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
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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 distructiveness 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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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 distructiveness 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 prefered 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 Manhatton, Montana in June 1969. Isolation of the fungus involved plating surface sterilized[1/] 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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1/ 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_2O
Glucose	18.00
Yeast extract, Difco	3.00
Peptone, Difco	5.00
$K_2 H PO_4$	1.36
$K H_2 PO_4$	1.68
$Mg SO_4$	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 consistant 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 throughly 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.

	Infection Percentage			
	wounded roots		nonwounded roots	
Inoculum source	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 varing 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[a/]	Percent infected tillers per row[a/]
Nonwounded check	0.0 d	0.0 d
Wounded check	3.3 d	2.6 d
500 conidia/ml[b/]	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

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

b/ 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

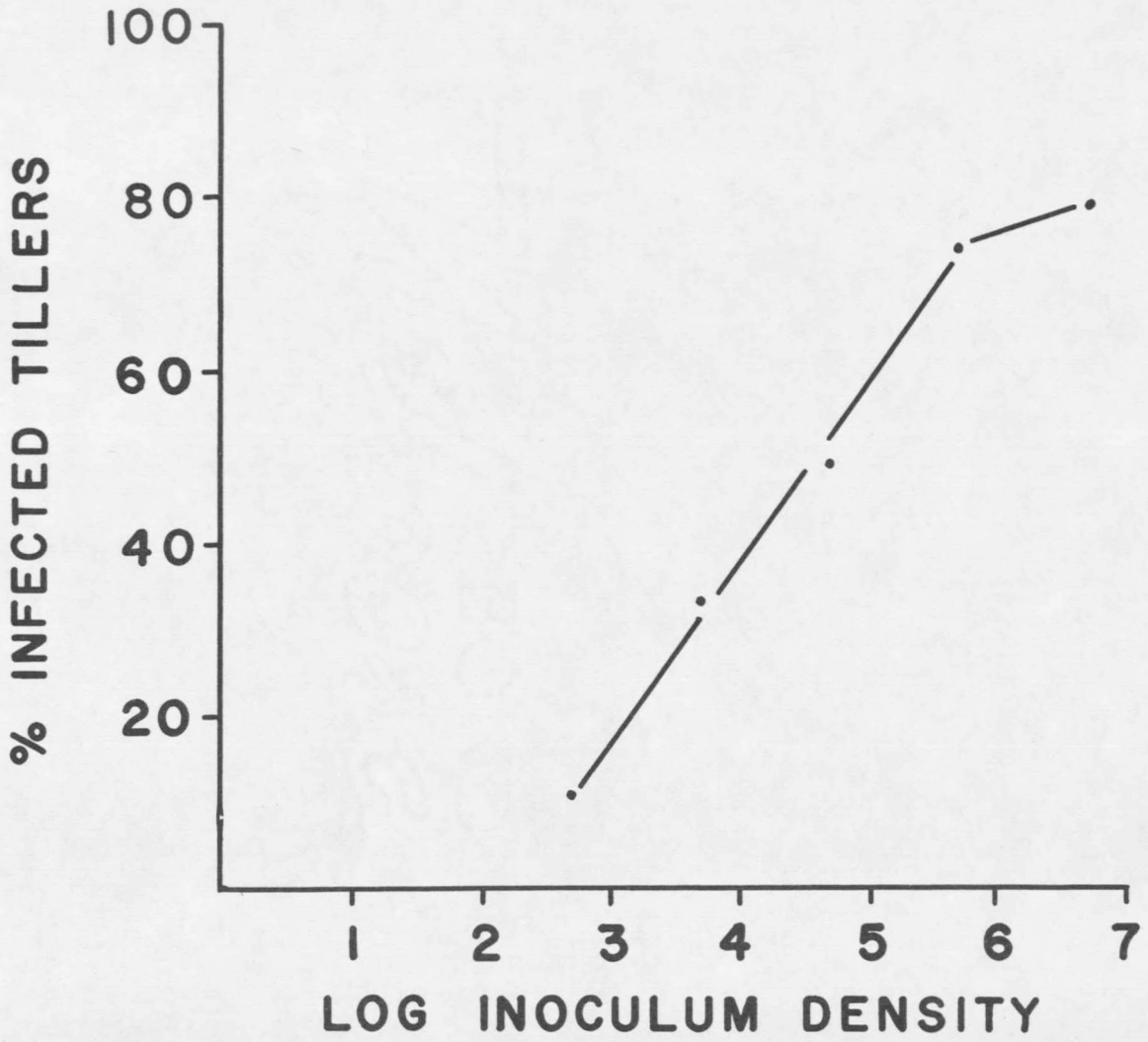

Figure 1 Effect of inoculum density (spores/ml) of Cephalosporium gramineum on infection of Lancer winter wheat.