Etiology and epidemiology of the barley stripe disease (Pyrenophora graminea) in a semi-arid environment
by Sally Gwillim Metz

A thesis submitted in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in PLANT PATHOLOGY
Montana State University

Abstract:
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Studies on spread of the disease from these foci showed that irrigation near heading increased the percent infection of seed with barley stripe threefold. In a dry environment, percent infection decreased to nearly zero.

Commercially grown cultivars in Montana were screened for resistance to three isolates of P. graminea. Betzes, Erbet, Shabet, and Steptoe were highly resistant, while Horsford, Larker, and Ingrid were susceptible.

Registered and experimental fungicides were evaluated in vitro for sources of potential seed treatments to replace Ceresan, which formerly was used to control the disease. None were as effective as Ceresan, but several experimental fungicides have good potential.

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by

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A thesis submitted in partial fulfillment
of the requirements for the degree

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ABSTRACT

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INTRODUCTION

Barley leaf diseases were not recognized as a problem in north-central Montana until the early 1970's. Farmers and agronomists on the Fairfield Bench in northcentral Montana became concerned in 1973 when they estimated losses in yield up to 50%, along with the loss in quality of their malting barley.

The Fairfield Bench is a large, 28,000 ha (70,000 acre) irrigation district. Growers have changed from a crop/fallow system to one of intensive continuous cropping. Nearly 14,000 ha (35,000 acres) of malting barley are grown under flood irrigation, some sprinkler irrigation, and minimum tillage. The other hectarage is composed of wheat and forages.

To intensify the problem of continuous barley, the malting companies dictate which cultivar will be grown to the extent that a near monoculture exists. From 1973-1975, Shabet was the preferred cultivar. This preference changed to Klages by 1977. Continuous barley grown under irrigation and minimum tillage set the scene for a tremendous disease outbreak in the mid 1970's. When mature plants were examined, the disease was so severe that it resembled barley stripe caused by *Pyrenophora graminea*; however, several organisms could have been involved: *P. graminea*, *P. teres*, or *Rhynchosporium secalis*.

Initially, the primary objective of this study was to determine the causal agent of the malting barley disease in northcentral

Montana. Since *P. teres* was incriminated, and since it was being studied at Montana State University by another student, I conducted further investigations on *P. graminea*. These studies were devoted to determining whether or not *P. graminea* was present in Montana, developing inoculation techniques, screening cultivars for resistance to the disease, and evaluating chemical seed treatments.

LITERATURE REVIEW

Barley leaf stripe is incited by the fungus *Pyrenophora graminea* Ito et Kurib. (*Drechslera graminea* (Rab.) Shoem., syn. *Helminthosporium gramineum* Rab.). The imperfect form of *P. graminea* is placed in the Hyphomycetes (Dematiaceae) based on the presence of brown septate conidiophores and large brown phragmoid conidia borne laterally and terminally on the conidiophore (Talbot, 1973). Most species of *Drechslera* have cylindric conidia that are nearly as wide at the apical septum as at the basal septum. For those species with cylindric conidia, the widest point of a conidium is near the middle with the exception of *P. teres* in which it is near the inflated basal cell. The contour of conidia is usually smooth without enlarged cells and consequent constrictions at the septa, again with the exception of *P. teres*. Conidium length is more variable than width. For example, in 15 collections of *P. graminea* the average length varied 46% while the width varied 29% (Shoemaker, 1962).

Conidia of *P. graminea* are characteristically 30-100 μ long by 11-24 μ wide with up to six septa being present (Talbot, 1973). Cells are shorter than wide (Shoemaker, 1962). Conidia are subhyaline to yellow brown (Talbot, 1973).

In contrast, *P. teres* is characterized by conidia which are slightly constricted at the septa and have an inflated basal cell (Shoemaker, 1962; Talbot, 1973). They are subhyaline to light

yellowish-brown, measuring 95-120 by 19-21 μ with 4-6 septa (Shoemaker, 1962). Cultures are characterized by tufts of mycelium (Shoemaker, 1962).

The *Pyrenophora* species attacking barley often cause confusion among scientists. Shoemaker (1962) points out several characterizing differences: "*P. teres* causes net blotch, *P. graminea* causes a systemic stripe disease, and *P. tuberosa* causes a conspicuous, elliptical leaf spot. Conidia of *P. teres* are consistently longer than those of *P. graminea*, usually have an inflated basal cell, and rarely produce any secondary conidia. *P. tuberosa* conidia are longer than those of *P. teres*, lack the inflated basal cell, and regularly have secondary conidiophores."

The perithecial stage of *P. graminea* is rarely reported in the literature. Smedegard-Petersen (1973) reports finding elongated sclerotium-like bodies superficially or partially embedded on barley straw from plants heavily infected with leaf stripe. They measure 576-728 by 442-572 μ and are very similar to perithecia of *P. teres*, having dark, rigid setae on the surface. The majority of perithecia remain immature, but as in *P. teres*, conidia are formed abundantly on setae of the perithecia (Smedegard-Petersen, 1973). Shoemaker (1962) reports that *P. graminea* often has only four ascospores per ascus and they are larger than those of *P. teres*.

Symptoms of barley stripe disease may begin to appear soon after emergence of seedlings, but often are not evident until six weeks after planting. The disease may exhibit small chlorotic, elongate spots which develop into white or yellow stripes extending the length of the leaves (Stakman and Rodenhiser, 1929; Drechsler, 1923). One blade may exhibit 5-7 stripes which turn dark brown with time (Drechsler, 1923). Infected plants become straw colored before normal maturity (Suneson, 1950; Weniger, 1932) and, if severely infected, are stunted, often being only half as tall as healthy plants (Stakman and Rodenhiser, 1929; Drechsler, 1923). As the disease progresses, the foliage of the entire plant splits, giving it a shredded appearance. Leaves lack mechanical rigidity as evidenced by their drooping and contorted positions (Drechsler, 1923). The spike seldom becomes visible or protrudes abnormally from the leaf sheath. Viable seed is seldom produced (Suneson, 1950; Drechsler, 1923) or, if produced, is shriveled (Weniger, 1932). Development of the disease is favored by cool (Aberg, 1945; Stakman and Rodenhiser, 1929), dry (Prasad et al., 1976; Machuzhenko, 1977; Knudsen, personal communication) weather early in the growing season.

Barley stripe is a problem in northern Africa, the middle east, northwestern Europe, and the southeastern United States. In addition to barley, *P. graminea* was reportedly isolated from wheat and inoculated onto wheat, rye, and oats by Johnson (1914), although his

descriptions indicate that he may have been dealing with *Cochliobolus sativus*. Rasulev and Kravtsova (1971) report that *P. graminea* infects wild barley, wheat and rye in the USSR.

Because of its systemic nature, the infection process of *P. graminea* is likened to that of loose smut (*Ustilago nuda*). Unlike barley stripe, loose smut is present in the embryo, establishes itself in the growing point and spreads to each young part of the plant as it is differentiated (Skoropad and Arny, 1956). As more histological studies are completed, it becomes apparent that *P. graminea* is found in the hull and pericarp, but not in the embryo or endosperm. During germination of susceptible varieties, the fungus penetrates through the coleorhiza, the sheath enclosing the embryonic root, in both artificially inoculated (Skoropad and Arny, 1956) and naturally infected (Platenkamp, 1977) seed. Skoropad and Arny (1956) report that penetration occurs by means of appresoria and hyphal pegs whereas Platenkamp (1977) states that hyphae grow intercellularly through the parenchymatous regions and usually intercellularly through meristematic regions. After mycelium becomes established in the entire seedling culm, rapid elongation of the seedling results in fragmentation of the hyphae and establishment of isolated centers of infection in the internodes. In older host tissues, most of the xylem elements contain mycelium, and many are mechanically plugged (Skoropad and Arny, 1956).

Cultural practices, seed treatments, and resistant cultivars may all be used to control barley stripe. Increased soil fertility or any other condition favoring vigorous plant growth apparently decreases the percentage of diseased plants developing from naturally infected seed (Leukel et al., 1933).

Originally, the hot water seed soak was used to control barley stripe (Weniger, 1932; Arny and Leben, 1956). Later, organic mercury seed treatment with Ceresan was highly effective (Reddy and Burnett, 1930; Stakman and Rodenhiser, 1929; Leukel et al., 1933; Weniger, 1932; Arny and Leben, 1956), but it was withdrawn from the market by the Environmental Protection Agency in the early 1970's.

Presently there is controversy concerning the effectiveness of carboxin and carboxin plus thiram in controlling barley stripe. Kingsland (1969) reports a decrease from 98 to 7 infected tillers per 3.05 meters of row with use of seed treated with carboxin (75% WP= Vitavax^{R}) at 120 mg/45.5 Kg (4 oz./100 lbs). Prasad et al. (1976) report no effective control of barley stripe with carboxin. In a field test, Kline (1972) finds that effective control is obtained with 240 mg/22.9 Kg (8 oz/bu) carboxin plus thiram. Other fungicides have been tried with varying degrees of success (Kingsland, 1972; Kline and Roane, 1972).

Growth regulator type herbicides, e.g., 2,4-D, inhibit the development of *P. graminea* in culture but have no effect on infection of barley in the field (Pall and Bobes, 1971; Kharchenko and Shkylar, 1977).

The most desirable form of control is the use of resistant cultivars. There has been effort throughout the world to determine the susceptibility of barley cultivars based on percent infection. Reactions of various cultivars are given by: Shands and Arny, 1944; Nilsson, 1975; Kline, 1971,1972; Atheya, 1973; Rai et al., 1975; Suneson and Santoni, 1943; Arny, 1945; and Suneson, 1950.

The gene action of resistance is not known. Speculation ranges from a single gene with incomplete dominance (Suneson, 1950), genes with modifying factors (Arny, 1945), to a collection of six different genes (Suneson, 1950). This may depend on the cultivar being studied. No work has been done on determining gene action since the 1940's.

Numerous inoculation techniques and modifications of them have been developed for *P. graminea*, all of which work to one degree or another. *P. graminea* does not sporulate readily in culture; therefore, most inoculation techniques involve the use of mycelium rather than spores. Teviotdale and Hall (1976b) perfected Srinivasan et al.'s (1971) original technque of inducing sporulation by adding leaf pieces to the agar medium by adjusting the lighting and temperature conditions. However, the number of spores produced is not high enough

to make it a feasible inoculation technique. Suneson and Houston (1942) use male-sterile barley for the study of floral infection. They use sporulating leaves from naturally infected plants as the inoculum and report infection rates of up to 89.5%. Soaking the barley kernels in spore suspensions is another inoculation method which has been used (Tapke, 1946; Johnson, 1914).

Arny and Shands (1942) developed the 'autoclaved wheat kernel' technique, which produces 80% infection in susceptible varieties. Equal quantities of wheat kernels and water are autoclaved and mycelial suspension is added. After the fungus grows for four days, surface sterilized seed is added to the inoculum in the flask and the entire mixture is plated after four more days.

Increased infection rates are correlated with the removal of the hull (Johnson, 1925; Teviotdale and Hall, 1976a; Mohammad and Mahmood, 1974a). Most often the seed is dehulled with acid (Shands, 1934; Mohammad and Mahmood, 1974a), but care must be taken not to reduce germination. After dehulling, the seed is placed between two layers of actively growing mycelium and kept at cool (0°C) temperatures for 10-14 days. Germinating the seed between two layers of mycelium may also be done successfully without dehulling (Houston and Oswald, 1948; Nilsson, 1975).

Net blotch, caused by *Pyrenophora teres* Drechs. (*Drechslera teres* (Sacc.) Shoem., syn. *Helminthosporium teres* (Sacc.)) is a common

disease of barley, occurring wherever the crop is grown in temperate, humid regions of the world (Dickson, 1956). It differs from *P. graminea* in that it is a leaf infecting organism rather than being a systemic, seed-borne one. In the later stages of development, symptoms of the two may be confused.

The usual symptoms produced by *P. teres* occur on the blade and sheath of leaves. The initial lesions appear as minute spots. These spots increase in size, and form narrow, dark brown, longitudinal and transverse streaks, producing the characteristic net-like pattern (Smedegard-Petersen, 1971). Chlorosis and necrosis of the adjacent tissue follows. *P. teres* f. sp. *maculata* produces dark brown, elliptical lesions measuring up to 3x6 mm in size, surrounded by chlorotic zone (Smedegard-Petersen, 1971, 1976). The development of symptoms is dependent on the host variety and pathogenicity of the isolate (Smedegard-Petersen, 1971).

Primary infection of net blotch may result from seed-borne inoculum (Shipton et al., 1973) with low temperatures (10-15°C) causing the highest levels of infection. The degree to which seed-borne inoculum contributes to epidemics is not well understood. Piening (1968) believes that low levels of infection (about 1%) could be significant in the development of secondary infections if climatic conditions were suitable.

Loss estimates on barley from net blotch are variable, with higher losses associated with cool, damp weather. In the greenhouse, infection and destruction of two-thirds of the barley leaves cause losses up to 53.3%. Yield reductions are greater in poor soil (43.2%) than in fertile soil (13.1%)(Piening and Kaufmann, 1969). Workers in New Zealand suggest that a direct correlation exists between the percentage of the flag leaf infected and the associated loss (Hampton and Arnst, personal communication).

Net blotch is most readily controlled through cultural practices: rotation, stubble destruction and resistant cultivars (Shipton et al., 1973). Organic mercurials were formerly used as seed treatments for effective control of the primary inoculum (Shipton et al., 1973). The use of foliage cover sprays is being investigated as a means of control (Shipton et al., 1973).

Chapter 1

IDENTIFICATION OF THE *Pyrenophora* SPECIES INFECTING BARLEY ON THE FAIRFIELD BENCH

Samples of seed from seven heavily infested barley fields on the Fairfield Bench were obtained from Power Farmers' elevator, Power, Montana, and Eisenman Seed Company, Fairfield, Montana. These seed lots were tested in the laboratory, greenhouse, growth chamber, and field to determine if they were infested with *P. graminea* or other related fungi.

MATERIALS AND METHODS

Seed Lots

In the laboratory, the seed was surface sterilized in 0.5% NaOCl plus surfactant (1 drop Ivory soap per 100 ml) for 10 minutes, placed directly on barley leaf piece agar (Teviotdale and Hall, 1976a; Appendix Table 1), and incubated for 5 days in the growth chamber (17-18°C, 23 hr. photoperiod supplied by Westinghouse 20 W cool white fluorescent bulbs).

One hundred fifty seeds of each lot were also planted in the greenhouse in 8 cm pots, 5 seeds/pot. The soil was a pasteurized mixture of loam, sand, and peat moss (1:1:1). Natural lighting was supplemented by incandescent lights to give a 12 hour daylength. Plants were examined for systemic infection four weeks after planting.

To determine if low temperatures during germination and growth would affect the results, 100 seeds of each lot were planted in flats (20x30 cm) and placed in a winter wheat vernalizing chamber for four weeks. Temperatures were 11°C night, 16°C day and lighting was provided by fluorescent bulbs giving an 8-10 hr. day. Plants were examined for systemic infection four weeks after planting.

Inoculation Procedures to Determine the Species of *Pyrenophora* Involved

To further determine which *Pyrenophora* species were involved in the Fairfield Bench problem, three different inoculation procedures were tried, two recommended for *P. graminea* and one for *P. teres*.

The cultures of *Pyrenophora* isolated from barley leaves grown on the Fairfield Bench were grown on potato dextrose agar in a growth chamber at 17-18°C, under Westinghouse cool white fluorescent bulbs with a 23 hr. photoperiod. All cultures sporulated profusely.

The layered mycelium technique was conducted by growing cultures of the Fairfield isolates as indicated previously, placing surface sterilized Shabet barley seed between 2-10 day old cultures and incubating them in the refrigerator for two weeks. Germinating seeds were then planted in loam soil in the greenhouse.

The wheat kernel inoculation was conducted according to the specifications of Arny and Shands (1942). Equal amounts of wheat and water were autoclaved, mycelium was added and allowed to grow for

four days and then barley kernels were added and the entire mixture was allowed to incubate for four days. The mixture was planted and grown in the greenhouse as indicated previously.

The foliar spray inoculation procedure used by Krupinsky (1976) and Eyal and Scharen (1977) for the inoculation of wheat with *Septoria nodorum* was followed for these inoculations. Two 8 cm pots, six seeds/pot were planted for each variety one week prior to inoculation. Seven day old culture plates were scraped, added to 50 ml distilled water, blended for three minutes on low setting of a Waring blender, and strained twice through cheesecloth. Two drops of a surfactant (Tween 20) were added to 20 ml of inoculum (200,000-225,000 mycelial fragments plus conidia/ml) which was atomized onto six pots as they rotated on a turntable. Pots were transferred to a dew chamber for 48 hours and then placed on a greenhouse bench. Symptoms were read six days later.

State-wide Survey for *P. graminea*

A survey to determine if *P. graminea* was currently found in Montana was conducted in the spring and summer of 1977. Plants were collected from the Agricultural Research Center plots as well as from farmers' fields on the Fairfield Bench. Samples were rated positive or negative for *P. graminea* based on leaf symptoms and/or cultural morphology of fungi isolated from the leaves. Isolations were performed on all samples from the Fairfield Bench.

RESULTS AND DISCUSSION

Seed lots from the Fairfield Bench showed a 2-56% infestation with a *Pyrenophora* species as indicated by isolations from the seed (Table 1-1). The Karl seed lot had 2% infestation, while the Shabet seed lots ranged from 38-56%. However, the seed lots produced no systemically infected plants when grown in the greenhouse, cold growth chamber, or in the field. If the seed had been infested with *P. graminea*, some systemically infected plants should have developed. *Pyrenophora* could not be isolated from the plants with fine white stripes and the stripes disappeared with age.

The *Pyrenophora* organism involved did not produce systemically infected plants from seed but virulently attacked the leaves of barley when inoculated by atomization (Table 1-2). This was interpreted to mean that *P. teres* was the causal organism of the Fairfield Bench problem and *P. graminea* was not.

P. graminea was never isolated from plants in farmers' fields on the Fairfield Bench, although it was observed consistently in several research center plots of barley grown from seed originating in northwestern Europe and isolated plants were found in some Bozeman bulk hybrids (Table 1-3). However, net blotch (*P. teres*) was repeatedly observed on the Fairfield Bench, especially where the previous season's stubble remained on the ground.

Table 1-1. Percent seed borne infestation by *Pyrenophora teres* found in Fairfield seed lots

Seed Lot	Cultivar	Total # Seeds Tested	# Seeds Infested	% Infestation
1	Shabet	101	52	51.5
2	Shabet	109	42	38.5
3	Karl	100	2	2.0
4	Shabet	101	40	39.6
5	Shabet	101	44	43.5
863	Shabet	123	69	56.1
865	Shabet	100	51	51.0

Table 1-2. Reaction of barley seedlings to *Pyrenophora* isolates from the Fairfield Bench

Cultivar or Line	Known Reaction[1] to *P. teres*	Isolates 76-114	76-113	76-64	863
CI19819	R	R	R	MR	R
Atlas 46	S	S	MS	S	S
Unitan	R	MR	MS	MR	MR
Betzes	S	S	S	S	S
Klages	MS	S	S	S	S
Firlbecks III	MR	MR	MR	MR	MR

[1]Reaction to a mixture of Montana and Mid-East isolates of *P. teres*, Harold Bockelman, Montana State University (personal communication).

Table 1-3. Plots in which *P. graminea* was observed in disease survey, Montana, 1977

Date Collected	Location	Cultivar, Nursery in in which observed	Origin of seed
6/15	Huntley	RP43971, Hockett's intrastate yield trial	England
6/16	Moccasin	RPB43971, Hockett's intrastate yield trial	England
		Rovaniemi 70-B, Kushnak's genetic material	Finland
6/29	Bozeman	RPB43971, Hockett's intrastate yield trial	England
		RPB45671, Hockett's intrastate yield trial	England
		Karl, Hockett's intrastate yield trial	Bozeman 1976
		Sanalta/Betzes, Hockett's bulk hybrid	Bozeman 1976
		Dekap/Betzes, Hockett's bulk hybrid	Bozeman 1976
		Munsing/Betzes, Hockett's bulk hybrid	Bozeman 1976
7/26	Bozeman	Lami, Metz increase	Denmark
		Edda II Metz increase	Sweden
	Sidney	RPB43971, Hockett's intrastate yield trial	England
8/3	Bozeman	Incr. 71 - Bomi, Eslick increase	Denmark

Chapter 2

NATURAL INCREASE IN INFECTION OF *P. graminea* IN RPB-43971 BARLEY HARVESTED AT MONTANA AGRICULTURAL RESEARCH CENTERS IN 1977

The statewide survey for *P. graminea* resulted in finding one seed lot, RPB-43971 from the intrastate barley yield nursery, which had a significant infection of barley stripe. The objective of this experiment was to determine the rate of disease increase from one year to the next in seed infected with barley stripe when grown under different environmental conditions.

MATERIALS AND METHODS

RPB-43971 was introduced in Bozeman in March 1976 from Rothwell Plant Breeders in England. A portion of the RPB-43971 seed lot grown and harvested in Bozeman 1976 was saved and used as a standard for the experiment. The rest of the seed was planted at Agricultural Research Centers throughout the state as part of a randomized block design with other varieties. Moisture conditions varied from location to location. Plot size varied but the row spacing was consistently 30 cm. The plants grown from seed harvested from these plots were tested for percent systemic infection and compared to the standard.

Lots of 100 seeds each from plots harvested at the Agricultural Research Centers in 1977 were planted in flats (20x30 cm) and placed in a randomized block design. Reference to standard greenhouse

conditions in this and later experiments will refer to the following:

Soil:	1 part Bozeman silt loam:1 part sand
Watering:	Minimum
Light:	9-14 hr. photoperiod achieved when necessary by supplemental fluorescent or metal halide lamps
Temperature:	Cool - 13-21°C.

Approximately six weeks after planting, percent of plants systemically infected was visually determined.

Statistical analysis consisted of analysis of variance for randomized block and LSD. The mean of replications 2 and 3 was substituted for the missing values in replication 1 for Huntley irrigated and Havre dryland. Replications were performed sequentially instead of simultaneously in the greenhouse. The replication values are based on unequal subplot means (Sidney, Moccasin, Huntley-irrigated, Havre and control = 3 subplots; Huntley dry and Bozeman = 4 subplots; Kalispell = 5 subplots). The subplots are actually the replications grown in the interstate yield trial as a randomized block.

RESULTS AND DISCUSSION

The results show that environment has a marked influence on increase or decrease of *P. graminea* in Montana. An increase in the amount of infected seed correlated with irrigation prior to heading. Plots which were irrigated in Huntley and Kalispell produced a significantly higher level (p=.01) of infection than the standard (Table 2-1). In areas which were dry (Sidney and Havre; Table 2-2), there were significantly lower levels (p=.05) of infection than in the standard. No significant change was observed in the seed from all other locations.

Table 2-1. Natural infection of *P. graminea* of RPB-43971 seed at Montana Agricultural Research Centers in 1977.

Location	Irrigation	Rep 1	Rep 2	Rep 3	$\bar{X}$
Kalispell	Sprinkler	8.40	13.00	15.60	12.33**
Huntley irrigated	Flood	--	12.00	10.33	11.16**
Moccasin	None	4.67	3.00	6.00	4.56
Bozeman	Sprinkler	5.00	3.25	2.50	3.58
Huntley	None	1.25	1.50	1.75	1.50
Havre	None	--	0.33	0.00	0.17*
Sidney	None	0.33	0.00	0.00	0.11*
1976 Standard		3.00	4.67	2.67	3.45

* LSD significantly different from the control at p=.05 = 2.84.
** LSD significantly different from the control at p=.01 = 3.94.

Table 2-2. Environmental and agronomic data for 1977 from the Agricultural Research Centers

Location	Heading Date RPB-43971	Type of Irrigation	Date of Irrigation	pp[1] ± 4&14 Days	Heading	Ave % Infection RPB-43971
Kalispell	June 24	Sprinkler	June 7	.09	.12	12.33**
Huntley	June 19	Flood	June 2 June 21	.81	.90	11.16**
Moccasin	June 21	None	None	.85	1.38	4.56
Bozeman	July 3	Sprinkler	June 21	.86	1.12	3.58
Huntley	June 20	None	None	.81	.90	1.50
Havre	July 16	None	None	.32	.39	0.17*
Sidney	Irregular	None	None	--[2]	--	0.11*
1976 Standard	--	--	--	--	--	3.45

[1]Precipitation

[2]Precipitation unknown

*LSD significantly different from the control, p=.05=2.84

**LSD significantly different from the control, p=.01-3.94

Chapter 3

INOCULATION PROCEDURES FOR *P. graminea*

Three inoculation procedures for the infection of barley with *P. graminea* have been reported in the literature: wheat kernel (Arny and Shands, 1942), layered mycelium (Nilsson, 1976; Mohammad and Mahmood, 1974), and floral infection (Suneson and Houston, 1942; Teviotdate, 1976a). Effectiveness of these three procedures was compared simultaneously because of the influence of environmental conditions.

MATERIALS AND METHODS

Barley Kernel

Arny and Shands' (1942) wheat kernel inoculation was modified by using autoclaved barley seed as the inoculum substrate. Autoclaving times were also varied. Erlenmeyer flasks (250 ml) were prepared by adding equal amounts of Shabet barley (30 g) and distilled water (30 ml). Flasks were capped with aluminum foil only and autoclaved 22 minutes at 121°C followed by a slow release of pressure (total time in autoclave 45-50 min.). The flasks were removed from the autoclave as soon as possible. They were shaken to loosen the seed mat within 2 hours following removal from the autoclave. Flasks were also shaken after cooling overnight and then three plugs of *P. graminea* mycelium cut with a #1 cork bore were added. Incubation was at room temperature (20°C) for four days with minimal additional light (4 hr.) since

excessive heat and/or light caused the flasks to sweat and the fungus to be killed.

Seed lots (100 seeds each) were placed in test tubes and surface sterilized in 0.5% NaOCl plus 1% 95% EtOH 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 4-5 hr. Seeds were then aseptically added to shaken flasks and the mixture was incubated 4-5 days. Flasks were shaken every other day to prevent clumping. Before seeds had produced more than 1.25 cm (1/2 inch) of growth, the entire mixture was planted in flats filled with a 1:1 mixture of pasteurized Bozeman silt loam and sand.

Layered Mycelium

Barley kernels in lots of 100 were surface sterilized, as described above, dried and placed on the mycelial mat of a 10 day old petri dish medium of actively growing *P. graminea*. Another mat of fungal culture was removed and placed upside down on top of the seeds. Lids were then replaced and the petri dishes stored in a plastic bag to prevent desiccation, and kept at 4°C for two weeks. When the radicle reached 0.5-0.75 cm, the barley kernels were planted.

Floral Inoculation

In the summer of 1977, inoculation/pollination was done according to Suneson and Houston (1942) in a male sterile barley population, 77-B-622852, of Dr. R. T. Ramage's at the agronomy farm near Bozeman, Montana. The inoculum was prepared by incubating naturally infected dried leaves of Lami barley (Knudsen, personal communication) on moist filter paper overnight in a growth chamber at 20°C with continuous fluorescent light. Sporulating leaves were rubbed vigorously over the male sterile barley head, and the head was subsequently pollinated within three minutes of inoculation. No attempt was made to increase the natural humidity of the field. Heads were harvested in the fall and kept as individuals throughout the experiment.

Simultaneous Comparison of Inoculation Technique

The floral inoculation, barley kernel inoculum, and layered mycelium were prepared as described, using isolations from naturally infected Lami plants. Bulk seed from the male-sterile population, 77-B-622852, was used for barley kernel and layered mycelium inoculations.

All treatments were planted simultaneously in the greenhouse as previously described.

Vacuum Treatment

Inoculation of barley kernels was attempted with a spore suspension and vacuum treatment. Spores were produced by Teviotdale and Hall's (1976b) method using mycelium placed on barley leaf piece agar exposed to near uv lights.

Several *P. graminea* isolates from the mid-East were separately tested on two susceptible barley varieties, Edda II (Nilsson, 1975) and Lami (Kudsen, Royal Vet & Agr. Univ., Copenhagen, Denmark, personal communication). The inoculum contained approximately 200,000 mycelial fragments and a small quantity of spores per milliliter. Using the technique of Tapke and Bever (1946) for covered smut, as modified by Johnston and Mathre (1977), seeds were added to the spore suspension and placed under a vacuum which was broken every 15 minutes for 1-1/2 to 2 hours. Seed was then dried, planted in the greenhouse, and examined three weeks later for systemic infection.

RESULTS AND DISCUSSION

Many factors affected the success of barley seed inoculation with *P. graminea*. A highly susceptible variety, correct environmental conditions, a virulent strain of the pathogen and art were necessary.

The older literature (Arny, 1945; Shands and Arny, 1944) reported Sanalta and Oderbrucker as being susceptible varieties. They were used in preliminary tests comparing barley kernel inoculation and

layered mycelium. No differences were found between the two inoculation procedures. Infection percentages ranged from 40-60%.

The comparisons of inoculation procedures were not replicated. Seed source, inoculum source, and environment were held constant while the inoculation procedure varied. The heads which were inoculated at flowering time were classified as individuals because it was not known how the genotype would react. However, all heads were susceptible. Bulk seed from the same population was used for barley kernel and layered mycelium inoculations. Layered mycelium produced the highest infection rate (Table 3-1), 64%, while barley kernel inoculum produced 49% infection and floral inoculation 36%. The heterogeneity chi-square indicated significant differences among all treatments.

Efforts to product systemic infection using the vacuum treatment were unsuccessful with no infection being observed three weeks after planting.

Table 3-1. Comparison of three inoculation techniques for *P. graminea*

Inoculation Method	# Plants Observed	# Plants Infected	% Infection[1]
Layered mycelium	152	98	64.5
Barley kernel	465	227	48.8
Floral inoculation	1012	360	35.6

[1]Heterogeneity X^2 for three treatments = 57.52**, for layered vs barley = 11.26**, and for barley is floral = 23.33**.

Chapter 4

BARLEY CULTIVAR SCREENING

Barley cultivars grown commercially in Montana were screened for resistance to *P. graminea* to determine if we have the genetic resources for resistance should barley stripe become a problem in Montana.

MATERIALS AND METHODS

Twenty-one barley cultivars grown commercially in Montana plus two susceptible checks (Edda II - highly susceptible; Lami - intermediate) were tested against three isolates of *P. graminea* obtained from barley in Morocco (ELS), Moccasin, Montana (ROV), and Huntley, Montana (RPB). The isolates obtained from Montana were from the barley cultivars Rovaniemi 70-B and RPB-43971, in which the seed was produced in Finland and originated in England, respectively.

Two replications over time of the cultivar testing experiment were performed using the barley kernel inoculation procedure as previously described. Standard greenhouse procedures described earlier were followed. During replication 1, a temperature range of 12-16°C was maintained, while in replication 2, greenhouse ambient temperatures were somewhat higher, with a temperature range of 16-21°C. The inoculation period on replication 2 was 36 hours shorter than that of replication 1.

Data were analyzed by analysis of variance (Table 4-1), least significant difference, standard error of a mean, and coefficient of variation.

RESULTS AND DISCUSSION

The cultivar by isolate interaction was significant (p=.05). This indicates that cultivars react differently to isolates; therefore, a large number of isolates should probably be used in a screening program. The lower infection rate of the second replication is attributed to the shorter inoculation period and higher greenhouse temperatures.

There is a high degree of variability in the intermediate class, although the highly susceptible as well as the highly resistant varieties gave consistent results. Steptoe, Erbet, Shabet, and Betzes consistently produced a low level of infection when inoculated with *P. graminea*. An infected plant was never found in Betzes; therefore, it appeared to exhibit an immune reaction. Two isogenic lines of Betzes, Shabet and Erbet, also carried an extremely high level of resistance to *P. graminea*.

Edda II, the susceptible check, was consistently highly infected. Horsford, Larker, and Ingrid were the only Montana cultivars which were consistently heavily infected, while several other Montana

cultivars had a wide range of susceptibility - Vanguard (5.0-95), Piroline (0.5-97), Klages (0-60).

Table 4-1. Analysis of variance for average percent infection by *P. graminea* in commercially grown Montana barley cultivars[1]

Source	df	ms	F
Total	137	--	--
Cultivar	22	3303.70	17.29**
Isolate	2	6527.09	34.16**
Cultivar x Isolate	44	610.761	3.20**
Replication	1	5016.12	26.25**
Error	68	191.09	

[1]Refer to Table 4-2, column 1.

Table 4-2. Response of commercially grown Montana barley cultivars to three isolates of *P. graminea*

Cultivar	Average % Infection[1]	Individual Isolates % infection		
		ROV[2]	RPB[3]	ELS[4]
Edda II (susceptible check)	83.00	75.00	88.50	85.50
Horsford	75.33	54.50	75.50	96.00
Vanguard	59.00	5.00[5]	95.00	77.00
Larker	51.50	39.00	51.00	64.50
Ingrid	51.17	44.50	57.00	52.00
Piroline	50.83	0.50	97.00	55.00
Karl	42.83	16.00	80.00	32.50
Klages	41.00	0.00	63.00	60.00
Lami (intermediate check)	31.67	26.50	35.00	33.50
Palliser	26.67	23.50	25.50	31.00
Hector	25.17	10.50	42.00	23.00
Firlbecks III	25.00	21.00	33.00	21.00
Freja	23.67	23.50[5]	4.00	43.50
Unitan	23.17	8.50	22.50	38.50
Lud	21.00	7.50	11.00	44.50
Hypana	16.67	1.50	6.00	42.50
Compana	11.00	11.00	1.50	20.50
Dekap	11.00	0.00	25.00	8.00
Georgie	9.16	0.00	3.00	24.50
Steptoe	4.83	2.00	3.50	9.00
Erbet	1.16	0.50	0.00	3.00
Shabet	0.83	0.00	0.50	2.00
Betzes	0.00	0.00	0.00	0.00

[1] LSD .05 = 15.96, s.e. $\bar{X}$ = 5.64, c.v. = 46.36

[2] LSD .05 = 22.44, s.e. $\bar{X}$ = 7.65, c.v. = 67.16

[3] LSD .05 = 28.12, s.e. $\bar{X}$ = 9.59, c.v. = 38.05

[4] LSD .05 = 34.49, s.e. $\bar{X}$ = 11.76, c.v. = 44.13

[5] Missing value formula used to calculate rep 1 (Vanguard rep 2 = 0; Freja rep 2 = 18)

Chapter 5

FUNGICIDES FOR THE CONTROL OF *P. graminea*

Ceresan, the organic mercury seed treatment fungicide formerly used to control barley stripe, was banned from the market in the mid 1970's by the Environmental Protection Agency. Therefore, an alternative seed treatment needed to be identified. Both registered and experimental fungicides were evaluated.

MATERIALS AND METHODS

In Vitro Tests

Media containing fungicides was prepared by adding 10, 100, or 1000 µg/ml active ingredient to V-8 juice agar (Appendix Table 2) after autoclaving. A separate test also included 20, 200, 2000 µg/ml. Plugs (4 mm diameter) of *P. graminea* mycelium were placed in contact with the agar plates and incubated at room temperature (20°C). Diameter measurements of resultant fungal growth were taken 72 hours later. Subjective decisions were used in determining whether the mycelium appeared alive or dead. A zero reading indicated no growth and apparent death. To determine if plugs which exhibited no growth were dead, they were transferred to V-8 juice agar plates without fungicide and incubated for 48 hrs.

Eight experimental fungicides were obtained from chemical companies. Chemagro provided BAY KWG 0519

(β-(4-chlorophenoxy)-α-(1,1-dimethylethyl)-1H-1,2,4 triazde-1-ethanol) and liquid and powder forms of BAY MEB 6447 (1-(4-chlorophenoxy)-3,3-dimethyl-1-(1H-1,2,4 triazol-1-yl)-2-butanone). R-28921 and R-37343 were obtained from Stauffer, Rohm and Haas formulates RH2161, and Polyoxin is produced in Japan by Nihon Nohyaku Company, Limited. UniRoyal formulates H-719(3-carboxanidido-4,4,5-(trimethylfuran).

Nine registered fungicides were evaluated in vitro for control of *P. graminea*: Arasan (thiram)(tetramethylthiuram disulfide); Benlate (methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate); Captan (cis-N-((trichloromethyl 1)thio)-4-cyclohexene-1,2-dicarboximide); Ceresan (ethyl mercury p-toluene sulfananilide); Daconil 2787 (tetrachloro-isophthalonitrile); DB Green (maneb + lindane); Demosan (1,4-dichloro-2,5-dimethoxybenzene); Vitavax (carboxin)(5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxamide); and Vitavax 200 (carboxin + thiram).

Effect of Seed Treatment on Percent *P. graminea* in RPB-43971 Barley

A preliminary investigation of seed treatments was studied in the greenhouse. The seed lot RPB-43971 grown at Kalispell in 1977 was treated with five different fungicides at the rate of 120 ml chemical formulation per 45.5 kg barley (4 oz/100 lbs). This seed lot was used because it was known to have a 12% natural infection level. Standard greenhouse procedures were followed. Five weeks after planting, the percent systemically infected plants was determined.

RESULTS AND DISCUSSION

In vitro tests with registered and experimental fungicides resulted in identifying several effective fungistats and only one effective fungicide. Ceresan was the only fungicide which completely killed the mycelium of *P. graminea*. Vitavax 200, Polyoxin, and RH 2161 sometimes killed the mycelium, as determined by no growth 48 hours after transferring mycelial plugs from agar plates containing fungicides to V-8 juice plates to which no fungicide had been added. All other fungicides which apparently killed the fungus only stopped it temporarily, thus acting as fungistats.

At 1000 µg/ml active ingredient Vitavax, Demosan, and Vitavax 200 effectively reduced or stopped growth (Tables 5-1 and 5-2). At 10 µg/ml a. i. BAY KWG 0519, BAY MEB 6447, Polyoxin, and RH 2161 were effective in their control of *P. graminea* (Table 5-3). No registered or experimental fungicide was as effective as Ceresan, the seed treatment formerly used for the control of *P. graminea*.

The preliminary seed treatment study (Table 5-4) indicated that Vitavax and PMA at 120 ml/45.5 kg (4 oz/100 lb) may be effective in controlling naturally occurring barley stripe infection. A heterogeneity chi-square showed that PMA and Vitavax have a homogeneous effect. Vitavax 200 and H-719 did not lower the percent infection in

the same population of infected plants. The check, Vitavax 200 and H-719 exhibit homogeneity. Plantvax does not group with any of the other treatments.

Table 5-1. Effectiveness of registered fungicides in controlling growth of *P. graminea*, in vitro

Fungicide	µg/ml active ingredient	Colony Diameter (mm)			
		Rep 1	Rep 2	Rep 3	$\bar{X}$
Arasan	10	30.2	28.2	30.5	29.7
	100	14.5	10.7	10.0	11.7
	1000	9.2	9.0	7.2	8.5
Benlate	10	27.2	26.5	26.7	26.8
	100	11.2	11.0	10.7	11.0
	1000	8.0	7.0	7.7	7.6
Captan	10	28.5	29.7	27.0	28.4
	100	26.0	26.5	25.2	25.9
	1000	8.0	8.7	9.2	8.7
Ceresan	10	0.0	0.0	0.0	0.0
	100	0.0	0.0	0.0	0.0
	1000	0.0	0.0	0.0	0.0
Daconil 2787	10	23.5	20.0	21.2	21.6
	100	14.7	14.5	13.7	14.3
	1000	13.7	13.5	12.2	13.2
DB Green	10	31.2	29.5	27.5	29.4
	100	14.7	14.7	14.7	14.7
	1000	6.0	6.0	5.7	5.9
Demosan	10	11.0	10.2	11.5	10.9
	100	7.0	7.2	8.0	7.4
	1000	0.0	0.0	0.0	0.0
Vitavax	10	25.2	25.2	24.7	25.1
	100	5.5	5.7	5.0	5.4
	1000	0.0	0.0	0.0	0.0
Check	0	30.0	30.7	30.0	30.2
	0	32.0	32.0	30.5	31.5
	0	30.2	29.7	31.5	30.7

LSD (.01) = 1.22
Standard error $\bar{X}$ = 0.519