



The effect of *Cephalosporium gramineum* on yield components of various winter wheat genotypes  
by Robert Howard Johnston

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of  
MASTER OF SCIENCE in Botany

Montana State University

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**Abstract:**

Conidiospore concentrations of five million spores of *Cephalo-sporium gramineum* per ml applied at the rate of 100 mls of spore suspension per foot of row of winter wheat (*Triticum aestivum*, L.) resulted in high uniform infection percentages. Inoculation of vernalized plants in the three-four leaf stage, by root wounding and subsequent inoculation, placed the plant under heavy disease stress in both field and greenhouse tests. Varying levels of resistance and/ or tolerance to this pathogen were observed. Under greenhouse conditions, a line row component of "Crest", a Montana developed cultivar looked particularly favorable as a possible source of tolerance. It was reduced 36.8% in yield compared to 67% reduction in a susceptible cultivar, Lancer. , Data indicated this source of tolerance may come from P.I. 178383. Infected plants were stunted, had reduced yield, and had higher levels of protein than healthy plants. The yield components most affected by this disease were number and weight of seeds (kernels) formed per head. Loss of kernel weight due to shriveling reduced the amount of carbohydrate present and resulted in a relative increase in percent protein. The number of heads produced by infected plants was never significantly different from the number of heads produced by healthy plants. Yield losses were as high as 78 percent. This gives an indication of the potential destructiveness of this pathogen under severe disease conditions. Use of the tolerance of P.I. 178383 may be of value in developing *C. gramineum* tolerant winter wheat cultivars.

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COMPONENTS OF VARIOUS WINTER WHEAT GENOTYPES

by

ROBERT HOWARD JOHNSTON

A thesis submitted to the Graduate Faculty in partial  
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of

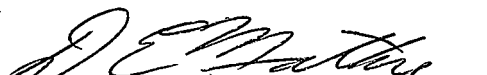
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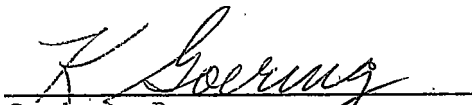
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## TABLE OF CONTENTS

	Page
VITA . . . . .	ii
ACKNOWLEDGMENT . . . . .	iii
TABLE OF CONTENTS . . . . .	iv
LIST OF TABLES . . . . .	vi
LIST OF FIGURES . . . . .	ix
ABSTRACT . . . . .	x
INTRODUCTION AND LITERATURE REVIEW . . . . .	1
GENERAL METHODS . . . . .	5
Isolation and culture of the pathogen . . . . .	5
Vernalization of winter wheat . . . . .	5
Method of wounding. . . . .	7
Method of inoculation . . . . .	7
Symptom expression. . . . .	8
Harvest . . . . .	9
Analysis of protein . . . . .	9
PROCEDURES AND RESULTS . . . . .	10
Infection vs. Inoculum Source . . . . .	10
Effect of Inoculum Density. . . . .	13
Time of Inoculation . . . . .	21
Varietal Response to <u>C. gramineum</u> . . . . .	24
Minor Gene Lines . . . . .	25

	Page
Field Test - 1969-70. . . . .	29
Field Test - 1970-71. . . . .	45
DISCUSSION . . . . .	51
SUMMARY. . . . .	55
LITERATURE CITED . . . . .	57

## LIST OF TABLES

			Page
Table	I	Composition of modified Eckerts growth media. . . . .	6
Table	II	Infection of Lancer winter wheat by <u>Cephalosporium gramineum</u> as related to source of inoculum . . . . .	12
Table	III	Effect of conidial inoculum density of <u>Cephalosporium gramineum</u> on infection of Lancer winter wheat. . . . .	14
Table	IV	Effect of inoculum density of <u>Cephalosporium gramineum</u> on yield and thousand kernel weight in Lancer winter wheat . . . . .	17
Table	V	Effect of inoculum density of <u>Cephalosporium gramineum</u> on number of seeds and percent shriveled kernels in Lancer winter wheat . . . . .	18
Table	VI	Effect of inoculum density of <u>Cephalosporium gramineum</u> on plant height, number of heads per row and percent protein in Lancer winter wheat. . . . .	19
Table	VII	Variation in infection by <u>Cephalosporium gramineum</u> of four winter wheat cultivars. . . . .	26
Table	VIII	Variation in infection by <u>Cephalosporium gramineum</u> of nine winter wheat genotypes. . . . .	28
Table	IX	Effect of <u>Cephalosporium gramineum</u> on yield of nine winter wheat genotypes. . . . .	30
Table	X	Effect of <u>Cephalosporium gramineum</u> on yield per head of nine winter wheat genotypes . . . . .	31

Table	XI	Effect of <u>Cephalosporium gramineum</u> on percent yield reduction and the number of heads per row of nine winter wheat genotypes . . . . .	32
Table	XII	Effect of <u>Cephalosporium gramineum</u> on the number of seeds formed per row of nine winter wheat genotypes. . . . .	33
Table	XIII	Effect of <u>Cephalosporium gramineum</u> on the percent shriveled kernels per row of nine winter wheat genotypes. . . . .	34
Table	XIV	Effect of <u>Cephalosporium gramineum</u> on thousand kernel weight of nine winter wheat genotypes . . . . .	35
Table	XV	Effect of <u>Cephalosporium gramineum</u> on the average number of seeds formed per head of nine winter wheat genotypes. . . . .	36
Table	XVI	Effect of <u>Cephalosporium gramineum</u> on plant height of nine winter wheat genotypes . . .	37
Table	XVII	Effect of <u>Cephalosporium gramineum</u> on percent protein of nine winter wheat genotypes. .	38
Table	XVIII	The effect of <u>Cephalosporium gramineum</u> on the number of infected plants per row and reduction in yield in six winter wheat cultivars . . . . .	41
Table	XIX	The effect of time of field inoculation of <u>Cephalosporium gramineum</u> on yield, test weight, percent protein and thousand kernel weight of six winter wheat cultivars. . . . .	42
Table	XX	The effect of time of inoculation of <u>Cephalosporium gramineum</u> on yield of six winter wheat cultivars. . . . .	43
Table	XXI	The effect of time of inoculation of <u>Cephalosporium gramineum</u> on test weight in six winter wheat cultivars . . . . .	44



	Page
Table XXII	The effect of <u>Cephalosporium gramineum</u> on the percent white heads per row and percent reduction in yield for five winter wheat cultivars. . . . . 47
Table XXIII	The effect of wounding and inoculation of <u>Cephalosporium gramineum</u> on yield of five winter wheat cultivars. . . . . 48
Table XXIV	The effect of wounding and inoculation of <u>Cephalosporium gramineum</u> in percent protein in five winter wheat cultivars. . . . . 50

## LIST OF FIGURES

	Page
Figure 1. Effect of inoculum density (spores/ml) of <u>Cephalosporium gramineum</u> on infection of Lancer winter wheat. . . . .	16
Figure 2. Effect of time of inoculation of <u>Cephalosporium gramineum</u> on yield of eight winter wheat cultivars . . . . .	23

## ABSTRACT

Conidiospore concentrations of five million spores of Cephalosporium gramineum per ml applied at the rate of 100 mls of spore suspension per foot of row of winter wheat (Triticum aestivum, L.) resulted in high uniform infection percentages. Inoculation of vernalized plants in the three-four leaf stage, by root wounding and subsequent inoculation, placed the plant under heavy disease stress in both field and greenhouse tests. Varying levels of resistance and/or tolerance to this pathogen were observed. Under greenhouse conditions, a line row component of "Crest", a Montana developed cultivar looked particularly favorable as a possible source of tolerance. It was reduced 36.8% in yield compared to 67% reduction in a susceptible cultivar, Lancer. Data indicated this source of tolerance may come from P.I. 178383. Infected plants were stunted, had reduced yield, and had higher levels of protein than healthy plants. The yield components most affected by this disease were number and weight of seeds (kernels) formed per head. Loss of kernel weight due to shriveling reduced the amount of carbohydrate present and resulted in a relative increase in percent protein. The number of heads produced by infected plants was never significantly different from the number of heads produced by healthy plants. Yield losses were as high as 78 percent. This gives an indication of the potential destructiveness of this pathogen under severe disease conditions. Use of the tolerance of P.I. 178383 may be of value in developing C. gramineum tolerant winter wheat cultivars.

## INTRODUCTION AND LITERATURE REVIEW

The stripe disease of winter wheat caused by the fungus Cephalosporium gramineum Nisikado and Ikata was first described in Japan in 1934 by Nisikado et al. (9). Since that time the disease has been described in Washington (1), New York (18), Montana (13), Illinois (6) and Michigan (15).

Cephalosporium stripe is characterized by brownish xylary stripes on the culm, leaf sheath and leaves of infected plants. As infection occurs, the stripe moves acropetally, starting near the crown. This striping is later accompanied by chlorosis and necrosis of the leaf tissue. Chlorosis usually parallels the brownish stripe and results in prominent symptoms. Several weeks before normal ripening, the spikes of highly susceptible cultivars turn white. As senescence approaches, the lower internodal regions of the culm darken, turning almost black in extremely susceptible cultivars. Microscopic examination of infected tissues reveals the presence of mycelia and numerous conidia within the xylem vessels (3, 7, 9). Dye movement studies indicate a reduction in water and nutrient movement through the plant (11, 16). This vascular plugging has been attributed to direct plugging by the fungus (3), to indirect plugging by pectin plugs (16), and in some cases to amorphous metabolic byproducts (12, 16). Leaf tissue necrosis is probably directly related to vascular plugging, although the fungus is known to produce a seedling inhibiting toxin

which may play an undetermined role in tissue necrosis (7). Bruehl suggested that upward movement of harmful metabolites may be responsible for the initial brownish discoloration of the vascular vessels (3).

The host range of the pathogen includes many genera within the family Gramineae. Bruehl indicated 29 species within 16 genera were susceptible when artificially inoculated (4). Under natural conditions winter wheat is the preferred host (3) although reports from Illinois indicate that the pathogen has been found naturally on barley, oats, and rye (6).

Most workers agree that wounding is a prerequisite for infection (3, 5, 10, 11, 16). Disease-free seed planted in infested soil will produce disease free plants. This observation was noted under both greenhouse (3, 16, unpub.) and field conditions (unpub.). Infection of winter wheat has been shown to be a result of root breakage by soil heaving during the spring (10, 11, 16).

It has been shown that the fungus overwinters in straw residue (5, 8, 9) which can serve as a source of inoculum for one to three or more years (8, 11, 16). Infected seed serves only to introduce the fungus into new areas and appears to play an unimportant role in inoculum build up (9). Control measures have been primarily concerned with crop rotation and destruction of refuse (9), both of which serve

to decrease straw residue and hence inoculum. Pool's work with autumn root growth showed that infection could be greatly reduced if the fall planting was delayed until the soil temperature at a depth of 4 inches was below 55°F (11). Apparently planting in a cool soil minimized autumn root growth and resulted in few roots being broken during soil heaving in the spring. Late planting is not always practical under Montana conditions due to extremely variable environmental factors. Since the abovementioned control measures are not always feasible, the use of resistant or tolerant cultivars, if any exist, would be the preferred control measure.

From an economical standpoint, *Cephalosporium* stripe results in reduced yield (2, 3, 11). Unpublished data collected under greenhouse conditions indicate yield reductions in the range of 40-60%, although under natural conditions this range should be lower. Plant Pathologists in Montana indicated an annual loss of 2-3% during the 1967 through 1969 growing periods. Yield is a collective term which is affected by both genotype and environment. Each of the yield components, the number of seeds per head, number of tillers per plant, and weight per seed, contributes to the final outcome . . . yield. In a search for resistant or tolerant cultivars, the researcher must, by necessity, weigh each factor as it is affected by the disease and select those which in the end result in the least yield reduction.

The purpose of this thesis was twofold. Primary efforts were aimed towards developing methods of working with the pathogen that would result in consistent reproducible disease. When this goal was achieved, a program of cultivar testing was initiated to determine which yield components are adversely affected under disease conditions. The longrange goal of this work is to find genes for resistance or tolerance which can be utilized in a winter wheat variety development program.

## GENERAL METHODS

Isolation and culture of the pathogen.- Conidiospore suspensions of C. gramineum, when used for inoculum, were found to give high infection percentages and good uniformity of disease development within experiments. The culture used throughout the course of this work was isolated from a diseased winter wheat plant found near Manhattan, Montana in June 1969. Isolation of the fungus involved plating surface sterilized<sup>1/</sup> diseased tissue on acidified corn meal agar plates. After approximately two weeks growth the fungus was transferred onto corn meal agar slants where it was maintained by annual transfer onto fresh slants. For experimental purposes, the fungus was grown in modified Eckert's media (17) (Table I) at room temperature. Cultures were agitated on a Burrell wrist action shaker. The degree of agitation was low and resulted in very slight splashing within the flask. This method resulted in conidial concentrations of over 100 million per ml within one to two weeks.

Vernalization of winter wheat.- In greenhouse studies vernalization was accomplished by first germinating Ceresan-treated seeds at room temperature on moist paper toweling in plastic shoe boxes for approximately two days. At this time the radicle was just emerging

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<sup>1/</sup> Surface sterilization involved washing tissue pieces in a 10% sodium hypochlorite solution for approximately two minutes.



Table I. Composition of modified Eckert's growth media (17).

Chemical	gram per liter H <sub>2</sub> O
Glucose	18.00
Yeast extract, Difco	3.00
Peptone, Difco	5.00
K <sub>2</sub> H PO <sub>4</sub>	1.36
K H <sub>2</sub> PO <sub>4</sub>	1.68
Mg SO <sub>4</sub>	0.50

and the shoe boxes were placed in a dark 4°C cold room. With the monthly addition of water, the seedlings were maintained in this manner for approximately sixty days. At the end of this period, the one-two leaf seedlings were transplanted into greenhouse soils.

Method of wounding.- Artificial inoculation in both field and greenhouse studies required wounding the root system of the plant. This was accomplished by cutting the root system with a knife (greenhouse studies) or with a sharpened straightened hoe (field studies). This cut was made at approximately a 45 degree angle starting 2-3 inches from the base of the plant. The angle was toward the plant, the slice approximately 4-6 inches deep. This cut was sufficiently deep to feel the roots being cut and torn.

Method of inoculation.- Generally inoculation was accomplished by pouring a fungal spore suspension into the soil slice at the rate of 100 mls of suspension per foot of row. Experimental procedure varied, in that some experiments involved simply pouring the inoculum into the soil slice. In other cases, the inoculum container was elevated above the soil slice several feet and the inoculum was allowed to pass through a length of 3/8 inch diameter rubber tubing. This method resulted in the inoculum entering the soil slice forcibly and possibly with deeper penetration than in the previously described

method. Both methods resulted in high infection percentages.

Spore concentrations varied between experiments. For this reason, the concentration used in each experiment will be included with the data pertinent for that experiment. Spore concentrations in the range of 100 million to 200 million were easily obtained in modified Eckert's medium within a two week growth period. These concentrations were diluted with tap water to obtain the spore concentrations reported for each experiment. Spore concentrations were determined using a haemocytometer (Levy-Hauser A-2906).

Symptom expression.- After inoculation in the greenhouse, disease symptoms appeared in approximately 14 days. Under field conditions, symptom expression was slower and required 3-4 weeks to develop.

Infection percentages and rate of symptom advance were determined only by observation. Since this particular disease is not easily confused with other wheat diseases, a bioassay of diseased plants to substantiate observational data was considered unnecessary. This conclusion was obtained from a preliminary survey in which the fungus was isolated from all plants recorded as diseased.

Pool (11) indicated the winter wheat cultivar Lancer was extremely susceptible to *Cephalosporium* stripe. This cultivar was used in all experiments as a susceptible check.

Harvest.- Harvest of greenhouse material was conducted on an individual plant basis. This material was thrashed in a single head thrasher in which air movement was kept to a minimum to prevent the loss of light shriveled kernels. The chaff was separated from the seed by winnowing. Field material was thrashed on an individual row basis using a Vogel plot thrasher. Air flow was reduced to prevent the loss of shriveled kernels. This seed was cleaned by repeated passes through a seed cleaner, also with reduced air flow.

Analysis of protein.- Analyses for percent protein were conducted by the Montana State University Cereal Quality Laboratory using the method of Udy (19, 20).

## PROCEDURES AND RESULTS

### Infection vs. Inoculum Source

Methods.- A primary goal of this work was to develop a method of inoculation that would result in consistent reproducible results. In earlier work, it had been observed that when *Cephalosporium* infected straw was plated on agar, the fungus grew primarily out of the ends of the straw pieces. Microscopic examination revealed that sporulation was also concentrated at the ends of straw pieces. It was assumed that the same phenomenon may exist in the soil and that the degree of fungal growth, sporulation and percent of infection may be correlated. To test this hypothesis and to determine whether inoculation using infected straw pieces or a conidial suspension would give the best results, the following experiment was devised.

Three infected straw lots of 150 grams each were cut into size classes of 1/8 inch, 1/2 inch and one inch. Each size class was subdivided into six lots of 25 grams each and placed into separate 17.8 cm diameter pots. A sufficient amount of soil was added to fill each pot and then each was thoroughly mixed. Twelve days later, 150 mls of a conidiospore suspension (35 million/ml) was added to each of another six soil filled pots. Six pots of uninfested soil were used as a control. Two days after the addition of the spore suspension, each pot was planted with six plants of Lancer winter wheat vernalized for 5.5 months. Twelve days later, the root system in three pots from

each group were wounded. The remaining three pots from each group served as a nonwounded control.

Results.- This experiment was terminated 67 days after planting, each plant being observed for infection. The results of this experiment are summarized in Table II. It is evident that infection occurred in both wounded and nonwounded plants. Since infection was expected only with wounding, it is postulated that wounding of nonwounded plants may have occurred during transplanting of seedlings from the vernalization boxes into soil. The highest degree of infection with the most uniformity resulted when the soil was infested with a conidiospore suspension. The data given in Table II suggests that a positive relationship exists between the number of exposed straw ends and the percent infection. The volume of straw in each size class was constant (150 grams), but the number of exposed ends followed a logarithmic progression. For example, 1 inch = 2 X ends, 1/2 inch = 4 X ends and 1/8 inch = 16 X ends. In this case, X would be a constant related to the volume of straw.

The variation and low infection percentages obtained with infected straw pieces made their use undesirable for further greenhouse tests. Therefore, conidiospore suspensions were used for further experiments.

Table II. Infection of Lancer winter wheat by Cephalosporium gramineum as related to source of inoculum.

Inoculum source	Infection Percentage			
	wounded roots		nonwounded roots	
	range	mean	range	mean
None	0	0.0%	0	0.0%
1/8" straw pieces	20.0-66.7%	50.0%	0	0.0%
1/2" straw pieces	0.0-66.7%	44.4%	0.0-16.7%	11.1%
1" straw pieces	0.0-66.7%	33.3%	0	0.0%
Conidiospores	100	100.0%	16.7-33.3%	22.2%

## Effect of Inoculum Density

Methods.- To determine the concentrations of spores that would give good infectivity and uniformity within experiments, vernalized Lancer winter wheat was inoculated with varying concentrations of conidia. One greenhouse bench, which had just been filled with fresh Bozeman silt loam that had not been in grain production for over 50 years, was divided into thirds, with each being a replication. Each replication was a completely randomized block with one row each of the following treatments: 1) nonwounded check, 2) wounded check, 3) 500 spores/ml, 4) 5,000 spores/ml, 5) 50,000 spores/ml, 6) 500,000 spores/ml, and 7) 5,000,000 spores/ml. There were ten plants in each treatment with the rows spaced 20 cm apart. When the plants were 36 days old they were wounded and inoculated. To be sure that each plant received the same volume of inoculum, an automatic pipetting device was used to deliver 20 mls of suspension into the soil slice directly beneath each plant. At the time of inoculation, the plants were in the three leaf stage.

Results.- The plants were read for percent infection in the soft dough stage. In Table III percent infection is reported as either the number of infected plants per number of total plants per row or by the number of infected tillers per number of total tillers



Table III. Effect of conidial inoculum density of Cephalosporium gramineum on infection of Lancer winter wheat.

Treatment	Infection	
	Percent infected plants per row <sup>a/</sup>	Percent infected tillers per row <sup>a/</sup>
Nonwounded check	0.0 d	0.0 d
Wounded check	3.3 d	2.6 d
500 conidia/ml <sup>b/</sup>	10.4 cd	11.0 c
5,000 conidia/ml	31.9 bc	33.8 b
50,000 conidia/ml	48.2 b	49.5 b
500,000 conidia/ml	75.6 a	74.7 a
5,000,000 conidia/ml	78.1 a	79.8 a

<sup>a/</sup> Means followed by the same letter are not significantly different at the 5% level of probability - Duncan's multiple range test.

<sup>b/</sup> Each plant received 20 ml of inoculum.

per row. Both columns of this table show similar results. This would be expected if each plant produced the same number of tillers and if every tiller per infected plant was infected.

The means from the last column of Table III are plotted in Figure 1. Means from both columns would have given similar results. This figure shows that percent infection is linear with the log of the inoculum density until the concentration of 500,000 spores/ml is reached. At this point, the graph begins to plateau indicating higher concentrations of spores will not appreciably increase percent infection under the conditions which influenced this greenhouse experiment.

Various plant characteristics were affected by infection (Tables IV, V, and VI). In Table IV it is evident that wounding has a slight effect on the plant (8.7% yield reduction due to wounding), but this is very minimal with respect to the yield reduction caused by the highest spore concentration (75.8% yield reduction). A spore concentration of at least 50,000 spores per ml was necessary to have a significant effect on yield. Apparently, variation within plants was large enough to have as much effect on yield as did lower (500 and 5,000 spores/ml) spore concentrations. Since the number of heads per treatment did not vary significantly (Table VI), the reduction in yield (Table IV) is due to factors affecting both the number of seed

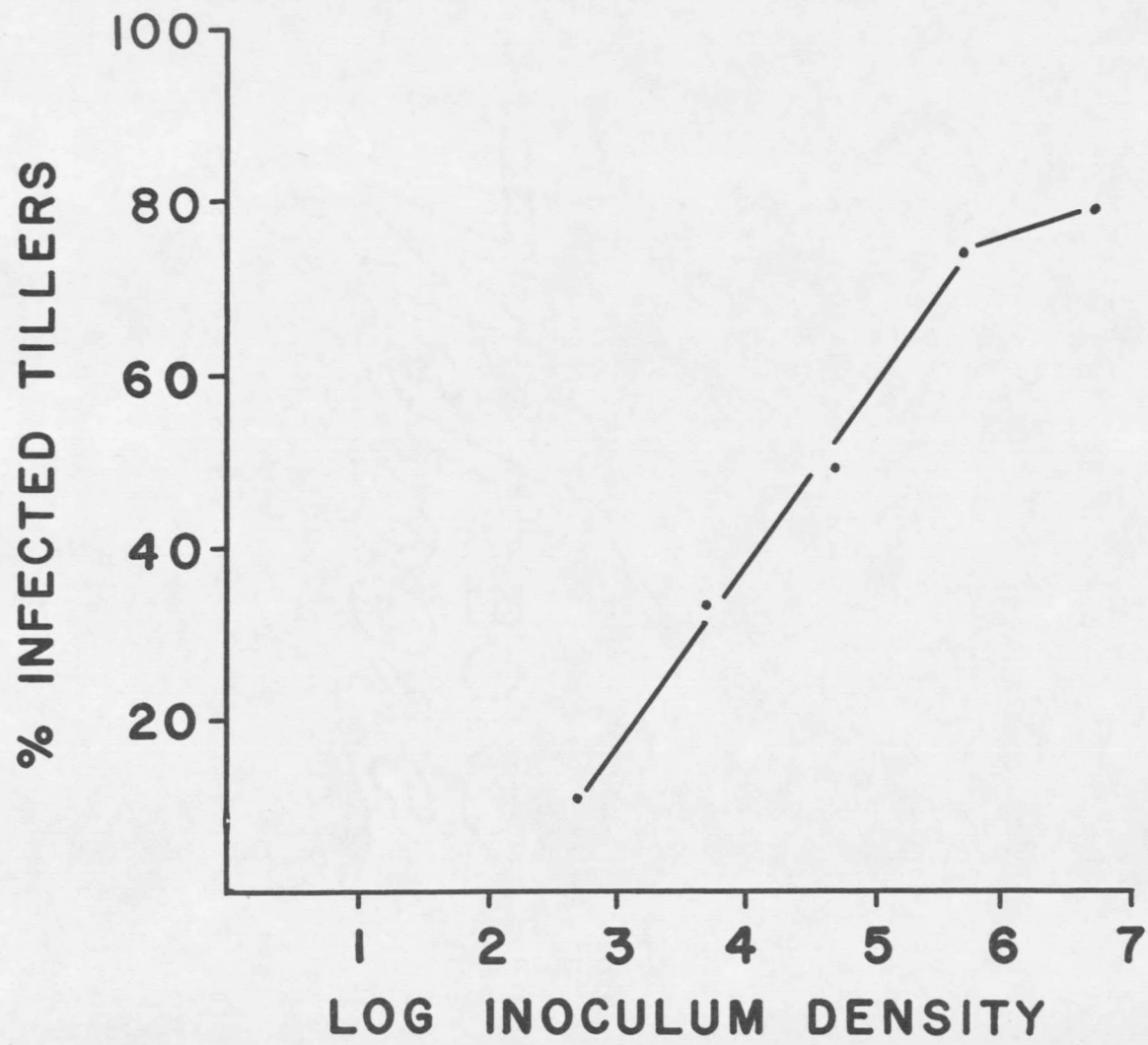


Figure 1

Effect of inoculum density (spores/ml) of Cephalosporium gramineum on infection of Lancer winter wheat.

Table IV. Effect of inoculum density of *Cephalosporium gramineum* on yield and thousand kernel weight in Lancer winter wheat.

Treatment	Plant Characteristics			
	Yield per row (grams) <sup>a/</sup>	Ave. yield per head (grams) <sup>a/</sup>	% reduction in yield respective nonwounded check <sup>a/</sup> to	1,000 kernel weight (grams) <sup>a/</sup>
Nonwounded check	29.06 a	0.68 a	-	35.2 a
Wounded check	26.57 b	0.65 a	8.7 c	34.2 a
500 spores/ml <sup>b/</sup>	24.08 c	0.58 ab	17.2 c	33.1 ab
5,000 spores/ml	24.93 bc	0.62 a	12.9 c	31.7 ab
50,000 spores/ml	17.55 d	0.48 b	40.3 b	27.2 bc
500,000 spores/ml	9.76 e	0.28 c	66.8 a	21.6 cd
5,000,000 spores/ml	6.76 e	0.20 c	75.8 a	18.5 d

<sup>a/</sup> Means followed by the same letter are not significantly different at the 5% level of probability - Duncan's multiple range test.

<sup>b/</sup> Each plant received 20 ml of inoculum.

Table V. Effect of inoculum density of Cephalosporium gramineum on number of seed and percent shriveled kernels in Lancer winter wheat.

Treatment	Plant Characteristics			
	Number of seeds per row <sup>a/</sup>	Number of shriveled seeds per row <sup>a/</sup>	% shriveled seeds per row <sup>a/</sup>	Ave. number of seed per head <sup>a/</sup>
Nonwounded check	823.3 a	39.7 b	4.9 c	19.3 a
Wounded check	779.0 a	68.0 b	8.5 c	19.0 a
500 spores/ml <sup>b/</sup>	729.3 a	74.3 b	9.9 c	17.2 a
5,000 spores/ml	786.3 a	190.0 ab	24.7 bc	19.8 a
50,000 spores/ml	643.0 ab	246.7 a	39.1 ab	17.5 a
500,000 spores/ml	467.0 bc	289.0 a	56.8 a	13.3 b
5,000,000 spores/ml	325.0 c	174.0 ab	61.8 a	9.9 b

<sup>a/</sup> Means followed by the same letter are not significantly different at the 5% level of probability - Duncan's multiple range test.

<sup>b/</sup> Each plant received 20 ml of inoculum.

Table VI. Effect of inoculum density of Cephalosporium gramineum on plant height, number of heads per row and percent protein in Lancer winter wheat.

Treatment	Plant Characteristics		
	Plant height <sup>a/</sup> in cm	Average number <sup>a/</sup> of heads per row	% protein <sup>a/</sup>
Nonwounded check	78.2 a	42.7 a	16.7 d
Wounded check	71.1 ab	41.0 a	17.0 cd
500 spores/ml <sup>b/</sup>	70.1 abc	42.3 a	17.1 cd
5,000 spores/ml	75.4 ab	40.0 a	17.6 bc
50,000 spores/ml	68.6 bc	36.3 a	17.7 bc
500,000 spores/ml	59.7 c	34.3 a	18.3 ab
5,000,000 spores/ml	59.7 c	32.3 a	19.1 a

<sup>a/</sup> Means followed by the same letter are not significantly different at the 5% level of probability - Duncan's multiple range test.

<sup>b/</sup> Each plant received 20 ml of inoculum.

per head and the weight of seed from each head (Table V and Table IV, respectively). As the data in Tables IV and V indicate, both the number of seeds per head and the thousand kernel weight are reduced significantly with increased spore concentrations. Spore concentrations are positively related with the percent shriveled kernels. Shriveling would directly effect the weight of each kernel and hence, thousand kernel weight. Since the number of tillers formed per treatment do not differ significantly (Table VI), the variation in the number of seeds per row (Table V), is directly related to the number of seeds formed per head (Table V).

Plant height is reduced significantly by disease resulting from spore concentrations of 50,000 or greater (Table VI). Plants inoculated with the highest spore concentration were reduced in height 16% from the wounded check. Percent protein (Table VI) is positively related to inoculum density. Apparently this disease reduces the endosperm/germ (carbohydrate/protein) ratio which effectively increases percent protein. This would be expected from the degree of shriveling and confirms data presented by Spalding et al (16).

From these data it was concluded that spore concentrations of 5 million spores/ml would place the plant under severe disease stress and would serve as an effective tool for screening winter wheat cultivars for tolerance and/or resistance. Although this concentration

is quite high and may not be representative of field conditions, cultivars that may prove resistant or tolerant at this concentration should give excellent response under natural field conditions.

#### Time of Inoculation

Methods.- Once the concentration of the inoculum had been chosen, it was necessary to determine at what stage of growth the plant should be inoculated so as to maximize the disease.

An experiment was designed to test eight cultivars of winter wheat inoculated at two different growth stages. This experiment consisted of a nonwounded check, an early inoculation (4-5 leaf stage) and a late inoculation (just heading) using the cultivars Lancer, Chinese 166, Winalta, Crest, Cheyenne, Nugaines, Froid and Warrior. Each cultivar was represented by 20 plants per 62 cm row. Rows were spaced 18 cm apart. Due to lack of space, this trial was nonreplicated.

The early inoculation, using a spore concentration of 5.2 million spores/ml, was performed thirty-five days after the date of planting. Since this experiment was nonreplicated and would yield only a minimum amount of useable data, it was decided that the late inoculation would be by hypodermic injection. Several researchers had reported using this method (4, 11). Using a spore concentration of



50 million spores per ml, each tiller of each plant was inoculated at a point approximately one inch above the soil surface, 49 days after the date of planting. Although the volume of inoculum injected into each tiller was difficult to determine, because of loss around the leaf sheaths, the amount injected was in the range of one-three mls per tiller.

Results.- The degree of infection severity differed among cultivars. On a scale of one-five<sup>1/</sup>, Crest rated 1-2 while all others rated a minimum of 4.

Since the experiment was nonreplicated, only the data on yield will be reported. However, measurements of percent protein, thousand kernel weight, percent shriveled kernels and plant height were consistent with the data given in the experiment on inoculum density. Infection severity seems to be related with the degree of yield reduction. Figure 2 shows that the yield reduction in Crest was much less than in all other cultivars. It is interesting to compare the yield reduction, due to early inoculation, of Crest and Lancer (1% and 93%, respectively).

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<sup>1/</sup> one = 0-20% infection, two = 20-40% infection, three = 40-60% infection, four = 60-80% infection, and five = 80-100% infection.

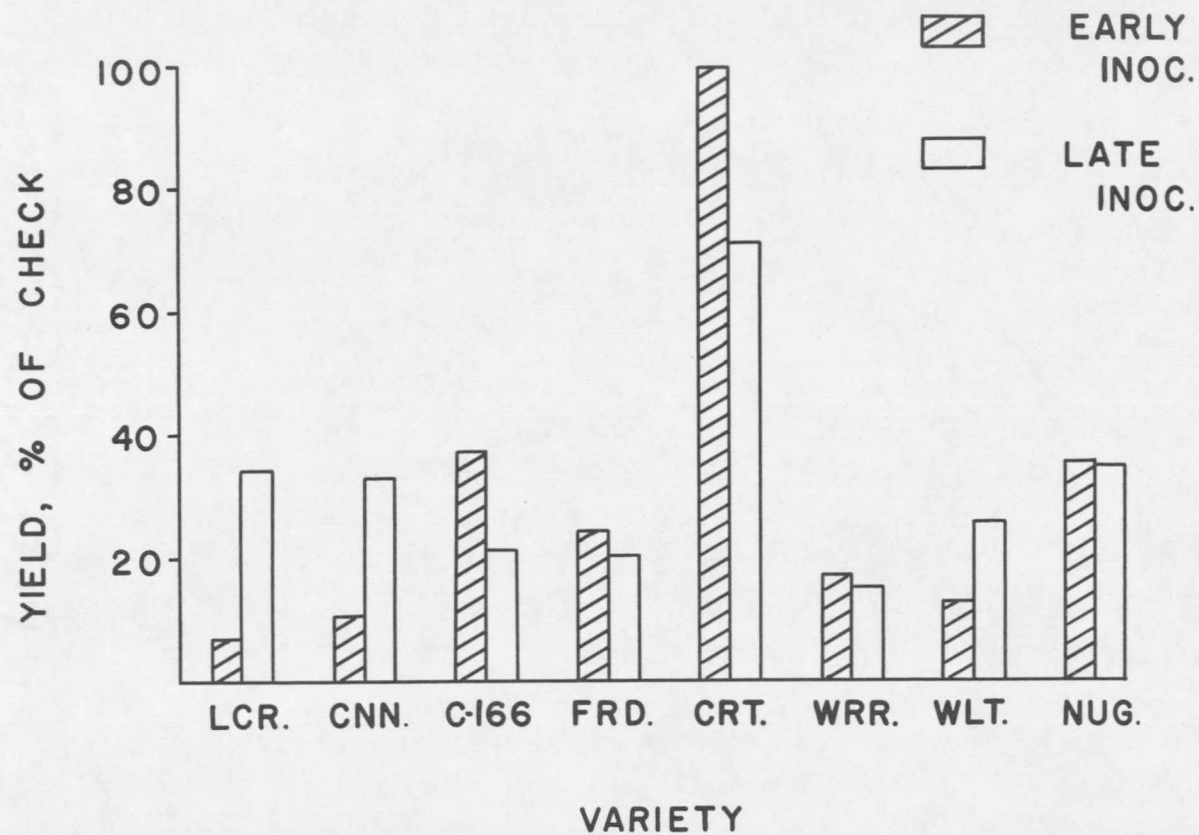


Figure 2

Effect of time of inoculation of *Cephalosporium gramineum* on yield of eight winter wheat cultivars. Early inoculation, 4-5 leaf stage; Late inoculation, just heading stage. LCR = Lancer, CNN = Cheyenne, C-166 = Chinese 166, FRD = Froid, CRT = Crest, WRR = Warrior, WLT = Winalta, NUG = Nugaines.

The low infection values of Crest indicated that this cultivar may have genes for resistance or tolerance to *Cephalosporium*.

#### Varietal Response to *C. gramineum*

Methods.- Crest winter wheat is a Montana release consisting of bulked line row components, with Westmont and P.I. 178383 as the parents. Since the previous experiment indicated that Crest could have tolerance to *Cephalosporium*, it was of importance to determine from which parent this tolerance was derived.

A split plot experimental design with three replications was the basis of this experiment. The following treatments were used as main plots: a nonwounded check, an early wounding, an early inoculation, a late wounding and a late inoculation. The cultivars Lancer, Crest, Westmont and P.I. 178383 were used as subplots. Each individual subplot was represented by 20 plants per 62 cm row. The rows were spaced 16 cm apart.

Wounding and inoculation of plants took place 29 and 43 days (early and late dates, respectively) after the date of planting.

Results.- Due to extremely varied growth and development of plants within replications, it was felt that data collected from harvested material would not be representative of actual conditions. For this

reason, the experiment was not harvested. Data on percent infection appeared to be valid since infection within and between replications was uniform. Analysis of variance indicated that variation due to inoculation date and the date x cultivar interaction was nonsignificant. For this reason, the values listed in Table VII are averaged over both dates of inoculation. As indicated, infection varied between cultivars. Lancer, the susceptible check was significantly higher in percent infection than the other cultivars. Although P.I. 178383 and Westmont are significantly different, Crest is not significantly different from either parent.

#### Minor Gene Lines

Methods.- Earlier observation of Crest under disease conditions revealed that certain plants were more susceptible to Cephalosporium stripe than other plants. Crest is a bulked cultivar, composed of 26 line row components. Earlier work indicated that P.I. 178383 has genes for resistance to stripe rust, dwarf bunt and Fusarium root rot (14, and R.J. Cook personal communication). Sharp and Volin's (14) work indicated that P.I. 178383 and Crest contain one major gene and varying numbers of minor genes, which condition resistance to stripe rust. The original line row components of Crest were known to contain one major gene and from 0 to 3 minor genes. Minor genes are assumed

Table VII. Variation in infection by Cephalosporium gramineum of four winter wheat cultivars.

Cultivar	Percent infection <u>a,b/</u>
Lancer	93.7 a
Westmont	80.2 b
Crest	80.0 bc
P.I. 178383	67.4 c

a/ % infection - average over both times of inoculation =

$$\frac{\text{Number infected plants}}{\text{Number total plants}} \times 100$$

b/ Means followed by the same letter are not significantly different at the 5% level of probability - Duncan's multiple range test.

to condition a nonspecific resistance to many races of a pathogen. (14, 21) and may be involved in conditioning resistance to other pathogens as well. If the latter is true, it appeared feasible that the variation in susceptibility of Crest might be related to the minor gene composition of each individual plant. To test this hypothesis, genotypes containing known numbers of major and minor genes for stripe rust resistance were tested for tolerance to *Cephalosporium*.

A split plot experimental design with three replications was used. A wounded check and inoculated plants were main plots. The genotypes<sup>1/</sup>Lancer, Moro, Crest LRC 42<sup>2/</sup>, Crest LRC 44, Crest LRC 34, MT 01500, MT 01301, MT 00902 and MT 00703 were represented as individual rows randomized within each main plot. Each 62 cm row consisted of 10 plants. Rows were spaced 24 cm apart. Wounding and inoculation of plants with 5 million spores per ml was performed 36 days after planting.

Results.- When the plants were in the soft dough stage, they were examined for percent infection. As noted in Table VIII, percent

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<sup>1/</sup> The genetic background of these cultivars is listed in Tables VIII through XVII.

<sup>2/</sup> LRC = Line row component.

Table VIII. Variation in infection by Cephalosporium gramineum of nine winter wheat genotypes.

Genotype	Genetic background <sup>a/</sup>	Infection		
		% Infection <sup>b,e/</sup>	% Infection <sup>c,e/</sup>	% Infection <sup>d,e/</sup>
Lancer	--	96.6	93.8	100.0
Moro	1M, 0m	86.6	88.5	96.4
Crest LRC 42	1M, 1m	96.6	94.4	96.4
Crest LRC 44	1M, 2m	83.3	81.4	93.8
Crest LRC 34	1M, 3m	85.9	82.4	100.0
MT 01500	0M, 0m	73.3	69.6	88.7
MT 01301	0M, 1m	76.6	79.6	91.7
MT 00902	0M, 2m	83.3	82.4	92.1
MT 00703	0M, 3m	90.0	86.7	96.2

<sup>a/</sup> M = number of major genes, m = number of minor genes conditioning resistance to Puccinia striiformis West.

<sup>b/</sup> Infected plants/total plants.

<sup>c/</sup> Infected tillers/total tillers.

<sup>d/</sup> Infected tillers/total tillers/infected plants.

<sup>e/</sup> Analysis of variance indicated differences among genotypes was nonsignificant.

infection was analyzed three separate ways, 1) infected plants/total plants, 2) infected tillers/total tillers, 3) infected tillers/total tillers/total plants. An analysis of variance indicated that the difference in infection among genotypes was nonsignificant in all cases.

After harvest, various factors which affect yield and grain quality were compiled and analyzed. These results are presented in Tables IX through XVII.

All genotypes were significantly reduced in total yield (Table IX) and yield per head (Table X). As indicated in Table XI, percent reduction in yield ranged from 36.8% (Crest 34) to 77.7% (Moro). The number of seed per row was affected only in highly susceptible genotypes (Table XII). Yield appears to be affected primarily by plumpness and weight per kernel (Table XIII and XIV), and the number of seeds per head (Table XV). The number of heads formed was not affected by the pathogen. This was indicated by a nonsignificant genotype times treatment interaction and further confirms data from the experiment on inoculum density (Table VI).

#### Field Test - 1969-70

Methods.- Field testing of cultivars was initiated in the fall of 1969. Although the cultivars tested were artificially inoculated,



Table IX. Effect of Cephalosporium gramineum on yield of nine winter wheat genotypes.

Genotype	Genetic background <sup>a/</sup>	Yield (grams/row)	
		Wounded check <sup>b/</sup>	Inoculated <sup>b/</sup>
Lancer	--	21.8 cd	6.8 bc
Moro	1M, 0m	35.7 a	7.4 bc
Crest LRC 42	1M, 1m	15.4 e	5.5 bc
Crest LRC 44	1M, 2m	19.3 de	10.6 ab
Crest LRC 34	1M, 3m	18.6 de	11.1 ab
MT 01500	0M, 0m	26.4 c	15.2 a
MT 01301	0M, 1m	16.6 de	4.0 c
MT 00902	0M, 2m	18.0 de	9.7 b
MT 00703	0M, 3m	32.6 a	9.5 bc
Mean for treatments		22.7	8.9

<sup>a/</sup> M = number of major genes, m = number of minor genes conditioning resistance to Puccinia striiformis West.

<sup>b/</sup> Within treatment means followed by the same letter are not significantly different at the 5% level of probability. Between treatment means not underscored by the same line are significantly different at the 5% level of probability - Duncan's multiple range test.

Table X. Effect of Cephalosporium gramineum on yield per head of nine winter wheat genotypes.

Genotype	Genetic background <sup>a/</sup>	Yield/head (grams)	
		Wounded check <sup>b/</sup>	Inoculated <sup>b/</sup>
Lancer	--	0.73 d	0.22 c
Moro	1M, 0m	1.37 a	0.41 ab
Crest LRC 42	1M, 1m	0.72 d	0.24 c
Crest LRC 44	1M, 2m	0.73 d	0.40 ab
Crest LRC 34	1M, 3m	0.74 d	0.48 a
MT 01500	0M, 0m	0.73 d	0.39 ab
MT 01301	0M, 1m	0.85 c	0.20 c
MT 00902	0M, 2m	0.77 cd	0.35 b
MT 00703	0M, 3m	1.03 b	0.35 b
Mean for treatments		0.85	0.34

<sup>a/</sup> M = number of major genes, m = number of minor genes conditioning resistance to Puccinia striiformis West.

<sup>b/</sup> Within treatment means followed by the same letter are not significantly different at the 5% level of probability. Between treatment means not underscored by the same line are significantly different at the 5% level of probability - Duncan's multiple range test.

Table XI. Effect of Cephalosporium gramineum on percent yield reduction and the number of heads per row of nine winter wheat genotypes.

Genotype	Plant Characteristics		
	Genetic background <sup>a/</sup>	% reduction in yield <sup>b/</sup>	Number of heads per row <sup>b/</sup>
Lancer	--	67.0 abc	30.0 b
Moro	1M, 0m	77.7 a	22.0 c
Crest LRC 42	1M, 1m	60.1 abc	21.5 c
Crest LRC 44	1M, 2m	40.6 c	26.8 bc
Crest LRC 34	1M, 3m	36.8 c	24.7 bc
MT 01500	0M, 0m	42.5 c	36.7 a
MT 01301	0M, 1m	75.9 ab	20.0 c
MT 00902	0M, 2m	45.0 bc	25.2 bc
MT 00703	0M, 3m	69.8 abc	29.5 b

<sup>a/</sup> M = number of major genes, m = number of minor genes conditioning resistance to Puccinia striiformis West.

<sup>b/</sup> Means followed by the same letter are not significantly different at the 5% level of probability - Duncan's multiple range test.

Table XII. Effect of Cephalosporium gramineum on the number of seeds formed per row of nine winter wheat genotypes.

Genotype	Genetic background <sup>a/</sup>	Seeds/row	
		Wounded check <sup>b/</sup>	Inoculated <sup>b/</sup>
Lancer	--	637.0 cde	438.3 bc
Moro	1M, 0m	1076.7 a	472.3 abc
Crest LRC 42	1M, 1m	<u>449.0 e</u>	<u>303.7 c</u>
Crest LRC 44	1M, 2m	<u>648.0 cd</u>	<u>540.7 ab</u>
Crest LRC 34	1M, 3m	<u>608.3 cde</u>	<u>501.7 ab</u>
MT 01500	0M, 0m	<u>736.3 bc</u>	<u>664.0 a</u>
MT 01301	0M, 1m	<u>542.3 de</u>	<u>374.0 bc</u>
MT 00902	0M, 2m	<u>496.0 de</u>	<u>465.0 bc</u>
MT 00703	0M, 3m	848.7 b	476.3 abc
Mean for treatments		671.4	470.7

<sup>a/</sup> M = number of major genes, m = number of minor genes conditioning resistance to Puccinia striiformis West.

<sup>b/</sup> Within treatment means followed by the same letter are not significantly different at the 5% level of probability. Between treatment means not underscored by the same line are significantly different at the 5% level of probability - Duncan's multiple range test.

Table XIII. Effect of Cephalosporium gramineum on the percent shriveled kernels per row of nine winter wheat genotypes.

Genotype	Genetic background <sup>b/</sup>	% Shriveled kernels/row <sup>a/</sup>	
		Wounded check <sup>c/</sup>	Inoculated <sup>c/</sup>
Lancer	--	1.5 a	79.4 bc
Moro	1M, 0m	2.2 a	95.8 ab
Crest LRC 42	1M, 1m	3.9 a	85.7 abc
Crest LRC 44	1M, 2m	5.2 a	53.3 de
Crest LRC 34	1M, 3m	2.1 a	50.0 e
MT 01500	0M, 0m	12.4 a	60.5 de
MT 01301	0M, 1m	10.2 a	100.0 a
MT 00902	0M, 2m	0.8 a	68.5 cd
MT 00703	0M, 3m	0.4 a	78.2 c
Mean for treatments		4.3	74.6

<sup>a/</sup> % shriveled kernels =  $\frac{\text{Number shriveled kernels per row}}{\text{Number total kernels per row}} \times 100$

<sup>b/</sup> M = number of major genes, m = number of minor genes conditioning resistance to Puccinia striiformis West.

<sup>c/</sup> Within treatment means followed by the same letter are not significantly different at the 5% level of probability. Between treatment means not underscored by the same line are significantly different at the 5% level of probability - Duncan's multiple range test.

Table XIV. Effect of Cephalosporium gramineum on thousand kernel weight of nine winter wheat genotypes.

Genotype	Genetic background <sup>a/</sup>	Thousand kernel weight (grams)	
		Wounded check <sup>b/</sup>	Inoculated <sup>b/</sup>
Lancer	--	34.2 bc	15.4 c
Moro	1M, 0m	33.2 bcd	15.7 c
Crest LRC 42	1M, 1m	33.8 bc	16.8 bc
Crest LRC 44	1M, 2m	29.6 e	19.8 ab
Crest LRC 34	1M, 3m	30.9 cde	22.5 a
MT 01500	0M, 0m	36.0 ab	22.2 a
MT 01301	0M, 1m	30.4 de	10.6 d
MT 00902	0M, 2m	36.3 ab	20.2 a
MT 00703	0M, 3m	38.5 a	20.0 ab
Mean for treatments		33.7	18.1

<sup>a/</sup> M = number of major genes, m = number of minor genes conditioning resistance to Puccinia striiformis West.

<sup>b/</sup> Within treatment means followed by the same letter are not significantly different at the 5% level of probability. Between treatment means not underscored by the same line are significantly different at the 5% level of probability - Duncan's multiple range test.

Table XV. Effect of Cephalosporium gramineum on the average number of seeds formed per head of nine winter wheat genotypes.

Genotype	Genetic background <sup>a/</sup>	Seeds/head	
		Wounded check <sup>b/</sup>	Inoculated <sup>b/</sup>
Lancer	--	21.4 def	14.4 f
Moro	1M, 0m	41.3 a	26.4 a
Crest LRC 42	1M, 1m	21.2 ef	13.7 b
Crest LRC 44	1M, 2m	24.4 cd	20.0 cde
Crest LRC 34	1M, 3m	23.9 cde	21.4 bc
MT 01500	0M, 0m	20.5 ef	17.4 def
MT 01301	0M, 1m	27.9 b	18.6 cde
MT 00902	0M, 2m	21.3 def	16.8 ef
MT 00703	0M, 3m	26.8 bc	17.4 def
Mean for treatments		25.4	18.5

<sup>a/</sup> M = number of major genes, m = number of minor genes conditioning resistance to Puccinia striiformis West.

<sup>b/</sup> Within treatment means followed by the same letter are not significantly different at the 5% level of probability. Between treatment means not underscored by the same line are significantly different at the 5% level of probability - Duncan's multiple range test.

Table XVI. Effect of Cephalosporium gramineum on plant height of nine winter wheat genotypes.

Genotype	Genetic background <sup>a/</sup>	Plant height (cm)	
		Wounded check <sup>b/</sup>	Inoculated <sup>b/</sup>
Lancer	--	79.9 b	63.5 b
Moro	1M, 0m	59.8 f	49.1 c
Crest LRC 42	1M, 1m	67.6 de	45.6 c
Crest LRC 44	1M, 2m	64.3 e	63.3 b
Crest LRC 34	1M, 3m	71.5 cd	64.3 b
MT 01500	0M, 0m	74.5 c	62.8 b
MT 01301	0M, 1m	72.1 cd	60.8 b
MT 00902	0M, 2m	73.0 c	61.3 b
MT 00703	0M, 3m	87.2 a	71.1 a
Mean for treatments		72.2	60.2

<sup>a/</sup> M = number of major genes, m = number of minor genes conditioning resistance to Puccinia striiformis West.

<sup>b/</sup> Within treatment means followed by the same letter are not significantly different at the 5% level of probability. Between treatment means not underscored by the same line are significantly different at the 5% level of probability - Duncan's multiple range test.



Table XVII. Effect of Cephalosporium gramineum on percent protein of nine winter wheat genotypes.

Genotype	Genetic background <sup>a/</sup>	% Protein	
		Wounded check <sup>b/</sup>	Inoculated <sup>b/</sup>
Lancer	--	17.3 bc	19.3 ab
Moro	1M, 0m	14.9 e	18.7 bc
Crest LRC 42	1M, 1m	17.5 b	18.8 b
Crest LRC 44	1M, 2m	<u>18.4 a</u>	<u>19.0 b</u>
Crest LRC 34	1M, 3m	17.4 bc	19.3 ab
MT 01500	0M, 0m	17.8 b	19.5 ab
MT 01301	0M, 1m	17.5 b	19.4 ab
MT 00902	0M, 2m	16.6 cd	19.9 a
MT 00703	0M, 3m	15.9 d	18.1 c
Mean for treatments		17.0	19.1

<sup>a/</sup> M = number of major genes, m = number of minor genes conditioning resistance to Puccinia striiformis West.

<sup>b/</sup> Within treatment means followed by the same letter are not significantly different at the 5% level of probability. Between treatment means not underscored by the same line are significantly different at the 5% level of probability - Duncan's multiple range test.

it was the intent of this work to test cultivars under uncontrolled environmental conditions that would more nearly simulate natural conditions than would greenhouse tests.

A split plot experimental design, which was replicated three times, was the basis for an experiment in which the cultivars Winalta, Lancer, Cheyenne, Itana, Crest and Nugaines were used as main plots. The following treatments were used as subplots; a nonwounded check, an early wounded check, an early inoculation, a late wounded check, and a late inoculation. Each treatment was represented by a single row 6.1 meters long. The rows were spaced 30.5 cm apart and planted at the rate of 67.3 kg/ha. The cultivars were planted on September 23, 1969 with the first date of wounding and inoculation on May 14, 1970. A spore concentration of 11.8 million spores/ml was used to inoculate the plants, which were in the 3-4 leaf stage. The second date of inoculation occurred 13 days later, on May 27, 1970. A spore concentration of 13.1 million spores/ml was used to inoculate the plants which were now in the 4-5 leaf stage.

Results.- Symptom development was followed on 10 tillers from each cultivar, which were initially tagged when symptoms first appeared. These tillers were examined approximately once a week until the flag leaf turned necrotic. Correlation analyses of rate of symptom development vs. yield reduction for each cultivar indicated that

variation in symptom development was too large to be closely correlated with yield reduction.

The number of infected plants per row was determined after the plants had flowered (Table XVIII). Infection of Lancer was significantly higher than for all other cultivars. The data in this table also indicates that yield in the early inoculated treatment was significantly reduced from the yield in the late inoculated treatment. However, all cultivars were reduced in a similar manner, as indicated by a nonsignificant treatment times cultivar interaction. As shown in Table XIX, a significant yield difference existed between treatments. Both dates of inoculation were reduced significantly from their respective checks. These data would indicate that percent reduction in yield was due to both wounding and disease.

Individual cultivar response for yield and test weight (Tables XX and XXI) differed among cultivars. As indicated in Table XX, Winalta and Nugaines were not reduced in yield significantly by either wounding or the disease. All other cultivars show a significant variation from the nonwounded check. Test weight data from Table XXI shows a similar pattern with the exception of Cheyenne which in this case, was not affected significantly in test weight. Thousand kernel weight and percent protein differed among cultivars but not among treatments (Table XIX).

Table XVIII. The effect of Cephalosporium gramineum on the number of infected plants per row and reduction in yield in six winter wheat cultivars.

Cultivar	Plant Characteristics			
	# of infected plants per row <sup>a/</sup>	% reduction in yield; early inoculation <sup>a,b,c/</sup>	% reduction in yield, late inoculation <sup>a,b,c/</sup>	Average % reduction in yield <sup>a/</sup>
Lancer	59.5 a	31.9 a	25.9 a	28.9 a
Cheyenne	39.7 b	36.8 a	28.8 a	32.8 a
Itana	32.0 b	38.1 a	25.8 a	32.0 a
Crest	26.7 b	34.0 a	17.9 a	25.9 a
Winalta	38.3 b	24.3 a	20.1 a	22.2a
Nugaines	25.3 b	19.6 a	15.1 a	17.3 a

<sup>a/</sup> Means followed by the same letter are not significantly different at the 5% level of probability - Duncan's multiple range test.

<sup>b/</sup> Percent reduction in yield due to the early inoculation was significantly (5%) higher than the percent reduction in yield due to the late inoculation.

<sup>c/</sup> % reduction respective to nonwounded check.

Table XIX. The effect of time of field inoculation of Cephalosporium gramineum on yield, test weight, percent protein and thousand kernel weight of six winter wheat cultivars.

Treatment	Plant Characteristics <sup>a/</sup>			
	Yield (grams) <sup>b/</sup>	Test weight (lbs/bu) <sup>b/</sup>	% protein <sup>b/</sup>	Thousand kernel wt (grams) <sup>b/</sup>
Nonwounded check	803.4 a	63.6 a	13.9 a	33.9 a
Wounded check May 14	716.6 b	63.3 b	13.9 a	33.6 a
Wounded check May 27	728.9 b	63.5 a	13.9 a	34.6 a
Inoculated row May 14	546.9 c	62.8 c	13.9 a	34.1 a
Inoculated row May 27	617.3 d	63.0 c	13.8 a	34.0 a

<sup>a/</sup> Means followed by the same letter are not significantly different at the 5% level of probability - Duncan's multiple range test.

<sup>b/</sup> Values given are averaged over all six cultivars.

Table XX. The effect of time of inoculation of Cephalosporium gramineum on yield of six winter wheat cultivars.

Treatment	Yield (grams)					
	Crest <sup>a/</sup>	Winalta <sup>a/</sup>	Lancer <sup>a/</sup>	Cheyenne <sup>a/</sup>	Itana <sup>a/</sup>	Nugaines <sup>a/</sup>
Nonwounded check	761.0 a	749.0 a	831.0 a	890.3 a	808.0 a	781.0 a
Wounded check May 14	724.7 a	560.3 a	840.7 a	743.0 ab	730.3 ab	700.3 a
Wounded check May 27	623.3 ab	665.0 a	763.0 a	793.7 a	751.0 a	777.3 a
Inoculated row May 14	491.7 b	565.3 a	566.3 b	553.7 c	449.3 c	605.0 a
Inoculated row May 27	622.3 ab	597.7 a	614.7 b	622.3 bc	599.7 bc	647.3 a

<sup>a/</sup> Means followed by the same letter are not significantly different at the 5% level of probability - Duncan's multiple range test.

Table XXI. The effect of time of inoculation of Cephalosporium gramineum on test weight in six winter wheat cultivars.

Treatment	Test Weight (lbs/bu)					
	Crest <sup>a/</sup>	Winalta <sup>a/</sup>	Lancer <sup>a/</sup>	Cheyenne <sup>a/</sup>	Itana <sup>a/</sup>	Nugaines <sup>a/</sup>
Nonwounded check	62.1 a	64.1 a	63.6 a	63.6 a	64.0 a	63.6 a
Wounded check May 14	61.7 a	63.8 a	63.5 a	63.6 a	63.7 ab	63.2 a
Wounded check May 27	61.7 a	64.1 a	63.6 a	63.8 a	64.0 a	64.0 a
Inoculated row May 14	60.8 b	63.2 a	62.5 b	63.5 a	63.5 a	63.3 a
Inoculated row May 27	61.3 ab	63.7 a	62.4 b	62.9 a	63.7 ab	63.7 a

<sup>a/</sup> Means followed by the same letter are not significantly different at the 5% level of probability - Duncan's multiple range test.

## Field Test - 1970-71

Methods.- Another experiment was designed to compare the reaction to C. gramineum of P.I. 178383, Westmont and Crest under field conditions since Crest and P.I. 178383 had performed well in greenhouse experiments. The cultivars Crest, Westmont, P.I. 178383, Cheyenne and Lancer were used as main plots in a split plot experimental design, which was replicated five times. The treatments nonwounded check, wounded check and inoculated row were used as subplots within each main plot. Each treatment was represented by a single 3.1 meter row. The rows were spaced 30.5 cm. apart and planted at the rate of 170 seeds per 3.1 meters. All rows also received 90 kg/hectare of 18-46-0 fertilizer at the time of planting on September 15, 1970.

On May 23, 1971 the inoculated row was wounded, inoculated with  $10.8 \times 10^6$  spores per ml and then rewounded. The wounded check was also wounded twice. The plants were in the four leaf stage. Five days after inoculation, fifty tillers each from P.I. 178383, Crest, and Lancer were hypodermically injected with  $15 \times 10^6$  spores/ml and tagged. Symptom development was followed in these plants.

Results.- Correlation analyses of data collected from tagged plants indicated Lancer was the only cultivar in which symptom expression could be correlated with yield. Days from inoculation until symptom



expression occurred in the flag leaf, was used as the independent variable. Yield constituted the dependent variable. The correlation coefficient for Lancer was .38 (positive) which was significant at 5%. However, the independent variable accounted for only 14.2% of the variation. Correlation coefficients from P.I. 178383 and Crest were nonsignificant. The variation accounted for by the independent variable was less than 1% in both cases. These data would indicate that the rate of symptom expression is not closely correlated with yield reduction.

At the time the kernels were starting to fill, each cultivar was examined for the number of white (blighted) heads per row (Table XXII). Percent infection could not be accurately determined because of extensive tillering and high plant populations. Percent white heads ranged from 7.7% (Lancer) down to .079% (P.I. 178383), almost a 100-fold difference.

Reduction in yield was approximately the same for all cultivars (Table XXII). Percent reduction in yield, although greater than in the previous field experiment, indicates that all cultivars tested exhibited approximately equal levels of tolerance. However, this experiment yielded some unexpected results. As indicated in Table XXIII, with every cultivar the wounded check out-yielded the non-wounded check. This is a complete reversal from the data collected in

Table XXII. The effect of *Cephalosporium gramineum* on the percent white heads per row and percent reduction in yield for five winter wheat cultivars.

Cultivar	Plant Characteristics		
	Number of white heads per inoculated row <sup>a/</sup>	Percent white heads per inoculated row <sup>a/</sup>	% reduction in yield-respective to wounded check <sup>a/</sup>
Lancer	57.8 a	7.72 a	43.9 a
Westmont	29.0 b	4.54 b	40.0 a
P.I. 178383	0.8 c	0.08 c	38.5 a
Crest	35.2 ab	5.46 ab	43.5 a
Cheyenne	21.8 bc	3.36 b	46.4 a

<sup>a/</sup> Means followed by the same letter are not significantly different at the 5% level of probability - Duncan's multiple range test.

Table XXIII. The effect of wounding and inoculation of Cephalosporium gramineum on yield of five winter wheat cultivars.

Treatment	Yield (grams)					Ave. for all cultivars <sup>a/</sup>
	Lancer <sup>a/</sup>	Westmont <sup>a/</sup>	P.I. 178383 <sup>a/</sup>	Crest <sup>a/</sup>	Cheyenne <sup>a/</sup>	
Nonwounded check	271.6 a	241.2 a	235.2 a	234.8 a	245.8 b	245.7 b
Wounded check	341.6 b	267.0 a	270.6 a	276.4 a	325.8 a	296.3 a
Inoculation	188.4 c	160.8 b	162.4 b	154.0 b	169.8 b	167.1 c

<sup>a/</sup> Means followed by the same letter are not significantly different at the 5% level of probability - Duncan's multiple range test.

the field experiment the previous year. The reason for this is unknown.

In all cultivars, the inoculated row yielded significantly less than the wounded check.

Percent protein (Table XXIV) varied between treatments for Westmont, P.I. 178383 and Cheyenne. Lancer and Crest were not significantly affected by either wounding or the disease. When averaged over all treatments, percent protein for the inoculated row was significantly higher than for either check.

Table XXIV. The effect of wounding and inoculation of Cephalosporium gramineum on percent protein in five winter wheat cultivars.

Treatment	Percent Protein					Ave. for all cultivars <sup>a/</sup>
	Lancer <sup>a/</sup>	Westmont <sup>a/</sup>	P.I. 178383 <sup>a/</sup>	Crest <sup>a/</sup>	Cheyenne <sup>a/</sup>	
Nonwounded check	15.3 a	17.0 a	14.9 b	16.4 a	15.7 a	15.9 b
Wounded check	15.3 a	16.4 b	14.5 c	16.6 a	15.9 ab	15.7 b
Inoculation	15.6 a	16.8 ab	15.7 a	16.6 a	16.2 b	16.2 a

<sup>a/</sup> Means followed by the same letter are not significantly different at the 5% level of probability - Duncan's multiple range test.

## DISCUSSION

The cultivar Crest appeared to be tolerant to C. gramineum under greenhouse conditions (Fig. 2, Table VII). Under field conditions though, this cultivar gave results similar to other susceptible cultivars. Data from both field experiments (Table XVIII and XXII) indicated that under the environmental conditions which influenced these experiments, all the cultivars tested exhibited approximately equal levels of tolerance or susceptibility to the pathogen. Differences between field and greenhouse experiments may be related to the stress placed on the plant. Perhaps under greenhouse conditions, inoculation of plants was more thorough and the controlled environmental conditions more conducive to disease development. Under severe disease stress, Crest could be tolerant while other cultivars would exhibit greater susceptibility.

Disease development during the first field experiment appeared to be representative of disease development with natural inoculum. During the second field experiment, however, *Gephalosporium* stripe did not follow the normal path of symptom development. After the initial discoloration of the vascular bundles, entire leaves turned necrotic almost immediately. The characteristic leaf chlorotic pattern associated with the disease failed to develop. White (blighted) heads became evident almost two weeks before they would normally. This unusual symptom development could be related to the abnormally hot dry weather

which prevailed during this growing season. This rapid loss in photosynthetic capacity may indicate why the percent yield reduction in the second field experiment was much higher than in the first field experiment (Tables XVIII and XXII).

The data would indicate that complete resistance to the pathogen does not exist in the cultivars tested. However, the degree of susceptibility varied among cultivars (Table VII). As this table indicates, Crest and both of its parents were infected significantly less than Lancer, the susceptible check. Although Crest did not differ significantly from either parent, P.I. 178383 was significantly lower than Westmont in the percentage of infected plants. These data would indicate that under high inoculum densities, low levels of resistance may exist in P.I. 178383 and perhaps Crest. Pool (11) has also indicated that low levels of resistance may exist. Bruehl, (5) on the other hand, indicates that no resistant varieties are known. With high levels of inoculum density, varying levels of tolerance were found. Table VIII indicates that all genotypes tested exhibited approximately equal levels of infection. However, Crest 34 appeared to be more tolerant to C. gramineum than all other genotypes. Factors such as average number of seeds formed per head (Table XV) and the number of seeds per row (Table XII) were not significantly affected by the disease. This genotype was only reduced in yield by 36.8% compared

to 67.0% for Lancer, the susceptible check (Table XI).

The hypothesis that minor genes for stripe rust resistance may also condition tolerance to C. gramineum does not appear to be valid. It was postulated that increasing numbers of minor genes for stripe rust resistance might condition increasing levels of resistance to C. gramineum. From Table XI, it is evident that the genotype with the greatest number of minor genes did not always give the least yield reduction. In this case, disease severity is related to factors other than minor gene response to stripe rust. Very likely, genetic sources of tolerance to C. gramineum are completely unrelated to genetic resistance to stripe rust.

The data from the inoculum density and minor gene experiments would indicate that the decreased yield from infected plants is directly related to the decreased weight and numbers of seed formed per head. This is in agreement with data presented by Pool (11) and Bruehl (2). Loss of kernel weight is evident in the percent shriveled kernels and reflected in the thousand kernel weight. These light kernels would also intensify the yield loss, due to the fact that during harvest many of these light kernels would be expelled from the combine with the chaff. In contrast to Bruehl's data (1), excessive tillering in diseased plants was not observed. In fact, at no time was the difference between inoculated and healthy plants significant.



This is consistent with Pool's data (11).

This disease also has a significant effect on plant height and percent protein. Stunted plants were usually reduced in height by 20% relative to the healthy control. Previous reported data (11) are in close agreement. Percent protein is increased due to kernel shriveling. This alters the carbohydrate to protein ratio and results in a relative increase in percent protein.

Although artificial inoculation gave good results in both field and greenhouse experiments, studies of disease development and effects on yield should also be conducted under conditions of natural inoculation. At this time, the degree of correlation between artificial and natural inoculation has not been determined. Cultivars susceptible to the pathogen under artificial conditions may exhibit varying levels of resistance under natural conditions. In this case, resistance could be related to the ease in which roots are broken during soil heaving in the spring. If the degree of correlation is found to be high, then either artificial or natural inoculation techniques could be used in a winter wheat breeding program. It is requisite though, that this correlation be high before artificial inoculation techniques are employed in a cultivar improvement program.

## SUMMARY

Under greenhouse conditions inoculum density studies with Cephalosporium gramineum indicated that conidiospore suspensions of 500,000 per ml and 5,000,000 per ml applied at the rate of 100 mls per foot of row of winter wheat, would affect various yield components in a similar manner. A spore concentration of at least 50,000 per ml was necessary to cause a percent yield reduction which varied significantly from the wounded check. Therefore, in most greenhouse studies, an inoculum density of 5,000,000 spores per ml was used to test the reaction of winter wheat cultivars to this pathogen. It was felt that this higher conidiospore concentration would place the plant under severe disease stress and serve as an effective screening tool. Inoculation of plants in the three-four leaf stage resulted in high levels of infection in both field and greenhouse studies.

Although the percent infection for the cultivars tested was always high, varying levels of resistance were observed in Crest and its parents, P.I. 178383 and Westmont. These three lines had significantly fewer infected plants per row than Lancer a susceptible cultivar with P.I. 178383 more resistant than Westmont.

With high levels of infection, varying levels of tolerance were observed. Testing of three line row components of Crest indicated approximately equal infection percentages for all three, with the percent yield reduction varying from 36.8 percent to 60.1 percent

while Lancer was reduced in yield by 67.0 percent.

Plants infected by C. gramineum are characterized by stunting, kernel shriveling, increased protein and reduced yield. The increase in protein appears to be dependent on the degree of kernel shriveling, which directly reduces the endosperm-germ (carbohydrate-protein) ratio and results in a relative increase in percent protein. Yield of diseased plants was most dependent on the number and weight of seed formed per head. In all greenhouse experiments, the thousand kernel weight of infected cultivars was always significantly lower than the thousand kernel weight of healthy controls. The number of seed formed per head was affected only in those genotypes that are highly susceptible to C. gramineum. The number of tillers produced by diseased plants was never significantly different from the number of tillers produced by the healthy control. The percent yield reduction varied widely among the cultivars tested. Highly susceptible cultivars were reduced in yield by as much as 78 percent.

During the course of this work, the wheat cultivars tested were all artificially inoculated. In the future, this work must be compared to results obtained under conditions of natural inoculation. If the degree of correlation is high, either artificial or natural inoculation methods, or both, could be used in a program for developing C. gramineum tolerant plants, using P.I. 178383 as one possible source of tolerance.

#### LITERATURE CITED

1. Bruehl, G.W. 1956. Prematurity blight phase of Cephalosporium stripe disease of wheat. Plant Disease Reporter 40(3): 237-241.
2. Bruehl, G.W. 1956. Cephalosporium stripe disease of wheat in Washington. Phytopathology 46(3): 178-180.
3. Bruehl, G.W. 1957. Cephalosporium stripe disease of wheat. Phytopathology 47(11): 641-649.
4. Bruehl, G.W., and J.W. Strobel. 1957. Cephalosporium gramineum of wheat in Washington. Phytopathology 47(1): 5.
5. Bruehl, G.W., P. Lai, and O. Huisman. 1964. Isolation of Cephalosporium gramineum from buried naturally infested host debris. Phytopathology 54(8): 1035-1036.
6. Gerdeman, J.W., and R.O. Weibel. 1960. Cephalosporium stripe on small grains in Illinois. Plant Disease Reporter 44(11): 877.
7. Ikata, S., and J. Kawi. 1938. Studies in the stripe disease of wheat. Bulletin of the Agricultural Experiment Station, Okayamaken 111 p. Review of Applied Mycology 17: 593.
8. Lai, P., and G.W. Bruehl. 1966. Survival of Cephalosporium gramineum in naturally infested wheat straw in soil in the field and in the laboratory. Phytopathology 56(2): 213-218.
9. Nisikado, Y., H. Matsumoto, and K. Yamauti. 1934. Studies on a new Cephalosporium, which causes the stripe disease of wheat. Ber. Ohara Insts. Landwirtsch. Forsch., Kurashiki, Japan, 6: 275-306. The Review of Applied Mycology 13: 623-624.
10. Pool, R.A.F., and E.L. Sharp. 1966. A relationship between autumn root growth of winter wheat and infection by Cephalosporium gramineum. Phytopathology 56(8): 895.
11. Pool, R.A.F. 1967. Cephalosporium stripe of winter wheat: disease processes and effects. Montana State University, Ph.D. Thesis.
12. Pool, R.A.F., and E.L. Sharp. 1969. Possible association of a polysaccharide and an antibiotic with the disease cycle of

Cephalosporium stripe. *Phytopathology* 59(11): 1763-1764.

13. Sharp, E.L. 1959. Two previously unreported fungi on cereals in Montana. *Plant Disease Reporter* 43: 12-13.
14. Sharp, E.L. and R.B. Volin. 1970. Additive genes in wheat conditioning resistance to stripe rust. *Phytopathology* 60(7): 1146-1147.
15. Smith, N.A., R.P. Scheffer, and A.H. Ellingboe. 1966. Cephalosporium stripe of wheat prevalent in Michigan. *Plant Disease Reporter* 50(3): 190-191.
16. Spalding, D.H., G.W. Bruehl, and R.J. Foster. 1961. Possible role of pectinolytic enzymes and polysaccharide in pathogenesis by Cephalosporium gramineum in wheat. *Phytopathology* 51(4): 227-235.
17. Tolmsoff, W.J. 1965. Biochemical basis for biological specificity of dextro (p-dimethylaminobenzenediazo sodium sulfonate), a respiratory inhibitor. University of California, Davis, Ph.D. Thesis.
18. Tyler, L.J., and L.E. Dickens. 1957. Cephalosporium leaf stripe disease of winter wheat. *Plant Disease Reporter* 41(5): 384.
19. Udy, D.C. 1956. Estimation of protein in wheat and flour by ion-binding. *Cereal Chemistry* 33(3): 190-197.
20. Udy, D.C. 1957. Improved milling and collection of small samples for protein analysis by dye-binding. *Cereal Chemistry* 34(3): 389-394.
21. Van der Plank, J.E. 1968. Disease Resistance in Plants. New York: Academic Press. 206 p.

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