AN ABSTRACT OF THE THESIS OF

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Title: THE APPLICATION OF MATHEMATICAL MODELS IN THE EPIDEMIOLOGY OF FOOT ROT OF WHEAT CAUSED BY CERCOSPORELLA HERPOTRICHOIDES FRON

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Robert L. Powelson

Inoculum that is uniformly distributed in a soil system can be represented by a tetrahedron with the apices representing spores. If tetrahedra are arranged to form a perfect lattice, a cubic close-packed lattice would result. Using this model distance (D) between spores in the soil can be calculated using the following equation: $D = 1.1225\left(\frac{V_s}{N}\right)^{1/3}$ where (V_s) represents the volume of the soil and (N) the number of spores or inoculum density. Distances between spores decreases most rapidly up to 10,000 spores/g of soil. Inoculum density as well as distance between spores is not linearly related to the probability of infection for a wide range of inoculum densities (200 to 50,000 spores/g of soil). The relationship is a decaying exponential function and asympotically it approaches zero for extremely large distances or very low inoculum densities. This function as it

soil. There was an additional one week delay before the maximum amount of sporulation occurred on the other three soils.

The relative amount of sporulation on the different soils and sand was also evaluated under field conditions. In general, the rate as well as the amount of sporulation was suppressed by the soils when compared with sand and there was no difference among the soils. A major fall sporulation pulse and a minor spring sporulation pulse were observed. The conidia produced by the fall pulse are the major source of inoculum for soil-borne infections. Spring infections resulting from inoculum produced in the fall probably do not occur because conidia of C. herpotrichoides do not survive for longer than four months.

Field plots were established at Sherman Experiment Station, Moro, Oregon, Pendleton Experiment Station, Pendleton, Oregon and La Grande, Oregon. The soil was infested with inoculum densities of 2000, 10,000, 30,000 and 50,000 spores/g of soil. At the La Grande location sand was used as well as the parent soil. The amount of infection produced when the infection court was surrounded with infested sand was significantly greater than with the parent soil. Regression lines drawn from points on an arithmetic plot as well as a log probit plot of the data suggest that synergism may be responsible for the number of infections observed with sand. With the parent soil at the La Grande location and at the other locations independent action

approaches a limit asympotically can be considered linear over a small range of distances or inoculum densities.

Three different soils, sand, sandy loam and silt loam, were infested with conidia of Cercosporella herpotrichoides at inoculum densities of 2000, 10,000, 30,000 and 50,000 spores/g of soil. Regression lines drawn from points on an arithmetic plot of the data suggest that infection of wheat by soil-borne inoculum of C. herpotrichoides is under the influence of the rhizosphere. There was a decrease in disease incidence with the two soils when compared with sand. Regression lines drawn using the multiple-infection transformation suggest a significantly greater number of spores of C. herpotrichoides are required to incite disease in wheat with the silt loam soil than in the sandy loam soil or sand. The rate of carbon immobilization in the soils was greater than in sand indicating that the decrease in disease incidence in the soils is related to the biological activity of the soils. Microbial competition for nutrients may be occurring in the rhizosphere with a resultant shrinkage of the rhizosphere influence.

The influence of four different soils and sand on the rate, amount and time of sporulation of C. herpotrichoides on infected stubble was evaluated. Under controlled environmental conditions the soils suppressed the quantity of spores produced, but had no effect on the rate of spore production. The peak in sporulation occurred after three weeks when stubble was incubated on sand and the sandy loam

of propagules was demonstrated. There were no differences in the number of spores required for one infection per wheat plant at Sherman Experiment Station or with the parent soil at La Grande, but fewer spores were required to produce one infection at Pendleton Experiment Station.

Infections by soil-borne inoculum of C. herpotrichoides provide a means of maintaining a resevoir of carry over inoculum sources during years unfavorable for above ground epidemic development.

The Application of Mathematical Models in the Epidemiology of Foot Rot of Wheat Caused by Cercosporella herpotrichoides Fron

by

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THE APPLICATION OF MATHEMATICAL MODELS IN THE EPIDEMIOLOGY OF FOOT ROT OF WHEAT CAUSED BY *CERCOSPORELLA HERPOTRICHOIDES* FRON

CHAPTER 1

THE RELATIONSHIP BETWEEN INOCULUM DENSITY AND INFECTION OF WHEAT BY *CERCOSPORELLA HERPOTRICHOIDES* FRON IN DIFFERENT SOILS

Disease incidence and severity are affected by the inoculum potential of pathogenic propagules in the soil (Dimond and Horsfall, 1965; Baker, Maurer, and Maurer, 1967). Inoculum potential, which measures the ability of a pathogen to infect its host, has been defined by Martinson (1963) to be a function of inoculum density, available nutrients, environmental factors and the genetic capacity of the host and pathogen.

Because disease does not increase in a simple linear way with an increase in inoculum, mathematical models have been proposed for the transformation of data to obtain a linear relationship between amount of inoculum and amount of disease. Gregory (1948) suggested that the number of infections is not simply related to the percentage of diseased plants and thus proposed a multiple infection transformation which converts percent disease to number of infections and corrects for the probability of more than one infection per plant occurring at higher inoculum densities. A logarithmic-probability transformation has been advocated by some (Wilcoxon and McCallan, 1939; Horsfall, 1956). In

this transformation the percentage of diseased plants in probit units is linearly related to the inoculum density when plotted on a log-probit grid. Baker (1965; 1968) and Baker et al. (1967) proposed models based on arithmetic or logarithmic transformations for both amount of disease and inoculum density. From the slopes obtained from these transformations, inferences about the type of host-parasite interaction could be made as well as various conclusions about mechanisms of biological control. In the construction of his mathematical models, Baker employed solid geometry using a lattice of equilateral tetrahedra with each apex of a tetrahedron representing a propagule in the soil. The sides of the tetrahedron represented the distance between propagules. A tetrahedron was selected for his models because it represents the simplest three-dimensional figure possible even though tetrahedra do not entirely fill space. Spheres and cubic lattices were eliminated because spheres do not fill space as well as tetrahedra and distances across diagonals are not equal to the sides in all forms of cubic lattices.

Our objectives were to determine the relationship of inoculum density to disease incidence and the effect of different soil types on this relationship. Cercosporella herpotrichoides Fron, causal agent of eyespot or foot rot of wheat, was selected because of the differences in disease incidence observed with different soils across the wheat growing regions of Oregon.

Materials and Methods

Soils from cultivated fields of similar wheat cropping and foot rot history and washed, white sand were infested with various inoculum densities of C. herpotrichoides and the disease incidence in wheat was determined. Location of soil samples and soil characteristics are listed in Table 1. Soil analyses were made by the Soil Testing Laboratory, Oregon State University, Corvallis, Oregon. Random soil samples were taken from the top six inches and bulked. The soils were air dried, screened through a sieve (15 meshes/20 mm) and stored at 21 C. Soils were wetted to 50% moisture holding capacity (MHC), incubated at 21 C for two weeks to establish a biological equilibrium and then infested with a washed conidial suspension of C. herpotrichoides. The various inoculum densities were established by adding the conidia in enough water so that the resultant MHC was 75%. The soils were then thoroughly mixed by hand for uniform conidial distribution. Estimates of soil bulk density were made for calculations of the mean distance between spores for each soil.

The susceptible variety Nugaines, Triticum aestivum, a semi-dwarf winter wheat was used. Seeds were surface sterilized for two min with a 20% commercial Chlorox solution and then rinsed with distilled water. Ten seeds were then germinated in a S/P Seed-Pak growth pouch (6 1/2 X 6 3/4"). The seeds were germinated in the dark

Table 1. Soil characteristics and location.

Soil	Location	pH	Organic Matter	Nitrogen NO_3	Nitrogen Total	Percent Moisture by Weight 1/3 ATM	Percent Moisture by Weight 15.0 ATM
			%	ppm	%		
Walla Walla silt loam	Pendleton Experiment Station, Pendleton, Oregon	7.5	2.97	5.49	2.92	32.75	9.52
Imbler sandy loam	Grande Rhonde Valley Oregon	7.0	1.18	5.46	1.32	19.52	5.94
Sand	---	6.4	0.06	0.94	0.18	1.39	0.48

for four days at 21 C to ensure an elongated coleoptile, thus providing a more normal and adequate infection court. For the next 20 days, the plants were kept in a growth chamber on a 12 hr day at 15 ± 1 C and a 12 hr night at 10 ± 1 C until the nonvernalized wheat seedlings were in the two leaf stage. After 24 days the infested soils were added to the growth pouch trough to cover the base of the tillers to a depth of 15 mm. After inoculation, the plants were returned to the growth chamber. The plants were pruned back to a height of 15 cm every two weeks.

When the plants were three months old, they were removed from the growth pouches and the amount of infection was determined. Because lesion development was slight, the lower 15 mm of the tillers in contact with the soil were washed in running water for 24 hr and plated on streptomycin water agar. After 10 days incubation at 10 C, the sections were washed in 0.5 ml of 30% Fabil staining solution (Noel, 1964) using a Vortex mixer for 10 sec. The presence of Cercosporella spores in the washing solution was considered indicative of infection. A minimum of 50 plants for each inoculum density per soil were observed for foot rot infection. The experiment was repeated twice with similar results.

Results and Discussion

Spore distance studies

For plant pathogens in the soil, both the position and the density of the inoculum profoundly influence disease incidence. If the simplest three-dimensional figure representing propagules in a three-dimensional medium is a tetrahedron as proposed by Baker (1965) and Baker et al. (1967) and if these tetrahedra are arranged to form a perfect lattice with "rotational invariant" properties of this lattice, a cubic close-packed lattice structure would result (Figure 1). An illustration of this model would be a sodium chloride crystal; the sodium ions would be analogous to the spores, ignoring the chloride ions. In this model each apex of the tetrahedron represents a spore in the soil with each spore corresponding to the center of a sphere in a cubic close-packed lattice. Each and every spore in this arrangement would have 12 nearest neighboring spores at a distance $D = \sqrt{2}a$. The six next-nearest neighbors would be at a distance of 2a; however, to a first approximation these will have very little influence on the central spore.

The distance between apices of the tetrahedron or spores is directly proportional to the cube root of the volume of the small cube; $V = a^3$. Since the volume of a tetrahedron inscribed in a cube is one-third the volume of a cube, the proportionality constant can be calculated. Thus the volume of the tetrahedron (V_t) is a function of the

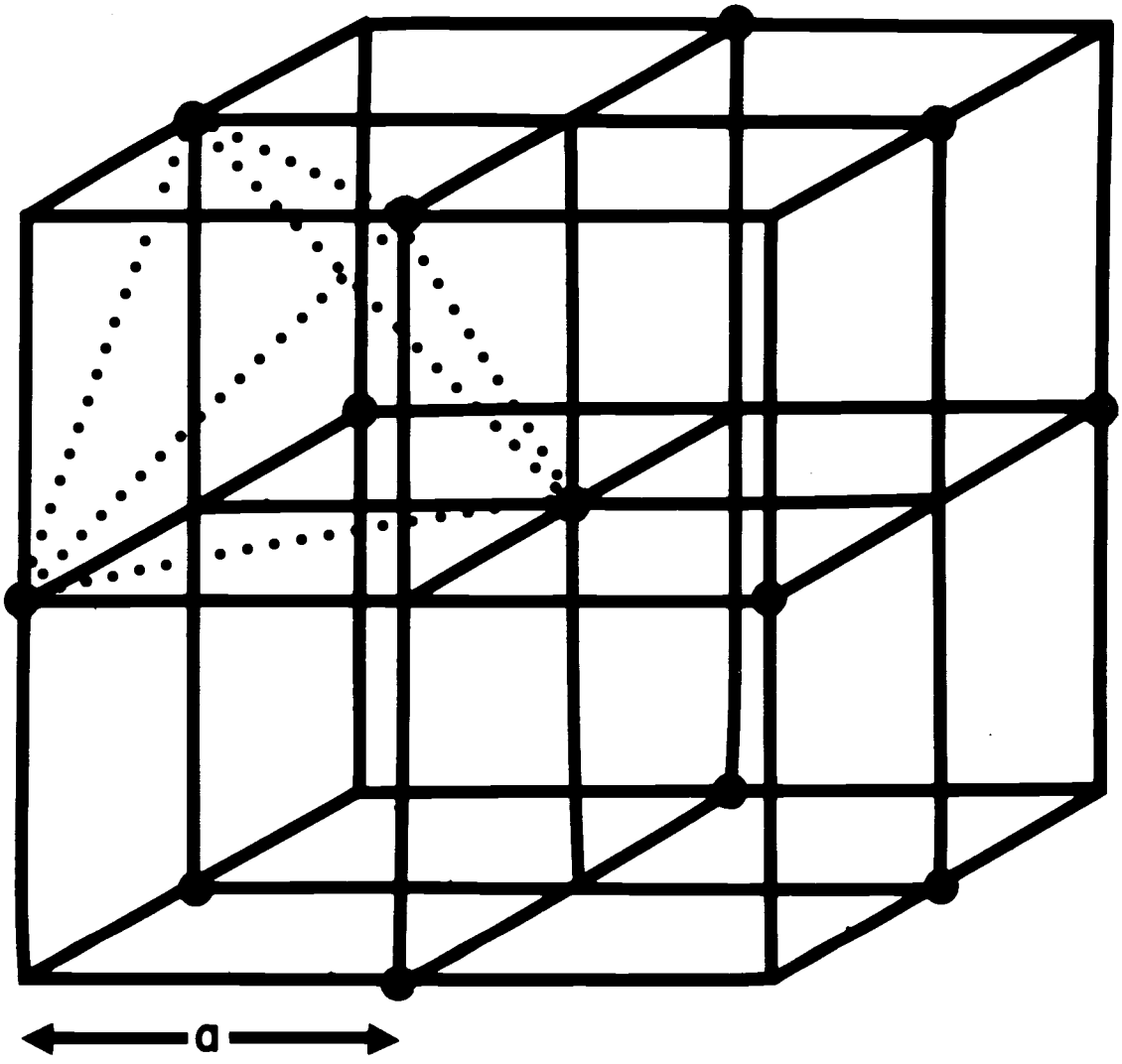


Figure 1. A cubic close-packed lattice structure with the solid circles representing spores. Each spore would be shared by eight tetrahedra and each tetrahedron is inscribed in a small cube with length a.

length of an edge of a tetrahedron and is described by the following formula:

$$V_t = \frac{\sqrt{2}}{12} D^3 \qquad \text{I}$$

$$= 0.11785\ D^3$$

where (D) is the distance between apices of the tetrahedron.

In order to relate the number of spores (N) to the total volume of the soil (V_s) and the distance between spores (D), the number of spores per tetrahedron and the number of tetrahedra in a given volume of soil must be determined. Since the volume of a tetrahedron (V_t) only occupies one-third the available space in a cube, the number of tetrahedra (N_t) in a cubic close-packed lattice in a given volume of soil can then be determined by the following equations:

$$N_t = \frac{V_s}{3\ V_t} \qquad \text{II}$$

Because each spore is shared by eight tetrahedra, and each tetrahedron is drawn from four spores, the number of spores per tetrahedron is one-half or a tetrahedron represents one-half a spore. Thus:

$$N = 1/2 N_t \qquad \text{III}$$

where (N) is the number of spores. Solving equations I, II, and III for D as a function of V_s and N:

$$D = 1.1225 \left(\frac{V_s}{N} \right)^{1/3} \qquad \text{IV}$$

Thus the distance between nearest spores can readily be determined by this equation if the volume of the soil and the number of spores in that volume is known and if it is assumed that the spores are packed in a cubic close-packed lattice. The result obtained in equation IV does not agree with the results of others (Baker and McClintock, 1965; Stienstra and Lacy, 1972):

$$D' = 2.0369\left(\frac{V_s}{N}\right)^{1/3} \qquad V$$

Implicit in the derivation of equation V is that a lattice of tetrahedra occupies all available space and that one tetrahedron represents one spore. In our equation, $D = 1.1225\left(\frac{V_s}{N}\right)^{1/3}$, D differs from D' in equation V by a factor equalling the cube root of six: $D' = (6)^{1/3} D$.

Baker and McClintock (1965) calculated that there was not a linear relationship between number of propagules per unit volume of soil and distance between propagules. Using Baker's equation (equation V) and a soil bulk density of 1.4, they showed that an increase in inoculum density decreases distance between propagules most rapidly up to 2000 to 3000 propagules/g of soil. Using equation IV and a soil bulk density of 1.4 for sand, distance between spores decreases most rapidly up to 10,000 spores/g of sand (Figure 2). The magnitude of decrease in distance between propagules was very small as the inoculum density increased above 20,000 spores/g of sand. For each of the soils assayed, there was no significant difference in distance between spores for each

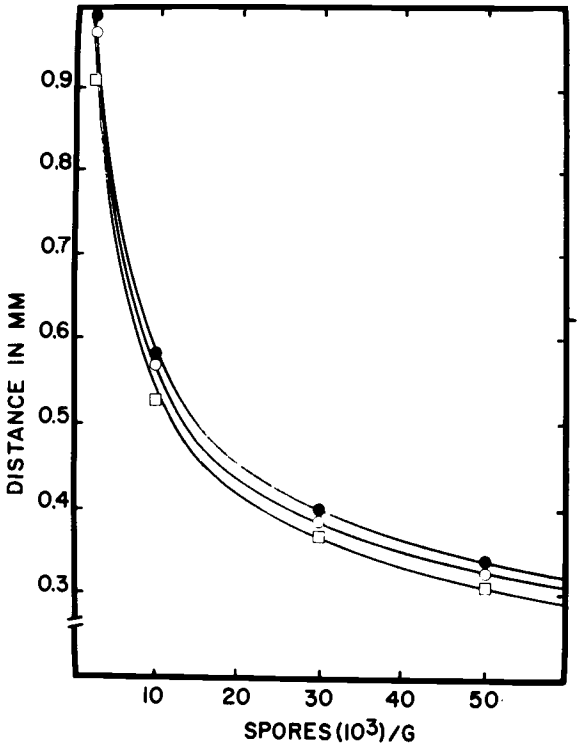


Figure 2. Relationship between inoculum density and distance between spores with three different soils: sand (solid circles); sandy loam (open circles); and silt loam (open squares).

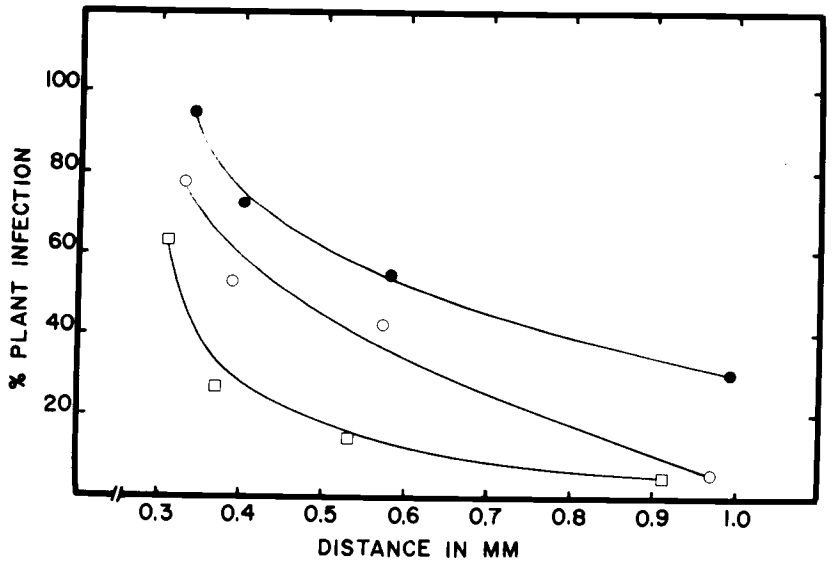


Figure 3. Relationship between disease incidence and distance between spores (inoculum density) with three different soils: sand (solid circles); sandy loam (open circles); and silt loam (open squares).

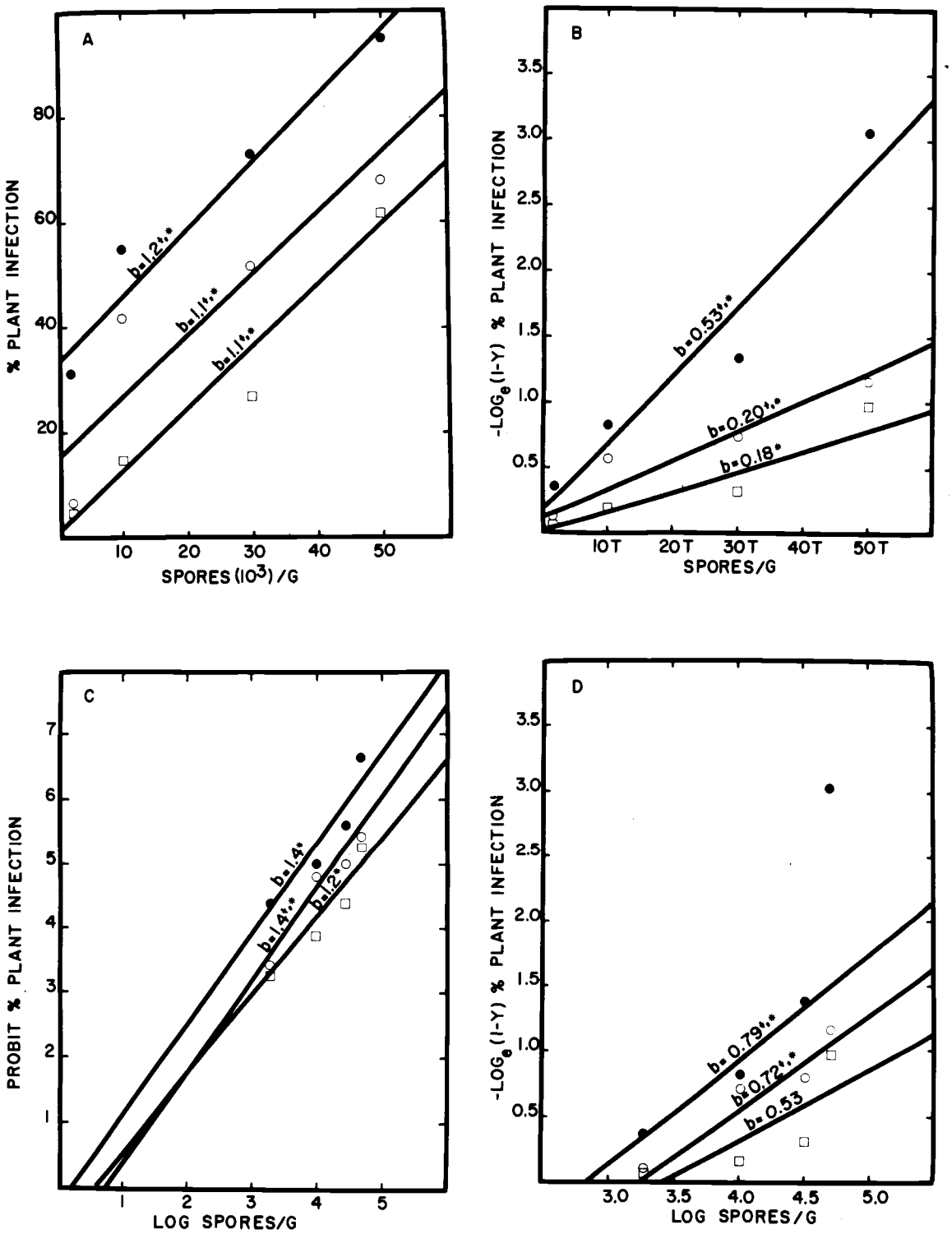
inoculum density signifiying that the different soils had no influence on the position of the propagules.

Percentage of disease when plotted against the calculated distance between spores was approximately linear over the inoculum range of 2000 to 30,000 spores/g of soil for all soils (Figure 3), whereas a more linear relationship was obtained for the entire inoculum density range when percent infection was plotted against inoculum density (Figure 4-A). Because infection of wheat by C. herpotrichoides occurs by independent action of spores, the equation of S. D. Garrett (Baker, 1971) based on the probability (P) that a host will be infected by a density (d) of spores can be used to determine the percent success of individual propagules in an infection court when the inoculum density-infection curve is known. For an ED_{50} value for P, the equation is:

$$p = 1 - 0.5^{1/d} \qquad \text{VI}$$

If the probability of infection is plotted as a function of distance between propagules as well as the number of propagules per unit volume for an inoculum density range of 200 to 10,000 spores/g of soil, the relationship is linear. The same relationship is true for the inoculum density range of 10,000 to 50,000 spores/g of soil. But for the entire inoculum density range, the distance between propagules as well as the number of propagules per unit volume is not linearly related to the probability of infection.

Figure 4. The relationship of inoculum density and disease incidence with three soil types: sand (solid circles); sandy loam (open circles); and silt loam (open squares). A) Arithmetic plots; B) semilogarithmic transformation; C) log-probit transformation; D) log-log transformation. The correlation coefficients and the regression coefficients are significantly positive at the 5% level of significance for those marked + and *, respectively.


A
% PLANT INFECTION
SPORES (10³)/G
b=1.2t,*
b=1.1t,*
b=1.1t,*
B
-LOGe(1-Y) % PLANT INFECTION
SPORES/G
b=0.53t,*
b=0.20t,*
b=0.18*
C
PROBIT % PLANT INFECTION
LOG SPORES/G
b=1.4*
b=1.4t,*
b=1.2*
D
-LOGe(1-Y) % PLANT INFECTION
LOG SPORES/G
b=0.79t,*
b=0.72t,*
b=0.53

Natural field populations of soil-borne pathogens have been reported to range from 250 to 3000 propagules/g of soil (Tolmstoff and Young, 1957; Nash and Snyder, 1962; Chinn, Sallans, and Ledingham, 1962; Maier, 1965; Evans, Snyder and Wilhelm, 1966) and recently Ashworth et al. (1972) have reported that populations of Verticillium albo-atrum as low as 3.5 microsclertia/g of soil can produce 100% infection in cotton. If the density of inoculum in the soil is within the limits reported, 250 to 3000 spores/g of soil, and if the distance between spores decreases most rapidly up to 10,000 spores/g of soil (Figure 2), the flattening out of disease response curves can not be attributed to the decrease in distance between spores as proposed by Stienstra and Lacy (1972). Thus the decrease in slope of disease response curves at high inoculum densities is probably due to lack of available infection sites as stated by Van der Plank (1963).

Effect of Inoculum Density on Disease Incidence

Increase in inoculum densities from 2000 to 50,000 spores/g of soil for each soil assayed caused an increase in disease incidence (Figure 4-A). The infection pattern of Cercosporella foot rot fits Baker's (1967) Model I type of pathogen-host relationship; nonmotile inoculum around a fixed infection court. Host infection by soil-borne inoculum of C. herpotrichoides may occur through the coleoptile or the base of the outer leaf sheaths. Usually a single distinguishable lesion

per tiller is produced. If the spores germinate in the rhizosphere, the influence of the infection court would be in the form of a hollow cylinder. The rhizosphere in this study would be analogous to that portion of the leaf sheath or coleoptile below the soil surface. The addition of increased inoculum into this volume should result in a proportional increase in disease. Thus the slope resulting from plotting disease incidence as a function of inoculum density should be 1.0 (Baker, 1968). The relationship between number of infections and inoculum density for each of the soils in our study was linear. The slopes ranged from 1.1 to 1.2 (linear regression analysis); correlation coefficients were 0.90 to 0.97. The slopes were not significantly different at the 5% level of probability (analysis of covariance), but when the elevation of each slope was compared, there was a significant difference ($p = 0.05$) for the sand - soil comparisons. However, the sandy loam - silt loam comparison was not significantly different (analysis of covariance). Thus the position of the curve, not the slope was altered by the different soils. The slopes obtained were approximately 1.0 for the soils suggesting a rhizosphere influence. The significant difference in elevation of the slopes between the sand and each of the soils indicated that the disease potential in sand was greater.

As the inoculum density increases, the probability for multiple infections also increases (Gregory, 1948). Determination of percent disease does not measure multiple infections on individual plants. The

multiple infection transformation of Gregory (1948) converts percentages into number of infections. The semi-logarithmic transformation suggests adequate corrections for multiple infections (Figure 4-B). A regression line drawn from points on the slope for each of the soils passes near the origin suggesting independent action of propagules. If there is a rhizosphere influence, a slope of 1.0 is predicted according to Baker's models (Baker, 1971). With a slope greater than 1.0 the regression line would pass to the right of the origin indicating synergism (Van der Plank, 1963).

Inferences can be made about the number of spores/g of soil required to produce on the average of one infection per plant for each of the soils (Figure 4-B). The two soils, especially the silt loam, had a suppressive effect on infection when compared with the sand. Sixteen thousand spores/g of sand were required for one infection whereas 25% and 40% more spores/g of soil were required to produce one infection per wheat plant with the sandy loam soil and silt loam soil, respectively.

Pathogen suppression by soils has been observed with other diseases. Burke (1965) reported that Fusarium root rot of bean was suppressed in different soils and that this suppressive factor was microbiological. Increased biological buffering due to an increase in numbers and types of competitive organisms was responsible for the suppression

of Fusarium wilt of sweet potatoes in different soils (Smith and Snyder, 1971).

A logarithmic-probability plot (Figure 4-C) gave a near linear relationship between percent infection and the logarithm of the inoculum density for the soils. The slopes ranged from 1.2 to 1.4 (linear regression analysis); correlation coefficients were 0.92 to 0.98. The slopes did not differ significantly at the 5% level of probability, but the position of the curves was significantly different ($p = 0.05$) for the sand-soil comparison; however, the sandy loam - silt loam comparison was not significantly different (analysis of covariance). With Baker's mathematical models, if synergism is responsible, in part, for the number of plants infected, slopes greater than 2.0 would indicate synergism for a rhizosphere effect and slopes greater than 1.31 near ED_{50} for a rhizoplane effect (Baker, 1971).

The relationship between multiple infections plotted as a function of log inoculum density was linear over the inoculum range 2000 to 30,000 spores/g of soil for each of the soils (Figure 4-D). The slopes for this range of spore densities were 0.21 to 0.79 (linear regression analysis); correlation coefficients were 0.96 to 0.99. If slopes are computed for the entire inoculum density range for both soils but not for sand (percent infection at 50,000 spores/g of sand does not fit the line), b values were 0.72 and 0.53 for the sandy loam and the silt loam, respectively; correlation coefficients were 0.97 and 0.79, respectively.

According to Baker (1971), the expected b values for a rhizosphere influences would be 1.0 and 0.67 for a rhizoplane effect.

Baker (1971) suggested that the position of the curves in a logarithmic-probability plot reflects how efficient propagules are in causing disease; i.e., the farther to the right the curve is, the more propagules required to produce an infection. Thus fewer conidia of C. herpotrichoides were required to incite plant infection in sand than with the different soils (analysis of covariance) and the two soils did not alter the efficiency of spores of C. herpotrichoides to incite disease (Figure 4-C).

Dimond and Horsfall (1965) contend that the slope and position of the linear dosage response curve, when mortality of spores is plotted against doses of fungicides on a log probit grid, will give an estimate of the mode of action of the fungicides tested; i.e., if the slopes between two fungicides differ significantly, the mode of action of the fungicides are different. If we assume an inoculum density-disease curve is analogous to the dosage response curve of fungicides as has been suggested (Dimond and Horsfall, 1965; Baker, 1971), different parameters such as different soils, different planting depths, or different varieties, etc. could be compared. If the above assumption is valid, conclusions may be drawn on the effect of soil types on infection of wheat by C. herpotrichoides: (i) the pathogenic process did not differ significantly in the different soils and (ii) the virulence of the

pathogen was not affected because there were no visual differences in the degree or extent of lesion formation or amount of sporulation from lesions when incubated. However, the amount of infection of wheat by C. herpotrichoides did differ with the soils tested and this difference may be related to the biological activity of these soils.

Effect of Soil Type on Spore Germination

To determine if there were fungistatic effects (Lockwood, 1964; Hsu and Lockwood, 1971) responsible for the differences observed in disease incidence with the different soils, the percent germination and extent of germ tube elongation were determined for each soil. The same technique used by Byther (1968) to determine percent germination in soils was used except the soils were preincubated at 75% MHC for two weeks to establish a biological equilibrium. Water agar, sprayed with a spore suspension and sprayed slides not covered with soil but incubated on moistened filter paper in a Petri dish served as the controls. Four hundred spores per reading were counted for percent germination and germ tube elongation.

Limited germination occurred in all three soils indicating some sensitivity to soil fungistasis (Table 2). Failure of Byther (1968) to preincubate his soils could account for the higher percent germination he observed with C. herpotrichoides. No difference was noted in extent of germ tube elongation among the different soils, but there was a

Table 2. Influence of soil type on percent germination and germ tube elongation.

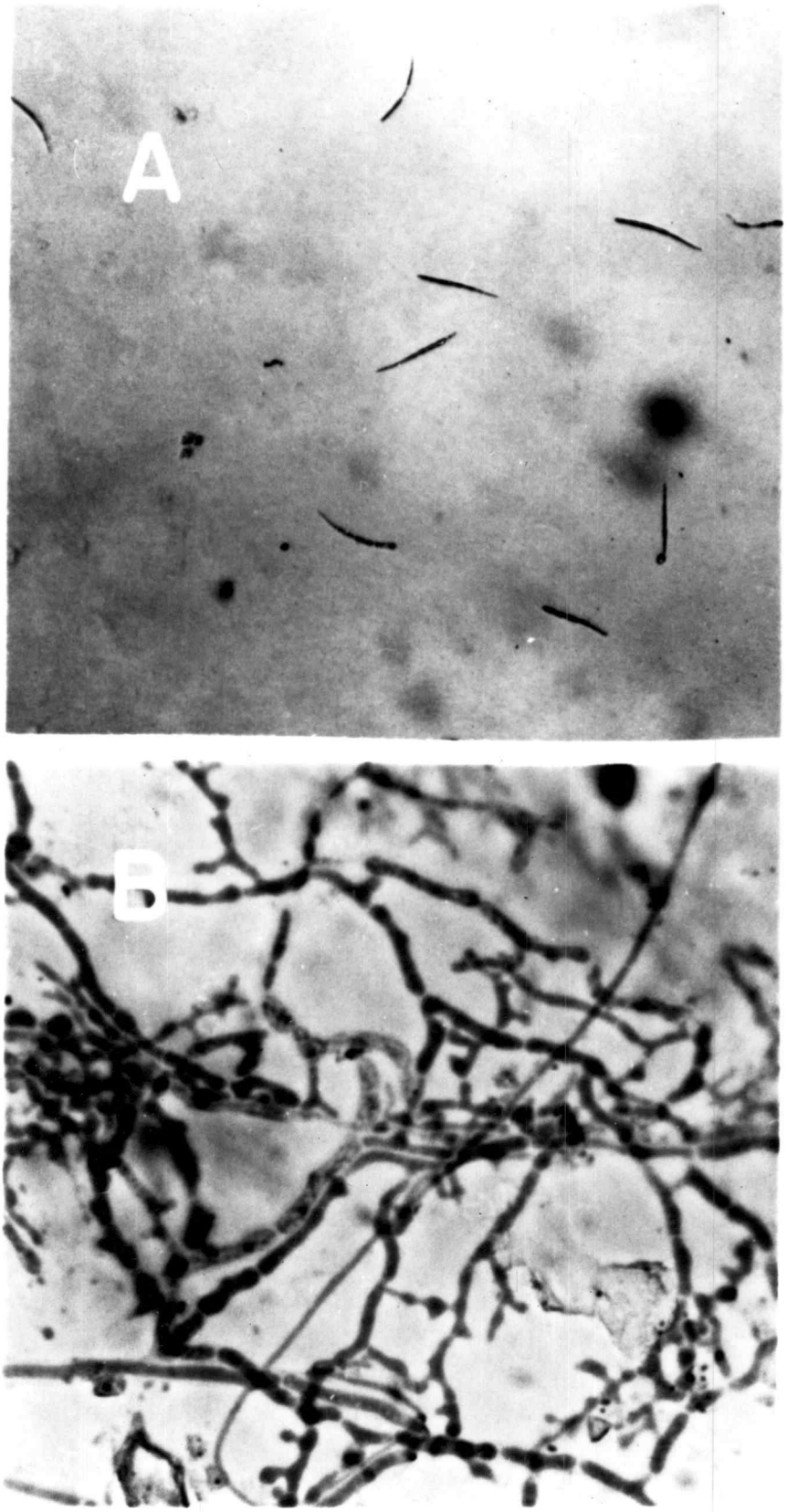
	Sand		Sandy Loam		Silt Loam		Water Agar		Glass Slide
Days	% Germ	Elongation (μ)	% Germ	Elongation (μ)	% Germ	Elongation (μ)	% Germ	Elongation (μ)	% Germ
2							94	78	24
7	10	16	10	20	10	22			
15	12		10		10				
22	9	20	8	18	9	22			
29	9	21	12	29	13	17			

definite inhibition of hyphal growth in the soils when compared to the water agar control. The amount of lysis was the same for the sandy and the silt loam soils, 25 to 30% after seven days, whereas lysis in sand was 5 to 10%.

Soils that were preincubated at 75% MHC for two weeks were added to bags made from 20 by 100 mm dialysis tubing. A bag filled with sterile distilled water was used as a control. The surface of the bags was sprayed with a conidial suspension of C. herpotrichoides and incubated in a moist chamber at 10 C. After 72 hr extensive germination and hyphal elongation were evident on the surface of the dialysis tubing containing the soils, but very little germination and no hyphal elongation were observed on the bags containing the sand or the sterile distilled water (Figure 5-A and B).

The limited amount of germination and hyphal elongation observed on the glass slides exposed to the soils as well as the dialysis tubing containing the moistened sand or the distilled water may be attributed to a nutrient gradient away from the spores. The extensive germination and hyphal elongation observed when the soils were placed in the dialysis tubing resulted from a nutrient gradient from the soil to the surface of the dialysis tubing. This suggests that the nutrients in the soils at 75% MHC diffused to the surface of the tubing providing an energy source for spore germination. In the other situation, the only nutrients available would be those provided by the spores themselves,

Figure 5. Extent of germination of spores of Cercosporella herpotrichoides on surface of dialysis tubing bags containing A) sand (X100) and B) sandy loam soil (X400) after four days incubation in a moist chamber at 10 C.


A
B

with a nutrient gradient away from the spores. These observations suggest that spores of C. herpotrichoides probably do not contain a reserve of nutrients to support extensive germination and hyphal elongation, thus indicating a requirement for an exogenous source of nutrients for germ-tube outgrowth.

Biological Activity of the Soils

Our studies indicated that spores of C. herpotrichoides required an exogenous energy source for spore germination. According to Baker (1968) pathogens requiring exogenous sources of carbon and nitrogen for spore germination and penetration are good candidates for control through competition. Usually a very biologically active soil will create a situation in which nutrients, especially simple carbon substrates, become immobilized, thus limiting the pathogen (Powelson, 1969).

Because germination and hyphal elongation did not differ among the soils studied, the rate of carbon immobilization in the different soils was used to determine if there were differences in the biological activity of the soils.

The soils were adjusted to 50% MHC and incubated at 10 C for two weeks. Sucrose, 10 mg C/g of moist soil, was added to each of the soils so that the final MHC of each soil was 75%. The soils were then assayed for carbon immobilization using the silica gel diffusion

technique of Powelson and Pyott (1966). Verticillium dahliae Kleb. was chosen as the assay organism because it is known to require an exogenous source of carbon and nitrogen for germination (Powelson, 1970) and produces a clear growth zone on silica gel. Five g of soil containing the sucrose amendment were placed on one third of the surface of the silica gel plate which had previously been seeded with spores of V. dahliae. A plug 5 mm in diameter containing 1 mg KNO_3/ml was placed 30 mm from the soil front. As a result of diffusion of sucrose from the soil and nitrogen from the plug, a zone of intense sporulation occurred in a region of optimum concentrations of these nutrients as long as diffusible sucrose was available. There were two samples of each soil for each observation period. As the carbon was immobilized, the growth zones moved closer to the soil until diffusible sucrose was no longer available (Figure 6-A, B, C and D). Rate of movement and absence of zones of sporulation were indicators of carbon immobilization (Table 3).

Rate of immobilization was most rapid with the silt loam series with complete carbon immobilization occurring by day 42. There was approximately an additional four week delay before complete immobilization occurred with the sandy loam soil. No noticeable immobilization of carbon was evident with the washed sand. Because a high C:N ratio was established in the soils with the addition of sucrose, an immediate increase in the soil microflora was expected. With this

Figure 6. Differences in the growth zones produced in silica gel medium seeded with Verticillium dahliae when soils containing 10 mg/C from sucrose were incubated before being placed on the plates A) sand - 70 days; B) sandy loam - 34 days; C) sandy loam - 49 days; and D) sandy loam - 70 days.

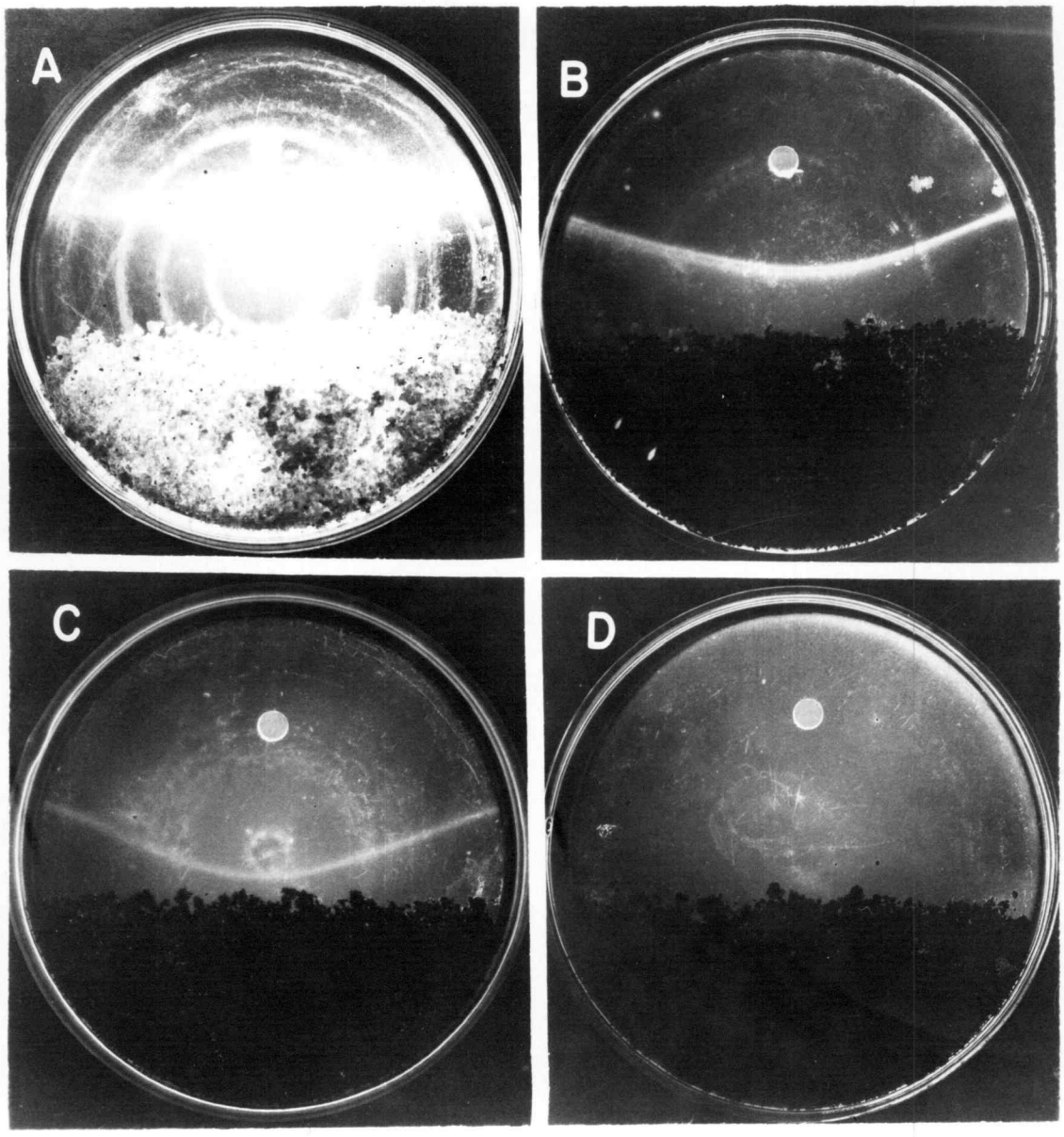

A
B
C
D

Table 3. Influence of soil type on rate of carbon immobilization as measured by intensity and movement of growth zones on seeded silica gel plates.

	Sporulation			Distance (mm) of growth zone from soil line		
Days	Sand	Sandy loam	Silt loam	Sand	Sandy loam	Silt loam
0	+++*	+**	+	21	22	23
3	+++	+	+	22	24	26
6	+++	+	+	21	22	23
10	+++	+	+	24	25	20
14	+++	+	+	21	20	20
20	+++	+	+	21	20	18
27	+++	+	+	21	18	11
34	+++	+	-+	23	12	1
42	+++	+	0	22	9	0
49	+++	+	0	23	4	0
56	+++	-+	0	22	1	0
63	+++	0	0	22	0	0
70	+++	0	0	21	0	0
77	+++	0	0	22	0	0

* a broad, diffuse growth zone

** a narrow, intense growth zone

increase in heterotrophic flora, large quantities of CO_2 would result and the NO_3 in the soil would be utilized. This situation would persist until carbon was no longer available.

With the absence of available carbon, zones of sporulation disappeared. The rate of immobilization was most rapid in the silt loam

soil suggesting that this soil was more biologically active; i. e., the microflora were more competitive or more efficient in carbon utilization.

Rate of carbon immobilization occurred most rapidly in the silt loam soil (Table 3) suggesting a very biologically active soil, whereas the sandy loam series was less active biologically. In sand, which is considered nearly biologically inert, there were no noticeable changes in the intensity of sporulation or movement in zones of sporulation, indicating lack or absence of carbon immobilization. There was a negative correlation (-0.97) between the average amount of disease and the rate of carbon immobilization for sand and both soils. The lesser the amount of disease, as illustrated with the silt loam soil, the faster the rate of carbon immobilization. Sand which demonstrated no carbon immobilization after ten weeks had the greatest incidence of disease for all inoculum densities.

If biological control is occurring via competition in the silt loam and the sandy loam soils, reduction in inoculum density by antibiosis or lysis in the rhizosphere or on the rhizoplane or by competition for nutrients on the rhizoplane should result in a decrease in disease incidence (Baker, 1968). If the aforementioned mechanisms reduce disease incidence, the effect should be similar to that of adding fungicides. Slopes, if plotted on a non-log basis, would be shallower than the reference slope, and if plotted on a log-log basis, would be a series

of parallel lines (Baker, 1968). The parallel slopes of approximately 1.0 on a non-log basis obtained for the soils, which suggest a rhizosphere influence, do not reflect biological control acting at the level of antibiosis or lysis or competition for nutrients on the rhizoplane. Control, though, may be acting at the level of competition for nutrients in the rhizosphere. This may explain the series of parallel lines when the data are plotted on a non-log basis (Figure 4-A) and the series of shallower slopes when the data are plotted on a log-log basis (Figure 4-D). Theoretically control could be possible through shrinking the rhizosphere. In competition of this type, essential nutrients in the rhizosphere would be immobilized. The rate of carbon immobilization may be reflective of the competitiveness of the microflora for the carbon substrates. Benson and Baker (1970) using their model soil system demonstrated that competition for carbon sources in the rhizosphere could promote biological control of bean root rot.

A rhizosphere influence was responsible for the amount of infection in wheat by soil-borne inoculum of C. herpotrichoides. In order to produce the same amount of infection with a rhizoplane effect, more inoculum would have been required. Because a greater number of spores were required for infection to occur with the different soils (Figure 4-B and C), the suppressive effect of the soils was probably due to the shrinking of the rhizosphere. As evidenced by the carbon

immobilization study, microbial competition for nutrients was probably the mechanism involved in the shrinking of the rhizosphere.

CHAPTER II

THE INFLUENCE OF SOILS ON SPORULATION AND INFECTION BY CERCOSPORELLA HERPOTRICHOIDES FRON

Epidemics of foot rot of wheat, caused by Cercosporella herpotrichoides Fron, occur sporadically in certain wheat growing regions of the Pacific Northwest. The incidence of this disease is dependent, in part, on weather conditions, inoculum levels and cultural practices.

Above ground infections occur from rain-splashed conidia produced from infected stubble lying on the soil surface. Lesions usually develop at the base of the plant (Ponchett, 1958; Drath and Rapilly, 1967; Byther, 1968; Glynne, 1969; Rowe, 1972). Conidia are produced from infected stubble underground (Fehrmann and Schrodter, 1971) as well as on the soil surface. Below ground infections can occur from soil-borne inoculum of C. herpotrichoides (Byther, 1968; Chapter 1) as well as from conidia lying on the soil surface Scheinpflug, 1964). In eastern Oregon where foot rot of wheat is a problem, most of the wheat acreage is seeded with deep furrow drills. During the fall and winter months, these furrows become filled as a result of wind and water erosion. Inoculum produced from infected stubble can thus become soil-borne and come in contact with below ground tissues as a result of erosion.

The purpose of this study was to determine the role of soil-borne inoculum of C. herpotrichoides on the epidemiology of foot rot of wheat.

Materials and Methods

The relative amount of sporulation from naturally infected stubble on four different soils plus white, washed sand was determined in the laboratory under controlled environmental conditions. The soils used were from four locations in Oregon with different soil types and climate (Table 4). Soil samples were taken from the top six inches at each location and bulked. The soils were then air dried, screened through a sieve (15 meshes/20 mm) and stored at 21 C. The screened soils and the sand control were uniformly wetted up to 75% moisture holding capacity (MHC) and incubated at 21 C for one week to establish a biological equilibrium. Twenty sections of infected wheat stubble one cm long were then placed on the surface of each of the soils and sand. The stubble and soil were then misted with distilled water so there would be good contact between the stubble and soil. The soil containers were covered with polyethylene to maintain a water saturated atmosphere and incubated in the dark at 10 C. The amount of sporulation was determined by washing the stubble in 0.5 ml distilled water using a Vortex mixer for 10 sec and counting the number of spores in the wash water using a haemocytometer. The stubble pieces

Table 4. Soil type and rainfall at the field locations.

Location	Abbr.	Soil Type	Rainfall/yr (in.)
La Grande, Oregon	CASE	Imbler sandy loam	19.01
Pendleton Experiment Station Pendleton, Oregon	PES	moist Walla walla silt loam	21.11
Sherman Experiment Station Moro, Oregon	SES	Walla Walla silt loam	10.24
North Willamette Experiment Station Aurora, Oregon	NWES	Willamette sandy shot loam	47.3

were returned to the soil surface after each reading. Readings were made at weekly intervals for eight weeks.

An evaluation of the relative amount of sporulation on naturally infected stubble was made at one location in western Oregon (NWES) and two locations in eastern Oregon (SES and PES). One cm sections of naturally infected stubble were laid on the surface of screened soils and sand that were contained within a 50 mm high ring cut from #10 cans. The rings were covered with hardware screen (8 meshes/25 mm) to prevent the stubble pieces from blowing away. At monthly intervals the number of spores produced on 10 stubble pieces was determined for each soil. Each section was agitated in 0.5 ml of a 30% Fabil solution (Noel, 1964) using a Vortex mixer for 10 sec. After washing the stubble was discarded and the number of spores present in the wash solution was determined with a haemocytometer. At each location there were the parent soils, sand, and soil from one other location.

In October 1971 plots were established in fields of Nugaines wheat in eastern Oregon at three locations: CASE, PES and SES. Random samples of soils for inoculum density studies were taken from the top six inches at each location and bulked. White, washed sand was also used at the CASE location. The soils were air dried, screened through a sieve (15 meshes/20 mm) and stored at 21 C. Before infestation with a washed conidial suspension of C. herpotrichoides, the soils were moistened uniformly to 50% MHC and

incubated at 21 C for two weeks to establish a biological equilibrium. The soils were then infested at four inoculum densities: 2000, 10,000, 30,000 and 50,000 spores/g of soil.

Paper cups (1 oz) with the bottoms removed were placed over individual wheat seedlings in the two leaf stage. Each cup was filled to a depth of 20 to 25 mm with 20 to 25 g of infested soil from its respective location. The plots had been seeded with a deep furrow drill. To secure the cups and to simulate erosion of soil into the furrows that occurs under field conditions, soil from the sides of the furrow was packed around the cups (Figure 7). The plants were harvested at the heading stage of growth, 10.1 on the Freekes scale (Large, 1954) and examined for visible underground lesions. The percent infection both on a plant and tiller basis was determined.

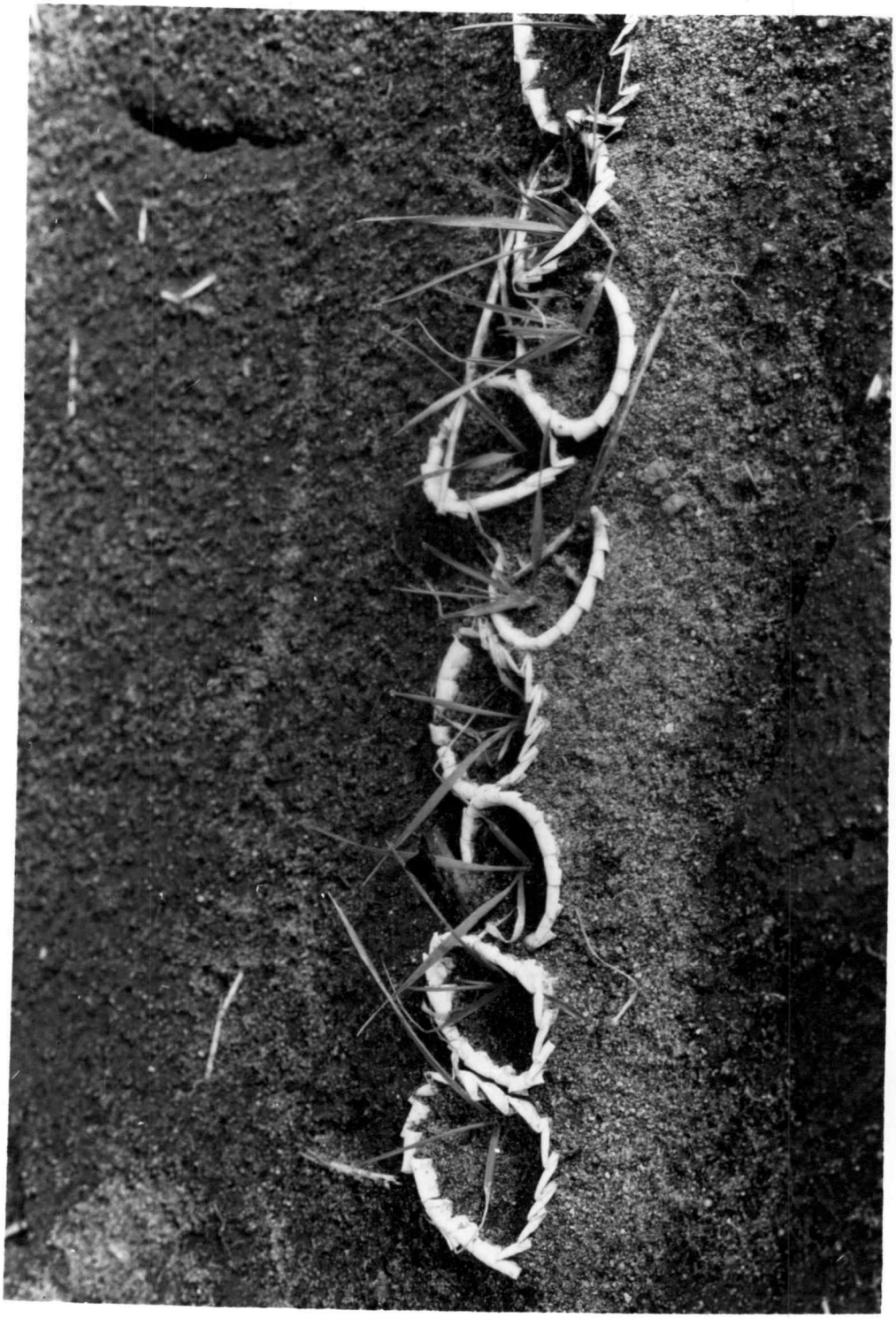
Precipitation and temperature information was taken from data recorded at La Grande, Pendleton Experiment Station, Sherman Experiment Station, and North Willamette Experiment Station (U. S. Weather Bureau, 1971, 1972).

Results

Effect of Soil Type on Sporulation

Under controlled environmental conditions in the laboratory there was a linear increase in spore production from naturally infected

Figure 7. Field inoculation technique using paper cups filled with soil infested with Cercosporella herpotrichoides.



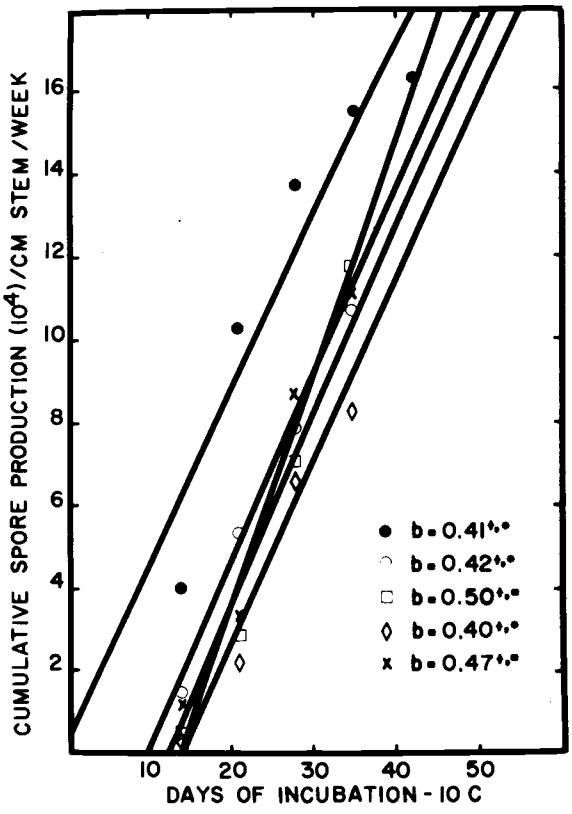
stubble when incubated at 10 C on soil and sand when cumulative spore production was plotted as a function of time (Figure 8). There was no significant difference in the rate of spore production among the soils and sand (analysis of covariance). Differences in relative amounts of sporulation were observed. The number of spores produced on infected stubble on sand was significantly greater ($p = 0.05$) than the other four soils, and the number of spores produced on infected stubble on soil from CASE and NWES was significantly greater ($p = 0.05$) than the amount produced on soil from SES (analysis of covariance). Thus the number of spores produced and not the rate of spore production was altered by the different soils.

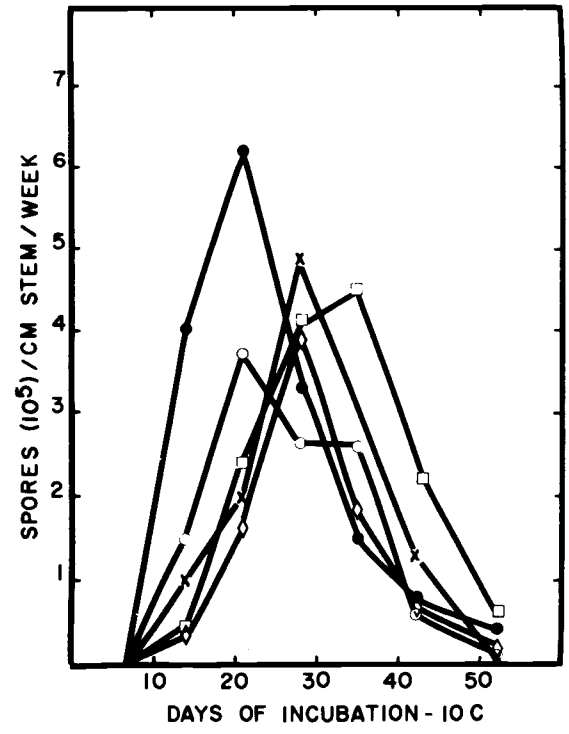
Under optimum conditions of temperature and moisture, the onset of sporulation did not occur until after the first week (Figure 9). The soils fell into two groups with respect to peaks in spore production. On sand and on the sandy loam soil from CASE, the peak in sporulation was reached by the end of the third week whereas there was an additional one week delay before the maximum amount of sporulation was reached on the other three soils.

The seasonal sporulation potential on wheat stubble naturally infected by C. herpotrichoides was also determined under field conditions at PES, SES and NWES locations. Maximum levels of sporulation diminished after 60 days for the soils and sand at NWES and decreased to minimal levels after 100 days (Figure 10-A). At PES and SES there

Figure 8. Rate of spore production by Cercosporella herpotrichoides from infected stubble incubated at 10 C on five different soils: sand (solid circles); CASE (open circles); PES (open squares); SES (diamonds); and NWES (X). Correlation coefficients and regression coefficients are significantly positive at the 5% level of probability for those marked + and *, respectively.

Figure 9. Sporulation of Cercosporella herpotrichoides from infected stubble on five different soils incubated at 10 C: sand (solid circles); CASE (open circles); PES (open squares); SES (diamonds); and NWES (X).


CUMULATIVE SPORE PRODUCTION (10^4)/CM STEM/WEEK
2
4
6
8
10
12
14
16
10
20
30
40
50
DAYS OF INCUBATION - 10 C
b = 0.41
b = 0.42
b = 0.50
b = 0.40
b = 0.47


SPORES (10^5)/CM STEM/WEEK
1
2
3
4
5
6
7
10
20
30
40
50
DAYS OF INCUBATION - 10 C

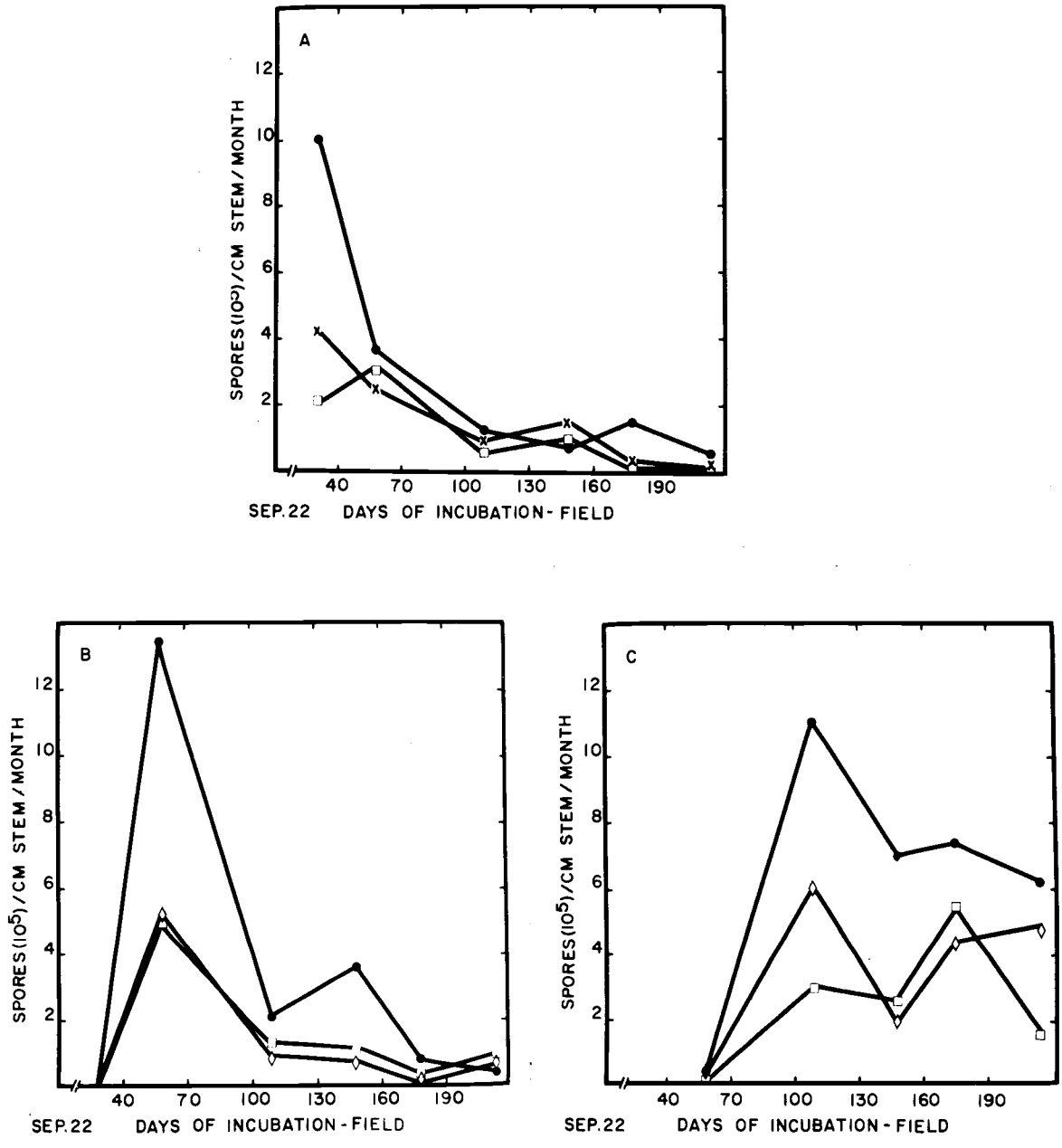


Figure 10. Sporulation of Cercosporella herpotrichoides from stubble incubated in the field on different soils at three locations: sand (solid circles); PES (open squares); SES (diamonds); NWES (X). A) North Willamette Experiment Station (NWES); B) Pendleton Experiment Station (PES); C) Sherman Experiment Station (SES).

were two sporulation pulses; a major one in the late fall or early winter and a mild one in the spring (Figure 10-B and C). The fall sporulation pulse at PES preceded the one at SES by two months whereas the spring sporulation pulse occurred at approximately the same time. At both locations the fall sporulation pulse was more dramatic than the spring pulse.

Cumulative spore production on wheat stubble naturally infected with C. herpotrichoides from October to April was determined for each of the soils and sand at each location. From the onset of optimum conditions for sporulation, moderately cool temperatures and wet weather (Rowe, 1972) the rate of spore production was linear for each of the soils and sand at each location (linear regression analysis) (Figure 11-A, B and C). There was a significantly greater ($p = 0.01$) rate of sporulation from the infected stubble on sand when compared with the soils at the PES and SES locations, but there was no difference in rate of sporulation between the soils (analysis of covariance). At NWES there was no significant difference in rate of sporulation between the sand and the soils or between soils. Differences were observed on relative amounts of sporulation from the soils and sand at each location. At NWES there was a significantly greater number of spores ($p = 0.01$) produced on sand when compared with the soils, and the sandy shot loam at NWES produced more spores than did the silt loam soil of PES ($p = 0.01$). The number of spores produced by sand

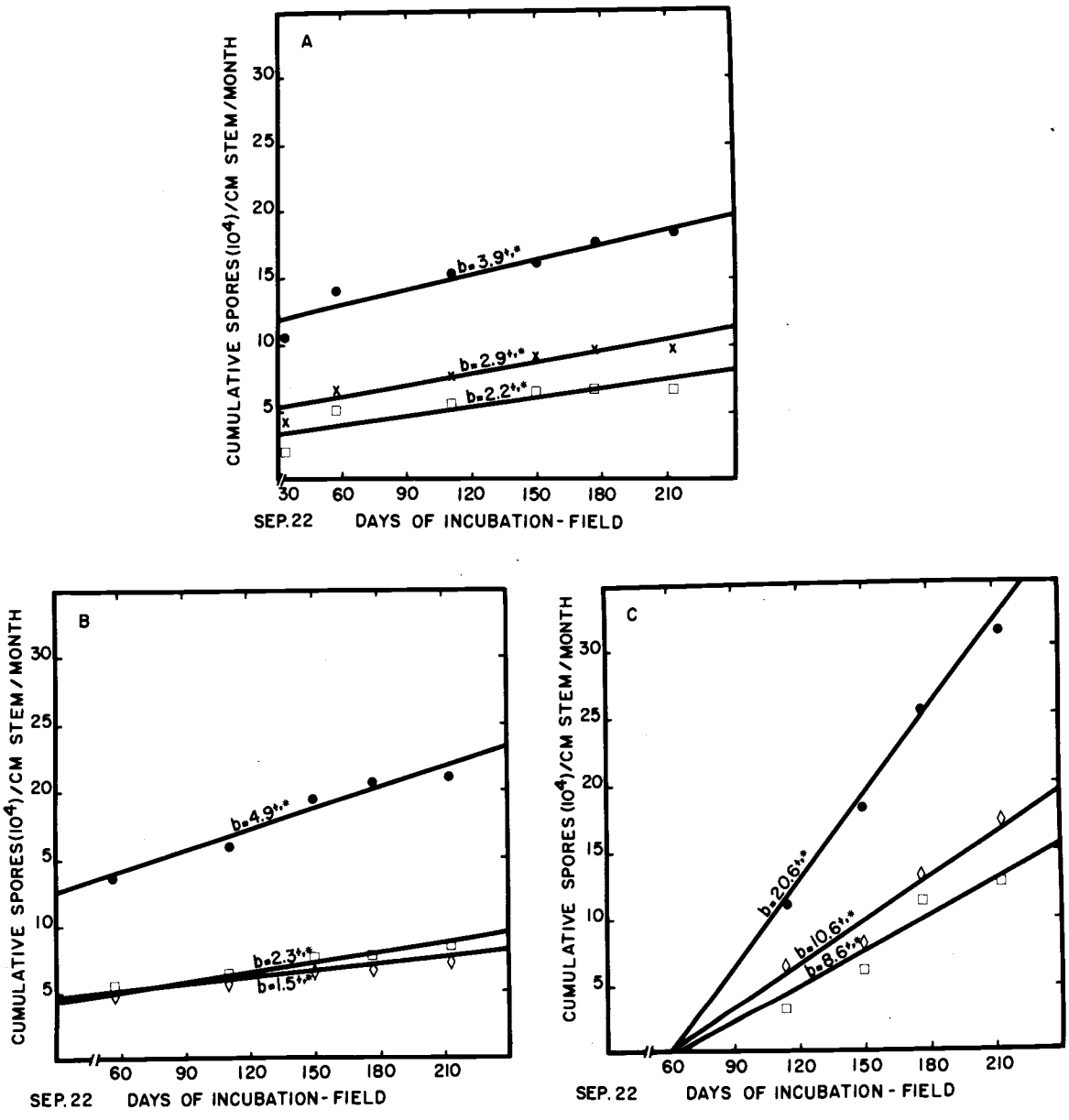


Figure 11. The rate of spore production by Cercosporella herpotrichoides from infected stubble incubated in the field on different soils at three locations: sand (solid circles); PES (open squares); SES (diamonds); NWES (X). A) North Willamette Experiment Station (NWES); B) Pendleton Experiment Station (PES); C) Sherman Experiment Station (SES).

at SES was significantly greater ($p = 0.01$) from the amount produced by the soils and the soils differed significantly ($p = 0.05$) from each other in their amount of sporulation. Sporulation from stubble on sand at PES was significantly greater than on the soils ($p = 0.01$); however, there was no significant difference between the soils at this location.

Survival of Conidia

Because conidia of C. herpotrichoides produced on infected stubble may be splashed by rain onto the soil surface and may come into contact with the host as soil-borne inoculum, it is important to know how long these potential infective units survive. Therefore, survival of conidia in the parent soil at each location was determined. A washed spore suspension of three-week-old conidia of C. herpotrichoides was sprayed onto glass slides and allowed to air dry. Immediately after drying the slides were placed vertically into screened soil and covered with one inch of soil. The slides were removed from the soil monthly and examined for the number of viable conidia. Conidia were considered lysed when they were weakly or no longer stained with Fabil staining solution, otherwise they were considered viable. Using the equation reported by Byther (1968), survival curves were determined from observations on lysed and viable conidia. A few spores of C. herpotrichoides remained viable in the

different soils for approximately four months (Figure 12). However, fifty percent of the spores, especially those that did not germinate, underwent autolysis within 30 days, and by the middle of February only 10% of the conidia remained viable. The different soils had no effect on the rate of survival.

Because infected stubble is the primary inoculum source, additional observations were made on the length of survival of conidia on stubble in contact with the soil. The four soils and sand were moistened to 75% MHC and incubated at 21 C for one week to establish a biological equilibrium. Sections of infected stubble 1 cm long were then placed on the surface of the soils, and the containers were covered with polyethylene and kept in the dark at 10 C for 63 days. Percent lysed spores was: sand, 0%; CASE, 25%; NWES, 21%; PES, 80% and SES, 93%. The length of viability of conidia differed markedly between the sandy soils and the silt loam soils.

Effect of Inoculum Density on Disease Incidence

Infection by soil-borne conidia of C. herpotrichoides was demonstrated at all three locations. Infections occurred on tillers in contact with the infested soil (Figure 13). At PES and for both the sand and parent soil at CASE, of those plants infected, 92% of the infections had penetrated the leaf sheaths and became established in the culm tissue. At SES only 60% of the infections had penetrated into the culm tissue.

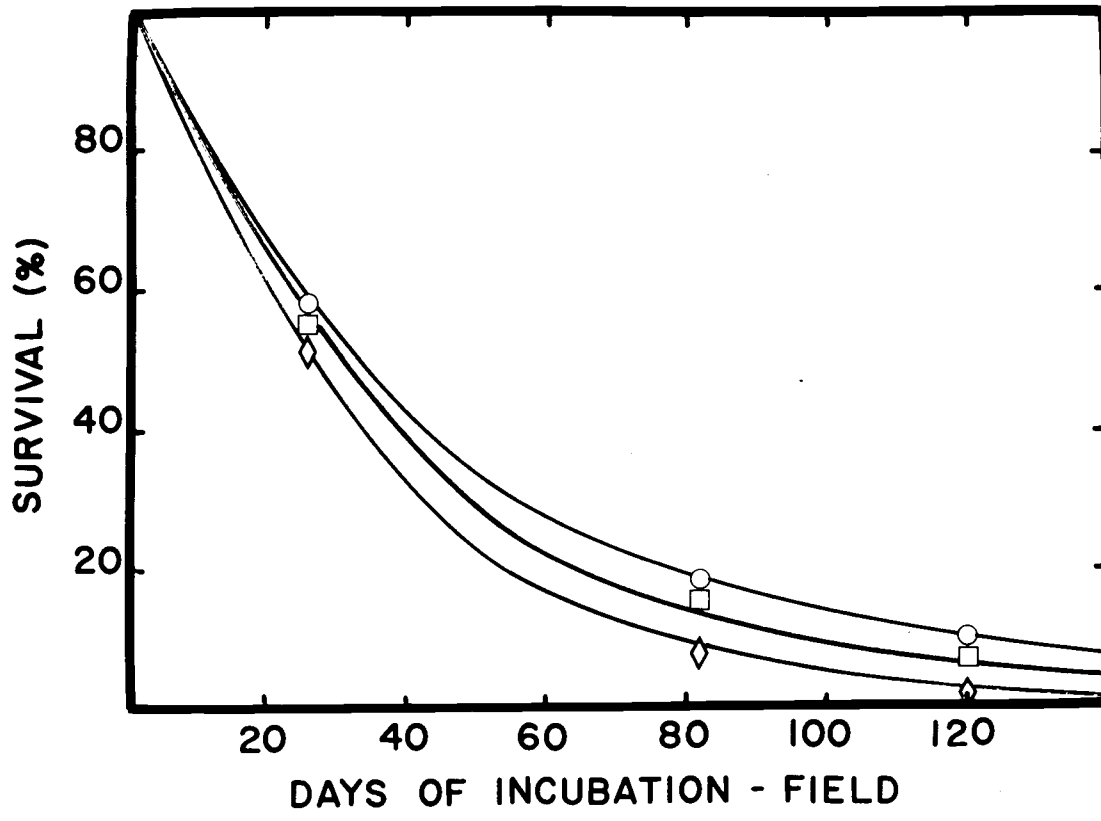


Figure 12. Influence of different soils on survival of conidia of Cercosporella herpotrichoides: CASE (open circles); PES (open squares) and SES (diamonds).

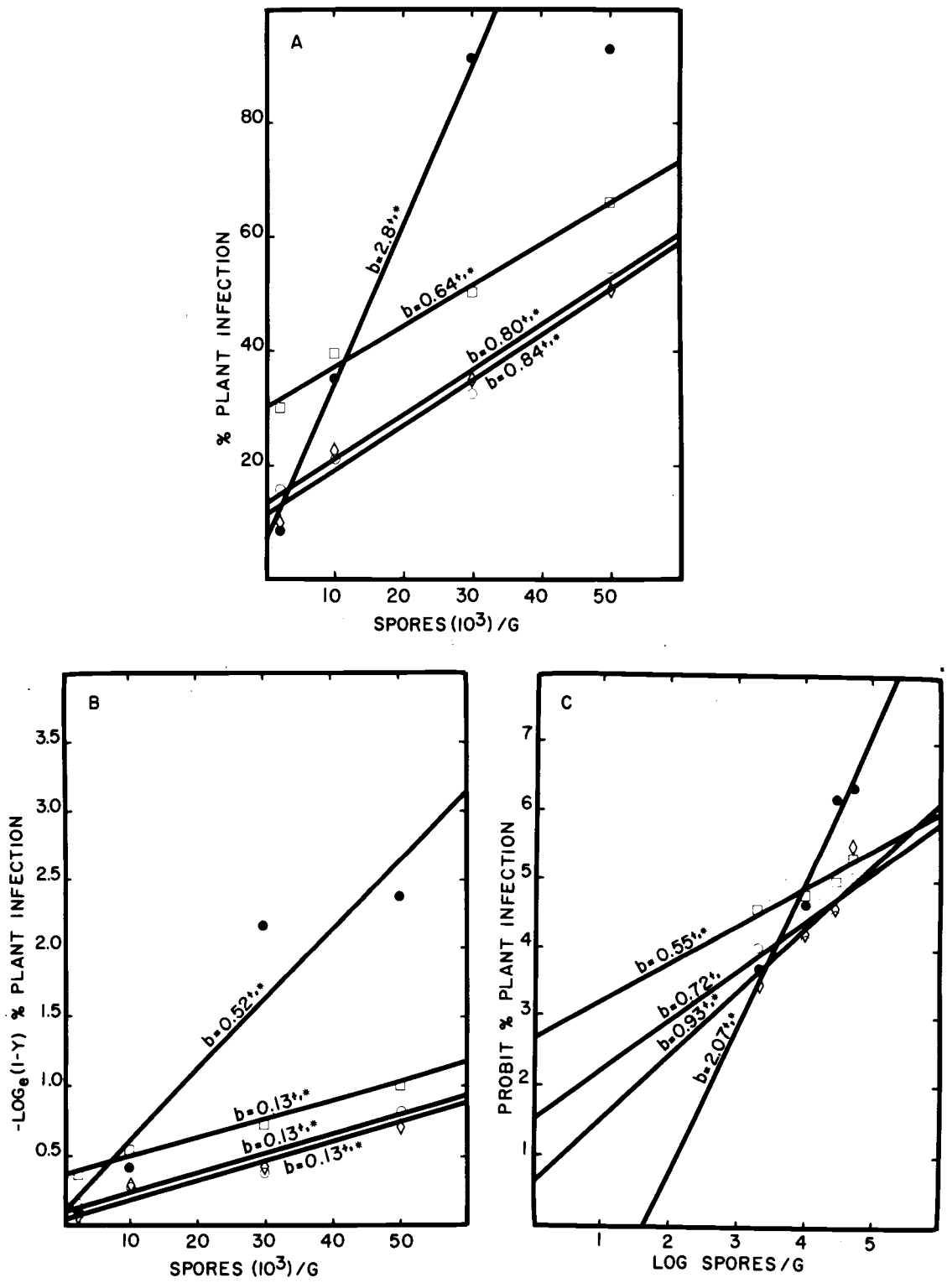
Figure 13. Symptoms resulting from infection of wheat by soil-borne inoculum of Cercosporella herpotrichoides.

Actual death of the wheat plants due to infection was observed only with sand at the CASE location. This amounted to an average of 43% kill with the greatest percent death occurring with the higher inoculum densities of 30,000 and 50,000 spores/g of sand. Field observations indicated that this was due to severe fall infection which resulted in increased susceptibility to winter kill (Lange-De La Camp, 1966).

Increases in inoculum density from 2000 to 50,000 spores/g of soil caused a linear increase in percent plant infection for all three soils at the three locations. A linear relationship was obtained for the inoculum density range of 2000 to 30,000 spores/g of sand when plotted as a function of percent infection (Figure 14-A). The slopes for each of the soils ranged from 0.64 to 0.84 whereas it was 2.8 for sand (inoculum density range of 2000 to 30,000 spores/g of sand) (linear regression analysis). The slopes for the soils did not differ significantly but the slope obtained for sand ($b = 2.8$) differed significantly ($p = 0.05$) from all three soils (analysis of covariance). The position of the regression line for sand differed from PES, SES and CASE ($p = 0.05$) and the position of the regression line for PES differed significantly ($p = 0.05$) from SES and CASE (analysis of covariance).

Because the probability of multiple infections increases as the inoculum density increases, the multiple infection transformation of Gregory (1948) was used to correct for the faster rate of increase in number of infections observed as the inoculum increased (Figure 14-B).

Figure 14. The relationship of soil-borne inoculum of Cercosporella herpotrichoides and disease incidence at various inoculum densities at three locations: sand, La Grande, Oregon = CASE (solid circles); Imbler sandy loam, La Grande, Oregon = CASE (open circles); Walla walla silt loam, Pendleton Experiment Station = PES (open squares); Walla Walla silt loam, Sherman Experiment Station = SES (diamonds). A) Arithmetic plot; B) semi-logarithmic transformation; C) log-probit transformation.


A
% PLANT INFECTION
SPORES (10³) /G
b=2.8†,*
b=0.64†,*
b=0.80†,*
b=0.84†,*
B
-LOGe(1-Y) % PLANT INFECTION
SPORES (10³) /G
b=0.52†,*
b=0.13†,*
b=0.13†,*
b=0.13†,*
C
PROBIT % PLANT INFECTION
LOG SPORES/G
b=0.55†,*
b=0.72†,
b=0.93†,*
b=2.07†,*

The regression line drawn from points on the slope for each of the soils and sand passed near the origin suggesting independent action of propagules. According to Van der Plank (1963) a slope greater than 1.0 would pass to the right of the origin indicating synergism.

A logarithmic-probability plot (Figure 14-C) gave a near linear relationship between the logarithm of the inoculum density and percent infection. The slopes for the soils ranged from 0.55 to 0.93 whereas sand had a slope of 2.07 (inoculum density range of 2000 to 50,000 spores/g of sand) (linear regression analysis). The slope for sand as well as its position differed significantly ($p = 0.05$) from each of the soils. There were no significant differences between slopes or their position when all soil combinations were compared. According to Baker (1971) slopes greater than 2.0 would indicate synergism operating at the rhizosphere level and slopes greater than 1.31 at the ED_{50} would indicate synergism operating at the rhizoplane level.

Discussion

Controlled environmental studies on cumulative spore production from infected stubble showed a significant difference in number of spores produced among soils, but there were no differences in rates. Therefore, the soils tended to suppress the quantity of spores produced, but not the rate at which the spores were produced. Thus the time and rate of spore production from infected stubble lying on the

soil surface is probably more dependent on weather conditions than on soil type.

Sporulation of C. herpotrichoides from infected stubble occurs in the field during periods of favorable temperatures and wet weather (Rowe, 1972). In the wheat growing regions of western Oregon, ideal conditions favoring active sporulation occur nearly continuously from October to April whereas in central and eastern Oregon, ideal conditions prevail sporadically. At NWES in western Oregon the peak in sporulation occurred by November and the inoculum was exhaused by December (Figure 10-A). The rate of spore production on naturally infected stubble under continuous incubation of 100% RH and 10 C as reported by Rowe (1972) is similar to the results found at NWES for all soils. Hence, the late date of seeding which is practiced in western Oregon coupled with the early onset of ideal conditions for sporulation means that inoculum sources would nearly be exhausted by the time when the wheat plants were susceptible to infection.

In central and eastern Oregon where favorable conditions for sporulation occur sporadically, the inoculum source is not exhausted and thus spores are available for infection from October to April. There appears to be a major fall and spring infection period (Hartz, 1969; Fehrmann and Schrodter, 1971; Rowe, 1972) and this can be correlated with fall and spring sporulation pulses. The annual "rhythm of sporulation" with peaks in the autumn and spring has been reported

by Hartz (1969) with his studies on conidial production from straw infected with C. herpotrichoides. The spring sporulation pulse, even though it is of a lesser intensity than the fall pulse, provides inoculum for above ground infections and is important epidemiologically if late date of seeding is practiced. However, below ground infections resulting from inoculum produced in the spring are probably of no consequence because of the lack of erosion at this time of year.

Under field conditions the silt loam soils at PES and SES had a suppressive effect on both the rate and amount of sporulation. In the laboratory there was a greater amount of lysis of spores on stubble when in contact with these two soils. The rate as well as the amount of sporulation on sand at SES was significantly greater ($p = 0.01$) than sporulation on sand at PES or NWES (Figure 11-A, B and C). This difference was probably due to the failure to record spores splashed away by rain. At SES the January sample, that accounted for the major proportion of spores counted, was taken previous to a major rainfall whereas at PES and NWES, samples were taken most of the time after a period of intense rainfall.

Inoculum is produced on infected stubble both underground (Fehrmann and Schrodter, 1971) as well as on the soil surface, with the highest levels of sporulation occurring in the fall (Figure 10-A, B and C). Rain and wind erosion into the deep furrow rows occurs in the fall, thus inoculum produced in the fall can come in contact with a

below ground infection court. Because the furrows are usually eroded in by spring, soil-borne infections by inoculum produced in the spring probably does not occur. Even if some erosion occurred in the spring the number of plants infected would be minor because: (i) the spring sporulation pulse is of a small intensity and (ii) significant numbers of conidia of C. herpotrichoides do not survive for longer than four months when buried in the soil (Byther, 1968; Figure 12); therefore, spring infection by soil-borne conidia produced in the fall would probably not occur.

When percent infection and inoculum density were plotted arithmetically or as a probability-logarithmic transformation, there was a linear relationship for the sandy loam soil at CASE (Figure 14-A and C). For sand at the same location, however, percent infection was linear only for the inoculum density range of 2000 to 30,000 spores/g of sand. The slope as well as its position obtained for sand in both of these transformations ($b = 2.8$ and 2.07, respectively) was significantly different ($p = 0.05$) from the sandy loam soil. If these differences between sand and the sandy loam soil are real, conclusions may be drawn on the mode of action of C. herpotrichoides on infection of wheat in sand and the sandy loam soil: (i) C. herpotrichoides was significantly more pathogenic in sand than in the sandy loam soil and/or (ii) the virulence of the pathogen may have been greater in sand since death of the wheat plants occurred. However, of those plants that survived,

there were no visual differences in degree or extent of lesion formation on a tiller basis when sand and the soil treatments were compared.

Controlled environmental studies in the laboratory on the infection of wheat by soil-borne conidia of C. herpotrichoides showed that the inoculum was under the influence of the rhizosphere (Chapter 1), and according to Baker (1972) a slope greater than 2.0 on a probability-logarithmic plot is expected for a rhizosphere influence if synergism is responsible for the higher number of infections. Since environmental conditions could not be responsible for the differences observed, the significant difference between the slope and its position for the sand when compared with the sandy loam soil in both transformations suggest that synergism may be responsible for the higher number of infections observed when the infection court environment is sand. Byther (1966) reported that anastomosing of conidia of C. herpotrichoides occurred when high spore concentrations were sprayed onto glass slides and buried in the soil. If lysis is a factor limiting the density of C. herpotrichoides spores in soil as demonstrated with survival studies (Byther, 1968; Figure 12), the distance between spores could be drastically increased in a relatively short period of time. Also, if anastomosis of conidia occurs in sand because the distance between propagules is not reduced by lysis and if anastomosis is responsible for a higher inoculum potential, synergism may be operative.

However, because of the high inoculum densities used in this study and the lack of demonstratable synergism in the sandy loam soil, synergism probably does not occur in nature with this pathogen.

If the position of the regression line in an arithmetic plot of percent infection and inoculum density reflects the relative amount of disease, then there was a significant difference ($p = 0.05$) in the amount of disease at PES when compared with SES and CASE, but not between the latter two locations (Figure 14-A).

The semi-logarithmic transformation of Gregory (1948) can be used to determine the number of propagules required to produce on the average of one infection per wheat plant. In sand 18,000 spores/g were required to produce one infection whereas 65,000 spores/g of soil were required to incite one infection when the infection court was surrounded by the sandy loam soil (Figure 14-B). The sandy loam soil had a suppressive effect on infection when compared with the sand. There were no difference in number of spores required for one infection per wheat plant at SES or the parent soil at CASE but fewer spores were required to produce one infection at PES than at the other two locations.

The lower portion of the wheat plant beneath ground with its scenescing coleoptile and leaf sheaths provide the principle sites for infection by soil-borne inoculum of C. herpotrichoides. Lesions were observed both on below ground leaf sheaths as well as on the culm

tissue. Even though the degree of severity of infection was the same for PES and CASE, there was a significant difference in the amount of disease between these two locations. There was no difference in amount of disease between SES and CASE locations, but there was differences in severity of disease.

Under controlled environmental conditions there is good evidence that soil reduces the inoculum potential of C. herpotrichoides when compared with sand (Chapter 1). However, under field conditions the difference in suppressiveness of soils can be modified. The differences observed, therefore, in field studies on percent infection as well as number of spores required to produce an average of one infection per wheat plant at PES when compared with SES and CASE locations may be reflective of the weather patterns at these locations. Because significant numbers of conidia of C. herpotrichoides do not survive for longer than four months, weather patterns from October through January need be considered. The soil environment is less subject to changes in temperature and moisture than the above ground environment. The soil temperature during the infection period from October to the end of January would probably stay in the optimal temperature range for infection for a greater length of time. Moisture for infection would be limiting only until after the first substantial rainfall. If sporulation from surface borne stubble is reflective of the amount of moisture in the soil, then soil moisture was never limiting at PES and

was delayed until the beginning of November at CASE and the end of November at SES.

Under field conditions, the soil is probably not the dominant factor responsible for the incidence of below ground infections of wheat by C. herpotrichoides. However, under certain environmental conditions the suppressiveness of the different soils contribute to the lower levels of below ground infections realized in the different wheat growing regions of the Pacific Northwest. Thus during seasons when conditions are unfavorable for epidemic development above ground, a significant amount of disease can occur below ground. Hence, the amount of below ground infection would be dependent, in part, on the climatic conditions as well as the soil type. Finally, soil-borne infection of wheat by C. herpotrichoides is probably important epidemiologically in that it serves as a means of maintaining a resevoir of inoculum for future epidemics when environmental conditions become favorable for above ground infections.

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