# CEPHALOSPORIUM LEAF STRIPE OF WHEAT: DECLINE AND MODE OF INFECTION

By

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ABSTRACT

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PART I: Decline of Cephalosporium leaf stripe

A field previously reported to be exhibiting declining disease incidence was selected for this work and was studied in its eighth and ninth years of wheat monoculture. Three similar fields with 0, 3 and 4 years of wheat monoculture were also studied. Soil was reciprocally transferred between these fields, and Cephalosporium gramineum soil populations as well as disease incidence were monitored. Both were low in the newest and oldest fields, and high in the fields of intermediate age. Addition of C. gramineum, as oat seed inoculum, increased disease in the newest and oldest fields only and resulted in an inverse relationship between the years of wheat monoculture and disease incidence. Results in the second year of testing were identical except that additional inoculum produced uniformly high levels of disease in all four fields. In the second year, population levels were too variable to allow the substantially larger populations in the two fields of intermediate age to be statistically significant. There was no reduction in disease incidence from reciprocal transfers of soil. Fields supporting 4 or 5 years of wheat monoculture did not appear to show spontaneous disappearance of disease. It was concluded that the field with 9 years

of continuous wheat is maintaining its decline status, but that disease decline may not result in other fields under continuous wheat monoculture.

PART II: Mode of Infection

Various methods of measuring the effect of root exudates on Cephalosporium gramineum were developed. It was found that exudates from frozen roots increased germination, growth, and conidiogenesis above that of non-frozen root exudate. Conidia held in a fungistatic condition, due to the influence of non-sterilized soil, were able to germinate and penetrate root tissue when exudate from roots that had been frozen was present. Hyphae were shown to grow deep into host root tissue. Various methods of freezing roots allowed C. gramineum to colonize plants and cause symptom production. Evidence is presented that such roots were unbroken, thereby implicating an active penetration process. Roots held at low temperatures (2 C), but not frozen, did not become infected. An inexpensive and easy-to-use apparatus was designed to simulate field freezing. This freezer allowed non-vernalized plants to be frozen without crown death because only root tissue came into contact with frozen soil. Freezing and inoculating grain varieties with known resistances resulted in disease incidence similar to those found in the field. It is concluded that active penetration after freeze stress is an important means of infecting plants. Field observations tended to support this conclusion.

To my wife, son, parents, and brothers

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# LITERATURE REVIEW

## General

In 1934 Nisikado et al., described a new disease of wheat in Japan (57), caused by an undescribed species of Cephalosporium. Nisikado and Ikata named the fungus Cephalosporium gramineum Nis. and Ika. and the disease Cephalosporium leaf stripe. Cephalosporium leaf stripe (CLS) has since been described in many grasses, including the genera Dactylis, Avena, Bromus, Elymus, Secale, Hordeum, Triticum, Agropyron, and Arrhenatherum (8, 36). Cephalosporium leaf stripe is a serious problem in Japan (57), Europe (29, 75), and the United States (52, 55, 69, 79, 81). In the United States, Cephalosporium leaf stripe is of particular importance in Washington (7), Kansas (94), and Montana (53); in Montana it is the most important soil-borne disease of wheat (52). In 1966, CLS was observed in Michigan by N. A. Smith, R. P. Scheffer, and A. H. Ellingboe (79).

In 1963, Bruehl showed that C. gramineum also formed a sporodochial stage on dead, infested wheat stubble in the field (9). Colonies of this fungus on agar were identical to those formed by C. gramineum and inoculation of wheat produced the symptoms of Cephalosporium leaf stripe. Bruehl identified the fungus as Hymenula cerealis Ellis and Everh. and stated that this name should have precedence over C. gramineum (9). He did, however, recommend that Cephalosporium leaf stripe remain the name of the disease. Therefore, the causal organism has two imperfect names:

Cephalosporium gramineum for its parasitic stage, and Hymenula cerealis for its saprophytic stage. To date, no perfect stage has been described for this fungus. For clarity, C. gramineum will be retained as the name of the fungus in this paper.

Parasitic stage

It is generally agreed that C. gramineum invades its hosts through the root system, (4, 8, 53, 56, 57, 62, 76). Once in the vascular tissue, systemic colonization of all vegetative portions of the plant occurs; however, the fungus is restricted to the xylem as long as the plant is alive (86). When introduced directly into root xylem, at room temperature, the fungus moves through the crown into the lower leaf sheaths within 4-6 days (86). Stripe development begins approximately one week after (86), when long chlorotic bands extend the length of the leaves (7, 8, 57) encompassing darkened vascular bundles. Acropetal symptom development follows an early senescence of lower leaves resulting in stunting, poorly filled or unfilled seed heads, and decreased flour quality (53, 55). Cephalosporium leaf stripe can result in a 70-80% yield reduction in severely infected plants (40, 67, 76).

In 1938, Ikata and Kawai (38) showed that certain culture filtrates of C. gramineum stunted wheat seedlings, while others produced stripe symptoms which later became necrotic. Bruehl (8) suggested that symptom development was most likely a result of xylary plugging by mycelium and physiological disfunction due to toxic metabolites produced by the fungus. Spaulding et al. (80) found that C. gramineum produces pectinases and cellulases, and were able to demonstrate pectinase activity in diseased but not healthy plants. They also found that diseased tissues had a lessened water content and attributed this to increased viscosity

of the water and xylary plugging by a polysaccharide produced by the fungus. They felt that hyphae and pectin plugs in the xylem elements further inhibited water movement, since acropetal and lateral dye movement could not occur in stripe tissue. They concluded that such "vascular distress" may contribute to cellular dysfunction and death. Work by Pool and Sharp (61) supported this conclusion by showing that a polysaccharide produced by C. gramineum cultures restricted dye movement in the xylem.

Wiese (86) suggested that a diffusable substance(s) and not pectin or hyphal plugging of the xylem was responsible for interveinal stripe development. He found that such occlusions followed rather than preceded lateral extension of leaf striping. Electron micrographs of infected vascular bundles showed an accumulation of an electron dense material surrounding conidia, which, after liberation, was found to line the walls of infected vessels. Wiese felt this may be visual evidence of the diffusable product responsible for the restriction of lateral water and/or nutrient movement.

Kobayashi and Vi (41) identified a toxin from culture filtrates of C. gramineum which caused symptoms much like those of naturally infected plants. This compound, Graminin A, was also found to have antibiotic properties effective against some bacteria and fungi. Creatura (21) found that Graminin A caused stomates to open wider and respond more slowly to water potential changes than stomates not treated with this compound. This response preceded stripe development and was more pronounced under conditions of water stress. She showed that differences in stomatal activity were not a function of differences in the leaf water status. Her work indicates that a toxin, Graminin A, and not diffusable metabolites are responsible for the early pathogenesis.

Saprophytic stage

After plant scenescence, C. gramineum begins its Hymenula cerealis stage characterized by random saprophytic growth from the xylem and the colonization of cortical tissues. The hyphae emerge through openings (i.e. stomates) in the plant residues, and phialides form at hyphal tips (92). Rapid conidiogenesis along with mucopolysaccharide production results in a mass of tightly adhering phialospores (13, 15, 61, 92). Wiese and Ravenscroft (89) found these conidia to have a half-life of 0.5 to 2.5 weeks in moist field soil held at 25 C, or 26 weeks when held at 7 C. When colonized plant material was present the population was renewed as long as the integrity of the host material was maintained. For example, infested wheat straw on the soil surface was able to renew the colony forming units (probably conidia) in soil each fall and winter, for approximately three years. Such straw was less efficient with each succeeding year. Buried straw, however, was a source for population renewal for only one year. The population density of C. gramineum buried in infested straw was approximately 11-32% of the original population after 18 months (42). Pool and Sharp (62) observed survival for as long as 40 months in infested residue. Trichoderma spp. were predominant colonizers of degenerating C. gramineum-infested straw; however, C. gramineum can exclude Trichoderma and other fungi for at least 13 weeks (12). Thus, C. gramineum is an effective substrate possessor (11, 12, 43). There is some evidence that antibiotic production may play a role in excluding other fungi from previously infested wheat straw (14). Effective substrate possession is not unusual among fungi and may be of particular importance to pathogens of weak saprophytic ability (12).

Survival during the fall and winter until host infection occurs is, therefore, a function of spore production and longevity as well as effective substrate possession.

The development of a selective medium (green wheat agar) in 1973 (88) allowed for the first time quantitative detection of C. gramineum propagules in the soil. Wiese and Ravenscroft (89) showed that colony forming units in the field rose from near zero in July to a peak of approximately 100,000 colony forming units/g soil by mid-winter (November-January). This was followed by a decrease to near zero by May. They found that removal of plant residues or treatments which hastened residue decay greatly reduced detectable C. gramineum populations. Population levels were progressively lower when wheat residue was disked, plowed, or removed, respectively, from the field.

Disease decline

Soils are known to vary in their 'hospitality' to microorganisms. Chemical (17, 18, 48), physical (19, 30, 65), and microbial (3, 18, 20, 47, 48, 49) factors all influence the success of a fungal species to establish and maintain its presence in the soil ecosystem. In the case of soil-borne pathogens, these factors can affect disease incidence and severity. It is well known that sterilized soil stimulates growth of introduced, weakly-pathogenic fungi. This usually results in an increase in disease severity, as compared with non-sterilized soils which are suppressive to such unchecked growth. Consequently, all soils exhibit biological control of disease, to a greater or lesser extent, when compared to sterilized soil. Of more practical significance are those naturally occurring soil systems which are indistinguishable from fields with high disease incidence, yet suppress soil-borne diseases to

economically acceptable levels. There are several good examples of soils which have evolved to a suppressive state (1, 5, 28, 31, 33, 34, 71, 72, 82, 84, 93).

Once a field becomes infested with C. gramineum, continued wheat monoculture normally results in a build-up of C. gramineum populations. Recently, Wiese and Ravenscroft (91) showed a long-term decrease in pathogen population and disease incidence with continued wheat monoculture. This phenomenon was observed over an eight-year period and resulted in disease levels too low to be used for disease studies. Addition of infested straw, the naturally occurring source of inoculum, was ineffective in significantly changing the disease status of this field (Personal communication, A. V. Ravenscroft). Soils exhibiting a spontaneous decrease in disease incidence with continued monoculture are sometimes referred to as 'decline' soils, and the disappearance of the disease may be called 'disease decline.' The most studied example of this phenomenon is take-all decline (TAD) of wheat.

Gaeumannomyces graminis is the causal organism of take-all disease of wheat, a disease of great significance in temperate climates where wheat is intensively cultured (83, 87) particularly in the Pacific Northwest (18). As early as 1898, Roediger noted a decrease in infection in previously infested land (34). Glynne et al. in 1935 (25) observed that soil continually planted to wheat was not conducive to take-all. It wasn't until 1964 that Slope and Cox (34) obtained experimental evidence that there was a decline of take-all over time. In 1972, Pope showed a decrease in infectivity in soils which 12.5% 'decline' soil had been added (63). Pearson et al. (60) showed that as little as 0.0001% (by wt.) of decline soil could decrease disease in non-decline soils.

In the field, fumigation eliminated the decline factors, but it was reinstated by adding 1% (by wt.) suppressive soil (73).

Attempts to link the suppressiveness to specific factors has so far met with only mixed success. Early work attributed TAD to the microbiological status of the soil (34). Antagonistic microorganisms were thought to be responsible for decline since exposure to 60 C for 30 min., or chemical sterilants removed the decline factor (73). Vojinovic (34) found that populations of bacterial colonies and actinomycetes antagonistic to G. graminis increased after the decay of host tissue. This was particularly true for diseased host tissue.

G. graminis is known to respond tropically to wheat root exudates (69), increasing the effectiveness of a given inoculum concentration. Pope and Jackson showed that the efficiency of this response decreased in decline soils, as compared with non-decline soils (64). They attributed this to either a depletion of exudates due to increased microbial competition, or to 'signal jamming' by metabolites produced by a new rhizosphere population. Zogg and Jaggi (96) demonstrated an increase in numbers of bacteria and actinomycetes in soil supplemented with G. graminis hyphae. Sixty percent of the microorganisms isolated were antagonistic to G. graminis. Fluorescent Pseudomonas were suspected of being responsible for TAD since they : 1) are common in wheat rhizospheres (particularly in TAD soil) (51) and on the rhizoplane of G. graminis-lesioned roots (20), 2) have the same cardinal temperature of inactivation as decline soil (20), 3) are as effective at reinstating decline conditions in bioassays (73) as known decline soil (20), and 4) are efficient antagonists of G. graminis on agar (68).

Reduced virulence and viral infection of G. graminis were hypothesized as factors in decline. Cunningham suggested that less virulent strains of the pathogen were important in TAD (34). Lapierra et al. (44) and Lemaire et al. (45) found that G. graminis isolates from decline fields were infected with a virus causing reduced virulence and poor culturability. However, Rawlinson et al. (66) in a follow-up study could not demonstrate causality between viral infection of G. graminis and virulence, sectoring, growth, or perithecia formation. Virus infection is not now thought to be important in the establishment of hypovirulence (3, 66).

Other explanations for TAD such as volatile, plaque-forming substances (74), saprobic/pathogenic population shifts (34), changes in the $NH^{+}_{4}$-N:$NO^{-}_{3}$-N ratios (6), and cross protection by weakly- or non-pathogenic fungi (22, 95) have not received general acceptance.

Cephalosporium leaf stripe decline has not been reported in any of the major wheat growing areas outside of Michigan, where wheat monoculture is commonly practiced. Its only documented occurrence is at the Michigan State University farms in East Lansing, Michigan.

## Control

No chemical means of controlling this disease are currently available; nor does chemical control appear likely in the near future, due to the low cash value of the crop and the intravascular nature of the fungus. Proper residue management (e.g., deep plowing) and crop rotation have proven to be quite effective in reducing C. gramineum populations. However, in some states crop rotation is not economically feasible due to the limited number of crops which can be grown. In addition to saving

fuel and unnecessary machinery wear, wheat residues must be left on the surface of some soils to prevent excessive water loss and to prevent erosion.

In lieu of residue management, resistance is the best potential method of controlling the disease. In Montana, where CLS is the most important soil-borne disease of wheat, over 1,000 lines have been screened for resistance, using artificially infested field plots (52). No immune varieties have been found, but various levels of resistance have been identified.

Mode of infection and disease resistance

Several papers have focused on the nature of resistance to CLS. Inoculation for these studies usually involves root severing followed by root dip, or direct injection of conidia via a hypodermic needle into the stem (8, 39, 40, 54, 56). This circumvention of extra-xylary tissues is thought to mimic the natural infection process since it is generally agreed that C. gramineum is incapable of direct penetration of roots and that wounding is first necessary. Although root feeding wireworms have been shown to effect such injury, spring heaving due to diurnal freezing and thawing is regarded as the single most important source of wounding. Spring wheat, although susceptible, is virtually disease free, even in infested fields. Likewise, susceptible wheat grown under greenhouse conditions in infested soil rarely becomes infected unless roots are mechanically severed and drenched with a conidial suspension. That the predisposition factor occurs during winter field conditions was shown by Bruehl in 1957 (8). After germinating wheat seeds on cultures of C. gramineum and planting them outdoors in the fall, 100% infection

resulted by the following spring. Only 6% of the plants became infected when grown in the greenhouse.

Early fall planting and fertilization have been shown to increase the size of the root systems and, consequently, the number of roots broken per plant due to heaving (10, 62). Such treatment increased the level of Cephalosporium leaf stripe when rated the following summer (62). More recently, it was observed that the earlier wheat was sown, the more disease occurred up to a point, after which disease incidence began to decrease (90). Mathre and Johnson (54) were unable to demonstrate active growth by C. gramineum from soil to newly cut or crushed roots which presumably simulated naturally occurring heaving damage. To explain the mode of fungal entry from the soil into the xylem, they proposed that conidia were 'vacuumed up' by mass flow with water as it entered the broken xylem elements. Resistance, therefore, would be an intra-xylary phenomenon.

Morton and Mathre (56) identified three types of resistance to CLS: 1) a reduction in the number of diseased plants in a population, 2) a reduction in the number of diseased tillers within a plant, 3) a reduction in the rate and severity of disease development within a plant. The latter two responses have been observed in only one wheat cultivar.

# PART I: DECLINE OF CEPHALOSPORIUM LEAF STRIPE

# INTRODUCTION

Cephalosporium leaf stripe, caused by the soil-borne fungus Cephalosporium gramineum, affects winter wheat in many of the wheat growing regions of the world including the United States (52, 55, 69, 79, 81). Among the commercial wheat cultivars, levels of resistance are insufficient to combat the disease and avoid yield losses. Cultural practices, however, can play a major role in the survival of C. gramineum in the soil. Recently, Wiese and Ravenscroft (91) reported that extended wheat monoculture may lead to a Cephalosporium leaf stripe 'decline'. During the latter part of their eight year study, C. gramineum populations and leaf stripe symptoms decreased dramatically. Similar phenomena have been observed for take-all of wheat (25, 34, 35, 71, 93) and various other soil-borne fungal diseases (28, 31, 33, 72, 82, 84). The purpose of this study was to describe the relationship between leaf stripe incidence, C. gramineum populations, and years of continuous wheat production and to determine if disease suppression may be a transferable trait.

# MATERIALS AND METHODS

## Field selection

Fields at Michigan State University, East Lansing, as previously described (91) with 0, 3, 4 and 8 years of continuous wheat production were used. Soybeans were previously grown in the 0 and 3-year-old wheat fields, oats in the 4-year-old field and corn in the 8-year-old field. The wheat cultivars (Triticum aestivum L.) grown in each of the fields were Ionia, Genesee, or Yorkstar, all equally susceptible to Cephalosporium leaf stripe (91).

Field maintenance

The pH of all soils was maintained between 6.0 and 6.8 as recommended by the Michigan State University Soil Testing Laboratory. No irrigation or pesticides were applied except for 2,4-dichloro-phenoxyacetic acid (2,4,-D) in the spring to reduce broad-leaf weeds. Each autumn, preplant tillage operations included plowing the previous wheat stubble 20-25 cm deep with a fixed moldboard plow followed by a spring-tooth harrow.

Planting

Planting was done in October. The cultivar 'Genesee' was used exclusively. Seeds were planted 3.6 cm deep in 15.5 cm rows.

Inoculum preparation

Isolates of Cephalosporium gramineum Nis. & Ika. (=Hymenula cerealis Ellis & Everh.) from Montana (supplied by D. Mathre) and Michigan were used in the 1977-78 and 1978-79 field experiments, respectively.

One hundred and fifty g of oat seeds were autoclaved in 100 ml water for 30 min. Ten ml of a conidial suspension grown in liquid culture for 7 days were added to each jar. After shaking and incubation at room temperature for 1-2 months, the inoculum was allowed to dry at room temperature (53).

Monitoring Cephalosporium gramineum populations

Soil samples were taken in all uninoculated plots during the winters of 1977-78 and 1978-79. Three samples, taken from the top 7.5 cm (3 in.) of soil from each plot, were mixed and assayed in the laboratory using wheat leaf agar (88). C. gramineum propagules/g soil were determined by adding approximately one to two g soil from the subsample mixture to one liter of water and shaking vigorously. One ml aliquots of this suspension

were pipetted onto each of two assay plates and the number of C. gramineum colonies determined after 4-7 days incubation at 22 C. These two values were averaged and adjusted to represent the number of propagules per dry g of soil.

Soil transfers and infestation

1977-78 season

A completely randomized, split block design was used in these experiments. Fields with 0, 3, 4 and 8 years of wheat were divided into 12, 1.2 x 6.1 m (4 x 20 ft) plots. Nine of these were amended with soil from the other fields and three plots remained unaltered. For example, field 0 had three plots amended with soil from field 3, three plots amended with soil from field 4, and three plots amended with field 8 soil. Fields 3, 4, and 8 were treated likewise. Soil was transferred at a rate of $1.3 \times 10^2$ kl/ha (95 liters/plot). If soil amendments did not change disease incidence or population levels from that in unamended plots, these data were combined to increase the number of replications for further analysis. One half of each plot was infested with C. gramineum grown on oat seed. Oat inoculum (4.7 g m row) was placed 3.6 cm deep between the rows using a Planet Junior hand planter 2 weeks after planting.

1978-79 season

The same fields, now with 1, 4, 5, and 9 years of continuous wheat, were used. All fields had 9, 1.2 x 6.1 m plots which were monitored for C. gramineum populations and rated for disease incidence. Half of 3 plots in each field had added oat seed inoculum. Soil transfers were made from field 9 to field 4 only at two rates; $1.3 \times 10^2$ and $3.9 \times 10^2$

kl/ha. Three plots of each transfer rate was rototilled about 20 cm deep, and half of each plot was infested with oat inoculum one week after planting (4.7 g/m row). This inoculum was placed directly over the furrow, on the soil surface, then covered with a thin layer of soil.

Disease rating

Disease incidence was assessed at anthesis and reported as percentage of plants with leaf stripes. One hundred plants were rated for each treatment.

## RESULTS

In 1977-78, the natural C. gramineum population was significantly lower in field 8 than in fields 3 and 4, whereas field 0 was intermediate (Table 1). Leaf stripe caused by natural C. gramineum inoculum was correlated with the inoculum density (Table 1). When additional inoculum was added (in the form of colonized oat seed), there was no increase in disease in fields 3 and 4; however, fields 8 and 0 showed marked disease increases. This apparently reflects a difference in the conduciveness to disease of these soils, since field 0, a field with a very low population of C. gramineum, exhibited the greatest amount of disease (Table 1).

Natural C. gramineum populations were not affected by the introduced soils (Table 2), nor was the resultant disease incidence, either with or without supplemental oat seed inoculum (Tables 3 and 4).

In the 1978-79 season population change in relation to years of wheat monoculture had too much variability to be significantly different though the same tendency to higher populations in fields 4 and 5 was seen (Table 5). However, in fields with only natural inoculum, disease

TABLE 1. Effect of number of years of continuous wheat on natural *Cephalosporium gramineum* populations and infection caused by natural and oat seed inoculum of *C. gramineum*.

| Field[b] | Natural population[c] (X $10^3$/g dry soil) | Plants showing stripe symptoms, %[a] | |
|---|---|---|---|
| | | Natural inoculum | Natural plus oat seed inoculum |
| 0 | 2.7 ab | 9 a | 33 d |
| 3 | 10.2 b | 15 bc | 21 c |
| 4 | 7.5 b | 17 bc | 18 bc |
| 8 | 1.0 a | 6 a | 13 b |

[a]Mean of 12 replicates with 100 plants per replicate. Values followed by the same letter do not differ significantly ($P = 0.05$).

[b]The field is represented by the number of years of wheat monoculture in that field.

[c]Mean of 6 soil bioassays made from each treatment between November 1977, and June 1978. Samples were taken from plots without supplemental inoculum.

TABLE 2. Effect of soil transfers between fields with different periods of wheat monoculture on populations of *Cephalosporium gramineum*.

| Donor fields[b] | Natural Population[a] (X $10^3$/g dry soil) | | | |
|---|---|---|---|---|
| | 0 | 3 | 4 | 8 |
| 0 | 1.9 a | 4.2 a | 4.0 a | 0.6 a |
| 3 | 2.3 a | 1.6 a | 10.4 a | 1.3 a |
| 4 | 5.9 a | 20.6 a | 12.1 a | 1.2 a |
| 8 | 0.5 a | 13.5 a | 4.0 a | 0.4 a |

[a]Mean of six assays conducted between November 1977 and June 1978. Values in each column followed by the same letter do not differ significantly ($P = 0.05$).

[b]$1.3 \times 10^2$ kl/ha soil was transferred from donor to recipient fields and raked evenly over the surface of each plot within one week of planting.

TABLE 3. **Effect of soil transfers between fields with different periods of wheat monoculture on disease caused by natural populations of Cephalosporium gramineum.**

| Donor fields[b] | Plants showing stripe symptoms, %[a] | | | |
|---|---|---|---|---|
| | 0 | 3 | 4 | 8 |
| 0 | 4 a | 15 a | 15 a | 4 a |
| 3 | 9 a | 13 a | 21 a | 7 a |
| 4 | 13 a | 23 a | 15 a | 7 a |
| 8 | 9 a | 9 a | 18 a | 4 a |

[a]Disease was due to naturally occurring C. gramineum populations. Mean of three replicates with 100 plants per replicate. Values in each column followed by the same letter do not differ significantly ($P = 0.05$).

[b]$1.3 \times 10^2$ kl/ha soil was transferred from donor to recipient fields and raked evenly over the surface of each plot within one week of planting.

TABLE 4. Effect of soil transfers between fields with different periods of wheat monoculture on disease caused by natural populations of Cephalosporium gramineum supplemented with oat seed inoculum.

| Donor fields[b] | Plants showing stripe symptoms, %[a] | | | |
|---|---|---|---|---|
| | 0 | 3 | 4 | 8 |
| 0 | 33 a | 22 a | 14 a | 11 a |
| 3 | 32 a | 20 a | 24 a | 17 a |
| 4 | 41 a | 24 a | 17 a | 12 a |
| 8 | 27 a | 18 a | 18 a | 13 a |

[a]Mean of three replicates with 100 plants per replicate. Values in each column followed by the same letter do not differ significantly ($P = 0.05$).

[b]$1.3 \times 10^2$ kl/ha soil was transferred from donor to recipient fields and raked evenly over the surface of each plot within one week of planting.

TABLE 5. Effect of period of wheat monoculture on natural Cephalosporium gramineum populations and on disease caused by natural and supplemental inoculum of C. gramineum.[a]

| Field | Natural population[b] (X $10^3$/g dry soil) | Plant showing stripe symptoms, % Natural inoculation[c] | Natural plus oat seed inoculum[d] |
|---|---|---|---|
| 1 | 3.2 a | 8.3 a | 92.3 c |
| 4 | 60.0 a | 59.6 b | 92.0 c |
| 5 | 17.4 a | 58.0 b | 96.3 c |
| 9 | 1.9 a | 8.3 a | 97.0 c |

[a]Values followed by the same letter do not differ significantly ($P = 0.05$).

[b]Mean of three soil bioassays from November 1978 to March 1979, from plots without supplemental inoculum.

[c]Mean of nine replicates with 100 plants per replicate.

[d]Mean of three replicates with 100 plants per replicate.

TABLE 6. Effect of incorporating wheat soil from a field exhibiting Cephalosporium leaf stripe decline into a field not in disease decline, as measured by disease incidence and population levels.

| Amount of transferred soil[b] | Plants showing stripe symptoms, %[a] | | Population[d] ($X\ 10^3$/g dry soil) |
|---|---|---|---|
| | Natural[c] inoculum | Natural plus oat[c] seed inoculum | |
| No additional soil | 60 a | 92 b | 8.6 a |
| $1.3 \times 10^2$ kl/ha | 68 a | 98 b | 13.7 a |
| $3.9 \times 10^2$ kl/ha | 92 b | 99 b | 11.2 a |

[a]Values followed by the same letter do not differ significantly ($P = 0.05$).

[b]The soil was incorporated into each plot with a rototiller to a depth of 20 cm (8 in.), within one week of planting.

[c]Mean of three replications with 100 plants per replication.

[d]Mean of three determinations of natural populations made between November 1978 and April 1979.

incidence showed a pattern identical to that of the 1977-78 season, i.e., increasing then decreasing disease levels with years of monoculture (Table 5). Without oat inoculum, fields 4 and 5 had significantly greater disease than fields 1 and 9. The oat-inoculated plots were so heavily diseased that no differences could be distinguished between fields (Table 5).

In the 1978-79 season, soil transfers were made only from field 9 to field 4 to further test if the apparent suppressiveness in field 9 was a transferable trait. Soil transfers did not change the population level or disease incidence except for an enhancement of disease when soil at the rate of $3.9 \times 10^2$ kl/ha was transferred to plots in field 4 (Table 6). The oat seed inoculated plots gave uniformly high levels of disease. The natural pathogen populations were unaffected by these soil transfers (Table 6).

## DISCUSSION

Fields that had been planted to wheat for 3 or 4 years had higher Cephalosporium gramineum populations and disease incidence than fields sown to wheat for 0 or 8 years. The low pathogen density in field 8 resulted from a long-term population decline (91). Field 0, not previously planted to wheat, supported a low population of C. gramineum due to the lack of a susceptible host. This trend appeared to be repeated in the following season, in the same fields, since disease incidence was again higher in the two fields of intermediate age. However, the population differences were not statistically significant due to great variability in the data.

Cephalosporium gramineum populations have two characteristics which make their quantification difficult; 1) extreme fluctuations in numbers

over relatively short periods of time, and 2) localization of spore production. Cephalosporium gramineum propagules increase from near zero propagules/g soil in mid-summer, to peak populations by mid-winter (89). These progagules are produced in foci (sporodochia) which, until spores become disseminated by rain, are highly localized. This makes soil population determinations highly variable for sites even inches apart. For example, in one plot sampled on January 9, 1979, and again on March 1, 1979, the population changed from zero to $1.0 \times 10^6$ propagules/g soil, respectively. Apparently no infested straw was in the first sample and straw with sporodochia was in the second. Since these samples were collected from frozen ground with the aid of an axe to loosen the soil, it was difficult to collect a more homogenous sample.

It may be assumed that in 1978 the high level of disease developing in field 0, when oat seed inoculum was used (Table 1), was caused by a population density equal to, or less than, that established in fields 3, 4 and 8. This assumption can be made since the native pathogen population was low in field 0 and the amount of artificial inoculum was uniform between all fields. Therefore, in the summer of 1978 the field with the fewest years of continuous wheat gave the greatest disease response to a given amount of added inoculum. This implies that disease incidence is inversely related to years of wheat monoculture, a correlation supportive of the idea of disease decline. This trend was not observed the following year due to uniformly high levels of disease in all oat inoculum-supplemented plots (Table 5). Interestingly, this indicates that a high incidence of disease can be established, even in fields exhibiting a 'decline'.

The transferability of a 'decline factor' was not demonstrated. Moreover, the increase in disease (but not pathogen population) when 'decline' soil was transferred to a 'conducive soil' is not easily explained since the 'decline' soil had a low population of the pathogen.

There appear to be several factors involved in determining Cephalosporium leaf stripe incidence in any given year, viz. i) the previous winter's population density, ii) environmental conditions and, iii) the suppressiveness of the soil. The field which had nine years of continuous wheat maintained the characteristics of field exhibiting a disease decline. However, there are not enough data to understand the long term disease pattern of Cephalosporium leaf stripe, or indeed if there is a generalized pattern. It is possible that pathogen populations and disease fluctuations over time may be a characteristic of individual fields regardless of seemingly identical cultural practices.

# PART II: MODE OF INFECTION

# INTRODUCTION

Cephalosporium leaf stripe is a disease of winter wheat caused by the soil-borne fungus Cephalosporium gramineum. This disease is present in many of the major wheat growing regions of the world (7, 8, 16, 29, 57, 69, 75, 79, 94) including the United States. Cephalosporium leaf stripe is particularly destructive in Washington (7, 8) and Kansas (99), and is the most serious of all soil-borne wheat diseases in Montana (52). Over 1,000 wheat lines have been screened for disease resistance to Cephalosporium leaf stripe in Montana alone (52). Most of the basic studies on resistance assume that C. gramineum enters passively through severed roots, as conidia, drawn into the plant by transpiration water (54). That winter stresses predispose wheat to infection is now well established (8, 58, 59, 62, 90); however, other forms of winter injury have not been studied in regard to Cephalosporium leaf stripe. Previous work at Michigan State University has uncovered evidence that other forms of winter stress may play a major role in the predisposition of plants to infection (2). This paper presents evidence that increased root exudation resulting from freeze stress causes C. gramineum to germinate and actively penetrate wheat roots, resulting in disease.

# MATERIALS AND METHODS

## Fungus culture

Cephalosporium gramineum Nis. and Ika., (=Hymenula cerealis Ellis & Everh.) was used throughout this work. The isolate came from a naturally infested wheat field in East Lansing, Michigan, unless otherwise stated. Stock cultures were maintained on potato-dextrose agar (PDA) at 4 C. The fungus was routinely passed through wheat plants to insure against loss of pathogenicity.

Source of wheat seed

Winter wheat seed (Triticum aestivum (L.) cv. 'Genesee') was obtained from the Michigan Crop Improvement Association.

Sterilization of seeds

Wheat seeds were sterilized by treating for 5 min. in 70% ethanol followed by 5 min. in 5.25% sodium hypochlorite amended with 0.5 ml of Tween 20 per 100 ml solution. After four washes in sterile distilled water, seeds were germinated in approximately 5 ml of sterile 0.1% nutrient broth in petri dishes.

Media

The pathogen was cultured on the following media: i) potato-dextrose agar, ii) water agar prepared with 2% agar, and iii) peptone/yeast extract medium (PYM) (18.0 g glucose, 3.0 g yeast extract, 5.0 g peptone, 1.4 g $K_2HPO_4$, 1.7 g $KH_2PO_4$, 0.5 g $MgSO_4$, and one liter of water). This medium was used for liquid culture. Solid PYM was prepared with 2% agar.

Green leaf agar (GLA), as developed by Wiese and Ravenscroft (88), was used throughout this work for the selective isolation of C. gramineum from soil and plant material. Leaves from 30-day-old wheat plants grown in the greenhouse or growth chamber were autoclaved for 5 min at 130 C and 20 lb pressure (100 g leaves: 1 liter water). The liquid was poured through two layers of cheesecloth and the volume was adjusted to one liter. This extract was stored at -10C until needed or was used immediately. Two percent agar was added to solidify the medium. Prior to solidification, 0.9 or 1.1 g $CuSO_4 \cdot 5H_2O$ was added per liter.

Method for studying soil-imposed fungistasis

A method was devised utilizing agar on soil to test the effect of different soils (or other planting media) and root treatments on conidial fungistasis. Soil was passed through a 1 mm sieve, and ca. 10 g was spread on the bottom of a petri dish. A filter paper (Whatman #1) was placed over the soil and enough distilled water was pipetted on the filter paper to wet the soil thoroughly and to assure good contact between the soil and filter paper. A 6 cm x 6 cm x 1 mm square of 2% water agar was placed on top of the filter paper. Conidia applied to the surface of the agar were observed by taking sample 8 mm diam. disks from the agar with a cork borer and examining them under the microscope. Wheat seedling roots were placed on the agar to assess the effect of root exudates on conidial germination.

In some studies, it was desirable to have a system in which non-sterile field soil could be used while maintaining sterility of the conidia and wheat roots (Figure 1). The center of a 'ring' cut from 10 ml of solidified 2% water agar, with a central opening 2.5 cm diam., was filled with saturated (31% moisture), non-sterile field soil. A sterile Millipore filter (0.22 um, 4.7 cm diam.) was placed over the soil with the edge extending beyond the outer portion of the agar ring. Disks of agar (8 x 1 mm) bearing conidia of C. gramineum on the upper surface were placed on the center portion of the filter over the soil. Conidia treated in this way did not germinate as long as the disks remained in contact with the filter-covered soil (Table 1). The agar disks did not become contaminated with other microorganisms. Conidia placed on water agar alone without soil readily germinated, producing copious numbers of conidia.

FIGURE 1. Sterile chamber used to study soil-imposed fungistasis. All components within the petri dish are sterile except for the soil. The bacterial-proof membrane filter maintained the sterility of the agar disks.

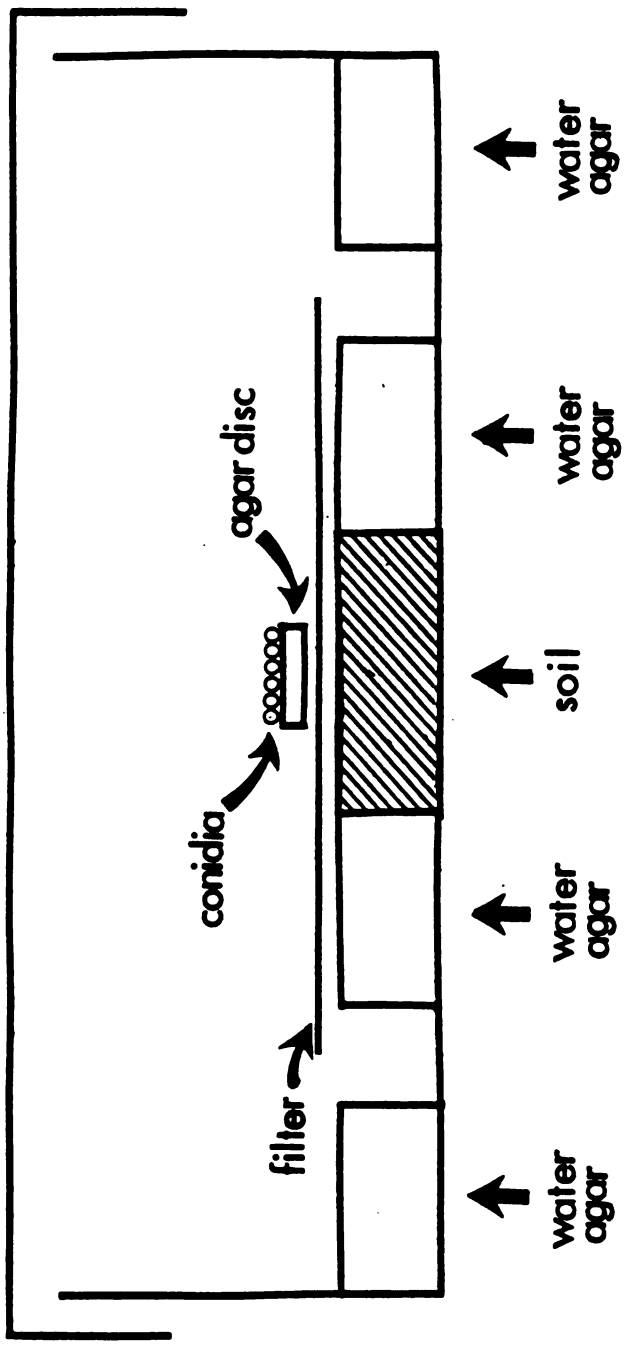
water agar
water agar
agar disc
conidia
soil
water agar
filter
water agar

FIGURE 1.

Electron microscopy

Root segments were cut into short (1-2 cm) pieces and placed in a 5% gluteraldehyde solution at pH 7.2 overnight. After two washes in 0.1 M phosphate buffer (pH 7.2) the root tissue was soaked in 1.0% osmium tetroxide overnight. Two washes in 0.1 M phosphate buffer (pH 7.2) preceded an ethanol dehydration series. The tissue was freeze-fractured by placing in liquid nitrogen and striking repeatedly with a razor blade until all pieces were approximately 2-3 mm in length. The tissue was critical-point dried using a Sorval critical-point dryer (Ivan Sorvall, Inc., Norwalk, Conn.), and was fixed to a stub using double stick tape. Specimens were gold coated to a thickness of approximately 120 nm using a sputter coater (Mini-coater, Film-Vac, Inc., Englewood, NJ). A Super Mini II scanning electron microscope (International Scientific Instruments, Japan) was used to visualize the samples.

Artificial wheat freezing methods

The freeze chamber used was supplied through the courtesy of C. R. Olien, Department of Crop and Soil Sciences, Michigan State University. It consisted of an insulated cabinet coupled with a large refrigeration unit. Temperatures were controlled by setting a time clock to trigger a motor driven valve which allowed increased amounts of coolant to be released. Fans were used to transfer cold air from the refrigeration unit to the freeze chamber cabinet. The minimum temperature desired was then held for the appropriate amount of time before the timer turned off the cooling unit, allowing the material to thaw.

The equipment used in the previous method is expensive in cost, time, maintenance, and space. Consequently, an inexpensive and easy-to-use method was developed (Figure 2). A lid was constructed for a 527.5 liter (18.61 cu. ft.) chest style freezer (Frigidaire) from 3/4" plywood in which 33, 10 cm (4 in.)-diameter holes were drilled. Plants grown in one liter plastic cups (32T Sweethart Plastics, Inc., Wilmington, MA 01889) containing greenhouse mix as a growth medium, were inserted in these holes so that approximately two inches of the cup remained above the plywood. All cups were saturated with water before the freezer was turned on. Decreasing pot temperatures were monitored using a thermister with a strip-chart recorder (Esterline Minigraph temperature recorder, George R. Peters Assoc., Troy, MI 48084).

When the freezer was set at -18 C, soil temperatures fell at well within the rates established for simulating field conditions (Figure 3). Soil temperature within the freeze chamber was equilibrated at -5 C $\pm$ 1.5 approximately 2.5 cm above the bottom center of the cups. The soil was frozen to within 7.5 cm of the soil surface, but not above, thus allowing the crown tissues to remain unfrozen. Hardened and unhardened plants were tested in this chamber without plant death occurring, which allowed continued incubation and disease rating after exposing plants to freeze stress.

Cation exchange chromatography and bioassay procedures

Procedures followed were similar to those of McKeen (37). Pasteur pipettes (0.5 cm diam.) were packed with 1 cm of glass wool followed by 1 ml of wet Dowex 50-8 cation exchange resin. Ten ml of 1 N hydrochloric acid was used to saturate the column followed by a water wash. When the pH of the water remained unchanged after passage through

FIGURE 2. Chest freezer method of simulating freeze stress.

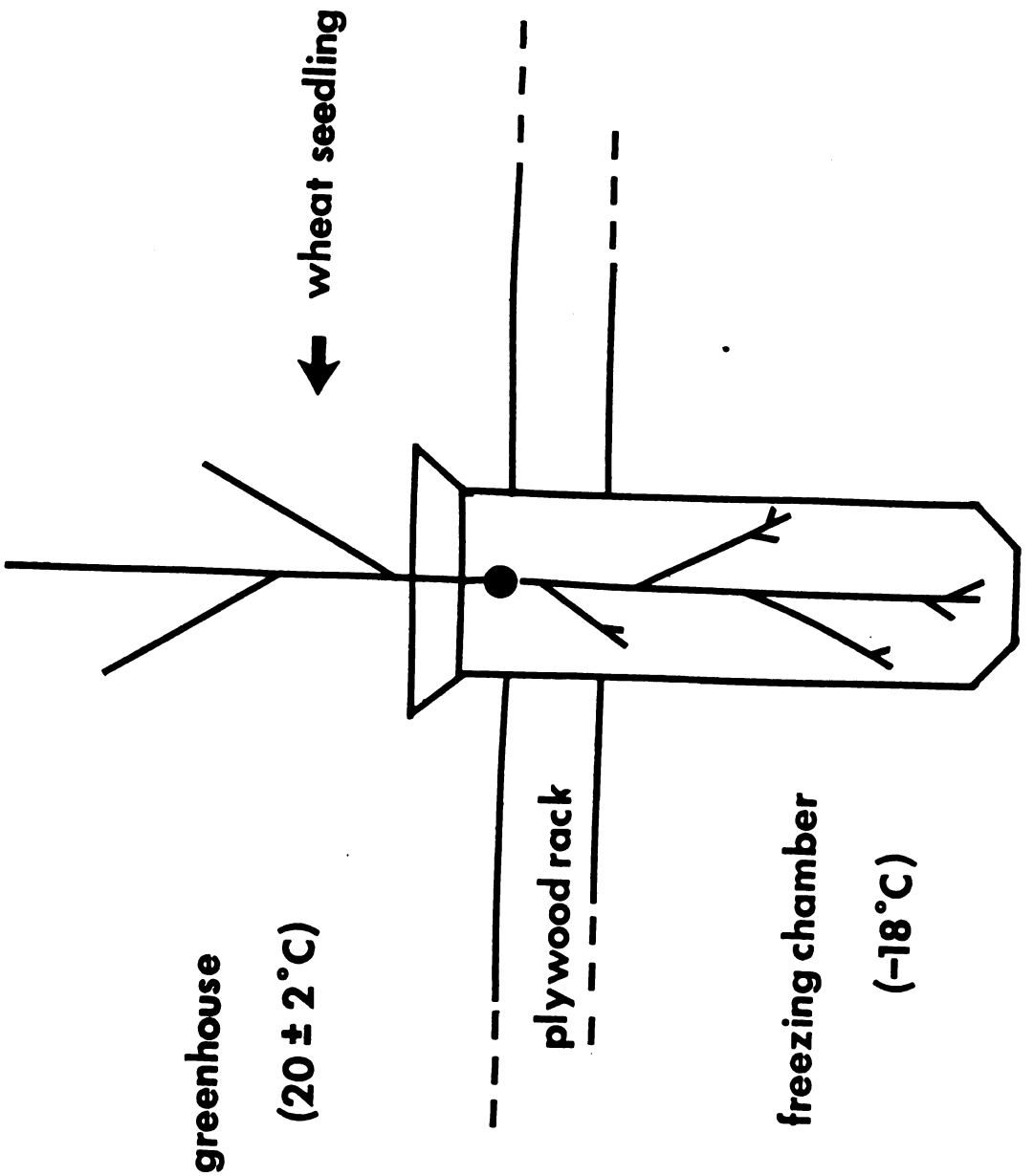
wheat seedling
greenhouse
(20 ± 2°C)
plywood rack
freezing chamber
(-18°C)

FIGURE 2.

FIGURE 3. Soil temperature dynamics 2.5 cm from bottom center of cups containing a greenhouse mix undergoing freezing and thawing in the chest freezer. The data are from a typical freeze experiment.

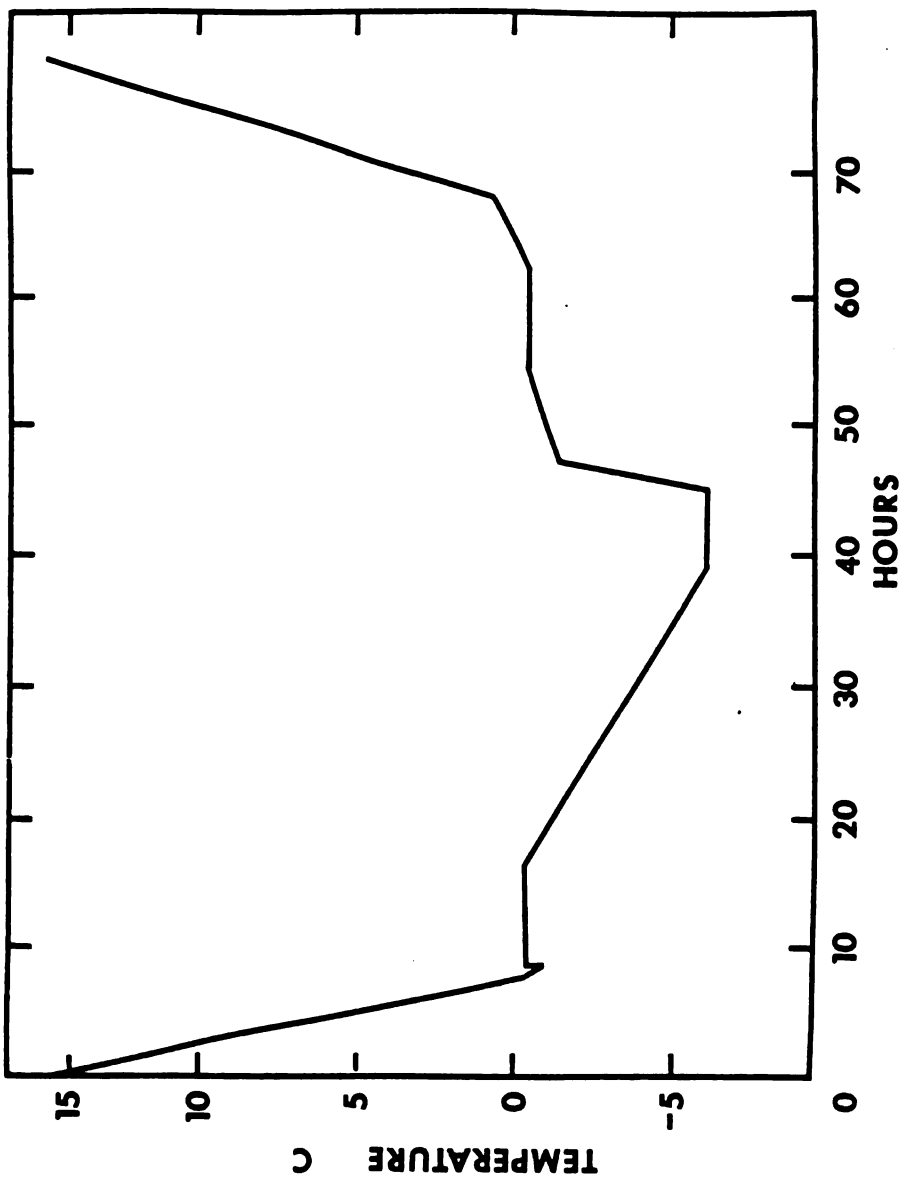


FIGURE 3.

the column, the root exudate sample was applied followed by five bed volumes of water. The material which passed through constituted the neutral and anionic fraction. The column was subsequently eluted with five bed volumes of 2M ammonium hydroxide. This eluate comprised the cationic fraction. The different fractions were vacuum evaporated to dryness at 40 C before resuspension.

Root exudates were aseptically collected from wheat seedlings grown axenically in syringes for 11 days before freezing to -1.0 C. Roots remained alive as determined by vital staining with (3-(4,5-dimethylthiazol-Z-Yl)-2,5-diphenyl-2H-tetrazolium bromide) (MTT). Before staining, the frozen roots were white and succulent, similar to unfrozen roots. Freezing was effected in the chest freezer. The plywood lid was modified to accept 2.5 cm diam. syringes rather than pots. Exudates collected 24 h before and 24 h after freezing constituted the non-frozen and frozen root exudates, respectively. The exudates were incubated overnight in methanol (2 x the volume of exudate) and vacuum evaporated to dryness at 40 C in a water bath. The dried material was resuspended in 1 ml water and passed through the cation exchange column then redried under vacuum at 40 C. The exudates were resuspended in 0.1 M phosphate buffer per ml exudate to yield a solution of pH 6.5 containing exudate equivalent to that from 0.027 g dry root/ml. Five µl of exudate were placed with 5 µl of a washed conidial suspension (ca. $10^6$ conidia/ml) on an agar disk, incubated 1 hr on a sterile membrane filter covering soil, then incubated for 12 hr with no soil contact. Results were reported as percent germination based on the number which germinated on PDA without the influence of soil (Table 5).

Quantification of sugar in wheat root exudate

Thirty ml plastic disposable syringes containing 3 ml sterile Hoagland's solution were used as containers for the seedlings. Sterile serum bottle caps covered the small orifice and sterile cotton plugged the large orifice. Seedlings were grown until the first true leaf touched the cotton plug. The cotton was removed, and the seedlings were elevated so that the seed and root remained within and the shoot was outside the syringe. The cotton was then aseptically replaced and syringes were placed in conetainer trays (Ray Leach's Conetainer Nursery, Canby, OR 97013). Twenty-five ml of sterile Hoagland's solution was injected into each syringe, through the serum bottle cap. Fresh sterile Hoagland's solution was periodically added to maintain a constant level.

The concentration of sugars in root exudate was determined by the procedure of Dubois et al. (23). Two ml of root exudate was combined with 1 ml of 5% phenol in water in 16 mm diameter test tubes. Five ml of concentrated (95%) sulfuric acid was quickly added the hot liquid was left to stand for 10 min. before shaking and incubating in a 25 C water bath for 25 min. Optical density was read at 480 mμ and compared to a standard curve made from known concentrations of glucose (Figure 4). Results are reported in glucose equivalents (μg/ml).

## RESULTS

The development of a sterile agar disk method for studying Cephalosporium gramineum conidia and soil fungistasis

In order to study the effect of nutrients (i.e., root exudates) on conidial germination, a system which allowed indefinite sterility and intimate contact of conidia with non-sterilized soil was developed (Figure 1).

FIGURE 4. Standard optical density curve for various concentrations of glucose.

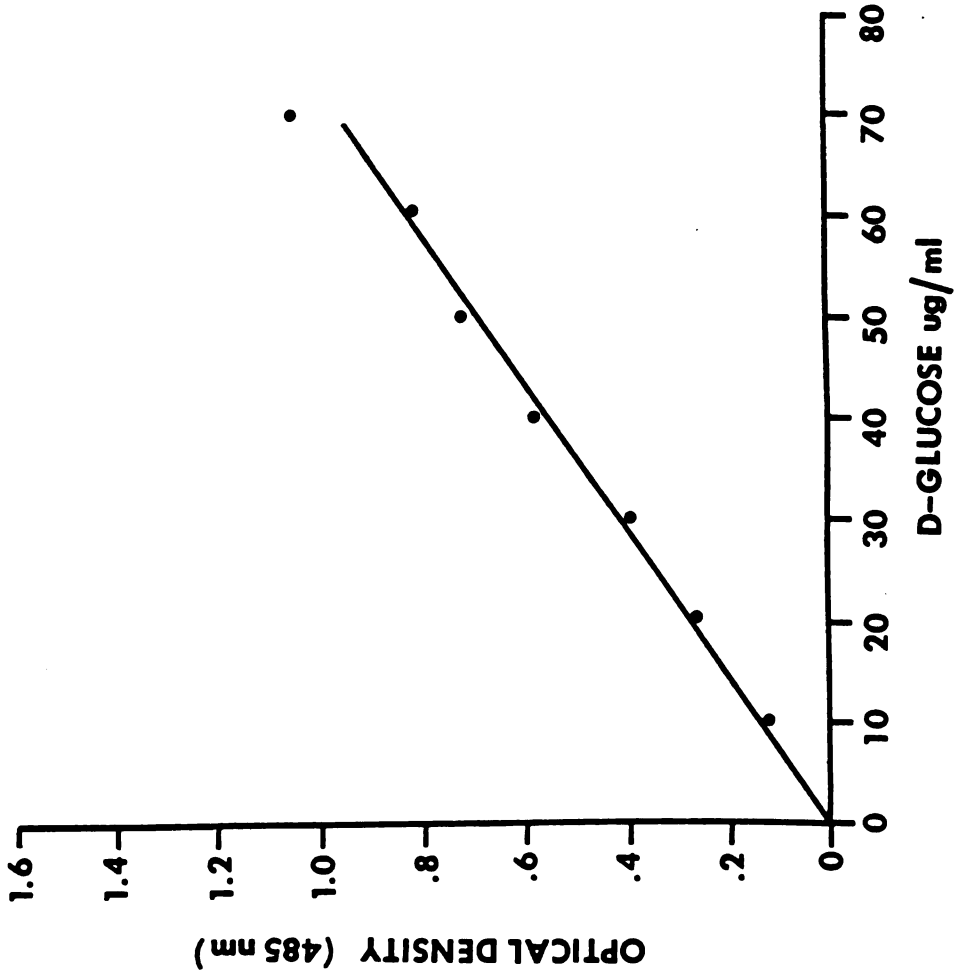
OPTICAL DENSITY (485 nm)
1.6
1.4
1.2
1.0
.8
.6
.4
.2
0
0
10
20
30
40
50
60
70
80
D-GLUCOSE ug/ml

FIGURE 4.

The conidia were placed on water agar disks (8 x 1 mm) which had previously been placed on a membrane filter (0.22 μm) covering non-sterilized soil. Conidia were placed on agar disks immediately or five days after the disks were placed on the filter. In half of the five-day treatments, disks were removed prior to addition of conidia to determine if there was a residual effect from the soil contained in the agar. Conidia in sterile distilled water and on water agar disks alone (without soil) served as controls. The conidia were incubated for 24 hr on all disks before germination was counted. Because there was poor germination in this experiment, germination was determined by counting the number of germinated conidia per microscope field (Table 7). This method allowed for rapid counting of large numbers of conidia since each field contained approximately 200 conidia.

Non-sterilized soil imposed fungistasis upon the test conidia whether or not there was a prior incubation of the disks on the soil. Disks incubated for five days on non-sterile soil, then removed, retained the capacity to limit germination even though they were not in contact with the soil during the germination period. Incubation on sterilized soil or on water agar which had not been in contact with soil allowed significantly more germination than non-sterilized soil. There was no marked effect of varying the length of contact with sterile soil. Sterile distilled water did not support germination.

Another experiment was designed to more precisely define the relationship between the time of incubation of agar disks on soil and the expression of fungistasis. Disks were incubated on soil for 0, 2, 6, 12 or 24 hr, after which they were aseptically placed in sterile plastic petri plates without soil. One-half of the disks carried conidia

TABLE 7. Germination of Cephalosporium gramineum conidia after 24 hr incubation on agar disks covering soil.

| | Treatment | Soil | Germination[a] |
|---|---|---|---|
| 1. | Agar in contact with soil 5 days before addition of conidia; agar remained on soil for conidial incubation. | Natural | 0 a |
| 2. | As 1, except agar removed after 5 days | Natural<br>Sterile | 6 a<br>88 b |
| 3. | Conidia applied to agar at same time agar was applied to soil | Natural<br>Sterile | 0 a<br>68 b |
| 4. | Water agar contol | -- | 211 c |
| 5. | Sterile distilled water | -- | 0 a |

[a]The numbers represent the number of conidia germinated per microscopic field (approximately 1 $cm^2$). Values followed by the same letter do not differ significantly ($P = 0.05$).

before incubation on soil, the remainder had conidia placed on them at the conclusion of these incubation periods and were allowed to germinate for 24 hr (Table 8). The longer disks were incubated on soil, the greater was the inhibition. Except for the longest incubation period (24 hr), there were no differences in germination between conidia which were on the agar disks while in contact with soil and those which were not. When the conidia, which had been on the disks during the soil incubation, were allowed to germinate an additional 24 hr after removal from the soil, significantly more germination occurred. This shows the conidia were still viable.

In another experiment the effect of a richer nutritive substrate on germination of conidia in contact with agar disks over soil was investigated. A conidial suspension (0.5 ml, $10^6$ conidia/ml) was added to the surface of agar disks prepared from 2% PDA, water agar, and washed water agar. These were immediately placed on a membrane filter which covered field soil as above. Disks prepared as above and incubated on a membrane filter without soil served as controls. After 24 hr incubation on soil, the percentage germination and germ tube lengths were determined.

Germ tube length, as well as percentage germination, were greater on PDA disks than on water agar or washed water agar disks. When non-autoclaved field soil was present, 0, 2, and 13% of the conidia germinated on the washed water agar, water agar, and PDA, respectively. Control disks gave 43, 53, and 56% germination, respectively.

Wheat roots as a source of nutrition for Cephalosporium gramineum

a. Effect of total wheat root contents on conidiogenesis

To determine the effect of wheat root contents on C. gramineum conidiogenesis, 10 g of roots from 67-day-old wheat plants were blended

TABLE 8. Germination of Cephalosporium gramineum conidia on agar disks covering soil after various incubation periods.

| Length of disk incubation on soil, hr | Length of conidial incubation on disk, hr | Germination %[a] | |
|---|---|---|---|
| | | Conidia on disks during soil incubation | Disks incubated on soil prior to addition of the conidia |
| 0 | 24 | 20 ef | 16 de |
| | 48 | TMG[b] | |
| 2 | 24 | 20 ef | 18 def |
| | 48 | 30 gh | |
| 6 | 24 | 9 c | 12 cd |
| | 48 | 26 fg | |
| 12 | 24 | 4 b | 8 bc |
| | 48 | 18 def | |
| 24 | 24 | 0a | 7 bc |
| | 48 | 13 de | |
| Amended with PDB after 0 hr incubation | 24 | 41 ij | |
| Amended with PDB after 24 hr incubation | 48 | 43 j | 35 h |

[a]Numbers followed by the same letter do not differ significantly ($P = 0.05$).

[b]TMG = Too much growth for determining germination percent.

in a Serval Omnii mixer in 50 ml distilled water at high speed for 5 minutes. The slurry was filtered through two layers of cheesecloth and the final volume was adjusted to 100 ml with distilled water. Two g of agar were added before autoclaving, then this wheat root extract agar was poured into petri plates (10 ml per plate) and allowed to solidify. Conidia were spread evenly over the surface of the agar. After nine days incubation (ca. 22 C) conidial numbers were determined. Five randomly selected disks of agar (8 x 1 mm) were placed in 0.25 ml of 5 N HCl in test tubes and heated in a water bath at 80 C for 1 min. After liquification of the agar, conidia were counted using a haemocytometer. Two percent water agar treated as above but without wheat root extract served as a control.

Agar made from wheat root extract greatly stimulated conidial production. There was a 665-fold increase in conidial numbers in the wheat root extract agar compared to a 160-fold increase in water agar alone (Table 9).

b. Effect of exudates from non-injured wheat roots on conidial germination

Roots are known to exude nutrients which can stimulate the germination of spores under the influence of soil imposed fungistasis (49, 70). The following experiments were designed to measure the effect of wheat seedling root exudation on C. gramineum conidial germination under such conditions. Four-day-old wheat seedlings, grown aseptically, were placed on the agar surface at the same time conidia were added, or in another experiment, the roots were allowed to grow under the agar for five days before addition of the conidia to the surface of the agar. Both systems were designed to allow exudates from the roots to diffuse

TABLE 9. Numbers of Cephalosporium gramineum conidia produced on wheat root agar and water agar after 9 days incubation.

| Incubation time (days) | Conidia per petri plate[a] | |
|---|---|---|
| | Wheat root agar | Water agar |
| 0 | $2.1 \times 10^3$ | $2.1 \times 10^3$ |
| 9 | $1.4 \times 10^6$ | $3.4 \times 10^5$ |

[a]Conidial numbers were determined using a haemocytometer after liquification of agar disks.

into the agar and be available to C. gramineum conidia. Non-sterilized soil or sand was used to induce fungistasis in these experiments. The conidia were examined daily for four days by removing agar disks (8 x 1 mm) and observing under the microscope. The results of both experiments gave identical results.

Conidial germination over the soil or sand was limited to short germ tubes (<30 μm), regardless of the proximity to roots. Germination occurred within 12 hr on water agar alone, producing copious growth. By 24 hr new conidia were being produced.

c. Effect of exudates from wheat seedling roots subjected to freezing and mechanical injury on conidial germination.

Roots placed under stress such as freezing soil temperatures, are known to exude more than non-stressed roots (50). The effect of nutrients from frozen roots on conidial germination was examined by removing 14-day-old wheat plants from the sand potting medium, severing the roots from the shoots, and freezing the roots (-11 C) for 15 hr in distilled water. After a 9 hr thaw at 22 C, the roots were removed and the concentration of the resulting exudate solution was adjusted to be equivalent to that from $3.3 \times 10^{-3}$ g dry root per ml water. Roots obtained as above from identical plants were either left intact or were cut into 1 cm lengths after severing from the shoot. These roots were soaked for 24 hr in distilled water at 22 C without freezing and, as before, adjusted to $3.3 \times 10^{-3}$ g dry root equivalents per ml water. Equal volumes (ca. 1.0 ml) of each of these three exudates were added to 1 ml of a conidial suspension ($10^6$/ml) and placed in individual wells of acid-washed, sterilized depression slides. After incubating, overnight germination was determined. Sterile distilled water served as a control.

A marked stimulation of germination occurred with frozen exudate as compared with exudate prepared from cut or uncut roots or sterile distilled water (Table 10).

The effects of exudate from frozen wheat roots on the germination of spores in soil-imposed fungistasis was also investigated. Sterile, 4-day-old wheat seedlings were placed on water agar disks so that roots came into contact with C. gramineum conidia previously placed on the upper surface. The agar disks rested on a membrane filter which covered non-sterile field soil (Figure 1). Seedling roots were frozen immediately before placing them on the agar disks. After various incubation times, the conidia were observed under the microscope for germination.

Freezing was effected by adding enough sterile distilled water to cover the roots of the seedlings which had previously been placed in sterile 50 ml beakers. The beakers were covered with Parafilm and placed into an ethanol bath. The temperature of the ethanol was lowered by the addition of liquid nitrogen. A stirring bar kept the temperature changes uniform throughout the ethanol. The water temperature dropped from 20 C to -5 C in 12 min. After an additional 3 min at -5 C, all beakers were removed and allowed to thaw at room temperature. Due to contact of the crown with the frozen water, the frozen plants were killed. Non-frozen roots were treated as above, except that they were incubated at 20 C for 15 min. Conidia placed on disks without frozen roots served as controls.

Frozen wheat seedling roots placed on agar disks over soil stimulated abundant germination of C. gramineum conidia and vigorous growth of mycelium (Figure 5). Non-frozen roots stimulated sparse germination, whereas, there was no germination in the absence of roots, even after a

TABLE 10. Effect of exudate from wheat roots treated in various ways on conidial germination and growth of Cephalosporium gramineum on sterile depression slides.

| Exudate source[a] | Germination, %[b] | Germ tube length, m[b] |
|---|---|---|
| Frozen intact roots | 23 b | 27.0 d |
| Non-frozen cut roots[c] | 5a | 2.5 c |
| Non-frozen intact roots | 3a | 0.5 b |
| Water | 0a | 0.0a |

[a]Exudate was collected from 14-day-old wheat plants grown in sand. Roots were frozen for 15 hr at -11 C; all other roots were held at 22 C. All roots were in water for a total of 24 hr.

[b]Values in each column followed by the same letter do not differ significantly ($P$ = .05)

[c]Roots were cut with a razor blade into 1 cm lengths before placing in water for 24 hr.

FIGURE 5 (A to G). Light and electron micrographs of Cephalosporium gramineum germination on agar covering soil and growth through wheat root tissue.

(A to D). Nomarski interference contrast micrographs of C. gramineum conidia on agar disks which were placed on a membrane filter covering unsterilized soil and incubated for 24 hr. A) No wheat roots present; conidia remain ungerminated (145 X). B) As A, except that no soil was present below the filter (145 X). C) As A, but with a sterile, non-frozen wheat root stimulating some conidial germination (145 X). D) As C, except that the wheat root had been frozen (145 X). Note the vigorous hyphal growth.

(E to G). Scanning electron micrographs of C. gramineum penetration and growth within a frozen wheat root after 3 days' incubation. E) Cross section of a frozen wheat root. Hyphae have penetrated the epidermis and cortex, but the stele has not been colonized (117 X). F) Hyphae penetrating the cell walls of the cortex (118 X). G) Hyphal tips displaced from their original point of contact (arrows) with a cortical cell wall during sample preparation, showing apparent sites of penetration (4880 X).

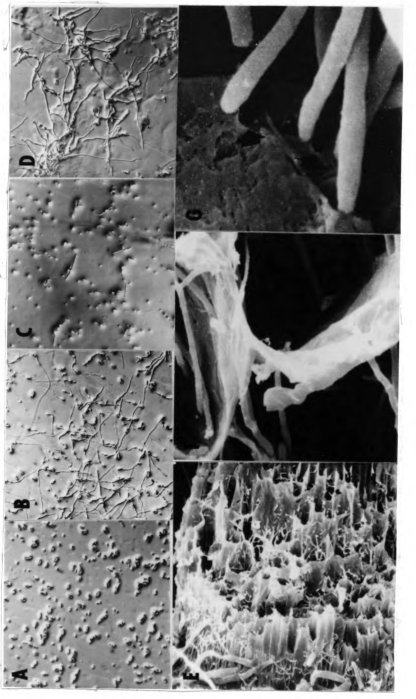


FIGURE 5.

six-day incubation. Electron micrographs of freeze-fractured, 10-day-old frozen wheat roots showed hyphal penetration and growth deep into the root tissues. Therefore, not only was there stimulation of germination and growth, overcoming soil-imposed fungistasis, there was active penetration and colonization of roots. Non-frozen roots were not examined with the electron microscope.

Further electron micrography studies were conducted on roots frozen to -10 C in syringes (Figures 6 and 7) into which conidia were introduced. After germination, hyphae grew randomly along the surface of the root before penetrating the epidermis, either intra- or intercellularly. Penetration of the epidermis was usually preceded by a swelling of the hypha, probably serving as an appressorium at the point of entry. In some cases a somewhat pointed projection from the hyphae, apparently serving as a penetration peg, was observed to enter the epidermal cell first. Entry made by hyphae without such a structure was also seen. Once in the epidermis, growth was more directed through the cortex toward the axis. Upon endodermal contact the hyphae spread over the surface of this tissue layer in a manner similar to that before epidermal penetration. Growth through the endodermis was not observed at the time intervals sampled. Either few entry points into the stele were made, and were therefore missed, or they occurred after the tenth day of incubation. Non-frozen roots were also examined, but hyphal penetration was not found to extend beyond the epidermis.

d. Root exudate fractionation.

In order to quantitate the effect of freezing on exudation from wheat roots a chemical index of exudation was sought. Common and abundant components of root exudate are amino acids and sugars (70).

FIGURE 6. Electron micrographs of Cephalosporium gramineum penetration into wheat root epidermis following freezing (-10 C) in syringes and 10 days' incubation with C. gramineum conidia.

A, typical growth along the surface of a wheat root (675 X). B, A close-up of 'A' (2380 X). Note the appressorium-like swellings at the points of entry (arrows). A penetration peg-like structure initiating an intracellular entry point is visible on the left-center of the micrograph. The other two arrows point at intercellular entry points.

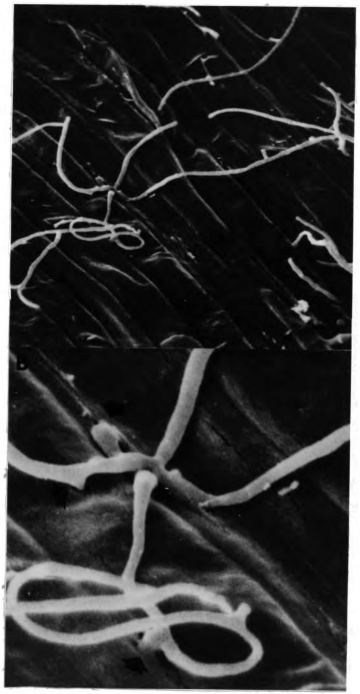

FIGURE 6.

FIGURE 7. Electron micrograph of Cephalosporium gramineum growth over the surface of wheat root endodermal tissue (476 X) following freezing (-10 C) in syringes and 10 days incubation with C. gramineum conidia. The cortex has been stripped away to allow the visualization of this phase of colonization.

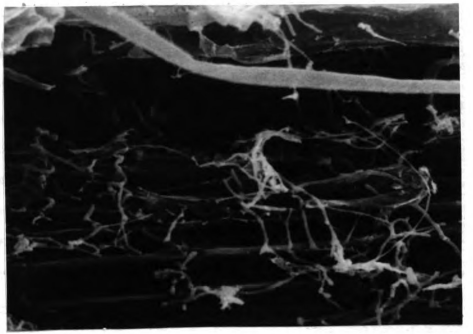

FIGURE 7.

Measuring the changes in these compounds should reflect changes of membrane permeability. Therefore, exudates were separated into two fractions, i) the cationic and ii) the anionic plus neutral fractions, and these were tested for ability to stimulate conidial germination. The procedure used should separate the amino acids into the cationic fraction and the sugars into the neutral-anionic fraction.

Results indicated that the anionic-neutral fraction contained the stimulatory component in both frozen and non-frozen root exudate. The cationic fraction stimulated no more germination than the water control (Table 11).

Root exudation after artificially imposed freeze stress should be similar to that occurring under natural conditions if the freeze kinetics closely approximate those found in the field. Wheat seedlings were aseptically grown in syringes containing sterile Hoagland's solution for 20 days at 18 C and hardened for 20 days at 2 C. The seedlings were then frozen following established freeze-stress procedures (59) in the chest freezer at a rate of -0.5 C/hr. A minimum temperature of -10 C was followed by a slow thaw (0.5 C/hr). Root exudates were collected three days before and three days after freezing. These solutions were adjusted to contain the equivalent of exudate from 0.002 g dry root per ml water. Two percent agar (w/v) was then added before autoclaving and pouring into petri plates (10 ml/plate). One ml of conidia (approximately $10^5$ conidia/ml) was added to disks (8 x 1 mm) of exudate agar and incubated at 22 C for 1.5 days. Germination, length of germlings, hyphal branching, and conidial production were determined at the end of the incubation period. Glucose equivalents were determined in the exudate.

TABLE 11. Effect of fractionated exudate from frozen or non-frozen wheat roots on germination of *Cephalosporium gramineum* conidia.

| Test material[a] | Root treatment[b] | Fraction | Germination %[c] |
|---|---|---|---|
| Exudate | Non-frozen | Cationic | 41 ab |
| Exudate | Non-frozen | Anionic + Neutral | 71 c |
| Exudate | Frozen | Cationic | 43 ab |
| Exudate | Frozen | Anionic + Neutral | 75 c |
| Water | Without roots | Cationic | 28 a |
| Water | Without roots | Anionic + Neutral | 37 ab |
| Water Control | Without roots | No column used | 31 a |
| Buffer Control | Without roots | No column used | 37 ab |
| Water Agar | Without roots | No column used | 57 bc |

[a]Root exudates were aseptically collected from wheat seedlings grown 11 days in water and frozen to -1.0 C.

[b]Non-frozen = sample collected 24 hr before roots were frozen; Frozen = sample collected 24 hr after roots were frozen.

[c]Germination is adjusted to a percentage of those germinating on PDA. Actual germination on PDA = 33%. Numbers followed by the same letter do not differ significantly ($P = .05$).

The percentage germination was unusually low for all samples, and no differences were detected (Table 12). The lengths of germlings were not statistically different due to excessive variability. Vigor of growth, as measured by hyphal branching and conidial production were significantly greater in the exudate from frozen roots than in that collected before freezing. There was approximately a 77-fold increase in glucose equivalents in exudate from frozen roots as compared with before freeze exudate.

Disease incidence in relation to mechanical and freeze-induced injury

Root severing due to winter heaving has been cited as the major predisposing factor for infection of wheat by C. gramineum (54). Other forms of freeze-induced root injury which cause an increase in root exudation, but no root breakage, have not been examined in regard to disease incidence.

The following experiments were designed to partition several components of winter injury and evaluate them as predisposition factors for disease.

a. Mechanical severing of roots (heaving simulation).

Wheat seedlings grown for 10 days in sand were carefully removed and washed in tap water. One-half of the root system was removed by cutting with a razor blade perpendicular to the axis of the plant. The plants were transplanted into sieved (5 mm) field soil which had 1-6 x $10^3$ colony forming units (cfu) of C. gramineum per g soil. The population consisted of the naturally occurring C. gramineum propagules with additional conidia added from liquid cultures grown in the laboratory. After eight weeks incubation in the greenhouse, no symptoms were observed.

TABLE 12. Response of Cephalosporium gramineum to exudates from frozen and non-frozen, axenically grown wheat roots.[a]

| Exudate source[b] | Plant age, days | Germination %[c] | Length of germlings, µm | Hyphal branches, No. | Conidial production index[d] | Glucose equivalents, g/ml[e] |
|---|---|---|---|---|---|---|
| Freeze-stressed roots | | | | | | |
| Before freeze | 37 | 1.8 a | 151.7 a | 1.6 a | 0.37 ab | 2.3 a |
| After freeze | 40 | 3.3 a | 239.6 a | 2.5 b | 2.00 c | 155.0 b |
| Non-freeze-stressed roots | 40 | 2.7 a | 151.1 a | 0.0 a | 0.00 a | 2.0 a |
| Control (water agar) | -- | 1.9 a | 199.8 a | 0.5 a | 0.60 b | -- |

[a]Values in each column followed by the same letter do not differ significantly ($P = .05$).

[b]Exudates were collected from aseptically grown, hardened wheat plants. Freeze stress was induced by a temperature of -10 C. Root exudates were collected three days before and three days after freezing. They were adjusted to contain the equivalent of exudate from 0.002 g dry root/ml, and solidified with agar (2% by wt.).

[c]Washed conidia were pipetted onto disks of root exudate agar and left to incubate 1.5 days before counts were made.

TABLE 12. (Continued)

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[d]Conidial production indices were: 0 = 0 new conidia, 1 = 0-5, 2 = 5-10, and 3 = more than 10 new conidia per germling.

[e]As determined by the phenol-sulfuric acid colorometric test.

In another experiment, 10-day-old wheat seedlings were grown and root-injured as above and the seedlings were i) planted with no additional conidia in unsterilized field soil; ii) root-dipped in a conidial suspension ($2.4 \times 10^6$ conidia per ml) and planted in field soil; iii) planted directly in an area (ca. 2.5 cm diam. x 1.0 cm deep) of field soil to which five ml of the conidial suspension had been mixed; and iv) planted in autoclaved soil treated as in (iii). The naturally occurring populations of C. gramineum were approximately $8.3 \times 10^2$ CFU per g soil. Plants were grown in a greenhouse and observed for symptom development.

Root severing was most effective as a predisposing factor when roots were subsequently dipped in a conidial suspension. Placing cut roots into naturally or artificially infested soil, either autoclaved or non-autoclaved, gave similar low rates of infection (Table 13).

b. Artificial freeze-induced injury

Six-week-old wheat plants grown in one liter pots of sand at 20 C, were hardened for three weeks at 2.5 1 C in a lighted growth chamber. Cephalosporium gramineum conidia were added as a drench to each pot ($67 \times 10^3$ CFU per g sand). Pots were then maintained either in the growth chamber at 2.5 1 C, or were subjected to decreasing temperatures for 24 hr until the air temperature reached -15.0 C. The soil temperature reached was not measured. The refrigeration was then turned off and the pots allowed to thaw in the closed freeze chamber. All plants were moved to a greenhouse and observed for symptom development. This procedure has been established as an accurate simulation of winter stress encountered in Michigan (C. R. Olein, personal communication).

Plants with cut roots had the highest percentage infection, with those held at 2.0 C having more infection (77%) than those at -15.0 C

TABLE 13. Leaves colonized with Cephalosporium gramineum after cut wheat roots were either dipped in a conidial suspension or placed in soil infested with C. gramineum conidia.

| Inoculation procedure[a] | Soil treatment | Leaves colonized, %[b] |
|---|---|---|
| Natural infestation | Non-sterile | 3 a |
| Natural infestation and plant roots dipped in conidial suspension | Non-sterile | 62 b |
| Additional inoculum | Sterilized | 7 a |
| Additional inoculum | Non-sterile | 2 a |

[a]Roots of 10-day-old wheat seedlings were cut in half (perpendicular to the axis) and plants were transplanted in soil which was either sterilized or non-sterilized. Natural infestation = $8 \times 10^2$ CFU/g soil. Plant roots dipped = cut roots were immersed in a conidial suspension ($2.4 \times 10^6$ CFU/ml) for 3 min before planting. Additional inoculum = 5 ml of a conidial suspension ($2.4 \times 10^6$ conidia/ml) was mixed into a small area of soil (ca. 2.5 cm diam. x 1.0 cm deep).

[b]Percent colonization was determined by leaf isolations on green leaf agar. Values followed by the same letter do not differ significantly, ($P = .05$).

(39%) (Table 14). In addition, symptoms developed more rapidly on plants with cut roots. Plants exposed to 2 C showed symptoms after 21 days and those exposed to -15.0 C after 32 days. Of plants with non-cut roots, only those subjected to -15.0 C gave symptoms (14%) which appeared after 46 days. Such cold temperature treatments had no significant effect on numbers of C. gramineum colony forming units. Populations were assayed using GLA 24 hrs after 18 C or -15 C. The means of three experiments were 13 x $10^4$ and 8 x $10^4$ CFU/g soil for the 18 C and -15 C treatments, respectively.

c. Natural freeze-induced injury

Seeds were sown October 6, 1978, 3.7 cm deep in a field which had had three consecutive wheat crops. When the plants were 2.5 to 5.0 cm tall they were removed from the soil and placed in 10 cm diameter plastic pots. Five plants were placed in each pot using the field soil as a planting medium. The pots were then buried so that the soil in the pots was level with the surrounding field soil. After various incubation periods, pots were removed from the field, placed in a growth chamber and incubated for 50 days. Symptom development was recorded as the percentage of plants which yielded symptoms (Table 15). The first disease appeared in the January 13 sample after which infection increased with each successive sampling date. The soil temperature was monitored using a thermister at a depth of 5.0 to 7.5 cm and a strip chart recorder (Model M00206A4-5-002, Esterline Angus, Box 24000, Indianapolis, IN 46224). In addition, soil temperature data collected by Dr. D. E. Linvill, Michigan State University from an area approximately 0.8 km from the experimental field were also consulted. The elevation and soil type were similar to those found in the experimental plots.

TABLE 14. Effect of exposure of wheat plants growing in sand to freezing conditions on development of Cephalosporium leaf stripe symptoms.

| Root treatment[a] | Temperature, °C | Infected leaves, %[b] | Days after addition of conidia to first symptom expression[b] |
|---|---|---|---|
| Non-cut | +2.0 | 0 a | ---- |
| Cut | +2.0 | 77 d | 21 a |
| Non-cut | -15.0 | 14 b | 46 c |
| Cut | -15.0 | 39 c | 32 b |

[a]Roots were cut by severing ½ of the sand/root ball with a razor blade, then replacing the soil in the pot before drenching with conidia. Roots were drenched with conidia just before placing the plants into the freezing chamber. Plants were frozen by reducing the ambient temperature at approximately 0.5 C/hr until -15.0 C (air temperature) was reached.

[b]Numbers are the means of three separate experiments. Values in each column followed by the same letter do not differ significantly ($P = .05$).

TABLE 15. Effect of exposing wheat plants to various periods of winter conditions in relation to development of Cephalosporium leaf stripe symptoms.[a]

| Date pots were transferred from field to growth chamber | Plants with symptoms, % |
|---|---|
| 11/20 | 0 |
| 1/13 | 2 |
| 3/7 | 11 |
| 5/3 | 28 |
| Control[b] | 34 |

[a]Seeds were sown on October 6, 1978

[b]Plants were left in the field and rated for disease at anthesis.

Temperatures never dropped below freezing at the depths monitored. The first freezing temperature occurred on December 31, and the soil remained frozen for the next 31 days. The soil froze periodically until March 17 after which no further freezing occurred. It is important to note that the first freezing event occurred before the first symptom development (Table 15). In addition, snow covered the ground during the period preceding the first disease occurrence. These conditions are not conducive to heaving since the buffering capacity of the snow prevents diurnal freeze/thaw cycles. In addition, disease incidence increased steadily with time and did not appear to be related to conditions favorable to heaving. These results agree with those of Wiese and Ravenscroft (unpublished) who found in a four year study that disease incidence appeared to progress linearly with time and was not necessarily related to spring heaving.

d. Cultivar screening procedures for resistance determination

One of the most important potential applications of freeze tests is to allow screening of cultivars for resistance. An inexpensive apparatus was designed for this purpose which effects rapid and reproducible soil freeze profiles and, at the same time, operates within the established soil temperature rate changes. This 'chest freezer method,' termed as such since the freeze apparatus is constructed from a chest freezer with a plywood pot rack, was evaluated using three cultivars of wheat and one of Agrotriticum. Seeds were obtained from D. E. Mathre from the University of Montana and were planted in 10 cm plastic cups. A greenhouse soil-oat seed inoculum mixture (20:1, v/v) was used as the planting medium and the plants were grown for one month in the greenhouse

before freezing (-5.0 C) the cups. Plants were then incubated in a growth chamber at 18 C for 50 days and observed for symptoms.

There was a good correlation between infection percentages in this test and those found in the field (personal communication, D. E. Mathre) (Table 16). The most resistant plant, Agrotriticum, had significantly less disease than did the most susceptible cultivar (WKP3496). A cultivar with an intermediate level of resistance (WKP3526) fell between the least and most susceptible cultivars, as did WKP3512. The latter was reported to be as susceptible as WKP3496, but low germination produced poor stands which may have affected the results. Genesee, previously untested for its resistance level in comparison to the cultivars in this test, appeared to be quite resistant.

In another experiment, roots of 24-day-old seedlings, previously grown in sand, were severed and dipped in one of various concentrations of conidia. Concentrations ranged from $4.2 \times 10^6$ to $4.2 \times 10^2$ conidia per ml. Subsequently, plants were placed in greenhouse soil and rated for symptom development after 25 days (Table 17).

Disease incidence differed among the varieties. As in the freeze test, Agrotriticum and Genesee showed the fewest symptoms. However, the remaining cultivars did not seem to correspond to their respective, field determined, resistance levels.

## DISCUSSION

Mathre and Johnston (54) concluded that hyphae from infested wheat straw was unable to grow and infect wheat roots due to insufficient response to root exudate. They hypothesized that passive entry of conidia into the root system took place in the spring, after heaving had severed these roots. Once root xylem was exposed, conidia, presumed to be drawn

TABLE 16. Evaluation of a modified chest freezer as a method to screen plants for Cephalosporium leaf stripe.

| Cultivar | Known relative resistance[a] | Plants with symptoms, % |
|---|---|---|
| Agrotriticum | High resistance | 14 a |
| WKP3526 | Intermediate resistance | 38 ab |
| WKP3512 | Intermediate resistance | 34 ab |
| WKP3496 | Low resistance | 54 b |
| Genesee | Unknown | 20 a |

[a]Resistance levels were determined by D. E. Mathre, University of Montana, in fields supplemented with oat inoculum of Cephalosporium gramineum.

TABLE 17. Effect of various concentrations of Cephalosporium gramineum conidia used as a root dip on wheat plants of known resistances after mechanical severing of their roots

| Conidial conc. per ml x 4.1 | Number of plants showing symptoms[a] | | | | |
|---|---|---|---|---|---|
| | Most resistant[b] | | Least resistant | | Unknown resistance |
| | Agrotriticum | WKP3526 | WKP3512 | WKP3496 | Genesee |
| $10^6$ | 0 | 5 | 5 | 3 | 0 |
| $10^5$ | 0 | 4 | 2 | 0 | 1 |
| $10^4$ | 0 | 1 | 0 | 0 | 0 |
| $10^3$ | 1 | 1 | 0 | 0 | 0 |
| $10^2$ | 0 | 0 | 0 | 0 | 0 |

[a]Numbers represent the number of plants showing symptoms per pot with five plants per pot and one pot per treatment.

[b]Resistance levels were determined by D. E. Mathre, University of Montana in fields supplemented with oat seed inoculum of C. gramineum.

in with transpiration water, would enter the vascular tissue, reproduce, and colonize the plant. Such a mechanism would, in a fundamental way, separate Cephalosporium gramineum from other vascular fungal pathogens such as Fusarium sp. and Verticillium sp. which gain entry by active penetration and growth into root tissue.

The present work has shown that active growth and penetration of wheat roots can occur in natural, unsterilized soil without root breakage. Table 18 compares various aspects of active and passive infection.

Fungistasis was shown to be overcome by the increase in root exudation following freeze stress. Root breakage was not the cause of exudation enhancement since microscopic examination did not reveal such injury; in addition, cut roots did not release sufficient exudates to account for this effect.

Changes in the nutrient content of root exudate as a result of freezing resulted not only in increased germination but, perhaps more importantly, increased vigor of growth and conidium production. The end result was an increase in inoculum potential. Garrett defines inoculum potential as "the energy of growth of a parasite available for infection of a host, at the surface of the host organ to be infected" (24). In this case the energy of growth goes from near zero, in the completely fungistatic condition, to an energy level sufficient to effect infection.

After conidial germination, runner hyphae grew over the root surface which initiated penetration points on the epidermis. Once penetration occurred, the epidermis and root cortex did not appear to serve as barriers to colonization. Details of endodermal and xylary penetration are not known since the process was not microscopically visualized.

TABLE 18. Comparison of active and passive infection processes.

| TRAIT | ACTIVE | PASSIVE |
|---|---|---|
| 1. Infection court | Entire rhizoplane | Severed end of root xylem |
| 2. Predisposition factor | Freezing | Heaving (diurnal freezing) |
| 3. Latent period (time from predisposition to symptom expression) | 30-50 days | 10-30 days |
| 4. Relationship to snow cover | Can take place under snow (does not require active transpiration) | Probably can't take place under snow (requires active transpiration) |
| 5. Relationship to *C. gramineum* population peak | Can occur at any time after initial freeze, normally encompassing peak population period | Can occur only after heaving, usually in spring, well after peak population period |
| 6. Resistance | Apparently is correlated to 'field resistance' | Not well correlated to 'field resistance' |

Therefore, the potential infection court in active penetration is the entire rhizoplane, and the volume of soil which can contribute propagules is that distance from the root surface in which the root exudates can effectively support hyphal growth to the root. This volume would be much larger than that sampled by passive infection, in which each root can be broken in only one location to form an infection court. The volume of soil sampled in passive infection would be that distance from the xylem elements that transpiration water can draw in conidia. Wheat crowns have been shown to screen out colloidal particles of gold (personal communication, C. R. Olien) moving through xylem from the roots to the shoots. If broken roots are unselectively sampling soil particles at least the size of C. gramineum conidia, it would be of interest to know how long a newly broken root would have adequate negative pressure before becoming plugged with debris.

Smith and Olien (77) found that recovery in winter barley from winter stress was reduced by disease incited by Fusarium roseum f. sp. cerealis. They showed that freezing predisposed the crown tissue to disease resulting in less adventitious root development. Crown lesions, caused by ice crystal damage, were believed to be the site of penetration. In the present work it is not known if increased root exudation alone is responsible for disease occurrence, or if structural or physiological changes due to freeze injury were also involved.

C. gramineum populations in soil change radically with time, reaching a peak between December and January (95). Therefore, the likelihood of a given volume of soil yielding infective propagules also changes with time. Snow cover, which is common in Michigan wheat fields during periods of peak C. gramineum populations, greatly reduces an already

slow transpiration rate. Consequently, this would reduce the efficiency of an infection mechanism depending on conidial uptake via the transpiration stream. Conversely, snow cover allows the soil to warm, increasing the proportion of the root which is at temperatures compatible for growth of C. gramineum. Although C. gramineum is described as a slow grower even at its optimal temperature (20 C), its ability to grow at low temperatures (5-7 C (9)) may afford it a competitive advantage during the periods in which the soil is beginning to thaw. Therefore, in Michigan, environmental and population parameters are more favorable for active penetration than for passive entry.

In my work, wheat seedlings with severed roots, planted in an infested greenhouse mix, yielded very low infection. However, plants with severed roots were readily infected by applying a conidial suspension to saturated sand. Apparently, the soil type as well as the moisture content of the soil is important to the success of passive infection.

The freeze screening technique used in this study separated one variety of Agrotritichum sp. and three wheat cultivars according to their field determined resistances. A test utilizing root severing as the predisposition factor also resulted in differences in resistance; however, the latter results were not as well correlated with field observations.

Germination and growth in response to an external source of nutrients is common among soil fungi whether acting as saprophytes or pathogens. However, successful saprophytism for C. gramineum is dependent upon prior parasitic possession of substrate, not infestation by conidial germination from soil. Germination would be counter-productive for

passive infection since this would increase the size of propagules and make them less easily taken-up in the transpiration stream. Consequently, germination and growth in soil in response to exudates is useful only as a mechanism for active infection of plants. Therefore, the development and retention of characteristics which allow germination, growth, and infection in natural soils argues that there is a selective advantage to these traits. Hence, active penetration is an important component in the life cycle of C. gramineum.

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