# Cephalosporium Stripe of Winter Wheat: Infection Processes and Host Response

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## ABSTRACT

Factors influencing the infection of winter wheat (*Triticum aestivum*) by *Cephalosporium gramineum* were examined. Hyphae that could serve as infecting propagules were not observed to develop from infected straw in natural soil. Conidia were able to serve as infecting propagules when roots were severed at depths down to at least 30 cm below the soil surface. Higher infection percentages were noted when conidia contacted wounded root ends immediately after the roots were severed, but infection could take place as long as 16 days after root wounding. When individual severed roots were inoculated with known numbers of conidia there was a linear relationship between the $\log_{10}$ number of conidia and percent plants infected. When individual roots of two genotypes of differing susceptibility were inoculated, there was a difference in the slope of the lines describing the percentage plants infected versus $\log_{10}$ number of conidia used. Inoculation of plants of various genotypes indicated that differences in degree of susceptibility were greatest when single roots were inoculated with $5 \times 10^5$ conidia as compared to a massive root slice inoculation or above-ground injection of conidia.

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The stripe disease of wheat (*Triticum aestivum* L.) which is caused by the fungus *Cephalosporium gramineum* Nisikado and Ikata was first reported in Japan in 1934 (12). Since then it has been observed in Washington (2), Montana (18), Illinois (7), New York (21), Michigan (20), Kansas (23), England (19), Scotland (8), and Italy (6). It is primarily a disease of winter wheat, although the pathogen has been isolated from diseased barley, oats, and various grasses (3, 6, 7, 23). Infected plants are usually stunted, the heads are often sterile or contain shriveled kernels, and yield reductions per infected plant are often as high as 70% (10).

Entry of the pathogen into the plant is believed to occur primarily through root wounds (1, 3, 14, 15). Under most circumstances, the wounds are thought to be caused by heaving of the soil during spring thaws although other forms of injury may also be effective; e.g., wire worm attack (19). Infection of injured roots is believed to occur in the spring since no symptoms are observed in the fall and the pathogen is isolated infrequently prior to spring (1). If fall root development is retarded, disease incidence should be lower since fewer root wounds would occur during the heaving process in the spring. Pool's (14, 15) observations in Montana substantiate that seeding when soil temperatures are relatively high and/or fertilization with phosphate greatly increases the amount of fall root growth and the incidence of this disease.

The response of various winter wheat genotypes to infection has not received extensive investigation. Some workers have reported that cultivars differ in susceptibility; however, these observations usually have been made following natural infection where inoculum density has not been controlled, or where distribution of inoculum in the test area may not have been uniform (14, 24). Other environmental conditions could also have caused escapes; e.g., uneven freezing and thawing in spots leading to uneven root damage and apparent, but not real, differences in susceptibility. Bruehl (3) initially reported that some cultivars were resistant to aboveground hypodermic injection with a "rich" conidial suspension, but a later report (17) indicated that these same cultivars were susceptible to natural infection.

The purpose of this study was to examine in more detail the etiology of this disease as related to the infection processes. In particular, we wanted to determine: (i) whether hyphae or conidia (or perhaps both) can act as infecting propagules, (ii) the inoculum density necessary for infection, (iii) the sites of infection as related to depth of root wounds, (iv) the duration of susceptibility of root wounds, and (v) the effect of host genotype on infection and disease development.

MATERIALS AND METHODS.—*Infecting propagules.*—The ability of *Cephalosporium gramineum* to grow as mycelium in soil and thus serve as an infecting propagule was studied as follows: Disks of mycelium (6-mm diameter) from the leading edge of a 1-week-old-colony growing on water agar (WA) or Difco corn meal agar (CMA) were placed on glass microscope slides coated on one side with water agar (WA), CMA, or Wiese's wheat leaf extract-copper sulfate medium (LEM) (22). In addition, *C. gramineum*-infected wheat straws were cut into 6- to 10-mm lengths, split in half longitudinally, and also used as a source of inoculum. The infested slides were covered with autoclaved (121 C for 90 minutes) or nonautoclaved Bozeman silt loam soil and placed in sealed petri dishes. The plates were incubated at room temperature 22 ± 2 C or at 10 C. In addition, some slides were infested, but not covered with soil and incubated at 100% relative humidity (RH). The moisture content of the soil was not allowed to fall below −5 bar water potential, a level previously shown to be noninhibitory to the growth and survival of *C. gramineum* (5). After 1 week, the soil was brushed from the agar surface and the slides were examined microscopically for evidence of hyphal growth and sporulation.

Lancer (C.I. 13547) winter wheat was used to determine if the presence of host roots would influence mycelial growth from infected straw pieces. These plants were grown in Bozeman silt loam in polystyrene boxes (27 cm × 34 cm × 9 cm) under fluorescent lights (18-hour day, 6-hour night) at 23 C. Each box had a removable side, that allowed access to the root zone. Infected straws 6-10 mm long were placed on WA-coated glass slides and placed next to roots that were either (i) severed, (ii) crushed with a pair of forceps, or (iii) left uninjured. Some slides were placed in areas not associated with roots. After 1 and 4 weeks the slides were removed and examined microscopically for evidence of *C. gramineum*.

*Infection of roots.*—The number of conidia required to initiate infection was studied using vernalized Lancer wheat growing in wooden boxes (8 cm × 50 cm × 40 cm) which had one removable side. Individual roots, one per plant, were severed at a depth of 10-15 cm and immediately inserted into capillary tubes (inside diameter 1.0 mm) containing a known number of conidia produced in shake culture (10). The capillary tubes were constricted at one end to prevent loss of the conidial suspension prior to uptake by the plant. Three replications of three plants each received 10, $10^2$, $10^3$, $10^4$, or $10^5$ conidia per plant. In most cases, the plant root would absorb the conidial suspension within a few seconds. A second experiment compared Lancer and Winalta (C.I. 13670) using single root inoculations of $10^2$, $10^3$, $10^4$, or $10^5$ conidia per plant with three replications of five plants each.

The depth at which roots can be infected and symptoms produced was studied using the wooden growth boxes described above. The boxes contained a sandy loam-peat mixture used to grow Lemhi (C.I. 11415) wheat plants. When the plants were in the three-to-five-leaf stage, one side of the growth box was removed and the roots severed with a knife at 10, 20, or 30 cm below the soil surface. A heavy conidial suspension ($5 \times 10^7$ conidia/ml) produced as described previously (10) was poured over the severed roots.

Vernalized plants of Lancer, Winalta, or P.I. 178383 were used to determine the length of time that severed roots are susceptible to infection. These plants were grown in a sandy silt loam in the polystyrene boxes described above. When the roots had reached the bottom of the boxes, they were severed with a knife at a depth of 10-15 cm. The area surrounding the severed roots was inoculated with 100 ml of a conidial suspension of 5,000 or $5 \times 10^6$ conidia/ml at 0, 1, 2, 4, 8, or 16 days after the roots were severed. Three replications of three plants each were used for each treatment.

*Host response.*—To determine the response of several host genotypes to infection, vernalized plants of Lancer,

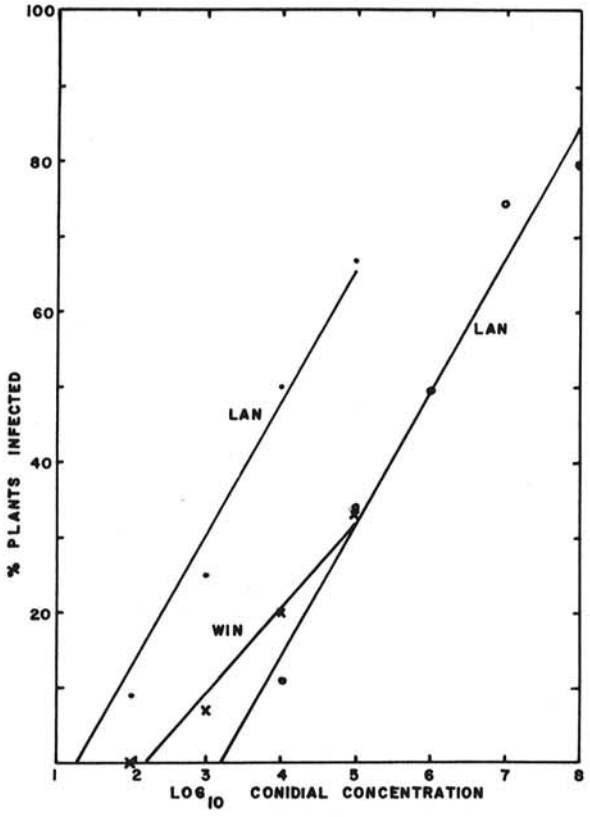


**Fig. 1.** Relationship between conidial concentration and infection of winter wheat by *Cephalosporium gramineum*. ·————· Single root inoculation of Lancer. x————x Single root inoculation of Winalta. o————o Root slice inoculation of Lancer, data from Johnston and Mathre (11).

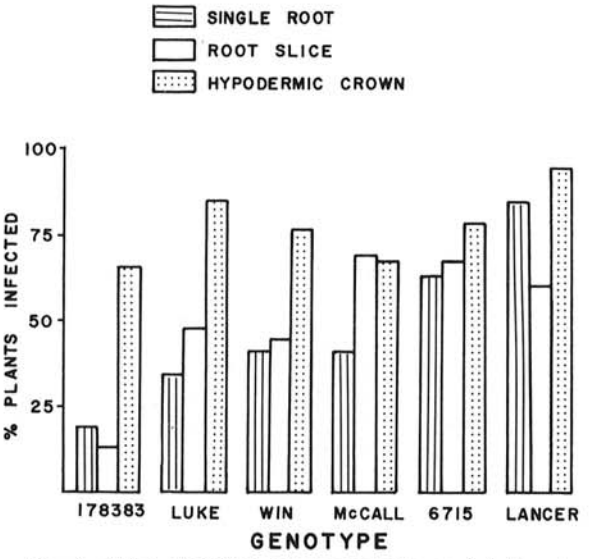


**Fig. 2.** Effect of winter wheat genotype on infection by *Cephalosporium gramineum* in relation to method of inoculation. Single roots were inoculated with $10^5$ conidia/plant; root slice inoculation involved $5 \times 10^5$ conidia/ml; hypodermic crown inoculation utilized $5 \times 10^5$ conidia/ml.

Luke (C.I. 14586), Winalta, McCall (C.I. 13842), P.I. 178383, or MT 6715 in the four-leaf stage were inoculated three different ways: (i) a hypodermic injection into the primary tiller about 3 cm above the soil line using a conidial suspension of $5 \times 10^5$ conidia/ml, (ii) a root slice inoculation accomplished by severing all of the roots of a plant about 5 cm below the seed followed by application of 100 ml of a conidial suspension of $5 \times 10^5$ conidia/ml to each box, or (iii) a single root inoculation using the capillary tube technique described above with $10^5$ conidia per plant. Three replications of six plants each were used for each treatment.

RESULTS.—*Propagule production.*—Since *Cephalosporium gramineum* has been shown to be a poor saprophytic competitor in soil, it is believed to survive from year to year only within straw pieces colonized parasitically (4). This being the case, the question arises as to how, and in what form, this pathogen moves from the interior of the infected straw to and ultimately into the host root. One possibility is for the fungus to grow as mycelium from the straw and infect the root via infection hyphae. Another possibility would be for the fungus to grow to the surface of the infected straw and sporulate, thus releasing conidia into the soil which could serve as the infecting propagules. To see if hyphae of *C. gramineum* would grow through soil from sources of inoculum, we placed inoculum in the form of naturally infected wheat straws or mycelium growing on WA in either autoclaved or nonautoclaved soil. Hyphae grew 4-5 mm in 1 week onto slides coated with CMA or WA with abundant sporulation along the hyphae in autoclaved soil, while hyphal growth was reduced 50-90% in nonautoclaved soil and little or no sporulation was observed. In air at 100% RH, the mycelial growth and sporulation were only slightly greater than that observed in autoclaved soil. This data would indicate that soil per se is not an inhibitory factor to mycelial growth and sporulation but that a fungistatic factor is operational in natural soil which inhibits both processes, but particularly sporulation.

The fungistatic factor in soil that affects many soilborne fungi is overcome by the presence of host root exudates. Therefore, we examined the response of *C. gramineum* to wheat roots to see if their presence would stimulate growth of hyphae in natural soil. Infected straw pieces on WA-coated slides were exposed to wheat roots that were either intact, crushed, or severed. After 1 and 4 weeks of exposure, no *C. gramineum* hyphae were detected growing from the straw pieces regardless of the condition of the root to which they were exposed. This also suggests that hyphae growing from inoculum sources in the soil are not serving as infective propagules.

*Inoculum density.*—Since Ravenscroft and Wiese (16) reported that large numbers of conidia of *C. gramineum* could be detected in the upper 6 cm of soil in naturally infected wheat fields in Michigan, the infecting propagules most likely are conidia. These are probably produced on straw near the soil surface and are then washed down into the upper soil layers during the fall and winter. We therefore investigated inoculum density (i.e., numbers of conidia per unit volume of soil) as a factor in infection.

When the total root mass of a wheat plant is severed and inoculated, there is no way of knowing or controlling

the number of infection sites that occur. Hence, differences in infection between cultivars might be related more to the number of roots produced, and hence, potential infection sites, than to any inherent internal resistance mechanism. Use of a capillary tube containing a known number of conidia circumvented this potential problem of infection sites since only one site would be involved.

With a susceptible cultivar such as Lancer, we observed a linear relationship between the $\log_{10}$ number of conidia applied to a single root and the percentage of plants infected (Fig. 1). A similar relationship was apparent for the moderately susceptible cultivar Winalta. However, more conidia (between 100 and 1,000) were required to initiate symptoms in Winalta than in Lancer (between 10 and 100 conidia). Although the data points in Fig. 1 did not fall exactly on the regression line, they fell well within the 95% confidence belt calculated for each. The slope of the line describing infection of Lancer was 17.5. When data were used from an earlier paper (10) that concerned massive root inoculation of Lancer with known numbers of conidia, the slope of the line describing infection was 17.8, a value not significantly different, $P = 0.05$, from that for single root inoculation. However, an inoculum dose approximately 100 times higher was required to initiate infection with massive root inoculation than for single root inoculations with the capillary tube. The slope of the line describing single root inoculation of Winalta was 11.2, a value significantly different ($P = 0.05$) from that for single root inoculation of cultivar Lancer.

*Host genotype effects.*—The use of controlled inoculum densities to determine if there are differences in susceptibility to *C. gramineum* has not been done until recently. Studies in the greenhouse (9) and in the field (11) indicated that, while no winter wheat cultivars yet tested are immune to *C. gramineum*, some genotypes are more susceptible than others. This difference in susceptibility was manifested by variation in the percentage of plants infected and in yield reduction. Cultivars P.I. 178383 and Luke (a soft white wheat which has P.I. 178383 parentage) were chosen because they exhibited few symptoms in the field when planted early in the fall in the presence of a high level of oat kernel inoculum (11). Winalta and McCall appeared to be of intermediate susceptibility in these same tests, while MT 6715 and Lancer were highly susceptible. Our greenhouse tests were set up to determine whether differences in susceptibility are related to infection phenomena.

When the six genotypes were inoculated above ground by hypodermic injection, all had infection percentages above 60% (Fig. 2). It is also interesting to note that even though the inoculation took place only in the primary tiller, all tillers subsequently became infected. This indicates that the pathogen was able to move down into the crown where the secondary tillers are formed.

Single root inoculations resulted in infection percentages ranging from 18% for P.I. 178383 to 83% for Lancer. Inoculation of the total root mass by use of the root slice technique resulted in infection percentages ranging from 13% for P.I. 178383 to 69% for McCall.

When symptom development on a plant was combined with infection percentage to give a severity score, the greatest degree of separation in susceptibility of the six genotypes occurred using the single root inoculation (Table 1). P.I. 178383 was the least susceptible regardless of the inoculation technique while Lancer was the most susceptible. The intermediate cultivars varied in ranking of susceptibility depending on the method of inoculation used. When the symptom severity score for the three inoculation techniques is added together, the ranking of cultivars from least to most susceptible is in the same order as that using only the single root inoculation; i.e. P.I. 178383 = 3.04, Luke = 4.63, Winalta = 4.66, McCall = 6.52, MT 6715 = 8.08, and Lancer = 10.18. There was a significant ($P = 0.05$) correlation ($r = 0.95$) between symptom severity for the root slice inoculation and single root inoculation, while a significant correlation did not exist between hypodermic inoculation and single root inoculation ($r = 0.76$) or root slice inoculation ($r = 0.74$).

TABLE 1. Effect of winter wheat genotype on severity of symptoms from Cephalosporium stripe as related to inoculation technique

| Genotype | Average symptom severity[a] — Inoculation technique: Single root | Root slice | Hypodermic injection |
|---|---|---|---|
| P.I. 178383 | 0.71 c | 0.27 b | 2.06 b |
| Luke | 1.25 bc | 0.94 ab | 2.44 ab |
| Winalta | 1.47 bc | 0.80 b | 2.39 ab |
| McCall | 2.75 ab | 1.71 ab | 2.06 b |
| MT 6715 | 2.75 ab | 2.33 a | 3.00 ab |
| Lancer | 3.83 a | 2.41 a | 3.94 a |

[a]Severity scored as follows: 5 = heads bleached white; 4 = symptoms in the flag leaf; 3 = symptoms in the first leaf below the flag leaf; 2 = symptoms in the second leaf below the flag leaf; 1 = symptoms in the third leaf below the flag leaf; 0 = no symptoms.
[b]Column means followed by the same letter are not significantly different, $P = 0.05$, by Duncan's multiple range test.

TABLE 2. Duration of susceptibility of severed roots of winter wheat to infection by *Cephalosporium gramineum*

| Inoculum density | Plants infected[a] (%) — Inoculation time – days after severing: 0 | 1 | 2 | 4 | 8 | 16 |
|---|---|---|---|---|---|---|
| $5 \times 10^6$ conidia/ml | 100 | 42 | 27 | 18 | 19 | 28 |
| $5 \times 10^3$ conidia/ml | 30 | 26 | 11 | 20 | 10 | 17 |

[a]Mean for the entries Lancer, Winalta, and P.I. 178383.

*Duration of wound susceptibility.*—If the ends of roots broken during the spring heaving process are the main entry points for conidia of *C. gramineum*, the time span over which these broken ends can serve as infection courts may be another important phenomenon governing infection. At the high inoculum level ($5 \times 10^6$ conidia/ml), all plants were infected when inoculation followed wounding within 5 minutes (Table 2). However, the efficiency of infection dropped off rather sharply when inoculation occurred 1 or more days after the roots were severed. After 2 days the level of infection remained fairly constant at about 25% for the high inoculum level and 15% for the lower inoculum level. Severed roots were susceptible for at least 16 days after injury. This was the

maximum length of time tested, so the root ends may be susceptible for a longer period. It is also noteworthy that inoculum density did not have as great an effect on percentage infection when inoculation occurred 4 or more days after root wounding as compared to the shorter time periods.

*Wounding depth.*—Deep plowing of infected straw to a depth of 30 cm has been shown to provide an excellent means for the control of Cephalosporium stripe (14). While the mechanism by which this technique is functional is unknown, two possibilities seem tenable. Either buried straw would decompose so rapidly as to preclude inoculum production or, alternatively, root infection for some reason may not be possible at soil depths of 30 cm. To test the latter hypothesis, root zones were severed at soil depths ranging from 10 to 30 cm and inoculated. Within 23 days, all plants in all treatments were infected and showed definite aboveground symptoms. There was, however, a correlation between the rate of symptom appearance and the depth at which root injury had occurred. Nineteen days after inoculation, all plants in which roots were severed at the 10-cm depth showed symptoms of Cephalosporium stripe, whereas 60% of those wounded at a 20-cm depth, and only 20% of those at a 30-cm depth displayed symptoms. Hence, the infection process does not seem to be inhibited at increasing soil depths.

DISCUSSION.—Several workers have shown that infection by *C. gramineum* can occur if wheat seed is germinated on cultures of this pathogen (13, 17). However, under natural conditions this association of mycelium and the host is not likely to occur. Our observations on the development of mycelium from inoculum sources in natural soil would seem to preclude hyphae as the infecting propagules. While mycelial growth was profuse in autoclaved soil, a fungistatic effect on mycelial growth and sporulation was observed in natural soil. Ravenscroft and Wiese (16) noted that high levels of conidia were produced from sporodochia on straw at the soil surface in Michigan wheat fields during the fall, winter, and spring. Thus, conidia that have washed down into the soil appear to be the most likely natural form of infecting propagule. We visualize the infection process as being one where conidia are "vacuumed" into xylem vessels exposed when roots are severed in the spring by frost heaving of soil.

The depth at which roots are severed does not seem to be a critical factor in infection since infection occurred when roots were severed as deep as 30 cm below the soil surface. However, under natural field conditions, most of the infection sites will probably be in the first 15 cm since this is the zone in which most of the inoculum is located (16, 24), and where most of the broken roots occur (14). The fact that the severed root ends are most susceptible to infection on the day that they are broken also suggests that viable conidia must be present in the soil at this time. If such were not the case and the conidia had to be produced in response to exudates from broken roots, the efficiency of infection would be greatly reduced as evidenced by the decline in infection when roots were inoculated several days after the roots were severed (Table 2). It is also possible that conidia might germinate in the vicinity of broken root ends with infection then occurring from developing hyphae. This aspect is currently being investigated.

Bruehl (3) initially reported that some cultivars of winter wheat were resistant to aboveground hypodermic inoculation. Rivera and Bruehl (17) later reported that these same cultivars were susceptible to natural infection. However, Pool (14) and Yunoki and Sakurai (24) did report that under natural field conditions some cultivars were less susceptible than others. Our experiments with controlled levels of inoculum in the greenhouse (Fig. 1, 2, Table 1) (9) and in the field (11), suggest that differences in susceptibility do exist, and that inoculum density is an important factor which affects the host response. A cultivar of intermediate susceptibility (e.g., Winalta) did not respond as strongly to increased inoculum levels as did the highly susceptible cultivar Lancer. This difference in susceptibility may be responsible for the difference in the slope of the dosage-response curves (Fig. 1). This suggests that perhaps sporulation within the xylem vessels of a host of lower susceptibility is diminished compared to that in a host of high susceptibility.

Our work on method of inoculation also suggests that if resistance (or decreased susceptibility) is expressed in a plant, that the root portion of the plant is involved. Note that P.I. 178383 was more susceptible to aboveground inoculation than to root inoculation (Fig. 2). Using data (11) on the response of the six cultivars tested to inoculum in the field, a significant ($r = 0.87$) correlation was observed between the severity of symptoms observed for the greenhouse root slice inoculation and for the field response.

Until better sources of resistance than P.I. 178383 are located, the use of controlled levels of inoculum introduced through the host root system may permit the development of winter wheat genotypes that are significantly less susceptible to *C. gramineum* than some of our currently productive and widely grown cultivars.

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