MONTANA STATE UNIVERSITY

# Comparative virulence of Pyrenophora graminea Ito et Kurib isolates and the inheritance of resistance to P. graminea in barley

by Richard Lee Ruff

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:
Studies were continued to identify genes for resistance in barley to barley leaf stripe disease (causal organism : Pyrenophora graminea Ito et Kurib.). Because of the variability of infection, inoculation techniques were studied. Eight media were developed to compare substrate effects upon infection of a barley cultivar's seedlings by a P. graminea isolate. Although infection mean differences of the cultures were not significant, cultures grown on diffusate from germinated barley seed had slightly increased infectivity.

Virulence of 24 P. graminea isolates was tested to determine the spectrum of resistance in three barley cultivars. Artificial inoculation of seeds was by the layered mycelium method. Both of the highly susceptible cultivars, 'Lami' and 'Summit', were found to have a low level of resistance to some of these isolates. 'Betzes' seeds inoculated with 18 of the isolates resulted in plants without symptoms. However, up to 16% symptomatic seedlings were found after inoculation with six other isolates. Specific resistance in these cultivars to the isolates was inferred.

The Turkish cultivars, Tokak and Yesilkoy, were each reciprocally crossed to Betzes to study the inheritance of resistance in barley to leaf stripe disease. Tokak and Betzes are highly resistant to infection, and Yesilkoy has a low level of resistance. Seeds of the crosses were produced through the F3 and BC F2 generations and inoculated by the layered mycelium method. Seedlings were grown for six weeks and divided into a class with disease symptoms or a class without symptoms.

Seedlings' reactions from the F1 and F2 generations of the Betzes X Tokak crosses fit models indicating that a single dominant gene was inherited from each parent. The lack of fit of the data in the BC F2 families indicated a modification of the dominant genes models. Seedlings' reactions indicated that a recessive gene was inherited from Yesilkoy. Qualitative gene action with multiple genes or quantitative gene action was inferred from the results of seed inoculations. A method to calculate the effects of penetrance and expressivity on expected gene ratios was discussed.

# COMPARATIVE VIRULENCE OF *PYRENOPHORA GRAMINEA* ITO ET KURIB ISOLATES AND THE INHERITANCE OF RESISTANCE TO *P. GRAMINEA* IN BARLEY

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A thesis submitted in partial fulfillment
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of

Master of Science

in

Plant Pathology

MONTANA STATE UNIVERSITY
Bozeman, Montana

December 1984

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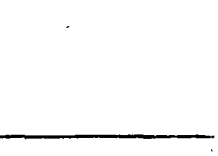
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## ACKNOWLEDGMENTS

I express sincere appreciation and gratitude to the following persons and agencies (not in any particular order):

Dr. A. L. Scharen, for his continual encouragement, confidence, and personal concern as well as material facilities;

Dr's. E. H. Hockett, J. H. Riesselman, and E. L. Sharp for their assistance and advice during my graduate studies;

the staff and fellow graduate students of the Department of Plant Pathology for their assistance, abbetment, and perseverance;

my mother and father, family, and friends for emotional, moral, and physical support;

the Ground of my being for life;

US-AID and the USN for financial support.

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# ABSTRACT

Studies were continued to identify genes for resistance in barley to barley leaf stripe disease (causal organism: Pyrenophora graminea Ito et Kurib.). Because of the variability of infection, inoculation techniques were studied. Eight media were developed to compare substrate effects upon infection of a barley cultivar's seedlings by a P. graminea isolate. Although infection mean differences of the cultures were not significant, cultures grown on diffusate from germinated barley seed had slightly increased infectivity.

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Seedlings' reactions from the $F_1$ and $F_2$ generations of the Betzes X Tokak crosses fit models indicating that a single dominant gene was inherited from each parent. The lack of fit of the data in the BC $F_2$ families indicated a modification of the dominant genes models. Seedlings' reactions indicated that a recessive gene was inherited from Yesilkoy. Qualitative gene action with multiple genes or quantitative gene action was inferred from the results of seed inoculations. A method to calculate the effects of penetrance and expressivity on expected gene ratios was discussed.

# CHAPTER 1

## INTRODUCTION

Barley leaf stripe disease is seed-borne and systemic in barley, Hordeum vulgare L. The fungus, Pyrenophora graminea Ito et Kurib. is the causal organism. The anamorph stage is Drechslera graminea (Rab. ex Schlect.) Shoem., synonym Helminthosporium gramineum Rab. ex Schlect. Barley leaf stripe is a potentially threatening disease in barley growing areas thoughout the world. Early in this century, leaf stripe was one of the most significant, yield reducing diseases of barley. In the 1930's, the introduction of organic mercury seed treatments decreased the commercial importance of leaf stripe. Since the 1970's restricted use of these seed treatments, due to their long term effects in nature, has led to increased disease incidence.

The Pathogen. Ito (1930) described Pyrenophora graminea as the teleomorph stage of Drechslera graminea on barley in Japan. The description was based on

cultural characters and on the pathogenicity of the fungus. Alexopoulos and Mims (1979) placed the genus in the family Pleosporaceae, subclass Loculoascomycetidae. Pseudothecia of *P. graminea* are rarely reported in the literature. Smedegaard-Petersen (1973) described sclerotium-like bodies on barley straw collected from leaf stripe infected fields. The sclerotium-like bodies were superficially submerged and elongated with dark, rigid setae on the surface. Asci and ascopsores were seldom found, and no physical characteristics were given. Using inoculum from ascospore cultures, typical leaf stripe symptoms apparently developed on plants grown from inoculated seed (Smedegaard-Petersen, 1973).

Shoemaker (1962) described the conidial characteristics of the anamorph, *D. graminea*. Conidia were light green to medium yellowish brown. Cells within the conidia were shorter in length than width. Conidia measured 55-85 X 16-20 microns. Secondary conidiophores regularly formed on both apical and basal cells (Shoemaker, 1962).

Historically, the form genera *Drechslera* and *Bipolaris* were in the form genus *Helminthosporium*. *Drechslera* and *Bipolaris* form species were later removed from the *Helminthosporium* form genus. These two form

genera were differentiated from each other essentially by two characters, conidium shape and germination pattern. Alcorn (1983) questioned the validity of separating the two form genera by the germination of conidia. He reported wide variation in the number of cells which germinated in each conidium. Alcorn (1983) proposed the point of origin of the germ tube from the basal cell and the germ tube's direction of growth in relation to the long axis of the conidium as distinguishable characteristics. D. graminea produces germ tubes growing laterally from the basal cell of the conidium.

The Host. Research suggests that the progenitors of barley grew in western Asia (U. S. Department of Agriculture, 1979). The world center for diversity in barley is Ethiopia. Barley, H. vulgare, belongs to the grass family, Gramineae, tribe Hordeae. Cultivated barley is diploid with a basic chromosome number of seven.

The barley kernel consists of the caryopsis, lemma, palea, and rachilla. The caryopsis consists of the pericarp, integuments, endosperm, and embryo. As the kernel germinates, the embryonic root, or radicle, first emerges through the coleorhiza. Later, the primordial shoot, or epicotyl, emerges.

Barley grows best in well-drained, loam and clay-loam soils with a pH of at least 6.0. It does not tolerate flooded soils. Barley grows best where the ripening season is long and cool, and rainfall is moderate. In the lower latitudes or the temperate zones, barley is often grown during the cool season. Where drought, summer frost, or alkaline soils are encountered, barley is one of man's most dependable cereal crops. In recent years an average of 91 million hectares per year have been devoted to barley production worldwide (U. S. Department of Agriculture, 1979; Mathre, 1982).

The Host-Pathogen Relationship. Barley kernels become infected with the leaf stripe organism during development. The embryo is not infected. Platenkamp (1976) found mycelium in the pericarp of ungerminated kernels. She described infection of the coleorhiza as the kernel germinates. From the coleorhiza, the mycelium grew into the apical meristem. Teviotdale and Hall (1976a), using naturally infected seed, found mycelium in the seedling apex after 21 days of germination at 6 C. Platenkamp (1976) found hyphae thoughout the leaves and stems of 12-day-old plants naturally infected and grown at 20 C.

Once the mycelium invades the culm, rapid elongation of the internodes breaks the hyphal strands, and infection loci are established in the elevated plant tissues. The stem apex is invaded just before the spike emerges from the boot (Skoropad and Arny, 1956). With high humidity at the time of heading, conidia are produced on leaves of infected plants. These conidia are windblown to nearby heads where infection of the developing kernels occurs.

Low soil temperatures (6-14 C) during germination and early growth of seedlings enhance disease development. Prasad, Leonard, and Murphy (1976) found higher disease incidence with an intermediate soil moisture (-7.1 bars) compared with a wet soil (-1.0 bar) or a dry soil (-12.9 bars). Irrigation near heading enhanced kernel infection in the field (Metz and Scharen, 1979).

Disease symptoms may appear soon after emergence of seedlings, but they are often not evident until six weeks post-planting. One or more long, chlorotic stripes develop parallel to the leaf ribs, often extending from the base to the tip of the leaf. Later the yellow stripes turn brown or grey as the tissue becomes necrotic. The leaf blade may split and have a frayed

appearance. Growth of infected seedlings is often retarded, and plant death may occur at an early stage. In the field, heavy attacks arrested spike emergence, and barren spikes or improperly developed kernels resulted (Smedegaard-Petersen, 1976). Yield reductions of 0.6% to 1.0% for each percent of leaf stripe infected plants are reported (Richardson, Whittle, and Jacks, 1976; Tekauz, 1983).

Early attempts to control leaf stripe involved cultural practices and seed treatments. The hot water seed soak was an early control method. With the advent of organic mercury seed treatments, the disease was effectively controlled. Organic mercury is not currently available for seed treatments. Johnston, Metz, and Riesselman (1982) found promising results in tests of leaf stripe control with systemic chemicals. The use of these chemicals in many areas of continued leaf stripe disease occurrence, for example North Africa and the Near East, is not practical due to infrastructure problems. Control of leaf stripe disease by increasing host resistance is a viable alternative to chemical control.

This study was initiated to determine available genes in barley for resistance to leaf stripe disease. A preliminary objective was to improve the artificial

inoculation techniques of the disease organism onto barley kernels. This preliminary study is discussed in chapter one. In chapter two, data are presented on the variation in virulence of *P. graminea* isolates from several sources. Any attempts to develop resistant barley cultivars will have to take this variation into account. In the third Chapter of this thesis, experiments are discussed on the determination of genes for resistance to the disease. Two reciprocal crosses were made, and progeny were tested for resistance in the backcross (BC) and $F_1$ to $F_3$ generations. Results of inoculation tests indicated dominant and recessive gene action; however, these effects were not confirmed in BC $F_2$ and $F_3$ generations of crossed material.

# CHAPTER 2

## MEDIA EFFECTS ON INFECTION STABILITY OF *PYRENOPHORA GRAMINEA* ISOLATES WHEN ARTIFICIALLY CULTURED

### Introduction

One problem with artificial inoculation of *P. graminea* is the variability in infection. *P. graminea* does not readily sporulate in culture, although researchers report various methods to induce sporulation of the fungus in culture (Paxton, 1922; Houston and Oswald, 1946; Teviotdale and Hall, 1976b; Sengupta and Singh, 1979; Tekauz and Chiko, 1980; Grbavac, 1981). Even with these methods, conidia are not produced in enough abundance for use as an inoculum source. In the literature (Arny and Shands, 1942; Houston and Oswald, 1948; Nilsson, 1975; Metz, 1978; Smedegaard-Petersen and Jorgensen, 1982; Konak, 1983; Tekauz, 1983) mycelia, with or without conidiospores, are reported to be the predominant inoculum either grown on a cereal kernel substrate or on artificial media.

Shands and Dickson (1934) reported variation in hyphal-tip cultures of *P. graminea*. They reported differences in culture morphology on PDA (potato dextrose agar), in pathogenicity tests, and in symptoms when single spore cultures were transferred by hyphal-tips. Shands and Arny (1944) and Arny (1945) used hyphal-tip cultures of *P. graminea* that had been maintained on artificial media for over twelve years. These cultures were stable and virulent on a large number of barley varieties.

Nilsson (1975) compared the virulence of newly isolated *P. graminea* cultures and old cultures grown on PDA. Virulence of the stored cultures was determined after two and nine months. Nilsson showed that some cultures lost little virulence, while others completely lost the ability to induce disease symptoms on plants. Metz and Scharen (1979) identified barley cultivars with various reactions to different *P. graminea* isolates. The cultivars varied in their disease reaction among replications. They proposed genetic factors, environmental conditions, or the inoculation technique as causes for the variations in reaction.

Konak (1983) reported a dramatic loss in virulence (from 95% to 2% seedling infection) of specific *P.*

graminea isolates inoculated to a single barley cultivar. J.G.N. Davidson (personal communication) found culturing pure isolates of P. graminea impossible. Therefore, fresh isolates were obtained from infected leaves for each experiment. However, different isolations made from a single leaf at the same time in the same way differed in virulence (0%-100% infection). Such variability between experiments produces results that are not comparable.

As a preliminary task to my inheritance study, I attempted to devise a method to decrease the infection variability of the barley leaf stripe disease organism. I concentrated on the growth substrate used to culture P. graminea prior to inoculation. With the cooperation of other researchers, eight media were developed for comparing the effects of the substrate upon infection of a specific barley cultivar. After culturing a P. graminea isolate on these media for eight months, only small differences were detected in infection percentages.

## Materials and Methods

A single conidiospore isolate of P. graminea was taken from naturally infected leaf tissue grown from breeder's barley seed, RPB 43971 (obtained from Mr. V.

Stewart, Northwest Agricultural Research Center, Kalispell, Montana). The isolate was first grown on water agar medium. Two weeks later, a single 6 mm plug of mycelia and medium from the perimeter was transferred to the eight media that follow: 1. water agar; 2. barley leaf piece agar (Teviotdale and Hall, 1976a); 3. potato dextrose agar (PDA) amended with 2 grams spring wheat bran per liter of water; 4. PDA amended with 20 grams spring wheat bran per liter of water; 5. PDA amended with germinated barley seed diffusate (Appendix Table 12); 6. PDA amended with germinated barley seed extract (Appendix Table 13); 7. V8 juice; and, 8. barley leaf extract (as in Konak [1983] but with 30 g/l fresh barley leaves). Individual cultures were transferred by a single, 6 mm, mycelial bore to their respective media every two weeks from January to August 1983. A single conidiospore was again isolated in August and transferred to the same media. These newly isolated cultures were compared to the above cultures in an inoculation test.

Inoculum for the inoculation tests was prepared by autoclaving 30 g 'Ingrid' barley, C. I. 10083, with 30 cc distilled water in 1 pt Mason jars for 30 minutes at 121 C (Arny and Shands, 1942; Metz and Scharen, 1979; Konak, 1983). The jars were shaken within two to three hours

after removing from the autoclave to loosen the seed mats, and then they were allowed to cool overnight. The sterile barley kernels were inoculated with three 6 mm bores of mycelium and medium per jar. At this time approximately 4 cc sterile, distilled water was added to the jars, and the substrate was mixed well. Noninoculated checks were included in each experiment. The autoclaved barley kernels in jars were treated the same; however, the kernels were not inoculated with P. graminea mycelia. The jars containing inoculated and noninoculated kernels were incubated at 12-14 C (12 h light/dark cycles) for five to seven days.

'Summit' seed used in the experiments was obtained from Dr. E.A. Hockett, USDA, Montana State University. Prior to inoculating the 60-seed lots with P. graminea mycelia, the seeds were surface disinfected for three to five minutes in 10% Clorox (0.5% sodium hypochlorite) + 2% ETOH. Surface disinfected seed lots were allowed to dry on paper towels over night in a Microvoid IIC clean air chamber. The seed lots were then mixed with the previously prepared barley kernel inoculum.

One pt Mason jars of P. graminea mycelia on kernel substrate and a Summit seed lot were then placed in a refrigerator at 4 ± 2 C. The barley seeds were allowed to

germinate in this substrate for 12-14 days at which time most seeds had noticeable shoots. The entire mixture of germinated kernels, mycelia, and substrate was then planted in a 1:1 sand:soil mixture in 12 cm plastic pots. The pots were placed in a growth chamber at 16 C / 2 C ± 1 C (12 h light/dark cycle). Emergence of the seedlings was evaluated at 10-14 days after planting. Number of symptomatic plants was read at three, four, and six weeks.

The disease readings taken six weeks after planting were recorded as percent symptomatic plants of total emerged plants in each replication. Analyses of variance were calculated for each experiment and for the four experiments conducted over time, including the reisolation. Analyses were conducted on the Superbrain II microcomputer with statistics programs written by R. E. Lund (1983).

## Results and Discussion

Table 1 lists the infection means (two replications each) of *P. graminea* grown on each medium for the four experiments. No significant differences (LSD 0.05) between means of the cultures grown on different media were noted in any of the planting times. In both 29

August plantings no significant differences (LSD 0.05) were calculated between any of the means of the cultures and the noninoculated check. These data indicate that the substrates did not affect the stability of virulence of this P. graminea isolate when serially cultured over eight months.

Table 1: Per cent of barley seedlings infected as a result of inoculation with cultures of Pyrenophora graminea isolate RPB grown on different media.

| Media | Planting Date 3 Mar | 5 Jun | 29 Aug | 29 Aug[a] |
|---|---|---|---|---|
| Noninoculated | 00.0 | 00.0 | 00.0 | 00.0 |
| Water agar | 30.0 | 38.0 | 2.0 | 1.0 |
| B L P A[b] | 20.0 | 44.0 | 1.2 | 2.0 |
| PDA + 1[c] | 20.0 | 32.0 | 00.0 | 2.0 |
| PDA + 10[d] | 35.0 | 28.0 | 3.9 | 3.9 |
| PDA + seed dif.[e] | 38.0 | 51.0 | 2.0 | 2.8 |
| PDA + seed ext.[f] | 35.0 | 32.0 | 1.3 | 1.0 |
| V 8 juice | 30.0 | 44.0 | 2.9 | 0.93 |
| B L Ext.[g] | 24.0 | 45.0 | 00.0 | 3.6 |
| Means[h] | 25.0 | 35.0 | 1.5 | 1.5 |

[a] Trial using reisolated P. graminea cultures.
[b] Barley Leaf Piece Agar (Teviotdale and Hall, 1976a).
[c] PDA amended with 2 g/l spring wheat bran.
[d] PDA amended with 20 g/l spring wheat bran.
[e] PDA amended with germinated seed diffusate (see appendix Table 12).
[f] PDA amended with germinated seed extract (see appendix Table 13).
[g] Barley leaf extract medium (Konak, 1983, but with 30 g/l fresh barley leaves).
[h] Means of symptomatic seedlings per total emerged seedlings for each planting date.

A two-factorial analysis of variance for the experiments conducted over time was also calculated. Percentage of emerged plants that had symptoms was one factor, and the date that the experiments were conducted was the second factor. The results, including a mean comparison using a LSD of 0.05, are given in Appendix Table 14. The LSD (0.05) between culture means over time in this analysis indicated that the inoculum grown on PDA amended with germinated, barley seed diffusate had the highest percentage of infected plants. However, the eight month cultured test and the reisolation test (both planted on 29 August) resulted in extremely low infection levels on the susceptible cultivar, Summit. These low infection levels tended to increase the significant differences found between the cultures over the timed experiments as indicated by the relatively large F-value for planting dates in the analysis of variance.

Prasad, Leonard, and Murphy (1976) found that differences in soil water potential affected percent diseased plants grown from artificially inoculated seeds. Highest infections were in plants grown in soils of intermediate water potential (average of -7.1 bars). Infections decreased greatly in plants grown in soils with high water potentials. In my experiments water was

added to large pans containing up to 21 pots, and the water was absorbed by the soil. Without monitoring the soil water potential, high potentials were quite possible during emergence of the inoculated seeds. This may explain the overall low infection levels obtained in the plants in the last two planting dates.

Tekauz (1983) found varying levels of infection within replications of his tests with artificially inoculated barley seed. He proposed that the lack of good contact between the wheat kernel inoculum and the seed, due to clumping of the substrate, may have decreased infection levels. Also, he suggested minor environmental variations and possible partial contamination as causes for the variability in infection between replicates. In continuing experiments, I have isolated low levels of bacteria from the autoclaved barley kernels. Although mycelia were present on all the substrates at the time of planting in these experiments, partial contamination that would hinder infection is possible.

This comparison of culture media was begun with three P. graminea isolates. However, one isolate proved difficult to maintain on all the substrates. A second isolate gradually lost the ability to grow on some of the

substrates so that by October 1983, it was being maintained on only three of the substrates. Therefore, data from only one isolate were analyzed. No significant differences between the infection means of the cultures were detected for any of the isolates.

In the inoculation method used in this study, the inoculated seed was treated harshly. First, the seed was surface disinfected, then dried, and then placed with P. graminea inoculum growing on a kernel substrate. After germination, the seeds were tansplanted into a planting mixture. Any injury of these germinated seeds during transplanting resulted in decreased emergence. The production of inoculum on a substrate that can be mixed with the planting mixture would be beneficial. The surface disinfected seed could then be planted directly into the inoculum and planting mixture. Such an inoculum production method has been proposed recently (Miles and Wilcoxson, 1984).

The purpose of this study was to develop a more practical, efficient, and reliable method of artificially inoculating the leaf stripe organism onto barley seeds. Konak (1983) presented results indicating that P. graminea grew more profusely on a rich medium, his barley leaf extract medium. More recently, J.G.N. Davidson

(personal communication) stated that virulence was independent of the isolation method or the medium (V8 juice and water agar). My attempt was to extend the spectrum of substrates used. Since *P. graminea* becomes active and systemically invades the plant during germination, I amended a proven medium, PDA, with the diffusate and extract of germinated barley seed. Although mean differences of the cultures were not highly significant, an indication was obtained that diffusate from germinated barley seed did increase culture pathogenicity. These results may provide impetus for continued research into better inoculation methods with *P. graminea*.

# CHAPTER 3

## VIRULENCE OF *PYRENOPHORA GRAMINEA* ISOLATES ON SPECIFIC BARLEY CULTIVARS

### Introduction

Christensen and Graham (cited in Stakman and Christensen, 1960) in a 1934 bulletin were the first to detail variation in virulence among single spore isolates of *Pyrenophora graminea* S. Ito & Kuribay. Virulence of 24 *P. graminea* isolates inoculated on 'Peatland', C. I. 2613, barley varied from almost 0% to 77% infection in a greenhouse experiment.

Arny (1945) detailed physiologic specialization among three *P. graminea* isolates tested on five barley cultivars. The results showed that one isolate was pathogenically distinct from two other isolates.

Kline (1971) reported resistance levels to barley leaf stripe disease in 82 winter barley cultivars. Kline wrote "Isolates did not differentially attack cultivars, and there was no evidence of pathogenic specialization among these isolates on these cultivars."

Several researchers have hypothesized physiologic specialization in pathogenic isolates of P. graminea. These researchers include Nilsson (1975) in Sweden; Metz & Scharen (1979) in Montana, USA; Tekauz and Chiko (1980) in Canada; and, Knudsen (1980) in Denmark. Knudsen (1980) stated that resistance in barley to his population of P. graminea was mainly determined by quantitative factors which would support the hypothesis of physiological specialization in the fungus.

Smedegaard-Petersen and Jorgensen (1982) conducted experiments on 28 barley cultivars using 15 P. graminea, single spore isolates collected in Denmark. Artificial inoculation of seeds was by the layered mycelium technique. They found considerable variation in virulence of P. graminea isolates on specific barley cultivars. The isolates also showed variation in virulence to all cultivars. They proposed that the separation of P. graminea isolates into different pathogenic races was indicated by these data.

Konak (1983) reported on virulence tests utilizing the barley cultivar 'Summit' with 27 P. graminea isolates obtained from Montana, USA, Turkey, and Ethiopia. Inoculation of seed was by a modified barley kernel

inoculum method. Variation in virulence of these isolates was 0% to 95.3% infection.

Virulence studies on *P. graminea* reported in this paper describe the variation in virulence of 24 *P. graminea* isolates from several sources on three barley cultivars that exhibit varying degrees of resistance.

## Materials and Methods

Leaf samples from plants symptomatic of barley leaf stripe disease were collected in Tunisia, Egypt, Syria, Turkey, and Montana, USA. Leaf pieces were placed on moistened filter paper or water agar in Petri dishes after surface sterilization in 10% Clorox solution (approximately three minutes). After conidiophore and conidia production, masses of spores were transferred to water agar. Then, after 16-24 hours germination at approximately 15 C, a single conidium was transferred to barley leaf piece agar (BLPA) (Teviotdale and Hall, 1976a). Cultures were allowed to grow on BLPA for 12-16 days at 15 C, after which a single, six mm bore from the perimeter of growing mycelia was placed in autoclaved barley kernels (30 g 'Ingrid', C. I. 10083, barley + 36 cc distilled water). Mycelia grew on the barley kernels for 9-12 days at 15 C. Then, a single barley kernel with

mycelia was placed on PDA + 4 (4 g/l wheat bran) medium in each plastic Petri dish. Cultures were again allowed to grow at approximately 15 C for 12-15 days.

Three barley cultivars were used in these experiments: 'Betzes', C. I. 6398, originally obtained from Dr. E.A. Hockett, was increased at the Post Farm, Bozeman, Montana, and harvested in September, 1982; Summit seed, originally from North American Plant Breeders, was increased at Mesa, Arizona and harvested in April, 1983; and, 'Lami' seed, obtained from Ms. Sally Metz, was increased at Mesa and harvested in April, 1983. Forty-seed lots were surface sterilized for 4-5 minutes in 10% Clorox plus 2% ETOH, after which the seeds were dried in a Microvoid IIC clean air chamber for eight to twelve hours.

Inoculation was by the layered mycelium method (Shands, 1934; Houston and Oswald, 1948; Nilsson, 1975). Individual seed lots (40 seeds each) were placed on top of a layer of actively growing mycelia on PDA + 4 medium. Another layer of mycelia growing on the medium was placed over the top of the seeds. Petri dishes were sealed with Parafilm strips and placed in a growth chamber at approximately 8 C (12 h light/dark cycle) for seven days. At that time the seeds had germinated, and radicles were

0.5 to 1.5 cm long. The seeds were removed from the Petri dishes and planted in a 1:1 sand:soil (pasteurized Bozeman silt loam) mixture in aluminum trays (20 X 30 cm, 3 seed lots in each divided tray). The trays were returned to the same growth chamber. Water was applied as required to maintain a moist soil surface. After four weeks, plants were removed to a greenhouse maintained at 16-20 C. Symptomatic plants were first evaluated at four weeks post-planting and every five to six days thereafter until eight weeks post-planting. Symptomatic plants were pulled at each evaluation. Percent symptomatic plants of the total plants emerged from each seed lot were recorded for analysis.

Three different split plot experiments using the same procedures were conducted, each with eight *P. graminea* isolates, a check *P. graminea* isolate (RPB), and a noninoculated check. The check isolate, RPB, had been serially transferred for more than eight months on BLPA (see Chapter 1). The noninoculated check was surface sterilized seed lots germinated in an agar sandwich as above, but with no fungus. The noninoculated checks had no symptomatic plants in any of the experiments. Each isolate-cultivar combination consisted of three replications in each experiment. Analysis of the data

was by a split plot design with the isolates as the main plot and cultivars as the subplot. Analyses were calculated with a Superbrain microcomputer using programs written by R.E. Lund (1983).

## Results and Discussion

In this paper, the variation in virulence of *P. graminea* isolates from Montana (numbered isolates), Tunisia (TN- isolates), Egypt (E- isolates), Syria (S- isolates), and Turkey (TK- isolates) is reported. Percent symptomatic plants of total emerged plants is given for each isolate X cultivar interaction (average of three replications each) in Tables 2, 3, and 4. Percent emergence of the cultivars differed in the experiments. Over the three experiments, average emergence for Lami was 82.9%, for Summit, 82.1%, and for Betzes, 59.9%. The Betzes seed lot had poor emergence throughout the experiments. Analysis of covariance with percent emergence as the independent covariable did not increase precision, however. The severity of symptoms was not taken into account. Rather, any symptomatic plant was evaluated as infected and pulled.

Table 2: Barley plants infected by *Pyrenophora graminea* as a percentage of total plants emerged (average of three replications).

| Isolates | Cultivars 'Lami' | 'Summit' | 'Betzes' | Isolate Means |
|---|---|---|---|---|
| Noninoculated Ck. | 0.0 A[a] | 0.0 A | 0.0 A | 0.0 |
| E-8 | 3.0 A | 0.0 A | 1.2 A | 1.4 |
| TN-1 | 6.6 A | 4.9 A | 0.0 A | 3.8 |
| 24-216 | 19.1 A | 2.0 A | 0.0 A | 7.0 |
| 33-267 | 22.5 AB | 9.6 A | 0.0 A | 10.7 |
| TN-9 | 44.1 BC | 1.0 A | 16.4 A | 20.5 |
| TN-6 | 47.6 C | 2.7 A | 0.0 A | 16.8 |
| 24-212/19 | 49.4 CD | 4.9 A | 0.0 A | 18.1 |
| 33-313 | 71.5 DE | 2.0 A | 6.7 A | 26.8 |
| RPB | 73.4 E | 3.5 A | 4.5 A | 27.1 |
| Cultivar means | 33.7 | 3.1 | 2.9 | |

[a] Different capital letters on two isolate X cultivar interactions indicate a significant difference between means (LSD 0.05).

Table 3: Barley plants infected by *Pyrenophora graminea* as a percentage of total plants emerged (average of three replications).

| Isolates | Cultivars 'Lami' | 'Summit' | 'Betzes' | Isolate Means |
|---|---|---|---|---|
| Noninoculated Ck. | 0.0 A[a] | 0.0 A | 0.0 A | 0.0 |
| TN-4 | 0.0 A | 1.0 A | 1.2 A | 0.7 |
| E-9 | 0.0 A | 0.0 A | 0.0 A | 0.0 |
| S-2 | 10.2 AB | 0.0 A | 0.0 A | 3.4 |
| E-17 | 37.3 BC | 25.8 AB | 0.0 A | 21.1 |
| E-14 | 45.7 CD | 10.4 AB | 1.5 A | 19.2 |
| E-10 | 49.7 CD | 11.7 AB | 0.0 A | 20.5 |
| E-11 | 66.8 D | 20.2 AB | 0.0 A | 29.0 |
| E-19 | 96.2 E | 36.2 B | 1.3 A | 44.6 |
| RPB | 100.0 E | 11.0 AB | 3.7 A | 38.2 |
| Cultivar means | 40.6 | 11.6 | 0.8 | |

[a] Different capital letters on two isolate X cultivar interactions indicate a significant difference between means (LSD 0.05).

Table 4: Barley plants infected by Pyrenophora graminea as a percentage of total plants emerged (average of three replications).

| Isolates | Cultivars 'Lami' | 'Summit' | 'Betzes' | Isolate Means |
|---|---|---|---|---|
| Noninoculated Ck | 0.0 A[a] | 0.0 A | 0.0 A | 0.0 |
| TK-18 | 0.0 A | 0.0 A | 0.0 A | 0.0 |
| E-29 | 0.9 A | 0.0 A | 0.0 A | 0.3 |
| TK-8 | 1.1 A | 0.0 A | 0.0 A | 0.4 |
| TK-14 | 1.7 A | 1.0 A | 0.0 A | 0.9 |
| TK-13 | 5.7 A | 0.9 A | 0.0 A | 2.2 |
| TK-11 | 8.2 A | 0.0 A | 0.0 A | 2.7 |
| S-7 | 31.3 B | 6.4 A | 0.0 A | 12.6 |
| TK-9 | 32.6 B | 4.0 A | 0.0 A | 12.2 |
| RPB/MT6 | 94.3 C | 0.0 A | 0.0 A | 31.4 |
| Cultivar means | 17.6 | 1.2 | 0.0 | |

[a] Different capital letters on two isolate X cultivar interactions indicate a significant difference between means (LSD 0.05).

Virulence of these isolates on the cultivar Lami varied from 0% to 100% infected plants. Lami is highly susceptible to P. graminea (Metz and Scharen, 1979; Knudsen, 1980; Smedegaard-Petersen and Jorgensen, 1982). However, infections on Lami did vary between isolates which indicated a level of resistance to some isolates.

Summit has been reported to be highly susceptible to P. graminea (Metz, 1978; Konak, 1983). The data presented here, however, indicate a low level of resistance in Summit to P. graminea. The Summit seed lot used in these tests was harvested from a leaf stripe disease infected nursery. Intense roguing was undertaken in this nursery prior to heading, and plants lacking symptoms were selected for bulked seed harvest. Selection may explain the low levels of infection when the isolates were inoculated onto this Summit seed lot.

Betzes barley had been reported to have 0% infection by leaf stripe disease (Kline, 1972; Metz and Scharen, 1979; Tekauz and Chiko,1980), until Konak (1983) described symptomatic plants in artificial inoculation experiments. Virulence of the P. graminea isolates used in these experiments on Betzes resulted in 0% to 16% infected plants. Eighteen of the 24 isolates tested produced no disease symptoms when inoculated on Betzes.

Konak (1983) proposed that Betzes has at least one dominant gene for resistance. Inoculation tests with Betzes crosses will be reported later.

The data presented here indicate that differential interactions occurred between specific barley cultivars and P. graminea isolates from infected barley plants. The significant isolate X cultivar interaction in the analysis of variance for each experiment (P-values less than 0.01 for each experiment) indicated differential, cultivar-specific virulence. These differences in cultivar-specific, isolate virulence were apparent in experiment 1 (Table 2) where a difference in isolate X cultivar infection ranking occurred. With most isolates tested, cultivar ranking from susceptible to resistant reaction was Lami, Summit, and Betzes, respectively. Isolates 33-313 and TN-9 reversed the Summit and Betzes ranking such that Betzes was less resistant than Summit. No significant differences between these means were found. The LSD's between Summit and Betzes infection means for isolates TN-9 and 33-313 had t-values between 0.10 and 0.20, and greater than 0.50, respectively.

Smedegaard-Petersen and Jorgensen (1982) also described variation in virulence of different P. graminea isolates on barley cultivars. Even with relatively

susceptible cultivars, these workers reported variation in virulences of specific isolates from "full susceptibility to immunity." Data of Smedegaard-Petersen and Jorgensen (1982), along with those reported in this paper and by other researchers (Arny, 1945; Metz and Scharen, 1979; Knudsen, 1980), indicate the occurrence of specific resistance to *P. graminea*.

From these data it can be concluded that sources of resistance in barley to leaf stripe disease are available. These sources must be tested for resistance to *P. graminea* isolates specific to the intended growing area if they are to be utilized effectively in cultivar development programs.

# CHAPTER 4

## INHERITANCE OF RESISTANCE IN THREE BARLEY CULTIVARS TO TWO *PYRENOPHORA GRAMINEA* ISOLATES

### Introduction

Barley leaf stripe disease was efficiently controlled by organic mercury seed treatments for a number of years. Mercury seed treatments have decreased in use because of the environmental effects of the fungicides. Since the 1970's leaf stripe disease has again increased in occurrence. The disease has recently been positively identified in New Zealand, and a recommendation for treatment of new seed lines was made (Arnst, Sheridan, and Grbavac, 1978). In Montana, USA, a potential increase in infected seed lots from 1% to 43% within three years was predicted (Metz and Scharen, 1979). In Denmark, leaf stripe is endemic with levels of 20% to 25% infection reported, and rare infections of up to 70% have been observed (Tekauz, 1983). Tekauz (1983) also reported widespread occurrence of the disease in

Canada with infections ranging from a trace to 3% diseased plants.

Current movement in the agricultural chemical industry is toward specific, systemic fungicides. Fungicides for the control of leaf stripe disease are becoming available (Johnston, Metz, and Riesselman, 1982; Smedegaard-Petersen and Jorgensen, 1982). However, because of economic and environmental concerns and with the development of integrated crop management systems (Nissen and Juhnke, 1984), resistant cultivars remain important components of the production formulae. Also, many barley producing areas lack the needed distribution, marketing, and treatment facilities, to chemically treat farmers' seed. Cultivars resistant to prevalent diseases are an important means of increased production in those areas.

The primary emphasis in my research was to identify genes in barley which would provide resistance to barley leaf stripe disease. Crosses between three Turkish and American cultivars were made. Seed was produced through the $F_3$ and backcross $F_2$ generations. In this chapter, I give details of experiments with the artificial inoculation of plants from these crosses and discuss results.

## Materials and Methods

In the summer of 1982 reciprocal crosses between 'Betzes' (C. I. 6398) and 'Tokak' (C. I. 8655), and Betzes and 'Yesilkoy' were made at the Arthur Post Research Center, Bozeman, MT. At the same time, backcrosses were made onto $F_1$ plants of the Betzes X Tokak and Betzes X Yesilkoy crosses. The $F_{(1)}$ seeds were provided by Mr. Cahit Konak (1983). The seeds from these crosses were harvested in September 1982. Four to ten seeds (approximately 50% of the seed from a crossed head) from four parent plants of each cross were planted in Mesa, AZ in the fall of 1982. $F_{(2)}$ and backcross (BC) $F_{(2)}$ seeds were harvested in April 1983. $F_{(2)}$ seeds were planted in Bozeman in the spring of 1983, and $F_{(3)}$ seeds were harvested in September.

Inconsistencies in the designation of crossed seed and generations often occur in papers. Ahokas (1976) attempted to standardize designation of generations. He proposed that the generation number of seed be placed in parentheses. The plants grown from that seed have the same generation number, but the parentheses are removed.

Inoculation experiments were begun in January 1984. The layered mycelium method was used as described in Chapter 2. Seeds of bulked and specific-plant parents,

$F_1$, $F_2$, and $F_3$ generations from one cross with at least one susceptible cultivar were inoculated with a single spore isolate of *P. graminea* at the same time. Seeds of parents, BC $F_1$, and BC $F_2$ generations from the two recurrent parents backcrossed onto the $F_1$ plants with at least one susceptible cultivar were also inoculated with a single spore isolate of *P. graminea* at the same time. A check *P. graminea* isolate, RPB (see Chapter 1), was inoculated onto check cultivar seeds in all experiments. Inoculated seeds were germinated in the layered mycelium for 10-12 days with *P. graminea* isolate MT6 and for 6-7 days with isolate Kaya. Both of these isolates were obtained from infected 'Summit' leaves inoculated by Konak (1983).

After germination in the layered mycelium, seed lots were planted in a 1:1 sand:soil mixture and placed in a growth chamber. The growth chamber used for parents, $F_{(1)}$, $F_{(2)}$, and $F_{(3)}$ seed lots was set at a constant temperature of 8 $\pm$ 2 C with a 12 h/12 h light/dark cycle. Lights were fluorescent tubes (Sylvania cool white, 95 W) with an intensity of 3.0-3.6 X $10^4$ ergs/$cm^2$sec. The growth chamber used for parents, BC $F_{(1)}$ and BC $F_{(2)}$ seed lots was set at a constant temperature of 9 $\pm$ 1 C with a 12 h/12 h light/dark cycle. Lighting was also provided

by fluorescent tubes (Sylvania cool white, 195 W) with an intensity of 2.8-4.0 X $10^4$ ergs/$cm^2$sec. Plants were allowed to grow for three weeks in the growth chambers, and then they were transferred to a greenhouse. Temperatures in the greenhouse were no lower than 18 C. However, during the summer months, midday temperatures sometimes did exceed 30 C. Additional lighting was provided with halogen lamps as needed to provide a minimum of 12 h days.

Diseased plants and total emerged plants were counted at 4, 5, and 6 weeks post-planting. Any plant showing striping typical of leaf stripe disease was counted as infected and destroyed. Rare leaves with indefinite or atypical symptoms were transferred to barley leaf piece agar (BLPA) or moistened filter paper and observed for sporulation. Percentage of diseased plants per total emerged plants was recorded for each seed lot. Lots varied in number of seed from 10 seeds or less for the $F_1$ generation to 100 seeds in some of the bulked parent, $F_2$ and $F_3$ generations.

## Results

Emergence of the seedlings varied among the seed lots in all trials conducted. This variation was

lessened when the seed lots were germinated in the layered mycelium for a shorter time. Inoculation with P. graminea isolate MT6 was for 10-12 days, whereas this time was reduced to 6-7 days with the Kaya isolate. Emergence percentages were higher in the inoculations using this shorter time period. For example, in the Tokak X Betzes and reciprocal crosses the average emergence of barley seedlings from 357 seed lots when inoculated with isolate MT6 was approximately 59%. With the same crosses after inoculation with isolate Kaya, the average emergence of barley seedlings was about 77% (221 seed lots).

Methods were sought to increase the precision of seedling infection percentages using emergence percentages as an added variable. Seeds from the generations of crossed seed were limited in number. So, tests were conducted on parent and check cultivar bulked seeds (Appendix Table 17). Comparing the six-weeks emergence of seedlings, a drop of 6% to 7% emergence occurred with the inoculation of seeds of the highly susceptible cultivar, Summit, and in the resistant cultivar, Tokak, versus the noninoculated seeds of the respective cultivars. An analysis of covariance was calculated on these results with the percent emergence of

seedlings as an independent covariable and the percent infection of seedlings as the dependent variable (Appendix Table 18). In the analysis of this data, as well as similar data sets, the percent emergence of seedlings was not significant. Precision was not increased in the analysis of covariance with the addition of the covariable. Therefore, percentages of emerged seedlings were considered random in this study.

Betzes - Tokak Crosses

Betzes and Tokak were both resistant to infection by P. graminea (4.9% and 7.4% infection of seedlings, respectively, in these trials). Konak (1983) proposed that each of these cultivars had one dominant gene for resistance. This proposal was based on the inoculation of 115 $F_2$ seedlings with P. graminea isolate MT6. $F_{(1)}$ seeds were inoculated with two P. graminea isolates in trials with seeds from check cultivars, bulk parents, and specific parents (seeds from the female and male plants used for the cross) (Table 5). No symptomatic plants were found in the Betzes X Tokak nor reciprocal crosses when $F_{(1)}$ seeds were inoculated with the isolate MT6. Results were similar when the same crosses were inoculated with the isolate Kaya, except for one symptomatic plant in the Betzes X Tokak $F_1$ trial. The leaves from this single

plant produced Pyrenophora-type conidia after isolation on BLPA. Some of the seedlings from specific parents of these crosses also had no, or a few, symptomatic plants in these inoculation trials.

Table 5: Number of barley seedlings derived from check cultivars, parents, and progeny of two crosses with and without symptoms after inoculation with two isolates of Pyrenophora graminea.

| Seed Source | Isolate | No Symptoms | Symptoms | Total |
|---|---|---|---|---|
| Betzes X Tokak trial[a] | | | | |
| Summit - Ck | MT6 | 73 | 15 | 88 |
| Lami - Ck | MT6 | 59 | 31 | 90 |
| Betzes - bulked | MT6 | 86 | 1 | 87 |
| Tokak - bulked | MT6 | 65 | 1 | 66 |
| Betzes - spec.[b] | MT6 | 19 | 1 | 20 |
| Tokak - spec. | MT6 | 25 | 0 | 25 |
| B X T, $F_1$[c] | MT6 | 2 | 0 | 2 |
| Betzes - spec. | MT6 | 37 | 0 | 37 |
| Tokak - spec. | MT6 | 57 | 5 | 62 |
| B X T, $F_1$ | MT6 | 2 | 0 | 2 |
| Betzes - spec. | MT6 | 58 | 0 | 58 |
| Tokak - spec. | MT6 | 76 | 9 | 85 |
| B X T, $F_1$ | MT6 | 14 | 0 | 14 |

Table 5 (cont'd).

| Seed Source | Isolate | No Symptoms | Symptoms | Total |
|---|---|---|---|---|
| **Tokak X Betzes trial** | | | | |
| Summit - Ck | MT6 | 69 | 26 | 95 |
| Lami - Ck | MT6 | 48 | 27 | 75 |
| Tokak - bulked | MT6 | 52 | 1 | 53 |
| Betzes - bulked | MT6 | 24 | 0 | 24 |
| Tokak - spec. | MT6 | 33 | 1 | 34 |
| Betzes - spec. | MT6 | 56 | 4 | 60 |
| T X B, $F_1$ | MT6 | 6 | 0 | 6 |
| Tokak - spec. | MT6 | 41 | 1 | 42 |
| Betzes - spec. | MT6 | 47 | 3 | 50 |
| T X B, $F_1$ | MT6 | 2 | 0 | 2 |
| Tokak - spec. | MT6 | 30 | 3 | 33 |
| Betzes - spec. | MT6 | 11 | 0 | 11 |
| T X B, $F_1$ | MT6 | 8 | 0 | 8 |
| **Betzes X Tokak trial** | | | | |
| Summit - Ck | Kaya | 4 | 46 | 50 |
| Betzes - bulked | Kaya | 59 | 4 | 63 |
| Tokak - bulked | Kaya | 53 | 7 | 60 |
| Betzes - spec. | Kaya | 27 | 5 | 32 |
| Tokak - spec. | Kaya | 32 | 1 | 33 |
| B X T, $F_1$ | Kaya | 10 | 0 | 10 |
| Betzes - spec. | Kaya | 26 | 5 | 31 |
| Tokak - spec. | Kaya | 24 | 10 | 34 |
| B X T, $F_1$ | Kaya | 10 | 0 | 10 |
| Betzes - spec. | Kaya | 36 | 4 | 40 |
| Tokak - spec. | Kaya | 46 | 5 | 51 |
| B X T, $F_1$ | Kaya | 14 | 1 | 15 |

Table 5 (cont'd).

| Seed Source | Isolate | No Symptoms | Symptoms | Total |
|---|---|---|---|---|
| Tokak X Betzes trial | | | | |
| Summit - Ck | Kaya | 2 | 32 | 34 |
| Lami - Ck | Kaya | 1 | 68 | 69 |
| Tokak - bulked | Kaya | 56 | 5 | 61 |
| Betzes - bulked | Kaya | 45 | 0 | 45 |
| Tokak - spec. | Kaya | 40 | 0 | 40 |
| Betzes - spec. | Kaya | 42 | 0 | 42 |
| T X B, $F_1$ | Kaya | 14 | 0 | 14 |
| Tokak - spec. | Kaya | 19 | 3 | 22 |
| Betzes - spec. | Kaya | 36 | 5 | 41 |
| T X B, $F_1$ | Kaya | 7 | 0 | 7 |
| Tokak - spec. | Kaya | 36 | 5 | 41 |
| Betzes - spec. | Kaya | 38 | 3 | 41 |
| T X B, $F_1$ | Kaya | 11 | 0 | 11 |

[a] Each trial was conducted at a different time.
[b] Parents marked 'spec.' give reactions of seedlings from the specific female and male plants that were crossed to produce the associated $F_1$ progeny.
[c] The $F_1$ seedling reactions are given following their specific female and male parent plants.

$F_2$ populations of the Betzes X Tokak cross and its reciprocal were inoculated with *P. graminea* isolates MT6 and Kaya. Each $F_2$ population was composed of $F_2$ seedlings derived from five or less $F_1$ plants which were derived from a single mother plant. Inoculations of $F_{(2)}$ seeds with isolate MT6 produced results which fit a 15:1 genetic ratio which was expected for the two dominant

genes model. The reactions of $F_2$ seedlings from individual $F_1$ plants of the cross Betzes X Tokak and its reciprocal were fit to the 15:1 ratio, and a homogeneity Chi-square ($X^2$) was calculated for each cross (Table 6).

Table 6: Number of specific parent and $F_2$ barley seedlings with and without symptoms after inoculation with Pyrenophora graminea isolate MT6 and Chi-square ($X^2$) values when fit to a 15:1 ratio.

| Seed Source | No Symptoms | Symptoms | Total | $X^2$ | df | p>[a] |
|---|---|---|---|---|---|---|
| Betzes X Tokak | | | | | | |
| Betzes - spec.[b] | 28 | 0 | 28 | | | |
| Tokak - spec. | 50 | 2 | 52 | | | |
| 402-a | 27 | 4 | 31 | 2.34 | 1 | 0.10 |
| 402-b | 21 | 2 | 23 | 0.23 | 1 | 0.50 |
| 402-c | 27 | 0 | 27 | 1.80 | 1 | 0.20 |
| 402-d | 18 | 1 | 19 | 0.03 | 1 | 0.70 |
| Subtotal | 93 | 7 | 100 | 4.40 | 4 | 0.25 |
| Betzes - spec. | 19 | 1 | 20 | | | |
| Tokak - spec. | 25 | 0 | 25 | | | |
| 405-a | 62 | 2 | 64 | 1.07 | 1 | 0.30 |
| 405-b | 55 | 2 | 57 | 0.11 | 1 | 0.70 |
| 405-c | 34 | 0 | 34 | 2.27 | 1 | 0.10 |
| 405-d | 36 | 4 | 40 | 0.96 | 1 | 0.30 |
| Subtotal | 187 | 8 | 195 | 4.41 | 4 | 0.25 |
| Total | 280 | 15 | 295 | 8.81 | 8 | 0.25 |
| | | | | -0.68 | 1 | 0.25 |
| Homogeneity | | | | 8.13 | 7 | 0.25 |

Table 6 (cont'd).

| Seed Source | Symptoms | No Symptoms | Total | $X^2$ | df | p>[a] |
|---|---|---|---|---|---|---|
| Tokak X Betzes | | | | | | |
| Tokak - spec. | 55 | 2 | 57 | | | |
| Betzes - spec. | 37 | 0 | 37 | | | |
| 416-a | 69 | 2 | 71 | 1.43 | 1 | 0.20 |
| 416-b | 67 | 3 | 70 | 0.46 | 1 | 0.50 |
| 416-c | 62 | 2 | 64 | 1.07 | 1 | 0.30 |
| 416-d | 67 | 2 | 69 | 1.53 | 1 | 0.10 |
| 416-e | 51 | 4 | 55 | 0.10 | 1 | 0.70 |
| Subtotal | 316 | 13 | 329 | 4.59 | 5 | 0.25 |
| Tokak - spec. | 41 | 1 | 42 | | | |
| Betzes - spec. | 24 | 0 | 24 | | | |
| 417-a | 69 | 3 | 72 | 0.52 | 1 | 0.30 |
| 417-b | 47 | 3 | 50 | 0.01 | 1 | 0.90 |
| 417-c | 66 | 2 | 68 | 1.21 | 1 | 0.20 |
| 417-d | 65 | 5 | 70 | 0.09 | 1 | 0.70 |
| Subtotal | 247 | 13 | 260 | 1.83 | 4 | 0.75 |
| Total | 563 | 26 | 589 | 6.42 | 9 | 0.50 |
| | | | | -3.39 | 1 | 0.05 |
| Homogeneity | | | | 3.03 | 8 | 0.90 |

[a] Probability level of a larger Chi-square value.
[b] Disease readings of seedlings from the specific parents of the $F_2$ populations are given preceeding the $F_2$ data. Seedlings from the bulk parents and check cultivars disease readings are given in Table 5 respective to the specific cross and isolate.

After inoculation with P. graminea isolate Kaya, $F_2$ seedlings derived from the Betzes X Tokak and reciprocally crossed $F_1$ plants did not fit a one, two, or three, dominant gene model. $F_2$ seedlings derived from six individual $F_1$ plants from the Betzes X Tokak cross

fit a 15:1 ratio, but the progeny from three other $F_1$ plants had quite high Chi-square values (Table 7). $F_2$ seedlings derived from eleven $F_1$ plants from the Tokak X Betzes cross also were not homogenous for the expected ratio. $F_2$ seedlings from six of these eleven $F_1$ plants did fit the 15:1 ratio.

Table 7: Number of specific parent $F_2$ barley seedlings with and without symptoms after inoculation with Pyrenophora graminea isolate Kaya and Chi-square ($X^2$) values when fit to a 15:1 ratio.

| Seed Source | No Symptoms | Symptoms | Total | $X^2$ | df | p>[a] |
|---|---|---|---|---|---|---|
| Betzes X Tokak | | | | | | |
| Betzes - spec.[b] | 37 | 4 | 41 | | | |
| Tokak - spec. | 57 | 8 | 65 | | | |
| 402-b | 41 | 3 | 44 | 0.02 | 1 | 0.75 |
| 402-c | 50 | 6 | 56 | 1.90 | 1 | 0.10 |
| 402-d | 25 | 3 | 28 | 0.74 | 1 | 0.25 |
| Betzes - spec. | 27 | 5 | 32 | | | |
| Tokak - spec. | 32 | 1 | 33 | | | |
| 403-b | 114 | 3 | 117 | 2.71 | 1 | 0.10 |
| 403-c | 73 | 27 | 100 | 73.48 | 1 | [e] |
| Betzes - spec. | 36 | 4 | 40 | | | |
| Tokak - spec. | 46 | 5 | 51 | | | |
| 405-a | 54 | 13 | 67 | 19.78 | 1 | [e] |
| 405-b | 110 | 5 | 115 | 0.71 | 1 | 0.25 |
| 405-c | 76 | 13 | 89 | 10.61 | 1 | [e] |
| 405-d | 57 | 3 | 60 | 0.16 | 1 | 0.50 |

Table 7 (cont'd).

| Seed Source | No Symptoms | Symptoms | Total | $X^2$ | df | p>[a] |
|---|---|---|---|---|---|---|
| Tokak X Betzes | | | | | | |
| Tokak - spec. | 19 | 3 | 22 | | | |
| Betzes - spec. | 36 | 5 | 41 | | | |
| 414-a | 38 | 0 | 38 | 2.53 | 1 | 0.25 |
| 414-b | 13 | 2 | 15 | [c] | | |
| 414-c | 9 | 1 | 10 | [c] | | |
| 415-a[d] | 72 | 16 | 88 | 21.38 | 1 | [e] |
| 415-b | 87 | 8 | 95 | 0.76 | 1 | 0.25 |
| 415-c | 48 | 10 | 58 | 11.96 | 1 | [e] |
| Tokak - spec. | 39 | 5 | 44 | | | |
| Betzes - spec. | 16 | 1 | 17 | | | |
| 416-b | 85 | 5 | 90 | 0.07 | 1 | 0.75 |
| 416-c | 89 | 8 | 97 | 0.66 | 1 | 0.25 |
| 416-e | 84 | 0 | 84 | 5.60 | 1 | [e] |
| 417-b[d] | 86 | 8 | 94 | 0.82 | 1 | 0.25 |
| 417-c | 85 | 3 | 88 | 1.21 | 1 | 0.25 |

[a] Probability level of a larger Chi-square value.
[b] Disease readings of seedlings from the specific parents of the $F_2$ populations are given preceeding the $F_2$ data. Seedlings' reactions from the bulk parents and check cultivars are given in Table 5 respective to the specific cross and isolate.
[c] Chi-square values were not calculated for populations consisting of less than 16 plants.
[d] Specific parents of these populations were not tested.
[e] P-values less than 0.05 were not calculated ($X^2>3.84$).

$F_1$ plants from backcrosses to both parents were inoculated with both MT6 and Kaya isolates (Table 8). A few symptomatic plants were found after these inoculations. With a dominant gene derived from each

parent, a 1 (resistant):0 (susceptible) ratio is expected in the backcrossed $F_1$ populations.

Table 8: Number of barley seedlings from bulked parents and backcrossed $F_{(1)}$ seeds with and without symptoms after inoculation with two Pyrenophora graminea isolates.

| Seed Source | Isolate | No Symptoms | Symptoms | Total |
|---|---|---|---|---|
| Betzes - bulked | MT6 | 87 | 1 | 88 |
| Tokak - bulked | MT6 | 77 | 12 | 89 |
| Betzes/Tokak X Tokak, $F_1$[a] | MT6 | 20 | 1 | 21 |
| Betzes/Tokak X Betzes, $F_1$ | MT6 | 21 | 1 | 22 |
| Betzes - bulked | Kaya | 69 | 2 | 71 |
| Tokak - bulked | Kaya | 62 | 4 | 66 |
| Betzes/Tokak X Tokak, $F_1$ | Kaya | 37 | 0 | 37 |
| Betzes/Tokak X Betzes, $F_1$ | Kaya | 22 | 2 | 24 |

[a] Data from the backcrossed $F_1$ generation include seedlings derived from three $F_1$ plants for each backcross.

Segregation occurred among the BC $F_2$ families when they were tested with P. graminea isolates MT6 and Kaya. Families in the backcrossed $F_2$ generation did not fit the expected ratios resulting from the two dominant genes model (Table 9). The BC $F_2$ families were classified either as resistant or as segregating. The percentage

infection of seedlings from the recurrent parent was the upper limit of the resistant class. Families with greater infection percentages than the recurrent parent were classified as segregating. The distribution of these backcrossed $F_2$ families was definitely skewed towards higher susceptibility than either parent which indicates gene action other than the dominant genes model.

Table 9: Number of backcrossed $F_2$ families resistant and segregating after inoculation with two Pyrenophora graminea isolates.

| Seed Source | Families | | |
|---|---|---|---|
| | Resistant | Segregating | Total |
| Betzes/Tokak X Tokak - MT6 | | | |
| Tokak[a]: Infection = 10.2% | | | |
| 420[b] | 1 | 4 | 5 |
| 421[b] | 3 | 3 | 6 |
| 422[b] | 1 | 5 | 6 |
| 423[b] | 0 | 4 | 4 |
| Total | 5 | 16 | 21 |
| Betzes/Tokak X Betzes - MT6 | | | |
| Betzes: Infection = 3.1% | | | |
| 427[c] | 2 | 4 | 6 |
| 428[c] | 1 | 5 | 6 |
| 429[b] | 2 | 4 | 6 |
| 430[b] | 3 | 3 | 6 |
| Total | 8 | 16 | 24 |

Table 9 (cont'd).

| Seed Source | Families: Resistant | Segregating | Total |
|---|---|---|---|
| Betzes/Tokak X Tokak - Kaya | | | |
| Tokak: Infection = 6.7% | | | |
| 420[d] | 1 | 4 | 5 |
| 421[d] | 1 | 5 | 6 |
| 422[d] | 1 | 5 | 6 |
| 423[d] | 0 | 5 | 5 |
| Total | 3 | 19 | 22 |
| Betzes/Tokak X Betzes - Kaya | | | |
| Betzes: Infection = 4.2% | | | |
| 427[e] | 0 | 5 | 5 |
| 428[e] | 0 | 2 | 2 |
| 429[e] | 2 | 4 | 6 |
| 430[e] | 3 | 3 | 6 |
| Total | 5 | 14 | 19 |

[a] The average infection percentage of the respective recurrent parent in each backcross trial was used as the upper limit of the resistant class.
[b] Seed lots consisted of 20-35 seeds each.
[c] Seed lots consisted of 10-20 seeds each.
[d] Seed lots consisted of 60-80 seeds each.
[e] Seed lots consisted of 35-80 seeds each.

Betzes - Yesilkoy Crosses

Betzes is highly resistant to infection by *P. graminea*, and Yesilkoy has a much lower level of resistance (6.4% and 18.1% infection of seedlings, respectively, in these trials). $F_{(1)}$ seeds of the Betzes X Yesilkoy and reciprocal crosses were tested for disease reaction after inoculation with two *P. graminea* isolates (Table 10). No symptomatic $F_1$ plants were observed after

inoculation with isolate MT6. When inoculated with isolate Kaya, a few of the $F_1$ plants from the Betzes X Yesilkoy and reciprocal crosses exhibited typical disease symptoms. These symptomatic plants were placed on BLPA or moistened filter paper, and typical Pyrenophora sp. conidia were produced.

Table 10: Number of barley seedlings derived from check cultivars, parents, and progeny of two crosses with and without symptoms after inoculation with two isolates of Pyrenophora graminea.

| Seed Source | Isolate | No Symptoms | Symptoms | Total |
|---|---|---|---|---|
| Betzes X Yesilkoy trial[a] | | | | |
| Summit - Ck | MT6 | 70 | 14 | 84 |
| Lami - Ck | MT6 | 28 | 58 | 86 |
| Betzes - bulked | MT6 | 48 | 0 | 48 |
| Yesilkoy - bulked | MT6 | 71 | 17 | 88 |
| Betzes - spec.[b] | MT6 | 54 | 1 | 55 |
| Yesilkoy - spec. | MT6 | 66 | 30 | 96 |
| B X Y, $F_1$[c] | MT6 | 12 | 0 | 12 |
| Betzes - spec. | MT6 | 79 | 0 | 79 |
| Yesilkoy - spec. | MT6 | 26 | 3 | 29 |
| B X Y, $F_1$ | MT6 | 10 | 0 | 10 |
| Betzes - spec. | MT6 | 79 | 4 | 83 |
| Yesilkoy - spec. | MT6 | 22 | 1 | 23 |
| B X Y, $F_1$ | MT6 | 19 | 0 | 19 |

Table 10 (cont'd).

| Seed Source | Isolate | No Symptoms | Symptoms | Total |
|---|---|---|---|---|
| Yesilkoy X Betzes trial | | | | |
| Summit - Ck | MT6 | 52 | 44 | 96 |
| Lami - Ck | MT6 | 11 | 83 | 94 |
| Yesilkoy - bulked | MT6 | 64 | 10 | 74 |
| Betzes - bulked | MT6 | 62 | 3 | 65 |
| Yesilkoy - spec. | MT6 | 47 | 8 | 55 |
| Betzes - spec. | MT6 | 38 | 0 | 38 |
| Y X B, $F_1$ | MT6 | 7 | 0 | 7 |
| Yesilkoy - spec. | MT6 | 38 | 4 | 42 |
| Betzes - spec. | MT6 | 44 | 2 | 46 |
| Y X B, $F_1$ | MT6 | 5 | 0 | 5 |
| Betzes X Yesilkoy trial | | | | |
| Summit - Ck | Kaya | 15 | 52 | 67 |
| Lami - Ck | Kaya | 3 | 65 | 68 |
| Betzes - bulked | Kaya | 63 | 7 | 70 |
| Yesilkoy - bulked | Kaya | 64 | 12 | 76 |
| Betzes - spec. | Kaya | 58 | 7 | 65 |
| Yesilkoy - spec. | Kaya | 21 | 11 | 32 |
| B X Y, $F_1$ | Kaya | 12 | 0 | 12 |
| Betzes - spec. | Kaya | 60 | 6 | 66 |
| Yesilkoy - spec. | Kaya | 6 | 3 | 9 |
| B X Y, $F_1$ | Kaya | 15 | 2 | 17 |
| Betzes - spec. | Kaya | 41 | 11 | 52 |
| Yesilkoy - spec. | Kaya | 31 | 11 | 42 |
| B X Y, $F_1$ | Kaya | 12 | 0 | 12 |

Table 10 (cont'd).

| Seed Source | Isolate | No Symptoms | Symptoms | Total |
|---|---|---|---|---|
| Yesilkoy X Betzes trial | | | | |
| Summit - Ck | Kaya | contaminated | | |
| Lami - Ck | Kaya | 2 | 66 | 68 |
| Yesilkoy - bulked | Kaya | 45 | 16 | 61 |
| Betzes - bulked | Kaya | 62 | 2 | 64 |
| Yesilkoy - spec. | Kaya | 18 | 2 | 20 |
| Betzes - spec. | Kaya | 53 | 6 | 59 |
| Y X B, $F_1$ | Kaya | 4 | 0 | 4 |
| Yesilkoy - spec. | Kaya | 20 | 24 | 44 |
| Betzes - spec. | Kaya | 56 | 3 | 59 |
| Y X B, $F_1$ | Kaya | 3 | 2 | 5 |
| Yesilkoy - spec. | Kaya | 7 | 2 | 9 |
| Betzes - spec. | Kaya | 53 | 11 | 64 |
| Y X B, $F_1$ | Kaya | 7 | 0 | 7 |
| Yesilkoy - spec. | Kaya | 42 | 16 | 58 |
| Betzes - spec. | Kaya | 32 | 5 | 37 |
| Y X B, $F_1$ | Kaya | 5 | 0 | 5 |

[a] Each trial was conducted at a different time.
[b] Parents marked 'spec.' give results of seedlings from the specific female and male plants that were crossed to produce the following $F_1$ seedlings.
[c] The $F_1$ seedling reactions are given following their specific female and male parent plants.

Disease reactions of $F_2$ seedlings from these crosses were variable. $F_2$ seedling reactions of populations from the Yesilkoy X Betzes cross did fit an expected Chi-square ratio of 13:3 after inoculation with isolate MT6 (Table 11). This ratio indicated the expected dominant gene inheritance from Betzes and a recessive gene from

Betzes or Yesilkoy. A homogeneity Chi-square was calculated, and the $F_2$ populations of this cross were found homogeneous. $F_2$ seedling reactions of populations from the reciprocal cross (Betzes X Yesilkoy) did not fit any expected ratios when inoculated with isolate MT6. The populations of $F_2$ seedlings from six of the eight $F_1$ plants did fit an expected Chi-square ratio of 13:3, however.

Populations of the inoculated Betzes X Yesilkoy $F_{(2)}$ seeds fit an expected 13:3 ratio when tested by the Chi-square method and inoculated with isolate Kaya (Appendix Table 11). Calculation of the homogeneity Chi-square indicated that the $F_2$ populations were homogeneous, and the populations were grouped together. The $F_2$ populations of seedlings from the reciprocal cross (Yesilkoy X Betzes) did not fit any dominant gene ratios after inoculation with isolate Kaya.

Table 11: Number of specific parent and $F_2$ barley seedlings with and without symptoms after inoculation with two Pyrenophora graminea isolates and Chi-square ($X^2$) values when fit to a 13:3 ratio.

| Seed Source | No Symptoms | Symptoms | Total | $X^2$ | df | p>[a] |
|---|---|---|---|---|---|---|
| Yesilkoy X Betzes - MT6 | | | | | | |
| Yesilkoy - spec.[b] | 48 | 8 | 56 | | | |
| Betzes - spec. | 47 | 4 | 51 | | | |
| 408-a | 76 | 11 | 87 | 2.13 | 1 | 0.10 |
| 408-b | 82 | 12 | 94 | 2.21 | 1 | 0.10 |
| 408-c | 35 | 6 | 41 | 0.46 | 1 | 0.25 |
| Subtotal | 193 | 29 | 222 | | | |
| 409-a[c] | 62 | 16 | 68 | 0.16 | 1 | 0.50 |
| 409-b | 68 | 11 | 79 | 1.21 | 1 | 0.25 |
| 409-c | 33 | 6 | 39 | 0.29 | 1 | 0.50 |
| 409-d | 30 | 7 | 37 | 0.01 | 1 | 0.90 |
| Subtotal | 193 | 40 | 233 | | | |
| Total | 386 | 69 | 455 | 6.47 | 7 | 0.25 |
| | | | | -3.84 | 1 | 0.05 |
| Homogeneity | | | | 2.63 | 6 | 0.75 |
| Betzes X Yesilkoy - MT6 | | | | | | |
| 396-a[c] | 17 | 5 | 22 | 1.74 | 1 | 0.10 |
| 396-b | 44 | 8 | 52 | 0.22 | 1 | 0.50 |
| 396-c | 100 | 10 | 110 | 1.62 | 1 | 0.10 |
| 396-d | 84 | 6 | 90 | 3.36 | 1 | 0.05 |
| Betzes - spec. | 24 | 1 | 25 | | | |
| Yesilkoy - spec. | 20 | 2 | 22 | | | |
| 398-a | 66 | 1 | 67 | 8.84[e] | 1 | [d] |
| 398-b | 10 | 1 | 11 | | | |
| 398-c | 30 | 2 | 32 | 1.35 | 1 | 0.10 |
| 398-d | 25 | 3 | 28 | 0.15 | 1 | 0.50 |

Table 11 (cont'd).

| Seed Source | No Symptoms | Symptoms | Total | $X^2$ | df | p>[a] |
|---|---|---|---|---|---|---|
| Yesilkoy X Betzes - Kaya | | | | | | |
| 408-a[c] | 64 | 14 | 78 | 0.03 | 1 | 0.75 |
| 408-b | 57 | 18 | 75 | 1.36 | 1 | 0.10 |
| 408-c | 51 | 25 | 76 | 9.98 | 1 | [d] |
| Yesilkoy - spec. | 42 | 16 | 58 | | | |
| Betzes - spec. | 32 | 5 | 37 | | | |
| 409-a | 60 | 13 | 73 | 0.04 | 1 | 0.75 |
| 409-b | 65 | 11 | 76 | 0.91 | 1 | 0.75 |
| 409-c | 54 | 21 | 75 | 4.21 | 1 | [d] |
| 409-d | 46 | 28 | 74 | 17.70 | 1 | [d] |
| Yesilkoy - spec. | 18 | 2 | 20 | | | |
| Betzes - spec. | 53 | 6 | 59 | | | |
| 411-a | 68 | 10 | 78 | 1.80 | 1 | 0.10 |
| 411-b | 75 | 4 | 79 | 9.02 | 1 | [d] |
| 411-c | 70 | 11 | 81 | 1.42 | 1 | 0.10 |
| Betzes X Yesilkoy - Kaya | | | | | | |
| Betzes - spec. | 41 | 11 | 52 | | | |
| Yesilkoy - spec. | 31 | 11 | 42 | | | |
| 397-a | 58 | 19 | 77 | 1.66 | 1 | 0.10 |
| 397-b | 17 | 3 | 20 | 0.18 | 1 | 0.50 |
| 397-c | 20 | 5 | 25 | 0.03 | 1 | 0.75 |
| 397-d | 14 | 5 | 19 | 0.71 | 1 | 0.25 |
| Subtotal | 109 | 32 | 141 | | | |

Table 11 (cont'd).

| Seed Source | No Symptoms | Symptoms | Total | $X^2$ | df | p>[a] |
|---|---|---|---|---|---|---|
| Betzes X Yesilkoy - Kaya (cont'd) | | | | | | |
| 398-b[c] | 26 | 3 | 29 | 1.35 | 1 | 0.10 |
| 398-c | 76 | 14 | 90 | 0.60 | 1 | 0.25 |
| 398-d | 44 | 9 | 53 | 0.11 | 1 | 0.50 |
| Subtotal | 146 | 26 | 172 | | | |
| Total | 255 | 58 | 313 | 4.64 | 7 | 0.50 |
| | | | | -0.01 | 1 | 0.90 |
| Homogeneity | | | | 4.63 | 6 | 0.50 |

[a] Probability level of a greater Chi-square value.
[b] Disease readings of the specific parents of the $F_2$ populations are given preceeding the $F_2$ data. Seedlings from the bulk parents and check cultivars disease readings are given in Table 10 respective to the specific cross and isolate.
[c] Specific parents of this $F_2$ population were not tested.
[d] P-values less than 0.05 were not calculated ($X^2>3.84$).
[e] Chi-square values were not calculated for populations consisting of less than 16 plants.

The populations of BC $F_1$ plants from these crosses did segregate as expected for a one dominant gene (Betzes) and one recessive gene model (Table 12). One symptomatic BC $F_1$ plant from the backcross Betzes/Yesilkoy X Betzes seeds was observed when inoculated with isolate MT6. With the backcross to Betzes, all plants grown from the BC $F_{(1)}$ seeds are expected to be resistant. In the populations of seedlings from the backcross to Yesilkoy, a ratio of three resistant BC $F_1$ plants to one susceptible BC $F_1$

plant is expected. Seedlings' reactions of BC $F_1$ populations which had been backcrossed to Yesilkoy fit an expected 3:1 ratio (P-value >0.50) after inoculations with both the MT6 and Kaya isolates.

Table 12: Number of barley seedlings from check cultivars, bulked parents, and backcrossed $F_{(1)}$ seeds with and without symptoms after inoculation with two Pyrenophora graminea isolates.

| Seed Source | Isolate | No Symptoms | Symptoms | Total |
|---|---|---|---|---|
| Summit - Ck | MT6 | 30 | 84 | 114 |
| Lami - Ck | MT6 | 13 | 92 | 105 |
| Betzes - bulked | MT6 | 65 | 3 | 68 |
| Yesilkoy - bulked | MT6 | 81 | 12 | 93 |
| Betzes/Yesilkoy X Betzes, $F_1$[a] | MT6 | 29 | 1 | 30 |
| Betzes/Yesilkoy X Yesilkoy, $F_1$ | MT6 | 28 | 9 | 37 |
| Summit - Ck | Kaya | 18 | 58 | 76 |
| Lami - Ck | Kaya | 0 | 75 | 75 |
| Betzes - bulked | Kaya | 62 | 3 | 65 |
| Yesilkoy - bulked | Kaya | 56 | 17 | 63 |
| Betzes/Yesilkoy X Betzes, $F_1$ | Kaya | 23 | 0 | 23 |
| Betzes/Yesilkoy X Yesilkoy, $F_1$ | Kaya | 31 | 9 | 40 |

[a] Data from the backcrossed $F_1$ generation include seedlings derived from three or four $F_1$ plants for each backcross.

With the one dominant gene and one recessive gene model, BC $F_2$ families are expected to segregate in a ratio of two homozygous resistant families to two segregating families. The BC $F_2$ families did not fit the expected ratios. The BC $F_2$ families were separated into either resistant or segregating classes. The average infection percentage of seedlings from the recurrent parent was the upper limit of the resistant class. Families with greater infection percentages than the recurrent parent were classified as segregating. The BC $F_2$ families were found homogeneous for fitting a 1:1 ratio, so these BC $F_2$ families were grouped together (Table 13).

Table 13: Number of backcrossed $F_2$ families resistant and segregating after inoculation with two *Pyrenophora graminea* isolates.

| Seed Source | Families | | |
|---|---|---|---|
| | Resistant | Segregating | Total |
| Betzes/Yesilkoy X Yesilkoy - MT6 Yesilkoy: Infection = 13.0%[a] Four $F_1$ Plants[b] | 9 | 11 | 20 |
| Betzes/Yesilkoy X Betzes - MT6 Betzes: Infection = 5.0% Four $F_1$ Plants[b] | 8 | 11 | 19 |
| Betzes/Yesilkoy X Yesilkoy - Kaya Yesilkoy: Infection = 11.6% Four $F_1$ Plants[c] | 7 | 10 | 17 |
| Betzes/Yesilkoy X Betzes - Kaya Betzes: Infection = 5.7% Four $F_1$ Plants[c] | 10 | 14 | 24 |

[a] The average infection percentage of seedlings from the respective recurrent parent in each backcross trial was used as the upper limit of the resistant class.
[b] 30-40 seedlings were read from each BC $F_1$ plant.
[c] 60-80 seedlings were read from each BC $F_1$ plant.

## Discussion

Konak (1983) proposed that Betzes and Tokak each had at least one dominant gene for resistance. He could not determine whether or not these genes were at the same locus. The data of seedlings from the $F_1$ and $F_2$ generations do not refute that Betzes and Tokak each have

one dominant gene for resistance when inoculated with P. graminea isolate, MT6. However, because seedlings from specific parents and the $F_2$ generation fit the same expected ratio, no conclusions as to gene action are proposed. The infection reactions of seedlings from the BC $F_2$ families indicate that the suggested dominant genes are not at the same locus, and that genes other than two dominant genes are involved in this cross.

Dominant gene action is also one possibility in the Betzes-Tokak crosses when seed is artificially inoculated with the P. graminea isolate Kaya. Only one symptomatic $F_1$ plant was observed from the inoculated seeds derived from this cross and its reciprocal.

The $F_2$ populations from the Betzes X Tokak and reciprocal crosses do not fit a dominant gene ratio when artificially inoculated with the isolate Kaya. The populations which do not fit a dominant gene ratio exhibited greater than expected numbers of infected plants. Although only a few BC $F_1$ plants from the Betzes/Tokak backcrosses were infected with isolate Kaya, the BC $F_2$ families from these backcrosses consisted of greater than expected numbers of infected seedlings. These findings indicate that any dominant gene action is modified by other factors which segregate in advanced

generations. The lack of higher numbers of $F_3$ and BC $F_2$ families from these crosses prevent the analyses required to determine oligogenic or quantitative gene action.

Betzes was also crossed reciprocally with Yesilkoy in this study. Dominant gene action was again not refuted in the $F_1$ plants and $F_2$ populations from these crosses, but the similarity of parents' and their progenies' reactions in these crosses prevented conclusions as to gene action. No infected $F_1$ plants were found after artificial inoculation with P. graminea isolate MT6. A few infected $F_1$ plants occurred after inoculation with the isolate Kaya.

The $F_2$ populations from the Betzes X Yesilkoy and reciprocal crosses were variable in disease reaction. When inoculated with the isolate MT6, $F_2$ populations from the Yesilkoy X Betzes cross did fit an expected 13:3 ratio. The $F_2$ populations from the reciprocal cross did not fit an expected ratio when inoculated with the MT6 isolate. Conversely, after inoculation with the isolate Kaya, the $F_2$ populations from the Betzes X Yesilkoy cross fit an expected ratio of 13:3. Populations of $F_2$ seedlings from the reciprocal cross did not fit an expected ratio after inoculation with the Kaya isolate. BC $F_1$ and BC $F_2$ seedlings' reactions supported the model

with Betzes providing a single, dominant gene, and Yesilkoy providing a single, recessive gene.

Genetic penetrance is the frequency of a recognizable effect in individuals carrying a gene (Allard, 1960). Expressivity is the degree of manifestation of the genetic character (Allard, 1960). Although these concepts have been discussed in inheritance studies (Metcalfe and Helgason, 1962; Boyd, 1966; Khan, 1969; Alon, et al., 1974), a method to calculate such effects is not widely accepted. Wells (1958) proposed one method of calculating such effects in an inheritance study of the Ustilago hordei (Pers.) Lagerh.-Hordeum vulgare L. interaction. Another method has been used in relation to the inheritance of barley leaf stripe disease resistance (Konak, 1983; E.A. Hockett, personal communication).

Parent seedlings' reactions could not easily be distinguished from $F_1$ and $F_2$ seedlings' reactions in these crosses. These results may be due to the penetrance and expressivity of the resistance genes. A method of calculating penetrance and expressivity effects is presented in Appendix Table 20. Calculations with these modified expected ratios confirmed the dominant and recessive gene model proposed for the $F_2$ populations from

the Yesilkoy X Betzes cross as previously presented. The calculated ratio also fit the results of the $F_2$ populations from the Betzes X Yesilkoy cross when inoculated with isolate MT6. The calculated ratios using this method did not increase the fit of the results from the Betzes X Yesilkoy and reciprocal crosses after inoculation with the Kaya isolate.

These results with P. graminea isolate MT6 lend support to the dominant gene hypothesis in Betzes proposed by Konak (1983). A distinct dominant gene is also proposed for the cultivar Tokak. Results from this study with the cultivar Yesilkoy indicate that a recessive gene for resistance is present. The occurence of greater than expected numbers of susceptible plants in the BC $F_2$ families and a limited number of $F_3$ famlies (Appendix Table 19) indicates that a more complex gene action is involved. The $F_3$ families from these crosses were not analyzed because of the limitation in the number of families tested. This limitation suggests a major hindrance in the study of resistance in barley to leaf stripe disease. Multiple gene and quantitative gene action have been proposed in resistant cultivars. To be able to propose and determine such gene models, advanced

generations of crossed seed with sufficient numbers of lines must be analyzed.

A great amount of growth chamber space was required to artificially inoculate barley seeds with the fungus and to grow the seedlings. To verify multiple gene action, advanced lines might better be inoculated and grown to maturity in the field. One would be able to have sufficient replications in such a study to statistically increase the precision of infection percentage means. Also, one would be able to read the plants for disease through their complete life cycle. Multiple disease readings would facilitate the method of determining resistance proposed by Scott and Rosenkranz (1982; Rosenkranz and Scott, 1984). Field inoculations of a non-native pathogen (in Montana) present problems. Either the isolation of such a disease nursery or the planting of the nursery in another area may provide a solution.

The gene actions determined here agree with a finding presented by Isenbeck (cited in Arny, 1945). Isenbeck found $F_3$ lines that were more susceptible than either parent in a cross between two resistant varieties. Arny (1945) also found dominant gene action in the inheritance of resistance to leaf stripe disease. He

also proposed incomplete dominance along with a number of factors, partial dominance, and a modifying gene for resistance. Suneson (1950) proposed a gene providing incomplete dominance and two recessive genes for resistance to leaf stripe disease. Nilsson (1975) has also proposed major gene control for resistance in barley to leaf stripe disease.

Knudsen (1980) found continuous variation in the disease reaction of 145 barley cultivars that were naturally inoculated with P. graminea (to a population of the pathogen). Tekauz (1983) has suggested that the high resistance to leaf stripe disease in Betzes is not monogenic. Although near isogenics of Betzes ('Shabet', 'Erbet') are resistant to the disease (Metz and Scharen, 1979), a cultivar derived from a cross using Betzes and a more susceptible parent ('Domen') was susceptible ('Klages'). Results presented here indicate that more than single, dominant gene action is involved in Betzes, especially after inoculation with the P. graminea isolate, Kaya.

In conclusion single, dominant genes in Betzes and Tokak and a recessive gene in Yesilkoy for resistance to P. graminea were proposed. The gene action seems to be more complex than these preliminary models. Genes for

resistance to *P. graminea* are available in barley, but the statement of Shands and Arny (1944) remains valid: "Before assuming a varietal response an investigator should artificially inoculate the varieties in which he is interested, using local cultures..."

# CHAPTER 5

## SUMMARY

This study on the inheritance of resistance in barley to Pyrenophora graminea Ito et Kurib. was a continuation of previous research conducted at Montana State University (Metz and Scharen, 1979; Johnston, Metz, and Riesselman, 1982; Konak, 1983). Results of the studies on inheritance have been inconclusive. Variability of infection percentages with different inoculation methods has been reported. Therefore, a more efficient and stable inoculation method was sought.

Konak (1983) reported that P. graminea grew more profusely on a rich medium. I used this medium and several other media to test the stability of virulence of P. graminea isolates when the isolates were cultured continuously on the media over time. Two of the media tested were amended with wheat bran. Since the mycelium of P. graminea is dormant within the pericarp of the barley kernel, the enhancement of growth on wheat bran seemed plausible. P. graminea systemically infects

barley plants during emergence. Therefore, both a diffusate and an extract of germinated, barley seeds were used as an amendment for two other media. Other media used were standard culturing media (WA, V8 juice, and barley leaf piece agar). Although no significant differences between means of the cultured isolate were found, it was indicated that the diffusate of germinated barley seed was beneficial to infectivity by the P. graminea isolate tested.

Variability in disease reacton of specific barley cultivars has also been reported in this host-pathogen interaction. I collected a number of P. graminea isolates from MT, USA, North Africa, and the Near East. Twenty-four of these isolates were artificially inoculated to three barley cultivars. No specific conclusions were reached as to different levels of virulence associated with particular regions. The variability of the disease reaction in the cultivar Summit indicated that a low level of resistance to some isolates of P. graminea is present in this cultivar.

Differential interactions between specific barley cultivars and P. graminea isolates were recorded. The differences between means of the isolate X cultivar interactions were significant in an analysis of variance.

These results support hypotheses of specific resistance in barley to certain virulence groups of *P. graminea*.

The primary purpose of my thesis research was to identify genes for resistance in barley to infection by *P. graminea*. The mechanisms of resistance in barley to this disease are not understood. To study inheritance of resistance three parents were crossed, and plants were tested from the parent to the $F_3$ and BC $F_2$ generations. A standardized inoculation method and controlled environment were used in testing seed lots for either the presence or absence of disease symptoms. Results from the $F_1$ and $F_2$ generations did not refute the presence of dominant gene action in Betzes and Tokak and recessive gene action in Yesilkoy. However, with the increased susceptibility of seedlings in the BC $F_2$ families, inheritance of resistance in Tokak and Betzes was determined to be more complex than the dominant genes model.

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# APPENDIX

Table 14: Potato dextrose agar plus diffusate of germinated, barley seed.

| | |
|---|---|
| 6 80-seed lots | Summit barley seed |
| 39 g | BBL Potato dextrose agar |
| 1000 cc | Distilled water |

1. Surface disinfect the seed lots 1 min in 50% ETOH; then, 3-4 min in 10% Clorox.
2. Dry the seed lots 8-12 h on paper towels in a clean air chamber.
3. Place each 80-seed lot on filter paper in a Petri dish; add 5 cc sterile distilled water; cover with filter paper and Petri lid.
4. Let seeds germinate at room temperature for 3 days.
5. Soak germinated seed 10 min in 25 cc distilled water; strain liquid through two layers of cheese cloth.
6. Add germinated seed diffusate to flask measuring the liquid as part of the one liter water.
7. Autoclave medium 20 min at 121 C.

Table 15: Potato dextrose agar plus extract of ground, germinated, barley seed.

| | |
|---|---|
| 6 80-seed lots | Summit barley seed |
| 39 g | BBL Potato dextrose agar |
| 1000 cc | Distilled water |

1. Surface disinfect the seed lots 1 min in 50% ETOH; then, 3-4 min in 10% Clorox.

2. Dry the seed lots 8-12 h on paper towels in a clean air chamber.

3. Place each 80-seed lot on filter paper in a Petri dish; add 5 cc sterile, distilled water; cover with filter paper and Petri lid.

4. Let seeds germinate at room temperature for 3 days.

5. Add 25 cc distilled water to each seed lot and grind with a mortar and pestle; blend mixture for 3 min at high speed in a blendor (Waring, e.g.); strain mixture through two layers of cheese cloth.

6. Add ground extract to flask measuring the liquid as part of the one liter water.

7. Autoclave medium at 121 C.

Table 16: Two-factorial analysis of variance of cultured isolate experiments and comparison of culture means.

| Source | df | S. S. | M. S. | F-value | P-value |
|---|---|---|---|---|---|
| Planting Dates | 3 | 1.57 | 0.52 | 99.34 | 0.00 |
| Media | 8 | 0.27 | 0.03 | 6.42 | 0.00 |
| Dates X Media | 24 | 0.31 | 0.01 | 2.49 | 0.007 |
| Error | 36 | 0.19 | 0.005 | | |

Means Comparison: LSD at 0.05 (using error mean square)

| | | |
|---|---|---|
| Noninoculated | 0.00[a] | A |
| PDA + 2[b] | 0.13 | B |
| PDA + 20[c] | 0.17 | BC |
| B L P A[d] | 0.17 | BC |
| PDA + seed ext.[e] | 0.17 | BC |
| Water agar | 0.18 | BC |
| B L Ext.[f] | 0.18 | BC |
| V 8 juice | 0.19 | BC |
| PDA + seed dif.[g] | 0.23 | C |

[a] In the ANOVA, 0.00% was entered as $1.0 \times 10^{-4}$.
[b] PDA amended with 2 g/l spring wheat bran.
[c] PDA amended with 20 g/l spring wheat bran.
[d] Barley leaf piece agar (Teviotdale and Hall, 1976a).
[e] PDA amended with ground, germinated, barley seed, extract (Appendix Table 13).
[f] Barley leaf extract medium (Konak, 1983, but with 30 g/l fresh barley leaves).
[g] PDA amended with germinated, barley seed, diffusate (Appendix Table 12).

Table 17: Comparison of emergence percentages of barley seedlings from the parent and check cultivars.

| Cultivars | Germ. | 6-week Emerg.[a] | Noninoc. Emerg.[b] | Inoc. Emerg. | Infection[c] |
|---|---|---|---|---|---|
| Summit | 98.7 | 97.3 | 95.0 | 88.5 | 64.9 |
| Lami | 97.9 | 94.0 | 90.0 | 88.9 | 88.8 |
| Betzes | 91.9 | 80.2 | 85.4 | 81.9 | 4.2 |
| Tokak | 90.0 | 91.9 | 91.2 | 83.9 | 7.3 |
| Yesilkoy | ---- | ---- | 85.0 | 87.6 | 17.5 |

[a] The 6-week emergence is the percentage of emerged plants that had germinated in the germination test.
[b] Noninoculated emergence is the percentage of emerged plants 6 weeks old after seed lots had been placed in the layered PDA + 4 medium without fungus.
[c] Average infection percentages of emerged plants are given from the same seed lots that were counted for the inoculated emergence.

Table 18: Analysis of covariance with emergence percentages as the covariable and infection percentages as the dependent variable of seedlings from the parent and check cultivars.

| Source | df | S. S. | M. S. | F-value | P-value |
|---|---|---|---|---|---|
| Emergence[a] | 1 | 0.012 | 0.012 | 0.711 | 0.406 |
| Cultivars | 4 | 6.789 | 1.697 | 107.900 | 0.000 |
| Isolates[b] | 2 | 0.066 | 0.033 | 2.096 | 0.140 |
| Cv's X Isolates | 8 | 0.182 | 0.023 | 1.443 | 0.221 |
| Error | 29 | 0.456 | 0.016 | | |

[a] An arc sin transformation of the percentage data was made before calculating the analysis of covariance.
[b] Isolates used in the inoculation of seed were *P. graminea* isolates MT6, Kaya, and RPB. A noninoculated check was not included in the calculations.

Table 19: Number of $F_3$ families resistant and segregating after inoculation with two Pyrénophora graminea isolates (30-80 seedlings were read in each family).

| Seed Source | Families | | |
|---|---|---|---|
| | Resistant | Segregating | Total |
| Betzes X Tokak - MT6 | | | |
| Betzes[a]: Infection = 0.0% | | | |
| Tokak[b]: Infection = 3.7% | | | |
| 402-a[c] | 35 | 5 | 40 |
| Tokak X Betzes - MT6 | | | |
| Tokak[b]: Infection = 3.3% | | | |
| Betzes: Infection = 0.0% | | | |
| 416-a | 3 | 37 | 40 |
| Betzes X Tokak - Kaya | | | |
| Betzes: Infection = 9.8% | | | |
| Tokak[b]: Infection = 12.3% | | | |
| 402-a | 24 | 16 | 40 |
| Betzes[b]: Infection = 10.0% | | | |
| Tokak: Infection = 9.8% | | | |
| 405-a | 16 | 24 | 40 |
| Tokak X Betzes - Kaya | | | |
| Tokak[b]: Infection = 11.4% | | | |
| Betzes: Infection = 5.9% | | | |
| 416-a | 14 | 26 | 40 |
| 416-b | 20 | 20 | 40 |
| Betzes X Yesilkoy - MT6 | | | |
| Betzes: Infection = 3.0%[d] | | | |
| Yesilkoy: Infection = 19.1% | | | |
| 398-a | 12 | 28 | 40 |
| Yesilkoy X Betzes - MT6 | | | |
| Yesilkoy: Infection = 14.2% | | | |
| Betzes: Infection = 8.0% | | | |
| 408-a | 16 | 24 | 40 |

Table 19 (cont'd).

| Seed Source | Families | | |
|---|---|---|---|
| | Resistant | Segregating | Total |
| Betzes X Yesilkoy - Kaya | | | |
| Betzes[e]: Infection = 10.0% | | | |
| Yesilkoy[e]: Infection = 15.8% | | | |
| 398-a | 13 | 24 | 37 |
| 398-b | 17 | 20 | 37 |
| Yesilkoy X Betzes - Kaya | | | |
| Yesilkoy[e]: Infection = 26.2% | | | |
| Betzes[e]: Infection = 3.1% | | | |
| 408-a | 1 | 38 | 39 |
| Yesilkoy: Infection = 27.6% | | | |
| Betzes: Infection = 13.5% | | | |
| 409-a | 18 | 20 | 38 |

[a] Disease readings of seedlings from the specific parents are given preceeding the $F_3$ data. Seedlings' reactions from the bulk parents and check cultivars are given in Tables 5 and 10 respective to the specific cross and isolate.

[b] The greater infection percentage of the specific parents was the upper limit of the resistant class.

[c] Data from inoculation of $F_2$ seedlings was given in Tables 6, 7, and 11 respective to the specific cross and isolate.

[d] The infection percentage of seedlings from the resistant parent, Betzes, was used as the upper limit of the resistant class.

[e] Specific parents were not tested due to insufficient seed numbers. Infection percentages of bulked seed lots are given.

Table 20: Calculations of genetic ratios with the effects of penetrance and expressivity.

---

Yesilkoy X Betzes - MT6

Lami (check): Infection = 88.3%
Yesilkoy: Average of bulked and specific parent infections = 13.0%
Betzes: Average of bulked and specific parent infections = 4.20%

In a 13:3 ratio the 13 resistant plants have a dominant gene in 12 of the resistant plants (from Betzes) and a double recessive in one of the resistant plants (from Yesilkoy).

Therefore, the percentage of resistant plants that are infected and show symptoms can be calculated.

$$\frac{12(0.0420) \pm 1(0.1297)}{13} = 0.0487$$

Susceptible plants without symptoms (escapes) are also found.

$$1.000 - 0.883 = 0.117$$

When more susceptible plants are without symptoms than resistant plants that are with symptoms, a subtraction is made.

$$0.117 - 0.0487 = 0.06823$$

This fraction of symptomless, susceptible plants is then taken from the susceptible class and added to the resistant class.

$$13:3 = 0.8125:0.1875$$

or, with penetrance and expressivity calculations:

13:3 becomes
0.8125 + (0.1875 X 0.0683):0.1875 - (0.1875 X 0.0683)
= 0.8253:0.1747

Table 20 (cont'd.).

Betzes X Yesilkoy - MT6

Lami (check): Infection = 67.4%
Betzes: Average of bulked and specific parent infections = 1.92%
Yesilkoy: Average of bulked and specific parent infections = 15.7%

Calculations follow as above for an expected 13:3 ratio.

$$\frac{12(0.0192) \pm 1(0.157)}{13} = 0.0298$$

1 - 0.674 = 0.326
0.326 - 0.0298 = 0.296 ( the fraction of susceptible plants that appear resistant)

13:3 = 0.8125:0.1875

or, with penetrance and expressivity calculations:

13:3 becomes
0.8125 + (0.1875 X 0.296):0.1875 - (0.1875 X 0.296)
= 0.868:0.132

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