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

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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by

Cahit Konak

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Cahit Konak

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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TO MY FATHER

VITA

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

Cahit received his elementary education in Susurluk, part of his high school education in Bursa, Turkey, and then graduated from high school in Balikesir, Turkey, in 1962. He attended the Aegean University in Izmir, Turkey, where he received a B.S. degree in Agronomy in September, 1967.

Cahit served in the Turkish Military from 1968 to 1970. After the military, he worked for a malting and brewing company and later the Agricultural Research Institute in Izmir. He worked as a trainee for CIMMYT (International Maize and Wheat Improvement Center) and Montana State University in 1979. He returned to Montana State University to attend graduate school and will complete an M.S. degree in Plant Pathology in March, 1983.

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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 intracelluarly 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 Arny (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 Arny (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 Arny, 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 succeptible 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, laways 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 x 10^4 erg/cm^2 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 x 10^4 erg/cm^2 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.