two from each row, were taken in each population. Each plant was rated for reaction type and percent leaf area affected (as described in Chapter 5). The leaves at the second and third nodes were evaluated and an average value recorded. Ten plants of each parent cultivar were also rated.

Each of the populations and their parents were also screened for resistance in a controlled-environment room to a single isolate, Pt R (as described in Chapter 4). At least 80 plants of each population and 10 plants of each parent cultivar were rated for reaction type and percent of the leaf area affected (as described in Chapter 5).

Results and Discussion

Frequency distributions for the F_2 data were plotted and the

means and standard deviations of the F_2 populations and parent cultivars were computed. Comparison of the field and growth chamber data showed that the F_2 populations and their parents generally had lower 'Disease Index' means and were less variable under field conditions than in the growth chamber (Table 8-1). The lower 'Disease Index' means observed in the field could have been due to mature plant resistance or to environmental conditions that were less than optimum for disease development. This was relatively unimportant compared to the variability of the populations. The fact that each of the 20 F_2 populations was more variable in the growth chamber tests may be valuable to the breeder. The variability observed in the F_2 populations tested in the growth chamber would not have been due to the environment because it was kept relatively constant and at optimum conditions. Therefore, the variability must have been due to genetic causes. This indicated that plants with low levels of resistance due to minor genes were expressing that resistance in the seedling stage. Some of these plants probably showed their resistance in the field, but it was not easily recognizable. Consequently, more transgressive segregation could be recognized in the growth chamber than in the field. Therefore, selection for transgressive segregates would have been easier in the growth chamber than in the field.

Most of the F_2 populations has 'Disease Index' means that were between the means of the parents in both field and growth chamber data. One F_2 population had means that were less than the low-parent means in both the field and growth chamber. This was the population from the cross Mona-Arivat x Atlas 68. This was good evidence for transgressive segregation. Three F_2 populations showed evidence of transgressive segregation in the growth chamber, but not in the field. These were from the crosses Hector x Manker, Palliser x Vanguard, and Atlas 68 x Mona-Arivat. Three F_2 populations showed evidence for transgressive segregation in the field, but not in the growth chamber. These were from the reciprocal crosses Compana x Atlas 68 and Atlas 68 x Compana and from the cross Karl x Betzes. The F_2 populations of the reciprocals Atlas 68 x Mona-Arivat and Mona-Arivat x Atlas 68 were interesting. The former showed transgressive segregation in the growth chamber, but not in the field, while the latter showed transgressive segregation mostly in the field, and only slightly in the growth chamber. The cross Mona-Arivat x Atlas 68 was more resistant than the reciprocal in the field, while the reverse is true in the growth chamber. This probably involved the penetrance and expressivity of the resistance gene(s) in Atlas 68.

It is interesting to note that these segregations would have probably gone unnoticed if only qualitative ratings were used.

Table 8-1. Comparison of field and growth chamber screening of F₂ barley populations and their parents by 'Disease Index' values.

Cross	'Disease Index' Values				
	Field		Growth Chamber		
	$\bar{x}$	SD	$\bar{x}$	SD	
<u>Klages x Larker</u>	P ₁ :	22.4	10.2	47.0	10.3
	P ₁ ¹ :	13.6	4.7	48.0	12.1
	F ₂ ² :	15.9	4.4	57.9	20.4
<u>Vanguard x Palliser</u>	P ₁ :	38.5	10.1	39.4	12.6
	P ₁ ¹ :	14.0	5.7	49.0	8.8
	F ₂ ² :	21.6	4.4	49.6	18.7
<u>Vanguard x Ingrid</u>	P ₁ :	38.5	10.1	39.4	12.6
	P ₁ ¹ :	15.1	3.4	28.6	7.2
	F ₂ ² :	19.9	4.9	33.9	14.6
<u>Vanguard x Freja</u>	P ₁ :	38.5	10.1	39.4	12.6
	P ₁ ¹ :	10.4	3.9	21.5	7.2
	F ₂ ² :	21.2	4.0	34.7	10.3
<u>Karl x Betzes</u>	P ₁ :	36.0	11.4	21.6	13.1
	P ₁ ¹ :	46.0	17.1	42.5	8.6
	F ₂ ² :	23.4	5.0	35.7	20.7
<u>Hector x Freja</u>	P ₁ :	50.0	18.4	49.0	8.8
	P ₁ ¹ :	10.4	3.9	21.5	7.2
	F ₂ ² :	29.6	8.2	26.5	10.3
<u>Hector x Manker</u>	P ₁ :	50.0	18.4	49.0	8.8
	P ₁ ¹ :	12.8	5.6	43.2	12.4
	F ₂ ² :	26.0	6.0	34.0	11.4
<u>Palliser x Vanguard</u>	P ₁ :	14.0	5.7	49.0	8.8
	P ₁ ¹ :	28.5	10.1	39.4	12.6
	F ₂ ² :	24.8	4.4	29.3	11.3

Table 8-1. Cont.

Cross		'Disease Index' Values			
		Field		Growth Chamber	
		$\bar{x}$	SD	$\bar{x}$	SD
<u>Hypana x Atlas 68</u>	P ₁ :	15.6	4.8	24.3	15.2
	P ₂ :	41.0	14.0	7.5	6.6
	F ₂ :	21.8	4.3	24.4	17.7
<u>Betzes x Ingrid</u>	P ₁ :	46.0	17.1	42.5	8.6
	P ₂ :	15.1	3.4	28.6	7.2
	F ₂ :	29.3	6.7	45.1	16.3
<u>Ingrid x Klages</u>	P ₁ :	15.1	3.4	28.6	7.2
	P ₂ :	22.4	10.2	47.0	10.3
	F ₂ :	33.0	6.7	42.3	13.7
<u>Ingrid x Vanguard</u>	P ₁ :	15.1	3.4	28.6	7.2
	P ₂ :	38.5	10.1	39.4	12.6
	F ₂ :	26.2	4.6	33.0	12.8
<u>Compana x Atlas 68</u>	P ₁ :	54.5	14.9	39.5	6.4
	P ₂ :	41.0	14.0	7.5	6.6
	F ₂ :	29.1	8.0	11.8	13.2
<u>Firlbeck's III x Freja</u>	P ₁ :	13.2	6.5	30.5	15.2
	P ₂ :	10.4	3.9	31.5	7.2
	F ₂ :	21.1	3.2	42.6	10.2
<u>Lud x Palliser</u>	P ₁ :	10.8	4.6	41.9	23.0
	P ₂ :	14.0	5.7	49.0	8.8
	F ₂ :	22.7	3.6	48.5	18.6
<u>Freja x Vanguard</u>	P ₁ :	10.4	3.9	21.5	7.2
	P ₂ :	38.5	10.1	39.4	12.6
	F ₂ :	23.9	3.6	40.3	14.5
<u>Manker x Hector</u>	P ₁ :	12.8	5.6	43.2	12.4
	P ₂ :	50.0	18.4	49.0	8.8
	F ₂ :	31.3	8.4	44.9	14.0

Table 8-1. Cont.

Cross		Disease Index Values			
		Field		Growth Chamber	
		$\bar{x}$	SD	$\bar{x}$	SD
<u>Atlas 68 x Compana</u>	P ₁ :	41.0	14.0	7.5	6.6
	P ₁ ¹ :	54.5	14.9	39.5	6.4
	F ₂ ² :	26.1	7.6	16.3	13.6
	F ₂ :				
<u>Atlas 68 x</u> <u>Mona-Arivat</u>	P ₁ :	41.0	14.0	7.5	6.6
	P ₁ ¹ :	12.8	4.9	26.6	11.3
	F ₂ ² :	20.4	2.6	3.7	6.9
	F ₂ :				
<u>Mona-Arivat x</u> <u>Atlas 68</u>	P ₁ :	12.8	4.9	26.6	11.3
	P ₁ ¹ :	41.0	14.0	7.5	6.6
	F ₂ ² :	8.9	3.0	7.0	8.2
	F ₂ :				

Chapter 9

DISCUSSION

Since *P. teres*, the causal organism of net blotch disease of barley, is so highly variable, the development of resistant cultivars should be aimed at resistance that will be effective against a wide range of virulence types. There are several known sources of resistance to *P. teres*, most are single, dominant genes. Bjarko (1979) found at least five different genes for resistance. The sources of these genes, and other sources of resistance, have been incorporated into recurrent selection populations at Montana State University (Bockelman, et al., 1981) in an attempt to develop broad based resistance to net blotch.

Broad based resistance can be established with both major and minor genes. The use of multiple major genes, those that impart a high level of resistance to a specific virulence type or race, can be highly effective in disease control. Modern wheat cultivars that are resistant to stem rust, caused by *Puccinia graminis* Pers. ex Pers. subsp. *graminis* f. sp. *tritici* (Erikss. and E. Henn.), in various parts of the world, utilize multiple genes for specific resistance rather than single genes alone (Watson, 1970). Late blight of potatoes, caused by *Phytophthora infestans* (Mont.) de Bary, is commonly controlled by cultivars with several major and minor genes for resistance (Nelson, 1978; Watson, 1970).

The success of such genotypes probably depends on several factors. One is the reduced probability that a strain of the pathogen can arise that has gained two or more genes for virulence. A related factor, proposed by van der Plank (1968), is that strains with several genes for virulence tend to lose fitness, so cultivars with several genes for resistance may be more stable in their reaction to disease. The possibility that genes will act in additive or complementary relationships to enhance the level of resistance shown by the particular genes alone could be important in the success of a broad based resistance.

The use of major, specific genes alone has some disadvantages to the use of major and minor genes together or minor genes alone. Most of the virulence changes noted in the past have been due to the use of cultivars with specific genes for resistance. Though resistance provided by multiple major genes can be highly effective, it may be rendered ineffective by growing lines in which the genes occur singly. It has been noted that in wheat, the progressive accumulation of genes for resistance to stem rust has been paralleled by the evolution of strains with several corresponding virulence genes (Watson, 1970). Similar evolution of complex strains has occurred with the late blight fungus through a series of steps (Nelson, 1978; Watson, 1970).

The use of multiple major genes can be effective in disease control; however, resistance due to the additive effects of minor, nonspecific genes is more likely to provide a permanent resistance (Hooker, 1967; Watson, 1970). Resistance of this type has been called various names such as general, horizontal, minor gene, quantitative, nonspecific, and others. It is characterized by having no affinity with specific resistance, which is recognized by hypersensitivity and a correspondence between host and pathogen genes. Nonspecific resistance is generally under polygenic control and results from complex systems of many different characters in the plant that have effects on the host-pathogen relationship. Its effect on plant disease includes factors such as reduced number and size of lesions, reduced sporulation and longer latent period. Resistance of this type is usually very stable and long lasting because the mechanisms that control resistance are under polygenic control and are not specifically related to genes for virulence in the pathogen; so, they tend to stabilize the pathogen population rather than affect the origin of new strains (Robinson, 1973; van der Plank, 1968).

Epidemiologically, major gene, specific resistance delays the onset of an epidemic, whereas minor gene, nonspecific resistance slows it down once it has started. For many diseases, crops could

be adequately protected by moderate levels of nonspecific resistance.

The effectiveness of minor gene, nonspecific resistance in preventing yield losses was shown by Sharp and Volin (1970). They showed that a wheat line with only one detectable minor gene for resistance to stripe rust, caused by *Puccinia striiformis* Westend. var. *striiformis*, gave a susceptible reaction under epidemic conditions in the field, yet the severity ratings increased at a slower rate than the susceptible checks. The yield of this line was not reduced, whereas susceptible checks were reduced more than 50% in yield.

The combination of both major and minor genes for resistance in a cultivar can be valuable. If and when the major genes 'break down,' the level of resistance imparted by the minor genes will protect the cultivar from severe damage. Late blight of potatoes is a prime example of the use of both major and minor genes. Another is stripe rust of wheat. Lewellen, et al. (1967) showed that the resistance in wheat to a specific pathogenic type was caused by both major and minor genes conditioning resistance. Lines were isolated from the cross PI 178383 x Itana that had one, two, and three minor genes (Sharp and Hehn, 1967). The minor genes acted additively, with three genes conditioning a higher degree of resistance than two, and two a higher degree of resistance than one.

The major gene was epistatic to the minor genes, but in the absence of the major gene the minor genes conditioned moderate resistance. Sharp and Volin (1970) showed that the minor genes were nonspecific in that they reacted similarly to several different virulence types. They also showed that the variety Moro, which contains only the major resistance gene from PI 178383, was susceptible to a particular isolate that had the corresponding virulence gene, whereas Crest and PI 178383, which contain the major gene plus some minor genes, were intermediate in reaction. A line from PI 178383 x Itana was resistant to this same isolate. It was apparent that the susceptible cultivar Itana had contributed one minor gene which had additive effects with those from PI 178383 to condition a high level of resistance.

Resistance that is inherited polygenically might be expected to show transgressive segregation. Indeed it has been shown in a number of cases. Hooker (1967) showed that transgressive segregation for resistance to corn rust, caused by *Puccinia sorghi* Schw., was not common. Toxopeus (1961) showed that it occurs in potatoes for late blight resistance. Krupinsky and Sharp (1979) demonstrated transgressive segregation for resistance to stripe rust in wheat. Bjarko (1979) found evidence for transgressive segregation in barley for net blotch resistance. Scharen and Bryan (1979) found transgressive segregation for resistance to *Septoria nodorum* in spring wheat.

Since the increased resistance associated with transgressive segregation depends on the additive effects of minor genes, it might be expected that crosses between cultivars of diverse origin would most likely result in transgressive segregation. Surely it would be more likely between diverse cultivars than in crosses between related cultivars that have a large proportion of their genes in common. Many crop cultivars are likely to possess minor genes effective against pathogens even though they may appear susceptible. It often takes special environmental conditions to detect these genes. Detection may be complicated if major genes are present that are epistatic to the minor genes.

The use of nonspecific minor genes in developing resistant cultivars may not be as difficult as expected. From the standpoint of manipulation in a breeding program, it is easier to deal with a trait governed by one or a few genes than with one governed by many genes. Also, the expression of nonspecific resistance is usually, but not always, less clear-cut than that of specific resistance, so it is harder to recognize and classify plants with nonspecific resistance. On the other hand, since minor gene resistance is usually nonspecific, it is not necessary to screen material with an array of virulence types; the resistance will be expressed similarly regardless of the virulence type used.

Furthermore, minor gene resistance is largely due to additive gene action; dominance and epistasis effects are usually variable and of less importance and heritability values are moderate to high (Simmons, 1972). Consequently, minor gene, nonspecific resistance can be manipulated easily in a breeding program.

This study was fairly broad in scope. Little was known about the occurrence of additive resistance in barley to net blotch. Bjarko (1979) found evidence for it in five F_2 populations that he tested. This study was initiated to follow up on Bjarko's findings, and determine if acceptable levels of resistance could be attained from crosses between susceptible cultivars.

F_2 plants 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 resulting F_4 progenies were screened for seedling resistance to the same isolate. Some of the best F_4 lines were also screened to a combination of isolates representing all five Montana virulence types. The resistance of the lines was similar to both the single isolate and the combination of isolates. This indicates that the resistance is nonspecific in action which is typical of polygenically controlled, additive resistance.

A method of quantifying resistance was established that provided a way to detect small differences in levels of resistance. Differences in the resistance of lines were detected by using this method, that were not detected by qualitative methods.

Many of the F_4 lines had high levels of resistance to all of the Montana virulence types. These high levels of resistance were not observed in the F_2 and F_3 generations, indicating that the resistance is not due to dominant gene action, but to additive gene action.

The use of commercially acceptable cultivars as the parents in the crosses avoided the undesirable agronomic traits that are commonly associated with sources of resistance. The F_4 lines that were established in this study could probably be used to develop agronomically acceptable cultivars with a minimum of selection. These cultivars would have the advantage of nonspecific resistance to net blotch which is likely to be very long lasting and stable.

Comparisons of the disease reaction of 20 F_2 populations in the field (mature plant stage) and growth chamber (seedling stage) indicate that transgressive segregation for resistance is more likely to be detected in the seedling stage. This has important implications in breeding programs. If early selection for

resistance is desired, it would be best accomplished in the seedling stage. This would require growing many plants to maturity in the greenhouse which can be a problem. An alternative approach would be to bulk the F_2 and F_3 generations and start selection in the F_4 . High levels of resistance were observed among and within the F_4 progenies that were tested, so bulked F_4 populations should contain numerous plants with detectable resistance. Therefore, selection could be made without limiting the genetic variability of the population. This should insure the selection of agronomically valuable traits as well as resistance.

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