



The inheritance of resistance of barley (*Hordeum vulgare* L.) to *Pyrenophora graminea* Ito et Kurib
by Cahit Konak

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Plant
Pathology

Montana State University

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Abstract:

Barley stripe disease, caused by the fungus pathogen *Pyrenophora graminea* Ito et Kurib, causes significant losses to the barley crop in Turkey and the U.S.A. This research was initiated to study crosses between some important Turkish barley cultivars and some American barley cultivars that had been identified as resistant to *P. graminea*. A major goal of the research was the understanding of the inheritance of resistance to *P. graminea* in barley.

Because of the nature of barley stripe, it was necessary to improve laboratory and greenhouse techniques for culturing the fungus and for inoculating populations of the host. In the course of these studies, three inoculation techniques were used, modified and improved. These were the barley kernel method, the layered mycelium method, and the soil inoculation method.

Five American barley cultivars and seven Turkish barley cultivars were crossed reciprocally and progeny were increased to the F₂ generation. Populations of F₂ progeny were inoculated with selected cultures of *P. graminea* and ratios of resistant to susceptible reactions were analyzed to determine the numbers of genes acting and the mode of gene action.

A very high level of variability was revealed in cultures of *P. graminea*. In some cases, a remarkable loss of pathogenicity was observed. Some ideas as to causes of the variability were discussed.

It is also speculated that some variable results reported in the literature may have been due to variation in the pathogen.

Analysis of the F₂ barley populations revealed several different genes for resistance to *P. graminea* exist in the American and Turkish barleys studied, and that several different kinds of gene action may also be present in the hybrid progeny.

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Plant Pathology

MONTANA STATE UNIVERSITY
Bozeman, Montana

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of a thesis submitted by

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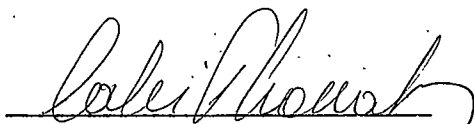
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TO MY FATHER

VITA

Cahit Konak was born to Omer and Nuzhet Konak on April 25, 1944, in Susurluk, Turkey.

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ABSTRACT

Barley stripe disease, caused by the fungus pathogen Pyrenophora graminea Ito et Kurib. causes significant losses to the barley crop in Turkey and the U.S.A. This research was initiated to study crosses between some important Turkish barley cultivars and some American barley cultivars that had been identified as resistant to P. graminea. A major goal of the research was the understanding of the inheritance of resistance to P. graminea in barley.

Because of the nature of barley stripe, it was necessary to improve laboratory and greenhouse techniques for culturing the fungus and for inoculating populations of the host. In the course of these studies, three inoculation techniques were used, modified and improved. These were the barley kernel method, the layered mycelium method, and the soil inoculation method.

Five American barley cultivars and seven Turkish barley cultivars were crossed reciprocally and progeny were increased to the F_2 generation. Populations of F_2 progeny were inoculated with selected cultures of P. graminea and ratios of resistant to susceptible reactions were analyzed to determine the numbers of genes acting and the mode of gene action.

A very high level of variability was revealed in cultures of P. graminea. In some cases, a remarkable loss of pathogenicity was observed. Some ideas as to causes of the variability were discussed. It is also speculated that some variable results reported in the literature may have been due to variation in the pathogen.

Analysis of the F_2 barley populations revealed several different genes for resistance to P. graminea exist in the American and Turkish barleys studied, and that several different kinds of gene action may also be present in the hybrid progeny.

CHAPTER 1

INTRODUCTION

Barley stripe, caused by the fungus Pyrenophora graminea is an important disease of barley in temperate regions of the world. The disease was widespread in the U.S.A. in the early part of this century, but the use of mercury seed dressings after 1920 gradually reduced the disease to one of relative unimportance.

The disease became potentially important again due to the banning of the organic mercury seed treatment fungicides from the market in the mid 1970s. Ceresan (having organic mercury as its active ingredient), was the only fungicide which completely killed the mycelium of P. graminea. No fungicide has been developed to replace Ceresan up to this day. Seven experimental fungicides were reported to control the disease completely (Johnston et al., 1982), but none is yet approved for general use.

Environment has a marked influence on the increase or decrease of P. graminea. Irrigation practices are increasing and irrigation prior to heading time in a semi-arid climate increases the spread of the disease for the next year. Conversely, everything which increases vigorous plant growth, decreases the severity of the disease.

Control of barley stripe with resistant varieties is the cheapest way, but difficult because P. graminea is a highly variable organism that occurs as numerous virulence types. The probability of asexual

recombination through heterokaryosis and parasexuality appears high. Because it is such a variable organism, long lasting resistance may be difficult to achieve.

The introduction of susceptible varieties can sharply increase the amount of the disease in a region. Summit, a two-row spring barley developed in England, was responsible for such increases in some regions of the U.S.A. According to Johnston et al. (1982), regional testing in the United States began in 1972. When Summit was grown in 1978 as breeder seed, the plants were known to carry a trace of barley stripe. In 1979, in Montana, after its release as a variety, Johnston et al. (1982) found an irrigated field which contained 30 percent infected plants. Laboratory assay of the seed harvested from this field determined that the infection level in the seed was greater than 90 percent. In two growing seasons, the infection level increased from a trace to 30 percent to greater than 90 percent.

Genetic resistance to this fungus is available. The cultivars Betzes, Shabet, and Erbet were found resistant (Metz and Scharen, 1979). It appears that resistance is easy to transfer to susceptible varieties.

Most of the research on barley stripe was done in the early part of this century. In recent years, due to the banning of Ceresan from the market, increased research activities on the different aspects of P. graminea have been undertaken. The purpose of this research was to determine the inheritance of resistance to barley stripe in selected cultivars, and to elucidate the gene action involved.

CHAPTER 2

LITERATURE REVIEW

The Pathogen: Pyrenophora Graminea

Ito (1930) described Pyrenophora graminea Ito et Kurib as the perfect stage of the fungus on Hordeum sativum L. in Japan. The imperfect form of P. graminea is Drechslera graminea (Rab.) Shoem., and its synonym is Helminthosporium gramineum. The imperfect form of P. graminea belongs to the genus of Hyphomycetes (Dematiaceae) recognized chiefly by the presence of brown septate conidiospores and large brown phragmoid conidia borne laterally and terminally on the conidiophore (Talbot, 1973).

Conidia of Drechslera spp. are essentially cylindrical at maturity (Shoemaker, 1962). The conidia length and diameter of conidia are 30-110 μm and 11-24 μm , respectively. Conidia develop at the apical pore of a conidiophore and are first spherical, soon becoming obovoid, then more or less cylindrical after further elongation. The mature spore is divided into 5-7 cells that are longer than wide or isodiametric. The outer wall of the conidium is fairly rigid, pigmented slightly, and quite thin. The inner wall is amorphous, usually hyaline, and often appears thick in dried conidia. The septa in Drechslera conidia are quite obvious. Germination is by the production of one or more germ-tubes from any cell (Shoemaker, 1962). The conidia of P. graminea are sub-hyaline to yellow brown (Talbot, 1973).

The conidiophore produces a conidium at an apical pore and by sub-apical growth, grows past the scar and forms a new apex. The conidiophore base is usually enlarged and either globose or tapered into the stalk. The stalk is uniform in diameter, fairly straight, erect, simple or rarely branched, and septate at regular intervals. The width at the apex ranges from 5-15 μm , and the length is from 25-385 μm . Conidiophores usually arise singly from the host but are sometimes grouped (Shoemaker, 1962).

P. graminea is bisexual and heterothallic (Smedegard-Petersen, 1976). Pseudoperithecia of P. graminea are rarely reported in the literature and there seems to be some uncertainty as to their existence. Thus, Dickson (1956) stated that the association between the conidial and a perfect stage of this pathogen has not been confirmed. The perfect stage was first described by Ito (1930) and confirmed by Smedegard-Petersen (1973). Smedegard-Petersen reported that the sclerotium-like bodies were superficial or partly submerged, elongated with dark rigid setae on the surface. They measured 576-728 x 442-572 μm and were very similar to the pseudoperithecia of Pyrenophora teres. Some of the pseudoperithecia contained asci and ascospores but the majority remained immature. Conidia were formed abundantly on setae of the pseudoperithecia. Smedegard-Peterson also found pycnidia of P. graminea on infected straw and in cultures formed on sterilized corn leaf segments placed on Sach's agar. The pycnidia and pycnospores, which were morphologically similar to those formed by P. teres, measured 70-176 μm and 1.4-3.2 x 1.0-1.6 μm , respectively. Attempts to germinate

pycnospores on potato dextrose agar were successful in about half of the isolations.

Symptoms

Typically the young leaf at emergence or soon after, shows the presence of the fungus in one or more stripes that form on the leaf surface (Smith, 1929; Drechsler, 1923; Stakman and Rodenhiser, 1929). The disease may exhibit small chlorotic, elongate spots which develop into white or yellow stripes extending the length of the leaves (Drechsler, 1923; Stakman and Rodenhiser, 1929; Tekauz and Chiko, 1980). In some instances, the stripes consist partially of necrotic tissue when first observed, while in other cases at first only chlorosis is evident. Stripe symptoms are not always observed on all leaves of infected plants during an experiment (Tekauz and Chiko, 1980). Infected seedlings are more or less retarded in growth and death of the plant often occurs at an early stage (Smedegard-Peterson, 1976). On leaves of surviving plants, 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 gray as the tissue becomes necrotic (Smedegard-Peterson, 1976) and the leaf blade may split free and attain a frayed appearance (Drechsler, 1923; Smedegard-Peterson, 1976). Infected plants are frequently reduced in height (Drechsler, 1923; Stakman and Rodenhiser, 1929; Smedegard-Peterson, 1976). In heavy attacks, ear emergence from the sheath may be arrested (Drechsler, 1923; Suneson, 1950; Smedegard-Peterson, 1976) and barren ears or improperly developed kernels may result (Weniger, 1932; Smedegard-Peterson, 1976).

Pathological Histology

The pathogen-host tissue relationship with P. graminea has been studied by few workers and results of the histology of the susceptible reaction are controversial. Germination of the conidiospores is accomplished by the production of one or more germ tubes from any cell (Shoemaker, 1962). P. graminea was found to be a seed transmitted disease by Ravn (1900). He further mentioned that this fungus infects seedlings during germination but cannot infect them in later growth stages. According to Ravn, the pathogen established itself first in the growing point then infected all shoots and leaves. Smith (1929) reported that hyphae from the pericarp layer penetrated the epidermis of the coleoptile by means of an appressorium and a hyphal peg, grew into the coleoptile, then into the leaves and stem apex in the later stages of pathogen development.

Stelzner (1934) found that mycelium penetrates through the coleorhiza and the roots, sometimes without formation of appressoria, then grows through the scuteller node and continues toward the top of the plant. Skoropad and Arny (1956) reported that the fungus penetrated coleorhizae by means of appressoria and hyphal pegs. It advanced intracellularly through meristematic areas. They also mentioned that spread of mycelium in the host occurred through the lumina of xylem vessels. Teviotdale and Hall (1976) noted that the critical stage for infection of the germinating embryo began when the coleoptile reached the apex of the seed and continued until the seedling emerged from the soil. Inoculum located in the pericarp and seed coat over the embryo was most effective in producing the seed-transmitted stripe. The

presence of the fungus in barley seed demonstrated that the percentage of seed transmission was always less than the amount of infected seed. The most impressive and most recent research to explain this situation was done by Platenkamp (1976). She worked with naturally inoculated barley seeds and reported that the only important source of inoculum was the resting mycelium in and around the tracheid mass at the embryo end of the vessel in the pericarp of the seed. The apical part of the coleorhiza became infected by hyphae from this mycelium during the germination of both seed and resting mycelium. Stelzner (1934) and Skoropad and Arny (1956) observed nearly the same thing; infection by P. graminea started in the coleorhiza. This finding disagreed with Smith (1929) who had described the hyphae as entering the scuteller node by way of roots or scutellum, and the infection taking place through the coleoptile. She further proposed that hyphae grew intercellularly into the coleorhiza without formation of appressoria. Part of the hyphae become trapped in the scutellum, part are stopped in the scutellar node, possibly by a pathological reaction which is a degeneration of hyphae in the basal part of the coleorhiza and in the scutellar node. This explanation agrees with the suggestion of Skoropad and Arny (1956), that the resistance to barley stripe is due to a form of hypersensitive reaction. After 12 to 14 days the plant could be completely invaded.

Another important thing to understand about the infection of seedlings is the time it takes the fungus to reach the different parts of the plant. Teviotdale and Hall (1975) reported that fungus was present in coleorhiza and scutellum after seven days at 6C; no infection was observed in the stem or shoot apex. The total number of seedling

parts invaded after 28 days of germination at 6 C was distinctly greater than the number invaded after seven days. Platenkamp (1976) found that after two days, mycelium grew on and into coleorhizae. Invasion of the scutellum usually was not found before the plants were at least 14 days old. Invasion of fungus mycelia from the scutellum to the coleoptile occurred in one case after four days, but in other cases not until after eight or nine days.

Factors Affecting Infection and Symptom Expression

The epidemiology of P. graminea can be better understood by studying the effects of temperature and soil moisture on transmission of the pathogen from naturally infected seed and the effect of temperature and relative humidity at heading time.

The effect of temperature on the development of barley stripe has been investigated by several researchers. Ravn (1900) reported that more stripe occurred in early plantings of barley grown from diseased seed than in later plantings from the same seed lot. Johnson (1925) stated that low soil temperatures favored infection under controlled experimental conditions. The greatest infection occurred at temperatures from 10 C to 12 C and very little infection occurred at soil temperatures higher than 20 C. Leubel et al. (1933) concluded that disease development was favored by soil temperatures 15 C or below during the period of emergence and temperatures above 20 C inhibited the disease. They also found that the temperature during pre-emergence was the most critical in determining disease development. Shands (1934) found that the optimum temperature for the mycelial growth of barley

stripe on potato-dextrose agar was near 25 C with a maximum above 32 C, and a minimum considerably below 8 C. In studying the influence of incubation temperature upon stripe development, the highest percentage of disease occurred at 20 C when inoculated seedlings were incubated four days and later grown in a greenhouse at 16 C. The percentage of stripe tended to be higher when the plants were transferred from low to high temperatures than a transfer from high to low temperatures. In 1976, Prasad et al. found that day temperatures of 6-14 C favored disease, but that greatest disease development occurred when night temperatures were higher (14-22 C for the cultivar N.C. 526, and 22-30 C for the cultivar Keowee) than the optimum day temperatures. Prasad et al. (1976) also suggested that disease development may depend on the balance between growth rates of the host and the fungus. Conditions such as high night temperatures that adversely affect the host but not the fungus might increase disease development even after the seedlings have emerged from the soil. It was further noted that plants in wet soil (-1.0 bars) grew most rapidly and had the lowest level of disease. However, the slowest growing plants, those in dry soil (-12.9 bars), had less disease than those in soil of intermediate water potential (-7.1 bars). Teviotdale and Hall (1976) stated that seed transmission of P. graminea was increased when soil temperatures were below 12 C, and reduced or prevented when temperatures were above 15 C in field plantings of naturally infected seed. The disease was detectible in a greater number of embryos with increased germination time at 6 C. Tekauz and Chiko (1980) reported that the radial growth of P. graminea for 1237 colonies on 10 percent V-8 juice agar was maximal at 25 C.

Growth at 2.5 C was slight, at 10 C approximately 50 percent of maximum, and there was no growth at 35 C.

P. graminea infection can be separated into two critical stages. The first stage is the infection of the flower at heading time and establishment of mycelium in the pericarp. The second stage is the infection at the growing point and eventually the whole plant. Thus far, only the factors affecting infection of the growing point and the entire plant were reviewed. The factors affecting the susceptibility of different cultivars and the susceptibility at different growth stages have been investigated by several researchers. Teviotdale and Hall (1976) in a study of the inoculation of developing barley, demonstrated that infection can occur at any stage of plant development, from before head emergence through the soft dough stage. Susceptibility followed a decreasing trend from flowering to hard dough stage. Infection of developing seed occurred over a temperature range of 10 to 33 C and in a wide variety of moisture conditions. Free moisture was not required. Knudsen (1980) confirmed that plants at all growth stages - from the time of heading to the hard dough stage - may become infected by P. graminea and that the most severe infection occurred after inoculation during early stages of spike development. Metz and Scharen (1979) found that an increase in the amount of infected seed was correlated with irrigation prior to heading. When disease assays were made in areas that were low in soil moisture, there were significantly lower levels of infection as compared to assays made in irrigated areas.

Pathogenic Variation

The first report of physiologic specialization in P. graminea was by Arny (1945). Kline (1971, 1972) tested resistance to barley stripe in winter and spring barley cultivars by using different isolates. He could not find evidence of pathogenic specialization among those isolates, but there was variation among isolates in their capacity to cause infection. Mohammad and Mahmood (1973) tested 73 cultivars and seven isolates collected from different parts of India. Isolates varied in their infective ability and also were physiologically specialized. Nilsson (1975) and Metz and Scharen (1979) both mentioned the existence of different virulence groups. Knudsen (1980) found physiologic specialization and compared a number of varieties which were common in different investigations. There was little correlation among the different investigations. Certain varieties were ranked immune in one investigation and highly susceptible in another and vice versa even though similar methods of inoculation were used. It was concluded that those facts supported physiological specialization of P. graminea (Knudsen, 1980).

Smedegard-Peterson (1976) studied the inheritance of genetic factors controlling the expression of lesion symptoms, pathogenicity and sexual compatibility in crosses between P. teres and P. graminea. In the progeny, four distinct classes of lesion types were distinguished, namely two parental types and two recombinant types. The presence of four phenotypes indicated that the capacity in P. graminea to produce stripe lesions is determined by two allelic pairs in two loci.

Smedegard-Petersen (1976) backcrossed the hybrids to the parental isolates and found that pathogenicity in P. graminea on the cultivar Wing is determined by a single allelic pair. Smedegard-Petersen (1976) also reported that the capacity of producing net, spot, and stripe lesions in P. teres and P. graminea is determined by three allelic pairs. It was further concluded that the net and spot forms of P. teres as well as P. graminea should be considered as forms of one and the same biological species.

Inheritance of Resistance

Varietal differences in resistance to P. graminea were reported by Suneson and Santoni (1943), Shands and Army (1944), Kline (1971, 1972), Mohammad and Mahmood (1973), Rai et al. (1975), Nilsson (1975), Metz and Scharen (1979), and Knudsen (1980). The varieties were shown to have different levels of resistance, ranging from immune to highly susceptible.

There is not much agreement among investigators about inheritance of resistance to P. graminea. Most studies of the pathogen resulted in inconsistent results. Isenbeck (1930) found that resistance was governed by several dominant factors. Different types of resistance were reported in different cultivars by Army (1945). In the crosses with the cultivar Oderbrucker, resistance appeared to be dominant and three factors were probably involved. In the crosses with the cultivar Lion, dominance was not definite and a number of factors seemed to be important. In two other crosses, one major and one modifying factor pair were involved. Suneson (1950) stated that resistance was governed by a collection of six different genes. Nilsson (1975) proposed a major

gene control of the resistance. Knudsen (1980) used a different method from the previous worker's and evaluated offspring varieties from each of the parents "Vada" and "Emir" for their field resistance to a population of pathogens instead of to single spore isolates. He found that resistance was determined by quantitative factors.

Inoculation Techniques

P. graminea invades the host plant by means of floral infection. The pathogen is seed-borne. It is known that seedling infection either does not occur or plays only an insignificant part in the life history of the pathogen. P. graminea does not sporulate readily in culture; therefore, most inoculation techniques involve the use of mycelium rather than spores. A number of inoculation techniques have been developed for this pathogen.

Inoculation of germinating seed was first tested by Ravn (1900). Dehulled barley grains of susceptible varieties were germinated in contact with mycelium. Shands (1934) placed the seeds between layers of potato dextrose agar on which the fungus was growing and planted them in soil after an overnight incubation. This method is described in detail by Houston and Oswald (1948) who inoculated the seeds by germinating them between layers of the four-day old cultures. The most efficient inoculation was achieved with this method at 12 C for 12 hours. The same method was used with small modifications by Mohammad and Mahmood (1974), Nilsson (1975), and Metz and Scharen (1979).

High percentages of infection were obtained when the barley seeds were inoculated by contact with wheat kernel inoculum prepared by autoclaving equal parts by weight of the wheat seeds and distilled water

(Shands, 1934). Arny and Shands (1942) modified this method. They used 15 grams of wheat and 15 cc of water which were put in flasks and autoclaved for 45 minutes. Two cc of mycelial suspension were then added to the flasks. Surface sterilized barley seed was added to the inoculum in the flasks after four days of incubation. The seeds were allowed to germinate for four days at room temperature, and the mixture was planted in the soil. This method was used with modifications by Kline (1971-72), Nilsson (1975), Metz and Scharen (1979), and Tekauz and Chiko (1980).

Successful methods of floral infection were first described by Shands (1934). Before his investigations, some researchers attempted to use floral infection for inoculation but were unable to produce a consistently high percentage of disease. The heads were sprayed with a suspension of conidia obtained from diseased plants. Suneson and Houston (1942) used male sterile barley to study floral infection. Sporulating leaves from naturally infected plants were used as the inoculum. Metz and Scharen (1979) incubated naturally infected dried leaves on moist filter paper overnight in a growth chamber and rubbed them over male sterile barley. They obtained an infection level of 36 percent. Knudsen (1980) used naturally infected spreader rows of a susceptible variety to investigate field resistance of several cultivars.

Inducing Sporulation in Culture

P. graminea sporulates abundantly on the leaves and glumes of plants growing under field conditions. However, for most researchers the fungus has failed to sporulate when grown on artificial media. Many

researchers have attempted to induce sporulation in culture. The first positive report came from Paxton (1922) who obtained conidia on corn-meal agar from single-ascospore culture. Houston and Oswald (1946) recorded sporulation within 48 hours on agar cultures of the fungus that were stored outdoors to expose them to diurnal changes of the environment. Pillai et al. (1979) used the modified technique of Houston and Oswald (1946) and were successful in obtaining sporulation. This modified technique was comprised of subculturing ten day old cultures on potato dextrose agar and placing a cellophane disc on the surface of the agar. The culture was incubated at room temperature (18-25 C) for seven days and then exposed to outdoor conditions. Sengupta and Singh (1979) developed a method of using potato dextrose agar containing a two percent hot water extract of rice straw. Incubation was done at 25 ± 1 C in diffused light. The culture sporulated profusely by the fifth day. Teviotdale and Hall (1976b) reported that P. graminea grown on barley leaf-piece agar repeatedly sporulated when exposed to relatively high-intensity light at room temperature (26 ± 1 C) followed by a reduction in temperature.

Tekauz and Chiko (1980) induced sporulation by transferring single conidia to test tube slants of 10 percent V-8 juice agar medium and incubating them at 20 C and a 12 h photoperiod for 10 days.

Control

Barley stripe was a very important disease and was first reported in the early 1900s, but the increased use of mercury seed treatments after 1920 gradually reduced the disease to one of relative unimportance. After the banning of mercury fungicides from the market,

the disease became important again and researchers were forced to think about efficient ways to control the disease. Cultural practices, seed treatments, and resistant varieties can be used to control the disease.

Increased soil fertility or any other condition favoring vigorous plant growth decreases the percentage of diseased plants that develop from naturally infected seed (Leukel et al., 1933). Irrigation applied when plants are near heading stage was shown to be conducive to infection (Metz and Scharen, 1979). At the same location with no irrigation, the infection rate dropped to nearly zero.

Control of disease using genetically resistant cultivars is the most efficient method of control. A number of varieties have been screened for barley stripe resistance (Suneson and Santoni, 1943; Shands and Army, 1944; Kline, 1971, 1972; Mohammad and Mahmood, 1973; Rai et al., 1975; Nilsson, 1975; Metz and Scharen, 1979; Knudsen, 1980). The cultivar Betzes was found to be resistant by Metz and Scharen (1979), Kline (1972) and Tekauz and Chiko (1980). The cultivars Shabet and Erbet, which are derivatives of Betzes (Hockett and Eslick, 1972), and the cultivar Steptoe were found to be highly resistant to P. graminea (Metz and Scharen, 1979).

A high incidence of barley stripe in the United States and in some other countries has stimulated the screening of numerous registered and experimental seed treatment fungicides for efficacy in controlling the disease. Kline and Roane (1972) reported that twice the recommended rate of carboxin plus thiram (recommended rate 88.5-118 ml/cwt) provided control of P. graminea. Bartlett and Ballard (1975) recommended that a combined liquid formulation of guazatine plus mazalil (0.6 + 0.04 g

active ingredient per kg barley seed) be used as an effective alternative to organomercury. Magnus (1979) confirmed the result of Bartlett and Ballard (1975) and stated that compounds containing Imazalil gave satisfactory stripe control. Contradictory to Kline and Roane's (1972) work, Metz and Scharen (1979) found that Vitavax 200 (carboxin plus thiram) was an effective fungistat at 1000 µg/ml active ingredient in petri dishes, but when tested in the greenhouse on naturally infected barley seed, gave little control. Vitavax 75 WP (carboxin) and PMA (phenyl mercury acetate) were found to give effective control of barley stripe in petri dishes by the same researchers. Johnston et al. (1982) showed that seven experimental fungicides completely controlled barley stripe. They were Nuarimol, Prochloraz, Fenapromil, Imazilil, CGA 64251, Triadimenol and Gus 215.

CHAPTER 3

PATHOGENICITY TESTS OF SOME P. GRAMINEA ISOLATES

A collection of isolates was tested for pathogenicity on a susceptible barley cultivar. These tests were necessary in order to select a small number of isolates that infected a large percentage of test seeds, produced clear symptoms, but did not kill seedlings before emergence.

MATERIALS AND METHODS

Twenty-seven different isolates obtained from Montana, Turkey, and Ethiopia were tested against the susceptible cultivar Summit (Mt 729, RPB 261-70). Seeds of the cultivar Summit were provided by E. A. Hockett from the Foundation seed increase. The isolates used were: 24 from Montana; two from Turkey; and one from Ethiopia. The isolates were kept in test tubes of potato dextrose agar (PDA), and transferred at 14 day intervals. Cultures were never allowed to attenuate, always being reisolated from infected leaves after two transfers on agar. The barley kernel inoculum method was used (Metz and Scharen, 1979). It is a modification of the wheat kernel inoculation method (Arny and Shands, 1942) in which autoclaved barley seed is used as the inoculum substrate. Erlenmeyer flasks (250 ml) were prepared by adding equal amounts of Ingrid barley (30 g) and distilled water (30 ml). Flasks were capped with aluminum foil and autoclaved 22 minutes at 121 C followed by a slow

release of pressure (total time in autoclave 45-50 minutes). Flasks were shaken to loosen the seed mat within two hours following removal from the autoclave. Then eight plugs of P. graminea mycelium cut with a no. 2 cork borer were added. Incubation was at 15 C for ten days with twelve hour dark and light periods.

One-hundred-twenty-five seeds were placed in test tubes and surface sterilized in 0.5 percent NaOCl for a minimum of three minutes. The sterilant was decanted, and unrinsed seeds were spread on dry paper towels in a clean air chamber where sterile air was allowed to blow over the seed for 4.5 hours. Seeds were then aseptically added to shaken flasks and the mixture was incubated 12-14 days at 15 C until some seeds were visibly sprouting. The entire mixture was planted in rows in flats (36 x 25 x 8 cm) filled with a 1:1 mixture of pasteurized Bozeman silt loam and sand. Two replications were planted at two separate times.

RESULTS AND DISCUSSION

The isolates of P. graminea used in this study and their pathogenicity to the cultivar Summit are shown in Table 1. Isolates were different in pathogenicity. The highest incidence of infection was obtained from isolate Montana 10 (Mt 10) (95.3 percent). The isolates which caused a high level infection in the average of the two planting times were: Mt 8 (91.3 percent); Mt 17 (88.5 percent); Eth 465 (79.2 percent); Mt 10 (76.6 percent) and Mt 16 (76.4 percent). Isolate Mt 14 was non-pathogenic (0.0 percent). Maintenance of pathogenicity in cultures that were kept on artificial media became a problem. As shown in Table 1, isolates in general tended to lose their pathogenicity.

Isolate Mt 5 decreased from 93.8 percent to 54.8 percent and the decrease was from 93.9 percent to 42.3 percent in isolate Mt 6. The average infection level was 67.4 percent in the first replication. It dropped to 37.9 percent in the second replication.

The pathogenicity of isolate Mt 12 increased from 12.1 percent to 66.7 percent. But in the second replication, germination percentage was very low (4.8 percent). Some cultures remained stable. Also, some isolates were lost. This subject will be discussed in detail in Chapter 4.

Table 1. Pathogenicity of the isolates collected from Montana, Turkey, and Ethiopia on the cultivar Summit.

Isolates	No. of Seeds Planted	No. of Seeds Germinated	% Germination	No. of Diseased	% Diseased	% Avg. Diseased
Check I ^{1/}	125	76	60.8	--	--	--
	125	111	88.8	--	--	--
Check II ^{2/}	125	110	88.0	--	--	--
	125	119	95.2	--	--	--
Mt 1	125	98	78.4	69	70.4	73.2
	125	46	36.8	35	76.1	
Mt 2	125	94	75.2	35	37.2	31.9
	125	30	24.0	8	26.7	
Mt 3	125	114	91.2	1	0.9	1.7
	125	57	45.6	2	3.5	
Mt 4	125	104	83.2	57	54.8	54.8
	--	--	--	--	--	
Mt 5	125	99	79.2	93	93.8	74.3
	125	31	24.8	17	54.8	
Mt 6	125	99	79.2	93	93.9	68.1
	125	97	77.6	41	42.3	
Mt 7	125	109	87.2	94	86.2	69.3
	125	84	67.2	44	52.4	

Table 1. (cont'd).

Isolates	No. of Seeds Planted	No. of Seeds Germinated	% Germination	No. of Diseased	% Diseased	% Avg. Diseased
Mt 8	125	94	75.2	89	94.7	91.3
	125	70	56.0	61	87.9	
Mt 9	125	95	76.0	61	64.2	67.8
	125	49	39.2	35	71.4	
Mt 10	125	86	68.8	82	95.3	76.6
	125	19	15.2	11	57.9	
Mt 11	125	96	76.8	85	88.5	53.2
	125	95	76.0	17	17.9	
Mt 12	125	91	72.8	11	12.1	39.4
	125	6	4.8	4	66.7	
Mt 13	125	89	71.2	76	85.5	49.3
	125	76	60.8	10	13.2	
Mt 14	125	100	80.0	0	0	0
	125	106	84.0	0	0	
Mt 15	125	110	88.0	18	16.4	10.7
	125	97	77.6	5	5.1	
Mt 16	125	98	78.4	72	73.1	76.4
	125	68	54.4	54	79.4	
Mt 17	125	107	85.6	94	87.8	88.5
	125	28	22.4	25	89.3	
Mt 18	125	101	80.8	86	85.1	51.3
	125	34	27.2	6	17.6	
Mt 19	125	109	87.2	86	78.9	62.6
	125	41	32.8	19	46.3	
Mt 20	125	96	76.8	76	79.2	52.6
	125	65	52.0	17	26.1	
Mt 21	125	108	86.4	67	62.0	38.2
	125	69	55.2	10	14.5	
Mt 22	125	45	36.0	42	93.3	48.5
	125	80	64.0	3	3.7	

Table 1. (cont'd).

Isolates	No. of Seeds Planted	No. of Seeds Germinated	% Germination	No. of Diseased	% Diseased	% Avg. Diseased
Mt 23	125	90	72.0	18	20.0	14.7
—	125	64	51.2	6	9.4	
Mt 24	125	88	70.4	70	79.5	60.3
	125	73	58.4	30	41.1	
Cum 50	125	70	56.0	65	92.8	55.1
	125	46	36.8	8	17.4	
Kaya	125	85	68.0	73	85.4	59.4
	125	70	56.0	23	32.8	
Eth 465	125	93	74.4	83	89.2	79.2
	125	26	20.8	18	69.2	

^{1/} Check I: Germination of cultivar Summit seeds in the barley kernel inoculum substrate without inoculum.

^{2/} Check II: Germination of cultivar Summit seeds in the soil.

CHAPTER 4

INOCULATION PROCEDURES: STUDIES OF INOCULATION TECHNIQUES
AND DEVELOPMENT OF CULTURE METHODS FOR P. GRAMINEA

The pathogen, P. graminea, is seed borne, but it invades the barley plant by means of floral infection.

Many tests have been conducted to determine a practical and efficient method of inoculation of barley with the barley stripe organism. Layered mycelium (Shands, 1934; Houston and Oswald, 1948; Mohammad and Mahmood, 1974; Nilsson, 1975; and Metz and Scharen, 1979), barley kernel inoculum or wheat kernel inoculum (Shands, 1934; Arny and Shands, 1942; Kline, 1971-72; Nilsson, 1975; Metz and Scharen, 1979; and Tekauz and Chiko, 1980) and floral infection (Shands, 1934; Suneson and Houston, 1942; Metz and Scharen, 1979; and Knudsen, 1980) methods have been reported in the literature.

Barley kernel inoculum, layered mycelium methods and a soil inoculation method were compared in the present study. In addition, further studies on the barley kernel inoculum method were made and a better medium was sought because of some growth and sporulation problems of P. graminea on the artificial media.

MATERIALS AND METHODS

The susceptible barley cultivar Summit was tested against five isolates of P. graminea obtained from Montana, Turkey, and Ethiopia. The experiment was planted in four replications. Isolates used were:

one from Turkey, one from Ethiopia, and three from Montana. Montana isolates Montana 6, Montana 10 and Montana 17 were obtained from Dr. E. A. Hockett's barley experimental plots in 1981 from plot numbers: 41-1627 (Rupal/Tr 20b), 41-1304 (Mt 547354/Mt 31972), and 41-1339 (Rupal/Mt 547354), respectively. Cultures were isolated from infected leaves, then transferred no more than twice on PDA before being isolated again from infected leaves.

Two checks were used in the test of the three inoculum methods. For Check I, seeds of the cultivar Summit were germinated on wet paper towels and kept in plastic bags. This method was used for all three experiments. Check II was different in each of the three experiments. Summit seeds were germinated in 12 days in the inoculum substrate without inoculum in the barley kernel inoculum method. In the layered mycelium method, Summit seeds were germinated in 14 days between the layers of agar medium without the fungus. In the soil method, seeds of the cultivar Summit were planted directly into the soil without inoculum, and they emerged in 12-14 days.

Barley Kernel Inoculum

The barley kernel inoculum method was described in Chapter 3. The same methods were used with a small modification in autoclaving time which was increased to 30 minutes from the original 22 minutes.

Layered Mycelium

Barley kernels in lots of 125 were surface sterilized, as described in the barley kernel inoculum method, dried and placed on the mycelial mat of a 10 day old potato dextrose agar (PDA) medium with actively

growing P. graminea. Another identical fungal culture was removed and placed upside down on top of the seeds. Lids were then replaced and the petri dishes were sealed with a parafilm band to prevent desiccation and were kept at 4 C for two weeks. Then the barley kernels were planted in rows in flats filled with a 1:1 mixture of pasteurized Bozeman silt loam and sand. The flats were placed in a growth chamber with a 12-hour daily photoperiod ($2.2-3.3 \times 10^4$ erg/cm² sec) at 8 ± 3 C (dark/light). The disease notes were taken two months from the planting date.

Soil Method

Autoclaved barley seed was used as the inoculum substrate. Erlenmeyer flasks (1000 ml) were prepared by adding equal amounts of Ingrid barley (180 g) and distilled water (180 ml). Flasks were capped with aluminum foil and autoclaved 30 minutes at 121 C. The flasks were shaken to loosen the seed mat within two hours following removal from the autoclave. Then 15-20 plugs of P. graminea mycelium, cut with a no. 2 cork borer, were added. Incubation was at 15 C for 10 days with 12 hour light/dark periods.

One-hundred-twenty-five seeds were placed in test tubes and surface sterilized in 0.5 percent NaOCl for a minimum of three minutes. Sterilant was decanted, and unrinsed seeds were spread on dry paper towels in a clean air chamber where sterile air was allowed to blow over the seed for 30 minutes. The inoculum plus substrate from the erlenmeyer flasks was divided into three parts by weight. Each part of inoculum plus substrate was spread into ten rows in flats filled with a 1:1 mixture of pasteurized Bozeman silt loam and sand. Seeds were then planted into the same ten rows. The flats were placed in a growth

chamber with a 12-hour daily photoperiod ($2.2-3.3 \times 10^4$ erg/cm² sec) at 8 ± 3 C (dark/light). Disease notes were taken two and one-half months from the planting date.

Statistical analysis. An analysis of variance was conducted to compare disease reactions of the five different isolates on the cultivar Summit with three different inoculum methods (Steel and Torrie, 1960). A student-Newman-Keuls test was used in the studies of inoculation techniques.

Modification in the Barley Kernel Inoculum

Shands (1934) and other researchers used equal amounts of water and barley or wheat seeds to prepare an inoculum substrate. In this study, barley kernel inoculum of P. graminea was prepared using 30 g of barley placed in a glass pint canning jar and moistened with 35 ml of distilled water. The jars were capped with the regular canning lid in which a 12.7 mm hole had been punched. A 7 cm disc of Whatman no. 4 filter paper was placed over the lid and was held in place by the regular screwband (Mathre and Johnston, 1975). The jars were autoclaved for 60 minutes at 121 C. The temperature was increased slowly to prevent cracking of the jars. After autoclaving, the seed mat was left to cool for at least three hours. Then 12 plugs of P. graminea mycelium were cut with a no. 2 cork borer and added to the jars. The inoculated seed was then incubated at 15 C for 10 days, with a 12 hour dark and 12 hour light period. After a 6-10 day incubation period, the inoculated barley seeds were used to inoculate other jars of seed. The second transfer of inoculum was allowed to incubate for four to eight days.

To begin the test of cultivar resistance, 125 seeds were placed in test tubes and surface sterilized in a 0.5 percent solution of NaOCl for a minimum of three minutes. Sterilant was decanted, and unrinsed seeds were spread on dry paper towels in a clean air chamber where sterile air was allowed to blow over the seed for four to five hours. Seeds were then aseptically added to flasks and mixed with a sterile spatula. At the same time, 4 ml of sterile distilled water were added to the mixture. The mixture was incubated for 10 days with a 12-hour daily photoperiod at 12-15 C (dark/light). During incubation the seed mixture was mixed again to distribute the moisture equally before the seeds germinated. The entire mixture was then planted in rows into flats which were filled with a mixture of 70 percent sand and 30 percent pasteurized Bozeman silt loam.

Development of Culture Methods

Studies of culture media involved adding barley leaf extract to the different mixtures. To prepare barley extract, 75 g of leaves from two-month-old barley plants were put into a blender and ground with 200 ml of distilled water. The barley extract was strained through cheese cloth. The other ingredients of the medium were added to barley extract to bring the total amount to one liter. To compare growth of the fungus in different media, single spores of isolate Mt 6 were grown on water agar, water agar plus barley extract, potato dextrose agar, malt-yeast agar, malt-yeast agar plus barley extract, minimal medium and minimal medium plus malt-yeast agar plus barley extract. Formulae for the minimal medium plus malt-yeast agar plus barley extract is given in the Appendix, Table 8.

RESULTS AND DISCUSSION

The average infection levels were 59.5 percent in the barley kernel inoculum, 54.1 percent in layered mycelium and 18.2 percent in the soil method (Table 2). No significant differences were found between infection percentages of barley kernel inoculum and layered mycelium. The infection percentage for the soil method was significantly lower than in the other two methods. The average check emergence percentage in the soil method was expected to be higher than that of the other methods. However, the average control emergence percentages were 81.6 percent in the layered mycelium method, 61.0 percent in the barley kernel inoculum method, and 43.3 percent in the soil method. There were no significant interactions between cultures and methods and also no significant differences between replications (Appendix, Table 9). It was thought that the seeds did not emerge well because of the very heavy soil used. However, the average germination percentage in the soil method was 90.2 percent in a later experiment when the soil composition was corrected.

There was variation between the replications in germination percentages and in the percentage of diseased plants in both the barley kernel inoculum and the layered mycelium method (Table 4).

Germination percentages varied from 94 percent to 2 percent among the replications and disease percentages varied from 94 percent to 33 percent for the isolate Mt 6 in the barley kernel inoculum method. A similar situation occurred with the other isolates. There was large variation in disease percentages among the replications although germination percentages were quite high in the layered mycelium method

Table 2. Comparison of three inoculation procedures with five cultures of *P. graminea* on barley, cultivar Summit. All numbers represent two replications. (See also Appendix, Tables 9 and 11.)

Isolates	No. Seeds Planted	No. Seeds Emerged	% Germ.	No. Seeds Diseased	% Diseased
<u>I. BARLEY KERNEL INOCULUM</u>					
Check I ^{1/}	500	497	99.4	0	0
Check II ^{2/}	500	228	45.6	0	0
Mt 6	500	295	59.0	252	85.4 a ^{5/}
Mt 10	500	349	69.8	115	32.9 b
Mt 17	500	312	62.4	238	76.2 a
Kaya	500	343	68.6	127	37.0 b
Eth 465	500	227	45.4	176	77.6 a
AVERAGE			61.0		59.5
<u>II. "SANDWICH" OR LAYERED MYCELIUM</u>					
Check I ^{1/}	500	497	99.4	0	0
Check II ^{3/}	500	231	46.2	0	0
Mt 6	500	417	83.4	279	66.9 ab
Mt 10	500	456	91.6	136	29.8 c
Mt 17	500	405	81.0	226	55.8 b
Kaya	500	342	68.4	152	44.4 bc
Eth 465	375	318	84.8	256	80.5 a
AVERAGE			81.6		54.1
<u>III. SOIL METHOD</u>					
Check I ^{1/}	500	497	99.4	0	0
Check II ^{4/}	500	468	93.6	0	0
Mt 6	250	87	34.8	41	47.1 a
Mt 10	375	240	64.0	12	5.0 b
Mt 17	500	159	31.8	60	37.7 ab
Kaya	500	295	59.0	28	9.4 b
Eth 465	250	31	12.4	7	22.5 b
AVERAGE			43.3		18.2

^{1/} Percentage of seeds germinated in wet paper towelling (not included in analysis).

^{2/} Percentage of seeds emerged, first germinated in inoculum substrate less fungus then planted in soil.

^{3/} Percentage of seeds emerged, first germinated in two layers of agar medium less fungus then planted in soil.

^{4/} Percentage of seeds emerged in soil without fungus or substrate.

^{5/} Numbers followed by the same letter are not different at the 0.05 percent level according to the Student-Newman-Keuls test.

Table 3. Variation in the germination and disease percentages in the cultivar Summit using the barley kernel inoculum and layered mycelium method. (See also Appendix, Table 11.)

Isolates and Replications		% Germination	% Infection
BARLEY KERNEL INOCULUM METHOD			
Mt 6	A	93.6	81.1
	B	97.6	86.8
	C	2.4	33.3
	D	42.4	94.3
Mt 10	A	79.2	3.0
	B	89.6	0.0
	C	58.4	73.9
	D	52.0	89.2
LAYERED MYCELIUM METHOD			
Mt 6	A	96.8	81.8
	B	96.0	64.1
	C	80.0	80.0
	D	60.8	30.2
Mt 10	A	98.4	43.2
	B	97.6	18.8
	C	79.2	0.0
	D	89.6	52.6

compared to the generally lower germination levels in the barley kernel inoculum method. In addition, the growth of the fungus in the barley kernel inoculum substrate was different between the replications of the different isolates. Germination levels were also variable.

The variation between replications of isolate Mt 10 for both germination and infection percentages (Table 3) may have been due to the variation present in experimental conditions and procedures as well as variation in the isolate itself. It is thought that the lower percentage values may be disregarded since subsequent experiments using the altered procedures outlined below eliminated the anomalous values.

The barley kernel inoculum method could be easily modified to minimize the amount of variation present. It was concluded that the amount of moisture and oxygen in the flasks was affecting the variation in germination and pathogenicity. The moisture content in the flasks may have changed due to the differential moisture loss in the flasks during autoclaving. The amount of oxygen in the flasks may have changed due to respiration of the fungus and the seeds during the incubation period.

Based on these considerations, the amount of water in the flasks was increased from 30 ml to 39 ml to shorten germination time and a hole was punched in the canning lid to allow for aeration. Following this increase in the amount of water in the media, and the allowance for aeration, there was little variation in the growth of the fungus. In addition, seeds germinated at the same time except for some small varietal differences. With this new method, germination was much faster; seeds germinated in four to six days instead of 10 to 14 days. Seeds were kept in the jars 10 days to increase the level of infection. This operation tended to decrease plant survival because the tender growing shoots were vulnerable to mechanical damage at the time of transplanting. It has been shown that a longer germination time results in a higher level of infection (Nilsson, 1975; Teviotdale and Hall, 1976a). In this case, germination time could have been lengthened by germinating the seeds at lower temperatures instead of 12-15 C as was used in these studies. These studies are still under investigation.

The fungus grew more vigorously on water agar plus barley extract than on water agar. Mycelium was not thick on either medium. Rankings

of the media according to the amount of fungus growth from the least to the most are: water agar, water agar plus barley extract, potato-dextrose agar, minimal medium, malt-yeast agar, malt-yeast agar plus barley extract and minimal medium plus malt yeast agar plus barley extract. Growth of the fungus in the different media showed that P. graminea grew more profusely on rich medium. Using barley extract increased the vigor of growth, but sporulation could not be detected.

CHAPTER 5

INHERITANCE OF RESISTANCE TO P. GRAMINEA IN CROSSES BETWEEN
AMERICAN AND TURKISH BARLEY CULTIVARS

Losses caused by the barley stripe disease stimulated research on the development of cultivars resistant to P. graminea in the early part of this century. But most work on breeding for resistance was abandoned with the advent of Ceresan and other organic mercury seed dressings, which controlled the disease completely at very low cost.

After removal of the mercury seed dressings from the market in the mid-1970s because of their danger to people and the environment, genetic resistance to the barley stripe disease became important again. In order to understand the ways that resistance could be used in a barley breeding program, it was necessary to study the inheritance of resistance to P. graminea in those barley cultivars and lines that exhibited such resistance. The present study was designed to examine the resistance found in some American and Turkish barleys, and to determine the similarities and differences in resistance found when the Turkish and American cultivars were crossed.

MATERIALS AND METHODS

Turkish cultivars Tokak, Cum 50, Yesilkoy, Zafer, Kaya, Gem CI 7243 and Yercil were crossed reciprocally with the American cultivars Betzes CI 6398, Erbet CI 13826, Shabet CI 13827, Ershabet CI 15722 and Steptoe CI 929 in Bozeman, Montana, during the summer of 1981. Twenty F_1 seeds

of each cross (Appendix, Table 13), were planted in Mesa, Arizona, during the winter of 1981-82. In April, 1982, F₂ seeds were harvested from the five most vigorous F₁ plants in each row. These F₂ seeds were brought back to Bozeman for testing.

Parent cultivars were tested for resistance to P. graminea (cultured as described on page 18) by use of the soil inoculation method and the modified barley kernel inoculum method as described in Chapters 2 and 3. Modifications were necessary in the soil method because of space limitations in controlled environment growth chambers. Previously, flats of inoculated plants were kept in the growth chambers for two-and-one-half months. Trials showed that if flats of plants were kept in the growth chamber a shorter period, they could be moved to the greenhouse and infection would continue to develop normally. Therefore, in the tests of parent cultivars, the plants were kept in the growth chambers for 35 days, then transferred to the greenhouse where the day length was natural and the temperature was maintained at 20 ± 3 C. Disease readings were made two-and-one-half months after planting. Seedlings were examined one by one for disease symptoms and were categorized as diseased or healthy.

In the modified barley kernel inoculum method, as described in Chapter 3, the inoculated seeds were planted in flats of soil, then the flats were kept for 25 days in the growth chambers. Parent cultivars and F₂ populations were tested using this method. Disease assessment was made in the greenhouse two months after planting.

Five single-spore isolates of P. graminea were used in the soil inoculum test of parent cultivars. Isolates used were: Montana 6,

Montana 10, and Montana 17, which had been obtained from experimental plots of Dr. E. A. Hockett, and isolates Kaya from Turkey and Ethiopia 465, obtained from Ethiopia.

F₂ populations were tested with the kernel inoculum method, and cultures Montana 6, Montana 10, and Kaya. These cultures had been observed to remain more stable and lose less of their pathogenicity than the other cultures.

Statistical analysis. An analysis of variance (Appendix, Table 9) was conducted to compare disease reactions of the five different isolates on the twelve parent cultivars (Steel and Torrie, 1960). A Student-Newman-Keuls test was used in the test of parent varieties. The Chi-square method was used in the test of F₂ generations (Steel and Torrie, 1960).

RESULTS AND DISCUSSION

Four levels of resistance were found when testing five different isolates with the soil inoculum method (Appendix, Table 11). The first group included Betzes, Steptoe, Tokak, Erbet, Cum 50, Yesilkoy, Shabet, and Gem. Zafer, Kaya and Yercil were in second, third and fourth groups, respectively. Infection percentages of two selected isolates on parent varieties inoculated with the isolates Mt 6 and Mt 10 are shown in Table 4. Betzes which was found immune in previous studies (Kline, 1972; Metz and Scharen, 1979; and Tekauz and Chiko, 1980), was five percent infected with the isolate Mt 6 but was not infected with isolate Montana 10. Other cultivars behaved as expected from previous observations of resistance or susceptibility.

Table 4. Percentage of plants, out of 125 planted, displaying symptoms when inoculated by the soil with two different P. graminea isolates.

Parents	Isolates	
	Mt 6	Mt 10
Betzes	5 R ^{1/}	0 R
Step toe	2 R	0.8 R
Tokak	4 R	1 R
Erbet	5 R	1 R
Cum 50	7 MR	4 R
Yesilkoy	7 MR	4 R
Shabet	9 MR	2 R
Ershabet	9 MR	2 R
Gem	10 MR	6 MR
Zafer	22 MS	19 MS
Kaya	16 MS	40 S
Yercil	47 S	60 S

^{1/} R = resistant	5 percent or less
MR = moderately resistant.	6-15 percent
MS = moderately susceptible.	16-30 percent
S = susceptible	31 percent or more.

F₂ populations were inoculated by means of the barley kernel inoculum method with isolate Mt 6, and analyzed using Chi-square so that the ratios of segregation were determined (Table 5).

In the cross between Betzes and Tokak, it appears that at least two dominant genes are involved and they may be the same genes because there is no segregation. The cross between Betzes and Yercil did not fit any ratio. In the cross between Shabet and Zafer it appears that two dominant genes are involved. In the crosses among Shabet and Yercil and Erbet and Zafer it appears that one dominant and one recessive gene are involved. The cross Erbet and Yercil fits the 3:1 ratio. This explains that Erbet has at least one dominant gene for resistance and the susceptible cultivar Yercil does not have any genes for resistance. Based on this cross, previous predictions of gene number and types of

Table 5. Chi-square values of eight F_2 barley populations when inoculated by the barley kernel inoculum method with P. graminea isolate Mt 6.

F_2 Generations	No. Plants		Observed Ratio	Expected Ratios			$p > \frac{1}{2}$
	Symptom	No Symptom		3:1	15:1	13:3	
1. Betzes x Tokak	107	8	13.4:1	--	.097	--	.750
2. Betzes x Yercil	47	29	1.6:1	--	--	--	no fit
3. Shabet x Zafer	90	8	11.2:1	--	.612	--	.250
4. Shabet x Yercil	80	12	6.7:1	--	--	1.967	.100
5. Erbet x Zafer	90	12	7.5:1	--	--	3.267	.050
6. Erbet x Yercil	79	23	3.4:1	.327	--	--	.500
7. Ershabet x Tokak	110	4	27.5:1	--	1.462	--	.100
8. Ershabet x Cum 50	68	4	17.0:1	--	.059	--	.750

$\frac{1}{2}$ Probability level.

resistance can be confirmed. Though the cross Betzes and Yercil does not fit any ratio, it is known that Yercil does not have any genes for resistance. This suggests that Betzes may have at least one dominant gene for resistance. Also, it can be said that Shabet has at least one gene for resistance because Shabet gave 15:1 and 13:3 ratios when crossed with susceptible varieties Zafer and Yercil, respectively.

However, evidence is weak; Zafer might have minor genes for resistance when we compare obtained ratios of Zafer crosses with ratios of Yercil crosses when one resistant parent was used. The crosses Ershabet and Tokak, and Ershabet and Cum 50 gave disease percentages similar to the parent varieties; therefore, these three cultivars Ershabet, Tokak, and Cum 50 may have the same dominant genes for resistance.

In addition to the classic calculation of expected value, another method (personal communication, E. A. Hockett) was used to calculate an additional expected ratio based upon penetrance and expressivity

(Allard, 1960 and Khan, 1969). Penetrance can be defined as the ability of a gene to be expressed in individuals which carry it and expressivity is concerned with the manner of expression (Allard, 1960). The results obtained show that studies of the inheritance of host resistance to P. graminea may be complicated by environmentally induced variation in host reaction. For example, the large difference between the levels of infection using the same isolate - 98 percent infection with the first planting and 58 percent infection with the second planting - could possibly be attributed to penetrance. Levels of stripe symptom expression in plants inoculated with the same isolate at the same planting time were from completely striped to non-striped but when isolations were made, the fungus was found to be present even in the symptomless plants. Likewise, levels of stunting varied from plants that were very short to plants of normal height, and in both cases the fungus was present when isolations were made. Both of these phenomena might be attributed to expressivity. Calculations of expected values according to penetrance and expressivity are shown in Appendix, Table 12.

The expected ratios and Chi-square values of F_2 populations when inoculated with the P. graminea isolate Montana 6 were calculated (Table 6). The crosses were made using resistant x resistant, resistant x susceptible, and resistant x moderately susceptible parents. In the cross between Betzes and Tokak, a dominant gene in each cultivar was assumed to be involved. Both parents may have the same gene or different genes for resistance since the data do not distinguish between these two possibilities. The cross between Betzes and Yercil confirms that Betzes has a dominant gene for resistance, but the data do not fit

Table 6. Expected ratios and Chi-square values of eight F₂ barley populations when inoculated by the barley kernel² inoculum method with *P. graminea* isolate Mt 6.

F ₂ Generations	No. Plants Non-Inf.	No. Plants Inf.	Observed Ratio	Expected Ratio	Ratio ^{1/}	X ²	p> ^{2/}
1. Betzes x Tokak (AAbb) (AAbb or aaBB) ^{7/}	107	8	13.4:1	23:1	No seg.	2.060	.100
				14:1	(15:1)	.0006	.980
				20:1	(63:1)	1.283	.250
2. Betzes x Yercil (aabb)	47	29	1.6:1	5:1	(3:1)	29.269	No fit
3. Shabet x Zafer (AAbb) (aabb)	90	8	11.2:1	7:1	(3:1)	1.488	.100
				8:1	(15:1) ^{3/}	.997	.250
				6:1	(15:1) ^{4/}	2.686	.100
				9:1	(63:1)	.445	.500
4. Shabet x Yercil	80	12	6.7:1	4:1	(3:1)	1.828	.100
				8:1	(15:1)	.258	.500
5. Erbet x Zafer (AAbb)	90	12	7.5:1	10:1	(3:1)	.762	.250
				12:1	(15:1) ^{5/}	2.469	.100
				8:1	(15:1) ^{6/}	.084	.750
				14:1	(63:1)	4.668	No fit
6. Erbet x Yercil	79	23	3.4:1	5:1	(3:1)	3.709	.050
7. Ershabet x Tokak (AAbb)	110	4	27.5:1	18:1	No seg.	.704	.250
				12:1	(15:1)	2.751	.050
8. Ershabet x Cum 50 (AAbb or aaBB)	68	4	17.0:1	12:1	No seg.	.399	.500
				9:1	(15:1)	1.524	.100
				12:1	(63:1)	.486	.250

^{1/} Expected ratio was calculated taking into account penetrance and expressivity.

^{2/} Probability level.

^{3/} Calculations of the expected value assuming the cultivar Shabet had two dominant genes for resistance.

^{4/} Calculations of the expected value assuming both cultivars Shabet and Zafer had one dominant gene for resistance.

^{5/} Calculations of the expected value assuming the cultivar Erbet had two dominant genes for resistance.

^{6/} Calculations of the expected value assuming both cultivars Erbet and Zafer had one dominant gene for resistance.

^{7/} Possible genotypes. Capital letters indicate dominant genes; small letters indicate recessive genes.

a 3:1 segregation for resistance. From the cross between Shabet and moderately susceptible parent Zafer it appears that at least one dominant gene is involved with resistance. The cross between Shabet and Yercil indicates that Shabet has at least one dominant gene for resistance, and it is believed that Yercil does not have any genes for resistance. In the cross between Erbet and Zafer, it appears that at least one dominant gene is involved with resistance. In the cross between Erbet and Yercil, one dominant gene segregation confirms that Erbet has at least one dominant gene. It is not possible to distinguish between Betzes and Tokak, and Ershabet and Tokak, and Ershabet and Cum 50, whether they have the same genes or two different genes at two loci. Generally the data fits the hypothesis of a single dominant gene for resistance in Betzes, Shabet, Erbet and Ershabet. This is to be expected since they are closely related. There are no genes for resistance in Yercil and Zafer as supported by the data.

An unusual observation was made in this study of both parent cultivars and F_2 generations. Stripes on the leaves, stunting and rosetting are common symptoms of the barley stripe disease. These symptoms have been described by several researchers (Smith, 1929; Drechsler, 1923; Stakman and Rodenhiser, 1929; Weniger, 1932; Suneson, 1950; Smedegard-Petersen, 1976, Tekauz and Chiko, 1980). In the present study, I worked with barley seedlings, and observed that most of the diseased plants tillered while healthy seedlings did not tiller. The number of secondary tillers varied from one to four. Some plants which tillered and were stunted did not have the stripe symptoms (Figure 1). Three tillered, stunted plants, without stripe symptoms, were selected

and isolations were made from the upper and lower parts of the plants. A fungus grew from the isolated tissue taken from both lower and upper parts of the culm. The cultural characteristics of the fungi were identical to P. graminea when they were transferred to minimal salt plus malt-yeast agar plus barley extract medium which was regularly used in the experiment. But the identity of the fungus has not been proved according to Koch's postulates because of time limitations. It is, however, my strong belief that the isolated fungus is P. graminea. This shows that the plants can be diseased without expressing the usual stripe symptoms. Also it might be evidence for an escape mechanism of the plants from the disease. However, some plants without stripe symptoms were kept growing for an additional two-and-one-half months during which time at least one tiller from most plants died. This might be a further indication that the tillers were diseased even though they did not exhibit usual symptoms.

GENERAL SUMMARY

The primary objective of this research was to determine the gene action of resistance to P. graminea in barley. The experimentation was started by testing the virulence of 29 isolates of P. graminea obtained from different locales around the world. Isolates were found to have different levels of virulence varying from 0 to 95 percent infection. During the course of the experiments, three isolates lost their ability to grow in culture and to infect barley seed. Other isolates attenuated differently and at different times. Pinkerton and Strobel (1976) found

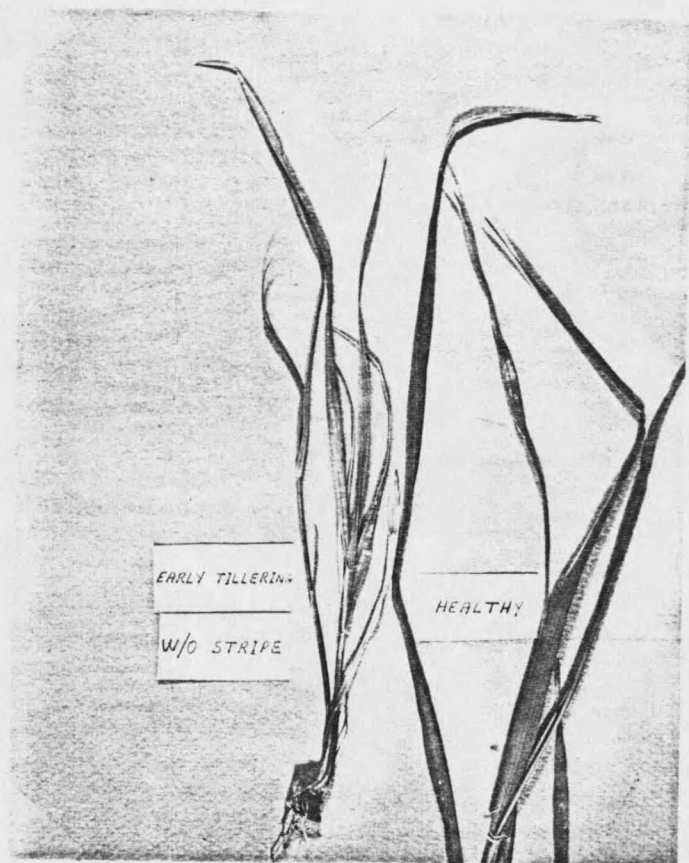


Figure 1. Tillered and stunted plant without stripe symptom.

attenuation of cultures of Helminthosporium sacchari grown in synthetic medium. When the isolates were replicated some of them tended to lose their pathogenicity. These observations agree with the findings of Nilsson (1975) who reported that pathogenicity of the fungus when used for inoculation experiments, and increased on artificial substrate, might lose its pathogenicity slightly or very rapidly.

This situation and the failure of P. graminea to sporulate when grown on artificial media encouraged the search for a new medium. The fungus sporulated on autoclaved leaves. It was thought that mixing of

barley extract in different media might encourage the fungus to sporulate. P. graminea grew more profusely on a rich medium, and lost its growth ability differently in different media. The final medium which was used was minimal medium plus malt-yeast agar plus barley extract (Appendix, Table 8). It was found that the fungus can be kept for a longer time on this medium than on the other media without attenuation.

A method was tried as suggested by Nilsson (1975) to prevent loss of pathogenicity in the isolates. This method required frequent reculturing of the fungus from beginning plant material to guarantee good infection in stripe tests. Attenuated cultures were eliminated when the fungus was recultured on artificial medium. Six single spores of each culture were reisolated from plants infected with five different isolates. Some of the spores of some isolates did not grow in artificial medium and only two normal-appearing isolates were obtained out of five isolates. Subsequently 15-20 single spores were isolated from each piece of tissue. Approximately half of them grew. Some of the single-spore cultures were different from each other in the amount of growth and pigmentation in culture (Figure 2). As they were transferred, they continued to change. Similar changes in culture were observed on Cochliobolus sativus (Sharp, 1962) and Phytophthora infestans (Caten and Jinks, 1967).

These observations suggest that P. graminea, when cultured on artificial media, may exhibit attenuated growth and/or may undergo genetic changes as have been discussed earlier. In the virulence test of isolates (see Table 1) the fungus showed a general decline in

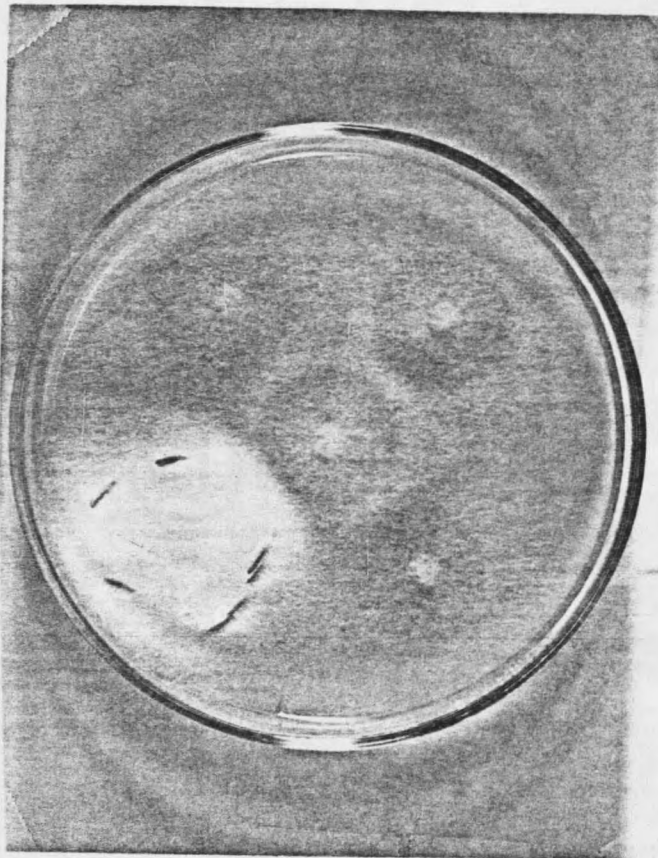


Figure 2. Variation in growth of P. graminea in artificial medium.

virulence as measured by the number of infected seedlings resulting from inoculation of a susceptible barley cultivar. This occurred after the cultures had been maintained on artificial media for two months. It is known that the virulence of a fungus pathogen can be related to other morphological or physiological changes that may occur in culture (Cochrane, 1958). It is not now known whether or not attenuated cultures of P. graminea lose their virulence. This question needs to be resolved.

Proof of the remarkable loss in pathogenicity of the P. graminea cultures under study is documented in Table 7. Cultures Mt 6 and Mt 10 were used to inoculate seeds of the susceptible cultivar "Summit" over a period of 1981 and 1982. In the summer of 1981, the percentages of infection obtained with cultures Mt 6 and Mt 10 were 94 and 95, respectively. By October of 1982, these percentages had declined to six and two, respectively. The decline continued even after the cultures were replaced with the reisolated cultures from infected leaves. Even with this decline in virulence, the parent cultivars were tested on September 1, 1982, with isolate Mt 6 having 24 percent infected plants and the F₂ populations were tested with the same isolate on September 24, having 30 percent infected plants, making the two experiments comparable.

Table 7. Percentage of infected plants (cv. Summit) in different inoculation times.

Different inoculation times	% inf. plants	
	Mt 6	Mt 10
Summer 1981	94	95
Fall 1981	42	58
June 1982	85	33
Sept. 1, 1982	24	3
Sept. 24, 1982	30	0
Dec. 13, 1982	6	2

The isolates were kept in test tubes containing PDA, and transferred at 14 day intervals between the Summer 1981 and Fall 1981. The reason for change in the virulence of the fungus within these time periods could be either attenuation or genetical change or both. After June 1982, isolates had been reisolated from plant material before they

changed on the artificial media. It appears that this was the genetic change. Genetic change can occur by heterokaryosis, parasexuality and mutation in this case. This loss of virulence on plants is contradictory to the results of Nilsson (1975) who reported that virulence of the fungus could be effectively maintained by reisolating from leaves.

Following pathogenicity investigation of the different isolates, it was necessary to identify which was the most effective inoculation technique. To determine which technique would be the most effective, three inoculation methods were compared using the same seed source, five different isolates and controlled environmental conditions. The three techniques used were as follows: (1) barley kernel inoculum method, (2) layered mycelium method, (3) soil inoculation method. When these three methods were compared (Table 2), the infection rate using barley kernel inoculum method was 59.5 percent, 54.1 percent with the layered mycelium method, and 18.2 percent with the soil method. No significant differences were found between the barley kernel inoculum method and the layered mycelium method. The soil inoculum method was significantly lower than the other two methods. Infection rates reported using the barley kernel inoculum method in previous studies were 67 percent (Nilsson, 1975) and 48.8 percent (Metz and Scharen, 1979). Infection rates found for the layered mycelium method in previous works were 83 percent (Nilsson, 1975) and 64.5 percent (Metz and Scharen, 1979). Infection percentages in Nilsson's studies were found higher than this study in both barley kernel inoculum and layered kernel inoculum method. Infection percentages in Metz' and Scharen's studies were lower in the

barley kernel inoculum method and higher in the layered mycelium method than this study. In the previous studies only one isolate was used while in this study five different isolates were used. The infection percentage of the most virulent isolate was 85.4 percent with the barley kernel inoculum method and 80.5 percent with the layered mycelium method in this investigation.

Three important considerations for inoculation methods are: efficiency, homogeneity, and practicality. In the virulence tests of the isolates and in the comparison of the three inoculation methods, there was much variation in seed germination and percentages of plants with symptoms with regard to the barley kernel inoculation method. Though germination percentages in the layered mycelium method were satisfactory, the percentages of plants with symptoms were changing drastically over time of inoculation. The barley kernel inoculum method was modified by making a hole in the jar cover, then covering it with filter paper to allow air exchange. The amount of water used was increased from 30 ml to 39 ml. Increased water and oxygen is believed to increase growth of fungus and germination of seeds. In the previous barley kernel inoculum method, seeds germinated in 10-14 days at temperatures between 14-17 C. After modification, they germinated in 4-6 days at temperatures between 12-15 C. For maximum infection, a long germination time is needed. Cooler temperatures would probably lengthen the germination time.

Inheritance of resistance to P. graminea was studied in crosses between American and Turkish barley cultivars. In this preliminary study only F₂ generations were observed. For better understanding of

inheritance of resistance, observation of F_3 and F_4 generations and progenies resulting from back crossing the F_1 plants to their parents is necessary.

Twelve different varieties tested had different levels of resistance to five isolates of *P. graminea* (Appendix, Table 11). Based on statistical analysis, the cultivars Betzes, Steptoe, Tokak, Erbet, Cum 50, Yesilkoy, Shapet, Ershabet were categorized as resistant and Zafer, Kaya and Yercil were categorized as susceptible. Though Betzes was immune in three previous studies (Kline, 1972; Metz and Scharen, 1979; and Tekauz and Chiko, 1980), infection on this cultivar was observed with the isolates Mt 6 and Mt 17 (Appendix, Table 11).

The expected segregation values were calculated according to the classical Chi-square method and a special formula of Chi-square (Snedecor, 1956) by taking into account penetrance and expressivity. According to the Chi-square method, it was found that resistant varieties Betzes, Tokak, Shabet, Erbet, Ershabet, and Cum 50 have at least one gene for resistance. The moderately susceptible variety Zafer may have some minor genes and the susceptible variety Yercil does not have any gene for resistance. When penetrance and expressivity were taken into account the results were almost the same. However, indication of having more than one gene was stronger in this method, and also there was strong evidence that the moderately susceptible cultivar Zafer has some minor genes.

This preliminary experiment gave results that were somewhat similar to those of Army (1945) who found different types of resistance ranging from control by one to control by three dominant genes, a modifying

factor and complete to incomplete dominance. However, in this experiment, evidence for incomplete dominance could not be found. Also, these results agree in some respects with results of Nilsson (1975), who predicted a major gene control of resistance and with results from Isenbek (1930), who reported resistance to be controlled by several dominant factors. No evidence was found concerning the control of resistance by a collection of six different genes (Suneson, 1980) and quantitative inheritance (Knudsen, 1980).

This study showed several inconsistencies in results. Three factors were identified that may have caused inconsistencies: (1) variation in the inoculum method used, (2) attenuation of the fungus on artificial media, and/or (3) genetic change of the fungus. Interaction of two or three of these factors could create even more complexities. Variation in the barley kernel inoculum method was lowered to a more acceptable level as the study progressed, but further study is needed to fully explain this variation. The effect of attenuation of P. graminea on artificial media also requires further study. In addition, a better artificial medium is needed that will support growth of the fungus for a longer time and result in sporulation. The potential spontaneous variability that was found within single spore isolates and their populations also needs further study.

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APPENDICES

Table 8. Minimal medium + malt yeast agar + barley leaf extract.

35 g	Bacto Czapek Dox Broth.
2 g	Yeast Extract
2 g	Malt Extract
2 g	Sucrose
20 g	Agar
75 g	Fresh Barley Leaves
1000 ml	Distilled H ₂ O

Preparation of the barley leaf extract: 75 g fresh barley leaves are mixed with 200 ml of distilled water in a blender. The mixture is sieved through a cheese cloth.

Table 9. Analysis of variance of comparison of three inoculation procedures for P. graminea on barley.^{1/}

Source	df	ms	F
Reps	1	67.51	.2559
Isolates	5	2585.0	9.795**
Methods	2	4067.0	15.41**
Methods x isolates	10	274.6	1.041
Methods x planting times	2	120.3	.4557
Isolates x planting times	5	644.0	2.440
Methods x Isolates x planting times	10	274.7	1.041
Error	32	263.9	

^{1/} Refer to Table 2.

Table 10. Comparison of three inoculation procedures for P. graminea on barley.

Isolates and Reps.	No. Seeds Germinated ^{1/}	% Germinated	No. Seeds Infected	% Infection	
<u>I. BARLEY KERNEL INOCULUM</u>					
Check I ^{2/}	A	123	98.4	--	--
	B	124	99.2	--	--
	C	125	100.0	--	--
	D	125	100.0	--	--
Check II ^{3/}	A	73	58.4	--	--
	B	68	54.4	--	--
	C	38	30.4	--	--
	D	49	39.2	--	--
Mt 6	A	112	89.6	95	84.8
	B	122	97.6	106	86.8
	C	3	2.4	1	33.3
	D	53	42.4	50	94.3
Mt 10	A	99	79.2	3	3.0
	B	112	89.2	0	0.0
	C	73	58.4	54	74.0
	D	65	52.0	58	89.2
Mt 17	A	99	79.2	76	76.8
	B	117	93.6	105	89.7
	C	38	30.4	16	42.1
	D	58	46.4	41	70.1
Kaya	A	115	92.0	43	37.4
	B	117	33.6	17	14.5
	C	58	46.4	35	60.3
	D	53	42.4	32	60.3
Eth 465	A	79	63.2	64	81.0
	B	72	57.6	63	87.5
	C	62	49.6	42	67.7
	D	14	11.2	7	50.0

Table 10. (cont'd).

Isolates and Reps.	No. Seeds Germinated ^{1/}	% Germinated	No. Seeds Infected	% Infection	
<u>II. LAYERED MYCELIUM</u>					
Check I ^{2/}	A	123	98.4	--	--
	B	124	99.2	--	--
	C	125	100.0	--	--
	D	124	100.0	--	--
Check II ^{4/}	A	89	71.2	--	--
	B	90	72.0	--	--
	C	13	10.4	--	--
	D	39	31.2	--	--
Mt 6	A	121	96.8	99	81.8
	B	120	96.0	77	64.2
	C	100	80.0	80	80.0
	D	76	60.8	23	30.3
Mt 10	A	123	98.4	54	43.9
	B	122	97.6	23	18.8
	C	99	79.2	0	0.0
	D	112	89.6	59	52.7
Mt 17	A	117	93.6	81	69.2
	B	120	96.0	59	49.2
	C	62	49.6	2	3.2
	D	106	84.8	84	79.2
Kaya	A	108	86.4	61	56.5
	B	60	48.0	25	41.7
	C	86	68.8	28	32.5
	D	88	70.4	38	43.2
Eth 465	A	110	88.0	103	93.6
	B	--	--	--	--
	C	95	76.0	75	78.9
	D	113	90.4	78	69.0

Table 10. (cont'd).

Isolates and Reps.	No. Seeds Germinated ^{1/}	% Germinated	No. Seeds Infected	% Infection	
<u>III. SOIL INOCULATION</u>					
Check I ^{2/}	A	123	98.4	--	--
	B	124	99.2	--	--
	C	125	100.0	--	--
	D	125	100.0	--	--
Check II ^{5/}	A	109	87.2	--	--
	B	116	92.8	--	--
	C	122	97.6	--	--
	D	121	96.8	--	--
Mt 6	A	37	29.6	19	51.4
	B	50	40.0	22	44.0
	C	--	--	--	--
	D	--	--	--	--
Mt 10	A	99	79.2	1	1.0
	B	81	64.8	1	1.2
	C	60	48.0	10	16.6
	D	--	--	--	--
Mt 17	A	97	77.6	37	38.1
	B	19	15.2	11	57.9
	C	29	23.2	11	37.9
	D	14	11.2	1	7.1
Kaya	A	125	100.0	12	9.6
	B	119	95.2	5	4.2
	C	14	11.2	0	0.0
	D	37	29.6	11	29.7
Eth 465	A	--	--	--	--
	B	--	--	--	--
	C	14	11.2	2	14.3
	D	17	13.6	5	29.4

^{1/} 125 seeds were planted in each replication.

^{2/} Percentage seeds germinated in paper toweling.

^{3/} Percentage of seeds emerged, first germinated in inoculum substrate less fungus then planted in soil.

^{4/} Percentage of seeds emerged, first germinated in two layers of agar medium less fungus then planted in soil.

^{5/} Percentage of seeds emerged in soil without fungus or substrate.

Table 11. Germination percentages and infection percentages of parent varieties to five different P. graminea isolates when inoculated with the soil method.

Parents	Isolates	% Germ. ^{1/}	% Inf.	Avg % Inf.
1. Betzes CI 6398	Mt 6	93.6 ^{2/}	5.1	4.9
		82.4 ^{3/}	4.8	
	Mt 10	84.0	0.0	0.0
		88.0	0.0	
	Mt 17	91.2	5.6	5.5
		88.0	5.4	
	Eth 465	95.2	0.0	0.0
		94.4	0.0	
	Kaya	98.4	0.0	0.0
		96.0	0.0	
2. Shabet CI 13827	Mt 6	88.0	10.0	8.9
		90.4	7.9	
	Mt 10	89.6	2.6	2.2
		88.0	1.8	
	Mt 17	95.2	10.4	9.1
		91.2	7.8	
	Eth 465	77.6	1.0	0.5
		84.0	0.0	
	Kaya	80.0	0.0	0.0
		94.4	0.0	
3. Erbet CI 13826	Mt 6	95.2	3.3	5.4
		96.0	7.5	
	Mt 10	91.2	0.8	1.6
		99.2	2.4	
	Mt 17	85.6	0.9	1.7
		91.2	2.6	
	Eth 465	94.4	0.0	0.0
		93.6	0.0	
	Kaya	93.6	0.0	0.0
		81.6	0.0	

Table 11. (cont'd).

	Parents	Isolates	% Germ. ^{1/}	% Inf.	Avg % Inf.
4.	Ershabet CI 15722	Mt 6	94.4	6.8	9.0
			85.6	11.2	
		Mt 10	80.8	3.9	2.4
			88.8	1.0	
		Mt 17	93.6	0.0	0.9
	81.6	1.9			
Eth 465	92.8	0.0	0.0		
	84.0	0.0			
Kaya	98.4	0.0	0.0		
	98.4	0.0			
5.	Steptoe CI 929	Mt 6	91.2	1.7	2.1
			92.0	2.6	
		Mt 10	91.2	0.8	0.8
			86.4	0.8	
		Mt 17	92.8	0.0	0.0
82.4	0.0				
Eth 465	96.0	0.0	0.0		
	96.8	0.0			
Kaya	96.0	0.0	0.4		
	88.8	0.9			
6.	Tokak	Mt 6	96.8	4.9	4.1
			95.2	3.3	
		Mt 10	94.4	0.8	1.2
			92.8	1.7	
		Mt 17	92.0	0.8	0.8
92.0	0.8				
Eth 465	96.0	1.6	1.2		
	92.0	0.8			
Kaya	97.6	0.8	0.4		
	94.4	0.0			

Table 11. (cont'd).

	Parents	Isolates	% Germ. ^{1/}	% Inf.	Avg % Inf.
7.	Cum 50	Mt 6	94.4	8.4	7.2
			91.2	6.1	
		Mt 10	83.2	5.7	4.3
			78.4	3.0	
		Mt 17	92.0	5.2	3.9
89.6	2.6				
Eth 465	98.4	0.0	0.8		
	94.4	1.6			
Kaya	87.2	0.9	1.4		
	83.2	1.9			
8.	Kaya	Mt 6	86.4	14.8	16.0
			92.8	17.2	
		Mt 10	85.6	42.0	40.2
			87.2	38.5	
		Mt 17	90.4	7.0	7.9
90.4	8.8				
Eth 465	91.2	14.9	15.1		
	99.2	15.3			
Kaya	95.2	2.5	2.5		
	--	--			
9.	Yesilkoy	Mt 6	94.4	8.4	7.0
			97.6	5.7	
		Mt 10	90.4	4.4	4.3
			75.2	4.2	
		Mt 17	88.8	1.8	1.3
84.0	0.9				
Eth 465	86.4	2.7	2.3		
	77.6	2.0			
Kaya	80.8	0.9	0.4		
	83.2	0.0			

Table 11. (cont'd).

Parents	Isolates	% Germ. ^{1/}	% Inf.	Avg % Inf.
10. Zafer	Mt 6	86.4	25.9	21.8
		89.6	17.8	
	Mt 10	93.6	19.6	18.9
		96.0	18.3	
	Mt 17	98.4	10.5	9.3
97.6		8.1		
Eth 465	Kaya	93.6	5.9	5.3
		84.8	4.7	
11. Yercil	Mt 6	96.8	0.8	3.3
		95.2	5.8	
	Mt 10	88.8	54.0	46.6
		81.6	39.2	
	Mt 17	88.8	49.5	60.0
81.6		70.5		
Eth 465	Kaya	96.8	24.7	22.0
		95.2	19.3	
Kaya	Eth 465	81.6	36.2	38.9
		92.0	41.7	
Kaya	Eth 465	90.4	4.4	3.1
		80.8	1.9	

Table 11. (cont'd).

Parents	Isolates	% Germ. ^{1/}	% Inf.	Avg % Inf.
12. Gem CI 7243	Mt 6	88.0	9.0	10.6
		91.2	12.2	
—	Mt 10	92.8	6.0	5.5
		77.6	5.1	
—	Mt 17	92.0	4.3	3.8
		94.4	3.3	
—	Eth 465	92.8	1.7	2.1
		92.0	2.6	
—	Kaya	76.8	0.0	0.4
		93.6	0.8	

^{1/}125 seeds were planted in each planting time.

^{2/}First planting.

^{3/}Second planting.

Table 12. Calculation of the expected ratios according to penetrance and expressivity.

Calculation of the expected value in the case of one dominant gene involved:

Erbet x Yercil

5% Inf. 47% Inf.

$\frac{A}{E} \frac{A}{E}$ $\frac{a}{y} \frac{a}{y}$

F_2	1	$\frac{A}{E} \frac{A}{E}$	5S + 95R	-----	5S + 95R
	2	$\frac{A}{E} \frac{a}{Y}$	2 (5S + 95R)	-----	10S + 190R
	1	$\frac{a}{Y} \frac{a}{Y}$	47S + 53R	-----	$\frac{47S + 53R}{62S + 338R}$

Expected ratio 338:62
5.4:1

Calculation of the expected value in the case of two dominant genes involved (each parent has one gene):

Betzes x Tokak

5% Inf. 4% Inf.

$AAbb$ $aaBB$

F_2	9	$A_ B_$	9(4S + 96R)	-----	36S + 864R
	3	$A_ bb$	3(5S + 95R)	-----	12S + 285R
	3	$aaB_$	3(4S + 96R)	-----	15S + 288R
	1	$aabb$	47S + 53R	-----	$\frac{47S + 53R}{110S + 1490R}$

Expected ratio 1490:110
13.5:1

Table 12. (cont'd).

Calculation of the expected value in the case of three dominant genes involved:

Betzes x Tokak
 5% Inf. 4% Inf.
 — AAbbdd aaBBDD

F_2	27 A_ B_ D_	27(4R + 96S)	-----	108S + 2592R
	9 A_ B_ dd	9(4R + 96S)	-----	36S + 864R
	9 A_ bb D_	9(4R + 96S)	-----	36S + 864R
	3 A_ bb dd	3(5R + 95S)	-----	15S + 285R
	9 aa B_ D_	9(4R + 96S)	-----	36S + 864R
	3 aa B_ dd	3(4R + 96S)	-----	12S + 288R
	3 aa bb D_	3(4R + 96R)	-----	12S + 288R
	1 aa bb dd	53R + 47S	-----	$\frac{47S + 53R}{302S + 6098R}$

Expected ratio 6092:302
 20.1:1

Calculation of the expected value in the case of no segregation (some dominant gene in both parents):

Betzes x Tokak
 5% Inf. 4% inf.
 $\frac{A_ A_}{B_ B_}$ $\frac{A_ A_}{T_ T_}$

F_2	1 $\frac{A_ A_}{b_ b}$	1(4S + 95R)	-----	5S + 95R
	2 $\frac{A_ A_}{B_ T}$	2(4S + 96R)	-----	8S + 192R
	1 $\frac{A_ A_}{T_ T}$	1(4S + 96R)	-----	$\frac{4S + 96R}{17S + 383R}$

Expected ratio 383:17
 22.5:1

Table 13. Crosses between Turkish and American barley varieties and number of F_1 seeds obtained.

Crosses	No. F_1 Seeds
1. Betzes x Tokak	76
2. Betzes x Cum 50	58
3. Betzes x Kaya	58
4. Betzes x Yesilkoy	72
5. Betzes x Zafer	69
6. Betzes x Yercil	83
7. Betzes x Gem	83
8. Shabet x Tokak	32
9. Shabet x Cum 50	83
10. Shabet x Kaya	42
11. Shabet x Yesilkoy	100
12. Shabet x Zafer	78
13. Shabet x Yercil	70
14. Shabet x Gem	41
15. Erbet x Tokak	12
16. Erbet x Cum 50	58
17. Erbet x Kaya	68
18. Erbet x Yesilkoy	91
19. Erbet x Zafer	80
20. Erbet x Yercil	72
21. Erbet x Gem	60
22. Ershabet x Tokak	44
23. Ershabet x Cum 50	67
24. Ershabet x Kaya	70
25. Ershabet x Yesilkoy	75
26. Ershabet x Zafer	84
27. Ershabet x Yercil	64
28. Ershabet x Gem	76
29. Steptoe x Tokak	44
30. Steptoe x Cum 50	52
31. Steptoe x Kaya	51
32. Steptoe x Yesilkoy	31
33. Steptoe x Zafer	31
34. Steptoe x Yercil	20
35. Steptoe x Gem	116
36. Tokak x Betzes	86
37. Tokak x Shabet	89
38. Tokak x Erbet	148
39. Tokak x Ershabet	91
40. Tokak x Steptoe	63

Table 13. (cont'd).

Crosses	No. F ₁ Seeds
41. Cum 50 x Betzes	61
42. Cum 50 x Shabet	55
43. Cum 50 x Erbet	89
44. Cum 50 x Ershabet	90
45. Cum 50 x Steptoe	104
46. Kaya x Betzes	85
47. Kaya x Shabet	49
48. Kaya x Erbet	58
49. Kaya x Ershabet	44
50. Kaya x Steptoe	69
51. Yesilkoy x Betzes	45
52. Yesilkoy x Shabet	84
53. Yesilkoy x Erbet	71
54. Yesilkoy x Ershabet	70
55. Yesilkoy x Steptoe	80
56. Zafer x Betzes	48
57. Zafer x Shabet	26
58. Zafer x Erbet	52
59. Zafer x Ershabet	39
60. Zafer x Steptoe	47
61. Yercil x Betzes	31
62. Yercil x Shabet	95
63. Yercil x Erbet	48
64. Yercil x Ershabet	73
65. Yercil x Steptoe	86
66. Gem x Betzes	23
67. Gem x Shabet	46
68. Gem x Erbet	83
69. Gem x Ershabet	55
70. Gem x Steptoe	64

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