Cephalosporium stripe of winter wheat: disease processes and effects
by Robert Alfred Frank Pool

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of
DOCTOR OF PHILOSOPHY in Botany
Montana State University

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Although no immune wheat varieties were discovered, a number of selections were found to have varying degrees of resistance.

A large molecular weight, viscous, extracellular fungal polysaccharide was shown to be present in the infected wheat and to be a contributing factor in restricting fluid movement in wheat leaves.

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ABSTRACT

The effect of Cephalosporium gramineum infection in winter wheat was studied relative to the environmental conditions which affect growth of the wheat and the incidence of disease. Studies in the field and with controlled environment chambers showed that warm soil temperatures and ample moisture availability contribute to growth of the wheat roots and an increase in disease incidence. Fertilizer at the time of planting increased Cephalosporium stripe disease as it simultaneously enhanced fall root growth. Wheat with a greater autumn root mass was more subject to root damage in the spring thaws. Numerous breaks in the roots allowed multiple entrance points for the pathogen and a greater possibility for infection of any one plant.

Although no immune wheat varieties were discovered, a number of selections were found to have varying degrees of resistance.

A large molecular weight, viscous, extracellular fungal polysaccharide was shown to be present in the infected wheat and to be a contributing factor in restricting fluid movement in wheat leaves.

INTRODUCTION

The fungus, Cephalosporium gramineum Nisikado et Ikata, incites a systemic disease of winter wheat. Cephalosporium stripe disease is a relatively new problem but is one of the more important soil-borne diseases of wheat in Montana. The observed increases in disease incidence with early planting dates, added fertilizer, and excessive stubble cover require further study. A possible water relationship to disease or symptom development as well as the possibility of resistant varieties should be investigated.

In this investigation three phases of the host-environment-parasite interaction were studied. In the first phase, gross field observations were made of disease incidence throughout the state. Cultural practices involving the use of fertilizer, stubble cover, and different planting dates were related with disease incidence and yield. A number of varieties and lines of wheat were surveyed in a search for resistance to natural infection.

Based on several observations in the field, a second aspect of the problem involved controlled environment studies. A constantly changing profile simulated autumn conditions, and a diurnal temperature profile of 45/72 F (dark/light) simulated early summer field conditions in observing different reactions of the host. Varying controlled humidity conditions were also employed in studying the pathogen and its interaction with the host and the environment.

The third approach to the problem concerned investigation of a polysaccharide fungal byproduct possibly involved in the disease syndrome. In this phase of the study, physical and chemical means were employed in an

effort to characterize the molecule.

LITERATURE REVIEW

Cephalosporium gramineum Nisikado et Ikata incites the systemic Cephalosporium stripe disease of winter wheat. The disease was first discovered in Japan in 1931 and described in 1934 (Nisikado et al., 1934; Waldee, 1949), but it may have been detected as early as 1924 in Minnesota (Henry, 1924; Orton, 1931; Grey & Nobel, 1960) when a Cephalosporium sp. was isolated from winter wheat. Cephalosporium gramineum was first reported to be in the state of Washington in 1955 (Bruehl, 1956b) and in Montana in 1956 (Sharp, 1959). It has also been reported in New York (Tyler & Dickens, 1957), Illinois (Gerdeman & Weibel, 1960), and Michigan (Smith et al., 1966) as well as Scotland (Grey & Nobel, 1960), and England (Slope, 1962). The disease may also be present in Yugoslavia (D. Sutić, personal communication) and in Canada (D. Stelfox, personal communication).

The imperfect fungus genus Cephalosporium is of world wide distribution being found in oceanic muds to depth of 220 meters and in water to 1127 meters (Johnson & Sparrow, 1961). Airborne conidia have been found off the pacific coast (Rittenberg, 1940), and at altitudes of 9000 feet in the arctic (Pady & Kapica, 1953).

The cephalosporia cause diseases in animals, including humans, as well as plants (Pisano, 1963).

Cephalosporium gramineum occurs occasionally in several grasses and will infect artificially a large number of grasses (Nisikado et al., 1934; Bruehl, 1957). It has not been found in spring wheat under natural conditions, but has been naturally found in barley, oats, and rye (Gerdeman & Weibel, 1960).

The well established leaf symptoms of the disease start with a mottled chlorosis suggestive of wheat streak mosaic virus. This is followed by one to several thin necrotic lines, chlorotic striping with accompanying necrosis, or a necrotic line which is eventually accompanied by chlorosis. The early maturing heads are poorly filled with light weight shrunken kernels. The vessels are filled with mycelium and conidia, and the infected plants are stunted (Nisikado et al., 1934; Ikata & Kawai, 1938; Waldee, 1949; Bruehl, 1956a & b; Slope, 1962; Slope & Bardner, 1965). In many respects the disease resembles the black bundle disease of corn caused by Cephalosporium acremonium (Reddy & Holbert, 1924; Nisikado & Higuti, 1939), and the brown stem rot of soybeans caused by Cephalosporium gregatum (Hildebrand, 1952; Chamberlain & McAlister, 1954; Dunleavy & Weber, 1967).

Wheat varieties with some possible resistance have been noticed (Nisikado et al., 1934; Bruehl, 1957) and a few have been used in Japan with partial success (Waldee, 1949). On the other hand, Bruehl (1964) stated that no resistant winter wheats are known. The low incidence of natural infection in many other graminae would indicate that they may be partially resistant.

The disease may be seedborne (Nisikado et al., 1934) and may serve to introduce the organism into an area. The main method of overwintering of the fungus is apparently on infected wheat straw (Nisikado et al., 1934; Bruehl & Strobel, 1957; Bruehl & Lai, 1966). Oteino (1961) suggests that infection takes place through the scutellum and, as such, injury to the

host is not necessary. However, other workers have found that it is necessary to injure the host in order to obtain infection (Bruehl, 1957; Spalding et al., 1961; Rivera & Bruehl, 1963). Natural infection can occur by root damaging insects such as wireworms (Slope & Bardner, 1965) or through other physical injury such as drought cracks of the soil or freezing and thawing (Bruehl, 1957; Spalding et al., 1961; Bruehl, 1964; Pool & Sharp, 1966). The natural mode of entry for the pathogen is probably through the roots (Ikata & Kawai, 1938; Pool & Sharp, 1966) though this channel of entry is disputed by Otieno (1961). Recent attempts to isolate the fungus from the roots of diseased plants have been unsuccessful (Bruehl 1957; Otieno, 1961) although it was identified in the roots (Ikata & Kawai, 1938) and was isolated from roots in this study. The fungus has been isolated from all the above ground portions of wheat except the awns (Bruehl 1957; Bruehl, 1964; Lai & Bruehl, 1966). Spalding et al. (1961) speculated that the pathogen could persist possibly two to three years in infected host material. Lai & Bruehl (1966) have shown a survival for 24 months in different soils under field conditions but not under controlled laboratory conditions. They also found that C. gramineum survives longer in straw at 10 C than at 20 C.

Spalding et al. (1961) showed that there is less moisture in the upper 1/3 of infected culms than in the same region of healthy culms. They also indicated that ripening culms and heads loose moisture more rapidly in infected than in healthy plants.

Cephalosporium stripe disease is most often found in areas receiving drainage water (Bruehl, 1957) or in soils of high winter moisture (Spalding

et al., 1961). However, Bruehl (1956a) suggested that low soil moisture levels in the field possibly obscured leaf striping and disease diagnosis since desiccation of blighted culms would arrest development of the pathogen and the discoloration would not develop. Controlled humidity studies indicate that low humidities favor the survival of the organism in infected straw at high temperatures (Ikata & Kawai, 1938), but at moderate temperatures of 10 to 20 C, Lai & Bruehl (1966) found no influence of soil moisture content on the survival of C. gramineum in buried infected straws.

Cephalosporium gramineum has been reported to cause severe damage to conductive tissue of wheat culms (Nisikado et al., 1934; Bruehl, 1957; Spalding et al., 1961; Otieno, 1961). Such damage could interfere with the uptake and distribution of water by wheat plants. Spalding et al (1961), using a 1% aqueous solution of Eosin Y, showed that lateral movement in diseased plants was restricted and that malfunctioning of the vascular pathway was complete even in the early stages of stripe formation. They also demonstrated a yellow dark amorphous material in the vessels in addition to mycelium (Nisikado et al., 1934; Otieno, 1961) and some pectin plugs.

Spalding et al. (1961) demonstrated that C. gramineum produces polygalacturonase, pectinesterase, and cellulases and that these were not important in pathogenesis. It was further shown that C. gramineum cannot utilize cellulose as a carbon source to any extent (Spalding et al., 1961; Lai & Bruehl, 1966).

When a culture medium in which C. gramineum had grown was divided into several fractions (Spalding et al., 1961), it was found that an unpurified fraction containing an alcohol insoluble polysaccharide was not readily taken up by excised wheat culms. They were unable to explain the reduction in fluid uptake caused by this polysaccharide fraction.

The economic importance of this disease is readily evident when one considers that entire plants often die before heading, heads may die in the boot, or the heads may emerge but blight prematurely thus restricting kernel filling (Bruehl, 1956b). The infected grain, being low in starch, has a high protein to carbohydrate ratio (Spalding et al., 1961). Such characteristics are often associated with wheat grown under dryland conditions.

MATERIALS & METHODS

The winter wheat most extensively used in this investigation was Cheyenne (C.I. 8885). The Cephalosporium gramineum culture used was isolated from wheat grown near Creston, Montana, in 1965.

Infected plants were identified by inspection of the leaves and sheaths for continuous chlorotic and necrotic striping. Presence of the organism was verified by plating suspect samples on potato dextrose agar containing 30 ppm streptomycin and 0.25 ml/liter of a 1% solution of rose bengal. After a few weeks at 50 F, Cephalosporium gramineum colonies were recovered from the agar around infected samples. After the plants had headed out, infected tillers were detected by the early maturing or white heads. This assay method was corroborated by the leaf striping and by plating leaf and culm samples on potato dextrose-rose bengal-streptomycin agar.

Except where otherwise indicated, the procedure for inoculating plants was to sever the roots in the soil with a straight blade. This cutting operation left a narrow trench into which a broth culture of the fungus was poured. The media used for growing cultures for the field studies in 1965 is outlined in Table I. The undertaking of chemical studies necessitated simplification of the culture medium. In Table II is given the broth used for the field inoculation in 1966 as well as the controlled environment and chemical studies. A 30 day old culture was used for the field inoculation studies. A ten day old culture was used in the other inoculation studies.

FIELD EXPERIMENTS

Field studies were conducted at Bozeman (sandy loam soil), Missoula (clay loam soil), and at the Central Branch Station at Moccasin, Montana,

Table I Broth culture used for field pathogenicity studies.

Chemical	grams per liter
Sucrose	15
$(NH_4)_2SO_4$	5
K_2HPO_4	1.3
KH_2PO_4	1.0
$MgSO_4 \cdot 7H_2O$	0.5
Peptone	0.5
Thiamine HCl	10^{-5}
Pyridoxine HCl	10^{-5}
Calcium Pantothenate	10^{-5}

Trace elements: Fe, Zn, Co, Mn, Mo, Cu, Ca

Table II Broth cultures used for inoculation and polysaccharide studies

Chemical	grams per liter
Sucrose	15
K_2HPO_4	1.3
KH_2PO_4	1.0
$(NH_4)_2SO_4$	1.0
$MgSO_4 \cdot 7H_2O$	0.5

(Judith clay loam with a calcarious base).

I. Distribution of Disease throughout the State

A survey was made of the state in 1965, 1966, and 1967 in a search for a possible distribution pattern of the disease. A few additional samples were supplied by County Extension Agents.

II. Pathogenicity Tests at Bozeman and Missoula

At Bozeman, in 1965, 261 lines of Westmont x P.I. 178383 and 39 lines of Itana x P.I. 178383 were surveyed for differences in susceptibility to natural infection. The center 16 feet of a 18 foot single row, non-replicated planting was used. The infected plants were counted in July and the white heads counted in August. In another test, 24 varieties and 9 bulk selections (Table VIII) were planted in four replications, each replication consisting of three 18 foot row plots. Infected plants were determined in June and July on the basis of leaf symptoms and in August on the basis of prematurely blighting heads. On July 2, 1965, three selections (Itana, Rio, and Burt x Itana-113) were tagged on individual tillers exhibiting different degrees of advance of the symptoms. A record was kept of the youngest leaf of the tiller exhibiting visible symptoms. The stages of growth of the plants were recorded by use of the Feekes scale (Large, 1954). Sixty plants each of Rio and Itana were tagged: ten with no visible symptoms, ten with symptoms advanced no higher than the lowest or 5th leaf, and likewise ten each with symptoms advanced to the 4th, 3rd, 2nd, and flag or 1st leaves. Twenty plants of Burt x Itana-113 selection, which had no visible symptoms, were tagged. Ten plants each with symptoms

advanced to the 5th, 4th, 3rd, and 2nd, leaves were tagged. Only one plant of Burt x Itana-113 could be found in 192 feet of row with symptoms advanced to the flag leaf.

Due to a very low incidence of infection at the Bozeman experimental nursery in 1966, the natural infection study was conducted in a nursery at Missoula, Montana. This nursery contained 16 varieties planted in 18 foot single row plots in six replications (Table VIII). A 16 foot section was counted in each row. A high number of misplaced labels invalidated the experiment involving the tagging of individual tillers. Leaf symptom counts and white head counts made in May and July respectively, were used to compare the susceptibility of the various varieties to natural infection. Field observations were made at Missoula in 1967 in a field adjacent to that of the 1966 study and consisted of 15 varieties planted in 4 row plots with four replications (Table VIII). The field was surveyed in May for leaf symptoms of the Cephalosporium stripe disease.

III. Cultural Experiments at Moccasin

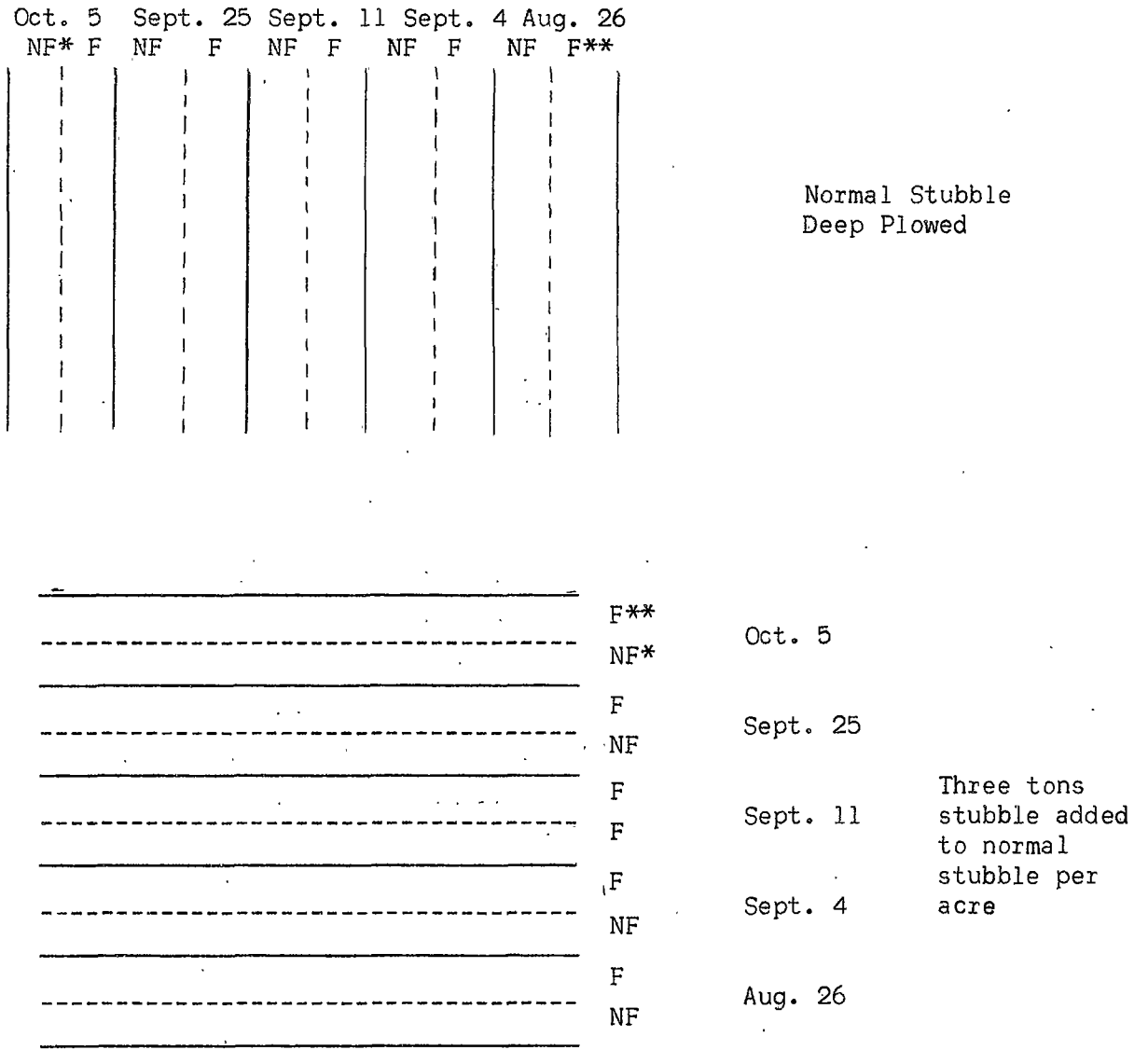
At Moccasin, Montana, the susceptibility of ten varieties to natural and artificial infection was tested in 1965 (Table VII). For each variety, three adjacent relatively uniform 12 foot rows were used. One row was injured and inoculated as described with one liter of the C. gramineum culture. A second row was injured but not inoculated. The remaining row served as an uninjured control. Disease readings were made in June and July comparing the different treatments. The plants were harvested in August, 1965. The grain from the injured control and non-injured control

rows was kept separate but not subdivided. The plants from each inoculated row were divided into two groups. One group consisted of infected plants and the other group of non-infected plants. Fifty heads were randomly selected and hand threshed from these infected and non-infected groups. The remaining samples were threshed in a head thresher with no fan and hand-winnowed to save the small kernels.

At Moccasin the effect of fertilizer, date of planting, and amount of stubble was investigated in 1965 and 1966 using Cheyenne winter wheat. These factors were studied to determine their relationship to natural and artificial infection with C. gramineum. Artificial inoculation was effected in the spring by cutting the roots in 12 feet of uniform row with a straightened hoe and pouring a broth inoculum into the slit in the ground made by the hoe.

In 1965, five different planting dates were used in conjunction with two fertilized treatments and two stubble treatments (Figure I). For each of the planting dates and fertilizer conditions within the high stubble treatment, three 12 foot rows were carefully compared for Cephalosporium stripe disease. One row had the roots injured as previously described and was inoculated with approximately one liter of culture. A second row was injured but not inoculated. The third row served as an uninjured, non-inoculated control. Disease readings were taken for these rows in June, July, and at harvest in August. Detailed artificial inoculation studies were not conducted on wheat planted in the deep plowed field. The yield from 32 foot rows for each of the 18 treatments

Figure I Planting plan for date of seeding-stubble-fertilizer experiment at Moccasin, Montana, 1964.



* Not Fertilized

** Fertilized

was obtained. The 32 foot rows were threshed in a plot thresher. The 12 foot rows were threshed in a head thresher without a fan, and hand winnowed to save the small, light kernels.

The 1966 Moccasin study consisted of 3 planting dates with two stubble treatments and two fertilizer treatments (Figure II). For this study, five adjacent rows of plants for each of the 16 different treatments were studied as follows:

1) Plants injured
   A) Two liters of inoculum (4.83×10^6 spores/cc)* followed by two liters distilled water.
   B) Two liters of autoclaved inoculum followed by two liters distilled water.
   C) Non-inoculated control.
2) Plants not injured
   A) Two liters of inoculum and two liters distilled water poured on the base of the plants.
   B) Non-inoculated control.

The number of infected plants per row was determined in June and white head counts were made in July. The plants were threshed in a head thresher using a low air flow and hand-winnowed to save the light kernels.

Since there appeared to be a high correlation between yield and date of planting, an analysis was made of the soil temperatures associated with

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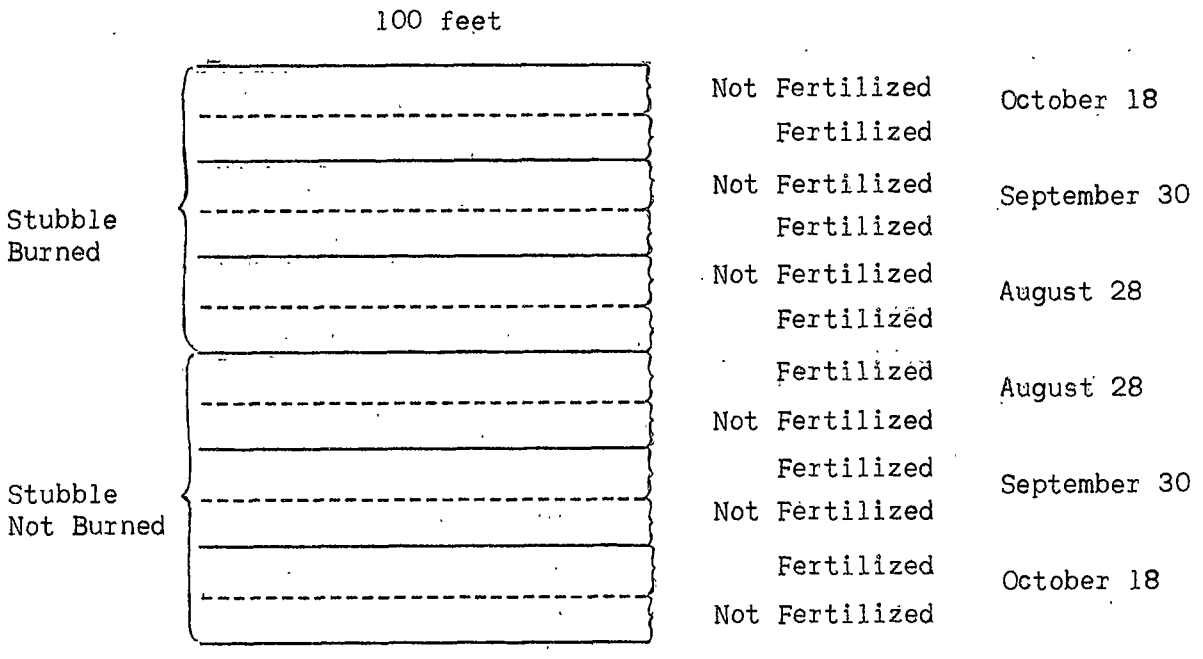
* Conidiospore counts were made using a No. 508 Levy-Hausser Counting Chamber.

Figure II Planting plan for date of seeding-stubble-fertilizer experiment at Moccasin, Montana, 1965.



the different planting dates. On the basis of this information, spring root lengths were measured on March 19, 1966, and April 2, 1967, by digging trenches in the plots and taking measurements with a meter stick.

A soil ice layer was measured in March, 1966, in the plots. No winter ice layer was observed in the 1967 test field.

CONTROLLED ENVIRONMENT STUDIES

Controlled environment studies were conducted in an ISCO model E environment chamber equiped with G.E. cool-white lamps. Unless otherwise indicated, the temperature profile consisted of 12 hours of light (3000 FC) at 72 F, 69% relative humidity, and 9 hours of darkness at 45 F, 89% relative humidity. Between the night and day conditions there was a 1.5 hour interim during which 1/3 of the lights were on (1000 FC) and the chamber was maintained at 55 F and 75% relative humidity. Except where otherwise indicated, four inch clay pots were filled with Bozeman topsoil mixed with peat moss 3:1 (soil:peat moss). Cheyenne winter wheat harvested from a single 32 foot row in 1965 was planted 25 seeds per pot. Three pots were planted for each treatment of an experiment and each experiment was replicated three times. After 14 days the seedlings were thinned to a uniform 20 plants per pot. Plants to be kept on maximum water uptake were generally placed in common water containers, five pots per tray. Two weeks prior to inoculation they were transfered to petri dishes which were kept filled with water. Pots with restricted soil moisture were maintained for seven days in separate petri dishes filled with water. After seven days they were only watered once daily at 9:30 AM by placing 25 ml of water into

the petri dish. The plants were inoculated by cutting the roots between the two rows with a knife. Ten ml of inoculum was poured into the cut area followed by ten ml of distilled water. The control plants received ten ml of autoclaved inoculum followed by ten ml of distilled water. After inoculation, all plants were maintained in separate petri dishes. The pots were at all times randomly placed throughout the chamber and sub-irrigated.

I. Soil Moisture Relationship to Disease Development

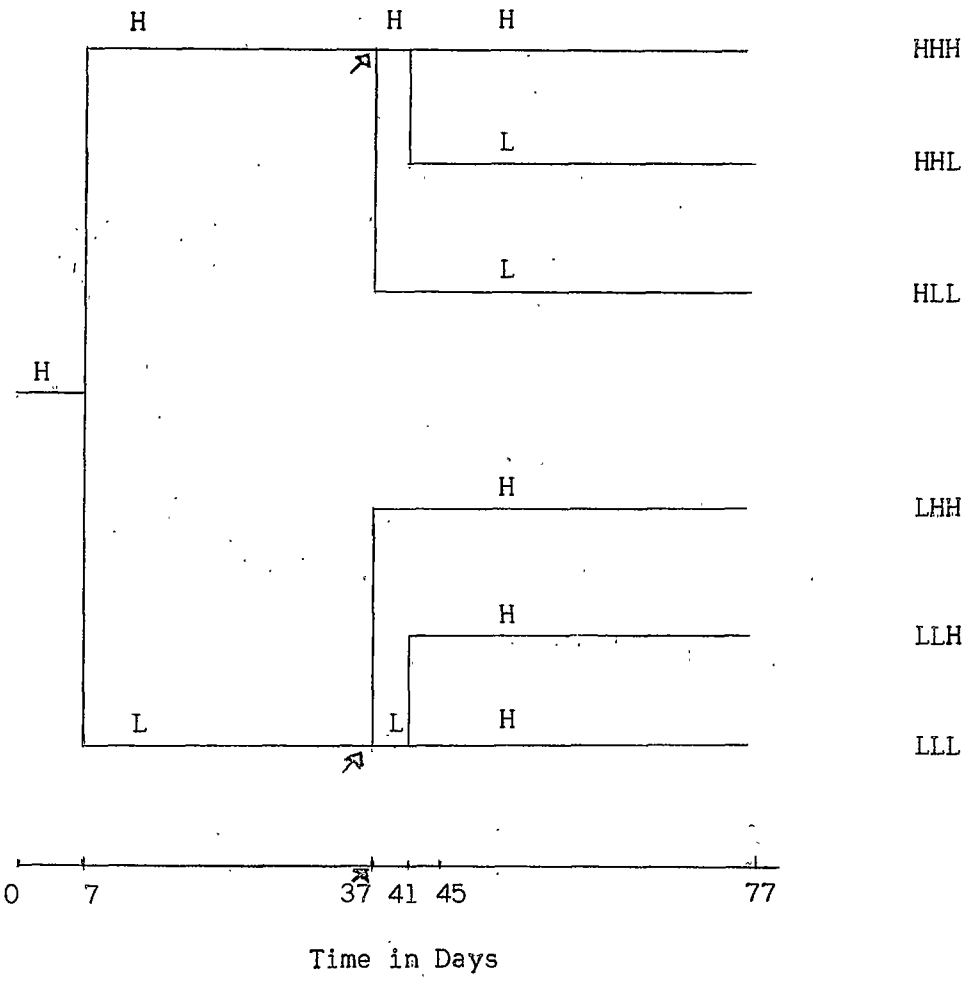
A. Soil Moisture before Vernalization

An experiment was conducted to test a possible relationship between soil moisture and the incidence of disease. The objectives of this test were to determine 1) if soil moisture before inoculation influences the infection process, and 2) if soil moisture after inoculation influences the infection process. A flow sheet, Figure III, diagramatically illustrates the conditions of the experiment. When seeded, the pots of Cheyenne were placed in shallow pans of water in the environment chamber and treated as previously described. At seven days they were separated into high and low moisture groups. At 37 days (four leaf stage) the plants were inoculated. The first replication received 3.6×10^7 spores per ml; the second replication received 3.2×10^7 spores per ml; and the third replication received 4.7×10^7 spores per ml.

Immediately after inoculation, one third of the pots on restricted soil water were saturated with water by sub-irrigation. After four days another third of the pots was saturated with water. The remaining

Figure III Flow sheet of controlled soil moisture conditions prior to vernalization.

Flow Sheet	Title of Treatment



H - Pots in water maintaining the soil at field capacity. Soil moisture 35%

L - Pots with limited moisture availability. Soil moisture 20%.

↗ - Inoculation of plants in all pots

plants received a restricted water ration. These conditions were maintained until the experiment was terminated 40 days after the plants had been inoculated. These treatments were labeled LHH, LLH, and LLL respectively.

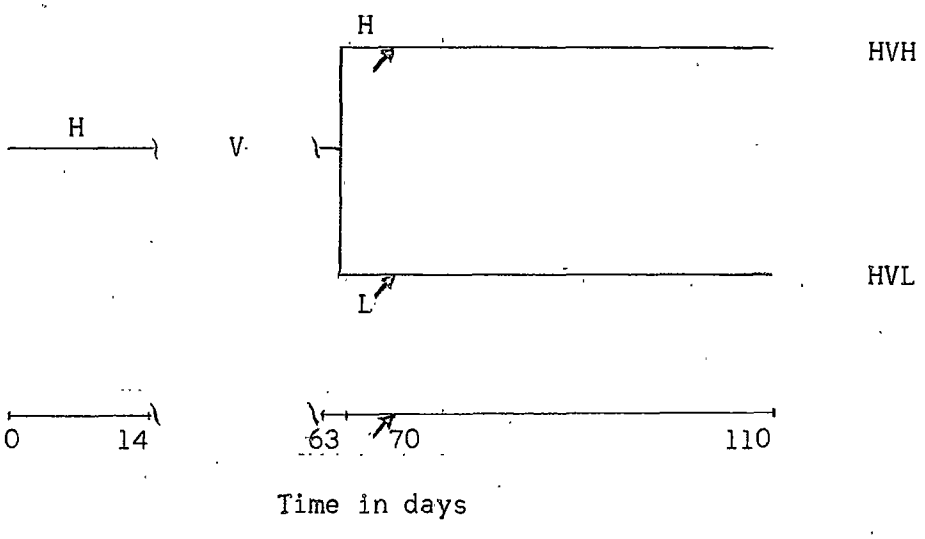
The pots maintained at maximum moisture capacity prior to inoculation were treated in a similar manner. Immediately after inoculation, one-third of the pots were kept without water for 4 days to allow the soil to partially dry. They were then maintained on a restricted water ration of 25 ml per day. The other pots remained in petri dishes filled with water. Four days after inoculated, another third of the plants were deprived of water for four days, then allowed only 25 ml of water per day. The remaining pots were kept in the petri dishes full of water. These conditions were maintained until the experiment was terminated 40 days after the plants were inoculated. These treatments were labeled HLL, HHL, and HHH, respectively.

B. Soil Moisture after Vernalization

An experiment was conducted to assess the effect of soil moisture on disease inception after the plants were vernalized. Figure IV illustrates the conditions of the experiment. The potted seedlings were maintained at high moisture availability in the environment chamber for 14 days (two leaf stage) at which time they were transfered to the vernalization chamber. After vernalizing for 49 days, they were separated into two groups and placed in petri dishes in the environment chamber. For seven days one group of pots was kept in dishes of water while the other group was given 25 ml of water per day. The pots were inoculated as described above. The

Figure IV Flow sheet of controlled soil moisture conditions after vernalization.

Flow Sheet	Title of treatment



H - Pots in water maintaining the soil at field capacity. Soil moisture 35%

L - Pots with limited moisture availability. Soil moisture 20%

V - Plants being vernalized.

↗ - Inoculation of plants in all pots.

first replicate received 4.1×10^7 spores per ml; the second replicate received 3.1×10^7 spores per ml; and the third replicate received 3.3×10^7 spores per ml. The plants were retained at the same soil moisture availability after inoculation as for the week preceeding inoculation. The experiment was terminated after 110 days (40 days after inoculation).

C. Effect of Vernalization on the Host-Parasite Interaction

An experiment was designed to see if fall infected plants can outgrow the disease. The procedure used in this test was to grow seedlings under high moisture conditions for the entire experiment. At the four leaf stage (37 days), the plants were inoculated. Replicate one received 5.6×10^7 spores per ml; replicate two received 5.2×10^7 spores per ml; and replicate three received 4.6×10^7 spores per ml. Immediately after inoculation, one-third of the plants were placed in the vernalization chamber. After four days, another third was placed in the vernalization chamber, and 15 days after inoculation the remaining plants were placed in the vernalization chamber. Each set of plants was vernalized for 49 days then returned to the environment chamber and was maintained under high moisture conditions for another 40 days. The number of plants exhibiting symptoms was determined when the pots were removed from the vernalization chamber and at the end of the experiment.

D. Adsorption of Cephalosporium gramineum Conidiospores to Dry Soil

It is possible that the variations in the degrees of infection realized in some experiments may be the result of a differential adsorption of conidiospores to the soil rather than some difference in the host itself.

To test this possibility, pots were seeded in the usual manner being separated into high and low soil moisture groups at seven days. At the four leaf stage (37 days), all of the soil was washed from the roots of the plants. The roots were then blotted dry and allowed to air dry at room temperature for one hour. The plants were then repacked into the pots with fresh, dry soil. The leaves of the plants were trimmed. At this point the plants which had been allowed free water were divided into two groups. One group was saturated and set in a pan of water for four hours. The other group was left dry in the soil. The plants which had been allowed only 25 ml of water per day were similarly divided and watered. After inoculation, the plants in dry soil were sub-irrigated with 25 ml of water. After 40 days at either high or low soil moisture availability, the plants were inspected for disease incidence and discarded. The first replicate was inoculated with 5.6×10^7 conidiospores per ml; the second with 5.2×10^7 spores per ml; and the third with 4.6×10^7 spores per ml.

E. Soil Moisture Determinations

Soil moisture content was determined using an Ohaus moisture determination balance Model 6010. Soil samples were taken at three depths in the pots. Ten (10.00) gram soil samples were heated 15 minutes with a Westinghouse infrared heat ray lamp. A pan temperature of approximately 650 F was obtained with the lamp one inch from the pan and using 140 watts. The soil samples were not charred by this threatment. All plants were removed prior to soil moisture determinations.

II. Seedborne Infection

An experiment was conducted to determine if the disease was seedborne but possibly not expressed until after the plants were vernalized. Twenty seeds of Cheyenne from known infected parent plants were planted in each of five pots. The plants were kept for 37 days in the environment chamber, then vernalized 49 days at 40 F. Forty days after being returned to the environment chamber, the plants were checked for Cephalosporium stripe disease. The pots received an optimum amount of water throughout the experiment.

III. Relationship between Date of Planting and Root Growth

Cephalosporium gramineum infection appears to be more severe when the grain is planted early rather than late in the fall. A controlled environment experiment was conducted in which 1964 autumn field conditions of Moccasin, Montana, were simulated. Judith clay loam soil from a wheat field that had been in summer fallow one year was placed in six wooden boxes. The boxes measured 8 x 20 x 21 inches I. D. The six boxes were wrapped in a single plastic envelope and fitted into an ISCO model E environment chamber measuring 30.5 x 47 inches I. D. Field soil temperature and day length conditions were simulated by daily programing the chamber. A single row of Cheyenne winter wheat was planted in each box at the rate of 60 pounds per acre. Twice a week the plants were given one liter of water. The dates of planting varied to coincide with three field dates used in 1964 (August 26, September 4, and September 25). One box at each planting date was fertilized with $Ca_3(PO_4)_2$ as 20 pounds

of P_2O_5 per acre. Soil temperatures were measured with a continuously recording soil-air thermograph. The simulated field conditions were maintained for 2.5 months until freezing temperatures were reached. At this point the side of each box was opened and the soil gently washed from the roots. Root lengths were measured for each of the six conditions. As an indication of the extent of branching, the air dried weight of the root system plus two mm. stem was obtained.

IV. Longevity of the Organism

To assess the longevity of C. gramineum in wheat straws, naturally infected straws were cut into two inch lengths and placed on wire screens inside air-tight vessels. Controlled relative humidities were maintained within the containers by the use of various saturated salt solutions (Table III). One set of samples was kept in the dark at 68 ± 1 F. Another set was placed in a chamber programed for a 35/65 (dark/light) diurnal profile (Sharp, 1965). Samples were periodically removed and tested for growth of C. gramineum. In another test, infected straws were buried in soil containing finely chopped residues of either wheat, barley, oats, alfalfa, or safflower, both sterilized and non-sterilized. For each treatment, 11.4 grams of residue were thoroughly mixed with soil in seven inch clay pots (equivalent to two tons/acre). The pots were placed in the greenhouse where moisture conditions within the pots alternated between wet and dry. Samples of the wheat straws were periodically tested for C. gramineum.

Table III Solutions used for controlled relative humidities.

Chemicals used for Saturated salt solutions	Relative humidity
$CaCl_2$	0%
$LiCl \cdot H_2O$	15%
$KC_2H_3O_2$	20%
$MgCl_2 \cdot 6H_2O$	33%
$K_2CO_3 \cdot 2H_2O$	43%
$Na_2Cr_2O_7 \cdot 2H_2O$	52%
$NaBr \cdot 2H_2O$	58%
$NaNO_2$	66%
NaCl	76%
KBr	84%
$Na_2HPO_4 \cdot 12H_2O$	95%
Distilled H_2O	100%

V. Greenhouse Experiments

Experimental conditions in the greenhouse varied from 65 F to 95 F with a day length from eight hours and 40 minutes to 15 hours and 20 minutes. Replicates of greenhouse experiments were run simultaneously in attempts to avoid the effects of these extreme conditions.

A. Rate of Advance of Symptoms

Ten varieties and selections were planted in vermiculite - 15 plants per pot in each of two pots. After 14 days in trays of water they were vernalized at 40 F. They were then planted in a greenhouse bench in single four foot rows with two replicates. The bench soil was a mixture of Judith clay loam, Bozeman top soil, peat, and a wind blown silt which collects in the Three Forks area of Montana. The plants were watered daily. After the plants had been in the greenhouse for 28 days, the roots were injured and each row was inoculated with 40 ml of broth containing 2.4×10^7 conidiospores per ml. This was followed by 40 ml of distilled water. The number of infected plants was determined at 21 and 39 days after inoculation and when harvested.

B. Infection Process

Cheyenne winter wheat was vernalized for at least 12 weeks to induce tillering, placed in the greenhouse for one week and then inoculated. Inoculation was effected by 1) cutting the roots and adding ten ml of inoculum solution followed by ten ml of distilled water, 2) hypodermically injecting one tiller at a point two inches above the ground, 3) subepidermally injecting the second leaf from the top of the plant, or

4) infecting the plant above the upper most node. Information was obtained concerning the rate and direction of movement of the organism in the host.

CULTURE OF THE ORGANISM

In the initial isolations from the longevity test, straw samples were surface sterilized for at least two minutes in a solution containing equal parts of Chlorox (sodium hypochlorite 5.25%) and 95% ethanol. They were then plated on potato dextrose agar containing rose bengal (1.0 ml/liter of a 1% solution) and streptomycin (30 ppm) (Paharia and Kommedahl, 1956). Readings were made after the plates had been incubated one to two weeks at 68 F. In later tests the technique was modified to gently washing the straw samples in distilled water rather than using surface sterilization. Additionally, the rose bengal was reduced from 1.0 ml/liter to 0.25 ml/liter, the incubation temperature lowered to 50 F and the incubation interval, prior to making a final evaluation, lengthened to two months.

Broth culture of C. gramineum was conducted at room temperature in quantities ranging from ten ml to 40,000 ml. Unless otherwise indicated, the samples were shaken three or four times during the day. The medium used for field inoculation studies (Table I) was later modified (Table II) and used for the inoculation of potted plants and the polysaccharide studies. Since the broth cultures were conducive to budding of the conidiospores, the inoculum potential was estimated by the use of a Number 508 Levy-Hausser counting chamber.

CHEMICAL STUDIES

I. Polysaccharide Study

Separation of a fungal polysaccharide from the C. gramineum broth culture was initiated by centrifuging the suspension at 15,000 x g. for two minutes to remove the cells. An equal volume of 95% ethanol was added to the supernatanat and the precipitated polysaccharide collected on a glass rod (Spalding, et al., 1961) and placed in 95% ethanol. The transparent precipitate was washed once with 95% ethanol then dissolved in distilled water. It was reprecipitated with an equal volume of 95% ethanol for a total of four precipitations. Prior to the fourth precipitation, the sample was vacuum filtered through water washed Whatman Number Two filter paper. After the fourth precipitation, the polysaccharide was dissolved in deionized, distilled water and 10 to 25 ml samples were dialized overnight against two 2-liter changes of deionized distilled water. The sample was again precipitated with 95% ethanol, redissolved, filtered, reprecipitated, and analyzed.

The polysaccharide was hydrolyzed by autoclaving (15 psi) for two hours in 1N HCl, 1N H_2SO_4, or 3.3N H_2SO_4. The hydrolysis products were separated by descending paper chromatography using n-butanol:acetic acid:water::4:1:5 or n-butanol:pyridine:water::3:1:1. The method of Trevelyan (1950) was used to detect the products of acid hydrolysis. Thin layer chromatograms were run on silica gel G:aluminum oxide G:water::12 g:12 g:40 ml. The solvent system used was formic acid:methyl-ethyl-ketone:tertiary butanol:water::15:30:40:15. Detection was effected by the

method of Stahl (1965) using:

anisaldehyde	0.5 ml
95% ethanol	9.0 ml
36N sulfuric acid	0.5 ml
glacial acetic acid	0.1 ml

The plates were heated to 100 C for five to 10 minutes and the sugars were identified by $R_{glucose}$ and specific color reactions. Since many of the paper chromatograms were allowed to run off the paper, $R_{glucose}$* was used rather than R_f.

The percentage of carbohydrate in the molecule was determined by the phenol-sulfuric test (Koch et al., 1951; Dubois et al., 1956; Hodge and Hofreiter, 1962). A known volume of the polysaccharide solution was placed on a pre-weighed coverslip and dried to a constant weight over Drierite ($CaSO_4$). The μg per ml carbohydrate expressed as glucose was determined for the polysaccharide solution. Glucose was used as a standard. Colorimetric readings were made using a Spectronic 20 at 490 mμ.

Molecular weight estimation was made using Sephadex G-200. From a 35 ml column, 23 cm high, 1.5 ml fractions were colected using an LKB** automatic fraction collector. The flow rate was six to nine ml per hour. The void volume was measured using blue dextran (M.W. 2×10^6) and occurred in tube number seven with slight trailings in tubes six and eight. The polysaccharride was detected in the effluent by precipitation with ethanol or by the phenol-sulfuric test.

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* $R_{glucose}$ = migration distance of specimen/migration distance of glucose

** LKB, Produkter AB, Stockholm 12, Sweden

Analysis for sugar linking was made using β-glucosidase* and α-amylase**. In the first test with α-amylase, 100 ml of polysaccharide solution was mixed with 10 ml of 1N sodium acetate. The pH was adjusted to 6.9 using HCl, and 0.3 ml of α-amylase was added. In the first test with β-glucosidase, 100 ml of polysaccharide was mixed with 11 ml of 1N sodium acetate. The solution was brought to pH 6.8 using acetic acid, and 1.0 mg of β-glucosidase was added. The samples were incubated for 24 hours at room temperature with occasional shaking of the flasks.

In the subsequent test, 120 ml of polysaccharide was mixed with 13 ml of 1N sodium acetate and the pH adjusted to 6.8 with acetic acid. To one 40 ml fraction, 0.6 mg of β-glucosidase was added. To a second 40 ml fraction 0.15 ml of α-amylase was added. The third fraction served as a control. The samples were incubated for 24 hours at room temperature on a Burrell wrist action shaker.

Control viscosities were determined before the incubation period. After the incubation, viscosities were determined on all samples. Additionally, the products were tested for ethanol precipitation and reducing sugars before and after the incubation period.

The molecule was tested for lipid content by extracting with anhydrous ether for 18 hours. The ether fraction was dried using N_2 and thin layer chromatographed using hexane:ethyl ether:acetic acid::80:20:1

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* Beta Glucosidase (Almond), Sigma Chemical Company, St. Louis, Missouri

** Alpha Amylase 2x Crystalline, Nutritional Biochemicals Corporation, Cleveland, Ohio