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
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Abstract:
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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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ROBERT ALFRED FRANK POOL

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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

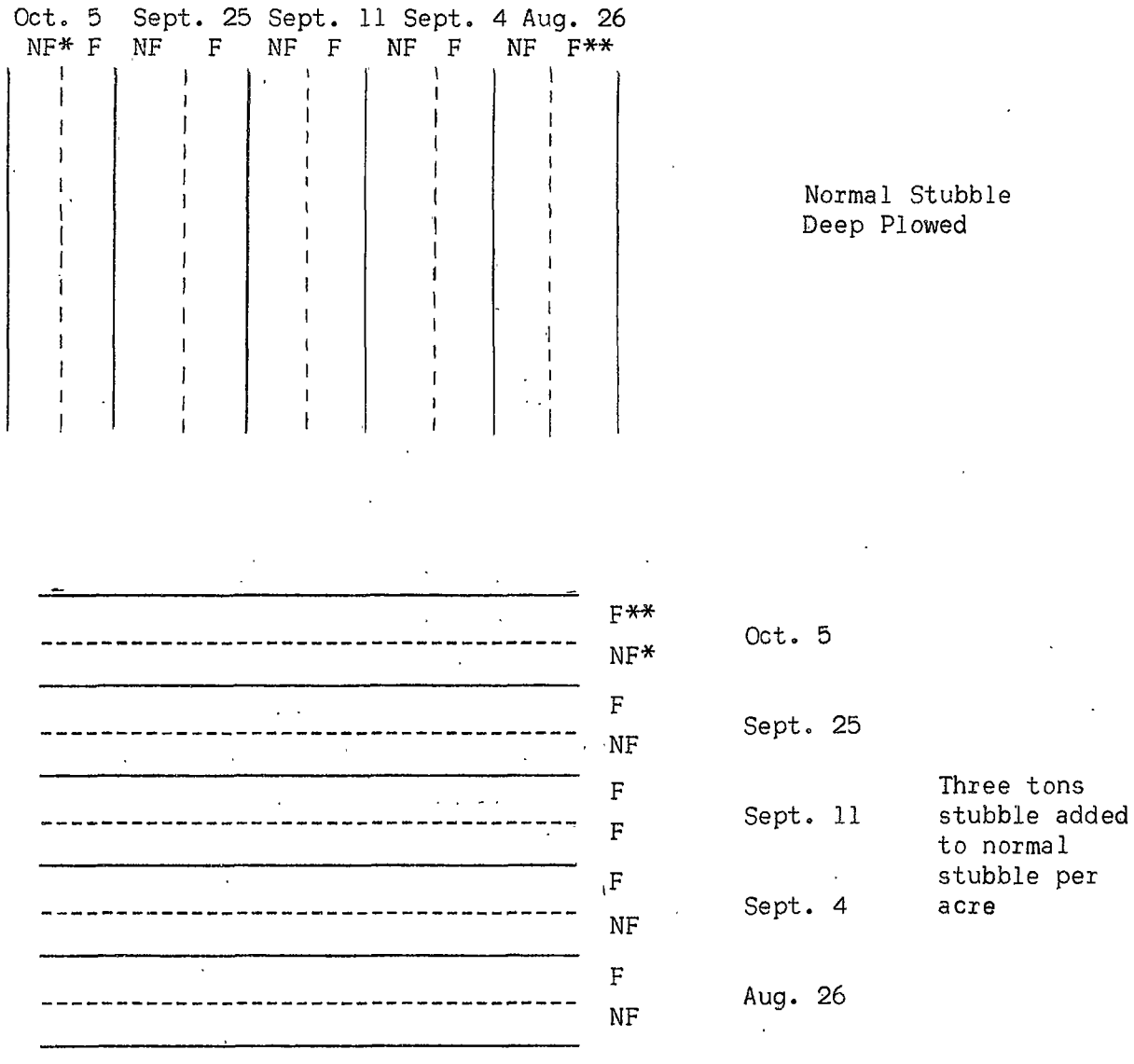
At Moccasin, Montana, the susceptibility of ten varieties to natural and artificial infection was tested in 1965 (Table VII). For each variety, three adjacent relatively uniform 12 foot rows were used. One row was injured and inoculated as described with one liter of the C. gramineum culture. A second 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.

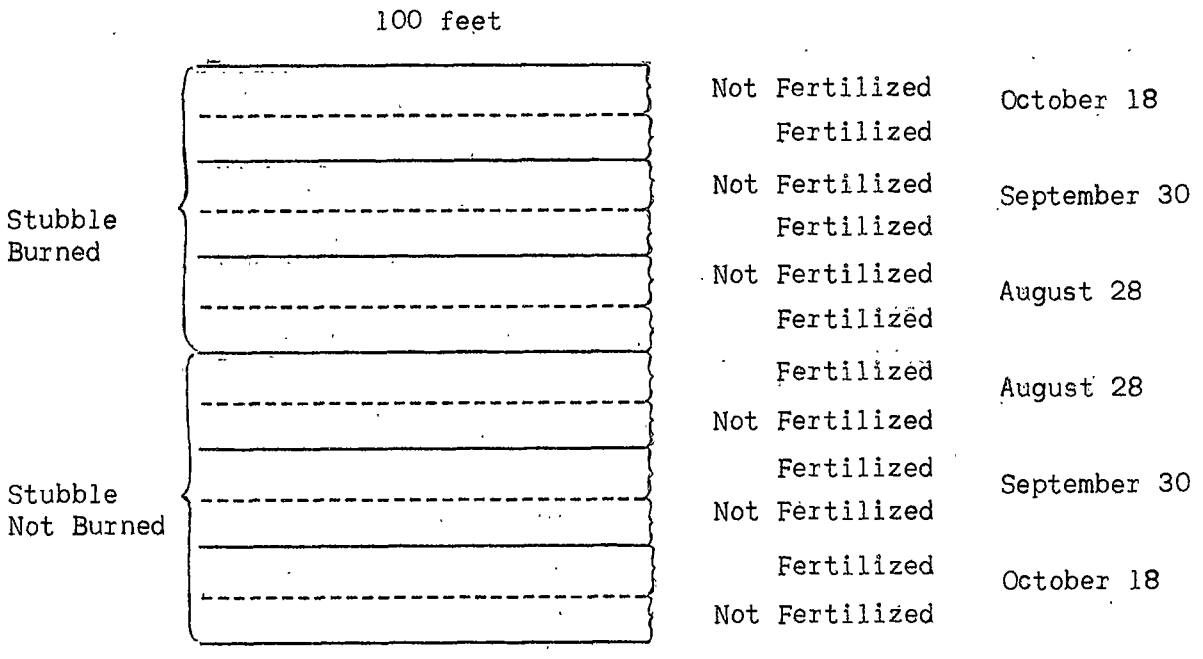
The number of infected plants per row was determined in June and white head counts were made in July. The plants were threshed in a head thresher using a low air flow and hand-winnowed to save the light kernels.

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

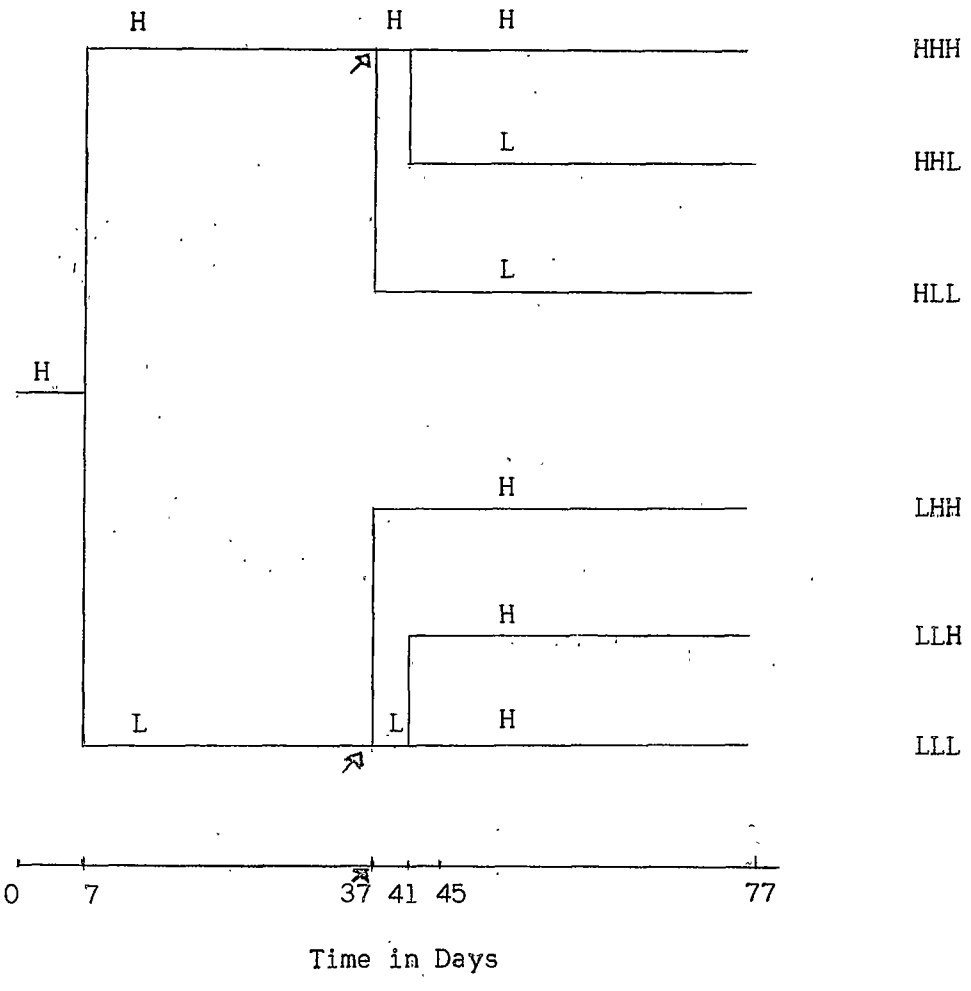
A. Soil Moisture before Vernalization

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.

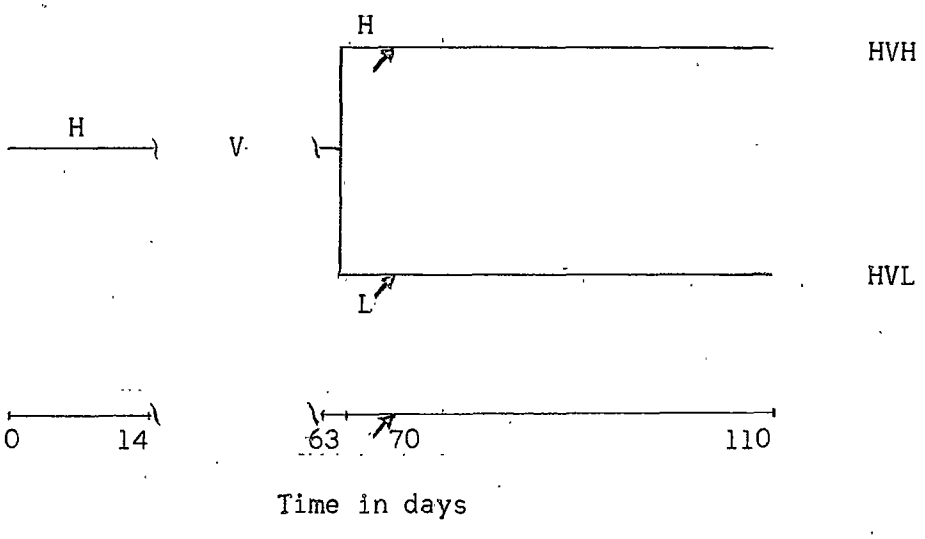
The pots maintained at maximum moisture capacity prior to inoculation were treated in a similar manner. Immediately after inoculation, one-third of the pots were kept without water for 4 days to allow the soil to partially dry. They were then maintained on a restricted water ration of 25 ml per day. 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

solvent system. The sample was developed with sulfuric acid-potassium dichromate and heated to 100 C for 10 minutes.

The polysaccharide was identified in infected straw. The straw was ground either in a mortar and pestle or a Sorvall Omnimixer. One gram (1.0 g) dry straw was mixed with 100 ml of water, or 30.0 grams of fresh tissue was mixed with 175 ml of water. The ground sample was autoclaved 20 minutes at 15 psi., then placed at room temperature overnight. Cellular debris was removed by filtration through water washed J. Green Number 488 filter paper. The eluent was then filtered through a millipore filter. The fresh tissue samples were run through Amberlite IRA-401 ion exchange resin charged with 6N HCl. This treatment effected removal of most of the pigments. The samples were then concentrated to three to five ml on a flash evaporator - temperatures 50 ± 1 C and 4 ± 1C, vacuum 24 ± 1 inches of water. Samples of 0.5 ml were filtered through a 35 ml column of Sephadex G-200 at a flow rate of four tubes per hour, 1.5 ml per tube as previously described. The pooled samples from tubes five through 12 were concentrated using a flash evaporator. The sample was further concentrated to 0.5 ml by a stream of nitrogen. One ml of 95% ethanol was added, the mixture shaken and observed for a precipitate. The sample was then centrifuged for one minute in an International Clinical Centrifuge, the supernatant discarded, and the precipitate washed with 1.5 ml of 95% ethanol. The precipitate was dissolved in one ml of distilled water and tested for sugars by the phenol-sulfuric test.

The purified polysaccharide was tested as a carbon source for the growth of Cephalosporium gramineum. Minimal broth solution (Table II)

was prepared using a solution of the polysaccharide rather than sucrose as the carbon source. In one test, 100 ml of a 0.1 mg/ml polysaccharide was used to make a 100 ml broth. The inoculum was obtained from an agar culture. In two subsequent tests, 50 ml of 0.3 mg/ml polysaccharide was used as the carbon source. The broth was divided into four fractions. Inoculation was effected by a tube dilution method. Two loops full of a one month old broth culture was introduced into the first tube. Two loops full from the first tube were used to inoculate the second tube and two loops full from the second tube were used to inoculate the third tube. The fourth tube served as a non-inoculated control. A set of control tubes was composed of the broth lacking any carbon source. The media was autoclaved 20 minutes at 15 psi. before the inoculations. Incubation was at room temperature with the flasks being shaken several times a day.

II. Toxin

In a zone surrounding agar colonies of C. gramineum, growth of a number of organisms was inhibited. By using the NaOH-IKI-soluble starch technique of Thomas (1961), a molecule was detected with a reaction similar to that described for penicillins and cephalosporins. Loop transfers of 24 hour nutrient agar cultures of Alcaligenes faecalis, Bacillus subtilis, Salmonella typhimurium, Aerobacter aerogenes, and Pseudomonas aeruginosa were made into nutrient agar. These agar suspensions were poured into petri dishes containing six mm of agar plugs of a possible C. gramineum toxin. The six mm plugs were obtained proximal to two month old cultures of C. gramineum grown on PDA-rose bengal-streptomycin agar.

RESULTS

DISTRIBUTION OF DISEASE THROUGHOUT THE STATE:

In Figure V is indicated the distribution of C. gramineum throughout Montana in 1965, 1966, and 1967. Superimposed on each of these maps is the rainfall data during the winter wheat growing season for that year. As indicated in these figures, Cephalosporium stripe disease is prevalent in the western, wet, mountainous part of the state, but not in the dry eastern half of the state.

LONGEVITY STUDY

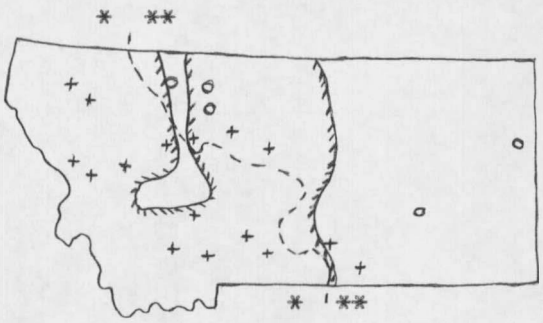
Cephalosporium gramineum was isolated from infected straw which had been retained under various humidity conditions for as long as 45 months. In the earlier isolations (0-12 months), readings were made after one week and marked positive (+) if C. gramineum was isolated from any of the samples. Later samples (after 12 months) were recorded for the percentage of samples from which colonies of C. gramineum grew. In some of the more recent samplings, it has taken two months before cultures of C. gramineum could be detected from the plated straws. This may indicate a weakened or dormant condition of the fungus.

When retained at a constant 68 F in the dark for 45 months (Table IV), infected straw suspended in jars at 0% to 43% and 95% to 100% relative humidity retained some viable C. gramineum. However, between 52% to 84% relative humidity, no viable cultures could be recovered.

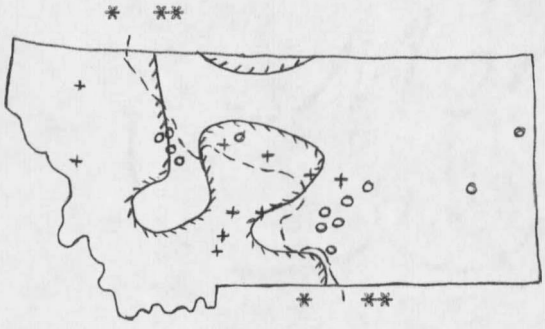
At a 35/65 F diurnal profile, infected straws in jars with 0% to 52% and 95% relative humidities retained some recoverable C. gramineum organism for 40 months (Table V). Between 58% and 84% relative humidities, no

Figure V Distribution of Cephalosporium stripe disease of wheat in Montana.

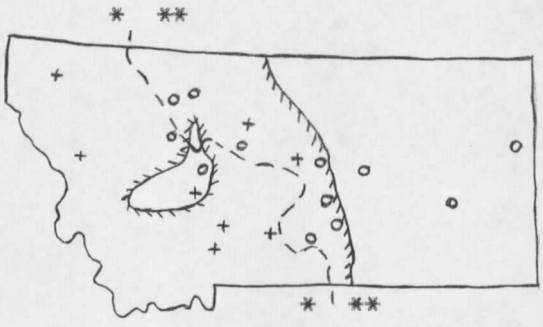
A. 1965



B. 1966



C. 1967



\+ disease definitely found
o disease definitely not found
* mountains
** plains
mm greater than 10 inches of rain during winter wheat growing season September through June

Table IV Survival of C. gramineum in naturally infected straws held at a constant 68 F under 12 different relative humidites.

Relative humidity	Vapor pressure deficit	Samples from which C. gramineum was recovered											
		storage time months											
		1*	3	6	9	12	15	22	26	30	34	40	45
0	17.5	+	+	+	+	+	+**	100	88***	91	100	100	93
15	14.9	+	+	+	+	+	+	100	80	50	25	66	57
20	14.0	+	+	+	+	+	+	63	78	100	78	67	100
33	11.7	+	+	+	+	+	+	100	100	100	78	100	93
43	10.0	+	+	+	+	+	+	56	66	66	56	33	50
52	8.4	+	+	+	+	+	+	0	11	50	0	0	0
58	7.4	+	+	+	+	+	+	0	37	13	0	0	0
66	6.0	+	+	****									
76	4.2	+	+	+	+	+	+	0	50	0	0	0	14
84	2.8	+	+	+	-	-	+	0	50	13	11	0	0
95	0.9	+	-	-	-	-	+	0	100	-100	78	85	54
100	0.0	+	-	-	-	-	+	56	25	78	25	66	28

* sample surface sterilized in 5.2% sodium hypochlorite: 95% ethanol::1:1
medium PDA, streptomycin 30 ppm, 1% rose bengal 1 ml/liter

** technique modified - straws non-surface sterilized, but washed in distilled water

*** medium modified - PDA, streptomycin, 1% rose bengal 0.25 ml/liter

**** straws accidently dropped into chemical solution

Table V Survival of C. gramineum in naturally infected straws at 12 different relative humidities under a 35/65 (night/day) cycle.

Relative Humidity	Vapor Pressure Defecit mm Hg	Samples from which C. gramineum was recovered										
		Storage time, months										
		1*	3	6	9	12	17	21	25	29	35	40
0	4.8-15.9	+	+	+	+	+**	100	100***	100	100	64	100
15	4.1-13.9	+	+	+	+	+	100	100	100	100	92	93
20	3.8-12.7	+	+	+	+	+	0	100	100	88	75	86
33	3.2-10.6	+	+	+	+	+	50	100	100	100	80	93
43	2.7- 9.0	+	+	+	+	+	100	100	89	50	92	64
52	2.3- 7.6	+	+	+	+	+	50	100	63	36	25	14
58	2.0- 6.7	+	+	+	+	+	50	50	22	13	8	0
66	1.6- 5.4	+	+	+	+	+	0	25	42	50	0	0
76	1.2- 3.8	+	+	+	+	+	0	11	21	11	0	0
84	0.7- 2.5	+	+	+	-	+	30	33	10	13	0	0
95	0.2- 0.8	+	+	+	-	+	13	33	50	66	33	50
100	0.0	+	+	+	-	-	44	13	42	66	17	0

* Sample surface sterilized in 5.25% sodium hypochlorite:ethanol::1:1
Medium - PDA, streptomycin 30 ppm, 1 ml/liter of 1% rose bengal

** Technique modified - straws non-surface sterilized, but washed in distilled water

*** Medium modified - PDA, streptomycin 30 ppm, 0.25 ml/liter of 1% rose bengal

colonies were recoverable. The vapor pressure deficit for this temperature profile varied from a high deficit at 3 PM to a low deficit at 3 AM.

When naturally infected straws were buried in soil containing various plant residues (Table VI), virulent C. gramineum was recovered after 35 months from all of the residues used except barley. Cephalosporium gramineum survival in infected straw declined with storage in these various soil residues and controlled humidity conditions. A more rapid decline in survival was recorded by Lai and Bruehl (1966) when different soils were used.

The information obtained from this study indicated that C. gramineum can survive for longer than three years in infected straw. Possibly if the technique of culturing the organism was further modified, the pathogen could be recovered from those conditions where it is presently no longer detectable.

HOST RESPONSES

I. Resistant Varieties and Severity of Symptoms

The tests conducted at Bozeman, Missoula, and Moccasin verify (Nisikado, et al., 1934) that varieties differ in their resistance to the disease. The extremes of resistance obtained in 261 lines of Westmont x P.I. 178383 (Figure VI) and 39 lines of Itana x P.I. 178383 (Figure VII) indicate possible sources of genetic resistance. Each of the lines used was selected for favorable agronomic characteristics of one type or another. The relative resistance appears to be related to some of these desirable field properties.

Table VI Survival of C. gramineum in naturally infected straw buried in several crop residues.

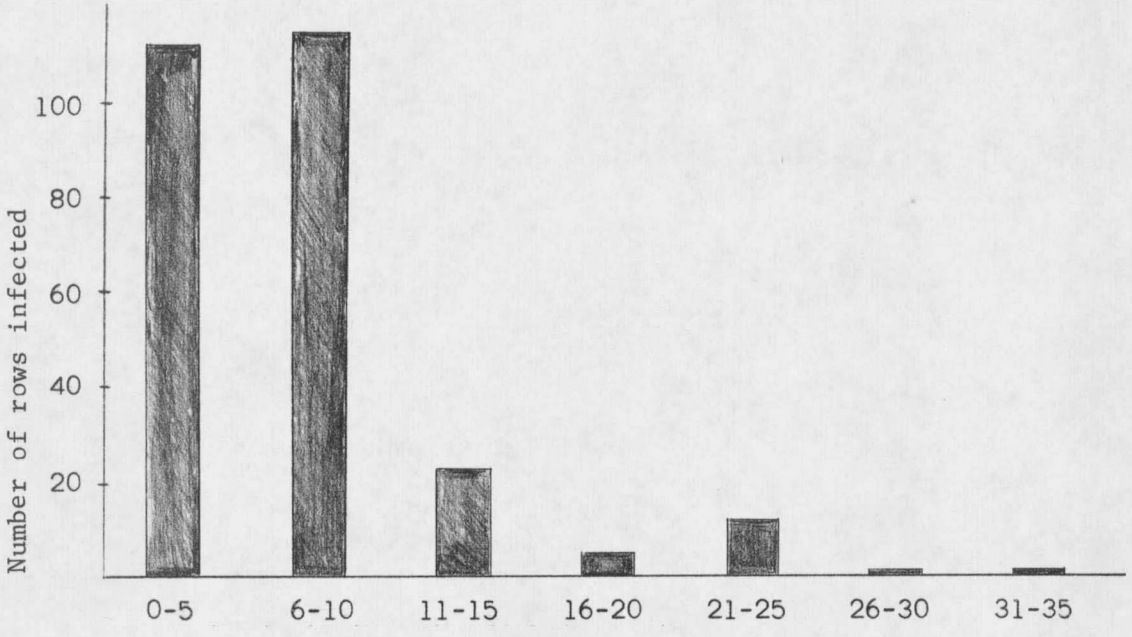
Residue	Samples from which C. gramineum was recovered								
	Storage time months								
	4*	6	8	10	17	21	25	35	40
Wheat	+	+	-	75**	0	0***	50	25	16
Wheat, soil sterile	+	+	-	75	25	35	50	17	5
Barley	+	+	-	75	38	56	50	0	0
Barley, soil sterile	+	+	-	100	38	38	20	0	0
Oats	+	+	-	50	36	13	7	17	6
Oats, soil sterile	+	+	-	75	0	33	36	8	0
Alfalfa	+	+	-	25	58	13	25	25	0
Alfalfa, soil sterile	+	+	-	25	45	63	11	17	0
Safflower	+	+	-	75	50	38	67	8	6
Safflower, soil sterile	+	+	-	25	82	63	13	8	0

* surface sterilezed in 5.25% sodium hypochlorite:95% ethanol::1:1
medium PDA, streptomycin 30 ppm, 1% rose bengal 1ml/liter

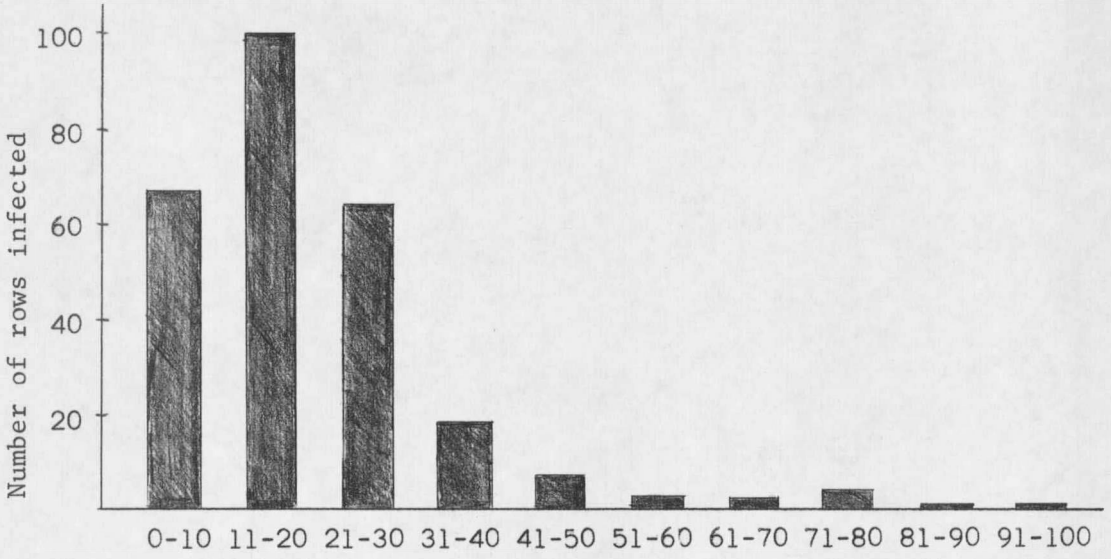
** technique modified - straws non-surface sterilized, but washed in distilled water

*** medium modified - PDA, streptomycin, 1% rose bengal 0.25 ml/liter

Figure VI Differences in degrees of resistance of 261 crosses of Westmont x P.I. 178383 to natural infection of C. gramineum.

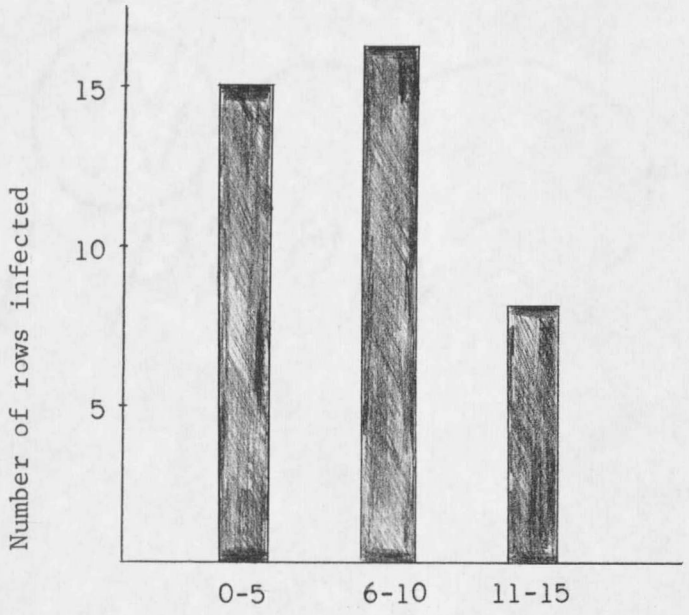


Number of plants infected per row. July, 1965

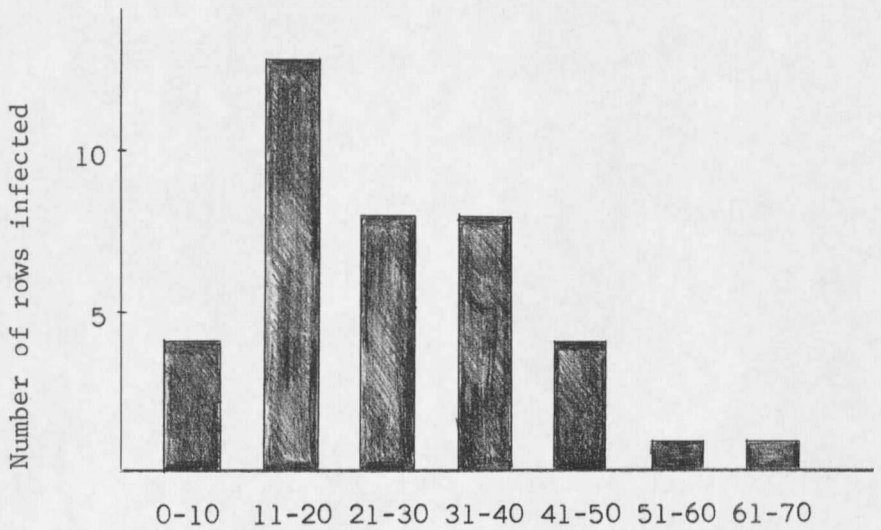


Number of heads infected per row. August, 1965.

Figure VII Differences in degrees of resistance of 39 crosses of Itana x P.I. 178383 to natural infection of C. gramineum.



Number of plants infected per row. July, 1965.



Number of heads infected per row. August, 1965.

As an example of a resistance study, the 10 selections tested at Moccasin in 1964-1965 are listed in Table VII. The order is based on several symptoms counts as well as yield data. A comparison is also made of the degree of discoloration of the fresh mature straw at the time of harvest. The mature straw symptoms are an indication of the decreased yield in infected heads but with the possible exception of Lancer, which was severely discolored below the nodes, the symptoms in the mature straw are not a reliable indication of the susceptibility of a variety. After being stored at room temperature for one year, the infected straw from all ten varieties became grey while the control straws did not discolor.

In Table VIII the lines and selections tested at Bozeman, Mocassin, and Missoula are listed in an approximate order of susceptibility to natural infection by C. gramineum. The number of varieties planted in common in these various nurseries facilitates such a comparison. The difference in susceptibility or resistance between adjacent selections is negligible. The divisions into more and less susceptible groups are not rigid but used to indicate varietal differences in resistance. The importance in this data is that some varieties are consistently more resistant to natural infection with C. gramineum than are other varieties.

The typical pattern of symptom development is a mottled chlorosis of the leaves followed by the appearance of one or several chlorotic lines which progress from the tip to the sheath. A necrotic line subsequently develops in the sheath and progresses up the sheath to the leaf tip. However, in one strain (Im 462-N10 x It 686(17)/P.I. 178383 #19) the first symptom of disease is a single necrotic line extending through the sheath

Table VII Varieties and selections of winter wheat used in a resistance study at Moccasin, Montana.

Variety	C. I. number	Infected plants per 12 foot row		Mature straw symptoms	Yield*
		June 15	July 15	September**	
Winalta	13670	0	2	5	92.0
Gaines	13448	4	3	3	98.6
Cheyenne	8885	4	6	8	68.7
Gage	13532	5	8	2	98.2
Itana	12933	4	7	7	59.4
Scout	13546	8	9	9	66.5
Yogo x Cheyenne		12	15	1	93.9
Warrior	13190	15	5***	5	88.8
W. H. Bulk****		22	23	6	99.2
Lancer	13547	18	37	10	71.6

Selections are listed from most to least resistant to C. gramineum.

* $\frac{\text{gm per 50 infected heads which reach maturity}}{\text{gm per 50 healthy heads}}$

** Arbitrary scale:
1) least color deviation from uninfected control
10) most discoloration of straw

*** Infected plants difficult to detect

**** Winter hardiness bulk selection

Table VIII Relative resistance of various winter wheat varieties and selections to natural infection by C. gramineum.

Variety	Location*				
	A	B	C	D	E
Moderately Resistant (3-6%)					
Winalta	+	+	+		+
Burt x Itana-120				+	
Tendoy				+	
Burt x P.I. 178383 (C-63-11)			+	+	
Columbia x Utah-115				+	
Columbia x Utah-116				+	
Burt x Itana-113				+	
Omar^2 x P.I. 178383 (Sel 172)		+			
Wanser			+		
Moro			+		
Moderately Susceptible (5-7%)					
McCall			+		
Westmont^2 x P.I. 178383 (13-5-17)		+			
Westmont^2 x P.I. 178383 (7-14-5)		+			
P.I. 178383				+	
NRN 10-BVR 14 x Burt^5				+	
Burt x Itana-112				+	
Cheyenne	+	+	+	+	+
Columbia				+	
Karkof				+	
Rego		+	+		+
Gaines	+	+	+		
Susceptible (7-14%)					
Westmont		+	+	+	
(Re x Rio x Cnn^2) x Cnn^3				+	
Hussar x Cheyenne				+	
Colorow				+	
Itana x Karkof-17 sel 1-26-1				+	
Burt	+	+			
Im 462-N10 x It 686 (17) P.I. 178383 #19	+	+			
Itana-65				+	
Itana	+			+	+
Gage					+
Scout	+				+

Table VIII Continued

	A	B	C	D	E
Very Susceptible (9-17%)					
Wasatch x Karkof-17 Sel 18-5				+	
Delmar	+	+	+	+	
Rio				+	
Westmont[2] x P.I. 178383 (8-10-8)		+	+		
Burt x P.I. 178383 (C-61-9)		+			
Bulk selections				+	+
Yogo x Cheyenne	+				+
Warrior		+	+		+
Omar		+	+		
Nugaines			+		
Lancer	+	+	+		+

* A Greenhouse tested

B Tested at Missoula, Montana, 1965

C Tested at Missoula, Montana, 1966

D Tested at Bozeman, Montana, 1964

E Tested at Moccasin, Montana, 1964

\+ Location at which variety was tested

to the leaf tip. Eventually, the line is accompanied by a lateral chlorosis. This unusual symptom expression may indicate a less compatible host-parasite relationship in the early stages of infection which possibly could be utilized in breeding for disease resistance.

The development of symptoms within a host varies with the means of inoculation. When the culm or a leaf is hypodermically inoculated, symptoms are expressed within eight to ten days. However, when the plant is inoculated by root injury, symptoms are not expressed for 15 to 20 days. Varieties differ also in the rate of development of symptoms. Ten varieties are compared in Table IX for the rate of advance of symptoms. In some varieties, such as Lancer, symptoms develop relatively early, whereas in other varieties such as Winalta, symptom development is relatively slow.

When wheat is hypodermically inoculated, the necrotic line occurs above the inoculation point but only for a short distance below. Similarly, C. gramineum is found above the area of inoculation but in only a small area below this point. This may indicate that the infection spreads only in an upward direction.

II. Loss Due to Infected Plants

A study was conducted to assess the factors which contribute to the losses associated with Cephalosporium stripe disease. Under greenhouse conditions, Bruehl (1956a) noticed excessive tillering in infected plants, "double or more," with the exception of the variety Baart. He suggested that infection in an early stage of development of the plant would result in the increased tillering noticed. The tillering pattern of three field

Table IX A comparison of the rate of advance of symptoms in several varieties*.

Variety	Percent Infection		
	21 Days**	39 Days	Harvest
Burt	46	61	75
Cheyenne	50	67	85
Delmar	42	77	92
Gaines	35	70	87
Im 462-N10 x It 686(17) P.I. 178383 #19	27	70	75
Itana	45	80	95
Lancer	70	74	96
Scout	43	86	91
Winalta	38	73	86
Yogo x Cheyenne	58	92	96

* Vernalized plants grown in greenhouse benches, inoculated by root injury

** Days after inoculation

grown selections is presented in Table X. It can be seen that regardless of the stage of infection in June, the number of tillers reaching maturity is essentially the same in all infected plants of one variety. Infected plants may have more or less tillers reaching maturity than healthy plants. Burt x Itana-113 tillers to approximately the same extent whether infected or not. Rio and Itana have less tillers when naturally infected than when healthy. A lower number of tillers reaching maturity in infected plants would certainly contribute to a lowering of yield.

The grams of grain from 50 randomly selected heads from 12 field grown varieties is compared in Table XI. One can readily note the low yield from infected heads relative to healthy heads. The yield per head of Itana and Scout is severly reduced in infected plants, whereas the loss per head in Gaines and Gage is negligible. The severe decrease in yield in infected plants is readily evident.

The yield relative to the state of advance of symptoms was compared for two selections. Rio and Burt x Itana-113 were specifically tagged in June as previously described. Irrespective of the severity of symptoms two months before harvest, approximatley the same amount of grain was obtained from all infected tillers which produced heads (Table XII). Less grain is produced from infected than healthy tillers. The length of the heads from 3 varieties is compared in Table XIII. The heads were measured from the start of the lowermost floret to the tip of the uppermost floret, exclusive of the awns. There was no significant difference in the length of infected and healthy heads. This indicates that the low yield realized from infected plants is largely due to a lesser filling of the heads. The

Table X Tiller development in relation to disease symptom development.

Variety	Leaf stage to which symptoms had advanced by June*					
	Uninfected control	5	4	3	2	Flag leaf
	Number of tillers reaching maturity					
Rio	3.8	3.0	2.8	2.7	3.1	2.6
Itana	3.9	2.3	2.8	2.7	3.4	2.8
Burt x Itana-113	2.6	3.5	1.9	3.1	3.3	2.0**

* The field grown plants were tagged in June, 1965, when symptoms had reached the leaf indicated.

** Only one plant had symptoms advanced to the flag leaf in June.

Table XI Grams of grain from 50 randomly selected heads from 12 wheat varieties naturally infected and not infected with C. gramineum.

Variety	grams/50 heads		
	diseased straw	clean straw	diseased/clean
Gage	33.4	27.5	1.21
Gaines	27.6	28.0	0.98
Winter hardiness bulk	24.2	24.4	0.95
Yogo x Cheyenne	21.6	23.0	0.94
Winalta	17.4	18.9	0.92
Warrior	21.4	24.1	0.89
Lancer	22.2	31.0	0.72
Scout	27.6	41.5	0.67
Cheyenne	20.2	32.3	0.62
Itana	23.3	39.2	0.59
Burt x Itana-113	19.3	50.9	0.38
Rio	12.2	41.2	0.30

Table XII Comparison of the yield from tillers specifically tagged when the symptoms had advanced to different stages in June.

Variety	Grams of grain per head of specifically tagged tillers					
		Leaf Infected when Tagged				
	Uninfected control	5	4	3	2	Flag Leaf
Rio	0.82	0.19	0.29	0.20	0.27	0.28
Burt x Itana-113	1.02	0.59	0.30	0.31	0.41	0.29

Table XIII Comparison of length of heads of infected and non-infected plants.

Variety	Length of heads exclusive of awns in cm.					
		Leaf Infected				
	Non-infected control	5	4	3	2	Flag leaf
Rio	5.9	5.4	5.9	5.3	5.7	5.7
Itana	5.8	5.9	4.9	5.2	5.7	5.5
Burt x Itana-113	6.7	6.3	6.2	5.7	6.4	6.3

photograph (Figure VIII) illustrates the range of sizes and degree of filling of infected and healthy heads from Burt x Itana-113. A comparison of the size of the kernels and yield is also illustrated.

III. Amount of Stunting

In Table XIV the amount of stunting of Rio, Itana, and Burt x Itana-113 is compared between healthy plants and infected plants. In Figure IX the difference in height of healthy and infected plants is compared. The difference in stunting among infected plants showing different degrees of infection in June was not significant. The infected plants were approximately three-fourths as high as control plants in all cases. The stunting of diseased plants is significant at the 1% level.

ENVIRONMENTAL INFLUENCE

From field observations, it appeared that the environment may play an important role in disease development. In the western mountainous half of the state there was a higher rainfall and greater incidence of disease than in the eastern plains. In fields which were not severely infested, diseased plants were most often found in low spots in the field. These areas may have been only a few inches lower than the remainder of the field, but they tended to collect snow and water and consequently be somewhat wetter than the rest of the field.

I. Effect of Soil Moisture

Based on the field observations indicating a possible relationship between soil moisture and disease development, a series of soil moisture

Figure VIII A comparison of healthy and infected heads of wheat for degree of filling, size of kernels, and yield.

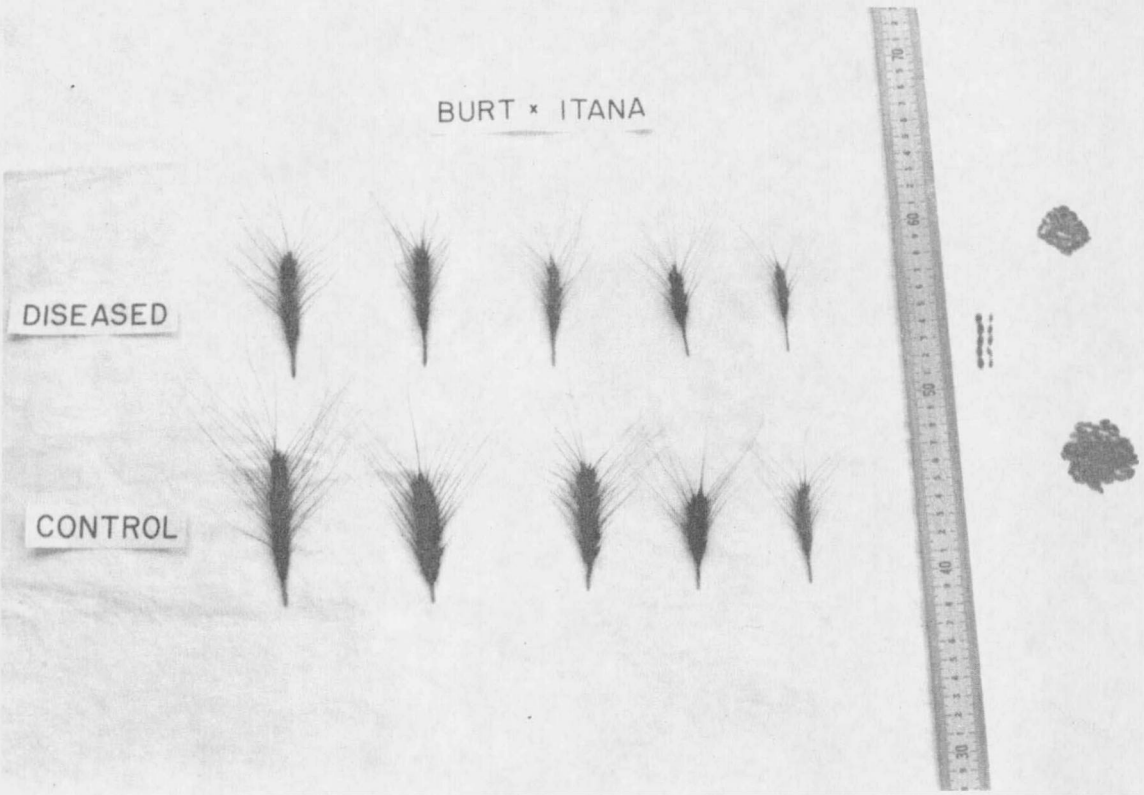


Table XIV Average heights in cm. of wheat naturally infected and non-infected with C. gramineum.

Variety		Leaf stage to which symptoms had advanced by June*					
		Non-infected control	5	4	3	2	Flag leaf
Rio	A	102**	68	77	73	71	75
	B	105	69	77	76	72	78
Itana	A	100	84	75	75	79	75
	B	96	85	77	79	81	71
Burt x Itana-113	A	92	65	70	65	64	(71)
	B	91	66	69	66	65	(73)

A - Average height of all tillers on the plants

B - Average height of the tagged tillers

* Plants tagged in June, 1965, when the disease symptoms were advanced to the leaf indicated.

** Height when harvested, measured from the top of the roots including the heads but exclusive of the awns.

Figure IX A comparison of the height of healthy and C. gramineum infected wheat.

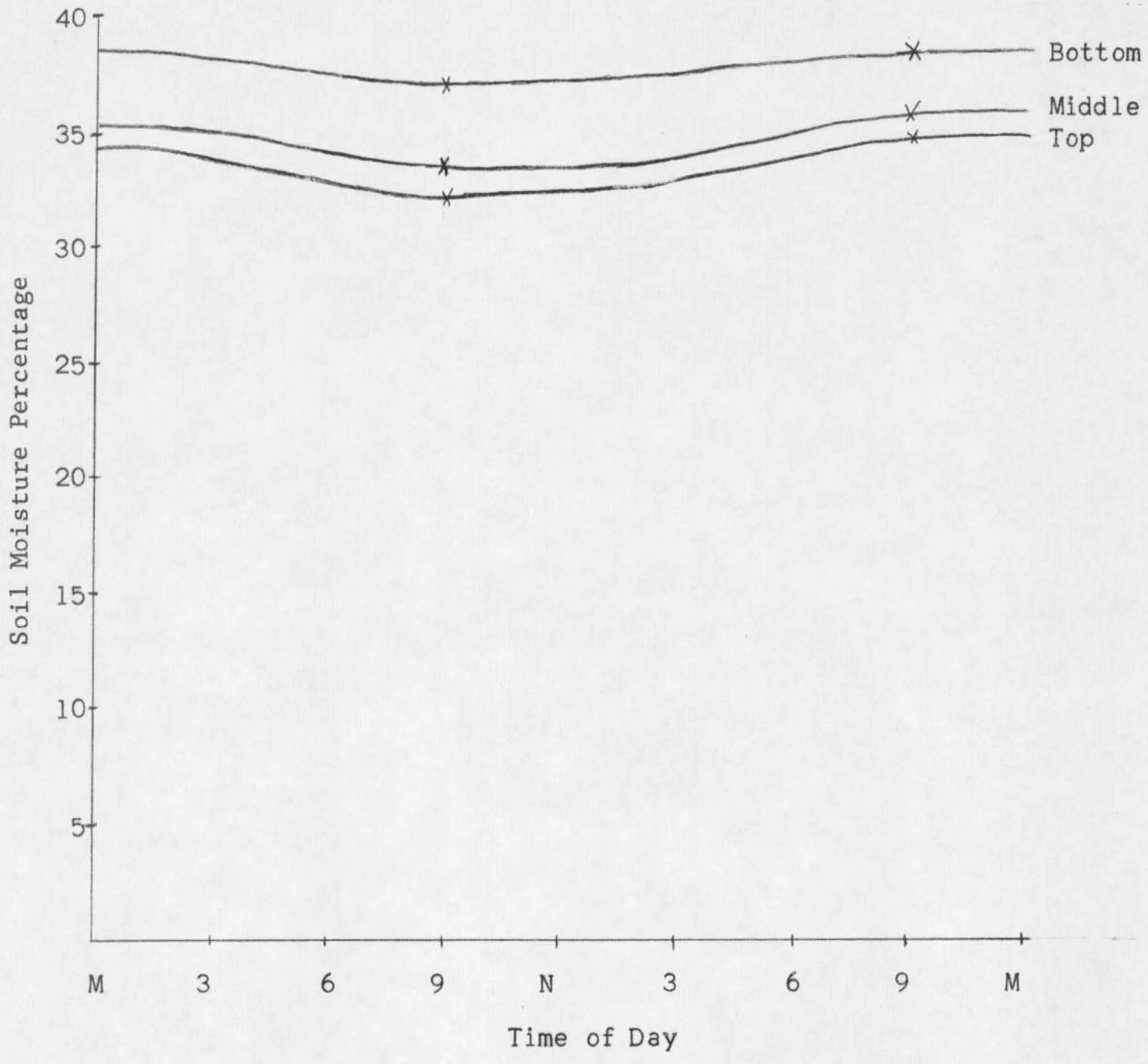


experiments were conducted. Soil moisture determinations were made on an Ohaus moisture determination balance at 14, 40, and 52 days after planting. There was no significant difference in soil moisture content between these sampling dates within any one watering profile. The soil in pots maintained continuously in water fluctuated by about 2% between nine AM and nine PM in a diurnal cycle (Figure X). The top of the pots was slightly dryer (32-35%) than the middle (34-36%). The middle was about 3% dryer than the bottom (37-38%). These differences between the various regions of the pot were not considered to be important to the availability of free moisture to the plants.

The soil moisture profile in pots allowed only 25 ml of water per day showed extreme diurnal fluctuations (Figure XI). This was related directly to the daily watering procedure. The top of the pots contained about 9% water showing a slight diurnal fluctuation. The soil at the bottom of the pot reached a low of 21% moisture before watering. This rapidly increased to better than 30% about 1.5 hours after being watered. By nine PM the moisture content of the soil dropped to about 25%. The soil in the center of the pot cycled to a lesser extreme than the bottom but still fluctuated between 18% and 23% moisture. In discussing the various experiments, the pots of plants allowed only 25 ml of water per day will be considered to have a soil moisture content of 20%.

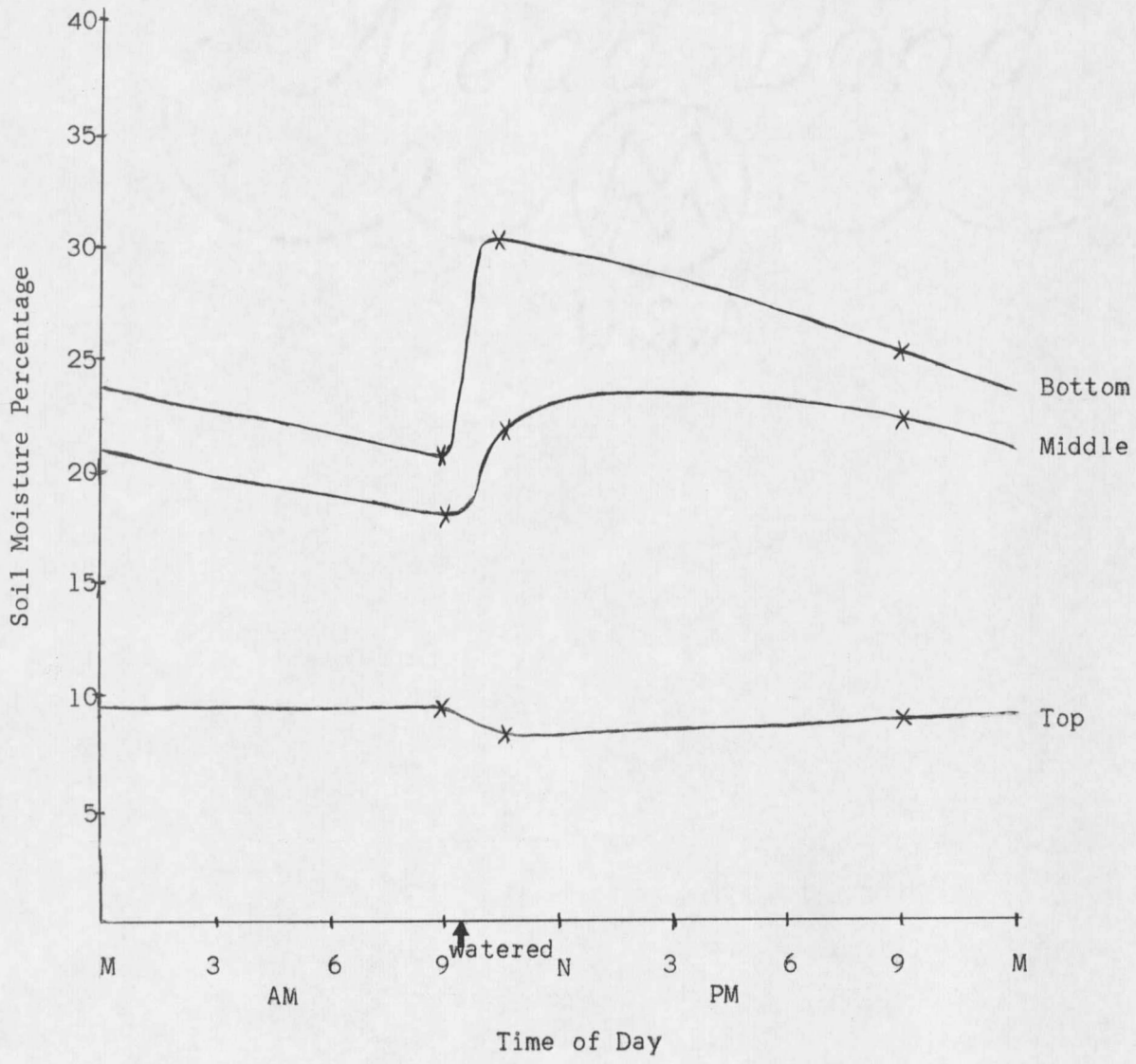
The effects of high (35%) and low (20%) soil moistures preceeding and/or following the inoculation of vernalized and non-vernalized plants was tested (Table XV). By using a randomized block analysis of variance (Table XVI) and Duncan's New Multiple-Range Test (Table XVII), it was

Figure X Daily soil moisture profile for pots of wheat abundantly watered.*



* Twenty plants per four inch clay pot set in petri dishes filled with water.

Figure XI Daily soil moisture profile for pots of wheat with restricted water availability.*



* Twenty plants per four inch clay pot set in petri dishes, sub-irrigated with 25 ml of water per day.

Table XV Infected plants following injury and inoculation through the roots.

Soil moisture maintained while growing	Soil moisture at inoculation	Soil moisture maintained after inoculation	Number of infected plants
		NON-VERNALIZED	
A			
35%	35%	20 or 35%	5.4
20%	20%	20 or 35%	1.4**
B			
35%	35%	20%	4.6
20%	20%	35%	1.7*
C			
35%	35%	35%	4.7
20%	35%	35%	2.7
35%	6.5%	20%	0.3
20%	6.5%	20%	0
		VERNALIZED	
35%	35%	35%	9.9
35%	20%	20%	3.9**

Twenty plants per four inch pot

* significant difference at 5% level

** significant difference at 1% level

Table XVI Analysis of variance of soil moistures preceeding and following inoculation of non-vernalized plants.

Source of variation	Degrees of freedom	Mean square	F value
Experiments	2		
Treatments	5	3.7556	6.95**
Error	10	0.5402	
Total	17		

** Significant at the 1% level

Table XVII A statistical comparison* of infected plants for two pre-inoculation soil moistures and three post-inoculation soil moistures.

Treatment	LLL**	LLH	LHH	HLL	HHL	HHH
Mean	1.598	2.112	2.411	3.541	3.749	4.519
p at 5% level						

* Duncan's New Multiple-range test

** See text:
L - 20% soil moisture
H - 35% soil moisture
First letter is pre-inoculation soil moisture
Second letter is immediate post-inoculation soil moisture
Third letter is soil moisture from four days after inoculation until termination of the experiment

shown that the soil moisture content immediately after inoculation or four days after inoculation did not affect disease incidence. There was however, a greater incidence of disease with high soil moistures (35%) prior to inoculation, than with low soil moistures (20%) during the same time period.

A set of experiments was conducted to test the effect of pre-inoculation soil moistures on disease incidence after the plants were vernalized. The plants were vernalized by being kept for a minimum of seven weeks at a constant 40 F with a 12 hour night/day (dark/light) cycle. The plants were grown and vernalized with available free water (soil moisture 35%). For one week following vernalization they were sub-irrigated either freely (soil moisture 35%) or allowed restricted moisture (soil moisture 20%). After inoculation the plants were retained on these soil moisture conditions. The differences in the number of plants infected between the two soil moisture treatments were subjected to a randomized block analysis of variance. Vernalized plants maintained on a high soil moisture availability (35%) prior to inoculation with C. gramineum were more susceptible to infection than those retained on a low soil moisture (20%) during the same time interval. The differences were significant at the 1% level of confidence.

Since the conidia might be adsorbed to the soil, an experiment was conducted in which plants were grown at 20% or 35% soil moisture but were repacked in soil at 6.5% or 35% moisture four hours before inoculation (Table XV,C). Altering the soil moisture prior to inoculation nullified most of the effects of the conditioning soil moistures. This indicated

that there was much adsorption of the pathogen to the dry soil. However, the preconditioning moistures do apparently afford some physiological resistance to disease since there was a greater infection in plants grown initially at 35% soil moisture than in plants grown at 20% soil moisture.

To test for the effect of vernalization on disease development at the time of inoculation, a group of plants was inoculated by the root injury technique. One-third of the plants was immediately placed at 40 F to vernalize. Four days later another third of the plants was placed at 40 F. Fifteen days after inoculation the remaining third was placed at 40 F to vernalize. There was no difference between treatments in the number of plants which were infected 40 days after being removed from the vernalization chamber. As expected the effect of vernalization is insignificant in Cephalosporium gramineum infection. This indicates that infection of the wheat can take place during any phase in the growth of the plant. These experiments indicated that soil moisture plays a significant role in development of Cephalosporium stripe disease.

II. Date of Planting

Field measurements made at Moccasin, Montana, showed that there were less infected plants following late planting (October) rather than early planting (August). The average soil temperature for the first 14 days after planting, the percent infection, and yield associated with natural infection are indicated in Table XVIII. The decrease in number of diseased plants and increase in yield was closely associated with the average soil temperature for the initial 14 days following seeding. In the earlier

Table XVIII Average soil temperature at a depth of eight cm. for the first 14 days after planting*, the yield, and percent infection with C. gramineum. Moccasin, Montana.

Planting Date	Average Temp.F 14 days	Percent Infection	Yield bu/acre
1964			
August 26	55	4.5	19
September 4	55	37.5	26
September 11	55	22.3	33
September 25	50	11.7	30
October 5	49	5.2	36
1965			
August 28	60	0	19
September 30	53	0	35
October 18	54	0.7	25

* Fertilized, in stubble

plantings the soil was warmer and the incidence of disease was generally higher than in the later plantings. The low incidence of infection in the 1965 material was probably due to the drier growing conditions in that season.

A measurement of root lengths of field plants seeded at different dates indicated that earlier seeded wheat has longer roots in the spring than late seeded grain. The root length for Cheyenne wheat planted at different dates in 1965 and 1966 are shown in Table XIX. The Judith clay loam soil in which these experiments were conducted had a cement-like base which was virtually impermeable to the plant roots. The roots spread out on this layer making measurement of the longer roots difficult.

III. Effect of Fertilizer

Since warm autumn soil temperatures contribute to root growth, the added effect of fertilizer applied in the drill rows was tested. There was an interaction between the date of planting and effect of fertilizer - 44 lb. $Ca_3(PO_4)_2$ as 20 lb. P_2O_5 per acre. This interaction decreased the yield and increased the incidence of disease. Fertilizer added in the drill rows with the early (August) plantings contributed to a decrease in the yield over the non-fertilized wheat (Table XX). Added fertilizer in the later plantings (October) caused an increase in the yield of wheat over the non-fertilized plants. The P_2O_5 fertilizer slightly increased the incidence of C. gramineum infection in all dates of planting, but in the later planting dates, the increased yield more than compensated for the slight increases in disease incidence.

Table XIX Root lengths in the spring of plants seeded at different dates in the fall. 1965, 1966.

Date Planted	Fertilization*	Root Depth (inches)		Depth of Cement**** Layer (inches)	
		N**	B***	N**	B***
1965 - Spring lengths measured in March, 1966.					
Aug. 28	Fertilized	15(C)****	16(C)	15	16
	Not Fertilized	15(C)	17(C)	15	17
		23		--*****	
Sept. 30	Fertilized	15(C)	14(C)	15	14
	Not Fertilized	13(C)	14(C)	13	14
Oct. 18	Fertilized	13	8	15	16
	Not Fertilized	10	7	12	15
1966 - Spring lengths measured in April, 1967.					
Aug. 24	Fertilized	24(C)	17(C)	24	17
	Not Fertilized	23(C)	20(C)	23	20
Sept. 15	Fertilized	18(C)	24(C)	18	24
	Not Fertilized	18(C)	19(C)	18	19
Oct. 6	Fertilized	12	11	18	19
	Not Fertilized	12	12	18	18

* 44 lb. $Ca_3(PO_4)_2$ per acre added in drill rows.

** Normal stubble cover.

*** Normal stubble cover burned off.

**** Impermeable subsoil on which roots layer.

***** No cement-like layer in one corner of the field.

Table XX Fertilization as it affects infection and yield.

Planting Date	Fertilized		Non-Fertilized	
	Percent Infection	Yield bu/acre	Percent Infection	Yield bu/acre
Aug. 26	37	15	37	22
Sept. 4	28	26	20	31
Sept. 11	45	31	--	--
Sept. 25	20	31	16	28
Oct. 5	9	32	10	27

Cheyenne winter wheat inoculated with C. gramineum.

In an effort to clarify the effects of fertilizer and soil temperature associated with the different planting dates, the field environment conditions at Moccasin in the fall of 1964 were simulated using an environment chamber. Cheyenne winter wheat was planted in Judith clay loam soil at three different time intervals and either fertilized or not fertilized. Measurements of the root lengths and root weights (Table XXI) indicated that fertilizer increased root mass for the earliest date of planting but did not significantly affect the root system for the other two dates of planting. The effect of fertilizer in increasing root length was significant at the 1% level for the August 26 date. The increase in root weight due to fertilizer was significant at the 5% level for the earlier date.

The most striking factor in the environment was the rapid change in soil temperature encountered in the autumn. The average soil temperatures at eight cm. in the nurseries for the first 14 days following seeding in 1964 and 1965 are given in Table XVIII. When compared on a weekly basis, Table XXII, the soil temperature in 1964 declined after the third seeding (Sept. 11) and did not reach a weekly high above 51 F again that fall. In 1965 on the other hand the soil temperature did not remain below 50 F until all plants had been growing at least one month.

Generally the soil at the Moccasin station freezes each winter. In the spring the ground thaws from the bottom and top with diurnal freezing of the surface few centimeters. A typical example of the spring profile of the soil is illustrated in Figure XII. An ice layer was observed between 23 and 61 cm. in the nursery. The surface two cm. of soil was freezing

Table XXI Length and weight of roots of Cheyenne winter wheat grown in an environment chamber simulating field conditions at Moccasin, Montana, 1964.

Simulated Planting Date	Fertilized 44 lb. $Ca_3(PO_4)_2$/acre	Not Fertilized
LENGTH*		
Aug. 26	217	174
Sept. 4	155	147
Sept. 25	68	77
WEIGHT**		
Aug. 26	135	97
Sept. 4	74	71
Sept. 25	17	17

p greater than 1% between dates

* Length in cm.

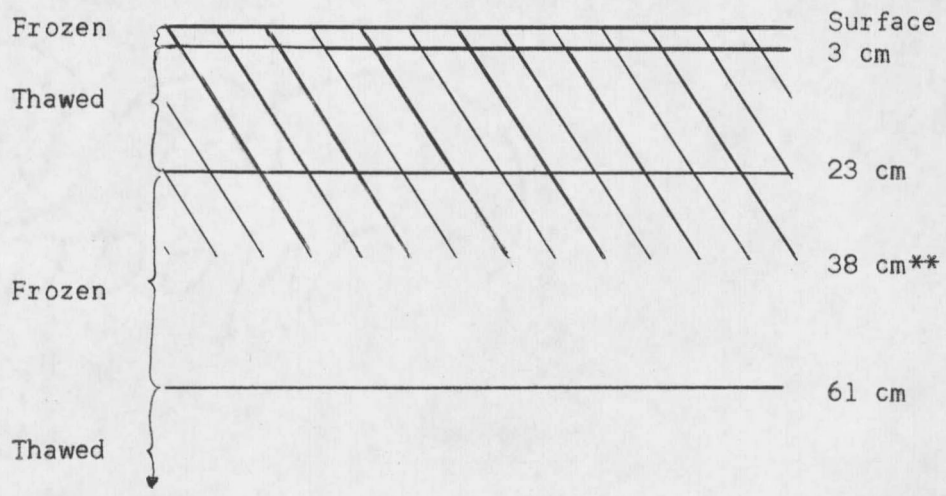
** Air dry weight in gm.

Table XXII Average soil temperatures at a depth of eight cm. during successive seven day intervals. Moccasin, Montana.

Starting Date	Average Temperature F*	
	1964	1965
August 26	56	--
September 2	54	60
September 9	54	54
September 16	54	47
September 23	51	48
September 30	50	51
October 7	51	57
October 14	46	51
October 21	45	51
October 28	45	51
November 4	41	49
November 11	36	40
November 18	31	38

* Stubble fields

Figure XII Typical spring soil profile at Moccasin, Montana*.



* Morning of March 19, 1966.

** Shaded area is usual topsoil area.

and thawing daily at this time. The heaving of the soil with the spring thaws would tend to disturb the roots of the plants. If the extremities of the roots were held solid, as in a block of ice, there would be considerable tension on the roots as the plants were moved in the upper heaving soil. Those plants with longer roots would tend to be held longer and subject to more heaving strain than similar plants with a less developed root system. Also, with a more branched root system, the plants would be held in the ice by more roots and therefore subject to breakage at more points.

IV. Influence of Stubble Cover

Since C. gramineum has been demonstrated to survive for at least 45 months in infected straw, the amount of infected stubble present in a field should be related to the incidence of disease. When infected stubble was added at the rate of three tons per acre to the normal field stubble, there was a significant increase in the incidence of infection under nine different planting conditions (Table XXIII). When the natural stubble was removed by burning (Table XXIV), there was no significant difference in disease incidence from the control attributable to differences in stubble for six different seeding conditions. Burning the stubble would also be expected to lower the disease incidence by reducing inoculum potential. The lack of a difference in this latter threatment might have been due to a lower incidence of C. gramineum disease in wheat in the entire state during the 1965-1966 growing season.

Table XXIII Effect of planting date, stubble cover, and fertilizer on *C. gramineum* natural infection and yield on 12 foot rows of winter wheat. Moccasin, Montana, 1964.

Planting Date	Fertilizer*	Stubble**		Plowed***		Temperature**** F
		Percent Infection	Yield bu/acre	Percent Infection	Yield bu/acre	
Aug 26	Fertilized	4.5	18	22.5	46	55
	Not Fertilized	5.7	30	10.0	37	
Sept. 4	Fertilized	33.4	26	2.2	47	55
	Not Fertilized	14.7	33	0.9	48	
Sept. 11	Fertilized	9.6	33	1.5	51	55
Sept. 25	Fertilized	11.7	30	0.0	37	50
	Not Fertilized	3.1	26	1.4	32	
Oct. 5	Fertilized	4.3	36	0.0	51	49
	Not Fertilized	2.4	25	0.0	33	

* 44 lb. $Ca_3(PO_4)_2$ per acre

** Added stubble, three tons per acre

*** Normal stubble deep plowed

**** Average soil temperature (stubble) at eight cm. for the first 14 days after seeding

Table XXIV Effect of planting date, stubble cover, and fertilizer on C. gramineum natural infection and yield on 12 foot rows of winter wheat. Moccasin, Montana, 1965.

Planting Date	Fertilizer*	Stubble**		Burned***		Temperature**** F
		Percent Infection	Yield bu/acre	Percent Infection	Yield bu/acre	
Aug. 28	Fertilized	1.1	20	3.1	17	60
	Not Fertilized	3.0	24	0.4	24	
Sept. 30	Fertilized	0.2	39	1.9	36	53
	Not Fertilized	0.4	43	1.8	34	
Oct. 18	Fertilized	0.3	31	1.2	32	54
	Not Fertilized	0.4	25	0.7	31	

* 44 lb. $Ca_3(PO_4)_2$ per acre

** Normal stubble cover

*** Normal stubble cover burned off before plowed

**** Average soil temperature at eight cm. for the first 14 days after seeding

With the exception of the latest (October) planting, wheat grown in added stubble was advanced at least one stage on the Feekes scale (Figure XIII) (Large, 1954) from plants grown in normal stubble (Table XXV & Table XXVI). However, as previously described there was not as great a difference in the autumn soil temperatures in 1965 as there was in 1964. An insulation effect of the stubble was evident when the soil temperature profiles of the high and low stubble treatments were compared. A high stubble level in the field tended to dampen the extremes of soil temperature fluctuations that were recorded in the low stubble treatments. When the soil temperatures dropped below 40 F, soil with more stubble was several degrees warmer than soil with reduced stubble.

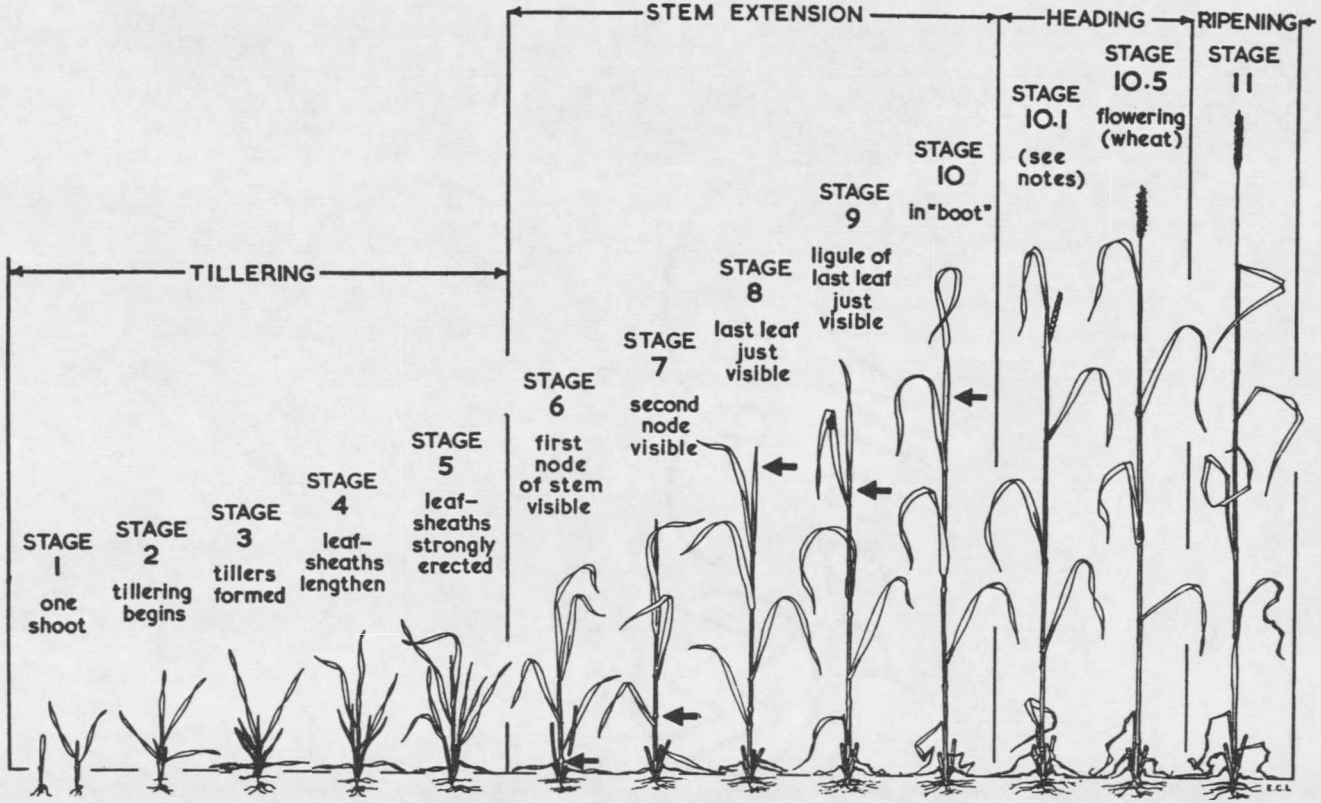
SOURCES OF INOCULUM

All parts of infected plants can serve as important sources of inoculum. Cephalosporium gramineum has been cultured from roots, culms, leaves, glumes, and awns of infected plants. Only one of three hundred seeds from known infected plants was infected after the seedlings were vernalized. These results corroborate Bruehl's work indicating that seeds alone are not an important inoculum source for an epidemic, but they can introduce the inoculum into an area. All infected plant residue can act as an inoculum source, including infected floral parts which remain attached to the seed.

CHEMICAL STUDIES

In the broth and agar culture methods employed in this study of C. gramineum, two prominent phenomenon were observed. The broth culture soon

Figure XIII Growth stages in cereals.



From Large (1954): *by courtesy of H.M. Stationery Office, London*

Table XXV Growth stage* of Cheyenne winter wheat measured in June, 1965.

Date Planted	Stubble**		Plowed***	
	Fertilized	Not Fertilized	Fertilized	Not Fertilized
8/26/64	S-9	S-9	S-8	S-8
9/4/64	S-9	S-8	S-7	S-6
9/11/64	S-9	---	S-7	---
9/25/64	S-8	S-8	S-6	S-5
10/5/64	S-6	S-5	S-6	S-5

* Large, 1954

** 3 Tons/acre added to normal cover

*** Normal cover deep plowed

Table XXVI Growth stage* of Cheyenne winter wheat measured in June, 1966.

Date Planted	Plowed**		Burned***	
	Fertilized	Not Fertilized	Fertilized	Not Fertilized
8/28/65	S-10.1	S-10	S-10.1	S-10.1
9/30/65	S- 9+****	S- 9	S- 9+	S- 9+
10/18/65	S- 9+	S- 9+	S- 9+	S- 9+

* Large, 1954

** Normal cover

*** Normal stubble cover burned off before plowed.

**** Sheath of last leaf extended, no swelling of the boot.

became viscous and retained this viscosity after removal of the organisms by centrifugation and/or filtration through a millipore filter. In agar culture, a few organisms, notably Helminthosporium sp. and Alternaria sp., were very restricted in growth near Cephalosporium gramineum colonies. The phenomenon in the broth culture was due to a polysaccharide. The observations relative to growth in agar were presumed to be due to a toxin.

I. Polysaccharide

A relatively dilute solution (0.1 mg/ml) of the extracellular fungal polysaccharide when precipitated with an equal volume of 95% ethanol would redissolve if not removed from the solution within five to 15 minutes. The polysaccharide was not recoverable once it had redissolved in ethanol. The molecule is stable, however, either dissolved in water or precipitated in 95% ethanol.

The redissolving phenomenon would occur with the crude culture filtrate, the purified polysaccharide dissolved in water, the crude or purified solution boiled 60 minutes, and the crude or purified solution autoclaved 30 minutes at 15 psi. The heating treatments indicate that the phenomenon is not enzymatic in nature and that the molecule appears to be heat stable. Concentrated solutions of the purified polysaccharide did not noticeably redissolve.

The extracellular fungal polysaccharide was examined for a number of physical and chemical properties (Table XXVII). The Biuret test for proteins was negative. When added to the aqueous polysaccharide solution, the copper sulfate solution coagulated as if it were chelating with the polysaccharide.

Table XXVII Properties exhibited by the polysaccharide purified from Cephalosporium gramineum culture filtrate.

Property	Tests employed
Non-proteinaceous	Biuret test - negative 260/280 ratio - same as blank
Non-reducing	Nelson's test - negative Benedict's test - negative $AgNO_3$-NaOH-$Na_2S_2O_3$ - negative Potassium ferricyanide test - negative
Carbohydrate containing	Molisch test - positive Phenol-sulfuric test - negative
Non-chitinous	Water soluble Chitosan test - negative
Molecular weight greater than 200,000	Sephadex G-200
No exposed β 1-4 links	β-glucosidase, 24 hours
No exposed α 1-4 links	α-amylase, 24 hours
Not amylose	No blue color with IKI
Viscosity 510-550	Cannon - Ubbelohde Semi-micro Viscometer Number 75-K921
Heat stable	Autoclave 15 psi for 30 minutes Boil 60 minutes
Non-lipid	Ether extraction
Contains glucose, sucrose, may contain rhamnose, glucuronate	HCl hydrolysis, chromatograph H_2SO_4 hydrolysis, chromatograph
Biologically active	Suitable carbon source for growth of C. gramineum
Difficultly soluble dried from ethanol	Insoluble in water, 24 hours Insoluble in 1N HCL, 24 hours Insoluble in conc. HCl, 24 hours Insoluble but breaks up in 1N NaOH, 24 hours Soluble in 40% NaOH

Since this reaction occured at all concentrations of polysaccharide tested the results were always negative for protein.

Using a Beckman D U spectrophotometer, the absorption spectra of the polysaccharide was determined in the visable and ultra-violet regions. The absorption spectrum paralleled that of the water solvent used. The 260/280 ratio of both the polysaccharide and the water solvent was 1.15, further indicating that the molecule is non-proteinaceous.

Nelson's test, Benedict's test, the silver nitrate-sodium hydroxide-sodium thiosulfate test, and potassium ferricyanide test were used to detect the reducing ability of the polysaccharide. All of these tests were negative, indicating the absence of a detectable number of reducing groups.

Ether extraction from the purified and the hydrolyzed polysaccharide did not reveal any lipids by thin layer chramatography.

The Molisch test and the phenol-sulfuric test were positive, thereby indicating that the material is carbohydrate. By quantitation of the latter test, the molecule was determined to be 82% to 110% carbohydrate expressed as glucose.

A comparison was made of the absorption spectrum of 40 μg/ml glucose and 40 μg/ml of the polysaccharide after a phenol-sulfuric reaction. No difference between the two was detectable at 480 or 490 mμ. A peak in the polysaccharide absorption at 480 mμ would have indicated the presence of pentose sugars.

Paper and thin layer chromatographs of the acid hydrolized polysaccharide revealed large quantities of glucose. A spot corresponding

in $R_{glucose}$ with rhamnose was demonstrated in the three solvent systems used. The spot may not be rhamnose, however, since the thin layer detection proceedure is color specific. A positive reaction for rhamnose is dark green whereas the corresponding spot from the hydrolysate stained blue and quickly turned to red. Sucrose was identified by its non-reducing nature and $R_{glucose}$ in paper chromatographs and by its $R_{glucose}$ and color in thin layer. However, the material detected might not have been sucrose since fructose was not detected after hydrolysis. A number of slow migrating, unidentified products of hydrolysis were also detected. Some of these slow migrating reducing spots were probably partial hydrolysis products of the polysaccharide. One such spot which was consistently present corresponded closely in migration habit with glucuronic acid, but was not positively identified.

A negative chitosan test (Rawlins and Takahashi, 1952) and the solubility properties indicated that the molecule was not chitin. Control tests on Puccinia striiformis infected leaves and Boletus sp. were positive. A positive chitosan test was obtained in Cephalosproium gramineum infected leaves, but the purified polysaccharide gave a negative test.

A molecular weight of greater than 200,000 was determined for the polysaccharide by exclusion from Sephadex G-200. In the 35 ml column used, the blue dextran standard (M.W. 2×10^6) was eluted in tube number seven (1.5 ml/tube) with slight tailing in tubes number six and eight. The polysaccharide was detectable by ethanol precipitation only in tube number seven when 0.3 ml containing 0.1 mg/ml polysaccharide was used.

A phenol-sulfuric test using 1.0 of the 1.5 ml in each fraction gave a peak at tubes number seven and eight. Larger concentrations would allow detection of a precipitate in tubes number six, seven, eight, and nine.

An elemental analysis of the water dried polysaccharide run by Schwarzkopf* Laboratories indicated 11.91% Hydrogen, 38.21% Carbon and 49.88% Oxygen with no remainder. The simplest ratio of C:H:O for these figures is 119:32:29. Considering the amount of glucose liberated with acid hydrolysis of the molecule, the C:H:O ratio would indicate possible unusual linkages as well as much water of hydration.

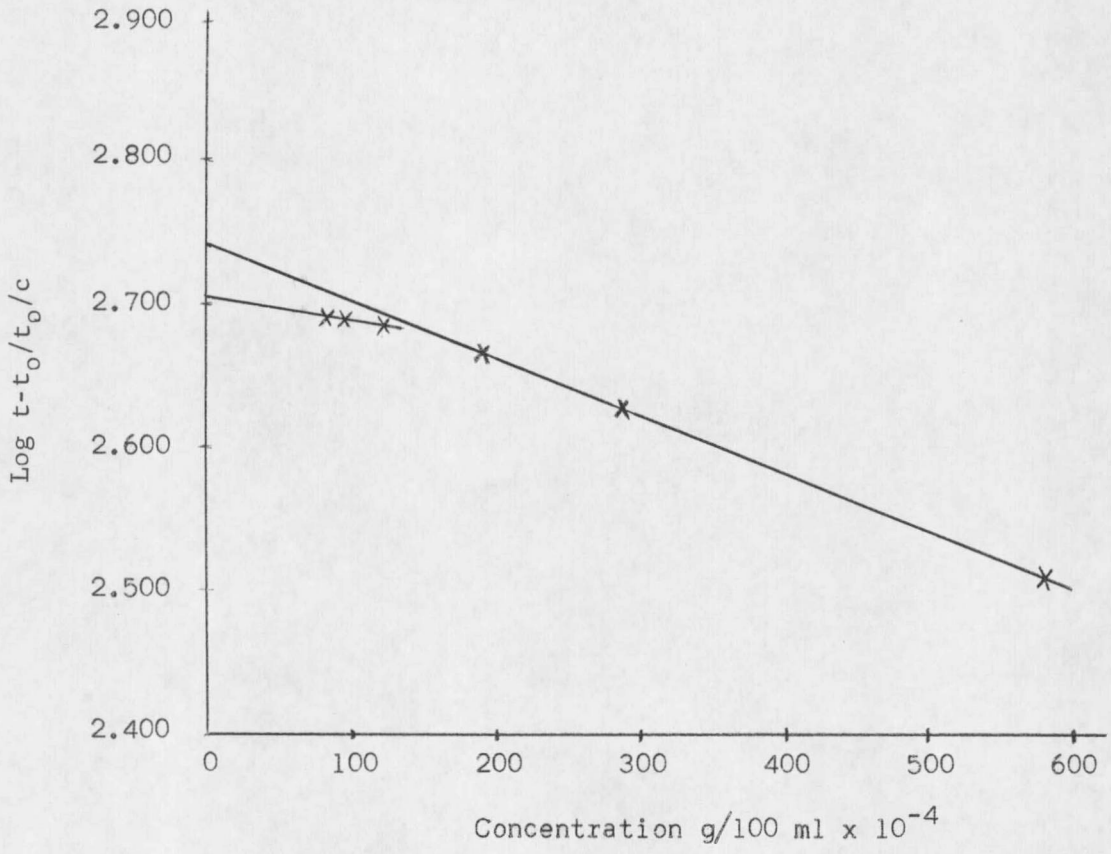
Attempts to digest the polysaccharide from a water solution were unsuccessful. Neither α-amylase nor β-glucosidase were effective in lowering the viscosity of the polysaccharide solution after 24 hours incubation. Also, there were no reducing sugars detectable by the potassium-ferricyanide test after these digestions. The molecule was recoverable from the digestion solutions by precipitation with an equal volume of 95% ethanol. The use of these two enzyme systems indicated that there are no exposed α1-4 and no exposed β1-4 glucosidic linkages.

Intrinsic viscosity of the molecule was estimated using a Cannon Ubbelohde Semi-micro Viscometer Number 75-K921 at a temperature of 30 C. An intrinsic viscosity of 510 to 550 was determined. The method used in this determination involved plotting log $t-t_o/t_o/c$ versus concentration in gm/100 ml x 10^{-4} (Figure XIV).

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* Schwarzkopf Microanalytical Laboratory, New York

Figure XIV Viscosity determination of extracellular C. gramineum polysaccharide.



t = time molecule

t_o = time solvent

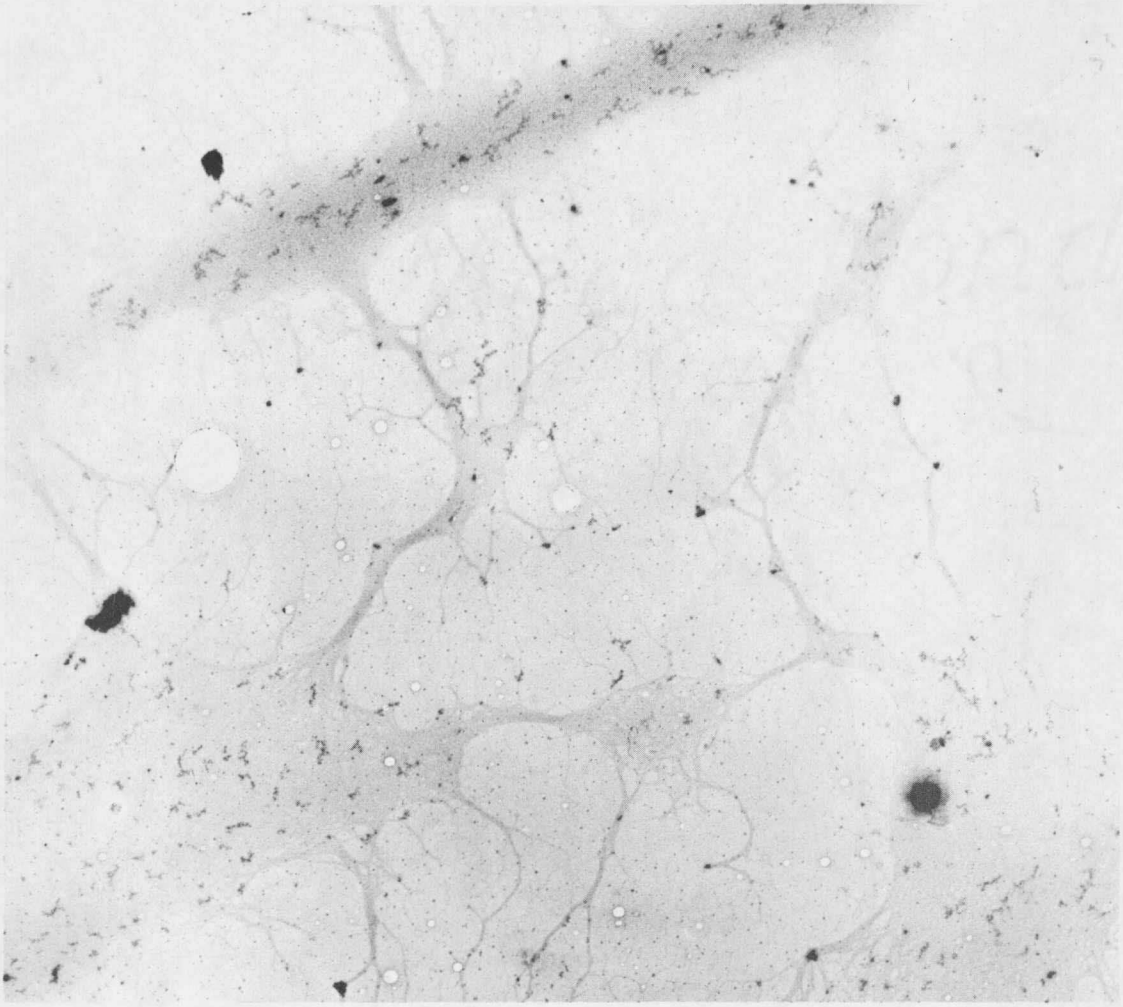
c = concentration in gm/100 ml

Electron micrographs of the molecule dried from water revealed an amorphous material, apparently quite cohesive as shown in Figure XV. This stringy ultra-structure is reminicent of the viscous polysaccharide which precipitates in ethanol. Electron micrographs of the precipitated polysaccharide dried from 95% ethanol showed only a massive electron dense material.

The precipitate dried from alcohol in addition to being electron dense, was quite insoluble. It would not redissolve in water and was insoluble in 1N HCl, 2N HCl, conc. HCl and 0.5N NaOH within 24 hours. In 1N NaOH the precipitate broke up in 24 hours but did not dissolve to any extent. It was soluble in 10N NaOH.

Separation of the polysaccharide from infected straw was effected by taking advantage of its large molecular weight. The aqueous filtrate from infected leaves was concentrated and sieved through Sephadex G-200. A characteristic precipitate was observed with 95% ethanol in the concentrated Sephadex eluate from infected but not from control plant samples. There was a slight precipitate in the uninfected control plants, though of a more solid nature than the polysaccharide precipitate. The precipitates were washed once with 95% ethanol, dissolved in 1.0 ml of water and tested for carbohydrate by the phenol-sulfuric test. The precipitate from infected plants contained about ten times as much carbohydrate as that obtained from healthy tissue. On the basis of these tests, the fungal polysaccharide was shown to be present in infected but not in non-infected wheat.

Figure XV Electron micrograph* of polysaccharide dried from water.



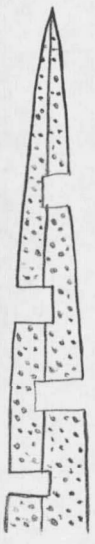
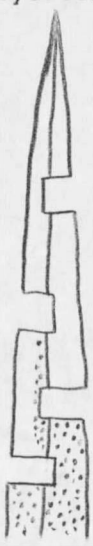
* Zeiss EM-9A
Magnification 15,000x

In tests using the polysaccharide as the carbon source, C. gramineum grew profusely. Within three weeks after inoculated, the first and second tube dilutions contained abundant conidia. There was no appreciable growth in any of the control solutions. After two months incubation, there were several hundred times as many conidia in the tubes containing the polysaccharide as in the control tubes. An increase in conidia in the control tubes suggested that the fungus might be able to fix atmospheric carbon dioxide, but further work is needed to establish this possibility. However, the ability of C. gramineum to utilize the purified polysaccharide as an available carbon source was established.

Spalding et al. (1961) used Eosin Y to show malfunctioning of the vascular pathway in striped leaves. The physical characteristics of the fungal polysaccharide are such that it could be the agent responsible for blocking the lateral movement in infected leaves. To test this possibility, leaves from healthy vernalized Cheyenne wheat were excised under water and immediately placed in test tubes containing 2.5 ml of either the polysaccharide (0.1 mg/ml) or glucose (0.1 mg/ml). After 18 to 24 hours, the leaves were placed in 1% aqueous Eosin Y. Segments of the leaf were removed at various levels to block upward flow artificially and to force a lateral movement of dye (Figure XVI).

After one hour, the dye had penetrated most major vein areas in the control plant. The dye was excluded from the lateral areas of the leaves which had been in the polysaccharide. After two hours in the dye, the extreme lateral areas of the test leaves were still not stained. The control and test leaves were entirely stained after six hours in Eosin Y.

Figure XVI Restriction of fluid movement in wheat leaves.

	Hours in 1% Eosin Y		
	1	2	3
A. Glucose - 0.1 mg/ml*			
B. Fungal Polysaccharide - 0.1 mg/ml*			

* Cut end of leaf in solution 24 hours

These tests indicated that the purified polysaccharide is capable of blocking lateral movement of fluids in wheat leaves.

II. Toxin

Attempts were made to biologically identify the growth inhibiting substance associated with Cephalosporium gramineum agar cultures (Table XXVIII). Even though there was inhibition of Aerobacter aerogenes on nutrient agar, failure to inhibit the growth of Salmonella typhimurium, and Pseudomonas aeriginosa indicated that the material was not cephalosporin N (Gottshall et al., 1951; Hobby et al., 1956; Reedy and Shaffer, 1956; Goldberg, 1959; Jackson et al., 1965). The crude material inhibited the growth of Alcaligenes faecalis indicating that it is probably not one of the cephalosporin P series (Burton and Abraham, 1951). The inhibition of Bacillus subtilus may indicate that the material is cephalosporanic acid (Miller, 1962). However, since there was inhibition of Alcaligenes faecalis, Bacillus subtilus, Escherichia coli, and Staphylococcus aureus, it is probable that the bacterial inhibiting substance is cephalosporin C (Stauffer et al., 1966).

Alternaria sp. and Helminthosporium sp. were tested on czapek agar under similar conditions as used with the bacteria. These fungi were not inhibited adjacent to the agar plugs, indicating that the diffusible antibacterial agent is not affecting the fungal growth. A Pencillin sp. which exhibited a moderate inhibition adjacent to C. gramineum was grown on agar containing the fungal polysaccharide. The Penicillin sp.exhibited a peculiar raised growth pattern typical of a toxic response. It is

Table XXVIII Inhibition of organisms by C. gramineum cultures*.

Organism	Radius of inhibition zone (mm)**	Stimulation of growth
Salmonella typhimurium	0***	-
Pseudomonas aeriginosa	0	-
Aerobacter aerogenes	6	+
Alcaligenes faecalis	7	-
Bacillus subtilus	3	+
Escherichia coli	6	-
Staphylococcus aureus	6	-
Alternaria sp.	0****	-
Helminthosporium sp.	0	-

* Six mm PDA agar plugs from one month old C. gramineum cultures.

** From edge of agar plug.

*** Nutrient agar.

**** Czapek agar.

therefore probable that the C. gramineum polysaccharide is responsible for the inhibition of Alternaria sp. and Helminthosporium sp. when these are grown adjacent to Cephalosporium gramineum cultures.

DISCUSSION

Cephalosporium stripe disease of winter wheat was recently recognized as a problem in the northwestern United States. Within the last two to four years it has become the most important soil borne disease in Montana. Preliminary evidence indicates that the degree of infection and destruction attributable to this disease will cause losses that exceed all other soil borne disease losses in winter wheat in Montana in 1967. As an example, Rego in a field ten miles from Bozeman had 5 to 10% infection in the 1964-1965 season. In the 1966-1967 season after one year of summer fallow, the wheat was approximately 60% infected with C. gramineum.

As previously described, Cephalosporium stripe disease was found mostly in the western mountainous area of Montana. The disease was not found in the far northern areas of central Montana. This area is relatively dry and many of the fields have been recently taken from range. In addition there are large areas of open range between these fields. In some of the mountain valleys in the high infection areas of the state, the disease was not detectable. These fields are well isolated by mountains. The restriction of movement of infected plant debris probably keeps the inoculum potential quite low in the isolated areas. Infected plants were possibly present in these fields but in such low numbers that they were not detected.

Cephalosporium gramineum has been isolated from all of the residue of infected plants including roots and awns. These plant parts are spread across the field with the normal harvesting operation. Within a few years, the inoculum would be spread over an entire field from a point source of introduction. Such dispersion has been observed with Cephalosporium

gregatum (Hildebrand, 1952). The wind carrying infected chaff would aid in spreading inoculum from one area to another.

It would appear that one could effectively remove much of the inoculum by burning the stubble. This practice is not common in Montana, however, since the stubble is generally needed to retain the snow and prevent winter kill. Deep plowing the stubble would temporarily remove the inoculum but studies of the longevity of the organism in straw indicate that this straw would still be infected when brought to the surface with the next deep plowing.

There are conflicting reports relative to the importance of seed as an inoculum source for this disease. Bruehl's report (1957) that seeds are not an important inoculum source was based on non-vernalized seedlings. Nisikado et al (1934) reported that seed was an important source of infection and introduction of the organism into an area. Their report did not mention whether the tests were based on vernalized plants or not. The present study which involved vernalizing plants from possible infected seed is in agreement with the conclusion by Bruehl that seeds are not an important inoculum source in our area in causing an epidemic. If it is considered that from seedborne infection alone, one plant of every 200 is infected and there are about eight plants per foot, one plant would survive and be infected with C. gramineum for every 24 foot of row in which infected seed is used. This is less than 1% infection. But, the infected straw would now be available as an additional inoculum source in the field. If parts of the floret remained attached to the seed, as often happens in infected plants, then the fungus would be present directly at the base of

the plant.

It is possible that the differences attributed to the importance of seed as an inoculum source could be due to varietal differences used in the different tests. The time interval between harvesting and sowing the seed may also be important. Cephalosporium acromenium in corn can be seed borne but the viable fungi in infected seed are reduced with age (Pisano, 1963). The year old seed used in the C. gramineum tests may have been old enough that high levels of seedborne infection were not detected.

Varietal differences in resistance to C. gramineum have been demonstrated in the field tests for natural infection. Such resistant varieties were used in Japan to combat this disease (Ikata, 1939). The tillering differences demonstrated are also probably varietal differences in the response of the plants to infections. Bruehl indicated that infected plants tiller more than non-infected plants. This appears to be the case when space planted but not when field planted. However, under our field conditions the smallest dead tillers could have broken off before the plants were harvested.

Bruehl also found that not all tillers of an infected plant were infected. That is contrary to the findings in this study in which all tillers of naturally infected plants contained the organism and expressed symptoms. In attempting to resolve this conflict it was noticed that an emerging tiller was often infected and died before the first leaf had fully expanded. In other instances, a tiller may have reached the four leaf stage before symptoms appeared. Thus for at least a month the tiller appeared to be non-infected. Furthermore it was found that if a plant was hypodermically

inoculated into the stem, the fungus would readily progress toward the tip of the plant, but movement of the fungus toward the roots was very limited. If a single leaf was inoculated, the fungus could be only reisolated from that one leaf. If the point of inoculation was above a node, tillers arising below this node did not become infected. Apparently the organism does not readily progress down the conducting tissue in a living plant.

The means of natural infection could presumably be important in the yield loss attributable to this disease. Stem inoculation would cause less damage than root inoculation if a surface vector is involved in transmission of the disease. Slope and Bardner (1965) have shown that wireworms have a role in the infection process of C. gramineum in England. It is possible that nematodes might have a similar role in Montana. Since nematodes would be expected to damage the roots of spring wheat as well as winter wheat, natural Cephalosporium stripe of spring wheat should be observed. Spring wheat has not been found to be naturally infected with C. gramineum. Both nematodes and wireworms would attack the roots. Attempts to get aphids to act as aerial vectors for Cephalosporium gramineum have so far been unsuccessful. Otieno (1961) has shown that under high inoculum potential, C. gramineum can invade winter wheat through the scutellum. The importance of this mode of entry relative to entry through root damage should be investigated further. In this laboratory, the inoculation of seedlings in soil has not resulted in infection unless the seedlings were first injured in some way.

Seedlings as young as 7 days grown in petri dishes have become infected by briefly dipping the cut primary root into inoculum. Fourteen day old

seedlings in dishes or soil were also infected by inoculating the sheath. Bruehl (1957) indicated that the rosette stage of winter wheat exhibits some resistance to Cephalosporium stripe disease. Such resistance was not observed in this study. The wheat was susceptible at all ages after seven days. It was suspected that plants infected before vernalization might outgrow the disease under favorable conditions after vernalized. Such was not found to be the case. Regardless of the time inoculation took place relative to vernalization, the wheat became infected.

Field and controlled environment experiments confirmed the work of Ikata (1934) and Bruehl (1957) that late planting leads to less disease. It was demonstrated that soil temperatures at the time of planting were a major factor affecting the root growth of the wheat. Wheat planted early (August) had longer root systems with more extensive branching than later planted wheat (October). The August planted wheat was in warm soil in the autumn longer than the October planted wheat. During the winter, cool soil temperature slowed down the root growth of all the wheat. The differences in root mass from the several autumn planting dates and fertilizer treatments persisted until after the ground was thawed in the spring. The soil ice layer demonstrated in 1966 is probably present sometime during the winter most years. With the spring diurnal freezing and thawing of the surface of the field and the associated heaving of the soil, the plants would be stressed at the interface between frozen and non-frozen soil both at the surface and at the lower ice layer. Under such strain, the roots would be subject to breakage. The longer roots would be held fast in the lower ice layer for a greater length of time and

thus be subjected to these strains more often than shorter roots. With more extensive branching, there would be more rootlets under stress, more points subject to breakage and consequently more available entrance points for the pathogen.

The suggestion that heaving of the soil damages the roots, thus providing the primary mode of entrance for the pathogen can be questioned. However, the incubation period is two to four weeks after root inoculation with a relatively warm (45/72 F) diurnal cycle. In plants inoculated just prior to being vernalized, symptoms were expressed in the majority of plants after eight weeks at 40 F. With a longer delay between inoculation and the onset of vernalization, more plants expressed symptoms after vernalization.

In June, field plants have been exposed to temperatures above 40 F for at least eight weeks. Yet, plants advanced to S-9 on the Feekes scale are often just exhibiting the first symptoms of infection of the lowest leaves. Attempts to locate infected plants in the field in late December from August planting have been unsuccessful. When grown out or sectioned onto PDA, plants with possible symptoms were not found to contain C. gramineum. Similarly, inspection of August and September planted nurseries in March and early April did not disclose any C. gramineum infected plants. It is therefore highly probable, as suggested previously, that infection takes place in the spring.

The soil moisture relationship to susceptibility has previously been described. Whenever infection may occur, plants grown in low soil moisture availability were physiologically more resistant to infection than plants

grown in abundant soil moisture. Relatively dry soil also tended to adsorb or at least restrict the movement of the non-motile conidia. The available inoculum potential was therefore effectively reduced. The practical result of this situation can be seen in the distribution of the disease in the state. In the eastern half of the state where there is generally a low rainfall, the disease has not been detected. More than likely wheat grown on restricted soil moisture availability has a vascular system with smaller elements. The movement of the pathogen through the host might then tend to be restricted. These dry land plants would also have a lower transpiration rate which would further restrict the passive movement of the fungus and its byproducts through the host. The parasite would then be excluded from the plant as the injured roots, to which the fungus might be confined, died as a result of injury and low moisture availability. A further investigation into these areas could be profitable.

The extracellular fungal polysaccharide investigated could play a role in restricting the availability of water to the plant. There was a direct correlation noticed between the number of conidia in a culture and the amount of polysaccharide produced. It is possible that this polysaccharide is a conidial byproduct which has been noticed as the mucus surrounding the conidial head in culture (Buchanan, 1911). This point should be investigated further. By its size (M.W. greater than 200,000) and intrinsic viscosity (510-550) the mere presence of this molecule in any concentration in the vessels would restrict water movement to some extent. Such a restriction of water movement in vessels was postulated by the findings of Oteino (1961) who described xylem plugs of unknown composition

in infected plants. Spalding et al. (1961) demonstrated a restriction to fluid movement in C. gramineum infected leaves. In the present study, the purified fungal polysaccharide was shown to be capable of retarding the fluid movement in wheat leaves. It is possible that in the procedures used to purify the polysaccharide, the molecule was partially hydrolyzed. A larger molecule present in the plant would restrict water flow even more than was demonstrated. Any restriction in fluid movement would be expressed as a characteristic dry land syndrome. Infected plants generally have such a syndrome. The infected plants are shorter, mature earlier, and the heads are less filled than healthy plants.

This fungal polysaccharide has been recovered from infected but not healthy plants as evidenced by column chromatography. The mere presence of recoverable amounts of this large, viscous molecule would imply that there is some area within the host where water movement is restricted. The plugging of the small lateral leaf veins prior to the onset of chlorosis (Spalding et al., 1961) is probably caused by this polysaccharide.

It is possible that the polysaccharide is a dextran. The absence of exposed α 1-4 and β 1-4 glucosidic links, the size and viscosity, and the large amount of glucose released upon hydrolysis all contribute to this possibility. The rhamnose and possible glucuronic acid detected upon hydrolysis could be other fungal products separated as artifacts of the purification procedure. They might be constituents of the molecule or merely be attached by hydrogen bonding. No dextranase was available to test this polysaccharide.

Cephalosporium gramineum cultures exhibit a zone of inhibition around the colony. By using a biological assay technique, cephalosporin C. or possibly cephalosporanic acid, has been suggested as the bacterial inhibitor. It is possible that the C. gramineum polysaccharide was responsible for inhibition of Alternaria sp. and Helminthosporium sp. but this point should be further investigated.

Cephalosporium stripe disease development might have a logical pattern. The fungus invades the host wheat through some root injury. Plants grown with high soil moisture availability are more susceptible to infection and the available inoculum potential is higher than in dry soil. The presence of the fungus mycelium and conidia in the wheat restrict water movement through the vessels but to only a limited extent. The extracellular water soluble fungal polysaccharide moves passively up the transpiration stream. In the larger vessels its restriction to water movement is not an important factor. However, as this molecule is carried to the small interveinal elements it severely restricts water movement. The restriction of water curtails photosynthetic activity thus decreasing sugar production. Through influences on enzymatic activity, the water deficit stimulates hydrolytic reactions (Couch, Purdy, & Henderson, 1967) which eventually lead to senesence and death of the restricted cells. The cells most affected would be adjacent to the veins thus the leaves would exhibit a chlorotic striping symptom. Chlorosis of the leaves progresses from the tip to the sheath as would be expected under the conditions outlined. The necrotic line progresses up the sheath to the leaf tip and may be caused by some other factor.

The decrease in sugar production would affect the entire plant and contribute to the observed stunting. The sugars that are produced would move down the phloem to the roots. This may contribute to the healthy looking condition of the roots of infected plants. With water movement in the small elements leading to the developing head restricted by the fungal polysaccharide, and with limited supplies of plant sugars, growth in the head would be restricted. If the restriction occured early enough in the development of the head, the last internode would fail to elongate and the head would die in the boot. Restriction at a later growth stage would result in lack of development of kernels, failure of the partially developed kernels to fill out, and premature drying of the heads. When the heads ripened, these partially filled kernels would shrivel. All of these symptoms are characteristic of the disease.

The lack of filling of heads and small kernel size are probably the most important factors which contribute to the reduced yield. The number of tillers produced by a plant are important, but if they do not head, the plant is unnecessarily taxed. On infected plants, a large number of heads die in the boot or partially emerged and yield little or no grain. Infected heads that do fill out seldom produce as many kernels as healthy plants resulting in a definite yield loss. Those kernels which are produced are generally shriveled. This gives them a large surface to volume ratio and they are easily discarded with the chaff. This loss with the chaff is most easily demonstrated by running heads from infected plants through a head thresher with a normal air flow or recovering the shriveled kernels

by standing behind a combine. It is easily seen that the size and quantity of grain is severely affected by this disease. It is probable that the quality of grain is affected although there was no statistical difference in the viability of the grain recovered from infected and healthy plants.

SUMMARY & CONCLUSIONS

Cephalosporium stripe disease of winter wheat was found in the western mountainous half of Montana but not in the plains of the far northern and eastern parts of the state. The field conditions most suitable for disease development were high degrees of stubble, planting early and fertilizing with $Ca_3(PO_4)_2$ in the drill rows, and high soil moisture. Conversely, there was a lower disease incidence if the grain was planted later in the fall when the soil temperature was lower, when stubble was reduced in the field, and when the field was well drained. Under these latter conditions, fertilizer at the time of planting may facilitate more infection but the overall yield in the field more than compensates for the losses due to increased disease.

Infected plants could often be found in low, water collecting spots in lightly infected fields. Under controlled environment conditions, a greater percentage of plants in soils with higher soil moisture (35%) at the time of injury and inoculation became infected than plants in soil with a lower moisture content (20%). This condition was apparently not related to the state of maturity of the plant since plants as young as one week old or with just the first leaf showing could become infected.

In addition to the above environmental conditions favoring disease development, varietal differences in degree of susceptibility have been demonstrated. Several Burt x Itana crosses showed considerable degrees of resistance as indicated by leaf and head symptoms. The difference in discoloration of mature straws appeared to be generally unrelated to the susceptibility of a variety. In a few cases of extreme susceptibility, such as the variety Lancer, there was much culm discoloration in mature

infected straw.

The loss in yield due to Cephalosporium stripe disease was proportional to the degree of infection. Diseased plants do produce a few viable kernels per head but they are shriveled and readily blown out with the chaff when the heads are threshed.

Infected plants were easily identified in the field by the chlorotic and necrotic lines in the green leaves, sheath and on the stem. When headed out, the diseased plants were shorter (75% of the healthy) and the heads matured early giving a white head syndrome. The disease as evidenced by necrotic symptoms readily passed up but not down infected tissue from the site of hypodermically inoculated plants. The organism could not be isolated much below the inoculation level but was readily isolated above this point. As such, the organism apparently could not progress down the stem and cross into other tillers unless the secondary tillers arose reasonably close to the inoculation point. The field findings indicated that if one tiller was infected, all became infected. This implies that infection was taking place at or below the area of tiller initiation.

It is of interest that the chlorosis of leaves starts at the tip of the leaf and progresses back to the sheath, but the necrotic line starts at the node and works up the sheath and to the leaf tip. This indicates that possibly two factors are working to produce these disease symptoms. It was also directly below the nodes that the most severe discoloration of the mature straw was noticed.

A transparent precipitate was obtained by precipitating the crude culture extract with an equal volume of 95% ethanol. It was a non-lipid,

non-protein containing, heat stable carbohydrate. It was non-chitinous, contained no exposed α1-4 or β1-4 glucosidic linkages and was slightly soluble when dried from ethanol. It was composed primarily of glucose residues linked to a molecular weight in excess of 200,000 with an intrinsic viscosity of 510 to 550.

This non-proteinaceous polysaccharide though probably not an antifungal agent had several peculiar chemical properties. It was water soluble and readily precipitated in 50% ethanol, but if left in the ethanol it would redissolve and not again be obtained as an ethanol precipitate. It was detectable as a precipitate at 5×10^{-6} gm/ml and was nearly a gel at 2.5 mg/ml.

One of the most important properties of this polysaccharide is that it can serve as the sole carbon source in vitro for the fungus. It was isolated from infected straw and as such it might serve as the carbon source to keep the organism alive for extended periods.

An antibacterial byproduct of Cephalosporium gramineum was detected. By biological assay it was shown not to be cephalosporin N or cephalosporin P. It has some properties of cephalosporin C but a detailed analysis was not conducted.

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